

# FLUORIDE RELEASE AND ANTI-BACTERIAL ACTIVITY OF DIFFERENT BIOACTIVE RESTORATIVE MATERIALS: AN *IN VITRO* COMPARATIVE STUDY

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## ABSTRACT

**INTRODUCTION:** Anti-bacterial properties of restorative materials through fluoride release enhance their inhibition ability against bacterial development, and reduce recurrent caries incidence.

**OBJECTIVES:** The aim of the current *in-vitro* research was to assess and compare fluoride release and anti-bacterial activity of three distinct bioactive dental restorations, such as resin-modified glass ionomer (RMGI), giomer, and Activa bioactive composite at different time intervals *in vitro*.

**MATERIAL AND METHODS:** 60 disc-shaped specimens were prepared and based on the kind of dental restoration materials, and were grouped into three equivalent major groups (20 specimens each group): RMGI (Fuji II LC), giomer (Beautiful II), and enhanced RMGI (Activa bioactive composite). Every main group was divided into two sub-groups based on the evaluator criteria, such as fluoride release ( $n = 15$ ) and anti-bacterial action ( $n = 5$ ). Based on storage duration, every fluoride release sub-group was then sub-divided into 3 equivalent divisions: 24 hours, 1 month, and 3 months (5 specimens each group). An ion-specific electrode was used to quantify fluoride. Anti-bacterial activity was recorded following one day according to sizes of inhibition zone.

**RESULTS:** Fluoride release and anti-bacterial activity were statistically higher in Fuji II followed by Activa, while Beautiful II showed the least fluoride release. The fluoride release amount of all groups was greatest in the first 24 hours, and decreased significantly with time (1 month > 3 months).

**CONCLUSIONS:** Given the context of this research, RMGI presents better fluoride release and anti-bacterial activity than Activa and giomer.

**KEY WORDS:** anti-bacterial activity, bioactive restoration, fluoride release.

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## INTRODUCTION

According to investigations evaluating fluoride release, the mineral phase of the teeth surface develops a calcium fluoride-like coating. This layer makes it easier for fluorapatite or fluoro-hydroxyapatite to precipitate, which encourages re-mineralization and stops additional mineral phase degradation [1]. It is undeniable that fluoride release plays a positive function in maintaining

the teeth and oral health. The most often discovered materials with the term “fluoride” are glass ionomers or glass silicates. Due to unique chemical adherence to the teeth structures, high biocompatibility, and fluoride release, glass ionomers are mostly utilized. Despite the benefits, such materials have low aesthetics, slow setting process, and low mechanical qualities [2].

The inherent property of different oral bacteria and restorations material are the main factors in the progn-

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sis of restorative procedure. According to certain claims, the release of specific ions, such as fluoride, can greatly enhance anti-bacterial properties of restorative materials, hence lowering the occurrence of recurrent caries, which is the primary cause of the failure of teeth restorations [3, 4].

One of the solutions developed was hybrid materials that combine technologies of glass ionomers and composites. Compomers, giomers, resin-modified glass ionomer cements (RMGICs), and subsequently bioactive resin composites are the major types of such hybrid materials [5]. These materials were developed to maintain the benefits of traditional glass ionomers and composite resins while solving some of their problems. Among the methods of acid-base reaction and free radical polymerization, these materials' curing processes vary [6].

RMGICs (Fuji II) are hybrid materials, in which the course of their entire setting reaction maintain a large acid-base interaction [7]. Because of their enhanced physical and mechanical qualities in comparison with conventional glass ionomer restorations, they gained clinical preference. Additionally, another advantage is their capacity to store and release fluoride directly, thus protecting teeth surfaces that are prone to caries in high-risk caries patients [8].

A brand new class of hybrid aesthetic restoration materials is called "giomer" (Beautifil II). Its composition includes surface pre-reacted glass ionomer filler particles (S-PRG). Their manufacturer claims it has fluoride releasing and recharging potential, in conjunction with the resin composite's excellent physical qualities [9].

A novel class of bioactive restoratives has just been introduced as a restorative dentistry strategy (Activa) that is enhanced RMGIs. This restorative material is claimed by the manufacturer to be the first bioactive dental material that replicates the physical and chemical characteristics of real teeth using an ionic resin matrix and bioactive fillers. Additionally, they asserted that Activa releases higher fluoride ions compared with conventional glass ionomers [10].

