Artery vessel fabrication using the combined fused deposition modeling and electrospinning techniques

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Abstract

Purpose – The purpose of this paper is to prepare a new combined method of rapid prototyping, fused deposition modeling (FDM) and electrospinning for the fabrication of coronary artery bypass graft (CABG).

Design/methodology/approach – A dynamically optimum design of blood vessel graft was constructed using FDM and electrospinning. Fabrication of 3-D CABG model was constructed using pro-engineer based on the optimum hemodynamic analysis and was converted to an stereolithography file format which was imported to the Magic software where it was edited to a high-resolution contour. The model was then created from acrylonitrile butadiene styrene which was used as a collector for electrospinning fabrication. For the electrospinning thermoplastic polyurethane was dissolved with hexafluoroisopropanol. The voltage applied for electrospinning was 15 kV where the solid FDM model was used to collect nanofibers at fixed distance.

Findings – The properties of the fabricated vessel agreed well with those of human artery. The proposed method can be effectively used for the fabrication of an optimized graft design. This proposed method has been proved as a promising fabrication processes in fabricating a specially designed graft with the correct physical and mechanical properties.

Originality/value – The proposed method is novel and combines the advantages of both FDM and electrospinning techniques.

Keywords Polyurethane, Blood vessels, Heart, Rapid prototypes, Nanotechnology

Paper type Research paper

1. Introduction

Coronary bypass grafting is an invasive surgery in which the surgeon creates new routes (bypass) around narrowed and blocked arteries, allowing sufficient blood flow to deliver oxygen and nutrients to the heart muscle. In the majority of the cases, coronary artery bypass graft (CABG) is implemented using autogenous vein or the internal mammary artery. However, in some patients, autogenous vessel may not be available for use due to quality or absence due to previous operations. In these cases, prosthetic or artificial grafts are the only option.

Unfortunately, the clinical results for prosthetic graft in CABG so far are poor compared to autogenous vessel and they lack the ability to repair and revascularise and are potentially more thrombogenic than autologous grafts. Moreover, methods to achieve a clinically effective material have frequently involved incorporating biological components in porous biomaterials scaffolds. The selection of the biomaterials is based on biocompatibility, ease of manufacturing into the desired 3-D architecture and matching of the resultant mechanical properties with that of the target tissue. Biomaterials that have been widely tested include natural materials, such as collagen, agarose, silk and fibrin and synthetic polymers, such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL) and their copolymers and polyurethanes (PUs).

Concurrently, design of the scaffold architecture remains a challenging task. To be effective, the scaffold must be capable of regulating morphology and function of adherent cells, without compromising tissue-specific mechanical properties. In the case of anisotropic structural tissues such as blood vessels, achievement of suitable mechanical properties and the design

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and manufacturing of the vessel that totally or partially mimics the physical property of natural tissue is a challenging task. Current fabrication techniques are not sufficiently suitable to control scaffold structure to modulate mechanical properties and surface characteristics, and hence, a new manufacturing technique is needed.

The main advantage of rapid prototyping (RP) is to create an uncharacteristic product with complicated features easily and quickly with a high degree of accuracy. However, selecting a proper RP to fabricate the coronary artery blood vessel can simplify the fabrication process, which depends on the raw materials of vessel and the require properties of tissue mimicking. It is difficult to fabricate the vessel directly if the raw materials need longer curing time for solidification. Furthermore, the operating temperature of the system is too high to incorporate biomolecules into the scaffold, therefore limiting the biomimetic aspects of the scaffold produced. Moreover, the material deposited solidifies into dense filaments, blocking the formation of microporosity. Microporosity is an important factor in encouraging revascularization and cell attachment (Kyeong *et al.*, 2006).

Moreover, one of the most important factors in implant vessel is to allow biological activities such as cell adhesion, migration, growth and differentiation to attain a proper integration between cells and implant for vascularization and endothelialization of the vessel (Zhang *et al.*, 2005). Most of these human organs are deposited on fibrous structures with the fibril/fiber size realigning from nanometer realigning to millimeter scale. So nanofibers have now been extensively used to mimic these natural tissue matrixes.

