Front. Mater. Sci. China 2009, 3(4): 359–366
DOI 10.1007/s11706-009-0067-3

RESEARCH ARTICLE

# A novel approach via combination of electrospinning and FDM for tri-leaflet heart valve scaffold fabrication

**Rui CHEN**[1], **Yosry MORSI**[2], **Shital PATEL**[2], **Qin-fei KE**[1], **Xiu-mei MO** (✉)[3]

1 College of Textiles, Donghua University, Shanghai 201620, China
2 Faculty of Engineering and Industry Science, Swinburne University of Technology, Melbourne, Victoria, 3122, Australia
3 College of Chemistry and Chemical Engineering and Biological Engineering, Donghua University, Shanghai 201620, China

**Abstract** In this paper, a novel combination method of electrospinning and rapid prototyping (RP) fused deposition modeling (FDM) is proposed for the fabrication of a tissue engineering heart valve (TEHV) scaffold. The scaffold preparation consisted of two steps: tri-leaflet scaffold fabrication and heart valve ring fabrication. With the purpose of mimicking the anisotropic mechanical properties of the natural heart valve leaflet, electrospun thermoplastic polyurethane (ES-TPU) was introduced as the tri-leaflet scaffold material. ES-TPU scaffolds can be fabricated to have a well-aligned fiber network, which is important for applications involving mechanically anisotropic soft tissues. We developed ES-TPU scaffolds as heart valve leaflet materials under variable speed conditions and measured fiber alignment by fast Fourier transform (FFT). By using FFT to assign relative alignment values to an electrospun matrix, it is possible to systematically evaluate how different processing variables affect the structure and material properties of a scaffold. TPU was suspended at certain concentrations and electrospun from 1,1,1,3,3,3-hexafluoro-2-propanol onto rotating mandrels (200–3000 rpm). The scaffold morphological property and mechanical anisotropic property are discussed in the paper as a function of fiber diameter and mandrel RPM. The induction of varying degrees of anisotropy imparted distinctive material properties to the electrospun scaffolds. A dynamic optimum design of the heart valve ring graft was constructed by FDM. Fabrication of a 3D heart valve ring was constructed using pro-engineer based on optimum hemodynamic analysis and was converted to an STL file format. The model was then created from PCL which was sewed and glued with electrospun nanofibrous leaflets. This proposed method was proven as a promising fabrication process in fabricating a specially designed graft with the correct physical and mechanical properties.

**Keywords** tissue engineering heart valve, fused deposition modeling, electrospinning, thermoplastic polyurethane, nanofiber

## 1 Introduction

Heart valve disease, a significant cause of mortality worldwide, occurs when the heart is unable to pump out an adequate volume of blood. Blood backs up, engorges the veins in the lungs and other parts of the body, and causes a congestion of fluid in body tissues. Heart disease can affect any of the valves in the heart, but is most prevalent in the aortic heart valve [1]. In 2008, the American Heart Association Statistics Committee gave their report that in the United States alone, heart valve disease was the sole cause of mortality in 20260 deaths, and of these deaths aortic valve disease was responsible for 12265 [2], and there is an urgent need for artificial heart valve replacement. Now the most common and efficient treatment for valvular disease is the implantation of a prosthetic valve. However, until now, a non-thrombogenic, non-calcifying prosthetic which maintains normal valve mechanical properties, hemodynamic flow and exhibits sufficient anti-fatigue properties has not been designed. Currently, adults who undergo replacement of diseased valves by either mechanical prosthetic or biological valves (including the porcine aortic valve and bovine pericardial xenograft, cadaveric allograft, or pulmonary-aortic autograft) generally have enhanced survival and quality of life [3]. Nevertheless, each of these valves has its limitations. Many researchers are therefore exploring tissue engineering (TE) strategies towards the development of a heart valve equivalent.

Received August 21, 2009; accepted September 16, 2009

E-mail: xmm@dhu.edu.cn

The technology of TE aims to generate new tissue or substitute for malfunctioning ones and could well become an alternative method to whole organ transplantation [4,5]. It has been agreed that an optimal tissue engineering heart valve (TEHV) would consist of insert materials covered with a cellular coating, developed for the purpose of improving the valve performance [6]. TEHV have potential growth and anti-thrombogenic factors, suitable scaffold materials with adequate mechanical properties and an optimized degradation rate to provide a medium in which the interstitial cells and endothelial cells can grow.

