Diminution of Internal Bacterial Contamination of External Dental Implants Using Silver Nanoparticles

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Abstract- *The field of nanotechnology is one of the most active research areas in modern biotechnology. Nanoparticles exhibits new or improved properties based on specific characteristics such as size distribution and morphology. There have been impressive developments in the field of nanotechnology in the recent past years, with numerous methodologies developed to synthesize nanoparticles (1-100 nm) of particular shape and size depending on specific requirements. New applications of nanoparticles and nanomaterials are increasing rapidly. The intrinsic properties of metal nanoparticles are mainly determined by size, shape, composition, crystallinity and morphology. Nanoparticles, because of their small size, have distinct properties compared to the bulk form of the same material, thus offering many new developments in the field of biosensors, biomedicine and bionanotechnology. Nanotechnology is also being utilized in the medicine for diagnosis, therapeutic drug delivery and the development of treatments for many diseases and disorders. Since the dental implant/abutment interface cannot totally seal the passage of microorganisms, the interior of implant becomes a reservoir of pathogenic microorganisms that produce and maintain chronic inflammation in the tissues around implants. Silver nanoparticles (nano-Ag) are potent and broad-spectrum antimicrobial agents. The aim to this study was to evaluate the capacity of nano-Ag to prevent the contamination of the implant internal surface by Escherichia coli, Staphylococcus aureus and Salmonella typhi, caused by the implant/abutment microgap infiltration.*

Keywords- Silver Nanoparticles; Implant/Abutment unit; Escherichia coli; Staphylococcus aureus and Salmonella typhi.

I. INTRODUCTION

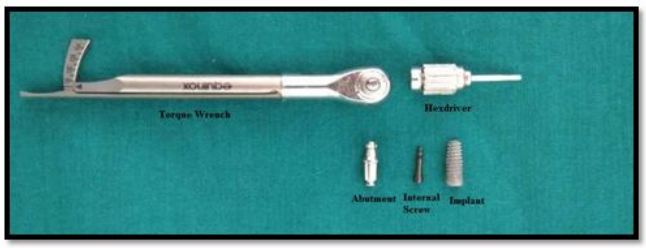
Nanotechnology plays an increasingly crucial role in many key technologies of the new millennium. The application of nanoscale materials and structures, usually ranging from 1 to 100 nm, is an emerging area of nanoscience and nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine and water treatment [1]. Studies have shown that the antibacterial activity of AgNPs is both size and dose dependent and the possible mechanism of action of AgNPs was stated as that AgNPs adhere, penetrate into the bacterial cell wall and thereby inhibiting the regular functions [2]. New technology advances in reducing silver compound chemically to nanoscale sized particles have enabled the integration of this valuable antimicrobial into larger number of materials-including plastics, coatings and foams as well as natural and synthetic fibers. Dental tissue is comprised of mineralized tissues, namely, enamel and dentin, with a soft dental pulp as its core. Enamel is one of the hardest materials found in the body. It is composed of inorganic hydroxyapatite and a small amount of unique noncollagenous proteins, resulting in a composite structure [3]. Due to the limited potential of self-repair, once these dental tissues are damaged due to trauma or bacterial infection, the only treatment option that is available to repair the damage is the use of biocompatible synthetic materials [4]. Most of the synthetic implants are subjected to the hostile microenvironment of the oral cavity and thus have a limited lifespan and functionality. Thus, there is a need to develop biofunctional materials that not only aid in dental restoration but also mimic some of the native tissue functionally. Long term success of implant-based treatment depends on control of mechanical and biological factors [5]. Mechanical factors include the static and dynamic occlusal load on the prosthetic crowns and implants. In turn, biological factors also play a very important role in the short and long term success of a dental implant [6]. It is known that peri-implant infections can produce discomfort to the patient and also accelerate bone loss [7]. The internal colonization of the implant surface by microorganism is eventually inevitable. The interior of implant becomes a reservoir of pathogenic microorganisms, promoting and maintaining a chronic inflammation in tissues around the implants [8]. This condition causes bone loss and may lead to failure of the implant treatment [9].

