Growth inhibition of glass ionomer cements on mutans streptococci

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Abstract

This study was conducted to identify the factors involved in the antibacterial activity of glass ionomer cement (GIC) on mutans streptococci. The antibacterial effect of GIC was estimated using agar plates infected with strains of S. mutans *and* S. sobrinus. *The effect of pH and fluoride release of GIC on mutans streptococci was studied under acid and neutral pH. Strains of* S. sobrinus *were more sensitive to GIC antibacterial activity, the difference being statistically significant ($P < 0.001$). The GIC Fuji II LC, Fuji II type II, Vitremer, Vitrebond, and Ketac-Cem were the most active materials in this study. The inhibition activity was associated with GIC fluoride release; 140 ± 25 ppm were required to inhibit* S. sobrinus *6715. Inhibition activity was not associated with changes in pH after setting of these materials. (Pediatr Dent 16:346–49, 1994)*

Introduction

Tooth decay is perhaps the most common and expensive bacterial disease in humans. Studies carried out in vivo and in vitro have shown that *Streptococcus mutans*, and to a lesser extent *S. sobrinus*, are isolated from plaque samples of crowns and roots, and are the main factor in dental caries.[1,2]

Recurrent dental caries has been associated with deteriorating restorative materials, providing a potential pathway to the cariogenic strains of mutans streptococci. Glass ionomer cements (GIC) inhibit growth of mutans streptococci, its antibacterial effect believed to be due to fluoride release in vitro.[3-5] The antibacterial activity of GIC may be due to the low pH of the cement before setting and/or its fluoride release.[6] Studies in vivo have shown that GIC reduced the prevalence of dental caries in a child population[7] and levels of mutans streptococci in human plaque samples.[8,9] However, these studies did not provide information as to whether the antibacterial effect reduced the level of *S. mutans*, *S. sobrinus*, or both. GICs are used increasingly in pediatric dentistry as preventive alternatives to other restorative materials, and new GICs contain fluoride and have superior physical properties. This study compared the bacterial inhibition, pH, and fluoride release of four glass ionomer liners/bases and seven restorative materials on strains of *S. mutans* and *S. sobrinus*.

Methods and materials

Bacterial strains and growth conditions

The GICs used in this study are shown in Table 1. Indicator bacterial strains used to determine the growth inhibition activity of GIC on mutans streptococci included: *S. mutans* MT8148, NG71, and GS5 (serotype *c*); *S. mutans* MT703R (serotype *e*); *S. mutans* OMZ175 (serotype *f*). *S. sobrinus* MT4532, MT6223, [6715 (serotype *g*) strains also were included in this study]. These strains were grown in brain heart infusion broth (BHI™, Difco Laboratories, Detroit, MI) for 18 hr at 37°C. All tested strains were grown and subcultured once a week on trypticase soy agar (TSA™, BBL Microbiology Systems) plates anaerobically. The origin of these mutans streptococci strains was reported previously.[10] The GICs used in this study were prepared according to manufacturers' recommendations.

Antibacterial effect of GIC on agar plates was estimated by adding 200 µl of an overnight culture (BHI) broth of the indicator strain [about $2x10^7$ colony forming units (cfu) to 3 ml of sterile saline]. This suspension was poured onto the surface of TSA plates. Excess was removed by pipette and plates were dried for 10 min at room temperature. The visible light-cured (VLC) materials were polymerized with a 40-sec irradiation with a

Table 1. GIC used in this study

Materials	*Symbol*	*Manufacturer*	*Batch No.*
GIC Liners/Bases			
Ketac-Cem	KC	Espe	032790
Photac-Bond-Aplicap	PBA	Espe	0008
Fuji Lining LC	FLLC	GC	270803
Vitrebond	VB	3M	881111
GIC Restorative			
Fuji II Type II	FII	GC	920624
Fuji II LC	FIILC	GC	130224
Miracle Mix	MM	GC	920304
Ketac-Fil-Aplicap	KFA	Espe	022A28
Ketac-Silver	KS	Espe	P064
VariGlass	VG	Caulk	921231
Vitremer	VM	3M	19930119

Espe/Premier, Norristown, PA; GC Corporation, Tokyo, Japan; 3M, St. Paul, MN; Caulk, Milford, DE.

