Assessment of Antimicrobial Efficacy of Silver Nanoparticles Versus Calcium Hydroxide as Intra Canal Medication (In vivo Study)

Soha A Abdou, Medhat A. Kataia, Magdy M.M. Ali, Reham El Sayed Hassan and Sahar I. Negm

Introduction

Microorganisms are the main causative factors in the development of necrosis of the dental pulp and the formation of periapical lesions. Although we try to clean and irrigate the root canal system properly, some bacteria still remain entrapped in the dentinal tubules. It has been shown that, if the canal is not dressed with a disinfectant between visits, microorganisms will multiply rapidly within days to near the original numbers [1].

Several studies demonstrated that, some of microorganisms were resistant to the most commonly used intra canal medication calcium hydroxide Ca (OH)§ [2]. Recent studies have focused on using nanoparticles to disinfect root canals. Silver is an effective antimicrobial agent with low toxicity and broad spectrum activity Among the various options available.

AgNPs which are one of the most commercialized nanomaterials. They are widely applied as biocides for their strong antimicrobial activity [3]. It seems valuable to evaluate the antimicrobial efficacy of AgNPs as intra canal medication and compare it with the commonly used intra canal medication, Ca (OH)§.

Materials and Methods

Preparation of Silver Nanoparticles: Silver nanoparticles (AgNPs) have been prepared by chemical reduction method as reported by Turkevich et al. [4] and, Lee and Meisel [5]. A solution of silver nitrate (AgNO3) has been used as Ag§ ions precursor. The color of the solution slowly turned into grayish yellow, indicating the reduction of the Ag§ ions to Ag nanoparticles.

Patient Selection and Classification: Twenty patients medically free with necrotic pulp tissues of single rooted teeth with no periapical lesion, their age ranges from 20 to 50 years of both sexes were selected. Patients were
randomly divided into two groups according to type of medicament used. The medicaments used were Ca(OH)₂ paste and 0.03 mg/ml AgNPs paste.

**Endodontic Treatment Was Done in Two Visits.**

**The First Visit:** After approval of the ethics committee of Minia University, access cavities were prepared using sharp round bur mounted on a high-speed hand piece. The first microbiological samples were taken after using H-files size 30. The files were inserted into sterile Wasserman tubes containing 1 ml of sterile brain heart infusion (BHI) broth. The tubes were incubated at 37°C for one day before culturing. Working length was determined by periapical radiographs using bisecting angle technique. Mechanical preparations were completed using REVO-S rotary system mounted in endodontic motor. Irrigation was done using normal saline at all procedure steps. AgNPs was placed inside the canals using sterile plastic syringe. The access cavities were closed using sterile cotton pellet and temporary filling.

**The Second Visit:** After seven days, intra canal medicaments were removed by irrigation with 10 ml of sterile normal saline. The second microbiological samples were taken and cultured. After confirmation of the length of the master cone by periapical radiographic X-ray; obturation was done. The obturation was done using Revo-S gutta percha points.

* MICRO MEGA.
** Motor X smart E-Cube, Korea.

**Antimicrobial Effect Evaluation:** After incubation of microbiological samples, BHI broth was spread on different types of agar plates. After culturing, the blood and Sabouraud agar plates were incubated aerobically at 37°C for two days. The Wilkins agar plates were incubated anaerobically at 37°C for five days. The colonies were identified by Culture characteristics [6], Microscopic examination, Biochemical reactions (Catalase test) [7] and Polymerase chain reaction (PCR).

**PCR:** DNA extraction was done to the samples according to Qiagen DNA extraction protocol for bacteria (DNeasy blood and tissue handbook 2016) followed by PCR amplification with specific primers using hot start master mix method [8]. Detection of amplified products was done using Agarose gel electrophoresis [9] and Real time curve analysis [10].

**Counting of the Types of Microorganisms:** After incubation, serial dilution was done to 1/100000 for all specimens. Colonies of each microorganism were counted from the plates and the colony forming units CFU/ml were calculated according to the following equation: - CFUs / ml = number of colonies × dilution × volume of infected BHI broth [11].

