XI_SPLC

CRS_Granada_2016

XI Spanish-Portuguese Conference on Controlled Drug Delivery

"Revolutionary Approaches in Nanomedicine Development"

Granada, Spain, 21th-23th of January 2016

Conference Book of Abstracts

CRS Spanish-Portuguese Local Chapter

COLEGIO OFICIAL DE FARMACÉUTICOS DE GRANADA

FACULTAD DE FARMACIA UNIVERSIDAD DE GRANADA

UNIVERSITAS GRANATENSIS 1531

HefaGRa

http://congress2016.splc-crs.org

EDITORIAL: Ediciones Sider S.C.
e-mail: edicionessider@yahoo.es
Impreso en : imprime, pasaje de recogidas n6 - 958 26 79 16 Granada

AUTOR: Spanish-Portuguese Local Chapter of the Controlled

TITULO: XI Spanish-Portuguese Conference on Controlled Drug Delivery “Revolutionary Approaches in Nanomedicine Development”

ISBN: 978-84-943468-8-0

DEPOSITO LEGAL: GR 41-2016

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"Revolutionary Approaches in Nanomedicine Development"

January 21 – 23, 2016. Granada (Spain)

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XI Spanish-Portuguese Conference on Controlled Drug Delivery
"Revolutionary Approaches in Nanomedicine Development"
January 21 – 23, 2016. Granada (Spain)

PROGRAMME

Site: **Ilustre Colegio Oficial de Farmacéuticos de Granada**. Calle San Jerónimo, 18, 18001 Granada, Spain. Phone: +34-958 80 66 21.

Day 1. January 21st, 2016 (Thursday)
15.30 h. – 17.00 h. Registration and Poster Mounting.

17.00 h. – 17.15 h. Welcome.
Mrs. Pilar Aranda Ramírez. Rector of the University of Granada.
Mrs. Ana del Moral García. Dean of the Faculty of Pharmacy of the University of Granada.
Mr. Antonio Mingorance Gutiérrez. President of the *Consejo Andaluz* de *Colegios Oficiales de Farmacéuticos*.
Mr. Manuel Fuentes Rodríguez. President of the *Ilustre Colegio Oficial de Farmacéuticos de Granada*.
Mrs. María Adolfina Ruiz Martínez. President of the SPLC-CRS.

17.15 h. – 18.15 h. Plenary 1 (P1). "Magnetic Nanoparticles in Drug Delivery and Disease Diagnostics. Present and Perceived Future". Mr. Ángel V. Delgado. Department of Applied Physics, University of Granada, Spain.
Chairman: Mr. José Luis Pedraz Muñoz (University of the Basque Country).

18.15 h. – 19.00 h. PhD Thesis Award Ceremony (Co-sponsored SPLC-CRS and Faculty of Pharmacy of the University of Granada). "Optimization of cell microencapsulation in terms of biosafety, biomimesis and applicability in therapeutic targets of central nervous system". Mr. Edorta Santos Vizcaíno. Laboratory of Pharmacy and Pharmaceutical Technology, University of the Basque Country, Spain.
Chairmen: Mrs. María Adolfina Ruiz Martínez and Mrs. Ana del Moral García.

19.00 h. Guided tour. "Historic centre of Granada city".

20.00 h. Welcome drink at the "Hotel AC Palacio de Santa Paula" (Calle Gran Vía de Colón, 31, Granada).

Day 2. January 22nd, 2016 (Friday)

9.00 h. – 10.00 h. Plenary 2 (P2). "Taking the most of nanotechnology-based drug delivery for anticancer therapy". Mr. João Nuno Moreira. Center for Neuroscience and Cell Biology (CNC), Faculty of Pharmacy, University of Coimbra, Portugal.

Chairman: Mrs. Mireia Oliva Herrera, University of Barcelona, Spain.

10.00 h. – 11.00 h. Session I. Discovery and Development of Nanomedicines.

Chairmen: Mr. Manuel Guzman Navarro (University of Alcalá de Henares) and Mrs. Araceli Rita Delgado Hernández (University of La Laguna).

IL1. "Protecting pharmaceutical inventions". Mr. Juan Antonio Muñoz Orellana. Oficina de Transferencia de Resultados de Investigación. University of Granada, Spain.

IL2. "Is the zebrafish a suitable research model for drug discovery in nanomedicine?" Mr. Juan María Alfaro Sánchez. Commercial Manager and Scientific Advisor, Neuron Bioservices, Spain.

IL3. "Carbosilane dendritic systems. A synthetic platform for the development of nanomedicines". Mr. Francisco Javier de la Mata. Department of Organic Chemistry and Inorganic Chemistry. University of Alcalá de Henares. Ambiox Biotech – Nanotechnology & AIDS, Spain.

11.00 h. – 11.30 h. Coffee break and poster viewing.

11.30 h. – 13.30 h. Session II. Young Section SPLC-CRS.

Chairmen: Mrs. Matilde Durán Lobato (University of Sevilla), Mr. Juan Aparicio Blanco (Complutense University of Madrid), Mrs. Ana Olivera Fernández (University of Santiago de Compostela), and Mrs. Sandra Cristina Campos de Jesus (University of Coimbra).

IL4. "A Personal Perspective of the Controlled Release Society" Mrs. María José Alonso Fernández. Department of Pharmacy and Pharmaceutical Technology, University of Santiago de Compostela, Spain.

IL5. "Multivariate Development of Chitosan Nanoparticles for the Delivery of Complex Therapeutic Human Enzymes" Mr. Paulo Roque Lino. Research Institute for Medicines, Faculty of Pharmacy, University of Lisbon, Portugal.

IL6. "Nanomedicine applied in cancer treatment: experimental and clinical assays" Mrs. María del Carmen Leiva Arrabal. Department of Anatomy and Human Embriology, University of Granada, Spain.

Oral Communications.

O1. Melanoma cancer vaccine based on multifunctional nanoparticles for antigen delivery and immunomodulation. Authors: Carina Peres, Joana M. Silva, Liane Moura, Luís Graça, Rogério Gaspar, Verónique Preat, Helena F. Florindo.

O2. Locally-controlled release of Palladium-labile prodrug for ovarian carcinoma treatment. Authors: Ana Maria Pérez-López, Belén Rubio-Ruiz, Thomas Bray, Sam Myers, Craig Fraser, Asier Unciti-Broceta.

O3. Microencapsulated solid lipid nanoparticles as a novel hybrid system for pulmonary antibiotic administration. Authors: D.P. Gaspar, L.M.D. Gonçalves, P. Taboada, C. Remuñán-López, A.J. Almeida.
O4. Nanoparticle nose-to-brain transport across an in vitro nasal model. Authors: Oihane Gartziandia, Susana Patricia Egusquiaguirre, John Bianco, José Luis Pedraz, Manoli Igartua, Rosa María Hernandez, Véronique Préat, Ana Beloqui.
O5. Graphene oxide nanoparticles coated with serum improves in vitro and in vivo the functional activity of microencapsulated C_2C_{12} myoblasts. Authors: Laura Saenz del Burgo, Jesús Ciriza, Argia Acarregui, Haritz Gurruchaga, Rosa María Hernández, Gorka Orive, Jose Luis Pedraz.
O6. Detection of intracellular apoptotic activity using polystyrene nanoparticles. Authors: Rosario M. Sánchez-Martín, Juan Manuel Cardenas-Maestre, Ana M. Perez-Lopez, Mark Bradley.
O7. Anxiolytic-like effect of liposomal formulation containing Nimodipine in mice. Authors: Lina C. G. e A. I. Moreno, Rivelilson M. Freitas, Juan M. Irache, Nereide S. Santos Magalhães.
O8. Determination of the most suitable cell load for mesenchymal stem cell D1 microencapsulation systems: in vitro and in vivo study. Authors: T. López-Méndez, E. Santos, F.J. Blanco, J.L. Pedraz, G. Orive, R.M. Hernández.

13.30 h. – 15.00 h. Lunch at "El Rincón de Lorca" (Calle Angulo, 3, Granada).

15.00 h. – 16.00 h. Plenary 3 (P3). "Ultrasound-enhanced drug delivery using nanoparticle-stabilized microbubbles". Mrs. Ruth Schmid. SINTEF Materials and Chemistry, Sector for Biotechnology and Nanomedicine, Department of Polymer Particles and Surface Chemistry, Norway.
Chairman: Mrs. María José Alonso Fernández (University of Santiago de Compostela).

16.00 h. – 17.45 h. Session III. New Strategies in the Treatment of Cancer and Infectious Diseases.
Chairman: Mrs. Maria Manuela de Jesus Guilherme Gaspar (University of Lisboa).
IL7. "Nanomedicine in the future of pediatric oncology". Mr. Ángel Montero Carcaboso, Department of Pediatric Hematology and Oncology. Hospital Sant Joan de Déu. Barcelona, Spain.
IL8. "Combination of Chemical Therapies and Gene Silencing for the Development of Novel Acanthamoeba Treatments". Mr. Jacob Lorenzo Morales. University Institute of Tropical Diseases and Public Health of The Canary Islands (IUETSPC), University of La Laguna, Tenerife, Spain.

Oral Communications.
Chairmen: Mrs. Mª. Consuelo Montejo Rubio (CEU San Pablo University), and Mrs. Carmen María Évora García (University of La Laguna).
O9. Lipid peroxidation and protein carbonylation in rat liver after treatment with citrate-stabilized gold nanoparticles. Authors: Carlos López-Chaves, Cristina Sánchez-González, Juan Soto-Alvaredo, María Montes-Bayón, Jorg Bëttmer, Juan Llopis.

O10. The role of helper lipids in the intracellular disposition and transfection efficiency of niosome formulations for gene delivery to retinal pigment epithelial cells. Authors: G. Puras, E. Ojeda, M. Agirre, J. Zarate, S. Grijalvo, R. Eritja, L. DiGiacomo, G. Caracciolo, J.L. Pedraz.

O11. Development of polymeric vaccine delivery nanosystems for HIV/AIDS control through dendritic cell modulation. Authors: A.I. Matos, C. Peres, A.E. Ventura, Ana S. Viana, P. Borrego, L.C. Silva, N. Taveira, H.F. Florindo.

O12. Cytotoxicity evaluation of Cannabidiol-loaded lipid nanocapsules on the human glioma cell line U-373. Juan Aparicio-Blanco, Ana Isabel Torres-Suárez.

O13. Formulation, characterization and controlled release studies of vitamins microparticles prepared with different biopolymers by spray drying technique. Authors: Berta N. Estevinho, Ioana Carlan, Fernando Rocha.

O14. Poly(anhydride) nanoparticles for oral immunotherapy against peanut allergy. Ana Brotons-Cantó, Nekane Martín-Arbella, Jose Matías, Carlos Gamazo, Juan M. Irache.

O15. A novel nanotechnology tool to assess drug promiscuity in cancer. Teresa Valero, Victoria Cano-Cortés, Asier Unciti-Broceta, Rosario Sánchez-Martín.

O16. PEG-modified PLA nanoparticles for site-specific functionalization. João Conniot, Sheiliza Carmali, Teresa S. Barata, Liana C. Silva, Steve Brocchini, Helena F. Florindo.

O17. Preliminary studies for the development of a new formulation for patients on oncologic treatment. Roser Sanz Casañas, Beatriz Clares Naveros, Mireia Mallandrich, Ana C. Calpena Campmany.

17.45 h. – 18.15 h. Coffee break and poster viewing.

18.15 h. SPLC-CRS General Assembly.

21.00 h. Conference dinner at “La Chumbera” (Camino del Sacromonte, 107, Granada).

Day 3. January 23rd, 2016 (Saturday)

9.00 h. – 10.00 h. Plenary 4 (P4). “Recent advances on topical, transdermal and nail drug delivery”. Mrs. Mª Begoña Delgado Charro, Department of Pharmacy and Pharmacology, Faculty of Sciences, University of Bath, Great Britain.

Chairman: Mrs. Alicia López Castellano, CEU Cardinal Herrera University, Spain.

10.00 h. – 11.30 h. Session IV. Advanced Nanotechnologies in Biomedicine.

Chairman: Mr. António José Leitão Neves Almeida (University of Lisboa).

IL9. “Targeted non-viral vectors for gene delivery”. Mrs. María Concepción Tros de Ilarduya Apaolaza, Department of Pharmacy and Pharmaceutical Technology, University of Navarra, Spain.

IL10. “Medical devices as drug delivery platforms”. Mrs. Carmen Isabel Álvarez Lorenzo. Deparment of Pharmacy and Pharmaceutical Technology, University of Santiago de Compostela, Spain.

IL11. "Nanomedicines for modulating diabetes: nanoscale tools against macroscale disease". Mr. Bruno Sarmento. Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal.

Oral Communications.

Chairmen: Mrs. Ana Isabel Torres Suárez (Complutense University of Madrid), and Mrs. Rosa Mª Hernández Martín (University of the Basque Country).

O18. Bacteriocin-loaded microparticles: a promising approach to the treatment of infectious diseases. Authors: Mª José Martín, Raúl Cebrián, Beatriz Clares, Mª Adolfina Ruiz, Mercedes Maqueda, José L. Arias.

O19. PCL/chitosan nanoparticle adjuvant ability for HBsAg vaccines. Authors: Sandra Jesus, Edna Soares, Olga Borges.

O20. Development and optimization of gelatin scaffolds with dual action for tissue engineering and regenerative medicine. Authors: Pello Sánchez, José Luis Pedraz, Gorka Orive.

O21. Deformable liposomes increase vicenin-2 human skin penetration *ex vivo*. Authors: Ana Luiza Scarano Aguillera Forte, María José Vieira Fonseca, Norberto Peporine Lopes, Teresa María Garrigues Pelufo, Ana Melero Zaera.

O22. Doxorubicin and Edelfosine lipid nanoparticles act synergistically against osteosarcoma cell lines. Y. González-Fernández, E. Imbuluzqueta, M. Zalacain, L. Sierrasesúmaga, A. Patiño-García, M. J. Blanco-Prieto.

O23. Paromomycin liposomes vs. Ambisome®: *in vitro* and *in vivo* studies against *Leishmania infantum* infections. Authors: Maria Manuela Gaspar, Georgina Gomes Alves, Helena Castro, Ana Tomás, M. Eugénia Meirinhos Cruz.

O24. Understanding the interface of edible nanoemulsions to modulate the bioaccessibility of neuroprotective antioxidants. M. Plaza-Oliver, M.V. Lozano, V. Rodríguez-Robledo, L. Castro-Vázquez, J. González-Fuentes, P. Marcos, M.J. Santander-Ortega, M.M. Arroyo-Jiménez.

11.30 h. – 12.00 h. Coffee break and poster viewing.

12.00 h. – 13.15 h. Session V. Advanced Nanoplatforms in the Management of the Disease.

Chairmen: Mrs. Mª Dolores Ramona Torres López (University of Santiago de Compostela) and Mrs. Mª Rocío Herrero Vanrell (Complutense University of Madrid).

IL12. "Nanostructured materials for improved drugability of neuropeptides and immune-based interventions" Mr. David Pozo Pérez. Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER), Spain.

IL13. "Advances and opportunities of nanocarriers in the inflammatory process". Mrs. María Luisa González Rodríguez. Department of Pharmacy and Pharmaceutical Technology, University of Seville, Spain.

IL14. "Nanosystems in medical imaging: advances and prospects". Mr. Mazen M. El-Hammadi. Department of Pharmaceutics and Pharmaceutical Technology, Damascus University, Damascus, Syria.

13.15 h. – 13.30 h. Closing Ceremony. Oral and Poster Communication Prizes.

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PLENARY LECTURES

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PLENARY LECTURES

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Magnetic nanoparticles in drug delivery and disease diagnostics. Present and perceived future

Ángel V. Delgado

Department of Applied Physics, University of Granada, Spain.
e-mail: adelgado@ugr.es

Introduction: The number of biomedical applications of magnetic nanoparticles has grown exponentially in recent years. They participate of the main features of nanomaterials in this field: small size, access to places of the body far from their injection site, possible specificity, to mention just a few. On top of these, the nanostructures based on magnetizable (ferro- or ferrimagnetic) particles have the additional advantage of controlling their position, degree of aggregation, and even triggering their activity by means of external magnetic fields. This leads to specific fields of application of magnetic nanoparticles (MNPs) in the diagnostic and treatment of diseases including (the list is not extensive) [1-3]: drug delivery, magnetic embolization, enhanced resonance imaging, hyperthermia,… In a recent editorial in ACS Nano [4], P.S. Weiss warned researchers in the field of nanoscience and nanotechnology: "*We are in danger of letting science fiction writers define what is nano. That could be a disaster of epic proportions*". In other words, we must be aware of the limits and challenges of nanotechnology, make them clear to the society and promote progress on them. In this contribution, it is intended to describe such a progress in my (and others´) group(s), and to fix some of the open questions still remaining.

Materials and Methods: It will be described some of the magnetic particles which have been envisaged for pharmaceutical applications. These will include magnetite, maghemite, iron, or iron/magnetite. In addition to the obvious magnetic characterization, size, shape, and stability will also be considered. These magnetic basic units will be coated with the "active" shell, capable of making them biocompatible, reducing the action of the phagocytic mononuclear system on them, and serving as storage for the therapeutic load. Many different coatings have been tested, both polymeric [poly(D,L-lactide), alkylcyanoacrylates, chitosan, PLGA] and inorganic (silica, gold) in nature. The completeness of the coating will be mostly evaluated by means of zeta potential and hydrodynamic size determinations using dynamic light scattering techniques. The use of these particles in drug delivery will be tested with a wide range of drugs, and again, zeta potential determinations will be used, jointly with spectrophotometric measurements, as a suitable tool for loading and release properties. If there is a field where both European and American Administrations have approved the use of magnetic nanoparticles, this is contrast-enhanced magnetic resonance imaging (MRI). It will also be shown some results obtained with our particles in this application. Finally, the technique of magnetic hyperthermia will be presented, and the achievements possible will be discussed.

Results and Discussion: Our data, obtained both *in vitro* and *in vivo*, will show that magnetic nanovectors can be used for the transportation and release of Doxorubicin, Gemcitabine, 5-Fluorouracyl, Methotrexate, Bevacizumab, Paclitaxel, etc. A realistic point must be made here: only liposomes have been approved as drug delivery vehicles based on nanoparticles, and they are non-magnetic. Hence, attention will be paid to existing *in vitro* and *in vivo* tests of the suitability of these transport devices. Another field where approval exists is MRI. Results will be discussed, considering how we can improve on existing commercial contrast agents. Finally, it will be considered our results regarding magnetic hyperthermia, regarding its implementation and testing, as well as materials selection for this purpose.

Conclusions: As expected messages from this presentation, we will first focus on the versatility of magnetic particle preparation, concerning size, composition, and even shape. We will also show that the collection of available particles is magnified when the possible coatings are studied. The contrast in magnetic resonance is certainly afield with real perspectives of improving and the same can be said about hyperthermia. The presentation will also include an overview of existing information about the toxicity of these nanovehicles, and to what extent such potential toxic effects refrain the responsible authorities of approving the use of more than just a few designs.

Acknowledgements: Financial support from Junta de Andalucía, Spain, under Project PE-2012-FQM-0694 is gratefully acknowledged.

References:

[1] Durán JDG, Arias JL, Gallardo V, Delgado AV. J Pharm Sci **97** (2008) 2948.
[2] Davies ME. Mater Res Bull **37** (2012) 828.
[3] Petros RA, DeSimone JM. Nature Rev Drug Discov **9** (2010) 615.
[4] Weiss, PS. ACS Nano **9** (2015) 3397.

Taking the most of nanotechnology-based drug delivery for anticancer therapy

João Nuno Moreira

Center for Neuroscience and Cell Biology (CNC), Faculty of Pharmacy, University of Coimbra, Portugal.
e-mail: jmoreira@ff.uc.pt

Cancer remains as stressful condition and a leading cause of death in the western world. Actual cornerstone treatments of cancer disease rest as elusive alternatives, offering limited efficacy with extensive secondary effects as a result of severe cytotoxicity in healthy tissues. The advent of nanotechnology brought the promise to revolutionize many fields including oncology, proposing advanced systems for cancer treatment. Drug delivery systems rest among the most successful examples of nanotechnology. Throughout time they have been able to evolve as a function of an increased understanding from cancer biology and the tumor microenvironment. Marketing of Doxil® unleashed a remarkable impulse in the development of drug delivery systems. Since then, several nanocarriers have been introduced, with aspirations to overrule previous technologies, demonstrating increased therapeutic efficacy besides decreased toxicity. This contribution will provide an insightful vision on nanotechnology-based strategies for cancer treatment, approaching them from a tumor biology-driven perspective.

Ultrasound-enhanced drug delivery using nanoparticle-stabilized microbubbles

Ruth Schmid

SINTEF Materials and Chemistry, Sector for Biotechnology and Nanomedicine, Department of Polymer Particles and Surface Chemistry, Norway.
e-mail: ruth.b.schmid@sintef.no

Introduction: For many diseases, there is a high need for novel site focused treatments, as the most efficient drugs often lead to severe systemic toxicity or never reach the diseased tissue due to low transport through biological barriers. Focused ultrasound applied at the disease site has proven promising as a non-invasive method to facilitate the transport of membrane impermeable compounds [1]. Gas-filled microbubbles (MBs), clinically in use as ultrasound contrast agents, are known to greatly amplify the biophysical effects of ultrasounds. However, as the effectiveness of MBs depends on several variables, it is essential to develop MBs that are tailored for their therapy, instead of using commercially available contrast agent MBs. Currently, there are no such products on the market.

Materials and Methods: PEGylated multifunctional nanoparticles (NPs) of poly(alkylcyanoacrylates) (PACAs) were prepared using the mini-emulsion polymerization method [2], and gas MBs were stabilized by self-assembly of the NPs at the gas-water interface [2].

Results and Discussion: We have developed a technology platform consisting of MBs stabilized by poly(ethylene glycol) (PEG)-coated polymeric NPs (Figure 1) [2], incorporating multiple functionalities, including imaging and therapy, in a single system. The NPs, containing lipophilic drugs and/or contrast agents, are composed of the widely used PACAs and prepared in a single step. These NPs exhibit very attractive properties for diagnosis and therapy, including satisfying drug-loading capacity, designable biodegradability, low toxicity, long circulation time in blood, as well as easy, cost-efficient, reproducible and scalable production. MBs stabilized by the NPs are subsequently prepared by self-assembly of the particles at the MB gas/liquid interface. We have recently shown that these MBs can act as contrast agents for conventional ultrasound imaging with comparable properties to the commercial SonoVue MBs (Bracco). Then, by precise tuning of the applied ultrasound pulse, the MBs may oscillate and subsequently burst, opening biological barriers and releasing the NPs constituting the shell, resulting in increased local deposit of NPs into target tissue [3]. Using these novel MBs in combination with focused ultrasound, the blood-brain barrier (BBB) could be successfully and safely opened transiently and the NPs could cross the BBB and deliver a model drug in the central nervous system [4]. The novel technology platform could hence be used both to image the distribution of bubbles and to increase the delivery of the NPs to target cells, while limiting the exposure to healthy tissue.

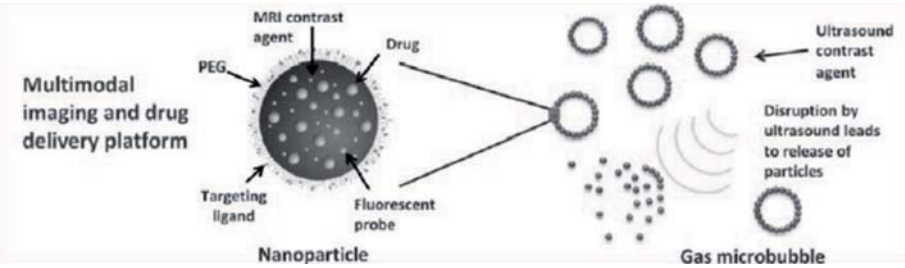

Figure 1. Multifunctional technology platform for ultrasound-enhanced drug delivery.

Conclusions: A novel multifunctional technology platform consisting of NP-stabilized gas MBs has been developed, which together with focused ultrasound opens for enhanced drug delivery through biological barriers both into tumors and into the brain.

Acknowledgements: Financial support from the Norwegian Research Council through various grants is gratefully acknowledged.

References:

[1] Frenkel V. Adv Drug Deliv Rev **60** (2008) 1193.
[2] Mørch Ý, Hansen R, Berg S, Åslund AK, Glomm WR, Eggen S, Schmid R, Johnsen H, Kubowicz S, Snipstad S, Sulheim E, Hak S, Singh G, McDonagh BH, Blom H, de Lange Davies C, Stenstad PM. Contrast Media Mol Imaging **10** (2015) 356.
[3] Eggen S, Fagerland SM, Mørch Ý, Hansen R, Søvik K, Berg S, Furu H, Bøhn AD, Lilledahl MB, Angelsen A, Angelsen B, de Lange Davies C. J Control Release **187** (2014) 39.
[4] Åslund AK, Berg S, Hak S, Mørch Ý, Torp SH, Sandvig A, Widerøe M, Hansen R, de Lange Davies C. J Control Release **220** (2015) 287.

Recent advances on topical, transdermal and nail drug delivery

M. Begoña Delgado Charro

Department of Pharmacy and Pharmacology, Faculty of Sciences, University of Bath, Great Britain.
e-mail: b.delgado-charro@bath.ac.uk

Introduction: Topical and transdermal drug delivery represent a niche yet continuously active area of drug development [1]. This work aims to present some representative recent research in three areas: *i*) combined transdermal iontophoresis of naltrexone and buprenorphine and anti-Parkinson drugs; *ii*) formulation and imaging approaches in nail drug delivery; and, *iii*) tape stripping as a dermatopharmacokinetic approach to predict transdermal absorption and assess topical bioequivalence [2, 3].

Materials and Methods: *i*) Transdermal *in vitro* iontophoresis used dermatomed porcine skin and constant direct, current delivered through Ag/AgCl electrodes. The receptor and donor phases were manipulated to investigate the effect of pH, molar fraction, electro-osmotic flow and drug competition. The drug delivered to the receptor phase, stratum corneum and viable tissue was measured. *ii*) *In vitro* release (IVRT) and *in vitro* permeation (IVPT) experiments used artificial membranes and nail clippings from healthy volunteers. The delivery and depth of penetration of antifungal drugs and markers into and across the nail were determined using standard diffusion experiments and confocal and Raman imaging experiments. The latter was also used to assess the effect of water, DMSO and propylene glycol on the nail plate. *iii*) Following ethical approval, two *in vivo* tape-stripping, FDA funded, studies compared the uptake of diclofenac and acyclovir from several topical drug products. Other ongoing projects investigate the potential use of tape-stripping data to predict transdermal absorption. For this, *in vitro* studies with nicotine, buprenorphine and testosterone commercial formulations combined standard permeation with tape-stripping experiments.

Results and Discussion: The results from the iontophoretic experiments were in agreement with previous data. More interestingly, they revealed the key role of a drug molar fraction, rather than its nominal concentration, in determining the drug transport number and therefore, its iontophoreric transdermal flux. Iontophoresis of the naltrexone-buprenorphine combination revealed that transport interactions between two drugs can go far beyond those expected due to charge carrying competition. IVRT experiments with nail formulations provide an excellent, easy tool with which to determine whether a vehicle is able to maintain the active in a deliverable state for sufficient periods of time and fast screening of potential formulations. The combined use of nanoparticle vehicles with microneedles may allow forming drug reservoirs in the nail plate from which delivery can take place for longer periods of time. Imaging technologies have been successfully applied to establish penetration profiles of drugs and solvents, particularly revealing the non-Fickian uptake of water, DMSO and propylene glycol. *In vivo* tape stripping was able to differentiate between diclofenac topical products expected to perform differently. Preliminary, *in vitro* tape-stripping results are consistent with the kinetics of nicotine, buprenorphine and testosterone following transdermal administration.

Conclusions: Transdermal and topical drug delivery remains an active research and development area. The last decade had seen significant advances in our understanding of the mechanisms of skin and nail absorption and underlined the need: *i*) to characterize the so-called residual phase from which delivery actually takes place; and, *ii*) of developing tools with which to predict bioavailability and topical bioequivalence therefore reducing the need for clinical trials.

Acknowledgements: The financial support from the FDA, Science Without Borders, MRC, Parkinson Disease Society UK, MedPharm, MRC and Parkinson's UK is gratefully acknowledged.

References:

[1] Wiedesrsberg S, Guy RH. J Control Release **190** (2014) 150.
[2] Chiu WS, Belsey NA, Garrett NL, Moger J, Delgado-Charro MB, Guy RH. Proc Natl Acad Sci USA **112** (2015) 7725.
[3] N'Dri-Stempfer B, Navide WC, Guy RH, Bunge AL. Pharm Res **26** (2009) 316.

XI Spanish-Portuguese Conference on Controlled Drug Delivery

"Revolutionary Approaches in Nanomedicine Development"

January 21 – 23, 2016. Granada (Spain)

INVITED LECTURES

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Protecting pharmaceutical inventions

Juan Antonio Muñoz Orellana

Oficina de Transferencia de Resultados de Investigación. University of Granada, Spain.
e-mail: orellana@ugr.es

In a highly competitive market such as pharmaceuticals, a main aspect of technological development is associated with the protection of inventions against exploitation by competitors. In general, only 1 in 10,000 molecules with activity reaches the market, and requires ≈ 15 years of development and an average investment between 350 and 5,000 M$ [1]. Thus, without having some guarantees about the exclusiveness on their holdings, the investment involves such a high risk that investments hardly will occur.

Possibly the most suitable mechanism for reducing the risk associated with copies or imitations is patenting. A patent allows excluding competitors from the use and commercialization of inventions. In this field, it is very important to know what kind of inventions can be protected. Not only active principles are patentable. So are different chemical selections like stereoisomers or enantiomers, compositions, drug delivery systems, second and further medical uses of known drugs, and even patterns of administration, once they meet the patentability requirements, and Novelty and Inventive Step [2, 3] together with a sufficient disclosure.

But beside patents, there are also other not so well-known mechanisms like CCPs, data exclusivity and market protection related with the conduct of clinical trials, market exclusivity for orphan drugs and pediatric rewards that provide other kind of competitive advantages.

These protection mechanisms should be considered from the initial phase of development, and especially when technology has born in Universities and public research institutions.

References:

[1] Harper M. The cost of creating a new drug now $5 billion, pushing big pharma to change. http://www.forbes.com/sites/matthewherper/2013/08/11/how-the-staggering-cost-of-inventing-new-drugs-is-shaping-the-future-of-medicine/. Accessed 14 Dec 2015.
[2] Spanish Trademarks and Patents Office (http://www.oepm.es).
[3] European Patent Office (http://www.epo.org).

Is the zebrafish a suitable research model for drug discovery in nanomedicine?

Juan María Alfaro Sánchez

Neuron Bio, Granada, Spain.
e-mail: jmalfaro@neuronbio.com

Various *in vivo* biological models have been proposed for studying the interactions of nanomaterials in biological systems. Unfortunately, the widely used small mammalian animal models (rodents) are costly and labor intensive, and generate ethical issues. Recently, there has been increasing interest in the scientific community in the interactions between nanomaterials and non-mammalian organisms, which are now being recognized as valid models for the study of human disease [1].

The zebrafish embryo is considered as one of the best animal models for biomedicine studies and especially in testing nanomaterial´s toxicity, as zebrafish possess the most homologous genome degree with higher resemblance to the human genome, thus corresponding to human embryo functional biological behavior [2].

The virtually transparent embryos of zebrafish and the ability to accelerate genetic studies by new technologies such as CRISPR/Cas-9, have led to the widespread use of zebrafish in the detailed investigation of vertebrate gene function and increasingly the study of human genetic disease [3-5]. Comparison to the human reference genome shows that ≈ 70% of human genes have at least one obvious zebrafish orthologue, increasing to 82% when it is compared specifically to disease-related genes [6].

The invited lecture will be focused on the zebrafish´s capacities to help biotech and pharmaceutical companies in the discovery and development of new drugs and nanodrugs. Advantages and disadvantages of the use of this animal model in nanomedicine will be analyzed.

References:

[1] Giannaccini M, Guschieri A, Dente L, Raffa V. Nanomedicine **10** (2013) 703.
[2] Bar-Ilan O, Albercht RM, Fako VE, Furgeson DY. Small **5** (2009) 1897.
[3] Golzio C, Willer J, Talkowski ME, Oh EC, Taniguchi Y, Jacquemont S, Reymond A, Sun M, Sawa A, Gusella JF, Kamiya A, Beckmann JS, Katsanis N. Nature **485** (2012) 363.
[4] Panizzi JR, Becker-Heck A, Castleman VH, Al-Mutairi DA, Liu Y, Loges NT, Pathak N, Austin-Tse C, Sheridan E, Schmidts M, Olbrich H, Werner C, Häffner K, Hellman N, Chodhari R, Gupta A, Kramer-Zucker A, Olale F, Burdine RD, Schier AF, O'Callaghan C, Chung EM, Reinhardt R, Mitchison HM, King SM, Omran H, Drummond IA. Nat Genet **44** (2012) 714.
[5] Roscioli T, Kamsteeg EJ, Buysse K, Maystadt I, van Reeuwijk J, van den Elzen C, van Beusekom E, Riemersma M, Pfundt R, Vissers LE, Schraders M, Altunoglu U, Buckley MF, Brunner HG, Grisart B, Zhou H, Veltman JA, Gilissen C, Mancini GM, Delrée P, Willemsen MA, Ramadža DP, Chitayat D, Bennett C, Sheridan E, Peeters EA, Tan-Sindhunata GM, de Die-Smulders CE, Devriendt K, Kayserili H, El-Hashash OA, Stemple DL, Lefeber DJ, Lin YY, van Bokhoven H. Nat Genet **44** (2012) 581.
[6] Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, Collins JE, Humphray S, McLaren K, Matthews L, McLaren S, Sealy I, Caccamo M, Churcher C, Scott C, Barrett JC, Koch R, Rauch GJ, White S, Chow W, Kilian B, Quintais LT, Guerra-Assunção JA, Zhou Y, Gu Y, Yen J, Vogel JH, Eyre T, Redmond S, Banerjee R, Chi J, Fu B, Langley E, Maguire SF, Laird GK, Lloyd D, Kenyon E, Donaldson S, Sehra H, Almeida-King J, Loveland J, Trevanion S, Jones M, Quail M, Willey D, Hunt A, Burton J, Sims S, McLay K, Plumb B, Davis J, Clee C, Oliver K, Clark R, Riddle C, Elliot D, Threadgold G, Harden G, Ware D, Begum S, Mortimore B, Kerry G, Heath P, Phillimore B, Tracey A, Corby N, Dunn M, Johnson C, Wood J, Clark S, Pelan S, Griffiths G, Smith M, Glithero R, Howden P, Barker N, Lloyd C, Stevens C, Harley J, Holt K, Panagiotidis G, Lovell J, Beasley H, Henderson C, Gordon D, Auger K, Wright D, Collins J, Raisen C, Dyer L, Leung K, Robertson L, Ambridge K, Leongamornlert D, McGuire S, Gilderthorp R, Griffiths C, Manthravadi D, Nichol S, Barker G, Whitehead S, Kay M, Brown J, Murnane C, Gray E, Humphries M, Sycamore N, Barker D, Saunders D, Wallis J, Babbage A, Hammond S, Mashreghi-Mohammadi M, Barr L, Martin S, Wray P, Ellington A, Matthews N, Ellwood M, Woodmansey R, Clark G, Cooper J, Tromans A, Grafham D, Skuce C, Pandian R, Andrews R, Harrison E, Kimberley A, Garnett J, Fosker N, Hall R, Garner P, Kelly D, Bird C, Palmer S, Gehring I, Berger A, Dooley CM, Ersan-Ürün Z, Eser C, Geiger H, Geisler M, Karotki L, Kirn A, Konantz J, Konantz M, Oberländer M, Rudolph-Geiger S, Teucke M, Lanz C, Raddatz G, Osoegawa K, Zhu B, Rapp A, Widaa S, Langford C, Yang F, Schuster SC, Carter NP, Harrow J, Ning Z, Herrero J, Searle SM, Enright A, Geisler R, Plasterk RH, Lee C, Westerfield M, de Jong PJ, Zon LI, Postlethwait JH, Nüsslein-Volhard C, Hubbard TJ, Roest Crollius H, Rogers J, Stemple DL. Nature **496** (2013) 498.

Carbosilane dendritic systems. A synthetic platform for the development of nanomedicines

Francisco Javier de la Mata

Department of Organic Chemistry and Inorganic Chemistry, University of Alcalá de Henares, Madrid, Spain; and Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
e-mail javier.delamata@uah.es

Introduction: Our research is focused in the use of carbosilane dendrimers for biomedical applications. These dendrimers present a hydrophobic inner structure made of C-C and Si-C bonds, and thus they have to be turned water-soluble for nanomedicine design. We have reached this goal introducing either cationic or anionic groups on their surface. In the particular case of cationic dendrimers, they have shown to be active as bactericides and as delivery systems for nucleic acids [1], whereas anionic dendrimers turn out to be effective antiviral agents [2].

Materials and Methods: All compounds described in this work have been prepared following different but well-known chemical approaches, e.g. click chemistry reactions. All dendritic derivatives have been fully characterized using different analytical and physicochemical techniques depending of its nature.

Results and Discussion: A synthetic platform for the synthesis of dendritic nanosystems based on a carbosilane skeleton is presented for the preparation of macromolecules with different topologies and using different synthetic approaches. Following these procedures, we have prepared spherical dendrimers, dendrons, hyperbranched polymers and dendritic nanoparticles that have been conveniently functionalized for their use in different biomedical uses (Figure 1). Different applications of these macromolecules will be shown as well as the transfer technology process that we have carried out with some of them. In particular, we will be focused in the use of these nanodendritic systems as antiviral agents and its possible use as topical microbicides for the prevention of HIV infection.

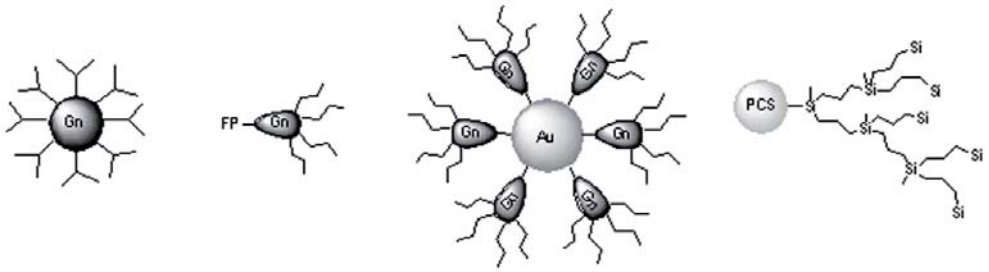

Figure 1. Dendritic nanosystems based on a carbosilane skeleton that can be used for biomedical applications.

Conclusions: A new technology for the preparation of nanoscopic carbosilane dendritic systems has been developed. After suitable functionalization, these carbosilane dendritic systems offer good properties to be used in different biomedical applications. In particular, these compounds have shown a great antiviral activity and could be used for the development of a topical microbicide for the prevention of HIV infection.

Acknowledgements: CTQ2014-54004-P (MINECO), and Consortium NANODENDMED ref S2011/BMD-2351 (CM) (University of Alcalá de Henares, Spain). CIBER-BBN is an initiative funded by the VI National R&D&i Plan 2008–2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions and financed by the Instituto de Salud Carlos III with assistance from the European Regional Development Fund.

References:

[1] (a) Chonco L, Bermejo-Martín JF, Ortega P, Shcharbin D, Pedziwiatr E, Klajnert B, de la Mata FJ, Eritja R, Gómez R, Bryszewska M, Muñoz-Fernandez MA. Org Biomol Chem **5** (2007) 1886. (b) Ortega P, Copa-Patiño JL, Muñoz-Fernandez MA, Soliveri J, Gomez R, de la Mata FJ. Org Biomol Chem **6** (2008) 3264. (c) Fuentes-Paniagua E, Hernández-Ros JM, Sánchez-Milla M, Camero MA, Maly M, Pérez-Serrano J, Copa-Patiño JL, Sánchez-Nieves J, Soliveri J, Gómez R, de la Mata FJ. RSC Adv **4** (2014) 1256.

[2] (a) Chonco L, Pion M, Vacas E, Rasines B, Maly M, Serramía MJ, López-Fernández L, De la Mata J, Alvarez S, Gómez R, Muñoz-Fernández MA. J Control Release **161** (2012) 949. (b) Sepúlveda-Crespo D, Lorente R, Leal M, Gómez R, De la Mata FJ, Jiménez JL, Muñoz-Fernández MÁ. Nanomedicine **10** (2014) 609.

A personal perspective of the Controlled Release Society

María José Alonso Fernández

Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), University of Santiago de Compostela, Spain; Department of Pharmacy and Pharmaceutical Technology, University of Santiago de Compostela, Spain; and Health Research Institute of Santiago de Compostela (IDIS), Spain.
e-mail: mariaj.alonso@usc.es

I have been a member of the Controlled Release Society (CRS, Inc.) since the beginning of my carrier in the early 90´s. My intention at that time was not simply to learn about the newest discoveries in the drug delivery field, but to be part of a global scientific society; a society that involves the whole world with a great variety of scientific profiles, going from pharmaceutical technology and biopharmaceutics, to engineering but also the most advanced chemistry and biology. I have no doubt that my whole carrier has been totally influenced by my affiliation to this society, and that this was mainly due to the networking I could gain from being part of it. In this sense, I have always being proud of having the opportunity to work and discuss with people like Patrick Couvreur, Robert Langer, Nicholas Peppas, Tomas Kissel, Paolo Colombo and so many others who have paved the way of this society and, thus, of the drug delivery field. And the greatest opportunity I got, while being a scientific advisor of the CRS Inc., was the one of starting with my Portuguese friends, Eugenia Cruz representing them, the Spanish-Portuguese Local Chapter of the Controlled Release Society (SPLC-CRS). Now, after more than two decades of trayectory, I am glad to see how our drug delivery community has grown around this chapter quite significantly and I am very thankful to all friends who have contributed to it.

Now we are in the *e*-learning times and some people may wonder about the necessity to be affiliated to a scientific society, while you may find everything in the web. However, in my view of how the scientific community works, it is clearer than ever, that it is very important to become a very active and visible member of a scientific society. This is the reason why the current CRS offers the possibility of being highly involved in a number of committees to all types of scientists. If offers, as well, the chance to closely interact with industry; an interaction that in the current times, maybe more important than ever. It helps young scientists to find their carriers and identify job opportunities. And the most important thing: it offers everyone the chance to influence the society in the way to would like it to be. The products of the CRS Inc. are not solely the Annual Meeting and several other meetings and workshops that you may offer to organize, but also the journals, the books and, specially all the networking and *e*-learning possibilities, which are just there for all of you to express yourselves and contribute.

During my presentation, I would like to present my view of the current CRS activities and to try to open a discussion about the way Meeting attendees might be willing to contribute to the CRS and the SPLC-CRS. I would also like to summarize how this society has influenced my scientific activity. My ultimate goal would be to persuade, mainly young scientists, to be very active in the communication and networking possibilities that both the CRS and the SPCR-CRS offer to them.

Multivariate development of chitosan nanoparticles for the delivery of complex therapeutic human enzymes

Paulo Roque Lino

Nanostructured Systems for Overcoming Biological Barriers Group, and Metabolism and Genetics Group, Research Institute for Medicines (iMed.ULisboa), Universidade de Lisboa, Portugal.
e-mail: proquelino@ff.ulisboa.pt

Introduction: Comparing with small chemical drugs, protein pharmaceuticals possess a high specificity and high potency at relatively low concentrations [1]. Though promising in a biotechnological perspective, their application to the treatment of human diseases is usually limited due to their inherent complex nature and usual structural instability. From the potential spectrum of therapeutic protein addressable disorders, phenylketonuria (PKU; OMIM #261600) stands out as the most prevalent disorder of amino acid metabolism. Caused by a deficient activity of human phenylalanine hydroxylase (hPAH; EC 1.14.16.1), the development of an enzymatic replacement therapy (ERT) has been hindered by the decreased physicochemical stability of hPAH and loss of function throughout pharmaceutical production and storage. We previously described the ability of low molecular weight polyols to modulate hPAH´s *in vitro* stability profile [2]. Herein is reported the development of a delivery system based on chitosan (CS) and further modified with hyaluronic acid (HA) and/or cyclodextrins (CDs) to nanoencapsulate hPAH trough ionotropic gelation with tripolyphosphate (TPP) and modulate protein stability and the systems´ functional, cyto, haemo, and immunocompatibility profile. The rationale was to use a mild method to entrap the tetrameric hPAH in a polyol-like matrix that would allow an efficient rescue of the protein´s structure and function, by avoiding solvents, high shear stress, and high temperature.

Materials and Methods: The particulate system was obtained by ionic gelation, and optimized with modulation of CS:TPP ratio, pH, and presence of protein´s buffer in either CS and TPP solution. Subsequent modification with different HA and CD underwent taking into account particle average size, polydispersity, surface charge, particle yield production by CS incorporation, protein encapsulation efficiency, loading capacity, TEM morphology, cell viability, nanoparticle internalization, and haemocompatible profile [degree of haemolysis, platelet activation, coagulation activation (partial thromboplastin time, PTT, and activated partial thromboplastin time, aPTT), and complement activation]. Denaturing electrophoretic profile (discontinuous denaturing polyacrylamide gel electrophoresis, SDS-PAGE), specific enzymatic activity, and protein thermal stability were assessed under standard conditions.

Results and Discussion: Throughout a systematic design approach based on previously defined critical formulation parameters, we successfully modified a nanoparticulate carrier system with several HAs and CDs. Having gained extensive insights into how each component of the colloidal system behaved, an optimum balance between the beneficial properties of system modification and protein loading resulted in an overall maintenance and/or improvement of the tested parameters. The selected functional nanoformulations were apparently suitable for different administration routes. By merging *in silico*, *in vitro*, and *ex vivo* data, we arrived at comprehensive new insights into how free and enzyme-loaded colloidal systems may be systematically tailored towards a safe and effective nanobiointerface with a given tissue. The polyol-like nature of the nanobiomaterials may have been critical to improve enzyme stabilization as well as the tested biocompatibility of the developed formulations. Furthermore, an in-depth knowledge of how this nanoparticulate polymeric systems behave and how cellular viability, haemostasis and the complement response may be activated or dissipated according to the therapeutic need at hand, considerably improve their drug delivery potential. Within a regulatory setting, these results contribute to clarify some of the general nanotoxicological concerns, perhaps wrongly attributed to an entire group of heterogeneous and flexible materials where CS is included. Each final colloidal system should be regarded with its own specificities.

Conclusions: We provided a mechanistic development platform to formulate and deliver the complex hPAH and open exciting new perspectives towards future preclinical assays to address the still unmet disorder PKU, and potentially other therapeutically complex ERT addressable clinical presentations.

Acknowledgements: Co-authors of the work: João Leandro, Lara Figueiredo, Mariana P. Amaro, Lídia M.D. Gonçalves, Paula Leandro, António J. Almeida. Financial support from Fundação para a Ciência e a Tecnologia, Portugal, Projects PTDC/QUI/64023/2006, PTDC/EBB-BIO/101237/2008 and PEST-OE/SAU/UI4013/2011 to iMed.ULisboa and grants SFRH/BSAB/1210/2011 to AJ Almeida and SFRH/BD/47946/2008 and the National PKU Alliance Post-Doc Fellowship to PR Lino is gratefully acknowledged.

References:

[1] Wang W. Int J Pharm **185** (1999) 129.
[2] Nascimento C, Leandro J, Lino PR, Ramos L, Almeida AJ, de Almeida IT, Leandro P. Appl Biochem Biotechnol **162** (2010) 192.

Nanomedicine applied in cancer treatment: experimental and clinical assays

María del Carmen Leiva Arrabal

Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain; and Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
e-mail: mcleiva@ugr.es

Introduction: Despite advances in cancer therapy, current treatments are unspecific. Most antitumor cytotoxics act against tumor and healthy cells, producing high toxicity and side effects. New therapeutic strategies try to discriminate between both types of cells, taking advantage particular characteristics of the tumor site. In this context, nanotechnology may improve anticancer treatment, making drugs more specific for tumor tissues, increasing the plasma half-life of the drug, and minimizing toxicity. For this reason, nanotechnology applied to oncology is recently under great development. A representative example is paclitaxel (PTX). This is an active taxane against lung, breast, and ovarian cancer which presents poor solubility and great toxicity. In addition, Cremophor®, a toxic solvent, is needed for its formulation. The use of NPs can avoid PTX degradation, decreases side effects, maximizes PTX accumulation in tumor cells for a more specific response and, even, inhibits tumor multidrug resistance (MDR) [1, 2]. In fact, the commercial formulation Abraxane® has achieved great outcomes, and other clinical trials with PTX-loaded nanoparticles (NPs) are in process. In relation with this new cancer therapy approach, we have developed new solid lipid NPs of tripalmitin loaded with PTX (Tripalm-PTX) which have been analyzed to establish their antitumor activity in comparison with the free drug. Results of this new formulation are presented.

Materials and Methods: Tripalm-PTX was developed and its cytotoxicity was studied in MCF7 human breast tumor cells compared to the plain NPs (Tripalm). After treatment (48 h, twice), cell viability (%) was measured by the sulforhodamine B method, comparing free PTX and Tripalm-PTX. Cell uptake of NPs marked with fluorescein isothyocianate (FITC) was assayed by fluorescent microscopy and flow citometry at 4, 24 and 48 h. In addition, cell cycle modulation was studied after 48 h of treatment exposure by flow cytometry, in MCF7 samples which had previously undergone serum deprivation. Finally, inhibition of MDR mediated by P-glycoprotein was studied. It was used a resistant colorectal tumor cell line (HCT-15) for 2 experiences: firstly a cytotoxicity assay as previously described using free PTX, Tripalm-PTX, and their combination with Verapamil (a P-glycoprotein inhibitor); secondly, a flow cytometry study to assess the accumulation of rhodamine B into cells after exposure to Tripalm.

Results and Discussion: Tripalm showed no toxicity against MCF-7 cells. Tripalm-PTX treatment in MCF7 cells showed a higher cytotoxicity than free PTX, achieving a 14-fold lower IC_{50}. Internalization assay demonstrated a time-dependent incorporation of NPs into the cells, with a poor incorporation of FITC in solution. Moreover, cell cycle study showed a greater accumulation of cells in phase sub-G_1 after Tripalm-PTX treatment (61.2%). MDR inhibition assays demonstrated that Tripalm increased rhodamine B cell retention by P-glycoprotein channels blockage, improving PTX cytotoxicity in HCT-15 resistant cells.

Conclusions: Our findings suggest that incorporation of PTX into tripalmitin NPs represents an improvement in breast cancer treatment. The use of these systems to transport PTX may avoid side effects, enhance antitumor efficacy and inhibit MDRs, which may generate a great benefit.

Acknowledgements: Financial support from Junta de Andalucía, Spain, under Project P11-CTS-7649 and PI-0049 is gratefully acknowledged. Co-authors of the work: Rafael Contreras-Cáceres, Raúl Ortiz, Amelia Díaz, Enrique Cobos, José C. Prados, Consolación Melguizo, Juan Manuel López-Romero.

References:

[1] Jabir NR, Tabrez S, Ashraf GM, Shakil S, Damanhouri GA, Kamal MA. Int J Nanomedicine **7** (2012) 4391.
[2] Feng L, Mumper RJ. Cancer Lett **334** (2013) 157.

Nanomedicine in the future of pediatric oncology

Ángel Montero Carcaboso

Preclinical Therapeutics and Drug Delivery Research Program, Developmental Tumor Biology Laboratory, Fundació Sant Joan de Déu, Barcelona, Spain; and Department of Pediatric Hematology and Oncology, Hospital Sant Joan de Déu, Barcelona, Spain.
e-mail: amontero@fsjd.org

Many pediatric tumors are characterized by unique genetic or biomarker alterations. Examples of pediatric tumor specific alterations are the Ewing´s sarcoma EWS/FLI-1 fusion gene, the high expression of the disialogangloside (GD2) in neuroblastoma, osteosarcoma and rhabdomyosarcoma, or the K27M mutation in the H3.3 histone of diffuse intrinsic pontine gliomas.

Nanotechnology could take advantage of such homogeneous features of pediatric diseases to enable active drug targeting to specific pediatric tumor cells. However, preclinical studies in pediatric oncology are challenging because pediatric solid tumor models are scarce. The translational research program at the Hospital Sant Joan de Déu contributes to generate clinically relevant models of pediatric solid tumors, including patient-derived xenografts, which are useful for the validation of novel nanotechnology approaches, the identification of biomarkers, and the design of clinical trials.

Combination of chemical therapies and gene silencing for the development of novel *Acanthamoeba* treatments

Jacob Lorenzo Morales

University Institute of Tropical Diseases and Public Health of the Canary Islands, University of La Laguna, Canary Islands, Spain.
e-mail: jmlorenz@ull.edu.es

Introduction: Free-living amoebae of *Acanthamoeba* genus are causal agents of a severe sight-threating infection of the cornea known as *Acanthamoeba* keratitis (AK). Moreover, the number of reported cases worldwide is increasing year after year, mostly in contact lens wearers. AK has remained significant, despite our advances in antimicrobial chemotherapy and supportive care. In part, this is due to an incomplete understanding of pathogenesis and pathophysiology of the disease, diagnostic delays and problems associated with chemotherapeutic interventions mainly due to the ability of this pathogen to form a highly resistant cyst stage.

Material and methods: In our laboratory, we aim to develop novel therapeutics against *Acanthamoeba* infections by combination of *si*RNA-based gene silencing and chemotherapy. The current understanding of *Acanthamoeba* infections and molecular mechanisms associated with the disease, as well as virulence traits of *Acanthamoeba* may be potential targets for improved therapeutic interventions that ought to be exploited.

Results/Conclusions: The obtained results so far in our laboratory are being presented in this communication. In the case of *Acanthamoeba*, we have recently established a novel therapy based on statins which was elucidated using the combination of *si*RNAs/chemotherapy approach.

Acknowledgements: This work was supported by the grants RICET (project no. RD12/0018/0012 of the programme of Redes Temáticas de Investigación Cooperativa, FIS), Spanish Ministry of Health, Madrid, Spain, the Project PI13/00490 "Protozoosis emergentes por amebas de vida libre: aislamiento, caracterización, nuevas aproximaciones terapéuticas y traslación clínica de los resultados" from the Instituto de Salud Carlos III, and Project ref. AGUA3 "Amebas de vida libre como marcadores de calidad del agua" from CajaCanarias Fundación. Co-authors of the work: Carmen Mª Martín-Navarro, María Reyes-Batlle, Ines Sifaoui, Atteneri López-Arencibia, Basilio Valladares, Enrique Martínez-Carretero, José E. Piñero. ALA and MRB were funded by Becas de Investigación Obra Social La Caixa-Fundación CajaCanarias para Postgraduados 2014". IS was funded by Ayudas para estancias investigadores- ULL, 2015. JLM was supported by the Ramón y Cajal Subprogramme from the Spanish Ministry of Economy and Competitivity RYC-2011-08863.

Targeted non-viral vectors for gene delivery

María Concepción Tros de Ilarduya Apaolaza

Department of Pharmacy and Pharmaceutical Technology, University of Navarra, Spain.
e-mail: ctros@unav.es

Gene therapy has emerged as a promising approach for the treatment or prevention of acquired and genetic diseases. At the present time, major somatic gene transfer approaches employ either viral or non-viral vectors. Viral vectors show high gene transfer efficiency, but are deficient in several areas, including the induction of a host inflammatory and immune response. Some of these problems can be circumvented by employing non-viral vehicles, such as cationic liposomes or polymers. The complexes they form with deoxyribonucleic acid (DNA) are defined as "lipoplexes" or "polyplexes", respectively, and constitute the most promising alternative to the use of viral vectors for gene therapy. The targeting capacity of these systems is also a key point for the successful of gene therapy strategies.

Our objective is to provide a framework for the future design and synthesis of optimal non-viral vectors for gene therapy. The structure, charge, and formulation of these vehicles is also related to the stability, and consequently to the efficiency of gene transfection. It will be presented in this contribution the design, development, and evaluation of different targeted non-viral systems (lipoplexes and polyplexes) formulated in the presence of several ligands, e.g. transferrin, asialofetuin, folic acid or hyaluronic acid.

Acknowledgements: Co-authors of the work: Koldo Urbiola, Laura Blanco-Fernández, N. Düzgüneş, Carmen Ortiz Mellet, José M. García Fernández.

Medical devices as drug delivery platforms

Carmen Isabel Álvarez Lorenzo

R+D Pharma Group, Department of Pharmacy and Pharmaceutical Technology, University of Santiago de Compostela, Spain.
e-mail: carmen.alvarez.lorenzo@usc.es

Introduction: Drugs and medical devices have typically followed different development criteria because they are intended to pursue dissimilar aims. Drugs are expected to exert their therapeutic effect at a local or systemic level through certain biological/biochemical pathways. By contrast, medical devices to be inserted/implanted in the body are sought to play a physical role in the diagnosis or the therapy (in some cases just to facilitate the application of a probe or the administration of a drug), and in the replacement of a tissue, organ or function of the body. Drug-eluting medical devices are designed to obtain synergistic features [1, 2]; namely, the incorporation of the drug enables the tuning of the host/microbial responses to the device and the management of device-related complications. On the other hand, the medical device acts as platform for the delivery of the drug for a prolonged period of time just at the site where it is needed and, consequently, the efficacy and the safety of the treatment, as well as its cost-effectiveness are improved.

Materials and Methods: Nanotechnology enables the modification of the inner structure or the surface of the medical device to endow medical devices with ability to host drugs/biological products and to regulate their release. The molecular imprinting technology aims to arrange the polymer components in a conformation suitable for creating domains that act as receptors for a certain substance [3]. To do that, the substance is added to the monomers soup and acts as a template causing their arrangement as a function of the affinity. After synthesis of the network, the template-functional bonds are reversibly broken revealing the imprinted cavities; that is, pockets of the size and with the most adequate chemical groups to host again the template molecules. As in the *in vivo* recognition processes, multiple-point interactions between the template molecule and various functional monomers are required. Methods for surface modification with stimuli-responsive polymers or with networks that can selectively recognize certain biomarkers or cells have been implemented for feed-back modulated release of the active substance [4]. A wide range of chemical-, irradiation- and plasma-based techniques for grafting of nanoscale brushes and networks that are sensitive to changes in temperature, pH, light, ionic strength, or concentration of certain biomarkers, from a variety of substrate materials is currently available.

Results and Discussion: Nanostructuring of the matrix of the devices allows formation of domains that can act as artificial receptors for the drug, exhibiting affinity-regulated mechanisms for the hosting and the release of the drug [2, 3]. Surface functionalization of medical devices has already been shown useful for the release on-demand of drugs and biological products, being switchable on/off as a function of the progression of certain physiological or pathological events [5], e.g. healing, body integration, biofouling, or biofilm formation.

Conclusions: Although *in vivo* tests are still limited, improved knowledge of the interactions among the medical device, the functionalized bulk or surface, the drug and the body, and application of revolutionary nanotechnology approaches are expected to pave the way to the design of drug-eluting medical devices with optimized and novel performances.

Acknowledgements: Work supported by MINECO (SAF2011-22771 and SAF2014-52632-R) and FEDER.

References:

[1] Concheiro A, Alvarez-Lorenzo C. In: Smart Materials for Drug Delivery. Royal Society of Chemistry, vol. 2, RSC, UK, 2013, pp. 313-348.
[2] Concheiro A, Alvarez-Lorenzo C. Adv Drug Deliv Rev **65** (2013) 1188.
[3] Gonzalez-Chomón C, Concheiro A, Alvarez-Lorenzo C. Ther Deliv **4** (2013) 1141.
[4] Alvarez-Lorenzo C, Bucio E, Burillo G, Concheiro A. Expert Opin Drug Deliv **7** (2010) 173.
[5] Segura T, Puga AM, Burillo G, Llovo J, Brackman G, Coenye T, Concheiro A, Alvarez-Lorenzo C. Biomacromolecules **15** (2014) 1860.

Nanomedicines for modulating diabetes: nanoscale tools against macroscale disease

Bruno Sarmento

Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), Porto, Portugal; and Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde (CESPU), Instituto Universitário de Ciências da Saúde, Porto, Portugal.
e-mail: bruno.sarmento@ineb.up.pt

Diabetes and cancer are common diseases with tremendous impact on health worldwide. Interestingly, both are diagnosed within the same individual more frequently than would be expected by chance, even after adjusting for age. Both diseases are complex with multiple subtypes. Current treatment regimens, with outstanding pharmacological active molecules, are providing significant advances in controlling symptomatology and in efficient treatments to patients, but suffering from pharmacokinetic issues. And here is where nanotechnology raises as a valuable tool to provide better biodistribution profiles for drugs following no-invasive administration. In this presentation, it will be demonstrated the feasibility of nanomedicines to improve the bioavailability and efficacy of antidiabetic and anticancer biopharmaceutical drugs.

Diabetes mellitus is a high prevalence and one of the most severe and lethal diseases in the world. Antidiabetic peptides as insulin or glucagon-like peptide-1 (GLP-1) are commonly used to treat diabetes in order to proportionate a better life condition. However, due to bioavailability problems, their most common route of administration is the subcutaneous route. This invasive delivery route is, still, the most efficient, but less desired by patients. Non-invasive delivery systems have potential to overcome the most pressing problem regarding effective treatment of diabetic patients - therapy compliance, thus being considered as a convenient alternative, but it faces important challenges. Therefore, the nanoencapsulation of antidiabetic peptides into nanoparticles is presented as a good strategy to improve bioavailability. In the last years, different strategies and nanocarriers have been used.

Cancer fight using chemotherapy or gene therapy is another area where nanoparticles show much promise by selectively gaining access to tumor due to their small size and modifiability. Surface engineered nanoparticles are of upmost impact towards the active targeting for cellular structures they may provide. Particular outcomes are expected to molecular therapy using small interfering ribonucleic acid (*si*RNA), which has shown great potential in the treatment of cancer by silencing vital genes in tumor cells, adapting it to specific tumor types and customizing it to personalized therapy.

In our research group, we have developed and characterized lipid and polymeric nanoparticles containing insulin [1] and/or GLP-1 [2], following their evaluation as medical products to control diabetes upon oral or pulmonary delivery. Our non-invasive technologies have demonstrated a clear efficacy in lowering the blood glucose levels in diabetic animal models. Not only nanoparticles have demonstrated to be able to cross biological barriers, but also provide a sustained release of their peptide payloads. No inflammation, cytotoxicity or tissue damage have been associated with chronic use of such nanoparticles, giving promising clinical application in a near future.

We have also developed an epidermal growth factor receptor (EGFR)-targeted chitosan nanosystem for silencing mitotic checkpoint regulator protein gene as a strategy to efficiently induce cell death in EGFR overexpressing cancer cells [3]. Our systems showed time-dependent enhanced and selective intracellular internalization of EGFR-targeted nanoparticles compared to non-targeted systems. We also nanoencapsulated *si*RNA to specifically silence efflux proteins responsible for cancer drug resistances, in a synergic action for cancer treatment.

Acknowledgements: Financial support from Fundação para a Ciência e Tecnologia (FCT), Portugal, COMPETE, CESPU and University of Helsinki and Northeastern University is gratefully acknowledged.

References:

[1] Andrade F, das Neves J, Gener P, Schwartz S Jr, Ferreira D, Oliva M, Sarmento B. Nanomedicine **11** (2015) 1621.
[2] Araújo F, Shrestha N, Shahbazi MA, Liu D, Herranz-Blanco B, Mäkilä EM, Salonen JJ, Hirvonen JT, Granja PL, Sarmento B, Santos HA. ACS Nano **9** (2015) 8291.
[3] Nascimento AV, Singh A, Bousbaa H, Ferreira D, Sarmento B, Amiji MM. Mol Pharm **11** (2014) 3515.

Nanostructured materials for improved drugability of neuropeptides and immune-based interventions

David Pozo Pérez

Laboratory for Advanced Therapies in Neuroprotection and Immune Regulation, Department of Cell Signaling, Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER), Seville, Spain; and Department of Medical Biochemistry, Molecular Biology and Immunology, Faculty of Medicine, University of Sevilla, Spain.
e-mail: david.pozo@cabimer.es

Among the molecules in need of lead optimization, ligands for G protein-coupled receptors (GPCRs, also known as seven-transmembrane domain receptors, 7-TM receptors) are of particular interest, as GPCRs are involved in virtually all physiological processes, with at least 40% of drugs currently in the market thought to modulate GPCRs. A relevant number of endogenous ligands for GPCRs are neuropeptides. Neuropeptides and their receptors provide a mechanistic basis for the mutual biochemical language between the endocrine, the immune, and the nervous systems. Potential advantages of treatments targeting neuropeptide systems in comparison to classical neurotransmitter systems and ion channels revolve around the subject of efficacy as well as the reduced likelihood of side effects, thus making them attractive candidates for the development of new clinical applications for various disorders. In the present communication, it is disclosed the advantages of two nanotechnology approaches for improved vasoactive intestinal peptide (VIP)-based drug delivery systems as a proof of concept, one based on VIP-functionalized gold nanoparticles (NPs) and the other exploiting VIP-functionalized liposomes as a targeting agent to transport drugs to human prostate cancer cell lines.

Even though there have been several important improvements in the development of neuropeptide therapeutics, they have shown limited success, mainly due to poor bioavailability after protease degradation. Thus, the development of small, non-peptide mimic ligands have resulted in molecules that often have reduced affinity and selectivity compared to their endogenous ligand counterparts. On the other hand, the development of inhibitors of specific proteases increases the chances of adverse effects. In this sense, despite its potential, the neuropeptide VIP, as a paradigm of other peptide-based therapeutics, is still not available for treating clinical problems. For these reasons, an alternative strategy was investigated that simultaneously: *i*) targets the protease substrate (neuropeptide) instead of the protease; and, *ii*) makes use of the entire neuropeptide molecule to retain its full biological activity. Remarkably, although it has been hypothesized that surface functionalization of proteins and bioactive peptides on noble metallic nanoclusters might protect from protease degradation, so far there are no formal proofs in this sense. The aim was to prove that coating gold NPs with the neuropeptide VIP impairs the hydrolytic activity of extracellular proteases, leading to VIP-mediated functional responses after harsh conditions resembling the extracellular circulating proteases milieu. This is the first study to address the potential protection from protease degradation upon gold NP functionalization of a given peptide.

