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SHORT COMMUNICATION

Evaluation of antimicrobial efficacy of nano coated silver-titania metallic plates against selective pathogens

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ABSTRACT

Aim: Nanotechnology is an increasingly growing field with its current application in Science and Technology for the purpose of manufacture of novel materials at the nanoscale level. Silver-Titania nanoparticles ($AgTiO_2$-NPs) have been known to have inhibitory and bactericidal effects.
Methodology and Results: In the present study, stable silver-titania nanoparticles coated metallic blocks were prepared for testing their efficacy against selected bacterial pathogens like *Escherichia coli* and *Staphylococcus aureus*. In the experimental part, the bacterial pathogens were inoculated on silver-titania nanoparticle coated blocks and the treatment was carried out in '0' time and '24' h interval and were enumerated.
Conclusion, significance and impact of study: The results were compared with the control (uncoated metallic blocks) and analyzed by using Japanese Industrial Standard (JIS Z2801:2000) method. From this study, it was concluded that silver-titania nanoparticles has inhibitory effect on bacterial pathogen tested.

Keywords: Silver-Titania nanoparticle, *Escherichia coli*, *Staphylococcus aureus*, JIS

INTRODUCTION

Human beings are often exposed to microorganisms in the living environment and are prone to infection. Antibiotics are the most commonly prescribed medications in modern medicine which cure disease by killing or damaging bacteria. The determinations of the activity of these antibiotics are crucial to the successful outcome of antimicrobial therapy. The resurgence of antibiotics is playing a significant role in the emergence of resistant bacteria.

Silver has been effectively used as an antimicrobial agent for centuries; the recent progression in interest for this element particularly focuses on the increasing threat of antibiotic resistance, caused by the abuse of antibiotics (Panacek *et al.,* 2006; Sambhy *et al.,* 2006). Intensified research in antibacterial material of various natural and inorganic substances has been found to be exquisite (Kim *et al.,* 1998; Cho *et al.,*2005). The desired application of the material is attained by its peculiar property when the dimension of a material is reduced to the atomic level (Burrell *et al.,* 1999; Gleiter *et al.,* 2000). The development of nanoscale techniques for silver production may assist the resurgence of the medical use of silver, especially in applications to eliminate pathogens (Chio *et al.,* 2005). Silver nanoparticles have a large outer geometry to volume ratio (Ichinose *et al.,* 1992), which meliorate their antimicrobial capability to interact with other substances, even at a low concentration (Lee *et al.,* 2003; Li *et al.,* 2006). Biotechnology and nanotechnology has integrated and emerged as bionanotechnology for developing biosynthetic and environmental- friendly technology for synthesis of nano materials.

The Japanese Industrial Standard (JIS) has been prepared for standardizing the method for evaluating antimicrobial efficacy in antimicrobial products shown in the report on Life-Related Processed Products with New Functions (Antimicrobial Products): December 1998 (so-called "Guidelines for Antimicrobial Products"). This standard specifies the exactitude testing methods to evaluate antimicrobial activity and antimicrobial efficacy in bacteria on the surface of antimicrobial products.

MATERIALS AND METHODS

Preparation of silver-titania coated metallic blocks

The silver-titania coating was carried out using Praxair plasma spray system with SG100 torch (USA). The feedstock powder, silver-titania nanocomposite was

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prepared by spray drying. The silver content was varied from 1 % - 5 %. The feedstock material was carried into the torch by carrier gas (Argon). The plasma spray coating was carried out by injecting the feedstock material (silver-titania nanocomposite powder) into the hot plasma stream where the particles were heated and accelerated at high velocity, and impacted on to the metallic substrate. The melted particles were rapidly solidified, flattened and formed splats. The silver-titania coating was formed by the build-ups of these splats. The plasma spraying parameter is listed (Table 1). The produced silver-titania coated samples identification was listed (Table 2).

Table 1: Plasma spray parameters used to produce the silver-titania coatings

Operating System	Value
Plasma spray power	12 kW
Primary gas (Ar)	80 psi
Secondary gas (He)	50 psi
Carrier gas (Ar)	30 psi
Spraying distance	80 mm
Powder feed rate	2 rpm

Table 2: Labeling and identification of coated samples

Sample Name	Sample ID
Silver (1 %)-titania	ST1
Silver (3 %)-titania	ST3
Silver (5 %)-titania	ST5

Study on antimicrobial activity of nano particle

Sample size and Test Organism

In this study, a total number of three samples at different concentrations of test samples and control samples (without coating) were prepared and tested against selective bacterial pathogens *viz*. *Escherichia coli* and *Staphylococcus aureus.*

Experimental Set Up

In this investigation, each test piece was placed separately in a sterilized Petri dish with the coated side facing upwards for inoculation. Each test piece was instilled with about 0.4 mL of the inoculum *viz. E. coli or S. aureus* ($2.5 \times 10^5 - 1.0 \times 10^6$ cells/mL) and covered with a square poly ethylene film of 40 mm ± 2 mm, to help the inoculum to spread evenly over the test piece. The petri dishes with the coated and uncoated test pieces were incubated at 37 °C ± 1 °C for 24 h ± 1 h. For the '0' h test pieces, after the standard procedure described, the covering film was removed immediately, and washed with 10 mL of the SCDLP broth.

