Evaluation of the Antibacterial Activity Against *Streptococcus mutans* and Solubility of Different Dental Luting Cements *In Vitro*

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The aim of this *in vitro* study was to evaluate the antibacterial and solubility properties of four luting cements in artificial saliva at varying pH and clarify the composition by chemical analysis. Samples of bioceramic (C1), two resin-modified glass ionomer (C2 and C3), and zinc phosphate (C4) cements were stored on media with *Streptococcus mutans* and artificial saliva (pH 4.6 and pH 6.5). The disc diffusion method was used to evaluate antibacterial activity. In the solubility test, cement discs were stored in artificial saliva (pH 4.6 and pH 6.5) and distilled water (pH 7). After 48 and 120 hours, the solubility of the cement specimens was determined. Chemical analysis was performed by X-ray fluorescence. The growth inhibition zones of *S. mutans* induced by C4 were the largest in any pH values tested ($p < 0.05$). The solubility of C4 was significantly higher than the solubility of other cements. C2 had the smallest inhibition zones and was least soluble in all media during the observation period, but without significant differences. After 48 hours, the solubility of C4 was significantly higher at pH 4.6 compared to control. The solubility of the cements, excluding C2, was significantly higher after 120 hours at pH 6.5. A significant association was found between larger amount of zinc oxide in cement composition and a larger zone of inhibition. Lower solubility was associated with higher amounts of aluminium and silicon oxides in the cement. Thus, cement containing higher amounts of zinc oxides had the highest antibacterial activity. In the solubility test, cements were more soluble in acidic media than in neutral media, and lower solubility was detected in cements containing more aluminium and silicon oxides. In clinical practice, C1 or C3 may be suggested when there is a higher risk of caries due to more desirable combination of antibacterial activity and lower solubility, for a lower caries risk C2 might be suggested.

Keywords: bacterial sensitivity test, solubility, dental cement, *Streptococcus mutans*, dental caries.

1. INTRODUCTION

The main purpose of luting cements is to ensure prosthesis retention and seal the microgap. The success and clinical longevity of restorations depend on the antibacterial activity and low solubility of the chosen cement. Secondary caries is one of the main factors influencing the failure of fixed dental restorations [1]. A microgap between the tooth preparation margin and fixed prosthesis creates conditions for plaque accumulation and colonization of *Streptococcus mutans*, which leads to secondary caries [2]. A variable pH of oral fluids while interacting with luting cements may lead to a loss of dimensional stability [3]. These changes could compromise the cement structure and increase the possibility of secondary caries formation in a tooth-restoration interface. The demand for more biocompatible and bioactive properties of luting cements increases the need for studies of the biomechanical properties of luting cements. Glass ionomer cements (GICs) are widely used in clinical practice due to their biocompatibility [4], adhesiveness to natural tooth structures [5], ability to release fluoride, and rechargeability [6]. Fluorides effectively suppress oral bacteria growth by inhibiting enzymes that play important roles in the glycolytic cycle [7, 8]. However, more than 90 % of fluorides are not released due to firmly embedding in the cement while setting [9, 10]. Enhancing the antibacterial potential of GIC with antimicrobial additives allows residual microorganisms underneath the restorative cement to be eliminated [11]. Over the years, GICs have evolved into resin-modified glass ionomer and calcium aluminate glass ionomer hybrid cements that provide dental clinical practice with luting cements with improved biophysical properties. Calcium aluminate GIC is considered to be a bioactive cement due to its ability to release calcium, phosphate, and hydroxide ions [12]. According to Unosson et al., the higher pH induced by calcium aluminate GICs could be related to its antibacterial properties [2]. However, how the antibacterial and solubility properties of luting cements are linked to their chemical composition is unclear. The aim of the present study was to determine and compare the antibacterial effect and solubility of different cements in artificial saliva at varying pH *in vitro*. We also aimed to analyse the chemical composition of the cements and evaluate the relationship between antibacterial properties and solubility.

2. EXPERIMENTAL DETAILS

The investigated luting cements (bioceramic cement (C1), resin-modified GIC (C2), resin-modified GIC (C3),

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zinc phosphate cement (C4)) are shown in Table 1. A total of 156 samples of cement, including 36 in a control group, were prepared for antibacterial testing. All cements were mixed according to the manufacturers' instructions and transferred to plastic moulds 1.5 ± 0.2 mm deep and 8 ± 0.2 mm in diameter. The moulds were pressed by a plastic plate and the excess cement was removed. After setting, the cement discs were removed from the plastic moulds and measured using a mechanical calliper (accuracy 0.02 mm). The samples were visually assessed for the presence of pores or excess cement. *Streptococcus mutans* (strain NCTC 10449) was used to determine the growth inhibition activity of the investigated cements [2]. *S. mutans* was inoculated in modified Tryptone Soya broth (mTSB; Merck, Milipore, USA) and cultured anaerobically in a shaking water bath at 160 rpm at 37 °C for 24 hours. Bacterial cells were collected and centrifuged at 8000 rpm for 45 seconds. After suspension in sterile saline, the optical density of the *S. mutans* culture was measured using a spectrophotometer. *Salivarius mitis* agar media was prepared according to the manufacturer's instructions and poured into Petri dishes.

