International Journal of Pharmaceutics 430 (2012) 335–341

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Sustainable release of vancomycin, gentamicin and lidocaine from novel electrospun sandwich-structured PLGA/collagen nanofibrous membranes

Dave W. Chen [a,b,1], Yung-Heng Hsu [a,b,1], Jun-Yi Liao [c], Shih-Jung Liu [a,*], Jan-Kan Chen [d], Steve Wen-Neng Ueng [b]

[a] Department of Mechanical Engineering, Chang Gung University, Tao-Yuan,Taiwan
[b] Department of Orthopedic Surgery, Chang Gung Memorial Hospital, Tao-Yuan,Taiwan
[c] Graduate Institute of Medical Mechatronics, Chang Gung University, Tao-Yuan,Taiwan
[d] Department of Physiology and Pharmacology, Chang Gung University, Tao-Yuan,Taiwan

ARTICLE INFO

Article history:
Received 13 October 2011
Received in revised form 21 March 2012
Accepted 5 April 2012
Available online 12 April 2012

Keywords:
Electrospinning
Nanofibrous membranes
Polylactide–polyglycolide (PLGA)
Collagen
Vancomycin
Gentamicin
Lidocaine
Cell behavior

ABSTRACT

This study investigated the in vitro release of vancomycin, gentamicin, and lidocaine from novel electrospun sandwich-structured polylactide–polyglycolide (PLGA)/collagen nanofibrous membranes. For the electrospinning of biodegradable membranes, PLGA/collagen and PLGA/vancomycin/gentamicin/lidocaine were separately dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). They were then electrospun into sandwich structured membranes, with PLGA/collagen for the surface layers and PLGA/drugs for the core layer. After electrospinning, an elution method and HPLC assay were employed to characterize the in vitro release rates of the pharmaceutics over a 30-day period. The experiment showed that biodegradable nanofibrous membranes released high concentrations of vancomycin and gentamicin (well above the minimum inhibition concentration) for 4 and 3 weeks, respectively, and lidocaine for 2 weeks. A bacterial inhibition test was carried out to determine the relative activity of the released antibiotics. The bioactivity of vancomycin and gentamicin ranged from 30% to 100% and 37% to 100%, respectively. In addition, results indicated that the nanofibrous membranes were functionally active in responses in human fibroblasts. By adopting the electrospinning technique, we will be able to manufacture biodegradable biomimetic nanofibrous extracellular membranes for long-term drug delivery of various pharmaceuticals.

1. Introduction

Treating severe, easily infected burn wounds is not an easy task. Infectious organisms preferentially target wounds beneath dressing materials, leading to possibly serious infections that require removal of the wound dressing and excision of cutaneous wounds (Warden et al., 1982; Mi et al., 2002). Inhibition of bacterial multiplication and invasion is especially important in contaminated wounds. Traditionally, topical antimicrobial should be applied 1–2 times daily to the injured area to reduce infections. However, patients often suffer from discomfort with the application of topical drugs and a substantial nursing effort is required for the replacement of wound dressings. Infection can be reduced using dressings that incorporate antibiotics (Elsner and Zilberman, 2009; Teo et al., 2011; Elsner et al., 2011). In addition, the replacement of dressings and the damage inflicted on the newly formed epithelium can be minimized. Antibiotic-loaded wound dressings (Zilberman and Elsner, 2008) made out of biodegradable polymeric membranes have advantages in several ways. First, biodegradable membranes provide bactericidal concentrations of antibiotics for the prolonged time needed to completely treat the particular infection. Second, variable biodegradability from weeks to months may allow many types of infections to be treated. Third, the biodegradable membranes dissolve, thus there is no need for removal; and lastly, because the biodegradable membranes dissolve slowly, the soft tissue or bone defect will slowly fill with tissue, so there is no need for reconstruction.

Electrospinning is a simple and effective nanofabrication method for preparing nanofibrous membranes with diameters ranging from 5 to 500 nm, which are 10^2–10^4 times smaller than those prepared by the traditional method of solution or melt spinning. In the electrospinning process, a polymeric solution placed inside a syringe is pushed out from a metal capillary that is connected to a high voltage power supply (Sill et al., 2008). Nanofibers

* Corresponding author at: Biomaterials Lab, Mechanical Engineering, Chang Gung University, 259, Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan 333, Taiwan. Tel.: +886 3 2118166; fax: +886 3 2118558.
E-mail address: shihjung@mail.cgu.edu.tw (S.-J. Liu).
[1] These authors have equal contribution to this study and are co-first authors of this paper.

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2012.04.010

are collected in the form of a non-woven matrix on a grounded collector after solvent evaporation. By adopting appropriate process parameters such as solvent, polymer concentration, and flow rate, electrospun nanofibers with various diameters can be obtained (Noh et al., 2006; Min et al., 2004; Yoo et al., 2008; Li et al., 2002; Matthews et al., 2002; Powell et al., 2008; Rho et al., 2006; Chen et al., 2008). Electrospun drug-loaded nanofibrous membranes potentially offer advantages over conventional ones. Local antibiotics and anesthetic have the advantage of delivering high drug concentrations to the precise area required, and the total dose of antibiotic applied locally is not normally sufficient to produce toxic systemic effects. With its large surface area and microporous structure, the membrane can quickly start signaling pathways and attract fibroblasts to the dermis layer, which can excrete important extracellular matrix components such as collagen and growth factors to repair damaged tissue.

