Antimicrobial Effects of Silver Nanoparticles Coated Leather on Diabetic Foot Ulcer

Research Article

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Abstract

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Background: Lower limb infection is among significant causes of mortality, morbidity, and prolonged treatment in diabetic patients. Lately, silver nanoparticles, found in various medical applications such as silver coated medical devices, have been introduced as an effective antimicrobial agent. Considering the fact that the antibacterial activity of silver nanoparticles has been established, the aim of study was to determines the efficacy of these particles in reducing the number of microorganisms which grow on diabetic foot ulcer and subsequently prevent diabetic foot infections.

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Methods: In this case-control study aerobic and anaerobic microorganism were taken from diabetic foot infections of patients visiting the outpatient diabetes clinic by micro debridement. Microorganisms were recognized through standard microbiological methods using kits such as tio glycolat, blood Agar, MacConkeys and chocolate Agar. The natural leather and nanoparticle-coated leather were then inserted in culture media. After 24 hours, samples were count by Muller.

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Results: Two hundred twenty two strains of 24 different types of bacteria were isolated from 95 diabetic foot ulcers, from among which 94 [42.3%] and 128 [57.7%] strains were respectively gram negative and gram positive. The number of bacteria that grew on the nanoparticle-coated leather was significantly lower.

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Conclusion: Considering the reduced number of aerobic, anaerobic, gram positive and negative bacteria that grew in diabetic foot ulcers when using nanoparticle-coated leather culture, it could be concluded that the use of such silver coats leather in diabetic foot shoes may prevent diabetic foot infections considerably.

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Keywords

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Diabetes; Diabetic foot ulcer; Bactericidal agent; Silver nanoparticles; Nanobiotechnology

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Introduction

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The lifetime risk of developing foot ulcer in diabetic patients is about 25% [1]. The high rate of infection in such ulcers is considered as an important cause of amputation in 25–50% of diabetics [2–7] and therefore is associated with morbidity and mortality in a large number of individuals [8].

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Nanotechnology is an emerging science and with growing use particularly in developing new materials at nanoscale levels [9]. From among different types of available nanomaterials, nanosilver is proved to be most effective material which has good antimicrobial properties against bacteria, viruses and other eukaryotic micro-organisms [10]. In other words, silver nanoparticles offer scientists a vast number of opportunities to re-explore the biological properties of already known antimicrobial compounds [11]. As a result, the use of such compounds is gaining more popularity these days and nanosilver can be found in clothing, food containers, wound dressings, ointments, implant coatings, and many other items [12]. Since there is not any study evaluating the effect of nanosilver on diabetic foot ulcers infection, the current study aimed to determine the efficacy of these particles in lowering the number of microorganisms which grow on diabetic foot ulcers.

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Method and Material

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The case-control study has been approved [code: E-0023] by the ethics committee of Endocrinology and Metabolism Research Institute [EMRI] and conducted in shariati hospital of Tehran University of Medical Sciences [TUMS] in Iran from January 2009 to December 2010. Ninety five of diabetic patients with diabetic foot infection who admitted at the outpatient diabetes clinic and clinically diagnosed with diabetic foot infection whether superficial infected ulcers and osteomyelitis were included in the study.

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The patients randomly selected and signed informed consent form after explaining the aim of research.

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They should be antibiotic free during last week. Patients who took oral, topical and injected form of antibiotics within this period of time were excluded.

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Aerobic and Anaerobic cultures were taken from their diabetic foot ulcers through micro debridement. Diabetic foot wounds were washed with normal saline; the specimens were obtained from the base of the ulcer, deep wound or needle aspiration of the abscess. The specimens were sent to the microbiology laboratory in thioglycollate tubes and incubated at 37°C for 24 hours. Following the gram stain, the cultures on blood agar and Mac Conkey agar were incubated under aerobic and anaerobic conditions at 37°C for 48 hours.

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Leather was provided by Charm Sazi Mojtahedi Co. [Tabriz Iran]. The samples for further treatment were prepared in the size of 2.0 cm² and coated with nanosize silver. Silver nanoparticle suspension was supplied by PlasmaChem GmbH Company in form of Colloidal solution, 0.1 mg/mL.

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The leather sample did not pretreat before they were subjected to nanosilver coating. The concentration of silver nanoparticles has not been measured because we did not use any mediator substances for coating nanosilver. So it seems that the concentration of silver nanoparticles was a little more than the nanosilver optimum size. The nanosilver optimum size was 10 nm. The silver antibacterial coated on the surface of leather by pad-dry cure method.

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Then natural leather and nanoparticles coated leather were inserted in culture Medias. All 222 strains of 24 different types of bacteria were then placed on these two culture media. After 24 hours, the samples were count by Mueller-Hinton agar.

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Variables were suggested in descriptive data. Categorical data put into a table in form of frequencies and percentages. All data were analyzed with SPSS 18.

