



Synergistic acceleration in the osteogenic and angiogenic differentiation of human mesenchymal stem cells by calcium silicate–graphene composites

Ming-You Shie^{a,b,c}, Wei-Hung Chiang^d, I-Wen Peter Chen^e, Wen-Yi Liu^{a,f}, Yi-Wen Chen^{a,g,*}

^a 3D Printing Medical Research Center, China Medical University Hospital, Taichung City, Taiwan

^b School of Dentistry, China Medical University, Taichung City, Taiwan

^c Department of Bioinformatics and Medical Engineering, Asia University, Taichung City, Taiwan

^d Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei, Taiwan

^e Department of Applied Science, National Taitung University, Taitung City, Taiwan

^f Department of Laboratory Science and Technology, China Medical University, Taichung City, Taiwan

^g Graduate Institute of Biomedical Sciences, China Medical University, Taichung City, Taiwan

ARTICLE INFO

Article history:

Received 8 September 2016

Received in revised form 16 November 2016

Accepted 7 December 2016

Available online 4 January 2017

Keywords:

Graphene

Calcium silicate

Osteogenesis

Angiogenesis

Human marrow stem cells

ABSTRACT

Recent exciting findings of the biological interactions of graphene materials have shed light on potential biomedical applications of graphene-containing composites. Owing to the superior mechanical properties and low coefficient of thermal expansion, graphene has been widely used in the reinforcement of biocomposites. In the present study, various ratios of graphene (0.25 wt%, 0.5 wt% and 1.0 wt%) were reinforced into calcium silicate (CS) for bone graft application. Results show that the graphene was embedded in the composites homogeneously. Adding 1 wt% graphene into CS increased the young's modulus by ~47.1%. The formation of bone-like apatite on a range of composites with graphene weight percentages ranging from 0 to 1 has been investigated in simulated body fluid. The presence of a bone-like apatite layer on the composites surface after immersion in simulated body fluid was considered by scanning electron microscopy. In vitro cytocompatibility of the graphene-contained CS composites was evaluated using human marrow stem cells (hMSCs). The proliferation and alkaline phosphatase, osteopontin and osteocalcin osteogenesis-related protein expression of the hMSCs on the 1 wt% graphene-contained specimens showed better results than on the pure CS. In addition, the angiogenesis-related protein (vWF and ang-1) secretion of cells was significantly stimulated when the graphene concentration in the composites was increased. These results suggest that graphene-contained CS bone graft are promising materials for bone tissue engineering applications.

© 2016 Published by Elsevier B.V.

1. Introduction

Autograft possesses all the characteristics indispensably for new bone formation, osteogenesis, osteoconductivity, and osteoinductivity and has been considered as the gold standard in the medical applications. Alternatively, the bone cements have shown great potential for hard tissue regeneration via cell proliferation simulation and calcified tissue formation [1–3]. These bioactive materials development for hard tissue regeneration brought many beneficial advantages in the field of bone substitute. Moreover, the bioactive materials exist large amount of surface areas that can be active and directly bonded to the nature bone tissues. Calcium silicate (CS)-based materials have received great attention in recent years due to its excellent bioactivity when comparing to calcium phosphate-based materials. Recently, several

researchers demonstrated that CS could have assisted to the hard tissue formation and regeneration because of the Si ion release and fast apatite formation ability of the CS-based material [4–6]. In our previous study, a fast setting CS cement was successfully produced with a mixture of CaO, SiO₂, and Al₂O₃, which showed a significant setting time reduction. The CS cement presents good osteoconduction and reduces inflammation in primary human dental pulp cells (hDPCs). Moreover, previous reports show that CS-based materials are able to promote osteogenesis differentiation from stem cells, such as human mesenchymal stem cells (hMSCs), hDPCs and human periodontal ligament cells (hPDLs) [7–10]. However, the poor fracture toughness and wear resistance of CS-based materials limit the implant performance and life span in a load-bearing environment [11]. In order to improve mechanical property of CS, several studies had been implemented by combining CS with other materials, e.g. polymers and carbon-based materials, for morphological and functional modifications [12,13]. In particular, the mechanical performance and biocompatibility of CS had been improved significantly by reinforcement with carbon-based nanomaterials in several reviews [14–16].