One of the most important issues to be considered in TEHV is the development of the scaffold fabrication technique that mass-produces the biodegradable scaffold capable of standing the complex, mechanical and dynamic environmental conditions necessarily to stimulate cell growth. Micro-porous synthetic extracellular matrixs (ECMs) can regulate the organization of cells seeded into the matrix and the subsequent proliferation of the cells to form new tissues. A variety of processing techniques are available to fabricate synthetic ECMs from synthetic polymers, and various biodegradable polymers have been processed into a variety of configurations including fibers, porous sponges and tubular structures [7].

Fused deposition modeling (FDM) uses a moving nozzle to extrude a fiber of polymeric material (x- and y-axis control) from which the physical model is built layer-by-layer. The model is lowered (z-axis control) and the procedure repeated. Although the fiber must also produce external structures to support overhanging or unconnected features that need to be manually removed, the pore sizes in tissue engineering scaffolds are sufficiently small enough for the fiber strand to bridge across without additional support structures. The FDM process was found to provide good control and reproducibility of the desired degree of porosity and 3D microstructure. FDM also offers flexibility and ease of varying the microstructure to meet specific structural and functional requirements for the scaffold structure. In combination with computed tomography (CT) measurement techniques, FDM makes it possible to manufacture a custom prosthesis that precisely fits the patient. Morsi et al. used the process for developing a 3D scaffold of the TE of the heart valve [8]. However, the FDM operates at high temperatures (120°C), which eliminates the incorporation of biological molecules into the process, as high temperature induces protein inactivation and low yield. It is recognized that there is a narrow processing parameter window for the application of biodegradable polymers with FDM [9,10].

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 the kind of polymer, viscosity, surface tension, jet charge density, temperature and humidity control the electrospinning process, especially the diameter and morphology of the resulting fibers [11]. Recently, researchers have found that the nanofibrous structure formed via the electrospinning method improves the function in in vitro tissue regeneration and decrease the formation of scar tissue [12]. Thus, the scaffolds prepared from the electrospinning method can be considered to mimic the native ECM.

As far as we know, many kinds of polymer and biological materials have been electrospun for biomedical scaffolds, but the mechanical property cannot meet the request of native ECM. Thermoplastic polyurethanes (TPUs) are a widely used class of polymers 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 [13]. Conventional TPUs are among the 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 [14]. The TPU used in this research is a kind of medical-grade, aliphatic, polyether-based TPU. It is biodegradable and the bio-stability is better than poly (ester urethane).

The aim of this research is to present a novel methodology for the application of fused deposition modeling rapid prototyping technology and electrospinning nanofabrication technology for scaffold fabrication of tri-leaflet heart valves. The morphology, fiber diameter distribution, fiber alignment and mechanical properties are carefully characterized for future valve grafts applications.

## 2 Materials and method

### 2.1 Designing the solid model for the heart valve ring

A 3D representation of the heart valve ring model was constructed using pro-engineer, based on hemodynamic analysis and converted to an STL file format which was imported to Magics software. In Magics software the 3D volume of the heart valve ring was edited and the resulting distortions and errors due to partial volume effects were corrected. Furthermore, morphology operations, Boolean operations, and cavity fill were used to generate a high resolution contour suitable for the application in hand.

### 2.2 Rapid processing

In this study, the heart valve ring was produced by the state of the art FDM machine (specially designed by Fochif Company, Shanghai), with PCL ($M_n$= 80000, Sigma-Aldrich) grain material. Insight software was used to prepare the model for fabrication. The model orientation was chosen and a model support which holds the model together during the building process was created. The determination of the optimal part’s orientation is essential for all layered manufacturing (LM) processes. However, the way in which a part's orientation affects the manufactured part is process-dependent. Supports can be internal or external. External supports are needed to support overhanging features, and internal supports are essential in order to support the top surfaces of hollow parts. Depending on the machine specifications, a slice algorithm was invoked in order to break the 3D model into a series of slices with finite thickness. The task of slicing involves intersecting a CAD model (or the associated STL file) with a horizontal plane. Slicing transforms the process planning tasks from the model to the layer domains. While the computation of layer thicknesses requires information about the geometry of the whole CAD model, the output from the slicing procedure is the layer thickness values of the individual slices for the manufacture of the complete CAD model.

Subsequently, a tool-path plan which determines the motion of the extruder head was also generated for each layer. Path planning is a pure layer domain task. Path planning can be thought of as consisting of two components controlled by interior and exterior path planning. While determining the geometric path and the process parameters associated with the path is solved in interior path planning, exterior path planning controls the accuracy of the external geometry of the manufactured layer. For most processes, there is a sequence in which material is deposited in order to form the inside of the layer. Interior path planning is therefore required for nearly all LM processes.

