Vascular smooth muscle cell interactions with elastin-like polypeptide modified surfaces

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Introduction: In small diameter (<6mm) vascular grafts, complications that arise include thrombogenesis and neointimal hyperplasia, with the latter resulting in occlusion of the graft due to vascular smooth muscle cell (VSMC) migration to the interior of the graft and subsequent VSMC proliferation. The use of elastin-like polypeptides (ELP), consisting of the repeat sequence VGVAPG, have previously been shown to induce non-thrombogenicity, with lower levels of platelet adhesion and fibrinogen adsorption associated with ELP4 coated surfaces (1). Work has been reported on the use of a bioactive fluorinated surface modifier (BFSM) to modify the surface of polycarbonate polyurethane (PCNU) surfaces, imparting the material with desired cell adhesive characteristics (2). When BFSMs include an elastin cross-linking peptide (ECP), it is possible to cross-link ELP4 to the surface of the modified biomaterial. Electrospinning is a technique used in tissue engineering for the generation of nanoscale diameter fibers. The morphology of these surfaces can mimic aspects of a 3-D extracellular matrix (ECM) (3). In this study the BFSM surface modification technique was used to modify polyurethane substrates, allowing them to be cross-linked with ELP4 in order to investigate VSMC response to the materials with respect to adhesion, proliferation and viability. It is hypothesized that VSMC adhesion, proliferation and viability will be enhanced on ELP4 cross-linked surfaces when compared to non-modified controls.

Materials and Methods: ECP-BFSM modified PCNU films were prepared by dissolving PCNU and ECP-BFSM (0.05wt%) in dimethyl formamide (DMF), placing 150µL of the solution in wells of a 96-well polypropylene plate and casting the films under humid conditions at 55°C for two weeks. Electrospun membranes were generated by dissolving PCNU and ECP-BFSM (0.05wt%) in 1,1,1,3,3,3-hexafluoro-isopropanol (HFP). The solution was injected through a needle and subjected to a voltage of 12kV at a flow rate of 0.05mL/hr. The formed fibers were collected on a rotating mandrel, charged to -6kV, rotating at 1150rpm in order to achieve aligned nanofibers. To cross-link ELP4 to the surface of these materials they were first treated with 200µL of 10wt% hydrazine monohydrate in a 50:50 mixture of methanol and distilled water for 24 hours. The surface was then treated with 200µL of 1mg/mL of genipin (GP) dissolved in distilled water for two hours, followed by a 24 hour treatment with 200µL of 1mg/mL GP and 1mg/mL ELP4 dissolved in distilled water. Prior to seeding, the films and membranes were sterilized by incubating at 37°C with 200µL of sterile phosphate buffer solution (PBS) supplemented with 100U/mL penicillin and 100mg/mL streptomycin. Human VSMCs (HUVS-112D, ATCC® Cat. No. CRL-2481™) were then seeded on 0.05wt% ECP-BFSM modified PCNU, ELP4 cross-linked and unmodified PCNU flat films and electrospun membranes and were studied over a period of 7-days by assessing viability (Live/Dead, DNA), proliferation (Live/Dead, DNA, SEM) and adhesion/morphology (SEM).

Results and Discussion: VSMCs demonstrated greater adhesion (**Fig. 1**) and viability (Live/Dead assay, data not shown) on surfaces cross-linked with ELP4 as compared with unmodified PCNU films and 0.05wt% ECP-BFSM modified PCNU films. This was apparent in both a serum (**Fig. 1** and **Fig. 2**) and non-serum environment (data not shown).

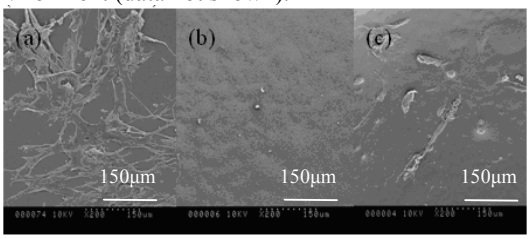

Fig. 1 *SEM images of VSMCs after 7 days are shown on (a) ELP4 cross-linked, (b) 0.05wt% ECP-BFSM modified PCNU and (c) unmodified PCNU flat films.*

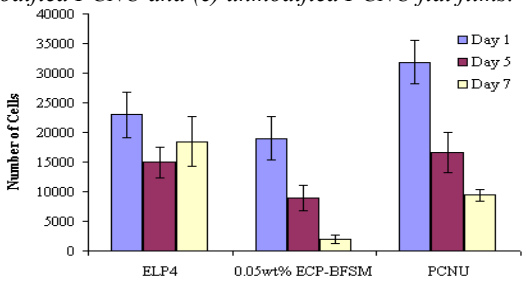

Fig. 2 *DNA data for different surfaces over a 7-day period, corresponding to samples in* **Fig. 1**.

VSMC response to the different surfaces was further confirmed by DNA measurements (**Fig. 2**) for ELP4 cross-linked surfaces versus 0.05wt% ECP-BFSM modified surfaces (i.e. no ELP4) and unmodified PCNU surfaces after a 7-day period. The poor retention of VSMCs on ECP-BFSM surfaces is not surprising given the low adhesive nature of the fluorine chemistry associated with the BFSM in the absence of ELP4. PCNU surfaces initially exhibited adhesion but this was not maintained over 7 days, emphasizing the necessary role of matrix proteins in maintaining cell adhesion. These results demonstrate a preference of VSMCs for ELP4 cross-linked surfaces.

Conclusion: VSMCs demonstrated a favorable response to ELP4 cross-linked surfaces when compared to controls, as indicated by increased adhesion and viability. This approach to applying select peptides of elastin to surfaces simulates the findings of previous studies examining VSMC interactions with elastin and elastin derivatives (4). Current work is looking at VSMC contractile and synthetic phenotypic markers for electrospun and flat film materials.

References: 1) Woodhouse K.A. *et al.* Biomaterials 2004;25:4543-4553. 2) Ernsting M.J. *et al.* Biomaterials 2005;26:6536-6546. 3) Boudriot U. *et al.* Artificial Organs 2005;30(10):785-792. 4) Leach J.B. *et al*. Acta Biomaterialia 2005;1:155-164.

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