1. Abstract

The aim of the project is to optimize the parameters of a polymer deposition Rapid Prototyping (RP) machine in order to make compatible scaffolds of biodegradable polymers.

The polymers used are PLA (PolyLactic Acid dissolved in Chloroform or 1,4-dioxane) and Chitosan (dissolved in Acetic Acid). Both are well known in the field of Tissue Engineering (TE) due to their interesting properties, such as biodegradability and biocompatibility.

The RP technique will allow us to easily control the porosity and the interconnectivity of the pores, two very important characteristics for the development of successful scaffolds. However, some improvements must be done in order to obtain real 3D-scaffolds. The modification of several parameters of the deposition of the polymer such as the printing speed, the polymer flow, the height of a single line, the polymer concentration and of course the design of the scaffold, will lead to the fabrication of real 3D-scaffolds.

After a presentation of the TE research field, a brief introduction of conventional methods and RP methods will be given. Then we will focus on our polymer deposition machine and the parameters optimized for finally ending on our results obtained with this RP method.

SEM micrograph of a scaffold made by this RP technique (200x).
Fabrication of 3D-porous scaffolds by Rapid Prototyping method
2. Summary

1. Abstract .......................................................................................................................... 1

2. Summary ........................................................................................................................ 3

3. Introduction .................................................................................................................... 5

3.1. Tissue Engineering .................................................................................................... 5

3.2. The Support system: Scaffold .................................................................................... 7

3.3. Conventional techniques ............................................................................................ 9

3.3.1. Particulate leaching ............................................................................................... 9

3.3.2. Gas foaming .......................................................................................................... 10

3.3.3. Thermal Induced Phase Separation (TIPS) .......................................................... 12

3.3.4. Emulsion freeze drying ......................................................................................... 13

3.4. Rapid Prototyping (RP) ............................................................................................. 15

3.4.1. 3D printing (3DP) .................................................................................................. 15

3.4.2. Stereolithography (SL) .......................................................................................... 17

3.4.3. Selective Laser Sintering (SLS) ............................................................................ 18

3.4.4. Fused Deposition Modeling (FDM) ....................................................................... 20

3.4. Aim of the project .................................................................................................... 25

4. Experimental part .......................................................................................................... 27

4.1. Materials .................................................................................................................... 27

4.2. RP machine ............................................................................................................... 28

4.2.1. Technic specs ....................................................................................................... 28

4.2.2. Pump specs ........................................................................................................... 28

4.2.3. CAD tool .............................................................................................................. 29

4.3. Rapid Prototyping ..................................................................................................... 33

4.3.1. Air-syringe pump ................................................................................................. 33

4.3.2. Smart pump .......................................................................................................... 44

4.3.3. Displacement pump .............................................................................................. 47

4.4. PLA in chloroform versus PLA in 1,4-dioxane ......................................................... 48

4.5. Interferometry ........................................................................................................... 49

4.6. Scanning Electron Microscopy (SEM) ....................................................................... 56

5. Conclusions ................................................................................................................... 61

6. Acknowledgments ......................................................................................................... 62

7. References ..................................................................................................................... 63
3. Introduction

3.1. Tissue Engineering

Tissue Engineering (TE) is an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function [1]. Three strategies have been adopted for the creation of new tissues [2]:

- Isolated cells or cell substitutes. This approach avoids the complication of surgery, allows replacement of only those cells that supply the needed function, and permits manipulation of cells before infusion. Its potential limitations include failure of the infused cells to maintain their function in the recipient, and immunological rejection [2].

- Tissue-inducing substances. The success of this approach depends on the purification and large-scale production of appropriate signal molecule, such as growth factors, and, in many cases, the development of methods to deliver these molecules to their target [2].

- Cell placed on or within matrices. In closed systems, the cells are isolated from the body by cellular membrane that allows permeation of nutrients and waste products but prevents large entities such as antibodies or immune cells from destroying the transplant. These systems can be implanted or used as extra corporeal devices. The principle of TE is to culture cells into a matrix and incorporate it into the body. The matrices are made of natural materials such as collagen or from synthetic polymers. Immunological rejection may be prevented by immunosuppressive drugs [2].

![Figure 1. Schematic description of TE procedure.](image-url)
The strategy for Tissue Engineering is divided in six steps (Figure 1). In the first step, cells need to be extracted from the patient. Then, it is necessary to isolate the desired cell type from the crude cell extract. The isolation is essential because this is a selection of cells we want to use and grow for our TE application. In many cases, the population of the desired cells is very low, thus it is essential to growth and expand the population of these cells \textit{in vitro}. This step is very important when working with stem cells because the stem cells need to be maintained in their pluripotent state while their population is increased. Once we have sufficient number of cells, they are implanted in a matrix, called scaffold. The scaffold offers a suitable environment to permit cells to stick, growth, and develop in the right direction. This proliferation is now controlled and catalyzed by the scaffold design and coating. Finally, the scaffold with its inner and outer cells is ready to be implanted into the patient by the surgeon.
3.2. The Support system: Scaffold

Scaffolds designed to regenerate body tissues by seeding cells into porous matrices may allow the cell growth and tissue remodeling through a combination of structure and chemical signaling. In comparison to cell culture on 2D plates, culturing cells in 3D scaffolds results in a microenvironment more closely resembling that found in vivo, such that the seeded cells respond to mechanical and biological cues from the 3D locations to promote cell adhesion, proliferation and differentiation. Suitable tissue scaffolds are subjected to certain design criteria [3]. For example scaffolds should have features such as high porosity, interconnected pore structure and biocompatible surface chemistry. Besides being non-antigenic, non-carcinogenic, non-toxic, non-teratogenic and biocompatible, it is crucial for the material to have controlled biodegradability or bioresorbability so that the scaffold can eventually replaced by cells native to the host [3].

In Table 1, the preferred scaffolds pore size for different cells types are summarized [4]:

<table>
<thead>
<tr>
<th>Tissue regeneration</th>
<th>Cell size (µm)</th>
<th>Preferred pore diameter (µm)</th>
</tr>
</thead>
</table>

The requirements for the achievement of successful scaffolds for TE application are the following [11]: (1) the scaffold must present a large surface area and proper surface chemistry to favor cellular attachment, differentiation and proliferation; (2) It should have interconnecting pores of appropriately sized to favor tissue integration and vascularization; (3) the scaffold needs sufficient mechanical properties for handling the implantation in the intended site.

Many materials, both natural and synthetic, have been postulated as suitable candidates for their use as scaffolds for TE [4]. These materials as summarized in Table 2:
The major goal in scaffold manufacturing is to maintain control over its macro-structural (mechanical properties and porosity) and micro-structural (pore size, pore distribution and pore interconnectivity) properties. Therefore, the key requirements for a controlled process include [11]: (1) the processing procedures and conditions should not adversely affect the material properties and subsequent clinical utility of the scaffolds; (2) the process accurately controls the macro and microstructure; (3) the scaffolds are reproducible; (4) the process should be flexible enough to fabricate architecture for different tissues and organs.

Numerous techniques have been developed to manufacture scaffolds to meet the above mentioned prerequisites. Such techniques include the conventional manual-based fabrication techniques as well as the use of computer-aided fabrication techniques [11].

