

Preparation and Characterization of Electrospun PLGA/silver Composite Nanofibers for Biomedical Applications

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The main objective of the present paper is to represent a new class of Poly Lactic-co-Glycolic Acid (PLGA) nanofibers containing silver (Ag) nanoparticles (NPs) by electrospinning process. Silver nanoparticles were well loaded without any chemical and structural modifications into PLGA polymer matrix to form an organic-inorganic nanocomposite. Syntheses of silver NPs was carried out by exploiting reduction ability of N, N-dimethylformamide (DMF) solvent which is mainly utilized in decomposition of silver nitrate precursor into silver NPs. Typically, a sol-gel consisting of different concentration of AgNO₃ in PLGA has been electrospun, so, silver NPs have been created in the nanofibers. Observable beads can be noticed with high silver nitrate contents. TEM image of one of the modified nanofiber confirmed that NPs are present in/on the nanofiber. Overall, the size of the nanofibers was within the range of 50 to 100 nm, while the size of silver nanoparticles was within range 5 to 10 nm. The said realized results strongly recommend the use of obtained nanofiber mats as antimicrobial agents in biomaterials or water purifying systems.

Keywords: electrospinning, nanofibers, PLGA, silver nanoparticles

1. INTRODUCTION

Metal ions and metal compounds have been extensively studied in various fields like antimicrobial filters, wound dressing material, water disinfection, sensors, chemical and gas filtration, protective cloth and air filtration, etc. Antimicrobial agents which are used in industrial purposes have included quaternary ammonium salts, metal salts solutions, and antibiotics. Unfortunately, some of

these agents are toxic or of poor effectiveness, which makes them not suitable for application in health foods, filters, and textiles, and for the exclusion of pollution. Among nanoparticles used for these purposes, the metallic nanoparticles are considered the most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest for researchers due to the growing microbial resistance against metal ions, antibiotics, and the development of resistant strains [1, 2]. Thus, the incorporation of nanoparticles into polymer nanofiber attracts the interest of researchers who work in biomaterial and drug delivery fields. Different types of nanomaterials like copper, zinc, titanium, magnesium, gold [3], alginate [4] and silver have been developed but silver nanoparticles (Ag NPs) have proved to be most effective as they exhibit potent antimicrobial efficacy against bacteria, viruses and eukaryotic micro-organisms. Ag NPs is used as a disinfectant drug [2]. Silver is a non-toxic, non-tolerant disinfectant that can reduce many bacterial infections significantly [5]. Silver ions and silver compounds have been extensively studied in various fields like antimicrobial filters, wound dressing material, water disinfection, sensors, chemical and gas filtration, protective cloth and air filtration, etc. It is considered the most toxic element to microorganisms in the following sequence: Ag>Hg>Cu>Cd>Cr>Pb>Co>Au>Zn>Fe>Mn> Mo>Sn [6]. Several reports suggest that the electrostatic interaction between the negatively charged bacterial cells and positively charged nanoparticles is considered crucial for the nanoparticles to form as bactericidal material [7]. The silver cation, Ag^+ being a potent antimicrobial agent binds to and damages the bacterial cells at multiple sites. The mechanism of action for Ag^+ is to strongly bind to electron donor groups containing sulphur, oxygen, or nitrogen and bring about structural and functional changes in the cell.

On the other hand, there has been increasing interest in ultrafine polymer fibers for biomedical applications. In particular, drug-impregnated, biodegradable, ultrafine fibers are very effective for topical drug administration and wound healing of the unique properties of the ultrafine fibrous webs such as high surface-to-volume ratios, small pore sizes, and high porosity. There are several techniques used for the production of polymeric ultrafine fibers, such as electrospinning technique [8 -17]. This technique has evinced more interest in recent years because of its versatility and potential for applications in diverse fields. It is popular for its simple process operation, high performance in nanofiber fabrication and low-cost setup of required devices, composed of a high voltage generator, a syringe pump, and a grounded collector. The procedure involves applying a very high voltage to a capillary and pumping a polymer solution through it. Nanofibers of polymer collected as a nonwoven fabric on a grounded plate below the capillary. Several polymers have been successfully produced into ultrafine (nano /micro) fibers by electrospinning such as PVA, PEO, PLLA, PGA, PLGA, PCL silk, fibrinogen, collagen, gelatin, and chitosan. Due to its good biodegradability, biocompatibility and proper mechanical properties, Poly Lactic-co-Glycolic Acid (PLGA) is considered as one of the most popular biodegradable polymers approved by the U.S. Food and Drug Administration [18]. PLGA has been widely investigated for its applicability in drug-delivery applications, surgical implants and tissue engineering scaffolds [16, 17]. Many studies have been concerned with the characterization of the biological behavior of 2D PLGA scaffolds for tissue replacement and drug delivery system [19, 20-23, 16, 17]. Little studies have been concerned with investigating the changes in the physical and thermal properties of electrospun PLGA nanofibers compared to the bulk material. These properties may be

