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Alternative methods of sterilization on films of polymers: Poly(-L-lactic acid) (PLLA), Poly(L-lactic acid-co-glycolic acid) (PLGA) and Poly(-LD-lactic acid) (PLDLA), for bioresorbable vascular scaffolds models.

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Abstract. Biodegradable polymers have been the subject of study for more than three decades because of their unique characteristics such as: biocompatibility and non-immunogenic and non-toxic properties, revealing their great acceptance in living organisms and being used as fastening elements in materials such as prostheses, sutures, drug encapsulation matrices and several important applications. The Poly(-lactic acid) (PLLA and PLDLA) and its glycolic acid copolymer (PLGA), present great biocompatibility. A problem when using polymers in bioengineering is sterilization process, which should enable the inactivation of a wide variety of microorganisms without affecting the properties of the materials of the device sterilized. Most of the processes used have limitations for use in thermo sensitive and chemo sensitive materials. Among the alternatives are ultraviolet radiation (UV) and plasma of hydrogen peroxide. This project tested these two alternatives methods, in films and tubes of these polymers. After the process of sterilization, no changes were found in thermal properties evaluated by differential scanning calorimetric analysis (DSC) and termogravimetric analysis (TGA). The mechanical properties of the PLLA, PLDLA and PLGA materials after the sterilization processes, also presents no changes, by UV and plasma, indicating the stability of samples to these processes.

Keywords. *Biomaterials, UV, Hydrogen peroxide plasma.*

Introduction. Synthetic biodegradable polymers have been used since the 1970s for biomedical applications in the fabrication of temporary implantable devices. Biomaterials have gained clinical attention in recent years due to their acceptance in living organisms and have been used as fixation elements in biomaterials for bone replacement, such as suture threads and drug encapsulation matrices in controlled release systems. Among the most used bioabsorbable polymers in the medical and dental areas, Poly (-L-lactic acid) (PLLA), Poly (lactic acid co-glycolic acid) (PLGA) and Poly (-LD-lactic acid) (PLDLA). Monomers have commercial availability as raw material and already have standardization adopted for use in the biomedical area [1, 2].

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These biomaterials have been used for more than three decades for both their biocompatibility and non-immunogenic and non-toxic properties. When they degrade, they generate as a product the lactic acid, which is a natural compound present in all animals [3].

A major problem when working with polymers in bioengineering is choose the sterilization process. By definition, the sterilization process should enable the inactivation of a wide variety of microorganisms, including toxins and resistant bacterial spores. This process differs from that of disinfection due to the ability to destroy virtually all forms of microorganisms. Sterilization processes are divided into physical and chemical methods. Physical methods utilize steam under pressure, such as autoclaving, or dry heat, such as the greenhouse. These methods have as principle the denaturation of proteins, enzymes and degradation of the compounds, generating death of the microorganisms and inactivation of toxins, including the spores more resistant. Chemical methods may use immersion in liquids such as glutaraldehyde or paracetic acid. Other gaseous chemical methods use ethylene oxide, hydrogen peroxide plasma and ultraviolet (UV) radiation. These methods are also effective in destroying microorganisms and spores with chemical denaturation of proteins, but do not use elevated temperatures for this [4, 5, 6].

Among the methods currently used, some limitations are found: In the sterilization by ionizing radiation, chemical changes can be produced in some materials, mainly in polymeric biomaterials. Gaseous sterilants, however, are increasingly used in devices incompatible with damp heat or dry heat. Among them, ethylene oxide (EtO) and formaldehyde are the most used. However, they present limitations associated with toxicity and difficult removal of their waste after the end of the process, which may compromise efficacy and safety. However, the chemical solutions of glutaraldehyde and formaldehyde are not recommended due to the process of removing the residues from the highly toxic and corrosive sterilizing solution, causing chemical changes in the materials, especially in biopolymers, and very low safety during the process [7].

Among the alternative methods under development for safe sterilization of thermosensitive and chemosensitive materials are ultraviolet radiation and plasma of hydrogen peroxide.

