

INITIATOR-FREE PHOTOCROSSLINKING OF ELECTROSPUN BIODEGRADABLE POLYESTER FIBER BASED TUBULAR SCAFFOLDS AND THEIR CELL AFFINITY FOR VASCULAR TISSUE ENGINEERING*

Lin Ye^b, Xin Wu^{a+}, Xue Geng^b, Yong-hong Duan^a, Yong-quan Gu^a, Ai-ying Zhang^b,
Jian Zhang^{a**} and Zeng-guo Feng^{b**}

^a Xuanwu Hospital, Capital Medical University, Beijing 100053, China

^b School of Materials Science and Engineering, Beijing Institute of Technology, Beijing 100081, China

Abstract Random copolyester of poly(ϵ -caprolactone-co-L-lactide) (PCLA) with a 50:50 feeding molar ratio was synthesized *via* the ring-opening polymerization and functionalized by the end-capping reaction with acryloyl chloride. The resulting acrylated PCLA was then fabricated into small diameter tubular scaffolds by electrospinning technique and the formed scaffolds were followed by photocrosslinking under UV irradiation in the absence of photoinitiator. The mechanical strengths including tensile, suture retention and burst pressure were greatly enhanced after the photocrosslinking. The *in vitro* degradation data clearly revealed that the mechanical properties of the crosslinked scaffolds still remained after one month degradation in PBS solution, while those of the non-crosslinked ones lost heavily. The cytotoxicity assay on the mouse fibroblast L929 cells was conducted *via* MTT measurement. Furthermore, the observation on endothelial and fibroblast cell adhesion and proliferation was also made by using scanning electron microscopy (SEM). The initiator-free photocrosslinked tubular scaffolds show the potential to be used in vascular tissue engineering.

Keywords: Vascular tubular scaffold; Photo-crosslink; Electrospinning; Mechanical property; *In vitro* degradation.

INTRODUCTION

Despite many years of efforts and various attempts, small-diameter (less than 6 mm in inner diameter) vascular grafts have not been clinically applied, mainly due to thrombus- and excess tissue growth-caused occlusion after grafting^[1–4]. As a prominent protocol in regenerative medicine, vascular tissue engineering (TE) has offered an alternative using biodegradable tubular scaffold for the mechanical support, shape, and cell-scale architecture of neo-tissue construction *in vitro* or *in vivo* as seeded cells expand and organize.

As for the *in vivo* vascular TE strategy, besides the patency, the mechanical properties of the scaffold are also indispensable for the success of artificial vascular grafts during the reconstruction process^[5]. The mechanical strengths including tensile, bursting pressure, suture retention and the like not only afford the appropriate strength to resist the impact of blood pressure and exhibit good compliance in accordance with the throb of blood fluid, but also provide physical-chemical signals to communicate with the seeded cells^[6]. Consequently, the poor mechanical property may result in the early failure of artificial graft, for example, bad

* This work was financially supported by the ‘863’ Project of Minister of Science and Technology of China (Nos. 2006AA02A134 and 2007AA021905) and basic research foundation of Beijing Institute of Technology (No. 20080942001).

** Corresponding author: Jian Zhang (张建), E-mail: zhangjianxw@yahoo.cn

Zeng-guo Feng (冯增国), E-mail: sainfeng@bit.edu.cn

⁺ The first two authors contribute equally to this work.

Received December 16, 2009; Revised February 3, 2010; Accepted February 11, 2010
doi: 10.1007/s10118-010-9188-5

compliance may lead to the formation of pseudoaneurysm, because diameter will become bigger and the wall will get thinner under cyclic deformations arising from the throb of blood fluid. However, as the crosslink structure is highly favorable over non-crosslink structure to resist cyclic stress relaxation, the crosslinked tubular scaffold is expected to maintain its mechanical property for long term so that it is durable enough to withstand high pressure and flow environments in blood vessel without collapse until neo-tissue develops and matures *in vivo*.

To crosslink the initial porous scaffold can substantially enhance its mechanical strengths so that the excellent mechanical properties of biological grafts are mimicked. It has been recognized as one of the necessary and key techniques to prepare vascular TE scaffolds. For instance, the collagen and other natural materials are fabricated into tubular scaffold by electrospinning, and then are crosslinked by using crosslink agent in order to enhance their mechanical strengths. Glutaraldehyde is frequently used to crosslink collagen or acellular scaffolds, but its residue toxicity is extremely concerned^[7, 8].

Photo-induced crosslinking exhibits many advantages such as fast polymerization, mild reaction condition, easy manipulation, low toxicity and so on^[9]. Among these, the mild reaction condition under which photocrosslinking occurs has promoted investigators to broadly utilize photopolymerization as a means for biomaterial preparations. In theory, photopolymerization can provide an effective and benign method for the *in situ* photocrosslinking that could improve the mechanical properties of scaffold without damaging its morphology and biological properties. Another advantage of this strategy is that the sterilization and crosslinking process are carried out simultaneously while the UV irradiation is hired to carry out the photocrosslinking process. However, during the process adding photo-initiators often arouses the risk of residue toxicity^[10]. An appropriate solution is that the initiator-free photocrosslinking is applied. Greiner and co-workers introduced thienyl acrylate groups into PVA, and then the electrospun thio-PVA was photocrosslinked without using any photoinitiator, but the synthetic route of thienyl chloride was tedious^[11].

