Biomineralization in vitro and in vivo studies of a novel nanohydroxyapatite/superhydrophilic vertically aligned carbon nanotube nanocomposites

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INTRODUCTION AND AIMS

Hydroxyapatite is used as a biomaterial for the regeneration of bone tissue because its chemical and crystallographic similarities with the main inorganic component of natural bone. Vertically-aligned multi-walled carbon nanotubes (VAMWCNTs) are of particular interest in regenerative medicine. Template-induced hydroxyapatite has wide-ranging prospects in applied fields of bone regenerative medicine. Thus, a combination of these two excellent materials might be used in bone tissue engineering applications. We have recently shown a new method to obtain crystalline HA/VAMWCNT composites using direct electrodeposition process. Here, we have shown an new and fast method to obtain HA/VAMWCNT nanocomposites. From this, we introduce a new biomimetic mineralization routine employing the HA/VAMWCNT nanocomposites as highly stable template materials. The biomineralization was obtained after HA/VAMWCNT nanocomposites soaking in a synthetic body fluid (SBF) known to favor the "in vitro" and "in vivo" calcification process.

METHODS

NANOCOMPOSITES PRODUCTION

- VAMWCNT films were produced as a thin film, using a microwave plasma chamber at 2.45GHz on Ti substrate (10x10x1mm) with Fe catalyst.
- Superhydrophilic VAMWCNT composites were obtained by the incorporation of oxygen-containing groups using a pulsed-direct current plasma reactor with an oxygen flow.
- The fabrication HA/VAMWCNT nanocomposites was performed through a direct electrodeposition of the thin HA films on the VAMWCNT scaffolds using the Ca/P electrolytes.

BIOLOGICAL STUDIES

- A simulated body fluid (SBF) (5x) solution was used for "in vitro" bioactivity study.
- The adhesion of the human osteoblast cells on the HA/VAMWCNTs nanocomposites were monitored up to 7 days.
- Adult, male, C57BL/6 rats (22-28g body weight) were used in this study. All rats were daily examined for signs of infection or discomfort and water and food for 9 weeks.
- Nine weeks after implantation of the disks, rats were sacrificed by Halotano inhalation.

RESULTS AND DISCUSSION

- Figure 1b shows the massive growth of the apatite crystallite clusters with a globular-like shape on the HA/VAMWCNT nanocomposites. Details of the densification of superhydrophilic VAMWCNT films after 21 days incubation in SBF are shown in figure 2d compared to nanocristals formed after HA electrodeposition (Figure 1c).
- Figure 2A presents reflections in 25.9° (002) and three peaks in the region around 31.6° (triplet), characteristic of the planes (211), (112) and (300), and 34.0° (200) (JCPDS card number: 9-432). In this diffraction, the peak intensity of calcite is due to the biomimernalization in vitro process. Figure 2B is the FT-IR ATR analysis. The multiples located around 1000 cm⁻¹ are attributed to phosphate modes. Carbonate bands are detected at 879, 1415, and 1455 cm⁻¹. Figure 3c shows a picture of the local and Fig. 3d shows the histological analysis after sixteen weeks of surgery for the samples around. It shows that the HA/VAMWCNTs were integrated entirely into new bone tissue at sixteen weeks after surgery (Fig. 3d).

Figure 1. (a) SEM image of the cross section of HA film on top of the VAMWCNT. (b) FEG-SEM images show the bioactivity of HA/VAMWCNT nanocomposites after soaking for 21 days in SBF. The cross section shows the densification of HA/VAMWCNT nanocomposites. (c) Details of HA nanocrystals formed after biomineralization process.

Figure 2. Bioactivity characterization of HA/VAMWCNT nanocomposites using: (A) X-ray diffraction patterns of the superhydrophilic VAMWCNT films. HA/VAMWCNT nanocomposites obtained by electrodeposition technique and soaked in SBF for 21 days. (B) FTIR (ATR) optical technique shows the biological apatites obtained on the HA/VAMWCNT nanocomposites before and after 21 days of soaking in SBF.

Figure 3. Human osteoblast cells adhesion on plates like HA/VAMWCNT composites after 7 days (a and b). Histological appearance of lamellar bone formation in response to HA/VAMWCNTs (c) and a picture of local surgery (d) implants in the calvarium rats at sixteen weeks after surgery.

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