useful for predicting the degradation rate and selecting the suitable sterilization technique for the PLGA nanofibrous scaffolds.

The present study is aimed at fabricating and characterizing biodegradable PLGA nanofibers containing Ag NPs polymeric matrix scaffolds which are considered suitable for soft tissue replacement through using the electrospinning technique without adding any foreign reducing agent. Moreover, evaluation of the obtained nanofiber matrices for morphological properties and thermal properties were investigated. The main objectives of this study are (1) fabricating of PLGA nanofibers and PLGA nanofibers containing Ag NPs using the electrospinning technique, (2) studying the morphology of electrospun nanofibers and finally (3) studying the changes in the physical and thermal properties of PLGA and PLGA/Ag nanofibers compared to the bulk material.

2. EXPERIMENTAL PROCEDURES

Poly (DL-lactide-co-glycolide) (PLGA) (Lot Number 2012031203B with L/G ratio 75:25, IV 0.59 and weight-average molecular weight of 70,000) and other chemicals which used as solvents (Tetrahydrofuran, THF and dimethylformamide, DMF) used in the experiment were purchased from NaBond Company, China. All chemicals were used directly without any further purification. 14 wt. % PLGA was dissolved in a mixed solution of DMF and THF with a weight ratio of 1/1 at 60 °C. Subsequently, the solution was filled in a 20 mL NORM-JECT Luer Lok tip plastic syringe having an 18 gauge stainless-steel needle with 90° blunt end. The electrospinning setup included high voltage power supply, purchased from the NanoNC, Inc. (S. Korea), and a nanofiber collector of aluminum foil that covered a laboratory produced roller with the diameter of 12 cm. The collector was placed at 14 cm tip to collector distance (TCD). During electrospinning, a positive high voltage of 20 kV was applied to the needle; and the solution feed rate of 0.5 mL/h was maintained using a KDS 200 syringe pump purchased from the KD Scientific Inc. (Holliston, MA). The electrospinning setup is shown in Fig. 1. The electrospun PLGA–Nanosilver nano-fibrous could be readily peeled off from the aluminum foil, and the obtained nano-fibrous were stored before the subsequent characterizations.

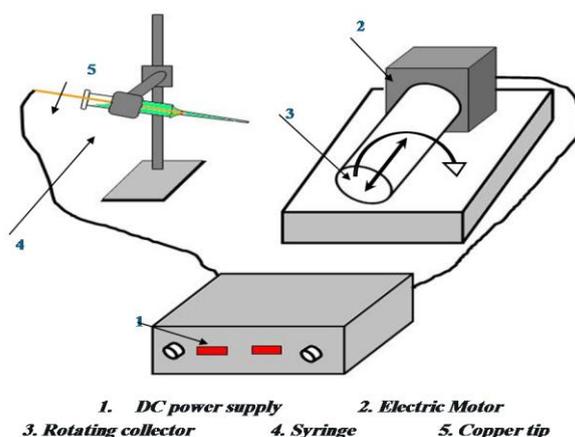


Figure 1. Schematic of the electrospinning apparatus

The thermal degradation behaviors of PLGA Polymer and PLGA nanofibers were studied with a thermogravimetric analyzer (TA Instruments, Q500 TGA, United States); instrument in the temperature range from 20 °C to 800 °C under nitrogen at a flow rate of 40 ml/min and at a heating rate of 10 °C/min.

The surface morphology of PLGA Polymer and PLGA nanofibers were observed with FE-SEM (JEOL GSM-6610LV) at an accelerated voltage of 25 10 kV. The fracture surfaces were vacuum-coated with platinum for SEM.

