Correlations of *In Vitro* and *In Vivo* Degradation Tests on Electrospun Poly-DL-Lactide-Poly(ethylene glycol) Fibers

Shuhui He, Xiaohong Li, Ye Yang, Guoqing Jia, Jie Zou

Key Laboratory of Advanced Technologies of Materials, Ministry of Education of China, School of Materials Science and Engineering, Southwest Jiaotong University, Chengdu 610031, People's Republic of China

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ABSTRACT: Although electrospun fibrous scaffolds have shown promise in biomedical applications, the degradation behaviors after *in vitro* and *in vivo* incubations and their correlations have not been clarified till now. The present study aims to comprehensively investigate the effects that *in vitro* cell inoculation and *in vivo* implantation have on the degradation, when compared with commonly processed degradation in buffer solutions. During the investigational time period, there was no significant difference in the degradation behaviors after incubation of fibrous mats into phosphate buffer saline, simulated body fluid and cell culture media. After cell inoculation into the degradation system, significantly higher mass loss, lower molecular weight reduction, and less significant increase in the molecular weight polydispersity were detected. The tight attachment of cells on fibers further enhanced the degradation process after cell seeding on the fibrous mats. When compared with *in vitro* cell-free degradation, the subcutaneous implantation of fibrous mats led to a significantly higher degradation rate at the initial stage, but slower degradation at the later stage. It was indicated that the degradation behaviors after *in vivo* implantation were close to those after cell culture on fibrous mats, thus providing an effective *in vitro* tool to predict *in vivo* degradation profiles of electrospun fibers. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 125: 2207–2215, 2012

Key words: degradation behaviors; electrospun fibers; cell-mediated degradation; *in vivo* implantation

INTRODUCTION

Electrospinning has become the most popular technique in recent years by the use of static electricity to draw fibers from a polymer solution or melt. Electrospinning generates uniform and continuous fibers with diameters down to a nanoscale dimension, which closely mimic the size scale of fibrous proteins found in natural extracellular matrix (ECM), and have a very high fraction of surface available to interact with cells. The porous feature possesses microscale interconnected pores, which are essential to transport the oxygen and nutrient supply for cell growth.[1] The loosely bonding between fibers is beneficial for cell migration and well distribution in the whole fibrous mats, and to expand tissue functionality.[2] Moreover, using innovative collectors and matrix mixtures, scaffolds with aligned fibers, improved mechanical properties or diversified functional moieties can be produced.[3] Therefore, electrospun fibers have gained popularity with the tissue engineering community as potential scaffolds for regeneration of cartilage, bone, skin, and blood vessels.[4]

Drug delivery with polymer nanofibers is based on the principle that the drug dissolution rate increases with increased surface area of both the drug and the corresponding carrier if necessary.[5] Electrospun fibrous membrane containing drugs can be applied locally for wound healing or disease treatment, or postprocessed into other kinds of drug formulations. A local therapy has many potential advantages such as circumventing possible adverse effects resulting from systemic administration, decreasing dose or number of dosages, and maintaining local agent levels within a desirable range.[6] The great flexibility of selecting materials allows the incorporation of not only antibiotics and anticancer drugs, but also cells, DNA, and proteins that can be delivered site-specifically and in a controlled and sustained manner over periods of time from days to weeks and even months.[7]

Biodegradability is a critical feature for drug delivery carriers and tissue engineering scaffolds. The release rate and effective lifetime of drug delivery devices can be modulated through the degradation of fiber matrices.[8] As for the tissue engineering scaffolds, the fiber degradation is essential to enhance

Correspondence to: X. Li (xhli@swjtu.edu.cn).
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the ingrowth of new tissue, while the degradation rates should be matched with the rate of neo-tissue formation so as to provide a smooth transition of the load from the scaffold to the tissue.[9] Once the new tissue forms or the drug release completes, the carriers are completely degraded and absorbed by the body, leaving no trace in most cases. Therefore, the biodegradability should be individually tailored for specific applications when considering using electrospun fibers in biomedical areas.

Electrospun poly-DL-lactide-poly(ethylene glycol) (PELA) fibers have shown advantages as carriers for drug delivery and tissue engineering scaffolds.[10] When compared to the commonly used electrospun poly(DL-lactide) (PDLLA) fibers, indicating a hydrophobic surface with water contact angle of $132.2^\circ \pm 1.5^\circ$,[11] the inoculation of hydrophilic poly(ethylene glycol) (PEG) segments improved the hydrophilicity of electrospun fibers to create a better environment for cell attachment. Electrospun PELA fibers were used as carriers for controlled release of anticancer drug,[12] proteins,[13] and plasmid DNA in our previous study.[14] The accumulated PEG segments created a hydrophilic microenvironment for drug molecules, resulting in less significant burst release, larger entrapment efficiency and higher specific activity retention than electrospun PDLLA fibers. An initially burst release of lysozyme as low as 6.2% followed by gradual release for 33 days was indicated from PELA fibers.[15] Electrospun PELA fibrous mats with loading of basic fibroblast growth factor (bFGF) induced full recovery of diabetic skin ulcer, complete reepithelialization, and regeneration of skin appendages, because of the sustained release of bFGF and enhanced cell infiltration into fibrous mats.[16]

Although electrospun PELA scaffolds have shown promise in tissue engineering and drug delivery applications, the degradation behaviors after *in vitro* cell inoculation and *in vivo* implantation have not been clarified up to now. The degradation condition might be an effective factor on the degradation kinetics of scaffolds.[17] Therefore, the purpose of this study was to obtain insight into the *in vitro* and *in vivo* degradation behaviors of electrospun PELA scaffolds, when compared with commonly processed degradation in phosphate buffer saline (PBS). Another objective of this study was to determine an optimal *in vitro* investigation method to predict *in vivo* behaviors through comparing the degradation in different incubation conditions.

