INFLUENCE OF LASER SINTERING PARAMETERS ON THE FABRICATION OF SCAFFOLDS FOR TISSUE ENGINEERING

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Abstract. *Tissue engineering seeks to repair tissue and organs by the implantation of scaffolds, which are three-dimensional structures that provide support for adherence and cellular development. This field is widely investigated due to high demand for organs and tissues transplants. Currently, fabrication of scaffolds with appropriate macro- and microstructures is a technological and engineering challenge, as conventional scaffolds fabrication processes have limitations in producing it with controlled pore size and defined architecture. Laser sintering is a potential alternative for the production of highly porous scaffolds with complex geometries and interconnected pores. This study investigated the influence of the main laser sintering parameters of biocompatible polymeric material poly (vinyl alcohol) (PVA) in scaffolds properties, through microstructural characterization and measurement of porosity. Laser power, scan lines spacing, layer thickness, part bed temperature and laser scan count was varied. Results showed that the variation of the laser sintering parameters influences the amount of energy supplied to the samples and, therefore, has great influence on the final quality of scaffolds microstructure and porosity. Higher energy levels provided better necking formation of the PVA particles and, as consequence, the samples showed satisfactory results in terms of porosity and microstructure.*

Keywords: *Scaffold, Poly(vinyl alcohol), Laser Sintering.*

1. INTRODUCTION

Tissue Engineering is an interdisciplinary field that aims to regenerate damaged tissues and organs by implanting scaffolds, three-dimensional structures that provide support for cell adhesion and development. This field is widely investigated due to the high demand for transplants of organs and tissues, a demand that exceeds the availability of organs for transplants. The transmission of diseases between the donor and the recipient, as well as the possibility of immunological rejection to the organ donated also are factors that drive the investigations in the field of Tissue Engineering (Wiria et al., 2010). Therefore, performing transplants from organs or tissues of the recipient's own body is a possibility to eradicate such problems.

Currently the development of scaffold fabrication techniques with appropriate macro and microstructures for Tissue Engineering is a technical and engineering challenge to be overcome (Griffith and Naughton, 2002). Conventional manufacturing processes (gas foaming, fiber bonding, solvent casting and particulate leaching) produce scaffolds for cell adhesion and development (Yang et al., 2001). However, these methods have their limitations in the production of scaffolds with pores of controlled dimensions and defined architecture.

ASTM F2792-12a (American Society for Testing and Materials) defines Additive Manufacturing as materials bonding processes, usually layer by layer, by which three-dimensional objects are formed from CAD (Computer Aided Design) models (ASTM, 2012). Laser sintering (LS), an additive manufacturing technology, enables the production of scaffolds with complex, highly porous geometries with interconnected pores Gibson et al. (2015). This is perceived as a viable alternative for obtaining scaffolds suitable for Tissue Engineering, (Wiria et al., 2010).

It is desirable that the scaffolds have a high porosity (with pore interconnectivity, with defined architecture and size), a defined roughness and mechanical properties suitable for its future application. For the polymer materials, properties such as biocompatibility, biodegradation, bioreabsorbency, hydrophilicity or hydrophobicity and crystallinity are desired, (Shuai et al., 2013b). The poly (vinyl alcohol) (PVA) was the polymer of choice for this work, because it satisfies all the requirements when it comes to the desired properties.

2. OBJECTIVES

The objective of this work was to investigate the influence of the main parameters (laser scan speed, laser scan spacing, layer thickness, laser power and laser scan count) of the laser sintering process of poly (vinyl alcohol) (PVA) polymer material on the properties of scaffolds (microstructure and porosity) through microstructural characterization and porosity measurement and analysis of the aforementioned properties of the scaffolds after degradation tests.

