The antibacterial and antifungal effect of Silver Nanoparticles and silver hydroxyapatite nanoparticles on Dental Ceramic

Cherif A Mohsen*, Manal R. Abu-Eittah**, Raiesa M M Hashem***

* Professor & Chairman, Crown & Bridge Department, Faculty of Dentistry, Minia University.
**. Associate Professor, Crown and Bridge Department, Faculty of Dentistry, Minia University.
***Assistant lecturer, Crown & Bridge Department, Faculty of Dentistry Minia University, B.D.S, M.Sc., Minia University

Abstract: Aim of the study: investigation of the antibacterial and antifungal effect of silver nanoparticles and silver hydroxyapatite nanoparticles on Dental Ceramic. **Materials and Methods:** Silver nanoparticle and silver hydroxyapatite nanoparticles of 40ug/ml concentration was added to dental porcelain in a ratio 1:100 and manipulated. A total of 45 samples were constructed and classified into three groups (15 samples for each). Group 1(Control group containing samples without any modification), Group II (silver nanoparticles modified porcelain samples) and Group III (silver hydroxyapatite nanoparticles modified porcelain samples). Samples were inserted in the Petri dishes against each type of the microorganism, (Escherichia coli (gram –ve bacteria), staphylococcus aureus (gram +ve bacteria) and candida albicans (yeast)) 5 samples for each. The colonies forming units, CFU were counted after 7 days. Results were tabulated and statistically analyzed. **Results:** group II and III exhibited antimicrobial effect toward gram-negative (E. coli), gram-positive (S. aureus) as well as to the yeast (C. albicans). Where the microbial percentage inhibition in group II was (68.5 ± 9.5 for staph. aureus, 81.0 ± 3.7 for E. coli, 70.9 ± 9.3 for C. albicans) and that for group III was (68.4 ± 5.8 for Staph. aureus, 79.4 ± 3.8 for E. coli, 70.1 ± 6.0 for C. albicans). The effects of the tested groups (II and III) were more pronounced on E-coli than staphylococcus aureus. **Conclusion**: The addition of silver nanoparticles and silver hydroxyapatite nanoparticles to porcelain was proved to be a very effective method in inhibition of bacterial growth via decreasing the colonizing activity, and reduced much the size of the very few, formed colonies. The antibacterial effects of both tested materials were more pronounced on gram –ve than gram +ve bacteria.

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Introduction

Ceramic materials have been used in dentistry for well over 200 years. They are the most biocompatible dental restorative materials due to their chemical stability. Leakage at the crown margins mainly means the lack of adhesion or failure of seal at the tooth-restoration interface, which might lead to dental caries, pulp sensitivity, necrosis, periodontal disease, esthetic problems and compromise the restoration's longevity. Therefore, there is a great need to improve the longevity of restorations by incorporating bioactive agents to combat microbial destruction and recurrent caries while sustaining the load-bearing capability. [1, 2]

The development of materials containing substances with antimicrobial activity has been applied in a variety of biomedical applications. Silver or silver ions have long been known to exhibit powerful antimicrobial activity and strong bactericidal effects against as many as 16 species of bacteria. For this reason, silver-based compounds have been used extensively in many bactericidal applications as water purification, wound care, bone prostheses, reconstructive orthopedic surgery, cardiac devices, catheters and surgical appliances. Silver has been used in ionized and elementary forms, as nanoparticles. [3)], [4]

Silver nanoparticles have been proved to be most effective as they have good antimicrobial efficacy against bacteria, viruses, and other micro-organisms. As a result of their small size, nanoparticles may offer advantages to the biomedical field through improved biocompatibility. Additionally, it appears that bacteria are far less likely to acquire resistance against metal nanoparticles than other conventional and narrow spectrum antibiotics. This is thought to occur because metals may act on abroad range of microbial targets, and many mutations should occur for the microorganisms to resist their antimicrobial activity. [5]

In dentistry, both inherent properties of restorative materials and oral bacteria are believed to be responsible for restoration failure. Secondary caries that often occurs at the interface between the restoration and the cavity preparation is primarily caused by demineralization of tooth structure due to invasion of plaque bacteria (acid producing bacteria) in the presence of fermentable carbohydrates. [6]

Marginal discrepancies and rough or defective cement surfaces have been proven to be responsible for plaque accumulation, which is the primary causative factor in the etiology of periodontal disease and caries. Leakage at the crown margins mainly means the lack of adhesion or failure of seal at the tooth-restoration interface, which might be the pathway to the pulp chamber. Therefore, marginal discrepancies and leakage might lead to periodontal disease, Secondary dental caries, pulp sensitivity and necrosis, and esthetic problems such as staining or marginal discoloration, and they might ultimately result in failure of restorations. [7]

