Platelet inhibition and endothelial cell adhesion on stable elastin-like polypeptide enriched biomaterials

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Introduction: Biomaterials for vascular graft applications require low blood thrombogenic properties which are stable over time. Elastin derived peptides have previously been shown to have low thrombogenic character[1] and provide a support structure for vascular cell growth[2]. However, physical adsorption of elastin-like polypeptides (ELPs) to biomaterial surfaces has previously been shown to result in surface protein desorption[1]. Fluorinated surface modifying additives containing peptides can be blended in a base polyurethane and migrate to the surface of the material[3]. Moreover, these additives have also been shown to be used as vehicles for the delivery of elastin crosslinking peptides which can act as a site for covalent crosslinking of ELPs[4]. Herein, the stability and platelet interactions following blood contact of these ELP surface modified materials is reported. Endothelial cell adhesion to these biomimetic materials is also investigated as a means of assessing the potential for the materials to be applied in vascular graft applications.

Materials and Methods: *Synthesis and Purification:* Elastin crosslinking peptide bioactive fluorinated surface modifiers (ECP-BFSMs) were synthesized and their surface enrichment in a polycarbonate-urethane (PCNU) base polymer has been reported[4]. *Elastin Crosslinking:* ECP peptide expressed on the PCNU surfaces was deprotected using a 10wt% solution of hydrazine monohydrate in a 50:50 mixture of ddH_20 and methanol in order to expose the lysine amine groups for subsequent ELP crosslinking. Elastin-like polypeptides-4 (ELP4), synthesized using recombinant techniques in E. coli[1], were crosslinked to the deprotected ECP-BFSM film surfaces using genipin as a crosslinker and were allowed to react for 24 hours. X-ray photoelectron spectroscopy (XPS) was used to evaluate the amount of ELP4 on the surfaces, both before and after 24 hour desorption in 2% sodium dodecyl sulfate (SDS) as a measure of the coating stability. *Blood Studies:* The degree of platelet adhesion to the ELP4 x-linked films was previously characterized using radiolabelled platelets from human blood in a cone and plate device which provides material-blood contact under a laminar flow regime[4]. In the current study, scanning electron microscopy of film surfaces following blood contact under identical flow conditions was used to investigate platelet morphology. *Cell Culture:* Human umbilical vein endothelial cells (HUV-EC-C, CRL1730, ATCC) were grown on the ELP4 x-linked films and compared to the base PCNU and the ECP-BFSM modified PCNU materials to investigate cell adhesion and viability using a live/dead assay in conjunction with confocal microscopy.

Results and Discussion: XPS results (Table 1) demonstrate an increase in nitrogen surface content associated with the ELP4 coatings (ELP4 x-linked and ELP4 adsorbed). After treatment with 2% SDS to assess ELP4 stability, the ELP4 adsorbed to PCNU surface shows a percent reduction of nitrogen of 3.4% vs. 1.2% for ELP4 x-linked. This suggests an enhanced stability of the ELP4 coating on the ELP4 x-linked materials.

Materials	C	N	O
PCNU	84.1%	1.1%	14.8%
ECP-BFSM	79.6%	3.2%	17.2%
ELP4 x-linked	71.0%	7.0%	22.0%
ELP4 x-linked treated with 2%SDS	70.5%	5.8%	23.7%
ELP4 adsorbed to PCNU	71.5%	5.0%	23.6%
ELP4 adsorbed to PCNU treated with 2%SDS	73.5%	1.6%	24.9%

Table 1. XPS results of material surfaces (C-carbon, N-nitrogen and O-oxygen).

Scanning electron micrographs after contact with reconstituted whole blood under flow indicate a highly activated platelet morphology with platelet spreading and the formation of platelet clusters on the PCNU surfaces while the other surfaces (ECP-BFSM, ELP4 x-linked and ELP4 adsorbed) show scarce areas of platelets with a more rounded morphology with pseudopodia extensions.

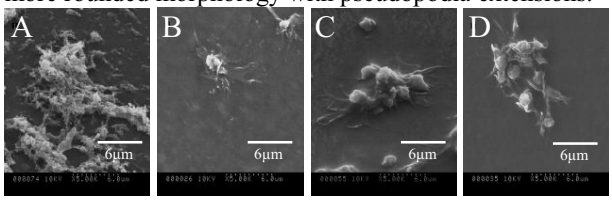

Figure 1. Scanning electron micrographs of A) PCNU, B) ECP-BFSM, C) ELP4 x-linked and D) ELP4 adsorbed to PCNU after contact with blood under flow conditions.

Preliminary HUVEC seeding experiments on the ELP4 x-linked materials demonstrated higher initial cell adhesion over ECP-BFSM and PCNU surfaces (Figure 2).

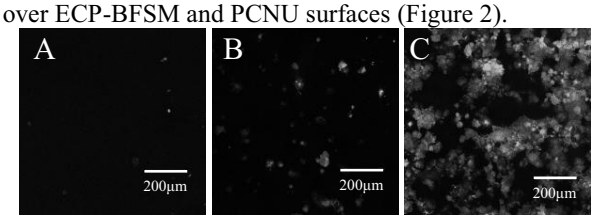

Figure 2. Confocal images of live/dead stained HUVEC seeded surfaces after 1 day. A) PCNU, B)ECP-BFSM, C) ELP4 x-linked. Lighter areas represent cell dense regions.

Conclusions: In conclusion, ELP4 x-linked materials have been shown to provide a stable ELP4 enriched surface under harsh desorption conditions using 2% SDS. In addition, ELP4 x-linked materials have been shown to inhibit platelet activation under flow conditions by morphological assessment, while also enhancing HUVEC attachment. Thus, ELP4 x-linked materials may be conducive towards re-endothelialization for use in vascular graft applications.

References: [1]Woodhouse K.A. et al., Biomaterials 2004;25(19):4543-53. [2]Dutoya S. et al., Biomaterials 2000;21(15):1521-9. [3]Ernsting M.J. et al., Biomaterials 2005;26(33):6536-46. [4]Blit P.H. et al., WBC2008, Amsterdam, NE. Paper#2102.

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