"A HYBRID NATURAL-SYNTHETIC CHITOSAN-BASED HYDROGEL AS SCAFFOLD FOR MAMMALIAN CELL CULTURE FOR TISSUE ENGINEERING APPLICATIONS"

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INTRODUCTION. The polysaccharide, Chitosan (CTS) is structurally analogous to glycosaminoglycans (GAG) such that it exhibits numerous interesting biological, physical and chemical properties that mimic the extracellular matrix (ECM). CTS is an attractive biopolymer that can readily be modified to produce novel materials for Tissue Engineering (TE) applications. Biomaterials used as scaffold for cell culture are typically biodegradable and they have similar mechanical and structural properties to those of the ECM in different tissues.

The **objective** of this work is twofold: 1) to test *In-vitro* two novel hydrogels: (Chitin-*g*-Glycidyl Methacrylate)-Xanthan (CTS-g-GMA)-X and (Chitin-*g*-Glycidyl Methacrylate)-Xanthan crosslinked with glutaraldehyde (CTS-g-GMA)-X-GL as scaffold for mammalian cells, and 2) to analyze their morphology and viability to assess the use of these biomaterials as potential scaffolds for tissue engineering applications.

METHODOLOGY. Biomaterial synthesis: A preparation method is followed according to [1,2]. **Cells culture:** A hydrogel film is placed in a 24-well polystyrene plate and its pH is adjusted at 7.2. Cells from Enteric Nerve System (**ENS**) [3] and neuronal cells from mice brain (**NEU**) [4] were seeded onto the polymer at 0.9 X 10^4 cells mL^{-1}. Skin from mice (**SK**) [5] and Chondrocytes from human cartilage (**CT**) were seeded at 2 X 10^6 cells mL^{-1}. Standard culture media is used for all cultures in a humidified atmosphere at 37°C, H_r=70% with 5% CO_2 for 24 h. **Cell viability:** A Calcein–AM and Eth–D1 in LIVE/DEAD® Viability/Cytotoxicity Kit is used according to the manufacturer's specifications and analyzed by confocal microscopy LSM510–META. Finally all cell cultures, in 24-well polystyrene plate, are treated with 4% paraformaldehyde for 32 h and imaged by Confocal and Environmental Scanning Electron Microscopy (ESEM) using an XL-30-ESEM at 20 kV. A control experiment is run under the same above conditions except no biopolymer is used.

RESULTS AND DISCUSSION. (CTS-g-GMA)-X hydrogels prove useful in maintaining cell viability after 24 h of culture for ESN and NEU by Viability and Cytotoxicity assays, as shown in Figures 1b and 1c, respectively. It is known that a crosslinked material greatly reduces its ability to swell in aqueous media. This is the case for (CTS-g-GMA-)-X-GL which is more rigid than its non-crosslinked analog. Figures 1c and 1f show that (CTS-g-GMA-)-X-GL is not an appropriate scaffold at ECM conditions for ESN and NEU cell cultures. On the other hand, a lower swelling capacity that exhibits the crosslinked (CTS-g-GMA)-X-GL biomaterial provides a good scaffold for both CT and SK cells as shown in Figures 1i and 1l, respectively. These cells maintain their viability and morphology; they also showed cellular adhesion to the biopolymer (see Figure 2). Figures 1h and 1k show that the non-crosslinked biomaterial, (CTS-g –GMA)-X, maintain cell viability but not their typical morphology. It is observed that ESN seeded onto (CTS-g-GMA)-X may help to induce differentiation to neural cells as shown in Figure 1b. It still remains to be studied if these materials maintain *In vitro* cell phenotype for longer culture times or if they go through a differentiation or de-differentiation process.

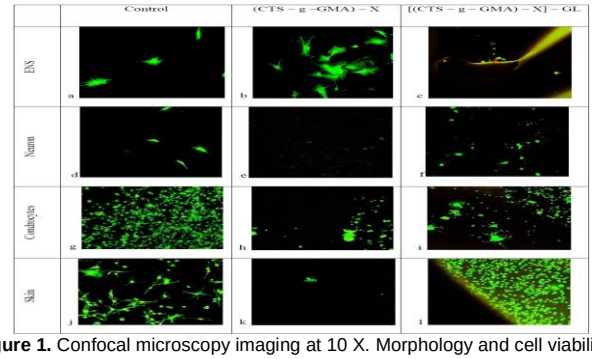

Figure 1. Confocal microscopy imaging at 10 X. Morphology and cell viability after 24 hours of culture onto each polymer. Control in polystyrene six-well plate (a, d, g and j). Cell cultures onto (CTS-g-GMA)-X (b, e, h and k), and [(CTS-g-GMA)-X]-GL (c, f, i and l). Living cells are stained with Calcein (green).

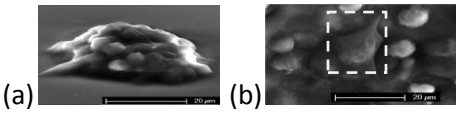

Figure 2. ESEM imaging of crosslinked [(CTS-g-GMA)-X]-GL seeded with two different cell types. (a) images of chondrocytes isolated from human cartilage, CT (1500 X).Typical rounded morphology and adhesion to the biopolymer is maintained; (b) Polymer seeded and cultured for 24 hrs with mice skin cells (SK) at 1200 X. Cell adhesion and cytoplasm can be observed (see center of image).

CONCLUSION. Non-crosslinked biomaterials have proven suitable for In vitro ENS cultures. The crosslinked one provides a good scaffold for in vitro SK and CT cultures. This study shows that chitosan-based biomaterials can be chemically fine-tuned to mimic ECM for cell growth and proliferation. The next step is to conduct verification of some specific cellular markers to demonstrate cell phenotype and not only cell morphology and viability.

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