THIOLATED CHITOSAN DERIVATIVES AS MUCOADHESIVE COATING MATERIALS FOR SOLID LIPID NANOPARTICLES

Richard Wibel[1], Arne Matteo Jörgensen[1], Patrick Knoll[1], Christian Steinbring[1], Andreas Bernkop-Schnürch[1*]

[1] Department of Pharmaceutical Technology, University of Innsbruck, Institute of Pharmacy, Center for Chemistry and Biomedicine, 6020 Innsbruck, Austria

Introduction: Thiolated polymers strongly adhere to mucus by forming covalent disulfide bonds[1]. However, strong mucus interaction restricts the penetration beyond the outer loose mucus layer. Less reactive S-protecting ligands enable diffusion before forming disulfide bonds and, thus, allow them to reach deeper into the mucus[2].

Objectives: In the present study, chitosan (CS) bearing free thiols (CS-Cys), 6-MNA-protected thiols (CS-Cys-MNA), and L-cysteine-protected thiols (CS-Cys-Cys), respectively, were synthesized. The CS derivatives were applied as coating materials to solid lipid nanoparticles (SLN). The resulting SLN were compared with each other in terms of stability, mucus interaction and diffusion as well as in terms of mucosal residence time.

Materials and Methods: CS was thiolated by introducing L-cysteine via amide bond formation. Free thiol groups were protected with highly reactive 6-mercaptonicotinic acid and less reactive L-cysteine, respectively, via thiol/disulfide exchange reactions. After applying the polymers as coating materials, the mucus interaction was investigated via rheological experiments. Moreover, mucus diffusion was examined via the rotating tube assay and single particle tracking. The mucosal residence time of the formulations was determined on *ex vivo*-tissue.

Results and Discussion: Strength of mucus interaction followed the rank order plain < CS < CS-Cys-Cys < CS-Cys < CS-Cys-MNA whereas mucus diffusion followed the rank order CS-Cys < CS-Cys-Cys < CS < CS-Cys-MNA < plain. In accordance with lower reactivity, CS-Cys-Cys-coated SLN were immobilized to a lower extent than CS-Cys-coated SLN while CS-Cys-MNA-coated SLN dissociated from their coating material resulting in a similar diffusion behavior as plain SLN. Consequently, CS-Cys-Cys-coated SLN and CS-Cys-MNA-coated SLN showed highest retention on porcine intestinal mucosa.

Conclusions: Two different strategies to achieve a synergism of mucoadhesion and diffusion were enabled in this study: (1) Application of L-cysteine as S-protecting ligand to decrease reactivity of the thiolated polymer and (2) application of a coating material with strong mucus interaction but lower affinity to the plain particle allowing a dissociation of the particle from the coating material..

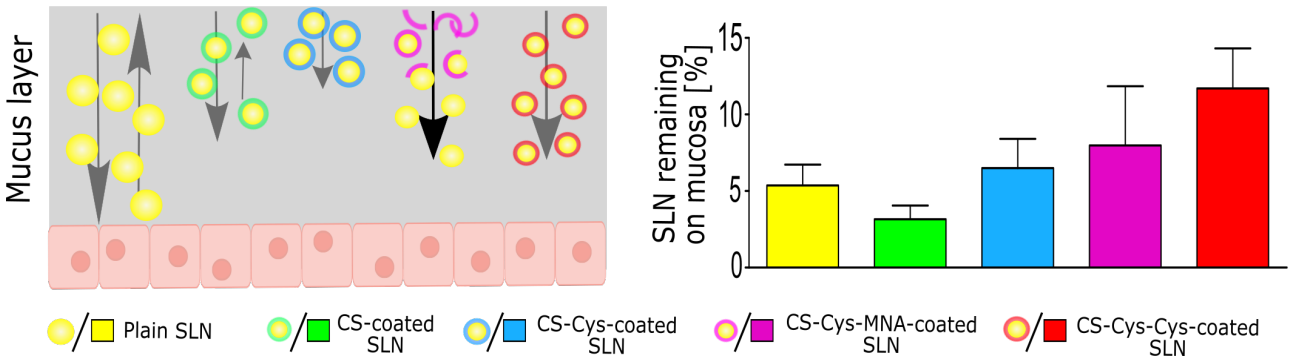


Figure 1: Schematic depiction of penetration of the SLN formulations into the mucus layer and the remaining SLN amount on intestinal porcine mucosa after 120 minutes of continuous rinsing (n ≥ 3 ± SD).

References:

1. C. Leichner, et al., *Advanced Drug Delivery Reviews*, 151-152, (2019).
2. P. Knoll, et al., *Acta Biomaterialia*, 135, (2021).

TACKLING THE CHALLENGE TO MANAGE ATOPIC DERMATITIS WITH NANOGELS

Barbosa, A. I.[1, 2]; Lima, S. C.[1]; Reis, S. [1]

[1]LAQV, REQUIMTE, Faculty of Pharmacy, University of Porto, Portugal.
[2]ICBAS School of Medicine and Biomedical Sciences, University of Porto, Portugal.

