A New Medical Dressing with Silver Nanoparticles to Treat Diabetic Foot Patient

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ABSTRACT

In this study, we prepared medical dressings with silver nanoparticles to treat diabetic foot patient by photochemical reduction process of silver ions using sodium citrate and UV radiation. Antibacterial activity of dressing with silver nanoparticles against bacteria associated with diabetic foot was studied by disk diffusion method. It was observed that the bacteria did not grow on the culture medium where the medical gauze was impregnated with silver nanoparticles.

KEYWORDS: Silver; diabetic foot; Antibacterial activity.

INTRODUCTION

Diabetes is one of the most prevalent diseases in the worldwide that affects all ages. This condition occurs when the pancreas produces less than of its normal amount of insulin, or when the body cannot effectively use the insulin it produces. Insulin is the hormone that responsible of regulating blood sugar levels. The chronic hyperglycemia of diabetes is associated with long-term complications in the eyes, kidneys, nerves, and blood vessels [1].

Type 2 diabetes is a common illness that has a significant impact on human health and widespread widely in the world, so there are many medical researchers interested in diabetic and focused in pathological cases associated with this disease, such as diabetic foot and gangrene. Diabetic foot is term referring to many pathologies in the foot that result directly from diabetes or its long-term complications [2]. Nearly 6% of people with diabetes suffer from foot disease includes infection, ulceration, or damage tissues of the foot [3].

It can affect patients’ quality of life, social participation and livelihood [4]. Between 0.03% and 1.5% of patients with diabetic foot, require an amputation [5].

Uncontrolled diabetes contributes to the development of neuropathy and peripheral arterial disease by complex metabolic pathways [6]. Diabetes also implicated in Charcot arthropathy, which involves progressive destruction of joints, bones, and soft tissues, most commonly in foot and ankle [7]. A combination of neuropathy, repeated micro trauma, abnormal loading of foot, and metabolic abnormalities of bone cause inflammation, which lead to osteolysis, dislocation, fractures, and deformities [8].

The basic nature and chronicity of foot problems together deter interest in the foot among health care professionals, such problem lack the acute or dramatic presentation of other medical condition and may progress to a serious state in the absence of any symptoms rate in diabetic clinic may be as low as 12% and that doctors are unlikely to examine their patient feet unless the patients...
remove their sock and shoes before the consultation begins [9].

**Bacteria associated with diabetic foot ulcers**

Several studies have been conducted on the bacteria associated with diabetic foot ulcers, and the results have been different and sometimes conflicting [10]. Some studies found that *Staphylococcus aureus* is the main pathogen [11], while in others it is found that Gram-negative aerobic bacteria (*Pseudomonas spp.*) are the dominant pathogen [12]. Alternatively, planting those samples in an appropriate way to isolate these bacteria. However, some studies, which used appropriate methods, showed that anaerobes have a limited role [13], and this difference in the results of the aforementioned studies is due to the geographical diversity, and the degree and severity of the infections covered in these studies [14].

**Staphylococcus aureus bacteria**

It belongs to the family staphylococaceae, which includes 36 bacterial species. The most pathogenic is *Staphylococcus aureus* and constitutes 20-30% of the bacterial group of skin and mucous membranes in healthy individuals. It is a spherical selective of anaerobic spores 1.5-0.5µm in diameter in the form of grape clusters, Gram-positive, immobile, non-sporegenic, capless, able to tolerate salinity. Furthermore, it is able to grow in a medium containing 10% NaCl and it can grow at a temperature ranging between 10-42°C, positive for catalase, oxidase and coagulant, dilute gelatin and return nitrate to nitrite, do not release H2S and do not release indole. Fermentation of mannitol sugar when grown on Mannitol Salt Agar medium gives its distinctive characteristic [15]. This bacterium causes diseases ranging from minor diseases such as skin infections, abscesses and boils, to serious diseases such as pneumonia and endocardial sepsis and septicemia. It is also considered as one of the most important causes of wound infection, burn infection, eye infection, ear infection, urinary tract infection, respiratory tracts infection, and osteomyelitis [16].