II. PROCEDURE

In the present study two groups were established: one experimental group with implants that received the application of various AgNPs on the internal surface of the implant before mounting the abutment and a positive control group with implants that received the application of sterile distilled water instead of AgNPs suspension on the internal surface of the implant. In these two groups, the implant/abutment units were immersed in the bacterial suspensions. The bacteria used were *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi.* All abutments and screws were sterilized before use and all the materials were handled in aseptic condition during the study to avoid any external contamination.

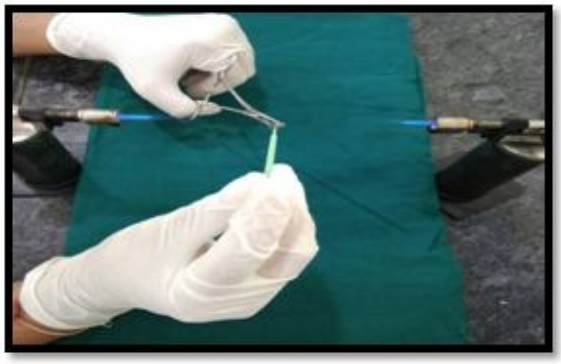
In the experimental group; AgNPs suspension was applied to the implant's inner cavity, where the threads for fixing the abutment screw are located as show in plate-2. As shown in plate-3 and 4, abutment were seated carefully on the implants bodies and screwed with 10N torque using torque wrench. The implant/abutment units were then immersed individually into sterile glass screw cap tubes, containing 5 ml of bacterial suspensions as revealed in plate-5 and plate-6. This volume was enough to cover the implant/abutment interface. In the positive control group sterile distilled water was coated in the implants inner cavity instead of AgNPs suspension. The implant-abutment units were immersed in the bacterial suspensions as in the experimental group. Both the groups were incubated at 37ºC for 24 h. After the incubation, all implant/abutment units were removed from the screw cap tubes (with bacterial suspensions or sterile distilled water). The abutments were carefully removed from the implants using the hexdriver and as shown in plate-7, the surface of the implants inner cavity was sampled with sterile brush to detect the bacterial contamination. The sampling was done on the nutrient agar plates as shown in plate-8, which was incubated for 24 h at 37ºC.

Plate-1: Implant Components



Various implant components that were used during the experimental study, where torque wrench was used to apply the 10 N torque on the implant/abutment unit, hexdriver was used to carefully remove the abutments from the implants and the internal screw is used for fixing the abutment on implant.

Plate-2: Application of AgNPs on the inner cavity of Implant



Using the sterile cotton brush different AgNPs were applied on the internal surface of the implants in positive group and similarly in control group sterile distilled water was applied.

Plate-3: Mounting of the Abutment

The mounting of abutment on the implant surface after application of AgNPs and sterile distilled water.

Plate-2.4: Appying 10 N Torque

After fixing the abutment on the implants, 10N torque is applied on the implant/abutment unit using the torque wrench.

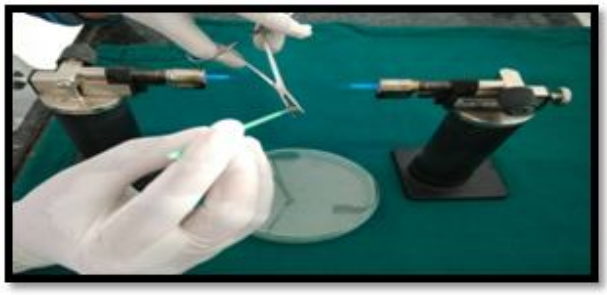
Plate--5: Plunging of Implant/Abutment unit in the bacterial suspension

After proper fixing of implant/abutment unit, it is plunged in the bacterial suspension for 24 h.

