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ABSTRACT

The auhtors report 3D biomimetic scaffolds using polymer blend of polyurethane and cellulose for cardiac tissue engineering. The biocompatible and biodegradable polyurethane is designed, synthesized and characterized. By incorporating 10 wt% of naturally ordered cellulose into the polyurethane and electrospinning them into 3D scaffold, the scaffold exhibits good biocompatibility and mechanical property to support and accommodate constant cycles of contraction/expansion of cardiac tissue. The biocompatibility is further improved using scaffold fabricated from aligned fibers due to synergistic effects between cells and ordered macromolecules. The anisotropic structured scaffold is mimicked the extracellular matrix and has therapeutic potential in reconstruction of damaged myocardium.

GRAPHICAL ABSTRACT
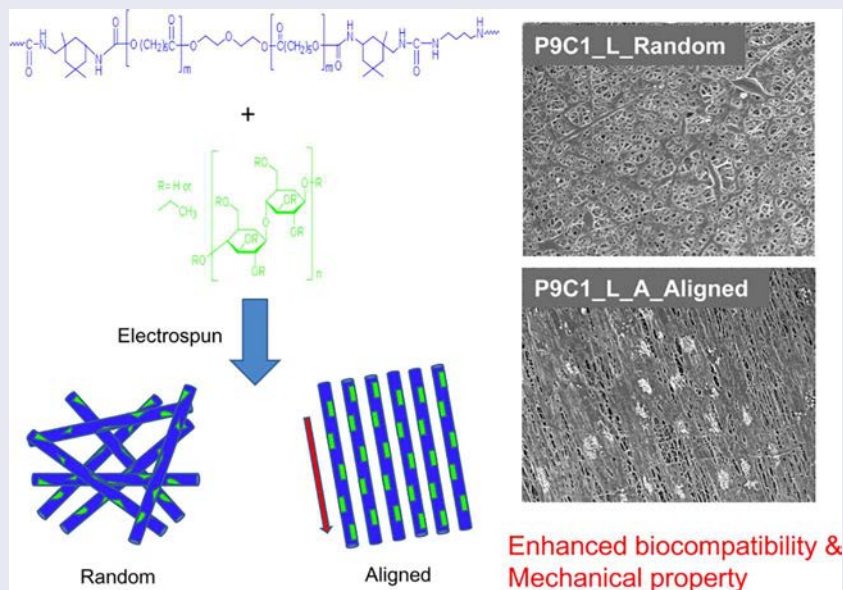
1. Introduction

Cardiac disease is one of the main causes of death in humans [1]. Tissue engineering has been demonstrated as a feasible therapeutic strategy for heart disease [1–8]. The technology employed 3D scaffold to guide cellular regulation and growth for regeneration and reconstruction of damaged myocardium. The scaffold needs to be a biocompatible, biodegradable, and biomimetic structure of cardiac extracellular matrix (ECM) with optimal mechanical properties and good interactions with cells. The myocardium bears mechanical loads along a certain direction, which results in an anisotropic structure of aligned fibers in native heart tissue [4]. At each cardiac cycle, both contractile and expansive forces are produced. Thus, a scaffold with high strength and flexible elastic property is highly desirable to meet the requirement for sustaining constant cyclic stresses.

The electrospinning process [9–12] is a very versatile technique to prepare 3D fibrous structures with interconnected porous feature to facilitate the flow of nutrients. The size of the fiber can be tuned to promote cell retention, proliferation, and differentiation. Most importantly, the electrospinning technique enables the fabrication of the aligned fibrillar structure to direct cell orientation and growth [12].

Polyurethanes are one of the promising elastomers for cardiac tissue engineering due to their excellent mechanical properties [5,12–17]. However, the fibers prepared from electrospun polyurethanes often have features of non-uniform with beads, intertwining and sticky together due to their soft and elastic characteristics [12,16]. It limits the construction of 3D interconnected porous structures thus deteriorate nutrients taken by cardiomyocytes. The issue has been resolved by blending molecularly rigid cellulose into commercial available polyurethane made from aromatic diisocyanate and polyether diol. They are structurally mimicked the anisotropic nature of extracellular matrix (ECM) of heart muscles. The 3D scaffolds exhibit good biocompatibility and mechanical properties [17]. However, this polyurethane is not biodegradable.

The biodegradable polyurethanes for cardiac tissue engineering have been reported previously and most of them are synthesized from aromatic isocyanate reacting with polyester polyol [18–22]. The degraded aromatic moiety is toxic to human body [19,22]. For nontoxic and biocompatible polyurethane, the aliphatic isocyanates such as 1,4-diisocyanato butane and 1,6-diisocyanato hexane are used in synthesis instead of aromatic isocyanate [23–25]. The drawback of long aliphatic chain moiety of polyurethane is that it is crystallized easily and becomes difficult to process. Amino acid based aliphatic diisocyanate (2,6-diidocyanato methyl caproate) and chain extender (phenylalanine) were used to synthesize biodegradable polyurethane with polycaprolactone diol for cardic tissue engineering application [12,16,26]. However, their mechanical properties are inferior than those of aromatic based polyurethane. Here, we report the success of synthesis of biodegradable aromatic free, noncrystalline cyclic polyurethane that can be easily dissolved in common solvent and fabricate into 3D scaffolds using electrospinning. This new class of polyurethane exhibits good biocompatibility and mechanical properties. The properties of this polyurethane are noticeably enhanced by incorporating of naturally ordered cellulous and alignment of fibers. The effects on the changes of properties by varying the amount of cellulous and fiber alignment are systematically investigated and discussed. The new biodegradable and biocompatible scaffold fabricated from this novel polymer blend has potential to be used in the regeneration and reconstruction of damaged myocardium.

