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Development of PLLA-based Bio Inks Applicable in the Medical Field

Sara Crivellin[1], Samuel Diógenes Azevedo de Souza[1], Mylena Stefani[1], Maria Ingrid Rocha Barbosa Schiavon[1], André Luiz Jardini[1], Rubens Maciel Filho[1*], Viktor Oswavldo Cárdenas Concha[2]

Abstract

Some synthetic polymers, such as Poly (L-lactic acid) (PLLA), are interesting to use in 3D bioprinting because they can have adaptable and resilient mechanical properties that also facilitate bioprinting processes. PLLA is a biocompatible polymer which makes it very interesting for medical applications, it is an aliphatic polyester obtained from α-hydroxy acids in different ways: ring-opening polymerization, and direct polycondensation. The use of one path over the other depends on the molecular mass to be obtained. In this work, the final polymer was obtained through the direct polycondensation route. After direct polycondensation, the PLLA produced was purified by solubilization in chloroform followed by precipitation in ethanol. Purified PLLA was produced using 0.5 g of polymer, 4 g of chloroform, and 1 g of glycerol was added to it. PLLA-based bio-ink was produced by 3D bioprinting using the extrusion technique. Before extrusion, the produced and purified material was solubilized with ethyl acetate, a non-toxic and biocompatible compound with the following proportions: PLLA 20% $m*v^{-1}$ in 10 ml (95% ethyl acetate and 5% acetone $v*v^{-1}$. Finalizing, the polymer obtained was characterized by Fourier Transform Infrared Spectroscopy (FTIR), using the Bruker VERTEX 70v equipment in transmission mode with laser radiation of 633 nm wavelength, and the structure obtained with the 3D-printing was analyzed via scanning electron microscopy (SEM) to assess its surface morphology. The structure produced showed interesting properties to act as a temporary delivery device and may well hold some drugs to act as a drug delivery device.

Keywords: Polymer, Poly (L-lactic acid), L-lactide, medical application, Additive manufacturing.

***Author for Correspondence**
Rubens Maciel Filho
E-mail: rmaciel@unicamp.br

[1]Student, Department of Chemical Engineering, State School of Chemical Engineering National Institute of Science and Technology in Bio fabrication (Biofabrics'), State University of Campinas, Brazil
[2]Student, Department of Chemical Engineering, Federal University of Sao Paulo, Diadema, Sao Paulo, Brazil.

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INTRODUCTION

Poly(L-lactic acid) (PLLA) is known as one of the best biodegradable biopolymers used in medicine, with applications ranging from simple sutures to stents and temporary orthopedic fixation devices [1, 2]. The polymer can be synthesized by three conventional routes, as was showed in Figure 1, namely ring-opening polymerization (ROP), azeotropic dehydration condensation (CDA), and direct polycondensation (DP) [3].

ROP is the most efficient production route that consists of opening the cyclic dimer of L-lactic acid, L-lactide (Horváth et al. 2021). The use of a metallic class catalyst (zinc, titanium, and mainly tin octanoate (OctStII)) results in the formation of high molecular weight PLLA [4].

