

Effect of antibacterial agents on the surface hardness of a conventional glass-ionomer cement

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ABSTRACT

In atraumatic restorative treatment (ART), caries removal with hand excavation instruments is not as efficient as that with rotary burs in eliminating bacteria under the glass ionomer cements (GICs). Thus, different antibacterial agents have been used in recent studies to enhance the antibacterial properties of the GICs, without jeopardizing their basic physical properties. Objective: The objective of this study was to evaluate the effect of antibacterial agents on the surface hardness of a conventional GIC (Fuji IX) using Vickers microhardness [Vickers hardness number (VHN)] test. Material and Methods: Cetrimide (CT), cetylpyridinium chloride (CPC) and chlorhexidine (CHX) were added to the powder and benzalkonium chloride (BC) was added to the liquid of Fuji IX in concentrations of 1% and 2%, and served as the experimental groups. A control group containing no additive was also prepared. After the completion of setting reaction, VHN measurements were recorded at 1, 7, 15, 30, 60, and 90 days after storage in 37°C distilled water. A one-way ANOVA was performed followed by a Dunnett t test and Tamhane T2 tests and also repeated measurements ANOVA was used for multiple comparisons in 95% confidence interval. Results: VHN results showed significant differences between the control and the experimental groups at all time periods ($p < 0.05$ for all). Significant differences were observed between all study periods for individual groups ($p < 0.05$). After 7 days, VHNs were decreased in all experimental groups while they continued to increase in the control group. BC and CHX groups demonstrated the least whereas CT and CPC groups exhibited most adverse effect on the hardness of set cements. Conclusions: Despite the decreased microhardness values in all experimental groups compared to the controls after 7 up to 90 days, incorporating certain antibacterial agents into Fuji IX GIC showed tolerable microhardness alterations within the limitations of this *in vitro* study.

Key words: Glass ionomer cement. Antibacterial agents. Hardness.

INTRODUCTION

The success of the recently developed atraumatic restorative treatment (ART) procedure relies on the performance of glass ionomer cements (GICs). The ART technique consists of excavating infected dentine caries with hand instruments, followed by sealing the cavities and adjacent fissures with the GIC^{8,22}. In this procedure, the conventional hand-mixed GICs are known as the most commonly recommended dental material because of their fluoride and aluminum releasing patterns and also low pH characteristics during the adhesion reaction^{6,10,11,14,24,27,28}. In a

previous study, it was reported that using manual instruments and restoring the cavities with GICs might have a possibility to control the caries disease⁹.

However, when conventional hand-mixed and fluoride containing GICs are used for sealing cavities, it may be questionable whether caries inhibition process would occur under the restorations. Cavities treated by ART may have residual infected dentine and if a GIC is unable to arrest the carious process, the restoration could fail^{26,28-30}. Additionally, caries removal using the hand excavation technique is not always as effective as rotary burs in terms of eliminating bacteria^{11,12}. Thus, when GIC restorations

are placed, residual bacteria may remain viable for up to 2 years, and the resulting infected dentin may cause restoration failure^{29,30}.

To address this problem, several studies have been undertaken to enhance the antibacterial properties of the GICs. These ideas may constitute new suggestions for improving antibacterial properties of GICs without jeopardizing their basic physicochemical characteristics in the current literature^{1,3,18,23,25}. Chlorhexidine (CHX) is a widely used antibacterial agent, and has been used to enhance the antibacterial properties of GICs^{13,18,23,25}. In addition, other cationic disinfectants such as cetrимide (CT), cetylpyridinium chloride (CPC) and benzalkonium chloride (BC) have been incorporated into the GICs. In a previous study, Botelho^{1,3} (2005,2003) showed that the addition of these agents to the conventional GIC Fuji IX in various concentrations had significant antimicrobial effects. However, incorporation of antibacterial agents may cause basic mechanical changes in the physical properties of GICs^{2,4,18,23,25}. In this regard, Botelho² (2004) also showed that the addition of CT, CPC, BC and CHX in concentrations of 1 to 4% to Fuji IX reduced the compressive strength at 7 days. Such a reduction in strength is likely to affect the clinical performance of the cement. Botelho⁴ (2005) went on to use CHX, BC and CT at 1 to 5% final concentrations with dentin conditioner and found that in most cases, the bond strength of Fuji IX to dentin was unaffected. The one exception was BC at 5%, where there was a slight decrease in bond strength.