2. Experimental

Isophorone diisocyanate (IPDI; 97%), 1,4-diamino butane (DAB; 99%), poly(ε-caprolactone) diols (PCL diols; 99 + %, mol. wt. 2000), acetone (99.5%), tetrahydrofuran (THF; 99.5%), dimethylacetamide (DMAc; 99.5%), and anhydrous dimethyl formaldehyde (DMF; 99.5%) and dimethyl sulfoxide (DMSO; 99.5%) were obtained from Acros Chemical Company. Dibutyltin dilaurate (DBTDL; 95%) and ethyl cellulose (EC, 99 + %) were purchased from Aldrich Chemical Company.

Dulbecco's Eagle's medium, phosphate-buffered saline (PBS), fetal bovine serum, and penicillin/streptomycin were purchased from Gibco (Invitrogen, USA). A paraformaldehyde solution of 4% (w/v) was purchased from Merck, Germany. A yellow tetrazole: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, (MTT), Teflon O-ring

(Z504688-1), Triton X-100, 4′,6-diamido-2-phenylindole (DAPI, 1 µg/mL) were obtained from Sigma, USA. The tissue culture plates (TCPS) were purchased from Corning, USA. Rabbit polyclonal antibody α-actin and rabbit polyclonal antibody connexin-43 were obtained from Cell Signaling, USA. Antibodies of fluorescein isothiocyanate (FITC) and tetramethylrhodamine isothiocyanate (TRICT) were obtained from Abcam (USA).

2.1. Synthesis and characterization of biodegradable polyurethane

0.944 g of PCL diols was placed in a three neck flask, the moisture of diols was removed under vacuum (10^{-2} Torr) at 80°C for 12 h, then 30 mL of anhydrous DMF was added into the flask. A 0.2 mL of IPDI was dried first under vacuum at room temperature for 3 h, then the IPDI was added into the flask together with 0.5 mg of DBTDL catalyst. The mixture was reacted at 50°C for 5 h. A 1 wt% of DAB in DMF was added slowly into the flask and reacted 17 h at room temperature. The reaction product was precipitated by adding distil water. The polyurethane was collected by filtration and dried in vacuum oven at 40°C for 17 h. The chemical structure of the polyurethane was characterized by FTIR (Spectrum 100, Perkin Elmer). The molecular weight of polyurethane was determined by gel permeation chromatography (GPC, Viscotek). The glass transition temperature of polyurethane was evaluated by differential scanning calorimetry (DSC, TA Instrument Q200).

2.2. Fabrication of scaffolds from electrospun fibers

The solutions of polyurethane were prepared by dissolving polyurethane in dimethylacetamide (2/3, v/v) at the weight concentrations of 15%, 20%, 25% respectively. The solutions of polymer blends were prepared by dissolving the certain ratio of blend in a co-solvent of tetrahydrofuran and dimethylacetamide (2/3, v/v) at the concentration of 25% by weight. The apparatus of electrospinning process was used to fabricate scaffolds, and consists of a syringe pump (KDS-100, KD Scientific, Taiwan), a power supply (You Shang Technical Corp., Taiwan), and a metal collector. The polymer solution was feed at a flow rate of 0.5 to 1 mL/h using a 3 mL syringe (24-gauge, Terumo, USA). The distance between the collector and the tip of the needle was fixed for the plate collector at 15 cm and the drum collector at 10 cm. Under an optimal high voltage (in the range of 20 to 70 volts), the solution drop was become a "Taylor cone" and ejected as fibers onto the metal collector. A metal plate (20 cm in diameter) at a rotating rate of 350 rpm was used to collect isotropic fibers. A drum collector (5 cm in diameter and 20 cm in length) at 2800 rpm was used to collect aligned fibers. Both collectors were covered by aluminium sheets before the collection of fibers, so the collected fibers can be easily removed. As-electrospun scaffolds were dried in a vacuum oven (10^{-3} Torr) at 40°C for 17 h to remove residual solvent.

The samples of electrospun fibrous scaffold were denoted by a general formula of PxCy_fiber size, where P is polyurethane, C is cellulose, and the x and y indicate the mixing

ratio of polyurethane to cellulous. Three sizes of fibers were fabricated with the diameter around 100 nm (S), 500 nm (M), and 1000 nm (L) and expressed as PxCy_S, PxCy_M, and PxCy_L, respectively. The denotation of pristine polyurethane film was set as PU_F, and polyurethane scaffolds were set as PU_S, PU_M, and PU_L for different sizes of fibers. The aligned fibers were set as PU_X_A where X was the size of fiber.

2.3. Characterization of scaffolds

The scanning electron microscope (SEM; JSM-6700 F, JEOL, Japan) operated at 10 KV and 8 KV were used respectively to study the fibrous structure and the cell morphology of samples. The sizes of fiber (average and standard deviation) were determined by selecting more than 20 pieces of fibers in the SEM images for both isotropic and aligned fibers. For the sample preparation of cell morphology studies, the cells loaded fibrous scaffolds, after culturing for certain time, were washed by PBS three times and fixed by paraformaldehyde solution for 30 min in ambient. The fixed cells were washed further by PBS three times and then freeze-dried.

