DENTAL MATERIALS 33 (2017) 525–535

Available online at www.sciencedirect.com

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Growth and accelerated differentiation of mesenchymal stem cells on graphene-oxide-coated titanate with dexamethasone on surface of titanium implants

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ARTICLE INFO

Article history:
Received 17 October 2016
Received in revised form
21 February 2017
Accepted 3 March 2017

Keywords:
Titanium implants
Graphene oxide
Dexamethasone delivery
Cell proliferation
Osteogenic differentiation

ABSTRACT

Objective. In this study, the objective is to construct graphene-oxide-coated titanate on titanium foils as drug vehicle to enhance cell proliferation and osteo-differentiation of rat bone mesenchymal stem cells (rBMSCs).

Methods. Graphene oxide (GO) sheets obtained using the modified Hummer's method and characterized by AFM were coupled with bioactive titanate on Ti implants (GO-Ti) pretreated by alkali, followed by reduction (rGO-Ti). They were characterized by Raman spectroscopy, XPS, SEM, FTIR and contact angle. After dexamethasone (DEX) was loaded onto them (DEX-GO-Ti and DEX-rGO-Ti), cell proliferation of rBMSCs on them was evaluated by CCK-8 and F-actin staining, and differentiation through alkaline phosphatase activity, mRNA expression, and calcium nodules.

Results. The obtained GO sheets were monolayers from AFM. Raman spectra exhibited two prominent peaks at D and G bands, and the I(D)/I(G) ratios increased from 0.96 to 1.68 after reduction. XPS proved the existence of oxygenated functional groups for GO-Ti and the reduction of their intensity for rGO-Ti. From SEM, GO and rGO were evenly coated on nanostructures. DEX-GO-Ti absorbed most amount of DEX and released in a sustained manner. CCK-8 results showed that DEX-GO-Ti showed excellent performance on promoting cell proliferation. RMBSCs on DEX-GO-Ti presented greatly high expression of calcium, proteins and mRNA related to osteogenic differentiation.

Significance. GO coated titanate nanostructrues on surfaces of Ti foils by a simple self-assembly method, showed excellent vechiles for DEX. The construct promoted proliferation and accelerated osteogenic differentiation of rBMSCs, and would be prosperous for their further applications.

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http://dx.doi.org/10.1016/j.dental.2017.03.001
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1. Introduction

Titanium (Ti)-based materials have demonstrated their superiority among metallic materials in the biomedical field, such as orthopedics and dentistry, due to their excellent mechanical strength and favorable biocompatibility [1]. However, there are some disadvantages of current Ti-based implant materials that may hinder their further applications. For example, the biological inertness of Ti may induce the generation of fibrous tissue, which causes micro-movement between the bone and the interface, and ultimately, leads to the failure of implants. Various trials have been proposed to address this issue, and one of the current methods is to spontaneously deposit on the surface hydroxyapatite, similar to the composition of mineral part of the bone or osteoinductive ions [2,3]. The hydroxyapatite coating has been shown to greatly improve the bioactivity of implants and accelerate osteogenic differentiation, but the poor mechanical properties and its weak bonding to the substrate usually affect the long-term reliability under load [4].

In the past few years, graphene, a flat monolayer of carbon atoms closely packed into a honeycomb shaped two-dimensional lattice, has attracted increasing attentions for its wide applications in biomedicine, such as drug delivery carriers, imaging agents and tissue engineering substrates due to their outstanding physicochemical, optical and mechanical properties [5]. Being only one atom thick, it introduces the least amount of artificial material possible and has a large number of remarkable properties, which can confer beneficial properties on implants to minimize the possibility of inflammatory responses caused by the implantation of foreign materials [6]. Graphene has the highest Young's modulus (0.5–1 TPa) among any known material and is not brittle [7,8]; the excellent mechanical properties endow it to play a key role in hard tissue engineering [9]. The derivative, graphene oxide (GO) consists of hydrophobic π domains in the core region and ionized groups around the GO edges. These are features that substantially enhance its interactions with proteins through hydrophobic and electrostatic interactions [10] and they could potentially enhance the specific differentiation of mesenchymal stem cells [11]. Evidences have shown that GO promoted the adhesion, proliferation and mineralization in the presence of osteogenic chemical inducers [12,13]. It was reported that the strong noncovalent binding abilities of graphene allowed it to act as a preconcentration platform for osteogenic inducers, which further accelerated osteogenic differentitation [11].

