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EXPERIMENTAL BIOCERAMIC SEALER MODIFIED BY DIFFERENT CONCENTRATIONS OF MAGNESIUM HYDROXIDE NANOPARTICLES; ANTIBACTERIAL EFFECT AND FLOW. (AN IN VITRO STUDY)

Hagar Mostafa Talaat Mohamed [*], Dalia Ali Ahmed Moukarab [**]
and Asmaa Abdel-Hakeem Metwally Idreese [***]

ABSTRACT

Objective: This study aims to evaluate the antibacterial effect and flow of a bioceramic sealer modified by different concentrations of nano magnesium hydroxide (NMH).

Methods: NMH were characterized using transmission electron microscope and X-ray diffraction analysis. Then, added to Ceramoseal HBC by 0.025%, 0.05% and 0.1% in weight. for direct contact antibacterial test (DCT), the tested materials were added to wells of a 96-well plate. Bacterial suspension was added to each well, incubated for 1hour and 24 hours and transferred to agar plates for colony forming unit to be counted. Flow test was applied according to ISO standards and the diameters of compressed discs of tested materials were measured. The data were statistically analyzed using One way ANOVA test and Student t-test.

Results: For DCT test, all tested groups showed antibacterial effect compared to the positive control. At 1 hour, group I showed the highest antibacterial effect. At 24 hours, group I and IV were the same antibacterial effect that is higher than group II and III. Regarding flow, group I was highest flow and group IV was the lowest but all results were more than 17 mm.

Conclusion: NMH modified sealers showed antibacterial efficacy and their flow still according to ISO requirements.

KEY WORDS: *Ceramoseal HBC,* Nano magnesium hydroxide, Antibacterial, Flow

* Demonstrator in Biomaterials Department, Faculty of Dentistry, Assiut University, Assiut, Egypt
** Associate Professor of Endodontics, Head of Endodontic Department, Faculty of Dentistry, Minia University, Minia, Egypt.
*** Lecturer of Dental Materials, Biomaterials Department, Faculty of Dentistry, Minia University, Minia, Egypt.

INTRODUCTION

Endodontic treatment is aiming to provide a hermetic sealed system inside the root canal and prevent any future bacterial infection. It includes canal preparation, disinfection then obturation of the canals using a core filling material and endodontic sealers. [1]

Endodontic sealers have a very important role as obturating materials. They act as a binding paste between the rigid core and dentinal walls, fill the spaces, voids, lateral and accessory canals thus create a monoblock that is necessary for success of endodontic treatment. [2]

Different types of sealers are available according to their composition: zinc oxide eugenol, glass ionomer, calcium hydroxide, silicone, resin based and bioceramics. [3]

Bioceramic sealers have become widely used in endodontics over the last years. The main compositions of these sealers are calcium silicate and/or calcium phosphate. These materials make the sealers have antimicrobial, biocompatible and bioactive properties stimulating new hard tissue deposition. [3]

Recently, nanomaterials have been used as additives to improve the properties of the endodontic sealers. The nanoscale size of these materials and their increased surface area to volume ratio have improved the efficacy and clinical service of many existing endodontic materials. [4]

Nano magnesium hydroxide (NMH) are metallic nanomaterials with various biomedical applications due to their biocompatibility and low cost. These materials were introduced in dentistry owing to their bioactive properties and their antimicrobial activity against oral bacteria. [5]

Complete bacterial eradication from the root canal system during chemo-mechanical preparation is difficult. Therefore, it is very important for endodontic sealers as obturating materials to have antimicrobial effect thus killing residual bacteria and prevent bacterial reinfection. [6]

Flow of endodontic sealers determines their penetration into the root canal system and contributes to the interlocking between sealer and dentine that is important for a successful obturation. [7]

Therefore, this study evaluated the antibacterial effect and flow properties of a bioceramic sealer modified by different concentrations of NMH. The null hypothesis was that there is no significant difference between the tested materials in terms of antibacterial activity and flow

MATERIALS AND METHODS

This in-vitro study was approved by Research Ethical Committee of Faculty of Dentistry, Minia University (RHDIRB2017122004) with protocol number (707/2023) at meeting number (94)

Preparation of NMH

Magnesium hydroxide nanoparticles were purchased from Nano Gate company. They were sensitized by sol-gel technique as reported by ***Wahab et al. 2007***[8]. 0.2M of magnesium nitrate was prepared then dissolved in 100 ml of deionized water. While stirring, 0.5M of sodium hydroxide solution was added to the magnesium nitrate solution drop by drop. After few minutes, magnesium hydroxide precipitated in the bottom of the beaker. Stirring was continued for 30 minutes. Then, the formed precipitate filtered and washed using methanol to remove any ionic impurity. Centrifugal of precipitate was done at 5000 rpm/min for 5 minutes and dried at room temperature.