Plate-6: Implant/Abutment Unit Immersed in bacterial suspension

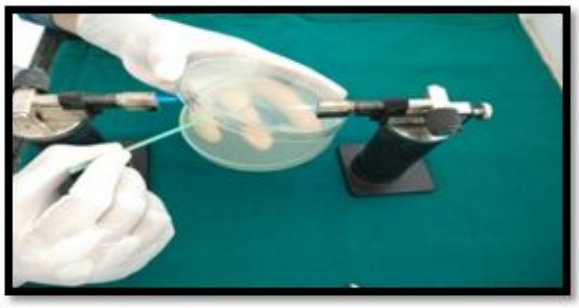
This implant/abutment unit was immersed in the bacterial suspension and incubated for 24 h at 37ºC

Plate-7: Testing for presence of Bacteria



After 24 h the abutment is removed from the implant using the hexdriver and the internal surface of the implant was checked for the presence of bacterial growth.

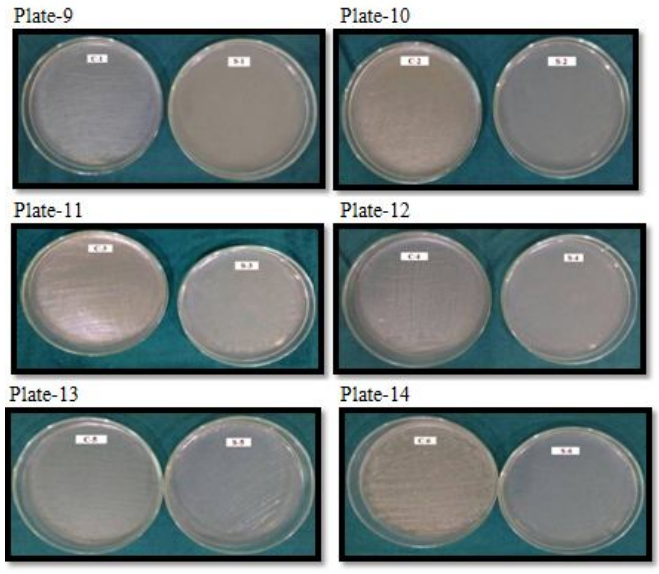
Plate-8: Streaking on Nutrient Agar Plate



The sampling was done of the implant internal surface on the nutrient agar plates to confirm the presence of bacterial growth or whether the bacterial growth was inhibited.

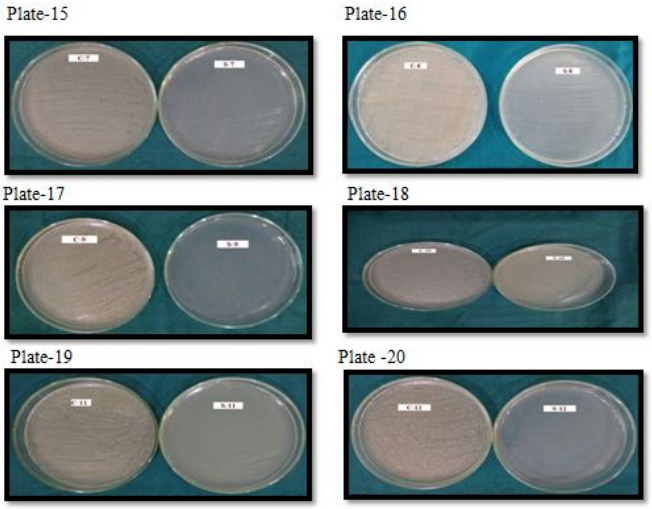
III. RESULT AND DISCUSSION

After incubation period the nutrient plates from experimental group indicated as S-1 to S-18, in plates-9 to plate-26, showed no growth of bacteria and growth was observed in the plates from positive control group, indicated as C-1 to C-18, in plates-9 to plate-26. Absence of bacterial growth in the experimental group signifies that the AgNPs were effective for inhibiting the bacterial growth inside the implants after 24 h in contact with bacterial suspensions.



