In vitro analysis of a local polymeric device as an alternative for systemic antibiotics in Dentistry

Abstract: The development of a biodegradable material with antimicrobial properties for local applications is required in the prevention and treatment of infectious diseases. The objective of this study was to produce blends of poly-L-lactide acid (PLLA) synthetic polymer associated with several antimicrobials, as an alternative in the prevention and treatment of infections, as well as to evaluate its cytotoxicity, release of antimicrobials and inhibit bacteria growth. Blends of PLLA added with 20% Amoxicillin, Metronidazole, Clindamycin or Azithromycin were used to produce Films (F) or Meshs (M) by casting and electrospinning methods, respectively. Standardized discs of the films and meshes were stored in buffer solutions (pH 5 or 7.4) and aliquots were analyzed by high performance chromatography (HPLC) during 168 hours. Cytotoxicity on human gingival fibroblasts was tested after 24, 48 and 72h by MTT reaction. The antimicrobial capacity was determined against P. gingivalis and S. pyogenes. The specimens were weighed after 3 and 6 months of storage for degradation analysis. SEM was performed to control interfaces and degradation. Antimicrobials presented a continuous and exponential drug release. Analysis showed that both M and F were able to inhibit S. pyogenes and P. gingivalis growth, indicating the release of active antimicrobial agents. The products were not toxic to the fibroblasts. Amoxicillin-film showed more degradation than PLLA at both pHs (p < 0.05), whereas Azithromycin-meshes were more degraded than PLLA at pH 7.4 (p < 0.05). PLLA association with antimicrobials is biocompatible and may represent a potential tool for the local delivery of antimicrobials.

Keywords: Biocompatible Materials; Polymers; antimicrobial, electrospinning.

Introduction

Indiscriminate administration of systemic antimicrobials by health professionals is one of the main reasons of the rise of multi-resistant isolates. In this aspect, the choice of a local delivery drug therapy may result in an efficient treatment of localized infections, whereas systemic formulations could be selected only for potentially aggressive infections. This is an important and desirable situation, since it avoids the selection of resistant strains in the human normal microbiota, and decreases financial expenditure with drugs.
Reducing and/or eliminating undesirable effects of a drug and promoting release at specific sites, as well as minimizing the undesirable systemic effects are ways to improve the action of a drug. A single dose of drug via polymeric biomaterials is an alternative to maintain the antimicrobial concentration in the therapeutic range constant for an extended period, which may increase the clinical efficacy and decrease the toxicity.

Biomaterials carrier systems can be presented in various forms such as solvent-controlled systems, reservoir systems and chemically controlled systems. The use of drug resorbable delivery systems had its beginning in the last decades. The application of antimicrobial agents to biomaterials had an important increase in the medical area, especially in Dentistry, where the removal of biofilms is important for an effective treatment.

Several studies indicate the use of systemic antibiotics in the treatment of severe periodontitis. Systemic antimicrobials are also useful in Implantology and Surgery, where the placement of a biomaterial in a microorganisms-rich site may result in possible contamination of the receiver site and the biomaterial, leading to technical failure in the surgical use of implants and/or grafts.

However, their effect on the microbiota of other human sites and the selection of resistant strains have been poorly evaluated. The systemic use of antimicrobial agents can cause gastrointestinal side effects, hypersensitivity and undesirable drug interactions. Furthermore, the antimicrobials should reach high concentrations in the biofilms, where bacteria present several mechanisms of drug resistance.

Since the active antimicrobials should reach adequate concentrations in the specific site of action and these products should remain locally active or be retained for a prolonged time, polymeric carriers for antibiotics would be an interesting alternative for their local use.

A possible method for preventing bacterial colonization involves biomaterial’s surface modification, implemented locally by coating with a biocompatible, resorbable polymeric layer, which may release antimicrobials only in the desired site. This material could be adapted to each specialty, satisfying the needs of both the professional and the patient.

Thus, this study investigated local delivery systems to be used in different dental situations.