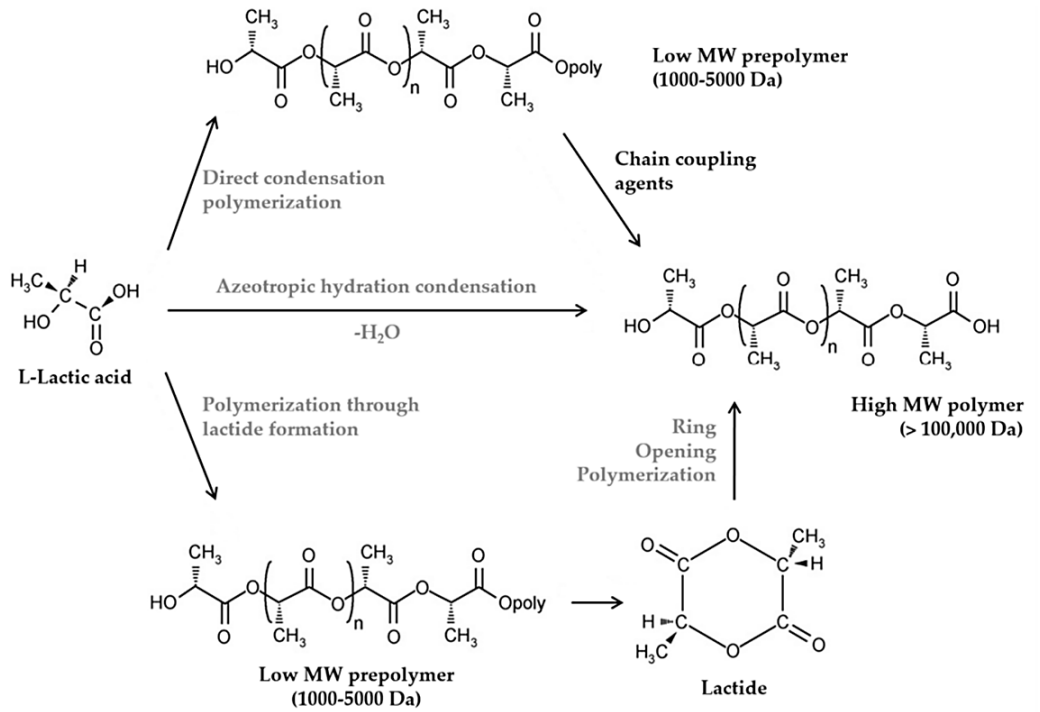

Figure 1. Productive routes for the synthesis of PLLA (Source: Adapted from Muller et al., 2017).

CDA in azeotropic distillation where dissociated water can be removed during the polycondensation process may lead to obtaining a high molecular weight polymer at the end of the process [5]. The main problem with this technique is that purification requires large amounts of organic solvents to remove water, such as toluene and xylitol [5].

DP is the simplest process compared to the other techniques mentioned [6]. The technique consists of three steps that involve, firstly, the dehydration of L-lactic acid, polycondensation of the oligomers, and the removal of water during the polycondensation reaction [7].

After its synthesis step, the polymer needs to be processed, thus allowing its application and meeting any specific medical requirement. One such processing technique is 3D printing provided by the manufacture of bioinks [8].

This technique has a low production cost, low quality of structure in relation to other bioprinting techniques, fast printing speed in both polymers and composites with viscosities between 3.5 to 12 mPa*s^{-1} [9]. Figure 2 shows inkjet bioprinting.

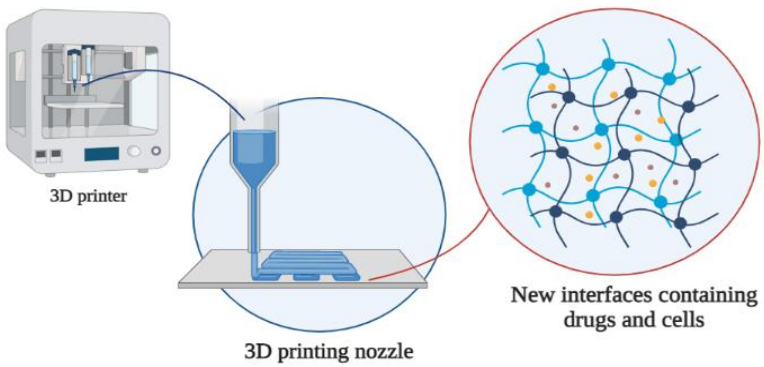

Figure 2. Experimental unit.

MATERIALS AND METHODS

L-lactic acid 85% (Synth), Tin Octanoate 2 (Sigma-Aldrich), 1-dodecanol (Vetec), Chloroform (Synth), Ethanol (Sigma-Aldrich), ethyl acetate, and 5% acetone: are the components considered in this work.

Experimental unit

To perform the synthesis of PLLA, the DP reactor was built as shown in Figure 2.

Raw PLLA Synthesis

The L-lactic acid was dehydrated for a period of 3 h at 130°C and 300 mmHg under constant stirring. At the end of this step, the temperature was increased to 160°C, and the pressure was increased to 500 mmHg. Then, the OctStII catalyst 1% w/v and the co-catalyst 1-dodecanol 0.025% w/v were added, which were kept under constant stirring for 72 h. The crude polymer obtained was formed into a mold and stored in a desiccator inside the refrigerator. The raw polymer obtained is shown in Figure 3.

Initially, 20 g of the crude polymer was removed, and it was solubilized in 60 ml of chloroform for 24 h under constant stirring. Subsequently, the polymer was precipitated by adding ethanol until the solution became cloudy. The remaining solution was filtered, and the refined polymer was macerated to a clarified white powder as shown the Figure 4.