In the present study, a sandwich-structured nanofibrous matrix was produced via electrospinning to develop biodegradable and biomimetic drug-eluting dressings. The materials used to prepare the membranes included polylactide–polyglycolide (PLGA), collagen, vancomycin, gentamicin and lidocaine. Collagen is a natural extracellular matrix (ECM) component of many tissues, such as skin, bone, tendon, ligament, and other connective tissues. Among the isotypes of collagen, type I is the principal structural and functional protein and is composed of two $\alpha 1$ chains and one α_2 chain. The underlying α chains that form these natural polymers are arranged into a repeating pattern that forms a coiled structure. The specific complement of α subunits present within the fibril defines the material properties of the natural polymer (Matthews et al., 2002). Scaffolds that are intended for cell culture purposes need to exhibit mechanically supportive properties for cellular morphogenesis. The mechanical strength of electrospun collagen nanofibers can be enhanced with the addition of PLGA materials (Yang et al., 2009). Polylactide–polyglycolide (PLGA) is in the class of synthesized biodegradable and biocompatible copolymers, from which resorbable sutures, resorbable surgical clips, and controlled-release implants are made. PLGA also falls within the class of copolymers that have been used for implantable and injectable controlled-release, drug delivery systems (Kumbar et al., 2008; Williams, 1982). These copolymers, with a history of safe use, have been approved for human use. After being introduced into the body, PLGA material induces only a minimal inflammatory response and biodegrades through the hydrolysis of its ester linkages to yield biocompatible lactic and glycolic acids (Ali et al., 1993; Kobayashi et al., 1992).

Vancomycin is a glycopeptide antibiotic that functions by inhibiting the formation of the bacterial cell wall. It is effective against gram-positive bacteria, especially in recalcitrant staphylococcal infections that are unresponsive to penicillin or cephalosporin antibiotics. Gentamicin, on the other hand, is an antibiotic complex elaborated by fungi of the genus Micromonospora and is effective against many gram-negative bacteria, especially Pseudomonas species, as well as certain gram-positive bacteria, particularly *Staphylococcus aureus*. Lidocaine is an anesthetic with sedative, analgesic, and cardiac depressant properties and is applied topically in the form of base or hydrochloride salt as a local anesthetic.

To prepare the nanofibrous drug-eluting membranes, PLGA/collagen and PLGA/vancomycin/gentamicin/lidocaine combinations were separately dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). The blended solutions were electrospun into sandwich structured membranes, with PLGA/collagen for the surface layers and PLGA/drugs for the core layer. After electrospinning, an elution method and an HPLC assay were employed to characterize the in vitro release rates of the pharmaceutics over a 30-day period. A bacterial inhibition test was carried out to determine the bioactivity of the released antibiotics. Furthermore, to assay the cytocompatibility and cell behavior of electrospun sandwich-structured nanofibers, the interaction between normal human fibroblasts and the nanofibrous matrix were studied.

2. Materials and methods

2.1. Materials

The poly (D,L)-lactide-co-glycolide (PLGA) used was commercially available material (Resomer RG 503, Boehringer, Germany) and had a ratio of 50:50 and an intrinsic viscosity of 0.4. Collagen from bovine achilles tendon, type I, and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) were purchased from Sigma–Aldrich (Saint Louis, MO, USA). The drugs used included commercial grade vancomycin hydrochloride, gentamicin sulfate and lidocaine hydrochloride (Sigma–Aldrich, Saint Louis, MO, USA).

2.2. Electrospinning

The electrospinning setup utilized in this study consisted of a syringe and needle (the internal diameter 0.42 mm), a ground electrode, an aluminum sheet, and a high voltage supply (Liu et al., 2010). The needle was connected to the high voltage supply, which could generate positive DC voltages and current up to 35 kV and 4.16 mA/125 W, respectively. PLGA/collagen (280 mg/140 mg, w/w) and PLGA/vancomycin/gentamicin/lidocaine (240 mg/35 mg/35 mg/35 mg, w/w/w/w) were dissolved in 1 ml of HFIP each. For the electrospinning of sandwich-structured nanofibers, a predetermined volume of PLGA/collagen solution (Table 1) was delivered and electrospun by a syringe pump with a volumetric flow rate of 3.6 ml/h, followed by the electrospinning of PLGA/vancomycin/gentamicin/lidocaine solution with a volumetric flow rate of 1.2 ml/h as the core layer, and finally by the spinning of another PLGA/collagen layer. Fig. 1 shows schematically the electrospinning of sandwich-structured nanofibrous membranes. The distance between the needle tip and the ground electrode was 9, 12, or 15 cm, and the positive voltage applied to polymer solutions was 17 kV. Different combinations of blended solutions were used for preparation of the

Table 1
Processing parameters used in the experiments.

Sample	Composition			Membrane thickness (mm)
	PLGA/collagen solution (ml)/layer thickness (mm)	PLGA/drugs solution (ml)/layer thickness (mm)	PLGA/collagen solutions (ml)/layer thickness (mm)	
1-4-1	1/0.015	4/0.077	1/0.015	0.107
2-4-2	2/0.030	4/0.077	2/0.030	0.138
1-7-1	1/0.015	7/0.133	1/0.015	0.164
2-7-2	2/0.030	7/0.133	2/0.030	0.193

The distance between the needle tip and the ground electrode was 12 cm.
PLGA/collagen layer: 0.015 mm/ml; PLGA/drugs layer: 0.019 mm/ml.

