Applied Surface Science 293 (2014) 124–131

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Sodium functionalized graphene oxide coated titanium plates for improved corrosion resistance and cell viability

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ARTICLE INFO

Article history:
Received 8 August 2013
Received in revised form
17 December 2013
Accepted 20 December 2013
Available online 30 December 2013

Keywords:
Sodium
Graphene oxide
Titanium
Orthopedic implants
Corrosion
Biocompatibility

ABSTRACT

Surface functionalization is an important process that has been adopted to well explore the applications of nanomaterials. In this context, we demonstrate the sodium functionalized graphene oxide (NaGO) as an excellent candidate for increasing the life time of titanium (Ti) based ortho-implants. As-prepared aqueous dispersion of NaGO was used to assemble NaGO sheets on commercially pure Ti (CpTi) plates by heat controlled spin coating. The resulting wrinkled NaGO sheets play a dual role in implant material, i.e., passive layer against corrosion and biocompatible scaffold for cell viability. The preparation, physicochemical properties, and biocompatibility of NaGO coatings formed on CpTi were reported. The electrochemical polarization studies demonstrate the relative susceptibility of control GO and NaGO coatings to corrosion, which outline that the NaGO coating act as a geometric blocking layer and hence prevent the implant surface from contacting corrosive media. The immunofluorescence and cell proliferation studies performed using human dermal fibroblasts cells showed that NaGO coatings significantly ($P < 0.05$) enhanced the cellular viability for longer in vitro culture period (15 days) than control GO and pristine CpTi.

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1. Introduction

Biocompatible materials are crucial for drug delivery, tissue engineering and implant devices. A variety of materials, including metallic, polymer nanocomposites, and carbon-based nanostructures have been studied intensively for this application [1–3]. Particularly, two-dimensional networks of graphene oxide (GO) have recently shown substantial promise [4–7]. The strategy of functionalizing nanomaterials on the surface of bio-active substrate to form advanced coating holds great potential to revolutionize the future of nano-biomaterial applications [8,9]. Reports have shown that due to its aromatic scaffold nature graphene and GO are potential for promoting the cell behavior including attachment, growth, proliferation and differentiation. This can promote the local concentration of extracellular matrix (ECM) including collagen, laminin and fibronectin by non-covalent binding [10]. However, studies must be extensively performed in order to take advantages of properties of GO for fabricating advanced biomaterial scaffolds. For instance, a simple chemical modification and substrate stiffness can provide dynamic changes in intra-cellular structures and phenotype [10–12]. Thus, it is of potential interest to consider surface functionalized GO nanomaterial substrates as a template or scaffold for growing therapeutically important cell lines. In this regard, surface roughness, corrosion resistance (against aggressive electrolyte environment), and viability and mitochondrial activity of cell cultures on the nanomaterial coated substrate are the important factors to be taken into account.

An approach of utilizing functionalized nanomaterial coatings is certainly an optimized strategy for not only achieving synergistic effects, but also to improve biocompatibility and corrosion resistance [13]. A combination of functional nanomaterial is a feasible

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0169-4332/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.apsusc.2013.12.114

means of overcoming the drawbacks of low cell adhesion, low cell proliferation and lack of cell viability on the conventional bio-active substrates [13,14]. Among the alkali metals, elemental sodium (Na) is an essential macronutrient (major mineral source) required to animals for regulating many physiological processes such as, regulation of blood volume, blood pressure, osmotic equilibrium and pH [15]. In addition to potassium (K), Na components are widely used as a vehicle in the pharmaceutical industries to promote the bioavailability of therapeutic agents [16]. Furthermore, Na^+ channel is an active membrane regulates several pathophysiological processes [17]. Due to its fundamental biological role, learning more about their synergistic property with novel GO material will be of considerable interest. Park et al. found that the metal ions can electrostatically interact with oxygen functionalities such as epoxy, hydroxyl and carboxyl groups in the basal plane and edges of GO sheet [18]. They have anchored Ca^{2+} and Mg^{2+} ions on GO papers by filtering the aqueous solution of calcium chloride and magnesium chloride, respectively. Likewise Na^+ ions also be anchored on GO via solution blending procedures.

Fibroblasts are the well-known model system that has been actively used for several biochemical and physiological processes [19,20]. Dermal fibroblast cells exist within the dermis layer of skin responsible for generating connective tissue and play a vital role in wound healing [21]. Due to its functional role researchers are intensively attempting to generate mature dermal fibroblasts to treat burn wounds. In some extent, fibroblasts have been used to promote the survival rate for human stem cells, which easily undergo cell apoptosis [22]. Therefore, studying the interaction between dermal fibroblast cells and Na^+ functionalized GO coating will be important to advance the development of biomaterial scaffold.