Among the different techniques to discourage initial bacterial attachment to exposed hard material surfaces, the anti-bio adhesion coatings, antibiotic releasing coating and silver incorporation. Silver incorporation has attracted much attention since several forms of silver have been demonstrated to inhibit the growth of a broad spectrum of microorganisms. Although the antimicrobial properties of silver have been known for centuries, we have only recently begun to understand the mechanisms by which silver inhibits bacterial growth. [8, 9, 10, 11]

Spacciapoli et al., in (2001) [12] demonstrated the use of silver nitrate for the treatment of periodontal pathogens. They found that Silver nitrate more efficient than antibiotics for the treatment of oral cavity of periodontal infections. Furno et al., in (2004) [13] have used a variety of methods to test the efficacy of silver nanoparticles impregnated on silicon elastomer. However, it was found that the antibacterial efficiency of silver nanoparticles reduces after washing. Silver nanoparticles can be used for the impregnation of medical devices and lead to promising antimicrobial activity.

Ahn et al., in (2009) [14] compared the experimental composite adhesives (ECAs) containing silica nanofillers and silver nanoparticles with two conventional adhesives (composite and resin-modified glass ionomer [RMGI]) to analyze surface characteristics, physical properties and antibacterial activities against cariogenic streptococci. They found that ECAs can help in preventing enamel demineralization around their surfaces without compromising physical properties. Burqers R et al., in (2009) [15] evaluate the antibacterial activity of a resin composite material loaded with silver microparticles against Streptococcus mutans. They found that the addition of micro particulate silver to a resin composite material increased the surface hydrophobicity and reduced the number of adhering streptococci. Simultaneously it increased the percentage of dead and inactive cells on the composite surface. Thus, silver additives seem to demonstrate anti-adherence activity as well as a bactericidal effect.\ Liao et al., in (2010) [16] found that silver nanoparticle-modified titanium is a promising material with an antibacterial property that may be used as an implantable biomaterial.

Kaiiin et al., in (2011) [17] studied the incorporation of silver nanoparticles into yttria-stabilized zirconia (YSZ) ceramic for eliminating microorganism adhesion on dental restoration graft. Inductively coupled plasma-mass spectrometry (ICP-MS) revealed that YSZ block containing 0.0047 wt% nanosilver, which is safe to mammalian cell, can inhibit the growth of Escherichia coli, Streptococcus mitis and Candida albicans. The pristine YSZ disc had no effect on bacterial growth. This study suggests that silver nanoparticles incorporated into YSZ blocks possess a broad spectrum antimicrobial effect and may help prevent biofilm formation on their surfaces to improve implant survival rate. **Abu-Eittah. M R., in (2013)** [18] Investigated the anti-bacterial effect of acrylic - based provisional fixed prosthodontics material modified with silver nanoparticles against aerobic gram-ve (E-coli), and anaerobic gram +ve (Entero coccus faecalis) bacteria. The result showed an inverse proportion between the concentrations of added AgNps and the CFU count in both studied microorganisms. The effect of studied concentrations were to some extent, more effective against E- coli than Entero coccus faecalis $p<0.05$.

Shahin Kasraei et al., in (2014) [19] evaluated the antibacterial properties of composite resins containing 1% silver and zinc-oxide nanoparticles on Streptococcus mutans and Lactobacillus. They found that composites containing nano zinc-oxide particles or silver nanoparticles exhibited higher antibacterial activity against Streptococcus mutans and Lactobacillus compared to the control group ($p < 0.05$). There were no significant differences in the antibacterial activity against Lactobacillus between composites containing silver nanoparticles and those containing zinc-oxide nanoparticles.

Therefore the aim of this study was to evaluate the effectiveness of incorporation of silver nanoparticles into dental ceramic and testing its antibacterial effect against Escherichia coli (E-coli, gram –ve species), staphylococcus aureus (gram +ve bacteria) and candida albicans (yeast).

Materials and methods

1-Preparation of control samples (group I)

A split tephlon disc of 5 mm diameter and 2 mm thickness was used as a mold for the samples. The porcelain powder was mixed with porcelain molding liquid to form a past. A moistened brush was used to apply each porcelain layer in small increments until it reached its step level in the mold. The samples were dried by heating slowly in the open entrance of the furnace. This is carried out in order to drive off excess

water before it has a chance to form steam. Once the compact has been dried. The porcelain samples were fired in a porcelain furnace,Firing protocol (drying for 5 minutes, rate of temperature increase: 50°C/minute, vacuum value: 730 mmHg, firing initiation temperature: 680°C, firing finishing temperature: 930°C for 30 minutes then rapid cooling was done in porcelain furnace).

2-Preparation of modified porcelain samples (group II and III).

The Silver nanoparticles powder and silver Hydroxyapatite nanoparticles powder was weighed with an electronic sensitive balance and added to the powder of the dental porcelain in a ratio 1:100. And manipulated as previously explained in control samples.

