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ORIGINAL ARTICLE

Incorporation of *astrocaryum vulgare* (tucuma) oil into PCL electrospun fibers

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Abstract

The aim of this study was to incorporate tucuma oil (Astrocaryum vulgare) into PolyCaprolactone (PCL) electrospun fibers and evaluate its physicochemical properties and cell viability. FTIR and DRX confirmed that tucuma oil (TO) does not affect the chemical properties of PCL and that the oil was loaded into the PCL microstructure, while TGA analysis showed that the oil increased the thermal stability of the polymeric fibers. SEM showed that the addition of the oil modified fibers structure by reducing the average fiber size from 5.5 µm to 1.7 µm for TO loaded samples. Cell viability assay demonstrated an increment on cell proliferation from 80% of pure PCL to 100% for samples containing TO. Therefore, it can be concluded that tucuma oil can be incorporated into PCL to form fibers by electrospinning, without meaningful changes in its physicochemical properties and increasing its biocompatibility.

Keywords: *cytotoxicity, vegetal oil, fibers.*

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1. Introduction

There are several studies focusing on the manufacture of polymeric fibers and one of the most used processes is electrospinning[1-8]. This technique has such as being able to produce scaffolds with a controlled fiber diameter, high surface area, and porous structure[1,9-12]. The ability to reproduce and manipulate the electrospinning process in vitro on a spatiotemporal scale similar to that of native tissue provides a great potential of clinical success[6,13,14]. The wide range of commercially available biomaterials, as well as the strategies adopted in tissue engineering and regenerative medicine, favor the search for new products and methodologies to obtain these[15-17].

In line with this approach, the manufacture of scaffolds from absorbable, hydrolytically degradable polymers belonging to the aliphatic polyester class is being widely investigated for the use in tissue engineering[18-24]. Their inherent biocompatibility properties and the possibility of undergoing hydrolysis in the body make these biomaterials suitable for the tissue reconstruction process. In addition to these, one can list its ease of processing and modulation of degradation rate, mechanical and visco-elastic properties[25].

Electrospun fibers are extremely attractive in the biomaterials field due to their large surface area to volume ratio[26]. Its potential applications include tissue engineering scaffolds, drug delivery media, wound healing, filtration media, composites[8,27,28], among others. It is known that the properties and internal molecular structure of polymers are strongly affected by their processing conditions. Thus, understanding the processing - structure - property relationship is of great importance for the development of polymeric fibers that meet the demands of the desired application[29].

Several studies have been seeking viable alternatives for the use of absorbable polymers containing natural oils as those oils may enhance the material properties without significantly alter the structure of the polymeric matrix. In this idea, the tucuma oil shows great application possibilities, due to the small toxicity presented and efficiency in the controlled drug delivery[30].

The tucuma palm (*Astrocaryum vulgare*), which is found in the Amazon Rain forest, is considered a pioneer of expressive growth, fire resistant with ability to sprout after burning and mainly inhabits the poultry and pastures. The kernel of the palm tree is externally covered with an oily orange canopy from which the oil is extracted[31]. Among tropical seeds, tucuma palm is an economical source of vegetal oils[32] and it is also abundant in the northwest, north, and central-west regions of Brazil[33]. Besides that, the simplicity of the extraction process to obtain the oil, which is mainly based on mechanical cold-pressing[34], also turns it into a promising biotechnological resource. In this view, tucuma oil has been already used in the biomedical field due to the high fatty acids content and the fact it has several carotenoids as bioactive compounds[35]. Based on the aforementioned, its incorporation into electrospun biomaterials trends to improve biocompatibility in addition to physical-chemical properties[36-40].

In this work, a study for obtaining PCL fibers with the addition of tucuma oil by the electrospinning process was carried out. The proposal aimed to evaluate whether the tucuma could be electrospun together with the PCL and whether this product would present good biocompatibility for its application as scaffolds or dressings for pressure injuries. Different amounts of tucuma oil were added to the PCL and its impact on morphology, physical and chemical properties, and its cytotoxic effect in cell media were studied.

2. Materials and Methods

2.1 Electrospinning methodology

For the preparation of a homogeneous polymer solution, 15% (w/v) PCL was dissolved in acetone at 50 °C using a hot plate and it was magnetic stirred until the polymer was completely solubilized. This solution was transferred to a 5 mL syringe with an 18 gauge needle. After some tests, the experimental parameters were optimized to 10 kV of potential difference and 10 cm distance between the tip of the needle containing the polymeric solution and the target. The tucuma oil was added into polymeric PCL solution before the electrospun process on the concentrations describe in Table 1.

