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Research Article

ANTIMICROBIAL EFFICACY OF SILVER NANOPARTICLES AGAINST PERIODONTAL PATHOGENS

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A B S T R A C T

Background: Plaque accumulation on the teeth surfaces and the prosthetic or orthodontic appliances presents a serious challenge for the maintenance of oral health. Silver nanoparticles can be incorporated in the restorative materials to prevent plaque formation and progression of periodontal diseases.
Aim: To evaluate the antimicrobial efficacy of silver nanoparticles by calculating minimum inhibitory and bactericidal concentration of silver nanoparticle.
Materials and method: Antimicrobial efficacy of nanosilver was evaluated against specific periodontal pathogens (P.gingivalis, A.actinomycetemcomitans, P.intermedia). Minimum inhibitory (MIC) and bactericidal concentration (MBC) was determined using broth dilution assay. A commercially available silver nanoparticles gel (0.02mg/gm) was used for the study.
Results: P.gingivalis, A.actinomycetemcomitans were sensitive to silver nanoparticles. However, P.intermedia was found to be resistant. Conclusion: Nanosilver has anti-bacterial effect against P.gingivalis, A.actinomycetemcomitans. Future studies are needed to explore the applicability of these silver based antimicrobial agents in clinical dentistry.

INTRODUCTION

Scientific research has provided overwhelming evidence that many oral infections are attributed to specific bacterial etiology. These bacterial infections are linked with certain host factors, most important is plaque. There is always a tendency for the accumulation of dental plaque on the surfaces of the tooth, restorations, prostheses and orthodontic appliances, surgical dressings and suture materials.

Bacterial plaque further leads to secondary caries under the restoration, gingival inflammation in relation to prosthetic and orthodontic appliances that may progress to periodontitis. Periodontitis is a chronic inflammatory disease, which is characterized by destruction of the tooth supporting apparatus. Nowadays, attention has been directed towards increasing the antimicrobial properties of these restorative and surgical materials. Many researchers have used different approaches to achieve the same. But their goals have been not achieved till the emergence of recent developments in nanotechnology, which provides a promising insight into the management of oral diseases[1].

The overall results of previous studies indicated a relatively beneficial effect for biomaterials containing silver nanoparticles (AgNps) particularly in skin wound healing. Silver has been known to be a disinfectant for several centuries and has been widely used in the treatment of clinical diseases. Silver nanoparticles exhibit a powerful broad antibacterial spectrum effective against wound pathogens such as bacteria, fungi and moulds[2,3] and have anti-inflammatory properties as well[4]. Compared to conventional antibiotics, silver nanoparticles can exert superior activity by better contact with microorganisms, higher interaction with bacterial membranes and DNA, inhibitory effects on the respiratory chain in bacterial mitochondria and genesis of free radicals, enhancing their bactericidal activity [5].

AgNps can be easily mixed into textiles, films and molded plastic products and has found usage in medical environments, medical devices, plastics, fabrics, paints, and polymeric food contact material approved by the U.S. Food and Drug Administration. In addition, it has high physical and chemical stability along with superior discoloration resistance during processing or use. In light of the advantages and effects of nanosilver as reported by previous studies, the ***aim*** of the present *in vitro* study was to evaluate the antimicrobial efficacy, for silver nanoparticles against the periodontal pathogens, *P.gingivalis, A.actenomycetemcomitens and P.intermedia.*

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MATERIALS AND METHODS

For the present study, a commercially available nanocrystalline silver gel (0.02mg/gm) was used. The antibacterial efficacy of silver nanoparticles was tested against the periodontal pathogenic strains of Porphyromonas gingivalis (ATCC No: 33277), Aggregatibacter actinomycetemcomitans (ATCC No: 43718) and Prevotella intermedia (ATCC No: 25611). MIC and MBC tests to determine antibacterial efficacy were performed at Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum, Karnataka.