Beside the potential of noble metal NPs, the use of liposomes is recognized as a promising strategy for improving the delivery of anticancer drugs to tumours. Conventional chemotherapy is the most applied treatment for many cancers but has low specificity and limited effectiveness due to its severe side effects. The effectiveness of doxorubicin is limited due to its high toxicity and side effects, and strong multidrug resistance response in cancer cells after repeated administration. Biomarkers that differentiate malignant tissues from normal tissues can be used as targets for this purpose and one of these attractive molecular targets is VIP receptors which are overexpressed in human prostate cancer cell lines compared to normal prostate tissue. In particular, in human and rat prostatic carcinoma, VIP receptors are mainly VPAC1 receptors, and the same occurs in the prostate cancer cells lines LnCAP and PC-3. VIP-functionalized phospholipid liposomes were used to encapsulate doxorubicin in order to deliver it to these tumour cells. The aim of this study was to assess the potential of VIP as a ligand for prostatic carcinoma targeting by liposomal nanocarriers. Moreover, it was evaluated the effect of a peptide coupling method on the cellular uptake, cytotoxicity and apoptosis of doxorubicin-loaded liposomes. It was also addressed *in vivo* experiments in a preclinical setting to evaluate the VIP active driven targeting of the prostate cancer cells by liposomes.

Besides the implications in the field of neuropeptides, this study places the concept of surface functionalization in the broader perspective of novel proteins escaping from extracellular proteases, which could represent a major driven force and an added value to steer the research in the field of engineering NPs and peptide-derived treatments. Moreover, we have developed a reliable method to synthesize homogenous, stable and properly characterized VIP-functionalized liposomes. VIP-functionalized liposomes demonstrated significant cellular binding and uptake by VIP receptor expressing cells (PC-3, DU-145, and LnCAP) in contrast to unconjugated liposomes. VIP-functionalized liposomes showed higher cell-death efficacy in VIP receptor expressing cells than unconjugated liposomes, being confirmed by cytotoxicity and apoptosis studies. *In vivo* biodistribution studies showed VIP-functionalized liposome accumulation in liver, kidneys and spleen, whereas unconjugated liposomes showed preference for liver and kidneys after 12 h of intravenous injection.

Acknowledgements: Co-authors of the work: Soledad Lopez, Maria Leal, Manuel Pernia-Leal, Rebecca Klippstein.

Advances and opportunities of nanocarriers in the inflammatory process

María Luisa González Rodríguez

Department of Pharmacy and Pharmaceutical Technology. University of Seville, Spain.
e-mail: malugoro@us.es

Inflammation represents a highly complex network of cellular and sub-cellular components aimed to defend the host against deleterious stimuli. Macrophages are a major cellular component present in the inflammatory milieu. They are recruited by further downstream signals to aid in resolution and repairing during the acute process. Failure to cease the injurious stimuli leads to chronic inflammation. During the chronic phase, macrophages are continuously recruiting and releasing inflammatory mediators such as chemokines, cytokines, lipid mediators, proteases, and reactive oxygen species. These mediators cause detrimental effects to the host, thus leading to the initiation, exacerbation, and progression of several infectious and non-infectious diseases. They also display protective functions in chronic inflammatory diseases and, depending on the pathological environment, these cells adapt to different phenotypes which exacerbate or resolve the disease. Therefore, macrophages represent promising targets for the treatment and diagnosis of inflammatory diseases.

The presentation will be focused on the analysis of the role of macrophages in inflammatory diseases, e.g. rheumatoid arthritis, osteoarthritis, cystic fibrosis, and diabetes, with an insight into potential molecular targets. Apart from resident macrophages in the area of disease onset, circulating monocytes and macrophages are continuously recruited to meet the demands of inflammatory response and expression of chemokines, cytokines, and cell adhesion molecules. Thus, considerable emphasis has been laid upon targeted therapies against them, to treat inflammatory diseases. Although they can readily phagocytose nanoparticles, they have been used as Trojan horses to deliver drugs and imaging agents to the disease site. One of the most exploited approaches is enabling phagocytosis of loaded nanovehicles by them, which is then passively targeted to the site of inflammation due to mounting immune response. Actually, the active targeting approach has become more popular where the surface of the delivery vehicle is decorated with a ligand that selectively interacts with their target receptors.

Among the remarkable approaches that have been developed for the targeted therapy of inflammatory diseases, liposomes show great potential for selective drug delivery to inflamed barriers. This targeted therapy has evidently been underexploited against inflammatory diseases even though the approach has clearly shown promising results. Hence, it will be finally discussed the potential of different types of liposome-based approaches to devise macrophage specific targeted therapy, highlighting their progresses into one of the most extensively and clinically advanced drug delivery platforms used.

Nanosystems in medical imaging: advances and prospects

Mazen M. El-Hammadi

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Damascus University, Damascus, Syria.
e-mail: mazenhammadi@yahoo.co.uk

Recently, the interest in medical and molecular imaging has considerably increased. Latest advances in imaging have made possible the gaining of much more in-depth details about complex biological processes, disease progress, and body's interactions with drug molecules. In addition, combined imaging and therapy, as in the so-called theranostic systems, allows a real-time monitoring of therapeutic procedures in the human body.

Nanosized systems used in biomedical research show unique characteristics and have proven to be highly versatile platforms that can be structurally designed to exhibit specific functions and achieve precise tasks in the organism. Thus, NPs and other nanosystems hold great promise as biomedical imaging, diagnostic, and theranostic tools. Nanoprobes can be used to improve targeting, optimize *in vivo* pharmacokinetics and increase circulation time, minimize non-specific tissue uptake and reduce toxicity, and enhance imaging sensitivity and brightness of the imaging agents. Additionally, nanosystems can be loaded with a combination of two, or more, imaging agents with different properties allowing synergetic multimodal imaging.

In this presentation, an overview of the developments and major applications of nanosystems as imaging agents (contrast agents, dyes, probes, or labels) will be provided. The key properties of the most important *in vivo* imaging techniques will be discussed and the ability of nanosystems to enhance the imaging output explored. Finally, the future opportunities and grand challenges of the field are also addressed.

XI Spanish-Portuguese Conference on Controlled Drug Delivery

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Melanoma cancer vaccine based on multifunctional nanoparticles for antigen delivery and immunomodulation

Carina Peres[1,2], *Joana M. Silva*[1,3], *Liane Moura*[1], *Luís Graça*[2], *Rogério Gaspar*[1], *Verónique Preat*[3], *Helena F. Florindo*[1]

[1]Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Portugal.
[2]Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Portugal.
[3]Louvain Drug Research Institute, Advanced Drug Delivery & Biomaterials, Université Catholique de Louvain, Belgium.
e-mail: cperes@ff.ul.pt

Introduction: Cancer cells are able to overcome the general body immune response. Therefore, the vaccine design aiming the induction of an integrated T-cell mediated immunity is one of the most used strategies for the last years to improve cancer immunotherapy [1]. Dendritic cells (DCs) are the most potent antigen presenting cells (APCs) in the human body for the stimulation of naïve T-cells, playing a key role in the fight of the immune system against cancer cells. After recognizing, taking up and processing the antigen, DCs migrate into T-cell areas of secondary lymph nodes and present antigen to T cells. The interaction among B cells, T cells and mature DCs results in broaden and integrated immune response, crucial to get a cytotoxic effect that will lead to tumor rejection and regression [2]. Nanoparticles (NPs) are emerging as safe alternatives for the induction of anticancer immune responses in several tumor models. By encapsulating the TAA in NPs, it is possible to stimulate tumor-specific cytotoxic T lymphocyte (CTL) responses and direct the immune response towards the Th1 pathway, which are vital for tumor-protective immunity [3]. Our strategy aims to combine the antigen carry capacity of NPs and the specific targeting and maturation of DC receptors *in vivo*, by developing an antigen-specific therapeutic melanoma cancer vaccine based on poly(D,L-lactide-*co*-glycolide (PLGA) and poly(ε-caprolactone) (PCL) carriers able to target mannose receptors or Toll-like receptors (TLRs) at DCs.

Materials and Methods: Biodegradable NPs (PLGA/PEG-*b*-PLGA/PEG-*b*-PCL or mannose-PEG-*b*-PCL) were prepared by the double emulsion-solvent evaporation method entrapping melanoma antigens and the TLR ligands (TLRls) CpG and Poly(I:C). The size of NPs was determined by dynamic light scattering (DLS) and Atomic Force Microscopy (AFM). A lectin-binding assay was developed to confirm the presence of mannose residues at NP surface. Three different APC lines were used to characterize NP-APC interactions using flow cytometry and confocal microscopy. We evaluated the efficacy of our nanoparticulate cancer vaccine by assessing its antitumor immune responses in the B16F10 melanoma mouse model, both at therapeutic and prophylactic settings.

Results and Discussion: NPs with 140 nm presented high loading capacities of antigens and adjuvants. NPs displayed mannose residues available for binding at the NP surface. Macropinocytosis, clathrin-mediated endocytosis, caveolin- and lipid raft-dependent endocytosis are involved in NP internalization. NPs demonstrated both endolysosomal and cytosolic localizations and a tendency to accumulate nearby the endoplasmic reticulum, suggesting capacity to drive antigens to be presented through MHC class I and II molecules to both CD8+ and CD4+ T cells, favoring a complete and coordinated immune response. In both therapeutic and prophylactic settings, the combination of mannose-grafted NPs loaded with one melanoma antigen and both TLRls caused the highest tumor growth reduction, mainly when MHC class I and class II peptides were used.

Conclusions: In this work, we report the successful development of a biodegradable polymeric nanocarrier that has a great potential to be used for cancer immunotherapy. We were able to demonstrate that mannose-grafted NPs have a superior antitumor effect *in vivo*, suggesting their higher capacity to target APCs. The great versatility of this carrier allowed the combination of NPs containing different tumor antigens, which demonstrated the highest antitumor effect, mainly when MCH class I and class II antigens were combined.

Acknowledgements: The authors are grateful to Fundação para a Ciência e a Tecnologia, Ministério da Ciência e da Tecnologia Portugal for iMed.ULisboa grant UID/DTP/04138/2013, research projects (PTDC/SAU-FAR/119389/2010 and UTAP/ICDT/DTPFTO/0016/2014), PhD grants (SFRH/BD/64295/2009 to JM Silva; SFRH/BD/87591/2012 to C Peres) and Post-doc research grant SFRH/BPD/94111/2013 to L Moura. Fonds de la Re-cherche Scientifique Médicale, Belgium also supported this work.

References:

[1] Silva JM, Videira M, Gaspar R, Préat V, Florindo HF. J Control Release **168** (2013) 179.
[2] Hubbell JA, Thomas SN, Swartz MA. Nature **462** (2009) 449.
[3] Silva JM, Vandermeulen G, Pinto SN, Rodrigues C, Afonso CA, Viana A, Jérôme C, Videira M, Silva LC, Préat V, Florindo HF. Nanomedicine (Lond) **9** (2014) 2639.

Locally-controlled release of Palladium-labile prodrug for ovarian carcinoma treatment

Ana Maria Pérez-López, Belén Rubio-Ruiz, Thomas Bray, Sam Myers, Craig Fraser, Asier Unciti-Broceta

Edinburgh Cancer Research UK Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, UK.
e-mail: aperezl@staffmail.ed.ac.uk

Introduction: The social and economic relevance of cancer worldwide is continuously growing. The devastating effects of difficult-to-treat cancers do not only suppose an overwhelming affliction to hundreds of thousands of patients and families, but also represent a significant use of the Health Systems resources. It has recently developed a unique strategy to release anticancer drugs inside tumours using a purely chemical method (so-called biorthogonal organometallic (BOOM) prodrug activation) [1,2], aiming to reduce the side effects of chemotherapy. The strategy is based on the use of palladium (Pd^0), a biocompatible metal, which possesses unique catalytic properties. By intratumoral implantation of a Pd^0-functionalized device (e.g. by minor surgery), cytotoxic drugs can be locally generated in the area surrounding the insert at levels sustained by the controlled flow of a systemically-administered prodrug. This strategy would enable increasing dosing of drug while reducing adverse side effects in distant tissues and organs. The first report of this novel strategy described the Pd^0-mediated generation of the clinically-approved anticancer 5-FU from an inactive precursor [1], which allows the specific activation of drugs in the presence of palladium. Herein, we report the generation of biologically inert precursor of cytotoxic Olaparib by introducing Pd^0-cleavable propargyl group in a critical position for its activity. The activation *in situ* showed antiproliferative effect in ovarian cancer cells.

Materials and Methods: Pd^0-functionalized resin was prepared from NovaSyn TG amino resin HL (0.39 mmol NH_2/g) as previously described [1]. Olaparib (0.12 mmol) was dissolved in dry DMF (1.5 mL) with DBU (0.14 mmol) under N_2 atmosphere. Propargyl bromide (0.12 mmol) was added dropwise to the mixture and stirred overnight at room temperature. The solvents were then removed in vacuo, the crude redissolved with CH_2Cl_2 (10 mL), and washed with H_2O (10 mL) and CH_2Cl_2 (3 × 10 mL). The combined organic layers dried over $MgSO_4$, filtered, and concentrated in vacuo to be purified by Merck TLC Silica gel 60 F254 plates semipreparative using 5% MeOH in DCM. Propargyl-Olaparib (100 μM) were dissolved in PBS (1 mL) with 1 mg of Pd^0-resin and shaken at 1400 rpm and 37 ºC in a Thermomixer. Reaction crudes were monitored at 0, 6 and 24 h by analytical HPLC (Agilent) using an UV detector at 280 nm to avoid the detection of PBS salts. Cells were seeded in a 96 well plate format at the appropriate cell concentration (1,500 cells/well for PEO1 and PEO4 cells) and incubated for 48 h before treatment. Each well was then replaced with fresh media containing Olaparib, Propargyl-Olaparib and Propargyl-Olaparib/Pd^0 resin combination, and incubated for 5 days. PrestoBlue cell viability reagent (10%, v/v) was added to each well and the plate incubated for 2 h. Fluorescence emission was detected using a PerkinElmer Victor2 multilabel reader (excitation filter at 540 nm and emissions filter at 590 nm).

Results and Discussion: Olaparib is a PARP inhibitor, inhibiting poly ADP ribose polymerase (PARP), an enzyme involved in DNA repair. It acts against cancers in people with hereditary BRCA1 or BRCA2 mutations, which include some ovarian, breast, and prostate cancers [3]. The absence of the (2H)-phthalazinone hydrogen of Olaparib, which is essential for hydrogen bonding interactions with the isoenzyme PARP1/2, reduces the efficacy to synthesize poly (ADP-ribose) chain. Treatment of Olaparib with propargyl bromide in the presence of DBU formed the corresponding alkylated prodrug in moderate yield. The alkylated prodrug was evaluated as a bioorthogonal candidate in ovarian carcinoma cells after confirming its activation by Pd^0 beads in PBS. Analysis of the toxigenic effect displayed by prodrug/catalyst combination clearly ranked the propargyl moiety protected drug as an effective Olaparib-generating precursor in the presence of Pd^0 resin, exhibiting analogous cytotoxic activity to that of the unmodified drug.

Conclusions: The chemical masking of the (2H)-phthalazinone of Olaparib led to a significant reduction of the drug's cytotoxicity activity to generate a Pd-labile drug precursor. Cell viability confirmed the bioorthogonal generation of antiproliferative Olaparib through the combined treatment of extracellular Pd^0 resin and the propargyl precursor in ovarian cancer cell culture.

Acknowledgements: This project was also supported by MSD-Scottish Life Sciences Fund postdoctoral award (for AMPL) and Cancer Research UK. BRR thanks the Martin Escudero Foundation for her fellowship.

References:

[1] Weiss JT, Dawson JC, Macleod KG, Rybski W, Fraser C, Torres-Sánchez C, Patton EE, Bradley M, Carragher NO, Unciti-Broceta A. Nat Commun **5** (2014) 3277.
[2] Weiss JT, Dawson JC, Fraser C, Rybski W, Torres-Sanchez C, Bradley M, Patton E, Carragher NO, Unciti-Broceta A. J Med Chem **57** (2014) 5395.
[3] Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, Ashworth A, Carmichael J, Kaye SB, Schellens JH, de Bono JS. N Engl J Med **361** (2009) 123.

Microencapsulated solid lipid nanoparticles as a novel hybrid system for pulmonary antibiotic administration

D.P. Gaspar[1,2], *L.M.D. Gonçalves*[1], *P. Taboada*[3], *C. Remuñán-López*[2], *A.J. Almeida*[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Portugal.
[2]Nanobiofar Group, Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Santiago de Compostela, Spain.
[3]Condensed Matter Physics Department, Faculty of Physics, University of Santiago de Compostela, Spain.
e-mail: dianagaspar@ff.ulisboa.pt

Introduction: Tuberculosis (TB) is an infection that generally remains localized in the lung, so the development of strategies for delivering antitubercular drugs directly to the lung via the respiratory route currently gaining interest. The advantages of inhaled therapy include direct drug delivery to the diseased organ, targeting to alveolar macrophages harbouring the *Mycobacterium tuberculosis,* reduced systemic toxicity and improved patient compliance. Research efforts have demonstrated the feasibility of drug delivery systems employing nanoparticles to serve as inhalable antitubercular drug carriers [1]. However, these nanocarriers may be unfeasible for pulmonary administration because their instability and poor delivery efficiency due to their low-inertia [2]. Herein we describe a novel hybrid pulmonary delivery system based on microencapsulated solid lipid nanoparticles (SLNs) containing rifabutin (RFB) for lung TB treatment, intended for administration as a normal dry powder inhaler (DPI).

Materials and Methods: The RFB-SLNs were prepared using a modification of a previously described method [3]. Using a spray-drying technique, RFB-SLNs were incorporated in microspheres using mannitol and trehalose aqueous solutions [4]. Full physicochemical and *in vitro* characterizations of RFB-SLNs and RFB-SLNs-loaded microspheres were performed.

Results and Discussion: A RFB-SLN preparation technique was employed, avoiding the use of organic solvents or sonication, common to other currently used methods. The SLN showed a particle size within the nano-range scale with narrow size distribution and a negative surface charge (≈ -24mV), with high incorporation of RFB (> 90%), being easily internalized by human monocytes (THP-1 cell line), which is an important feature because *M. tuberculosis* is an intracellular parasite. After RFB-SLN incubation with lung cells (Calu-3 and A549), no evidence of acute cytotoxicity was observed. Microspheres containing RFB-SLNs were prepared with inert excipients, using spray-dried. Microcopy analysis showed that microspheres are spherical with well-defined limits and a smooth surface. Moreover, it suggested that SLN are efficiently encapsulated in microspheres and homogenously distributed throughout the excipients matrices. Due to the low density (ρ), aerodynamic diameter (d_{aer}) of the dry powders were low being in the optimal range particle size for SLNs achieving the alveolar region (1-5 μm) [2, 4]. The dry powders performance during aerosolization and the *in vitro* drug deposition were tested using a twin-impinger approach in the form of a DPI, where it was demonstrated that formulations can reach the deep lung, which is corroborated with the suitable aerodynamic characteristics of them. Isothermal titration calorimetry (ITC) revealed that SLNs have higher affinity for mannitol than for trehalose, which is in agreement with the larger hydration shell and interaction strength with water presented by the latter. This implies a greater ability of trehalose to incorporate SLNs in more rigid and packed structures requiring lower concentrations. Upon microsphere dissolution in aqueous media, SLNs are readily recovered, maintaining the nano-range size and shape. It is expected that when these systems reach the deep lung, they will readily dissolve, maintaining the SLN *in vitro* RFB release behaviour (> 95% over 24h).

Conclusions: A hybrid micro- and nano-particulate platform has been developed to improve pulmonary delivery of SLNs incorporated in a DPI, thus opening up a wide range of treatment applications for TB with potential application to other lung diseases.

Acknowledgements: This work was supported by FEDER and Fundação para a Ciência e Tecnologia, Portugal (grants SFRH/BD/89520/2012, SFRH/BSAB/1210/2011, strategic project PEst-OE/SAU/UI4013/2011, and research project PTDC/DTP-FTO/0094/2012). P.T. thanks Ministerio de Economía y Competitividad (MINECO) and Xunta de Galicia for research projects MAT 2013-40971-R and EM 2013-046, respectively. C.R-L. gratefully acknowledge support from the Spanish Institute of Health "Carlos III" (Project FIS PS09/00816) and Xunta de Galicia (Project PGIDIT, 09CSA022203PR, NANOPULMOGENIC; Project Competitive Reference Groups, 2014/043-FEDER).

References:

[1] Pandey R, Khuller GK. J Antimicrob Chemoth **55** (2005) 4.
[2] Al-Qadi S, Grenha A, Carrión-Recio D, Seijo B, Remuñán-López C. J Control Release **157** (2012) 3.
[3] Estella-Hermoso de Mendoza A, Campanero MA, Lana H, Villa-Pulgarin JA, de la Iglesia-Vicente J, Mollinedo F, Blanco-Prieto MJ. Nanomedicine (Lond) **7** (2012) 5.
[4] Grenha A, Seijo B, Remuñán-López C. Eur J Pharm Sci **25** (2005) 4.

Nanoparticle nose-to-brain transport across an *in vitro* nasal model

Oihane Gartziandia[1,2,3], *Susana Patricia Egusquiaguirre*[1,2], *John Bianco*[3], *José Luis Pedraz*[1,2], *Manoli Igartua*[1,2], *Rosa María Hernandez*[1,2], *Véronique Préat*[3], *Ana Beloqui*[3]

[1]NanoBioCel Group, Laboratory of Pharmaceutics, School of Pharmacy, University of the Basque Country (UPV/EHU), Spain.
[2]Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
[3]Université Catholique de Louvain, Louvain Drug Research Institute, Advanced Drug Delivery and Biomaterials, Belgium.
e-mail: oihane.gartziandia@ehu.es

Introduction: Drug access to the CNS is hindered by the presence of the blood-brain barrier (BBB), and the intranasal route has risen as a non-invasive route to transport drugs directly from nose-to-brain avoiding the BBB [1]. Nanoparticles (NPs) have been described as efficient shuttles for direct nose-to-brain delivery of drugs [2]. There are few studies describing NP nose-to-brain transport. The aim of this work was: *i*) to develop, characterize and validate an olfactory mucosa *in vitro* model; and, *ii*) to study the transport of polymeric- and lipid-based NPs across this model to estimate NP access into the brain using cell penetrating peptide (CPPs) moieties: Tat and Penetratin (Pen).

Materials and Methods: Nanostructured lipid carriers (NLC) and poly(D,L-lactide-*co*-glycolide) (PLGA) NPs were prepared using the melt-emulsification technique [3], and a water-in-oil-in-water (w/o/w) double emulsion solvent evaporation method [4], respectively. For the characterization of NPs, the mean diameter and size distribution were measured by dynamic light scattering, and the zeta potential was determined by laser Doppler micro-electrophoresis. The *in vitro* stability of all the NP formulations was tested first in transport buffer (HBSS) and secondly, in cerebrospinal fluid (CSF), to assess a foreseen *in vivo* application of NPs. The olfactory mucosa nasal *in vitro* model was developed following the previous experience of our group [5], and cell monolayer integrity was assessed measuring the trans-epithelial electrical resistance (TEER) of 3 different cell ratios every 2 days during 21 days, to select the optimum cell ratio and day to perform the transport studies. To evaluate the cytotoxicity of the different formulations in olfactory mucosa cells, cell viability was assessed by MTT assay after the incubation of cells with the afore mentioned NPs (0.01-1.5 mg/mL). Transport of NPs across olfactory cells was studied quantitatively by fluorescence measurement and qualitatively by confocal laser scanning microscopy, for which DiR-labeled NPs were employed.

Results and Discussion: In this study 4 lipid-based NPs (NLC, CS-NLC, Tat-CS-NLC, Pen-CS-NLC) and 4 polymeric NPs (PLGA, PEI-PLGA, Tat-PLGA, Pen-PLGA NPs) were prepared. All NLC formulations exhibited a mean particle size between 100 and 139 nm, and a zeta potential between -20 and +45 mV, depending on their surface characteristics. Regarding PLGA NPs, mean particle sizes were found to be ≈ 200 nm. The zeta potential of these NPs was ≈ -20 mV, and as with NLC, the linkage of the CPPs to the NPs led to a significant change in the surface charge, from negative to positive values (≈ +5 mV). All tested PLGA and NLC formulations were stable in HBSS and in CSF, and biocompatible with the olfactory mucosa cells. Nevertheless, 0.7% of PLGA NPs was able to cross the olfactory cell monolayer, whereas 8% and 22% of NLC and chitosan-coated NLC (CS-NLC) were transported across the nasal *in vitro* model, respectively. Thus, our finding suggests that lipid-based NPs could result more appropriate than polymeric NPs for the intranasal administration. Moreover, the incorporation of CPPs to CS-NLC surface significantly increased their transport, reaching 46% of transported NPs. Although the cationic and CPP coated NPs showed higher transport than uncoated PLGA NPs, no significant differences were observed on PLGA transport across the nasal cell monolayers whatever the formulation ($p > 0.05$), and the values ranged from 0.7 to 7% of transported NPs.

Conclusions: In this study a nasal *in vitro* model has been validated for the first time to evaluate NP nasal-to-brain delivery. This model is a valuable tool to evaluate the potential of different NPs to access the brain through the nasal route. All in all, CPP-CS-NLC seem to be promising brain shuttles via nose-to-brain. However, to further confirm CPP-CS-NLC nasal-penetration, these *in vitro* results should be verified *in vivo*.

Acknowledgements: This project was partially supported by the "Ministerio de Economía y Competitividad" (SAF2013-42347-R), the University of the Basque Country (UPV/EHU) (UFI 11/32), and FEDER funds. O. Gartziandia thanks the University of the Basque Country for a fellowship grant.

References:

[1] Illum L. Eur J Pharm Sci **11** (2000) 1.
[2] Mistry A, Stolnik S, Illum L. Int J Pharm **379** (2009) 146.
[3] Gartziandia O, Herran E, Pedraz JL, Carro E, Igartua M, Hernandez RM. Colloids Surf B Biointerfaces **134** (2015) 304.
[4] Egusquiaguirre SP, Manguán-García C, Pintado-Berninches L, Iarriccio L, Carbajo D, Albericio F, Royo M, Pedraz JL, Hernández RM, Perona R, Igartua M. Eur J Pharm Biopharm **91** (2015) 91.
[5] des Rieux A, Fievez V, Théate I, Mast J, Préat V, Schneider YJ. Eur J Pharm Sci **30** (2007) 380.

Graphene oxide nanoparticles coated with serum improves *in vitro* and *in vivo* the functional activity of microencapsulated C_2C_{12} myoblasts

Laura Saenz del Burgo[†], *Jesús Ciriza*[†], *Argia Acarregui, Haritz Gurruchaga, Rosa María Hernández, Gorka Orive, José Luis Pedraz*

NanoBioCel Group, Laboratory of Pharmaceutics, School of Pharmacy, University of the Basque Country (UPV/EHU), Spain; and Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
e-mail: laura.saenzdelburgo@ehu.eus [†]These authors contributed equally

Introduction: Alginate cell microencapsulation is widely used in research as a delivery system for therapeutic drugs. The structure of the capsule and its semipermeable membrane enable the flow of nutrients and gases between its outer and inner side, as well as the waste outlet and release of therapeutic molecules produced by encapsulated cells [1, 2]. Our research group has previously studied the microencapsulation of genetically modified C_2C_{12} myoblasts to secrete erythropoietin (EPO), and showed that the release of EPO lasted for at least 210 days [3, 4]. In spite of the great promise of the cell encapsulation technology, there are still several challenges that need to be solved. The major one aims to decrease the number of dying cells inside the microcapsules. Graphene oxide (GO), a chemically modified highly oxidized form of graphene [5], promotes the proliferation of different cell types either on 2D or 3D matrices. However, a more detailed *in vitro* characterization of GO containing scaffolds needs to be performed. Because of the unique properties of this material, we proposed to combine these nanoparticles with cell microencapsulation technology. Consequently, we incorporated GO into alginate microcapsules, characterized their physical properties and tested its *in vitro* and *in vivo* biocompatibility with EPO producing C_2C_{12} myoblasts for future clinical applications.

Materials and Methods: Murine C_2C_{12} myoblasts engineered to secrete EPO were immobilized into alginate-poly-L-lysine-alginate (APA) microcapsules using a pneumatic atomization generator (Cellena®). GO was suspended either in water, fetal bovine serum (FBS) or bovine serum albumin (BSA) and mixed with alginate (final concentrations: 10-500 µg/mL GO). Early apoptosis was quantified with Annexin-V-FITC apoptosis detection kit and viability with the LIVE/DEAD® viability/cytotoxicity kit, both by flow cytometry. CCK8 assay was performed to analyze the metabolic activity and the cell membrane integrity was determined by the release of lactate dehydrogenase (LDH). For adsorption studies, EPO and GO were incubated for 24 h and the non-adsorbed protein was detected by Elisa. For *in vivo* studies, syngeneic C3H mice were implanted with APA microcapsules and blood samples were collected weekly for 2 months. Hematocrit was determined using a standard microhematocrit method and expressed as mean ± standard deviation.

Results and Discussion: We have determined that 25-50 µg/mL of GO is the most suitable concentration for microencapsulation of C_2C_{12}-EPO myoblasts at a density of 4×10^6 cells/mL of alginate in 160 µm diameter size microcapsules, since this rank provides lower cell apoptosis and death, and better metabolic activity and membrane integrity results. However, EPO release decreased when encapsulated C_2C_{12}-EPO myoblasts in GO-alginate microcapsules were analyzed, even at the lowest GO concentration, due to the EPO adsorption to GO nanoparticles. Therefore, we tested if pre-incubation of GO nanoparticles with FBS or BSA might represent an alternative by giving a protein coating to GO nanosheets, which would attenuate their capacity to adhere the therapeutic protein. FBS-coating avoided completely the adsorption of EPO to GO and enhanced, even more than non-coated GO nanosheets, the viability, membrane integrity and EPO release of encapsulated cells, while reducing cell apoptosis and death. Finally, C_2C_{12}-EPO myoblasts encapsulated in alginate microcapsules containing FBS-coated GO nanoparticles showed the highest hematocrit levels after implantation *in vivo* compared to alginate microcapsules without GO or non-coated GO.

Conclusions: Altogether, our results show that GO concentrations between 25 and 50 µg/mL increase the viability, metabolic activity and membrane integrity of encapsulated C_2C_{12}-EPO myoblasts. Moreover, the coating of GO nanoparticles with FBS effectively avoids the adsorption of the therapeutic protein by GO, enhancing myoblasts viability within APA microcapsules and, therefore, increasing the release of the therapeutic protein *in vitro* and *in vivo*.

Acknowledgements: Financial support from University of the Basque Country UPV/EHU (UFI 11/32), the Basque Country Government and the Ministry of Science and Innovation is gratefully acknowledged. Authors wish to thank the intellectual and technical assistance from the Platform for Drug Formulation (NANBIOSIS) CIBER-BBN.

References:

[1] Ciriza J, Saenz del Burgo L, Virumbrales-Muñoz M, Ochoa I, Fernandez LJ, Orive G, Hernandez RM, Pedraz JL. Int J Pharm **493** (2015) 260.
[2] Orive G, Santos E, Pedraz JL, Hernández RM. Adv Drug Deliv Rev **67-68** (2013) 3.
[3] Murua A, Orive G, Hernández RM, Pedraz JL. J Control Release **137** (2009) 174.
[4] Orive G, De Castro M, Ponce S, Hernández RM, Gascón AR, Bosch M, Alberch J, Pedraz JL. Mol Ther **12** (2005) 283.
[5] Goenka S, Sant V, Sant S. J Control Release **173** (2014) 75.

Detection of intracellular apoptotic activity using polystyrene nanoparticles

Rosario M. Sánchez-Martín[1,2], *Juan Manuel Cardenas-Maestre*[3], *Ana M. Perez-Lopez*[3], *Mark Bradley*[3]

[1]Department of Medicinal and Organic Chemistry, University of Granada, Spain.
[2]Pfizer - Universidad de Granada - Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Granada, Spain.
[3]School of Chemistry, University of Edinburgh, United Kingdom.
e-mail: rmsanchez@ugr.es

Introduction: Monitoring enzymatic activity in biological systems is important in many fields of cell biology, medicinal chemistry, and chemical biology. For long time, these enzymatic activity studies were performed using either recombinant proteins or cell lysate. Nowadays, there is a high trend to develop novel approaches to allow enzymatic activity assays *in situ*. The capability to analyze the activity of a particular enzyme inside the cell, in its natural cellular environment, will provide valuable information and also display a more realistic picture of the genuine behavior of an enzyme. There is a demand in the research community of intracellular stable multifunctional probes to monitor the *in situ* intracellular enzymatic activity during longer periods of time without need of reloading [1]. Previously, we reported the application of polymeric nanoparticles (NPs) as a cellular delivery and tracking device capable of introducing a range of biological and chemical modalities into cells [2-4]. Herein, a novel application of these multifunctional polymeric materials is described based on their use as accurate *in situ* intracellular sensors by conjugation of an internal control (that gives an extra feature to both evaluate cellular uptake efficiency and track probes over time). In particular, a specific application of these multifunctional microspheres as sensors of caspase-3/7 proteases by conjugation of peptide substrate of caspase-3 (DEVD) with a fluorogenic reporter (7-amino-4-trifluoromethylcoumarin, AFC) to Cy5-labeled NPs is described [5].

Materials and Methods: Amino-functionalized polystyrene NPs (460 nm) were prepared by dispersion polymerization. Functionalization with poly(ethylene glycol) and bifuntionalization with Fmoc-Lys spacer-Dde (OH) was carried out. Labeling with fluorophore Cy5 and conjugation of the DEVD-AFC substrate were done following a Fmoc solid phase protocol. Human cervical carcinoma (HeLa) cells were treated with different concentrations of the Cy5-DEVD-nanoprobe, grown for 24 h prior to the induction of apoptosis with staurosporine (ST). NPs (without the fluorogenic substrate) and Ac-DEVDAFC (without a carrier system) were used as controls. Efficiency of uptake and sensing capacity were analysis by flow cytometry.

Results and Discussion: In order to provide an internal control (that gives an extra feature to both evaluate cellular-uptake efficiency and track probes over time) together with the sensing measurement, a protocol based on the use of an orthogonal protected structure for dual functionalization of microspheres has been successfully developed. DEVD-AFC was attached to fluorescent labeled microspheres and evaluated for *in situ* detection and monitoring of enzymatic activity. Results indicated that this probe was hydrolyzed successfully by caspases-3/7, resulting in the release of AFC. No fluorescence was observed in cells loaded with Cy5 if apoptosis was not induced, confirming cleavage selectivity. This probe allows quantifying amount of enzyme per cell based in AFC/Cy5 fluorescence intensity ratio.

Conclusions: A fluorogenic substrate specific for caspase-3/7 was attached to Cy5 fluorescent labeled bifunctional polymeric particles and the resulting conjugate was successfully employed to monitor *in situ* intracellular apoptotic activity by flow cytometry. This chemical probe allows cells to be monitored and the detect apoptosis at different time points.

Acknowledgements: This project has received funding from the 7th European Community Framework Program (FP7-PEOPLE-2011-CIG-Project Number 294142).

References:

[1] Valeur B, Berberan-Santos MN. Molecular Fluorescence: Principles and Applications, 2nd Ed. Wiley-VCH, Weinheim, Germany, 2012.
[2] Unciti-Broceta A, Díaz-Mochón JJ, Sánchez-Martín RM, Bradley M. Acc Chem Res **15** (2012) 1140.
[3] Cardenas-Maestre JM, Panadero-Fajardo S, Pérez-López AM, Sánchez-Martín. J Mater Chem **21** (2011) 12735.
[4] Yusop RM, Unciti-Broceta A, Johansson EM, Sánchez-Martín RM, Bradley M. Nat Chem **3** (2011) 239.
[5] Cárdenas-Maestre JM, Pérez-López AM, Bradley M, Sánchez Martín RM. Macromol Biosci **7** (2014) 923.

Anxiolytic-like effect of liposomal formulation containing Nimodipine in mice

Lina C. G. e A. I. Moreno[1,2,3], *Rivelilson M. Freitas*[2], *Juan M. Irache*[1], *Nereide S. Santos Magalhães*[3]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Navarra, Spain.
[2]Laboratory of Experimental Neurochemistry Research, Federal University of Piaui, Brazil.
[3]Immunopathology Keizo-Asami Laboratory, Federal University of Pernambuco, Brazil.
e-mail: linaclaramoreno@gmail.com

Introduction: Anxiety is an emotional state that is part of human existence. However, this condition may progress to pathology when it occurs disproportionately to the triggering event that causes it or when there is no apparent reason for its onset [1]. The pharmacological treatment of pathological anxiety consists of the use of benzodiazepines, buspirone and antidepressants. Although these drugs show great efficacy, their administration has many side effects such as amnesia, induction of dependence and sedation [2]. Nimodipine (NMD) has high lipophilicity and hence easily crosses the blood-brain barrier. This drug is used in the treatment of brain ischemia, besides being useful in the therapy of mood disorders and senile dementia, and in displaying anticonvulsant properties [3]. However, the administration of NMD has a number of limitations: low bioavailability, low aqueous solubility and photosensitivity. The goal of the present study was thus to develop a liposomal formulation containing NMD (NMD-Lipo) and to evaluate anxiolytic activity using models of anxiety (open-field, light and dark and elevated plus-maze test).

Materials and Methods: NMD-Lipo was prepared using the method of hydrating the lipid film. NMD-Lipo was produced using the lipids soybean phosphatidylcholine and cholesterol (117.6 mM) at 8:2 ratio and drug concentration of 1 mg/mL. These constituents were dissolved in a mixture of chloroform:methanol (3:1, v/v) under magnetic stirring. The solvents were removed by vacuum evaporation resulting in a thin lipid film. This film was then hydrated with 10 mL of pH 7.4 phosphate buffer solution, and the resulting was then subjected to sonication to obtain small unilamellar liposomes. After 24 h of production, NMD-Lipo was characterized by evaluating its features: macroscopic aspects, pH, particle size, polydispersity index, zeta potential, drug content and encapsulation efficiency. The anxiolytic-like effect was evaluated using 3 classic models of anxiety: the open-field, de light and dark and the elevated plus-maze test.

Results and Discussion: The data of NMD-Lipo properties indicates that the liposomes are homogeneous small unilamellar vesicles (SUV) with size of ≈ 107 nm, a surface charge of -5.32 ± 1.29 mV and a payload of 0.98 ± 0.58 mg/mL with an encapsulation efficiency of 99%. The administration of NMD-Lipo (at the doses of 0.1, 1 or 10 mg/Kg) has no sedative or muscle relaxant effect in animals, since there was no reduction in the number of crossings, grooming and rearings. The increased residence time of the animals treated with NMD-Lipo in the bright field is a reflection of the anxiolytic-like activity of the formulation. Furthermore, the reduction in residence time of rodents treated with the combination of flumazenil and NMD-Lipo in the illuminated box suggested that NMD-Lipo acted on benzodiazepine receptors. The increase in the number of entries and length of stay in the open arms of mice treated with NMD-Lipo suggested the anxiolytic activity of the formulation. On the other hand, the reduction in number of entries (as well length of stay in open arms) of rodents treated with a combination of flumazenil and NMD-Lipo suggested that NMD-Lipo act on benzodiazepine receptors [4].

Conclusions: This study suggests that the administration of a liposomal formulation containing NMD did not produce sedation and muscle relaxation in mice, showing anxiolytic-like activity in the open-field, the light and dark and the elevated plus-maze tests. Results of the treatment with NMD-Lipo were significantly better than the rodents treated with non-encapsulated NMD, suggesting that the liposomes promoted a controlled release effect. The decrease in the anxiolytic effect of NMP-Lipo in animals pretreated with flumazenil suggests that the formulation acts on benzodiazepine receptors.

Acknowledgements: This work was supported by the FACEPE, the CNPq and the CAPES (Brazil).

References:

[1] Salomons AR, Kortleve T, Reinders NR, Kirchhoff S, Arndt SS, Ohl F. Behav Brain Res **209** (2010) 241.
[2] Bartley CA, Hay M, Bloch MH. Prog Neuropsychopharmacol Biol Psychiatry **45** (2013) 34.
[3] Sun C, Wang J, Liu J, Qiu L, Zhang W, Zhang L. AAPS PharmSciTech **14 (**2013) 332.
[4] Moreno LCGAI, Oliveira GZS, Cavalcanti IMF, Santos-Magalhães NS, Rolim HML, Freitas RM. Pharmacol Biochem Behav **116** (2014) 64.

Determination of the most suitable cell load for mesenchymal stem cell D1 microencapsulation systems: *in vitro* and *in vivo* study

T. López-Méndez[1], E. Santos[1], F.J. Blanco[2], J.L. Pedraz[1], G. Orive[1], R.M. Hernández[1]

[1]NanoBioCel Group, Laboratory of Pharmaceutics, School of Pharmacy, University of the Basque Country (UPV/EHU), Spain; and Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
[2]CIBER-BBN-Bioscaff Cartílago, INIBIC-Hospital Universitario A Coruña, Spain.
e-mail: tania.lopez@ehu.eus

Introduction: Cell encapsulation technology has provided a wide range of promising therapeutic treatments for different types of diseases, such as diabetes, bone and cartilage defects, cancer or anemia [1]. In recent years, mesenchymal stem cells (MSCs) are being widely studied due to their interesting characteristics. Firstly, MSCs have the property of being hypoimmunogenic, which is of great importance in the field of transplantation of organs, tissues and/or cells. Moreover, they are able to secrete a variety of cytokines and growth factors of therapeutic interest (VEGF, IGF-1, IL-10, TGF-β, etc.), which produce an immunomodulatory effect on the environment of the MSCs and stimulate endogenous repair mechanisms [2]. Furthermore, the combination of MSCs with biomaterials, such as alginate, allows the modification and control of certain characteristics of this cell type to create different therapeutic profiles [3]. Due to the lack of studies describing the influence of different MSC D1 densities in cell microencapsulation systems, the objective of this investigation is to determine the most appropriate cell load for microencapsulation systems that incorporate MSCs D1, to obtain a high viability that can be maintained over time, a proliferation rate that allows cell renewal within the capsule, and a constant secretion of the therapeutic molecule.

Materials and Methods: MSCs D1 line was obtained from ATCC (D1 ORL UVA [D1]) and genetically modified to secrete murine erythropoietin (Epo). Alginate-poly-L-lysine-alginate (APA) microcapsules were elaborated using an electrostatic droplet generator with brief modifications of the procedure designed by Lim and Sun [4]. Cell loads used in the present study were 2×10^6 (Group 1), 5×10^6 (Group 2) 10×10^6 (Group 3) or 20×10^6 (Group 4) cells/mL. Morphological analyses of all batches of microcapsules were made by observation under a Nikson TSM microscope. The viability of cells was tested with the Live/Dead kit (Life Technologies) following manufacturer's indications. The exact number of living cells per capsule was analyzed by flow cytometry using *Trucount tubes* and Live/Dead kit to differentiate living and dead cells. Encapsulated MSC D1 supernatants were assayed for Epo secretion using the ELISA kit. Bromoxiuridine (BrdU) study was done to test the proliferation capacity of cells, and a cell counting kit-8 (CCK-8) was used to analyze the metabolism of encapsulated cells. Animal studies were carried out according to the ethical guidelines established by our Institutions, under an approved animal protocol. Adult female C57BL/6J mice were implanted subcutaneously with a total volume of 300 µL of cell loaded microcapsules. At days 15, 30 and 45 after implantation, 3 animals from each group were sacrificed and capsules were explanted. The aforementioned viability assays, Epo secretion quantification, CCK-8 and BrdU tests and histological studies were performed with the explanted microcapsules. In order to detect significant differences between the groups, an ANOVA test was used. All statistical computations were performed using SPSS 29 (SPSS, Chicago, USA).

Results and Discussion: *In vitro* results obtained in metabolism and Epo production assays show a general upward trend in all groups, with significant differences in some points due to the more pronounced increase in the lower density groups. Differences can be noticed also in viability assays, especially in the last day of the study, when the percentage of living cells per capsule was higher in the group of 2×10^6 cells/mL, similar between the groups of 5×10^6 and 10×10^6 cells/mL, and smaller in the group of 20×10^6 cells/mL. These data agree with the images obtained by microscopy using the Live/Dead kit. Altogether, these results suggest that, even if there is a general increase in the total cell number in all groups, the reduced space into the capsule does not allow drastic increases in the higher cell density groups, resulting in lower percentages of viability and smaller changes in cell metabolism and Epo production. The results obtained *in vivo* show a similar trend in general terms but, the enriched environment found when implanted in the animal tissue appears to promote cell proliferation in all groups which leads, first in the higher cell density groups, to a scenario of uncontrolled growth and possible implant failure. Results obtained in the BrdU test, comparing the *in vitro* and *in vivo* studies, confirm this hypothesis showing faster proliferation patterns for all the groups when implanted.

Conclusions: Although *in vitro* a higher cell density allows a more controlled release of the therapeutic molecule, *in vivo* there are other factors that primarily affect cell proliferation that must be considered and controlled for optimum results.

Acknowledgements: T. López-Méndez thanks the Government of the Basque Country for the PhD fellowship.

References:

[1] Santos E, Pedraz JL, Hernández RM, Orive G. J Control Release 170 (2013) 1.
[2] Wang Y, Chen X, Cao W, Shi Y. Nat Immunol 15 (2014) 1009.
[3] Rowley JA, Madlambayan G, Mooney DJ. Biomaterials 20 (1999) 45.
[4] Lim F, Sun AM. Science 210 (1980) 908.

Lipid peroxidation and protein carbonylation in rat liver after treatment with citrate-stabilized gold nanoparticles

Carlos López-Chaves[1], *Cristina Sánchez-González*[1], *Juan Soto-Alvaredo*[2], *María Montes-Bayón*[2], *Jorg Bëttmer*[2], *Juan Llopis*[1]

[1]Department of Physiology, University of Granada, Spain.
[2]Department of Physical and Analytical Chemistry, University of Oviedo, Spain.
e-mail: carloschaves@ugr.es

Introduction: Thanks to their physical and chemical properties, gold nanoparticles (GNPs) have been widely used by many biomedical applications such as antitumor therapies or diagnostic tools. However, some aspects like their storage, distribution or toxicity in biological samples are still unclear. Thiobarbituric acid reactive substances (TBARSs) and protein carbonyl groups are accepted markers of the amount of lipids and proteins that have been oxidized by highly reactive free radicals [1]. The aim of this study was to evaluate the toxicity of citrate-stabilized GNPs in proteins and lipids on liver of rats treated with GNPs by intraperitoneal administration.

Materials and Methods: 16 male Wistar rats (Charles River Laboratories, L'Abresde, France) were divided into 4 study groups (4 rats per group): 1 control group without treatment; 1 group treated with 10 nm-sized GNPs; 1 group treated with 30 nm- sized GNPs and 1 group treated with 60 nm- sized GNPs. Rats were daily injected 0.4 mL of 50 ppm GNPs solution (RM 8011, RM 8012 and RM 8013; National Institute of Standards and Technology, USA) via intraperitoneal. The experiment lasted 9 days. All rats were fed with AIN-93 diet. After anaesthetising, rats were sacrificed and organs were stored at -80 ºC. Liver samples were homogenized in phosphate buffer. Lipid peroxidation was measured by an adaptation of Ohkawa´s method [1]. Protein carbonyl groups were determined after using Cayman Chemical® colorimetric assay kit following manufacturer´s protocol.

Results and Discussion: There was a significant increase in lipid peroxidation in all treated groups compared to control group. This increase was slightly superior in the group treated with 10 nm-sized GNPs. Furthermore, these increases were also observed in protein carbonyl groups in rats treated with 10, 30 and 60 nm-sized GNPs compared to control rats.

Conclusions: Treatment with 10, 30 and 60 nm-sized GNPs increased the content of protein carbonyl groups and lipid peroxidation. The fact that the group treated with the smallest size GNPs had a slightly higher peroxidation together with their higher number of particles per mL lead us to consider that there was an increase in the internalization of these nanoparticles on the liver.

Acknowledgements: Spanish Ministry for Science and Innovation for the financial support (grant number CTQ2011-23038) and Spanish Ministry for Education, Culture and Sport, grant reference FPU13/00062.

References:

[1] Ohkawa H, Ohishi, Yagi K. Anal Biochem **95** (1979) 351.

The role of helper lipids in the intracellular disposition and transfection efficiency of niosome formulations for gene delivery to retinal pigment epithelial cells

G. Puras[1,2], *E. Ojeda*[1,2], *M. Agirre*[1,2], *J. Zarate*[1,2], *S. Grijalvo*[2,3], *R. Eritja*[2,3], *L. DiGiacomo*[4], *G. Caracciolo*[4], *J.L. Pedraz*[1,2]

[1]NanoBioCel Group, Laboratory of Pharmaceutics, School of Pharmacy, University of the Basque Country (UPV/EHU), Spain.
[2]Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
[3]Institute of Advanced Chemistry of Catalonia, IQAC-CSIC, Spain.
[4]Department of Molecular Medicine, "Sapienza" University of Rome, Italy.
e-mail: gustavo.puras@ehu.es

Introduction: Niosomes as gene delivery vectors are commonly based on non-ionic surfactants, cationic lipids and helper lipids. The final gene expression will depend on the capacity of the niosomes to enter the cell and the pathway employed to deliver its cargo into the cell nucleus. Different endocytic vias mediate the intracellular process and the final cargo delivery, where the most studied are clathrin-mediated endocytosis (CME), caveolae-mediated endocytosis (CvME) and macropinocytosis [1-3]. Also, particle transport mechanisms can determine the final intracellular fate of the vector, e.g. lysosomal degradation. Such pathways have their particular characteristics and their intervention in the cellular uptake, and further internal processing of the vectors will depend on many factors, e.g. size, surface charge, morphology and composition. Therefore, it is necessary to study all these factors to develop more efficient non-viral vectors. Thus, we carried out a comparative study of 4 different niosome formulations based on the same cationic lipid and non-ionic tensoactive, but different helper lipid, to determine its role in the formulations and the transfection process mediated by nioplexes upon the addition of pCMS-EGFP reporter plasmid. Niosome formulations only differed in the helper lipid: squalene (Sque), cholesterol (Cho), squalane (Squa) or none helper lipid (None).

Materials and Methods: Niosomes prepared by oil-in-water (o/w) emulsion technique, and nioplexes were characterized in terms of size and zeta potential. The capacity of the niosomes to condense, release and protect the DNA against enzymatic degradation was evaluated by agarose gel electrophoresis. *In vitro* experiments were performed in retinal pigment epithelial cells (ARPE-19) cells by flow cytometry to assess the transfection efficiency of nioplexes and cell viability. Additionally, we carried out cell uptake studies at 1 h after the addition of the nioplexes. To comprehend the internalization process, we analyzed cell trafficking of the formulations in different entry pathways (CME, CvME, macropinocytosis) and lysosomal compartment. Colocalization was analyzed through Mander's overlap coefficient between the nioplexes (niosomes/Cy3 stained DNA) and the above stained endocytic pathways.

Results and Discussion: Results showed that the helper lipid does affect the physicochemical properties of both bare and DNA-loaded niosomes. Among the helper lipids studied, Sque was very promising since it boosted transfection efficiency of nioplexes in ARPE-19 cells. The ability to condense, protect and release DNA was thoroughly evaluated to rationalize efficiency data. Also, relevant transfection-limiting barriers such as cellular uptake and endosomal escape/lysosomal degradation were mechanistically investigated by confocal fluorescence microscopy. Collectively, our data pointed out that the helper lipid has a deep influence on the cellular uptake of nioplexes that, in turn, affects the final fate and transfection efficiency. Niosomes elaborated with Sque as helper lipid showed the highest percentages of transfected cells. Such transfection efficiency could be attributed to their high cellular uptake and the particular entry pathways used, where macropinocytosis pathway and lysosomal elution played an important role. Therefore, these studies bring new insights into the role of helper lipids towards the development of highly efficient niosome formulations as non-viral gene delivery vectors for gene therapy.

Conclusions: Our results suggest that helper lipid composition is a crucial step to be considered in the design of niosome formulation for gene delivery applications, since clearly modulates the internalization mechanism and consequently the final transfection efficiency.

Acknowledgements: This work was partially supported by the University of the Basque Country UPV/EHU (UFI 11/32), the Spanish Ministry of Education (Grant CTQ2010-20541, CTQ2010-14897), the Basque Government (Department of Education, University and Research, predoctoral BFI-2011-2226 grant). Technical and human support provided by SGIker (UPV/EHU) is gratefully acknowledged.

References:

[1] Zhao F, Zhao Y, Liu Y, Chang X, Chen C, Zhao Y. Small **7** (2011) 1322.
[2] Marchini C, Pozzi D, Montani M, Alfonsi C, Amici A, Amenitsch H, Candeloro De Sanctis S, Caracciolo G. Langmuir **26** (2010) 13867.
[3] Cardarelli F, Pozzi D, Bifone A, Marchini C, Caracciolo G. Mol Pharm **9** (2012) 334.

Development of polymeric vaccine delivery nanosystems for HIV/AIDS control through dendritic cell modulation

A.I. Matos[1], C. Peres[1], A.E. Ventura[2], Ana S. Viana[3], P. Borrego[4], L.C. Silva[1,2], N. Taveira[4], H.F. Florindo[1]

[1]Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Portugal.
[2]Centro Química-Física Molecular, Instituto Superior Técnico, Universidade de Lisboa, Portugal.
[3]Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Portugal.
[4]Unidade dos Retrovírus e Infeções Associadas, Centro de Patogénese Molecular, Faculdade de Farmácia, Universidade de Lisboa, Portugal.
e-mail: aimatos@ff.ulisboa.pt

Introduction: Even with the antiretroviral therapy progress, the control of HIV infection remains a worldwide concern. Despite being the most challenging goal, the development of safe and highly immunogenic HIV vaccines is considered the most prominent prophylactic strategy in HIV-AIDS research [1]. To prevent HIV infection and impair the dissemination of the virus, dendritic cells (DCs) can trigger effective long-lasting adaptative immune responses by inducing both: *i*) humoral response through the induction of virus-specific neutralizing antibodies at entry site; and, *ii*) cellular immune response with the stimulation of cytotoxic T lymphocytes (CTLs) able to kill already infected cells [2]. A promising approach is based on the development of polymeric-based nanocarriers to protect and thus promote the delivery of HIV-specific immunogens to those highly specialized antigen presenting cells (APCs) [3]. This study aimed the development and characterization of different types of poly(ethylene glycol)-poly(D,L-lactide-*co*-glycolide) (PEG-PLGA) nanoparticles (NPs) to deliver entrapped HIV-1 gp41 peptides (T20 and T1249) to DCs.

Materials and Methods: Polymeric NPs were prepared by the double emulsion (w/o/w) solvent evaporation method. Polyvinyl alcohol (PVA), block copolymer Pluronic® and glycol chitosan (Cs) were included in formulation composition to assess their potential adjuvant effect and/or improve antigen entrapment and NP stability. HIV-1 gp41 peptides, T20 and T1249, were entrapped or co-entrapped into NPs. Characterization of NPs was performed in terms of size, zeta potential and surface morphology by Dynamic Light Scattering, Laser Doppler Electrophoresis and Atomic Force Microscopy, respectively. Entrapment efficiencies (EE %, w/w) and loading capacities (LC, μg/mg of NP) of the entrapped antigens were quantified by intrinsic fluorescence. DCs were used to evaluate the *in vitro* cytotoxicity of NPs by the AlamarBlue® assay and the cellular uptake by Flow Cytometry and Confocal Microscopy. The peptide integrity was also assessed by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis.

Results and Discussion: PEG-PLGA NPs presented a mean size in the range of 170-180 nm, with low polydispersity index (PdI) values (≤ 0.1) and a surface charge close to neutrality. NP size, spherical shape and surface morphology were also evalauted by AFM. These antigen delivery systems presented high EE (> 75%) and LC (> 15 μg/mg of NP), and no cytotoxic effect on DCs (CRL-11904) up to 72 h of incubation, in the range of tested concentrations (0.125-1 mg/mL). Moreover, PEG-PLGA NPs were extensively taken up by DCs after 40 h of incubation, especially those composed by Cs or PL. Confocal microscopy also confirmed the internalization of NPs. According to NP physicochemical characteristics and internalization pattern by DCs, PEG-PLGA_Cs formulation was selected for further studies. The structure of the HIV-1 peptides entrapped into PEG-PLGA_Cs NPs was maintained intact, not being affected by the double emulsion procedure.

Conclusions: The results herein described show that the stable PEG-PLGA-based NP formulations prepared by the w/o/w solvent evaporation method constitute a promising strategy for the successful delivery of HIV-1 antigens to DCs, aiming the prophylactic and/or therapeutic vaccine development.

Acknowledgements: The authors acknowledge FCT for the financial support (PTDC/SAU-FAR/119389/2010, UID/DTP/04138/2013) and COMBI-VIH from Gilead Genese Program 2014.

References:

[1] Date A, Destache C. Biomaterials **34** (2013) 6202.
[2] Himeno A, Akagi T, Uto T, Wang X, Baba M, Ibuki K, Matsuyama M, Horiike M, Igarashi T, Miura T, Akashi M. Vaccine **28** (2010) 5377.
[3] Caputo A, Castaldello A, Brocca-Cofano E, Voltan R, Bortolazzi F, Altavilla G, Sparnacci K, Laus M, Tondelli L, Gavioli R, Ensoli B. Vaccine **27** (2009) 3605.

Cytotoxicity evaluation of cannabidiol-loaded lipid nanocapsules on the human glioma cell line U-373

Juan Aparicio-Blanco[1], *Ana Isabel Torres-Suárez*[1,2]

[1]Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, Complutense University of Madrid, Spain.
[2]University Institute of Industrial Pharmacy, Complutense University of Madrid, Spain.
e-mail: juan.aparicio.blanco@ucm.es

Introduction: Over the last decade, cannabinoids have progressively attracted increasing interest as potential anticancer therapies since they are mostly well tolerated and devoid of systemic side effects of conventional chemotherapy agents [1]. Intravenous administration may allow release times to be greatly prolonged; however, given their very low water solubility, the intravenous administration of cannabinoids remains a challenge. Therefore, lipid nanocapsules, which consist of an oily core provided with a surfactant shell, thanks to their nanometric size and lipophilic nature may well play the key role to serve as a nanoplatform for intravenous cannabinoid delivery. In this work, we determined the release profile of cannabidiol (CBD), the main non-psychotropic cannabinoid, from lipid nanocapsules; thereupon we evaluated the effect of this nanoplatform on the human glioma cell line U373, and evidenced their internalization thanks to the indocarbocyanine derivative fluorescent dye 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO) [2].

Materials and Methods: Lipid nanocapsules (LNC) were prepared according to the phase inversion technique (PIT) [3]. Briefly, all components are mixed and three cycles of progressive heating and cooling are applied under magnetic stirring, to invert the O/W emulsion into W/O emulsion by passing through a phase-inversion zone. During the last cycle, an irreversible shock is induced with cold water, leading to the formation of LNCs. Cannabidiol-loading represented 15% (w/w) of oil content. Labrafac® WL 1349 (caprylic-capric acid triglycerides) was provided by Gattefossé S.A. (France). Solutol® HS15 (mixture of free polyethylene glycol 660 and polyethylene glycol 660 hydroxystearate) and Lipoïd® S75 (soybean lecithin at 70% of phosphatidylcholine) were gifts from BASF and Gmbh (Germany), respectively. NaCl was supplied by Panreac. De-ionized water was obtained from a MilliQ® Purification System (Millipore, France). CBD was provided by THC Pharma GmbH (Germany). For *in vitro* release assays, SpectraPor® dialysis membranes, molecular weight cut-off 50 kDa were purchased from Spectrum Labs Inc. (USA). Phosphate buffer solution pH 7.4 added with polysorbate 80 was chosen as release medium to mimic physiological conditions and to ensure sink conditions. DiO was purchased from Molecular Probes® (Thermo Fisher Scientific). The cytotoxic effect of the CBD-LNCs on the human glioblastoma cell line U-373 MG was determined, after 4 and 7 days of incubation, by the MTT colorimetric test. The U-373 MG cell line was cultured in a complete medium (DMEM with glutamine, non-essential amino acids, fetal bovine serum 10%, Penicillin/Streptomycin and Amphotericin B) at 37 ºC and 5% CO_2. In the cytotoxicity study, the cells were seeded in 24-well plates at the density of 5×10^3 viable cells/well. The results were expressed as cells viability percentage with respect to the control cells (cells without any treatment). The effect of free CBD on the cell line was also determined. The internalization of LNC was evidenced by confocal laser microscopy with a LEICA SP5 (LEICA, Germany) using LEICA LAS AF software. Images were recorded using a 63 fold/1.4 NA objective. Fluorescence originating from DiO, which was excited by a 488 nm laser, was recorded from 500 to 690 nm for fluorescence profiling in overlapping 15 nm steps.

Results and Discussion: 50 nm-sized CBD-loaded LNCs were prepared according to the expanded phase inversion method, with a drug loading of 60 mg of CBD/g of LNC and with encapsulation efficiency ≈ 80%. LNC efficiently extended CBD release over fifteen days under the assayed conditions. The 50% inhibitory concentration of the free drug substance on the human glioma cell line was between 30-35 μM. When the drug substance is encapsulated, CBD exhibits antitumor effect at least up to a week, according to the *in vitro* release profile. Given a theoretical concentration, CBD encapsulated shows higher efficacy than the free CBD in solution. The encapsulation of a fluorescent dye evidenced the internalization of LNC after few hours into the U373 MG cell line.

Conclusions: This work evidences that LNCs enhance CBD activity against a brain tumor cell line *in vitro*, so they may well serve as adequate cannabinoid-carriers against glioblastoma multiforme. Furthermore, the fact that the activity would remain after medium removal seemed to indicate an efficient internalization of LNCs in the early stages, which was demonstrated by confocal microscopy.

Acknowledgements: Financial support from Ministry of Education, Spain, FPU13/02325, and from CAM-UCM Financial Program, Research Group 910939, Parenteral Administration of drug products, are gratefully acknowledged.

References:

[1] Velasco G, Sánchez C, Guzmán M. Nat Rev Cancer **12** (2012) 436.
[2] Aparicio-Blanco J, Torres-Suárez AI. J Biomed Nanotech **11** (2015) 1283.
[3] Heurtault B, Saulnier P, Pech B, Proust, JE, Benoit JP. Pharm Res **19** (2002) 875.

Formulation, characterization and controlled release studies of vitamins microparticles prepared with different biopolymers by spray drying technique

Berta N. Estevinho, Ioana Carlan, Fernando Rocha

LEPABE, Departamento de Engenharia Química, Faculdade de Engenharia da Universidade do Porto, Portugal.
e-mail: berta@fe.up.pt

Introduction: Microencapsulation, among others techniques, is applied to achieve controlled release of compounds [1]. In the present work, the microencapsulation and release profiles of vitamins microencapsulated by a spray drying process with different biopolymers (considering their biological advantages) are studied. The spray drying process is flexible and produces particles of good quality [1]. Vitamins are important micro nutritional compounds which are involved in many biochemical functions in the human body but are not synthesized by our body; so, they have to be supplied through diet. However, vitamins are very sensitive to the food/pharmaceutical processing. Thus microencapsulation can be used as an alternative to minimize the factors that interfere with the stability of the vitamins, allow for controlled release and mask their undesirable taste. Two different vitamins were used: vitamin B_{12}, considering that is the most chemically complex and the largest of all the vitamins, and vitamin C that is the most common vitamin in the food industry. The release mechanisms vary with the type of encapsulating agent used, the method of preparation, and the environment where the release occurs. There are several models that can describe the release of the active compound. In an ideal system the release may follow zero-, half- or first-order kinetics. In general, the real release of the active compound may be slightly different from these ideal models [2, 3], and because of that, more complex mathematical models attempt to describe the phenomena, namely, the Higuchi and the Korsmeyer-Peppas equations [2,3].

Materials and Methods: The microparticles containing the soluble vitamins were prepared by a spray drying technique, using a spray dryer BÜCHI B-290 (Flawil, Switzerland) with a standard 0.5 mm nozzle. Three different biopolymers were used to prepare microparticles with vitamins: chitosan, a modified chitosan (water soluble) and sodium alginate. The mixture solution (encapsulating agent and vitamin) were then fed to the spray dryer, under the following conditions: solution and air flow rates, air pressure and inlet temperature were set at 4 mL/min (15%), 32 m^3/h (80%), 6.0 bar and 120 ºC, respectively. The concentration of the vitamin in the fed solution to the spray dryer was 2% (w/w). The structural analysis of the surface of the microparticles was performed by scanning electron microscopy (Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M) at Centro de Materiais da Universidade do Porto (CEMUP). The evaluation of release profiles of the vitamin B_{12} and vitamin C was made by an UV method, in an UV-Vis spectrophotometer (SCANSPEC SP110070 from SCANSCI) at 361.4 nm and 260.6 nm, respectively.

