Alternative Cutaneous Substitutes Based on Poly(L-co-D,L-lactic acid-co-trimethylene carbonate) with Schinus terebinthifolius Raddi Extract Designed for Skin Healing

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ABSTRACT: The search for new therapies and drugs that act as topical agents to relieve pain and control the inflammatory processes in burns always attracted interest in clinical trials. As an alternative to synthetic drugs, natural extracts are useful in the development of new strategies and formulations for improving the quality of life. The aim of this study was to develop a wound dressing using poly(L-co-D,L-lactic acid-co-trimethylene carbonate) (PLDLA-TMC) containing Schinus terebinthifolius Raddi (S.T.R.). S.T.R. is a native Brazilian plant known for its strong anti-inflammatory responses. The membrane of PLDLA-TMC + S. terebinthifolius Raddi was prepared at different concentrations of S.T.R. (5, 10, 15, and 50%). The Fourier transform infrared results showed no change in the PLDLA-TMC spectrum after S.T.R. addition, whereas the swelling test showed changes only in PLDLA-TMC + S.T.R. at 50%. The wettability measurements showed a mass loss due to the decrease in the contact angle in all samples after the S.T.R. addition in the polymer, whereas the S.T.R. release test showed a linear delivery pattern. The scanning electron microscopy analysis showed that S.T.R. was homogeneously distributed at only 5 and 10%. Tensile tests demonstrated an increase in Young’s modulus and a reduction in the elongation till rupture of PLDLA-TMC after the addition of S.T.R. The biocompatibility in vitro evaluation with rat fibroblast cells seeded in the membranes of PLDLA-TMC + S.T.R. showed that although S.T.R. interfered in cell morphology, all concentrations tested showed that cells were able to adhere and proliferate during 7 days. Thus, S.T.R. at 50% was chosen to be tested for in vivo trials. The histological and immunohistochemistry results revealed an accelerated skin healing at 7 days after controlled secondary burns were introduced in the dorsal skin, with a striking total recovery of the epidermis and high rates of molecular activation of cell proliferation. Due to the known biocompatibility properties of PLDLA-TMC and its stable release of S.T.R., we strongly recommend S.T.R.-containing PLDLA-TMC as a curative device to favor skin healing.

INTRODUCTION

The skin acts as an important barrier of protection, and lesions are often common, but depending on the injury complexity, tissue destruction may occur due to coagulation, denaturation of proteins, or ionization of cellular content leading to tissue death. The skin rupture may lead to complications such as liquid loss, which can generate severe dehydration and hypovolemia, bloodstream infection, hypothermia, immunity weakness, scar formation, edema, breathing problems, pain, and many other commitments. When skin damage is severe enough to induce some of those complications, an effective therapy is mandatory to bring back its function and patient self-esteem. The wound healing is an important physiological process to restore the integrity of skin after trauma, either by accident or even by an intent procedure. The normal wound-healing process involves three successive overlapping phases: hemostasis/inflammation, proliferation, and remodeling. Some aberrations of the wound-healing process, such as excessive wound healing (hypertrophic scar and keloid) or chronic wound (ulcer), impair the normal physical function.4,5

■ INTRODUCTION

The skin acts as an important barrier of protection, and lesions are often common, but depending on the injury complexity, tissue destruction may occur due to coagulation, denaturation of proteins, or ionization of cellular content leading to tissue death. The skin rupture may lead to complications such as liquid loss, which can generate severe dehydration and hypovolemia, bloodstream infection, hypothermia, immunity weakness, scar formation, edema, breathing problems, pain, and many other commitments. When skin damage is severe enough to induce some of those complications, an effective therapy is mandatory to bring back its function and patient self-esteem.
Biomaterials designed to improve skin healing must possess mechanical and chemical properties that could reconstitute, even partially, the skin function, but to conduct the healing toward the regeneration, the bioengineered construct should control the natural inflammation process in the course and accelerate tissue proliferation and remodeling. Nowadays, a large number of wound-care products are available commercially, like creams, solutions, dressings, and even skin tissue-engineered substitutes. Thus, dressings based on polymers act as an effective method for the treatment of skin wounds, presenting a good ratio between the cost and clinical benefits.6 In this direction, the most applied polymers in the dressing manufacture are poly(glycolic), poly(lactic acid) (PLA), poly(acrylic acid), poly(e-caprolactone), poly(vinylpyrrolidone), poly(vinyl alcohol), and poly(ethylene glycol).7 These polymers have already shown in vivo or in vitro healing properties directed to favor epithelization of the damaged area and induced cell adhesion and proliferation, respectively.8 However, among the polymers listed, one should highlight the poly(lactic acid) (PLA). This polymer belongs to the class of aliphatic polyester, a thermoplastic polymer that presents ester bonds in the main chain, which makes it susceptible to degradation by hydrolysis. Since lactic acid is a chiral molecule with two optically active forms (l-lactic acid and d-lactic acid), the polymerization of these monomers leads to the formation of two different types of PLA. One of them is the poly(l-lactic acid) and the other is poly(l-co-d,l-lactic acid) copolymer (PLDLA), which are semicrystalline and amorphous polymers, respectively.9,10

