Composites Part B 104 (2016) 26–34

Contents lists available at ScienceDirect

Composites Part B

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

Biological activity of electrochemically synthesized silver doped polyvinyl alcohol/graphene composite hydrogel discs for biomedical applications

M.M. Abudabbus [a], I. Jevremović [a], A. Janković [a], A. Perić-Grujić [a], I. Matić [b], M. Vukašinović-Sekulić [a], D. Hui [c], K.Y. Rhee [d, **], V. Mišković-Stanković [a, d, *]

[a] Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000, Belgrade, Serbia
[b] Institute of Oncology and Radiology of Serbia, Pasterova 14, 11 000, Belgrade, Serbia
[c] Department of Mechanical Engineering, University of New Orleans, New Orleans, LA, 70148, United States
[d] Department of Mechanical Engineering, Kyung Hee University, Yongin, 449-701, South Korea

ARTICLE INFO

Article history:
Received 9 April 2016
Received in revised form
8 July 2016
Accepted 17 August 2016
Available online 20 August 2016

Keywords:
Polymer-matrix composites (PMCs)
Chemical properties
Electron microscopy
Non-destructive testing

ABSTRACT

Silver/poly(vinyl alcohol), Ag/PVA, and silver/poly(vinyl alcohol)/graphene, Ag/PVA/Gr, composite hydrogel discs were produced by freezing/thawing, followed by electrochemical reduction of Ag^+ ions inside respective matrices, at constant voltage in specially designed electrochemical cell. Raman, FT-IR and FE-SEM characterized interactions between PVA, graphene and silver nanoparticles. Ag/PVA and Ag/PVA/Gr discs classified as non-cytotoxic towards peripheral blood mononuclear cells (PBMC) according to MTT cytotoxicity test, while exhibiting antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The slow silver release and high remaining silver content of ~67–68 wt % after 28 day immersion in simulated body fluid confirmed that both composites can preserve their sterility over time.

1. Introduction

The study and preparation of metal particles on the nanometer scales have attracted considerable interest in both fundamental and applied research. Difference in physical and mechanical properties of metal nanosized particles is prominent compared to macroscopic materials. Silver nanoparticles (AgNPs) in particular, can be used in wound dressings and antimicrobial materials [1–10]. AgNPs exhibit enhanced antibacterial properties compared to bulk silver due to high surface area and high fraction of surface atoms in contact with bacteria or fungi [11–13]. However, lack of sufficient chemical and physical stability limits the application of AgNPs on wound care products to some extent [14–16]. A wide range of protective and stabilizing agents has been used to increase the stability and dispersion of metal nanoparticles in aqueous media, successfully controlling the particle's nanostructure [8,10,17–20]. One of them is polyvinyl alcohol (PVA), a water-soluble synthetic polymer with high hydrophilicity, biocompatibility and non-toxicity. PVA hydrogels have attracted great interest in the fields of tissue engineering and regenerative medicine as matrices for repairing and regenerating a wide variety of tissues and organs [21]. Recently, research has focused on composites of PVA and graphene, especially due to their improved mechanical strength [22–29]. Highly pure metal nanoparticles as well as the possibility of precise control of particle size can be achieved by electrochemical synthesis adjusting several parameters, such as applied potential, current density, reaction temperature, and swelling solution [1,3,6,30,31]. Furthermore, absence of undesired products is especially desirable property and good for future application in synthesis of metal nanoparticles on a large scale. Also, chemical methods for synthesis of nanoparticles involve the usage of reducing agents, which can be toxic to living organisms, whereas the electrochemical route of nanoparticles synthesis involves use of mild reaction conditions and non-toxic reaction precursors and is therefore especially attractive for biomedical applications. Different electrochemical methods like

* Corresponding author. Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000, Belgrade, Serbia.
** Co-corresponding author. Department of Mechanical Engineering, Kyung Hee University, Yongin, 449-701, South Korea.
E-mail addresses: rheeky@khu.ac.kr (K.Y. Rhee), vesna@tmf.bg.ac.rs (V. Mišković-Stanković).

http://dx.doi.org/10.1016/j.compositesb.2016.08.024
1359-8368/

potentiostatic [32–34], galvanostatic [35], pulsed sonoelectrochemical technique [36,37], single and double pulse [38] deposition methods have been shown to be very promising in the preparation of a variety of materials with nanometer dimension.