2.2 Characterization

2.2.1 X-ray diffraction (XRD)

The characterization of the crystalline planes of the samples was performed using a Bruker D2 PHASER diffractometer. Couple Two Theta / Theta scan with 1482 steps of 1 second, 2θ from 5° to 70° and increment of 0.05° per second. The search was done at the International Center for Diffraction Data (ICDD) Database, file PDF2014 - PDF-2 Release 2014 RDB.

Table 1. Parameters used in the electrospinning technique.

Sample	Oil Concentration (µg/mL)	Distance (cm)	Electric potential (kV)
PCL	0	10	10
PCLT100	100	10	10
PCLT250	250	10	10
PCLT500	500	10	10

2.2.2 Fourier-transform infrared spectroscopy (FTIR)

The FTIR technique was used to verify the presence of functional groups in the PCL matrix. The assay was performed on a Spectrometer FTIR/NIR, model FRONTIER, brand PERKINELMER, with ATR methodology and resolution of 8 cm^{-1} and scanning from 4000 to 650 cm^{-1}.

2.2.3 Thermogravimetric analysis (TGA)

Thermal behavior of samples was evaluated under N_2 atmosphere with flow rate of 20 mL/min and heating rate of 10 °C/min. The testing was performed from 30 to 600 °C using one Perkin Elmer instrument, model TGA 400.

2.2.4 Scanning electron microscopy (SEM)

The morphology and size of the fibers were characterized by scanning electron microscopy (SEM), using a Hitachi, model tm3000 (secondary electron – SE). The software ImageJ was used to measure the diameter of the fibers. Fifty measurements were performed per sample

2.2.5 Cell viability (MTT)

The MTT assay for cell viability was used to verify the cytotoxicity of the obtained scaffolds by using an experimental protocol similar to that described by Wilms et al.[41].

Blood Collection for toxicological tests: Peripheral blood samples were obtained from three discard samples from the Clinical Analysis Laboratory of the Franciscan University, under the approval of the Institution's Human Ethics Committee (CAAE: 31211214.4.0000.5306) with no identification data. Samples were obtained by venipuncture using Vacutainer®-type heparin tubes, which were used to separate the Peripheral Blood Mononuclear Cell (PBMCs).

Treatments: A culture medium containing only the cells was used as a negative control, while the PCL, PCL100, PCL250, and PCL500 samples were incubated with PBMCs in an environment with 5% CO_2 at 37 °C for 24h. Moreover, the results were represented as a function of optical density and then, the cell viability was calculated through spectrophotometry at the 570 nm wavelength, according to Equation 1:

$$Cell\,Viability(\%) = \frac{OD570e}{OD570b} x100 \quad (1)$$

Where:

OD570e: mean value for optical density of 100% of the extract of the test samples.

OD570b: mean value for optical density of the blanks.

2.3 Statistical analysis

For the analysis of the SEM images, the IMAGE J software was used, where 50 measurements were taken from each group. Subsequently, the data were entered at the software OriginPro 8.1. The one-way ANOVA test was used to see if there was a significant difference between the measurements where p would have to be less than 0.05.

Also, for the MTT assay for cell viability, the treatments were compared to the negative control through one-way ANOVA to check for statistical differences between the groups and Dunnett's post-hoc test to compare their means. The 95% confidence interval was used and $p<0.05$ were considered significant.

3. Results and Discussions

Figure 1 shows the diffractograms selected for PCL and the PCL mixed with tucuma oil at 250 µg/mL and it shows it has the same structural characteristics. When the PCL/tucuma is analyzed at different concentrations, a crystal structure is obtained[30]. Diffractogram peaks present between 2Θ ranging from 21° to 24° are typical of PCL.

The FTIR-ATR spectrum of tucuma oil, PCL and PCL + tucuma oil are shown in Figure 2 and it contains characteristics

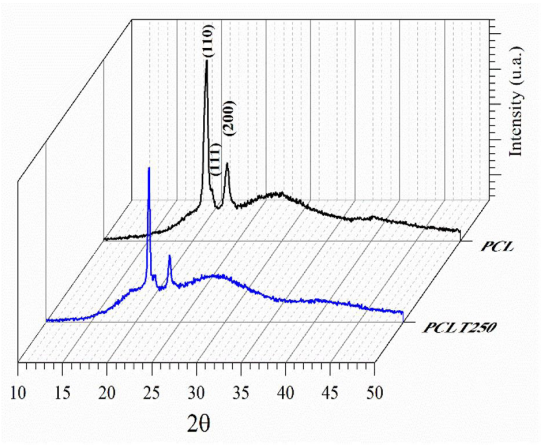


Figure 1. Diffractograms of pure PCL and PCL samples with 250 µg/mL of tucuma oil.

