RESEARCH ARTICLE OPEN ACCESS

Biocompatibility of Poly (L-Lactic Acid) Synthesized In Polymerization Unit By Cytotoxicity And Hemocompatibility Assay And Nanofibers Production

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ABSTRACT

The absorbable polyacid is one of the most used and studied materials in tissue engineering. This work synthesized a poly (L-lactic acid) (PLLA) through ring-opening polymerization and produced nanofibers by the electrospinning process. The PLLA was analyzed by FTIR and the cytotoxicity was evaluated by the MTT assay and Live/Dead®. The hemocompatibility was tested by platelet adhesion and hemolytic activity assay. The tests were performed in contact with human mesenchymal cells at varying times. The high rates of cell viability and proliferation shown by MTT and Live/Dead® tests demonstrate that this PLLA is a non-toxic material and the hemocompatibility assay revealed that the biomaterial was also biocompatible. It was achieved as well the successful production of electrospinning nanofibers, which can be converted for specific biomedical applications in the future.

Keywords **-** biomaterials; material synthesis; PLLA; cell culture; MTT test; tissue-engineering; biofabrication

I. INTRODUCTION

For the last few years, there was a growing interest in the field of biomaterials and tissue engineering. This interest is a result of the indispensable role of those in the medical field. Biomaterials are biocompatible and bioactive, with the intent to interact with biological systems, as well as restore functions of living tissues and organs on human or animal individuals [1-4].

Tissue engineering is a new field of study that utilizes the basic concept and technique of life science and engineering, providing an understanding of the co-relationship between the structure and function of a body tissue, aiming to create a substitute of it for transplantation, therefore to maintain, improve or restore the function of human body. Biomaterials are usually used in tissue engineering and defined as any substance, except food and medications, that can be used for a length of time as part of a system that aims to treat or to restore any tissue, organ, or body function [5, 6]

The polymers compose a very broad class of material and there had been developments in the biomedical field for use of those polymeric materials. These biomaterials must show appropriate mechanical properties as well as biocompatibility, to be used in human body. Thus, they should not present any local or systemic adverse biological response. The material should be non-toxic, non-carcinogenic, non-antigenic and non-mutagenic. In blood applications, they must also be non-thrombogenic [7].

There are three categories of tests for assessing cytotoxicity that are listed in ISO 10993-5 (ISO: No. 10993-1 (2009) Biological evaluation of medical devices) (extract test, direct contact test, indirect contact test). It is possible to apply a wide variety of experimental protocols to conclude about the cytotoxicity of the materials. The choice of one or more of these categories, depends on the nature of the sample to be evaluated, the potential site of use, and the nature of the use. [8]

The development of synthetic materials, textured polymers and metals, as well as their increased use in medicine makes the research of biomaterials hemocompatibility pertinent. Complications arise from the polymorphism and diversity of the different materials, as well as the static and dynamic test models and the patient's individual biologic factors. First, methods, models, tests as well as preanalytical factors have to be standardized according to the current knowledge in medicine laid down in the ISO 10993. Hemocompatibility tests are a prerequisite for

materials used in biomedical devices such as treatments including venous catheters, stents, vena cava filters, vascular grafts and prosthetic heart valves. Those are a few examples of biomaterials that are introduced into the vascular system and brought into contact with blood, and thus, it is vital to assure the hemocompatibility of it . [9]

Nowadays, many biomaterials are used in the medical area. We can find bioabsorbable screws and pins, biodegradable peripheral stents and suture materials. Frequently these materials are made of poly(L-Lactide) (PLLA) and others compounds which contains that polymer. Polylactides in overall and in specific poly (L-lactide) (PLLA) may be seen as second-generation biomaterials[1-4]. Different variants of temporary devices have been used in the medical field, and the most used are polyesters composed of derivatives of α-hydroxy acids such as poly (L-lactic acid) (PLLA), poly (D-lactic acid) (PDLA), poly (DL-lactic acid) (PDLLA), poly (glycolic acid) (PGA) and polycaprolactone (PCL). The polymer is degraded through simple hydrolysis, breaking the molecule into small units, and so its products can then be eliminated from the body through natural metabolic pathways, such as citric acid cycle, or through renal excretion [10]. The hydrolysis initially promotes the fall of the molar mass, mainly in the amorphous regions. As the water diffuses into the device and fragmentation occurs to decrease in mechanical strength and further reduced weight due to hydrolysis and enzymatic attack [11].Thus, no surgical removal of the materials is necessary and over the time, the new tissue can be shaped substituting the mechanical purpose of the implant itself.