## OBJECTIVES

From a previous review, it was postulated that it would be important to evaluate and compare fluoride release capacity and anti-bacterial activity of RMGI (Fuji II LC),

giomer (Beautifil II), and enhanced RMGI (Activa bioactive composite). The null hypothesis was that there would be no variation in fluoride release potential and anti-bacterial activity among the tested restoration materials.

## MATERIAL AND METHODS

Three different bioactive restoration materials were employed in this research, and are shown in Table 1.

### SAMPLE GROUPING

Using specifically designed standardized divided Teflon molds, 60 samples were created: 3 mm thickness and 6 mm diameter for fluoride release, and 2 mm thickness and 10 mm diameter for anti-bacterial activity. Specimens were grouped into three primary groups (20 specimens each group) based on the kind of restoration material, i.e., Fuji II, Beautifil II, and Activa. Based on the assessment criterion ( $n = 15$  for fluoride release,  $n = 5$  for anti-bacterial activity), each major group was divided into 2 sub-groups. Based on the storage duration, every fluoride release sub-group was then separated into three equivalent time periods, such as 24 hours, 1 month, and 3 months ( $n = 5$ ).

### SPECIMENS PREPARATION

Sterilized microscope glass slide and celluloid strip were put on top of specially constructed Teflon mold, which was then loaded with tested restorations specimens using a sterilized gold-plated tool (Miltex, stainless Italy, 70-204 EELT 4) according to the manufacturer's instructions. To avoid forming of an oxygen-inhibited layer, the second celluloid strip was used to cover the upper part of the mold [12]. To ensure secure filling of the prepared samples and extruding of the excess material, a new glass slide and 500 gm pressure were put above the new Mylar strip for 30 seconds [13] (Figure 1). The utilized pressure and microscope slide were eliminated from the top surfaces before curing. Polymerization was performed using LED light-curing device (Elipar S10, 3M-ESPE, USA; wavelength 455 nm  $\pm$  10 nm, light intensity 1,200 mW/cm<sup>2</sup>) for 20 seconds

**TABLE 1.** Brand name, material specification, the material's constitution, place of manufacturing, and batch number

Brand name and material specification	Composition	Manufacturer (batch No.)
Fuji II LC (RMGIC)	2-HEMA, polyacrylic acid, and water. 58 wt% fluoro-aluminum silicate	GC, Tokyo, Japan (2103252)
Beautifil II (Giomers)	Bis-GMA, UDMA, Bis-MPEPP, TEG-DMA. 83.3 wt% fluoro-silicate glass	Shofu, Kyoto, Japan (041824)
Activa (Enhanced RMGIC)	A blend of diurethane and other methacrylates with modified polyacrylic acid. 55.4 wt% bioactive glass and sodium fluoride	Pulpdent, Watertown, MA, USA (180419)

HEMA – hydroxy-ethyl methacrylate, Bis-GMA – bisphenol-A-diglycidyl-methacrylate, UDMA – urethane dimethacrylate, Bis-MPEPP – bisphenol A polyethoxy methacrylate, TEG-DMA – triethylene glycol dimethacrylate, RMGIC – resin-modified glass ionomer cement

in each group according to materials manufacturers' instructions. Guiding light curing unit's tip was held perpendicular to the celluloid strips on the mold's top surface, which was kept centered and in close contact with the celluloid strips to standardize curing distance. After light curing, the cylindrical formed samples were taken out of their molds and rinsed continuously with running water for 1 minute, followed by measurements of their diameter and thickness with a digital caliper. After that, each sample was polished using Sof-Lex polishing system (3M-ESPE, St. Paul, MN, USA) for removing the surface layer's resin-rich coating [14].

