European Polymer Journal xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Biocompatibility and hemocompatibility evaluation of polyether urethanes synthesized using DBU organocatalyst

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ARTICLE INFO

Article history:
Received 15 April 2016
Received in revised form 1 August 2016
Accepted 10 August 2016
Available online xxxx

Keywords:
Polyurethanes
Organocatalysis
Hemocompatibility
Biocompatibility
Lysine diisocyanate

ABSTRACT

Biomaterials must fulfill some requirements before moving into in vivo application. In vitro test is usually conducted as a preliminary screening evaluation. Although most of the studies are focused in the cytotoxicity, interactions between blood elements and the biomaterials or hemocompatibility must also be considered. Aliphatic polyurethanes have been always considered ideal candidates for in-vivo application due to their versatility. However, the utilization of metal catalyst to promote the polymerization have limited their use. Recently, some organocatalysts have shown to be competitive to tin based catalyst for the preparation of polyurethanes and have relaunched their use in biomedicine. In the present study we carried out the organocatalyzed polymerization of 5 commercially available isocyanates, hexamethylene diisocyanate, isophorone diisocyanate, *trans*-1,4-cyclohexylene diisocyanate, 4,4′-methylenebis(cyclohexyl isocyanate) and L-lysine diisocyanate to analyze the cytotoxicity and hemocompatibility of the resultant polymers as a function of the employed diisocyanate. The diisocyanates were polymerized with hydroxy end-capped oligomeric poly (tetramethylene glycol) (PT2K) as the long chain diol and 1,3-propanediol as the short chain diol. We demonstrated that from selected diisocyanates, lysine diisocyanate based polyurethanes possessed lower cytotoxicity and better hemocompatibility than the other polyurethanes. In comparison with a well known blood compatible polymer such as poly(2-methoxyethyl acrylate), the lysine diisocyanate based polyurethanes showed remarkable values in terms of cytotoxicity and platelet adhesion, but major levels of protein adsorption.

1. Introduction

The increasing use of synthetic materials in medical devices makes the research of biomaterials' hemocompatibility very relevant in order to reduce adverse effects of the host cells such as implant rejection [1–3]. One of the polymer families that are continuously gaining attention is the family of polyurethanes (PUs) due to their excellent mechanical properties and potential hydrolytic degradation [4–6]. In addition, properties of PUs can be easily tailored by varying the nature of the reagents used in the synthesis [7,8], and as a result, these materials have been designed for a variety of medical devices, such

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http://dx.doi.org/10.1016/j.eurpolymj.2016.08.008
0014-3057/

as catheters, cardiac assisting devices, artificial heart, cardiovascular biomaterials, hemodialysis bloodline sets, center venous catheters, and intravenous bags [9,10].

A crucial feature that must be considered in medical devices is the biocompatibility with the host cells to reduce adverse effects [11,12]. Biomaterials must fulfill stringent requirements with respect to their interactions with blood element and the materials should contain minimal or acceptable hemocytotoxicity to be used in vivo [13–16]. These materials should not induce coagulation activity or exhibit thrombus formation. An ideal material would have appropriate mechanical properties combined with a good biocompatibility on the short term as well as during degradation [17]. For instance, synthetic grafts have been associated with excellent long-term results when treating large-diameter arteries. Meanwhile these results have been disappointing when they are used to replace small-diameter (<6 mm) arteries due to problems related to platelet adhesion [18].

To date, in spite of the excellent properties and biocompatibility of PUs, serious concerns have been raised about their applicability due to the utilization of toxic metal catalysts [19,20]. Organotin catalysts are considered the gold standard to promote the polymerization of aliphatic isocyanates. However, their high stability against hydrolysis and oxidative degradation combined with the difficulties to remove them from PUs have limited the use of these materials in nanomedicine [21]. In addition, some European Union regulations have restricted the use of organotin-based compounds, and the use of alternative catalyst would reduce problems associated with regulatory issues [22]. Therefore, in the last decade significant efforts have been dedicated to the synthesis of PUs using tin-free approaches. In this fashion, organocatalysts have emerged as an efficient alternative for the synthesis of PUs for biomedical application [23]. Cramail et al. demonstrated the efficiency of bicyclic alkylated guanidines to catalyze the synthesis of polyurethanes [24]. Along these lines, some of us showed the extraordinary capacity of organic acids to catalyze the isocyanate alcohol reaction, showing the ability to prepare organocatalyzed PUs [25–27]. Albeit, these promising results, to the best of our knowledge no reports have described the relationship between the biocompatibility, particularly hemocompatibility, and the structural features of organocatalyzed polyurethanes.

