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Silver Sulfadiazine-loaded PVA/CMC Nanofibers for the Treatment of Wounds Caused by Excision

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Abstract: Polyvinyl alcohol/carboxymethylcellulose/silver sulfadiazine composite nanofibers (PVA/CMC/SSD) containing different proportions of each ingredient were prepared by electrospinning and characterized by Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), thermo-gravimetric analysis (TGA), powder X-ray diffraction analysis (XRD), atomic absorption spectroscopy (AAS), and ultraviolet-visible (UV-Vis) spectroscopy. The obtained nanofibers were uni-axially formed with randomly oriented longitudinal axes without entanglement, and their mean diameters ranged from 160 to 210 nm. Silver sulfadiazine, on the other hand, was appeared as a non-uniform dispersion of spherical nanoparticles ranging from 35 to 75 nm over the surface of nanofibers. Efficacy of the silver sulfadiazine loaded nanofibers for treatment of wounds caused be excision in rabbits in a period of 14 days was evaluated and it was found that the extent of skin repair in the applied dressing depends on the total Ag content. The dressing composed of 73.6:24.6:1.8 weight percent of PVA/CMC/SSD provided the best result in comparison with a control dressing made form PVA/CMC (88.8:11.2 %). In vitro cell viability of fibers on HSF-PI 18 fibroblast like cells was assessed by MTT assay. Antibacterial activity of the prepared nanofibers was also evaluated against gram-negative *Escherichia coli* and *Pseudomonas aeroginosa,* and gram-positive *Staphylococcus aureus*. PVA/CMC/SSD samples, was shown to have significant inhibition effect in comparison with drug references including penicillin G, chloramphenicol and erythromycin as positive controls (P<0.05).

Keywords: Electrospinning, Polyvinyl alcohol, Carboxymethyl cellulose, Silver sulfadiazine, Wound

Introduction

In recent decades, various techniques have been used for the production of polymeric continuous nanofibers such as drawing, template, phase-separation and electrospinning process. Among the methods of production of nanofibers, electrospinning is one of the best techniques due to its suitable price to prepare continuous fibers [1]. Newly, electrospun nanofibers have been applied as wound dressing, because nanofibrous matrix fabricated by electrospinning is similar to a structure which mimics native extracellular matrix (ECM) [2]. Several polymer-based nanofibers have been applied as wound dressing, including polyvinyl alcohol, gelatin, carboxymethyl cellulose, and collagen [3] which has been proved to be effective in improving the skin healing process [4]. Polyvinyl alcohol has a number of desirable characteristics that makes it a good material for dressing wounds. For example it has sufficient mechanical strength and high flexibility, non-toxic nature, and in addition water swells it [5]. Also electrospun carboxymethyl cellulose (CMC) nanofibers have attracted attention for medical purposes because of its excellent biocompatibility and biodegradability. In addition, PVA blended with polysaccharides such as CMC and some other synthetic polymers are attractive because of abundance, ease of modification, and good biocompatibility [6]. CMC blended with PVA acts as a model drug carrier because PVA can reduce repulsive forces within its charged solution [7]. There are many types of materials which are useful for prevention from colonization of various types of bacteria and wound infection, such as Ag NPs and silver sulfadiazine (antibiotic agent) which should be incorporated into the polymeric nanofibers matrix [8]. Composite fibers have been synthesized by electrospinning a dispersion of Ag NPs in a polymer solution and have been widely used for treatment of wounds as dressing. Silver sulfadiazine (SSD) drug has been widely applied for healing of burn-wound over the past decades [9]. Gelatin/Polyurethane nanofibers scaffold containing silver sulfadiazine (SSD) was prepared, dried at room temperature (NFSSD-2) and dried by using lyophilization (NFSSD-1), and was used as burn-wound healing scaffold on animal models, in comparison with gauze. It was found that although there were marginal differences in the burn-wound closure rate of the experimental groups, but NFSSD-2 showed the best burn-wound healing effect in all of the groups [10].

Morsi *et al.* have prepared SSD loaded chitosan/carbopol mixture based hydrogels, to form cubosomal hydrogels (cubogels) for topical treatment. In vivo study was conducted on rats to predict the effectiveness of the newly prepared cubogels in comparison with the commercially available cream Dermazin. The results showed that prepared cubogels were successful in the treatment of deep second degree burn which may result in better patient compliance [11]. Lee *et al.* have successfully manufactured chitosan/polyurethane blended fiber sheets containing silver sulfadiazine (SSD) by

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electrospinning technique. The electrospun fibers were tested for their antibacterial activity against gram-negative (*P. aeruginosa*), gram-positive (*S. aureus*) and Methicillin-resistant *S. aureus* (MRSA). They proved that chitosan/ Polyurethane/SSD fiber sheets have displayed a strong antimicrobial activity by inhibition of bacterial growth and prevention of infection during the wound healing process. The results indicated that this material would be good as a wound dressing [12]. Ullah *et al.* prepared an antibacterial wound dressing with silver sulfadiazine loaded on zein nanofiber mats by electrospinning. In order to investigate antibacterial properties, Bacillus and *E. coli* bacteria strains were used as Gram-positive and Gram-negative strains respectively. The antibacterial activity of zein nanofibers loaded with drug was observed with both strains of bacteria in comparison to a control. Excellent antibacterial efficacy was attributed to the sample with SSD [13]. Keeping in mind, increased inflammation observed with SSD application is caused by the cream base itself. This superficial inflammation increases protease activity on the wound surface, which is very harmful to a viable healing wound such as bed wound [14] but electrospun nanofibers containing SSD dressing can minimize these deleterious effects by controlled release system during the treatment of wounds [15]. Recently, Gao *et al.* reported preparation and characterization of silver sulfadiazine-loaded polyvinyl alcohol hydrogels and showed their effective antibacterial activity as wound dressing [16].