### SAMPLES STORAGE FOR FLUORIDE RELEASE INVESTIGATION

Each sample was immersed in a plastic box filled with 5 ml of de-ionized water at 37°C (triple distilled water, anion H + cation OH = H<sub>2</sub>O free of minerals). Water was prepared especially for the experiment by Cairo University's Faculty of Pharmacy, Pharmaceutical Department. Boxes were vigorously jolted after 1 day, and the water was then removed and analyzed. The samples were submerged once more in 5 ml of fresh de-ionized water, which was replaced daily for further equiposing. Measurements of fluoride released were performed after one day, one month, and three months storage periods. An ion-specific electrode (FC 301 B, Hanna Company, Italy) was attached to a mobile fluoride meter with a microprocessor (HI 98401, Hanna Company, Italy) for fluoride detection. The meter was calibrated using two basic fluoride-calibrated liquids, i.e., HI 70701 (10 mg/l Fl liquid) and HI 70703 (100 mg/l Fl liquid), at a heat of 20 ± 3°C to examine the electrode potential. The temperature of de-ionized water was measured and adjusted using a temperature probe (HI 7662, Hanna Company, Italy), which was linked to the meter. Readings were displayed on the liquid crystal display's lower portion (LCD). To achieve an accurate and consistent value of every measurement, a reference electrode (HI 7663, Hanna Company, Italy) was connected to the fluoride meter and submerged in de-ionized water during fluoride assessment [13].

For the creation of a context of steady ion concentration for fluoride quantification, a 4 ml storage solution of every specimen and 1 ml de-ionized water employed for washing were added to 0.5 ml of available total ionic strength adjustment buffer II solution TISAB II (HI 4010 05, Hanna Company, Italy) at a proportion of 10 : 1. For rendering fluoride accessible for analysis, we used TISAB II with 2% 1.2 cyclohexane diamine tetra acetic acid, which is a metal chelating agent that selectively breaks fluoride from polyvalent cations. Afterwards, it was put into a Teflon pot (beaker) that was uniquely made with a lid with three openings. Three electrodes were kept apart from one another and the base of the beaker. To provide an accurate fluoride assessment in de-ionized water,



FIGURE 1. Packing the restoration into the mold



FIGURE 2. Fluoride-specific ion electrode

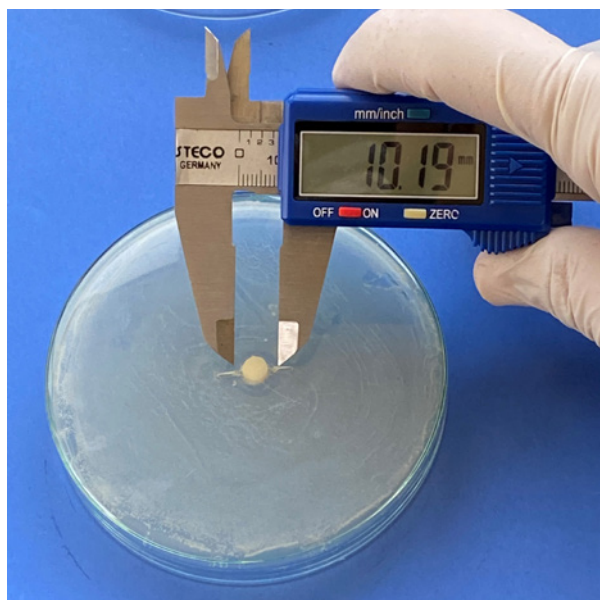
the electrodes were fully immersed within the solution. Values were displayed in ppm on the top section of LCD (Figure 2).

### SAMPLES STORAGE FOR TESTING ANTI-BACTERIAL ACTION

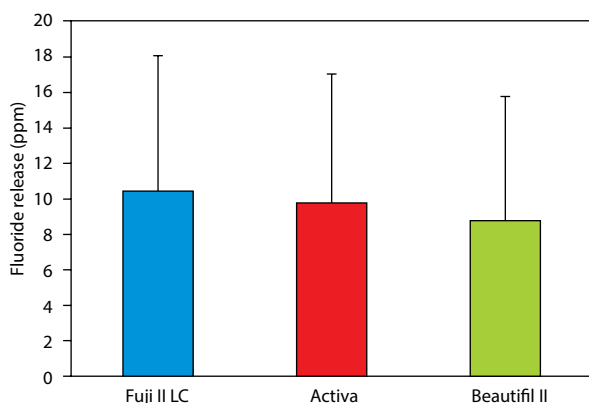
The Microbiological Resources Centre, MIRCEN, Cairo, Egypt, provided *Streptococcus mutans* ATCC 25175 type strain that was employed in the research. Bacteria were inoculated into brain heart infusion broth (BHI, Oxoid, Basingstoke, England) and cultivated over night at 37°C. The inoculum (100 l) was scrubbed on trypticase soy agar and allowed to be arid for ten minutes after being adjusted to match the turbidity of 0.5 McFarland standards [15]. Inhibition zone of 3 distinct restorations used in this study (Fuji II, Beautiful II, and Activa) were measured after anaerobic incubation at 37°C for 24 hours. Inhibition zones for the reproduction of bacteria were determined in mm units using an electronic digital caliper [16] (Figure 3).