3. MATERIALS AND METHODS

The experiments were carried out on the laser sintering machine Sinterstation 2000 (3D Systems Inc.), located in the Renato Archer Information Technology Center in Campinas, São Paulo [partner of this project]. The material used in the experiments was the polyvinyl alcohol powder (Mw 89,000-98,000 g / mol, 99 +% hydrolyzed) purchased from Sigma-Aldrich. A Tescan VEGA3 LMU scanning electron microscope (SEM), located at PUCPR, was used to analyze the surface microstructure of the samples that were submitted to the gold coating process.

A Scanning Electron Microscope (SEM) was used to analyze the results. The operation of the SEM occurs as follows: an electron gun is fired, emitting a beam of electrons (through a difference of electric potential) through electromagnetic condensing lenses, which aims to reduce the final diameter of the electron beam. After leaving the cannon and passing through the lenses, the electron beam hits the sample and is deflected. This deflection is then captured by the detectors that transmit the signal to the display system. The latter is responsible for transmitting the formed image (Maliska, 2004). The methodology covered in this work comprised three stages. The steps occurred as follows:

Step 1 - The preparation and characterization of the microstructure and porosity of the laser sintered test samples was conducted. Then, an estimate of the pore volume of the samples was performed. The estimation was made by relating the samples density and volume, as per the ASTM F2450-10 standard. The pore volume, V, was calculated according to Eq. (1).

$$V_p = V_t - \frac{m_s}{\rho_s} \tag{1}$$

Where V_t is the total volume, m_s the mass of scaffold and ρ_s the relative density of the scaffold. The mass of the sample, m_{sa}, was measured and the density of the PVA, ρ_{pva}, was obtained experimentally. The volume of the test sample, V_{sa}, was determined by measuring the sample size and calculated through Eq. (2), the volume of a cylinder.

$$V_{sa} = \pi r^2 h \tag{2}$$

Where r is the scaffold radius and h is the height of the scaffold.

Step 2 - The fifth step was not completed, because it was not possible to manufacture the scaffolds with different internal geometries due to the lack of availability of the laser sintering machine, located in the city of Campinas. In addition, the non-performance of the experimental tests in PUCPR is due to the fact that the EOS P396 machine, German manufacturing, approved in Institutional Project of the FINEP Announcement Infrastructure Research in Community Universities - 01/2013, was not installed at the time due to the delay of the execution of the pneumatic and electrical infrastructures, as well as the availability of training by the German manufacturer.

Step 3 - The sixth step also could not be completed because it depended on the completion of the fifth step to be performed.

4. RESULTS

After the theoretical steps (1, 2 and 3) the polyvinyl alcohol material was analyzed before and after sintering. As can be seen in Fig. 1, the PVA powder, before passing through the sintering process, is composed of spherical particles of various sizes.

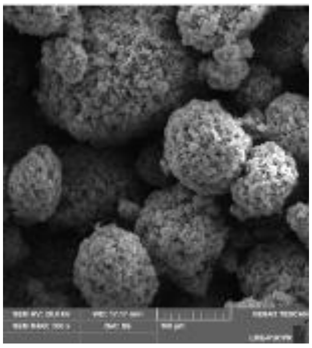


Figure 1. Micrograph of PVA powder before sintering.
Scale bar = 100 µm.

During the fourth stage of this work the test samples were produced in two phases by laser sintering of the PVA powder.