<table>
<thead>
<tr>
<th>Biomaterials</th>
<th>Degradation time</th>
<th>Degradation mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural polymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>2–24 weeks (depending on the crosslinking degree)</td>
<td>Enzymatic degradation [12]</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Half life weight: 10–56 days for 52%–62% de-acetylation degree (DD), more than 84 days for DD &gt; 72%</td>
<td>Enzymatic degradation [13]</td>
</tr>
<tr>
<td>Synthetic Polymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly (L-lactic acid) (PLLA)</td>
<td>2–12 months</td>
<td>Hydrolytic mechanism [8]</td>
</tr>
<tr>
<td>Poly (glycolic acid) (PGA)</td>
<td>4–6 months</td>
<td>Hydrolytic mechanism [8]</td>
</tr>
<tr>
<td>Poly (caprolactone) (PCL)</td>
<td>1–2 years</td>
<td>Hydrolytic mechanism [14]</td>
</tr>
<tr>
<td>Bio-ceramics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite (HA)</td>
<td>In the order of years, poor degradation</td>
<td>Dissolution, resorbed by osteoclast [15]</td>
</tr>
<tr>
<td>Tricalcium phosphate (TCP)</td>
<td>8–24 weeks</td>
<td>Dissolution, resorbed by osteoclast [16]</td>
</tr>
</tbody>
</table>
3.3. Conventional techniques

3.3.1. Particulate leaching

A particulate-leaching method was developed to prepare highly porous biodegradable polymer membranes [17]. It involves the casting of polymer/porogen composite followed by the dissolution of the porogen (Figure 2). Polymer porous membranes of controlled porosity, surface/volume ratio, and crystallinity were prepared with porogen particles, such as sodium chloride or sodium citrate particles. The porosity increased with the porogen weight fraction, and the median pore diameter increased as the porogen particle size increased. Foams produced via this method were 99.9 wt% porogen free and had porosities as high as 93% and average pore diameters up to 150 µm [18].

![Figure 2. Schematic description of particulate leaching method [19].](image)

It is a simple and user friendly method, suitable with a range of biomaterials and no special equipment is needed. However there is a non uniform pore size distribution resulting in density differences. Moreover it is difficult to achieve full interconnectivity and large pore interconnections. Finally organic solvents are typically required which is tricky for biocompatibility [19].
Using this technique, macro-porous composite scaffolds comprising resorbable Calcium Phosphate (CaP) particles embedded within a poly (lactide-co-glycolide acid) (PLGA) 75/25 matrix were prepared (Figure 3). Scaffolds have a nominal macro-pore size in the range of 800–1800 µm with porosity between 81–91%. These scaffolds can be used as an alternative to the trabecular bone graft [20].

3.3.2. Gas foaming

It is a technique to fabricate macro-porous sponges from synthetic biodegradable polymers using high pressure carbon dioxide processing at room temperature [21],[22]. Solid discs of polymer were saturated with CO₂ by exposure to high pressure CO₂ gas for hours at room temperature. The solubility of the gas in the polymer was then rapidly decreased by reducing the CO₂ gas pressure to atmospheric levels. This created a thermodynamic instability for the CO₂ dissolved in the polymer discs, and resulted in the nucleation and growth of gas cells within the polymer matrix (Figure 4). Polymer sponges with large pores (approximately 100 µm) and porosities of up to 93% could be fabricated with this technique. The porosity of the sponges could be controlled by the preform production technique, and mixing crystalline and amorphous polymers [23].
This is a simple method with suitable range of biomaterials and no special equipment needed. Fully interconnecting pores and large pore interconnections can be fabricated. Moreover, no organic solvents are required. However, the scaffolds obtained by this technique present poor mechanical properties due to their foam structure.

Open-pore biodegradable foams with controlled porous architectures were prepared by gas foaming micro-particulate of poly (ε-caprolactone) (Figure 5). Disc-shaped samples were saturated with CO$_2$ in a high-pressure vessel (HiP), at 65 bar and at a saturation temperature of 70°C for 3 h and subsequently cooled to the ambient temperature. The pressure was then quenched to ambient pressure [24]. The control of porosity (in the range 78–93%) and pore size (up to 10 µm) have been achieved [24].
3.3.3. Thermal Induced Phase Separation (TIPS)

Thermal-induced phase separation (TIPS) has been shown to be an excellent technique to make micro-porous polymeric membranes [25]. After a polymer is dissolved in a solvent at a high temperature, phase separation by spinodal decomposition is induced by cooling or quenching the solution (Figure 6). The TIPS process is classified mainly into two types such as solid-liquid (S-L) TIPS, where the polymer crystallizes out of the solution, and liquid–liquid (L-L) TIPS, where the solution separates into a polymer-rich continuous phase and a polymer-lean droplet phase [26]. Phase separation continues until the polymer-rich phase becomes immobilized by gelation, glass transition, or crystallization. Once this occurs, the structure is effectively frozen into place and the solvent can be removed from the film. The dried film can then serve as a micro-porous membrane or scaffold [25]. Porosity up to 90% and pore size in the range of 5 to 600 µm can be obtained [25].

![Figure 6. Schematic description of spinodal decomposition method](..)

It is a simple method, suitable with a range of biomaterials and no special equipment is needed. Fully interconnecting pores and large pore interconnections can be fabricated if spinodal decomposition is totally achieved. Moreover, organic solvents are still used and causes problem of biocompatibility [27].
Porous scaffolds of poly(lactide-co-glycolide acid) (PLGA)/nano hydroxyapatite (NHA) in 1,4-dioxane were fabricated by thermally induced phase separation technique (Figure 7). First, NHA powders were dispersed in 1,4-dioxane/water mixture by ultrasonication. Then, PLGA was added into the solution. The PLGA/NHA compounds were heated at 65°C in the ultrasonicator until the mixed solution turned into homogeneous. Then sample was heated to 15°C above the measured cloud-point temperature, after which it was quickly dipped into bath maintained at the desired quenching temperature (5 or 9°C). The sample was removed from the quenching bath after (0.5, 2, 4, 6 or 8 h) to observe the coarsening effect. The annealed sample was immediately immersed in liquid nitrogen to be fast frozen. At last, the sample was freeze-dried at 60°C and 14 Pa for at least 48 h to remove the solvents, yielding the porous scaffolds [28]. Regular and highly interconnected macro-porous (100–150 µm) are obtained. These scaffolds are developed for efficient bone tissue engineering [28].

3.3.4. Emulsion freeze drying

The method consists on the formation of an emulsion by homogenization of a polymer solvent solution and water, rapidly cooling the emulsion to lock in the liquid-state structure, and removing the solvent and water by freeze-drying (lyophilization) (Figure 8) [29]. Foams with porosity in the range 91 - 95% were obtained with an average pore diameter ranging from 13 to 35 µm with good interconnections between the pores. However larger pore diameters bigger than 200 µm were also found.
Figure 8. Schematic description of emulsion freeze drying method.

This is a simple method with suitable range of biomaterials and no special equipment needed. Fully interconnecting pores and large pore interconnections can be fabricated. No organic solvents are required. Nevertheless, the foams obtained with this method have bad mechanical properties.

Figure 9. SEM micrograph of the porous HA/PHBV composite scaffold [30].