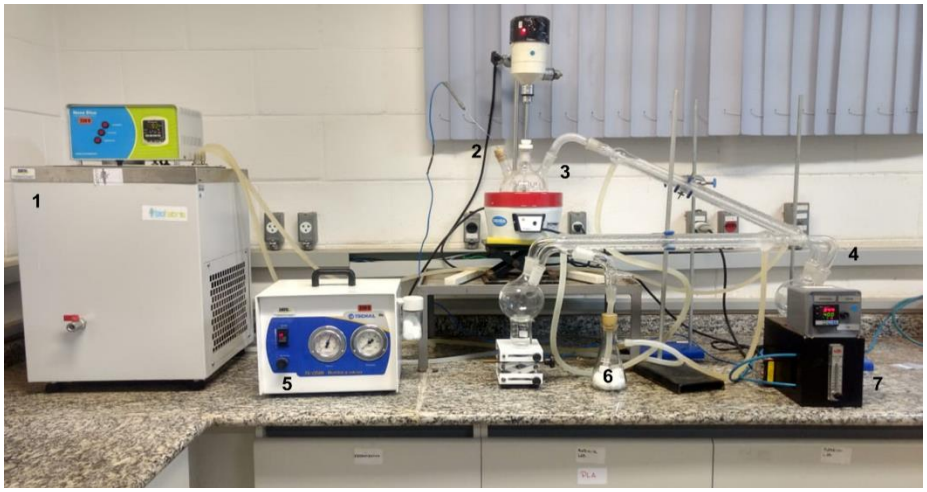

Figure 3. Experimental unit (1) water bath; (2) thermocouple; (3) main reactor with a heating mantle; (4) by-product reservoir; (5) vacuum-pressure pump; (6) trap; (7) thermal controller.

Figure 4. The raw PLLA produced.

It is worth mentioning that the same process was repeated for the remaining crude PLLA (equivalent to 111.21 g) and all the purified PLLA was weighed and stored in the desiccator inside the refrigerator. Figures 5 and 6 show the refined PLLA at the end of the process.

Additive Manufacturing

For the printing process, 8 g of purified polymer was vested in a solution with 10 mL of ethyl acetate, mixed for 24 h in constant agitation, and put in a syringe. A cylinder with 2.5 cm diameter and 1 cm height was drawn, adjusted in the 'Cura software', and saved as .gcode achieve. The .gcode draw of the piece was applied in the 'Pronterface software' which each parameter involving the printing was set. The Figure 7 shows the drawing of the work.

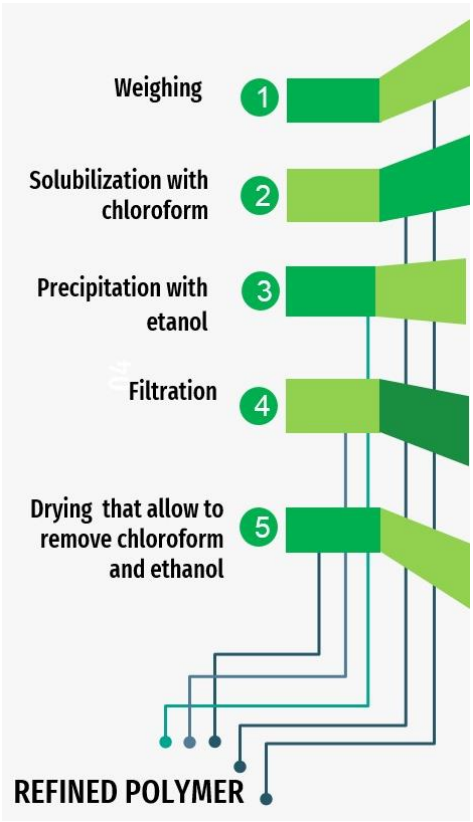

Figure 5. Diagram of purification process.

Figure 6. Purified PLLA.

