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Research Paper

Durability evaluation of biopolymer coating on titanium alloy substrate

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ABSTRACT

For this study, a commercially available phosphorylcholine (PC) polymer was applied to Ti6Al4V ELI. A multivariate approach to design a statistically significant array of experiments was employed to evaluate and estimate optimization of PC-immobilization process factors. The seven process factors analyzed were (1) power level for RFGD plasma treatment, (2) duration of plasma treatment, (3) concentration of PC solution used to coat samples, (4) rate at which samples were dipped in/out of the solution, (5) temperature for curing, (6) relative humidity level during curing, and (7) duration of curing. Imaging and analysis of the coating were done via fluorescence microscopy (FM), confirming the uniform coverage of PC polymer on titanium substrate. The process factors were evaluated by three measured responses: initial thickness, coating durability and degree of cross-linked coating, which were assessed by FM, a spray test and extraction in IPA, respectively. Variations in PC solution concentration showed no impact on fouling resistance of the resultant coating. It was hypothesized that the PC-application process factors could be optimized to yield favorable outcomes in durability and degree of cross-linked coating responses. The resulting statistical model indicates that PC solution concentration, dip rate, and cure temperature are the three greatest singular effects on both durability and degree of cross-linking. In addition, plasma treatment of the substrate with O_2 was effective in enhancing the degree of cross-linking of the polymer surface.

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1. Introduction

Titanium and its alloys, in addition to stainless steels, are widely used in surgical implant applications. Cardiovascular treatments, such as vascular stents, heart valves, and ventricular assist devices (VADs), have continued to promote the development of surface modification methods in order to reduce platelet deposition on metallic surfaces (Liu et al., 2004). Anticoagulation and antiplatelet pharmacologic therapies are frequently used on patients implanted with such apparatuses due to the risk of thrombosis and thromboembolism (Colli et al., 2007; John et al., 2008).

To improve the thromboresistance of titanium alloys in contact with the cardiovascular system, various surface coatings have been developed such as diamond-like carbon (DLC), titanium nitride (TiN), heparin, silicone, and 2-methacryloyl-oxyethel phosphorylcholine (MPC) (Sin et al., 2005). Exceptional biocompatibility and hemocompatibility have been shown on

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biomimetic surfaces synthesized from phospholipid polymers [phosphorylcholine (PC)] by reducing protein adsorption and platelet adhesion, as the PC head group is prevalent on the outside of biological cell membranes (Hayward and Chapman, 1984; Ishihara et al., 1990, 1998; Lewis 2000; Lewis et al., 2001).

In addition to coatings, physical and chemical surface treatments have been utilized on titanium alloys including passivation, polishing, oxidation, vapor deposition, silanization, glow discharge plasma treatment, and ion implantation (Liu et al., 2004). Plasma treatment and ion implantation have been shown to increase the hardness and wear resistance of metallic substrates (Ueda et al., 2003). Moreover, plasma treatment has been shown to improve biocompatibility and increase adhesion of different materials (Yasuda and Gazicki, 1982). Radio frequency glow discharge (RFGD) plasma treatment is frequently used for surface cleaning, and only introduces ions and electrons, as opposed to bulk materials (Ueda et al., 2003). The deposited ions can act as functional groups to covalently attach other polymers or biomolecules (Chevallier et al., 2001). Covalent linkage of PC to a substrate is more stable than physically adsorbed PC. Thus, modification of a surface in order to promote the covalent connection of PC to a metallic substrate is highly desirable for long-term medical implant applications.

Inorganic coatings on metallic surfaces, such as diamond-like carbon (DLC), titanium-oxides, and nitrides, which show evidence of mechanical and chemical stability, and high inertness, are employed to increase hemocompatibility of the base materials used in surgical devices. The hydrophobicity and low surface roughness exhibited on DLC-coated substrates have shown a higher proportion of albumin than fibrinogen in thrombogenic studies (Jones et al., 2000). However, DLC can be difficult to apply to the enclosed or undercut surfaces present on many implantable devices. Surface adhesion of DLC to substrates has been shown to be low without plasma pre-treatment (Ozeki et al., 2010), as with MPC-coatings.