Currently, electrospinning manufacturing technique 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 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 (Nair *et al.*, 2004). Recently, researchers have found that the nanofibrous structure formed by electrospinning method will improve the *in vitro* tissue regeneration function and decrease the formation of scar tissue (Webster *et al.*, 2004). Therefore, the scaffolds constructed from electrospinning technique can be tailored to totally or partially mimicking the native extracellular matrix (ECM) and the physical properties of the vessel. To date, representative polymers including synthetic ones such as PLA (Kim *et al.*, 2003; Yang *et al.*, 2005), PGA (Boland *et al.*, 2001), poly(lactic-co-glycolic acid) (Ayutsede *et al.*, 2005), PCL (Li *et al.*, 2005; Yamane *et al.*, 2005) and natural ones such as collagen (Matthews *et al.*, 2002), chitosan (Desai *et al.*, 2008), gelatin (Huang *et al.*, 2004) and silk (Min *et al.*, 2004) have been electrospun into nanofibers.

Thus, far, several vascular scaffolds have been developed based on a variety of hydrolytical polymers. Hong *et al.* (2009), for example, investigated poly(ester urethane) urea and phospholipids polymer blend for small diameter, fibrous vascular conduit. Kim *et al.* (2003) used electrospun PCL nanofibers with anisotropic mechanical properties as vascular scaffold. Tillman *et al.* (2009) employed PCL-collagen compound scaffolds for vascular reconstruction. Yin *et al.* (2009) used silk fibroin/gelatin blend nanofibers for biomedical scaffold application.

However, despite the huge amount of research carried out in electrospinning of various kinds of polymers and biological materials for biomedical scaffold, there is still a need to optimize the mechanical and physical properties of the vessel to mimic the 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 best implants, catheters and prosthetic heart valve leaflets (Pedicini and Farris, 2003). 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 PU (Tatai *et al.*, 2007). The TPU used in this research is of medical grade, aliphatic and polyether based which can degrade and its biostability is better than poly(ester urethane). In this paper, an indirect fabrication method based on the combined RP technology and electrospinning is proposed for manufacturing blood vessel suitable for coronary artery bypass. The 3-D graft computer-aided design (CAD) model with specific features is designed and fabricated by RP and is used as a collector for electrospinning technique.

2. Design the solid model

Reconstruction of a 3-D representation of CABG model constructed using pro-engineer based on the hemodynamic analysis was converted to a stereolithography (STL) file format which was imported to Magics (2004) software. In Magics software, the 3-D volume of CABG was edited; 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.

3. RP process

3.1 Fused deposition modeling

Fused deposition processing builds a 3-D object layer by layer from a CAD design. Filaments of 200 μm nominal diameter were fed into a liquefier head via computer-driven rollers. The fused deposition modeling (FDM) machine has a second nozzle that extrudes support material and builds support for any structure that has an overhang angle of less than 45° from horizontal as a default. The material extruded out of the heated liquefier head in the FDM system is deposited in the form of a fine bead of material, referred to as a "road".

The process of deposition in each layer starts with a road of material of defined width and thickness being deposited to define the perimeter(s) or boundary (ies) of the given part layer. Once the perimeters are defined, the internal portion of the layer is filled by roads of defined width and thickness. The raster fill approach is used most frequently due to its speed and the ability to change the direction of raster motion in adjacent layers. Typically, alternate layers are built with raster directions at 90° to one another. Such a strategy results in maximum packing of material and a minimum of voids between roads and layers.

3.2 Rapid processing in this research

Insight software is 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. Note that the determination of the optimal part orientation is essential for all layered manufacturing (LM) processes. 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. 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. Interior path planning is therefore required for nearly all LM processes. The tool-path information was then converted into machine codes for physical model fabrication, as shown in Figures 1 and 2.

