Development of porous composite membrane of PLLA/PEG/HA for tissue engineering

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Composites membranes of poly(lactic acid)/poly(ethylene glycol)/hydroxyapatite (PLLA/PEG/HA), presenting uniform porosity distribution and characteristics appropriate for guided bone regeneration (GBR), were prepared in this work. The appropriate combination among polymer components (PLLA:PEG-1000, 2:3) and a biocompatible reinforcing ceramic filler (HA, 20 wt%), resulted in the development of composite devices presenting new mechanical and biological characteristics, ideal for bone matrix substitution, which allows cell growth. The membranes were prepared with a uniform porous (4 m) structure suitable for cells penetration, anchorage, differentiation and proliferation. The homogeneous porosity in membranes was achieved by self-organization during solvent evaporation process, due to phase-separated structure of the composites. The in vitro culture of osteoblasts on PLLA/PEG/HA membranes indicated good biocompatibility.

Introduction

Tissue engineering is an emerging field that provides an alternative solution to the treatment of diseases. With the development of guided bone regeneration (GBR), barrier membranes have been used for bone regeneration in osseous defects. Non-resorbable barriers were soon challenged by resorbable materials to avoid secondary surgery for removal. During recent years, several degradable polymer devices have been developed and employed clinically [1,2]. However, when compared with non-resorbable expanded poly(tetra-fluoroethylene) (ePTFE) membranes as standards, polymer devices have nearly exclusively fallen short of the ePTFE with respect to bone formation [3,4].

These membranes safeguard the injured area from invasion of conjunctive epithelium tissues, thus prioritizing the bone cell growth[5,6], and, addicionally, protect and preserve the bloodstream in the region of the wound stimulating the bone repair process[7]. Among the most important desirable characteristics of membranes, for bone tissue engineering, biocompatibility, osteoconductivity and absorption time compatible with bone regeneration are cited as fundamentals. However, although collagen membranes have an excellent biocompatibility, they carry the risk of bovine spongiform encephalopathy transmission [7]. Moreover, pure collagen membranes are not osteoinductors and are quickly resorbed, threatening the barrier function.

In this work we describe the synthesis and characterization of hydrogel/hydroxyapatite composite prepared from a polymer blend of PLLA/PEG-1000 (at different proportions) and hydroxyapatyite (HA) (20wt%) should have potential applications in tissue engineering. We hypothesize that a porous composite membrane composed by a resorbable polymer, a hydrophilic polymer and a osteoconductive ceramic (20% w/w) is a promising biomaterial to mimic the physicochemical and biological activities of the bone regeneration[5].

Experimental

The membranes were prepared by casting of chloroform dispersions of polymers (PLLA and different concentrations of PEG) and HA upon a glass plate. Each dispersion was dried under hood, followed by vacuum oven drying for 24 hours. The biocompatibility was determined *in vitro*, by culturing fibroblasts on the membranes. A FMM1 cell line was used, and prior to seeding, cells were cultured in Dulbecco's modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum and antibiotic and antimycotic 1% solution, at 37 ◦C under humidified atmosphere, 5% CO_2 and 95% air. Surfaces, cross-sections and biocompatibility were examined using a scanning electron microscope (SEM) (Phillips XL30). Mechanical properties were evaluated by Dynamic Mechanical Thermal Analyzer DMTA V (Rheometrics Scientific).

Results and Discussion

In Figure 1, the SEM shows a homogeneous porosity for the PLLA/PEG/HA membrane, with diameters of pores around 4 m, which is in the range appropriate for cells fixation and growth [7].

The DMTA analyses of the PLLA/PEG/HA (d_1) dry membrane, showed a storage modulus of 82.20 MPa (25 C), higher than that observed for dry commercial porous collagen membrane, 0.88 MPa (25 C) (Figure 2). At physiological temperature (37 ºC), the storage modulus were 52.00 MPa and 1.23

MPa for the PLLA/PEG/HA and collagen membranes, respectively.

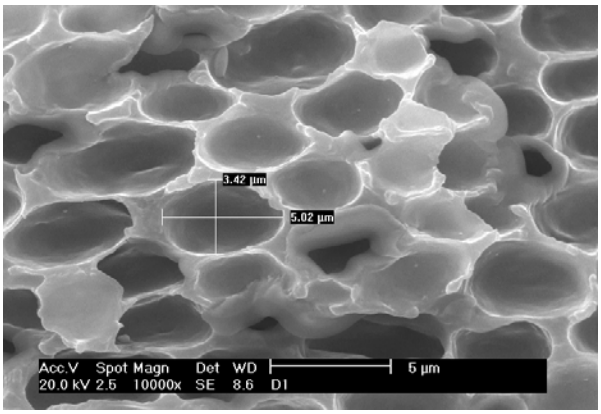

Figure 1. Transversal area of dry PLLA/PEG/HA membrane presenting uniform pore sizes.

