Antibacterial Efficacy of Nanoparticle-Incorporated Root Canal Sealer against Common Endodontic Pathogens - An *in vitro* Study

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Authors’ contributions

This work was carried out in collaboration between both authors. Author SH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author IN guided through the entire process. Both the authors read and approved the final manuscript.

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ABSTRACT

Infection caused by microorganisms play a crucial role in the induction of inflammation of pulpal as well as the periapical tissues. Factors like improper disinfection or obturation of the root canal space contributes to the failure of the root canal treatment. The aim of the study was to modify the contents of MTA-based sealer with nanoparticles and check for its antibacterial efficacy against *E. faecalis*. Silver nanoparticles (10 nm) were incorporated in MTA based sealer at various concentrations to form the test product. The antibacterial efficacy of the modified sealer was tested by well diffusion test on *E. faecalis*. The zone of inhibition (mm) was checked for each test product. Analysis of the results showed significant diameters of zones of inhibition (mm) as compared to sealer without nanoparticles. The zone of inhibition increased with the increase in the concentration of silver nanoparticles. The silver nanoparticle incorporated sealer can be used in the clinical setup to prevent reinfection of the root canal system and ensure the success of the root canal treatment.

Keywords: Antibacterial; MTA; nanoparticles; root canal treatment; sealer.
1. INTRODUCTION

Dental caries and trauma are the leading cause of dental pain that indicates the pulpal and/or periapical inflammation and infection [1]. Endodontic management is the treatment of choice in such cases [2]. The success or failure of a root canal therapy depends on the complete elimination and disinfection of the root canal system. Inability to prevent reinfection lead to the failure of root canal therapies sooner or later [3,4]. Hence, a three dimensional seal of the canal system that includes the root canal, its accessory canals and abnormal anatomy, if any is extremely important and forms a goal of the root canal therapy. Obturation is the process where the disinfected and prepared root canals are filled with suitable materials as close to the Cemento-enamel junction is possible [5]. Root canal sealers in conjunction with solid core filling materials aid in the sealing of the canal system three dimensionally [6]. Sealers help overcome the limitations of Gutta percha by filling minute microscopic space between the dentinal wall and gutta percha. Major functions of a root canal sealer are to seal canal systems against bacterial in growth from oral cavity, entombment of leftover viable microorganisms and complete obturation of the canal system at a submicroscopic level to prevent stagnant fluid from accumulation and serving as bacterial nutrition [7].

The ideal properties of sealers include establishment of hermetic seal, minimal cytotoxicity to periodontal ligament cells, tackiness when mixed to provide adhesion, radiopacity to be seen on a radiograph, lack of shrinkage and staining of tooth structure [8]. Root canal sealers that possess superior sealing ability and antimicrobial activity would hence be beneficial in clinical aspects [9] as they can not only prevent bacteria from re-entering and re-infecing the canal system but also inactive the remaining viable bacteria of the canal system post-obturation.

Root canal sealers commonly used belong to the categories of zinc oxide eugenol (ZOE), epoxy resin (ER) and calcium hydroxide (CH), based on the basic composition of the sealers [10]. Calcium silicate based cement consisting of added metal oxides lead to the development of Mineral Trioxide Aggregate (MTA) that has bioactive properties [11]. Apart from being extremely biocompatible, it is seen to stimulate tissue repair and induce mineralization [12,13]. These reasons make MTA a suitable material to be used as a root canal sealer as it fulfills almost of the criteria for a material to be called an ideal root canal sealer.

Silver has gained fame of being an antimicrobial agent and is being incorporated to check for its antimicrobial efficiency against various species of microorganisms important as per the dental perspective [14].

We have numerous highly cited publications on well-designed clinical trials and lab studies [15–30]. This have provided the right platform for us to pursue the current study. Our aim was to evaluate the antimicrobial efficacy of a MTA-based sealer against the common pathogen associated with the failure of root canal therapy when incorporated with silver nanoparticles In vitro.