PLLA has gained great attention because of its excellent biocompatibility and mechanical properties. It also presents a diversification of applications, since simple changes in their physical and chemical structure may make it useful in different areas. Depending on the application and its final destination, is possible to get different products using specific polymerization routes. However, its long degradation time coupled with the high crystallinity of its fragments can cause inflammatory reactions in the body. In order to overcome this, PLLA can be used as a material combination of L-lactic and D, L-lactic acid monomers, being the latter rapidly degraded without formation of crystalline fragments during this process [12].

Prior works done by our group [13, 14] have shown that the PLLA can be obtained using different routes (Figure 1). In general, three methods can be used to produce high molecular mass PLLA of about 100,000 Daltons: (a) direct condensation polymerization; (b) azeotropic dehydrative condensation and (c) polymerization through lactide formation, the ring-opening polymerization [15]. Currently, direct condensation and ring-opening polymerization are the most used production techniques.

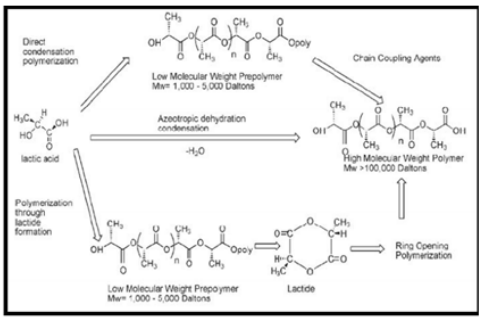


Figure 1. PLLA different synthesis methods [14]

While there are different ways to manufacture PLLA, its production is not easy. The synthesis of PLLA demands severe control of conditions such as temperature, pressure and pH, just as well as the use of catalysts and long polymerization time, which lead to a high-energy consumption to achieve the final product.

PLLA not only is a biodegradable and biocompatible material, it also presents mechanical response similar to collagen, as well as a semi crystalline structure, with an excellent elastic capacity, characteristics that makes the polymer a perfect candidate for electrospinning [16-19]. Electrospinning of polymer solutions has been extensively used in the last few years to produce polymeric fibers of Nano-dimensions [20].

The electrospinning fibers have exceptional properties due to the biomimetic features in consequence of the fiber caliber, with less than 1000 nanometers in diameter, as well as due to the high surface area/volume ratio [21]. In the electrospinning process, a capillary tube or syringe and a metallic electrode are used, associated to a grounded high voltage supply in the polymeric solution. An electrical tension is applied and when the electrostatic forces overcome the solution's surface tension, the hemispherical surface of the capillary's drop (Taylor's cone) elongates and an electrically charged jet of polymeric solution is generated. During the jet's trajectory to the collector, the solvent evaporates; thus solid nanofibers are formed and collected in the form of non-woven mats on the metallic collector. The variables of this process are many: solutions concentration (which determines the solutions viscosity), solvent type, applied electrical field, ionic salts addition (which can increase the solution's electrical conductivity), flow rate, temperature and others [20]. This work will present evidence about the properties and synthesis of PLLA tablets and nanofibers, as well as evaluate the biocompatibility of it, through cytotoxicity assays.