Ultraviolet radiation has a microbiocidal effect only when used with sufficient intensity and exposure time. It presents diverse applications as in the air sterilization, surfaces and in packaging in the alimentary and pharmaceutical industries. The wavelengths below 200 nm are inefficient for this application, since the radiations in the range of 210 and 330 nm can be considered efficient as germicides because they are absorbed by proteins and nucleic acids, causing chromosome disruption, genetic mutations and inactivation of enzymes that lead to cell death. In general, ultraviolet radiation has proven to be a faster, more reliable, effective, economical and environmentally safe way to treat surfaces and liquids, with limitations in the use of devices with complex geometries that have areas of difficult radiation exposure, or few transparency [8].
STERRAD® Plasma Hydrogen Sterilization (Sterrad Sistem® - Johnson & Johnson®) uses a combination of plasma and hydrogen peroxide vapor (H_2O_2), at low temperature and without toxic residues. Hydrogen peroxide is bactericidal, bactericidal, tuberculiscidal, sporocidal and fungicidal and acts through the production of free radicals that damage lipid membranes, DNA

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and other essential cellular components. The STERRAD® sterilization cycle consists of the injection of hydrogen peroxide vapor into the treatment and emission chamber of microwaves that generate plasma with free radicals that have the capacity to denature proteins, leading to cell death. Unlike the UV method, plasma can reach regions of devices with more complex geometries [9].

Degrading processes in polymeric materials can cause chain breakage, branching and molecular weight distribution changes, which reflect changes in their properties. In view of the above, the present project has its importance in the evaluation of the possible changes in chemical and mechanical properties, in the biocompatible polymers of interest, due to the sterilization processes, by UV and plasma, determining the best strategies of choice among the available alternatives.
This project is about the development of films and tubes, as bioressorbable vascular scaffolds models, of the biocompatible polymers poly (-L-lactic acid) (PLLA), poly (lactic acid co-glycolic acid) (PLGA) and poly (-LD-lactic acid) (PLDLA), for sterilization by UV and plasma hydrogen peroxide and to evaluate the presence of chemical, structural and mechanical properties.

Materials and methods.

Bioresorbable Polymers: Films of PLLA (molar mass distribution rate ~140.000 Da - Laboratory of Biomaterials of the Pontifical Catholic University of São Paulo), PLDLA (molar mass distribution rate 100.000-160.000 Da - Laboratory of Biomaterials of the Pontifical Catholic University of São Paulo) and PLGA (molar mass distribution rate ~66.000 Da -Sigma-Aldrich®) polymers were obtained using solvent evaporation method. Then, 2.5 g of polymer was dissolved in 50 ml of chloroform under constant stirring and at room temperature for 1 hour. After complete dissolution, polymers were deposited in Petri dishes, previously silanized, to facilitate the subsequent removal of the formed film. The silanization of plate was carried out with silicone oil in an oven at 200 ° C for 2 hours, followed by cooling and removal of excess oil with surface washing with detergent. The plates containing solution were capped with exhaustion at room temperature and, after complete solvent evaporation, after 3 to 4 days average, films were removed from the plates and stored under vacuum at room temperature.

Samples of PLLA tubes were prepared by the dip coating method, using deposition of polymer film on surface of guide metal cylinders of suitable diameter, in the order of 2 to 5 millimeters, approximating the dimensions of bioresorbable vascular scaffolds. The deposition of the film was carried out by dipping the cylinder in solution of polymer, followed by evaporation of solvent, even used in making of the films. The procedure was repeated the number of times necessary to obtain the desired thickness for tubes.

Sterilization by ultraviolet radiation: Safety cameras equipped with ultra violet emission lamps, UV-C type, were used for sterilization. The films were exposed to radiation for 1.5h on each face, in direct contact with the radiation. After this period the films were again stored in a vacuum chamber at room temperature until tested.

