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Optimization of Peptide Modified Gold Coated Polyurethanes as Potential Substrates for Endothelial Cell Growth

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Introduction

Over 350 000 vascular graft replacements are performed in the United States each year[1]. While the use of artificial materials has proved adequate for large diameter (>5mm) implants problems with long-term patency of small diameter (<5mm) grafts have yet to be overcome. Graft failure is often due to activation of the intrinsic mechanism for blood clotting due to blood contact with the artificial surface. This can lead to thrombus formation, emboli and occlusion of the blood vessel.

Attaching the naturally occurring endothelial cell layer to the lumen of the artificial vessel could provide the antithrombotic mechanism normally present *in vivo*. To provide this protection, the lumen of the artificial blood vessel needs to be made amenable to endothelial cell growth. To this end, it is proposed that the use of gold coated polyurethanes for the chemisorption of cell adhesion peptides, via the thiol moiety of cysteine, could be used to promote endothelial cell growth. In this study, cysteine and three synthetic peptides, were chemisorbed to gold coated polyurethane surfaces. The modified surfaces were characterized and the effect of amino acid concentration and chemisorption time on the surfaces was analyzed. Quantification of the chemisorbed cysteine via an enzyme linked assay was also attempted.

Materials and Methods

The polyurethane was prepared based on the method of Santere *et al*[2]. Methylenedi-p-phenyl diisocyanate, polypropylene glycol and ethylendiamine were used in a molar ratio of 2:1:1 respectively. The polyurethane produced had a molecular weight of approximately 76 kDa. Films of polyurethane, about 2mm in thickness, were coated with gold by evaporation to a thickness of approximately 1000Å.

5mm diameter disks were punched from the films. The surfaces and glassware used in the chemisorption were cleaned by boiling in an ammonia hydrogen peroxide solution for 10 minutes at 80°C. The surfaces were then incubated in a buffered solution of cysteine ranging in concentration from 0 to 0.5 M for 1 to 125 hours at room temperature.

Characterization of the surfaces was performed using advancing water drop contact angles, scanning electron micrography (SEM), X-ray photoelectron spectroscopy (XPS) and an enzyme linked assay (ELA). Nuclear magnetic resonance imaging (NMR) and ultraviolet spectroscopy (UV) were used for verification of the chemical reactions taking place in the enzymatic assay.

The ELA utilized the high affinity of the egg white protein avidin for the vitamin biotin. Biotin was attached to cysteine by reaction with the free amino group of the amino acid. The biotinylated cysteine was then separated from the reaction by-products and unreacted reagents by solid phase extraction. A Sep-Pak® Light C18 cartridge (Waters, Milford, MA) was conditioned with acetonitrile and buffer. The biotin/cysteine reaction mixture loaded onto the column and the column was eluted with increasing concentrations of acetonitrile, retaining the fraction containing only the biotinylated cysteine. The biotinylated amino acid was subsequently diluted and non-biotinylated cysteine added to give the desired concentration with 10% biotinylated amino acid. After incubation the surfaces were removed from solution, rinsed, and placed in solution with β-galactosidase conjugated avidin. The surfaces were again removed from solution, rinsed, and placed in solution with o-nitrophenyl β-D-galactopyranoside. The absorbance at 405nm was monitored.

Results and Discussion

Water contact angles measured on the modified surfaces showed significant differences compared with the cleaned surfaces.

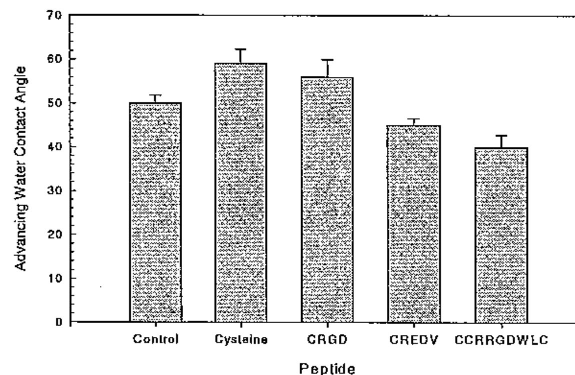

Figure 1: Advancing water contact angles of surfaces modified with cysteine and synthetic peptides. Control is cleaned gold surface. Error bars are standard deviations.

SEMs of the surfaces before and after chemisorption showed significant cracking and non-uniformities in the gold surface. This was also apparent in the XPS spectra which showed significant C, O and N peaks before chemisorption, likely resulting from the underlying polymer and carbon contamination of the gold surface. The XPS spectra also showed definite effects of incubation time and cysteine concentration on the chemisorption process. Significant changes in the nitrogen spectra, indicating the presence of a free amino group from the amino acid, were detected (see Fig. 2 (a) and (b)). Over 1 atomic % sulfur could be detected on the surface after incubation in $5x10^{-4}$ M cysteine for 25 hours (see Fig. 2 (c)).

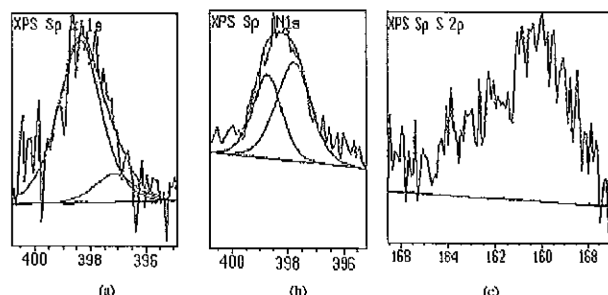

Figure 2: XPS spectra of gold surfaces and chemisorbed with cysteine (a) clean gold, (b) and (c) $5x10^{-4}$ M cysteine, 125 hrs.

The ELA also demonstrated that there was significant chemisorption of cysteine with a maximum amount being chemisorbed after 25 hours in a concentration of $5x10^{-4}$ M.

Conclusion

These studies demonstrate that surface chemistry can be altered using the chemisorption technique and that optimum chemisorption conditions exist. In the future the surfaces will be modified with cell adhesion peptides and their ability to support cell growth measured.

References

[1] Ratner, BD J Biomed Mater Res **27**: 837, 1993.
[2] Santerre, JP, ten Hove, P and Brash, JL J Biomed Mater Res 26: 39, 1992.