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Antibacterial Effects of Agicoat Silver Crystalline Nanofibers on Wound Infection Agents

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Background: Drug resistance is a major concern in the treatment of burn wounds. The present study aimed to evaluate the effects of silver crystalline nanofibers on the bacteria isolated from burn wounds at Imam Musa Kazim Hospital in Isfahan, Iran and compare the findings with the samples collected from the skin of healthy adults with standard collection bacteria.

Methods: Agicoat nanosilver dressing was used as the antibacterial agent. The antibacterial effects of the silver nanoparticle solution and silver crystalline nanofibers were assessed using the well diffusion method. In addition, the macrodilution method was applied to determine the MIC and MBC of the silver crystalline nanofibers.

Results: No significant difference was observed in the growth inhibition zone of the silver nanoparticle solution and crystalline nanofibers. Comparison of the effects of silver crystalline nanofibers on wound infection bacteria and healthy skin bacteria (MBC=0.128 mg/ml) were similar although the effects were more significant than the effects of tetracycline. Moreover, the standard bacterial strains were more sensitive to the nanofibers (MBC= 0.032). The antibacterial properties of the silver crystalline nanofibers reduced after the washing process (10 times; $P < 0.001$).

Conclusion: According to the results, the silver crystalline nanofibers had significant antibacterial properties on burn wound infection bacteria.

1. Introduction

Recently, biotechnologists have paid special attention to the wide applications of nanoparticles, which are speculated to play a pivotal role in medicine in the future. Researchers have also succeeded in the production of various metallic compounds from silver, zinc, mercury, copper, nickel, and titanium, which have exhibited several antibacterial properties to prevent the growth of bacteria and fungi, as well as other pathogens. Despite the antimicrobial properties these compounds, they may also have harmful properties, which limit their application [1].

Skin infections could be caused by opportunistic normal flora or be transmitted through patients or hospital personnel due to the use of contaminated tools, such as bed linens and nurse uniforms. Therefore, elimination of microorganisms from the sources of infection and timely treatment of infected patients are essential to the prevention of skin infection transmission [2, 3].

In terms of medical application, silver is considered superior to other antimicrobial agents and antibiotics owing to its advantages, which enable the use of this compound and its derivative nanoparticles against infectious microbial agents [4].

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Silver nanocrystal dressing is single-layer gauze coated with silver nanocrystals, which are established on highly flexible nylon fiber network by the chemical reduction method. This layer exerts antimicrobial and anti-inflammatory effects through the slow and steady release of silver ions with an extremely high surface coefficient on a vast surface, thereby increasing efficacy and diminishing the need for high concentrations of antibiotics and disinfectants (e.g., povidone-iodine), while eliminating the need for the early replacement of wound dressing. Another remarkable advantage of these products is the proper provision of oxygen and moisture to the wound. Color change of the fibers from dark green to light green (toward white) after the release of the silver ions is an indicator for the changing of the dressing [5,6]. Dressings have broad applications in the treatment of burn wounds, diabetic wounds, crushed/chronic wounds, wounds in the patients undergoing chemotherapy, as well as those with osteomyelitis and skin transplantation. Wound healing is a dynamic and complex process, which is promoted by environmental conditions. With technological advancement, more than 3,000 products have been made available for the treatment of various wounds, each of which targets a different aspect of the healing process [7,8].

Considering that a large number of hospitalized burn patients in Iran are reported to have secondary infections caused by multiple antibiotic-resistant bacteria and candida and the urgent need for antimicrobial textiles with few side-effects as convenient alternatives to other antibiotic treatments [9], the present study aimed to modify mineral nanostructures with antibacterial properties through the incorporation of a nanocrystal system following their fixation on nylon fibers and investigate the effects of the synthesized nanofibers on the bacteria isolated from normal skin flora and burn wounds.

2. Materials and Methods

2.1. Bacteria

Clinical isolates of the bacteria that were identified using biochemical tests were obtained from the wounds of the patients hospitalized at Imam Musa Kazim Hospital in Isfshan, Iran during 2018-2019. Other bacteria were isolated from the skin of healthy adults. The bacterial isolates were identified using conventional biochemical methods, and two strains of standard bacteria were also obtained from the Persian culture collection (Iran).

2.2. Silver Nanofibers

Agicoat dressing was used as the antibacterial agent, which was manufactured using the chemical vapor deposition method [10].

