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SELECTIVE LASER SINTERING OF POLY(VINYL ALCOHOL) WITH THE PURPOSE OF PRODUCING SCAFFOLDS FOR *IN VITRO* STUDIES

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Abstract. *In Additive Manufacturing (AM) parts are produced by adding material layer-by-layer. AM technologies play an increasingly important role in the biomedical industry, like in tissue engineering (TE). In TE new and functional living tissue is fabricated using living cells, which are usually associated with a scaffold or 3D structure to guide tissue development aiming at tissue regeneration. AM technologies offer excellent potential for fabricating TE scaffolds due to their ability to create structures with complex geometry and internal architecture with a high degree of automation, good accuracy and reproducibility, as opposed to conventional scaffold fabrication methods. The main goal of this research project is to investigate the Selective Laser Sintering (SLS), an AM technology, of poly(vinyl alcohol) (PVA) polymer material, through experimental tests, with the purpose of fabricating scaffolds for the application in in vitro studies. The influence of the number of scans on the surface microstructure and PVA material properties is being investigated. The number of scans varied from 1 to 6. It is possible to conclude that the number of scans has a strong influence on surface microstructure of the laser sintered samples and could also change PVA material properties after SLS. In vitro cell culture tests showed that the laser sintered PVA is biocompatible with human osteoblast cells.*

Keywords: *Selective Laser Sintering, Tissue Engineering Scaffolds, PVA*

1. INTRODUCTION

Selective Laser Sintering (SLS) is an additive manufacturing technology in which a laser beam is used to sinter powder materials layer-by-layer to produce solid 3D objects. A 3D CAD file is created and then the system software uses this file to slice the part in many layers, each one representing a 2D cross-section of the virtual model. Next, in the SLS machine, the first layer of powder is spread and leveled on the building platform using a roller or a blade. Then, a laser beam is directed onto the powder bed by a laser scanning system, causing the particles to fuse together, following the cross-sectional profile of the digital model, resulting in a sintered layer. The building platform moves down, another layer of powder is spread and leveled on the building platform, the laser beam scans over the powder bed, sintering this layer and binding it to the previous layer. This process repeats until the part is completely built.

SLS offers good potential for fabricating tissue engineering scaffolds as it can produce porous scaffolds with complex geometry, channels and controlled porosity and, in the case of polymers, it is solvent free; it may be applied as an alternative to conventional scaffold fabrication methods to overcome their limitations.

Poly(vinyl alcohol) (PVA) is a biocompatible and non-toxic material, has good hydrophilicity and chemical stability, being one of the most biomaterials used in biosensing and more recently in biomedical application (Reis *et al.*, 2006). In addition, it has been successfully laser sintered by Chua, *et al*., 2004, Tan, *et al*., 2005, Wiria, *et al*., 2008, Wiria, *et al*., 2010, Shuai, *et al*., 2013a and Shuai, *et al*., 2013b. Chua *et al*. and Wiria *et al*., showed that positive results can be obtained by adjusting the SLS main processing parameters, namely the laser power, scan speed and part bed temperature. Besides, they demonstrated the viability of using PVA as a material for fabricating TE scaffolds. However, Shuai, *et al*., 2013a and Shuai, *et al*., 2013b, showed that the laser beam power necessary to sinter the samples vary strongly among different equipment, and that particles with different geometry and sizes also affect the final result.

The purpose of this research work, then, is to investigate the production of PVA scaffolds by SLS for *in vitro* studies, a relatively new and growing area in which scaffolds could be used in the design of 3D models for *in vitro* disease model or tissue equivalent for safety and effectiveness tests. The specific objective is to investigate the influence of the number of scans on scaffold surface microstructure and on PVA material properties: degree of crystallinity and chemical stability.

2. METHODOLOGY

2.1 Material and SLS machine

Poly(vinyl alcohol) powder material (M_w 89000-98000 g/mol, 99+% hydrolyzed) (Sigma-Aldrich company) was used for this research. The experiments were carried out on the SLS machine Sinterstation 2000 (3D Systems, Inc.).

2.2 Fabrication of samples

Samples were produced as circular discs with diameter of 9 mm and height of 2 mm. The number of scans was varied from 1 to 6 while the other SLS process parameters were kept constant (Table 1).

Table 1. SLS process parameters.

