In vivo evaluation of the effects of calcium phosphate collagen fibrous material on bone repair

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The selection of the appropriate material is an important step toward the construction of tissue-engineered products. Composition and presentation of the material determine the success of the bone implant and influence cell adhesion, proliferation and differentiation. In this context, bioceramics have been emerging as a promising material to be used as bone grafts. Calcium phosphate (ACP) usually appears as an intermediate phase during the formation of other calcium phosphate forms, including hydroxyapatite (HA). It has the advantage of being biocompatible and osteoconductive and has a self-setting nature, which makes them injectable, allowing the use of a minimally invasive surgical procedure during clinical use. In addition, the incorporation of an organic component into the inorganic phase of the material is strongly desirable to better approximate the bone graft substitute to the structure of bone tissue. In this context, some authors have been introducing collagen to the ceramics, which might represent a promising way of improving the biological performance of the material. In this context, the aim of this study was to assess the characteristics of the CaP and collagen composites, in 2 different compositions (powder and fiber form), via scanning electron microscopy (SEM), pH and calcium release evaluation after immersion and to evaluate the performance of these materials on bone repair process in a tibial bone defect model. For this, four different formulations CaP powder (CaPp), CaP powder with collagen (CaPp/Col), CaP fibers (CaPf) and CaP fibers with collagen (CaPf/Col) were developed. Forty young adult male Wistar rats (12 weeks old; weight 295 ± 29g) were used for the in vivo experiment. The surgery procedure was consisted of inducing a bone defect on both tibias of rats. After 2 weeks of implantation, rats were sacrificed by CO2 asphyxia. The tibias were collected for analysis. Scanning electronic microscopy (SEM), fourier transform infrared spectroscopy (FTIR), calcium uptake and pH evaluation was used for characterization analysis. Histological analysis and immunohistochemistry were used to evaluate the biological interaction of materials and bone tissue. SEM images indicated that both material forms were successfully coated with collagen and that CaPp and CaPf presented HCA precursor crystals on their surface. Although presenting different forms, FTIR analysis indicated that CaPp and CaPf maintained the characteristic peaks for this class of material. Additionally, the calcium assay study demonstrated a higher calcium uptake for CaPp compared to CaPf for up to 5 days. Furthermore, pH measurements revealed that the collagen coating prevented the acidification of the medium, leading to higher pH values for CaPp/Col and CaPf/Col. Histology showed that, in CaPp, a delimited area of the defect, which was filled with CaP particles of different sizes distributed throughout the defect region. In this group, among the CaP particles newly formed bone and granulation tissue was observed. In CaPp/Col similar results to the CaPp group were found. For CaPf, a reduced amount of material was observed, with granulation tissue and newly formed bone around the material particles and filling the area occupied by the material compared to CaPp and CaPp/Col. For CaPf/Col, bone defect was filled mainly with newly formed bone and granulation tissue. It is possible to observe that, the material fibers were almost completely degraded. Runx2 immunoexpression was predominantly detected in the granulation tissue at the edges of the bone defect and around the materials for all groups. In some samples, osteoblasts cells were also immunomarked for all groups. Similarly to Runx2, RANKL immunoexpression was mainly verified in the granulation tissue at the edges of the bone defect and around the materials for all groups. In summary, the results indicated that the fibrous CaP enriched with the organic part (collagen) glassy scaffold presented good degradability and bone-forming properties and also supported Runx-2 and RANK-L expression. Additional studies should be performed to provide more information concerning the late stages of material absorption and bone formation.