2.3. Antimicrobial Evaluation of the Silver Nanoparticle Solution and Crystal Nanofibers Using the Agar Diffusion Method

The qualitative well diffusion method was used to compare the antimicrobial effects of the raw silver nanoparticle solution (no fiber combination) and silver nanofibers based on antimicrobial material diffusion on agar. A microbial suspension equivalent to McFarland standard No.0.5 was prepared from the clinical isolates, and the bacteria isolated from healthy skin were inoculated to Müeller-Hinton agar (MHA) plates.

At the next stage, a serial dilution was prepared from raw antimicrobial materials and added to the wells on the culture medium. In addition, standard disks were soaked in the silver nanocrystal solution and placed beside the bacterial culture. The inoculated media were incubated at the temperature of 37 ºC for 24 hours. The same method was also applied to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Moreover, tetracycline (15 mg/ml) was used as the positive control since this broad-spectrum antibiotic has proven effective in the elimination of various wound infections in the selected hospital. Normal saline was also used as the negative control [11].

2.4. Antimicrobial Evaluation of the Silver Crystal Nanofibers Using the Macrodilution Method

Initially, various concentrations of the silver nanoparticles were prepared in test tubes, and the bacterial isolates and standard strains were inoculated in accordance with standard protocols [3,12].

2.5. Analysis of the Antimicrobial Activity of the Washed Silver Crystal Nanofibers

To perform the washing process, pieces of the antimicrobial nanofibers were placed in a flask containing distilled water with the temperature of 40°C and neutral soap (Johnson's kids' soap) for three minutes on a magnetic heater. After 30 minutes, the fibers were discharged from the temperature of 40 °C and placed in a flask containing distilled water for rinsing. Afterwards, the fibers were rinsed in sterile distilled water approximately five times, and the water was removed using sterile forceps. Following that, the fibers were placed in a sterile plate in an oven at the temperature of 50 °C for drying .

To investigate the durability of the antibacterial properties of the fibers, they were cut to the size of blank discs (diameter: 6 mm). The antibacterial effects of the nanofibers were determined using the agar disk-diffusion method on the MHA media containing the isolated bacteria. The diameters of the growth inhibition zones (mm) were measured and reported after incubation at the temperature of 37 °C for 24 hours. In this process, the disks containing tetracycline (15 mg/ml) and normal saline were used as the positive and negative controls, respectively [13].

3. Results and Discussion

3.1. Antibacterial Activity of the Silver Crystal Nanofibers

The bacterial isolates collected from the skin of healthy adults and burn wounds of the patients at the selected hospital were identified using conventional biochemical tests and used to assess the antibacterial activity of the silver crystal nanofibers using the agar disk-diffusion method (Figure 1) (Table 1).

According to the obtained results, the silver nanocrystal solution and dressing had similar effects on the isolated *Staphylococcus aureus* and *Pseudomonas aeruginosa* from burn wounds ($P > 0.01$); however, the diameter of the growth inhibition zone was greater in case of *S. aureus*. Despite the difference in the size of the growth inhibition zones, no significant differences were observed in the effects of the silver nanocrystal solution and dressing against the isolated *S. aureus* and *S. epidermidis* from the healthy adults and the three standard bacterial strains ($P > 0.01$).

According to the findings, the maximum diameter of the growth inhibition zone was observed with the use of the silver nanofibers on the *S. aureus* and *S. epidermidis* isolated from normal skin. Moreover, the silver nanofibers in solution and nanocrystal dressings could control the normal bacteria of the skin, which attested to the fact that nanoparticles lack the selective property to distinguish between pathogenic bacteria and normal skin flora.

According to the information in Table 1, the silver nanofibers in solution had more significant effects on the standard bacteria compared to the other bacterial strains. However, this finding was expected since standard collection bacteria are not usually antibiotic-resistant.

According to the literature, gram-positive bacterial strains are slightly more sensitive to some antimicrobial nanofibers, and this reaction could be partly attributed to the differences between the structure of gram-positive and gram-negative bacterial cell walls, as well as the type and conformation of the applied nanoparticle; this is consistent with the results of the present study. In another research, Shin et al. (1999) reported that 0.01 and 0.05 mg/ml of chitosan oligomers in polypropylene fibers could decrease the growth of more than 90% of *S. aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* [14]. In terms of the evaluation of the antimicrobial properties of fibers and selected bacteria, the findings of the mentioned research are in line with the results of the present study. In this regard, McGee et al. (2005) confirmed the antimicrobial activity of Sylgard fibers, which completely inhibited the growth of the isolated *S. aureus* from several wounds [15].