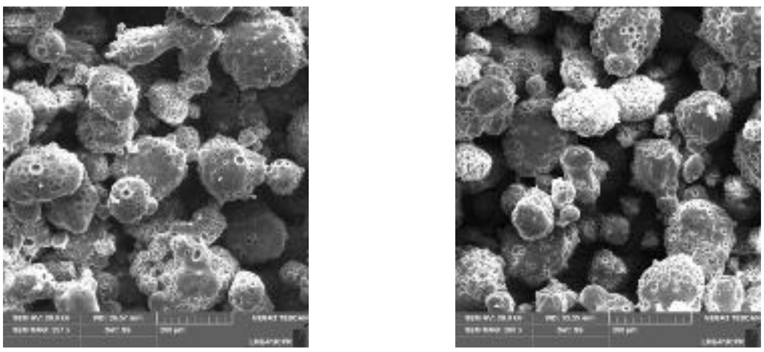
4.1. Phase 1

In the first phase, experiments were carried out in order to find the set of parameters through which it was possible to produce samples, since PVA is not a standard material for laser sintering. During the manufacture of the samples, the laser scanning speed was maintained at 2000 mm/s. Other parameters such as temperature on the construction platform, laser power, spacing between laser scanning lines, layer thickness and feeder temperature were varied as shown in Tab. 1.

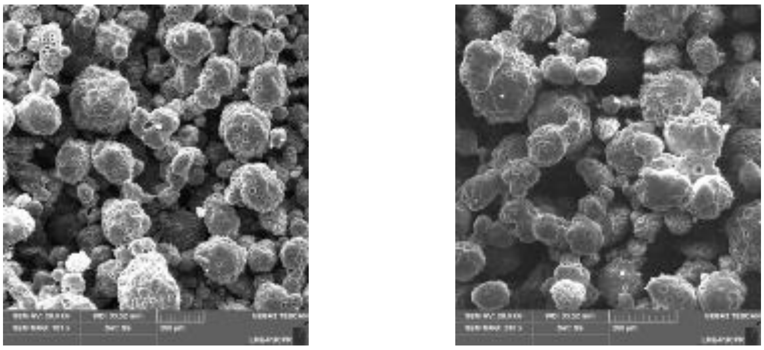
Table 1. Phase 1 sintering parameters.

<table>
<tr><th>Sample Set</th><th>Building Platform Temp. (°C)</th><th>Scan Speed (mm/s)</th><th>Power (W)</th><th>Scan Spacing (mm)</th><th>Layer Thickness (mm)</th><th>Feeder Temp. (°C)</th></tr>
<tr><td>1</td><td rowspan="6">65</td><td rowspan="20">2000</td><td>22</td><td>0,12</td><td>0,15</td><td rowspan="4">TF = 40 °C</td></tr>
<tr><td>2</td><td>26</td><td>0,12</td><td>0,15</td></tr>
<tr><td>3</td><td>22</td><td>0,12</td><td>0,22</td></tr>
<tr><td>4</td><td>26</td><td>0,12</td><td>0,22</td></tr>
<tr><td>5</td><td>22</td><td>0,12</td><td>0,22</td><td rowspan="2">TF = 65 °C</td></tr>
<tr><td>6</td><td>26</td><td>0,12</td><td>0,22</td></tr>
<tr><td>7</td><td rowspan="12">70</td><td>24</td><td>0,12</td><td>0,22</td><td rowspan="3">TF = 70 °C</td></tr>
<tr><td>8</td><td>26</td><td>0,12</td><td>0,22</td></tr>
<tr><td>9</td><td>28</td><td>0,12</td><td>0,22</td></tr>
<tr><td>10</td><td>22</td><td>0,12</td><td>0,22</td><td rowspan="9">TF = 60 °C</td></tr>
<tr><td>11</td><td>24</td><td>0,12</td><td>0,22</td></tr>
<tr><td>12</td><td>26</td><td>0,12</td><td>0,22</td></tr>
<tr><td>13</td><td>22</td><td>0,10</td><td>0,22</td></tr>
<tr><td>14</td><td>24</td><td>0,10</td><td>0,22</td></tr>
<tr><td>15</td><td>26</td><td>0,10</td><td>0,22</td></tr>
<tr><td>16</td><td>22</td><td>0,14</td><td>0,22</td></tr>
<tr><td>17</td><td>24</td><td>0,14</td><td>0,22</td></tr>
<tr><td>18</td><td>26</td><td>0,14</td><td>0,22</td></tr>
<tr><td>19</td><td rowspan="2">90</td><td>35</td><td>0,12</td><td>0,15</td><td rowspan="2">TF = 50 °C</td></tr>
<tr><td>20</td><td>26</td><td>0,08</td><td>0,15</td></tr>
</table>

In Fig. 2 it can be seen that, after being sintered, the particles from sets 2, 3, 4 and 7 partially melted the outer portion and there was little necking between the PVA particles in the samples. This suggests that the low energy density delivered through different set of parameters during the sintering of the test samples may be the reason why adequate sintering has not been achieved.