**Follow up and Prognosis:** Follow up was done for all patients after 1 month and 3 months by clinical examination and radiographic evaluation.

**Statistical Analysis:** Data were presented as mean, standard deviation (SD), frequency and percentage. Dependent t-test used to compare between before and after intra canal medication for Log₁₀ CFU/ml. Wilcoxon signed rank test was used for detection of each microorganism.

**RESULTS**

**Nanoparticles Characterization Results**

**Determination of Size and Shape of Silver Nanoparticles AgNPs:** The particle size and shape were determined using Transmission electron microscope (TEM) which was performed on JEOL JEM-2100 at an accelerating voltage of 200 kV. The average size of the particles was 50 ± 5 nano microns (nm) and the particles were spherical in shape.

**Determination of Optical Properties of AgNPs:** The optical properties of the prepared nanoparticles were characterized using UV-Vis absorption spectra which were obtained on an Ocean Optics USB2000+VIS-NIR Fiber optics spectrophotometer. Optical Properties (Abs.): εₘₐₓ = 401 nm.

**In vivo Antimicrobial Effect of the Tested Intra Canal Medication:**

**By Colony Forming Units CFUs:** In Ca(OH)₂ group: There was a statistically significant decrease in number of colony forming units CFUs between before and after treatment with Ca(OH)₂ with 17.95 % percentage reduction. In cocc, bacilli and candida; there was a statistically significant decrease in number of CFUs between before and after treatment with 23.29, 21.13 and 23.90 % percentage reduction, respectively (Table 1, Fig. 1).
Table 1: Mean and standard deviation (SD) for colony forming units (log$^{10}$ CFUs/ml) for different microorganisms

<table>
<thead>
<tr>
<th>Time</th>
<th>Cocci Group I (Ca(OH)$_2$)</th>
<th>Cocci Group II (AgNPs)</th>
<th>Bacilli Group I (Ca(OH)$_2$)</th>
<th>Bacilli Group II (AgNPs)</th>
<th>Candida Group I (Ca(OH)$_2$)</th>
<th>Candida Group II (AgNPs)</th>
<th>Total Group I (Ca(OH)$_2$)</th>
<th>Total Group II (AgNPs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>7.3</td>
<td>6.8</td>
<td>7.1</td>
<td>7.1</td>
<td>6.57</td>
<td>5.5</td>
<td>7.8</td>
<td>7</td>
</tr>
<tr>
<td>SD</td>
<td>0.95</td>
<td>1.4</td>
<td>1.29</td>
<td>1.45</td>
<td>1.29</td>
<td>1.38</td>
<td>1.23</td>
<td>1.89</td>
</tr>
<tr>
<td>Mean</td>
<td>5.6</td>
<td>1.3</td>
<td>5.6</td>
<td>3.2</td>
<td>5</td>
<td>3.29</td>
<td>6.4</td>
<td>4</td>
</tr>
<tr>
<td>SD</td>
<td>2.41</td>
<td>1.77</td>
<td>1.58</td>
<td>1.4</td>
<td>2.1</td>
<td>1.25</td>
<td>1.51</td>
<td>1.25</td>
</tr>
<tr>
<td>% of reduction</td>
<td>-23.29%</td>
<td>-80.88%</td>
<td>-21.13%</td>
<td>-54.93%</td>
<td>-23.90%</td>
<td>-40.18%</td>
<td>-17.95%</td>
<td>-42.86%</td>
</tr>
<tr>
<td>p-value</td>
<td>0.025*</td>
<td>&lt;0.001*</td>
<td>0.003*</td>
<td>&lt;0.001*</td>
<td>0.026*</td>
<td>0.195 NS</td>
<td>0.01*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*=significant, NS=Non-Significant

Fig. 1: Bar chart showing the mean colony forming units (log$^{10}$ CFUs/ml) for different microorganisms

By PCR:

**Effect of Intra Canal Medication Type on Identified Microorganisms:** In both intra canal medication groups, there was no statistically significant difference in types of microorganisms before and after treatment.

