Increase of mineral nodules and alkaline phosphatase levels in osteoblasts cultures by using disordered carbon nanotubes and titanium discs.

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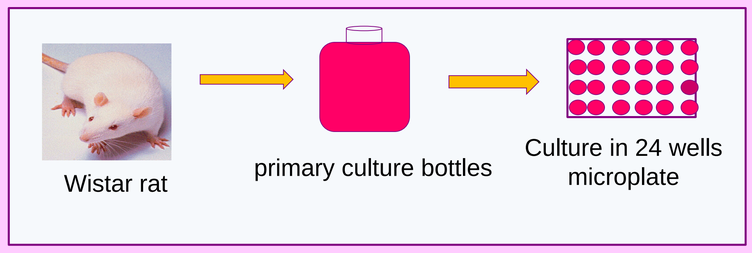
INTRODUCTION

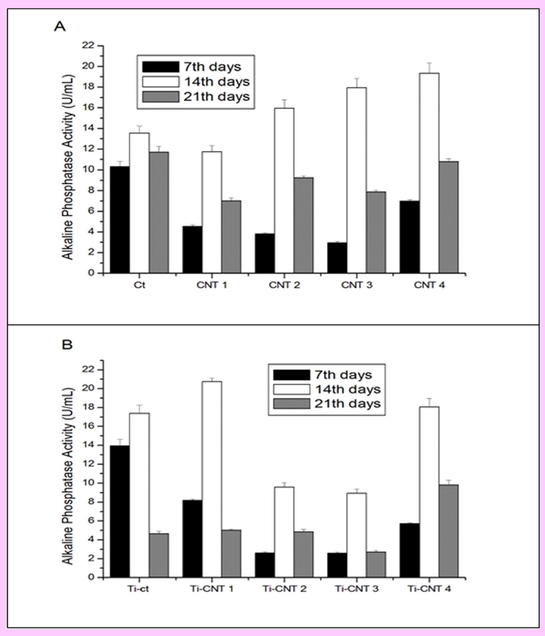
The accumulation of mineral composed mainly by calcium and phosphate ions that form hydroxyapatite crystals is known as biomineralization process. Currently, titanium materials are the most widely used for dental implants and also for the repair of osseous defects, due to its high biocompatibility. However, sometimes it is desirable an additional positive response of the biological tissues to the treatment being used, and the use of titanium together with other biomaterials such as CNTs has been studied to achieve this purpose. Therefore, the aim of this study was to investigate the *in vitro* influence of different carbon nanotubes (with or without titanium surfaces) on osteoblast culture growth and on the formation of bone-like nodules using a primary osteoblastic cell line derived from rat bone marrow cells.



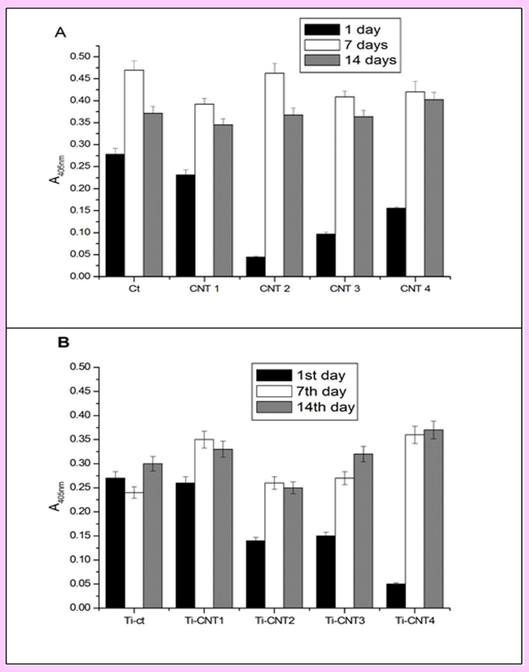
METHODS

The four sets of CNTs with diameter distribution size and disorder relatively large were prepared employing (Co, Mn) and (Fe) as catalysts, two sources of carbon precursors (methanol and ethanol) and NaCl substrate. Alkaline phosphatase activity and formation of mineral nodules were evaluated after addition of CNTs in different phases of cell growth.