Despite the good mechanical properties shown by the PLDLA, its application as a wound dressing requires improved elasticity, once it is necessary to be flexible enough to be molded on the injury skin. In this scenario, to increase the tenacity and flexibility of the desired curative device, trimethylene carbonate (TMC) was incorporated into the PLDLA.11 TMC is an aliphatic elastomer and when incorporated into the PLDLA, the resulting polymer is turned flexible. Poly(l-lactic acid-co-TMC) (PLDLA-TMC) produced by our group is more flexible than PLDLA according to the charge applied. To improve healing properties, since the use of PLDLA-TMC as a polymeric dressing is not enough to increase skin recovery, such as therapeutic qualities like antiseptic or anti-inflammatory characteristics, PLDLA-TMC was added to the natural plant extract of Schinus terebinthifolius Raddi (S.T.R.). Many studies have shown the antioxidants, anti-inflammatory, antitumor, and antibacterial power of plants12,13 and, consequently, their use as a natural remedy for this purpose is gaining attention in traditional medicine.14 S.T.R. is a plant that belongs to the Anacardiaceae family.15 Many research studies have evidenced the anti-inflammatory, antimicrobial, and cicatrizing properties of S.T.R.15–18 These properties relate to the presence of tannins and essential oils in both its branches and leaves, whereas the tannins are the ones responsible for the cicatrizing action. In addition, the presence of flavonoids in the bark of S.T.R. potentiates this effect due to their anti-inflammatory action.19

Thus, the wound treatment attracts a high level of attention and it is economically demanding to treat extensive skin burns. Since the commercial skin dressing sold nowadays is very expensive, limiting adequate treatment to only few people who can pay for it, the aim is to produce a dressing based on PLDLA-TMC with S. terebinthifolius Raddi powder extract, which could prove to be of great commercial interest.

## MATERIALS AND METHODS

### Synthesis of PLDLA-TMC. Poly(l-co-d,l-lactic acid-co-trimethylene carbonate) [PLDLA-TMC (50/50)] was synthesized in the Biomaterials Laboratory at PUC-SP according to Motta and Duek.20 Appropriate amounts of l-lactide, d,l-lactide, and TMC (L/d,l/TMC = 35:15:50 w/w/w) were mixed and poured into glass ampoules. The molar ratio of Stannous octoate (Sn(Oct)2) added to the monomer was 1:5000. After being sealed under vacuum, the ampoules were immersed in an oil bath at 130 °C for 48 h. At the end of the reaction, PLDLA-TMC was dissolved in chloroform and precipitated in methanol. It was done to purify the PLDLA-TMC obtained. The terpolymer was dried under vacuum for 8 h at 45 °C to eliminate the residual solvent.

### Obtention of the S.T.R. Powder. The milled bark of S.T.R. tree was purchased from Zé Franco Ervas Medicinas store, located in the city of Sorocaba, SP, Brazil. The S.T.R. powder extract was obtained by the percolation extraction method. The powder (250 g) from the bark of S.T.R. was mixed with 70% ethanol (1:2.0, v/v) and left standing for 1 h. Then, the mixture was transferred to a percolator. The extraction was done at room temperature with a flow rate of 5 mL/h until the percolation was exhausted. Through the rotary evaporation process, the solvent was removed; the humid extract obtained was dried in a vacuum oven at 35 °C for 72 h.

