Journal of Industrial and Engineering Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

In vitro investigation of electrophoretically deposited bioactive hydroxyapatite/chitosan coatings reinforced by graphene

Marija Đošić[a], Sanja Eraković[b], Ana Janković[b], Maja Vukašinović-Sekulić[b], Ivana Z. Matić[c], Jovica Stojanović[a], Kyong Yop Rhee[d,*], Vesna Mišković-Stanković[b,d,**], Soo-Jin Park[e,***]

[a] Institute for Technology of Nuclear and Other Mineral Raw Materials, Bulevar Franš d'Eperea 86, 11000 Belgrade, Serbia
[b] Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11 000 Belgrade, Serbia
[c] Institute of Oncology and Radiology of Serbia, Pasterova 14, 11 000 Belgrade, Serbia
[d] Department of Mechanical Engineering, Kyung Hee University, Yongin 449-701 South Korea
[e] Department of Chemistry Inha University, Incheon 402751, South Korea

ARTICLE INFO

Article history:
Received 16 August 2016
Received in revised form 13 November 2016
Accepted 5 December 2016
Available online xxx

Keywords:
Coating materials
Composite materials
Corrosion
Electrochemical impedance spectroscopy
X-ray diffraction
Scanning electron microscopy
SEM

ABSTRACT

Graphene (Gr) and natural polymer chitosan (CS) were introduced to hydroxyapatite (HAP) to produce a three-component composite coating, which was fabricated by cathodic electrophoretic deposition on Ti substrates in an ethanol suspension. These HAP/CS/Gr coatings were characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), X-ray photoelectron spectroscopy (XPS) and electrochemical measurements and found that the graphene into HAP/CS composites significantly improves their morphology, thermal stability, and bioactivity. Both HAP/CS and HAP/CS/Gr composite coatings are classified as non-cytotoxic when tested against healthy peripheral blood mononuclear cells (PBMC), while antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* could not be verified.

Introduction

The application of orthopedic and dental implants as bone replacements has improved the medical treatment of bone diseases. The general idea was that implant materials have to be biocompatible, so wide range of materials has been developed in the past decades. Ceramics and glasses, as implant materials, have a wide range of applications since their chemical composition could be adjusted in such a manner as to obtain desired properties of implant, e.g. chemical response of the surrounding tissue and degradation rate [1–3].

Natural bone is a nanocomposite consisting of a mineral phase (bone-like apatite) and a polymer matrix (collagen). Therefore synthetic or natural-synthetic composites are promising candidates for tissue engineering applications [4,5]. Hydroxyapatite (HAP) has a chemical structure similar to the mineral part of natural bone, and is bioactive, enabling the formation of bone-like apatite on its surface [6–8]. On the other hand, HAP by itself is brittle, which limits its applicability [9]. For this reason, the development of bioactive composites with both HAP and polymers is of great scientific interest.

Chitosan is a semicrystalline homopolymer made up of β (1→4) linked *N*-acetyl-D-glucosamine and D-glucosamine subunits [10]. Due to its antimicrobial activity, chemical stability, and biocompatibility, chitosan has been utilized in biomedical implants [11,12]. It can stimulate bone repair and regeneration by promoting cellular differentiation. All the above-mentioned unique properties combined with the chitosans ability to provide support for viable human osteoblasts and chondrocytes, make it suitable for future engaging in bone and cartilage tissue engineering [13]. Normally, it is insoluble in aqueous solutions when the pH is above 7. However, in dilute acids, when the pH falls below 5, free amino groups are protonated making the chitosan molecules fully soluble [14]. Its cationic nature in aqueous solutions makes this polymer attractive for the fabrication of

* Corresponding author. Fax: +82 31 202 6693.
** Co-corresponding author at: Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11 000 Belgrade, Serbia. Fax: +381 11 3370 387.
*** Co-corresponding author. Fax: +82 32 867 5604.
E-mail addresses: rheeky@khu.ac.kr, joveedu@naver.com (K.Y. Rhee), vesna@tmf.bg.ac.rs (V. Mišković-Stanković), sjpark@inha.ac.kr (S.-J. Park).

http://dx.doi.org/10.1016/j.jiec.2016.12.004
1226-086X/

composite coatings using electrophoretic deposition. Chitosan also has an excellent film forming ability [12]. By itself it has poor mechanical strength, but incorporation of nanofillers leads to improved physical and chemical properties. Therefore chitosan is ideal for a composite, due to covalent bonding and physisorption of filler material to the polymer matrix [15]. The presence of chitosan in composite coatings improves coating adhesion. A HAP/chitosan composite shows improved flexibility relative to pure HAP, allowing easier design of implants [16]. The improvement in the mechanical properties can be attributed to interfacial bonding between the polymer matrix and HAP [17]. Additionally, incorporation of HAP in the composite structure increases the osteoconductivity of the material, improving its bioactivity and bone-bonding ability [18,19].

Some properties of chitosan, including its mechanical properties and conductivity, can be modified by the incorporation of graphene, which controls the swelling rate of the polymer [20]. Graphene is composed of a single layer of sp^2-hybridized carbon atoms arranged in a two-dimensional honeycomb lattice. Graphene and its related derivatives can be used for biomedical applications (drug and gene delivery, cancer therapy, biosensors, etc.) [21] since it can be applied as a reinforcement for composites due to its excellent thermal and mechanical characteristics, including a large specific surface area and electrical conductivity [22–24]. It has been reported that during implant integration, osteoblast adhesion and proliferation were stimulated by the electrical conductivity of graphene [23]. The inclusion of graphene into polymer or ceramic matrices strongly influences the mechanical, thermal, and electrical properties of the starting material [6,25–29].

