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Research Article

Nanosilver Application in Dental Cements

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Streptococcus mutans is the microorganism mostly responsible for initiation of tooth decay and also for the progression of an established lesion. Silver has been used for its antibacterial properties for many years, in different forms: ionised and elementary forms, as silver zeolites or as nanoparticles. The purpose of this study was to evaluate the antibacterial activity of three dental cements modified by nanosilver. Three cements were used: Sealapex, RelyX ARC, and Vitrebond. The cements were incorporated with 0.05 mL of silver nanoparticles solution. Control groups were prepared without silver. Six Petri plates with BHI were inoculated with *S. mutans* using sterile swabs. Three cavities were made in each agar plate (total = 18) and filled with the manipulated cements. They were incubated at 37°C for 48 h, and the inhibition halos were measured. The paired *t*-Test was used for statistical analysis ($P < 0.05$). No inhibition halos were obtained for Sealapex and Rely X, but Vitrebond showed bactericidal activity without silver and enhanced effect with silver incorporation.

1. Introduction

The initial adhesion of specific oral bacteria to tooth surfaces or artificial dental substrata is both the primary and the essential prerequisite for the development of cariopathogenic biofilms [1–4]. Within the complex formation of such biofilms, *Streptococcus mutans* is primarily responsible for the initiation of tooth decay as well as for the progression of an established lesion [5]. Although the prevalence of primary caries has been on decline worldwide since early 1980s, secondary caries remains an unresolved problem in restorative dentistry [6]. Ideally, the restorative materials should exhibit antibacterial properties to limit the adhesion and proliferation of pathogens at a very early stage and, therefore, prevent secondary caries [5].

In Dentistry there is a wide range of cements with different applications where the antimicrobial activity is relevant. Antibacterial activity of dental luting cements is a very important property when applying dental crowns, bridges, inlays, onlays, or veneers, because bacteria may be still present on the walls of the preparation or gain access to the cavity if there is microleakage present after cementation [7]. In endodontic infections, it is impossible to completely eliminate the microorganisms from the root canal system in all cases [2, 8]. Consequently, the use of root canal filling materials with antibacterial activity is considered beneficial in the effort to further reduce the number of remaining microorganisms and to eradicate the infection [8, 9]. Though the endodontic infection has predominance of anaerobic bacteria it is of polymicrobial nature and the antimicrobial spectrum of action of these materials should be investigated [9]. Among all the dental restorative materials, glass-ionomer cements (GICs) are found to be the most cariostatic and somehow antibacterial due to release of fluoride, suppressing caries formation [6, 10]. Although numerous efforts have been made on improving antibacterial activities of dental restoratives, most of them have been focused on slow release of various incorporated low-molecular-weight

Table 1: Description of the experimental groups.

Groups	Quantity of silver (in volume)	Quantity of silver (in volume)
(1) Endodontic cement (Sealapex)	0.05 mL	0 (control)
(2) Resin luting cement (Rely X ARC)	0.05 mL	0 (control)
(3) Glass ionomer cement (Vitrebond)	0.05 mL	0 (control)

antibacterial agents such as antibiotics, zinc ions, silver ions, iodine and chlorhexidine [6, 10, 11].

Silver has been used for its bactericidal properties for many years [5, 12] It has been used in water purification, wound care, bone prostheses, reconstructive orthopedic surgery, cardiac devices, catheters and, surgical appliances [3, 12–21]. The antimicrobial, antifungal and antiviral action of silver or silver compounds is proportional to the amount of released bioactive silver ions (Ag^+) and its availability to interact with bacterial or fungal cell membranes [13, 22]. Silver has been used in ionised and elementary forms, as silver zeolites or as nanoparticles [3]. Nanoparticles are insoluble particles that are smaller than 100 nm in size [19]. Their advantage is on the smaller-sized particles that show stronger antibacterial activity due to their higher surface area to volume ratio [20].

In dentistry, some studies of the antibacterial effect of dental materials incorporating silver were made [5, 11, 23–25]. Yoshida et al. [23] showed that a resin composite incorporated with silver-containing materials had a long term inhibitory effect against *S. mutans.* Incorporating silver zeolite in endodontic filling materials increases the material bactericidal effect against some microorganisms [24, 25]. Composite resins modified by microparticulated silver revealed antiadherence activity and bactericidal effect against *S. mutans* [5]. Orthodontic adhesives with nanosilver particles had their antibacterial capacity raised without compromising physical properties [11].

