

# Bioactive Electrospun Polymer Scaffold Tuned to the Selective Adhesion of Human Blood Outgrowth Endothelial Cells for Cardiovascular Applications

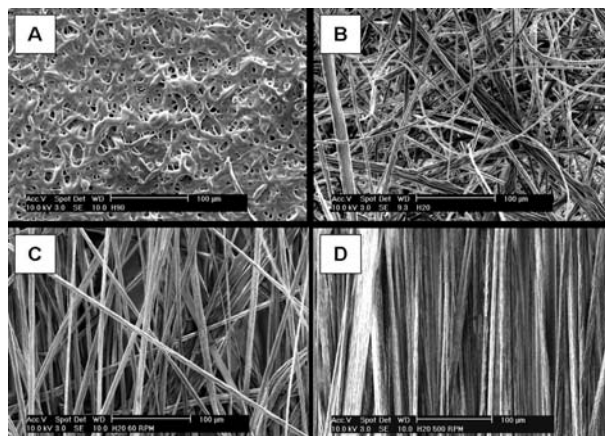
The function of many blood contacting biomedical devices is compromised by thrombus development at the biomaterial/blood interface. No surface except a healthy endothelium is fully blood compatible. However, a confluent and functioning endothelial cell layer on a biomaterial surface has not yet been successfully achieved in humans. We propose that human blood outgrowth endothelial cells (HBOECs)—adult circulating stem cells with the capacity for differentiating into endothelial cells—are an attractive cell source for the generation of a functioning endothelium on a biomaterial surface. This document describes the fabrication of electrospun scaffolds containing endothelial cell- and HBOEC-specific ligands. When exposed to blood it is hoped that the HBOEC-specific ligand will scavenge these adult stem cells from circulation, and the bound cells will spread, divide, differentiate and eventually generate a blood-compatible interface.

## Experimental methods

The polymer used in this research is a random methacrylic terpolymer polymerized from hexyl methacrylate (HMA), methyl methacrylate (MMA), and methacrylic acid (MAA). Methacrylates were chosen for their ease of synthesis and biocompatibility. By specifying the molar ratio of HMA and MMA, physical properties of the polymer can be controlled. MAA is incorporated in small concentrations (2 mole %) to impart carboxylic acid functionality to the polymer chains [1]. The material has been shown to resist hydrolytic and oxidative degradation *in vitro* [2]. Selective cell adhesion can be achieved through the covalent incorporation of cell-specific peptide ligands: the RGD tripeptide unit for endothelial cells, and novel human blood outgrowth endothelial cell specific ligands found through phage display screening [3]. The ligands are covalently immobilized to the biomaterial through chain transfer chemistry, a novel method for biofunctionalization [4]. To maximize the cellular response, the polymer material is electrospun into a fibrous structure inspired by the native extracellular matrix.

## Results/Discussion

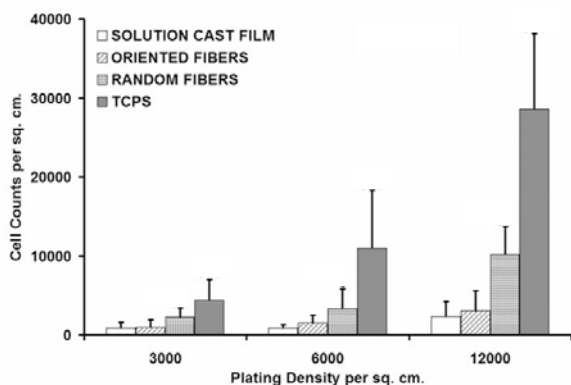
Varying the composition of the terpolymer provides a simple method to control the topography of the electrospun scaffolds. Fibers can be drawn into alignment by electrospinning onto a rotating collector, and the degree of fiber alignment can be controlled by varying the rotation speed of the collector. Scanning electron micrograph (SEM) images of various scaffold morphologies are shown in Figure 1.



**Figure 1.** SEM images of various scaffold topographies: (A) slightly porous, (B) highly porous, (C) partially aligned, (D) highly aligned.

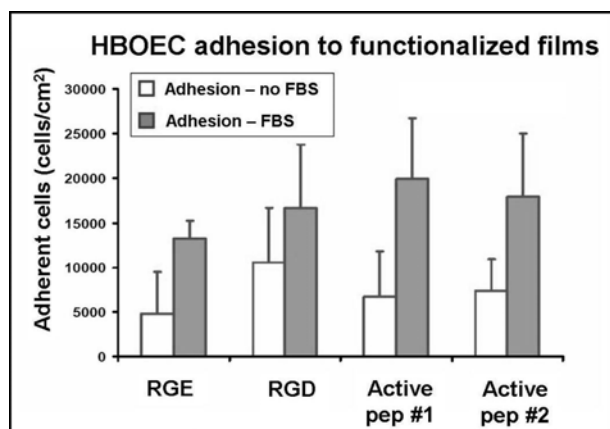
Ligands used in this study were terminated with a GGGSC spacer group. Cysteine (C) is unique among the amino acids in that it contains a thiol bond (S-H), a chemical group that can induce chain transfer during free radical polymerization. By adding the cysteine-terminated peptides to the reaction vessel during polymerization, chain transfer occurs, resulting in end grafting of the peptide ligands to the polymer chains. This novel biofunctionalization method has the additional advantage of synthesizing and functionalizing the material in a single step. Both endothelial cell- and HBOEC-specific ligands have been successfully incorporated into the polymer material and subsequently electrospun without ligand degradation.

Endothelial and human blood outgrowth endothelial cell adhesion studies have been performed on unfunctionalized random fiber surfaces and aligned fiber surfaces, with tissue culture polystyrene and polymer film surfaces used as controls. The adhesion results for the HBOECs are shown in Figure 2. Processing the material into fibrous constructs increases the initial cell adhesion, with random fiber surfaces adhering more cells than the aligned fiber surfaces. Furthermore, it was shown that the HBOECs are more aggressive in their attachment to and proliferation on the electrospun scaffolds, supporting the idea that these adult stem cells are an attractive source for the generation of an endothelium in comparison with more mature endothelial cells.



**Figure 2.** Initial cell adhesion of HBOECs to unfunctionalized scaffold surfaces after 6 hours of incubation.

Cell adhesion to the biofunctionalized polymer is also being explored. HBOECs were incubated with terpolymer surfaces functionalized with RGD, RGE (a proven non-adhesive peptide unit), and two HBOEC-specific peptides found through phage display screening. The results of this experiment are shown in Figure 3.



**Figure 3.** Initial cell adhesion of HBOECs to functionalized scaffold surfaces after 6 hour of incubation.

No statistically significant difference in cell adhesion was found between the surfaces. However, the ligand concentration in the functionalized materials was low (~1  $\mu\text{mol pep/g polymer}$ ). Current work includes increasing the ligand density in the terpolymer scaffolds, with the expectation that the higher ligand density will result in stronger surface/cell affinity. Also, the adsorption of proteins from body fluid or cell culture media can act to inhibit the specificity of the surface and result in the non-specific attachment of undesired cell types. Non-fouling character will be incorporated into the polymer material through the incorporation of oligo(ethylene glycol) (oEG) in order to maximize the desired bioactivity of the surface.

### Conclusions

The methacrylic terpolymer used in this study can be electrospun into random fiber scaffolds with a variety of topographies. Furthermore, by using a rotating collector, scaffolds composed of aligned fibers can be generated. Peptide ligands also have been successfully incorporated into the material via a novel biofunctionalization technique. Future work includes increasing the ligand density in the scaffolds and the incorporation of non-fouling character to increase the desired cell/surface interactions.

### References

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