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Sterilization by plasma of hydrogen peroxide: The films were subjected to plasma sterilization of hydrogen peroxide for 45 minutes in a plasma chamber (Sterrad Sistem® - Johnson & Johnson®). According to the manufacturer's guidelines: the films were packed in polypropylene permeable packages inside the equipment, passed through the first vacuum cycle at 0.3mmHg for 10 minutes at room temperature; Second cycle with 58% hydrogen peroxide injection for 6 minutes. Third cycle with diffusion of hydrogen peroxide for 12 minutes; Fourth cycle with the emission of microwaves to form the plasma for 12 minutes, being able to reach the maximum temperature of 50 ° C; Fifth cycle consisting of series of ventilation, vacuum and repressurization until reaching atmosphere and room temperature for approximately 5 minutes. After this period, the films were again stored in a vacuum chamber at room temperature until tested.

Evaluation of mechanical properties: The mechanical properties were evaluated by tensile stress strain test on samples, obtained from the cut of the prepared films. The cutting was done in hydraulic press with specific mold. The samples had their dimensions obtained by digital micrometer (Scarret ® -796). The tensile strain tensile tests of the PLLA films were performed on the MTS TRYTON 250® at a speed of 100mm / min. The tests of the PLDLA and PLGA films were performed in universal test equipment (Instron® 3369) at a speed of 500mm / min, as the MTS equipment did not allow the maximum elongation measurement, which, for these polymers, was higher than 50 mm.

Evaluation of thermal properties: The thermal properties were evaluated by thermogravimetric analysis (TGA) (TA Instruments®, TGA Q500), which was performed using an inert atmosphere with a flow of Nitrogen at a flow rate of 20 ml / min, with a scanning from ambient temperature to 600 °C with a speed of 10 °C per minute.

In thermal differential scanning calorimetry (DSC) analysis (TA Instruments®, Q-series), samples were submitted to the heating-cooling-heating cycle, with a heating rate of 10 ° C per minute and a cooling rate of 5 °C per minute in an inert atmosphere of Nitrogen with flow Of 20ml / min.

Confirmation of sterility: PLLA cylindrical specimens coated with PLDLA or PLGA after sterilization by plasma or UV were cultured in polypropylene tubes in 2.5 ml of Luria-Bertami (LB) culture medium (Sigma Chemical Company In incubator with shaking at 37 °C for 24 hours. Positive controls were done with non-sterilized specimens and negative controls with sterile culture media. These studies were adapted from the Brazilian standards ISO11135: 2007 and ABNT NBR15245: 2005, which refer to the ethylene oxide method currently used in the sterilization of these scaffolds.

Results and discussion.

The PLLA film obtained by solvent evaporation showed a whitish coloration characteristic of crystalline polymer. On the other hand, the PLDLA and PLGA films were presented as transparent, characteristic of amorphous polymers. The films presented values of thickness

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between 0.5-0.39 mm, with very low standard deviation, which confirms the uniformity of thickness of the samples, obtained by the method of solvent evaporation.

Thermal properties: Thermal properties evaluated by thermogravimetric analysis (TGA) are presented in Fig. 1, which comprise the mass percentages as a function of temperature, for PLLA, PLDLA and PLGA, before and after sterilization by UV and plasma, respectively. From the TGA curves the degradation temperatures for each polymer were determined, shown in Table 1.

