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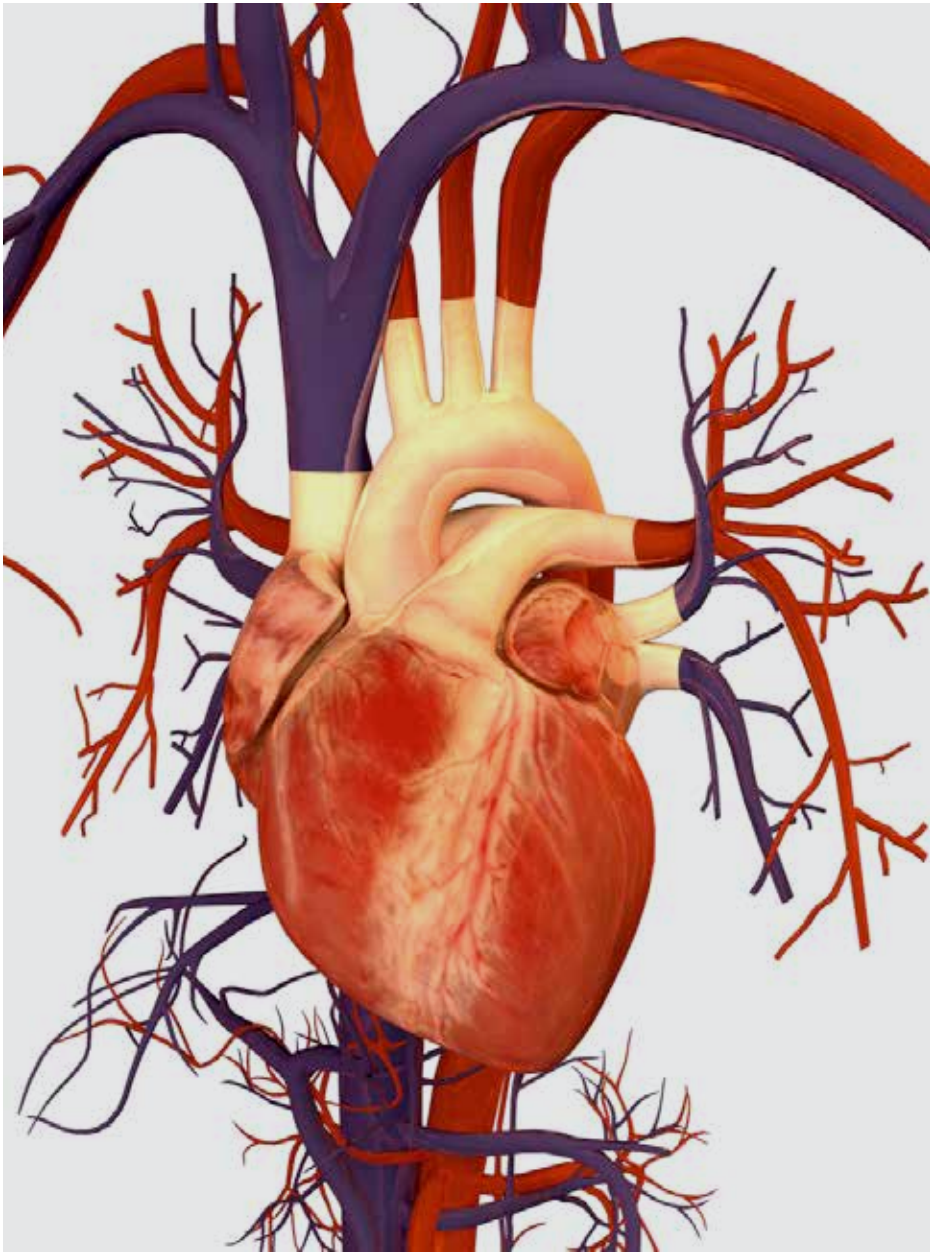
Design of a polymeric replica of the human aortic arch for *in vitro* experimentation

Introduction

The aorta is the most important blood vessel in the body, which carries highly oxygenated blood from the heart to the different organs. It is about 30 cm long (Dominguez, 2015) with a diameter of 2,5 cm in the ascending section (Aaronson & Word, 2012).

Aneurysms are the main aortic disease (Heart disease and aortic aneurysm, 2015), which affects 27% of men over 65 years. Current treatments for aneurysms are open surgery and endovascular repair (EVAR) (Pons, 2015; Greenhalgh et al., 2004). In both cases, the damaged fragment is substituted or channeled with a Dacron® or Teflon® graft, inert materials with properties different to those of the vascular tissue replaced. Moreover, EVAR is only available for aneurysms in the descending aorta.

