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# Effect of saliva contamination on the sealing properties of glass ionomer sealants placed over Er,Cr:YSGG laser-etched fissures

## *ABSTRACT*

***Aim*** *This study investigated the effect of laser pretreatment in reducing the microleakage of conventional (GIC) and resin-modified glass ionomer (RMGIC) sealants on saliva-contaminated enamel.*
***Materials and methods*** *Study Design: 80 extracted non-carious third molars were randomly assigned to two groups (n=40/each): Group A enamel pretreatment with erbium, chromium:yttrium, scandium, gallium, garnet (Er,Cr:YSGG) laser; and Group B no pretreatment. Each group was divided into two subgroups (n=20/each) based on presence/absence of saliva contamination following laser pretreatment. In subgroups: fissures were sealed with GIC (n=10) or RMGIC (n=10). Microleakage was evaluated quantitatively using an image analysis toolkit (ImageJ), and the data were statistically analysed.*
***Results*** *In the absence of laser pretreatment, the GIC sealant demonstrated significantly lower microleakage values than RMGIC counterparts on both uncontaminated and saliva-contaminated enamel ($p<0.001$, Mann–Whitney U test).*
***Conclusion*** *Among the tested combinations, GIC sealant with Er,Cr:YSGG pretreatment may be the best approach for sealing pits and fissures, when saliva contamination is inevitable before sealant application.*

***Keywords*** *Atraumatic restorative technique (ART); Fissure sealant; Glass ionomer; Laser pretreatment; Microleakage; Sealants.*

## Introduction

Dental caries is a common infectious disease, and it can be prevented or arrested in its early stages. Fluoride application for caries prevention has led to a decrease in caries on the smooth surfaces of teeth, whereas caries lesions on occlusal surfaces still have a high prevalence because of their complex morphologies [Fejerskov, 2004; Marthaler, 2004]. Studies confirm that sealing occlusal pits and fissures is a highly effective preventive method for occlusal caries lesions [Courson et al., 2011; Ferrazzano et al., 2016].

Fissure sealants are mainly resin-based [Ripa, 1993; Frencken and Wolke, 2010] and bond micromechanically to the enamel via phosphoric acid pretreatment. Resin-based sealants are technique-sensitive materials that require effective moisture control for proper placement and long-term retention. Indeed, most resin-based sealant failures occur in partially erupted teeth, which render moisture contamination inevitable due to the difficulties in achieving proper isolation [Dennison et al., 1990]. Another important drawback of many resin-based sealants is the incorporation of bisphenol A-glycidyl methacrylate (BPA), an endocrine disruptor that mimics estrogen and alters hormonal function [McKinney et al., 2014].

For many years, glass ionomer cements have been used for sealing pits and fissures of partially-erupted permanent molars [Beauchamp et al., 2009]. Glass ionomer sealants bond chemically to tooth structures, are less sensitive to moisture, and thus, perform better than resin-based sealants in saliva-contaminated fissures [Borsatto et al., 2004; Barja-Fidalgo et al. 2009]. In addition, glass ionomer cements release fluoride and can be recharged topically with fluoride to sustain their caries-preventive effect. Irrespective of clinical retention rates, glass ionomer sealants are as effective as resin-based sealants in preventing occlusal caries [Liu et al. 2014].

As with any resin-based material, the sealing efficacy of resin-based sealants can be jeopardised over the time by microleakage [Ripa, 1993; Dennison et al., 1990]. In addition to phosphoric acid-etching of enamel, which is the gold standard for enamel pretreatment [Beauchamp et al., 2009; Manhart et al., 2004], additional methods such as the use of an intermediate adhesive layer, enameloplasty, air abrasion and laser pretreatment have been tested to improve the retention and sealing properties of fissure sealants [Haznedaroglu et al., 2014]. Among these methods, laser etching has the potential to improve the sealing efficacy of both resin-based and glass-ionomer based sealants [Jaberi et al., 2012; Tirali et al. ,2013], which may be an advantageous strategy in sealing partially erupted teeth in children with isolation problems. The present study aimed to investigate the effect of laser pretreatment on the sealing efficacy of conventional and resin-modified glass ionomer sealants, with particular interest in saliva-contaminated enamel. The tested null-hypothesis was that laser pretreatment had no effect on the sealing efficacy of conventional and resin-modified glass ionomer sealants placed on saliva-contaminated enamel.

## Materials and methods

This study was approved by the Institutional Review Board of Baskent University (Project no: D-DA 16/04) and supported by the Baskent University, Turkey Research Fund.