Introduction: Atopic dermatitis (AD) is one of the most widespread and burdensome inflammatory skin diseases, with an expressive 25% prevalence in infancy, but it also affecting about 7-10% of adults worldwide. Despite the several available treatments for AD, it is necessary to give response to the numerous manifestations of the disease and regulate its dry skin condition. Hence, hydrogels have proven to be good topical alternatives to regular ointments or creams for AD due to their high-water content, improved drug delivery, responsiveness to stimuli, versatility in terms of preparation and possible combination with other drug delivery systems (DDS). Trending nanodelivery strategies focus on unmet AD clinical needs, either by combining different bioactive compounds, or by their incorporation in different DDS. That is the case of nanogels, which are bioactive compound-loaded nanoparticles and nanovesicles that were incorporated in hydrogel matrices as a new strategy for the design of topical formulations, tuned to overcome the hurdles of skin inflammatory diseases.
Objectives: This study aimed to design and characterize nanogels for the topical delivery of betamethasone, an effective corticosteroid for AD treatment. To address this challenge betamethasone was loaded in two different types of nanostructured lipid carriers and further embedded in a hydrogel matrix composed of k-carrageenan and poly(vinyl) alcohol for further physicochemical characterization, full skin permeation profile and cellular biocompatibility.
Materials and Methods: The physicochemical characterization of the designed nanogels involved rheological analysis, Fourier-transform Infrared Spectroscopy, and morphological determinations by scanning electron microscopy. The *ex vivo* skin permeation profile was determined using Franz diffusion cells with porcine ear skin as barrier, due to its human counterpart similarity in terms of function and morphology. The cellular biocompatibility towards fibroblasts and keratinocytes was also addressed.
Results and Discussion: High drug content values of betamethasone were achieved for the differently prepared lipid nanoparticles, creating a good platform for the corticosteroid delivery in skin layers. Rheological determinations of all nanogels proved favourable pseudoplastic behaviour and good resistance to deformation. After 24h in contact with skin, approximately 60% of the initial amount of betamethasone is permeated, and 20% remains retained in skin layers, which is favourable to maximize local efficacy of the corticosteroid. Several nanogels' concentrations were tested proving higher biocompatibility towards fibroblasts (up to 100 mg mL^{-1}) than keratinocytes (up to 25 mg mL^{-1}).
Conclusions: Nanogels can represent an interesting strategy to design new therapeutic approaches for AD.

Acknowledgements: This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through grants UID/QUI/50006/2020, from the European Union (FEDER funds through COMPETE POCI-01-0145-FEDER-030834) and National Funds (FCT, Fundação para a Ciência e Tecnologia) through project PTDC/QUI-COL/30834/2017. SCL thanks funding from FCT/MEC (CEECIND/01620/2017) financed by national funds. AIB thanks her funding from project PTDC/QUI-COL/30834/2017 and from FCT/MEC (SFRH/BD/147038/2019). To all financing sources, the authors are greatly indebted.

Praziquantel drug release from montmorillonite using enhanced sampling methods

Borrego-Sánchez, A.[1]; Debnath, J.[1]; Parrinello, M.[1]

[1]Istituto Italiano di Tecnologia (IIT), Italy.

Introduction: Praziquantel is the drug of choice in the treatment of Schistosomiasis. However, this drug has a low aqueous solubility. The improvement of the drug dissolution profile would help optimizing its pharmacological effects and reduce adverse reactions. Clay minerals have been used as excipients for this purpose, showing that these low-cost materials increase the solubility and improve the dissolution profile of the drug.[1,2,3] However, the physicochemical mechanism related to the dissolution process is unknown.

Objectives: The objective of our research was to establish an accurate computational strategy capable of identifying the parameters that control drug release and to calculate drug dissolution rates. In such a way, one would eventually be able to design improved drug delivery systems.

Materials and Methods: The models used for simulating the praziquantel drug adsorbed on the surface and in the interlayer space of the clay are displayed in Figure 1. The number of water molecules was chosen to reproduce the dosage form in an aqueous medium as much as possible. Periodic boundary conditions were applied. The LAMMPS program[4] interfaced with PLUMED[5] was used, and the interatomic interactions were described by the CVFF Interface force field.[6] When needed to explore long-time scales, we used the recently developed GAMBES method[7].

Results and Discussion: Standard molecular dynamics simulations showed that the praziquantel is released from the montmorillonite surface in the nanosecond time scale. In contrast, GAMBES simulations showed that the drug is released from the interlayer space of the clay on the microsecond time scale. These simulations on the surface and interlayer models have allowed identifying some important features of the drug release mechanism. In particular, we found the aromatic part of the praziquantel is the last part that loses the interaction with the clay.

Conclusions: A fast desorption was observed in agreement with previous experimental studies. GAMBES and the computational setup established in this work are a useful tool to obtain the release rate of the drug from the excipient and to describe the mechanism. In future studies, this tool shall allow us to do a fast screening of a great number of drug-clay systems, select the systems with the desired release kinetics, and reduce the need for experimental tests, thus saving time and cost.

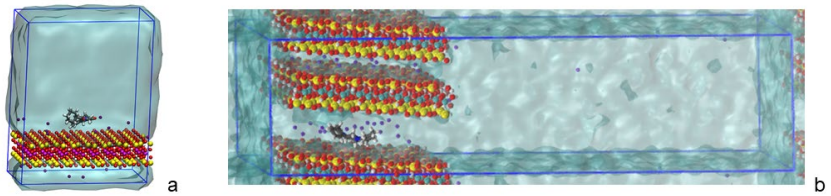


Figure 1. Praziquantel adsorbed on the surface (a) and in the interlayer space (b) of the montmorillonite in aqueous solution.

References:
1. Borrego-Sánchez *et al., Appl. Clay Sci.* 160, 173-179 (2018).
2. Borrego-Sánchez *et al., Pharmaceutics* 12, 914 (2020).
3. Borrego-Sánchez *et al.,* International patent WO2020012044 (2020).
4. Plimpton, *J. Comput. Phys.* 117, 1-19 (1995).
5. Tribello *et al., Comput. Phys. Commun.*185, 604-613 (2014).
6. Heinz *et al., Langmuir* 29, 1754-1765 (2013).
7. Debnath, Parrinello, *J. Phys. Chem. Lett.* 11, 5076-5080 (2020).

Acknowledgements: This research was funded by European Commission Horizon 2020 Framework Programme, H2020-MSCA-IF-2019, MSCA-IFEF-ST, grant nº 895355, METADRUG.

FAST DISSOLVING TRILAYERED POLYMERIC MICRONEEDLE PATCH FOR MAXIMISING ROSE BENGAL-LOADED TRANSFEROSOMES DERMAL DELIVERY

Demartis, S[1,2]; Volpe-Zanutto, F.[2]; Paredes, A.J.[2]; Jahan, S.A.[2]; Gavini, E[3].; Donnelly R.F[2].