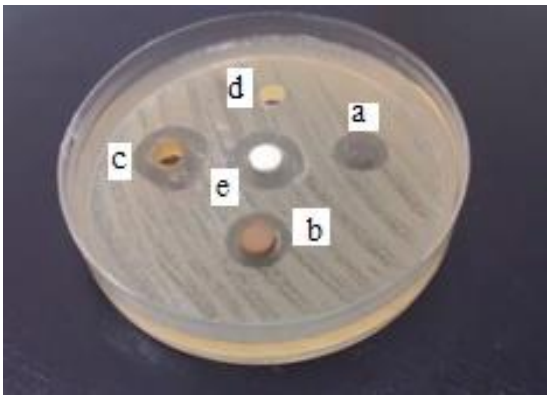

Figure 1: Comparison of Antibacterial Activity of Silver Crystalline Nanofibers Using Disk-Diffusion Method with Nonosilver in Solution; a) Silver Crystal Nanofibers in Solution; b) Disk Containing Silver Nanocrystals; c) Pure Silver Nanocrystal Solution; d) Negative Control (normal saline); e) Positive Control (tetracycline)

3.2. Antibacterial Effects of the Silver Nanocrystals Based on the Tube Macrodilution Method

In the present study, tube macrodilution analysis was performed in order to determine the MIC and MBC of the silver nanoparticle fibers on bacterial isolates, and the obtained results indicated that the MIC on 16 isolates of *S. aureus*, which were collected from the burn wounds of hospitalized patients at the selected hospital, changed from 0.008 to 0.128 mg/ml, while the MBC values on the mentioned isolates changed from 0.016 to 0.256 mg/ml.

Comparison of the quantitative results regarding the effects of the silver nanocrystal fibers at various dilutions on 16 isolates of *Pseudomonas aeruginosa*, which were collected from the burn wounds of hospitalized patients at the selected hospital, using the broth macrodilution method indicated similar results to the *S. aureus* isolates. In case of 16 *S. aureus* isolates obtained from healthy skin, the broth macrodilution analysis demonstrated that the MIC values changed from 0.016 to 0.128 mg/ml, while the MBC values on these isolates changed from 0.032 to 0.256 mg/ml. As for 16 *S. epidermidis* isolates, which were collected from healthy skin, the MIC values changed from 0.016 to 0.064 mg/ml, while the MBC values changed from 0.032 to 0.128 mg/ml.

Evaluation of the effects of various dilutions of the silver nanocrystal fibers on standard bacterial strains using the broth macrodilution method showed the MIC and MBC concentrations on S. aureus PTCC 1431 to be 0.032 and 0.064 mg/ml, respectively, while these values were estimated at 0.064 and 0.256 mg/ml on *P. aeruginosa* PTCC 1310, respectively.

Table 1: Mean Diameter of Growth Inhibition Zones of Isolated Bacteria from Burn Wounds and Healthy Skin and Standard Bacterial Strains in Pure Silver Nanocrystals, Silver Nanocrystal Dressings, and Disks Containing Silver Nanocrystal Solution (S: sensitive; [-]: bacterial growth not observed; mean inhibition zone diameter: >16 mm)

Isolate	Sensitivity to antimicrobial agent (inhibition zone diameter)				
	Silver nanocrystal solution	Silver nanocrystal dressings	Silver nanocrystal solution on disks	Tetracycline (positive control)	Normal saline (negative control)
Staphylococcus aureus from burn wounds	S (16.1 mm)	S (14.8 mm)	S (15 mm)	S (13.3mm)	-
Pseudomonas aeruginosa from burn wounds	S (15.4 mm)	S (13 mm)	S (14.2 mm)	S (12.5 mm)	-
Staphylococcus aureus from healthy skins	S (15.8 mm)	S (13.3 mm)	S (14mm)	S (12 mm)	-
Staphylococcus epidermidis from healthy skins	S (15mm)	S (14.9 mm)	S (15.7 mm)	S (12.1mm)	-
Staphylococcus aureus (PTCC 1431)	S (14.1mm)	S (13.4 mm)	S (15.3 mm)	S (13.3 mm)	-
Pseudomonas aeruginosa (PTCC 1310)	S (13.8 mm)	S (14.2mm)	S (15 mm)	S (12 mm)	-

Although the MIC and MBC values of nanoparticles on some burn wound infection bacteria were higher compared to some of the bacteria isolated from healthy skin in the current research, no significant difference was observed in the mean effects of the silver nanoparticles on these three bacterial strains ($P < 0.001$). In this regard, the findings of Hayes (2005) indicated that the MIC of AEM 5700 antimicrobial fibers to be 10 μg/ml for *S. aureus* and *Enterococcus faecalis* [16].