Currently electrospinning is emerging as the choice of interest for constructing vascular TE tubular scaffolds. It is a fiber spinning technique that produces polymer ultrafine fibers of nanometer to micrometer size in diameters through the action of an external electric field imposed on a polymer solution or melt^[12–15]. The ultrafine fibers prepared by electrospinning provide unexpected high surface area to volume ratios, porous structure and other adequate characteristics for vascular tubular scaffolds^[16–19]. A series of biodegradable copolyesters (PCLAs) of ϵ -caprolactone (ϵ -CL) and L-lactide (LLA) were synthesized *via* the ring-opening polymerization and fabricated into vascular tubular scaffold by electrospinning technique as previously reported^[20, 21]. However, a preliminary result showed that those electrospun vascular scaffolds collapsed soon after they were implanted *in vivo* due to low resistance against environmental tissue pressure. It mandated that a more durable scaffold is required to withstand the pressure and flow environments without collapse until vascular tissue develops and matures *in vivo*. Aimed at fabricating a more durable scaffold with powerful mechanical properties in this context, a random biodegradable copolyester of ϵ -CL and LLA with a 50:50 feeding molar ratio was synthesized and end-capped by using acryloyl chloride. Then the acrylated polyester was fabricated into small diameter tubular scaffolds by electrospinning technique and the formed scaffolds were followed by the initiator-free photocrosslink conducted by UV irradiation. Furthermore, the biocompatibility of the crosslinked tubular scaffolds was evaluated by both cytotoxicity assay and cell adhesion and proliferation observation. The long term maintenance of the mechanical strengths of the resulting tubular scaffolds was also manifested by *in vitro* degradation.

MATERIALS AND METHODS

Materials

ϵ -Caprolactone (ϵ -CL) (Aldrich, USA) was purified by distillation under reduced pressure after being dehydrated by CaH_2 . L-lactide (LLA) (Tianyuan biomaterial company, Fushun, China) was recrystallized from ethyl acetate and dried for 24 h at 40°C under vacuum before polymerization. Stannous 2-ethyl hexanoate (Sigma, USA) was used as received. 1,4-Butanediol (BDO) (Beijing Chemical Reagents Company, China) was

purified by distillation under reduced pressure. Acryloyl chloride (Alfa-Aesar, USA) and dimethoxyphenylacetophenone (DMPA) (Fluka, Switzerland) were received without purification. All of other reagents and solvents used were of analytical grade.

Preparation of Acryloyl Terminated PCLA

Copolyester (PCLA) of ϵ -CL and LLA having a feeding molar ratio of CL:LLA = 50:50 was synthesized according to previous reports^[20, 21]. PCLA (5 g) was dissolved in 10 mL THF along with the proton scavenger triethylamine (TEA) (0.101 g, 1×10^{-3} mol) to which acryloyl chloride (0.091 g, 1×10^{-3} mol) in 5 mL THF was added dropwise in ice-water bath. The white precipitate was formed gradually and the reaction was maintained at 0–4°C for 6 h. The precipitate was filtered and the solvent was removed by rotation evaporation. The product was dissolved in chloroform and precipitated in methanol. Finally the product was dried in vacuum oven at room temperature with yield 95%. FTIR/cm⁻¹: 3047 (vinyl hydrogen), 2906 (alkyl), 1759 (carboxyl), 1635 (C=C), 1112 (C—O); ¹H-NMR/ δ : 6.45, 6.15, 5.90 (vinyl group), 5.15 (CH, LLA), 1.56 (CH₃, LLA), 4.10, 2.31, 1.62 and 1.38 (five methylene groups in CL). According to ¹H-NMR spectrum, about 90% PCLA was terminated by acryloyl groups.

Photocrosslinked Membrane Preparation

The photocrosslinking of the acrylated PCLA was initiated with UV irradiation (315–380 nm, 150 W) at room temperature. To highlight the effect of changing the initiator concentration and crosslinking time on the mechanical properties, four compositions containing 0 mol%, 0.5 mol%, 1 mol% and 2 mol% DMPA were irradiated with UV light for 5, 20, 40 and 60 min, respectively. The acrylated PCLA (1.0 g) and a pre-determined amount of DMPA were both dissolved in 10 mL CH₂Cl₂ solution. The resulting homogeneous solution was transferred into a PTFE model to vaporize at room temperature for 24 h to give a clear membrane. It was dried in vacuum oven at room temperature for another 24 h. For photocrosslinking, the dried membrane was placed under UV light with a distance of 7 cm from the lamp for a certain period of time. The membrane was turned over with an interval of 5 min during the process.

Tensile Measurements

The polymer membrane was cut into dumbbell-shaped samples and their rectangle part was 20 mm $\times$ 5 mm with 0.5 mm thickness. The tensile measurement of the compression-molded membrane samples was done on a WD4005 tensile testing machine (Kexing Experimental Instrument Research Company, Changchun, China) equipped with a maximum 2 kN load cell at a crosshead speed of 100 mm/min according to ASDM standard. The results reported were the mean values for five replicates.

Gel Fraction Determination

The crosslinked PCLA membrane (10 mm $\times$ 10 mm, rectangle) was immersed in excess CH₂Cl₂ at room temperature. The membrane weight was measured and defined as original weight before immersing. After two days, the samples were taken out and dried in vacuum oven to remove solvent thoroughly. The membrane weight was measured again and defined as dry weight. Based on the original (W_0) and dry weight (W_d), the gel fraction (GF) of photocrosslinked PCLA membrane was calculated using the following equation.

$$GF = W_d/W_0 \times 100\% \quad (1)$$

Fabrication of Crosslinked Tubular PCLA Scaffold by Electrospinning

A custom-made electrospinning apparatus was constructed using a high-voltage supplier (BGG 40 kV/2 MA, BMEI Co., Ltd, China), a syringe pump (BT01-100, Longer, China), a syringe with a stainless steel blunt-ended needle and a custom-made rotating mandrel-type collector or the plate collector. The acrylated PCLA copolymer was dissolved in CH₂Cl₂ at a final concentration of 19 wt%. The solution was delivered at a constant flow rate (5 mL/h) with an air gap of 20 cm between the metal collector and the needle tip at a driving voltage of 20 kV. The dropped polymeric solution was spun on a rotating metallic mandrel collector and as-spun fibers were mutually bonded to form seamless tubular scaffold. After the tubular scaffold was formed, it was rotated under

UV light for 40 min for photopolymerization.

