ELECTROSPUN SCAFFOLDS CONTAINING PGlPDL NPs SYNTHESIZED VIA E-ROP FOR TISSUE ENGINEERING APPLICATIONS

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ABSTRACT

Developing materials for designing tissue engineering devices is a challenging effort, as they must meet specific requirements for this application, such as adequate mechanical properties, morphology, biocompatibility, and hydrophilicity. The incorporation of nanoparticles into the scaffolds offers several advantages, such as the incorporation of drugs or marker agents to promote enhanced healing. Herein, we synthesized the copolymer poly(globalide-co-pentadecalactone) via e-ROP as a starting material to produce nanoparticles, which were subsequently incorporated into poly(vinyl alcohol) (PVA) fibers through electrospinning. The proven biocompatibility of hydrophilic PVA, combined with the versatility of PGlPDL NPs, are promising for designing various tissue engineering devices. The designed scaffolds with incorporated nanocarriers have potential to act as a multifunctional platform, providing the mechanical support required for cells and enabling controlled drug release, marking a significant step forward in the development of more effective medical treatments.

Keywords: Enzymatic ring-opening polymerization; *Candida Antarctica* Lipase B; Tissue engineering; Polymer nanoparticles; Electrospinning.

1 INTRODUCTION

Tissue engineering scaffolds act as an artificial extracellular matrix (ECM), providing biomechanical support for cell growth, vascularization, proliferation, and differentiation. For this reason, materials designed for this application must have proper mechanical characteristics, such as morphology, tension strength, and porosity, and proper hydrophilicity and biodegradability when it is required [1,2]. Concerning the manufacturing processes, electrospinning has emerged as a powerful tool for designing scaffolds with topographical features and easy processability, allowing a high control fiber diameter and scaffold porosity, resembling the natural ECM [3]. Bioactive agents, such as growth factors or biologically active drugs can be incorporated into these scaffolds aiming to accelerate the healing process via stimulation of cell differentiation or by reducing the inflammatory response through a drug release. The incorporation can be done via blend formation, different manufacturing processes such as coaxial electrospinning, or by dispersing nanocarriers loaded with these materials into the fibers [4,5].

Polymer nanoparticles embedded into electrospun mats aiming to design scaffolds for tissue engineering have been merely reported in the literature, in contrast to magnetic or inorganic nanoparticles [6–9], usually employed to design theranostic or anti-bacterial devices. Polyester nanoparticles are suitable materials to act as nanocarriers due to their high stability, bioavailability, biodegradability and enhanced targeting properties, allowing a controlled release of their loaded content [10]. Moreover, they can be synthesized via green routes involving biobased monomers and high-yield and enzyme-catalyzed reactions under mild conditions, placing these materials as excellent candidates for nanocarrier development.

Herein, we aim to prepare 3D scaffolds via electrospinning made with poly (vinyl alcohol), (PVA), a hydrophilic and biocompatible polymer, with embedded nanocarriers of poly(globalide-co-pentadecalactone) nanoparticles (PGlPDL NPs) for application as a tissue engineering device in wound dressings. PGlPDL was synthesized via enzymatic ring-opening solution copolymerization using NVZ-435 (immobilized Candida Antarctica Lipase B) as the catalyst. Nanoparticles of PGlPDL were produced via miniemulsification and solvent evaporation methods and dispersed in a PVA solution prior to electrospinning. The designed scaffolds can act as a multifunctional platform to combine mechanical support required for the cells with a controlled drug release through the incorporation of the nanocarriers into the scaffolds, being one step forward in the development of more effective medical treatments.

2 MATERIAL & METHODS

Dichloromethane P.A. 99.8% (DCM), propanone P.A. and ethanol P.A. were purchased from Vetec Química Fina Ltda. (Rio de Janeiro – Brazil). Toluene P.A was purchased from Dinâmica Química Contemporânea Ltda. (São Paulo – Brazil). All solvents were used without any purification. The enzyme Novozym 435 (lipase B from Candida Antarctica immobilized on cross-linked polyacrylate beads) was donated by Novozymes A/S (Barigui – Brazil). The esterification activity of the enzyme was 28.5 U/g. [11] Enzymes were dried under vacuum before polymerization (65 °C, 24h), and stored at a desiccator over silica and 4Å molecular sieves. ω-pentadecalactone (ω-PDL) was purchased from Sigma Aldrich (São Paulo – Brazil), and globalide (Gl) was donated by Symrise (São Paulo – Brazil). Both monomers were dried before polymerization, for 24 h at 80 °C under vacuum conditions and

stored in a desiccator over silica and 4 Å molecular sieves. Poly (vinyl alcohol) (PVA) (Mw=72.000 g/mol, hydrolysis degree 87-89%) was purchased from Dinâmica Química Contemporânea Ltda. (São Paulo – Brazil). Sodium dodecyl sulfate (SDS) was purchased from Neon Comercial (Suzano, São Paulo, Brazil).

