*Journal of
Nanoscience and Nanotechnology*
Vol. 13, 1–5, 2013

Novel Electrospun Nanotholits/PHB Scaffolds for Bone Tissue Regeneration

Lauro Xavier Filho[2], Gabriel Molina Olyveira[1,*], Pierre Basmaji[2],
and Ligia Maria Manzine Costa[1]

[1] *Department of Nanoscience and Advanced Materials-UFABC, Rua Santa Adélia, 166,
Santo André-SP, 09291-210, Brazil*
[2] *Innovatec's-Biotechnology Research and Development, São Carlos-SP, 13560-042, Brazil*

Nanotholits is an osteoinductor or be, stimulates the bone regeneration, enabling bigger migration of the cells for formation of the bone tissue regeneration mainly because nanotholits are rich in minerals considered essential to the bone mineralization process on a protein matrix (otolin) as hydroxiapatite. In order to improve its biodegrability and bioresorption in new platforms for tissue engineering, it was electrospun PHB/nanotholits from aqueous solutions of this polymer at concentrations of nanotholits 1% (w/v) and compared morphological and thermal properties with PHB/nanotholits casting films. Electrospun PHB/nanotholits mats presents more symmetric nanopore structure than casting films mats observed by SEM images mainly because the orientation of pores along the longitudinal direction of the electrospun fibers. Nanotholits influences in PHB electrospun/casting was analyzed using transmission infrared spectroscopy (FTIR). TGA showed similar thermal properties but DSC showed distinct thermal properties and crystallinity process of the developed bionanocomposite mainly because of different processing.

Keywords: Bionanocomposites, Electrospinning, Bone Tissue Regeneration.

RESEARCH ARTICLE

1. INTRODUCTION

The development of materials for tissue repair or replacement of lost organs or tissues has been encouraged by the progress of research in the field of biomaterials, contributing significantly to improving the quality and life expectancy of the population.[1] Currently, there is a significant number of metallic biomaterials, ceramics, polymers and composites, as options for use in various fields of medicine. Experience with biomaterials represent a major advance in implant, but still requires detailed studies of their bioactivity and biocompatibility in the human body.[2]

Rapid technological development has enabled great advances in this area, increasing the effectiveness of dental implants, representing an important role in rebuilding functional/aesthetic patient. Thus, the constant advancement of implant has given encouragement to the research of biomaterials for this purpose as well as the reactions that occur in tissue-implant interface.[3]

Despite many advances in tissue engineering (TE), scientists still face significant challenges in repairing or replacing soft tissues such as tendons,ligaments, skin, liver, nerve and cartilage to improve the quality of people life. Conventional therapeutic treatments targeted to reconstruct the injured tissues or organs have some limitations such as donor limitations and graft rejections.[4] When it comes to organ and tissue regeneration, it is fundamental to the study of behavior and cellular differentiation induced by the structure, composition and presence of biological elements of the media, to enhance the supports and advances in cell culture techniques, which might allow reproductive tissues and organs in all its complexity.[5]

In this context, our group has shown the ability to regenerate bone nanocomposites with otoliths. Nano-otholits is an osteoinductor or be, stimulates the bone regeneration, enabling bigger migration of the cells for formation of the bone tissue. Otoliths of Cynoscion acoupa are small particles, composed of a combination of a gelatinous matrix and calcium carbonate, present in the ear internal bony fishes and are part of a system which acts as a sensor of depth and balance, so as a detector of sound vibrations. The Cynoscion acoupa is commercialized in all the coast of Brazil. The Cynoscion acoupa of the fish demonstrated to be an important source of collagen too; the membranes of collagens can be gotten from the acid extraction and posterior saline precipitation. These features allow its

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[*] Author to whom correspondence should be addressed.

1533-4880/2013/13/001/005 doi:10.1166/jnn.2013.7191

applications in scaffold for tissue regeneration, medical applications and nanocomposites.[6] Olyveira et al.[7] reported the first otolith/collagen/bacterialcellulose nanocomposites as a scaffold for bone regeneration. The success of the subsequent transplantation of the engineered *in vitro* construction is due to the properties of the materials but also to the osteoprogenitor cell sources. In addition, Olyveira et al. reported that otoliths showed biocompatibility with pulp tissues *in vivo* and the response of pulp tissue was successful.[8] It can be concluded that direct pulp capping with the preparation of otoliths, similar to calcium hydroxide, preserves the vitality, stimulates the formation of mineralized tissue barrier and induces reparative pulp response. This biomaterial represents a promising biomaterial for use in human dental medicine in the future.