**DISCUSSION**

A new silver nanotechnology has become favourable due to its effects against biofilms [12]. The
AgNPs interact with multiple targets in the microbial cell, such as cell membrane, enzymes and plasmids, simultaneously providing the bacteria least capacity to gain resistance [13]. The size of AgNPs was 50 nm as the size of the particle was related to the antimicrobial activity; the smaller particles give more bactericidal effects than larger particles [14-16]. Concentration of AgNPs used in this study was 0.03 mg/ml. As reported by Xinping [3] this concentration had the most effective antimicrobial properties with least toxicity. Ca(OH)₂ was chosen as the base line because of its prevalent clinical application [17, 18]. Despite the limitations of Ca(OH)₂, however its antimicrobial action as intra canal medication is well documented [18, 19].

All microbiological steps which were done in this study were done under laminar air flow to prevent contamination [3]. Polymerase chain reaction (PCR) was done for accurate confirmation of the types of microorganisms [20, 21]. The medicaments were removed using saline before bacterial sampling because it has no any antimicrobial effect so it does not affect the results of the study [22, 23]. Counting the colony forming units (CFUs) was performed because it allows the calculation of the quantity of microorganisms within the root canals [24].

The results of the present study showed that the percentage reduction of numbers of microorganisms before and after treatment with 0.03 mg/ml AgNPs paste group was significantly higher than Ca(OH)₂ paste group. This may be attributed to high affinity of silver to negatively charged molecules within the bacterial cells which inactivate critical functions of bacterial cells and prevent bacterial growth and biofilm formation. Silver nanoparticles are effective against a broad range of pathogens, which form biofilm, such as E. coli, Strep. pneumoniae, S. aureus and Actinomyces niger [25]. The antimicrobial efficacy of Ca(OH)₂ is related to hydroxyl ion release in an aqueous environment, thick mixtures paste of Ca(OH)₂ may not be ideal as intra canal dressings [26]. Also the possibility of neutralizing the high alkalinity of Ca(OH)₂ by the dentin and biofilm matrix of bacteria, which lead to reduced antibacterial effect [27, 28]. This result is in accordance with the results of Javidi [29] who stated that Ca(OH)₂ alone was unable to eliminate E. faecalis sufficiently even after 1 week. However, combining Ca(OH)₂ with silver nanoparticles effectively eliminated E. faecalis after only one day. Also Sadeghi [30] stated that nanosilver solution had antimicrobial properties against Actinomyces viscosus and Streptococcus sanguinis. Zhang [31] concluded that AgNPs alone or with Ca(OH)₂ had an obvious inhibitory effect on the biofilm of E. faecalis. The result of this study is in contrast with the results of Mozayeni [32] who concluded that Nanosilver gel was not effective against E. Faecalis. This may be attributed to the different synthesis procedure of nanosilver particles and the fact that the added gel may have inhibited the release of nanoparticle ions.

But for Candida, there was no statistically significant decrease in number of CFUs between before and after treatment in AgNPs paste group. This result is in accordance with the results of Mozayeni [33] who concluded that antifungal efficacy of Ca(OH)₂ and 2% CHX gels was significantly higher than that of nanosilver gel in all tested samples. And with the results of Ballal [34] who concluded that Ca(OH)₂ showed higher efficacy at the first 24 hours against Candida albicans. The result of this study is in contrast with the results of Baker [14] who stated that 8 mg/cm² AgNPs had antimicrobial activity against Candida albicans. This may be due to using different concentration of AgNPs.

There was no statistically significant difference in types of microorganisms before and after treatment except for Enterococcus which appeared after treatment significantly in 0.03 mg/ml AgNPs group. This may be attributed to the detection of a bacterial species after treatment by molecular methods may result from the detection of DNA molecules still present in the specimen, which does not mean cell viability [35].

CONCLUSIONS

- The antimicrobial effect of 0.03mg/ml AgNPs paste was better than Ca(OH)₂ paste.
- AgNPs paste seems to be a valuable option in the battle against different types of bacteria.

REFERENCES