### 2.3 Materials used in electrospinning

Thermoplastic polyurethane (Tecoflex EG-80A) was purchased from Noveon and used 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) as solvent, which was brought from Daikin Industries Ltd. (Japan).

### 2.4 Electrospinning fabrication methodology

TPU was dissolved with HFIP at a concentration of 6%. When TPU was dissolved completely, it was fed into a plastic syringe with a needle (inner diameter, 0.21 mm). A syringe pump (789100C, cole-pamer, USA) was used to feed the solution to a needle with a feed-rate of 1.5 mL/h. Electrospinning voltage was applied to the needle at 15 kV using a high-voltage power supplier (BGG6-358, BMEI Co. Ltd., China). A mandrel was used to collect nanofibers at fixed distance (18 mm from the needle tip).

As to fabrication of the electrospun nanofibrous leaflet scaffold with anisotropic mechanical properties, a grounded mandrel ($D = 6$ cm) was chosen instead of foil to collect nanofibers and fabricate the porous tubular scaffold. The length and thickness of the tubular scaffold can be determined by the length of the mandrel and electrospinning time. The basic experimental schematic illustration used is shown in Fig. 1. Aligned electrospun polyurethane nanofibers were formed onto the target from 200 to 3000 rpm/min. Scaffolds were allowed to dry overnight at room temperature and then placed under vacuum for 48 h at 30°C. Then the tubular matrices were cut as flat membranes and prepared for leaflet fabrication.

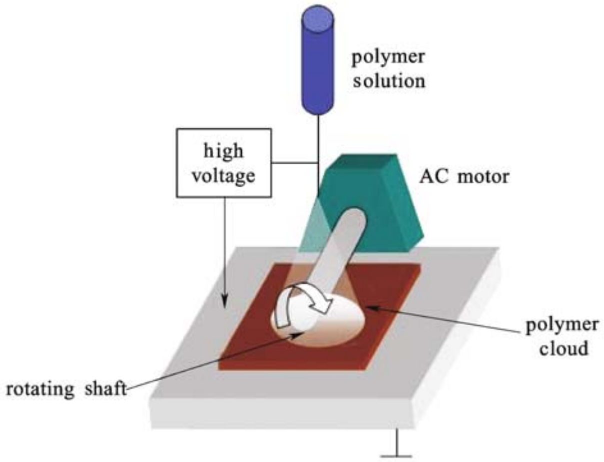

**Fig. 1** Collecting electrospun fibers on a rotating mandrel

### 2.5 Combination of fabrication techniques

In this paper we used FDM for exact fabrication of the hemodynamically optimized heart valve ring mold made from PCL. Then the electrospun material with aligned nanofibers were cut and glued with the heart valve ring. The combination of the heart valve ring and electrospun materials is shown in Fig. 2.

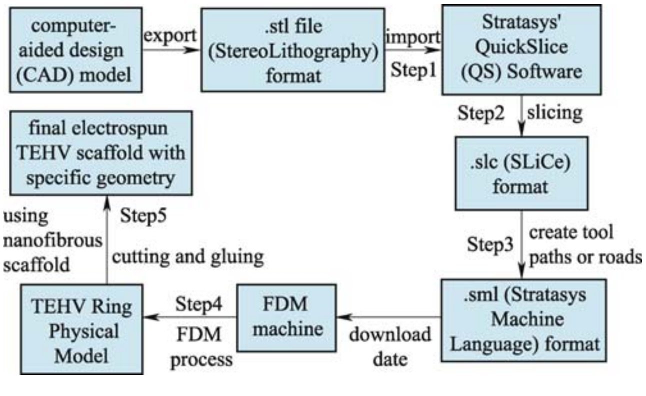

**Fig. 2** Summary of basic FDM process combined with electrospinning process.

Step 1: Import CAD data in STL (STereoLithography) format into QuickSlicet;

Step 2: Slicing of the CAD model into horizontal layers and conversion into a .slc (SLiCe) format;

Step 3: Creation of the deposition path for each layer and conversion into a .sml (Stratasys Machine Language) format for downloading to the FDM machine;

Step 4: FDM fabrication process using a filament modeling material to build the actual heart valve ring physical part in an additive manner layer-by-layer;

Step 5: Combination of heart valve ring and electrospinning materials.

### 2.6 Electrospun nanofibers characterization

The valvular scaffold was carefully removed off the stainless collector. The morphology of the cross section was observed using SEM (JEOL, JSM-5600, Japan) at an accelerated voltage of 15 kV.

