

INFLUENCE OF HYDROXYAPATITE CONCENTRATION ON ADHESION AND DIFFERENTIATION OF CALVARIA BONE CELLS.

Karem Noris Suárez(1), Jan Peterson(1), Alpidio Boada(2), Gema González (3)

(1)Departamento de Biología Celular, Universidad Simón Bolívar. Caracas, Venezuela. (2), Inst. Estudios Científicos y Tecnológicos, Universidad Simón Rodríguez. Caracas, Venezuela. (4)Lab. Ciencia e Ing. de Materiales, Dpto. de Ingeniería, Instituto Venezolano de Investigaciones Científicas. Caracas, Venezuela. Email: gemagonz@ivic.ve, knoris@usb.ve

Bone tissue is made up of a mineral phase (hydroxyapatite) and an organic phase (collagen, bone sialic protein, osteopontin, etc). This tissue is in constant regeneration through a cyclical process with the participation of the osteoblast, bone cells responsible of the synthesis of the matrix proteins after osteoclast have remove the previous bone tissue (Howship's lacunae). Once the osteoblasts have synthesized the bone organic matrix and the mineralization process progresses they suffer morphological changes until the most differentiate stage is reached and are named osteocytes. They is characterized by very little cytoplasm with long prolongations, connecting these cells between themselves in the mineralized matrix.

The use of hydroxyapatite (HA) as a biomaterial, to repair or substitute bone tissue [1], has been widely recognized due to its similarity with bone, which is formed by approx. 70%wt. and 50%vol. of this material [2]. Biomaterials must have an attractive surface to respond to the requirements of the tissue implanted, such that they can be considered bioactive [3]. Powder Hydroxyapatite (PHA) is a material used as bone filler for fracture repair. In the present study we have examined the effect of HAP concentration on osteoclast adherence by *in vitro* bioactivity characterization. Nanocrystalline powder of hydroxyapatite was synthesized by a precipitation method of $\text{Ca}(\text{OH})_2$ and NH_4HPO_4 at room temperature and characterized by x-ray diffraction and transmission electron microscopy. Bioactivity tests were performed employing bone cells obtained from neonatal rats calvariae, following the method described by Suarez *et al.* [4]. Osteoblastic cells (3×10^5 cells/dish) were placed on 12-well culture plates. Suspensions of DMEM culture medium with different PHA concentrations were prepared. 0.5 ml of suspension was used for each PHA concentration tested, these were 0, 10, 100 and 1000 μg . A film of PHA was obtained on the surface of each well. Adhesion was evaluated after 6 h by cell counting employing a hemocytometer. These tests were performed by triplicate. The morphology was evaluated by Scanning electron microscopy (SEM) after 24h of cultured in 100 μg of PHA. Electron microscopy sample preparation was performed by cell fixation in 2.5 % phosphate-buffered glutaraldehyde pH 7.4 at 4 °C by 1h, followed by 2 washes in the same phosphate-buffered and finally fixed with 1% OsO_4 at 4 °C by 1h. after rinse in distilled water a dehydration procedure was carried out employing consecutive increasing amounts of ethanol (70%, 80%, 90%, 95% y 100%) and then the samples were dried in a critical point drier equipment Hitachi HCP-2. Gold coating was performed with a EMS 350 sputter equipment. The samples were observed in a scanning field emission electron microscope Hitachi S-4500 operating at 10 keV. The analysis of cellular adhesion after 6 h of cell culture in different proportions of PHA showed that cell adhesion decrease as a function of PHA concentration. The lower concentration tested (10 μg) inhibits adhesion in 20% compared to the control sample, while for 100 μg and over, adhesion of the osteoblastic cells is inhibited 80% (Fig. 1). The morphology of the cells after 24 h test can be observed in the micrographs, showing an osteocyte-like appearance, similar to that described in the literature. Cells with scarce cytoplasm and long prolongations connecting to neighbor cells can be observed in Fig. 2. These results suggest that high concentrations of PHA

inhibit osteoblastic cell adhesion and induces morphological changes suggesting the formation of the osteocytic phase of these cells. The osteocytic phase corresponds to the stage of major differentiation of bone cells, in this condition cell loss its proliferation ability and also the capacity to synthesize proteins forming the bone matrix. This state of differentiation of bone cells is considered the most specialized one and therefore correspond to terminal cells. In conclusion, PHA inhibits cellular adhesion as function of the concentration and induces cell differentiation to the osteocytic stage of the osteoblastic cells derived from calvaria.

References

- [1] A.J., Salinas et al (2000) *Biomaterials*. 21, 251
- [2] L.L., Hench, J., Wilson (1993). *Advances Series in Ceram.* 1,181.
- [3] F. H., Lin, M. H. HON. (1989) *Journal Austr. Ceram. Soc.* 25, 41
- [4] K., Noris Suarez, et al. (2005) *Revista Latinoamericana de Metalúrgia y Materiales*. En prensa.

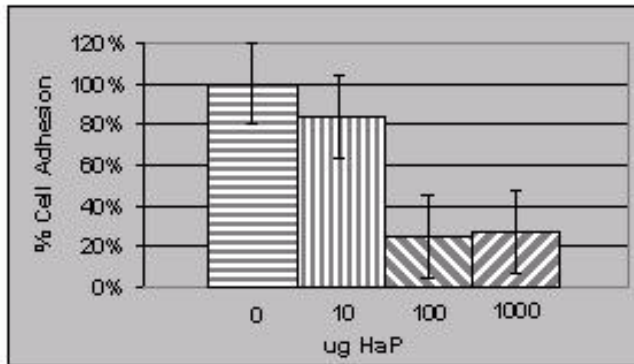


Figure 1. Cell adhesion (%) shown for bone cells after six hours of incubation with increasing amount of HAP. Triplicate test was done for each condition

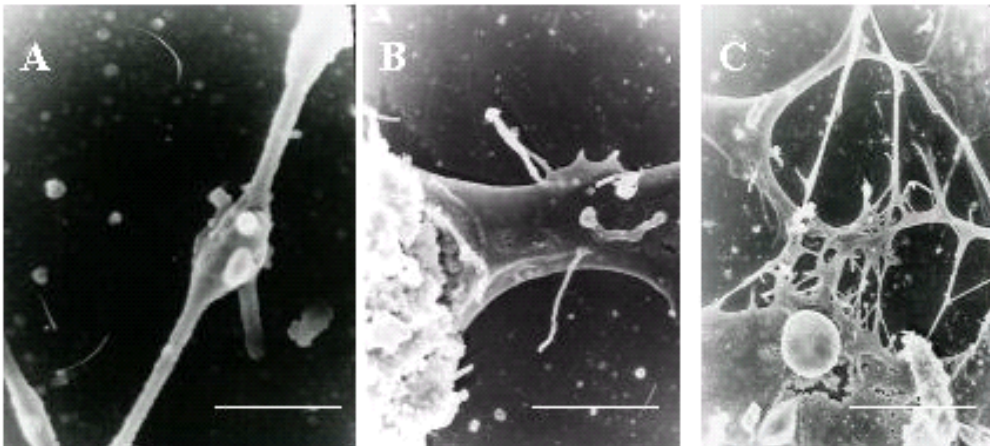


Figure 2. A.- Detailed of contact between two prolongations. B.- Detail of structure observed on cell-like osteocyte membrane surface, contacting HAP, resembling minuscule prolongations (arrows head).C.- several cells contacting through a structure of cellular prolongations. Cells show osteocyte-like appearance. Bars 1 μ m in A and B; bar = 4 μ m in C.