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Original Research Article

Antibacterial effect of titanium dioxide with or without silver as additive on staphylococcus aureus from clinical samples

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ABSTRACT

Introduction: Antibiotic resistance has now become a perpetual global problem due to the emergence and reemergence of old and new multiresistant bacteria that there is a constant need of newer drugs to combat this phenomenon. Nanoparticles are now considered for their antibacterial effect and those such as Titanium dioxide have a good mechanical properties and biocompatibility, especially being non toxic to human skin and cost effective. This study was done to show the antibacterial effect of Titanium dioxide with and without Silver as addendum on Staphylococcus aureus strains from clinical samples.

Materials and Methods: Staphylococcus aureus isolated from various clinical samples was inoculated onto peptone water and further inoculated onto glass slides coated with TiO2 annealed at 200^0C, 400^0C and onto TiO2 with 0.1%, 0.3%, 0.6% and 0.8% silver as additive at 1 hour intervals each. The growth was observed after 18 hours of incubation.

Results: The highest antibacterial effect was observed within 2 hours of treatment with 0.8% Ag on TiO2 nanoparticles, while it took longer with the other concentrations of silver. With TiO2 and 0.1% Ag, it took at least 7 hours of treatment for complete antibacterial effect though within the first hour itself the effect was observed.

Conclusion: Though TiO2 nanoparticles in pure form have significant antibacterial effect, there is a considerable increase in its antibacterial effect when silver is added as an additive, with higher concentrations of Ag having more effect.

1. Introduction

Staphylococci are gram positive cocci arranged singly or in grape like clusters. Some Staphylococci produce an enzyme called coagulase, based on which they are broadly divided into coagulase positive Staphylococci chiefly consisting of Staphylococcus aureus and coagulase negative Staphylococci. There are nearly 40 species of Staphylococci which are pathogenic to humans and animals such as S. aureus, S. saprophyticus, S. epidermidis, S. intermedius, S. equorum to name a few.[1]

Of the different species of Staphylococcus, Staph. aureus is known to be a pathogenic organism associated with many infections. It is produces coagulase which is one of the virulent characters of Staph aureus. The other staphylococci do not produce coagulase and are mainly non pathogenic. Coagulase negative staphylococci (CONS) such as staphylococcus epidermidis and Staphylococcus saprophyticus are present as a normal flora of the skin and mucous membrane, but of late are known to cause many nosocomial infections.[2–4]

Staph aureus is found to be a regular inhabitant of the skin, nares, and respiratory tract of man.[5] It is one of the most common organisms associated with a wide variety of infections including skin and soft tissue infections like pimples, boils, abscesses, carbuncles, impetigo, folliculitus, urinary tract infections, cellulitis, scalded skin syndrome, endocarditis, toxic shock syndrome, bacteriemia,

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septicaemia, osteomyelitis, meningitis, pneumonia.[6] It has been reported that infected prosthetic inplants and devices are one of the major sources of staph infections.

Uses of indwelling devices is one of the routine procedures in surgeries since the recent past. However, infections to these devices leading to complications is a major object of concern. It has been reported that more than 2 million diseases in the United States of America, which is the cause for morbidity and mortality are due to indwelling devices thus requiring longer hospital days and repeated surgical procedures.[7]

Although antimicrobials are of paramount importance to fight diseases, their regular abuse has lead to the development of multi drug resistant bacteria. Antibiotic resistance has now become a perpetual global problem due to the emergence and reemergence of old and new multiresistant bacteria that there is a constant need of newer drugs to combat this phenomenon.[8] The continued occurrence of drug resistance has given rise to the importance of alternate strategies for treatment of such microorganisms. Since most of the bacterial colonies occur as a surface mediated process, a promising alternative can be with the use of methods which are aimed at modulating the interfacial interactions between the surfaces such as indwelling devices and the host tissues. Of late there is a lot of interest in the field of nanomedicine wherein different types of nanoparticles are used for their antibacterial effect.

Titanium dioxide (TiO_2) has a wide range of application. It has found its use in water cleansing, self cleaning glasses and in air purifiers. It has good mechanical properties and biocompatibility, especially being non toxic to human skin and cost effective. Thereofr they have been found in orthopedic prostheses, orthodontics, joint replacement and so on.[9–11] Silver, since time immemorial has been known for its antimicrobial properties, but its potential had not been utilized till of late, after the reduction of the size to nano level. This resulted in an increased surface area, thereby acting as antimicrobial agents to even drug resistant bacteria.