As to the investigation of the relationship between fiber diameter and rotary speed, a mandrel ($D = 2$ cm) rotating at high speed varying from 200 to 3000 rpm was used to produce nanofibers with controllable orientation. After 30–60 min, electrospun nanofiber mats were taken off from the mandrel and sputter coated with gold and observed with SEM at a voltage of 15 kV. Measuring of fiber alignment was achieved using a 2D fast Fourier transform approach. Grayscale 8-bit images were cropped to 2048×2048 pixels for analysis. Image J software (NIH, USA) supported by an oval profile plug-in was used to conduct 2D FFT analysis. Pixel intensities were summed up along a radius from the center to the edge of the image to quantify the relative contribution of objects oriented in that direction. All alignment data were normalized to a baseline value of 0 and plotted in arbitrary units from 0 to approximately 0.25.

The specimens of polyurethane nanofiber films for mechanical tests were obtained through cutting a thin nanofiber tube which was collected by a rotating mandrel with a diameter of 6.0 cm. Mechanical measurements were achieved by applying tensile test loads to these specimens. In this study, three specimens were prepared according to the method described by Huang et al. for each proportion [15]. First, a white paper was cut into templates with width × gauge length = 10 mm × 50 mm, and double-sided tapes were glued onto the top and bottom areas of one side. Secondly, the aluminum foil was carefully peeled off and single-sided tapes were applied onto the gripping areas as end-tabs. The resulting specimens had a planar dimension of width × gauge length = 10 mm × 30 mm. Mechanical properties were tested by a materials testing machine (H5K-S, Hounsfield, England) at a temperature of 20°C and a relative humidity of 65% and an elongation speed of 10 mm/min.

### 2.7 Statistical analysis

Values (at least triplicate) were averaged and expressed as means±standard deviation (SD). Each experiment was made for two or three times.

## 3 Results and discussion

### 3.1 Morphology of the leaflet scaffold

Electrospinning the polymer solution onto a stationary or rotating mandrel at varying velocities yielded scaffolds that exhibited both structurally isotropic and highly anisotropic fiber networks. From Fig. 3(a) and (b), it can be seen that the random specimens and those electrospun onto a mandrel with low tangential velocities (in the range of 200–2000 rpm) exhibited fairly isotropic networks, with no discernible difference between the flat sheets. Aligned fiber networks developed when the mandrel velocity equaled 3000 rpm/min or greater, with a very noticeable increase in alignment as the mandrel velocity was further increased (Fig. 3(c)).

Nanofiber diameters were calculated from the diameters of 100 nanofibers, each sample of which was directly measured from SEM photographs. From the result of the aligned electrospun polyurethane nanofibers, we could get the conclusion that from the rotating speed ranging from 200–2000 rpm the fiber diameters were decreased with the rotating speed increasing. On the other hand, the electrospun polyurethane diameters were increased, with the rotating speed exceeding 2000 rpm. Thus, the rotating speed of 2000 rpm was the critical point for the fiber diameter distribution.

### 3.2 Measurement of fiber alignment

It is difficult to measure stress, strain and alignment and to describe the relationships among these parameters in a small diameter tubular fibrous scaffold for their successful application in tissue engineering. In this work, a large diameter mandrel instead of a small one was used to collect nanofibers at different mandrel speeds. Then the membranes constructed with these fibers were measured for alignment degree and mechanical properties.

The FFT was used to characterize fiber alignment as a function method for eletrospinning. Figure 4(a)–(c) shows the raw output of the 2D FFT alignment analysis of aligned nanofibers. Under the conditions used in this experiment, the surface of the mandrel was moving at a velocity of approximately from 0.223 to 3.142 m/s. Pixel intensities and the distribution of the intensities of this output image correlate to the directional content of the original image. The slender profiles of the silhouette in the raw output of the 2D FFT indicate fiber alignment. Figure 4(d)–(f) shows radial plots of the summations of relative pixel intensity at a radius versus the angle (°) caused by the inherent symmetry of the raw 2D FFT output. From the results, the peak numerical values increased with the rotary speed. A taller, narrower peak at 90° and 270° indicates the general direction in which the fiber population is oriented.