II. MATERIALS AND METHODS

1. Manufacturing of PLLA

1.1. Poly-lactic acid synthesis

The synthesis of PLLA was conducted by bulk polymerization by adding L-lactide monomer into a glass reactor containing the catalyst Sn (Oct) 2 (Sigma). The proportion monomer/catalyst was 0.5%. The mixture was immersed in an oil bath at 140°C for 2 hours under nitrogen flow. The produced polymer was dissolved in chloroform, CHCl3 (Merck), precipitated in ethyl alcohol and dried in a vacuum oven at 60°C for 12 hours. After the synthesis were manufacture tablets of PLLA and nanofibers.

1.2. Manufacturing PLLA *tablet*

The PLLA tablets of 2mm x 5mm were produced by compression of the raw material on an hydraulic press.

1.3. Manufacturing of PLLA nanofibers by *Electrospinning*

For the production of the PLLA nanofibers, the polymer was dissolved with acetone and chloroform. The solution was then loaded in a 10ml syringe, connected to a polyamide cylinder, attached to a 0,7mm hypodermic needle as a nozzle. The flow rate of the jet (8ml/h) was managed using a syringe pump. To charge the solution, a 15kV high voltage power-source was used. The distance between the needle and the collector plate was of 17cm. Meaning to enhance the experimental settings, a default electrospinning setup was used as shown in Figure 2.

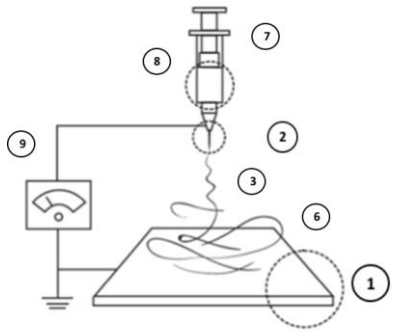


Figure 2. Diagram of the Electrospinning apparatus
1. Metallic Target; 2.Needle; 3.Nanowire;
6.Nanofiber mat; 7, Syringe Infuser; 8.Polymer;
9.High Voltage Source.

1.4. Fourier Transform Infrared Spectroscopy (FTIR)

The functional groups of the produced PLLA were analyzed by Fourier Transform Infrared Spectroscopy (FTIR), and paralleled with standard commercial polymer, PLLA (PURAC). The samples were evaluated by ATR accessory SMART mode OMNI-SAMPLER in an infrared Fourier transform spectrometer Thermo Scientific Nicolet 6700. The spectra were analyzed in the mid-infrared range 4000-675cm-1, obtained from the intensities absorption bands of molecules expressed in transmittance.

1.5. PLLA nanofiber morphology

To analyze the nanofiber morphology, images of the PLLA nanofibers were documented in the Scanning Electron Microscope (SEM) (LEO Electron Microscopy 440i). The nanofiber mat was sputter coating with gold, and then analyzed by SEM. The images were then processed with an image software (Image Pro Plus; Media Cybernetics Inc., USA).

1.6. Isolation of mesenchymal stem cells derived from adipose tissue

The human adipose tissue was obtained by liposuction procedure. It was washed with phosphate-buffered saline (PBS) to remove any connective tissue and red blood cells present. The adipose tissue was added to a 50-ml Falcon tube, followed by digestion for 30 minutes at 37 °C with 20 mg collagenase type 1A (Sigma, St. Louis, MO, EUA), 200 mg of bovine serum albumin (BSA), 20 ml of Dulbecco's Modified Eagle's Medium Low Glucose (DMEM-LG) and 10 µl of gentamicin. After tissue digestion, 10 ml of fetal bovine serum (FBS) was added to neutralize the enzymatic activity, and the cells were centrifuged at 1500 rpm for 15 minutes, resuspended in 10 ml DMEM with 10% FBS, seeded into culture plate and incubated at 37 °C with 5% atmospheric CO2. After 24 hours, the medium was changed every three days until the cells reached 70% of confluence. After four passages, the Adipose tissue-derived stem cells (ADSCs) (1x103 cells/ml) were characterized and seeded into a 96-well plate and incubated with DMEM-LG containing 10% FBS at 37 °C for 24 hours.