In this novel study, for the first time, we designed SSD embedded nanofibers of PVA/CMC with different contents and confirmed its positive feasibility as wound dressing. Also, the release behavior of nanofibers incorporated with SSD was studied in phosphate buffer solution (PBS, pH 7.4). In vitro study of the samples incubated at 37±1 °C for 24 h, were perrformed to determine their antibacterial properties against three types of bacteria strains: gram-negative *P. aeruginosa*, *E. coli* and gram positive *S. aureus*. Finally, the wound healing efficacy of the PVA/CMC nanofibers containing SSD was assessed by an in vivo rabbit model in view appearance of wound surface. Therefore, it is clear that this research will be useful in industrial scale for applications of PVA/CMC/SSD nanofibers as wound dressings for healing wounds [17].

Experimental

All of the chemicals including PVA (99 %, M=125000 $g \cdot mol^{-1}$), and NaCMC (viscosity of 1000-2000 cps) were purchased from Sigma-Aldrich and was used as received. Silver sulfadiazine (99 %) was dedicated by Sobhan Darou, Rasht, Iran.

Bacteria and Culture

The bacteria *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Pseudomonas aeroginosa* (ATCC 15442) were purchased from American Type Culture Collection and used as test microorganisms. The strains were cultured on Nutrient Broth medium and incubated at 37 °C for 24 h. Statistical analyses were performed by using SPSS version 19 in Windows 7. One-way ANOVA followed by the Duncan test at a confidence level of 95 % was used. All experiments were done in triplicate and data presented as the mean±SD.

Instrumentation

XRD patterns were recorded on a SPOE STAT-IP with mono chromatized Cu Kα radiation at 40 kV in the range of 2θ 5-70 °. SEM imaging was performed on an EM-3200 scanning electron microscope. FT-IR spectra were recorded in KBr disks on an ALPHA Bruker instrument. UV-Vis spectra were recorded on a Photonix Ar2017, Iran. DSC analysis was performed on a Netzsch 200F23 instrument. Atomic adsorption analysis was performed by a Yang-Lin AAH-8000 instrument. The electrospinning process was performed on a Profile apparatus, Iran.

Preparation of Polymer Solutions

10 % w/v solution of polyvinyl alcohol in deionized water was prepared via stirring at 80 °C for 6 h (viscous solution, A). Sodium carboxymethyl cellulose (CMC) was added gradually to deionized water at 90 °C and stirred for 4 h to prepare a homogeneous viscous solution (5 % w/v, B). Silver sulfadiazine (SSD) was dissolved in deionized water under ultrasound irradiation for 45 min to prepare the required concentration (C).

Preparation of PVA, PVA/CMC, and PVA/CMC/SSD Nanofibers

For preparation of PVA nanofibers, the following parameters were used: Rate of injection: 0.5 m*l*/h, applied voltage 18.0 kV, distance of needle from the ground collecting drum: 11 cm.

For preparation of PVA/CMC nanofibers, 80/20 mixture of solutions A and B was stirred magnetically for 12 h at 60 °C and then injected at 0.7 m*l*/h from a needle at the distance of 11 cm at 18.0 kV.

To prepare PVA/CMC/SSD nanofibers with different compositions, the required volumes of solutions A and B was cooled down to 40 °C, and then silver sulfadiazine solution (C) was added dropwise during 2 h, and the obtained homogeneous solution was injected at the rate of 0.4 m*l*/h from a needle at the distance of 11 cm at 19 kV.

After 5 h, the mat of the aforementioned nanofibers was collected and dried at 60 °C overnight.

In vitro Drug Release from PVA/CMC/SSD Nanofibers

In vitro release behavior of PVA/CMC nanofibers loaded with SSD (1.6, 1.7 and 1.8 % w/w) was studied in 20 m*l* of phosphate buffer solution (PBS, pH 7.4) in an Erlenmeyer. The samples were incubated at 37±1 °C for 14 days with

continuous shaking (120 rpm). At systematic time intervals (3, 5, 10, 18 h and 1, 2, 5, 7, 9, 11, 13 d), 4 m*l* of the solution was sampled and the same amount of fresh PBS was replaced to maintain a constant volume. Each sample was filtered and the amount of released drug was determined via measurement of the absorbance of the filtrate at the wavelength of 254 nm by using a UV-Vis spectrophotometer.