In the current research, the results of the analysis of variance (ANOVA) regarding the mentioned data indicated that the variations in the MIC and MBC were significant in eight different concentrations of the antimicrobial agent in the samples of burn infection bacteria, healthy skin bacteria, and standard bacterial strains ($P < 0.01$). Moreover, changes based on the type of the bacteria had significant differences. In a study by Imani et al. (2011), the antibacterial effects of CrO and COFe2O4 nanoparticles on *S. aureus* were compared, and the obtained results indicated that the CrO nanoparticle had more significant antibacterial effects on gram-positive bacteria compared to the $COFe_2O_4$ nanoparticle [17].

In another study in this regard, Qaziasgar and Kermanshahi (2009) investigated the antimicrobial effects of the fibers produced by Isfahan polyacryl factory (Iran) on the *P. aeruginosa* isolated from the wounds of 54 hospitalized patients in Isabn-e Maryam Hospital in this city. According to the findings, the MIC of the fibers on bacteria was 1-3 μg/ml, while it was estimated at 0.001 μl/ml with an antimicrobial agent solution. In addition, the interaction between these antimicrobial agents on the isolated bacteria from wounds was reported to have a better synergistic response [18].

According to the current research, despite the higher concentrations that were required for the elimination of bacteria, no significant differences were observed between the antimicrobial properties of the silver nanocrystal solution, disks, and dressings containing silver nanocrystals in most of the treated bacteria. However, some differences could be due to the absorption of the nanosilver crystals onto the nylon fibers and its washing process.

3.3. Effect of Washing on the Stability of the Antibacterial Properties of the Silver Crystalline Nanofibers

Figure 2 depicts the effect of washing on the stability of the antimicrobial properties of the silver nanocrystal dressings based on the agar disk-diffusion method in the fibers after washing with Johnson neutral soap (10 times).

According to the results of the present study regarding the effect of washing on the stability of the antimicrobial properties of the silver nanocrystal dressing, the antimicrobial properties reduced and were eliminated after 10 times of washing with Johnson neutral soap. Therefore, it could be concluded that washing led to the gradual release of the silver nanocrystals and reduction of its antimicrobial properties. This is in line with the findings reported by Vincent and Vigo [13] and Lee et al. (2003) [19], in polyester fibers containing silver nanoparticles) ($P < 0.001$).

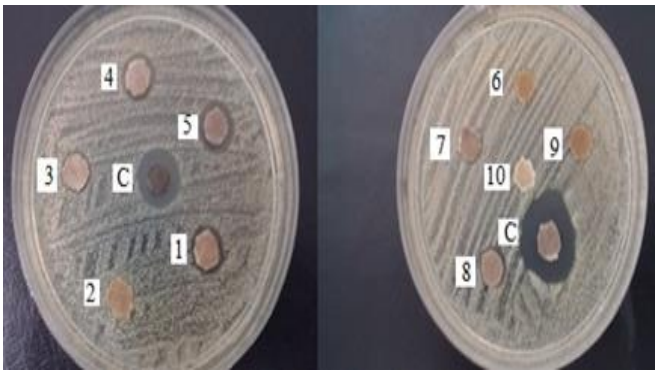

Figure 2: Inhibition Zone Diameter of *Staphylococcus aureus* under Effect of Nanosilver Bandages after 1-12 Times of Washing (C: control without nanoparticles)

4. Conclusion

According to the results, the antibacterial effects of the silver nanocrystal solution and silver nanocrystal dressing had no significant difference in the investigated bacteria. On the other hand, the nanoparticles exerted more significant inhibitory effects as opposed to tetracycline. Therefore, these nanodressings could be applied as clinical bandages or in the clothes of patients, physicians, nurses, and hospital personnel after proper clinical examinations in-vivo on experimental animals with burn wounds.

Authors' Contributions

F.G., and M.D., designed the project and performed laboratory data collection and data analysis. M.M., contributed to the study design and data analysis.

Conflict of Interest

None declared.

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