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Preparation and characterization of coaxial electrospun thermoplastic polyurethane/collagen compound nanofibers for tissue engineering applications

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ABSTRACT

Collagen functionalized thermoplastic polyurethane nanofibers (TPU/collagen) were successfully produced by coaxial electrospinning technique with a goal to develop biomedical scaffold. A series of tests were conducted to characterize the compound nanofiber and its membrane in this study. Surface morphology and interior structure of the ultrafine fibers were characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM), whereas the fiber diameter distribution was also measured. The crosslinked membranes were also characterized by SEM. Porosities of different kinds of electrospun mats were determined. The surface chemistry and chemical composition of collagen/TPU coaxial nanofibrous membranes were verified by X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectrometry (FTIR). Mechanical measurements were carried out by applying tensile test loads to samples which were prepared from electrospun ultra fine non-woven fiber mats. The coaxial electrospun nanofibers were further investigated as a promising scaffold for PIECs culture. The results demonstrated that coaxial electrospun composite nanofibers had the characters of native extracellular matrix and may be used effectively as an alternative material for tissue engineering and functional biomaterials.

1. Introduction

The regeneration of damaged or lost tissue requires that certain function reparative cells assemble three-dimensionally around and inside the supporting scaffold. This is normally carried via a series of biological activities such as adhering, migrating, growing and differentiating to attain a proper integration between cells and scaffold for synthesizing the new tissue [1]. The technology of tissue engineering (TE) aims to generate new or substitute and could well become an alternative method for organ transplantation [2,3]. So far as we know, the TE methods have been applied to different types of tissue and organ such as skin [4], bone [5], liver [6], intestine [7], heart valve [8], muscle [9] and tongue [10].

Most of these human organs deposited on fibrous structures with the fibril/fiber size realigning from nanometer to millimeter scale. So nanofiber has now been extensively used to mimic these natural tissue matrixes. Various fabrication methods have been used including electrospinning [11,12], phase separation [13,14] and self-assembly [15,16]. At present, electrospinning is the most prevalent process that can create nanofibers through an electrically charged jet of polymer solution or polymer melt. Different processing parameters such as type of polymer, viscosity, surface tension, jet charge density, temperature and humidity control the electrospinning process, especially the diameter and morphology of resulting fibers [17]. Recently, researchers have found that the nanofibrous structure formed by electrospinning method would improve the function of tissue regeneration *in vitro* and decrease the formation of scar tissue [18]. Therefore scaffolds constructed by electrospinning method can be used to mimic the native extracellular matrix (ECM).

Effective use of polymer nanofibrous scaffolds for tissue engineering relies not only on the construction of the fibers, which can mimic the physical structure of the native extracellular matrix, but also on the biochemistry characteristics of the materials used. One method of functionalizing nanofiber is realized by employing an advanced coaxial electrospinning technology. Through combination of different materials in the axial or radial direction, novel properties and functionalities for nanoscale devices can be anticipated. The unique core–shell structure offers a number of potential benefits. For example, the core materials should provide certain properties required by the tissue to be repaired, while the shell materials could be tailored to provide or endow additional properties, such as biocompatibility or hydrophilic properties. The major advantage of this core–shell nanostructure is the potential to obtain a combination of properties of different kind of materials.

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Native ECM is the complex of poly-protein and polysaccharide with nanofibrous structure. Among the natural biopolymers, collagen has been widely used as poly-protein in TE for its excellent biocompatibility and non-immunogenicity, but its mechanical property cannot achieve the request of native ECM. Thermoplastic polyurethanes (TPUs) are a widely used class of polymer with excellent mechanical properties and good biocompatibility, and have been evaluated for a variety of biomedical applications such as coating materials for breast implants, catheters, and prosthetic heart valve leaflets [19]. Conventional TPUs are among biomaterials not intended to degrade but are susceptible to hydrolytic, oxidative and enzymatic degradation *in vivo*. While the susceptibility of TPU to such degradation is a problem for long lasting biomedical implants, it can be deliberately exploited to design biodegradable polyurethane [20]. The TPU used in this research is one kind of medical-grade, aliphatic, polyether-based TPUs. It is biodegradable and its bio-stability is known to be better than poly(ester urethane).

As candidate materials, pure TPU and collagen have already been electrospun into nanofibers as biomaterials [21,22]. Moreover, they have biological benefits and have been used as TE scaffold. However, complete analysis of coaxial electrospun TPU and collagen compound nanofiber has not been conducted yet. In this study, TPU and collagen were selected as the scaffold material and coating reagent and a coaxial electrospinning technique was employed to produce the individually surface-coated nanofibers. The primary objective of this study was to investigate the efficacy of using surface functionalized nanofibers in regulating cell–scaffold interactions by using pig illiac endothelial cells (PIECs) as the sample cells for soft tissue engineering applications.

2. Materials and methods

2.1. Materials

The polymer of thermoplastic polyurethane (Tecoflex EG-80A) was purchased from Noveon, Inc. (USA) and collagen (mol. wt., $0.8–1 \times 10^5$ Da) was purchased from Sichuan Ming-rang Bio-Tech Co. Ltd. (China). The two materials both used 1,1,1,3,3,3,-hexafluoro-2-propanol (HFP) as solvent, which was brought from Daikin Industries Ltd. (Japan).

2.2. Scaffold fabrication by coaxial electrospinning

In our experiment, different solvents were used to investigate the formation of an appropriate Taylor cone in the process of coaxial electrospinning. In this study, the collagen and TPU were both dissolved in HFP solvent separately. The collagen solution was made in a weight ratio of 8 wt.% and TPU solutions were made with different concentrations which varied from 3 to 6%. The prepared solutions were resolved using multipoint heating magnetic stirring apparatus with sufficient stirring at room temperature.

Briefly, the basic experimental schematic illustration consisted of syringe-like apparatus with an inner needle coaxially placed inside with an outer one as shown in Fig. 1. Two immiscible liquids are injected at appropriate flow rate through this coaxially steel needles arranged. Both needles are connected to the same electrical potential. A high electrospinning voltage was applied between the needle and ground collector using a high voltage power supply. The electric field generated by the surface charge caused the solution drop at the tip of the needle to distort into a Taylor cone. With an increase in the supplied high voltage to a threshold value, a steady coaxial compound fluid jet with an external meniscus surrounding the inner one was formed and ejected out of the Taylor cone. The fluid jet was then reducing into sub-micrometer scale as a consequence of bending instability. After evaporation of the solvents during the course of flying, the thin jet was deposited on the collector which resulted in a bi-component composite fibrous membrane.

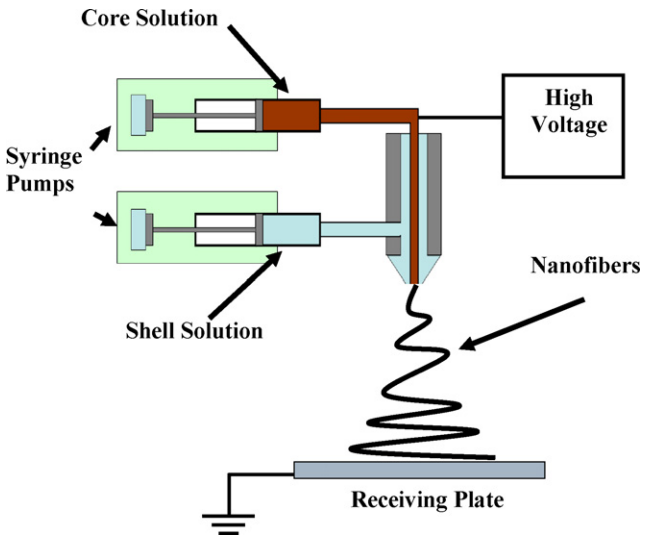

Fig. 1. Schematic diagram of a set-up for coaxial electrospinning.

