J Dent Mater Tech 2024; 13 (4): 162-168
https:// 10.22038/jdmt.2024.79502.1628

JDentMaterTech

Original Article Open Access

Biofilm formation and surface characteristics of conventional glass ionomer, resin-modified glass ionomer, and GC gold hybrid restorative material

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Abstract

Objective: The present study aimed to evaluate the surface roughness, microhardness, and biofilm formation of conventional glass-ionomer cement (GIC) and two GIC-based restorative materials.

Methods: Twenty-four samples were prepared from each of the following restorative materials: Group 1) GIC, Group 2) resin-modified glass-ionomer cement (RMGIC), and Group 3) GC Gold Hybrid restorative material. The surface roughness and microhardness of specimens were evaluated using a surface profilometer and a Vickers microhardness tester, respectively. Half of the samples in each group were exposed to *Streptococcus mutans* suspension and the other half to *Streptococcus oralis* suspension. The bacterial colonies were counted using a digital colony counter. Data were compared using the Kruskal Wallis and Wilcoxon signed rank tests (α=0.05).

Results: The surface roughness and microhardness values were significantly different among the groups (P = 0.001). RMGIC showed significantly lower surface roughness and significantly higher microhardness among the groups (P<0.05). There was a significant difference in *S. mutans* biofilm formation among the groups (P< 0.001), but *S. oralis* biofilm was not significantly different (P=0.063). GC Gold Hybrid had a significantly higher S. mutans biofilm formation compared to other groups (P<0.05). The formation of *S. oralis* biofilms was significantly higher than that of *S. mutans* biofilms in all materials (P < 0.05).

Conclusions: RMGIC had the most favorable surface properties among the groups. GC Gold Hybrid had a higher bacterial adhesion and less favorable surface properties, which might increase the rate of secondary caries around the restoration.

Keywords: Colony count, Glass ionomer cement, Hardness, Restorative material, *Streptococcus mutans*, Surface characteristics

Introduction

Dental restorative materials facilitate adherence and accumulation of oral microorganisms. The dental plaque or biofilm on restorative material surfaces contains numerous bacteria involved in the demineralization process, which can lead to secondary caries along the margins of the restoration (1). Among the Streptococcus species that predominate the biofilms, *Streptococcus mutans* (*S. mutans*) adheres to tooth and restorative material surfaces and hence is considered the primary cause of secondary caries formation (2). *Streptococcus oralis* (*S. oralis*) is a pioneer species that serves as an anchor for intermediate and late pathogenic colonizers and thus contributes to the formation of biofilm (3). The ability of dental restorative materials to attract bacterial adhesion is affected by different surface characteristics, such as surface roughness, surface free energy, and chemical composition (4). High surface roughness of restorative materials is a preparatory factor for microbial colonization and a risk factor for intra-oral diseases (5).

Microhardness is a direct indicator of a material's resistance to deformation, scratching, and wear, which are vital factors for the durability of dental materials in the oral environment (6). Microhardness testing is particularly valuable for optimizing material formulation and predicting its clinical longevity. On the other hand,

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Accepted: 28 April 2024. Submitted: 10 September 2024.

biofilm formation causes erosion in resin composite and GICs by producing acidic byproducts, increasing the surface roughness and lowering the microhardness.

Glass ionomer cement (GICs) are a group of materials with a thermal expansion like that of dentin, minimal cytotoxicity, and good biocompatibility (7). The material undergoes slight hygroscopic expansion following water absorption, resulting in marginal gap closure, reduced microleakage, and reduced risk of recurrent caries (8). These cements are bioactive because the fluoride released by the material inhibits demineralization and promotes remineralization of the adjacent tooth tissues (9). The compositions of GICs have continually been modified to improve the mechanical properties, polishability, aesthetic appearance, and moisture resistance. Newer restorative materials, such as resin-modified GIC (RMGIC), EQUIA Forte GIC, and GC Gold Hybrid, were introduced with improved physical properties compared to the conventional GICs.

RMGICs are modified GICs that have resin components. RMGICs are more resistant to microleakage, have stronger bonding to the tooth structure, and are less soluble than conventional GICs (10). Furthermore, they are less prone to crack formation (10).