### Preparation of PLDLA-TMC Membranes. PLDLA-TMC membranes impregnated with S.T.R. were prepared by the solvent evaporation method. The concentration of the polymer in these membranes was 10% (w/v) (polymer weight/solvent volume), and the S.T.R. concentration varied from 0 (positive control), 5, 10, 20 to 50% (w/w) (polymer weight/S.T.R. weight). After complete polymer solubilization in CHCl3, it was poured on a plate inside a glass box saturated with chloroform for 12 h and dried at room temperature. The samples were identified as PLDLA-TMC (as a positive control and S.T.R. free), PLDLA-TMC + 5%S.T.R., PLDLA-TMC + 10%S.T.R., PLDLA-TMC + 20%S.T.R., and PLDLA-TMC + 50%S.T.R. (Table 1).

### Table 1. Composition of the Membranes of PLDLA-TMC or with S.T.R.

| sample                  | chloroform (g) | PLDLA-TMC (g) | S.T.R. (g) |
|-------------------------|----------------|---------------|------------|
| PLDLA-TMC               | 7.0            | 0.7           | 0          |
| PLDLA-TMC + 5%S.T.R.    | 7.0            | 0.7           | 0.035      |
| PLDLA-TMC + 10%S.T.R.   | 7.0            | 0.7           | 0.070      |
| PLDLA-TMC + 20%S.T.R.   | 7.0            | 0.7           | 0.140      |
| PLDLA-TMC + 50%S.T.R.   | 7.0            | 0.7           | 0.350      |

### Thermal Analysis. Thermogravimetric analysis (TGA) was performed under a nitrogen atmosphere in a TGA 55 instrument (TA Instruments). Samples of approximately 10 mg were analyzed. Samples were heated from room temperature to 500 °C at a heating rate of 10 °C/min and a nitrogen gas flow rate of 50 mL/min.

### Differential Scanning Calorimetry (DSC). The DSC analysis was performed with a DSC 250 instrument (TA Instruments). DSC samples of approximately 10 mg were weighed and measured against an empty pan as a reference. Measurements were carried out under a 80 mL/min nitrogen flow rate according to the following protocol: first heating from 25 to 200 °C at 10 °C/min, first cooling (quenching after first...
heating) from 200 to \(-30\) °C at \(50\) °C/min and 2 min of isotherm, and second heating from \(-30\) to 200 °C at 10 °C/min. In particular, the first heating scan was used to erase any prior thermal history of the sample and the second heating scan was used to evaluate glass temperature \(T_g\).

Swelling Test. All polymeric tests were carried out under the same conditions. All samples were cut in 20 (l) \(\times\) (w) \(\times\) 0.3 (t) mm³. The bath temperature and maximum immersion time considered were 37 °C and 110 h, respectively. The absorption measurements were done under immersion in phosphate-buffered saline (PBS) at pH = 7.4. After removing the specimen from PBS, it was dried to remove the surface moisture and weighed by a high-precision analytical balance to evaluate the mass change during the swelling. The percentage of weight variation for each specimen was calculated from its initial weight \(w_0\) and its weight after absorption \(w_f\), as follows in eq 1:

\[
\text{swelling} = \frac{w_f - w_0}{w_0} \times 100
\]

\(S.\ terebinthifolius\) Raddi Release Test. The release of S.T.R. was performed in a triplicate of membranes \([30 \ (l) \times 10 \ (w) \times 0.3 \ (t) \text{ mm}^3]\) with different S.T.R. contents, submerged individually in 4 mL of PBS at 37 °C. After 1 h of the start of the test, the supernatant (2 mL) was removed and measured by a UV spectrophotometer (Fento 800 XI) at 480 nm. All aliquots were rejected after each measurement, and then each PBS was filled in each specimen after the reading point. The analysis was repeated once a day until day 8. The amount of S.T.R. released was calculated from a standard curve containing a known content of S.T.R.

Contact Angle Measurement. The wettability of the surfaces was evaluated by measuring the static contact angles of polar liquid (dH₂O) droplets on the membrane surface of each sample. Contact angles were measured by a Rammé-Hart 100-00 goniometer. The results were obtained as medians of eight measurements at four random regions of the membranes.

Scanning Electron Microscopy (SEM). The surface of each sample was gold sputter-coated and analyzed by SEM (JEOL JSM 6010) at an acceleration voltage of 3 kV under detector of secondary electrons. Low-magnification images of the surface morphology of each sample were evaluated.

Tensile Testing. The ASTM D 638 standards were used for all tensile tests. The Universal Testing Machine (INSTRON EMIC 23–30) with tensile grips at 25 °C with a strain rate of 10 mm/min was configured for all analyses executed. For each sample, tests were done in quadruplicate.