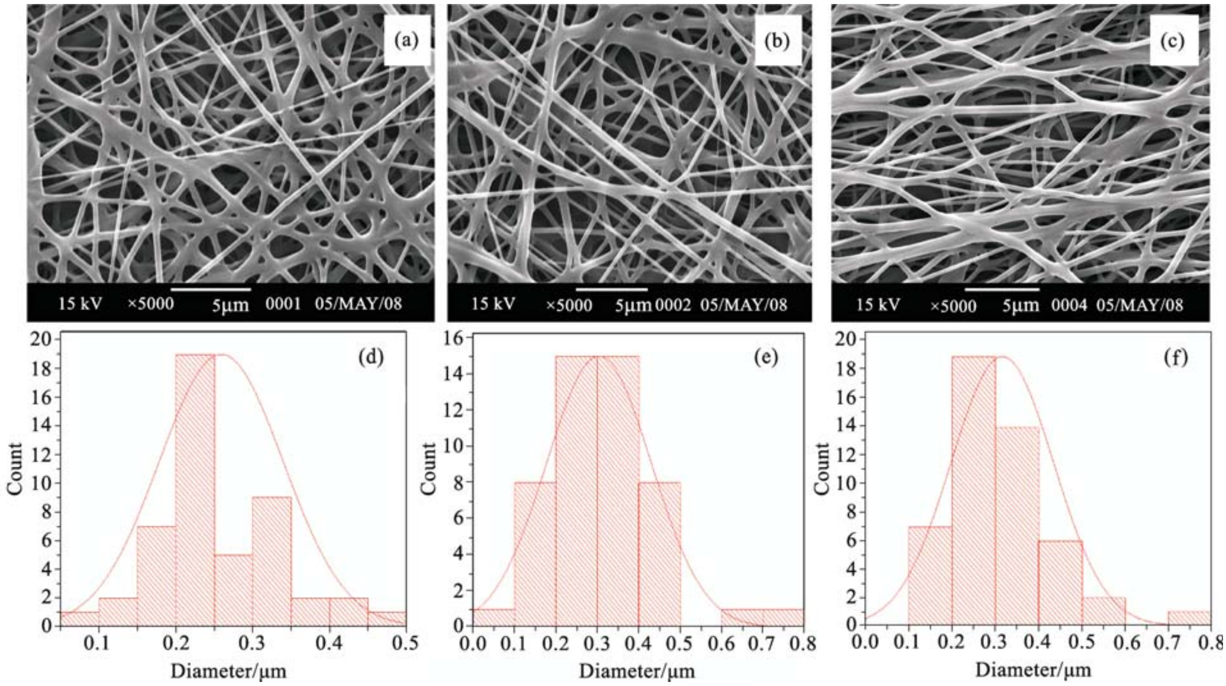

**Fig. 3** SEM images of electrospun polyurethane nanofibers with **(a)** rotating speed of 200 rpm and **(d)** fiber diameter distribution; **(b)** rotating speed of 2000 rpm and **(e)** fiber diameter distribution; **(c)** rotating speed of 3000 rpm and **(f)** fiber diameter distribution

