Examination of Optimal Concentrations of Poly (Lactic-co-glycolic Acid) in Hexafluoroisopropyl Alcohol for Guided Bone Regeneration Membrane Prepared by Electrospinning

Daisuke Isaji,¹ Takehiro Watanabe,² Takahiro Takahashi,² Hiroki Sato,² Hiroshi Nakada,² Yasuhiro Tanimoto,³ Megumi Fuse,⁴ Suguru Kimoto,² and Yasuhiko Kawai²

¹Nihon University Graduate School of Dentistry at Matsudo, Removable Prosthodontics, Matsudo, Chiba 271–8587, Japan
Departments of ²Removable Prosthodontics, and ³Dental Biomaterials, Nihon University of Dentistry at Matsudo, Matsudo, Chiba 271–8587, Japan

⁴Liberal Arts (Chemistry), Nihon University of Dentistry at Matsudo, Matsudo, Chiba 271–8587, Japan

Article History

Received 25 November 2019

Accepted 2 December 2019

Abstract

Purpose: Guided bone regeneration (GBR) has recently become widespread with the expansion of indications for implants, and GBR membranes are broadly categorized as non-resorbable or resorbable membranes. Resorbable membranes do not require surgical removal and they have excellent biocompatibility. However, its mechanical properties are inferior to those of non-resorbable membranes; therefore, it is challenging to handle during surgery and can easily cause infection. Hence, in this study, we evaluated a new resorbable membrane using poly(lactic-co-glycolic acid) (PLGA) and hexafluoroisopropyl alcohol (HFIP).

Methods: The membrane was prepared by electrospinning using PLGA and HFIP as the solvent; we then examined the concentration ratio of PLGA to HFIP for optimal mechanical properties and cell activity. The mechanical properties of the membrane were evaluated using scanning electron microscopy (SEM), fiber diameter, Fourier-transform infrared spectroscopy analysis, and a tensile test; membrane cell activity was evaluated using water contact angles and alkaline phosphatase staining.

Results: SEM revealed that the prepared PLGA membranes were composed of uniform, randomly oriented fibers with diameters ranging from 0.5 μm to 2.1 μm . The fiber diameter measurements and tensile tests showed that the fiber diameter and mechanical strength increased as the PLGA concentration increased. However, in terms of cell activity, membranes with high PLGA concentrations did not have a good effect on cells. Moreover, the concentration with the highest cell activity was 20 wt%.

Conclusion: This study suggested that 20 wt% is the most suitable PLGA concentration for HFIP when making a PLGA membrane by electrospinning.

Keywords :

guided bone regeneration,
electrospinning,
hexafluoroisopropyl alcohol,
poly (lactic-co-glycolic acid)

Introduction

Guided bone regeneration (GBR) has recently become an essential therapeutic procedure for bone and peri-implant defects, and it is also used for bone augmentation procedures prior to implant placement (1, 2). GBR requires a barrier membrane to prevent the invasion of soft

tissue and to create a space for guiding new bone growth into the bone defect (3). GBR membranes are necessary for the success of GBR and must be biocompatible, flexible, and have sufficient mechanical strength. GBR membranes are grouped into two categories—non-resorbable membranes and resorbable membranes—according to their degradation characteristics (4).

The non-resorbable membranes, such as polytetrafluoroethylene membrane and titanium membrane, have ex-

Correspondence to :

Daisuke Isaji

E-mail : mada16004@g.nihon-u.ac.jp

cellent space-making ability with adequate mechanical strength. However, a second surgical procedure is required to remove the non-resorbable membrane after the bone has healed.

The resorbable membrane is made of collagen, synthetic polymer, and inorganic ceramic; hence, there is no need for a second surgery. In addition, collagen allows superior cell adhesion and proliferation compared to other materials. However, the mechanical strength is low and the space between the membrane and the defect site cannot be maintained during surgery. Therefore, many researchers have explored thin GBR membranes with various combinations of synthetic biodegradable materials using traditional fabrication methods, such as solvent casting, a functionally graded multilayer(5), electrospinning, a platelet-rich fibrin membrane(an autologous membrane), and heating press.

