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Evaluation of the Antibacterial Effect of Bioactive Dental Restorative Materials: *in vitro* Study

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ABSTRACT

Aims: Due to their remarkable good sealing ability and compatibility, bioactive dental restorative materials possess promising characteristics that can assist in reducing secondary caries incidence. Another characteristic that would be beneficial for these materials in promoting protection against secondary caries formation is the antibacterial property. Therefore, the purpose of this study was to evaluate the antibacterial properties of two bioactive dental restorative materials: Theracal LC and Activa Bioactive Restorative and compare them with three conventional restorative materials.

Methods:

Direct contact test with *Streptococcus mutans* (*S. mutans*) was used to evaluate the antibacterial properties of the dental materials. Samples (4 mm X 4 mm diameter) were made from each material and placed in a well of the 24-well plate along with 500 μl of a solution containing 1 x 10^5 CFU/ml of *S. mutans*. Chlorhexidine was used as a positive control whereas no addition was used as a negative control. The plate was incubated for 24 hours at 37°C. *S. mutans* growth was evaluated by measuring the optical density at 590 nm.

Results:

The results showed that both bioactive and conventional dental restorative materials possess weak antibacterial effects. The highest median of inhibition was reported for chlorhexidine (96.28 %), followed by glass ionomer cement (GIC) (41.89 %). The lowest median of inhibition was reported for composite resin (6.41 %). These results indicate that GIC had higher antibacterial effects in comparison with the other bioactive and conventional

materials. Kruskal-Wallis reported a p-value of 0.007, indicating a significant difference between the antibacterial effects of the materials. Pairwise multiple comparisons with an adjusted p-value of 0.005 were done with Man-Whitney U. A p-value of 0.003 was reported for the comparison between GIC and Activa Bioactive materials.

Conclusion:

The bioactive and conventional dental restorative materials tested in this study possess weak antibacterial effects. GIC showed the highest antibacterial effect among the materials tested while composite showed the weakest effect. Future studies should investigate the antibacterial effects of the bioactive dental materials in vivo in order to evaluate their interaction with bacterial species involved in dental caries, and other oral environmental factors such as saliva and pH.

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INTRODUCTION

Dental caries is a widespread disease that is very common in children and adults [1]. The disease is induced by changes in the ecological equilibrium of the oral environment that results in localized demineralization of the teeth hard tissues by the action of acidogenic bacteria species that ferments carbohydrates to produce acid byproducts [2, 3]. Disruption in the balance between the various bacterial species in the oral cavity induced by frequent intake of fermentable carbohydrate and lactic acid production lead to a drop in the pH of the oral environment, which promotes a higher presence of acidogenic and acid-tolerant bacteria and reduces the growth of other species that are associated with healthy enamel [3].

Several bacterial species have been claimed to be associated with caries lesion initiation and progression [4, 5]. Examples of these species include *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus* [4, 5]. *S. mutans* species have been investigated thoroughly in the literature as the most associated bacteria in caries initiation and development [4, 5]. The cariogenic properties of *S. mutans* have been attributed to various characteristics such as the ability to produce lactic acid from fermenting carbohydrate resulting in low pH of the dental plaque around the teeth surfaces [5]. Also, the ability to attach to the teeth surfaces, which is mediated by extracellular polysaccharide that the bacteria synthesize from sucrose [5, 6].

Management of dental caries depends on how far the lesion affected the teeth tissues. White spots lesions or localized demineralization with no surface cavitation are managed with preventive treatment measures such as topical fluoride and fissure sealants application [7]. However, lesions extended in the enamel and dentine that did not reach the pulp or affected

its vitality irreversibly are treated by the removal of the destructive carious tissues and placement of dental restorative materials to replace the lost tissues [7]. However, the restorative approach does not guarantee that the restored teeth tissues will not be infected again as the recurrence of the lesion around the restorative filling materials remains possible. In this case, the lesion is known as secondary or recurrent caries [8].

Secondary caries or recurrent caries refers to the lesion that occurs over time at the margins between an existing restoration and tooth hard tissues [8]. Several studies have reported that the most common cause of restorations failure and replacement is secondary caries regardless the locations of the restored teeth, the sizes, and the number of surfaces of the dental fillings [8-10]. Moreover, Secondary caries was reported as the major reason for failure and replacement of fluoride-releasing filling materials such as glass ionomer cement (GIC), resin-modified GIC, and compomer [9, 10].

Secondary caries results in the need to replace the filling materials frequently and that is one of the aspects that makes the dental treatment highly expensive [11]. Thus, in an attempt to overcome the problem of restorations failure due to secondary caries, researchers investigated the dental restorative materials for any potential antimicrobial effect [12-17]. Also, they explored the possibilities of creating dental materials with genuine inhibition properties through the addition of some components known to have an antibacterial effect such as chlorhexidine and antibiotics [18]. The antimicrobial activity of dental materials renders them an advantage in preventing bacterial growth and thus reducing the incidence of recurrent caries [12, 17, 19].