In this work, we explore the formation and characteristics of Na functionalized GO (NaGO) coatings on commercial pure titanium (CpTi) substrates. Physiochemical properties of NaGO coatings were evaluated by Raman and X-ray photoelectron (XPS) spectroscopic techniques and contact angle measurements. Morphology was observed through field emission scanning electron microscope (FE-SEM), high resolution transmission electron microscope (HR-TEM) and atomic force microscope (AFM). Susceptibility of prepared NaGO coating material toward corrosion under simulated body fluid (SBF) solution was determined in comparison with pristine CpTi and control GO (CGO) on CpTi, respectively. Human dermal fibroblast (HDFn) cells were used as the model system to understand the fundamental biocompatibility of NaGO coating. Further, the specific role of Na functionalization on GO in relation to surface roughness and hydrophilicity was also discussed.

2. Experimental details

2.1. Materials

Graphite powder (45 μm), potassium permanganate ($KMnO_4$), hydrogen peroxide (H_2O_2) and staining dyes (Rhodamine labeled phalloidin (RLP) and 4′,6-diamidino-2-phenylindole (DAPI)) were obtained from Sigma Aldrich, U.S.A. Sodium chloride, and sulfuric acid were obtained from Daejung, Korea. Unless otherwise stated, all chemicals were reagent grade, and used as received. HDFn cells and supplemented medium 106 were purchased from Invitrogen. Cell proliferation reagent WST-1 assay kit obtained from Roche. Bio-implant grade CpTi was purchased from Nilaco Corporation, Japan.

2.2. Preparation of graphite oxide

Graphite oxide was prepared by modified Hummers method described elsewhere in the literature [23]. Briefly, 2 g of graphite powder was stirred with 50 ml H_2SO_4 in an ice bath for 30 min, 7.0 g of $KMnO_4$ was added gradually under stirring. Then the reaction mixture was brought to 35 °C and stirred for next 2 h. Subsequently the reaction mixture was placed in an ice bath. Excess deionized (D.I.) water was poured rapidly, and 35% H_2O_2 was added drop wise until the gas formation ceases, completely. The contents were filtered using 0.2 μm nylon membrane filter, and dried under vacuum for 12 h at room temperature (R.T., ∼25 °C) to afford brown graphite oxide powder.

2.3. Preparation of NaGO

0.75 g of graphite oxide was stirred with 30 ml aqueous filtrate of saturated sodium chloride (SSC) solution at R.T. for 24 h. Then filtered, washed thrice with deionized (D.I.) water, and dried under vacuum at R.T. for 12 h. Control sample was obtained by stirring same quantity of graphite oxide with 30 ml D.I water. The 0.2 g of SSC treated and control graphite oxide samples were sonicated (40 kHz) with 20 ml D.I. water for 3 h, and centrifuged at 3000 rpm for 30 min to afford the dispersions of NaGO and CGO with the solid content of 3.0 mg/ml, respectively.

2.4. Formation of NaGO coatings on CpTi

CpTi plates were cut into small pieces (2 cm × 2 cm) and cleaned by sonicating with D.I. water for an hour, dried under vacuum at 75 °C for 12 h. CGO and NaGO dispersion were deposited on cleaned CpTi at 50 °C through heat controlled spin coating (HCSC) method, described in our previous report [24]. A three step spin coating procedure, 200 rpm–300 s; 500 rpm–20 s; 2000 rpm–160 s, was used. Samples were dried under vacuum at 75 °C for 5 h. Uncoated CpTi, and CGO and NaGO coated CpTi were coded as Bare CpTi, CGO-CpTi and NaGO-CpTi, respectively.

2.5. Physiochemical characterization of NaGO coatings

FE-SEM images of CGO-CpTi and NaGO-CpTi were obtained by a JSM-7500F (JEOL) field emission scanning electron microscope with 15 kV accelerated voltage. The HR-TEM images were obtained by JEOL JEM 2100F. AFM studies were carried out in an intermittent air mode using Bio-AFM: Nanowizard II, (JPK Instruments) equipped with silicon probes (Tap 150-G). CA measurements were performed by scissile drop method in a contact angle goniometer (GBX New Technologies Development, Korea). ∼10 μl droplet of D.I. water was suspended from the tip of a microliter syringe. Images were collected with the camera and exact contact angle (θ) was determined from images of droplets using drop shape analysis software. The reported θ value is an average of at least 5 individual measurements performed at different locations of the specimen. XPS studies were performed using an angular resolved electron analyzer with a monochromatic Al Kα source (model Theta Probe, Thermo Fisher Scientific). The emitted electrons were detected at the angles between 23° and 83°. Raman spectra was recorded with a Micro-RAMAN system (Ramboss-500i) using a 100X objective lens at R.T., with a 633 nm He–Ne laser beam and 1800 lines/mm grating.

2.6. Electrochemical characterization

A conventional three electrode cell was used for the electrochemical measurements: reference electrode = Ag/AgCl; counter electrode = platinum foil; working electrode = test material. Potentiodynamic polarization studies were carried out for the test specimens in simulated body fluid solution (SBF). The procedure followed for the preparation of SBF solution was adopted from earlier report [13]. A potentiostat (model PGSTAT 302N, AUTOLAB, the

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