Biodegradable polymer-based scaffolds containing hydroxyapatite (HA) particles can be very useful for bone tissue engineering. In this investigation, HA nano-particles were incorporated in poly(hydroxybutyrate- co-valerate) (PHBV) polymer to fabricate, via emulsion freeze drying process, osteoconductive composite scaffolds (Figure 9). The scaffolds produced are highly porous and exhibit interconnected porous structures with pore sizes ranging from 10 to 200 µm [30].
3.4. Rapid Prototyping (RP)

These conventional techniques are incapable of precisely and repeatedly controlling the microstructure of the scaffold in terms of pore size, geometry interconnectivity, and spatial distribution of pores. The scaffolds fabricated with these techniques have a foam structure, which is difficult for cells to migrate through because of the coarse microstructure and poor interconnectivity. Moreover, some of them use organic solvents which are difficult to remove [11].

To overcome the drawbacks of the conventional techniques, Rapid Prototyping (RP) methods are attracting the interest of the TE community. The RP techniques allow the fabrication of very complex 3D structures in a layer-wise fashion in a reproducible way [31].

RP also known as Solid Freeform Fabrication (SFF) is a group of techniques that creates three-dimensional (3D) objects through repetitive deposition and processing of material layers using computer-controlled equipment. It is based on the 2D cross-sectional data obtained from slicing a computer-aided design (CAD) model of the object [32]. They have the advantage of being able to build structures with customized shapes and better control over localized pore morphologies, porosities, and material composition to suit the requirements of multiple cell types arranged in hierarchical structures. However, most of these techniques were originally conceived to produce engineering prototypes rather than bio-functional structures [3].

3.4.1. 3D printing (3DP)

The 3DP technology was developed at the Massachusetts Institute of Technology (MIT) [33],[34]. Basically, the 3DP is a layered fabrication process, in which the sliced 2D profile of a computer model is printed on a fresh layer of powder via deposition of a suitable binder (Figure 10). Successive 2D profiles are then printed on a freshly laid layer of powder until the whole model is completed. The printed binder would join the respective profiles of each layer together. The specimen is completed upon removal of the unbound powder and suitable post-processing [35]. In the past, this technique was mainly used to fabricate drug delivery devices [36]. Recently, the capability of creating complex 3D structures attracted tissue engineers to apply the technology to design and fabricate scaffolds [32].
With this method, it is possible to obtain porosity between 45 - 60%, with pore size in the range of 45-1600 µm [38]. The layer-by-layer process allowed fabrication of complex architectures with excellent resolution. However, one of the main drawbacks of this technique is the high cost of the equipment needed. Moreover, significant times are required to manufacture scaffolds with suitable thickness [38].

With this 3D printing method, it is possible to fabricate cylindrical scaffolds of copolymers polylactide–coglycolide (PLGA, 85L/15G) in a suitable solvent like dichloromethane (Figure 11). Interconnected porous channels of about 800 µm and micro-porosities of 45–150 µm were achieved [32].
3.4.2. Stereolithography (SL)

Stereolithography is an additive fabrication process using a liquid UV-curable photopolymer and a UV laser to build structures a layer at a time (Figure 12). This relies on a photosensitive monomer resin which polymerizes and solidifies when exposed to UV light. Due to the absorption and scattering of the beam, this reaction only takes place near the surface [39]. An SL machine consists of a build platform (substrate) which is mounted in a vat of resin and UV helium-cadmium or argon ion laser. The first layer of the part is printed on the resin surface by the laser using information obtained from the 3D solid CAD model. Once the contour of the layer has been scanned and the interior either hatched or filled, the platform is next lowered to the base of the vat to coat the part thoroughly. It is then raised such that the top of the solidified is level with the surface and a blade wipes the resin leaving exactly one layer of resin above the part. The part is then lowered to one layer below the surface and left until the liquid has settled [40]. This is done to ensure a flat, even surface and to inhibit bubble formation. Then, the next layer may be scanned and fabricated.

![Schematic description of stereolithography method](image)

Figure 12. Schematic description of stereolithography method [37].

Large porosity could be obtained with this method (~90%), with pore size in the range of 20-1000 µm [41]. An accurate control over pore size and interconnectivity can be achieved. The layer-wise fashioned process allowed fabrication of complex and anatomically-shaped structures. However, the machinery required are very expensive and only polymers compatible with UV curing can be used [41].
In this example, Poly(propylene fumarate) (PPF) was used as the material, diethyl fumarate (DEF) was used as the solvent, and bisacrylphosphrine oxide (BAPO) was used as the photo-initiator (Figure 13). Pore size up to 1000 µm with good interconnectivity was obtained with this SL method [42].

3.4.3. Selective Laser Sintering (SLS)

SLS uses a fine powder of material which is heated with CO\textsubscript{2} laser of power in the range of 25–50 W such that the surface tensions of the grain are overcome and they fuse together (Figure 14). Before the powder is sintered, the entire bed is heated to just below the melting point of the material in order to minimize thermal distortion and facilitate fusion to the previous layer [43]. Each layer is drawn on the powder bed using the laser to sinter the material. Then the bed is lowered and a powder-feed chamber raised. A new layer of powder is deposited and spread by a counter rotating roller. The sintered material forms the desired structure while the unsintered powder remains in place to support the structure and may be cleaned away and recycled once the process is completed [44]. There is a large range of materials available for this process, basically any material which can be pulverized may be employed, such as ceramics and polymers.
Only porosity up to 40% could be obtained, with pore size in the range of 30-2500 µm [45]. Once again, an accurate control over pore size and interconnectivity can be achieved and the layer-by-layer process allowed fabrication of complex and anatomically-shaped structures. However, the machinery equipment is expensive. Resolution limitations occur at lower pore sizes. Furthermore the biomaterials need to come in powder form with tight controlled particle size. Thus, this technique is mainly applicable to ceramic materials [45].

Porous polycaprolactone (PCL) scaffolds were computationally designed and then fabricated via SLS technique (Figure 15). Pores ranged from 1.75–2.5 mm in diameter, producing
scaffolds with designed volumetric porosity ranging from 63 to 79%. PCL scaffolds fabricated via SLS are being studied for bone and cartilage TE application [46].

3.4.4. Fused Deposition Modeling (FDM)

FDM works also on an additive principle by laying down material in layers (Figure 16). A plastic filament or metal wire is unwound from a coil and supplies material to an extrusion nozzle which can turn on and off the flow. The nozzle is heated to melt the material and can be moved in both horizontal and vertical directions by a numerically controlled mechanism, directly controlled by a computer-aided design software package. In a similar manner to stereolithography, the model is built up from layers as the material hardens immediately after extrusion from the nozzle [47].

![Figure 16. Schematic description of Fused Deposition Modeling method [37].](image)

Large porosity up to 80% can be achieved, with pore size in the range of 100-2000 µm. [48],[49],[50]. The exact control of pore size and interconnectivity can also be obtained. The layer-wise fashion process allowed fabrication of complex pore architectures and anatomically-shaped structures with good resolution. But since the technique uses polymer melts, it is limited to thermoplastic polymers. Low pore sizes are difficult to obtain while maintaining high porosity [48],[49],[50].
For example, polybutylene terephthalate (PBT) polymer were fused deposed in order to produce porous scaffolds (Figure 17). Pore size with a range of 100-1000 µm and average porosity of 65% were obtained with this FDM method. These scaffolds can be used as an alternative to trabecular bone [51].

A summary of biomaterials used in RP fabrication of TE scaffolds and the achieved mechanical properties are given in the Table 3 [52]:

Table 3. Mechanical properties of the scaffolds fabricated using RP techniques.