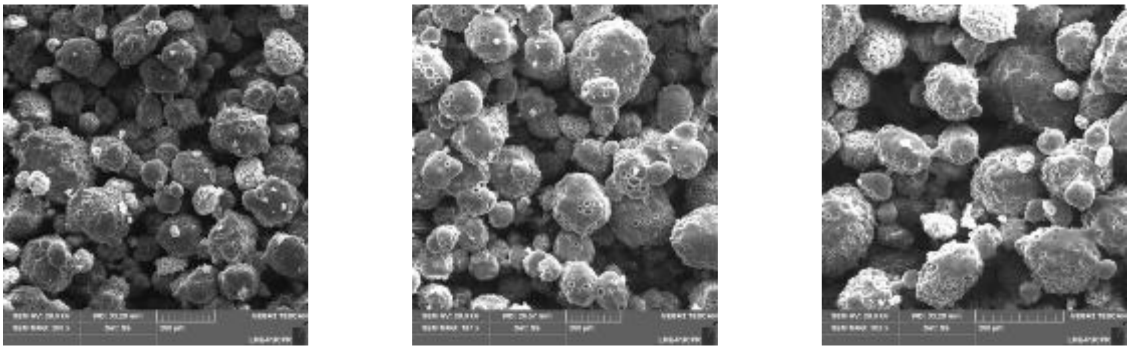
(a) (b)



(c) (d)

**Figure 2. Laser sintered samples: (a) Set 2, (b) Set 3, (c) Set 4, (d) Set 7.
Scale bar = 200 µm.**

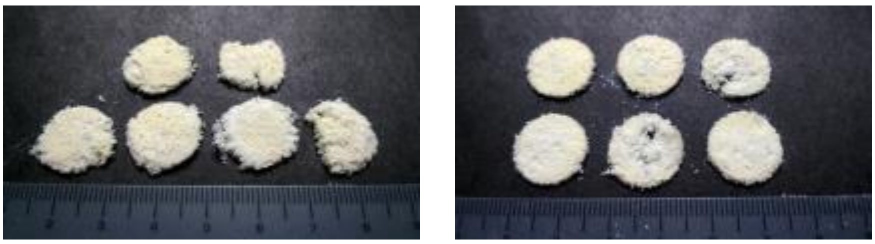
The same occurred with the samples of sets 10, 15 and 18, as can be seen in Fig. 3.



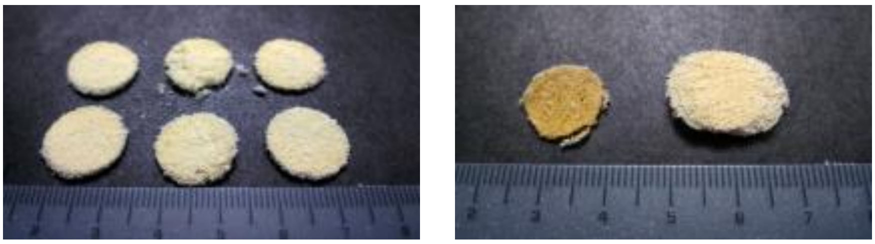
(a) (b) (c)

**Figure 3. Laser sintered samples: (a) Set 10, (b) Set 15, (c) Set 18.
Scale bar = 200 µm.**

During the first phase it can be seen that almost all of the test samples were fragile and, in some cases, fragmented, as evidenced in Fig. 4. However, one sample of set 19 presented a better result when compared to other samples because the layer was sintered six times and, therefore, exposed to greater amounts of energy.



