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Full length articles

Short Communications

IN VITRO ANTI-BACTERIAL AND BIOLOGICAL PROPERTIES OF MAGNETRON SPUTTERED SILVER NANOPARTICLES CONTAINING TITANIUM IMPLANTS

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ABSTRACT

Bacterial infection followed by implant fixation is a common complication after surgery. In order to reduce the incidence of implant-associated infections, several biomaterial surface treatments have been proposed. In this study, the effect of *in vitro* antibacterial activity of magnetron sputtered silver nanoparticles was studied. Sputtering was done using radiofrequency magnetron sputtering technology. Film applicator coating assay was used to assess the antibacterial effect of the coated titanium implants. SEM examination revealed successful deposition of silver nanoparticles on the titanium surface. The average diameter of the nanoparticles was 40-60 nm. SEM examination before incubation and after incubation of bacteria was done. The bactericidal ratio between the uncoated and coated implant was determined.

Key words: Infection, silver nanoparticles, antibacterial effect

Introduction

Infections associated with orthopaedic implants are a challenge to the long-term "survival" rate of implants and may cause the treatment to fail.[1,2] The titanium surfaces of implants are appropriate for microbial colonization and biofilm formation.[3]Moreover, there are boundaries to the use of aseptic surgical techniques and prophylactic antibiotics. Therefore, strategies involving the delivery or incorporation of antibiotics such as gentamicin and vancomycin on some titanium implants have been tried to reduce infections.[4, 5]Non-conventional antibiotics have been developed in an attempt to handle these problems since there is increasing concerns on antibiotic-resistant pathogens in orthopaedic field. Silver is effective against a broad spectrum of bacterial and fungal species, including strains that are resistant to antibiotics.[6] Silver nanoparticles are considered to be even more active due to theirlarge surface area to volume ratio.[7] Previous work has indicated that silver nanoparticles embedded in titanium may be highly effective in inhibitingboth *Staphylococcus aureus* and *Escherichia coli*.[8]

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In veterinary practice, a common complication following open reduction and internal fixation using plates and screws to treat compound fracture is osteomyelitis. Compound fractures are very difficult to treat since they are already contaminated and infected with microorganisms. The common organism found in compound fracture is *Staphylococcus aureus* organism. This study is conducted in an attempt to eradicate infection in compound fracture and as a prophylactic tool.

MATERIALS AND METHODS

Preparation of titanium plate

Commercially available pure titanium implants were ultrasonically cleaned with acetone and distilled water. In this study, 2.0 mm titanium reconstruction implants were used to test the antibacterial effect of silver nanoparticles.

Deposition of silver nanoparticles on the titanium surface

Silver nanoparticles were deposited on the titanium surface using radiofrequency magnetron sputtering technology. Sputter coating machine delivers uniform amount of silver nanoparticles on the titanium surface.

Operating Characteristics of a sputtering machine

Parameters used to coat a titanium implant in a radiofrequency magnetron sputter coating machine are shown in the below table.

Table 1: Parameters required to surface coat an implant (Moseke et al. 2011)

Parameter	Details
Target	Silver
Power	40 W
Deposition time	8 min
Inert gas	Argon
Size of the nanoparticle	40-60 nm
Base pressure	10^{-5}
Working pressure	2×10^{-3}
Characterisation	SEM

Characterization of the silver nanoparticles

The morphology of the silver nanoparticles coated surface of the titanium implants was characterized using SEM (HITACHI S-4800, Tokyo, Japan).

Antibacterial tests

The antibacterial activity of the silver nanoparticles coated titanium surface was tested against the Gram-positive *Staphylococcusaureus* (MTCC).

Zones of inhibition (ZoI) tests were carried out to decide the degree of silver ion discharge from the coated titanium surface. In the ZoI test, the concentration of *Staphylococcus aureus*was adjusted to 1x 10^6 colony-forming units (CFUs)/mL and spread evenly on Luria-Bertani medium agar plates. Titanium coated with silver nanoparticles implant and the non-coated control titanium implant were placed on the above prepared agar plates separately. The plates were incubated at 37°C in an aerobic petri dish for 24 hours and photographed to record the results.

Film applicator coating (FAC) was used to test the antimicrobial effect by directly incubating microbial cells on Ti-nAg surfaces. In the FAC assay the concentration of each bacterial strain was adjusted to $1x10^4$ cells/mL in phosphate buffer solution and Luria Bertani Medium. The titanium implants were then placed on these culture plates and kept in wet boxes. Then 10 µL bacterial suspensions were applied to the Ti-nAg and the titanium control implants, respectively. Following incubation at 37°C in an aerobic petri dish for 24 hours, the bacterial suspensions on the coupons were then transferred separately into tubes containing 10 mL of sterilized PBS, followed by vigorous vortex mixing for 5 minutes. Following this 10 µl of bacterial solutions from the mixtures were then spread on Luria Bertani medium broth-agar plates. The plates were incubated aerobically for 24 hours. The viable cells on each of the plates were counted by quantifying the CFUs.