For the mechanical properties of scaffolds, a tensile instrument (Criterion 42.503, MTS) equipped with a load ranging from 5 to 250 N was used. The rectangular shape of fibrous scaffolds (10 mm in width, 60 mm in length, and 60 μm in thickness) were used and tested in ambient at a crosshead speed of 50 mm/min until break. Five samples were tested to obtain the average and the standard deviations of measured data.

The biodegradability of the scaffolds was determined by immersing the scaffolds into PBS for 20 weeks at 37°C. Then the changes of weight and pH were monitored every week. Each sample weight of 10–15 mg was used in the test. Five samples were measured for each kind of scaffold to obtain the average and standard deviation of the biodegradability.

2.4. Biocompatibility evaluation of scaffolds

The biocompatibility of scaffolds was evaluated using H9C2 rat cardiac myoblasts cells. The culture media was made from 10 mL Dulbecco's modified Eagle's medium which contained 4.5 g/L glucose, 10% PBS, 10% fetal bovine serum, and 1% penicillin/streptomycin. The circular samples (15 mm in diameter) were used in cell culture. They were placed in a tissue culture plates with 24 wells. A Teflon O-ring was placed on top of the fibrous scaffold to prevent it from floating. All of scaffolds and Teflon rings were treated with alcohol (70%), irradiated by UV for 17 h. and further washed with PBS. A 1 mL medium solution containing 3×10^4 cells/mL was put into each well. Then the culture plate was kept in the culture incubator at 5% CO_2 and 37°C. For the control group, the same conditions were used without scaffolds. The cell viability in both control groups and experimental groups were evaluated by the colorimetric method of MTT. The solution of MTT was prepared at a concentration of 2 mg/mL in PBS, sterilized by filtration (Millipore) and kept in dark. After the cell culturing, the aged culture medium was removed, 0.2 mL MTT solution was added in each well, and incubated at 37°C for 3 h. Afterward,

the MTT solution was removed, then 0.2 mL dimethyl sulfoxide was added into each well to dissolve the purple formazan crystals by using an orbital shaker (OS701, KS, Taiwan) for 15 min. The ELISA plate reader (ELx 800, BIO-TEK, Winooski, VT) was used to measure the optical density of 0.1 mL purple solution in a culture plate of 96 wells at 570 nm. For the statistical analysis of measured data, statistical significances were calculated by utilizing the one-way analysis of variance in Student's t-test. The significant difference was determined at p value of <0.05 (*), 0.01(**), and 0.001(***).

2.5. Immunocytochemistry

The immunocytochemistry was used to identify the cytoskeletal phenotype and gap junction of cultured H9C2 cells. After four days of cell culturing, the cells were fixed in a 0.4 mL solution of 4% (w/v) paraformaldehyde for 15 min, the paraformaldehyde solution was decanted, the sample with PBS was washed three times. Then a 0.4 mL of 0.5% Triton X-100 solution was added into sample and mixed for 30 min. The samples were then washed with PBS again two times and blocked with 0.4 mL of 5% nonfat milk for 60 min. The primary antibodies of rabbit polyclonal antibody α-actin (diluted 1:400) and rabbit polyclonal antibody connexin-43 (diluted 1:8000) were added into samples respectively and mixed well in 4°C for 17 h. The secondary goat antibody of FITC (diluted 1:20) for α-actin and TRICT (diluted 1:60) for connexin-43 were used to react with the cells respectively for 2 h in ambient. Cells were counterstained by DAPI. The immunostained cells were evaluated using a fluorescence microscope (Zeiss Axiovert 200 M) under indirect fluorescence.

3. Results and discussion

3.1. Design, synthesis, and characterization of biodegradable aromatic free polyurethane and characteristics of ethyl cellulose

Polyurethane (PU) with linkages of ester and urea was designed and synthesized in this research. The two linkages are chosen for the labile functionality of degradation and balanced pH after the biodegradation, because the ester is degraded into acid and the urea is degraded into amine. The cyclic isophorone diisocyanate (IPDI) instead of aromatic diisocyanate was used for the hard segment of the polymer to have good mechanical strength but eliminate the issue of aromatic toxicity. Triethylene oxide poly(ε-caprolactone) diols (PCL) was used for the soft segment of the polymer to provide elastic property to accommodate constant deformation of cardiac tissue. The reaction scheme of polyurethane synthesis is shown in Scheme 1. Two moles of IPDI were reacted with one mole of triethylene oxide PCL with molecular weight of 2000 at 50°C for 5 h using dibutyl tin dilaurate (DBTDL) as catalyst to form the prepolymer first. Then 1 mol of 1,4-diamino butane (DAB) was added in and reacted for 17 h at room temperature to obtain final product. The reaction was monitored by IR to show the decreasing of isocyanate peak of 2260 cm^{-1} and the presence of N-H stretching at 3374 cm^{-1}. The final product was confirmed by IR that shows no
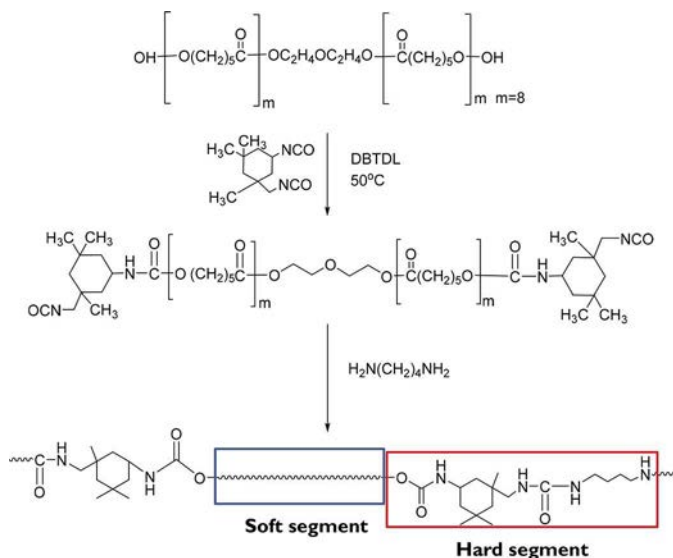
Scheme 1.   Synthesis of biodegradable polyurethane.

