In vitro biomineralization of carbon nanotube/hidroxyapatite composites obtained by electrodeposition process

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Statement of Purpose: The development of porous materials for sustained three-dimensional growth of cells, such as scaffolds composed of vertically-aligned multi-walled carbon nanotubes (VACNT) is of particular interest in regenerative medicine and tissue engineering, because they can be potentially tailored to mimic natural extracellular matrix in terms of structure, chemical composition and mechanical properties [1]. Template-induced hydroxyapatite (HA) has broad prospects in applied to bone repair. There is a resurgent interest in controlling HA crystal nucleation, crystallinity and growth for assembling composite materials analogous to those produced by nature, involving biomineralization process [2]. Thus, it becomes very attractive a combination of these two excellent materials for applications in bone tissue engineering [3]. In this way, this work presents a new method for the electrodeposition of HA crystalline nano platelet films on VACNT. The HA is obtained crystalline directly from growth process whithout any thermal annealing. The as grown HA films have shown exceptional bioactivity and citocompatibility.

Methods: VACNT films were produced as a thin film, using a microwave plasma chamber at 2.45GHz on Ti substrate (10x10x1mm) from Ni catalyst. Superhydrophilic VACNT composites were obtained by the incorporation of oxygen-containing groups using a pulsed-direct current plasma reactor with an oxygen flow rate of 1 sccm, at a pressure of 85 mTorr, -700 V and with a frequency of 20 kHz [3]. The fabrication VACNT/HA composites was performed with a direct electrodeposition of the thin HA films on the VACNT scaffolds using $Ca(NO_3)_2.4H_2O + (NH_4).2HPO_4$ electrolyte applying a constant potential of -2.0 V for 30 minutes, with the solution temperature maintained at 75 ^oC. The bioactivity of HA/VACNT composites were investigated using simulated body fluid [4] solution (SBF). X-ray diffractometry (XRD), Raman spectroscopy, FT-IR, scanning electron microscopy (SEM) and energy dispersive x-rays (EDX) analysis investigate d the structural characteristics, semi-quantitative analysis and morphology of VACNT/HA composites, before and after soaking with SBF. Human osteoblast adhesion on HA/VACNT composites were evaluated by SEM and fluorescence microscopy (FM).

Results: Figures 1a-b shows SEM images of the lamellar and HA platelet crystals formed on VACNT films (Fig 1a). Notice that a thin HA film (thickness of 3.5 µm) grows without affecting the alignment of VACNT films (Fig 1b). The SEM images of Fig. 1c-d show the biomineralization and bioactivity process of HA/VACNT composites after soaking in SBF for 21 days . The top surface appears less rough due to the blending of adjacent HA platelets to form a continuous globular-like apatite film (Fig. 1c). The cross-section image (Fig.1d) reveals HA film spreading down deep into the VACNT to form a consolidated composite with homogeneous apatite layer along the entire length of VACNT.

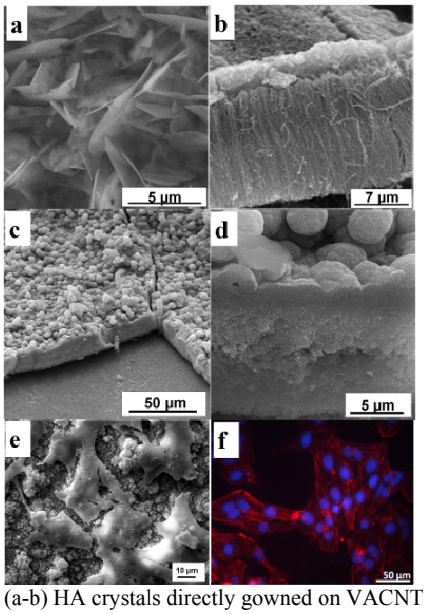

Figure 1. (a-b) HA crystals directly gowned on VACNT films by electrodeposition process. (c-d) In vitro biomineralization process after 21 days soaked in SBF. (e-f) Human osteoblast adhesion on HA/VACNT composites.

Figures 1e and 1f show the SEM and FM images of human osteoblasts adhesion on the crystalline platelet-like HA surfaces after 7 days. The cells spread with no preferential direction, acquiring a flat roughly circular form with active formation of membrane projections all over the cell surfaces (Fig.1e). Figure 1e highlighted the exceptional cell adhesion with a high density of verystraight actin filaments along all the cell directions.

Conclusions: The HA electrodeposition on VACNT produced highly crystalline films, without any thermal treatment. These HA films show remarkable bioactivity properties that accelerate the *in vitro* biomineralization process and show excellent human osteoblast adhesion.

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