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Procedia CIRP 89 (2020) 92–97

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CIRP BioManufacturing Conference 2019

Real bifurcated vascular grafts manufacturing for tissue engineering

Antonio J. Guerra[a], Caterina de Marie[b], Elisabetta Ceretti[b], Joaquim Ciurana[a]*

[a] *Department of Mechanical Engineering and Civil Construction, Universitat de Girona, C/Maria Aurèlia Capmany 61, 17003 Girona, Spain*
[b] *Department of Mechanical and Industrial Engineering, Via Branze 38, 25123 Brescia, Italy*

* Corresponding author. Tel.: +34 972 418 265 / E-mail: quim.ciurana@udg.edu (Joaquim Ciurana)

Abstract

In medicine, atherosclerosis is a life threatening sickness. When stents cannot be use due to the higher narrowing of the vessel, bypass surgery has to be done. While autologous tissue is the graft of choice in most surgical bypass procedures, the next best option is the use of synthetic vascular graft. Literature has reported numerous works about electrospinning and 3D-printed vascular graft but most of the works are limited to straight and simple shapes. The generation of real complex vascular graft is still an open challenge.
This work aims at designing and prototyping real bifurcated vascular grafts. 3D-printing process (FDM) was employed to create the vascular graft mould with poly-vinyl alcohol (PVA) as material. Dip coating process (DCP) was used to generate a uniform, thin, mechanically resistant and impermeable layer to ensure the blood flow. Finally, electrospinning process (EP) was employed for coating the graft with a porous and uniform layer that ensures the cell grown and then the regeneration of the autologous vessel.
The present manuscript describes a novel process to obtain real bifurcated vascular grafts and analyses the effects the different process parameters have on the final dimensional accuracy of the graft.

Keywords: 3D-Printing, Electrospinning, Additive Manufacturing, Vascular Graft, Polymer, Manufacturing.

1. Introduction

In medicine, vascular problems are a life threatening sickness. Vascular diseases include numerous pathologies many of which are related to atherosclerosis, a condition that is developed when a substance called plaque builds up in the walls of the arteries. The vessel wall becomes markedly thickened by accumulating cells and surrounding material. The vessel narrows and blood flow is reduced, thus decreasing the oxygen supply. Nowadays certain procedures are used in clinics to treat atherosclerosis. The most common is Angioplasty, a therapy where a special tubing (stent) is threaded up to the coronary arteries. The stent is a small tubular mesh whose function is to open a narrowed arterial vessel and reduce the chance of a heart attack. Many previous research had been focused in these devices [1]–[4]. The second atherosclerosis treatment is the coronary artery bypass graft surgery (CABG). The clinical gold standard is the use of autologous vessels, such as saphenous vein or internal mammary artery. However, the possible damaged conditions of these vessels, due to the presence of cardiovascular disease, and the need for repeated surgery procedures limit the use of autologous grafts [5].

To overcome the aforementioned problems, vascular tissue engineering represents a promising approach by developing synthetic functional grafts with morphological, mechanical and biological properties similar to those of native vessels [6]. Nowadays, there is a tremendous requirement for developing small diameter vascular grafts [7], [8] and vascular graft with real and complex shapes. Artificial grafts represent a consolidated solution for the replacement of large and

10.1016/j.procir.2020.05.123

medium diameter vessels solving the present limitations of autologous vessels use [7]–[10].

Literature has reported numerous works about of electrospun and 3D-printed vascular graft but most of the works are limited to straight and simple shapes. Until now, technologies such as electrospinning have been used to fabricate scaffolds for three-dimensional cell culture [11] as well as small diameter tubular scaffolds. Some authors directly electrospun on a rotating mandrel as a collector [12]–[14] and others rolled two-dimensional meshes [15]. However, when scaffolds must be patient-personalized with a specific design with real and complex shapes, the need for more customizable structures becomes evident.

