



# Graphene oxide decorated electrospun gelatin nanofibers: Fabrication, properties and applications



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## ABSTRACT

Gelatin nanofiber fabricated by electrospinning process is found to mimic the complex structural and functional properties of natural extracellular matrix for tissue regeneration. In order to improve the physico-chemical and biological properties of the nanofibers, graphene oxide is incorporated in the gelatin to form graphene oxide decorated gelatin nanofibers. The current research effort is focussed on the fabrication and evaluation of physico-chemical and biological properties of graphene oxide-gelatin composite nanofibers. The presence of graphene oxide in the nanofibers was established by transmission electron microscopy (TEM). We report the effect of incorporation of graphene oxide on the mechanical, thermal and biological performance of the gelatin nanofibers. The tensile strength of gelatin nanofibers was increased from  $8.29 \pm 0.53$  MPa to  $21 \pm 2.03$  MPa after the incorporation of GO. In order to improve the water resistance of nanofibers, natural based cross-linking agent, namely, dextran aldehyde was employed. The cross-linked composite nanofibers showed further increase in the tensile strength up to  $56.4 \pm 2.03$  MPa. Graphene oxide incorporated gelatin nanofibers are evaluated for bacterial activity against gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacteria and cyto compatibility using mouse fibroblast cells (L-929 cells). The results indicate that the graphene oxide incorporated gelatin nanofibers do not prevent bacterial growth, nevertheless support the L-929 cell adhesion and proliferation.

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## 1. Introduction

Electrospun nanofibers from natural polymers attract wide attention in biomedicine due to the proven biocompatibility, biodegradability, non-cytotoxicity and the structural resemblance of the nanofibers with native extracellular matrix [1]. When compared with synthetic polymers used for the construction of three-dimensional scaffolds, natural polymers such as gelatin, chitosan and others play a significant role in determining many cellular functions [2]. Gelatin is a protein obtained by the denaturation of collagen, which is a major structural protein in human body. Gelatin is biocompatible, biodegradable, non-toxic and non-antigenic material. We recently reported different strategies for improving the mechanical and biological properties of gelatin nanofibers [3–6]. However, the mechanical performance of gelatin-based nanofibers, which is one of the key properties of the tissue engineering material, is found to be not very promising. The present work is an attempt to improve the mechanical performance of gelatin nanofibers without compromising the biological activities.

The mechanical performance of natural polymers can be tuned by non-covalent/covalent functionalization [7]. Graphene is a spectacular

material that has attracted enormous research interest due to their distinct and extraordinary properties [8]. Graphene is a single-atom-thick layer of  $sp^2$  bonded carbon atoms arranged in a regular hexagonal lattice similar to graphite [9]. In recent years, substantial research work has been conducted to improve the mechanical and functional properties of polymeric materials via incorporating graphene or graphene oxide (GO) as reinforcing filler [10–12]. However, incorporation of GO in natural polymer based nanofibers and the effects on biological performance of the nanofibers are not studied extensively. Thus, we prepared GO-gelatin composite nanofibers to combine the effect of both GO and gelatin by the simple and versatile electrospinning technique. We could achieve an improvement in the mechanical and biological performance of GO-gelatin composite nanofibers. Graphene and GO are being extensively investigated as promising candidates for biomedical research such as bio sensing, cell growth and differentiation [13].

GO incorporated polymer nanofibers exhibit enhanced optical, electrical and biological performance based on the properties of both GO and the polymer matrix. A good dispersibility of GO in the polymer solution is essential for obtaining composite nanofibers. The nature of the bonding interaction between the filler and matrix at the interface has significant impact on the properties of the composites. Most of the dispersion methods produce composites, where the polymer matrix and the filler interact through dispersive forces. GO is highly dispersible in

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water and fully exfoliated GO sheets are formed in water. Hence, a uniform GO suspension can be obtained. The edges and basal planes of GO are composed of oxygen functional groups that increase the hydrophilicity of GO and enhance the water dispersibility. GO incorporated polymer nanofibers are reported in the literature for various applications. GO loaded poly ( $\epsilon$ -caprolactone) for muscle tissue engineering applications has been reported recently [14]. Electrospinning of GO incorporated polyvinyl alcohol (PVA) nanofibers is reported and enhancement in mechanical property and biological performance towards MC3T3-E1 osteoblast cells are investigated [15]. Electrospun poly(D, L-lactic-co-glycolic acid) (PLGA)/collagen nanofibers decorated with GO is reported to enhance the cell attachment and proliferation of human dermal fibroblast cells, which revealed its efficiency to be used as scaffold for tissue regeneration [16]. Significant improvements in the thermo mechanical and surface chemical properties of electrospun PLGA nanofibers are achieved by incorporating GO nanosheets to PLGA nanofibers [17]. Polyacrylonitrile (PAN)–GO composite nanofibers are explored as effective candidates for applications such as super capacitors and energy production and storage devices [18]. GO incorporated electrospun polyamide 66 (PA 66) nanofiber membrane is fabricated for obtaining transparent conductive thin films [19]. Upadhyay et al. have reported GO incorporated poly methyl methacrylate (PMMA) nanofibers with improved thermal stability and oxygen gas barrier property [20]. GO is also used as an effective reinforcing agent for natural polymeric materials. GO incorporated protein and polysaccharide based bulk composites are reported to be suitable for tissue engineering, wound dressing and drug delivery applications [21–23]. GO loaded gelatin films are found to promote bone mineralization, cell adhesion and proliferation [24]. Studies on GO incorporated natural polymeric nanofibers are rare to find in the literature. Recently, a comparative study of the mechanical properties of GO incorporated gelatin film and GO incorporated electrospun nanofibers is reported [25].