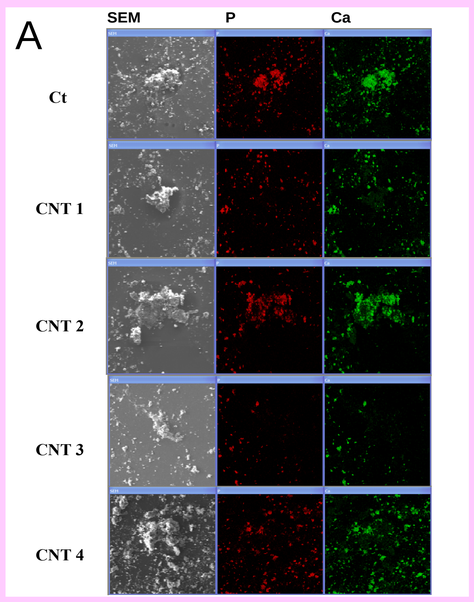
RESULTS

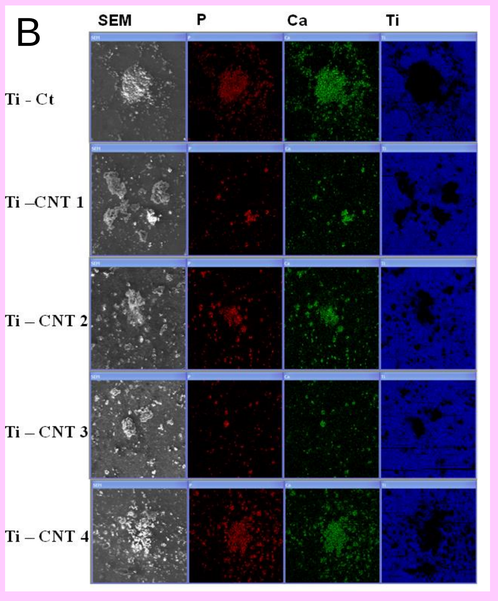


Levels of alkaline phosphatase activity in osteoblasts cultures incubated on the 1st, 7th and 14th day with the different types of CNTs and monitored after 7 days of culture. (A) cells grown on plastic surface and (B) cells grown on titanium discs.



Number of bone-like nodules evaluated in osteoblasts culture after being incubated on the 1st, 7th and 14th day of growth with the different types of CNTs. The nodules were detected at the 21st day of culture. (A) Cells grown on plastic surface and (B) cells grown on titanium discs.

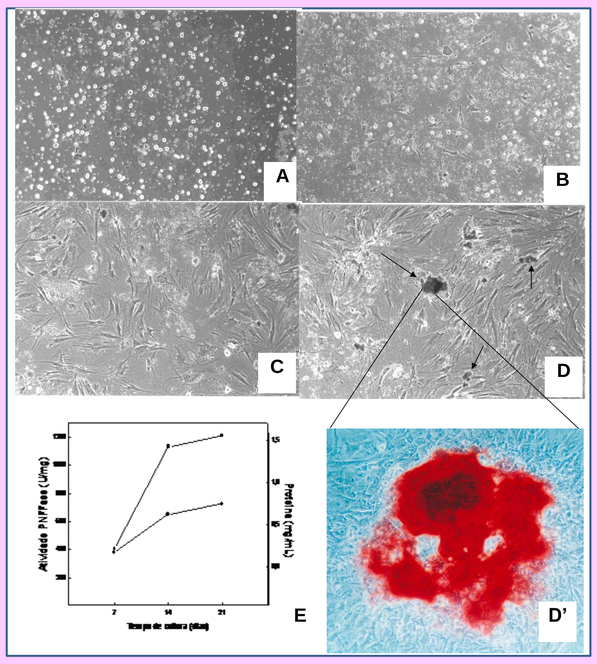




Mapping of bone-like nodules for Calcium, Phosphorus and Titanium in cells grown in the presence of CNTs incubated on the 1st day and grown until the 21st day. (A) Cells grown on plastic surface and (B) cells grown on titanium discs.

Ca/Pi molar ratios observed for the nodules analyzed in the above figures.

	Growth Condition	Control	CNT1	CNT2	CNT3	CNT4
Ca/Pi	Plastic	1.397	0.931	1.298	0.822	0.801
	Titanium	1.616	1.947	1.285	1.068	1.635



Culture of osteoblastic cells after (A) 24 h, (B) 4 days, (C) 10 days, (D) 14 days and (D') colored with alizarin red. Magnification: 10x (arrows indicate mineralized matrix). (E) Effect of time of culture of osteoblastic cells in PNPPase activity and protein concentration for the membrane fraction rich in alkaline phosphatase.

CONCLUSION

The use of CNTs with osteoblasts in the initial phase of growth results in a decrease in the levels of alkaline phosphatase activity, as well as in the formation of mineral nodules, whereas the same CNTs, when incubated during the stationary phase of growth, provide increased alkaline phosphatase activity. The addition of CNTs in cultures grown on titanium disks, in general, proved more advantageous than for cells grown on plastic surface. Thus, this study provides information for the application of different types of CNTs associated with titanium in processes of biomineralization stimulation, suggesting that depending on the CNT type there is an interaction between CNTs and Ti that favors the formation of mineral nodules on Ti surface.

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