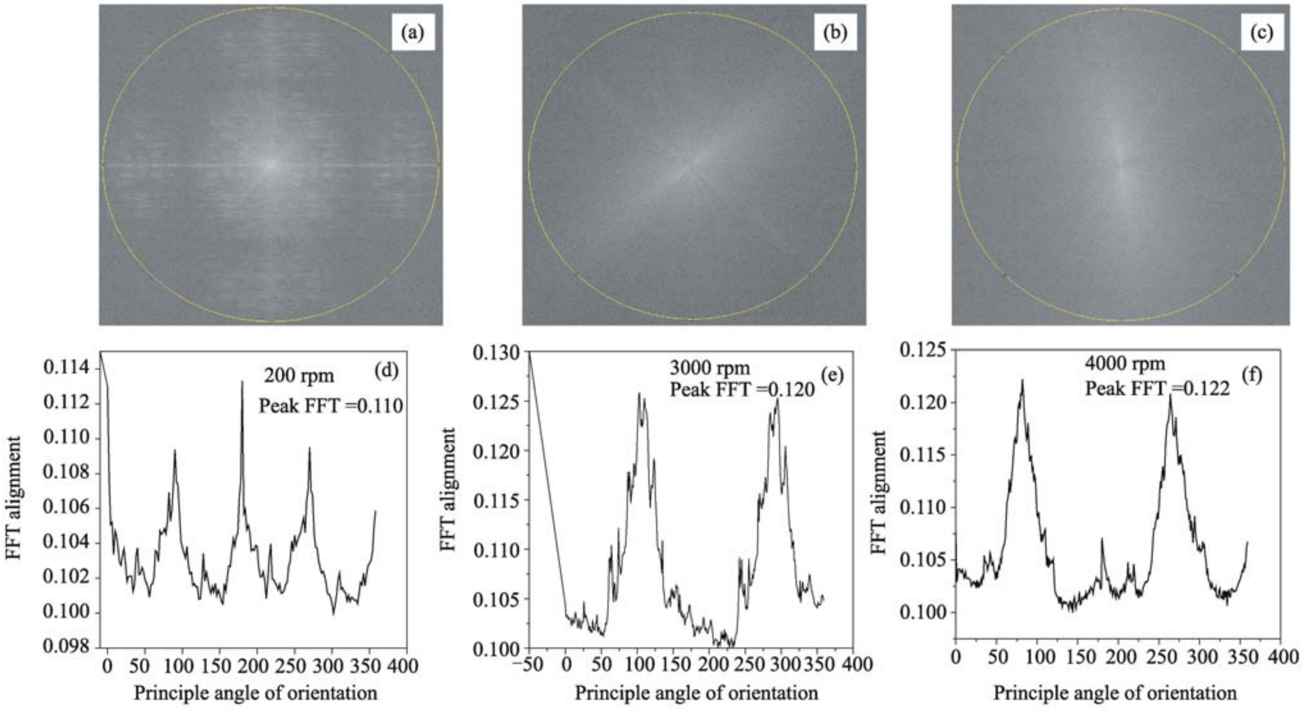

**Fig. 4** Alignment degree of electrospun fibers collected by a rotating mandrel with different rotary speeds: **(a)** 200 r/min, **(b)** 2000 r/min and **(c)** 3000 r/min shows the raw output of the 2D FFT alignment analysis of electrospun nanofibers; **(d)(e)(f)** radial plot of the summations of relative pixel intensity

### 3.3 Mechanical properties

To simulate the anisotropic mechanical behaviors of natural soft tissues, the mechanical properties of aligned nanofibers are important for their successful application in soft tissue engineering. TPU nanofibrous mats with different rotating speeds were electrospun into 0.5 mm thick fiber mats to measure their mechanical properties. Figure 5 shows the tensile stress-strain curve of TPU nanofiber mats with various rotary speeds (from 200 to 3000 rpm). The electrospun TPU material at a low rotary speed (below 1000 rpm) gives a characteristic response for elastomeric materials sigmoidal in shape. It shows a very soft and flexible characteristic, with a low Young's modulus and a high elongation at a break of 365%. With the increase in rotary speed, the initial modulus of the mats become large. This phenomenon indirectly implies that rotary speed is an important process parameter and it can impart anisotropic mechanical properties to scaffolds. From the stress-strain typical curves, we can see that with rotary speeds increasing, the materials' mechanical properties change from elastomeric to plastic.

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 valves and muscles. Different natural tissues have different mechanical properties. For example, heart valves of humans and porcines have different mechanical tensile stresses and ultimate strains along the circular and axial directions. From the analysis, we can adjust the scaffold mechanical property to meet the requirement in practice through changing the rotary speeds. The mechanical behaviors also demonstrated that the high velocity spun scaffolds exhibited high anisotropic mechanical properties closely resembling the native aortic heart valve tri-leaflet.

### 3.4 TEHV scaffold combination with FDM and electrospinning

The tubular scaffold prepared for leaflet fabrication was carefully cut according to the geometry of the tri-leaflet and glued with the FDM heart valve ring. It was then dried under vacuum. Figure 6 shows that the heart valve ring was fabricated by FDM, and Fig. 6 shows electrospun fibrous leaflet materials compounded with the FDM ring. As shown in Fig. 6, the whole scaffold showed good