Powder bed temperature	80 °C
Laser scan speed	2000 mm/s
Scan spacing	0,12 mm
Powder layer thickness	0,46 mm
Laser power	26 W

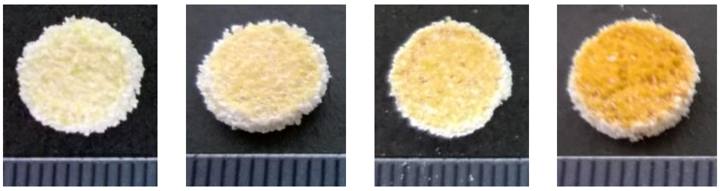
Microstructure analysis of the surfaces of the samples after the SLS process was performed using the Tescan VEGA3 LMU scanning electron microscope. In addition, it was used to analyze the sample surfaces after the cell culture tests. The degree of crystallinity of the PVA powder and PVA after the SLS process was qualitatively analyzed using the Shimadzu XRD-7000 X-ray diffractometer. The measurements were conducted using step scan in which the angle (2θ) was set between 4-60° and step size at 0,02°. Fourier Transform Infrared Spectroscopy (FTIR) spectra were used to qualitatively analyze the chemical stability of PVA after the SLS process. The spectra were measured with a Bruker's Vertex 70 FTIR in the wavenumber range of 4000-600 cm^{-1}, with resolution of 4 cm^{-1} and 16 scans, using the Attenuated Total Reflectance (ATR) mode.

2.3 Cell culture

For cell culture tests, laser sintered samples with number of laser scans 4 and 6 were used. However, as a result of the sintering process, small powder particles remain entrapped inside the samples. Since this lose particles could leave the sample during cell culture and therefore prevent cell growth, they have to be removed from the sample prior cell culture. For that the samples were ultrasonically washed in deionized water for 20 minutes, after what they were left to dry for 24 h at 40 °C in a furnace. The samples were then sterilized by immersion in 70% ethanol for 24 hours and then washed with phosphate-buffered saline (PBS) solution. Osteoblastic cells derived from human osteosarcoma, SaOs-2, were deposited on the samples at a concentration of 1×10^4 cells/mL in a McCoy#s 5A culture medium. Cell culture was carried out on a 24-well cell culture plate in an incubator at 37 °C containing 5% CO_2. Cell growth analysis was performed after 3 days of culture.

3. RESULTS AND DISCUSSION

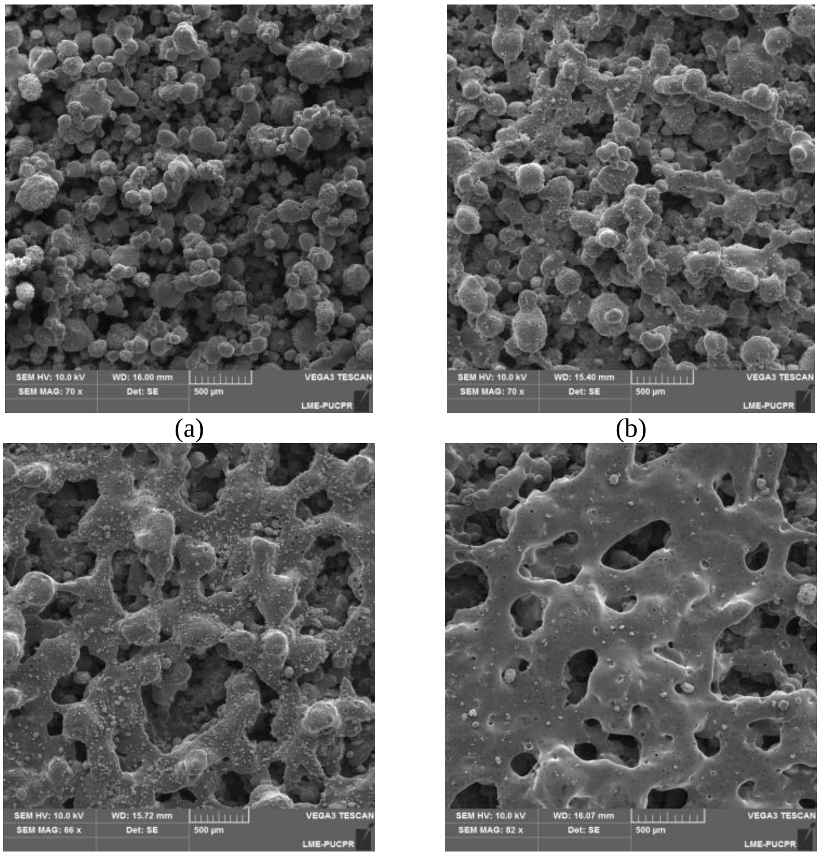
Samples resultant from the sintering process on PVA, performed with a laser power of 26 W, are shown in Figure 1. The most evident difference between them is the color changes that turn more brownish as it goes from Fig. 1(a) to 1(d). This color change is a result of the number of laser scans used to prepare each sample; in this case 1, 2, 4 and 6 lasers scans, respectively.