Results and Discussion: Spherical microparticles with a regular shape and a mean diameter ≈ 3 μm were produced using all the biopolymers tested (chitosan, modified chitosan and sodium alginate). The microparticles formed with chitosan had a very rough surface, and the microparticles formed with modified chitosan and sodium alginate had a smooth surface. Comparing the SEM images with the release profiles, a slow release was associated to a rougher surface (microparticles with chitosan). For both of the vitamins the release was total. The total amount of the vitamin was released in different times depending on the encapsulating agents used. Considering the release equations presented before, the ones that best adapted to the experimental results are kinetic of zero order and Korsmeyer-Peppas equation. The experimental results adjusted to kinetics of zero order presented good correlation coefficient (r^2 = 0.889 - 0.986). Through the Korsmeyer-Peppas equation, it is possible to identify the mechanisms involved in the controlled release, which are not governed by the Fick´s law. The Korsmeyer-Peppas equation (applied to $Qt/Qf < 0.6$) was adjusted to the experimental results, presenting correlation coefficients ranging from 0.842 to 0.951. The parameter *n* was higher than 0.85 (super case-II transport) for all the cases excepting for the release of the vitamin B_{12} from the modified chitosan microparticles (n = 0.773) meaning an Anomalous transport.

Conclusions: The microparticles were prepared by a spray drying technique with a mean diameter ≈ 3 μm, for all the biopolymers tested (chitosan, modified chitosan and sodium alginate). The release equations that best adapt to the experimental results are the kinetic of zero order and the Korsmeyer-Peppas equation.

Acknowledgements: The authors wish to thank Fundação para a Ciência e a Tecnologia (FCT) for the postdoctoral grant SFRH/BDE/15649/2007 (B. Estevinho).

References:

[1] Estevinho BN, Rocha F, Santos L, Alves A. Trends Food Sci Technol **31** (2013) 138.
[2] Pothakamury UR, Barbosa-Cánovas GV. Trends Food Sci Technol **6** (1995) 397.
[3] Sankalia MG, Mashru RC, Sankalia JM, Sutariya VB. Eur J Pharm Biopharm **65** (2007) 215.

Poly(anhydride) nanoparticles for oral immunotherapy against peanut allergy

Ana Brotons-Cantó, Nekane Martín-Arbella, Jose Matías, Carlos Gamazo, Juan M. Irache

Departments of Pharmacy and Pharmaceutical Technology and Microbiology, IdiSNA, University of Navarra, Spain.
e-mail: abrotons@alumni.unav.es

Introduction: Among food allergies, peanut (*Arachis hypogea*) has attracted great research attention because it is a long-life allergy with high prevalence and it is often severe [1]. Management of peanut allergy is based on avoidance peanut-containing products and use of self-injectable epinephrine. In this context, oral immunotherapy is one of the most promising treatments to overcome this disease [2]. For this purpose, one possible strategy may be the use of poly(anhydride) nanoparticles (NPs), based on the use of the copolymer of methyl-vinyl-ether and maleic anhydride. These poly(anhydride) NPs have been recently identified as innate immunity inducers, activating the complement system and Toll-like receptors (TLRs), mainly TLR2 and TLR4 [3]. The aim of this work was the development of NPs as an adjuvant and allergen-delivery system for peanut allergen immunotherapy.

Materials and Methods: NPs loaded with peanut extract (PE) were prepared by a solvent displacement method and dryed by spray-drying [4]. After the physicochemical characterization, particles were tested by oral administration in a mice model of peanut allergy (ICR). Animals were sensitized against peanut by oral gavage with peanut proteins and cholera toxin, and one sensitized vaccinated with either free peanut extract and the NP-based vaccine, and challenged to provoke an anaphylactic shock.

Results and Discussion: NPs achieved an encapsulation efficiency of 50%, mean size ≈ 200 nm, negative zeta potential and low polydispersity index. The efficacy of the formulations was evaluated in peanut allergic mice treated with 1 mg peanut protein 3 times (Figure 1). NPs were able to diminish the anaphylaxis symptoms induced in mice by the intraperitoneal administration of 2 mg PE (i.e. hypothermia, piloerection and stillness) compared to free peanut solution and induce a balanced Th_1/Th_2 response.

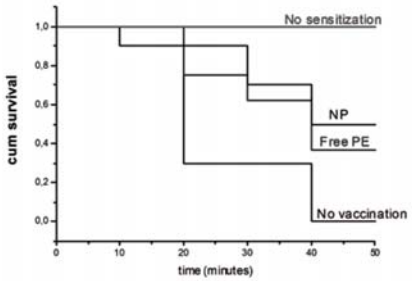

Figure 1. Cumulative survival vs. time (min) of the experimental groups.

Conclusions: Taken together, our findings indicate that poly(anhydride)-based NPs are efficient stimulators of immune responses and promising adjuvants and allergen-delivery systems applied for immunotherapy.

Acknowledgements: This research has received funding from the European Community through the FP7-2011-NMP-280761 "ALEXANDER" project. ABC also thanks "Asociación de Amigos de la Universidad de Navarra" for the scholarship support.

References:

[1] Nguyen-Luu NU, Ben-Shoshan M, Alizadehfar R, Joseph L, Harada L, Allen M, St-Pierre Y, Clarke A. Pediatr Allergy Immunol **23** (2012) 133.
[2] Rebouças Jde S, Irache JM, Camacho AI, Esparza I, Del Pozo V, Sanz ML, Ferrer M, Gamazo C. Eur J Pharm Biopharm **82** (2012) 241.
[3] Tamayo I, Irache JM, Mansilla C, Ochoa-Repáraz J, Lasarte JJ, Gamazo C. Clin Vaccine Immunol **17** (2010) 1356.
[4] Arbós P, Wirth M, Arangoa MA, Gabor F, Irache JM. J Control Release **83** (2002) 321.

A novel nanotechnology tool to assess drug promiscuity in cancer

Teresa Valero[1,2], *Victoria Cano-Cortés*[2], *Asier Unciti-Broceta*[1], *Rosario Sánchez-Martín*[2]

[1]Edinburgh Cancer Research UK Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, UK.
[2]Pfizer - Universidad de Granada - Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Parque Tecnológico de Ciencias de la Salud (PTS), Granada, Spain.
e-mail: teresa.valero@genyo.es

Introduction: Among currently available treatments for cancer, the use of promiscuous tyrosine kinase inhibitors (TKI) has gained unprecedented popularity during the last decade [1, 2]. This promiscuous kinase inhibitor targets Bcr-Abl, Src family, receptor tyrosine kinases and TEC family kinases [3], targeting the well-conserved ATP pocket. However, this intrinsic promiscuity makes it very difficult to elucidate which target(s) of Dasatinib is(are) responsible for the phenotypic effect observed in each cancer type. On one hand, inhibitor´s promiscuity may be advantageous if it circumvents resistance mechanisms. On the other hand, it may represent a major drawback, depending on the result of the inhibition, if it leads to severe side effects, which limit the dosage used and therefore the efficiency of the treatment. The elucidation of the full spectrum of targets of this and other kinase inhibitors in each cell type is crucial to develop personalized selective therapies. We aim to offer a new tool to make possible the interrogation of a full kinome for a certain drug. The central objective of this contribution is to develop a reliable, *in situ*, ratiometric, and cost-efficient method based on nanotechnology to detect protein kinases which are targeted by promiscuous kinase inhibitors such as Dasatinib, and to prove its functionality on living systems. As a proof of concept Dasatinib was conjugated to fluorescently labeled nanoparticles (NPs).

Materials and Methods: Amino-functionalized microspheres (460 nm) with 2% divinylbenzene (DVB) cross-linking were prepared by a nemulsifier-free emulsion polymerization (styrene, DVB, VBAH, AIBN·2 HCl, V5O initiator, water) [4, 5]. PEGylation of polystyrene NPs was performed following a Fmoc solid phase protocol using Oxyma/DIC as coupling reagents. Following a multifunctionalization strategy based on protection-deprotection the fluorophore Cy5 was conjugated. Finally, we followed a bio-orthogonal functionalization strategy in order to modify Dasatinib so that it could be conjugated to NPs and efficiency was demonstrated by Zeta potential (Zetasizer Nano, Malvern Instruments) and cleavage followed by high resolution mass and NMR. NP uptake by MDA-MB-231 cell line was assessed using a Fortessa flow cytometer and analyzed by Flowjo XV, imaging of the uptake process over one week was recorded by Incucyte™ (ESSEN BioScience). Cell biocompatibility was assessed by MTT test and kinase adsorption by western blot. Statistic analysis was performed using Graphpad Prism software.

Results and Discussion: Uniform, monodisperse amino-functionalized microspheres (460 nm) were successfully functionalized and characterized by size and zeta potential. Bio-orthogonal functionalization of Dasatinib allowed its conjugation with these NPs. The efficiency of conjugation was demonstrated by different methods: *i*) Zeta potential showed a decrease of potential compared to amino-functionalized NPs; and, *ii*) parallel reactions on resins allowed us to cleave conjugated modified Dasatinib, to isolate it and to characterize it by high resolution mass and NMR. Dasatinib-bearing NPs were biocompatible and they did not show any significant reduction in proliferation compared to controls as showed by MTT viability test over 1 week. Cellular uptake was as efficient as control NPs as showed by flow cytometry experiments with a MNF50 comparable to control NPs and previous reports of this group [6]. Imaging experiments over 1 week using the Incucyte™ technology demonstrated a continuous increase in cellular uptake dependent on cell migration. Finally, western-blot analysis of NP adsorbed kinases showed an important influence of corona effect.

Conclusions: We have successfully conjugated a reference kinase inhibitor drug, Dasatinib, to crosslinked polystyrene NPs. These NPs are uniform, monodisperse, biocompatible and show an efficient cellular uptake. Moreover, the influence of corona effect has to be considered. This versatile method will serve potentially to elucidate the action mechanism of any known drug in an easy and cost-efficient way.

Acknowledgements: Funding from the European Union´s 7th Framework Program for research, technological development and demonstration under grant agreement no. 267226, TALENTIA POSTDOC, and CEI BioTic Granada, University of Granada (MECD).

References:

[1] Knight ZA, Lin H, Shokat KM. Nat Rev Cancer **10** (2010) 130.
[2] Das J, Chen P, Norris D, Padmanabha R, Lin J, Moquin RV, Shen Z, Cook LS, Doweyko AM, Pitt S, Pang S, Shen DR, Fang Q, de Fex HF, McIntyre KW, Shuster DJ, Gillooly KM, Behnia K, Schieven GL, Wityak J, Barrish JC. J Med Chem **49** (2006) 6819.
[3] Araujo J, Logothetis C. Cancer Treat Rev **36** (2010) 492.
[4] Unciti-Broceta A, Johansson EM, Yusop RM, Sánchez-Martín RM, Bradley M. Nat Protoc **7** (2012) 1207.
[5] Sanchez-Martin RM, Muzerelle M, Chitkul N, How SE, Mittoo S, Bradley M. Chembiochem **6** (2005) 1341.
[6] Unciti-Broceta JD, Cano-Cortés V, Altea-Manzano P, Pernagallo S, Díaz-Mochón JJ, Sánchez-Martín RM. Sci Rep **5** (2015) 10091.

PEG-modified PLA nanoparticles for site-specific functionalization

João Conniot[1,2], *Sheiliza Carmali*[2], *Teresa S. Barata*[2], *Liana C. Silva*[1], *Steve Brocchini*[2], *Helena F. Florindo*[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal.
[2]EPSRC Centre for Innovative Manufacturing in Emergent Macromolecular Therapies, UCL School of Pharmacy, London, UK.
e-mail: jmoconniot@ff.ulisboa.pt

Introduction: Dendritic cell (DC)-based cancer vaccines are expected to elicit a specific immune response against tumor cells. It is thus important to design an efficient platform that can allow DC targeting and modulate the intracellular trafficking of antigens [1]. Based on previous work [2], the goal was to develop PLA-PEG-*bis*-sulfone polymeric nanoparticles (NPs) that can be site-specifically functionalized with ligands, such as antigen-binding fragments (Fabs), to accomplish specific targeting of DCs.

Materials and Methods: PLA-PEG-*bis*-sulfone **3** was synthesized by covalent coupling of *bis*-sulfone-PEG-amine (3 and 10 kDa) **1** with 2 kDa *N*-hydroxysuccinimide PLA ester **2**. Preliminary characterization of PLA-PEG-*bis*-sulfone **3** was performed through ^{1}H-NMR and FTIR spectroscopy.

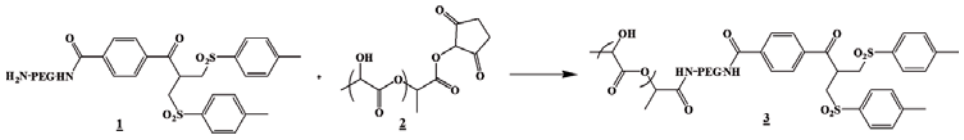

Figure 1. Schematic representation of PLA-PEG-*bis*-sulfone **3**.

To assess the ability of compound **3** to conjugate with Fabs, ranibizumab was used as model. Reduced ranibizumab was added to a solution of PLA-PEG-*bis*-sulfone **3** and incubated at room temperature overnight. SDS-PAGE electrophoresis was performed to evaluate the conjugation. Compound **3** was then used to formulate NPs by state of the art double emulsion solvent evaporation method, using PVA as surfactant and a mixture of PLA-PEG-*bis*-sulfone **3** and PLA (1:9). mPEGPLA/PLA NPs (1:9) were used as control. NPs were recovered by centrifugation and washed to remove excess of surfactant. Mean diameter and polydispersity index (PdI), surface charge and morphology of NPs were analyzed through DLS, zeta potential and TEM, respectively. Finally, reduced Fabs were allowed to react with *bis*-sulfone moieties of PLA-PEG-*bis*-sulfone/PLA NPs, under gentle stirring overnight. The association of Fabs to PLA-PEG-*bis*-sulfone/PLA NPs was assessed by Ellman's reagent assay.

Results and Discussion: ^{1}H-NMR and FTIR analysis indicated that PLA-PEG-*bis*-sulfone **3** was synthesized. Characteristic NMR shifts were observed at 1.58-1.64 ppm, corresponding to PLA, 3.48-3.7 ppm for PEG moiety and the distinctive signal of the *bis*-sulfone group between 7 and 8 ppm. Compound **3** was found able to conjugate with reduced Fabs, which was concluded from SDS-PAGE gel analysis by the presence of a band between 65 and 70 kDa, corresponding to PLA-PEG-*bis*-sulfone **3** (12kDa) conjugated with Fab (50 kDa). mPEG-PLA/PLA (1:9) and PLA-PEG-*bis*-sulfone (1:9) formulations led to spherical NPs with ≈ 250 nm in size (PdI < 0.2). Surface charge of NPs was negative in 0.1 M PBS + 0.15 M NaCl and close to neutrality in the buffer used to perform the conjugation. Neutral surface charge is important to decrease electrostatic interactions of Fabs and NP's surface. The association of Fabs to PLA-PEG-*bis*-sulfone/PLA NPs was assessed using Ellman's reagent, in a preliminary assay. The association of Fabs to PLA-PEG-*bis*-sulfone/PLA NPs was ≈ 80% and superior to the determined for mPEG-PLA/PLA NPs, which were used as negative control.

Conclusions: This work showed that PLA-PEG-*bis*-sulfone **3** is able to conjugate to reduced Fabs and it is suitable to formulate spherical NPs. PLA-PEG-*bis*-sulfone/PLA and mPEG-PLA/PLA NPs presented identical morphology, size variability and surface charge. The conjugation of Fabs to PLA-PEG-*bis*-sulfone/PLA NPs presented promising results. The method will be further characterized and optimized. At the end, NPs will be used for DC targeting to potentiate antigen delivery to these phagocytic cells, which will present them to T cells, promoting tumor cell eradication.

Acknowledgements: Financial support from Fundação para a Ciência e a Tecnologia, Ministério da Ciência e da Tecnologia, Portugal (PhD Grant SFRH/BD/87150/2012 to João Conniot and Pest-OE/SAU/UI4013/2011). Funding from the UK Engineering & Physical Sciences Research Council (EPSRC) for the EPSRC Centre for Innovative Manufacturing in Emergent Macromolecular Therapies is gratefully acknowledged.

References:

[1] Conniot J, Silva JM, Fernandes JG, Silva LC, Gaspar R, Brocchini S, Florindo HF, Barata TS. Front Chem **2** (2014) 105.
[2] Brocchini S, Godwin A, Balan S, Choi JW, Zloh M, Shaunak S. Adv Drug Deliv Rev **1** (2008) 60.

Preliminary studies for the development of a new formulation for patients on oncologic treatment

Roser Sanz Casañas[1], *Beatriz Clares Naveros*[2], *Mireia Mallandrich*[1], *Ana C. Calpena Campmany*[1]

[1]Department of Pharmacy and Pharmaceutical Technology, Biopharmaceutical and Pharmacokinetic Unit, University of Barcelona, Spain.
[2]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
e-mail: rossancas@gmail.com

Introduction: Oral mucositis is a common condition in oncologic patients receiving neck and head radiotherapy and/or chemotherapy. It is a painful disorder associated with erythematous and ulcerative lesions in the oral cavity, pharynx and esophagus, which might compromise the oncologic treatment and patient´s quality of life [1, 2]. Several therapeutic approaches have been used to prevent and manage oral mucositis with different degree of efficacy proven [1]. A phase III randomized study concluded that doxepin (Dxp) rinse reduces the pain associated to oral mucositis [3]. In this work, we present the preliminary development of a Dxp semisolid formulation, which might be used for pain relief in oncologic patients with oral mucositis. Studies comprised rheological characterization and drug release studies.

Materials and Methods: 5 different test formulations were prepared at 5% Dxp concentration each. 3 permeation enhancers were chosen to promote drug delivery to the oral mucosa and were incorporated alongside Dxp into Orabase™ (O) to conform the semisolid test formulations. Chosen enhancers were myristylic alcohol (A), menthol (M) and transcutol (T). T was added at a 10% level to 3 of the test formulations while A and M were separately used at 5% concentration in 2 of the formulations each (one in combination with T and the other one as the sole enhancer for the formulation). 5 placebo formulations prepared alike (but without Dxp) and a plain O formulation were tested for rheological characteristics with a HAAKE rheometer Rheo Stress 1, using a plate-plate geometry (6 cm in diameter). Drug release studies were performed for the 5 semisolid Dxp formulations using Franz Diffusion Cells. They consisted of the donor and the receptor compartments with a polysulfone membrane clamped between them (diffusion area: 2.54 cm^2). The receptor medium was PBS under constant stirring. Cell temperature was kept at 32.0 ± 0.5 ºC. Test formulations were studied by triplicate. The cumulative amount of Dxp permeated through the membrane was calculated from the Dxp concentration in the receptor medium and plotted as a function of time. Dxp was quantitatively determined with a previously validated UV-Vis spectrophotometric method (λ = 208 nm) [4].

Results and Discussion: Rotational viscosimetry tests confirmed the pseudoplastic behavior of all tested formulations as viscosity values decreased with increasing share stress rates. The presence of at least one enhancer significantly decreased viscosity (viscosity values at 25 ºC and 50 s^{-1} (Pa.s): O = 6.9 ± 0.1, OM= 4.2 ± 0.2, OA = 4.1 ± 0.7, OT = 1.5 ± 0.1, OMT = 1.0 ± 0.1, OAT = 0.9 ± 0.1). Results for the oscillatory test (plate gap of 0.5 mm; constant shear stress of 1 Pa) showed a decrease on both the elastic and viscous behavior of the formulations containing either M or A; such decrease being more accentuated when T was included in the formulation. As regards the release studies, cumulative amounts released from the test formulations where determined and fitted into a Higuchi´s square root model (as it best described the release pattern according to AIC). The estimated release rate constant for the model (*kH*) showed higher values for the formulations containing T (*kH* values from 60.9 $\mu g \cdot h^{-0.5}$ for ODA, to 211.3 $\mu g \cdot h^{-0.5}$ for ODT). Amodelistic parameters such as the area under the curve (AUC) and the mean release time (MRT) were also estimated for each test formulation, confirming that the formulations reaching higher degrees of release were the ODT (AUC = 2.619 µg) and the ODMT (AUC = 2.098 µg); both with a comparable MRT (1.4 h for ODT and 1.5 h for ODMT). In general, formulations with higher release rates presented lower viscosity values although a direct relationship could not be established. Other factors might also have an influence on the effect and should be taken into account, i.e. the hydrophilic and lipophilic nature of each enhancer, the partition coefficient, the solubility, etc.

Conclusions: Permeation enhancers modify physical characteristics of semisolid formulations and their drug release rate. The present work confirms that viscosity plays a role in the drug release pattern and should be considered when developing new formulations. Additionally, the work also confirms the formulations containing T alone or in combination with M as the most promising ones for further research in the field of oral mucositis pain relief.

Acknowledgements: Lyda Halbaut (University of Barcelona, Spain) is gratefully acknowledged for the support provided with the rheological analysis.

References:

[1] Saunders DP, Epstein JB, Elad S, Allemano J, Bossi P, van de Wetering MD, Rao NG, Potting C, Cheng KK, Freidank A, Brennan MT, Bowen J, Dennis K, Lalla RV. Support Care Cancer **21** (2013) 3191.
[2] Lalla RV, Sonis ST, Peterson DE. Dent Clin North Am **52** (2008) 61.
[3] Leenstra JL, Miller RC, Qin R, Martenson JA, Dornfeld KJ, Bearden JD, Puri DR, Stella PJ, Mazurczak MA, Klish MD, Novotny PJ, Foote RL, Loprinzi CL. J Clin Oncol **32** (2014) 1571.
[4] Sanz R, Clares B, Mallandrich M, Casals I, Bellido D, Calpena AC. Curr Pharm Anal **11** (2015) 269.

Bacteriocin-loaded microparticles: a promising approach to the treatment of infectious diseases

Mª José Martín[1,†], *Raúl Cebrián*[2,†], *Beatriz Clares*[1,3,4], *Mª Adolfina Ruiz*[1,3,4], *Mercedes Maqueda*[2], *José L. Arias*[1,3,4]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[2]Department of Microbiology, University of Granada, Spain.
[3]Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
[4]Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain.
e-mail: mjmv@ugr.es [†]These authors contributed equally

Introduction: Bacteriocins are ribosomally synthesized (poly)peptides with a more than interesting bactericidal effect [1]. Additional properties are a low toxicity and a lack of cross-resistance to antibiotics. Therefore, bacteriocins are promising tools for the treatment of different infectious diseases. Specifically, bacteriocin AS-48 is a cyclic peptide antibiotic produced by *Enterococcus faecalis*, characterized by a wide spectrum of activity against different microorganisms (*Listeria* spp., *Bacillus* spp., *Staphylococcus* spp., *Clostridium* spp., *Mycobacterium* spp., *Escherichia coli*, and *Salmonella* spp., to cite just a few). Unfortunately, the potential clinical use of this peptide can be hindered by the development of antimicrobial resistances [2]. Pharmaceutical technology may emerge as a revolutionary approach to minimize this potential problem. In this line, and in order to optimize the administration and antibacterial activity of AS-48, it is presented in this work the complete development and characterization of two microparticulate systems.

Materials and Methods: Microparticles loaded with bacteriocin AS-48 were formulated by external gelation, using alginate and an exopolysaccharide (EPS). A water-in-oil emulsion was prepared using as internal phase an aqueous solution of EPS (extracted from *Halomonas Maura*), sodium alginate, and AS-48. Different concentrations of these components were investigated: from 1 to 3% (w/v). A vegetable oil was used as external phase. Finally, $CaCl_2$ was added to the resultant emulsion in order to assure the gelling of the particles. The microparticles were then centrifuged (Eppendorf Centrifuge 5804, Germany) and stored at 4 ºC. Geometry of the microparticles was investigated by optical microscopy (Olympus BX40 microscope equipped with an Olympus SC35 camera, Japan). AS-48 entrapment efficiency was determined by high-performance liquid chromatography (HPLC). To that aim, an adequate amount of particles was treated with phosphate buffered saline (pH 7) and then incorporated to a SiliaBond® C18. Elution at 30%, 60%, and 100% in acetonitrile:isopropanolol (1:2) was done. Active fractions were lyophilized and purified (Jupiter HPLC 300 C4 column, Phenomenex Inc., Spain). The antimicrobial activity of bacteriocin AS-48-loaded microparticles was tested against *Listeria monocytogenes* by means of a drop methodology.

Results and Discussion: The bacteriocin AS-48-loaded microparticles were characterized by a spherical shape, a mean size between 120 and 200 μm, and an AS-48 entrapment efficiency between 35 and 45%. The greater entrapment efficiencies were possible when a 1:1 alginate:EPS mixture was used. *In vitro* studies demonstrated a significantly stronger inhibition activity against *Listeria monocytogenes* than controls. These findings were in concordance with the HPLC results.

Conclusions: A reproducible technique has been proposed to obtain bacteriocin AS-48-loaded microparticles that may be adequate for the oral route of administration. Further *in vitro* and *in vivo* studies are under development to define the potential clinical use of this formulation.

Acknowledgements: Financial support from project BS11-2015 (Campus de Excelencia Internacional BioTic, Granada, Spain) is gratefully acknowledged.

References:

[1] Cotter PD, Ross RP, Hill C. Nat Rev Microbiol **11** (2013) 95.
[2] Montalbán-López M, Sánchez-Hidalgo M, Valdivia E, Martínez-Bueno M, Maqueda M. Curr Pharm Biotechnol **12** (2011) 1205.

PCL/chitosan nanoparticle adjuvant ability for HBsAg vaccines

Sandra Jesus, Edna Soares, Olga Borges

Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra, Portugal; and Center for Neuroscience and Cell Biology, University of Coimbra, Portugal.
e-mail: sjesus.mg@gmail.com

Introduction: Despite the existence of a prophylactic vaccine against hepatitis B virus (HBV), recent data from World Health Organization (WHO) showed that hepatitis B infection remains a major health problem [1]. To date, several particle-based delivery systems for hepatitis B surface antigen (HBsAg) were tested as an alternative to HBV licensed vaccines. Due to their nanoparticulate nature, small size and shape, nanoparticles (NPs) improve antigen recognition by the immune system, particularly from antibody presenting cells (APCs), resulting in increased antigen uptake and presentation, stimulation of cytokine production, upregulation of co-stimulatory molecules, migration of mature APCs to the draining lymph nodes and enhanced T cell stimulatory capacity [2, 3]. In order to develop an improved HBV vaccine, PCL and chitosan, two well-known biodegradable polymers, were chosen to create a blend polymeric vaccine delivery system with immunostimulatory properties. PCL/chitosan NPs were tested *in vitro* and *in vivo* to evaluate its underlying immunological mechanism of action and its influence on the immune response generated.

Materials and Methods: PCL/chitosan NPs were prepared by simple nanoprecipitation. Briefly, an organic phase (0.2% PCL diluted in acetone) was added dropwise to an aqueous phase (0.1% chitosan and 5% Tween® 80), previously placed under a high speed homogenizer. The mixture was performed at a ratio of 1:3 (v/v) in a final volume of 18 mL, followed by NP maturation under magnetic swirl for 45 min. NPs were isolated by centrifugation at 16000 × g, for 75 min at 4 °C with a 200 µL glycerol bed, which as further eliminated by dialysis of the suspension against water for 48 h. Final formulations were achieved by simple incubation of PCL/chitosan NPs with HBsAg and/or HBsAg encoding plasmid. Particle size and surface charge were analyzed by dynamic or electrophoretic light scattering, respectively. *In vitro* mechanistic assays using cell cultures (*β*-hexosamidase release and TNF-*α* production) were performed under LPS-free conditions. Vaccination studies were performed in C57BL/6 mice using different strategies to evaluate: *i*) PCL/chitosan NPs adjuvant ability; *ii*) PCL/chitosan NPs dose effect; *iii*) subcutaneous and intranasal administration routes; and, *iv*) the antigen dose effect. Mice serum samples, nasal and vaginal washes were analyzed for anti-HBsAg specific immunoglobulins. Mice spleen cells were cultured with or without the antigen to evaluate cytokine production. Immune response evaluation was based on enzyme-linked immunosorbent assay (ELISA).

Results and Discussion: Water suspended PCL/chitosan NPs (± 200 nm, ± 25 mV) did not induce TNF-*α* secretion from mononuclear cells isolated from human peripheral blood but they induced *β*-hexosaminidase release from a human mast cell line (HMC-1). Vaccination studies with HBsAg encoding plasmid adsorbed on PCL/chitosan NPs conducted to a negligible immune response, a result we speculate to be related to the final formulation highly negative zeta potential. Alternatively, the recombinant HBsAg protein adsorbed on the surface of NPs administered subcutaneously led to a strong humoral immune response (IgG titers), superior to the response generated by vaccination with the commercial vaccine Engerix-B®, at the same dose ($p < 0.05$). Also, NPs induced increased IFN-*γ* and IL-17 production suggesting a Th1/Th17 cellular immune response different from what was observed with free HBsAg. These increased immune responses were proved to be dependent on the NPs dose, supporting its adjuvant immunostimulatory ability. Intranasal vaccination with HBsAg adsorbed PCL/chitosan NPs showed advantages since an antigen dose equivalent to the one used for the subcutaneous route (1.5 µg antigen/mouse) was able to induce humoral immune responses.

Conclusions: The work performed showed PCL/chitosan NPs are mast cell activators and are immunological adjuvants for HBsAg. The delivery system was able to increase the humoral and cellular immune responses to the recombinant antigen. Furthermore, the nanocarrier stimulation of Th1 and Th17 cytokines together with the higher IgG titers may provide better protection than the existing alum-adjuvanted HBV vaccines.

Acknowledgements: Work supported by Portuguese Foundation for Science and Technology (FCT) - project PTDC/SAU-FAR/115044/2009, by the Center for Neuroscience and Cell Biology strategic project UID / NEU / 04539 / 2013 and by FCT fellowship DFRH - SFRH/BD/81350/2011.

References:

[1] World Health Organization. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. March 2015.
[2] Akagi T, Baba M, Akashi M. In: Kunugi S, Yamaoka T (Eds.). Polymers in Nanomedicine. Springer Berlin Heidelberg (2012) pp. 31-64.
[3] Awate S, Babiuk LA, Mutwiri G. Front Immunol **4** (2013) 114.

Development and optimization of gelatin scaffolds with dual action for tissue engineering and regenerative medicine

Pello Sánchez[1,2], José Luis Pedraz[1], Gorka Orive[1]

[1]NanoBioCel Group, Laboratory of Pharmaceutics, School of Pharmacy, University of the Basque Country (UPV/EHU); and Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
[2]Arthroscopic Surgery Unit Research, Hospital Vithas San José, Vitoria-Gasteiz, Spain.
e-mail: gorka.orive@ehu.es

Introduction: The aim of tissue engineering and regenerative medicine is to develop biocompatible and biodegradable systems suitable not only to interact with cellular receptors and hence, suitable to support therapeutic cells but also to deliver growth factors in a controlled manner. Such a system may also serve as temporary replacement of damaged extracellular matrix allowing host cell migration [1]. Gelatin is a protein derived from collagen, the most abundant protein in connective tissue. Besides having the characteristics of collagen, including biodegradability and cell adhesion capacity, due to the hydrolysis of collagen chains in manufacturing of gelatin, it can be dissolved in water increasing its biocompatibility. Depending on the extraction and manufacturing method of collagen proteins, the resultant gelatin has different isoelectric points (pI). Type A gelatin derived from acid-cured tissue has a pI of 7.0-9.0 whereas type B derived from lime-cured tissue has a pI of 4.7-5.2, becoming a good biomaterial to retain and deliver growth factors [2]. Traditionally, glutaraldehyde (GA) has been used as crosslinking agent but due to its high cytotoxicity there is a need to explore new candidates. Genipin is a compound extracted from the *Gardenia jasmoides* fruit with a comparable capability of crosslinking but lower toxicity than GA [3]. In this work we developed/optimized and evaluated dual function of gelatin-genipin scaffolds to act as cell supporter and growth factors delivery carriers.

Materials and Methods: Type B gelatin powder with a strength of ≈ 225 bloom number was dissolved in DI water for 45 min at 40 ºC under constant agitation. At the same time, a genipin solution was prepared by dissolving 100 mg of genipin in 10 mL of DI water. Once the gelatin solution was homogeneous, the required amounts of the solution of genipin were added to reach final concentrations of 0.1%, 0.2% and 0.3% (w/v). Three solutions were mixed at 40 ºC and under stirring until the color turned green (≈ 15 min). Later, 5 mL of each solution were put into 100 mm petri dishes and were left crosslinking during 72 h at room temperature. After crosslinking, the scaffolds were punched and cylinders of 8 mm in diameter were plunged into ethanol 70% solution during 10 min. Afterwards, scaffolds were washed twice in PBS and freeze-dried. Thereafter, swelling ratios were determined using a gravimetric method; Swelling ratio = (Wwet-Wdry) / Wdry. Biomechanical properties were determined analyzing young´s modulus, Poisson distribution and permeability. Surface morphology of scaffolds was characterized by SEM. The toxicity was evaluated by two methods following the "Biological evaluation of medical devices guideline (ISO 10993): cytotoxicity on extracts and cytotoxicity by direct contact" guideline. Cell adhesion and morphology of D1 MSCs and L-929 fibroblast was studied using fluorescent dyes to mark actin microfilaments and *ds*DNA. VEGF and Sonic hedgehog (SHH) were used to study the capacity of release from genipin 0.2% scaffolds.

Results and Discussion: Following the manufacturing process, three different scaffolds with increasing concentration of genipin were obtained. Both the swelling ratio and the young´s module increased on a par with concentration of genipin. Nevertheless, permeability decreased and Poisson distribution remained constant. The toxicity assays demonstrated the absence of cytotoxicity of any scaffold. D1 and L-929 cell adhesion was better only in genipin 0.2% scaffold (no differences with control in D1). Nevertheless, fibroblast adhesion was half of D1 adhesion. Due to the results of those assays, growth factors control release was studied in genipin 0.2% scaffold. After an initial burst release, VEGF delivery was prolonged during 10 days. In case of SHH, it was a burst release in first 24 h but not a constant delivery. In both cases, a great amount of growth factor was retained into the scaffold.

Conclusions: In this work we demonstrated that gelatin-genipin scaffolds are suitable to support therapeutic cells and to deliver growth factors in a controlled manner.

Acknowledgements: The authors acknowledge the support from the Arthroscopic Surgery Unit, Vitoria-Gasteiz, Spain; and AGRENVEC, Madrid, Spain.

References:

[1] Billet T, Vandenhaute M, Schelfhout J, Van Vlierberghe S, Dubruel P. Biomaterials **33** (2012) 6020.
[2] Young S, Wong M, Tabata Y, G. Mikos A. J Control Release **109** (2005) 256.
[3] Manickam B, Sreedharan R, Elumalai M. Curr Drug Deliv **11** (2014) 139.

Deformable liposomes increase vicenin-2 human skin penetration *ex vivo*

Ana Luiza Scarano Aguillera Forte[1,2], *María José Vieira Fonseca*[1], *Norberto Peporine Lopes*[1], *Teresa María Garrigues Pelufo*[2], *Ana Melero Zaera*[2]

[1]Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Brazil.
[2]Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Valencia, Spain.
e-mail: aanaluiza2@gmail.com

Introduction: Currently, several studies suggest the applicability of plant extracts in cosmetic formulations to protect the skin against UV-induced damage and skin ageing [1]. *Lychnophora salicifolia* (ELS) extract has sundry compounds which present proven antioxidants and anti-inflammatory properties, such as, vicenin-2 and chlorogenic acid [2]. However, a conventional topical formulation might not be able to produce the desired protective effect on long exposure to sunlight because antioxidant compounds have to pass through stratum corneum (SC) and reach the deeper layers of the skin in sufficient amounts to protect them appropriately [3]. Thus, the incorporation of plant extracts in deformable liposomes can target and prolong the delivery of this compound in the deeper skin layers.

Materials and Methods: ELS-loaded deformable liposomes were prepared by lipid film-hydration method using phosphatidylcholine and Tween® 20 (4:1, w/w). For gel preparation, hydroxyethylcellulose was added in an ELS solution, ELS solution added Tween® 20 in the same liposomes proportion, or the freshly prepared ELS liposomal dispersion under constant stirring by means of a magnetic stirrer. The final concentration of ELS in all formulations was 2.5% (w/w). The vicenin-2 distribution in the different human skin layers was evaluated using Saarbrücken model [4]. Human skin samples were obtained from cosmetic surgery with the volunteers' agreement. 100 mg of each formulation were placed in aluminum chambers which are fixed over the skin. The whole system was incubated for 2 and 4 h at 32 ºC. After the incubation, the system was opened and the rests of formulation were cleaned. Then the SC was completely removed by tape-stripping method. The rest of skin was frozen at -80 ºC and segmented in 40 µm cuts parallel to the surface. Vicenin-2 in the stripes and skin cuts was extracted using methanol:water (70:30) and analyzed by HPLC. Four replicates were performed for each formulation and incubation time. To ensure the total recovery, a mass balance was accomplished, in which every part of the experiment, including the rest of formulation applied, were extracted and analyzed.

Results and Discussion: Total recovery for all applied formulations fit the limits of recovery of 85-115% provided by the Scientific Committee on Consumer Safety (SCCS). The results showed that in comparison to the other formulations, the gel containing ELS-entrapped in deformable liposomes allowed a larger quantity of vicenin-2 to penetrate through the SC, especially after 4 h of incubation. On the other hand, the gel containing ELS added Tween® 20 accumulated vicenin-2 in SC layers after 2 h incubated and, only in the 4 h incubation experiment, it was possible to observe this compound in the skin cuts.

Conclusions: In this study, we observed that ELS-entrapped deformable liposomes can increase vicenin-2 penetration in deeper human skin layers *ex vivo*. Besides, the effect cannot be only attributed to the Tween® 20 which is part of liposome constitution. Then, deformable liposomes might be a viable way to increase skin penetration of antioxidants and anti-inflammatories from natural sources.

Acknowledgements: Financial support from FAPESP, CNPq and Capes is gratefully acknowledged.

References:

[1] Tundis R, Loizzo MR, Bonesi M, Menichini F. Curr Med Chem **22** (2015) 1515.
[2] Gobbo-Neto L, Santos MD, Kanashiro A, Almeida MC, Lucisano-Valim YM, Lopes JL, Souza GE, Lopes NP. Planta Med **71** (2005) 3.
[3] Nichols JA, Katiyar SK. Arch Dermatol Res **302** (2010) 71.
[4] Melero A, Garrigues TM, Alós M, Kostka KH, Lehr CM, Schaefer UF. Int J Pharm **378** (2009) 101.

Doxorubicin and Edelfosine lipid nanoparticles act synergistically against osteosarcoma cell lines

Y. González-Fernández[1,2,3], E. Imbuluzqueta[1,2], M. Zalacain[2,3], L. Sierrasesúmaga[2,3], A. Patiño-García[2,3], M.J. Blanco-Prieto[1,2]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Navarra, Spain.
[2]Centro de Ingeniería Biomédica and IdiSNA, University of Navarra, Spain.
[3]Laboratory of Pediatrics, University Clinic of Navarra, Spain.
e-mail: mjblanco@unav.es

Introduction: Osteosarcoma (OS) is the most common primary bone malignancy representing 5% of total pediatric cancers, the eighth in general incidence among childhood cancers. Due to its aggressive local growth pattern and its high propensity to metastasize, mainly to the lungs, it represents one of the leading causes of pediatric cancer death [1, 2]. OS treatment is based on a neo-adjuvant multiagent chemotherapy followed by a surgical resection and adjuvant chemotherapy. Unfortunately, during the past decades, the advances in OS treatment have been scarce. Doxorubicin (DOX) is an anthracycline antibiotic used as a first-line antineoplasic agent in the treatment of OS. However, its low efficacy when used as a single treatment together with its narrow therapeutic index, severe adverse effects and the development of multidrug resistances, have led researchers to investigate new treatment combinations and alternative forms of administering DOX for cancer therapy [3]. Therefore, the objective of the present work was to design, formulate and evaluate in OS cell lines, the efficacy of lipid nanoparticles (LNs) containing DOX and Edelfosine (EDF), a new antitumoral agent that has shown efficacy in several cancer cell lines including OS [4], in order to improve the treatment of OS.

Materials and Methods: EDF and DOX were encapsulated into Precirol® LNs following the hot melting homogenization method, in which the drug is melted together with the lipid and emulsified with a water phase containing 2% Tween 80® [4, 5]. The developed LNs were characterized in terms of size, polydispersity index (PdI), zeta potential and encapsulation efficiency. The antitumor activity of free and encapsulated drugs, alone and in combination, was studied using the MTS assay in a commercialized continuous OS cell line, U-2 OS (ATCC® HTB-96™), as well as in primary OS cell lines extracted from the primary bone tumor and lung metastases of OS patients. Results were expressed in terms of inhibitory concentration 50 (IC_{50}) values. Finally, drug internalization was analyzed by UHPLC-MS/MS after exposure of the cells to the different treatments.

Results and Discussion: Optimized EDF and DOX formulations presented sizes less than 150 nm, PdI bellow 0.22 and negative zeta potentials with high encapsulation efficiencies ≈ 90%. Cytotoxicity studies revealed that EDF was more effective treating the metastatic cells than the primary bone tumor cells (IC_{50} values of 11-15 μM *vs*. 37-66 μM, respectively), which was attributed to its higher entrance via lipid rafts overexpressed in metastatic cells. Moreover, the encapsulation into LNs significantly increased the internalization of EDF in all OS cell lines, reducing its IC_{50} value up to 4 times. On the other hand, OS cells were found to be very sensitive to the action of DOX, with IC_{50} values in the nM range. Importantly, despite the inherent sensitivity of the cells to the drug, its entrapment into LNs led to a higher cellular internalization, especially in the metastatic cell line in which it achieves twice the concentration of the free drug, allowing an improvement in DOX efficacy of up to 2.4 times. Finally, drug combination studies demonstrated that EDF and DOX, either as free drugs or encapsulated, act synergistically when both drugs are administered as a combination therapy.

Conclusions: LNs containing EDF and DOX have been successfully developed. Entrapment into LNs led to a higher cell internalization of the drugs, which, in turn, improved their antitumor efficacy. Moreover, the co-administration of EDF and DOX produced a synergistic effect, allowing to reduce the individual drug doses required to achieve the same antitumor effect. Taken together, the results of this study suggest that the combination of EDF and DOX and their administration in LNs could be useful in the treatment of primary and metastatic OS.

Acknowledgements: Financial support from Asociación Española Contra el Cáncer (AECC, CI14142069BLAN), Fundación Caja Navarra (CAN, 70565), and Centro de Ingeniería Biomédica (CBIO) are gratefully acknowledged.

References:

[1] Ottaviani G, Jaffe N, Cancer Treat Res **152** (2009) 3.
[2] González-Fernández Y, Imbuluzqueta E, Patiño-García A, Blanco-Prieto MJ, Curr Pharm Design **21** (2015), doi: 10.2174/1381612821666151027152534.
[3] Thorn CF, Oshiro C, Marsh S, Hernandez-Boussard T, McLeod H, Klein TE, Altman RB. Pharmacogenet Genomics **21** (2011) 440.
[4] González-Fernández Y, Zalacain M, Imbuluzqueta E, Sierrasesumaga L, Patiño-García A, Blanco-Prieto MJ. J Drug Deliv Sci Tec (2015) doi: 10.1016/j.jddst.2015.08.004.
[5] Blanco-Prieto MJ, Hermoso de Mendoza AE, Campanero Martinez MA, Villa Pulgarin JA, Varela Miranda RE, Mollinedo Garcia F. Patent ES2388963A1 (2012).

Paromomycin liposomes *vs*. Ambisome®: *in vitro* and *in vivo* studies against *Leishmania infantum* infections

Maria Manuela Gaspar[1], *Georgina Gomes Alves*[2], *Helena Castro*[2,3], *Ana Tomás*[2,3], *M. Eugénia Meirinhos Cruz*[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Portugal.
[2]IBMC, Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal.
[3]ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal.
e-mail: mgaspar@ff.ulisboa.pt

Introduction: Infectious diseases constitute an immense global threat, being responsible for 15 millions of deaths per year worldwide. Over the last decades, liposomes, the most studied and successful drug delivery system allowed improving the pharmacologic and therapeutic properties of several molecules. One example is the case of aminoglycosides that due to an inappropriate biodistribution and/or pharmacokinetic profiles render them not satisfactory for medical use. Paromomycin (PRM) is an aminoglycoside with a broad spectrum *in vitro* activity against protozoa and mycobacteria. However, it is poorly absorbed into systemic circulation after oral administration and when parenterally injected undergoes rapid clearance being excreted in urine. PRM encapsulated in liposomes revealed a preferential targeting of the antibiotic to the liver and spleen relative to free PRM, which was translated into an enhanced therapeutic effect in a visceral *Leishmania* murine model and absence of toxicity [1]. The present work aims at demonstrating *in vitro* and *in* vivo the antileishmanial effect of PRM-loaded liposomes comparatively to Ambisome®.

Materials and Methods: PRM-loaded liposomes were prepared by the dehydration-rehydration method, followed by extrusion to reduce and homogeneize the nanoformulations mean size [1]. *In vitro* activity of PRM and Amphotericin B in free and liposomal forms were evaluated in bone marrow-derived macrophages infected with *L. infantum* amastigotes. PRM in free and liposomal forms and Ambisome® were tested in a *L. infantum* murine model and the therapeutic effect was evaluated in terms of parasite burden (PB) [1].

Results and Discussion: *In vitro* tests revealed that liposomal formulations displayed a very high intracellular activity towards infected macrophages. In particular for PRM the IC_{50} values were 100 and 2.5 μM for the antibiotic in the free and liposomal forms, respectively. For Amphotericin B formulations the IC_{50} were 19 and 6 nM but the free form was toxic to uninfected macrophages. With respect to the biological effect against *L. infantum* parasites, a 4 and 6 log reductions in spleen and liver were obtained for mice treated with PRM-loaded liposomes *vs*. control group, while no statistically significant differences were observed for mice treated with free PRM. Moreover, PRM liposomes and Ambisome® showed similar therapeutic effects in terms of PB reductions (higher than 99.9%) in fighting this disease.

Conclusions: The obtained results demonstrate the potential of PRM-loaded liposomes as a valuable strategy for treatment of *L. infantum* infections. Moreover, PRM-loaded liposomes represent a promising alternative to Ambisome®.

Acknowledgements: This work was supported by FCT Portugal, (POCI/SAU-FCB/58355; PEst-OE/SAU/U14013). Gilead Sciences, Inc. (USA) provided the Ambisome® for the animal studies.

References:

[1] Gaspar MM, Calado S, Pereira J, Ferronha H, Correia I, Castro H, Tomás AM, Cruz ME. Nanomedicine **11** (2015) 1851.

Understanding the interface of edible nanoemulsions to modulate the bioaccessibility of neuroprotective antioxidants

M. Plaza-Oliver, M.V. Lozano, V. Rodríguez-Robledo, L. Castro-Vázquez, J. González-Fuentes, P. Marcos, M.J. Santander-Ortega, M.M. Arroyo-Jiménez

Cellular Neurobiology and Molecular Chemistry of the Central Nervous System group. Faculty of Pharmacy; and Regional Centre of Biomedical Research (CRIB), University of Castilla-La Mancha, Spain.
e-mail: mvictoria.lozano@uclm.es

Introduction: Currently, the food industry is trying to implement the valuable knowledge acquired in the pharmaceutical field related to the design of new nanostructures able to overcome the oral barrier. This diet prevention approach is based on the use of nanostructures to encapsulate active therapeutic molecules for increasing and homogenising their intestinal absorption [1, 2]. The interface of nanostructures determines the system stability and its interaction with enzymes. Aware of this, we have studied the role that the interface composition of different soybean nanoemulsions (SB-NEs) has on the *in vitro* bioavailability of the neuroprotective antioxidant α-tocopherol. For that purpose 3 SB oil formulations were designed. These formulations were based on the emulsifier lecithin but modified with 2 non-ionic surfactants Pluronic® F-68 (PF68) or Pluronic® F-127 (PF127) yielding: *i*) SB-NE (only lecithin on the interface); *ii*) SB-NE PF68 (lecithin plus PF68); and, *iii*) SB-NE PF127 (lecithin plus PF127).

Materials and Methods: SB-NEs were formulated by emulsifying SB oil with lecithin following a modification of the solvent displacement technique [3]. The incorporation of PF68 or PF127 to the external aqueous phase led to the surfactant protected SB-NEs, i.e. SB-NE PF68 and SB-NE PF127. Size, ζ-potential and colloidal stability of the different prototypes in the gastrointestinal tract was evaluated using a DLS Malvern device (Zetasizer Nano ZS90). The intestinal digestion of SB-NEs was quantified by titrating the fatty acids released after their enzymatic degradation. Finally, the bioaccessibility of the encapsulated α-tocopherol was determined by analyzing the amount of antioxidant present in the mixed micelles produced by the intestinal digestion of the systems.

Results and Discussion: The physicochemical characterization showed that SB-NEs had narrow size distributions with hydrodynamic mean size values in the rage of 195-205 nm for all the prototypes formulated. Their negative superficial charge decreased in magnitude relative to SB-NE values by the presence of the PF68 or PF127 surfactants. The interface composition was involved in the stabilization mechanisms, such as electrostatic repulsion and steric stabilization, which allowed all SB-NEs to be stable along the gastrointestinal tract. Nevertheless, interface composition displayed a strong effect on the SB-NEs lipolysis in the intestinal milieu, showing that SB-NE as well as SB-NE PF68 were clearly digested; meanwhile SN-NE PF127 was totally stable under these conditions. These results suggested that SB-NE was the formulation that would most likely achieve significant *in vitro* bioaccessibility values. Indeed, it was possible to obtain 38 ± 1% of bioaccessibility; a value that was maximized after reducing 4-fold the lecithin content in SB-NEs, remarkably reaching 71 ± 3% of bioaccessibility of the encapsulated α-tocopherol. Despite showing similar physicochemical properties, it was the SB-NE with the lowest lecithin content the one that achieved the highest bioaccessibility probably due to the facilitated access that lipase had towards the nanoemulsion interface to induce its intestinal digestion.

Conclusions: Surface composition is a key point to take into consideration for the design of bioaccessible nanosystems. The inclusion of bioactive molecules, such as α-tocopherol in nanoplatforms may improve its absorption pattern, but an adequate design of the system interface could maximize its properties, as shown in this work by reaching 70% bioaccessibility for α-tocopherol.

Acknowledgements: Authors thank the financial support given by the PEII-2014-040-P research project from the Junta de Comunidades de Castilla-La Mancha, as well as by the GI20153002 research project from the Vicerrectorado de Investigación, University of Castilla-La Mancha. Authors are also grateful to Dr. M. Rocío Fernández Santos for the support given.

References:

[1] Acosta EJ. Nanotechnology in the Food, Beverage and Nutraceutical Industries. Woodhead Publishing, Amsterdam (2012).
[2] McClements DJ, Xiao H. Food Funct **3** (2012) 202.
[3] Lozano MV, Torrecilla D, Torres D, Vidal A, Domínguez F, Alonso MJ. Biomacromolecules **9** (2008) 2186.

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Carbon nanotubes functionalized with cationic carbosilane dendrons pyrene modified at focal point as new nanocarriers for anticancer *si*RNA

Carlos E. Gutiérrez[1,2], *Marina Buyanova*[3], *Evgeny Apartsi*[3], *Aliya G. Venyaminova*[3], *Rafael Gómez*[1,2], *F. Javier de la Mata*[1,2]

[1]Department of Organic and Inorganic Chemistry, University of Alcala, Alcalá de Henares, Spain.
[2]Networking Research Center for Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
[3]Laboratory of RNA Chemistry, Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia.
e-mail: emilio.gutierrez@edu.uah.es

Introduction: Carbon nanotubes (CNTs) possessing chemical passivity and compatibility with biomacromolecules and cells are investigated actively in different fields of nanotechnology and biomedicine. However, previous studies have shown that the surface functionalization of CNTs with hydrophilic systems is a powerful method for increasing the hydrophilicity, thereby increasing their solubility and biocompatibility [1]. A wide variety of both, covalent and non-covalent, functionalization methods have been developed. CNTs can be considered innovative nanocarriers within the emerging platforms developed for therapeutic and diagnostic purposes in cancer nanotechnology [2]. Here in, we have synthetized new cationic carbosilane dendrons with a pyrene unit at the focal point for the functionalization of CNTs, single (SWNT) and multi-walled (MWNT), by non-covalent approach, through π-π* stacking interactions. These systems are used as new nanocarriers of nucleic acids such as anticancer *si*RNA. The characterization of these compounds was performed by usual techniques. The formation of nanoconjugates with anticancer Mcl-1-siRNA and their physicochemical properties were determined.

Materials and Methods: Due to the poor solubility of commercial CNTs, they were oxidized to obtain MWNT-COOH and SWNT-COOH. To avoid electrostatic interaction between oxidized nanotubes and the cationic groups of the dendrons, single and multi-walled CNTs with NH_2-PEG_{1600}-NH_2 and HMDA were functionalized using covalent method, to obtain four systems SWNT-PEG, SWNT-HMDA, MWNT-PEG and MWNT-HMDA [3]. The pyrene group was introduced by reaction of 1-pyrenebutyric acid with carbosilane dendrons containing a C-Br bond at the focal point and of periphery with NMe_3^+ was performed using a thiol-ene reaction. These dendrons were mixed increasing the concentration from 0 mg/L to 196 mg/L with the four CNTs systems in 50 and 100 mg/L for each essay and the adsorption occurs by sonication during 20 minutes. The amount of adsorbed dendron was measured by fluorescence spectroscopy at 381 nm. Mcl-1-siRNA was used to prepare nanoconjugates with the dendron-CNTs systems, the physicochemical characterization were performed using electrophoresis in agarose gel, fluorescence techniques and atomic force microscopy (FAM).

Results and Discussion: After the CNTs functionalization, fluorescence measurements determined that the adsorption of the dendrons were higher on the SWNT-PEG system explaining that SWNT systems were more water-soluble than MWNT and analogously SWNT-PEG systems were more soluble than SWNT-HMDA. The increasing of CNTs amount in solution caused the decrease of pyrene group fluorescence intensity. For this reason, the fluorescence quenching is interpreted as an evidence of pyrene adsorption on the nanotube surface. The optimum ratio CNTs/Dendron-Pyr for a 100% adsorption is 1:1 at concentration 50 mg/L of each. It was also observed that the third generation dendron is adsorbed to the CNTs faster than lower generations and also the best to retain anticancer Mcl-1-*si*RNA at low concentrations.

Conclusions: In this work new systems based on single and multi-walled CNTs functionalized with cationic carbosilane dendrons pyrene modified at focal point of different generations were synthetized and characterized. These hybrids are able to form nanoconjugates with anticancer Mcl-1-*si*RNA, being those dendronized with the third generation dendron the most appropriate. Therefore, these nanoconjugates can be considered as a new family of carriers for anticancer *si*RNA to be explored in the future.

Acknowledgements: Ministerio de Economía y Competitividad (Spain), Ref CTQ2014-54004-P, and Consortium NANODENDMED ref S2011/BMD-2351 (Comunidad de Madrid).

References:

[1] Bottini M, Rosato N, Bottini N. Biomacromolecules **12** (2011) 3381.
[2] Herrero MA, Toma FM, Al-Jamal KT, Kostarelos K, Bianco A, Da Ros T, Bano F, Casalis L, Scoles G, Prato M. J Am Chem Soc **131** (2009) 9843.
[3] (a) Apartsin EK, Novopashina DS, Nastaushev YV, Ven'yaminova AG, Nanotechnologies in Russia **7** (2012) 99; (b) Apartsin EK, Buyanova MY, Novopashina DS, Ryabchikova EI, Filatov AV, Zenkova MA, Venyaminova AG. ACS Appl Mater Interfaces **6** (2014) 1454.

Synthesis, anticancer activity and reactivity with relevant biomolecules of carbosilane metallodendrimers based on arene ruthenium (II) complexes

Marta Maroto-Díaz[1,2], Benelita T. Elie[3,4], Pilar Gómez-Sal[1], Jorge Pérez-Serrano[5], Rafael Gómez[1,2], Maria Contel[3,4], F. Javier de la Mata[1,2]

[1]Department of Organic and Inorganic Chemistry, University of Alcala, Alcalá de Henares, Spain.
[2]Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
[3]Department of Chemistry, Brooklyn College and The Graduate Center, The City University of New York, USA.
[4]Biology PhD Program, The Graduate Center, The City University of New York, USA.
[5]Department of Biomedicine y Biotechnology, University of Alcala, Alcalá de Henares, Spain.
e-mail: marta.maroto@uah.es

Introduction: In the search of more effective and selective potential anticancer metallodrugs different approaches have been pursued, including the study of ruthenium compounds [1]. One of the main drawbacks of cancer chemotherapeutics is the lack of selectivity which induces severe adverse effects on healthy tissues and organs. For that reason, it would be desirable to develop carrier platforms that could improve the efficacy and reduce toxicity of cancer treatment by specific delivery of the therapeutic agents to the tumor sites. In the field of nanoscopic delivery systems, carbosilane dendrimers have been studied in different biomedical applications, such as non-viral vehicles for the delivery of nucleic acids (oligonucleotides or *si*RNA) [2]. However, their application as delivery vehicles for metal-fragments in cancer therapy has not yet been described. Recently, the antitumoral properties of some metallodendrimers based on DAB or PPI scaffolds have been described, including η^6-*p*-cymene-ruthenium (II) systems [3].

Materials and Methods: The new organometallic carbosilane dendrimers were fully characterized by nuclear magnetic resonance (NMR). The antiproliferative properties of the new ruthenium complexes in several human cancer cell lines were assayed by monitoring their ability to inhibit cell growth using the MTT or XTT assays. We followed the interaction of selected compounds with plasmid pBR322 DNA by electrophoresis in agarose gel and with *Calf Thymus* DNA (CT DNA) by circular dichroism (CD). We selected highly cytotoxic water-soluble first generation dendrimer as a model to evaluate the mechanism of cell death in triple negative MDA-MB-231 cancer cells. MDA-MB-231 viability, necrosis, and apoptotic activity were analyzed by the ApoTox-Glo Triplex. The interaction of selected compounds with HSA and with Cathepsin B was followed by Fluorescence Spectroscopy.

Results and Discussion: New organometallic carbosilane dendrimers (first and second generation) and their corresponding non-dendritic mononuclear derivatives were prepared by reaction of precursor $[Ru(\eta^6\text{-}p\text{-cymene})Cl_2]_2$ with carbosilane dendrimers functionalized with *N*- donor monodentate ligands such as NH_2- and pyridine, or with *N,O-, N,N*-chelating imine ligands. Most compounds display significant antiproliferative activities in the low micromolar range and the first generation ruthenium dendrimers were found to be the most active compounds. The interaction of selected metallodendrimers with DNA was weak and electrostatic in nature. Initial mechanistic studies indicate that the MDA-MB-231 cell death type for selected water-soluble first generation metallodendrimers is through both apoptototic and necrotic pathways. Except for water-soluble non-dendritic monoluclear derivate the Stern-Volmer plot suggests the presence of different binding sites in the protein HSA with different binding affinities. First generation ruthenium dendrimers inhibit Cathepsin-B which may suggest potential antimetastatic properties of these compounds.

Conclusions: We report here on the synthesis, anticancer activity and reactivity with relevant biomolecules of new ruthenium η^6-*p*-cymene-based carbosilane dendrimers ruthenium derivatives. These facts point to a mode of action of these ruthenium derivatives different from that of cisplatin.

Acknowledgements: CTQ2014-54004-P (MINECO), and Consortium NANODENDMED ref S2011/BMD-2351 (CM) to the University of Alcalá (Spain). College (The City University of New York) authors thank the National Cancer Institute (NCI) for grant1SC1CA182844 (to M. C.). CIBER-BBN is an initiative funded by the VI National R&D&i Plan 2008–2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions and financed by the Instituto de Salud Carlos III with assistance from the European Regional Development Fund.

References:

[1] (a) Bergamo A, Gaiddon C, Schellens JHM, Beijnen JH, Sava G. J Inorg Biochem **106** (2012) 90; (b) Bergamo A, Sava G. Dalton Trans **40** (2011) 7817.
[2] Serramía MJ, Álvarez S, Fuentes-Paniagua E, Clemente MI, Sánchez-Nieves J, Gómez R, de la Mata J, Muñoz-Fernández MA. J Control Release **200** (2015) 60.
[3] (a) Govender P, Antonels NC, Mattsson J, Renfrew AK, Dyson PJ, Moss JR, Therrien B, Smith GS. J Organomet Chem **694** (2009) 3470; (b) Govender P, Renfrew AK, Clavel CM, Dyson PJ, Therrien B, Smith GS. Dalton Trans **40** (2011) 1158; (c) Govender P, Sudding LC, Clavel CM, Dyson PJ, Therrien B, Smith GS. Dalton Trans **42** (2013) 1267.

Composition of the encapsulating solutions conditions cell behaviour within alginate poly-L-lysine-alginate (APA) microcapsules

Ainhoa Gonzalez-Pujana, Edorta Santos, José Luis Pedraz, Gorka Orive, Rosa María Hernandez

NanoBioCel Group, Laboratory of Pharmaceutics, School of Pharmacy, University of the Basque Country (UPV/EHU), Spain; and Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
e-mail: rosa.hernandez@ehu.es

Introduction: Cell encapsulation technology consists in the immobilization of cells that produce therapeutic factors [1]. For that purpose, cells are enclosed in a polymeric biocompatible matrix, surrounded by a semipermeable membrane. Hence, a bidirectional diffusion of oxygen, nutrients and the therapeutic factor is allowed while protecting the implant from host's immune response. Consequently, a sustained and "de novo" release of the therapeutic factor is obtained. This technology has led to the development of many promising therapeutic treatments for a wide range of disorders such as diabetes [2], hemophilia B [3] or anemia [4]. Over the last decades, the immobilization of cells within alginate-poly-L-lysine-alginate (APA) microcapsules has been subjected to intensive research. However, some basic parameters in this technology need to be studied more profoundly in order to gain control over the system, and thus, to understand its performance. The aim of this work was to study the influence encapsulating solutions that embed the biomaterials have on the capsules. Adequate pH and osmolarity values are of paramount importance to maintain the cell viability; and this purpose can be fulfilled following two different strategies. On the one hand it is possible to add electrolytes, and thus mimic cell's physiological environment. On the other, the addition of inert molecules may promote the integrity of the capsule as they are formed by electrostatic interactions. In order to elucidate which strategy is more beneficial for the whole biosystem, we compared two batches of microcapsules which only differed from the solutions used in the encapsulation process: a first batch containing physiological concentrations of electrolytes (Biological group); and a second one, consisting of inert solutions based on mannitol (Technological group).

Materials and Methods: D1-MSCs genetically modified to release EPO were immobilized into APA microcapsules using an electrostatic droplet generator with slight modifications of the procedure designed by Lim and Sun [5]. Briefly, cells were harvested from 2D cultures and suspended in a solution of 1.5% (w/v) sodium alginate obtaining a cell density of 5×10^6 cells/mL. The resulting suspension was extruded through a 0.35 mm needle at a 5.9 mL/h flow rate. Once beads were formed they were collected in a gelling solution and maintained in agitation for 10 min in order to ensure a complete ionic gelation. Beads were then washed and suspended in 0.05% PLL solution for 5 minutes. A second coating was performed with a 0.1% alginate solution for 5 min. The resulting capsules were cultured in complete medium at 37 ºC in a 5% CO_2/95% air atmosphere. The process was carried out under aseptic conditions. To assess the viability of the immobilized cells calcein/ethidium assay, flow cytometry and CCK8 assay were performed. In order to evaluate cell proliferation and EPO production ELISA assays were carried out. In order to detect significant differences between the two groups Student's t-test was used. In the case of non-normally distributed data, Mann-Whitney non-parametric analysis was applied. When there were more than two groups to compare, one way ANOVA test was performed. All statistical computations were performed using SPSS 29 (SPSS, Chicago, IL).

Results and Discussion: The use of different solutions led to significant differences between the two groups of microcapsules. The Technological batch proliferated exponentially during the study, and therefore, the metabolic activity and secretion of the therapeutic factor increased likewise. On the contrary, the proliferation of the Biological group was linear, and so was the increase in the rest of the parameters. These data are supported by the micrographs taken at different stages of the study, showing the formation of enormous aggregates in the Technological group; a trend that was not observed in the Biological one. Altogether, these results suggest that the composition of the encapsulating solutions plays a pivotal role in the final characteristics of the microcapsule, and therefore, in cell behaviour.

Conclusions: In this work, it has been proven that the composition of the solutions containing the biomaterials is a crucial factor in the encapsulation process. By only varying the solutions in which the biomaterials were included, two completely different groups of microcapsules were obtained. The fact that these two groups showed significant differences in cell proliferation, metabolic activity and production of the therapeutic factor gives us idea of the relevance this point has when it comes to designing the appropriate microcapsules for different aims.

Acknowledgements: This project has been supported by the Basque Government (IT-407-07).

References:

[1] Hernandez RM, Orive G, Murua M, Pedraz JL. Adv Drug Deliv Rev **62** (2010) 711.
[2] De Vos P, Faas MM, Strand B, Calafiore R. Biomaterials **27** (2006) 5603.
[3] Hortelano G, Al-Hendy A, Ofosu FA, Chang PL. Blood **87** (1996) 5095.
[4] Murua A, Orive G, Hernandez RM, Pedraz JL. Adv Exp Med Biol **670** (2010) 54.
[5] Lim F, Sun AM. Science **210** (1980) 908.

