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Myoglobin Attachment on Apatite Surface

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Abstract – The proteins-biomaterial interaction is the first and mainly event for integration between the implanted material and biological tissues. Carbonate hydroxyapatite is the most similar inorganic material of bone and has been widely tested as bone grafts. In this work myoglobin was used as a protein model because its native structure is well known and its efficiency in bind and store oxygen in the cells. The myoglobin adsorption efficiency on hydroxyapatite sample was 70% higher then CHA 7% sample. The results showed that the carbonated content inhibited the protein adsorption. Cells experiment performed on HA and Myo-HA sample showed good attachment after 7 days.

Myoglobin protein is responsible for binding and releasing oxygen in the muscles through the heme group that holds an iron ion. Oxygen is essential to aid wound healing of tissues, including skin, muscle and bone (1). Current techniques to optimize oxygen delivery to tissues include the use of local devices placed directly over the wound (topical). The aim of this work is to prepare an oxygen delivering scaffold using hydroxyapatite (HA) and carbonate hydroxyapatite (CHA) with different carbonate content (1% and 7% w/w) adsorbed with myoglobin for optimal wound healing. Myoglobin is a globular protein with a molecular weight of 17.800 Da, isoeletric point of 7.4 and dimensions of 2.5 x 3.5 x 4.5 nm^3. Horse skeletal muscle myoglobin was purchased from Sigma, HA and CHA samples were prepared according to Mavropoulos et al 2002 (2).

The adsorption of myoglobin onto HA and CHA was performed at 37°C stirring 0.05 g of the samples with 4mL of myoglobin solution at different concentrations in phosphate buffer pH 6.8. The adsorption experiments were accomplished in triplicate and after 24 hours the amount of protein remaining in solution was measured by UV spectrometry at 409 nm. The amounts of adsorbed proteins were calculated from solution depletion. Immediately after the sorption the Myo-HA and Myo-CHA samples was washed with phosphate buffer and submitted to desorption experiments to know the amount of protein that was not effective adsorbed.

Figure 1 show the myoglobin adsorption amounts on HA and CHA samples over 24 hours. The results revealed that HA and CHA 1% presented similar adsorption efficiencies and that the adsorption capacities of these materials were not reach. CHA 7% samples were less efficient, the amount of protein adsorbed reached the maximum capacity when 0.5 mg myoglobin/mL was used. For higher concentration no significant change in the adsorption was observed. This result suggested that the PO_4^{3-} substitution by CO_3^{2-} in some point affect the myoglobin adsorption perhaps due to its preference for absorbing at PO_4^{3-} sites".

Figure 2 shows the Myo-HA disks cultured with human osteoblastic cells (SaOs2) at 3th passage after 7 days. It was observed that the myoglobin adsorbed on hydroxyapatite surface did not inhibit the cell adhesion and proliferation.

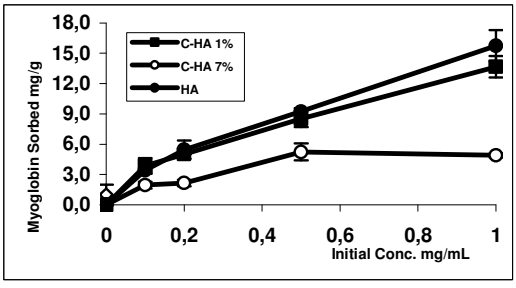

Figure 1: Myoglobin adsorption efficiency for HA, C-HA 1% and C-HA 7%.

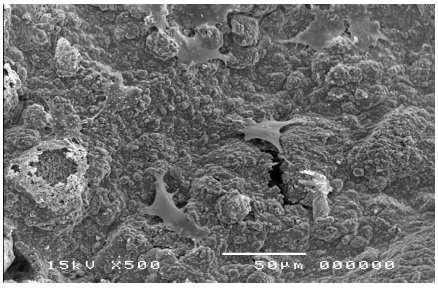

Figure 2: SEM micrographs of osteoblast cells on Myo-HA disks after 7 days in culture.

References

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