Characterization of nanoparticles:

Transmission Electron Microscope (TEM)

TEM was performed using JEOL JEM-2100 electron microscope at an accelerating voltage of 200 kV.

Xray Diffraction (XRD)

XRD pattern was done by XPERT-PRO Diffractometer system, with 2 theta (20^o - 80^o), with Minimum step size 2 Theta: 0.001, and at wavelength ($K\alpha$) = 1.54614^o.

Mixing of the bioceramic sealer with nanoparticles

NMH was mixed with the Ceramoseal HBC bioceramic sealer at concentrations of 0.025%, 0.05% and 0.1% in weight. These concentrations were determined following a pilot study evaluated the flow of the material at a decreasing concentration from 1%. These NMH concentrations didn't affect the setting of the material. Mixing was done by manufacturer using ultrasonic homogenizer for five minutes to insure thorough homogenization and proper dispersion of nanoparticles inside the sealer.

Antibacterial efficacy of NMH

NMH powders were investigated for their antimicrobial activity by dissolving them in 1ml of distilled water creating suspensions having the same concentrations of tested sealers (0.025%, 0.05% and 0.1%). then, their antibacterial activity was tested *Enterococcus Faecalis* (ATCC 29212) using direct contact test (DCT).[9]

Study design

Bioceramic sealer paste was divided into four groups regarding the percentage of added nanoparticles. Group I: Bioceramic sealer without added nanoparticles. Group II: Bioceramic sealer + 0.025% NMH. Group III: Bioceramic sealer + 0.05% NMH. Group IV: Bioceramic sealer + 0.1% NMH. Each group was tested for antibacterial effect and flow properties.

Antibacterial test using direct contact test (DCT)

Antibacterial effect was tested following the methodology of Zhang et al. 2009[9]. Tested materials were transferred to sterile insulin syringes. wells of a 96-well microtiter plate were coated with 100 μL of tested materials using the insulin syringes (10 sample of each group). Then, 10 μL bacterial suspension of *Enterococcus Faecalis* (ATCC 29212) made with a standard density of 0.5 on the Macfarland (1.5 x 10^8/ml) added to each well. 10 μL bacterial suspension was applied to the walls of uncoated wells as a positive control. The plate was incubated for 1 hour and 24 hours in a microbiological oven at 37° C. After that, 240 μL Tryptic Soy Broth (TSB) was added to each well and mixed using a pipette for 1 minute. Then, the bacterial suspension was transferred then diluted serially using TSB. Number of living bacteria was determined by culturing 20 μL of aliquots onto plates of Tryptic Soy Agar (TSA) after 10-fold serial dilutions. Plates were incubated at 37° C for 24 hours and formed colonies were counted and CFU/ml were calculated at the two different times in the experiment. experiments were done in triplicate.

Flow test

Flow test was applied according to ***ISO 6876/2012***[10] standard. A 0.05 mL of the tested materials applied to the center of a glass plate (60 x 60 x 3 mm) using a graduated syringe (10 samples for each group). After 180 ± 5 s, another glass plate weighing 20 ± 2 gram and 100-gram load were applied on the top of the material. After 10 minutes of manipulation, the load was removed and the largest and the smallest diameters of the sealer disks were measured using a caliper. When the difference between the two diameters less than 1 mm, the mean value between them was recorded. If the difference between the two diameters was more than 1 mm or the disc was observed to be not uniform circle, the test was repeated. The mean of 10 replicates for each group was taken and considered as the result of the test. Values were recorded for each group to be analyzed statistically.

STATISTICAL ANALYSIS

Statistical analysis of the data

Data analysis was performed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Normality of distribution was tested by Shapiro-Wilk test. Data was described by mean and standard deviation. Significance of the results was judged at the 5% level.

One way ANOVA test was used to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons. Student t-test was used to compare between two studied groups.

RESULTS

Characterization of nanoparticles:

Transmission Electron Microscope (TEM)

The TEM image of Magnesium hydroxide revealed that the prepared Mg(OH)2 in nanoscale with average size 80± 10nm and spherical like shape as shown in figure (1).