Animals

New Zealand white rabbits of both sexes, weighing 1700±25 grams were used in this study. The rabbits were purchased from Pasteur Institute of Iran (Tehran, Iran). The rabbits were kept individually in the cages (61 cm×77 cm× 46 cm) and allowed to feed on a standard, commercial, pellet diet supplemented with fresh vegetables and tap water. Food and water was available ad libitum during the experimental period. The test animals were kept in a holding room at a temperature of 22±2 °C and a humidity of 55 percent. The full thickness wound was made in the skin of the rabbits. Hairs of the back of the rabbits were fully shaved. The animals were held in standard crouching position. A template measuring 20×20 mm^2 was placed on the stretched skin by using a fine tipped pen and this area was locally anaesthetized with a subcutaneous injection of lidocaine 2%. The wounds were made by excising the skin, within the border of template to the level of loose subcutaneous tissue using a size 15 scalpel blade and a forceps. Four excisional wounds with diameter of 7 mm were made in the back of each animal. Then, one wound was covered with pure PVA/ CMC (as control) and the rest was covered by three different SSD-loaded nano-fibers (entries 4, 11, 12 in Table 1). The numbers of 5 rabbit were used in this study. All treatments were applied once a day until complete healing was achieved. The rabbits were returned to the cages after each treatment and their cages were changed daily kept clean to avoid wound infection. To determine wound healing, every 24 hours, each rabbit was held in the standard crouching position and the outline of the wound was traced on a transparent plastic sheet using a fine tipped pen. Measurement errors were minimized by repeating each measurement three times and using the average of measurements in all calculations. All animal procedures were performed according to the Animals (Scientific Procedure) Act, 1986. The protocol was approved by the university of Guilan ethical committee.

Cell Seeding and MTT Assay

Discs (5 mm in diameter) of the neat PVA, PVA/CMC, and PVA/CMC/SSD (entries 4, 11, 12 in Table 1) nanofiber mats were sterilized under ultraviolet light for 1 h on each side, then decontaminated by soaking in 70 % (v/v) ethanol for 2 h. They were subsequently washed with sterile PBS and pre-soaked in complete medium containing RPMI 1640 (Bioidea, Glutamax high glucose), 10 % heat-inactivated fetal bovine serum (Gibco) and 1 % antibiotics (Bioidea, 100X penicillin/streptomycin solution) for 24 h. For seeding, the HSF-PI 18 fibroblast like cells (NCBI Code: C194, Pasteur Institute of Iran) were trypsinized (0.05 % trypsin-EDTA, Bioidea), centrifuged and resuspended in a complete culture medium. Aliquots of 50 μ*l* containing 1.5×10^4 cells were then seeded on top of each nanofiber disc. Cell nanofiber constructs were incubated (37 °C, 95 % humidity and 5 % CO_2) for 48 h. HSF-PI 18 cells were cultured in plastic dishes as control, with the same cell number as seeded on nanofiber discs.

Cell proliferation and nanofiber cytotoxicity was measured using the MTT assay kit (Bio-Idea, Houston, TX) at 2 days (24 h, and 48 h) after cells seeding. The test was performed using manufacturers recommended protocol. Briefly, the medium was removed and replaced with 100 μ*l* ready to use RPMI and then 10 μ*l* of MTT solution was added to each well. This was incubated for 4 h (5 % CO_2, 37 °C, 95 % humidity). The solution was then removed and 50 μ*l* dimethylsulphoxide (DMSO, 99.5 %; Sigma, St. Louis, MO) was added to dissolve the formazan products. The plate was incubated for 15 min at room temperature with gentle shaking. The absorbance was read by an ELISA plate reader (Biotek, Winooski, VT) at 490 nm with a reference wavelength of 630 nm. Cell viability was calculated using the following equation:

Cell viability (%): [(A490 − A630) of treated cells / (A490 − A630) of control cells] × 100.

All experiments were repeated at least three times, and the results are presented as mean± standard deviation (SD).

Antibacterial Function of the Nanofibers

In order to determine the antibacterial effects of nanofibers on gram-negative *E. coli* and *P. aeroginosa* and gram positive *S. aureus*, the bacteria were cultured in Muller-Hinton agar and incubated at 37 °C for overnight and their growth inhibition effects was measured. The surface of culture medium was swabbed with suspension of bacteria. The nanofibers were then cut of the round pieces with 5 mm diameter. Anti-bacterial assessment was performed by the disc diffusion method. The discs were placed on agar plates containing bacteria. Each plate was incubated at 37 °C for overnight. The clear zone was formed around SSD-loaded nanofibers and their antibacterial activities were evaluated via comparison with the non-loaded SSD nanofibers (pure PVA) which was considered as control. All experimental data were fetched from triplicate samples with individual nanofibers and were expressed as the mean±standard deviation. Statistically significance differences of samples were examined using the Student's test. The significance level was set at $P<0.05$.

Results and Discussion

A variety of PVA/CMC/SSD composites were prepared and their proportions are summarized in Table 1. UV-Vis spectra of all samples were recorded from a 50 ppm solution of the composite in water. Presence of a moderate peak as a shoulder at 275 nm is attributed to the carbonyl group of PVA, arising from $\pi \rightarrow \pi^*$ transitions, which is related to the extent of hydrolysis of vinyl acetate and oxidation during synthesis of PVA. Strong absorptions at lower than 200 nm are attributed to acetate group [18], and solvent cut-off effects. Increasing of the CMC and SSD content, on the other hand, resulted in an increase of the PVA matrix absorption in the $n \rightarrow \pi^*$ region due to agglomeration of these components in solution [19].