Amongst these techniques, electrospinning is a resourceful and cost-effective technique that can be used to synthesize continuous nanofibers(6). This technique can be used for soluble or fusible polymers alone, or polymers can be modified with additives such as particles or enzymes to get the desired properties. The resultant ultrafine fibers exhibit many interesting features, such as a high surface area, tailorable porosity in the range of a submicron- to nanoscale, and greater potential for surface functionalization. In addition, electrospinning fibers are considered to be an excellent candidate for a variety of biomedical purposes, including wound dressings, drug delivery, and tissue engineering scaffolds. In electrospinning, poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), and poly-L-lactic acid have each been used for research as a biodegradable polymer. In particular, PLGA attracts considerable attention because its degradation rate can be adjusted by altering the ratio of lactic acids to glycolic acids(7).

In electrospinning, the biodegradable polymer is dissolved before jetting. Solvents used for dissolution include dichloromethane(8), acetone(9), chloroform(9), tetrahydrofuran(10), and hexafluoroisopropyl alcohol(HFIP)(11). Among these, HFIP can produce more stable fibers in terms of fiber dimensions, mechanical properties, and cytotoxicity(12).

For these reasons, it was decided to use PLGA as the biodegradable polymer and HFIP as the solvent when preparing the membrane by electrospinning. However, it

Table 1. Preparation of polymer solutions.

Group	PLGA (wt%)	HFIP (wt%)
PLGA 10wt%	10	90
PLGA 15wt%	15	85
PLGA 20wt%	20	80
PLGA 25wt%	25	75
PLGA 30wt%	30	70

Abbreviations: PLGA: poly(lactic-co-glycolic acid); HFIP: hexafluoroisopropyl alcohol

is necessary to conduct a basic study on the optimal concentrations of PLGA and HFIP related to the mechanical properties and cell activity of the PLGA membrane. The purpose of this study was to evaluate the mechanical properties of the membrane based on changes in fiber diameter, tensile test results, and Fourier-transform infrared spectroscopy(FT-IR)analysis when the composition ratio of PLGA in the HFIP was changed. Furthermore, we evaluated the cell activity based on the change in cell behavior of the water contact angle and alkaline phosphatase(ALP) activity.

Materials and Methods

Materials

Five polymers of PLGA 75/25(inherent viscosity 0.55–0.75 dL/g; DURECT Corporation, AL, USA)at concentrations of 10, 15, 20, 25, and 30 wt%, respectively, in HFIP (Wako, Osaka, Japan)were prepared and kept at 25°C overnight until the solutions became homogeneous(13).

Preparation of polymer solutions

PLGA solution was prepared by dissolving PLGA in HFIP. The compositions of the various composites are listed in Table 1.

Electrospinning(Fig. 1)

The electrospinning set-up(IMC-1639; Imoto, Kyoto, Japan)consisted of a high voltage power supply(Matsusada Precision Inc., Shiga, Japan)and a plastic syringe(Terumo Corp., Tokyo, Japan)fitted with a 21-G stainless steel needle(Terumo Corp., Tokyo, Japan). The PLGA solution was continuously supplied at flow rates of 1.5 mL/h through the needle using a syringe pump. The distance between the needle tip and the ground electrode

Fig. 1. Schematic illustration of the electrospinning apparatus.

($d=9$ cm; a stainless-steel sheet on a drum whose rotation speed could be varied) was 10 cm. Upon application of a high voltage of 15 kV between the needle tip and the collector, the PLGA solution was evaporated and fibrous PLGAs were deposited on the ground electrode at 25°C (13). The obtained fibrous PLGA was dried overnight at 25°C (14).