EXPERIMENTAL

Materials

PEG (M_w = 6 kDa) was purchased from Sigma-Aldrich Inc. (St. Louis, MO). PELA containing 10% of PEG were prepared by bulk ring-opening polymerization using stannous chloride as initiator.[18] The molecular weight (M_n) of 51.9 kDa and polydispersity index (PDI) of 1.90 were determined by gel permeation chromatograph (GPC, Waters 2695 and 2414, Milford, MA) with a Styragel HT 4 column (7.8×300 mm^2) using polystyrene beads as standard. The mobile phase was tetrahydrofuran (THF, Fisher Scientific, Fair Lawn, NJ) using a regularity elution at a flow rate of 1.0 mL/min. Ultra-pure water from a Milli-Q biocel purification system (UPI-IV-20, Shanghai UP Scientific Instrument, Shanghai, China) was used. All other chemicals and solvents were of reagent grade or better, and purchased from Changzheng Regents Company (Chengdu, China) unless otherwise indicated.

Fabrication of electrospun PELA fibrous mats

Electrospun PELA fibrous mats were obtained as described previously.[19] Briefly, PELA solution in acetone was added in a 2 mL syringe, attached with a clinic-shaped metal capillary. An oblong counter electrode was located about 15 cm from the capillary tip. The flow rate was controlled within 0.6 mL/h by a precision pump (Zhejiang University Medical Instrument Company, Hangzhou, China) to maintain a steady flow from the capillary outlet. The applied voltage was controlled within the range of 20 kV using a high voltage statitron (Tianjing High Voltage Power Supply Company, Tianjing, China). The fiber collections were vacuum dried at room temperature for 2 days to completely remove any solvent residue prior to further use.

Characterization of electrospun PELA fibrous mats

The morphology of electrospun fibers was examined by scanning electron microscope (SEM, FEI Quanta 200, The Netherlands) equipped with field-emission gun and Robinson detector after vacuum-coated with a thin layer of gold to minimize charging effect. The fiber diameter was measured from SEM images, and five images were used for each fibrous sample. From each image, at least 20 different fibers and 100 different segments were randomly selected and their diameter was measured to generate an average fiber diameter by using the tool of Photoshop v8.0.[19]

Degradation of electrospun PELA fibrous mats *in vitro*

Electrospun PELA fibrous mats were sterilized before degradation tests, and mounted by a cell-culture ring designed by Zhu et al. to avoid shrinkage and contraction during incubation.[20] The sterilization was conducted by commercial ^{60}Co γ-radiation, ultraviolet irradiation, 70% ethanol treatment, or electron-beam

irradiation using linear accelerator (Precise™, Elekta, Crawley, UK) with a total dose of 80 cGy. Fibrous mats of around 15 mg were put into in a 12-well tissue culture plate (TCP), and 2.0 mL of PBS, simulated body fluid (SBF) or Dulbecco's modified Eagle's medium (DMEM) were added into each well before incubation at 37°C. SBF was prepared based on previous report,[21] and DMEM was from Gibco BRL (Rockville, MD). The degradation media were refreshed every 1 week.

In another group of wells degradation tests were performed in the presence of *in vitro* cell culture. NIH3T3 cells were from American Type Culture Collection (Rockville, MD), and cultured in DMEM containing 10% fetal bovine serum (FBS) with 4 m*M* L-glutamine, 4.5 mg/mL glucose, 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco BRL, Rockville, MD), and incubated at 37°C and 5% CO_2 humid atmosphere. Cells were cultured either on TCP or on fibrous mats, which were named cell/T and cell/F groups, respectively. In the cell/F group, 200 μL of cell suspension (2×10^4 cells/mL) was seeded onto fibrous mats and placed in a 12-well TCP. The cell-seeded mats were incubated at 37°C in a humidified atmosphere for 4 h to make cells diffuse into and adhere to the scaffold before the addition of 1.8 mL of culture medium into each well. In the cell/T group, cells were seeded at 4000 cells/well in 2.0 mL DMEM before inputting the fibrous mats. After 7 days of incubation, cells on TCP or fibrous mats were trypsinized and reseeded onto TCP or fibrous mats. The above process was repeated till the end of degradation study. At predetermined intervals, the degradation media were removed and then electrospun mats were rinsed with distilled water, and dried in vacuum desiccator.