## STATISTICS EVALUATION

By examining data distribution using Kolmogorov-Smirnov and Shapiro-Wilk tests, quantitative findings were checked for normality. Inhibition zone diameter and fluoride release information displayed a typical (parametric) pattern. Numbers of the mean, standard deviation (SD),



**FIGURE 3.** Electronic digital caliper calculates the inhibition zone diameter



**FIGURE 4.** A bar graph displaying data of the mean and standard deviation according to fluoride release of the three materials regardless of time

**TABLE 2.** The mean, standard deviation (SD) values, and results of two-way ANOVA test of comparison between fluoride release (ppm) of the three material types regardless of time

Fuji II LC		Activa		Beautifil II		p-value	Effect size (partial eta squared)
Mean	SD	Mean	SD	Mean	SD		
10.44 <sup>A</sup>	7.6	9.71 <sup>B</sup>	7.35	8.76 <sup>C</sup>	6.98	< 0.001*	0.604

\*Significant at p-value ≤ 0.05. Different superscripts indicate statistically significant differences between materials.

median, and range values were provided as statistics. One-way ANOVA was used for parametric values to contrast inhibition zone diameters of the three materials. Influence of restoration type, time, and their relationships on average fluoride release was examined using a two-way ANOVA test. Pair-wise contrasts were evaluated using Bonferroni post-hoc test where the ANOVA test was significant. When Kruskal-Wallis test was significant, pair-wise comparisons were done with Dunn test. Cut-off for significance was chosen at p-value ≤ 0.05. Statistical analysis was carried out using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, New York, USA).

## RESULTS

### FLUORIDE RELEASE

The results showed there was a statistically significant variation among the mean fluoride release of the three materials regardless of the storage periods ( $p < 0.001$ , effect size = 0.604). According to pair-wise comparison, Fuji II LC demonstrated a statistically significantly greatest mean fluoride release. Statistics indicated that the mean value for Activa was smaller, while a statistically significantly least mean fluoride release was demonstrated by Beautifil II (Table 2 and Figure 4). Regarding the mean fluoride release at different times, the three materials varied statistically significantly:  $p < 0.001$ , effect size = 0.983 for the Fuji II LC group;  $p < 0.001$ , effect size = 0.982 for the Beautifil II group; and  $p < 0.001$ , effect size = 0.98 for the Activa group. Pair-wise comparisons showed that the greatest means of fluoride release was found after 24 hours. The mean fluoride release after one month demonstrated statistically significantly lesser mean scores. The statistically significantly lowest mean fluoride release was found after three months (Table 3 and Figure 5).

### ANTI-BACTERIAL EFFECT

According to anti-bacterial effect results, there was a zone of inhibition observed with each group, and a statistically significant difference existed among mean inhibition zone diameters in each group ( $p < 0.001$ , effect size = 0.955). Pair-wise comparisons showed that a statistically significantly greatest mean inhibitory zone diameter was observed in the Fuji II LC group. The mean value

**TABLE 3.** The mean, standard deviation (SD) values, and results of two-way ANOVA test of comparison between fluoride release (ppm) at different times within each material

Time	Fuji II LC		Activa		Beautiful II	
	Mean	SD	Mean	SD	Mean	SD
24 hours	19.82 <sup>A</sup>	0.74	18.62 <sup>A</sup>	1.02	17.35 <sup>A</sup>	0.81
1 month	9.64 <sup>B</sup>	0.67	9.21 <sup>B</sup>	0.30	7.99 <sup>B</sup>	0.73
3 months	1.96 <sup>C</sup>	0.19	1.31 <sup>C</sup>	0.21	0.93 <sup>C</sup>	0.04
<i>p</i> -value	< 0.001*		< 0.001*		< 0.001*	
Effect size (partial eta squared)	0.983		0.982		0.98	

\*Significant at *p*-value ≤ 0.05. Different superscripts in the same column indicate statistically significant differences between times.