Some components of various dental materials have been investigated and claimed to have antimicrobial properties [17, 19-22]. Examples of those, the potential role of fluoride released from some materials such as GIC, resin-modified GIC, and polyacid-modified composite resin (compomer) in preventing or reducing the incidence of recurrent caries [17, 19, 21, 22]. Also, the alkaline properties that some dental materials possess were reported to contribute to the bacterial inhibitory effect of several dental materials [20].

Bioactivity

The interaction between teeth hard tissues and the restorative dental materials was thought to be passive. However, in the last decades, researchers investigated the interactions that occur at the interface between some dental materials and teeth hard tissues and found that the interfacial layer is an area of active interactions [23, 24]. In the recent years, there has been a significant interest in a specific property of some dental materials, which is bioactivity [23, 25, 26]. Bioactivity was defined by Hench et al. as "A bioactive material is one that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material" [27].

In term of dental materials, the bioactivity has been referred as the ability to produce hydroxyapatite crystals at the interface between the surface of the materials and the teeth tissues in the presence of simulated tissue fluid [28-30]. The hydroxyapatite crystals are formed as a result of the interaction between the calcium ions released from the bioactive materials and phosphate from the simulated tissue fluids. These mineral crystals deposit at the interface between dentine and the material surface [28]. Several bioactive materials were introduced and

are being used in the restorative and endodontic dentistry. Examples of bioactive materials that are used in the dental field include tricalcium silicate-based materials, calcium aluminate-based materials, and bioactive resin-based materials [28, 31, 32].

The interactions between a dental material and teeth hard tissues through adhesion and chemical bond are properties already seen in the conventional dental material GIC. GIC exchanges and releases ions at the interface with dentine [33-35]. Also, it was found to play a potential role in remineralization of dentine through fluoride release [34, 35]. However, in term of bioactivity GIC showed no bioactivity [31]. Studies investigated the bioactivity of GIC in comparison with bioactive calcium aluminate and calcium silicate-based materials and showed that despite ions release and exchange between GIC and the tooth, GIC failed to form hydroxyapatite crystals at the interface with the dentine [24, 31]. Also, it was found that the interface between GIC and dentine was composed only of infiltration of polyacrylic acids and tartaric acid components of GIC into dentine [24].

Bioactive Dental Materials and Their Antibacterial Properties

1. Theracal LC

Theracal LC (Bisco Inc, Schamburg, IL, USA)**:** is a resin-modified calcium silicate material that has been developed as a liner or base for direct and indirect pulp capping uses [36]. The material consists of calcium silicate components which represent about 40-50% of the

material. Also, it contains a resin component that includes several monomers such as urethane dimethacrylate (UDMA), bisphenol A-glycidyl methacrylate, (BisGMA). Other constituents of Theracal LC include fumed Silica as thickening agents, and barium sulfate or bismuth oxide for radiopacity [36]. Theracal LC is characterized by an alkaline pH. The manufacturer claims that pH of Theracal LC remains at 8.0 or higher even after 170 days [36].

The bacterial inhibition activity of Theracal LC was investigated in one study against different *Streptococci* species including *S. mutans*. It was reported that Thercal LC demonstrated favorable antibacterial effect against *S. mutans* when tested in agar diffusion test [37].

Theracal LC induces an alkaline medium due to its high pH that approximately approaches 10-11 initially [38]. Theracal LC releases calcium ions and hydroxyl ions [38]. Hydroxyl ions release leads to an alkaline environment, which was correlated with the antimicrobial properties [20]. High pH and hydroxyl ions induce a detrimental effect on bacterial growth, division, and metabolism as they interfere with the enzymatic activities of the bacterial cell membrane [20].

2. <u>Activa Bioactive Restorative</u>

Activa Bioactive Restorative (Pulpdent Corporation, Watertown, MA, USA) is a new bioactive composite resin-based material that is available in the market as a liner, base, and restorative material. The material consists of an ionic resin matrix, a resin with rubberized and shock-absorbing properties, and fillers of GIC. Activa Bioactive Restorative is claimed

by the manufacturer to possess bioactive properties and release more fluoride than GIC does [32]. The dynamic interaction of the material with teeth and saliva leads to an exchange of ions. The material releases and recharges calcium, phosphate, and fluoride ions [32]. Activa is considered a smart material, a property of which the material is influenced by the dynamic changes in the pH of the oral environment leading to release and recharge of ions [32].

Although no previous studies have investigated antimicrobial activity of Activa Bioactive Restorative, the manufacturer claimed that the material has antibacterial properties resulted from the phosphate acid group in the ionic resin component [32]. Moreover, despite the absence of antimicrobial studies of the material, the claim that it releases fluoride suggests potential antimicrobial properties similar to that seen with GIC [17, 19, 32, 39].