Characterization of Electrospun PCLA Scaffold

Netzsch PC-200 was used for an analysis of the thermal behavior of the polymers at a heating rate of 10 K/min. The polymer samples (6–10 mg) were heated from -100°C to 100°C in stainless steel pans. The results of the second run were used for the glass transition temperature (T_g) investigation.

The porosity of electrospun microfiber tubular scaffolds was measured by using mercury porosimetry (Autopore IV 9510, Micromeritics Instrument Corp., USA).

The tubular scaffold was cut along axis direction and made into dumbbell-shaped samples, and the tensile measurement was done as mentioned above in “Tensile Measurements” section.

To determine the suture retention strength of the tubular scaffolds, the samples were cut into a rectangular shape in 5 mm width, 15 mm length, and 0.3 mm thickness. After soaking the specimen in phosphate buffered saline (PBS, pH 7.4) at room temperature for 12 h, one end of the specimen was fixed to the stage clamp of the uniaxial load test machine and the opposite end was connected to another clamp by a common suture material. The suture was placed 2 mm from the edge of the specimen using a tapered noncutting needle. The distance between the clamps was 2 cm. The specimen was pulled at a crosshead speed of 8 mm/min until either the suture ripped or the scaffold fractured. Suture retention strength, which was defined as fracture strength, was obtained.

The burst pressure strength for the electrospun PCLA scaffolds was measured by increasing the pressure within the tubular scaffold until failure occurred. A pressure transducer catheter (thin latex tube) was inserted through a cannulating fixture at one end of the tubular scaffold. A gas or water pump was connected through a custom cannula at the other end of the scaffold. The pressure was gradually increased until failure occurred and the pressure change was recorded.

In vitro Degradation

Degradation studies were conducted in PBS. The tubular scaffold was cut into rectangular non-woven membranes with 10 mm $\times$ 10 mm. The initial weights and molecular weights of the samples were recorded, and they were placed in test conical flasks containing 10 mL PBS solution and incubated at 37°C for the period of the study. The PBS solution was changed once a week. At pre-set time points, the samples were removed and then lyophilized for 3 days to remove traces of water and weighed. Mass loss ratio (M_{loss}) was measured by determining the mass loss of the sample over the period of the study according to the following equation.

$$M_{\text{loss}} = (W_0 - W_t) / W_0 \times 100\% \quad (2)$$

Where W_0 is the original weight and W_t represents the weight of samples after t weeks' degradation.

The molecular weight of degradation samples was measured by GPC, and the morphology changes were observed by SEM.

Cytotoxicity Assay

For test on extract study, films of dimension 0.5 cm² were incubated in 1 mL of media for a period of 24 h. The L929 fibroblast cells were treated with this extract and the cell viability evaluated using MTT Assay. The *in vitro* tetrazolium-based colorimetric assay (MTT) is a rapid colorimetric method based on the transformation of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT) to purple formosan crystals by mitochondrial enzymes of metabolically active cells. The amount of formosan generated is directly proportional to the number of viable cells.

The mouse fibroblast L929 cells were cultured in 96-well flat-bottomed plates at 1×10^3 cells/mL concentrations. After 24 h culture at 37°C in a humidified atmosphere with 5% CO₂, a cell monolayer was obtained. Extraction media (DMEM with the supplements of 10% FBS, 100 U/mL penicillin and 0.1 mg/mL streptomycin) were prepared by immersing samples for 24 h cut from electrospun tubular PCLA scaffolds and the control culture was done with the complete DMEM (DMEM with the supplements of 10% FBS and penicillin streptomycin). The cells were allowed to proliferate for 5 days, and cells with medium alone served as control. After the plates were incubated for 24 h at 37°C in 5% CO₂ atmosphere, the extract was removed and

100 μL of MTT (MTT, Sigma Chemical Co., St. Louis, Missouri USA) working solution was introduced into each well and incubated at 95% humidified atmosphere at 37°C for 4 h. After the reagent was removed and the material was rinsed with PBS, 100 μL of DMSO was added to each well and incubated for 5 min at 37°C in a shaker incubator till the converted dye dissolved completely. The absorbance of the resulting solution in each well was recorded at wavelength of 570 nm (LP400 Pasteur, France) (24H, 72H, 120H).