(a) (b)



(c) (d)

Figure 4. Laser sintered samples: (a) Set 1, (b) Set 5, (c) Set 7, (d) Set 19.

The formation of necking, shown in Fig. 5, in the sample of set 19 suggests that the amount of energy supplied to the test samples is a decisive factor in the quality of the final pieces.

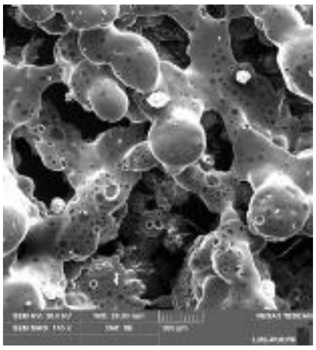


Figure 5. Sintered PVA – Set 19.
Scale bar = 200 µm.

4.2. Phase 2

After the manufacturing and study stages of the first phase samples, the laser power was increased up to the allowed limit of the machine, 40 W, in order to improve the degree of sintering, providing the maximum energy to the powder. However, as can be seen in Fig. 6, the result was similar to those found in phase 1.

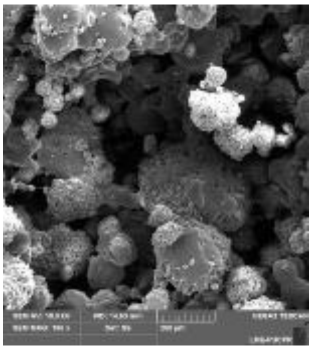


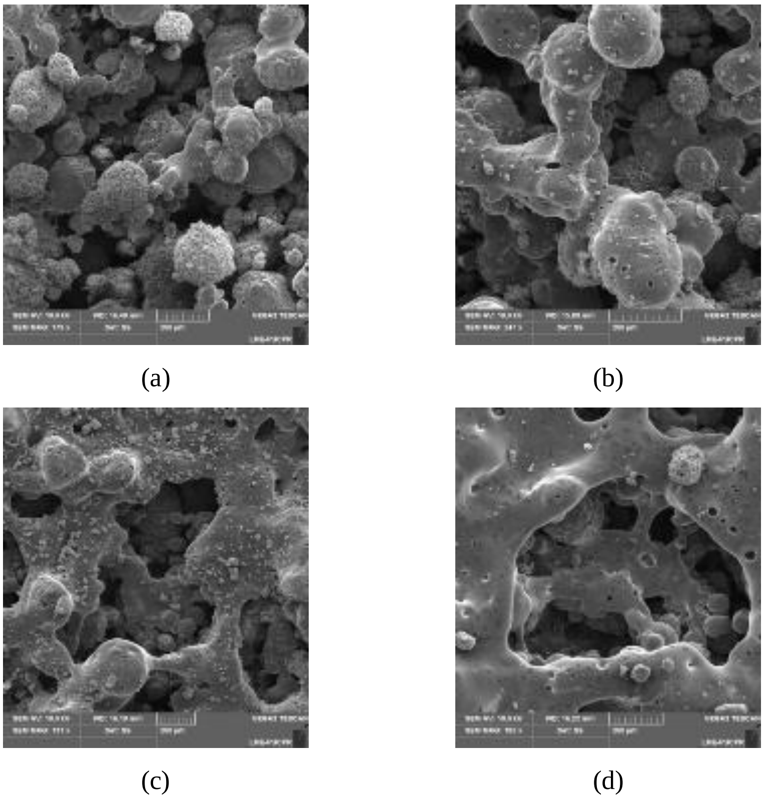
Figure 6. Sintered PVA – 40W.
Scale bar = 200 µm.

Following up, the process parameters such as building platform temperature, laser scanning speed, laser scan spacing and layer thickness, as shown in Tab. 2, were kept constant. In order to increase the amount of energy delivered to test samples, only laser power (26 W and 32 W) and laser scan count (1, 2, 4 and 6) were varied.