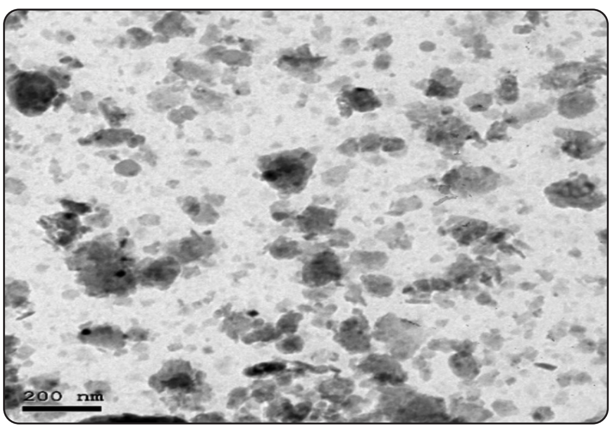

Fig. (1) Shows the TEM image of NMH

X-ray Diffraction (XRD)

The XRD pattern is shown in figure 2. The patterns are consistent with the pattern of crystalline magnesium hydroxide Nanoparticles. The XRD of NMH which was verified using the JCPDS card no. 00-007-0239, shows broad peaks with high intensities of NMH crystallites with a maximum peak from (101) crystal plane at 2 Theta of 38.017 degrees.

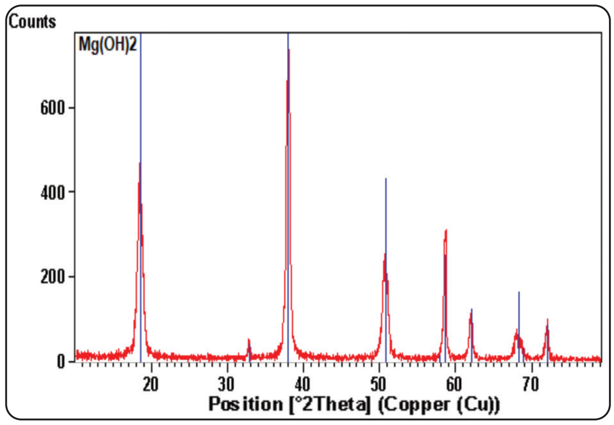

Fig. (2) Shows XRD analysis.

Antibacterial test for the solutions of NMH

The mean bacterial count of the tested groups was ranged between 500x 10^3 and 600x 10^3 at 1 hour, while it was ranged between 28.20x 10^3 and 210x 10^3 at 24 hours. All tested groups showed statistically significant antibacterial effect compared to the positive control at both time intervals.

At 1 hour; the highest antibacterial effect was found in group III while group I was the lowest and the results were statistically significant.

At 24 hours; groups III was the highest antibacterial effect while group I was the lowest. There was statistical significant difference in all groups.

For comparing between 1 hour and 24 hours in each group Student t-test was used. There was statistical significant increase in the antibacterial effect at 24 hours' time of contact compared to 1 hour in all tested groups.

The mean antibacterial effect and SD of tested groups are shown in table (1) and figure (3).

TABLE (1) Mean and SD of bacterial count (CFU/ml) at 1h and 24h time intervals.

Groups / Time	Positive control	Group I	Group II	Group III	p_1
1 hour	3500.0[a] ± 0.0	600.0[b] ± 35.36	510.0[c] ± 65.19	500.0[c] ± 35.36	<0.001*
24 hours	3500.0[a] ± 0.0	210.0[b#]±12.25	112.0[c#] ±13.04	28.20[d#] ± 2.49	<0.001*
p_2	–	<0.001*	<0.001*	<0.001*	

p1: p value for comparing between the four studied groups in each time

p2: p value for comparing between 1 hour and 24 hours in each group

Significance level p≤0.05, *significant ***Means with the same superscript letter are not significantly different.***

#: 1 hour Significant with 24 hours

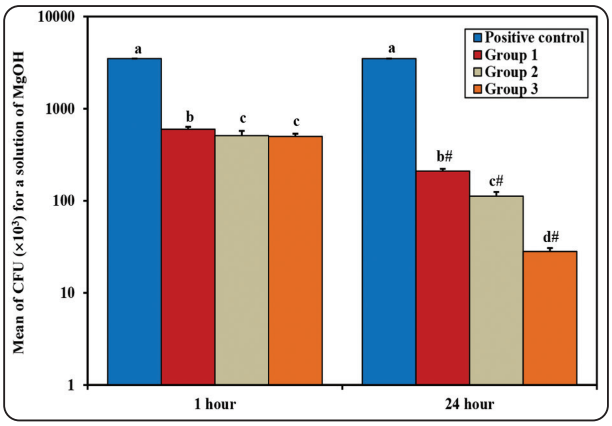

Fig. (3) Bar chart illustrating mean value of bacterial count (CFU/ml) of tested groups.