It is well known that electrophoretic deposition (EPD) of composite coating on the metal substrate is very suitable method for obtaining potential hard tissue implants [6–8,30]. Great number of papers are available dealing with different systems based on graphene oxide, hydroxyapatite and/or chitosan composites with the aim to be applicable as biomaterials [9,20,24,31]. It has been postulated that graphene-based materials cause physical damage on bacterial membranes upon direct contact, resulting in the release of intracellular contents. This rupture is originated by the blade-like action of low thickness graphene-based materials (a few nanometers) having sharp edges. The EPD of graphene oxide/hydroxyapatite/chitosan composite coatings with rather high graphene oxide content (e.g. 0.0, 1.0 and 1.7 wt%) is reported in the literature [30]. Using graphene instead of graphene oxide in composite coatings for biomedical application has great benefits due to high-purity chemical in the case of pristine graphene with no remnants from the synthesis (e.g. GO, rGO). Additionally, smaller quantity of graphene component in composite coatings is beneficial due to the potential final application as biomaterial for hard tissue implantation.

With the aim of producing a biomaterial capable of mimicking natural bone characteristics, composite coatings based on HAP, chitosan, and graphene were electrophoretically deposited on titanium substrates. The focus of this research was to employ graphene in small quantity (0.01 wt%) for obtaining high quality three-component composite coatings, which is highly beneficial due to potential application as biomaterial. Additionally, the influence of chitosan and graphene on the hydroxyapatite in the three-component composite coatings was performed through detailed analysis of the hydroxyapatite crystallographic parameters (e.g. *d*-spacing, the unit cell parameters *a* and *c*, the unit cell volume). The goal is to obtain a non-cytotoxic hybrid ceramic-polymer coating, reinforced with graphene nanosheets that will simultaneously ensure bioactivity as well as increase corrosion resistance in in vitro conditions.

Experimental

Materials

HAP (nanopowder, <200 nm particle size) and chitosan (medium molecular weight) were supplied by Sigma-Aldrich. 99.2%-pure graphene nanopowder (AO-3) was purchased from Graphene Supermarket, USA. The average thickness of the graphene nanoflakes was 12 nm, with approximately 30–50 layers overlapping. Titanium foil from Sigma Aldrich (0.25 mm thickness, 99.7% trace metals basis) was used as a substrate for electrophoretic deposition. Ti samples of different dimensions were used for different measurements: 25×10 mm for surface analysis, 40×20 mm for impedance spectroscopy, and 10×5 mm for cell-based assays. Mechanical pretreatment of Ti metal plates was carried out by polishing with grit emery paper and wet 0.3 μm alumina, followed by ultrasonication in acetone for 15 min. After polishing, plates were stored in ethanol to match final deposition conditions.

Electrophoretic deposition

Electrophoretic deposition was done in 100 mL of prepared absolute ethanol suspensions. Depending on the sample, suspensions contained: 1 wt% of nanosized HAP (for HAP coating deposition); 1 wt% of nanosized HAP and 0.05 wt% of chitosan (for HAP/CS coating deposition); 1 wt% of nanosized HAP, 0.05 wt% of chitosan, and 0.01 wt% of graphene (for HAP/CS/Gr coating deposition); or 0.05 wt% of chitosan (for CS coating deposition); all at an approximate pH of 2.4 which was adjusted by the addition of 6 M HCl. All prepared suspensions underwent several 30-min rounds of sonication, followed by vigorous stirring with a magnetic stirring rod in order to maximize the homogeneity of the final suspension [32].

A three-electrode cell was used for cathodic electrodeposition. During depositions, suspensions were continuously stirred. A Ti plate served as a working electrode, while two platinum panels were used as counter electrodes, each placed parallel to the Ti electrode at a distance of 1.5 cm to ensure uniform coating on both sides of the Ti foil. Coatings were deposited using a constant voltage of 60 V and a deposition time of 3 min for HAP/CS/Gr and HAP/CS, and 30 s for HAP, at room temperature. Pure chitosan coatings were deposited on Ti at an applied voltage of 30 V and a deposition time of 12 min. Electrodeposited coatings were air dried for 24 h at room temperature [32].

Characterization

The surface morphology of electrodeposited coatings was analyzed by field-emission scanning electron microscopy (FE-SEM) using LEO SUPRA 55 (Carl Zeiss, Germany) microscope operated at an acceleration voltage of 200 kV. Fourier transform infrared spectroscopy (FT-IR) was carried out using KBr pellets in a Spectrum One spectrophotometer (Perkin Elmer, USA). The scan was carried out in the range of 450–4000 cm^{-1} with a spectral resolution of 0.5 cm^{-1}. X-ray photoelectron spectroscopy (XPS) was carried out using a K-Alpha spectrometer (Thermo Scientific, USA) equipped with Al Kα X-ray radiation (1486.6 eV) and a micro-focused monochromator. Elemental depth profiling was performed using Ar ion sputtering. Thermogravimetric analysis (TGA) was conducted by TGA Q5000 IR/SDT Q600 (TA instruments, Eden Prairie, MN, USA) from 30 °C to 1000 °C under N_2 (50 mL/min), at a heating rate of 20 °C/min. A Philips PW 1051 powder diffractometer with Ni-filtered Cu K_α radiation ($\lambda = 1.5418$ Å) was employed to assess the phase composition of electrodeposited coatings before and after immersion in simulated body fluid (SBF). X-ray

Please cite this article in press as: M. Đošić, et al., In vitro investigation of electrophoretically deposited bioactive hydroxyapatite/chitosan coatings reinforced by graphene, J. Ind. Eng. Chem. (2016), http://dx.doi.org/10.1016/j.jiec.2016.12.004