Table 2. Phase 2 sintering parameters.

Building Platform Temperature (°C)	80
Laser Scan Speed (mm/s)	2000
Laser Scan Spacing (mm)	0,12
Layer Thickness (mm)	0,46
Laser Power (W)	26, 32
Laser Scan Count	1, 2, 4, 6

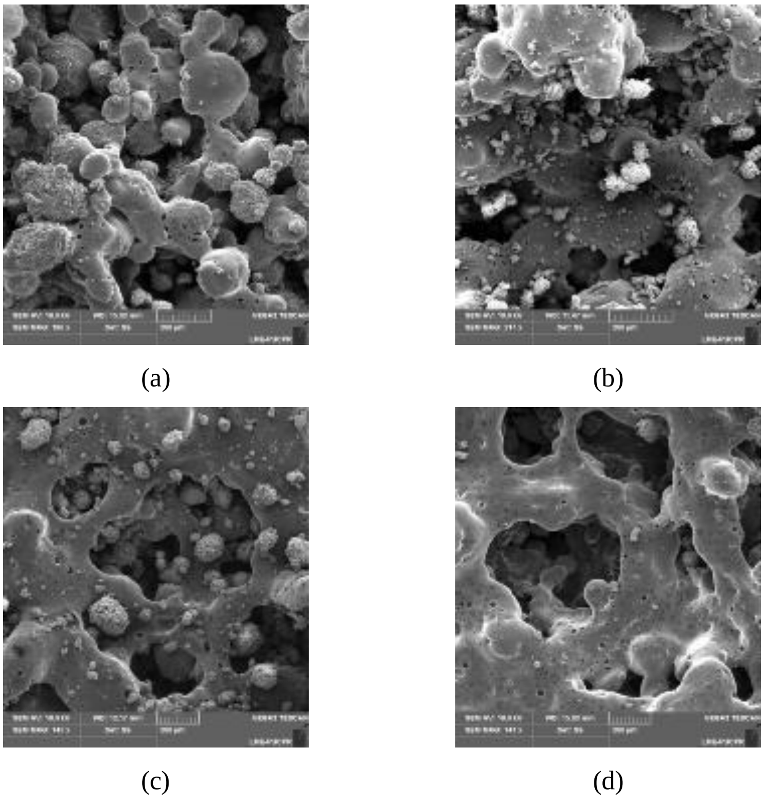
In Fig. 7 it can be observed that the greater the number of times the laser sweeps the sample at the same laser power (26 W), the greater the number of bonds between the PVA powder particles.



(a) (b) (c) (d)

Figure 7. Samples sintered at 26 W: (a) 1 time, (b) 2 times, (c) 4 times, (d) 6 times. Scale bar = 200 µm.

Similar results were found for the sintered test samples with 32 W laser power, as evidenced by Fig. 8.

