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Antibacterial Activity of Bioactive Glass 45S5 and Chitosan Incorporated as Fillers into Gutta Percha

Ahmed I AL-Jobory*, Raghad AL-Hashimi

Department of Aesthetic and Restorative Dentistry, College of Dentistry, University of Baghdad, Baghdad, Iraq

ABSTRACT

Aim: To evaluate the anti-bacterial activity of bioactive bioglass 45S5 and Chitosan incorporated as fillers in gutta percha against Enterococcus faecalis.

Methods: The anti-bacterial activity of bioactive bioglass 45S5 and Chitosan incorporated as fillers in gutta percha against Enterococcus faecalis were investigated by measuring the inhibition zone that represent the sensitivity or toxicity of materials against microorganism.

Results: there is highly significant difference between control and new modified gutta percha at all specimens, while the control group showed no sensitivity to microorganism.

Conclusion: Bioactive bioglass BG45S5 and chitosan showed in vitro highly antibacterial effects when mixing with gutta percha against Enterococcus faecalis comparing to commercial (control) gutta percha.

Key words: Gutta percha, Bioactive bioglass BG45S5, Chitosan, Anti-bacterial, Enterococcus faecalis, Inhibition zone

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Corresponding author: Ahmed I AL-Jobory

e-mail✉: drahmedaljobory@tu.edu.iq

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INTRODUCTION

The principal objectives of endodontic therapy are to clean and shape the root canal system and to fill the canal system completely in three dimensions. This aimed to prevent penetration of bacteria and their products into the periapical tissues and develop a hermetic seal [1]. Inadequate obturation of root canal system results in failure of endodontic therapy [2]. A better understanding of root canal anatomy, and improved materials, root canal therapy is achieving an increasingly high overall success rate [3].

However, bacteria inside the root canal system have a significant impact on this success rate. When a tooth is infected before treatment, the success of root canal therapy drops to 79%, as compared to the 93% success rate of root canal treated teeth without apical periodontitis [4]. A few bacterial species, predominantly facultative anaerobes [5,6], are responsible for causing apical periodontitis observed in root canal failures [7]. Root canal failures result either from these microorganisms leaking into the canal after its obturation or from bacteria not eliminated during therapy. The most common genera found to be responsible for root canal failures are *Enterococcus, Fusobacterium, Propionibacterium*, and *Actinomyces* [8]. Removing all bacteria in the canal before obturation has proven to be difficult. Therefore, improving the cleaning and disinfection phase of treatment is of crucial importance and has been the impetus for the advancement of instrumentation and irrigation [9,10].

A limited number of studies in the literature have explored the advantages of using obturation materials with antimicrobial properties to prevent bacterial contamination after endodontic procedures [11-13]. Standard gutta-percha has been shown to have an intrinsic effect on very few bacteria species [11,14].

Research has also been con-ducted on the antibacterial activity of gutta-percha points

containing established root canal medications, including calcium hydroxide, zinc oxide, chlorhexidine, and iodine-polyvinylpyrrolidone alone or used in combination. However, none of the gutta percha tested possessed an inhibitory activity strong or broad enough to disinfect root canals contaminated with common endodontic pathogens, including *E. faecalis* [12,14]. Chitosan similar in structure to cellulose composed of one monomer of glucose [15]. It is a polysaccharide comprising copolymers of glucosamine and N-acetyl glucosamine. It can be derived by partial deacetylation of chitin from crustacean shells [16]. It is an environmentally friendly polymer [17]. Chitosan is soluble in diluted acid, in addition, chitosan is stabilized by H–bond network in the solid, providing good mechanical properties [18]. Chitosan has a wide variety of applications such as microbial biosorption or metal removal for wastewater treatment [19].