Degradation of electrospun PELA fibrous mats *in vivo*

All animal procedures were approved by the University Animal Care and Use Committee. The sterilized electrospun fibrous mats were rolled into a cylindrical shape of 15 mm length. Animals were anesthetized upon intraperitoneal injection of pentobarbital (45 mg/mL), and the femoral hair was removed with hair clippers. Six incisions were made in the back using a blade, and subcutaneous pockets were created by incision using blunt scissors. After implanting fibrous samples subcutaneously, the incision was then sutured. The animals awoke 1–2 h after the operation, behaved as usual, and had normal appetite. After 1, 2, and 4 months, implanted scaffolds were retrieved with surrounding tissues. All the fresh tissues attached to the matrices were removed without damage to the underlying fibrous matrices. The explants were rinsed with ethanol/water (70 : 30, v/v) and distilled water, and then vacuum dried at room temperature overnight.

Characterization of degradation behaviors of PELA fibrous mats

The degradation behaviors were estimated from gross views, microscopic morphologies, and mass loss of fibrous mats, and from changes of M_n and PDI of matrix polymers. The visual images of fibrous mats after degradation were determined with a CCD camera, and the microscopic morphological change was estimated from SEM observation as aforementioned. The recovered fibrous mats from the degradation experiments were dissolved in THF, and the molecular weight and PDI were determined using GPC as described earlier. The mass loss was determined gravimetrically by comparing the dry weight of recovered polymers at a specific time with the initial weight.

Statistical analysis

All experiments were performed with $n = 4$ and the values were presented as mean ± standard (SD). Whenever appropriate, a two-tailed Student's *t*-test was used to discern the statistical difference between groups. Probability values of $P < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Characterization of electrospun PELA fibrous mats

The electrospinning parameters were adjusted until nonbeaded and uniform fibers could be obtained.[19] The SEM image of nonwoven electrospun PELA fibrous mat is shown in Figure 1, indicating a randomly interconnected structure, smooth morphology with bead-free and very porous. The mean diameter of electrospun fibers was 870 ± 95 nm.

Effect of sterilization on electrospun PELA fibrous mats

The challenge remains to achieve an efficient and nondestructive sterilization procedure for polyester scaffolds that preserves their 3D structure, which was critical to tissue engineering and drug delivery applications.[22] Sterilization techniques were optimized in current study to minimize damages to the delicate 3D fibrous geometry. Ethanol at concentrations of 60–80% (v/v) was usually used to treat electrospun fibrous mats for cell culture study.[23] But it was classified as a disinfectant, rather than a sterilization agent, because of its inability to destroy hydrophilic viruses or bacterial spores.[24] In addition, shrinkage and brittleness of fibrous mats were

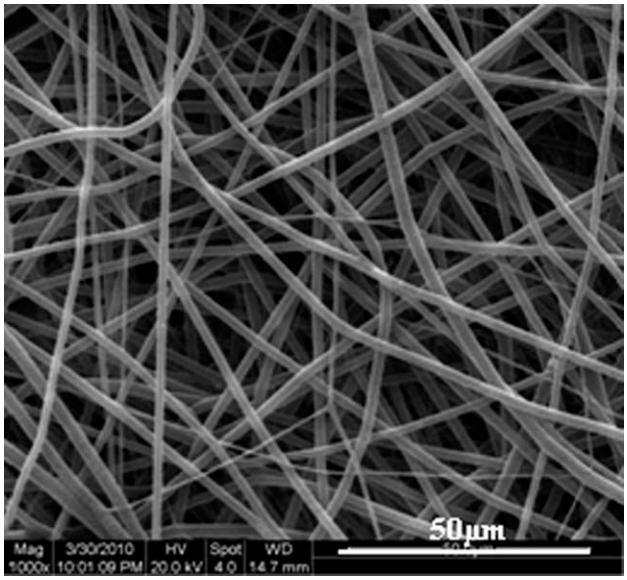

Figure 1 SEM image of electrospun PELA fibrous mats.

observed in current study after ethanol treatment. To achieve a better sterilization method of fibrous mats for tissue engineering purposes, Rainer et al. reported that ultraviolet irradiation was one of the most suitable techniques for an effective sterilization while preserving chemical and morphological features of electrospun fibers.[25] But it indicated less effective to sterilize thick fibrous mats in the current study, and bacteria contamination occurred after 3–4 days of incubation in cell culture medium. γ-irradiation was thought to be the most effective means of sterilization or decontamination, and used previously for sterilization of electrospun fibrous mats.[26] As shown in Figure 2(a), significant morphological changes were observed in sterilized fibers by commercial γ-irradiation, containing flat fibers with fusion points between fibers. The obtained fiber matrix indicated M_n of 26.8 kDa and PDI of 2.32, and there was around 50% of molecular weigh-loss after γ-irradiation. Thus, γ-irradiation was unsuitable to sterilize electrospun PELA fibers, because of the significant degradation of matrix polymers and fusion of fibers. Alternatively, electron-beam irradiation with a total dose of 80 cGy was adopted in current study, indicating efficient sterilization and no significant effect on the matrix polymers and fibrous morphology [Fig. 2(b)]. The obtained fibrous mats indicated M_n of 46.4 kDa and PDI of 1.98, and were used for the degradation study.

