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COMPOSITE GRAFT USING CHITOSAN, HYDROXYAPATITE AND BONE MARROW FOR BONE REGENERATION IN A RABBIT MODEL.

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Introduction: Bone grafting is a surgical procedure by which new bone or a replacement material is placed into spaces between or around broken bone (fractures) or holes in bone (defects) to aid in healing. Bone grafts are required to comply with certain criteria of biocompatibility. Among them it is important to mention, favoring neovascularization, stimulating neo-bone formation and avoiding host excessive immune response [1]. It is also favorable that the graft could be easily manufactured, in large quantities and at low cost [2,3].

Objective: The present study was intended to design and manufacture a new multilayered flexible porous bioceramics using chitosan and hydroxyapatite. This graft, together with the use of cells and growth factors obtained from the patient`s own bone marrow, would allow the development of a new biocompatible bone implantable material.

Materials and Methods: Chitosan multilayered graft material (polysaccharide), derivatized with phosphate groups and mineralized with calcium phosphate (hydroxyapatite). 10 adult male New Zealand White Rabbit, between 2 and 3 months old, 3 -3.5 kg in weight, divided in 5 groups of 2 individuals were used. The animals were then anesthetized via isoflurane inhalation and operated under sterile conditions. A 4.5-mm diameter drill bit was used to perform a 1cm deep hole in each tibia, and then these holes were filled with the multilayered chitosan graft pre-incubated for 2 minutes with the rabbit's own bone marrow aspirate. Tibias were subject to a seriated X-ray imaging (Fig.1) and postmortem histopathological studies to assess the *in vivo* biocompatibility at 1, 2, 4, 8 and 16 weeks of study.

Results: We obtained a multilayered implantable porous graft with hydratation of only 25%, flexible, resorbable, with a direct structural and functional connection between the host's bone and the surface of the graft and also growth of bony tissue into the graft, does yielding a satisfactory osteointegration and osteoconduction, all of this with a minimal inflammatory response. At 16 weeks of implantation the graft showed complete regeneration of the tibial defect (Fig.2)

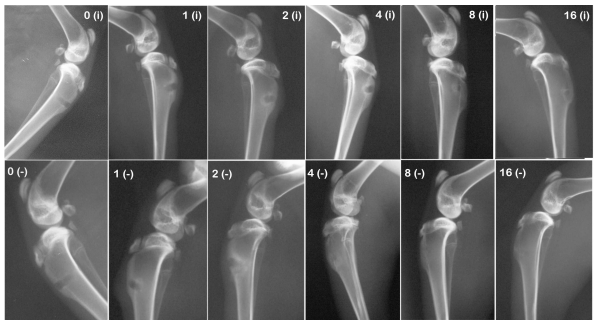

Fig. 1. Seriated X-ray images of tibial defects with (+) and without (-) chitosan graft.

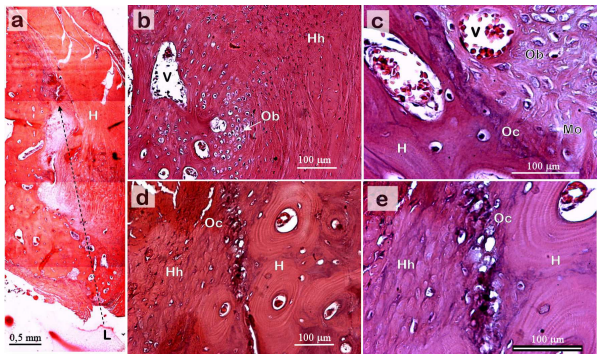

Fig.2. 16 weeks histopathological study of rabbit tibial defect at 40x magnification (a) showing the linear path of the drill bit (L), and at 400x magnification (b-e) showing bone (H), new bone (Hh), osteoclasts (Oc), osteoblasts (Ob) and bone matrix (Mo).

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