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Fabrication and characterization of wound dressings containing gentamicin/silver for wounds in diabetes mellitus patients

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Abstract

In this study, the dual functional wound dressings for diabetic patients were fabricated with the help of gentamicin/ cellulose acetate by electrospinning. The resultant nanofibers were functionalized with silver nanoparticles (AgNPs) on its surface by a simple facile wetting process. Herein, gentamicin (Ge) was used for the objective to heal the infection inflammation and Ag NPs were loaded for the antibacterial properties. The resultant CA/Ge/Ag nanofibers were characterized for morphology analysis, chemical interactions examination, release behavior, structure analysis and antibacterial efficiency by scan electron microscope/ transmission electron microscope, FTIR/XPS, ICP study, XRD study and disc diffusion method of antibacterial, respectively. Based on characterization results it was concluded that all samples of CA/Ge/Ag nanofibers showed the antibacterial properties and appreciable release behavior.

1. Introduction

At present, the prevalence, disability and mortality of diabetes mellitus have been ranked third in chronic non-communicable diseases. The number of people who die each year from diabetes mellitus and its complications is the fifth leading cause of death in the world. Diabetes mellitus has caused heavy medical burdens and large indirect social costs while causing major health problems, which has aroused widespread concern in society. Diabetes mellitus is a metabolic disease characterized mainly by hyperglycemia. The main hazards of diabetes mellitus are manifested in complications, one of which is that skin ulceration is not easy to heal. The main reason is that the diabetic patients themselves have weak body resistance and tissue repair function is impaired. However, the healing of the incision tissue, from the growth of granulation tissue to the formation of collagen fibers, requires protein and other tissue factors [1, 2]. However, due to abnormal sugar utilization, diabetic patients cannot effectively convert glucose into protein and other substances. Hyperglycemia causes hypertonic tissue cells, which is not conducive to cell survival. These lead to impaired tissue regeneration and repair function, coupled with the high glucose environment in the patient’s body is a good medium for bacteria, which can easily lead to wound infection, which makes the wound more difficult to heal [3–6].

In order to accelerate wound healing of diabetic mellitus patients, this paper mainly studies wound dressing of diabetic patients. Wound dressings are materials used to cover sores and wounds. It can be widely divided into passive dressings (traditional dressings), interactive dressings and bioactive dressings (closed dressings). Traditional wound dressings are mainly dry gauze and oil gauze, which cannot be used for infectious wounds, and have no promoting effect on wound healing, so they cannot be used for wounds of diabetics. The bioactive dressings are made of polymer materials and biological materials processed by high-tech methods, which is a hot spot in the research and development of wound dressings [7, 8].
Among medical dressings, polymer materials such as nanofibers are promising due to their light weight and porous structure. Nanofibers are easy to manufacture and can be manufactured in a variety of ways, such as stretching, template synthesis, self-assembly, micro phase separation, and electrospinning. Electrospinning is jet spinning of polymer solution in strong electric field. The electro spun nanofiber membrane can control the evaporation of water and promote the liquid elimination ability \cite{9,10}. And because of its large specific surface area to volume ratio, enhanced chemical reactivity and scalable and cost-effective manufacturing methods, it has become a research interest in a wide range of industries.

Here, we proposed a dual nature composite for healing of diabetic wound and antibacterial activity through gentamicin and Ag nanoparticles, respectively. This bifunctional composite nanofiber membrane was prepared in which cellulose acetate and gentamicin are mixed into a solution and electrospun to obtain a nanofiber membrane. The silver nanoparticles are functionalized on the surface of the nanofiber membrane by a simple wetting process and reduced in situ under physiologically mild conditions. Acetate fiber is a readily available man-made fiber, which is low in cost, non-toxic, easy to decompose, and has a high use rate in medical fibers. Gentamicin is a commonly used antibiotic with good thermal stability. It is mainly used to treat bacterial infections, especially those caused by Gram-negative bacteria. The combination of antibiotics and wound dressings can effectively inhibit the proliferation of bacteria, prevent and fight infections \cite{11,12}, and promote wound healing. However, diabetic patients have poor immunity and are highly susceptible to infection, but diabetics have poor immunity and are highly susceptible to infection. Silver is an ideal natural antibacterial material that can reduce the bacterial load on the wounds, accelerate wound healing, and treat infections. And silver is effective against a variety of aerobic, anaerobic, Gram-positive, Gram-negative, fungal and viral \cite{13,14}. Therefore, we introduce silver ions on the surface of the nanofiber membrane to improve the antibacterial properties of the dressing.

In this report, CA/Ge/Ag nanofibers were fabricated and characterized by SEM & TEM for morphology analysis, FT-IR & XPS were done for examine the chemical interactions between CA, gentamicin and silver. Release behavior was studied by ICP. Antibacterial activity was done by disc diffusion method.

2. Experimental

2.1. Materials

Gentamicin salt (GEN) and cellulose acetate \((\text{CA}) 30000 \text{gmol}^{-1}\) had supplied by Sigma-Aldrich Co., Ltd (Louis, Missouri, USA). Acetone (99.5%), Sodium hydroxide \((\text{NaOH}, \text{Purity of 97.0%})\), Silver nitrate \((\text{AgNO}_3, \text{purity of 99.8%})\), and dimethylformamide 98% (DMF) were provided by Wako Pure Chemical Industries, Ltd (Osaka, Japan).

