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Analysis of functionalized polyethylene terephthalate with immobilized NTPDase and cysteine

Vignesh Muthuvijayan [a], Jun Gu [a], Randy S Lewis [b,*]

[a] School of Chemical Engineering, Oklahoma State University, 423 Engineering North, Stillwater, OK 74078, USA
[b] Department of Chemical Engineering, Brigham Young University, 350 Clyde Building, Provo, UT 84602, USA

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Abstract

Polyethylene terephthalate (PET) was functionalized to introduce carboxyl groups onto its surface by a carboxylation technique. Surface and bulk properties, such as possible surface deterioration, surface roughness and the mechanical strength of the carboxylated polymers, were studied and compared with those of aminolyzed and hydrolyzed PET. Atomic force microscopy studies showed that unlike aminolysis and hydrolysis, which increased the surface roughness significantly due to cracking and pitting, the surface roughness of unmodified and carboxylated PET were comparable. While hydrolysis and aminolysis of PET resulted in significant loss of strength, tensile testing revealed that unmodified and carboxylated polymers had similar strength. The development of mechanically stable, functionalized PET would vastly improve the biomedical applications of this polymer. To understand the potential for improving biomedical applications, biologically active molecules, namely nucleoside triphosphate diphosphohydrolase (NTPDase) and cysteine, were immobilized on the carboxylated PET using amide bonds. NTPDase was also immobilized to aminolyzed PET using imine bonds, while cysteine was immobilized on aminolyzed PET using both imine and amide bonds. Attachment of NTPDase and cysteine was verified by analyzing the NTPDase activity and the cysteine surface concentration. The stability of these immobilizations was also studied.

Keywords: Polyethylene terephthalate; Surface modification; Surface analysis; Nitric oxide; Blood compatibility

1. Introduction

Polyethylene terephthalate (PET) is a linear, aromatic polyester that has been shown to have the desirable mechanical strength and durability for biomedical applications [1,2]. PET has a wide range of medical applications, including vascular prostheses [3–6], heart valve sewing cuffs [7–9], implantable sutures [10,11] and surgical mesh [12,13]. As a number of PET applications involve contact with blood, hemocompatibility is a major concern. Surface modification offers an effective approach to improve hemocompatibility. However, PET is an inert polymer and lacks active functional groups on the surface to attach biologically active molecules. Hence, surface functionalization of PET would aid in immobilizing biomolecules which could potentially improve hemocompatibility. Currently, various different techniques, including hydrolysis [2,14,15], reduction [16], glycolysis [17], aminolysis [2,14,18–21] and amination [1], are used to introduce reactive functional groups on PET surfaces.

Although these techniques offer effective means of introducing reactive groups, surface modifications can restrict the surface and bulk properties of PET. Loss of mechanical strength is a common problem associated with surface modification due to degradation of the polymer [1,15,16,22,23]. This results in reducing the durability of PET in implants and other long-term biomedical applications. Even with these limitations, many studies have demonstrated immobilization of active biomolecules [5,6,16,19,21,22,24–26]. These studies have also showed improved hemocompatibility [6,19,21], infection resistance [5,24–26] and cell adhesion

* Corresponding author. Tel.: +1 801 422 7863; fax: +1 801 422 0151.
E-mail address: randy.lewis@byu.edu (R.S. Lewis).

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[22]. Hence, identifying a surface modification technique that does not alter the mechanical strength of PET would overcome the limitations currently associated with surface modification.

In addition to techniques such as aminolysis and hydrolysis, carboxylation has been performed to introduce reactive carboxyl groups onto PET surfaces [27]. However, the surface and bulk properties of carboxylated PET have not been explored. Understanding the effects of carboxylation on the surface and bulk properties could potentially provide an effective PET surface modification. For this purpose, this study has analyzed carboxylated PET to understand the effects of the surface modification on surface and bulk properties, namely possible surface deterioration, surface roughness and mechanical strength. In addition, two other techniques to introduce functional groups to the PET surface, namely aminolysis and hydrolysis, were analyzed for comparison. Comparing the properties of modified PET to the properties of unmodified PET provides valuable information. As well as assessing the effect of PET modification on surface and bulk properties, it is also critical to analyze whether biologically active molecules can be attached to the modified polymer.