Figure 3 shows simplified cross-sections and measurements used in the calculation of the length of the anastomoses for a typical angle of 40°. In this model, the cross-section of the graft changes gradually along its length from a circular to an elliptical shape, where the elliptical intersection between the graft and host artery is further compressed to fit the smaller artery at the anastomosis based on the assumption of Wijesinghe *et al.* (1998),

Figure 1 Support and slices in insight

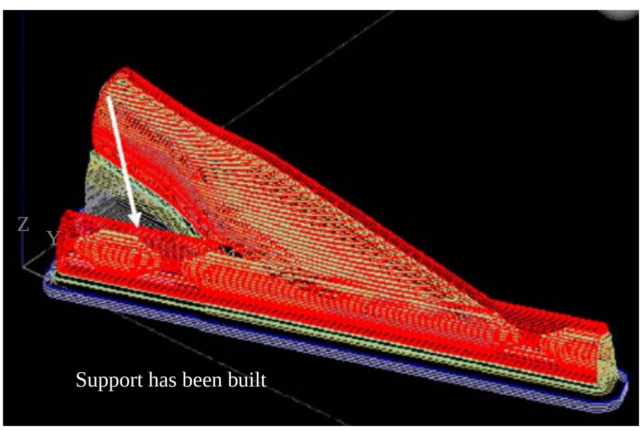


Figure 2 Tool paths are being checked for validity

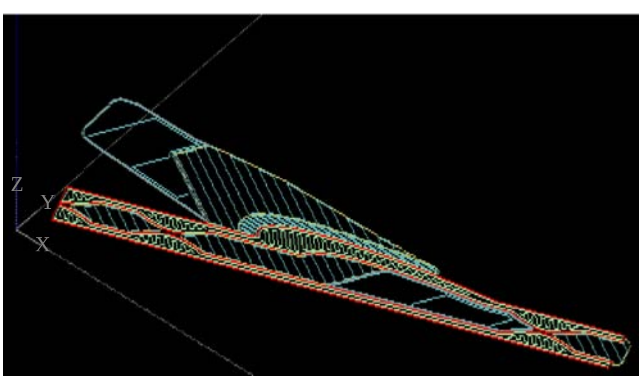
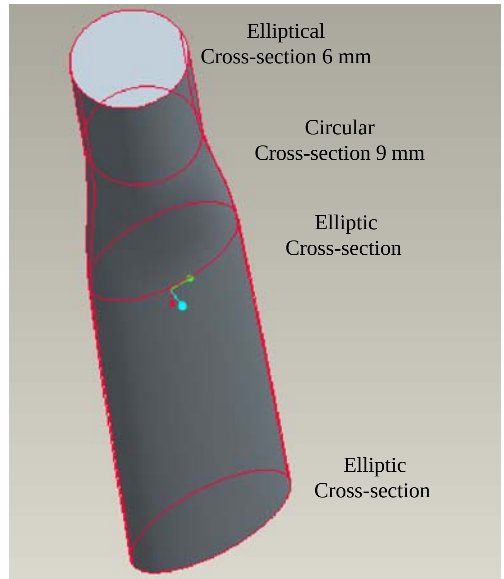


Figure 3 The solid model of the bypass graft with four of its cross-sections and the altering surface profile along its length



so that the cross-section area of the ellipse has to be the same as the area of the circular cross-section.