Transfection of cSLN:pDNA lipoplexes induces transient luciferase gene expression in HEK293T cell line

Anna Fàbregas[1], Silvia Prieto[2], Marc Suñé-Pou[1], Josep Mª Suñé-Negre[1], Encarna García-Montoya[1], Pilar Pérez-Lozano[1], Josep Ramon Ticó[1], Montserrat Miñarro[1], Carlos Suñé[2]

[1]Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Barcelona, Spain.
[2]Departament of Molecular Biology, Instituto de Parasitología y Biomedicina "López-Neyra". Consejo Superior de Investigaciones Científicas (IPBLN-CSIC), Spain.
e-mail: msunepou@gmail.com

Introduction: Cationic solid lipid nanoparticles (cSLNs) have been designed as a non-viral gene delivery system for the treatment of several diseases. The use of cSLNs as non-viral vectors for gene delivery can be achieved by including cationic lipids in the formulation, thus providing a positive surface potential that favors their binding to negatively charged nucleic acids. Although transfection efficiency is higher for viral systems**,** the main advantages of cSLNs among other gene delivery systems include a low immunogenicity and good biocompatibility, thereby reducing toxicity effects. The transfection of cell lines by cSLN:pDNA lipoplexes is focused on the possibility to express proteins encoded in plasmids (pDNA) sequences, which can be detected by different methodologies such a bioluminescence assay in the case of pDNA containing a LUC (luciferase) gene insert.

Materials and methods: The cSLNs were synthesized using hot microemulsification method [3]. The cSLN formulation consists of stearic acid as the matrix lipid, octadecylamine as the cationic lipid and the surfactant Poloxamer 188 [1]. The cSLNs showed a particle size between 120-130 nm, measured by laser diffraction, and zeta potential values greater than 30 mV, measured by laser Doppler microelectrophoresis. Different pDNA quantities (100, 500 and 1000 ng) of pDNA containing the LUC gene were mixed with 15 µL of cSLNs, and immediately incubated for 45 min at room temperature to generate the cSLN:pDNA lipoplexes. The optimal binding efficiency of the cSLN:pDNA lipoplexes was previously established at 25:1 (cSLN:pDNA) (v/v). HEK293T cells grown in M6 plates were transfected with these lipoplexes. Calcium phosphate as transfection reagent was used as a control. Expression of the LUC protein was determined by a bioluminescence assay using *Dual Luciferase Assay* kit. Bioluminescence due to luciferase expression (in relative light units, RLU) was measured with a standard manual luminometer.

Results and Discussion: Luciferase activity (expressed en RLU) in cells transfected by means of cSLNs was detected in a dose-response manner. cSLN transfection efficiency appeared limited compared to the calcium phosphate. The luciferase activity levels with the cSLN were approximately 10% of that detected with calcium phosphate. The low transfection efficiencies might be attributable either to their ability to deliver DNA into cells or, less probably, to the cytotoxicity of the nanoparticles.

Conclusions: Although with reduced efficiency, our results showed that the produced cSLNs are able to deliver plasmids into cells, supporting potential use for *in vivo* applications as non-viral transfection reagents. These data encourage further studies aimed at improving the current formulation to increase the transfection efficiency of nucleic acids.

References:

[1] Fàbregas A, Sánchez-Hernández N, Ticó J, García-Montoya E, Pérez-Lozano P, Suñé-Negre J, Hernández-Munain C, Suñé C, Miñarro M. Int J Pharm **473** (2014) 270.
[2] De Jesus MB, Zuhorn IS. J Control Release **201** (2015) 1.
[3] Vighi E, Montanari M, Hanuskova M, Iannuccelli V, Coppi G, Leo E. Int J Pharm **440** (2013) 161.

Dendronized cationic gold nanoparticles: synthesis and *si*RNA binding

Cornelia E. Peña-González[1], Javier Sánchez-Nieves[1,2], Olga A. Krasheninina[3,4], Evgeny K. Apartsin[3], Alya G. Venyaminova[3], Rafael Gómez[1,2], F. Javier de la Mata[1,2]

[1]Department of Organic and Inorganic Chemistry, University of Alcala, Spain.
[2]Networking Research Center for Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
[3]Laboratory of RNA Chemistry, Institute of Chemical Biology and Fundamental Medicine SB RAS, Russia.
[4]Novosibirsk State University, Russia.
e-mail: cornelia.pena@uah.es

Introduction: Gold nanoparticles (AuNPs) are extensively used as multifunctional carriers of therapeutic nucleic acids, for instance, small interfering RNA (*si*RNA), because of their unique properties such as excellent biocompatibility, well-defined surface chemistry, low toxicity and easy functionalization [1]. A powerful approach to obtain nanoparticles for *si*RNA delivery is the passivation of AuNPs with a ligand possessing positive charges at the surface. The modified AuNPs bind *si*RNA *via* electrostatic interactions forming non-covalent complexes (nanoplexes) and promote their delivery across the cell membrane [2]. The coverage of AuNPs by dendrons allows to increase transfection efficiency by tuning the charge and to reduce their toxicity. The efficiency of cellular uptake and/or the subsequent release of *si*RNA from endosomes are improved due to the hydrophobicity of the skeleton of dendrons on the AuNPs surface [3]. We have designed cationic carbosilane dendritic molecules that act efficiently as RNA delivery systems, being also able to cross blood brain barrier due to combination of positive charge and lipophilicity of carbosilane skeleton [4]. Herein, we report the synthesis and characterization of AuNPs capped with cationic carbosilane dendrons and the physicochemical study of the nanoplexes formation with anticancer Bcl-xL *si*RNA.

Materials and Methods: An aqueous solution of cationic carbosilane dendrons was added dropwise to an aqueous solution of $HAuCl_4$. Afterward, $NaBH_4$ in water was added dropwise, and the mixture was stirred for 4 h. Nanoparticles were purified by dialysis (MWCO 10,000). Dendronized AuNPs were characterized by nuclear magnetic resonance (NMR), transmission electron microscopy (TEM), thermogravimetric analysis (TGA), UV, elemental analysis, and zeta potential (ζ) measurements. The ability of AuNPs to form nanoplexes with the anticancer pro-apoptotic Bcl-xL *si*RNA were performed using agarose gel electrophoresis, fluorescence spectroscopy and fluorescence polarization assay.

Results and Discussion: Dendronized AuNPs were obtained by reaction of their precursors in water. The mean size of AuNPs was found by TEM. The Au/dendron relationship was obtained by the analysis of TEM, TGA, and elemental analysis data. UV spectroscopy showed the band corresponding to the plasmon resonance at 530-540 nm. The ^{1}H-NMR of dendronized nanoparticles have resonances similar to those of dendrons but clearly broaden, although the signal belonging to the $-CH_2S$ group was not observed. These two phenomena are related with the proximity of the metallic surface. The ζ measured in aqueous solution at neutral pH was > 30 mV. The ability of AuNPs to form nanoplexes with *si*RNA was assessed. Nanoplexes were formed at different dendron/*si*RNA or AuNPs/*si*RNA charge ratios and studied by agarose gel electrophoresis and fluorescence spectroscopy. The electrophoregrams show different profiles of Bcl-xL retention depending on the generation dendron present in AuNPs. The fluorescence spectroscopy data confirms the data obtained. The displacement of *si*RNA from nanoplexes by heparin has been studied to determine the stability of nanoplexes in blood during systemic delivery. We have shown that *si*RNA is completely displaced from complexes in the presence of 60-80 mg/L heparin. These findings suggest that the nanoplexes formed cannot be destroyed by the blood glucosaminoglycans (< 2.5 mg/L in human plasma), however, the formation of nanoplex is reversible that indicates the possibility of *si*RNA release.

Conclusions: In this work we synthetized AuNPs functionalized with cationics carbosilane dendrons. These AuNPs were characterized by a number of physicochemical techniques. We proved the formation of nanoplexes by agarose gel electrophoresis and fluorescence assays. Moreover, we demonstrated that *si*RNA can be released from the nanoplexes upon displacement with heparin.

Acknowledgements: CTQ-2014-54004-P (MINECO), and Consortium NANODENDMED ref S2011/BMD-2351 (CAM) to UA. CIBER-BBN is financed by the ISC III with assistance from the European Regional Development. FP7-PEOPLE-2012-IRSES-GA-2012-316730, EU-Belarus-Russia Network in Nanomaterials-Driven Anti-Cancer Gene Therapy. NANOGENE.

References:

[1] Bottini M, Rosato N, Bottini N, Biomacromolecules **12** (2011) 3381.
[2] Ghosh PS, Kim CK, Han G, Forbes NS, Rotello VM. ACS Nano **2** (2008) 2213.
[3] Fröhlich E. Int J Nanomedicine **7** (2012) 5577.
[4] (a) Serramía MJ, Álvarez S, Fuentes-Paniagua E, Clemente MI, Sánchez-Nieves J, Gómez R, de la Mata FJ, Muñoz-Fernández MA. J Control Release **200** (2015) 60; (b) Fuentes-Paniagua E, Serramía MJ, Sánchez-Nieves J, Álvarez S, Muñoz-Fernández MA, Gómez R, de la Mata FJ. Eur Polym J **71** (2015) 61.

Ultraflexible Cyclosporin A liposomes: *in vitro* skin absorption studies

Juan José Carreras[1], Adrián Sala[1], Willian E. Tapia[1], Teresa Mª Garrigues[1], Ana Luiza Scarano Aguillera Forte[1,2], Ana Melero[1]

[1]Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Valencia, Spain.
[2]Faculty of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Brazil.
e-mail: aanaluiza2@gmail.com

Introduction: Inflammatory skin diseases, such as psoriasis and atopic dermatitis, affect a high percentage of the population. Nevertheless, the current available therapies can only reduce the symptoms, but not the causes. Cyclosporine A is a very effective drug for these skin illnesses. However, it is used as a second line therapy because of its serious systemic adverse effects [1]. Topical administration is limited in this case by the inability of Cyclosporine A to diffuse and distribute through and within the epidermis to reach the dermis. Ultraflexible Cyclosporine A liposomes have been formulated to facilitate the permeability of this drug [2, 3]. In addition, the ability to diffuse through the stratum corneum and dermis access has been evaluated.

Materials and Methods: Two types of ultraflexible liposomes containing, Tween® 20 and Tween® 80, have been prepared by film-method followed by extrusion or sonication [4], to reduce their size and homogenize the particle dispersion. Ethosomes were prepared by the Touitoui method [5], followed by extrusion. The permeability tests were performed using heat separated epidermis [6], obtained from human abdominal skin, by means of Franz diffusion cells [7]. Four different formulations of ultraflexible liposomes were tested, as well as solutions of the drug with the same concentrations of the permeability enhancers used to flexibilize the liposomes.

Results: Liposomes and enhancers increase the solubility of Cyclosporine A in aqueous medium and facilitate the diffusion of drug through skin. After 1.5 hours, detectable concentrations of Cyclosporine A were found in the receptor compartment only when the liposomes were used. The profiles show access to deeper skin layers but no steady state conditions are reached, except for the ethosomes, meaning that it is a retarded process.

Conclusions: These results allow predicting an increase in skin penetration of the drug to the target site mediated by ultraflexible liposome, while reducing the risk of systemic side effects.

References:

[1] Rosmarin DM, Lebwohl M, Elewski BE, Gottlieb AB. J Am Acad Dermatol **62** (2010) 838.
[2] El Maghraby GM, Barry BW, Williams AC. Eur J Pharm Sci **34** (2008) 203.
[3] El Maghraby GM, Williams AC, Barry BW. Int J Pharm **196** (2000) 63.
[4] Yamaguchi T, Nomura M, Matsuoka T, Koda S. Chem Phys Lipids **160** (2009) 58.
[5] Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. J Control Release **65** (2000) 403.
[6] Kligman AM, Christophers E. Arch Dermatol **88** (1963) 702.
[7] Ng SF, Rouse JJ, Sanderson FD, Meidan V, Eccleston GM. AAPS PharmSciTech **11** (2010) 1432.

Clodronate modulates the antigenic profile and inhibits the maturation and biomineralization potential of osteoblast-like cells

Francisco Javier Manzano-Moreno[1,2], Javier Ramos-Torrecillas[2], Elvira de Luna-Bertos[2], Candela Reyes-Botella[1,2], Olga García-Martínez[2], Concepción Ruiz[2]

[1]Biomedical Group (BIO277), Department of Stomatology, School of Dentistry, and Instituto de Investigación Biosanitaria, University of Granada, Spain.
[2]Biomedical Group (BIO277), Department of Nursing, Faculty of Health Sciences, and Instituto de Investigación Biosanitaria, University of Granada Spain.
e-mail: elviradlb@ugr.es

Introduction: Bisphosphonates (BPs) are a class of pharmacological agents chemically related to inorganic pyrophosphate, in which the oxygen linking the phosphates has been replaced by carbon; they are mainly used in the treatment of bone disorders. Non-nitrogen-containing BPs are used in the treatment of osteoporosis. However, the action mechanisms of these compounds on bone and their effects on osteoblasts are not fully understood [1-3]. The aim of this study was to evaluate the role of osteoblasts in bisphosphonate-related osteonecrosis of the jaw (BRONJ) by studying the effects of different concentrations of clodronate, a non-nitrogen-containing bisphosphonate, on osteoblast growth, differentiation, and antigenic profile.

Materials and Methods: Osteoblasts were incubated in culture medium with 10^{-5}, 10^{-7} or 10^{-9} M of clodronate. Proliferative capacity of the osteoblasts was determined by spectrophotometry (MTT) at 24 h of culture. Flow cytometry was used to study antigenic profile (CD54, CD80, CD86, HLA-DR). Cell differentiation was evaluated at 7, 15, and 21 days by the study of nodule formation and alkaline phosphatase activity (ALP) at 24 h by spectrophotometric assay.

Results and Discussion: Non-nitrogen-containing bisphosphonates such as clodronate increase the proliferation of MG-63 osteoblast-like cells and decrease their differentiation capacity, generally at low doses, and modulate the expression of co-stimulatory molecules associated with immune function. This effect alters the physiology of osteoblast, which has been also been described in nitrogen-containing BPs such as alendronate, ibandronate and pamidronate [4].

Conclusions: Non-nitrogen-containing bisphosphonates such as clodronate alter the physiology of osteoblast which could contribute to impair the bone regenerative capacity, and to the development of BRONJ. Further research is needed in order to develop new drugs in the osteoporosis treatment that avoid serious problems related with BPs such as BRONJ.

Acknowledgements: This study was supported by the research group BIO277 (Junta de Andalucía) and Department of Nursing (University of Granada, Spain).

References:

[1] Im GI, Qureshi SA, Kenney J, Rubash HE, Shanbhag AS. Biomaterials **25** (2004) 4105.
[2] Marx RE. J Oral Maxillofac Surg **61** (2003) 1115.
[3] Idris AI, Rojas J, Greig IR, Van't Hof RJ, Ralston SH. Calcif Tissue Int **82** (2008) 191.
[4] Manzano-Moreno FJ, Ramos-Torrecillas J, De Luna-Bertos E, Reyes-Botella C, Ruiz C, García-Martínez O. Clin Oral Investig 19 (2015) 895.

Poly(D,L-lactide-*co*-glycolide) nanoparticles for targeted brain drug delivery in the cerebral tuberculosis disease

P. Marcianes-Moreno[1], *S. Negro*[1,2], *L. Garcia-Garcia*[3], *E. Barcia*[1,2], *C. Montejo*[4], *A. Fernández-Carballido*[1,2]

[1]Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad Complutense de Madrid, Spain.
[2]Instituto Universitario de Farmacia Industrial, Universidad Complutense de Madrid, Spain.
[3]Cartografia Cerebral, Instituto Pluridisciplinar, Universidad Complutense de Madrid, Spain.
[4]Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad CEU-San Pablo, Spain.
e-mail: patri_1905@hotmail.com

Introduction: Central nervous system (CNS) tuberculosis is caused by *Mycobacterium tuberculosis.* It is one of the most serious forms of this infection, acting as a prominent cause of morbidity and mortality in developing countries [1-2]. It is manifested in a variety of forms as tuberculous meningitis, tuberculoma, and tubercular abscess [3]. Its treatment is complex due to increasing incidence of multidrug resistances [4]. Furthermore, the blood-brain barrier (BBB) plays an important role limiting strategies of therapy; therefore the development of controlled delivery systems is currently one of the most promising strategies to cross the BBB [5]. The aim of this work was to elaborate rhodamine (Rh) nanoparticles prepared with labrafil, as surface modifier, in order to improve their passage through the BBB.

Materials and Methods: Rh nanoparticles (NPs) were prepared by the nanoprecipitation method, using poly(D,L-lactide-*co*-glycolide) RG® 502 (PLGA) as polymer. The ratio Rh:PLGA used was 1:20. Briefly, PLGA was dissolved in acetone by vortexing. This mixture was added dropwise into PVA under stirring. The resulting suspension was then evaporated with rotavapor to completely remove acetone. The NP suspension obtained is washed and centrifuged at 15,000 rpm for 30 min. Finally, the dispersed solution was freeze-dried for 24 h. Moreover, Rh-PLGA-labrafil NPs were prepared by the same method but incorporating 3.5 mg of labrafil into the organic phase. The shape and surface morphology of NPs were analyzed by scanning electron microscopy. The mean diameter and size distribution of NPs were analyzed using a Zetatrac® Ultra at 25 ºC. An *in vivo* study was carried out in male Wistar rats weighing 250-300 g. Animals were anesthetized and injection of the corresponding formulation was performed in the tail vein. At 30 min or 60 min after administration animals were sacrificed and brains removed. Sections of cerebral cortex were analyzed by confocal microscopy.

Results and Discussion: SEM images revealed that NPs prepared without surfactant, either loaded or unloaded with Rh, were spherical with smooth surfaces, whereas NPs elaborated with labrafil showed more aggregation due to the presence of surfactant on the surface. Formulations did not show crystals on the surface indicating that Rh is encapsulated within the polymer matrix. Mean sizes of Rh-PLGA NPs and Rh-PLGA-labrafil NPs were 150.5 ± 5.1 µm and 156.3 ± 6.0 µm, respectively. It is known that smaller sizes facilitate the passage of NPs through the BBB, being necessary a size < 200 nm. Span values of Rh-PLGA NPs (range 0.7-1) reflect a slight polydispersion of the particle populations in all formulations indicating that the method employed in the elaboration of the particles allows obtaining monodisperse distributions. The *in vivo* study shows that Rh-PLGA NPs exhibit lower fluorescence than Rh-PLGA-labrafil NPs at 30 and 60 min. For Rh-PLGA-labrafil NPs the highest fluorescence was obtained at 30 min. At 60 min, a slight decrease in fluorescence intensity was observed probably due to the passage of Rh released from the NPs to the blood by means of P-glycoprotein.

Conclusions: *In vivo* studies confirm that the incorporation of labrafil in the formulation improves the passage of nanoparticles across the BBB.

Acknowledgements: UCM research group 910939.

References:

[1] Bartzatt R. Cent Nerv Syst Agents Med Chem **11**(2011) 321.
[2] Saifullah B, Hussein MZ, Hussein Al Ali SH. Int J Nanomedicine **7** (2012) 5451.
[3] Garcia-Monco JC. Handb Clin Neurol **121** (2014) 485.
[4] van der Harst JJ, Luijckx GJ.. Curr Pharm Des **17**(2011) 2940.
[5] Vera M, Barcia E, Negro S, Marcianes P, García-García L, Slowing K, Fernández-Carballido A. Int J Pharm **473** (2014) 518.

Biocompatibility and *in vitro* catalytic properties of palladium nanoparticles

Belén Rubio-Ruiz, Ana Maria Pérez-López, Thomas Bray, Jason T. Weiss, Asier Unciti-Broceta

Edinburgh Cancer Research UK Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, UK.
e-mail: v1bruiz2@exseed.ed.ac.uk

Introduction: Due to their size, structure versatility and optoelectronic properties, metal nanoarchitectures are attracting interest for a number of biomedical applications. To date, iron, gold, silver and platinum nanostructures are the most extensively studied examples but due to the highly complex and multifactorial interactions with biological systems their widespread application is impeded. Palladium (Pd^0) is a noble metal with remarkable catalytic, mechanic and electronic properties. Despite of these unique characteristics, Pd^0 nanostructures have not been widely exploited in the nanomedicine field [1]. In this work, we have studied the biocompatibility of Pd^0 nanoparticles obtained by a green synthetic procedure and its catalytic capacity to mediate bioorthogonal organometallic (BOOM) reactions inside the cells.

Materials and Methods: *Synthesis of Pd^0 nanoparticles*. Pd^0 nanoparticles were synthesized by adding brown solid of $Pd(NO_3)_2 \cdot 2H_2O$ (2 mmol) and white powder of ascorbic acid (6 mmol) into a mortar and grinding at room temperature. Black powders of Pd^0 nanoparticles were produced successfully after drying in vacuum at 80 °C for 8 h [2]. *Synthesis of Probe 1*. Probe 1 was prepared from rhodamine 110 chloride as previously described [3]. *Study of toxicity of Pd0 nanoparticles in cancer cell culture*. Human lung adenocarcinoma A549 cells were seeded in a 96 well plate format and incubated for 48 h before treatment. Increasing quantities of Pd^0 nanoparticles (100 μM-3 mM) were incubated with cells for 5 days and cell viability determined by PrestoBlue™ assay. *Study of in vitro catalytic efficiency of Pd^0 nanoparticles*. Probe 1 (25 μM) was incubated in PBS with increasing concentrations of Pd^0 nanoparticles (up to 2 mM). The appearance of fluorescence was monitored for 12 h using a PerkinElmer Victor2 multilabel plate reader (excitation filter at 480 nm and emissions filter at 535 nm).

Results and Discussion: Using a green method we have produced Pd^0 nanoparticles by grinding solid reactants directly for only a few minutes. Biocompatibility studies in cancer cell culture demonstrate the innocuousness of these Pd^0 nanoparticles at high concentrations (IC_{50} = 2 mM) after 5 day incubation. Catalytic properties of the Pd^0 nanoparticles were assayed by means of a Pd^0-sensitive pro-fluorophore (probe 1) prepared from rhodamine 110. The fluorescent signal detected after incubation of both Pd^0 nanoparticles and probe 1 confirmed the catalytic functionality of the Pd^0 nanoparticles.

Conclusions: We have prepared Pd^0 nanoparticles that are biocompatible in cancer cell culture and catalytic effective *in vitro*. Future experiments will be focused on the evaluation of the catalytic functionality of these nanoparticles in living systems as devices for performing BOOM reactions inside the cells.

Acknowledgements: BRR thanks the Martin Escudero Foundation for her fellowship. This project was also supported by MSD-Scottish Life Sciences Fund postdoctoral award (for AMPL) and Cancer Research UK.

References:

[1] Dumas A, Couvreur P. Chem Sci **6** (2015) 2153.
[2] Zhang A, Liu M, Liu M, Xiao Y, Li Z, Chen J, Sun Y, Zhao J, Fang S, Jia D, Li F. J Mater Chem A **2** (2014) 1369.
[3] Weiss JT, Dawson JC, Macleod KG, Rybski W, Fraser C, Torres-Sánchez C, Patton EE, Bradley M, Carragher NO, Unciti-Broceta A. Nat Commun **5** (2014) 3277.

Ropinirole-loaded PLGA microspheres revert rotenone-induced neurodegeneration

E. Barcia[1,2], *L. Boeva*[1], *K. Slowing*[3], *L. García-García*[4], *A. Fernández-Carballido*[1,2], *S. Negro*[1,2]

[1]Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad Complutense de Madrid, Spain.
[2]Instituto Universitario de Farmacia Industrial, Facultad de Farmacia, Universidad Complutense de Madrid, Spain.
[3]Departamento de Farmacología, Facultad de Farmacia, Universidad Complutense de Madrid, Spain.
[4]Unidad de Cartografía Cerebral, Instituto Pluridisciplinar, Universidad Complutense de Madrid, Spain.
e-mail: ebarcia@ucm.es

Introduction: Ropinirole (RP) is a specific D2 and D3 dopamine receptor agonist indicated for the treatment of Parkinson´s disease (PD) both as monotherapy and in combination with levodopa in advanced stages [1, 2]. It is hypothesized that pulsatile rather than continuous stimulation of dopamine receptors in PD may induce motor fluctuations; thereby expecting that these responses could be avoided by continuous stimulation of the dopamine receptors. For this, a controlled release formulation in the form of PLGA microparticles is developed and evaluated in an animal model.

Materials and Methods: RP-loaded microspheres were prepared by the solvent-evaporation technique from an O/W emulsion. The ratio drug:polymer was 120 mg RP:400 mg PLGA 502. A rotenone (RT)-induced animal model of PD is developed in male Wistar rats by means of daily intraperitoneal (i.p.) doses of RT (1 mg/Kg dissolved in sunflower oil), and the efficacy of the new controlled delivery system is evaluated regarding brain histology and immunohistochemistry. Control animals received the vehicles used (sunflower oil or saline) (G1). The other animal groups received RT alone (G2), RT and an amount equivalent to 15 mg/Kg of RP-loaded microparticles given on days 15th and 30th of the study (G3), or RT and RP in saline given i.p. daily at a dose level of 1 mg/Kg starting on day 15th of the study (G4). At the end of the study period (45 days) animals were killed by decapitation, brains removed and histology and histochemical assessments were made [histological Nissl-staining (cresyl violet)], GFAP (glial fibrillary acidic protein) immunohistochemistry, and TH (tyrosine hydroxylase) immunohistochemistry.

Results and Discussion: Nissl-staining showed signs of neuronal damage at the level of the substantia nigra (SN) in RT-treated rats (G2). RT produced a marked reduction in the number of dopaminergic neurons in the SN (≈ 40%). An intense gliosis was observed in the SN after 45 days of RT administration with this astrocytic response being almost completely reverted by RP-loaded PLGA microparticles. The group receiving RP in saline reduced the astrocytic response however exhibiting a light but observable glial activation as observed by GFAP immunolabeling. As expected gliosis was not found in control rats. Several studies have reported that RT triggers a moderate or null reduction of TH-immunoreactive neurons in the substantia nigra pars compacta (SNpc) [3]. In our case and in agreement with these reports, the TH immunoreactivity in the SNpc was also very lightly reduced. This reduction was reverted by RP when formulated within PLGA microspheres.

Conclusions: The results obtained in our study confirm the potential interest of the new controlled delivery system developed for RP (PLGA microspheres) which have demonstrated to be effective in reverting neurodegeneration in an animal model of PD.

Acknowledgements: This work was supported by a research project Fundación Mutua Madrileña (FMM2012) and by the Complutense University research group 910939.

References:

[1] Bogan RK, Fry JM, Schmidt MH, Carson SW, Ritchie SY. Mayo Clin Proc **81** (2006) 17.
[2] Nyholm D. Clin Pharmacokinet **45** (2006) 109.
[3] Lapointe N, St-Hilaire M, Martinoli MG, Blanchet J, Gould P, Rouillard C, Cicchetti F. FASEB J **18** (2004) 717.

The potential of HA-NCs for the delivery of small hydrophobic and large hydrophilic anticancer drugs

Ana Olivera[1], Ana Cadete[1], Ángela Molina[2], Gema Moreno[2], Mª José Alonso[1], Dolores Torres[1]

[1]Center for Research in Molecular Medicine and Chronic Diseases (CIMUS) and Department of Pharmacy and Pharmaceutical Technology, University of Santiago de Compostela, Spain.
[2]Department of Biochemistry, Universidad Autónoma de Madrid, Spain.
e-mail: anaoliverafernandez@gmail.com

Introduction: Hyaluronic acid (HA) is attracting increasing attention for the design of targeted drug delivery systems [1, 2]. In this report, we describe the optimization of HA nanocapsules (HA-NCs) for the intracellular delivery of therapeutic compounds. HA-NCs were formulated by a self-emulsification method, which makes them suitable for the manipulation and formulation of very sensitive molecules [3]. The model compounds were nile red (NR), as asmall molecular weight hydrophobic drug, and a protein as a large molecular weight hydrophilic drug.

Materials and Methods: HA-NCs were prepared using a recently developed self-emulsification method. These NCs were composed of Miglyol® 812N and Tween® 80 as theoil core, Solutol® HS15 with a PEGylated chain as the interfacial surfactant and a cover shell constituted by a hydrophobically-modified HA. NR-loaded HA-NCs were prepared by dissolving NR in the oil phase. Protein-associated HA-NCs were prepared by the same method by incubating blank NCs with a protein solution. NCs were characterized in respect to size, polydispersity index (PDI) and zeta potential. Stability assays were performed using different media (human blood plasma, PBS and RPMI culture medium) by incubating loaded HA-NCs with the respective medium and measuring the physicochemical characteristics at different time points. Cellular uptake was done using different cancer cell lines and was evaluated by confocal microscopy.

Results and Discussion: After formulation optimization, empty HA-NCs showed a particle size ≈ 130 nm and a negative zeta potential. Irrespectively of the model drug, a high encapsulation efficiency was obtained. In both cases, empty and loaded HA-NCs maintained their stability after the incubation with PBS, RPMI culture medium and human blood plasma. Confocal microscopy images showed the ability of HA-NCs to transport the protein and the fluorophore to the intracellular compartment.

Conclusions: We have developed a versatile system for anticancer drug delivery which consists of an oil core for the loading of hydrophobic anticancer drugs and a polymeric surface for protein association. The delivery of proteins for specific intracellular targeting is an ambitious approach where polymeric NCs appear as promising carriers for accurate delivery. The proposed system offers the additional advantage of co-delivering both drugs to reach their intracellular targets.

Acknowledgements: Financial support from Instituto de Salud Carlos III, Spain, under Project PI13/00132 and from Xunta de Galicia (Competitive Reference Groups-FEDER Funds Ref 2014/043) is gratefully acknowledged. A.C. acknowledges the Collaboration Grant received from Spanish Government.

References:

[1] Oyarzun-Ampuero FA, Rivera-Rodríguez GR, Alonso MJ, Torres D. Eur J Pharm Sci **49** (2013) 483.
[2] Teijeiro C, McGlone A, Csaba N, Garcia-Fuentes M, Alonso MJ. In: Torchilin V (Ed.). Handbook of Nanobiomedical Research (2014) chapter 7.
[3] Date AA, Desai N, Dixit R, Nagarsenker M. Nagarsenker. Nanomedicine (Lond) **5** (2010) 1595.

Immune checkpoint blockade in melanoma by new targeted Doxorubicin immunoliposomes

María Merino[1], *Ana M. Contreras-Sandoval*[1], *Sara Zalba*[3], *Kepa Berraondo*[2], *María J. Garrido*[1]

[1]Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Navarra, Spain.
[2]Division of Gene Therapy and Hepatology. Center for Applied Medical Research (CIMA), University of Navarra, Spain.
[3]Laboratory Experimental Surgical Oncology, Department of Surgery, Erasmus MC Cancer Institute, Rotterdam, The Netherland.
e-mail: mmerino@alumni.unav.es

Introduction: Immunotherapy has emerged in recent years as a new strategy to treat various kinds of diseases. In oncology, the majority of solid tumours are characterized by the up-regulation of several types of membrane molecules involved in different proliferation signalling pathways, like PD-L1 (Programmed cell Death Ligand 1). This molecule is engaged PD-1, a receptor expressed in T cells, to transmit an inhibitory signal that maintains self-tolerance. However, cancer is able to use this pathway to suppress tumour immunity and then, proliferate. The use of monoclonal antibodies (mAbs) against PD-1 and PD-L1, two relevant immune checkpoints, is the new therapy to promote antitumor efficacy by T-cells infiltration into tumour [1]. In monotherapy, these mAbs show low efficacy, representing a limitation that leads to their combination with other therapeutic agents. Doxorubicin (Dox) acts mainly by inhibiting DNA topoisomerase II, but its immunogenic properties, reported in literature, contribute to enhance cell death, and then, the antitumor efficacy [2]. However, its toxicity, which results in numerous adverse effects, has been controlled by its encapsulation in different liposomal formulations available in clinic (Doxil™, Caelix™ and Evacet™) [3]. Therefore, the development of immunoliposomes encapsulating Dox, and conjugated with a ligand capable of recognizing a specific antigen overexpressed in cancer cells, provides a selective nanosystem for improving efficacy and decreasing side effects. The aim of this project was the development of PD-L1 targeted Dox liposomal formulation and its pharmacokinetic/pharmacodynamic evaluation in an *in vitro*/*in vivo* platform using a melanoma cell line.

Materials and Methods: Dox liposomes were prepared by the film-hydration method [4], combined with a pH gradient [5]. Briefly, lipids, HSPC:CH:DSPE-PEG_{2000} (1.85:1:0.12 M ratio) were dissolved in a chloroform:methanol solution (9:1, v/v). The film, obtained after evaporation of organic solvents at 65 ºC, was hydrated with ammonium sulphate buffer at pH 5.5. The liposomal solution was extruded (100 nm of pore membrane) several times to obtain a homogeneous population of liposomes. They were washed in hepes buffer at pH 7.4 and incubated in a thermoshaker with Dox (1:0.15 M ratio). This formulation (LPDOX) was washed at pH 6.7 and purified by centrifugation. PD-L1 targeted Dox liposomes (LPDOXFab') were formulated according to the post-insertion method. DSPE-PEG_{2000}-Mal micelles were prepared in hepes buffer at pH 6.7 and incubated with anti-PD-L1-Fab' fragments (30:1 M ratio) overnight at 4 ºC. Afterwards, Fab' conjugated micelles were incubated with previous preformed LPDOX. This targeted formulation was purified by centrifugation at pH 6.7. Characteristics such as particle size, PDI and zeta potential were analyzed by laser diffractometry. Dox EE was measured by fluorescent spectrophotometry and coupling efficiency quantified with MicroBCA™ kit. Drug release profile was measured at pH 7.4 for 24 h. For *in vitro*/*in vivo* studies, B16 OVA melanoma cell line was used. Cytotoxicity (IC_{50}) for free and encapsulated drug was obtained after 24 h exposure. Dox cell incorporation was measured after 4 h by flow cytometry to evaluate the impact of the different formulations in drug availability. Currently, a melanoma mouse model is being used to assay antitumor efficacy.

Results and Discussion: The post-insertion method allowed us to develop PD-L1 targeted Dox liposomes. Particle size for targeted and non-targeted liposomes was ≈ 157 ± 1.8 nm associated with a low PDI (0.084 ± 0.035). The EE was 95.6 ± 7.5% whereas ligand conjugation was 40.8 ± 7.4%. Accumulative drug release for both types of formulations reached 35% at physiological pH during 24 h, supporting their use in intravenous route. The IC_{50} reached the highest cytotoxicity with free Dox (0.08 ± 0.03 μM) followed by LPDOX (2.7 ± 0.52 μM) and LPDOXFab' (3.8 ± 0.52 μM), which did not differ statistically, supporting the similar Dox intracellular accumulation. *In vivo* experiments, where the immune system is implicated, have to be done to evaluate the real advantage that may provide the blocking of this immune checkpoint. This study is currently been carried out by our group, as well as the caption of images from cultured cells treated with fluorescent liposomes, in order to see the location of LPDOXFab'.

Conclusions: PD-L1 targeted Dox liposomes have been successfully developed by the post-insertion method. *In vitro* studies revealed similar behaviour of targeted and non-targeted formulations. However, the role of the immune response is being assayed in an *in vivo* model, as well as the blocking mechanism of the anti-PD-L1-Fab'.

References:

[1] Robert C, Soria JC, Eggermont AM. Eur J Cancer **49** (2013) 2968.
[2] Casares N, Pequignot MO, Tesniere A, Ghiringhelli F, Roux S, Chaput N, Schmitt E, Hamai A, Hervas-Stubbs S, Obeid M, Coutant F, Métivier D, Pichard E, Aucouturier P, Pierron G, Garrido C, Zitvogel L, Kroemer G. J Exp Med **202** (2005) 1691.
[3] Chang HI, Yeh MK. Int J Nanomedicine **7** (2012) 49.
[4] Zalba S, Navarro I, Trocóniz IF, Tros de Ilarduya C, Garrido MJ. Eur J Pharm Biopharm **81** (2012) 273.
[5] Barenholz Y. J Control Release **160** (2012) 117.

Hepatitis B vaccine: chitosan-based delivery system for efficient HBsAg oral immunization

Edna Soares[1,2], Sandra Jesus[1,2], Olga Borges[1,2]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra, Portugal.
[2]Center for Neuroscience and Cell Biology, University of Coimbra, Portugal.
e-mail: sjesus.mg@gmail.com

Introduction: Hepatitis B virus infection is a serious global health burden where sexual transmission is a major problem. The WHO encourages the development of oral vaccines to simplify transport, storage and administration in developing countries, and to elicit mucosal immunization preventing sexual dissemination. Antigen encapsulation in polymer-based particles with enhanced immune responses is decisive for future vaccine development [1, 2]. Chitosan (Chi) is a biopolymer with immunostimulatory and mucoadhesive features matching the needs for an efficient subunit antigen vaccination through oral mucosa. Sodium alginate (Alg) may be applied to protect chitosan particles from the harsh gastrointestinal tract environment [3].

Materials and Methods: Particles were prepared by a coacervation/precipitation technique previously described with minor modifications [4, 5]. Briefly, sodium sulfate (0.625%, w/v) was added dropwise to a chitosan solution (0.1%, w/v in sodium acetate buffer 25 mM, pH 5) during few seconds under vortex high speeding, to form Chi particles. Alg-coated particles (ChiAlg) were obtained by mixing equal volumes of Chi particles and Alg solution (1%, w/v in sodium acetate buffer 25 mM, pH 5). ChiAlgCa particles are ChiAlg particles with calcium chloride crosslink (60 µL, 18 mM). Cytotoxicity was verified in colorectal epithelium (Caco-2) and mast (HMC-1) cell lines. The β-hexosaminidase release of HMC-1 cells was performed as previously described [6]. *Ex vivo* studies with mice intestinal ileal loops were executed to evaluated Peyer's Patches (PPs) particles uptake. *In vivo* immunization studies with Chi and ChiAlgCa encapsulated Hepatitis B surface Antigen (HBsAg) were carried out following two schemes: *i*) one subcutaneous (1.5 µg HBsAg) and two oral immunizations (20 µg HBsAg each); and, *ii*) three oral immunizations (20 µg HBsAg each). Mice serum samples, feaces and vaginal washes were analyzed for anti-HBsAg specific immunoglobulins. All immune response evaluation was based on enzyme-linked immunosorbent assay.

Results and Discussion: Chi particles were positively charged in water (+19.9 ± 1.6 mV) with a mean diameter of 612 ± 28 nm. ChiAlg particles were larger (1053 ± 194 nm) than Chi particles, although smaller than ChiAlgCa ones (1523 ± 70 nm). All particles displayed reduced cytotoxicity for HMC-1 and differentiated Caco-2 cells. Caco-2 cell viability decreased significantly for particles concentrations < 500 µg/mL. Chi particles could activate mast cells, pointing them as immune modulators. Thus, these particles were indeed good candidates as an adjuvant delivery system. Chi, ChiAlg and ChiAlgCa FITC-labeled particles were efficiently internalized by ileal PPs. PPs uptake is a relevant feature for oral vaccine delivery as they represent key players of the mucosal immune host response toward gut antigens and bacteria. Any ideal vaccine delivery system should interact with APCs, and in the present works, all developed particles were internalized by peripheral blood mononuclear cells. *In vivo* studies reveal that all mice from group *i* developed high titers of HBsAg specific systemic antibodies and 60% of group *ii* responded to HBsAg immunization. Both groups developed a Th2-biased immune response. All groups developed mucosal immunity: anti-HBsAg sIgA detected in faeces and vaginal washes.

Conclusions: The developed formulation for oral HBsAg immunization was able to induce both systemic and mucosal immunity, mostly important for sexually transmitted Hepatitis B. The subcutaneous priming appears to be crucial for an entire number of responders.

Acknowledgements: Work supported by Portuguese Foundation for Science and Technology (FCT) - project PTDC/SAU-FAR/115044/2009, by the Center for Neuroscience and Cell Biologystrategic project UID / NEU / 04539 / 2013, and by FCT fellowship DFRH - SFRH/BD/81350/2011.

References:

[1] Koppolu B, Zaharoff DA. Biomaterials **34** (2013) 2359.
[2] Czerkinsky C, Holmgren J. Curr Top Microbiol Immunol **354** (2012) 1.
[3] Borges O, Borchard G, de Sousa A, Junginger HE, Cordeiro-da-Silva A. Int J Pharm **337** (2007) 254.
[4] Borges O, Cordeiro-da-Silva A, Romeijn SG, Amidi M, de Sousa A, Borchard G, Junginger HE. J Control Release **114** (2006) 348.
[5] Borges O, Cordeiro-da-Silva A, Tavares J, Santarém N, de Sousa A, Borchard G, Junginger HE. Eur J Pharm Biopharm **69** (2008) 405.
[6] Staats HF, Kirwan SM, Choi HW, Shelburne CP, Abraham SN, Leung GY, Chen DY. Medchemcomm **4** (2013) 1.

Retinal structure improvement after non-viral gene therapy in a *Retinoschisin*-deficient mouse model

P.S. Apaolaza[1], A. del Pozo-Rodríguez[1], M.A. Solinís[1], J. Torrecilla[1], U. Friedrich[2], B.H.F. Weber[2], A.R. Gascón[1]

[1]Pharmacokinetic, Nanotechnology and Gene Therapy (PharmaNanoGene) group, Lascaray Research Center, Faculty of Pharmacy, University of the Basque Country UPV/EHU, Spain.
[2]Institute of Human Genetics, University of Regensburg, Germany.
e-mail: alicia.rodriguez@ehu.eus

Introduction: X-Linked Juvenile Retinoschisin (XLRS) is an inherited retinal disease in which a mutation of the gene codifying retinoschisin (RS1) protein, leads to premature loss of vision in males [1]. The availability of a RS1-deficient mouse model, which shares important diagnostic features with the human disease, eases the research and improvement of therapeutic interventions [2]. Given the drawbacks of viral vectors, such as immuno-oncogenicity, the development of non-viral vectors is a promising alternative to attempt the treatment of XLRS with gene therapy. Among non-viral vectors, solid lipid nanoparticles (SLNs) are one of the most effective lipid-based carriers [3]. The purpose of this research is the evaluation of SLN-based vectors complexed with protamine (P) and dextran (DX) (DX-P-DNA-SLN) bearing either a plasmid encoding the human *RS1* gene under the control of a murine opsin promoter (mOPS), which exhibits specificity for photoreceptors (PR), or an ubiquitous promoter, CMV. Besides, promoter-RS1 units were included in a pCMV-GFP plasmid to assess the biodistribution of the expression of both genes in the retina.

Materials and Methods: The plasmids containing the CMV or mOPS promoters and the *RS1* units were developed by enzymatic digestion and straightaway inserted into the pCMV-GFP plasmid [4]. DNA was mixed with an aqueous solution of P and DX and kept in contact with an aqueous solution of SLNs prepared by an emulsification-evaporation technique [3]. The vectors bearing the pCMV-GFP_CMV-RS1 or pCMV-GFP_mOPS-RS1 plasmids were intravitreally injected in a single dose in the left eye of 14 days old mice (p14). The right eye of each mouse was kept as non-treated control. After 2 weeks, enucleated eyes were embedded in OCT (Tissue-Tek), fast frozen immersed in dry ice and cryosectioned (14 μm-thick). Transfection was evaluated by immunohistochemistry. 6 sections per eye were examined under confocal microscope (Olympus Fluoview FV500). Finally, the structure of the retina was analyzed by Masson´s trichrome staining technique and the thickness of the retina and outer nuclear layer were measured [5].

Results and Discussion: *RS1* was detected along all the retinal layers, from the ganglion cells to the retinal pigment epithelium, with minor differences in the distribution profile depending on the promoter. *RS1* is secreted in multiple cell types and spreads from the site of expression; therefore its biodistribution in several layers does not necessarily mean that it was produced there [5]. In addition, the disorganized retina in the diseased mice favours the migration of the formulation through the retina, and thus the access to different cell layers. With both promoters, retinal structure improved owing to the levels achieved in the PR layer, our target in this disease. Transfection levels in PR and *RS1* production with the formulation containing the mOPS promoter were comparable to those detected with the same formulations bearing the *RS1* gene under the control of the CMV promoter. However, the change of CMV by the mOPS promoter led to a modest improvement in the retinal recovery at the studied short-times after administration. Using viral vectors, it has been detected greater structural and functional improvement when PR and no other retinal cells produced *RS1*, but the highest effect was detected from month 4 after injection [1]. Therefore, further studies could confirm whether the recovery detected in our study with the formulations containing the mOPS promoter results in a higher rescue of the retina at longer times.

Conclusions: Our results prove that *RS1* gene transfer by non-viral SLN-based formulations administered intravitreally, a safer route, leads to a widespread location of the *RS1* protein in the retina and partially restores retinal structure in a *RS1*-deficient model. This work points at lipid-based non-viral vectors as a promising and feasible alternative to the viral vectors for retinal disorders.

Acknowledgements: This work was supported by the Basque Government´s Department of Education, Universities and Investigation (IT-341-10). We thank the University of Basque Country (UPV/EHU) for the grant awarded to P.S.A., and by grants from the Deutsche Forschungsgemeinschaft (DFG) to B.H.F.W. (WE1259/12-4).

References:

[1] Byrne LC, Oztürk BE, Lee T, Fortuny C, Visel M, Dalkara D, Schaffer DV, Flannery JG. Gene Ther **21** (2014) 585.
[2] Molday RS, Kellner U, Weber BH. Prog Retin Res **31** (2012) 195.
[3] del Pozo-Rodríguez A, Delgado D, Solinís MA, Gascón AR, Pedraz JL. Int J Pharm **261** (2007) 268.
[4] Friedrich U, Stöhr H, Hilfinger D, Loenhardt T, Schachner M, Langmann T, Weber BH. Hum Mol Genet **20** (2011) 1132.
[5] Apaolaza PS, Del Pozo-Rodríguez A, Torrecilla J, Rodríguez-Gascón A, Rodríguez JM, Friedrich U, Weber BH, Solinís MA. J Control Release **217** (2015) 273.

Silencing of Hepatitis C virus replicon by SLN-based vectors containing a *sh*RNA to inhibit the internal ribosome entry site (IRES)

Josune Torrecilla[1], Ana del Pozo-Rodríguez[1], Paola S. Apaolaza[1], María Ángeles Solinís[1], Beatriz Berzal-Herranz[2], Cristina Romero-López[2], Alfredo Berzal-Herranz[2], Alicia Rodríguez-Gascón[1]

[1]PharmaNanoGene Group, Faculty of Pharmacy. Lascaray Research Center. University of the Basque Country UPV/EHU, Spain.
[2]Instituto de Parasitología y Biomedicina "López-Neyra" (IPBLN-CSIC), Spain.
e-mail: alicia.rodriguez@ehu.eus

Introduction: The development of new therapies for refractory Hepatitis C virus (HCV) genotypes represents a major public health objective [1]. HCV is an ideal candidate for the therapeutic approach of RNA interference (RNA*i*) using short hairpin RNA (*sh*RNA). A major challenge for RNA*i* therapy is the development of suitable delivery systems [2], such as solid lipid nanoparticles (SLNs) which are one of the most promising non-viral vectors for gene therapy [3]. In this work, we evaluate the capacity of SLN-based vectors to inhibit HCV replication in the human hepatoma cell line supporting a subgenomic HCV replicon (Huh-7 NS3-3') [4], and also deepen the cell internalization mechanisms and the intracellular disposition of SLN-based vectors, and the relationship with their efficacy. Finally, the biocompatibility of the vectors in terms of hemaglutination capacity and hemolytic activity was also evaluated.

Materials and Methods: SLNs were prepared by the solvent emulsification-evaporation technique, and the final nanocarriers were prepared with SLNs, protamine (P), hyaluronic acid (HA) and *sh*RNA74 plasmid (HA-P-DNA-SLN) [4]. After treatment of the Huh-7 NS3-3' cells with the nanocarriers at different *sh*RNA74 doses, intracellular HCV subgenomic replicon RNA was quantified by qRT-PCR. The effect of temperature on cell uptake and subsequent silencing efficacy was studied by pre-incubating Huh-7 cells at 4 or 37 ºC for 30 min prior to the addition of the vectors. To identify the endocytic mechanism, a co-localization study was performed with Nile Red-labelled SLNs and AlexaFluor® 488-Cholera Toxin, Transferrin or LysoTracker® (markers of caveolae, clathrins and lysosomes, respectively). To explore the involvement of CD44 in the cellular entry of the vectors, cell internalization and silencing assays were done after pre-incubation of the cells for 30 min with a solution of HA (3 mg/mL) mixed with normal growing media (1:3 ratio). Hemagglutination and hemolysis were studied by adding the vectors to an erythrocyte suspension at 1:1 (v/v) ratio. Hemagglutination was observed under an optical microscope and for the hemolysis assay; the absorbance (540 nm) of the released hemoglobin was measured.

Results and Discussion: The vectors (231 ± 11 nm and +28.73 ± 0.46 mV) reduced the HCV replication in the Huh-7 NS3-3'. The highest inhibition (50%) was achieved with the highest *sh*RNA74 dose (3 µg). At 4°C, cell uptake decreased in comparison to 37 °C, which confirms cell internalization by endocytosis. Huh-7 cells present clathrin and caveolae/lipid raft-mediated endocytosis mechanisms, which were used by the vectors. The clathrin route leads to the vectors to lysosomes, and the co-localization of the vectors with LysoTracker® confirms that this mechanism participates in the internalization of the vectors. Consequently, formulations are exposed to the lysosomal activity, which facilitates the release of the plasmid from the vector. The silencing rate at 4 °C was two-fold lower than at 37 °C, but the cell uptake decreased only slightly; thus, the energy-dependent entry mechanisms seems to be a highly effective route. The blockage of the CD44 receptor led to a decrease in the cell uptake of the vectors, although the silencing efficacy was not affected. In this sense, when we blocked the CD44 receptor, the energy-dependent mechanisms, effective for gene transfection, could be induced and therefore the silencing activity remained. Finally, our formulations showed no hemagglutination capacity, probably due to the stealth properties of HA, and absence of hemolytic activity, also indicative of stability in bloodstream.

Conclusions: This study shows the capacity of HA-SLN vectors to reduce the replication capacity of HCV in hepatocytes. Silencing rate was related to the capacity of the vectors to enter the cells, being the energy-dependent mechanisms the most efficient ones. Vectors were shown to be biocompatible and useful for systemic administration.

Acknowledgements: This work was supported by the Basque Government's Department of Education, Universities and Investigation (IT-341-10), by the Department of Industry (Saiotek) S-PE11UN035 and by the Spanish Ministerio de Economía y Competitividad (BFU2012-31213). The work is partially supported by FEDER funds from the EU.

References:

[1] Preciado MV, Valva P, Escobar-Gutierrez A, Rahal P, Ruiz-Tovar K, Yamasaki L, Vazquez-Chacon C, Martinez-Guarneros A, Carpio-Pedroza JC, Fonseca-Coronado S, Cruz-Rivera M. World J Gastroenterol **20** (2014) 15992.
[2] Torrecilla J, Rodríguez-Gascón A, Solinís MÁ, del Pozo-Rodríguez A. Biomed Res Int **2014** (2014) 161794.
[3] Rodríguez-Gascón A, del Pozo-Rodríguez A, Solinís MA. In: Martin F (Ed.), Gene Therapy – Tools and Potential Applications (2013) pp. 3-33.
[4] Lohmann V, Körner F, Koch J, Herian U, Theilmann L, Bartenschlager R. Science **285** (1999) 110.
[5] Torrecilla J, del Pozo-Rodríguez A, Apaolaza PS, Solinís MÁ, Rodríguez-Gascón A. Int J Pharm **479** (2015) 181.

Formulation of sustained release metformin tablets with a novel hydrogel

Rebeca Simancas Herbada[1], *Luis Miguel Vázquez Costal*[1], *Juan Aparicio Blanco*[1], *Jorge Sánchez Retama*[2], *Enrique López-Cabarcos*[2], *Ana Fernández Carballido*[1,3], *Ana Isabel Torres-Suarez*[1,3]

[1]Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, Complutense University of Madrid, Spain.
[2]Department of Chemistry Physics II, Faculty of Pharmacy, Complutense University of Madrid, Spain.
[3]University Institute of Industrial Pharmacy, Complutense University of Madrid, Spain.
e-mail: rebecasimancas@ucm.es

Introduction: Sustained-release tablets can be coated or uncoated tablets that usually contain special excipients in order to extend the time of release of the active ingredient, looking for increased dosing interval and therefore better adherence to treatments. Many of sustained-release tablets are based on the use of hydrogels to control drug release rate after administration. Its three-dimensional structure based on long main chains linked together by shorter molecules, provides characteristics of both, solid and liquid compounds [1]. Hydrogels possess the ability to accommodate large numbers of water molecules between chains forming gels in presence of aqueous fluids, such as physiological fluids, due to the relaxation of the polymer chains while maintaining the shape and integrity. In this work we describe the synthesis of a new hydrogel derived from acrylic acid: a poly(magnesium acrylate) hydrogel (PAMgA), and its use for the formulation of tablets of a highly hidrosoluble drug as metformin.

Materials and Methods: The synthesis of the hydrogel is carried out in two stages: *i*) synthesis of the magnesium acrylate monomers (AMgA); and, *ii*) polymerization reaction to obtain the PAMgA hydrogel [2, 3]. The first step was the synthesis of the monomer which was carried out by a neutralization reaction: 2 mol of acrylic acid were added dropwise to a solution of magnesium hydroxide under constant stirring. Secondly, the synthesis of PAMgA was done by the free radical polymerization method, using ammonium persulfate (PSA) as iniciator and TEMED as a catalyst. The hydrogel synthesized had a concentration of 5 mM of cross-linker agent (PSA). Finally the polymer was washed with distilled water and lyophilized, and the powder obtained was used to elaborate tablets. Tablets with 500 mg of metformin hydrochloride and 500 mg of PAMgA, as excipient, were elaborated in an eccentric tableting machine. The drug dissolution from tablets was evaluated using USP method 2. For this assay two different media were used: simulated gastric fluid (pH 1.2, FaSSGF) and simulated intestinal fluid (pH 6.8, FaSSIF). The samples obtained at different sampling times were filtered and the active ingredient content was measured by UV spectophotometry at 232 nm.

Results and Discussion: The monomer obtained had a clean and transparent appearance, with a slightly yellowish color. Ones AMgA was formed, the polymerization reaction took place, with PSA and TEMED. The hydrogel obtained had long segments between linking points in the monomer (AMgA) chains, due to PSA concentration (5 mm). The viscous and white substance was lyophilized and a white powder was obtained. Tablets containing the drug were white, rounded and unscored, with an average weight of 971 ± 6.51 mg, a diameter of 14.15 ± 0.03 cm and hardness of 62 ± 1 N. Regarding the dissolution rate, metformin showed a lower dissolution rate from tablets at pH 6.8; reaching an 80% of drug released at the end of the assay, 24 h; whereas 90% of the drug was released after 24 h in media FaSSGF (pH 1.2). These differences could be due to the increase of the pH and the different ions and enzymes that contains the media. In both media, tablets provided a controlled release of the drug throughout the assay, being the drug release slightly higher at initial sampling times.

Conclusions: In this work we have shown that PAMgA hydrogel is a good excipient for sustained drug delivery devices for oral administration, because of its good compresibility and its capability to control the release of a highly hidrosoluble drugs in contact to aqueous media.

References:

[1] Rubio-Retama J, Tamimi FM, Heinrich M, López-Cabarcos E. Langmuir **23** (2007) 8538.
[2] Cheddadi M, López-Cabarcos E, Slowing K, Barcia E, Fernández-Carballido A. Int J Pharm **413** (2011) 126.
[3] Jantratid E, Janssen N, Reppas, Dressman JB. Pharm Res **25** (2008) 1663.

BMP-2, cells and microfracture to repair a chondral lesion

Raquel Vayas[1], Ricardo Reyes[2], María Rodríguez-Évora[2], Carlos del Rosario[2], Carmen Evora[2], Araceli Delgado[2]

[1]Traumatology Service, University Hospital Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain.
[2]Department of Chemical Engineering and Pharmaceutical Technology, Faculty of Pharmacy, University of La Laguna, Spain.
e-mail: adelgado@ull.es

Introduction: Focal cartilage defects are a common cause of knee symptoms and disability and may progress to osteoarthritis, a degenerative joint disease. Peculiarly, the cartilage is avascular, nutrient and oxygen supply being constrained to diffusion so that it does not heal spontaneously. The limits of the microfracture (MFX) treatment as well as the high cost and the need for two operations of the autologous chondrocyte implantation (ACI) and ACI-related procedures have encouraged the search for alternative strategies utilizing single-step procedure. The aim of the present study was to evaluate the treatment with two doses (3-12 μg) of bone morphogenetic protein 2 (BMP-2), a chondrogenic growth factor, with bone marrow mesenchymal stem cells (MSC) and with microfracture (MFX), as well as its combinations in a focal knee cartilage defect model in rabbits. An injectable formulation of microspheres in Pluronic® F-127 was used as delivery system for BMP-2 encapsulation as well as for cell scaffolds.

Materials and Methods: The microspheres of PLGA (Resomer® 504, Evonik) containing BMP-2 (Noricum, Spain) were prepared by a double emulsion (water/oil/water) process. Batches for encapsulation yield and GF release assay were prepared with ^{125}I-BMP-2. The formulations were prepared individually by dispersing 7 ± 0.5 mg of microspheres containing 3 or 12 μg of BMP-2 in 300 μL of 1.5% Pluronic® F-127 in saline. This procedure was performed in a 1 mL syringe. The formulation was incubated at 4 ºC for 90 min before injection. To prepare the pre-seeded formulations, 20 μL of a cell suspension in PBS (1.25×10^7 cells/mL) were added to the microsphere suspension and incubated at 37 °C under orbital shaking (100 rpm) for 90 min before administration. The chondral defect was performed on mature (6 months old) male New Zealand rabbits (3-4 Kg), anaesthetized intramuscularly with ketamine (35 mg/Kg) and xylazine (5 mg/Kg). MSC were isolated from another individual from the same litter as the animals to be implanted. The cells were expanded at ≈ 80% of confluence and were frozen for later experimental use. Seven experimental groups were considered for histological evaluation. The defect of 4 femurs per experimental group and time point (12 and 24 weeks) were prepared for histological evaluation. The histological findings were scored by 2 independent evaluators, using an established scoring system of 6 parameters for osteochondral repair, as described by Wakitani *et al.* [1] and modified by Tokuhara *et al.* [2]. For immunohistochemistry purpose, sections were immunolabeled with anti-Col II and anti-Col X polyclonal antisera and with an anti-aggrecan monoclonal antibody (Millipore, Spain).

Results and Discussion: The mean volume diameter of the microspheres was 55 μm (80% in the range of 31-81 μm) and BMP-2 encapsulation efficiency was 70.5 ± 6.8%. The formulation was easily injected and the administered effective dose of BMP-2 was 70 ± 10% of the loaded dose. Most pre-seeded cells were viable after incubation, just before injection. ≈ 75% of the BMP-2 *in vivo* was released in the first 48 h and 90% was delivered after 6 days. As MFX is the most popular technique for repairing cartilage injury, groups of animals treated with MFX, MSC and BMP-2, and groups treated with the combination of BMP-2 and MFX and BMP-2 together with MSC were included, to compare responses. After 24 weeks, the response of all the groups was statistically similar, except the response of control group and the group treated with MFX. The group treated with MFX reaches 40-45% of the maximum score while the response of the groups implanted with MSC, BMP-2 and BMP-2 combined with MSC or MFX were in the range of 67-79%. MSC and BMP-2 induced the same level of cartilage repair. However, bearing in mind that cells constitute the component that typically introduces greater variability in the system response [3], together with the need for cell isolation and expansion before implantation, their clinical application might be limited against the growth factor formulated in a controlled release system.

Conclusions: Our results support the use of BMP-2 and MSC for repairing a chondral injury, but did not justify the use of the proposed combinations. The use of high doses of BMP-2 is not justified because the induced cartilage repair with doses of 3 and 12 μg were statistically similar.

Acknowledgements: Financial support from Ministerio de Economía y Competitividad, Spain, under Project MAT201455657-R is gratefully acknowledged.

References:

[1] Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, Goldberg VM. J Bone Joint Surg Am **76** (1994**)** 579.
[2] Tokuhara Y, Wakitani S, Imai Y, Kawaguchi A, Fukunaga K, Kim M, Kadoya Y, Takaoka K. Int Orthop **34** (2010**)** 761.
[3] Martin I, Miot S, Barbero A, Jakob M, Wendt D. J. Biomechanics **40** (2007) 750.

Development of synthetic exosomes for cancer therapy

Abi J. Vázquez-Ríos[1], Belén López-Bouzo[1,2], S. Grijalvo[3], Carmen Abuín[1], A. Avinyo[3], R. Eritja[3], Rafael López-López[1], María de la Fuente[1]

[1]Nano-oncologicals Lab, Translational Medical Oncology Group, Health Research Institute of Santiago de Compostela (IDIS), SERGAS, Santiago de Compostela, Spain.
[2]Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), Santiago de Compostela, Spain.
[3]Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), CIBER-BBN Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Barcelona, Spain.
e-mail: abivazquezrios@hotmail.com

Introduction: Exosomes are extracellular vesicles (40-100 nm) secreted by various cell types. Given their role in cell communication, carrying genetic material and proteins selectively, exosomes have many of the characteristics of an ideal drug delivery system. However, cells release relatively low amounts of exosomes and purification is cumbersome [1]. Furthermore, their use in therapy requires much deeper knowledge of its composition and function to ensure the safety of this approach. That is why by studying the composition and characteristics of natural exosomes and making use of nanotechnology, we want to develop and characterize liposomes (synthetic exosomes) that simulate natural exosomes, structurally and functionally, with a controlled composition and a higher yield of production, with the purpose of selectively targeting associated therapeutics to tumor cells.

Materials and Methods: Exosomes were isolated from tumor cells and plasma by serial differential ultracentrifugation [2], and characterized by transmission electron microscope (TEM), dynamic light scattering (DLS), Western blot (WB), and proteomic analysis. Synthetic exosomes were formed by the injection of ethanol in water technique [3], loaded with different cargos (nucleic acids, drugs and fluorophores) and characterized by their size (DLS), superficial charge (laser doppler anemometry, LDA) and morphology (TEM). Cell uptake studies of both exosomes and synthetic exosomes were performed in different cancer cell lines by means of confocal laser-scanning microscope (CLSM) after 4 h of incubation. The cytotoxicity of exosomes and synthetic exosomes was assessed by the MTT assay. A model protein was associated into the lipid vesicles.

Results and Discussion: size analysis showed that isolated exosomes from human plasma and cancer cell lines (SW480 and PC-3) had a mean particle diameter ranged from 80 to 100 nm, a slight negative surface charge and a narrow size distribution. Their analysis by TEM revealed the cup-shape characteristic morphology of exosomes and confirmed their nanometric size. The presence of exosomal protein markers, e.g., CD9, CD81 and Alix, was confirmed by Western blot and by proteomic analysis. Proteins involved in cell adhesion, cell communication and transport of the exosomes were also identified in our exosomes. Liposomes were formed by lipid compounds characteristics of exosomes, i.e. PC:CH:SM:Cer in a ratio based on a thorough lipidomic assay of exosomes isolated from prostate cancer cells [4]. Conditions were carefully adjusted to obtain liposomes with similar particle size and zeta potential (mean particle size = 100 ± 8 nm; PdI = 0.2; ζ potential = -7 ± 2 mV), and morphology to natural exosomes. To demonstrate their potential in drug delivery, exosomes and synthetic exosomes were both loaded with different therapeutic cargoes (hydrophobically modified nucleic acids and curcumine) and their physicochemical properties were determined. In all cases, and irrespective of the type of molecule, synthetic exosomes showed higher encapsulation efficiencies. Preliminary results show that it is also possible to functionalize them with proteins that have been previously identified in exosomes by proteomics. With respect to the evaluation of their properties in cell culture, synthetic exosomes showed not toxicity at the concentrations tested in MTT assays. Uptake studies revealed that cancer cells internalized efficiently both types of exosomes (natural and synthetic ones). However, according to our results, synthetic exosomes seems to be more efficient in transporting their cargo to tumor cells.

Conclusions: We have successfully achieved the preparation of liposomes with a similar lipidic composition, physicochemical properties and morphology to natural exosomes. These synthetic exosomes demonstrated to be effective for the association of exogenous nucleic acids and hydrophobic drugs, and are able to interact with tumour cells more efficiently than natural exosomes without causing toxicity. Next experiments will be aimed to incorporate exosomal proteins involved in cell communication, adhesion and specific targeting for an enhanced targeted delivery of antitumor drugs.

Acknowledgements: Financial support given by the Carlos III Health Institute-ISCIII (CP12/03150).

References:

[1] Théry C, Zitvogel L, Amigorena S. Nat Rev Immunol **2** (2002) 569.
[2] Vlassov AV, Magdaleno S, Setterquist R, Conrad R. Biochim Biophys Acta **1820** (2012) 940.
[3] Laouini A, Jaafar-Maalej C, Limayem-Blouza I, Sfar S, Charcosset C, Fessi H. J Colloid Sci Biotechnol **1** (2012) 147.
[4] Llorente A, Skotland T, Sylvänne T, Kauhanen D, Róg T, Orłowski A, Vattulainen I, Ekroos K, Sandvig K. Biochim. Biophys. Acta **1831** (2013) 1302.

Release assay of cannabidiol from polymeric microspheres: sampling and separation method

María Isabel Morales Calero, Ana Isabel Fraguas Sánchez, Ana Isabel Torres Suarez

Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, Complutense University of Madrid, Spain.
e-mail: mamora09@ucm.es

Introduction: *In vitro* release assays are an important tool in developing new modified-release dosage forms and in their quality control. The different characteristics of the dosage forms and route of administration will determine the methodology of the assay. While the release assays of drugs formulated into immediate dosage forms are well described in pharmacopoeias, in case of extended-release formulations, mainly for parenteral administration, little or no information about the conditions of the release assay can be found in official compendia. Currently, the more used methods to study drug release from parenteral dosage forms are sampling and separation, continuous flow method and dialysis method [1]. The main aim of this work is the study of the release of an insoluble and unstable drug, cannabidiol (CBD, a natural cannabinoid currently used in the treatment of pain in cancer and of spasticity in multiple sclerosis), formulated in biodegradable polymeric microspheres for parenteral administration, by using the sampling and separation method.