GC Gold Hybrid, also known as GC Gold Label Hybrid Restorative GIC, contains highly reactive, ultrafine fluor aluminosilicate glass particles (11). GC Gold Hybrid has been reported to have a higher compressive and flexural strength than conventional GIC and RMGIC, making it more suitable for posterior load-bearing areas (12). GC Gold Hybrid contains a combination of fillers, where voluminous glass fillers are supplemented with small, highly reactive fillers. Additionally, it has a unique nano-filled resin coating on the filler particles (13). According to the manufacturer, GC Gold Hybrid features GC Advanced Glass Hybrid technology, which combines two types of Fluoro-Almino-Silicate (FAS) glass and two types of polyacrylic acid to improve its physicochemical properties.

Evaluating the physical and mechanical properties of different types of GICs is important when choosing GICs as restorative materials. Some studies have evaluated the surface characteristics of GIC and RMGIC (5, 14-16). However, no previous study has evaluated GC Gold Hybrid surface characteristics and biofilm adhesion in comparison with GIC and RMGIC. Therefore, the present study aimed to investigate *S. mutans* and *S. oralis* biofilm formation, surface roughness, and microhardness of GIC, RMGIC, and GC Gold Hybrid. The null hypothesis was that no differences would be observed in biofilm formation, surface roughness and microhardness between these restorative materials.

Materials and methods

Study design

The protocol of the present in vitro study was approved by Institutional Review Boards (IRBs) (ref: 249/IRB-IBSEC/SIST) of Sathyabama Dental College and Hospital.

Sample size estimation

The sample size was determined using G*Power 3.1.9.7 software according to the findings of a study by Fatima et al. (17). The type I error was fixed at 5%, and the power of the study was fixed at 95%. The minimum sample size was estimated at 24 samples per group.

Samples preparation

Three groups of material were investigated in the present study: Group 1) conventional glass-ionomer restorative cement (GIC; Fuji IX Extra, GC Corp., Tokyo, Japan), Group 2) resin-modified GIC (RMGIC; GC Gold Label 2 LC, GC Corp., Tokyo, Japan), and Group 3) GC Gold Hybrid (GC Gold Label Hybrid Restorative GIC, GC Corp., Tokyo, Japan). Twenty-four specimens of each material group were prepared using sterile Teflon molds (8 mm in diameter and 3 mm deep) following the manufacturer's instructions. After 48 hours, all samples were polished for 20 s with a Soflex fine polishing disc (3M Espe, USA) using a slow-speed handpiece at 15,000 rpm. Following the polishing procedure, all samples were rinsed with distilled water and dried at room temperature. Then, they were incubated in distilled water at 37 °C for 24 hours.

Surface roughness evaluation

A mechanical profilometer (Mitutoyo Corp., Tokyo, Japan) with a 2 µm contact style was used to test the surface roughness. The measurement length was 1.5 mm, and the cut-off distance was 0.8 mm. On each sample, three different areas on the middle and sides were assessed to determine the average surface roughness (Ra) value.

Microhardness testing

A Vickers microhardness tester (HMV-G31DT, Shimadzu, Tokyo, Japan) was used with a 300 g load cell and a 15 s holding period. A microcomputer and specialized software were connected to this apparatus to analyze

Table 1. Average surface roughness (µm) and surface microhardness (VHN) values in the study groups

Study groups	Surface roughness	Microhardness
	Mean± SD	Mean± SD
Conventional GIC	0.25±0.07 [b]	91.04±6.80 [b]
RMGIC	0.14±0.06 [a]	101.73±9.26 [c]
GC Gold Hybrid Restorative GIC	0.39±0.05 [c]	75.40±5.89 [a]
P-value	0.001*	0.001*

SD: Standard deviation; GIC: glass-ionomer cement; RMGIC: resin-modified glass-ionomer cement
*Values less than 0.05 represent a significant difference between groups according to the Kruskal-Wallis test.
In each column, the different lowercase, superscript letters represent a significant difference between the materials at P < 0.05

the images at 40x magnification. The microhardness was measured and was expressed as Vickers hardness number (VHN).

Biofilm formation

S. mutans (MTCC 890 strain) and *S. oralis* (MTCC 2696 strain) were purchased from Microbial Type Culture Collection and Gene Bank (MTCC) (CSIR-Institute of Microbial Technology, Chandigarh, India). The bacterial isolates were inoculated into 10 ml of brain heart infusion (BHI) broth (HiMedia Laboratories Pvt. Ltd., Mumbai, India) and incubated at 37°C for 24 hours. The culture turbidity was adjusted to match the McFarland standard scale of 0.5 to obtain 1.5 x 10^8 colony-forming units (CFU/ml).

The GIC samples were sterilized by immersing them in 100 mL of 70% ethanol for three consecutive days (18). Then, they were placed in sterile distilled water overnight to remove any residual ethanol.