In this experiment, the outer needle and the inner needle were connected through two silicones tubes to syringes which contained the shell solution and the core solution. A high voltage DC power supply (BGG6-358, BMEICO LTD., China) which could generate 20 kV was employed in the present work and a grounded metallic screen was used to collect the ultrafine fibers. The distance between the spinneret and the collector was 130–50 cm, respectively. The electrospinning was done at ambient temperature with humidity of 55%. The core and shell flow rates were 0.8 and 1.2 ml/h respectively and the resulting non-woven fibrous mats had a thickness of around 0.10–0.20 mm. The mats were then placed into a vacuum oven for 24 h to remove residual solvent.

2.3. Morphology characterization of coaxial TPU/collagen nanofibers

The morphologies and diameters of the nanofibers electrospun by pure and coaxial of TPU to collagen were determined with SEM (JEOL, JSM-5600 Japan) at a accelerated voltage of 15 kV. Verification of core–shell structure was characterized by TEM (H-800, Hitachi) at 100 kV, and the samples for TEM observations were prepared by collecting the nanofibers onto carbon-coated Cu grids. The diameter range of the fabricated nanofibers was measured via SEM images using image visualization software ImageJ 1.34s (National Institutes of Health, USA). Average diameter and diameter distribution were determined by measuring one hundred random nanofibers from the SEM images. Surface properties of the nanofibers were examined using a nanoscope atomic force microscope (Digital Instruments), in the tapping mode and expressed as height and phase images.

2.4. Crosslinking and water resistant test

The crosslinking process was carried out by placing coaxial electrospun membrane (collagen (shell)/TPU (core) (8 wt.%/3 wt.%)) together with a supporting aluminum foil in a desiccator using glutaraldehyde (GTA) (25% water solution) with different process time. An optimized extent of crosslinking was determined by testing the dissolubility of crosslinked scaffolds immersed in 37.0 °C deionized water for 72 h, then dried in vacuum at room temperature for 1 week. This experimental condition was selected in order to

simulate a real situation of coaxial electrospun nanofibers in physiological applications such as for tissue engineering scaffolds or release carriers. Then the samples were dried in a vacuum for 1 week and then observed by SEM.

2.5. Surface atomic chemistry analysis

Surface chemistry analysis of the electrospun scaffolds were also analyzed using X-ray photoelectron spectroscopy (XPS) (Escalab 250; Thermo Scientific Electron, East Grinstead, UK) equipped with Mg Kα at 1486.6 eV and 150 W power at the anode. A survey scan spectrum was taken and the surface elemental compositions relative to carbon was calculated from the peak height with a correction for atomic sensitivity.

2.6. The porosity of electrospun mats

The electrospun mats of TPU, collagen and coaxial compound collagen/TPU were prepared. The thickness of the nanofiber mat was measured with a micrometer (Shanghai, China). The apparent density and porosity were calculated according to the following equations [23]:

$$\text{Apparent density of nanofiber mats}\,(\text{g/cm}^3) = \frac{\text{Mass of nanofibrous mast}\,(\text{g})}{\text{Thickness of nanofibrous mats}\,(\text{cm}) \times \text{Nanofiber mats area}\,(\text{cm}^2)}$$

$$\text{Porosity of nanofiber mats}\,(\%) = \left(1 - \frac{\text{Naonfiber mats apparent density}\,(\text{g/cm}^3)}{\text{Bulk density fo raw TPU/Collagen}\,(\text{g/cm}^3)}\right) \times 100\%$$

2.7. FTIR spectra

Electrospun TPU nanofibers, collagen nanofiber and TPU/collagen with core/shell structure were prepared for the FTIR test on AVATAR 380 FTIR instrument (Thermo Electron, Waltham, MA). All spectra were recorded by an absorption mode in the wavelength range of 4000–500 cm^{-1}.

2.8. Mechanical measurement

Mechanical measurements were carried out by applying tensile test loads to samples which were prepared from electrospun ultra fine non-woven fiber mats. In this study, all these tests were performed in an ambient temperature at 20 °C and a relative humidity of 65%. Four specimens of each sample were prepared according to the method described by Huang et al. [24]. First, a white paper was cut into templates with a planner dimension of width × gauge length = 10 mm × 30 mm and then double side tapes were glued onto the top and bottom areas of one side. Secondly, the aluminum foil was carefully scraped off and single side tapes were applied onto the griping areas as end-tabs. Finally, the resulting species were tested on a commercial materials testing machine (H5K, Hounsfield, England) with a load cell of 10 N, and the elongation speed is 10 mm/min.

2.9. Viability and morphology study of PIEC on nanofiber mats

Pig iliac endothelial cells (PIECs) were cultured in DMEN medium with 10% fetal serum, and 100 units/ml streptomycin in humidified incubator under standard culture conditions (5% CO_2, content at 37 °C), and the medium was replaced every 3 days. Mats of complex nanofibers of TPU and collgen were electrospun on 14 mm circular glass coverslips. After the coverslips with nanofibers were prepared already, they were dried in vacuum for over 1 week to release the residua solvents. Then the mats were placed in desiccator to crosslink using glutaraldehyde (25% water solution) steam for 2 days. Where after the compound nanofibers mats were dried in vacuum for over 2 weeks to release the residual glutaradehyde. And the next step is to fix the coverslips into 24-well plates with stainless ring. Before seeding cells, fiber scaffolds were sterilized with 75% alcohol solution, which were placed with phosphate-buffered saline solution (PBS) after 2 h.

Cells viability on the nanofibers was determined by MTT method. Briefly, the cell and nanofiber complex was incubated with 5 mg/ml 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-diphenytetrazoliumromide (MTT) for 4 h. Thereafter the culture media were extracted and added 150 μl dimethyl sulfoxide (DMSO) to have low speed surge for about 10 min. When the crystal was sufficiently resolved, aliquots were pipetted into the wells of a 96-well plate and placed into an Enzyme-labeled Instrument (MK3, Thermo, USA), and the absorbance at 490 nm for each well was measured.

For the cell viability test, endothelium cells were seeded onto nanofiber mats ($n = 3$) at a density of 5×10^4 cells/cm^2. On days 1, 3, 5 and 7 after cell seeding, unattached cells were quantified by MTS kit (C0009, Beyotime Institute of Biotechnology, China) and Enzyme-labeled Instrument (MK3, Thermo, USA).

After 24 h of culturing, the electrospun fibrous scaffolds with cells (density is 1.5×10^5 cells/cm^2) were examined by SEM. To prepare the cell-cultured samples for SEM observation, the scaffolds were rinsed twice with PBS in the first place, followed by fixation with 4% glutaraldehyde water solution for 2 h and then the samples were rinsed twice with PBS and thereafter dehydrated in graded concentrations of ethanol (30, 50, 70, 80, 90, 95 and 100). Finally, they were dried in vacuum overnight. The dry cellular constructs were coated with gold sputter and observed under the SEM at a voltage of 15 kV.

