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Electrospun poly(D,L-lactic acid)/gelatin membrane using green solvent for absorbable periodontal tissue regeneration

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Abstract Electrospinning was performed using an eco-friendly solvent composed of acetic acid, ethyl acetate and distilled water to investigate the effect of gelatin concentration on mechanical properties and cytotoxicity of absorbable poly(D,L-lactic acid) (PDLLA)/gelatin blend membrane. The tensile stress, strain at break, and WUC of the PDLLA/gelatin (97/3) scaffold at 26 wt% concentration were determined to be 3.9 ± 0.7 MPa, 37 ± 1.3 %, and 273 ± 33 %, respectively. FT-IR results revealed that PDLLA and gelatin were bound only by van der Waals interactions. The cell viability of PDLLA/gelatin membranes containing 0 %, 1 %, 2 %, 3 %, and 4 % gelatin were more than 100 %, which makes all membranes highly suitable as a barrier membrane for absorbable periodontal tissue regeneration due to their marketed physical properties and biocompatibility.

Key words Absorbable periodontal tissue regeneration, Barrier membrane, Poly(D,L-lactic acid) (PDLLA), Gelatin, Electrospinning, Synthetic graft

1. Introduction

Periodontitis is a disease in which the tissue around the teeth becomes inflamed and destroys the gingiva and bone that support the teeth. Without proper treatment, the alveolar bone that supports the teeth can be destroyed. As gingivitis progresses, the gums and teeth separate from periodontal pockets [1-6]. Periodontal regeneration is known as regeneration of tissues supporting teeth, such as cementum, periodontal ligament and alveolar bone. Absorbable periodontal tissue regeneration membrane is widely used in clinical applications in areas where bone quality is poor in the implant placement site or surrounding areas. In case of tooth loss, a bone graft material is placed, an implant is placed on top of it, and an absorbable periodontal regeneration membrane is placed in the form of a shield to protect the bone graft material from the outside. Otherwise, rapidly growing gingiva easily grows into the alveolar bone [1-6].

The periodontal regeneration membrane is synthesized using absorbable biodegradable materials and is decomposed in the body after a customized duration after implantation. Absorbable barrier membrane is widely used in dentistry because it does not require secondary surgery compared to non-absorbable Ti or poly(tetrafluoroethylene) film [7]. Synthetic implant materials used to induce periodontal tissue regeneration use synthetic biocompatible polymers such as polylactic acid (PLA) and are mainly manufactured using 3D printing or electrospinning [2,4,6]. The hydrophobicity of PLA can be solved by alloying it with natural polymers such gelatin, chitosan, starch, and collagen [1-6]. The hydrophilicity and decomposition of the guided tissue regeneration membrane play an important role in periodontal regeneration as the rate of degradation should match the rate of new tissue formation [1].

Biopolymeric scaffolds composed of synthetic and natural polymers are important in tissue engineering due to their suitable mechanical properties, biocompatibility and biodegradability. Nanofibrous PLLA (poly(L-lactic acid)/gelatin scaffolds with specific ratios of PLLA and gelatin exhibit appropriate mechanical properties and biological functions. Because PDLLA (poly(D,L-lactic acid) has low crystallinity, low mechanical properties and fast degradation rate, it can be applied as a drug delivery system or scaffold material for tissue engineering. Racemic mixtures of equimolar compositions between PLLA and PDLA have been demonstrated to yield stereopolymer blends of PLLA/PDLA with tensile strength, elastic modulus and elongation at break superior to those of

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the PLLA and PDLA enantiomers [2,4]. The development of electrospun hybrid polymers is hampered by the limited number of green solvents that can be used to dissolve both synthetic and natural polymers [8-14]. Fluorinated alcohols with strong groups ($C_3H_2F_6O$ (1,1,1,3,3,3-hexafluoro-2-propanol, HFIP) and $C_2H_3F_3O$ (2,2,2-trifluoroethanol, TFE)) are widely used as solvents, but polymers are rapidly decomposed, expensive, and toxic [1-6]. Non-toxic aqueous solvents have emerged as solvents for dissolving and electrospinning natural polymers [10,11]. Green solvents of acetic acid, ethyl acetate and distilled water were used in this study. Hydrophilicity and cell proliferation of the hybrid membrane can be achieved by adding gelatin to PDLLA [15]. The effect of polymer concentration on the physical properties and water uptake capacity (WUC) of the PDLLA/gelatin membrane was investigated. In addition, cytotoxicity was examined to evaluate the biocompatibility of the PDLLA/gelatin membrane.