[1] School of Pharmacy, Queen's University Belfast, Medical Biology Centre, Belfast, UK;
[2] PhD program in Chemical Science and Technology, Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italy;
[3] Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italy

Introduction: Rose Bengal (RB) is a photosensitiser yet intrinsically cytotoxic drug employed in melanoma therapy. The cutaneous administration route is preferred to treat superficial lesions, but the RB chemical-physical profile hampers skin permeation and cell crossing [1]. We developed RB-loaded transferosomes (RBTF) to enhance RB dermal delivery, successfully increasing RB amount permeating the epidermis and the anticancer activity, probably due to a higher cell uptake [2]. Still, the entire delivery of intact RBTF must be assured to maximise the therapeutic efficiency. Dissolving polymeric microneedle (DMNs) can painlessly pierce the outermost skin layers and deposit their cargo directly in the deepest strata, bypassing the stratum corneum barrier.
Objectives: Herein, we combined the delivery strategy of RBTF with three-layered DMNs to offer an RBTF dosage form (RBTF-DMNs) able to maximise the dermal delivery of intact RBTF.
Materials and Methods: RBTF were prepared by a modified reverse-phase evaporation technique [2] and characterised in dimensional profile and zeta potential (NanoBrook Omni particle sizer and zeta potential analyser), and morphology (TEM analysis). RBTF-DMNs was fabricated by a three-layer mould casting technique: RBTF were loaded only in the first layer, the tips, made of a 40% w/w of PVA (9-10 KDa) and PVP (58 KDa) (1:1); the second layer consisted of a PVP K90 30% w/w cast on the top of the tips, and the third layer was a 3D-printed baseplate. RBTF-DMNs were observed by optical and multiphoton microscopy; mechanical properties, including resistance to the compression force and *in-vitro* and *ex-vivo* penetration ability, were evaluated. *In-vitro* penetration ability was tested using the Parafilm M® skin simulant model and the *ex-vivo* on full-thickness neonatal porcine skin [3]. Finally, RBTF-DMNs was dissolved in water to release RBTF. The redissolved RBTF (red.RBTF) were re-characterized, and the results were compared with the original RBTF to assess the efficiency of the new RBTF delivery platform.
Results and Discussion: RBTF-DMNs were sharp, of a pyramidal shape with a height of 750 µm. The compression test reported a reduction of 9.77±0.18% in the height of the tips; the penetration ability study showed an *in vitro* insertion depth of around 375 µm (3 Parafilm M® sheets) and efficient *ex-vivo* penetration visualised by 3D-reconstructed multiphoton micrograph. To obtain successful DMNs, a tips height reduction below 10% and penetration of at least two Parafilm M® layers are required. Analyses of the dimensional profile and zeta potential of RBTF and red.RBTF revealed that average size significantly increased (p value<0.01) (62.91±5.28 nm vs 216.03±83.60 nm), leaving the polydispersity index (0.27±0.04 vs 0.24±0.07) and the zeta potential (-38.47±0.19 vs -38.20±0.12) stable. Red.RBTF are in the same dimensional range as the RBTF formulation previously tested [2], which efficiently enhanced RB epidermis permeation. Furthermore, TEM analyses revealed double-layer and spherical red.RBTF (micrographs not reported) and often measure a smaller vesicle diameter detected by the particle sizer. The overall analyses suggest a good stability of RBTF during the integration process in RBTF-DMNs.
Conclusions: The RBTF-DMNs casting technique and the materials employed were able to form tips supported by a 3D-printed baseplate robust enough to pierce full-thickness porcine skin, leaving mostly unchanged the RBTF characteristics. In this view, RBTF-DMNs could significantly promote the intact RBTF accumulation in the dermis compared to epidermal permeation providing an efficient strategy to enhance RB anti-melanoma activity.

References:
[1] Demartis S, *et al. Dyes and Pigments.* 188, 109236 (2021)
[2] Demartis S, *et al. Molecular Pharmaceutics.* 18, 11 (2021).
[3] Li M, *et al. International Journal of Pharmaceutics.* 612, 121295 (2021).

Acknowledgements: S.D. thanks Regione Autonoma Sardegna and Queen's University Belfast for the opportunity to carry out this research.

SELF-PROPELLED NANOMOTORS FOR BACTERIAL BIOFILM ERADICATION

Díez, P.[1,2,3*]; Escudero, A.[1,2,4]; Ziemyte, M.[5]; Ferrer, M.D.[5]; Murguía, J.R.[1,2,3,4*]; Mira, A.[5] and Martínez-Máñez, R.[1,2,3,4*]

[1]Unidad Mixta de Investigación en Nanomedicina y Sensores. Universitat Politècnica de València, Instituto de Investigación Sanitaria La Fe, 46026, València, Spain. [2]Instituto Interuniversitario de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat Politècnica de València, Universitat de València, Spain. [3]CIBER de Bioingeniería, biomateriales y nanomedicina (CIBER-BBN), 28029 Madrid, Spain. [4]Unidad Mixta UPV-CIPF de Investigación en Mecanismos de Enfermedades y Nanomedicina, València, Universitat Politècnica de València, Centro de Investigación Príncipe Felipe, 46012 València, Spain. [5]Genomics & Health Department, FISABIO Foundation, Valencia, Spain.

