EUROPEAN ACADEMIC RESEARCH
Vol. VII, Issue 10/ January 2020

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

PLLA Synthesis and Membrane Production through Rotary Jet Spinning Process
Poly (L-lactic acid) Synthesis and Physico-chemical Analysis

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Abstract

Poly (L-lactic acid) (PLLA) is a well-established polymer, which is widely used in a wide range of fields; however, it is a high cost material, presenting great difficulties for its purchase. Therefore, the present work proposes PLLA synthesis in the laboratory, as an alternative to turn this more feasible; its processing through rotary jet spinning to obtain fibrous membranes; its physico-chemical analyses for material characterization; and, in vitro cell viability analyses with osteoblasts and fibroblasts. Scanning Electron Microscopy (SEM),

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Thermogravimetric Analysis (TGA), Energy Dispersive Spectroscopy (EDS), and Live/Dead® assays were the characterization analyzes of the obtained membrane. PLLA membranes had initial degradation at 200 °C, and none harmful chemical elements were present. In vitro test verified the compatibility of the membranes with two cell lines, indicating a possible and potential application of these membranes in the medical field.

Key words: Physico-chemical analysis, PLLA, Poly (L-lactic acid), rotary jet spinning.

1. INTRODUCTION

Poly (L-lactic acid) (PLLA) is a thermoplastic polyester made with lactic acid (mixed function organic compound – carboxylic acid and alcohol) (Figure 1). The lactic acid can be produced using renewable sources through bacteria fermentation process of starch-rich vegetables such as beets, maize, sugarcane, and cassava, making PLLA biodegradable [1].

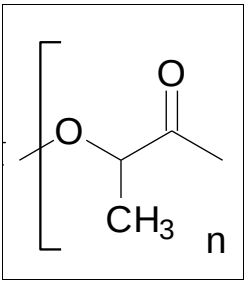

Figure 1 – PLLA Chemical Structure

In contact with the human body, PLLA is hydrolyzed to lactic acid, which under aerobic conditions it is metabolized in water and carbon dioxide, and then it is finally excreted by the body. Although it does not endanger the human body, PLLA is brittle and rigid, which limit the application areas [1-2].

The use of PLLA in medical field has considerably advanced in recent years due to its properties and cost when compared to other

available polymers on the market. The PLLA biodegradability and biocompatibility characteristics make it a biopolymer, expanding its application fields [3-4].

Even though PLLA is more affordable than other polymers, it is still a high-value polymer and difficult to find on the market. In this way, new production routes of this polymer have been investigated in the literature, seeking to cease industrial dependence, in order to reduce the cost for research and development of new materials from the PLLA.

Rotary jet spinning process consists of a simple method of obtaining polymeric fibers, which oriented or random fibers is possible to be obtained, from micro to nanometric scale, and a large quantity of fibers with a small solution amount. The method is described as a process, in which a polymeric solution is exposed to a high rotation speed, forming a long polymeric jet that extends to its solidification, producing fibers [5-6].

With smaller pores and a higher surface contact area than other membranes, the use of rotary jet spun membranes has considerably grown due to diverse application possibilities in the medical field, such as artificial skin production, dressings for wounds healing, balloon angioplasty, intragastric balloons, scaffolds, neural connections, among others [3].

In light of the foregoing, the present work aims at synthesizing PLLA polymer; producing PLLA membranes via rotary jet spinning process; and, studying their physico-chemical and cell viability properties through Scanning Electron Microscopy (SEM), Thermogravimetric Analysis (TGA), Scanning Electron Microscopy (EDS), and Live/Dead® assays, in order to evaluate the potential of PLLA membranes to be applied in the medical field.

2. MATERIALS AND METHODS

2.1 Materials

PLLA used in this work was synthesized through ring opening polymerization of L-lactide (Purac® – Purasorb L®) with Tin(II) 2-ethylhexanoate (95%) as a catalyst and 1-Dodecanol (98%) as an

initiator, both from Sigma Aldrich®, at the National Institute of Biofabrication (INCT-Biofabris/UNICAMP) as described elsewhere [7].

