ELECTRICAL STIMULATION PROMOTES OSTEOBLAST FUNCTIONS PERTINENT TO OSTEOGENESIS

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INTRODUCTION
Bone repair, (occurring, for example, in osteotomies in animal models) can be accelerated through the use of electrical stimulation (1). Such literature reports provided evidence that electrical stimulation promotes bone healing; however, the mechanisms responsible for osteogenesis under electrical stimulation are still not fully understood.

It is our hypothesis that cellular/molecular responses, specifically those of osteoblasts, play a critical role in bone healing under electrical stimulation. The present study used in vitro cellular models, current-conducting polylactic acid/carbon nanotube (PLA/CNT) composites, and a custom-made laboratory system of our own design to demonstrate that electrical stimulation affects both cellular and molecular functions of osteoblasts relevant to osteogenesis.

MATERIALS AND METHODS
Multi-walled carbon nanotubes (CNT) produced by the electric arc method (2) were blended (20 % w/w) with polylactic acid (PLA; molecular weight 100,000). The resulting PLA/CNT composite substrates (each 4 cm in diameter) were sterilized in a 70% ethanol solution prior to experiments with cells.

Rat calvarial osteoblasts were isolated and characterized as previously described (3). Cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum under standard cell culture conditions (that is, a 37 °C, humidified, 5 % CO2 / 95 % air environment).

Osteoblasts cultured on the PLA/CNT composite substrates were then exposed to electrical stimulation (consisting of an alternating current of 10 mA at a frequency of 10 Hz with a 50 % duty cycle) using a laboratory system of our own design; the cells were exposed to the stimulus for 6 hours daily for up to 21 consecutive days. Controls were osteoblasts maintained under standard cell culture conditions, but no electrical stimulation, for similar periods of time.

After 6 hours, osteoblast mRNA was extracted from lysed cells using standard procedures; Reverse Transcription Polymerase reaction techniques were used to examine expression of Alkaline Phosphatase, Osteopontin, Osteocalcin, Collagen Type I, and Transforming Growth Factor b-1. In addition, after 2 days, cell proliferation was measured by counting stained osteoblasts in situ using fluorescence microscopy. Lastly, after 21 days, calcium content in the extracellular matrix was extracted and quantified using a commercially available kit.

RESULTS
Compared to controls (that is, osteoblasts under no electrical stimulation but otherwise similar experimental conditions), exposure of osteoblasts to alternating current (10 mA at a frequency of 10 Hz with a 50 % duty cycle) stimulation resulted in upregulation of mRNA expression of Collagen Type I and of Transforming Growth Factor b-1after 6 hours, a 46% increase (p<0.03) in cell proliferation after 2 days, and a 300% increase (p<0.02) in the amount of extracellular calcium after 21 days.

DISCUSSION
The major result of the present study is evidence that alternating electric current (10mA at 10 Hz) stimulation of osteoblasts results in enhanced cellular/molecular functions pertinent to the composition of both the organic and inorganic phases of bone. These responses were functions of time of exposure to the electric stimulus and ranged from enhanced Collagen Type I and Transforming Growth Factor-b1 gene expression after exposure for 6 hours, to enhanced cellular proliferation after exposure for 6 hours daily for 2 consecutive days, and to enhanced deposition of calcium-containing mineral in the extracellular matrix after exposure for 6 hours daily for 21 consecutive days.

In addition to contributing valuable information to bone cell physiology, these results provide insight into the mechanism(s) involved in bone healing under electrical stimulation (delivered through metal electrodes) which has been observed in animal models and reported in the literature (1). In this respect, the present study has made another contribution: novel polylactic acid/carbon nanotube composites, which are biodegradable and biocompatible substrates, may be used to deliver electric current stimulation to cell and, eventually, to tissues. Nanocomposites are materials with unique electrical and mechanical properties; to date, however, their promise and potential in bone repair, healing, and regeneration remain unexplored.

REFERENCES

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