isocyanate peak and increasing in N-H bending of 1640 cm^{-1} (Figure S1). The weight average molecular weight of polyurethane is in the range of 150–200 kDa, which was determined by GPC. It is desirable to use matched molecular weight for each polymer in the blend to have consistent and predictable mechanical strength. Therefore, the ethyl cellulose (EC) of 164 kDa, which has a comparable molecular weight with polyurethane, was chosen to blend with the polyurethane throughout this research. The glass transition temperature (Tg) of polymer was determined by DSC that showed −65°C for polyurethane and 126°C for ethyl cellulose (Figure S2). Both polymers are suitable for biological application because there is no dimensional change associated with phase transition in the human body temperature of 37–40°C. Both pristine polyurethane and blends of polyurethane and ethyl cellulose (PU/EC) are electrospun into 3D fibrous scaffolds for the study of biodegradability and biocompatibility.
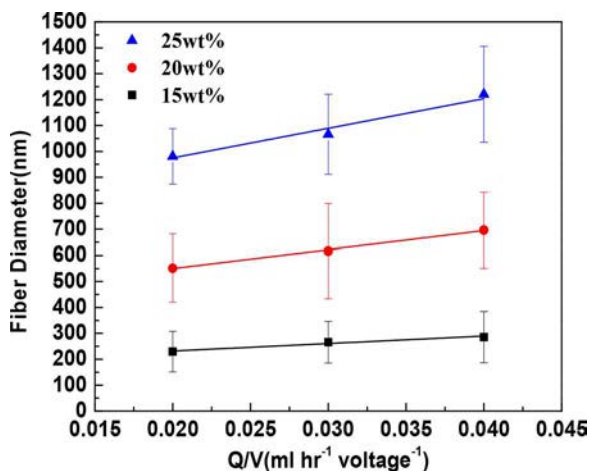

Figure 1.   Plots of fiber diameter of D versus flow rate divide by applied voltage of Q/V at different polymer concentrations (i.e., 15, 20, and 25 wt%). The selected 20 fibers in the SEM images were used to determine the average and standard deviation of fiber diameter.

3.2. Fabrication and characterization of 3D fibrous PU/EC scaffolds

The fiber diameter of 3D scaffolds significantly influences the mechanical properties of scaffolds and the growth of cells (retention, cell proliferation, and so on) [2,16]. We used the electospinning process to fabricate fibers, the diameter of the fibers can be easily controlled by changing the solution concentration and by varying the process parameters of flow rate (Q) and applied voltage (V). At a fixed solution concentration, the relationship between fiber diameter and process parameters can be expressed in the following equation [17]:

$$\log D = C_1 \log\left(\frac{Q}{V}\right) + \log C_2 \qquad (1)$$

where C_1 is a constant term and C_2 is related to χ, γ, and ϵ (γ: surface tension of solution, ε: dielectric permittivity, χ: dimensionless term responsible for the normal displacements). Figure 1 shows a plot of the fiber diameters of D verse the flow rate over the applied voltage of Q/V at three different solution concentrations. The results are consistent with the linear relationship shown in Eq. 1. Thus this relationship is served as the guidance to process the fibers with desired diameter of ~100 nm, ~500 nm, and ~1000 nm for three samples series of S, M, and L, respectively.

We used the same guidance to control the fiber diameter of polymer blend in the range of 1000 nm. Three weight blending ratios of polyurethane and ethyl cellulose: 5/5, 7/3, and 9/1 were used and made into the scaffolds of P5C5_L, P7C3_L, and P9C1_L, respectively. Figure 2 shows the SEM images of representative scaffolds accompanied by the scaffold fabricated from aligned fibers. The fibers in PU/EC scaffolds are less curvature than the fibers in pristine PU, which results from the ordered molecular structure of cellulose [26–28]. The large size of fibers form a 3D network with more porosity than that of small size fibers when the same thickness and area of scaffold was examined by SEM and used for culture. The more porous scaffold will be more suitable for the growth of cells due to the ease of nutrients flow (more discussion in section 3.3).