Freshly-extracted impacted human third molars (n=80) were collected under informed consent and stored in saline solution at 4°C for up to 1 month. The sample size was calculated using open source software (GPower, Germany, http://www.gpower.hhu.de) [Faul et al., 2007] and the power for the study was 0.80, with an effect size of 0.6 and $\alpha$ error of 0.07 for 10 teeth in each subgroup. After surface debridement with a hand scaling instrument, pits and fissures were cleaned with a low-speed water-cooled rotating brush. The teeth were randomly assigned to one of two main groups (n=40/each) with respect to the pretreatment protocol applied to pits and fissures: Group A pretreatment with erbium, chromium:yttrium, scandium, gallium, garnet (Er,Cr:YSGG) laser; Group B no pretreatment.

### Laser pretreatment

An Er,Cr:YSGG hydrokinetic laser system (Millenium System, Biolase Technology Inc., San Clemente, CA, USA) was used. The power output was set at 3.5 W (output parameters: wavelength, 2.78 μm; pulsed-with duration, 140 to 200 μs; repetition rate, 20 Hz). Air and water was sprayed through the hand-piece at a level of 85% water and 90% air to prevent enamel surfaces from overheating. The laser beam was delivered in non-contact mode, positioned perpendicular to the fissures at a distance of 1 mm from the surface. The duration of exposure depended on the time needed to evenly guide the laser beam across the pits and fissures to be irradiated. Fissures were then rinsed and air-dried.

### Saliva contamination

All laser-pretreated teeth were further divided into two subgroups according to the presence or absence of saliva contamination (20 teeth/subgroup) before sealant placement. For teeth randomly assigned to the saliva-contaminated groups, occlusal surfaces were contaminated for 10 s with 0.01 mL of fresh human whole saliva using a micropipette. Saliva was collected from one non-smoking donor. The contaminated enamel was left undisturbed, then air-dried after 10 s to simulate contamination under clinical conditions.

### Sealing procedures

Fissures of 10 randomly-selected saliva-contaminated teeth were sealed with a resin-modified glass ionomer cement (Vitremer, 3M ESPE, Seefeld, Germany) and the other 10 with a conventional glass ionomer cement (self-cure glass ionomer, Riva SDI, Bayswater, Australia). The glass ionomer cements were prepared according to the manufacturers' instructions and placed according to the ART manual [Frencken et al., 1997]. In the uncontaminated subgroup, the teeth were sealed as with their saliva-contaminated counterparts. The same experimental protocol was applied for all teeth that did not receive Er,Cr:YSGG laser pretreatment (group B).

### Microleakage test

A conventional dye-penetration test was utilised for quantitative evaluation of microleakage. Following sealing of fissures, the teeth were placed in deionised water at 37°C for 24 hours. Thereafter, teeth were subjected to thermocycling 1000 times at 5–55°C with a 15 s dwell time and a 10 s transfer time. The apices were sealed with sticky wax and the teeth were coated with two consecutive layers of nail varnish up to 1 mm from the sealant margins. Samples were then immersed in 0.5% basic fuchsin solution (Wako Pure Chemical Industry, Osaka, Japan) for 24 hours. Thereafter, samples were thoroughly rinsed under tap water until no dye was observed, air-dried and embedded in epoxy resin (Struers, Copenhagen, Denmark). Three parallel longitudinal sections were made through the occlusal surfaces in the bucco-lingual direction, using a water-cooled low-speed diamond saw (Isomet, Buehler, IL, USA). Each section was digitally photographed at 12X (1280×1024 resolution) under a stereo-microscope (Olympus, Tokyo, Japan). The images were transferred to a Macintosh computer in TIFF format. An open-source image analysis software (Image J, V.1.42, National Institutes of Health, Bethesda, MD) was used to measure (in mm) the extent of buccal and lingual dye penetration along the enamel-sealant interface. The microleakage value for each section was calculated by dividing the

| | No Saliva contamination | | Saliva Contamination | |
|---|---|---|---|---|
| | GIC | RMGIC | GIC | RMGIC |
| No Laser Pretreatment | 1.14 (0.51)$^{a1}$ | 1.70 (0.31)$^{b1}$ | 1.35 (0.16)$^{a1}$ | 1.89 (0.24)$^{b1}$ |
| Laser Pretreatment | 0.40 (0.27)a$^{2}$ | 0.30 (0.16)$^{a2}$ | 0.97 (0.52)$^{b2}$ | 2.41 (0.93)$^{c1}$ |

TABLE 1 Microleakage values (mm) with respect to the tested glass ionomer sealant, presence/absence of saliva contamination and laser pretreatment. For each row, values with the same superscript letter indicate microleakage scores that are not significantly different at p>0.0125. For each column, values with the same superscript number indicate microleakage scores that are not significantly different at p>0.0125. GIC=conventional glass ionomer cement; RMGIC=resin-modified glass ionomer cement.