Conventional Dental Materials

1. Glass Ionomer

GIC has been used for many decades in dentistry. It has multiple applications such as restorative filling material, liner/base, and luting cement [40]. The material consists of a powder of calcium or strontium fluoroaluminosilicate glass that interacts through an acid-base reaction with liquid of polyalkenoic acids such as acrylic acid, itaconic acid, or maleic acid [40]. The material has been recommended to use in patients with high risk of caries because it possesses anti-cariogenic properties as reported in the literature [16, 19, 41, 42].

The antimicrobial effect of GIC has been examined extensively in the literature [14, 16, 42, 43]. GIC's bactericidal properties were explored in clinical and in vitro studies [14, 16, 42, 43]. Agar

diffusion test was used in several in vitro studies to investigate the inhibitory effect of GIC against several bacterial species such as *S. mutans*, *Streptococcus oralis*, *Streptococcus salivarius*, and *Enterococcus faecalis* [14, 16, 43]. The results of these studies indicate that GIC possesses antibacterial effect [14, 16, 43]. Furthermore, similar results were indicated by a clinical study done by Tegginmani et al., in which the investigators compared the levels of *S. mutans* in plaque adjacent to carious teeth before and after their restorations with GIC. A decrease in the number of *S. mutans* was found in the dental plaque adjacent to GIC restored-teeth [42].

Fluoride release was correlated with the antibacterial effect of GIC [17, 19]. The rate of fluoride release from GIC increases when the pH drops to an acidic level of 4 in the first weeks of setting [44]. Fluoride ions were claimed to influence the bacterial acidogencity through reducing lactic acid production from *S. mutans* adjacent to glass ionomer–restored areas even in an acidic pH [17, 19]. Fluoride also affects carbohydrate metabolism process and acid production in the bacterial cells by inhibiting the enzymatic activity of enolase, H+/ATPase, and sugar transport [45].

Another factor that was related with GIC's inhibitory effect is low pH of the material during setting [21]. During the setting reaction of the GIC, the material exhibits very low pH that reaches 2.74 immediately after mixing and 4.17 around 9 minutes after mixing [46]. The growth of *S. mutans* was shown to be inhibited completely at pH of 4.8 [21]. In contrast to the studies that indicated the antibacterial effect of GIC, an in vitro study done by Yesilyurt et al.

claimed that GIC has only microbial inhibition properties in its unset state. Once the material set, it does not exhibit any antimicrobial activity [47].

2. Compomers

The term compomers or poly-acids modified composite referrers to a class of materials that combines some components of composite resin and GIC. This material consists of di-methacrylate monomers and fillers of calcium-aluminum fluorosilicate glass [48, 49]. Compomer was created to overcome the drawbacks of both composite resin and GIC such as low fluoride release in composite and low mechanical strength in GIC [49, 50]. However, the material is closer in properties to composite resin than to GIC [49].

The inhibition of bacterial growth by compomer was investigated in multiple studies [13, 21, 51, 52]. In vitro studies using agar diffusion and direct contact tests tested the inhibitory effect of compomer and reported that it had potential antibacterial effects against *S. mutans* [13, 51]. Compomer is characterized by the ability to release fluoride ions. The initial rate of fluoride release from the material increases at low pH of 4 in the first 2-3 weeks of setting [44]. Fluoride release has been indicated as an essential factor in the antibacterial behavior of fluoride-releasing restorative materials through its role in reducing bacterial acidogencity and lactic acid production [17, 19].

In contrast, other studies have reported that compomer had no antibacterial effects when tested on *S. mutans* [21, 52]. The results were related to the low fluoride release and no pH drop during setting of the material [21]. Moreover, it was reported that despite the fluoride release from compomer, the material failed to induce any inhibition effect against *S. mutans* [52].

3. Composite Resin

Since their development, composite resins have been used frequently as restorative filling materials because of their esthetic properties [53]. Composite resin is composed of resin matrix of Bis-GMA (bisphenol-A-glycidyl dimethacrylate) and other polymerizable monomers. Also, it has filler component of silica or ceramics [54]. The material has versatile applications in dentistry, it is used as a restorative material, cavity liner, fissure sealant, and luting cement [53]. However, in spite of composite resin's frequent use as filling materials to restore carious teeth, its properties in preventing or reducing recurrent caries are questionable [13, 21].

Several studies investigated the antibacterial effect of composite resin [13, 21, 43, 55]. However, some studies have reported that composite resin failed to show any inhibition results against *S. mutans* when tested by agar diffusion and direct contact tests [13, 21, 43, 55]. The authors suggested that the lack of the inhibition effects was related to the absence of the material's acidity during setting and the failure to release fluoride [21].