After setting, each dish of nutritional media was inoculated with the chosen optical density (OD600 = 5) of *S. mutans* culture equivalent to a concentration of 5 × 109 CFU/ml. Each Petri dish was divided into four parts, one for each luting cement sample. The cement discs in each plate were covered with artificial saliva (Glandosane, Germany). Half of each cement sample was covered with artificial saliva of pH 4.6 and the other half with the saliva of pH 6.5. The Petri dishes with samples were incubated under anaerobic conditions (7 – 15 % CO_2) in an anaerobic jar (Merck, Milipore, USA) at 37 °C for 48 hours and 120 hours. The control group was incubated under the same conditions but without artificial saliva. The antibacterial effect of the luting cements was assessed using the disc diffusion method. The zone of inhibition (IZ) was measured with a calliper (accuracy ± 0.02 mm) after 48 hours (IZ48) and 120 h (IZ120). The IZ was assessed by measuring three lines (Fig. 1): two lines were perpendicular to each other, intersecting in the centre of the cement disc, and the third line formed a 45° angle with each perpendicular line [14]. These lines were used to measure the distance where no bacterial growth was visually observed. The lines marking the IZ for each sample were measured three times and the average calculated.

A total of 216 cement disc samples were prepared for solubility testing. Standardized plastic moulds with a depth of 3 ± 0.2 mm and diameter of 8 ± 0.2 mm were used for the cement samples. The cement discs were prepared and adjusted in the same way as for the testing of antibacterial properties. The mass of each cement sample was measured using an electronic scale (KERN EW 220-3NM, Germany; accuracy ± 0.001 g). The initial mass of the investigated cement samples was 0.205 ± 0.005 g. The cement discs were visually assessed for pores and excess. Solubility testing was carried out as described by Chopra et al. [15]: 27 samples of each different cement were stored for 48 h (SL48): 9 cement discs in artificial saliva at pH 4.6, 9 in artificial saliva at pH 6.5, and 9 in distilled water at pH 7. Another 27 samples of each different cement were stored for 120 hours (SL120) at 37 °C: 9 cement discs in artificial saliva at pH 4.6, 9 in artificial saliva at pH 6.5, and 9 in distilled water at pH 7. After 2 and 5 days, the cement samples were removed from the media and dried in a desiccator for 1 hour. The dried samples were weighed using the electronic scale. The solubility of the cement was assessed by measuring the weight loss. The solubility was calculated as follows [15]:

Weight loss = Initial weight – Final weight

Solubility = Weight loss × 100/Initial weight

The chemical composition of the luting cements was determined using a wavelength dispersive X-ray fluorescence (WD-XRF) spectrometer (Rigaku ZSX Primus IV) equipped with a Rh target, end window, and 4 kW X-ray tube. Cylindrical cement samples with specific binder 37 mm in diameter and 3 mm in height were prepared using a hydraulic press with 200 kN capacity (Herzog TP20). The measurements were taken at 36.6 °C under a vacuum. The scan was set to identify all detectable elements > 0.0001 % by mass.

Table 1. Luting cements

Cement type, product, manufacturer	Composition	Dispensing method
Bioceramic cement (C1) „Calibra Bio", Denstply Sirona	Powder: calcium aluminate ($CaAl_2O_4$), glass powder, inert strontium fluoride. Liquid: water, polyacrylic acid, tartaric acid, lithium chloride, nitrilotriacetic acide acid/trisodium salt.	Capsule
Resin-modified GIC (C2) „FujiPlus™", GC Corporation	Powder: fluoro-alumino-silicate glass, Liquid: 2-hydroxyethylmethacrylate, distilled water, polyacrylic acid, urethanedimethacrylate.	Hand-mix
Resin-modified GIC (C3), „FujiCEM™ Evolve", GC Corporation	Fluoro-alumino-silicate glass, polycarboxylic acid, metacrylate monomers, water.	Auto-mix syringe
Zinc phosphate cement (C4), „Hoffmann's Cement", Hoffmann Dental Manufaktur	Powder: zinc oxide, magnesium oxide. Liquid: orthophosphoric acid, polyacrylic acid.	Hand-mix

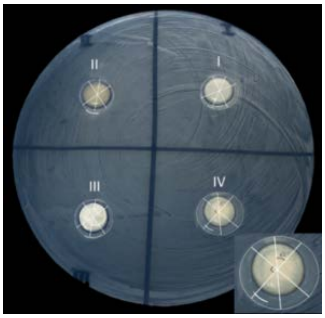

Fig. 1. Disc diffusion test. Inhibition zones around cement samples

3. STATISTICAL ANALYSIS

After 48 and 120 hours, data on IZs and solubility were evaluated using two-way analysis of variance (ANOVA) and a general linear model (GLM) with cement and pH as fixed factors and a block as a random factor. A normal distribution of data and homogeneity of variance were controlled by Shapiro-Wilk's and Levene's tests, respectively. Post-hoc comparisons of means among cements were tested using Tukey's test at a significance level of $P < 0.05$. At the cement level, SL48 and SL120 were compared by an unpaired t-test. Relationships between IZs, solubility, and chemical composition were determined using a simple linear Spearman's rank correlation. A multiple stepwise regression analysis with backward variable elimination was used to determine whether separate compounds in the chemical composition were significant predictors of the IZs and solubility of the luting cements. Before statistical analysis, all variables were log-transformed. Statistical analyses were performed using STATISTICA 5.5.

3. RESULTS

The chemical compositions of the four tested cements are summarized in Table 2. X-ray phase analysis revealed that the concentration of ZnO was highest (86.5 %) for C4. For C1, C2, and C3, the concentration of SrO constituted 75.7 %, 42.5 %, and 63.7 % of the cement mass, respectively. The concentrations of SiO_2 (29.9 %) and Al_2O_3 (13.5 %) were higher in C2 compared to C1, C3, and C4. F_2 was only found in C3.