2.10. Statistical analysis

Statistics analysis was performed using origin 7.5 (Origin Lab Inc, USA). Values (at least triplicate) were averaged and expressed as means ± standard deviation (SD). Statistical differences were determined by the analysis of one-way ANOVA and differences were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Solvent selection

In the electrospinning system, there are a number of parameters affecting fiber morphology and fiber diameter, such as polymer concentration/viscosity, applied voltage, needle diameter and the delivery rate of polymer solution [25]. Additionally, the solvent used to dissolve the polymer has a significant effect on the spinnability of the polymer solution and fiber morphology. In this study, we used HFP as the solvent for the electrospinning process and fabrication of the fibrous scaffolds. HFP is an ideal organic solvent. It allows full extension of the polymer and it evaporates completely after the fiber formation process without leaving any residue on the formed fibers [26]. In the non-optimal conditions, we found that when using TPU solution (N,N-dimethylformamide or tetrahydrofuran as solvent) and collagen (HFP as solvent) in coaxial electrospinning process, white deposition appeared at the bottom of the coaxial spinneret, and the electrospinning process couldnot be carried through. Conversely, when we used HFP as solvent for both TPU and collagen, the Taylor cone at the bottom of the spinneret was clear and did not have any impurity or deposi-

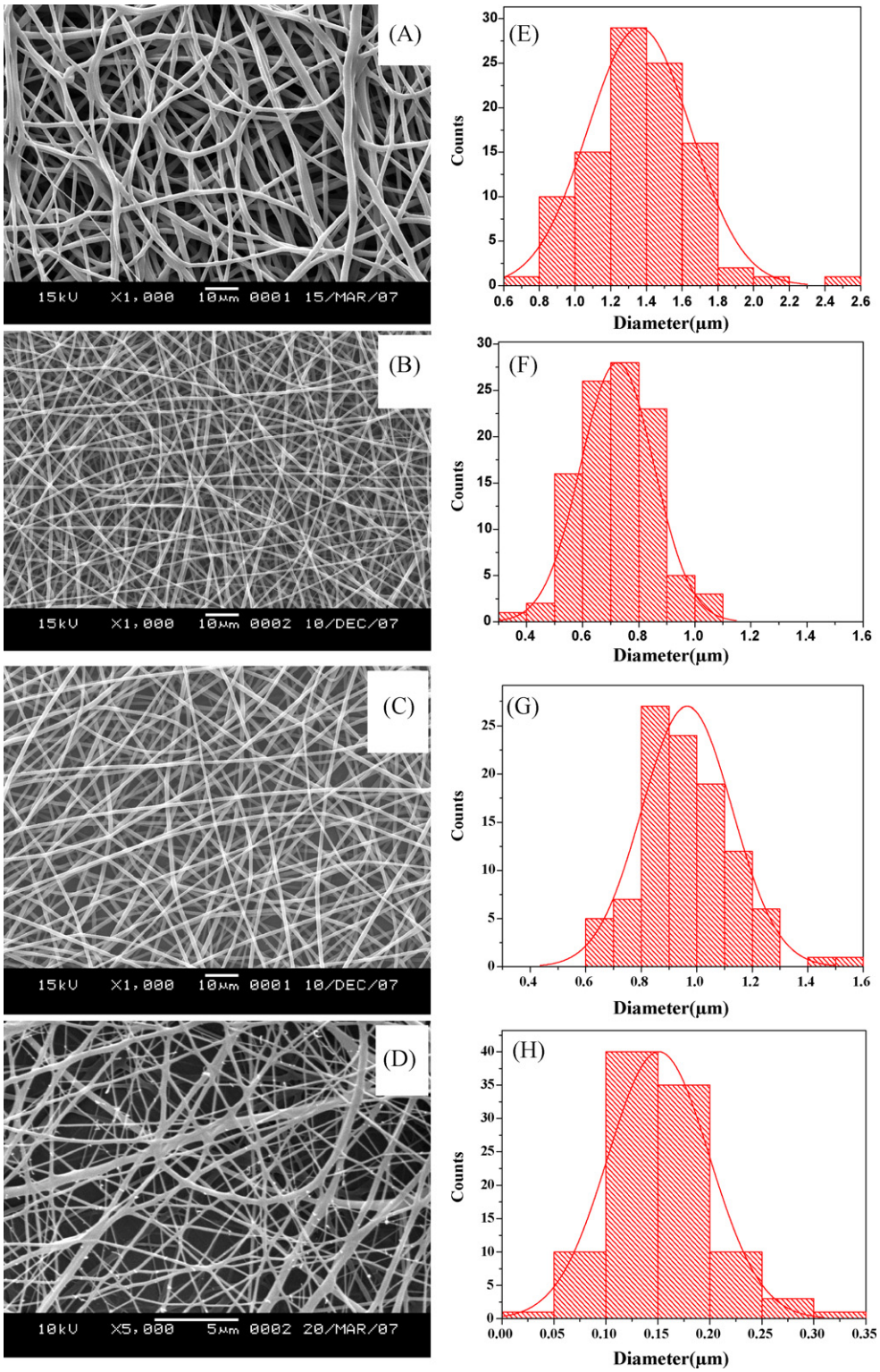

Fig. 2. (A and E) SEM images and their nanofiber diameter distribution of pure TPU; (D and H) SEM images and their nanofiber diameter distribution of pure collagen; (B and F) SEM images and their nanofiber diameter distribution coaxial electrospun nanofibers of collagen (shell)/TPU (core) (8 wt.%/3 wt.%); (C and G) SEM images and their nanofiber diameter distribution coaxial electrospun nanofibers of collagen (shell)/TPU (core) (8 wt.%/6 wt.%).

tion. Therefore, HFP was selected as a proper solvent for coaxial electrospinning of collagen and TPU blends.

3.2. Morphology of coaxial electrospun nanofibers

As stated above, polymers of TPU and collagen were dissolved in HFP separately. When they were prepared, the two solutions were put into the coaxial spinneret. Through observing the structure of almost conical electrified menisci consisting of an outer meniscus surrounding an inner one, we successfully fabricated composite nanofibers with core–shell structure.

The SEM photographs of pure TPU, collagen and TPU/collagen with core–shell structure composite nanofibers were shown in Fig. 2, whereas the diameters distributions were further deter-

Table 1
Numerical statement of average diameters and standard deviation ($n = 100$).

Samples	TPU	Collagen (shell)/TPU (core) (8 wt.%/3 wt.%)	Collagen (shell)/TPU (core) (8 wt.%/6 wt.%)	Collagen
Average diameter (μm)	1.33	0.72	0.96	0.15
Standard deviation (μm)	0.289	0.13	0.163	0.048

mined and results were also exhibited in Fig. 2. Numerical statements of average diameters and standard deviation were summarized in Table 1. Nanofiber diameters were calculated from the diameter of 100 nanofibers each sample which was directly measured from SEM photographs. From the result of fiber diameter distribution, it could be observed that TPU had the largest number of average diameter, while collagen had the smallest number of average diameter. Diameter of core–shell structure nanofiber was in the range of 700–800 nm.

It has been known that in electrospinning, the force which causes the stretching of the solution was due to the repulsive forces between the charges on the electrospinning jet [27]. Collagen is a kind of typical amphiprotic macromolecule electrolyte. When collagen was affiliated, more ions were formed in the compound spinning solution. So a higher discharge density could be carried by the electrospinning jet. The conductivity of the solution could be increased by the addition of ions. On the other hand, the increased charge carried by the solution would increase the stretching of the solution. Moreover, the increase in the stretching of the solution also assisted to yield fibers of smaller diameter.

