Fabrication and enhanced mechanical properties of porous PLA/PEG copolymer reinforced with bacterial cellulose nanofibers for soft tissue engineering applications

Mustafa Abu Ghalia, Yaser Dahman*

Department of Chemical Engineering, Ryerson University, Toronto M5B 2K3, Ontario, Canada

ABSTRACT

A new class of polylactic acid (PLA)/polyethylene glycol (PEG) copolymer reinforced with bacterial cellulose nanofibers (BC) was prepared using a solvent casting and particulate leaching methods. Four weight fractions of BC (1, 2.5, 5, and 10 wt%) were incorporated into copolymer via silane coupling agent. Mechanical properties were evaluated using response surface method (RSM) to optimize the impact of pore size, porosity, and BC contents. Compressive strength obtained for PLA/PEG-5 BC wt% was 9.8 MPa, which significantly dropped after developing a porous structure to 4.9 MPa. Nielson model was applied to investigate the BC stress concentration on the PLA/PEG. Likewise, krenche and Hapli-Tasi model were employed to investigate the BC nanofiber reinforcement and BC orientation into PLA/PEG chains. The optimal parameters of the experiment results found to be 5 wt% for BC, 230 μm for pore size, and 80% for porosity. Scanning electron microscopy (SEM) micrograph indicates that uniform pore size and regular pore shape were achieved after an addition of BC-5% into PLA/PEG. The weight loss of copolymer-BC with scaffolds enhanced to the double values, compared with PLA/PEG-BC % without scaffolds. Differential Scanning Calorimetric (DSC) results revealed that the BC nanofiber improved glass transition temperature (T_g) 57 °C, melting temperature (T_m) 171 °C, and crystallinity (χ %) 43% of PLA/PEG reinforced-BC-5%.

1. Introduction

The research on the repair and regeneration of damaged tissue focuses on creating new approaches to tissue replacement and overhaul [1]. Tissue engineering shows a great promise for overcoming this issue [2]. Polymer biomaterial is one of the most desirable material that can be used as a scaffold for tissue regeneration. However, it should satisfy a number of requirements. These involve biocompatibility, degradability, non-toxic material, with appropriate pore size, porosity, as well as supporting cell attachment, and proliferation. Toughness and elasticity are considerably important mechanical properties for soft tissue, such as muscle, bladder, skin, and blood vessel [3]. The polymers applied in tissue engineering are dominated by synthetic biodegradable polyester such as; poly (lactic acid) (PLA), polyglycolide (PGA), and their blends. Nonetheless, these polyesters have long degradation rate, low flexural modulus, and hydrophobicity, which considered as

influential subjects to confront the substantial biomedical requirements. The biomaterial used for the repair soft tissues should be able to reproduce the inherent tissue elasticity. The polyether compound such as poly(ethylene glycol) (PEG) has been studied for soft tissue engineering. PEG is a well-recognized nontoxic polymer and exhibits much greater elasticity than PLA [4]. The choice of a hydrophilic PEG to be combined with PLA in the fabricated of soft-tissue engineering scaffolds is typically based on accelerating degradation, improve elasticity, and biocompatibility [5].

Biocellulose (BC) nanofibers are potentially considered as one of the utilizing natural resources to employing into the degradable polymer. Lately, several scientists are attracted to the BC nanofibers, due to the superiority in biocompatibility and mechanical properties. BC can be prepared from fermentation process throughout a specific type of bacterial strains. The unique structure of BC is formed from the pure form of cellulose nanofibers, which contains a high degree of crystallinity associated with a high degree of polymerization [6]. These exceptional properties also possess potential forerunner for development technologies in the various significant area, such as tissue engineering, sustainable polymeric biomaterials, and green nanocomposites [7].

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* Corresponding author.
   E-mail address: ydahman@ryerson.ca (Y. Dahman).

BC nanofiber reinforced into PLA nanocomposite has gained much consideration because of their low density, inexpensive, non-abrasiveness, very low toxicity and degradable properties. Several reports [6–8] revealed that the mechanical and thermal properties of degradable polymers are enhanced when using an appropriate compatibilizer with the cellulose nanofibers. The non-uniform dispersion can cause inferior tensile strength and high water absorption. Hence, the suitable interface compatibilizer agent between the PLA matrices and BC nanofibers would lead to improving BC distribution. Meanwhile. Reduce surface polarity between BC content and PLA leads to improve dispersion of BC nanofibers [9,10].

In recent years, surface modification of (BC) by grafting coupling agent into PLA has demonstrated a special interest in reducing the number of hydroxyl groups and enhance the surface hydrophobicity of BC nanofiber, [11]. Silane chains can enhance interaction between PLA matrices and BC nanofibers [12]. Moreover, the presence of PEG in PLA blends lead to improving hydrophilicity and accelerate its degradation rate. Various silanes are potentially considered as effective agents in enhancing the interface between PLA/PEG blends and BC nanofibers. This involves the alkoxysilanes forming bonds with BC hydroxyl function groups. Additionally, the organofunctional group is spontaneously reacting with PLA through covalent bonding [13,14].