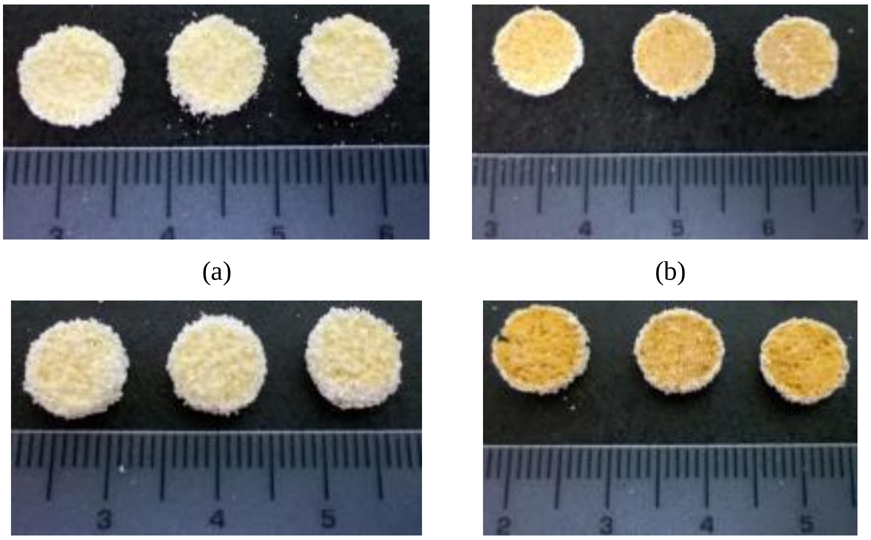


(a) (b) (c) (d)

Figure 8. Samples sintered at 32 W: (a) 1 time, (b) 2 times, (c) 4 times, (d) 6 times.

Scale bar = 200 µm.

The direct proportionality between the laser scan count and the amount of bonding between the particles suggests an increase in the amount of energy delivered to the samples. This increase in the amount of energy provided an improvement in the mechanical properties of the test samples, which became less fragile and ceased to crumble, as well as a change in the color of the samples. This can be seen in Fig. 9.



(a) (b) (c) (d)

Figure 9. Samples sintered at powers of: 26 W - (a) 1 time, (b) 4 times; 32 W - (c) 1 time, (d) 4 times.

During the studies of the samples in the second phase it was also noticed that with the increase of the laser power, for sintered samples with same laser scan count, the greater the number of bonds between the PVA powder particles and the higher the level of sintering of the deepest layers of powder. This implies that there was an increase in the amount of energy supplied to the test samples, as occurred with the increase in energy provided by the greater laser scan count.

4.2.1 Pore volume estimative

After the first and second phase studies, an estimate was made of the pore volume of the samples manufactured in phase 2, that is, with 26 W and 32 W laser power and with laser scan count of 1, 2, 4 and 6. The results for the different samples are presented in Tab. 3 and can be better visualized in the graph of Fig. 10.

Table 3. Results of pore volume estimation.

		Pore Volume (%)
26 W	1x	99,975 ± 0,002
	2x	99,970 ± 0,003
	4x	99,965 ± 0,001
	6x	99,965 ± 0,0003
32 W	1x	99,972 ± 0,0005
	2x	99,968 ± 0,001
	4x	99,963 ± 0,0012
	6x	99,962 ± 0,0005

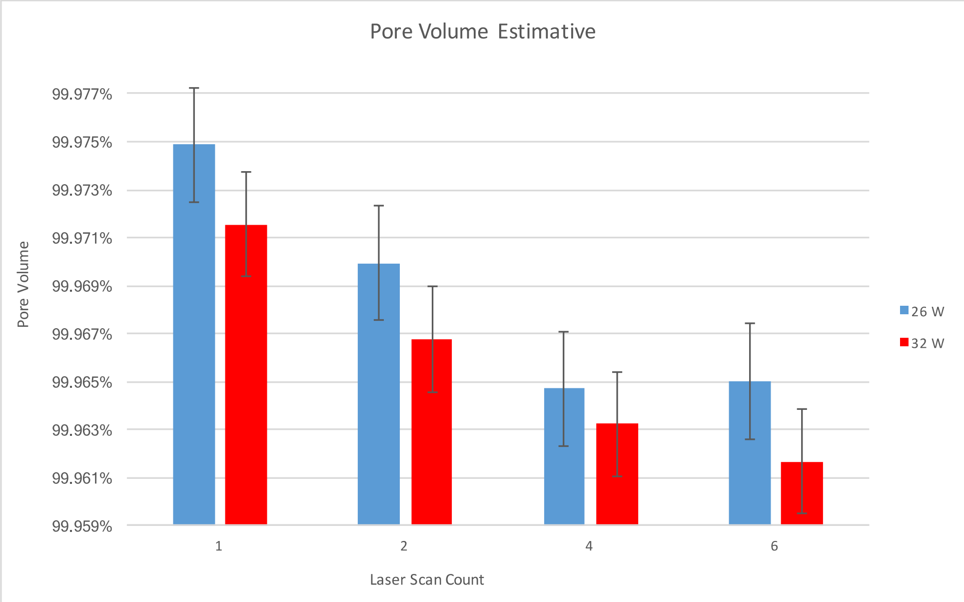


Figure 10. Estimation of the pore volume of the samples sintered in phase 2.