Due to the few studies regarding the bactericidal effect of nanosilver particles and dental materials, the purpose of this study was to evaluate the antibacterial activity of three dental cements modified by nanosilver particles.

2. Material and Methods

Three cements were used (Table 1): Group 1 (G1): endodontic filling cement (Sealapex, Sybron-Kerr, Romulus, MI, USA), Group 2 (G2): resin luting cement (RelyX ARC, 3 M-ESPE, Seefeld, Germany), and Group 3 (G3): glass ionomer cement for cavity lining (Vitrebond, 3 M-ESPE, Seefeld, Germany). An amount of 0.05 mL of silver nanoparticles was incorporated in a colloidal solution. The control groups were prepared without the incorporation of silver (C1-Sealapex, C2-Rely X ARC, and C3-Vitrebond). The nanoparticles were incorporated to the already mixed cements, during manipulation. All the materials were manipulated according to the manufacturer instructions. The ones that needed polymerization (RelyX and Vitrebond) were photocured with a LED unit (LED radii-cal, SDI, Australia) with a controlled wave length of 440–480 nm for 60 seconds.

The silver nanoparticles were prepared by the research group of the Physics Institute of Federal University of Goias (UFG), by reduction of silver nitrate. This method can be described by the reaction:

$$AgNO_3 + NaBH_4 \longrightarrow Ag + \frac{1}{2}H_2 + \frac{1}{2}B_2H_6 + NaNO_3 \quad (1)$$

The bactericidal activity was evaluated by the Agar Diffusion Test. Six Petri plates with 20 mL of Brain Heart Infusion (BHI, Difco Laboratories, Detroit, MI, USA) were inoculated with 0.1 mL of *Streptococcus mutans* (ATCC 25175). With sterile swabs, the microbial suspension was spread on the medium, obtaining growth in junction. Three cavities of 4 mm in diameter and 4 mm in depth were made in each agar plate (total = 18) with a copper coil and were then completely filled with the manipulated cements. Each agar plate corresponded to one of the experimental groups. The plates were incubated at 37°C for 48 h, after that the bacterial inhibition halos diameter were observed and measured with the computer program Image J (Version 1.43u, Wayne Rasband, National Institutes of Health, USA). The halos diameters were measured through their pictures using the cavities' size as scale. The diameters were obtained in three measures in each halo. All assays were carried out under aseptic conditions.

Size of the prepared nanosilver particles was also studied using a ZetaSizer Nanoseries from Sympatec Co. (Clausthal-Zellerfeld, Germany). When the particles or molecules are illuminated with a laser, the intensity of the scattered light fluctuates at a rate that is dependent on the size of the particles as smaller particles are "kicked" further by the solvent molecules and move more rapidly. Analysis of these intensity fluctuations yields the velocity of the Brownian motion and hence the particle size using the Stokes-Einstein relationship.

The statistical analysis was carried out with the software SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, EUA). Kolmogorov-Smirnov test was used to verify the standard sample distribution of data and Levene test was used to verify the homogeneity of variance. As data presented normal standard and homogeneous variances and considering the data dependency, the paired *t*-Test was chosen. The significance level in all analysis was of 0.05.

3. Results

The means and standard deviations for inhibition halos in mm are shown in Table 2. After incubation, the glass ionomer cement was the only cement that showed inhibition halos with and without the addition of nanosilver particles (Figures 1 and 2). The other two cements did not show any

Table 2: Means and standard deviations (±SD) of the halos measurements in mm for the glass ionomer cement.

Vitrebond	Mean	Standard deviation	P
Inhibition without silver (C3)	20.68	0.73	<0.0001
Inhibition with silver (G3)	22.28	0.76	<0.0001

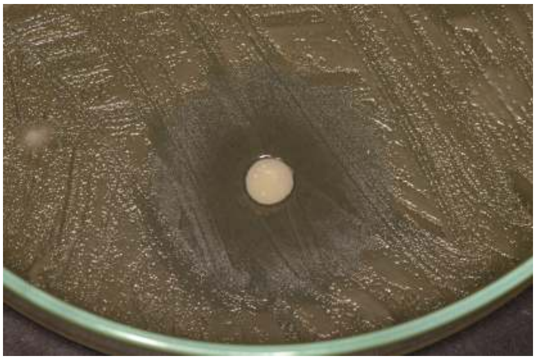

Figure 1: Inhibition halo formed around the specimen of glass ionomer cement without silver nanoparticles.