The GIC samples were placed in a sterile container containing 20 ml of BHI broth. Then, the samples in each group were divided into two subgroups (n=12). Half of the samples received 10 µL of the *S. mutans* culture and the other half received 10 µL of the *S. oralis* culture. Then, the containers were incubated at 37 °C in a shaker incubator, which formed a dynamic setting for 72 h. Afterwards, the GIC samples were rinsed to remove the planktonic cells.

The biofilm that formed on the surfaces of the GIC samples was gently scraped using a sterile surgical blade and collected in 1 ml of sterile isotonic saline. Next, 10 µL of the saline containing the biofilms was added to Mutans Sanguis Agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India) to culture *S. mutans*, and another 10 µL to Brain Heart Infusion Agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India) to culture *S. oralis*. The plates were incubated in a candle jar (5-10% CO_2) for 24 hours to allow colony formation in the agar plates. Then, the colonies were counted using a digital colony counter and expressed in CFU/ml.

Statistical analysis

SPSS 20.0 (IBM Inc., NY, USA) was used to perform the statistical analysis. The normal distribution of the data was assessed using the Shapiro-Wilk test. Since data had a non-normal distribution, the Kruskal-Wallis test was used to compare the microhardness, surface roughness, and biofilm levels in the study groups. Pairwise comparisons were made using Mann–Whitney U test. Wilcoxon signed-rank test was used to compare the two bacterial biofilms. Values lower than 0.05 were considered statistically significant.

Results

Table 1 provides the roughness and microhardness values of the different groups. The Kruskal-Wallis test revealed a significant difference in roughness values among the groups (P = 0.001). RMGIC (0.14±0.06 µm) had a significantly lower surface roughness than the other groups (P < 0.05). Moreover, the roughness of the conventional GIC (0.25±0.07 µm) was significantly

Table 2. Average *S. Mutans* and *S. Oralis* biofilm formation (CFU/ml) in the study groups

Study groups	*S. Mutans*	*S. Oralis*	p-value
	Mean ± SD	Mean ± SD	
Conventional GIC	500.00 ± 338.44 [a]	25375.00 ± 23778.22	0.002**
RMGIC	341.67 ± 290.63 [a]	12608.33 ± 14309.34	0.003**
GC Gold Hybrid Restorative GIC	2175.00 ± 3101.06 [b]	79591.67 ± 178450.47	0.002**
P value	<0.001*	0.063	

SD: Standard deviation; GIC: glass-ionomer cement; RMGIC: resin-modified glass-ionomer cement
*Values less than 0.05 represent a significant difference between groups according to the Kruskal-Wallis test.
In the column, the different lowercase, superscript letters represent a significant difference between the materials at P < 0.05.
**Values less than 0.05 represent a significant difference in biofilm formation between the two microorganisms according to the Willcoxon signed rank test.

lower than that of the GC Gold Hybrid (0.39±0.05) (P < 0.05; Table 1).

The surface microhardness was also statistically different among the groups (P = 0.001; Table 1). Pairwise comparisons revealed that RMGIC (101.73±9.26 VHN) had a significantly higher microhardness value than the other groups (P < 0.05). Furthermore, the microhardness of conventional GIC (91.04±6.80) was significantly higher than GC Gold Hybrid (75.40±5.89) (P < 0.05; Table 1).

Table 2 and Figure 1-A-C provide the average biofilm formation in the study groups. *S. mutans* biofilm formation was significantly different among the groups (P < 0.001). GC Gold Hybrid (2175.00±3101.06 CFU/ml) had a significantly higher S. mutans biofilm formation than RMGIC (341.67±290.63 CFU/ml) and GIC (500.00±338.44 CFU/ml) (P < 0.05; Table 2). However, the conventional GIC had a comparable *S. mutans* biofilm formation with RMGIC (P = 0.832).

S. oralis biofilm formation (Figure 1-D-F) was not significantly different among the groups (P = 0.063; Table 2). The amount of *S. oralis* biofilm colonies in each material group was significantly higher than that of the *S. mutans* (P < 0.05; Table 2).

Discussion

The results of the present study indicated that the GIC, RMGIC, and GC Gold Hybrid had significantly different surface microhardness and roughness values. Moreover, the *S. mutans* biofilm formation was significantly different among the groups; therefore, the null hypothesis was rejected. The highest surface microhardness, lowest surface roughness, and lowest *S. mutans* biofilm formation were observed in RMGIC, followed by the conventional GIC and GC Gold Hybrid, respectively.

