Current Research in Dentistry

Research Article

Comparative Analysis of Surface Microhardness and Antimicrobial Activity of Conventional and Bioactive Composite Materials for Dental Restorations

Bruno Fongaro Dalbosco, Rafael da Silva Vanolli, Poliana Maria de Faveri Cardoso, Maria Ritha Veiga Colognese, Carlos Eduardo Misiak Godoy, Julio Katuhide Ueda and Veridiana Camilotti

Department of Restorative Dentistry, School of Dentistry, State University of West Parana, Cascavel, Brazil

Article history
Received: 18-03-2025
Revised: 31-07-2025
Accepted: 20-08-2025

Corresponding Author:
Maria Ritha Veiga Colognese
Department of Restorative Dentistry, School of Dentistry, State University of West Parana, Cascavel, Brazil
Email: mariarithacolognese@gmail.com

Abstract: This study evaluated the fluoride release capability, antimicrobial activity, and surface microhardness of bioactive composite resins, critical properties for the effectiveness and durability of dental materials in restorative procedures. Conventional and bioactive composite resins with varying viscosities were tested. Specimens of each resin type were prepared and stored in physiological saline solution for 24 hours to simulate oral conditions. Surface hardness was measured using a Knoop hardness test, assessing material resistance to indentation. Antibacterial activity was evaluated by immersing the specimens in a culture medium containing *Streptococcus mutants*, a common cariogenic bacterium, for 24 hours. After immersion, the specimens were washed to remove non-adherent bacteria, allowing for an accurate count of viable bacterial colonies. Results showed that bioactive composite resins exhibited lower surface microhardness compared to conventional resins lacking Giomer technology. However, bioactive resins demonstrated significant antibacterial activity. This suggests that while bioactive resins may be softer, their ability to inhibit bacterial growth could offer clinical benefits. The integration of Giomer technology in composite resins is a promising strategy to enhance antimicrobial properties and potentially reduce secondary caries. Nonetheless, the observed decrease in surface microhardness highlights a trade-off that may impact the material's longevity and wear resistance. Further research is necessary to optimize the balance between antimicrobial efficacy and mechanical strength in advanced dental materials.

Keywords: Bioactive Composite Resins, Antimicrobial Activity/Surface Microhardness/Fluoride Release, Restorative Dentistry

Introduction

The demand for an ideal restorative material with desirable characteristics represents a constant challenge in the field of dentistry. This material should exhibit satisfactory physical, mechanical, and chemical properties, along with excellent aesthetic properties. In this context, the ability to release fluoride emerges as a highly relevant attribute, as this ion possesses recognized antimicrobial properties in the prevention of dental caries.

The relationship between fluoride release capacity and antimicrobial activity has been a subject of scientific interest, as the release of this ion by composite resins plays a significant role in inhibiting bacterial growth and preventing biofilm formation. In this regard, Giomer technology stands out as a promising approach for the development of bioactive restorative materials. This technology is based on the incorporation of glass particles with pre-activated surface (S-PRG), which allows the release of six types of ions with bioactive properties. However, although the ionic interaction of this material with the oral environment is beneficial for antibacterial activity, it may compromise its structure and surface hardness, resulting in inferior mechanical properties.

Finally, the clinical relevance of bioactive restorative materials in dental practice becomes evident. The development of giomer technology represents a potential alternative to enhance the antimicrobial properties of composite resins, with significant clinical benefits. However, these materials have limitations related to their

physical and mechanical properties. Therefore, the hypothesis of this study is that there is a positive correlation between the fluoride release capability of composite resins and antimicrobial activity, while surface hardness may exhibit a negative correlation with antimicrobial activity.

Materials and Methods

Pilot Test

A flowchart summarizing the sequence of the pilot tests was created (Figure 1). The sample size calculation was performed using G*Power software, considering an effect size of 0.5, an alpha error probability of 0.05, and a statistical power of 0.80.

Microhardness Test

After the pilot test with five specimens of each resin, the sample calculation was performed using the Bioestat 5.0 software. A sample size of 12 per group was determined with P<0,05. Thus, two bioactive composite resins, Shofu Beautifil and Shofu Beautifil Flow (SHOFU Dental ASIA-Pacific Pte. Ltd., Kyoto, Kyoto, Japan), and two conventional composite resins, Opus Bulk Fill and Opus Bulk Fill Flow (FGM Dental Group, Joinville, Santa Catarina, Brazil), were selected.