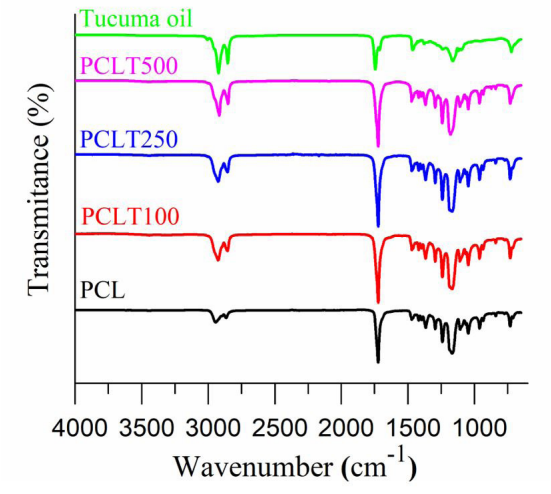


Figure 2. Infrared spectroscopy of PCL and PCL samples with 100 µg/mL, 250 µg/mL and 500 µg/mL of tucuman oil.

bands of a vegetable oil[42-45]. These bands are described in Table 2, according to the findings of Leonardi et al.[42], Ali et al.[44] and Gomez et al.[45]. Bands in the region between 2980 and 2830 cm^{-1} are characteristic of the asymmetric and symmetric stretching modes of the C-H methylene group. This molecule is present both in PCL and in vegetable oils, such as tucuma. The carbonyl C=O aliphatic stretching was observed in 1733 cm^{-1} and 1744 cm^{-1} to PCL and PCl + tucama oil, respectively.

It was observed that the incorporation of tucuma oil in PCL resulted in an increase in the intensity of the bands related to the methylene group, since all samples of PCL + tucuma presented greater intensity in these bands when compared to pure PCL. Nevertheless, it is not possible to see a significant difference in these intensities when the PCL + tucuma oil spectra are compared between them. Thus, the FTIR results suggest that the quantity of 100 µg/mL is sufficient to promote alterations in PCL properties.

Figure 3 shows the SEM images from PCL and PCL with tucuma oil. The medium diameter from PCL fibers was the 5.3 ± 3.7 µm and the PCL fibers with tucuma oil were 1.72 ± 0.90 µm, 2.6 ± 1.3 µm, 2.9 ± 1.2 µm, to samples with 100 µg/mL, 250 µg/mL and 500 µg/mL, respectively. The ANOVA test showed a significant difference among PCL fibers diameter and all samples with tucuma oil. The same result was observed between and PCL plus 100 µg/mL and PCL plus 500 µg/mL ($p < 0.05$), despite of difference between fibers diameter from PCL plus 100 µg/mL and PCL plus 250 µg/mL the ANOVA test did not find a significant difference when comparing them ($p = 0.29$). These results were different from the work from Felgueiras et al.[46], where there was not statistical change in fiber diameters when an essential oil was added to polymer fibers. However, them are in accord with the showed by Tampau et al.[47] and Hasanpour Ardekani-Zadeh and Hosseini[48], where the addition of oil furthered the diameter fibers reduction, when compared to polymer without oil. This comportment could be explained by change of physical-chemical characteristics

Table 2. ATR-FTIR spectrum analysis for all samples[35,37,38].

Wave number (cm^{-1})	Attributed band	Reference
3006	=CH Stretching	Leonardi et al.[42]
2922	CH_2 Assymetric stretching	Leonardi et al.[42]
2853	CH_2 Symetric stretching	Leonardi et al. [42]
1743	C=O Aliphatic strecthing	Leonardi et al.[42]
1710	C=O Stretching	Leonardi et al.[42]
1650	C=C Stretching	Gomez et al.[45]
1464	CH_2 scissoring deformation	Leonardi et al.[42]
1417	In-plane deformation of C-O-H	Leonardi et al.[42]
1377	Symetric flexion of CH	Leonardi et al.[42]
1239	C-O Stretching	Leonardi et al.[42]
1161	C-O (esther) Stretching	Leonardi et al.[42], Ali et al.[44]
1117		
1095		
721	CH_2 Rocking deformation	Leonardi et al.[42], Ali et al.[44]