Table 2. Chemical composition of tested cements

Chemical compound	Chemical composition, %			
	Bioceramic cement (C1)	RM-GIC (Fuji Plus) (C2)	RM-GIC (Evolve) (C3)	Zinc phosphate (C4)
F_2			0.597	
Na_2O	*	*	0.0777	*
MgO	0.153	0.502	0.0332	0.669
Al_2O_3	7.50	13.5	3.35	1.19
SiO_2	4.80	29.9	6.99	0.835
P_2O_5	3.27	2.57	0.744	9.12
SO_3	0.513	2.18	0.327	0.0291
K_2O	0.449	2.10	0.208	0.198
CaO	4.08	3.45	0.357	0.915
Fe_2O_3	0.323	1.22	0.131	0.0374
CuO	0.0218			
ZnO	3.22	0.566		86.5
SrO	75.7	42.5	63.7	0.501
TiO_2		1.43	0.306	
Cl_2			0.048	
Yb_2O_3			23.1	
NiO				0.0128
* Traces of Na_2O (< 0.0001 % by weight)				

With regard to the IZs, GLM revealed overall significant differences among the tested cements ($F = 21.8$, $P = 0.0001$), but the pH of the artificial saliva ($F = 2.1$, $P = 0.135$) and the block ($F = 1.9$, $P = 0.178$) showed no significant influence. The interaction of cement × pH was not significant (GLM, $F = 0.9$, $P = 0.525$). No IZs were observed in the control group. The only significant differences among tested cements were found in IZ48 (two-way ANOVA, $F = 54.7$, $P = 0.0001$) and IZ120 (two-way ANOVA, $F = 57.3$, $P = 0.0001$). At pH 4.6, IZ48 and IZ120 were significantly larger with C4 than the other cements (Fig. 2). At the same pH, IZ48 and IZ120 were significantly larger with C3 than C2 (Fig. 2 a). At pH 6.5, IZ48 and IZ120 had a similar pattern as at pH 4.6 (Fig. 3).
At pH 6.5, IZ48 and IZ120 were significantly larger with C1 than C2 (Fig. 3).

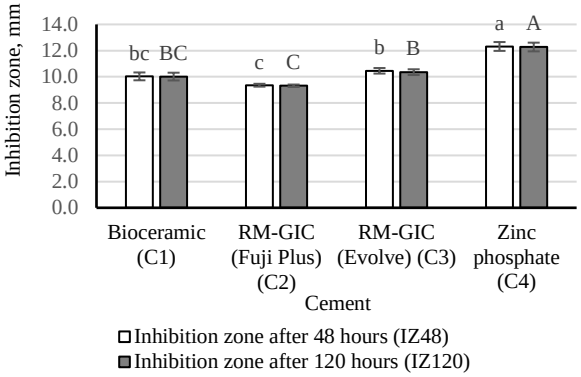

Fig. 2. Inhibition zone after 48 and 120 hours in pH 4.6. Each bar shows the mean for 15 replicates ± standard error (SE). Different letters indicate significant differences among tested cement ($P<0.05$, Tukey's (HSD) test)

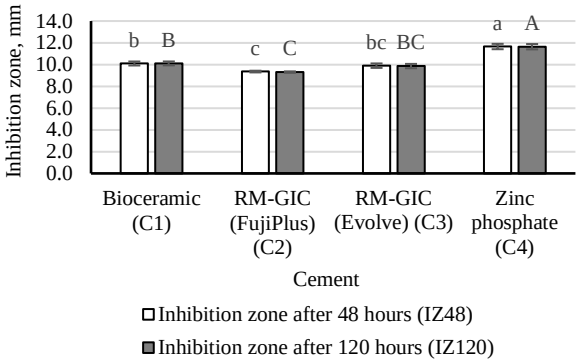

Fig. 3. Inhibition zone after 48 and 120 hours in pH 6.5. Each bar shows the mean for 15 replicates ± standard error (SE). Different letters indicate significant differences among tested cement ($P<0.05$, Tukey's (HSD) test)

With regard to the solubility of the cement, we found overall significant differences among the tested cements (GLM, $F = 23.2$, $P = 0.0001$), and the pH had a significant influence (GLM, $F = 9.0$, $P = 0.0001$). The interaction of cement × pH was significant (GLM, $F = 2.2$, $P = 0.014$). The block effect was not significant (GLM, $F = 1.6$, $P = 0.193$). Two-way ANOVA revealed significant differences in SL48 ($F = 39.6$, $P = 0.0001$) and SL120 ($F = 34.2$, $P = 0.0001$) among the tested cements. We also found significant differences in SL48 (two-way ANOVA, $F = 16.3$, $P = 0.0001$) and SL120 (two-way ANOVA, $F = 3.3$, $P = 0.044$) between artificial saliva (pH 4.6/pH 6.5) and distilled water (pH 7.0, control). The interaction (cement × pH) was significant only for SL48 (two-way ANOVA, $F = 3.4$, $P = 0.005$). At pH 4.6/pH 6.5, SL48 and

SL120 were significantly higher with C4 than the other tested cements (Fig. 4, Fig. 5). With C4, SL48 was significantly higher at pH 4.6 than pH 7.0 (Fig. 4).

At pH 7.0, SL120 was significantly higher with C4 than C3 (Fig. 5). With C1, SL120 was significantly higher at pH 6.5 than pH 7.0 (Fig. 5). At pH 6.5, SL120 was significantly lower for C2 and C3 than the other cements (Fig. 5). For C1, C3, and C4 at pH 6.5, SL120 was significantly higher than SL48 (Fig. 6). At pH 4.6 and pH 7.0, we found no significant differences between SL48 and SL120 at cement level (unpaired t-test, $P > 0.05$).

