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THE IMPACT OF SILVER NANOPARTICLES LOADED IN CHLORHEXIDINE ON RADICULAR DENTIN MICROHARDNESS (AN IN-VITRO STUDY)

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ABSTRACT

Aim: This study compared the effects of silver nanoparticles (AgNPs) , 2% chlorhexidine gel and silver nanoparticles loaded in 2% chlorhexidine gel on radicular dentin microhardness.

Methods: 30 extracted human permanent single rooted teeth were selected. Teeth were decoronated then verticaly sectioned, one section for each root was included. Random distribution of samples was performed as follows: 2% chlorhexidine gel, AgNPs, and AgNPs loaded in 2% chlorhexidine, n=10 for each group. Baseline microhardness was measured using Vickers microhardness test, then medicaments were added for 2 weeks. Microhardness was assessed post-application. The variation in microhardness values was expressed in percentile values. Statistical analysis was carried out using One Way ANOVA, followed by Tukey test. Significance level was set at 0.05. T-Test was used for the statistical analysis of the difference between the pre-treatment and the post-treatment microhardness values.

Results: AgNPs and AgNPs loaded in 2% chlorhexidine significantly raised dentin microhardness, while 2% chlorhexidine significantly reduced it, ($P < .05$).

Conclusion: AgNPs and AgNPs loaded in 2% chlorhexidine displayed a reinforcing effect while 2% chlorhexidine weakened radicular dentin microhardness.

KEY WORDS: AgNPs, radicular microhardness, 2% chlorhexidine, nanoparticles.

INTRODUCTION

Root canal treatment aims primarily at eradicating or maximally reducing bacterial loads that would predispose to apical periodontitis and /or periapical lesions. Moreover, bacterial biofilms present a constant challenge that mandates extra eradication measures to prepare the canal environment for healing. Several chemomechanical measures have been devised in order to achieve this target. This includes mechanical instrumentation, irrigation as well as intracanal medications.

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Antibacterial medications have been a measure of control against resistant infections that would need further steps beyond the regular cleaning and shaping. Several agents have been used in solution and medication forms with variable efficiencies against bacterial strains. Chlorhexidine is a major agent used in solution form as an irrigant and in paste form as a medication. When used at a 2 % concentration, it proved to be effective against E-fecalis bacteria, a major contributor to resistant infection cases [1–3].

With the evolution of nanotechnology and the possibility of developing nanoscale antibacterial agents, a broader spectrum of options became available. Several agents were tested in the nanoform against various bacterial species and showed promising results. Their high surface are to volume ratio provided them with higher reactivity. Silver nanoparticles (AgNPs) are among the most explored agents for their broad antimicrobial spectrum against various bacteria, viruses, and fungi. In endodontics, they have been explored as potential retrofill materials, canal sealers, intra-canal medications, and irrigants [4–9]. Adding silver nanoparticles to chlorhexidine was attempted in different research trials in order to provide additional antibacterial effects [10].

Prolonged periods of contact of the different medications with radicular dentin have shown variable degrees of detrimental effects on dentin microhardness and degrees of mineralization, which in turn have been directly related to the concentration and duration of contact [2,11]. This in turn necessitates the assessment of the impact of these different substances on root dentin microhardness. Therefore the aim of this study was to compare the impact of silver nanoparticles in conjunction with chlorhexidine , to chlorhexidine and silver nanoparticles separately on root dentin microhardness.

MATERIALS AND METHODS

Specimens selection

Thirty fully developed permanent single rooted teeth extracted for periodontal reasons were sampled from the oral and maxillofacial surgery department, Faculty of Dentistry, Ain Shams University. Teeth were cleaned using an ultrasonic scaler and calculs deposites were removed. They were disinfected by immersion in 5% NaOcl solution. They were decoronated to 16mm then kept in distilled water. Cleaning and shaping was then performed using ProTaper rotary files till F4 (Dentsply, Maillefer) while maintaining apical patency and irrigating with 2.5% sodium hypochlorite after each file change.

Preparation of samples

A 0.3-mm IsoMet saw under continuous cooling was used to vertically split root fragments, yielding 60 halves. The more regular halves were selected then inserted in self cure acrylic resin. Root surfaces were smoothed by means of 400, 600, 800 &1200 grit polishing papers under constant water supply.

Chlorhexidine gel preparation

2% Chlorhexidine gel was made available by adding up chlorhexidine (JK Dental, Egypt) to hydroxymethylcellulose and natrosol. A gel form was obtained by implementing 4mg of polymer for each 1 ml of chlorhexidine.

Preparation of nano-based medicaments

100ppm AgNPs solution was set by the chemical reduction method (5). CHX-loaded AgNPs (Nano-Tech Egypt for Photo-Electronics, Cairo, Egypt) solution was devised through the electrostatic attraction approach by functionalizing the silver nanoparticles which then became electrostatically linked to the positive charges of CHX. 100 ppm AgNPs were dispersed in 100 ml of CHX to yield a final concentration of 100mg/L, then the electrostatic binding between CHX and AgNPs was allowed by overnight mixing [12]. Propylene glycol was used as

a vehicle providing a gel-like consistency.

