Osteogenic Induction on Single-Walled Carbon Nanotube Scaffolds

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ABSTRACT

We demonstrated recently that CNT scaffolds support proliferation of mature osteoblasts as well as production of mineralized bone in vitro. More specifically, we showed that osteoblasts grown on single-walled (SW) CNTs retain plasma membrane electrical functions involved in secretory activities. Here we studied osteoinductive properties of SWCNT scaffolds as they support the growth of osteoblastic precursors, their differentiation into mature osteoblasts, and expression of membrane proteins involved in secretory processes. We used electrically neutral ("as prepared", AP-) SWCNTs, and SWCNTs chemically modified with carboxyl (-COOH, net negative electric charge), polyethylene glycol (PEG, electrically neutral), and poly-(m-amino-benzene sulfonic acid, PABS, net positive and negative electric charges) functional groups. We found that PEG-SWCNT and PABS-SWCNT showed the highest upregulation of ALP activity as a measure of osteoinduction. In addition, we found that AP-SWCNTs induced the expression of voltage-gated chloride channels ClC-3 and ClC-5 involved in secretory activities in hFOB osteoblasts. SWCNT preparations might be seen as osteoinductive materials with great potential for use in bone regeneration and repair.

1 INTRODUCTION

One main goal in bone tissue engineering is to create bone substitutes that will be incorporated successfully to native bones. Surgical procedures for bone repair utilize a variety of materials as prosthesis, grafts, and fillers. Common problems leading to multiple surgeries arise from quick degradation, poor contact, or rejection of the implant. Development of new biomaterials for bone tissue regeneration with improved biocompatibility, physical properties, and resistance to resorption is of crucial importance for the treatment of bone defects.

We propose the use of carbon nanotube (CNT)(1) scaffolds to stimulate the growth of osteoblasts and production of bone matrix in vitro(2). Production of bone materials is the result of secretory activities by osteoblasts, the bone-forming cells found on bone surfaces(3). In the fractured and wounded bone, successful repair and/or regeneration depends in large part on the capacity of osteoblast precursors to proliferate and fully differentiate into mature, secretory osteoblasts(4). In cases of large affected areas, the use of an adequate scaffold material that facilitates osteoblast proliferation, differentiation and secretion is of crucial importance(5). Compatibility of the regenerated bone with the existing bone will be determined by the chemical composition, physical properties, and nanotopography of the scaffold used to induce bone formation.

The unique physical-chemical characteristics of carbon nanotubes (CNT) make them a potentially ideal material for applications in bioengineering(6). With the purpose to identify CNT preparations with osteoinductive properties, we measured the proliferation and differentiation of mouse MC3T3 E1 and human hFOB preosteoblasts cultured on glass coverslips coated with SWCNTs.

2 MATERIALS AND METHODS

2.1 Materials

Bone morphogenetic protein-2 (BMP-2), ascorbic aid, and β−glycerophosphate were obtained from Sigma.

2.2 Cell culture

MC3T3 E1 preosteoblastic cells we grown for 20 days in differentiating and mineralizing medium containing 10 mM β-glycerophosphate, 50 μg/ml ascorbic acid, and 100 ng/ml BMP-2 in DMEM (Sigma), with the addition of 5% fetal bovine serum (Sigma) and antibiotics, at 37°C in a 5 % CO_2 humidified incubator. Human FOB preosteoblast differentiation was induced in DMEM medium at 39ºC. Cells were grown on glass coverslips and CNT-sprayed coverslips in 35-mm plastic culture dishes. The culture medium was changed every 3-4 days.

We studied osteoblast proliferation on CNTs in 5 day-old cultures. By day 5, control cells grown on glass reached confluence. Cell growth was calculated on the basis of number of cells per field of observation at a magnification of x 200 with an Olympus IX50 inverted microscope. Cell counts were performed with phase contrast and fluorescence microscopy.

2.3 CNT preparations

AP-SWNTs and nitric acid-treated SWCNTs (SWCNT-COOH) were obtained from Carbon Solution

Inc. (Riverside, CA), and SWCNTs chemically functionalized with poly-(*m*-aminobenzene sulfonic acid) (SWCNT-PABS) and poly ethylene glycol (SWCNT-PEG), were obtained by previously published synthetic methods.(7) SWCNT-COOH, SWCNT-PABS, and SWCNT-PEG were chosen for the present study on the basis of their net negative, zwitterionic, and neutral electric charge, respectively, at the pH of the experiment. Carbon nanotube coated glass coverslips were prepared as described previously.(8) Briefly, CNT samples (100 μg/mL) were sonicated in solvent (water for functionalized CNTs, and 95 % ethanol for AP-SWCNTs and AP-MWCNTs, multi-walled CNTs) for about 2 hours, and the resulting dispersion was sprayed onto pre-heated (ca. 80°C) glass coverslips. Sprayed coverslips were allowed to dry in air and used for cell culture after a sterilization procedure with UV irradiation overnight.

2.4 Alkaline phosphatise (ALP) activity

Cell cultures were assessed for total ALP expression using the pNPP substrate (Sigma) according to the manufacturer's protocol.

