Production and Characterization of Gelatin Based Electro-spun Nano-fibres as Burn Wound Dressings

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ABSTRACT

Silver sulfadiazine is used to prevent and treat infections of second- and third-degree burns. It kills a wide variety of bacteria. In this study silver sulfadiazine was used in gelatin based electro-spun nano-fibers with various drug to polymer ratios (0, 5, 10, 15 and 20 \%). SEM, EDX and FTIR analysis showed that the continuous, bead-free, fine fibers containing silver sulfadiazine as an antibiotic drug were successfully produced. The release profiles of the loaded drug from the produced nano-fibrous dressings were evaluated by an in vitro elution method. It was observed that the sample with 10 wt \% of gelatin has had the optimum trend of release. Moreover, antibacterial activity of the dressings was evaluated against the pathogenic micro-organisms S. aureus and E. coli in the nutrient agar solid medium. It was obvious that all the samples had antibacterial activity against these two bacteria. The produced silver sulfadiazine loaded gelatin based electro-spun nano-fibrous dressings have the potential for being used in the wound healing applications.

1. Introduction

The first and principle protective layer of the body which acts as a barrier in the case of assault of the exterior factors towards the body is the skin. However, when faced with some dramatic injuries, such as severe burns or chronic impacts, it may be wounded. In the case of burning, many advantageous skin constructive agents such as proteins may be damaged or even degraded and eventually begin losing their three-dimensional shape and the amount of them may be broken down which results in cell and tissue damage [1-6].

Moreover, in the aforementioned situation, disruption of the normal functioning of the skin will occur, namely: oxygen supply may decline, skin will be unable to prevent water loss through evaporation and to maintain temperature control, all of which results in the formation of a suitable environment for rapid growth and proliferation of pathogenic microorganism [5,7]. In these cases, the natural restoration processes such as epithelialization and collagen construction in wound healing process are defective [8].

Cutaneous wound healing is a process which
Hamedani, Fazeli, Barzin

includes stages of hemostasis, inflammation, tissue restoration and re-modeling with scar formation [9]. There are various factors which influence the healing process, one of which is bacterial colonization and infection of the wound [10].

Healing these severe and simply contaminated and infected burn wounds is challenging. In this regard, wound dressings play a crucial role since they present various advantages by providing a preserving effect, an appropriate micro medium for tissue restoration and by conserving the bacterial infection [9, 11, 12]. From the traditional point of view, the injured area must be eluted with a topical antimicrobial agent one to two times a day so that the produced infections become less noticeable day by day. However, this application of the antimicrobial agent usually causes discomfort for the patients. On the other hand, although it is true that the traditional dressings provide a slight preservative effect against bacteria, as soon as the wound exudates elute the other surface of the dressing, this ability will certainly be lost. Besides, these dressings will dehydrate the wound bed since they do not have the ability to maintain a moist environment, therefore, they will be stuck to the wound and cause further damages when being removed [13,14]. However, accumulation of the exudates may lead to an appropriate environment for proliferation of the bacteria and pathogenic microorganisms. Consequently, this issue inspired the researchers to enhance the current wound dressings which afterward leads to the production of dressings with therapeutic effects [13]. The newly formed dressings had the ability to release some types of drugs such as antibiotics and anesthetics. Moreover, in order to reduce the damages to the newly formed epithelium which occur while changing the former dressing with the new one, bio-resorbable dressings were presented [12, 15, 16].

Electro-spun Nano fibrous wound dressings have addressed the aforementioned properties to some extent. In fact, electro-spinning is a remarkable method for producing nano-fibres with diameters ranging from 5 to 500 nm [12]. In this process, a polymer solution is placed inside a syringe which is connected to a metal capillary. A high voltage supply is connected to the capillary. When the electrostatic forces overcome the surface tension of the polymeric solution, a fibre jet is driven out of the droplet, which has formed at the tip of the capillary and accelerated toward a grounded collector. During this path, evaporation of solvent will occur and finally solid fibres will be collected from the collector [17]. Electro-spun drug–loaded nano-fibrous dressings provide various characteristics such as large surface to volume ratio and micro-porous structure which assist them in approaching the structure of extra cellular matrix. Moreover, they can deliver sufficient amount of the required drug to the exact area and their porous structure provides them the breathing ability and oxygen permeable characteristics [8, 12, 16].