Morphological changes of PELA fibrous mats after *in vitro* incubation

To evaluate the degradation profiles of electrospun fibers under different conditions, the gross views and microscopic morphologies, mass loss, molecular weight reduction, and PDI changes were estimated. Figure 3 shows the gross views of fibrous mats after 2, 5, and 8 weeks of incubation. There was no significant change after 2 weeks of incubation. Apparent shrinkage was found for fibrous mats after 5 weeks of incubation in PBS, SBF, and DMEM, while slight breakage of fibrous mats occurred in cell/F and cell/T groups. At week 8 after incubation, broken fibrous mats were indicated for all the fibrous mats, and significant fragmentation was observed for fibers of DMEM, cell/F, and cell/T groups.

Figure 4 shows SEM morphologies of fibrous mats after incubation under different conditions. When compared with the fibrous morphology before degradation as shown in Figure 2(b), the fiber size increased and the space between fibers shrank for

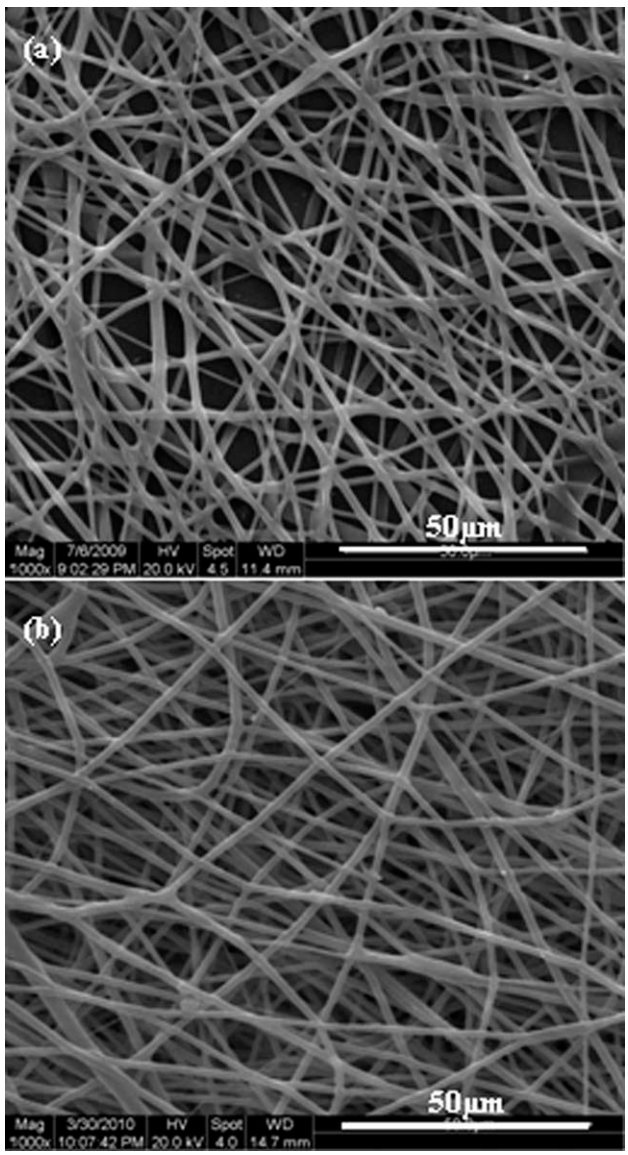

Figure 2 (a) SEM images of electrospun PELA fibrous mats after sterilization by commercial γ-irradiation and (b) electron-beam irradiation with a total dose of 80 cGy.

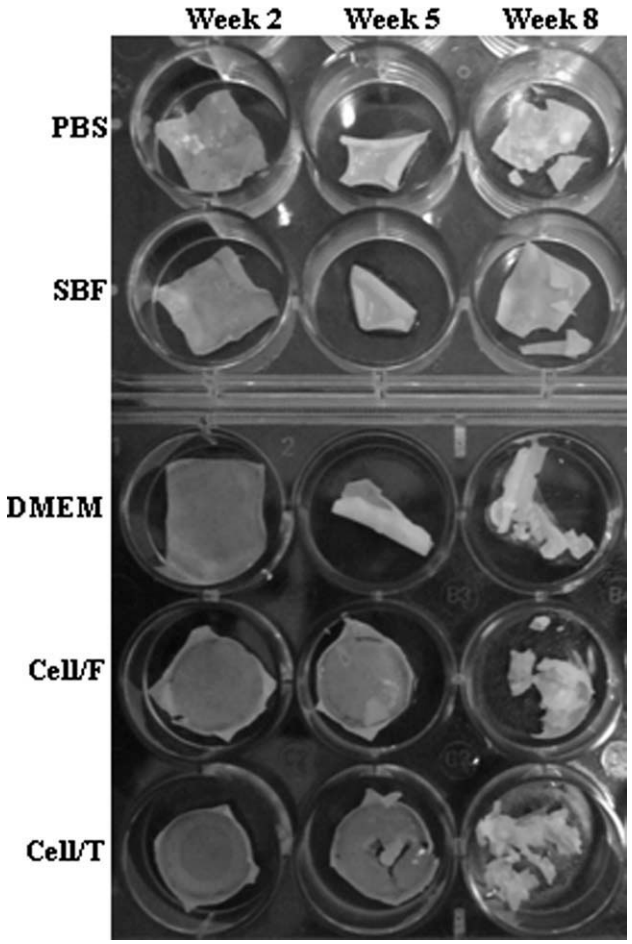

Figure 3 Gross views of electrospun PELA fibrous mats after degradation in PBS, SBF, and cell culture medium (DMEM), and after cell culture on fibrous mats (cell/F) and tissue culture plates (cell/T) for 2, 5, and 8 weeks.

all samples, and curliness and congeries of fibers were easily found after 5 weeks of incubation. No significant difference was observed among the fibrous mats after incubation under different conditions. The electrospinning process caused the inner stress and high degree of alignment and orientation of polymer chains, and the shrinkage was due to the chain relaxation of matrix polymers after incubation into the medium with elevated temperature.

