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A comparative study of polyethylene terephthalate surface carboxylation techniques: Characterization, *in vitro* haemocompatibility and endothelialization

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ABSTRACT

Surface functionalization of polymers is crucial for improving biocompatibility and haemocompatibility, which correlates to improved performance of medical devices. Here, we have evaluated the effect of four PET surface carboxylation techniques on the antifouling property, haemocompatibility, and endothelialization. Surface carboxylation was achieved by formaldehyde + bromoacetic acid treatment (PET-1[COOH]), methacrylic acid grafting (PET-2[COOH]), NaOH hydrolysis + $KMnO_4$ oxidation (PET-3[COOH]), and oxygen plasma treatment + acrylic acid grafting (PET-4[COOH]). The carboxyl densities on these surfaces were 0.4, 23.2, 31.9, 16.4 nmol/cm^2, respectively. XPS and FTIR spectroscopy confirmed the introduction of carboxyl groups. Water contact angle results showed that hydrophilicity increased with an increase in surface carboxyl density. SEM images confirmed that these modifications didn't cause any surface deterioration. AFM studies showed an increase in surface roughness of the carboxylated PET. Tensile testing showed that these modifications did not affect the bulk properties. Compared to control, PET-3[COOH] has a 9-fold reduction in BSA adsorption. Haemocompatibility studies showed significantly reduced %hemolysis and platelet adhesion on the carboxylated PET. Cell culture studies revealed that endothelial cell (EA.hy926) attachment increased with increase in surface carboxyl density. PET-3[COOH] showed the most improved haemocompatibility and endothelial cell attachment. These results clearly show that the method of functionalization has a significant impact on the haemocompatibility and cell attachment.

1. Introduction

Polyethylene terephthalate (PET) is one of the most widely used synthetic polymer for blood-contacting medical devices such as vascular grafts, surgical sutures, catheters, and heart valve sewing cuffs [1]. The main advantages of using PET is due to its characteristic mechanical strength, processability, and biological inertness towards the host immune system. Despite these benefits, PET has poor blood compatibility and low cell attachment due to its inert hydrophobic surface. This is a serious limitation for small diameter vascular grafts [2,3]. Such biocompatibility issues and thrombus formation will lead to failure of devices. The first step of communication for any contact of the biomaterial is the interaction between the foreign material surface and the host biological system. Hence, surface properties of the material play an important role in the biocompatibility of the medical devices. Over the last two decades, PET surface has been modified with various techniques and molecules, to improve haemocompatibility and cell attachment. These surface modification techniques, viz., physical coatings [4], chemical modifications [5,6], and plasma deposition [7,8], were all extensively studied for the blood-material interaction and cell attachment.

Most of the research in improving the blood compatibility of PET surface involves attachment of anti-thrombogenic molecules such as heparin [9,10], hirudin [11], sulfated polysaccharides [12], etc. But, no biomolecule coating can impart anti-thrombogenic properties as effectively as the natural endothelial cell lining. This made the focus of the research field to shift towards enhancing endothelialization of the polymer surfaces. Due to its inert surface, PET has been coated with biomolecules such as RGD (Arg-Gly-Asp) peptides [13], gelatin [14], collagen and chondroitin sulfate [15] to improve the attachment of endothelial cells. The polymer surfaces favoring the adhesion of endothelial cells will make the material-host system interface into an endothelial lining-host interface, leading to enhanced haemocompatibility, and better implant success.

Although many studies have explored different bioactive molecules, and their potential in enhancing haemocompatibility and

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endothelialization of PET, most of these studies don't evaluate the different surface functionalization techniques. As the method used for introducing functional groups to the PET surface directly affects the amount of bioactive molecules attached, it is critical to compare different methods available for functionalization. As PET has a hydrophobic surface that shows a poor adhesion of endothelial cells [16], choosing surface modification techniques that can enhance the polymer hydrophilicity could provide an inherent advantage in endothelial cell attachment [17].

In our work, we have chosen to introduce carboxyl groups to PET surfaces, as this would enhance surface hydrophilicity. This should, in turn, help in endothelial cell attachment. Here, we have compared four different methods of carboxylation, with each having its own chemistry of modification. The surface carboxyl group density and its stability were estimated by toluidine blue O (TBO) assay. Surface properties of these carboxylated PET (PET-[COOH]) were characterized using X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), atomic force microscopy (AFM), and static water contact angle measurement. As previous studies have shown that introduction of functional groups on the inert PET surface by chemical modification can alter the mechanical strength [18], we have compared the bulk properties of the modified and unmodified PET. We have further studied the effects of different surface carboxyl density and surface topography attained from these methods on Bovine serum albumin (BSA) adsorption, haemocompatibility, and endothelial cell attachment.

2. Experimental section

2.1. Materials

PET films with a thickness of 100 μm were obtained from Sumilon Polyester Ltd. (India). Acetic acid, formaldehyde, sulfuric acid, hydrochloric acid, bromoacetic acid, sodium hydroxide, methacrylic acid, acrylic acid, potassium persulfate, ammonium ceric nitrate, and toluidine blue O were purchased from HiMedia Laboratories. All the chemicals used for the experiments were high analytical grade. Bovine serum albumin, lactate dehydrogenase (LDH) activity assay kit, and MTT assay kit were purchased from Sigma-Aldrich. Endothelial cells (EA.hy926) were kindly gifted by Dr. Cora-Jean S. Edgell, University of North Carolina Lineberger Comprehensive Cancer Center.

