LARGE-PORE SILICA NANOCARRIERS FOR ANTIANGIOGENIC TREATMENT AGAINST AGE-RELATED MACULAR DEGENERATION

Amelia Ultimo[1], Mar Orzaez[2], Maria Jose Santos-Martinez[1], Ramón Martínez-Máñez[3], María Dolores Marcos[3], Félix Sancenón[3], Eduardo Ruiz-Hernández[1]

[1] School of Pharmacy, Trinity College Dublin, Dublin 2, Ireland; [2] Centro de Investigación Príncipe Felipe, Valencia, 46012, Spain; [3] Instituto Interuniversitario de Reconocimiento Molecular y Desarrollo Tecnológico, Valencia, 46022, Spain

Background: Wet age-related macular degeneration (AMD) is a progressive disease that leads to central vision loss due to damaged retinal pigment epithelium (RPE) and photoreceptors in the central area of the retina. Wet AMD occurs in patients who develop subretinal choroidal neovascularization, mostly produced by an abnormal expression in the RPE of the vascular endothelial growth factor (VEGF). Current approaches for the treatment of AMD present considerable issues such as important side effects and low patient compliance. The encapsulation of anti-VEGF drugs in suitable nanocarriers with better penetration, higher retention times and sustained release would be of great interest. The objective of this work is the development of a drug delivery system for the topical administration of anti-VEGF siRNA molecules based on large-pore mesoporous silica nanoparticles (LP-MSNs). siRNA is loaded into the LP-MSNs pores, while the nanoparticles' external surface is functionalized with polyethylenimine (PEI) chains, which act as pore capping ensembles that allow the siRNA controlled release and promote endosomal escape to facilitate its cytosolic delivery.

Methods: LP-MSNs were functionalised to obtain three different sets of materials. The first one, S1, was loaded with the fluorescent dye rhodamine B and capped with PEI chains, to perform cargo release assays and verify PEI capping ability; S2 was covalently functionalised with rhodamine B isothiocyanate through 3-aminopropyltriethoxysilane chains, and externally capped with PEI, and employed to study particles cytotoxicity, cellular uptake and hemocompatibility; finally, S3 was loaded with anti-VEGFA siRNA and capped with PEI, and used for VEGF silencing in ARPE-19 retinal cells. The materials were characterised using standard techniques such as transmission electron microscopy, dynamic light scattering, zeta potential measurements and N_2 adsorption-desorption analysis. siRNA quantification was performed using a NanoDrop 2000 spectrophotometer. Nanoparticles hemotoxicity was tested with red blood cells and in platelet rich-plasma using a Quartz Crystal Microbalance with Dissipation monitoring (QCM-D).

Results: Spherical monodispersed dendrimer-like nanoparticles with an average size of 105 nm and center-radial large pores of about 17 nm were obtained. The release studies demonstrated that the cargo remains protected inside the pores in the absence of an adequate triggering stimulus. The siRNA-loaded nanodevices reduced VEGF expression, demonstrating the developed nanocarrier capacity to provide siRNA protection, endosomal escape and consequent cytoplasmic release. Nevertheless, although bare LP-MSNs showed negligible toxicity in several cell lines and in erythrocytes in previous assays, coated nanoparticles affected cells viability and induced hemolysis and platelet aggregation, probably due to the positively charged external PEI layer.

Conclusions: Our results represent a first step for the development of topically administered nanovehicles based on LP-MSNs for the sustained attenuation of VEGF in the RPE by siRNA. The successful results obtained in VEGF silencing in ARPE-19 cells demonstrate that although further modifications are needed for improving their biocompatibility, the designed nanodevices present a great potential for nucleic acid delivery, holding great promise for the next stages of the project.