<table>
<thead>
<tr>
<th>RP process</th>
<th>Biomaterials</th>
<th>Range of material properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D Printing</td>
<td>Dextran / gelatin</td>
<td>Compressive modulus: 0.059–0.102 MPa [32]</td>
</tr>
<tr>
<td></td>
<td>HA</td>
<td>Compressive strength: up to 22 MPa [38]</td>
</tr>
<tr>
<td></td>
<td>Poly(urethane) (PU)</td>
<td>Young’s modulus: 580 MPa [52]</td>
</tr>
<tr>
<td>Selective Laser Sintering</td>
<td>PCL</td>
<td>Compressive modulus: 52–67 MPa [53]</td>
</tr>
<tr>
<td></td>
<td>PCL/HA</td>
<td>Compressive modulus: 33–102 MPa [54]</td>
</tr>
<tr>
<td>Fused Deposition Modeling</td>
<td>PCL</td>
<td>Compressive modulus: 4–77 MPa [55]</td>
</tr>
<tr>
<td></td>
<td>Alumina</td>
<td>Compressive strength: 50 MPa [56]</td>
</tr>
</tbody>
</table>

FINAL SUMMARY TABLE [19]:
<table>
<thead>
<tr>
<th>Scaffold Name</th>
<th>Porosity &amp; Pore size</th>
<th>Schematic</th>
<th>Advantages &amp; disadvantages</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particulate leaching</td>
<td>Porosity up to 95% 5C - 1800 µm</td>
<td><img src="#" alt="Schematic" /></td>
<td>Advantages: Simple and easy method, suitable with a range of biomaterials and no special equipment is needed. Disadvantages: Density differences result in non-uniform pore size distribution. Difficult to achieve full interconnectivity and large pore interconnections. Organic solvents typically required.</td>
<td><img src="#" alt="Image" /></td>
</tr>
<tr>
<td>Gas forming</td>
<td>Porosity up to 95% 5-200 µm</td>
<td><img src="#" alt="Schematic" /></td>
<td>Advantages: Simple method, suitable with a range of biomaterials and no special equipment is needed. Full interconnecting pores and large pore interconnections can be fabricated. No organic solvents are needed. Disadvantages: Incapable of controlling the microstructure of the scaffold in terms of pore size, geometry, interconnectivity, and spatial distribution of pores. Foam structure, which is difficult for cells to migrate through, has low mechanical properties.</td>
<td><img src="#" alt="Image" /></td>
</tr>
<tr>
<td>Conventional method</td>
<td>Porosity ~ 80% 5-600 µm</td>
<td><img src="#" alt="Schematic" /></td>
<td>Advantages: Simple method, suitable with a range of biomaterials and no special equipment is needed. Full interconnecting pores and large pore interconnections can be fabricated if porosity decomposition is achieved. Disadvantages: Organic solvents typically required. Foam structure, which is difficult for cells to migrate through, has low mechanical properties.</td>
<td><img src="#" alt="Image" /></td>
</tr>
<tr>
<td>Emulsion freeze drying</td>
<td>Porosity up to 95% Average pore size 13-35 µm up to 200 µm</td>
<td><img src="#" alt="Schematic" /></td>
<td>Advantages: Simple method, suitable with a range of biomaterials and no special equipment is needed. Full interconnecting pores and large pore interconnections can be fabricated. Disadvantages: Incapable of controlling the microstructure of the scaffold in terms of pore size, geometry, interconnectivity, and spatial distribution of pores. Foam structure, which is difficult for cells to migrate through, has low mechanical properties. Organic solvents typically required.</td>
<td><img src="#" alt="Image" /></td>
</tr>
<tr>
<td>Fabrication method</td>
<td>Porosity</td>
<td>Pore size</td>
<td>Advantages</td>
<td>Disadvantages</td>
</tr>
<tr>
<td>--------------------</td>
<td>----------</td>
<td>-----------</td>
<td>------------</td>
<td>---------------</td>
</tr>
<tr>
<td>3D Printing</td>
<td>&lt; 45-60%</td>
<td>45-1600μm</td>
<td>- SFF techniques have accurate control over pore size and interconnectivity over conventional/autoclonal approaches. The layer-by-layer process allows fabrication of complex and anatomically-shaped structures. No toxic components used in post-processing.</td>
<td>- Expensive machinery required. Resolution limitations at lower pore sizes. Biomaterials need to come in powder form with controlled particle size. Weak bonding between powder particles, rough surface.</td>
</tr>
<tr>
<td>Stereolithography</td>
<td>&lt; 90%</td>
<td>20-1000μm</td>
<td>- Accurate control over pore size and interconnectivity. Layer-by-layer process allows fabrication of complex and anatomically-shaped structures. High accuracy, good mechanical strength and broad range of materials.</td>
<td>- Expensive machinery required. Polymeric materials are only compatible with UV curing.</td>
</tr>
<tr>
<td>Selective Laser Sintering</td>
<td>&lt; 80%</td>
<td>30-2500μm</td>
<td>- Accurate control over pore size and interconnectivity. Layer-by-layer process allows fabrication of complex and anatomically-shaped structures. High accuracy, good mechanical strength and broad range of materials.</td>
<td>- Expensive machinery required. Resolution limitations at lower pore sizes. Biomaterials need to come in powder form with tight controlled particle size, mainly applicable to ceramic materials. High temperature sintering process needed and trapped powder is difficult to be removed. Polymeric materials require UV curing.</td>
</tr>
<tr>
<td>Fused Deposition Modeling</td>
<td>&lt; 80%</td>
<td>100-2000μm</td>
<td>- Accurate control over pore size and interconnectivity. Layer-by-layer process allows fabrication of complex and anatomically-shaped structures with good resolution. Low costs.</td>
<td>- Since the technique uses polymer melts, it is limited to thermoplastics. Low pore sizes difficult to achieve while maintaining high porosity.</td>
</tr>
</tbody>
</table>
3.1. Aim of the project

The aim of the project was to optimize the parameters of a Rapid Prototyping machine in order to make compatible scaffolds of biodegradable polymers. The polymers used were PLA (PolyLactic Acid) dissolved in chloroform and 1,4-dioxane; and Chitosan dissolved in acetic acid.

Moreover, the aim was not only to make these scaffolds but especially to obtain real 3D scaffolds. The RP technique allows us to easily control the porosity and the interconnectivity of the pores, two very important characteristics for the development of successful scaffolds. However, some improvement must be done in order to obtain real 3D scaffolds. The modification of several parameters of the deposition of the polymers, such as the printing speed, the polymer flow, the height of a single line, the polymer concentration and of course the design of the scaffold, will lead to the fabrication of real 3D-scaffolds.
4. Experimental part

4.1. Materials

PLA (PolyLactic Acid) is a biodegradable polymer extensively used in TE (Table 2).

\[
\text{PLA formula: } \text{CH}_3 \text{OCH} \cdot \text{C} \text{O} \text{n}
\]

In our study, we use a poly lactic acid copolymer : Poly(95DL/L) lactic acid (PURAC, Netherlands) made of 95% of poly(L-lactic acid) and 5% of poly(D-lactic acid) in order to optimize the biodegradability.

PLA was dissolved at different concentrations in different solvents like chloroform or 1,4-dioxane. For example, a 2.5% PLA solution was obtained by dissolving 0.25 g of PLA in 10 mL of CHCl₃. In Table 4, a summary of the different concentrations of polymer is given.