In this work, silver/polyvinyl alcohol (Ag/PVA) and silver/polyvinyl alcohol/graphene (Ag/PVA/Gr) composite hydrogel discs were produced by electrochemical reduction of Ag^+ ions at a constant voltage, by *in situ* synthesis of silver AgNPs inside PVA and PVA/Gr hydrogels, previously crosslinked by successive freezing and thawing cycles. The aim was to optimize the electrochemical synthesis parameters (swelling time of hydrogel in electrolyte solution, implementation time and applied voltage) and to investigate the cytotoxicity, antibacterial activity, silver release and sorption properties of prepared Ag/PVA and Ag/PVA/Gr hydrogel discs. These materials are aimed for medical applications as wound dressing, soft tissue implants and drug carriers.

2. Experimental

2.1. Materials

Chemicals used were all p.a. grade, PVA powder (''hot soluble'', fully hydrolyzed $M_w = 70000–100000$ Sigma, St. Louis, US), $AgNO_3$ (M. P. Hemija, Belgrade, Serbia), KNO_3 (Centrohem, Stara Pazova, Serbia), graphene powder (Graphene Supermarket, USA). In all the experiments ultra-pure water from Milli-Q system (Millipore, Billerica, MA, USA) was used.

2.2. Preparation of Ag/PVA and Ag/PVA/Gr composite hydrogel discs

PVA powder was first dissolved in hot dH_2O (90 °C) in order to obtain PVA aqueous solution with a concentration of 10 wt % PVA. To prepare a PVA/Gr colloid dispersion, graphene was added to dissolved PVA under vigorous stirring to yield a final concentration of 10 wt % PVA and 0.01 wt % Gr. Subsequently, dispersion was cooled down to room temperature and sonicated for 30 min to ensure uniformity.

After cooling down, the PVA solution and PVA/Gr colloid dispersion were poured into a Petri dish (height = 5 mm), and subjected to successive freezing and thawing in 5 cycles, where one cycle involved freezing for 16 h at −18 °C and thawing for 8 h at 4 °C. The obtained hydrogels were cut into small discs ($d = 10$ mm). The swelling of the PVA and PVA/Gr hydrogels was performed by immersing the pre-weighed hydrogel discs in solutions of different $AgNO_3$ concentration (0.25, 0.5, 1 and 3.9 mM $AgNO_3$ and 0.1 M KNO_3) at 25 °C, during different periods of swelling time (between 24 h and 72 h) in order to determine the optimal time of swelling at room temperature in the dark.

Swollen PVA and PVA/Gr hydrogels were used to produce Ag/PVA and Ag/PVA/Gr composites, respectively, by electrochemical reduction of Ag^+ at a constant voltage, by *in situ* synthesis of silver nanoparticles inside a PVA and PVA/Gr matrix, respectively [39]. Briefly, specially designed electrochemical cell was employed, with two Pt plates horizontally placed face-to-face, as working and counter electrodes. The hydrogel discs were placed in between two electrodes in the special glass holder. The polarity of the electrodes was changed after the half of the implementation time, in order to reduce the formation of thin layer of metallic Ag at the surface of the hydrogel in the contact with the working electrode and to ensure the synthesis of Ag nanoparticles inside the hydrogel matrix. The following parameters were varied: applied voltage (50 V–200 V) and implementation time (2 min–4 min), using MA 8903 Electro-Phoresis Power Supply (Iskra d.d., Ljubljana, Slovenia). The electrodes were cleaned by rinsing with HNO_3 (1:1) during 5 min and dH_2O water after each experiment.