**Pseudomonas aeruginosa bacteria**

It belongs to the family Pseudomonadaceae, which includes ten genera, the most important of which was the genus *Pseudomonas*. One of the most important pathogenic species of *Pseudomonas* is *Pseudomonas aeruginosa*. They are gram-negative bacilli, characterized by their odor resembling the smell of bitter almonds, because of the liberation of acetophenol compounds from tryptophan. Its dimensions range from 0.5-1.5 µm in length and 0.5-1 µm in width as a fluidized gelatin, aerobic obligate bacilli or in chains, motile by one or more polar flagella, positive for catalase and oxidase, do not release indole, and produce water-soluble pigments such as pyoverdin and pyocyanin [17]. These bacteria are capable of reducing nitrate to nitrite releasing nitrogen gas, not releasing H2S gas, but releasing ammonia gas, do not have capsule, but some pathogenic species form a layer of polysaccharides, not fermenting for sugars, and not rancid, and able to grow at a temperature of 42 degrees celsius. It may be killed by boiling or completely drying, and the phenolic compounds and glutaraldehyde compounds are considered effective antiseptics against these bacteria, they are responsible for many diseases such as infections of wounds, burns, urinary infections and infections associated with cystic fibrosis [18].

**Development of the phenomenon of bacterial resistance to antibiotics**

During the second half of the last century, antibiotics were adopted as a treatment for many diseases caused by bacteria, which began to develop resistance to these antibiotics, as a result of the wrong and indiscriminate use of the latter) the public. Where the European Medical Agency estimates that 25,000 people die in Europe annually because of infections caused by bacteria resistant to antibiotics. The World Health Organization also reported that about 440,000 cases of tuberculosis resistant to a wide range of antibiotics appear annually, and cause at least 150,000 deaths. According to the Centers for Disease Control and Prevention in the United States of America, antibiotic-resistant bacteria cause two million cases of sepsis annually [19]. At the present time, 70% of the germs that cause infections in hospitals are considered to be resistant to at least one of the known and used antibiotics in treatment. A study conducted in Mexico and Iran showed that more than 70% of strains of *Escherichia coli* have become resistant to ampicillin antibiotics, vancomycin-resistant *Enterococcus*, *sulfamethazol*Methicillin-resistant *Staphylococcus aureus* spp, some *Enterobacteriaceae* resistant to
carbapenems or fluoroquinols, and multidrug-resistant *Pseudomonas aeruginosa* is one of the most important and dangerous antibiotic-resistant bacteria [20].

**Silver as nanoparticles**

(Ag0), oxides (mainly Ag2O), or in ionic forms (Ag+) show an activity against bacteria [21]. For long periods, silver has shown broad-spectrum activity against bacteria, fungi, and viruses when used as antimicrobial agent. Nowadays, nanosilver is being use in many medical devices, including surgical ones as well as a disinfectant against hospital wastewater, due to its strong antibacterial activities [22]. Sliver has an advantage in comparison to other antimicrobial agents, which shows a high toxicity to microorganism while low toxicity to mammalian cells [23]. However, there are some arguments on the toxicity of the released silver ions against microorganisms [24]. Some studies suggested the safe usage of sliver with a maximum permitted dosage [25]. However, in the case of a typical application like wound healing, silver has to retain inside a solid support to apply properly over the affected area.

Photochemical reduction by UV radiation is one of the fastest reduction and environmental friendly procedure to produce Ag nanoparticles in the cellulosic matrix, which has not been investigated much yet [27]. In our study, we showed the fabrication of silver nanoparticles deposited on medical gauze by photochemical reduction process using sodium citrate and UV radiation and antibacterial activity of medical gauze impregnated with silver nanoparticles against bacteria associated with diabetic foot was studied using the disk diffusion method.