Physical characterization

1 Morphology

The detailed structure of respective PLGA membranes was imaged by scanning electron microscopy (SEM) (S-3400N; Hitachi, Tokyo, Japan) at an acceleration voltage of 15 kV. Before SEM observation, all of the samples were cut from a PLGA membrane (1 cm $\times$ 1 cm), and carbon deposited. The electrospinning fibers were analyzed manually by measuring the fiber diameters of 10 randomly selected fibers. Before undertaking these measurements, the contrast and threshold of each image were optimized to ensure that the fibers in the top layers were in focus. The measurements were performed using Adobe Photoshop 5.0 software (Adobe, San Jose, CA, USA).

2 Tensile test

The PLGA membranes were prepared with a test specimen punching blade No. 6 (KOBUNSHI KEIKI CO., LTD, Kyoto, Japan). The tensile tests were performed using an EZ-Test (AGS-H; SHIMADZU, Kyoto, Japan) at a crosshead speed of 10.0 mm/min (10). All tests were conducted at 25°C. Six measurements were taken at different samples, and the average value was obtained with the standard deviation.

3 Water contact angles

Water contact angles indicating the wetting ability of the materials were measured by the Drop Master (DM300, Kyowa Interface Science Co., Ltd, Saitama, Japan) at 25°C. The 1 $\times$ 1-cm freshly prepared PLGA membranes were kept at 25°C for 24 h, and then the membranes were attached to a glass slide for contact angle measurements. A single droplet of distilled water (2 μ L) was applied to the membrane surface, and the contact angle was measured after 5 seconds (7). Seven measurements were taken at different locations, and the average value was obtained with the standard deviation.

4 FT-IR analysis

The PLGA membranes were characterized by Nicolet iS 5 FT-IR (Thermo Fisher Scientific K.K., Tokyo, Japan). The infrared spectra of the samples were measured over a wavelength range of 4,000–500 cm^{-1} . All spectra were taken in the spectral range by the accumulation of 64 scans with a resolution of 4 cm^{-1} (10).

Biological characterization

1 Cell culture and proliferation assay

Mouse preosteoblasts (MC3T3-E1, RIKEN BioResource Center, Tsukuba, Japan) were cultured in Dulbecco modified α -MEM containing a 10% buffer solution (HEPES, Thermo Fisher Scientific K.K., Tokyo, Japan) and 1% penicillin and streptomycin solution ($\times 100$) (Wako, Osaka, Japan) at 37°C in 5% carbon dioxide (15).

The culture medium was changed every 3 days. For biological experiments, cultured cells were detached by trypsinization-EDTA (0.25% Trypsin-EDTA, Thermo Fisher Scientific K.K., Tokyo, Japan), suspended in new culture medium, and used for designed experiments (16).

2 ALP staining

The PLGA membranes were first cleaned three times with phosphate-buffered solution followed by isopropanol rinsing. Samples were then sterilized by ultraviolet irradiation for 20 minutes. Specimens measuring 3 $\times$ 3 cm were placed into empty wells of a 6-well culture plate. MC3T3-E1 cells were then seeded into the wells at a density of 10,000 cells per well. The plate was incubated at 37°C for up to 1 week. After 1, 4, and 7 days of incubation, ALP staining was performed using the TRAP/ALP Stain Kit (Wako). After the staining, non-adherent cells

Fig. 2. SEM images showing the morphology of respective PLGA concentrations. (Original magnification 1,000x.)

were removed by washing three times with PBS. Next, adherent cells were collected by mild trypsinization and counted using a Coulter counter (Beckman Coulter, Tokyo, Japan). The experiments were performed in quintuplicates. The results are presented as the percentage of adherent cells (2).

Statistical analysis

All values are expressed as the mean $\pm$ standard deviation. The statistical comparisons were made via analysis of variance, and the Bonferroni post-hoc test was used to evaluate differences among the groups (PASW Statistics Version 18.0, SPSS, Chicago, IL, USA). The results were considered to be statistically significant at $P < 0.05$.

Results

Histological microscope observation

SEM results are shown in Fig. 2. SEM revealed that respective PLGA membranes were composed of uniform, randomly oriented fibers. Three-dimensional, interconnected pores formed between the fibers and were distributed throughout the membranes. In addition, the 10 wt% PLGA membrane (Fig. 2a) was partially observed as beads because of its small fiber diameters, and the 30 wt% PLGA membrane (Fig. 2e) was partially observed

as merged fibers because of its thick fiber diameters.