The antibacterial effect in each group was determined as bactericidal ratio and was calculated as follows:

Bactericidal ratio (%) = [(CFU of CG - CFU of EG)/CFU of CG] × 100%

CFU represents colony-forming unit; CG - titanium implants (control group) and EG - silver nanoparticles coated titanium implants (experimental group).

RESULTS AND DISCUSSION

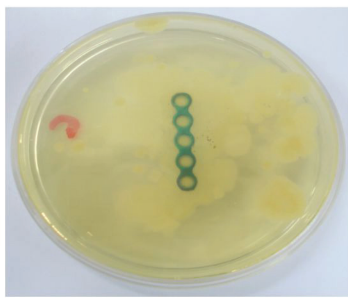
Zones of inhibition (ZoI)

There was no zone of inhibition noticed around either the silver nanoparticles coated titanium plate or the non-coated plate.

The Zones of Inhibition (ZoI) test was inconclusive in both control and silver nanoparticles coated titanium implants. This result may be due the Zone of Inhibition was based on the escape of silver ions from the surface the inhibition of the bacterial growth depends on a sufficient concentration of silver ions in the surrounding aqueous environment and the elemental silver has a very low rate of dissolution in an aqueous environment, it is possible that the silver dissociated from the titanium surface did not reach a concentration sufficient to inhibit bacterial growth.[9]

dissociated from the titanium surface did not reach a concentration sufficient to inhibit bacterial growth.[9]

Figure 1



CONTROL

Figure 2

COATED

Fig 1 and 2: Zone of inhibition tests showing no discernible line of inhibition in both control and silver nanoparticles coated titanium implants

Film applicator coating (FAC) assay

Film applicator coating (FAC) assay was done to determine the bactericidal ratio of silver nanoparticles coated titanium surface. The coated titanium implant showed remarkable antibacterial effect against *Staphylococcus aureus* organism. The bactericidal ratio was found to be 95 per cent.

Bactericidal ratio (%) = [(CFU of CG - CFU of EG)/CFU of CG] × 100%

The mean ± SD values obtained from the study was depicted in the bar diagram below.

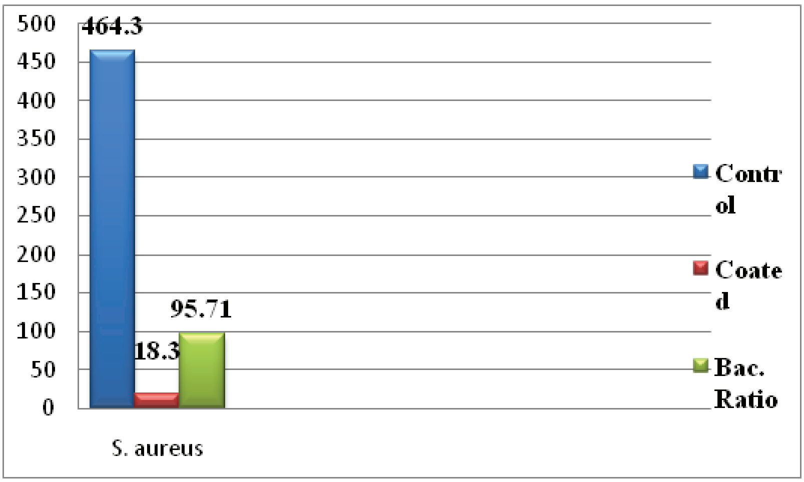


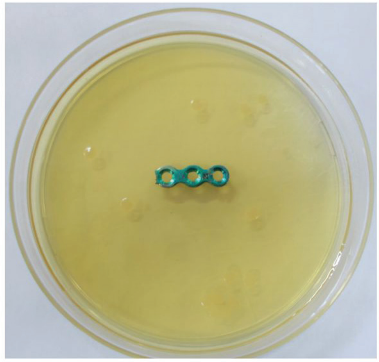
Figure 3: Counts of CFU (mean ± SD) and bactericidal ratio obtained from FAC