In this study, electro-spinning method has been utilized to produce various nano-fibrous wound dressings based on gelatin and different amount of silver sulfadiazine (SSD) as an antibiotic drug suitable for burn wounds. Then the effect of the composition of the spinning solution on the morphology, anti-bacterial activity and drug release of the samples was investigated so that the optimum composition of the polymer and SSD could be determined.

2. Materials and methods
2.1. Materials
Gelatin with molecular weight of 250 lb/lbmole and batch No. 1266061 was purchased from Gelatin Capsule Iran Company. Silver sulfadiazine (SSD) with batch No. AGSM 111 was prepared from Shenyang Funing Co. Glutaraldehyde used for cross linking of the samples was obtained from Merck Co. Staphylococcus aureus (S.aureus) bacteria with ID No. 1113 and Escherichia coli (E.coli) bacteria with ID No. 1338 were provided from Zakaria Razi Laboratory Institute.

2.2. Electro-spinning equipment
The electro-spinning set up utilized in this study was CO881007NYI model from Asian Nanostructure Company with the ability to provide high voltage up to 32 kV. The apparatus consisted of a syringe pump and a needle with internal diameter of 0.64 mm, a flatten collector and a high voltage supply. An electrode which transferred the electro-static forces from the high voltage supply was connected to the needle.

2.3. Preparation of the samples
In order to obtain drug loaded gelatin nano-fibres, solutions with 3 distinct weight percentages of polymer concentrations (8, 10 and 12 wt %) and various drug to polymer ratios (0, 5, 10, 15 and 20 %) were produced. It should be mentioned that the samples were named as Gelx-y in which x denotes the weight percentage of the polymer in the spinning solution and y demonstrates the drug to polymer weight ratio.

For the electro-spinning of gelatin nano-fibrous dressings, each of the mentioned solutions were electro-spun for 1 hour, by a syringe pump with a volumetric flow rate of 0.2 ml/h, applied voltage of 22 kV and capillary to collector distance of 17 cm.

The produced SSD loaded electro-spun nano-fibres were further crosslinked with glutaraldehyde vapour in a desiccator for 24 hours. Then the samples were placed in a vacuum oven with the temperature of 30 °C for 12 hours in order to remove the excess glutaraldehyde vapour [12].

2.4. Characterization methods
Conductivity of the spinning solutions was measured using a conductimeter model CRISON GLP 32.

The morphology of electro-spin nano-fibres was observed by scanning electron microscope (SEM) Vega 2 tescan after gold coating. The average diameter of the fibres was determined by analyzing the SEM images using ImageJ software.

Existence of the drug among the electro-spun fibres without being chemically changed or destroyed was investigated by Fourier transform infrared spectroscopy (FTIR) Nexus 870 setup.

In order to evaluate the distribution of the drug among the fibres (through the distribution of silver particles), Energy-dispersive X--ray analysis (EDX) model INCA, Oxford Instruments Company, was applied.

2.4.1. Gel content measurements
Gel content is a criteria for determination of the insoluble fraction of a cross linked polymer. The higher the gel content the less the amount of the soluble chains (un-cross linked ones) would be. In order to determine the gel content of the samples after exposure to Glutaraldehyde vapour, the cross linked dressings with known weights (denoted as md1) were put in test tubes containing 10 ml of phosphate buffer saline solution (pH 7.4)
each and incubated for 24 hours at 37 °C [18]. Then the specimens were withdrawn from the solutions and placed inside an oven for 20 minutes to dry. Then the weights of the dried samples (denoted as md2) were measured with an accuracy of 0.0001 gr and the gel content was determined according to the following equation:

\[
\text{Gel content \%} = \left(1 - \frac{m_{d1} - m_{d2}}{m_{d1}} \right) \times 100
\]

