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Fabrication of novel high performance ductile poly(lactic acid) nanofiber scaffold coated with poly(vinyl alcohol) for tissue engineering applications

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ABSTRACT

Poly(lactic acid) (PLA) nanofiber scaffold has received increasing interest as a promising material for potential application in the field of regenerative medicine. However, the low hydrophilicity and poor ductility restrict its practical application. Integration of hydrophilic elastic polymer onto the surface of the nanofiber scaffold may help to overcome the drawbacks of PLA material. Herein, we successfully optimized the parameters for *in situ* deposition of poly(vinyl alcohol), (PVA) onto post-electrospun PLA nanofibers using a simple hydrothermal approach. Our results showed that the average fiber diameter of coated nanofiber mat is about 1265 ± 222 nm, which is remarkably higher than its pristine counterpart (650 ± 180 nm). The hydrophilicity of PLA nanofiber scaffold coated with a PVA thin layer improved dramatically (36.11 ± 1.5°) compared to that of pristine PLA (119.7 ± 1.5°) scaffold. The mechanical testing showed that the PLA nanofiber scaffold could be converted from rigid to ductile with enhanced tensile strength, due to maximizing the hydrogen bond interaction during the heat treatment and in the presence of PVA. Cytocompatibility performance of the pristine and coated PLA fibers with PVA was observed through an *in vitro* experiment based on cell attachment and the MTT assay by EA.hy926 human endothelial cells. The cytocompatibility results showed that human cells induced more favorable attachment and proliferation behavior on hydrophilic PLA composite scaffold than that of pristine PLA. Hence, PVA coating resulted in an increase in initial human cell attachment and proliferation. We believe that the novel PVA-coated PLA nanofiber scaffold developed in this study, could be a promising high performance biomaterial in regeneration medicine.

1. Introduction

Biodegradable synthetic polymer fibers have attracted massive interest as effective substitute materials in regeneration medicine applications [1,2]. Specifically, poly(lactic acid) (PLA) is one of the most widely used synthetic polymers in this field due to the non-toxicity of lactic acid, which is naturally present in the human body, and FDA-approved [3–6]. However, there are some major limitations such as the hydrophobic nature and poor ductility of PLA which hinder its practical use as substitute materials in tissue regeneration [3]. It is known that the surface wettability reflects the adhesion, growth of cells, and protein absorption on the surface of the material [3,6]. Some researchers noticed that the porous scaffolds fabricated from PLA are floating in cell culture medium [7]. Thus, the hydrophobic nature of PLA is a serious problem in a predominantly hydrophilic bioenvironment where the cells fail to have initial attachment to the implanted scaffolds.

Mechanical properties play a crucial role in determining the *in vivo* performance of the scaffolds in the tissue engineering field, such as vascular graft system [4], bone implants, and wound dressing [8]. The scaffold has to be composed of a durable biomaterial capable of withstanding physiological hemodynamic forces while maintaining structural integrity until mature tissue forms *in vivo*.

Electrospun nanofibers represent an emerging class of biomimetic nanostructures that can act as proxies of the native tissue, while

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providing topographical and biochemical cues that promote tissue healing. Development of advanced nanofiber scaffold in the second half of the 20th century resulted in a marked revolution in advanced structural materials [9] mainly because of the brittleness characteristics of PLA nanofibers as a potential scaffold material [4,10,11].

Many efforts have been invested to address the aforementioned limitations of PLA nanofibers. For instance, poly blend nanofibers prepared by simple pre-mixing solution or melt-blending of PLA with organic polymers [4,11–15] such as thermoplastic starch (TPS) were reported [14]. However, microscopic observations revealed non-uniform dispersed PLA inclusions within the TPS matrix. Pitarresi et al. [16], produced electrospun nanofiber scaffolds by employing PHEA-g-PLA copolymer as a starting material. However, these scaffolds didn't provide significant biocompatible properties. Pi et al. [15], noticed the occurrence of microphase separation in the coating layer through the film drying because of the dissimilarity of PLA and polyethylene oxide (PEO). This is because PEO is water soluble, while PLA is water-insoluble. Therefore, the preparation of wettable and ductile PLA scaffold using a facile approach for biomedical applications remains a challenge. As a result, changing the mechanical properties of post-electrospun PLA nanofibers from brittle/rigid to ductile using a simple, cost-effective approach is one of the main challenges in the present study. Successful development of a hydrophilic and ductile PLA nanofiber scaffold will open a new era towards the designing of high performance advanced biomaterials in the field of tissue engineering. In this paper, we successfully developed a facile strategy that is based on a surface modification of the post-electrospun (PE) PLA nanofibers through deposition of a hydrophilic and elastic layer on each single PLA nanofiber using a simple hydrothermal route. The novelties of this paper are: a) optimizing of a simple and cost-effective hydrothermal process for fabrication of a novel PLA based-scaffold with unique morphology and mechanical and biological properties; b) overcoming the lacks of pristine PLA; and c) introducing the amine groups in the fibers for improving the cell adhesion and proliferation in tissue engineering.