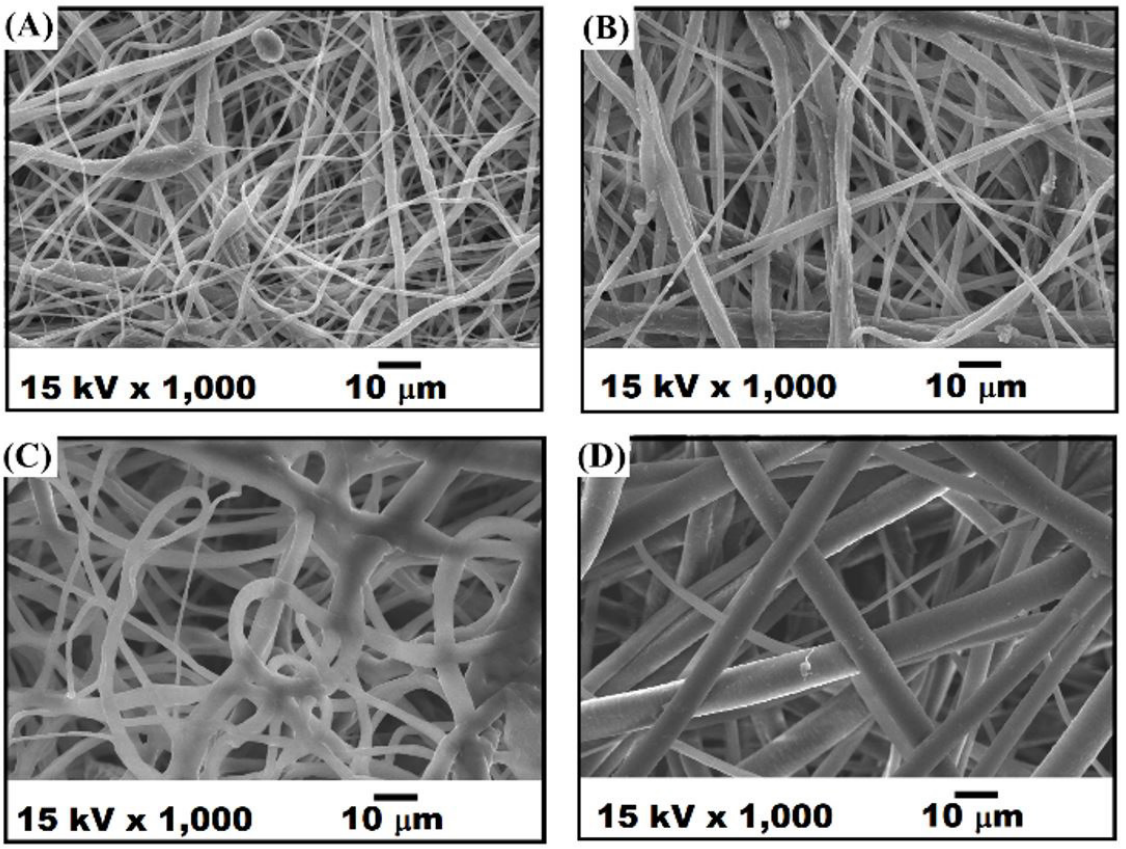
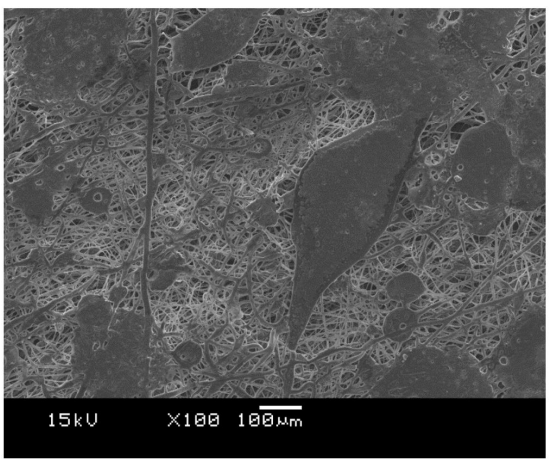


Figure 3. SEM of samples with different concentrations of oil and pure PCL, (A) 100 μg/mL, (B) 250 μg/mL, (C) 500 μg/mL and (D) PCL.

of the polymer solution by tucuma oil that modifying the chain entanglements, responsible for fiber formation[49]. Figure 4 shows the SEM image to PCL plus 500 μg/mL tucuma oil where can be see a region in the sample that polymeric fibers collapsed. This could be justified by the insufficient evaporation of solvent due the large quantity of tucuma oil[49]. This result could explain the increased of diameter fibers from samples loaded with more tucuma oil, where minus tucuma oil has finner fibers and great quantity of tucuma oil has thicker fibers (1.72 ± 0.90 μm to PCL plus 100 μg/mL and 2.9 ± 1.2 μm to PCL plus 500 μg/mL).