Materials and Methods: CBD is highly lipophilic and unstable in aqueous medium. Poly(ε-caprolactone) (PCL) is the polymer used to prepare the microspheres loaded with CBD, by following the emulsion and solvent evaporation method [2]. The microspheres were characterized in terms of size distribution (by laser diffraction technique), shape and state of aggregation (by scanning electron microscopy, SEM), and CBD loading and encapsulation efficiency (by HPLC). *In vitro* release of CBD from microspheres was determined by the sampling and separation method, using two different media: PBS, commonly used for these *in vitro* assays, and simulated body fluid (SBF) [3], trying to represent the conditions that occur *in vivo*. Polysorbate 80 (0.5%, w/v) was added to each medium in order to maintain sink conditions. Samples were incubated in a water bath at 37 ºC with shake at 100 rpm for 20 days. At time intervals, the complete release medium was withdrawn and replaced by fresh one. In the removed medium, CBD concentration was determined by HPLC. CBD inside the microspheres was also determined at the same sampling times. To that aim, an HPLC analytical procedure was previously validated by our group [4].

Results and Discussion: The yield of the microencapsulation process was 73.66%. Non-aggregated spherical microspheres were obtained, with an average size of 46 µm and span of 1.58; that allows their parenteral administration with a 25 gauge needle. The encapsulation efficiency calculated, higher than 100%, indicates a high encapsulation of the drug and losses of polymer during microencapsulation process. When PBS was used as release medium, CBD was released slightly faster than when SBF was used. By SEM, higher erosion of the microspheres was observed in PBS than in SBF. In the case of biodegradable microspheres, drug release is the consequence of diffusion together erosion processes, in such a way that the higher erosion could explain the faster release of CBD in PBS. On the other hand, the microspheres formed deflocculated sediments in PBS whereas the formation of flocculated sediment was observed in SBF. With both media, the burst effect was similar. After burst release, there is a prolonged release of CBD for 7 days in PBS and for 10 days in simulated body fluid. The amount of CBD inside the microspheres after 20 days of assay was lower than 1%.

Conclusions: *In vitro* release assay by sampling and separation method requires simpler equipment, although sample preparation may be quite complex, especially in the case of poorly soluble and unstable drugs. The composition of the release medium can modify the results of the assay because of the different behavior of the suspension of microspheres as a consequence of the agitation system, and the different erosion rate of the polymer.

References:

[1] D'Souza SS, DeLuca PP. Pharm Res **23** (2006) 460.
[2] Hernán Pérez de la Ossa D, Ligresti A, Gil-Alegre ME, Aberturas MR, Molpeceres J, Di Marzo V, Torres Suárez AI. J Control Release **161** (2012) 927.
[3] Simon A, de Almeida Borges VR, Cabral LM, de Sousa VP. AAPS PharmSciTech **14** (2013) 425.
[4] Martín-Sabroso C, Tavares-Fernandes DF, Espada-García JI, Torres-Suárez AI. Int J Pharm **458** (2013) 188.

Inhibition of aminopeptidase activity of human osteoblasts by NSAIDs

G. Lucena[1], C. Reyes-Botella[2], O. García-Martínez[3], J. Ramos-Torrecillas[3], E. De Luna-Bertos[3], C. Ruiz[3]

[1]Biobanco. Sistema Sanitario Público de Andalucía, Junta de Andalucía, Granada, Spain.
[2]Biomedical Group (BIO277). Department of Stomatology, School of Dentistry, University of Granada, and Instituto de Investigación Biosanitaria (ibs.Granada), Spain.
[3]Biomedical Group (BIO277). Department of Nursing, Faculty of Health Sciences. University of Granada, and Instituto de Investigación Biosanitaria (ibs.Granada), Spain.
e-mail: elviradlb@ugr.es

Introduction: Aminopeptidases (APs) are important for the physiology of prokaryotic and eukaryotic cells but they are often involved in a lot of pathological processes. In tumor tissues it is detected an overexpression of APs, hence their inhibitors have been proposed as an attractive toolin combination with chemotherapy [1]. Furthermore, cyclooxygenase-2 (COX-2) and their metabolites appear to be involved in the increased expression of theses enzymes [2]. Human osteoblasts in culture have APs associated with cell membranes. The objective of the study has been to identify the effect of two nonsteroidal anti-inflammatory drugs (NSAIDs), meloxicam and piroxicam, on the AP activity in human osteoblasts in culture.

Material and Methods: Human osteoblasts were obtained by culture of trabecular bone sections from healthy volunteers. The cells were identified by their morphological and biochemical characteristics, and antigenic profile. Cultured human osteoblastswere treated with concentrations ranging from 1 and 500 μM of meloxicam and piroxicam, for 24 h and untreated cells were used as a control group. AP activity was measured fluorometrically using, as substrates, the aminoacyl-*β*-naphthylamides (aaNAs): AlaNA, LeuNA, ArgNA, PheNA, LysNA, GlyNA, and MetNA. Enzymatic activity was expressed as pmol of aaNA hydrolyzed per min per 10^4 cells/mL. Results are expressed as percent of activity compared to control group. Statistical significance was evaluated by ANOVA test.

Results and Discussion: Meloxicam and piroxicam inhibit AP activity against different substrates studied, with an IC_{50} of between 70 and 75 micromolar. This effect has clinical interest because some studies have shown that inhibition of aminopeptidase activity inhibits the angiogenesis and prevents invasiveness of cancer cells, reducing the incidence of tumor metastasis and stoping secondary tumor formation [3]. De Monte et al. showed that meloxicam and lornoxicam, NSAIDs belonging to the class of “oxicams”, inhibit a isoforms of human carbonic anhydrase (CA) at low micromolar concentrations [4]. The multiple pharmacological effects of the sulfonamide anti-inflammatory agents could be ascribed to the dual inhibition of CA and COX enzymes, supporting the evidence that inflammation and hypoxia pathways are involved in cancer onset and progression. Perhaps these are not the only mechanism of action of these drugs.

Conclusion: Meloxicam and piroxicam significantly inhibit APs and since these are directly involved in tumor process would be interesting to look for suitable dosage forms to be used for that purpose for combination with chemotherapy.

References

[1] Piedfer M, Dauzonne D, Tang R, N’Guyen J, Billard C, Bauvois B. FASEB J **25** (2011) 2831.
[2] Guan PP, Guo JW, Yu X, Wang Y, Wang T, Konstantopoulos K, Wang ZY, Wang P. Sci Rep **5** (2015) 10412.
[3] Hitzerd SM, Verbrugge SE, Ossenkoppele G, Jansen G, Peters GJ. Amino Acids **46** (2014) 793.
[4] De Monte C, Carradori S, Gentili A, Mollica A, Trisciuoglio D, Supuran CT. Curr Med Chem **22** (2015) 2812.

Development of Donepezil-loaded polymeric nanoparticles of poly(ε-caprolactone) for the treatment of Alzheimer´s disease

Amanda Cano[1], Marcelle Silva de Abreu[1], Mª Antonia Egea[1], Marta Espina[1], Ana Calpena[2], Mª Luisa García[1]

[1]Department of Physical Chemistry, Faculty of Pharmacy, University of Barcelona, Spain.
[2]Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Barcelona, Spain.
e-mail: amanda90cf@gmail.com

Introduction: Alzheimer´s disease (AD) is the most common neurodegenerative disorder, which accounts for 60-70% of dementias [1]. It is characterized by loss of short term memory and other mental abilities, according as neurons die and different areas of the brain atrophy. The etiology of AD is complex and largely unknown [1]. One of the drugs most used in the treatment of this disease is Donepezil, a reversible inhibitor of acetylcholinesterase, recently considered effective in severe AD [2]. This drug is capable of producing improvement in cognitive function and overall clinical status [3]. To enhance drug bioavailability centrally and decrease its peripheral side effects a nanostructured controlled drug delivery system was designed, able to cross the blood-brain barrier [4]. This work describes the development, optimization and characterization studies of Donepezil polymeric nanoparticles of poli(ε-aprolactone) (PCL) for the treatment of AD.

Materials and Methods: Donepezil HCl was obtained from Nutragreenlife Biotechnology Co. Ltd. (Hong Kong, China), PCL, polyvinyl alcohol (PVA) and Tween® 80 were obtained from Sigma-Aldrich (Japan). Instruments used were Z-Sizer nanoseries Malvern Instruments Ltd. (UK), Transmission electron microscope JEOL 1010, Turbiscan® Lab expert model, UPLC Acquity™ Waters 2695 Alliance. Polymeric nanoparticles were prepared by solvent displacement method [5], using mixtures of PVA and Tween® 80 as surfactant. Optimization of the samples was made by a 2^3 factorial design. Particle morphology was analyzed by transmission electron microscopy. Encapsulation of the drug was determined by ULPC method, as well as a validation of the technique. The analysis of the interactions between the drug and the polymer was performed by spectroscopic methods. Stability of the selected formulation was also evaluated.

Results: Selected formulation show a particle size lower than 200 nm, suitable for crossing the blood-brain barrier. Zeta potential values were of the order of -14 mV, improving stability of the sample by repulsion of the particles. Transmission electron microscopy studies showed spherical particle morphology. Encapsulation value was ≈ 75%. Validation of the UPLC method proved the linearity, precision and accuracy of the technique. No covalent bonds between the drug and the polymer were observed. Short term stability studies showed no significant changes in the sample in the backscattering profile over time.

Conclusion: In this work we have developed Donepezil-loaded polymeric nanoparticles of PCL intended for the treatment of AD. These particles have suitable morphometric characteristics to cross through the blood-brain barrier, improving the effectiveness and reducing the side effects of this drug. Additional studies are needed to further develop this formulation and to determine their physicochemical and pharmaceutical characteristics, as well as *in vitro* and *in vivo* studies to know its effectiveness.

Acknowledgements: Financial support from Generalitat de Cataluña, Spain, under Project MAT2014-59134-R is gratefully acknowledged.

References:

[1] Xu PX, Wang SW, Yu XL, Su YJ, Wang T, Zhou WW, Zhang H, Wang YJ, Liu RT. Behav Brain Res **264** (2014) 173.
[2] Yamagata B, Watanabe T, Tomioka H, Kobayashi H, Nakano Y, Mimura M. Regul Pept **163** (2010) 137.
[3] Gandía L, Alvarez RM, Hernández-Guijo JM, González-Rubio JM, de Pascual R, Rojo J, Tapia L. Anticolinesterásicos en el tratamiento de la enfermedad de Alzheimer. Rev Neurol **42** (2006) 471.
[4] Sahni JK, Doggui S, Ali J, Baboota S, Dao L, Ramassamy C. J Control Release **152** (2011) 208.
[5] Lboutounne H, Chaulet JF, Ploton C, Falson F, Pirot F. J Control Release **82** (2002) 319.

Novel genipin-cross-linked double poly-L-lysine membranes provide highly resistant capsules for imaging in the far-red fluorescence range

Edorta Santos[1,2], María Virumbrales-Muñoz[2,3], Iñaki Ochoa[2,3], Gorka Orive[1,2], Rosa María Hernández[1,2], Luis Fernández[2,3], José Luis Pedraz[1,2]

[1]NanoBioCel Group, Laboratory of Pharmaceutics, School of Pharmacy, University of the Basque Country (UPV/EHU), Spain.
[2]Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
[3]Group of Structural Mechanics and Materials Modelling (GEMM), University of Zaragoza, Zaragoza, Spain.
e-mail: joseluis.pedraz@ehu.eus

Introduction: The immobilization of therapeutic cells within polymeric matrices coated with semi-permeable membranes have demonstrated good preclinical results in the treatment of diverse pathologies so far [1]. Alginate-poly-L-lysine-alginate (APA) system still remains the design of choice for most scientists in this field. However, among the main limitations this capsule type presents, there are their poor mechanical properties and the impossibility to visualize the localization of the capsules once implanted in the body [2]. In practice, such features lead to few biosafety guarantees that must be solved before the definitive leap to clinic. Thus, the aim of this work was to produce highly resistant capsules capable of bearing strong pressures, at the same time that they can be monitored *in vivo* by means of non-invasive tools. For that purpose, we proposed a novel capsule design based on a genipin-cross-linked double poly-L-lysine (GDP) membrane. Genipin is a naturally occurring protein cross-linker known for showing several advantages over the use of other cross-linkers [3]. In addition, cross-linked genipin emits fluorescence in the far-red range when excited at 590 nm, which may be harnessed to track the position of implanted capsules *in vivo*. In the present study, we tested different genipin concentrations and exposure times in order to find the most suitable combination rendering the maximum fluorescence intensity while preserving cell integrity and function. Additionally, we studied the reaction kinetics and the effect of this novel formulation on the mechanical properties of the capsule.

Materials and Methods: Microcapsule obtaining was carried out by using an electrostatic droplet generator with slight modifications of the procedure designed by Lim and Sun [4]. Briefly, 1.5% alginate suspension was extruded through a 0.35 mm needle at a 5.9 mL/h flow rate. Once beads were formed they were collected in a gelling solution. Beads were then washed and coated according to the desired capsule design as follows: AP → classical alginate-poly-L-lysine capsules; APG → alginate-poly-L-lysine capsules cross-linked with genipin; APGP → APG capsules with a second coating of poly-L-lysine (PLL); GDP → APGP capsules subjected to a second cross-linking reaction. The typical second 0.1% alginate coating was omitted to explore more thoroughly the effect of PLL and genipin. In order to study the impact of the different coatings, D1-MSCs genetically modified to release EPO were immobilized at a cell density of 5×10^6 cells/mL. In order to assess the viability of the immobilized cells calcein/ethidium assay, flow cytometry and CCK8 assay were performed. In order to evaluate the EPO production, an ELISA assay was carried out. Mechanical properties were assessed by means of the explosion assay, compression assay in a chip and atomic force microscopy (AFM). Fluorescence (FL) intensity and reaction kinetics were studied by using confocal microscopy and a FL plate reader. Data are presented as mean ± SD. In order to detect significant differences between different groups, one way ANOVA test was performed. All statistical computations were performed using SPSS 29 (SPSS, Chicago, IL).

Results and Discussion: Obtained results showed that 0.1% genipin concentration and 5 min exposure time produced the maximum FL signal, and thus cross-linking rate, while cell viability remained unaltered. The double cross-linked coating did not affect either cell viability or cell function, giving results comparable to those of the AP capsules. In contrast, the double membrane provided the capsules with remarkably superior mechanical properties as evidenced by swelling, compression and AFM assays. The cross-linking of the second coating in GDP capsules produced a 6-fold increase in FL signal when comparing to APG and APGP groups, probably due to the availability of more PLL for the amino cross-linking. This is crucial to serve as a good quality monitoring tool with a proper signal/noise ratio *in vivo*. On the other hand, obtained data revealed that the cross-linking reaction took about 72 h to fully complete, obeying a first order reaction pattern. Whereas the double genipin coating increased the final FL signal, the double PLL coating gave as a result a more rapid reaction comparing to only one PLL coating.

Conclusions: The present approach provides meaningful results showing that GPD capsules present notably increased mechanical properties, while produce a strong and optimized FL signal for *in vivo* imaging. Altogether, these data suggest that GDP capsules may be a suitable candidate to replace classical APA capsules in a near future.

References:

[1] Orive G, Santos E, Poncelet D, Hernández RM, Pedraz JL, Wahlberg LU, De Vos P, Emerich D. Trends Pharmacol Sci **36** (2015) 537.
[2] Santos E, Pedraz JL, Hernández RM, Orive G. J Control Release **170** (2013) 1.
[3] Wang C, Lau TT, Loh WL, Su K, Wang DA. J Biomed Mater Res B Appl Biomater **97** (2011) 58.
[4] Lim F, Sun AM. Science **210** (1980) 908.

Pharmacokinetic of GDNF from PLGA loaded microspheres after intravitreal injection in rabbits

Rocío Herrero-Vanrell[1,2], Cristina García-Caballero[1,2], Elena García[3,4], Luis E. Pablo[3,4], Alicia Arranz-Romera[1,2], Vanessa Andrés-Guerrero[2], Irene T. Molina-Martínez[1,2], Julián García-Feijoo[2,5], Vicente Polo[3,4], Irene Bravo-Osuna[1,2]

[1]Department of Pharmacy and Pharmaceutical Technology (Research Group UCM 920415), Faculty of Pharmacy, Complutense University of Madrid, Spain.
[2]Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Madrid, Spain.
[3]Servicio de Oftalmología, Hospital Universitario Miguel Servet de Zaragoza, Spain.
[4]Instituto de Investigación Sanitaria IIS – Aragón, Spain.
[5]Servicio de Oftalmología, Hospital Clínico San Carlos de Madrid, Spain.
e-mail: rociohv@farm.ucm.es

Introduction: Successful therapy of diseases affecting the back of the eye requires maintained concentrations of the active substance during a long period of time in the intraocular target site [1]. The sustained release of the Glial cell line derived neurotrophic factor (GDNF) from a novel formulation of Vit E-poly(D,L-lactide-*co*-glycolide) (PLGA) microspheres (MPs) has demonstrated to promote retinal ganglion cells (RGCs) survival in an animal model of glaucoma (unilateral elevation of IOP after episcleral injection of a hypertonic solution in rats) for at least 3 months [2]. Nevertheless, the pharmacokinetic of the neurotrophic factor after intravitreal injection (ITV) has not been studied. The objective of the present work was to evaluate the GDNF levels after a single ITV injection of GDNF/Vit E-PLGA MPs.

Materials and Methods: GDNF and the ELISA (enzyme-linked inmunosorbant assay) kit for GDNF quantification were purchased from R&D (Minneapolis, USA). PLGA 50:50 (Resomer® 503) was supplied by Boehringer Ingelheim (Pharma Co., Germany) and α-tocopherol acetate (Vit E) was obtained from Sigma-Aldrich (Schnelldorf, Germany). Adult female New Zealand white rabbits (2-3 Kg, SAEA, Zaragoza University, Spain) were used for the *in vivo* studies (n = 30). Animal experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic Vision Research. GDNF/Vit E-loaded PLGA MPs were prepared using a s/o/w emulsion-solvent evaporation technique. The microspheres were characterized in terms of morphology, particle size and particle size distribution, GDNF loading and *in vitro* release studies. Animals received a single ITV injection (50 µL) of GDNF Vit E-PLGA MPs (4%, w/v) (24 eyes), unloaded Vit E-PLGA MPs (4%, w/v) (24 eyes), GDNF solution (50 ng/mL) (3 eyes) and balanced salt solution (BSS) (7 eyes). At pre-set times (24 h, and 1, 4, 6, 12 and 24 weeks) the eyes were enucleated and the intravitreal levels of GDNF were quantified. All values were expressed as mean ± standard deviation (mean ± SD), except mean concentration values from vitreous samples that were graphically represented by using the standard error of the mean (mean ± SEM). Data were analyzed by using Statgraphics software (StatPoint Technologies, Inc., Warrenton, USA). A p-value < 0.05 was considered to be statistically significant.

Results and Discussion: Microspheres ranged from 20 to 40 µm in size and were spherical in shape with porous surfaces. The neurotrophic factor (74.74 ng GDNF/mg MPs) was released *in vitro* in a controlled fashion up to 24 weeks. Due to an eye response to the ITV injection, non-loaded PLGA MPs caused a small but significant initial increase of the endogenous levels of GDNF (10.24 ± 4.03 pg/mL) compared to non-injected eyes (3.69 ± 1.60 pg/mL). High intraocular GDNF levels were observed for the first month after injection (726.87 ± 66.42 pg/mL values at 4 weeks) in animals receiving GDNF/Vit E-PLGA MPs. After 2 months, constant levels of GDNF were observed until the end of the study (20.36 ± 7.60 pg/mL values at 6 months), and resulted significantly higher than the basal GDNF levels [2].

Conclusions: A single injection of the novel formulation of GDNF/Vit E-PLGA MPs provides significantly higher levels than the basal values of the neurotrophic factor in the vitreous cavity for at least 6 months.

Acknowledgements: Spanish Ministry of Economy and Competitiveness for financial support (MAT 2013-42127-R), RETICs 65288, UCM Research Group 920415 (GR3/14) and PI12/02285.

References:

[1] Herrero-Vanrell R, Bravo-Osuna I, Andrés-Guerrero V, Vicario-de-la-Torre M, Molina-Martínez IT. Prog Retin Eye Res **42** (2014) 27.
[2] Checa-Casalengua P, Jiang C, Bravo-Osuna I, Tucker BA, Molina-Martínez IT, Young MJ, Herrero-Vanrell R. J Control Release **156** (2011) 92.

Folic acid-PLGA-nanoparticles designed for CBD ovarian cancer cells targeting

A.I. Fraguas Sánchez[1], *A.I. Torres Suárez*[1,2], *A. Fernández Caballido*[1,2]

[1]Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, Complutense University of Madrid, Spain.
[2]University Institute of Industrial Pharmacy, Complutense University of Madrid, Spain.
e-mail: aifraguas@ucm.es

Introduction: In the last decades cannabidiol (CBD), the major non-psychoactive cannabinoid presents in *Cannabis sativa* plant, has emerged as a potential anticancer drug in several kinds of tumors, such as breast cancer, prostate cancer or gliomas [1]; given its ability to inhibit or reduce the proliferation, adhesion, migration and invasion of tumor cells and to induce apoptotic effects [2, 3]. Despite of its potential therapeutic interest, it is difficult to develop an effective formulation of CBD, due to its high liposolubility and its stability problems [4]. Nanotechnology may resolve these questions and also may increase its antitumor activity. The non-selective distribution of the drug limits the effectiveness of conventional anticancer treatments, increasing the toxicity and diminishing antitumor effectiveness. In this regard, the use of labeled nanoparticles (NPs) with specific ligands to cancer cells as carriers of chemotherapeutic agents may improve the drug selective distribution at tumor level, decreasing the adverse effects and enhancing the anticancer activity. One of the most effective ligands is folic acid, which plays an essential role in cell proliferation and whose receptors are overexpressed in numerous tumor cells [5], mainly in ovarian cancer. The aim of this work was to evaluate at first time the antiproliferative activity of CBD in ovarian cancer, using SKOV-3 cell line as model, to develop biodegradable NPs loaded with CBD and labeled with folic acid.

Materials and Methods: Cell proliferation studies were performed using SKOV-3 cell line as model of ovarian cancer. Cells were seed in 24-well-plates and treated after 24 h of seeding with CBD (2-25 μM) during 24 and 48 h. Then, cell viability was determined by MTT. The induction of apoptosis and the generation of reactive oxygen species (ROS) were also evaluated. Biodegradable NPs, made of poly(D,L-lactide-*co*-glycolide) Resomer® (PLGA-RG-502®), were obtained by nanoprecipitation technique. Folic acid was conjugated on the NP surface in the presence of EDC and NHS [6]. The amount of labeled folic acid was quantified by HPLC and obtained NPs were characterized in terms of morphology, particle size, zeta potential, drug loading and entrapment efficiency.

Results and Discussion: CBD exhibited an antiproliferative effect in ovarian cancer cells, with an inhibitory concentration 50 (IC_{50}) of 21.43 μM after 48 h of incubation. In this work it has been reported that the induction of apoptosis is one of the mechanisms responsible for antitumor activity of CBD in ovarian cancer cells. However, the generation of ROS was not observed, showing CBD an antioxidative effect. CBD-loaded NPs were spherical with a non-porous and uniform surface, and showed a mean particle size (expressed as volume diameter) below 200 nm, a surface zeta potential of -14.2 mV and a high drug loading and entrapment efficiency. Folic acid-labeled NPs also showed a particle size below 200 nm and a surface zeta potential of -1.33 mV.

Conclusions: Due to the antiproliferative activity of CBD in SKOV-3 cells, this cannabinoid may be an useful therapeutic tool in the treatment of ovarian cancer. The developed NPs showed a particle size below 200 nm and a high drug loading, and could be a good strategy to target selectively CBD to ovarian cancer cells and to increase its anticancer activity.

Acknowledgements: This work is part of the activity of the research group "Parenteral administration of drugs" (Ref 910933). The authors wish to thank the Ministry of Education of Spain the granting of an University Staff Training (FPU) fellowship to AI Fraguas (FPU14/06441).

References:

[1] Hermanson DJ, Marnett LJ. Cancer Metastasis Rev **30** (2011) 599.
[2] Chakravarti B, Ravi J, Ganju RK. Oncotarget **5** (2014) 5852.
[3] Massi P, Solinas M, Cinquina V, Parolaro D. Br J Clin Pharmacol **75** (2013) 303.
[4] Mechoulam R, Hanus L. Chem Phys Lipids **121** (2002) 35.
[5] Nicolas J, Mura S, Brambilla D, Mackiewicz N, Couvreur P. Chem Soc Rev **42** (2013) 1147.
[6] Grabrucker AM, Garner CC, Boeckers TM, Bondioli L, Ruozi B, Forni F, Vandelli MA, Tosi G. PLoS One **6** (2011) 17851.

Synthesis and characterization of cationic carbosilane dendrimers and study of their interaction with dexamethasone

A. Barrios-Gumiel[1], F.J. de la Mata-de la Mata[1], J. Sánchez-Nieves[1], J. Rodríguez-Villanueva[2], M. Guzmán-Navarro[2]

[1]Department of Organic and Inorganic Chemistry, University of Alcala; Networking Biomedical Research Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
[2]Department of Biomedical Sciences, U.D. Pharmacy & Pharmaceutical Technology, University of Alcala, Spain.
e-mail: manuel.guzman@uah.es

Introduction: Dendrimers belong to a new generation of nanosystems that have aroused great attention due to its high potential as a vehicle for drug delivery [1]. One of the most interesting attributes is the multivalency present on their surface allowing introduction of different functional groups on it. Cationic dendrimers are interesting for topical ocular application through a great interaction with corneal epithelium mucin (negatively charged in physiological conditions), leading to mucoadhesive nanosystems able to increase drugs residence time on the outer eye surface [2]. Dendrimers with peripheral hydroxyl groups has shown selective tropism for microglia cells when they are activated, for example when inflammatory process occurs at retinal level [3]. Dexamethasone-21-phosphate (Dxm-21-P) is a synthetic corticosteroid with anti-inflammatory and immunosuppressive properties. It is used topically for corneal and conjunctive allergic-inflammatory pathologies and by systemic and intraocular routes for ocular degenerative diseases affecting the posterior segment of the eye such as glaucoma or macular degeneration associated with age.

Materials and Methods: Second generation of carbosilane dendritic systems $G_2O_3(SNMe_2(CH_2CH_2OH)I)_{12}$ and $G_2O_3(SNMe_3I)_{12}$ were synthesized according to the methodology described in the literature [4]. Dxm-21-P, pharmacopoeia quality, was obtained from Sigma-Aldrich (Spain). The characterization and interaction studies of dendritic systems and Dxm-21-P were performed by ^{15}N-, ^{29}Si-, ^{1}H- and ^{13}C-NMR and diffusion optimized spectroscopy (DOSY) (Varian Instruments, Unity-300, Mercury-300, Gemini-200 and Bruker-400 models) and by fluorescence spectroscopy (Shimadzu RF 540). Particle size and zeta potential (ζ) were evaluated by dynamic light scattering (DLS, Malvern Zetasizer 2000). Cytotoxicity studies were accomplished in 2 culture cell lines (HeLa cells and J774 macrophages) by MTT assay.

Results and Discussion: Dendrimers decorated with ammonium functions, $G_2O_3(SNMe_2\ HCl)_{12}$, were synthesized starting with thiolene reactions of the vinil-terminated dendrimer ($G_2O_3V_{12}$) with $HS(CH_2)_2NMe_2$ HCL. In order to synthesized dendrimers with cationic NMe_3^+ and $[NMe_2(CH_2CH_2OH)]^+$ moieties, first we proceeded to neutralize $G_2O_3(SNMe_2\ HCl)_{12}$ derivatives with an excess of NaOH. By following addition of excess MeI or $HO(CH_2)_2I$, $G_2O_3(SNMe_3I)_{12}$ (4222,20 Da) and $G_2O_3(SNMe_2(CH_2CH_2OH)I)_{12}$ (4589,79 Da) were obtained. Hydrodynamic diameters obtained by DLS were 82.97 ± 2.57 μm and 57.58 ± 10.89 μm for $G_2O_3(SNMe_2(CH_2CH_2OH)I)_{12}$ and $G_2O_3(SNMe_3I)_{12}$) respectively, suggesting the formation of molecular aggregates [5]. Their ζ value was close to -25 mV and decreased by adding Dxm-21-P to the aqueous dendrimer´s dispersion. The DOSY-NMR assays with $G_2O_3(SNMe_2(CH_2CH_2OH)I)_{12}$ and $G_2O_3(SNMe_3I)_{12}$ have shown a similar diffusion behaviour when they were put together with Dxm-21-P solutions, demonstrating a saturable interaction between dendrimer and drug at 1:1 molar ratio. Furthermore, these 2 dendrimers exhibited fluorescent properties, allowing analytical quantification and Dxm-21-P-dendrimer interaction evaluation. $G_2O_3(SNMe_3I)_{12}$ cytotoxicity is lower than produced by $G_2O_3(SNMe_2(CH_2CH_2OH)I)_{12}$, being cell viability > 90% for all tested concentrations. However, $G_2O_3(SNMe_2(CH_2CH_2OH)I)_{12}$, dendrimers with terminal hydroxyl groups were non-viable at or above 5 μM concentrations (viability < 25%).

Conclusions: Based on the results obtained by DOSY-RMN, ζ values and fluorescence quantum efficiency assays, it can be concluded that an electrostatic interaction between dendrimers and Dxm-21-P take place. These nanosystems may have therapeutic applications as drug carriers for topical ocular formulations, allowing a sustained drug release that it is not achieved by conventional aqueous formulations (eye drops).

References:

[1] Wu LP, Ficker M, Christensen JB, Trohopoulos PN, Moghimi SM. Bioconjugate Chem **26** (2015) 1198.
[2] Bravo-Osuna I, Noiray M, Briand E, Woodward P, Molina-Martinez IT, Herrero-Vanrell R, Ponchel G. Pharm Res **29** (2012) 2329.
[3] Iezzi R, Guru BR, Glybina IV, Mishra MK, Kennedy A, Kannan RM. Biomaterials **33** (2012) 979.
[4] Fuentes-Paniagua E, Hernández-Ros JM, Sánchez-Nieves J, Solivery J, Gómez R, De la Mata FJ. RSC Adv **4** (2014) 1256.
[5] Souza J, Dias K, Silva S, Rezende L, Rocha M, Emery F, Lopez R. J Control Release **200** (2015) 115.

Hyaluronic acid films for acetazolamide-cyclodextrin complexes

Javier A. Calles[1,2,3], *María Julia Mora*[4], *Gladys Granero*[4], *Marcela Longui*[4], *Yolanda Diebold*[1], *Enrique M. Vallés*[1], *Santiago D. Palma*[4]

[1]Institute of Applied Ophthalmo-Biology, University of Valladolid, Spain.
[2]Pilot Plant of Chemical Engineering, Universidad Nacional del Sur, Argentina.
[3]Department of Biology, Biochemistry and Pharmacy, Universidad Nacional del Sur, Argentina.
[4]Department of Pharmacy, Universidad Nacional de Córdoba, Argentina.
e-mail: jcalles@ioba.med.uva.es

Introduction: Acetazolamide (ACZ) is an effective carbonic anhydrase reversible inhibitor. Currently, it is orally administered for glaucoma treatment because of its capacity of decreasing intraocular pressure (IOP). However, it is necessary to administer large oral doses to achieve desired IOP reduction. Thus, a large number of side effects related to its systemic distribution like diuresis and systemic acidosis are usually generated in patients [1]. Some of these unwanted systemic side effects could be avoided by administering ACZ topically in the eye. However, its low aqueous solubility and low corneal permeability limit intraocular bioavailability. The aim of this work was the design and characterization of crosslinked hyaluronic acid (HA) films loaded with ACZ-hydroxypropyl-β-cyclodextrin (HPβCD)-triethanolamine (TEA) complexes for topical ocular administration.

Materials and Methods: The inclusion complexes were obtained by dissolving ACZ, HPβCD and TEA, to achieve 1:1:1 molar ratios, in Milli® Q water. The solutions were sonicated, filtered, frozen and then lyophilized. HA crosslinked films were synthesized from HA/IT/PEGDE solutions using Milli® Q water as solvent. The amount of each reagent was adjusted to achieve 1:1:2 molar ratios and 2% (w/w) HA concentration. The crosslinking reaction proceeded for 24 h under mild stirring at room temperature (RT) following previously described method [2]. Crosslinked hydrogels were cast at RT under an extraction hood in 7.0 cm diameter Petri dishes. ACZ-HPβCD-TEA complexes were incorporated within crosslinking reaction. ACZ final concentration was 0.3% w/w. Physicochemical properties were determined by swelling, stress-strain, Fourier transform infrared spectrometry, X-ray, X-ray fluorescence and scanning electron microscopy (SEM). Biopharmaceutical performance was studied by *in vitro* biocompatibility with human corneal epithelial cells (HCE) and *in vitro* drug release studies. Swelling was determined by immersion in distilled water at RT. Swelling ratio was calculated as the increase in weight or diameter of immersed films. Stress-strain properties were studied in 4×1 cm rectangular samples using an Instron 3369 tester in traction mode at 2 mm/min at RT. X-ray diffraction and X-ray fluorescence spectrometry analyses were performed in an X-ray diffractometer Philips PW1710 and Philips MagiX spectrometer, respectively. FTIR analysis was performed in a Nicolet SXC FTIR spectrometer. SEM images were obtained in a Leo EVO-40XVP microscope. Samples were frozen in liquid nitrogen and manually fractured. The HCE cell line was used to determine whether or not loaded films were biocompatible after 24 h exposure in terms of cell viability and proliferation by XTT and Alamar Blue tests, respectively. Drug delivery was studied in a dissolution dispositive [3], and analyzed by HPLC.

Results and Discussion: Experimental findings showed good mechanical properties for ACZ-loaded crosslinked films. Swelling studies showed stable behavior of immersed samples, maintaining size along experiment. Crosslinking process did not alter HA ductility. Crosslinked loaded and unloaded films showed plastic deformation as well as unmodified HA films. Morphological study by SEM revealed the presence of dispersed pores in cryofractured surfaces. FTIR analysis of films revealed C=O (1680 cm^{-1}) and C=N (1553 cm^{-1}) bands from ACZ; suggesting that ACZ is not complexed. X-ray diffractograms showed no differences between loaded and unloaded films. However, X-ray fluorescence showed sulfur signal increased in 0.33% for loaded films. Proliferation rates and viability of HCE cell line were not affected after 24 h exposure to ACZ loaded systems. *In vitro* drug delivery profiles showed a fast and extensive release of ACZ from films. This limited drug retention could be attributed to a decomplexation between ACZ and CDs produced within the long crosslinking process.

Conclusions: In this work we have shown the capability to formulate ACZ into a topical solid dosage form, avoiding solubility issues and bringing the possibility of overcome side systemic effects related to oral administration. Despite limited drug retention, probably due to a decomplexation of ACZ, CDs seems to contribute to ACZ incorporation in films. Further studies are guaranteed.

Acknowledgements: Financial support from UNS, UNC, CONICET, FEDER-CICYT MAT2013-47501-C02-1-R (Ministry of Economy and Competitiveness, Spain).

References:

[1] Kaur IP, Smitha R, Aggarwal D, Kapil M. Int J Pharm **248** (2002) 1.
[2] Calles JA, Tártara I, López-García A, Diebold Y, Palma SD, Vallés EM. Int J Pharm **455** (2013) 48.
[3] Calles JA, Palma SD, Vallés EM. INPI Expedient: 20130100220 (2013) Argentina.

Formulation and characterization of Eudragit®/Ciprofloxacin microparticles for colon delivery

Begoña Muñoz Ortega, Mª Teresa Marin Boscá

Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Granada, Spain.
e-mail: bego2515@gmail.com

Introduction: Delivery systems controlling drug release only in the colon holds great promises since they improve the utilization of drugs and decrease the dosing times compared with conventional forms. The two major types of the so-called inflammatory bowel disease (IBD), Crohn´s disease (CD) and ulcerative colitis (UC), are characterized by the chronic inflammation of the gastrointestinal tract (both the large and small bowel). The distinction between CD and UC is based largely on the distribution of inflammation. Reducing the size of drug delivery systems to the micro- or nano-meter scale might increase the colonic residence time. A size-dependent accumulation of microparticles (MPs) and nanoparticles (NPs) can be observed specifically in the inflamed intestinal regions. Ciprofloxacin (CF) was selected because of its extensive coverage for intestinal flora, relatively favorable side-effect profile and preliminary data suggesting its efficacy in the treatment of active CD [1]. The aim of the present study was to prepare polymeric MPs using different acrylic compounds, namely Eudragit® [2]. Eudragit® RL (ERL), Eudragit® RS (ERS), and a mixture of both 1:1 were used. Spray-drying [3] was selected as preparation method of CF/Eudragit® MPs. Our purpose was to design a CF delivery system via oral route for the treatment of inflammatory bowel disease and compare the differences on particle size, drug content and drug delivery profiles of the prepared MPs to select the best formulation.

Materials and Methods: ERS, ERL or a mixture of both was dissolved in ethanol absolute. Then CF was added and kept stirring for 2 h. The resultant solution was spray dried in a Mini Spray Dryer B-290 (Büchi). The solvent in the droplets created by atomization was evaporated inducing the formation of solid MPs from the drops under the conditions of: inlet air temperature 120 ºC, outlet air temperature 80 ºC and spray feed rate 5 mL/min. The dry product was then separated in a cyclone and settled down into a collector. The encapsulation efficiency (%) was determined dissolving 10 mg of MPs into 25 mL of pH 1.2 HCl büffer and kept under stirring overnight. Then several dilutions were made and analyzed spectrophotometrically at 276 nm for CF content, using a calibration curve based on standard solutions. Particle size was detected by Mastersizer 2000LF with a sample dispersion unit via minimum liquid volume Hydro 2000 mP (A). The yields of preparation were determined by weighing the product of spray-dried MPs with respect to the weight of the initial polymer and the drug used. The rate of drug release from the MPs was studied in phosphate buffered saline (PBS) dissolution medium and was determined in a dissolution apparatus using the USP Apparatus I in order to simulate the colon conditions [4]. A total of 35 mg of MPs were suspended in 1000 mL of PBS, pH 7.4, 37 ºC, at 50 rpm agitation rate. The samples were estimated spectrophotometrically at 276 nm by using a Perkin Elmer UV/Vis double beam spectrophotometer and cumulative percentage drug release was calculated.

Results and Discussion: For the 3 studied drug:polymer proportions (2:1, 1:1 and 1:2), MPs with a 1:1 and 1:2 proportions were smaller than those obtained with ratio 2:1. In general, as polymer content increased, the encapsulation efficiency slightly decreased. This can be due to the fact that ERL being more permeable than ERS due to the presence of more quaternary ammonium groups leading to insufficient encapsulation of drug in polymeric matrix. Drug release studies at pH 7.4 (PBS) showed that for the three drug:polymer proportion studied, the release of CF from the MPs with ratio 2:1 was poorly controlled. When the polymer amount increased in the MPs (1:1 and 1:2 ratios) the dissolution rates were slower, being the last those of the best dissolution behavior. In general, at the same drug:polymer proportion, the percent of drug release in 24 h was smaller when the formulation contained ERS, than those containing ERL or mixture of both.

Conclusions: From the results of the present study, it can be concluded that CF-loaded MPs prepared using ERL with a drug:polymer ratio 1:2, was the formulation with the best drug delivery profile, with an adequate particle size, for its sustained release in the colon for the treatment of IBD.

References:

[1] Hulten K, Almahharawi A, El-Zaatari FA, Graham DY. Dig Dis Sci **45** (2000) 445.
[2] Kim CK, Kim MJ, Oh KH. Int J Pharm **106** (1994) 213.
[3] Esposito E, Cervellati F, Menegatti E, Natruzzi C, Cortesi R. Int J Pharm **242** (2002) 329.
[4] Gupta VK, Gnanarajan G, Kothiyal P. Pharma Innov **1** (2012) 14.

Nanofibers containing *Aloe vera* and EGF for wound healing

I. García-Orue[1], G. Gainza[1], C. Vairo[1], F.B. Gutierrez[2], J.J. Aguirre[2], A. Delgado[3], C. Evora[3], J.L. Pedraz[1,4], R.M. Hernandez[1,4], M. Igartua[1,4]

[1]NanoBioCel Group, Laboratory of Pharmaceutics, School of Pharmacy, University of the Basque Country (UPV/EHU), Spain.
[2]Hospital Universitario de Álava (HUA) Txagorritxu, Vitoria-Gasteiz, Spain.
[3]Department of Chemical Engineering and Pharmaceutical Technology. School of Pharmacy. University of La Laguna, Spain.
[4]Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
e-mail: manoli.igartua@ehu.es

Introduction: Due to the ageing population, the incidence of chronic wounds is increasing alarmingly, becoming a major health burden. Those wounds are unable to restore the anatomic and functional integrity of the skin within 6 weeks of standard medical treatment [1]. A new approach for the treatment of chronic wounds are the nanofibrous dressings, which can replace the natural ECM until it is repaired, due to their similar morphological characteristics. Nanofibers can be produced by electrospinning, a technique that uses a high voltage electrical field to generate polymer fibers [2]. To improve the efficacy of the dressings, drugs can be incorporated into the nanofibers. In this study recombinant human epidermal growth factor (*rh*EGF) and *Aloe vera* extract have been incorporated into PLGA nanofibers. EGF is a wound healing mediator and its exogenous administration has proven to promote wound healing [1, 3], and *Aloe vera* extract stimulates the proliferation and activity of fibroblast [4].

Materials and Methods: To prepare the electrospun nanofibers, first of all PLGA 50:50 was dissolved in hexafluoroisopropanol and emulsified by vortexing for 3 min with an aqueous solution of polyvinyl alcohol containing *Aloe vera* and *rh*EGF. Then, this emulsion was electrospun horizontally, at a flow rate of 1 mL/h, on a rotating collector (200 rpm) located at 12 cm from the needle, under 12 kV power supply. Besides of those nanofibers (NF-*rh*EGF), nanofibers containing only *Aloe vera* (NF-ALOE) and nanofibers of PLGA (NF-PLGA) were also produced. The morphology of the nanofibers was analyzed by SEM. Porosity was calculated as $(\%) = \left(1 - \frac{\rho_{app}}{\rho_{true}}\right) \times 100$; where ρ_{true} is the real density (assessed by a helium pycnometer) and ρ_{app} is the apparent density (calculated dividing the obtained mass by the calculated volume). Membrane thickness was measured from photographs obtained by stereo microscopy. The amount of *rh*EGF incorporated in the fibers was determined by ELISA. The *in vitro* bioactivity of the *rh*EGF and *Aloe vera* released from the electrospun was assayed in Balb/C fibroblast. Briefly, cells were incubated for 48 h with free *rh*EGF and the released medium of NF-EGF, NF-ALOE and NF-PLGA. Then, a CCK8 assay was done to evaluate the cell proliferation. The *in vivo* wound healing study was performed in db/db mice [5]. Two full thickness wounds of 0.8 cm diameter were created in the back of each mouse. The treatment was topically administered on days 1 and 4, and the experimental groups were the following: *i*) non-treated; *ii*) free *rh*EGF; *iii*) NF-PLGA; *iv*) NF-ALOE; and, *v*) NF-*rh*EGF. On day 8 the mice were sacrificed. The effectiveness of the treatment was assessed by measuring the wound area on days 1, 4 and 8. Based on the result of the Levene test for the homogeneity of variances, the means were compared through one-way ANOVA, with application of Student-Newman-Keuls post-hoc, or Mann-Whitney U test.

Results and Discussion: SEM images showed that the electrospun membranes were composed of uniform and randomly oriented fibers with an average fiber diameter of 519 ± 198 nm. Membrane thickness was 26.9 ± 0.9 µm and the porosity was 66.7%. The amount of *rh*EGF in the membrane was 12.42 ± 2.74 µg/mg. Results obtained *in vitro* showed that even if the free *rh*EGF and the NF-ALOE groups improved fibroblast proliferation compared to control, the NF-EGF achieved a significantly higher proliferation, revealing a beneficial effect of the combination. Those findings were consistent with the *in vivo* results, where the NF-EGF accelerated significantly wound closure comparing to the other groups at days 4 and 8. The group treated with NF-ALOE improved wound closure on day 8, but in lesser extent than NF-EGF.

Conclusions: In this work we have shown that the combination of *rh*EGF and *Aloe vera* into PLGA nanofibers improves fibroblast proliferation and accelerates wound healing *in vivo*. Nevertheless, results of the histological studies, currently being carried out, are needed to assess the effect of the dressings in reepithelization, collagen deposition or resolution of the inflammation.

Acknowledgements: I. García-Orue thanks the Basque Government for the fellowship grant.

References:

[1] Gainza G, Pastor M, Aguirre JJ, Villullas S, Pedraz JL, Hernandez RM, Igartua M. J Control Release **185** (2014) 51.
[2] Gümüşderelioğlu M, Dalkıranoğlu S, Aydın RS, Cakmak S. J Biomed Mater Res A **98** (2008) 461.
[3] Gainza G, Bonafonte DC, Moreno B, Aguirre JJ, Gutierrez FB, Villullas S, Pedraz JL, Igartua M, Hernandez RM. J Control Release **197** (2015) 41.
[4] Hashemi SA, Madani SA, Abediankenari S. Biomed Res Int **2015** (2015) 714216.
[5] Michaels J 5th, Churgin SS, Blechman KM, Greives MR, Aarabi S, Galiano RD, Gurtner GC. Wound Repair Regen **15** (2007) 665.

Effect of triglyceride molecular weight of oils on nanoemulsion formulation and *in vitro* colloidal behaviour

M. Plaza-Oliver, M.J. Santander-Ortega, V. Rodríguez-Robledo, L. Castro-Vázquez, J. González-Fuentes, P. Marcos, M.V. Lozano, M.M. Arroyo-Jiménez

Cellular Neurobiology and Molecular Chemistry of the Central Nervous System group. Faculty of Pharmacy; and Regional Centre of Biomedical Research (CRIB), University of Castilla-La Mancha, Spain.
e-mail: mvictoria.lozano@uclm.es

Introduction: Nanoemulsions (NEs) are widespread drug delivery systems with excellent properties for the encapsulation of hydrophobic drugs. Among their benefits, NEs are not only remarkable for their excellent capacity for improving the solubility of poorly soluble drugs, but also for inducing enhanced permeation as well as increased bioaccessibility of their cargo active molecules [1]. The selection of the oil has been traditionally done according to the affinity of the drug towards the oil. Nevertheless, oil selection is also important in the formulation process and subsequently will determine the final properties of the NE obtained [2]. Hence, we have selected 3 oils, i.e. wheat germ oil, coconut oil and palm oil, with respectively different state of matter, liquid, semi-liquid and solid, that will yield wheat germ NEs (WG-NEs), coconut NEs (CN-NEs) and palm NEs (P-NEs). The inclusion of oils with such diverse properties required the optimization of the process, initially beginning from suitable organic solvents mixtures for oil solution till the formulation of NEs with satisfactory physicochemical properties. The selection of the oils pursued not only to obtain adequate platforms for the encapsulation of bioactives but also to confer these nanostructures with neuroprotective properties, due to the content in tocopherol, tocotrienols and omega-3 of the oils chosen.

Materials and Methods: NEs were formulated by emulsifying WG oil, CN oil or P oil with lecithin following a modification of the solvent displacement technique [3], to obtain WG-NE, CN-NE and P-NE. In order to ensure NE stability the presence of the surfactant Pluronic® F-127 was required in the external aqueous phase. Size, ζ-potential and colloidal stability of the different prototypes in the gastrointestinal tract was evaluated using a DLS Malvern device (Zetasizer Nano ZS90). The intestinal digestion of SB-NEs was quantified by titrating the fatty acids released after their enzymatic degradation.

Results and Discussion: The solvent displacement method consists of the controlled aggregation of hydrophobic components, which are previously dissolved in appropriate organic solvents, when those organic solvents diffuse towards an external aqueous phase under magnetic stirring. Thus, it is imperative the solubilization of the hydrophobic components of the formulation, in this case WG, CN or P oil plus lecithin. Once we selected the right ethanol/acetone ratio for each prototype we proceed to formulate and characterize the systems. All the prototypes had narrow size distributions with hydrodynamic mean size values in the rage of 210-235 nm and negative superficial charge of ≈ -35 mV, conferred by lecithin. Nevertheless, P-NE was the formulation with the smaller size values (210 nm) and CN-NE the one with highest inter-batch variability. The stability in simulated gastrointestinal media showed that all the systems were stable, despite their slight increase of the size values, which are completely assumable for the oral route. Lipolysis degradation rate was different among the formulations tested, achieving the highest values for CN-NE (≈ 41%), meanwhile it reached ≈ 30% for WG-NE and 26% for P-NE after 1 h of digestion. These differences can be attributed to the different inner oil composition that would affect the arrangement of the surfactant on the interface, which is the frontline for enzymatic degradation.

Conclusions: Oil selection is an important step in the suitable design of NE formulations. Parameters such as drug affinity or toxicity are the first to come to mind, but oils will also determine the formulation process, the physicochemical properties of the NEs and finally their biological performance and their fate. These preliminary studies give hints about the effect of WG, CN and P oils in the properties of the NEs obtained.

Acknowledgements: Authors thank the financial support given by the PEII-2014-040-P research project from the Junta de Comunidades de Castilla-La Mancha, as well as by the GI20153002 research project from the Vicerrectorado de Investigación, University of Castilla-La Mancha. Authors are also grateful to Dr. M. Rocío Fernández Santos for the support given.

References:

[1] Plaza-Oliver M, Baranda JF, Rodríguez Robledo V, Castro-Vázquez L, Gonzalez-Fuentes J, Marcos P, Lozano MV, Santander-Ortega MJ, Arroyo-Jimenez MM. Int J Pharm **490** (2015) 209.
[2] Bouchemal K, Briançon S, Perrier E, Fessi H. Int J Pharm **280** (2004) 241.
[3] Lozano MV, Torrecilla D, Torres D, Vidal A, Domínguez F, Alonso MJ. Biomacromolecules **9** (2008) 2186.

PLGA microspheres for intravitreal injection: size *versus* drug release

J. Rodríguez-Villanueva[1], R. Herrero-Vanrell[2], I.T. Molina-Martínez[2], I. Bravo-Osuna[2], M. Guzmán-Navarro[1]

[1]Department of Biomedical Sciences, U.D. Pharmacy & Pharmaceutical Technology, University of Alcala, Spain.
[2]Department of Pharmacy and Pharmaceutical Technology (Research Group UCM 920415), Faculty of Pharmacy, Complutense University of Madrid, Spain.
e-mail: manuel.guzman@uah.es

Introduction: Drug release kinetic from multiparticulate drug delivery systems is governed by a large number of factors, that are related with the particle´s properties, e.g. size, surface area, porosity, morphology and internal structure, and characteristics of the release medium [1]. An accurate understanding of the relationship between size and drug release profiles is essential to yield useful microspheres (MSs) for therapeutic applications [2]. Dexamethasone (Dxm) is a corticosteroid widely used in the clinical practice for the treatment of pathologies affecting the posterior segment of the eye, where sustained drug concentrations are required for extended periods of time. To that aim, relatively large MSs are preferred [3]. However, intravitreal injection of particles needs small gauge needles to produce as little injury as possible. Recently, a new generation of biodegradable PLGA MSs loaded with Dxm, vitamin E (vitE) and human serum albumin (HSA) has been developed. Our method allows preparation of MSs with sizes ranging from 2 to 20 µm and from 20 to 40 µm. Taking into account the relevance of particle size in formulations destined to intraocular administration, this work focuses on the evaluation and comparison of Dxm release behavior between these 2 fractions in 3 different formulations of MSs containing Dxm (F1), Dxm/vitE (F2) and DXM/vitE/HSA (F3).

Materials and Methods: MSs were obtained by an emulsion-solvent extraction/evaporation method [4]. To prepare F1 MSs, parent Dxm, finely ground, was added to a PLGA DCM solution (1:10 w/w Dxm/PLGA) and then, this s/o system was emulsified (5000 rpm, 1 min, in Polytron® PT 3000, Kinematica AG) with an aqueous PVA solution (2%, w/v) to form a dispersed s/o/w system. MSs consolidation was performed in a PVA solution (0.1, w/v) with magnetic stirring for 3 h and then filtered through nylon filters (Gilson Inc.) to obtain 2 size fractions of MSs: from 2 to 20 µm and from 20 to 40 µm. After washing twice with Milli Q® water, MSs were frozen (-80 ºC) and freeze-dried (Cryodos® Telstar). To prepare F2 MS formulation, vitE was added to the s/o disperse system; and for F3, 20 µg of freeze-dried FITC-HSA were mixed with vitE by sonication (UIS250v, Braun Labsonic) for 30s. MS particle size and particle distribution were assessed by laser light diffractometry (Microtrac SRA®). For *in vitro* release studies, 2 mg of freeze-dried MSs were suspended in 2 mL of phosphate buffer solution (PBS, pH 7.4) in low-binding Eppendorf® tubes, containing 0.02% Tween 80® and 0.05% sodium azide. Tubes were maintained at 37 ± 1 ºC on a water bath for 30 days. The tubes were centrifuged (5000 rpm, 10 min) every 24 h for the first 3 days, and every 72 h until the end of the study. For each sample, supernatant was collected and Dxm quantifying according by a HPLC methodology previously validated. MS morphology was observed before and after Dxm release assays by SEM (Zeiss DSM 950®).

Results and Discussion: Spherical particles were obtained with a mean size of 18.1 ± 0.5 µm for F2 to 20 µm and 27.5 ± 0.8 µm for that ranged 20 to 40 µm. A biphasic release profile was observed in all MS formulations (F1, F2 and F3) regardless of size. Release studies showed a high particle size dependency: after 30 days, while F1 and F2 MSs, from 2 to 20 µm, released almost 100% of their drug loading (96.31 ± 2.76% and 88.96 ± 4.32%, respectively), drug released from F1 and F2 MSs, from 20 to 40 µm, resulted 37.85 ± 5.93% and 44.56 ± 1.44%, respectively. F3 showed significant differences with both F1 and F2, being the release 55.80 ± 1.31% (2 to 20 µm) and 18.58 ± 1.82% (20 to 40 µm). No significant differences were seen on the burst effect between MSs of the same composition but different particle size, showing fraction 20 to 40 µm of F3 the lowest drug release in these first 5 h (1.16 ± 0.09 µg). Drug release kinetics were fitted to a bi-exponential equation including diffusion and erosion processes. being K_1 and K_2 the first-order rate constant that characterize these mechanisms.

$$\frac{\Delta Q_t}{\Delta t} = a \cdot e^{-K_1 \cdot t} + b \cdot e^{-K_2 \cdot t}$$

K_1 constant resulted lower on F3 MSs compared with F1 and F2, for both size fractions; and, K_2 exhibited statistically significant differences due to particle size, showing a double value for all MS formulations sizing 2 to 20 µm.

Conclusions: MSs with a particle size ranging between 20 and 40 µm demonstrated a more adequate behavior when Dxm sustained release is needed for extended periods of time, while 2 to 20 µm fraction could be suitable for acute outbreaks. F3 MSs provided a more sustained drug release, highlighting that HSA performs a modulating action.

Acknowledgements: Research was supported by Nanofarmacia project (FGUA) and research group of Comunidad Madrid UCM920415(GR3/14).

References:

[1] Hadef I, Rogé B, Edwards-Lévy F. Biomacromolecules **16** (2015) 2296.
[2] Riddhi MD, Rakesh KP. Am J PharmTech Res **3** (2013) 362.
[3] Checa-Casalengua P, Jiang C, Bravo-Osuna I, Tucker BA, Molina-Martínez IT, Young MJ, Herrero-Vanrell R. J Control Release **156** (2011) 92.
[4] Rodríguez J, Herrero-Vanrell R, Molina I, Bravo-Osuna I, Guzmán M. Int J Pharm (in press).

Formulation and characterization of solid lipid nanoparticles, nanostructured lipid carriers and nanoemulsions for an antihistaminic drug

Sukran Damgali[1], María Luisa García L.[2], María Antonia Egea[2], Marta Espina[2], Ana Cristina Calpena C.[3], Melike Uner[1]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, Istanbul University, Turkey.
[2]Department of Physical Chemistry, Faculty of Pharmacy, University of Barcelona, Spain.
[3]Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Barcelona, Spain.
e-mail: sdamgali@gmail.com

Introduction: Solid lipid nanoparticles (SLNs) are alternative carrier systems to emulsions, liposomes and polymeric nanoparticles that are produced by replacing the liquid lipid (oil) of an o/w emulsion by a solid lipid or blend of solid lipids. SLNs are composed of 0.1 to 30% (w/w) solid lipid dispersed in an aqueous medium, and if necessary stabilized with preferably 0.5 to 5% (w/w) surfactant. Nanostructured lipid carriers (NLCs) are the second generation of lipid nanoparticles that are produced using blends of solid lipids and liquid lipids, preferably in a ratio of 70:30 up to a ratio of 99.9:0.1. NLCs were developed to overcome some potential limitations associated with SLNs. Compared to SLNs, NLCs show a higher loading capacity for a number of active compounds, a lower water content of the particle suspension, and avoid/minimize potential expulsion of active compounds during storage [1]. Nanoemulsions (NEs) can be considered to be conventional emulsions that contain very small droplets [2]. This project is a basic investigation to be able to design and create lipid nanoformulations which are planning to be used for subcutaneous injection and the influence of surfactant of these placebo lipid nanoformulations.

Materials and Methods: Lipid nanoformulations consist of 2 phases which are lipid and aqueous. One of the most critical parameters for nanoformulations is to choose the lipids. Stearic acid and lauroglycol were chosen as a solid and a liquid lipid, respectively. To find the optimum formulation, samples were prepared with different amounts of lipids, and different kinds and amounts of surfactants. For this purpose, 5 different surfactants (Cremophor® EL, Pluronic® F-68, Plantacare® 2000, Tyloxapol®, Pluronic® F-68:Plantacare® 2000), 2 different amounts (1.5 and 2%) of surfactants and 2 different amounts (6 and 10%) of lipids were studied. The ratio of stearic acid:lauroglycol was kept to 7:3. The lipid and the aqueous phases are mixed under high speed (20,000 rpm). In addition, the pressure and cycle times of the high pressure homogenizer were also investigated. 3 different homogenization pressures (600, 1000, and 1500 bar) and 2 different cycle numbers (3 and 4 cycles) were performed. Particle size and the zeta potential were determined by photon correlation spectroscopy (PCS) and electrophoretic mobility, respectively.

Results and Discussion: The concentration of ingredients affects the particle size of the lipid nanoparticles. A high concentration of the surfactants strongly reduces the surface tension and facilitates particle partition during homogenization. The decrease in particle size is connected with a high increase in surface area. The effect of homogenization process cannot be explained by a single physical phenomenon. There are changeable parameters such as homogenization pressure, temperature, cycle number. At working high pressures, shear stress, cavitation, turbulence, impingement, and temperature increase have all a potential effect on samples. By increasing homogenization pressure, particle size of SLN and NLC formulations were also increased (except the formulations with Plantacare® 2000). On the contrary, the droplet size of NE formulations was decreased (except the formulations with Plantacare® 2000).

Conclusions: Selecting lipids and surfactants based on compatibility helps to formulate SLNs, NLCs and NEs with good stabilities and desirable sizes. The surfactant concentration has an undeniable impact on the particle and droplet size distribution. A high surfactant concentration favors a lower particle and droplet size. Incorporation of a suitable surfactant is one of the critical points to provide desirable lipid nanoformulations. There is a possibility of the changes of the results after putting drug into the optimized nanoformulations. In such a case, some parameters may be changed.

Acknowledgements: Financial support for this project is taken from The Scientific and Technological Research Council of Turkey (TUBITAK), under the International Doctoral Research Fellowship Program (project 1059B141400866).

References:

[1] Pardeike J, Hommoss A, Müller RH. Int J Pharm **366** (2009) 170.
[2] Mcclements DJ. Soft Matter **8** (2012) 1719.

Storage of microencapsulated mesenchymal stem cells

Haritz Gurruchaga, Jesús Ciriza, Laura Saenz del Burgo, Argia Acarregui, Rosa María Hernández, Gorka Orive, José Luis Pedraz

NanoBioCel Group, Laboratory of Pharmaceutics, School of Pharmacy, University of the Basque Country (UPV/EHU), Spain; and Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.
e-mail: laura.saenzdelburgo@ehu.eus

Introduction: Cell microencapsulation permits the immobilization of desired cell and its protection from the immune system. This technology is being studied as drug and cell delivery system to treat different diseases [1]. However, different aspects need to be improved to reach the clinics, such as the preservation process that will facilitate the *on demand* access of patients to the treatment in a clinic far from the manufacturer, and also will reduce the costs of this technology. Several parameters should be considered on encapsulated cell cryopreservation such as the time and temperature during the cryopreservation process or the cryoprotectant (CPA) solutions used [2-4]. Two different freezing methods are being used with specific freezing rates and CPAs. On the one hand, the slow freezing protocol, that uses slow cooling rates and low concentration of CPAs and, on the other hand, the vitrification that uses fast cooling rates and high concentration of CPAs. Our goal was to compare several penetrating and non-penetrating CPAs at different concentrations to elucidate the optimal CPA solution for the conventional cryopreservation of encapsulated mesenchymal stem cells (MSCs). Next, the conventional cryopreservation protocol was compared with vitrification.

Materials and Methods: D1MSC-Epo cells were immobilized into alginate-poly-L-lysine-alginate (APA) microcapsules of 180 µm using a pneumatic atomization generator (Cellena®) and APA microcapsules of 500 µm using an electrostatic droplet generator. In the first experiment, to know the best CPA solution for conventional cryopreservation, 180 µm capsules were performed. Briefly, the slow cooling protocol was 4 ºC 20 min, -80 ºC 24h and liquid N_2 immersion and the solutions tested were: DMSO 10% (D10), DMSO 5% (D5), glycerol 10% (G10), trehalose 10% (T10), DMSO 5% + trehalose 5% (D5T5). After thawing, samples were tested by the subsequent *in vitro* assays: metabolic activity measured by CCK-8 cell viability kit; fluorescence micrographies were taken with an epifluorescence microscope after dying cells with the Live/Dead kit; osmotic resistance of capsules measured by a treatment with sodium citrate and differentiation assays for osteogenic and adipogenic differentiation. Afterwards, an *in vivo* assay was performed with the bests CPA solutions in syngenic Balb/c mice: microcapsules containing D1MSC-Epo cells were subcutaneously implanted, blood was collected from the submandibular area and hematocrit levels were characterized using a microhematocrit method. A second set of experiments was performed with 500 µm capsules to compare the conventional cryopreservation and vitrification. In these experiments, the best CPA solution for conventional cryopreservation protocol was compared with a described CPA solution in vitrification protocol [5], performing the aforementioned *in vitro* assays.

Results and Discussion: In the first set of experiments, CPA solutions that were able to maintain cell viability and metabolic activity were D10, D5 and D5T5. Thereby, these solutions were tested *in vivo*. In this case, the only CPA solution able to raise the hematocrit levels similarly to the cryopreserved group was D10. In the second set of experiments, we compared D10 solution with a described vitrification protocol. Cell viability and capsule integrity decreased significantly in the vitrified group.

Conclusions: The present research work suggests that D10 is the most suitable cryoprotectant solution, among the assessed ones, for the slow cooling cryopreservation of microencapsulated MSCs. Moreover, the conventional cryopreservation seems to be more appropriate to preserve the microencapsulated cells compared to the studied vitrification protocol.

Acknowledgements: Authors thank the University of the Basque Country (UPV/EHU) for the granted fellowship and financial support from the University of the Basque Country UPV/EHU (UFI 11/32), the Basque Country Government, and the Ministry of Science and Innovation. Authors thank the intellectual and technical assistance from the Platform for Drug Formulation (NANBIOSIS) CIBER-BBN.

References:

[1] Orive G, Santos E, Pedraz JL, Hernández RM. Adv Drug Deliv Rev **67-68** (2014) 3.
[2] Fahy GM. Reprod Biomed Online **14** (2007) 709.
[3] Murua A, Orive G, Hernández RM, Pedraz JL. Biomaterials **30** (2009) 3495.
[4] Pravdyuk AI, Petrenko YA, Fuller BJ, Petrenko AY. Cryobiology **66** (2013) 215.
[5] Gurruchaga H, Ciriza J, Saenz Del Burgo L, Rodriguez-Madoz JR, Santos E, Prosper F, Hernández RM, Orive G, Pedraz JL. Int J Pharm **485** (2015) 15.
[6] Ahmad HF, Sambanis A. Acta Biomater **9** (2013) 6814.

Formulation and characterization of high solubility Ebastine solid dispersions

Césareo Jiménez de los Santos, José I. Pérez-Martínez, Mª José Cózar-Bernal, José R. Moyano

Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Seville, Spain.
e-mail: cozar@us.es

Introduction: In the pharmaceutical field, the vast majority of new API candidates are poorly water soluble. In order to overcome their low solubility and thus enhance the dissolution rate and bioavailability, different strategies were applied. Some examples are micronization, nanosupensions, cocrystals, polymorph screening, cyclodextrin complexes, micellar solubilization or formulation as solid dispersions [1-3]. In recent years, the "old" strategy of solid dispersions as remediation of the bioavailability problems of poorly water soluble drugs is back, thanks to the optimization of industrial processing techniques such as hot melt extrusion and others. Ebastine is a H1-antihistaminic drug characterized by a low solubility that limits its dissolution process and bioavailability. According to the BCS classification, Ebastine is a class II drug (low solubility/high permeability), and thus becoming a candidate for improvement of solubility by pharmacotechnical methods. In this work, solid dispersions of poorly water soluble Ebastine with some common hydrophilic carriers (PEG and Gelucires) were elaborated by fusion method. The influence of excipient and drug:carrier ratio load was characterized by thermal analysis (differential scanning calorimetry, DSC, and hot-stage microscopy, HSM). Finally, the *in vitro* dissolution rate behavior was evaluated by USP methods, followed by a kinetic fit of the obtained experimental data.

