Materials Science and Engineering C 70 (2017) 121–131

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

On the biological performance of graphene oxide-modified chitosan/polyvinyl pyrrolidone nanocomposite membranes: *In vitro* and *in vivo* effects of graphene oxide

Nafiseh Mahmoudi [a], Abdolreza Simchi [a,b,*]

[a] Department of Materials Science and Engineering, Sharif University of Technology, P.O. Box 11155-9161, Azadi Avenue, 14588 Tehran, Iran
[b] Institute for Nanoscience and Nanotechnology, Sharif University of Technology, P.O. Box 11365-9466, Azadi Avenue, 14588 Tehran, Iran

ARTICLE INFO

Article history:
Received 27 May 2016
Received in revised form 2 August 2016
Accepted 24 August 2016
Available online 28 August 2016

Keywords:
Electrospinning
Graphene oxide
Composite membrane
Biological performance
Animal model

ABSTRACT

Nanofibrous structures that mimic the native extracellular matrix and promote cell adhesion have attracted considerable interest for biomedical applications. In this study, GO-modified nanofibrous biopolymers (GO) were prepared by electrospinning blended solutions of chitosan (80 vol%), polyvinyl pyrrolidone (15 vol%), polyethylene oxide (5 vol%) containing GO nanosheets (0–2 wt%). It is shown that GO nanosheets significantly change the conductivity and viscosity of highly concentrated chitosan solutions, so that ultrafine and uniform fibers with an average diameter of 60 nm are spinnable. The GO-reinforced nanofibers with controlled pore structure exhibit enhanced elastic modulus and tensile strength (150–300%) with a controllable water permeability to meet the required properties of natural skins. Potential use of the GO-modified biocomposites for tissue engineering is demonstrated in mesenchymal stem cell lines extracted from rat's bone marrow. The biocompatibility assay and SEM imaging reveal that the nanofibrous structure promotes the attachment and maintained characteristic cell morphology and viability up to 72 h. *In-vivo* evaluations in rats show that a faster and more efficient wound closure rate (about 33%) are attained for the 1.5% GO nanofibrous membrane as compared with control (sterile gauze sponges).

1. Introduction

Polymeric nanofibers have recently emerged as a promising material with numerous possibilities for various biomedical and industrial applications. Very large surface area-to-volume ratio with high porosity and small pore size have made polymeric nanofibers attractive for tissue engineering, medical prostheses, wound dressing and drug delivery as well as water filtration and sensing applications [1]. The protein components of the extracellular matrix (ECM) in the tissue, which regulate many aspects of cell behavior, are fibrous with diameters ranging from 50 to 150 nm depending on tissue type [2,3]. The organization of protein fibers such as collagens, elastin, keratin, laminins, fibronectin and vitronectin provides a high level of mechanical strength and offers locations for cell adhesion and proliferation [4]. Therefore, polymeric nanofibers which closely match the structure and function of ECM fibers are of great interest in tissue engineering. Studies have shown that structural features and type of nanofibrous materials directly influence the attachment, proliferation, migration and growth of cell [5].

Three production methods of electrospinning, self-assembly and phase separation are commonly used to fabricate fibrous scaffolds for tissue engineering [3,6,7]. The electrospinning is the most simplest and cost-effective method to prepare long and continuous fibers with diameter ranging from macro-scale to nano-scale [8]. As a result, fabrication of ultrafine fibers from a verity of natural and synthetic polymers by electrospinning of polymer solutions has extensively been investigated [9,10]. Studies have shown that electrospun nanofibrous scaffolds offer numerous advantages over other scaffolds because of their innate properties in view of porosity, fiber morphology and strength as well as their resemblance to the native topographical features of ECM [9,11,12]. Meanwhile, natural polymers are more favored because of their superior biocompatibility and resorbable degradation products [13]. In order to exploit the favorable biological properties of natural polymers and the mechanical strength of synthetic polymers, hybrid nanofibers represent a major advancement in tissue engineering [14].