Recently, Olyveira et al.[9] obtained a new platform for catalysis, drug and cell delivery with electrospun bacterial cellulose. In this work, bacterial cellulose mats was acetylated and after processing by electrospinning to produce artificial symmetric nanoporous. However, one better knowledge in new platforms with biopolymeric surface changes is necessary for studies with scaffolds in tissue engineering, for example platforms polymer/biopolymer. One of the advantages of the e-spinning process over the conventional film-casting technique is the highly porous nature of the electrospun (e-spun) fiber mats which exhibit much greater surface area that assumingly could allow drug molecules to diffuse out from the matrix much more conveniently,[10] when these fibrous materials are used as carriers for delivery of drugs. E-spun CA fiber mats have been explored as affinity membranes,[11] antimicrobial membranes,[12] three-dimensional (3D) structures resembling the urinary bladder matrix (UBM),[13] and drug-delivery membranes.[14]

In this work, it was electrospun PHB with nanotholits from aqueous solutions of this polymer at concentrations of nanotholits 1% (w/v) and compared with PHB/nanotholits casting films.Nanotholits influences in morphology and thermal behavior was analyzed.

2. MATERIALS AND METHODS

2.1. Materials

Poly(3-hydroxybutyrate) (PHB; $\text{Mw} = 300,000$ g.mol^{-1}-Sigma Aldrich), chloroform (anhydrous, $\geq$ 98%-Synth-BR). Otoliths were supplied by VIAFARMA LTDA (Brazil).

2.2. Methods

PHB scaffolds were prepared with a proportion of 1% (w/w) of otholits. Solution was prepared at a concentration of 1% w/w of otholits in chloroform. The solution was stirred on magnetic stirrer at room temperature for 3 hours. After this period the solution was processed by electrospinning using voltage of the 20 Kw and 12 cm distance from the needle until collector. Nanofibers mats were collected in grounded metal collector and casting films were collected in petri disher.

2.3. Characterization

2.3.1. Scanning Electron Microscopy (*SEM*)

Scanning electronic microscopy images were performed on a PHILIPS XL30 FEG. The samples were covered with gold and silver paint for electrical contact and to perform the necessary images.

2.3.2. Transmission Infrared Spectroscopy (*FTIR, Perkin Elmer Spectrum* 1000)

Influences of otholits in PHB was analyzed in the range between 250 and 4000 cm^{-1} and with resolution of 2 cm^{-1} with samples.

2.3.3. Differential Scanning Calorimetry (*DSC*)

To analyze the crystalline and thermal behavior of the material after polymer processing, calorimetric experiments were carried out with the help of differential scanning calorimetry (DSC 822 Mettler Toledo, Switzerland). The samples were first cooled at −90 °C and heated until 250 °C at a rate of 10 °C/min, in nitrogen atmosphere.

2.3.4. Thermogravimetric Analysis (*TGA*)

Thermogravimetric analysis was carried out for bionanocomposite using a NETZSCH TG 209F1. The samples were heated from 25 °C to 800 °C, at 10 degree/min in inert (nitrogen) atmosphere. The weight of all specimens was maintained around 10 mg.

3. RESULTS

3.1. SEM

PHB/nanotholits mats were characterized by SEM. Figures 1(a) and (b) shows, as an example, SEM images of PHB membrane samples are shown in Figure 1. Pores uniformly distributed throughout the fibers can be observed with a low size dispersion and average size around 20–100 nm. As to electrospinning, previous studies have demonstrated that the deposition rate of fibers during electrospinning is in the order of several meters per second, the solution jet was elongated up in less than a second, and the elongation rate can reach up high velocity, which leads to a dramatic increase of the surface-area-to-volume ratio in milli-seconds. The orientation of pores along the longitudinal direction of the electrospun fibers is attributed to the rapid stretching effect during electrospinning.[15, 16]