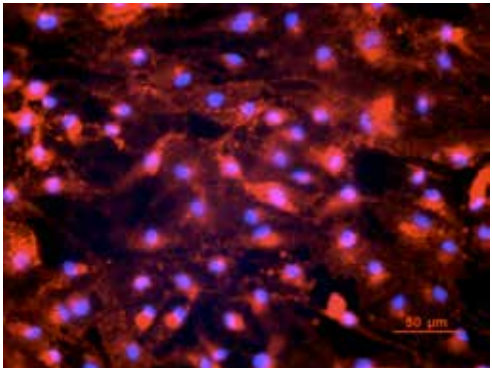
A scaffold is an artificial 3D support, typically made with polymeric biomaterials, which helps in tissue regeneration providing the structural support for cells and growth factors (Tissue engineering and regenerative medicine, 2015; Chan & Leong, 2008). Therefore, scaffolds with similar characteristics to the aorta might be an alternative treatment for aneurysms. On the basis that a scaffold built with a polymer with similar characteristics to the aorta might improve patient's quality of life by helping the regeneration of the tissue and restoring the original properties of the healthy vessel, the goal



of this project is to manufacture an *in vitro* replica of the ascending aorta, based on a medical image, which could be built tailored to every patient.
Previous projects at the IQS School of Engineering had studied the best biomaterial for creating scaffolds of the human vasculature and, more specifically, the aorta. After considering the aorta mechanical characteristics, polycaprolactone (PCL) (Sánchez, 2015) was chosen. Therefore, this project has focused on creating the first prototype of a scaffold of the human ascending aorta using PCL and 3D printing technology. To achieve that, I followed three steps:
1. Seeking the proper support to give the suitable shape to the PCL.
2. Finding a way to eliminate the support without damaging the PCL structure.
3. Confirming cellular adhesion and adequate morphology.

Materials and methods
Porosity is a very important characteristic of the scaffold. It can be modified using chemicals called porogen. The salt leaching method was chosen as the most suitable one to create porous scaffolds. Applying this method to our case, PCL was dissolved in chloroform and sodium chloride (NaCl) was added to the solution. The solvent evaporated and a polymer-salt composite was obtained. The NaCl particles were then dissolved in water, leaving the pores in the PCL structure.
To shape the scaffolds, we used Sr-30 3D printed molds. These molds were designed with SolidWorks®, and based on a clinical image obtained from a healthy patient (Pons, 2015). That ensured that the scaffolds could be built tailored to each patient. The scaffolds were obtained covering Sr-30 3D printed molds with the PCL solution and then dissolving the molds.
To seed cells in the PCL scaffold, normal human dermal fibroblasts (NHDF) were cultured *in vitro* and injected inside the scaffold. The whole structure was fastened inside a 37ºC, 5% CO_2 incubator and placed on a slowly rotating apparatus to ensure seeding homogeneity.

Results and discussion
Even though the main goal of the project was the manufacture of an *in vitro* replica of the human ascending aorta, I started trying to create PCL replicas of the carotid bifurcation, as they were simpler than the aortic arch and, therefore, easier to achieve. To shape the PCL I used wax molds, which I covered with different layers of the PCL solution. The first issue found was that wax was soluble in chloroform, so when the PCL-chloroform solution was applied on the wax, it dissolved, leaving holes in the PCL structure that was drying on it.



A physical barrier between the wax mold and the PCL solution was the first option tried to make PCL and wax compatible. Different materials, nonsoluble in chloroform, nonpermeable, cheap, easy to model and also easy to remove without damaging the PCL were considered. After some tests, animal intestine was chosen as the best material to create this barrier.
In the following set of experiments, we tried to create the PCL structures covering the wax molds with the animal intestine and then applying four layers of PCL-chloroform solution. Three different combinations were tested: wax support with a layer of lamb intestine, wax support with a layer of lamb intestine and pork intestine, wax support with two layers of lamb intestine and pork intestine. In any of these cases the PCL replica of the carotid bifurcation was achieved. That led to the conclusion that the animal intestine was not impermeable enough to isolate the PCL-chloroform solution from the wax, therefore different strategies to isolate the PCL-chloroform solution from the wax needed to be found.
After failing with intestine, the following idea focused on trying a combination of two materials, plastic wrap and intestine. Plastic wrap was impermeable and isolated the wax mold from the PCLchloroform solution. Moreover, it was cheap, very moldable and nonsoluble in chloroform. Its main issue, the fact that it could be not

