

CHITOSAN NANOCAPSULES AS TRANSMUCOSAL CARRIERS FOR ORAL PEPTIDE DELIVERY

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The oral administration of peptides and proteins remains a great challenge for pharmaceutical scientists (1). Among the approaches that have been explored so far in this direction, the use of colloidal carriers represents a promising strategy (2). The hypothesis behind this approach was that colloidal carriers are able to protect the encapsulated peptide from enzymatic degradation in the gastrointestinal fluids and, to enhance its absorption across the intestinal epithelium.

With this idea in mind, we have developed a new colloidal carrier based on the polysaccharide chitosan. The selection of chitosan was based on its capacity to interact with epithelia, due to its mucoadhesive and enhancing permeability properties. The nanocarriers (chitosan nanocapsules) consisted of an oily core surrounded by a chitosan wall and were prepared in two steps. Firstly, we prepared a nanoemulsion by the solvent displacement technique (3) and secondly, this colloidal system was coated with chitosan by simple incubation in the polymer solution (4). The coating of the oily nanodroplets was feasible due to ionic interactions established between the negatively charged nanodroplets and the positively charged chitosan, which allowed the stabilization of the system (Fig 1).

Salmon calcitonin, a model peptide, was associated to the nanostructures. The encapsulation efficiency was high and affected by the nanostructure composition. Chitosan nanocapsules were able to greatly enhance the oral absorption of the peptide as shown by the important and long lasting pharmacological effect (decrease in the calcemia levels) observed in rats. Given the lack of efficacy of the salmon calcitonin solution and the uncoated nanoemulsion, the success of the chitosan nanocapsules should be attributed to the presence of the chitosan corona around the systems.

Previous ex-vivo studies aimed at elucidating the mechanisms of action of the chitosan nanocarriers at improving the peptide absorption were performed in an enterocyte model cell line (Caco-2 cells). These studies suggested that the presence of the chitosan coating did not affect the internalization of the carriers by the cells, and that the transepithelial permeability was not enhanced by the nanocarriers at the studied concentrations. Therefore, as the main mechanism of action of the chitosan nanostructures remains unclear, we decided to investigate the role of the mucus in their interaction with epithelia. Thus, the nanocarriers were put in contact with a co-culture of Caco-2 cells and mucus-secreting cells (HT29-M6 cells), and the interaction was followed by confocal scanning laser microscopy. Confocal images evidenced a more important interaction of chitosan nanocapsules with the cells (Fig. 2), as compared to the uncoated nanoemulsion. Besides, chitosan nanocapsules interacted more importantly with the mucus-secreting cells than with Caco-2 cells. This marked binding of chitosan nanocapsules to the cell monolayers, especially to the mucus-secreting cells, could be the explanation for their ability to enhance the absorption of the associated peptide.

Figure 1: Transmission electron micrographs of: A) uncoated nanoemulsion and B) and C) chitosan nanocapsules.

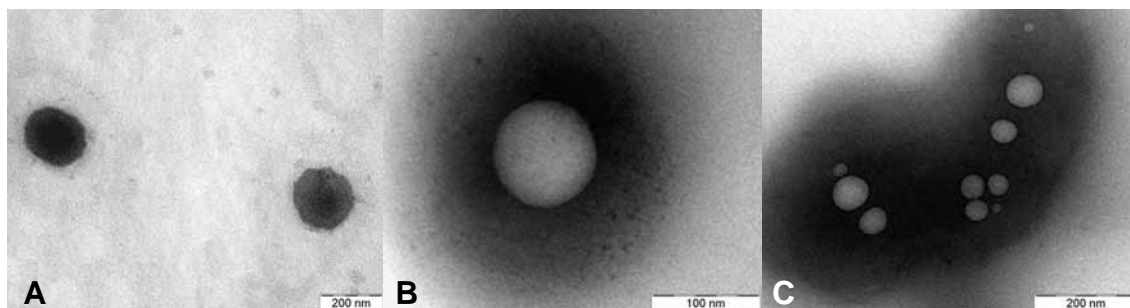
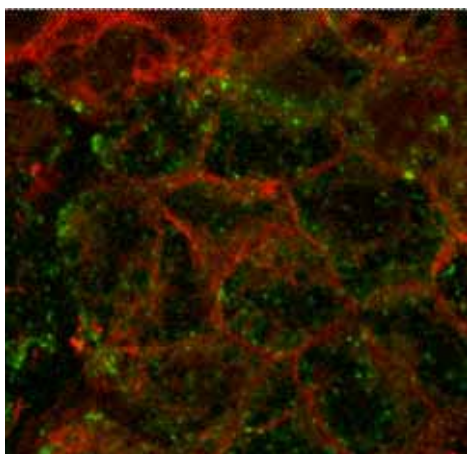


Figure 2: Montage of 12 confocal scanning microscopy horizontal cross sections showing the association of fluorescent chitosan nanocapsules (green) to the Caco-2 cells (red). Step size in z-axis of 0.5 μm .



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