Spatial and temporal control of osteoblastic cells proliferation on electroconductive carbon nanotube-based bone grafts

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Abstract

Biomaterials can still be reinvented to become simple and universal bone regeneration solutions. Following this roadmap, "smart" bone grafts have been designed with new functionalities able to stimulate specific bone cells responses. Regarding the beneficial effects of endogenous electrical signals in natural bone, electron conductivity emerge as an exciting functionality. As opposed to natural piezoelectric bone, electroconductive bone grafts have key advantages: external control over the level and duration of stimulation; confinement of exogenous electrical fields on their surface leading to spatial and temporal control of bone tissue regeneration. Following this, the present work aims to: (1) process MWCNTs-based bone grafts; (2) assess the α-MEM-conductive bone grafts interactions under (or not) electrical fields; (3) evaluate *in vitro* the efficiency of conductive bone grafts in delivering electrical stimulus to osteoblastic cells.

Biologically safer carbon nanotubes (CNT) [1-3] presenting outstanding characteristics - non-metallic phases, bioactive, high aspect-ratio and ultimate electrical conductivity - were used here as fillers to obtain highly conductive biomaterials. Calcium phosphate (CaP)/CNT powders show high interaction being the CNTs decorated with CaP particles (Fig. 1a). Microstructures of fracture (Fig. 1b) and polished surfaces (Fig. 1c) show that CNT are well dispersed combining individual CNT (Fig. 1d) and controlled sized agglomerates (<10 μm) (Fig. 1c). This CNT 3D network gives an electrical percolation threshold (Pc) in the range of 0.9-1.8 vol.% (Fig. 1e). Pursuing the main goal of this work, the selection of the CNT loading should be: low to preserve the biological profile of the matrix; high to give composites with higher conductivity than the biological milieus. The 2.5 wt.% loading is the one that matches this two requisites (Figs. 1e,f).

In an *in vivo* scenario, it is expected that this composite formulation induces the locally increase of the conductivity and confines the exogenous electrical fields on its surface. To evaluate this, two set of experiments were performed in α-MEM (12 ml). The presence of six CaP samples show an increase of 0.15 % of the impedance of the medium (Figs. 2a,c). Conversely, six CaP/CNT (2.5 wt.%) samples decrease the impedance in 1.26 % (Figs. 2b,c). Scanning vibrating electrode (SVET) measurements were accomplished under a constant electrical field E_{xx} of 3 $mV.cm^{-1}$/100 μA, accordingly to the configuration of Fig. 2d. At the borders, it can be seen that the conductive sample induces less distortion of the E lines than the dielectric one (Figs. 2e,f). Also, the E_{yy} component, perpendicular to the sample surface, is maximized for the conductive sample (Figs. 2 g-j).

The current-voltage response of ion channels in osteoblastic cells is shown in Fig. 3a. An action potential of +10 mV for 5 ms is enough to induce a maximum peak of current in the cell. This is followed by a depolarization to the resting state during 20 ms (red line in Fig. 3a). This biological data was used as reference to select the AC electrical signal parameters for the stimulation experiments (Fig. 3b). *In vitro* stimulation of MG63 osteoblastic cells was accomplished in a home-made apparatus (Fig. 3c, current circuit highlighted by blue arrows) using 12 ml of α-MEM solution and six samples per culture plate (same conditions seen in Fig. 2). The frequency was kept constant at 40Hz and the electrical field (5.6 and 15.3 $mV.cm^{-1}$)/current density (91 and 167 $\mu A.cm^{-2}$)/current (100 μA and 200 μA) and time (15 and 30 min) were varied. Potential and density current distributions of the stimulation area of the culture plate (Figs. 3c,d) are presented for the 200 μA stimulus condition in Figs. 3e,f. It can be seen that the samples (black dotted line in Figs. 3e,f) were uniformly stimulated. MTT assay in Fig. 4g shows that electroconductive CaP/CNTs templates under electrical stimulus accelerate the proliferation of osteoblastic cells. For all the stimulation conditions the cell population is higher than the control (non-stimulated material) (Fig. 3g). Conversely, for the dielectric materials the stimulus delivering is less efficient, showing responses equal or lower than the control (Figs. 3h,i). Interestingly, these observations corroborate the results of Fig. 2. SEM and CLSM microscopy images (Figs. 3j,k) show no evident differences in cells morphology between the two conditions and for the three materials.

In conclusion, osteoblastic cells were efficiently stimulated on CNT-based bone grafts. MTT assays showed almost 300% increase in cell proliferation, relatively to the non-stimulated condition, after only 3

days of daily stimulation time of 15 min. These exciting observations are intimately related with the locally increase of the conductivity and the confinement of electrical fields on the surface of the conductive material.