PLLA polymer was chosen due to its excellent biocompatibility, proven by applying materials in biomedicine and full biodegradability characteristics throughout a large time lapse (6 months to 2 years), which would include various dental applications, such as microchips in Periodontics, membrane grafting protection and bone repair, coating dental implants and prosthetic components. It should be noted that this is already a material used in Biomedicine, approved by the FDA and European Regulatory Agencies for food and some surgical applications such as drug-release systems.

The production of PLLA by the electrospinning technique is able to create a membrane that mimics the extracellular matrix, for the use in dental clinical situations that already use membranes for protecting grafts and bone defects. The electrospinning technique allows greater control of parameters in order to obtain a larger surface area for optimal release of antimicrobial drugs, and orients fibers with properties superior to the film produced by deposition.

Although the use of antimicrobials associated with biomaterials have been studied for dental purposes, including the development of electropum mats with Metronidazole associated to PLLA, studies regarding the association of other commonly used antimicrobials, such as Amoxicillin, Azithromycin or Clindamycine, with PLLA in electropum mats or even in film were not found in the literature, which has posed a methodological challenge to this study. Therefore the aim of this study was to investigate drug release of resorbable polymeric reservoir, cytotoxicity and antimicrobial capacity and degradation of new PLLA (poli-l-lactide acid) devices. The systems were composed of films or meshes of blends of PLLA and Amoxicillin, Azithromycin, Clindamycin or Metronidazole, as an alternative to systemic antimicrobial.

Materials and Methods

Study design

Eight materials were developed, four of which are processed as films and four as electropum mats composed of a blend of PLLA and Amoxicillin,
Azithromycin, Clindamycin or Metronidazole. This \textit{in vitro} study was conducted using polymeric disc matrices (\(n = 180\)), with diameter of 15 or 6 mm, in films or meshes (electrospinning) types. Disks were submitted to drug release test (chemical step), cytotoxicity of human fibroblasts (cell phase) and antibacterial efficacy (microbiological phase). A structural degradation analysis (SEM) is also performed. All tests were performed in triplicate (Figure 1).

**Material development**

**Fils**

A 5 wt\% PLLA (poly-L-lactide) solution was prepared in 10 mL of chloroform. After complete dilution of the polymer in solvent the drug was is added in mass, with a standard concentration of 20\% in relation to the PLLA mass. This solution was maintained under vortexing until the solubilization of the antimicrobial to obtain a homogeneous solution, poured through layers in Petri dishes (1 mL) and subsequently maintained at room temperature overnight to evaporate the solvent. The dried material was kept 24h in a vacuum desiccator to remove any trace of molecules of solvent that could be trapped into the structure. After this step, films were cut into discs with 15mm microtomes for chemical and cellular tests and with 5mm for microbiological assay. For Amoxicillin, 0.05 mol/L CTAB (surfactant) solution was used in 5 wt\% PLLA and 20\% drug.

**Meshes (Electrospinning)**

A 5 wt \% PLLA solution was prepared (5 mL) in chloroform and dimethylformamide (9:1). After complete solubilization the drug was added in mass to PLLA solution, with a standard concentration of 20\% in relation to the PLLA mass and maintained under stirring to obtain an homogeneous solution, and subsequently subjected to electrospinning in an apparatus consisting of an infusion pump (Harvard Apparatus, model 975, USA), a power supply (Glassman High Voltage, Inc., EH Series high-voltage, UK) and a metallic collector (aluminum plate). A needle of 0.584mm diameter and a syringe with an effective volume of 10ml was used. At the voltage of 25 kV, and with a flow rate of 4ml/h, the distance from the needle to the collector was 18 cm. The mesh was dried in a vacuum desiccator to

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**Figure 1.** Study design.
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remove any trace of molecules of solvent that could be trapped into the structure. The mesh obtained was cut to 6 or 15 mm diameters. For Amoxicillin, 0.05 mol/L CTAB (surfactant) solution was used in a 5 wt% PLLA and 20% drug.