Surface hardness is an important factor in controlling wear resistance and thus can be used as an indication of likely long-term durability of materials¹⁵. Recently, it has been shown that microhardness is a valid measure of the surface properties of GICs^{7,19-21,31}. In Vickers microhardness [Vickers hardness number (VHN)] measurements, the hardness number increases as surface hardness increases²¹.

This study was undertaken to obtain information on the effects of incorporating CT, CPC and CHX into the powder of Fuji IX and BC into the liquid of Fuji IX. Microhardness was determined as an indication of the effect of these additives on the mechanical properties and hence the likely durability of the cements. These various additives were used at concentrations of 1% and 2%, and the values were compared to those of a control group of additive-free Fuji IX samples.

MATERIAL AND METHODS

All experiments were conducted using the conventional hand-mixed GIC Fuji IX (GC, Tokyo, Japan). CT (Serva, Heidelberg, Germany), CPC (Amresco, Ohio, USA) and CHX (Serva) were added to the powder, while BC (Serva) was added to the liquid at 1% and 2% final concentrations by mass.

This produced a total of 9 groups, 8 experimental and 1 control (Figure 1). Cements were mixed at powder/liquid ratios of 3.6:1 in all cases. Samples with dimensions of 5 mm diameter and 2 mm depth were prepared by placing freshly mixed cement pastes in metal moulds and allowing them to cure for the appropriate length of time.

Microhardness test

Eight GIC samples (5 mm in diameter and 2 mm deep) were prepared for each group. The GIC samples were prepared according to the manufacturers' directions and a polyester strip was used to cover the cement for 7 min until the initial reaction was completed. Slight pressure was applied and the bulk of extruded excess cement was removed.

Cements were covered with varnish and after the completion of the setting reaction, samples were placed into the plastic moulds containing distilled water and stored at 37°C for 90 days. VHN measurements were made at 1, 7, 15, 30, 60, and 90 days with the standard microhardness tester (HNV-700, Shimadzu Corp., Tokyo, Japan) on the top of the surface of each specimen and recorded. Vickers diamond indentations were performed under a load of 300 g and 15 s. Each sample was subjected to three indentations located 200 µm far from each other, and the mean VHN value was recorded. The diagonal length of the impressions were measured and the hardness (H) was calculated according to the standard formula $H=1.854P/d^2$.

Statistical analysis

To determine significant differences between the groups, a one-way ANOVA was performed followed by Dunnett t test and Tamhane T2 tests. To obtain the significant differences during the study period, repeated measurements ANOVA was used for multiple comparisons of the individual groups at 95% confidence interval using SPSS for Windows 15.0 (SPSS, Inc., Chicago, IL USA).

CONTROL GROUP	EXPERIMENTAL GROUPS	
Fuji IX	1% Cetrимide	+Fuji IX
	2% Cetrимide	+Fuji IX
	1% Cetylpyridinium chloride	+Fuji IX
	2% Cetylpyridinium chloride	+Fuji IX
	1% Benzalkonium chloride	+Fuji IX
	2% Benzalkonium chloride	+Fuji IX
	1% Chlorhexidine	+Fuji IX
	2% Chlorhexidine	+Fuji IX

Figure 1- Groups of specimens

Table 1- Mean Vickers hardness number (VHN) values of all groups±standard deviations (sd) during the study period after storage in distilled water