2. Cytotoxicity evaluation

2.1. Cells incubation

ADSCs suspensions (3x106 cells/ml) were inoculated into a 96-wells cell culture plate and incubated at 37 °C for 24 h. After this period, the PLLA samples (2mm x 5 mm) were cultured with the ADSCs for 24, 48 and 72 hours. DMEM-LG containing 0.5% phenol was used as the positive control for toxicity (CT+), whereas DMEM-LG containing 10% FBS was used as the negative control for toxicity (CT-). After these periods, the medium was removed and the cells were treated in accordance with the assays decribed below.

2.2. Cell viability assay by MTT

The modified MOSMANN method [22] was chosen to perform the MTT assay. After

incubation periods, the samples and the medium were removed and the cells were washed with 200 µl of PBS followed by a wash with 200 µl of DMEM-LG. Next, 200 µl of thiazolyl blue tetrazolium bromide solution (MTT, Sigma) in DMEM-LG (0,5 mg/ml) was added, and the plate was incubated in the dark for 4 hours at 37 °C. The MTT solution was withdrawn, and 200 µl of dimethyl sulphoxide (DMSO) was added to determine the absorbance values, at an absorption curve of λ= 595 nm (FilterMax F5 Multi-Mode Microplate reader, Molecular Probes). The absorbance values of the results were expressed as optical density (OD) as the mean ± standard deviation. The comparison between the values was made with the method Least Significance Difference (LSD) test of Fisher and parametric data analysis One-way ANOVA. Analysis with p <0.05 were considered significant. Analyzes were performed using StatView software (SAS Institute Inc., Cary, NC, USA).

2.3. Evaluation the morphology by Live/Dead®

The qualitative test for biocompatibility Live/Dead® fluorescence assay kit (Molecular Probes) was used to qualify the ADSC viability. After periods of incubation, the PLLA samples and the medium were removed and the cells were washed with 200 µl of PBS. Then, the cells were treated with a solution of Calcein AM and Ethidium homodimer-1 according to the manufacturer's instructions. The cells were incubated at 37 °C at room temperature for 30 min and then washed and maintained in PBS. The cells were observed by inverted fluorescence microscopy (Nikon E800) with a specific program (Image Pro-Plus software).

3. Platelet Adhesion

Platelet rich plasma (PRP) was prepared by centrifugation (1500 rpm, 15 min) of fresh rat whole blood. 50 µl of fresh PRP was added to each sample placed in a 24-well plate and incubated for 2 h at 37◦C. After washing with NaCl (0,9%) aqueous solution (10 min, 3 times), the samples were fixed using 2.5 v% glutaraldehyde solution overnight at 4◦C in the dark. Afterwards, the samples were washed again with NaCl (0,9%) aqueous solution and then subsequently immersed in 30%, 50%, 75%, 90%, 95%, and 100% (v/v) ethanol/water solutions for 10 min each. The morphologies of the platelets on different samples were observed by SEM after drying and sputtering with gold. The amounts of platelets on the samples were determined by counting platelets in six different areas.

4. Hemolytic Activity Assay

Rats blood sample was collected with Alsever buffer. The erythrocytes were separated for sedimentation process and washed three times on NaCl (0,9%) solution on 1200 rpm centrifugation per 5 minutes. On 15 ml falcon tubes were added 5 ml of NaCl (0,9%) and 100 µl of washed erythrocytes for negative control of hemolytic activity, for positive control was used 5ml of distillated water and 100 µl of washed erythrocytes and for the samples was done 5 ml of NaCl (0,9%), 100 µl of washed erythrocytes and solid PLLA of 500 mg each, all tests, for controls and samples, were made in triplicate. The solutions were incubated at 37ºC on water bath, after 1 hour, it was checked the absorbance of the supernatant at λ= 540 nm (FilterMax F5 Multi-Mode Microplate reader, Molecular Probes). The HR was calculated using:

$$HR = \frac{(AS545 - AN545)}{(AP545 - AN545)} \times 100$$

Where AS545 is the absorption value of the samples, AN545 is the absorption value of the negative control, and AP545 is the absorption value of the positive control.

