Rapid Assembly of Perfusable and Vascularizable Modular Constructs for Hepatic Tissue Engineering

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Statement of Purpose: The limited ability to vascularize and perfuse thick, cell-laden tissue constructs has hindered efforts to engineer complex tissues and organs, especially liver. To reproduce the functionality of a liver, there is a need to engineer tissue constructs that mimic the innate architecture and complexity with perfusable channels. The field of modular tissue engineering aims to address this limitation by fabricating constructs from the bottom up, with the objective of recreating native tissue architecture and promoting extensive vascularization. Using this strategy, we have developed methods for fabricating modular tissue prototypes by assembling ECM based, microcapsule modules. The interior environments of these modules can be tuned with ECM gels or microcarriers. Cells can be seeded internally and externally, and the modules subsequently assembled into 3D tissue constructs possessing parenchymal and vascular components. The modular constructs so assembled are permeated by a network of interconnected, endothelial cell-lined channels that allow in vitro perfusion, and may facilitate rapid and extensive vascularization *in vivo*. The long term goal of this work is to engineer functional, transplantable liver tissue from isolated hepatocytes. In this study we report the assembly and metablic performance characterization of 3D liver constructs with perfusable, endothelialized channels.

Methods: Primary rat hepatocytes were encapsulated in collagen-augmented microcapsules produced by complex coacervation between chondroitin 4-sulfate (CSA) and carboxymethyl cellulose (CMC) with chitosan. Briefly, cells were suspended in a polyanion solution (4 wt% CSA, 1.5 wt% CMC, 0.1 wt% type I collagen). Droplets of this solution were generated and collected in stirred chitosan solution (0.6 wt% high MW chitosan in 1% acetic acid). Ionic interactions between oppositely charged polymers generated the encapsulating membrane. Encapsulated hepatocytes ($20x10^6$ cells/mL of CSA/CMC) were maintained in cylindrical perfusion chambers under both packed bed and fluidized bed conditions. For fluidized perfusion, capsules were fluidized by continuous upward flow of medium. For packed bed cultures, the capsules were first fused together using a sequential washes with diluted versions of the chitosan and CSA/CMC solutions described above, and then subjected to downward flow of the medium in a continuous circulation flow circuit. The flow rates were adjusted to maintain physiological pressure differences (<100 mmHg) across the chamber (4-5 mL per minute). The perfusion systems were maintained at 37°C for 1-2 weeks and medium was changed every 2-3 days. Medium samples were analyzed for urea and albumin synthesis by the hepatocytes. To generate endothelialized channels in fused constructs, the external surfaces of capsules were coated with collagen-I (0.2 mg/mL) and seeded with HUVECs prior to capsule fusion with the diluted polymer washes.

Results/Discussion: HUVECs seeded on the collagen coated CSA/CMC capsules attached well and formed a viable monolayer within 24 hours (Fig. 1, A-C). SEM images 48 hours after seeding showed a well spread and smooth endothelial monolayer (Fig. 1D).

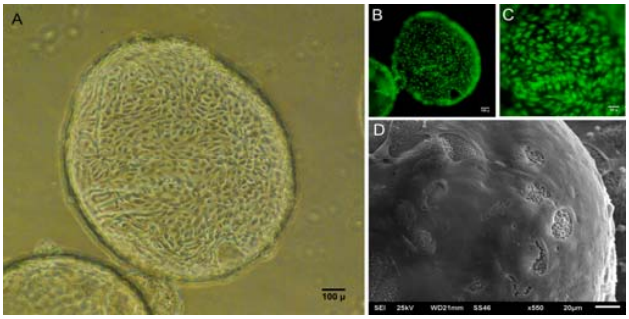

Fig. 1: Phase, fluorescence and SEM images of HUVECs on collagen coated CSA/CMC/Chitosan capsules.

The urea and albumin synthesis rates of the encapsulated hepatocytes maintained in perfusion culture conditions (Fig. 2, C-D) were compared to rates of identical cells in standard collagen sandwich dish cultures (Fig. 2A). Perfusion cultures maintained the functionality of encapsulated hepatocytes and healthy spheroids were seen in most capsules (Fig. 2B). Albumin and urea synthesis rates in both types of perfusion cultures (Fig. 2, G-H) approached those of the collagen sandwich cultures (Fig. 2, E-F).

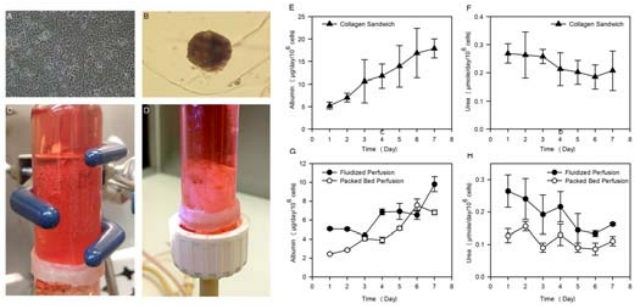

Fig. 2: Control (A, E, F) and encapsulated fluidized and packed bed (B-D, G, H) hepatocyte cultures.

Conclusions: These results demonstrate that GAG-based microcapsules can be fused to form 3D liver organoids with perfusable, interconnected channels that can also be endothelialized to facilitate neovascularization in-vivo. This finding establishes a technology foundation for subsequent rapid assembly of three-dimensional, tissue density liver 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.

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