Preparation and characterization of resorbable hydrogels membranes used for bone tissue engineering

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New composites membranes of poly(acid lactic)/poly(ethylene glycol)/hydroxyapatite (PLLA/PEG/HA), drawn with characteristics appropriate for guided bone regeneration (GBR), were prepared by casting from chloroform solutions. The combination of the components, PLLA (resorbable polymer), PEG (polymer with affinity to the biological fluids) and HA (ceramic with good mechanical and biological properties), confers to the membranes new mechanical and biological characteristics, ideal for mimicking the bone matrix and encouraging the cell growth. The obtained PLLA/PEG/HA membranes showed a porous morphology, similar to the bone matrix, suitable for cells penetration, anchorage, differentiation and proliferation. Adherence of cultured fibroblasts on PLLA/PEG/HA membranes indicated biocompatibility.

Introduction

Guided bone regeneration (GBR), sub-area of tissue engineering, is a well-established therapy to repair bone defects. This therapy praises the use of a membrane as physical barrier to re-cover the zone of bone defect. These membranes protect the injured area from invasion of connective and epithelial tissues, thus allowing the bone cell regeneration in this area. [1, 2] These membranes also act protecting and preserving the blood clot also essential for bone process repair. [3]

Non-degradable membranes, as expanded polytetrafluoroethylene (e-PTFE) and polystyrene, have been used in GBR. However these materials require removal in a secondary operation after bone regeneration. To avoid this problem, membranes from biodegradable materials, for example collagen and synthetic biodegradable polymers, have been investigated. [4]

Amongst the diverse desirable characteristics of the membranes for bone tissue engineering and GBR, biocompatibility; osteoinduction (anchorage of cells and differentiation in osteoblasts), time of absorption compatible with bone regeneration are cited as fundamentals. A sole material cannot supply the bone regeneration process with all these characteristics. For example, collagen membranes have an excellent biocompatibility; however, these membranes have an antigenic effect and carry the risk of bovine spongiform encephalopathy transmission. Moreover, pure collagen membranes are not osteoinductors and are quickly resorbed, jeopardizing the barrier function[5].

Taking into account these difficulties, in this work, we describe the synthesis and characterization of hydrogel/hydroxyapatite composites with potential application for bone tissue engineering, especially for GBR. We hypothesize that a composite membrane composed by a resorbable polymer, a hydrophilic polymer and an osteoconductive ceramic would be the ideal biomaterial for mimicking the physicochemical and biological activities of the bone matrix and the bone cell growth.

Experimental

The membrane composites were prepared by casting of a chloroform dispersion of polymer (PLA or PLA-co-PCL/PEG) and hydroxiapatite on a glass plate. This dispersion was dried under hood and then in a vacuum oven for 24 hours. The biocompatibility was determined *in vitro,* by culturing fibroblasts on the membranes. A FMM1 cell line was used, which was established from a human gingival fragment and obtained after a gingivectomy for prosthetic reason. Cells were cultured in Dulbecco's modified Eagle medium (DMEM, Sigma Chemical Co), supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution. Cells were incubated at 37 ◦C in humidified, 5% CO_2 and 95% air atmosphere. [6] Scanning Electron Microscopy (SEM) was used for examining the morphology (porosity) of membranes (PLLA/PEG/HA) and the cells growth.

Results and Discussion

The SEM pictures (Figure 1) show an optimal and homogeneous porosity of the barrier membrane (PLLA/PEG/HA) in its whole extension, with pores diameter of 8.5 μm, essential for nutrient supply and surface cell adaptation [6]

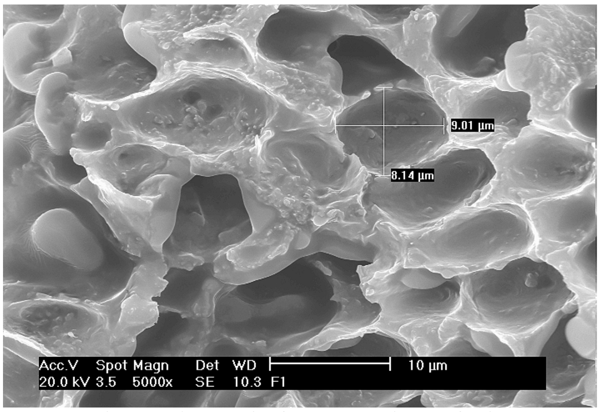

Figure 1. Electronmicrograph of a transversal area of a dry PLLA/PEG/HA membrane.

The DMTA analysis of the a dry PLLA/PEG/HA membrane, after two weeks, showed a storage modulus of 77.80 MPa (25 °C), higher than that observed for the commercial porous collagen membrane of 0.88 MPa (25 °C) (Figure 2). At physiological temperature (37 °C) the storage modulus for collagen and PLLA/PEG/HA membranes were 1.23 and 3.31 MPa, respectively. However, in general the mechanical characteristics of both membranes were similar.