## 3.3. Mechanical properties of scaffolds

We investigated the mechanical properties of these newly developed scaffolds systematically to determine their potential in cardiac tissue engineering application. Adequate mechanical properties of elasticity and toughness are required to accommodate the constant cycles of contraction and expansion of heart function. The mechanical properties of scaffold were determined by stress-strain tests. The representative stress-strain curves are shown in Figure 3 and data are summarized in Table 1. The mechanical properties of PU pristine film are also included for the purpose of comparison. The modulus and tensile strength of the film are higher than that of fibrous scaffold regardless the diameter of fibers, because the width of the film is much larger than the diameter of fibers. Conversely, the elongation of film is lower than the fibrous scaffold as expected. The size effect on the mechanical properties of scaffold also is observed with the larger diameter of fibers resulting in higher mechanical strength. The scaffold with aligned fibers
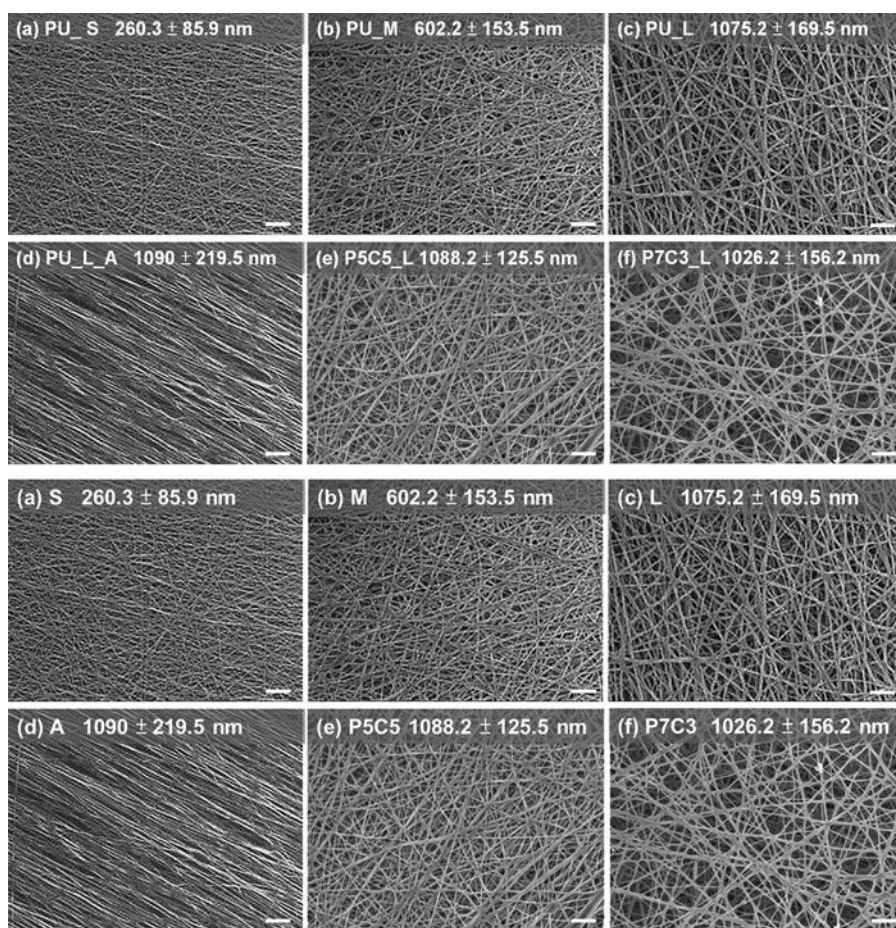
Figure 2. SEM photos of various electrospun fibrous scaffolds: (a) PU_S, (b) PU_M, (c) PU_L from random fibers of polyurethane, (d) PU_L_A from aligned fiber of polyurethane, (e) P5C5_L, and (f) P7C3_L from fibers of polymer blends. The average value and standard deviation of fiber diameter were shown from the calculation of 20 measured diameters of fibers in the SEM photo (scale bar: 10 μm).

exhibits close to five times of modulus, but only half of the elongation as compared with those scaffolds made from large size fibers. After the alignment, the fibers of scaffold are densely packed into highly ordered structure with high strength [29]. By incorporating 10 wt% of cellulose into the polyurethane (P9C1_L), the modulus of the scaffold is increased very effectively four times as compared with pristine PU

scaffold (PU_L). The increase is due to the rigid and stiff semicrystalline characteristics of cellulose [28]. The modulus of the P9C1_L scaffold is doubled further after alignment (PU_L_A). The more cellulose is presented in the PU, the higher the mechanical strength is but the lowered the elongation is as expected. Such high stiffness is not suitable for cardiac tissue engineering application. Thus, the sample P9C1_L, the
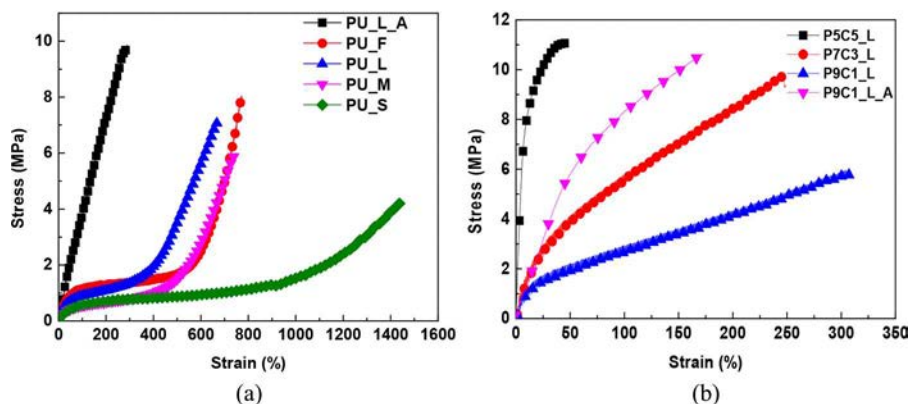
Figure 3. Plots of stress-strain curves of various scaffolds with different fiber size, alignment measured by using (a) pristine polyurethane film (PU_F), isotropic fibers with different size (PU_S, PU_M, PU_L) and aligned fiber (PU_L_A). (b) Polymer blend of polyurethane and cellulose, isotropic fibers with different blending ratio (P5C5_L, P7C3_L, P9C1_L), and aligned fibers (P9C1_L_A).

