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Influence of the addition of β-TCP on the morphology, thermal properties and cell viability of poly (lactic acid) fibers obtained by electrospinning

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ABSTRACT

Electrospinning is a simple and low-cost way to fabricate fibers. Among the various polymers used in electrospinning process, the poly (lactic acid) (PLA) stands out due to its excellent biodegradability and biocompatibility. Calcium phosphate ceramics has been recognized as an attractive biomaterial because their chemical composition is similar to the mineral component of the hard tissue in the body. Furthermore, they are bioactive and osteoinductive and some are even quite biodegradable. The beta-tricalcium phosphate (β-TCP) particles were synthesized by solid state reaction. Different contents of β-TCP particles were incorporated in polymer matrices to form fibers of PLA/β-TCP composites by electrospinning aiming a possible application as a scaffold for tissue engineering. The fibers were characterized by scanning electron microscopy (SEM), infrared (FTIR), differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA). The average diameter of the fibers varied in the range of 260–519.6 ± 50 nm. The presence of β-TCP particles promoted changes on thermal properties of the fibers. The composite with 8 wt-% of β-TCP showed a low degree of crystallinity and can be used for application in tissue engineering. The cell viability was analyzed by reduction of the methyl tetrazolium salt by the pyruvate dehydrogenase enzymatic complex present in the matrix of mitochondria (MTT test). All PLA fiber groups, with different contents of β-TCP, showed cytocompatibility ability with non-cytotoxicity effect and bioactive properties using SBF assay.

1. Introduction

Tissue engineering has emerged as a tool for functional restoration of organ and tissue damaged or lost due to disease or trauma, and constitutes regeneration of organs and living tissue, through the selection of the patient's own tissue, which are dissociated and cultured cells on biological or synthetic media, known as scaffolds [1].

Among the biodegradable polymers used in the production of these scaffolds, stands out the synthetic aliphatic polyesters such as poly (lactic acid) (PLA), poly (glycolic acid) (PGA) and polycaprolactone (PCL) [2,3]. However, these polymers have some limitations such as low bioactivity, hydrophobic surface and long term degradation in vivo [4].

Among ceramic materials, calcium phosphate bioceramics are recognized as an attractive biomaterial because they have similar chemical composition to the mineral component of bone. Moreover, they possess bioactive, biodegradable and osteoinductive properties. Among the ceramics of calcium phosphate, beta-tricalcium phosphate (β-TCP) $[Ca_3(PO_4)]_2$ stands out for its osteoconductive activity [5].

In an attempt to overcome these limitations of the polymer, we search the incorporation of inorganic particles in the polymer matrix or coating of polymer matrix [6]. It is expected to obtain with the calcium phosphate incorporation into the polymer matrix an improvement in the hydrophobic characteristic of the polymer, as well as an improvement in their bioactive property.

The use of electrospun fibers in biomedical applications has increased of late because these fibers offer a range of attractive features such as large surface areas, high porosities and ease of incorporation of functional components (drug, gene, enzyme, etc.), making them ideal candidates for tissue engineering applications [6]. Electrospinning is regarded as a simple and versatile top-down approach for fabricating uniform ultra-fine fibers in a continuous process and at long length scales [7].

PLA fibers and its composites have been obtained successfully in the literature as shown by the works of Pirani et al. [8], Zhou et al. [9], Harrosh et al. [10], and Neto et al. [11]. Recently, Tammaro et al. [12] studied the fabrication and characterization of hybrid meshes based on poly (L-lactide-co-D,L-lactide) (DL-PLA) and β-tricalcium phosphate (β-TCP) by electrospinning. Their results demonstrated the both pure PLA and hybrid non-woven meshes exhibited a good thermal stability

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and a continuous degradation in simulated body fluid medium. The viability assay revealed an excellent cytocompatibility of the fabricated non-wovens and enhanced alkaline phosphatase (AP) activity of MC3T3-E1 cells. The composite non-woven meshes demonstrated their potential for applications in tissue engineering. In another study, Torres et al. [13] optimized the production of PLA ultrafine fibers by electrospinning incorporating low levels of hydroxyapatite (HA). As a result, the blend of PLA with HA caused a considerably enhanced cell proliferation.

Here, we generated electrospun fibers of neat PLA and PLA/β-TCP composites with different contents of β-TCP. The influence of the β-TCP particles on the morphological and thermal properties as well as cell viability was analyzed.

2. Materials and methods

2.1. Synthesis and characterization of β-TCP particles

The β-TCP powder was synthesized by solid state reaction of a stoichiometric mixture of $CaCO_3$ (Synth, Brazil) and $CaHPO_4$ (Synth, Brazil) at 1050 °C for 6 h [14]. The synthesized powder was ball milled during 48 h and then ground in a high energy ball mill (rotation 250 rpm, alumina balls with 2 mm of diameter) for 4 h. The resulting powder was analyzed by X-ray diffraction (XRD) to identify the crystalline phases and by laser diffraction (Cilas, model 1190 L) to determine the particle size. The XRD (PANalytical, model X'pert Powder) operated at 40 kV/25 mA (CuKα, λ = 0.154 nm). The scanning 2θ ranged between 10 and 50° with a step scanning of 0.02 °/s.

