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# In Vitro Validation of High Capacity Mesoporous Silica Nanocarriers for Retinal Delivery of siRNA

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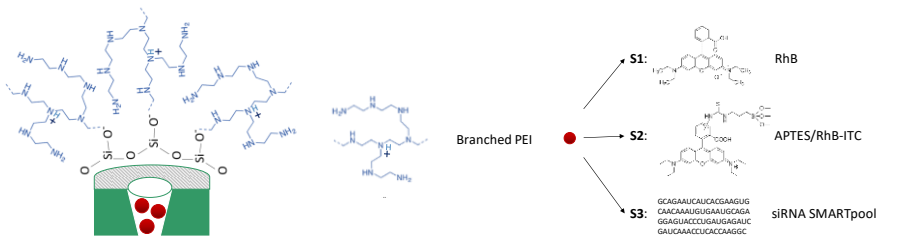
The main objective of this work is the preliminary development of large pore mesoporous silica nanoparticles (LP-MSNs) as carriers for siRNA delivery to address retinal neovascularization, mostly driven by an abnormal expression of vascular endothelial growth factor (VEGF).[1] Optimal means of VEGF inhibitors delivery remain to be determined to provide long-term suppression of improper ocular angiogenesis.[2,3] Highly efficient carriers are required for efficient siRNA transport, cellular uptake and intracellular release. Here, functionalized nanodevices based on LP-MSNs loaded with anti-VEGF siRNA and externally coated with polyethyleneimine (PEI) chains, that allow the controlled release of the cargo while facilitating endosomal escape, have been synthesized.




**Scheme 1.** Three different types of functionalized nanoparticles were developed: **S1**, with rhodamine B dye loaded pores, which allowed performing cargo release assays and verifying PEI ability as stimuli-responsive capping agent; **S2**, covalently functionalised with rhodamine B isothiocyanate through 3-aminopropyltriethoxysilane chains, employed to study nanoparticles cytotoxicity, cellular uptake and hemocompatibility of the nanoparticles; and **S3**, the final solid loaded with anti-VEGFA siRNA, developed for VEGF silencing.

LP-MSNs were synthesized using CTATos as a template, later removed by calcination at 550ºC. Representative transmission electron microscopy (TEM) images of the obtained nanoparticles are presented in Figure 1a. Cargo release assays were performed to check the capping ability of PEI in the absence of a specific stimulus (Fig. 2). Purified lysosomal extract was used as release medium, as we speculate that cargo release would occur in late endosomes and lysosomes thanks to proton sequestration by PEI and membrane disruption.[4] A haemolysis test with red blood cells and a platelet aggregation assay with platelet-rich plasma (PRP), monitored using a Quartz Crystal Microbalance with Dissipation technology (QCM-D), were carried out to study the particles hemotoxicity (Fig. 3). Finally, the human retinal pigment epithelial cell line ARPE19 was selected for the system in vitro evaluation. Nanoparticles uptake was followed by confocal microscopy (Fig. 4a) and cytometry (Fig. 4b), then nanoparticles cytotoxicity (Fig. 4c) and silencing performance (Fig. 4d) were studied by WST-1 test and ELISA, respectively.

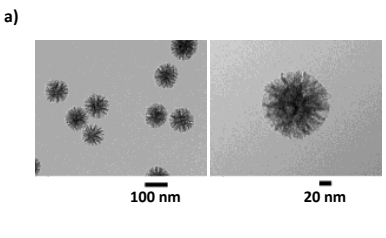



**Figure 1.** a) Representative TEM images of calcined LP-MSNs. b) LP-MSNs size distribution obtained by measurements taken from TEM images.

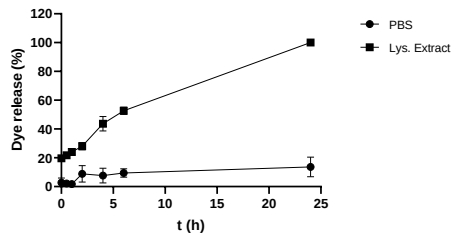



**Figure 2.** Normalized release profiles of RhB from S1 nanoparticles in absence or in presence of purified lysosomal extract, obtained by measuring RhB fluorescence at 580 nm at different times.