Over the past few years, additive manufacturing (AM) technology, used partially by three-dimensional printers, has emerged as a potential technique to manufacture polymeric grafts and scaffolds [16]. Manufactured 3D products can mimic the physiological architecture of some specific anatomical regions such as, for example, blood vessels. However, most works focused their research on AM methodologies such as melt-drawing [17], [18], rather than 3D printers. Thus new efforts have to be done to elucidate the effect of process parameters on filaments and scaffold features [16], [19], [20] and produce scaffolds with real and complex shapes using 3D printers.

This work aims to develop real bifurcated vascular grafts that can be implantable. 3D-printing technology based on fused deposition modelling (FDM) was employed to create the vascular graft mold. Then, dip coating process (DCP) was used to generate a uniform, thin, mechanically resistant, and impermeable layer to ensure the blood flow and support the pressures that vessels apply on the graft. Finally, electrospinning process (EP) was employed to coating the graft with a porous and uniform layer that ensure the cell grown and then help the regeneration of the autologous vessel. Poly-vinyl alcohol (PVA) was used as material to manufacture the vascular graft molds. PVA is a water-soluble synthetic polymer with a 200 ºC of melting point. PVA allow us to generate a soluble mold that can be removed after dip coating process. Polycaprolactone (PCL) was used as material for the dip coating and electrospinning processes.

2. Materials and Methods

To produce real bifurcated, implantable, and bioabsordable vascular grafts the following methodology was performed: (I) vascular graft design, (II) vascular graft mold fabrication (III) vascular graft mold coating by dip coating, (IV) vascular graft mold dissolution, and (V) vascular graft coating by electrospinning.

2.1. Vascular graft design

A vascular graft (Fig. 1) was designed with the computer-aided design (CAD) software SolidWorks® 2014. The graft was saved in STL file format and transferred to Repetier Host® Software to stablish the manufacturing parameters. Grafts are defined by: diameters of the bifurcated branches both left and right branches (DBLB, DBRB), and diameter of the main branch (DMB).

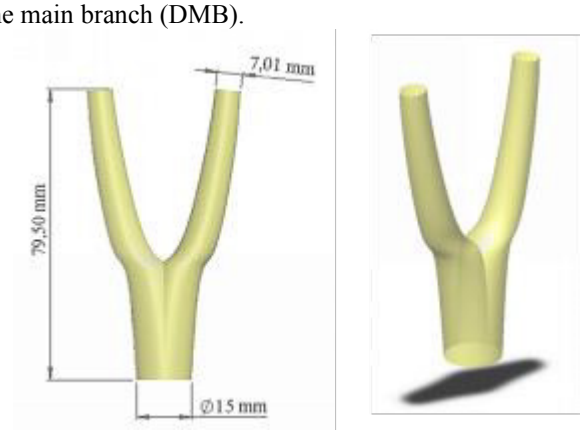

Fig. 1. Vascular graft

2.2. Vascular graft core fabrication

Vascular graft molds (Fig. 2a) were manufacture in a Sigma® (BCN 3D Technologies, Barcelona, Spain) (Fig. 2b) by 3D-printing with a fused deposition modelling (FDM). The machine provides 12.5 µm of precision in the X and Y axis and 1 µm in the Z axis with a 0.4 mm of nozzle diameter. Poly (vinyl alcohol) (PVA, RepRap S.A. Spain) was used as material for the manufacturing of vascular graft molds. PVA is a water-soluble synthetic polymer with a 200 ºC of melting point.

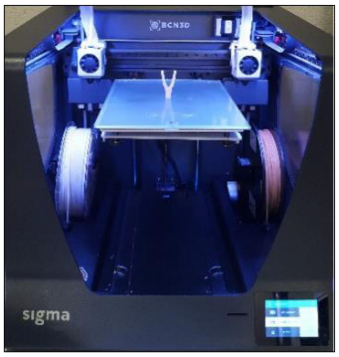

Fig. 2. 3D printer Sigma

Experiments to find the best 3D-printing process parameters in terms of dimensional precision were performed. Printing flow rate (FR% 80%: 120%), Printing temperature (PTª, 195 ºC: 225 ºC), and Printing speed (PS, 16 mm/s: 24 mm/s) were the parameters under investigation. Printing layer height (PLH, 0.1 mm), and Printing bed temperature (PBTª, 40 ºC) were kept constant. Employing a full factorial design with 3 factors and 3 levels, 81 replicas were printed (27 x 3 replicas).

