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Multifunctional network-structured film coating for woven and knitted polyethylene terephthalate against cardiovascular graft-associated infections

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ABSTRACT

Multifunctional network-structured polymeric coat for woven and knitted forms of crimped polyethylene terephthalate PET graft was developed to limit graft-associated infections. A newly synthesized antibacterial sulfadimethoxine polyhexylene adipate-*b*-methoxy polyethylene oxide (SD-PHA-*b*-MPEO) di-block copolymer was employed. Our figures of merit revealed that the formed coat showed a porous topographic architecture which manifested paramount properties, mostly bacterial anti-adhesion efficiency and biocompatibility with host cells. Compared to untreated grafts, the coat presented marked reduction of adhered Gram-positive *Staphylococcus epidermidis* previously isolated from a patient's vein catheter by 2.6 and 2.3 folds for woven and knitted grafts, respectively. Similarly, bacterial anti-adhesion effect was observed for *Staphylococcus aureus* by 2.3 and 2.4 folds, and by 2.9 and 2.7 folds for Gram-negative *Escherichia coli* for woven and knitted grafts, respectively. Additionally, adhesion and growth characteristics of L929 cells on the modified grafts revealed no significant effect on the biocompatibility. In conclusion, coating of PET with (SD-PHA-*b*-MPEO) is a versatile approach offers the desired bacterial anti-adhesion effect and host biocompatibility.

1. Introduction

Damaged blood vessels or heart valves are usually replaced using polyethylene terephthalate PET (Dacron®) grafts due to various advantages mainly; tensile ability, strength, stability, and low production cost (Desai et al., 2011; Ozaki et al., 2014). Particularly, woven and knitted forms of crimped PET (Fig. 1) are mostly used to replace the large diameter blood vessels and cover the heart valve swing ring and vascular stent (Ozaki et al., 2014).

However, so far prosthetic valve endocarditis (PVE) or prosthetic vascular graft infection (PVGI) are the leading cause of short-term patency of PET grafts due to their susceptibility to bacterial infection and biofilm formation (Ginalska et al., 2007; Sabe et al., 2013; Tayebjee et al., 2013). The development of infection starts primarily by bacterial adhesion onto the graft surface *via* weak, reversible, physical forces followed by an adhesion cascade to secure successful attachment. The adhered bacteria proliferate and develop an irreversible attachment, forming dense microbial communities called biofilm (Rodrigues, 2011). Studies on pathogenic organisms responsible for PET graft infections have shown that *Staphylococcus aureus* is the infecting organism in ~70% of the cases, while *Staphylococcus epidermidis* is involved in ~30% resulting in prolonged hospitalization, graft failure, and patient death (Holmes et al., 2012; McCann et al., 2008). To overcome the aforementioned problems, surface modification of PET using polymeric film for drug delivery becomes thus one essential technique for further applications (Ginalska et al., 2005; Niekraszewicz et al., 2011). The film offers the opportunity to load and release various drugs to treat the main complications like inflammatory responses (Valence et al., 2013), thrombosis (Hirlekar et al., 2010), and bacterial infection (Cai et al.,

Abbreviations: PET, polyethylene terephthalate; PHA-*b*-MPEO, polyhexylene adipate-*b*-methoxy polyethylene oxide; PET-Net, net-coated polyethylene terephthalate.

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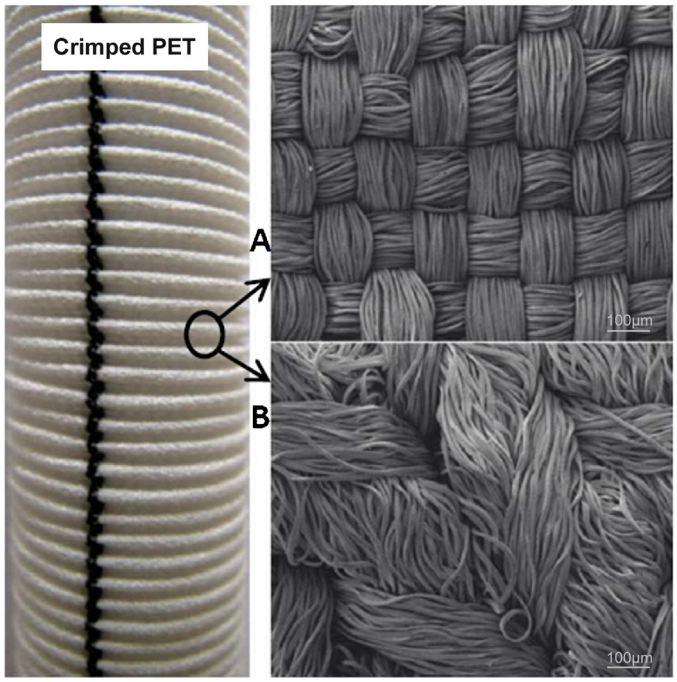

Fig. 1. SEM micrographs of woven (A) and knitted (B) forms of crimped PET grafts. The multifilament PET threads in woven form are fabricated in an over-and-under pattern, while the PET threads in the knitted form are looped. The crimped technique, shown in the photographic picture, is utilized to increase the flexibility, distensibility, and kink-resistance of grafts.