In general, we found that when the core concentration of TPU solution was between 3% and 6%, smooth nanofibers could be obtained. Either lower or higher concentration would lead significant beads on the surface of nanofibers. In addition, it has been found that chain entanglements in the solution play an important role in increasing fiber diameter and changing fiber morphology. And the increase of the solution concentration would result in great polymer chain entanglements. Thus, if the concentration is too low, bead-only structure will be produced due to a lack of chain entanglements in the solution [28]. Conversely, too high concentration will make it very difficult to pump the solution through the syringe needle. Moreover, when the concentration is too high, the solutions may dry at the tip of the needle before the electrospinning Taylor cone can be initiated. It was also noted that the shell concentration couldnot be too high, because a lower viscosity tended to facilitate the formation of more uniform fibers with a narrower fiber diameter distribution [29]. Thus, both the shell and the core solutions could influence the surface quality and morphology of the resulting composite nanofibers [30]. We also got the conclusion that one of the important parameters affecting the fiber morphology in the shell–core structure was the core concentration, whose appropriate value was found to be between 3% and 6% in this work. From the fiber diameter distribution, it was clear that the fiber diameter increased along with the increase of core concentration of TPU solution.

Fig. 3 showed the transmission electronic micrographs of the shell–core structure of the composite ultrafine fibers. The sharp core–shell interfaces with core phase TPU polymer encased by collagen shells were exhibited. This implied that the coaxial electrospinning was fast enough to prevent the mixture of the core fluid with shell fluid. We also found that some fibers displayed only monolayer structures instead of the core–shell structures. This could be attributed to the fact that the core fluid jet out of coaxial capillaries might be split into a number of sub-jets during the electrospinning process [29,31]. Therefore, we could observe that overall fiber diameters were not uniform no matter what concentration was used in core concentration.

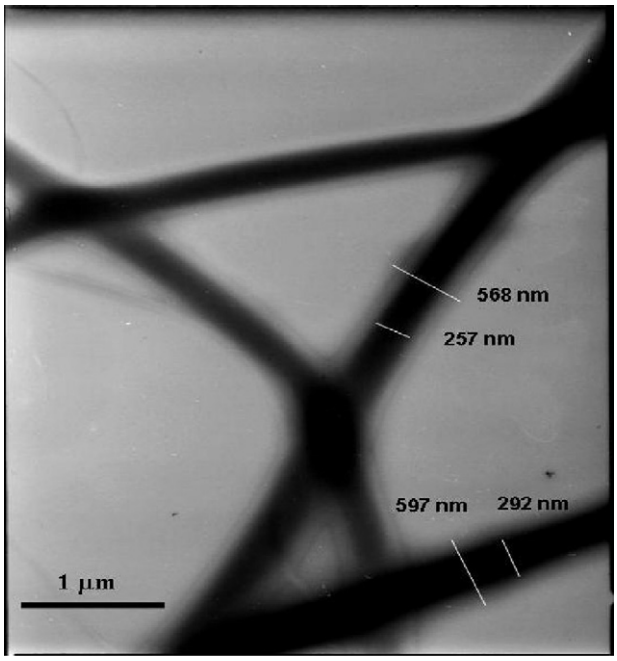

Fig. 3. Transmission electron microscopy image of the collagen (shell)/TPU (core) ultrafine fibers.

With the aim of observing the surface morphologies of TPU, collagen and coaxial electrospun TPU/collagen, atomic-force microscopy was employed using a height mode (Fig. 4). From the analysis of AFM images, it could obviously be observed that the surface of TPU nanofibers was much smoother than the surface of collagen nanofibers. The surface of coaxial compound nanofibers exhibited rough morphology compared with the surface of TPU. From surface morphologies of coaxial electrospun nanofibers, we could get the conclusion that collagen component might exist on the surface of compound nanofibers. On the other hand, the rough surface would be benefit for cell adhesion and proliferation. With the aim of obtaining surface modification function of nanofibers, coaxial electrospinning fabrication technique is an optimized approach.

3.3. Morphology of crosslinked coaxial electrospun nanofibers

Many chemicals such as formaldehyde, glutaraldehyde, carbodiimide and dextran dialdehyde, have been used to chemically modify collagen for biomedical applications. Amongst, glutaraldehyde (GTA) is by far the most widely used chemical, due to its high efficiency in stabilizing collagenous materials. The objective of this part study was to make collagen/TPU coaxial electrospun nanofibers water insoluble through a GTA crosslinking treatment so as to preserve their fibrous morphology and enhance scaffold potential applications.

SEM micrographs of uncrosslinked coaxial electrospun TPU/collagen (collagen (shell)/TPU (core) (8wt.%/3 wt.%)) were shown in Fig. 5(A), and it was soluble or swollen in water. To potentially use electrospun TPU/collagen coaxial electrospun nanofibers for tissue engineering, crosslinking was an efficient

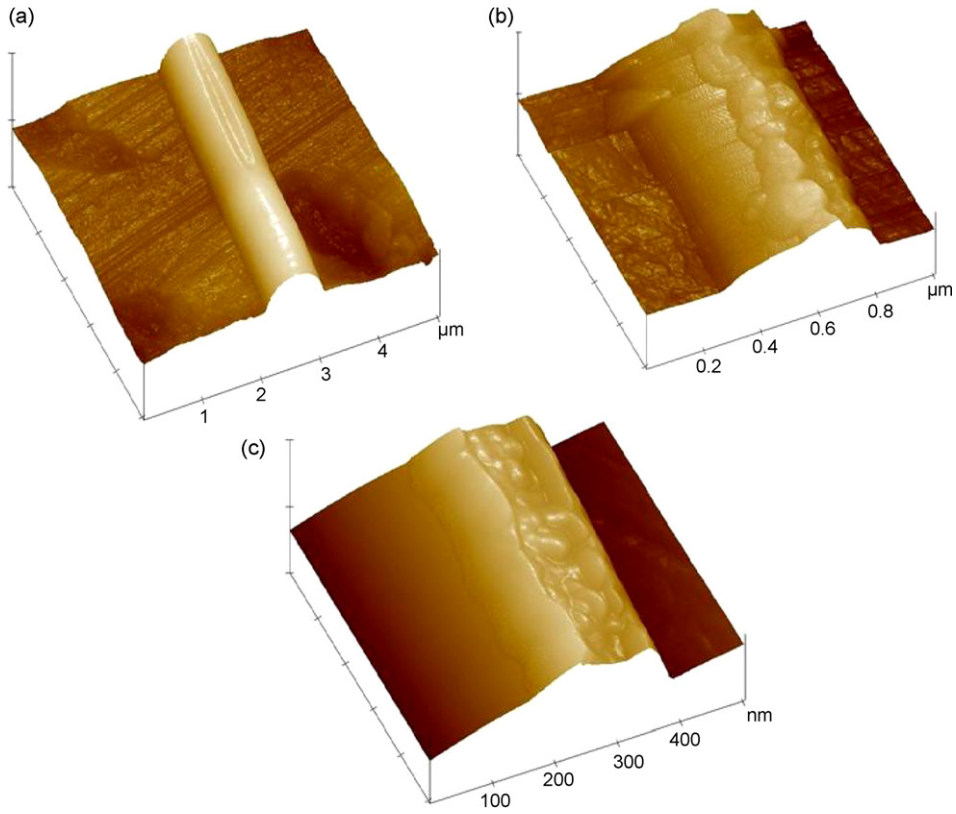

Fig. 4. AFM images represented by height model: (a) TPU fiber, (b) collagen fiber, (c) coaxial collagen (shell)/TPU (core) (8 wt.%/3 wt.%).

way to improve the dissolvability. SEM images of TPU/collagen coaxial electrospun membranes after crosslinked with GTA vapor for different process time interval were also exhibited in Fig. 5 as well. With increasing crosslinking time, nanofibers became thicker and melt into each other which would lead to reducing porosity. For the water resistant test in water, micrographs of crosslinked nanofibers for 12 h completely disappeared, crosslinked nanofibers for 24 and 48 h still remained fibrous structure. Crosslinked nanofibrous scaffolds were required to process good stability, suitable porosity and pore diameters for cell growth. Thus, we chose 48 h as an appropriate crosslinking time condition for cell culture.