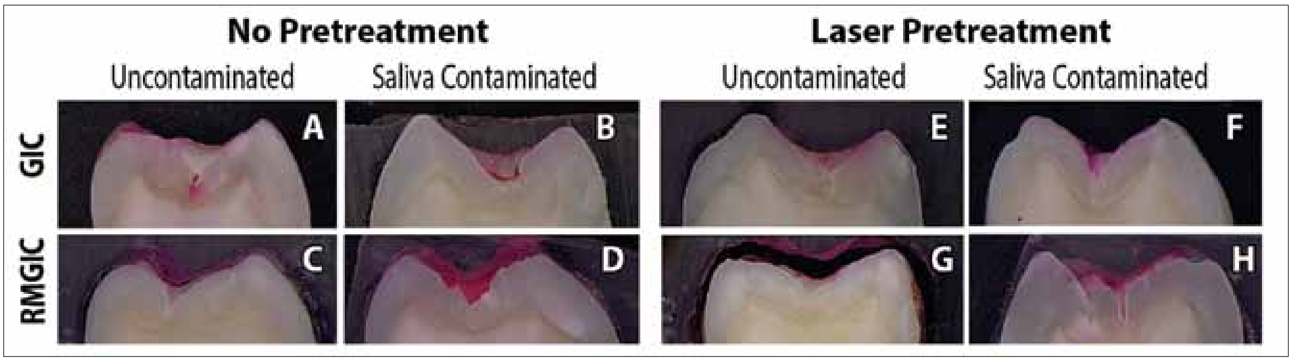

FIG. 1 Representative micrographs of the test groups: A: Non pretreated, uncontaminated conventional glass ionomer cement; B: Non pretreated, saliva contaminated conventional glass ionomer cement; C: Non pretreated, uncontaminated resin-modified glass ionomer cement; D: Non pretreated, saliva contaminated resin-modified glass ionomer cement; E: Laser pretreated, uncontaminated conventional glass ionomer cement; F: Laser pretreated, saliva contaminated conventional glass ionomer cement; G: Laser pretreated, uncontaminated resin-modified glass ionomer cement; H: Laser pretreated, saliva contaminated resin-modified glass ionomer cement. 12X, Original magnification. GIC=conventional glass ionomer cement ; RMGIC= resin-modified glass ionomer cement

sum of buccal and lingual dye penetration values by the sum of the lengths of buccal and lingual enamel-sealant interfaces. The measurements were made by a single calibrated operator (BC) blinded to test groups. The microleakage value for each specimen and for each tooth and subgroup was calculated as the mean±SD.

### Statistical evaluation

Data analysis was performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, USA). Normality of the continuous variable distribution was determined using the Kolmogorov-Smirnov test. The Levene test was used to evaluate homogeneity of variance. Statistical significance of median values between groups was evaluated using the Mann-Whitney U test. For all possible multiple comparisons, Bonferroni correction was applied to control Type I error. According to the Bonferroni correction, a p value less than 0.0125 was considered statistically significant.

## Results

The microleakage values with respect to the tested glass ionomer sealant, presence/absence of saliva contamination and laser pretreatment are presented in Table 1. Representative sections of the experimental groups are shown in Figure 1.

Among the test groups, the lowest microleakage values were observed in the laser-pretreated, saliva-uncontaminated teeth sealed with resin-modified and conventional glass ionomer cements, respectively ($p<0.001$).

In the absence of laser pretreatment and saliva contamination, the conventional glass ionomer sealants demonstrated significantly lower microleakage values than the resin-modified glass ionomer sealants ($p=0.002$; Mann–Whitney U test). Likewise, when the samples were contaminated with saliva in the absence of laser pretreatment, the conventional glass ionomer sealants demonstrated significantly lower microleakage values than the resin-modified sealants ($p<0.001$).

When laser pretreatment was employed on uncontaminated enamel, both sealant materials demonstrated similar microleakage values ($p=0.684$), while on saliva-contaminated enamel; the conventional glass ionomer sealant demonstrated significantly lower microleakage values than their resin-modified counterparts ($p<0.001$).