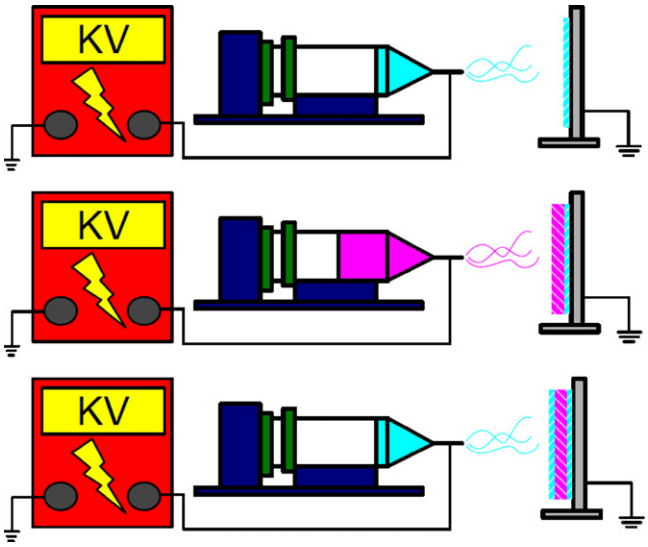

Fig. 1. The set up for the electrospinning of sandwich structured membranes.

nanofibrous membranes, i.e. 1 ml of PLGA/collagen solution, 4 ml of PLGA/vancomycin/gentamicin/lidocaine solution, and 1 ml of PLGA/collagen solution (denoted 1-4-1 in Table 1) produced a membrane of 0.107 mm in thickness. Other combinations of the blended solutions used are also listed in Table 1. All experiments were carried out at room temperature. After electrospinning, the specimens were placed in a vacuum oven at 40 °C for 72 h for the solvents to evaporate.

2.3. Characterization of nanofibers

The morphology of electrospun nanofibers was observed on a scanning electron microscope (SEM; Hitachi S-3000N, Japan) after gold coating. The average diameter and diameter distribution were obtained by analyzing SEM images using a commercial image analysis program (Optimas version 5.22, USA).

2.4. Water contact angle

Water contact angles of nanofibrous membranes were measured by a water contact angle analyzer (FTA-125, First Ten Angstroms, USA). Samples of 1 cm by 1 cm were cut from the membranes and placed on the testing plate, after the distilled water was carefully dropped on the surface of samples. The contact angles were measured by a video monitor. Both the contact angles of PLGA/collagen and PLGA/drugs membranes were measured.

2.5. In vitro release behaviors

An in vitro elution method was employed to determine the release characteristics of various drugs from the nanofibrous membranes. A phosphate buffer, 0.15 mol/L (pH 7.4), was used as the dissolution medium. Samples with an area of 2 cm by 3 cm (approximately 218 mg each) were cut from the electrospun membranes and were incubated in 1 ml of phosphate buffered saline at 37 °C for 24 h. The dissolution medium was collected and analyzed at 24-h intervals. The phosphate buffer (1 ml) was replaced every 24 h until the sample was fully dissolved.

The vancomycin concentrations in the buffer for the elution studies were determined by a high-performance liquid chromatography (HPLC) assay. The HPLC analyses were conducted on a Hitachi L-2200 Multisolvent Delivery System. A SYMMETRY C_8, 3.9 cm × 150 mm HPLC column (Waters) was used for separation of the antibiotics. The mobile phase contained 0.01 mol heptanesulfonic acid (Fisher Scientific UK Ltd.) and acetonitrile (Mallinckrodt, USA) (85/15, v/v). The absorbency was monitored at 280 nm and the flow rate was 1.4 ml/min. All samples were assayed in triplicate and sample dilutions were performed to bring the unknown concentrations into the range of the assay standard curve. A calibration curve was made for each set of the measurements (correlation coefficient >0.99). The elution product can be specifically identified and quantified with high sensitivity using the HPLC system. The concentrations of eluted gentamicin and lidocaine were also determined using the same HPLC assay.

2.6. Bioactivity of released antibiotics

Bioactivity of the released vancomycin on *S. aureus* (ATCC65389) was determined using an antibiotic disk diffusion method in a Nutrient Broth (beef extract, peptone, Difco Laboratories). Eight micro liters of solution from each daily buffer sample was pipetted on 6-mm disks. The disks were placed on nutrient agar plates (beef extract, peptone, agar, Difco Laboratories) and seeded with a layer of *S. aureus*, and the zones of inhibition were measured with a micrometer after 16–18 h of incubation at 35 °C. A calibration curve was first determined by six different standard concentrations (0.01, 0.1, 1, 10, 100, 1000 mg/ml). The released concentration of vancomycin was then determined by interpreting the curve. The bioactivities of the incubated vancomycin on *S. aureus* (ATCC65389) were determined by:

$$\text{Bioactivity}\ (\%) = \frac{\text{diameter of sample inhibition zone}}{\text{diameter of maximum inhibition zone}} \quad (1)$$

The relative activity of gentamicin was performed with the same scheme against *Escherichia coli* (ATCC25922).