Enumeration of Test Organism

The count of viable cells of bacteria was proceeded immediately in the washings by serial dilution method. About 1.0 mL of each of the washings from each dilution was dispensed into sterilized nutrient agar and incubated them at 37 °C ± 1 °C for 24 h to 48 h. For the test piece after 24 h incubation, the test bacteria was washed out in a similar manner as done in '0' time treatment and the serial dilution procedure was carried out to count viable cells of bacteria in the washings as in the previous step. After incubation, the number of colonies (30 to 300 colonies/plate) from each serially diluted Petri dish was counted and calculated according to the formula. The efficacy of antimicrobial products was judged from the value of antimicrobial activity according to the JIS standards. The value of antimicrobial activity obtained by the testing methods of this standard shall not be less than 2.0 for testing the antimicrobial efficacy of antimicrobial products (JIS Z 2801:2000).

Antimicrobial efficacy of silver-titania nanoparticle

When the test has been effective, calculate the value of antimicrobial activity according to the formula given below and the values were recorded.

$$R = [\log (B / A) - \log (C / A)] = [\log (B / C)]$$

Where,
R = value of antimicrobial activity
A = average of the number of viable cells of bacteria immediately after inoculation on the untreated test piece
B = average of the number of viable cells of bacteria on the untreated test piece after 24 h
C = average of the number of viable cells of bacteria on the antimicrobial test piece after 24 h

The antimicrobial efficiency was measured as the percentage reduction using the numbers of initial and surviving bacteria on the test samples based on the sample, using the following formula:

$$\text{Antimicrobial efficiency (\%)} = \frac{A - C}{A} \times 100$$

Where, 'C' and 'A' are the numbers of surviving bacteria (cells/mL) for the samples incubated for 24 h and 0 h, respectively and the results were presented in Table 3 (Jung *et al.,* 2009).

RESULTS

The feedstock material used in this study was prepared using different concentration of silver-titania nanoparticles. The samples include 3 different concentrations *viz*, ST1, ST3 and ST5 were prepared (Table 1 and 2) and the treatment was carried out of 3 samples in each set for "0" h and "24" h in *E. coli* and *S. aureus* respectively and the viable bacterial cells (Table 3) were analysed by JIS method.

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Table 3: Antimicrobial Efficacy against *Staphylococcus aureus*

Batch No.	A	B	C	R Value (log B / C)	Antimicrobial Efficacy (%)
ST1	35×10^4	43×10^6	32×10^2	4.12	99.08
ST3	38×10^7	75×10^4	36×10^1	3.32	99.99
ST5	75×10^4	133×10^4	55×10^2	2.38	99.27

The value of the antimicrobial activity according to the formula:
R = [log (B / A) – log (C / A)] = [log (B / C)]

DISCUSSION

The results showed that the growth of *E. coli* was completely inhibited (absolute efficiency) in all the concentrations tested, whereas in *S. aureus* the antimicrobial activity was above the range of 2 in all the samples tested and the antimicrobial efficacy (%) lies between 99.08 % (ST1) to 99.99 % (ST3). Earlier studies proved that silver nanoparticles have known to exhibit inhibitory and bactericidal effects. It is generally recognized that silver nanoparticles may attach to the cell wall, thus disturbing cell-wall permeability and cellular respiration. The nanoparticles may also penetrate inside the cell by interacting with phosphorus and sulfur-containing compounds such as DNA and protein and thereby causing damage (Castellano *et al.,* 2007). Sambhy *et al.* (2006) suggested a possible contribution to the bactericidal activity of silver nanoparticles is the release of silver ions from particles.

Yoksan and Chirachanchai (2010) studied the antimicrobial activity of silver nanoparticles in polysaccharide-based films against *E. coli* and *S. aureus,* as model Gram-negative and Gram-positive bacteria, respectively, by an agar diffusion method. Kim *et al.* (2007) results suggest that the antimicrobial effect of Ag nanoparticles may be associated with characteristics of certain bacterial species and substantiated that the Ag nanoparticles tested in *E. coli* showed effective growth inhibition than *S. aureus.* Gram positive and Gram negative bacteria exhibit heraldry in their membrane structures, the most distinctive of which is the thickness of the peptidoglycan layer.

Maness *et al.* (1999) demonstrated that TiO_2 materials have effective role against the microbial agents and have been successfully adopted. Sudana *et al.* (2003) elucidated the mechanism for photo killing of *E. coli* cells on titanium dioxide (TiO_2) thin film and suggested that the TiO_2 photo catalyst, when placed under visible light radiation generate oxidising substances against microbes and moulds. Wang *et al.* (2006) observed that the silver-ion-exchanged titanium phosphate films were effective in prohibiting the growth of *E. coli* and, hence were found to be used as antibacterial coatings.

CONCLUSION

From this study it is concluded that silver-titania nano coated metallic blocks possess relatively high antimicrobial activity against the bacterial pathogens tested. Silver-Titania (3%) metallic blocks showed the antimicrobial efficacy of 99.99 % in *S. aureus* tested. Nanosilver (nano Ag) is one of the most effectively used nanomaterials because of its strong antibacterial properties. In comparison with silver ion there is deficient information concerning the biological effects of nano Ag. Nano scale techniques for silver production may assist the resurgence of the medical use of silver, especially given that pathogens showing increasing resistance to antibiotics. Silver nano particles are also considered as candidate for coating medical devices.

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