Organic coatings are also used on metallic surfaces to improve hemocompatibility. PEO (Chen et al., 2005), PMEA (Gunaydin et al., 2002), PEG (Zhang et al., 2001), and other polymer coatings have been shown to reduce platelet adhesion and aggregation, and protein adsorption. However, durability, adhesion, the modification method, and efficacy impact the value of coatings, and must be considered on long-term surgical implants. While biomimetic organic polymers, such as PC, show promise for improvement of hemocompatibility, techniques to optimize immobilization of the material onto metals significant to implantable devices have not been widely evaluated for durability.

Characterization of PC material coated on substrates has been reported using X-ray photoelectron spectroscopy (XPS) (Hayward et al., 1986), atomic force microscopy (AFM) (Clarke et al., 2001), scanning electron microscopy (SEM) (Lewis et al., 2002), and Fourier-transform infrared (FTIR) microscopy (Lewis et al., 2004). Furthermore, spectroscopic ellipsometry (SE) and fluorescence microscopy (FM) analysis techniques have been used to analyze the presence, distribution, thickness and swelling of PC films on titanium, polypropylene, silicone, and other materials (Tang and Lu, 2001; Wang et al., 2005). Using FM analysis, the intensity of the reflected light is shown to be proportional to the thickness of the PC film, as quantified by SE. The linear relationship is depicted in Fig. 1.

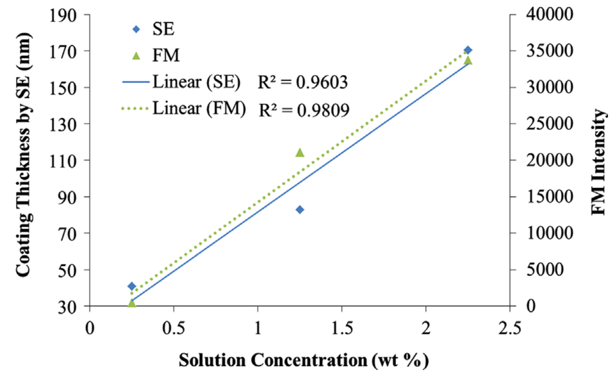

Fig. 1 – PC film thickness and FM intensity correlation as a function of solution concentration.

The primary objective in this study was to examine the process of titanium alloy (Ti6Al4V) surface modification for hemocompatibility, and assess which variables had the greatest impact on durability and integrity of the surface coating. A simple surface modification process was developed implementing RFGD plasma treatment. The parameters for plasma treatment, surface coating and curing of PC polymer were varied for statistical analyses. A technique was developed to aggressively erode the surface coating and to investigate the removal of the coating. Additionally, other analyses were carried out on surface coating thickness, coverage, degree of cross-linking by FM, surface wettability, and surface coating biocompatibility via *in vitro* protein adsorption analyses. A commercially available software was implemented in order to create a feasible design of experiments (DOE) test matrix, and to model and analyze each response.

2. Materials and methods

2.1. *Materials*

Titanium alloy, Ti6Al4V extra low interstitial (ELI) as per ASTM standard F136, was procured (Titanium Metal Supply, Poway, CA), machined to an average surface roughness (Ra) of 400 nm, passivated per ASTM standard F86, and polished to Ra of 100 nm with 400, pumice, 6 μm, and 1 μm grade ceramic aluminum oxide radial bristle disks (3 M, St. Paul, MN). Digesil NC (RPM Technology, Reno, NV) was used as a silicone stripper post-polishing. A phospholipid copolymer, PC 1036, was obtained from Vertellus Biomaterials (Hampshire, UK). Rhodamine 6G (Sigma-Aldrich, St. Louis, MO) was used as a staining agent for fluorescent visualization.

2.2. *Surface pretreatment*

After polishing, coupons were rinsed with distilled water and isopropyl alcohol (IPA), cleaned ultrasonically for 90 min in a 20 wt% phenol solution in dichloromethane (DCM), and solvent washed ultrasonically two times for 90 min each in DCM. The Ti6Al4V surfaces were pretreated by O_2 plasma with RFGD (Plasma Technology Systems, Belmont, CA) for

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