Surface hardness is a critical parameter for evaluating dental materials' durability and degradation rate (19). The findings of the current investigation indicated that the microhardness value of RMGIC surpassed that of the GIC and GC Gold Hybrid. This finding may be related to the high-quality bond between the resin matrix and glass particles in RMGIC, which may positively affect surface properties (20). Higher microhardness of RMGIC may encourage its application in posterior load-bearing regions. In contrast to the outcomes of this study, Mittal et al. (13) reported that GC Gold Hybrid had a favorable clinical performance when used to restore primary molars. However, they did not compare the findings with those of RMGIC or GIC. The study by Malhotra et al. (12) found that GC Gold Hybrid outperformed RMGIC and GIC regarding flexural and compressive strength. However, a statistical comparison was not performed in their study.

The present study revealed that the restorative materials with higher surface roughness had higher bacterial biofilm formation, especially *S. mutans* biofilm. Several other studies have also indicated that a higher surface roughness enhances the surface affinity for

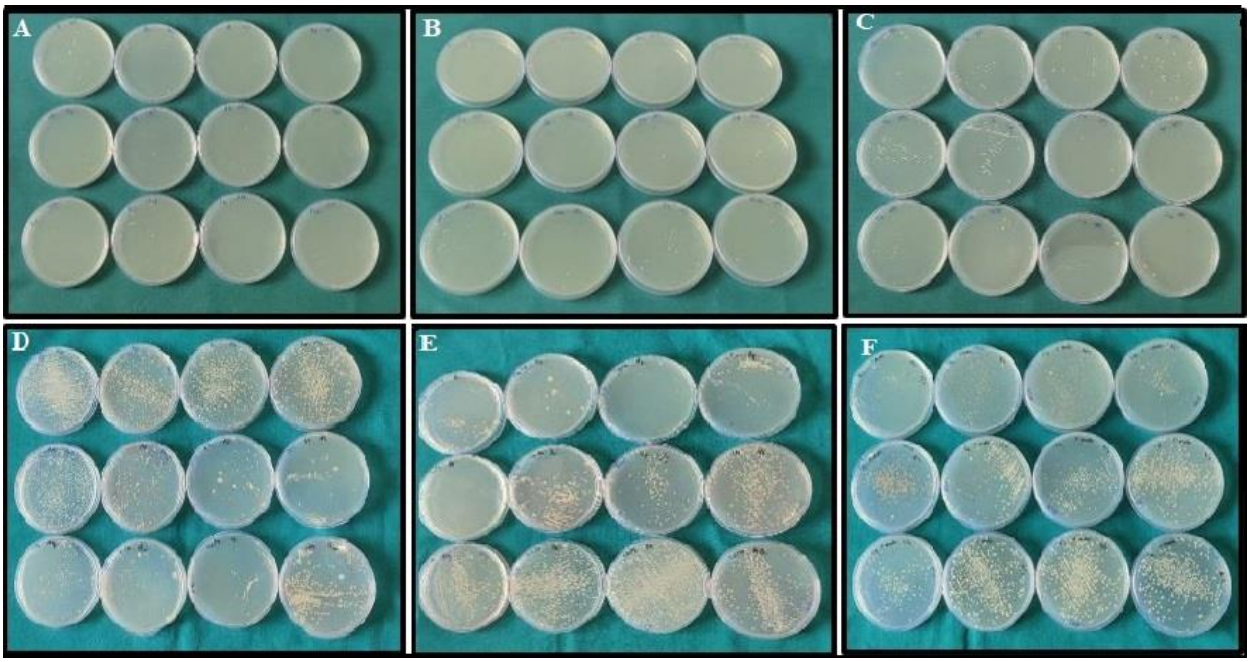

Figure 1: A-C) Colonies of *S. mutans* formed after 72 hours in conventional GIC, RMGIC, and GC Gold Hybrid Restorative GIC, respectively, and D-F) Colonies of *S. oralis* formed after 72 hours in conventional GIC, RMGIC, and GC Gold Hybrid Restorative GIC, respectively.

salivary proteins, bacterial adhesion, and biofilm formation (21). In contrast, Eick et al. (22) reported no significant correlation between surface roughness and the *S. mutans* adhesion.

Among the tested materials, RMGIC had the lowest surface roughness. Similarly, Kelten et al. (20) reported that RMGIC had the lowest surface roughness and number of adherent *S. mutans* compared to different glass ionomer-based materials, including giomer, amalgomer, and glass carbomer. These restorative materials combine the characteristics of glass ionomer and composite resins (giomer), amalgam (amalgomer), and carbomer (glass carbomer).

