The Development of Collagen Hydrogel Infused-Polymeric Scaffolds for Creating Vascularised Tissues *In Vitro*

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Statement of Purpose: The ability to create thick tissues is a major tissue engineering challenge, requiring the development of a suitable vascular supply. The current research aims to create *in vitro* vascularised tissues that can be readily connected to the host's vasculature following implantation. This paper presents the development of a methodology that combines novel polymer scaffold architectures that help to increase mass transfer for increased tissue mass viability and collagen hydrogel cell delivery to enhance cell growth and proliferation in scaffolds [1].

Methods: Biodegradable 5% PLGA(75:25) polymer scaffolds with various internal micro- and macro-architectures were manufactured using two techniques: (1) a controlled chemical processing technique that created highly structured porous scaffold geometries with macro-lumens, and (2) a polymer and porogen casting and porogen leaching method, incorporating porogen-fusion and macro-lumenation of the scaffold. Bone marrow stromal cells, human dermal fibroblasts, and NIH3T3 fibroblasts, all co-cultured with human microvascular cells, were seeded into the scaffolds using static, centrifugal, a combination of static and centrifugal cell seeding (CCS), as well as using collagen gel cell delivery to seed the cells into the scaffolds [2]. The hybrid constructs were cultured for one month statically and dynamically in roller bottles, and the resulting constructs were structurally analyzed using SEM and cryohistology methods for parameters such as scaffold architecture, cell distributions, and any unique tissue formations within the construct [3].

Results / Discussion: 8mm diameter biodegradable 5% PLGA(75:25) scaffolds displaying isotropic pore/scaffold geometries were manufactured. These scaffolds were created with ten 200µm lumens in each scaffold. As shown in Figure 1, under SEM observation these scaffolds were composed of rod-like & thin plate structures. A second set of 8mm diameter biodegradable 5% PLGA(75:25) scaffolds were manufactured using a porogen fusion technique and porogen leaching. The pore sizes created were between 53 and 106µm, and also between 106µm and 215µm. These scaffolds were created with five 200µm lumens. As shown in Figure 2, under SEM observation, these scaffolds showed randomly-oriented platelet-like scaffold wall structures.

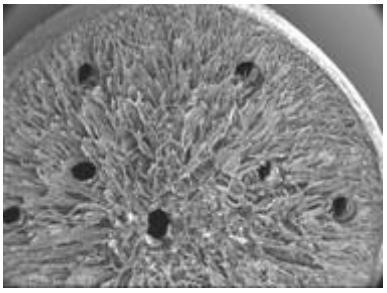

Figure 1: SEM of 5% PLGA(75:25) scaffold manufactured with ten 200 µm macro-lumens and isotropic pore structure.

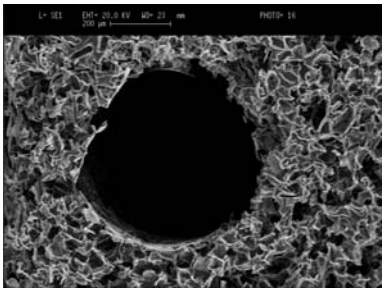

Figure 2: SEM of a macro-lumen and the surrounding 5% PLGA(75:25) scaffold with 53-105 µ m diameter pores created by porogen leaching.

A combined CCS and static cell seeding method was used to seed cells into porous scaffolds. This method showed efficiencies averaging 80% (StDev = 9) cell seeding efficiency compared to the lower efficiencies of the static or CCS methods alone. Use of collagen hydrogel for cell infusion into the scaffolds displayed a high seeding efficiency equivalent or higher than the combined CCS and static cell seeding method.

Dynamically cultured scaffolds showed more cell viability in the interior of the scaffold compared to statically cultured scaffolds. Cell layers formed on the surface of the lumen of the scaffolds. Figure 3 shows a histological image of a collagen hydrogel-infused porous scaffold cultured with human microvascular cells after culturing for 1 month.

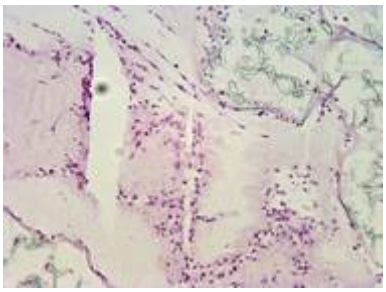

Figure 3: H&E stained collagen hydrogel-infused porous scaffold containing human microvascular cells after 1 month static culture.

Conclusions: The combination of CCS and static cell seeding gave high cell seeding efficiencies for both scaffold designs. It was seen that the use of collagen hydrogel cell delivery and dynamic culture gave good cell viability and distribution. Future studies will develop and analyze the use various combinations of culturing conditions with different cell/hydrogel/scaffold designs.

References:

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