Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 4 (2008) 976–988
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Segmented poly(esterurethane urea)s from novel urea–diol chain extenders: Synthesis, characterization and in vitro biological properties

P.C. Caracciolo [a], A.A.A. de Queiroz [b], O.Z. Higa [c], F. Buffa [a], G.A. Abraham [a,*]

[a] *Instituto de Investigaciones en Ciencia y Tecnología de Materiales, INTEMA (UNMdP-CONICET), Av. Juan B. Justo 4302, B7608FDQ Mar del Plata, Argentina*
[b] *Department of Physics and Chemistry, Instituto de Ciências Exatas, Universidade Federal de Itajubá (UNIFEI), Av. BPS. 1303, 37500-903 Itajubá, Minas Gerais, Brazil*
[c] *Instituto de Pesquisas Energéticas e Nucleares-IPEN/CNEN-SP, São Paulo, Brazil*

Received 25 September 2007; received in revised form 7 December 2007; accepted 4 February 2008
Available online 2 March 2008

Abstract

This work describes the preparation, physicochemical characterization, mechanical properties and in vitro biological properties of two bioresorbable aliphatic segmented poly(esterurethane urea)s (SPEUU) based on poly(ε-caprolactone) diol (PCL diol), 1,6-hexamethylene diisocyanate and two novel urea–diol chain extenders. To strengthen the interactions through hydrogen bonding in the hard segments of SPEUU, novel chain extenders containing urea groups were synthesized and used in the SPEUU formulation. The different chemical structures of the chain extenders modulated the phase separation of soft and hard segments, as demonstrated by the thermal behavior. The hard segment association was enhanced using a diurea–diol chain extender. The biological interactions between the obtained materials and blood were studied by in vitro methods. Research on the protein adsorption, platelet adhesion and thrombus formation is presented. Studies of protein adsorption onto polymeric surfaces showed that SPEUU adsorbed more albumin than fibrinogen. Studies on platelet adhesion and thrombus formation of SPEUU-coated coverslips indicated the antithrombogenic behavior of these surfaces. The synthesized SPEUU revealed no signs of cytotoxicity to Chinese hamster ovary cells, showing satisfactory cytocompatibility.

Keywords: Segmented poly(esterurethane urea)s; Chain extender; Blood compatibility; Cytotoxicity

1. Introduction

Having been used in the medical field for almost half a century, segmented polyurethanes (SPU) remain one of the most common groups of biomaterials applied to the fabrication of implantable devices, owing to the possibility of tailoring their physical properties [1]. The development of biodegradable and biocompatible SPU and polyurethane networks with labile moieties susceptible to hydrolysis in the polymer backbone has been a subject of considerable interest in recent years [2–5]. The highly variable chemistry of SPU may be used to generate polymers that exhibit a wide range of physical and chemical properties. In this sense, a careful selection of monomers can lead to a biodegradable SPU whose properties can be tailored to many different biomedical applications. Depending on their mechanical properties, chemical composition and surface characteristics, biodegradable SPU can potentially be used in designing cardiovascular implants, drug delivery devices, non-adhesive barriers in trauma surgery, injectable augmentation materials and tissue adhesives [1,6].

Nowadays, the use of polyurethanes in tissue–organ regeneration scaffolds for replacement or repair of a wide range of biological tissues is an area of intensive research,

* Corresponding author. Tel.: +54 223 481 6600; fax: +54 223 481 0046.
E-mail address: gabraham@fi.mdp.edu.ar (G.A. Abraham).

1742-7061/$ - see front matter © 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2008.02.016

and some examples of their uses in medicine are in articular cartilage repair, cancellous bone graft substitutes, artificial skin, cardiovascular tissue engineering, meniscal reconstruction and meniscal prostheses.

The unique and versatile properties of SPU elastomers are directly related to their two-phase microstructure, with hard domains acting as thermally reversible crosslinking points, and soft domains constituting the flexible segments [7]. Polyester soft segments can be used to provide hydrolytically labile segments. When appropriately designed, chain extenders are able to impart specific properties to the material. Thus, labile groups may be introduced in the hard segments through the use of appropriate short-chain compounds [8,9], and the incorporation of urea linkages strengthens hard segment interactions through the hydrogen bonding of urea groups in adjacent chains [10].