Materials and Methods: Ebastine was a gift from Andromaco (Spain). PEG_{6000} was purchased to Panreac (Spain). Gelucire 44/14 was a gift by Gattefossè (France). Solid dispersions were prepared by fusion method. An appropriate amount of Ebastine (10, 20, 40 and 60%, w/w) was added over the molten carriers (60 ºC). After slow solidification to ambient temperature, samples were milled to powder and sieved (500 μm). Physical mixtures in the same drug:carrier ratios were elaborated as reference materials. DSC was performed by using a Mettler equipment (FP 85TA cell furnace, FP 80HT control unit and FP 89HT control software). Samples were accurately weighed and sealed in pierced aluminium crucibles, and analyzed under static air atmosphere in the range of 40 to 260 °C at a heating rate 10 °C/min. HSM studies were performed using a Mettler FP 82HT hot plate with a Mettler FP80HT control unit. The hot plate was attached to an Olympus BH-2 microscope, equipped with an Olympus C-5060 ADU adapter and Olympus Camedia C-5060 camera. The starting temperature was 40 °C, increased at 5 °C/min to a maximum of 260 °C. Dissolution studies were performed in a SOTAX mod. CH-4123 apparatus (Switzerland). Powder samples, equivalent to 20 mg of Ebastine, were placed in the vessels with 900 mL of distilled water in paddle at 50 rpm. Experiments were performed in triplicate at 37 °C and the samples were collected at 5, 10, 20, 30, 45, 60 and 120 min without replacing the dissolution media, and filtered through 0.45 μm (Millipore filter). Drug concentration was measured spectrophotometrically at λ = 258 nm (Agilent 8453 spectrophotometer).

Results and Discussion: DSC studies revealed that pure Ebastine melts with a sharp endothermic peak at 95 ºC. Thermal analysis of binary system shows the disappearance of endothermic effect of Ebastine in solid dispersions, only appearing at high drug ratios (40 and 60%), indicating that solubilizing limit of the carrier was reached. Surprisingly, this phenomenon can be also observed in the physical mixtures. However, a direct observation through HSM techniques revealed that during heating process, the previous melt of carrier in physical mixtures promotes the *in situ* dissolution of drug, being impossible to observe its fusion due to the inexistence of a drug solid phase. Dissolution studies revealed that solid dispersion techniques improved the dissolution process for all the binary systems. The kinetic fit of the dissolution profiles indicated that Ebastine:PEG_{6000} systems follow a Hixson Crowell kinetic, whereas Ebastine:Gelucire 44/14 systems preferentially fit Higuchi kinetics. An explanation of this behavior would be the additional surfactant properties of Gelucire when compared with PEG_{6000}. After comparing dissolution parameters as dissolution efficiency at 30 and 60 min (DE30 and DE60) and mean dissolution time (MDT), it can be concluded that the Ebastine:Gelucire 44/14 solid dispersions at 10 and 20% shown the better results.

Conclusions: The release from solid dispersions shows a significant improvement of solubility and dissolution rate when compared with reference material (drug alone and physical mixtures), in particular the systems at 10 and 20% drug:carrier ratios, opening a way to develop oral formulations with improved bioavailability.

References:

[1] Kawabata Y, Wada K, Nakatani M, Yamada S, Onoue S. Int J Pharm **420** (2011) 1.
[2] Leuner C, Dressman J. Eur J Pharm Biopharm **50** (2000) 47.
[3] Hajare AA, Jadhav Asian PR. J Pharm Tech **2** (2012)116.

Unraveling the therapeutic potential of targeted synergistic drug combination against nucleolin-overexpressing ovarian cancer cell lines

Ana Filipa Cruz[1,2], *Nuno A. Fonseca*[1,3], *Susana F. Sampaio*[1,4,5], *Vera Moura*[1,3], *Ramiro D. Almeida*[1], *Sérgio Simões*[1,2], *João Nuno Moreira*[1,2]

[1]CNC – Center for Neurosciences and Cell Biology, University of Coimbra, Portugal.
[2]FFUC – Faculty of Pharmacy, Pólo das Ciências da Saúde, University of Coimbra, Portugal.
[3]TREAT U, SA - Parque Industrial de Taveiro, Lote 44, Coimbra, Portugal.
[4]Institute for Interdisciplinary Research, University of Coimbra (IIIUC), Portugal.
[5]Doctoral Programme in Experimental Biology and Biomedicine, University of Coimbra, Portugal.
e-mail: anafilipafcruz@gmail.com

Introduction: Ovarian cancer is the fifth major cause of cancer death in females and, since the mid-1970s, the incidence of ovarian cancer in females aged 15-39 has increased ≈ 56%. The high mortality rate, results from a late-stage diagnosis and tumor recurrence after platinum therapy [1]. There is evidence that a putative population of cells, called cancer stem cells (CSCs), is involved not only in recurrence but also in tumorigenicity, metastasis and drug resistance. Several markers, including CD44 and EpCAM, have successfully been used to identify putative CSC sub-populations in ovarian tumors [2]. Another important aspect to consider is the fact the PI3K/AKT/mTOR pathway (which plays a critical role in the malignant transformation of human tumors and their subsequent growth, proliferation, and metastasis) is frequently overactivated in ovarian cancer [3]. We have previously developed a lipid-based nanoparticle (NP) containing doxorubicin (DOX) and functionalized with the nucleolin-binding F3 peptide. Nucleolin overexpression has been demonstrated on the surface of both breast cancer (putative CSC and non-stem cancer cells) and endothelial cells [4]. The F3 peptide-targeted pH-sensitive lipid-based NP was recently modified to contain a synergistic drug combination of C6-ceramide (a pro-apoptotic lipid described to inhibit the PI3K/Akt signaling cascade) and DOX. Following the promising results in breast cancer [4], the aim of this work was to test the potential of this strategy against ovarian cancer.

Materials and Methods: The specific interaction was assessed upon incubation of ovarian cancer cells with rhodamine-labeled F3 peptide-targeted (F3-L), non-specific peptide targeted (NS-SL) or non-targeted (NT-L) liposomes for 1 h and 4 h at 37 ºC and 4 ºC. To study the interaction of the targeted NPs with putative ovarian CSCs, cells were incubated with calcein-loaded F3 peptide-targeted (F3-L[Calcein]), non-specific peptide targeted (NS-L[Calcein]) or non-targeted (NT-L[Calcein]) liposomes for 4 h at 37 ºC, subsequently stained with anti-CD44/PECy5 and anti-EpCAM/PE antibodies and immediately analyzed by flow cytometry. Cytotoxicity was assessed by the rezasurin assay, upon incubation of ovarian cancer cells with serial dilutions of F3 peptide-targeted liposomes containing DOX: C6-ceramide-loaded liposomes, F3-L[C6Cer:DXR] (or non-targeted counterparts). In addition, p-Akt and Akt expression levels were evaluated by western blot analysis.

Results and Discussion: *In vitro* studies with ovarian cancer cell lines demonstrated a significant improvement in cellular association and internalization of the F3 peptide-targeted formulation (F3-L), relative to the non-targeted counterparts. Moreover, there was a significant increase of the cellular association of F3-L liposomes with the putative CSC enriched population. These results are in accordance with the marked increase of cytotoxicity enabled by F3-L[C6Cer:DXR], including relative to F3 peptide-targeted liposomes containing only DOX. At the molecular level F3-L induced down-regulation of p-Akt levels, which supports C6-ceramide mediated inhibition.

Conclusions: Taken together, these results pointed out the therapeutic potential arising from the intracellular delivery of the developed synergistic drug combination against ovarian cancer.

Acknowledgements: AFC is recipient of a FCT fellowship (reference SFRH/BD/85554/2012) and a student of the Pharmaceutical Sciences PhD program from FFUC. Work was supported by QREN/FEDER/COMPETE (Ref. 30248/IN0617) and strategic project UID/NEU/04539/2013.

References:

[1] Guddati AK. Med Oncol **29** (2012) 3400.
[2] Wei X, Dombkowski D, Meirelles K, Pieretti-Vanmarcke R, Szotek PP, Chang HL, Preffer FI, Mueller PR, Teixeira J, MacLaughlin DT, Donahoe PK. Proc Natl Acad Sci USA **107** (2010) 18874.
[3] Mabuchi S, Kuroda H, Takahashi R, Sasano T. Gynecol Oncol **137** (2015) 173.
[4] Fonseca NA, Gomes-da-Silva LC, Moura V, Simões S, Moreira JN. J Control Release **196** (2014) 122.

Cellular distribution of citrate-stabilized gold nanoparticles after intraperitoneal administration

Carlos López-Chaves[1], Juan Llopis[1], Juan Soto-Alvaredo[2], María Montes-Bayón[2], Jorg Bëttmer[2], Cristina Sánchez-González[1]

[1]Department of Physiology, University of Granada, Spain.
[2]Department of Physical and Analytical Chemistry, University of Oviedo, Spain.
e-mail: carloschaves@ugr.es

Introduction: Despite all the benefits, the widespread use of gold nanoparticles (GNPs) might have an important impact on living organisms [1]. However, up to now there is little knowledge about the effect of these particles in human health in terms of distribution or bioaccumulation. The study aims to give new insights how GNPs are distributed inside the cell (*in vitro* and *in vivo*) after a short period of exposure. This approach will therefore provide important data of the relationship between the metal distribution as mentioned above and potential physiological influence.

Materials and Methods: HepG2 cell line was obtained from the Cell Culture Resource Centre (University of Granada, Spain). The cells were precultured in 25cm^2 culture flasks in RPMI-1460 medium (containing 2 mM L-glutamine and 2.0 mg/mL sodium bicarbonate) supplemented with 10% (v/v) foetal bovine serum (FBS). Cells were treated with 10 ppm 10 nm-sized GNPs solution for 24 h. After exposure, the medium was removed and cells were rinsed with phosphate buffered saline (PBS). 4 male Wistar rats (Charles River Laboratories, L'Abresde, France), were daily injected 0.4 mL of 50 ppm 10 nm-sized GNPs solution (RM 8011, National Institute of Standards and Technology, USA) via intraperitoneal. The experiment lasted 9 days. All rats were fed with AIN-93 diet. After anaesthetising, rats were sacrificed. Cell culture and liver samples were fixed with 1% glutaraldehyde and post-fixed with 1% osmium tetroxide followed by dehydration with ascending series of alcohol before embedding samples in araldite. Ultra-thin sections were cut and doubly stained with uranyl acetate and lead citrate.

Results and Discussion: GNPs were observed inside lipid drops in the cells in both cell culture and liver samples. Some of them were also observed in cytoplasm. No structural or functional abnormalities were found.

Conclusions: After treatment with 10 nm-sized GNPs, cells were able to incorporate them inside cell and metabolize and integrate them in lipid drops without altering functionality. Thus, GNPs are a promising tool for many biomedical applications due to their physical properties and their innocuousness.

Acknowledgements: Spanish Ministry for Science and Innovation for the financial support (grant number CTQ2011-23038) and Spanish Ministry for Education, Culture and Sport, grant reference FPU13/00062.

References:

[1] Alex S, Tiwari A. J Nanosci Nanotechnol **15** (2015) 1869.

Targeting solid tumors: nanoformulations of a potent metallodrug

Mariana A. Nave[1], Rui E. Castro[1], Cecília M. Rodrigues[1], Angela Casini[2], Graça Soveral[1], Maria M. Gaspar[1]

[1]Research Institute for Medicines (iMed.ULisboa), University of Lisbon, Portugal.
[2]School of Chemistry, Cardiff University, United Kingdom.
e-mail: mgaspar@ff.ulisboa.pt

Introduction: Aquaporins (AQPs) are a family of small transmembrane proteins that facilitate the transport of water and small solutes such as glycerol across the cell plasma membrane. Aberrant expression of several AQPs has been recently correlated to different tumor types, such as melanoma and colorectal cancers [1]. Metallodrugs have been reported as potent and selective human AQP_3 inhibitors that can be further explored for *in vivo* studies [2]. Pursuing this idea, we are now developing and evaluating an appropriate nanolipidic system, liposomes, aiming to stabilize metallo-compounds as AQP_3 inhibitors and to improve their targeting to tumor sites [3]. One of these compounds, $[Cu(phen)Cl_2]Cl$ (Cuphen), was incorporated in liposomes and its cytotoxic effect was evaluated *in vitro* against melanoma and colon cancer cell lines. *In vivo* toxicity evaluation of Cuphen nanoformulations was also performed.

Materials and Methods: Cuphen was incorporated in liposomes, with long-circulating properties (polyethylene glycol, PEGylated), by the dehydration-rehydration method followed by an extrusion step, in order to reduce and homogenize the lipid nanoformulations [3]. Cuphen-loaded liposomes were characterized in terms of mean size, polydispersity index, zeta potential and encapsulation parameters using different lipid compositions. The cytotoxic activity of Cuphen in free and liposomal forms, against several cell lines (A431, MNT-1, HaCaT B16F10 and C26), was performed using MTS and Guava ViaCount assays [4]. The hemolytic activity of Cuphen in free and liposomal forms using EDTA-preserved peripheral human blood from voluntary donors was determined as described elsewhere [3]. The *in vivo* effect of Cuphen formulations was evaluated following intravenous administration at a dose of 1.5 mg/Kg of body weight, 3 times a week.

Results and Discussion: Homogeneous Cuphen-loaded liposomal formulations were obtained (mean size of 140 ± 15 nm and polydispersity index < 0.15) and Cuphen incorporation was favored by fluid and neutral phospholipids (i.e. PC-based vesicles). Cytotoxicity *in vitro* studies of Cuphen in the free form revealed IC_{50} values < 3 μM. Similar results were obtained after drug encapsulation in liposomes reflecting the preservation of its cytotoxic effect. Moreover, unloaded liposomes did not exert cytotoxic effect towards any of the tested cell lines. Cuphen in free and in liposomal forms did not show hemolityc activity in the range of concentrations tested (0.2 to 200 μM). The intravenous administration of Cuphen in free and liposomal forms was well tolerated, without significant changes in biochemical and pathological parameters in the animals ($p > 0.05$).

Conclusions: In this work we have shown that Cuphen is a potent inhibitor of cellular viability with IC_{50} values in the micromolar range. When incorporated in liposomes, Cuphen is able to exert its effect against all the cell lines under study, and unloaded liposomes presented no citotoxicity. In addition, *in vivo* studies showed that Cuphen incorporated in long-circulating liposomes did not show toxic effects. In view of these promising results, the establishment of a melanoma and colon murine xenograft models to validate the therapeutic effect of Cuphen formulations is the next set of experiments.

Acknowledgements: This work was partially supported by iMed.ULisboa (UID/DTP/04138/2013).

References:

[1] Verkman AS, Hara-Chikuma M, Papadopoulos, MC. J Mol Med **86** (2008) 523.
[2] Martins AP, Ciancetta A, de Almeida A, Marrone A, Re N, Soveral G, Casini A. ChemMedChem **8** (2013) 1086.
[3] Gaspar MM, Calado S, Pereira J, Ferronha H, Correia I, Castro H, Tomás AM, Cruz ME. Nanomedicine **11** (2015) 1851.
[4] Mansoor TA, Borralho PM, Luo X, Mulhovo S, Rodrigues CM, Ferreira MJ. J Nat Prod **77** (2014) 1825.

Development of natural and semi-synthetic flavanones loaded PLGA-nanoparticles as anticancer agents

Berenice Andrade-Carrera[1], Beatriz Clares[2], Mireia Mallandrich[3], Verónica Noé[4], Ana Cristina Calpena[3], María Luisa García[5], María Luisa Garduño-Ramírez[1]

[1]Centro de Investigaciones Químicas, Instituto de Investigación en Ciencias Básicas y Aplicadas, Universidad Autónoma del Estado de Morelos, México.
[2]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[3]Department of Biochemistry and Molecular Biology, University of Barcelona, Spain.
[4]Department of Pharmacy and Pharmaceutical Technology, University of Barcelona, Spain.
[5]Department of Physical Chemistry, University of Barcelona, Spain.
e-mail: lgarduno@uaem.mx

Introduction: Cancer is caused by abnormalities in the genetic material of cells. Different carcinogenic, physical, chemical and/or biological agents can provoke these abnormalities. Nowadays million of people died for this disease. Therefore, search for new alternatives for prevention and treatment of cancer is extremely important to minimize this unwanted human mortality. Natural products have been reported as important sources that could produce potential chemotherapeutical tools. Over 50% of anticancer approved drugs were from natural resources. *Eysenhardtia platycarpa* is a small tree distributed in Southern Mexico. It is also known as "taray", "palo dulce" (sweet wood), and "palo azul" (blue wood). It is well-known in traditional herbal medicine and used for the treatment of kidney diseases, bladder infections and diabetes mellitus, as well as diuretic agent [1]. This study aimed to elaborate poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles (NPs) loading the flavanone (2*S*)-5,7-dihydroxy-6-prenylflavanone (**1**) and new derived compounds from this by acethylation (**1a**), methylation (**1b**), cyclization (**1c**) and vinyloge-cyclization (**1d**).

Materials and Methods: From the methanolic extract of *E. platycarpa* leaves flavanone (**1**) was isolated. From this natural flavanone, semisynthetic compounds (**1a** to **1d**) were obtained by acethylation, methylation, cyclization and vinyloge-cyclization, respectively. These compounds were characterized by 1D and 2D ^{1}H and ^{13}C high-resolution solid-state NMR spectroscopy with a Varian INOVA-400 instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C). PLGA NPs containing flavanone **1** and **1a** to **1d** (NPs**1**, NPs**1a**-**1d**) (1.5 mg/mL) were elaborated by the solvent displacement technique [2]. Mean particle size (Z-ave), zeta potential (ZP) and polydispersity index (PI) were determined with a Zetasizer Nano ZS (Malvern Instruments, UK). Morphological and structural properties were examined by transmission electron microscopy (TEM) with a Zeiss 902 CEM 902 (Zeiss, Spain). Cytotoxicity was evaluated in MiaPaca-2 cell line isolated from pancreatic cancer (10, 50, 75 and 100 μM), according to the method described by Mosmann [3].

Results and Discussion: Spherical shaped NPs with Z-ave ranging from 141.6 ± 0.77 to 205.2 ± 0.26 nm and PI values between 0.058 ± 0.053 and 0.101 ± 0.031 were obtained. ZP values ranged from -10.6 ± 0.23 to -6.48 ± 0.405 mV. Cytotoxicity assay results showed that NPs**1a** and NPs**1b** exhibited higher efficacy (% cell growth inhibition of 98.24 ± 1.01 and 91.53 ± 1.05, respectively) when compared with NPs**1** at the highest concentration. On the other hand, NPs**1c** and NPs**1d** were inactive in all tested concentrations.

Conclusions: The molecular design strategy applied in this work, and concretely, acethylation and methylation of the lead flavanone improved the cytotoxic efficacy in MiaPaca-2 cell line. It was hypothesized that this effect could be probably due to the increment of the lipophilicity due to an acetyl moiety.

Acknowledgements: The authors would like to thank CONACyT, Mexico for the scholarship 253949, the Ministry of Science and Innovation of Spain with the project MAT201459134R and Laboratorio Nacional de Estructuras de Macromoléculas de la Universidad Autónoma del Estado de Morelos, Mexico (LANEM).

References:

[1] Domínguez-Villegas V, Domínguez-Villegas V, García ML, Calpena A, Clares-Naveros B, Garduño-Ramírez ML Nat Prod Commun **8** (2013) 177.
[2] Domínguez-Villegas V, Clares-Naveros B, García-López ML, Calpena-Campmany AC, Bustos-Zagal P, Garduño-Ramírez ML. Colloids Surf B Biointerfaces **116** (2014) 183.
[3] Mosmann T, J Immunol Methods **65** (1983) 55.

Formulation and characterization of nanospheres Pioglitazone for treatment of Alzheimer´s disease

Marcelle Silva de Abreu[1], *Amanda Cano*[1], *Ana Cristina Calpena*[2], *Marta Espina*[1], *María Luisa García*[1]

[1]Department of Physical Chemistry, University of Barcelona, Spain.
[2]Department of Pharmacy and Pharmaceutical Technology, University of Barcelona, Spain.
e-mail: marcelle.ievv@hotmail.com

Introduction: Alzheimer´s disease (AD) is a complex neurodegenerative disorder of multifactorial etiology, in which there are involved genetic factors, inflammatory responses, environmental factors and aging [1]. It is well-known that the protein TAU and the β-amyloid peptide are molecules involved directly with the AD and constitute an adaptive cellular strategy to the oxidative stress [2]. The main obstacle to transport drugs to the brain is the blood-brain barrier (BBB), acting as an immune and metabolic barrier. There is strong evidence that the conditions caused by abnormalities of insulin, increases the risk of cognitive impairment related to age in AD. Pioglitazone (PGZ) is an oral antidiabetic from thiazolidinediones, agonist of the peroxisome proliferator-activated receptors (PPARs), that can play an important role on mechanisms of neurodegenerative diseases [3, 4]. The main goal of this work was the association of the PGZ to nanospheres (NSs) from poly(D,L-lactide-*co*-glycolide)-poly(ethylene glycol) (PLGA-PEG) for the treatment of AD.

Materials and Methods: NSs of PGZ were prepared by the solvent displacement technique using polyvinylalcohol as surfactant, a matrix of PLGA-PEG, and acetone as organic solvent. In order to determine the influence of several factors such as the pH of the aqueous phase, the initial concentration of the stabilizer and matrix concentration on the physicochemical properties [particle size, polydispersity index (PI), zeta potential (ZP)] of NSs a factorial design 2^3 has been done. Morphometry parameters were determined by dynamic light scattering (DLS), ZP by electrophoretic mobility, drug loading efficiency by ultra-performance liquid chromatography (UPLC) and morphology by transmission electron microscopy (TEM). NSs were prepared with 2 different pH values of the aqueous phase (4.5 and 5.5), at a concentration of PGZ of 1 mg/mL, PVA of 20 and 30 mg/mL, and PLGA-PEG of 7.0 and 9.0 mg/mL. *In vitro* release studies of PGZ from NSs and free drug were performed in amber glass Franz-type diffusion cells with dialysis cellulose membranes between the donor and the receptor compartment (13 mL DMSO/water; 3:7 v/v), keeping sink conditions in the whole experiment at 37.0 ± 0.5 ºC, under continuous stirring. Samples of 300 µL were withdrawn at selected time intervals for 72 h and replaced with the same volume of fresh receptor medium. The concentration of released drug was measured in triplicate by UPLC. 4 different kinetic models (zero order, first order, hyperbola and Korsmeyer-Peppas functions) were used to fit the experimental data obtained from drug release experiments.

Results and Discussion: Average size of PLGA-PEG-PGZ nanospheres ranged from 246 to 280 ± 5nm. It was observed that the IP decreases at low concentrations of PVA and PLGA-PEG. The ZP, which is a measure of the surface charge of the particles that influences the stability and mucoadhesion, increased (in absolute value) at low concentrations PLGA-PEG and PVA. According to the objective of this study, the optimized formulation showed a size about 250.2 ± 1.1 nm, PI of 0.224 ± 0.021, ZP of -5.57 ± 0.33 mV and high association efficiency (98.14%). The morphology of the optimized particles was spherical with few aggregates being their average size similar to those obtained by DLS. The release profiles of PGZ from NSs, exhibited a quicker release at the beginning, due to the amount of PGZ present on the surface of the NSs, followed by a slow release phase. This behavior may be attributed to the fact that the drug trapped inside the NSs slowly diffuses out into the release medium (modified). From the values of Akaike´s information (AIC) and coefficient of determination (r^2) (93.4 and 0.9535, respectively), the release curves of NSs and the free drug solution fit properly to the kinetics hyperbola model.

Conclusions: In this work PGZ-loaded PLGA-PEG NSs have been developed by interfacial deposition procedure, as an alternative for multifactorial treatment of the Alzheimer´s disease.

Acknowledgements: CAPES (Coordination for the Improvement of Higher Education Personnel) Brazil and the Spanish Ministry of Science and Innovation grant (MAT2014-59134R) for financial support.

References:

[1] Liu L, Chan C. Ageing Res Rev **15** (2014) 6.
[2] Anand R, Gill KD, Mahdi AA. Neuropharmacology **76** (2014) 27.
[3] Chen J, Li S, Sun W, Li J. PLoS One **10** (2015) 1.
[4] Sato T, Hanyu H, Hirao K, Kanetaka H, Sakurai H, Iwamoto T. Neurobiol Aging **32** (2011) 1626.

Development of polymer-based combination therapeutics for the treatment of castration-resistant prostate cancer (CRPC)

Sonia Vicente-Ruiz[1], Ana Armiñan[1], Irene Casanova[2], Elena Gallon[1], Julie Movellan[1], María García[2], David Charbonnier[1,3], José A. López-Guerrero[2], María J. Vicent[1,3]

[1]Polymer Therapeutics Lab., Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain.
[2]Fundación Instituto Valenciano de Oncología, Spain.
[3]Screening Platform, Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain.
e-mail: sruiz@cipf.es

Introduction: Prostate cancer (PCa) is the first most common cancer in men and leads to 10% of cancer deaths in Europe [1]. Therefore, identification of biomarkers for aggressive PCa would facilitate therapeutic targets. A potential biomarker is the presence of a fusion gene between TMPRSS2 (androgen-dependent serine protease) and ERG (transcription factor belonging to the ETS family) causing overexpression of TMPRSS2-ERG (T2E) transcript, which is presented in 50% of diagnosed PCa, always indicating the presence of cancer [2, 3]. This fusion gene defines a particular subtype of PCa. Androgen receptor (AR) and insuline growth factor 1 receptor (IGF1R) are involved in PCa [4]. Preliminary results shown a reciprocal feedback loop regulation between both pathways, when a route is inhibited it activates the other and viceversa [5], but the combination of an IGF1R inhibitor (monoclonal antibody mAb) with an anti-androgen drug (Abiraterone) results in synergistic effects in T2E positive cells [6]. For this reason and together with the already known benefits of the use of nanomedicine as anticancer treatments, and in particular polymer therapeutics [7], we aim to design a polymer-based therapy based on the combination of both drugs to block synergistically PCa tumor growth [8].

Materials and Methods: An antibody drug conjugate [poly-L-glutamic acid (PGA)- mAb] was synthesized and fully characterized. This immunoconjugate was evaluated in a panel of PCa cell models (VCap, LNCap, PC3, RWPE1, DU145 and 22RV1) and compared with the parent drug.

Results and Discussion: Only VCap cells showed response to both compounds due to the presence of T2E fusion gene. More importantly, no significant differences on cell viability were observed upon conjugation to PGA, indicating that the synthetic approach used was safe and do not reduced antibody performance. In order to design the above-mentioned combination approach, selectivity against T2E was a major goal apart from synergism. Therefore, to achieve this proof a VCap derived cell line with silenced T2E fusion gene was developed. mAb , Abiraterone as free single drugs and its combination, PGA- mAb conjugate and its combination with endocrine therapy were evaluated against both VCap cell lines. This study clearly demonstrated that, antitumor activity was directly correlated with the presence of T2E fusion gene since the silencing of tERG was always accompanied by an increase of survival. Important to note, the performance of PGA - mAb conjugate in combination with free endocrine drug showed an enhanced selectivity towards the presence of T2E fusion gene in comparison with the combination of free antibody. Studies are being carrier out in order to elucidate this behavior.

Conclusions: The combination of PGA-mAb conjugate with endocrine drug could be a promising therapy in TMPRSS2-ERG PCa patient subtype, as an enhancement in activity as well as selectivity has been observed in T2E positive models when compared with the parent free antibody combination.

Acknowledgements: This study is funded by Plan Nacional I+D; SAF2013-44848-R and FPI grant BES-2014-068439.

References:

[1] Siegel R, Naishadham D, Jemal A. CA Cancer J Clin **62** (2012) 10.
[2] Barbieri CE, Demichelis F, Rubin MA. Histopathology **60** (2012) 187.
[3] Tomlins SA, Bjartell A, Chinnaiyan AM, Jenster G, Nam RK, Rubin MA, Schalken JA. Eur Urol **56** (2009) 275.
[4] Aggarwal RR, Ryan CJ, Chan JM. Urol Oncol **31** (2013) 522.
[5] Carver BS, Chapinski C, Wongvipat J, Hieronymus H, Chen Y, Chandarlapaty S, Arora VK, Le C, Koutcher J, Scher H, Scardino PT, Rosen N, Sawyers CL. Cancer Cell **19** (2011) 575.
[6] Mancarella C, Casanova-Salas I, Calatrava A, Ventura S, Garofalo C, Rubio-Briones J, Magistroni V, Manara MC, L· pez-Guerrero JA, Scotlandi K. Oncotarget 6 (2015) 16611.
[7] Duncan R, Vicent MJ. Adv Drug Deliv Rev **65**(2013) 60.
[8] Greco F, Vicent MJ. Adv Drug Deliv Rev **61** (2009) 1203.

Comparative release study and kinetic modelling of formulations of Ketorolac

Mireia Mallandrich[1,2], *Francisco Fernández-Campos*[1], *Ari Barreto*[1], *Salima El-Moussaoui*[1], *Beatriz Clares*[3], *María L. García*[2,4], *Ana C. Calpena*[1,2]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Barcelona, Spain.
[2]Institut de Nanociència i Nanotecnologia, IN2UB. Barcelona, Spain.
[3]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[4]Department of Physical Chemistry, University of Barcelona, Spain.
e-mail: mireia.mallandrich@ub.edu

Introduction: Ketorolac tromethamine (KT) is a non-steroidal anti-inflammatory drug (NSAID) with a potent and moderate non-opioid analgesic activity. It is currently administered orally and intramuscularly for pre- and post-operative pain treatment [1]. A topical or transdermal preparation is non-invasive, maintains blood levels for extended periods of time; thereby eliminating the gastrointestinal adverse effects of KT, and also allows for local action. Hydrogels are three-dimensional cross-linked polymeric networks that exhibit the ability to swell and retain a large amount of water, without dissolution [2]. Biodegradable polymeric nanoparticles (NPs) are widely used to enhance the bioavailability of topically administered drugs. Poly(D,L-lactide-*co*-glycolide) (PLGA) is the most extensive biodegradable polymer used due to its biocompatibility, mucoadhesiveness and non-antigenic nature [3-5]. The purpose of this work was to study the release behaviour of 2 formulations of KT for topical administration.

Materials and Methods: 3 formulations were elaborated, 2 hydrogels and a nanostructured formulation. Both hydrogels were prepared at a concentration of 2% of KT (w/v), and NPs at the concentration of 0.5%. On one hand, sodium alginate hydrogel (SA-HG) was produced by dissolving SA (4%) in a KT solution and kept in a water bath at 37 ºC for 24 h, and Pluronic® F-127 hydrogel (P-HG), which was made by dissolving the polymer (18%) in KT solution and kept in a water bath at 4 ºC for 24 h. On the other hand, polymeric NPs (152.1 ± 0.2 nm, PdI: 0.142 ± 0.02) were prepared by the double emulsion technique. *In vitro* release studies were performed on Franz-type cells, using dyalisis membranes, over a period of 24 h, after which, samples were analized by HPLC method with UV detector to quantify the amount of drug released from the formulations. Data analysis was carried out by non-linear regression software (GraphPad Prism v.5) applying mathematical modelling (zero-order, first-order, Higuchi and Korsmeyer-Peppas) to depict release behaviour. Best fitting profile was selected based on the lowest AIC (Akaike Information Criteria) value, CV% and goodness-of-fit.

Results and Discussion: P-HG released 100% of KT from the formula. In opposite, SA-HG presented a poor release of KT with only 0.94%. NP formulation was positioned midway between the 2 hydrogels with a release of KT of 67.61%. In accordance with aforementioned criteria, first-order was the best model to describe the release profile for all formulations. The first-order kinetic profile shows that KT is rapidly released from the formulation and reaches the maximum amount released at 5 h.

Conclusions: NPs and P-HG exhibit good biopharmaceutical properties for topical delivery. However, an optimization of the SA-HG formulation might be carried out to increase the release of KT from it.

Acknowledgements: This research has been supported by the Ministry of Science and Innovation of Spain MAT2011-26994 and MAT 2014-59134R. The authors would like also want to acknowledge B. Siha and O. Pereda for her technical support.

References:

[1] El-Setouhy DA, El-Ashmony A, Mohy S. J Pharm Pharmacol **62** (2010) 25.
[2] Ahmed EM. J Adv Res **6** (2015) 105.
[3] Dos Santos KC, da Silva MF, Pereira-Filho ER, Fernandes JB, Polikarpov I, Forim MR. Nanotechnol Sci Appl **5** (2012) 37.
[4] Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. J Control release **161** (2012) 505.
[5] Nagarwal RC, Kant S, Singh PN, Maiti P, Pandit JK. J Control release **136** (2009) 2.

Design and characterization of sodium hyaluronate nanoparticles

Herminia Castán, Mª Adolfina Ruiz, Mª Dolores Muñoz, Mª Encarnación Morales

Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
e-mail: castan@correo.ugr.es

Introduction: Hyaluronic acid (HA) is a naturally occurring glycosaminoglycan distributed throughout the extracellular matrix. The structure of HA consists of repeating disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine. This is an important compound in connective tissues, and thus biocompatible and biodegradable. In addition, HA can play the role of reservoir for tissue repair and may promote or maximize tissue healing, and can be used in several clinical applications [1, 2], e.g. in the formulation of drug delivery systems for cancer treatment thanks to the fact that diverse tumor cells overexpress the HA receptor CD44 [3]. If this is the case, self-assembled polymeric nanoparticles (NPs) based on HA are very attractive as tumor-targeting drug carriers in cancer treatment. However, the choice of the anionic polymer to be used could greatly influence the specificity, stability, and size of the assembled NPs [4]. The aim of this work is the development and characterization of NPs surface coated by sodium hyaluronate for cancer treatment. It is expected that these hyaluronate sodium-based NPs could selectively target cancer cells, thus improving the internalization of the loaded drug.

Materials and Methods: The emulsification/external gelation technique has been used to obtain hyaluronate sodium-based NPs. The aqueous phase containing different amounts of sodium hyaluronate and Tween® was added to an oil phase, and the mixing was stirred at 1000 rpm for 24 h. Under continuous stirring, calcium chloride 5% (w/v) was added. The obtained emulsion was then subjected to a cleaning procedure involving a cycle of sedimentation and redispersion in distilled water. The presence of the shell, particle size and the width of the size distribution were determined by photon correlation spectroscopy (PCS) and by high resolution transmission electron microscopy (HRTEM). The surface electrical properties were analyzed by zeta potential (ζ) determinations (Malvern Zetasizer 2000, Malvern Instruments, UK) [5].

Results and Discussion: Hyaluronate sodium-based NPs were characterized by a spherical shape, and a suitable and moderately monodisperse size (mean particle size: 169.93 ± 2.41 nm). However, when the concentration de hyaluronate sodium was greater, the size of the NPs was 383.53 ± 49.08 nm. Results of ζ determinations showed that NPs 0.06% and 5% are stable (-29.6 and -27.3 mV, respectively). Hence, size and ζ of the hyaluronate sodium-based NPs were dependent on the composition, with an increase in size and in surface charge with increasing HA content.

Conclusions: It has been shown that it is possible to obtain hyaluronate sodium-based NPs by following the emulsification/external gelation technique. Although the efficiency of the coating was demonstrated by chemical characterization and surface analysis of the NPs, if compared with the NPs with 0.5%, it can be observed that the concentration of polymer influenced the NP size. These results revealed the promising potential of hyaluronate sodium-based NPs as a stable and effective drug delivery nanosystem for cancer treatment.

Acknowledgements: Financial support from Inves Biofarm (Granada, Spain).

References:

[1] Menzel EJ, Farr C. Cancer Lett **131** (1998) 3.
[2] Almond A. Cell Mol Life Sci **64** (2007) 1591.
[3] Choi KY, Min KH, Na JH, Choi K, Kim K, Park JH, Kwon IC, Jeong SY. J Mater Chem **19** (2009) 4102.
[4] Duceppe N, Tabrizian M. Biomaterials **30** (2009) 2625.
[5] Jafari Malek S, Khoshchehreh R, Goodarzi N, Khoshayand MR, Amini M, Atyabi F, Esfandyari-Manesh M, Tehrani S, Mohammad Jafari R, Maghazei MS, Alvandifar F, Ebrahimi M, Dinarvand R. Colloids Surf B Biointerfaces **122** (2014) 350.

Release and permeation studies of Doxepin from mucoadhesive films

Herminia Castán, Mª Adolfina Ruiz, Mª Dolores Muñoz, Mª Encarnación Morales

Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
e-mail: castan@correo.ugr.es

Introduction: Oral mucositis is one of the most common side effects of radiotherapy and/or chemotherapy [1]. This is a debilitating condition that appears as a result of the cytotoxic effects of chemotherapy drugs and radiation in the oral mucosa [2], thus being one of the most important causes of the increased use of drugs for pain treatment. In this line, an alternative to analgesics are tricyclic antidepressants[e.g. doxepin hydrochloride, DH] since they may induce an analgesic local effect [3]. Oral administration of DH has also side effects related to its main activity as antidepressant, especially after parenteral administration. Therefore, the administration of DH directly to the oral cavity could be an interesting approach against oral mucositis, and the objective of this study was to develop DH-based mucoadhesive films.

Materials and Methods: Films were prepared using a solvent evaporation process. To improve the performance of the patch and release characteristics, glycerol was used as plasticizer and polyvinylpyrrolidone as a film-forming polymer. The procedure consisted in dispersing SCMC and HPMC in water (at 4% and 3%, respectively) and chitosan in an acetic acid aqueous solution (1.5%, v/v). Then, adequate plasticizers were added under continuous stirring to obtain a suitable viscosity dispersion. The mixtures were added onto a petri dish (5 g/petri dish), then stored at 4 ºC for 48 h, and finally dried at 25 ºC during 24 h. For the permeation study, it was used a reverse chicken skin after removal of superficial fat content (mucosal tissue model). These skin panicles were mounted in a stainless steel discs of 4 cm in diameter and a central aperture of 2 cm in diameter (which allowed the exposure of the mucosa during the release assay). Franz-type diffusion cells FDC-400 cell (Vidra-Foc, Barcelona, Spain) used in this experiment consisted of 2 compartments with a membrane clamped between the donor and receiver chambers. The receptor phase was phosphate buffer (pH 6.8). Finally, DH was determined spectophotometrically (λ = 293 nm).

Results and Discussion: It was observed a similar DH release profile for the HPMC and chitosan systems during the first 6 h of assay, with a quick drug release in the 1st h (41.06 ± 7.77%, for HPMC, and 47.47 ± 7.3%, for chitosan). This could be the consequence of a burst effect, frequently observed in different polymeric vectors [4-6], and which possibly comes from the relaxation of polymer chains that swell up in the presence of humidity. For the SCMC systems, it was observed a sustained release during the first 24 h (63.45 ± 11.54%). This may be due to the electrostatic interaction occurring between the anionic polymeric chains and the drug (positively charged at the pH of medium). Orabase® preparations (5%) showed a latency time of 15 min with a release of 2.3%. In the permeation study, the systems presented a sustained permeation, and HPMC led to a higher percentage of DH permeated. Furthermore, Orabase® presented only 0.6% of permeation, hence demonstrating the effectiveness of the systems formulated (DH permeation through the oral mucosa). Also, with the 3 formulations studied, SCMC (19.91%), HPMC (69.5%), and chitosan (24.17%), it was increased the amount of drug permeated in comparison to the drug solution (8.26%).

Conclusions: DH release from the formulations was similar and significantly higher than those of the Orabase® preparations. It was confirmed that the systems showed sustained drug permeation and that the HMPC system induced a higher drug permeation. Moreover, the formulations did not present any significant latency period. Therefore, all the films resulted in DH release profiles appropriate for the treatment of pain in oral mucositis.

References:

[1] Katranci N, Ovayolu N, Ovayolu O, Sevinc A. Eur J Oncol Nurs **16** (2012) 339.
[2] Rodríguez A, Torres D, Robles M, Pachón J, González D, Gutiérrez JL. Int J Oral Max Surg **41** (2012) 225.
[3] McCleane G. Anesthesiol Clin **25** (2007) 825.
[4] Nafee NA, Ismail FA, Boraie NA, Mortada LM. Int J Pharm **264** (2003) 1.
[5] Wong CF, Yuen KH, Peh KK. Int J Pharm **178** (1999) 11.
[6] McCarron PA, Woolfson AD, Keating SM. J Pharm Pharmacol **52** (2000) 1451.

Polystyrene nanoparticles as delivery system: effect of functionalization on the formation of protein corona

Victoria Cano-Cortés, Mª Paz Ruíz-Blas, Juan José Díaz-Mochón, Rosario María Sánchez-Martín

Department of Medicinal and Organic Chemistry, University of Granada; and Pfizer - Universidad de Granada - Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Spain.
e-mail: vccortes@ugr.es

Introduction: One of the principal goals of nanomedicine is the development of an efficient delivery system able to transport the bioactive cargo in an efficient manner. In previous studies our research team has synthesized monodisperse amino-functionalized polystyrene nanoparticles (NPs), and tested them on a wide number of different cell lines, including primary cultures and embryonic stem cells [1]. An efficient uptake has been achieved by using these NPs loaded with various cargos such as nucleic acids, functional proteins and bioactive molecules [2-4]. Promising biological applications that these NPs have shown make of key importance to carry out a study of the interactions between these NPs with biological systems. In the biological environment, NPs are surrounded by a complex mixture of serum´s extracellular proteins that are adsorbed onto the NP surface forming protein corona, thereby altering its function. It is crucial to modulate these interactions when NPs are designed to ensure that the desired effect is achieved. We aim to study how functionalization of the NPs with different functional groups together with the conjugation of different biomolecules, such as peptides, antibodies (Abs), nucleic acids, etc., can affect the formation of the corona.

Materials and Methods: Amino-functionalized polystyrene NPs (460 nm) were prepared by dispersion polymerization [5], and functionalized with poly(ethylene glycol) and Fmoc-Lys spacer-Dde (OH) [6]. Following different conjugation strategies, NPs were functionalized with biomolecules, in particular, a homing peptide, an Ab and a transcription factor were conjugated. Different functionalized and non-functionalized NPs (NPs/mL) [7] were incubated with different culture media: Dulbecco´s modified Eagle medium (DMEM), RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), glutamine (4 mM) and antibiotics (penicillin and streptomycin, 100 units/mL), in reduced-serum medium (Opti-minimal essential medium, Opti-MEM™) OptiMEM™ and 2 buffers enriched to promote cell growth, TDB (trypanosome dilution buffer) and PBS supplemented with glucose for 3 and 24 h stirred (1400rpm) and 37 °C. After the incubation period, the NPs were washed twice with sterile PBS to remove non-binding proteins. To separate proteins of the NPs, they were re-suspended in 2× Laemmli simply buffer and then incubated under stirring at 95 ºC for 5 min. The NPs were centrifuged and supernatants were loaded on SDS/PAGE gel. Finally, the gel was stained with Coomassie blue solution and the image was processed.

Results and Discussion: A monodispersed population of 460 nm amino-functionalized cross-linked NPs was obtained by dispersion polymerization. PEGylation of NPs was performed following a Fmoc solid phase protocol and using Oxyma/DIC as coupling reagents. This PEGylation reduces the formation of the protein corona. Bifunctionalization of the NPs was achieved by using an orthogonally protected lysine Fmoc-Lys spacer-Dde (OH) and different conjugation strategies were optimized to link diverse biomolecules to NPs. The non-functionalized NPs shown similar protein corona regardless of the nature of the culture medium, while functionalized NPs with different biomolecules exhibited considerable decrease formation of protein corona. Furthermore, protein corona was not formed when the NPs were incubated with TDB and PBS with glucose.

Conclusions: We have shown how the functionalization of NPs decreases the formation of protein corona, ensuring the NP´s functionality in biological applications.

Acknowledgements: This project has received funding from the 7th European Community Framework Program (FP7-PEOPLE-2011-CIG-Project Number 294142 and FP7-PEOPLE-2012-CIG-Project Number 322276).

References:

[1] Tsakiridis A, Alexander, LM, Gennet N, Sánchez-Martín RM, Livigni A, Li M, Bradley M, Brickman JM. Biomaterials **30** (2009) 5853.
[2] Unciti-Broceta A, Díaz-Mochón JJ, Sánchez-Martín RM, Bradley M. Acc Chem Res **15** (2012) 1140.
[3] Cárdenas-Maestre JM, Pérez-López AM, Bradley M, Sánchez Martín RM. Macromol Biosci **7** (2014) 923.
[4] Cardenas-Maestre JM, Panadero-Fajardo S, Pérez-López AM, Sánchez-Martín RM. J Mater Chem **21** (2011) 12735.
[5] Unciti-Broceta A, Johansson EMV, Yusop RM, Sánchez-Martín RM, Bradley M. Nat Protoc **7** (2012) 1207.
[6] Cabeza L, Cano-Cortes V, Rodríguez MJ, Vélez C, Melguizo C, Sánchez-Martín RM, Prados JC. J Nanopart Res **17** (2015) 37.
[7] Unciti-Broceta JD, Cano-Cortés V, Altea-Manzano P, Pernagallo S, Díaz-Mochón JJ, Sánchez-Martín RM. Sci Rep **5** (2015) 10091.

Polymeric nanoparticles loaded with Paclitaxel: a new innovative therapy for the treatment of lung cancer

Julia Jiménez-López[1,2], Lucía Martín-Banderas[3], Raúl Ortiz[1,4], Mª Carmen Leiva[1,2], José C. Prados[1,2], José Manuel Baeyens[5], Consolación Melguizo[1,2]

[1]Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain.
[2]Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
[3]Department of Pharmacy and Pharmaceutical Technology, University of Seville, Spain.
[4]Department of Health Science, University of Jaén, Spain.
[5]Department of Pharmacology, University of Granada, Spain.
e-mail: mcleiva@ugr.es

Introduction: Lung cancer is the tumor with the highest mortality rate worldwide. Paclitaxel (PTX), an antimitotic agent which causes the kidnapping of the cell in the G_2 phase of the cell cycle, is widely used as a treatment of choice for treating this type of cancer [1, 2]. This drug is typically formulated in Cremophor® excipient to avoid its insoluble nature. However, classical formulations induce many undesirable effects, among which stands its high toxicity. In addition, PTX has poor stability and low tumor specificity [3]. A potential strategy to overcome these limitations is the use of nanoparticles (NPs) as drug delivery system [2, 4]. The aim of this work is to develop polymeric NPs loading PTX as a new strategy to the treatment of lung cancer.

Materials and Methods: PTX-loaded polymeric NPs were synthetized with a modified nanoprecipitation method [5]. Proliferation assays were performed to compare the effect of PTX free and biodegradable polymeric NPs loaded with the drug (NP-PTX). We used the human lung tumor cell lines (LTCL) A549, the murine LTCL LL2, and the human no-tumor cell line L132. For this purpose, cell lines were seeded into 24-well plate and were incubated with a concentration range [1-55 nM] of PTX and NP-PTX for 96 h. Medium was replaced after 48 h and new drugs were added. Cytotoxicity studies with blank NPs were performed. Cytotoxic activity was determinate with sulforhodamine B assay. In addition, A549 cell line was incubated with the same concentration of nile red (NR) and NR-loaded NPs at different incubation times (0.5, 1, 2 and 4 h) for cellular uptake studies. Results were analyzed with fluorescence microscopy. Finally, multicellular tumor spheroids were obtained from A549 cells cultured in 96-well plates previously pre-coated with 50 µL 1% agarose. After 3 days, MCTS were treated with the corresponding IC_{50} dose of free PTX and NP-PTX and blank NPs at the same concentration during 96 h. MCTS growth was monitored with imaging microscopy, measuring the longest and shortest diameter to calculate the volume of MCTS.

Results and Discussion: Proliferation assays showed a significant reduction of the IC_{50} dose with NP-PTX (3.63, 2.69 and 2.96 times lower in A549, LL2 and L132, respectively) compared with free PTX. The safety of NPs was compared to the cytotoxicity assays where no inhibition was observed in the growth of any cell lines. Intracellular uptake studies with NR and NR-loaded NPs by cytometer and fluorescence microscopy showed that NPs improved the incorporation of NR inside the cells at different incubation times. Finally, MCTS assays showed a significant reduction in the volume of spheroids when they were treated with free PTX and NP-PTX. However, this reduction at 9th day was 46% in spheroids treated with free PTX and 73% in those treated with NP-PTX, which is a significant improvement of the treatment of MCTS. No significant difference was observed between MCTS untreated and treated with blank NPs.

Conclusions: New polymeric NPs associated with PTX could be a new strategy for the treatment of lung cancer, which would increase the therapeutic window while would reduce side effects.

Acknowledgements: Financial support from Junta de Andalucía, Spain, under Project P11-CTS-7649 and PI-0049 is gratefully acknowledged. LMB is specially grateful to "V Plan Propio de Investigación" (University of Seville).

References:

[1] Uesato S, Yamashita H, Maeda R, Hirata Y, Yamamoto M, Matsue S, Nagaoka Y, Shibano M, Taniguchi M, Baba K, Ju-ichi M. Planta Med **80** (2014) 452.
[2] Surapaneni MS, Das SK, Das NG. ISRN Pharmacol **2012** (2012) 623139.
[3] Ma P, Mumper RJ. J Nanomed Nanotechnol **4** (2013) 1000164.
[4] Babu A, Templeton AK, Munshi A, Ramesh R. J Nanomater **2013** (2013) 1.
[5] Martín-Banderas L, Alvarez-Fuentes J, Durán-Lobato M, Prados J, Melguizo C, Fernández-Arévalo M, Holgado MÁ. Int J Nanomedicine **7** (2012) 5793.

A preliminary study poly(ε-caprolactone) and poly(butylcyanoacrylate) magnetic nanoparticles to improve the chemotherapeutic cancer treatment

Laura Cabeza[1,2], José L. Arias[1,2,3], Gloria Perazzoli[1,2], Raúl Ortiz[1,4], Mª Carmen Leiva[1,2], Consolación Melguizo[1,2], Beatriz Clares[2,3], Ángel V. Delgado[5], José C. Prados[1,2]

[1]Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain.
[2]Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
[3]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[4]Department of Health Science, University of Jaén, Spain.
[5]Department of Applied Physics, University of Granada, Spain.
e-mail: mcleiva@ugr.es

Introduction: Chemotherapeutic cancer treatment, especially in advanced cancer, shows serious limitations. Main causes of cancer treatment failure are: the low specificity of these drugs toward tumor tissues, the specific characteristics of their metabolism, and/or the difficulty in the drug administration [1, 2]. The use of nanoparticles (NPs) associated to antitumor drugs may improve the cancer response. For instance, magnetic NPs may help to increase the drug specificity. On the other hand, NPs tend to accumulate in tumor tissues because of the enhanced permeability and retention (EPR) effect [3]. The incorporation of magnetic cores into NPs, allows directing them to tumor tissues by using a magnetic field [4]. The aim of this work is to test the cytotoxicity of two magnetic nanopolymers [poly(ε-caprolactone), PCL, and poly(butylcyanoacrylate), PBCA] containing magnetite (Fe_3O_4) cores, and their utility in the treatment of tumors.

Materials and Methods: PCL NPs and PBCA NPs with a magnetic core were obtained. Cell cytotoxicity of the NPs was determined using proliferation assays on the human colon cancer cell line T84 and the human non-tumor cell line from colon tissue CCD18. These cell lines were treated with different concentrations of magnetic PCL NPs (Fe-PCL) and magnetic PBCA NPs (Fe-PBCA) (ranging from 0.05 to 100 µg/mL) and with free Fe_3O_4 during 48 and 72 h. The percentage of proliferation was measured by the sulforhodamine B method. Untreated cells were used as negative control. We studied cell uptake of magnetic NPs by transmission electron microscopy (TEM) on cells treated with 10 µg/mL of magnetic NPs for 5, 15, 30, 60 and 120 min. Free magnetic NPs were also observed by TEM. NP uptake was also studied with the Prussian blue staining, by dyeing the iron inside cells, and light microscopy. For the *in vivo* studies, the leukemia cell line L1210 was used to generate subcutaneous tumors on DBA/2 mice. Mice were treated with magnetic NPs (5 mg/Kg) and magnets were used for 2 h to direct the NPs to the tumors. Another group of mice was not exposed to the magnet. After that, mice were sacrificed, tumors were embedded in paraffin and sections of 3 µm were dyed by the Prussian blue staining.

Results and Discussion: Magnetic NPs showed no cytotoxicity in any cell line at 48 and 72 h of exposition. Only some toxicity was detected in CCD18 cells when treated with free Fe_3O_4. TEM analysis showed the spherical shape of magnetic NPs. They could be observed into the cells after a short exposition times and even after 60 and 120 min. Prussian blue staining showed blue areas, thus indicating the presence of cells with iron inside after exposure to the magnetic NPs in comparison with cells that were not treated. *In vivo* studies showed a greater presence of magnetic NPs in tissues exposed to the magnet. These results demonstrated the high biocompatibility of the Fe-PCL NPs and the Fe-PBCA NPs. Moreover, a greater accumulation of NPs in tumor tissues shows that by using a magnetic field, magnetic NPs may accumulate into the tumor tissue where they could release the loaded drug.

Conclusions: Fe-PCL NPs and Fe-PBCA NPs could be ideal nanocarriers of chemotherapeutic drugs to improve cancer treatment, showing a high biocompatibility and a great accumulation in tumor interstitium when guided by a magnetic field.

Acknowledgements: Financial support from projects P11-CTS-7649, PI-0049, and PE-2012-FQM-694 (Junta de Andalucía, Spain) is gratefully acknowledged.

References:

[1] Adams JD, Flora KP, Goldspiel BR, Wilson JW, Arbuck SG, Finley R. J Natl Cancer Inst Monogr **15** (1993) 141.
[2] Carvalho C, Santos RX, Cardoso S, Correia S, Oliveira PJ, Santos MS, Moreira PI. Curr Med Chem **16** (2009) 3267.
[3] Maeda H. Bioconjug Chem **21** (2010) 797.
[4] Reddy LH, Arias JL, Nicolas J, Couvreur P. Chem Rev **112** (2012) 5818.

Uptake efficiency and biological effects of functionalized cross-linked polystyrene nanospheres in human melanoma cancer stem cells subpopulations

Saúl A. Navarro[1,2], Victoria Cano-Cortés[3,4], Cynthia Morata-Tarifa[1,2,5], Rosario M. Sánchez-Martín[3,4], Juan Antonio Marchal[1,2,6]

[1]Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain.
[2]Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
[3]Department of Medicinal and Organic Chemistry, University of Granada, Spain.
[4]Pfizer - Universidad de Granada - Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Granada, Spain.
[5]Braman Family Breast Cancer Institute, Sylvester Comprehensive Cancer Center, University of Miami, USA.
[6]Department of Human Anatomy and Embryology, University of Granada, Spain.
e-mail: vccortes@ugr.es

Introduction: Cancer stem cells (CSCs), a rare rate of cells inside tumors, are responsible for the initiation and sustained growth of tumors, the resistance to therapy, progression, relapse and metastasis. The abilityof CSCs to move to other sites in the body and to initiate a new tumor mass, place these cells as an important factor in the formation of metastases [1]. In addition, it has been described that *mi*RNAs are important regulators of CSCs, since in these cases an aberrant expression of *mi*RNAs leads to increased levels of self-renewal and decreased levels of apoptosis, 2 characteristic phenomena of CSCs [2]. Preliminary results have determined novel *mi*RNAs that are up-regulated in melanoma aldehyde dehydrogenase ($ALDH^+$) CSCs regarding the tumor population control. Our group has designed and developed a new chemical method for preparing a range of functionalized nanoparticles (NPs), monodisperse and cross-linked with various sizes available depending on the biological application (100 nm - 2 μm). Recently, we have optimized the release of these NPs efficiently in human mesenchymal stem cells [3]. Our main objective was to evaluate the uptake efficiency and biological effects of several functionalized cross-linked polystyrene nanospheres in human melanoma $ALDH^+$ CSCs subpopulations.

Materials and Methods: Functionalized cross-linked polystyrene NPs were synthesized and fluorescently labeled for delivering into cells. For the assays, we used naked polystyrene nanospheres and 2 NPs obtained following a Fmoc solid phase protocol using Oxyma/DIC as coupling, VCC 506.3 (-COOH) and VCC 506.4 ($-NH_2$) labeled both with Cy5. 5×10^4 G-361 human melanoma cells were seeded on 24-well plates. 24 h later 6.5×10^3 naked polystyrene 460 nm NPs re-suspended in medium were added on the cell culture. After 8 h of incubation, medium with NPs was removed. 72 h later cells were used for determination of ALDH activity, cell cycle distribution and apoptosis by flow cytometry. In addition, G-361 cells were separated by differential trypsinization and then sorted based on their ALDH activity and by specific surface markers by FACS and seeded on 6-well low adherence plates with spheres medium. 24 h later, 5×10^4 $ALDH^+$ cells were seeded on 24-well low adherence plates per well with spheres medium, and different concentrations of NPs/cell were added to the cell culture and incubated 1-8 h. After incubation, the efficiency of NP internalization was determined by flow cytometry (FACS CANTO II) and data analysis (FACS DIVA software).

Results and Discussion: Incubation with NPs did not cause a significant change in the percentage of $ALDH^+$ cells. Furthermore, entry of the NPs does not affect the expression of surface markers, apoptosis levels or cell cycle profile of $ALDH^+$ cells. Our results indicate that these NPs do not alter the physiological state of cells and therefore can be used in subsequent vehiculization studies. The internalization rate of VCC 506.4 NPs was higher, almost 100% in the shortest incubation time, and faster than in VCC 506.3 NPs.

Conclusions: Functionalized cross-linked polystyrene nanospheres are not toxic and do not alter the physiological state or cell morphology of melanoma CSCs subpopulations. In addition, we demonstrate the high uptake of these NPs in CSCs and how NPs do not affect the stem characteristics of these tumor initiating cells. Our results encourage *in vitro* and *in vivo* studies about the therapeutic efficacy of functionalized cross-linked polystyrene NPs as vehicles for drugs and/or anti-*mi*RNAs targeting CSCs.

Acknowledgements: Financial support from Fundación Progreso y Salud, Consejería de Salud (Junta de Andalucía, project nº. PI-0533-2014) and the 7th European Community Framework Program (FP7-PEOPLE-2011-CIG-Project Number 294142) is gratefully acknowledged.

References:

[1] (a) Clevers H. Nat Med **17** (2011) 313; (b) Valent P, Bonnet D, De Maria R, Lapidot T, Copland M, Melo JV, Chomienne C, Ishikawa F, Schuringa JJ, Stassi G, Huntly B, Herrmann H, Soulier J, Roesch A, Schuurhuis GJ, Wöhrer S, Arock M, Zuber J, Cerny-Reiterer S, Johnsen HE, Andreeff M, Eaves C. Nat Rev Cancer **12** (2012) 767.
[2] Garg M. World J Stem Cells **4** (2012) 62.
[3] Sanchez-Martin R, Cano-Cortés V, Marchal JA, Perán M. Methods Mol Biol **1058** (2013) 41.

Treatment of retinitis pigmentosa: ocular absorption of glutathion (GSH)

María Sebastian Morello[1], *María Aracely Calatayud Pascual*[1], *María Miranda*[2], *Cristina Balaguer Fernández*[1], *Alicia López Castellano*[1]

[1]Departamento de Farmacia, Instituto de Ciencias Biomédicas, Universidad CEU Cardenal Herrera, Valencia, Spain.
[2]Departamento de Biomedicina, Instituto de Ciencias Biomédicas, Universidad CEU Cardenal Herrera, Valencia, Spain.
e-mail: maria.sebastian@uch.ceu.es

Introduction: Retinitis pigmentosa is a group of degenerative diseases that decrease vision, frequently leading to blindness. This disease is characterized for the loss of photoreceptor density and loss of other retinal cells. Main causes of this disorder are genetic defects that normally the most affected cells are rods. Most common symptoms are decreased vision in low light and tunnel vision. Actually, the treatment that is used is not so effective but there are several studies that maybe give more time or safe patient´s vision. One of each is the administration of antioxidants [1, 2]. The aim of this work is to study *in vitro* the absorption of glutathione (GSH) through the eye cornea and sclera, and to include this molecule in the therapeutic arsenal of retinitis pigmentosa.

Materials and Methods: *In vitro* absorption studies were carried out for 3 h with ocular membranes. Such membranes were obtained from hybrid rabbits eyes. To carry out the experiments, diffusion Franz cells were used. The ocular membrane was fixed between the 2 compartments of Franz cells. The donor compartment was filled with GSH 10 mg/mL isotonic solution and the receptor compartment with buffer solution (pH 7.4). Samples (180 µL) were taken from the receptor compartment at predetermined times and then GSH was analyzed by a validated HPLC method [3].

Results and Discussion: *In vitro* studies with both membranes, showed the possible absorption of GSH through them. When we used sclera, it was shown that GSH absorption was significantly higher than the absorption obtained with the corneas ($p < 0.05$), and we detected glutathione disulfide (GSSG), the oxidized form of the molecule, in the samples obtained of the sclera experiments. In the studies performed with the sclera, detectable concentrations of GSH were observed after 30 min, whereas in studies with corneas no detectable concentrations of antioxidant were detected after 60 min.

Conclusions: The antioxidant GSH is able to cross the barriers that exist between ocular surface and retina. Further studies will be needed to test the use of ocular promoting strategies, and carried out to reduce the start up time of absorption of GSH.

References:

[1] Sanz MM, Johnson LE, Ahuja S, Ekström PA, Romero J, van Veen T. Neuroscience **145** (2007) 1120.
[2] Sternberg P Jr, Davidson PC, Jones DP, Hagen TM, Reed RL, Drews-Botsch C. Invest Ophthalmol Vis Sci **34** (1993) 3661.
[3] Reed DJ, Babson JR, Beatty PW, Brodie AE, Ellis WW, Potter DW. Anal Biochem **106** (1980) 55.

Preparation and characterization of Venlafaxine-PLGA nanoparticles for brain delivery

María Dolores Cayero-Otero, Lucía Martín-Banderas, Mercedes Fernández-Arévalo

Department of Pharmacy and Pharmaceutical Technology, University of Seville, Spain.
e-mail: mcayero@us.es

Introduction: The blood-brain barrier (BBB) is one of the strongest security systems of the human body, preventing a wide range of substances present in the bloodstream (including numerous drugs) from freely diffusing and penetrating into the central nervous system (CNS). Therefore, crossing the BBB is a major challenge for the efficient drug delivery in specific areas of the brain. Fortunately, BBB exhibits capillary endothelial cells with transport mechanisms mediated by receptors [1], and there are several strategies to cross the BBB [2]. Many targeting ligands have been developed to improve brain drug delivery, such as transferrin (Tf). Tf has been widely applied to enhance the cellular uptake of drug-loaded nanoparticles (NPs) since Tf-receptors are overexpressed in brain capillary endothelium and glioma cells [3]. Venlafaxine (VLF) is a dual action antidepressant that can inhibit the re-uptake of serotonin and norepinephrine, thus increasing the levels of these neurotransmitters in the synaptic cleft between neurons in the brain [4]. Oral therapy has a number of drawbacks, such as slow onset of action, side effects like tachycardia, increased blood pressure, dizziness, dry mouth, sexual dysfunction and bioavailability of 40-45%. Additionally, VLF has a plasma half-life of 4-5 h, therefore needing frequent administration to maintain an effective therapeutic plasmatic level [5].

Materials and Methods: VLF-loaded NPs were prepared by emulsion SEV and NPP. In the SEV technique, an emulsion was prepared by mixing VLF aqueous suspension (20-25%, w/w) containing PVA (0.5%, w/v) with a 4% (w/v) PLGA in ethylacetate using a homogeneizer. The solvent was evaporated under magnetic stirring (4 h). In NNP, PLGA (1.5%, w/v) was co-disolved with Span 60® in acetone containing VLF (10%, w/w). This solution was added dropwise by using a syringe pump, under magnetic stirring, to 9 mL of a Pluronic® F-68 aqueous solution (0.5%, w/v). Acetone was evaporated at room temperature during 4 h. In both methods, NPs were collected by ultracentrifugation (10000 rpm, 4 ºC, 30 min); size and zeta potential (ZP) were previously measured. Geometry was examined by transmission electron microscope. The encapsulation efficiency was determined by HPLC, by quantifying the amount of drug in the supernatant. Finally, NPs were washed and lyophilized. Then, Tf was conjugated on the surface of VLF-loaded NPs using a surface-activation method (carbodiimide method). In brief, VLF-loaded NPs were dispersed in PBS followed by addition of EDC and NHS solutions dropwise. The activation was carried out by agitating for 4 h; activated NPs were recovered by ultracentrifugation. For conjugation of Tf, the activated NPs were dispersed in PBS followed by dropwise addition of Tf (1 mg/mL). After agitation for 2 h, the NPs were incubated overnight at 4 ºC. Finally, Tf-conjugated VLF-loaded NPs were recovered by ultracentrifugation and the supernatant was collected for indirect quantification of conjugation efficiency.

Results and Discussion: Particle size, PdI and ZP of NPs were characterized by DLS. VLF-NPs prepared by SEV had a mean size of 253.02 ± 13.10 nm with a ZP of -3.30 ± 1.74 mV. PdI of 0.043 ± 0.03 indicated monodisperse stable system. After conjugation, size increased about 24% and ZP decreased around 26%. Tf-conjugated VLF-loaded NPs showed a mean size of 312.94 ± 27.93 nm with a PdI less than 0.2 and a ZP of -2.40 ± 0.56 mV. NPs had an average encapsulation efficiency of up to 65.5 ± 2.65% and the conjugation efficiency was 91.86 ± 0.72. VLF-loaded NPs prepared by NPP exhibited a mean size of 403.32 ± 36.10 nm with a PdI of 0.82 ± 0.14 and a ZP of -30.85 ± 4.68 mV. The conjugation was not effective in this method because the NPs tended to aggregate. The NPs had an encapsulation efficiency of 27.11 ± 5.10%. TEM analysis revealed that VLF-loaded NPs prepared by both methods had a solid structure and spherical shape.