The minimum inhibitory concentration of vancomycin on *S. aureus* (ATCC65389) was determined using an antibiotic tube dilution method in Cation supplemented Mueller–Hinton Broth (Difco Laboratories). Vancomycin was diluted serially twofold in tubes containing 0.5 mL of the Cation Supplemented Mueller-Hinton Broth. The minimum inhibitory concentration of gentamicin to *E. coli* (ATCC25922) was also determined by the same method.

2.7. Cell cultures of electrospun nanofibers

Cytotoxicity of the nanofibrous membranes was examined by MTT assay (Roche, Germany) of cell viability. Electrospun nanofibers were hole punched and put onto 24-well culture plates. Human fibroblasts obtained from foreskins of patients (1–3 years of age) undergoing surgery were seeded (5×10^3 cells/well) in Dulbecco's Modified Eagle's Medium (DMEM) at 37 °C under 5% CO_2/95% air conditions until cell confluence. Cell viability was monitored at 1, 3, 7, 14 and 21 days by MTT assays and quantified using an ELISA reader.

2.8. Statistics and data analysis

Data was collected from the samples and were analyzed by one-way analysis of variance (ANOVA). Differences were considered statistically significant for P values <0.05.

3. Results and discussion

3.1. Electrospinning of PLGA/collagen nanofibers

The fabrication of drug-eluting nanofibrous matrices via electrospinning is highly desirable because the core PLGA/drugs nanofibrous membrane can provide sustainable release of pharmaceuticals (Meng et al., 2011a,b; Kontogiannopoulos et al., 2011),

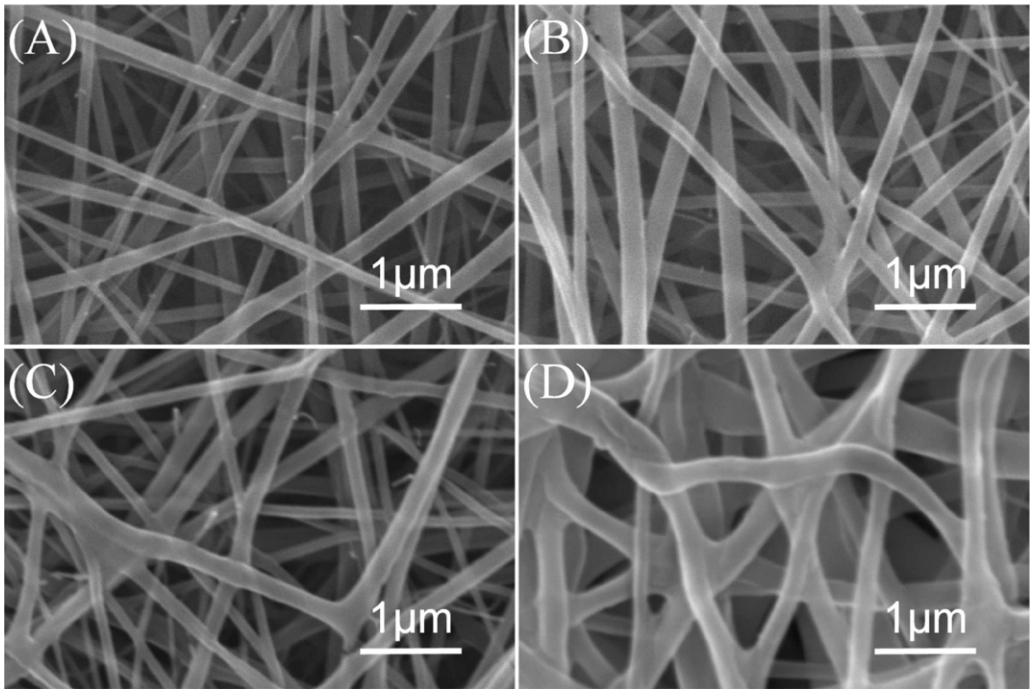

Fig. 2. SEM images of electrospun PLGA/drugs nanofibers with a distance of (A) 9 cm, (B) 12 cm, (C) 15 cm, and (D) electrospun PLGA/collagen nanofibers.

while the surface PLGA/collagen nanofibers acts as a scaffold for cell growth and proliferations (Li et al., 2002; Matthews et al., 2002; Powell et al., 2008). In addition, PLGA is non-toxic, eliciting minimal inflammatory response and can be eventually absorbed without any accumulation in the vital organs (Kumbar et al., 2008). Collagen is a principal structural component of the ECM matrix. In this study, continuous PLGA and collagen nanofibers were obtained by electrospinning. By adopting different distances between the needle tip and the ground electrode, nanofibers of various diameters could be successfully fabricated. Fig. 2A–C shows the SEM micrographs of PLGA/drugs nanofibers electrospun using different distances (magnification of 20,000×). The diameters of the spun nanofibers PLGA/drugs ranged from 37 nm to 203 nm, from 55 to 222 nm, and from 40 to 259 nm for distances of 9, 12, and 15 cm, respectively. Evidently, the diameters of the nanofibers increased to some extent with the distance. On the other hand, Fig. 2D shows the SEM micrograph of PLGA/collagen nanofibers. The diameters of the nanofibers for PLGA/collagen ranged from 185 to 314 nm. The image characterization of the nanofibers in Fig. 2D suggests that the fiber diameter increases with the presence of collagen in the blended solutions. Furthermore, the porosity of all of nanofibrous matrix was high.

