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New poly(ester urea) derived from L-leucine: Electrospun scaffolds loaded with antibacterial drugs and enzymes

Angélica Díaz [a], Luis J. del Valle [a], David Tugushi [b], Ramaz Katsarava [b], Jordi Puiggalí [a,*]

[a] Departament d'Enginyeria Química, Universitat Politècnica de Catalunya, Av. Diagonal 647, Barcelona E-08028, Spain
[b] Institute of Chemistry and Molecular Engineering, Agricultural University of Georgia, 13 km. David Aghmashenebeli Alley, Tblisi 0131, Georgia

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ABSTRACT

Electrospun scaffolds from an amino acid containing poly(ester urea) (PEU) were developed as promising materials in the biomedical field and specifically in tissue engineering applications. The selected poly(ester urea) was obtained with a high yield and molecular weight by reaction of phosgene with a bis(α-aminoacyl)-α,ω-diol-diester monomer. The polymer having L-leucine, 1,6-hexanediol and carbonic acid units had a semicrystalline character and relatively high glass transition and melting temperatures. Furthermore it was highly soluble in most organic solvents, an interesting feature that facilitated the electrospinning process and the effective incorporation of drugs with bactericidal activity (e.g. biguanide derivatives such as clorhexidine and polyhexamethylenebiguanide) and enzymes (e.g. α-chymotrypsin) that accelerated the degradation process. Continuous micro/nanofibers were obtained under a wide range of processing conditions, being diameters of electrospun fibers dependent on the drug and solvent used.

Poly(ester urea) samples were degradable in media containing lipases and proteinases but the degradation rate was highly dependent on the surface area, being specifically greater for scaffolds with respect to films. The high hydrophobicity of new scaffolds had repercussions on enzymatic degradability since different weight loss rates were found depending on how samples were exposed to the medium (e.g. forced or non-forced immersion). New scaffolds were biocompatible, as demonstrated by adhesion and proliferation assays performed with fibroblast and epithelial cells.

1. Introduction

Poly(ester urea)s (PEUs) have been proposed as a new class of α-amino acid-based polymers with bioabsorbable properties. These polymers can be easily prepared from bis(α-amino acid)-alkylene diester monomers, which can undergo either nonspecific (chemical) or specific (enzymatic) hydrolysis due to the presence of two ester linkages per elemental unit in the molecule. The first syntheses were reported in the late 1970s by Huang et al. [1] and yielded low molecular weight powdery samples (M_n close to 2000 g/mol). Later, Yoneyama et al. synthesized high molecular weight PEUs by condensing the above diester–diamine monomers with non-physiological diisocyanates [2]. In order to avoid the use of diisocyanates, other syntheses based on polycondesation processes through active carbonates (e.g. di-*p*-nitrophenyl carbonate) were investigated [3]. However, presumably intramolecular cyclization with hydantoin formation, which represent a chain scission process, led to low molecular weight polymers.

Problems were solved when an acid chloride of carbonic acid (phosgene, diphosgene, triphosgene) was entered into the polycondensation reaction with a di-*p*-toluenesulfonic acid salt of a bis(α-amino acid)-alkylene diester (Fig. 1a) [4]. In the interfacial polycondensation reaction, the nucleophilic amino group was readily revealed by addition of an inorganic base, such as NaOH, $NaHCO_3$ and Na_2CO_3. This method provides high-yield, high-molecular weight PEUs potentially useful for biomedical applications because of their advantageous mechanical, chemical and biodegradation properties over well-known, chemically similar poly(ester amide)s also derived from α-amino acids [5]. For example, the PEU derived from carbonic acid, L-leucine, and 1,6-hexanediol (named 1L6, as indicated in Fig. 1a) has tensile strength at yield, elongation at break and Young's modulus of 21 MPa, 114% and 622 MPa, respectively [4]. Its melting temperature is 114 °C and its glass transition temperature is 47 °C. New PEUs were proposed to be useful as implantable surgical devices such as vascular stents and hard tissue replacement implants, and also for delivery of a variety of pharmaceutical and biologically active agents to humans and other mammals.

Micro/nanofiber nonwoven scaffolds produced by electrospinning have shown great potential for tissue engineering applications because of their typically high surface area and porosity. Electrospinning is a well-known electrostatic technique that uses a high voltage field to charge the surface of a polymer solution droplet at the end of a capillary

* Corresponding author.
E-mail address: Jordi.Puiggali@upc.edu (J. Puiggalí).