Conclusions: PLGA NPs prepared by solvent emulsion evaporation method were superior to those produced by NNP. Compared with NNP, emulsion SEV technique resulted in NPs with a more suitable size to penetrate the BBB, a higher homogeneity and higher encapsulation efficiency. Furthermore, ligand conjugation to NPs prepared by NNP was not successful due to aggregate forms.

Acknowledgements: Financial support from Junta de Andalucía, Spain, and "V Plan Propio de Investigación" of the University of Seville.

References:

[1] Yan F, Wang Y, He S, Ku S, Gu W, Ye L. J Mater Sci Mater Med **24** (2013) 2371.
[2] Martín-Banderas L, Holgado MA, Venero JL, Álvarez-Fuentes J, Fernández-Arévalo M. Curr Med Chem **18** (2011) 5303.
[3] Cui Y, Xu Q, Chow PK, Wang D, Wang CH. Biomaterials **34** (2013) 8511.
[4] Gutierrez AM, Stimmel LG, Aiso YJ. Clin Ther **25** (2003) 2138.
[5] Troy SM, Parekar VD, Fruncillo RJ, Chiang ST. J Clin Pharmacol **35** (1995) 404.

PLA/PEG-*b*-PLA particles: promising peptide carriers

Merari Tumin Chevalier[1,*], *Mónica Cristina Garcia*[2,*], *Daniela Gonzalez*[3], *Alvaro Jimenez-Kairuz*[2], *Vera Alvarez*[1]

[1]Grupo de Materiales Compuestos de Matriz Polimérica (CoMP), INTEMA - Facultad de Ingeniería, Universidad Nacional de Mar del Plata, Argentina.
[2]Unidad de Investigación y Desarrollo en Tecnología Farmacéutica (UNITEFA)-CONICET. Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina.
[3]Department of Chemical and Biomolecular Engineering, University of Nebraska-Lincoln, USA.
e-mail: merari.tumin.chevalier@gmail.com [*]Both authors contributed equally to this work.

Introduction: The last decade has seen significant technological progress in the development of new pharmaceutical platforms to improve current pharmacotherapy. In particular, therapeutically effective and patient-compliant drug delivery systems continuously lead researchers to design novel tools and strategies. The primary method of accomplishing drug controlled release has been through incorporating the actives within polymers. Particularly, polymeric micro- and nanoparticles as drug carriers can be made from a wide variety of natural and synthetic polymers. Due to particle´s ability to improve the pharmacotherapy treatment, such devices are being extensively studied and used as drug carriers and controlled release systems in the field of biomaterials, medicine and pharmacy. Considering poly(D,L-lactide) (PLA) capability for high loading drugs and its ability to modulate drug release, this work attempts to study the physicochemical and biopharmaceutical properties of PLA/PEG-*b*-PLA polylisine particulated carriers.

Materials and Methods: Microparticles were obtained by a proprietary electrohydrodynamic technology (Bio-Target Inc., USA; LNK Chemsolutions LLC, USA). Briefly, an organic solution containing all the necessary components was processed using this technology resulting in a dry collection of the specified microparticles. Particles were characterized by scanning electron microscopy (SEM), thermo-gravimetric analysis (TGA), differential scanning calorimetry (DSC), and Fourier transform infrared spectroscopy (FTIR). UV-Vis spectrophotometry was used to determine the encapsulation efficiency (EE) of the obtained system. Polylisine release from particles was performed in a bicompartimental diffusion device (Franz´s cells) mounted with a semisynthetic cellulose membrane. The donor compartment was filled with 0.5 mL of particles dispersed in 1% BSA in PBS and kept in contact with 16 mL of receptor medium at 37 °C. Ringer solution was used as receptor media. Samples of 1 mL of receptor medium were withdrawn at predetermined time intervals and replaced with equal quantities of fresh medium. The amount of drug released was determined by high performance liquid chromatography (HPLC).

Results and Discussion: Results obtained showed that the particles exhibit thermal stability (Figure 1). The TGA showed a thermal decomposition of the particles at 275 °C. DSC showed an endothermic event at 58 °C attributed to the glass transition temperature of PLA. There are not differences in the thermal analysis between particles loaded with polylisine and control. The particles have a high percentage of polylisine loaded; the EE calculations yielded a value of 81%.

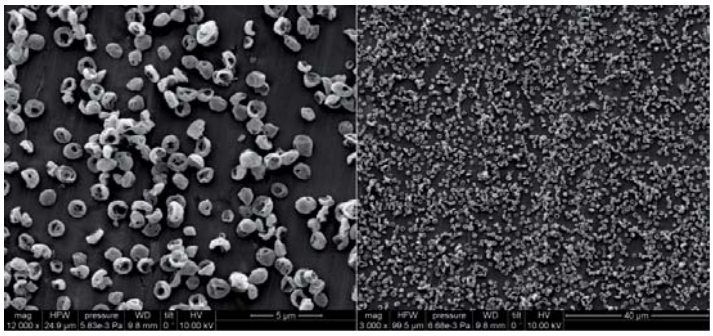

Figure 1. SEM micrographs of polylisine-loaded PLA/PEG-*b*-PLA microparticles.

Conclusions: The properties of these particles are encouraging for the design of controlled drug release systems for peptides that could improve existing therapies in the field of health.

References:

[1] Tiwari G, Tiwari R, Sriwastawa B, Bhati L, Pandey S, Pandey P, Bannerjee SK. Int J Pharm Investig **2** (2012) 2.

Anticonvulsant effects of liposomal formulation containing nimodipine in mice

Lina C. G. e A. I. Moreno[1,2,3], *Rivelilson M. Freitas*[2], *Juan M. Irache*[1], *Nereide S. Santos Magalhães*[3]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Navarra, Spain.
[2]Laboratory of Experimental Neurochemistry Research, Federal University of Piaui, Brazil.
[3]Immunopathology Keizo-Asami Laboratory, Federal University of Pernambuco, Brazil.
e-mail: linaclaramoreno@gmail.com

Introduction: Nimodipine (NMD) has been shown to have an inhibitory action on seizures and brain damage in rodents [1]. However, the pharmaceutical applicability of this drug is limited by its low solubility in gastrointestinal fluids and its high first-pass effect in the liver, which leads to low bioavailability [2]. These difficulties can be overcome through the use of liposomes [3]. The aim of the present study is to evaluate the anticonvulsant activity of liposomes containing NMD (NMD-Lipo) on pilocarpine-induced seizures.

Materials and Methods: NMD-Lipo were prepared using the method of hydrating the lipid film. The possible anticonvulsant activity was tested by the pilocarpine model. Mice were divided into 22 groups, with each group containing 12 animals and were treated with saline, diazepam, free NMD (at the doses of 0.1, 1 and 10 mg/Kg), NMD-Lipo (at the doses of 0.1, 1 and 10 mg/Kg) and empty liposomes with and without combination with pilocarpine in a dose of 400 mg/Kg. After the treatments, the animals were recorded in 30 cm × 30 cm chambers with: appearance of peripheral cholinergic signs (miosis, piloerection, chromodacryorrhea, diarrhea, and urination), stereotyped movements (continuous sniffing, paw licking, and rearing), tremors, seizures, status epilepticus, and mortality rate, during 24 h.

Results and Discussion: Free NMD at the dose of 0.1, 1 and 10 mg/Kg did not reduce the occurrence of peripheral cholinergic signs, stereotypic movements, and tremors. Moreover, free NMD at the doses of 0.1, 1 and 10 mg/Kg was unable to prevent the installation of the seizure and reduce the mortality rate in mice. On the other hand, NMD-Lipo at doses of 0.1, 1, and 10 mg/Kg did not reduce the occurrence of peripheral cholinergic signs, but decreased stereotypic movements and tremors in the mice. NMD encapsulated into liposomes at all doses tested was able to prevent the occurrence of 100% of the seizures. None of the mice pretreated with NMD-Lipo and subsequently given with pilocarpine died. As expected, empty liposomes showed no anticonvulsant activity, suggesting that the liposomes potentiate the anticonvulsant effect of NMD [4].

Conclusions: The administration of the liposomal formulation at the doses of 0.1, 1, and 10 mg/Kg was able to reduce stereotypic movements and tremors. Moreover, NMD-Lipo prevented the installation of 100% of the pilocarpine-induced seizures and prevented the death of 100% of the mice treated with pilocarpine, showing even better results than the rodents treated with diazepam. The results of the anticonvulsant activity suggest that NMD has a dose-dependent effect.

Acknowledgements: This work was supported by the FACEPE, the CNPq and the CAPES (Brazil).

References:

[1] Martinez Y1, N'Gouemo P. Brain Res **1366** (2010) 211.
[2] Bege N, Renette T, Endres T, Beck-Broichsitter M, Hänggi D, Kissel T. Eur J Pharm Biopharm **84** (2013) 99.
[3] Moreno LC, da Silva Oliveira GZ, Cavalcanti IM, Santos-Magalhães NS, Rolim HM, de Freitas RM. Pharmacol Biochem Behav **116** (2014) 64.
[4] Moreno LC, Cavalcanti IM, Satyal P, Santos-Magalhães NS, Rolim HM, Freitas RM. Neurosci Lett **585** (2015) 38.

Transcorneal permeation of Pranoprofen loaded-nanoparticles

Cristina Cañadas-Enrich[1], Ana C. Calpena Campmany[1], Helen L. Alvarado Bonilla[1], Roser Sanz Casañas[1], Alexander Parra Coca[1], Guadalupe Abrego Escobar[2]

[1]Biopharmaceutical and Pharmacokinetic Unit, Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Barcelona, Spain.
[2]Department of Chemical Analysis, Faculty of Chemistry and Pharmacy, University of El Salvador, El Salvador.
e-mail: rossancas@gmail.com

Introduction: Pranoprofen (PF) is a non-steroidal anti-inflammatory drug which can be used as a safe and effective alternative anti-inflammatory treatment following strabismus and cataract surgery [1, 2]. Although this drug has shown high anti-inflammatory and analgesic efficiency, the pharmaceutical use of PF is limited by its inadequate biopharmaceutical profile. PF is commercially available as eye-drops. However, this conventional dosage form cannot be considered optimal in the treatment of ocular diseases as less than 5% of the administered drug penetrates the cornea and reaches intraocular tissues [3]. To improve these inconveniences, we formulated PF in poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles (NPs) as a new delivery system suitable for the ocular route. The formulations were developed and optimized by the solvent displacement technique [4]. Particle size determined by photon correlation spectroscopy (PCS) was ≈ 350 nm with polydisperisity index values in the range of monodisperse systems (< 0.1) and the formulation had a net negative charge (zeta potential: -7.41 mV) [5]. Optimized NPs were selected for studies regarding morphological characterization and *ex vivo* corneal permeation.

Materials and Methods: The optimum formulations were those prepared with PLGA (9.5 mg/mL) and PF (1.5 mg/mL) dissolved in 5 mL of acetone. The organic phase was poured, under moderate stirring, into 10 mL of an aqueous solution of PVA (10 mg/mL). The pH of the aqueous phase was adjusted to 5.5. The NP suspension was concentrated to 10 mL under reduced pressure. Morphological examination of the PF-loaded NP formulation was performed using a transmission electron microscopy (TEM). *Ex vivo* corneal permeation experiments were done with corneas obtained from New Zealand rabbits. Corneas were fixed between the donor and receptor compartment of Franz diffusion cells (corneal area available for permeation: 0.64 cm^2). The receptor compartment was filled with freshly prepared bicarbonate Ringer´s (BR) solution. The compartment was kept at 37.0 ± 0.5 ºC under continuous stirring. The PF-loaded NP formulation was tested against the commercial eye drops (Oftalar®, PF 0.1%). 1 mL of each sample was placed in the donor compartment. 300 µL aliquots were withdrawn from the receptor compartment at fixed times and replaced by an equivalent volume of BR solution. At the end of the study, the corneas were tested for retained drug by extracting the PF with a fixed volume of methanol: water (50:50, v:v) under sonication. The amounts of PF permeated (Q_p) and retained (Q_r) through the cornea were analyzed by HPLC. PF permeation parameters, such as permeability coefficient (K_p) and lag time (t_l) were calculated from the amounts permeated across cornea.

Results and Discussion: TEM image revealed that the tested PF-loaded NPs were of spherical shape with smooth surface, with an average diameter of ≈ 300 nm without aggregation. Difference in the diameter when measured by PCS (350 nm [5]) or TEM (300 nm) could be allocated to the PCS measuring the "hydratated diameter" of the NPs in the aqueous medium, which is often bigger than the actual size of the NPs [6]. On the other hand, PF permeation parameters estimated from the corneal permeated amounts in the comparative study using PF-loaded NPs and Oftalar® were, K_p: 9.42 × 10^{-2} cm/h and t_l: 3.76 × 10^{-1} h, and K_p: 3.46 × 10^{-2} cm/h and t_l: 4.19 × 10^{-1} h, respectively. The Q_r and Q_p of PF values obtained from the PF-loaded NPs and Oftalar® after 6 h of study were: Q_p: 33.11 %/cm^2 and Q_r: 83.78 %/cm^2/g, and Q_p: 13.53 %/cm^2 and Q_r: 52.55 %/cm^2/g, respectively.

Conclusions: According to the results obtained from the physicochemical characterization and morphological examination of the optimized PF-loaded NP formulation, it can be concluded that these systems show an average size appropriate for ocular administration. Additionally, the permeation profile of the formulation revealed higher amounts of permeated drug with the PF-loaded NPs than those obtained with Oftalar®. In the same way, amounts of PF retained in the cornea were higher for the NPs. Therefore, the PF-loaded NPs might be a suitable and effective system for ocular application of PF.

Acknowledgements: This research has been supported by the Ministry of Science and Innovation of Spain MAT 2011-26994 and MAT 2014-59134R.

References:

[1] Akyol-Salman I, Leçe-Sertöz D, Baykal O. J Ocul Pharmacol Ther **23** (2007) 280.
[2] Sawa M, Masuda K, Nakashima M. IOL RS **13** (1999) 193.
[3] Araújo J, Gonzalez E, Egea MA, Garcia ML, Souto EB. Nanomedicine **5 (**2009) 394.
[4] Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Int J Pharm **55** (1989) R1.
[5] Abrego G, Alvarado HL, Egea MA, Gonzalez-Mira E, Calpena AC, Garcia ML. J Pharm Sci **103** (2014) 3153.
[6] Song X, Zhao Y, Hou S, Xu F, Zhao R, He J, Cai Z, Li Y, Chen Q. Eur J Pharm Biopharm **69** (2008) 445.

Magnetically responsive polyethylenimine nanocomposites: formulation conditions, composition and magnetic behaviour

Raúl Megías[1], *José L. Arias*[1,2,3], *Ángel V. Delgado*[4], *Margarita López-Viota*[1]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[2]Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain.
[3]Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
[4]Department of Applied Physics, University of Granada, Spain.
e-mail: gcetern@correo.ugr.es

Introduction: Magnetic colloids have been widely used in biomedicine for many different purposes, e.g. drug delivery, hyperthermia, and magnetic resonance imaging [1]. Nowadays, gene therapy is taking advantage of magnetic nanosystems to improve its efficiency (by the so-called magnetofection), because traditionally used viral vectors present poor transfection efficiencies and little safety for therapeutic purposes [2]. The aim of this work is to obtain a magnetic responsive non-viral vector based on 2 well-known materials, i.e. polyethylenimine (PEI, the "gold standard" of non-viral vectors) and magnetite (Fe_3O_4, successfully used in biomedicine during the last decades) [3].

Materials and Methods: Fe_3O_4 nanoparticles (NPs) were obtained by co-precipitation of ferrous salts in basic media. Later, a PEI aqueous solution (5%, w/w) was added, under magnetic stirring to the Fe_3O_4 NP aqueous dispersion. The mixture was then neutralized and stored at 4 ºC. The obtained Fe_3O_4/PEI (core/shell) NPs were characterized by photon correlation spectroscopy (PCS), microscopy analysis (SEM, STEM, and EDX), X-ray diffractometry, and determination of magnetic hysteresis cycles.

Results and Discussion: Fe_3O_4/PEI NPs were characterized by a small particle size (110.1 ± 2.1 nm) and by a strong positive surface electrical charge (66.8 ± 8.6 mV). Microscopy analysis defined the spherical particle shape, and that the Fe_3O_4 cores were satisfactorily embedded within the PEI matrix (Figure 1). X-ray diffractometry showed the crystalline structure of the Fe_3O_4 cores, necessary to assure the magnetic responsiveness, even when they are covered by the PEI shell. Hysteresis cycle determinations demonstrated the adequate magnetic properties of the Fe_3O_4/PEI NPs.

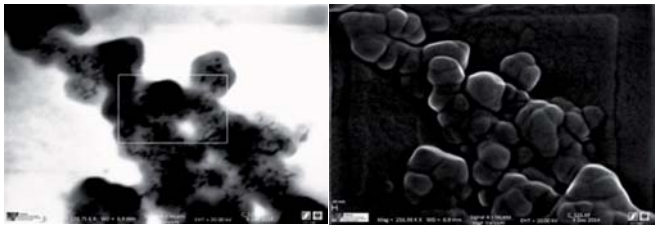

Figure 1. STEM (left) and SEM (rigth) pictures of Fe_3O_4/PEI NPs.

Conclusions: We have developed a reproducible methodology for the formulation of Fe_3O_4/PEI NPs. The preliminary physicochemical characterization of the nanocomposites ascertained their potential application in the biomedical field.

Acknowledgements: Financial support from project PE-2012-FQM-694 (Junta de Andalucía, Spain) is gratefully acknowledged.

References:

[1] Reddy LH, Arias JL, Nicolas J, Couvreur P. Chem Rev **112** (2012) 5818.
[2] Arsianti M, Lim M, Marquis CP, Amal R. Langmuir **26** (2010) 7314.
[3] Plank C, Zelphati O, Mykhaylyk O. Adv Drug Deliv Rev **63** (2011) 1300.

Tamoxifen-loaded PLGA nanoparticles: preparation and characterization

Merari Tumin Chevalier[1], *Nicoletta Rescignano*[2], *Jose Maria Kenny*[2], *Vera Alvarez*[1]

[1]Grupo de Materiales Compuestos de Matriz Polimérica (CoMP), INTEMA - Facultad de Ingeniería, Universidad Nacional de Mar del Plata, Argentina.
[2]Institute of Polymer Science and Technology, CSIC, Madrid, Spain.
e-mail: merari.tumin.chevalier@gmail.com

Introduction: Therapeutically effective and patient-compliant drug delivery systems continuously lead researchers to design novel tools and strategies. In particular, polymeric biodegradable nanoparticles (NPs) are submicron-size entities which can be made from a wide variety of natural and synthetic polymers. The existing anticancer agents do not greatly differentiate between the cancerous and normal cells, leading to systemic toxicity and adverse effects, significantly limiting the maximum permissible dose of the active agent. Drug permeation into cancer cells from conventional formulations is very poor due to an ineffective biodistribution and quick elimination. Depending upon the dose and the concentration, Tamoxifen (Tmx) has several side effects [1], such as endometrial carcinoma for postmenopausal women, liver cancer, venous thrombosis, pulmonary emboli, and ocular effects (retinopathy and corneal opacities). Considering the attractive features of PLGA for drug delivery [2], such as protection of drug from degradation, possibility of sustained release, chance to modify surface properties to provide stealthness and/or better interaction with biological materials, and possibility to target NPs to specific organs or cells, this work attempts to obtain and study PLGA NP as Tmx carriers.

Materials and Methods: Poly(D,L-lactide-*co*-glycolide) (PLGA) was used as matrix biodegradable polymer, the drug of choice to carry within the polymer was Tmx, poly(vinyl alcohol) (PVA) and poloxamer 188 were used as surfactants, chloroform ($CHCl_3$) was used as a solvent. NPs were prepared using the simple emulsion technique. Briefly, 0.2 g of PLGA were dissolved in 5 mL of $CHCl_3$ for 2 h by magnetic stirring. This solution was then mixed with an organic solution of Tmx. The resulting solution was sonicated with 2% (w/v) of a surfactant aqueous solution for 30 min using the tip sonicator (Vibra Cell Sonics mod. VC 750). To promote the formation of a stable suspension of NPs and to avoid aggregation, the generated double emulsion was transferred in 200 mL of 0.2% (w/v) PVA aqueous solution. After allowing the suspension to stabilize, a rotary evaporator was used to remove the organic solvent. The NPs were collected by centrifugation and were washed twice with distilled water. The obtained NPs were fully characterized regarding morphology and size using scanning electron microscopy (SEM) and dynamic light scattering (DLS). Thermal analysis was performed by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). High-performance liquid chromatography (HPLC) was used to determine the encapsulation efficiency (EE) of the Tmx in the polymeric nanocarrier. Additionally, Fourier transform infrared spectroscopy (FTIR) was also performed to check the chemical identity and interactions in the drug delivery system.

Results and Discussion: Tamoxifen-loaded PLGA NPs were successfully obtained showing an average size ≈ 300 nm. Morphologically, a quasi-spherical regular shape was observed for all of the PLGA NPs, demonstrating that the selected experimental conditions allow control of the formation of the polymeric particles, their dimensions, and hence their properties. A suitable EE of Tmx and thermal stability was also observed.

Conclusions: It was possible to conduct a reproducible method for the preparation of PLGA NPs loaded with Tmx. They can serve as drug delivery systems to carry and modulate the release of Tmx, decreasing side effects and optimizing its therapeutic effect.

References:

[1] Maji R, Dey NS, Satapathy BS, Mukherjee B, Mondal S. Int J Nanomedicine **9** (2014) 3107.
[2] Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. J Control Release **161** (2012) 505.

Folic acid-decorated and PEG-coated PLGA-based nanoparticles for targeted treatment of colon cancer

Mazen M. El-Hammadi[1,2], *Ángel V. Delgado*[3], *José L. Arias*[1,4,5]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[2]Department of Pharmaceutics and Pharmaceutical Technology, University of Damascus, Syria.
[3]Department of Applied Physics, University of Granada, Spain.
[4]Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain.
[5]Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
e-mail: mazenhammadi@yahoo.co.uk

Introduction: Colorectal cancer is the third most commonly diagnosed cancer and the fourth leading cause of death, with approximately one million new cases accounting for 9% of all cancer incidences. Controlling colon cancer and increasing survivals rate is an objective that necessitates the development of more efficient therapeutic tools capable of defeating advanced or recurrent colon cancer, which is incurable by conventional chemotherapy approaches. A targeting delivery carrier, such as the one that combines poly(D,L-lactide-*co*-glycolide) (PLGA) with poly(ethylene glycol) (PEG) and folate (FOL), is expected not to only enhance the pharmacological efficiency of encapsulated 5-fluorouracil (5-FU) (one of the first line cytotoxic agents employed in the chemotherapy of colon malignancy), but it can also overcome the anticancer´s drawbacks including minimizing its potential toxicity, increase its biological half-life, and overcoming the development of drug resistances [1].

Materials and Methods: PLGA-PEG-FOL conjugate was synthesized in a 4-step process [2, 3]. Briefly, PLGA was first activated with *N*,*N*'-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) and then conjugated with an excess of PEG-*bis*-amine. Next, the PLGA-PEG conjugate was bound to FOL previously activated with DCC and NHS. Nanoparticles (NPs) of PLGA, PLGA-PEG, and PLGA-PEG-FOL were fabricated by a nanoprecipitation solvent evaporation technique using a probe sonicator. In brief, an organic solution containing adequate amounts of PLGA in acetone were added at once to an aqueous solution of PVA and sonication was started immediately. Acetone was completely evaporated using a rotary evaporator. The aqueous dispersion was then centrifuged for 60 min at 10,000 rpm, the supernatant removed, and the NPs re-suspended in water. To select the optimal formulation, several PVA concentrations and solvent ratios (of the acetone organic solution) were tested. Sonication conditions were also studied by changing sonication time and sonicator output. NPs were characterized, drug loading capacity of the produced NPs was evaluated, and drug release studied.

Results and Discussion: Structures of the new conjugates were confirmed using ^{1}H-NMR. Under the optimal parameters for the formulation, the average particle size was 181.3 nm with an ideal polydispersity index of 0.058. PLGA-PEG and PLGA-PEG-FOL NPs loaded with 5-FU had an average particle size of 145.3 nm and 149.2 nm with a polydispersity index of 0.066 and 0.075, respectively. These particle sizes were much smaller compared with the unmodified 5-FU-loaded PLGA NPs. Zeta potential values for all produced formulas were negative and ranged between -16 to -28 mV. TEM images confirmed that all prepared NPs had similar morphologies with a spherical shape and a smooth surface. The loading percentage increased linearly with the amount of the drug and the maximum drug loading achieved was 5.4%. The *in vitro* release of 5-FU from the NPs was carried out in PBS (pH 7.4) at 37 ºC using a membrane dialysis method. 5-FU-loaded PLGA NPs, 5-FU-loaded PLGA-PEG NPs, and 5-FU-loaded PLGA-PEG-FOL NPs exhibited a similar drug release profile; an initial burst release, with ≈ 25-30% and 52-60% of the drug released after 1 and 6 h, respectively, followed by a sustained, linear drug release.

Conclusions: In this work we synthesized PLGA NPs coated with PEG, functionalized with FOL, and loaded with 5-FU. The produced NPs had favorable characteristics and a prolonged drug release profile. *In vitro* and *in vivo* experiments are required to assess the anticancer activity of the FOL-decorated NPs.

Acknowledgements: Financial support of the Erasmus Mundus – JOSYLEEM Program is acknowledged as is the post-doctoral fellowship to Mazen M. El-Hammadi. Research leading to these results has received funding from projects FIS PI11/02571 (Instituto de Salud Carlos III, Spain) and PE-2012-FQM-694 (Junta de Andalucía, Spain).

References:

[1] Holgado MA, Alvarez-Fuentes J, Fernández-Arévalo M, Arias JL. Curr Drug Targets **12** (2011) 1096.
[2] Liang C, Yang Y, Ling Y, Huang Y, Li T, Li X. Bioorg Med Chem **19** (2011) 4057.
[3] Saxena V, Naguib Y, Hussain MD. Colloids Surf B Biointerfaces **94** (2012) 274.

Physicochemical properties influencing the success of the transmembrane gradient method for loading liposomes

Mª de la Oliva Guerra-Morillo, Mª José Cózar-Bernal, Antonio Mª Rabasco, Mª Luisa González-Rodríguez

Department of Pharmacy and Pharmaceutical Technology, University of Seville, Spain.
e-mail: malugoro@us.es

Introduction: Usual methods to encapsulate drugs into liposomes involve aggressive conditions such as high temperatures, organic solvents or vortexing. The transmembrane gradient method assures high drug encapsulations, avoiding these conditions. This can be used to encapsulate proteins and labile drugs. Recently, it has been used for cancer therapy amongst other applications. The process is based on the capability of the anion NH_4^+ to dissociate to a proton and neutral ammonia (NH_3) gas, having a very high permeability coefficient. This dissociation depends on the pH. The higher the pH is, the higher is the dissociation of the ion NH_4^+. The drug loading requires the presence of NH_3 inside the liposome, diffusing across the membrane to the extraliposome aqueous phase, leaving behind the excess of protons and creating a proton gradient. Then, the drug will diffuse through the membrane and reach the intraliposome aqueous phase in its uncharged form. Once it is inside, is protonated and uses the excess of protons (elevating the pH), renewing the dissociation of NH_4^+ to NH_3 and H^+, and starting again. The type (low molecular weight inorganic, organic, or polymeric) and valence of anion that forms the ammonium salt can be used to control the drug release rate from the liposome. Moreover, the physicochemical properties of the drugs as well as their molecular weight are determinant to achieve the exchange. Thus, the objective of this work is the study of the influence of some properties of the drugs to reach good results during this process [1].

Materials and Methods: Liposomes were prepared following the thin layer evaporation method. The thin film was reconstituted with an acetate ammonium solution and a dialysis against 150 mL of HEPES buffer was performed to create an ionic gradient outer and inner the vesicles. During the dialysis, conductivity was measured to confirm the exchange of ions between the inside and the outside. After that, the liposomes were reconstituted with solutions of the different drugs, which were selected with different degree of ionization. The liposomes were then maintained at 4 ºC to keep stability. Afterwards, entrapment efficiency was measured by HPLC. Zeta potential, size and polydispersity index of each batch are also measured.

Results and Discussion: The ionic gradient was confirmed by conductivimetry measurements. The best encapsulation was reached by Theophylline (47.8%, pKa 8.7) > Acetazolamide (30%, pKa 7.2) > Sumatriptan (25.7%, pKa 9.2) > Metformin (9.8%, pKa 12.4). In addition to pKa, also other properties, such as water solubility and partition coefficient (K_p) may be taken into account. It is well-established that K_p can help to predict transmembrane passive diffusion. In these liposomes, where the internal aqueous phase is different from the external medium due to different composition and pH of these aqueous phases, the K_p of drug supports the influx into the liposomes. Herein, the K_p acts to reduce the partition into the membrane, so reducing the desorption rate. This justified a higher entrapment of Theophylline (log P -0.02) compared to Acetazolamide (logP -0.26). On the other hand, the higher water solubility of Theophylline (22.9 mg/mL) with respect to Acetazolamide (2.79 mg/mL) increases the concentration gradient inside and outside of vesicles, favouring the drug entrapment. Size characterization of vesicles gave values between 200 and 220 nm, without significant differences among batches. Zeta potential values were from -17 mV to -23 mV, indicating high stability of liposomes formulated.

Conclusions: From the study, a multifactorial influence of the physicochemical properties of the drug to be entrapped has been demonstrated. In addition to pKa, other properties such as water solubility and K_p must be considered.

Acknowledgements: Financial support from CACOF is gratefully acknowledged.

References:

[1] Haran G, Cohen R, Bar LK, Barenholz Y. Biochim Biophys Acta **1151** (1993) 201.
[2] Zucker D, Marcus D, Barenholz Y, Goldblum A. J Control Release **139** (2009) 73.

Development and characterization of Levodopa-loaded liposomes for treating Parkinson´s disease: stability studies

Elena García-Esteban, Mª José Cózar-Bernal, Antonio Mª Rabasco, Mª Luisa González-Rodríguez

Department of Pharmacy and Pharmaceutical Technology, University of Seville, Spain.
e-mail: malugoro@us.es

Introduction: Parkinson´s disease is a neurodegenerative disorder. The clinical use of numerous drugs for the treatment of central nervous system diseases is limited by their incapability to cross the blood-brain barrier. Actually, the most attractive and versatile technique for delivery drugs into the brain parenchyma involves the use of drug carriers, such as liposomes. Levodopa (L-dopa) is the drug of choice for treating the Parkinson´s disease. However, this drug has low pharmacokinetic and bioavailability properties. From a physicochemical point of view, L-dopa is oxidized in aqueous solution hence, the addition of antioxidant is crucial. Superoxide dismutase (SOD) is an antioxidant enzyme that plays a significant role in the removal of free radicals from the organism. Furthermore, it has been observed that patients treated with L-dopa have low plasma levels of SOD endogenous as a result of oxidative stress that produce dopamine metabolism. The aim of this work was to develop and characterize a formulation based on liposomes loaded with L-dopa and SOD to improve the stability of L-dopa.

Materials and Methods: L-dopa-loaded lipid vesicles were prepared by thin layer evaporation and were composed of phosphatidylcholine (PC), cholesterol (CH) and stearylamine (6.7:2.8:0.5 molar ratio). Lipids were dissolved in chloroform and a thin lipid film was achieved by rotary evaporation. Then, the lipid film was hydrated with a L-dopa solution (2 mg/mL) in Hepes buffer pH 6.2, following 5 cycles of vortex, consisting in stirring for 1 min and heating at 58 ºC for 5 min, until vesicle formation. Unilamellar vesicles were obtained by extruding the above samples through 800 nm pore sized polycarbonate membrane filters. SOD-loaded lipid vesicles were prepared following the same procedure than L-dopa-loaded liposomes. Vesicles were composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), cholesterol (CH) and stearylamine (6:3:0.5 molar ratio). The lipid film was hydrated with SOD solution (0.5 mg/mL) in Hepes buffer pH 7.4. Lipid vesicles were characterized before and after undergoing the process of extrusion, using morphological analysis, particle size, polydispersity index and zeta potential. The encapsulation efficiency (EE, %) of L-dopa was determined by HPLC. SOD enzyme activity (% act.) was determined by the xanthine/xanthine oxidase method. Then, 4 batches of liposomes were prepared to study L-dopa stability with/without antioxidant and to elucidate the contribution of the antioxidant in the stability of the formulation with time. Batch 1: liposomes L-dopa; batch 2: liposomes L-dopa + liposomes SOD; batch 3: liposomes L-dopa + standard SOD; batch 4: liposomes (L-dopa + SOD). Finally, we quantified the EE of L-dopa and % activity of SOD at time 0, 3, 6 and 10 days.

Results and Discussion: A decrease of the EE of L-dopa over time was obtained (Figure 1). The components of the lipid vesicles (PC, CH) suffer degradation, hydrolysis and oxidation processes, which increased the permeability of the lipid bilayer, leading to the release of the encapsulated drug. Although the presence of SOD into the formulations affects to the L-dopa entrapment in the initial time, however, this enzyme acts stabilizing the drug with time in all the batches. Also, a degradation process of SOD was observed with time (Table 1), attributable to the acidic pH of the formulation that affects the stability of the enzyme.

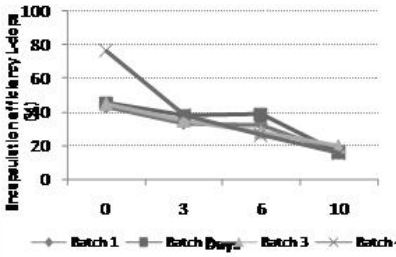

Figure 1. EE (%) *vs*. time (days) of the batches.

Table 1. Activity of SOD (%) as a function of time.

Batch	Activity (mean value ± standard deviation)			
	0 days	3 days	6 days	10 days
2	26.1 ± 0.2	25.2 ± 0.1	4.6 ± 0.1	0
3	46.4 ± 0.2	8.5 ± 0.1	0	0
4	0	0	0	0

Conclusions: Among all formulations, the presence of both drugs into different liposome samples was desirable, and maintenance of L-dopa with time was positive for further use in next formulations. In addition, we will proceed to lyophilize samples to improve the stability of formulations with time.

Acknowledgements: Financial support from CACOF is gratefully acknowledged.

References:

[1] Dai Q, Xia Y, Sun C, Weng M, Chen J, Wang J, Chen J. Chem Engin J **245** (2014) 359.
[2] Kumar K, Jyothi T, Nagendar S, Devipriya S, Vijaya G, Sudhakar A. Pharmanest **4** (2011) 2.
[3] Maestrelli F, González-Rodríguez ML, Rabasco AM, Mura P. Int J Pharm **312** (2006) 53.

Calcein release behavior from gold nanoparticle-loaded liposomes

N. Naitlho, Mª José Cózar-Bernal, Antonio Mª Rabasco, Mª Luisa González-Rodríguez

Department of Pharmacy and Pharmaceutical Technology, University of Seville, Spain.
e-mail: amra@us.es

Introduction: The design of a drug delivery system depends upon different parameters. One of the most noticeable factors is the drug release profile which determines the site of action, the concentration of the drug at the time of administration and the period of time that the drug must remain at a therapeutic concentration [1]. Heat-sensitive liposome technology is a potential method to produce triggered systems for controlled drug delivery. The temperature required for gel-to-liquid crystalline phase transition in the liposomes can be adjusted by lipid composition and/or by adding gold nanoparticles (Au NPs). This property has been used for example in cancer treatment, where the slightly higher temperature in the tumor triggers drug release from liposomes with a phase transition below 41 ºC [2]. In this work, we have elaborated liposomes with Au NPs adsorbed onto the vesicle surface for further characterization and evaluation of temperature and the Au NP effect on drug release profiles using calcein as model drug.

Materials and Methods: Cholesterol (CH), L-α-phosphatidylcholine (PC) and didodecyldimethylammonium bromide (DDAB) were used as lipid phase for preparing liposomes by the thin layer evaporation technique [3]. A mixture of 26 mg PC, 0.66 mg CH and 4.6 mg DDAB was dissolved in chloroform. The solvent was then removed under reduced pressure in a rotary evaporator at 58 ºC, thus obtaining a thin film of dry lipid on the flask wall. The film was hydrated by adding 3 mL of calcein solution (0.1 mg/mL in PBS) under vigorous mechanical shaking with a vortex mixer until vesicle formation. Complexes of liposomes with Au NPs were prepared by adding the Au NP dispersion to the liposome sample, in different ratios and stirred with vortex. Calcein release assay was performed by a dynamic dialysis technique [4]. In this method, 0.5mL of calcein-loaded liposomal dispersion was dropped into a cellulose acetate dialysis bag (Spectra/Por®, M_W cutoff 12.000; Spectrum, Canada) immersed in 30 mL of PBS buffer (pH 7.4), and magnetically stirred at 37 ºC and 42 ºC. Aliquots taken at time intervals from the receiver solution were replaced with equal volumes of buffer. Calcein fluorescence was assayed by a microplate reader with a 96 black opaque, being the excitation and monitoring wavelengths 480 and 515 nm, respectively. The mean vesicle size was measured by dynamic light scattering analysis (DLS, Mastersizer, Malvern Instruments, Malvern, UK). TEM analysis (Philips CM 10, Philips, USA) was used to examine the ultrastructure of Au NP-loaded liposomes.

Results and Discussion: We characterized the complexes of liposomes with Au NPs using TEM. Images show that Au NPs complexed satisfactorily to the liposomes, and DLS analysis indicated that the size of the complexes was almost the same as intact liposomes. Release tests indicated that the presence of Au NPs onto liposomes increased the release rate of calcein. The triggered calcein release was more pronounced with Au NP-loaded liposomes than with liposomes alone. Heat is conducted more efficiently to the lipid molecules, thereby inducing the phase transition and calcein release. On the other hand, the temperature-induced calcein release from Au NP-loaded liposomes was demonstrated. AuNP-loaded liposomes at 37 ºC showed similar release rates and higher total amount of drug released than liposomes subjected to 42 ºC. Probably, the influence of the bilayer composition must be modified to efficiently trigger drug release by the temperature.

Conclusions: In this work we have shown that the anchoring of Au NPs to liposomes occurred satisfactorily, and we demonstrated that the content release from liposomes with embedded Au NPs was higher than in liposomes without them. Further studies will be focused on the modification of bilayer composition to accelerate the drug release at a temperature conditioned by the thermal conditions of the pathological area.

Acknowledgements: This work is part of the project CTQ2014-57515-C2-1-R (Nanostructured interfaces derived DNA for molecular recognition and biomedical applications) funded by the Ministerio de Economía y Competitividad (2014).

References:

[1] Maherani B, Arab-Tehrany E, Kheirolomoom A, GenyD. Biochimie **95** (2013) 2018.
[2] Lindner LH, Eichhorn ME, Eibl H, Teichert N, Schmitt-Sody M, Issels ID, Dellian M. Clin Cancer Res **10** (2004) 2168.
[3] Villasmil-Sánchez S, Drhimeur W, Salas Ospino SC, Rabasco AM, González-Rodríguez ML. Drug Dev Ind Pharm **36** (2010) 667.

Co-loaded niosomes encapsulating Timolol and Acetazolamide: preparation and characterization

C.M. Arroyo, Mª José Cózar-Bernal, Antonio Mª Rabasco, Mª Luisa González-Rodríguez

Department of Pharmacy and Pharmaceutical Technology, University of Seville, Spain.
e-mail: amra@us.es

Introduction: Ocular barriers constitute an obstacle for topical treatment of ophthalmic diseases. Liposomes and derivatives offer an interesting alternative to overcome these limitations. Their continuous evolution brings improved features such as stability or deformability, with the aim to cross intact the corneal membrane. Niosomes are derivated vesicles composed mainly of hydrated non-ionic surfactants in addition to, in many cases, cholesterol. Niosomes are osmotically active, chemically stable and have long storage time compared to liposomes; they have high compatibility with biological systems and low toxicity because of their non-ionic nature; they can entrap lipophilic drugs into vesicular bilayer membranes and hydrophilic drugs in aqueous compartments, and even doubly charged vesicular systems can be developed to strengthen its effectiveness and potential. The objective of this study was to analyze the effect of surfactant ratios, the presence of charged lipids and cholesterol content on encapsulation of both drugs simultaneously into the same vesicle. Also, the physicochemical properties of formulations were analyzed.

Materials and Methods: Thin-film hydration method (TFH) was used to entrap Timolol and Acetazolamide into vesicles. Non-ionic surfactants used were Span® 60 and Brij® 72. Five factors: ratio Span® 60/Brij®72, incorporation of charged agents (stearylamine and dicethylphosphate), amount of stearylamine, amount of non-ionic surfactants, and amount of cholesterol were selected as causal factors. The vesicle size, polydispersity index, zeta potential and the percentage of entrapped drugs were the responses (dependent variables) for each formulation evaluated. All of these responses were assessed in triplicate. The encapsulation efficiency was determined by HPLC (Elite LaChrom); size, polydispersity index and zeta potential were analyzed by dynamic light scattering (Zetasizer nano). In addition, *in vitro* permeation studies were performed.

Results and Discussion: From characterization studies, the optimal ratio of Span® 60/Brij® 72 50/50 was the better as in terms of percentage of both drugs entrapped into the vesicles as size and zeta potential properties. Also, the encapsulation percentage was higher in the presence of stearylamine: 58% for Acetazolamide and 67% for Timolol. The presence of this positively charged lipid contributes to reducing the encapsulation efficiency. With this composition, niosomes made with Timolol and Acetazolamide had the highest percentages. Therefore, the co-incorporation of both drugs favored the trapping of Acetazolamide. *In vitro* permeation studies showed that when both drugs were administered without nanocarrier, a quick release was obtained compared with drug-loaded vesicles, which makes patent the role of vesicles in controlling drug release.

Conclusions: Niosomal systems are defined as efficient alternatives for treating ophthalmic diseases, favoring the permeation and exerting a control over drug release, allowing in the future improved effectiveness of treatments.

References:

[1] Moghassemi S, Hadjizadeh A. J Control Release **185** (2014) 22.
[2] Haj-Ahmad RR, Elkordy AA, Chaw CS. Curr Drug Deliv **12** (2015) 628.

Polyglutamate-based combination therapy in the treatment of advanced breast cancer

Juan J. Arroyo[1], Coralie Deladriere[1], Esther Masia[1,2], Vicent J. Nebot[1], Alison Paul[3], Ana Armiñán[1], María J. Vicent[1,2]

[1]Polymer Therapeutics Lab., Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain.
[2]Screening Platform, Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain.
[3]School of Chemistry, University of Cardiff, United Kingdom.
e-mail: jarroyo@cipf.es, jjarroyoc@gmail.com

Introduction: Polymer therapeutics encompass a diversity of complex and rationally designed supramolecular drug delivery technologies in which a water-soluble multivalent polymer carrier is decorated with covalently bound bioactive species. These nanomedicines are engineered to navigate the complex *in vivo* environment, and to incorporate functionalities for achieving target specificity, control of drug concentration and exposure kinetics at the tissue, cell, and subcellular levels. Coupling low molecular weight anticancer drugs to polymeric carriers through a cleavable linker is an effective method to improve the therapeutic index of clinically established agents, enhancing antitumor effect and reducing severe side effects [1, 2]. The use of polymer-drug conjugates in combination therapy offers the best opportunity to enhance antitumor effect and to reduce the severe side effects [2, 3]. In our systems, we combine a chemotherapeutic with an endocrine agent in the proper synergistic ratio by binding to the same polymer carrier through protease cleavable or pH sensitive linkers, ensuring the simultaneous uptake by the target cells bringing significant advantage versus single treatments. We have previously reported the first polymer-drug conjugate of this type: the non-biodegradable HPMA copolymer bearing the combination of the aromatase inhibitor aminoglutethimide (AGM) with the anthracycline Doxorubicin (Dox) [3, 4]. *In vivo* proof of concept of this conjugate, HPMA copolymer-AGM-Dox has already been achieved in an orthotopic 4T1 murine metastatic breast cancer model and its mechanism of action has been studied [5]. In order to further improve the features of our system, we moved to a biodegradable and multivalent carrier poly(L-glutamic acid) (PGA) that allows not only to increase the conjugate molecular weight, being able to enhance the loading possibilities, but also to reduce the immunogenicity and to increase the tumor targeting due to the EPR effect [5].

Materials and Methods: A novel PGA-AGM-Dox conjugate family was developed including compounds with different drug linkers and drug ratios looking for synergism. Drugs were attached to the polymer through protease-labile linkers, including the cleavable tetrapeptide GFLG well-known to be degraded by cathepsin B_1 (in order to allow direct comparison with model HPMA conjugate) as well as pH-labile hydrazone linkers, known to be degraded at the lysosomal acidic environment.

Results and Discussion: Conjugates have been deeply characterized by different physicochemical techniques (NMR, DLS, SLS, SANS, etc.) to elucidate structural and conformational features in solution as well as drug release kinetics. This family of conjugates has shown the capacity of self-assemble in aggregates ≈ 100 nm in aqueous solution, showing differences in both their critical aggregation concentration and shape as evidenced by DLS, SANS and cryo-TEM. *In vitro* evaluation of drug release kinetics, cellular uptake and cell viability allowed a screening for the best candidates achieving drug synergism in both MCF-7ca and 4T1 cell lines. The conjugates showing the greatest *in vitro* activity were chosen for *in vivo* evaluation in an orthotopic 4T1 murine tumor model, and their bioavailability and biodistribution have been studied.

Conclusions: We have found physicochemical descriptors for qualitative determination of structural-activity relationships in biological settings, demonstrating that PGA-based combination conjugates structural and conformation features in solution may play an active role, leading to differences in the biological performance [6].

Acknowledgements: This work is supported by ERC Consolidator Grant-MyNano, Spanish Ministry of Science and Innovation (SAF2013-44848-R, INNPACTO program IPT-2012-0712-010000, Programa I3), GENTxGENT and Generalitat Valenciana (ACOMP2013/186).

References:

[1] (a) Vicent MJ, Duncan R. Adv Drug Deliv Rev **65** (2013) 60; (b) Canal F, Sanchis J, Vicent MJ. Curr Opin Biotech **22** (2011) 894.
[2] Greco F, Vicent MJ. Adv Drug Deliv Rev **61** (2009) 1203.
[3] (a) Greco F, Vicent MJ, Gee S, Jones AT, Gee J, Nicholson RI, Duncan R. J Control Release **13** (2007) 459; (b) Vicent MJ, Greco F, Paul A, Griffiths PC, Nicholson RI, Duncan R. Angew Chem Int Ed Engl **44** (2005) 4061.
[4] Deladriere C, Armiñán A, Masiá E, Lucas R, Vicent MJ. *Ready for submission*.
[5] (a) Vicent, MJ, Barz M, Canal F, Conejos-Sanchez I, Duro-Castano A. P201131713 (2012); (b) Conejos-Sanchez I, Duro-Castano A, Birke A, Barz M, Vicent MJ. Polym Chem **4** (2013) 3182.
[6] Deladriere C, Arroyo JJ, Masiá E, Nebot VJ, Armiñán A, Vicent MJ. *Ready for submission*.

Development of 5-Fluorouracil-loaded chitosan nanoparticles against colon cancer

Mazen M. El-Hammadi[1,2], *Francisco S.M. Franco Frazão*[1], *Ángel V. Delgado*[3], *Consolación Melguizo*[4,5], *José C. Prados*[4,5], *Mª Adolfina Ruiz*[1], *José L. Arias*[1,4,5]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[2]Department of Pharmaceutics and Pharmaceutical Technology, University of Damascus, Syria.
[3]Department of Applied Physics, University of Granada, Spain.
[4]Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain.
[5]Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
e-mail: mazenhammadi@yahoo.co.uk

Introduction: 5-Fluorouracil (5-FU) is one of the most used drugs for the colon cancer treatment. Due to its structure, 5-FU can affect the nucleoside metabolism, therefore is incorporated into ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), leading to cytotoxicity and cell death. The major drawback of this drug are the resistance associated with its utilization, thus, the response in advanced colon cancer is only ≈ 10-15% [1]. This work aimed at improving the efficiency of 5-FU, as an agent of colon cancer treatment, by loading into the biodegradable polymer chitosan.

Materials and Methods: Chitosan nanoparticles (NPs) were prepared by a coacervation method [2, 3]. Briefly, 12.5 mL of sodium sulphate (20%, w/v) were added to 50 mL of chitosan (1%, w/v) in an aqueous solution of acetic acid (2%, v/v), containing Pluronic® F-68 1% (w/v), at an addition rate of 2.5 mL/min and under mechanical stirring (1200 rpm) for 1 h. Afterwards, NP dispersion was centrifuged at 10,500 rpm for 60 min. Drug-loaded NPs were prepared by adding the drug to the chitosan solution before starting the preparation procedure. Drug loading was determined indirectly by measuring the free unloaded 5-FU in the supernatant collected after centrifugation of the NP dispersion, by UV-Vis spectrophotometry at 266 nm. Mean particle size and zeta potential (ζ) of the NPs were measured by photon correlation spectroscopy and electrophoresis, respectively. 5-FU-loaded chitosan NPs were tested in apoptosis and chemoresistant SW480 human carcinoma cell line (University of Granada, Spain).

Results and Discussion: The average particle size was found to be 205.9 ± 23.8 nm and the ζ mean value was 15.2 ± 3.6 mV. Drug loading increased from 0.09 to 8.89% as the starting concentration of 5-FU was raised from 10^{-4} to 0.01 M, and the entrapment efficiency followed an exponential curve growing with 5-FU increasing concentration. The NPs did not demonstrate a significant toxic effect on the tested colon cancer cell line. This may be due to the poor stability exhibited by the prepared chitosan NPs.

Conclusions: We successfully fabricated and characterized 5-FU-loaded chitosan NPs. Although a good drug loading percentage was obtained, no substantial antitumor effect was observed against the SW480 human carcinoma cell line. Nevertheless, optimization of the nanoformulation to improve its stability may open possibilities for the application of chitosan-based NPs in the treatment of colon cancer.

Acknowledgements: Financial support of the Erasmus Mundus – JOSYLEEM Program is acknowledged as is the post-doctoral fellowship to Mazen M. El-Hammadi. Research leading to these results has received funding from projects FIS PI11/02571 and PI11/01862 (Instituto de Salud Carlos III, Spain), and PE-2012-FQM-694 (Junta de Andalucía, Spain)

References:

[1] Arias JL. Molecules **13** (2008) 2340.
[2] Arias JL, Reddy LH, Couvreur P. Biomacromolecules **12** (2011) 97.
[3] Yang Y, Wang S, Wang Y, Wang X, Wang Q, Chen M. Biotechnol Adv **32** (2014) 1301.

Polymeric nanoparticle-based vaccine: targeting dendritic cells and the tumor microenvironment

Carina Peres[1,2,3], *Ana I. Matos*[1], *Ana S. Viana*[4], *Luís Graça*[2], *Véronique Préat*[3], *Helena F. Florindo*[1]

[1]Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Portugal.
[2]Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Portugal.
[3]Louvain Drug Research Institute, Advanced Drug Delivery & Biomaterials, Université Catholique de Louvain, Belgium.
[4]Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Portugal.
e-mail: cperes@ff.ulisboa.pt

Introduction: Despite progress accomplished on cancer prevention, detection and treatment, breast cancer is still one of the most devastating diseases affecting the life of many people around the world [1]. Conventional therapies used for breast cancer do not target specifically the tumor affecting both malignant and normal cells, which contributes to several side effects. Cancer vaccines have been used as an alternative therapeutic strategy and have already shown promising results [2]. However, only a small number was able to lead to an effective tumor regression, which can be explained by the immunosuppressive properties of tumor microenvironment induced namely by the release of potent immunosuppressor molecules, such as transforming growth factor (TGF)-β1 [3]. Therefore, the elimination of both the tumor itself and the tumor microenvironment, without adversely affecting the desired antitumor effector cells, seems to be an ideal therapeutic strategy to eradicate this disease. Thus, the aim of the present study is to develop a polymeric NP-based cancer vaccine to deliver incorporated tumor-associated antigens and/or *si*RNA to target dendritic cells (DCs) and for immunomodulation by silencing immune-suppressive TGF-β1 genes within breast tumor site.

Materials and Methods: Antigen or *si*RNA-chitosan complexes encapsulated in poly(D,L-lactide) (PLA) NPs have been formulated by a double emulsion solvent evaporation method. These NPs were coated with polyvinyl alcohol (PVA) or with block copolymer Pluronic® to improve stability under physiological conditions. In order to potentiate tumor targeting, NP surface was modified by hyaluronic acid (HA), a targeting moiety that specifically recognizes CD44 receptor, overexpressed on several tumor cells. NP size, surface charge (zeta potential, ZP) and morphology were analyzed by Dynamic Light Scattering, Laser Doppler Electrophoresis and Atomic Force Microscopy (AFM), respectively. Antigen entrapment efficiency (EE) and loading capacity (LC) were quantified by HPLC, while *si*RNA EE and LC were calculated using PicoGreen® reagent. Finally, cell viability was determined by Alamar Blue® assay.

Results and Discussion: Overall, the different PLA NPs showed a mean hydrodynamic diameter close to 200 nm, a polydispersity < 0.2, and a slightly negative surface charge. Moreover, low standard deviation (SD) values were obtained for the three parameters (Z-Ave, PdI and ZP), attesting the high reproducibility of the method used for NPs preparation. The entrapment of α-Lac or *si*RNA in NPs did not alter their stability since no significant differences were detected in Z-Ave, PdI and ZP, between plain and antigen/*si*RNA-loaded NPs. In addition, these parameters were showed to be independent of the glycol chitosan derivative used to complex the antigen/*si*RNA, since no significant differences were observed between the values obtained for the two types of nanoparticulate systems. Moreover, Z-Ave, PdI and ZP seem to be independent on both external phase surfactant, and HA coating, since they present similar values. Size, shape and surface morphology of NPs were further characterized by AFM. This analysis revealed particles with smooth surface, spherical shape, and sizes close to 180 nm, which corroborates well with DLS data. Moreover, AFM images indicate that NPs formulated with PVA and Pluronic® have similar properties, such as the non-targeted NPs and the targeted ones. The entrapment efficiency was calculated indirectly by the determination of the amount of antigen that remained free in the supernatants collected during the three washings and centrifugations steps. HPLC suggests a high antigen EE for the different nanosystems prepared, reaching values between 80 and 93% (w/v). Also the assay using PicoGreen® reagent demonstrated the high capacity of these nanoplatforms to encapsulate *si*RNA, obtaining values of EE higher than 90%. PLA NPs did not have any significant cytotoxic effect on cancer cells 4T1 and DCs JAW SII, over a wide range of concentrations, at 24, 48 and 72 h post incubation. Indeed, cell viability is close to 100% for the lowest NPs concentration tested and remained higher than 80% for the highest tested amount of NPs.

Conclusions: The results presented herein allow to state that the formulation method followed for PLA-based NP preparation is highly reproducible and that this nanoparticulate system constitutes a promising platform for the delivery of TAA and immunomodulators to different cells within tumor microenvironment. *si*RNA-NP knockdown capacity is currently under evaluation by Western blotting and confocal microscopy.

Acknowledgements: This work was supported by FCT (Portugal - PTDC/SAU-FAR/119389/2010, UTAP-ICDT/DTP-FTO/0016/2014 and SFRH/BD/ 87591/2012), and by iMed.ULisboa (UID/DTP/04138/2013).

References:

[1] Boyle P, Bernard L. World Cancer Report 2008. IARC Press (2008).
[2] Devaud C, John LB, Westwood JA, Darcy PK, Kershaw MH. Oncoimmunology **2** (8) (2013).
[3] Kissick HT, Sanda MG. Curr Opin Immunol **35** (2015) 15.

Application of Pd^0-functionalized resins for the *in situ* activation of cytotoxic drugs as selective anticancer therapy

Belén Rubio-Ruiz, Ana Maria Pérez-López, Jason T. Weiss, Asier Unciti-Broceta

Edinburgh Cancer Research UK Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, UK.
e-mail: v1bruiz2@exseed.ed.ac.uk

Introduction: Chemotherapy is the treatment of cancer with antineoplastic drugs, which commonly operates by killing cells that divide rapidly, one of the principal features of cancer cells. However, the most effective cytotoxic drugs are limited by lack of selectivity since non-cancerous cells are also heavily affected [1]. To mitigate unwanted side effects, many efforts have been made on the design of cancer specific strategies such as prodrugs, which are inactive precursors of cytotoxic agents that are biochemically converted into their active forms in a specific biological setting [2]. Pd^0 nanoparticles are safe for biological applications, highly catalytic, and can be readily generated and trapped in an amino-functionalized polymer matrix [3]. Our research is focused on the application of these polymer entrapped Pd^0 nanoparticles to cleave protecting groups used to inactivate a clinically-used cytotoxic agent, thus restoring the drug' pharmacological properties *in situ* as selective strategy against cancer.

Materials and Methods: *Synthesis of Pd^0-Resins*. Pd^0-functionalized resins were prepared from NovaSyn TG amino resin HL (0.39 mmol NH_2 / g) as previously described [3]. *Synthesis of Prodrugs 1-5*. Using an alkylation strategy to mask functional groups essential for the cytotoxic mode of action of a well-known-clinically-used drug, different Pd^0-sensitive prodrugs were developed. *Pd0-catalysed dealkylation of prodrugs in cancer cell culture*. Human lung adenocarcinoma A549 cells were seeded in a 96 well plate format and incubated for 48 h before treatment. Each well was then replaced with fresh media containing: Pd^0-resins (1 mg/mL); Prodrugs 1-5 (1-100 μM); drug (1-100 μM); or combination of 1 mg/mL of Pd^0-resins + prodrug (1-100 μM). All experiments (including the untreated cells) contained 0.1% (v/v) of DMSO and were performed in triplicates. After 5 days of treatment, PrestoBlue™ cell viability reagent (10%, v/v) was added to each well and incubated for 1 h. Fluorescence emission was detected using a microplate reader and results normalized.

Results and Discussion: Cell viability studies confirmed that alkylation of a commercial cytotoxic drug with different Pd^0-sensitive moieties yielded a number of biochemically stable inactive derivatives (prodrugs 1-5). Specifically, prodrug 5 displayed a ≈ 100-fold reduction in cytotoxic activity relative to the unmodified drug (EC_{50} drug = 4.85 μM *vs*. EC_{50} prodrug 5 > 500 μM) in human lung adenocarcinoma A549 cells. While Pd^0-resins and prodrug 5 displayed no cytotoxicity separately, combination of both exhibited equivalent antiproliferative properties to unmodified drug in A549 cancer cells, underlining the *in vitro* efficacy of this activation strategy.

Conclusions: Our results show that the cytotoxic properties of the drug used were successfully eliminated and selectively restored in cancer cell culture by extracellular Pd^0 catalysis. The *in vitro* efficacy shown by this masking/activation strategy underlines its potential to develop a bioorthogonally activated prodrug approach and supports further *in vivo* investigations.

Acknowledgements: BRR thanks the Martin Escudero Foundation for her fellowship. This project was also supported by MSD-Scottish Life Sciences Fund postdoctoral award (for AMPL) and Cancer Research UK.

References:

[1] DeVita VT Jr, Chu E. Cancer Res **68** (2008) 8643.
[2] Rautio J, Kumpulainen H, Heimbach T, Oliyai R, Oh D, Järvinen T, Savolainen J. Nat Rev Drug Discov **7** (2008) 255.
[3] Weiss JT, Dawson JC, Macleod KG, Rybski W, Fraser C, Torres-Sánchez C, Patton EE, Bradley M, Carragher NO, Unciti-Broceta A. Nat Commun **5** (2014) 3277.

Focal immune response via Palladium-mediated histamine release: artificially-controlled immune system boost and its effect against cancer

Ana Maria Pérez-López, Belén Rubio-Ruiz, Marc Vendrell, Asier Unciti-Broceta

Edinburgh Cancer Research UK Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, UK.
e-mail: aperezl@staffmail.ed.ac.uk

Introduction: The key reason why tumours escape of control of the immune system is that, unlike pathogens, they do not express potent tumour rejection antigens (TRAs). The development of effective cancer vaccines would require the identification of potent and broadly expressed TRAs as well as effective adjuvants to stimulate a robust and durable immune response [1]. An alternative approach to vaccination is to provoke an artificial immune response by recruiting immune cells to cancer tissues, through the production of chemical factors such as histamine [2]. Accumulating histamine only in the proximity of the cancer cells, TRAs will be recognized by the massive recruitment of immune cells. To release histamine specifically in tumoral tissues, we have developed a unique strategy to uncage an inactive histamine precursor in a locally-controlled manner using a purely chemical method, so-called bioorthogonal organometallic (BOOM) prodrug activation [3]. The strategy is based on the use of solid palladium (Pd^0), a biocompatible metal which possesses unique catalytic properties. A number of alkylated-histamine were synthesized by masking amine groups essential for the histamine receptor interactions. The efficacy of this unprecedented histamine uncaging strategy to induce local chemotaxis of immune cells was studied in cancer cell culture by extracellular Pd^0 catalysis.

Materials and Methods: Pd^0-functionalized resins were prepared from NovaSyn TG amino resin HL (0.39 mmol NH_2/g) as previously described [3]. Alkylated/carbamate histamine precursors were synthesized using propargyl bromide/propargyl chloroformate/di-*t*-butyl dicarbonate in basic conditions in order to protect amine groups of histamine. Pro-histamine precursor (100 μM) were dissolved in PBS (1 mL) with 1 mg of Pd^0-resins and shaken at 1400 rpm and 37 ºC in a Thermomixer. Reaction crudes were monitored at 0, 3, 6, and 24 h by analytical HPLC (Agilent) using an UV detector at 280 nm to avoid the detection of PBS salts, and by fluorescence emission after histamine blue activation (histamine fluorogenic sensor) [4], using a PerkinElmer Victor2 multilabel reader (excitation filter at 340 nm and emission filter at 460 nm). RAW 264.7 cells were seeded in a 24 well plate format at the appropriate cell concentration (30,000 cells/well) and incubated in coverslips for 48 h before treatment. Each coverslip was then moved to a 6 well plate containing media with histamine, pro-histamine and pro-histamine/Pd^0 resin combination, and incubated for 7 days for chemotaxis assay. All conditions were normalized to the untreated cells (100%) and curves fitted using GraphPad.

Results and Discussion: Histamine is an organic nitrogenous compound involved in local immune responses as well as regulating physiological function in the gut and acting as a neurotransmitter (e.g. chemotaxis). This chemotactic response could be stimulated using pro-histamine precursors after their activation by Pd^0 beads in the proximity of the tumor. Pro-histamine precursors were successfully synthesized with appropriate reagents in the presence of the suitable base formed the corresponding histamine derivatives in moderate yields. To evaluate the stability of pro-histamine precursors in PBS, we were using histamine blue, which react with histamine *in situ* to generate a fluorescence structure able to emit at 460 nm. The alkylated-imidazole histamine derivative is a promise candidate for bioorthogonal activation by Pd^0 beads *in vivo* base on the macrophages chemiotaxis activation results.

Conclusions: The chemical protection of histamine as an alkylated/carbamate precursor led to a variety of histamine precursors with high stability in biocompatible conditions. The activation of chemotaxis in the proximity of a tumor *in vivo* could develop a new strategy against cancer using biocompatible Pd^0 resin device.

Acknowledgements: This project was also supported by MSD-Scottish Life Sciences Fund postdoctoral award (for AMPL) and Cancer Research UK. BRR thanks the Martin Escudero Foundation for her fellowship.

References:

[1] Pastor F, Kolonias D, Giangrande PH, Gilboa E. Nature **465** (2010) 227.
[2] Petit-Bertron A, Machavoine F, Defresne MP, Gillard M, Pierre C, Mistry P, Scheneider E, Dy M. PLOS One **4** (2009) 6504.
[3] Weiss JT, Dawson JC, Macleod KG, Rybski W, Fraser C, Torres-Sánchez C, Patton EE, Bradley M, Carragher NO, Unciti-Broceta A. Nat Commun **5** (2014) 3277.
[4] Kielland N, Vendrell M, Lavilla R, Chang YT. Chem Commun **48** (2012) 7401.

Innovative strategy to deliver protein-adsorbed SLNs using the pulmonary route

D.P. Gaspar[1,2], *C. Serra*[3], *L.M.D. Gonçalves*[1], *P. Taboada*[4], *A.J. Almeida*[1], *C. Remuñán-López*[2]

[1]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Portugal.
[2]Nanobiofar Group, Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Santiago de Compostela, Spain.
[3]Centre for Scientific and Technological Support to Research, University of Vigo, Spain.
[4]Condensed Matter Physics Department, Faculty of Physics, University of Santiago de Compostela, Spain.
e-mail: dianagaspar@ff.ulisboa.pt

Introduction: Being highly vulnerable molecules, therapeutic proteins present short *in vivo* half-lives, due to degradation by enzymes. Solid lipid nanoparticles (SLNs) have been proposed as carriers for therapeutic peptides and proteins for drug transport to the lung epithelium. However, the smaller the particle, the more difficult it becomes to achieve high drug encapsulation efficiency. To circumvent this problem and the possible drug degradation during the particle formulation process, the drug can be adsorbed onto the particle surface [1]. Nevertheless, nanoparticles utility for pulmonary application is severely hindered causes them to escape from lung deposition. To overcome this problem, the production of spray-dried powders containing nanoparticles have been recently reported [2]. Herein we developed a new hybrid microencapsulated SLNs containing a model protein drug (papain, PAP) for pulmonary administration.