separated from the PCL, could be solved with the second material, the intestine. The intestine would be set all over the plastic wrap and would prevent it from sticking to the PCL. An experiment to confirm the effectiveness of this method was carried out and finally a replica of the carotid bifurcation could be achieved.
In summary, a good method to give the PCL the appropriate shape had been found. It consisted in covering a wax mold with plastic wrap and animal intestine. This structure was then soaked in a PCL-chloroform solution four times to create four layers of PCL and it was then air-dried.
The same method found was used to create the replicas of the human aorta. Sr30 molds were also soluble in chloroform, so they were isolated from the PCL-chloroform solution with animal intestine too. In this case, plastic wrap was not necessary, as Sr30 was less soluble than wax, in chloroform. To build the PCL replica of the aortic arch, the Sr30 molds were covered with the animal intestine and five layers of PCL solution.
Once the PCL had dried on the Sr30 mold, it was needed to dissolve the Sr30 support to keep only the PCL structure. According to the enterprise who manufactures Sr30, it dissolves in a pH 13 NaOH solution. When we tried it, PCL broke in these conditions, as it could not stand such a basic solution for much time. Therefore we needed to find another solvent to dissolve the Sr30 which did not damage the PCL. After continued research, we could not find much information about the solubility of Sr30, so we carried out a solvents test, that is to say that we tested the effect of different solvents on PCL and Sr30, looking for one that dissolved the Sr30 without dissolving or modifying the PCL (Bordes et al., 2010). The best option found was ethanol. Another parameter that we checked was to confirm that the structures which had been in contact with the solvent and the dissolved Sr30 were not toxic for cells. A toxicity test which consisted in putting in contact little fragments of this structures (washed in ethanol a different number of times) with cultured cells was performed. The results were good and the following time the Sr30 mold was dissolved in ethanol. Finally the Sr30 mold could be dissolved without damaging the PCL structure and the scaffold of the human aortic arch was obtained (Figure 1: photo of the PCL replica of the human ascending aorta).
To test cell adhesion, normal human dermal fibroblasts (NDHF) were seeded in PCL carotid bifurcation structures twice. In the first seeding ($1.6 \cdot 10^6$ cells), cell adhesion was observed under the fluorescence microscope, although it was limited and heterogeneous over the walls, with isolated crowded areas and empty spaces. On the other hand cells seemed well attached and they had grown with the appropriate conformation. Based on this observations, we can suggest the hypothesis that cells are comfortable in this environment. Based on the observations under the fluorescence microscope, we could also confirm that the PCL structure had

pores of about 25μm in diameter. A new set of experiments was designed with a higher seeding concentration. In the second seeding, the number of cells was doubled ($5.06 \cdot 10^6$ cells) and this time cell adhesion over the walls of the PCL structure increased compared to the previous experiment. The distribution of the cells along the structure was also more homogeneous (Figure 2: photo taken with the fluorescence microscope).

Conclusions

1. The PCL structures with the shape of the arch of the aorta can be obtained by covering Sr-30 3D printed molds with five layers of PCL solution. Chloroform in the PCL solution can be isolated from the Sr-30 using lamb and pork intestine.
2. A pH 13 sodium hydroxide solution to dissolve the Sr-30 mold has been discarded. Instead, the Sr-30 molds can be dissolved with ethanol without affecting the PCL integrity. Nonetheless, more than 5 L of ethanol were used to dissolve each structure, therefore more research will be needed in order to find a way to reduce this amount.
3. Homogeneous cell adhesion in the PCL 3D structure was verified under the fluorescence microscope. Cells had the appropriate morphology in the PCL environment.

Bibliography

— DOMINGUEZ, P. *Optimización de un soporte de Policaprolactona para modelar arterias humanas*. TFG. (IQS, 2015). —Aaronson, Philip I.; Ward, Jeremy P. T. *The Cardiovascular System at a Glance*, 2012. — *Heart disease and aortic aneurysm*. [On line.] <http://www.webmd.com/heart-disease/heart-disease-aortic-aneurysm> [Date accessed: 2015-08-20] — PONS, R. *Fluidodynamic markers of aortic aneurysm progression*. TFM. (IQS, 2015). — GREENHALGH, R. M.; BROWN, L. C.; KWONG, G. P. S.; POWELL, J. T.; THOMPSON, S. G. «Comparison of endovascular aneurysm repair with open repair in patients with abdominal aortic aneurysm (EVAR trial 1), 30-day operative mortality results: randomised controlled trial». *Lancet,* 364 (2004), p. 843-848. — *Tissue engineering and regenerative medicine*. [On line.] <http://www.nibib.nih.gov/ science-education/science-topics/tissue-engineering-and-regenerative-medicine> [Date accessed: 2015-09-10] — CHAN, B. P.; LEONG, K. W. «Scaffolding in tissue engineering: general approaches and tissue-specific considerations». *European Spine Journal*, 17 (2008), p. 467-479. — SÁNCHEZ, M. *Diseño de un soporte polimérico para reemplazar tejidos vasculares*. TFM. (IQS, 2015). — BORDES, C. *et al.* «Determination of poly(μcaprolactone) solubility parameters: Application to solvent substitution in a microencapsulation process». *Int. J. Pharm.*, 383 (2010), p. 236-243.