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3D-printed tubular scaffolds for vascular tissue engineering

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Abstract

Biomedical engineering is the application of engineering principles and design concepts to medicine and biology for healthcare purposes. Much of the work in biomedical engineering consists of research and development, spanning a broad array of subfields like biomechanics, genetic, neural, pharmaceutical, medical devices and tissue engineering among others. Tissue engineering has emerged as a key discipline for organ and tissue regeneration, in which compatibility with final user and a fast regeneration are still major challenges. Recently, three-dimensional printing has arose as an alternative system for producing biomaterials devices, such as scaffolds for tissue engineering. The proliferation of murine fibroblasts NIH/3T3, widely used in tissue engineering studies, into *polycaprolactone* scaffolds has been assayed in this paper. This work aim to analyze the effect of different architectures of 3D tubular scaffolds with empty core on fibroblast proliferation, focusing the tissue engineering application. We determined the effect of manufacturing process parameters and scaffold design onto the cell proliferation. From results, it can be concluded that manufacturing parameters (printing speed, temperature, and flow rate) affected fibroblast growth rate up to 40-65 %. Narrow pores produced by an increased material extrusion showed less fibroblast growth, possibly due to a hindered oxygen and nutrient exchange. Nevertheless, future experiments using different designs and scaffold's architecture must be done.

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Keywords: Tubular Scaffold; 3D-Printing; Biodegradable; Vascular; Tissue Engineering

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 [9].

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 [10]. Nowadays, there is a tremendous requirement for

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developing small diameter vascular grafts [5], [6]. Artificial grafts represent a consolidated solution for the replacement of large and medium diameter vessels solving the present limitations of autologous vessels use [5]–[8].

Until now, random technologies such as electrospinning were 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, 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 of works focused their research on AM methodologies such as melt-drawing [17], [18], forgetting 3D printers. Thus new efforts have to be done to elucidate the effect of process parameters on filaments and scaffold features [16], [19], [20].

A further important matter is the three-dimensional cell culture on tubular grafts. Nowadays, fibroblasts cells are widely used as biological model in tissue engineering applications and, especially, in tubular scaffolds culture [15], [21], [22]. Fibroblasts belong to the connective tissue, specialized in the secretion of extracellular matrix and the architectural structure of the organism. The connective tissue and, therefore fibroblasts, are present in most body systems and apparatus and they play a key role in tissue repair. All these previous reasons explained their broad use in tissue engineering projects. Despite that fact, most previous research did not focus on cell seeding system on tubular scaffolds [12], [13]. For instance, structures were only seeded on one lumen surface [18] or scaffolds were first seed on monolayer non-tubular form and then meshes were rolled [15]. Moreover, produced scaffolds have to be optimized to allow a suitable fibroblast cell culture.

In the present work, an innovative 3D-Printed Tubular Scaffold to small diameter vascular graft is presented. The machine presented in this work allows the production of 3D Tubular Scaffold of different diameters in just one step, increasing the precision of final graft. The effect of printing process parameters over the tubular scaffolds structure is studied and related to the subsequent fibroblast cell culture.

2. Materials and Methods

2.1. Material

Polycaprolactone (PCL) CAPA 6500® supplied by Perstorp was used as the material because of its properties **(Table 1).** PCL is biodegradable polyester with a low melting point (60ºC) and a glass transition of about -60ºC.

Table 1. Polycaprolactone Properties

Molecular Weight	Melting Temperature	Yield Stress	Young Modulus	Strain Break	Degradation Time
50000 g/mol	60 ºC	17.5 MPa	470 MPa	>700 %	> 24 Months

2.2. 3D-printed tubular scaffold fabrication

Tubular Scaffolds were manufactured by 3D printing employing a novel 3D Tubular Printer designed and implemented by our research groups **(Fig. 1)**. This 3D Additive Manufacturing Machine is based on the Fused Filament Fabrication (FFF) method. The filament is melted into the extruder nozzle and deposited onto a rotatory platform. The machine provides a precision of 0.9375 μm in the X axis, 0.028125º in the W axis, 0.3125 in the Z axis, and 0.028125º in the extruder. The nozzle provides 0.4 mm of diameter.

