Cellular / Molecular Responses of Electrically Stimulated Osteoblasts Cultured on Novel Polymer / Carbon Nanophase Substrates

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INTRODUCTION

Bone repair (occurring, for example, in osteotomies in animal models) can be accelerated through the use of electrical stimulation (Black *et al*, 1983). However, the underlying cellular/molecular-level mechanisms of osteogenesis under these conditions are not fully understood.

In order to elucidate some of these events, the present *in* vitro study investigated select cellular/molecular functions of rat calvarial osteoblasts (the bone-forming cells) cultured on current-conducting polylactic acid/carbon nanotube (PLA/CNT) composites following exposure to electrical stimulation.

MATERIALS AND METHODS

Preparation of Polymer / Nanophase Composite Substrates: Multi-walled carbon nanotubes (CNT) produced by the electric arc method (Ajayan, 1999) were added (20 % w/w) to a solution of dissolved polylactic acid (PLA; molecular weight 100,000) in chloroform. The polymer solution was sonicated for 15 minutes, allowed to air dry for 48 hours, and vacuum-dried at room temperature for 24 hours. These non-porous, composite substrates (each 4 cm in diameter) were sterilized in a 70% ethanol solution for 15 minutes prior to experiments with cells.

Cell Culture: Osteoblasts were isolated and characterized as previously described (Puleo *et al.*, 1991). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1 % penicillin/streptomycin under standard cell culture conditions (that is, a 37 °C, humidified, 5 % CO_2 / 95 % air environment). Cells of population number 2 - 3 were used in the experiments.

Alternating Current System: Osteoblasts were exposed to externally applied electrical stimulation using a laboratory system of our own design. Briefly, a stainless steel electrode was suspended into the supernatant media at a distance of 0.5 cm from osteoblasts cultured onto the surface of individual current conducting PLA/CNT substrates. An HP8110A pulse/function generator provided the electrical stimulus, consisting of an alternating current of 10 μA at a frequency of 10 Hz with a 50 % duty cycle.

Proliferation of Osteoblasts Under Electrical Stimulation: Osteoblasts (6,000 cells in DMEM, supplemented with 10% fetal bovine serum) were cultured on the PLA/CNT composite substrates under standard cell culture conditions for 24 hours. These cells were exposed to alternating electric current stimulation for 6 hours daily for 2 consecutive days. Controls were osteoblasts maintained under standard cell culture conditions, but no electrical stimulation, for similar periods of time. At the end of the experiments, cells were fixed with 10 % formalin, stained with Hoechst 33258, and counted *in situ* using fluorescence microscopy.

Formation of Mineral Deposits by Osteoblasts Under Electrical Stimulation: Osteoblasts (38,000 cells in DMEM, supplemented with 10% fetal bovine serum, 50 μg/mL ascorbic acid, and 10 mM β-glycerophosphate) were cultured on the PLA/CNT composite substrates under standard cell culture conditions for 48 hours. These cells were exposed to alternating electric current stimulation for 6 hours daily for 21 consecutive days. Controls were osteoblasts maintained under standard cell culture conditions, but no electrical stimulation, for similar periods of time. At the end of the 21 days, calcium content was assayed using a commercially available kit and following manufacture's.

Select Gene Expresion by Osteoblasts Under Electrical Stimulation: Osteoblasts (200,000 cells in DMEM, supplemented with 10% fetal bovine serum) were cultured on the PLA/CNT composite substrates under standard cell culture conditions for 48 hours. These cells were exposed to alternating electric current stimulation for 6 hours daily for 1, 7, 14, and 21 consecutive days. Controls were osteoblasts maintained under standard cell culture conditions, but no electrical stimulation, for similar periods of time. Cells were lysed and mRNA was extracted from these cells using standard procedures; Reverse Transcription Polymerase reaction techniques were used to examine expression of genes for alkaline phosphatase, bone sialoprotein, collagen type I,

osteocalcin, osteonectin, osteopontin, osteoprotegerin, and transforming growth factor-β.

RESULTS

Compared to controls (that is, osteoblasts under control (no electrical stimulation) conditions, exposure of osteoblasts to the electrical stimulation regime employed in the present study resulted in a 46% increase in cell proliferation, in a 300% increase in the amount of extracellular calcium during mineral deposition, and in differential gene expression of alkaline phosphatase, bone sialoprotein, collagen type I, osteocalcin, osteonectin, osteopontin, osteoprotegerin, and transforming growth factor-β.

DISCUSION

The present study provides evidence that electrical stimulation delivered through current-conducting biomaterials, such as PLA/CNT composites, modulates osteoblast functions responsible for the chemical composition of the organic and inorganic phases of the bone matrix. In addition, this research elucidates cellular/molecular-level mechanisms responsible for osteogenesis under electrical stimulation. Such functions can have major consequences for bone repair, healing, and regeneration under electrical stimulation.

REFERENCES

Black, J. *et al.*, *Bioelectrochem. and Bioenerg.*, *173*, (1984) 323. Ajayan, P.M., *Chem. Rev.*, *99*, (1999) 1787. Puleo, D.A. *et al.*, *J. Biomed. Mater. Res.*, *25*, (1991) 711.