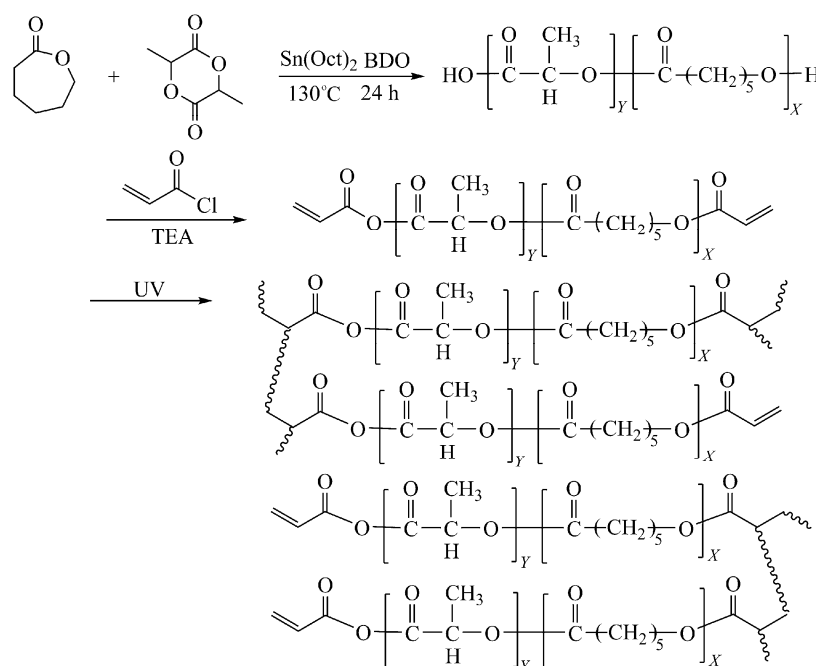
Cell Adhesion and Proliferation

Endothelial cells (ECs) were obtained from Chinese National Institute for the Control of Pharmaceutical and Biological Products. For cell culture studies, the photocrosslinked tubular scaffolds were cut along axis direction to form two-dimensional fiber structures. Briefly, they were sterilized in ethanol at 30°C and incubated at 37°C for 30 min in culture medium prior to cell seeding. To evaluate the initial stage of *in vitro* cellular interactions with vascular scaffolds, the scaffolds were seeded with ECs and fibroblast L929 in culture, respectively. Both cell types were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS and 1% penicillin and streptomycin at 37°C in 5% CO_2 , and the culture medium was changed every day. Biological activity of the polymeric scaffolds was determined by testing the ability of cells to proliferate on the scaffold material. Both cell types (2×10^4 cells/ cm^2) were seeded onto the scaffolds (10 mm $\times$ 10 mm) in the wells of a 24-well culture plate. After being cultured at 37°C in 5% CO_2 atmosphere for 3 days, the cellular scaffolds were detected through SEM.

RESULTS AND DISCUSSION

Photocrosslinking Procedure

The preparative route of photocrosslinked PCLA is shown in Scheme 1. At first hydroxyl end-capped PCLA was prepared *via* the bulk ring-opening copolymerization of ϵ -CL and LLA catalyzed by stannous 2-ethyl hexanoate. Then its acrylation was carried out *via* the reaction with acryloyl chloride using TEA as proton scavenger^[22]. Finally the acrylated PCLA was fabricated into tubular scaffold by electrospinning followed by the initiator-free photocrosslink under UV irradiation. In theory this strategy is versatile for photocurable polyesters with various compositions, but it was found to be especially effective for a random PCLA composed of a 50:50



Scheme 1 Synthetic route of photocrosslinked PCLA

feeding molar ratio of ϵ -CL to LLA. Because the bulk photo-crosslinking was carried out here, the transmission of UV light or the transparency of the polymeric films is crucial to perform the photocrosslinking process. If the crystallization structure is present and large enough in polymer bulk, the polymeric films usually are semitransparent or opaque in which the transmission of UV light is hindered by diffuse reflection effect so that the photocrosslinking in the interior is hard to be completely accomplished. In our previous work, the photocrosslinking of four arm acrylated PCL films in the opaque state was conducted under UV light, and the tensile strength enhancement was only about 40% due to the limited transmission of UV light into the opaque films^[23]. Herein the selected PCLA film appears clearly transparent allowing for the transmission of UV radiation to fulfill the photopolymerization in the interior of the polyesters. Additionally it is indeed characteristic for a random copolymer with amorphous structure as evidenced by the following DSC measurement.

As for vascular tissue engineering application, this selected PCLA was designed to be fabricated into small diameter tubular scaffolds by electrospinning technique and the formed scaffolds were followed by the photopolymerization under UV irradiation. Taking account the smooth electrospinning process, the molecular weight of the PCLA was predetermined to be about 50000 Daltons. However, this value may be too high for a photocurable prepolymer to form a tight crosslinking network structure due to low crosslink density resulting from the low reactivity available for the terminal functional groups of high molecular weight precursor. In general the electrospinning process requires the electrospun material having a relatively higher molecular weight. As a result, the molecular weight of around 50000 Daltons was a compromise between the crosslink density of crosslinking network and the molecular weight of electrospinning material. Although the crosslink density was low, the mechanical properties of the resulting crosslinked PCLA films and fabrics were evidently improved after photocrosslinking as demonstrated by the following characterization.

As the cytotoxicity of photoinitiator residue is severely concerned, it is desirable that the photocrosslinking is performed in the absence of any photoinitiator. DMPA is a low bio-toxicity photoinitiator. For comparison, the photocrosslinking was carried out with or without adding DMPA. The tensile strength and elongation at break of the crosslinked PCLA membranes with varying amounts of DMPA are shown in Table 1. As can be seen, after exposure to UV radiation for 1 h, whether or not adding DMPA, all the membranes had almost the same tensile strength. It clearly indicated that the acrylated PCLA comprising a 50:50 feeding molar ratio of ϵ -CL to LLA can be successfully initiator-free photocrosslinked to provide the high tensile strength as compared with those containing DMPA. The maximum tensile strength was reached in 40 min, whereas the elongation was decreased with increasing the UV radiation time.

Also shown in Table1, the gel fraction of the crosslinked PCLA membranes in the absence of DMAP was increased from 0.28 (5 min) to 0.65 (60 min) as the crosslink network structure was gradually created with increasing the photocuring time. However, for the crosslinked PCLA containing DMAP, the values were only a little higher than that without adding DMAP implying their denser crosslinking structure. Taking into account the trade-off for the mechanical property and the cytotoxicity, the photocrosslinking process without DMAP is obviously preferable.

Table 1. Investigation on photocrosslink parameters*

Sample No.	DMPA (wt%)	Radiation time (min)	Tensile strength (MPa)	Elongation at break (%)	Gel fraction (%)
1	0	0	13.2 $\pm$ 3.2	1315 $\pm$ 54	0
2	0	5	17.0 $\pm$ 1.3	1007 $\pm$ 63	28 $\pm$ 16
3	0	10	16.9 $\pm$ 0.4	971 $\pm$ 18	36 $\pm$ 6
4	0	20	18.8 $\pm$ 3.2	958 $\pm$ 100	30 $\pm$ 2
5	0	40	21.6 $\pm$ 3.9	1069 $\pm$ 149	59 $\pm$ 3
6	0	60	21.4 $\pm$ 0.8	1165 $\pm$ 73	65 $\pm$ 6
7	0.5	60	21.1 $\pm$ 3.8	982 $\pm$ 27	76 $\pm$ 8
8	1	60	20.7 $\pm$ 3.7	963 $\pm$ 26	76 $\pm$ 10
9	2	60	19.7 $\pm$ 2.4	839 $\pm$ 71	73 $\pm$ 6

* The error is standard deviation.