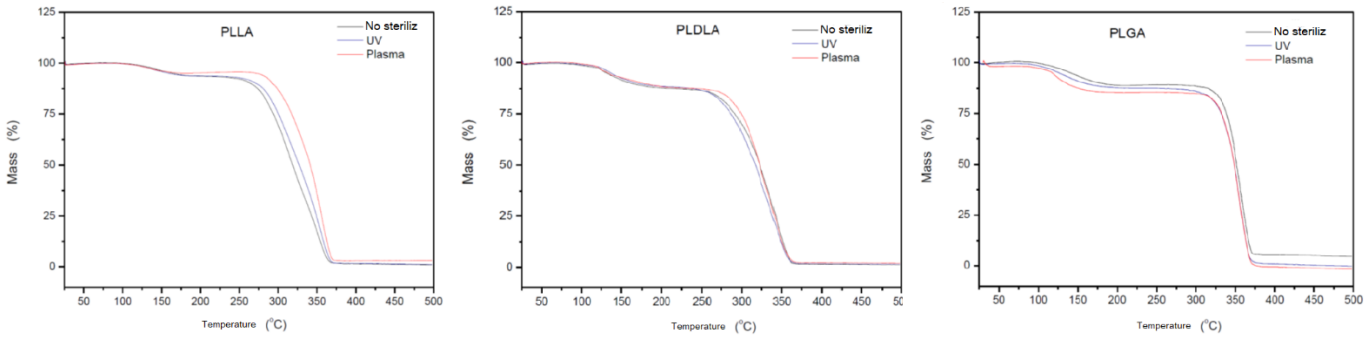

Figure 1. The results of thermogravimetric analysis (TGA), which comprise the mass percentages as a function of temperature, for PLLA, PLDLA and PLGA, before and after sterilization by UV and plasma, respectively.

Table 1. Degradation temperatures for each polymer determined.

Polymer:	Lost of water	Degradation temperature [°C]
PLLA	Yes	350,00
PLLA UV	Yes	350,00
PLLA Plasma	Yes	350,00
PLDLA	Yes	350,00
PLDLA UV	Yes	350,00
PLDLA Plasma	Yes	350,00
PLGA	Yes	360,15
PLGA UV	Yes	360,15
PLGA Plasma	Yes	360,15

The differential thermal analyzes (DSC) presented the results shown in the graphs, Fig. 2, for PLLA, PLDLA and PLGA, before and after sterilization by UV and plasma, respectively.

From the DSC curves it was possible to determine the glass transition (Tg), crystallization (Tc) and melt (Tm) temperatures for the polymers before and after sterilization. The values are presented in Table 2.

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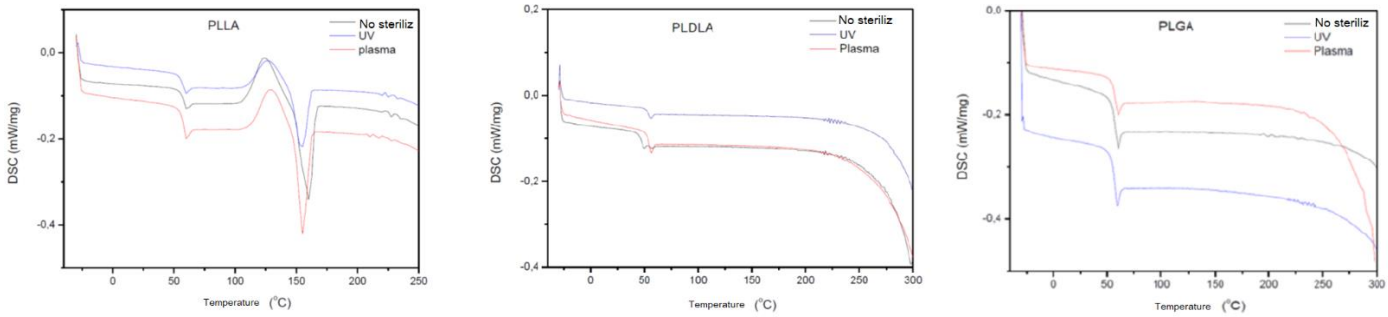

Figure 2. Results of differential thermal analyzes (DSC), for PLLA, PLDLA and PLGA, before and after sterilization by UV and plasma, respectively.

Table 2. Glass transition (Tg), crystallization (Tc) and melt (Tm) temperatures for each polymer, before and after sterilization.

Polymer:	Cristalization temperature [°C]	Glassy transition temperature [°C]	First melting temperature [°C]	Second melting teperature [°C]
PLLA	124,36	50,10	151,2	NA
PLLA UV	124.12	50,11	150,7	NA
PLLA Plasma	125,07	50,08	150,8	NA
PLDLA	NA	49,80	NA	NA
PLDLA UV	NA	50,62	NA	NA
PLDLA Plasma	NA	50,63	NA	NA
PLGA	NA	50,97	NA	NA
PLGA UV	NA	50,58	NA	NA
PLGA Plasma	NA	50,57	NA	NA

The PLLA has crystalline characteristics, whereas PLDLA and PLGA have amorphous morphology, since for PLLA it was possible to identify well defined melting temperature and PLDLA and PLGA it was not possible to identify this melting temperature.