2.5 Chloride channel (ClC) expression

ClC expression was detected with standard Western blotting, using anti-ClC-3 and anti-ClC-5 antibodies from Santa Cruz Biotechnology.

3 RESULTS AND CONCLUSIONS

Osteoblast preparations reached confluency by day 4-5 (Fig. 1). We assessed osteogenic differentiation of MC3T3 E1 cells by measuring alkaline phosphatase (ALP) activity after 20 days in culture. We found that PEG-SWCNT and PABS-SWCNT showed the highest upregulation of ALP activity as a measure of osteoinduction. This suggests that electrically neutral and zwitterionic CNTs are better inducers of bone formation than negatively charged scaffolds.

AP-SWCNT scaffolds as well as the control on plastic dishes equally supported the growth of differentiated hFOB osteoblasts for up to 7 days (Fig. 2). We found that AP-SWCNTs induced the expression of voltage-gated chloride channels ClC-3 and ClC-5 involved in secretory activities in hFOB osteoblasts(9). We conclude that AP-SWCNT as well as some chemically modified SWCNT scaffolds possesses osteoinductive properties that induce differentiation of preosteoblasts. Electrically neutral AP-SWCNT preparations support the proliferation of preosteoblastic cells and their differentiation into mature osteoblasts, as well as the expression of the molecular machinery involved in secretory activities.

SWCNT preparations might be seen as osteoinductive materials with great potential for use in bone regeneration and repair.

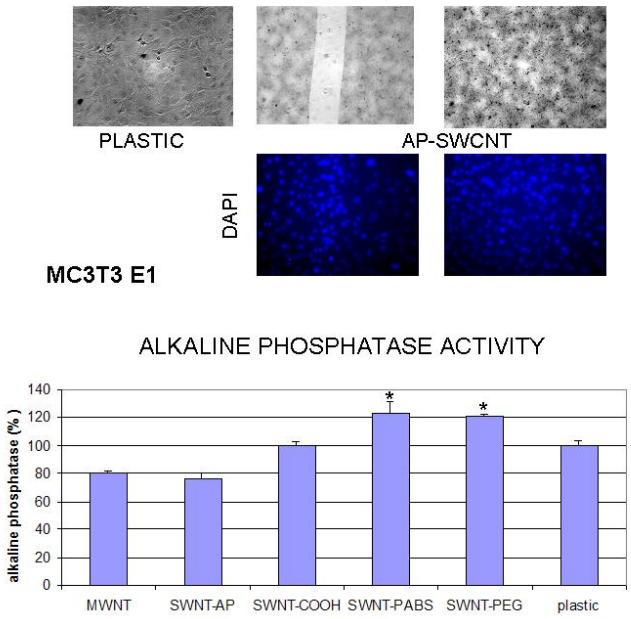

Figure 1. MC3T3 E1 osteoblasts cultured on AP-SWCNT and plastic as control. DAPI was used to stain cell nuclei. Alkaline phosphatase activity was measured with pNPP substrate on differentiated osteoblasts grown on different SWCNT preparations.

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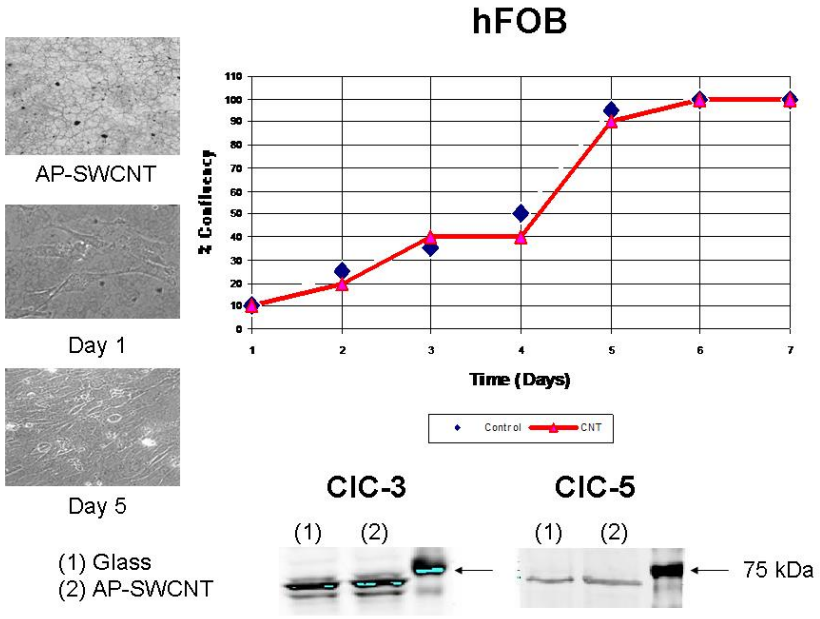

Figure 2. Growth curve of differentiated hFOB osteoblasts grown on plastic (control) and AP-SWCNTs. Western blots for the expression of chloride channels ClC-3 and ClC-5.

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