Fiber diameter

Results of the fiber diameter are shown in Fig. 3. The fiber diameter thickened in the following order: 10 wt% ($0.53 \pm 0.29 \mu\text{m}$), 15 wt% ($0.71 \pm 0.21 \mu\text{m}$), 20 wt% ($1.45 \pm 0.57 \mu\text{m}$), 25 wt% ($1.83 \pm 0.24 \mu\text{m}$), and 30 wt% ($2.10 \pm 0.35 \mu\text{m}$).

There was a significant difference between the 10 wt% and 20 wt%, and 25 wt%, 30 wt% groups (all, $P < 0.05$). Additionally, there was a significant difference between the 15 wt% and 20 wt%, and 25 wt%, 30 wt% groups (all, $P < 0.05$). There was also a significant difference between the 20 wt% and 30 wt% groups ($P < 0.05$). The largest difference in fiber diameter was found between the 10 wt% and 30 wt% groups, with an average difference of $1.57 \mu\text{m}$.

Water contact angles

Results of the water contact angles are shown in Fig. 4. The water contact angles increased in the following order: 15 wt% ($93.40 \pm 8.11^\circ$), 10 wt% ($93.71 \pm 4.91^\circ$), 30 wt% ($93.99 \pm 9.70^\circ$), 20 wt% ($94.0 \pm 9.29^\circ$), and 25 wt% ($94.5 \pm 7.92^\circ$). There was no significant difference in the water contact angles of the prepared PLGA membranes ($P >$

Fig. 3. Comparison of Fiber diameter of different concentration of PLGA. (n=10)

Fig. 5. Comparison of Tensile strength of different concentration of PLGA. (n=6)

Fig. 4. Comparison of Water Contact Angle of different concentration of PLGA. (n=7)

Fig. 6. FT-IR spectroscopy of respective PLGA concentration. The spectrum of PLGA shows peaks at 865 cm^{-1} . And HFIP shows peaks at 1722 cm^{-1} . (a) PLGA 30 wt% (b) PLGA 25 wt% (c) PLGA 20 wt% (d) PLGA 15 wt% (e) PLGA 10 wt%

0.05).

Tensile test

The tensile test results are shown in Fig. 5. The tensile strength increased in the following order: 10 wt% (9.34 ± 0.44 MPa), 15 wt% (10.23 ± 0.79 MPa), 20 wt% (11.32 ± 1.07 MPa), 25 wt% (11.85 ± 0.60 MPa), and 30 wt% (12.67 ± 1.38 MPa). There was a significant difference between the 10 wt% and 20 wt%, and 25 wt% and 30 wt% groups (all, $P < 0.05$). Moreover, there was a significant difference between the 15 wt%, 25 wt%, and 30 wt% groups ($P < 0.05$). There was also a significant difference between the 20 wt% and 30 wt% groups ($P < 0.05$). The largest difference in tensile strength was found between the 10 wt% and 30 wt% groups, with an average difference of 3.33 MPa.

FT-IR analysis

The FT-IR spectra of respective PLGA membranes are shown in Fig. 6. The FT-IR spectra of respective PLGA membranes showed that the structure of nanofibers contained all the peaks corresponding to PLGA. Moreover, as the concentration of PLGA increased, the peak representing PLGA increased and the peak representing HFIP decreased.

ALP staining

The measured number of adherent cells after 1, 4, and 7 days of culture on respective PLGA membranes are shown in Figs. 7 and 8. After 1 day, the 10 wt%, 15 wt%, and 20 wt% groups had significantly more cultured cells than the 25 wt% and 30 wt% groups ($P < 0.05$). After 4 days, the 30 wt% group had significantly fewer cultured

Fig. 7. Alkaline phosphatase staining of respective PLGA concentrations after 1, 4 and 7 days. (n=5)

cells than the other groups ($P < 0.05$). There was no significant difference between the other groups ($P > 0.05$). After 7 days, the 20 wt % group had significantly more cultured cells than the 15 wt% and 30 wt% groups ($P < 0.05$). Additionally, the 30 wt% group had significantly more cultured cells than the 15 wt% and 25 wt% groups ($P < 0.05$).