2.3. Vascular graft mold coating

Vascular graft molds printed with the best 3D-printing process parameters were coated by dip coating process to obtain a thin, uniform, mechanically resistant, and

impermeable layer that ensures the correct blood flow and vessel pressures. Dip coating techniques can be described as a process where the substrate to be coated is immersed in a liquid and then withdrawn with a well-defined withdrawal speed under controlled temperature and atmospheric conditions (Fig. 3). The process is defined by the start-up time, withdrawal speed, evaporation time, number of cycles, and solution concentration. Polycaprolactone (PCL) CAPA 6500® (Sigma-Aldrich, St. Louis, MO, USA) was used as material for the dip coating process. PCL is biodegradable polyester with a low melting point (60ºC). Acetone (PanReac AppliChem, Gatersleben, Germany) was the non-toxic solvent chosen.

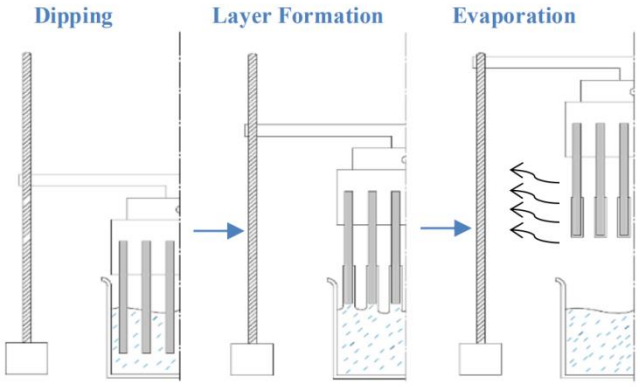

Fig. 3. Dip coating process methodology

To find dip coating process parameters that give us a uniform, thin, mechanical-resistant, and impermeable layer a series of experiments were performed. Based on previous works [21] the solution concentration (SC 8%: 15%), and number of cycles (NC, 4: 8), were investigated. Evaporation time of 10 seconds and withdrawal speed of 250 mm/min were kept constant based on previous knowledge. Employing a full factorial design with 2 factors and 3 levels were coated.

2.4. Vascular graft mold dissolution

Vascular graft moulds were dissolved in water overnight at 40ºC in oven. Once the PVA was completed removed, grafts were rinsed in water 5 times.

2.5. Vascular graft electrospinning fabrication

Finally, vascular PCL grafts coated with the best dip coating parameters were coated by electrospinning process. Employing a Spraybase® machine (Spraybase, Dublin, Ireland) (Fig. 4a). Again, Polycaprolactone (PCL) CAPA 6500® was used as material for the electrospinning process. Acetone (PanReac AppliChem, Gatersleben, Germany) was the non-toxic solvent chosen.

Based on previous works [11] the parameter selected for the electrospinning process were the following: 15% w/v of solution concentration placed in a plastic syringe (BD Plastipak, Franklin Lakes, NJ, USA), 7.5 kV of fixed voltage, 6 mL/h of flow rate, and 30 mm of distance between the emitter (18G needle with 0.8 mm of nozzle diameter) and stationary collector.