In Vitro Biocompatibility Tests. Rat fibroblast cells were purchased from Sigma-Aldrich. Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum and antibiotics (Sigma-Aldrich) was used for routine cell cultivation. When cells reached passages between three and five, the assays were performed. Prior to the assays, 0.8 mm diameter rounded membranes were UV-C sterilized and soaked in pure DMEM for 1 h in 48-well plates. The cell concentration of \(2 \times 10^4\) per well was seeded in all of the membranes. Cells were also seeded in Thermanox coverslips (Nunc Inc.) as a negative control. The cellular proliferation and viability were evaluated after 1, 3, and 7 days. After this period, cells were subjected to fluorescence live imaging by direct labeling of calcein green AM to identify live cells and ethidium homodimer (Ethd-1) to identify the dead ones (Thermo Scientific) by laser scanning confocal microscopy (LSCM) (model TCS SP8; Leica, Germany). Samples were observed in a photomultiplier tube mode using a 488 nm laser line to detect emission signals in the range of 500–540 nm.

In Vivo Assays. In this test, 30 Wistar rats of both sexes of approximately 3 months of age, raised in the biotery of the Medical and Health Sciences Faculty at Pontifical Catholic University of São Paulo (PUC-SP), under ethical committee approval number 2016/61 were divided into three groups, according to the treatment applied: a negative control group (only lesions without a polymeric membrane), a positive control group (PLDLA-TMC membrane only), and a membrane group containing 50% (w/w) of S.T.R. (PLDLA-TMC + 50%S.T.R.). The concentration of 50% (w/w) S.T.R. was chosen for in vivo studies based on the previous in vitro assays. For each study group, five animals were assigned, and the implants were analyzed 7 and 14 days after the surgical intervention (burn injury). After implantation, animals were raised in individual cages until euthanization. Surgeries were performed after neuromuscular blocking by ketamine/xylazine anesthesia. After fur trichotomy on the region of interest (cervical back near down calvaria), the burn injury was done in a skin area of 4 cm² (2 × 2 cm²). The skin was subjected to burn for 10 s under water immersion at 70 °C. After the recovery period, the animals were euthanized by halothane inhalation and the skin lesion area was removed and fixed in formaldehyde at 10% and processed for histology and immunohistochemistry. The early inflammatory responses and tissue healing were investigated by antibody-peroxidase labeling to identify granulocytic leukocytes (myeloperoxidase, MPO), macrophages (CD68), and cellular proliferation (proliferating cell nuclear antigen, PCNA).

## RESULTS AND DISCUSSION

Scanning Electron Microscopy. To verify the distribution and dispersion of S.T.R. in PLDLA-TMC, the SEM analysis was conducted. **Figure 1** shows the surface of PLDLA-TMC with different concentrations of S.T.R. The morphology of the PLDLA-TMC + S.T.R. surface showed subtle differences among samples. The smooth and dense presentation of the PLDLA-TMC matrix was changed...
by the presence of small tactoids of S.T.R. For PLDLA-TMC + 5% S.T.R., the size of tactoids is smaller than for PLDLA-TMC + 50% S.T.R. For example, in the sample of PLDLA-TMC + 5% S.T.R., the size of tactoids is smaller than in PLDLA-TMC + 50% S.T.R., i.e., as the concentration of S.T.R. increases, the size of the tactoids also increases. This is because high concentration of S.T.R. causes the agglomeration of the fibers on the surface of PLDLA-TMC. The largest tactoid size observed for higher concentrations of S.T.R. is due to the agglomeration of these particles when added to PLDLA-TMC. This particle agglomeration occurs due to the hydrophilic characteristic of S.T.R. particles and the hydrophobic characteristic of PLDLA-TMC. Therefore, this difference in the polarity of the constituents causes the difficulty to disperse the particles of S.T.R. by PLDLA-TMC.

