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Physical and Chemical Properties of Poly (l-lactic acid)/Graphene Oxide Nanofibers for Nerve Regeneration

Authors: Hayriye Öztatlı[1] and Duygu Ege[1]
Affiliation: [1] Institute of Biomedical Engineering, Boğaziçi University, Istanbul, Turkey

ABSTRACT
The development of biodegradable polymeric nanofiber scaffolds for a potential effort to repair injured nerve cells is of great interest in nerve tissue engineering applications. Poly (L-lactic acid) (PLLA) has been widely used in nerve conduit studies due to its biocompatibility, easily shaped properties and degradation to low toxic lactic acid. However, its hydrophobicity and lack of binding sites for cellular activities restricts its use as implants. In this regard, this study involves the incorporation of graphene oxide (GO) into PLLA nanofibers for enhancing mechanical properties, electrical conductivity and hydrophilicity of PLLA to make it suitable for a potential peripheral nerve regeneration application. For this purpose, PLLA and PLLA/GO nanofibers were prepared via electrospinning. The processing parameters and solution parameters were optimized to adjust physical and mechanical properties of nanofiber in terms of size, porosity and biologically active affinity for cellular interaction. The morphology and composition of the developed electrospun fibers were characterized via, Scanning Electron Microscopy (SEM), Raman Spectroscopy, tensile testing and contact angle measurements. The morphological results showed that using chloroform/DMF ratio of 8/2 for 7wt% PLLA led to the formation of bead free and thinner PLLA fibers than fibers produced from other concentration of PLLA. Moreover, the addition of the GO resulted in decrease of the average diameter of PLLA fibers from 828 nm to 490 nm and the thinnest nanofiber structure was obtained by addition of 10 v/v % GO. The sonication time of GO highly enhanced the porosity of the nanofibers, namely the porosity of the nanofibers increased with increasing sonication time. Raman Spectroscopy exhibits peaks at bands of 1775, 873 and 1455 cm^{-1} that are attributed to C=O stretching, C-COO stretching and CH_3 asymmetric deformation respectively for PLLA and 1379 and 1599 cm^{-1} which represent structural imperfections and sp2 domain of carbon atoms respectively for GO. Hence, Raman peaks confirmed that GO was mixed in PLLA nanofibers. The incorporation of GO significantly improved the tensile strength from 2.25 MPa of pure PLLA to 8.13 MPa, 10.44 MPa and 12.93 MPa with 5, 7.5 and 10 v/v% of GO addition, respectively. The results revealed that the addition of GO led to enhanced chemical and physical properties of fibers which is promising for nerve regeneration applications.

1. INTRODUCTION

Peripheral nerve injury is a commonly occurring health issue and 3% of all trauma patients suffer from this medical condition [1]. Artificial conduits are used for over a century for the treatment of such injuries [2]. The current gold standard of nerve conduits is use of autografts. However, the use of autografts has disadvantages including requirement of a secondary surgery, high-cost and impairment of the originating site of the graft. Nerve engineering shows promise to substitute autografts and eliminate associated disadvantages. In order to optimize the clinical performance of nerve conduits, choice of material, topographical and chemical cues need to be optimized [3, 4].

Synthetic materials such as polyglycolic acid, PLLA, polycaprolactone and polyhydroxybutyrate have great promise due to ability to sustain suitable mechanical properties, easy availability and consistent reproduction [4–6]. Among these synthetic polymers, electrospun PLLA nanofibers have been previously studied for nerve regeneration applications however it has some disadvantages such as hydrophobicity and lack of surface

cell recognition sites. As GO is highly hydrophilic and electroconductive, it has previously shown to induce neural growth and differentiation. Although, a high concentration of GO is cytotoxic, nanofibers comprising of small amounts of GO and PLLA has high potential to led to neural tissue regeneration [7, 8].

