Selecting process parameters in RepRap additive manufacturing system for PLA scaffolds manufacture

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Abstract

Additive manufacturing (AM) technologies may offer a viable and simpler alternative to fabricate scaffolds directly from patients’ data. Existing commercial AM machines are currently being modified to improve their accuracy and capabilities. However, high costs, material restrictions, and difficulty to study process parameters should be considered. In this context, this work focuses on the study and optimization of a novel open-source and low-cost 3D printer machine, called RepRap, employed to fabricate biocompatible scaffolds. Several process parameters and porosity were studied. Lastly, the RepRap printer was considered a versatile, inexpensive, flexible, and simple machine to efficiently fabricate scaffolds.

1. Introduction

The development of accurate constructs made of a matrix or scaffold and living cells to repair and regenerate damaged tissue is a current challenge in the tissue engineering field [1]. Many complications concerning high costs, low efficiency, and long fabrication times have been associated with scaffold manufacturing technologies, and therefore, the progression and implementation of scaffolds at a high scale has been restricted [2]. In this context, additive manufacturing technologies, recently emerged as an innovative set of technologies to produce 3D products, may offer a viable and simpler alternative to fabricate these constructs while controlling efficiently their architecture [3]. In fact, several additive technologies have already been used to design and manufacture scaffolds for medical applications, including fused deposition modeling (FDM), stereolithography (SLA), Inkjet printing, and selective laser sintering (SLS) [4-6].

In terms of tissue engineering, these additive manufacturing technologies enable the fabrication of customized scaffolds directly from the patient, by using a scanner to import the 3D data needed, and employing a range of materials such as polymers or ceramics. For this purpose, existing additive manufacturing machines are currently being modified to improve their accuracy and capabilities [7, 8]. However, many contradictions still need to be considered, including the high costs associated with these commercial machines, their material restrictions, and the difficulty to study process parameters.

The optimization of process parameters is a major challenge to obtain adequate scaffold morphology and biomechanical behavior, very important to improve cells adhesion and proliferation, and therefore, tissue regeneration. Indeed, appropriate porosity, pore size, pore shape, and mechanical strength are required to achieve cell growth and matrix formation [9]. Open-source extruders, like the RepRap machine, allow a thorough study of several process parameters involved in the fabrication of scaffolds, such as, deposition velocity,
layer thickness, nozzle tip size, filament distance, deposition pattern, and speed movement. Therefore, a precise control over this manufacturing process is possible. In fact, the selection of correct process parameters will have a direct influence on the morphology and biomechanical performance of these constructs manufactured with the RepRap machine.

This work focuses on the study and optimization of a novel open-source and low-cost 3D extruder machine, called RepRap, employed to fabricate Poly-L-lactic Acid (PLA) scaffolds. Porosity and mechanical strength were analyzed under several conditions, and optimal process parameters were determined to ensure the best performance.

2. Materials and Methods

2.1. Material

A 1.75mm Poly-L-lactic Acid (PLA) wire (Faberdashery Ltd., United Kingdom) with a density of 1240 Kg/m³ was used to produce rectangular scaffolds. PLA is a resorbable polymeric material, widely used in clinics, proven to be biocompatible and free of toxicological and immunological hazards.

2.2. 3D printer machine

An open-source RepRap Prusa 3D printer (MakerGear, USA) was selected to fabricate three-dimensional scaffolds (Fig. 1) due to its capability to be modified and optimized by the user, and because it is an affordable, flexible, and manageable machine.

![Fig. 1. Prusa RepRap 3D printer](image)

This printer consists of a thermoplastic extruder positioned within a computer-controlled Cartesian platform. Once the material is supplied to the extruder, a certain temperature and pressure, exerted by a gear, causes the change of the filament diameter from 1.75 to 0.35 mm, which is ejected through a nozzle and finally deposited onto a heated platform.

The software associated with this 3D printer is based on a firmware, which interprets G-codes file formats from a host computer-aided manufacturing (CAM) software installed in a computer. Accordingly, the 3D models designed, either using computer-aided design (CAD) software or others, in a STL or TXT file formats are transferred to the host software which converts them into G-code files able to command and control the machine in order to obtain the final 3D objects printed.