Chitosan (CS), a partially *N*-deacetylated derivative of chitin, is a natural polymer which has exclusively been studied for tissue engineering applications [15,16]. Chitosan is well known for its biodegradability, biocompatibility and antibacterial activity [17,18]. It is also nonantigenic and biologically renewable [19]. Therefore, fabrication of CS nanofibers with controllable size and directional alignment has attracted significant attention for the development of scaffolds with structures close

* Corresponding author at: Department of Materials Science and Engineering, Sharif University of Technology, P.O. Box 11155-9161, Azadi Avenue, 14588 Tehran, Iran.
E-mail addresses: nafiseh_mahmoudi@mehr.sharif.ir (N. Mahmoudi), simchi@sharif.edu (A. Simchi).

http://dx.doi.org/10.1016/j.msec.2016.08.063
0928-4931/

to ECMs. During the last decade, the electrospinning of CS has intensively been researched [20]. In order to improve its poor spinnability and mechanical strength, blending with synthetic polymers such as poly (ethylene oxide) (PEO), poly (vinyl alcohol) (PVA), poly(L-lactide) (PLA), poly(glycolic acid) (PGA), poly(ε-caprolactone) (PCL), poly(vinyl pyrrolidone) (PVP) has been performed [21–23]. The main concern remains to study more with CS-based nanofibers is deal with their mechanical response, long-time structural integrity and biological functionality in aqueous environment upon *in vivo* experiments [24]. In this paper, an approach based on electrospinning to fabricate chitosan-based nanofibers containing GO nanosheets is presented. The fiber size is controllable in the range of 60 to 230 nm, which is in the range of fibrous structure of the natural ECMs. The processing conditions and mechanisms that would affect the structure and properties of nanofibrous scaffolds are demonstrated. Relatively small amount of PEO (5 vol%) was added to the GO/CS solutions in order to reduce viscosity and made the solution spinnable at high polymer concentrations. PVP (15 vol% of CS) was also introduced aiming to improve the physico-mechanical properties of CS scaffolds, as shown elsewhere [25]. Electrospun PVP-based nanofibers including PVP/CS have been used in drug-delivery and tissue engineering applications [26–29]. Both PEO and PVP are synthetic biocompatible polymers that have been approved for use in food and biomedical applications [25,30,31]. In spite of utilizing relatively high amount of PEO/PVP and/or employing nonionic surfactant and co-solvents to ease spinnability of CS [32], we show that GO nanosheets can be used, which would provide many opportunities for better utilization of bio-based materials. Graphene and graphene oxide, which is a chemically functionalized derivative of graphene sheets with hydroxyl and epoxide groups on the basal plane and carboxyl groups at the edges, have recently attracted considerable attention for many potential applications such as glucose sensors [30], biocomposites [31], supercapacitors [33], reduction of oxygen [34], biomedical devices [35], drug delivery and tissue engineering [35–39]. This wide potential application of graphene oxide in medicine is attributed of its unique structure and superior chemical stability, excellent mechanical property, good biocompatibility and bactericidal potential [35,40,41]. Wang et al. [42] investigated the production of graphene oxide/poly(vinyl alcohol) composite nanofibers *via* electrospinning. They showed that thermal and mechanical properties of the fibers (with 100–500 nm sizes) were improved by GO nanosheets. No results on physical characteristics such as permeability and biological performance were reported. More recently, Jin et al. [43] studied the effect of GO on the mechanical properties of PAN nanofibers mats. In addition to enhanced mechanical strength, they showed that the mats were biocompatible with good cell-materials interactions, which could be promising for cell culture scaffold with electrical stimulation. In a more recent study [43], they fabricated polymer cored reduced GO nanofibers by electrospinning of a polymer inside a GO suspension and heat-driven self-assembly. They showed that the fibers were biocompatible while their nanoscale architecture played a critically constructive role in supporting cellular activities.