2. Experimental

2.1. Materials

PDLLA (Resomer® R207S) and gelatin from porcine skin (Type A) were purchased from Sigma-Aldrich, USA. Acetic acid (99.5 %) and ethyl acetate (99.5 %) were used as they were purchased from Samchun Pure Chemical Co., Ltd., Korea. A green solvent for PDLLA/gelatin was prepared by mixing acetic acid, ethyl acetate and distilled water (3:2:1 volume ratio) [10,11].

2.2. Electrospinning

Membranes were fabricated using an electrospinning equipment consisting of a syringe pump (KDS-200, Stoelting Co., USA), a BD metal needle, a grounded collector, and a high-voltage power supply (ES30P-5W, Gamma High Voltage Research Inc., USA) [4,6,11,12]. The PDLLA/gelatin solution was drawn into a 10 mL BD luer-lock syringe attached to a syringe pump and fed to a 20 gauge metal needle at a flow rate of 1 mL/h. PDLLA/gelatin fibers were collected at a voltage of 20 kV and a distance of 15 cm using a rotating drum collector with a diameter of 9 cm and a length of 20 cm. The rotational speed of the mandrel-type collector and the transverse speed of the needle have been described elsewhere [8-11]. PDLLA containing various gelatin concentrations ranging from 0 to 4 wt% was prepared by dissolution in green solvent using a magnetic stirrer for 48 h at room temperature. The solution viscosity was measured using a viscometer (DV 1M, Brookfield, USA). Fourier-transform infrared spectroscopy (FT-IR, Spectrum Two, PerkinElmer, UK) was examined to study the chemical bonding of the PDLLA/gelatin polymer [8-11,15-17]. The surface morphology of the membrane was examined by using an SEM (S-3000H, Hitachi, Japan) and an optical stereomicroscope (SV-55, Sometech, Korea). Fiber diameter was evaluated using an optical microscope equipped with *iSolution Lite image* software [8-11]. The tensile strength of the membrane was examined using an Instron 5564 with a crosshead speed of 10 mm/min. Specimens were prepared in dumbbell shape according to ASTM D-638 (type V). All experiments were performed at least 5 times. The WUC of the membrane is determined by a previously reported equation [10,11,14]. The experimental results were expressed as mean±standard deviation, and $p < 0.05$ was considered statistically significant [10,11].

2.3. Cytotoxicity (cell viability)

The extract test method was conducted on the PDLLA and PDLLA/gelatin membranes to evaluate the potential of cytotoxicity on the base of the International Organization for Standardization (ISO 10993-5) [10,11,18,19]. Membranes were aseptically extracted from the medium and the ratio of the membranes to extraction vehicle was 6 cm^2/mL. Detailed experimental procedures are described elsewhere [8-11,15-21].

3. Result and Discussion

The tensile strength and failure strain of various PDLLA membranes over a range of PDLLA concentrations from 20 wt% to 30 wt% are shown in Fig. 1. A uniform and continuous PDLLA fiber without beads was observed. In the present study, a PDLLA solution with a concentration of 26 wt% was investigated. However, the strength of the 26 wt% PDLLA membrane was not sufficient for application in periodontal tissue regeneration. A 4-layer PDLLA membrane with a thickness range of 0.2 to 0.4 mm was prepared for strength enhancement. The strength of the 4-layer PDLLA membrane increased from 2.9 MPa to 3.6 MPa, but the WUC decreased from 100 % to 77 %. Gelatin is a polypeptide derived from the hydrolysis of collagen and has been used as a biological polymer due to its water solubility and low