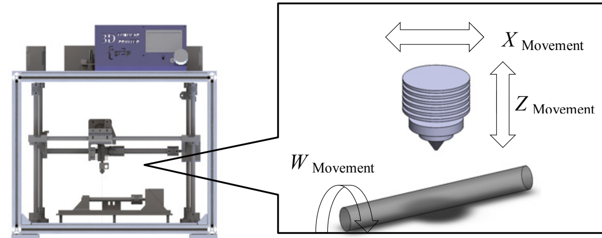
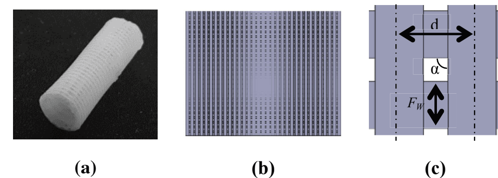


Fig. 1. 3D Printing Methodology System

The tubular scaffold model used for the experiments has been based on previous studies of our groups [23], [24]. The scaffolds are defined by the distance between filaments *(d = 0.5 mm)*, the deposition angle *(α = 90º)*, and the filament width *(F_W = 0.4 mm)*. Scaffolds were manufactured with 4 layers, the first one was designed totally impermeable to ensure blood circulation. The other three layers were designed employing the traditional scaffold design to ensure cell attachment **(Fig. 2).**



(a) **(b)** **(c)**

Fig. 2. (a) Real 3D-Printed Scaffold **(b)** 2D View **(c)** Cell

2.3. Design of experiment

Screening experiments were carried out to find the correct process parameters level. The process parameters studied were nozzle temperature ($N_{Tª}$: 120/220 ºC), fluid flow rate ($F_{R\%}$: 80/120 %) and printing speed (P_S: 200/1600 mm/min). According to screening experiments, parameters were selected and the DOE was developed based on a *Central Composite Design* (CCD) DOE with 6 center points* and alpha 1.682 **(Table 2).**

Table 2. CCD Design of Experiment

Sample	1	2	3	4	5	6	7	8	9	10
$N_{Tª}$	180	180	180	180	220	220	220	220	169.5	230.5
$F_{R\%}$	90	110	90	110	90	110	90	110	100	100
P_S	500	500	1500	1500	500	500	1500	1500	1000	1000

Sample	11	12	13	14	15*	16*	17*	18*	19*	20*
N_{T^a}	200	200	200	200	200	200	200	200	200	200
$F_{R\%}$	100	100	84.7	115	100	100	100	100	100	100
P_S	237	1762	1000	1000	1000	1000	1000	1000	1000	1000

2.4. Scaffold structure characterization

Tubular Scaffold structure (Filament width, F_W, and Cell Area, C_A) were analysed by Optical Microscopy with Microscope Nikon SMZ – 745T attached to a digital camera CT3 ProgRes for the collection of digital images**.** These images were processed using the Image J® Software 1.5F. The main intention behind the scaffold structure study was to visualize the relationship between the printing process parameters with the physical characteristic of the scaffolds and the subsequent cell culture.

2.5. Scaffold cell culture

2.5.1. Cell line

Murine NIH/3T3 fibroblasts were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). NIH/3T3 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Walthman, MA, USA) supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% sodium pyruvate, 50 U/mL penicillin and 50 μg/mL (HyClone, Logan, UT, USA). Cells were maintained in a 5% CO_2 humidified incubator at 37ºC.

2.5.2. Scaffold sterilization

Tubular scaffolds were sterilized with 70% ethanol/water solution overnight, washed twice with PBS (Gibco, Walthman, MA, USA) and finally exposed to UV light for 30 minutes as we previously done for non-tubular scaffolds [23].