* Corresponding author at: 3D Printing Medical Research Center, China Medical University Hospital, Taichung City, Taiwan.

E-mail address: evinchen@mail.cmu.edu.tw (Y.-W. Chen).

Graphene is a single-atom-thick layer of sp^2 hybridized carbon atoms arranged in a honeycomb lattice [17]. Graphene-based materials exhibit several spectacular and extraordinary properties such as high surface area, excellent thermal and electrical conductivity, high optical transparency, and mechanical strength. Therefore, several researches have been proposed or are under development in areas including electronics, biological engineering, filtration, lightweight composite, photovoltaics and energy storage [18]. Graphene oxide (GO), a chemically modified graphene offers potential for the ton scale production, consist of hydrophobic p domains in the core region and ionized groups around the GO edges. The biocompatibility of graphene-based materials has been confirmed in a suitable amount for bio-applications of tissue engineering, drug delivery [19], bioimaging [20], and photo-thermal therapy [21] in recent years. Wang et al. [22] concluded that graphene-based materials under a low percentage of 20 $\mu\text{g/mL}$ (2 wt%) did not present toxicity to human fibroblast cells, and a dose > 50 $\mu\text{g/mL}$ (5 wt%) shows the cytotoxicity such as decreasing cell adhesion. Recently, most of in vitro studies indicate that graphene with a suitable percentage can promote stem cell adhesion, growth, expansion, and differentiation [23–25]. Nayak et al. stated that the presence of graphene based materials remarkably accelerates the differentiation of hMSCs at a rate comparable to differentiation under the influence of additional growth factors, such as BMP-2. Based on previous literatures, we believe that graphene is excellent candidates to be used as an addition material for the scaffold manufacture for bone tissue engineering [26].

Furthermore, Mukherjee and his colleagues have reported that graphene-based materials exhibit angiogenic properties allowing the formation of new capillaries from the pre-existing vasculature [27]. In order to investigate the endothelial cell proliferation, the endothelial cells were incubated with graphene-based materials in different concentrations for 24 h. Their results demonstrated the synthesis and characterization of graphene-based materials exhibiting pro-angiogenic activity, observed by several in vitro and in vivo assays including the cell proliferation, tube formation, intracellular reactive oxygen species (ROS) formation and NO signalling. In addition, Park et al. have studied and demonstrated that graphene-based materials can effectively enhance the secretion of growth factors including VEGF, FGF-2, and hepatocyte growth factor (HGF) which are the major growth factors that induce angiogenesis [28]. Those investigators exhibited the angiogenesis and blood vessel regeneration potential of graphene based materials no matter in vitro or vivo assays.

In fact, the vascularization was supplied the oxygen and nutrients exchanged to the cells during bone remodelling and various studies has been demonstrated angiogenic differentiation is a prerequisite for osteogenic in vivo [29,30]. Thus, a key factor in critical bone defects regeneration is vascularization around the materials. In this study, considering the importance of osteogenesis and angiogenesis, we incorporated graphene with CS cement as a composite to promote bone and capillaries tissue formation in bone regeneration. The graphene and CS materials in composites are expected to synergistically direct the differentiation of hMSCs toward osteogenic lineage. Furthermore, graphene is assumed to enhance the angiogenesis and induce the capillaries formation. The osteogenesis properties will be evaluated by quantitative measurement on bone nodule formation for hMSCs in order to explore the capabilities of CS/graphene composite and suitability to apply this composite scaffold in tissue engineering for bone and blood vessel in bone.