In contrast to the Zone of Inhibition test, the silver nanoparticles coated titanium plate surface unveiled a robust antibacterial property. Following 24-hour incubation of bacteria on Silver nanoparticles coated titanium plate surface (Ti-nAg surface) resulted in a depletion of bacteria which is more than 95 per cent. Ti-nAg specimens significantly inhibited the growth of both Staphylococcus aureus than non-coated Titanium specimen. The incongruity between ZoI and FAC tests may be due to the difference of the antibacterial mechanism. The former relied on leaching of the silver ions from the surface, whereas the latter mediated an inhibitory effect by direct contact. The SEM examination of anti-adhesive test showed that there was much less bacteria adherent to Ti-nAg surface than to the non-coated control titanium surface. This was due to the direct contact inhibition and the anti-adhesive properties of the Ti-nAg surface. Therefore it was suggested that the silver nanoparticles coated titanium plate (Ti-nAg) surface reduces the risk of bacterial colonization. This was in accordance to the study done by Chen et al. (2006) where he observed that deposition of silver-hydroxyapatite (Ag-HA) coatings on titanium implant surfaces by magnetron sputtering exhibited high antibacterial activity against S. aureus. Juan et al. (2010) found similar observation where he proposed that the silver nanoparticles modified titanium surface provided effective contact with microorganism and exhibited strong antibacterial properties against S. aureus organism. Silver nanoparticles coated titanium surface showed strong antibacterial properties against infection causing organisms which was in accordance with Zhao et al. (2011), Goodman et al. (2013) and Jematet al. (2015).

Figure 3

CONTROL

Figure 4



COATED

Fig 4 and 5: Film applicator coating assay tests showing bacterial growth in control and silver nanoparticles coated titanium implants

Characterization of the silver nanoparticles on titanium surface

The Scanning Electron Microscopy (SEM) micrographic pictures showed silver nanoparticles uniformly deposited on the titanium surface, with a diameter of approximately 40 - 50 nm at 500 x magnificationsand at 1000 x magnifications. Some of the silver nanoparticles formed aggregates/agglomerates together.

In the control titanium surface after the incubation there was numerous amount of *Staphylococcus aureus* organism present at 500 x magnification. In the silver nanoparticles coated titanium implant there was absence of *Staphylococcus aureus* organism at 500 x magnifications.

A noticeable change after the coating of titanium implants with silver nanoparticles was appreciable amount of silver macroscopically deposited uniformly over the implant. The SEM micrograph showed silver nanoparticles sparingly deposited on the titanium surface, with a diameter of 40-60 nm. This finding was similar to the study done by Lkhagvajav et al. (2011) where he demonstrated colloidal silver nanoparticles with a size range of 25 to 45 nanometer exhibiting antibacterial activity against *S. aureus* and *E. coli* organisms. This was contrary to the study done by Morones*et al.* (2005) where he opined that the bactericidal properties of the silver nanoparticles were size dependent and direct interaction with the bacteria occurred preferentially at a diameter of 1 to 10 nanometer size.

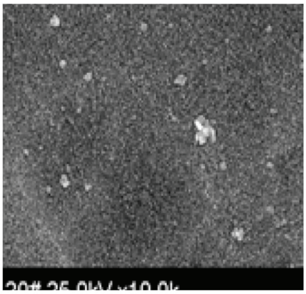


Figure 6: SEM micrograph of silver nanoparticles coated titanium

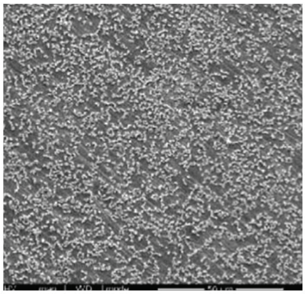


Figure 7: SEM micrograph of control titanium showing numerous bacterial growth

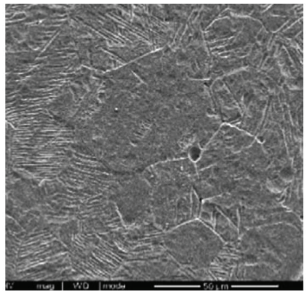


Figure 8: SEM micrograph of silver nan oparticles coated titanium showing no or absence of bacterial growth

CONCLUSION

In the present study, silver nanoparticles coated titanium implants were prepared using radiofrequency magnetron sputtering technology. Deposition was successful which was confirmed by Scanning Electron Microscope. SEM images showed uniform deposition of silver nanoparticles on the titanium surface. The average diameter was 40-60 nanometers. Film applicator coating assay and Zone of inhibition tests were performed and the anti-bactericidal effect of the nanoparticles was studied. The bactericidal ratio of the silver nanoparticles coated titanium implants was 95.41 percent. Nanoparticles showed remarkable antibacterial properties against *Staphylococcus aureus* organism. These data suggests that silver nanoparticles coated titanium implants will be a promising tool in preventing implant associated infections in near future.

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