As shown in Figure 4, at week 8 after incubation in PBS and DMEM, fibers were fused into porous films without distinct fiber structure. There were larger amount of nanoparticles deposited on fibers after 8 weeks of incubation in SBF in comparison with those at week 5. As indicated in the previous study, there was no calcium phosphate precipitate formed on electrospun PDLLA fibers during incubation in SBF, because of the lack of bioactive groups to induce nucleation and growth of calcium phosphate.[27] However, the formation of carboxyl end groups after extensive degradation of PELA backbone should induce surface nucleation and promote apatite formation. It was indicated that carboxyl groups were initially combined with calcium ions through electrostatic attraction, and then phosphoric ions, which were considered to promote the nucleation and subsequent growth of calcium phosphate on the degraded fibers.[28] For the cell/T and cell/F groups, fibers became flat and collapsed from their previous cylindrical shape after 8 weeks of incubation, and significant rupture and conglutination of fibers could be found (Fig. 4).

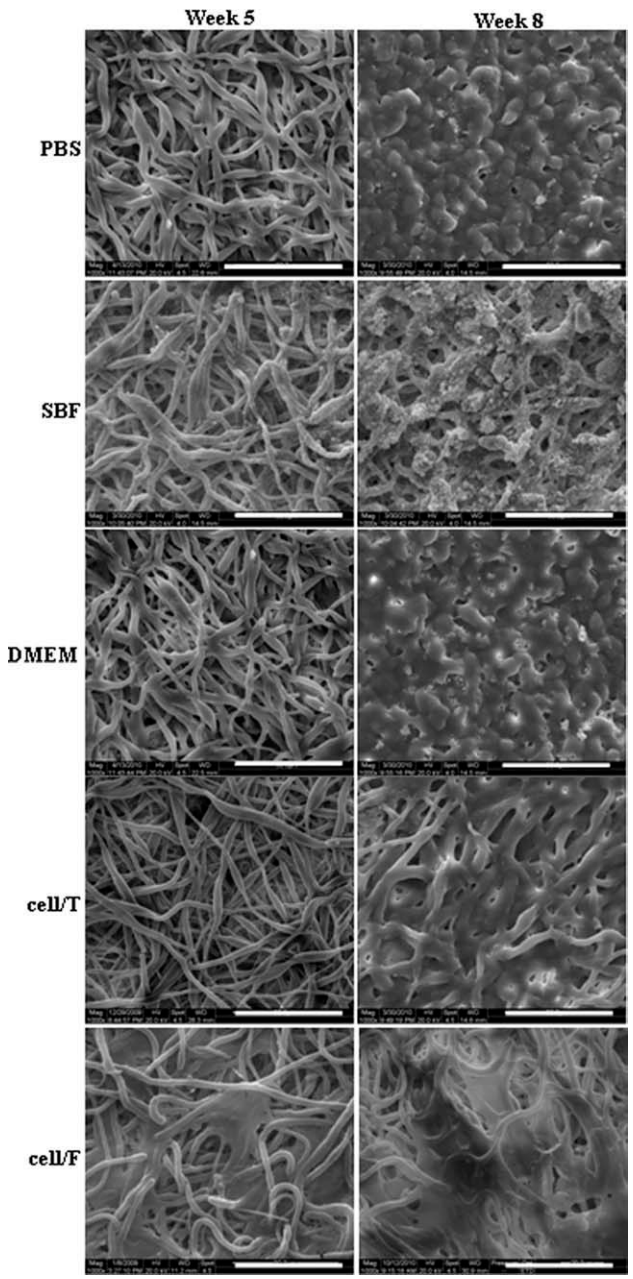

Figure 4 SEM images of electrospun PELA fibrous mats after degradation in PBS, SBF, and cell culture medium (DMEM), and after cell culture on fibrous mats (cell/F) and tissue culture plates (cell/T) for 5 and 8 weeks. Bars represent 50 μm.

Degradation profiles of PELA fibrous mats after *in vitro* incubation

Gravimetric evaluation of the mass loss of fibrous mats is summarized in Figure 5(a). The mass loss was slow in the early stage and became more significant in the later stage of degradation, due to the dissolve of degraded oligomers into the medium. There was no significant difference during the initial 2 weeks of incubation among different degradation conditions. However, the difference in the mass residual became larger during the following incubation. The mass residuals for cell/T and cell/F groups were significantly lower than fibers after incubation in PBS, SBF, and DMEM ($P < 0.05$). There were around 24.7%, 23.8%, 25.2%, 29.9%, and 31.2% of mass loss after 8 weeks incubation of fibers in PBS, SBF, DMEM, cell/T, and cell/F, respectively.