SIGNIFICANCE OF THE RESEARCH

Bioactivity was suggested in many studies to be a significant beneficial characteristic in endodontic and restorative dentistry [28, 56]. Researchers correlated biocompatibility and sealing ability with bioactivity. It was reported that bioactive materials have remarkable good sealing ability related to hydroxyapatite crystals deposits at the interface between the teeth tissues and the materials [28]. Moreover, it has been suggested that bioactive materials could

play a crucial role in secondary caries prevention [56]. Bioactive materials have an appetite-forming property, which could assist in closing the marginal gaps at the interface between the restorative materials and the teeth tissues and act as a protective measure in preventing secondary caries formation [56]. Another characteristic that would contribute in preventing secondary caries is the antibacterial property [15, 16]. Thus, the necessity of further investigating this property of bioactive materials is well–established.

To our knowledge, no previous studies have investigated the antibacterial properties of the new bioactive material. (Activa Bioactive Restorative) Moreover, the antibacterial effect of Theracal LC against *S. mutans* was investigated in only one study by using agar diffusion test [37]. Thus, this is the first study that aims to test the antibacterial activity of the selected bioactive materials by direct contact test in plates.

RESEARCH AIMS AND HYPOTHESIS

The purpose of this study was to evaluate the antibacterial properties of two bioactive dental restorative materials: Theracal LC and Activa and compare them with three conventional restorative materials: GIC, compomer, and composite resin. To achieve this goal, direct contact test with *S. mutans* bacteria was used.

We hypothesized that the selected bioactive restorative materials would have greater inhibitory effect against *S. mutans* than that of the conventional restorative materials.

MATERIALS AND METHODS

Research Design

The research was conducted as an *in vitro* laboratory study with the purpose of investigating and comparing the antibacterial effect of select bioactive and conventional dental restorative materials. The study was conducted in the microbiology laboratory at Tufts University school of Dental Medicine and was approved by Tufts University Health Science Campus Institutional Review Board (IRB).

Materials and Preparation

1. Strain and Medium

Streptococcus mutans (ATCC® 25175™) was used in this research. The bacterial strain was purchased from American Type Culture Collection (ATCC, Manassas, VA). Brain heart infusion broth (powder-BD 237500) and brain heart infusion agar (powder-BD 211065) were used as media to culture *S. mutans* and were obtained from Becton Dickinson.

2. Dental Materials

The antibacterial activity of five dental materials was investigated in this research.

The two bioactive materials used in the study:

1. Activa Bioactive Restorative (Pulpdent, Watertown, MA, USA).
2. Theracal LC (Bisco Inc, Schamburg, IL, USA).

The three conventional materials used in the study:

1. Glass ionomer cement (Ketac-Fil Plus Aplicap, 3M ESPE, USA).

2. Composite resin (Filtek Z250 restorative, 3M ESPE, USA).
3. Compomer (Dyract Extra Universal, Dentsply, Konstanz, Germany).

3. *S. mutans* Medium Preparation

The broth for culturing *S. mutans* was prepared according to the manufacturer' instructions by mixing 37.0 g of Brain Heart Infusion (BHI) broth powder (powder-BD 237500) with 1 liter of distilled water. The broth was sterilized in an autoclave for 15 minutes at 121°C and 15-30 psi.

The agar petri dishes were prepared by mixing 52.0 g of BHI agar powder (powder-BD 211065) with 1 liter of distilled water. Then, the agar solution was sterilized by an autoclave for 15 minutes at 121°C and 15-30 psi. The agar solution was allowed to cool down to 45-50°C before pouring into petri dishes. Both the BHI broth and the agar petri dishes were stored at 2-6°C.

4. *S. mutans* Culture

Inside the hood of a biosafety cabinet, the *S. mutans* strain was cultured by mixing a bacterial vial containing 1 ml of *S. mutans* with 4 ml of BHI broth in a sterile tube. The tube was incubated for 24 hours at 37°C in an incubator (Thelco Model 4 Gravity Convection Incubator).

A sterile inoculation loop was immersed in the incubated bacterial solution and used to load *S. mutans* to an agar petri dish in 3-4 stroke movements. The petri dishes were incubated for 24 hours at 37°C. To prepare the *S. mutans* solution that was used in the 24-well plate experiment, a single colony from the agar petri dishes was mixed with 4 ml of brain heart

infusion broth in a sterile tube. The tube was then incubated for 24 hours at 37°C. The growth of *S. mutans* in the culture medium was determined by measuring the optical density of the solution a spectrophotometer at 590 nm. To obtain *S. mutans* solution with 0.005 OD, 5 ml of BHI broth was mixed with 10μl of the incubated *S. mutans* solution. Then, 500 μl of the solution was mixed with 10 ml of BHI broth, producing a final solution with 1 X 10^5 colony forming unit per milliliter (CFU/ml) of *S. mutans*, which was used in all experiments [57].