On this background, the present work undertakes a detailed study of GO incorporated electrospun gelatin nanofibers and its physico-chemical and biological properties. GO is incorporated into gelatin nanofibers by dispersing GO in aqueous gelatin solution. The resulting homogeneous dispersion is electrospun to obtain GO incorporated gelatin (GO-GEL) nanofibers. Gelatin is a highly hydrophilic polymer and hence the gelatin nanofibers completely lose the fibrous morphology on exposure to the high degree of moisture. Even though GO can give structural reinforcement, it cannot provide the water resistance ability for gelatin nanofibers. Hence, an effective cross-linking treatment is required to improve the water stability [26]. Several cross-linking agents based on bifunctional molecules such as glutaraldehyde (GT) [26], carbodiimide [27], etc., are developed for gelatin matrices. In our previous studies, the efficiency of dextran aldehyde and sucrose aldehyde to cross-link gelatin nanofibers is reported [3,4,6]. In the current work, the GO-GEL nanofiber is cross-linked using dextran aldehyde. The GO-GEL and dextran aldehyde cross-linked GO-GEL (DA-GO-GEL) mats are well-characterized and their biological performances are evaluated.

## 2. Materials and methods

### 2.1. Materials

Expanded graphite (EG) (grade-3805) was provided by Asbury Carbons, USA. Gelatin Type A with Bloom number 225 was obtained from MP Biomedicals, Mumbai, India. Dextran ( $M_r \sim 35,000$ – $45,000$ ), sodium tetraboratedecahydrate (borax) and trinitro benzene sulfonic acid (TNBS) were purchased from Sigma Aldrich, USA. Sodium metaperiodate, sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate and acetic acid (AR grade), potassium permanganate, sulphuric acid and hydrogen peroxide were obtained from Merck, Mumbai, India. All the materials were used without further purification. Cellulose dialysis tubing with MWCO 3500 from Spectrum Laboratories Inc. CA, USA was used for the experiments. Double distilled water was employed

in all the experiments and MilliQ water (Millipore) was used for cell culture and other experiments.

### 2.2. Electrospinning of GO-gelatin composite nanofibers

Graphene oxide (GO) was prepared by oxidation of expanded graphite using modified Hummer's method [28]. EG was obtained by subjecting expandable graphite to thermal shock at  $1050^\circ\text{C}$  (For details refer supporting information). The dried, spongy GO was mixed with aqueous gelatin solution (30% w/v) in 8:2 (v/v) water/acetic acid mixture and ultrasonicated for 1 h to obtain the homogeneous solution for electrospinning. Solutions with GO loadings of 0.25 to 1% (w/w) were prepared. Pure gelatin (30% w/v) in water/acetic acid (8:2 v/v) was used as the reference sample for comparison. In both the reference and the GO loaded sample, the amount of gelatin was maintained same. The solution was loaded into 5 ml plastic syringe (Dispovan) capped with a needle of  $0.60 \times 25$  mm. A syringe pump was used to control the flow rate of the solution. The applied voltage was adjusted between 25 and 30 kV, flow rate was kept as 0.3 ml/h and collector speed was 1500 RPM. Fibers were collected on a grounded metal collector (wrapped with an aluminum foil) which was kept at a distance of 20 cm away from the needle.

### 2.3. Cross-linking of GO-gelatin (GO-GEL) nanofibers using dextran aldehyde

Dextran aldehyde was prepared by periodate oxidation of dextran under dark condition for 6 h at room temperature. Dialysis followed by freeze drying yielded dextran aldehyde as powder [3,5]. Dextran aldehyde (0.05 g) was dissolved in 10 ml of pure ethanol containing a minimum quantity of aqueous borax ( $300 \mu\text{l}$ , 0.02 M). About 0.5 g of the electrospun GO-gelatin mats was cut into rectangular pieces and placed into this cross-linking medium for 5 days at  $37^\circ\text{C}$  to obtain dextran aldehyde cross-linked GO-gelatin (DA-GO-GEL) mats.

### 2.4. Trinitrobenzenesulfonic acid (TNBS) assay

The cross-linking degree of DA cross-linked GO-GEL mats was determined by conventional TNBS assay [29]. About 5 mg each of as spun and cross-linked nanofibrous mats was treated with 1 ml of 4% sodium bicarbonate solution and 1 ml of 0.5% freshly prepared TNBS solution. The mixture was heated at  $40^\circ\text{C}$  for 2 h. To this mixture, 3 ml of 6 N HCl was added and temperature was raised to  $60^\circ\text{C}$ . Complete dissolution of the cross-linked mats was achieved within 1 h. The resulting solution was diluted with 5.0 ml of double distilled water, and the absorbance was measured spectrophotometrically (Agilent Technologies, Cary100 UV-Visible Spectrophotometer, USA) at 346 nm. Degree of cross-linking was calculated as follows.

Degree of cross – linking

$$= 1 - \left\{ (\text{absorbance}_{\text{cl}}/\text{mass}_{\text{cl}}) \times (\text{absorbance}_{\text{ucl}}/\text{mass}_{\text{ucl}})^{-1} \right\}$$

The subscripts cl and ucl stand for the cross-linked and uncross-linked nanofibrous mats.

### 2.5. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were obtained using Spectrum 100, Perkin Elmer, USA, with universal attenuated total reflectance accessory (UATR). The crystal used for UATR accessory is standard ZnSe and light path angle of incidence of  $45^\circ$ . For each spectrum, 32 scans were accumulated at  $4 \text{ cm}^{-1}$  resolution, in the scanning range of  $4000$ – $650 \text{ cm}^{-1}$ .

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