**Contact Angle Measurement.** The contact angle ($\theta$) measurement was done to analyze the surface of PLDLA-TMC and PLDLA-TMC containing the S.T.R. powder. It is an interesting technique to verify indirectly the distribution of the S.T.R. powder on the PLDLA-TMC surface. Since PLDLA-TMC has a dense structure, it was important to evaluate whether the S.T.R. powder was distributed on the polymer surface, which would justify its use as an efficient wound dressing. In Figure 2, it is possible to observe that the contact angle changes according to time. When comparing the contact angle value according to the time between PLDLA-TMC and PLDLA-TMC with different contents of S.T.R., one can note that PLDLA-TMC showed higher contact angle values among all samples analyzed. This is related to the hydrophobic characteristics of PLDLA-TMC. On the other hand, the presence of S.T.R. in PLDLA-TMC reduced the contact angle value compared to that in PLDLA-TMC, causing the surface of PLDLA-TMC to become more hydrophilic. This happened because the S.T.R. powder shows a hydrophilic characteristic. Such a reduction in $\theta$ value is remarkable since it proves that the S.T.R. powder was scattered over the membrane surface. This is an intended characteristic to develop an efficient dressing for S.T.R. delivery while in contact with the wounded skin.

In addition, despite adding higher concentrations of S.T.R., the sample PLDLA-TMC + 50% S.T.R. did not show the lowest value of the contact angle. Therefore, it could be an indication that at high concentrations of S.T.R. there was an aggregation of S.T.R. particles. Thus, there were not good distribution and dispersion of S.T.R. particles on the PLDLA-TMC surface. On the other hand, PLDLA-TMC + 10% S.T.R. and PLDLA-TMC + 20% S.T.R. showed the smallest value of the contact angle. Thus, it could be an indication that there were better distribution and dispersion of S.T.R. particles on the PLDLA-TMC surface. Therefore, the analysis of the measurement of the contact angle corroborated with the SEM images. That is, the higher the concentrations of S.T.R. added to PLDLA-TMC, the bigger the size of the tactoids formed due to the aggregation of the S.T.R. particles on the PLDLA-TMC surface.

A number of studies have shown that moderate hydrophilicity favors cell adhesion, growth, and spreading on such substrates as compared to hydrophobic or strongly hydrophilic substrates. In this case, this factor impairs changes in cell behavior for biomaterials, as already demonstrated elsewhere.

**Swelling Test.** This analysis evaluates how the wound dressing would behave when put in contact with the exudate from the skin during its healing. Figure 3 shows the weight change versus immersion time of samples in PBS at 37 °C.

![Figure 2. Contact angle measurements vs time.](image)

![Figure 3. Water uptake (%) vs time (h) of the PLDLA-TMC membrane and the PLDLA-TMC membrane with 5, 10, 20, and 50% of S.T.R. The early times up to 5 h are better depicted in the upper-right graph.](image)
However, PLDLA-TMC + 50%S.T.R. began to lose weight faster than other samples. This could be related to the presence of agglomerated (big) tactoids of S.T.R. on the surface of PLDLA-TMC. Due to the contact of the samples of PLDLA-TMC + S.T.R. with the PBS solution (aqueous medium) and the temperature of 37 °C for 110 h, microcracks formed on the surface and in the bulk of these samples, leading to peeling off and solubilization of S.T.R. particles from the PLDLA-TMC surface into PBS solution. Thus, it may justify the weight lost by all of the samples of PLDLA-TMC + S.T.R. since the beginning of the swelling test. Moreover, the presence of such agglomerated (big) tactoids could enable the faster release of S.T.R. particles in the solution than other samples. Therefore, this swelling behavior could corroborate with the SEM analysis and contact angle analysis.

In membranes with lower concentrations of S.T.R. (5, 10, and 20%), a more restrained PBS absorption was observed to occur in PLDLA-TMC. This slight increase of weight can be justified due to the simultaneous occurrence of the absorption process of PBS solution and the release of S.T.R. particles in the PBS solution, and in this case, the main effect is the PBS gain by the samples with 5, 10, and 20% S.T.R. In addition, at completion of the swelling test (110 h), these samples with 5, 10, and 20% S.T.R. showed a decrease in the sample weight when compared to the initial test time. Again, it could be related to the release of S.T.R. particles in the PBS solution.