Fig. 3 shows the result of water contact angle measurements. The contact angles were 117.7° and 89.4° for the PLGA/drugs and the PLGA/collagen nanofibers, respectively. While the PLGA/drugs nanofibers exhibited hydrophobic characteristics, the PLGA/collagen nanofibers showed a more hydrophilic behavior, mainly attributed to the presence of collagen.

3.2. Release behaviors of pharmaceuticals from nanofibrous membranes

The release characteristics of vancomycin, gentamicin, and lidocaine from the nanofibrous membranes were studied. Drug-eluting membranes of different compositions, namely 1-4-1, 2-4-2, 1-7-1, 2-7-2 were fabricated and tested. The release curve of vancomycin from the membranes is shown in Fig. 4, while the release behaviors of gentamicin and lidocaine are shown in Figs. 5 and 6, respectively. All release curves exhibited an initially high release during the first 3 days, followed by a more gradual and sustained release of the drugs. The elution of vancomycin showed a second peak in release at day 15. In addition, the experimental results in Figs. 4–6 suggest that biodegradable nanofibrous membranes released high concentrations of vancomycin and gentamicin (well above the

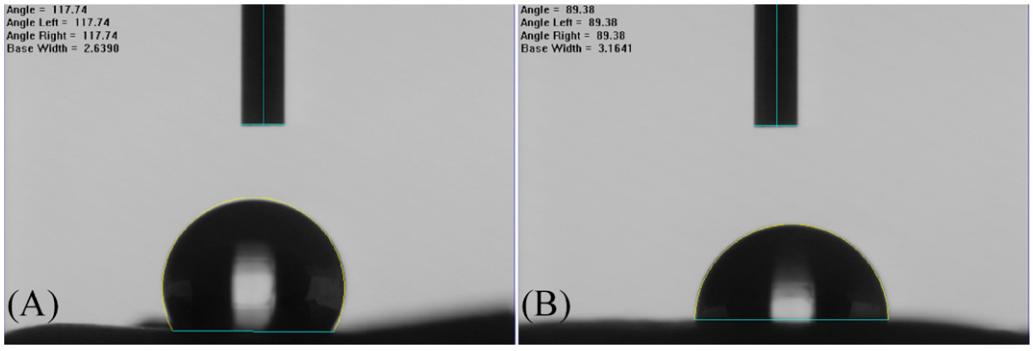

Fig. 3. Contact angle measure of (A) PLGA/drugs layer, and (B) PLGA/collagen layer.

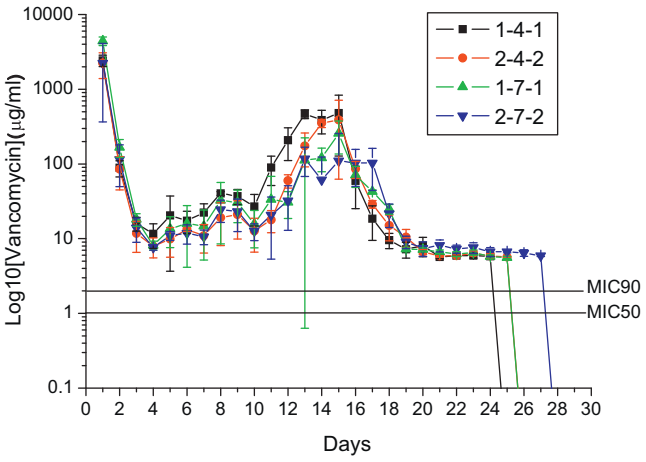

Fig. 4. Release characteristic of vancomycin from nanofibrous membranes.

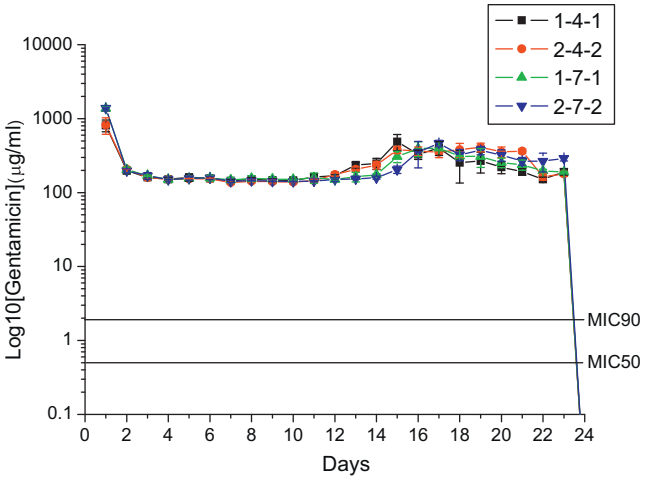

Fig. 5. Release characteristic of gentamicin from nanofibrous membranes.

minimum inhibition concentration) for 4 and 3 weeks, respectively, and lidocaine for 2 weeks. By increasing the volume of the PLGA/drugs nanofibers at the core (e.g., from 1-4-1 to 1-7-1), one can increase the released concentration, mainly due to the fact that the volume of drugs increased in the nanofibers. The release drug concentration increased accordingly. On the other hand, by