In other studies, Rezende et al., 2005 [10], determined the melting temperatures for membranes of these polymers by thermal analysis. In this way, complementary studies are being carried out in this sense. Also, no significant differences were found in the DSC analyzes, the peak patterns for the samples before and after the plasma and UV sterilizations, which represent the melting and degradation temperatures of the samples.

Mechanical properties: The values of elastic modulus (E), stress and strain in the flow as well as stress and strain at break were determined by the data acquisition software are presented in Fig 3. The mean values determined for each sample were calculated and plus standard deviations. The assessment of the difference between groups was performed by analysis of variance and a confidence interval of $p < 0.05$ was considered.

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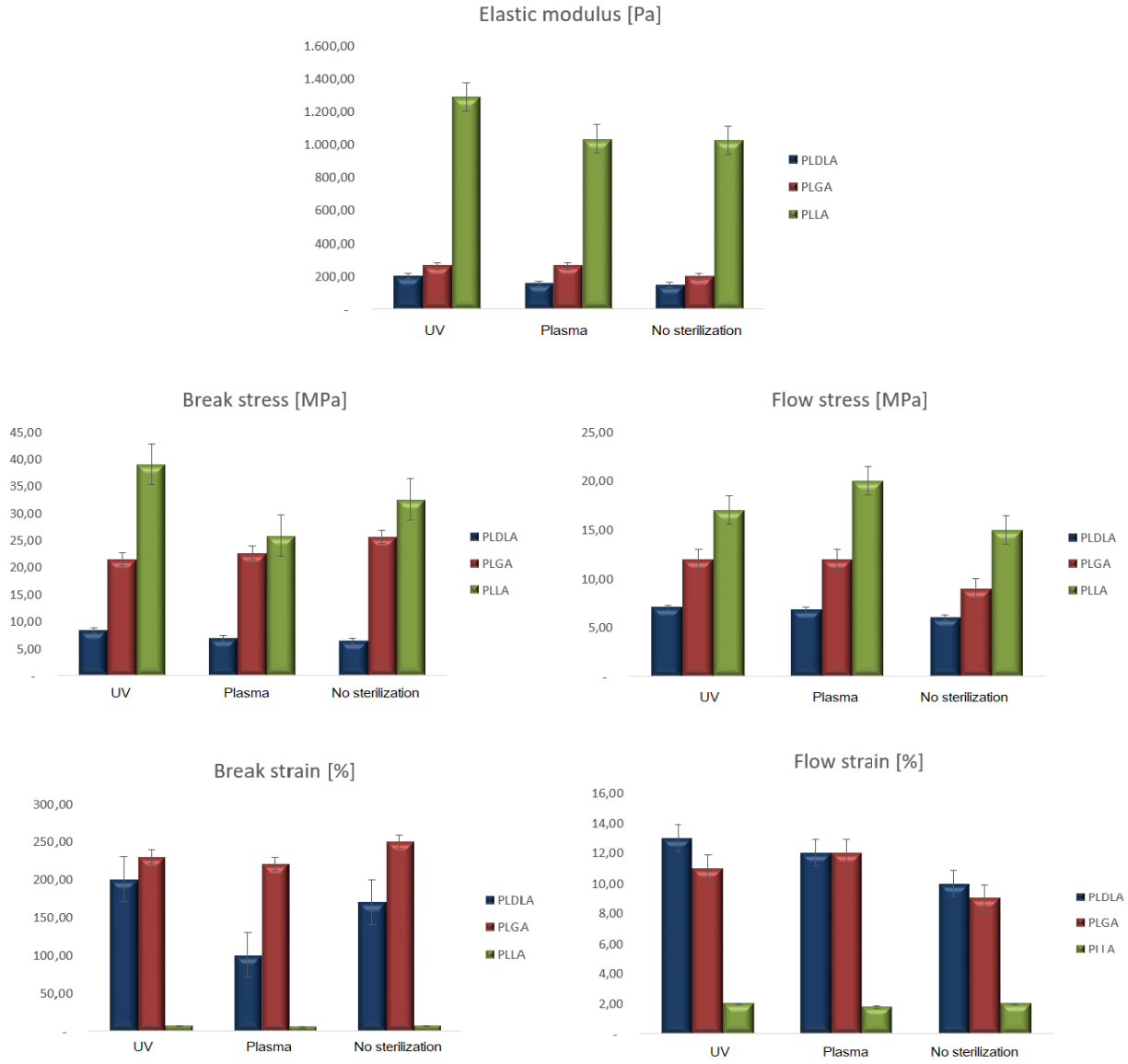