<table>
<thead>
<tr>
<th>Polymer Concentration W/V</th>
<th>Volume of solvent</th>
<th>Mass of PLA needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5%</td>
<td>10 ml</td>
<td>0.25 g</td>
</tr>
<tr>
<td>3%</td>
<td>10 ml</td>
<td>0.3 g</td>
</tr>
<tr>
<td>4%</td>
<td>10 ml</td>
<td>0.4 g</td>
</tr>
<tr>
<td>5%</td>
<td>10 ml</td>
<td>0.5 g</td>
</tr>
<tr>
<td>6%</td>
<td>10 ml</td>
<td>0.6 g</td>
</tr>
<tr>
<td>7.5%</td>
<td>10 ml</td>
<td>0.75 g</td>
</tr>
<tr>
<td>10%</td>
<td>10 ml</td>
<td>1 g</td>
</tr>
</tbody>
</table>
4.2. RP machine

4.2.1. Technic specs

The RP machine is developed and commercialized by “nScrypt, Inc”. This machine (Tissue Engineering 3D-300 series) is capable of printing 3D scaffolds from different materials (such as polymer, ceramic, composites and living cell solutions) with a large range of viscosities from 1 to 1,000,000 cps (centipoises). It is helped by a CAD (Computer Aided Design) tool, which permits the development of the design. The material can be deposited using one of the three different pumps (Figure 18).

4.2.2. Pump specs

Air-syringe pump: Also known as Air pressure pump. The air pressure is injected in a syringe containing the depositing solution. The syringe is fixed to the motion system, and can be plugged to a wide range of needles. Then, due to the pressure, the depositing solution is flowing via the needles, and printed on the substrate.

Displacement pump: Also known as positive displacement pump. In this case, a piston is plugged inside the syringe and presses the material solution out of the needle.
Smart Pump™: This is the most important and clever pump of the machine. It is capable of dispensing material with a wide range from 1 to 1,000,000 cps (centipoises) precisely with accurately controlled air pressure, timing, valve opening and dispensing height. The valve is moving in z direction. To understand the process of the smart pump, here is a sketch which describes the mechanism of operation (Figure 19):

Figure 19. Schematic description of the smart pump.

The patented valve technology creates a back pressure when the dispensing process finishes, causing the material to be pulled back into the print nozzle. This removes the material on the tip and creates a fresh start for the next print session. The print tip has a conical shape.

4.2.3. CAD tool

The CAD tool is a software that helps the user to create the desired design. Once it has been created, the design is sent to the RP machine and creates the desired structure. This is a very useful tool that allows the printing of very complex 3D shapes. Three steps are involved in the process for producing a scaffold: (1) Draw the design; (2) Configuration of the machine; (3) Launch of the RP.

(1) Drawing the design:

This is the very first step of the process. We have to draw the desired design (Figure 20) with a special software. It is like programming, a code is required to create the required 3D design.
**Fabrication of 3D-porous scaffolds by Rapid Prototyping method**

**Code explanations:**

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>pen airsyringe</code></td>
<td>Loads the desired pump. In this case, the air-syringe pump.</td>
</tr>
<tr>
<td><code>speed x</code></td>
<td>Write the desired values. The pressure is in Psi and the speed in mm/s.</td>
</tr>
<tr>
<td><code>pressure x</code></td>
<td>Write the desired values. The pressure is in Psi and the speed in mm/s.</td>
</tr>
<tr>
<td><code>move x y z</code></td>
<td>move of the pump in 3 dimensions. The values indices are in mm. The reference is moving with the printer. So it is not necessary to know the exact coordinate of each point. For example, printing a square looks like:</td>
</tr>
</tbody>
</table>

```
move 1 0
move 0 1
move -1 0
move 0 -1
```

For the smart pump, the code is a little bit different:

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>pen SMARTPUMP_1</code></td>
<td></td>
</tr>
</tbody>
</table>

```
speed 10
pressure 20
move 2 0 0
```

While working with the smart pump, the new important commands are “trigvalverel” and “valverel”. These commands allow opening and closing the valve with an incredible efficiency.

First of all, with the machine control, it is necessary to define the Open and Close position of the valve.
“trigvalverel x y”: Open the valve at the “previously defined position + x” at a speed of “y”.
For example if the define position of the valve is 1.80 mm, the valve will go to the position “x+1.80” mm at a speed of “y” mm/sec.

“valverel x y”: Close the valve at the “previously defined position + x” at a speed of “y”. x could be equal to zero if we want to stop completely the flow or it could be a positive value if we just want to reduce the stream.

Finally for the displacement pump, the only thing to change is the call of the pump, “pen DisplacementPump” is the command to load the displacement pump.

(2) Configuration of the machine:

Figure 21. Resource control panel.

The configuration of the machine is done by the “Resource Control panel” (Figure 21). This panel is the interface between the machine, the design software and the user. First of all, the machine must be connected and enable. The three motion systems and the pump chosen must be ready (Green “Ready” button). If not (Red “Fault” button instead), they must be initiated. Once this step is over, the needle of the pump must be placed just over the substrate. With the help of the motion control (X-Y-Z) and the related button (Positive-Negative), the right position is reached. The speed of these motions system can be change via the speed gauge. Then the machine is ready to print.
There is an extra step if the smart pump is being used. With the “Open” and “close” buttons we must calibrated the pump. So the method is the following: For low viscous solution (or wide aperture), put a low pressure (via the gauge “Material Feed Pressure”) between 10 or 20Psi, then open the valve slowly until you obtain a sufficient flow. Define the position as “Open position” in the “Pump Valve Config”, and then close slowly the valve until nothing is going out of the nozzle (even after cleaning it). Finally define the “Close position”.

Use the same method with viscous polymer (or fine aperture) but with increased pressure like 30 or 40 Psi.

If any problem occurs, the button “Fault acknowledge” must be pressed in order to reset every parameter and re-initiate the pumps and motion system.

(3) Launching the RP:

Figure 22. RunForm panel.

Once the machine is configured and the design ready. The “RunForm” panel is opened (Figure 22), and the “Cycle Start” button pressed. The program then will compile the data for a while and start the printing. While printing, the only parameter you can change is the pressure via the “Material Feed Pressure” gauge in the “Ressource control panel” (Figure 21)
4.3. Rapid Prototyping

4.3.1. Air-syringe pump

Firstly we worked with the air-syringe pump because we wanted to optimize many important parameters like the concentration, the printing speed, the choice of the needle, etc. The air-syringe pump has an easier code to plot and has less parameter to play with. Therefore, it is more suitable to begin with this pump and optimize as many parameters as we can, and then continue the work with the smart pump or the displacement pump.

4.3.1.1. Optimizing parameters

Concentration of polymer solution: Polymer concentrations in the range of 2.5%-10% were tried. Solutions concentrations from 2.5 to 6% gave good results. The machine pressure was sufficient to ensure a constant flow as well as a reasonable printing speed. The more concentrated is the solution, the more pressure we need to obtain a sufficient flow. In the same way, the more it is concentrated, the slowest we can print the scaffold because the printing speed and flow are oppositely correlated. With solution concentrations in the range of 6 to 7.5% PLA we could only make a scaffold with a very low speed (10 mm/s) and full pressure (100Psi) for example. Over 7.5%, the pressure is insufficient to allow a constant flow, and the printing speed is not fast enough to permit the building of height 3D structures in a reasonable time.

Therefore, the suitable range of concentration is between 2.5% and 6% PLA regarding on the application, the accuracy and fineness of the structure and the rapidity of the printing.