Chitosan used in different biomedical application due to its antibacterial effect, biocompatibility, and biodegradability. It is used in surgery in tissue engineering which concerned about the restoration of damaged organs using spontaneity scaffolds regeneration that mimic the native tissue [20]. Also, it is used in drug delivery and encapsulation of sensitive drugs, due to it's an interesting biodegradability, antibacterial activity and antifungal properties [21]. In addition, due to its renewability and bioavailability, since it is the second most abundant polymer in nature after cellulose, Chitosan is applicable in food packaging [22]. Bioactive glasses (BAG) are one such group of biomaterials which are used in the fields of dentistry and orthopaedics to repair or replace damaged bone [23]. A material is said to be bioactive, if it gives an appropriate biological response and results in the formation of a bond between the material and the tissue [24]. Bioactive glasses are composed of calcium and phosphate which are proportionally similar to the hydroxyapatite present within the bone [25]. They also have the unique ability to dissolve in biological fluids and release ions such as silica, sodium and calcium. This ionic dissolution facilitates hydroxyapatite formation and direct bonding to bone and soft tissues [26]. In addition, the quick dissolution with rapid change in pH of the surrounding medium enables these glasses to exhibit anti-bacterial properties [27]. They have a wide range of medical and dental applications and are currently used as bone grafts [24], scaffolds [28] and coating material for dental implants [24].

METHODOLOGY

Fabrication of new bioactive gutta percha

The total amount of the Gutta Percha (Dentsply Maillefer, Switzerland) was weighed by four digits sensitive balance ADAM AFA-210LC (UK) which was 1.5 gm. The filer weights were 1% for bioactive powder 45S5 (MO-SCI, USA) and chitosan (SHAANXI SANGHERB BIO-TECH INC, China) where they incorporated by replacement of 0.015 gms (for each filer) of the Gutta-Percha with same weights of powder. So, these percentages were clinically applicable (ISO 6876/2012 standards).

Fabrication of gutta percha

Gutta percha points (1.5 gms) were taken and placed in glass beaker. The beaker has been placed in electrical oven CARBOLITE (UK) at 200ºC for 15min. the beaker has been taken out of the oven, and then the gutta percha points became semi-soft. A small chloroform (Riedel-de Haën, German) amount (5ml) has been added to the beaker to solve the gutta percha with continues moving of solvent with glass stick till complete solvent of gutta percha and become like suspension of liquid [29].

Preparing of filers

The bioactive bioglass (with weights 0.015gms represent filler percentage 1%) was dissolved in formic acid (SCR-China) (5ml) and stirring for 3 days until all particles was dissolved. Then the chitosan (with weights 0.015gms represent filler percentage 1%) has been dissolved in 1.0% of the acetic acid (MERCK- German) (v/v) by using magnetic stirrer. The viscous chitosan was adding to previous viscous bioactive bioglass and mix by using a magnetic stirrer. All the viscous mixture of bioactive bioglass and chitosan was adding and mixed together and the total fillers weight was 0.03gm.

Mixing the gutta percha with filers

All the mixture of filler was adding slowly to solvent gutta percha and mixed with a magnetic stirrer till the materials acquired a semi viscous state, it placed in glass petri dishes until complete drying and setting.

Preparing of sample as a disk

A mold was design and fabricated to acquired materials to produce a 5mm width and 2mm height of materials. A 0.035gms of materials was selected and add to cylinder of fabricated devices and a constant scrowing for 1 min was applied to materials to obtain a homogenous disc for all groups (Figures 1-3).

Microbiological study

This study of microbiological was done in the Tikrit University, labs. Of Pharmacy College, to evaluate and test the antibacterial activity (inhibition zone) of the experimental material (New gutta percha) as a disk of 2mm height and 5mm width against selected *Enterococcus faecalis* microorganism.

Mueller hinton broth (MHB)

Based on the instructions of the manufacturer, the preparation of media was done by placing 21gm of powder in 1 L of the distilled water in graduated beaker. Autoclaving the solution at 1210C at pressure 15psi for 15minutes after the complete dissolution of the powder. Then the solution has been left for cooling at the room temperature and thereafter, tightly closed and kept in a refrigerator until being used (Figure 4).

Figure 1: Special mod ready to compress the mixture.

Figure 2: Special mod parts.

Figure 3: Disc of gutta percha (0.035 grms) with 5mm width and 2mm height.

Mueller hinton agar (MHA)

Based on the instructions of the manufacturer, the preparation of the media was done by placing 38.0gm of the MHA in 1 L of the distilled water and boiled in a graduated beaker until complete dissolving of the powder. Autoclaving the solution at 121c0 at pressure 15psi for 15min, then left for cooling at 45^0C-50^0C. The media has been poured in a pre-sterilized petridish and left to solidify at the room temperature and stored in a refrigerator to the point of usage (Figures 5 and 6) [30,31].