2.5.3. Tubular scaffold culture

After sterilization, tubular scaffolds were placed in 12-well non-adherent microplates (Sartstedt, Nümbrecht, Germany). Scaffolds were soaked with DMEM and incubated at 37ºC prior cell seeding to facilitate cell adhesion. After 30 min incubation period, remaining medium was removed and cell seeding process was performed with a final concentration of 5000 cells per scaffold, concerning the cell growth kinetics and the culture period. To ensure a homogeneous and uniform cell growth through the tubular structure a novel procedure was performed according the following diagram (**Fig. 3**).

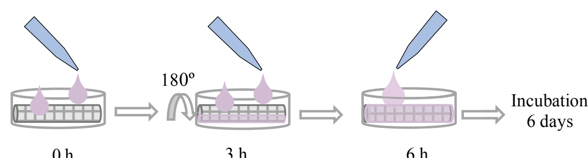


Fig 3. Fibroblast cell seeding methodology for tubular PCL scaffolds

First, a small cell suspension of 2500 cells on 50 μL was prepared. Two droplets of 25 μL were put drop by drop onto the upper surface. Scaffolds were incubated and after 3 hours, once cells were already adhered, structures were turned 180 degrees and seeding process was repeated now in the opposite face. Finally, scaffolds were incubated 3 more hours to allow cell attachment and then culture medium was added. This new methodology derives from a protocol already used for, in this case, non-tubular scaffolds [23].
Fibroblasts culture on tubular scaffolds was maintained during 6 days at 37ºC and 5% CO_2 atmosphere, and culture medium was changed every 3 days. Adherent two-dimensional (2D) controls were performed with same cell density (5000 cells/well).

2.5.4. Cell proliferation assay

MTT (3-4.5-dimethyl-2-thiazolyl)-2.5-diphenyltetrazolium bromide) assay was selected to test fibroblast proliferation on tubular scaffolds. MTT is a yellow tetrazolium salt which can be reduced by the mitochondria of viable cells. This metabolic transformation results in the production of water-insoluble purple crystals of formazan. Produced formazan can be solubilized by dimethyl sulfoxide (DMSO) into a coloured solution. Hence, absorbance of DMSO solution is related to the cell number.
After 6 incubation days, culture medium was removed and tubular scaffolds were put into new wells. They were incubated with 3 mL DMEM medium and 300 μL MTT (Sigma-Aldrich, Saint Louis, MO, USA) during 2 h and 30 min. DMSO was added and scaffolds were shacked to ensure a complete dissolution of resulting formazan crystals. Four 100 μL aliquots from each sample were pipetted into a 96-well plate and absorbance was measured at 570 nm through a microplate reader (Bio-Rad, Hercules, CA, USA). Adherent controls were equally processed.

3. Results and Discussion

This section shows the experimental results for the 20 samples. *Analysis of variance (ANOVA)* method was applied to test the statistical significance of the process parameters (N_{T^a}, $F_{R\%}$, and P_S) for the dimensional quality factors (F_W, and C_A). The analysis was carried out at a 95% confidence level ($\alpha = 0.05$)

3.1. Manufacturing Process: Scaffold structure

The effect of printing process parameters over the Scaffold's strut width and cell or pore area is presented in this section. Regarding the filament width all printing parameters influenced over it **(Fig. 4 a-c).** The increase of printing temperature produced a reduction of material's viscosity, raising its spread capability along the tubular printing bed and enlarging the filament width. Moreover, a printing speed increase also produced a filament width expansion. Finally, concerning the printing fluid flow rate, this parameter control the amount of material expelled by the nozzle. As expected, its rise produced an increase of filament width. Because cell area is related to filament dimensions, the enlargement of strut width resulted in a reduction of cell area. Depending on the employed printing process parameters different cell areas were obtained **(Fig. 4 d-f).**

Cell proliferation is known to be strongly related to pore area,

both the shape and the size, therefore they were analysed on the following section. The novel machine presented in this work has demonstrated its goodness to manufacture tubular scaffold with small diameter. Based on other experiments, it can be concluded that this machine also allows the fabrication of customized scaffolds for other purposes; vascular, tracheal, etc.