Dimensions of the line: Very concentrated solution will give a thick and large line size whereas a low concentrated solution will give small size line in height and width. The consequence is that with low concentrated polymer, we have to print many layers to reach a certain height. However with concentrated solution, we can only print few layers to reach the same height. The speed of the printing process is inversely related to the concentration of polymer. With concentrated solutions, we have to print slowly whereas with diluted solutions, we can print fast.

Drying time: It is coherent that with a low concentrated polymer, the drying time is longer than with a high concentrated polymer. With diluted polymer, the solution extruded will first have a cylindrical shape. But just after, it will become flat and expands on the substrate. Then if you have predicted a certain distance between each layer, this height should not be respected because the
scaffold does not have the final shape yet. If the predicted height is higher than the real one, the needle will not touch and not print the scaffold anymore.

Needle size: The needle size is a critical selection. Regarding on our design (size of pores and distance between each line) and our polymer concentration, we had to choose between a wide ranges of aperture. For coarse structures and concentrated solution, we could only use small gauge aperture (invert scale), and for fine design and low concentrated solution, we could only use very narrow apertures.

Thus, it is impossible to make fine design with concentrated polymer (because we cannot use small needles) and for some logical reason we will not make coarse structures with low concentrated solution (because it will need a too long printing time).

In Table 5 is given the dispensing tips available for my project:

<table>
<thead>
<tr>
<th>Tip type</th>
<th>Color</th>
<th>Gauge</th>
<th>Inside Diameter mm</th>
<th>Outside Diameter mm</th>
<th>Picture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision stainless steel tips</td>
<td>Red</td>
<td>25</td>
<td>0.25</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clear</td>
<td>27</td>
<td>0.20</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lavender</td>
<td>30</td>
<td>0.15</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yellow</td>
<td>32</td>
<td>0.10</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Smooth-flow tapered tips</td>
<td>Red</td>
<td>25</td>
<td>0.25</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>PTFE-coated tips</td>
<td>Orange</td>
<td>23</td>
<td>0.33</td>
<td>0.64</td>
<td></td>
</tr>
</tbody>
</table>

Accumulations: The main problem while using the air-syringe pump is the presence of accumulations in the final structure. Accumulations are the main problem of this RP technique. Here is a non exhaustive list of where (or when) they can appear:
Change of printing direction: There is accumulation in the angle of every change of direction (Figure 23).

Figure 23. Schematic representation of an accumulation in angle.

Cross of another printed line: In our design accumulations emerge at each cross (Figure 24).

Figure 24. Schematic representation of an accumulation in crossing.

Dust or impurities: Accumulations may occur when impurities or dust are deposited on the substrate.

Fast drying on the needle: The polymer first sticks to the needle but then it is deposited on the printing surface and creates a defect that will create an accumulation. To overcome this problem, the polymer concentration cannot be higher than 6%.

Model design is not perfect: For example if the height of a real line is different than the height of a line in the program, there is a mismatch that can create an accumulation if the needle is too high or too close to the substrate. Too high, the polymer will not flow out on the substrate and form a drop on the needle before falling on the scaffold and develop accumulations. Too close, the needle will touch the polymer newly printed and taking some parts away and accumulating them in another area. A way of solving this problem is to measure the height of one single line thanks to a profilometer.

So, there are many manners of making accumulation, a few can be fixed by playing with parameters, some by changing the design and many by changing the pump.

However we tried to fix some accumulation issues by reducing the flow or increasing the speed in certain area (Figure 25).
Increasing speed while changing direction for low concentrated polymer can prevent accumulation but it is not a solution. The machine cannot have differences of speed in very small areas.

Machine difficulty: In small areas, some problems can appear when we want to change the speed (Figure 26). For example while changing direction. We want a higher speed to prevent accumulation, we write our code, but while printing the machine makes strange noises and stop working. We do not know the cause of that but it only occurs when changing the speed in small areas (like changing three times the speed in less than a centimeter).

Pressure: We noticed that still without pressure, the polymer was still going out of the needle. The main disadvantage of the air-syringe pump is that it is not possible to control the stopping of the flow.
If you want to increase or decrease the pressure, it is possible but you have to wait before you see any results on your scaffolds. For example, if you need to decrease it in a certain area, it is almost impossible because you have to preview the decrease in another area regarding the printing speed (Figure 27).

If you want to stop the flow completely, it is impossible with certain needles or polymer concentrations (big diameter needles or low viscous polymers) because it is still flowing by capillarity. It could probably work if we put a negative pressure which would avoid anything to go out.

Substrate: We worked on three different substrates: A Teflon substrate, a PLA substrate and finally a glass substrate. The Teflon substrate did not work at all. The scaffolds could not be print because the polymer did not stick to it. Then we tried the PLA as substrate itself. It worked incredibly well, but it is very tough to process because it includes extruding a film of PLA with the RP machine. It might be the most suitable substrate for further studies. We finally worked with the glass substrate which was relatively well. The fixation is quite good but it remains problems in some particular areas.

Fixation problem: In many cases, the fixation of the scaffold on the glass substrate is a problem. It can happen both with concentrated polymer and low concentrated solutions (Figure 28).

For concentrated polymers, it is due to the fact that the printed line has a cylindrical shape. This cylindrical shaped printed polymer has a small contact area with the substrate. And so, while changing direction, the dispensing solution does not stick anymore at the substrate. It cannot really be solved because if you low the printing
speed while changing direction, you create accumulation problem. And due to the pressure trouble, you cannot really decrease the speed and the flow at the same time.

![Figure 28. Schematic representation of fixation issue.](image)

- For low concentrated solution it is due to the solvent evaporation. So the scaffold shrinks and does not stick anymore on the substrate while drying. It is a real issue when you need to overprint on the deposited scaffold. There is no real answer for that. Just do not use too liquid solution for the extrusion.
4.3.1.2. Results

Cuadricula 9

This scaffold (Figure 29, 30) was created using PLA 2.5% dissolved in chloroform with a needle size of 0.15 mm (Inside Diameter). The design was created in order to avoid some of the problems seen before with the air-syringe pump. It is a quite big design (4 cm$^2$ surface area), and so the polymer could dry before each layer, even if the printing was fast. The borders are quite large, that the polymer can stick better to the substrate and minimize the border accumulation effect.

![Figure 29. Design of cuadricula 9.](image)

This design (Figure 29) worked fine and we could reach almost hundred layers before stopping the deposition. Here, the limiting factor was the accumulations which occurred because the height of a real line was smaller than the line in the program and so after many layers, the needle did not touch the scaffolds, and it was printing by dots of accumulated polymer. (“if the needle is too high, the polymer will not flow out on the substrate and form a drop on the needle before falling on the scaffold and develop accumulations”). Also as we can see in the picture (figure 30), we had polymer accumulation in the borders, due to the change in direction. [Refer to the Appendix for the design code]
This design (Figure 31) is very clever because it prevent many accumulation troubles. The scaffold (Figure 32) was created with a 2.5% PLA solution dissolved in chloroform and a needle size of 0.20 mm. The pump is printing one small line then changes direction, and then prints again a small line, etc. This design avoids completely accumulations due to constant changing direction and caused by crossing lines. Each horizontal line is separated by 0.05 mm more than necessary. Further modifications of design were made (change the size of holes) in order to print with different concentrations. And finally modified the values of each square printed to create a bigger gap between each node and prevent extra accumulations.
This grid also allowed us to obtain a very nice and 3D scaffold. We could reach about 50 layers before stopping the deposition. The main difficulty here was to plot a design which prevents lines to stack each other. Due to the expansion of our solution, sometimes the gap was enough to ensure a small contact, but mostly it allow lines to stack one on the other. Moreover we had problems to avoid the mismatch between the height of a printed line and the height of a designed line. [Refer to the Appendix for the design code]

Figure 32. Digital picture of construct grid.
Cuadricula 14

“Cuadricula 14” is also a design (Figure 34) we have created to prevent accumulations. The particularity is that horizontal (or vertical) lines are printed in two times (Figure 33). The reason is that the length of changing direction is now twice longer than before, with the same size of pore. It should prevent many accumulations that normally occurred before.