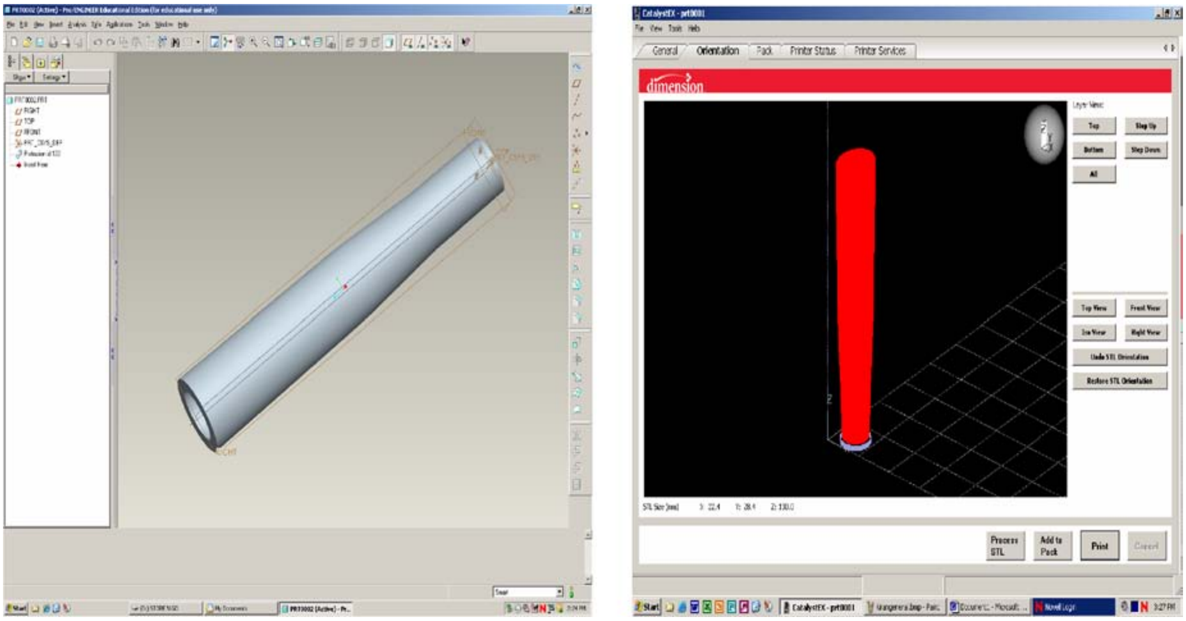
With the calculated parameters, the graft was modeled first with defined geometry comprised of four sections, as shown in Figure 3. Two sections are two circles with the same diameter, and the other two sections are ellipses. All sections must have the same area. The distances between the sections were varied graft by graft to make the transition of the surface as smooth and natural as possible at the site of the squeeze. In this technique, the host artery was generated to cut naturally through the graft at one end, where there is an elliptic cross-section. In this way, the grafting junction does not show an elliptic suture line (Figure 4) which allows the maximum compliance between the host and the graft in the way that both the host artery and the graft are not affected much by local high stress concentration by deformation. In addition, it permits the maximum flow through the junction. These two factors are expected to help reduce the problems of intimal thickening and restenosis at the junction.

3.3 Electrospinning fabrication methodology

PU was dissolved with hexafluoroisopropanol at a concentration of 6 percent. When PU has been dissolved completely, it was fed into a plastic syringe with a needle (inner diameter, 0.21 mm). A syringe pump (789100C, Cole-Pamer, Vernon Hills, IL) was used to feed the solution to 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 can be used to collected nanofibers at a fixed distance (18 mm from the needle tip).

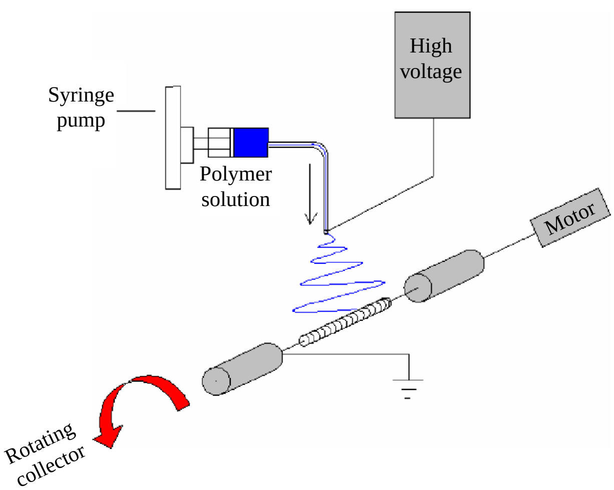
In order to fabricate small-diameter tubular scaffold, a grounded mandrel FDM model was chosen instead of foil to collect the nanofibers and fabricate the porous tubular scaffold. The length and thickness of tubular scaffold are determined by the length of mandrel and electrospinning time. The basic experimental schematic illustration used is shown in Figure 5. Aligned electrospun PU nanofibers were formed onto the target from 200 to 2,000 rpm. Scaffolds were allowed to dry overnight

Figure 4 (a) model of the bypass graft after intersected by the host vessel shows different sections along the graft and a non-standard suture line; (b) export options for STL files



(a) (b)