**Generation of aliquots and Drug release profile**

Polymeric materials were individually immersed in Falcon tubes with buffer solution pH 7.4 or pH 5.0 and stored at 37°C. These pHs were chosen due to the receptor site be considered neutral in normal situation pH (7.4) or more acidic in conditions of inflammation or infection (pH 5.0). Then, aliquots (300 μL) of the solutions were collected at 8, 24, 48, 72, 96, 120, 144 and 168 hours, in order to determine the release rate as a function of time by HPLC (high performance liquid chromatography performance). The HPLC analysis parameters were: flow rate of 1 mL/min for all antibiotics; mobile phase of 45% acetonitrile and 55% potassium phosphate buffer at pH 7.4 for Clindamycin, 95% water and 5% acetonitrile, Metronidazole 80% methanol and 20% potassium phosphate buffer at pH 7.4 for Azithromycin, and 4% acetonitrile and 96% potassium phosphate buffer at pH 3.5 for Amoxicillin; the absorbance was detected at the wavelength of 210 nm to Clindamycin and Azithromycin, 300 nm to Metronidazole and 230 nm to Amoxicillin.

**Antibacterial efficacy**

**Bacterial strains and agar diffusion method**

This assay determined the ability of antimicrobial agents incorporated into the polymer base to inhibit bacterial growth. The base polymers were prepared as standardized discs with 5 mm diameter or meshes of the same size. To determine the effectiveness of antimicrobial agents incorporated into the polymeric bases, reference strains of *Streptococcus pyogenes* (ATCC 10782) and *Porphyromonas gingivalis* (ATCC 33277) were used. The agar diffusion method was employed. The experiment was performed in triplicate. Tryptone soy broth was used for *S. pyogenes* and Triptone soy broth supplemented with hemin (0.5 mg/ml) and menadione (1 mg/ml) (Sigma Chemical Co., St. Louis, USA) (TSHK) for *P. gingivalis* cultures. Twenty four hour cultures of each bacteria were centrifuged, re-suspended to achieve 5 X 10^7 CFU/ml and used to inoculate on the surface of Müller Hinton agar for *S. pyogenes* and TSHK supplemented with 5% defibrinated blood agar plates for *P. gingivalis*, with the aid of a swab. The polymers disks containing the antimicrobial agents tested, and control discs (no addition of the agent), and commercial paper discs containing each of the antimicrobial agents were distributed on the agar surface, followed by incubation at 37°C for 48 hours aerobically for *S. pyogenes*, and for 5 days in anaerobic conditions (85% N₂, 5% CO₂ and 10% H₂) in an anaerobic chamber (Plas Labs, Lansing, MI, USA) for *P. gingivalis*. After growth, the inhibition zone was measured with a standard millimeter ruler, and photographed for filing and final comparison (DSLR camera, Nikon D3200, Nikon, Japan). The calculation of the diameter of the halos was done in Adobe Photoshop CS4 software (Adobe Systems Inc, USA).

**Cell cultures**

Keratinized tissue fragments were collected in Integrated Clinical of Dental School – University of Sao Paulo, according to the Ethics Committee approval Nº 736.009. The fragments were collected during the implant procedure with a reopening scalpel punch of 0.6 mm and then placed in culture medium for fibroblasts (Eagle Modified Dulbecco’s Medium, (DMEM). After approximately 15 days, cells reached 90% confluence. At this time, the gingival fragments were removed, followed by a trypsinization step. Cells were cultured in DMEM (DMEM, Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% fetal calf serum (Invitrogen, Burlington, ON, Canada) and 1% antibiotic-antimycotic solution (Sigma ®, Adrich, MO, USA). All procedures were performed in a laminar flow hood to maintain sterility of materials and substances used for cell cultivation. The cells were maintained in an incubator at 37 °C in a humidified atmosphere containing 95% air and 5% carbon dioxide. The culture medium was changed every 2 days and the progression of the culture was assessed by phase microscopy FGHs - Gingival fibroblasts cells in the third passage (P3) were counted in a hemocytometer (Fisher Scientific, Pittsburgh, PA, USA) and plated in 24-well
Carnaval TG, Gonçalves F, Romano MM, Catalani LH, Mayer MAP, Arana-Chávez VE, Nishida AC, Lage TC, Francci CE, Adde CA

plates. After the entire cultivation process, samples of pharmacological polymers were supplemented with 10% fetal bovine serum (FBS; Invitrogen, Burlington, ON, Canada) and 1% gentamicin (Sigma ®) and then incubated in solution rich in fibroblasts.

