Synthesis, characterization and biocompatibility of resorbable osteomimetic membranes of PLLA-co-PCL/PEG/HA-βTCP for bone tissue engineering

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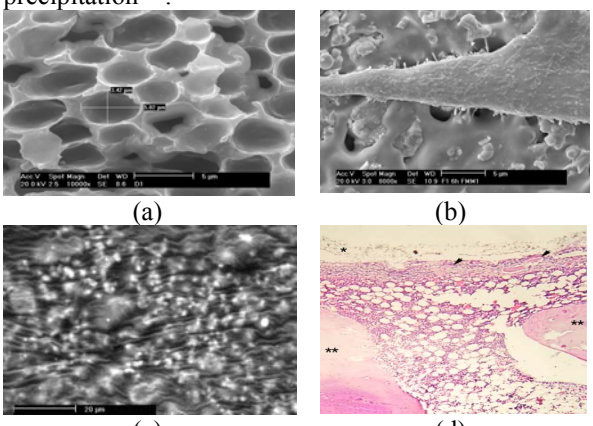
Introduction: In recent years several degradable polymer devices have been developed and used in the medical and odontological fields [1,2]. With the development of guided bone regeneration (GBR), barrier systems based on membranes have been used for bone regeneration in osseous defects. Non-resorbable barriers are being substituted by resorbable materials to avoid secondary surgery for their removal. Diverse characteristics are desirable in membranes while obtaining them for bone tissue engineering and include: biocompatibility, osteoconduction and time of absorption compatible with bone regeneration [3]. This study reports the synthesis and characterization of the composite PLLA-co-PCL/PEG/HA-βTCP based on porous membranes with potential application for tissue engineering and the results of *in vivo* and *in vitro* biocompatibility tests. A composite consisting of a resorbable polymer, another hydrophilic polymer and an osteoconductive ceramic can be an ideal biomaterial for mimicking the physico-chemical and biological activities of bone regeneration.

Materials and Methods: The composite was prepared by casting a chloroform dispersion of polymer and ceramic upon glass plate. The components of the membranes were: PLLA-co-PCL (40 wt.%), PEG-1000 (60 wt.%) and HA/βTCP 1:1 (20 wt.%). Biocompatibility was determined *in vitro,* 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. 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). The *in vitro* reactivity and degradation was carried out by soaking the samples in SBF solution kept at 37°C in stirred at 40 rpm for a maximum of 60 days and with an initial pH of 7,25. The solutions were renewed every week. After these periods the surfaces were examined in a SEM-EDS. Male New Zealand white rabbits (3–3.5 kg) were appropriately housed. Six animals were used in each study group. A 2.8 mm diameter hole was made in the medial lower right tibia along the axis of the bone with a saline-cooled drill. The hole was covered with a resorbable osteomimetic membrane PLLA-co-PCL/PEG/HA-βTCP. A lateral hole without the membrane was performed as a control and different time periods up to 1, 2, 3, 4, 6 and 8 weeks were used in this biocompatibility study. Histological analyses were carried out in hematoxylin-eosin paraffin-embedded sectioned tissues.

Results and Discussion: The micrographs (Fig.1-a) reveals homogeneous porosity in the membrane. The pore dimensions are close to 4 μm and these can facilitate ionic migration that happens initially between the surface of the membrane and blood plasma, influencing favorably the cell absorption dynamics [4]. These pores are also important in terms of capillarity and permeability of fluids, which could contribute to dissolution of the resorbable ceramic phase during degradation of polymeric matrix. Adherence assay of fibroblasts in culture (FMM1) on PLLA-co-PCL/PEG/HA-β-TCP membrane indicated a biocompatibility of the membrane (Fig.1-b). The calcium phosphate globules, confirmed by EDS, on surface of the membrane (Fig.1-c) can eased the osteoconduction process. The degradation of the membrane due to the presence of the β-TCP, promotes the supersaturation of the SBF solution and subsequent precipitation [4].



(a) (b) (c) (d)

Figure 1- SEM micrographs of membranes; (a) morphology of a transversal section; (b) FMM1 cell on surface 6 hours after plating; (c) surface after 60 days in SBF; (d) Hematoxylin-eosin, decalcified sections; original magnification X40 at 2 weeks; bone membrane (*), Cortical bone (**), newly formed bone (arrows).

Conclusions: The results indicate that the membranes have morphology and porosity that are suitable for cell penetration, anchorage, differentiation and proliferation. The degradation property of the membrane was compatible with cell adsorption process. The *in vitro* and *in vivo* assay indicate biocompatibility of this composite material.

References

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