3- Bacterial Adhesion test

A-Determination of minimum inhibitory concentration (MIC).

Five ml of silver nanoparticles of concentration 300 ug/ml was purchased from Nano tech, Egypt. They were diluted by distilled water to obtain the different concentrations used in determination of minimum inhibitory concentration (30, 40, 50, 75ug/ml). Thirty ug/ml Ag NPs (powder form) was used also in determination of MIC. A modified Kirby-Bauer disc diffusion method was used [(20)]. The MICs of Ag NPs was determined as the lowest concentrations that inhibited visible growth of the microbial strain.

B- Anti-Microbial test

One hundred µl of the tested microbial strain were grown in 10 ml of fresh media until they reached a count of approximately 105 cells/ ml. one hundred µl of the microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained using sterile cotton-tipped applicator sticks. Mueller-Hinton agar was used as growth medium as it results in good batch-to-batch reproducibility. Robertson cooked meat, 2% peptone, 3 % water, 2% yeast extract, 1% dextrose, and agar was used for gelling the components of the culture. The media was autoclaved before inoculation of the microorganisms. The plates were incubated at 37^0 and left undisturbed to grow for 48 hours.

For Escherichia coli, as these micro-organisms are anaerobes, reducing agents were added to the culture medium. A proper titer of 1% glucose, 0.1 thio-glycolate, 0.1% ascorbic acid, and 0.05% cysteine were added to the culture of Escherichia coli. The samples of control group and tested groups (group II and III) were placed in center of the plates. Plates were incubated at 37˚C for 7 days. Colonies forming units (CFU) which reflect bacterial cells viability and activity were counted. The antibacterial effect in each group was calculated as a percentage inhibition which calculated as follows:

Percentage inhibition of bacteria (%) = total CFU of control group.–total CFU of Ag modified samples in each experimental group. /CFU of control group. X 100. [(22)]

Results

The clear zone (inhibition zone) related to different concentrations of Ag nanoparticles in different studied microbial species was observed at concentration 40ug/ml and this concentration considered the minimum inhibitory concentration (MIC).

Pair-wise comparisons between the groups revealed that control group showed the statistically significantly highest mean count. There was no statistically significant difference between group II and group III; both showed the statistically significantly lowest mean count.

By comparison (CFU) in control group and tested group we can see that there was decrease in the size as well as the number of CFU count in tested group than in control group, and the tested group showed a distinct hallow surrounding the porcelain sample. Which means no microbial colonization around and over the porcelain sample as shown in figure (2).

Table (1): Mean, standard deviation and p-value of (CFU) count of the studied microbial species in different groups.

Micro-organism type	(CFU) in control group		(CFU) in (group II)		(CFU) in (group III)		
	Mean	SD	Mean	SD	Mean	SD	P-value
Staph. *aureus*	280.0^a	57.0	84.0^b	8.9	86.0^b	6.5	<0.001*
E. *coli*	260.0^a	65.2	48.0^b	8.4	52.0^b	8.4	<0.001*
C. *albicans*	320.0^a	75.8	88.0^b	8.4	92.0^b	8.4	<0.001*

**: Significant at P ≤ 0.05, Different superscripts are statistically significantly*

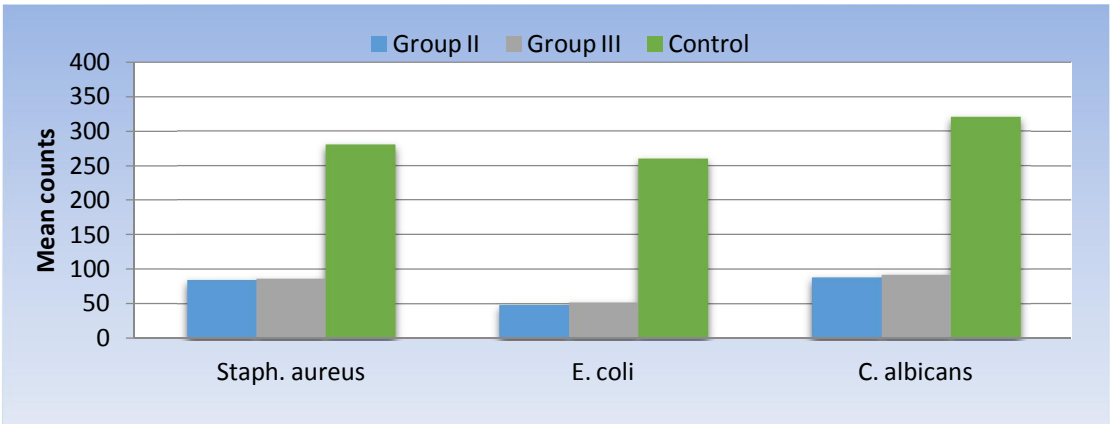

Figure (1): Mean (CFU) count of the studied microbial species in different groups.