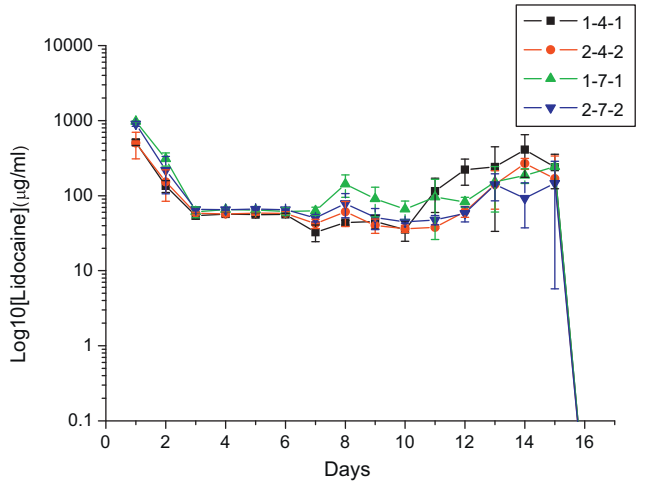

Fig. 6. Release characteristic of lidocaine from nanofibrous membranes.

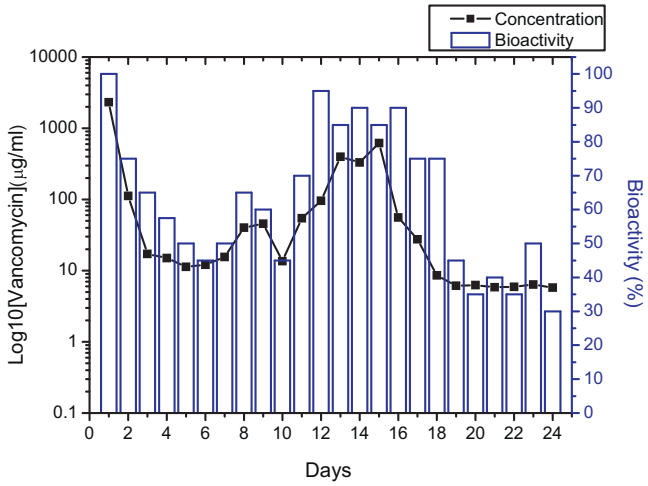

Fig. 7. Bioactivity of released vancomycin from nanofibrous membranes.

increasing the thickness of the surface layers (i.e. from 1-7-1 to 2-7-2), the initial burst release and the daily release rate could be reduced. This can be explained by the fact that the surface PLGA/collagen nanofibrous membrane acts as a barrier to the pharmaceuticals at the core nanofibers. The release rate thus decreases.

The bioactivity of eluted vancomycin on *S. aureus* (ATCC65389) and gentamicin on *E. coli* were determined using an antibiotic disk diffusion method. Figs. 7 and 8 show, respectively, the bioactivities of released vancomycin and gentamicin. The bioactivity of vancomycin ranged from 30% to 100% (Fig. 7), while the activity of gentamicin ranged from 37% to 100% (Fig. 8). The activities of the antibiotics remained high after the electrospinning process. This further testifies that the electrospinning can be an appropriate process for the preparation of drug-eluting membranes.

3.3. Cell adhesion and spreading of normal human fibroblasts

Previous studies have shown that collagen and electrospun collagen nanofibrous matrices were able to promote wound healing (Parry and Craig, 1988; Park et al., 2003; Hansbrough et al., 1989). As a potential wound-dressing material and scaffolding for tissue engineering, the nanofibrous matrix should promote cell growth and physiological function and should be able to maintain normal states of cell differentiation. The biological properties of an electrospun sandwich-structured matrix in cell culture experiments

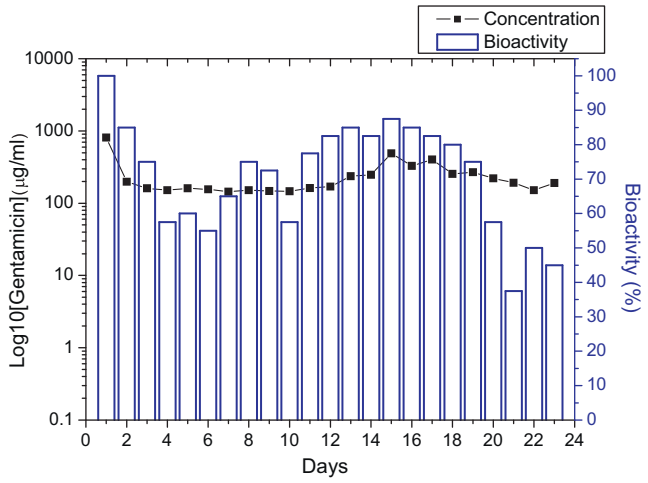

Fig. 8. Bioactivity of released gentamicin from nanofibrous membranes.