5. Dental Materials Sample Preparation

Cylindrical-shaped samples from each of the five dental restorative materials were made by using plastic split-mold with 4 mm X 4 mm diameter holes. The dental restorative materials were handled strictly according to the manufacturers' instructions. GIC samples were prepared by mixing the capsules that contain the powder and liquid of the material in a triturator for 8 seconds then allowed to self-set in the split-mold. A light-emitting diode (LED) light cure unit (Demi, Kerr) was used to polymerize all the resin-based materials. Compomer and composite samples were prepared by polymerizing each 2mm thickness of materials for 20 seconds. Activa Bioactive Restorative samples were made by light-curing each 4 mm thickness of the material for 20 seconds as the manufacturer instructed. Theracal LC samples were prepared by light-curing each 1 mm thickness of the material for 20 seconds. Theracal LC samples were light-cured for an additional 20 seconds per side because they exhibited a slight dissolution in aqueous solution. This dissolution of the material caused an increase in the optical density of the solution, which could lead to errors in measuring *S. mutans* growth.

Preliminary Experiment

A preliminary experiment was done at first to compare the effectiveness of two ways of disinfection of the samples. Two sets of samples made from each dental material were used: one set was disinfected by using 70% ethanol and followed by rinsing with sterile distilled water then kept overnight under ultraviolet light. The other set was only disinfected by 70% ethanol followed by rinsing with sterile distilled water. Both sets were tested in the 24-well plates. One sample was placed in each well along with 500μl of a solution that contained 1 x 10^5 CFU/ml of *S. mutans*. The results showed that both sets of materials gave almost similar optical densities and inhibition results, which indicated no noticeable difference between both methods of disinfection. Based on these results, dental samples that were used in all experiments were disinfected with 70% ethanol followed by rinsing in sterile distilled water.

24-well Plates Experiment

All material samples were tested in a 24-well plate within an hour of the materials setting. In each 24-well plate used in the experiment, two wells were occupied with 500 μl of BHI broth only to serve as a test for contamination. Two other wells were used as negative control and were filled with 500μl of the bacterial solution that contained 1 x 10^5 CFU/ml of *S. mutans*. For the positive control, two wells were filled with 500 μl of *S. mutans* solution and 20 μl of chlorhexidine. All remaining wells were filled with 500 μl of *S. mutans* solution and one sample from each dental material (one sample per well). Following that, the 24-well plate was incubated for 24 hours at 37°C. one hundred μl of each solution in the 24-well plate was placed in duplicate in a 96-well plate and the optical density determined by a

spectrophotometer at 590 nm. The optical density of each solution that contained the dental samples was measured and compared with the optical density of solutions from the positive control and negative control wells to evaluate the bacterial growth and inhibition results. The percent of inhibition of each dental material was measured by using the following equation:

Percent of inhibition= (1-(mean of the optical density of sample/mean of the optical density of the negative control)) *100.

The experiment was repeated ten times for GIC and compomer, fourteen times for composite resin and Activa Bioactive, and seventeen times for Theracal LC.

RESULTS

Descriptive statistics (means and standard deviations) were calculated for the optical densities of the *S. mutans* growth for the negative control, positive control, and all tested dental materials (Table 1& Figure 1). All bacterial solutions that contained the materials samples and chlorhexidine showed less growth of *S. mutans* in comparison with the negative control. The least mean of the optical density of *S. mutans* growth was reported for chlorhexidine samples (0.014± 0.009) followed by GIC samples (0.180± 0.119). The highest mean of the optical density of *S. mutans* growth was reported for the negative control (0.399± 0.149) followed by the composite resin samples (0.380± 0.215).

The mean optical density and standard deviation of *S. mutans* growth in the presence of Activa Bioactive Restorative, compomer, and Theracal LC were (0.315± 0.142, 0.245± 0.131, and 0.283± 0.138) respectively.

The distribution of the data was not normal for the percent inhibition of two of the tested materials, therefore the data was analyzed using Kruskal-Wallis test. The median and interquartile range of percent inhibition of chlorhexidine and all dental materials are reported in Table 2 & Figure 2. The highest antibacterial effect was reported for chlorhexidine with a median of inhibition of 96.28 %. Among the dental materials, GIC showed the highest median of percent inhibition (41.89 %), indicating a higher antibacterial effect in comparison with the other tested dental materials. On the other hand, composite resin showed the least median of percent inhibition (6.41 %), indicating the lowest antibacterial effect. The median percent inhibitions of Activa Bioactive, compomer, Theracal LC were 12.25%, 20.97%, and 27.22% respectively.

