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Electrospinning Complexly-shaped, Resorbable, Bifurcated Vascular Grafts

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Abstract

The use of vascular grafts is indicated in a wide range of medical treatments. While autologous tissue is the graft of choice in most surgical bypass procedures, the next best option is the use of synthetic vascular grafts. While significant advances have been reported in the use of electrospinning for vascular grafts both at *in vitro* and *in vivo* level, most of the work is limited to straight, tubular shapes with uniform diameters. In order to generate resorbable scaffolds with curving and bifurcated tubular shapes with non-uniform diameters, this study proposes combination of directed electrical field and dynamic positioning of electrospun fibers aimed at a custom, 3D printed mandrel. The proposed approach produced a woven membrane of electrospun fibers. In this study, the fibers used were polycaprolactone. They were spun onto a 3D printed (in ABS plastic) bifurcated tubular mandrel. Preliminary mechanical testing of these bifurcated grafts is reported, with maximum indentation force between 0.7 and 2.3 N. In tension tests, the scaffolds showed an average maximum strength of 0.60 MPa (no indexing condition in the B direction) and 1.37 MPa (indexing in the B direction).

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Keywords: tissue engineering, vascular grafts, electrospinning, bifurcated tubular scaffolds

1. Introduction

There is a wide range of medical treatments that require vascular grafts, for example surgical bypass for atherosclerosis of the major vessels, dialysis access in patients with end-stage renal disease, and pediatric congenital heart defects. Autologous tissue is the graft of choice in most surgical bypass procedures. When autologous tissue is not available in the patient, the next best option is the use of synthetic vascular grafts [1].

In the filed of vascular surgery, the end-to-side anastomosis procedure (i.e. connection of one vessel end to the side of another vessel) brings additional challenges [2]. Suturing at the roughly right angle connection is difficult and leaves the graft prone to failure at the suture site, which is under high flow pressure. Some devices and surgical procedures have been proposed to facilitate end-to-side anastomosis [2-3].

In addition, surgeons can use commercial bifurcated synthetic vascular grafts in order to approach the end-to-side anastomosis procedure. Examples of such synthetic vascular grafts are a) the GORE-TEX® Stretch Vascular Graft - Bifurcated (W. L. Gore & Associates, Newark, Delaware, USA), made out of stretched PTFE (commonly known as Teflon™), or b) the Vascutek® Gelsoft™ Bifurcate Grafts (Vascutek Ltd., Renfrewshire, Scotland, UK), based on polyester. However, these options are based on non-resorbable polymers and tubes of uniform dimensions. Current synthetic vascular grafts therefore present limitations, especially for cases involving vessels with bifurcations and small diameters [1].

The ideal bifurcated vascular graft would be tissue-engineered to better suit the patient. One limitation in achieving this goal is the limited availability of bifurcated tubular scaffolds based on resorbable materials.

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1.1. Related Work

While significant advances have been reported in the use of electrospinning for vascular grafts, both at *in vitro* and *in vivo* level, most of the work is limited to tubular shapes [1] [4-5]. Zhang and Chang developed a method to build interconnected tubular structures with electrospun mats. Their method is based on guides for the electric field and removable scaffold mandrels [6].

The present study shows a method to build bifurcated tubular scaffolds with electrospinning. The proposed method is based on both a special electric field collector designed to be inside a 3D printed object and special kinematics for positioning the scaffold mandrel relative to the electrospun fiber source nozzle.

1.2. Objective

The objective of this study is to validate a method combining electrospinning and 3D printed mandrels, with patient-specific shapes, for the fabrication of resorbable, bifurcated vascular grafts.

2. Materials and Methods

2.1. Conceptual Design

The basic design concept uses a directed electrical field and dynamic positioning of the construct, as shown in Figures 1 and 2. Given the correct positioning, we hypothesize that the electrospun fibers can generate a mat with a weave as complex, if not more so, than traditionally spraying onto a single diameter mandrel. In this case our test 3D mandrel includes a bifurcated tubular shape, as an internal electrical field collector pulls the fibers. It should be noted that the internal collector directs the electrical field towards the mandrel, while the fibers are actually deposited on the mandrel and the actual collector when using a porous design for the mandrel wall.

The mandrel can be 3D printed from inert material and removed, it can made out of resorbable polymer and be part of the final synthetic vascular graft, or it can be an object which is effectively dissolved within the external electrospun membrane. Alternatively, the mandrel could be removed, if the graft can stretch a bit, leaving only the electrospun membrane to form the graft. The internal electrical field collector is always removed from the construct, after electrospinning. Due to the continuous nature of the electrospun fibers, after the internal collector is removed, the electrospun mat says on the surface of the mandrel.