Figure 5 Collecting electrospun fibers on a rotating mandrel



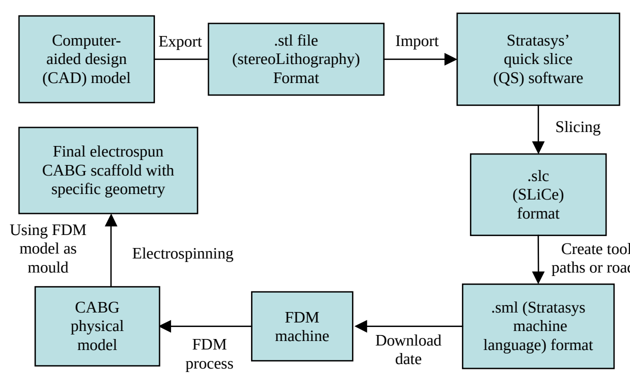
Sources: Feng *et al*. (2009); Chen *et al*. (2009)

at room temperature and then placed under vacuum for 48 h at 30°C.

4. Combination of fabrication techniques

In this paper, we used FDM for exact fabrication of the hemodynamically optimized artery graft to make exact CABG mould made from acrylonitrile butadiene styrene as the electrospinning ground collector. Normally, either sheet or cylinder is used as the ground cylinder for electrospinning. However, here the mould was fabricated by FDM technique so that the exact CABG geometry scaffold was used. Figure 6 shows the combined process. The combination manufacturing step consisted of 5 steps which were given in detail below. Steps 1-4 were the manufacturing process of rotation collector of bypass based on FDM. Steps 5 and 6 are the tubular nanofibrous scaffold manufacturing process based on electrospinning. Using electrospinning and rotary collecting method, we can get aligned nanofibrous tubular scaffold with exact optimum geometry. This is most the significant novelty in this research.

Figure 6 Summary of basic FDM process combined with electrospinning process



Manufacturing steps:

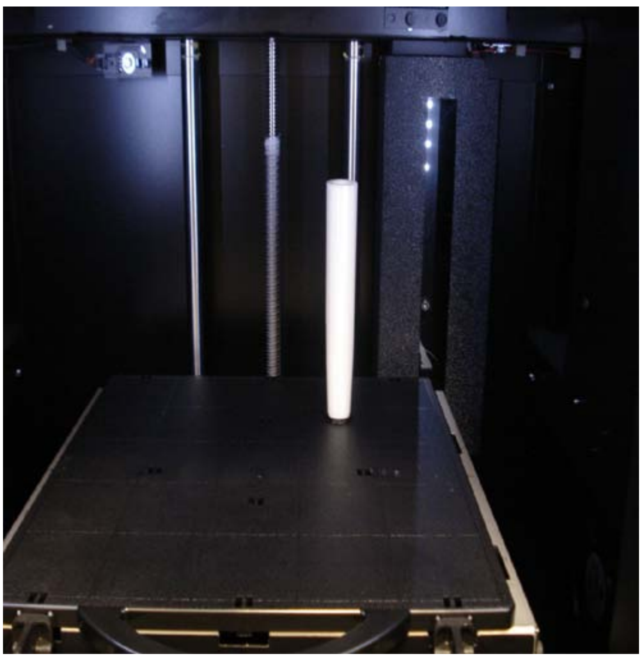
- *Step 1*: Import of CAD data in STL format into QuickSlicet.
- *Step 2*: Slicing of the CAD model into horizontal layers and conversion into a.slc (SLiCe) format.
- *Step 3*: Creation of deposition path for each layer and conversion into a.sml (Stratasys Machine Language) format for downloading to FDM machine.
- *Step 4*: FDM fabrication process using a filament modeling material to build actual CABG physical part in an additive manner layer by layer.
- *Step 5*: Using this FDM physical model as mould for electrospinning ground collector, the optimized specific geometry.

- *Step 6*: Removing the electrospinning nanofibrous scaffold from FDM mould.

5. Characterization of electrospun nanofibrous scaffold

The concept is based on the fact that the tubular scaffold is to be carefully removed off from the mould and then dried in the normal way under vacuum. Note that gelatin solution on the surface of the collector is normally used for ease of removal. After electrospinning process, we place the whole shaft in water to dissolve gelatin, so that there would be a small gap between the collector mould and the scaffold. Figure 7 shows CABG collector fabricated by FDM and Figure 8 the electrospun fibrous tubular scaffold with PU solution. As shown in Figure 8, the tubular scaffold showed good 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.

