In vitro and *in vivo* evaluation of hydroxyapatite nanopowders

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Introduction: Hydroxyapatite (HA) is one major constituent of inorganic components, bones and teeth, in human hard tissues, playing an important role in the functioning of these tissues responsibles for chewing, stability and support on the body [1]. The properties of the HA powders such as crystal morphology, crystallinity, thermal stability, and solubility have been shown to be strongly influenced by the manufacturing process for obtaining [2]. The addition of magnesium to apatite phase has attracted the researches due to its significant impact on the mineralization process and also its influence in the HA crystal formation and growth. The ultrasound radiation has showing to be a promising tool to prepare homogeneous and fine ceramic powders from aqueous solution. In this work, the citotoxicity test in different nanometric powder of HA was realized. The repair of bone defect was evaluated by test "in vivo".

Materials and Methods: The powders used during the experiment were HA – Stream Chemical (HAc) and HA synthesized in the laboratory by neutralization method as described in literature [2]**.** The synthesized powders were HA, HAMg036 (with 0,36% wt% of Mg^{2+}) and HAUSMg036 (with 0,36% wt% of Mg^{2+} and synthesized inside the ultrasound bath - USC 2850/Unique). Crystalline phases of the materials were analyzed by X-ray diffraction with monochromatic CuKa radiation (XRD, Siemens D500). The specific surface area of powders was measured by gas adsorption in N2 atmosphere (BET method, Micrometrics ASAP 2000, USA). To evaluate the bioactivity and biocompatibility of these samples, *in vitro* and *in vivo* testing were accomplished. The biocompatibility evaluation *in vitro* and *in vivo* was performed following the ISO 10993-5 guidelines. Cytotoxicity test was based on the quantitative assessment of surviving viable cells (CHO-k1 cell line) upon exposure to the samples extracts, by incubation with the supravital dye tetrazolium compound MTS [3].
The samples used for *in vivo* testing were separated in four groups. 2mm drilled holes were performed in the lateral side of right and left femur of the adults Wistar rats, in the right limb was used a sample in the hole meanwhile in the left limb was used nothing in the hole to perform him as a control to the sampled limb. The repair time observed was 4 weeks. The surgical and the euthanasia procedure followed the brazilian norms of animal experimentation. The histological analysis was performed in heamatoxylin-eosin paraffin-embedded sectioned tissues comparing the samples besides the sampled and control limbs.

Results and discussion: Figure 1 shows the XRD pattern of all the powders. The predominant phase was confirmed with JCPDS file nº 09-432 to be HA. The neutralization method associated with the ultrasound technique plus magnesium was effective to produce nanopowders. The BET analysis of all the samples leads to decreasing in specific surface area: 45.7 m^2/g (HAc), 31.6 m^2/g (HA8), 22,5 m^2/g (HAMg036) and 20,3 m^2/g (HAUSMg036).

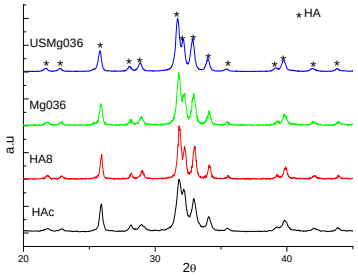

Figure 1 XRD of the HA samples

The cell viability determination is shown in Fig.2. The tested samples did not presented any cytotoxic effect similarly to the negative control ($IC_{50\%}$>100) showing their harmless character. The positive control (phenol solution) showed a cytotoxic effect over the cells ($IC_{50\%}$=55).

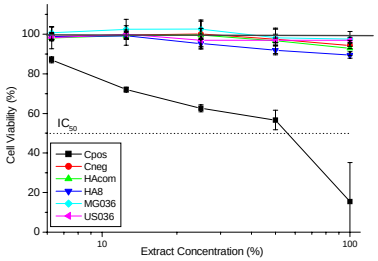

Figure 2 Cytotoxicity test of the HA samples

In the surgical procedure was observed that after the placement of the powder sample in the drilled hole the coagulation time increase retarding the formation of the clot. This fact may be related to the nanometric size of the powders, allowing increased reactivity. The results of the histological evaluation showed that the bone repair itself was successful in all groups and in sampled and control limbs.

Conclusion: The cytotoxicity test revealed the biocompatible nature of the HA powders which is a primary indication of their potential use as a biomaterial. The nanometric size of the studied powders interfered in the surgical procedure due to the high reactivity. However a satisfactory result of tissue repair was observed by histological analysis.

References

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