The structure of PLGA Polymer and PLGA nanofibers was analyzed by FT-IR spectra. Powder Samples were mixed with KBr to make pellets. FT-IR spectra in the absorbance mode were recorded using FT-IR spectrometer, and fiber sample was measured by sensor (Bruker, TENSOR Series FT-IR Spectrometer, Germany), connected to a PC, and the data was analyzed by IR Solution software. The whole analytical methods are standard in OPUS TM software.

3. RESULTS AND DISCUSSION

To prepare nanofibers by electrospinning, high voltage is applied to a polymer solution, where upon a charged jet is ejected from the needle and then undergoes extensive stretching and thinning during a rapid solvent evaporation stage. While the jet travels towards the grounded collector, polymer fibers are formed. The electrospinning process is governed by a variety of forces including the Coulomb force between the charges on the jet surface, the electrostatic force due to the external electric field, the viscoelastic force of the solution, the surface tension, the gravitational force, and the frictional force due to air drag [18]. Nanofibers of polymer collect as a nonwoven fabric that showed a number of unique characteristics such as large surface area-to-volume ratio and high porosity with very small pore size. These unique characteristics make them excellent candidates for tissue engineering scaffolds.

Fig. 2 shows the scanning electron micrographs of the PLGA polymers before and after electrospinning. The electrospinning process produced relatively smooth nanofibers. Beads or agglomerated nanofibers cannot be observed in the obtained mats. As can be seen in this figure, the solution has produced a smooth morphology for nanofibers with wide ranges of diameters. On the other hand, all fibers exhibited uniform diameters with several micrometers in length. It was also observed that most of the as-spun nanofibers are round and uniform over a length of several micrometers.

As shown in Figure 2 (b), pure PLGA produces smooth and bead-free nanofibers. Figure 3 shows SEM images for all nanofiber formulations. Observable beads can be noticed with high silver nitrate contents (Figure 3, a, and b). This could be attributed to low viscosity and/or high conductivity of the solution due to increasing silver content. Many experiments have shown that a minimum viscosity for each polymer solution is required to yield fibers without beads [24, 25]. At a low viscosity, it is common to find beads along the fibers deposited on the collection plate. When the viscosity increases, there is a gradual change in the shape of the beads from spherical to spindle-like until a smooth fiber is obtained [25, 26] as shown in Fig. 3, (c and d). At a lower viscosity, the higher

amount of solvent molecules and fewer chain entanglements will mean that surface tension has a dominant influence along the electrospinning jet causing beads to form along the fiber. When the viscosity is increased which means that there is a higher amount of polymer chains entanglement in the solution, the charges on the electrospinning jet will be able to fully stretch the solution with the solvent molecules distributed among the polymer chains. With increased viscosity, the diameter of the fiber also increases [27-30, 24]. This is probably due to the greater resistance of the solution which is stretched by the charges on the jet [28]. The surface tension has a part to play in the formation of beads along the fiber length. The viscosity of the solution and its electrical properties will determine the extent of elongation of the solution. This will in turn have an effect on the diameter of the resultant electrospun fibers.

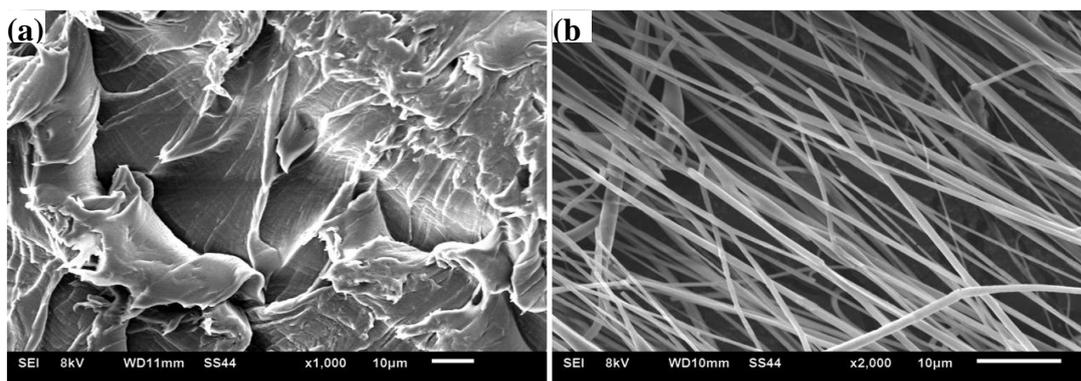


Figure 2. Scanning electron microscopy images of (a) the PLGA polymers and (b) nanofibers after electrospinning