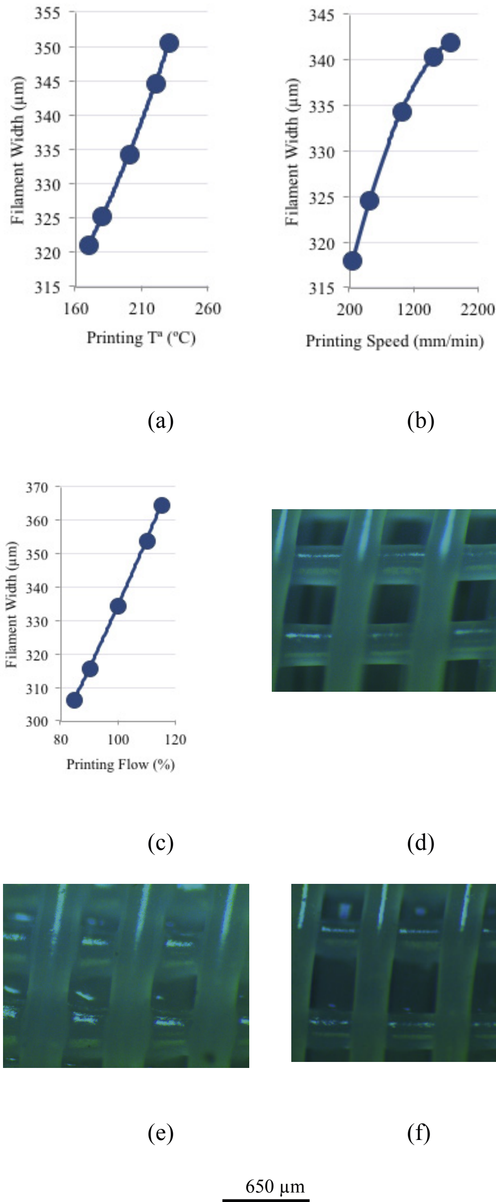


Fig. 4. (a-c) Main Effects Plot for Filament Width depending on **(a)** printing temperature when speed and flow were fixed (1000 mm/min and 100% respectively); **(b)** printing speed when temperature and flow were fixed (200ºC and 100% respectively); and **(c)** printing flow when temperature and speed were fixed (200ºC and 1000 mm/min). **(d)** Optical Image of E01 sample **(e)** Optical Image of E06 sample **(f)** Optical Image of E11 sample

Manufacturing process has shown a great stability being able to manufacture replicas with almost 100% of likeness. The correct selection of process parameters becomes crucial to achieve the CAD geometry design, where the printing temperature is the main parameter. The preceding parameter is in charge of material's viscosity and thus, the amount of material expelled by the nozzle. The creation of a mathematical model of the 3D Printing process would be an interesting tool to improve the parameter optimization process.

3.2. Fibroblast cell culture

On previous section, additive manufacturing process affected final scaffold geometry. In particular, printing temperature, speed and flow presented a significant impact on filament width and, therefore, pore area (**Fig. 4**). Scaffold pattern is proved to possess a great influence on cell proliferation. Consequently, fibroblasts culture was used to test tubular scaffolds suitability for cell applications. Fibroblasts were cultured on tubular scaffolds for 6 days following the novel methodology described in **Fig. 3**. After incubation period, an MTT cell proliferation assay was performed in order to quantify fibroblasts growth on each scaffold design. Purple formazan crystals, which indicated cell localization, were found through all tubular structure of scaffolds and not only on the upper surface (**Fig. 5 a-d**). Thus fibroblast culture was proven to possess a homogeneous nature when following the described cell seeding methodology.

Regarding cell efficiency on tubular scaffolds, all fibroblasts proliferation values were comprised between 40 and 65% compared to a two-dimensional control.