visible light-curing unit (Optilux 400™, Demetron Research Corp, Danbury, CT). The capsulated materials were triturated in high speed for 10 sec using a Var-i-Mix III™ (Caulk, Milford, DE). The GIC to be tested was mixed according to the manufacturer's specifications and then placed at a predesignated area (about 3 mm in diameter) onto the surfaces of TSA plates previously inoculated with *S. mutans* and were incubated anaerobically for 2 days. Under these conditions, the inhibition zone was recorded in mm and each experiment was carried out in triplicate. Mean and standard deviation of each experiment was recorded. The growth inhibition activity of GIC (Fuji II LC, Fuji Lining LC, Vitrebond, and Vitremer) on strains of *S. mutans* and *S. sobrinus* was analyzed by repeated measures analysis of variance (ANOVA).

Effect of pH and fluoride release

For measuring pH, GIC specimens were placed into sterile borosilicate glass tubes (13x100 mm, Baxter Scientific Products, McGaw Park, IL), light cured, and then 2 ml of double-distilled deionized (Milli-Q treated, Millipore) water (pH 6.2) or 2 ml of 50 mM Tris-HCl buffer (pH 8.0) was added. The tubes were incubated at 37°C during 48-hr period and pH was estimated using an electrode (43 pH meter™, Beckman Instruments Inc, Fullerton, CA). To inactivate the acid pH of GIC after setting, GIC samples were incubated in Tris-HCl buffer to adjust to pH 7.1–7.3. Fifty-µl samples then were added into holes in TSA plates previously infected with *S. sobrinus* 6715, and inhibition activity was estimated as described above. Fluoride release was measured with fluoride specific electrode (9609, Orion Research Inc., Boston, MA). A calibration curve was prepared with fresh standards and results expressed in ppm/mg.

Results

Growth inhibition of GIC (liners and bases) is shown in Table 2. Vitrebond, Fuji Lining LC, and Ketac-Cem showed the highest inhibition activity against all strains tested. However, agar plates infected with *S. sobrinus* showed more inhibition activity than plates infected with *S. mutans* ($P < 0.001$). Growth inhibition activity of GIC (restorative type) is shown in Table 3. Fuji II LC, Fuji II type II, Ketac-Fil, and Vitremer were the most active materials against all strains tested. However, all strains belonging to *S. sobrinus* were most sensitive to the antibactericidal activity of GIC restorative-type materials. Miracle Mix showed greater inhibition areas than Ketac-Silver cement against both *S. mutans* and *S. sobrinus* and their inhibitory activity was similar against both bacterial groups.

Figs 1 and 2 show changes in pH in 2-ml distilled water during 3-hr incubation at room temperature. Fig 1 shows the pH of GIC liners/bases and among these materials, Fuji Lining LC showed the lowest pH (4.2) followed by Photac-Bond-Applicap and Vitrebond (pH 5.2), and Ketac-Cem (5.5). Fig 2 shows changes in pH by GIC restorative type — VariGlass showed the lowest pH during this period. The materials Fuji II LC, Fuji II type II, and Miracle Mix showed a pH of about 4.2–4.3; Ketac-Fil Applicap and Ketac-Silver showed a pH of about 5.0–5.2; and Vitrebond and Vitremer showed a

Table 2. Antibacterial activity of GIC on mutans streptococci

Specie	Sero-type	GIC Liners/Bases FLLC	KC	PBA	VB
S. mutans					
MT8148	*c*	2.7 ± 0.6	1.8 ± 0.3	1.0 ± 0.0	3.8 ± 0.3
NG71	*c*	2.0 0.0	2.6 0.6	1.0 0.0	3.8 0.3
GS5	*c*	2.3 0.3	1.8 0.3	0.0 0.0	3.6 0.3
MT703R	*e*	2.2 0.7	2.5 0.5	0.3 0.3	3.6 0.3
OMZ175	*f*	1.5 ± 0.5	2.8 ± 0.3	0.0 ± 0.0	3.8 ± 0.3
S. sobrinus					
6715	*g*	3.8 ± 0.3	1.8 ± 0.3	0.3 ± 0.3	4.0 ± 0.0
MT4532	*g*	3.2 0.3	2.0 0.0	0.3 0.3	4.3 0.3
MT6223	*g*	3.2 ± 0.3	2.3 ± 0.3	0.0 ± 0.0	4.6 ± 0.5

Inhibition zone was determined in mm as described in methods.