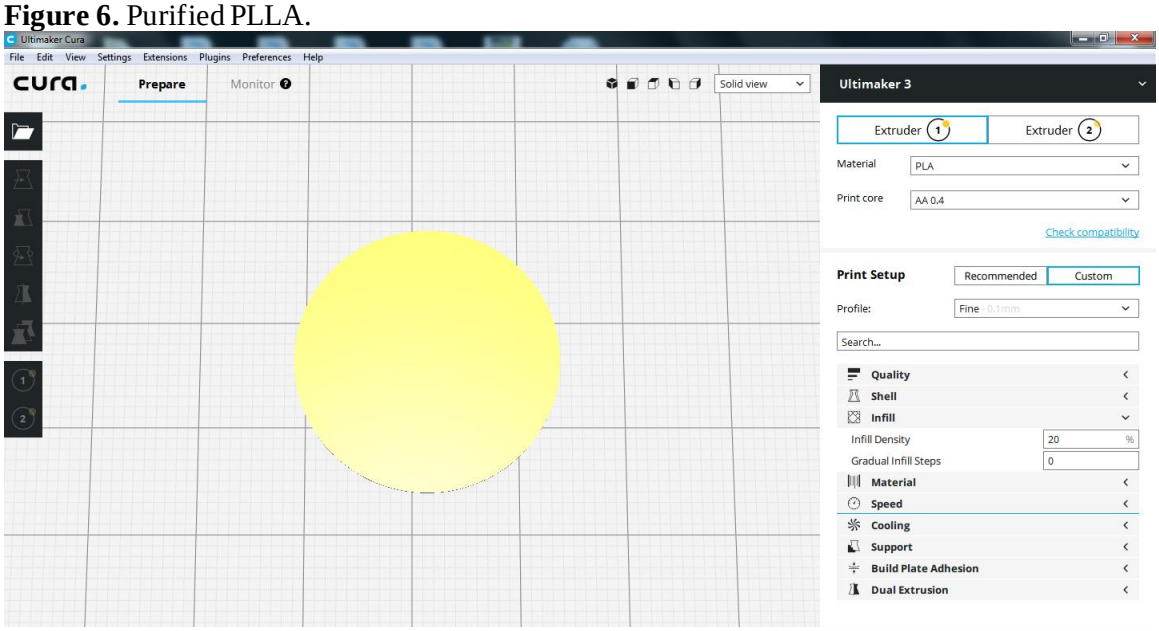

Figure 7. Development of the .gcode archive in Cura program.

Figure 8. PLLA-based bioink produced.

The 3D Biotechnology solutions (3DBS) printer was used to produce the bioink. Both the print speed and the flow rate was 10 $mm*s^{-1}$ and 1 $ml*min^{-1}$ adjusted at the Pronterface program. The Figure 8 shows the PLLA/ethyl acetate bioink.

CHARACTERIZATIONS OF THE POLYMER

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR measurements were performed in a Bruker VERTEX 70v spectrometer from the Cine group of Carbon Sci Tech Labs, located at the Faculty of Electrical and Computer Engineering (FEEC/Unicamp), in transmission mode with laser radiation of 633 nm wavelength. The sample was prepared by placing 0.2 mg of active material in a KBr pellet. The region under study was from 500 to 4000 cm^{-1} so the vibrational absorption bands were determined for crude PLLA and purified PLLA.

X-ray diffractor XRD

The X-ray diffractor used for the characterization of the PLLA was the Rigaku model MiniFlex 300/600 located at the Federal University of São Paulo (Campus Diadêma). The radiation used was

copper kα (λ=1.5 Å), the voltage of 40 kV, current of 40 mA, 2θ scan from 2° to 90° and scan speed of 5°*min^{-1}.

Scanning Electron Microscopy (SEM)

- The PLLA-based bioinks produced with the 3D-printing were analyzed with scanning electron microscopy (SEM), to see the morphology of the structure obtained in the superficial part.
- The surface morphology of PLLA-based bioinks, was analyzed by scanning electron microscopy (SEM) of the AES group of Carbon Sci-Tech Labs, located at the Faculty of Electrical and Computer Engineering (FEEC/Unicamp), using a Quattro ESEM model microscope from Thermo Scientific™.
- The same apparatus was used to analyze the atomic composition to verify the exact composition of the PLLA-based bioinks obtained through the extrusion. The sample was coated with a thin layer of iridium for metallization effects with a focus on acquiring better image resolution.

RESULTS AND DISCUSSION

Based on the measurements describes above, the FTIR of the crude and purified polymers (Figure 9) and the XRD of the respective polymers (Figure 10) were obtained. Table 1 presents the characteristic bands of the functions present in the PLLA.