## Discussion

In the present study, laser pretreatment reduced the microleakage of both conventional and resin-modified glass ionomer cement sealants in the absence of saliva contamination, necessitating partial rejection of the null hypothesis. Laser pretreatment also improved the sealing efficacy of the conventional glass ionomer cement on saliva-contaminated enamel. However, laser pretreatment had no significant effect on the sealing efficacy of the resin-modified glass ionomer sealant on saliva-contaminated enamel. Thus, the null hypothesis should be accepted in part.

Glass ionomer cement-based fissure sealants have lower retention rates than resin-based fissure sealants [Liu et al., 2014; Forss et al., 1994; Kumaran, 2013]. However, their caries-preventive effects are similar to those of resin-based ones [Frencken and Wolke, 2010; Liu et al., 2014], owing to the residual glass ionomer cement being retained in the deeper parts of the fissures and the fluoride release by the remnants of glass ionomer [Frencken and Wolke, 2010]. Conventional glass ionomer cements used as fissure sealants have demonstrated extensive microleakage in situ compared with resin-based ones [Ovrebö and Raadal, 1990]. Microleakage does not influence secondary caries in the short term, but the loss of marginal integrity due to microleakage leads to interfacial gap formation and subsequent biofilm formation around sealant margins, which may in turn initiate development of caries lesions [Cenci et al., 2008]. Thus, any technique/method that could reduce microleakage under glass ionomer cements would enhance their caries-preventive and therapeutic effect. Enamel pretreatment using enameloplasty and air abrasion have reduced the microleakage of conventional glass ionomer cements [Haznedaroglu et al., 2012], however both techniques may be difficult to apply in partially erupted teeth. Clinical studies have shown better performance of resin-based sealants on Er,Cr:YSGG-pretreated enamel, particularly when saliva contamination was inevitable before sealant placement [Baygin et al., 2012; Lepri et al., 2008]. It is, therefore, reasonable to implement laser pretreatment for glass ionomer sealant application, because the increased porosity of laser-etched enamel might contribute to micromechanical retention of the glass ionomer, whose chemical adhesion could be jeopardised after application on saliva contaminated enamel. Topaloglu and Alpoz [2010] showed that glass ionomer-based sealants had higher microleakage values compared to a light-cured resin-based fissure sealant under both saliva-contaminated and uncontaminated conditions. The authors concluded that this may be a result of no enamel pretreatment before GIC sealant application. To our knowledge, no previous study evaluated the possible effects of Er,Cr:YSGG pretreatment on the sealing efficacy of glass ionomer-based fissure sealants. In the present study, the use of the Er,Cr:YSGG laser significantly enhanced the sealing ability of the conventional glass ionomer-based sealant in both saliva-contaminated and uncontaminated conditions. The use of Er,Cr:YSGG was shown to improve the sealing efficacy of the conventional glass ionomer cement Ketac Fil Plus in class V cavities [Delme et al., 2006]. On laser-etched surfaces, the thermomechanical ablation process creates a microcrater-like appearance [Baygin et al., 2012; Lepri et al., 2008]. Such alteration of the surface micromorphology may contribute to improved sealing efficacy of the conventional glass ionomer sealant by increasing the surface area for chemical adhesion of the material with the tooth. In the present study, Er,Cr:YSGG pretreatment did not contribute to improved sealing in the resin-modified glass ionomer group with prior saliva contamination. While this finding requires elucidation using advanced diagnostic methods, it may be possible to assume that the increase in microleakage values may result from the sensitivity of resin-modified glass ionomer cements to extreme moisture contamination, even if the surface area for chemical adhesion is increased.

In the absence of laser pretreatment, the conventional glass ionomer sealants showed lower leakage values than resin-modified glass ionomer sealants in both saliva contaminated and uncontaminated conditions. Unlike their resin-modified versions, conventional glass ionomer cements are less vulnerable to moisture [Aboush et al., 1986; Korkmaz et al., 2010] whereas surface pretreatment is recommended for better adhesion for the resin-modified glass ionomer cements [Korkmaz et al., 2010; El-Askary et al., 2011].

Despite the experimental limitation of the present study, it can be concluded that it is beneficial to use a resin-modified glass ionomer as a fissure sealant along with laser pretreatment when there is no risk of saliva contamination before sealant application. If saliva contamination is inevitable, a conventional glass ionomer with Er,Cr:YSGG pretreatment appeared to be the best tested combination to seal pits and fissures.

### *Conflicts of interest*

The authors have no conflicts of interest relevant to this article.

### *Acknowledgments*

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