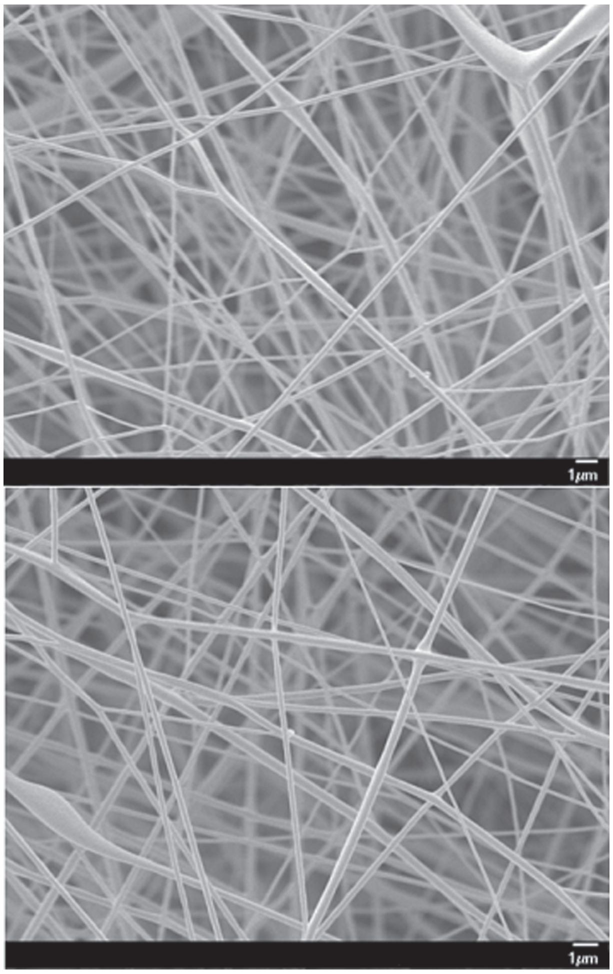

Fig. 1. Electrospinning PHB/otholits mats.

In Figures 1 and 2, it can be observed different sizes porous formation with electrospinning and casting polymers mats.

3.2. Interaction Between Nanotholits and PHB

Influence of nanotholits in PHB was analyzed in the range between 250 and 4000 cm^{-1} and with resolution of 2 cm^{-1} with FTIR analysis. The main features of the PHB in infrared spectroscopy are 1724 cm^{-1} (C=O) and a number of strong bands at wavenumber values between 1450–1000 cm^{-1} due to methyl (CH$_3$) and methylene (CH$_2$) deformations and C—O stretches; 2900 cm-1: CH stretching of alkane and It can be observed large CO$_2$ impurity in 2300 cm-1 at the time of measurement. ilustred in Figure 3 and 4. Besides in Figure 4 it can be observed otholits bands at 712 and 874 cm^{-1}; characteristic absorption bands of CaCO$_3$.[17,18] and some changes in these bands due desaglomeration of otholits in electrospinning process.

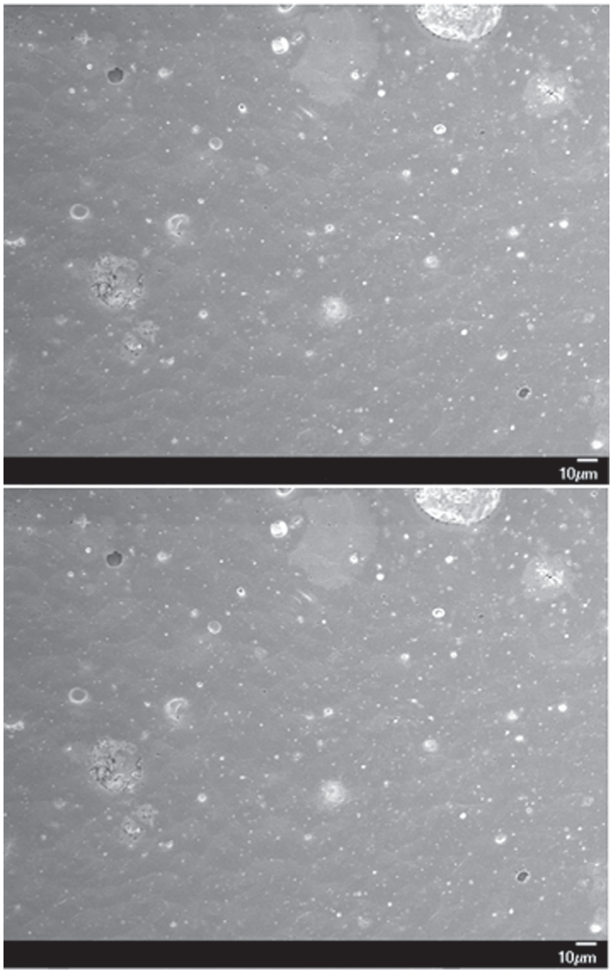

Fig. 2. Casting PHB/otholits mats.