3.4. Surface chemistry of coaxial nanofibers

The surface chemistry changes of blended nanofibers were verified by XPS spectroscopy. Fig. 6 showed the XPS survey scans of nanofibrous scaffold surfaces. Table 2 showed the atomic ratios of carbon, nitrogen and oxygen on pure collagen, TPU and coaxial collagen (shell)/TPU (core) (8 wt.%/3 wt.%) compound nanofibers.

As expected, the coaxial electrospun scaffold of collagen/TPU showed three peaks corresponding to C1s (binding energy, 286 eV), N1s (binding energy, 400 eV) and O1s (binding energy, 532 eV). The atomic ratios of carbon, nitrogen and oxygen on nanofibrous scaffolds calculated from XPS survey scan spectra were shown in Table 2. The oxygen content (20.29%) of TPU nanofiber surface was not changed by the incorporation of collagen (collagen/TPU, 19.46). On the other hand, nitrogen (13.76%) found on the coaxial electrospun nanofiber surface was much higher than TPU nanofiber (1.77%). This result illustrated that collagen was found to be present on the surface of coaxial electrospun nanofibers. However, it is hard to say that collagen was located on the surface of blended nanofibers, because both TPU and collagen have nitrogen content in their chemical structures. The existence of collagen molecules on the surface and inside the nanofibers provides sustained cell recognition signals with polymer degradation, which is important for the development of cell function.

Table 2
Atomic ratios of carbon, nitrogen, and oxygen on the surface of collagen nanofibers, TPU nanofibers, coaxial electrospun collagen/TPU blended nanofibers (collagen (8 wt.%)/TPU (3 wt.%)) calculated by X-ray photoelectron spectroscopy.

Substrate	Atomic percent		
	C	O	N
Collagen	66.24	19.96	13.8
TPU	78.13	20.29	1.77
Coaxial collagen/TPU	66.2	20.04	13.76

3.5. Porosity of nanofibrous mats

During the process of electrospinning, the thickness of different nanofibers was controlled by the deposition time if all the electrospinning parameters were fixed. The apparent density of coaxial electrospun nanofibrous mats and pure collagen and TPU were summarized in Table 3 ($n = 3$). We can see that all the apparent density of composite nanofibers was in the range of 0.30–0.40 g/cm^3 and it slowly increased when the TPU concentration decreased in this compound system.

As electrospun TPU coaxial with collagen nanofibous mats were having high pore size, using the apparent density of all different mats and the bulk density of TPU/collagen blends, we could calculate the porosity of the compound nanofibers. However, it was not easy to accurately measure the bulk density of the TPU/collagen blend for it was a high porous material and contained a mixture of different ingredient, so the bulk density of the blends were estimated to be 1.04 g/cm^3 on the basis of the bulk density of TPU. As shown in Fig. 7, we could observe that the porosity of TPU/collagen compound nanofibers had a big increase when the collagen content

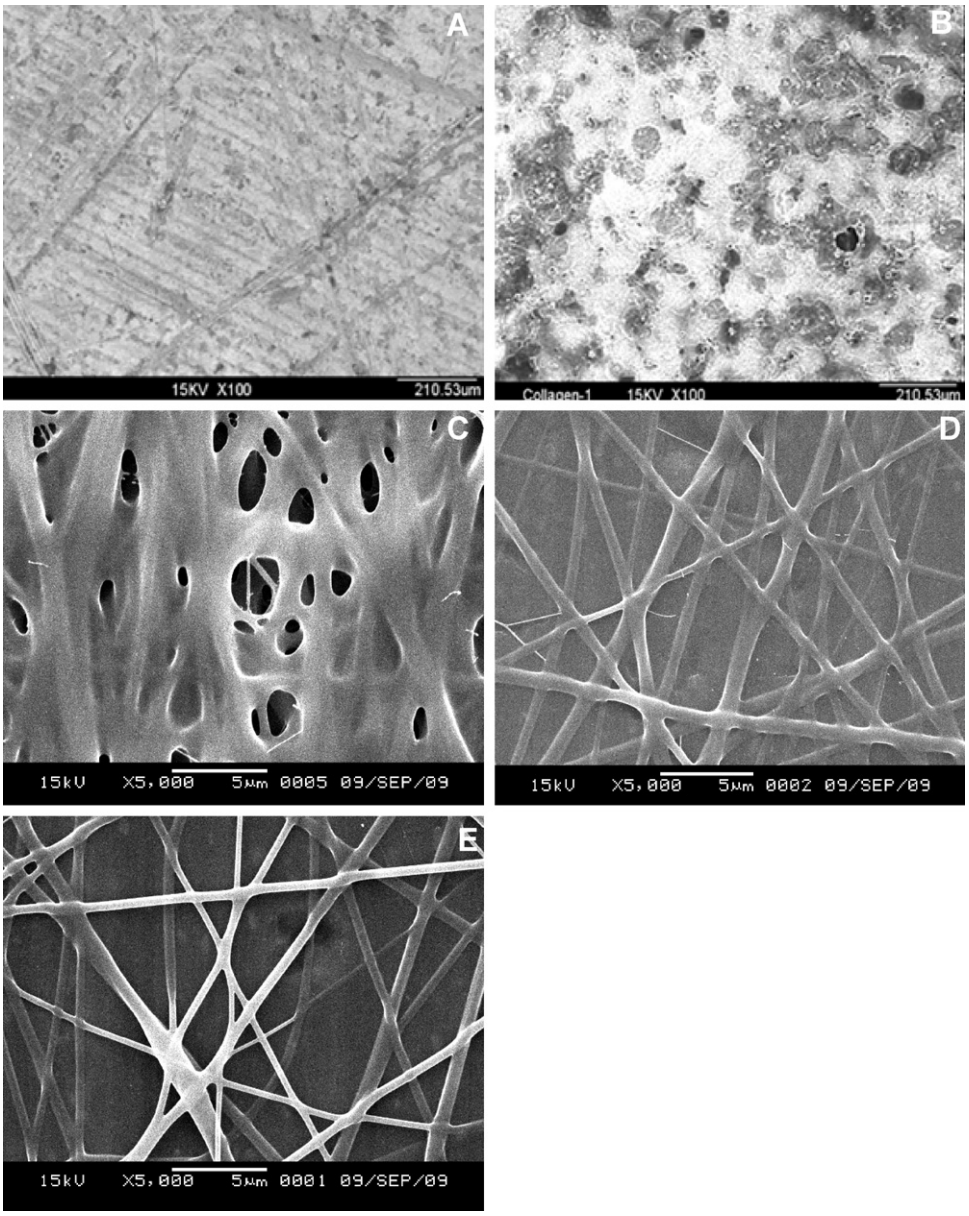

Fig. 5. SEM photographs of uncrosslinked and crosslinked TPU/collagen coaxial electrospun nanofibers. (A) Uncorsslinked, (B) corsslinked for 6 h, (C) crosslinked for 12 h, (D) crosslinked for 24 h and (E) crosslinked for 48 h.

increased in the system. The porosity increase of nanofibrous mats by adding collagen in the coaxial system could also be explained for the increase of solution conductivity [32]. From the fiber diameter analysis we could know that with the increase of the conductivity of the solution, smaller of the fiber diameter would be. On the other hand, with more electronic charges carried by nanofiber, there would be stronger repulsive forces among fibers during their deposition to the collector.