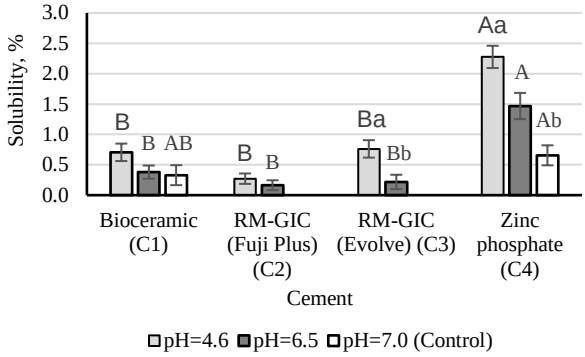

Fig. 4. Solubility after 48 (SL48) hours. Each bar shows the mean for 9 replicates ± standard error (SE). Different letters indicate significant differences among tested cement ($P<0.05$, Tukey's (HSD) test)

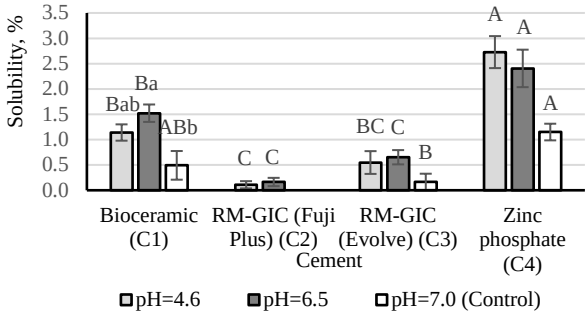

Fig. 5. Solubility after 120 (SL120) hours. Each bar shows the mean for 9 replicates ± standard error (SE). Different letters indicate significant differences among tested cement ($P < 0.05$, Tukey's (HSD) test)

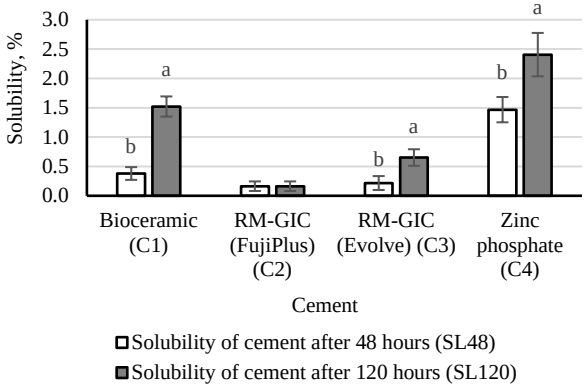

Fig 6. Solubility of different cement after 48 (SL48) and 120 (SL120) hours in pH 6.5. Each bar shows the mean for 9 replicates ± standard error (SE). Different letters indicate significant differences between SL48 and SL120 at cement level (unpaired *t*-test, $P < 0.05$)

Spearman's rank correlation showed that the IZ of luting cement positively correlated with ZnO concentration. In contrast, SrO and CaO concentrations negatively influenced the IZ. The solubility of luting cement negatively correlated with Al_2O_3 and SiO_2 concentration, but the SrO concentration negatively influenced only SL48 (Table 3). When chemical components were entered into the stepwise multiple regression model, ZnO concentration had a positive impact on the formation of an IZ ($\beta = 0.74$, $P = 0.0001$). However, Al_2O_3 and SiO_2 concentrations showed significant negative interactions with SL48 ($\beta = -0.68$, $P = 0.0001$) and SL120 ($\beta = -0.73$, $P = 0.0001$), respectively.

Table 3. Spearman's rank correlation of the chemical composition and antibacterial activity and solubility of luting cements

Chemical compound	Inhibition zone		Solubility	
	After 48 hours (IZ48)	After 120 hours (IZ120)	After 48 hours (SL48)	After 120 hours (SL120)
F_2	-0.01	-0.03	-0.17	-0.26*
Na_2O	-0.01	-0.03	-0.17	-0.26*
MgO	0.3**	0.31**	0.43***	0.38***
Al_2O_3	-0.72***	-0.72***	-0.63***	-0.65***
SiO_2	-0.69***	-0.70***	-0.67***	-0.83***
P_2O_5	0.48***	0.50***	0.57***	0.70***
SO_3	-0.72***	-0.72***	-0.63***	-0.65***
K_2O	-0.72***	-0.72***	-0.63***	-0.65***
CaO	-0.28**	-0.26**	-0.16	0.03
Fe_2O_3	-0.72***	-0.72***	-0.63***	-0.65***
CuO	-0.09	-0.07	-0.08	0.22*
ZnO	0.48***	0.50***	0.57***	0.70***
SrO	-0.33***	-0.32***	-0.40***	-0.19
TiO_2	-0.57***	-0.59***	-0.55***	-0.79***
CI_2	-0.01	-0.03	-0.17	-0.26*
Yb_2O_3	-0.01	-0.03	-0.17	-0.26*
NiO	0.66***	0.66***	0.69***	0.66***

Significant correlations are indicated by asterisks: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