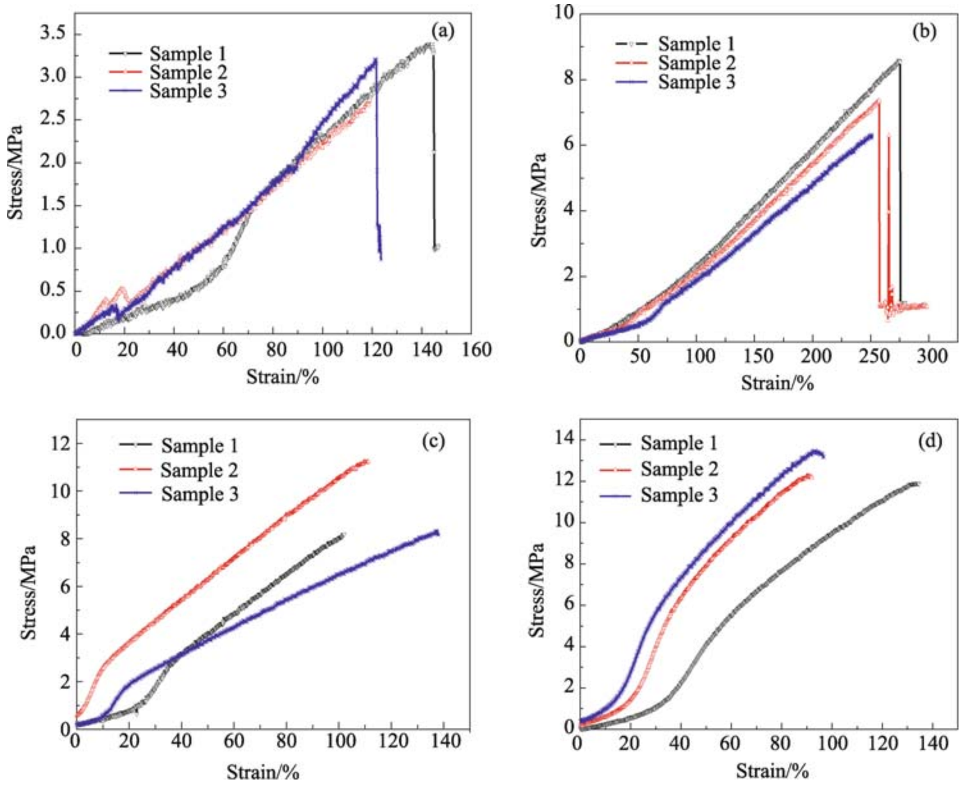

**Fig. 5** Axial mechanical properties of electrospun TPU with different rotary speeds: **(a)** 200 rpm; **(b)** 1000 rpm; **(c)** 2000 rpm; **(d)** 3000 rpm

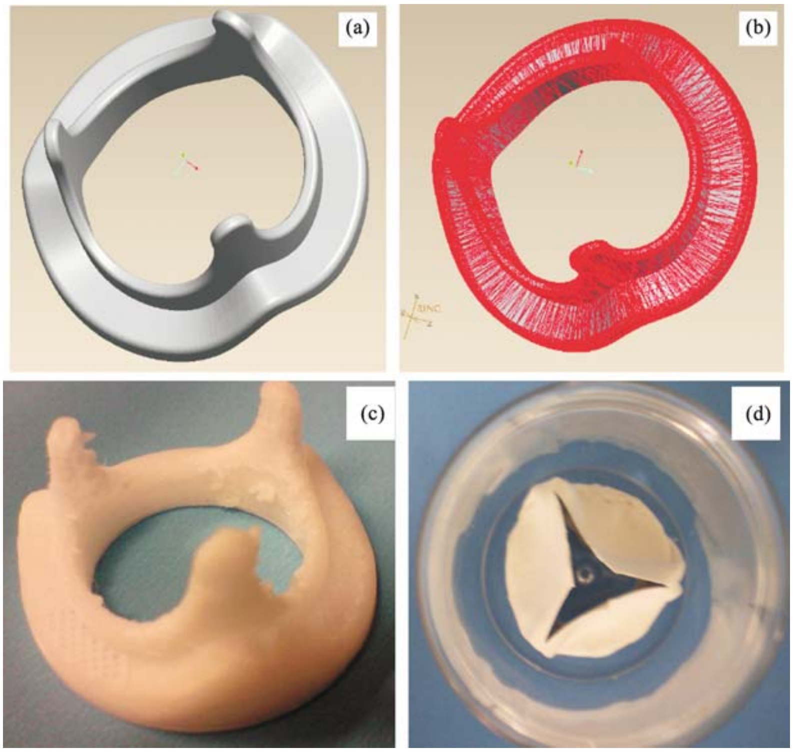

**Fig. 6** TEHV scaffold combination with FDM and electrospinning: **(a)** solid CAD model of heart valve ring; **(b)** STL file of heart vale ring (slicing of the CAD model into horizontal layers); **(c)** heart valve ring using PCL processed by FDM; **(d)** TEHV scaffold prepared by FDM ring and aligned nanofibrous mats

morphology and excellent elasticity. Compared with the primary scaffold, there was no visible structural distortion found after extending the cross section of the scaffold with a medical forceps.

One of the most important disadvantages of FDM is that the flexibility of the scaffold is inadequate. In this research work, with the purpose of improving flow mechanically, we used hemodynamic analysis to design the heart valve ring. The electrospinning materials are soft and have the mechanical ability of flexibility which is suitable for leaflet fabrication. By a combination of FDM and electrospinning technologies, we can prepare novel TEHV scaffolds in possession of good mechanical and hemodynamic properties. Further research will be focused on the fluid properties and endothelials with suitable seeding cells before implantation into the animal model.