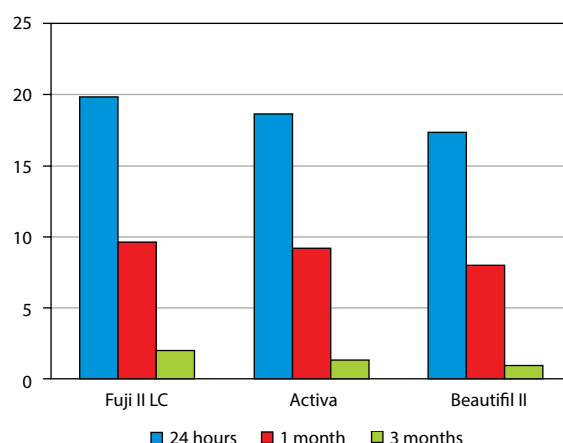
for the Activa group was statistically less. The statistically significantly least mean inhibitory zone diameter was seen in the Beautiful II group (Table 4 and Figure 6).

## DISCUSSION

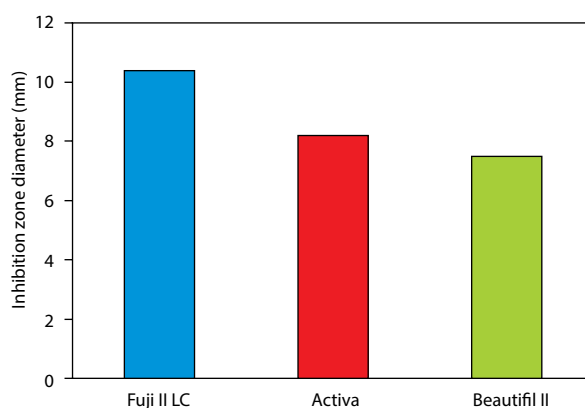
In recent years, there has been a sharp growth in the restoration of cavities with fluoride-releasing materials, which provide anti-cariogenic qualities and prevent the caries-causing bacteria metabolism, enhancing re-mineralization [17].

## FLUORIDE RELEASE

Since using an ion analyzer with an ion-specific electrode is simple, inexpensive, and does not need the utilization of complicated lab tools, it was chosen to determine the quantity of fluoride released. In addition, it provides a precise and guided approximation of the available fluoride found in a solution than spectrophotometry, ion chromatography, and capillary electrophoresis [18]. Our results showed that there were significant differences among the mean fluoride release of the three materials regardless of the time period. The maximum fluoride release was seen in Fuji II LC (RMGI) followed by Activa, while Beautiful II (giomer) presented the lowest fluoride release. This order could be explained by the fluoride release from restorations being affected by numerous variables, including the extent of the glass ionomer matrix layer encircling the glass filler in the set restoration [19]. The results of the current study are in line with a research by Garoushi *et al.* [9], who discovered that the greatest fluoride release values were observed



**FIGURE 5.** A bar graph displaying data of the mean and standard deviation of the three materials fluoride release as influenced by various storage periods



**FIGURE 6.** A bar graph displaying data of the mean and standard deviation of the inhibition zone diameters for each group

**TABLE 4.** The mean, standard deviation (SD) values, and results of one-way ANOVA test of comparison between inhibition zone diameters (mm) in the three groups

Fuji II LC		Activa		Beautiful II		<i>p</i> -value	Effect size (eta squared)
Mean	SD	Mean	SD	Mean	SD		
10.44 <sup>A</sup>	0.28	8.20 <sup>B</sup>	0.26	7.50 <sup>C</sup>	0.36	< 0.001*	0.955

\*Significant at *p*-value ≤ 0.05. Different superscripts indicate statistically significant differences between groups.

in RMGI followed by Activa, and the least one was giomer. Regarding RMGI, it had the highest fluoride release as HEMA monomer had a higher water absorption and a higher rate of ion release in aqueous conditions due to its watery base and existence of porosity within the structure [20]. Furthermore, the existence of a polymeric matrix in RMGI inhibits the acid-base interaction, increasing the permeability of materials. Additionally, RMGI has higher porosity, more water content, and Ca-Al-F-silicate glass fillers are more soluble, thus releasing more fluoride than giomer and Activa [21].