4. DISCUSSION

GICs and their hybrids are able to release fluoride ions in direct contact with saliva. This bioactive property assures an anticariogenic effect of the cement [16]. In the present study, all of the tested cements were found to have an antibacterial effect on *S. mutans*. The largest IZ was observed around the zinc phosphate cement, whereas smaller zones were observed around a resin-modified GIC (Evolve) and the bioceramic cement samples after 48 hours. A resin-modified GIC (Fuji Plus) had the smallest IZs of *S. mutans*. Similar studies have reported that both zinc phosphate and GICs have the highest solubility in the first 24 – 48 hours [17]. The higher initial solubility of the cements may also be influenced by the different pH values of the human saliva or other frequently used liquids. Although Liu et al. suggested that the release of zinc ions is more intense under acidic conditions (pH 4.5 and pH 5.5) [18], no significant differences in the formation of IZs were found in the present study at pH 4.6 and pH 6.5. Varying the pH of the artificial saliva did not influence the antibacterial effect, and this finding could be attributed not only to the small sample size tested, but also to the ability of *S. mutans* to metabolize carbohydrates (sucrose and dextrose) into

organic acids in the growth media. The degradation of carbohydrates allows the bacteria to release organic acids into the environment, thereby lowering the pH of the environment to a value (pH 4 or pH 5) that is more favourable for the bacteria to survive and grow [13]. The artificial saliva used in this study had no features of buffer solution, and the ability of *S. mutans* to release organic acids may have led to equilibration of the different media to a favourable acidic pH.

X-ray fluorescence revealed that the zinc phosphate cement with the highest zinc oxide concentration had the largest zones of inhibition. In the literature, zinc oxide has been extensively studied for its ability to inhibit bacterial growth [19]. Other studies have found that zinc ions directly affect the bacterial proteins involved in transmembrane proton translocation. Consequently, zinc ions lead to indirect inhibition of bacterial protease, which promotes bacterial adhesion to dental tissues [20, 21]. The antibacterial properties of zinc phosphate cement are linked to another important physical property, solubility. In an *in vitro* study, Chopra et al. observed that the highest solubility of zinc phosphate cement was associated with the formation of weak bonds between the zinc phosphate ions and the matrix [15]. Another compound that is known to have anti-caries properties is fluoride [22]. The glass ionomers, both conventional and resin-modified, include fluoride as an inherent part of the material [23]. In our study, chemical analysis of the cements showed that the only resin-modified GIC (Evolve) contained fluorides (~ 0.6 % of the cement weight). No fluoride compounds were found in the other investigated GIC hybrids. The anticariogenic effect of fluorides is more widely known due to their ability to remineralize tooth tissues. In addition, fluorides may also affect the metabolic activity of S. *mutans*. Fluorides inhibit bacterial enolase, which catalyses the conversion of 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic metabolism of *S. mutans* [24, 25]. By interfering with bacterial metabolism, fluorides inhibit bacterial growth and acid release [26]. A slightly different chemical composition of the calcium aluminate (bioceramic) cement was found during the *in vitro* study. Of all the cements investigated, the highest percentages of calcium oxide (4.08 %) and strontium oxide (75.7 %) were detected in the bioceramic cement. Released calcium ions locally neutralize the acids produced by the bacteria, increasing pH. As a result, in neutral or alkaline media, the growth of *S. mutans* is impaired [27, 28]. Strontium is commonly used in GICs to increase their radiocontrast properties. Studies suggest that strontium ions could also have an inhibitory effect on *S. mutans*, but the mechanism of the antibacterial effect of strontium ions on cariogenic bacteria has not been thoroughly investigated [29]. The indirect antibacterial effect of strontium is attributed to its ability to increase the pH of the environment where strontium ions are released. It has been observed that, during contact between cement glass particles and organic acids, the strontium ions are released and increase the pH of the media [30]. Liu et al. observed that a higher amount of strontium in strontium-bioactive glass cement has a higher bacterial inhibitory effect [31]. In contrast, Li et al. reported that the addition of a certain amount of strontium to bioactive glass in the cement could significantly reduce the IZ [32]. Based on the results of these studies, it can be assumed that calcium and strontium ions in bioceramic cement influence IZ formation, though a sufficiently high proportion of strontium oxide in the cement may also reduce the antibacterial activity.

A physiomechanical property, such as solubility, contributes to the ability of luting cement to release ions that inhibit bacterial growth. However, the excessive solubility of the cement may irreversibly modify its structure and, potentially, reduce the adhesion of the tooth to the prosthesis, increasing marginal permeability. The lower solubility of resin-modified GICs compared to conventional GICs has been analysed in the literature due to the formation of single, double, and cross bonds between the polymers and the resin matrix [33]. The addition of nanoparticles (silicon, aluminium) has been shown to contribute to a lower solubility of the cement. Felemban et al. reported that the addition of silicon particles to resin-modified GIC powder reduced the micro-permeability and solubility of the cement [34]. Lima et al. observed that aluminosilicate-based cements have more stable hydrolytic properties, which means that they are less soluble. In the same study, the solubility of cements containing more strontium was higher, possibly due to the rapid reaction of strontium with water [35]. In the current study, the resin-modified GIC (Fuji Plus) with the lowest solubility had the lowest mass percentage of strontium oxide compared to bioceramic and the other resin-modified GIC (Evolve). Although zinc phosphate cement had the lowest amount of strontium oxide compared to the other cements, the higher solubility of this cement may be due to the zinc oxide, which composes 90 % of the powder. We found a difference in the solubility between bioceramic and resin-modified GIC (Evolve) that could be caused by the differential release of ions. After 120 hours, at pH 4.6 and pH 6.5, the bioceramic cement had a higher solubility than the resin-modified GIC (Evolve). In a study by Santos et al., the release of fluoride and calcium ions from resin-modified glass ionomer solutions at varying pH was measured at specific time intervals. The release of fluoride was highest during the first 2 days, whereas the release of calcium ions was more stable and higher than fluoride during the same period of time [36]. Various studies have revealed that the solubility of cements is higher in acidic media [37 – 39] than in neutral or alkaline media. The results obtained in this study showed that zinc phosphate cement (at pH 4.6) and bioceramic cement (Calibra Bio) (pH 6.5) were more soluble than at pH 7. In the present study, both artificial saliva pH values were acidic, resulting in greater solubility of the cements in the artificial saliva than in the neutral media (distilled water). However, a larger sample would be needed to confirm this conclusion.

