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Annals of Medicine

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iann20

Nanoencapsulation as a novel delivery approach for therapeutic applications of gla-rich protein (GRP)

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To cite this article: Nuna Araújo, Carla Viegas, Jorge F. Pontes, Catarina Marreiros, Pedro Raimundo, Anjos L. Macedo, António Alves de Matos, Ana Grenha, Cees Vermeer & Dina Costa Simes (2021) Nanoencapsulation as a novel delivery approach for therapeutic applications of gla-rich protein (GRP), Annals of Medicine, 53:sup1, S23-S24, DOI: 10.1080/07853890.2021.1896900

To link to this article: https://doi.org/10.1080/07853890.2021.1896900

Published online: 28 Sep 2021.

of using a layer-by-layer (LbL) coating strategy, with alginate, chitosan and hyaluronic acid (ALG/CHI/HA) to get a controlled release of diclofenac from silicon based hydrogels.
Materials and methods: A lab-made silicon-based hydrogel intended for SCLs was loaded by soaking with the anti-inflammatory (diclofenac, DCF) and coated layer-by-layer (LbL) by immersion in solutions of alginate, chitosan and hyaluronic acid. Material properties such as transmittance, wettability, ionic permeability and swelling were studied. Drug release experiments were carried out under sink conditions (3 mL NaCl aqueous solution 130 mM, 36 °C, 180 rpm stirring). The coating stability and lysozyme-coating interaction was evaluated by quartz crystal microbalance with dissipation (QCM-D). A mathematical model was applied to predict the *in vivo* efficacy of the coated lenses. Chorioallantoic membrane (HET-CAM) tests were carried out to predict potential ocular irritation.
Results: The coating did not impair the studied physico-chemical properties, relevant for the application of the material in SCLs. HET-CAM tests did not suggest any potential for ocular irritation of both uncoated and coated samples. QCM-D data revealed the stability of the deposited layers and no adsorption of the protein. DCF release kinetics was controlled by the presence of the coating. The DCF concentration profile in the tear fluid estimated from the mathematical model predicts values above the half maximal inhibitory concentrations (IC_{50}) for cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) during more than 2 weeks, for the coated hydrogels, while for the uncoated such levels are only expected in the first day of release.
Discussion and conclusions: ALG/CHI/HA LBL coated silicon hydrogels present adequate properties to be used in DCF releasing SCLs. They reveal an antifouling behaviour against lysozyme, one of the most abundant protein in lacrimal fluid. *In vitro* studies suggest that such system has potential to be used in the production of efficient therapeutic SCLs.

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Acknowledgements

To Fundação para a Ciência e a Tecnologia for Diana Silva PhD Grant (PD/BD/114088/2015), and funding through the unit projects UID/QUI/00100/2019 for CQE and UID/BIM/04585/2019 for CiiEM, and the research project (STReoSTRAT) PTDC/CTM-BIO/3640/2014. This work was also partially funded by Spanish MINECO (SAF2017-83118-R), Agencial Estatal de Investigación (AEI) and FEDER.

Reference

[1] Silva D, Sousa HC, Gil MH, et al. Antibacterial layer-by-layer coatings to control drug release from soft contact lenses material. Int J Pharma. 2018;553(1–2):186–200.

DOI: 10.1080/07853890.2021.1896898

Nanoencapsulation as a novel delivery approach for therapeutic applications of gla-rich protein (GRP)

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ABSTRACT
Introduction: Gla rich protein (GRP) is a vitamin K dependent protein, shown to function as an inhibitor of pathological calcification and as an anti-inflammatory agent, with potential therapeutic use for age-related diseases such as osteoarthritis (OA) [1,2]. OA is a leading cause of disability and morbidity in the older population and constitutes a major worldwide challenge for our health system. Presently, there are no drugs approved that can prevent, stop, or even restrain progression of OA. GRP has been shown to be able to lower inflammation and mineralisation processes in the articular tissue. Chitosan/tripolyphosphate (TPP) nanoparticles were selected for this study due to their biocompatibility, biodegradability and capacity to overcome the problem of low solubility of GRP in physiological conditions. This study aims to produce and characterise chitosan/TPP nanoparticles as GRP-delivery vehicles and test its anti-inflammatory potential in human macrophages.