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

Figure 1. Laser sintered samples. Number of scans (a) 1, (b) 2, (c) 4 and (d) 6.

Although, usually, with SLS technique, only one laser scan is necessary to coalesce powder particles completely, this is not the case we found for PVA, for which the best results were obtained with 4 and 6 laser scans. For laser powers that did not induce materials degradation, limited to the range of 16 to 32 W, 1 or 2 laser scans did not merge the particles sufficiently to attribute cohesion between them and between layers. This is more evident in Figure 2, where SEM images of the surfaces of the laser sintered samples are shown, and from which it can be observed that the necking between particles increase with the increase of the number of scans while pore sizes decreases.



(c) (d)

Figure 2. SEM images of the laser sintered samples. Number of scans (a) 1, (b) 2, (c) 4 and (d) 6. Scale bar = 500 µm.

With 1 laser scan, Fig. 1(a), only partial fusion occurred resulting in the initial formation of necking between some particles, but the samples easily dismantle with the touch. With 2 laser scans, Fig 1(b), the necking formation increases, however, the particles are predominantly loose and the layers that are being sintered frequently slide over the layers beneath, due to low binding between them. With 4 laser scans, Fig. 1(c), in addition to wider necks between particles, what induced macropores formation, coalescence between the layers was obtained, although loose particles were still present. With 6 laser scans, Fig. 1 (d), the merging between particles resulted in complete coalescence, originating a mechanically stable structure with a minimum of loose powder particles.

Another aspect of the color change is that it can be related to material degradation due to SLS process. PVA, although its high fusion temperature of 180 °C, is known to undergo degradation at temperatures close to 90 °C, being the loss of crystallinity one of the major consequences. The diffraction pattern of PVA presents four main characteristic peaks, as shown in Fig. 3. Partial loss of crystallinity due to PVA exposure to high energy laser beam should be expected, since the powder particles external surface are melt, however, the crystalline characteristic of the particles core shouldn't be affected since only the outer cap is melted. The overall result is a reduction in the intensity of the peaks, what is observed in the diffractograms of all samples.