III. RESULTS AND DISCUSSION

Poly-lactic acid synthesis

The PLLA was synthesized by opening of the cyclic dimer of L-lactide in order to obtain high molecular weight polymer. The synthesis temperature was maintained at 140 °C thus avoiding, high temperatures, which lead to a depolymerization process which allows the decrease of the molecular weight of the polymer [25,26]. The obtained polymer had the PLLA molecular weight 86.93 g/mol.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analyses were made to determine the functional groups of the products obtained in order to understand more deeply what happens in the polymerization of Poly (lactic acid).

The standard PLLA and the one synthesized by our group, were structurally analyzed by spectroscopy in the infrared (FTIR). The infrared analyzes were performed to determine the functional groups of the product obtained, compared with the standard and verified the formation of the polymer. The spectrum of synthesized PLLA and standard PLLA is presented in Figure 3.

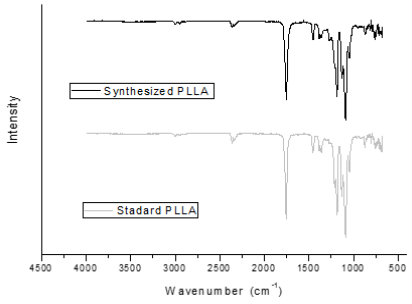


Fig. 3. FTIR spectrum of PLLA synthesized compared with PLLA standard.

In Figure 3 for the standard commercial PLLA, vibrations were observed symmetrical and asymmetrical valence of 1130.42 and 1044.92 cm-1, related to C-O the grouping COO; stretching of the C-H at 2999.47 and 2948.81 cm-1; COO stretching to 872 cm-1; valence vibration of C = O of COO at 1754 cm-1 and CH bending vibrations at 1387.86 and 1452 cm-1.

For PLLA obtained in the synthesis, were observed symmetric and asymmetric vibration valence 1130.53 and 1040.14 cm-1, related to C-O the grouping COO; stretching of the C-H bond at 2989.63 and 2946.87 cm-1, COO stretching to 870.76 cm-1, valence vibrations of C=O of COO at 1750 cm-1 and C-H bending vibrations at 1374.56 and 1345.14 cm-1.

The results demonstrated the similarity of the peaks relating to the absorption bands of each sample. Which shows the formation of the synthesized polymer PLLA through the studied pathway. The bands of functional groups are the same as those obtained in the standard sample and also those found in the literature shown by Nikolic [27], Motta and Duek [28]. And Lasprilla [29], confirming the formation of the polymer.

Cytotoxicity by MTT

Cytotoxicity test was performed to study the polymer biocompatibility. During the last few years, the interest of in vitro tests has increased, as an alternative to animal experimentation. The cytotoxicity is the harmful in vitro effect induced in the cell culture system by the presence of a certain substance or material, such as a biomaterial for instance. In this study we used the MTT assay (a direct and suitable assay for the quantitative in vitro biocompatibility evaluation), on which the metabolic activity and the rate of cell growth have indicated the degree of PLLA cytotoxicity in the cell culture.

MTT [30] is a yellow salt which is reduced by mitochondrial dehydrogenase activity of the enzyme resulting in a formazan salt purple. This reduction occurs only in living cells. Thus, cell viability can be determined by the intensity of purple color, which is proportional to the amount of formazan crystals formed.

After performing the MTT test, the absorbance values that were obtained, generated the curves below (Figure 4). The curves show the proliferation of mesenchymal cells in contact with the PLLA after 24, 48 and 72 hours.