The copolymerization of Gl and ω-PDL was carried out in a 25 mL vial in a proportion of 2:1 of the total monomers to the solvent (toluene). The monomers feed mass ratio (Gl: PDL) was fixed at 75:25. All the reagents were weighted precision balance (ATX224 Shimadzu, Japan). Novozym 435 was employed at 5 wt% to the total amount of monomers. After reaching the reaction temperature of 65 °C (sand bath), the reaction was carried out for 2 h under magnetic stirring. The reaction conditions were chosen based on previous studies [3,12]. After the polymerization the copolymer was purified: first, it was solubilized in dichloromethane (DCM); then, the enzyme was removed by filtration, and finally, the copolymer was separated from residual monomers and oligomers through precipitation in a cold solution of ethanol: acetone (3:1), in a proportion of 1:6 (v/v) related to the copolymer. After the precipitation, the copolymer was dried in an oven at 60 °C overnight. Typical yields of the copolymerization reactions were 70%. Monomer conversion and copolymer composition were determined using Nuclear Magnetic Resonance (‘H NMR, Bruker Company, USA) operating at 200 MHz. Samples weighing 10 mg were solubilized in 0.55 mL of CDCl3 (δ=7.26 for 1H NMR). Copolymer properties were evaluated in terms of Molecular Weight Distribution using Gel Permeation Chromatography (HPLC, model LC 20-A, Shimadzu do Brasil – Brazil); and thermal properties using Differential Scanning Calorimetry (DSC, Jade DSC Perkin Elmer®, USA), at a heating and cooling rate of 10 °C min^{-1}, in an inert atmosphere of nitrogen at 50 mL min^{-1}.

PGlPDL nanoparticles (NPs) were produced via miniemulsification followed by solvent evaporation. The organic phase was composed of 350 mg of the copolymer solubilized in 2.65 mL of dichloromethane (DCM), It was stirred up to complete solubilization. The aqueous phase comprised 15 mL of distilled water and 30 mg of Sodium Dodecyl Sulfate (SDS), which acts as a surfactant. The coarse emulsion was created by mixing the organic and aqueous phases under magnetic stirring. Miniemulsification was performed using an ultrasonic probe employed at the amplitude of 70%, in a 10s on/ 10s off pulse, for 3 min. After miniemulsification, the colloidal dispersion was heated at 50 °C and maintained under stirring and heating up to complete solvent evaporation (1h). The polymer NPs dispersed in water were then stored at 4 °C. Intensity average particle size and particle size distribution were measured using Dynamic Light Scattering (DLS – Malvern Instruments, Zetasizer Nano S).

For the electrospinning, 2 mL of the prepared NPs dispersion was mixed with 2 mL of an aqueous solution containing PVA at 20 wt%, for 2h, to reach a final PVA concentration of 10 wt%. The resultant solution containing the NPs was placed into a 5mL syringe, connected to a metallic needle. Operational conditions of the electrospinning were 10 kV of tension, 15 cm of distance tip-to-collector, and a flow rate of 1 $mL.h^{-1}$. After electrospinning (2h), scaffolds were dried overnight at 60 °C. A goniometer (Krüss ,DSA25E) was used to evaluate the scaffold's hydrophobicity, and the resultant morphology was investigated using Scanning Electron Microscopy (SEM) (VEGA3 TESCAN, Czech Republic).

3 RESULTS & DISCUSSION

The enzymatic ring-opening copolymerization between ω-pentadecalactone and globalide resulted in a copolymer with an average molecular weight (M_w) of 43.000 g/mol, number molecular weight (M_n) of 9220, and a polydispersity of 4.63, with monomodal distribution. Similar results were obtained by Tinajero-Diaz, Ilarduya and Muñoz-Guerra [13] in the copolymerization of Gl and ω-PDL, with Mn in the range of 9.000-12.000 g/mol for different copolymer compositions. Copolymer NMR spectrum after purification and thermogram are shown in Figure 1.