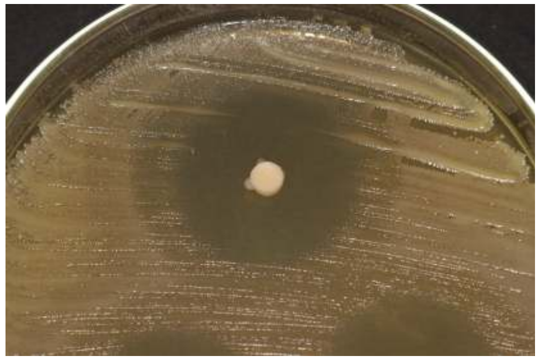

Figure 2: Inhibition halo formed around the specimen of glass ionomer cement with silver nanoparticles.

Figure 3: Specimen without inhibition halo, representative of Rely X ARC group without silver nanoparticles.

bactericidal effect against *S. mutans*, showing no inhibition halo (Figure 3).

The results showed that significant statistical differences were found between the size of the inhibition halos of C3 and G3 ($P < 0.0001$), suggesting that the addition of nanosilver particles increased the antibacterial activity of Vitrebond.

A dynamic light scattering was performed with the ZetaSizer machine to determine the particle sizes. The dimensions obtained showed a big difference in size between the particles, varying from 0.621 nm to 661 nm, with a mean value of 330 nm and a domination of particles above 400 nm. This great difference was confirmed by the polidispersity index equal to 1.

4. Discussion

The antimicrobial actions of silver, silver ions, and silver compounds have been extensively investigated in comparison with other metals [3–5, 22, 26]. Silver nanoparticles, either alone or as a composite with other agents, have shown particularly encouraging results as antimicrobial agents [4, 11, 15, 16, 18, 19, 27]. The application of nanoparticles to control biofilm formation within the oral cavity, as a function of their biocidal, antiadhesive, anti-inflammatory, antiviral, and delivery capabilities, is worthy of serious consideration [4, 15].

Bactericidal effect is a relevant characteristic when considering restorative materials. The three cements chosen for this study have different composition characteristics which lead to different interactions with silver, hence different results of antibacterial activity. Their diverse applications allowed covering several clinical situations in which this effect is desired. Ideally, luting cements should possess bactericidal properties that will prevent bacteria-induced pulpal irritation, tooth sensitivity, and recurrent caries [7]. Glass ionomer cements should release fluorides to suppress microleakage and secondary caries formation [6]. An endodontic sealer should have a strong, long-lasting antimicrobial effect to eliminate the microorganisms that have survived the chemomechanical instrumentation and improve the success rate of the endodontic treatment [8].

The bactericidal activity of silver nanoparticles has been attributed to mechanisms such as the release of Ag^+ [13, 16, 21, 28]. However, according to Chaloupka et al. [15], nanosilver has intrinsic antibacterial properties that do not depend on the elution of Ag^+. Silver causes bacterial membrane disruption probably due to the production of reactive oxygen species, including free radicals which might underlie and explain both the antibacterial activity of nanosilver and its potential toxicity to humans [11, 15, 18, 19, 23]. The active oxygen causes structural damage in bacteria, which is called an oligodynamic action [11, 18, 20, 24, 25]. Membrane disruption allocates nanosilver particles into cytoplasm causing subsequent damage of DNA and other phosphorus containing compounds, impairing the respiratory chain and cell division [15, 17, 19, 29]. Many of these studies have been carried out under *in vitro* conditions, and there is a clear need for further *in vivo* and *in situ* studies [4].