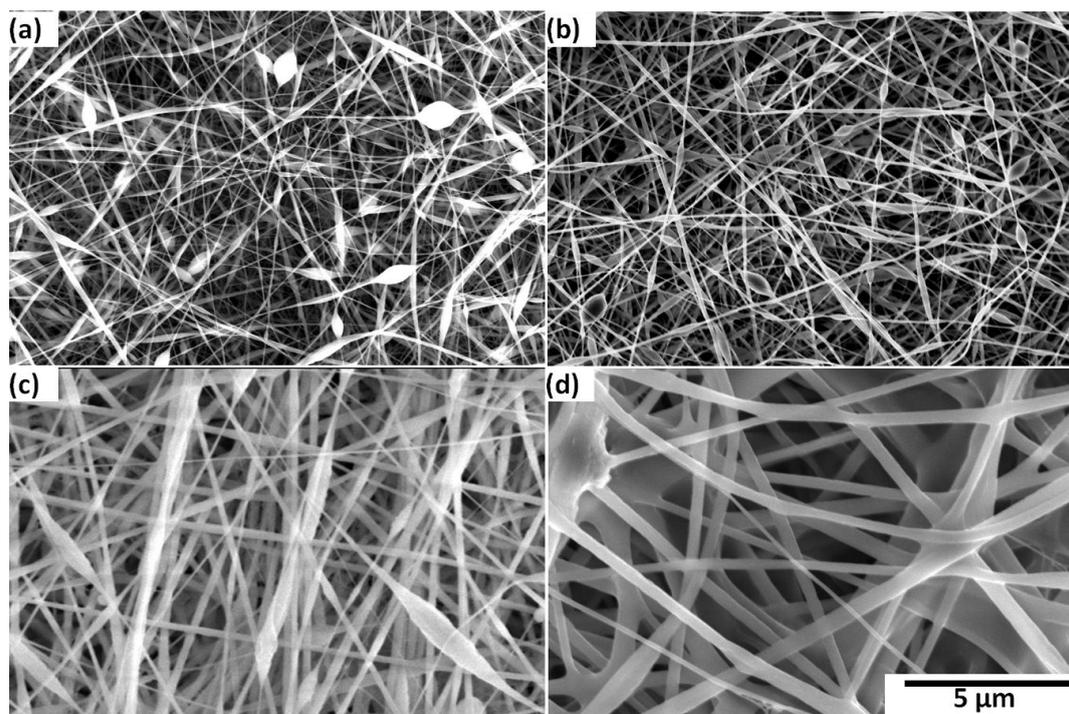


Figure 3. Scanning electron microscopy images of the PLGA nanofibers containing different amounts of silver nitrate, (a) 10%; (b) 7%; (C), 5%; and (d) 2 %

Furthermore, the electric conductivity of pure PLGA polymer is very low as they contain very few free ions, if any, which are responsible for the electric conductivity of solution. The presence of dissolved silver nitrate may increase the conductivity of the solvent. Thus if the conductivity of the solution is increased, more charges can be carried by the electrospinning jet. As previously mentioned, beads formation will occur if the solution is not fully stretched. Therefore, when a small amount of salt or polyelectrolyte is added to the solution, the increased charges carried by the solution will increase the stretching of the solution. As a result, smooth fibers are formed which may otherwise yield beaded fibers. The increase in the stretching of the solution will also tend to yield fibers of smaller diameter [31].

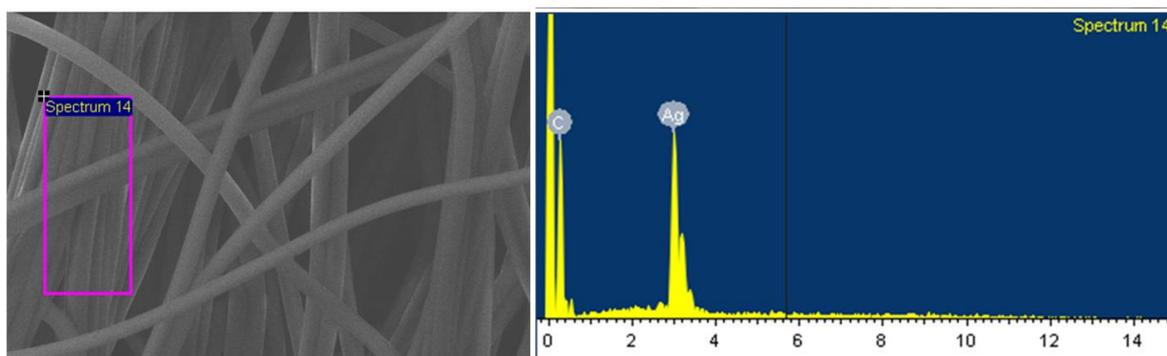


Figure 4. The energy dispersive spectrum (EDS) collected on the PLGA/Ag NPs

The energy dispersive spectrum (EDS) collected on the PLGA/Ag NPs sample (whose microstructure is illustrated in Fig. 3) distinctly identifies Ag as the elemental component in the fiber and is shown in Fig. 4. The other peaks belonging to carbon are generated from the PLGA.

Elementary analysis of *PLGA /Ag NPs* nanocomposite was carried out by using SEM-EDS. The results show that carbon and Ag were the principal element of PLGA/Ag NPs nanocomposite. EDS analysis thus provides direct evidence that Ag ions embedded in the PLGA/ silver nanocomposite. It is indicated that silver nanoparticles were well loaded without any chemical and structural modifications into *PLGA* polymer matrix to form an organic–inorganic nanocomposite.

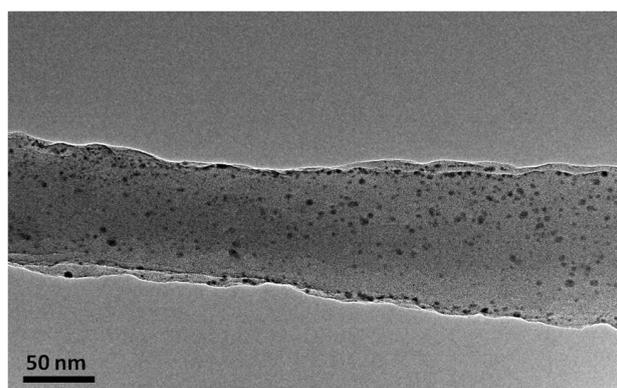


Figure 5. The TEM images of the PLGA/Ag NPs

Figure 5 shows high magnification TEM image of one of the modified nanofiber. It can be observed that NPs are present in/on the nanofiber. Overall, the size of the nanofibers are within the range of 50 to 100 nm, while the size of silver nanoparticle was within range 5 to 10 nm. Nearly spherical NPs can be seen. It can be also noticed when TEM images are compared with SEM ones that the NPs density is higher in TEM images than SEM for all silver nitrate contents. Given the fact that the electron beam in TEM analysis passes through the nanofibers, so, it can detect the silver NPs incorporated inside the nanofibers, however, in a case of SEM; only the surface morphology is investigated, consequently, we can say that the number of silver NPs inside the obtained nanofibers are so many compared with the NPs synthesized on the outer surface. However, in the TEM image (Figure 5) some NPs are clearly visible (the dark dots). This observation might add good feature to the prepared nanofiber matrix, since many authors have been proofed that PLGA is biodegradable, [8-11] so with time passing more silver NPs will appeared which enhances the antibacterial activity for the prepared nanofiber mats.

Infrared spectroscopy has been frequently used to investigate the conformational changes of *PLGA/Ag NPs*. FTIR spectra of the bulk PLGA polymer, PLGA nanofibers and PLGA/Ag nanofibers are shown in Fig. 6. Our observations showed that, in bulk PLGA polymer, PLGA electrospun nanofibers and PLGA/Ag nanofibers, the C=O stretch and the C–O stretch hovered around 1760 and 1050 cm^{-1} respectively. Both spectra exhibit carbonyl stretching demonstrating the presence of the ester group. Particularly, there was no carbonated component of mineralized PLGA/Ag. Bands around 3,000 cm^{-1} are present due to the alkyl groups. Similar peaks have been recorded for PLGA nanofibers [32, 33]. By comparing the spectra of bulk PLGA with PLGA nanofibers and PLGA/Ag nanofiber composites, it has been found that there are no changes in the FTIR spectra regardless of whether it is a bulk material or in the nanofiber form. Only the transmittance intensity of the PLGA nanofibers was increased may be as a result of the increase of unsaturated groups resulted from the cross-linking reactions.

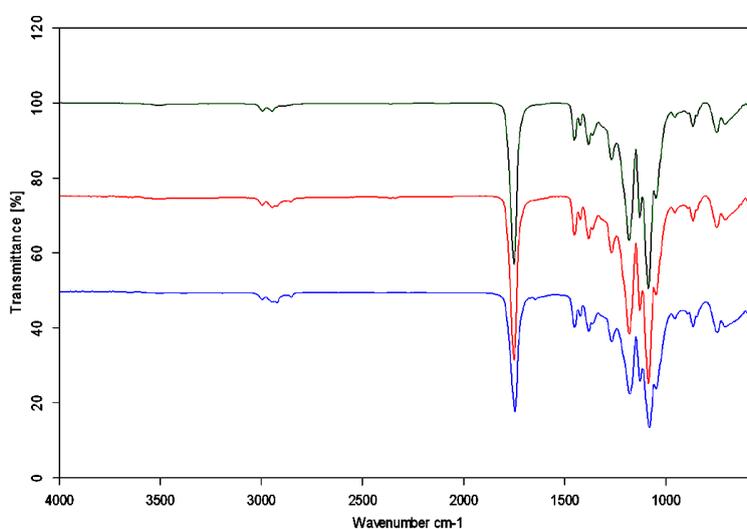


Figure 6 FTIR spectra of the (a) bulk PLGA polymer, (b) PLGA electrospun nanofibers and (c) PLGA/Ag nanofibers

The thermal stability of the samples were analyzed by thermogravimetric under N_2 atmosphere, from room temperature to 600 °C at a heating rate of 10 °C min⁻¹. Fig. 7, 8 and 9 show the (a) TGA and (b) DSC, of the bulk PLGA polymer, PLGA nanofibers and PLGA/Ag composite nanofibers, respectively. Compared with the TGA curves of bulk PLGA polymer, PLGA nanofibers and PLGA/Ag composite nanofibers show their thermal stability up to 240 °C. It can be seen that the weight of these materials remain unchanged until the temperature of analysis reaches 240°C. This weight loss mainly occurred in the range of 260-380°C, 240-365 °C and 230- 255 °C for bulk PLGA polymer, PLGA nanofibers and PLGA/Ag composite nanofibers, respectively with negligible change at temperature higher than 400°C. This weight loss indicates thermal decomposition or evaporation in the material. The present results indicated that the decomposition temperature of bulk PLGA material is higher than that of both PLGA nanofibers and PLGA/Ag composite nanofibers, nanofibrous PLGA sheet. This can be attributed to high surface area of the nanofibers and the present of Ag nanoparticles that could increase the thermal conductivity of the composites, as seen in Fig. 10. The minor weight loss was due to the loss of moisture and trapped solvent (water, DMF and THF) in the as-spun composite nanofibers while the major weight loss was due to the combustion of organic PLGA matrix.