Recently, our research group has successfully exploited a simple and inexpensive hydrothermal strategy for *in situ* deposition of inorganic compounds onto PE nanofibers and creating advanced high performance 3D composite scaffolds for medical implants [17,18]. Interestingly, our process has no side effect on the properties of the polymer fibers. Furthermore, it provides sufficient interfacial bonding between the polymer fibers and deposited compounds, and subsequently improves the mechanical properties of the scaffold. This novel process has been successfully used by our group to apply an *in situ* deposition of a wettable and elastic polymer thin layer on PE PLA nanofiber.

In this study, poly(vinyl alcohol) (PVA) was selected to conformal coat each single PLA nanofiber because it is a water-soluble synthetic polymer that possesses good biocompatibility, biodegradability, and excellent mechanical properties [4]. PVA, in general, has a high water content and tissue-like elasticity. The abundant hydroxyl groups on PVA can be readily modified to attach growth factors and adhesion proteins. We speculate that the hydrophilic properties of PVA molecules can create a good physical/chemical interaction with PLA nanofibers on the molecular level by forming strong hydrogen bonding throughout a hydrothermal treatment, and thereby enhancing the surface and mechanical properties of the PLA nanofibers. It is worthmentioning that exploiting our strategy can overcome the difficulty of mixing both PLA and PVA phases at the macromolecular level because PVA is more hydrophilic than PLA. Hence, the composite PVA/PLA nanofibrous scaffold that integrates the favorable wettability properties of PVA and elasticity as well as FDA approval of PLA is expected to significantly improve the material properties for tissue regeneration applications. Additionally, the cytocompatibility performance of the PLA nanofiber scaffold coated with a hydrophilic PVA thin layer using hydrothermal strategy was studied using the MTT test. The molecular interactions between PLA fiber and PVA molecules during the hydrothermal process were discussed in detail.

2. Experimental

2.1. Fabrication of nanofibers

The electrospinning setting used in the current research for fabrication of nanofibers and PLA pellets is a type of Ingeo Biopolymer 2003D, (a Nature Works, LLC (USA) product supplied by Green Chemical Co., Ltd., Korea) as described in detail in our previous report [19]. Briefly, the solution for electrospinning was prepared by dissolving PLA in dichloromethane (Junsei Chemical Co., Japan) at a concentration of 10 wt.%. The injection rate and the applied voltage were 0.5 ml/h and 18 kV, respectively. The collected nanofiber mat was placed in a vacuum oven for 24 h at 40 °C to remove any potential residual solvents.

A PVA-coated PLA electrospun mat was prepared following these procedures: (1) the PLA as-electrospun mat was cut into rectangular specimens (30×20 mm^2). (2) These specimens were immersed into optimized freshly prepared 1 wt.% PVA aqueous solution (it was observed that increasing the PVA solution concentration >1 wt.%, affects negatively the surface morphology of the nanofiber mats as shown in Fig. S1 of the supplementary materials). PVA solution viscosity at 1 wt.% was measured by a Brookfield, DV-III ultra programmable Rheometer at room temperature and the result was about 17 cP. (3) 40 ml of PVA solution containing the PLA electrospun nanofiber mat was placed in a Teflon-lined autoclave container and heat-treated at 150 °C for 30 min. Previous studies reported that PVA and PLA polymers have good thermal resistivity up to this temperature [20,21]. (4) After the reaction process is complete, the PVA-coated PLA mats were gently rinsed in distilled water to remove unattached PVA molecules from PLA nanofiber mats. (5) The resultant coated samples were left to dry at room temperature for four days until steady weight and then introduced into a vacuum oven (10 mbar) at 35 °C for 72 h. (6) The amount of PVA adhering to the surface was evaluated from the dry weight of the substrates.

2.2. Surface/material characterizations

The surface micrographs of the pristine and composite mats were characterized by a field emission scanning electron microscope (FESEM; Hitachi S-7400, Japan). The micrographs of the coated samples were taken at an accelerating voltage of 5 kV and with magnifications of 5 and 25 K. To measure the fiber diameters, the FESEM images were processed and analyzed by means of ImageJ software (National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/). FTIR (MB100 spectrometer, Bomen, Canada) analysis in a transmission mode was used to identify the functional groups thereby reflecting phase structure of pristine and hydrothermally treated samples. Thermogravimetric analysis (TGA) was performed by a TGA-DSC, Q-20 Perkin-Elmer Inc., USA, at a heating rate of 20 °C/min with a constant purge of N_2. Differential scanning calorimetry (DSC) data were obtained from a Perkin-Elmer Pyris Diamond DSC. Samples were scanned at a heating rate of 10 °C/min in N_2 environment. The Tg values were measured as the change of the specific heat in the heat flow curves.

2.3. Surface wettability measurements

Flat mats were used to evaluate the hydrophilicity of pristine PLA and PVA/PLA composite nanofiber (treated) mats, using the water contact angle (WCA) measurements. 3 μl of purified water (ultrapure grade) was pipetted out on top of the shiny side of 30×30 mm^2 mats positioned on the stage of a bench-type contact angle goniometer (GBX; Digidrop, France), ensuring that the membrane mat was completely flat. The micrographs were taken after 1 s and WCA measurement was recorded. To confirm the coating homogeneity and coating distribution of a PVA layer on the PLA membrane mat, the WCA was measured at five different positions on each flat mat surface.