2. MATERIALS AND METHODS

2.1 Test Product

Silver nanoparticles (10 nm) purchased from Sigma Aldrich Company was used in this study. Silver nanoparticles 20 µg/mL solution in aqueous buffer, contains sodium citrate as stabilizer. The endodontic sealer MTA-Fillapex, developed by Angelus (Londrina/Parana/ Brazil) and launched commercially in 2010 was mixed with 0.5%, 1.0%, 2.5%, 5%, and 10% of silver nanoparticles.

2.2 Bacterial Strain

Bacterial strain Gram positive Enterococcus faecalis ATCC 29212, was chosen based on their clinical and pharmacological importance. The bacterial strain was cultured on nutrient agar by using spread plate technique on Mueller-Hilton Agar (MHA) plates and was incubated for 24 hours at 37°C. The stock cultures were maintained on nutrient agar slants at 4°C. The test organism was prepared by inoculating colonies into 20 ml of nutrient broth in a 100 ml Erlenmeyer flask and incubated for 24 hours at 37°C.

2.3 Testing Groups

The testing groups included negative control only the medium without any test compound; sealer control, only the sealer without nanoparticles; sealer+silver nanoparticles at different concentrations (0.5, 1.0, 2.5, 5, 10%) of silver nanoparticles mixed with the sealer.
2.4 Antimicrobial Susceptibility Test

2.4.1 Well diffusion test

Seven uniform wells of size 6 mm were prepared on Mueller-Hilton Agar inoculated with E. faecalis. The experimental solutions were added to the respective wells on each plate. These plates were incubated for 24 hours at 37°C in an incubator. After the incubation period, plates were checked for zones of inhibition of bacterial growth and diameters of the zones achieved by each group against E. faecalis were recorded in millimeters (mm). All tests were carried out three times to ensure reliability, and the average of the three replicates for each test sample was calculated.

2.5 Statistical Analysis

Multiple comparisons were performed using one-way analysis of variance (ANOVA). Statistical significance was accepted at a level of p<0.05. Data were analysed using SPSS (Version 11).

3. RESULTS AND DISCUSSION

MTA Fillapex, developed by Angelus (Londrina/Parana/ Brazil) and launched commercially in 2010, is seen to comprise natural resin, salicylate resin, diluting resin, bismuth trioxide, nanoparticulated silica, MTA and pigments [31]. There have been claims that MTA Fillapex has a good antimicrobial efficiency [32–36]. This claim is in consistency with the findings of the present study as the unmodified test product was seen with a little zone of inhibition against the growth of E. faecalis. The test product was shown to have significant antimicrobial efficiency against E. faecalis with increasing concentration of silver nanoparticles. The incorporation of silver nanoparticles increased the zone of inhibition of the test products against E. faecalis. The diameter of the zone of inhibition is directly proportional to the percentage of silver nanoparticles added (Table 1 and Plate 1). The original test product without silver nanoparticles is the least. The silver ion was tested and found to be effective against a broad range of microorganisms. Silver ions have been used to control bacterial growth in a variety of medical applications. With the discovery of antimicrobial drugs, the use of silver as an antimicrobial substance was reduced. The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulfhydryl) groups and it has a deadly effect on bacterial enzymes, bacterial growth and cell division and results in damage of bacterial cell wall and contents [37]. The emergence of antibiotic resistant species, revived the use of silver as an antimicrobial agent. These silver particles are extremely efficient when delivered in particles of nano ranges. The nano range comprises particles with a size of 1 to 100 nm. The silver nanoparticles are stored in a liquid medium to prevent agglomeration and entrapment of the particles within the matrix. Lara et al. implied that the particle shape, size, distribution and agglomeration are important characteristics of nanoparticles [38]. These factors determine the distribution of the particles in-vivo, their biological fate, toxic effects, and targeting ability.

