Materials Science and Engineering C 79 (2017) 697–701

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

# Fabrication of chitosan/graphene oxide polymer nanofiber and its biocompatibility for cartilage tissue engineering

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ARTICLE INFO

*Article history:*
Received 11 April 2017
Received in revised form 30 April 2017
Accepted 10 May 2017
Available online 10 May 2017

*Keywords:*
Graphene oxide
ATDC5 cells
Nanofiber
Cartilage

ABSTRACT

In this work, Chitosan/Poly(vinyl alcohol) (PVA)/graphene oxide (GO) composite nanofibers were fabricated by electrospinning technique. The prepared nanofibers were characterized by using various spectroscopic and microscopic techniques. FESEM images have confirmed the uniform distributions of graphene oxide nanosheets in the nanofibers with self-assembly with chitosan/PVA chains. Additionally, the results of raman spectra confirmed the existence of GO sheets in the nanofibers. The tensile strength experiments revealed that the incorporation of GO increased the mechanical properties of nanofibers. Further the biocompatibilities of the chitosan/PVA/GO towards ATDC5 cells was studied in a cell proliferation assay after day 14. The obtained results revealed that the chitosan/PVA/GO (6 wt%) is found to deliver the most appropriate environment for the growth of ATDC5 cells when compared with chitosan/PVA/GO (4 wt%) and chitosan/PVA. Hence, the chitosan/PVA/GO (6 wt%) nanofiber can be used as possible substitute as an artificial cartilage.



## 1. Introduction

Graphene, a 2D carbon nanosheet with single or few layers, has gained scientific attention due to its extraordinary chemical and physical properties which leads to its use in prospective applications, such as catalysis [1], sensor [2], electronics [3], energy [4] and others [5]. Now a days, attention in the use of graphene hybrid materials in biology is found to be a new area of research with substantial potential [6,7]. In general, Graphene oxide (GO) can be synthesized by the oxidation of graphite, where epoxide and phenol hydroxyl groups are introduced at the edges and the –COOH groups at basal planes of GO. GO exhibits very good biocompatibility because of the presence of abundant oxygen functionalities. Also, GO can be easily soluble in water and other polar solvents.

Due to its biocompatibility, GO has a great potential applications in biology such as in drug delivery and tissue engineering. Graphene hybrid materials exhibit a tendency to agglomerate in a layer-by-layer because of its good inter planar interactions, indicating the considerable loss of part of its surface area. On the other hand, it is challenging to prepare single-layered GO dispersion with surface coated polymer matrix, making it to utilize its total surface area for applications. Hence, the usage of a suitable solvent to dissolve both the polymer matrix and filler is the most prominent method for the preparation of GO/polymer composite materials. Various GO/polymer composite materials have been fabricated in recent days. For instance, Pant et al. [8] reported the fabrication of nylon-6 spider-wavelike nano-nets, where GO was successfully dispersed in nylon-6 due to its ability to form hydrogen bonding among GO sheets and polymer molecules. On the other hand, the preparation of PVA/GO nanofibers was already reported by electrospinning techniques [9,10].

However, these fabrication methods involves the advantage of surface functionalities of GO that can form hydrogen bonding with the side chains of polymers used. But these nanofibers do not show the utilization of higher surface area of graphene oxide because of their curly nature, which can be enhanced by leaving the functional surface of graphene oxide. Also, the incorporation of GO as nanofiller increases the total mechanical strength of fabricated nanofiber because of its exceptional mechanical and elastic properties. Several hybrid and inorganic materials have been reported in literature for their use in gene delivery [11,12], optogenetics [13], therapeutics [14,15], Photodynamic therapy [16] and drug delivery [17,18]. Similarly, chitosan based hybrid materials are found to be considered as biomaterial for wound healing in tissue engineering applications. But the chitosan is less soluble in organic solvents and hence it is difficult to electrospun. However, the reported methods for the fabrication of polymer chitosan nanofibers [19–21] involved the use of hazardous solvents such as acrylic acid (AA), dichloromethane (DCM) and trifluoroacetic acid (TFA) which may discourage their use in biomedical applications.

In this work, we fabricated PVA/Chitosan nanofibers by using GO as fillers due to its antibacterial activity and biocompatibility [9,22]. In this work, we have used GO to form a stable homogeneous suspension with PVA/Chitosan system and then electro spun to obtain nanofibers. The as prepared nanofibers were characterized by using various microscopic

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http://dx.doi.org/10.1016/j.msec.2017.05.056
0928-4931/

and spectroscopic techniques. We also studied the in-vitro cytotoxicity of prepared nanofibers against ATDC5 cells to check the possibility of their use in cartilage tissue engineering applications.

## 2. Materials and methods

### 2.1. Materials

Potassium permanganate ($KMnO_4$, 99.0%), Chitosan (200,000 cps), Graphite flakes (100 mesh, 99%), glyoxal solution (40 wt% in $H_2O$) and Polyvinyl alcohol (PVA) (Mw = 85,000–124,000), Glutaraldehyde (25%), ethanol (≥99.5%) Acetic acid, hexamethyldisilazane, Sulfuric acid ($H_2SO_4$, 97%), 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H–tetrazolium (MTS), phosphoric acid and hydrogen peroxide ($H_2O_2$, 30 wt%) were purchased from Sigma Aldrich Ltd., shanghai.