**S.T.R. Release Test.** Figure 4 illustrates the release profile of S.T.R. from the PLDLA-TMC matrix with different S.T.R. concentrations versus time (h). As shown in Figure 4, the concentration of S.T.R. in PBS solution increases according to time. The release of S.T.R. from PLDLA-TMC is related to the solubility of S.T.R. in PBS solution (water medium) and to the swelling of PLDLA-TMC as it is soaked in PBS solution. However, the sample with higher concentrations of S.T.R. (PLDLA-TMC + 50%S.T.R.) showed a greater amount of S.T.R. released during the test. Moreover, the PLDLA-TMC + 50%S.T.R. sample showed a release rate of S.T.R. faster than other samples loaded with minor concentrations of S.T.R. One can observe in Figure 4 that the release profile of PLDLA-TMC + 50%S.T.R. shows a higher inclination as compared to all other curves on the initial time of the release test. Such a greater released value of S.T.R. and the faster release rate of S.T.R. of PLDLA-TMC + 50%S.T.R. than those of other samples with 5, 10, and 20% of S.T.R. are related to the presence of S.T.R. tactoids in the PLDLA-TMC + 50%S.T.R. These big tactoids can facilitate the easier release of these particles in the PBS solution than other samples with small S.T.R. tactoids (5, 10, and 20% S.T.R.). Therefore, according to the swelling and release tests, the PLDLA-TMC + 50% S.T.R. could be further a new dressing designed to control tissue repair since S.T.R. release was faster than that of other samples.

**Tensile Testing.** To evaluate whether the wound-dressing membranes containing S.T.R. presented desired mechanical properties to be used as a skin dressing, the tensile assays were conducted. Therefore, an ultimate wound bandage should possess desired mechanical properties and preserve its integrity during use and management and it should be highly flexible to easily adapt to the skin curves and movements. The mechanical properties of pure PLDLA-TMC and with different contents of S.T.R. were evaluated according to Young’s modulus and elongation at break (Figure 5).

![Figure 4](image1.png)
Figure 4. Release profile of S.T.R. from the PLDLA-TMC membrane.

![Figure 5](image2.png)
Figure 5. Young’s modulus and elongation at break (%) vs PLDLA-TMC with different concentrations of S.T.R.

The inclusion of S.T.R. within the membrane of PLDLA-TMC influences significantly its mechanical properties and generates a higher stiffness since there was an increase in Young’s modulus (2.3 MPa for PLDLA-TMC and up to 17.8 MPa for PLDLA-TMC + 50%S.T.R.) and a decline in the elongation at break (2190.2% for PLDLA-TMC to 718.4% for PLDLA-TMC + 50%S.T.R.). When PLDLA-TMC was filled with S.T.R., Young’s modulus augmented and the elongation at break decreased for all S.T.R. concentrations analyzed. Thus, the S.T.R. particles acted as a reinforcing filler that enhanced the mechanical property of PLDLA-TMC. In this way, the improvement in Young’s modulus of the membranes containing S.T.R. may be attributed to the interaction between the PLDLA-TMC matrix and the tactoids of S.T.R. that decreases the movement of macromolecules in the PLDLA-TMC membrane. The decline of the polymer elongation after adding S.T.R. did not invalidate its use as a bandage since the membrane still maintained a significant deformation property and could adapt to body movements and skin anatomy.

**Differential Scanning Calorimetry.** The results of DSC are presented in Figure 6 and Table 2. The T<sub>c</sub> of the PLDLA-TMC membrane was approximately 13.2 °C, and the T<sub>s</sub> values of the PLDLA-TMC + 5%S.T.R., PLDLA-TMC + 10%S.T.R., PLDLA-TMC + 20%S.T.R., and PLDLA-TMC + 50%S.T.R. membranes were approximately 14.6, 14.2, 15.7, and 16.4 °C, respectively. Despite the
chains was not significant. This means that the mobility of the small segments of polymeric chains was significantly affected after the addition of S.T.R. particles in PLDLA-TMC. Therefore, it is interesting from the point of view of application since to be used as a wound dressing, it needs to be highly flexible when the clinical purpose is concerned with the use on the skin. The PLDLA-TMC membrane did not present endothermic peaks that could be related to the melting points (T_m). In this way, it is indicative of that PLDLA-TMC shows an amorphous structure. Therefore, it can be seen that the addition of S.T.R. particles in PLDLA-TMC did not promote a crystalline organization.

**Figure 6.** DSC curves of (a) PLDLA-TMC membrane and the PLDLA-TMC membrane with (b) 5%, (c) 10%, (d) 20%, and (e) 50% of S.T.R.