3.3. Thermal Properties

3.3.1. *DSC*

The melting behavior and crystallization were carried out with electrospun mats and casting of PHB/otholits. It can

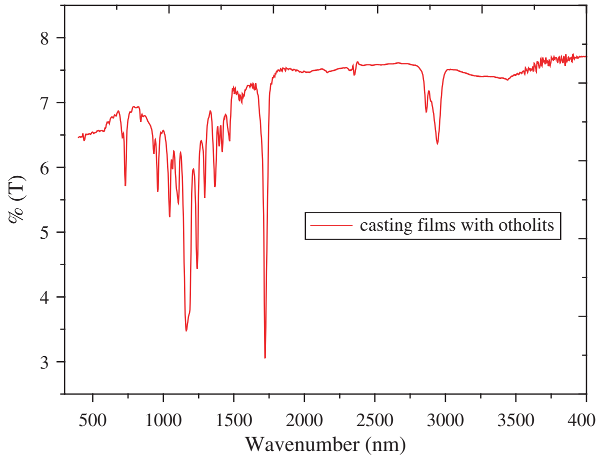

Fig. 3. FTIR of casting films with PHB/otoliths.

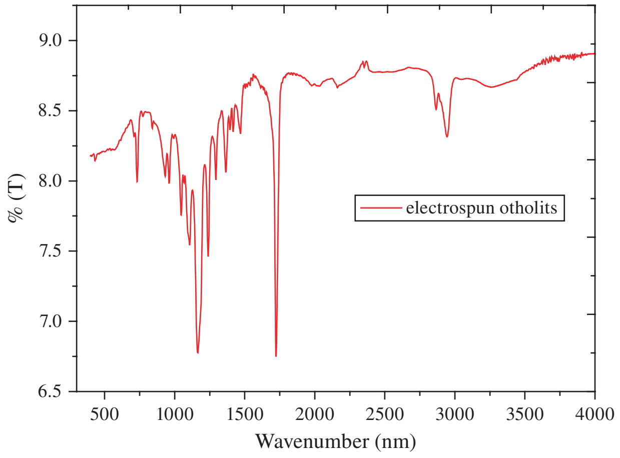

Fig. 4. FTIR of electrospinning films with PHB/otoliths.

be observed in Figure 5, fusion peak of PHB at 50 celsius degree and that otholits addition cause peak broading probably by the presence of crystals with different thicknesses and varying degrees of perfection because the addition of filler mainly in casting films than electrospinning films as comproved by peak broadening at 175 celsius degree. Besides, it can be observed in Figure 5 that system has fusion peaks (213 celsius degree) shifts to lower temperatures and with less crystals to merge, characteristic of a system with crystallinity process difficulty.

3.3.2. TGA

In order to analyze thermal behavior for composites are characterized typical weight loss versus temperature plots. The TGA and DTGA curves of samples are shown in Figures 6–7. In TG spectrum, it can be observed just one loss of weight peak, however, in DTG spectrum. It can be observed that some samples has more than one loss of weight peak. It is comproved similar thermal behavior with different polymer processing as observed Figures 6–7, with PHB degradation peak at 300 celsius degree in both

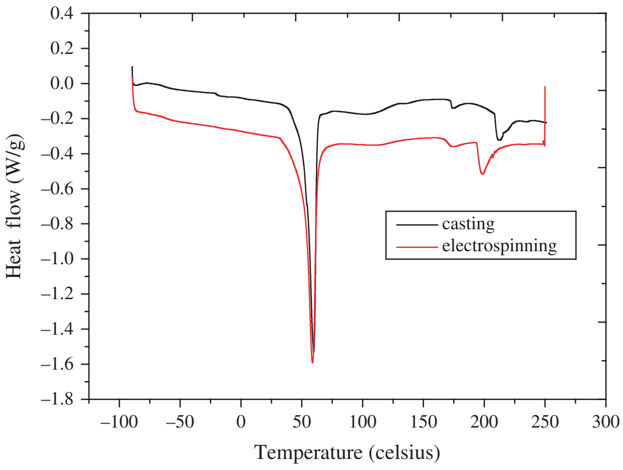

Fig. 5. DSC curves during heating with PHB/otoliths.

