Effect of scaffold morphology on the proliferation and colonization of seeded chondrocytes

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Statement of Purpose: The morphology and chemical composition of solid supports play an important role in the development of tissue constructs with the physical properties of the scaffolds influencing the ability of cultured cells to remain viable, proliferate and colonize. To achieve uniform cell colonization and cellular in-growth that is required from many tissue engineered equivalents, penetration into the thickness of the scaffold over the culture duration is desired. Larger pore sizes are also desired to overcome diffusional limitations in the mass transfer of nutrients. However in many tissue engineering applications that typically seed scaffolds with cells, the initial step, and culture, a higher cell density is often noted close to the scaffold face that was contacted with the cell suspension. Cell colonization of the scaffold often depends upon the pore size and pore connectivity and typically about 20% to 30% of the scaffold spatial volume is colonized at the end of 7 to 14 days in culture. The objective of our research is to: (1) evaluate cellular colonization in polymeric scaffolds with differing pore sizes and pore architecture as a function of initial seeding density and scaffold thickness and (2) evaluate the use of low intensity diffuse ultrasound (US or LIDUS, 0.25 mW/cm^2) to enhance cell migration and cellular colonization.

Methods: Scaffolds (CS) were prepared from a 1% solution of chitosan in 2% acetic acid via freeze-drying and lyophilization. Biomerix Biomaterial™ (PU) was provided as a generous gift by BIOMERIC INC. (Palo Alto, CA) and used without making any further modifications. Bovine chondrocytes that were isolated from shoulder joints of 6-month old calves using procedures detailed elsewhere[1] were seeded on indicated scaffolds at a seeding density of 5 x 10^5 cells/cm2 and 2x10^4 cells/cm^2, respectively and maintained in culture for 7 days. Scaffolds with a nominal diameter of 5-mm and thickness of 2.5-mm and 5.0-mm were used. Non stimulated and cell-seeded scaffolds served as controls. At the end of the culture period, viabilities were assessed by Live-Dead™ assay kit, cell structure was visualized by staining the actin cytoskeleton with phalloidin and nuclei with Sytox™. Cell numbers were estimated by staining whole mounts with Sytox™ and further visualization under a confocal microscope and the numbers of nuclei were counted in each 10 μ *z* section using ImageJ 1.43u NIH software. Measurements were taken from 3 independent sections of area from 3 scaffolds.

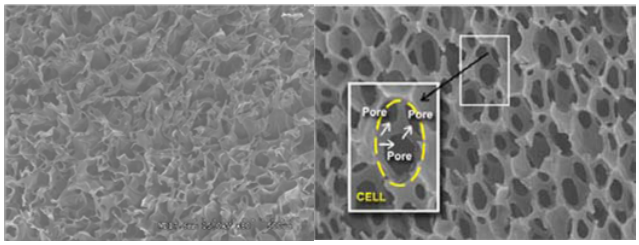

Figure 1: SEM of scaffolds used in this study. (A) CS and (B) PU.

Results

SEM morphologies of the scaffolds are shown in Figure 1 and pore sizes of 100 μm and 150 μm were estimated by mercury intrusion porosimetry for CS and PU, respectively. Relative high cellular viabilities were obtained. The number of cells as a function of depth into the scaffold is also shown in Figure 2/3. In the absence of any stimulation (Figure-2), it was observed that cells were able to penetrate up to 80 μm and 50 μm in depth when seeded on PU and CS, respectively. When subjected to LIDUS, increased penetration of CS was noted (Figure-3) at the end of 7-days in culture, and similar trends were also noted for PU scaffolds.

Conclusions

The open architecture of the PU scaffolds, which resembles a honeycomb structure with open and interconnected pores, perhaps contributed to the presence of cells at higher depths into the scaffolds. More interestingly, when stimulated by US, more cells were noted to colonize deeper into the scaffolds, when compared to non-stimulated controls. This observation was also supported by SEM microscopy, where scaffolds were dissected (in the middle) and the cut face was observed. Cells were only noted on specimens obtained from LIDUS stimulated groups

References

1. Noriega, S., et al., *Intermittent applications of continuous ultrasound on the viability, proliferation, morphology, and matrix production of chondrocytes in 3D matrices.* Tissue Eng, 2007. **13**(3): p. 611-8.

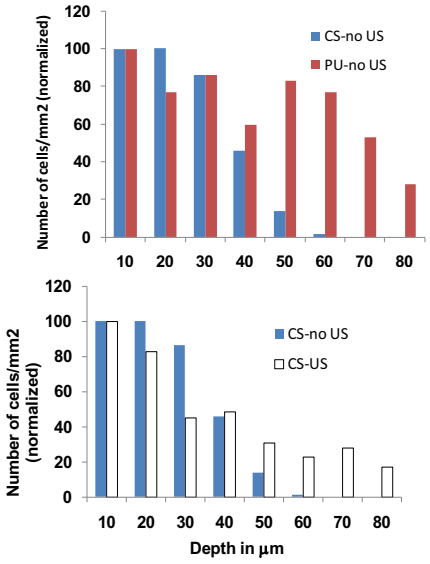

Figure 2/3: Number of cells/mm2 as function of depth into the scaffold. [2]: CS and PU and [3]: cells on CS with and without LIDUS