Electrospinning of Acrylated PCLA Prepolymer

The as-prepared PCLA precursor was dissolved in CH_2Cl_2 and electrospun into ultrafine fiber meshes or tubular scaffolds under 20 kV voltages. The formed tubular scaffolds possessed smooth inner and outer walls. In fact, the diameter of the tubular scaffolds and the wall thickness can be readily regulated by varying electrospinning parameters, such as time, the diameter of a rotating metallic mandrel collector, solution concentration, *etc.* The inner diameter of the formed tubular scaffold is about 5 mm and the wall thickness is around 0.1 mm. The porosity of the electrospun microfiber vascular tubular scaffolds was measured by using mercury porosimetrical analysis. As summarized in Table 2, their porosities are 44% and 49% before and after photocrosslinking. The pore diameter of both scaffolds ranges from 4–5 μm to 20 μm . The ability of the electrospun scaffolds to mimic the extra cellular matrix (ECM) is vital due to the fact that the nonwoven fibrous mats composed of sub-nanofibers are characteristic of a very high fraction of surface available to interact with cells, making them ideal for cell attachment. Although the porosity in the scaffolds herein is a little low, likely due to the less uniform distribution of the diameters of the electrospun fibers, the pores are perforate so that it can mimic ECM structure to allow the transportation and exchanging of nutrient and bio-signals.

Table 2. Characterization of PCLA scaffold before and after photocrosslink*

Scaffold type	T_g (°C)	Porosity (%)	Tensile strength (MPa)	Suture retention strength (N)		Bursting pressure strength (kPa)	
Non-crosslinked scaffold	−10.7	44	12.8 ± 2.9	Dry	7.6 ± 0.8	Air	120 ± 24
				Wet	7.5 ± 0.7	Water	62 ± 20
Crosslinked scaffold	−13.6	49	19.1 ± 2.1	Dry	8.0 ± 0.6	Air	142 ± 31
				Wet	8.3 ± 0.7	Water	123 ± 26

*The error is standard deviation.

The DSC curves of the acrylated PCLA membranes before and after the crosslinkage are depicted in Fig. 1. The amorphous structure as revealed in the uncrosslinked scaffolds certainly benefited the photopolymerization process. The T_g of the crosslinked PCLA sample is slightly higher than that of the uncrosslinked ones, likely due to the limited motion performance of acrylated PCLA by the crosslinked network structure after photocrosslinking. However, the increase of T_g herein is not so obvious for the high molecular weight of the acrylated PCLA precursor arisen from the low crosslink density.

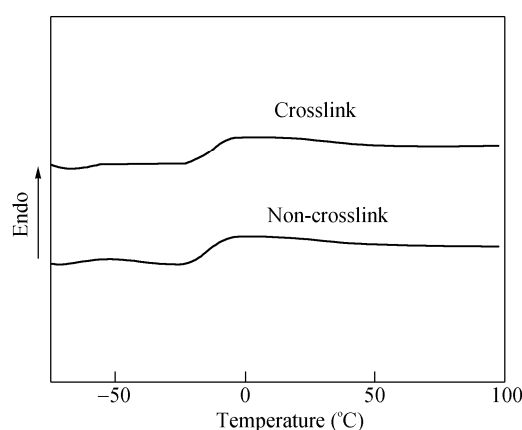


Fig. 1 DSC curves of PCLA before and after photocrosslink

Mechanical Properties of Tubular Scaffolds

For comparison, the tensile strengths of tubular scaffolds before and after UV irradiation were also measured. The tubular scaffolds were cut along the axis direction and made into dumbbell-shaped samples to be testified on tensile testing machine. Surprisingly, the tensile strength was found to enhance from 12.8 MPa to 19.1 MPa

after the photocrosslink, beyond the tensile strength of a porcine artery (1 MPa)^[24].

Suture retention strength is also a crucial factor in the fabrication of vascular TE scaffolds as it directly relates to the success of the graft implantation procedure. In order to mimic the *in vivo* surgical operation environment, the scaffold samples were immersed in PBS solution before testing. Then the dry and wet scaffold samples were evaluated, respectively, and the results are listed in Table 2. It was seen that the crosslinked scaffolds have the higher suture retention strength than the uncrosslinked ones, while the wet samples possess almost the same suture retention strength with the dry ones. It implies not only that the suture retention strength is greatly enhanced by the photocrosslinking procedure, but also that the crosslinked PCLA scaffold is more suitable to be sutured under the *in vivo* condition. In fact this suture retention strength is markedly higher than the acceptable ones for suturing during implantation, which is required to be greater than 2.0 N^[25]. As the suture retention strength is related to the thickness of samples, herein the thin scaffold sample around 0.1 mm in thickness was prepared for the suture retention strength measurements. Regarding the future implantation experiments in animal and clinic applications, the scaffolds involved are generally fabricated to be about 0.3–0.5 mm in thickness, and as a result the current suture retention strength is strong enough for vascular TE applications.

The burst pressure strength is associated with the pressure that a vascular graft can be subjected to before an acute leak develops and the graft fails. To determine whether the tubular scaffolds have adequate strength to endure physiologic forces, the burst pressure testing was performed to identify the maximum pressure that the scaffolds could endure before failure by using both water and air as pressure conducting media. The thickness of measured tubular scaffolds is around 0.5 mm in thickness, and the experimental results are summarized in Table 2. As can be seen, the photocrosslinked tubular scaffolds exhibited the increasing burst pressure strength as compared with those uncrosslinked ones. The photocrosslink imparts the scaffolds the excellent mechanical strength with the potential to be used as substitutes for native blood vessels. In addition, as water is an incompressible fluid, the burst pressure strength determined with water is generally lower than that with air as a compressible conducting media.

In vitro Degradation

The *in vitro* degradation was carried out in PBS solutions. The variations of the molecular weight and mass loss of photocrosslinked PLCA samples were monitored to trace the degradation process. Although GPC analysis cannot measure the molecular weight of photocrosslinked scaffolds because the crosslinked structure is unsolvable in any solutions, the molecular weight of the sol part can be measured after the filtration out the unsolvable gel part. As the crosslink structure would get destructed with the degradation progress, the molecular weight changes were really measured in the late period of degradation process.