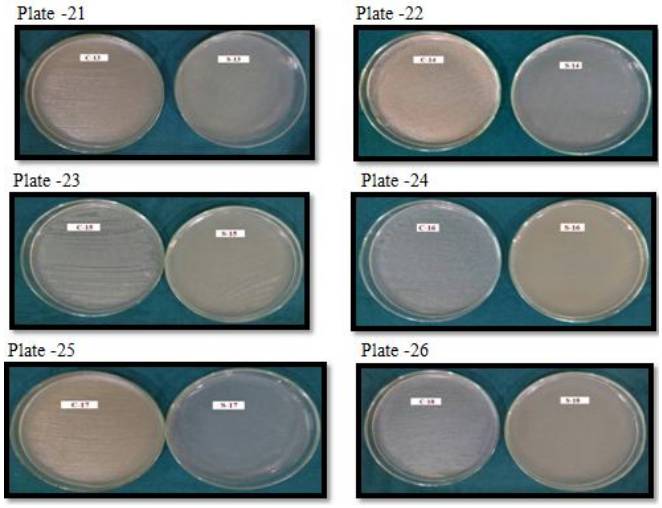
Antibacterial Activity of AgNPs at Internal Surface of Implant/Abutment Unit

Where; C-1 to C-6 are the control plates having bacterial growth (*E. coli*) whereas; S-1 to S-6, are the experimental plates where bacterial growth has been inhibited because of application of AgNPs.



Antibacterial Activity of AgNPs at Internal Surface of Implant/Abutment Unit

Where; C-7 to C-12 are the control plates having bacterial growth (*S. typhi*) whereas; S-7 to S-12 are the experimental plates where no bacterial growth is observed because of application AgNPs



Antibacterial Activity of AgNPs at Internal Surface of Implant/Abutment Unit

Where; C-13 to C-18 are the control plates showing bacterial growth (*S. aureus*) whereas; S-13 to S-18 are the experimental plates having no bacterial growth because of application AgNPs

The nutrient plates from experimental group showed no growth of bacteria and growth was observed in the plates from positive control group. Absence of bacterial growth in the experimental group indicates that the AgNPs were effective for inhibiting the bacterial growth inside the implants after 24 h in contact with the bacterial suspensions. The presence of microorganisms inside implants can be reservoir of peri-implant pathogens that may contact bone around the implant via the implant abutment interface [8]. This exposure of pathogens results in different types of inflammatory reactions in peri-implant soft tissues and can ultimately lead to bone loss and implant failure [10]. The presence of microorganisms in the implant/abutment microgap may not cause significant bone loss that would greatly compromise the rehabilitation in a short term [11]. However, it will be significant in an aesthetic location, as even minimal bone loss will affect the quantity and quality of bone surrounding implants and consequently, compromise the shape and contour of the overlying soft tissues [12]. This study attempts bringing evidence that silver nanoparticles can be an effective and convenient alternative in reducing the microorganism contamination at the implant/abutment interface, regardless of the design of the implant/abutment connection. The results showed that the silver nanoparticles suspension was effective for reducing the number of *E. coli, S. aureus and S. typhi,* cells inside the implants with external hexagonal connection after being placed in an environment contaminated with *E. coli, S. aureus and S. typhi,* after 24 h of incubation. Some possible mechanisms of action may be involved in the antimicrobial activity of silver nanoparticles. Nano-Ag can be interacting with components of the cell membrane of microorganisms, causing structural damage forcing the dissipation of protons and promoting cell death [13]. Nano-Ag has illustrated the ability to collapse the plasma membrane potential, causing a depletion of the levels of intracellular ATP [13]. Silver ions (Ag^+), release from the silver nanoparticles, can diffuse phosphorus of the DNA molecules resulting in the inactivation of replication and these ions can also react with sulphur-containing proteins, leading to the inhibition of enzyme function [14]. Silver is also known to inhibit a number of oxidative enzymes such as yeast alcohol dehydrogenase, affecting the bacterial cell survival [14]. It must be pointed out that the present *in vitro* test showed reduced contamination with *E. coli, S. aureus and S. typhi* in the presence of AgNPs, even though the implant/abutment units were not under any occlusal load. Higher bacterial contamination would be expected if the implants were under masticatory forces. Thus, mechanical cycling test must be performed to test the efficiency of this AgNPs in reducing or avoiding the contamination of the implant inner cavity by microorganisms when the implants are under occlusal loads. Within the limits of this study, it can be concluded that the application of AgNPs in the inner cavity of implants with the external hexagonal connections reduces the colonization of the test organisms on its internal surfaces, even when the abutment is screwed with a low torque.