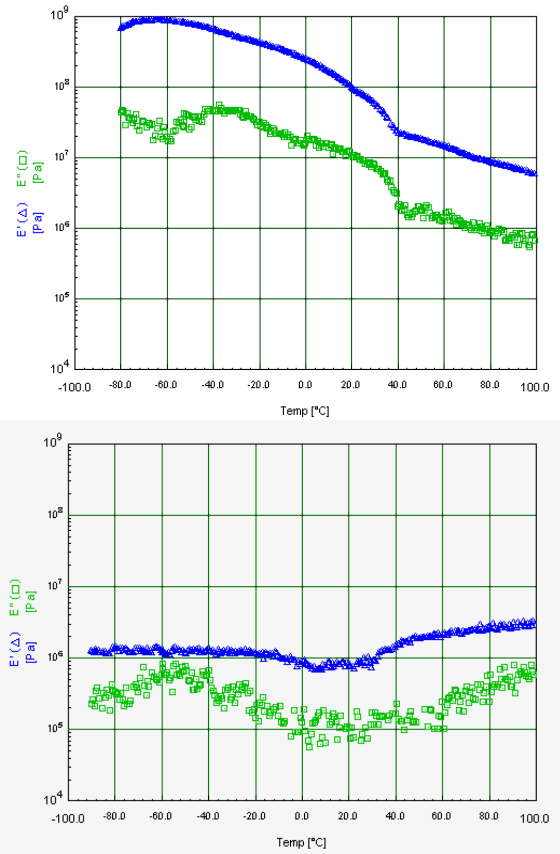

Figure 2. DMTA curves - Storage and loss modulus in function of temperature for a dry commercial collagen (bottom) and a dry PLLA/PEG/HA (top) membranes.

The porosity degree of membrane affected the magnitude of storage modulus of materials (collagen and PLLA/PEG/HA dry membranes). The high porosity along with the incorporation of ceramics and cold crystallization of PLLA, confers the membrane, with elapsing of the time (seven to nine weeks), a fragile behavior.

SEM analysis also allowed the observation of the adherence of fibroblasts on the membranes (PLLA/PEG/HA and collagen), demonstrating biocompatible characters of both membranes (Figure 3).

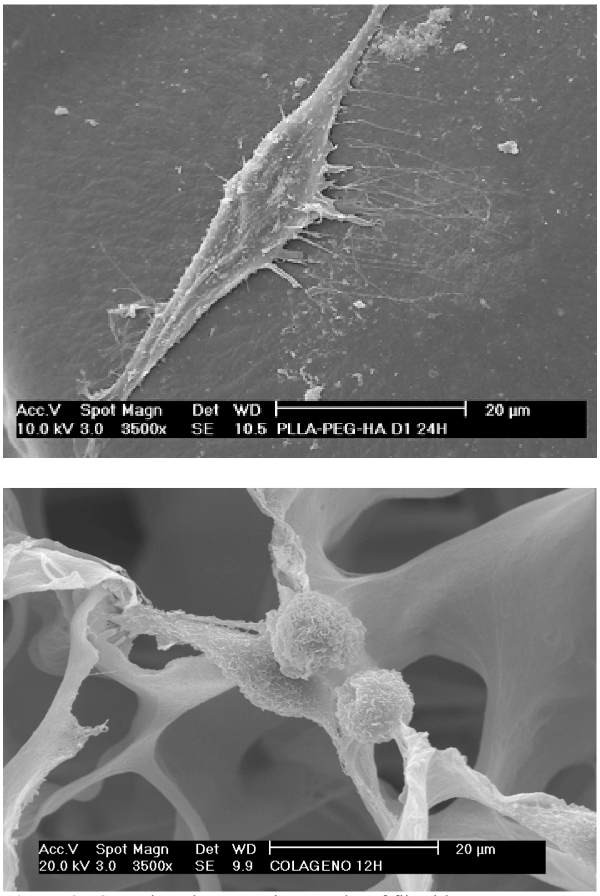

Figure 3 - Scanning electron micrographs of fibroblasts on PLLA/PEG/HA (top) and commercial collagen (bottom) dry membranes.

References

1. N. Claffey, S. Motsinger, J. Ambruster, J. Egelberg *J. Clin. Periodonto.* 1989, *16,* 12;
2. G. Greenstein, J. Caton *J. Periodontol 2000* 1993, *1,* 36;
3. S. Pitaru, H. Tai, M. Soldinger, O. Azar-Avidam, M. Noff *J. Periodontol Res.* 1987, 22 331;
4. I. Magnusson, C. Batich, B. Collins *J. Periodontol* 1988, 59, 1;
5. A. Linde, C. Thoren, C. Dahlin, E. *Sandberg J. Oral Max. Surg.* 1993, 51, 897;
6. T. Sigurdsson, R. Hardwick, G. Bogle, U. Wikesjo *J. Periodontol* 1994, 65, 356.