Determination of MIC and MBC

The double dilution method was used for the determination of antibacterial activity. Nutrient broth, brain heart infusion broth (BHI broth, HIMEDIA M210-500G) was prepared & added in test tubes. Stock solutions of silver nanoparticles were prepared in DMSO at concentration of 200µg/ml followed by two fold dilution at concentrations of (100, 50, 25….3.125µg/ml). The inoculums were added to the other wells with test antimicrobial compounds ranging from 100, 50, 25….1.6, 0.8, 0.4, 0.2µg/ml. The test tubes were then incubated at 37°C for 48 hrs and minimal inhibitory concentration were measured for the growth in the form of turbidity. The methodology also included positive control i.e. tubes containing inoculum and nutrient media, devoid of nanoparticles.

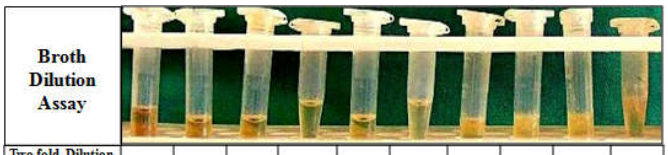
To determine MBC, from MIC dilution tubes of sensitive organisms, first 5 tubes were plated. Plates were incubated for 24 hrs, next day the colony count was taken.

Table 1 MBC results

Concentration of AgNps	100	50	25	12.5	6.25	3.12
P.gingivalis	+	+	+	+	+	+
A.actinomycetemcomitans	-	-	-	-	+	+

RESULTS

After 24 hrs of incubation, all the test tubes containing inoculums of *P. gingivalis, A.actinomycetemcomitans* and *P. intermedia* were observed for turbidity. Appearance of turbidity indicates bacterial growth. Turbidity was present in control group also, suggestive of microbial growth. *P.gingivalis* was sensitive for silver nanoparticles upto 3.125µg/ml dilution (Fig 1), beyond which turbidity was present, indicating the microbial growth.



Two fold Dilution of nanosilver (µg/ml)	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
Sensitivity of *P.gingivalis*	S	S	S	S	S	S	R	R	R	R

Figure 1 Minimum Inhibitory Concentration of nanosilver gel against *P.gingivalis* by broth dilution assay.

Similarly, *A.actinomycetemcomitans* was sensitive upto 6.25µg/ml dilution (Fig 2). MIC of nanosilver for *P.gingivalis* was 3.125µg/ml and 6.25µg/ml for that of *A.actinomycetemcomitans*. *P.intermedia* was found to be resistant as turbidity was present in all the test tubes because of microbial growth (Fig 3).

Two fold Dilution of nanosilver (µg/ml)	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
Sensitivity of *A.actinomycetem comitans*	S	S	S	S	S	R	R	R	R	R

Figure 2 Minimum Inhibitory Concentration of nanosilver gel against *A.actinomycetem comitans* by broth dilution assay

Two fold Dilution of nanosilver (µg/ml)	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
Sensitivity of *P. intermedia*	R	R	R	R	R	R	R	R	R	R

Figure 3 Minimum Inhibitory Concentration of nanosilver gel against *P. intermedia* by broth dilution assay.

MBC was done to see whether there was bacteriostatic or bactericidal effect of the silver nanoparticles at the MIC against the organism. If there is no growth indicates that it is bactericidal effect and it is bacteriostatic effect if growth is present. Thus, nanosilver found to be bacteriostatic for *P.gingivalis* as few colonies were present even at 100µg/ml concentration. AgNps are bactericidal for *A.actinomycetemcomitans* till 12.5µg/ml and bacteriostatic at MIC as growth is present at 6.25µg/ml.

DISCUSSION

The reliance on antibiotics to fight infections is now seen as a short term solution in light of the rise and spread of bacteria cells not affected by such drugs. Antibiotic resistance is a major danger and the development of novel strategies to prevent/treat infections is urgently needed[6,7] because new antibiotics are rarely discovered (the last new antibiotic class dates back to the 1980s) and it is widely accepted that it is only a matter of time before resistance is developed towards a new antibiotic. Moreover, it is important to prepare antibacterial drugs that are effective against already resistant cells not only unable to induce further resistance.