IV. CONCLUSION

The use of AgNPs can be an effective supplemental therapy to reduce bone loss around implants caused by microbiological factors. The results and the methodology of the present study create base of knowledge for further studies testing AgNPs against different bacteria associated with peri-implantitis. More *in vitro* and *in vivo* studies are required to prove that AgNPs can produce beneficial results in implant treatment.

V. ACKNOWLEDGEMENT

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REFERENCES

[1] D. Mandal, M.E. Bolander, D. Mukhopadhyay, G. Sarkar, and P. Mukherjee. "The use of microorganisms for the formation of metal nanoparticles and their application". Applied microbiology and biotechnology, 69(5), pp.485-492, 2006.

[2] S. Shrivastava, T. Bera, A. Roy, G. Singh, P. Ramachandrarao, and D. Dash. "Characterization of enhanced antibacterial effects of novel silver nanoparticles". Nanotechnology, 18(22), pp.225103, 2007.

[3] A. H. Yen, and P. C. Yelick. "Dental tissue regeneration–a mini-review". Gerontology, 57(1), pp.85-94, 2011.

[4] B. D. Ratner. "Replacing and renewing: synthetic materials, biomimetics, and tissue engineering in implant dentistry". Journal of dental education, 65(12), pp.1340-1347, 2001.

[5] J. Qian, A. Wennerberg, and T. Albrektsson. "Reasons for marginal bone loss around oral implants". Clinical implant dentistry and related research, 14(6), pp.792-807, 2012.

[6] S.P Passos, L. Gressler May, R. Faria, M. Özcan, and M. A. Bottino. "Implant–abutment gap versus microbial colonization: Clinical significance based on a literature review". Journal of Biomedical Materials Research Part B: Applied Biomaterials, 101(7), pp.1321-1328, 2013.

[7] R. Vázquez Álvarez, M. Pérez Sayáns, P. Gayoso Diz, and A. García García. "Factors affecting peri-implant bone loss: a post-five-year retrospective study". Clin Oral Implants Res. 26: pp1006-1014, 2015.

[8] V. K. Jansen, G. Conrads, and E. J. Richter. "Microbial leakage and marginal fit of the implant-abutment interface". International Journal of Oral & Maxillofacial Implants, 12(4): pp 527-540, 1997.

[9] A. P. Ricomini Filho, F.S.D.F. Fernandes, F.G. Straioto, W.J.D Silva, and A.A. Del Bel Cury. "Preload loss and bacterial penetration on different implant-abutment connection systems". Brazilian dental journal, 21(2), pp.123-129, 2010.

[10] I. Ericsson, L. G. Persson, T. Berglundh, C. P. Marinello, J. Lindhe, and B. Klinge, "Different types of inflammatory reactions in peri-impJ. lant soft tissues". Journal of Clinical Periodontology, 22(3), pp.255-261, 1995.

[11] T. Albrektsson, D. Buser, and L. Sennerby. "Crestal bone loss and oral implants". Clinical implant dentistry and related research, 14(6), pp.783-791, 2012.

[12] M. Tesmer, S. Wallet, T. Koutouzis, and T. Lundgren. "Bacterial colonization of the dental implant fixture–abutment interface: an in vitro study". Journal of Periodontology, 80(12), pp.1991-1997. 2009.

[13] C. N. Lok, C. M. Ho, R. Chen, Q. Y. He, W. Y. Yu, H. Sun, et al. "Proteomic analysis of the mode of antibacterial action of silver nanoparticles". Journal of Proteome Research. 5: 916-924. 2006.

[14] Y. Matsumura, K. Yoshikata, S. I. Kunisaki, and T. Tsuchido. "Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate". Applied and environmental microbiology, 69(7), pp.4278-4281, 2003.