The objective of the present work is to investigate the impact of the modified BC nanofibers orientation in PLA/PEG blends (porous and nonporous) on the mechanical properties. Subsequently, Response surface method is used to achieve the modeling process and predict the optimal condition of each factor; BC nanofiber loading, pore size, and porosity. In addition, the influence of these factors on the morphological study and hydrolytic behavior were also examined.

2. Materials

2.1. Materials

PLA was obtained through polycondensation process in our Lab with melting point 170–180 °C, and number average molecular weight (M_n) of 64,166 g/mol. Ethylene Glycol (EG) monomer (purity 99.7% and M_n = 62.07 g/mol). Chemical solutions such as 1,4 dioxane solution, M_n = 88 g/mol, purity 99.8%, and a triethoxy-vinylsilane coupling agent, purity 89%, M_n = 148 g/mol were purchased from Sigma-Aldrich. BC nanofibers were prepared as reported elsewhere [15] and were kept in the suspension solution of 42 g/L. Sodium Chloride (NaCl) Salt (Sigma Ultra 99.5%), Mn = 58.44 g/mol with well-defined particle crystal sizes was used for the formation of porous structure in scaffolds.

2.2. Synthesis of PLA/PEG copolymer

Step-growth polymerization was conducted for 80 ml of lactic acid with 20 ml of ethylene glycol as the diol in the absence of solvent, which added to the 1000 ml reaction vessel with a five neck head seal. The reactor was set in an oil bath equipped with mechanical stirring, as described in our previous research [16]. The copolymers were synthesized by direct polycondensation (DP) through three operations: distillation, oligomerization, and polymerization. The pressure was reduced incrementally, and then the polymerization temperature was set at 180 °C for the last 5 min of terminated polymerization. Class of chain extender (styrene-glycidyl acrylate copolymer) (1.25 wt %) was added during the polymerization, and the final sample was purified by ethanol and precipitated in an evaporating chamber.

2.3. Synthesis of BC nanofibers

BC nanofibers were prepared via saccharification and fermentation (SSF) based exclusively on the natural agricultural sources. These sources are used as the fermentation carbon basis; the samples treated with 1% diluted sulfuric acid and placed on the adequate fermentation process using *Gluconacetobacter xylenes bacterium*. The amount of BC produced was then stored for 1 week, and subsequently, the suspension solution was pretreated with the base solution (0.2 M NaOH) at 100 °C. In order to remove the produced BC nanofibers, the suspension solution washed and centrifuged five times. The final solution of BC nanofibers obtained as suspension solution with the concentration of 42 g/L [6,15].

2.3.1. Surface treatment of BC

Surface treatment of BC nanofiber was performed in the 1, 4 dioxane solution. BC (10 g) and coupling agent (1 g) were placed in a flask with the accurate volume of solvent. The procedure is described in the literature [17]. A certain quantity of triethoxy-vinilesilane coupling agent added in the flask. After agitation for 30 min, the flask closed with a plastic film and stored for 24 h at ambient temperature.

2.4. Preparation of the PLA/PEG copolymer-BC nanocomposites

The PLA/PEG was prepared in a reaction vessel equipped with an overhead stirring shaft. The copolymer and BC were heated at 60 °C for 2 h until the relative vapor content was less than 1.0 wt%. Afterward, the PLA/PEG heated for about half an hour at 110 °C, ensuring all PLA/PEG convert to the high viscous solution, and later on the BC suspension solution was mixed with a presence of dicumyl peroxide for 15 min at 170 °C and rotation speed 60 rpm. The BC nanofiber amounts were 1 wt%, 2.5 wt%, 5 wt%, and 10 wt%. The formulation for the samples is illustrated in Table 1. The BC treated coupling agent was grafted onto the PLA/PEG side chains and therefore form BC-reinforced PLA/PEG as illustrated in the reaction (Fig. 1). All the samples were poured using tensile prototype molding (2 mm thick) at 110 °C and subsequently kept for 24 h at ambient temperature under vacuum to eliminate the solvent and allow the copolymer chains to reconstruct [18].

2.5. Developing of porous structure

The NaCl powder was grounded and filtered within the range of 125 μm and 355 μm sieves, using Retsch mesh standard testing (Haan, Germany; DIN 4188 and ISO 3310). NaCl crystals were then added at a ratio of 2.5 g/gram of PLA/PEG-BC (1, 2.5, 5, and 10 wt%) and dissolved by heating at 80 °C in dioxane. The BC-reinforced-PLA/PEG/salt solution casting in prototype and put under vacuum for 24 h in order to eliminate the solvent. The sample was dry PLA/PEG -glued salt. Later on, the sample soaked in water for 12 h to filter out the salts and freeze dried in order to ready for further characterizations. The full removal of progeny leads to the formation of interconnected pore structure [17,18].

2.6. Fourier transform infrared spectroscopy (FTIR)

FTIR spectrometry was carried out in a Perkin-Elmer Spectrum 2000 FTIR (Ryerson University Chemistry Lab) with an attenuated total reflectance (ATR) crystal accessory (Golden Gate). All spectra were determined by five running scans at 2 cm^{-1} resolutions, ranges between 500 and 4000 cm^{-1}.