Silver nanoparticles are non-toxic at low concentrations and have a broad spectrum of antibacterial activity that includes many Gram positive, Gram negative and antibiotic drug resistant species like MRSA [39]. The mechanism of action of the silver nanoparticles could be attributed to the ability of the silver ion to bind to the negatively charged part of the bacterial cell wall. The basic function of the bacteria was disturbed causing a leak in the cytoplasmic contents and rupture of the cell membrane [40]. Further, the silver particles infiltrate within the cytoplasm causing interaction with the nuclear content and bacterial cell deaths. The MIC and MBC of silver nanoparticles against Enterococcus faecalis as determined is 5 mg/ml [41].

Correa et al [42], suggested the antimicrobial effectiveness of silver nanoparticles incorporated in various dental material. Silver nanoparticles at varying concentrations have been incorporated in a variety of materials like composite resin, adhesive system, acrylic resin, tissue conditioners, intracanal irrigants and medicaments, gutta percha, root canal sealers, MTA and titanium implants.

The root canal sealers play an important role in overcoming the limitations of the root canal filling materials. Various kinds of nanoparticulate matter are constantly being used to modify the conventional canal sealers so that the chemical, physical or antimicrobial properties of these sealers can be improved. Chitosan and zinc oxide nanoparticles added to sealers were seen
Table 1. Shows the inhibitory zone of the microorganism (in mm)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentration</th>
<th>Zone of inhibition (in mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>100µl</td>
<td>NI</td>
</tr>
<tr>
<td>Sealer Control</td>
<td>1%</td>
<td>2.6 ± 0.11</td>
</tr>
<tr>
<td>Sealer+ AgNP</td>
<td>0.5%</td>
<td>4.6 ± 0.28</td>
</tr>
<tr>
<td>Sealer+ AgNP</td>
<td>1.0%</td>
<td>7.8 ± 0.42</td>
</tr>
<tr>
<td>Sealer+ AgNP</td>
<td>2.5%</td>
<td>9.3 ± 0.35</td>
</tr>
<tr>
<td>Sealer+ AgNP</td>
<td>5.0%</td>
<td>12.4 ± 0.85</td>
</tr>
<tr>
<td>Sealer+ AgNP</td>
<td>10.0%</td>
<td>16.7 ± 0.71</td>
</tr>
</tbody>
</table>

NI means no inhibition zone. Each value is expressed as mean ± SD (n = 3). It can be inferred that the zone of inhibition increases proportionally with the concentration of the silver nanoparticles.

Plate 1. Zone of inhibition against E. faecalis
NC-Negative control; 1 – Sealer control; 2 - Sealer + AgNP(0.5%); 3- Sealer + AgNP(1.0%); 4- Sealer + AgNP(2.5%); 5- Sealer + AgNP(5.0%); 6-Sealer + AgNP(10.0%).

To effectively eliminate the bacterial biofilm and disrupts the biofilm structure. It was seen to be retaining this property through aging [43,44]. The antimicrobial efficiency of zinc oxide and silver nanoparticles incorporated zinc oxide eugenol based sealer proved silver nanoparticles have a superior antibacterial effect [45]. Also, silver nanoparticles incorporated within a zinc oxide based sealer with increasing concentration presented with results comparable and in consistency with the present study [46]. Zirconium oxide incorporated MTA seemed to have greater strength and radiopacity but mild antibacterial effect on E. faecalis [47]. Amongst other nanoparticles that have been incorporated in the root canal sealers, quaternary ammonium polyethyleneimine and bioactive glass [48]. The former nanoparticles improvised the antibacterial efficacy, while the latter promotes closure of the interfacial gap between root canal walls and core filling material. However, the antimicrobial effect of bioactive incorporated canal sealers is still under study.

4. CONCLUSION

The present In vitro study gives us a perspective of silver nanoparticles-modified MTA-based sealers to be efficient in controlling the growth of E. faecalis in laboratory environments. Also, the periodontal cell viability is seen to increase as the proportion of silver nanoparticles to sealer increases. However, more clinical oriented studies need to be done to check for other aspects like toxicity, discoloration or microleakage in the canal of this silver nanoparticles-modified sealer. Also, the antimicrobial efficiency over time needs to be checked.
CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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