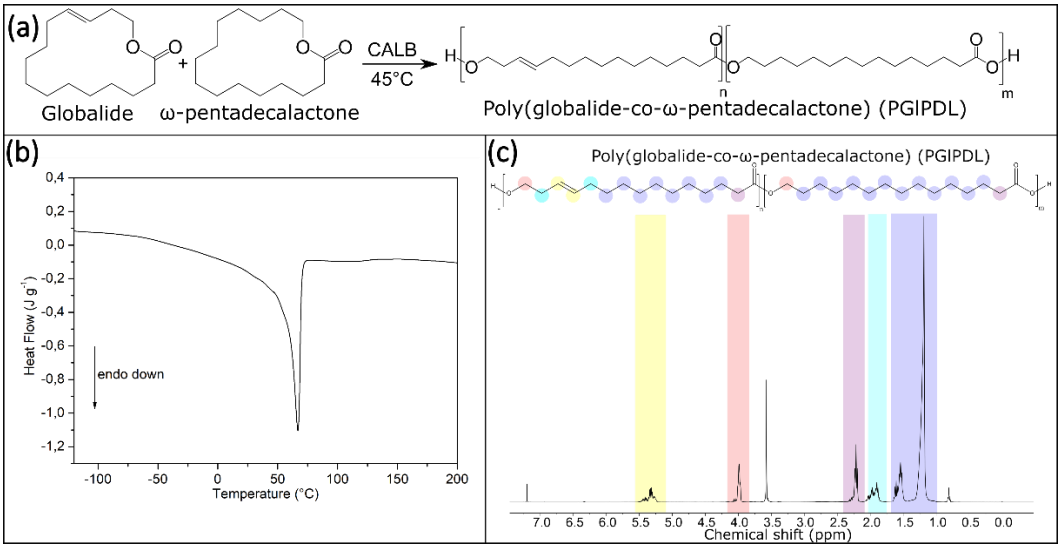

Figure 1 PGlPDL chemical and thermal properties. (a) Overall e-ROP between ω-PDL and Gl, (b) Thermogram from DSC analysis, (c) ^{1}H NMR spectrum

Copolymerization conversion was evaluated before purification, using NMR analysis, by comparing the methylene peaks between the monomer and the final copolymer. It reached 100% after 2h of reaction. Also, the comonomer feed ratio (75:25 Gl:PDL) was equal to the copolymer composition calculated based on the NMR spectrum, evidencing that there was no preference in interacting with the enzyme active site between the two comonomers. Regarding the thermal properties, the melting temperature of PGlPDL copolymer reached 63 °C, while the melting enthalpy was equal to 80 J g^{-1}. The degree of crystallinity was calculated based on a 100% crystalline sample of PPDL [14], and this value was equal to 34%. The presence of a single intermediate melting temperature in the copolymer serves as an indication of isomorphic crystallization, suggesting a random copolymer [15–18].

The copolymer miniemulsification followed by solvent evaporation led to the formation of nanoparticles with a mean particle diameter (D_p) of 160 nm and a polydispersity index (PDI) of 0.15, indicating a narrow particle size distribution. After the preparation of the NPs, the hydrophobic nanoparticles were incorporated into a PVA solution with a final PVA concentration of 10 wt%. Homogeneous fibers were obtained by electrospinning, with well-dispersed nanoparticles throughout the fiber extension.

The PVA matrix presented several suitable attributes for tissue engineering, such as biocompatibility and hydrophilicity. PGlPDL nanoparticles incorporated on PVA scaffolds can act as nanocarriers for several different bioactive hydrophobic molecules, such as drugs, growth factors, or dyes, for further application in drug delivery devices or theranostic agents. The hydrophobic character of these NPs may contribute to a prolonged release of the capsule content. Thus, the combination of PGlPDL NPs embedded into PVA fibers may constitute a promising platform for application as wound dressings with a controlled drug release that promotes enhanced healing of the damaged tissues.

4 CONCLUSION

In the present work, PGlPDL nanoparticles were satisfactorily produced via the solvent evaporation method, employing the PGlPDL copolymer (75:25 Gl) as the starting material, produced via enzymatic ring-opening copolymerization catalyzed by immobilized *Candida Antarctica* Lipase B. The resultant nanoparticles exhibited adequate properties in terms of crystallinity and hydrophobicity, for further application as nanocarriers for the prolonged release of drugs and/or marker agents, such as dyes. The incorporation of these nanoparticles into electrospun fibers was successfully performed by dispersing the NPs in a PVA solution prior to electrospinning. The proven biocompatibility of hydrophobic PVA, combined with the versatility of PGlPDL NPs, is promising for designing different tissue engineering devices. These devices could potentially be applied as wound dressings containing hydrophobic drugs or markers encapsulated in PGlPDL nanoparticles, which can prolong the release of these active molecules due to their hydrophobic character, thereby enhancing the wound healing process. This work highlights the versatility of enzymes in designing robust polymeric materials for the development of more effective medical treatments.

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