The results of this study provide a basis for extending the research using more sensitive methods. The antibacterial properties were assessed using the disc diffusion method, which is simplified and requires fewer financial resources, though its accuracy depends on the solubility of the cement. Cements that are more soluble or more penetrating into nutritional agar have larger IZs, but other cements, not penetrating or slowly penetrating, might have higher antibacterial effect during direct contact with microorganisms. Moreover, the IZs were measured visually (subjectively), which may have affected the results of the *in vitro* study. The lower solubility of resin-modified GIC (Fuji

Plus) resulted in the lower inhibitory effect of this cement, and this may have contributed to less accurate results regarding the cement's antibacterial properties. Due to the limited amount of material, only a few different pH values were selected, which may have influenced the results. Artificial saliva was prepared by modifying the pH in order to create conditions similar to the oral cavity. The artificial saliva preparations contained low levels of phosphates, but immunoglobulins and fluorides, which are essential in influencing both the solubility and antibacterial properties, were not detected. Furthermore, chemical analysis of the cement before and after antibacterial and solubility testing could provide more accurate results for the concentrations of ions released from the luting cements. Further and more precise studies (*in vitro* and *in vivo*) avoiding our study limitations could help in choosing cements according to specific clinical indications for the fixation of permanent prosthetic restorations.

5. CONCLUSIONS

All of the investigated cements exhibited antibacterial activity. Zinc phosphate cement had the largest inhibition zones of *S. mutans*. Resin-modified glass ionomer (Evolve) and bioceramic (Calibra Bio) cements had smaller IZs of *S. mutans*. However, the pH of artificial saliva did not have a significant effect on the antibacterial properties of the cements. Zinc phosphate cement demonstrated the highest solubility, whereas the lowest solubility was observed with resin-modified GIC (Fuji Plus), though the difference was not significant. In acidic media, the zinc phosphate cement was more soluble after 48 hours and the bioceramic cement (Calibra Bio) after 120 hours compared to the control. The higher amount of zinc oxide may be related to the higher solubility and antibacterial effect of the cement, whereas the higher alumina and silica content in the cement may be related to the lower solubility. To the best of our knowledge, this study is the first to show fluoride in only one of the examined cements (resin-modified GIC (Evolve)), though the literature claims that all GICs and their modifications contain fluoride. As few studies have assessed bioceramic (Calibra Bio) and resin-modified GIC (Evolve), our research contributes to advancing knowledge on the properties of these cements. In addition, our study forms the basis for future *in vivo* research that could help clinicians choose the best luting cement for specific clinical indications in prosthesis fixation. According to our study, bioceramic (Calibra Bio) or resin-modified GIC (Evolve) may be suggested when there is a higher risk of caries due to in clinical practice more desirable combination of antibacterial activity and lower solubility compared to zinc phosphate cement that only showed the highest antibacterial activity. When there is a lower caries risk, resin-modified GIC (Fuji Plus) might be suggested.

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REFERENCES

1. **Behr, M., Zeman, F., Baitinger, T., Galler, J., Koller, M., Handel, G., Rosentritt, M.** The Clinical Performance of Porcelain-fused-to-metal Precious Alloy Single Crowns: Chipping, Recurrent Caries, Periodontitis, and Loss of Retention *International Journal of Prosthodontics* 27 (2) 2014; pp. 153 – 160. https://doi.org/10.11607/ijp.3440
2. **Unosson, E., Cai, Y., Jiang, X., Lööf, J., Welch, K., Engqvist, H.** Antibacterial Properties of Dental Luting Agents: Potential to Hinder the Development of Secondary Caries *International Journal of Dentistry* 2012: pp. e529495. https://doi.org/10.1155/2012/529495.
3. **Bharali, K., Das, M., Jalan, S., Paul, R., Deka, A.** To Compare and Evaluate the Sorption and Solubility of Four Luting Cements after Immersion in Artificial Saliva of Different pH Values *Journal of Pharmacy & Bioallied Sciences* 9 (1) 2017: pp. S103 – S106. https://doi.org/10.4103/jpbs.JPBS_153_17
4. **Sidhu, S.K., Schmalz, G.** The Biocompatibility of Glass-ionomer Cement Materials. A Status Report for the American Journal of Dentistry *American Journal of Dentistry* 14 (6) 2001: pp. 387 – 396.
5. **Yoshida, Y., Van Meerbeek, B., Nakayama, Y., Snauwaert, J., Hellemans, L., Lambrechts, P., Vanherle, G., Wakasa, K.** Evidence of Chemical Bonding at Biomaterial-hard Tissue Interfaces *Journal of Dental Research* 79 (2) 2000: pp. 709 – 714. https://doi.org/10.1177/00220345000790020301
6. **Lee, S.Y., Dong, D.R., Huang, H.M., Shih, Y.H.** Fluoride Ion Diffusion from a Glass-ionomer Cement *Journal of Oral Rehabilitation* 27 (7) 2000: pp. 576 – 586. https://doi.org/10.1046/j.1365-2842.2000.00554.x
7. **Pradiptama, Y., Purwanta, M., Notopuro, H.,** Antibacterial Effects of Fluoride in Streptococcus mutans Growth *in Vitro* *Biomolecular and Health Sciences Journal* 2 (1) 2019: pp. 1 – 3. https://doi.org/10.20473/bhsj.v2i1.13232
8. **Lemos, J.A., Palmer, S.R., Zeng, L., Wen, Z.T., Kajfasz, J.K., Freires, I.A., Abranches, J., Brady, L.J.** The Biology of *Streptococcus mutans* *Microbiology Spectrum* 7 (1) 2019: pp. 10. https://doi.org/10.1128/microbiolspec.GPP3-0051-2018.
9. **Nicholson, J.W., Czarnecka, B.** Maturation Affects Fluoride Uptake by Glass-ionomer Dental Cements *Dental materials: official publication of the Academy of Dental Materials* 28 (2) 2012: pp. e1 – 5. https://doi.org/10.1016/j.dental.2011.10.011.
10. **Hasan, A.M.H.R., Sidhu, S.K., Nicholson, J.W.** Fluoride Release and Uptake in Enhanced Bioactivity Glass Ionomer Cement ('glass carbomerTM') Compared with Conventional and Resin-modified Glass ionomer Cements *Journal of Applied Oral Science* 27 2019: pp. e20180230. https://doi.org/10.1590/1678-7757-2018-0230
11. **Takahashi, Y., Imazato, S., Kaneshiro, A.V., Ebisu, S., Frencken, J.E., Tay, F.R.** Antibacterial Effects and Physical Properties of Glass-ionomer Cements Containing Chlorhexidine for the ART Approach *Dental Materials: Official Publication of the Academy of Dental Materials* 22 (7) 2006: pp. 647 – 652. https://doi.org/10.1016/j.dental.2005.08.003
12. **Engstrand, J., Unosson, E., Engqvist, H.** Hydroxyapatite Formation on a Novel Dental Cement in Human Saliva *ISRN Dentistry* 2012: p. 624056.