Groups	Storage time					
	1 day	7 days	15 days	30 days	60 days	90 days
Fuji IX	53.67±0.07 ^{A,1}	63.75±0.53 ^{A,2}	63.79±0.12 ^{A,3}	63.80±0.07 ^{A,4}	63.84±0.11 ^{A,5}	63.88±0.07 ^{A,6}
1% CT	50.07±0.07 ^{B,a,1}	55.05±0.07 ^{B,a,2}	54.08±0.71 ^{B,a,3}	53.63±0.78 ^{B,a,4}	53.27±0.89 ^{B,a,5}	52.98±0.89 ^{B,a,6}
2% CT	45.52±0.07 ^{B,b,1}	49.62±0.07 ^{B,b,2}	48.47±0.10 ^{B,b,3}	48.27±0.10 ^{B,b,4}	43.00±1.02 ^{B,b,5}	42.86±1.00 ^{B,b,6}
1% CPC	50.13±0.05 ^{B,a,1}	55.97±0.21 ^{B,a,2}	54.92±0.23 ^{B,a,3}	54.68±0.23 ^{B,a,4}	54.56±0.22 ^{B,a,5}	54.35±0.18 ^{B,a,6}
2% CPC	45.25±0.09 ^{B,c,1}	47.87±0.72 ^{B,c,2}	46.76±0.65 ^{B,c,3}	46.50±0.61 ^{B,c,4}	40.41±0.59 ^{B,c,5}	40.27±0.63 ^{B,c,6}
1% BC	53.30±0.07 ^{B,d,1}	62.45±0.35 ^{B,d,2}	61.42±0.47 ^{B,d,3}	59.73±0.65 ^{B,d,4}	59.45±0.45 ^{B,d,5}	59.32±0.43 ^{B,d,6}
2% BC	51.32±0.08 ^{B,e,1}	58.40±0.10 ^{B,e,2}	57.26±0.19 ^{B,e,3}	57.03±0.16 ^{B,e,4}	56.93±0.16 ^{B,e,5}	56.77±0.16 ^{B,e,6}
1% CHX	53.52±0.04 ^{B,f,1}	62.56±0.05 ^{B,d,2}	61.55±0.05 ^{B,d,3}	61.32±0.08 ^{B,f,4}	61.17±0.08 ^{B,f,5}	61.12±0.12 ^{B,f,6}
2% CHX	51.50±0.07 ^{B,g,1}	59.42±0.13 ^{B,f,2}	58.31±0.14 ^{B,f,3}	58.08±0.19 ^{B,g,4}	57.87±0.18 ^{B,g,5}	57.76±0.16 ^{B,g,6}

*In each column (each storage time period), values with different superscript uppercase letters indicate significant differences between control and experimental groups ($p < 0.05$). **In each column (each storage time period), values with different superscript lowercase letters indicate significant differences ($p < 0.05$) among experimental groups ($p > 0.05$). ***In each row (individual group) different superscript numbers indicate significant differences between all study periods ($p < 0.05$).

RESULTS

Table 1 presents the mean values (and standard deviations) of surface microhardness at 1, 7, 15, 30, 60, and 90 days for all groups. Vickers microhardness values in the control group were generally higher than those of the groups containing additives at most time intervals and for most of the additives. While BC and CHX groups demonstrated the closest, CT and CPC groups showed the most distant values to the Fuji IX control.

One-way ANOVA and the Dunnett t test showed that the differences were significant between the control and experimental groups in all cases ($p < 0.05$ for all). In addition, one-way ANOVA and the Tamhane T2 test revealed no significant differences between the 1% BC - 1% CHX groups at 7 and 15 days ($p > 0.05$) and 1% CT- 1% CPC ($p > 0.05$) groups at all time periods (Table 1).

Additionally, repeated measurements of ANOVA was indicated significant differences between all study periods for individual groups ($p < 0.05$). After 7 days, VHNs decreased to different extents in all study groups ($p < 0.05$), except for the controls ($p < 0.05$). At 60 and 90 days, the VHNs were decreased most markedly in the 2% CT and 2% CPC groups (Table 1).

DISCUSSION

As it is known, dentin carious lesions possess a wide microflora, it is clear that a mixture of antimicrobial agents that can be effective against all the microorganisms needed¹⁷. Additionally, cationic disinfectants may destroy the bacteria cell wall and kill the bacteria directly^{3,17}. Thus, since the progression of caries process under the GICs in

ART procedures are considered to be a problem this can be solved with the dental materials that inhibit bacterial growth^{1,3,17,18,23,25}.

For these purposes, cationic disinfectants have been incorporated into GICs to enhance their antibacterial properties^{1,3,13,18,23,25}. Though these have been shown to be effective as antibacterial additives, it is important not to jeopardize the basic physical properties of the GIC^{2,4,18,23,25}.