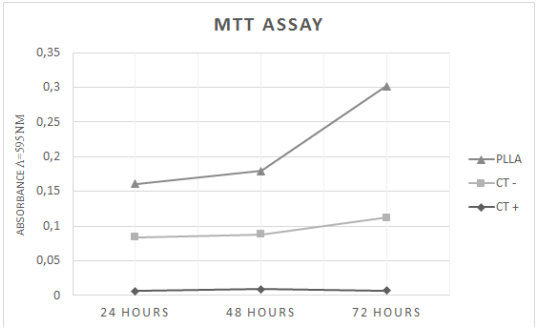


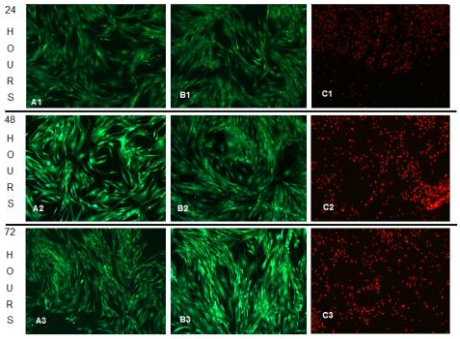
Figure 4. Kinetic curves of cell proliferation as measured by optical density (OD) from the MTT assay for mesenchymal cells cultured with PLLA, negative control for toxicity (CT-) and positive control for toxicity (CT+) for 24 hours, 48 hours e 72 hours.

According to the ANOVA test, there are no statistically significant differences between the PLLA and the (CT-) after 24 hours and 48 hours of culture ($p > 0.05$). However after 72 hours, the cells cultured with PLLA showed higher proliferation when compared to (CT-), considered statistically significant ($p < 0.05$).

These results show that the synthesized PLLA doesn't negatively affect the mesenchymal cell viability in the evaluated periods. This indicates that the material does not present cytotoxic behavior after 24, 48 and 72 hours, which it is in accordance with the MTT studies of Sarasua (2011) [31] and WU (2014) [32]. In the MTT tests made by Niu (2015) [33] and Liu (2014) [34], they evaluated polymers that showed no in vitro cytotoxicity as well, demonstrating correlation with our results.

Viability by LIVE/DEAD®

The Live/Dead® assay shows in a qualitatively matter the polymer biocompatibility when in contact with the cells. The cells were cultured with the biomaterial in three different times: 24, 48 and 72 hours. There was also the presence of positive control for toxicity (CT+) and negative controls of toxicity (CT-). The live cells reacted with the fluorescent marker SYTO® 9, staining the viable cells with green. On the other hand, un-viable cells were stained red, showing cell death. Figure 5 shows the results obtained by the Live/Dead® method by inverted fluorescence microscopy



. Figure 5. Images show cell viability on Live/Dead® test after 24 hours, 48hours and 72hours. A- Shows the negative control for toxicity (CT-), live cells in green fluorescence. B- PLLA, the cells present green fluorescence as negative control (CT-). C- Positive Control for toxicity (CT+), dead cells shown in red fluorescence.

Figures 5A1, 5A2 and 5A3 show the negative control for toxicity (CT-), on different times, where the viable cells with intact cellular membrane, were stained fluorescent green. On Figures 5B1, 5B2 and 5B3 show the cells in contact with PLLA for 24 hours, 48 hours and 72 hours respectively. The images of the cells in contact with PLLA appeared to have normal morphology on all times, just like the ones of (CT-) (Fig 5A and 5B). On the other hand, Figures 5C1, 5C2 and 5C3 show the positive control for toxicity (CT+), where it were stained red on all times, showing dead cells, with presence of debris and cell fragments.

The results obtained in this assay shows that the cells in contact with new PLLA demonstrated the same green fluorescence that the Negative Control (CT-), which reassure that the mesenchymal cells are alive and proliferating.

Bernstein (2012) [35], tested PLLA with different formats, such as screws and pins, and through his LIVE/DEAD® test, no cytotoxicity was detected, which corresponded with our results.

Our findings with the quantitative cytotoxicity assay MTT and qualitative cytotoxicity test LIVE/DEAD® showed that the mesenchymal cells in contact with the synthesized PLLA proliferated just as the ones of the negative control,showing the normal morphological characteristics of the cell. That demonstrates that our synthesized PLLA is a biocompatible material.