In this study, different volume fractions of GO incorporated PLLA were prepared with electrospinning to provide contact guidance for potential peripheral nerve regeneration. The physical and chemical properties and suitability of these nanofibers were characterized by Raman Spectroscopy, Scanning Electron Microscopy (SEM), contact angle measurements and mechanical tests.

2. EXPERIMENTAL

a. Materials

Poly (L-lactic acid) (PLLA) with inherent viscosity 2.4 dl/g was purchased from Purac Biomaterials, Netherlands. Chloroform, N,N-dimethylformamide (DMF), graphene oxide (GO) and isopropanol were of analytical reagent grade and obtained from Sigma Aldrich, USA.

b. Electrospinning

The filler content of GO by volumetric ratio of the solution was 5%, 7.5% and 10% (v/v). Initially, GO was diluted with DMF and ultrasonically treated with an ultrasonic homogenization instrument (Bandelin Sonopuls). Sonication was applied for 3, 5, 10 and 15 min. Then, 7 wt% of PLLA was added into the GO/DMF solution and stirred continuously at 120^{o}C. After complete dissolution of PLLA particles, the solution was diluted with chloroform and magnetically stirred at room temperature to obtain a homogenous PLLA/GO solution. PLLA and PLLA/GO solutions were separately electrospun using electrospinning system (NE 300, Inovenso, Turkey). The solution was fed into 10 mL syringe with 20 gauge stainless steel blunt needle. The needle tip to rotating mandrel collector distance was kept as 15 cm and the collector was covered with an aluminum foil. The electrospinning process was performed with a mandrel speed of 2064 rpm, 1 ml/h flow rate and 25kV applied voltage to obtain beads free smooth fibers.

c. Characterization of electrospun nanofibers

i. SEM

The surface morphology and the orientation of PLLA and PLLA/GO nanofibers were analyzed by using SEM (Philips XL30 ESEM-FEG/EDAX) at Boğaziçi University Research and Development Center Electron Microscopy and Microanalysis Unit. After coating the specimens with 50 nm gold using a sputter coater, the morphology of the nanofibers was examined with an accelerating voltage of 5 kV. Average diameters of the nanofibers were measured by using SEM images in conjunction with the image analysis software, Image J.

ii. Raman Spectroscopy

The chemical structure of PLLA and PLLA/GO nanofibers was examined with Raman Spectroscopy (Renishaw inVia Raman) at Boğaziçi University Advanced Technologies Research and Development Center. The excitation wavelength of 532 nm and 50X objective lens was used to obtain a Raman spectrum over the range of 500 cm^{-1} to 2200 cm^{-1}.

iii. Mechanical Analysis

The mechanical properties of nanofibers were analyzed using a Universal Test Machine (LR 5K Lloyd Instruments, UK) at Boğaziçi University Institute of Biomedical Engineering. The nanofiber specimens were cut into rectangular shapes of 60 x 10 mm^2 in length and width. Thickness of each sample was measured using electronic digital caliper. Ends of each specimen were placed vertically on mechanical gripping part of the tensile tester. Tensile tests were performed at a crosshead speed of 5 mm/min. The measurement was carried out for each nanofiber five times and the mean values were recorded.

iv. Wettability

The wettability of the PLLA and PLLA/GO nanofibers was characterized with a contact angle measuring system (CAM 101 KSV instruments) at Boğaziçi University Chemistry Department. Approximately 5 µL of deionized water was dropped on the nanofiber surface. Images of water on the surface were taken by an integrated camera system and water contact angle was measured and recorded. The measurement was repeated for five different spots of each nanofibers and average value of the measurements was reported.

3. RESULTS AND DISCUSSION

a. Morphology of Nanofibers

Figure 1 represents the average nanofiber diameter of PLLA/GO nanofibers with different
v/v % of GO and sonication time of GO.

