Adsorption of protein on titanium dioxide and titanium dioxide coated surface

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ABSTRACT

Biofunctions are required for metal implants introduced into stenotic blood vessels. These implants though required for dilation of the vessels, must exhibit blood compatibility and prevent the adhesion of platelets on its surface. Biomaterial grade titanium treated with polyethylene glycol as coating material was used for the experiment, with uncoated titanium as control. The adsorption of fibrinogen on both treated and untreated titanium oxide surfaces was studied. Whereas, there was significant adsorption of the fibrinogen protein on the untreated titanium oxide surface, none was recorded on the treated titanium oxide surface. Metal surface biofouling by proteins can be mitigated by the use polyethylene glycol coating on implants. This inhibition of proteins adsorption will prevent tissue growth, thereby allowing sliding movements in stenotic blood vessels.

Key words: Biofouling, Fibrinogen, Polyethylene glycol, Titanium, Titanium oxide

INTRODUCTION

Sliding lubrication in blood vessels is important when inserting guide wires and guiding catheters. Polyethylene glycol (PEG) is a biofunctional molecule that inhibits the adsorption of proteins therefore a successful immobilization of PEG to the metal surface is an important step in biofunctionalising the metal surface.

Titanium plays an important role as an implant material. It has become established as a common implant material because of its exceptional biocompatibility. Titanium base-metals with a stable and pure titanium oxide layer are bioinert to bioactive, meaning there are generally positive interactions between implant and tissue. In other words, protein adsorption and cell attachment takes place on titanium surfaces in a way that does not lead to a (strong) foreign body response. In view of the wide application of titanium for load bearing implants, there is a substantial interest to improve its acceptance by the body and to accelerate the healing process. The surface properties are therefore of prime importance, particularly in the early stages of implantation.

Titanium surface (as well as the surface of most other biomaterial) spontaneously adsorbs large amounts of various proteins from blood or other body fluids in an unspecific manner

[1], resulting in complex interactions with many different types of cells, platelets and bacteria such unspecific events may also contribute to foreign body responses inflammation [2]. Recently, novel approach has focused on eliminating nonspecific protein adsorption on the implant by adsorbing a layer of a protein-resistant co-polymer on the implant surface and simultaneously adding bioligands (e.g. small peptides) that elicit specific interactions with cells [3,4]. Polyethylene glycol (PEG) is the material of choice for imparting protein resistance to surfaces. By forming brush like structures; PEG creates a “stealth” repelling proteins, and cells and bacteria.

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Biofunctional ligands such as small peptide sequences can be added to the PEG chains in order to induce specific interactions [5] between the surface of the biomaterial and cells of the milieu. Polyethylene glycol coating in particular have been used to minimize surface biofouling of plasma proteins which induce the adhesion of platelets. When platelets adhere to a surface they start the signalling cascade that eventually leads to cross linking of fibrin and subsequently to blood clot formation. Therefore a surface that prevents platelets adhesion also prevents blood coagulation.

Cardiovascular implants such as artificial heart valves and vascular stents are made out of variety of materials. These diverse materials are needed to allow for the appropriate mechanical properties. All of these materials have different adhesion properties towards blood proteins, but all of them eventually induce blood clotting. Thus there is need to coat the implant with a material such as Polyethylene glycol which repels proteins and in the long run prevent blood coagulation.

In this study, the ability of Polyethylene glycol coated surfaces to repel protein was assessed through studies of the adsorption of fibrinogen (which is the main protein that reacts with platelets to begin the formation of fibrin). The results obtained from both the coated and uncoated titanium surfaces coated with PEG will minimize protein adhesion to the surface of the metal.

**MATERIALS AND METHODS**

**Materials**

Biomaterials grade Ti metal sample plates of length 1cm, width 1cm and thickness 1mm were used in this study. The Ti surfaces were ultrasonically degreased in benzene, acetone, and ethanol for 10minutes each, with deionized water rinsing between applications of each solvent. A passivation procedure was conducted by exposing the titanium samples to a 40% volume nitric acid solution at room temperature for 30minutes. All samples were then sterilized under UV light for a minimum of 24hours prior to each experiment.