MTT test
Assays were performed in 24-wells plates (Corning, New York, United States). Cells were cultured into the polymeric material, and analyzes were performed at 24, 48 and 72 hours after plating. The culture medium was removed and the wells washed with sterile PBS. The positive control was the gingival fibroblasts in the polystyrene plate, the negative control was a 1% solution of phenol in DMEM. Then 900 uL of culture medium with 10% FBS plus 10 µL of (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide) MTT (MTT, 5mg /mL in phosphate-buffered saline [PBS] sterile) was added to each well and incubated at 37°C for 3 hours. The solution was removed and wells were washed with PBS buffer. Then, they were added 100µL of DMSO (dimethylsulfoxide), at room temperature for 15 minutes to solubilize the crystal blue staining/violet, formed by the cleavage of the tetrazolium ring by the succinate dehydrogenase (SDH) of active mitochondria. The absorbance was measured by spectrophotometric ELISA reader apparatus (800 ELX - Universal Microplate Reader - Bio-Tek Instruments, CHF, USA), after solubilization, at a wavelength of 570nm.

Structural analysis

Digital photograph
Samples were photographed in order to ascertain the visual pattern configuration of the film and mesh and also of their aspects after degradation for 6 months.

SEM images
SEM was used to evaluate the morphology and characteristics of the polymer surface before and after degradation (1000x magnification and 5:00 Kx and ENT 1500kv).

Freeze-fracture
The freeze-fracture was performed on all films samples for the investigation of union phase polymer drug. The discs were transfered to nitrogen for freezing (-40° C) and were later broken with standard 3 mm forceps and analyzed by scanning electronic microscopy (LEO - 430-10-15 kV).

Degradation

Weight loss
All initial samples were previously dried and weighed in digital scale accuracy. After storage for 3 to 6 months in a potassium phosphate buffer solution with pH 7.4 or pH 5.0, the samples were dried in a vacuum chamber for disposal of liquid wastes and thereafter weighed to evaluate the loss of mass percentage in grams. All samples were subjected to SEM at baseline (prior to any type of storage in buffer solution), and also after 6 months of storage. It was not possible to carry out this analysis for samples with total degradation after the period.

Statistical Analysis
Statistical analysis was performed using MINITAB (version 17). Graphs and charts were produced in Excel 2010 software (Microsoft Excel 2013, Microsoft, Redmond, USA). For all evaluated items a confidence interval of 95 % (p < 0.05) was considered. After conference of normal range of means with the Anderson - Darling normality test, the analysis of variance of the averages (ANOVA - one way) was applied for antimicrobial inhibition zone and (ANOVA - two way) for MTT and degradation. When the mean difference (p < 0.05) was found, the complementary Tukey test was applied. For drug release, a descriptive analysis was performed. In this case, when there was a match between the standard deviations, data were considered similar (p > 0.05).

Results

Drug release
Figure 2 shows the exponential curves for the antimicrobial Metronidazole in patterns films and meshes, either pH 7.4 as at pH 5.0. Figure 2 also shows the release of Amoxicillin, Clindamycin and Azythromycin, respectively. Statistical analysis of charts provided that when there was an overlap of
standard deviations by different curves (pH 7.4 and pH 5.0), the groups were considered similar (p > 0.05).

