TISSUE DENSITY CULTURE IN GAG-BASED MICROCAPSULES AS A FOUNDATION FOR MODULAR TISSUE ENGINEERING

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Statement of Purpose: A primary Tissue Engineering objective is to create cell-based, tissue replacements, fabricated as a three-dimensional constructs. Such constructs promote cell-cell interactions, extracellular matrix (ECM) deposition and tissue level organization. Accomplishing these prerequisites with the biomaterials and the scaffold fabrication methods currently available remains a challenge. Modular tissue engineering is a scalable strategy of assembling tissue systems from microscale constructs containing parenchymal and vascular components. We have developed a method for fabricating 3D scaffolds from glycosaminoglycan (GAG)-based microcapsules. These GAG-based microcapsules can be internally and externally seeded with desired cell types and can be subsequently assembled to form 3D tissue constructs. In this study, we investigated the proliferation and organization of cells cultured in GAG-based microcapsules. The work sought to identify material formulations capable of providing a suitable culture microenvironment with the long term goal of engineering functional tissues using these modular scaffolds.

Methods: HTR-8/SVneo trophoblast (HTB) cells were encapsulated in microcapsules produced by complex coacervation between chitosan and GAGs. The cells were suspended in GAG solution (see below) and droplets of the solution were dispensed into a stirred chitosan solution (0.6% high MW chitosan and 3.8% sorbitol in 1% acetic acid) through a 24 gauge catheter. An insoluble ionic complex was formed at the droplet surface by the interaction of oppositely charged chitosan and GAGs. After washing and surface stabilization steps, microcapsules were maintained in DMEM/F12 culture medium (Sigma) supplemented with 10% FBS and antibiotics. The GAG formulations studied were: (a) 4% chondroitin 4-sulphate (CSA) with 1.5%. carboxymethylcellulose (CMC); (b) 4% CSA, 1.5 wt% CMC and 0.2% collagen type I; (c) 1% hyaluronic acid (HA) and 1% CMC; (d) 1% HA and 1% CSA. In separate cultures, HTBs were seeded on the outer surface of collagen coated, CSA capsules. Cell growth, viability, invasiveness and morphology were monitored using phase contrast and fluorescence microscopy, and histological analysis. ECM deposition by the cells was studied by immunohistochemistry.

Results/Discussion: HTBs grew rapidly and formed spheroids after day 3 in all test formulations. Cells continued to grow in all formulations and filled the capsules in all cases by day 30. However the growth pattern of HTBs and the integrity of capsules differed between formulations. Compact spherical aggregates were seen in CSA/CMC capsules (Fig. 1A) while the aggregates were irregular and significantly dispersed when an internal collagen matrix was included (Fig. 1B). Proliferation and cell spreading were also seen in cells seeded on the outer collagen coated surface of the CSA/CMC capsules (Fig. 2). Capsule wall thickness increased, and the integrity of the capsule was compromised in the HA/CSA formulation (Fig. 1C) due to HTB invasion into the capsule wall. HA/CMC formed very thin walled capsules, many of which collapsed after a week of culture (Fig. 1D). Notably, the CSA/CMC microcapsules were more intact and the HTBs were found to be less invasive in this formulation.

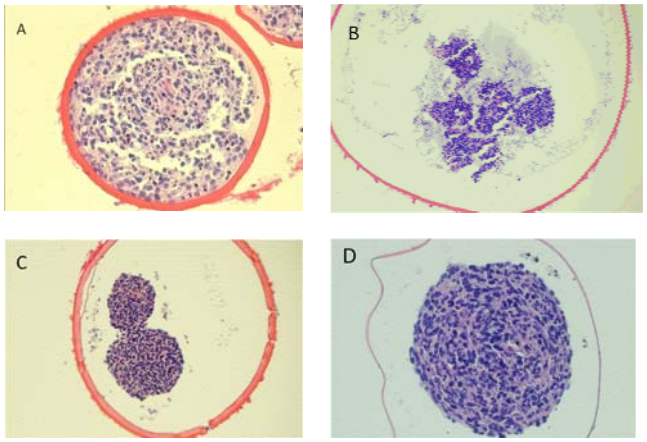

Figure 1: HTBs in GAG based Microcapsules

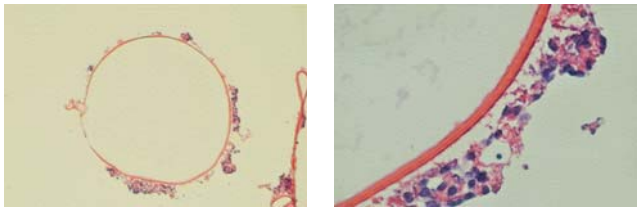

Figure 2: HTBs seeded outside microcapsules

Conclusions: These results demonstrate that GAG-based microcapsules provide a microenvironment suitable for developing and maintaining tissue density cultures for up to 30 days in vitro. This finding establishes a technology foundation for subsequent rapid assembly of three-dimensional, tissue density constructs. When coupled with growth of endothelial cells on the external capsule surfaces, these scalable systems are a promising platform for modular tissue engineering of several organ systems.