Bacterial culture

Enterococcus faecalis have been maintained in stock cultures that are frozen in brain heart infusion (BHI) broth. *E. faecalis* colonies have been inoculated in BHI broth at 370C for 24 hours. *Enterococcus faecalis* were obtain from Media hospital, Erbil, with ATCC 29212 (American Type Culture Collection).

Sensitivity of *E. faecalis*

Ten experimental materials were used to evaluate the sensitivity of the E.- faecalis to new gutta percha. The media of the MHA in the Petri-dish has been inoculated with 100.0µl of the *E. faecalis* suspension which has been prepared as 0.50 Mc-Farland (standard of turbidity 0.50). This number of standards includes about 1x108 of the bacterial cells per ml. The inoculum has been spread in all the directions through the use of the sterilized cotton swaps (Figure 7). Ten petri dishes were prepared, the plates were aerobically incubated for 18h-24h at a temperature of 370c. (Figure 8).

The inhibition zones that are clear zones of no bacterial growth have been measured over the diameter of every one of the petri dished with the use of the digital Vernier caliper, none of the zones have indicated a full bacterial resistance to the agent (Figures 9-11)[32].

RESULTS

The mean and standard deviation values of the inhibition zone of the *E. faecalis* are presented in Table 1. The result showed that the experimental gutta percha that mixed with chitosan and bioactive bioglass has best and power effect against *E. faecalis* (Figures 12 and 13).

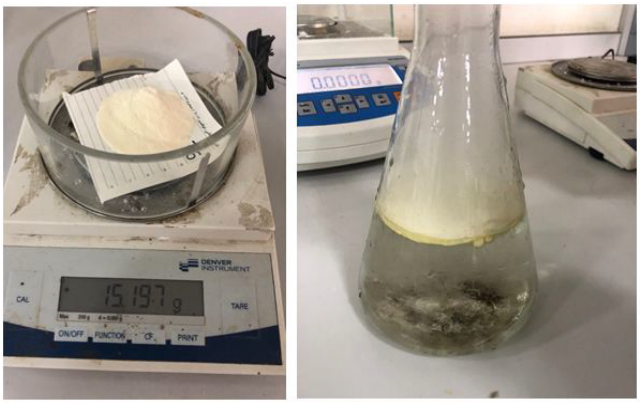

Figure 4: Preparation of mueller hinton broth (MHB).

Figure 5: Preparation of mueller hinton agar (MHA).

Figure 6: Pressure up gradually to 121 PSI.

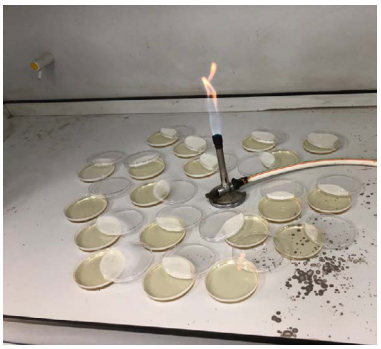

Figure 7: Petri-dishes are ready to inoculated with *E. faecalis*.

Figure 8: Incubation of plates.

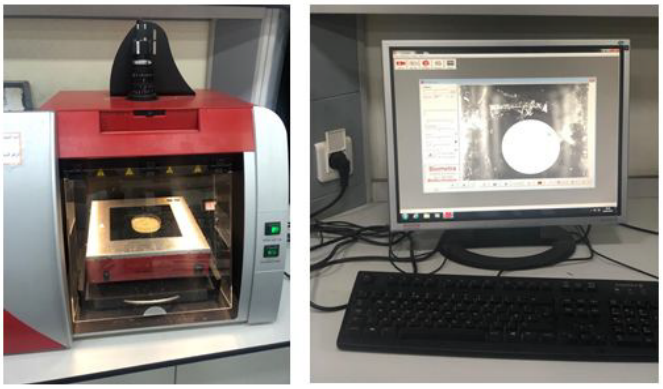

Figure 9: Biometra analytic device (BioDocAnalyze).

Figure 10: Biometra analytic device (BioDocAnalyze).