The test was conducted using a microscope equipped with 100× magnification, and the indentations were measured using integrated analysis software. The crosshead speed was set at 0.5 mm/s. The result for each specimen was obtained by the Knoop microhardness, according to the formula:

$$K = 14.229 \times \frac{F}{L^2}$$

Where:

K = Knoop Hardness Number (KHN)
14.229 = Constant derived from the geometry of the indenter
F = Applied load in gram-force (*gf*)
L = Length of the indentation (mm)

Using a silicone addition matrix with dimensions of 4x4 mm, the specimens were prepared. A glass plate was used as a base, followed by a strip of polyester and the placement of the matrix. Then, the composite resin was inserted, and another strip of polyester was placed on top. The assembly was pressed with another glass plate for 10 seconds. The glass plate was then removed, and photoactivation was performed on the polyester strip. Photoactivation was done using a GrandValo light curing unit (Indaiatuba, São Paulo, Brazil) for 40 seconds. Additionally, neighboring samples were always covered with gauze to keep them free from residual light while the adjacent specimen was being photo activated.

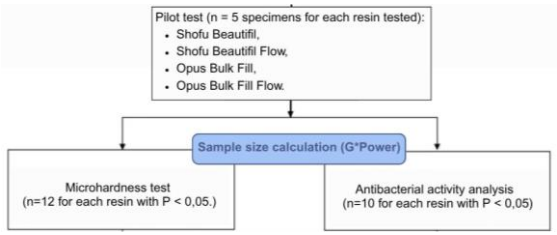

Fig. 1: Flowchart illustrating the sequence of pilot tests

Subsequently, finishing and polishing were carried out on the top and base surfaces of the specimens using the TDV finishing and polishing kit (Pomerode, Santa Catarina, Brazil), applying the sequence of the four sandpaper discs. Once this procedure was completed, the top and base of the specimens were marked for later analysis.

The specimens were stored in physiological saline solution and kept in an incubator at 37ºC (98.6ºF) for 24 hours. After this period, the specimens were rinsed under running water for 10 seconds and dried with an air jet for 10 seconds.

After 24 hours of fabrication, the specimens were mounted on the microhardness tester stage (FM 800 Future Tech Corp, Equilam, Tokyo, Japan) to measure the Knoop microhardness (HK) using a 25 gF load for 15 seconds. The readings were taken according to the instructions provided by Future Tech Corp, FM 800.

Antibacterial Activity Analysis

After conducting a pilot test with five specimens of each composite resin, the sample size was calculated using the Bioestat 5.0 software. A sample size of 10 specimens per group was determined with a significance level of p<0,05. The same composite resins used in the microhardness test were employed for this antibacterial analysis.

The specimens were prepared following the same protocol used in the microhardness test. The top surface of each specimen was identified, and they were stored in a plastic container with physiological saline solution at 37°C in an incubator for 24 hours.

For this study, the strain *Streptococcus mutans* CCT 7086 was used. *S. mutans* were reactivated in 5 mL of Brain Heart Infusion (BHI) culture medium and maintained under microaerophilic conditions at 37°C ±1 (98.6±1.8°F) for 18 hours. After growth, the suspension was centrifuged at 3000 rpm for 15 minutes (Excelsa II centrifuge, model 206-BL, FANEM), and the cells were washed twice with sterile saline solution. The product was suspended in BHI broth, and the turbidity of the material was adjusted to an absorbance of 0.15 read at 600 nm (FEMTO spectrophotometer), corresponding to a stock solution with a cell concentration of 108108 cells/mL.

The specimens were distributed in micro titer plates (10 wells) containing 1000 µl of BHI culture medium and S. mutans bacterial suspension. All specimens were fully submerged in the culture medium and incubated under microaerophilic conditions at 37°C ±1 (98.6±1.8°F) for 1 and 24 hours (Permution®, Curitiba, Paraná, Brazil). After the formation of the bacterial biofilm, the suspension from each well was aspirated, and the specimens were washed with 1000 µl of sterile Alkaline Phosphatase Buffer Solution (PBS). This procedure was repeated three times to remove non-adherent bacterial cells.