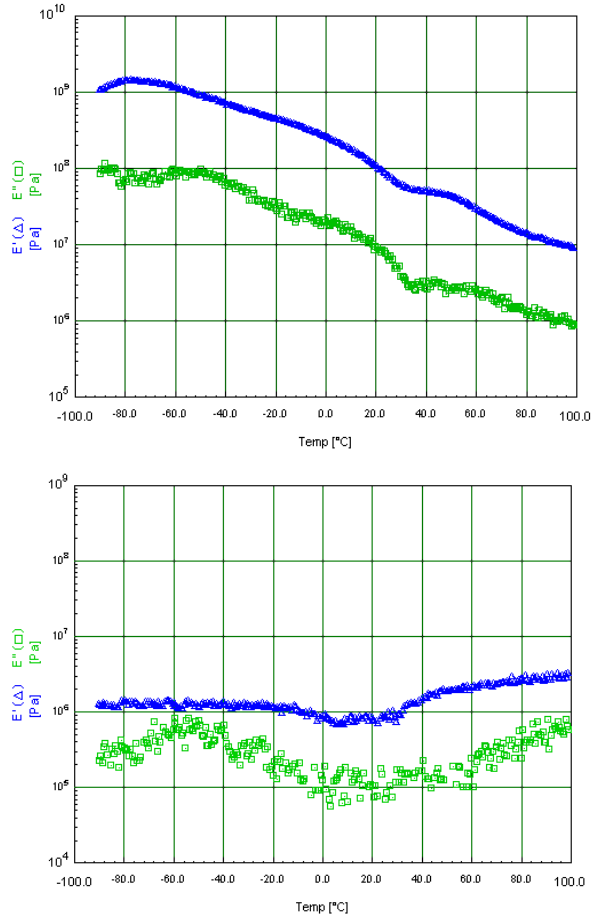

Figure 2 - DMTA curves of commercial collagen (bottom) and PLLA/PEG/HA dry membranes (top).

The PLLA/PEG/HA membrane showed an abrupt reduction in its storage modulus in the temperature range 25-37 C, while the storage modulus of the collagen membrane was practically constant.

The porosity degree in membranes affects its storage modulus E' magnitude. The uniform porosity and the thin walls observed by SEM, along with the ability of semicrystalline polymer chains to reorganize by cold crystallization explain its fragile mechanical behavior after seven weeks storage.

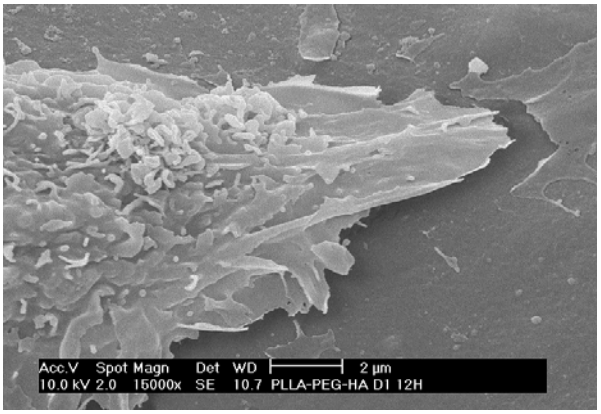

Figure 3.- Scanning electron micrograph of a FMM1 cell on the top of a PLLA/PEG/HA membrane (12 hours after seeding).

SEM of membranes after different culturing time revealed adherence of fibroblasts (FMM1) on PLLA/PEG/HA membrane, indicating its biocompatible character (Figure 3). For comparison, commercial collagen membranes were also seeded in a parallel experimental set, and demonstrated, as expected, good biocompatibility (Figure 4). The observed *filipodias* demonstrates adhesion on both types of membranes.

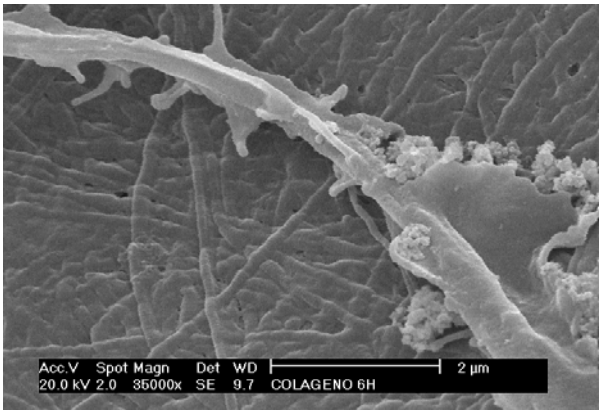

Figure 4 - Fibroblast on the top of a commercial collagen membrane (6 hours after seeding). Filipodias are fixed on membrane.

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