In general, silver nanoparticles have been observed to have the largest antibacterial effects with the smallest particle sizes, with average diameters under 10 nm being most effective [12, 16, 20, 27, 29]. Metal particles of small sizes (~ 5 nm) present electronic effects, which are defined as changes in the local electronic structure of the surface due to size [20]. These effects are reported to enhance the reactivity of the nanoparticle surfaces [3, 20]. Additionally, the higher proportion of surface atoms in smaller particle sizes allows a more effective release of silver ions from the nanoparticle surface [16]. The diameter that is measured in dynamic light scattering is called the hydrodynamic diameter and refers to how a particle diffuses within a fluid. This means that the size can be larger than measured by electron microscopy, for example, where the particle is removed from its native environment. In this study, the particles used were of 0.621 to 661 nm, with a polidispersity index of 1, indicating a high variation in the distribution of the particle sizes. According to Lok et al. [27], the 62 nm nanosilver exhibited the same activities as 9.2 nm nanosilver only when 9 times the total silver was added. Thus, one possible explanation for the lack of antibacterial effect in most of the cements studied is that the amount of bigger particles in this study was superior to the smaller ones.

In this study, Sealapex did not present any bacterial control. Though, other studies have reported its antimicrobial activity against a variety of microorganisms including *S. mutans* [9, 30, 31]. Studies have shown the antibacterial properties of calcium hydroxide P.A. (proanalysis) [30–32]. However, Sealapex presents 20% calcium oxide which hydrated transforms in calcium hydroxide, but with smaller quantities of hydroxyl ions than calcium hydroxide free in the tissues. This transformation needs water and in agar, which is a semisolid medium, it does not happen. The absence of prediffusion in this test probably justifies the lack of bactericidal activity of Sealapex. The prediffusion is defined by the 2 h that plates remain in room temperature before being incubated to permit the dissociation and diffusion of the calcium hydroxide contained in the endodontic cement [31].

In this test, Vitrebond was the only cement to present inhibition halo with and without nanosilver. Vitrebond is a glass ionomer cement that, according to the manufacturer, presents a high fluoride release which possibly influences *in vivo* and *in vitro* properties. Fluoride is widely used as an anticariogenic material in many dental products [7]. More than other dental restorative materials, glass ionomer cements have cariostatic characteristics due to the release of fluoride, which is believed to help reduce demineralization, enhance remineralization, and inhibit microbial growth [10]. The most important anticariogenic property of fluoride in these cements is the effect on cariogenic oral bacteria, especially on *S. mutans* [7]. That is in accordance with the findings of this study where Vitrebond presented good bactericidal activity against *S. mutans*. With silver addition this activity was significantly higher, while the presence of nanoparticles in the other cements did not lead to any antibacterial activity. This is probably due to the diffusion characteristics of Vitrebond which might have helped the diffusion of silver in the agar plates.

Rely X ARC is a resin luting cement frequently used for indirect restorations and posts cementation. The antimicrobial effect of resin composite materials with high concentrations of silver particles was demonstrated [5, 23]. However, in this study, no effect was observed in Rely X ARC. The diffusion test in agar is largely used in microbiology [25, 30]. However, the literature demonstrates that it does not establish reliable parameters to evaluate substances with different chemical characteristics such as dissociation and diffusion capabilities in semisolid medium, not expressing their real antimicrobial effect [30]. Additionally, this test can be considered inappropriate for the evaluation of antimicrobial effects of silver-loaded composite materials because they are poorly water soluble, have high molecular weight, and do not release silver ions into the culture media [5, 21]. According to Yoshida et al. [23] the inhibitory effect of three silver-containing resin composite materials against *S. mutans* is probably due to a direct contact with the bacteria and not to silver ion release.

Though positive results were found concerning nanosilver bactericidal effect, the inherent toxicity of nanoparticles must be considered. It happens because the particles have the same dimensions as biological molecules (e.g., DNA and proteins) and thus, may directly interact to damage DNA, denature proteins and enzymes and produce free radicals [15]. This is further compounded by the toxicity of elemental silver and biologically active silver ions [15, 20, 21]. Still, there are studies showing that the toxicity of silver nanoparticles is dependent on the size of the particles, the dose and the time of exposure [33–35]. Considering toxicity, two cements among the three studied are not used in direct contact with cells from the pulp or the periapical tissue. The resin cement and the glass ionomer cement, in the clinical situation, are only in contact with the dentin tissue. Also, the pulpal tissue contains a fluid which exerts a definite fluid pressure that is positively directed to the dentin walls, preventing movement of substances towards the pulp [36]. On the other hand, the teratogenicity of nanosilver in humans is unknown because no cases or studies have been reported in the literature. Thus, nanosilver assessment in humans for potential teratogenic effects is imperative.