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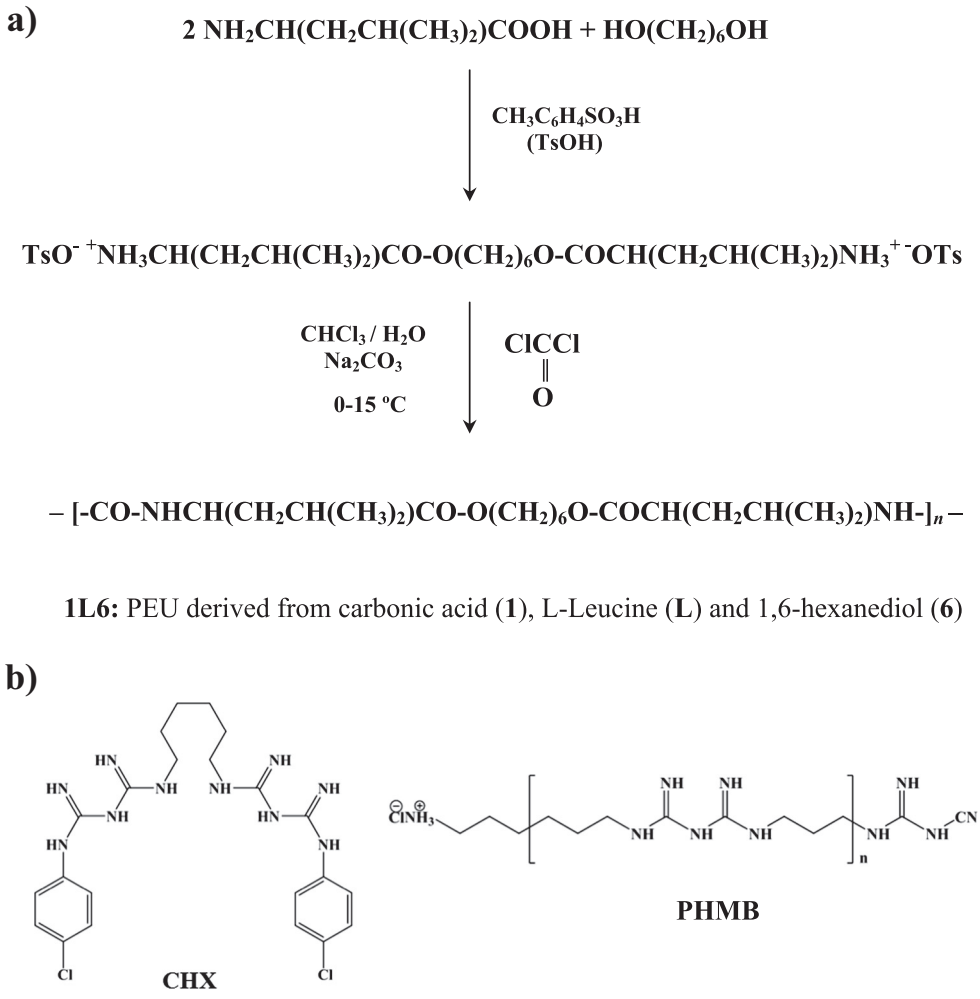

Fig. 1. a) Synthesis scheme for the poly(ester urea) derived from L-lysine, 1,6-hexanediol and carbonic acid. b) Chemical structures of selected antibacterial drugs: chlorhexidine (CHX) and polyhexamethylenebiguanide hydrochloride (PHMB).

tube and induce the ejection of a liquid jet towards a grounded target (collector) [6–9]. Morphology of fibers obtained in the collector depends on the solution properties (e.g. viscosity, dielectric constant, volatility and concentration) and operational parameters (e.g. strength of the applied electric field, deposition distance and flow rate), which should be conveniently addressed [10,11].

The unique properties of electrospun fibers have triggered a wide range of other potential applications [12], including composites [13,14], sensors [15], protective clothing [16], filtration membranes [17–20], magneto-responsive fibers and superhydrophobic membranes [21]. In addition, the electrospinning process provides a simple way to encapsulate drugs within a micro/nanofiber matrix that can lead to a controlled and sustained release. Several natural and synthetic biodegradable polymers have been successfully electrospun (e.g. polyglycolide [22], polylactide [22,23], polycaprolactone [24], collagen [25,26] and chitosan [26,27]).

The main goal of the present work is to explore the possibilities of PEUs, and specifically of the 1L6 sample, for preparing electrospun scaffolds. Furthermore, loading with anti-bactericidal agents having biguanide groups is explored, as well as the possibility of incorporating degrading agents such as a proteolytic enzyme like α-chymotrypsin. To the best of our knowledge, this is the first time that a poly(ester urea) has been assayed as an electrospinnable polymer, which is in itself an interesting topic because it adds to the range of materials useful for tissue engineering applications. Furthermore, development of antibacterial nanofibers through electrospinning is nowadays a relevant topic for wound dressing applications as has recently been reviewed by Gao et al. [28]. Different systems have been considered taking into account the substrate polymer (e.g., polylactide and polycaprolactone), the antibacterial agent (e.g., antibiotic, bactericide, silver and metal oxide nanoparticles and chitosan) and the applied procedure (incorporation of the agent in the electrospinning solution, coaxial electrospinning, previous encapsulation of the antibacterial agent, conversion of a precursor to its active form by a post-treatment and attachment of the active agent onto the fiber surface). Specific systems based on the use of bactericide agents are summarized in Table 1 [28], which also reveals the relevance of the use of biguanide derivatives.

Biguanide (Fig. 1b), commonly known as chlorhexidine (1,1′-hexamethylene-bis-5-(4-chlorophenyl) biguanide, CHX), is a widely employed antimicrobial agent [42]. Specifically, CHX is an important antiseptic, disinfectant, pharmaceutical and cosmetic preservative and antiplaque agent. Its high activity against microorganisms is provided by the presence of secondary amines that can be protonated, and therefore positively charged under normal pH conditions [43]. Immobilization of antimicrobial agents may reduce patient exposure to active agents and potentially increase the duration of antimicrobial efficacy [44,45]. One way to achieve immobilization is by loading micro/nanofibers of electrospun scaffolds with the desired drug.

Other chemical compounds bearing biguanide groups have been developed [46,47]. For example, polyhexamethylenebiguanide hydrochloride (PHMB) is a cationic oligomer having an average of 7–11 biguanide groups spaced by flexible hexamethylene segments (Fig. 1b). PHMB has