**Table 2.** Glass Transition Temperature (T_g) Values of Samples

| Sample                      | T_g (°C) |
|-----------------------------|----------|
| PLDLA-TMC                   | 13.2     |
| PLDLA-TMC + 5%S.T.R.        | 14.6     |
| PLDLA-TMC + 10%S.T.R.       | 14.2     |
| PLDLA-TMC + 20%S.T.R.       | 15.7     |
| PLDLA-TMC + 50%S.T.R.       | 16.4     |

**Figure 7.** TGA curves of (a) S.T.R. powder; of PLDLA-TMC membranes with (b) 5%, (c) 10%, (d) 20%, and (e) 50% of S.T.R.; and of (f) PLDLA-TMC membrane.

**Table 3.** TGA Results

| Sample                      | T_onset (°C) |
|-----------------------------|--------------|
| S.T.R. powder               | 35.5/238.9   |
| PLDLA-TMC                   | 285.8        |
| PLDLA-TMC + 5%S.T.R.        | 323.6        |
| PLDLA-TMC + 10%S.T.R.       | 320.8        |
| PLDLA-TMC + 20%S.T.R.       | 326.2        |
| PLDLA-TMC + 50%S.T.R.       | 320.3        |

The wide range of S.T.R. concentrations evaluated showed that the concentration of 10% presented a growth peak more than 100% as compared to PLDLA-TMC, although the cell morphology remained roundish at all cultured times. Nevertheless, when under 50%, cell growth was stabilized; therefore, the lower growth identified was followed by the spreading recovery. As observed in Figure 2, the contact angle of PLDLA-TMC itself as compared to all other S.T.R. concentrations was around 20° higher. This could be reflected by the same low adhesion pattern observed in all S.T.R.-containing samples (Figure 8). The contact angle cannot be

**In Vitro Biocompatibility.** As already shown by Jin et al.,

natural extracts can induce either cell death or proliferative behavior. Thus, the ideal concentration used is mandatory to determine how inductive the extract could be, i.e., whether it presents bioactive properties. Recently, it was shown that PLDLA-TMC containing Aloe vera could induce even at higher concentrations a strong increase in cell growth. Since the surface topography and the substances released can influence directly cell adhesion, no washing steps were performed for LCNSM live-imaging observations. In this way, as observed in Figure 8, the fibroblasts adhered to all PLDLA-TMC containing S.T.R. presented a morphology indicative of very low adhesion as compared to pure membranes of PLDLA-TMC. Despite this phenotype, which could be supposed to lead to cell death, it was possible to observe in Figure 9 that the counted cells from 1 to 7 days presented an overall cell growth followed by a late spread polygonal morphology. Rah et al. showed that cells that presented late spreading behavior were able to metabolize the bioactive substances delivered in the medium, preventing their death due to the reactive oxygen species.

The percentage of S.T.R. increase the tortuosity within the polymer membrane. Therefore, the presence of S.T.R. can retard the degradation in the samples with STR particles.
solely used as a parameter that can interfere in cell spreading; the surface topography and the substances released by the material are the main features that can influence cell behavior. As observed in Figure 1, the SEM showed that the more the concentration of S.T.R. in PLDLA-TMC, the rougher the surface was. Despite this condition, as depicted in Figure 4, the release profile showed that PLDLA-TMC + 5, 10, and 20% S.T.R. reached a plateau at around 50 h, and this continuous release is a strong indicative that can influence cell behavior.

Striking differences were observed in the release pattern of PLDLA-TMC + 50% of S.T.R. (threefold higher than the other concentrations), which corroborates that the release interferes in cell growth, as observed in Figure 9. Interestingly, the low cell growth was not indicative of cell death since no dead cells were identified by Ethd-1 (data not shown) on any sample. Nevertheless, after 7 days, cell spreading was identified at the highest S.T.R. concentration used. Thus, the late cell spreading, despite the continuous high S.T.R. release beyond 150 h, is clear evidence that cells were able to metabolize S.T.R., which represents a very low cytotoxicity effect even in very high concentrations.

S.T.R. is known for its anti-inflammatory responses, and based on the in vitro biocompatibility results, the PLDLA-TMC + 50% S.T.R. was chosen for further in vivo analysis. After 7 and 14 days of the controlled skin burn injury procedure and curative addition, the histological analysis revealed that epidermis was fully necrotic in negative controls but partially healed when pure PLDLA-TMC was covering the skin (Figure 10). Although a crust in the coagulation zone was observed in all negative and positive control curatives, after 7 healing days, practically, no crust was observed in PLDLA-TMC + 50% S.T.R. group, which was followed by an extended thin new epidermis containing cutaneous follicles. The dermis also showed major changes by the bioactive S.T.R. effect. The papillary dermis presented few inflammatory infiltrates as compared to controls after 7 days, whereas no inflammatory response was identified after 14 days. In addition, a new delicate smooth connective tissue containing collagen fibers bordering the neoepidermis was found in all healing areas.