Figure 7 CABG collector fabricated by FDM



The morphology of the cross-section was observed using a scanning electron microscope (SEM) (JEOL, JSM-5600, Japan) at an accelerated voltage of 15 kV. From the SEM images (Figure 9), it was easy to determine that the electrospun blend fibers maintained their structure in the scaffold and the fiber diameter was less than 1 μm. The diameter of the nanofibers were calculated from the diameter of 100 nanofibers, each sample of which was directly measured from the SEM photographs. Moreover, porosity of tubular scaffold was found to be high as well.

Mechanical measurements were achieved by applying tensile test loads to these specimens. Mechanical properties were tested by a materials testing machine (H5K-S, Hounsfield, England) at the temperature of 20°C, a relative humidity of 65 percent and an elongation speed of 10 mm/min.

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. The random specimens and those electrospun onto a mandrel with low tangential velocities (in the range 200-2,000 rpm) exhibited fairly isotropic networks, with no discernible difference between the flat sheet and the tubular scaffold.

Figure 10 shows typical stress-strain curves of different electrospun thermoplastic films (Samples 1-3) under tensile loading, whereas Table I presents the mechanical properties of the values. The electrospun TPU material gives a characteristic response for the elastomeric materials – sigmoid curve. It showed a very soft and flexible characteristic with low Young's modulus and high elongation at break of 160 percent. With the increase of rotating speed, the initial modulus of the mats became large. This phenomenon pointed that rotating speed employed a plastic property which is different from TPU. Therefore, the mechanical property can be adjusted to meet the requirement in practice through changing the rotating speed of TPU. Moreover, as it can be seen, the broken stress increases with increasing rotating speed, and the broken strain decreases as this is changing. The mechanical properties of nanofibers are important for their successful applications in blood vessel replacement and tissue engineering applications. TPU nanofibers were electrospun into 0.5-mm thick fiber mats to measure their mechanical properties. Compared with the

Figure 8 Electrospun fibrous tubular scaffold with PU solution

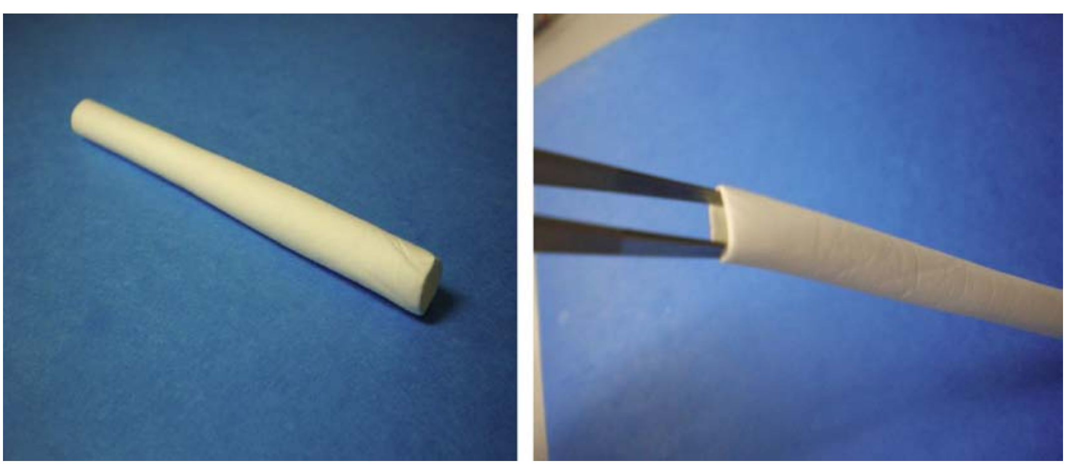


Figure 9 SEM images of electrospun PU under different magnification and their fiber distribution

