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MICROFLUIDICS FABRICATION OF SELF-ASSEMBLED MICROCAPSULES

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ABSTRACT

We report here a mild cell encapsulation method based on triggering the self-assembly of a multidomain peptide in presence of xanthan gum polysaccharide, which has been investigated in our group as an artificial matrix for the encapsulation of chondrocytic cells. Using a microfluidic device, we were able to produce microcapsules with homogenous size. The properties and performance of xanthan-peptide microcapsules were optimized by changing peptide/polysaccharide ratio and their effects on the microcapsule permeability and mechanical stability were analyzed. The effect of microcapsule formulation on viability and proliferation of encapsulated chondrogenic cells were also investigated. The encapsulated ATDC5 cells were metabolically active, showing an increased viability and proliferation over 21 days of *in vitro* culture, demonstrating the long-term stability of the developed microcapsules and their ability to support and enhance the survival of encapsulated cells over prolonged time.

INTRODUCTION

Cell microencapsulation is a technology with enormous clinical potential for the treatment of a wide range of human diseases. Xanthan gum is a polyanionic extracellular polysaccharide produced by the bacterium *Xanthomonas campestris*. This biopolymer is well known for its biodegradability and bioadhesive properties and also wound-healing effects (Hamcerencu M et al. 2007). Recently, xanthan derivatives (carboxymethylated xanthan and palmitoyl xanthan) were successfully used as artificial matrix for the encapsulation of chondrocytic cells (Mendes et al. 2010; Mendes et al. 2011). Xanthan microcapsules with long-term stability were generated and encapsulated cells showed high viability.

Self-assembling peptides are excellent structural units to form complex nanostructures that can recreate some of the architectural features of the natural extracellular matrix, as they can self-assemble into fibril nanostructures. The multidomain $K_2(QL)_6K_2$ peptide, with a central block of glutamine-leucine (QL) repeats and two flanking positively charged lysine (K), is able to self-assemble into cylindrical nanosctrures when charge is screened (Dong et al. 2007).

Microfludic devices have been recognized as potential tools for the fabrication of microcapsules, once this technology has the ability to control the microcapsule properties in terms of size and morphology, being a low stress inducing method suited for cell encapsulation.

Therefore, we investigated a mild cell encapsulation method based on triggering the self-assembly of multidomain $K_2(QL)_6K_2$ peptide in presence of xanthan gum polysaccharide, using microfluidics technology. The properties and microcapsule performance of Xanthan- $K_2(QL)_6K_2$ were optimized and the viability and proliferation of encapsulated chondrogenic cell line were investigated.

MATERIALS AND METHODS

The $K_2(QL)_6K_2$ peptide was synthesized by a solid phase approach following the Fmoc strategy. The mass of the crude product was analyzed by mass spectrometry and the peptide further purified by high-performance liquid chromatography (HPLC) .

Microcapsule formation and encapsulation of cells

Xanthan, was dissolved in PBS and mixed subsequently with a cell suspension. Using a microfluidics device, the

polymer solution and mineral oil were injected simultaneously using a sirynge and peristaltic pump, respectively. The Xanthan microcapsules were generated inside the microfluidic device by the shear flow of mineral oil at the bottleneck of the junction and collected in a vessel containing $K_2(QL)_6K_2$ solutions at different molarities, where self-assembly of the peptide and consequent microcapsule formation took place. The Xanthan-$K_2(QL)_6K_2$ microcapsules were washed in PBS and transferred to DMEM culture medium for cell culture. Encapsulated cells were maintained in culture for a period of 3 weeks and cell viability over time was assessed using the live/dead and Alamar Blue assays, whereas proliferation was determined by DNA quantification. The morphology of microcapsules was analyzed by scanning electron microscopy and light microscopy. The effect of peptide/polysaccharide ratio on the microcapsules permeability and mechanical stability was also analyzed.

RESULTS AND DISCUSSION

Hollow microcapsules with spherical shape were obtained from the self-assembly between Xanthan and $K_2(QL)_6K_2$ peptide. The microfluidic device allow us to obtain microcapsules with the diameter in the range of 390 ± 8 µm (lowest peptide concentration) to 585 ±18 µm (highest peptide concentration). Additionally, the microcapsule external surface appears to be quite smooth in the case of the capsules fabricated with the lowest peptide concentration while for the highest concentrations a more rough surface was observed. Consequently, the thickness of the membrane also increased with increasing peptide concentrations, affecting the permeability and mechanical resistence of the microcapsules.

The encapsulated ATDC5 cells were metabolically active, showing an increased viability and proliferation over 21 days of *in vitro* culture, demonstrating the long-term stability of the developed microcapsules and their ability to support and enhance the survival of encapsulated cells over prolonged time.

CONCLUSIONS

Combining self-assembling materials with microfluidic processing proved to be innovative approach to fabricate suitable matrices for cell encapsulation and delivery. Xanthan-$K_2(QL)_6K_2$ self-assembled microcapsules appear to be a promising alternative to the conventional biomaterials employed in cell encapsulation due the simplicity of fabrication. The optimized processing conditions enabled generating regular microcapsules with long-term stability and the ability to support the survival and function of encapsulated cells over prolonged time.

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