Evaluation of biological and confinement properties of a bone substitute to *in situ* preparation based on demineralized bone matrix for bone tissue regeneration

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***Abstract*—** Bone regeneration is the process by which the formation of new bone is stimulated. Bone fractures can originate at any time due to trauma, infections, tumors, congenital malformations or skeletal diseases. Currently there are different strategies to treat bone defects that in some cases, regeneration does not occur on its own. That is why they are treated with bone substitutes, which provide a necessary environment for the cells to synthesize new bone. The Demineralized Bone Matrix (DBM) is widely used as a bone implant due to its good properties, such as osteoinduction and bioactivity. However, the use of DBM is limited, because its presentation is powder, which is difficult to implant with precision and is susceptible to migrating to other sites through blood flow. That is why the DBM is commonly incorporated into a variety of vehicles or carriers. The objective of this project is to evaluate the bioactive and confinement properties of a bone substitute based on demineralized bone matrix (DBM). Also, structural and morphological properties were evaluated. Bone substitute was obtained from EIA Biomaterials Laboratory of EIA University and the DBM was facilitated by Tissue Bank Foundation.

Morphological and structural properties were evaluated by scanning electron microscopy (SEM), X-ray diffraction (DRX) and Fourier transform infrared spectroscopy with total attenuated reflection (FTIR-ATR). Water absorption capacity and degradation were also evaluated during three months. The cytotoxicity was evaluated by the MTT test. The bioactivity of the bone substitute was evaluated through immersion of the samples in simulated body fluid during four weeks. Confinement tests were performed on tibial fragments of a human donor with bone defects of determined size, to ensure that the substitute remains in the defect despite the continuous flow of fluid. According of the knowledge of the authors, the methodology for evaluating samples in a confined environment has not been evaluated before in real human bones. The morphology of the samples showed irregular surface and presented some porosity. DRX confirmed a semi-crystalline structure. The FTIR-ATR determined the organic and inorganic phase of the sample. The degradation and absorption measurements stablished a loss of 3% and 150% in one month respectively. The MTT showed that the system is not cytotoxic. Apatite clusters formed from the first week were visualized by SEM and confirmed by EDS as it showed in Figure 1. These calcium phosphates are necessary to stimulate bone regeneration and thanks to the porosity of the developed material, osteinduction and osteoconduction are possible. The results of the *in vitro* evaluation of the confinement of the material showed that the migration of the bone filling to other sites is negligible, although the samples were subjected to the passage of simulated body fluid as it is shown in Figure 2. The bone substitute, putty type, showed stability, is bioactive, non-cytotoxic and has handling properties for specialists at the time of implantation. The obtained system allows to maintain the osteoinductive properties of DBM and it can fill completely fractures in any way, however, it does not provide a structural support, that is, it should only be used to treat fractures without requiring a mechanical load.

***Keywords*—**Bone regeneration, cytotoxicity, demineralized bone matrix, hydrogel.

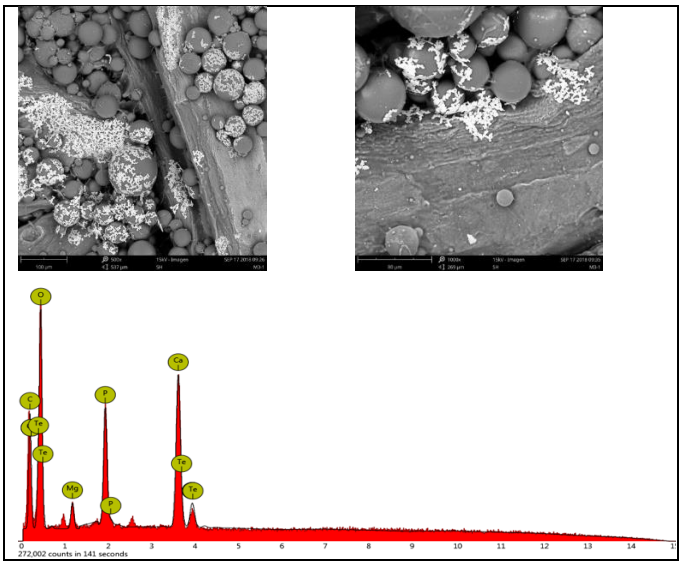


Figure 1. SEM-EDS Images of apatite cumulus formation after immersing the sample in simulated body fluid for a week.

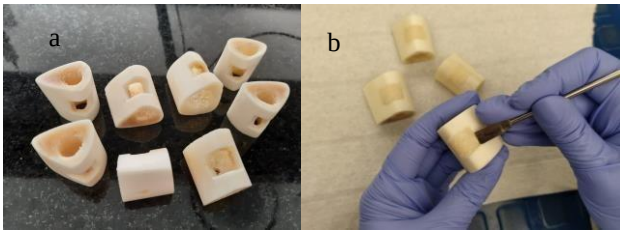


Figure 2. a) Biomedical Engineer Tibia fragments from human donor with bone defects of controlled size. b) Bone defects filled with putty for confinement testing.

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