All instruments and materials used during the sample handling, including forceps and Falcon tubes, were sterilized prior to use. Forceps were sterilized by autoclaving at 121°C for 15 minutes under 15 psi pressure, ensuring the elimination of all microbial contaminants. Falcon tubes were either pre-sterilized disposable tubes or subjected to chemical sterilization using 70% ethanol followed by exposure to UV light for 30 minutes in a laminar flow hood. These sterilization steps were strictly followed to prevent cross-contamination and ensure the reliability of microbiological analysis.

After washing, the specimens were removed from the wells using sterilized forceps and placed in Falcon tubes with 5 ml of Phosphate-Buffered Saline (PBS). The tubes were vortexed for 1 minute and then immersed in water in an ultrasonic bath for 8 minutes (Digital Ultrasonic Cleaner, produced by Kondortech, with a cleaning power of 160 W). This procedure was performed to disaggregate the biofilm and release the bacterial cells adhered to the specimens for viable cell counting in the resulting solution

The serial dilution method was used for counting, where several dilutions were made from the initial solution to determine the number of cells. From the resulting suspension after biofilm disaggregation, considered as the initial suspension for the counting procedure, seven dilutions (10-1, 10-2, 10-3, 10-4, 10-5, 10-6, 10-7) were made for each well of each investigated group. From the initial 100 µl solution, 900 µl of PBS was inoculated to obtain the 10-1 dilution, and then 100 µl was transferred from one microcentrifuge tube to another (each containing 900 µl of PBS), generating the subsequent dilutions.

From each dilution, 25 µl was pipetted onto appropriate culture medium (sucrose agar) contained in Petri dishes (90x15 mm). This inoculum was spread over the surface of the medium using a Drigalsky loop, always from the highest dilution to the lowest.

After distributing the different dilutions on the plates, they were incubated in an incubator at 37±1°C (98.6±1.8 °F) under microaerophilic conditions as described for 24 hours, allowing bacterial multiplication until visible colonies were formed. However, variations among bacterial strains can significantly affect results due to differences in growth rate, biofilm formation, antimicrobial resistance, and metabolism. Strains may also require different incubation conditions, impacting growth and CFU counts. These variations represent a limitation of the study that should be considered. however, efforts were made to minimize these differences to reinforce the validity of the results.

Each visible colony corresponded to one Colony-Forming Unit (CFU) after bacterial multiplication over time. To count and calculate the number of CFUs/mL, the dilution that yielded 30 to 300 colonies was used. The number of CFUs/mL was determined using the following Equation 1:

$$\frac{CFU}{ml} = \frac{c \times 10^n}{q} \quad (1)$$

Where:

c = Average number of colonies
n = Absolute value of the dilution at which the colonies will be counted
q = Amount pipetted for each dilution in the plate seeding, in mL (q = 0.025)

The mean values obtained in CFU/mL from the experimental groups were initially subjected to the normality test using the Shapiro-Wilk test, and based on the result of this normality prerequisite, p<0,05. Thus, observing the non-normal distribution of the sample data, the groups were subjected to the Kruskal-Wallis: Dunn test.

Results

The data analysis from the microhardness test is presented in Table 1. It was observed that the regular viscosity conventional composite resin showed significantly higher surface microhardness values, both at the top surface and the base surfaces ($p<0.05$). The other evaluated composite resins did not show significant differences among themselves. Regarding the surface type analysis, all evaluated composite resins exhibited significantly higher values for the top surface, except for Shofu Beautifil Flow composite resin, where the values between the top and base surfaces were statistically similar.

The analysis of the antibacterial activity data is presented in Table 2. It is possible to observe a significantly higher antibacterial activity for the bioactive resins compared to the conventional composite resins. Additionally, in intergroup analysis, the Beautifil Bulk Flowable Resin (Shofu) and Beautifil II Resin (Shofu) showed no significant statistical differences ($P>0.05$). Similarly, the Opus Bulk Fill Flow Resin (FGM) and Opus Bulk Fill Resin did not show statistical significance among the samples ($P<0.05$).