Much of the work till now has been done on the self-cleaning and water cleaning properties of the nanoparticles, buts their antibacterial effect on clinical samples is yet to be realized. We have therefore performed this study to show the antibacterial effect of Titanium dioxide with and without Silver as addendum on clinical samples.

2. Materials and Methods

This study was done by the Department of Microbiology of Mallareddy Institute of Medical Sciences for 1 and 1/2 years during June 2019 to Feb 2021. Prepared nanoparticles of Titanium dioxide annealed at 200^0C, at 400^0C, TiO_2 coated with 0.1% Ag, TiO_2 with 0.3% Ag, TiO_2 with 0.6% Ag, TiO_2 and with 0.8% Ag as addendum was procured from m/s NanoRam technologies, Bangalore (Fig: 1). 25 Staphylococcus aureus strains from various clinical samples were processed as per regular culture methods and antibiograms were put up as per CLSI protocol. All the samples with Staphylococcus aureus as a single growth was taken into the study. Culture positives with the other organisms were excluded from the study. 5 uncoated plain slides were taken as controls.

All the glass slides coated with the nanoparticles and uncoated plain slides which were taken as controls were covered individual with aluminium foil and sterilized in hot air oven at 160^0C, for 60 mins. The pipettes and tips to be used also were sterilized. 1 colony of Staphylococcus from the culture plate was taken and inoculated into sterile nutrient broth and incubated overnight at 37^0C. This was the working solution, which was adjusted to 0.5 MacFarland's standard the next day. 200ul of the working solution was taken and added to each slide with TiO_2 annealed at 200^0C, TiO_2 annealed at 400^0C, TiO_2 with 0.1% Ag, TiO_2 with 0.3% Ag, TiO_2 with 0.6% Ag, TiO_2 and with 0.8% Ag and uncoated plain slide. From each of these slides, 100ul was taken and added to 900 ul sterile nutrient broth to make a 1:10 dilution. Doubling dilutions from this was done using 100ul of the solution to 900ul of nutrient broth till a dilution of 1: ($1x10^7$) was attained. 100ul from each of these tubes was added to sterile Mueller Hinton plates, streaked. The plate was then coved with a dark paper to give a dark atmosphere and incubated at 37^0C for 18 hours. The slides were placed individually in sterile plates and also incubated at 37^0C. After each hour of incubation for a total of 8 hours, the slides were removed and the doubling dilution procedure was repeated on fresh MHA plates and incubated with dark paper cover at 37^0C.

The next day, the plates were observed for the growth of staphylococcus. The colonies present were counted with a colony counter and the CFU/ml was calculated accourding to the formula:

CFU/ml = dil x no of colonies.

Statistical analysis was done using Anova statistical method on SPSS software and Sigma 13 software.

3. Results

Staphylococcus strains showed a decrease of 2 log after the 4^{th} hour and Total inhibition of the bacterial growth was observed after 8 hours of incubation when treated with pure TiO_2 annealed at 200^0C. When treated with TiO_2 annealed at 400^0C, S. aureus showed a growth of 1.5 x 104 cfu/ml. after 7 hours of treatment, while after 8 hours, there was a total inhibition. However, when treated with TiO_2 doped with 0.1% Ag, there was significant reduction of the bacterial growth in the 1^{st} hour of treatment itself while a complete inhibition of growth was observed after 7 hours of treatment. With TiO_2 doped with 0.3% Ag, a growth of 4 log was observed in the strains after 4 hours of treatment, while total absence of growth was seen in after 4 hours of

treatment. In the case of TiO_2 doped with 0.6% Ag, 1 log growth was seen after 3 hours of treatment itself and absence was seen after 6 hours (Fig: 2). But with TiO_2 doped with 0.8% Ag, a very high antibacterial effect was observed with $1x10^2$ cfu/ml after 1 hour of treatment and no growth after that (Fig: 3).