https://doi.org/10.5402/2012/624056

13. **Baker, J.L., Faustoferri, R.C., Quivey, R.G.** Acid-adaptive Mechanisms of Streptococcus mutans– The More We Know, The More We Don't *Molecular Oral Microbiology* 32 (2) 2017: pp. 107 – 117. https://doi.org/10.1111/omi.12162.
14. **Kresnoadi, U., Santosa, I.K.B.** Antibacterial and Solubility Power Differences of Luting Cement, Resin Reinforced Glass ionomer, Bioactive, and Resin-based Composite in Dental Restoration *Eurasian Journal of Biosciences* 14 (2) 2020: pp. 3779 – 3783.
15. **Chopra, S., Bansal, P., Vilekar, A.M., Aatish, S., Sultan, M.** Evaluating the Solubility of Various Dental Cements in Artificial Saliva in Different Mediums at different Time Intervals: In-Vitro Study *European Journal of Molecular & Clinical Medicine* 7 (11) 2021: pp. 4692 – 4701.
16. **Do, Q., Nguyen, N., Huynh, T., Pham, H., Ho, T., Kieu, D., Huynh, N.** Study on the Fluoride-releasing Ability of Dental GIC Materials in Deionized Water and Artificial Saliva Environments *Vietnam Journal of Science, Technology and Engineering* 63 2021: pp. 7 – 14. https://doi.org/10.31276/VJSTE.63(1).07-14
17. **Vega-Jiménez, A.L., Rodríguez-Hernández, A.G., Piña-Barba, M.C., Ambrosio-Hernández, J.** Effect of Ions Released and pH of Two Glass Ionomer Cements in Human Gingival Fibroblasts *Odovtos International Journal of Dental Sciences* 21 (1) 2019: pp. 83 – 93.
18. **Liu, Y., Kohno, T., Tsuboi, R., Kitagawa, H., Imazato, S.** Acidity-induced Release of Zinc Ion from BioUnionTM filler and Its Inhibitory Effects Against *Streptococcus mutans Dental Materials Journal* 39 (4) 2020: pp. 547 – 553. https://doi.org/1010.4012/dmj.2019-061
19. **Lee, M.J., Seo, Y.B., Seo, J.Y., Ryu, J.H., Ahn, H.J., Kim, K.M., Kwon, J.S., Choi, S.H.** Development of a Bioactive Flowable Resin Composite Containing a Zinc-Doped Phosphate-Based Glass *Nanomaterials* 10 (11) 2020: pp. E2311. https://doi.org/10.3390/nano10112311
20. **Almoudi, M.M., Hussein, A.S., Abu Hassan, M.I., Mohamad Zain, N.** A Systematic Review on Antibacterial Activity of Zinc Against Streptococcus mutans *Saudi Dental Journal* 30 (4) 2018: pp. 283 – 291. https://doi.org/10.1016/j.sdentj.2018.06.003
21. **Phan, T.N., Buckner, T., Sheng, J., Baldeck, J.D., Marquis, R.E.** Physiologic Actions of Zinc Related to Inhibition of Acid and Alkali Production by Oral Streptococci in Suspensions and Biofilms *Oral Microbiology and Immunology* 19 (1) 2004: pp. 31 – 38. https://doi.org/10.1046/j.0902-0055.2003.00109.x
22. **Featherstone, J.D.** Prevention and Reversal of Dental caries: Role of Low Level Fluoride *Community Dentistry and Oral Epidemiology* 27 (1) 1999: pp. 31 – 40. https://doi.org/10.1111/j.1600-0528.1999.tb01989.x
23. **Sidhu, S.K., Nicholson, J.W.** A Review of Glass-Ionomer Cements for Clinical Dentistry *Journal of Functional Biomaterials* 7 (3) 2016: pp. E16 https://doi.org/10.3390/jfb7030016
24. **Takahashi, N., Washio, J.** Metabolomic Effects of Xylitol and Fluoride on Plaque Biofilm *In Vivo Journal of Dental research* 90 (12) 2011: pp. 1463 – 1468. https://doi.org/10.1177/0022034511423395
25. **Kitagawa, H., Miki-Oka, S., Mayanagi, G., Abiko, Y., Takahashi, N., Imazato, S.** Inhibitory Effect of Resin Composite Containing S-PRG Filler on Streptococcus mutans Glucose Metabolism *Journal of Dentistry* 70 2018: pp. 92 – 96. https://doi.org/10.1016/j.jdent.2017.12.017
26. **Manome, A., Abiko, Y., Kawashima, J., Washio, J., Fukumoto, S., Takahashi, N.** Acidogenic Potential of Oral Bifidobacterium and Its High Fluoride Tolerance *Frontiers in Microbiology* 10 2019: pp. 1099. https://doi.org/10.3389/fmicb.2019.01099