Based on these advantages, graphenes were poured or spin-coated on coupling agent-functionalized Ti for accelerating bone regeneration [14,15], yet it was inferior at in vivo osseointegration and drug delivery behavior. Titanate nanostructure with open pores on a titanium implant is favorable for cell growth and tissue [16,17]. GO sheets were electrochemically deposited on titanium as mechanical hardener and surface activity regulator, demonstrating improved compressive strength and enhanced cell viability, proliferation, differentiation and osteogenic activities of human osteoblast-like cells [12,18]. However, it is not easy to regulate the number of layers which play an important role in bio-functions [19,20] and likely to come off from the substrate due to the weak bonding via physical interaction. In addition, in situ local delivery of drugs and growth factors within implants is desirable to achieve specific differentiation [21]. For example, La et al. reported to load bone morphogenetic protein-2 (BMP-2), a well-known osteogenic factor, onto Ti substrates coated with GO, which enabled loading of large doses and the sustained release of the protein and promotes bone formation in vitro [22,23]. However, BMP-2 may lose bioactivity over a short time due to its short half-life, which limits its local delivery, and does not always exhibit efficacy in bone defect repair in vivo [24]. Dexamethasone (DEX) is a synthetic glucocorticoid usually applied in changing the expression levels of many proteins and enzymes contributed in bone differentiation [25]. In this study, GO sheets were incorporated into a hydrothermally prepared porous titanate scaffold on Ti implants through a simple assembly method, which were used as DEX delivery platforms for enhancing osteogenic differentiation of rat bone marrow stem cells (rBMSCs) characteristics of self-renewal, differentiation potential, and pluripotency [26].

2. Experimental

2.1. *Preparation of GO*

GO was prepared by oxidation and exfoliation of commercially available graphite (Alfa Aesar) by modified Hummers method [27]. Briefly, graphite flakes (Alfa Aesar) were pre-oxidized by concentrated H_2SO_4, $K_2S_2O_8$, and P_2O_5 by keeping the mixture at 80 °C for over 5 h. The mixture was then left to room temperature following by diluting with ultrapure water, filtering and washing thoroughly. The mixture was dried and re-oxidized by slowly adding H_2SO_4 and $KMnO_4$ under 0 °C with stirring. The mixture was then kept at 35 °C for ~2 h, and diluted slowly using ultrapure water. H_2O_2 was added to the obtained solution till the color changed into brilliant yellow. The solution was then filtered and washed by diluted HCl and ultrapure water for several times. To remove the residue ions in the samples, the samples were dialyzed in ultrapure water for over 1 week. The resulted GO was dried at 60 °C in a vacuum oven overnight. The dried GO was exfoliated in water by ultrasonication for 1–2 h to obtain the single layer GO dispersion (1.5 mg/mL), characterized by atomic force microscopy (AFM).

2.2. *Preparation and characterization of GO and rGO on Ti foils*

Titanium foils (99.7% Ti TA2, BaoTi Group Co. Ltd., $7 \times 7 \times 0.8\,mm^3$) were mechanically polished by water sandpaper (grades 400, 600, 800 and 1000) sequentially and then ultrasonically cleaned with acetone, ethanol and ultrapure water successively. Nanonetwork-structured sodium titanate thin films were prepared on the surface of titanium foil by the alkali-hydrothermal reaction. A typical preparation process was as follows: Ti foils were immersed in 20 mL of 10 M NaOH aqueous solution at 60 °C for 24 h and washed repeatedly with ultrapure water [17]. Then the titanium foils (remarked as Control and used as a reference material and a support for GO) were then immersed in 3% ethanol solution of 3-aminopropyltriethoxysilane (3-APTES, 3% ethanol solution of APTES) for 30 min, washed with ethanol and water

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