Pores play an important role in determining the physical and chemical properties of porous substrates and have adeterministic effect on the performance of membranes, catalysts, adsorbents, etc. On the other hand, the mats must have a large pore volume fraction as well as an interconnected pore work to permit the transport, multiplication and metabolites. High porosity is propitious to cell adhesion on the mat, promotes extracellular matrix regeneration. Thus, porosity is an essential factor for materials to mimic extracellular matrix in tissue engineering. From the porosity analysis of the coaxial compound nanofibrous mat and the observation of its microstructure, they exhibited high porosity and adequate pore size for cell ingrowths.

Table 3
Apparent density and porosity of different electrospun mats ($n = 3$).

Samples	TPU	Collagen/TPU (8 wt.%/6 wt.%)	Collagen/TPU (8 wt.%/3 wt.%)	Collagen
Apparent density (g/cm^3)	0.61 ± 0.02	0.36 ± 0.03	0.33 ± 0.03	0.29 ± 0.02
Porosity (%)	38–43	62–68	65–71	70–74

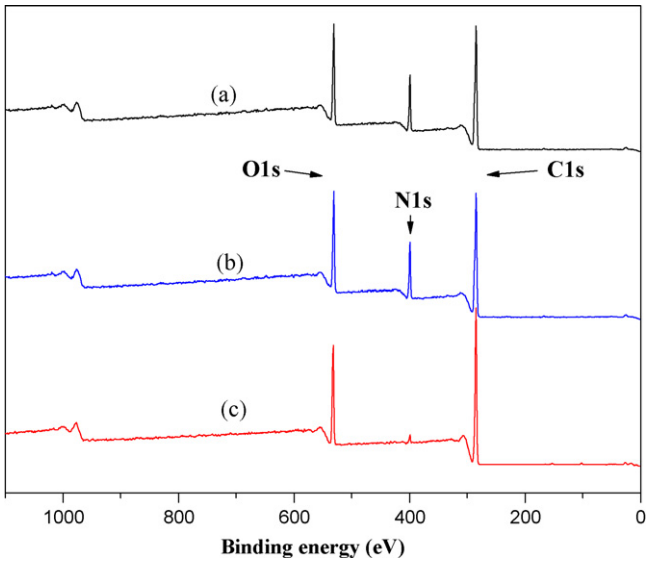

Fig. 6. X-ray photoelectron spectroscopy survey scan spectra of (a) collagen nanofiber, (b) coaxial electrospun collagen/TPU nanofiber and (c) TPU nanofiber.

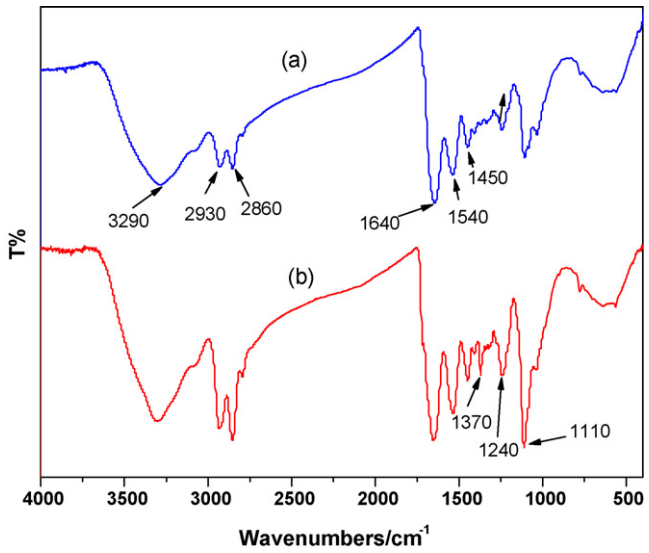

Fig. 8. FTIR spectra of coaxial collagen/TPU electrospun nanofibrous membranes. (a) Collagen (shell)/TPU (core) (8 wt.%/3 wt.%); (b) collagen (shell)/TPU (core) (8 wt.%/6 wt.%).

3.6. Chemistry characterization of coaxial TPU/collagen composite nanofibers

The chemical composition of TPU/collagen (Fig. 8) coaxial nanofibers were verified by Fourier transforms infrared spectroscopy (FTIR). The spectroscopy of electrospun thermoplastic polyurethane has characteristic absorption band at 3320, 2960, 1710, 1530, 1220, 1110 and 777 cm^{-1}, which represents υ (N–H), υ (C–H), υ (C=O), υ (C=C), υ (C–C), υ (C–O), υ (C–H) on substituted benzene, respectively [33]. Collagen displays characteristic absorption band at 1640, 1540, 1210 cm^{-1}, which are the amide I, II and III, respectively [34]. The amide I represents protein amide υ (C=O). The amide II is composed by δ (N–H) and υ (C–N) (60% and 40% contribution to the peak respectively). The amide III is made up of υ (C–N) and β (N–H).

Fig. 8 depicted the spectra of TPU/collagen coaxial nanofibers with different TPU concentrations. It could be seen that although the nanofibrous mats prepared in different core concentrations, these spectra were quite similar to each other and the peaks appear at the same bands. The difference among the three spectra is the intensity of some peaks. It was observed that the absorption peak at about 3290 cm^{-1} concerned with –OH and –NH stretching vibrations shift to a stronger wave number with increase of TPU (core) concentration. The results suggested that there might have the formation of hydrogen bond between the surface of collagen and TPU. The spectra showed both characteristics peaks of electrospun collagen and TPU. So this was the evidence to illustrate that there might be no other reaction between collagen and TPU. These two polymers kept structure independence in the coaxial structure. So far as we know, collagen and thermoplastic polyurethane have no toxicity and inflammation to human endothelial cells and tissues, and chemistry verification eliminates the apprehension of extra compound of the two biomaterials which may bring negative influence for tissue formation. And the hydrogen bond happened at the interface of two components would be effective for reinforcement of coaxial compound nanofibers.

