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Hydroxyapatite grafted carbon nanotubes and graphene nanosheets: Promising bone implant materials

Adebola Oyefusi [a], Opeyemi Olanipekun [a], Gururaj M. Neelgund [a], Deforest Peterson [b], Julia M. Stone [b], Ebonee Williams [b], Laura Carson [b], Gloria Regisford [c], Aderemi Oki [a,*]

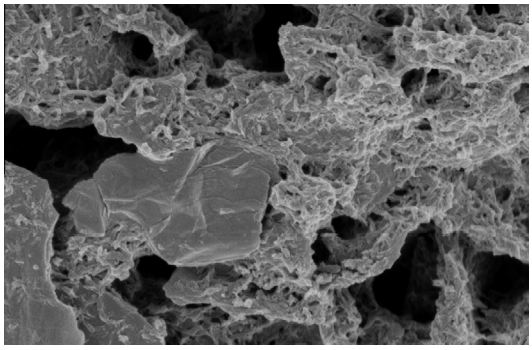
[a] Department of Chemistry, Prairie View A&M University, Prairie View, TX 77446, United States
[b] Cooperative Agricultural Research Center, Prairie View A&M University, Prairie View, TX 77446, United States
[c] Department of Biology, Prairie View A&M University, Prairie View, TX 77446, United States

HIGHLIGHTS

- Successful grafting of hydroxyapatite on CNTs and graphene.
- CNTs-HA and Gr-HA were used in proliferation of hFOB 1.19 cells.
- CNTs-HA and Gr-HA could be promising in bone tissue engineering.

GRAPHICAL ABSTRACT

Hydroxyapatite (HA) was successfully grafted to carboxylated carbon nanotubes (CNTs) and graphene nanosheets. The HA grafted CNTs and graphene nanosheets (CNTs-HA and Gr-HA) were further used to examine the proliferation and differentiation rate of temperature-sensitive human fetal osteoblastic cell line (hFOB 1.19).



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ABSTRACT

In the present study, hydroxyapatite (HA) was successfully grafted to carboxylated carbon nanotubes (CNTs) and graphene nanosheets. The HA grafted CNTs and HA-graphene nanosheets were characterized using FT-IR, TGA, SEM and X-ray diffraction. The HA grafted CNTs and graphene nanosheets (CNTs-HA and Gr-HA) were further used to examine the proliferation and differentiation rate of temperature-sensitive human fetal osteoblastic cell line (hFOB 1.19). Total protein assays and western blot analysis of osteocalcin expression were used as indicators of cell proliferation and differentiation. Results indicated that hFOB 1.19 cells proliferate and differentiate well in treatment media containing CNTs-HA and graphene-HA. Both CNTs-HA and graphene-HA could be promising nanomaterials for use as scaffolds in bone tissue engineering.

* Corresponding author. Fax: +1 936 261 3117.
E-mail address: aroki@pvamu.edu (A. Oki).

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Introduction

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH))_2$ (HA) is a bioceramic material often used for clinical bone grafting and implantation. HA has the ability to bond chemically with living bone tissue because of its chemical, compositional, biological, and crystal structure which are similar to native apatite in the human skeleton. Furthermore, the bioactivity and biocompatibility of HA enable osteoblast adhesion and proliferation. However, brittle HA is fragile in tension and offers low fracture toughness in comparison with natural bone. This drawback can be substantially minimized by strengthening and toughening HA with carbon nanomaterials while keeping its bioactivity [1].

Recently, carbon nanomaterials have attracted considerable attention due to their unique properties and wide range of applications [2–5]. Among the carbon nanomaterials, graphene (GR) is a new member with extraordinary electrical, thermal, and mechanical properties that have sparked current interest in materials science [6–8]. Carbon nanotube on the other hand have extensively been studied since its discovery. Its biocompatibility and bioactivity have also been tested and documented [9].

Till date, there are contradictory reports on the biomedical and biocompatibility of nanomaterials (particularly CNTs) due to its toxicity. However, the toxicity of CNTs has been shown to be reduced through chemical functionalization or coating with substances like polymers, hydroxyapatite or collagen [7,8,10]. Zanello et al. reported the mineralization of bone cells on chemically functionalized Single Walled Carbon nanotubes (SWCNTs) with HA. This being the first study on the potential use of SWCNTs as scaffold for bone growth [11]. Balani et al. also applied CNTs in HA coating using plasma spraying to improve the fracture toughness (by 56%) and enhance crystallinity (by 27%). The CNTs reinforced HA coating was further used to culture human osteoblast hFOB 1.19 cells to reveal its biocompatibility with living cells. Unrestricted growth of human osteoblast hFOB 1.19 cells has been observed near the CNTs regions assisted by CNTs surfaces to promote cell growth and proliferation [11–13].

