FIBERS OF POLY (LACTIC-CO-GLYCOLIC ACID) / POLY (ISOPRENE) BLEND FOR APPLICATION IN TISSUE ENGINEERING

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Abstract: Tissue engineering can be easily described as the in vitro seeding and attachment of human cells onto a scaffold in order to develop new tissue. The application of polymers as scaffolds may bring interesting properties, such as bioresorbability, biocompatibility, mechanical behavior closer to that presented by the original tissue. Poly (Lactic-co-Glycolic Acid) (PLGA) and Poly(Isoprene) (PI) were blended and conformed as fibers for this purpose. Was observed that both blending and dripping techniques have no major influence over material's chemical structures, with no residual solvents presented. For the blend in this proportion, PLGA and PI showed a partial miscibility. In the biological analysis, the material presented an increase in population after 14 days, being recommendable a further investigations for its application in biomedical field.

Keywords: PLGA, PI, Fibers, Fibroblast

Introduction

Tissue engineering can be easily described as the in vitro seeding and attachment of human cells onto a scaffold in order to develop new tissue/organ substitutes for facilitating the restoration and maintenance of biological functions. These supports should possess interconnecting pores of appropriate scale to favor tissue integration and vascularisation, encourage cellular attachment, differentiation and proliferation through an appropriate surface chemistry, and be made from material with controlled biodegradability or bioresorbability so that tissue will eventually replace the scaffold [1,2].

It may be suggested that, for anchorage-dependent cells, material surface parameters such as topography may play a major role in the formation and maintenance of tissue integrity, as well as in the organization, regulation of cell motility, and control of a large variety of cellular activities. Cells respond to differences in these parameters by changes in orientation, rate of movement, growth and differentiation, activation, and modulation of gene expression [3]. Thus, the use of fibers as scaffold may be benefic for certain cell lines like skeletal muscles, that require a geometric oriented environment in order to grow into elongated cells [4].

The application of polymers as scaffolds may bring interesting properties, such as bioresorbability, biocompatibility, mechanical behavior closer to that presented by the original tissue. Poly (Lactic-co-Glycolic Acid) (PLGA) is one of the most common biodegradable polymers used in the medical field since the 70's. The blend with Poly (Isoprene) (PI) generate a material with high amount of energy expend in plastic deformation, what may be ideal for restoration of soft tissues [5]. However, to apply the blend as a biomaterial, viability cell tests must be performed to detect the occurrence of toxic effects and the biological behavior of the material on the cellular level.

Materials e methods

In order to obtain the blend, PLGA (Mn=250,000) and PI (*Mn*=295,000) were dissolved in Chloroform (99.8%) in a 60%ww PLGA to 40%ww PI proportion, followed by homogenization and drying. For chemical characterization by FTIR, the spectrum of the polymeric blend in the 4000-400cm^{-1} region was obtained using the Perkin Elmer model Spectrum 1000 FTIR at room temperature (25°C). In order to investigate the polymers miscibility, differential scanning calorimetry (DSC) was carried on TA Instruments QS, between -80°C and 150°C.

For in vitro analysis, the material was conformed into fiber by dripping method [6]. The PLGA/PI blend was diluted in chloroform, concentration 1%, and induced to 500ml ethyl alcohol (99.8%) in mechanical rotation, in a rate of exposure of 400ml/h, at 25ºC. After dripping process, the fibers formed were left to dry in lyophilizer Terroni Enterprise II for 24h, vaccum, at. -40ºC. The dried fibers were then mounted into coverlips, in cylindrical shape Ø10mm #10µm. Human dermal fibroblasts were cultured in DMEM containing 1000 mg.l^{-1} D -glucose and 10% Fetal Bovine Serum, in a concentration of 15 x10^4 cell/well. Population density over the blend disks (treated group) and glass disks (control group) were observed after 1, 3, 7 and 14 days incubation. Both groups had surface washed with phosphate buffered saline, cells fixed with p-formaldehyde 4%, and cells dyed in Toluidine Blue 1% for 1 minute. Observations were carried in Leica DMBR optical microscope, with cell counting proceeded and statistical analysis using ANOVA (α=0,05).

Results and discussion

From the analysis of previously known molecular structure, were found on the infrared spectrum bands relative to PLGA in the C=O and C-O bonds, as shown in fig. 1. Due to the structure of the PI, there was variation of intensity of the bands relative to the vibrations C=C e =CH. Pandey et al (2008) reported that the use of chloroform in PLLA (Polylactic acid) blends and PGA (Polyglycolic acid) showed significant variation in polymer structure, in relation to materials used. This variation also indicated the formation of mixtures in the molecules, turning the material similar to a copolymer and no longer a blend [7]. The absorption of aliphatic bonds C-Cl in chloroform is observed between 850-550cm^{-1}, however, when several chlorine atoms are bonded to the same carbon atom, as is the case with chloroform (trichloromethane), the bands are more intense and located at the extreme limit of the highest frequency of this range [8]. For the blend, bands were observed at 756cm^{-1}, but it can also be deduced that the signals with low intensity are relative to the -C-H vibrations, given that this grouping is found in both polymer chains [9].

