International Journal of Biological Macromolecules xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Nano-gold assisted highly conducting and biocompatible bacterial cellulose-PEDOT:PSS films for biology-device interface applications

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ARTICLE INFO

Article history:
Received 4 May 2017
Received in revised form 7 September 2017
Accepted 17 September 2017
Available online xxx

Keywords:
Bacterial cellulose
Gold nanoparticles
Pedot:pss
Nanocomposites
Biocompatibility
Electrical conductivity

ABSTRACT

This study reports the fabrication of highly conducting and biocompatible bacterial cellulose (BC)-gold nanoparticles (AuNPs)-poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) (BC-AuNPs-PEDOT:PSS) composites for biology-device interface applications. The composites were fabricated using *ex situ* incorporation of AuNPs and PEDOT:PSS into the BC matrix. Structural characterization, using scanning electron microscopy (SEM), atomic force microscopy (AFM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), and x-ray diffraction (XRD) analysis, confirmed the uniform nature of the synthesized BC-AuNPs and BC-AuNPs-PEDOT:PSS composites. Four-point probe analysis indicated that the BC-AuNPs and BC-AuNPs-PEDOT:PSS films had high electrical conductivity. The composites were also tested for biocompatibility with animal osteoblasts (MC3T3-E1). The composite films supported adhesion, growth, and proliferation of MC3T3-E1 cells, indicating that they are biocompatible and non-cytotoxic. AuNPs and PEDOT:PSS, imparted a voltage response, while BC imparted biocompatibility and bio-adhesion to the nanocomposites. Therefore, our BC-AuNPs-PEDOT:PSS composites are candidate materials for biology-device interfaces to produce implantable devices in regenerative medicine.

1. Introduction

Cellulose is considered the material of choice for future applications because of its high stability and abundance. Bacterial cellulose (BC), produced by a class of acetic acid bacteria, is a unique biological material appearing as a net-like pellicle woven from ultrafine nanofibers with a diameter of approximately 100 nm [1]. Its distinguishing features include good biocompatibility, high hydrophilicity, water holding capacity (WHC), mechanical strength, and crystallinity, *in-situ* moldability, and good mass transport properties [1–3]. These exceptional properties have led to BC being applied widely as a constituent of food, paper, medical and optoelectronic devices, and auditory diaphragms [1,4–6]. In particular, because of its excellent biological properties, scientists have explored the biomedical applications of BC; for example, as a drug carrier [7], and as a tissue engineering scaffold to replace skin [8,9], blood vessels [1], cartilage [2], and corneas [10,11]. In addition to its intrinsic properties, the nanofibrillar and nanoporous structure of BC makes it a suitable matrix for the adsorption of organic polymers and inorganic nanomaterials, permitting the synthesis of multifunctional nanocomposites [12].

One such class of BC composites is obtained by incorporating conducting polymers (CP) into BC [13,14]. These nanocomposites are generally termed electroconductive hydrogels (ECHs) [15]. These polymeric blends combine the inherent properties of an electro-conductive polymer with that of a hydrogel, resulting in a combination of biological and electrical properties. ECHs are potential candidates for application in data collection, processing, and storage. In addition, the biocompatibility of these composites permits them to be used, for example, in molecular recognition, biocatalysis, and genetic probing. Therefore, ECHs are candidate materials for building biology-device interfaces in implantable devices for personalized and regenerative medicine [16].

Bio-polymers and conducting polymers have been combined to produce bio-device interfaces using electrochemical methods. The amino polysaccharide chitosan, electro-deposited on a gold-coated wafer, has been applied as a bio-device interface for biosensor

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http://dx.doi.org/10.1016/j.ijbiomac.2017.09.064
0141-8130/

assembly and in a bio-based redox capacitor [17]. Wang et al. [18], synthesized a biocompatible and electroconductive composite by blending polypyrrole (PPy) with poly (D,L) lactide (PDLLA). *In vivo* application of such ECHs in rats caused only very minor inflammation [18]. Poly (3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), an impressive conducting polymer, is well known for its good film-forming properties, tunable conductivity, and exceptional thermal stability [19]. PEDOT:PSS has been considered as the most successful conducting polymer in terms of its practical applications [20]. Shi et al. have reported various physical and chemical approaches to further enhance its electrical conductivity [19]. PEDOT:PSS has also been investigated as a potential conducting polymer for the synthesis of ECHs. For example, PEDOT:PSS was coated on the surface of silk, and the resulting material was tested for use in recording electrocardiographs, sensory-evoked signals, and electroencephalographs for experimental animals [21]. PEDOT:PSS has also been blended with gellan gum to synthesize functional ECHs [22]. BC, being a natural hydrogel, resembles the native extracellular matrix (ECM). Its 3-dimensional (3D) nanofibrous network, good biocompatibility, and high WHC, offers an excellent matrix for the fabrication of ECHs. The integration of biology with electronics (bioelectronics) has many potential applications. Briefly, they will enable development of handheld devices (biosensors) for multiple analyses in medical diagnosis, environmental sampling, lab on-a-chip devices, and smart fabrics, and will enhance energy conversion and harvesting [13]. Shi et al. [23] reported the synthesis of BC— Polyaniline (PAni) and BC-PPy ECHs, which responded well to voltage changes, providing signal amplification for analysis and detection. The ECHs were biocompatible and non-cytotoxic when tested in animal cells [23]. Thus, BC—CP electro-active hydrogels provide both electrical and biocompatible properties, permitting their application as bio-device interfaces in implantable devices and in regenerative medicine [24].