Figure 5(b) shows the decrease in molecular weight of matrix polymers versus the degradation time under different conditions. Significant difference was observed between cell involving conditions and other groups. The molecular weight loss was significantly higher for cell/F and cell/T groups than those after 5 weeks of incubation in PBS, SBF, and DMEM ($P < 0.05$). However, the molecular weight loss for cell-involving degradation samples turned to be lower during the following incubation. The molecular weight residuals were around 26.1%, 25.4%, 24.9%, 32.0%, and 36.8% after 8 weeks of incubation of fibers in PBS, SBF, DMEM, cell/T, and cell/F, respectively. Significantly higher molecular weight remaining was detected for the cell/F group than those under other degradation conditions ($P < 0.05$).

As shown in Figure 5(c), the molecular weight polydispersity indices (PDIs) of fiber matrices increased with the incubation time for all the degradation process. Larger PDIs were detected for cell-involving degradation process than those after incubation in PBS, SBF, and DMEM during 2 weeks of incubation. However, PDIs of matrix polymers indicated a decrease for the cell/F group and no apparent change for the cell/T group during the following incubation. After 8 weeks of incubation, PDIs increased from 1.98 to 2.86, 2.82, 2.79, 2.56, and 2.20 for fibers after incubation in PBS, SBF, DMEM, cell/T, and cell/F groups, respectively. Significantly, lower PDI was detected for the cell/F group than those under other degradation conditions ($P < 0.05$).

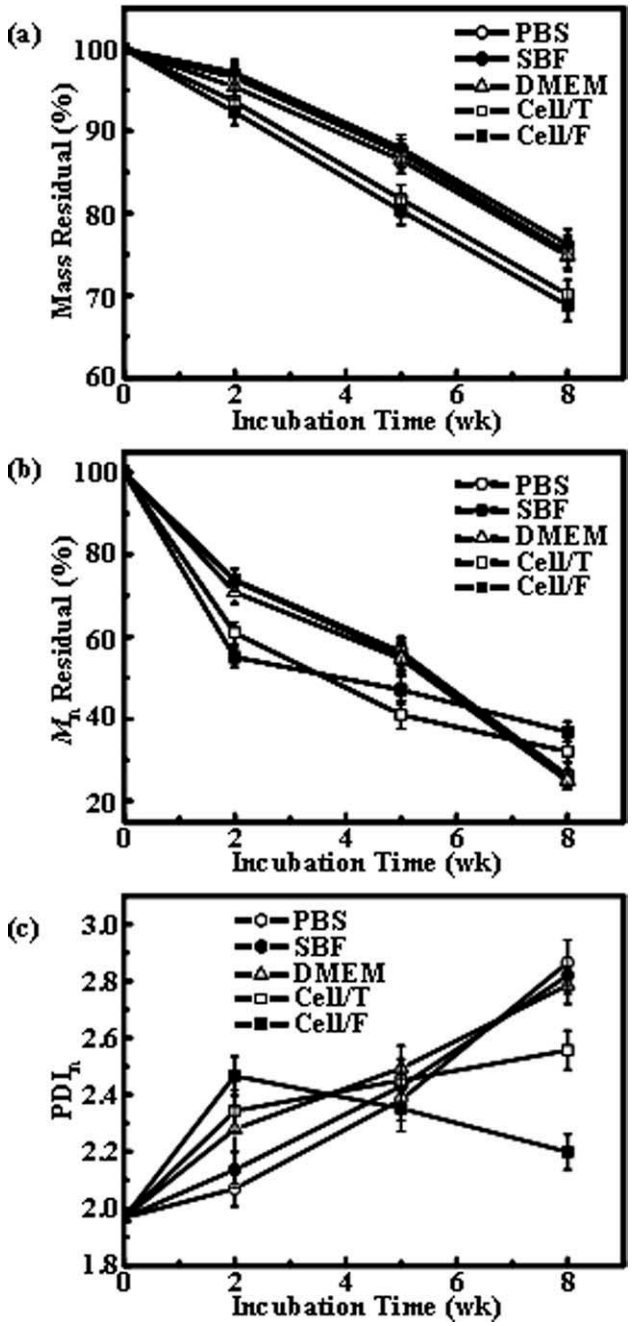

Figure 5 The mass residuals of (a) electrospun PELA fibrous mats, (b) the molecular weight reduction, and (c) polydispersity index (PDI) increase of matrix polymers after degradation in PBS, SBF, and cell culture medium (DMEM), and after cell culture on fibrous mats (cell/F) and tissue culture plates (cell/T) ($n = 4$).