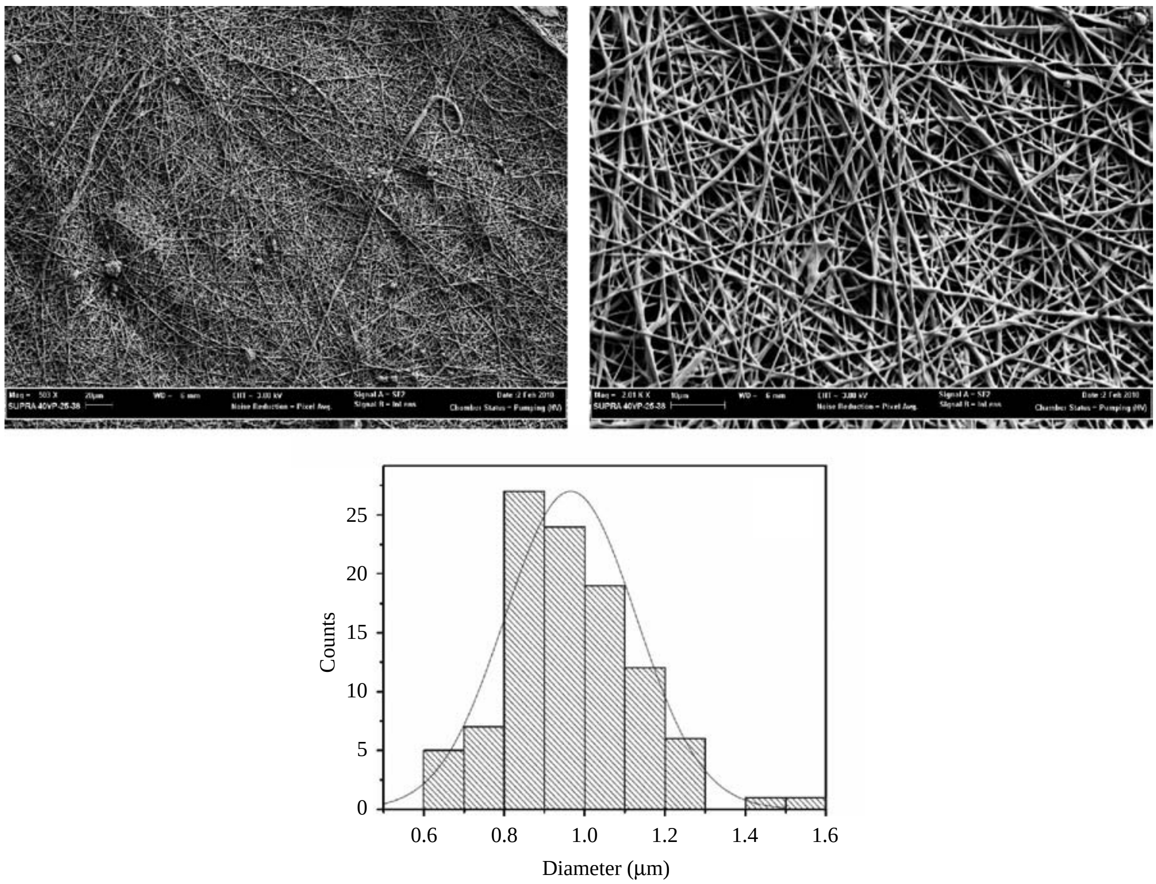


Figure 10 Typical stress-strain curve of electrospun tubular scaffold

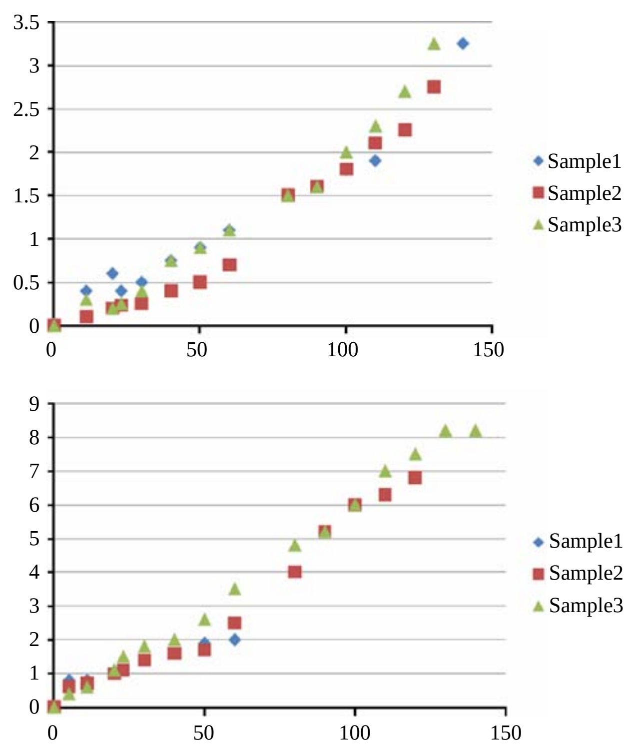


Table I Mechanical properties of TPU nanofibers films with different rotating speed

	1,000	2,000
Stress (MPa)	4.0 ± 0.48	5.8 ± 1.74
Strain (%)	135 ± 9.1	120 ± 20.4

mechanical property of natural human artery (Holzapfel *et al.*, 2005), the TPU stress and strain meet all the natural artery mechanical properties (natural human artery stress: 1.40 MPa; natural human artery strain: 100 percent).

To study the biocompatibility of the electrospun TPU material, it was conditioned with medium M199 overnight at 37°C in the incubator. Then, it was seeded with ovine endothelial cells at a cell density of 1×10^6 cells/cm^2. The material seeded with cells was cultured for a period of seven days at 37°C. It was observed that the endothelial cells adhered onto TPU material after 15 h of seeding, with the cells flattened and spread across the surface. The endothelial cells exhibited typical cobblestone morphology, as shown in Figure 11. As expected, the cell coverage increased significantly with an increase in the experimental time period. A marked increase was observed in the cell coverage as early as three days post seeding and this effect continued throughout the experimental time course of seven days.