Antibacterial test for the tested endodontic sealers

The mean bacterial count of the tested groups was ranged between 550x 10^3 and 1360x 10^3 at 1 hour, while it ranged between 3.40x 10^3 and 5.80x10^3 at 24 hours. All tested groups showed statistically significant antibacterial effect compared to the positive control at both time intervals.

At 1 hour; control group was the highest antibacterial effect while group IV was the lowest. Statistical significant difference between all tested groups was found.

At 24 hours; groups I and IV were the same antibacterial effect while group II and III showed significant lower antibacterial properties.

Comparing between 1 hour and 24 hours in each group, there was statistically significant increase in the antibacterial effect at 24 hours' time of contact compared to 1 hour in all tested groups.

The mean antibacterial effect and SD of tested groups are shown in table (2) and figure (4).

TABLE (2) Mean and SD of bacterial count (CFU/ml) at 1h and 24h time interval.

Groups / Time	Positive control	Group I (control)	Group II	Group III	Group IV	p_1
1 hour	3500.0[a] ± 0.0	550.0[e] ± 50.0	790.0[d] ± 74.16	1100.0[c] ± 100.0	1360.0[b] ± 134.2	<0.001*
24 hours	3500.0[a] ± 0.0	3.40[c#] ± 1.34	5.80[b#] ± 1.64	4.80[bc#] ± 0.84	3.40[c#] ± 1.52	<0.001*
p_2	–	<0.001*	<0.001*	<0.001*	<0.001*	

p1: p value for comparing between the four studied groups in each time

p2: p value for comparing between 1 hour and 24 hours in each group

Significance level p≤0.05, *significant ***Means with different superscript letters are significantly different.***

#: 1 hour Significant with 24 hours

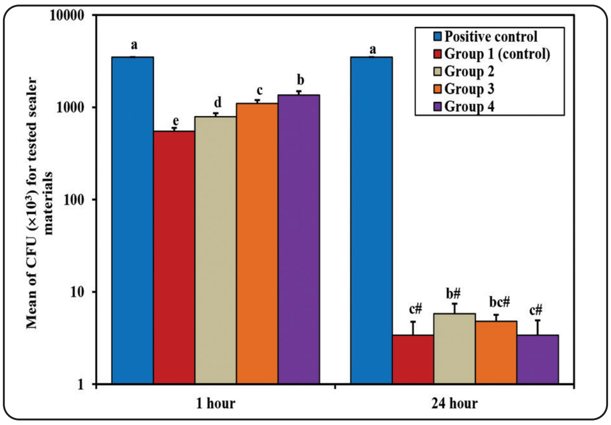

Fig. (4) Bar chart illustrating mean value of bacterial count (CFU/ml) of tested groups.

Flow test

The flow of the tested materials ranged between 17.93 and 21.83. Group I was the highest mean value; while the lowest value was recorded for group IV. The mean flow and SD of the tested groups are presented in table (3) and figure (5).

TABLE (3) Mean and SD value for flow test (mm).

Groups	flow test (mm)		P value
	Mean mm	SD	
Group I (Control)	21.83[a]	±0.97	<0.001*
Group II	19.90[b]	±1.34	
Group III	18.11[c]	±0.50	
Group IV	17.93[c]	±0.50	

Significance level p≤0.05, *significant

Means with the same superscript letter are not significantly different.

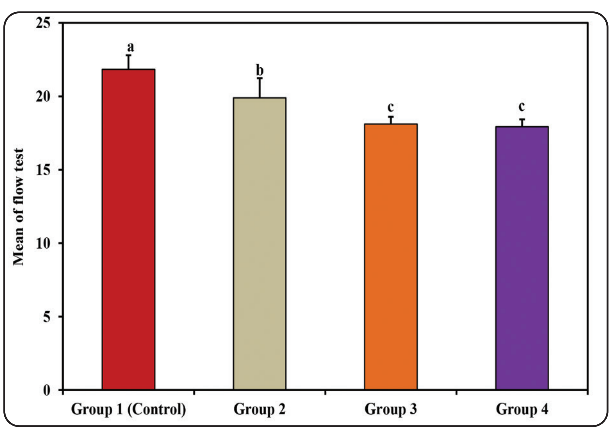

Fig. 5: Bar chart illustrating mean value of Flow test (mm).