Table 3. Antibacterial activity of GIC restorative materials on mutans streptococci

Specie	Sero-type	GIC Restorative Materials FIILC	FII	KFA	KS	MM	VG	VM
S. mutans								
MT8148	*c*	3.6 ± 0.5	3.5 ± 0.5	1.7 ± 0.6	0.5 ± 0.0	1.2 ± 0.3	0.0 ± 0.0	3.0 ± 0.5
NG71	*c*	3.2 0.6	3.3 0.6	2.7 0.6	0.5 0.0	1.2 0.3	0.0 0.0	3.0 0.5
GS5	*c*	3.6 0.5	3.5 0.5	1.8 0.3	0.5 0.0	2.0 0.7	0.0 0.0	3.2 0.3
MT703R	*e*	3.8 0.7	3.0 0.5	1.2 0.3	0.5 0.0	1.8 0.6	0.6 0.3	3.6 0.3
OMZ175	*f*	3.3 ± 0.5	3.8 ± 0.3	2.2 ± 0.3	0.5 ± 0.0	0.8 ± 0.6	1.0 ± 0.3	3.2 ± 0.3
S. sobrinus								
6715	*g*	4.6 ± 0.5	2.1 ± 0.3	1.3 ± 0.3	0.5 ± 0.0	1.2 ± 0.3	1.0 ± 0.3	4.0 ± 0.3
MT4532	*g*	4.6 0.5	2.5 0.0	1.0 0.0	0.5 0.0	1.2 0.3	0.0 0.0	4.3 0.3
MT6223	*g*	4.3 ± 0.6	2.0 ± 1.0	0.8 ± 0.3	0.5 ± 0.0	1.2 ± 0.3	0.0 ± 0.0	4.2 ± 0.3

Inhibition zone was determined in mm as described in methods.

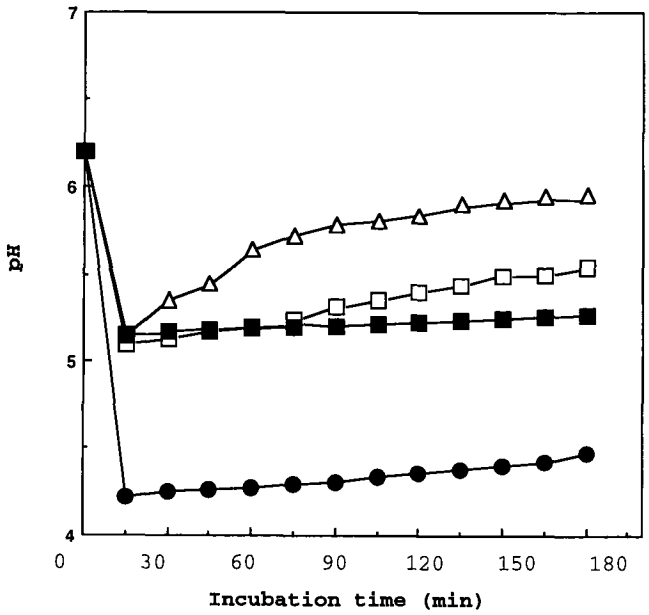

Fig 1. Changes in pH of GIC bases/liners in 2 ml of distilled water. ❑, KC; ●, FLLC; ■, PBA; and Δ, VB.

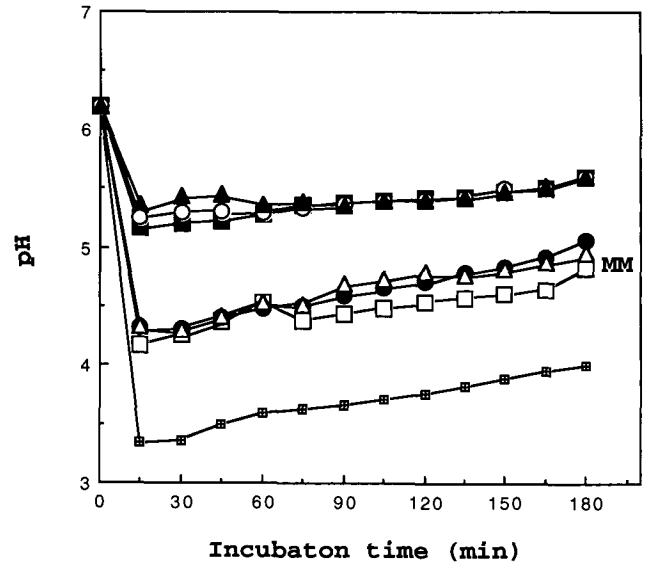

Fig 2. Changes in pH of GIC restorative type in 2 ml of distilled water. ●, FII type II; ❑, FIILC; Δ, MM; ○, KFA; ■, KS; ⊞, VG; and ▲, VM.

