Characterization of resorbable membranes based on polymer-ceramic composite (PLLA-co-PCL/PEG/β-TCP) for bone tissue engineering

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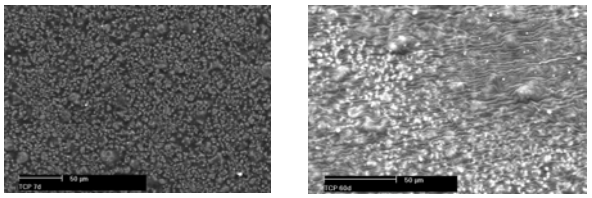
Introduction: The production of the composite (PLLA-co-PCL/PEG/β-TCP), poly (acid lactic-co-caprolactone) /poly(ethylene glycol)/β-tricalcium phosphate based porous membranes is specially important because this composite has characteristics that are suitable for guided bone regeneration (GBR)[1]. The bioresorbability of the ceramic (β-TCP) and of the polymer (PLLA) at physiologic pH allows its use as scaffolds in tissue engineering and in drug delivery systems [1]. This characteristic, along with biocompatibility and mechanical properties confer on this composite a specific biological reactivity that positively influences cell penetration, anchorage, differentiation and proliferation [2]. The aim of this investigation was to evaluate *in vitro* reactivity by exposing it to simulated body fluid (SBF), and to investigate their degradation/precipitation behavior and compatibility by cell culture (fibroblasts).

Materials and Methods: The polymeric phase of the membranes was synthesized according to previous study [3] and the phase ceramic used was β-TCP (Fluka, 21218). The process to obtain the composite, polymer (80wt. %)/ceramic (20wt. %) is described elsewhere [3]. The *in vitro* reactivity was carried out by soaking the samples in SBF solution maintained at 37°C in stirred at 40 rpm for a maximum period of 60 days. The initial pH was 7,25. The solutions were renewed every week. The surfaces of the samples, before and after immersion in SBF, were examined in a scanning electron microscope (SEM, Philips-XL 30) and Fourier Transform Infrared Spectroscope (FTIR, Perquin Elmer Spectrum GX). The pH of the solutions was monitored periodically. The *in vitro* biocompatibility was determined by culturing fibroblasts on the membranes. A cell line, FMM1 was used. Cells were cultured in Dulbecco's modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution. The cells were incubated at 37◦C in humidified 5% CO_2 and 95% air atmosphere. The surfaces, cross-sections and biocompatibility were examined using a scanning electron microscope (SEM-EDS).

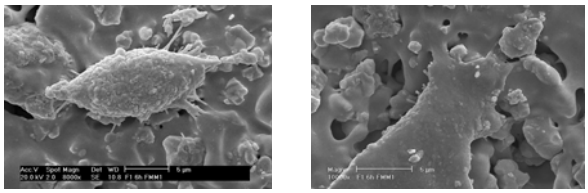
Results and Discussion: The surfaces of the membranes were examined in a SEM, before and after *in vitro* degradation and cell culture experiments. The migration of Ca and P ions of the ceramic phase, that constitute the membrane, created channels that enabled liquid penetration and promoted nucleation in SBF and accelerated polymeric degradation[4]. The SEM-EDS analysis revealed that the dissolution and precipitation process resulted in calcium phosphate globule formation. Micrographs of composite surfaces that were soaking in SBF for different duration are shown in Figure 1.



(a) (b)

Figure1- SEM micrographs of membranes surfaces after exposure to SBF for: (a) 7 days and (b) 60 days.

The dissolution behavior of the ceramic can be influenced by degradable and hydrophilic characteristics of the polymers. The affinity of the surface by liquids accelerated diffusion of ions and eased bond breakage of polymeric chains. Fractures were observed on the surfaces of samples after SBF experiments (Fig.1-b), due probably to continuous degradation process.



(a) (b)

Figure 2 - SEM of the FMM1 cell on the surface of the membrane (a) adherence of fibroblasts; (b) after 6 hours of plating.

Adherence assay of fibroblasts in culture (FMM1) on PLLA-co-PCL/PEG/β-TCP membrane indicate biocompatibility of the composite. This characteristic indicates that the procedure used to obtain it and the morphology of the membranes are well suited to cell adhesion.

Conclusions: The results of this study demonstrate that characteristics such as biocompatible composition, resorbable nature, morphology and hydrophilic characteristic of the membrane surface make this composite a candidate for use as a biomaterial in bone tissue engineering.

References:

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