2.2 Methods

2.2.1. Rotary jet spinning process

In order to perform the rotary jet spinning process, the PLLA was dissolved in chloroform [$CHCl_3$, 99%] from *Synth* (Brazil), at 25 °C, obtaining a final solution concentration of 0.2 g/L.

Laboratory of Biomaterials and Biomechanics (LABIOMEC/UNICAMP) manufactured the rotary jet spinning equipment that was used in this work. A speed of 6500 $r.min^{-1}$ at 25 °C was used.

2.2.2. Properties analysis of PLLA membranes

All membranes physico-chemical characterization analyzes followed the methodology described elsewhere [7], and were carried out in the Laboratory of Biomass Characterization, Analytical Resources and Calibration (LRAC/UNICAMP).

– *Scanning Electron Microscopy (SEM)*

The sample surface morphological analysis was carried out using the SEM technique in the LEO – scanning electron microscope (Oxford, Leo 440i, Cambridge, England), with a voltage of 20 kV and a current of 100 pA. In order to perform this analysis, the sample had to go through a metallic coating process with gold on a metallizer (SputterCoater EMITECH, K450, Kent, UK).

– *Energy Dispersive Spectroscopy (EDS)*

For the evaluation of the chemical elements in the fibrous membrane, the sample was analyzed by EDS coupled to SEM, using the LEO ElectronMicroscopy/Oxford, 6070 (Cambridge, Inglaterra), with a voltage of 20kV, and current of 600 pA to obtain spectra, under inert nitrogen atmosphere.

– *Thermogravimetric analysis (TGA)*

Thermal degradation temperature of PLLA membranes was verified by TGA (METTLER TOLEDO, TGA/DSC1 Schwerzenbach, Switzerland). Aproximately 7–10 mg of the sample were weighed on a

microanalytical balance (Mettler Toledo, MX5, Schwerzenbach, Switzerland) and placed in a 70 µL alumina crucible. The material was heated from 150 to 310 °C at a heating rate of 10 °C/min, under inert atmosphere with nitrogen (50mL/min).

– *Cell viability assay (Live/Dead®) of the membranes*

In order to obtain images by fluorescence microscopy, osteoblasts and fibroblasts cells, both with a concentration of $1x10^3$ cells/mL, were cultured with rotary jet spun PLLA for 24, 48 and 72 hours, which were labeled with a specific kit (Live/Dead® Viability cytotoxicity) according to the recommendations of the manufacturer. Cells were incubated in a solution containing 2 µM propidium iodide and 2 µM AM calcein for 30 minutes at 37 °C for labeling viable and non-viable cells.

Fibroblastic cell line (VERO) used in this study was provided by the Adolfo Lutz Institute (São Paulo, Brazil), cultured with Dulbecco's modified Eagle's medium with low glucose concentration (DMEM-LG-Gibco). Osteoblastic cell line (M3CT3-E1) used in this work were purchased from the ATCC, cultured with Eagle's minimal essential culture medium (MEM-ALFA, Vitrocell). Both mediums were supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin and streptomycin (Gibco), and maintained at 37 °C in an incubator (Sanyo Scientific, USA) with 5% CO_2 atmosphere and 95% ambient air.

3. RESULTS

3.1. *Rotary Jet Spinning Process*

The final product of rotary jet spinning process was a fibrous membrane of poly(L-lactic acid) synthesized in the laboratory, shown in Figure 1. The membrane presents a voluminous and continuous appearance, similar to cotton net.

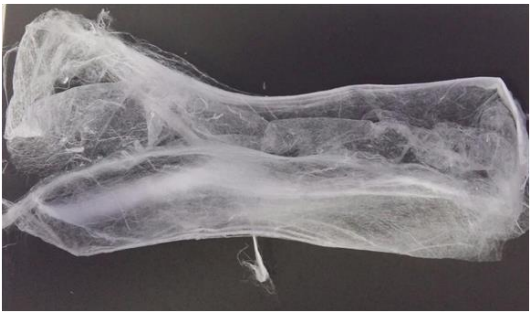

Figure 1 – Synthesized PLLA membrane obtained from rotary jet spinning process [2].

