Diffusion and cellular uptake delivery routes of active agents using multilayer microcapsules

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Statement of Purpose: There has been great interest in the development of nano- and micro-carriers of active agents, such as micelles, liposomes and polymersomes. Among them, the use of nanostructured microcapsules made using layer-by-layer (LbL) strategies has been studied. Multilayer microcapsules can deliver both hydrophilic and hydrophobic drugs in a controlled way by varying the number of assembled layers and by selecting molecules as building blocks that exhibit bioactivity and/or stimuli-responsiveness. Herein, nanostructured microcapsules of chitosan and elastin-like recombinamers (ELRs) – a class of genetically engineered macromolecules with temperature responsiveness – were produced using LbL assembly. Two distinct protein delivery routes were studied: (i) temperature-dependent diffusion to an aqueous medium and (ii) internalization by human mesenchymal stem cells (hMSCs). To evaluate the role of surface functionalization in the cellular uptake, ELRs modified with either RGD or a control of a scrambled RDG motif were used to assess the role of surface functionalization.

Methods: $CaCO_3$ microparticles were prepared by mixing Na_2CO_3 and $CaCl_2$ under heavy stirring. The mixture contained either FITC-BSA (route I) or DQ-ovalbumin – a protein conjugate that emits red fluorescence when intact and green when degraded (route II). The microparticles were immersed alternately in chitosan or ELR solutions, with a rinsing step in between, until 1 to 5 bilayers were assembled. Afterwards, the $CaCO_3$ cores were dissolved with EDTA. The microcapsules were evaluated by CLSM and SEM. For release studies, the capsules were suspended in PBS at 25 and 37 ºC and samples were taken every 24 hours for fluorescence measurements, during 14 days. For cellular uptake studies, hMSCs were incubated in microcapsule/cell ratios between 5:1 and 100:1 during 3 days at 37 ºC. Internalization efficacy was quantified by flow cytometry and DQ-ovalbumin degradation was followed by fluorescence microscopy.

Results: The release of FITC-BSA was distinct for each temperature: the protein quantity released was higher at 25 ºC than at 37 ºC (Figures 1A and 1B). For instance, microcapsules made of 1 bilayer released respectively 80% and 50% of the encapsulated protein. The role of the bilayer number was also evident: regardless of the temperature, the cumulative release was higher for capsules with 1 bilayer, evidencing the role played by the capsules' architecture in their permeability.

For the intracellular delivery of DQ-ovalbumin, SEM of RGD- and RDG-based microcapsules showed that both types of microcapsules were identical, being spherical with around 4 µm in diameter. Flow cytometry indicated that 63% of the hMSCs internalized RGD-functionalized microcapsules, while the nonfunctional analogue triggered internalization in around 53% of the cells. No statistical differences were found between both cases ($p>0.05$ using one-way ANOVA), suggesting that the exhibition of the RGD/RDG motifs does not influence significantly the incorporation of the microcapsules by the hMSCs. Intracellular processing of DQ-ovalbumin was assessed by qualitative fluorescence variation. In contrast to the internalization efficacy, it was found that cargo consumption was faster when encapsulated within the RGD-based microcapsules (Figures 1 C and 1D), evidencing a high bioavailability of DQ-ovalbumin when compared to the nonfunctional analogues.

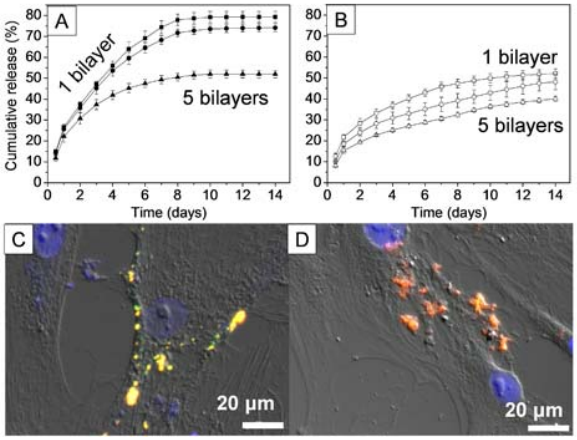

Figure 1. Cumulative release of BSA at (A) 25 ºC and (B) 37 ºC. hMSCs Intracellular degradation of DQ-ovalbumin encapsulated in (C) RGD and (D) RDG microcapsules.

Conclusions: Multilayered microcapsules based on chitosan and ELRs were studied as drug delivery vessels. Both diffusion and intracellular delivery were explored. The diffusion of the cargo could be tuned by varying temperature and the number of layers. Furthermore, the intracellular delivery of this class of microcarriers did not depend significantly on surface functionalization, but it influenced the fate of the cargo upon uptake. The developed multilayer microcapsules using biomimetic ingredients for diffusion and intracellular delivery let foresee new strategies in targeted therapies to achieve tunable drug administration and to increase the availability of molecules of interest in cells.

References: Costa RR. Small 2011;7:2640-2649. Costa RR. Nanomedicine: NBM 2013;9:895-902.