DISCUSSION

Bioceramic based root canal sealers have been lately used in endodontics due to their biocompatible properties and chemical composition which encourage forming a crystalline structure like natural tooth composition enhancing creation a strong chemical dentin sealer bond. [11]

Ceramoseal HBC is a premixed MTA based sealer containing nano hydroxyapatites in its composition in addition to calcium silicate and calcium oxides. This unique composition makes this sealer has an alkaline effect which encourages its antimicrobial effect. Moreover, this sealer has hydrophilic properties and utilizes dentin moisture forming a chemical interstitial bond with dentin that increases sealing and adaptation inside the root canal preventing future bacterial invasion. [12]

NMH are metallic nanoparticles known for their high antibacterial effects, biocompatible properties and their low cost. Inspired by the positive properties of these NPs, this study was conducted to test the effect of modifying Ceramoseal HBC endodontic by them at different concentrations. [13]

E. faecalis is among the main causes of endodontic failure. Many studies have reported its high presence in cases of endodontic failures and treatment resistant root canal infections. Their high pathogenicity is owing to their resistance to alkalinity, attachment to dentin, and poor nutrition requirements that increase their survival rate inside the root canal. [14]

DCT is used to evaluate the antibacterial activity of materials that are insoluble. It measures the effect of physical contact between test materials with bacteria. This overcomes the limitations of the Agar Diffusion test. The advantages of this method are reproducibility and quantitative results. [15]

NMH has been reported for its antimicrobial effects in many studies. Dong et al.[16] and Nakamura et al.[13] have demonstrated its high antibacterial properties against E.coli. Meng et al.[5] demonstrated its antibacterial effect against *S. mutans*. Okamoto et al.[17] also reported the inhibitory effects NMH against cariogenic bacteria (*S. mutans and S. sobrinus*).

Regarding the present study, NMH solutions having the same concentrations of tested sealers were tested for their antibacterial effect to investigate the effect of NMH alone. The results showed a strong antibacterial effect against *E. Faecalis* and the antibacterial effect of solutions were increased at longer time of contact which is consistent with previous studies. [5] [13] [16] [17]

A solution of 0.1% NMH showed significantly higher antibacterial effects than the other lower concentrations. This may be due to the antibacterial effect of NMH particles which is concentration dependent as there is larger amount of nanoparticles to come in direct contact with bacterial cell wall causing bacterial damage. [5]

For antibacterial test of the modified bioceramic sealers, tested sealers revealed higher antibacterial effect compared to the positive control group at the two time intervals. The results of 1 hour antibacterial test revealed that the control group has the highest antibacterial effect against bacteria. This antibacterial effect is due to the alkalinizing activity of fresh mixed sealers. The initially lower antibacterial activity of the modified groups at 1 hour contact may be attributed to that hydrophilic sealers have not yet completely dissolved and reacted with dentin moisture delaying the release of NPs which are responsible for damaging of larger bacterial count. Therefore, in 1 hour contact the bacterial death is mainly due to the alkalinizing effect of fresh sealers inside root canals. [18]

The increase of antimicrobial effect after longer time of contact may be due to the hydrophilic properties of the MTA based sealer and furthermore release of calcium and hydroxyl, which promote more alkalinizing activity. This also could explain the better antibacterial action of modified sealers after 24 hours, as there could be simultaneous further release of larger concentrations of NMH with longer times of contact with dentin. [19]

Flow of endodontic sealers determines its ability to infiltrate difficult areas to reach in root canal system such as accessory canals, isthmuses and dentin abnormalities. Flow of endodontic sealers is affected by their Particle size, film thickness, shear rate and diameter of the root canal. [20]

Endodontic sealers should have a moderate flow rate. Excessive flow increases the probability of sealers to reach the periapical system and inadequate flow reduces penetration of sealers into irregularities and accessory canals. According to ISO 6876/2012, the recommended flow of a root canal sealer shouldn't be less than 17 mm. [10,20]

Results from this study revealed that there is a decrease in the flow properties of tested groups as the NPs concentration is increased. Nevertheless, all tested materials values were higher than 17 mm as prescribed by ISO standards which supports previous studies. [10]

Therefore, the null hypothesis was rejected as NMH increased the antibacterial effects and decreased flow of the bioceramic sealer.

CONCLUSIONS

NMH has high antibacterial efficacy against *E. Faecalis*. The bioceramic sealer modified by NMH showed good antibacterial properties especially that modified with 0.1%NMH. All tested sealers were in accordance with the ISO specifications no 6876 limits regarding flow.

RECOMMENDATIONS

NMH solutions are recommended to be used as irrigants during endodontic treatments. 0.1% NMH modified sealers are recommended to be used in endodontics for their antibacterial properties. Further studies are required to test cytotoxic effects of NMH modified sealers. Also, it is recommended to evaluate the antibacterial properties of NMH modified bioceramic sealers for longer periods

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