Discussion

In this study, a membrane was prepared by electrospinning using PLGA as the biodegradable polymer and HFIP as the solvent. Moreover, the concentration of PLGA in the HFIP for optimal mechanical properties and cell activity was determined.

SEM and the electrospinning scaffolds fabricated using ultrafine fibers with diameters ranging from 100 nm to 5 μm met the conditions of extracellular matrix (ECM) mor-

phology mimicry. From the results of the tensile test, the mechanical strength increased as the concentration increased, and 30 wt% was the highest of all concentrations. Furthermore, from the result of the cell proliferation test, the 20 wt% concentration showed a higher proliferation ability than the other concentrations.

Electrospinning has been used in the fabrication of tissue engineering scaffolds, including nonwoven, three-dimensional, porous, and nanoscale fiber-based matrices (11). The characteristics of fiber scaffolds, such as those with a high surface area-to-volume ratio and those with a structural morphology similar to that of fibrous ECM, suggest that they may serve as effective scaffolds for tissue engineering.

PLGAs are biodegradable polymers, and the available forms, including PLA, PGA, and PCL, are widely used in the field of regenerative medicine. PLA has a high crys-

Fig. 8. Number of adherent cells after 1, 4 and 7 days of culture on respective PLGA concentrations. (n=5)

tallinity, and therefore, a slow degradation rate. However, it is hard, which affects its flexibility and conformity to tissue and moldability. PGA has a high degradation rate, and it is not strong enough for use as a tissue scaffold. PCL is biodegradable, biocompatible, and flexible; however, its scope of use is limited as it is scarcely used as a scaffold material because of its low melting point and slow degradation rate. The PLA/PGA ratio of PLGA can be altered to control its decomposition speed and mechanical properties. Furthermore, the United States Food and Drug Administration approved PLGA for use in the living body, ensuring that it is safe. Hoornaert et al. reported that pure PLA polymers degrade in about 7 months, and PLGA 50/50 and PLGA 70/30 copolymers degrade in about 2–4 weeks and 6–8 weeks, respectively (17).

HFIP is used as a solvent and synthetic intermediate. It is a colorless, volatile liquid, which is characterized by a strong pungent odor. HFIP is a polar solvent with strong hydrogen-bonding properties, and hence, it can be used to dissolve substances that function as hydrogen-bond receptors, such as amides and ethers.

We used PLGA and HFIP to fabricate membranes of differing PLGA concentrations by electrospinning. We conducted this study to determine the optimal concentrations of PLGA in HFIP for excellent mechanical properties in order to improve cell-cell contact, spreading, and proliferation.

By measuring the contact angle of the membrane surface at the various concentrations, it was demonstrated that there was no significant difference among the membranes. In addition, Arima et al. reported that a surface with a moderate contact angle (40–70°) can adsorb cell contact proteins competitively, thus contributing to cell contact (18). However, in this study, all the membranes had a mean angle of 93–95°. This finding suggests that the membrane surface is hydrophobic, which makes interaction with cells difficult.

Results of the tensile test revealed that the 30 wt% membrane exhibited 1.35 times higher tensile strength than the 10 wt% membrane. Furthermore, the mean value increased in the following order: 10 wt% (9.34 ± 0.44 MPa), 15 wt% (10.23 ± 0.79 MPa), 20 wt% (11.32 ± 1.07 MPa), 25 wt% (11.85 ± 0.60 MPa), and 30 wt% (12.67

± 1.38 MPa); this finding indicated that the tensile strength increased with an increase in the concentration of PLGA. This was a similar result to that reported by Chou et al. (19), in which an increase in PLGA content was accompanied by an increase in the average Young's modulus of a polymer blend fiber due to the high inherent rigidity of PLGA in the blend. In this study, PLGA was the only polymer used, which indicates that an increase in the concentration of PLGA was responsible for the increased tensile strength.