Metronidazole films have reached similar results when stored either at pH 7.4 or 5.0 over time. At pH 7.4, we observed a peak of release of 70.03% after 120 hours of storage, and the total release in acid pH occurred after 168 hours. The metronidazole polymer mesh showed higher release in the acidic pH than at the pH 7.4; demonstrating a peak release of 88% after 96 hours at pH 5.0 compared to 19% after 96 hours at pH 7.4.

The Amoxicillin curves showed similar patterns after storage in both conditions, with both a downward curve at pH 7.4 and up to 5.0. In Films, the peak was of 38.73% (8 hours) at pH 7.4 and 61.44% (144 hours) at pH 5.0. In Meshes, the peaks were 18.63% (168 hours) and 47.93% (96 hours), respectively.

Clindamycin presented the most balanced curves among different pHs. It had peak release of 68.42% (120 hours) and 76.47% (96 hours), both after the 4th day of storage. In contrast, the meshes showed a downward peak pattern of 81.10% (8 hours) and 72.76% (48 hours). This fact is very particular to the polymeric initial high rate of drug release and stability over time.

Azithromycin films showed a similar pattern and balanced pH at 7.4 and 5.0, with peaks of 32.53%, also similar with both peaks at 120 hours (82.85% and 73.15% respectively). Não tenho as figuras, mas achei esta parte tão difícil de entender.

Antibacterial efficacy

In order to evaluate whether the antimicrobial drugs released by the polymers were biologically active, inhibition zones around the polymers containing the antimicrobial agents were highlighted in S. pyogenes and P. gingivalis agar cultures.

Figure 3 represents the average in millimeters of antimicrobial inhibition areas for both microorganisms. The size of the inhibitory zone produced by all drug groups were compared (films x Mesh) among them using T-student test and with the positive control values (paper disc) by a descriptive statistical analysis. No significant differences were found (p > 0.05) between film and mesh within each antibiotic group. The size of inhibitory zones for azithromycin-films and aztyromicin-meshes observed for both strains was similar and larger than the zone produced by

![Graphs of drug release profile](image-url)
the standard control (paper disc), indicating a greater release of the drug by the polymer. Furthermore, the inhibitory zones of *P. gingivalis* produced by amoxicillin and azithromycin films and meshes and clindamycin-mesh were also larger than the standard paper discs, which demonstrated that the polymer provided greater release of antimicrobial agent than the paper (*p* < 0.05).

**Cytotoxicity**

The MTT reaction was performed in gingival fibroblasts to access the effect of all tested materials on cell viability. All drugs were compared to PLLA, positive control (polyesthere) and negative control (death, 1% phenol), and the significant values (*p* < 0.05) were described in Figure 4. All tested films and meshes presented no cytotoxic effects on fibroblasts, at 24 and 48 hours.

**Structural analysis**

The digital photography allowed the observation of different patterns in films and meshes samples. Moreover, the visual patterns were changed after degradation as sparks on films and as “dust” material in one mesh (Figure 5).

Each antimicrobial impregnation created individual chemical characteristics (Figure 6). As a result of

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**Figure 3.** Antibacterial efficacy against *S. Pyogenes* (aerobic) and *P. gingivalis* (anaerobic).

**Figure 4.** MTT reaction.
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Structural analysis of the interfaces (SEM) pictures were made. Both AM and AZ demonstrated a homogeneous pattern in the evaluated interface. CRIO.CL demonstrated the possible formation of since large structures could be observed immersed in the polymeric matrix. The MET presented a homogeneous structure with a crystalline structure at the interface, possibly formed by the antimicrobials (Figure 7). The comprehension of those characteristics was essential to map the profile of drug release, cytotoxicity and degradation.

SEM pictures also allowed finding fiber diameters using Image J software (Version 1.50a. National Institute of Health, Bethesda, Md, USA) (Table).

Degradation

Figure 8 represents the comparison between the mean percentage mass (mg) of preserved polymeric films after storage at pH 5.0 and 7.4, for 3 and 6 months, respectively. There were significant differences between Amoxicillin and PLLA stored at pH 5.0 (p < 0.05 0.007) and also at pH 7.4 (p < 0.05), with higher degradation of Amoxicillin blended materials.