2.2. Solutions and electrospinning

PLA from NatureWorks LLC, named Ingeo™ biopolymer 2003D, with 4.30% of D-lactic acid monomer was first dissolved in chloroform [$CHCl_3$, 99%] (from Vetec), after its complete dissolution, dimethylformamide (DMF) [C_3H_7ON, 99.8%] (from Synth) was added, forming a 60/40 v/v solution [11]. The PLA final concentration in this solution was 0.09 g/ml. This sample was named neat PLA. To produce the fibers of PLA/β-TCP composites, the particles of β-TCP was first mixed with chloroform, under ultrasound dispersion during 30 min. The biodegradable polymer was then added to the β-TCP suspension and after complete dissolution, the DMF solvent was added. The same ratio of solvents was also obeyed for the preparation of solutions containing the β-TCP particles (60/40 v/v). The final suspension was maintained under agitation for at least 12 h until complete homogenization. The final concentration of β-TCP in the solutions was 0.01, 0.05 and 0.08 g/ml. The samples were named PLA/β-TCP 1%, PLA/β-TCP 5%, PLA/β-TCP 8%, according to the β-TCP content. For the assembly of the electrospinning were used: a source of high voltage (Bertan® 230R), a static collector, one attached to an insulating rod claw holding a syringe (BD Yale®) containing the polymer solution, the needle (Inbras® 0.8 mm diameter and 25 mm length) and a glass syringe with a volume of 20 ml with the plunger. The working distance was maintained in 10 cm of the collector and the applied voltage was 12 kV.

2.3. Fibers characterization

2.3.1. Morphological

The morphology of the fibers was characterized by scanning electron microscopy (SEM) (ZEISS, model EVO MA10). The mean diameter of the fibers was measured using the ImageJ 6.0 software. The data were obtained of 30 fibers and expressed as mean ± standard deviation.

2.3.2. Microstructure

Sample chemical composition was investigated using attenuated total reflection (ATR) with iS5-Nicolet Thermo FT-IR spectrometer that has a scanning range of 400 to 4000 cm^{-1} and resolution of 4 cm^{-1}.

Table 1
SBF (5×) components and concentration.

Reagent	Quantity (mM)
NaCl	733.5
$MgCl_2 \cdot 6H_2O$	7.5
$CaCl_2 \cdot 2H_2O$	12.5
$Na_2HPO_4 \cdot 2H_2O$	5.0
$NaHCO_3$	21.0

2.3.3. Thermal behavior

The thermal behavior of the samples was analyzed by differential scanning calorimetry (DSC) using TA instruments, model DSC Q10 and samples weighting between 5 and 10 mg. A first heating was done from 25 to 200 °C, at 10 °C/min. After complete melting, the samples were cooled down at 10 °C/min, under nitrogen atmosphere, the second heating was done at 10 °C/min from 25 to 200 °C to obtain the glass transition temperature of the samples.

In order to determine the degree of crystallinity (*Xc*) of the samples was necessary to deduct the fraction that crystallized during heating in the DSC. Therefore to calculate the *Xc* Eq. (1) was used:

$$Xc(\%) = \frac{\Delta Hm - \Delta Hcc}{\Delta Hm^{\infty}} \times 100. \quad (1)$$

Where: ΔHcc = cold crystallization enthalpy, ΔHm = melting enthalpy of the sample and ΔHm = melting enthalpy of a 100% crystalline sample. For the PLA samples, ΔHm was 93 J/g [15].

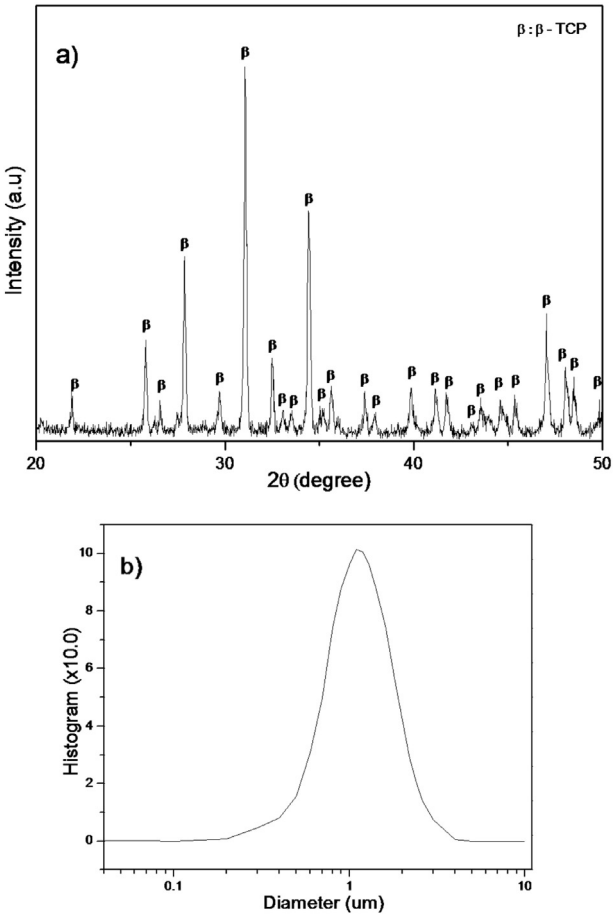

Fig. 1. Characterization of β-TCP powder synthesized by solid state reaction: a) XRD pattern and b) Granulometric distribution curve.