Classification of samples

30 samples were equally allocated according to the medication type into three groups (N=10).

- Group (1): 100 ppm AgNPs in propylene glycol
- Group (2): 2% CHX (Chemajet Chemical Company, Alexandria, Egypt).
- Group (3): CHX-loaded AgNPs gel (1:1).

Baseline microhardness measurement

Baseline microhardness of all samples before implementing medicaments were assessed using Vickers Diamond Micro-hardness tester (Wilson Buehler., USA). Three assessments were performed at the coronal, middle and apical thirds, at a depth of 1000 µm from the lumen, using a 200g load and a dwell time of 15s. One hardness value at each level was obtained by averaging the 3 readings for each canal third.

Microhardness determination after medication supplementation

A sterile cement spatula was selected to evenly distribute the medications over the entire surface. Root halves were stored for 2 weeks at 37ºC and 100% humidity. Samples were washed and dried after 2 weeks of medication usage. Vickers Diamond Micro-hardness tester was used to determine the microhardness change in Vickers Hardness Units (VHN). Three values were also taken at each canal third, at a depth of 1000 µm from the lumen.

Microhardness was determined as follows:
HV=1.854 P/d2
HV = Vickers hardness (Kgf/mm^2).
P = Load (kgf).
D = Average length of diagonals (mm).

Mean percentage of change in micro-hardness, was measured then calculated as follows:

V1-V2/V1*100

Where V1 = Preoperative VHN and V2 = Postoperative VHN

Statistical Analysis

T Test was used to analyze the differences between pre and post-treatment microhardness values, P-value was set at .05. The statistical comparison between the three experimental groups was carried out with one-way ANOVA, then multiple comparisons were performed using Tukey posthoc test, p= 0.05.

RESULTS

Non-significant differences were found between the baseline microhardness values of all groups, ($P > 0.05$). Two weeks of chlorhexidine application significantly reduced radicular dentin microhardness, while a significant increase was noted after AgNPs and AgNPs/chlorhexidine addition, ($P < 0.05$) (Table 1 , Fig. 1).

TABLE (1) Percentile changes in microhardness values 2 weeks post-medications application

	AgNPs	CHX+AgNPs	CHX	P value
Coronal	-5.46%^{b}	-3.51%^{b}	5.47%^{a}	P<0.05
Middle	-5.19%^{b}	-5%^{b}	5.02%^{a}	P<0.05
Apical	-4.3%^{b}	-3%^{b}	4.36%^{a}	P<0.05

Different superscripts indicate significant differences

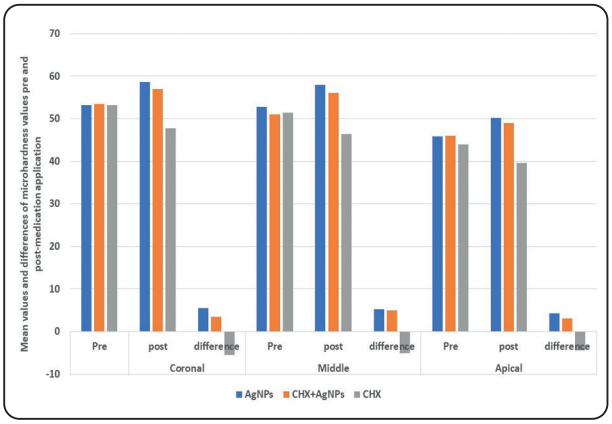

Fig. (1). Mean values and differences of microhardness values pre and post-medication application

DISCUSSION

Microbial colonies within the root canal system cannot be completely eliminated, rather reduced by a combination of chemical and mechanical means. Intra-canal antimicrobial medicaments are used as adjunctive measures to the cleaning and shaping procedures for this purpose. The relatively longer time of their application in contact with dentin highlights the importance of investigating their effects on dentin microhardness [4,12].

Nanotechnology and the introduction of nanobased medicaments with dimensions less than 100 nm paved the way for antibacterial agents capable of deeper intratubular penetration, and better reachability of the deep microbial biofilms that could attain from 300 to 1500 μm inside the dentinal tubules. Moreover, nano-based medications demonstrated higher chemical and biological reactivity by virtue of their higher surface area to volume ratio and higher charge density [5,13]. This would enable a more efficient encounter with the negatively charged bacterial cells. AgNPs were investigated for their effectiveness against bacterial antibiotic resistance, their anti-fungal and anti-viral potentials [8,10,14–18]. Their addition to other antimicrobials would enhance their efficiency, while reducing the bacterial tolerance and their cytotoxicity[10,16], that's why we included a group of AgNPs combined with CHX. This assembly was previously shown to possess enhanced antimicrobial effects [10,19]. CHX destroys the bacterial cell membrane, which allows the permeation of the nanoparticulate Ag+ inside the bacterial cells and enhances its effectiveness [14]. It displays the substantivity property by binding to the hydroxyapatite of the dental hard tissues [1,3].