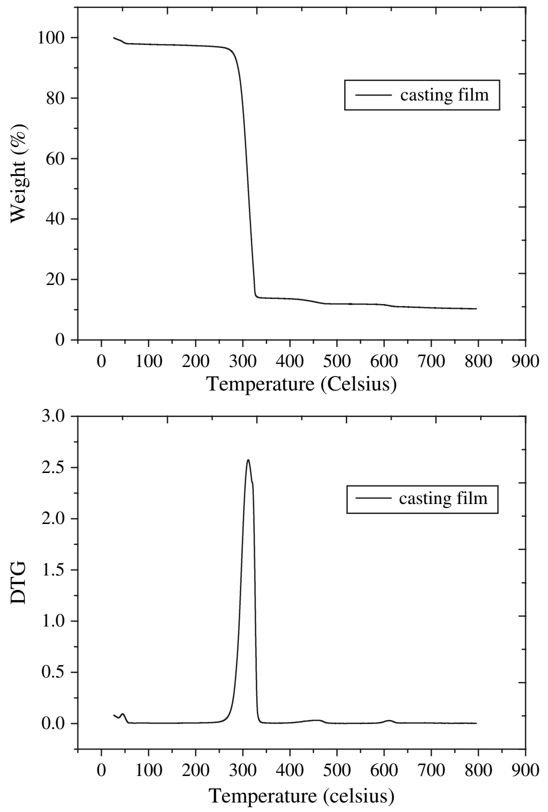

Fig. 6. TGA thermogram of casting films with PHB/otoliths.

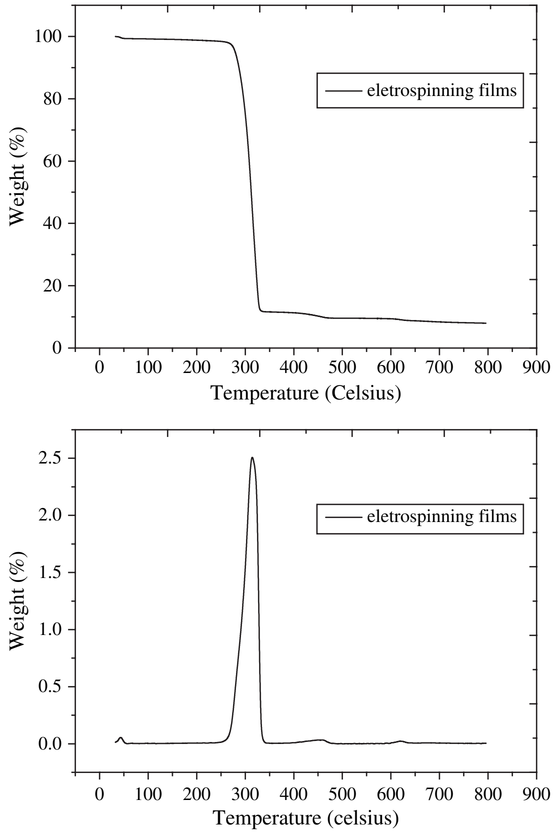

Fig. 7. TGA thermogram of electrospinning films with PHB/otoliths.

analyzed samples and no degradation peak for otholits because it has higher thermical behavior and decomposition at 900 celsius degree.[19]

4. CONCLUSION

PHB/otholits mats processed by casting and electrospinning techniques presents different morphological behavior. Electrospun sample presents more symmetric nanopore structure than casting films mats observed by SEM images mainly because the orientation of pores along the longitudinal direction of the electrospun fibers is attributed to the rapid stretching effect during electro-spinning. FTIR peaks support the hypothesis that electrospinning process makes desaglomeration of otholits and turn this system ideal mainly in tissue engineering because it increase total surface area of interaction between cells and membrane. Thermal properties showed that both systems has similar properties, with probably different crystalline strucuture in casting and electrospinning films.

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Received: xx Xxxx xxxx. Accepted: xx Xxxx xxxx.