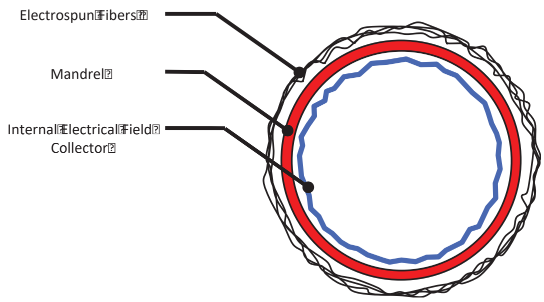


Figure 1. Scaffold cross section during electrospinning process, showing the internal electrical field collector.

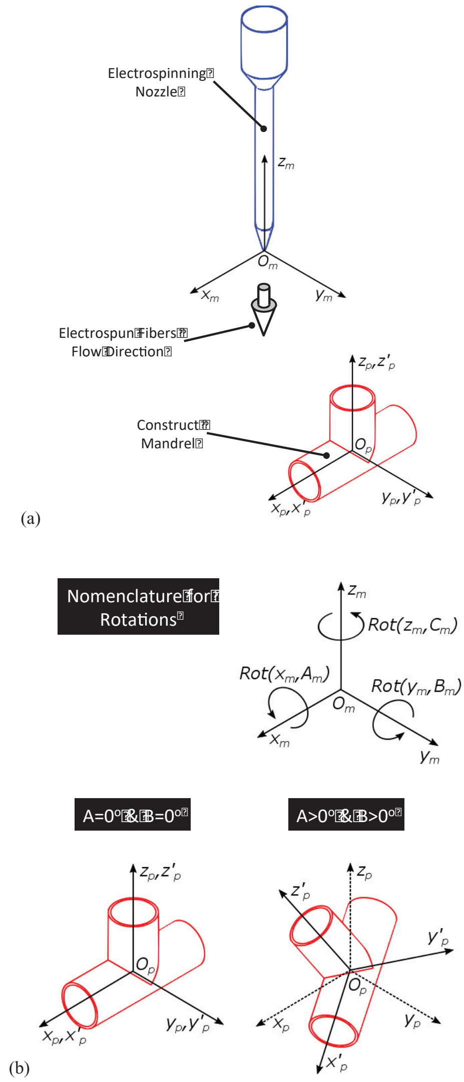


Figure 2. (a) Coordinate system for machine and part. (b) Nomenclature for part rotations.

Specific kinematics is proposed to position the mandrel with respect to the direction of the electrospun fibers' flow. Figure 2a shows the machine coordinate system (x_m, y_m, z_m,), defined with an origin (O_m) at the tip of the electrospinning nozzle. The reference point on the part (O_p) is located at the intersection of the bifurcated axes. The short tubular T shape is concentric with the z_p part axis.

The intended positioning system should provide two degrees of freedom in order to rotate the mandrel in the A direction (around the x_m machine axis), and, simultaneously, position the mandrel in the B direction (around the y_m machine axis), as shown in Figure 2b. The system should allow for continuous rotation and/or the indexing of specific angular positions. Ideally, the part origin (O_p) should be aligned with the electrospinning nozzle (i.e. x_m=0 and y_m= 0).

The combination of directed electrical field and dynamic positioning of the construct is expected to produce a semi-uniform mat of electrospun fibers on top of a bifurcated tubular mandrel.

2.2. Positioning Device

The proposed conceptual design was reduced to practice, including the positioning device shown in Figure 3. In this prototype, balsa wood and plastic were used in order to avoid interference with the electrospinning electrical field. Figure 3b shows how the orientation along the B direction is achieved through a simple compliant mechanism with flexible joints. The compliant mechanism is actuated through an axial motion along the main rotating axis A (green rod in Figure 3a). The positioning device control is based on Arduino technology.

Figure 3c shows the positioning device inside an acrylic enclosure. The motors are isolated from the main electrospinning chamber by an adjustable acrylic wall. The internal electrical field collector is formed with aluminum foil. Voltage is delivered to the internal collector through the slip ring system at the left side of the picture.