Platelet Adhesion

Detection of adhered platelets on biomaterial surface that has blood-contacting application is an important test to assess its thrombogenicity. Usually, for qualitative evaluation of platelet adhesion, after exposure to platelet-rich plasma (PRP) under standardized conditions the test surface is rinsed to remove non-adherent platelets and is analyzed under scanning electron microscopy (SEM) to detect morphology of adhered platelet and degree of aggregate formation.

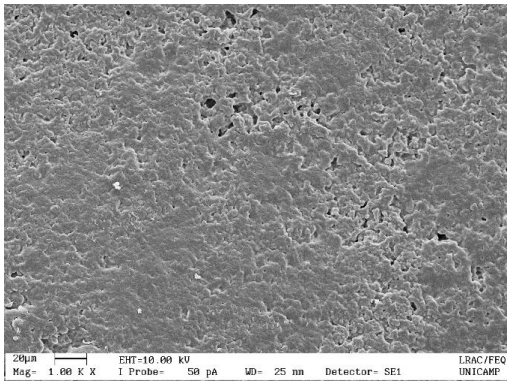


Figure 6. SEM image show platelet on PLLA surface 1000x.

Platelet adhesion on stents is also important for their application and should be inhibited as much as possible because many platelets adhering to the stents can induce severe thrombosis. The results showed in figure 6 that PLLA effectively inhibit the adhesion of platelets, thus reducing the potential risk of thrombosis.

Hemolytic Activity Assay

The hemolysis ratio is critical to evaluate the hemocompatibility of blood-contacting materials. Higher hemolysis ratios indicate more damage to the erythrocyte cells caused by the materials. According to the ISO 10993-4, materials that cause a hemolysis ratio of less than 5% are safe and can be used as blood-contacting materials. [36]

$$HR = \frac{0,385 - 0,386)}{2,205} \times 100 = 2,67\%$$

Where: 0,385= Negative Control absorbance
0,326= PLLA absorbance
2,205= Positive Control absorbance

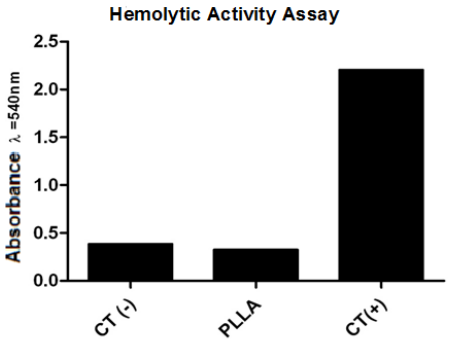


Figure 7. Comparison between Negative Control (CT-), Positive Control (CT+) and PLLA hemolytic activity assay results.

The results showed (figure 7) that PLLA presented lower hemolysis ratio of 2.67%, which was much lower than the safe value of 5%. The hemolysis ratio was satisfactory, which indicates that there is no damage to the erythrocyte cells caused by the materials.

PLLA Electrospinning

The electrospinning process was performed after the PLLA synthesis and the following realization of the cytotoxicity tests, and evaluation of it biocompatibility. PLLA nanofibers were produced, forming a mat as shown in Figure 8.

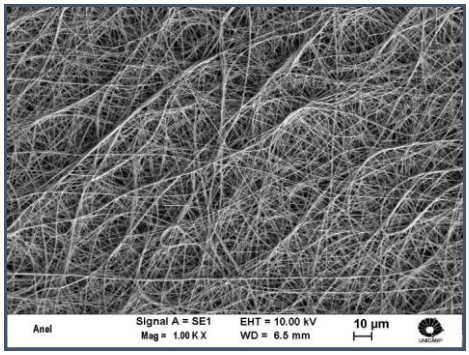


Figure 8. SEM image of the PLLA nanofibers mat. 1000x.

The nanofiber mat contains fibers that are extremely thin, and yet that keep their morphological structure. Those have a diameter of less than 1µm, as shown in Figure 9. To repair or restore the function of damaged or diseased tissue it is necessary many complex methods , employing a mix of knowledge and techniques of engineering, chemistry and cell biology. The use of PLLA and nanofibers to create a more efficient three-dimensional structure to be used in tissue engineering, is a well accepted method in the scientific community. The nanofiber scaffold has a high surface area per unit volume, as well an extremely interconnected pore network and fiber diameters that mimic the extracellular matrix environment.