Thermogravimetric analysis (Figure 5) showed that the tucuma oil incorporation (100-500 μg/mL) in PCL leads to a higher thermal stability. Moreover, the events in the region around 330 °C in Figure 5B, Figure 5C and Figure 5D may be occurring together with another thermal event, resulting in a wide endothermic peak around 423 °C. To verify this, the DTG curve for sample PC500 (Figure 6) was analyzed by least squares fit of gaussian functions and deconvolution revealed two peaks (392 °C and 424 °C). This result is consistent with the other samples (PCL100 and PCL250), however, it is noted that the endothermic peaks were moved to higher temperatures. This is due to the greater amount of tucuma oil added to the PCL.



'Figure 4. SEM of PCL500 sample.

The in vitro effect of tucuma-loaded fiber scaffolds on PBMCs donated by volunteers was investigated by MTT (Figure 7). Test results showed no cytotoxicity behavior in any of the evaluated samples. Pure PCL nanofibers showed a cell viability around 95.7% against PBMC cells, however,

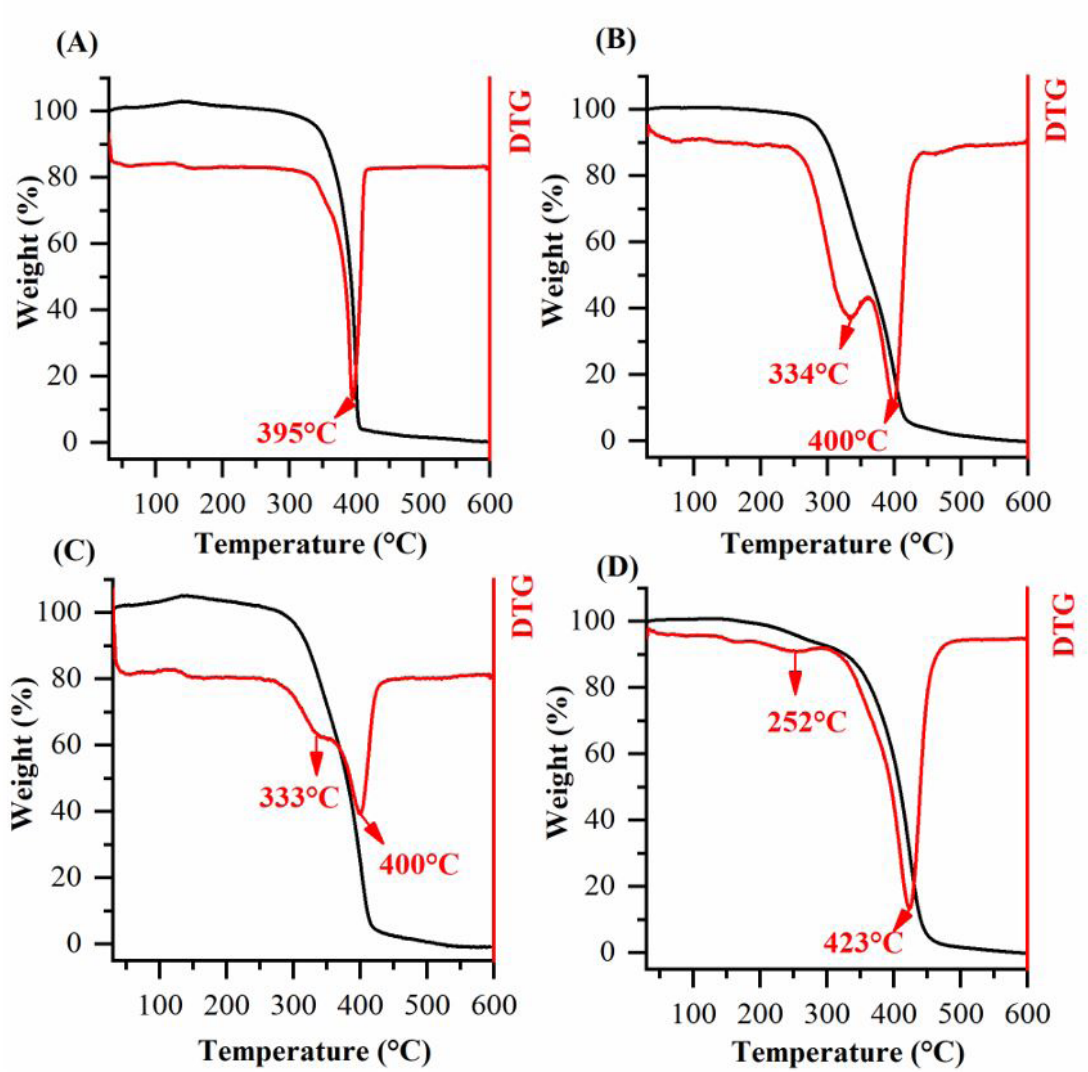


Figure 5. Termogravimetric analysis (A) PCL, (B) PCLT100, (C) PCLT250 and (D) PCLT500.

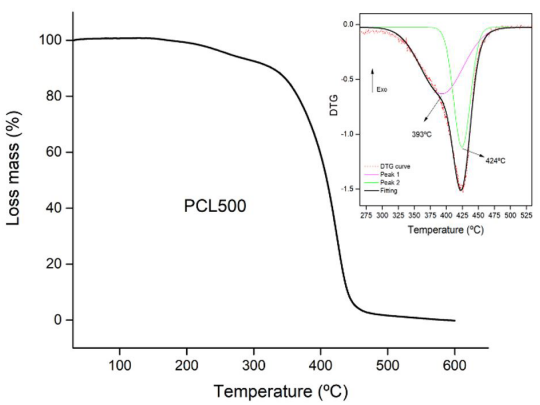


Figure 6. DTG curve for PCLT500 sample analyzed by least squares fit of gaussian functions.

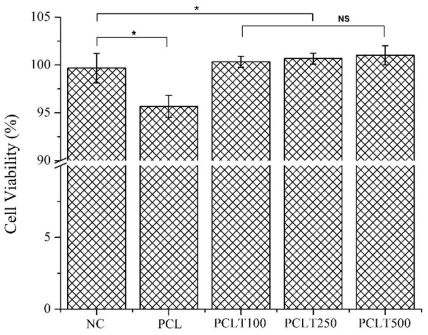


Figure 7. Cell viability assay of Peripheral Blood Monocytes (PBMC) Lymphocytes on PCL, 100, 250 and 500 µg/mL Tucuma-loaded PCL nanofibers after 24 hours ($p \leq 0.05$). Values are expressed as mean ± S.D. of three parallel measurements. (* - significant difference; NS - nonsignificant difference).

this result is significantly smaller when compared to negative control and to all tucuma-loaded PCL samples ($p \leq 0.05$). On the other hand, there was no significant difference in cell viability with all tucuma-loaded samples and negative control ($p \leq 0.05$). The results exhibited that tucuma oil could induce cell proliferation on PCL fiber mats. In agreement with the results obtained on this paper, Ongaratto et al.