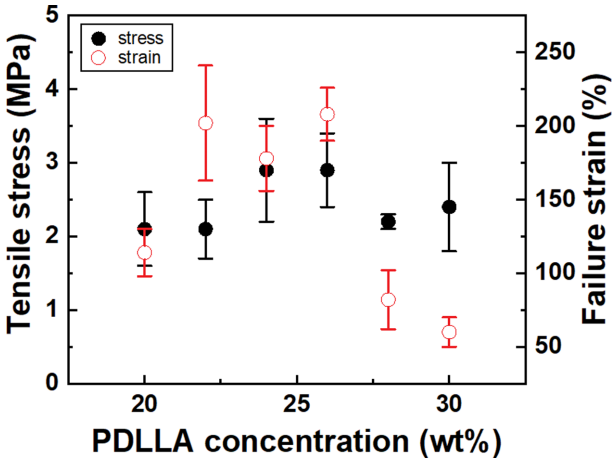

Fig. 1. Tensile strength and strain at break of various PDLLA scaffolds.

immunogenicity [10,11]. PDLLA/gelatin with a certain ratio of PDLLA and gelatin exhibits adequate physical properties and biological functions [8-15]. Electrospinning of the PDLLA/gelatin solution at a concentration of 26 wt% was investigated.

SEM images of the surfaces of PDLLA/gelatin membranes with different gelatin content are displayed in Fig. 2. Gelatin molecules with good dielectric constants are prone to charging during electrospinning [4,8]. Electrospinning jets containing higher gelatin content is therefore more likely to have a higher excess charge, resulting in thinner fibers [4]. The fiber diameter of PDLLA/gelatin membrane decreased from 936 nm to 610 nm as the gelatin concentration increased from 0 % to 4 wt%. Nanofibrous PDLLA/gelatin membranes containing specific ratios of PDLLA and gelatin exhibit better WUC due to the addition of hydrophilic gelatin. No noticeable difference in strength was observed with increasing gelatin concentration (Fig. 3). This may be because the gelatin concentration is so low that PDLLA