Introduction: Bacterial infectious diseases have become a global public health problem in recent years, causing elevated morbidity and mortality rates, particularly in immunocompromised patients. One of the main reasons for this is the microbial ability to adhere on biotic or abiotic surfaces and encase in a self-produced extracellular polymeric substances (EPS) matrix, composed of DNA, polysaccharides, and proteins, forming biofilms. EPS acts as a physical barrier hindering the penetration and diffusion of antibiotics. All of these favors the antimicrobial resistance (AMR), a growing global health problem in last years. In order to solve it, bio-inspired nanomotors have gained much attention recently due to their unique capabilities such as effective propulsion and cargo delivery. Among them highlight the Janus nanomotors made of platinum (Pt) and mesoporous silica nanoparticles (MSN), since Pt is responsible for self-propelled motion, acting as catalyst for the local decomposition of H_2O_2 (l) into H_2O (l) and O_2 (g), and MSN acts as nano container for antibiotics modulating their release through functionalization with gatekeepers.
Objectives: Design, synthesis, and validation of a multifunctional Janus Pt-MSN nanomotor fueled with H_2O_2 to eliminate bacterial biofilms, disrupting the EPS-matrix, and killing bacterial cells.
Materials and Methods: Janus Pt-MSN nanomotors were synthetized following a Pickering emulsion method, previously described by our group. Then, the MSN face was functionalized with benzimidazole moieties, loaded with the antibiotic Vancomycin, and capped with the protease ficin previously modified with β-cyclodextrin, constituting the pH-responsive gate-system. The nanomotors structure and functionality was analyzed using the standard nanomaterials characterization techniques: powder X-ray diffraction (PXRD), N_2 adsorption-desorption, thermogravimetric analysis (TGA), dynamic light scattering (DLS), transmission electron microscopy (TEM), scanning transmission electron microscopy coupled with electronic energy dispersive x-ray spectroscopy (STEM-EDX), motion analysis using NTA software in a Nanosight NS300, and controlled-release assays. Finally, the antimicrobial capabilities of the nanomotors were evaluated in real time in *S. aureus* biofilms, employing xCELLigence Real-Time Analyzer, colony counting assay, and confocal laser scanning microscopy (CLSM).
Results and Discussion: The results obtained in the assays performed showed that the developed nanomotors release vancomycin in response to acid pH (such as the pH present in the biofilm core), remaining capped at neutral pH. In addition, the nanomotors showed an enhanced diffusive movement in the presence of low H_2O_2 concentrations, superior to other nanomotors described in the literature. Regarding antimicrobial tests, the nanomotors achieved an 82% disruption of EPS biomass and a 3-log reduction in cell viability.
Conclusions: Our results demonstrate that the Janus Pt-MSN nanomotors developed fueled with low concentrations of H_2O_2, loaded with antibiotics and coated with proteases that degrade the EPS, have the ideal properties for the treatment of infections caused by biofilm-generating bacteria, which are difficult to eradicate. This is due to their multiple capabilities, such as autonomous movement, which together with the protease activity causes the detachment of the EPS, and the controlled and localized delivery of low concentrations of antibiotic, resulting in bacterial death, with the possible reduction of AMR.

Authors want to thank the Spanish Government for RTI2018-100910-B-C41 (MCIU/AEI/FEDER, UE) and CTQ2017-87954-P projects and the Generalitat Valenciana for support by project PROMETEO/2018/024. Díez,P. thanks the Instituto de Salud Carlos III and the European Social Fund for the financial support "Sara Borrell", (CD20/00120).

SELF-PROPELLED TARGETED NANOMOTOR FOR THE REDUCTION OF INFLAMMATORY RESPONSE

Hicke García, F.J [1]; Escudero Noguera, A.[1,2]; Lucena Sánchez, E.[1,2]; García Fernández, A.[1,2,3]; Díez Sánchez, P.*[1,3], Martínez Máñez, R.[1,2,3]

[1]Instituto Interuniversitario de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat Politècnica de València, Universitat de València, Spain; [2] Unidad Mixta UPV-CIPF de Investigación en Mecanismos de Enfermedades y Nanomedicina, Universitat Politècnica de València, Centro de Investigación Príncipe Felipe, Spain; [3] Unidad Mixta de Investigación en Nanomedicina y Sensores. Universitat Politècnica de València, Instituto de Investigación Sanitaria La Fe, Spain.

Introduction: The inflammatory response (IR) is a natural protective mechanism of the immune system that is triggered after an initial cellular injury. The IR is responsible for destroying, isolating, and localizing the injurious agent. However, a deregulated and excessive response is generated, causing damage, aggravating other diseases, and being an important risk factor for the development of long-term pathologies, such as atherosclerosis, cancer or Alzheimer's disease. One of the main agents involved in dysfunctional IR are macrophages, and specifically M1-type. These cells secrete many pro-inflammatory factors such as interleukins (ILs), tumour necrosis factor alpha (TNF-α), or reactive oxygen species (ROS), including H_2O_2. Therefore, one of the ways to address the problem of chronic inflammation could be intervene on this cell type, taking advantage of their main function, the internalization of foreign bodies.

Objectives: Synthesis, characterization, and evaluation of an innovative nanomotor based on Janus nanoparticles as a new therapeutic strategy to reduce chronic inflammation.

Materials and Methods: Janus nanomotors, based on the union of Platinum (Pt)-Mesoporous silica nanoparticles (MSN) were synthesized and characterized as previously reported by our group. The nanomotor are loaded with the anti-inflammatory drug dexamethasone into the pores of the mesoporous face, which are blocked by a molecular gate formed by PEG dithiol chains (PEG-DSS). Anti-CD169 monoclonal antibodies, are covalently attached to PEG. The anti-inflammatory behaviour of the proposed nanomotors will be validated *in vitro* in targeting, ROS measurement, internalization and cytotoxicity studies using the acute monocytic leukemia cell line THP-1, differentiated to M1-type macrophages. This will be followed by an *in vivo* study in a C57BL/6 murine model of subcutaneous air pouch.

Results and Discussion: The Anti-CD169 monoclonal antibodies are specific for the CD169 receptor of M1 macrophages, providing targeting to the inflamed areas where macrophages are abundant. Furthermore, once this zone is reached, the high levels of H_2O_2 (~0.11 μM) present will feed the nanomotor, achieving its self-propulsion through the catalytic decomposition on the Pt face, which is expected to enhance cell internalization into macrophages. Finally, the molecular gate of the nanomotor in the cell cytosol responses to the high glutathione concentrations, opening the pores and releasing dexamethasone. This strategy shows a dual effect: elimination of ROS by their use as fuel and the reduction of the macrophage population by the action of dexamethasone, leading to the remarkable decrease in chronic inflammation. So far, *in vitro* tests carried out have successfully demonstrated the ability of the nanomotor to self-propel through H_2O_2 decomposition and cargo release in response to glutathione, present in the cytosol. In addition, recent cytometry assays have shown specific targeting of the Anti-CD169 functionalized Janus Pt-MSN to M1-type macrophages.

Conclusions: Encouraged to the design system and the promising results obtained, we envision that our nanomotor could present good anti-inflammatory capabilities, specifically through reduction of TNF-α and IL-1 levels due to dexamethasone-controlled release, contributing to stop the development of severe disorders that involve inflammation.