For this work, Tonokip (open-source) was used as the firmware software and Repsnapper (open-source) as the CAM software to convert the scaffolds TXT files designed into G-codes.

2.3. Scaffolds design

A full factorial DOE, without repeatability, was carried out to obtain twenty-seven rectangular PLA prisms (Table 1). They were designed using Matlab software (MathWorks, USA) as TXT files. As shown in Table 1, each prism was produced by modifying several decisive parameters including deposition angles (a), slenderness (s), and distance between filaments (t). In addition, the number of layers in each specimen was also adapted to ensure a correct adherence between layers.

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| a | a1 | a1 | a1 | a1 | a1 | a1 | a1 | a1 |
| s | 0.36 | 0.36 | 0.36 | 0.5 | 0.5 | 0.5 | 0.8 | 0.8 | 0.8 |
| t | 0.55 | 0.75 | 1.05 | 0.55 | 0.75 | 1.05 | 0.55 | 0.75 | 1.05 |
20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 |
| a | a2 | a2 | a2 | a2 | a2 | a2 | a2 | a2 |
| s | 0.36 | 0.36 | 0.36 | 0.5 | 0.5 | 0.5 | 0.8 | 0.8 | 0.8 |
| t | 0.55 | 0.75 | 1.05 | 0.55 | 0.75 | 1.05 | 0.55 | 0.75 | 1.05 |
28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 |
| a | a3 | a3 | a3 | a3 | a3 | a3 | a3 | a3 |
| s | 0.36 | 0.36 | 0.36 | 0.5 | 0.5 | 0.5 | 0.8 | 0.8 | 0.8 |
| t | 0.55 | 0.75 | 1.05 | 0.55 | 0.75 | 1.05 | 0.55 | 0.75 | 1.05 |

On the contrary, several process parameters, such as the deposition velocity, were maintained constant throughout the experimentation. Three different deposition patterns were studied changing the deposition angles (a) layer by layer: 0°/90°, 45°/135°, and 0°/45°/90°/135° (Fig. 2). Three values of slenderness (s),
described as the relationship between the height of the scaffold and its base, were employed (Table 2).

Fig. 2. Deposition angles \((a)\): (a) 0/90°; (b) 45/135°; (c) 0/45°/90°/135°

Table 2. Slenderness values

| Height (mm) | Base (mm) | Slenderness \((s)\) (mm/mm) |
|------------|-----------|-----------------------------|
| 1          | 8         | 22                          | 0.36                       |
| 2          | 10        | 20                          | 0.5                        |
| 3          | 12        | 15                          | 0.8                        |

As shown in Fig. 3, three different distances between filaments \((t)\), determined by the shortest distance between two filaments located within the same layer, were used. Therefore, as a combination of the three possible values of the three parameters above-mentioned \((a, s, \text{ and } t)\), twenty-seven specimens were designed and further fabricated with the RepRap machine.

Fig. 3. Distance between filaments \((t)\)

2.4. Scaffolds additive manufacturing

All designed TXT files were transferred to the RepRap machine through the Repsnapper software. Several process parameters were optimized and established by doing some screening experiments in order to obtain the samples efficiently (Table 3).

Table 3. Process parameters used to fabricate scaffolds

| Parameters                  | Value       |
|-----------------------------|-------------|
| deposition velocity         | 2 mm/s      |
| nozzle tip size             | 0.35 mm     |
| diameter of filament        | 0.175 mm    |
| extrusion temperature       | 180 °C      |
| number of layers            | variable    |

2.5. Morphologic and Mechanical testing

All specimens were adequately measured with a digital caliper (with an error of 0.01mm) and weighed with an electronic balance. The porosity was determined as following: (1) 3D scaffolds volumes were measured \((X, Y, \text{ and } Z \text{ dimensions})\) as if they were solid volumes; (2) specimens were weighted and transformed to volumes by dividing with the material density value; (3) porosity was calculated as the difference between both volumes, as shown in the formula below:

\[
\text{Porosity} = \left( Vt - \frac{w}{\varphi} \right) \times 100
\]

where \(Vt\) is solid volume; \(w\) is the measured scaffolds weight; and \(\varphi\) is the PLA density (1240 Kg/m\(^3\)).