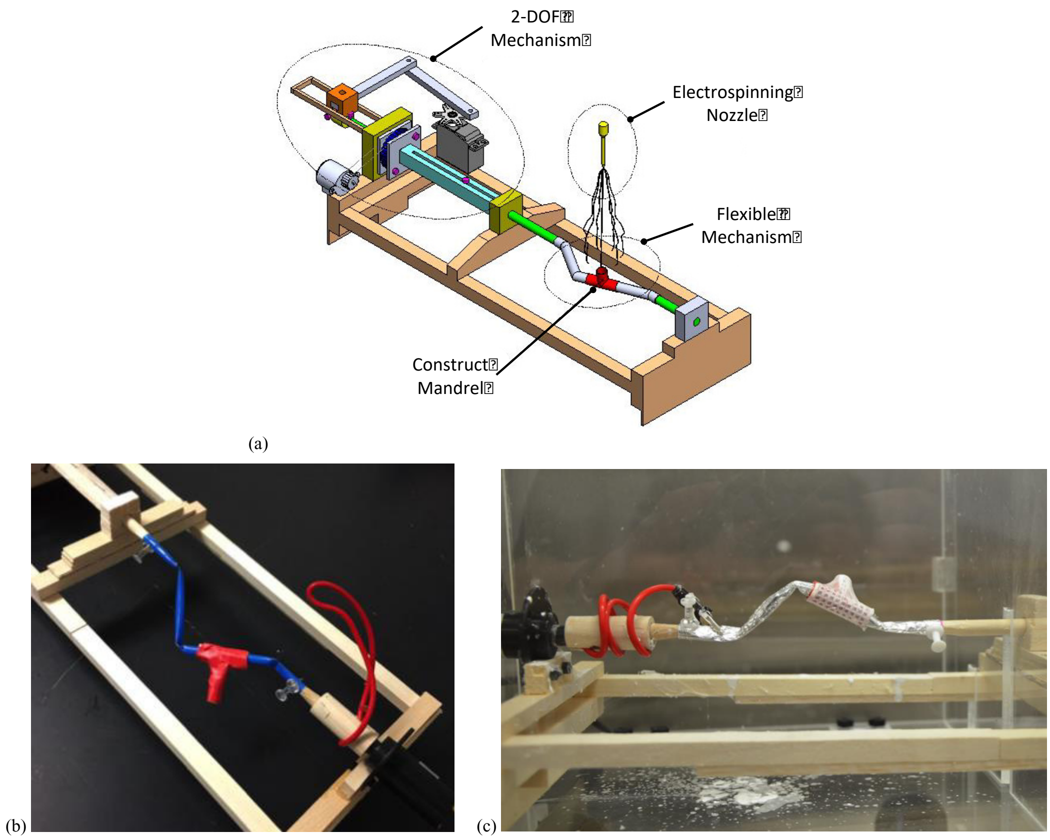


Figure 3. (a) CAD design of the positioning device; (b) positioning device with compliant links (in blue) holding the mandrel (in red) and slip ring (in black); (c) positioning mechanism inside the electrospinning chamber.

2.3. Process Parameters

2.3.1. Scaffold Mandrel

The mandrel was generated through Fused Modeling Deposition (FDM) with a Stratatys Fortus 400 machine and standard acrylonitrile butadiene styrene (ABS) material was used. The mandrel was designed with an open structure in order to facilitate electrical field flow. Internal diameters of 10.00 and 8.22 mm were used for the main tubular section and the T section, respectively (see Figure 4). Wall thickness is 1 mm. The pore size was chosen in order to have some structural integrity and facilitate a path for the electrical field towards the internal collector.

(a)

(b)

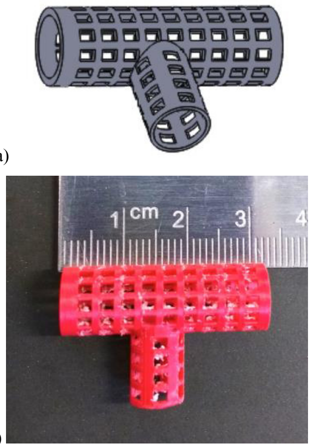


Figure 4. (a) Scaffold mandrel CAD model.
(b) Actual scaffold mandrel as FDM printed.

2.3.2. Electrospinning Process

The electrospinning solution is based on polycaprolactone (PCL) (at 10 %wt) and acetone, with stirring at 45°C for 4 hrs. The solution is delivered with a syringe infusion pump (model KS 100 (KD Scientific Inc., Holliston, MA).

The power source has a maximum voltage of 20 kV and maximum current of 250 μA (model ES20P-5W, Gamma High Voltage Research Inc., Ormond Beach, Florida, USA). The specific electrospinning parameters used are shown in Table 1, including the positioning and rotation kinematics.

For the experimental design, only two variable parameters were considered (see Table 1). Five replications were used for each condition. The positioning on the B direction for the condition with indexing was achieved by first positioning at B=0º. Then after 5 revolutions around the A axis, the B direction was indexed at B=60º. These two positions in the B direction were alternated for the duration of the electrospinning process (10 minutes).