The graph of Fig. 10 suggests that, as in Fig. 7 and Fig. 8, the pore volume decreases proportionally with the increase in the amount of energy supplied to the samples. This means that the more energy the samples are supplied with, the greater the necking between the particles and the smaller the pore volume of the scaffolds.

5. DISCUSSION

During the third stage of this work, several articles were studied in order to determine the parameters (building platform temperature, laser power, laser scan spacing, laser scan speed and layer thickness) to be used during the manufacturing process, via laser sintering, of scaffolds with suitable properties for Tissue Engineering.

The work in which the production of PVA scaffolds by laser sintering was investigated and presented in the articles "Selective laser sintering of biocompatible polymers for applications in tissue engineering" by Tan et al. (2005) and “Development of complex porous polyvinyl alcohol scaffolds: Microstructure, Mechanical, and Biological evaluations” by Shuai et al. (2013a), were adopted as reference for manufacturing of the test samples of this work.

During the manufacturing of the scaffolds in this work, it was not possible to vary the scan speed of the laser due to the limitations of the machine used in the process. This changed the approach in the production of scaffolds based on the articles taken as reference, because in both articles the laser scan speed is inferior to the speed used in this work. It was then necessary that several parameters were varied, so that their respective influences on the final result were measured. The results of these evaluations are the samples produced in the first phase.

The analyzes carried out in the first phase revealed that, in general, the samples produced were fragile, deformed easily and had results that were far from those observed in the articles produced by Tan et al. (2005) and Shuai et al. (2013a). However, when sintered more than once, thus exposed to the greatest amount of energy, the test sample from set 19 presented more satisfactory results. This led to the conclusion that most of the samples from the first phase were subjected to a low amount of energy when the layers were sintered only once, which did not allow adequate melting of the PVA powder particles.

In order to increase the amount of energy supplied to the test samples, in the second phase samples were produced by varying only laser power and laser scan count. The studies conducted have shown that both the increase in laser scan count and in laser power lead to an increase in the energy supplied to the test samples. This increase of energy resulted in test samples with three-dimensional porous structures and with interconnected pores, much like as in the articles produced by Tan et al. (2005) and Shuai et al. (2013a). The pore volume estimation showed that the more energy (the greater the number of sintered times and the higher the laser power), the smaller the pore volume the scaffolds presented.

6. CONCLUSION

The present work aimed to investigate the influence of the main parameters of the laser sintering process of the polymeric PVA material on the properties of the scaffolds, through microstructural characterization and porosity measurement. During the execution of this work studies on the foundations of the additive manufacture, laser sintering, polymers, scaffolds and scanning electron microscope were conducted. The preparation and characterization of the test samples and an estimation of the pore volume were performed. The studies carried out served as a basis for the analyzes conducted in the first and second phases of this work.

It was observed that, in general, the samples produced during the first phase were fragile and easily deformed, since they were submitted to a low amount of energy. In the second phase, it was noticed that both the increase in laser scan count and in laser power lead to an increase in the energy supplied to the test samples, causing changes in the surface microstructure of the scaffolds as well as a decrease in pore volume.

It is concluded that the variation of parameters such as building platform temperature, laser power, laser scan spacing, laser scan speed and layer thickness determine the amount of energy supplied to the test samples during the laser sintering process, and consequently determine the final quality of the surface microstructure and porosity of the scaffolds.

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