3.2. *Characterization of PLLA membranes*

- *Scanning Electron Microscopy (SEM)*

The optimal concentration selected for solution to be used in rotary jet spinning process and a solvent with suitable evaporation characteristics can be noted by observing the micrographs in Figure 2.

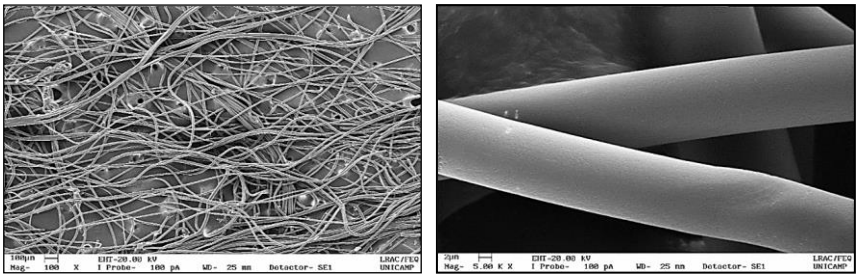

Figure 2 – Scanning electron micrographs of PLLA membranes at magnifications of 100x and 5000x.

PLLA fibers are scattered and overlapped in a randomly manner. The fibers do not present any visible defects, like pores. Only some contact marks between the fibers themselves and their junction can be seen, which are due to solvent evaporation time. The membrane also presented a smooth surface and constant diameter (Figure 2).

– *Energy Dispersive Spectroscopy (EDS)*

The EDS analysis was done to qualitatively evaluate the chemical elements present in the membrane, making sure that all the solvent had been evaporated during the rotary jet spinning process. Figure 3

represents the spectrum obtained in the EDS analysis, in which the elements carbon (C), oxygen (O), tin (Sn) and gold (Au) were found.

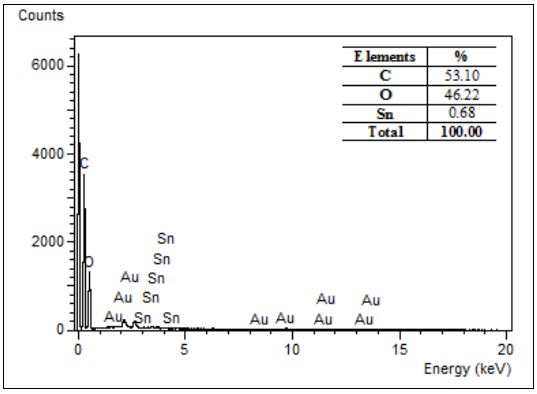

Figure 3 – Spectrum by EDS analysis [2].

The presence of gold is due to the metallization of the fibrous membrane, whereas the tin is due to the presence of the catalyst used to produce the polymer. The presence of carbon and oxygen were already expected to form the PLLA chain.

Carbon and oxygen are present in greater amounts, which together total 99.32%. The tin interference due to the catalyst is 0.68%; the gold presence belongs to the processing of the sample for analysis, being disregarded (Figure 3).

– *Thermogravimetric analysis (TGA)*

For a better interpretation of TGA results, the graph in Figure 4 was developed to relate the mass variation percentage with temperature (in degrees Celsius). The curve corresponds to the PLLA fibrous membrane mass loss.

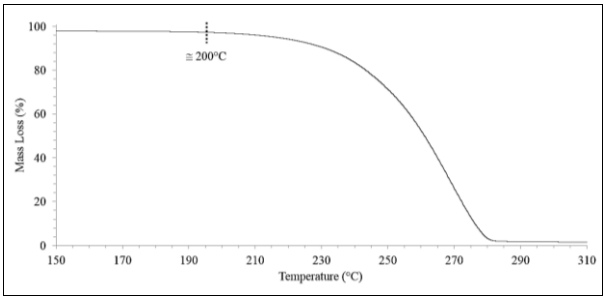

Figure 4 – PLLA membrane thermogravimetric curve.

The initial mass loss of the material starts approximately at 200 °C, which is adopted the initial degradation temperature, and stops around 285 °C, being the final degradation temperature, having practically no weight loss, indicating PLLA membrane complete degradation.