A p-value of 0.007 was reported for the Kruskal-Wallis, indicating a significant difference in the antibacterial effect between the materials (Table 2). Pairwise multiple comparisons using Man-Whitney U were performed to determine specifically which materials were significantly different when compared with each other (Table 3). The p-value was adjusted with Bonferroni correction and values less than 0.005 were considered statistically significant. The only significant results in the pairwise multiple comparisons were found between GIC and Activa Bioactive with a p-value of 0.003.

DISCUSSION

In this study, the antibacterial properties of two bioactive and three conventional dental restorative materials were investigated using the 24 well plate test, which is also known as direct contact test [58]. Direct contact test offers an advantage, in which the bacteria come in contact directly with the tested materials, in contrast to other testing methods such as agar diffusion test that depends on the diffusion of the soluble components of the tested materials into agar [58].

The results of this research suggest that the tested materials possess weak antibacterial effects. The highest median of inhibition was reported for GIC (41.89 %), indicating higher antibacterial activity in comparison with the other bioactive and conventional restorative dental materials. These results are in contrary to what we hypothesized. Kruskal-Wallis

reported a p-value of 0.007, indicating that the median of at least one material was statistically significant than the median of the other tested dental materials. Pair-wise comparisons with Man-Whitney U reported that the only significant difference among the dental materials was found between GIC and Activa Bioactive Restorative with a p-value of 0.003.

GIC results are in agreement with a study conducted by Klai et al. where it was concluded that various products of GIC exhibited some degree of antimicrobial effect by reducing the number of colony forming unit of *S. mutans*, indicating limited bacteriostatic properties but not bactericidal properties [59]. The investigators suggested that the inhibitory effects were related to the ability of the material to release fluoride as has been indicated extensively in the literature [17, 19, 59]. Fluoride has been reported to reduce the acidogencity of *S. mutans* by influencing their metabolism and lactic acid production [17, 19]. The weak-to-moderate inhibitory effects of GIC in this study are in contrast to multiple studies which indicated that GIC had potent antibacterial effects against *S. mutans* [14, 16, 17, 60]. Davidovich et al. reported that GIC completely inhibited *S. mutans* in direct contact test [60]. The difference in the antibacterial results between our study and what of Davidovich et al. could be related to the fact that the setting of the two experiments was different. Although both studies used direct contact test, the diameter of the dental samples might be different. The investigators did not exactly specify the diameter of the GIC samples they used in their study. The volume of the bacterial solution used was also different. In addition, the investigators used different GIC products than the one we used in this study. Variability in the fluoride release rate among

different products of GIC was reported in the literature, which could be a reason for the difference in the antibacterial effect [61].

Compomer's weak antibacterial effects also are consistent with what is reported in the literature [62, 63]. Matalon et al. reported that compomer had only short-term antibacterial activity against *S. mutans* that did not last after 24 hours of the material setting [62]. In addition, another study indicated that compomer failed to show any antibacterial effect [63]. Al-Naimi et al. reported that the release of fluoride from GIC was significantly greater than that of compomer and composite resin [64]. The difference in the fluoride-releasing rate could explain the disparity in the antibacterial behavior between the three materials. The absence of fluoride release from composite resin was reported by Vermeersch et al [21]. This could explain the very low antibacterial results of composite in this study. Our results are in agreement with findings reported in a study by Beyth et al.; composite exhibited minimal antibacterial effects by a short-term reduction in the number of *S. mutans* in direct contact test [55].

The bioactive materials tested in this study also showed weak antibacterial activity especially, Activa. Activa is similar in composition to composite resin as both materials contain monomers such as urethane dimethacrylate (UDMA) [65, 66]. Both materials showed weak antibacterial effects; the median of inhibition of Activa was 12.25% while for composite it was 6.41%. These low and close antibacterial results could be explained by the similarity in composition between the two materials [65, 66]. Moreover, resin-based fluoride–

releasing materials were reported to release a smaller quantity of fluoride in comparison with GIC [64].

The antibacterial effect of Theracal LC was found to be weak. The effect was lower than that of GIC but higher than the rest of the materials. Poggio et al. reported that Theracal LC showed similar effects when tested by agar diffusion test [37]. Although the method used in this study is different than the agar diffusion test, but it can support our results that the material possesses limited inhibitory effect against *S. mutans*. The antibacterial behavior of Theracal LC can be explained as a result of the high pH of the material and the release of hydroxyl ions [20, 38]. The alkaline pH and hydroxyl ions induce a detrimental effect on the enzymatic activities of the bacterial cell membrane [20].

