Novel Biological Scaffolds for 3-D Stem Cell Culture

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Statement of Purpose: The present study introduces novel 3-D porous and biological scaffolds made of fibrous porcine acellular dermal matrix for 3-D stem cell culture. The highly porous scaffolds provided a 3-D structure for maximizing cell penetration. The mechanical strength can be increased through cross-linking of acellular dermal fibers in the scaffolds. The objective of this study was to characterize the scaffolds and evaluate them for 3-D stem cell culture *in vitro*. The bone marrow stem cells were cultured on the scaffold for up to 4 weeks.

Methods: Scaffold fabrication and characterization Acellular porcine dermal fibers were used to form 3-D porous scaffolds. The porosity of the composite material was controlled by a crystallization process, which resulted in the porosity being greater than 95%. The scaffolds were then cross-linked with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) [1]. The scaffolds of 3mm thick were cut into 5mm (W) x 5mm (L) pieces and sterilized using e-beam irradiation. The surface morphology, tensile strength and thermal stability were evaluated using scanning electron microscopy (SEM JCM-500, JEOL Ltd, Tokyo, Japan), Instron Testing System 5865 (Instron Corp, Canton, MA) and differential scanning calorimetry (DSC Q2000, TA Instruments, New Castle, DE).

Cell culture Rat bone marrow mesenchymal stem cells were isolated and maintained in mesenchymal stem cell expansion medium (Millipore, Billerica, MA) with antibiotics (100U/ml of Pennicillin-G and 100µg/ml of Streptomycin) at 37^{o}C in a humidified atmosphere of 5% CO_2 in air. The scaffolds were hydrated in full culture media, and then placed in wells of a 12-well plate. $5x10^4$ cells were inoculated onto each scaffold and incubated on the scaffold for one hour in a 37^{o}C incubator to allow cells attach to the scaffold before 2mL of culture media was added to each well that contained a scaffold. Medium was replaced three times a week. Cell-scaffold constructs were fixed in 10% formalin, embedded and sectioned, and then stained with H&E. The stained sections were examined under light microscopy.

Results: The scaffolds prior to the cross-linking were found to be irrigation resistant, and maintained their integrity after being soaked 3 days in saline on a shaker. The scaffolds floated in the middle of the cell culture indicating that it has a lower density compared to the culture medium. It was observed that a dry scaffold could absorb liquid of the same volume of the scaffold. After hydration, no significant swelling was evident. The SEM analysis showed that the acellular dermal fibers formed 3-D architecture that exhibited high porosity and pores of about 100µm (Fig.1). The mechanical strength and elasticity were significantly enhanced after cross-linking with EDC (Table 1). The DSC analysis revealed that the scaffold's protein denaturation onset temperature increased from 49.0 (±0.9) ^{o}C to 72.8 (±0.2) ^{o}C after EDC cross-linking (Table 1).

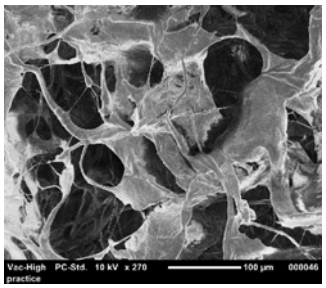

Figure 1 SEM micrograph of 3-D porous scaffold. Bar=100µm.

Table 1 Tensile Testing of Scaffolds

Treatment	Max Stress (MPa)	Elasticity (N/cm)
Non-crosslinked	0.09 ± 0.03	8.2 ± 1.5
Crosslinked	0.20 ± 0.03	26.2 ± 11.6

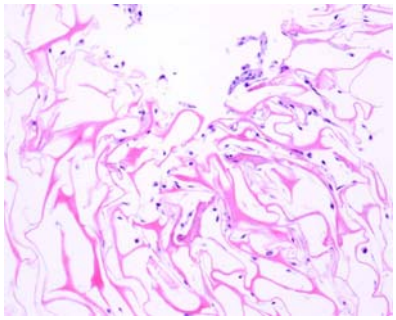

Figure 2. H&E stain of cross-section of cell-scaffold construct from 2-week culture.

The histological evaluation showed that the stem cells attached and proliferated on the scaffolds during the 4-week cell culture. On day one, cells were observed on the surface and inside the scaffold. After 2 weeks, the cells were present throughout the porous scaffolds (Fig.2). Multiple layers of cells were evident on the scaffolds from a 3-week culture.

Conclusions: We have developed a novel scaffold made of fibrous porcine dermal matrix that permitted rat stem cells to attach and proliferate. The scaffold has high porosity and has pore sizes that allow cell infiltration throughout the scaffold in 3-D stem cell culture. The thermal stability and mechanical strength of the scaffolds can be significantly modified using a cross-linking method with EDC. The preliminary rat stem cell culture studies showed that the novel scaffolds are promising substrates for 3-D stem cell culture and tissue engineering.

References: [1] Lee JM et al. J Mater Sci Mater Med. 1996; 7: 531-541.