GC Gold Hybrid had a higher surface roughness compared to RMGIC. Higher surface roughness might be due to the diverse particle size of GC Gold Hybrid, while RMGIC is comprised of homogenous particle sizes (4.5 to 4.8 µm) (23). In contrast to the present results, Komandla et al. (24) reported that GC Gold Hybrid had a significantly lower surface roughness compared to RMGIC before and after artificial toothbrushing. The reason might be that their study applied a resin coat (EQUIA Forte Coat) to the GC Gold Hybrid (24). The resin coat infiltrates the surface of restorative material, covering all the gaps, fissures, and porosities of GC Gold Hybrid, thus enhancing surface smoothness. However, studies have reported that applying protective coats on the glass ionomer restorations severely impedes the fluoride release from the restorations. Hence, it was advised that it is better not to coat the GIC materials when the fluoride release property is more important than other properties. In the present study, no coating was applied.

In the present study, the *S. mutans* biofilm colonization was significantly higher in GC Gold Hybrid than in the other groups. RMGIC showed a lower *S. mutans* biofilm formation than GIC; however, the difference was not statistically significant. In contrast to the outcomes of this study, Pedrini et al. (25) found a lower bacterial and fungal retention in RMGIC than the conventional GIC. Fúcio et al. (26) found that RMGIC had better efficacy in inhibiting *S. mutans* adherence than conventional GIC due to its greater pH. Although previous studies reported a higher pH in RMGIC compared to GIC, the present study found no significant difference in *S. mutans* biofilm formation between these materials. This finding could be attributed to the higher fluoride release from GIC, which may provide greater antibacterial effects (27). Both GIC and RMGIC had a lower *S. mutans* biofilm than GC Gold Hybrid. Lower biofilm formation further protects the restoration from degradation by the acid byproducts produced by the bacteria. Therefore, using RMGIC and GIC might reduce the likelihood of secondary caries, consequently enhancing the overall longevity of the restoration.

All restorative materials had a significantly higher *S. oralis* colony count than *S. mutans*. This result was expected since *S. oralis* is among the early bacterial colonizers (28). *S. oralis* adheres to dental hard tissues, bone, and other species in the initial biofilm (29). Although not statistically significant, GC Gold Hybrid showed the highest *S. oralis* count, possibly due to its rougher surface. Therefore, biofilm formation and maturation on the GC Gold Hybrid surface seems to be more probable due to the higher initial colonizers (*S. oralis*) and significantly greater *S. mutans* rate.

The present study had some limitations. The most important limitation was that it is not feasible to completely replicate the intraoral cavity within laboratory settings. Several factors such as the patient's oral health practice, diet, masticatory load, and immunological and salivary characteristics may affect biofilm formation and the surface properties of restorative materials (30). Further clinical investigations are warranted to evaluate the long-term clinical success of GC Gold Hybrid in comparison with the conventional GIC and RMGIC.

Conclusions

Within the limitations of this study, the following conclusions can be drawn:

1. RMGIC had significantly lower surface roughness and significantly higher microhardness than GIC and RMGIC. The formation of *S. mutans* biofilm was comparable between RMGIC and GIC, but significantly lower than that of GC Gold Hybrid. These findings indicate that RMGIC had the most favorable surface properties compared to the conventional GIC and GC Gold Hybrid.
2. GC Gold Hybrid had significantly higher surface roughness, lower surface hardness, and higher *S. mutans* biofilm formation than RMGIC and GIC. Therefore, the use of GC Gold Hybrid may increase the rate of secondary caries formation around the restorations compared to conventional GIC and RMGIC.
3. GIC, RMGIC, and GC Gold Hybrid showed comparable *S. oralis* biofilm formation.

Acknowledgement

The authors would like to thank Professor Dr Vaishnavi and associates at the white lab Saveetha Dental College

and Hospitals for their assistance in evaluating microhardness and surface roughness.

Conflicts of interest

Authors have no conflicts of interest to disclose.

Author contribution

A.M.V.S. carried out the study; V.R.C. conceived the ideas and led the writing; M.K. conceived the ideas and helped in microbiological evaluation; D.K. conceived the ideas in framing the methodology; S.K. inspected the research work; S.S. collected the data; S.S.K.K. analyzed the data; S.P. helped with plagiarism correction. All authors have read and approved the final manuscript.

Ethical approval

The protocol of the present study was approved by the Institutional Review Boards (IRBs) (ref: 249/IRB-IBSEC/SIST) of Sathyabama Dental College and Hospital.

Funding

No funding.

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