Table 1. Electrospinning process parameters.

Fixed Parameters	
Voltage, V [kV]	18
Electrospinning Nozzle, D_e [mm]	0.86
Solution Flow Rate, Q_e [ml/h]	7
Electrospinning Time, t_e [min]	10
Part Location (Nozzle Height), z_m [mm]	150 +/- sinusoidal (20 mm amplitude)
Part Location, x_m [mm]	0
Part Location, y_m [mm]	0 +/- sinusoidal (20 mm amplitude)
Part Rotation, ω_A [rad/s]	0.45
Variable Parameters	
Internal Electric Field Collector	On / Off
Mandrel Positioning in B Direction	Fixed at 0º / Indexing at 0º & -60º

2.4. Characterization of Bifurcated Tubular Scaffolds

2.4.1. Qualitative Analysis

The bifurcated scaffolds were analyzed via optical microscopy and SEM imaging. Overall density of electrospun membranes and scaffold integrity were evaluated for different process conditions.

2.4.2. Mechanical Properties

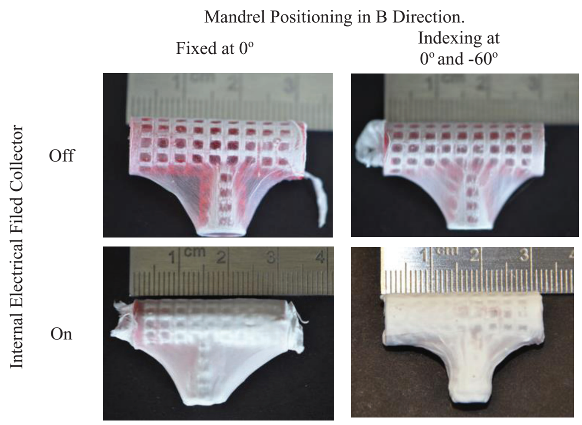
Preliminary evaluation of mechanical properties was conducted for the bifurcated scaffolds. As shown in Figure 3a, the construct location on the positioning device leaves the T section open to the electrospun fiber deposition. An indentation test was devised to test the maximum load allowed by the membrane of electrospun fibers generated at the end of the T section.

A universal machine Instron 3365 (Norwood, Massachusetts, USA) was used for indentation tests (5 N load cell and hexagonal rod with a flat end) and tension tests (1 kN load cell).

3. Results

Table 2 shows a representative sample scaffold for each combination of variable parameters. From a qualitative point of view there is a clear advantage in combining an internal electrical field collector with mandrel kinematics that combine rotation around x_m machine axis (A direction, as defined in Figure 1) and positioning around y_m machine axis (B direction, as defined in Figure 1). The overall mat shape tends to mimic the mandrel shape for all cases. However, with the use of internal electrical field collector and/or indexing in the B direction, there is a qualitative improvement in terms of both morphology and density. Figure 5 shows a representative sample with consistent weaving of a dense fiber, interwoven, and electrospun mat.

Table 2. Bifurcated tubular scaffolds for different experimental conditions.



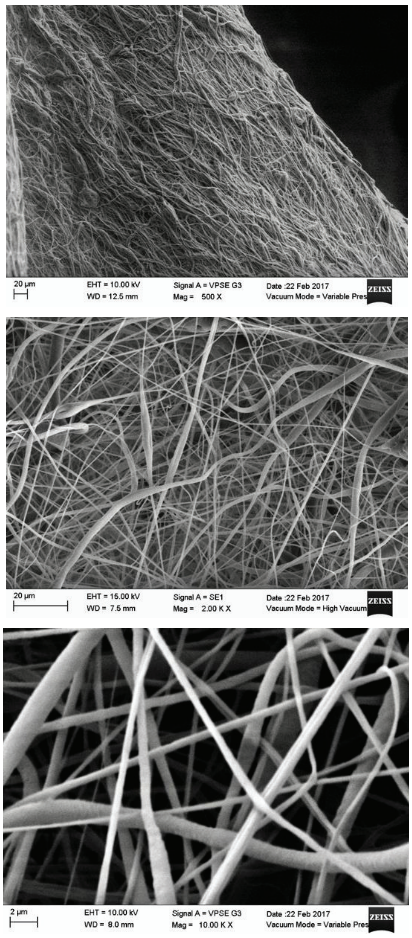


Figure 5. Electrospun fibers on bifurcated tubular scaffold (SEM imaging).

Quantification of the results is conducted in terms of mechanical properties. Only those samples with experimental conditions corresponding to the internal collector were tested for mechanical properties. The electrospun membranes generated without the use of an internal collector were too weak for this type of testing.