2. Materials and methods

2.1. Preparation of CS/graphene composite

The method used here for the preparation of CS powder has been described elsewhere [11]. In brief, reagent grade CaO (Sigma-Aldrich, St. Louis, MO), SiO_2 (High Pure Chemicals, Saitama, Japan), and Al_2O_3 (Sigma-Aldrich) powders were used as matrix materials (composition: 70% CaO, 25% SiO_2 , and 5% Al_2O_3). The oxide mixtures were then

sintered at 1400 °C for 2 h using a high-temperature furnace. The graphene was prepared via water-assisted liquid phase exfoliation of graphite [28]. Briefly, 50 mg microcrystalline graphite powder (325 mesh, 99.995% pure, purchased from Alfa Aesar, Ward Hill, MA) was immersed in *N*-methyl-2-pyrrolidone (NMP) mixture of water. The mass fraction of water in NMP was 0.2. The initial concentration of graphite was fixed at 5 mg/mL for exfoliation. The materials were batch sonicated for 6 h in a bath sonicator (Elma sonic P60H, Switzerland) at a fixed nominal power and frequency of 100 W and 37 kHz, respectively. Sample dispersions were hanged on for overnight in between sonication and centrifugation, and were centrifuged at 3000 rpm for 30 min using a Hettich, EBA20. The upper 75% of the colloidal supernatant were collected and dried in an oven to yield the graphene sheets. Then, the CS and graphene were ball-milled in ethyl alcohol using a centrifugal ball mill (S 100, Retsch, Hann, Germany) for 6 h. The nominal weight content of graphene in composites is listed in Table 1. The composite was mixed with water and molded in a Teflon mold (diameter: 6 mm, height: 3 mm). There was enough cement to fully cover each well of the 24-well plate (GeneDireX, Las Vegas, NV) to a thickness of 2 mm for cell experiments. All samples were stored in an incubator at 100% relative humidity and 37 °C for 1 day of hydration.

2.2. Setting time and strength

After the powder was mixed with liquid, the cements were placed into a cylindrical mold and stored in an incubator at 37 °C and 100% relative humidity for hydration. The setting time of the cements was tested according to International Standards Organization (ISO) 9917-1 standard. For evaluation of the initial and final setting time, each material was analyzed using Gilmore needles. The time was recorded when the needle failed to create a 1-mm deep indentation in three separate areas. After being taken out of the mold, the specimens were again incubated at 37 °C in 100% humidity for 1 day. The diametral tensile strength (DTS) testing was conducted on an EZ-Test machine (Shimadzu, Kyoto, Japan) at a loading rate of 1 mm/min. The maximal compression load at failure was obtained from the recorded load-deflection curves. The ten specimens were examined for each of the materials. After the samples were de-molded and incubated at 37 °C in 100% humidity for 1 day. The diametral tensile strength of the specimens was conducted on an EZ-Test machine (Shimadzu, Kyoto, Japan) at a loading rate of 1 mm/min. The maximal compression strength at failure was determined from the recorded load-deflection curves. At least 10 specimens from each group were tested.

2.3. Surface characterization

The phase composition of the cements was analyzed using X-ray diffractometry (XRD; Bruker D8 SSS, Karlsruhe, Germany), run at 30 kV and 30 mA at a scanning speed of 1°/min. The morphology of the cement specimens was coated with gold and examined under a scanning electron microscope (SEM; JSM-6700F, JEOL) operated in the lower secondary electron image (LEI) mode at 3 kV accelerating voltage. Raman measurements were performed using B&W Tek'si-Raman® spectrometer (B&W Tek Inc., Newark, Delaware). 300 mW of 785 nm radiation from a diode pumped laser was used for excitation. All the spectra reported here were collected under an exposure time set to a single 5 s accumulation in a range from 400 cm^{-1} to 1800 cm^{-1} , for a total of 905 data points. Rayleigh scattering was blocked using a holographic notch filter, and the tilted baselines of some SERS spectra were flattened using commercial Raman software (B&W Tek Inc.). The elemental compositions of the CS-graphene composites were characterized with an electron spectroscopy for chemical analysis (ESCA, PHI 5000 VersaProbe, ULVAC-PHI, Kanagawa, Japan).

Download English Version:

<https://daneshyari.com/en/article/5434981>

Download Persian Version:

<https://daneshyari.com/article/5434981>

[Daneshyari.com](https://daneshyari.com)