2.4.2. In Vitro release behaviors
An in vitro elution method was used to determine the release profile of the drugs from the produced nano-fibrous dressings. Phosphate buffer saline (PBS) solution with pH of 7.4 was used as the dissolution medium [12, 16]. Samples with dimensions of 1.5 cm by 2 cm were cut and incubated in 10 ml of the dissolution medium at 37 °C for 1, 2, 3, 4, 5, 6 and 24 hour intervals. The dissolution medium was totally collected at the aforementioned intervals and was replaced with 10 ml of the fresh solution [12].

The drug concentration in the obtained solutions was analyzed by Atomic Absorption technique (model SpectrAA-200) via determining the concentration of the silver component.

2.4.3. Antibacterial activity of the loaded dressings
The antibacterial activity of dressings containing SSD was evaluated against the pathogenic microorganisms S.aureus and E.coli. In vitro studies were performed in nutrient agar solid medium. The surface of the solid agar was inoculated with a suspension of S.aureus and E.coli separately in each Petri dish. Sections of the samples (0.5 Cm by 0.5 Cm) were placed on agar plates, allowing sufficient time for the drug to diffuse into the surroundings. The activity zones of all the samples were then measured and compared together [19].

3. Results and discussion
3.1. SEM images of the samples
The SEM images of all the scaffolds showed randomly oriented smooth and continuous fibres without any defects as beads. Fig. 1 shows the SEM images of SSD loaded electro-spun nano-fibrous gelatin scaffolds for Gel12 series as the example.

By utilizing the ImageJ software, the average diameter of each series of the fibres was measured from SEM images. Variation of the diameter of the nano-fibres with the amount of the loaded drug is shown in Fig. 2.

As can be seen in Fig. 2, at a specific drug to polymer ratio, the average diameter of the fibres has been increased by increasing the polymer concentration from 8 to 12 wt % in the spinning solution. In fact, at a specific electrostatic force, increase of the amount of the polymer concentration is synonymous with the decrease of the electrostatic force to pull the polymer solution which leads to producing fibres with higher average diameters.

Moreover, Fig. 2 shows that for each of the Gel8, Gel10 and Gel12 series, increases in the amount of the loaded drug from 0 to 5 % has led to decreasing the average diameter of the fibres, and further increase of the drug from 5 to 20 % caused the diameters to be increased.

It seems that there are two parameters affecting on the diameter of the produced fibres. One of them is conductivity of the spinning solution and the other is its viscosity. These parameters have two opposite effects on the fibre diameters in a way that increasing the viscosity of the solution
leads to increasing the fibre diameter, while in the fibre diameters [17,20-23]. Increasing the conductivity leads to decrease

Figure 1. SEM images of gelatin/SSD nano-fibres with 12 (wt %) polymer concentration: a) Gel12-0, b) Gel12-5, c) Gel12-10, d) Gel12-15, e) Gel12-20.

Figure 2. Variation of the average fibre diameters with the loaded drug.
Since silver sulfadiazine has the silver element, it was suggested that increasing the amount of the loaded drug would lead to increase in the conductivity of the solution and hence decrease the fibre diameter. On the other hand, increasing the amount of the loaded drug leads to increase in the amount of the total material (mass) per volume unit in the tip of the capillary, which for a specific electrostatic force is the same as decreasing the electrostatic force to pull the material and consequently increases the fibres diameter. Therefore, it seems that by increasing the amount of the drug up to 5 %, the increment of the conductivity is the dominant parameter but the amount of the drug more than 5 %, would cause the increment of the viscosity to be the effective parameter.

In order to confirm the validity of the suggested effect of the silver sulfadiazine on the conductivity of the spinning solution, conductivity of the samples Gel8-0 to Gel8-20 was measured and is shown in Table 1.