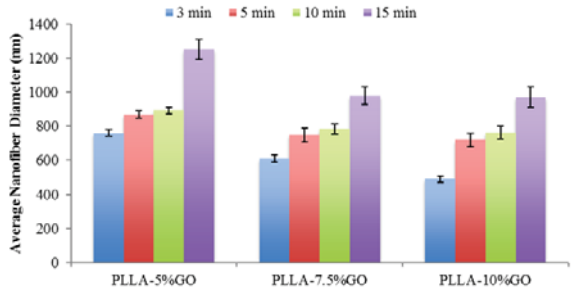
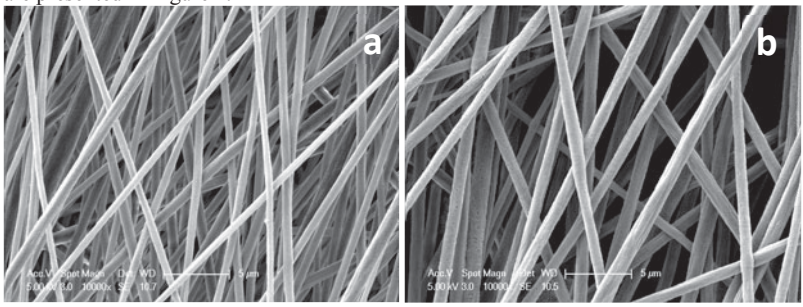


Figure 1. The average diameter graph of PLLA/GO nanofibers depending on the concentration and sonication time of GO (Results mean± standard error, n=40)

From Figure 1, it was observed that the average nanofiber diameter increases with increasing the sonication time of GO and decreasing the GO ratio in the nanofiber structure. SEM micrographs of electrospun PLLA and PLLA/GO nanofibers with 5, 7.5 and 10 % GO are presented in Figure 2.



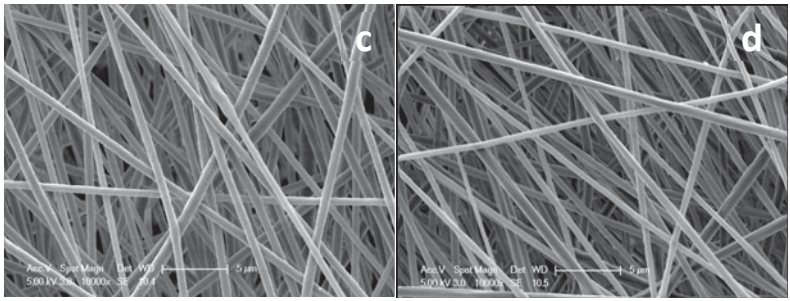


Figure 2. SEM (SE) images of a.) PLLA b.) PLLA-5%GO c.) PLLA-7.5%GO and d.) PLLA-10% GO nanofibers

Figure 3 depicts the average nanofiber diameters of PLLA and PLLA/GO nanofibers.

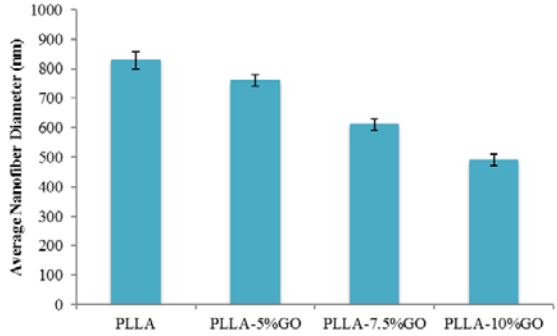


Figure 3. Average nanofiber diameter graph of nanofibers (Results mean± standard error, n=5)

The graph has shown that there was gradual decrease in the average nanofiber diameter with increasing volumetric ratio of GO solution. The average nanofiber diameter decreases from 828 nm for pure PLLA to 490 nm for PLLA/GO nanofibers with 10% GO ratio. This may be due to electrical conductivity of GO, which ultimately led to increase of the conductivity of the solution. Highly conductive solution carried more charge, which resulted in a stronger tensile force to applied voltage, so this facilitated the formation of thinner fibers.

b. Raman Spectroscopy of PLLA and PLLA/GO Nanofibers

Raman spectra of GO, PLLA and PLLA/GO nanofibers with different concentrations of GO were presented all together in Figure 4.