Before going to an in-vivo application, in vitro biocompatibility studies are required such as cellular, protein and platelet responses to the material. It is well established that platelet adhesion and protein adsorption on polymer surface are strongly determined by various external factors (temperature, pH, ionic strength), protein properties, and surface used for the coating [28]. Studies of polymer properties and biocompatibility involve the analysis of the surface hydrophobicity, platelet adhesion, protein adsorption and cytotoxicity. The measurement of both protein (fibrinogen) adsorption and platelet adhesion is crucial for determining the relative thrombogenic potential of each material [29,30]. For instance, Ren and coworkers found that by grafting polyethylene glycol onto the surface of polyurethane films the hemocompatibility can be enhanced [31]. They suggested that the blood compatibility is related with the balance of hydrophilicity/hydrophobicity. Similarly, polyurethanes can be modified with glycosaminoglycans such as hyaluronic acid or heparin to limit protein adsorption and platelet adhesion [32,33]. Although these results showed the excellent properties of surface modified polyurethane, a systematic study about the best isocyanate in terms of hemocompatibility is generally omitted.

In this work, we describe the organocatalyzed polymerization of 5 commercially available isocyanates and the cytotoxicity and hemocompatibility measurements of the resulting polyurethanes by cellular, protein and platelet responses to the material. We focused our study in aliphatic diisocyanates, because PU based on aromatic diisocyanates (TDI and MDI) produced carcinogenic aromatic diamines, such as toluene diamine (TDA) or 4,4́-methylene dianiline (MDA) upon degradation and are not ideal targets for nanomedicine [34]. Polyurethanes based on cycloaliphatic diisocyanates such as *trans*-1,4-cyclohexylenediisocyanate (CDI), 4,4́-Methylenebis(cyclohexyl isocyanate) (H_{12}MDI) and isophorone diisocyanate (IPDI) were compared with linear aliphatic diisocyanates such as hexamethylene diisocyanate (HDI) and lysine diisocyanate (LDI) in terms of hemocompatibility and citotoxicity.

2. Materials and methods

2.1. Materials

Hexamethylene diisocyanate (HDI, ⩾99.0%), isophorone diisocyanate (IPDI, 98%), *trans*-1,4-cyclohexylene diisocyanate (CMDI), 4,4′-methylenebis(cyclohexyl isocyanate) (HMDI, 90%), L-lysine diisocyanate were purchased from Sigma-Aldrich and used as received. 1,3-propanediol (98%) was dried by azeotropic distillation in benzene (99.8%) and poly(tetrahydrofuran)($M_n \sim 2000$) was dried by azeotropic distillation in toluene anhydrous (99.8%). 1,8-Diazabicycloundec-7-ene (DBU, 99%) was distillated under vacuum. Dry dichloromethane (DCM) (SeccoSolv®) was purchased from Merck Millipore and used as received. Poly(2-methoxyethyl acrylate) (PMEA; M_n 35,000, $Đ_M$ 2.6) and poly(ethylene terephthalate) (PET) sheets (thickness 125 μm) were used as negative and positive controls respectively in the platelet adhesion, protein adsorption test and WST cell proliferation assay.

2.2. Methods

^{1}H Nuclear Magnetic Resonance (NMR) spectra were recorded at room temperature on Bruker spectrometers operating at 300, 400 MHz or 500 MHz, using deuterated chloroform ($CDCl_3$) as solvent. Fourier transform infrared-attenuated total reflection (FTIR-ATR) spectroscopy was performed on Nicolet Magna 6700 spectrometer at a resolution of 2 cm^{-1}, and a total

Please cite this article in press as: A. Basterretxea et al., Biocompatibility and hemocompatibility evaluation of polyether urethanes synthesized using DBU organocatalyst, Eur. Polym. J. (2016), http://dx.doi.org/10.1016/j.eurpolymj.2016.08.008