Huang et al. reported that an electrospinning scaffold made of ultrafine fibers with a diameter of 100 nm to 5 μ m mimicked the ECM morphology (20). The values of SEM and fiber diameter in this study show that the conditions reported by Huang et al. were met. Therefore, the PLGA concentration possibly did not have an effect on ECM morphology mimicry. However, in membranes with low PLGA concentrations (10 and 15 wt%), beads were observed from thin fibers, and in membranes with high concentrations (30 wt%), fused fibers were observed from thick fibers. This finding suggests that the 20 wt% and 25 wt% membranes are suitable for reproducing the morphology of stable membranes.

The FT-IR analysis of the different membranes showed a peak at 865 cm^{-1} , which indicated the presence of PLGA in the membranes. A further increase in the PLGA concentration resulted in an increase in the intensity of this peak. This result is consistent with that in the report of Hiep et al. (10), indicating that the value of the PLGA FI-TR peak is dependent on the concentration of PLGA.

The results of the percentage of the number of adherent cells showed that the number of cells on the 25 wt% and 30 wt% membranes was significantly lower ($P < 0.05$) after 1 day of culture, and that of the 30 wt% membrane after 4 days of culture was significantly lower than those of the other membranes. However, the results after 7 days of culture showed that more growth occurred on the 20 wt% membrane when compared with the other membranes. This finding suggests that regardless of the type of solvent used by Chung et al. (12) during the electrospinning, cell growth was initially slow but increased with time. These results support the reports that the electrospinning structure can promote cell growth and that the effect of residual solvent on cell behavior is not significantly detrimental. Thus, cell growth was not af-

ected by the type of solvent; however, for membranes with thick fiber diameters, such as the 25 wt% and 30 wt% membranes, the PLGA concentration was low, making cell contact more difficult than in membranes with small fiber diameters.

The above findings suggest that small-diameter fibers have a high surface area-to-volume ratio and a high length-to-diameter ratio; hence, they are considered to have parameters that are beneficial for the attachment, growth, and proliferation of cells. The large surface area of the nanofibers, which can be obtained by electrospinning, promotes cell attachment and enables the control of cell proliferation. Furthermore, the tensile strength of the membrane was directly proportional to the PLGA concentration. Thus, the present result suggested that 20 wt% is the most suitable PLGA concentration for HFIP when making a PLGA membrane by electrospinning. Future studies should monitor the PLGA membrane over a longer period of time in order to study pH changes and conduct immersion tests.

Acknowledgments

This work was supported in part by KAKENHI (17K17221) from the Japan Society for the Promotion of Science.

Conflict of interest statement

No conflict of interest exists.

References

1. Lee SW, Kim SG: Membranes for the guided bone regeneration. *Maxillofac Plast Reconstr Surg*, 36: 239–246, 2014.
2. Hao J, Acharya A, Chen K, Chou J, Kasugai S, Lang NP: Novel bioresorbable strontium hydroxyapatite membrane for guided bone regeneration. *Clin Oral Implants Res*, 26: 1–7, 2015.
3. Shim JH, Huh JB, Park JY, Jeon YC, Kang SS, Kim JY, Rhie JW, Cho DW: Fabrication of blended polycaprolactone/poly(lactic-co-glycolic acid)/beta-tricalcium phosphate thin membrane using solid freeform fabrication technology for guided bone regeneration. *Tissue Eng Part A*, 19: 317–328, 2013.
4. Fu L, Wang Z, Dong S, Cai Y, Ni Y, Zhang T, Wang L, Zhou Y: Bilayer poly(lactic-co-glycolic acid)/nano-hydroxyapatite membrane with barrier function