Acknowledgements: This research was funded by the Ministerio de Ciencia, Innovación y Universidades (Spanish Government), the Agencia Estatal de Investigación (AEI) and European Union (projects RTI2018-100910-B-C41 and RTI2018-101599-B-C22-AR) (MCIU/AEI/FEDER, EU) and the Conselleria de Innovación, Universidades, Ciencia y Sociedad Digital, Generalitat Valenciana (Project PROMETEO 2018/024). F.J.H is grateful to the Spanish MEC for her FPU grant (FPU20/06021).

CANNABIDIOL AS A LIGAND FOR THE ACTIVE TARGETING OF SMALL-SIZED LIPID NANOCAPSULES

Pérez-López, Alexandre[1]; Aparicio-Blanco, Juan[1]; Martín-Sabroso, Cristina[1]; González-Matilla, Juan Francisco[2]; Torres-Suárez, Ana Isabel[1]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, Complutense University, Madrid, Spain.
[2]Department of Chemistry in Pharmaceutical Sciences, Faculty of Pharmacy, Complutense University, Madrid, Spain.

Introduction: Central Nervous System (CNS) diseases unfortunately remain one of the greatest health-care challenges of the twenty first century. This is mainly due to the difficulty of most current commercialized treatments to surpass the Blood Brain Barrier (BBB) and penetrate the CNS. In this context, the active targeting of small-sized lipid nanocarriers with targeted ligands is being explored as an appealing strategy to promote the passage through the BBB. Cannabidiol (CBD), the major non-psychoactive cannabinoid, has been selected as a potential targeting ligand because of his enhanced biodistribution across the BBB [1,2].

Objectives: the purpose of the present work was the covalently incorporation of CBD at the surface of small-size lipid nanocapsules (LNCs).

Materials and Methods: LNCs were covalently functionalized with CBD using “click chemistry” in an attempt to develop BBB-targeted nanocarriers. This term describes high yield reactions between terminal azyde and alkyne groups using cupper as catalyzer. For this purpose, three consecutive steps were followed. First, mono-alkylated CBD was synthesized using the Williamson Synthesis between pure CBD and propargyl bromide utilizing dry tetrahydrofuran as solvent. Then, azyde-LNCs were prepared by the phase inversion temperature technique [2], which is a solvent-free low-energy method based on the formation of nano emulsions. Briefly, Labrafac WL 1349 (caprylic-capric acid triglycerides), azyde-Kolliphor HS15 (obtained through the chemical reaction between commercial Kolliphor HS15 and sodium azyde), Lipoïd S75 (soybean lecithin at 70% of phosphatidylcholine), sodium chloride and ultrapure water were mixed in order to obtain monodisperse LNCs. Third, mono-alkylated CBD, copper sulfate and ascorbic acid were dissolved in a 15% (v/v) ethanol: water mixture and, subsequently, azyde-LNCs were added to perform the click reaction and obtain CBD-functionalized LNCs. Finally, non-functionalized LNCs and CBD-functionalized LNCs were qualitatively characterized with Nuclear Magnetic Resonance and quantitatively in terms of volume diameter, polydispersity index (PdI) and Z-potential using dynamic light scattering. The total functionalization rate was determined by quantitative UV spectroscopy at 328 nm of wavelength.

Results and Discussion: The functionalization of 20 nm azyde-LNCs with mono-alkylated CBD resulted in a statistically significant increase in the volume diameter compared to the non-functionalized LNCs, probably due to the presence of the cannabinoid on the surface of the LNCs. However, the functionalization process did not alter the PdI of the LNCs, being all of them highly monodisperse. Additionally, the decoration with CBD significantly decreased the Z-Potential profiles of CBD-functionalized LNCs, in agreement with the hypothesized superficial location of CBD. Finally, the total functionalization rate was determined to be 4.51 ± 3.95 %.

Conclusions:
1. “Click chemistry” allowed the active targeting of small-sized LNCs with CBD.
2. The functionalization with CBD increased the size and decreased the Z-potential of the LNCs.

References:
1. Aparicio-Blanco, J et *al., Mol Pharm.* 16, 1999 (2019).
2. Pisanti, S et al., *Pharmacol. Ther. 175, 133-175 (2017).*
3. Aparicio-Blanco, J et *al., J Biomed Nanotechnol*. 15, 1149 (2019).

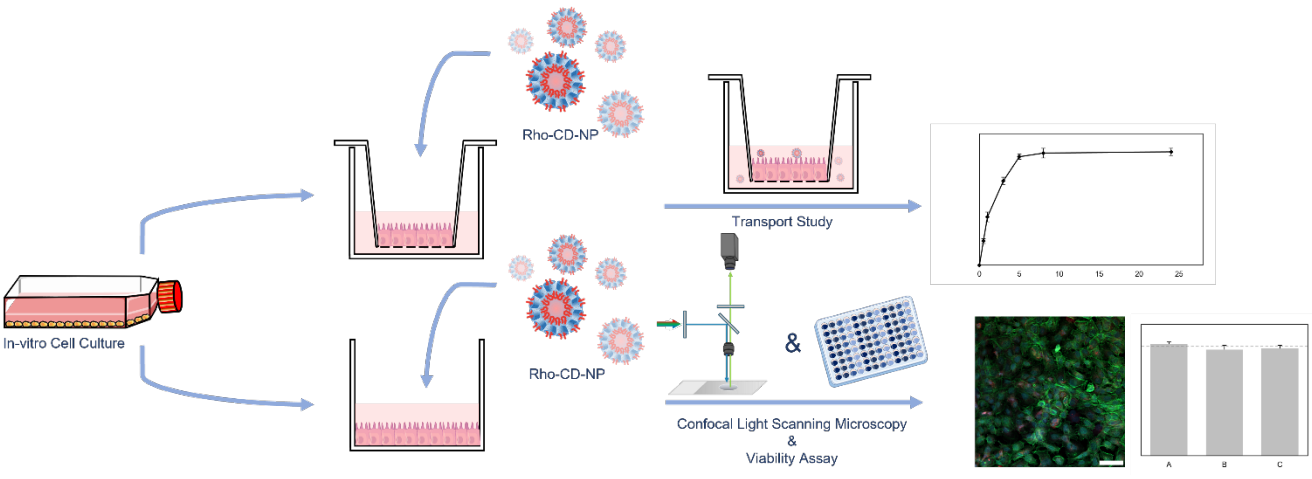
Acknowledgements: This work was funded by the Spanish Ministry of Science, Innovation and Universities research project PID2019-105531RB-I00/AEI/10.13039/501100011033: CBD functionalized lipid nanocarriers for the treatment of brain tumours.