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Results

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Two hundred twenty two strains of 24 different types of bacteria were isolated from 95 diabetic foot ulcers [Table 1]. One hundred thirty three of the strains [59.9%] were obtained from superficial wounds, whereas 89 others [40.1%] were from the depth of the wounds.

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Table 1: frequency of isolated bacteria from diabetic foot wounds (number of strains=222).

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From among the isolated strains 94 [42.3%] were gram negative and 128 [57.7%] were gram positive. Two hundred sixteen of these strains [97.3%] were aerobic and 6 [2.7%] of them were anaerobic. Table 2 outlines more detailed information on the characteristics of the microorganisms isolated from diabetic foot. The isolated strains were counted less than 5 including *streptococcus aureus*, *Citrobacter freundii*, *klebsiella pneumonia*, *Pseudomonas/sp*, *candidia*, *peptococcus*, *protus*, *diftroid*, *bacillus*, *micrococcus*, *Pseudomonas/Aureus*, *Bacteroides fragilis*, *Peptostreptococcus* categorized as “others” group.

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Table	Column 1	Column 2	Column 3
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Table 2: frequency (number and percentage) of Gram positive and Gram negative, depth, Aerobic and Anaerobic isolates.

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As it showed in figure 1, lower number of bacteria grew in nanoparticle-coated leather.

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Figure 1: Comparison number of strains in natural leather and nanosilver coated leather.

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Discussion

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The use of nanosilver products in the management of diabetic foot infections is expanding nowadays [12]. The present study revealed that fewer number of all different types of microorganisms responsible for diabetic foot infection grow on nanoparticle-coated leather comparison with natural leather without nanosilver.

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Most diabetic foot infections are polymicrobial as some five or seven different organisms are involved in the process. The microbiology of these wounds differs based on the extent of involvement [13-16] Aerobic gram-positive cocci, including *S. aureus*, *S. agalactiae*, *S. pyogenes* and coagulase-negative *staphylococci* are responsible for a large number of superficial foot wounds. As for, deep ulcers, particularly those which are extended to the fascia, gram negative bacilli such as *P. aeruginosa* and *Proteus* are also responsible. Infection with anaerobic organisms along with the above-mentioned pathogens should be kept in mind while managing wounds with extensive local inflammation and signs of systemic toxicity [17-21].

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Morones et al. [22], similarly, pointed out the antibacterial activity of silver nanoparticles against four types of gram negative bacteria, *E. coli*, *V. cholera*, *P. aeruginosa* and *S. typhus*. They stressed that the finding could be explained by the fact that silver nanoparticles attach to the cell membrane and release silver ions, disturbing its function and the penetration of bacteria [22]. Sondi and Salopek-Sondi [23] also reported the antimicrobial activity of silver nanoparticles against *E. coli*, a gram-negative bacterium [23].

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Similar to our study in which silver nanoparticles reduced the growth rate of gram negative diabetic foot microorganisms including *P. aeruginosa*, *E. coli*, *bacillus*, *protus*, *P. spironogesia*, *Klebsiella*, *Enterobacter*, *Acinetobacter* and *citrobacter* in leather-coated cultures, Panacek et al. [24] found high antimicrobial and bactericidal activity of silver nanoparticles against gram-positive and gram-negative bacteria including multi-resistant strains such as methicillin resistant *S. aureus* [24]. Shrivastava et al. [25], however, reported that synthesis of silver nanoparticles in the size range of 10-15 nm and its dose dependent effect on the Gram-negative and Gram-positive microorganisms [25]. According his study finding the dose dependent silver nanoparticles have marked activity against gram-negative organisms than the gram-positive organisms.

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Humberto et al. [26] also proved that silver nanoparticles are effective bactericidal agents even in fighting multidrug-resistant *P. aeruginosa*, *ampicillin-resistant E. coli* O157:H7 and erythromycin-resistant *S. pyogenes* [26]. Madhumathi [27] found that nanosilver composites have not only blood clotting capabilities but also bactericidal properties against *S. aureus* and *E. coli* [27]. These results further agree with previous findings which revealed that silver nanoparticles exert the same effect on gram positive and gram negative strains [28,29].

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Maleknia et al. [30] performed a study to assess the antibacterial properties of nanosized silver colloidal on wool fabric. They showed that nanosilver coated in wool fabrics not only has the antibacterial effect against *S-aureus* and *E-coli*, but also its efficiency was ever 96% after 20 washing time [30].

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Conclusion

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It could be concluded that silver nanoparticles with their unique chemical and physical properties might be used as an effective alternative for the available antibacterial agents for diabetic foot infection. Considering the diverse applications of wound dressings, medical device coatings, silver nanoparticles impregnated textile fabrics, silver nanoparticles could be used to prevent and treat diabetic foot ulcer.

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