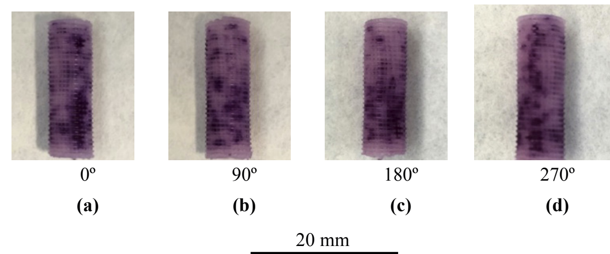


Fig. 5. Tubular scaffold after MTT cell proliferation assay. Four longitudinal faces of the same scaffold were visualized. Purple spots represent formazan crystals and, therefore, fibroblast localization after 6 culture days on tubular scaffolds.

Interestingly, all tested fabrication parameters (printing temperature, printing speed and printing flow) were proven to significantly affect fibroblast growth (p-value<0.000). Cell proliferation exhibited a decreasing trend when printing temperature, speed and flow increased (**Fig. 6**). Concerning the variable printing temperature, when speed (1000 mm/min) and flow (100%) were fixed, lower values of temperature showed an increased fibroblast growth, reaching a maximum of 58.5% of cell proliferation with 180ºC. Larger temperatures clearly attenuated fibroblasts kinetics, decreasing the cell proliferation to a 40%. A similar trend was found analysing the variable of printing speed. Fibroblast proliferation quickly decreased from 65% to 48% when increasing the speed close to 1000 mm/min. Finally, implanting temperature (200ºC) and speed (1000 mm/min), the variable of printing flow seems to possess less effect over cell growth. Only when flow

exceeded the 110%, fibroblasts proliferation decreased abruptly.

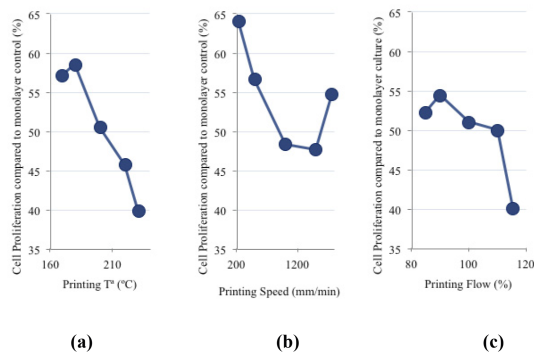


Fig. 6. Main Effects Plot for fibroblast cell proliferation depending on **(a)** printing temperature when speed and flow were fixed (1000 mm/min and 100% respectively); **(b)** printing speed when temperature and flow were fixed (200ºC and 100% respectively) and **(c)** printing flow when temperature and speed were fixed (200ºC and 1000 mm/min). Experiments were performed four times.

As it has demonstrated in previous results, high values of the measured parameters increased the strut width and decreased cell or pore area (**Fig. 4**). Therefore, the highest cell growth rates appeared with big pore areas. Smaller scaffold pores could hinder a perfect oxygen and nutrient exchange for the cells. Culture medium could not easily penetrate into the structure filaments and consequently cells get less oxygen and nutrients, essential for cell growth. This negative effect of narrowed pores on cell growth kinetics was already proposed by some previous studies [25], pointing out the significance of the pore area on cell behaviour.
In addition, the presence of a solid layer on scaffold structure (**Fig. 2**), unless facilitates cell adhesion and confer impermeability to scaffold, could also interfere in the medium transfer.

4. Conclusions

The novel 3D Tubular Printer presented in this work has demonstrated its viability for the manufacture of small-diameter tubular scaffold for tissue engineering. This machine allows the fabrication of customized scaffolds for different purposes such as vascular, tracheal and bones; in just one step.

Regarding fibroblasts seeding, a novel cell seeding methodology for tubular assemblages was described. The explained procedure achieved a homogeneous fibroblast culture through all the structure dimensions, reaching a more physiological model. Moreover, scaffold pattern had a significant effect on fibroblasts growth rate. Increased printing temperature, speed and flow values resulted in a reduced cell proliferation, presumably due to a minor oxygen and nutrient exchange on the small pores. Therefore, scaffold pore area could play a key role on fibroblasts growth as a result of a regulation of mass exchange.