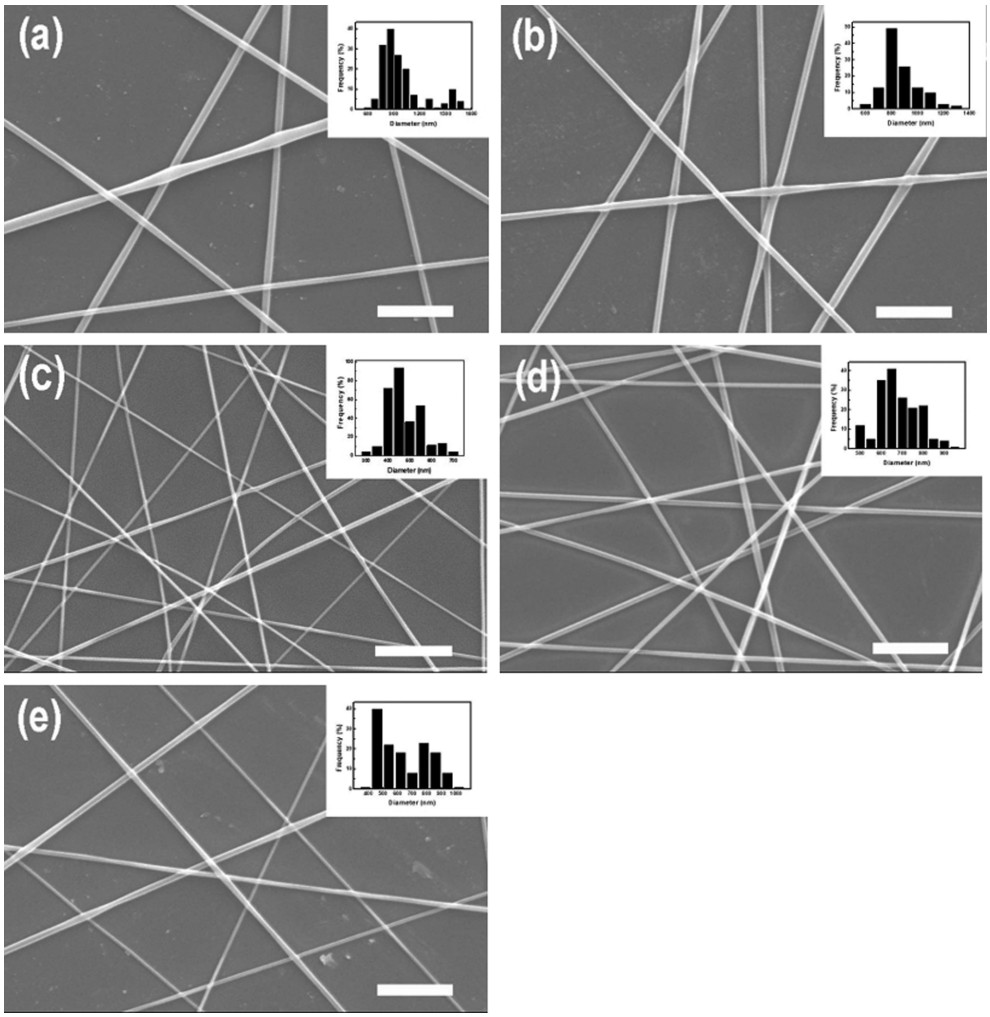

Fig. 2. SEM images of electrospun PDLLA/gelatin membranes containing different gelatin concentrations: (a) 0 wt%, (b) 1 wt%, (c) 2 wt%, (d) 3 wt% and (e) 4 wt%, respectively. Note that the scale bar is 10 μm.

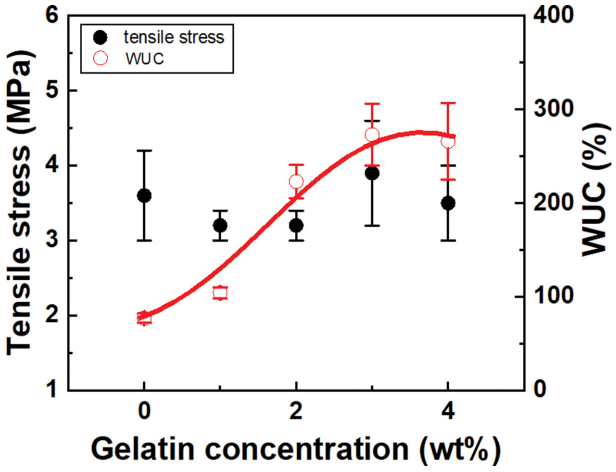

Fig. 3. Tensile stress and WUC of various PDLLA/gelatin scaffolds containing different gelatin concentrations.

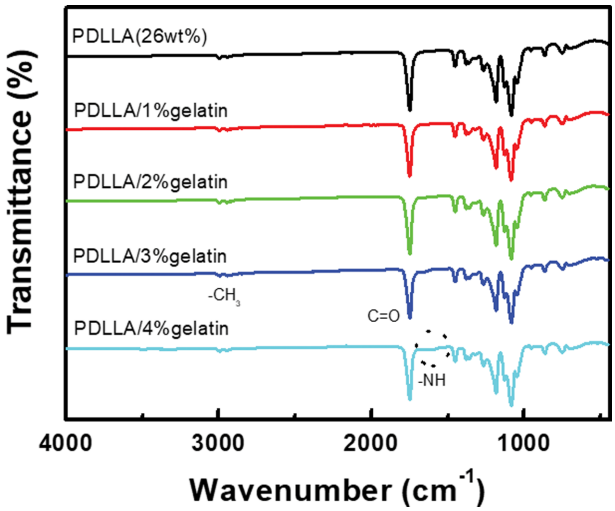

Fig. 4. FT-IR spectra of various 26 wt% PDLLA/gelatin scaffolds containing different gelatin concentrations.