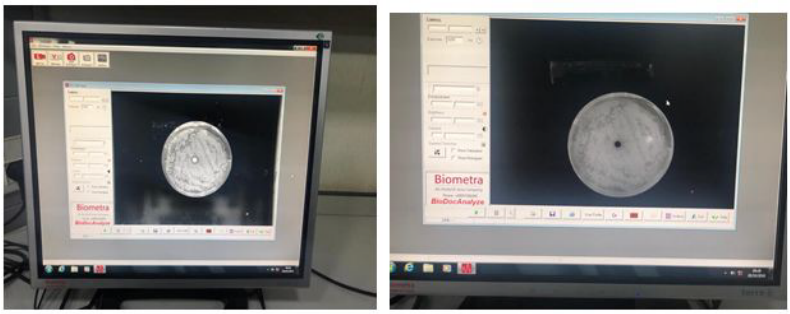

Figure 11: Analytic result by using different lights source.

Table 1: Descriptive statistics of inhibition zone.

Inhibition zone	Experimental gutta percha	Control gutta percha
1	3.4 cm	0
2	3.3 cm	0
3	3.5 cm	0
4	3.3 cm	0
5	3.4 cm	0
6	3.4 cm	0
7	3.5 cm	0
8	3.5 cm	0
9	3.7 cm	0
10	3.6 cm	0
Mean	3.46 cm	0
SD	0.21 cm	0

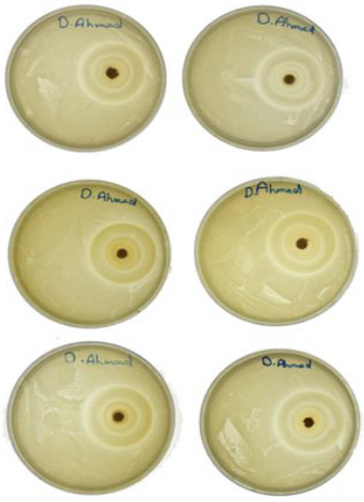

Figure 12: New gutta percha showing the inhibition zone.

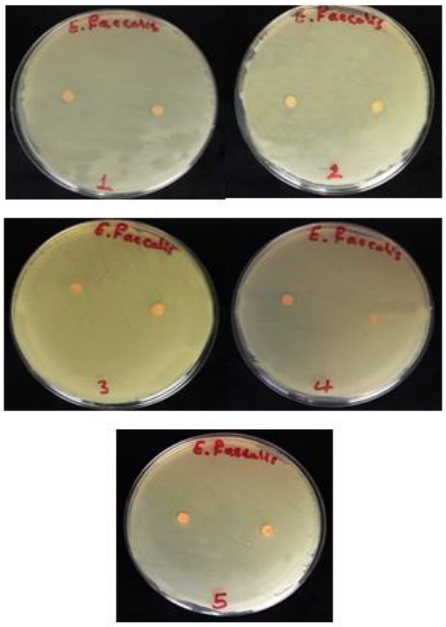

Figure 13: Control gutta percha showing the no inhibition zone.

DISCUSSION

In 1867, Bowman developed gutta percha as one of the significant filling materials, such material contains chlorhexidine and pastes of the calcium hydroxide and, while traditional GP is examined for their anti-bacterial activities via Jhamp et al. [33]. In addition, the results indicated that traditional Gutta percha hadn't had any anti-bacterial activities, while the microbial activity is verified via chlorhexidine Gutta percha as well as the Ca (OH)2 pastes. Evidences of small antibacterial activity with regard to the cones of gutta-percha is existing and it is because of zinc oxide (ZnO), the main cones' component [34]. The major factors related to endodontic treatment failure were persistent microbes in peri radicular region or root canal system. Dissimilar to the primary endodontic infections, that were polymicrobial and dominated via the Gram negative anaerobic rods, the dominant micro-organism which has been included in secondary infections were the facultative anaerobes such as the *Enterococcus faecalis*, such bacteria group is specified as major micro-organism existing in periapical lesions refractory and chronic apical periodontitis [35]. Long-term survival related to *E. faecalis* in the obturated root canals which are fundamentally depending upon the type of endodontic sealer as well as microbial gelatinase activities, for instance, the organism's virulence traits [36].

