simultaneously. Thus, arsonium-containing cationic lipids appear as promising versatile multi-faceted agents that could find various applications for the treatment of inherited as well as acquired diseases.

410. pDMAEMA-Based Polyplexes as Vectors for Retinal Gene Therapy

Diogo B. Bitoque,[1,2] Andreia P. Silva,[1] Margarita D. Romero,[3] Ana V. Oliveira,[1,2,5] Eduardo M. Lopes,[1,2] Ana M. Costa,[4] Gabriela A. Silva.[1,2]

[1]Centre for Molecular and Structural Biomedicine/Institute for Biotechnology and Bioengineering (CBME/IBB)), University of Algarve, Faro, Portugal; [2]Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal; [3]Department of Cell Therapy and Regenerative Medicine, Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER), Sevilla, Spain; [4]Department of Chemistry and Pharmacy, University of Algarve, Faro, Portugal; [5]PhD Program in Biomedical Sciences, Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal.

Methacrylate polymers have been described as highly biocompatible and are successfully used in medical applications1. Due to their cationic nature, these polymers can be used to form polyplexes with DNA for its delivery. This work aims to understand how feasible pDMAEMA (poly(2-(N,N'-dimethylamino)ethyl methacrylate)) is as a non viral gene delivery system to the retina. pDMAEMA-DNA polyplexes were prepared in PBS at 5, 7.5, 10, 12.5 and 20 nitrogen/phosphorous (N/P) ratio. Dynamic light scattering and zeta potential measurements confirmed positively charged nanosized polyplexes for all ratios. pDMAEMA was able to efficiently complex and protect DNA from DNase I degradation. The cytotoxicity was evaluated using two retinal pigment epithelium (RPE) cell lines: ARPE-19 and D407 and the HEK293 cell line (control). We have found that cytotoxicity of the uncomplexed polymer is concentration and time dependent, as expected, and negligible for the pDMAEMA-DNA polyplexes. Furthermore, for the concentrations to be used in vivo, the cytotoxicity was negligible, as shown by the absence of inflammation upon injection in the subretinal space of C57Bl6 mice. Transfection efficiency, as evaluated by fluorescence microscopy and flow cytometry, showed that the retinal cell lines were in fact transfected by these polyplexes, with the efficiency varying according to the ratio of N:P. These results suggest that pDMAEMA is a feasible candidate for non-viral gene delivery to the retina. 1. Ji Hoon Jeong, Sung Wan Kim, Tae Gwan Park (2007). Molecular design of functional polymers for gene therapy. Progress in Polymer Science, Volume 32, Issue 11: 1239 – 1274. Acknowledgements IBB/CBME, LA, FEDER/POCI 2010; Fundação para a Ciência e Tecnologia (PTDC-SAU-BEB-2008 to G.A.Silva, SFRH/BD/70318/2010 to A.V.Oliveira) and Marie Curie Reintegration Grant (PIRG-GA-2009-249314) under the FP7 program.

411. Chitosan-Hyaluronic Acid Hybrid Vectors for Retinal Gene Therapy

Ana V. Oliveira,[1,2,3] Diogo M. Bitoque,[1,3] Gabriela A. Silva.[1,3]

[1]Centre for Molecular and Structural Biomedicine (CBME/IBB, LA), University of Algarve, Faro, Portugal; [2]PhD Program in Biomedical Sciences, Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal; [3]Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal.

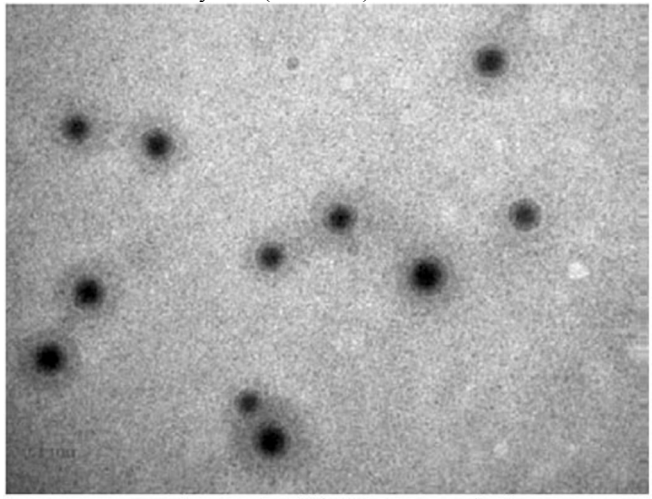
Our goal is to develop chitosan-based non-viral vectors optimized for ocular gene therapy. Therefore, chitosan-DNA polyplexes were prepared using a NH3:PO4 ratio of 5:1. These polyplexes were characterized regarding their morphology, stability and transfection efficiency on retinal pigment epithelial cells. The method to produce the polyplexes proved to be reproducible, consistently yielding polyplexes with a spherical morphology and sizes below 500 nm. These polyplexes have long-term stability and are capable of efficiently protecting DNA from DNase I degradation. However, transfection efficiency, evaluated by fluorescence microscopy and flow cytometry, showed these polyplexes to fall below commercial transfection reagents. The lower transfection efficiency observed for chitosan-DNA polyplexes is most likely due to reduced cell penetration and deficient plasmid release. Our endeavor to improve this lower transfection efficiency encompassed the preparation of polyplexes with different NH3:PO4 ratios and combining these with hyaluronic acid. The results for these hybrid polyplexes show a marked increase in the transfection efficiency. In addition, we are studying the effect of chemical modification of chitosan to promote timely and efficient DNA release. Overall, our hybrid systems show an improvement of the efficiency of chitosan-based nonviral vectors for gene therapy. Acknowledgements: IBB/CBME, LA, FEDER/POCI 2010; Fundação para a Ciência e Tecnologia (PTDC-SAU-BEB-2008 to G.A.Silva, SFRH/BD/70318/2010 to A.V.Oliveira) and Marie Curie Reintegration Grant (PIRG-GA-2009-249314) under the FP7 program.

412. Novel Nanoparticles To Encapsulate microRNA for the Treatment of Stroke

Li-Fang Wang,[1] Jenn-Shing Chen,[2] Yu-Lun Lo.[1]

[1]Kaohsiung Medical University, Kaohsiung, Taiwan; [2]National University of Kaohsiung, Kaohsiung, Taiwan.

microRNAs cannot be directly applied for clinical use in that they can be rapidly degraded and their cellular internalization is poor due to its negative surface charge. A variety of delivery systems, either viral or non-viral, have been investigated for microRNA delivery. Non-viral vectors have received growing interests due to many advantages such as they offer over the viral counterparts including improved safety, low immune responses, enabling repeated uses, and ease of production. In this report, an anionic natural polysaccharide-chondroitin sulphate (CS) was chemically linked to polyethylenimine (PEI) with a lower molecular weight of 10 kDa to minimize its cytotoxicity as a microRNA carrier for the treatment of ischemia stroke. It is noted the cytotoxicity of PEI reduced dramatically after modification as compared to the original PEI of molecular weight of 10K and 25K Da. The TEM image of CS-PEI and miRNA shows a spherical structure with a uniform size distribution. The size falls in a perfect range (about 100 nm), larger enough to escape the renal filtration (< 10 nm) but smaller enough to prevent uptake by the reticuloendothelial system (> 200 nm).



TEM images of CP(L)/miR-1xx at an N/P of 5