Surface Modification of Porous Scaffolds with Cell Adhesive Peptides for Tissue Engineering Applications

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Introduction: Bioactive fluorinated surface modifiers (BFSMs) have been developed to modify polymer surfaces with peptides for controlling cell function, and their use in flat film surfaces has been validated[1]. However, their surface affinity properties have not yet been studied for use in 3D scaffolds. The objective of this study was to assess the feasibility of using BFSMs in polyurethane scaffold materials by investigating their distribution throughout the scaffold, and evaluating the maintenance of smooth muscle cells (SMCs)[2] in an RGD (Arg-Gly-Asp)-BFSM modified polyurethane scaffold.

Methods:

Scaffold preparation: Tecoflex, a commercial biomedical polyurethane, was used as a model material. The polymer was dissolved in DMAC, together with BFSMs (BFSMs were prepared as previously reported[1]). 80 wt% $NaHCO_3$ particles (90% between 105-420 µm) were blended into the polymer mixture, cured by solvent drying at 55°C, followed by 40°C vacuum drying. The resulting 5 x 5 x 2 mm^3 scaffolds were immersed in distilled water to extract the salt.

Scaffold characterization: The pore morphology of the scaffolds was observed by scanning electron microscopy (SEM). The distribution of BFSMs in the scaffold was measured by 2-photon confocal microscopy. Dansyl labeled lysine-BFSMs were used for imaging purposes.

Cell culture: A-10 rat aortic smooth muscle cell line was seeded on both non-modified and RGD-BFSM modified Tecoflex scaffolds in 96-well plates, at a concentration of 20,000 cells per scaffold. The culture was maintained for 4 weeks, with medium changed every 2-3 days. At different times post seeding, scaffolds were stained with live/dead viability stain for 2-photon microscopy, and imaged by SEM.

Results / Discussion:

Pore morphology: Figure 1a shows that the interior of the scaffold is highly porous. The pores are well interconnected, and have an average size of 100-150 µm, which should result in the successful infiltration of SMCs.

BFSM distribution: Figure 1b is a 3-D image stack captured by the 2-photon microscope within the scaffold. Fluorescent BFSMs (bright regions) are observed to distribute throughout the porous structure.

Cell growth: Figure 2 illustrates the average number of cells present at a depth of 400 µm or more into the scaffold surface. Data collected weekly over a four week period show a significant increase in cell infiltration over time for the RGD-BFSM modified scaffold vs. the non-modified system. Figure 3a shows the SMCs adhering to the RGD-BFSM modified polymer surfaces (bright circles on fluorescent peptide regions). Cell viability was >70% at 4 weeks. Figure 3b depicts the cell density and morphology.

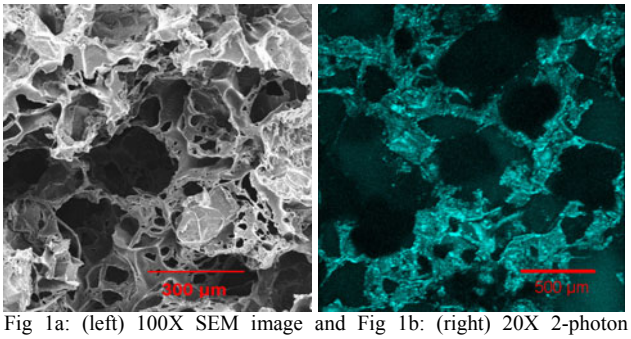
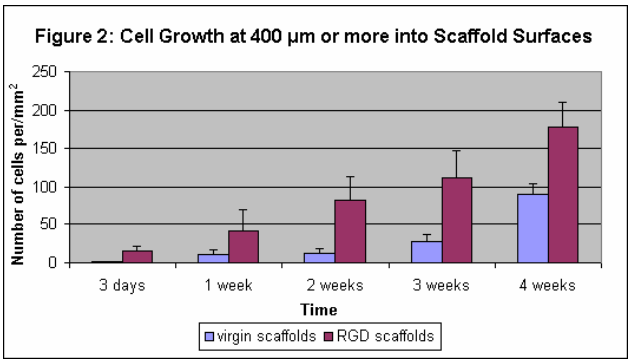


Fig 1a: (left) 100X SEM image and Fig 1b: (right) 20X 2-photon microscopy image (BFSM is bright region; pores are dark region). Both images show cross-sections of the scaffold.



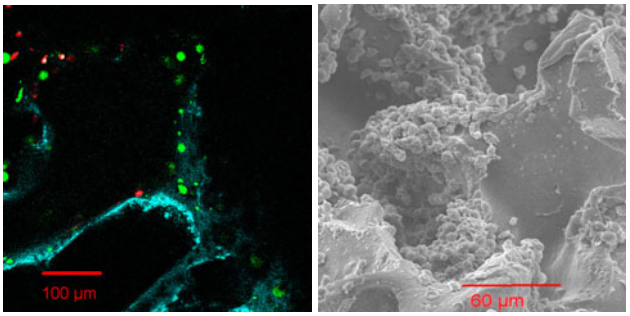


Figure 3a: (left) 20X 2-photon microscopy image showing the SMCs (cells show as bright region; pores show as dark region; BFSM show as grey background with superimposed cells) distributed on the RGD-BFSM modified polymer surfaces. Figure 3b: (right) 500X SEM image showing the adherent SMCs on the RGD-BFSM surfaces.

Conclusions: This study confirms that BFSMs distribute throughout the porous polyurethane surfaces and demonstrates that RGD-BFSMs promote greater SMC infiltration over prolonged periods inside the scaffold. The study indicates the potential use of the BFSM technology with biological moieties[2] for promoting controlled tissue growth in tissue engineering.

References:

1 Ernsting MJ. Biomaterials. 2005;26(33):6536-46
2 Fittkau MH. Biomaterials. 2005;26(2):167-74

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