Materials and methods: Nanoparticles of fluorescein-labelled chitosan/TPP with and without GRP (NG and NP, respectively) were prepared by ionic gelation [3]. Resulting NP and NG were characterised by dynamic light scattering, transmission electron microscopy (TEM) and flow cytometry. The anti-inflammatory activity of NP and NG was assessed in THP-1 cells differentiated to macrophages. Mac-THP-1 cells were pre-treated with both NP and NG, followed by LPS stimulation. Cell viability was assessed by the MTS cell proliferation assay, and levels of TNFα released to cell culture media were determined by ELISA.
Results: The average size determined for NG was increased relatively to the NP, while flow cytometry and TEM analysis indicate the presence of GRP in NG, suggesting an effective incorporation of human recombinant GRP. Flow cytometry studies confirmed the cellular uptake of nanoparticles by macrophages. The GRP-loaded nanoparticles were able to reduce the production of TNFα in LPS-stimulated macrophages.
Discussion and conclusions: The results confirm that chitosan/TPP nanoparticles are excellent drug delivery vehicles for GRP in macrophages and predict a wider therapeutic application in chronic inflammation-related diseases. GRP-containing nanoparticles will be further used in OA functional assays and the results will bring new knowledge on the role of GRP in the interplay between inflammation and mineralisation events associated with OA.

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Acknowledgements

The authors would like to acknowledge Portuguese Science and Technology Foundation (FCT) for the grant SFRH/BD/111824/2015 (N. Araújo), the transitional provision DL57/2016/CP1361/CT0006 and project UIDB/04326/2020 and UCIBIO UIDB/04378/2020. C. Marreiros is the recipient of a 0055 ALGARED + 5E fellowship.

References

[1] Cavaco S, Viegas CS, Rafael MS, et al. Gla-rich protein is involved in the cross-talk between calcification and inflammation in osteoarthritis. Cell Mol Life Sci. 2016;73(5):1051–1065.
[2] Viegas CSB, Costa RM, Santos L, et al. Gla-rich protein function as an anti-inflammatory agent in monocytes/macrophages: implications for calcification-related chronic inflammatory diseases. PLOS One. 2017;12(5):e0177829.
[3] Grenha A, Seijo B, Serra C, et al. Chitosan nanoparticle-loaded mannitol microspheres: structure and surface characterization. Biomacromolecules. 2007;8(7):2072–2079.

DOI: 10.1080/07853890.2021.1896900

Neuromodulation of lower limb motor pathways with trans-spinal direct current stimulation: an overview of current findings

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ABSTRACT
Introduction: The spinal cord (SC) is a complex structure containing several neuronal circuits related with motor function of the upper and lower limbs, operating under the influence of higher centres. Central nervous diseases can change the responses of the spinal motor circuits leading to its dysfunction. Over the last decade, there has been a growing interest in the study of trans-spinal direct current stimulation (tsDCS) as a potential therapeutic tool to modulate spinal circuits through the application of electric currents delivered non-invasively [1]. Computational modelling studies are potentially useful to optimise electrodes montage in order to target current delivery to specific spinal region and pathways [2].
The aim of this study is to describe the more effective tsDCS electrode montages to maximise the modulation effects in lower limbs, as derived from a narrative review of the literature.
Materials and methods: A literature narrative review was carried out through Pubmed database and manual search, considering the following selection criteria: research papers published in journals with impact factor in the areas of neuroscience, neurophysiology and biomedical engineering from 2008 to 2018; use of keywords related with the topic (tsDCS, trans-spinal, lumbar, thoracic, spinal cord, motor pathways, computational modelling); written in English. A qualitative analysis was performed over the literature selected considering the following items: electrode montage; tsDCS protocol; methods for assessing motor responses; observed changes; computational results; induced electric field (EF) distribution.
Results: Published studies showed different neuromodulation effects on the motor responses of the lower limb. Whereas some studies reported a reduction of spinal motoneurons excitability using anodal tsDCS, others described higher motor unit recruitment with cathodal stimulation (T10–T12), suggesting modulation of Ia-motoneuron (MN) synapse. Other