The GPC traces of degraded samples with and without photocrosslink are shown in Fig. 2. The peaks are shifted to low molecular weight side with the time evolution, indicating the degradation of both types of samples occurred. The trends of molecular weight loss and mass loss are shown in Fig. 3. It was found that the mass loss ratio (less than 10%) is much less than the molecular weight loss ones (more than 60%) during the whole degradation process. It clearly suggested that the bulk degradation mechanism is predominant in the degradation of both non-crosslinked and photocrosslinked scaffolds.

There were three stages evidenced in Fig. 3(a). In the first three weeks, the molecular weight loss ratios of both types of samples were comparable. In 4th and 5th weeks the value for the crosslinked scaffold became lower than the uncrosslinked ones. However, in the last four weeks, they behaved similarly again. Actually, in the first stage, the measured molecular weight of crosslinked scaffold only represented the molecular weight of its sol part because the crosslink structure was retained in the initial degradation period. Consequently both the crosslinked and non-crosslinked scaffolds showed the similar molecular weight loss trend. However, the crosslinked structure began to be destructed in the second stage so that the measured molecular weight change really represented the destruction of the polymeric crosslink network of whole photocrosslinked scaffold at that time. In the final stage, as the crosslinked structure was collapsed, the molecular weight loss behavior of both types of samples became similar again.

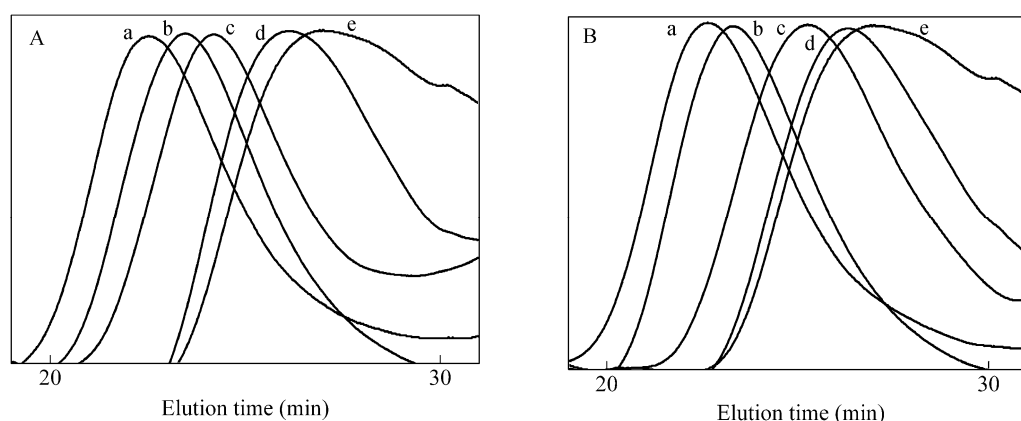


Fig. 2 GPC curves of degradation samples of non-crosslinked scaffold (A) and photocrosslinked scaffold (B) after (a) 1, (b) 3, (c) 5, (d) 7 and (e) 9 weeks

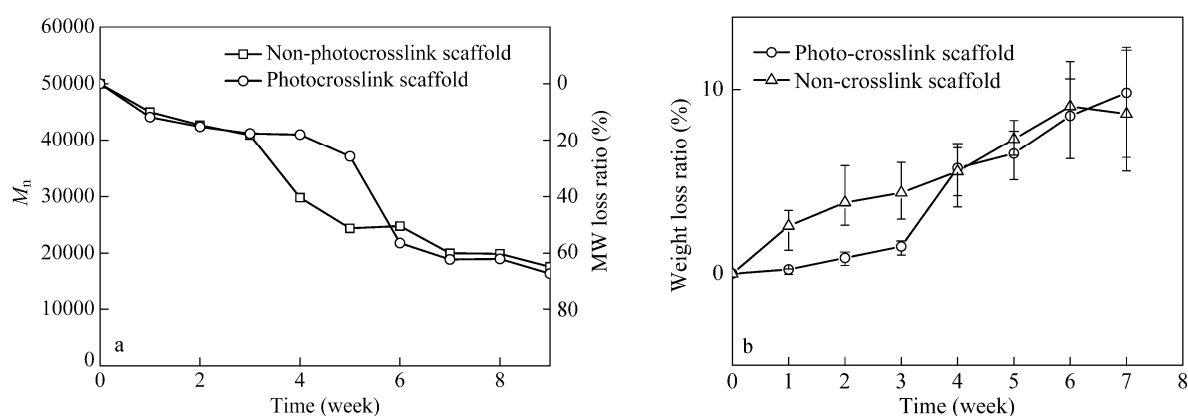


Fig. 3 Degradation curves of PLGA scaffolds before and after photocrosslinking
a) Molecular weight loss curves; b) Mass loss curves

The above analysis can also be used to explain the mass loss curves as shown in Fig. 3(b). As can be seen, the mass loss ratio of photocrosslinked PLGA scaffolds was much lower than that of non-crosslinked ones in the first stage, whereas they became close after the 4th week. According to the trend of molecular weight change in the degradation process, the crosslinked structure may remain in the first three weeks, and only sol part of the crosslinked scaffold contributed to the mass loss leading to a low mass loss ratio. In the 4th week, the crosslinked structure began to break down and the mass loss ratios of both the crosslinked and non-crosslinked scaffolds became close again. It was evident from molecular weight and mass loss variations that the crosslinked polymeric structure in the photocrosslinked PLGA scaffold was still retained before the 4th week. Consequently the mechanical properties of the photocrosslinked tubular scaffold would maintain for at least one month, while those for the non-crosslinked scaffold began to decrease very early.