The critical point is that GIC-antibacterial combinations should have optimum surface properties to resist occlusal loads^{18,25}. However, when antibacterial materials are incorporated to the GICs, alterations have been reported regarding their physical properties and it is generally accepted in the literature that the physical properties of GICs are deteriorated with the addition of foreign antibacterial particles^{2,18,23,25}. Investigators have highly recommended that providing acceptable modifications in powder or liquid ratios, in terms of composing suitable antibacterial concentrations, would aid the GIC-antibacterial structure to have comparable physical properties to those of the additive-free ones by constituting efficient antibacterial features^{2,18,23,25}. Thus, GIC-antibacterial combinations should be prepared in an acceptable way which could adequately reduce the bacteria without compromising the longevity of the restoration^{2,4,18,23,25}. Considering these factors, the antibacterial materials and their concentrations tested in this study were previously found sufficient in terms of reducing microorganisms^{1,3}. Moreover, incorporating CT, CPC and CHX into the Fuji IX's powder and BC to the liquid at 1 to 4% final concentrations, caused a decrease in compressive strength to varying degrees compared to the control

group². Furthermore, no data are available in the literature about the surface hardness alterations of such Fuji IX-antibacterial combinations compared to additive-free material, which could improve the durability of the restoration in clinical conditions.

Microhardness testing can be performed to evaluate the state of GICs at various storage times^{7,16,19,31}, since they are very sensitive to water sorption. Samples kept in contact with water, either in a humid atmosphere or completely immersed, are characterized by lower flexural strength, lower modulus of elasticity and softer surfaces than dry samples⁵. Thus, the present study also aimed at determining the surface hardness alterations according to Fuji IX-antibacterial combinations stored in water for up to 90 days.

Sanders, et al.¹⁸ (2002) reported that with regard to the Knoop hardness number (KHN), no significant differences were found between the Photac-fil and Photac-fil-CHX in the first week. At 6 weeks, KHNs increased in both groups, but this increase was clearer in the Photac-fil group compared to the Photac-fil-CHX group. Nevertheless, they concluded that at neither 24 h- nor 6 week-periods, the physical properties altered seriously. Their study was the first to analyze microhardness values in GIC-antibacterial combinations. In the current study, all experimental groups showed a decreasing trend in VHNs of the Fuji IX GIC after the 7-day period and this trend exhibited significant differences during these periods for all experimental groups. This may be attributed to the sensitivity of Fuji IX to the antibacterial agents used in this study compared to Photac-fil and/or to the medium in which the samples were stored.

In another study, Türkün, et al.²⁵ (2008) tested 0.5%, 1.25% and 2.5% diacetate or digluconate CHX concentrations with ChemFil Superior regarding the VHN. They found significantly reduced hardness in the 0.5% CHX digluconate and 2.5% CHX digluconate groups compared to ChemFil Superior at 24 h. After the 10 days setting period, all of the tested groups except for the 2.5% digluconate demonstrated hardness comparable to the original ChemFil Superior group. This is a consequence fact that there was no remarkable leakage of CHX particles out onto the surface. In this study, the VHNs for all groups increased between days 1 and 7. This increase was significantly different for all groups ($p < 0.05$). The results were explained by the well-known maturation reactions in these cements^{15,19,20}. Moreover, where additives were present, this increase was most marked in the 1% BC and 1% CHX groups. Also, between these groups, significant differences were not observed at 7 and 15 days periods ($p > 0.05$). Thus, it can be assumed that, these groups may exhibit similar outcomes when they are incorporated to the Fuji IX.

Additionally, in a previous study, it was reported

that the increase of VHN values of Fuji IX samples was more accentuated in the intervals between 1-7 days. Also, the increase was found to be more uniform between the 7 and 30 days¹⁹. However, in the present study, after the 7-day period, contrary to the previous findings^{18,25}, the VHNs decreased in all experimental groups ($p < 0.05$ for all individual group), whereas they continued to increase in the antibacterial free Fuji IX control group ($p < 0.05$) as reported elsewhere¹⁹. This may be attributed to the water storage conditions of the samples, and day 7 is the critical point for the Fuji IX-antibacterial samples. Okada, et al.¹⁶ (2001) reported that when Fuji IX was stored in water, it showed significantly increased VHNs between days 1 and 40. In the present study, water probably eroded the Fuji IX-antibacterial combinations and caused the dissolution of some components, which would certainly decrease the surface hardness. Thus, except for the control group, a plasticizing effect could have occurred in the experimental groups and the continuous setting reaction may have been influenced by the foreign particles in the matrix of the Fuji IX up to 90 days^{18,23,25}. This may indicate that certain antibacterial agents may have negative effects on the hardness properties of cements particularly in the ongoing cement reaction. Thus, to avoid from the clinically mechanical failure problems in GIC-antibacterial combinations these findings could be taken into account for future ART procedures. Regarding the surface hardness values, between the 1% CT and 1% CPC groups significant differences were not found at all time periods ($p > 0.05$). Moreover, 2% CT and 2% CPC had the greatest adverse effects on surface hardness values of the Fuji IX samples after 30 days (Table 1). These results suggest that CT and CPC groups are likely to be partner and less resistant to occlusal loads in clinical situations than the parent cement. This result may also support the previous findings of Botelho² (2004).

