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A new textured polyphosphazene biomaterial with improved blood coagulation and microbial infection responses

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ABSTRACT

A new poly[bis(octafluoropentoxy) phosphazene] (OFP) was synthesized for the purpose of blood contacting medical devices. OFP was further either developed into crosslinkable polyphosphazene (X-OFP) or blended with polyurethane (PU) as the mixture (OFP/PU) for improvement of mechanical property of polyphosphazene polymers. All the materials were fabricated as smooth films or further textured with submicron pillars for the assay of antimicrobial and antithrombotic properties. Results showed that crosslinkable OFP (X-OFP) and blends of OFP/PU successfully improved the mechanical strength of OFP and fewer defects of pillars were found on the textured polyphosphazene surfaces. The antithrombotic experiments showed that polyphosphazene OFP materials reduced human Factor XII activation and platelet adhesion, thereby being resistant to plasma coagulation and thrombosis. The bacterial adhesion and biofilm experiments demonstrated that OFP materials inhibited staphylococcal bacterial adhesion and biofilm formation. The surface texturing further reduced the platelet adhesion and bacterial adhesion, and inhibited biofilm formation up to 23 days. The data suggested that textured OFP materials may provide a practical approach to improve the biocompatibility of current biomaterials in the application of blood contacting medical devices with significant reduction in risk of pathogenic infection and thrombosis.

Statement of Significance

The thromboembolic events and microbial infection have been the significant barriers for the long term use of biomaterials in blood-contacting medical devices. The development of new materials with multiple functions including anti-thrombosis and antibacterial surfaces is a high research priority. This study synthesized new biostable and biocompatible polyphosphazene polymers, poly[bis(octafluoropentoxy) phosphazene] (OFP) and crosslinkable OFP, and successfully improved the mechanical strength of polyphosphazenes. Polymers were fabricated into textured films with submicron pillars on the surfaces. The antimicrobial and antithrombotic assays demonstrated that new materials combined with surface physical modification have significant reduction in risk of pathogenic infection and thrombosis, and improve the biocompatibility of current biomaterials in the application of blood-contacting medical devices. It would be interest to biomaterials and bioengineering related communities.

1. Introduction

The insertion or implantation of biomedical devices ranging from simple catheters to complex extracorporeal life support systems have been an indispensable component for modern medical care [1]. However, the use of these medical devices is complicated by the potential for thromboembolic events and microbial infection. For example, catheter-related bloodstream infections present significant medical problems associated with morbidity, mortality, and increased health care costs [2,3]. It was reported that the annual medical cost due to infection had been over $9.8 billion [4]. After decades of focused research and literally thousands of published papers, biomaterial associated thrombosis and

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microbial infection remain significant barriers to development and implementation of implantable medical devices [5,6]. The quest to design and fabricate new materials with multiple functions including anti-thrombosis and antibacterial surfaces remains a high research priority [7,8].

Biomaterial induced thrombosis is initiated by activation of the blood plasma coagulation cascade [9] along with platelet adhesion/activation [10] on the surface, while infection arises from pathogenic bacterial adhesion and subsequent biofilm formation [11,12]. Both of these clinical problems are related to the materials used in devices and it has been well established that surface properties of the material used in devices are directly related to both the *in vitro* and *in vivo* biological performance. Modification of material surface properties has represented a promising route to engineer biofunctionality at the material-blood interface without altering material bulk properties necessary for use in medical devices [13,14]. One effective approach is to utilize antifouling surface coating to alleviate the colonization of pathogens and resist biofilm formation, which generally contains germicides such as antibiotics [15], antimicrobial peptides [16], quaternary ammonium compounds [17], silver [18], and nitric oxide [19]. The surface coating is also used to suppress blood clotting by addition with anticoagulant drug such as heparin [20]. However, the addition of germicides in coating has faced some challenges such as apparent cytotoxicity, short lifetime, and potential for drug resistance [21]. Recently a new block copolymer has been developed for the surface coating and shows good dual functional antimicrobial and antifouling properties [22–24]. These materials avoid the use of conventional antibiotics and exhibit broad-spectrum antimicrobial properties against Gram-negative and Gram-positive bacteria and fungi. The antimicrobial polymeric systems have been considerably developed in the past decades and the details can be found in some excellent reviews [25–29]. However, the most important point that should be remembered is that the anticoagulant data of these materials is lacking for potential use in blood contacting medical devices.