pH of about 5.2 under conditions described in the methods. However, the inhibition activity was not correlated with the pH; no statistical difference was found in inhibition in experiments with acidic and neutral pH. The pH was monitored for 48 hr (data not shown), but no changes were noticed.

The GICs that showed the highest fluoride release were, Vitrebond, Fuji II LC, Fuji Lining LC, Ketac-Fil-Applicap, and Vitremer (Table 4). Also, these materials showed more antibacterial activity against all strains of mutans streptococci tested. There was a direct correlation between the amount of fluoride release and the area of bacterial inhibition ($R = 0.83$). When the pH was stabilized to 7.1–7.3 by using 50 mM Tris-HCl buffer (pH 8.0) and tested in TSA plates infected with indicator strains, only the GICs that released fluoride of 140 ± 25 ppm or more were active against strains of mutans streptococci.

Discussion

GICs have a degree of antibacterial activity on some strains of *S. mutans, S. sanguis, A. viscosus, S. salivarius, S. mitis,* and *L. casei,* but most studies used only one or two strains of each bacterial group.[3,6] They correlated the inhibition activity to the low pH of GIC after setting and/or fluoride release from these dental materials. Since *S. mutans* is associated with pit and fissure caries and *S. sobrinus* with smooth surfaces dental caries, and strains of these genetic groups have different cariogenicity in animal models,[1,2] it is important to include several strains of these bacterial groups to make conclusions on inhibitory activity.

All GICs investigated in this study possess some antibacterial effect — when freshly mixed — against at least one of the indicator strains used, but they may not be effective against the whole genetic group. These results suggest that low pH after setting is not associated with GIC inhibition activity. On the other hand, fluoride release was associated directly with the antimicrobial activity of GIC when the pH was adjusted to 7.1–7.3 by using 50 mM of Tris-HCl buffer (pH 8.0). Several investigators have demonstrated that fluoride has both a direct and indirect effect on the bacterial cell

Table 4. Fluoride release by glass ionomer cements

	Days						
GIC	*1*	*2*	*3*	*4*	*5*	*7*	*8*
Liners/Bases							
KC	49 ± 9	29 ± 7	27 ± 8	26 ± 8	25 ± 7	24 ± 7	22 ± 3
FLLC	253 15	228 10	208 15	176 12	155 10	131 11	114 10
VB	314 ± 18	306 ± 11	298 ± 10	290 ± 12	282 ± 10	270 ± 13	265 ± 10
Restorative Type							
KFA	198 ± 17	155 ± 15	145 ± 10	133 ± 15	121 ± 12	114 ± 15	106 ± 16
FIILC	257 20	229 12	212 15	180 18	163 17	142 15	131 17
VG	61 9	41 10	40 5	38 3	37 3	34 5	32 6
VM	210 ± 17	175 ± 12	167 ± 15	158 ± 12	140 ± 13	128 ± 12	116 ± 10

Results are expressed as ppm/mg.

of mutans streptococci, producing inhibition of acid production and electrolyte metabolism in vitro.[11,12] Our results indicate that fluoride at 140 ± 25 ppm concentration inhibits strains of mutans streptococci. Other ingredients, such as zinc, also could have played a role in bacterial growth inhibition.

Although, all GICs used in this study inhibit strains of *S. sobrinus* most effectively in vitro, the most effective inhibitor in the mouth could be GIC liners and bases. These are in close contact with dental caries and do not have the disadvantage of constant saliva flow to dilute fluoride concentration. GIC restorative materials may be effective for short time period, perhaps only a few days.

Conclusions

1. GIC restorative materials have antibacterial activity against all strains of mutans streptococci tested. However, GICs showed greater inhibition growth zones on TSA plates against strains of *S. sobrinus*.
2. The inhibition activity was associated with GIC fluoride release when pH was adjusted to neutrality; *S. sobrinus* 6715 inhibition required 140 ± 25 ppm or more of fluoride.

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