Considering the above-mentioned studies^{18,25} and within the limitations of this *in vitro* study, our findings could be interpreted as tolerable, particularly when using these antibacterial agents at these concentrations. Although significantly reduced hardness values were found in the experimental groups compared to the controls, they were able to be measured. All previous reports^{18,25} and our findings may give an indication about the incorporation of the antibacterial agents to the GICs resulting at softened but measurable surfaces compared to the additive-free material.

In addition, the VHNs obtained in the present study showed similar trends to that of compressive strength reported previously². Higher levels of additive had greater effect on the VHNs compared to the controls, showing that the decline in physical properties is related to additive concentration.

However, at levels of 1 and 2%, although significant differences were found, the decline was acceptable, and might not be sufficient to cause a large reduction in overall durability of the restoration in clinical conditions. Overall, it seems that the combination of CT, CPC, CHX and BC with Fuji IX at concentrations of 1% and 2% produces cements with tolerable setting and hardness properties for clinical use. Additionally, within the *in vitro* limitations of this study, adding CHX and BC to the Fuji IX GIC seems to provide appropriate physical properties compared to CT and CPC combinations.

CONCLUSION

Incorporation of the antimicrobial compounds CT, CPC, BC and CHX at 1% and 2% concentrations into the conventional GIC Fuji IX was found to have measurable effects on the surface hardness. The resulting cements had reduced hardness, as determined by VHN measurements, and hardness decreased after 7 days, unlike the additive-free control, which continued to increase in hardness up to 90 days. However, in general, all experimental groups exhibited tolerable physical properties. Moreover, 1% BC and 1% CHX groups were detected as the most suitable combinations whereas 2% CT and 2% CPC showed almost adverse effects. As a result of this *in vitro* study, the tested antibacterial materials could be used with Fuji IX at varying concentrations in ART procedures. Further investigations are needed to evaluate the most appropriate Fuji IX-antibacterial combination that may not affect the physical properties of Fuji IX in clinical situations.