Figure 3. Mechanical properties: elastic modulus, stress and strain in the flow, stress and strain at break, for PLLA, PLDLA and PLGA, comparing UV, Plasma and No sterilization samples.

Regarding the mechanical behavior, it was not possible to observe a significant difference between the samples before and after the sterilization process, in the tensile stress strain x tensile tests, showing that it do not interfere in these mechanical properties. The PLLA presented the highest values for elastic modulus and stress at rupture, presenting the highest resistance among the samples. This material also presented the highest hardness values on the Shore-A scale. The

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results obtained for the mechanical properties are in agreement with the thermal analyzes and microstructural characterization that showed the PLLA with characteristics of material with greater crystallinity.

On the other hand, the PLDLA and PLGA polymers had lower values, and very close to each other, for modulus of elasticity and maximum tensile stress, corroborating with the results of the thermal analysis and microstructural characterization that presented these polymers as amorphous structure materials.

Confirmation of sterility: The sterility of the samples was confirmed both for the UV process and for the plasma sterilization, according to the safety standards for ethylene oxide sterilization: ISO11135: 2007 and ABNT NBR 15245: 2005 [11, 12]. Safety standards for UV or Plasma sterilization for bioresorbable vascular scaffolds application have not been described in the literature. Due to this, the standards for ethylene oxide, which is widely used for this application, have been followed. The picture of the tube samples, with the dimensions near of the used in bioresorbable vascular scaffolds, used to this tests are in Fig 4.

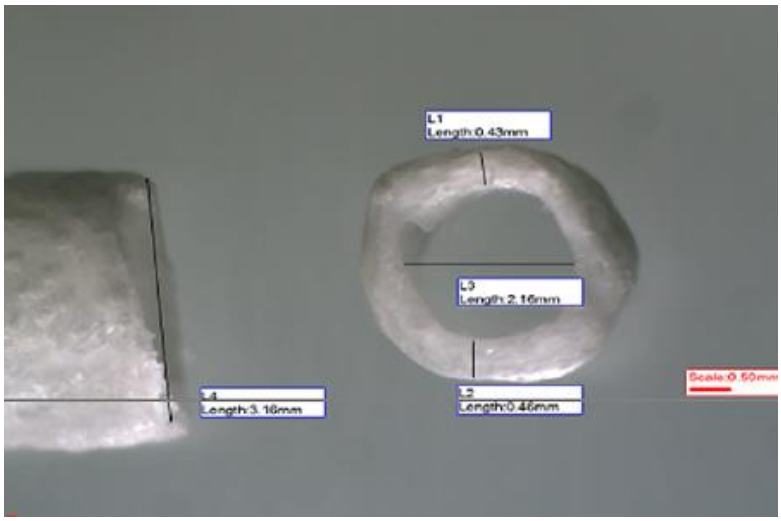

Figure 4. Tube samples and its scale measures.

Conclusion. In the thermal behavior, it was possible to identify Tg, Tc and Tm for PLLA and not for PLDLA and PLGA which characterize amorphous material.
As for the mechanical behavior of the polymers, PLLA showed higher values of elastic modulus and higher tensile strength than PLDLA and PLGA.
It was possible to conclude that there are no changes in the structure, thermal properties and mechanical properties of the PLLA, PLDLA and PLGA materials after the sterilization processes, by UV and plasma, indicating the stability of the samples to these processes.

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Disclosure. The authors report no conflicts of interest in this work.

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