This new design worked very well, and as “cuadricula 9”, we could reach around 100 layers before it stopped working fine. Also the surprising thing about this design is its ability to repair itself. For example when a dust come on the substrate and creates an accumulation, then three layers are printed before it goes once again at the same place, with of course the needle higher. This ability is very important and make this design one of the most successful we have produced. Just an important thing to care about while writing the design is the distance X (Figure 34) between these two lines. A too small distance will contact the two vertical lines (in our sketch) for example, and then create a gap of accumulated material.
The major limit of this design is the range concentration of polymer we can use. Scaffolds made of solutions over 4 or 5% PLA are extremely hard to print because each horizontal or vertical line we print must be higher than the previous one. The reason is to prevent crossing line accumulation and also to avoid the needle to touch the scaffolds. So after 3 layers, the needle goes once again in the same area, but cannot print anymore because it is too far from the previous level. It is only printing on the cross lines, and so accumulates. With lower polymer solution, this is also a limit, but the height of one single line is much lower, so after three layers, it is still close to the previous level. Moreover, the code is a little bit more complex to write and more time is needed to obtain a design. [Refer to the Appendix for the design code]
4.3.2. Smart pump

The control on the flow is the main advantage that had the smart pump over the other pumps. The patented valve technology creates a back pressure when the dispensing process finishes, causing the material to be pulled back into the print nozzle. This removes the material on the tip and creates a fresh start for the next print session. Thanks to this ability, we could prevent many problems seen before with the air-syringe pump.

For example we avoid the machine difficulty problem, the pressure trouble and also some accumulations issues.

4.3.2.1. Optimizing parameters

Concentration of polymer solution: As we tried the concentrations with the air-syringe pump before, we did not have to try again the ones not working. Some we only worked with solutions in the range between 2.5% and 6% concentrated PLA.

Pressure versus aperture issue: Opening and closing position are the new parameters of the smart pump with have to deal with. Before each use of the machine a calibration of these parameters must be done (section 4.2.3.CAD tools). A difficulty was to create a method to calibrate these parameters in order to have reusable codes afterwards. Because if not, you have different values of “Open/Close” position and then you have to change once again the “trigvalverel” or “valverel” code for each line or each layer you are printing. This could be a very tough work. It is not easy to first calibrate the Open and Close parameter and then to find the right values to obtain a sufficient flow playing with the pressure, the nozzle aperture and the values of “trigvalverel” and “valverel”.

Nozzle size: The main issue we had with the smart pump was the nozzle size. In fact we had a few ceramic nozzles (Figure 35) and their aperture dimension were not appropriate to the concentrations and design we had. Some nozzle were definitely too small (10 microns Outside Diameter) and some certainly too big (300 microns Inside Diameter). So we could not really create scaffolds with these nozzles. We could only work with concentrated solution of PLA and had to wait for the reception of an element that allowed us to put the air-syringe tips on the smart pump. We had the idea of putting the air-syringe tips on the smart-pump device. But unfortunately, the kit for doing it took a long time to arrive in the laboratory, and we could not really use the smart-pump with these needles.
Figure 35. Digital picture of the smart pump nozzle.

Accumulations: Some of the accumulation problems which occurred with the air-syringe pump could be overcome with the smart pump because it allows a better control of the flow and permits the creation of design much more complex. For example, accumulations while changing direction by reducing (or stopping) the flow in this area could be prevented. However the other accumulations issues could not be resolved that easily and using the smart pump did not change much the issues.

Width/Drying time/Fixation problem/Substrate: Exact same results than with the air-syringe pump.
4.3.2.2. Results

Cuadricula 18

This design (Figure 36) is quite similar to “cuadricula 9” but it has some modifications, and also it was created for the smart pump device application. This scaffold (Figure 37) was printed using a 2.5% PLA solution dissolved in 1,4-dioxane with a needle size of 0.10 mm. It has a distance between the lines of 250 µm, so this is the most complex done so far (theoretical pore size twice smaller than “cuadricula 9”).

The scaffold was printed quite well, but not enough high again. This time, the pressure and the flow (via the smart-pump behavior) was reduced during change in direction. Also, the design was modified. We have made longer non-crossing lines to let the smart pump reducing the flow as we wish. As you can see in this digital picture, it worked quite well. There are lower accumulations than “cuadricula 9” for example.
The main limiting problem here was the ceramic tip size. It was too large, so we could not really control the flow and make fine structure. Even when we stopped completely the flow, with low concentrated solution, it was still flowing out of the ceramic tip because it was too large. [Refer to the Appendix for the design code]

4.3.3. Displacement pump

This pump has no real interest when the smart pump is working well with good size of nozzle.

Here is an example of the code for an easy scaffolds design (Figure 38):

```plaintext
pen DisplacementP
ump speed 8 parameter 2 move 5 5
move 18 0 move 0 3
move 0 3 move 0 -18
move -18 0 move -3 0
move 0 3 move 0 18
move 18 0 move -3 0
move 0 3 move -18 0
move -18 0 move -3 0
move 0 3 move 0 18
move 18 0 move -3 0
move 0 3 move 0 3
move -18 0 move 0 0
move 0 3 move 0 0
move 18 0 move 0 0 0.15
```

Figure 37. Digital picture of cuadrícula 18.

Figure 38. Design of cuadrícula 1.
4.4. PLA in chloroform versus PLA in 1,4-dioxane

We used the same W/V concentrations to make our dissolutions with both solvents. Then with the air-syringe pump, we compared them at same concentrations. Table 6 summarizes the properties of each solvent used:

Table 6. Solvent properties.

<table>
<thead>
<tr>
<th>IUPAC name</th>
<th>1,4-dioxane</th>
<th>Chloroform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C₄H₈O₂</td>
<td>CHCl₃</td>
</tr>
<tr>
<td>Display</td>
<td>![Diagram of 1,4-dioxane molecule]</td>
<td>![Diagram of chloroform molecule]</td>
</tr>
<tr>
<td>Molar mass g/mol</td>
<td>88.11</td>
<td>119.38</td>
</tr>
<tr>
<td>Density g/cm³</td>
<td>1.033</td>
<td>1.48</td>
</tr>
<tr>
<td>Melting point °C</td>
<td>11.8</td>
<td>-63.5</td>
</tr>
<tr>
<td>Boiling point °C</td>
<td>101.1</td>
<td>61.2</td>
</tr>
</tbody>
</table>

The result is that more or less, at same concentration, they have same rheological properties. The 1,4-dioxane has better rheological because at same viscosity and concentration it dries slower than chloroform (higher boiling point). It is interesting to prevent drying in the needle (or right after going out of the needle) but it is problematic for the drying time on the substrate. The consequence is that it needs more time to dry. To avoid this problem, we had the idea of using a heating plate and put it below the substrate. The aim was to reach a certain temperature (around 40 °C) to allow the scaffold to dry faster and so to print the next layer sooner. More than 40-50°C could be dangerous first because of the degradation of PLA. Hydrolysis, responsible of the biodegradation of PLA is accelerated by the temperature. However 40°C seemed enough for drying the PLA dissolved in 1,4-dioxane. The main problem met was that the heating plate was not flat at all. With a non-flat surface, the printing of scaffold could not be done properly. I had to put some thickness compensations in order to obtain an almost flat heating plate.