**MATERIALS AND METHODS**

**Bacterial isolates**

Bacterial isolates associated with diabetic foot patients were obtained from Aleppo University Hospital and were genotyped according to the 1994 Bergy Specific Guide, and it was found that they belong to the bacteria of both species: *Pseudomonas aeruginosa* and *Staphylococcus aureus*

**Preparation method**

Medical gauze soaked with water and detergent solution at 50°C for 30min. Gauze was washed with distilled water, then a diluted solution of hydrogen peroxide 3% was added and heated to 50-60°C for 15min to remove possible impurities in the cotton gauze. 100ml of 0.01M silver nitrate solution was prepared and 25ml of 5% sodium citrate solution (a reference agent for silver ions) was added to it. Gauze treated with UV for 15-20 minutes, where the color changed from white to silver as a result of the conversion of silver ions to silver. Gauze was washed with 3% sodium chloride solution to remove unreactive silver ions followed by washing with distilled several times and dried at 40-50°C to save for subsequent tests.

**Antibacterial Activity Evaluation**

Antibacterial activity was evaluated by disk diffusion. Nutrient agar medium petri dishes were used to perform disk diffusion method. The medical gauze impregnated with silver nanoparticles was cut into a disk shape of 10 mm diameter and it was sterilized with a low power UV lamp for 5 min for each side. Then, these disks were placed on the agar plate inoculated with 10⁶ cfu/mL of bacterial suspension and incubated at 37 °C for overnight. The medical gauze without silver nanoparticles used as control. To measure antibacterial activities of our samples were observed by formation of inhibition under the granules. Given that agar is the place where medical gauze sticks to the skin, compared to medical gauze that is not impregnated with silver nanoparticles [28].

**Sensitivity of the tested bacteria to antibiotics**

The sensitivity of the studied bacteria to antibiotics was tested by Agar diffusion method, where it was grown on Mueller-Hinton Agar by brushing method, starting from a bacterial suspension with a Macfarland turbidity amount of 0.5 and antibiotic tablets were placed on the surface of the cultured dishes in the air incubator for 24 hours at 37°C. After the incubation period, the diameters of the bacterial inhibition halos were measured with a graduated ruler according to the Kirby-Bauer Method.

The diameters of the bacterial suppression halos were compared with the standard diameters based on the Clinical and Laboratory Standards Institute to determine whether the bacteria are resistant or sensitive to the studied antibiotics [29].
RESULTS AND DISCUSSION
It was observed that the bacteria did not grow on the culture medium where the medical gauze was impregnated with silver nanoparticle. It appeared completely transparent under the light for *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Figure 1), While the germs clearly grew on and under the medical gauze that was not impregnated with silver nanoparticles. This indicates the ability of silver nanoparticles in our experiment to inhibit the growth of bacteria.

![Figure 1. Antibacterial Activity. Blue arrow in the pictures indicates the medical gauze impregnated with silver nanoparticles.](image)

The releasing behavior of silver from the antibacterial substrate is an important issue because an excessive amount of silver release, either as particles or in ionic form could damage the human cells, limiting its applicability. On the other hand, if silver is strongly embedded and not released, poor antimicrobial activity will result.

**Sensitivity of the tested bacteria to antibiotics**
The tested bacteria in the study were resistant to at least three antibiotics, and the diameters of the inhibition halos against *Staphylococcus aureus* ranged between 12-28mm and 8-18 against *Pseudomonas aeruginosa* as shown in Table 1 and Figure 2.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th><em>Pseudomonas aeruginosa</em> (inhibition zone diameter mm)</th>
<th><em>Staphylococcus aureus</em> (inhibition zone diameter mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Imipenem</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
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<td>8</td>
</tr>
<tr>
<td>Ampicillin</td>
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<td>0</td>
</tr>
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<td>Cotrimoxazole</td>
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<td>0</td>
</tr>
<tr>
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<td>18</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

![Table 1. Resistance of tested bacteria](image)

**Figure 2. Sensitivity of the tested bacteria to antibiotics.**

**CONCLUSION**
We recommend the use of medical gauze impregnated with silver nanoparticles in the medical field in general, due to the ease of maintaining its sterility on the one hand, and its lack of high cost and its great ability to discourage the growth of germs where it adheres to the skin on the other hand, especially in diabetic foot patients due to their subjection to constant dressing change, with attention to the concentration that does not lead to human toxicity. The gauze impregnated with silver nanoparticles was superior to the effectiveness.

**REFERENCES**
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