As mentioned earlier, PET is used in many blood-contacting systems. In such an environment, contact with a foreign material such as PET activates platelets and leads to platelet aggregation. Covalent or ionic binding of anticoagulant or other biologically active molecules to the reactive functional groups on modified PET can potentially improve hemocompatibility. Cysteine has been shown to improve hemocompatibility when immobilized to aminolyzed PET [19]. Cysteine utilizes endogenous nitric oxide (NO) to inhibit platelet activation and aggregation [19,20]. Briefly, a rapid transnitrosation reaction occurs between S-nitrosoproteins (primarily S-nitrosoalbumin) and cysteine [28]. S-Nitrosocysteine formed is unstable and NO is released. Therefore, NO release from S-nitrosoalbumin is catalyzed by free cysteine [29]. The released NO acts as a potent inhibitor of platelet activation and aggregation [30]. Although hemocompatibility was enhanced by cysteine immobilization on aminolyzed PET, only about 50% platelet inhibition was achieved [19]. Modified properties of the aminolyzed PET and possible stability issues of immobilized cysteine could be some of the possible reasons for the incomplete inhibition. Therefore, in this study, cysteine was immobilized on aminolyzed and carboxylated PET to assess the effects of surface modification on polymer properties and to assess the stability of cysteine immobilization. Since polymer properties of hydrolyzed PET were assessed in this study and showed a loss of mechanical strength caused by hydrolysis, which is consistent with previous studies, cysteine immobilization on hydrolyzed PET was not studied [14,15,22,31].

Another biologically active molecule that could improve hemocompatibility is nucleoside triphosphate diphosphohydrolase (NTPDase). When blood comes in contact with a foreign material, irreversible platelet aggregation is effected by release of adenosine diphosphate (ADP) molecules in a concentration-dependent manner [32–34]. NTPDase is an enzyme that has been shown to inhibit ADP-induced platelet aggregation by hydrolyzing ADP to AMP and inorganic phosphate (Pi) [35–37]. NTPDase has been used to improve the hemocompatibility of polymers either by coating [38,39] or by covalent binding [40]. Although these techniques showed improved hemocompatibility, both protocols had their limitations. In case of coating polyurethane with NTPDase, rapid release of the non-specifically bound NTPDase could result in undesired effects. Also, once the coated NTPDase is washed off, the polymer would lose the hemocompatibility. Studies done by Marconi et al. [40] immobilized NTPDase to hydrolyzed PET, despite hydrolyzed PET showing loss of mechanical strength due to hydrolysis [14,15,22,31]. Based on these studies, immobilization of NTPDase to mechanically stable PET would potentially enhance hemocompatibility.

Similar to the cysteine in this study, NTPDase was also immobilized on aminolyzed and carboxylated PET. Attachment of NTPDase was assessed by studying the activity of immobilized NTPDase. The effectiveness of NTPDase immobilization on aminolyzed and carboxylated PET was assessed by comparing the NTPDase activity of the above-mentioned polymers with the activity observed by Marconi et al. [40] on NTPDase immobilized on hydrolyzed PET. Stability of NTPDase immobilization on these polymers was also studied.

Besides exploring the effects of three PET surface modification techniques, namely aminolysis, hydrolysis and carboxylation, this study also analyzes the immobilization of two biologically active molecules, NTPDase and cysteine, to aminolyzed and carboxylated PET. By performing these tasks, this study analyzes both aspects involved in developing a useful biomedical material, namely the physical properties of the modified polymer and the ability to immobilize biologically active molecules on the modified surface.

2. Materials and methods

2.1. Reagents

PET (thickness = 0.2 mm) was supplied by DuPont (Hopewell, VA). Ethylenediamine, glutaraldehyde, bromoacetic acid, sodium nitrite, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), 2-(N-morpholino)ethanesulfonic acid (MES), NTPDase (Grade VII containing the isoenzyme Desirée with low ATPase/ADPase ratio) and cysteine were purchased from Sigma (St. Louis, MO). Sodium hydroxide, sodium phosphate (monobasic and dibasic), sodium chloride, acetone, acetic acid, formaldehyde and other reagents were purchased from Fisher Scientific (Fair Lawn, NJ).

2.2. Surface modification

Before modifying the surfaces to introduce reactive functional groups, PET films were soaked in acetone for

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