NOVEL AMPHIPHILIC CYCLODEXTRIN NANOCARRIER FORMULATIONS FOR ORAL DRUG DELIVERY PERMEATE THE INTESTINAL BARRIER

Schreiner, J.[1]; Brettner, F.[1]; Vogel-Kindgen, S.[1]; Windbergs, M.[1]

[1]Institute of Pharmaceutical Technology and Buchmann Institute for Molecular Life Sciences, Goethe University, Frankfurt am Main, Germany

Introduction: Oral administration is the preferred route of drug delivery, though achieving high drug bioavailability is still challenging. Furthermore, the majority of newly developed chemical entities shows poor aqueous solubility and poor permeability. Formulation into nanocarriers can increase drug stability in the gastrointestinal tract and drug solubility as well as oral bioavailability. Besides established nanocarriers, amphiphilic cyclodextrin nanoparticles (CD-NPs) are currently emerging as novel drug delivery vehicles with a high potential.
Objectives: The aim of the present study was to investigate the potential of CD-NPs for oral drug delivery. For this purpose, we evaluated the cellular toxicity, cellular uptake, and intestinal permeation of different CD-NP formulations.
Materials and Methods: CD-NPs were fabricated by nanoprecipitation. Particle size and zeta-potential were determined by a Malvern Zetasizer Nano ZS. The cytotoxicity of CD-NPs was evaluated via MTT-assay. For uptake studies, enterocytes were incubated with fluorescently labeled CD-NPs. After incubation with the particles, cells were fixed, cell nuclei and cytoskeleton were stained with DAPI and Phalloidin-Alexa Fluor 488, respectively, and imaged using confocal light scanning microscopy. Permeability studies were performed using monocultures of enterocytes or co-cultures of enterocytes and mucus-secreting goblet cells. Cells were cultured for 21 days on transwell inserts before the addition of fluorescently labeled CD-NPs to the apical compartment. Fluorescence intensity in the basolateral compartment was measured for different time points.
Results and Discussion: All particles used in this study had a hydrodynamic diameter below 250 nm with a monodisperse size distribution (polydispersity index below 0.2) and negative zeta-potential (below -25 mV). The particles were stable in conditions mimicking the small intestinal fluid. Particle treatment had no significant impact on cell viability for all particle formulations in a concentration of up to 0.2 mg/mL. Using fluorescently labeled particles, we proofed the uptake of CD-NPs by enterocytes. More importantly, all formulations were able to permeate the intestinal barrier. There was no effect on the monolayer integrity as verified by stable TEER values before and after particle treatment. Comparing the permeation of the particles across monocultures of enterocytes and co-cultures of enterocytes and goblet cells, there was no significant difference in the apparent permeability coefficients. With the optimized formulations, P_{app} values of 5 x 10^{-7} cm/s were achieved.
Conclusions: The results demonstrate that CD-NPs are promising nanocarriers for oral drug delivery across the intestinal barrier. CD-NPs are easily taken up by intestinal epithelial cells and further show high mucosal permeability. Thus, amphiphilic CD-NPs can serve as a drug delivery platform for hydrophobic compounds to increase their intestinal permeation and bioavailability after oral administration.



LIPOSOMES LOADED WITH THE PNEUMOCOCCAL ENDOLYSIN MSLYS: FROM *IN VITRO* CHARACTERIZATION TO *EX VIVO* PERMEATION ACROSS THE TYMPANIC MEMBRANE

Silva, M.D.[1,2,3,4]; Paris, J.L.[2]; Ray, K.[3,4]; Gama, F.M.[1]; Silva, B.F.B.[2]; Remenschneider, A.[3], Sillankorva, S.[2]

[1]CEB−Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal; [2]INL−International Iberian Nanotechnology Laboratory, 4715-330 Braga, Portugal; [3]Department of Otolaryngology, Massachusetts Eye and Ear, 02114 Boston, MA, USA; [4]Wyss Institute for Biologically Inspired Engineering, 02115 Boston, MA, USA

Introduction: The increasing antibiotic resistance triggered interest in novel antimicrobials as well as delivery systems that allow a topical and targeted delivery. However, biological barriers, such as the skin or the tympanic membrane (TM), may hinder the success of the therapy. Drug permeation has been extensively studied in the context of transdermal delivery, but only recently started to be explored for transtympanic applications. *Ex vivo* Franz diffusion cell permeation tests have been used and validated for the permeation of compounds through the skin prior to *in vivo* studies, but their exploitation for transtympanic delivery is limited. Endolysins, peptidoglycan hydrolases derived from bacterial viruses, are attractive antimicrobials, with promising action against the otitis media pathogen *Streptococcus pneumoniae.* Liposomal systems, such as transfersomes or PEGylated liposomes, have been shown to enhance drug permeation across the TM. Here, we describe the *in vitro* characterization of the endolysin-loaded liposomal carriers as well as their *ex vivo* permeation through TMs.
Objectives: The main objective was to develop a delivery system containing an endolysin for a targeted transtympanic treatment of otitis media. To achieve this, it was necessary: i) to encapsulate the endolysin into liposomes for a controlled delivery; and ii) to evaluate the transtympanic permeation ability of the formulations.
Materials and Methods: Liposomes composed of 4 mM of L-alpha-lecithin and sodium cholate (5:1) (L:SC) or L-alpha-lecithin and PEG2000 PE (10:1) (L:PEG) loaded with the MSlys endolysin were prepared. The size, polydispersity index (PDI), zeta potential, stability, deformability, encapsulation efficiency, and *in vitro* MSlys release were determined. The cytotoxicity against fibroblasts and keratinocytes and the efficacy against pneumococcal planktonic and biofilm cells were also evaluated *in vitro*. Permeation studies were performed in Franz diffusion cells using porcine skin, sheep TMs, and cadaveric human TMs. The amount of MSlys permeated and its antipneumococcal activity were evaluated, and the protein integrity was analyzed by SDS-PAGE.
Results and Discussion: The MSlys endolysin was encapsulated into liposomes, with an average efficiency of about 35%. Liposomes with ca. 100 nm and relatively low PDI were produced, with L:PEG formulations being smaller and less polydisperse than L:SC. Both characteristics remained stable for one year at 4 ˚C. Liposomes were shown to be deformable and to provide a controlled release of MSlys over time following a first-order kinetics. No cytotoxicity was observed. Endolysin-loaded liposomes interacted with *S. pneumoniae* cells, reducing both planktonic and biofilm cultures. The potential of L:PEG over L:SC formulations to transport MSlys was demonstrated in preliminary transdermal assays. The permeation of MSlys across the TMs was enhanced when loaded in PEGylated liposomes. Samples were shown to significantly reduce pneumococcal cells after 2 h of permeation through the human TM. Nonetheless, loss of antipneumococcal activity after 4 h of permeation and protein hydrolysis at 48 and 72 h were observed.
Conclusions: This work reports the delivery of an endolysin through an intact TM using liposomes. However, further optimization is needed to expand the overall therapeutic efficacy of this strategy for use in otitis media.