Surface modification is also widely applied to the biomaterials that are currently used in medical devices to improve the biocompatibility. For example, polyurethane (PU) copolymers are among the primary materials used in a variety of blood-contacting medical devices due to their excellent mechanical properties [30–32]. Surface modification techniques have been applied to polyurethane biomaterials to improve their hemocompatibility, mostly focusing on improvement of thrombogenicity and anti-infection [33–35]. Over the past few years we have developed submicron textured PU surfaces consisting of ordered arrays of pillars which showed resistance to platelet adhesion [36] and bacterial adhesion/biofilm formation [37,38]. The experiments *in vitro* demonstrated their potential for the use in clinical applications to combat health-care infections and thrombosis caused by catheters or other implanted devices. However, it appears that the efficiency of antimicrobial and anti-thrombotic properties of PU materials with such surface topographical design still needs to be improved. The development of new antiadhesive biomaterials with a combination of surface modification technique to create surfaces with the highest probability for reduction of thrombosis and infection is important in health-care. In this study, we developed advanced polymers based on the polyphosphazene platform and applied a physical topographical surface modification to produce a specific surface which exhibits both excellenet antimicrobial anhesion and antithrombotic properties. The success of these biostable polymers is of interest for biomedical applications, specifically in blood-contacting medical devices that resist microbial colonization and blood coagulation.

Polyphosphazenes are macromolecules with a linear phosphorus-nitrogen backbone and with two organic or inorganic

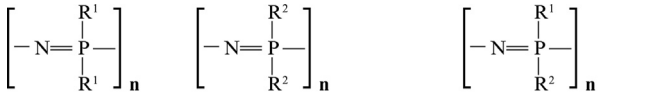

Fig. 1. A general structure of polyphosphazene. (R^1, R^2 : side groups).

side groups linked to each phosphorus (Fig. 1) [39,40]. The flexibility of the polyphosphazene backbone and the various side groups linked to the backbone have produced a large number of polyphosphazene polymers with a wide range of properties ranging from low temperature elastomers, films and fiber-forming polymers to rigid solids depending on the side groups [41–43]. These various properties of these polymers also make them highly attractive for life science and medical applications [44]. One example of the potential polyphosphazenes for the medical device fabrication is poly[bis(trifluoroethoxy)phosphazene] (TFE), which exhibits biocompatible, anti-inflammatory, and antithrombogenic properties [45]. Earlier work showed TFE having good blood compatibility due to high adsorption of serum albumin and the low adsorption of fibrinogen, and thereby effectively passivated against platelet adhesion [46]. Huang et al. conducted an *in vivo* test of TFE-coated stents in a porcine coronary stent model and found that TFE showed a long-term biocompatibility up to 6 months after implantation [47]. In a similar model, TFE-coated stainless steel stents were found to reduce thrombus formation and exhibit a trend toward less in-stent stenosis and inflammatory response than bare metal stents [48,49]. However, the main drawback of TFE is its poor mechanical property due to inherent chain flexibility of P-N backbone which results in the film easily being stretched and structural defects in the following physical surface modification. The alternative group of polyphosphazenes is bioerodible with amino acid–based substitute as side group. Such polymers undergo hydrolytic degradation through liberation of the amino acid and decomposition of the polyphosphazene backbone into low-toxicity and self-neutralizing phosphate and ammonia products [50]. Biodegradable polyphosphazenes of this type have been proposed as materials suitable for bone tissue engineering, drug delivery, and coatings [51–54].

The variety of side groups linked to polyphosphazene backbone also provides the possibility for the purpose of microbial infection resistance. Tian et al. [55] synthesized novel polyphosphazenes containing the fluoroquinolone antibiotic substituents, ciprofloxacin or norfloxacin, and found that antibiotics (<25 mol%) can be introduced to polyphosphazenes and 4–30% antibiotic release was measured in a six week hydrolysis study at 37 °C. As long as the antibiotic was released from these polymers, the antibacterial effect was observed. Recently, Lutzke et al. [56,57] synthesized poly(ethyl S-methylthiocysteinyl-co-ethyl cysteinyl phosphazene) and poly[bis(3-mercapto-3-methylbut-1-yl glycinyl) phosphazene], where the S-nitrosothiol functional groups were incorporated into the polyphosphazene backbone to form the biodegradable and nitric oxide releasing materials. Since nitric oxide can serve as an inhibitor of platelet adhesion and aggregation, and also as antimicrobial agent, such polymers exhibited great potential for applications in tissue engineering and having antithrombotic, antibacterial, and wound-healing properties. However, it should be recognized that these antimicrobial polyphosphazenes were all biodegradable and unsuitable for blood-contacting medical devices such as cardiovascular devices and catheters.

In this work we synthesized poly[bis(octafluoropentoxy) phosphazene] (OFP) based on the TFE platform, in which octafluoropentoxy side units are introduced into both sides of the backbone (Fig. 1) rather than the trifluoroethoxy groups. Such polyphosphazenes resemble classical fluorocarbon polymers such

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