COLOCALIZATION OF CELL ADHESION PROTEINS IN 3D TISSUE ENGINEERING SCAFFOLDS FABRICATED BY RAPID PROTOTYPING

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Statement of Purpose: Many groups have found differences in the pattern of cell spreading and expression of cell adhesion proteins on two-dimensional (2D) substrates such as glass coverslips and polymer films. However, only a few groups have explored cell adhesion and spreading in three-dimensional (3D) scaffolds, mainly those derived from biopolymers and tissues[1,2]. Analysis of cell adhesion on 3D scaffolds made from synthetic polymers such as polycaprolactone (PCL) appears to be limited. Understanding the process of cell adhesion in 3D polymer scaffolds leads to a better understanding of cell migration, proliferation, and differentiation within these scaffolds. PCL 3D rapid prototyped (RP) scaffolds and PCL 2D melt-pressed films, as well as glass coverslips for controls, were seeded with osteoblast-like cells and qualitatively analyzed for colocalization of vinculin, a focal adhesion marker, and actin, a cytoskeletal protein.

Methods: Two sets of RP scaffolds were fabricated. The first set was fabricated with a custom-designed fused deposition modeling (FDM) system at North Dakota State University (NDSU). The second set of scaffolds was fabricated using precision extruding deposition (PED), a variation of FDM, at Drexel University. Within the Drexel scaffolds, the struts within each layer were oriented 90° relative to the struts of the layer immediately below (unshifted, Fig. 1A). The NDSU scaffolds had the same architecture as the Drexel scaffolds, except the struts of each layer immediately below were shifted by one-half the pore width, so that the struts can be seen through the pores of the layer immediately above (shifted, Fig. 1B). On average, pores were 300 µm wide, struts were 200 µm wide, and porosity was between 55 % and 60 %. Scaffolds were sterilized in 70 % by volume ethanol, centrifuged in sterile phosphate-buffered saline (PBS), and pre-conditioned with culture media. Unshifted PCL scaffolds, shifted PCL scaffolds, PCL films, and glass coverslips (each n = 3) were seeded with MC3T3-E1 osteoblast-like cells. After static culture for 24 h at 37 °C, cells were fixed and fluorescently double stained for vinculin and actin. Samples were viewed with a confocal laser scanning microscope. Images of cells on each 2D and 3D substrate were captured and qualitatively analyzed for cell spreading and degree of colocalization. For 3D scaffolds, z-stack images were compressed to make one projected image.

Results / Discussion: Cells on glass coverslips were well-spread with a strong yellow signal at the ends of the actin fibers, where they meet the focal adhesions, indicating a large amount of colocalization (Fig. 2A). On the PCL films, cells were elongated and some colocalization was evident, mostly within the cell body (Fig. 2B) and sometimes at the ends of the stress fibers (not pictured). On the unshifted (Fig. 2C) and shifted (Fig. 2D) scaffolds, cells appeared spread and either curved around the polymer struts or very elongated. However, the actin and vinculin stain are rarely colocalized except in isolated cases. No qualitative differences between the two scaffold types were observed. As evident in the images, cells on all substrates contained a large amount of cytoplasmic vinculin, but this did not always translate to focal adhesions. Large focal contacts are normally colocalized with actin stress fibers, while immature focal complexes are not associated with the actin cytoskeleton[3]. Since colocalization areas are signal translation sites for the cells to migrate along surfaces, our results suggest that cells adhere, as well as migrate, well on glass, somewhat well on PCL films, and not as well on the 3D scaffolds. However, successful cell proliferation and differentiation has been demonstrated in other RP scaffolds[4,5]. This suggests a possible delay in focal adhesion development on 3D scaffolds, or that cell adhesion may work in a different fashion in 3D polymeric scaffolds, as has been shown in 3D biopolymer and tissue-derived scaffolds[1]. Future work involves quantitative measurement of colocalization.

References: 1. Cukierman et al., Science, 2001, **294**:1708-12. 2. Cukierman et al., Curr Opin Cell Biol, 2002, **14**:633-9. 3. Richards, Eur Cell Mater, 2003, **6(S 2)**:19. 4. Hutmacher et al., J Biomed Mater Res, 2001, **55**:203. 5. Rai et al., Biomaterials, 2004, **25**:5499.

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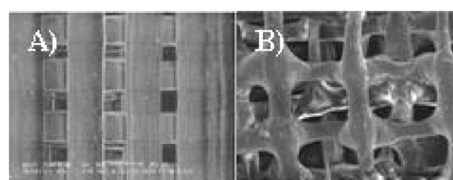

Figure 1

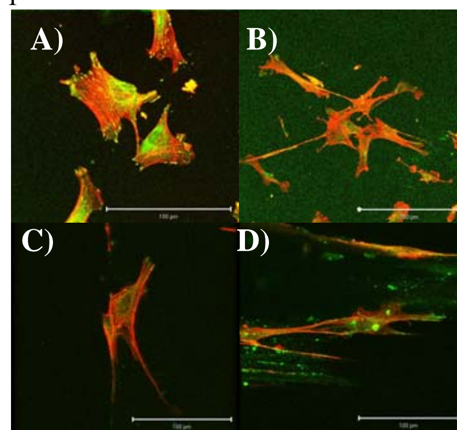

Figure 2