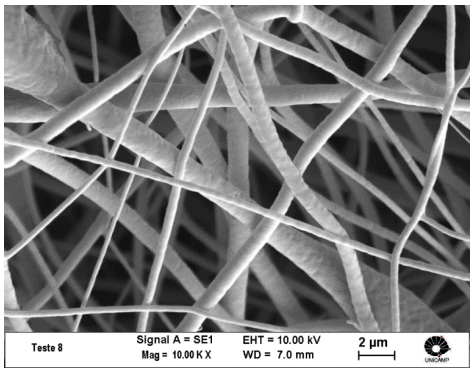


Figure 9. SEM image of the PLLA nanofiber mat. 10.000x.

With intention to show that the electrospinning process to produce the nanofibers wouldn´t be prejudicial to cell growth, the calorimetric assay of MTT [22] was performed with the PLLA nanofibers. The cytotoxicity of the nanofibers are shown in Figure 10.

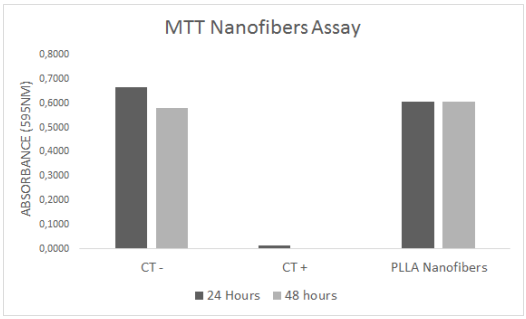


Figure 10. Kinetic curves of cell proliferation as measured by optical density (OD) from the MTT PLLA Nanofibers assay, negative control for toxicity (CT-) and positive control for toxicity (CT+) for 24 hours and 48 hours.

The graphics show the cytotoxicity of the mesenchymal cells in contact with the PLLA nanofibers after 24 hours and 48 hours. According to the ANOVA test, there are no statistically significant differences between the PLLA and the negative control of cytotoxicity (CT-) after 24 hours and 48 hours of culture ($p > 0.05$). Though, the cells cultured with the PLLA nanofibers showed higher proliferation when compared to the positive control of cytotoxicity (CT+), considered statistically significant ($p < 0.05$). Thus, the data shows that the electrospinning process wasn't harmful to the cells.

Considering the presented studies, it is possible to use the knowledge on the following tissue engineering (TE) tests with this material for clinical application, since it is essential to fabricate autologous TE skin substitutes, with sufficient mechanical strength for handling and suturing during surgical implantation and effective functionality for facilitating wound closure [37]. Composite scaffolds can also be created using electrospinning. For example, by sequentially spinning different polymer solutions, a scaffold with different layers can be constructed. Each layer can be tailored for specific cell adhesion and could be potentially beneficial for zonal articular cartilage or arterial vessel repair [38]. There had been different uses of the electrospinning technology in the biomedical field, the reasons are quite evident, such as the simplicity of the procedure in generating the large surface area- to- volume ratio of the material and the mechanical stability of the fibres that allows for its use in the biomedical field [39]. The improvement of PLLA production and electrospinning process generate new TE possibilities to be tested.

IV. CONCLUSION

This study has proved that the ring-opening polymerization is a viable process for the production of PLLA derivation from the lactic acid in these studied conditions. It also shows that this PLLA is a non-toxic polymer to the cell and hemocompatible, hence being then considered biocompatible. Thus, PLLA can be employed to the manufacture of nanofibers by the electrospinning process, which can be used for different purposes in the medical field.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support provided by the Scientific Research Foundation for the State of São Paulo (FAPESP Process 2008/57860-3) and National Council for Scientific and Technological Development (CNPq Process 573661/2008-1)..

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