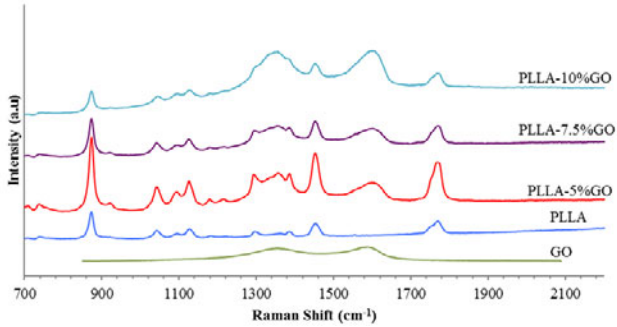


Figure 4. Raman spectrum of GO, PLLA and PLLA/GO nanofibers

Raman spectra of PLLA/GO nanofibers exhibited characteristic peak of both PLLA and GO. CH bending peaks at 1291 cm^{-1} broadened with increasing v/v % of GO in the nanofiber structures and there were shifts in G band from 1587 cm^{-1} for GO to 1599 cm^{-1}, 1597 cm^{-1} and 1597 cm^{-1} for PLLA/GO nanofibers with 5, 7.5 and 10% GO, respectively. These all indicated mixing of GO with PLLA chains in the composite nanofiber structures.

c. Mechanical Properties of Electrospun Nanofibers

The mechanical properties of PLLA and PLLA/GO nanofibers were investigated by tensile tests. The ultimate tensile stress of nanofibers were presented in the graph shown in Figure 5.

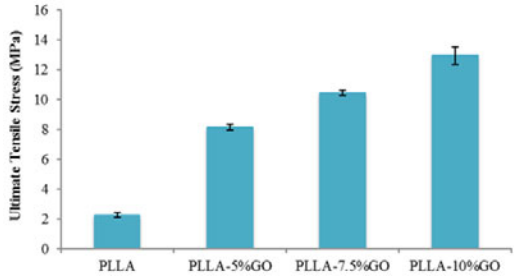


Figure 5. The ultimate tensile stress of PLL A and PLLA/GO nanofibers (Results mean± standard error, n=5)

As seen in the results, pure PLLA nanofibers had a tensile strength 2.25 MPa, whereas the incorporation of GO gradually increased the tensile strength of the composite nanofibers up to 12.93 MPa for PLLA/GO nanofibers with 10% GO.

d. Wettability of Electrospun Nanofibers

The captured photographical images of a drop of water on the surface of nanofibers at 3rd second were shown in Figure 6.

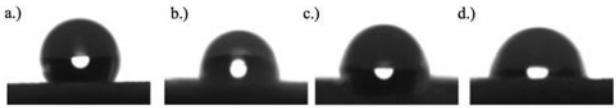


Figure 6. The water contact angle photographical images of a.) PLLA and PLLA/GO nanofibers with b.) 5, c.) 7.5 and d.) 10% GO

The effect of GO as filler on the surface wettability of PLLA and PLLA/GO nanofibers

was evaluated with the contact angle measurements of the deionized water droplets on PLLA and PLLA/GO nanofibers. As seen in Figure 6, hydrophobic PLLA nanofibers had an average water contact angle of 117.5^{o}. The contact angles were 91.3^{o}, 86.3^{o} and 84.7^{o} for 5, 7.5 and 10%GO, respectively which indicated increase of hydrophilicity with addition of GO in PLLA.

4. CONCLUSIONS

In this study electrospun PLLA/GO scaffolds were produced for potential peripheral nerve regeneration applications. PLLA and PLLA/GO nanofibers were prepared and characterized by SEM, Raman spectroscopy, tensile test and water contact angle measurements. The results confirmed that the addition of GO enhanced chemical, physical and mechanical properties of the nanofibers. Overall, the results of this study indicate that the produced GO/PLLA nanofibers has potential for nerve regeneration applications in terms of their chemical, physical and mechanical properties.

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