5. Conclusions

According to the results of this study, no inhibition halos were obtained for Sealapex and Rely X ARC, but Vitrebond showed bactericidal activity without the addition of nanosilver particles and enhanced effect with silver incorporation.

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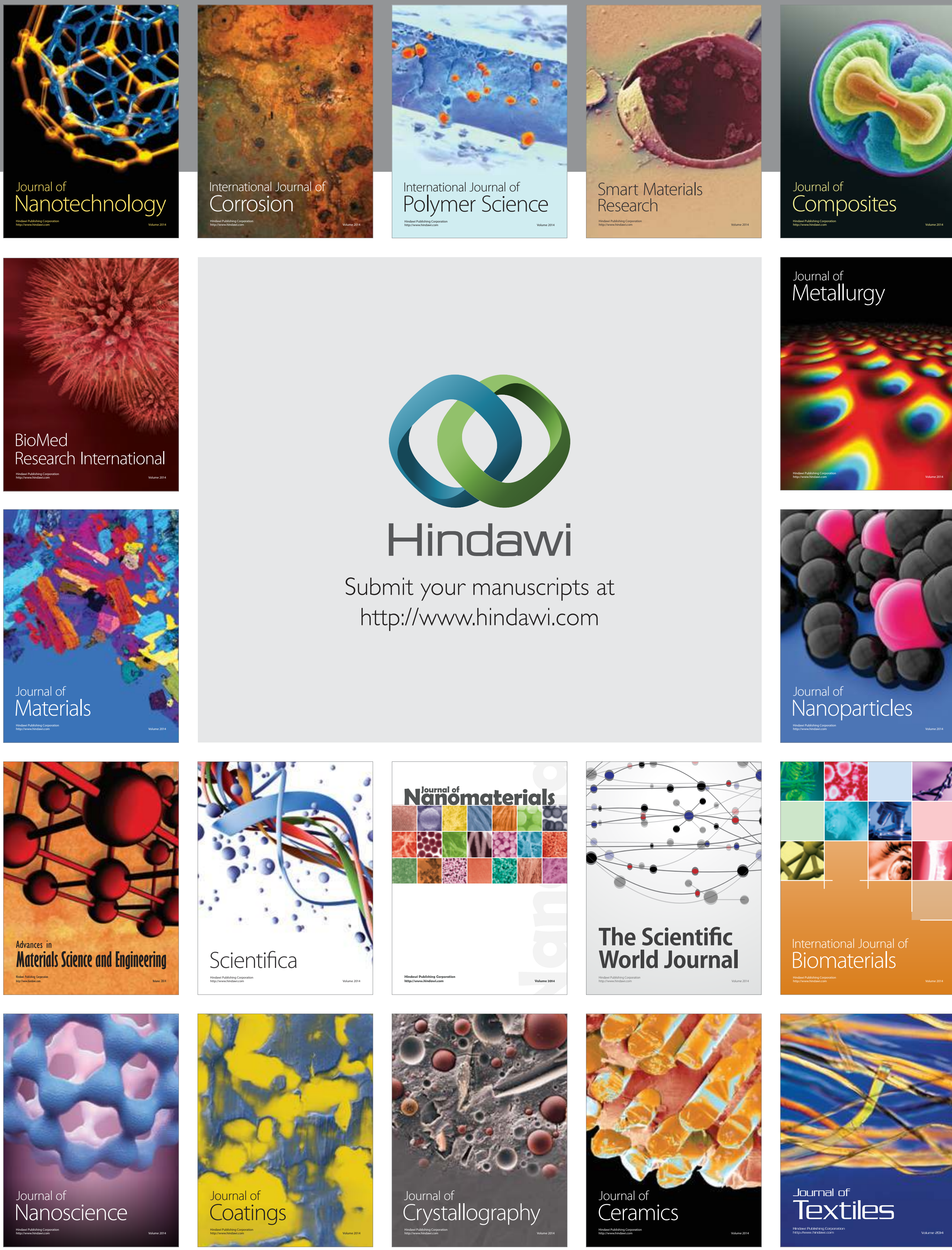
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