Table 1
Conductivity of the solution for the samples Gel8-0 to Gel8-20.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Conductivity $\mu m/cm$</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel8-0</td>
<td>1.02</td>
<td>0.045</td>
</tr>
<tr>
<td>Gel8-5</td>
<td>1.178</td>
<td>0.032</td>
</tr>
<tr>
<td>Gel8-10</td>
<td>1.245</td>
<td>0.034</td>
</tr>
<tr>
<td>Gel8-15</td>
<td>1.427</td>
<td>0.043</td>
</tr>
<tr>
<td>Gel8-20</td>
<td>1.618</td>
<td>0.047</td>
</tr>
</tbody>
</table>

As can be seen in Table 1, by increasing the amount of the silver sulfadiazine in the spinning solution, the conductivity of the samples has been increased.

3.2. FTIR and EDX analysis

In order to verify the existence of silver sulfadiazine among the gelatin fibres, the EDX picture was taken from the sample Gel10-15 as an example, which is shown in Fig. 3.

The small red spots in Fig. 3 indicate the silver element in the gelatin fibres. According to this figure, a good distribution of the drug among the wound dressing can be observed.

Moreover, FTIR analysis was performed on sample Gel12-20 as an example in order to be sure of the existence of the drug among the fibres after electro-spinning. Fig. 4 shows the FTIR spectra of this sample. According to this figure, a large and wide peak is observed at 3404 cm$^{-1}$ which is related to the -OH groups of gelatin and -NH$_2$ groups of silver sulfadiazine. The characteristic peaks observed at 1724, 1649 and 1264 cm$^{-1}$ are related to acidic C=O, amide C=O and C-N groups of gelatin respectively. Moreover, the characteristic peaks that appeared at 2931 and 1133 cm$^{-1}$ belong to -CH aromatic groups and sulfone groups of SSD respectively. Therefore, in the FTIR analysis of the sample Gel12-20, both gelatin and silver sulfadiazine peaks are clearly observed.
3.3. Gel content results
The crosslinked scaffolds were characterized to determine their gel content. The results are brought in Table 2. According to the obtained results, the gel content of all the samples have been more than 90% which means that all of them were successfully crosslinked using the aforementioned technique.

3.4. In Vitro release behavior of pharmaceuticals from nano-fibers scaffolds
The release characteristics of silver sulfadiazine from various cross linked samples were assessed by the method described in section 2.3.2. The cumulative release of SSD from the samples with 10 wt % of gelatin is shown in Fig. 5.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gel content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel8-0</td>
<td>99.43</td>
</tr>
<tr>
<td>Gel8-5</td>
<td>98.60</td>
</tr>
<tr>
<td>Gel8-10</td>
<td>99.41</td>
</tr>
<tr>
<td>Gel8-15</td>
<td>99.68</td>
</tr>
<tr>
<td>Gel8-20</td>
<td>99.63</td>
</tr>
<tr>
<td>Gel8-0</td>
<td>99.58</td>
</tr>
<tr>
<td>Gel8-5</td>
<td>99.49</td>
</tr>
<tr>
<td>Gel8-10</td>
<td>99.56</td>
</tr>
<tr>
<td>Gel8-15</td>
<td>99.58</td>
</tr>
<tr>
<td>Gel8-20</td>
<td>99.59</td>
</tr>
<tr>
<td>Gel8-0</td>
<td>99.78</td>
</tr>
<tr>
<td>Gel8-5</td>
<td>99.20</td>
</tr>
<tr>
<td>Gel8-10</td>
<td>99.33</td>
</tr>
<tr>
<td>Gel8-15</td>
<td>99.59</td>
</tr>
<tr>
<td>Gel8-20</td>
<td>99.40</td>
</tr>
</tbody>
</table>
As can be seen in this figure, the percentage of the released drug has been decreased with increasing the amount of the loaded SSD from samples Gel10-5 to Gel10-20 which can be attributed to the increase of the fibres diameter (and therefore decrease of the surface to volume ratio of the fibres) with increasing of the amount of the loaded drug as was observed before in the SEM section (Fig 2). Moreover, when the diameter of the fibres increases, it is more difficult for the drug to diffuse from the interior to the outside of the fibres, and therefore the amount of the released drug will be decreased. The same result has been presented in a study by Chen et al. in 2012, in evaluating the influence of fibres diameter on the amount of the released drug [24].