– *Cell viability assay (Live/Dead®) of the membranes*

The cell viability after 24, 48 and 72 hours of contact with PLLA membranes was determined by the Live/Dead® assay kit. Viable cells (green-labeled) and dead cells (red-labeled) were visualized by fluorescence microscopy (Figure 5).

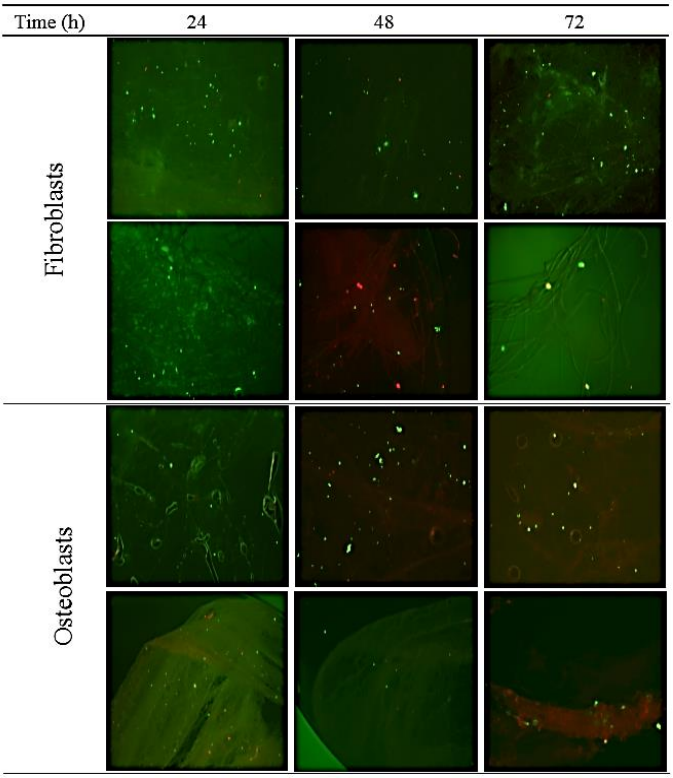

Figure 5 – PLLA membranes Live/Dead® assay of fibroblasts and osteoblasts after 24, 48 and 72 hours of contact (20x) [2].

Figure 5 demonstrates that a predominance of living cells (green) was found for both cells in 24, 42 and 78 hours of contact. The results

observed in the micrographs suggest that PLLA did not compromise cell viability and nutrients and oxygen transport to cells, but the adhered cells number to the membranes is low, showing the cells did not adhere to the membranes, as expected.

4. CONCLUSIONS

PLLA synthesis was carried in the laboratory, being a viable mechanism to produce the polymer, with a low cost, and not depending of commercial offers, since the technique proved to be executable and with adequate results.

PLLA membranes were successfully produced through rotary jet spinning processes, and from their characterization analysis and *in vitro* assay, demonstrated their properties and cell viability, being able to evaluate the potential of these membranes in future applications in the human being, not bringing problems for the human body.

The results for morphological and physico-chemical analyzes showed no significant differences between the synthesized and the commercial PLLA. The surface structure of rotary jet spun PLLA fibers has no defects along the fibers, and the interlacing between these fibers forms a fibrous membrane, which generates pores throughout the fibrous membrane, facilitating vascularization and cells gas exchange.

The chemical evaluation shows that PLLA membranes does not demonstrate toxicity, since there is no presence of toxic or harmful elements, and the solvent total exclusion was confirmed. The thermal analyzes also confirmed that the PLLA membranes have a high thermal capacity, in which the degradation temperature starts at approximately 200 °C.

Live/Dead® assays of the membranes in contact with osteoblasts and fibroblasts cells demonstrated that the PLLA membranes have no toxic effects to cells, but no adherence of cells to the membranes were noted, indicating that depending of membranes use, there is a necessity of using cell fixators.

Acknowledgments
The financial support was provided by grant #2008/57680-3, São Paulo Research Foundation (FAPESP), CAPES and the National Institute of Biofabrication (INCT-Biofabris).

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