27. **Gubler, M., Brunner, T.J., Zehnder, M., Waltimo, T., Sener, B., Stark, W.J.** Do Bioactive Glasses Convey a Disinfecting Mechanism Beyond a Mere Increase in pH? *International Endodontic Journal* 41 (8) 2008: pp. 670 – 678. https://doi.org/10.1111/j.1365-2591.2008.01413.x
28. **Galarraga-Vinueza, M.E., Passoni, B., Benfatti, C.A.M., Mesquita-Guimarães, J., Henriques, B., Magini, R.S., Fredel, M.C., Meerbeek, B.V., Teughels, W., Souza, J.C.M.,** Inhibition of Multi-species Oral Biofilm by Bromide Doped Bioactive Glass *Journal of Biomedical Materials Research A* 105 (7) 2017: pp. 1994 – 2003. https://doi.org/10.1002/jbm.a.36056
29. **Guida, A., Towler, M., Wall, J.G., Hill, R., Eramo, S.** Preliminary Work on the Antibacterial Effect of Strontium in Glass ionomer Cement *Journal of Material Science Letters* 22 2003: pp. 1401 – 1403. https://doi.org/10.1023/A:1025794927195
30. **Fredholm, Y., Karpukhina, N., Law, R., Hill, R.** Strontium Containing Bioactive Glasses: Glass Structure and Physical Properties *Journal of Non-Crystalline Solids* 356 2010: pp. 2546 – 2551. https://doi.org/10.1016/j.jnoncrysol.2010.06.078
31. **Liu, J., Rawlinson, S.C.F., Hill, R.G., Fortune, F.** Strontium-substituted Bioactive Glasses *In Vitro* Osteogenic and Antibacterial Effects *Dental materials: official publication of the Academy of Dental Materials* 32 (3) 2016: pp. 412 – 422. https://doi.org/10.1016/j.dental.2015.12.013
32. **Li, Y., Stone, W., Schemitsch, E.H., Zalzal, P., Papini, M., Waldman, S.D, Towler, M.R.** Antibacterial and Osteo-stimulatory Effects of a Borate-based Glass Series Doped with Strontium Ions *Journal of Biomaterials Applications* 31 (5) 2016: pp. 674 – 683. https://doi.org/10.1177/0885328216672088
33. **Agha, A., Parker, S., Parkinson, E.K., Patel, M.** Characteristics of experimental resin-modified glass-ionomer cements, containing alternate monomers to HEMA *Dental materials: official publication of the Academy of Dental Materials* 37 (10) 2021: pp. 1542 – 1552. https://doi.org/10.1016/j.dental.2021.08.003
34. **Felemban, N.H., Ebrahim, M.I.** Effects of Adding Silica Particles on Certain Properties of Resin-modified Glass-ionomer Cement *European Journal of Dentistry* 10 (2) 2016: pp. 225 – 229. https://doi.org/10.4103/1305-7456.178318
35. **Lima, R.B., Farias, J.F., Andrad, A.K., Silva, F.D., Duarte, R.M.** Water Sorption and Solubility of Glass ionomer Cements Indicated for Atraumatic Restorative Treatment Considering the Time and the pH of the Storage Solution *Revista Gaúcha de Odontologia* 66 2018: pp. 29 – 34. https://doi.org/10.1590/1981-86372018000100043100
36. **Santos, S.S., Delbem, A.C.B., Moraes, J.C.S., Souza, J.A.S., Oliveira, L.Q.C., Pedrini, D.** Resin-modified Glass ionomer Containing Calcium Glycerophosphate: Physico-mechanical Properties and Enamel Demineralization

Journal of Applied Oral Science: revista FOB 27 2019: pp. e20180188. https://doi.org/10.1590/1678-7757-2018-0188

37. **Yoshida, K., Tanagawa, M., Atsuta, M.** In-vitro Solubility of Three Types of Resin and Conventional Luting Cements *Journal of Oral Rehabilitation* 25 (4) 1998: pp. 285 – 291. https://doi.org/10.1111/j.1365-2842.1998.00228.x
38. **Marghalani, H.Y.** Sorption and Solubility Characteristics of Self-adhesive Resin Cements *Dental Materials: Official Publication of the Academy of Dental Materials* 28 (10) 2012: pp. e187 – 198.
39. **Giti, R., Vojdani, M., Abduo, M., Bagheri, R.** The Comparison of Sorption and Solubility Behavior of Four Different Resin Luting Cements in Different Storage Media *Journal of Dentistry Shiraz Iran* 17 (2) 2016: pp. 91 – 97.