# Encapsulation and Survival of a Chondrocytic Cell Line within a Polysaccharide Gum

Ana C. Mendes, Erkan T. Baran, Rui C. Pereira, Helena S. Azevedo, Rui L. Reis.

3B's Research Group - Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal; e-mail: ana.mendes@dep.uminho.pt
IBB – Institute for Biotechnology and Bioengineering, PT Government Associated Laboratory, Guimarães, Portugal

**Statement of Purpose:** A promising approach for cell delivery is the use of biocompatible materials that can form a gelled matrix and be injected into the body. There is a renewed interest in using biopolymers to obtain hydrogels as they usually undergo gelation under gentle conditions. Although alginate/poly-lysine polyelectrolyte complexes have been widely investigated for cell encapsulation, their mechanical properties and long-term stability are poor. Xanthan gum, a bacterial extracellular polysaccharide, has shown to be biocompatible, biodegradable and, at sufficiently high polymer concentration, it exhibits weak gel-like properties [1,2]. The aim of this work was to investigate the potential of xanthan as a new artificial matrix for the encapsulation of chondrocytic cell line and compare with other materials used for cell encapsulation. Towards this goal, we have developed a system to produce xanthan microcapsules and evaluated their ability to sustain the viability, function, and proliferation of encapsulated cells.

**Methods** Commercially available xanthan gum (from *Xanthomonas campestris)* was carboxymethylated following the procedure described by Liu et al [3]. The chemical structure of the CM-Xanthan (CMX) was characterized by FTIR, NMR and acid-base titration. To investigate the potential of CMX as matrix for cell encapsulation, CMX was dissolved (5 wt%) in Dulbecco's Modified Eagle Medium (DMEM) containing a cell suspension ($10x10^6$ cells/mL, ATDC5 cell line). The CMX capsules were formed by ionic crosslinking using an apparatus developed in our group. The system consists of one peristaltic pump which transports the polymer beads within a silicon tubing, generated by a syringe pump, in a mineral oil flow to the crosslinker solution (1.5% $CaCl_2$ and 0.9% NaCl). The CMX capsules remained in the crosslinking solution for 15 min under gentle stirring and then washed with PBS in order to remove traces of mineral oil. The collected capsules were coated with poly-L-lysine (PL, 0.1%) for 10 min and further transferred to DMEM culture medium. For comparison, alginate capsules were also produced following the procedure previously described for CMX capsules. Encapsulated cells were maintained *in vitro* culture for a period of 3 weeks. The viability of encapsulated cells over time was assessed using the live/dead and Alamar Blue assays, whereas proliferation was determined by DNA quantification. Light microscopy and histology were also used to monitor the morphology and examine the behavior of cells encapsulated in the over the time.

**Results:** Although xanthan exhibits weak gel-like properties, this polysaccharide is a nongelling gum. We have, therefore, performed carboxymethylation to introduce additional carboxylic groups into the xanthan structure and studied the formation and performance of xanthan capsules in ionic solutions. The produced microcapsules present a spherical shape, uniform size and diameter of about 500 µm (Fig. 1-a,c). Fig1-a illustrate fluorescence microscopy images showing encapsulation and survival of cells in the microcapsules (live cells shows the intensely fluorescent green calcein while dead cells should absorve propidium iodide and consequently produce a bright red fluorescence). The cells encapsulated in the CMX microcapsules remained viable over 21 days of *in vitro* culture and showed higher viability than cells within the alginate capsules (Fig. 1-b). Optical micrographs in Fig. 1-c show the behaviour of ATDC5 cells after 1 and 21 days of culture within CMX microcapsules, where a homogenous cell distribution can be observed. At day 21, the cells tend to form cellular aggregates demonstrating a chondrogenic phenotypic behaviour

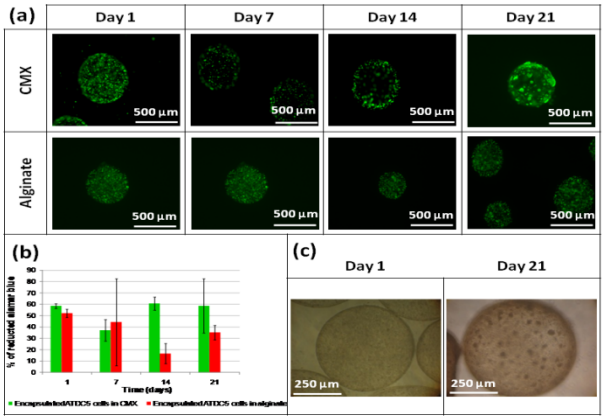


**Figure 1.** (a) Live/dead assay of ATDC5 cells cultured within CMX and alginate microcapsules. (b) Viability of encapsulated cells as function of time in culture using the Alamar Blue assay. (c) Optical microscopy images showing the morphology of ATDC5 cells encapsulated in CMX microcapsules.

**Conclusions:** CMX microcapsules appear to enhance the survival and normal morphology of ATDC5 cells. These results confirm the ability of using this material for cell encapsulation without interfering with cellular normal behavior. 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.

**References:** [1] Hamcerencu, M., Polymer, 2007, 48(7), 1921-1929. [2] Liu XF, Journal of Applied Polymer Science, 2001, 79, 1324-1335. [3] García-Ochoa, F, Biotechnology Advances, 2000, 18 (7), 549-579.

**Acknowledgements.** ACM acknowledges to FCT who supported this work though funding from SFRH/BD/42161/2007