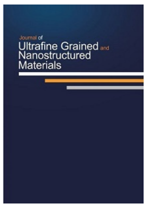

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Carbon nanotubes reinforced electrospun chitosan nanocomposite coating on anodized AZ31 magnesium alloy

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ABSTRACT

Chitosan based nanofibers containing carbon nanotubes were applied on AZ31 magnesium alloy via electrospinning. The magnesium substrate was initially anodized in a NaOH solution in order to improve the adhesion between the coating and substrate. Electrospinning parameters were optimized and homogenous nanofibers composite coatings were produced. Addition of carbon nanotubes reduced the size of the chitosan nanofibers. Also the size distribution of the fibers became more uniform. The chemical structure of the coatings was also evaluated by FTIR test and it verified stabilization of the nanofibers coating in a supersaturated sodium carbonate solution. Raman spectra showed the D and G band which corroborates the presence of Carbon Nanotubes in the coating.

***Keywords**: Magnesium alloy, Chitosan, Carbon nanotube, Nanofiber, Nanocomposite, Electrospinning.*

1. Introduction

Biomaterials including metals, ceramics and polymers are used in body for regeneration or replacement of damaged tissues. Metals due to their mechanical properties and load-bearing properties can be used as implants in hard tissues such as bone and teeth. Among metallic implants, magnesium and its alloys have great potential for using as bone implants due to their modulus of elasticity close to body bone and their biodegradability. Conventional implants due to their high modulus of elasticity, causing local stresses and finally implant failure. Also, the surgical re-operation is needed to remove them. However, magnesium and its alloy gradually degrade without creating toxic compounds in the body [1]. But these alloys have disadvantages that limit their application as implants. Among these weaknesses is intense activation of magnesium, which results in high corrosion rate in the physiological environment of the body. It causes the hydrogen gas releasing, poor tissue-implant interactions, and the loss of mechanical properties. The formation of hydrogen pockets as a result of these reactions can cause tissue necrosis. So, corrosion rate of these alloys should be controlled. One method is the application of biocompatible composites and nano-composites coatings made of biocompatible ceramics and polymers, which improves the biological properties and corrosion resistance of bone implants [2]. Electrospinning technique creates a polymeric-based coating nanofibers that has a high surface area to volume ratio and porosity similar to the outer cell matrix (ECM), which enhances cell adhesion and biocompatibility and controls corrosion rates of implant [3, 4]. Kim et al [5], enhanced corrosion resistance and biocompatibility of AZ31 Mg alloy by creating of PCL/ZnO nanofiber coating via electrospinning. Chitosan is a natural biopolymer that is used as coating because of its biocompatibility and biodegradability properties as well as its favorable mechanical properties [6-8]. The addition of carbon nanotubes is important to improve the properties including mechanical properties, conductivity, biocompatibility and increased cell adhesion to polymeric nanofibers for use as bone scaffolds [9, 10]. Farrokhi-Rad et al [11], created chitosan based coating containing CNT by electrophoretic deposition method. They concluded that CNTs reinforce the microstructure of the coating and prevent from their cracking. CNT incorporation in the coating improved mechanical properties and corrosion resistance of the metallic substrate. The main purpose of this study is optimizing formation of a CNT/ chitosan nanofiber composite coating on anodized AZ31 magnesium alloy.

2. Materials and methods

AZ31 magnesium alloy specimens with dimensions of 2 × 2.5 × 0.3 cm^3 were prepared. The specimens polished with SiC paper to 2000 grids and rinsed in acetone using ultrasonic waves for removing impurities and degreasing. The specimens were anodized in 1M NaOH solution at 3 Volts for 10 minutes. Pre-anodizing has been recommended for improving of the adhesion of the coating to the substrate [12]. Among chitosan solvents such as dilute hydrochloric acid, acetic acid, neat formic acid, dichloroacetic acid, trifluoroacetic acid (TFA) is more appropriate for the electrospinnig of chitosan. TFA forms salts with chitosan's amino groups and formation of these salts result in the destruction of the rigid interaction between the chitosan molecules and improves the electrospinnability of the solution. In addition, the high volatility of TFA is an advantage for faster solidification of the electrified jet of chitosan-TFA solution. Moreover, the addition of dichloromethane (DCM) volatile organic solvent (TFA / DCM solvent at 70/30 ratio) to the chitosan-TFA solution improves the homogeneity of the electrospun chitosan fiber [13, 14]. To prepare the electrospinning solution, at first 0.35 g chitosan (with degree of deacetylation of 85% and molecular weight of 120000) was slowly added to 5 cc TFA / DCM solvent at 70/30 ratio and dissolved for 24 hours using a magnetic stirrer. To prepare a CNT-containing solution, chitosan was gradually added to 4 cc TFA / DCM solvent at a ratio of 70/30 and dissolved for 24 hours using a magnetic stirrer. COOH Functionalized Multi Walled CNT (diameter: 50-80 nm, purity = more than 95%) was then dispersed in 1cc of the solvent using an ultrasonic probe at 50 W for 10 min and dropwise added to the chitosan solution on the stirrer to make the solution completely uniform. Electrospinning of composite nanofibers was performed under different conditions (for 15 minutes) in order to achieve optimum conditions of nanofibers on modified magnesium alloy.

Among electrospinning parameters, feed rate is very important that significantly affect the structure of the nanofibers. Therefore, 3 different feed rates of 0.2, 0.5 and 0.8 ml/h were examined for the deposition of the coating while the other parameters were kept constant (7% w/v concentration, distance=15 cm and voltage=20 kV). Applied voltage is another important factor. Hence, electrospinning was carried out at 3 different voltages of 15, 20 and 25 kV by keeping the other parameters constant (7% w/v chitosan concentration, distance=15 cm and feed rate=0.5 ml / h). Finally, to investigate the effect of the dip to collector distance, the composite nanofibers were electrospinned at 3 distances 10, 15 and 20 cm by keeping the other parameters constant (7% w/v polymer concentration, feed rate=0.5 ml / h, voltage=20kV).

If the chitosan-based nanofibers are not stabilized, the fibers will dissolve and lose their fiber structure when they expose to aqueous medium such as PBS and ethanol (during sterilization of the sample) [15, 16] . This is due to the fact that, when chitosan is dissolved in TFA, the chitosan Trifluoroacetate salts ($-NH_3^+CF_3COO^-$) are formed in two steps: (1) Protonation of the amino ($-NH_2$) groups of the chitosan and (2) Ionic interaction between the protonated amino ($-NH_3^+$) groups and Trifluoroacetate anions. This compound is soluble in aqueous solution. Therefore, after electrospinning for 75 minutes, the coatings were stabilized in 5 M supersaturated sodium carbonate solution (50 mL) for 10, 25 and 40 minutes to find the proper time. The structures of the coatings were studied by SEM. The sizes of nanofibers were calculated by image J software and the effect of carbon nanotubes on the morphology and size of nanofibers was also investigated. Chemical properties of the coatings were analyzed by FTIR using a US-made AVATAR Fourier Transform Infrared Spectrometer with 600-4000 cm^{-1} Scan Wave Range. Moreover, Raman spectra of nanofibers were achieved to confirm the presence of CNT in the fibers. These recorded in 1000-4200 cm^{-1} range with a TEKSAN N1-541 instruments (Nd:YAG laser source, λ= 532 nm and 0.7 Mw power).

3. Results and discussion

SEM images of the surfaces of (a) Mg alloy before anodizing and (b) Mg alloy after anodizing are shown in Fig.1. Anodizing increased the surface roughness that can result in a better adhesion of the coating to the substrate [12].Therefore, deposition were only applied on anodized specimens.

As mentioned earlier an important factor in electrospinning is the feed rate [14]. If it is too low, it may cause drying of the droplet in the needle tip. Also, it shouldn't be very high, because drop might have the opportunity to evaporate and exit from the syringe due to the heavy weight and results in nano-fibers containing beads. Fig. 2 a-c shows the SEM images of the coating deposited under different feed rates at a constant voltage (20 kV) and a working distance of 15 cm. At a rate of 0.2 ml/h due to the low mass solution in the tip of Syringe and presence of a strong electric field, the jet is not stable and is sprayed on the surface. Increasing of the feed rate to 0.5 ml/h stops throw drops from syringe and almost stable jet is created. Increasing of the feed rate to 0.8 ml/h results in the misbalance of the used solution and injected solution, and hence an unstable jet is created. So, it causes beads and heterogenic nanofibers. On the whole, the nanofibers produced at a feed rate of 0.5 ml/h, applied voltage of 20 kV and the needle tip to the

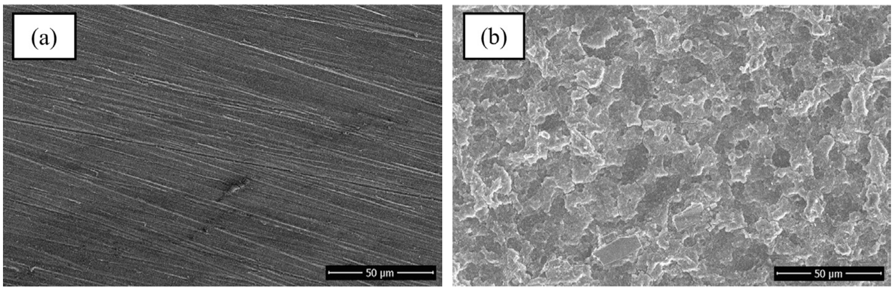

Fig. 1- SEM images of the surface of as received Mg (a) and after anodizing for 10 min (b).

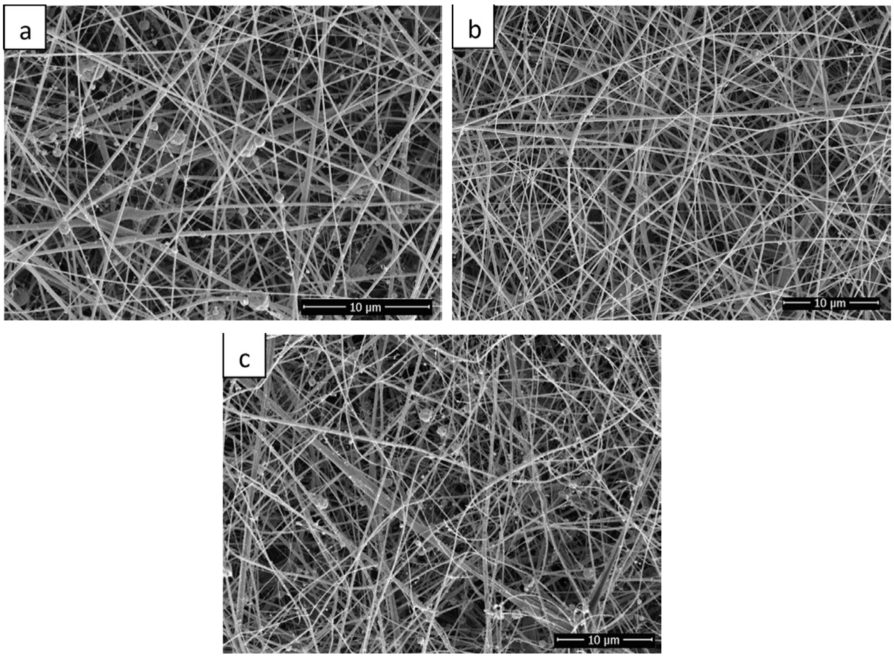

Fig. 2- FESEM images of composite nanofiber coatings achieved at feed rates of (a) 0.2, (b) 0.5 and (c) 0.8 ml / h (at 7% w/v chitosan concentration, 1% CNT with respect to chitosan concentration, distance=15 cm and voltage=20 kV).

collector distance 15 cm appears to have the most defect free and uniform structure. Therefore, the parameters used in this condition are considered as optimum.

For example, Fig.3 a shows that lower voltage (15 kV) does not have the ability to create stable jet and some heterogeneity and spraying is happened. Also, with increasing the voltage to 25 kV, nanofibers structure goes beyond optimal conditions resulting in the formation of beads and inhomogeneity (Fig. 3b).

As for the distance, its increase to 20 cm weakens the electric field and the instability of the primary jet in the syringe increases, resulting in an increase in the number of beads and heterogeneity in the nanofibers (Fig. 4b). In addition, at lower distance (10cm), due to the rapid spraying of fluid to the collector that is caused by the lack of proper evaporation, branching of nanofibers is happened (Fig. 4a).

The SEM image of the chitosan nanofibers coating created (under the above mentioned optimum condition) on magnesium alloy is shown in Figure 5. Comparison of Fig. 5 and the optimized composite coating (Fig. 2b) shows that both coatings have interconnected nanofibers with porous structure. According to Fig. 6 and 7 by adding carbon nanotubes, the average size of

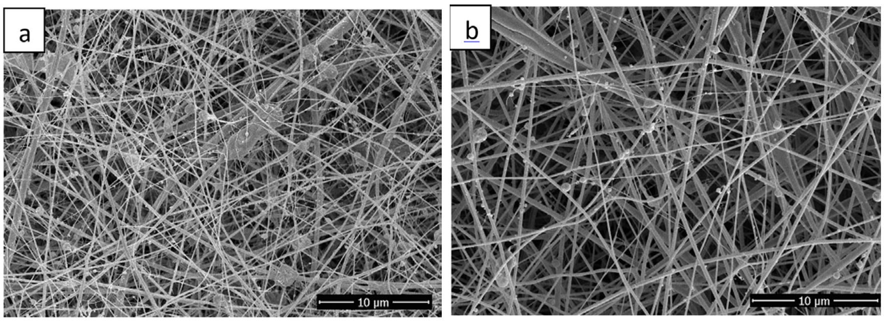

Fig. 3- FESEM images of composite nanofiber coatings achieved at voltage of (a) 15 and (b) 25 kV (at 7% w/v chitosan concentration, 1% CNT compare to chitosan concentration, distance=15 cm and feed rate= 0.5 ml / h).

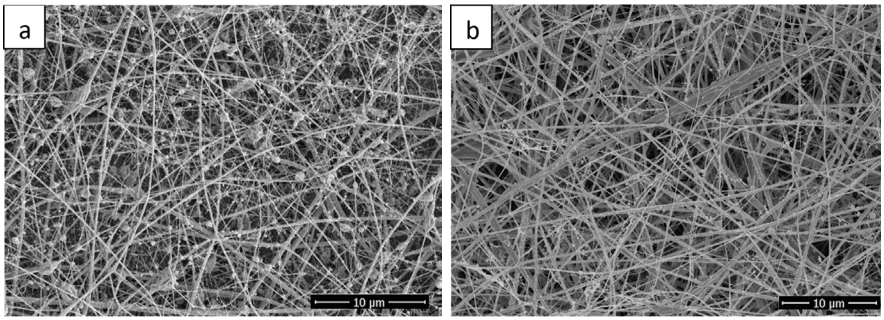

Fig. 4- FESEM images of composite nanofibers fabricated at tip to collector distance of (a) 10 and (b) 20 cm (at 7% w/v chitosan concentration, 1% CNT compare to chitosan concentration, 20 kV and 0.5 ml / h feed rate).

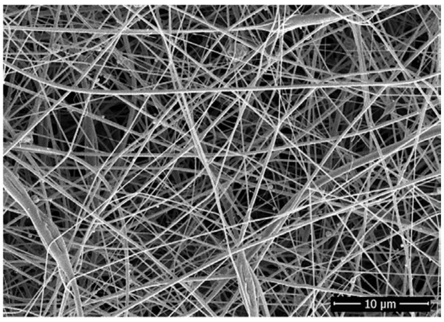

Fig. 5- FESEM images of chitosan nanofiber coating achieved at 7% w/v chitosan concentration, 1% CNT compare to chitosan concentration, 20 kV, 15 cm distance and 0.5 ml / h feed rate.

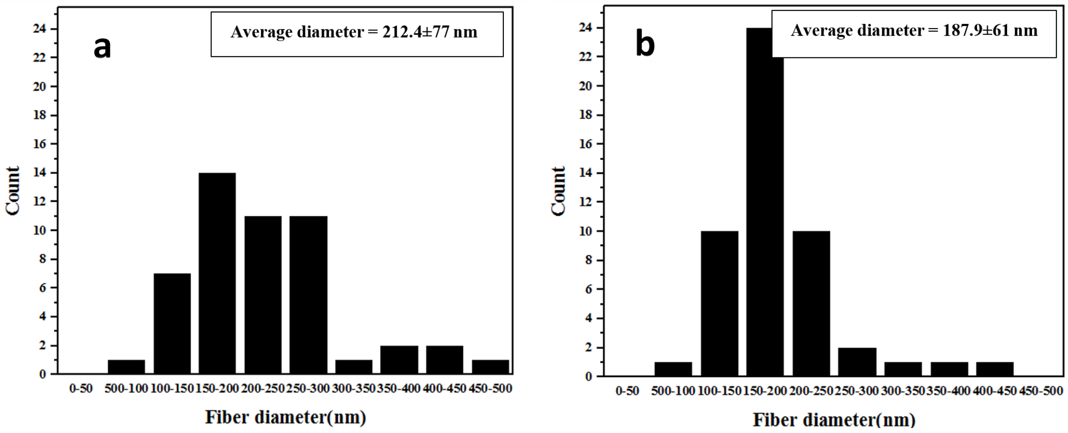

Fig. 6- Average and size distribution of nanofiber coatings; a) Chitosan fibers and b) composite fibers.

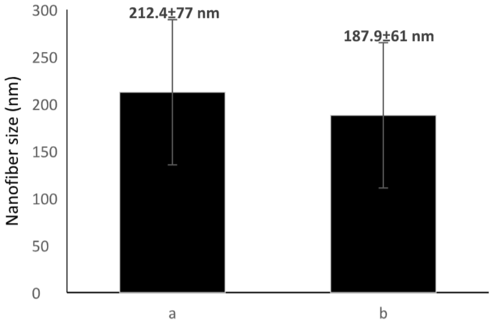

Fig. 7- Average diameter size of chitosan nanofibers (a) before addition of CNTs and (b) after addition of CNTs.

the nanofibers decreases from 212 nm to 188 nm and also the fiber size becomes somewhat more uniform. Composite nanofiber structure is denser. This is due to the effect of carbon nanotubes on the improved electrical conductivity of the solution that results in better electrospinning of the deposit [17].

As mentioned in the previous section, chitosan-based nanofibers must be stabilized after electrospinning. Fig. 8 a-d show SEM images of non-stabilized nanofibrous coating after contact with water (8a) and the coatings after stabilization in sodium carbonate solution for different times (8b-d). From Fig.8, it appears that 10 min is the optimum time for nanofibers stabilization with the least amount of changes in nanofibers structure. Fiber stabilization was confirmed by FTIR too.

Fig. 9 shows the spectra obtained from Fourier Transform Infrared Spectroscopy (FTIR) of chitosan nanofibers (1), and the fabricated composite nanofibers (2) together with the chitosan nanofibers after being stabilized (3). The addition of carbon nanotubes slightly changed the peak intensity. The relatively wide peak at 3450 cm^{-1} is related to the stretching of the primary amine and OH^- functional groups. The peaks at 1050 cm^{-1} and 1110 cm^{-1} corresponds to the saccharide structure of chitosan. The Chitosan-based Nanofibers stabilization can be explained as follows: when the nanofibers are in contact with Na_2CO_3(aq), the chitosan Trifluoroacetate salts ($-NH_3^+CF_3COO^-$) dissolves and leave $-NH_3^+$ groups on the chitosan chains. Neutralization of the $-NH_3^+$ groups occur rapidly such that the detached proton reacts with CO_3^{2-} ions to become HCO_3^- ions. Moreover, the detached proton can react with HCO_3^- ions to obtain carbonic acid. The characteristic peaks of chitosan fibers before stabilization are observed at 1530 cm^{-1} and 1670 cm^{-1}, which are related to the stretching of the protonated amine ($-NH3^+$) functional group. The presence of a strong peak at 1670 cm^{-1}, a carboxylate stretch at 1200 cm^{-1} (from TFA) and 3 peaks in the range of 725 cm^{-1} to 840 cm^{-1} indicate the presence of the salts in chitosan

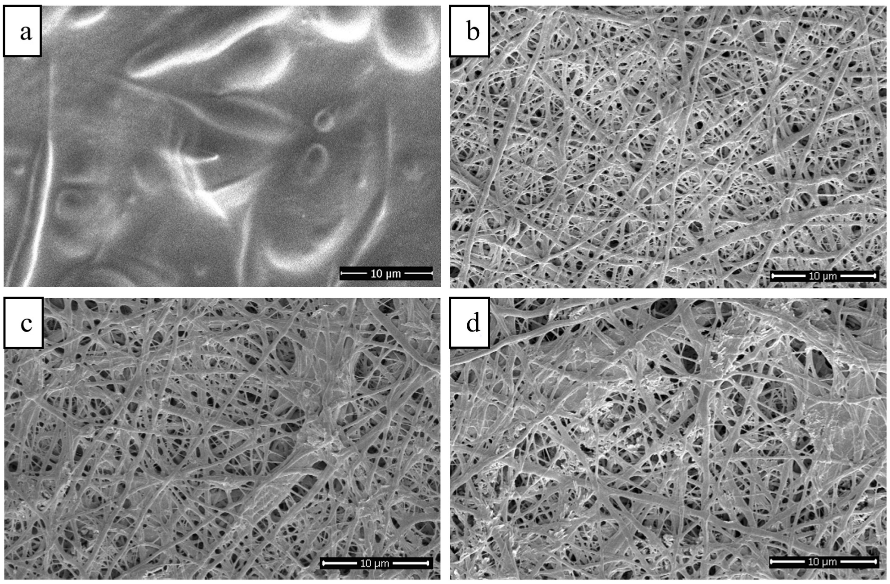

Fig. 8- FESEM images of non-stabilized specimen after contact with water (a) and stabilized specimens in sodium carbonate solution for (b) 10, (c) 25 and (d) 40 min. (Electrospinning time was 75 min).

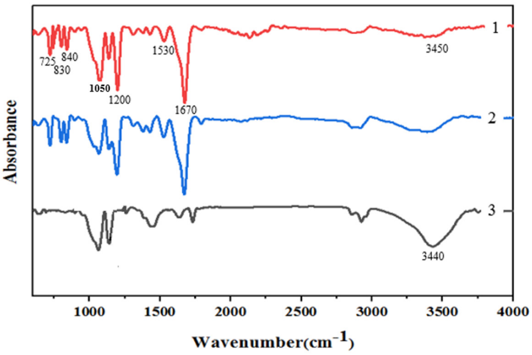

Fig. 9- FTIR spectra of nanofibers: (1) chitosan nanofibers, (2) nanocomposite fibers and (3) chitosan nanofibers after stabilization.

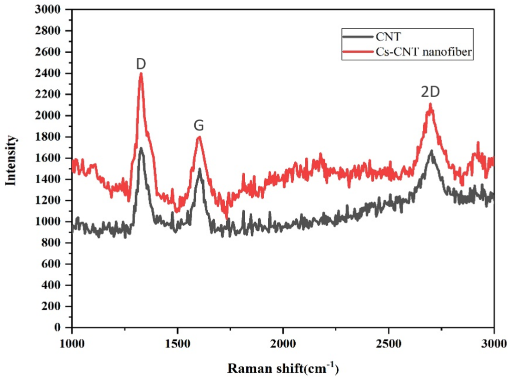

Fig. 10- Raman spectra: a) CNT and Chitosan-CNT composite coating.

fibers. But the spectrum of the nanofibers after stabilizing contains a strong peak at about 3440 cm^{-1} which is related to the stretching of the amine functional group (-NH_2) [16, 18]. It indicated that the ammonium ions (-NH_3^+) were converted to the amine (–NH_2) groups. The results verified the stabilization of the nanofibers.

The presence of CNT in nanofibers was confirmed by Raman Spectroscopy. As shown in Fig.10, the Raman spectra of nanocomposite fibers is almost similar to the spectra of CNT. Moreover, D-band, G-band and 2D-band peaks were detected at 1332, 1600 and, 2680 cm^{-1}, respectively indicated the presence of CNTs in structure of the coating [19]. The incorporation of carbon nanotubes in nanofibers can be clearly shown by TEM analysis [17, 20].

4. Conclusion

Homogenous and interconnected Chitosan/CNT nanofibrous coating was produced on anodized AZ31 magnesium alloy using conventional electrospinning technique. It was shown that the addition of CNT to chitosan nanofiber resulted in a reduction in size and a more uniform size of nanofibers. FTIR analysis showed that chitosan base nanofibers were correctly stabilized in 5M sodium carbonate solution for 10 min. Raman analysis indicated the presence of carbon nanotubes in the coating.

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