Chlorhexidine was prepared in a gel form by adding chlorhexidine gluconate to hydroxymethylcellulose and natrosol. Natrosol gel is a water-soluble biocompatible carbon polymer that can be easily removed from the root canal when flushed with distilled water [20]. The gel consistency can keep all the dentinal tubules open and can reduce smear layer formation and holds debris in suspension (rheological action). Furthermore, the gel formulation may sustain the "active principle" and substantivity of CHX in contact with the microorganisms for a longer time, thereby counteracting their growth [21].

Silver nanoparticles were prepared by the chemical reduction method. The preparation procedure of metallic nanoparticles consists of mixing two microemulsions containing metal salt and a reducing agent [5]. Propylene glycol was implemented as a vehicle to impart a paste-like consistency to the medications with no previously reported adverse effects on dentin microhardness [22]. This agent has been previously added to calcium hydroxide, to MTA and to root canal sealers for the same purpose of imparting a workable consistency as well as an antibacterial activity [23].

Microhardness was chosen as a measure for flexural and tensile dentinal strengths, and was compared to the baseline values before adding any material given that this feature is affected by the inherent dentinal properties e.g tensile strength, elastic modulus and the structural stability [24]. Knoop indenter microhardness test [25,26] and the Vickers indenter method [26,27,28] have been the main tests used to measure the hardness of dentin. Vickers microhardness test was selected for this study as it evaluates surface changes of deeper dental hard tissues, and has no sensitivity to surface conditions. Moreover, it is more sensitive to measurement errors. Vickers hardness number is calculated based upon two diagonals in comparison to only one in the knoop test, imparting more accuracy [22]. Vickers hardness testing device has also a smaller indenter tip that is convenient for small samples. The measurement parameters were set at 200g loads and 10 seconds as per Mathew et al [23].

Our findings revealed that two weeks of application of 2% CHX reduced the radicular dentin microhardness, this could be related to its disrupting effect on the bonds between collagen fibers and

hydroxapatite crystals. Likewise, Oliveria et al. [11] reported that adding 2% chlorhexidine for 15 minutes significantly lowered radicular dentin microhardness. Similarly, 1, 3 and 7 days of addition of 2% CHX exerted adverse effects on radicular microhardness at [23]. As a root canal irrigant, it also proved to reduce dentin microhardness with and without surface modifiers [29]. It was found to decrease Ca and P levels and dentin mcirohardness at concentrations of 0.2% and 2% when applied for 15 minutes [11]. Conversely, 2% CHX was found to be harmless on dentin microhardness and surface roughness when used as an irrigating solution [26]. Saghiri et al [30] also found out that 2% CHX solution did not adversely affect dentin microhardness. They attributed this finding to the inability of chlorhexidine to dissolve necrotic tissue and smear layer, which may result in a mechanical obstruction against the solution penetration and subsequently limited ability to reduce microhardness in deeper layers. Passos et al [31] explained the lowest values of the mechanical properties of dentin treated with 2% CHX by its cationic nature which enables it to bind anionic molecules, such as phosphate, present in hydroxyapatite. Moreover, it can induce changes in Ca-P relationship in the calcium carbonate complex of dentin.

AgNPs and AgNPs/CHX enhanced the dentinal microhardness values. This could be related to the precipitation of silver nanoparticles onto the dentinal surface and into dentinal tubules [12]. 3.8 % Silver diamine fluoride nanoparticles were found to reach a 40 mm intra-tubular depth [32]. Incorportation of silver nanoparticles into propylene glycol could provide another reason. Being a viscous material with a low surface tension, it was shown to carry higher amounts of Safranin O dye into dentinal tubules [32]. Drawing a parallel, the majority of the studies that investigated the impact of AgNPs on dentin microhardness were in alignment with this finding. Silver nanoparticle solution was also shown to be a good antibacterial agent that would not adversely affect dentin properties [33]. Likewise, dentine microhardness values increased when treated with graphene oxide combined with silver nanoparticles [12]. In a clinical study, dental sealant with silver nanoparticles reduced the mineral loss of children's first molars [34]. Additionally, when combined with fluoride, nano silver arrested the dentin caries of children in two clinical trials [35,36]. Treatment of enamel caries with nanosilver raised its microhardness [37]. Contrarily, when used as an irrigating solution for 15 minutes, AgNPs decreased dentin microhardness, which was probably attributed to the negative charge imparted to the particles by the manufacturing method [4].

Collectively, our findings could imply that the beneficial effects of silver nanoparticles on dentin microhardness surpassed the detrimental effects of chlorhexidine, when combined together. Moreover, the AgNPs-CHX combination medicament can be a used as an enhanced antibacterial agent with reinforcing effects on dentin microhardness.

CONCLUSION

Within the limitations of the present study, it could be concluded that the application of AgNPs and AgNPs-CHX as medicaments could improve radicular dentin microhardness.

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