As reported by Lau et al., adipose tissues derived from hypodermis and the pulp and root sheath of cutaneous follicles are the initial structures that trigger skin regeneration. It can be observed here that the adipose tissue is broadly identified in controls, whereas in curatives containing S.T.R., the skin lacks adipose cells and instead reveals a new epidermis migration originally from the follicle epithelial cells. Usually, the granulation tissue provides a provisional wound bed for re-epithelialization, but since S.T.R. strongly decreased the inflammatory response and prevented necrosis spreading to deep layers in the skin, apparently, S.T.R. also preserved the follicle pulp, which can explain the rapid follicle recovery and epidermis neoformation after 7 days. It is well known that the necrotic tissue spreads out from outer to inner areas due to the burn injuries until modulatory stem cell regulation and angiogenesis take place in the wound. Hypoperfusion after the injury is the main cause that can downregulate tissue homeostasis leading to necrosis, but when PLDLA-TMC was deposited covering the burn wound, the fluid loss was prevented while S.T.R. upregulated the inflammatory response. This can be corroborated by the immunohistochemistry results indicated in Figure 11. The specific molecular markers for inflammatory responses (MPO and CD68) showed strong labeling for MPO and CD68 in the crust, which is something expected to occur in coagulation, but in dermis when S.T.R. was present, no inflammatory markers were identified as compared to controls. Despite that PCNA identification was
evident in all samples, mainly in the basal layer of the new epidermis, in pure PLDLA-TMC samples, this labeling was spread in the new dermis after 7 days, whereas cell clusters positive for PCNA were strongly identified in the membranes containing S.T.R. at the same time. After 14 days, the negative and positive controls presented inflammatory infiltration in deeper layers of the dermis, even within the muscular endomysium.

Interestingly, in PLDLA-TMC + 50% S.T.R., no inflammatory response was identified, whereas PCNA immunoreactive cells were fully identified in the dermis despite low labeling in the basal epidermis layer. This result revealed that after 14 days, the newly formed epidermis was fully developed whereas the dermis was highly undergoing fibroblast proliferation and matrix synthesis. As reported by Kulac et al.,35 the burned rat skin showed strong PCNA labeling in extended re-epithelialization after only 4 days post injury in wounds treated with curcumin. In the same burn model, Ju et al.36 demonstrated that the dermis was faster regenerated when treated with the nanomatrix of silk fibroin, which reflected high collagen expression.

Taken together, the results of this study indicated that the PLDLA-TMC + 50% S.T.R., as compared with the controls, was bioactive and effective for modulating the healing of burn wounds in rats. The accelerated wound healing can be attributed to the inflammatory upregulating effects of S.T.R. resulting in the full development of the epidermis and high proliferation of dermal fibroblasts.

### CONCLUSIONS

In conclusion, the PLDLA-TMC + 50% S.T.R. had a desired elongation at break to be applied as a bandage since it could be molded in skin curves. Also, the release pattern was higher than that of other S.T.R. concentrations. In vitro biocompatibility revealed that despite low growth, the cells cultured were able to spread even at the highest S.T.R. concentrations. Due to all of these results, the PLDLA-TMC + 50% S.T.R. was chosen for the in vivo tests. This study suggests that PLDLA-TMC + 50% is efficient to be utilized in therapeutics for treating the injured skin by suppressing inflammation, stimulating re-epithelialization and dermal recovery, and subsequently reducing the wound-healing period. Therefore, the mechanical and thermal properties associated with the biologically inductive features make the PLDLA-TMC + 50% S.T.R. a promising candidate as a bandage to promote skin regeneration.
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Funding
The authors acknowledge the following financial support for the research, authorship, and/or publication of this article: FAPESP (grants 2015/06883-7), CNPq (grant 457422/2013-0), and FINEP (grant 01.14.0119.00).

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
The authors thank UNESP-Sorocaba for assistance in scanning electron microscopy and UFSCar-Sorocaba/PPGBMA for the permission to use the laser scanning confocal microscope, granted by Pró-Equipamentos/CAPES: 3420/2013-17 and 2610/2014-90.

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