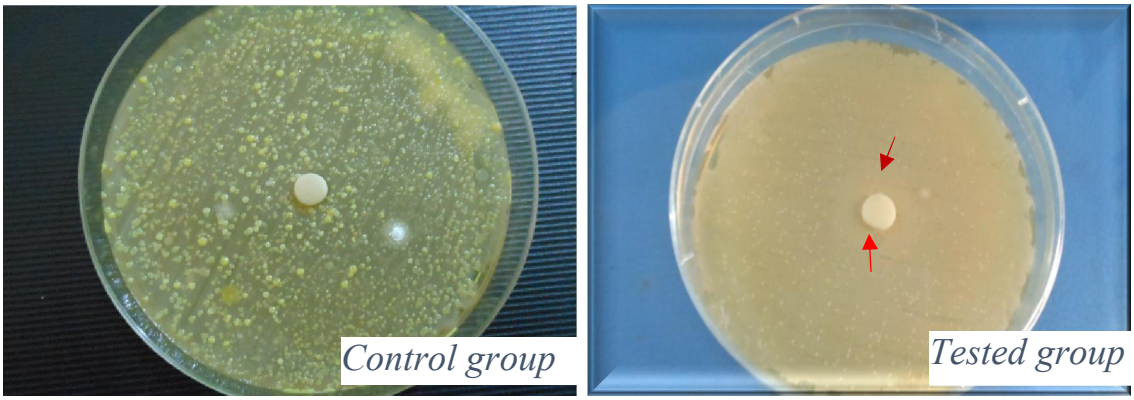

Figure (2): CFU after 7 days in control group and tested group at microbial culture.

Discussion

One of the major objectives of tooth restoration is the protection of exposed dentine against bacteria and their toxins. The interface between the restoration and dental hard tissue is an area of clinical concern as insufficient sealing can result in marginal discoloration, secondary caries, and pulpitis. [23, 24]

Numerous efforts have been made on improving antibacterial activities of dental restoration; most of them have been focused on release or slow-release of various incorporated low molecular weight antibacterial agents such as antibiotics, zinc ions, silver ions, iodine and chlorhexidine. [25]

The antimicrobial mechanism of silver nanoparticles is related to the formation of free radicals and subsequent free radical induced membrane damage. These free radicals may be derived from the surface of silver nanoparticles and responsible for the antimicrobial activity. Silver nanoparticles lead to the formation of "pits" in cell wall of the bacteria, and silver nanoparticles could enter into the periplasm through the pits and destroyed the cell membrane, then the silver nanoparticles could enter into the bacterial cell, which not only condensed DNA, but also combined and coagulated with the cytoplasm of damaged bacteria. In addition, silver nanoparticles resulted in the leakage of cytoplasmic component. Moreover, the silver nanoparticles could increase the decomposability of genome DNA. Silver has an important antimicrobial effect which is depended on superficial contact in these silver and can inhibit enzymatic system of the respiratory chain and later DNA synthesis.[9,10,11,26]

Many authors reported that gram –ve bacteria is more susceptible to the effect of silver nanoparticles than gram +ve bacteria. It was postulated that due to the thicker cell wall of gram-positive bacteria which have more peptidoglycan layer (~30nm) than gram-negative bacteria (~2-3nm with no cell membrane). In addition, peptidoglycan is negatively charged and silver ions are positively charged, more silver may get trapped by peptidoglycan in gram-positive bacteria than in gram-negative bacteria so more silver may get trapped and prevented from exerting the toxic effect on the interior of the bacterial cell. [27, 28, 29]

The results were in accordance with the results obtained by Liao Juan et al [16]. They studied silver nanoparticle-modified titanium (Ti-nAg) surface. Staphylococcus aureus and E- coli, were utilized to test the antibacterial effect of the Ti-nAg treated

surface. The results of their study revealed 94% of staphylococcus aureus and over 95% of Escherichia coli had been killed on the Ti-nAg surface, and the SEM examination of anti-adhesive efficacy test showed that there were less bacteria attached to TinAg surface than to a control surface of untreated Titanium. The inhibition of bacterial count was due to contact of bacterial cells with treated Ti nAg.

Conclusions

Under the limitations of this study, several conclusions could be detected:

1- The addition of silver nanoparticles and silver hydroxyapatite nanoparticles to porcelain was proved to be a very effective method in inhibition of bacterial growth via decreasing the colonizing activity, and reduced much the size of the very few, formed colonies.

2- The antibacterial effects of both tested materials were more pronounced on gram –ve than gram +ve bacteria.

3- No bacterial adhesion was detected on the samples in all types of microorganisms.

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