Due to the biological advantages of natural biopolymers, particularly CS as a promising wound healing material [44], many studies have devoted to fabricating CS-containing nanofibers [45]. For example, Liu et al. [46] synthesized poly(vinyl alcohol)/chitosan/graphene oxide electrospun nanofibers as a promising material for antibacterial biomedical applications. They showed that average diameter of the fibers decreased with increasing the GO content (up to 0.6%) from 200 nm to 123 nm while the tensile strength increased up to 25% and reached to about 3 MPa (which is much lower than natural skin [47]). Lu et al. [48] prepared CS/PVA nanofibers containing graphene and examined the wound healing process in animal models. They showed that the fibers containing graphene nanosheets (0.6%) healed completely at a faster rate as compared to others in both mice and rabbit. It was proposed that the presence of free electron in graphene does not affect the multiplication of eukaryotic cells, but inhibits the prokaryotic cell multiplication, thereby preventing the growth of microbes. More recently, Faria et al. [49] prepared antimicrobial electrospun biopolymer nanofiber mats functionalized with graphene oxide—silver nanocomposites. They electrospun PLGA and PLGA/CS fibers (diameter > 200 nm) and then functionalized their surfaces with GO and GO/Ag. Upon direct contact with bacteria cells, the mats effectively inactivated both Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacteria.

In the present study, chitosan-based nanofibrous membranes were prepared by direct electrospinning of concentrated polymeric solutions and composite suspensions. To adjust the mechanical strength of the nanofiber membranes close to the natural skin [47], we adopted a strategy to attain ultrafine fibers with an average diameter of 60 nm reinforced with well-exfoliated GO nanosheets. It should be noted that, due to high viscosity and low conductivity, electrospinning of concentrated CS solution is challenging and requires surfactants that might not be biocompatible [50]. To the best knowledge of the authors, fabrication of ultrafine nanofibers with high CS concentration (80% as the base polymer) which contain a high amount of well dispersed GO sheets (2%) has not reported yet. The other feature which distinct our work from previous studies is related to structural characterization of the nanofibrous membranes with regard to mechanical durability and water vapor permeability that fulfil the requirements of temporally skin grafts. In fact, previous investigations mainly focused on the effect of GO nanosheets on tensile strength and *in-vitro* biocompatibility. In the present work, we extended our studies on detailed characterization of physico-mechanical properties of nanofibrous mats and examined the novel materials in animal models. To the best knowledge of authors, *in-vivo* performance of ultrafine and highly concentrated CS-based nanocomposites containing GO nanosheets has been studied scarcely. It is shown that in rats, the developed membranes significantly enhance the wound healing process that determines the capacity of the mats for tissue engineering applications.

2. Material and methods

2.1. Materials

Chitosan (M_w = 190–310 kDa, DD ~85%), PVP (K-40, M_w = 40 kDa) and PEO (M_w = 900 kDa) were supplied by Sigma-Aldrich Co (USA). Graphite powder was purchased from CHEER Carbone Materials (China). All other reagents used were purchased from Merck Co. (Germany) with analytical grades.

2.2. Synthesis of graphene oxide

Graphene oxide nanosheets were synthesized from the graphite powder according to a modified Hummers' method, as explained in details elsewhere [51]. Briefly, 2 g graphite powder was added into 50 ml of H_2SO_4 in an ice-water bath. $KMnO_4$ (6 g) was then added and stirred for 2 h at 35 °C. After dilution with deionized (DI) water (350 ml), H_2O_2 (30%) was added and the resulting GO nanosheets were separated by using a centrifuge. The product was washed several times with HCl and DI water to attain a brownish powder.

2.3. Electrospinning of fibers

Solutions of chitosan (3% w/v) and PVP (5% w/v) were prepared in acetic acid (90%) and distilled water, respectively. These solutions were then mixed under magnetic stirring for 24 h at room temperature. To increase solution spinnability and to achieve nanofibers with high uniformity, PEO solution (5% w/v) was also added to the mixture. The final composition of polymer solution was CS:PVP:PEO 80:15:5vol%. The prepared GO was dispersed into distilled water using a sonicator (WiseClean WUC-D10H, Deutschland) overnight to yield a uniform suspension (5 g/l). Different contents of GO suspensions (0, 0.5, 1, 1.5 and