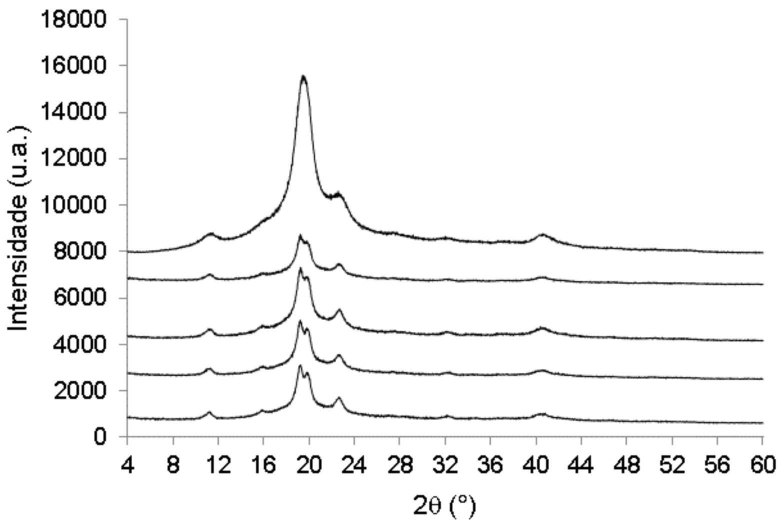


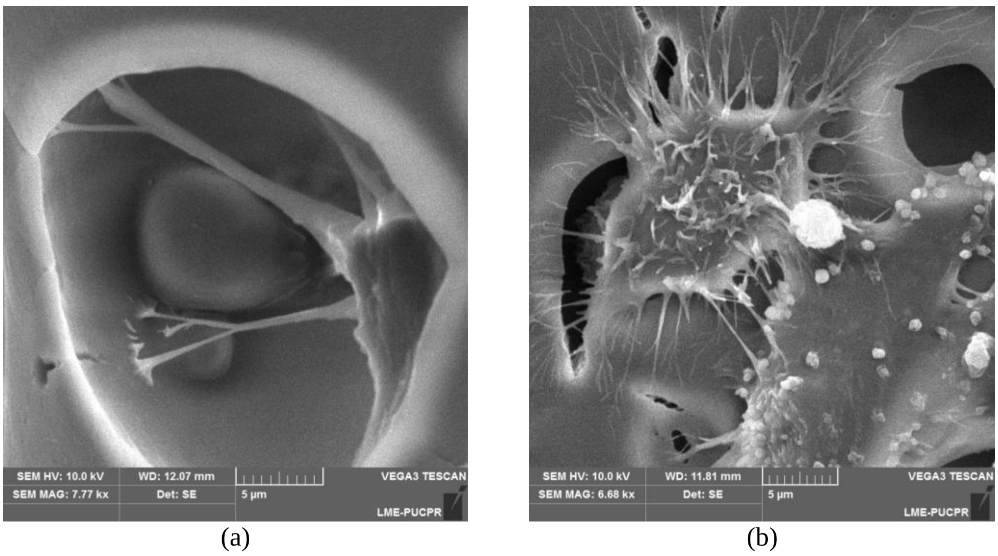
Figure 3. Diffraction spectra of the PVA powder and the laser sintered samples prepared with 1, 2, 4 and 6 laser scans, respectively. Scale bar = 500 µm.

Comparison of the results shows that there is no significant difference in the relative intensity between the four samples with different number of scans (1, 2, 4 and 6), that is, the peak intensities did not change with the increase of the number of laser scans. Differently from the report of SHUAI *et al.*, 2013a, no new peaks were observed, from which one may imply the absence of thermal degradation of PVA during the SLS process.

FTIR analysis (not shown) however, shows three main changes: the appearance of acetate group band at 1024 cm^{-1}, associated to the (C–O) stretching vibration, a band suppression at 920 cm^{-1}, which corresponds to the C–H vibration of PVA, and a band displacement at wavenumber 3427 cm^{-1}, attributed to the vibration of hydroxyl groups (O–H).

Despite the changes induced by the laser sintering process using laser power of 26 W and different number of laser scans (1, 2, 4 and 6), which could be considered as an important factor in the material degradation in culture medium, no changes, or signs of degradation, were observed in the samples exposed to the culture medium.

To evaluate the biocompatibility between the laser sintered PVA and SaOs-2 cells, samples fabricated with 4 and 6 laser scans, were subjected to cell culture tests using osteoblast SaOs-2 cells. The results are shown in Fig. 4, where it is possible to observe that, for both samples, after 3 days of cell culture, the SaOs-2 cells adhered well to the laser sintered PVA surface, spreading into the pores.



(a) (b)

Figure 4. SEM images of SaOs-2 cells after cell culture for 3 days. Number of scans (a) 4 and (b) 6. Scale bar = 5 µm.

The results indicate that the biocompatibility, expected between the laser sintered PVA and SaOs-2 cells, was not affected by the fact that PVA may have undergone thermal degradation, as the samples darkened with the increase in the number of scans, from 4 to 6.

4. CONCLUSIONS

The main goal of this research work was to study the selective laser sintering process of poly(vinyl alcohol) - PVA polymer, through experimental tests, in order to produce tissue engineering scaffolds to be used for *in vitro* studies. The influence of the number of laser scans on the microstructure on the surface of the samples and on the PVA material properties (degree of crystallinity and chemical stability) was investigated.

For the manufacture of parts from PVA powder material by SLS using the Sinterstation 2000 machine, laser power of 26 W and number of laser scans 1, 2, 4 and 6 it can be concluded that:

i) the number of laser scans has a great influence on the mechanical stabilility of the scaffold as well on the surface microstructure;

ii) the SLS process caused a decrease in the degree of crystallinity of the as-received PVA and no degradation occurred, however the color changes in the samples indicate a possible thermal degradation of the material;

iii) the SLS process may have changed the chemical composition of the as-received PVA, especially when more laser scans are performed;

iv) cell culture tests have shown that the laser sintered PVA material is biocompatible with osteoblastic cells derived from human osteosarcoma SaOs-2.

5. ACKNOWLEDGEMENTS

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7. RESPONSIBILITY NOTICE