Table 1. PVA/CMC/SSD composites with different proportions

Composition	PVA/CMC/SSD ratios (%)
1	96.5:2.0:1.5
2	95.5:3.0:1.5
3	94.3:5.2:0.5
4	93.2:5.2:1.6
5	93.0:3.0:4.0
6	90.4:2.0:6.8
7	87.8:2.8:9.4
8	81.8:17.6:0.6
9	81.4:17.4:1.2
10	81.3:17.5:1.2
11	81:17.3:1.7
12	73.6:24.6:1.8

[a]All of the samples were prepared according to the general experimental procedure.

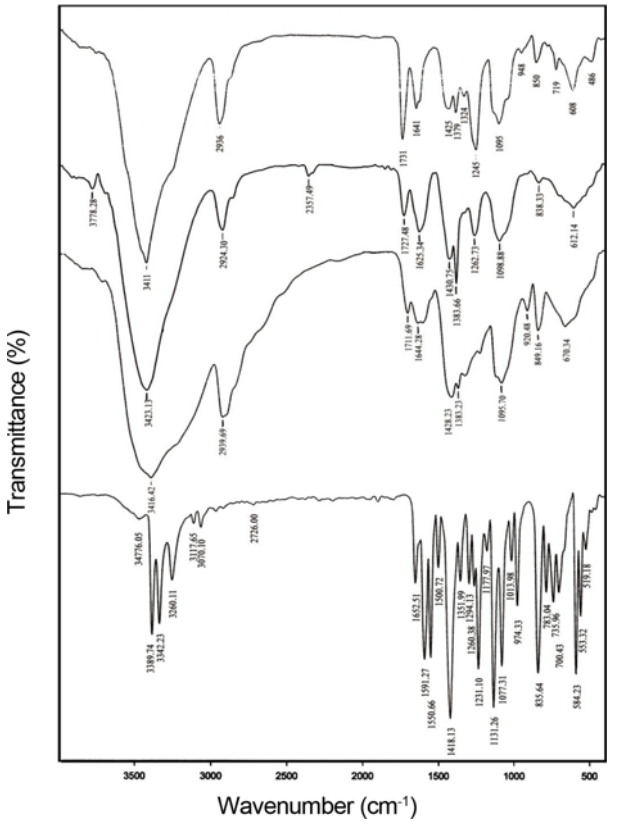


Figure 1. From top to bottom: FTIR spectra of Pure PVA mat, PVA/CMC (88.8:11.2), PVA/CMC/SSD (87.8:2.8:9.4) and SSD.

In the FTIR spectra of the PVA/CMC/SSD composites, characteristic vibrations of each component were observed with minor changes in the position and intensity of each band.

In the FTIR spectrum of PVA, the observed peaks (cm^{-1}) are attributable to the corresponding vibrations as follows [7, 20]: 3411 (br, OH stretching), 2936 (alkyl CH stretching), 1379 (symmetric O-CH_2 bending), 1095 (symmetric C-O-C bending), 850 (CH_2 rocking), 1731 (C=O stretching), 1425 (asymmetric O-CH_2 bending), and 608 (out of plane C-H bending). In the FTIR spectrum of PVA/CMC, in addition to the above mentioned vibrations, characteristic band of pyranose C-O was observed at 1628 cm^{-1}. When SSD was loaded onto the PVA/CMC, on the other hand, the pyranose stretching was broadened and shifted to 1643 cm^{-1} [21,22]. The overall shape of PVA/CMC/SSD spectrum, however, is more like the FTIR spectrum of PVA, due to low content of SSD. More or less, the same trend was observed in the case of other PVA/CMC/SSD composites. Generally, in all of them the O-H stretching was appeared at 3384-3446 cm^{-1}, which may be due to intermolecular hydrogen bonding between NH_2 of SSD and OH of pyranose ring [20]. Ethereal C-O-C stretching, on the other hand, in the range of 1094-1102 cm^{-1}, sharpened with the increase of SSD loading because of the absorption of pyranose ring at the same range.

Scanning electron microscopy (SEM) images of the PVA, PVA/CMC, and PVA/CMC nanofibers containing different loads of SSD (Table 1, entries 4, 7, 11, 12) are shown in Figure 2. Pure PVA nanofibers (Figure 2(a)) were formed as uni-axially aligned smooth fibers with randomly oriented longitudinal axes without entanglement, and mean diameters in the range of 190.0±30.0 nm. Addition of CMC to PVA nanofibers resulted in decrease of their diameter (Figure 2(b)). In the SEM images of the PVA/CMC/SSD nanofibers (Figure 2(c)-(f)), silver sulfadiazine was present as a non-uniform dispersion of spherical nanoparticles in the range of 35-75 nm. It can also be seen that disregard of polymer concentration, the diameter of nanofibers increases with increasing the applied voltage. This may be due to electrostatic stress which reduces the spinning speed of the fibers [10,23]. By increasing the concentration of silver sulfadiazine, the diameter of nanofibers was decreased.

