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XANTHAN-BASED AMPHIPHILE FOR CELL ENCAPSULATION

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ABSTRACT
Xanthan gum is a polyanionic extracellular polysaccharide produced by the bacterium *Xanthomonas campestris*. Recently, a xanthan derivative (carboxymethylated xanthan) was successfully used as artificial matrix for the encapsulation of chondrocytic. Self-assembling properties of amphiphilic molecules are being explored in several applications, once they have the ability to self-assemble in water into different shapes which can be suitably used to design templates for the synthesis of devices at the nanometer length scale, as for example capsules for cell encapsulation. In this context, xanthan, as a negatively charged biopolymer, was conjugated with hydrophobic molecules, such as palmitoyl chloride, in order to obtain polymeric amphiphiles**.** Our studies revealed that aqueous solutions of the amphiphilic palmitoyl xanthan could form gels in presence of calcium ions and self-assemble into spherical hollow-capsules that are suitable to generate regular microcapsules with long-term stability as well as with the ability to support the survival and function 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, a xanthan derivative (carboxymethylated xanthan) was successfully used as artificial matrix for the encapsulation of chondrocytic cells (Mendes et al. 2009). Xanthan microcapsules with long-term stability were generated and encapsulated cells showed high viability. Hydrophobized polysaccharides have emerged as a promising strategy in biomedical field due to the versatility to design functional structures through the spontaneous self-assembly in physiological conditions (Besheer et al. 2007 and Cheng et al. 2006). Therefore, we investigated a mild cell encapsulation method based on triggering the self-assembly of the amphiphile which is constructed by conjugation of hydrophobic palmitic acid to pH and ion sensitive xanthan. The properties and microcapsule performance of palmitoyl xanthan (PX) were optimized and the viability and proliferation of encapsulated chondrogenic cell line were investigated.

MATERIALS AND METHODS

Synthesis and characterization of palmitoyl xanthan

PX was synthesized by adding palmitoyl chloride dropwise to xanthan at different molar ratios (0.5, 0.75, 1.40 and 1.70). The mixture was vigorously stirred in an ice water bath and allowed to react overnight. The various conjugates were analysed for solubility and their structure investigated by FTIR, ^{1}H NMR, x-ray diffraction, and differential scanning calorimetry . The water-soluble PX conjugate was analysed by circular dichroism and dynamic light scattering.

Microcapsule formation and encapsulation of cells: PX was dissolved in HEPES buffer solution supplemented with $CaCl_2$. A murine chondrocytic cell line (ATDC5) was encapsulated into PX matrix using a novel micro-droplet generator[2]. The microcapsules were formed in physiological buffer solution (PBS) and subsequently coated with poly-l-lysine before transferring to culture medium. Encapsulated cells were maintained *in vitro* culture for a period of 3 weeks and the 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 and encapsulated cells was analyzed by scanning electron microscopy and light microscopy.

RESULTS AND DISCUSSION

From the various synthesized PX conjugates, the conjugate obtained with 1.7 xanthan/palmitoyl molar ratio was shown to be water-soluble and self-assemble in PBS. The PX can self-assemble into stable capsular hollow structures as a result of the dominating hydrophobic forces between alkyl chains that organize the molecules in multilayered lamellar structures. Those structures can further aggregate to form a shell as a result of polar-nonpolar repulsion between assembled alky chains and hydrophilic polysaccharide backbone (Fig. 1A). Poly-l-lysine coated microcapsules with an average diameter of 450 µm and homogenous size distribution were obtained (Fig. 1B). Fig. 1C shows that the encapsulated ATDC5 cells were able to reduce AlamarBlue® during 21 days of *in vitro* culture with slightly increasing rate as result of the increased cellular viability. In addition, the DNA quantification over time revealed a gradual increase of DNA as a result of improved cellular proliferation (Fig. 1D).

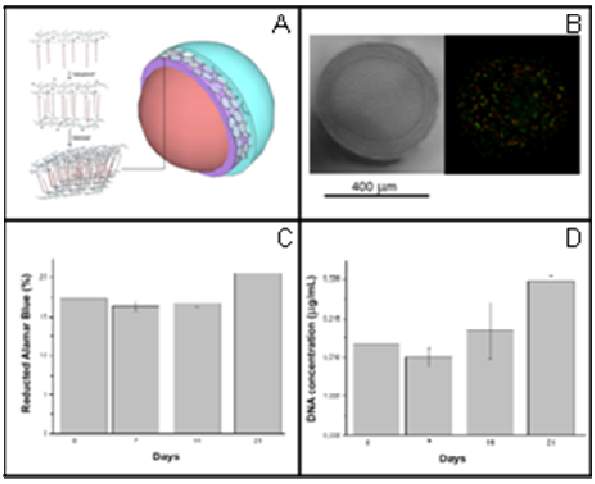

Fig. 1 Self-assembly mechanism for microcapsule formation (A). Optical and fluorescence microscopy images showing PX microcapsule morphology and live cells encapsulated into the PX matrix (B). Metabolic activity (C) and proliferation (D) of cells encapsulated into PX microcapsules over 21 days in culture.

CONCLUSIONS

Palmitoyl xanthan appears to be a promising alternative to the conventional biomaterials employed in cell encapsulation due the simplicity of conjugation. 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. Given these encouraging preliminary results, we are undertaking encapsulation experiments with human articular chondrocytes to be applied as cell-based therapies in cartilage tissue engineering approaches.

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