Table 1. Mechanical properties of various scaffolds fabricated from fibers with different size, cellulose amount, and alignment.

| Sample	Young's modulus (MPa)	Tensile strength (MPa)	Elongation (%)
PU_F	2.66 ± 0.60	8.04 ± 1.04	667.78 ± 27.12
PU_S	1.10 ± 0.19	4.67 ± 0.54	1220.2 ± 216.1
PU_M	1.27 ± 0.38	6.36 ± 0.47	814.25 ± 80.32
PU_L	1.44 ± 0.55	7.09 ± 0.88	748.33 ± 22.53
PU_A	7.03 ± 1.60	10.20 ± 1.93	302.55 ± 12.22
P9C1_L	5.98 ± 0.50	7.65 ± 0.70	307.2 ± 14.30
P7C3_L	17.96 ± 0.90	9.81 ± 0.80	244.9 ± 12.80
P5C5_L	111.9 ± 1.60	11.1 ± 1.20	45.33 ± 9.60
P9C1_L_A	11.92 ± 0.80	10.61 ± 1.60	173.96 ± 10.3

scaffold made from large-size fibers with appropriate 10 wt% of cellulose exhibits a suitable combination among Young's modulus, tensile strength, and elongation to be used as a durable ultra-thin (<100 μm) cardiac graft for the growth of cells. Furthermore, its high elongation implies the scaffold is flexibly complying with cardiac muscle.

3.4. Biodegradability and biocompatibility of scaffolds

To investigate the biodegradability of the scaffolds, we immersed the scaffolds into PBS buffer solution for 20 weeks at 37°C and monitored the changes of weight and pH every week. The results are shown in Figure 4. After 70 days saline solution immersion, the scaffolds fabricated from isotropic fibers (PU_S, PU_M, PU_L) lost about 15% of its weight, but the cast film (PU_F) and scaffolds made from aligned fibers (PU_L_A) had a weight loss of 10% and 6%, respectively (Figure 4a). The results can be easily understood from effective contact area between fibers and saline solution, the contact area of scaffold made from isotropic fibers is larger than that of film and scaffold made from densely packed aligned fibers. Thus, a faster degradation is occurred. The steady pH value of 7.5 ± 1.0 is observed (Figure 4b), which indicates that the developed scaffolds would not form unwanted by-products to influence the pH value and disturb the growth of cells.

The biocompatibility of scaffolds is related to the cell viability on the scaffold. The influences of fiber characteristics on the biocompatibility of scaffolds were studied. There were three characteristics: size of fibers, amount of cellulose in the blend, and the alignment of fibers. We did the evaluation of

cell viability by using MTT assay [17]. The assay analyzed the populations of the cardiac myoblast H9C2 cells after they were cultured on different scaffolds for the period of 4 h, one day, four days, and seven days, respectively. The active cells were reacted with MTT reagent and formed purple formazan crystals. The crystals were dissolved in DMSO to make a purple solution. By measuring the absorbance (O.D.) of the purple solution at 570 nm, the cell viability can be determined from the linear relationship between number of active cells and amount of crystals in cultured samples. The results are presented in Figure 5. From Figure 5a, every scaffold seeded with cells shows increasing cell populations over seven days, which is similar to the trend of cell growth on the two controls, TCPS and PU film, suggesting that our biodegradable PU scaffolds are biocompatible for the growth of cells. More interesting to find that the cell viability of scaffolds made from large size fibers (PU_L) is 1.5 times higher than the scaffold made from either small or medium size fibers for seven days. The TCPS and PU film also exhibit high biocompatibility. This high biocompatibility on the large dimension of fiber, TCPS, and PU film could be attributed to the cells that have more contact area for cell growth and differentiation. The results suggest that a certain diameter of ~1000 nm fibers is required for maintaining high viability of cells on scaffolds. Figure 5b shows the effect of cell growth by varying the amount of cellulous in the fibers of scaffold. The fastest rate of cell growth is observed for the scaffold containing highest amount of cellulose (P5C5_L) after a short interval of 4 h culturing. The results suggest that the compatibility of polyurethane can be improved with incorporating natural cellulose fibers [27,28]. The ordered morphology of cellulose might facilitate the growth of elongated cardiac myoblast H9C2 cell. However, the rate did not catch up with other samples containing less amount of cellulose (P7C3_L and P9C1_L) after culture for one, four, and seven days. The scaffold containing least amount of cellulose exhibited the highest compatibility. Apparently, the cell growth is very environment sensitive [29]. The cells are reaching for high biocompatibility of naturally ordered cellulose at the beginning, but the rigidity of cellulose restricts the movement of the cells and the rate slows down with time. Thus, the scaffold containing 10 wt% of cellulose (P9C1) shows the best compatibility. The effect of fiber alignment on the cell growth rate is shown in Figure 5c. The
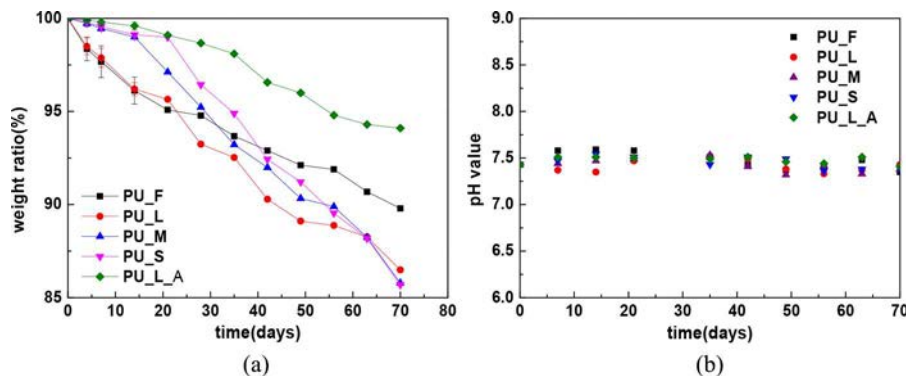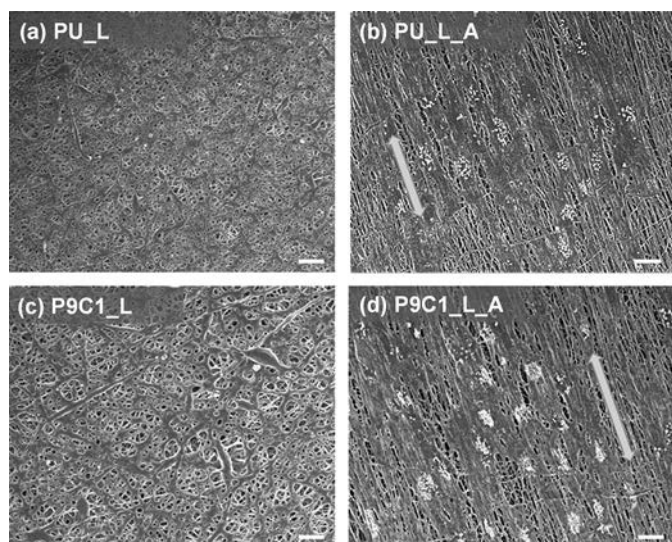