An efficacy of silver nanoparticles is primarily related to the fact that they attack more than one site in the bacteria, hence reducing the chance of developing bacterial resistance[8]. This property of Nanosilver particles has been exploited in medicine, dental materials, water treatment and cosmetics like sunscreen lotions.

Silver is a metal known for its broad spectrum antimicrobial property including antibiotic resistant strains[8]. In the microbiological field, it possesses attractive properties. In particular metallic nanoparticles are the most promising because they show good antibacterial properties due to their large surface area to volume ratio[9]. Silver nanoparticles are particles of silver ranging between 1 nm and 100 nm in size[5,10]. A crucial issue concerning silver nanoparticles is their tendency to form aggregates, losing their peculiar (antimicrobial) properties associated with the nanoscale. Polyelectrolytes in small concentration, have been used to stabilize the nanoparticles to prevent the aggregates formation[11,12].

Nanosilver attaches and anchors to the cell surface of the microbes, causing structural damage and markedly disturbing vital cell functions such as permeability, causing pits and gaps, depressing the activity of respiratory chain enzymes and leading to cell death. It inhibits the multiplication of micro-organisms and decreases the activity of matrix metalloproteinases thus decreasing inflammation associated with the wound[2,3].

The antimicrobial properties of silver nanoparticles are related to its oxidised form, silver ions. Thus, polymers that release silver ions in the oxidised form seem to have a strong antimicrobial activity and act as a reservoir of silver ions, releasing silver for the extended period of time[13,14]. Silver ions released from silver nitrate have been proven to be effective against Gram negative periodontal pathogens and Gram-positive streptococci[15]. Because silver therapy is of significant clinical benefit in the control of microbial infections, various forms of medical, biological and pharmaceutical preparations containing silver ions have been developed[16].

Goda Holla *et al*[17] 2012, showed antimicrobial efficacy of nanosilver gel (Novaron) against S.mutans, in an *in-vitro* study by a broth dilution assay. Santoshi Rani *et al*[18] 2015, studied efficacy of AgNps against periodontal pathogens in preventing guided tissue regeneration membrane colonization where nanosilver showed a comparable antimicrobial activity to doxycycline. Studies till date have reported the inhibitory properties of silver nanoparticles against human immunodeficiency virus-1 (HIV-1)[19]; *Staphylococcus aureus*[20] and *Escherichia coli*[21]. Likewise, M. Isabel González-Sánchez *et al*[8] showed the efficacy of AgNps against methicillin-resistant staphylococcus auareus (MRSA). They even cultured osteoblasts with nanosilver to evaluate in vitro cytotoxicity of the same. The results revealed that the presence of nanoparticles did not have a detrimental effect on the growth of osteoblast cells regardless of the cross-linking concentration and amount of nanoparticles used during the adsorption process. Such results were expected as generally silver nanoparticles do not exhibit cytotoxic properties.

Hence, further scope for research in prevention and treatment of periodontitis can be explored using the results of this study.

Limitations

Oral environment contains not only bacteria, but even viruses, fungi, yeasts, etc. Hence, efficacy of AgNps against this flora needs to be tested. This study had small sample size and hence the results cannot be generalized. Moreover, in vivo studies are required to validate the results of this in vitro study.

CONCLUSION

Silver nanoparticles have proved to have a inhibitory effect on the growth of *P.gingivalis* and *A.actinomycetemcomitans* in-vitro. Application of AgNps can be done as local drug delivery, can be incorporated in allograft or a local application for raw wound surfaces as a beneficial and preventive treatment option for periodontal diseases.

Future Prospective

Further studies are needed to evaluate the feasibility of incorporation of these particles in different restorative materials, their effect on physical properties and their clinical applicability.

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