

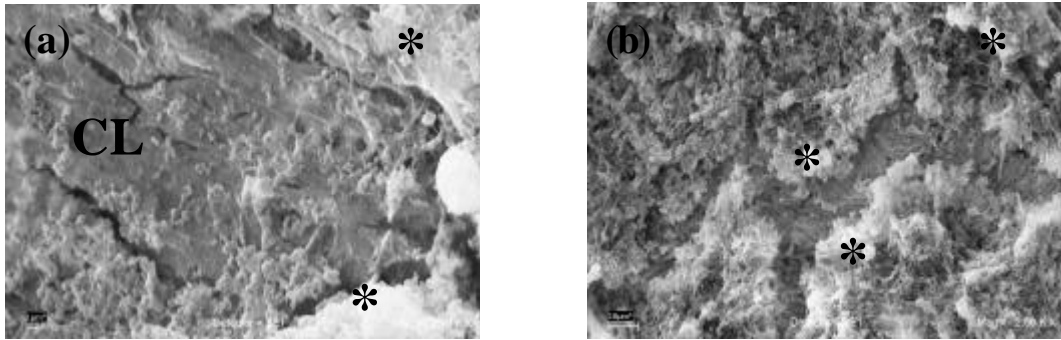
**IN VITRO MINERALIZATION OF HUMAN OSTEOBLASTIC CELLS ON AMORPHOUS CARBON COATINGS: AN ELECTRON AND IMMUNOFLUORESCENCE MICROSCOPY STUDY**

Sandra Elizabeth Rodil (1), René Olivares (2), José Guzmán (1), (1)Instituto de Investigaciones en Materiales, universidad Nacional Autónoma de México, Circuito exterior s/n, Ciudad Universitaria, 04510 México D. F., México; (2)laboratorio de Biología Celular, Facultad de Odontología, Universidad Nacional Autónoma de México, Ciudad Universitaria 04510 México D. F., México. Email: jguzm@servidor.unam.mx

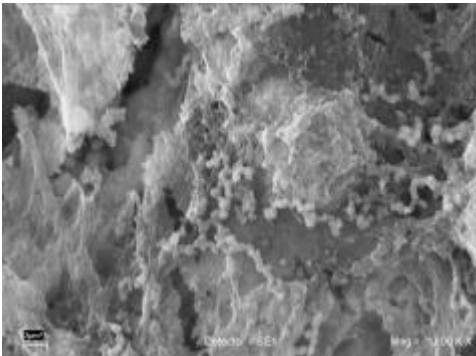
Osteoblast cells were isolated from human alveolar bone by a conventional explant technique. The isolated cells were culture on standard conditions and plated at a density of  $1 \times 10^4$ /well on amorphous carbon surfaces (15 mm in diameter) in 24-well culture plates. After an initial adhesion time of 3 hours, 600  $\mu$ L of culture medium were added. Once that cells attain confluence, mineralization inductive medium was added and samples were incubated for 7, 14 and 21 days by triplicate Following cultivation, one sample was prepared for observation in the scanning electron microscopy (SEM) and others for observation of some biochemical parameters of osteoblastic phenotypes: bone sialoprotein and osteocalcin.

Amorphous carbon coatings were deposited on stainless steel (316L) substrates by a conventional dc magnetron sputtering system, using a high purity graphite target and argon as the gas source. The purpose of the study is to determine the influence of the carbon surface on the formation of a mineralized matrix deposition by human osteoblasts cells. Figure 1 shows SEM pictures of the mineralized matrix after (a) 14 and (b) 21 days. SEM observations of these cultures showed the presence of continuous cell multilayers and fibrillar extracellular matrix with regions that seem to correspond to globular mineral deposits. However, for the simple observation and even by the aid of energy dispersive analysis it was not clear if the nodules were mineralized calcium phosphate deposits (see figure 2). As a confirmation optical images of active proteins associated to the novo bone formation and initial stages of the mineralization process were also obtained. Figure 3 shows immunofluorescence images of (a) bone sialoprotein (BSP) and (b) osteocalcin (OC, also known as bone gla protein, BGP). These two are major constituents of the non-collagenous proteins in the extracellular matrix of bone. BSP is specifically expressed by fully-differentiated osteoblasts and its expression is normally restricted to mineralized connective tissue of bones and teeth, where it has been associated with mineral crystal formation. Osteocalcin influences bone mineralization through its ability to bind to the mineral component of bone, hydroxyapatite and also functions in cell signaling for the recruitment of osteoclasts and osteoblasts, which have active roles in bone resorption and deposition, respectively.

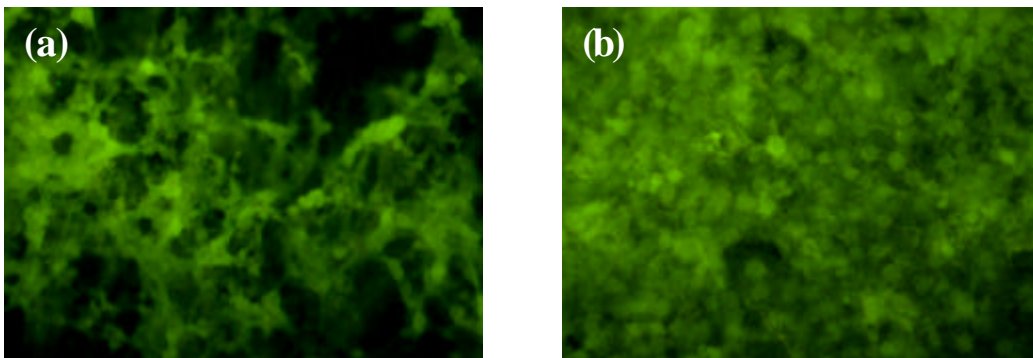
Comparison between SEM and immunofluorescence images allows us to conclude that the amorphous carbon surface promotes full differentiation of human osteoblasts and formation of the mineralized matrix.



**Figure 1.** Bars 1  $\mu\text{m}$  in (a) and (b); (a) Mineralized matrix (\*) and cellular layer (CL) in a-C after 14 days of incubation. (b) Mineralized matrix (\*) in aC after 21 days, the cellular layer is completely cover by the matrix.



**Figure 2.** Bar 2  $\mu\text{m}$ . Higher magnification of an aC sample after 21 days of culture. Small nodules embedded in the mineralized matrix.



**Figure 3.** Immunofluorescence images of the distribution of (a) bone sialoprotein and (b) osteocalcin.