Moreover, a proprietary robust resin system with energy absorbing elastomeric components is present in Activa that might affect the permeability, leading to a lower ability to fluoride release compared with RMGI [8]. Additionally, Activa is similar to a composite resin in composition, as both materials contain monomers, such as Bis-GMA and UDMA, which reduce the extent of fluoride release from glass fillers when subjected to storage media following light curing [22].

The results of a study by Mosallam *et al.* [23] are in line with the results of the present research in terms of Activa having lower fluoride release than RMGI. However, this result contradicted results of Shaymaa *et al.* study [8], where there was no significant variation in the fluoride release among Activa and RMGI found.

In addition, giomer displayed the least amount of fluoride release in this study. This may be due to gionomers not having any discernible acid-base reactivity, with minimal or without glass ionomer matrix phase. Water absorption is not essential in the acid-base interaction because S-PRG fillers already interacted with acid [24]. Moreover, the capability of S-PRG fillers using Bis-GMA/TEG-DMA resins in fluoride release in giomer was poor [25]. Also, giomer has an accumulated fluoride release of around 20% of the basic GIC, since PRG fillers include silane coupling [26]. Our results concur with those of Bansal *et al.* [27] study, as they found that compared with RMGI, giomer released less fluoride.

Furthermore, our results showed that Activa presents more fluoride release than giomer. These results support those of Ruengrungsom *et al.* [25], who found that Activa has fluoride-containing bioactive glass, which has an ion source of fluoride; therefore, when comes in touch with liquids, it could deteriorate and disintegrate, allowing fluoride release. Also, increasing fluoride release from Activa is affected by the acidity and hydrophilicity of their resin matrix. On the other hand, our results disagree with El-Bahrawy *et al.* [7], who observed that Activa has a fluoride release ratio that is equivalent to giomer and greater than RMGI.

De-ionized water was employed in this study as a storage media that is widely available and accurately depicts the fluoride release of products in the absence of interference of minerals or organic compounds, which could be found in PH-cycling solutions or saliva [7].

According to the time period, the fluoride release amount of all groups was greatest in the first 24 hours (burst effect) and decreased significantly with time. The burst effect might be due to the acid-base reaction [28], surface erosion, and fluoride's ability to permeate materials' pores and cracks. Furthermore, gross fluoride release occurs through the maturation phase because of interaction between the components and the storage media [29]. While the decreased fluoride release with age may be due to glass fillers slowly dissolving over age via material's pores [30]. This was partly in agreement with El-Bahrawy *et al.* [7], who showed the highest fluoride release of RMGI, giomer, and Activa in the initial 24 hours and decreased with time.

Regarding RMGI, the burst effect may be due to the first acid dissolving of the powder particle surfaces, in which a significant quantity of fluoride is incorporated into the interaction product matrix. Such fluoride quickly disperses from the matrix exposed to the material surface, and it is gradually replaced by fluoride migrating from the matrix under the surface [8].

Regarding Activa, with moisture present, Si-O-Si links in the silicate matrix break down, allowing the bioactive glass to dissolve and release fluoride quickly. Moreover, the presence of hydrophilic resins, such as TEG-DMA potentially results in bioactive glass particles hydrolytically disintegrating [31].

Indeed, to build up a firm layer of glass ionomer matrix for the material, the giomer employs PRG innovation. The significant amount of fluoride released in giomer during the initial 24 hours was due to the greater comprehensive acid-base interaction and the hydrogel layer formation of glass fillers [19]. These findings were consistent with many *in-vitro* investigations, which also demonstrated greater fluoride release in the initial 24 hours [19, 20, 25]. On the other hand, our findings partly conflicted with Garoushi *et al.* [9], who found that, although quantities of fluoride released from giomer and Activa do not initially cause a burst, they do stay mostly steady over time.