As it can be seen in Fig. 5, all the samples showed a burst release in the first 6 hours followed by a decrease in the slope of the drug release profile with time. It should be noted that the same trend was observed for the samples with 8 wt % gelatin (Gel8 series) and 12 wt % gelatin (Gel12 series) which are not shown here.

In order to evaluate the effect of the polymer concentration on the drug release profile, the samples with the constant drug to polymer weight ratio of 15 are compared in Fig. 6.

![Figure 5. Cumulative released SSD from crosslinked Gel10 series of the samples.](image)

It should be noted that although the drug to polymer ratio is constant, since the amount of polymer concentration is different, the total amount of the drug in the spinning solution would be different. So that the higher the polymer concentration, the higher the amount of the drug in the spinning solution. Therefore, it was expected that with increasing the amount of the polymer concentration, the released drug would be increased. But according to Fig. 6, increase of the polymer concentration up to 10 % caused the released drug to be increased whereas more increase in the polymer concentration has led to a drastic decline of the released drug. It seems that high concentration of polymer (more than 10 %) acts as a barrier for drug diffusion so that the release of the drug would be decreased. In other words, when the concentration of the polymer in the electrospinning solution goes higher than 10 %, the fibre mat becomes denser and the porosity
between the fibres severely decreases so that the polymeric chains do not allow the drug to diffuse easily through the fibres. In other words, the higher porosity of the fibres allows easy penetration of water into the nano-fibres and a greater release of drug out of them [25]. Therefore, polymer concentration less than 10 % supports the increasing porosity of the mat and increment of the amount of the released drug.

The same trend was observed for the samples with drug to polymer weight ratio of 5 and 10 which are not shown here. Therefore, it seems that samples containing 10 wt % of gelatin have the optimized amount of polymer for the highest release of SSD.

![Figure 6. Cumulative released SSD from samples with 8, 10 and 12 (wt %) of gelatin and constant drug to polymer weight ratio of 15.](image)

3.5. Antibacterial activity of the loaded drug

The antibacterial activities of the samples were evaluated against *E. coli* and *S. aureus* bacteria as described in section 2.3.3.

Figure 7 shows the antibacterial assay for the Gel8 and Gel10 series of the samples against *E. coli* and Gel12 series against *S. aureus* bacteria. Existence of hollows around the samples indicates the antibacterial activity of them. As can be seen in Fig. 7, all the samples showed antibacterial activity against these two bacteria.

4. Conclusions

In the present study, gelatin based dressings with 3 different polymer concentrations and various amounts of the loaded SSD drug were produced by electro-spinning technique. According to the SEM images, continuous bead free fibres were obtained. Moreover, it was found that increasing the amount of the loaded drug from 0 to 5 % has led to decrease in the average diameter of the fibres due to the increased conductivity of the spinning solution. But further increase of the drug from 5 to 20 % caused the diameters to be increased because of the increment of the viscosity. Investigation of the release kinetics of the dressings showed a burst release in the first 6 hours followed by a decrease in the slope of the drug release profile with time. Furthermore, for the entire drug to polymer ratios, the optimized amount of polymer concentration for releasing SSD was 10 wt % of gelatin.
Finally, antibacterial activity of the dressings was evaluated against the pathogenic microorganisms *E.coli* and *S.aureus* in the nutrient agar solid medium. It was obvious that all the samples had antibacterial activity against these two bacteria.

**Figure 7.** Antibacterial activity of the samples against *E.coli* and *S.aureus* bacteria.

**References**


Production and Characterization of Gelatin Based Electro-spun Nano-fibres as Burn Wound Dressings


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