Materials and Methods: The SLNs were prepared using a hot high shear homogenization method, as previously described [3]. To avoid the possible degradation of PAP caused by the high temperatures involved in SLN preparation, PAP was subsequently loaded by adsorption onto the SLN surface. The physical properties, morphology and structure of PAP-SLNs were analyzed. PAP adsorption process was also characterized. To prepare the PAP-SLN-loaded dry powders, suspensions of PAP-SLN in excipients were spray-dried using a laboratory-scale spray-dryer (Büchi® Mini spray-dryer, B-290, Switzerland). Full physical characterization of dry powders was performed.

Results and Discussion: As expected, PAP adsorption onto SLNs increased particle size, although remaining within nano-range scale, while decreasing the SLN negative surface charge. This is due to the adsorption process, which occurs through the electrostatic interaction between amino groups of PAP and the negative charged sites of SLN. TEM analysis demonstrated that PAP-SLNs had a spherical nanostructure as well as a compact appearance. It is also possible to state the nanoparticle-protein *corona*, a dynamic layer of proteins adsorbs to nanoparticles. The adsorption process followed a Freundlich type of adsorption isotherm over the PAP concentration range studied, suggesting the formation of protein multilayers on the nanoparticles surface [4]. Fourier transform infrared (FTIR), differential scanning calorimetry (DSC) and X-ray photoelectron spectroscopy (XPS) studies also confirmed the interaction between PAP and SLNs. The latter also allowed to state that PAP was efficiently adsorbed at the SLN surface. Nanoformulations were spray-dried with two different excipients (mannitol and trehalose) in a one-step spray-drying process with high production yields, originating spherical particles with well defined limits. Furthermore, PAP-SLN-loaded microspheres exhibited aerodynamic characteristics suitable to achieve deep lung deposition. The PAP desorption from dry powders was higher when compared with desorption from non-spray-dried SLNs indicating the spray-drying process may affect PAP adsorption onto the SLNs, favoring the protein desorption. Nevertheless, protein stability was kept throughout microsphere production, as assessed by SDS-PAGE. No changes in the migration of the PAP were detected when compared with the native protein. Also, no additional bands revealing fragmentation or aggregation were seen (presence of a single band at 23 kDa). The PAP enzymatic activity was not affected by the harsh preparation procedures demonstrating a stabilizing effect of the polyol excipients.

Conclusions: A novel, suitable and stable system containing microencapsulated PAP-adsorbed at the SLN surface has been developed with the purpose to improve pulmonary delivery of proteins with therapeutic effect.

Acknowledgements: This work was supported by FEDER and Fundação para a Ciência e Tecnologia, Portugal (grants SFRH/BD/89520/2012, SFRH/BSAB/1210/2011, strategic project PEst-OE/SAU/UI4013/2011 and research project PTDC/DTP-FTO/0094/2012). P.T. thanks Ministerio de Economía y Competitividad (MINECO) and Xunta de Galicia for research projects MAT 2013-40971-R and EM 2013-046, respectively. C.R-L. gratefully acknowledge support from the Spanish Institute of Health "Carlos III" (Project FIS PS09/00816) and Xunta de Galicia (Project PGIDIT, 09CSA022203PR, NANOPULMOGENIC; Project Competitive Reference Groups, 2014/043-FEDER).

References:

[1] Almeida A, Souto E. Adv Drug Deliv Rev **59** (2007) 6.
[2] Grenha A, Seijo B, Remuñán-López C. Eur J Pharm Sci **25** (2005) 4.
[3] Estella-Hermoso de Mendoza A, Campanero MA, Lana H, Villa-Pulgarin JA, de la Iglesia-Vicente J, Mollinedo F, Blanco-Prieto MJ. Nanomedicine (Lond) **7** (2012) 5.
[4] Florindo H, Pandit S,Gonçalves L, Alapar H, Almeida A. Int J Pharm **390** (2010) 1.

Synthesis and characterization of PLGA-lipid nanoparticles

Vanessa Sainz[1,2], *Ana Viana*[3], *Liana C. Silva*[1], *João A. Lopes*[1], *Steve Brocchini*[2], *Mire Zloh*[4], *Helena F. Florindo*[1]

[1]Department of Galenic Pharmacy and Pharmaceutical Technology, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal.
[2]Department of Pharmaceutics, UCL School of Pharmacy, United Kingdom.
[3]Centro de Química e Bioquímica (CQB), Faculdade de Ciências, Universidade de Lisboa, Portugal.
[4]Department of Pharmacy, University of Hertfordshire, United Kingdom.
e-mail: sainz@ff.ulisboa.pt

Introduction: Hybrid lipid-polymeric nanoparticles (NPs) that combine the advantages of their polymeric and lipid counterparts stand out as potential candidates for the development of biocompatible and biodegradable drug delivery systems. They present better entrapment efficiencies, cellular uptake and lead to controlled release profiles [1]. This study aimed to design a potential immune therapeutic hybrid lipid-polymeric nanoplatform able to deliver entrapped antigens and immune modulators to dendritic cells (DCs).

Materials and Methods: The poly(D,L-lactide-*co*-glycolide) (PLGA)-lipid hybrid NPs were prepared by the double emulsion-solvent evaporation method. Two different lipids, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphorylcholine (POPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphorylglycerol (DMPG), were used to modify the NP matrix. PLGA was dissolved in organic solvent and this organic phase was emulsified with the aqueous phase [ovalbumin (OVA) and/or PVA) by sonication for 15 s. OVA was used as a model antigen to attain the optimization of the NP formulation process. A second emulsion was performed adding a 1.25% (w/v) PVA solution to the previous w/o emulsion. This double emulsion was then added dropwise into 0.25% (w/v) PVA solution and stirred for 1h at room temperature. The NPs were recovered by centrifugation at 17500 rpm, 4 ºC for 45 min. These hybrid nanosystems were washed three times by centrifugation (17500 rpm, 4 ºC during 45 min) and finally suspended in Dulbecco´s phosphate-buffered saline. NP hydrodynamic diameter and polydispersity index (PdI) were determined by dynamic light scattering (DLS), while surface morphology was evaluated by atomic force microscopy (AFM). Zeta potential was determined by laser doppler electrophoresis. The entrapment efficiency (EE) and loading capacity (LC) of hybrid NPs were quantified by fluorescence using OVA Alexa Fluor® 647 conjugate as model antigen. Cell viability of murine immature dendritic cells (DCs; JAW SII cell line) in the presence of PLGA-lipid hybrid NPs was inferred using AlamarBlue® assay. The cells were incubated overnight at 37 ºC and 5% CO_2 and treated with different concentrations of NPs for 24, 48 and 72 h.

Results and Discussion: NPs were in a size range between 137.3 and 200.6 nm. Higher lipid/polymer ratios decreased nanoparticles´ size. Moreover, the mean diameter of those carriers was lower for NPs composed by neutral (POPC) and negatively charged lipids (DMPG). All nanoparticulate formulations presented a monodispersed population (PdI $\leq$ 0.068) and zeta potential close to neutrality (-6.70 and -2.37 mV). The entrapment of OVA did not change significantly the physicochemical properties of these hybrid nanosystems. In addition, high EE and LC were observed for both polymeric (EE: 69.91 ± 4.57%, w/v; LC: 3.52 ± 0.24 µg/mg), and hybrid lipid-polymeric NPs (PLGA/POPC – EE: 60.58 ± 4.89%, w/v; LC: 3.05 ± 0.25 µg/mg. PLGA/POPC/DMPG – EE: 84.06 ± 1.37%, w/v; LC: 4.21 ± 0.07 µg/mg). The AFM analysis showed that NPs present a spherical shape. The addition of lipids to the PLGA matrix resulted in smoother NP surfaces, which was more prominent when a mixture of POPC and DMPG was used. AFM images corroborate NP mean diameters obtained by DLS, but evidences higher PdI. In addition, *in vitro* cellular studies did not show a negative influence of these nanocarriers on the viability of DCs. The cell viability was close to 100% for all the concentrations of polymeric and hybrid nanosystems, after 24 h of incubation. No significant differences were observed after 48 and 72 h of incubation.

Conclusions: In this research we constructed a promising hybrid nanoplatform, which internalization mechanisms, intracellular trafficking and fate will be tested in the near future. This work showed that these nanocarriers constitute a promising strategy for antigen delivery and may be a potential tool for DC activation and effective maturation.

Acknowledgements: The authors are grateful to Fundação para a Ciência e a Tecnologia, Ministério da Ciência e da Tecnologia Portugal for iMed.ULisboa grant UID/DTP/04138/2013, research projects (PTDC/SAU-FAR/119389/2010 and UTAP/ICDT/DTPFTO/0016/2014) and PhD grant SFRH/BD/87869/2012.

References:

[1] Hadinoto K, Sundaresan A, Cheow WS. Eur J Pharm Biopharm **85** (2013) 427.

Optimization and stability study of the preparation of poly(D,L-lactide-*co*-glycolide) nanoparticles incorporating Morphine hydrochloride to prolong its blood circulation

Victoria Gómez Murcia[1], *Mercedes García Montalbán*[2], *Gloria Villora*[2], *Juan C. Gómez Fernández*[3], *Pilar Almela*[1]

[1]Pharmacy and Pharmaceutical Technology Section, Department of Pharmacology, IMIB-Arrixaca, University of Murcia, Spain.
[2]Department of Chemical Engineering, Faculty of Chemistry, University of Murcia, Spain.
[3]Department of Biochemistry and Molecular Biology A, IMIB-Arrixaca, University of Murcia, Spain.
e-mail: victoria.gomez2@um.es

Introduction: Nanoparticle (NP) formulations are widely used for extending drug release, and poly(D,L-lactide-*co*-glycolide) (PLGA) is often used as a material in their preparation due to its high biocompatibility and biodegradability. Double emulsion solvent diffusion method combines the advantages of the emulsification solvent diffusion and the double emulsion solvent evaporation technique, allowing the use of a double-emulsion procedure combined with a partially water-soluble organic solvent and biocompatible surfactants as Pluronic® F-68 and polyvinylalcohol (PVA). The lyophilization process is the most useful method to handle and stabilized NPs to ensure long-term preservation. In the present study, we have encapsulated morphine HCl in PLGA NPs, varying concentrations of the amount of surfactants, and we have studied their consequences for the properties and lyophilization stability of the resulting NPs.

Materials and Methods: NPs were prepared by a double emulsion solvent diffusion-ammonium loading technique. 1 mL of 240 mM ammonium sulphate was emulsified in 3 mL of ethyl acetate containing 3% PLGA by sonication over an ice bath using a probe sonicator, at 20 W output for 90 s. The resulting primary emulsion was added to 10 mL of deionized water, followed by sonication for 90 s over an ice bath to form the double emulsion. Ethyl acetate was eliminated by evaporation under reduced pressure using a rotary evaporator. 1 mL of 20 mg/mL morphine HCl solution was added to the emulsion under moderate stirring and the free drug was separated by ultrafiltration using centricon tubes. PVA or Pluronic® F-68 were used as a stabilizer, PVA was dissolved in the inner and the external aqueous phases while Pluronic® F-68 was dissolved in the organic phase. Effects of surfactant amount on the size, zeta potential and entrapment efficiency were investigated before and after lyophilization using glucose 20% as cryoprotectant to avoid possible modifications. Morphology studies were carried out with TEM. *In vitro* studies were performed in pH 7.4 phosphate buffer at 37 ºC. Adult male Swiss mice were used to study the pharmacokinetic *in vivo* following morphine intraperitoneal (i.p.) administration (30 mg/Kg). Plasma samples were analyzed using GC-MS.

Results and Discussion: When the lowest amount of Pluronic® F-68 NPs were used the lowest particle size (127.6 ± 3.2 nm) and the highest zeta potential (-29.4 ± 0.4 mV) were found, while when the lowest amount of PVA NPs (with size of 193.1 ± 1.6 nm and zeta potential of -1.2 ± 0.1 mV) was used a higher entrapment efficiency (76.6 ± 7.4) was observed. Primary nanosuspensions and rehydrated lyophilization formulations showed that the properties before and after were similar, with only slight variations with the final to initial size ratio not exceeding the limit of 1.5, with PI values in the range for monodisperse system (PI < 0.1). Optimal lyophilized NPs were obtained as no aggregations were observed with glucose 20% as cryoprotectant. *In vitro* studies showed an increased initial morphine burst release for the highest amount of PVA NPs in comparison to that seen for the lowest amount of Pluronic® F-68 formulation. During the initial 4-6 h 50% of 3% Pluronic® F-68 was released in comparison to 70% for when the highest amount of PVA NPs were used and the release behavior after lyophilization were similar than before in all formulations. TEM microscopy images obtained by both stabilizers were spherical and in the nanosize range. After 4 h, only morphine-loaded NPs i.p. administration produced measurable morphine plasma concentrations, with an almost linear decrease in morphine that continued steadily for 8 h. The area under the concentration (AUC) versus time curves were 476.6 ± 51.4 ng·h/mL and 1225.4 ± 30.1 ng·h/mL for free morphine and morphine NPs, respectively.

Conclusions: NPs stability with Pluronic® F-68 are better than with PVA. Lyophilization procedure was successfully achieved although slight change on NP size was observed. Finally, our NPs enhanced morphine bioavailability as blood AUC of morphine-loaded NPs was much higher than that of free morphine. We have developed a useful method to encapsulate morphine in order to obtain an extended delivery compared with free drug.

Acknowledgements: This work was supported by funds from University of Murcia (No. 368 to JCGF) and by Ministerio de Ciencia e Innovación (Grant SAF/FEDER 2013-49076-P) and Fundación Séneca (Grant 15405/PI 10).

References:

[1] Saez A, Guzmán M, Molpeceres J, Aberturas MR. Eur J Phapm Biopharm **50** (2000) 379.
[2] Shi C, Ahmad Khan S, Wang K, Schneider M. Int J Pharm **479** (2015) 41.

Different nanoparticles uptake behavior depending on changes in tumor metabolism

Patricia Altea-Manzano[1], *Juan D. Unciti-Broceta*[2], *Victoria Cano-Cortés*[1,3], *Miguel Martín Hernández*[1], *Rosario M. Sánchez-Martín*[1,3]

[1]Pfizer - Universidad de Granada - Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Spain.
[2]NanoGetic S.L., Parque Tecnológico Ciencias de la Salud (PTS), Granada, Spain.
[3]Departamento de Química Farmacéutica y Orgánica. University of Granada, Spain.
e-mail: patricia.altea@genyo.es

Introduction: Cancer cells must adjust their metabolism accordingly to enable this frenzied growth. These metabolic alterations facilitate resistance to cell death as well as biosynthesis of nucleotides and lipids, building blocks for growth [1]. The reprogrammed metabolism involves an enhanced nutrient uptake to supply energetic and improves biosynthetic pathways [2]. We focus on characterization of tumor cell lines undergoing a permanent metabolic challenge (switching off the mitochondrial oxidative metabolism by permanent mitochondrial DNA depletion, ρ^0 cells). We aim to address how these cells with extreme metabolic phenotypes may, nor just to adapt and survive, but indeed to maintain high proliferative rates. In addition, we use engineered nanoparticles (NPs) to study changes in uptake ratios under different metabolic conditions.

Materials and Methods: *Preparation of 200 nm Cy5-PEG-NPs*. PEGylated 200 nm NPs with Fmoc amine protecting group were coupled to Cy5 dye. Briefly, amino-methyl cross-linked polystyrene (PS) 200 nm NPs were coupled with Fmoc-1-amino-4,7,10-trioxa-13-tridecamine succinic acid using standard HOBt (1-hydroxybenzotriazole)/DIC (1,3-diisopropylcarbodiimide) chemistry. Fmoc protected 200 nm NPs were centrifuged (13000 rpm), washed 3 times with dimethylformamide (DMF) before deprotecting the Fmoc group with 20% piperidine in DMF with three consecutive treatments, 20 min each, at room temperature and shaken at 1400 rpm. Fmoc deprotected 200 nm NPs were then washed 3 times with DMF and coupled to Cy5. For the coupling, NPs were resuspended in DMF with DIPEA and commercially available Cy5 NHS ester was added. Coupling was performed overnight at room temperature and at 1400 rpm. Finally, Cy5-PEG-NPs were washed 2 times with DMF, 2 times washed with water, and finally resuspended in water. The effectiveness of the Cy5 conjugation was checked. *Cy5-PEG-NPs Uptake Study by flow cytometry*. 143B and ρ0206 cell lines were washed with phosphate buffered saline (PBS 1X), detached with trypsin/EDTA (0.25%, phenol red), counted and diluted with media to a final concentration of 10^5 cells per mL. 500 µL of each cell line suspension were plated in 24 well plates and incubated for 12 h. Then, media was replaced with a conditional media mixture containing specific number of Cy5-PEG-NPs to study uptake ratios. Cy5-PEG-NPs number was measured using the method describe above. As control were used unconjugated NPs, referred as naked NPs, and cells without NPs treatment. After different time point incubation with Cy5-PEG-NPs, the media was aspirated and cells were fixed with PFA 2%. Samples were analyzed via flow cytometry with a FACSCanto II flow cytometer (Becton Dickinson & Co., USA). Each experiment was done in duplicate per ratio and repeated 3 times per cell line. Dot plots and cytometry statistics were obtained using FlowJo software (percentage of positive population and median of fluorescence intensity) [3].

Results and Discussion: To further describe the uptake capability of cell lines, we used the MNF_{50} index, defined as the number of NPs capable of nanofecting 50% of the cell population. The influence of number of NPs added per cell (12500, 25000 and 50000 NPs per cell) and time of incubation (10, 20, 30, 40, 50, 60 and 75 min) were evaluated. Besides, a deeper study of the median of fluorescence intensity increments (ΔMFI = MFI sample/MFI untreated) of Cy5-positive cells gave more information about uptake capability of the different cells lines. This analysis revealed that uptake of these NPs was concentration and time dependent. We have demonstrated significant differences between both metabophenotypes regarding uptake ratios.

Conclusions: In this work we apply the novel parameter "multiplicity of nanofection" to match up the cellular uptake by reporting NPs number added per cell with the different culture medium conditions (lack of important nutrients in cancer cells like glucose, glutamine or pyruvate). Also, we have been able to observe how this has influence on cellular phenotype. In this line, one of the objectives proposed is to take advantage of related metabolic uptake differences to use functionalized NPs in biomedical applications.

References:

[1] DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. Cell Metab **7** (2008) 11.
[2] Boroughs LK, DeBerardinis RJ. Nat Cell Biol **17** (2015) 351.
[3] Unciti-Broceta JD, Cano-Cortés V, Altea-Manzano P, Pernagallo S, Díaz-Mochón JJ, Sánchez-Martín RM. Sci Rep **5** (2015) 10091.

5-Fluorouracil-loaded Pluronic® F-127-coated liposomes against colon cancer

Lourdes Valdivia[1], *José L. Arias*[1,2,3], *Mª José Martín*[1], *Laura Cabeza*[2], *Raúl Ortiz*[2,4], *Consolación Melguizo*[2,3], *José C. Prados*[2,3], *Ángel V. Delgado*[5], *Beatriz Clares*[1,2,3]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[2]Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain.
[3]Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
[4]Department of Health Science, University of Jaén, Spain.
[5]Department of Applied Physics, University of Granada, Spain.
e-mail: lvaldiviafdez@gmail.com

Introduction: Current chemotherapy against colon cancer is not fully effective, especially in advanced stages of the disease. Antitumor drugs can often generate severe side effects, compromising the patients´ quality of life. New therapeutic strategies based on drug-loaded nanoplatforms may maximize the outcomes of chemotherapy (and specificity), keeping to a very minimum the incidence of undesirable toxicities. In this sense, the use of liposomes is a good approach. In this line, a promising strategy to promote drug release from liposome-based nanocarriers specifically at the tumor interstitium is based on their conjugation with amphiphilic triblock copolymers, i.e. poloxamers or Pluronic®. They can be incorporated in the liposome membrane, thus enhancing its permeability [1, 2]. This work is focused on the development of Pluronic®-coated liposomes loaded with the anticancer agent 5-Fluorouracil (5-FU) against colon cancer.

Materials and Methods: Pluronic®-coated liposomes were obtained by modifying a reproducible thin film hydration technique [3, 4]. Briefly, L-α-phosphatidylcholine and Pluronic® F-127 were added to chloroform. This organic solution was then dried in a rotary evaporator under vacuum. A thin lipid film layer was obtained which was then dispersed under mechanical stirring in water until completely hydrated to finally obtain the liposome aqueous dispersion. 5-FU loading was achieved by including appropriate concentrations of the drug in the aqueous phase used to disperse the thin lipid film layer. All the formulations were prepared in triplicate. Particle size was determined by photon correlation spectroscopy (PCS) and high resolution transmission electron microscopy (HRTEM). Surface electrical properties were investigated by zeta potential (ζ) determinations as a function of pH and ionic strength. 5-FU loading (and release from the liposomal formulations) was determined by ultraviolet-visible (UV-Vis) spectrophotometry (266 nm). 5-FU release experiments were performed in triplicate following the dialysis bag method and under physiological conditions (pH 7.4, 37 °C). Finally, cytotoxicity of the liposomes was evaluated in human colon fibroblast CCD-18 and in human colon carcinoma T-84 cell lines. Studies were performed in triplicate for all the formulations.

Results and Discussion: Pluronic®-coated liposomes were characterized by an average diameter between 100 and 200 nm and by a spherical shape. 5-FU entrapment efficiency and 5-FU loading was found to be ≈ 11% and ≈ 10%, respectively. Electrophoretic analysis clearly demonstrated the great similarity between the ζ values of the drug-loaded liposomes and the blank liposomes, thus allowing to postulate that 5-FU was satisfactorily incorporated into the liposomal structure (drug absorption). In addition, 5-FU release showed a biphasic process involving an initial rapid 5-FU release phase, followed by a sustained drug release stage. Finally, 5-FU-unloaded Pluronic®-coated liposomes proved to be non-toxic in T-84 and CCD-18 cell lines, while drug loading to the liposomes facilitated an improvement of the antiproliferative effect of 5-FU against T-84 cells compared to 5-FU aqueous solutions.

Conclusions: A reproducible formulation methodology has been developed to obtain Pluronic®-coated liposomes. *In vitro* results postulate the potential application of 5-FU-loaded Pluronic®-coated liposomes against human colon cancer.

Acknowledgements: Financial support from projects P11-CTS-7649 and PE-2012-FQM-694 (Junta de Andalucía, Spain) is gratefully acknowledged.

References:

[1] Barenholz YC. J Control Release **160** (2012) 117.
[2] Zamboni WC. Oncologist **13** (2008) 248.
[3] Clares B, Biedma-Ortiz RA, Sáez-Fernández E, Prados JC, Melguizo C, Cabeza L, Ortiz R, Arias JL. Eur J Pharm Biopharm **85** (2013) 329.
[4] Clares B, Calpena AC, Parra A, Abrego G, Alvarado H, Fangueiro JF, Souto EB. Int J Pharm **473** (2014) 591.

Liposomes surface coated with Pluronic® F-127 for drug delivery applications: formulation and physicochemical characterization

Lourdes Valdivia[1], *Beatriz Clares*[1,2,3], *Mª José Martín*[1], *Mª Adolfina Ruiz*[1,2,3], *José L. Arias*[1,2,3]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[2]Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain.
[3]Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
e-mail: lvaldiviafdez@gmail.com

Introduction: Liposomes have been used to improve the efficiency of drug delivery to the disease site. Conventional liposomes are rapidly intercepted in blood by the mononuclear phagocyte system (MPS). To beat the challenge, poloxamers (Pluronic®) have been recently used to sterically stabilize liposomes. They are polyethylene oxide (PEO)-polypropylene oxide (PPO)-polyethylene oxide tri-block copolymers of different molecular weights. Poloxamers have an amphiphilic nature with hydrophobic (PPO) and hydrophilic (PEO) groups, making them extremely useful as emulsifiers and stabilizers [1, 2]. Here, we report the reproducible formulation of liposomes loaded with the antitumor agent 5-Fluorouracil (5-FU), Pluronic® F-127 coating was possible by simple surface adsorption upon liposome preparation.

Materials and Methods: Liposomes were prepared by following a reproducible thin film hydration technique [3]. 5-FU loading was possible by including the drug molecules in the aqueous phase used to disperse the thin lipid film layer. Finally, the 5-FU-loaded liposomes were incorporated drop-wise to an aqueous solution of Pluronic® F-127, mechanical stirring was continued for 2 h to assure a complete polymeric coating onto the liposome surface. All the formulations were prepared in triplicate. Particle size was investigated by high resolution transmission electron microscopy (HRTEM). Quantification of the amount of 5-FU loaded into (or released from) the liposomes was done by a simple ultraviolet-visible (UV-Vis) spectrophotometric method (266 nm) which was previously validated [3]. 5-FU release experiments were performed in triplicate following the dialysis bag method. In this study, the release medium was phosphate buffered saline (PBS, pH 7.4 ± 0.1, 37.0 ± 0.5 °C). At different time intervals, samples of 1 mL of the medium were withdrawn for UV-Vis spectrophotometric analysis, while an equal volume of PBS, maintained at the same temperature, was added after sampling to assure the *sink* conditions.

Results and Discussion: Liposomes exhibited an average diameter < 200 nm. No presence of particle aggregates or bulky sediments was observed in any of the formulations, and no appreciable change in particle geometry was detected by HRTEM after 3 months of storage in water at 4.0 ± 0.5 ºC. The incorporation of Pluronic® F-127 onto the liposome surface was visualized by HRTEM. 5-FU entrapment efficiency and 5-FU loading was found to be 11.8 ± 1.3% and 9.3 ± 1.5%, respectively. Finally, 5-FU release was characterized by a biphasic process: an initial rapid (burst) release (up to ≈ 15% in 24 h), followed by a sustained release phase (over a period of 196 h).

Conclusions: It has been developed a reproducible formulation methodology to obtain Pluronic®-coated liposomes for the efficient delivery of 5-FU, on the basis of a thin film hydration procedure. *In vitro* and *in vivo* studies are needed to clarify the potential of the nanoformulation in cancer treatment.

Acknowledgements: Financial support from projects P11-CTS-7649 and PE-2012-FQM-694 (Junta de Andalucía, Spain) is gratefully acknowledged.

References:

[1] Hossann M, Wang T, Wiggenhorn M, Schmidt R, Zengerle A, Winter G, Eibl H, Peller M, Reiser M, Issels RD, Lindner LH. J Control Release **147** (2010) 436.
[2] Tagami T, May JP, Ernsting MJ, Li SD. J Control Release **161** (2012) 142.
[3] Clares B, Biedma-Ortiz RA, Sáez-Fernández E, Prados JC, Melguizo C, Cabeza L, Ortiz R, Arias JL. Eur J Pharm Biopharm **85** (2013) 329.

Study of functionalized magnetic nanoparticles for biomedical applications

Patricia Jiménez[1], Margarita López-Viota[1], Raúl Megías[1], Julián López-Viota[2], Ángel V. Delgado[3]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[2]Vircell Microbiologists, Granada, Spain.
[3]Department of Applied Physics, University of Granada, Spain.
e-mail: mlvg@ugr.es

Introduction: Accumulation of magnetic nanoparticles into the site of action can be possible with the help of an magnetic field, then releasing the loaded drug in this desired area [1]. Functionalization of magnetic cores with a polymeric coating can increase their stability. In this work, it is described the synthesis and characterization of nanocomposites that may be used for biomedical purposes, e.g. magnetic resonance imaging (MRI), drug therapy and gene therapy. Careful design is needed to gain control of their shape, size, and surface properties. Concretely, these magnetic nanoplatforms were made of magnetic cores (magnetite, Fe_3O_4) embedded within a biodegradable matrix [poly(D,L-lactide-*co*-glycolide), PLGA, or polyethyleneimine, PEI].

Materials and Methods: Fe_3O_4 was prepared by chemical co-precipitation, adding $FeCl_3 \cdot 6H_2O$ (1 M) and $FeCl_2 \cdot 4H_2O$ (2 M) to NH_3 0.7 M (mean diameter: 25.2 ± 1.8 nm) [2]. Synthesis of Fe_3O_4/PLGA (core/shell) nanoparticles was performed by a double emulsion methodology after treating the magnetic cores with perchloric acid (2 M) [3], while Fe_3O_4/PEI (core/shell) nanoparticles were formulated by adding a PEI aqueous solution under mechanical stirring to Fe_3O_4 particles previously treated with citric acid monohydrate [4]. Characterization of shape and size was done by photon correlation spectroscopy (PCS), transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Surface electrical properties were analyzed by zeta potential (ζ) determinations as a function of pH and ionic strength. Stability of the nanocomposites was evaluated by determining ζ values in 1 mM sodium perchlorate medium, and by studying the release of Fe ions from the particles by inductively coupled plasma/optical emission spectrometry (ICP/OES). The stability was studied under physiological conditions in sterile NaCl solution 0.9% (w/v) (pH 7.4) by electrokinetic determinations and by determining the sedimentation kinetics.

Results and Discussion: Fe_3O_4/PLGA and Fe_3O_4/PEI composites were spherical nanoparticles under 200 nm. Electrokinetic measurements revealed an isoelectric point ≈ pH 8 for Fe_3O_4 particles and positive ζ values. On the opposite, Fe_3O_4/PLGA nanocomposites were characterised by an isoelectric point ≈ pH 4 and negative ζ values, while the Fe_3O_4/PEI nanoparticles didn´t exhibit an isoelectric point within the pH range investigated. Aging tests demonstrated that despite the Fe_3O_4 particles were stabilized with perchloric acid, they were partially dissolved and released Fe ions to the medium. On the contrary, the polymeric coating (PLGA or PEI) protected these cores against this process. The adequate stability of the nanocomposites (Fe_3O_4/PLGA and Fe_3O_4/PEI) under physiological conditions was confirmed by electrophoretic measurements and investigation of the sedimentation kinetics.

Conclusions: It was hypothesized that the Fe_3O_4/PLGA and Fe_3O_4/PEI nanocomposites could be advantageously used in biomedicine. In fact, the nanoparticles were characterized by a suitable geometry, controllable surface electrical charge, and *in vitro* stability.

Acknowledgements: Financial support from project PE-2012-FQM-694 (Junta de Andalucía, Spain) is gratefully acknowledged.

References:

[1] Fernández-Pacheco R, Valdivia JG, Ibarra MR. J Magn Magn Mater **311** (2007) 175.
[2] Massart R. IEEE Trans Magn **17** (1981) 1247.
[3] Nejati-Koshki K, Mesgari M, Ebrahimi E, Abbasalizadeh F, Fekri Aval S, Khandaghi AA, Abasi M, Akbarzadeh A. J Microencapsul **31** (2014) 815.
[4] Wang X, Zhou L, Ma Y, Li X, Gu H. Nano Res **2** (2009) 365.

Design, and technological and biological evaluation of matrix tablets containing a probiotic strain

Mª Teresa Sánchez, Mª Adolfina Ruíz, Mª Encarnación Morales

Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
e-mail: maritere389@gmail.com

Introduction: The industrial production and commercialization of probiotics has been increased during last decades. Habitually, a probiotic product should contain > 10^6 - 10^8 colony forming units (CFUs)/g or > 10^8 - 10^{10} CFU/dosage of viable cells [1]. The main problem associated with the administration of probiotics is to maintain the survival of these microorganisms under storage and in the gastrointestinal tract, thus there is a need for formulations capable of protecting the bacteria from environment and harsh stomach conditions. In this line, tablets can be easily designed using proper excipients to control the release, and bacteria adhesion and colonization at the ephitelial mucosa. Therefore, the aim of this work is to design and develop an oral probiotic tablet which satisfies the technological and viability requirements.

Materials and Methods: Tablet processing was done by direct compression of a powder mixture containing *Lactobacillus coryniformis* CECT 5711 and different polymers (sodium alginate, Methocel® k-15 M EP, Eudragit® L-100, ethylcellulose, and cellulose acetate phthalate, being used to assure a controllable release). Each mixture was filled into a die of 10 mm diameter, under pressure (ranging from 5 to 25 kN) and several compression times. The probiotic tablets were then evaluated according to the United States Pharmacopeia (USP) and *Real Farmacopea Española* (RFE) well-known methods to define their disintegration, tensile strength, friability, and mass uniformity characteristics [2, 3]. Cell viability inside the tablets was studied with microbial cultures on MRS Agar plates incubated at 37 ºC for 48 h. Colonies of bacteria were counted and converted to log CFUs, and the survival of the probiotics was analyzed. Finally, bacteria stability in these tablets during the storage period was investigated. To that aim, tablets were kept in hermetic containers at 4 ºC and at room temperature during one year.

Results and Discussions: Regarding the influence of the compression force on the formulation of the oral probiotic tablet, 15 kN of compression force was selected as optimal, while the combination of alginate sodium (25 mg), Methocel® K-15 M EP (100 mg), and Eudragit® L-100 (75 mg) was selected as the best tablet composition, according to the technological and viability assays. Viability inside tablets after compression was found to be more that satisfactory (5×10^9 CFU/tablet), if it is taken into account that a dose of 2×10^9 CFU/day of *L. coryniformis* CECT5711 can enhance the intestinal function [4], hence allowing an increment in several immunological parameters. In addition, once the tablets have passed the stomach acid medium, cell viability decreased in one log of CFU (5×10^8 CFU/tablet, an effective dose) [1]. Stability studies defined a cell viability of 10^9 CFU/tablet during 365 days and at different temperatures.

Conclusions: The probiotic tablet that has been formulated exhibits excellent technological properties, overcoming the problems associated to this microorganism and assuring an adequate survival for therapeutic purposes.

Acknowledgements: Probiotic support from Biosearch Life S.A. (Spain) is gratefully acknowledged.

References:

[1] Champagne CP, Ross RP, Saarela M, Hansen KF, Charalampopoulos D. Int J Food Microbiol **149** (2011) 185.
[2] Stadler M, Viernstein H. Int J Pharm **256** (2003) 117.
[3] Klayraung S, Viernstein H, Okonogi S. Int J Pharm **370** (2009) 54.
[4] Lara-Villoslada F, Sierra S, Martín R, Delgado S, Rodríguez JM, Olivares M, Xaus J. J Appl Microbiol **103** (2007) 175.

Effect of calcium chloride concentration on the microencapsulation of probiotics

Mª Teresa Sánchez, Mª Adolfina Ruíz, Mª Encarnación Morales

Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
e-mail: maritere389@gmail.com

Introduction: Microencapsulation allows packaging and protecting numerous materials from environmental and detrimental influences. Internal ionic gelation facilitates the formation of spherically structured systems with sizes of < 1000 μm, thanks to interactions between cations and negatively-charged biopolymers [1]. Alginates are widely used in food industry thanks to its ability to form stable gels through the ionic interaction between two adjacent G chains with Ca^{2+}, thus forming junction zones that stabilize the gel structure. Microencapsulation can be done to reduce cell losses during food processing and storage, as well as to enhance the viability of a given probiotic strain. However, it is important to ensure that the selected technique is compatible with microorganisms. The aim of this work was to study the effect of Ca^{2+} concentration on probiotic viability and microparticle´s morphology.

Materials and Methods: Microparticles were elaborated by following the emulsification/internal gelation method. The internal phase is formed by preparing a sodium alginate aqueous solution with *Lactobacillus gasseri* and calcium carbonate, while the external phase contained a vegetable oil and Span® 80. Glacial acetic acid was added to the emulsion to assure the solubilization of calcium carbonate. Finally, a calcium chloride solution was used to separate and harden the microparticles [2]. The method was carried out with 5 mL and 100 mL of a calcium chloride solution to know its effect on microparticle´s morphology, size, and microorganism survival. Particle geometry (size and shape) was evaluated by scanning electron microscopy. Microparticles were dispersed in a sodium citrate solution, and cell viability inside them was studied with microbial cultures on MRS Agar plates incubated at 37 ºC for 48 h. Colonies of bacteria were counted and converted to log colony forming units (CFUs), and survival of probiotic was analyzed. Finally, stability of bacterial cells into the microparticles during storage was investigated (tablets were kept in hermetic containers at 4 ºC for 3 months).

Results and Discussions: Synthesis (S1) contained 100 mL of a calcium chloride solution (5%, w/v), and S2 contained only 5 mL of this solution. Probiotic viability studies after the encapsulation procedure showed a viability loss of 0.5 log CFU in both cases. Particle size was similar in both cases, showing that 80% of particles had a size between 50 and 110 μm. After 90 days at 4 ºC, microparticles showed a viability loss of 2.6 ± 0.2 log CFU (S1) and 1.4 ± 0.2 log CFU (S2). Such a result could be explained by the effect that Ca^{2+} ions caused on alginate: these cations prompt the alginate compaction and the particles were denser [2]. Probably, due to this fact, the conservation was more difficult. Hence, S1 particles had a worse survival because of the higher amount of Ca^{2+} which caused higher compaction and bacteria death.

Conclusions: These probiotic microparticles had shown an excellent geometry for an oral and vaginal administration, thus Ca^{2+} concentration did not affected this property. However, *Lactobacillus* survival inside the microparticles showed higher loss in S1 particles than S2. Ca^{2+} concentration affected negatively the probiotic viability in contrast to previously published investigations [1], claiming that Ca^{2+} concentration has no negative effects on culture viability. Finally, 5 mL of a calcium chloride solution (5%, w/v) was selected as optimal in this encapsulation procedure, because it assured the greatest bacteria survival.

Acknowledgements: Probiotic support from Biosearch Life S.A. (Spain) is gratefully acknowledged.

References:

[1] Rosas-Flores W, Ramos-Ramírez EG, Salazar-Montoya JA. Carbohydr Polym **98** (2013) 1011.
[2] Burgain J, Gaiani C, Linder M, Scher J. J Food Eng **104** (2011) 467.

Formulation and characterization of glucose microspheres for cell therapy

Patricia Gálvez[1], Mª José Martín[1], Ana C. Calpena[2], Juan A. Tamayo[3], Mª Adolfina Ruiz[1], Beatriz Clares[1]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[2]Department of Pharmacy and Pharmaceutical Technology, University of Barcelona, Spain.
[3]Department of Medicinal and Organic Chemistry, University of Granada, Spain.
e-mail: beatrizclares@ugr.es

Introduction: The use of stem cells is currently one of the most promising areas in the design of new medicines. One of the main problems in cell therapy is related to the high percentage of cells alive being required at the time of administration (80%) and the short shelf-life of these cells. Different strategies to improve this fact have been studied. Some of them are cell encapsulation, scaffold-free delivery format, and the use of ingredients that can help in maintaining cell viability. To this respect, only few studies, have examined the influence of nutrient factors and excipients on the survival of stem cells. It is known that reduced levels of glucose and oxygen combined with other environmental conditions decreases the anabolic activity of cells [1]. On the contrary, high extracellular glucose or glycolytic intermediate concentrations can maintain cell viability to some extent whilst stimulating lactate production [2]. In this work, it is described the synthesis and characterization of biodegradable alginate microspheres secreting glucose, as an approach to enhance and prolong the functionality of stem cells.

Materials and Methods: Unloaded and glucose-loaded microspheres were elaborated by emulsification/internal gelation methodology with modifications [3]. Physicochemical characteristics of these microspheres were studied. The morphology was determined by optical microscopic, and particle size was analyzed by light diffraction. The surface electrical properties were evaluated by zeta potential (ζ) determinations. Fourier transform infrared spectrometry data were used for the characterization of the chemistry of the microspheres. The yield (%), loading capacity (LC, %), and encapsulation efficiency (EE, %) were also determined.

Results and Discussion: Gelation was achieved by gentle acidification of the alginate solution with or without glucose. This solution also contained an insoluble calcium salt. After the addition of an oil-soluble acid, calcium ions were released, thus obtaining spherical-shaped and small-sized microspheres. No aggregation was observed. Mean particle sizes ($LD_{4.5}$) of unloaded and glucose-loaded microspheres were 94.03 and 86.62 μm, with span factors of 1.06 and 0.77, respectively. Unloaded and glucose-loaded microspheres exhibited negatives ζ values: -31.29 and -32.54 mV, respectively. This negative charge could be attributed to the presence of polymeric terminal carboxylic groups on the particle surface. The inclusion of glucose did not significantly alter the electrical surface charge. The infrared spectra of the two types of particles showed that the characteristic bands of unloaded microspheres were maintained in the glucose-loaded microspheres, hence indicating that glucose did not induce subsequent modifications in the structure of alginate. The values of yield, LC, and EE were 70.29, 20.45, and 30.54%, respectively.

Conclusions: It is shown a comprehensive technological development of glucose-loaded microspheres as potential tools to improve the viability of a medicine based on stem cell.

Acknowledgements: Financial support from project MAT2011-26994 (MCNN – Ministerio de Ciencia e Innovación, Spain) is acknowledged.

References:

[1] Parry RV, Chemnitz JM, Frauwirth KA, Lanfranco AR, Braunstein I, Kobayashi SV, Linsley PS, Thompson CB, Riley JL. Mol Cell Biol **25** (2005) 9543.
[2] DhanasekaranM, Indumathi S, Rajkumar JS, Sudarsanam D. Cell Biochem Funct **31** (2013) 20.
[3] Martín-Villena MJ, Fernández-Campos F, Calpena-Campmany AC, Bozal-de Febrer N, Ruiz-Martínez MA, Clares-Naveros B. Carbohydr Polym **94** (2013) 1.

New emulsomes against malignant proliferation of epidermal keratinocytes for the treatment of multifocal actinic keratosis and skin squamous cell carcinoma

Eliana B. Souto[1,2], Slavomira Doktorovova[1,2], Amélia M. Silva[3,4], María L. Garcia[5,6], Beatriz Clares[7]

[1]Department of Pharmaceutical Technology, University of Coimbra, Portugal.
[2]Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Portugal.
[3]Department of Biology and Environment, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal.
[4]Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro, Vila-Real, Portugal.
[5]Department of Physical Chemistry, University of Barcelona, Spain.
[6]Institute of Nanoscience and Nanotechnology (IN2UB). University of Barcelona, Spain.
[7]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
e-mail: beatrizclares@ugr.es

Introduction: Multifocal actinic keratosis (AK) and skin squamous cell carcinoma (SSCC), a common cancer arising from malignant proliferation of epidermal keratinocytes, depend on patients´ characteristics (age, skin type, ethnicity), but also upon risk factors (mainly ultraviolet exposure) [1, 2]. Loss of mesenchymal Notch/CSL signaling is related to stromal atrophy and inflammation, and usually precedes epithelial tumors. A decrease of this signaling in stromal tissues is associated to multifocal pre-malignant AK lesions (well-known precursor lesions for SSCC), and it is related to changes in gene expression (which can be induced by ultraviolet exposure). We hypothesized the potential use of emulsomes against malignant proliferation of epidermal keratinocytes by delivering of selected anticancer drugs (5-Fluorouracil, Tretinoin, Imiquimod) [2]. Skin SSC is a rather common tumor associated to changes in epithelial and mesenchymal tissues, therefore being highly invasive and very likely to grow into deeper layers of skin, spreading into other parts of the organism. The lesions usually appear on sun-exposed areas of the body (e.g. face, lips, ears, and neck) and may also occur in scars or chronic skin sores. Notch signaling is involved in cell homeostasis while the activated Notch intracellular domain interacts with the DNA binding factor CSL (CBF-1), converting it into an activator of transcription [3, 4]. Differentiation of keratinocytes occurs via this pathway. Loss of the CSL/RBP-Jκ gene may induce skin cancer lesions, while suppression of the Notch/CSL signaling may occur in pre-malignant AK. Emulsomes are a new generation of drug delivery systems composed of an internal core of fats and triglycerides (stabilized by high concentration of lecithin), forming an o/w emulsion. They are nanosystems with the characteristics of both liposomes and emulsions. The solidified or semi-solidified internal oil core provides the adequate environment to load lipophilic drugs and to modify the release rate of hydrophilic compounds.

Materials and Methods: Effects of 5-Fluorouracil-, Tretinoin-, and Imiquimod-loaded emulsomes were tested on cultured primary human keratinocytes (HKCs) and on keratinocyte-derived SCC cells (SCC12 and SCC13 lines, and A431 cell line). The most effective combination was tested in HKCs and SCC cell lines for its ability to promote cell differentiation. 5-bromo-2´-deoxyuridine labeling and Alamar Blue tests were also done. The studies have been completed with *in vivo* tests in mice bearing multifocal pre-malignant keratinocytic lesions which progress into malignant SCCs over time was evaluated to assess Cre-mediated gene deletion.

Results and Discussion: Preliminary tests to drug-loaded emulsomes have been conducted on human skin to increase drug bioavailability. They successfully demonstrated their effectiveness to target epidermis, as reported for similar lipid carriers [5, 6]. The combinations have been tested for their anticancer effects in HKC and SCC cells, followed by ELISA assays, and RNA-sequencing analysis.

Conclusions: It has been demonstrated that the use of emulsomes is a suitable alternative to increase the bioavailability of anticancer drugs in pre-malignant AK and SCC lesions, with no tissue damage.

References:

[1] Costa C, Scalvenzi M, Ayala F, Fabbrocini G, Monfrecola G. J Dermatol Case Rep **9** (2015) 29.
[2] Filosa A, Filosa G. G Ital Dermatol Venereol **150** (2015) 379.
[3] Liu Z, Brunskill E, Varnum-Finney B, Zhang C, Zhang A, Jay PY, Bernstein I, Morimoto M, Kopan R. Development **142** (2015) 2452.
[4] Takazawa Y, Ogawa E, Saito R, Uchiyama R, Ikawa S, Uhara H, Okuyama R. J Dermatol Sci **79** (2015) 10.
[5] Alvarado HL, Abrego G, Souto EB, Garduño-Ramirez ML, Clares B, García ML, Calpena AC. Colloids Surf B Biointerfaces **130** (2015) 40.
[6] Clares B, Calpena AC, Parra A, Abrego G, Alvarado H, Fangueiro JF, Souto EB. Int J Pharm **473** (2014) 591.

Engineering of 5-Fluorouracil-loaded PLGA nanoparticles for cancer treatment

Elena Pretel[1], José L. Arias[1,2,3], Laura Cabeza[2,3], Cristina Lorente[1], Ángel V. Delgado[4], José C. Prados[2,3], Consolación Melguizo[2,3], Beatriz Clares[1]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[2]Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
[3]Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain.
[4]Department of Applied Physics, University of Granada, Spain.
e-mail: crislg@correo.ugr.es

Introduction: The introduction of nanotechnology in biomedicine has revolutionized the diagnosis and treatment of many diseases, e.g. cancer [1]. In the drug delivery arena, nanocarriers will protect the loaded drug from biological metabolization and elimination, and will make possible the highest therapeutic effect with minimal toxicity [2]. This work is devoted to the development of a reproducible methodology for the formulation of poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles (NPs) loaded with 5-Fluorouracil (5-FU). Size, electrical surface charge and toxicity characteristics of the NPs are also investigated.

Materials and Methods: PLGA NPs were formulated by a water-in-oil-in-water (w/o/w) double emulsion/solvent evaporation (DE/SEV) methodology [3]. In a few words, an aqueous solution of Pluronic® F-68 was added to a PLGA solution in chloroform under mechanical stirring. The resulting w/o emulsion was then added under mechanical stirring to an aqueous solution. As a result, a w/o/w emulsion was formed. Finally, mechanical stirring in a rotary evaporator was continued to obtain the colloid. Optimization of the synthesis conditions was performed by evaluating the effect of the stirring speed and time on NP production. Particle size was determined by photon correlation spectroscopy (PCS), high resolution transmission electron microscopy (HRTEM), and scanning electron microscopy (SEM). Characterization of the electrokinetic properties of the NPs was done by zeta potential determinations. Ultraviolet-visible absorbance measurements (8500 UV-Vis spectrophotometer, Dinko, Spain) were accomplished at room temperature to quantify 5-FU loading. 5-FU release from the NPs was performed in PBS (pH 7.4 ± 0.1) following the dialysis bag method. Finally, PLGA NPs were tested in human colon fibroblast CCD-18 and in human colon carcinoma T-84 cell lines by the 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl tetrazolium bromide (MTT) proliferation assay.

Results and Discussion: Particle size was not significantly determined by the mechanical stirring speed, being always adequate for the parenteral route of administration. However, PLGA NPs formulated using a sonicator showed the optimal size (≈ 110 nm, polydispersity index ≈ 0.2). In addition, these NPs were characterized by a negative surface charge (-30 ± 2 mV), probably defining an adequate stability under storage conditions (delay/absence of NP sedimentation). Maximum values of 5-FU entrapment efficiency and drug loading were ≈ 33% and ≈ 13%, respectively. 5-FU release from the NPs was a biphasic process with an initial fast (burst) drug release (up to 50% in 24 h), the remaining 5-FU molecules being released in a sustained way over the next 15 days. Results from the MTT assay indicated that (blank) PLGA NPs were not cytotoxic by themselves, while 5-FU-loaded NPs induced a greater antitumor effect in comparison with free 5-FU. These *in vitro* results were found to be reproducible when the NPs were kept under standard storage conditions during 30 days.

Conclusions: It has been developed a reproducible procedure to obtain 5-FU-loaded PLGA NPs with an adequate size for the parenteral route of administration, that can improve antitumor activity of the anticancer agent.

Acknowledgements: Research leading to these results has received funding from projects FIS PI11/02571 (Instituto de Salud Carlos III, Spain) and PE-2012-FQM-694 (Junta de Andalucía, Spain).

References:

[1] Couvreur P. Adv Drug Deliv Rev **65** (2013) 21.
[2] Arias JL. Mini Rev Med Chem **11**(2011) 1.
[3] Cózar-Bernal MJ, Holgado MA, Arias JL, Muñoz-Rubio I, Martín-Banderas L, Álvarez-Fuentes J, Fernández-Arévalo M. J Microencapsul **28** (2011) 430.

Study of a cellular suspension based on glucose-loaded microspheres and mesenchymal stem cells

Mª José Martín[1], Ana C. Calpena[2], Juan A. Tamayo[3], Mª Adolfina Ruiz[1], Beatriz Clares[1], Patricia Gálvez[1]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[2]Department of Pharmacy and Pharmaceutical Technology, University of Barcelona, Spain.
[3]Department of Medicinal and Organic Chemistry, University of Granada, Spain.
e-mail: mjmv@ugr.es

Introduction: The use of human mesenchymal stem cells (hMSCs) in regenerative medicine and advanced therapies has gained strong interest during the last years. Their anti-inflammatory, angiogenic, antithrombogenic, and antiapoptotic properties make them valuable tools for cell therapy. However, the clinical use of hMSCs requires a high viable number of cells (> 80%) at the time of administration [1]. Long-term clinical success will depend on cells remaining viable: failure of cell therapy has been attributed to an extensive cell death [2]. Selection of the excipients for the formulation of a cellular suspension has been studied to improve the short shelf-life of hMSCs, and it has been shown that glucose along with other nutrients such as Ringer´s lactate and albumin can assure cell viability [3]. In this work, it is described the formulation of a hMSC suspension with a new strategy based on the release of encapsulated glucose in biodegradable alginate microspheres to optimize cell viability.

Materials and Methods: Glucose-loaded microspheres were elaborated by emulsification/internal gelation with modifications [4]. Effects of glucose-loaded microspheres on cell viability were determined in 4 different media: with and without microspheres (*A* and *B*), with phosphate buffer solution (*C*), and a control suspension (*D*). Cell viability was determined every 6 h during 60 h by trypan blue dye exclusion staining and posterior counting of cells in a Neubauer chamber. Kinetic evaluation of cell viability was fitted to 5 different models: zero order, first order polynomial, second order polynomial, and dual with shoulder and plateau decay zero, to define the mechanism that statistically best represented the findings. Cell sterility was analyzed before being packaged and after the stability study with the microspheres, by direct inoculation in accordance with the European Pharmacopoeia. Morphological characteristics of the hMSCs in each medium were investigated by optical microscopy. Finally, a rheological characterization was performed at 25 ºC (rotational rheometer HAAKE Rheostress).

Results and Discussion: Glucose-loaded microspheres did not affect the stability of the cell suspension. Medium *A* showed that viability was > 90% for 30 h after packaging (91.5 ± 3.5%), and after 48 h viability declined < 80% (77.2 ± 2.7) as medium *B* (76.3 ± 3.5%). Medium *C* maintained the viability > 80% for less time, up to 36 h (82.9 ± 2.1%). Medium *D* showed that viability was > 80% for 48 h after packaging (80.2 ± 2.8%) and decreased more rapidly after 12 h. According to the smallest AIC and r^2 value ≈ 1, the second order polynomial model was the best to describe statistically the kinetic viability of media *A*, *B*, and *C*. First order polynomial was the most suitable for medium *D*. Sterility tests confirmed the absence of contaminating microorganisms. Morphological evaluation of the hMSCs in contact with the microspheres showed a homogeneous population with a fibroblast-like morphology before packing. Rheological studies in (unstirred) media demonstrated a Newtonian behavior and a lower viscosity value (1.01 ± 0.05 mPas), which indicated that there was migration of aggregates/flocculates. After shaking, they exhibited a pseudoplastic behavior, where aggregates were separated by shear forces breaking up the weak reversible flocculation. Viscosity value of the finished cell suspension was 1.53 ± 0.04 mPas.

Conclusions: It has been described a formulation of a cellular suspension with glucose-loaded alginate microspheres able to maintain the functionality and viability of hMSCs and the stability of cell suspensions.

Acknowledgements: Financial support from project MAT2011-26994 (MCNN – Ministerio de Ciencia e Innovación, Spain) is acknowledged.

References:

[1] Galvez P, Clares B, Hmadcha A, Ruiz A, Soria B. Br Med Bull **105** (2013) 85.
[2] Bayoussef Z, Dixon JE, Stolnik S, Shakesheff KM. J Tissue Eng Regen Med **6** (2012) e61.
[3] Gálvez-Martín P, Hmadcha A, Soria B, Calpena-Campmany AC, Clares-Naveros B. Eur J Pharm Biopharm **86** (2014) 459.
[4] Martín-Villena MJ, Fernández-Campos F, Calpena-Campmany AC, Bozal-de Febrer N, Ruiz-Martínez MA, Clares-Naveros B. Carbohydr Polym **94** (2013) 1.

Uptake of poly(ε-caprolactone) nanoparticles by A549 lung carcinoma cells

Cristina Galocha[1], *Mª José Martín*[1], *José L. Arias*[1,2,3], *Julia Jiménez-López*[2,3], *Mª Adolfina Ruiz*[1,2,3], *José C. Prados*[2,3], *Consolación Melguizo*[2,3], *Beatriz Clares*[1,2,3]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[2]Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
[3]Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain.
e-mail: galochacristina@gmail.com

Introduction: Paclitaxel (PTX) is one of the most active broad-spectrum chemotherapy agents in cancer treatment. Its major limitation is its low water solubility, being formulated in organic solvents of polyoxyethylated castor oil (Cremophor® EL) and dehydrated ethanol (50/50, v/v). Cremophor® EL is known to cause serious side effects that can severely limit the pharmacotherapy. In order to overcome the problem, PTX has been incorporated to numerous nanocarriers reporting several advantages over the standard-of-care therapy. Nanoparticles (NPs) carrying drugs may improve tumour uptake of drugs and reduce their toxic effects, thanks to the enhanced permeability and retention (EPR) effect characteristic of malignant tissues. It is accepted that an easy procedure to define the cellular uptake of drug-loaded NPs is based on the use of a fluorescent marker dye [1, 2]. In this work, it is investigated the utility and limitations of poly(ε-caprolactone) (PCL) NPs as an intracellular drug delivery system. To that aim, the NPs were loaded with Nile red, a fluorescent dye with an apolar character quiet close to that of PTX.

Materials and Methods: Nile red-loaded PCL NPs were prepared by double emulsion solvent evaporation [3]. Briefly, a solution of PCL and Nile red in dichloromethane was emulsified in polyvinyl alcohol to obtain an oil-in-water emulsion. This emulsion was then emulsified in a PVA aqueous solution to form a multiple water in-oil-in-water emulsion. Finally, the organic solvent was completely evaporated by mechanical stirring during 24 h, and the NPs were then washed twice and re-dispersed in an aqueous solution. The average particle size and size distribution were determined by photon correlation spectroscopy. Uptake of NPs by A549 lung carcinoma cells was investigated by fluorescence microscopy: cells were incubated with free Nile red and with Nile red-loaded PCL NPs at different incubation times (0.5, 1, 2, and 4 h).

Results and Discussion: A reproducible formulation based on PCL NPs as potential carriers of PTX has been developed. The NPs were characterized by a mean size ≈ 289 nm and by a polydispersity index of ≈ 0.4, being appropriate to facilitate a passive PTX targeting into the tumour interstitium. In addition, cellular uptake studies demonstrated an efficient NP incorporation into A549 cells. Therefore, it can be hypothesized the efficient delivery of PTX to these cancer cells. Finally, no cytotoxic activity was developed by the Nile red-loaded PCL NPs in the malignant cells.

Conclusions: *In vitro* results proved that PCL NPs could be advantageously used for the efficacious delivery of hydrophobic anticancer drugs, e.g. PTX, to tumour cells. More *in vitro* and *in vivo* studies are being done to define completely the potential use of the nanoformulation against lung cancer.

Acknowledgements: Financial support from projects PE-2012-FQM-694, P11-CTS-7649, and PI-0049 (Junta de Andalucía, Spain) is gratefully acknowledged.

References:

[1] Ma P, Mumper RJ. J Nanomed Nanotechnol **4** (2013) 1000164.
[2] Snipstad S, Westrøm S, Mørch Y, Afadzi M, Åslund AK, de Lange Davies C. Cancer Nanotechnol **5** (2014) 8.
[3] Kim BS, Kim CS, Lee KM. Arch Pharm Res **31** (2008) 1050.

Δ^9-tetrahidrocannabinol-loaded PLGA nanoparticles: *in vivo* evaluation of anticancer activity

Lucía Martín-Banderas[1], Josefa Álvarez-Fuentes[1], Inmaculada Rubio[1], José C. Prados[2,3], José L. Arias[2,3,4], Mª Carmen Leiva[2,3], Mª Ángeles Holgado[1], Mercedes Fernández-Arévalo[1]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Seville, Spain.
[2]Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain.
[3]Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
[4]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
e-mail: jlarias@ugr.es

Introduction: Nanoplatforms can optimize the efficacy and safety of chemotherapy, and thus cancer therapy. Δ^9-tetrahidrocannabinol (Δ^9-THC) has been described to inhibit tumor angiogenesis and cell growth in malignant tissues, thus causing cell death [1]. In this work, a reproducible methodology is described to prepare Δ^9-THC-loaded poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles (NPs) against lung cancer. The NPs were further improved by surface functionalization with the biodegradable polymers chitosan (CS) and poly(ethylene glycol) (PEG) to optimize the biological fate and antitumor effect. Vitamin E was incorporated to the formulations to enhance the stability of Δ^9-THC against oxidation [2].

Materials and Methods: The preparation of PLGA NPs by nanoprecipitation and the surface functionalization had been described previously [3]. 4 nanoformulations were synthesized, i.e. PLGA NPs, CS-coated PLGA NPs (CS-PLGA NPs), PEGylated PLGA NPs (PEG-PLGA NPs), and PEGylated CS-coated PLGA NPs (PEG-CS-PLGA NPs). *In vivo* anticancer studies were performed employing immunocompetent female C57BL/6 mice. All mice were subcutaneously injected in the right hind flank with 5×10^5 LL2 cells dispersed in 200 µL of PBS. When tumors were palpable, mice were treated every 3 days with: control (non-treated mice), free Δ^9-THC, PEG-PLGA NPs, and Δ^9-THC-loaded PEG-PLGA NPs (selected nanoformulations for this study given its promising *in vitro* antitumor effect). The treatment consisted in a peritumoral injection during 21 days of the formulations (Δ^9-THC equivalent doses: 5 mg/Kg of body mass). In the *in vitro* cell culture assays, Student´s *t*-test and one-way analysis of variance were used to define the significance between groups. Statistical analysis was performed using the Student´s *t*-test. The probability of mice survival (cumulative survival curves) was determined by the Kaplan Meier method, and the log-rank test was used to compare the fraction of surviving mice between groups ($\alpha = 0.05$). Data with $p < 0.05$ and $p < 0.001$ were considered as significant and very significant, respectively.

Results and discussion: The evaluated NPs had a mean size close to 590, 750 and ≈ 790 nm, when were incorporated onto their surface PEG, CS, and PEG-CS, respectively. The values of EE and DL were always > 90 and > 4%, respectively. The anticancer activity of free Δ^9-THC, PEG-PLGA NPs, and Δ^9-THC-loaded PEG-PLGA NPs was investigated in LL2 lung tumor-bearing immunocompetent C57BL/6 mice. No statistically significant differences in terms of antitumor effect were observed during the first 4 weeks of treatment. Compared to the control group and the free Δ^9-THC, Δ^9-THC-loaded PEG-PLGA NPs reported a 2.2-fold reduction in tumor volume on day 32, which was finally measured to be a 1.5-fold reduction on day 41 (end of the experiment). In any case, no statistically significant differences were defined between the groups in terms of tumor volume reduction and cumulative survival. On the other hand, no disparities were detected in the tumor necropsies of the animals included in the 4 study groups. Finally, the similarities found between the control group and the groups of mice under treatment in terms of evolution of body weight suggested the adequate *in vivo* safety margin of the formulations.

Conclusions: A reproducible nanoprecipitation technique has been proposed to prepare PLGA-based nanoformulations loaded with Δ^9-THC with adequate *in vivo* safety margin and high drug loading efficiencies. *In vivo* studies show the potential application of the nanoformulation in lung cancer chemotherapy. Supplementary surface modifications, i.e. decoration of NPs surface with ligands specific to receptors overexpressed onto the cancer cell membrane, may optimize the selectivity of these Δ^9-THC-loaded nanosystems toward tumor cells.

Acknowledgements: Proyect P09-CTS-5029 (Junta de Andalucía, Spain) and "V Plan Propio de Investigación" of the University of Seville.

References:

[1] Rocha FC, Dos Santos Júnior JG, Stefano SC, da Silveira DX. J Neurooncol **116** (2014) 11.
[2] Martín-Banderas L, Muñoz-Rubio I, Prados J, Álvarez-Fuentes J, Calderón-Montaño JM, López-Lázaro M, Arias JL, Leiva MC, Holgado MA, Fernández-Arévalo M. Int J Pharm **487** (2015) 205.
[3] Martín-Banderas L, Muñoz-Rubio I, Álvarez-Fuentes J, Durán-Lobato M, Arias JL, Holgado MA, Fernández-Arévalo M. Colloids Surf B Biointerfaces **123** (2014) 114.
[4] Gustafsson SB, Lindgren T, Jonsson M, Jacobsson S. Cancer Chemother Pharmacol **63** (2009) 691.

Engineering of magnetic solid lipid nanoparticles against colon cancer

María Muñoz de Escalona[1], Eva Sáez-Fernández[1], José C. Prados[2,3], Raúl Ortiz[2,4], Consolación Melguizo[2,3], Ángel V. Delgado[5], José L. Arias[1,2,3]

[1]Department of Pharmacy and Pharmaceutical Technology, University of Granada, Spain.
[2]Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Spain.
[3]Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS) – University of Granada, Spain.
[4]Department of Health Science, University of Jaén, Spain.
[4]Department of Applied Physics, University of Granada, Spain.
e-mail: jlarias@ugr.es

Introduction: Despite the well-known efficacy of 5-fluorouracil (5-FU) against a broad variety of solid tumours, the activity of this anticancer pyrimidine analogue is severely limited by a short plasma half-life and the development of drug resistances by malignant cells [1]. As a consequence, the required chemotherapeutic effect depends on the administration of high doses, concurrently determining a severe toxicity.

Materials and Methods: It was developed a methodology to prepare 5-FU-loaded magnetic solid lipid nanoparticles (MSLNs), based on a double emulsion/solvent evaporation technique [2]. Transmission electron microscopy and particle size analysis, infrared spectroscopy characterizations, electrophoretic assays, and blood compatibility studies were done. *In vitro* cytotoxicity studies were accomplished in human colon fibroblast CCD-18, and in human colon carcinoma T-84 and HCT-15 cell lines. Fluorescently labelled nanocomposites were prepared to analyze the uptake in all of these cell lines. Drug release analysis was done following the dyalisis bag methodology (at 37 ºC and pH 7.4). Finally, hysteresis cycle determinations and optical microscopy inspections were used to define the magnetic responsiveness.

Results and discussion: Spherical MSLNs were satisfactorily prepared (average size ≈ 180 nm). The complete physicochemical characterization ascertained the reproducibility of the formulation conditions in obtaining core/shell magnetic nanocomposites made of magnetite nuclei embedded within a glyceryl trimyristate solid matrix. The MSLNs were found to be hemocompatible *in vitro*, and underwent an intense uptake by CCD-18, T-84, and HCT-15 cells. Absence of cytotoxicity of blank (5-FU unloaded) MSLNs was found in these cells. Spectrophotometric determinations confirmed that the preparation procedure reported high drug loading values and a slow drug release profile. Finally, the adequate magnetic responsiveness of the nanocomposites for 5-FU delivery purposes was satisfactorily characterized *in vitro* by hysteresis cycle determinations and by optical microscopy inspections.

Conclusions: To the best of our knowledge, this is the first time that it is developed a magnetic solid lipid nanoplatform holding outstanding characteristics, that is, blood compatibility and lack of cytotoxicity, satisfactory cell internalization effectiveness, high 5-FU loading capability and little burst release, and magnetically targeted delivery, suggestive of their potential for the effective treatment of colon cancer.

Acknowledgements: **F**inancial support from FEDER, Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+i), Instituto de Salud Carlos III (FIS, Spain): projects PI11/01862 and PI11/02571; and from Junta de Andalucía (Spain, project PE-2012-FQM-694).

References:

[1] Goldstein DA, Zeichner SB, Bartnik CM, Neustadter E, Flowers CR. Clin Colorectal Cancer (2015) doi: 10.1016/j.clcc.2015.10.002.
[2] Nabi-Meibodi M, Vatanara A, Najafabadi AR, Rouini MR, Ramezani V, Gilani K, Etemadzadeh SM, Azadmanesh K. Colloids Surf B Biointerfaces **112** (2013) 408.

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