A mechanical compression test of the 3D structures was performed using an AG-X Autograph testing machine (Shimadzu, USA). A maximum load of 40kN and testing velocity of 5mm/s was applied following Standard UNE-EN ISO 604 for plastic materials. All the results were statistically analyzed using one factor ANOVA (IBM® SPSS® Statistics).

3. Results and discussion

3.1. Morphologic Analysis

A morphologic evaluation of the twenty-seven samples was carried out. As an example, Fig. 4 shows three of the scaffolds obtained.

The scaffolds were measured and weighed to determine the porosity and the reliability of the RepRap open-source machine. Table 4 shows theoretical and measured values obtained for scaffolds X (base), Y (depth), and Z (height) dimensions and weights.
The structures manufactured exhibited a weighted percentage error of 6.5% (X – axis), 2.5% (Y – axis), and 1.1% (Z – axis) concerning the dimensions of the scaffolds manufactured. Values below 10% are considered acceptable, therefore, the machine was deemed reliable. Nevertheless, a significant higher error (p<0.001) was obtained for X – axis compared to the other axis (Fig. 5), suggesting the need to optimize the accuracy in that axis.

Table 4. Theoretical and measured dimensional values and weights

| Slenderness (Z / X) | X / Y / Z theoretical dimensions (mm) | X / Y / Z measured dimensions (mm) |
|-------------------|---------------------------------------|-----------------------------------|
| 0.36              | 22.0 / 22.0 / 8.0                     | 23.1 / 22.2 / 8.2                 |
| 0.5               | 20.0 / 20.0 / 10.0                    | 21.1 / 23.1 / 16.3                |
| 0.8               | 15.0 / 15.0 / 12.0                    | 16.3 / 15.6 / 12.2                |

| Slenderness (Z / X) | Theoretical weight (mm³) | Measured weight (mm³) |
|-------------------|--------------------------|-----------------------|
| 0.36              | 2.80                     | 3.34                  |
| 0.5               | 3.02                     | 3.86                  |
| 0.8               | 2.20                     | 2.71                  |

The theoretical weight was calculated on Matlab software giving volume to the trajectory and considering a diameter of 0.35 mm for the extrusion nozzle and 1240 kg/m³ as the density of the PLA used. These results were compared to the values obtained by weighting all the specimens. The weighted percentage error obtained comparing theoretical and measured values was 24%. However, for samples with a starting deposition angle of 0°, the error decreased to 19% while for structures with a starting deposition angle of 45° the error was 34%.

Analyzing the scaffolds manufactured, the ones with deposition patterns of 45/135° and 0/45/90/135° showed higher weight values (3.47 mg and 3.56 mg respectively) than samples with 0/90° patterns (2.89 mg). Scaffolds weight (w) was also reduced in samples with increased distance between filaments (t=0.55, w=3.69 mg; t=0.75, w=3.30 mg; t=1.05, w=2.93 mg) indicating an augment of the pores size, and therefore an increase of void spaces.

A statistical analysis of how the measured weight and number of layers are affected by the three design parameters was performed. The results showed that both...
values (weight and number of layers) are influenced by the three design parameters \( p<0.003 \). The number of layers increased when slenderness and distance between filaments increased in order to assure good consistency of the final structures. Regarding the deposition patterns, the number of layers only decreased when the deposition angle started with \( 45^\circ \), indicating that this pattern allows the obtaining structures with a given height using the smallest possible number of layers.

Finally, porosity of all samples was measured.

![Graph showing porosity values as a function of deposition angle, slenderness, and distance between filaments.](image)

Fig. 6. Porosity values (%) as a function of deposition angle \( a \), slenderness \( s \), and distance between filaments \( t \). Where \( 1=a_1 \) \( (0/90^\circ) \), \( s_1 \) \( (0.36) \), \( t_1 \) \( (0.55) \); \( 2=a_2 \) \( (45/135^\circ) \), \( s_2 \) \( (0.5) \), \( t_2 \) \( (0.75) \); \( 3=a_3 \) \( (0/45/90/135^\circ) \), \( s_3 \) \( (0.8) \), \( t_3 \) \( (1.05) \)

Fig. 6 shows the average porosity values obtained for each parameter studied, where:

- deposition angles: \( 1 ) 0/90^\circ, (2) 45/135^\circ, (3) 0/45/90/135^\circ \)
- slenderness: \( 1 ) 0.36, (2) 0.5, (3) 0.8 \)
- distance between filaments: \( 1 ) 0.55, (2) 0.75, (3) 1.05 \)

A slight decrease of porosity percentage was encountered within the different deposition patterns \( a_1=39, a_2=29, a_3=26, p=0.041 \). No statistically difference was found for slenderness values \( s_0.36=36, s_0.5=29, s_0.8=29, p=0.327 \). However, a significant difference of porosity was found in specimens with different distance between filaments \( t_0.55=22, t_0.75=31, t_1.05=40, p=0.002 \). These results suggest that porosity depends on a design parameter. According, a reduction of the space between filaments may decrease the porosity of the final sample independently of the deposition pattern and slenderness.

3.2. Mechanical Behavior

A mechanical compression test of all twenty-seven structures was carried out. The mechanical behaviour test showed a stress – strain curves similar to that obtained in other works \([7, 10]\) with three different zones clearly marked, the first one indicating an initial stiff mechanical response (Fig. 7).

![Stress – strain curve zones obtained](image)

**Fig. 7. Stress – strain curve zones obtained**

The Young’s modulus of each scaffold was obtained as the slope of the zone 1 of the stress-strain curves. The results were grouped by design parameters (Table 5) and analyzed. Comparing Young’s modulus with the three deposition angles and with distance between filaments, no remarkable differences were perceived \( p=0.779 \) and \( p=0.493 \) respectively. However, when Young’s modulus were grouped by slenderness values, a significant difference was obtained \( p<0.001 \). The results indicated that scaffolds with big base geometries and small heights (low slenderness values) have reduced Young’s modulus compared with bigger scaffolds (high slenderness values).

### Table 5. Young’s modulus (MPa) of scaffolds manufactured

| Design Parameters | Young’s modulus (MPa) |
|-------------------|-----------------------|
| deposit angles (a) |                       |
| 0 – 90º           | 617 ± 292             |
| 45 – 135º         | 713 ± 305             |
| 0 – 45º- 90º- 135º| 674 ± 265             |
| slenderness (s)   |                       |
| s = 0.36          | 403 ± 113             |
| s = 0.5           | 632 ± 180             |
| s = 0.8           | 969 ± 164             |
| distance between filaments (t) | 705 ± 315             |
| t = 0.55          | 724 ± 238             |
| t = 1.05          | 576 ± 289             |

Concerning the relationship between porosity and scaffolds mechanical behavior, increasing porosity resulted in a significant reduction of scaffolds strength as shown in Fig. 8.

Data from mechanical analysis suggest that highly porous scaffolds present lower strength values, and therefore their performance would depend on the final application of the scaffolds. Moreover, it was possible to observe a strong relationship between slenderness and compression strength.
The analysis and evaluation of the results obtained in this work suggest that most of the parameters studied have a direct effect on the morphology and mechanical behavior of PLA scaffolds manufactured with the RepRap machine. An informative matrix was generated to schematize porosity and strength values of different scaffolds geometries (slenderness variable) obtained. As shown Fig. 9, the matrix plots the relationship between the porosity (X-axis) and Young’s modulus (Y-axis) of different slenderness values (s) for the RepRap machine.

4. Conclusions

This study is the first work done using the open-source RepRap 3D printer to fabricate scaffolds made of a biocompatible material. Due to the capabilities of this machine, three different parameters were studied to determine their influence on morphological and mechanical performance of the scaffolds.

A morphological analysis revealed a strong relationship between the three parameters studied (angle of deposition, slenderness, and distance between filaments) and scaffolds weight, as well as, the number of layers of the scaffolds. Moreover, a statistically significant influence of the distance between filaments was encountered on porosity values. Compressive mechanical tests indicated a negative correlation between porosity and Young’s modulus, and a strong relation between slenderness and Young’s modulus.

Results obtained demonstrated that the RepRap machine, considered a versatile, inexpensive, flexible, and simple machine, is able to efficiently fabricate scaffolds of PLA material. Further studies need to be done to determine the influence of other parameters in this manufacturing process, as well as, the use of other biocompatible materials.

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