Then we used this heating plate to dry faster low concentrated solution of PLA in chloroform. The results were good and the gain of time important. But still with all accumulations troubles we had, amplified by the non-flat plate, we could not reach high structure. Also we could not use the heating plate with the smart pump because it was too thick and the pump could not go high enough.
4.5. Interferometry

The best design done so far is “cuadricula 9”. We chose this scaffold because it was the higher of complex scaffold we obtained. It is around 200 µm high and has a distance of 500 µm between to printed line (in the CAD tool). It was created using PLA 2.5% dissolved in chloroform. As seen before, it was created using the air-syringe pump with a needle size of 0.15 mm.

The interferometry of the “cuadricula 9” was realized and the results treated via a software (Figure 39). The interferometer did not work well. The right interference needed to obtain a great 3D picture was not reached. Anyway, we obtained a picture, but we cannot measure the height of our structure.

The interferometry gives a good idea of the overall geometry of our scaffold. The height could not be measured, but we can obtain the values of pore size, distances between each printed line, and width of these lines (Figure 39 to 45).
Figure 40. Width of a line in x-direction / Cuadricula 9.

Figure 40 represents the measurement of a line width in x-direction. The width of this line is 0.147 mm. The inside diameter of the needle used is 0.15 mm. This means that in this case, the polymer did not expand when it was printed (in theory). But in reality it expanded while printed and shrunk when drying right after.
Figure 41 represents the measurement of a line width in y-direction. The width of this line is 0.180 mm. The inside diameter of the needle used is 0.15 mm. This means that in this case, the polymer expand when it was printed. In reality it has expand but shrunk right after. But the expansion was more important that the shrinkage.
Figure 42. Pore length in x-direction / Cuadricula 9.

Figure 42 represents the measurement of the pore length in x-direction. The length of this pore is 0.320 mm.
Figure 43 represents the measurement of the pore length in y-direction. The length of this pore is 0.312 mm. So we had lengths of 0.320 mm in x-direction and 0.312 mm in y-direction. This means that the surface area of one pore is almost 0.1 mm².
Figure 44 represents the measurement of the distance between two lines in x-direction. This distance measured is 0.474 mm and the distance plotted in the CAD tool is 0.5 mm. This can confirm the accuracy of the RP machine and the conversion of the design into the real scaffold.
Figure 45 represents the measurement of the distance between two lines in y-direction. This distance measured is 0.494 mm and the distance plotted in the CAD tool is 0.5 mm. This can confirm the accuracy of the RP machine and the conversion of the design into the real scaffold.
4.6. Scanning Electron Microscopy (SEM)

Like for the interferometry, we chose to see the micrograph of “cuadricula 9” because it was the higher of complex scaffold we obtained. It is around 200 µm high and has a distance of 500 µm between to printed line (in the CAD tool). I had two different scaffolds of the same design made in the same conditions. These scaffold was created using PLA 2.5% dissolved in chloroform with a needle size of 0.15mm (inside diameter). We obtained pictures of the “cuadricula 9” with different angles and approaches (Figure 46 to 51).

![Figure 46. SEM micrograph of Cuadricula 9 (50x).](image)

This micrograph (Figure 46) shows our sample, composed of three different faces (upper, profile and lower face from left to right).
Figure 47. SEM micrograph of lower face of Cuadricula 9 (200x).

Figure 48. SEM micrograph of lower face of Cuadricula 9 (400x).
Figure 47 and 48 show micrograph of the lower face of the scaffolds, at different magnifications. For doing picture of a non-conductive polymer with the SEM, we have to coat the sample with gold. This coating is supposed to be fine (20 nm), but it changed the dimensions of our scaffolds anyway. Sometimes we cannot make a difference between gold covering a pore and cold coating a line. By consequence the measurement made with the SEM are not accurate and must not be used as a suitable argument. Figure 47 and 48 summarize well what we are talking about. Between the two measurements, we do not know which one can define the real pore size. The smallest size is falsified by the gold coating and the biggest does not really represent the pore size.

Figure 49. SEM micrograph of upper face of Cuadricula 9 (200x).

Figure 49 show micrograph of the upper face of the scaffolds. First in this picture we can see better the gold coating done for the SEM imaging. In the upper square, the coating seems to be crackled, proving its existence. Also this micrograph gives a good argument to the theory of accumulation seen before. In this case, the accumulations occurred at the crossing line. In crossing position, the height of polymer is greater than in normal lines, and so after printing many layers, the needle does not print the scaffold except in crossing area. So, accumulations take place by print of dots of polymer in these crossing sites.
Figure 50. SEM micrograph of profile face of Cuadricula 9 (200x).

Figure 51. SEM micrograph of profile face of Cuadricula 9 (400x).
Figure 50 and 51 represent SEM micrograph of the profile face of our scaffolds. We cannot really measure the height of these scaffolds because they were not totally vertical in the SEM. However, as seen in figure 48, the figure 50 shows the accumulations dots that occur at crossing lines. These accumulations seem very high in comparison with the height of the scaffolds. The “cuadricula 9” is the best design so far, but still because it has crossing lines, accumulations take place. “Construct Grid” can be a good substitute to these troubles because it has no crossing sites, but it still has to be improved.

Figure 52 shows the SEM micrograph of “cuadricula 18”. Unfortunately the coating does not permit here to measure the pore size and the distance between the lines with accuracy. However, we measure a pore length of 65 µm, which means a pore area about 4000 µm². This scaffold is the most complex and accurate done so far. It has pore surface area 25 times smaller than “cuadricula 9” (0.1 mm² for “cuadricula 9”). The only problem is that no height was reached here.
5. Conclusions

The deposition of polymer using a RP machine is a very innovative and promising method for making biodegradable and biocompatible scaffolds. The technique is very powerful and every desired structure can be created if the parameters are well defined.

The goal of this project was to optimize these parameters. We could evaluate the influence of each but we could not realize true 3D scaffolds with height of more than 200-300 µm for fine structure. Of course it is possible to create higher coarse structure with more concentrated polymer and larger needles, but the pore area obtained are not suitable for a TE application. For “cuadricula 9” we obtained a pore size area of 0.1 mm$^2$, with a very good shape and reproducibility in the process. Moreover, “Cuadricula 18” showed the best of what the machine can afford in term of accuracy and complexity. It has a final pore surface area of 4000 µm$^2$ which means 25 times smaller than “cuadricula 9”.

Even if the process is not fully optimized, we have developed some interesting design to prevent some problems and facilitate the printing. These designs have a very complex and controlled structure and can easily be reproduced. Moreover, they prevent many problems that occur during the printing process but not all unfortunately.

Other improvements must be done in order to obtain real 3D structure with desired height. Lately we received new pieces which allow the plug of the air-syringe needles on the smart pump. It will show good results in the close future.
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References


Fabrication of 3D-porous scaffolds by Rapid Prototyping method