The dental materials are utilized for the obturation in the treatment of the root canal which is needed to be biocompatible and anti-bacterial, while the anti-bacterial activity which is related to cones of GP was indicated for being unimportant, such disadvantage making them less effective from the microbial infections following the process and should be enhanced. In addition, the materials of ZnO and $Ca(OH)_2$ were majorly indicated in dental practices as anti-microbials [37]. Bioactive bioglass BG 45S5 and chitosan have been shown as an effective medicament and their antimicrobial activity was proved, yet its action is limited via the short time for interaction [36-39].

In recent years, interesting in the antibacterial properties of bioactive glass was increasing. In our finding results, the experimental gutta percha with a great antibacterial effect than conventional (control) gutta percha, this due the filler effect of bioactive bioglass and chitosan. The antibacterial effect of bioactive bioglass was because of increasing the local pH after exchanging ions of Na with the protons in body fluid. Shifting to high alkaline environment was considered to be stressful for the bacteria, that are responding to changing ultrastructure and morphology, changing the expression pattern related to numerous proteins and genes. This may suggest to be the golden mechanism of bioactive glass to mediating the antibacterial action, this result found to be agreed with [40-44].

Another factor associated to the antimicrobial properties related to bioactive glass was the release of the ions of the calcium, silica and phosphate, resulting in perturbations regarding the bacteria's membrane potential and determine high osmotic pressure. The solutes' concentration in bacterial cytoplasm was typically high compared to that identified in surrounding environment, leading to positive pressure on cell membranes. Unexpected increment in the external solutes concentrations resulting in fast water efflux as well as pressure drop over cell membrane, which led to modified cell's size, cell shape, as well as membrane stress levels. These results are in accordance with the results obtained via [45-47]. The bioactive glass is showing activities against many clinically significant strains of bacteria: Gram negative and Gram positive species, also anaerobic and aerobic species [48-51].

Bioactive glass with many chemical compositions as well as many particle sizes. In particular, the particles' dimensions are determinant of anti-microbial activities. Actually, the decrease in the particle size and subsequent increment in the surface area might be enhancing bioglass contact with the aqueous environment, therefore augment ions' diffusion, local pH, and, finally, osmotic pressure, such results are in accordance with the results of Waltimo et al. [52] and Coraça-Huber et al. [53]. At the same time, chitosan NPs showed high antibacterial activity in comparison to their parental chitosan material because of the elevated ratio of surface to volume. It was indicated for showing antibacterial properties against the oral bacterial pathogens [54,55].

A lot of researches indicated that chitosan showed antimicrobial activity, yet the main approach isn't totally clarified [56]. The cationic nature is one of these hypotheses. Penetration of bacterial cell walls by chitosan and inhibit DNA transcription and the same time inhibit mRNA synthesis, these were done by chitosan's low molecular weight, whereas the high molecular weight chitosan has distinctive impact because it is binding to the negatively-charged components on bacterial cell wall, which change the permeability of the cells and blocking transport into cell [37,57,58]. Gram-negative bacteria affect more from this hypothesis because the cell wall of these bacteria had more negative charge than gram-positive bacteria [37,59].

Molecular weight, environmental pH value as well as the degree of acetylating related to chitosan will affect the binding to bacterial cell wall. In addition, the positive charge in the chitosan polymer will increase low pH which lead to strong binding to bacterial cell wall [59]. Younes et al. [60] in 2014 said that the degree of acetylation of chitosan in lower value and lower pH give better antibacterial activity to chitosan. However, this was very obvious toward Gram-negative bacteria.

There is a close relation between antibacterial activity as well as the hydrophilicity related to cell's wall, thus the action was specific and showing low toxicity towards the mammalian cells [61]. Another reason which made the chitosan more powerful because of the nanoparticles which used in this study. Antibacterial nanoparticulate were indicated for having high antibacterial activity compared to antibacterial powders. In addition, the charge density and high surface area of nanoparticulate, enabled for achieving high interaction degree with negatively charged surface related to the bacterial cells.

For our results the finding is agree with Mohan et al. [37] who indicated that chitosan impregnated gutta percha points exhibited high (and considerable) antifungal and antimicrobial activities, also enhanced the mechanical properties when put to comparison with commercial gutta percha points and using nano-technology for the dental applications for betterments in the clinical areas.

CONCLUSION

Bioactive bioglass BG45S5 and chitosan showed in vitro highly antibacterial effects when mixing with gutta percha against *Enterococcus faecalis* comparing to commercial (control) gutta percha.

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