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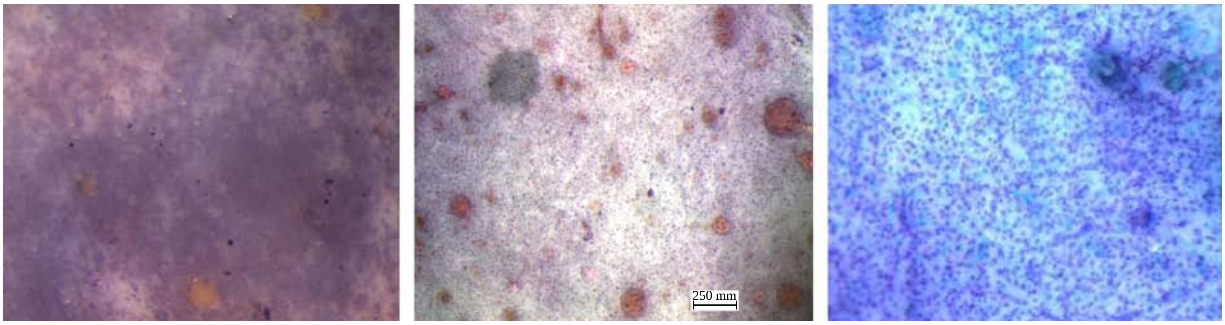
Figure 11 Endothelial cells covering TPU after 15 h, three days and seven days of culture



6. Conclusion

Coronary bypass grafting is widely used for the treatment of coronary heart diseases. However, in some cases due to limitations and the availability of the use of autogenous vessels, prosthetic or artificial grafts are needed. These grafts require a special design to ensure, compliance matching, revascularization and anti-thrombogenicity. These requirements present a challenge in the selection of biomaterials and manufacturing into the exact 3-D architecture and matching of the resultant mechanical properties with that of the target tissue host. The RP technique is widely used to construct uncharacteristic models based on computer tomography scan or CAD model with complicated features easily and quickly with a high degree of accuracy. The electrospinning can be effectively used to optimize the mechanical and physical properties of the vessel to mimic the native ECM. In this paper, we proposed a combined manufacturing technique, namely FDM and electrospinning, to fabricate the specifically designed artery graft for CABG applications. It is proved that the proposed method can clearly fabricate a specifically designed graft vessel.

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