2.2. Preparation of wound dressings

The 24 wt% CA was mixed in DMF and Acetone (6:4) and stirred for 6 hr. Then, different ratios of gentamicin salt were added in different concentration by weight; 0.125% & 0.25% and stirred for 4 hr. The solution was loaded to electrospinning for development of nanofibers with a high-voltage supply of 14 kV. The distance from the needle tip to the collector was 12 cm. The resultant nanofiber web was dried at room temperature for 1 hr. To remove the acetyl group from the composite, the web was dipped with 0.01 g/10 ml of NaOH for 1 h, washed with deionized water and dried at room temperature. After drying the web, the resultant dressings were loaded by Ag nanoparticles from in-situ facile method for the purpose of antibacterial applications. Herein, design of experiment was design in a way that loading of Ag nanoparticles on the surface was same/constant.

The nanofibers web was wetted with 0.01 g/10 ml of AgNO3 for 1 h. And washed with deionized water; Then wetted with 0.01 g/10 ml of NaOH for 1 h, washed with deionized water and dried at room temperature. The resultant nanofibers were characterized from different equipment.

2.3. Characterizations

The surface morphology of these nanofibers was evaluated by SEM (JSM-5300, JEOL Ltd, Japan) accelerated with the voltage of 12 kV and TEM (JEM-2100 JEOL Japan) accelerated with 200 kV. To examine the chemical interactions PT-IR spectra (IR Prestige-21 by Shinnadzu Japan) were done for the evaluation of chemical reaction or bonding with CA, Ge and Ag nanoparticles, X-ray photoelectron spectroscopy (XPS) was used. XPS was conducted on a Shinnadzu-Kratos AXIS-ULTRA HAS SV (Shinnadzu Co., Ltd) using an Al X-ray source, set at 10 kV and 15 mA. X-ray diffraction (XRD) was performed at room temperature using a rotaflex RTP300 (Rigaku Co., Japan) and operating at 50 kV and 200 mA. The antibacterial analysis of the CA nanofibers, CA/Ge nanofibers and CA/Ge/Ag nanofibers against Escherichia coli and B. Subtilis bacteria was determined by using agar diffusion test. The nanofibers of 12 mm diameter were placed for examination on agar plates seeded in log phase with bacterial cells and incubated at 37 °C overnight. This test was repeated three times on different
samples. The diameter of inhibition zone was examined in triplicate using ImageJ. The release behavior of the Ag and Ge from the CA/Ge/Ag and CA/Ge nano fibers was examined by an inductively coupled plasma (ICP) atomic emission spectrometer (SHIMADZU/ICPS, 10000IV, Japan) over a period of 72 h by immersing the nanofibers in deionized water and stirring very slowly at room temperature.

3. Results and discussion

3.1. Morphology analysis

In order to investigate the morphology of the CA nanofiber, CA/gentamicin nanofibers and CA/gentamicin/Ag nanofibers SEM images were studied as shown in figure 1. It was confirmed that all resultant nanofibers were successfully fabricated without beats and stabilized in morphology as shown in the figure 1. In order to further investigate the nanofibers diameter distributions, with the help of SEM images, average diameter of nanofibers was measured by ImageJ software with 50 different and random nanofibers. It was confirmed that nanofibers diameter was not affected by the addition of gentamicin but it was affected by the synthesis of Ag nanoparticles on the surface of CA/gentamicin as shown in the figures 1(c) and (e) because their diameter was higher than neat CA nanofibers and CA/gentamicin nanofibers. The diameter of neat CA nanofibers and CA/gentamicin nanofibers was 264 ± 28 nm and 320 ± 20 nm (0.125% Ge) & 320 ± 40 nm (0.25% Ge), respectively but after loading Ag nanoparticles it was 310 ± 30 nm and 320 ± 2 nm respectively.

In order to further investigation of the presence of silver nanoparticles in the CA/gentamicin/Ag nanofibers EDS spectra were studied as shown in the figure 2. It was confirmed that in the neat CA nanofibers and CA/gentamicin nanofibers there is no Ag amount as shown in the figures 2(a) and (b) but in the CA/gentamicin/Ag nanofibers (0.125% wt) and CA/gentamicin/Ag nanofibers (0.25% wt) there are appreciable amount of Ag in the nanofibers as shown in the figures 2(c) and (d). EDS spectra confirmed the successful synthesis of Ag nanoparticles on the surface of CA/Ge nanofibers.

The dispersion of nanoparticles on the surface of CA/GE/Ag nanofibers, again morphology of nanofibers was analyzed using a transmission electron microscope (TEM) with an accelerating voltage of 200 kV, as shown in figure 3. TEM images confirmed that on the surface of nanofibers there were nanoparticles in an appreciated amount. It can be seen from the TEM image that the coordination with Ag + ions and subsequent reduction leads to the successful synthesis of AgNP on the surface of nanofibers.