mainly contributes to the strength of the PDLLA/gelatin blend membrane [4]. However, the hydrophilicity of the PDLLA/gelatin blend membrane was dramatically improved by adding gelatin to PDLLA and reached a steady state with additional gelatin doping [4,8-15]. As the gelatin concentration increased from 0 % to 3 %, WUC increased from 77 % to 273 %, as displayed in Fig. 3. In this study, an eco-friendly PDLLA/gelatin membrane prepared with green solvents was successfully optimized. The tensile stress, strain at break, and WUC of the 97/3 PDLLA/gelatin scaffold at 26 wt% concentration were measured to be 3.9 ± 0.7 MPa, 37 ± 1.3 %, and 273 ± 33 %, respectively. A biodegradable commercial membrane (NeoDura) manufactured by Medprin Biotech GmbH (Germany) is widely used as a synthetic membrane. The strength, strain at break and WUC of NeoDura composed of PLLA and collagen were reported to be 3.7 ± 0.5 MPa, 55 %, and 455 %, respectively [15]. The low strain and WUC of the PDLLA/gelatin scaffold is most likely due to the slow solidification process of the aliphatic polymer chains in a green solvent [10,11]. Rapid chemical reactions to improve physical properties of scaffolds can be achieved using fluorinated alcohol solvents (HFIP, TFE) with strong functional groups [4,10-15], but further studies are needed.

FT-IR spectra of various 26wt% PDLLA/gelatin membranes containing different gelatin concentrations are shown in Fig. 4. PDLLA is a racemic mixture of PDLA and PLLA enantiomers, a chiral structure containing 4 different ligands. PDLLA's characteristic carbonyl group (1757 cm^{-1}) and methyl groups (-CH_3, 2995 cm^{-1}) were clearly visible. Amide I (1640 cm^{-1}) and II (1540 cm^{-1}) peaks representing gelatin were barely visible due to the low gelatin content. However, there were no new FT-IR peaks, indicating that PDLLA and gelatin were bound only by van der Waals interactions.

The cytotoxicity of PDLLA/gelatin membranes with gelatin concentrations ranging from 0 to 4 % determines toxicity. The cell viability of PDLLA/gelatin membranes containing 0 %, 1 %, 2 %, 3 % and 4 % gelatin was 100 %, 103 %, 114 %, 115 % and 105 %, respectively, compared to the negative control, indicating that all scaffolds were not cytotoxic under the condition of this study, as shown in Fig. 5. Among the scaffolds, the PDLLA/gelatin scaffold containing 3 % gelatin was highly suitable as a barrier membrane for absorbable periodontal tissue regeneration because of its marketed physical properties and biocompatibility.

4. Conclusions

Biodegradable PDLLA/gelatin membranes were electrospun using an eco-friendly green solvent to investigate physical properties and cytotoxicity of PDLLA/gelatin blend membrane as a function of gelatin concentration. The tensile stress, strain at break, and WUC of the optimized PDLLA/gelatin (97/3) scaffold at 26 wt% concentration were 3.9 ± 0.7 MPa, 37 ± 1.3 %, and 273 ± 33 %, respectively. FT-IR results revealed that no chemical bonding between PDLLA and gelatin chains was observed except for van der Waals interactions. Excellent cell viability and physical properties of the PDLLA/gelatin membrane containing 3 wt% gelatin suggested

