Cell Adhesion Peptide Modified Silicone Rubber: Potential for Use in Blood Contacting Applications

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Statement of Purpose: Biological responses at the blood-material interface, including non-specific protein adsorption, coagulation, platelet adhesion and activation significantly limit the use of currently available materials in blood contacting applications. Various strategies have been used to modify the surface of biomaterials to limit non-specific protein adsorption and promote the adhesion of endothelial cells, including modification with poly (ethylene oxide) (PEO) and cell adhesion peptides. In the current work, a novel method of modifying poly (dimethyl siloxane) (PDMS) with cell adhesion peptides tethered via a PEO spacer was used to improve blood protein and endothelial cell interactions. It is believed that these bioactive surfaces may induce desirable chemical and cellular responses in vivo, allowing for better control of proliferation, adhesion, and phenotype.

Methods: Polydimethylsiloxane (PDMS) disks for subsequent surface modifications were synthesized using standard methods. Si-H groups were created on the surface of the PDMS by reaction with poly methyl hydrosiloxane $(MeHSiO)_n$ (DC1107, Dow Corning) [1] as previously described. Subsequent modification with N-hydroxysuccinimide ester terminated polyethylene oxide (PEO-NHS, MW550) was achieved by two methods: In the first, direct attachment of PEO-NHS was achieved by means of initial synthesis of α-allyl-ω-*N*-succinimidyl carbonate-poly(ethylene glycol) (allyl-PEO-NHS) [2] and subsequent reaction with the Si-H modified PDMS surface via a platinum-catalyzed hydrosilylation reaction. The second reaction involved grafting of PEO-NHS by means of initial addition of allyl-PEO-OH to the Si-H modified PDMS and subsequent addition of N,N′-disuccinimidyl carbonate (diNHS). All surface modifications were confirmed by XPS, ATR-FTIR, and contact angle measurement. RGD containing cell adhesion peptides (GYRGDS, American Peptide) were radiolabeled with ^{125}I using the iodogen method. Subsequent exposure to the PEO-NHS modified surfaces in various concentrations resulted in the coupling of the peptides to the terminus of the PEO chain. Peptide surface densities were then quantified. Labeled (^{125}I) fibrinogen (ICN Pharmaceuticals, Irvine, CA) adsorption to the PEO-OH modified surfaces was quantified. Human umbilical vein endothelial cells (HUVECs, ATCC) were seeded on the PEO-RGD surfaces and quantified in terms of cell adhesion and proliferation by means of the CyQUANT proliferation assay (Invitrogen).

Results / Discussion: The modification of the silicone elastomer surfaces by PEO was demonstrated by the disappearance of the Si-H peak at 2165 cm^{-1} in the ATR-FTIR spectra upon modification. The appearance of broad peaks at ~2870 cm^{-1} and between 1600 and 1200 cm^{-1} corresponding to the CH_2-O and the OCH_2-CH_2 of PEO respectively further demonstrated successful attachment of PEO to the surface. A dramatic decrease in surface hydrophobicity as indicated by a decrease in the advancing water contact angle was also observed signaling the presence of the hydrophilic PEO molecule. XPS results revealed the presence of nitrogen on the peptide modified surfaces. Fibrinogen adsorption to PEO-OH modified PDMS was significantly reduced when compared to PDMS controls. Grafting of cell adhesion peptides to PEO-NHS modified PDMS resulted in a maximum surface peptide density of approximately 30 $pmol/cm^2$. It was also shown that desired peptide surface densities may be selectively generated on the surfaces based upon the peptide concentration in the reaction solution (Figure 1).

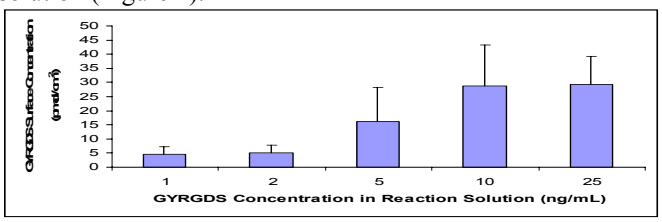

Figure 1. Cell adhesion peptide surface concentrations.

Seeding of HUVECs to the peptide modified surfaces resulted in eighty five percent cell adhesion compared to fifteen percent on PDMS controls (Figure 2).

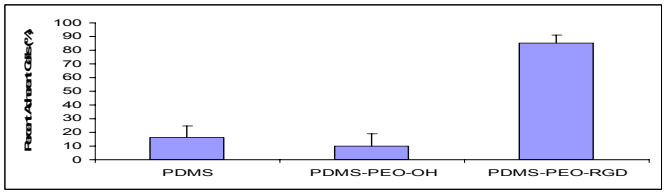

Figure 2. Percent HUVEC adhesion on RGD modified surfaces compared to controls.

Conclusions: PEO was successfully grafted onto the silicone elastomer surfaces using a platinum-catalyzed hydrosililation reaction between the surface Si-H groups and the allyl terminated PEO molecules. XPS, ATR-FTIR, and contact angle measurement confirmed the presence of PEO and cell adhesion peptides on the surfaces. PEO-OH surfaces demonstrated increased protein resistance when compared to PDMS controls. The NHS-PEO modified surfaces could be subsequently modified with cell adhesion peptides at various densities. Subsequent seeding of HUVECs demonstrated increased cell adhesion on the peptide modified surfaces compared to controls. The effect of varying cell adhesion peptide density on endothelial cell phenotype, proliferation and adhesion is currently under investigation.

References:

[1] Chen, H. Biomaterials. 2004;25(12):2273-82.
[2] Chen H. Bioconjugate Chem. Submitted 2005.

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