Acknowledgements: This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, the Wyss Institute for Biologically Inspired Engineering and from NIH NIDCD K08-DC018575 grant. MDS acknowledges the FCT grants SFRH/BD/128825/2017 and COVID/BD/152363/2022, and SS acknowledges funding by FCT through the individual scientific employment program contract (2020.03171.CEECIND).

BIODEGRADABLE MICROPARTICLES FOR THE LOCAL TREATMENT OF OSTEOMYELITIS

Touza, L.[1]; Garcia-Jamardo, S. [2]; Landin, M.[3]; Diaz-Rodriguez, P.[4]

[1]Department of Pharmacology, Pharmacy and Pharmaceutical Technology, I+D Farma (GI-1645), Faculty of Pharmacy, iMATUS and Health Research Institute of Santiago de Compostela (IDIS), [2]University of Santiago de Compostela, 15782, Santiago de Compostela; [3]Spain.

Introduction: Osteomyelitis (OM) is an infectious disease that affects the cortical and/or medullary portion of bone, resulting in an inflammatory process and the destruction of bone tissue. The most common cause is the spread of bacteria such as *Staphylococcus aureus* from an adjacent source of infection to the bone tissue after a trauma, surgery, or prosthesis insertion[1,2]. The standard pharmacological treatment is based on antibiotics (AB), used for long periods at high doses, with the consequent risk of systemic toxicity and the increase in bacterial resistance[3]. Alternatively, local release of AB has been pointed out as a potential therapeutic strategy, with AB-loaded polymethylmethacrylate (PMMA) beads being the most widely used system. This approach is highly successful in eradicating the infection but entails the need for a second surgical intervention for its removal since it is not biodegradable. The use of biodegradable polymers would overcome this limitation and would allow high local drug levels to be reached, systemic concentrations to be maintained at minimal levels, and the empty space resulting from surgical debridement of infected tissue to be filled[4].

Objectives: On this basis, the main objective of this work is to obtain and characterise biodegradable microparticles (MPs) using a synthetic copolymer, which allow the encapsulation and sustained release of an antimicrobial drug. The following specific objectives are proposed: 1) Development and selection of a reproducible technique to obtain ciprofloxacin (CIP)-loaded MPs with the desired properties. 2) Obtaining MPs loaded with the drug and their characterisation in terms of their physicochemical and loading properties.

Materials and Methods: A simple oil-in-water emulsion-solvent evaporation method technique (SE) was developed to obtain MPs of Resomer® 703 S, Poly (L-lactide-co-ε-caprolactone 70:30, PLLA-PCL), where the oil phase consisted of the polymer and CIP dissolved in dichloromethane (DCM), while the aqueous phase consisted of polyvinyl alcohol (PVA) in milliQ water. Additionally, to increase the drug encapsulation, as CIP is hydrophilic, two protocols of double emulsion-solvent evaporation (DE) were subsequently designed and compared. In both, the drug was added in a first aqueous phase of milliQ water at 60ºC, with or without gelatin. The oil phase and the second aqueous phase had the same composition as in SE. The MPs obtained were filtered, washed with milliQ water and vacuum-dried for 24 hours. Finally, they were characterised in terms of size and distribution by image analysis, and the encapsulation efficiency was evaluated by UV-Visible spectrophotometry. Their surface morphology was characterised by SEM and their thermal behaviour by DSC.

Results and Discussion: Both SE and DE techniques have allowed the obtention of white MPs with appropriate features. As expected, CIP-loaded MPs were just obtained by DE with and without gelatin due to their hydrophilicity, while for SE less than 10% of CIP was encapsulated. The mean size of the MPs varied within the range 1200-1400 µm for MPs with gelatin, and between 200-400 µm without gelatin. The sphericity of the MPs has a mean value of 0.80 out of 1 in both cases. The mean percentage of encapsulated CIP in the gelatin batches was 78%, while the percentage increased to 89% in the formulation without gelatin.

Conclusions: The results indicate that both DE developed methods show high encapsulation yield, loading high percentages of drug. The MPs developed show promise as therapeutic systems for the local treatment of chronic osteomyelitis, especially in those cases that require a surgical procedure.

References:

1. Dorati R. *et al*., Pharmaceuticals. 10: 96 (2017).
2. Lew D. *et al*., The Lancet. 364: 369-379 (2004).
3. Ikpeme, I. *et al*., International Journal of Clinical Medicine. 04, 07: 313-318 (2013).
4. Rutledge, B. *et al*., Clinical Orthopaedics & Related Research. 411: 280-287 (2003).

ANTIFUNGAL ACTIVITY OF DIFFERENT CLOTRIMAZOL GALENIC FORMULATIONS FOR TOPICAL ADMINISTRATION

Usach, I.; Martínez, P.; Peris, J.E.

Department of Pharmacy and Pharmaceutical Technology and Parasitology, University of Valencia, Spain.