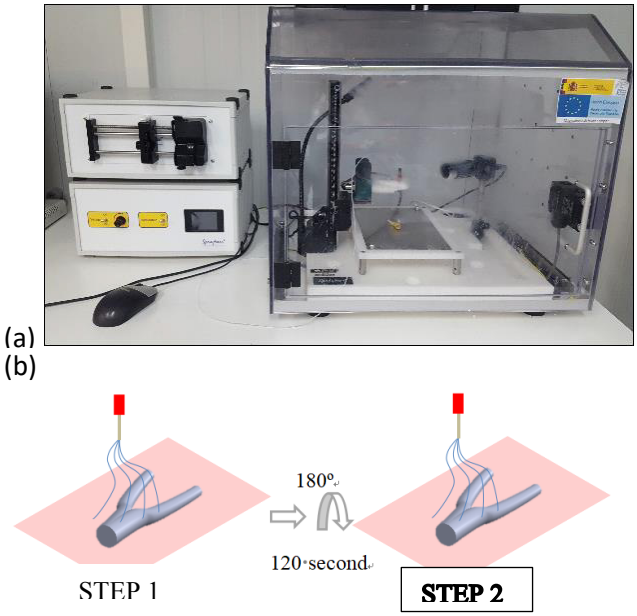

Fig. 4. Vascular graft electrospinning (a) Machine (b) Electrospinning methodology

The methodology followed to electrospun the graft was the following (Fig. 4b); (I) graft was placed into the stationary collector, (II) electrospinning parameters were set up, (III) graft was coated by electrospinning during 120 second while it was moved in XY directions to ensure the entire cover, and (IV) graft was turned after 120 second and the third step was repeated.

2.6. Characterization

2.6.1. Graft mold dimensional precision

Dimensional features (DBLB, DBRB, and DMB) of each of the 81 printed PVA samples were analyzed by optical microscopy with Nikon SMZ – 745T attached to a digital camera CT3 ProgRes. Image J® was used to process the images and collect the data. Micrometer Micromar® 40EWV was used to measure ST.

2.6.2. Coating precisions

Dimensional features after coating processes, both dip coating as electrospinning, were analyzed visually and organized as successful or not successful based on whether the sample was fully covered or not.

3. Results and discussions

3.1. Effect of FDM process on dimensional precision

3D Printing process parameters were analyzed and related with the final dimensional aspects achieved. In bioengineering the precision obtained in the manufacturing processes are critical and they have to be studied in deep. By regression analysis (MLR) the effects of FDM parameters,

(printing flow rate, printing temperature, and printing speed) were related with the scaffold's geometrical aspects, diameters of the bifurcated branches both left and right branches (DBLB, DBRB), and diameter of the main branch (DMB).