**Modification of Titanium Surface.**

The liquid polymer (PEG) was stored at -20°C until use Phosphate buffered saline (PBS) with a total ionic strength of 150mM and pH 7.4 was prepared using 8g of NaCl, 0.2g of KCl, 1.44g of Na₂HPO₄ and 0.25g of KH₂PO₄. Before use, the polymer solutions were thawed for 3 minutes at 37°C in a water bath and equilibrated at experimental conditions (25°C) for two minutes. The cleaned metals were then dipped in the PEG solutions for 30minutes and subsequently rinsed with PBS. After a second washing with deionized water, then dried under 1bar filtered nitrogen stream and packed in sterile sealed vials. This resulted in the formation of a monolayer of the polymer.

![Figure 1: Standard Protein Concentration Curve](image)

**Protein Adsorption**

Bovine serum fibrinogen was used as model protein in this study. 25mls each of protein solution (150µg/ml protein / saline solution) was pipette unto the treated and untreated metal respectively. The setup was allowed to remain for a period of 30minutess after which 0.04ml of protein solution was removed. The removed solutions were mixed with...
2.0ml Bradford reagent and inverted several times to mix properly. Then the absorbance was recorded at 595nm using a Corning 253 colorimeter. The protein concentrations were analysed using the Bradford protein assay. Each protein concentration was calibrated using a standard curve. The degree of adsorption was determined by subtracting the residual protein from the initially added protein. Measurements were performed in triplicate for each time point. Mean adsorption protein concentration between the different titanium surfaces were statistically analysed.

RESULTS AND DISCUSSION

Figure (2) shows the adsorbs of fibrinogen on untreated and treated TiO₂ metal surface at a concentration of 150µg/ml for 180minutes absorbance recorded a maximum of 0.06 for the untreated and a maximum of 0.00 for the treated metal surface.

Figure (2) show the effect of incubation time on the adsorption of fibrinogen on treated and treated TiO₂ metal surfaces. The result shows that virtually all of the fibrinogen of 150µg/ml (~100%) was adsorbed to the untreated TiO₂ metal within 180minutes. While for the treated TiO₂ metal surface no adsorption was recorded.

There are several factors that affect the adsorption of protein used in this study. The isoelectric point (IP) of Titanium oxide has been reported to be 4.4 - 6.2 [6,7]. At pH of 7.4, the oxide’s anionic character has been reported to attract a variety of cations, which consequently enable the surface to build electrostatically to a variety of proteins [6,8]. In addition, Titanium surfaces have been reported to consist of hydrophilic (polar) and hydrophobic (non-polar) components, with the average polar/nonpolar ratio being 0.21±0.07 [9]. A correlation between surface wettability and protein binding was reported as a result of the presence of water molecules. The amount of adsorbed proteins in other studies was reported to be significantly higher on the hydrophobic surfaces compared to hydrophilic surfaces [10].

The ability of polymer brushes to prevent the adsorption of proteins has been addressed theoretically i.e. (protein resistance requires the exclusion of the adsorption at the outer surface of the metal due to interactions). This has been shown practically by the surface of the PEG forming brush-like structures via attachment unto the TiO₂ metal surface, thus repelling the protein (fibrinogen) from its surface. Leading to the adsorption indicated in figure (2) and (3).
CONCLUSION

Results from the study show that the treatment of TiO$_2$ metal surface with PEG will help to repel plasma proteins (e.g. fibrinogen) from adhering onto the metal surface. Thus, when such metal surface coated implants are placed in the body, the presence of PEG prevents certain plasma protein interactions with the metal thereby reducing the effect of the foreign body responses and blood coagulation.

REFERENCES

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