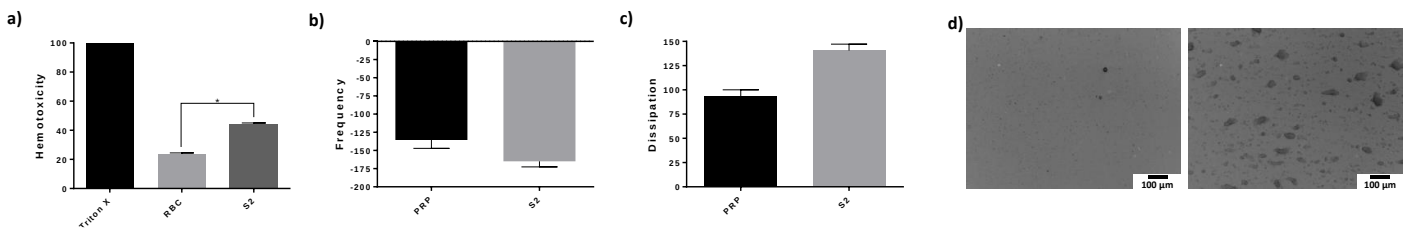



**Figure 3.** a) Haemolysis induced by S2 nanoparticles compared to red blood cells (RBC) in the absence of nanoparticles. Triton X-100 was used as positive control. Data are expressed as mean ± s.d. Asterisk indicates significant differences ($p < 0.001$) when t test was applied. b) Quantitative analysis of the effect of PRP in the absence or presence of S2 nanoparticles on the frequency of the quartz crystal as measured by the QCM-D (n=2). c) Quantitative analysis of the effect of PRP in the absence or presence of S2 nanoparticles on the energy dissipation as measured by the QCM-D (n=2). d) Representative micrographs of the surface of fibrinogen-coated polystyrene-coated quartz crystals as viewed by optical microscopy, showing platelet aggregates following the perfusion of PRP in the absence (left) or presence (right) of S2 nanoparticles.

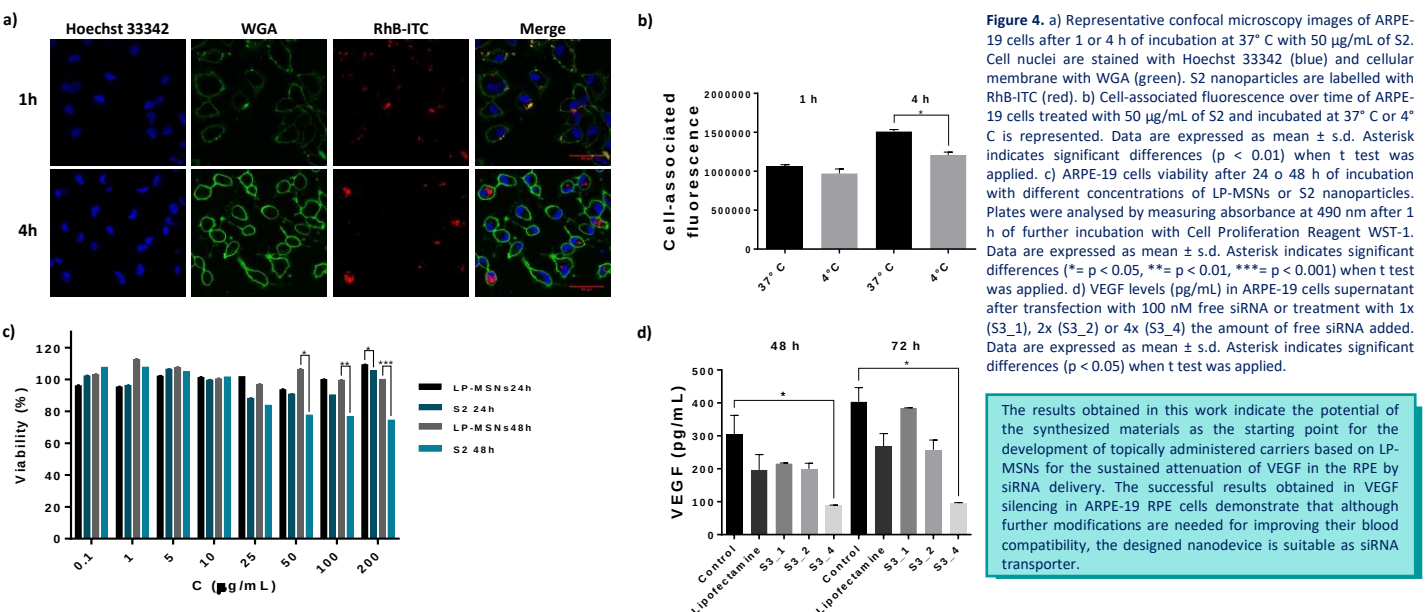



**Figure 4.** a) Representative confocal microscopy images of ARPE-19 cells after 1 or 4 h of incubation at 37° C with 50 µg/mL of S2. Cell nuclei are stained with Hoechst 33342 (blue) and cellular membrane with WGA (green). S2 nanoparticles are labelled with RhB-ITC (red). b) Cell-associated fluorescence over time of ARPE-19 cells treated with 50 µg/mL of S2 and incubated at 37° C or 4° C is represented. Data are expressed as mean ± s.d. Asterisk indicates significant differences ($p < 0.01$) when t test was applied. c) ARPE-19 cells viability after 24 o 48 h of incubation with different concentrations of LP-MSNs or S2 nanoparticles. Plates were analysed by measuring absorbance at 490 nm after 1 h of further incubation with Cell Proliferation Reagent WST-1. Data are expressed as mean ± s.d. Asterisk indicates significant differences (*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$) when t test was applied. d) VEGF levels (pg/mL) in ARPE-19 cells supernatant after transfection with 100 nM free siRNA or treatment with 1x (S3_1), 2x (S3_2) or 4x (S3_4) the amount of free siRNA added. Data are expressed as mean ± s.d. Asterisk indicates significant differences ($p < 0.05$) when t test was applied.

The results obtained in this work indicate the potential of the synthesized materials as the starting point for the development of topically administered carriers based on LP-MSNs for the sustained attenuation of VEGF in the RPE by siRNA delivery. The successful results obtained in VEGF silencing in ARPE-19 RPE cells demonstrate that although further modifications are needed for improving their blood compatibility, the designed nanodevice is suitable as siRNA transporter.

**Aknowledgements**
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