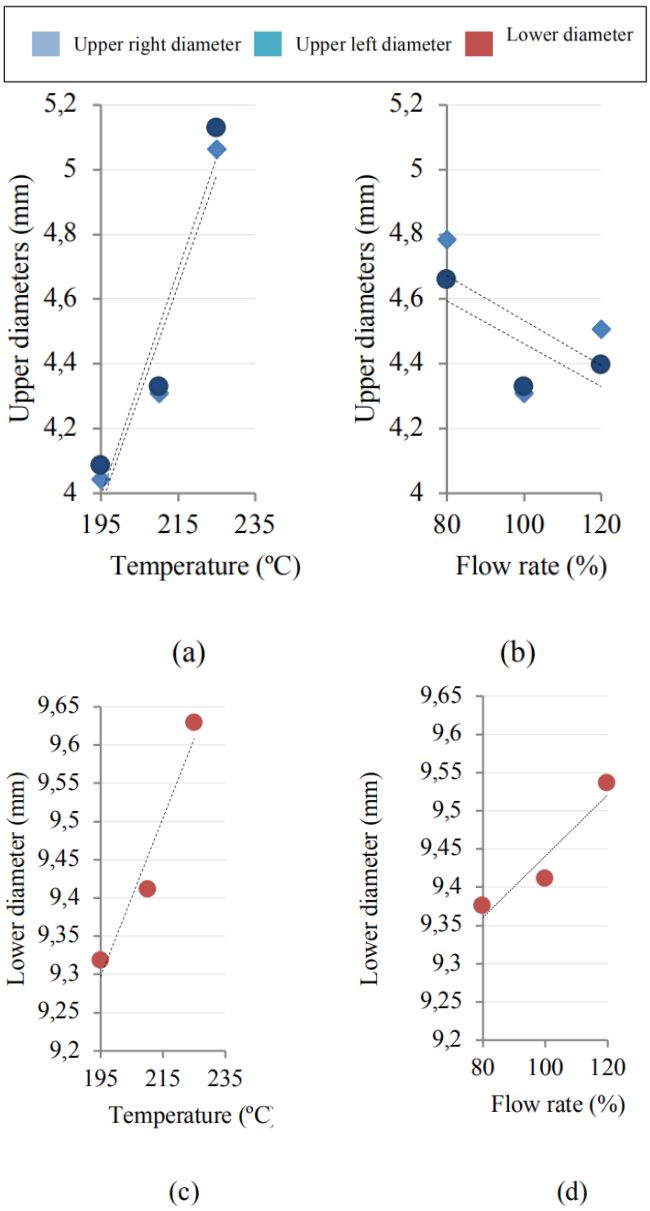

Fig. 5. Main effects plots for (a) printing temperature for upper diameters. Samples printed at 20 mm/s and 100 % of flow rate (b) flow rate for upper diameters. Samples printed at 20 mm/s and 210 ºc (c) printing temperate for lower diameter. Samples printed at 20 mm/s and 100 % of flow rate (d) flow rate for lower diameter. Samples printed at 20 mm/s and 210 ºc.

Results have proved the strong influence of printing temperature and printing flow rate ($p<0.05$). Printing speed did not show any statistical influence however slower speeds gave the best results.

The increase of printing temperature left an increase in the branches diameters, both the uppers as the lower (Fig. 5a and c). This effect was produced by the viscosity reduction suffered when printing temperature increases. The viscosity reduction makes that the printer nozzle expels greatest quantity of material, enlarging the filament width and the final branch diameters.

With respect of printing flow rate (Fig. 5b and d) in the lower branch the increase of printing flow rate produced an increase of the diameter and a reduction in the upper diameters. This could be produce by the union of three facts, (I) the increase of printing flow rate produces an increase in the quantity of material that nozzle expel, (II) the increase of printing flow rate reduce the cooling rate of the material making easy the spread of the material on the printing bed, and (III) the lower part of the scaffold supports the weight of the entire scaffold, fact that could produce a flattening of the lower part and therefore and enlargement of the diameter.

Regarding printing speed, it was observed that the increase of printing temperature raised the upper and lower diameters of the branches. This is produced by the increases in the inertias that the printer suffers when it works at higher speeds. The increase in these inertias produces a reduction of the precision.

In spite of the low precision obtained (about 60%), the printing parameters selected to print the samples for dip coating process were; printing temperature of 195 ºC, printing flow rate of 100%, and printing speed of 16 mm/s. These parameters were selected to minimize vessel diameter due to the subsequent processes will increase its dimensions.

3.2. Effect of dip coating process on scaffolds

Following section 3.2. Vascular graft mould coating, 9 samples were coated by dip coating (Table 1).

Table 1. Design of experiments for dip coating process.

	Samples								
Parameter	1	2	3	4	5	6	7	8	9
Concentration (%)	8	8	8	11.5	11.5	11.5	15	15	15
Cycles (#)	4	6	8	4	6	8	4	6	8

It was observed the strong influence of number of cycle on the coating process (Fig. 6). The best results, complete cover with low roughness, were obtained at 8 cycles and 15% concentration, however in term of superficial roughness were the obtained at 6 cycles and 8% concentration.

Results allow to conclude that controlling the polymer concentration and number of cycle different thicknesses and different superficial roughness can be obtained. Control over scaffold's wall thickness is crucial to make the scaffold resistant to blood pressure. Further studies that relate the coating process parameters with the pressure that the scaffold support could be useful.

3.3. Electrospinning process results

Sample 09 was selected for the electrospinning coating process for being the strongest after dip coating process based on the final wall thickness obtained. Scaffold's mold was successfully coated (Fig. 7) by electrospinning process. The complex shape of the mold makes toilsome the process.

Develop machines that help to cover this kind of complex shapes are still an open challenge.