One crucial issue that we faced in this study was the variability in results of the experiments of the tested dental materials although the same protocol was followed in all experiments. In some experiments, the materials showed some degree of inhibition while in others they showed weak or none. We believed that these variable results could be related to variations in making the dental samples such as polymerization of the materials (for example, the exact time of light-curing and how far the light-cure unit from the materials). Although we tried to follow the manufacturer's instructions precisely. For Theracal LC samples specifically, the variability could be related to polymerization issues. When the material was polymerized as the manufacturer instructed by light-curing each 1 mm thickness of the material for 20 seconds, the samples exhibited slight dissolution in aqueous solutions. The samples were then light-cured for an extra 40 seconds, 20 seconds per side to overcome the dissolution issue.

One of the limitations of this study includes that the dental materials were investigated only in vitro. An in vivo experiment using saliva and teeth as substrate might affect the results as they would simulate the oral environment. Moreover, this study only investigated the effect of the dental materials against one selected bacterial species (*S. mutans*). It did not include other bacterial species involved in the caries process such as *Lactobacillus* [5]. Also, another limitation is that a histological examination could not be used to evaluate the antibacterial effects of the materials. We tried to decalcify teeth after placing *S. mutans* in prepared cavities and restoring them with the dental materials. The teeth were decalcified in hydrochloric acid, but the dental materials did not soften or dissolve in the acid. Therefore, it was impossible to section the teeth for histological examination. Also, we tried to do gram staining for the restored teeth that had *S. mutans* in their cavities after incubating and cutting them into halves. However, it seemed that the teeth supported a greater growth of *S. mutans,* which made it impossible to identify which dental materials had less or more growth of *S. mutans* using the gram staining.

CONCLUSION

The dental restorative materials tested in this study showed weak antibacterial effect.

The highest antibacterial effect among the materials tested was obtained with GIC. This could be related to the material ability to release fluoride as was reported in the literature [17, 19]. The bioactive materials especially, Activa Bioactive Restorative showed very weak antibacterial properties. Such a weak inhibition would not be effective in preventing secondary caries formation.

We suggest that future studies should investigate the antibacterial effect of the dental materials in vivo in order to evaluate the interaction between the bioactive dental restorative materials, the bacterial species involved in dental caries, and the other oral environmental factors such as saliva and pH. Also, efforts should be directed toward creating and investigating new restorative dental materials with genuine antibacterial components.

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APPENDICES

Appendix A: Tables

	Optical Density**:** *Mean (Standard Deviation)*
S. mutans	0.399(0.149)
Chlorhexidine	0.014(0.009)
Activa Bioactive Restorative	0.315(0.142)
Composite resin	0.380(0.215)
Compomer	0.245(0.131)
GIC	0.180(0.119)
Theracal LC	0.283(0.138)

Table 1: The mean and standard deviation of the optical densities for negative control (*S. mutans*), positive control (chlorhexidine), and all the tested dental materials.

Material	*Median & Interquartile Range of Percent Inhibition*
Activa Bioactive	12.25 (25.19)*
Composite	6.41 (30.36)
Compomer	20.97 (21.80)
GIC	41.89 (36.74)*
Theracal LC	27.22 (45.26)
Chlorhexidine	96.28 (3.06)
P-value	0.007

Table 2: The median and interquartile range of percent inhibition of chlorhexidine and the dental materials.
* $p = 0.003$ for the post-hoc comparison between Activa Bioactive restorative and GIC.

Materials	***P-value of the pairwise comparison***
Activa Bioactive vs. Composite	0.834
Activa Bioactive vs. Compomer	0.143
Activa Bioactive vs. GIC	0.003*
Activa Bioactive vs. Theracal	0.029
Composite vs. Compomer	0.141
Composite vs. GIC	0.006
Composite vs. Theracal	0.038
Compomer vs. GIC	0.034
Compomer vs. Theracal	0.292
GIC vs. Theracal	0.366

Table 3: p-value of the Man-Whitney U pairwise comparisons of the inhibition difference between all tested dental materials against *S. mutans.*
(*) indicates the statistically significant values (adjusted P-value<0.005).

Appendix B: Figures

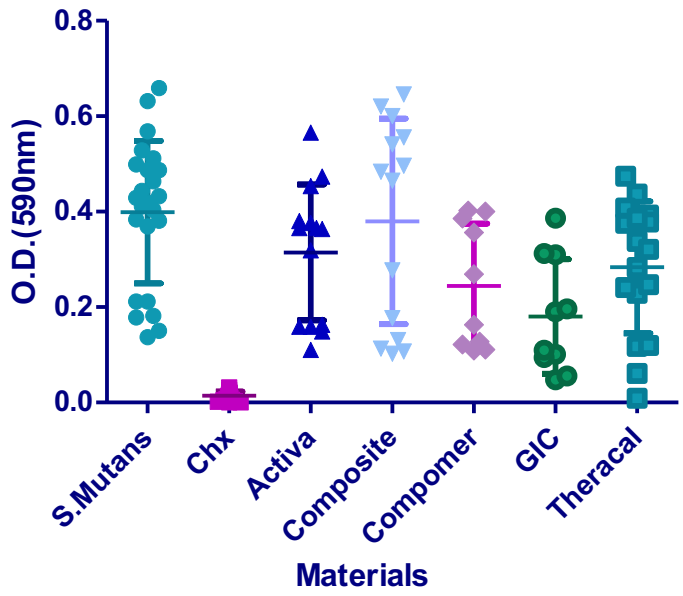

Figure 1: Mean of optical densities of *S. mutans* in the negative control (*S. mutans* only), the positive control(chlorhexidine) and the dental restorative materials.