## 4 Conclusions

Valve replacement is widely used for the treatment of heart valve disease. However, in some cases, due to limitations of the mechanical or biological xenograft valve, the availability of usable tissue engineering valve grafts are needed. These grafts require a special design to ensure compliance matching, tissue regeneration and anti-thrombogenicity. These requirements present a challenge in the selection of biomaterials and manufacturing into the exact three-dimensional architecture and matching of the resultant mechanical properties with that of the target tissue host. The rapid prototyping (RP) technique is widely used to construct an uncharacteristic model based on CT scan or CAD modeling with complicated features. The electrospinning can be effectively used to optimize the mechanical and physical properties of mimicking natural heart tri-leaflet valve ECMs. This study introduced a combined manufacturing technique, namely fused deposition modeling and electrospinning, to fabricate the specifically designed tri-leaflet scaffold applications. With the purpose of mimicking the anisotropic mechanical properties of the natural leaflet, aligned nanofibrous materials were introduced in the leaflet fabrication. The morphology, fiber alignment and anisotropic mechanical property were evaluated. It was proved that the proposed method can clearly fabricate a specifically designed graft valve scaffold. The next step for this research will be focused on the flow character investigation and the endothelialization in a specially designed bioreactor in vitro before the implantation to the animal model.

**Acknowledgements** This research was supported by the National High Technology Research and Developed Program (863 Program, 2008AA03Z305), Science and Technology Commission of Shanghai

Municipality Program (08520704600, and 0852nm03400), Natural Science Foundation of Shanghai (07ZR14001) and the 111 Project (B07024).

## References

1. Brody S, Pandit A. Approaches to heart valve tissue engineering scaffold design. Journal of Biomedical Materials Research - Part B Applied Biomaterials, 2007, 83(1): 16–43
2. Rosamond W, Flegal K, Furie K, et al. Heart disease and stroke statistics – 2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation, 2008, 117(4)
3. Kidane A G, Burriesci G, Cornejo P, et al. Current developments and future prospects for heart valve replacement therapy. Journal of Biomedical Materials Research - Part B Applied Biomaterials, 2009, 88(1): 290–303
4. Langer R, Vacanti J P. Tissue engineering. Science, 1993, 260 (5110): 920–926
5. Weinand C, Pomerantseva I, Neville C M, et al. Hydrogel-beta-TCP scaffolds and stem cells for tissue engineering bone. Bone, 2006, 38 (4): 555–563
6. Takagi K, Fukunaga S, Nishi A, et al. *In vivo* recellularization of plain decellularized xenografts with specific cell characterization in the systemic circulation: Histological and immunohistochemical study. Artificial Organs, 2006, 30(4): 233–241
7. Martina M, Subramanyam G, Weaver J C, et al. Developing macroporous bicontinuous materials as scaffolds for tissue engineering. Biomaterials, 2005, 26(28): 5609–5616
8. Masood S H, Singh J P, Morsi Y. The design and manufacturing of porous scaffolds for tissue engineering using rapid prototyping. International Journal of Advanced Manufacturing Technology, 2005, 27(3–4): 415–420
9. Landers R, Hübner U, Schmelzeisen R, et al. Rapid prototyping of scaffolds derived from thermoreversible hydrogels and tailored for applications in tissue engineering. Biomaterials, 2002, 23(23): 4437–4447
10. Landers R, Pfister A, Hübner U, et al. Fabrication of soft tissue engineering scaffolds by means of rapid prototyping techniques. Journal of Materials Science, 2002, 37(15): 3107–3116
11. Zong X H, Kim K, Fang D F, et al. Structure and process relationship of electrospun bioabsorbable nanofiber membranes. Polymer, 2002, 43(16): 4403–4412
12. Webster T J, Waid M C, McKenzie J L, et al. Nano-biotechnology: carbon nanofibres as improved neural and orthopaedic implants. Nanotechnology, 2004, 15(1): 48–54
13. Pedicini A, Farris R J. Mechanical behavior of electrospun polyurethane. Polymer, 2003, 44(22): 6857–6862
14. Tatai L, Moore T G, Adhikari R, et al. Thermoplastic biodegradable polyurethanes: The effect of chain extender structure on properties and *in-vitro*. Biomaterials, 2007, 28: 5407–5417
15. Huang Z M, Zhang Y Z, Ramakrishna S, et al. Electrospinning and mechanical characterization of gelatin nanofibers. Polymer, 2004, 45(15): 5361–5368