Biomedical segmented poly(esterurethanes) (SPEU) and segmented poly(esterurethane urea)s (SPEUU) based on PCL soft segments of different chain lengths and aliphatic diisocyanates, such as 1,4-butanediisocyanate (BDI), hexamethylene diisocyanate (HDI), L-lysine ethyl (or methyl) ester diisocyanate (LDI), 1,4-*trans*-cyclohexane diisocyanate (CHDI) and isophorone diisocyanate (IPDI), have been synthesized using a broad variety of chain extenders. There are several examples of chain extenders reported in the literature, including (i) short-chain diamines: 1,4-butanediamine or putrescine (BDA) [11–14], and amino acids or amino acid derivatives such as L-lysine ethyl ester or L-ornithine ethyl ester [5,15] and L-phenylalanine-based diester [16,17]; (ii) short-chain diols: 1,4-butanediol (BDO) [14,18,19], 1,4:3,6-dianhydro D-sorbitol or isosorbide diol [20], cyclohexane dimethanol [21], ethambutol dihydrochloride [22], 1,3-propanediol [23], 3-hexine-2,5-diol [2], and triblocks of BDO-BDI-BDO [14,24]; (iii) short-chain amino-diols such as 2-amino-1-butanol [20]; (iv) sulphydryl compounds such as 2-mercaptoethyl ether [20]; and (v) short-chain diurea–diphenols based on tyramine or tyrosine [10,25]. Water was also used to create hard segments of different lengths through urea-linkage formation [26].

It is well documented in the literature that the tissue compatibility of SPU depends upon the response of cells and enzymes on the material after implantation in the biological organism. The most important factors which affect SPU biocompatibility are the protein adsorption and cellular interactions that will occur at the biomaterial–tissue interface [27]. The study of in vitro and in vivo cellular behavior at the interface of synthetic materials used for implantable devices or cell culture scaffolds is fundamental. In vitro tests such as cytotoxicity, protein adsorption and blood-cell interaction with SPU may provide findings regarding the interactions of biomedical devices in biological media in a short period of time, which would contribute to minimize animal testing.

In this work, two linear SPEUU were synthesized from poly(ε-caprolactone) diol (PCL diol), HDI and two novel aliphatic urea–diol chain extenders prepared and characterized in the authors' laboratory. The physicochemical properties of the synthesized macrodiol, chain extenders and polymers are described, and the in vitro biological properties are addressed and discussed.

2. Materials and methods

2.1. Materials

ε-caprolactone, triethylene glycol (TEG), 2-aminoethanol (AE) and HDI were obtained from Aldrich, and used as received except for TEG, which was dried under vacuum at 60 °C for 24 h prior to use. LDI was kindly donated by Kyowa Hakko Kogyo Co., Ltd, Japan. Stannous 2-ethylhexanoate (Aldrich) and dibutyltin dilaurate (Fluka) were used as catalysts. *N*,*N*-dimethylacetamide (DMAc) (Aldrich) was kept over molecular sieves (4 Å) and distilled under vacuum. Human serum albumin (HSA) and fibrinogen (HFb) were purchased from Sigma. Protein solutions were freshly prepared in 10 mM phosphate buffered saline (PBS, pH 7.4) to a concentration of 1 mg ml^{-1} before each experiment.

2.2. Synthesis of SPEUU

2.2.1. PCL diol synthesis

Linear PCL diol with number-average molecular weight (M_n) 2200 Da was synthesized by charging a pre-determined amount of ε-caprolactone into a three-necked flask containing dried TEG. Stannous 2-ethylhexanoate (0.1 wt.%) was then added, and the reaction was carried out in bulk for 24 h with magnetic stirring in a nitrogen atmosphere at 130 °C.

2.2.2. Chain extenders synthesis

Two urea–diol chain extenders were synthesized from aliphatic diisocyanates (LDI or HDI) and AE at a molar ratio of 1:2. The reactions were carried out at 0 °C with magnetic stirring and nitrogen flow. The absence of an isocyanate peak (2250–2280 cm^{-1}) in the Fourier transform infrared (FTIR) spectrum confirmed complete conversion, which yields the chain extenders AE–L–AE and AE–H–AE, respectively.

2.2.3. SPEUU synthesis

SPEUU were synthesized via a standard two-step polymerization. Briefly, PCL diol was reacted with HDI in a 1:2.01 molar ratio at 80 °C in a nitrogen atmosphere. The pre-polymerization step proceeded for 1 h with stirring in the presence of dibutyltin dilaurate (0.1 wt.% of macrodiol). The prepolymers were subsequently reacted with either AE–L–AE or AE–H–AE chain extenders for 6 h at 80 °C. The resulting slurry was precipitated over cold distilled water, washed and dried under vacuum. The samples were designated PHL and PHH, depending on the chain extender used: AE–L–AE and AE–H–AE, respectively. Films were prepared by solution casting. SPEUU were dissolved in DMAc