Regarding meshes, differences occurred between Azithromycin and PLLA at pH 7.4 (p < 0.05) with higher degradation of the PLLA. Others comparisons between the same pHs showed no significant differences (p > 0.05). When comparisons were made within the same group depending on the material type (film or mesh), there was difference only in the PLLA group (p < 0.05), being the film less degradable than the mesh.

Discussion

For an effective elimination of pathogenic microorganisms, the antimicrobial agents should be available at the site of infection at appropriate concentrations for a sufficient period of time. Thus the major technological challenge of this study was to find criteria to evaluate the release of the antimicrobial drugs.

This study was based on two broad concepts: the reduction or elimination of the undesirable effects of antimicrobial drugs while increasing their therapeutic index and the creation of a drug-carrying device to specific sites (pharmacokinetics control and tissue distribution), which would minimize systemic toxic effects. We have developed a local system that delivers the antimicrobials to specific sites of interest that would possibly increase the specific pharmacological action at a site and decrease the dosage compared to systemic therapy.

Trying to create local situations where these biomaterials would be retained in the surgical or periodontal wound, they were stored in two different pH buffer solutions: pH 7.4 (neutral) and pH 5.0. The first simulates the blood pH that irrigates the local region; the latter simulates inflammatory and infectious conditions where released fluids and products of restorative chain lead to a more acidic environment. These choices could mimic situations, such as in the presence of lactic acid, where PLLA undergoes changes in its degradation properties.

The choice of PLLA as a carrier involves physicochemical and mechanical properties expected for the biomaterial such as stability in acid or slightly basic solutions and in biological media; accessibility to pure and impurities free; thermal stability at 37°C and under different atmospheres such as low PREDOX; processing capacity in films or meshes by electrospinning; not being toxic and inflammatory to human fibroblasts; and it allows sterilization with UV rays and is stable under a range of appropriate culture conditions.
Figure 6. Film and Mesh SEM characteristics.
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**Figure 7.** Freeze-fracture (1 = 1000 x and 2 = 5.00 kx).
The study photographs and microscopy images have shown that the biomaterials with the tested microbial agents present microscopic and macroscopic characteristics which would allow the protection of a possible wound, minimizing patient pain and local contamination, preventing the loss of fluids while maintaining a moist and irrigated wound. Furthermore, PLLA has good adhesion to mucosal and bone defects, with easy application and removal, and it does not interfere in the epithelial recovery and might even promote it.

The use of PLLA as shown here as a carrier of antimicrobial drugs has allowed the development of a control drug release device containing one of four antimicrobial agents (Amoxicillin, Azithromycin, Clindamycin, and Metronidazole), which releases the biologically active drugs over time and may prolong their effects at the infection sites.

The manufacture of meshes by electrospinning aims to create a plot that mimics the extracellular matrix observed in several dental clinic situations that are already using membranes as protection grafts and bone defects. Still, the use of technology by electrospinning allows greater control parameters for the purpose of obtaining oriented fibers and superior mechanical properties to the film made by deposition.

Almost all antimicrobials evaluated have demonstrated a higher release rate at acid pH, which is important in infectious and/or inflammatory clinical situation, in which fluids leave the site acidic. Moreover, PLLA degradation increases local acidosis, with consequent influence on drug release.

Concerning the polymer release properties, the initial “burst” release has been found only for Amoxicillin-film and Clindamycin-mesh, both at pH 7.4. This fact would

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Table. Fiber diameter.

<table>
<thead>
<tr>
<th>Element</th>
<th>PLLA</th>
<th>Amoxicillin</th>
<th>Azithromycin</th>
<th>Clindamycin</th>
<th>Metronidazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.861</td>
<td>0.268</td>
<td>0.517</td>
<td>0.401</td>
<td>0.485</td>
</tr>
<tr>
<td>2</td>
<td>0.789</td>
<td>0.268</td>
<td>0.259</td>
<td>0.268</td>
<td>0.416</td>
</tr>
<tr>
<td>3</td>
<td>0.717</td>
<td>0.268</td>
<td>0.323</td>
<td>0.401</td>
<td>0.416</td>
</tr>
<tr>
<td>Average</td>
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<td>0.366</td>
<td>0.357</td>
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<td>S.D</td>
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<td>0.000</td>
<td>0.134</td>
<td>0.077</td>
<td>0.040</td>
</tr>
</tbody>
</table>

S.D: standard deviation.