2012), while conserving the biocompatibility with biological tissues. In theory, casting of a polymeric film onto crimped PET grafts could be an ideal strategy to minimize the bacterial infection after implantation (Cai et al., 2012; Rodrigues, 2011). However, this method has severe limitations including relatively limited graft flexibility and tensile ability, which make it difficult to be sutured with the connecting tissues. Additionally, the film would be incised when sewing with surrounding tissue, which might weaken the desired effect of the coat (May-Pat et al., 2012; Niekraszewicz et al., 2011; Rodrigues, 2011). Hence, we report a priority to coating of crimped PET grafts by a multifunctional network-structured film using a novel biodegradable di-block copolymer polyhexylene adipate-*b*-methoxy polyethylene oxide (PHA-*b*-MPEO). The employed copolymer is composed of a hydrophobic block; polyhexylene adipate (PHA) and a hydrophilic block; methoxy polyethylene oxide (MPEO). For anti-bacterial effectiveness, the negatively charged and pH-sensitive sulfadimethoxine (SD) moiety was tethered to the copolymer backbone as schematically illustrated in Fig. 2. The synthesized di-block copolymer (PHA-*b*-MPEO) was previously characterized elsewhere (Assem and Greiner, 2011).

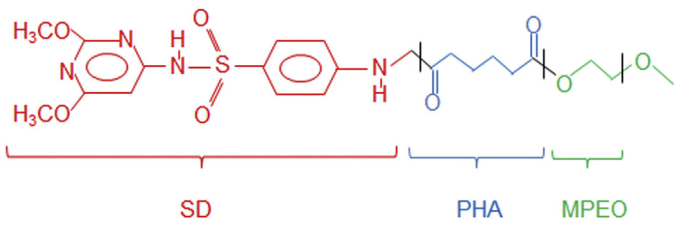

Fig. 2. The molecular structure of SD-PHA-*b*-MPEO di-block copolymer. The polymer is comprised of the hydrophobic block polyhexylene adipate; PHA, and the hydrophilic block methoxy polyethylene oxide; MPEO. The sulfadimethoxine (SD) moiety was immobilized to the chain end of the polymer backbone to impart anti-bacterial properties.

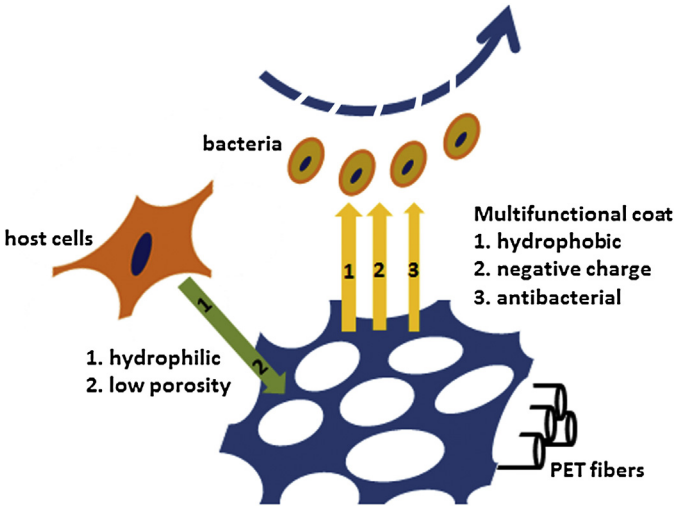

Fig. 3. The mechanism of the multifunctional network- structured film on bacteria adhesion and host cell behavior. The formed coat inhibits the bacterial adhesion due to the negatively charged, anti-bacterial sulfadimethoxine group, and the hydrophobic PHA block, in addition to the bacterial cell-repelling characteristics of MPEO block. Furthermore, the hydrophilic properties of MPEO and the formed porous coat enable the host cells to bridge the filaments.

The coating process was initiated by selective cleavage of the ester bond within PET surface using piranha solution to introduce a negatively charged hydroxyl group. In order to provide porous patterned film, we varied the humidity of the drying atmosphere and the polymer concentration during the process of film formation, and we took advantages of the repulsion forces between the modified surface and the employed polymer to equip a film that does not closely adhere to PET's surface. This versatile technique allows the fabrication of a network-structured film that does not tightly adhere to PET surface after polymer casting. The attempt to develop this coat was to maintain the essential graft's folding ability and to provide the desired local anti-bacterial effect and biocompatibility as schematically illustrated in Fig. 3.

2. Material and methods

2.1. Materials

Woven and knitted forms of crimped polyethylene terephthalate (PET, Dacron®) grafts (14 mm internal diameter, 0.45 mm wall thickness, 30 cm length, 12 filaments per yarn bundle) were kindly provided by Vascutek GmbH, Germany. The biodegradable amphiphilic sulfadimethoxine-polyhexylene adipate-*b*-methoxy polyethylene oxide (SD-PHA-*b*-MPEO) di-block copolymer was kindly supplied by Prof. Dr. Andreas Greiner, Department of Chemistry at University of Marburg, Germany. The bacterial strains; *S. epidermidis* (isolated from a vein catheter of a patient), *S.aureus* (ATCC 29213), and *Escherichia coli* (ATCC 25922) were supplied by Institute for Medical Microbiology and Hospital hygiene, Marburg University, Germany. Hydrogen peroxide H_2O_2 (30%) was purchased from Carl Roth GmbH, Germany. The mouse L929 fibroblasts cell line was obtained from DSMZ, Germany. Dulbecco's modified Eagle's medium (DMEM), Earle's balanced salt solution (EBSS), gamma irradiated fetal bovine serum (FBS), trypsin, streptomycin, penicillin, and amphotericin B were purchased from PAA Laboratories GmbH, Germany. L-glutamine was provided by VWR, Germany. All other used chemicals were of analytical reagent grade.