Herein we report the fabrication of CNTs-HA and Gr-HA, and characterization, including the proliferation and differentiation of bone cells (hFOB 1.19) in the media containing these nanocomposites.

Materials and method

All the reagents were purchased from Aldrich and used without further purification unless otherwise noted. All the aqueous solutions were prepared with ultrapure water obtained from Milli-Q Plus system (Millipore).

Synthesis of CNTs-COOH and graphene nanosheet

CNTs were functionalized as described before by refluxing in a 3:1 mixture of concentrated sulfuric acid and nitric acid under stirring at 70 °C for 24 h, followed by centrifugation and repeated washings with distilled water [9]. Graphene nanosheet was prepared by ethylenediamine (EDA) reduction of graphite oxide (GO) which was prepared from graphite powder according to the Hummers and Offeman method [14].

In situ deposition of HA over CNT-COOH and graphene nanosheets

The CNTs-HA and Gr-HA were prepared as earlier reported [9,15]. The GR nanosheets (50 mg) and carboxylated CNTs (200 mg) were separately dispersed in 50 mL deionized water (DI) water by sonication for 5 min. To this, an aqueous solution of $Ca(OH)_2$ (0.01 mol L^{-1}) was added and the suspension was stirred under ambient conditions for 1 h. Then pH of the suspension was adjusted to ≈9 by dropwise addition of H_3PO_4 under constant stirring. The resulting HA functionalized nanomaterials (Gr-HA and CNTs-HA) were centrifuged, washed with DI water and dried under vacuum at 40 °C for 7 h (Schemes S1 and S2).

Characterization

FT-IR spectra were recorded using Thermo-Nicolet IR 2000 spectrometer, TGA was performed with a TGA Q500 instrument under nitrogen environment at a heating rate of 10 °C/min. Powder XRD patterns were recorded on scintag X-ray diffractometer (PAD X), equipped with Cu Kα photon source (45 kV, 40 mA) at scanning rate of 3°/min. SEM measurements were carried out a JEOLJXA-8900 microscope.

Human fetal osteoblast cell culture

Human fetal osteoblastic cells (hFOB 1.19) were cultured in a 1:1 mixture of Dulbecco's Modified Eagle's Medium (DMEM) and F-12 Medium (Kaighn's Modification of Ham's F-12 Medium; ATCC, Manassas, VA, USA). The culture medium was supplemented with 10% Fetal Bovine Serum (FBS), 0.3 mg/mL G418 (geneticin) and 1% Penicillin/Streptomycin (ATCC, Manassas, VA, USA). The hFOB 1.19 cells were plated in a six-well plate at a concentration of 1.0×10^5 cells/well. The cells were allowed to reach 50–80% confluency after which the Gr-HA and CNTs-HA were added in duplicate to the cell culture medium in 200 and 400 ng/mL dosages. Control cells were grown in cell culture medium alone. The plates were incubated at 34 °C and 5% CO_2. The day of plating was considered as day 0 of the experiment and the treatment medium renewed every 2–3 days.

Cellular proliferation and differentiation

The fetal osteoblastic cell line, hFOB 1.19 cells were cultured for 12 days at 34 °C. However, at day 3, 6, 9 and 12, proliferating cells were assayed by to determine total protein quantification and osteocalcin expression. In addition, after proliferation for 6 days, one half of the samples were placed in another incubator at 39 °C to stimulate differentiation. On day 9 and 12, cells stimulated to differentiate were harvested and the total protein quantification and osteocalcin expression were also assayed cells and compared to cells continue proliferating at 34 °C.

Total protein analysis

The hFOB 1.19 cells were collected on day 3, 6, 9 and 12, and washed with Phosphate Buffered Saline (PBS) after removal of treatment medium and lysed by scrapping in 500 μL celLytic per well (10 mM Tris Cl, 0.2% NP-40, and 2 mM phenylmethylsulfonyl fluoride; Sigma Aldrich, USA). The extracted solution was centrifuged and the supernatant stored at −20 °C. The total protein quantification of the supernatant was determined by Bradford Protein Assay (Bio-Rad Laboratory Inc., Hercules, CA, USA). Bovine Serum Albumin (BSA) standards with concentrations of 0, 1, 2.5, 5, 10, 20 and 40 μg/mL and the unknown protein were placed in a 96 well plate using the 300 μL microplate assay protocol published by BIO-RAD DC Protein assay kit. The absorbance was measured at 595 nm with a Spectra Max microplate reader (SpectraMax M2, Molecular Devices Corporation, Sunnyvale, CA, USA) and SoftMax Pros software (SoftMax Pro 5, Molecular Devices Corporation, Sunnyvale, CA, USA).