Figure 4 shows the SEM images of both the crosslinked and non-crosslinked PLGA scaffolds in the *in vitro* degradation. In the first week, the ultrafine fibers of both types of scaffolds kept well integrated. In the 4th week, the original morphologies of the ultrafine fibers of the photocrosslinked scaffold (a) were still found, whereas some fibers of the non-crosslinked scaffold (b) began to be ruptured indicating the loss of mechanical strengths. This further demonstrated that the photocrosslinked scaffolds are more durable and can retain mechanical properties a longer time than the non-crosslinked ones. However, the rupture of ultrafine fibers was broadly observable in both scaffolds (c and d) in 9 weeks.

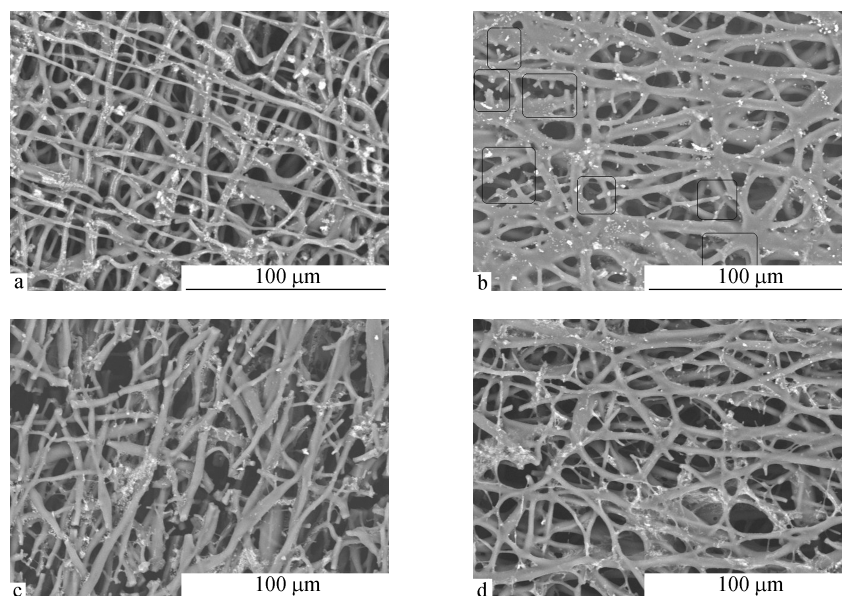


Fig. 4 SEM images of *in vitro* degradation of photocrosslinked (a, 4 weeks; c, 9 weeks) and non-crosslink (b, 4 weeks; d, 9 weeks) PCLA scaffolds

Cell Affinity

The cytotoxicity of the electrospun PCLA scaffolds before and after UV radiation was investigated by culturing L929 cells. Mitochondrial metabolic activity was observed by MTT assay and was detected in the cells cultured with extraction media from the crosslinked and non-crosslinked scaffolds with complete media used as a control. Statistically, there was no significant difference between these three groups ($p > 0.05$) as shown in Fig. 5. The cytotoxic level of both the crosslinked and non-crosslinked PCLA scaffolds was below level 1 as indicated in Table 3, further revealed that the initiator-free photocrosslinking process has no influence on the cytotoxicity of the acrylated PCLA biodegradable materials.

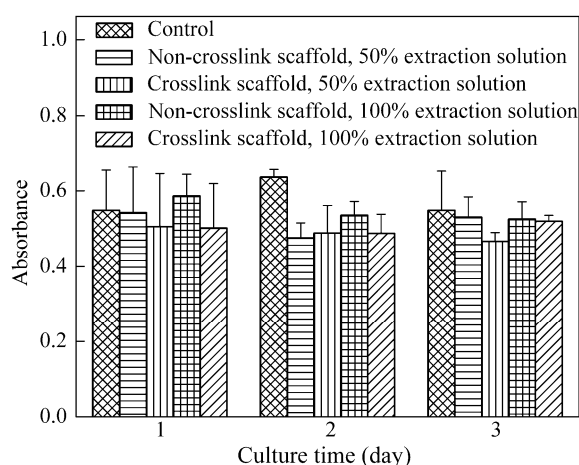
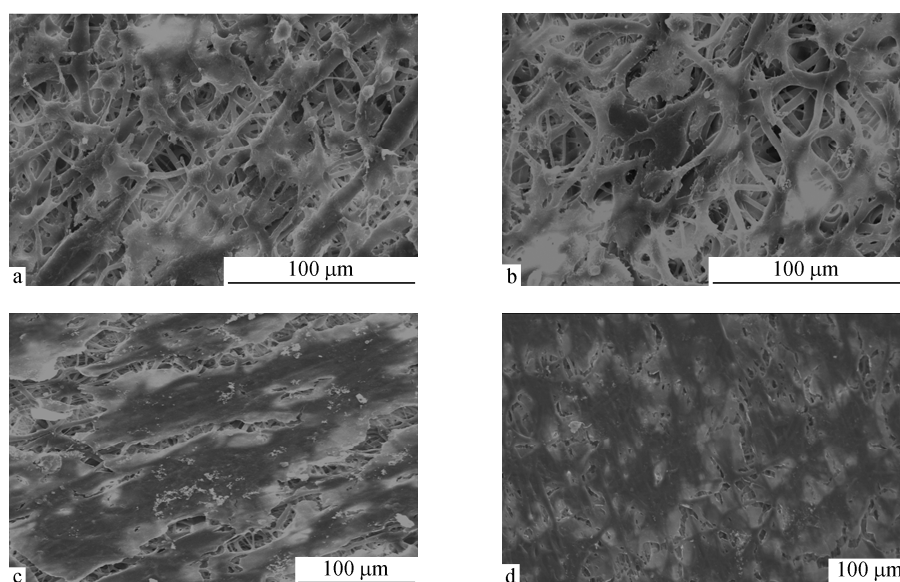


Fig. 5 The MTT values of non- and crosslink scaffolds

Table 3. Cytotoxic level of PCLA scaffolds before and after photocrosslinking

Extraction concentration		Time (h)		
		24	72	120
Non-crosslink scaffold	50%	0	1	0
	100%	0	1	0
Crosslink scaffold	50%	0	1	1
	100%	0	1	0