Figure 4. Biodegradability of PU scaffolds was monitored by changes of (a) weight and (b) pH by immersing scaffolds into PBS for 22 weeks.
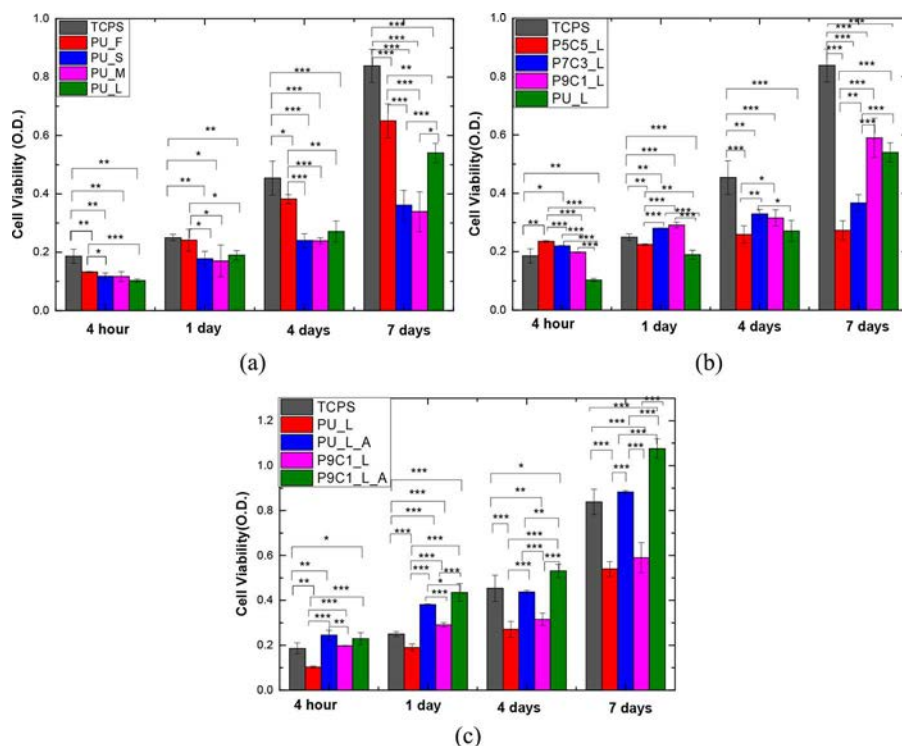
Figure 5. Growth rate of H9C2 cells on different types of scaffolds fabricated from (a) different size of PU fibers and PU film, (b) different amounts of cellulose in PU fibers, and (c) alignment of fibers.

scaffold fabricated from aligned fibers exhibits high rate of cell growth over the period of study. After seven days of culturing, a cell density is 1.12 times on the scaffold fabricated from aligned fibers (PU_A) as compared with the scaffold from random fibers (PU_L_A). Furthermore, the cell density becomes 2.07 times for the scaffold fabricated from aligned fibers containing 10 wt% cellulose (P9C1_L). We speculate the scaffold made from aligned fibers of polymer blend exhibits synergistic

Figure 6. SEM photos of the growth of cells on different scaffolds made from fibers of (a) isotropic polyurethane (PU_L), (b) aligned polyurethane (PU_L_A), (c) isotropic polymer blend (P9C1_L), and (d) aligned polymer blend (P9C1_L_A). The cells were cultured for four days (scale bar: 10 μm).