Results of some preliminary indentation testing are shown in Figure 6. Tension tests were conducted on the tubular sleeve of the bifurcated scaffold, after removal of the mandrel (see Figure 7). The tension tests showed average maximum strength of 1.37 MPa in samples with internal collector and indexing in B direction vs. 0.60 MPa in samples with internal collector and no indexing in B direction. The average scaffold membrane thickness was 170 µm vs. 150 µm, respectively.

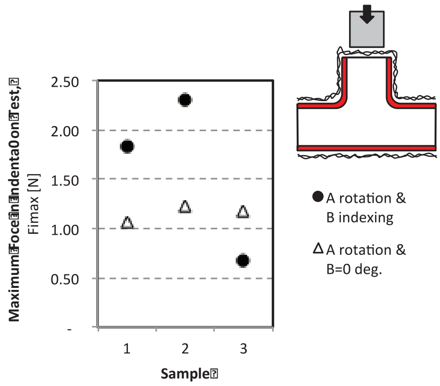


Figure 6. Indentation test for electrosupun membrane, only experimental condition with internal collector on.

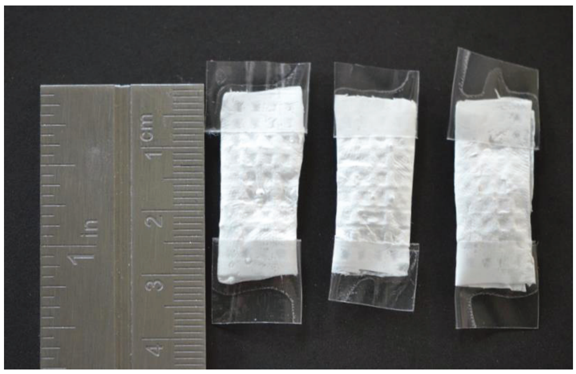


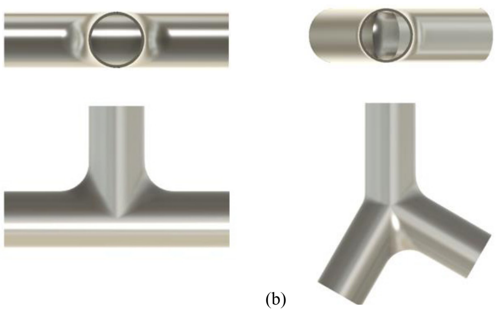
Figure 7. Sample preparation for tension tests.

4. Discussion

This study validates the use of a robotically controlled electrospinning fiber source and the electrical control of both the mandrel and the electrospun fiber source. The shape of our final scaffold corresponds well to the intended bifurcated tube (see Table 1). Based on these results, the approach to follow should maintain the use of internal electrical field collector and dynamic positioning of the mandrel.

Additional experiments will be required with larger number of replications in order to assess the process capability and statistical significance of mechanical properties resulting for different process parameters. Conventional tubular scaffolds have strength in the range of 3 and 4 MPa in order to be considered adequate for *in vivo* testing as vascular grafts inside models [7-8]. In this study, the resulting scaffolds showed maximum strength of at least 20% compared to that of conventional tubular scaffolds with similar electrospinning materials.

Once this first stage has been established, there are a number of potential improvements required in order to fabricate the currently desired bifurcated tubular scaffold shapes with useful mechanical properties. In addition to further testing different mandrel, scaffold materials and process parameters, more realistic shapes for the mandrel should be considered (as shown in Figure 8).



(a) (b)

Figure 8. (a) T shape scaffold. (b) Y shape scaffold.

5. Conclusions and Future Work

In order to generate resorbable scaffolds with bifurcated tubular shapes, this study proposes combination of directed electrical field and dynamic positioning of the mandrel. The proposed approach produced a mat of electrospun fibers on top of a bifurcated tubular mandrel. Preliminary mechanical testing of the bifurcated scaffolds is reported, with maximum indentation force between 0.7 and 2.3 N. In tension tests, the scaffolds showed an average maximum strength of 0.60 MPa (no indexing condition) and 1.37 MPa (indexing in the B direction).

In future work, the positioning of the electrospun fiber source and/or the mandrel requires further refinement in order to improve precision of continuous motion in A and B directions, as well as specific indexing angles at particular positions to control the weave. Further experimentation is required to establish process capability in terms of tolerances for the key dimensions of the bifurcated scaffold (such as diameter and thickness) and mechanical properties. Additional testing is required in order to assess the burst pressure strength of this kind of bifurcated scaffolds.

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Disclosure

This manuscript and its subject matter are the subject of pending patent application.

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