## ANTI-BACTERIAL ACTIVITY

Dental restorations have a benefit in inhibiting bacterial development, and therefore lowering the occurrence of secondary caries due to their anti-bacterial properties. Some components of various dental materials, such as fluoride, have been investigated and claimed to have anti-microbial properties [32]. *S. mutans* bacterial strain was utilized during the entire study based on its main etiological role in caries formation. Due to its connection to tooth cavities, *S. mutans*, a solitary bacterial biofilm, was used in previous studies on anti-bacterial dental restorations, since *S. mutans* is the greatest cariogenic and acidic bacteria, and is present in teeth plaque [33].

In our study, inhibition zones were observed for each group. This was in line with findings of Khalaf *et al.*

research [34], who discovered that RMGI, giomer, and Activa significantly inhibited the development of *S. mutans* in one day. They explained that this might be due to the release of fluoride, which inhibited bacterial metabolism and microbial proliferation. Additionally, the primary metabolic process of saccharolytic bacteria is glycolysis. The main theory for fluoride anti-microbial activity is that it inhibits glycolysis through affecting the absorption and breakdown of polysaccharides via bacterium cells. Moreover, it hinders the bacterium cells capacity to sustain pH equilibrium [35].

Our findings showed a significant variation among the inhibition zone diameters in each group. Furthermore, in the current research, RMGI had the highest fluoride release amount and anti-bacterial activity compared with other groups, demonstrating that variations in fluoride release amounts could be connected with variations in anti-bacterial properties, which have been indicated extensively in the literature. According to several research, the quantity of fluoride released is connected with anti-bacterial properties [36, 37]. Moreover, the highest anti-bacterial activity of RMGI may be due to the enhanced fluoride release and by offering a low pH; HEMA in liquid may help with the anti-bacterial action [38]. However, this result contradicted with Yesilyurt *et al.* [39] study, who observed that the anti-bacterial effect of GIC exclusively exhibits microbial inhibitory capabilities within the unset form due to decreasing pH during the setting reaction. After setting, the material shows no anti-bacterial action, this difference with our finding might be due to the difference in specimen size and the way of the tested device.

Comparatively, Activa in this study showed weaker anti-bacterial activity than RMGI, which was due to the bioactive composite resin material component; Activa has a modest acidity since it contains modified polyacrylic acid [40]. Our result was consistent with that of Walaa *et al.* [22], who showed poor anti-bacterial properties of Activa due to its lower fluoride release. However, Activa had higher anti-bacterial activity than giomer in our study. This agrees with Chaudhari *et al.* [35], who found that Activa releases more fluoride than giomer.

Regarding giomer, our results are in line with Tarasingh *et al.* [26] study, where giomer presented lower results than RMGI in inhibiting *S. mutans* due to lower fluoride release of giomer. However, these findings disagreed with Hotwani *et al.* [38], who claimed that giomer had a stronger anti-microbial property than RMGI against *S. mutans*. They explained that the RMGI contains higher resin that could provide an obstacle to fluoride diffusion and reduce the porosity, which may affect the amount of fluoride released. Based on our results, the null hypothesis was rejected, since Fuji II LC has a higher variation in fluoride release and anti-bacterial activity at variable storage periods than the other tested materials.

The limitations of this research are that the inhibition zone did not provide any information regarding

the survivability of *S. mutans* because it was unable to distinguish between the bacteriostatic and bactericidal activities. Additionally, an *in-vitro* test is not consistent with clinical reality, where different bacterial species may form intricate biofilms [41].

## CONCLUSIONS

Under the constraints of this research, we may conclude that the fluoride release is material- and time-dependent, and RMGI displayed the highest release of fluoride in de-ionized water at all time intervals as well as anti-bacterial activity compared with Activa and giomer. Anti-bacterial activity is correlated with fluoride release rate.

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## REGULATORY STATEMENT

The current study was carried out with the approval of the Operative Department, Al-Azhar University (Cairo, Boys, Egypt; 2019/02). There is no conflict regarding ethical considerations.

## CONFLICT OF INTEREST

The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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