### 2.2. Preparation of GO

The chemical preparation of GO was performed from graphite by following the reported modified Hummers approach [23]. About, 0.5 g of graphite was added to 0.5 g of sodium nitrate in a concentrated $H_2SO_4$ (98%, 23 mL of 12.1 M). The subsequently formed reaction mixture was then kept for stirring for about 15 min under ice bath conditions at zero degree temperature. Later, about 4 g of $KMnO_4$ was added to the above mixture slowly by maintaining the temperature at 20 °C. Then, the obtained reaction mixture was stirred at 40 °C constantly for about one and half hour in water bath and a 50 mL of deionized water was added until the color of the solution change to dark brown. Then, 6 mL of 30% $H_2O_2$ was added and diluted with 50 mL of deionized water. Then the obtained solution is washed with 5% HCl and double distilled water to attain neutrality in pH. Then the suspension is centrifuged and dried at 60 °C in an oven for about 45 mins. Later, GO dispersion is prepared by adding dried GO powder to deionized water to make 1 mg/mL and sonicated in an untrasonic bath and used for future experiments.

### 2.3. Fabrication of chitosan/PVA/GO nanofibers

Initially, homogeneous brown chitosan (8% weight) solution was prepared after electron beam lithography (EBI) treatment. PVA solution (20 wt%) was made by mixing PVA powder in double distilled water at 90 °C for about 24 h. On the other hand, PVA (2 g) and chitosan (5 g) were added to double distilled water and stirred vigorously to obtain a homogeneous solution. Then glyoxal, a cross-linking agent (6 wt%) was mixed to above solution and the pH of the solution is maintained between 2 and 3 using phosphoric acid. Later, 3 mL of GO dispersion (20 mg/mL) was mixed to Chitosan/PVA mixture and left for stirring for about 2 h. Then the obtained solution was electro spun at 18 kV to obtain a spun nanofiber where the tip-to-collector distance was maintained at 16 cm. The obtained spun nanofiber was kept in oven at 50 °C for about 24 h, followed by curing at 120 °C for about 10 min.

### 2.4. Characterization of nanoparticles

Fourier transform infrared (FTIR) spectrum for the fabricated chitosan/GO/PVA was taken using SHIMADZU, IRAffinity 1 instrument. FESEM, S-7400 Field emission scanning electron microscopy (Hitachi, Japan) was used to study the morphology of the fabricated nanofibers. On the other hand raman spectrum was recorded using RFS-100S FT-Raman spectroscopy (Bruker, Germany). AG-5000G, Shimadzu, Japan instrument was used to study the mechanical properties using a universal testing machine, where the machine is operated with a crosshead speed of 5 mm/min under room temperature conditions. The nanofiber samples for tensile strength measurements were prepared as per the ASTM Standard D 638 in the form of standard dumbbell shapes. Each sample was measured about five times.

### 2.5. Cell proliferation assay

A CellTiter 96® aqueous non-radioactive cell proliferation assay (MTS) was performed to know the biocompatibility of chitosan/PVA/GO nanofibers towards cartilage regeneration. Initially, mouse

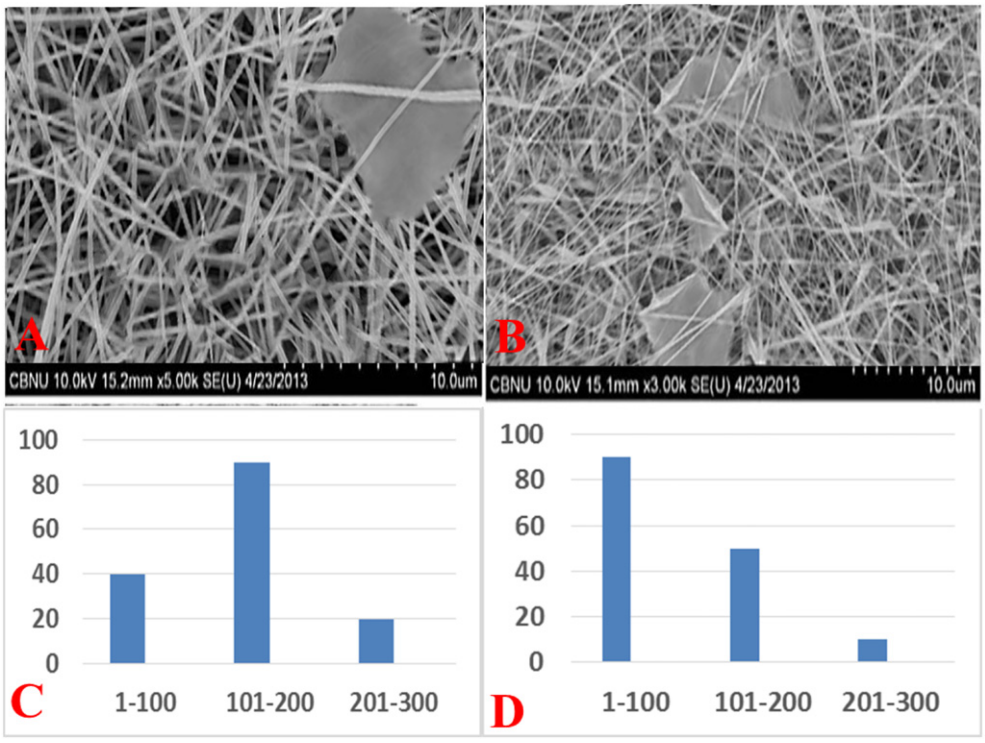


**Fig. 1.** FESEM images of Chitosan/PVA/GO nanofibers (4 wt%, 6 wt%) (A,B) and their size distributions (C,D).