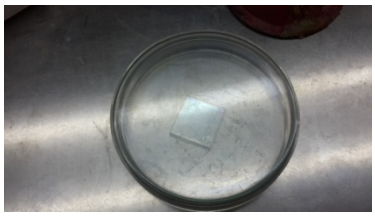

Fig. 1: Glass slide coated with TiO2

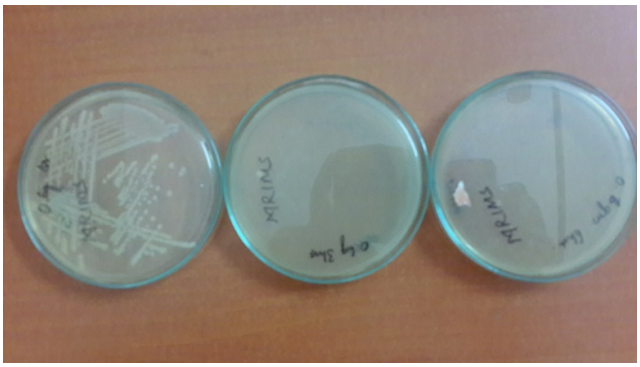

Fig. 2: Staphylococcus treated with TiO_2 doped with 0.6% Ag at base time, after 3 hour and after 6 hours

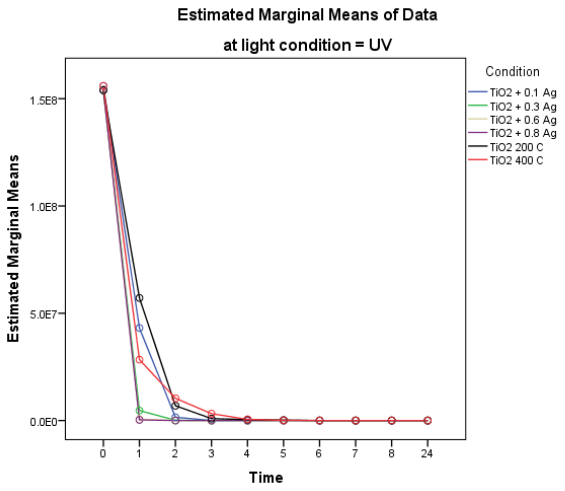

Fig. 3: Staphylococcus aureus treated with various concentrations of pure TiO_2 and TiO_2 doped with Ag

4. Discussion

In the present study, it was observed that when Staphylococcus aureus samples were tested with TiO2 annealed at 200^0C, there was a slight antibacterial, as compared to those treated with TiO2 annealed at 400^0C. However, when the organisms are treated with TiO2 annealed with varied concentrations of silver as additive, the bacterial effect is faster, with the best being TiO2 with 8% Ag as an additive.

In a study by Gupta et al. (2013), it was observed that Staph aureus, when tested with TiO2 doped with 3% an 7% concentrations of Ag, were completely killed. With lesser concentrations of Ag, there was lesser viability loss, showing the antibacterial effect of the nanoparticles. Also, the antibacterial effect of TiO2 in increasing concentrations of Ag showed better antibacterial effect compared to pure annealed TiO2, which was further more effective than crude TiO2. The annealed samples were more effective than the crude TiO2 as after the annealing process at 450 ^{0}C, the amorphous state of the TiO2 is converted to anatase and rutile phase with a band gap of 3.2 eV, which is antibacterial.[12]

Babaei et al (2016) studied the effect of TiO2 on Staph aureus and reported that there was a decrease in the population of S.aureus when they were grown in the presence of TiO2. They also researched the effect of contact time and concentration of TiO2 on the bacteria and observed that by in longer exposure time to UV radiation, there was an increase in the photocatalytic activity of the TiO2 on Staph and the number of CFU/ml thus reduced.[13] Sawai et al., (2003), Hernandez – Sierra et al (2008) and Liu et al (2009), reported the high antibacterial effect of nanpoparticles on Staphylococccus aureus.[14–16] Soderderg et al (1990) have stated that there was a clear antibacterial effect of 179 and 1790 ug/ml zinc oxide.[17]

It was observed that the bactericidal activity of the nanoparticles depended upon the size of the nanoparticle, its stability as well as the concentration of the growth medium. Since the bacterial pore size is larger than that of the nanoparticle, these nanoparticles can easily cross the cell membranes and cause damage.[18]

5. Conclusion

Titanium oxide has a very good antibacterial effect against Staphylococcus aureus in pure form. However, there is a considerable increase in its antibacterial effect when silver is added as an additive, with a higher concentration having more effect.

6. Source of Funding

None.

7. Conflict of Interest

None.

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