The correct selection of printing process parameters is crucial to achieve the designed scaffold structure and the consequent cell proliferation. In conclusion, tubular PCL scaffolds have a great potential for vascular tissue engineering applications. Low-cost machines such as 3D printers allow the production of customizable and small-diameter scaffolds, manufacturing the optimal design for the reference cells.

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6. References

[1] A. J. Guerra, J. Farjas, and J. Ciurana, "Fibre laser cutting of polycaprolactone sheet for stents manufacturing : A feasibility study," *Opt. Laser Technol.*, vol. 95, pp. 113–123, 2017.

[2] A. J. Guerra and J. Ciurana, "Effect of fibre laser process on in-vitro degradation rate of a polycaprolactone stent a novel degradation study method," *Polym. Degrad. Stab.*, 2017.

[3] N. Muhammad, "Laser Micromachining Of Coronary Stents For Medical Applications," 2012.

[4] S. Puértolas, D. Navallas, a. Herrera, E. López, J. Millastre, E. Ibarz, S. Gabarre, J. a. Puértolas, and L. Gracia, "A methodology for the customized design of colonic stents based on a parametric model," *J. Mech. Behav. Biomed. Mater.*, vol. 71, no. February, pp. 250–261, 2017.

[5] V. Catto, S. Far??, I. Cattaneo, M. Figliuzzi, A. Alessandrino, G. Freddi, A. Remuzzi, and M. C. Tanzi, "Small diameter electrospun silk fibroin vascular grafts: Mechanical properties, in vitro biodegradability, and in vivo biocompatibility," *Mater. Sci. Eng. C*, vol. 54, pp. 101–111, 2015.

[6] B. Marelli, A. Alessandrino, S. Farè, G. Freddi, D. Mantovani, and M. C. Tanzi, "Compliant electrospun silk fibroin tubes for small vessel bypass grafting," *Acta Biomater.*, vol. 6, no. 10, pp. 4019–4026, 2010.

[7] A. Rathore, M. Cleary, Y. Naito, K. Rocco, and C. Breuer, "Development of tissue engineered vascular grafts and application of nanomedicine," *Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology*, vol. 4, no. 3, pp. 257–272, 2012.

[8] V. Catto, S. Farè, G. Freddi, and M. C. Tanzi, "Vascular Tissue Engineering: Recent Advances in Small Diameter Blood Vessel Regeneration," *ISRN Vasc. Med.*, vol. 2014, pp. 1–27, 2014.

[9] S. Wang, Y. Zhang, H. Wang, G. Yin, and Z. Dong, "Fabrication and Properties of the Electrospun Polylactide / Silk Fibroin-Gelatin Composite Tubular Scaffold," pp. 2240–2244, 2009.

[10] D. Pankajakshan and D. K. Agrawal, "Scaffolds in tissue engineering of blood vessels," *Can. J. Physiol. Pharmacol.*, vol. 88, no. 9, pp. 855–873, 2010.

[11] M. Rabionet, M. Yeste, T. Puig, and J. Ciurana, "Electrospinning PCL Scaffolds Manufacture for Three-Dimensional Breast Cancer Cell Culture," *Polymers (Basel).*, vol. 9, no. 8, p. 328, Aug. 2017.

[12] F. Ahmed, N. Roy Choudhury, N. K. Dutta, L. Zou, and A. Zannettino, "Fabrication and characterisation of an electrospun tubular 3D scaffold platform of poly(vinylidene fluoride-co-hexafluoropropylene) for small-diameter blood vessel application," *J. Biomater. Sci. Polym. Ed.*, vol. 25, no. 18, pp. 2023–2041, Dec. 2014.