Thermogravimetric analysis of PVA/CMC, PVA/CMC/SSD, and SSD was also performed and revealed that in the

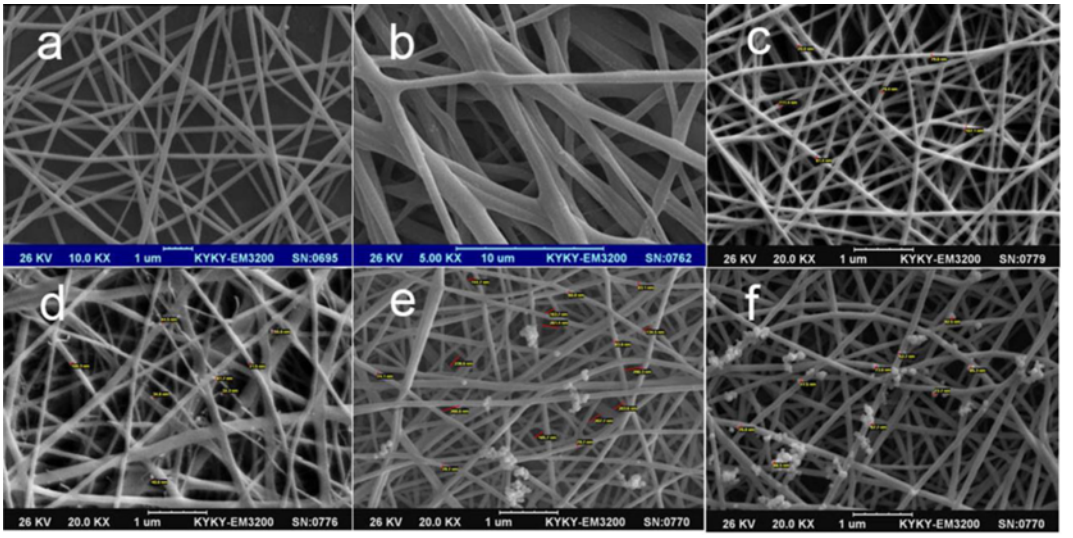


Figure 2. SEM micrographs of (a) pure PVA, (b) PVA/CMC (97.2:2.8), and (c-f) PVA/CMC/SSD composites with proportions listed in entries 4, 7, 11, 12 of Table 1.

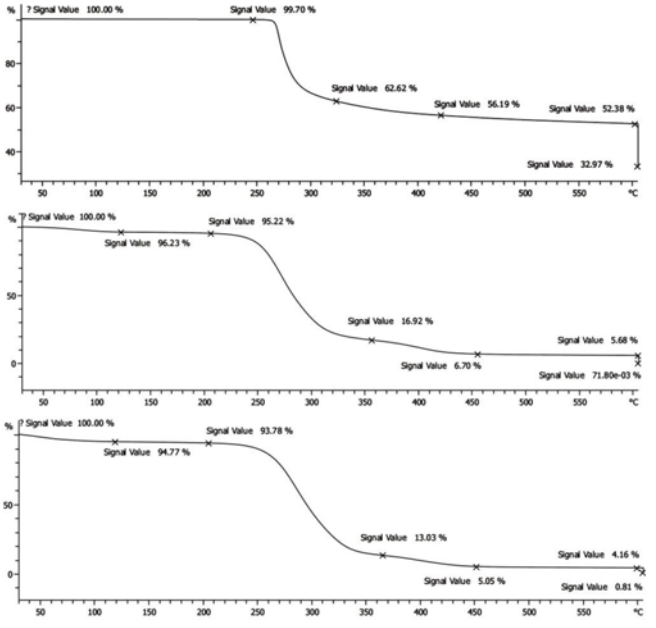


Figure 3. TGA analysis of SSD (up), PVA/CMC/SSD (87.8:2.8:9.4) (middle) and PVA/CMC (88.8:11.2) (bottom).

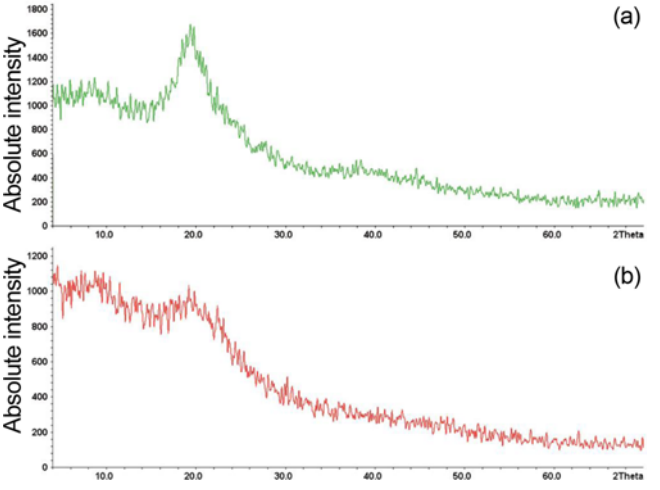


Figure 4. XRD patterns of PVA/CMC/SSD (a), and PVA nanofibers (b).

case of PVA/CMC, the main weight loss occurred at 205 °C (80.75 %) which is mainly due to carbonization process [24]. The remaining weight of 4.16 % at 559 °C, may be due to sodium oxide resulting from sodium carboxymethyl cellulose. The corresponding DSC curve, showed three endothermic minimums, with the most prominent valley at 185-233 °C [25]. Loading of silver sulfadiazine, moved the valley to 206-355 °C [26], and a remaining weight of 5.68 % at 604 °C is attributed to simultaneous presence of Na_2O and Ag_2O [12]. In the case of pure silver sulfadiazine, on the other hand, the main DCS valley appeared at 223-273 °C. These results are presented in Figure 3 and reveal that drug loaded fibers have higher thermal resistance.

