

Mechanism of Vascular Smooth Muscle Adhesion to Elastin-like Polypeptide Surface Enriched Materials

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Statement of Purpose: Vascular tissue engineering strategies hold great promise for addressing limitations with currently used small diameter (<6mm) vascular grafts. Biomaterial surface modification with elastin-like polypeptides (ELPs) represents a feasible approach for the bioengineering of the medial layer of a blood vessel due to the ELPs inherent low thrombogenicity¹ and ability to support vascular smooth muscle cell (SMC) attachment². Previously, a stable ELP4 surface enriched polyurethane material was developed through the use of a surface modifying additive approach in conjunction with surface cross-linking of a class of ELP molecules denoted as ELP4³. These ELP4 enriched materials have been shown to exhibit platelet inhibitory effects while simultaneously promoting endothelial and smooth muscle cell (SMC) attachment^{2,3}. This work will investigate the hypothesis that the cell surface elastin-laminin receptor (ELR) complex and the VGVAPG peptide site on ELP4 are implicated in the enhancement of SMC attachment to these novel ELP4 modified materials. In addition, the expression of filamentous actin within the attached cells will be examined to determine the specific influence of the ELR complex and VGVAPG sequences in ELP4 on SMC interactions with the ELP4 materials.

Methods: *Synthesis and Purification:* Elastin cross-linking peptide bioactive fluorinated surface modifiers (ECP-BFSMs) were synthesized and their surface enrichment in a polycarbonate-urethane (PCNU) base polymer has previously been reported². *Elastin Cross-linking:* The lysine moieties of ECP-BFSMs which migrated to the surface of PCNU materials were deprotected using a 10wt% solution of hydrazine in a 50:50 mixture of methanol and water in order to expose amine groups for ELP4 cross-linking. Recombinantly produced elastin-like polypeptide 4 (ELP4) were cross-linked to the deprotected ECP-BFSM film surfaces with genipin for 24 hours. *Cell Culture:* Human umbilical vein smooth muscle cells (HUVS-112D, ATCC) were grown on ELP4 cross-linked films and compared to their ECP-BFSM modified PCNU precursor as well as tissue culture polystyrene (TCPS). Competitive inhibition experiments were carried out to study the mechanism of cell adhesion to the ELP4 modified materials. A portion of the SMCs were pre-incubated for 1 hour in lactose (100mM), which disrupts the cell surface ELR complex. In addition, a portion of materials were incubated for 24 hours with V14 peptide (50µg/ml), which binds to VGVAPG sequences on ELP4, with the anticipation that this sequence is implicated in the observed cell adhesion. A Cyquant® assay was employed to determine the number of SMCs adhered to the materials after 1, 2 and 4 hours post-seeding. SMC seeded materials at 1 hour post-seeding were also stained with DAPI and Alexa488-phalloidin and imaged by confocal microscopy to display the cell nucleus and filamentous actin bundles within the cells.

Results: Smooth muscle cell adhesion to ELP4 cross-linked materials was significantly inhibited at 1 hour post-seeding when SMCs were pre-incubated in lactose (Fig.1A) and when V14 peptide (Fig.1B) was pre-incubated with the ELP4 cross-linked materials. No inhibition of cell adhesion was observed at any other time-point or for any other material with lactose or V14 peptide inhibitors. These results suggest that initial SMC adhesion to ELP4 cross-linked films may be mediated through the elastin-laminin receptor (ELR) complex binding to VGVAPG sequences on ELP4.

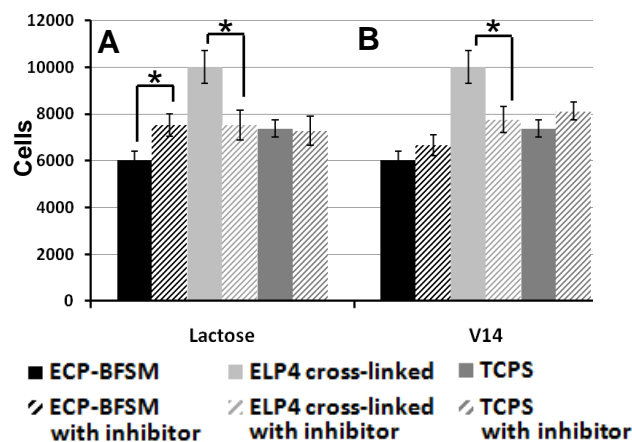


Figure 1. Effect of lactose (A) and V14 peptide (B) on SMC adhesion to ECP-BFSM modified PCNU, ELP4 cross-linked and TCPS materials at 1 hour post-seeding. *significant difference ($p < 0.05$).

Filamentous actin expression of smooth muscle cells seeded on the ELP4 cross-linked materials after 1 hour post-seeding (Fig. 2) demonstrated a higher degree of cell spreading and filamentous actin organization within the cells as compared to the other materials.

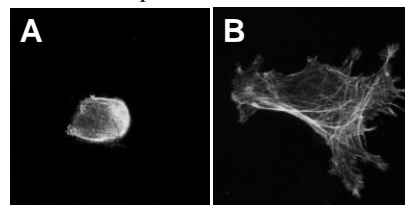


Figure 2. Actin filament images: A) ECP-BFSM modified PCNU and B) ELP4 cross-linked.

Conclusions: SMC adhesion to ELP4 cross-linked materials may initially be mediated through the non-integrin elastin-laminin surface receptor binding with VGVAPG sequences on ELP4. This initial interaction through the elastin-laminin receptor is proposed to be responsible for F-actin reorganization within the cell⁴.

References: ¹Woodhouse KA, Biomaterials 2004;25:4543-53. ²Battiston KG, SFB2009, San Antonio, USA. Paper#277. ³Blit PH, WBC2008, Amsterdam, NE. Paper#2102. ⁴Karnik SK, Development 2003;130:411-23.

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