2.3. Methods of characterization

2.3.1. Raman spectroscopy

HR-Raman analysis of Ag/PVA and Ag/PVA/Gr hydrogel discs was carried out using a Renishaw Invia Raman spectrophotometer equipped with a 514 nm argon laser. The intensity used was 10% of total power (50 mW). The spectral range of the analysis was carried out between 3500 and 100 cm^{-1}.

2.3.2. Field emission scanning electron microscopy (FE-SEM)

Surface morphology of Ag/PVA and Ag/PVA/Gr hydrogel discs was analyzed by FE-SEM (LEO SUPRA 55, Carl Zeiss, and Germany) operated at acceleration voltage of 200 kV and TESCAN MIRA 3 XMU.

2.3.3. Fourier transform infrared spectroscopy (FT-IR)

FT-IR analysis of Ag/PVA and Ag/PVA/Gr hydrogel discs was carried out using KBr pellets in a Perkin Elmer (spectrum one system) spectrophotometer. The scan was carried out in the range of 450 cm^{-1} to 4000 cm^{-1} with a spectral resolution of 0.5 cm^{-1} respectively.

2.3.4. Cytotoxicity

2.3.4.1. Preparation of peripheral blood mononuclear cells. Peripheral blood mononuclear cells (PBMCs) were washed three times in separating solutions (Histopaque-1077, Sigma Aldrich). Finally, cell slurry was re-suspended in nutrient medium (RPMI 1640, pH 7.2, supplemented with 10% heat-inactivated for 30 min at 56 °C fetal bovine serum (FBS), 3 mM l glutamine, 100 mg dm^{-3} streptomycin, 0.1 IU dm^{-3} penicillin and 25 mM HEPES).

2.3.4.2. Treatment of PBMC. PBMC were seeded in nutrient medium, in a 24-well plate in which either pure PVA, PVA/Gr hydrogel, Ag/PVA and Ag/PVA/Gr composite hydrogel discs were added. Ag/PVA and Ag/PVA/Gr composites were obtained by electrochemical reduction of PVA and PVA/Gr hydrogels preswollen in 0.25, 0.5, 1 and 3.9 mM $AgNO_3$ solution. All hydrogels were sterilized prior to experiments by UV irradiation for 30 min by placing them under UV-C lamp integrated in the laminar airflow hood. Pure PVA, PVA/Gr hydrogels, Ag/PVA and Ag/PVA/Gr composites in the form of cylinders ($d = 2$ mm, $\delta = 5$ mm) were placed in the 24-well plate. In order to study the effects of AgNP content, Ag/PVA and Ag/PVA/Gr composites obtained from PVA and PVA/Gr hydrogels swollen in solutions of varying $AgNO_3$ concentrations (0.25, 0.5, 1, 3.9 mM) were used. To each well, nutrient medium alone, serving as a blank, or the cell suspension was added. In the second experimental series, effects of Ag/PVA and Ag/PVA/Gr composite hydrogels on PBMC stimulated for proliferation were studied. Experimental set-up was the same except addition of mitogen phytohemagglutinin (PHA) that stimulates the proliferation of PBMCs.

2.3.4.3. Determination of target cell survival. The survival of target cells was determined using MTT test, in order to assess the activity of living cells by their mitochondrial dehydrogenase activity [40]. Standard MTT assay was employed based on reduction of the yellow salt - tetrazolium dye MTT (4,5 dimethyl thiozol − 2-yl) − 2,5 − diphenyl tetrazolium bromide) to its soluble formazan (purple colored crystals) by mitochondrial dehydrogenase activity of living cells. The absorbance was measured using 570 nm using Multiskan EX Thermo Labsystems (Thermoscientific, Waltham, MA, USA). Since the number of live cells is directly proportional to the absorbance of viable, metabolically active MTT treated cells, for calculation of the cell survival (S), absorbance of newly formed formazan was used: