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Fabrication and Characterization of Polyvinyl Alcohol/Gelatin/ Silver Nanoparticle Nanocomposite Materials

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Abstract: In this study, nanofibers containing silver nanoparticles were fabricated by electrospinnining. The blends of polyvinyl alcohol as a synthetic polymer and gelatin as a biopolymer were used to fabricate wound dressing material having antibacterial activity. Scanning electron microscope (SEM) analysis indicated that porous and interconnected nanofibrous structures were obtained for all blend compositions. X-Ray diffraction (XRD) results showed the existence of silver nanoparticles in the structures. The chemical changes related to crosslinking and the change of the structure in simulated body fluid were evaluated using Fourier transform infrared spectroscopy (FTIR). Antibacterial tests were performed to prove the antibacterial activity. The inductively coupled plasma optical emission spectrometry(ICP) results showed that controlled silver ion release was achieved. It was concluded that obtained nanofiber mats could be suitable candidates for wound dressing applications

Keywords: Silver Nanoparticles, Polyvinyl alcohol, Gelatin, Electrospinning, Wound Dressing

1. Introduction

Tissue engineering has evolved as a combination of engineering and life sciences principles to enhance tissue functions decades prior. The main direction of tissue engineering is to develop a suitable scaffold to mimic the extra cellular matrix (Shalumon et al., 2011). Tissue engineering provides a variety of techniques for scaffold production of which electrospinning is specifically noteworthy (Merkle et al., 2015). As of presently so numerous natural and synthetic polymers as well as their mixtures have been attempted in this case. A wide assortment of polymers are utilized in creating scaffolds using poly (vinyl alcohol), poly(ethyl oxide), poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid), poly(caprolactone), or natural ones such as collagen, gelatin, silk, chitin, chitosan, alginate, hyaluronic acid which are natural extracellular matrix (ECM) analogs, due to their non-toxicity, improved biocompatibility, cell attachment and proliferation ability. In addition, polymers containing nanoparticles, and even metals and ceramics are used to increase cell migration and proliferation (Linh et al., 2010; Shalumon et al., 2011; Yang et al., 2011). Since the utilize of natural polymers have certain drawbacks like low stability, poisonous degradation products which can be destructive to the cells, the natural polymers are regularly mixed with synthetic polymers. Moreover this have upgraded mechanical properties, degradation stability and improved affinity to the cellular components (Shalumon et al., 2011). In any case, cytotoxic solvents such as 2,2,2-trifluoroethanol, 1,1,1,3,3,3-hexafluoro-2-propanol, trifluoroacetic acid, formic acid and dimethylformamide are generally utilized for their electrospinning (Yang et al., 2011).

Among the synthetic polymers mentioned above, PVA is one of a few situations which electrospun in aqueous solutions successfully to achieve nanofibrous mats with robust mechanical properties. As a semi-crystalline, biocompatible, non-toxic, hydrophilic polymer which has well chemical and thermal stability with incredible electrospinnability, it is one of the appropriate polymers that are readily mixed with natural polymers and crosslinked to form hydrogels (Fathollahipour et al., 2015; Yang et al., 2011). However, due to inadequate cell recognition sites, the bioactivity of PVA is significantly limited (Yang et al., 2011). Having natural plenitude, excellent biocompatibility and inalienable biodegradability in physiological situations, gelatin (GE) has been broadly utilized and contemplated with respect to numerous biomedical applications, such as surgical treatments, bone, skin and cartilage tissue engineering, wound or burn dressings (Rujitanaroj et al., 2008; Yang et al., 2011). By combining the advantages of PVA and GE, PVA /GE hybrid scaffolds are expected to

have robust mechanical properties and biocompatibility, while promising cell adhesion and biodegradability properties are achieved. Moreover, pure gelatin nanofibrous scaffolds are too delicate to ever be dealt with in practice. For this reason, hybridization of PVA with gelatin will significantly increase the flexibility of gelatinous scaffolds (Yang et al., 2011). The scaffold material in our study is a mixture of a PVA and a GE nanofiber obtained by one-step electrospinning as a wound dressing material.

Various investigations have been carried out with silver salts and silver compounds showing them as promising materials for wound management. With regard to the antimicrobial action, Ag salts and Ag compounds have been utilized as wound treatments in various physical shapes, such as beads, gels, films and fibers (Chun et al., 2010). For this reason, Ag NPs / polymer composites, which function as a bactericide, have been applied to complicated cases of infected burns, purulent wounds and wound healing matrix (Nguyen et al., 2010). In this study, microwave-assisted synthesis was performed to obtain soluble starch coated silver nanoparticles as colloidal solution and at that point this solution was blended with PVA/GE polymer solution to fabricate a novel wound dressing material displaying antibacterial activity.

2. Materials and Method

2.1 Materials

Polyvinyl alcohol (PVA, 95.5-96.5 % hydrolyzed, 85000-124000) was obtained from Acros. glutaraldehyde 50 wt. % in H_2O and gelatin from porcine skin were purchased from Sigma Aldrich. Acetic acid (glacial), tryptic soy broth (TSB), and tryptic soy agar (TSA) were supplied from Merck. Other than these materials, deionized water was used in order to prepare solutions.

2.2 Preparation of scaffolds containing silver nanoparticles

Soluble starch coated silver nanoparticles were synthesized by the method based on our previous study (Aktürk et al., 2019). This nanoparticle solution with mean particle size of 21 nm obtained by microwave-assisted green synthesis was mixed with acetic acid at 30/70 %v/v to prepare gelatin solution of 22 % wt/v. PVA solution was prepared at 10% wt/v using deionized water at 85 ℃ by stirring for 4 h. PVA solution was added to gelatin-silver nanoparticle solution (GE-Ag) at weight ratios of 3:1, 3:3, 3:5, and 3:7. The as prepared polymer solutions were electrospun at voltage of 25 kV, at spinning distance of 17 cm and at flow rate of 1 ml/h to fabricate nanocomposites with different Ag, PVA and GE compositions. Then these nanofiber mats were crosslinked under glutaraldehyde vapor by using glutaraldehyde solution (25ml) at 37 ℃ for 24h.

2.3 Silver ion release investigations

Nanofiber mats were soaked in simulated body fluid (SBF) for time intervals of 1, 7, 14, and 28 days. The concentrations of silver ions in SBF solution were measured.

2.4 Antibacterial activity

The antibacterial activity of the silver-containing nanofiber mats against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 were investigated by disk diffusion method. Neat PVA/GE nanofiber mats were utilized as controls. Nanofiber mats were cut into disc with diameter of 30 mm and the crosslinking process was applied on them. Crosslinked nanofiber mats were sterilized under UV light for 2 hours (each for 1 hour). The bacteria were grown in TSB by incubation for 24 hours at 37 ° C. Then, bacterial concentrations of *E. coli* and *S. aureus* were set at 10^6 CFU / mL and at that point 100 µL of this bacterial suspension was spread to the TSA. Sterilized nanofiber mats were put on the inoculated TSA. After incubation at 37 °C for 24 hours, the antibacterial properties of the nanofiber mats were assessed by the formation of inhibition zones around the nanofiber mats diameter on each inoculated plate. All analyzes were performed in triplicate for each nanofiber mat.

2.4 Characterization of nanocomposite fiber mats

The surface morphology and microstructure of the nanocomposite fiber mats before and after immersion in SBF were observed by using scanning electron microscope (SEM). Before the observation, the surfaces of scaffolds were sputter coated (SC7620 sputter coater, Quorum Technologies Ltd, United Kingdom) with platinum for 120 s. For each experiment, the average fiber diameter and its standard deviation were analyzed by the help of an image visualization software (Image-J, National Institute of Health, USA) from about 50 measurements of the random fibers. X-ray Diffraction (XRD) (PANanalytical Xpert Pro) analysis was conducted to prove silver nanoparticles in nanofiber structure. The functional groups of the nanocomposite mats before and after crosslinking and before and after SBF immersion were investigated by Fourier-transform infrared (FT-IR) spectroscopy. FT-IR spectra were collected using Perkin Elmer Spectrum 100 model spectrometer in transmittance mode in the mid-IR region (4000-650 cm^{-1}). The concentrations of silver ions in the SBF solutions were measured by using inductively coupled plasma optical emission spectrometry (ICP-Perkin Elmer Optima 2100 DV model).

3. Results

Electrospinning was used to prepare highly porous nonwoven nanofibrous mats. Figure 1 shows SEM micrographs, average diameter and diameter distribution of the fiber mats made from PVA addition to GE-Ag blend at weight ratios of 3:1, 3:3, 3:5, and 3:7. As shown in Figure 1 defect free fibers are observed. When the PVA content increased, nanofiber mean diameter first decreased and then increased. Because the standard deviation of nanofiber size distributions are in a narrower range with the addition of PVA, it can be concluded that the nanofiber structures were formed more homogeneous by the aid of PVA.

The characteristics of Ag NPs in the nanofiber structures were investigated by using XRD measurements. The

representative XRD patterns of 3.1 PVA/GE-Ag and 3:7 PVA/GE-Ag nanocomposite mats are given in Figure 2. The 3:1 PVA/GE-Ag sample has diffraction peaks at 2Θ= 38.55˚, 44.804˚, 65.001˚, and 77.908˚, which belong to (111), (200), (220), and (311) crystal planes of Ag with cubic symmetry, individually. The 3:7 PVA/GE-Ag sample has these characteristic peaks at 38.783˚, 44.885˚, 64.998˚, and 77.944˚ (Celebioglu et al., 2014; Cheng et al., 2015; Islam and Yeum, 2013; Li et al., 2013; Mahanta and Valiyaveettil, 2012; Usman et al., 2016; Xiao et al., 2012; Zhang et al., 2016).

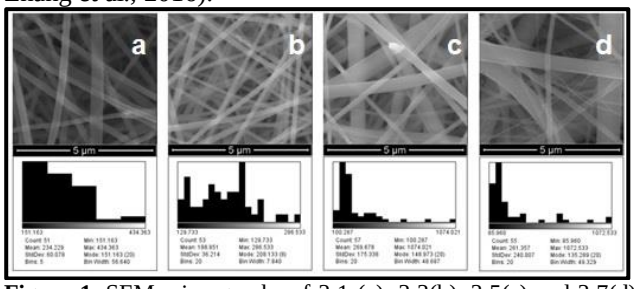

Figure 1. SEM micrographs of 3:1 (a), 3:3(b), 3:5(c) and 3:7(d) PVA/GE-Ag nanocomposites

For both nanofiber mats characteristic peaks of elemental Ag were observed, but, there were also additional peaks at about 28˚ corresponding to the indices (110) for Ag_3O_4 and 32˚ corresponding to the indices (120) for Ag_2O_2 which are the sign of oxidation of Ag (Celebioglu et al., 2014; Cheng et al., 2015). This is possibly originated from the further oxidation of Ag by PVA (Hong et al., 2006). With the addition of PVA into the structure, the intensity of the diffraction peak at around 19˚-21˚, which occurred due to the string inter and intra molecular hydrogen bonding corresponding with the crystalline peak of pure PVA, increased significantly (Cheng et al., 2015; Islam and Yeum, 2013; Lin et al., 2014; Park et al., 2011; Şimşek et al., 2016; Usman et al., 2016).

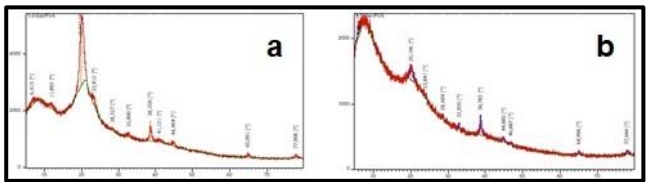

Figure 2. XRD spectra of 3:1 (a) and 3:7(b) PVA/GE-Ag nanocomposites

Because PVA and GE are water soluble, an e-spun GE and PVA nanofiber mat can partially or completely dissolve the when it comes in contact with an aqueous environment. When exposed to high ambient moisture, it may partially resolve and lose its fibrous structure (Rujitanaroj et al., 2008). Protection of nanofiber mat morphology and interfiber pores are critical for applications where the high surface area to volume ratio is advantageous (Destaye et al., 2013). More cross-linking is required to prolong the use of e-spun gelatin nanofibers in applications that need to be exposed to an aqueous medium or high humidity. Among the various chemical systems for cross-linking an e-spun gelatin (e.g., HDMI, EDC and GTA steam), GTA appears to be most economical and optimal material because it does not endanger the fibrous structure of the e-spun membrane (Rujitanaroj et al., 2008). The cross-linking reaction of PVA and GE was determined by using FTIR. The FTIR spectra of 3:1 PVA/GE-Ag and 3:7 PVA/GE-Ag nanocomposite mats were given in Figure 3.

The FTIR spectra of the nanofiber mats have the peaks of PVA which are characteristic broad spectrum at 3300 cm^{-1} (-OH stretching), 2916 cm^{-1} (symmetric -CH_2-), 1436 and 1087 cm^{-1} for C-O stretching and bending bonds, and 836 cm^{-1} for vibrational mode of C-C (Ghasemzadeh and Ghanaat, 2014; Kumar and Jaiswal, 2016; Martínez-Rodríguez et al., 2016). After the crosslinking reaction of PVA, the band at 1000-1140 cm^{-1} is observed due to the O-C-O vibration of the acetal group formation (Destaye et al., 2013). A new band at 1140 cm^{-1} is assigned to the symmetric C-C stretching mode in crystalline regions (Sui et al., 2012). This characteristic peak is well known to be sensitive marker for determining the degree of crystallinity of PVA. That is, the crystallization behavior of PVA is increased, which has an important role in preventing the dissolution of nanofibers in solutions (Sui et al., 2012). As seen in the spectra of 3:1 PVA/GE-Ag before and after crosslinking reaction, there is no differences for both structures except for the formation the band at 1143 cm^{-1} after crosslinking reaction. Due to the ratio of PVA is higher than GE at this nanofiber structure, the crosslinking reaction effect related to PVA crosslinking was seen more dominant.

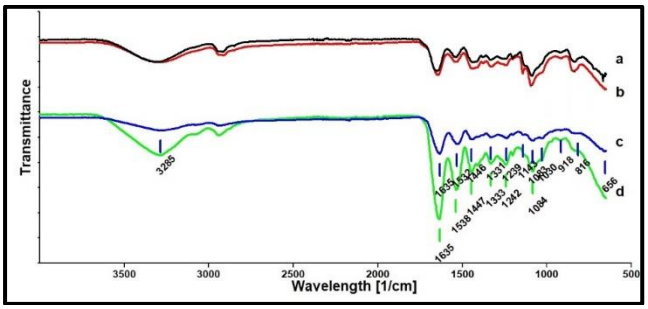

Figure 3. FTIR spectra of 3:1 PVA/GE-Ag before(a)/ after crosslinking (b) and 3:7 PVA/GE-Ag nanocomposites before(d)/after(c)crosslinking

The ordinary band assignments for gelatin detailed in the literature are amide A (N-H stretching vibration) found at around 3300 cm^{-1}; amide I (C=O stretch) found at 1640 cm^{-1}; amide II located between 1535 and 1554 cm^{-1} and amide III observed at 1240 cm^{-1}. The amide II and amide III groups result from the bending vibration of N-H groups and the stretching vibrations of C-N groups and interaction between these two modes (Gao et al., 2013). When the FTIR spectra of the 3:7 PVA/GE-Ag structure before and after crosslinking are examined, it is seen that the intensity of N-H group stretching vibration observed at 3285 cm^{-1} and the N-H group bending vibrations observed at 1635 cm^{-1} decrease relatively after cross-linking. The intensity of groups between 1250 and 1160 cm^{-1} decreased, which demonstrated the amount of C-N-C groups decreased (Lu et al., 2015). Additionally, the formation of the band at 1143 cm^{-1} was observed, which is the sign of the crosslinking reaction of PVA.

The success of crosslinking was checked by the immersion of nanofibrous mats in SBF. The SEM images of soaked nanofibers in SBF were given in Figure 4. As seen the images at day 0, the fibers fused together after cross-linking because of the existence of the water in moisture rich glutaraldehyde vapor (Gönen et al., 2016).

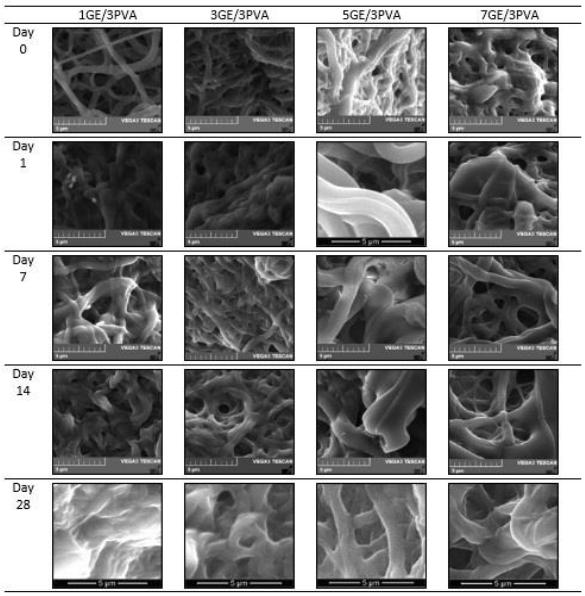

Figure 4. SEM micrographs of 3:1 (a), 3:3(b), 3:5(c) and 3:7(d) PVA/GE-Ag nanocomposites in SBF in different time intervals.

The change of chemical structures of 3:1 PVA/GE-Ag and 3:7 PVA/GE-Ag nanofiber mats were determined after SBF immersion for 28 days by using FTIR. As seen in the Figure 5, the 3:1 PVA/GE-Ag nanofiber mat was more effected than 3:7 PVA/GE-Ag nanofiber mat. It was observed that the peak at 1143 cm^{-1} showing the crystallinity of the 3:1 PVA/GE-Ag structure disappeared after the immersion in SBF.

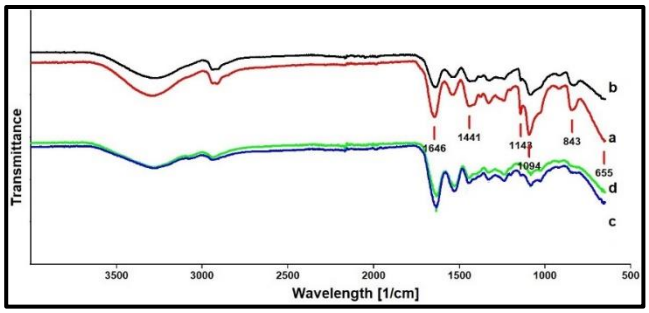

Figure 5. SEM micrographs of 3:1 (a), 3:3(b), 3:5(c) and 3:7(d) PVA/GE-Ag nanocomposites

The silver ion contents of the SBF solutions after the immersion of nanofiber composite mats were measured to determine the potential of the nanofiber mats for antibacterial and biomedical applications. During this method the ratio of nanofiber to SBF was taken as 0.25 mg/ml. Figure 6 illustrates the amount of silver ion releases into SBF as a function of time.

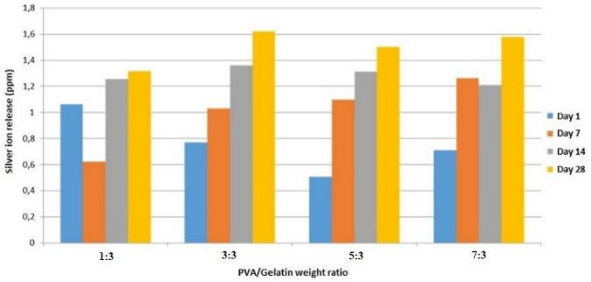

Figure 6. Silver ion release of nanocomposites in SBF in different time intervals.

Antibacterial tests were performed with 3: 7 PVA / GE-Ag sample since it has both stability in SBF and silver ion release below toxic level. The antibacterial properties of 3:7 PVA/GE-Ag nanofibers against gram-negative *E. coli* and gram-positive *S. aureus* were investigated by agar diffusion test measuring bacterial growth inhibition zone. Figure 7 shows images of the inhibition zones of the 3:7 PVA/GE-Ag nanofibers against *E. Coli* and *S. aureus* using 3:7 PVA/GE as the control.

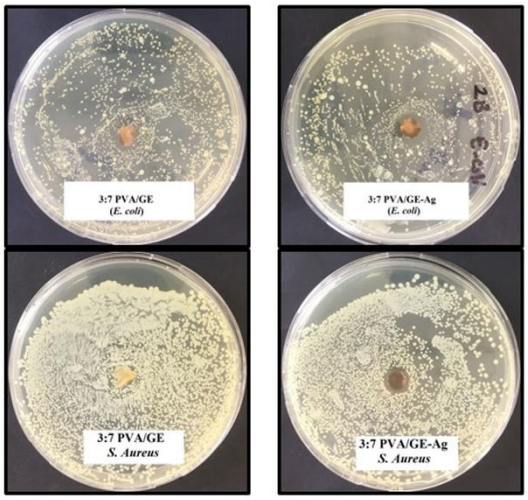

Figure 7. Antimicrobial activity of 3:7 PVA/GE-Ag nanofiber mats against *E. Coli* and *S. aureus*.

The inhibition zone of the surrounding Ag NP-containing fibers can be clearly observed. However, the PVA/GE nanofibers without AgNPs did not show any inhibition region. The results are dependent on the presence of antibacterial ability of AgNPs in the fibers. In particular, the 3:7 PVA/GE-Ag nanofibers were more effective against *E. coli* than *S. aureus.*

4. Discussion

A perfect wound dressing ought to be porous to preserve a wet environment and anticipate lack of hydration. Subsequently it ought to have high porousness, a high degree of swelling, and little weight loss (Li et al., 2013). To make the structure suitable for wound dressing applications silver nanoparticle containing GE/PVA nanofiber mats were cross-linked by saturated vapor from 50 vol% GTA aqueous solution for 24 h, followed by a heat treatment at 120 ℃ for 2h in this study. The formation of uniform fiber structures at nanofiber mats elucidate that the concentration and the viscosity of the electrospinning

solutions were at the optimal levels. The leaching behavior of PVA/GE-Ag nanofibers were evaluated using static method under equal conditions. Based on the SEM images, the more content of gelatin in the structure, there is smaller dissolution and more retention of the morphological structure of the crosslinked samples in SBF for 1, 7, 14 and 28 days. The Ag^+ ion concentration which is effective to inhibite the growth of bacteria was published at above 0.1 ppb and it was reported that below 2.3 ppm and 1.7 ppm have no effect on HaCat keratinocytes and human epidermal keratinocytes, respectively (Zhao et al., 2012). The maximum silver ion amount released from these nanofiber mats was 1.6224 ppm, which is under the toxic level. The accumulative Ag^+ release of all the nanofibers show similar trend. This recommends that the fibers ought to have sound antibacterial capacity and little cytotoxicity.

5. Conclusions

In this study, it was aimed to fabricate silver nanoparticle containing polyvinyl alcohol/gelatin nanofiber mats. The results showed that silver nanoparticles were incorporated in the structure successfully. The FTIR and SEM results showed that nanofiber structure of the blend at high gelatin ratio retained and there is no chemical changes after SBF immersion in SBF. The antibacterial tests performed with this nanofiber mat showed activity against *E. coli and S. aureus*. The obtained results demonstrates that this nanocomposite has the potential to use in wound dressing applications.

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