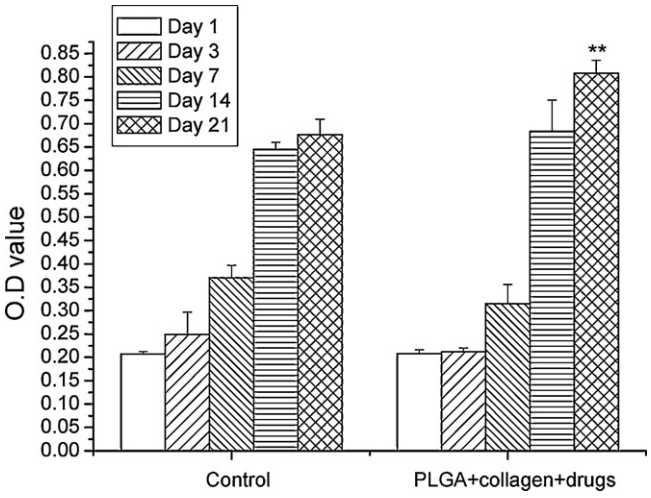

Fig. 9. Cytotoxicity tests from MTT assays of cell viability in nanofibrous drug-eluting membranes (**$P < 0.01$).

and the cytocompatibility of electrospun nanofibers were studied. Cytotoxicity tests from MTT assays of cell viability in nanofibrous matrix were studied, and the result is shown in Fig. 9. All nanofibers show no signs of cytotoxicity. However, the assay results suggest inferior cell viability in the nanofibrous drug-eluting membrane group at 3 and 7 days compared to the control group. This might be due to the fact that the antimicrobials released from the nanofibers harm viable tissue and delay the healing process. Furthermore, local anesthetics may also induce adverse reactions to the tissues. Cell growth and proliferation thus decreased. The possible influence of released pharmaceuticals on cell proliferation, however, gradually diminished at 14 and 21 days, as supported by the result in Fig. 9.

Kumbar et al. (2008) studied the electrospun fiber matrices composed of scaffolds of various fiber diameters and reported that human skin fibroblasts showed significantly higher proliferation on electrospun PLGA fiber matrices having fiber diameter in the range of 350–1100 nm. Despite the diameters of the electrospun nanofibers in this study (a range of 185–314 nm for the surface PLGA/collagen layer) being less than the reported optimal value, the fabricated nanofibrous matrices showed good cell adhesion in normal human fibroblasts. This might be due to the fact that the presence of collagen in the matrices promotes cell proliferation in the electrospun PLGA/collagen nanofibers. Rho et al. (Powell et al., 2008) reported that the electrospinning process may denaturalize the biological and structural properties of a natural protein such as collagen. The experimental results in this study suggested that the PLGA/collagen nanofibrous matrix remains intact during the processing procedure and can act as an excellent scaffold for cell adhesion and growth. In the literature (Williams, 1982; Kobayashi et al., 1992), the most concerning issue of the byproducts released with the degradation of PLGA is the acid. The experimental results of the cell cultures of the electrospun nanofibers and the bioactivity test of the released antibiotics in this study showed that the possible influence of released acid on cell proliferation or drug activity is negligible. Furthermore, electrospun nanofibrous membranes released sustainably high concentrations of vancomycin and gentamicin (well above the minimum inhibition concentration) in vitro for the period of time needed to treat infections, i.e. 2–4 weeks, and the bioactivity of vancomycin and gentamicin remained high after the electrospinning process. The results here suggest that electrospun biodegradable sandwich-structured nanofibers may be a good candidate as a drug-eluting wound dressing for infection treatment and skin regeneration.

4. Conclusions

In the present study, a sandwich-structured matrix produced by the electrospinning process was introduced for the application of wound dressing. The in vitro release of vancomycin, gentamicin, and lidocaine from electrospun sandwich-structured polylactide–polyglycolide (PLGA)/collagen nanofibrous membranes was investigated. The experimental results showed that biodegradable membranes released high concentrations of vancomycin and gentamicin (well above the minimum inhibition concentration) for 4 and 3 weeks, respectively, and lidocaine for 2 weeks. The bioactivity of vancomycin and gentamicin ranged from 30% to 100% and 37% to 100%, respectively. In addition, it was found that nanofibrous membranes were functionally active in responses in human fibroblasts. By adopting the electrospinning technique, we will be able to manufacture biodegradable biomimetic nanofibrous extracellular membranes for long-term drug delivery of various pharmaceuticals.