In the XRD pattern of PVA nanofibers (Figure 4(b)), characteristic reflections of semi-crystalline polyvinyl alcohol were observed [23]. Broad peaks at 2θ 19.8 and 22 are attributed to amorphous PVA. In the XRD pattern of PVA/CMC/SSD (Figure 4(a)), on the other hand, the intense peak of CMC at 2θ 19.2 is clearly visible. No specific reflection due to SSD however, was observed which may be originated from its much lower content than that of CMC.

EDS analysis was used to further approve the presence of Ag in the PVA/CMC/SSD nanocomposite. Figure 5 compares

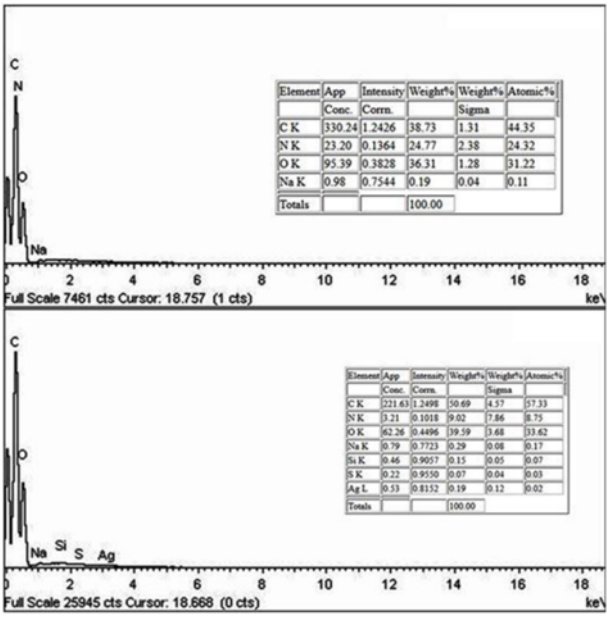


Figure 5. EDS analysis of PVA/CMC (88.8:11.2) (left), PVA/CMC/SSD (87.8:2.8:9.4) (right).

the EDS analysis of PVA/CMC and PVA/CMC/SSD and insets show the relative content of each element.

In vitro SSD Release from Nanofibers

The rate of drug release from PVA/CMC/SSD nanofibers may depend on various parameters such as thickness of nanofibers, ease of penetration of medium in polymer and type of interaction between drug and polymer. To get a better insight, profile of SSD release for PVA/CMC/SSD nanofibers with various SSD content (1.6, 1.7 and 1.8 wt%, entries 4, 11 and 12 of Table 1) was plotted versus time (Figure 6(b)). In the early stages (3 h after incubation), release of SSD was mainly controlled by diffusion from surface of the polymer according to the observed values (11.3, 10.6, and 11.1 %). After 24 h the amount of released SSD reached to 49, 51.5 and 55.5 %, and maximum release was obtained after 13 days (56.8, 71, 78 %). From Figure 6(b), it is clear that at the final stages, SSD release mainly depends on the SSD content of each nanofiber and this also affects the gradient of release at early days of incubation, because amount of released drug from PVA/CMC/SSD depends on the amount of the pores that are filled by PBS.

Indirect Cytotoxicity Evaluation

Biocompatibility is very important in wound dressing. For this reason, an MTT assay was performed to determine the cytotoxicity of the nanofibers. The in vitro cytotoxicity tests of single PVA, and PVA/CMC nanofibers with and without SSD toward HSF-PI 18 fibroblast (NCBI Code: C194) were evaluated using MTT assay for 1, and 2 days (Figure 7). As

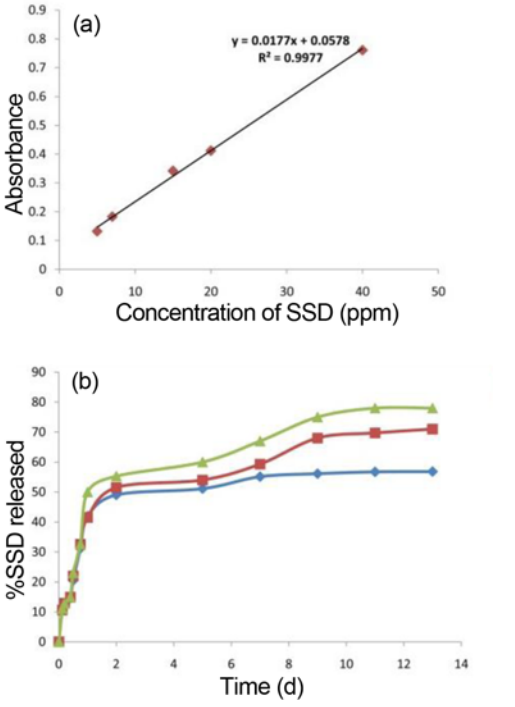


Figure 6. Calibration curve for determination of SSD at 254 nm (a), and SSD release profiles (b) for PVA/CMC/SSD nanofibers with various SSD contents (1.6, 1.7 and 1.8 wt%, entries 4, 11 and 12 of Table 1) versus time (days).