The Ag nanoparticles were loaded by a facile method in which immersion time for all samples was same, therefore Ag nanoparticles amount on nanofibers was same as shown in figure 3.

![Figure 1. SEM images of (a) neat CA nanofibers, (b) CA/Ge 0.125% wt Nanofibers, (c) CA/Ge/Ag Nanofibers (0.125%wt), (d) CA/Ge 0.25% wt Nanofibers & (e) CA/Ge/Ag Nanofibers (0.25%wt).](image-url)
3.2. ICP study

In order to investigate the release behavior of Ag nanoparticles and Ge from the CA/Ge/Ag nanofibers, inductively coupled plasma (ICP) study was done as shown in figure 4. In this study, 0.02 grams of CA/Ge &
Figure 4. Release profile of CA nanofibers, CA/Ge nanofibers, CA/Ge/Ag (0.125) nanofibers and CA/Ge/Ag (0.25) nanofibers.

Figure 5. (A) FT-IR spectra of (a) neat CA nanofibers, (b) CA/Ge 0.125% wt Nanofibers, (c) CA/Ge/Ag Nanofibers (0.125%wt), (d) CA/Ge 0.25% wt Nanofibers & (e) CA/Ge/Ag Nanofibers (0.25%wt), (B) XPS spectra for CA/Ge/Ag nanofibers having Ge 0.125% and CA/Ge/Ag nanofibers having Ge 0.25%.
CA/Ge/Ag nanofibers were solved in 5 ml of H2SO4 to calculate the amount of Ag nanoparticles and for the analysis of the Ag release the 1 g of CA/Ge nanofiber & CA/Ge/Ag nanofibers nanofibers were dissolved in deionized water and measured during 72 h. ICP study confirmed that resultant composites have the potential for release profile as shown in figure 4. Release was started within 5 h and maxim release was on 50 h after 50 h it was gradually reduced. It was observed that samples leached Ag into the deionized water 0.5 & 1.2 ppm for CA/Ge/Ag nanofibers; 0.125 & 0.25 respectively. The resultant graphs showed that in the CA/Ge/Ag nanofibers (0.25) the highest amount of Ag nanoparticles was released as shown in the figure 4. The ICP study confirmed that CA/Ge/Ag nanofibers; 0.125 & 0.25 nanofibers membranes are the significant and appreciable efficiency of antibacterial properties.

3.3. Chemical interactions
In order to investigate the chemical interactions between the CA, Ge and silver nanoparticles, FT-IR and XPS study were done as shown in the figure 5. XPS study was done to investigate the presence of Ag nanoparticles in the chemical structure of the CA/Ge/Ag nanofibers. It was confirmed that nanoparticles of Ag were present in form of Ag was chemically interacted at Ag3d5/2 & Ag3d3/2 with binding energy 366 eV and 372 eV [15], respectively as shown in figure 5(B). Similarly, Ag was interacted at S2p with binding energy 165 eV as shown in figure 5. In order to investigate the chemical interactions between cellulose acetate, Ge and Ag nanoparticles, FT-IR spectra were studied as shown in figure 5(A). It was observed that there was successful synthesized Ag nanoparticles on the surface of CA/Ge nanofibers because there was Ag peak at 1500 cm−1 in all CA/Ge/Ag nanofibers and also there were intense and well defined characteristic peaks asymmetric of SO2 at 1250 cm−1 and there were also characteristics peaks of NH bending of Ag at 1594 cm−1 and the characteristics peaks of C=O, C–CH2 and C–O–C of cellulose acetate at 1775, 1370 and 1100 cm−1 respectively in all resultant nanofibers [16].

3.4. Antibacterial test
In order to investigate the antibacterial efficiency of the neat CA nanofibers, CA/Ge nanofibers and CA/Ge/Ag nanofibers, antibacterial activity was examined by disk diffusion method against gram-negative E. coli and gram-positive B. subtilis bacteria. It was observed that neat CA nanofibers have no antibacterial efficiency but CA/Ge/Ag (0.125% Ge, 0.25% Ge) nanofibers have very great potential for antibacterial activity as shown in the figure 6. Silver nanoparticles has more potential for both gram negative bacteria and gram positive bacteria as shown in the figure 5, the inhibition zone of antibacterial activity against E. coli is greater than gram positive B. Subtilis bacteria (Khan et al 2018b; Khatri et al 2017).

4. Conclusion
Herein, the dual functional nanofibers composite was successfully fabricated by CA/Ge/Ag. Silver nanoparticles were successfully synthesized on the surface of cellulose acetate/Ge nanofibers web by in situ facile method. Herein, gentamycin (Ge) was used for the objective to heal the infection inflammation and Ag NPs were loaded for the antibacterial properties. The resultant CA/Ge/Ag nanofibers were characterized for morphology.
analysis, chemical interactions examination, release behavior, structure analysis and antibacterial efficiency. Based on characterization results it was confirmed that this composite can be used for antibacterial wound dressings because it has appreciable antibacterial properties and excellent antibacterial properties against *gram negative* E. coli, which is unique properties of silver nanoparticles for antibacterial activity and this composite has the good release profile for wound healing. It can fulfill the stated and implied needs of customer.

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