References

Ali, S.A.M., Doherty, P.J., Williams, D.F., 1993. Mechanisms of polymer degradation in implantable devices 2. Poly(DL-lactic acid). J. Biomed. Mater. Res. 27, 1409–1418.
Chen, J.P., Chang, G.Y., Chen, J.K., 2008. Electrospun collagen/chitosan nanofibrous membrane as wound dressing. Colloids Surf. A: Physiochem. Eng. Aspects, 183–188, 313-314.
Elsner, J.J., Zilberman, M., 2009. Antibiotic-eluting bioresorbable composite fibers for wound healing applications: microstructures, drug delivery and mechanical properties. Acta Biomater. 5, 2872–2883.
Elsner, J.J., Egozi, D., Ullmann, Y., Berdicevsky, I., Shefy-Peleg, A., Zilberman, M., 2011. Novel biodegradable composite wound dressings with controlled release of antibiotics: results in guinea pig burn model. Burns 37, 896–904.
Hansbrough, J., Boyce, S., Cooper, M., Forman, T., 1989. Burn would closure with cultured human keratinocytes and fibroblasts attached to a collagen-glycosaminoglycan substrate. J. Am. Med. Assoc. 262, 2125–2130.
Kobayashi, H., Shiraki, K., Ikada, Y., 1992. Toxicity test of biodegradable polymers by implantation in rabbit cornea. J. Biomed. Mater. Res. 26, 1463–1476.
Kontogiannopoulos, K.N., Assimopoulou, A.N., Tsivintzelis, I., Panayiotou, C., Papageorgiou, V.P., 2011. Electrospun fiber mats containing shikonin and derivatives with potential biomedical applications. Int. J. Pharm. 409, 216–228.
Kumbar, S.G., Nukavarapu, S.P., James, R., Nair, L.S., Laurencin, C.T., 2008. Electrospun poly(lactic acid-co-glycolic acid) scaffolds for skin tissue engineering. Biomaterials 29, 4100–4107.
Li, W.J., Laurencin, C.T., Caterson, E.J., Tuan, R.S., Ko, F.K., 2002. Electrospun nanofibrous structure: a novel scaffold for tissue engineering. J. Biomed. Mater. Res. 60, 613–621.
Liu, S.J., Kau, Y.C., Chou, C.Y., Chen, J.K., Wu, R.C., Yeh, W.L., 2010. Electrospun PLGA/collagen nanofibrous membrane as early-stage wound dressing. J. Membr. Sci. 355, 39–53.
Matthews, J.A., Wnek, G.E., Simpson, D.G., Bowlin, G.L., 2002. Electrospinning of collagen nanofibers. Biomacromolecules 3, 232–238.
Meng, Z.X., Xu, X.X., Zheng, W., Zhou, H.M., Li, L., Zheng, Y.F., Lou, X., 2011a. Preparation and characterization of electrospun PLGA/gelatin nanofibers as a potential drug delivery system. Colloids Surf. B: Biointerfaces 84, 97–102.
Meng, Z.X., Zheng, W., Li, L., Zheng, Y.F., 2011b. Fabrication, characterization and in vitro drug release behavior of electrospun PLGA/chitosan nanofibrous scaffold. Mater. Chem. Phys. 125, 606–611.
Mi, F.L., Wu, Y.B., Shyu, S.S., Schoung, J.Y., Huang, Y.B., Tsai, Y.H., Hao, J.Y., 2002. Control of wound infections using a bilayer chitosan wound dressing with sustainable antibiotic delivery. J. Biomed. Mater. Res. 59, 438–449.
Min, B.M., You, Y., Kim, J.M., Lee, S.J., Park, W.H., 2004. Formation of nanostructured poly(lactid-co-glycolid acid)/chitin matrix and its cellular response to normal human keratinocytes and fibroblasts. Carbohydr. Polym. 57, 285–292.
Noh, H.K., Lee, S.W., Kim, J.M., Oh, J.E., Kim, K.H., Chung, C.P., Choi, S.C., Park, W.H., Min, B.M., 2006. Electrospinning of chitin nanofibers: degradation behavior and cellular response to normal human keratinocytes and fibroblasts. Biomaterials 27, 3934–3944.
Park, S.N., Lee, H.J., Lee, K.H., Suh, H., 2003. Biological characterization of EDC-crosslinked collagen-hyaluronic acid matrix in dermal tissue restoration. Biomaterials 24, 1631–1641.
Parry, D.A.D., Craig, A.S., 1988. Collagen fibril during development and maturation and their contribution to the mechanical attributes of connective tissue. In: Nimni, M.E. (Ed.), Collagen, vol. 2. CRC Press, Florida, pp. 1–20.
Powell, H.M., Supp, D.M., Boyce, S.T., 2008. Influence of electrospun collagen on would contraction of engineered skin substitutes. Biomaterials 29, 834–843.
Rho, K.S., Jeong, L., Lee, G., Seo, B.M., Park, Y.J., Hong, S.D., Roh, S., Cho, J.J., Park, W.H., Min, B.M., 2006. Electrospinning of collagen nanofibers: effects on the behavior of normal human keratinocytes and early-stage wound healing. Biomaterials 27, 1452–1461.

Sill, T.J., von Recum, H.A., 2008. Electrospinning: applications in drug delivery and tissue engineering. Biomaterials 29, 1989–2006.
Teo, E.Y., Ong, S.Y., Chong, M.S.K., Zhang, Z., Lu, J., Moochhala, S., Ho, B., Teoh, S.H., 2011. Polycaprolactone-based fused deposition modeled mesh for delivery of antibacterial agents to infected wounds. Biomaterials 32, 279–287.
Warden, G.D., Saffle, J.R., Kravitz, M., 1982. A 2-stage technique for excision and grafting of burn wounds. J. Trauma 22, 98–103.
Williams, D.F., 1982. Biodegradation of surgical polymers. J. Mater. Sci. 17, 1233–1246.
Yang, Y., Zhu, X., Cui, W., Li, X., Jin, Y., 2009. Electrospun composites mats of poly[(D,L-lactide)-co-glycolide] and collagen with high porosity as potential scaffolds for skin tissue engineering. Macromol. Mater. Eng. 294, 611–619.
Yoo, C.R., Yeo, I.S., Park, K.E., Park, J.H., Lee, S.J., Park, W.H., Min, B.M., 2008. Effect of chitin/silk fibroin nanofibrous bicomponent structures on interaction with human epidermal keratinocytes. Int. J. Biol. Macromol. 42, 324–334.
Zilberman, M., Elsner, J.J., 2008. Antibiotic-eluting medical devices for various applications. J. Control. Release 130, 202–215.