Nanoparticles (NPs) made from noble metals, including gold NPs (AuNPs), exhibit good optical and electronic properties, in addition to chemical inertness and biocompatibility, increasing their application in functional bio-related materials [25], including those used in cell imaging [26], gene delivery [27], and photothermal therapy [28]. AuNPs have been immobilized on the surfaces of various materials, including sol-gel matrices, polymers, and other nanomaterials, providing a structural network for the immobilization of AuNPs onto the surface of an electrode. Nanocomposites of dopamine-enzyme-metal nanoparticles (Au or Pt) demonstrated better glucose detection than conventional multistep sensors [29]. Protein and enzyme biosensors have been fabricated by combining AuNPs with carbon nanotubes [30] and carbon nanospheres [31]. However, the supporting materials in these nanocomposites offer limited biocompatibility, which may limit their potential applications. Zhang et al. [32] reported the synthesis of BC-AuNPs nanocomposites for use as H_2O_2 biosensors. They concluded that BC, with its nanofiber network and excellent biocompatibility, would provide excellent support for fabrication of AuNPs biosensors [32]. Paula et al. synthesized gold coated BC-PANi composites for electroconductive applications. They observed a large increase in the electric conductivity of BC-PANi composites that incorporated Au nanoparticles [33]. They observed a more defined redox wave shape in the presence of AuNPs, which is an indication of better diffusional processes through the solid material. In addition, AuNPs promoted the formation of a phenazine structure by inducing an interchain redox reaction after electrochemical cycling. However, the authors did not report the biological aspects or their potential to be recommended for developing biological interface devices. In the present study, taking into account previous studies and our own experiences with these materials, we believe that the combination of AuNPs and PEDOT:PSS will result in multifunctional materials possessing high electrical conductivity and biocompatibility, with potential applications in biology-device interface materials.

In the present study, we report the synthesis of novel BC-AuNPs-PEDOT:PSS nanocomposites using an *ex situ* method. The structural and electronic properties of the obtained nanocomposites were evaluated using various methods. The biocompatibility of the nanocomposites was then investigated using animal osteoblasts. The excellent structural, electrical, and biological features of the nanocomposites suggested that they have potential for application as a biology-device interface for the production of implantable devices.

2. Materials and methods

2.1. BCE sheet production and synthesis of AuNPs

Gluconacetobacter hansenii PJK (KCTC 10505BP) culture and BC sheets were produced as described previously [34]. AuNPs were also synthesized following a previously described method with some modifications [35]. Briefly, 50 mL of 0.5 mM $HAuCl_4$ was placed in a beaker and heated to boiling. Five milliliters of 38.8 mM sodium citrate were added to the boiling $HAuCl_4$ solution. Initially, the color of the mixture was light yellow, which changed to purple and finally turned a dark, wine-like red.

2.2. Synthesis of BC-AuNPs nanocomposites

BC-AuNPs nanocomposites were synthesized through the *ex situ* penetration of AuNPs into a never-dried BC sheet. Briefly, never-dried BC was cut into small squares of equal mass and introduced into the AuNPs suspension and incubated with shaking at 150 rpm at room temperature for 48 h. The BC-AuNPs nanocomposite films were then rinsed with deionized (DI) water. Half of these films were dried at 80 °C, whereas the remaining films were used directly to synthesize BC-AuNPs-PEDOT:PSS composites.

2.3. Synthesis of BC-AuNPs-PEDOT:PSS nanocomposites

The obtained BC-AuNPs films were used to synthesize BC-AuNPs-PEDOT:PSS nanocomposites before drying. Briefly, never-dried BC-AuNPs composite films were incubated in the PEDOT:PSS solution (0.13%, weight/volume) for 48 h at ambient temperature with shaking at 150 rpm. The obtained BC-AuNPs-PEDOT:PSS composite films were washed with DI water and dried at 80 °C.

2.4. Characterization

Fourier transform infrared spectroscopy (FTIR) spectra of BC, BC-AuNPs, and BC-AuNPs-PEDOT:PSS nanocomposites were recorded using a Spectrum GX & Auto Image FTIR spectrophotometer [spectral range: 4000–400 cm^{-1}; beam splitter: Ge coated on KBr; detector: deuterated triglycine sulfate; resolution: 0.25 cm^{-1} (step selectable); PerkinElmer, Melville, NY, USA]. The samples were mixed with KBr and formed into pellets prior to FTIR analysis (IR grade; Merck, Darmstadt, Germany). Field-emission scanning electron microscopy (FE-SEM) images of the samples were obtained using Hitachi S-4800 (Tokyo, Japan) and Horiba EDX-350 instruments (Tokyo, Japan). Briefly, samples were fixed on a brass holder and then coated with osmium tetroxide using a VD HPC-ISW osmium coater (Tokyo, Japan) before FE-SEM observation. Transmission electron microscopy (TEM) of the AuNPs, BC, BC-AuNPs, and BC— AuNPs-PEDOT:PSS composite films was performed on a JEOL JEM-2100 microscope operated at ultra-high resolution (200 kV) and the particle size distribution was determined using ImageJ software. Topographical images of the native BC and the

Please cite this article in press as: S. Khan, et al., Int. J. Biol. Macromol. (2017), http://dx.doi.org/10.1016/j.ijbiomac.2017.09.064