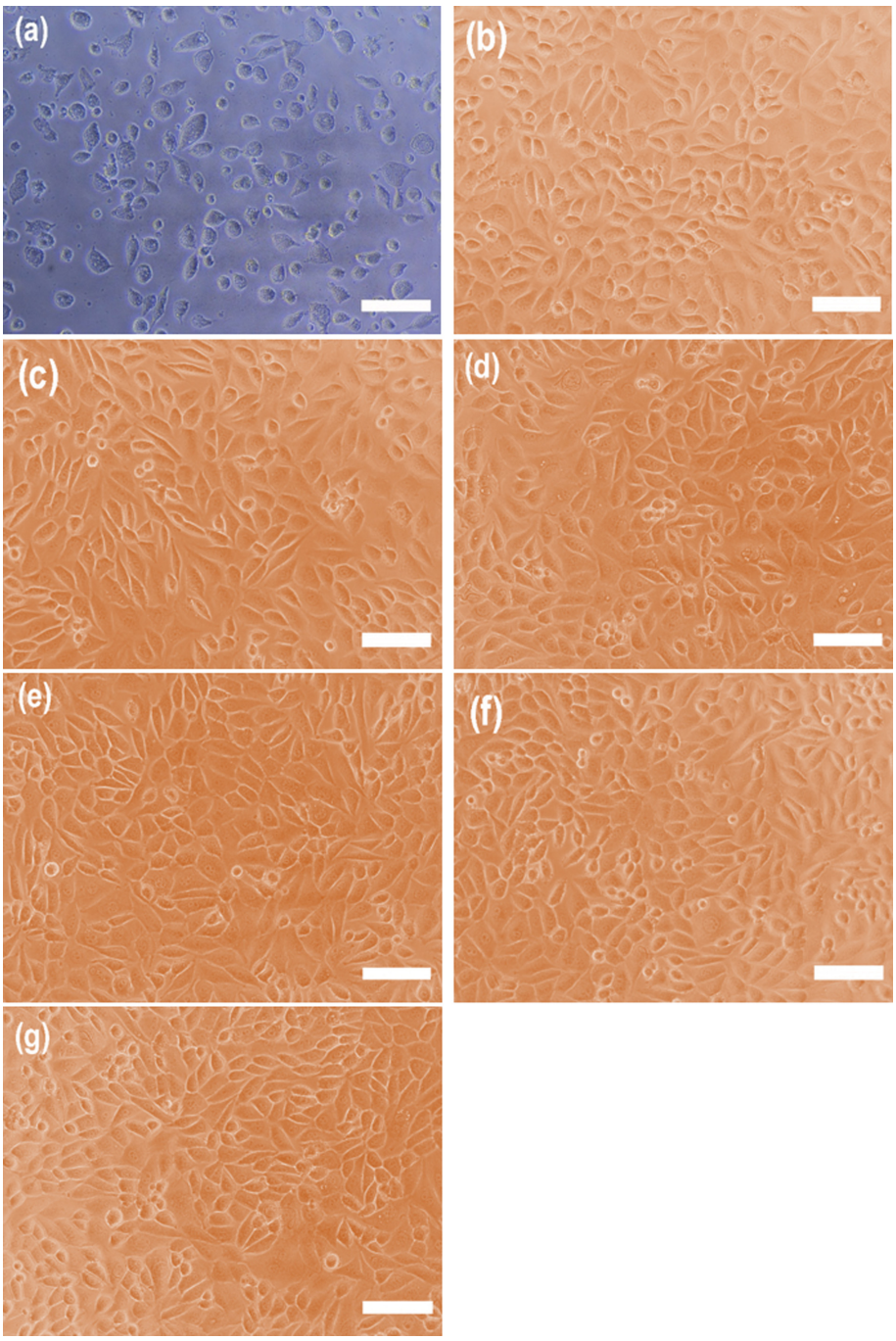

Fig. 5. Photographs of cell morphology: (a) positive control, (b) negative control, and the extracts of PDLLA/gelatin scaffolds as a function of gelatin concentration: (c) 0%, (d) 1%, (e) 2%, (f) 3%, and (g) 4% from EZ-cytox after exposing with the scaffold suspensions for 48 h. Note that the scale bar is 100 μm.

that it is highly suitable as a barrier membrane for absorbable periodontal tissue regeneration.

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