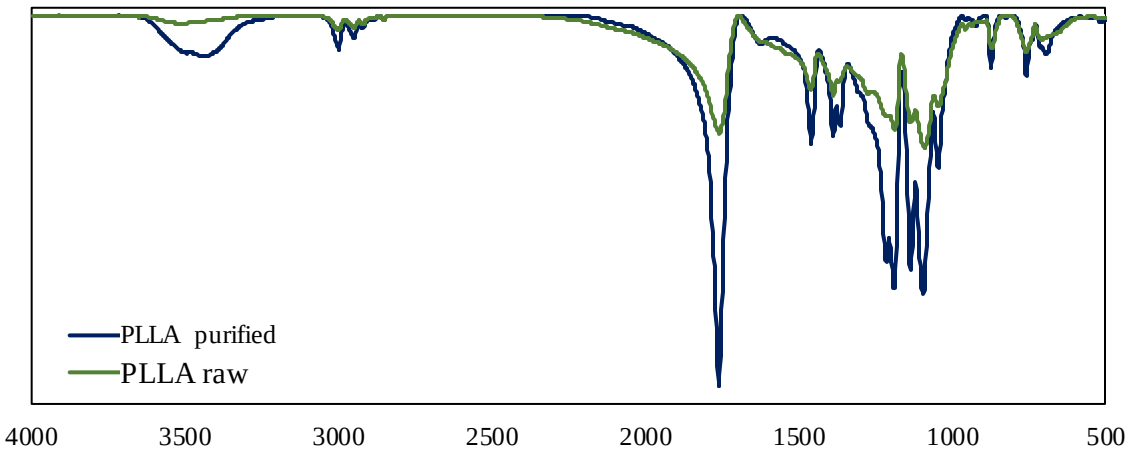

Figure 9. PLLA FTIR.

Table 1. Absorption bands of functional groups in the infrared region of PLLA.

Absorbance [a.u]	Chemical bond
2995,24	CH Asymmetrical stretch
2927,74	CH Symmetrical stretch
2854,45	CH
1749,31	-C= The carbonyl stretch
1456,15	CH_2 Angular twist
1382,86	CH Symmetrical deformation
1361,65	CH Asymmetric deformation
1182,28	(-C-O-) Stretch
1128,28	(-C-O-) Stretch
1085,85	(-C-O-) Stretch
1043,42	O-H Twist
869,83	(-C-C-) Stretch

According to Figure 10, the characteristic peaks of PLLA were found in the range of 2θ (14.5; 16.3; 18.7 and 21.9°) as in the literature [10]. Furthermore, the characterization analyses shown, it can be concluded that the PLLA is the polymer produced and the purified polymer coincides with the brute

one according to Figure 9 but admits an area of greater intensity as through the proposed process the impurities have decreased.

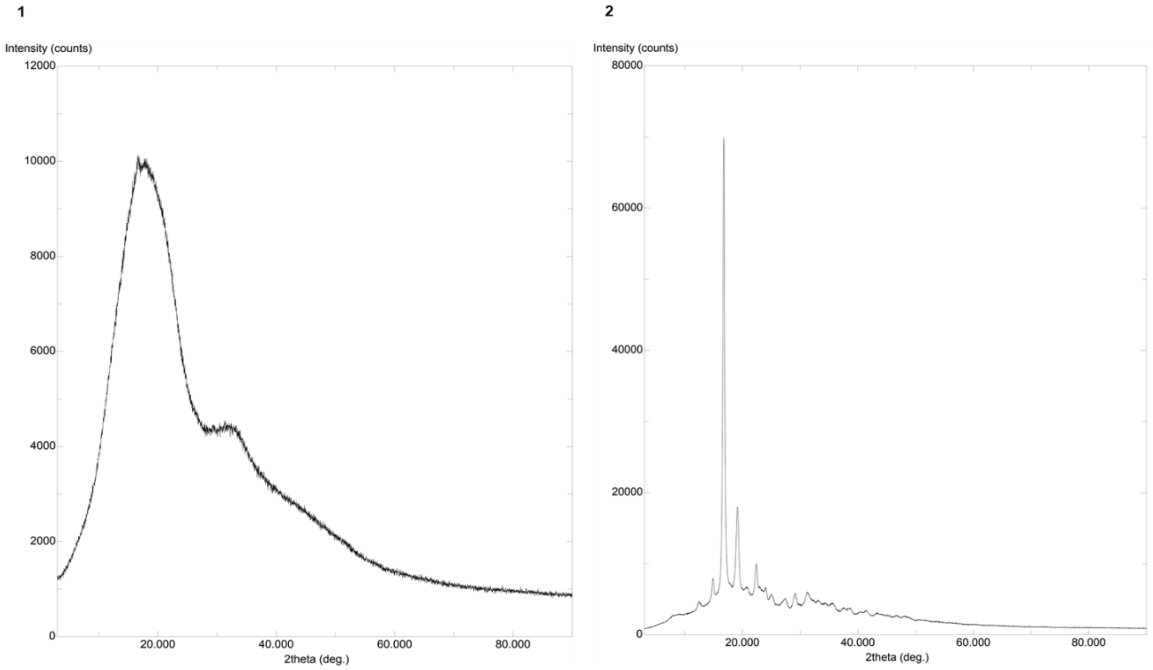

Figure 10. XRD of crude (1) and purified PLLA (2).