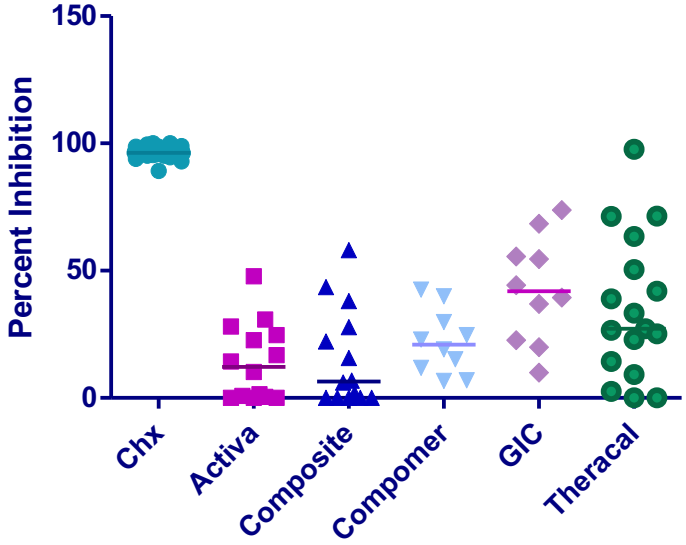

Figure 2: Median of percent inhibition of the positive control (chlorhexidine) and the restorative dental materials.

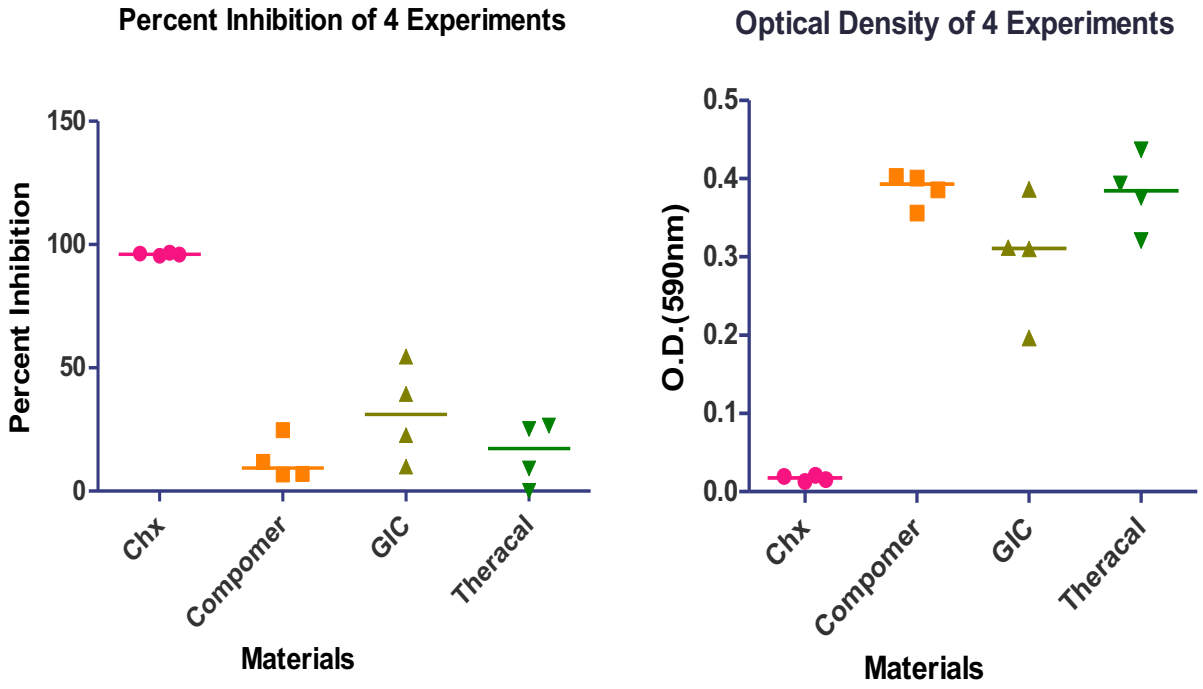

Figure 3: Median of percent inhibition and optical density of positive control (chlorhexidine) and three dental materials, compomer, GIC, and Theracal.
The results indicated above in the figure are from four experiments performed the same day.