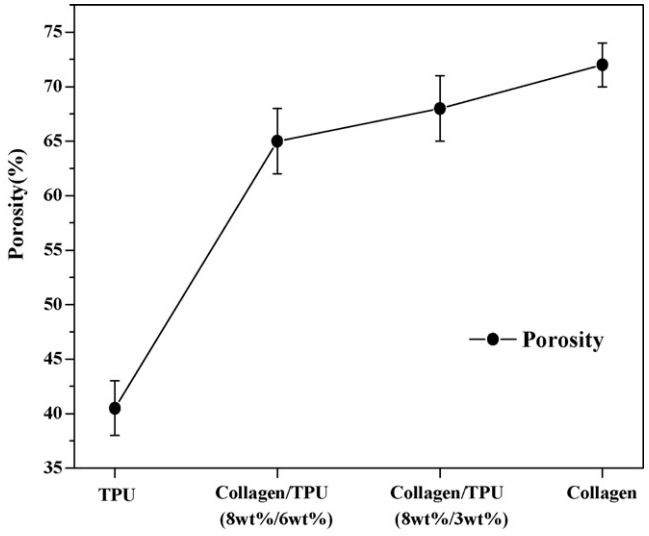

Fig. 7. The porosity of TPU/collagen coaxial nanofibrous mats. Date are representatives of three independent experiments and all date points are plotted as mean ± SD (n = 3).

3.7. Mechanical properties of TPU/collagen coaxial nanofibers

The mechanical properties of core–shell structure nanofibers are important for their successful applications in tissue engineering. Collagen and TPU compound nanofibers were electrospun into 0.5 mm thick fiber mats to measure their mechanical properties. Fig. 9 shows the typical stress–strain curve of collagen/TPU coaxial electrospun nanofibrous mats and pure TPU and collagen under tensile loading. The tensile strength and ultimate strain obtained from four independent tests were summarized in Table 4 (n = 4). The electrospun TPU material gave a characteristic response for elastomeric materials–sigmoid in shape. It showed a very soft and flexible characteristic with low Young's modulus and the high elongation at break of 300%. In contrast, the electrospun collagen materials exhibited plastic mechanical properties with high initial modulus and poor elongation at break of 15%. From the stress–strain curves of collagen/TPU coaxial electrospun mats, we could observe that by increasing the concentration of core TPU solutions, the strain of the materials became larger and the initial modulus became lower. Therefore, we could adjust the mechanical property to meet the requirement in practice through changing the TPU concentration in the coaxial system.

Table 4
Mechanical tensile properties of collagen/TPU coaxial nanofibrous scaffolds ($n = 4$).

Samples	Pure TPU	Pure collagen	Collagen/TPU (8 wt.%/3 wt.%)	Collagen/TPU (8 wt.%/6 wt.%)
Tensile stress (MPa)	7.7 ± 0.7	4.8 ± 0.7	4.53 ± 0.3	3.9 ± 0.24
Ultimate stain (%)	365 ± 24.5	13.7 ± 1.9	142.8 ± 7.2	145.6 ± ± 4.1

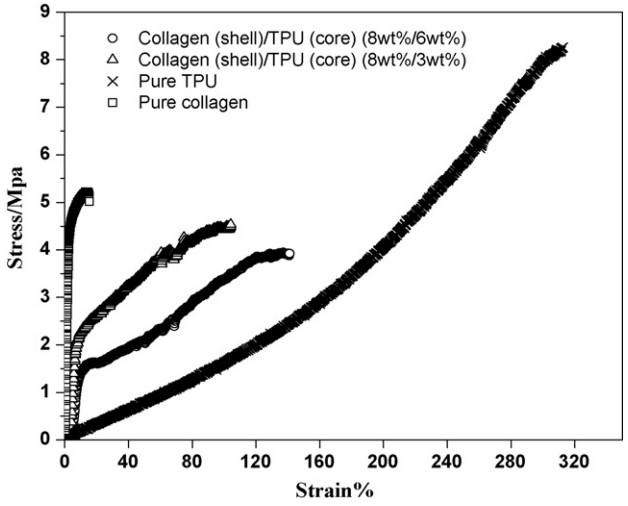

Fig. 9. Typical stress–strain curve for the electrospun TPU, collagen and TPU/collagen coaxial nanofibers.

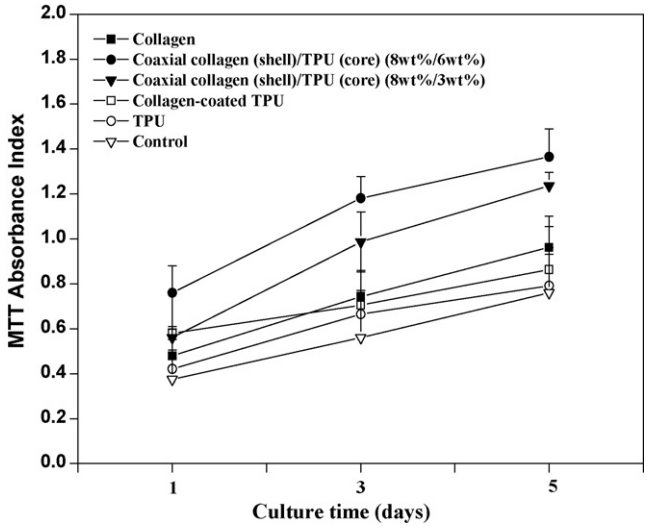

Fig. 10. Viability of PIECs cultured on TPU, coaxial electrospun collagen/TPU, collagen-roughly coated TPU and collagen nanofibers. PIECs cultured on the coverslips acted as a negative control. Date are representative of three independent experiments and all date points are ploted as mean ± SD, $^*P < 0.05$.

To design an ideal scaffold, various factors should be considered, such as pore size and morphology, mechanical properties versus porosity, surface properties and appropriate biodegradability. Of these factors, the importance of mechanical properties on cell growth is particularly obvious in tissues, such as bone, cartilage, blood vessels, tendons, heart valve and muscles. Different natural tissues have different mechanical properties. For example, the aortic heart valve of human and porcine has different mechanical tensile stress and ultimate strain [35]. TPU has high tensile strength, good tear and abrasion resistance, but its Young's modulus is very low. Collagen has high Young's modulus and excellent biocompatibility, but its tensile strength and strain are poor. In order to mimic the mechanical properties in the radial and circuit direction, we need to adjust the blend ratios of TPU and collagen. From the analysis, we can find that with the TPU concentration ingredient increasing, the Young's modulus, tensile strength and tensile strain changed. Coaxial nanofibers may have improved mechanical strength compared with pure non-crosslinked collagen nanofibers, which could combine the advantages of both synthetic and natural materials.

3.8. Viability of cells on coaxial collagen/TPU complex nanofibers

The proliferation date of PIECs on days 1, 3 and 5 after seeding on coaxial electrospun nanofibers of *in vitro* culturing were plotted in Fig. 10. The pure TPU nanofibers showed a significant level ($P < 0.05$) of slow increase in cell proliferation throughout the designed three times intervals as compared to the other nanofibrous scaffolds. In order to study the different core concentration of coaxial electrospun nanofibers and coating efficiency on cell proliferation, we designed our comparison to the nanofibers of pure TPU and collagen, coaxial collagen/TPU (with core concentrations 3, 6 wt.% individually) and collagen-coated TPU.