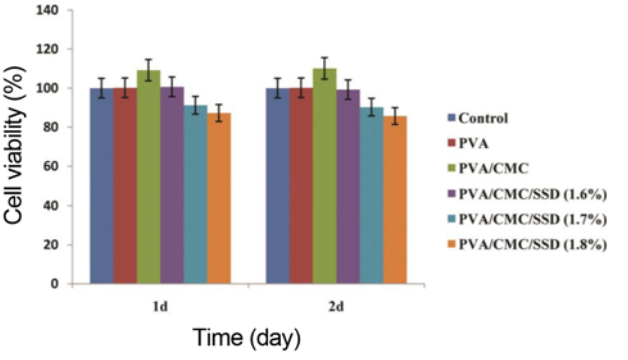


Figure 7. Cell viability in fibroblast for PVA, PVA/CMC, and PVA/CMC/SSD at varying concentration of SSD (1.6, 1.7 and 1.8 wt%) blend fibers mat (statistically significant $P<0.05$) cell. The viability of the control cells was set at 100 %.

can be seen, no significant cytotoxicity was observed with the neat PVA, but improvements in cells viability were observed in presence PVA/CMC blend nanofiber mat when compared with control ($P<0.05$). Also, it was shown that there is a slightly decrease in the cell viability with increase of the concentration of SSD into nanofibers matrix for 48 h. So that the PVA/CMC nanofiber containing SSD 1.8 wt% did not indicate significant cytotoxicity against cells and above 85 % of the cells remained after 48 h ($P>0.05$).

However, cell viability did not decreased significantly when cell fibroblasts were incubated with PVA/CMC/SSD nanofibers mat in various concentrations of the SSD (1.6-1.8 wt%) when compared with the control (P<0.05). These results suggest that the PVA/CMC/SSD nanofibers mat have excellent in vitro biocompatibility and are suitable for wound dressing applications.

Antibacterial Activities of the Nanofibers *in vitro*

Antibacterial activity of the prepared nanofibers was evaluated against gram-negative *Escherichia coli* and *Pseudomonas aeroginosa,* and gram-positive *Staphylococcus aureus*. As shown in Figure 8 for nanofibers (entries 4, 11, 12 Table 1) there were no significant difference (P<0.05) between diameter of inhibition zones in triplicate experimental data and their average diameters of the inhibition zones were nearly similar for each strain, individually.

Measuring the diameter of inhibition zone (mm) showed that the PVA and PVA/CMC nanofibers have no remarkable antibacterial activity (P<0.05), but PVA/CMC/SSD samples have been shown to have significant inhibition effect in comparison with drug references including penicillin G, chloramphenicol and erythromycin as positive controls (Table 2)(P>0.05). There was no significant difference between the antibacterial effect of PVA/CMC/SSD samples with mean SSD content of 1.7 % (P<0.05). While lower SSD contents drastically reduced antibacterial activity, higher loads of SSD did not improved antibacterial effect. However, SSD incorporated PVA/CMC exhibited significant activity against both *P. aeruginosa*, and *S. aureus* (P<0.05), in comparison with *E. coli*, thereby proving its applicability in protecting wound from established pathogens.

Table 2. Antibacterial tests of PVA, PVA/CMC, PVA/CMC/SSD, and positive controls on *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus*

Compound[a]	Applied volume: 30 μ*l*		
	Diameter of inhibition zone (mm)		
	E. coli	*P. aeruginosa*	*S. aureus*
PVA	-	-	-
PVA/CMC	-	-	-
PVA/CMC/SSD (93.2:5.2:1.6)	8±0.5	13.66±0.57	12±1
PVA/CMC/SSD (81:17.3 :1.7)	7.33±0.57	13.33±0.57	12.66±0.57
PVA/CMC/SSD (73.6:24.6:1.8)	6±0.0	13.33±0.57	12.66±1.15
Antibiotics[b]			
Penicillin G	0	0	0
Erythromycin	0	0	0
Chloramphenicol	20.6±1.2	22.6±0.5	21.7±0.6

[a]30 μ*l* of a 1.0 μg/μ*l* solution was used and [b]30 μg/disc.

Wound Healing by Nanofibers in the Rabbits

In order to study the wound healing properties of the obtained nanofibers, three kinds of nanofibers with different composition were selected. Three scars were coated with PVA/CMC/SSD nanofibers with proportions of entries 4, 11,