**Fig. 6** SEM pictures of cell adhesion and proliferation before (a, L929 cells; c, endothelial cells) and after (b, L929 cells; d, endothelial cells) photocrosslinking

To successfully engineer blood vessels that can be applied clinically, the vascular TE tubular scaffolds must possess excellent biocompatibility to ensure that seeded vascular cells remain viable and encourage host cell infiltration into the graft. These processes are required for the development of a viable, patent blood vessel from the scaffold. In this study, the basic cellular interactions between the non-crosslinked/crosslinked scaffolds and endothelial and fibroblast L929 cells in Fig. 6 clearly revealed that the scaffold material and photocrosslinkage do not affect cell viability in all the cases. Furthermore, these vascular tubular scaffolds enabled to support adhesion and proliferation of endothelial and fibroblast L929 cells in the initial stage *in vitro* as demonstrated by SEM observation. These findings indicated that the PCLA tubular scaffolds after the initiator-free photocrosslinking can be used in conjunction with vascular cells to create an engineered vessel that could withstand physiological vascular conditions. Further studies are currently conducted in an animal model to determine the applicability of this photocrosslinked PCLA scaffolding system.

CONCLUSIONS

Acrylated PCLA was prepared by the end-capping reaction of PCLA with acryloyl chloride. It was then fabricated into small diameter vascular graft scaffold by electrospinning technique followed by initiator-free photocrosslinking under UV light. The as-prepared vascular tubular scaffolds were found to possess the mechanical properties strong enough to adapt to the blood vessel environment. In addition, the cytotoxicity level of the non-crosslinked and crosslinked PCLA scaffolds was below level 1, and both endothelial and fibroblast L929 cells could be seeded on the surface of the scaffolds with good morphology. It showed the potential to be used as a promising scaffold biomaterial in vascular tissue engineering applications.

REFERENCES

- 1 Nerem, R.M. and Seliktar, D., *Annu. Rev. Biomed. Eng.*, 2001, 3: 225
- 2 Heureux, N., Germain, L., Labbe, R. and Auger, F.A., *J. Vasc. Surg.*, 1993, 17: 499
- 3 Suma, H., *Ann. Thorac. Cardiovasc. Surg.*, 1999, 5: 141
- 4 Hoerstrup, S.P., Zund, G., Sodian, R., Schnell, A.M., Grunerfelder, J. and Turina, M.I., *Eur. J. Cardiothorac. Surg.*, 2001, 20: 164
- 5 Lee, S.J., Liu, J., Oh, S.H., Soker, S., Atala, A. and Yoo, J.J., *Biomaterials*, 2008, 29: 2891
- 6 Teebken, O.E. and Haverich, A., *Eur. J. Vasc. Endovasc. Surg.*, 2002, 23: 475
- 7 Lee, S.W. and Belcher, A.M., *Nano Lett.*, 2004, 4: 387
- 8 Casper, C.L., Yang, W., Farach-Carson, M. and Rabolt, J.F., *Biomacromolecules*, 2007, 8: 1116
- 9 Wang, S., Kempen, D.H., Simha, N.K., Lewis, J.L., Windebank, A.J. and Yaszemski, M.J., *Biomacromolecules*, 2008, 9: 1229
- 10 Yang, L., Wu, W. and Xu, B.H., *Front. Mater. Sci. China*, 2008, 2(4): 430
- 11 Zeng, L., Hou, H.Q., Wendorff, J.H. and Greiner, W., *Macromol. Rapid Commun.*, 2005, 26: 1557
- 12 Greiner, A. and Wendorff, J.H., *Angew. Chem. Int. Ed.*, 2007, 46: 5670
- 13 Huang, Z.M., Zhang, Y.Z. and Kotaki, M., *Compos. Sci. Technol.*, 2003, 63: 2223
- 14 Demir, M.M., Yilgor, I. and Yilgor, E., *Polymer*, 2002, 43: 3303
- 15 Zhuo, H.T., Hu, J.L. and Chen, S.J., *Mater. Lett.*, 2008, 62: 2074
- 16 Agarwal, S., Wendorff, J.H. and Greiner, A., *Polymer*, 2008, 49: 5603
- 17 He, W., Ma, Z.W., Yong, T. and Teo, W.E., *Biomaterials*, 2005, 26: 7606
- 18 Kim, K., Yua, M.K., Zong, X.H., Chiu, J., Fang, D.F., Seo, Y.S., Hsiao, B.S., Chua, B. and Hadjiargyrou, M., *Biomaterials*, 2003, 24: 4977
- 19 Schindler, M., Ahmedb, I., Kamalb, J., Nur-E-Kamalb, A., Grafec, T.H., Chung, H.Y. and Meiners, S., *Biomaterials*, 2005, 26: 5624
- 20 Qiu, R.X., Qi, L.X., Peng, D.L., Han, J., Ye, L., Zhang, J. and Feng, Z.G., *Acta Polymerica Sinica(in Chinese)*, 2008, (12): 1142
- 21 Qiu, R.X., Li, C.M., Ye, L., Dong, J.D., Zhang, A.Y., Gu, Y.Q. and Feng, Z.G., *Biomed. Mater*, 2009, 4(4): 044105
- 22 Wang, S.F., Yaszemski, M.J., Gruetzmacher, J.A. and Lu, L.C., *Polymer*, 2008, 49: 5692
- 23 Wang, P.J., Geng, X., Ye, L., Zhang, A.Y. and Feng, Z.G., *Science & Technology Review*, 2009, 27(21): 50
- 24 Amiel, G.E., Komura, M., Shapira, O., Yoo, J.J., Yazdani, S. and Berry, J., *Tissue Eng.*, 2006, 12: 2355
- 25 Billiar, K., Murray, J., Laude, D., Abraham, G. and Bachrach, N., *J. Biomed. Mater. Res.*, 2001, 56: 101