The viability of cells cultured on complex nanofibers was compared with that of cells cultured on coverslips (control), pure collagen nanofibers, TPU nanofibers and collagen-coated TPU nanofibers. It was revealed that all the nanofiber mats had good cell viability than coverslips and cell viability had no obvious difference among the blend nanofiber mats at 24 h, and cell proliferation was very fast and the highest MTT absorbance index could reach 0.6. It was found that collagen/TPU nanofibers by both coating technologies exhibited very distinct and accelerated differences. The significance level increased to $P < 0.01$ at day 5 from $P < 0.05$ at day 1. Furthermore, coaxial electrospun nanofibers is significantly more favorable ($P < 0.05$) for cells proliferation than that of roughly collagen-coated TPU, pure TPU and control. There was no significant difference in the two different core concentrations compound nanofibers, but cell proliferation of core concentration of 6 wt.% exhibited a slightly better value than the concentration of 3 wt.%. Comparing the coaxial electrospun nanofibers with collagen nanofibers, the proliferation date and statistical test indicated that there is no significant difference among them. The fibers diameters, porosity and mehchanical property are very important for cell growth and migration. Among this system, the diameters, porosity and mechanical stress changed along with component in the blending system. Thus, compared with pure collagen and TPU, compound nanofibers could provide better growth condition for cell proliferation. In our studies, coaxial electrospnning of collagen to TPU might offer the most suitable qualification for cell culture.

Fig. 11 shows SEM morphology observations of PIECs on TPU (A), collagen (shell)/TPU (core) (8 wt.%/6 wt.%) (B), TPU with surface roughly collagen coating (C), pure collagen (D) mats after seeding for 24 h. As SEM images shown, cells could spread well both on mono-TPU, collagen nanofibers and TPU/collagen compound nanofibers. Visually, varied extents of cell spreading around the nanofibrous scaffolds of the TPU, collagen-coated TPU and coaxial TPU/collagen already indicate the differences in cell proliferation. The TPU nanofibers subjected to coatings (Fig. 10b and c) were attached with many more cells than those of the pristine TPU. This is consistent with the proliferation analysis previously. However, there is no such finding either in the raw TPU or in the collagen-coated TPU nanofibrous scaffold, indicating that a direct

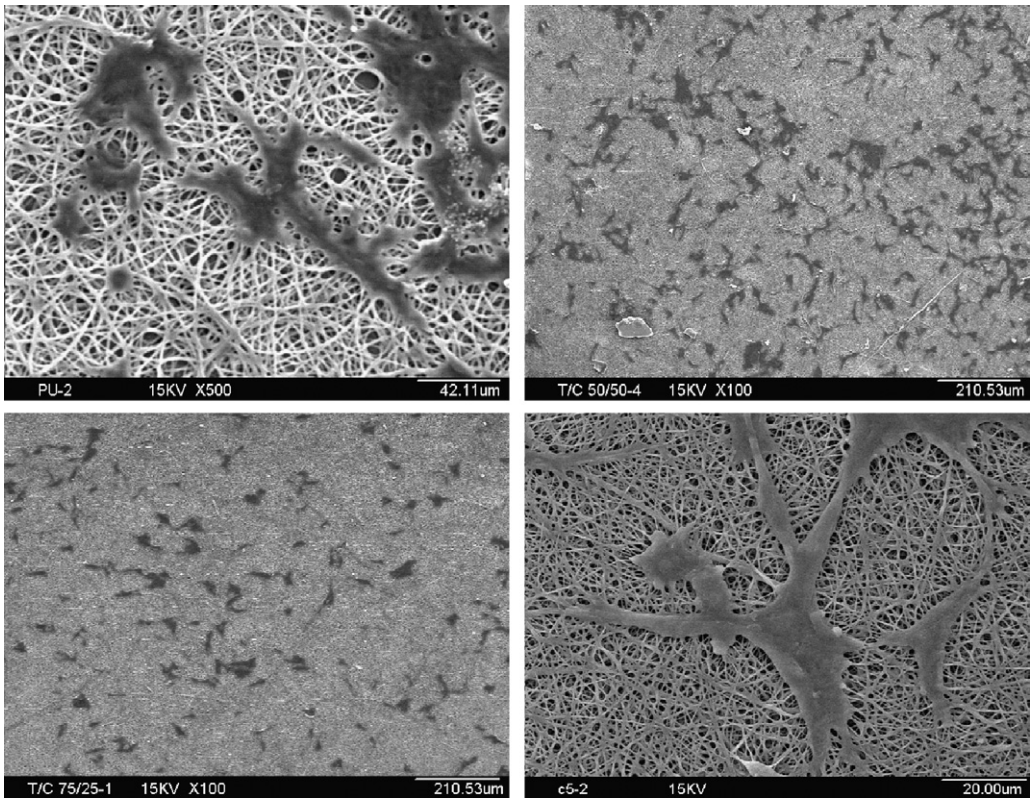

Fig. 11. SEM micrographs of PIECs cultured on fibrous substrates: (A) TPU fibers, (B) collagen (shell)/TPU (core) (8 wt.%/6 wt.%), (C) TPU with surface roughly collagen coating and (D) pure collagen fibers.

coating of collagen onto TPU would only be helpful to improve the cell proliferation behavior. Its action in favoring the cell-scaffold integration forming a 3D ingrowths in the scaffold is not apparent.

With respect to nanofibers for tissue engineering applications, our focus is the method to improve the cell–scaffold interaction and biocompatibility of nanofabrous scaffolds upon cell culturing. Various surface modifications have been used to improve the hydrophilicity and biochemistry. In our studies, the proliferation was promoted by TPU/collagen coaxial electrospun nanofibrous scaffolds. It has known that collagen can promote cell attachment and maintain characteristic morphology and viability [36]. The reason may be that collagen is the basic component of extracelluer matrix and is well known that have distinctive biological properties, including good biocompatibility and biodegradability. Thermoplastic polyurethane has good mechanical property and makes the scaffold maintain its nanofibrous shape. But the proliferation was not promoted by adding more collagen. It might be explained that pure collagen nanofibers became swollen into hydrogels if it was put into culture medium for too long time, and the mechanical strength got poor. So it couldnot maintain the nanofibrous shape which is suitable for cell proliferation. Thus, compared with pure collagen and TPU, coaxial electrospun nanofibers could provide better growth condition for cell proliferation. In our studies, we have successfully fabricated hybridization of synthetic and natural materials in the form of a core–shell structure. With synthetic polymer (better mechanical performance) as the core and structural component and natural biomacromolecules presented on the sheath for functional purposes, such nanofibers are advantageous for those where biocompatibility and mechanical properties are equally important. The cell viability experiment results have shown that coaxial electrospinning is an efficient surface coating of bioactive molecules with synthetic nanofibers.

4. Conclusions

In this study, the HFP was found as an appropriate solvent for collagen/TPU coaxial electrospinning. The preparation of this compound core–shell structured nanofiber is novel and to our knowledge has never been done before. The surface morphology and microstructure of the resulting composite nanofibers were characterized through SEM, TEM and AFM. The findings indicated that diameters of spun nanofibers were influenced by core concentration of TPU solution and the proper core solution was found to be an important parameter affecting the fiber morphology in the shell–core structure. With purpose to improve the stability of TPU/collagen coaxial electrospun nanofibers *in vitro* and *in vivo*, GTA vapor was introduced to crosslink the membrane and appropriate process time was defined as 48 h. Collagen content retained in the blend nanofibers was verified by XPS and FTIR test. Feasibility of polyurethane/collagen core–shell construct as an optimal TE scaffold materials was supported by its high porosity and adequate pore size. The mechanical behavior of the nanofibers membranes was also investigated and the collagen/TPU coaxial complex offers a potential tissue engineering scaffold and a promising functional biomaterial. PIECs proliferation *in vitro* demonstrated the feasibiltiy and efficacy of using core–shell composite nanofibers for improving cell–scaffold interactions. Currently we are going to investigate this concept further to explore the use of this method for constructing of complex sets of cellular interactions as biomimetic nanofibers in engineering tissues.

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