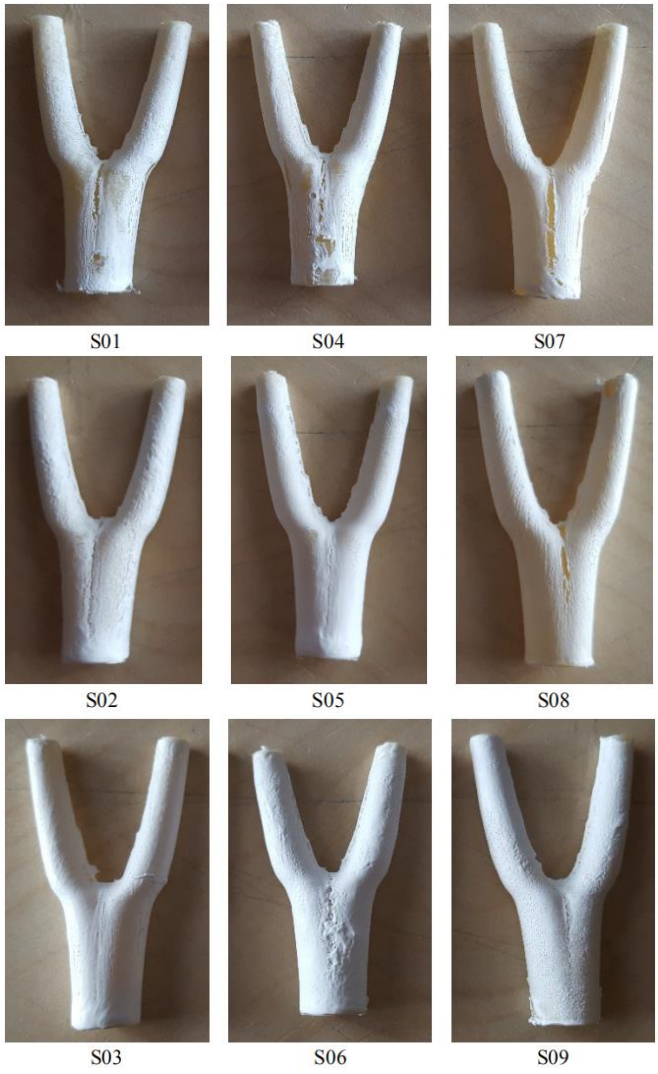

Fig. 6. Dip coating process results

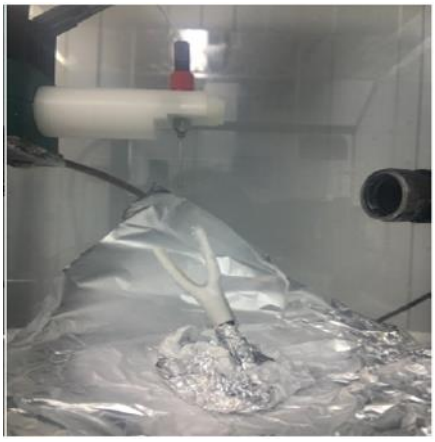

Fig. 7. Electrospun scaffold

4. Conclusions

The present work has introduced a novel process to manufacture real bifurcated vascular grafts.

This process is based on 5 steps: (I) graft design, (II) graft 3D printing process (FDM), (III) graft dip coating, (IV) graft electrospinning, and (V) FDM core dissolution.

FDM process has been able to produce the real bifurcated graft molds. However other 3D printing processes able to produce soluble objects should be study due to the low precision that FDM has produced. The molds were successfully coated by dip coating where the number of cycles and concentration proved to be the most influential parameters to obtain the desired wall thickness. Finally, the electrospinning process proved to be a good technique to cover the graft with a layer that mimic the body tissue. This last step was the most difficult due to the complex shape of the graft.

Further studies that analyze the pressure that this graft support and how cells grow through the scaffolds are necessary to know if this manufacturing process could be useful in the future to reduce the risk in the bypass surgeries.

Acknowledgements

The authors acknowledge the financial support from University of Girona (UdGG), the Ministry of Economy and Competitiveness (MINECO) (Spain) and Catalan Government, for its PhD scholarship and grants from DPI2016-77156-R and SGR 2017 - 00385.

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