References

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Figures

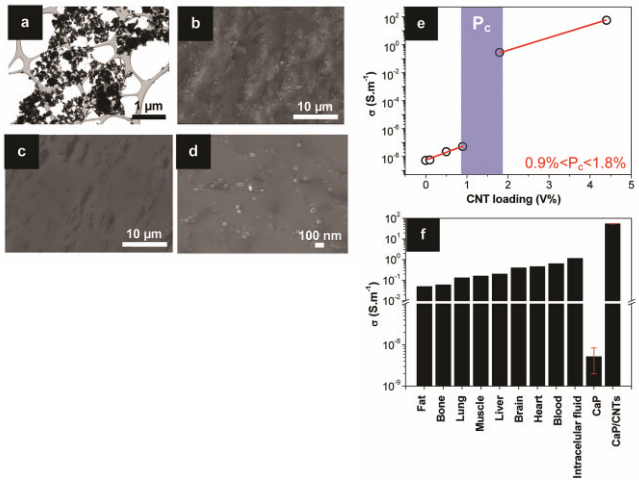

Fig. 1 Microstructure and electrical conductivity of CNT-based composites.

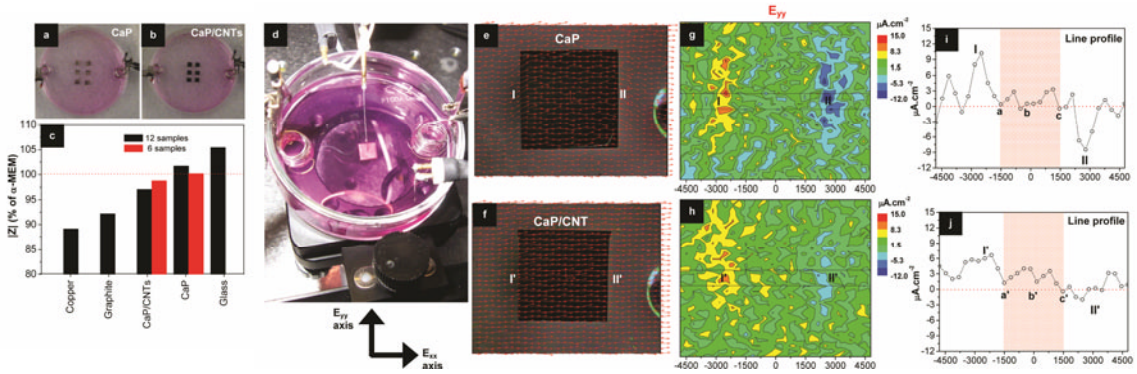

Fig. 2 α-MEM-MWCNTs-based bone grafts interactions under (or not) electrical fields.

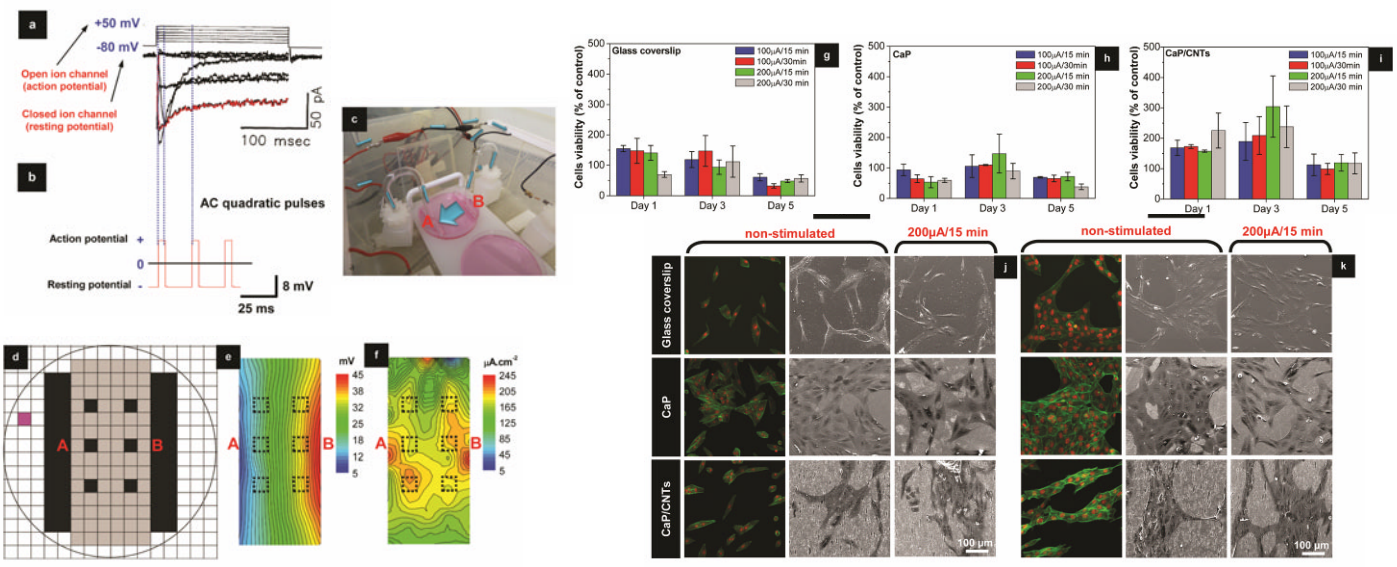

Fig. 3 *In vitro* evaluation of the efficiency of CNT-based bone grafts in delivering electrical stimulus to osteoblastic cells.