Introduction: Fungal skin infections caused by *Candida albicans* are common benign pathologies in clinical practice. The usual treatment of superficial candidiasis includes topical preparations of clotrimazole (CLT), considered effective and with good skin tolerance[1]. Some studies have been published aimed at improving the antifungal efficacy of CLT after topical application. Thus, formulations of nanoemulsions, ethosomes or three-dimensionally-structured hybrid vesicles have shown an improvement in antifungal activity compared to the reference commercial cream, Canesten®[2,3].

Objectives: The objectives of this work were the preparation of different galenic formulations of CLT, including a hydrogel, an O/W emulsion and liposomes, and the study of their effect against *C.albicans*, using Canesten® as a reference.

Materials and Methods: To prepare the hydrogel, a weighed amount of Carbopol 940 (0.18 % w/w) was gradually added to water. The gel was left to stand at 25 ºC overnight for complete swelling, afterwards the pH of the mixture was adjusted to 6-6.5 with NaOH 1 M. The O/W emulsion was formulated with an O/W non-ionic self-emulsifying base (22 % w/w), sweet almond oil (3 % w/w), BHT (0.02 % w/w), propylene glycol (5 % w/w) and water (≈70 % w/w). Liposomes were prepared mixing 50 mg of phospholipon 90G and 0.25 ml of a solution of CLT in DMSO (10 mg/ml). The mixture was hydrated with 4.75 ml of water and sonicated with 2 cycles with a high intensity ultrasonic disintegrator. CLT concentration in all formulations was 500 µg/ml. The antimicrobial activity of the active principle was evaluated by growth curves of *C.albicans* in the presence of different formulations of CLT and was compared with that obtained using Canesten®.

Results and Discussion: The drug-loaded liposomes showed an average size of 102.2 nm with a homogeneous size distribution (polydispersity index of 0.32) and a Z-potential of 0.07 mV. Growth curves showed that CLT dispersion, liposomes and hydrogel were more effective than Canesten® in the growing medium. By contrast, the effectiveness of the O/W emulsion was lower than that obtained with Canesten®. All these differences were statistically significant ($p < 0.05$).

Conclusions: The inhibitory capacity of CLT formulated as hydrogel and liposomes was better than that of the Canesten®. These formulations improve the delivery of CLT and may be considered as promising carriers for the treatment of topical fungal infections such as Candidiasis.

References:

1. Crowley PD et al., J. Appl. Microbiol. 117 (2014).
2. Soriano-Ruiz JL et al., Int J Pharm. 554 (2019).
3. Manca ML et al., Pharmaceutics. 11, 6 (2019).

DEVELOPMENT OF A SEMI-SOLID FORMULATION OF THREE ANTIOXIDANTS TO RELIEVE OF AND PROTECT FROM RADIODERMATITIS: PRELIMINARY STUDIES

Dokuzliyska LS[1], Guillot AJ[1], Ibáñez, A, Garrigues TM[1]

[1] DrugBiOp Research Group. Department of Pharmaceutical Technology and Parasitology, University of Valencia, Spain.

Introduction: Radiodermatitis is the skin's response to ionizing radiation exposure. It affects to the 95% of patients treated with radiotherapy for breast cancers. Effects can range from erythematous rash to desquamation and, even, necrosis. This response can cause significant pain and limitation of usual lifestyle activities. The pathophysiology of this condition seems to be caused by ROS-mediated cell damage. At present, the treatment is symptomatic and depends on the injury produced.

Objectives: The aim of this project is the design of a stable semi-solid topical form, able to deliver three actives in the dermis. The selection of actives was based on their properties as ROS scavengers: pterostilbene (PT), silibinin (SI) and nicotinamide riboside (NTR).

Materials and Methods: A solubility study with five of oily excipients most used in the Technology laboratory and two hydrophilic ones was performed. Five emulsions were prepared based on these results and were evaluated considering organoleptic characteristics and stability after the addition of the actives. Only one prototype was selected and further studied. It corresponds to a soapy glycerin cream. A conductivity test was carried out to determine the external phase. Subsequently, a stability test was performed in two phases: an accelerated test of 15 consecutive days in extreme temperature conditions (50ºC, room temperature and 2ºC) and a 90-day stability test. The parameters studied were organoleptic characteristics (appearance, colour and odour), determination of pH and phase separation after centrifugation at 3500 rpm during 15 min. Concentrations of the active were also measured after treatment of 0.5g of the emulsion with 4 ml methanol during 24h. The analysis of PT, SI and NTR was performed by UPLC coupled with mass spectrometry ACQUITY™ TQD. Studies of penetration were performed by means of the tape-stripping technique, using human skin samples. An incubation time of 1h was studied.

Results and Discussion. None of the solvents studied was able to dissolve the three actives at the same time. The best results were obtained for NTR in propylene glycol and PT and SI in ethoxy diglycol. The selected prototype is a w/o emulsion, with a very low conductivity (56 µS). Appearance, colour and odour were stable in the three conditions assayed, during the 90-day period studied. The pH values ranged from 6 to 7, regardless of the day considered ($p<0.05$). Values corresponding to the refrigerated sample were always lower (around 0.5 units) than those obtained with samples at ambience temperature or at 40ºC. There were not statistical differences (ANOVA, $\alpha=0.05$) among concentrations of any of the actives, considering time of sampling as the factor. All the actives studied were able to penetrate the stratum corneum; the profile of skin concentration vs depth is a mono exponential decay.

Conclusions: The w/o emulsion prepared is stable for 90 days, both physically and chemically. The preliminary studies presented here suggest the ability of the emulsion prepared as candidate to treat radiodermatitis. Further studies are needed to test toxicity and activity.

1. Kondziołka J, et al Overview of the Active Ingredients in Cosmetic Products for the Care of Skin That Has Been Exposed to Ionizing Radiation - Analysis of Their Effectiveness in Breast Cancer Radiotherapy. Clin Cosmet Investig Dermatol. 2021;14:1065-1076. (2021).
2. Isaac, V.L.B. et al Protocolo para ensaios físico-químicos de estabilidade de fitocosméticos (2008).
3. Melero A., In Vitro Human Skin Segmentation and Drug Concentration–Skin Depth Profiles. In: Turksen K. (eds) Permeability Barrier. Methods in Molecular Biology (Methods and Protocols), vol 763. Humana Press. (2011).