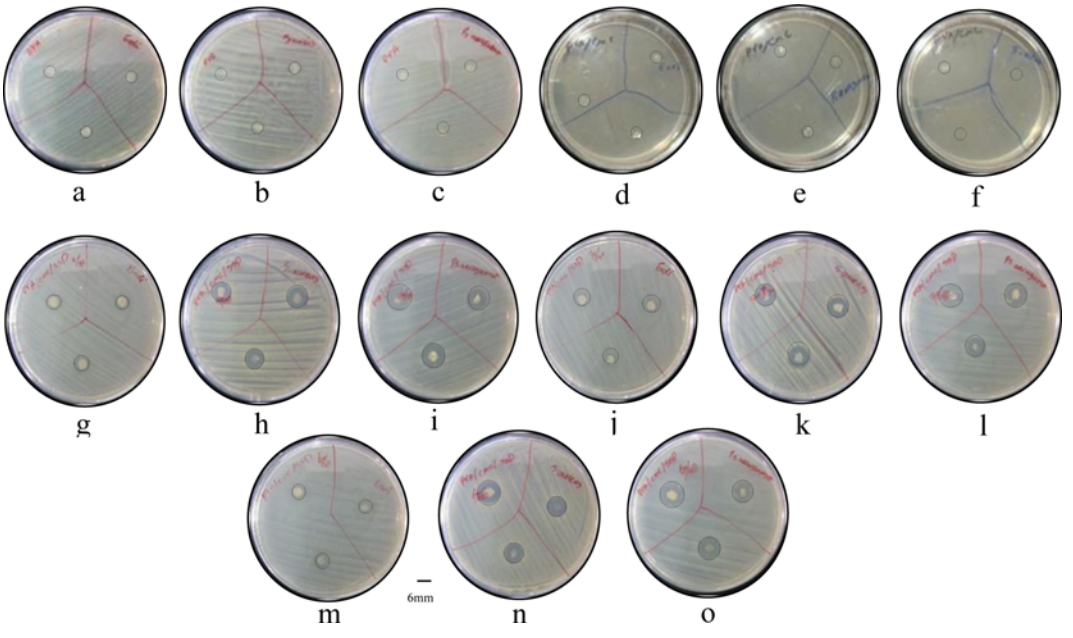


Figure 8. Antibacterial test against *E. coli*, *S. aureus*, and *P. aeroginosa*. The samples are PVA nanofibers (a, b, and c), PVA/CMC nanofiber (d, e, and f), and PVA/CMC/SSD (1.6, 1.7 and 1.8 wt%) nanofibers (g-o), respectively.

Table 3. Trend of wound healing in presence of PVA/CMC/SSD dressings containing 1.59 ppm (1), 1.63 ppm (2), and 2.11 ppm (3), against PVA/CMC (control)

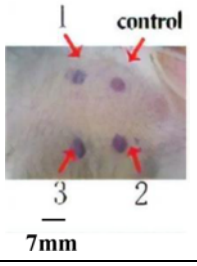
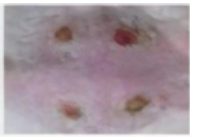
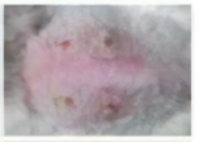
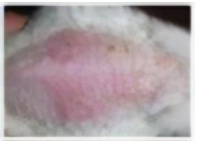
Ag content of dressing	Trend of wound healing during 14 days				
	Day 0	Day 4	Day 8	Day 12	Day 14
1.59 ppm 1.63 ppm 2.11 ppm Control: 0 ppm					

Table 4. Trend of wound healing in terms of mean wound diameter during 14 days

Wound no.	Mean of wound diameter±SD (mm)			
1 (1.59 ppm)	4.1±0.17	3.5±0.14	1.5±0.11	1.0±0.07
2 (1.63 ppm)	4.5±0.13	3.3±0.16	1.8±0.14	0.7±0.05
3 (2.11 ppm)	3.2±0.14	2.4±0.12	1.1±0.09	0.0±0.0
Control (0 ppm)	5.5±0.08	4.0±0.11	3.1±0.10	2.2±0.12
Days	4	8	12	14

12 from Table 1, and the 4th wound was covered by PVA/CMC (88.8:11.2) as control group. The dressings were changed every day until complete epithelialization. Table 3 shows process of wound healing during a period of 14 days. The images show that the extent of skin repair in the applied dressing depends on the total Ag content. To be more meaningful, the Ag content of each dressing was determined by atomic absorption spectroscopy (AAS) and the results are merged in the first column of Table 3. According to the data presented in Tables 3 and 4, nanofiber containing 2.11 ppm of silver nanoparticles indicated a significant decrease in wound size (P<0.05) during the 14 days, while in wounds treated with 1.59 ppm and 1.63 ppm of silver nanoparticles on day 8, no statistical significant difference in wounds size (P>0.05) was observed, when compared with the control wound. It is clear that after the 14th day, the dressing 3 with an Ag content of 2.11 ppm provided the best result. It was observed on day 14 that the wound reduction was above 98 % (P<0.05) for those dressed with 2.11 ppm, vs. 90 % and 86 % (P>0.05) with those dressed with 1.63 and 1.59 ppm, respectively. The same results were obtained when the mean±SD of wound diameters were measured as listed in Table 4.

Conclusion

In conclusion, an easy to prepare and effective wound dressing was invented for treatment of wounds caused by excision, based on silver sulfadiazine-loaded electrospun nanofibers of PVA/CMC. The obtained PVA/CMC/SSD composites also showed promising antibacterial activity against *P. aeroginosa* and *S. aureus.* Cytotoxicity analysis showed that the PVA/CMC nanofibers containing SSD were nontoxic. It is also suggested that PVA/CMC/SSD composites is an ideal candidate that can be used for wound healing applications.

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