Figure 8. Degradation characteristics.
be more frequently expected in meshes, due to their large surface area and the presence of pores which could favor the initial drug release. However, the absence of an initial peak of release may be desirable, in order to prolong drug release overtime.

Characteristics such as composition, morphology and nano-fiber degradation of the polymeric matrix may influence drug release by polymers. However, the release of antimicrobial drugs is not only dependent on the polymer characteristics, but factors related to the studied drugs such as their degree of hydrophilicity, molecular weight, optimum pH and possible physicochemical interactions with the polymer matrix should influence the kinetics of drug release.

Although all tested antimicrobials are soluble in gastric juice, the presence of some chemical characteristics turns them less compatible with the PLLA molecule and can have influenced the drug release. Metronidazole and amoxicillin present aromatic ring, unsaturated double bonds and OH groups which may have interfered in this feature. Furthermore, the polarity of the antimicrobial molecule could induce ionic strengths in the polymer solution during the process to produce the meshes, which induce loads that causes the reduction of nano-fibers and possible increase the surface area and drug release.

We have also observed that amoxicillin was not soluble in a polar solution like PLLA/chloroform, and its incorporations inside the polymer was only achieved using a surfactant CTAB. Despite the use of the surfactant, the amoxicillin meshes and films were still biocompatible, as shown by the MTT reaction (Figure 4).

Moreover, the drugs released by the polymers, either as meshes or films were biologically active, as shown by their inhibitory capacity in two selected species, *P. gingivalis* and *S. pyogenes*. Meshes and films produced inhibition zones in cultures of a strict anaerobic gram negative such as *P. gingivalis* and a facultative gram positive as *S. pyogenes*. These organisms were chosen due to spectrum of the tested drugs and their involvement in oral and other mucosa associated infections. *S. pyogenes* is is a human pathogen in a range of human diseases, from skin and mucosa infections to systemic purulent toxic-invasive diseases, which are usually treated with penicillin derivatives and/or clindamycin. Periodontitis occurs in response to a complex dysbiotic microbiota, where *P. gingivalis* is considered a key stone organism.

The selected drugs are commonly used in dentistry. Amoxicillin, like other penicillins inhibits peptidoglycan biosynthesis, which results in cell death. Metronidazole enters the cell by diffusion, and then its nitro group is reduced in anaerobic microorganisms. The nitro radical of metronidazole competes with the biological electron acceptors disturbing energy metabolism and then leading to cell death and amoxicillin and metronidazole are used for treatment of severe periodontitis as adjuvant to mechanical treatment. Clindamycin and Azithromycin are both bacteriostatic agents, which inhibit bacterial protein synthesis. Clindamycin achieves high levels in bone and both agents accumulate in phagocytic cells and are delivered in high concentrations to sites of infection. Furthermore, Azitromycin exerts immunomodulatory effects in chronic inflammatory disorders, including periodontitis. Thus, the observation of inhibition zones around the biomaterials incorporated with the four agents are indicative that their antimicrobial properties were preserved during the manufacturing of the meshes and films, and they remained biologically active. However, before their use to control localized infections in humans, several pre-clinical studies should be performed, including the determination of their properties to control biofilm formation and interfere in pre-formed biofilms, and determination of Azithromycin immunomodulatory properties. Furthermore, their efficacy should be evaluated in in experimental infection models in animals and their effect in selecting resistant strains in different infection sites should be also determined, especially due to their long-term effect.