REFERENCES

- 1- Botelho MG. The antimicrobial activity of a dentin conditioner combined with antibacterial agents. *Oper Dent.* 2005;30:75-82.
- 2- Botelho MG. Compressive strength of glass ionomer cements with dental antibacterial agents. *SADJ.* 2004;59:51-3.
- 3- Botelho MG. Inhibitory effects on selected oral bacteria of antibacterial agents incorporated in a glass ionomer cement. *Caries Res.* 2003;37:108-14.
- 4- Botelho MG. The microtensile bond strength of Fuji IX glass ionomer cement to antibacterial conditioned dentin. *Oper Dent.* 2005;30:311-7.
- 5- Cattani-Lorente MA, Dupuis V, Payan J, Moya F, Meyer JM. Effect of water on the physical properties of resin-modified glass ionomer cements. *Dent Mater.* 1999;15:71-8.
- 6- Coogan MM, Creaven PJ. Antibacterial properties of eight dental cements. *Int Endod.* 1993;26:355-61.
- 7- Ellakuria J, Triana R, Minguez N, Soler I, Ibaseta G, Maza J, et al. Effect of one-year water storage on the surface microhardness of resin-modified versus conventional glass-ionomer cements. *Dent Mater.* 2003;19:286-90.
- 8- Ercan E, Dülgeril ÇT, Soyman M, Dalli M, Yildirim I. A field-trial of two restorative materials used with Atraumatic Restorative Treatment, in rural Turkey: 24-month results. *J Appl Oral Sci.* 2009;17:307-14.
- 9- Frencken JE, Holmgren CJ. How effective is ART in the management of dental caries? *Community Dent Oral Epidemiol.* 1999;27:423-30.
- 10- Frencken JE, Pilot T, Songpaisan Y, Phantumvanit P. Atraumatic Restorative Treatment (ART): rationale, technique, and development. *J Public Health Dent.* 1996;56:135-40.
- 11- Frencken JE, Taifour D, Van't Hof MA. Survival of ART and amalgam restorations in permanent teeth of children after 6.3 years. *J Dent Res.* 2006;85:622-6.
- 12- Frencken JE, Van't Hof MA, Van Amerongen WE, Holmgren CJ. Effectiveness of single-surface ART restorations in the permanent dentition: a meta-analysis. *J Dent Res.* 2004;83:120-3.
- 13- Hoszek A, Ericson D. *In vitro* fluoride release and the antibacterial effect of glass ionomers containing chlorhexidine gluconate. *Oper Dent.* 2008;33:696-701.
- 14- Massara ML, Alves JB, Brandão PR. Atraumatic restorative treatment: clinical, ultrastructural and chemical analysis. *Caries Res.* 2002;36:430-6.
- 15- Nicholson JW. Chemistry of glass-ionomer cements: a review. *Biomaterials.* 1998;19:485-94.
- 16- Okada K, Tosaki S, Hirota K, Hume WR. Surface hardness change of restorative filling materials stored in saliva. *Dent Mater.* 2001;17:34-9.
- 17- Pinheiro SL, Simionato MRL, Imparato JCP, Oda M. Antibacterial activity of glass-ionomer cement containing antibiotics on caries lesion microorganisms. *Am J Dent.* 2005;18:261-6.
- 18- Sanders BJ, Gregory RL, Moore K, Avery DR. Antibacterial and physical properties of resin modified glass-ionomers combined with chlorhexidine. *J Oral Rehabil.* 2002;29:553-8.
- 19- Shintome LK, Nagayasu MP, Di Nicoló R, Myaki SI. Microhardness of glass-ionomer cements indicated for the ART technique according to surface protection treatment and storage time. *Braz Oral Res.* 2009;23:439-45.
- 20- Silva KG, Pedrini D, Delbem ACB, Cannon M. Microhardness and fluoride release of restorative materials in different storage media. *Braz Dent J.* 2007;18:309-13.
- 21- Silva RC, Zuanon AC, Esberard RR, Candido MS, Machado JS. *In vitro* microhardness of glass ionomer cements. *J Mater Sci Mater Med.* 2007;18:139-42.
- 22- Smales RJ, Yip HK. The atraumatic restorative treatment (ART) approach for the management of dental caries. *Quintessence Int.* 2002;33:427-32.
- 23- Takahashi Y, Imazato S, Kaneshiro AV, Ebisu S, Frencken JE, Tay FR. Antibacterial effects and physical properties of glass-ionomer cements containing chlorhexidine for the ART approach. *Dent Mater.* 2006;22:647-52.
- 24- Tobias RS. Antibacterial properties of dental restorative materials: a review. *Int Endod.* 1988;21:155-60.
- 25- Türkün LS, Türkün M, Ertuğrul F, Ateş M, Brugger S. Long-term antibacterial effects and physical properties of a chlorhexidine-containing glass-ionomer cement. *J Esthet Restor Dent.* 2008;20:29-44.
- 26- Van Amerongen WE. Dental caries under glass ionomer restorations. *J Public Health Dent.* 1996;56(Sp. issue 3):150-4.
- 27- Vermeersch G, Leloup G, Delmée M, Vreven J. Antibacterial activity of glass-ionomer cements, compomers and resin composites: relationship between acidity and material setting phase. *J Oral Rehabil.* 2005;32:368-74.
- 28- Weerheijm KL, Groen HJ. The residual caries dilemma. *Community Dent Oral Epidemiol.* 1999;27:436-41.
- 29- Weerheijm KL, Soet JJ, van Amerongen WE, Graaf J. The effect of glass-ionomer cement on carious dentine: an *in vivo* study. *Caries Res.* 1993;27:417-23.
- 30- Weerheijm KL, Kreulen CM, Soet JJ, Groen HJ, van Amerongen WE. Bacterial counts in carious dentine under restorations: 2-year *in vivo* effects. *Caries Res.* 1999;33:130-4.
- 31- Xie D, Brantley WA, Culbertson BM, Wang G. Mechanical properties and microstructures of glass-ionomer cements. *Dent Mater.* 2000;16:129-38.