- and osteogenesis promotion for guided bone regeneration. *Materials*(Basel), 10: 257, 2017.
5. Sam G, Pillai BR: Evolution of barrier membranes in periodontal regeneration-"Are the third generation membranes really here?". *J Clin Diagn Res*, 8: ZE14-17, 2014.
 6. Zafar M, Najeeb S, Khurshid Z, Vazirzadeh M, Zohaib S, Najeeb B, Sefat F: Potential of electrospun nanofibers for biomedical and dental applications. *Materials* (Basel), 9: 73, 2016.
 7. Song X, Ling F, Ma L, Yang C, Chen X: Electrospun hydroxyapatite grafted poly(l-lactide)/poly(lactic-co-glycolic acid) nanofibers for guided bone regeneration membrane. *Compos Sci Technol*, 79: 8-14, 2013.
 8. Ranganath SH, Wang CH: Biodegradable microfiber implants delivering paclitaxel for post-surgical chemotherapy against malignant glioma. *Biomaterials*, 29: 2996-3003, 2008.
 9. Kruse M, Walter P, Bauer B, Rutten S, Schaefer K, Plange N, Gries T, Jockenhoevel S, Fuest M: Electro-spun membranes as scaffolds for human corneal endothelial cells. *Curr Eye Res*, 43: 1-11, 2018.
 10. Hiep NT, Lee BT: Electro-spinning of PLGA/PCL blends for tissue engineering and their biocompatibility. *J Mater Sci Mater Med*, 21: 1969-1978, 2010.
 11. Li WJ, Cooper JA, Jr., Mauck RL, Tuan RS: Fabrication and characterization of six electrospun poly(alpha-hydroxy ester)-based fibrous scaffolds for tissue engineering applications. *Acta Biomater*, 2: 377-385, 2006.
 12. Chung S, Ingle NP, Montero GA, Kim SH, King MW: Bioresorbable elastomeric vascular tissue engineering scaffolds via melt spinning and electrospinning. *Acta Biomater*, 6: 1958-1967, 2010.
 13. Inami T, Tanimoto Y, Ueda M, Shibata Y, Hirayama S, Yamaguchi M, Kasai K: Morphology and in vitro behavior of electrospun fibrous poly(D,L-lactic acid) for biomedical applications. *Adv Mater Sci Eng*, 2013: 1-6, 2013.
 14. Rho KS, Jeong L, Lee G, Seo BM, Park YJ, Hong SD, Roh S, Cho JJ, Park WH, Min BM: Electrospinning of collagen nanofibers: effects on the behavior of normal human keratinocytes and early-stage wound healing. *Biomaterials*, 27: 1452-1461, 2006.
 15. Shim JH, Won JY, Park JH, Bae JH, Ahn G, Kim CH, Lim DH, Cho DW, Yun WS, Bae EB, Jeong CM, Huh JB: Effects of 3D-printed polycaprolactone/beta-tricalcium phosphate membranes on guided bone regeneration. *Int J Mol Sci*, 18: 899, 2017.
 16. Ezati M, Safavipour H, Houshmand B, Faghihi S: Development of a PCL/gelatin/chitosan/beta-TCP electrospun composite for guided bone regeneration. *Prog Biomater*, 7: 225-237, 2018.
 17. Hoornaert A, d'Arros C, Heymann MF, Layrolle P: Biocompatibility, resorption and biofunctionality of a new synthetic biodegradable membrane for guided bone regeneration. *Biomed Mater*, 11: 045012, 2016.
 18. Arima Y, Iwata H: Effect of wettability and surface functional groups on protein adsorption and cell adhesion using well-defined mixed self-assembled monolayers. *Biomaterials*, 28: 3074-3082, 2007.
 19. Chou SF, Woodrow KA: Relationships between mechanical properties and drug release from electrospun fibers of PCL and PLGA blends. *J Mech Behav Biomed Mater*, 65: 724-733, 2017.
 20. Yongcong F, Zhang T, Liverani L, Boccaccini AR, Sun W: Novel biomimetic fiber incorporated scaffolds for tissue engineering. *J Biomed Mater Res A*, 107: 2694-2705, 2019.