Effects of *in vitro* incubation conditions on the degradation profiles

As shown in Figure 5, during the investigational time period, there was no significant difference in the mass loss, molecular weigh residual, and PDIs of matrix polymers after incubation of fibrous mats in PBS, SBF, and DMEM ($P > 0.05$). After incubation into SBF, calcium phosphate precipitated on the fiber surface at the later stage of incubation (Fig. 4). However, the amount of precipitates was low and only present on the fiber surface, which led to a negligible effect on the degradation of fiber matrix based on our previous study.[29] When compared with PBS, the presence of serum in DMEM enhanced the wettability of electrospun fibers, which resulted in

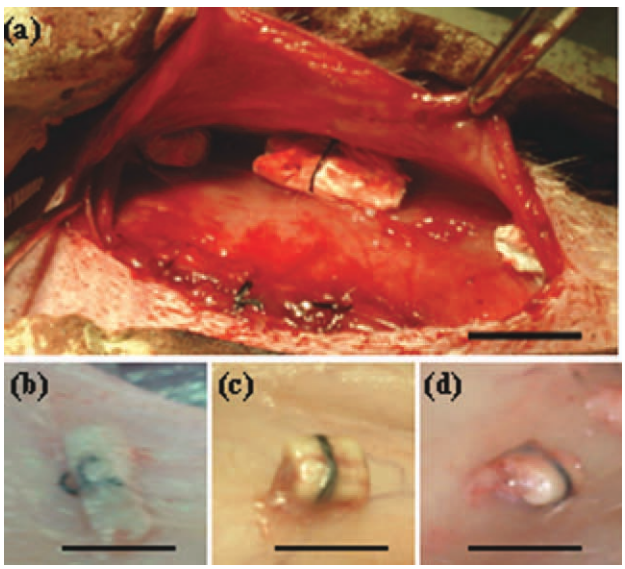

Figure 6 (a) Gross views of subcutaneously implanted electrospun PELA fibrous mats, and fibrous mats retrieved at weeks (b) 5, (c) 8, and (d) 16 after implantation. Bars represent 10 mm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

slightly higher mass loss [Fig. 5(a)] and molecular weigh reduction [Fig. 5(b)].

As shown in Figure 5, the inoculation of cells into degradation environment significantly affected the degradation profiles of electrospun fibrous mats. It was indicated that many *in vitro* factors could influence the biodegradation of polyesters, including pH, ionic strength of the medium, and enzymatic activity.[30] The high degradation rates of cell/F and cell/T groups in the early stage may be resulted from the pH value of degradation media decreased by the living of cells and the secretory production of cells.[31] So that the molecular weight residuals were lower and PDIs were higher than those of other groups. However, much higher amount of low-molecular-weight oligomers generated during the degradation process should be absorbed by cells, leading to the remaining of higher molecular weight part [Fig. 5(b)] and a slower increase in PDIs of matrix polymers [Fig. 5(c)]. In addition, the efficient removal of degraded oligomers alleviated the fiber fusion after involving cells into the degradation, while porous films without distinct fiber structure were indicated after incubation in PBS and DMEM for 8 weeks (Fig. 4).

In the cell/F group, cells were attached on the fiber surface spanning the gaps between electrospun fibers, as indicated by the flattened and spreading morphology covering the surface (Fig. 4). The tight attachment of cells on the fibers formed a sealing zone, into which acidic products and/or degradative enzymes were secreted.[31] The interfacial environment between cells and electrospun fibers enhanced the degradation, leading to a higher molecular weight loss [Fig. 5(b)] and larger PDIs of matrix polymers [Fig. 5(c)] for the cell/F group than those of the cell/T group after 2 weeks of incubation. In addition, the pseudopodia of cells not only attached to the material surfaces but actually penetrated into the fiber matrices along the degradation of matrix polymers. The increased resorbing area created a dynamic condition to remove degraded oligomers, leading to higher mass loss [Fig. 5(a)], higher molecular weight remaining [Fig. 5(b)], and lower PDIs of matrix polymers [Fig. 5(c)] for the cell/F group than those of the cell/T group after 8 weeks of incubation.

Degradation behaviors of PELA fibrous mats *in vivo*

Subcutaneous implantation is a commonly-used, standard model for investigating *in vivo* degradation. Figure 6(a) shows the image of subcutaneous implantation of fibrous mats, and Figure 6(b–d) show gross views of retrieved fibrous mats at weeks 5, 8, and 16 after implantation. At harvesting all the implants were covered by an intact fibrous tissue capsule. The degradation was evident on implanted material because of the disappearance of many fibers, and only around 1/4 of the fibrous cylinder remained at the implantation site at week 16 after implantation. The infiltration of tissues into fibrous mats destroyed the structural integrity, and the degraded fragments escaped from the implantation site.

SEM analysis was used to assay the morphological changes of electrospun PELA fibrous mats after

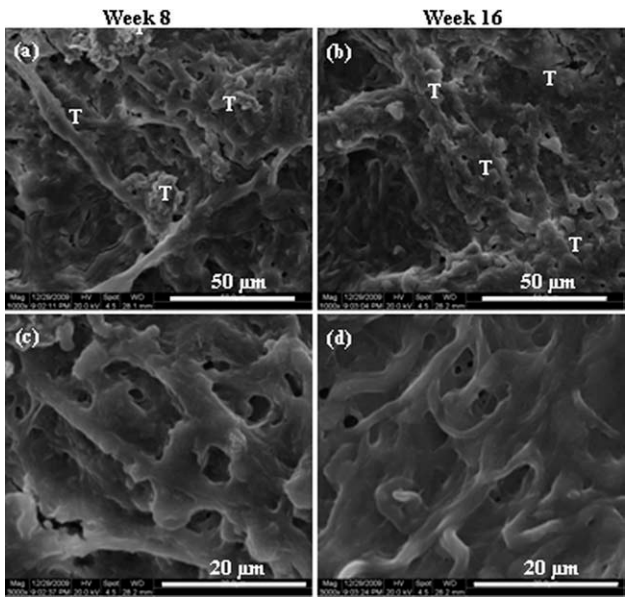

Figure 7 SEM images of fibrous mats retrieved at weeks 8 (a and c) and 16 (b and d) after implantation. "T" in a and b represents tissue infiltration into fibrous mats, c and d show higher magnification images.