The SEM analyses showed the superficial surface of the bioink. The structure is porous due to the UV rays used in the manufacturing process to dry the material, the goal to improve is to make the material more uniform and therefore increase the concentration of the ethyl acetate polymer solution for the extrusion process, continuing to use a solvent non-toxic and always focusing on a completely Green and sustainable process. The Figure 11 shows the PLLA bioink SEM.

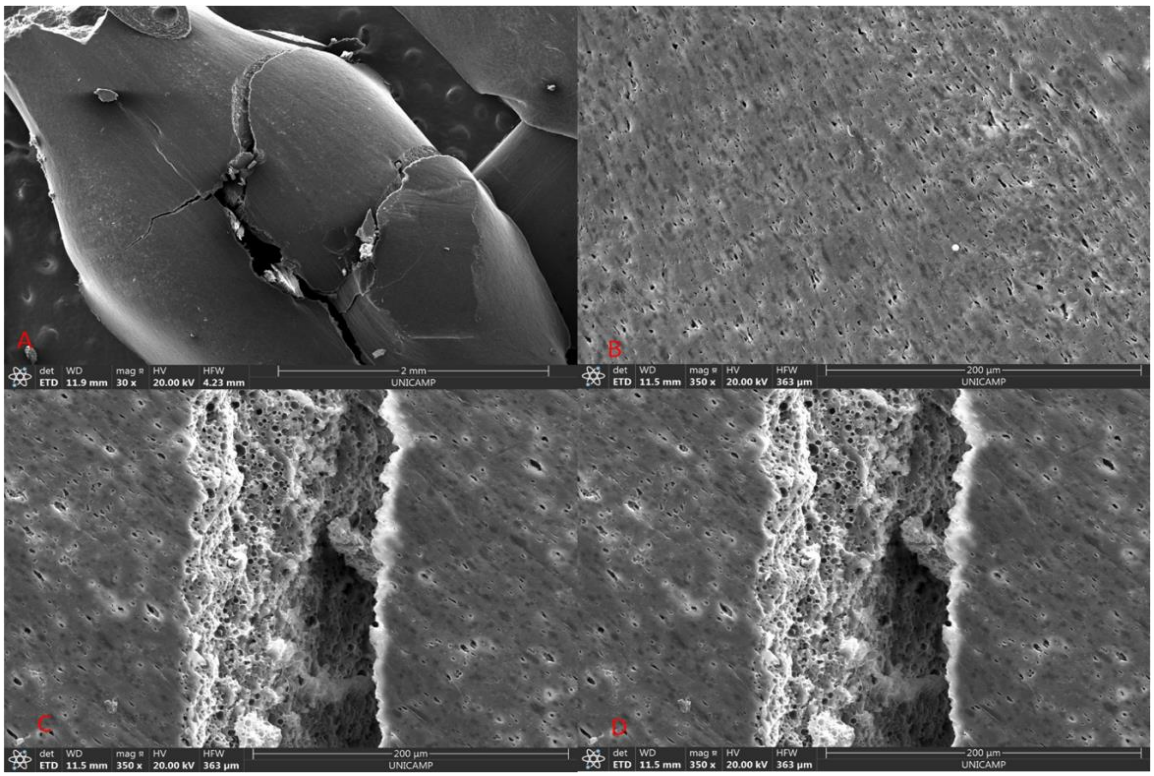

Figure 11. SEM analyses with the different resolutions: (A) 2 mm; (B) and (C) 200 µm; (D) 50 µm.

CONCLUSIONS

Based on the above, it was possible to produce and characterize the bio ink produced under conditions accepted for medical application. The FTIR and XRD analyzes performed, in addition to proving that the material obtained is PLLA as expected, it was possible to produce it under conditions of greater purity, a fact that should be studied and optimized in the future.

The SEM analysis found the porous morphology of the polymer that may play a role as a good matrix for loading a particular drug.

Acknowledgments

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