effects among naturally ordered cellulose and elongated PU fibers. That results in a favorable environment for enormous growth of elongated cardiac myoblast H9C2 cells. The elongated anisotropic cell growth mechanism has been confirmed by the SEM study of cell morphology. Figure 6a shows the elongated cell morphology (cultured for four days) on scaffold made from pristine polyurethane fibers (PU_L). The cell population is apparently increased with scaffold having aniso-tropic morphology which was fabricated from aligned PU fibers (PU_L_A; Figure 6b). The increased density of elon-gated cells is clearly observed for scaffold made from fibers containing cellulose (P9C1_L) as compared with pristine poly-urethane (PU_L; Figure 6c). The cell density and anisotropic property are further enhanced on scaffold made from aligned fibers (P9C1_L_A; Figure 6d). Furthermore, cells on scaffolds are found to be arranged along the aligned fibers with elon-gated morphology (Figures 6b and 6d), which are very distinct from the spindal-like cell morphology randomly distributed on isotropic scaffolds (cf. Figures 6a and 6c). The results indi-cate that the aligned fibers could provide topographic guid-ance for the growth and alignment of cells. The favorable adoption of cell growth directionally on aligned fibrous 3D structures that mimicked the *in vivo* cardiac anisotropy. Thus, the aligned fibrous 3D scaffold fabricated from the polymer blend of polyurethane and cellulose is suitable for cardiac tissue engineering.

Furthermore, we examined the phenotypic protein of car-diac myoblast expressions of cell morphology and functions on scaffolds of isotropic blend fibers (P9C1_L) and aligned blend fiber (P9C1_L_A) using immunocytochemistry. The muscle-specific intermediate filament protein of α-actin and
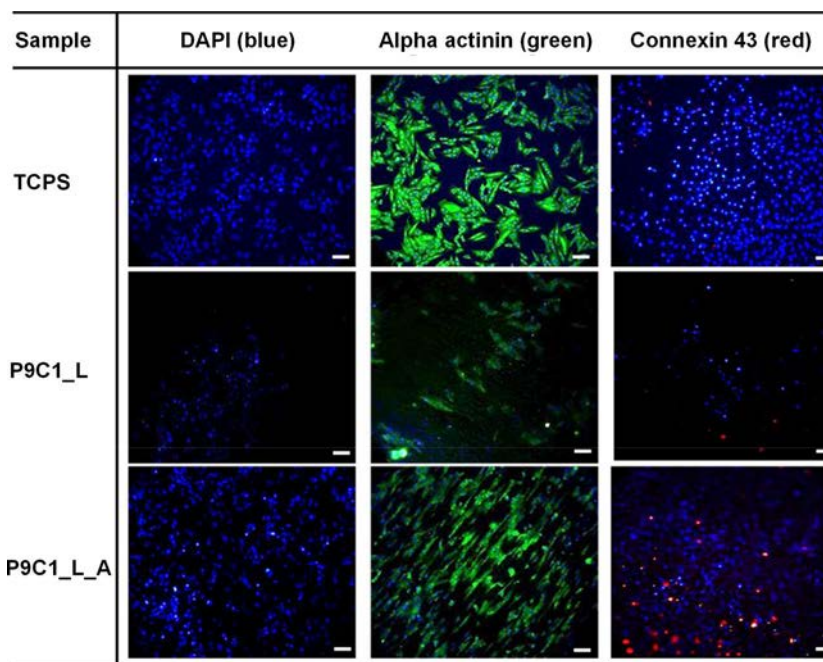
Figure 7. Immunofluorescent microscope photos of H9C1 morphology on different scaffolds after cells were cultured for four days (scale bar: 10 μm).

the constituent of cardiomyocyte gap junction of connexin-43 [9,17,32]. were used as immunofluorescence staining on the cells cultured for four days. The results are shown in Figure 7. The α-actin stained cells on P9C1_L are less spread and more isolated as compared with the cells on control (TCPS) and P9C1_L_A. For the cells on aligned fibrous scaffold, they are more populated and spread with nuclei surrounding by mature cytoskeleton. Moreover, the cells reveal extended phenotype with dense intercellular contacts. For the connexin-43 stained cells, the aligned fibrous scaffold (P9C1_L_A) shows the gap junctions of cells are more populated than that of control (TCPS) and isotropic fibrous scaffolds (P9C1_L). Thus, the results of immunofluorescence indicate the cells adopted preferably on the anisotropic fibrous 3D structures. The structures are mimicked the anisotropic nature of cardiac *in vivo*, that would promote adhesion and proliferation of cells for potential application in cardiac reconstruction and regeneration.

4. Conclusions

We report the success of synthesis of biodegradable polyurethane with the molecular weight of 150–200 kDa that can be easily dissolved in common solvent and fabricated into 3D scaffolds using electrospinning for cardiac tissue engineering. The polymer contains rigid isophorone segment and soft ethylene oxide polycaprolactone segment, terminated by urea linkage. This new class polyurethane exhibits good biocompatibility and mechanical properties. By incorporating 10 wt% of ethyl cellulose with a molecular weight of 164 kDa, comparable to polyurethane, a synergetic effect is observed, not only the modulus of the scaffold is increased by four times, but also its cell viability is increased by 1.13 times as compared with pristine polyurethane. The properties of this polymer blend scaffold are noticeably further enhanced by aligning the

electrospun fibers, the increases of 1.99 times for modulus and 2.13 times for cell viability are observed. The results are from highly ordered aligned fibers with anisotropic characteristics that are mechanically strong and morphologically compatible with the elongated shape of cardiac myoblast H9C2 cells. This new developed scaffolds with exceptional biocompatibility could meet the essential requirements for the survival and function of cardiac cells with native myocardium. Thus, the polymer blend of polyurethane and cellulose is a promising material for 3D scaffolds for the application in cardiac tissue engineering.

Funding

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