[13] Y. Wang, H. Shi, J. Qiao, Y. Tian, M. Wu, W. Zhang, Y. Lin, Z. Niu, and Y. Huang, "Electrospun Tubular Scaffold with Circumferentially Aligned Nanofibers for Regulating Smooth Muscle Cell Growth," *ACS Appl. Mater. Interfaces*, vol. 6, no. 4, pp. 2958–2962, Feb. 2014.

[14] L. Ye, J. Cao, L. Chen, X. Geng, A.-Y. Zhang, L.-R. Guo, Y.-Q. Gu, and Z.-G. Feng, "The fabrication of double layer tubular vascular tissue engineering scaffold *via* coaxial electrospinning and its 3D cell coculture," *J. Biomed. Mater. Res. Part A*, vol. 103, no. 12,

pp. 3863–3871, Dec. 2015.

[15] J.-J. Hu, W.-C. Chao, P.-Y. Lee, and C.-H. Huang, "Construction and characterization of an electrospun tubular scaffold for small-diameter tissue-engineered vascular grafts: A scaffold membrane approach," *J. Mech. Behav. Biomed. Mater.*, vol. 13, pp. 140–155, Sep. 2012.

[16] A. Giró-Perafita, M. Rabionet, T. Puig, and J. Ciurana, "Optimization of Poli(Ɛ-caprolactone) scaffolds suitable for 3D cancer cell culture," *Procedia CIRP*, vol. 49, no. The Second CIRP Conference on Biomanufacturing, pp. 61–66, 2016.

[17] Y. J. Tan, X. Tan, W. Y. Yeong, and S. B. Tor, "Additive Manufacturing of Patient-Customizable Scaffolds for Tubular Tissues Using the Melt-Drawing Method.," *Mater. (Basel, Switzerland)*, vol. 9, no. 11, Nov. 2016.

[18] L. Hou, J. Jin, J. Lv, L. Chen, Y. Zhu, and X. Liu, "Constitution and in vivo test of micro-porous tubular scaffold for esophageal tissue engineering," *J. Biomater. Appl.*, vol. 30, no. 5, pp. 568–578, Nov. 2015.

[19] J. De Ciurana, L. Serenó, and È. Vallès, "Selecting process parameters in RepRap additive manufacturing system for PLA scaffolds manufacture," in *Procedia CIRP*, 2013, vol. 5, pp. 152–157.

[20] H. Wang, S. Vijayavenkataraman, Y. Wu, Z. Shu, J. Sun, and J. Fuh Ying Hsi, "Investigation of process parameters of electrohydro-dynamic jetting for 3D printed PCL fibrous scaffolds with complex geometries," *Int. J. Bioprinting*, vol. 2, no. 1, Jan. 2016.

[21] S. A. Shaffiey, H. Jia, T. Keane, C. Costello, D. Wasserman, M. Quidgley, J. Dziki, S. Badylak, C. P. Sodhi, J. C. March, and D. J. Hackam, "Intestinal stem cell growth and differentiation on a tubular scaffold with evaluation in small and large animals," *Regen. Med.*, vol. 11, no. 1, pp. 45–61, Jan. 2016.

[22] J. Wang, Y. Wei, H. Yi, Z. Liu, D. Sun, and H. Zhao, "Cytocompatibility of a silk fibroin tubular scaffold," *Mater. Sci. Eng. C*, vol. 34, pp. 429–436, Jan. 2014.

[23] S. Palomeras, M. R abionet, I. Ferrer, A. Sarrats, M. L. Garcia-Romeu, T. Puig, and J. Ciurana, "Breast Cancer Stem Cell Culture and Enrichment Using Poly(ε-Caprolactone) Scaffolds.," *Molecules*, vol. 21, no. 4, p. 537, Jan. 2016.

[24] J. Martín Cañabate, "PCL scaffolds design and fabrication for 3D fibroblast culture," Final Project. 2016.

[25] C. J. Galban and B. R. Locke, "Analysis of cell growth kinetics and substrate diffusion in a polymer scaffold.," *Biotechnol. Bioeng.*, vol. 65, no. 2, pp. 121–32, Oct. 1999.