These biomaterials were not only biocompatible, but a higher level of viable fibroblasts were recovered over certain of these biomaterials when compared to PLLA. Amoxicillin-film and mesh resulted in higher levels of viable fibroblasts throughout the study, whereas increased levels were also observed for clindamycin-mesh, Azithromycin-film and mesh, and metronidazole mesh in the first two days.

This increased fibroblast growth may relate to the increased surface area afforded by microfibers, which mimics the extracellular matrix with pores and interconnections.
Considering the cytotoxicity, the association of PLLA and antimicrobials has shown to be biocompatible for human fibroblasts similar to the biocompatibility previously shown for PLLA.\textsuperscript{33}

This feature would be useful for the maintenance of a surgical wound and/or protected and moist bone defect. In fact, the polymeric mesh behaves like a three-dimensional substrate for cells, providing a suitable environment for adhesion and (cell growth).\textsuperscript{34}

Regarding the structural analysis images and degradation characteristics of the tested biomaterials, we conclude that the incorporation of antimicrobials in a blend promoted changes in fiber properties, composition, fiber morphology and degradation of the manufactured polymer.\textsuperscript{35,36,37}

Table confirmed that drug delivery systems produce polymeric fibers thinner than PLLA isolated, according to the authors investigations.\textsuperscript{36} Probably, the antimicrobials decreased the tension surface of solution and increased the molecular instability of union. It must be considered that although SEM pictures show micrometric fibers patterns, it has the same porosity and interconnections characteristics proposed for nano-fibers production.\textsuperscript{28}

The degradation in potassium phosphate buffer, either acidic or slightly basic, confirmed that this polymer undergoes simple hydrolysis of its ester type bonds, making unnecessary the presence of specific enzymes, avoiding inflammatory responses in the body.\textsuperscript{34}

Structural analysis showed that the antimicrobial is incorporated into the polymer structure in film or mesh, except for Metronidazole which formed external crystals structure which proved a glass temperature of 159°C to 163°C for this antimicrobial when combined with PLLA.\textsuperscript{8}

As expected, the pH of the storage solution has influenced polymer degradation\textsuperscript{8}. However, our data indicated that, after six months of storage, all developed polymers, except for Amoxicillin polymers, presented similar patterns of degradation independently on the pH (p > 0.05). These data contrast with others who reported that PLLA degradation would be accelerated by local acidosis\textsuperscript{8} and that PLLA incorporated with Amoxicillin showed decreased degradation under increased pH.\textsuperscript{38}

Finally, after analysis of drug release, antimicrobial inhibition, cytotoxicity, structural characteristics and degradation behavior, we can observe that the presentation film or mesh directly interferes with the polymer properties. In this regard the electrospinning may interfere with the alignment of polymer chains and stress\textsuperscript{27} by changing the glass transition temper to below the films.\textsuperscript{29} Moreover, the weft forming stitches with large surface area and pore interconnections are critical to the differences in film characteristics and meshes.

Under the limitations of the present study, we developed innovative polymers impregnated with the main antimicrobials used in Dentistry. These new biomaterials were shown to be stable, and able to release continuous levels of drugs for prolonged time, indicating their potential to prevent and control localized infections\textsuperscript{30}. Further studies considering in vivo and clinical analysis are still necessary before their use in humans. The use of drug release devices may contribute to rational drug use, leaving systemic antibiotics only for potentially harmful infections, which reduces the selection of resistant strains, as well as hypersensitivity installation, adverse reactions and financial spent on.\textsuperscript{2,8}

**Conclusion**

All developed polymers show potential for drug release, absence of cytotoxicity and antimicrobial activity compatible for use as a new device for sustained release therapy. PLLA is a biocompatible polymer whose association with all selected antimicrobials has shown to be safe, cytotoxicity and promising to release inhibitory doses against \textit{P. gingivalis} and \textit{S. pyogenes} microorganisms. The drug release profile is influenced by the chemical characteristic of the associated drug, polymer presentation (film and mesh) and pH storage solution. Films and meshes are able to be drug delivery system, the choice of submitting the application needs to be guided by clinical specialty.

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