[50] studied the cellular viability of different tucuma extract concentrations (5, 10, 50, 100 e 500 μg/mL) on PBMCs. The viability of the cells seeded on the pure PCL fibers mats was significantly lower than that of cells cultured on the control well, this result can be credited to the PCL fibers hydrophobic nature[51]. The incorporation of tucuman oil into PCL fiber mats leaded to an improvement on cell viability, which suggests that it possess in its chemical composition molecules such as the β-carotene[52] that can regulate the expression of genes responsible for cell proliferation and differentiation by controlling ROS production and lipid peroxidation[53-55].

4. Conclusions

PCL fibers incorporated with tucuma oil were successfully electrospun and were evaluated with respect to their chemical, physical and morphological properties as well as cytotoxicity. The incorporation of tucuma oil did not affected the PCL chemical structure, which was confirmed by XRD and FTIR. Also, the SEM analysis confirmed the fibrous network of PCL and the addition of tucuma oil into this microstructure. In respect to the morphologic properties, it was noted that the mean diameter of PCL fibers decreased with the addition of tucuma oil. Besides that, the incorporation of tucuma oil into the PCL matrix led to a higher thermal stability compared to pristine PCL. Regarding cytotoxicity, the incorporation of tucuma oil showed enhanced biocompatibility, once it increased the cell viability evaluated by an MTT assay and did not present cytotoxicity against PBMCs. Thus, PCL can be electrospun with tucuma oil to achieve a fibrous biomaterial with increased biocompatibility and interesting physical and morphological properties, without altering the PCL chemical structure.

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