TABLE I
Degradation Behaviors of Electrospun PELA Fibrous Mats After *In vivo* Implantation ($n = 4$)

Incubation time (week)	M_n (kDa)	PDI
5	22.7 ± 3.4	2.08 ± 0.05
8	17.3 ± 2.8	2.01 ± 0.06
16	11.0 ± 2.3	2.02 ± 0.05

subcutaneous implantation up to 16 weeks. The electrospun mats were rolled into fibrous cylinders for implantation, and body fluid, cells, and tissues should migrate into porous mats and spaces between layers of fibrous mats. The outer layers of the fibrous cylinder were removed, and fibrous layers at the inner part of the cylinder were observed by SEM. As shown in Figure 7, tissues migrated into the fibrous layers at week 8 after implantation, and a layer of tissue covered the fibrous mats at week 16 postimplantation. Fibers were swollen along with a decrease in the space between fibers of the extracted mats because of the degradation of matrix polymers. More structural definition loss of the fibers was observed at week 16 [Fig. 7(d)]. The fibrous mats were completely infiltrated with most pores having collapsed, while most fibers shrank and collapsed from their previous cylindrical shape.

Table I summarizes the change of molecular weights and PDIs of matrix polymers of the fibrous mats retrieved. There was about 75% of molecular weight loss at week 16 after implantation, while there was no marked difference in PDIs after implantation up to 16 weeks ($P > 0.05$). There were significant differences in the molecular weight and PDIs when compared with *in vitro* degradation data. As shown in Figure 5, there was around 60% of molecular weight remaining after 5 weeks incubation in cell-free degradation media. As a result of *in vivo* implantation, the typical response was the accumulation of cells, such as phagocytes and lymphocytes.[32] The cell secretions, such as acidic products and/or degradative enzyme may enhance the degradation of matrix polymers, resulting in significantly lower-molecular-weight residual of about 49% at week 5 after *in vivo* implantation, as compared with *in vitro* cell-free degradation media ($P < 0.05$). This showed the same tendency as previous reports, in which matrix polymers degraded significantly faster *in vivo* than in an *in vitro* environment.[33] However, at the later stage of degradation process, the accumulation of degradation products containing carboxylic end-groups in the fiber matrices, due to the lack of cell resorption and dynamic circulations during *in vitro* incubation, led to a higher degradation rate. A shown in Figure 5, there was around 25% of molecular weight remaining at week 8 after incubation in cell-free degradation media, which was significantly lower than about 37% for *in vivo* implantation ($P < 0.05$). In conclusion, as compared with *in vitro* cell-free degradation media, *in vivo* implantation indicated higher degradation rate at the initial stage, because of the tissue infiltration and cellular activation, and slower degradation at the later stage, due to the lack of autocatalysis effect of acidic oligomers accumulated.

The retarding of dynamic diffusion during *in vitro* incubation should be less significant when culture cells with fibrous mats. As shown in Figure 5 and Table I, there were about 47.1% and 48.9% of molecular weight remaining at week 5 for the cell/F group and after *in vivo* implantation, respectively. Similar results were also observed at week 8, which were about 36.8% and 37.2% for the cell/F group and after *in vivo* implantation, respectively. In addition, the molecular weight PDIs were around 2.0 for all the *in vivo* implanted samples, and no significant difference was found with electrospun fibers before degradation, because of the rapid diffusion of degraded oligomers. The cell/F group showed the molecular weight PDI of around 2.2 at week 8, which was the lowest among the *in vitro* incubation groups. Therefore, the degradation after cell culture on fibrous mats indicated similar profiles with *in vivo* implantation. An *in vitro* degradation study was essential to provide a methodological and theoretical basis for the understanding of *in vivo* degradation. The cell culture on fibrous mats provided cellular environment and dynamic diffusion condition, indicating an effective *in vitro* tool to predict *in vivo* degradation profiles of electrospun fibers.

CONCLUSIONS

Different degradation behaviors were determined on electrospun fibers after cell participation and *in vivo* implantation, when compared with commonly processed degradation in PBS. The inoculation of cells into degradation environment accelerated the degradation, and the tight attachment of cells on fibers further enhanced the degradation process after cell seeding on the fibrous mats. When compared with *in vitro* cell-free degradation media, the subcutaneous implantation of fibrous mats led to significantly higher degradation rate at the initial stage, but slower degradation at the later stage. In addition, the degradation after cell culture on fibrous mats indicated close results with *in vivo* implantation, thus providing an effective *in vitro* tool to predict *in vivo* degradation profiles of electrospun fibers. The obtained results should provide useful information on the *in vitro* and *in vivo* degradation rates of electrospun fibrous scaffolds and help researchers match them with specific purposes.

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