Endothelial Progenitor Cell Adhesion and Growth on Peptide-Coated Electrospun Scaffolds

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Statement of Purpose: Recent literature (1) has suggested that a key mediator of the endothelium repair mechanism is the bone marrow-derived mononuclear cell, endothelial progenitor cell (EPC). As these progenitor cells deteriorate or are depleted with age the patient becomes increasingly prone to vascular inflammation, thrombosis, and atherosclerosis. There is a clinical need to assess the number and quality of circulating EPC. But EPC are especially rare, difficult to define by surface antigens, and expand to colonies very slowly *in vitro*.

A more useful clinical assay would adhere only EPC and expand them quickly on 3D scaffolds embedded with growth factors and/or affinity tags specific for EPC. Affinity tags now exists in the form of peptides, isolated via phage display, that bind specifically to EPC but not adult endothelial cells (2). Tentative names are based on sequence, e.g. "SYQ", and "TPS". Candidate peptides have been incorporated into a novel non-fouling polymer. This polymer may be cast into films or electrospun into 3D nonwoven meshes. Such templates are being tested for their specific affinity for EPC and ability to rapidly expand them into colonies for a clinical assay of endothelial health.

Methods: Nonfouling terpolymer is produced by free radical polymerization of methacrylate monomers, methacrylated PEG, and a succinimide- or maleimide-methacrylate. The latter moieties bind cysteine-terminated peptides. For microscopy, the peptides are bound to functionalized glass slides with a heterobifunctional crosslinker. Thin films of peptide-modifed terpolymer are cast from solvent. 3D scaffolds are produced via electrospinning from an organic solvent.

EPC are procured from adult human peripheral blood by plating and the buffy coat on collagen well plates. EPC are cultured on the polymer to assess adhesion and growth responses to the peptides. Adhesion is measured by DNA assay and actin staining for static culture experiments. In dynamic experiments, in which cells are flowed over a range of defined shear rates, adhesion is measured visually by software analysis.

Results: The base methacrylate terpolymer is a poor surface for cell binding and features quite low fibronectin and albumin adhesion. Phage display peptides have been synthesized and incorporated into the methacrylate terpolymer in addition to a positive control sequence, RGD, and a negative control sequence, RGE. Endothelial cells (HUVEC) cultured on films of these polymers containing RGD show greater adhesion than even tissue culture polystyrene (Figure 1B). The base polymer itself exhibits very low cellular binding. Endothelial cells cultured on films (Figure 1A) bearing RGD (Figure 1D) stain strongly for actin compared to polystyrene (Figure 1C).

Preliminary data (Figure 2B) demonstrate EPC adhesion to glass slides coated with the promising peptides, "TPS" and "SYQ", equal to that on tissue culture polystyrene (TCPS) and greater than the negative control sequence, RGE. EPC adhere to surfaces coated with the phage display peptide, "TPS", even in the presence of serum albumin(Figure 2A).

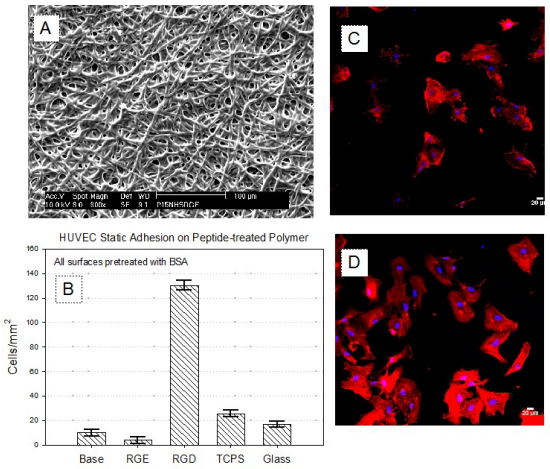

Figure 1. Electropun polymer scaffold (A), HUVEC adhesion to peptide-polymer films vs controls (B). Actin stain of HUVEC on polystyrene (C) and RGD-polymer (D)

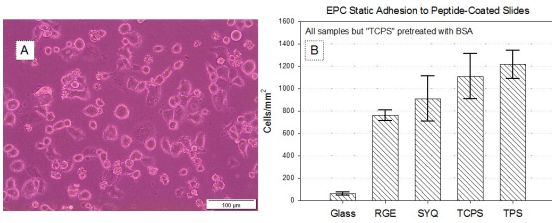

Figure 2. Brightfield micrograph of EPC adhering to TPS-slide (A), EPC static adhesion on peptide-coated slides in the presence of albumin, excepting polystyrene (B)

Conclusions: Methacrylate terpolymers feature minimal protein adsorption, are amenable to processing, and can incorporate virtually any short peptide or combination of peptides. Phage display peptides with specific affinity for human peripheral EPC have been synthesized and incorporated into several substrates for cell adhesion: modified glass slides, cast films, and electrospun scaffolds.

Increased adhesion of cells to the RGD binding sequence proves that the peptides remain active, even in the presence of blocking proteins. Meanwhile, depressed adhesion after incorporation of the RGE sequence demonstrates the minimal native binding affinity of the virgin terpolymer. Ongoing work aims to demonstrate selective EPC affinity for electrospun peptide scaffolds. This would permit rapid EPC expansion directly from blood samples and support graft endothelialization.

References:

1. Hirschi K. Arterioscler Thromb Vasc Biol. 2008; 28:1584-1595.
2. Veleva A. Biomaterials. 2008; 29:3656-3661.