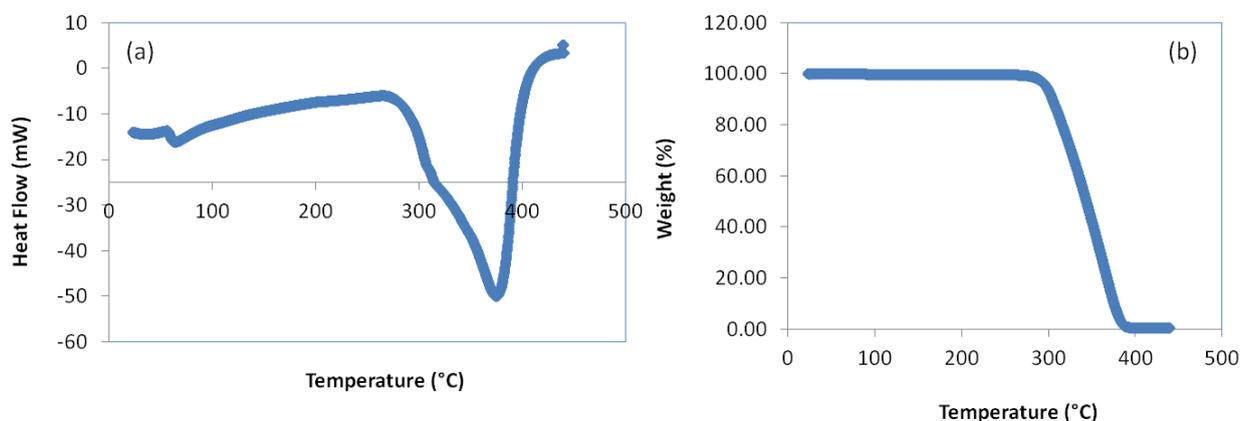


Figure 7. (a) TGA and (b) DSC of the bulk PLGA polymer

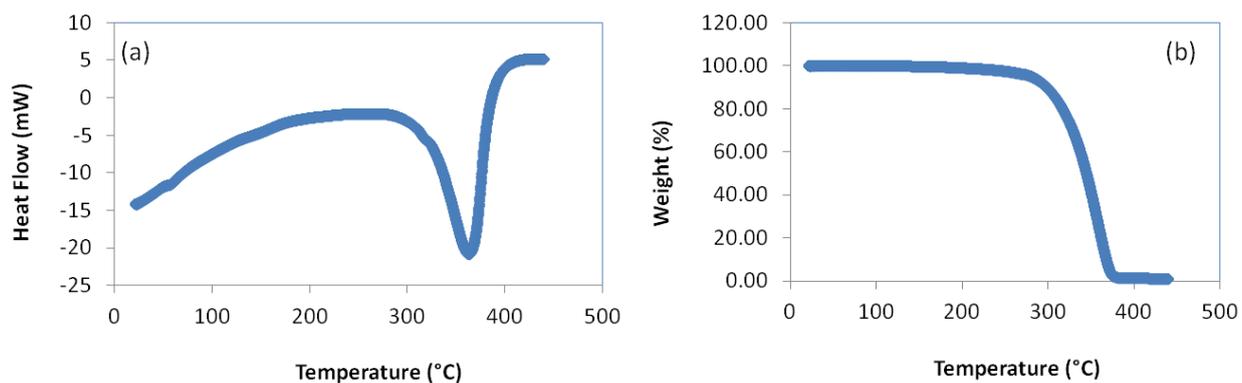


Figure 8. (a) TGA and (b) DSC of the PLGA nanofibers

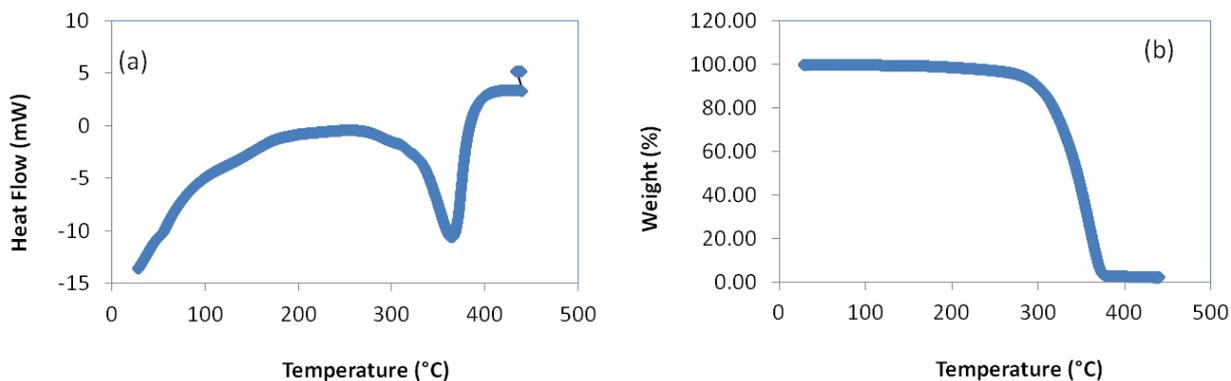


Figure 9. (a) TGA and (b) DSC of the PLGA/Ag composite nanofibers

The DSC thermogram confirms the amorphous nature of PLGA as it only shows the glass transition temperature (T_g) around 55°C and 51°C for bulk PLGA, PLGA nanofibers and PLGA/Ag composite nanofibers respectively. The T_g of the electrospun PLGA nanofibers and PLGA/Ag composite nanofibers is lower than that of the raw PLGA. This could be due to the improvement in the orientation of molecular chains in the electrospun polymer nanofibers as well as the huge surface area to volume ratio of electrospun fibers. In addition, the crystallinity of the fiber structure is expected to decrease appreciably when compared to the raw PLGA materials. In other words, the chain entanglement in bulk form is much higher when compared to the same polymer in nanofiber form. The results indicated also that the decomposition temperature of PLGA nanofibers decreased from 371°C to 360°C when compared with bulk PLGA.

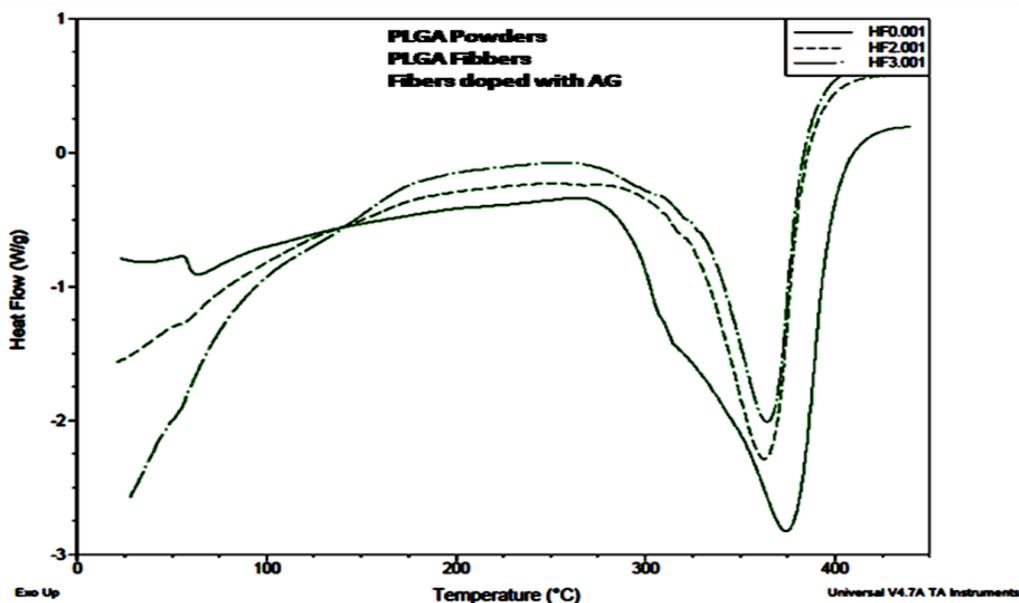


Figure 10. a comparison between TGA of the as-spun bulk PLGA polymer, PLGA nanofibers and

4. CONCLUSION

In this study we aimed at to representing a new class of Poly Lactic-co-Glycolic Acid (PLGA) nanofibers containing silver (Ag) nanoparticles (NPs) by electrospinning process. The following conclusion could be obtained:

1. Nano-biotechnologically engineered silver nitrate/ PLGA sol-gel can be easily electrospun to form smooth nanofibers.
2. Silver nanoparticles were well loaded without any chemical and structural modifications into PLGA polymer matrix to form an organic–inorganic nanocomposite.
3. SEM results confirmed well oriented nanofibers and good dispersion of pure silver NPs, TEM analysis indicated that, size of the nanofibers are was within the range of 50 to 100 nm, while the size of silver nanoparticles is was within range 5 to 10 nm. Nearly spherical NPs can be seen.
4. Observable beads can be noticed with high silver nitrate contents.
5. The obtained results show that the prepared silver NPs/ PLGA nanofibrous matrix could be properly employed as recommended candidate for many biological applications such as internal aid for water and air filters membranes and for prolonged antimicrobial wound dressing agents.

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