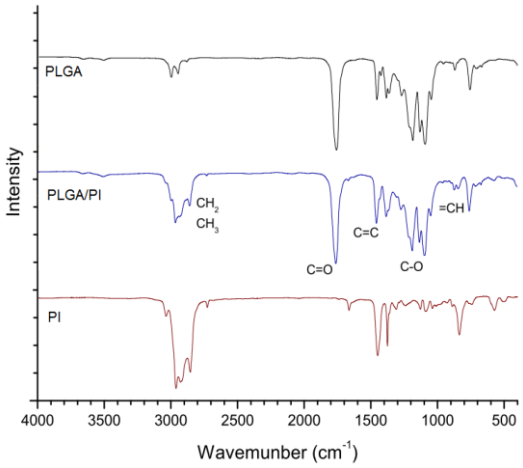

Figure1: FTIR spectra.

Through DSC analysis is possible to see (Fig. 2) that the glass transition temperatures (Tg) relative to raw PLGA and PI are 58,88°C and -65,74°C, respectively. For the blend, the Tg related with PI shows an expressive deviation, from approximately -66°C to around 25°C. Deviation also observed for the PLGA Tg, from 58,88°C to 48,95°C. This approximation of glass transition temperatures indicates a partial miscibility between the polymers [10,11].

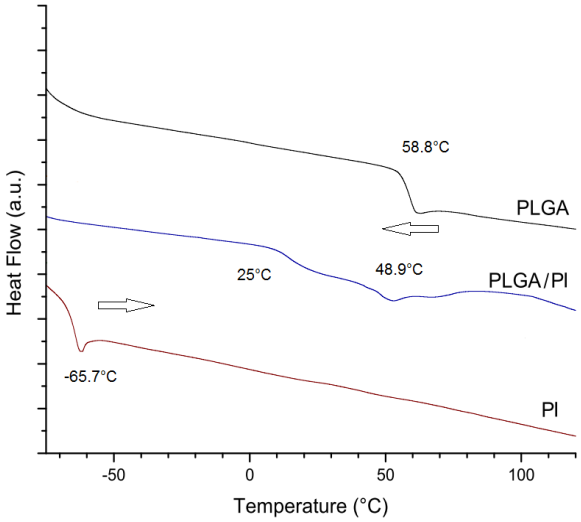

Figure 2: DSC thermogram.

The microscopic images shown in Fig. 3 were submitted to analysis and cell counting, generating the chart exposed in Fig. 4.

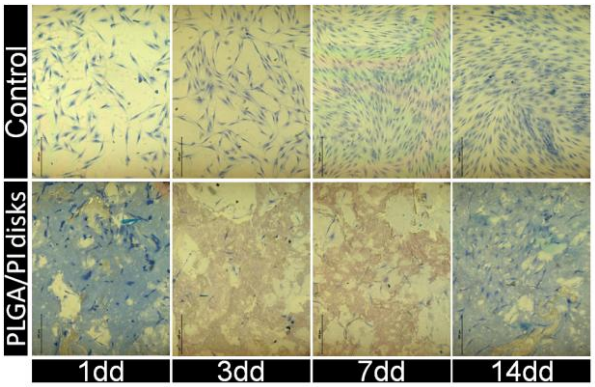

Figure 3: Microscopy of Human Dermal Fibroblasts

The cell counting showed that the population over the material is significantly smaller than the population presented in the control group for all the periods. However, while the control group showed a population peak at 7 days, the treated group showed a bigger population after 14 days, with significant growth when compared to day 1. Therefore can be assumed that the material may be tolerable for application as biomaterial in environments that don't require the material to be a growth factor for the cells, i.e., anti-adhesion membranes, bulks and fillings.

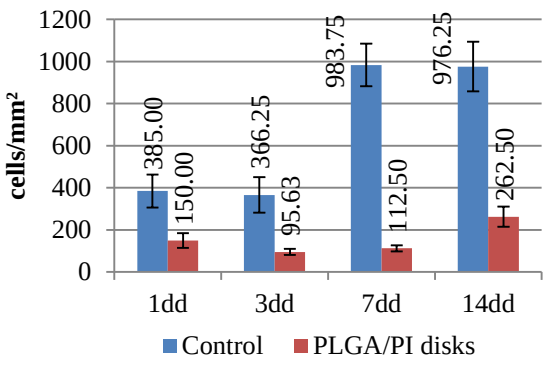

Figure 4: Cell population counting.

Conclusions

Was observed that both blending and dripping techniques have no major influence over material's chemical structures, with no residual solvents presented. For the blend in this proportion, PLGA and PI showed a partial miscibility. In the biological analysis, the material presented an increase in population after 14 days, being recommendable a further investigations for cell culture behavior and hypothetical biomedical applications.

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