2.2. Different methods of carboxylation

Unmodified PET samples (5 cm × 1 cm) were washed in acetone for 24 h, and vacuum dried. These PET films were used for further processing. Scheme 1 illustrates the schematic representation of the different methods of surface carboxylation of PET.

2.2.1. Method 1 (PET-1[COOH])

Surface carboxyl groups were introduced to the backbone of the PET chain using a previously described protocol [19]. Briefly, PET films were hydroxylated by 18.5% (v/v) formaldehyde in 1 M acetic acid for 4 h at room temperature. Subsequently, the samples were taken out and washed with an excess of deionized water. These hydroxylated PET films were treated with 1 M bromoacetic acid in 2 M sodium hydroxide for 18 h at room temperature. After that samples were washed thoroughly with an excess of deionized water.

2.2.2. Method 2 (PET-2[COOH])

In this method, cerium induced methacrylic acid grafting was done on the hydroxylated PET obtained after step one of method 1, for introducing carboxyl groups [20]. Briefly, the modified PET films were treated with 6% (v/v) MAA with 0.4 M sulfuric acid, and 0.007 M ammonium ceric nitrate. The reaction mixture was purged with nitrogen. Poly methacrylic acid (PMAA) grafting was performed at 60 °C for 2 h. The PMAA-grafted PET samples were washed with deionized water for 24 h to remove the unreacted monomers and homopolymers of MAA. Then, the carboxylated films were vacuum dried.

2.2.3. Method 3 (PET-3[COOH])

This method involves hydrolysis of PET and complete oxidation of alcohol groups formed on the surface [21]. Briefly, PET samples were hydrolyzed using 0.25 N sodium hydroxide in water/acetonitrile (1:1) solution for 10 h at 60 °C. After that, the hydroxyl groups were completely oxidized by potassium permanganate (5 g in 100 mL 1.2 N sulfuric acid) for 1 h at 60 °C. Then, the carboxylated PET films were washed with 4 N hydrochloric acid for 20 min. Deionized water was used to wash the carboxylated PET thoroughly to remove any residual acids on the surface.

2.2.4. Method 4 (PET-4[COOH])

Here, PET surfaces were treated with oxygen plasma to introduce reactive peroxide groups, to which acrylic acid was grafted [22,23]. The cleaned PET films were treated with argon plasma in a plasma reactor at 40 W power. The initial pressure was 5×10^{-5} mbar, and the argon flow rate was 10 sccm. Oxygen was supplied to the reactor at the deposition pressure of 3×10^{-3} mbar with a flow rate of 20 sccm. The exposure time was 10 min. Immediately after the plasma treatment, acrylic acid grafting process was started. The plasma treated samples were kept in 6% (v/v) acrylic acid solution with the initiator, 0.2% (w/v) potassium persulfate, and nitrogen was purged to start the polymerization process. The reaction was incubated at 60 °C for 2 h. Then, the samples were washed thoroughly with an excess amount of deionized water for 24 h to remove the unreacted monomer, and vacuum dried.

2.3. Estimation of surface carboxyl group

Toluidine blue O (TBO) staining assay was used for the determination of surface carboxyl group density. The carboxylated PET films were incubated in 0.5 mM TBO (pH = 10.0) for 6 h at 30 °C. After that, the films were taken out and washed with an excess of 0.1 mM sodium hydroxide to remove the unbound TBO molecules. The bound TBO on the carboxyl group was released using 50% acetic acid solution under mixing for 15 min. The concentration of TBO detached was measured using a spectrophotometer (V-630, Jasco) at 633 nm. The carboxyl density of the unmodified PET was also estimated and used as a control. The calculation of COOH surface density was based on the principle that one molecule of TBO binds with one carboxyl group [24].

2.4. Stability of surface carboxyl groups on modified PET

Long-term patency of the implant depends on the stability of the modified surfaces. Here, the stability of the introduced carboxyl groups was investigated by incubating the modified PET in phosphate buffer saline (PBS, pH = 7.4) at 37 °C for one week. The incubated samples were taken out each day, washed with deionized water, and estimated for surface carboxyl density using TBO assay as mentioned earlier.

2.5. Surface characterization of carboxylated PET films

XPS measurements were performed using Kratos Axis Ultra spectrophotometer. The spectra obtained were analyzed and fitted using CasaXPS software (Version 2.3.18PR1.0). The characteristic absorption peak for the carboxyl functional group was analyzed by FTIR (FT/IR-4000, JASCO). The samples were analyzed in the transmittance mode in the range of 400–4000 cm^{-1}. The surface topography of the unmodified and carboxylated PET films was observed using SEM (Quanta 200, FEI). The PET samples were sputter coated with gold before observing under SEM. AFM images were taken using a Pico plus 5500 ILM AFM (Agilent Technologies, USA) in the non-contact, acoustic AC