Table 1: Surface microhardness analysis of the composite resins

	Shofu Beautifil	Shofu Beautifil Flow	Opus Bulk Fill	Opus Bulk Fill Flow
Top	51.00 Aa	37.78 Ba	37.92 Ba	34.20 Ba
Base	40.20 Aa	30.03 Bb	33.79 Bb	26.70 Cb

Different uppercase letters in the same row indicate statistically significant differences between composite resin types (P<0.05) Different lowercase letters (a, b) in the same column indicate statistically significant differences between top and base surfaces of the same resin (P<0.05)

Table 2: Bacterial growth in CFU

Specimens	Shofu Beautifil	Shofu Beautifil Flow	Opus Bulk Fill	Opus Bulk Fill Flow
1	890	540	224000	213000
2	830	600	220000	209000
3	900	570	226000	211000
4	870	550	220000	210000
5	810	580	219000	208000
6	880	530	222000	208000
7	910	560	219000	205000
8	860	570	223000	215000
9	850	570	223000	213000
10	880	590	225000	210000

The statistical analysis between bioactive and conventional composites revealed significant differences in antibacterial activity among the groups. The bioactive resins (Shofu Beautifil and Shofu Beautifil Flow) exhibited significantly lower bacterial growth compared to the conventional resins (Opus Bulk Fill and Opus Bulk Fill Flow), indicating superior antibacterial properties. Statistical significance was determined using the Kruskal-Wallis test followed by Dunn's post hoc test, with a significance level set P<0,05.

Discussion

This study aimed to evaluate the surface microhardness and antimicrobial activity of different composite resins, comparing bioactive and conventional materials. The hypothesis proposed was that the bioactive composite resins would exhibit greater antibacterial activity, but lower surface hardness compared to conventional resins. The results confirmed this hypothesis, showing a significant inverse relationship between antimicrobial activity and surface microhardness.

The mean values obtained in CFU/mL from the experimental groups were initially subjected to the normality test using the Shapiro-Wilk test. Based on the result of this normality prerequisite (P<0,05), the data exhibited a non-normal distribution. Consequently, the groups were subjected to the Kruskal-Wallis test followed by Dunn's post hoc test to determine statistical significance among the groups.

The study has some limitations that should be considered. The in vitro nature of the study may not fully replicate the complexities of the oral environment, such as the effects of saliva, food, and daily wear on the materials. In addition, the absence of long-term durability tests, such as wear resistance or degradation under simulated oral conditions, limits the clinical relevance of the findings. Additionally, the study did not evaluate the long-term durability and performance of the composite resins, which are critical factors for clinical success. Future research should include long-term clinical trials to validate these findings and provide a more comprehensive understanding of the materials' behavior in the oral environment.

The results obtained in the present study confirmed the hypothesis raised, indicating a positive correlation between the fluoride release capacity of bioactive composite resins and their antimicrobial activity, while also confirming a negative correlation between surface hardness and antimicrobial activity. While this demonstrates a potential link between fluoride release and antimicrobial activity, further research is needed to isolate and analyze the specific contribution of fluoride release in different composite resin formulations.

In the analysis of the surface microhardness of the composite resins on different surface types, it was observed that Opus Bulk Fill Regular resin obtained the highest microhardness values on both surfaces, while Shofu Beautifil Flow resin obtained the lowest values. Therefore, the data obtained indicate that the regular viscosity conventional composite resin exhibits higher surface hardness compared to the other evaluated composite resins.

The difference in microhardness between the regular viscosity conventional composite resin and the high viscosity conventional composite resin can be attributed to the variation in the number of inorganic particles present in the resins. The lower number of inorganic fillers in the high viscosity resins can result in reduced mechanical properties, including lower surface hardness. On the other hand, the lower surface microhardness observed in bioactive resins compared to conventional resins is a result of the ionic interaction that these materials establish with the oral environment. While this ionic interaction is beneficial for antimicrobial activity, it can compromise the structure and hardness of the material, leading to inferior mechanical properties. Therefore, the difference in surface microhardness between regular viscosity conventional composite resins, high viscosity conventional composite resins, and bioactive composite resins is associated with the compositional characteristics of each material. It is also

important to highlight that finishing and polishing techniques may significantly affect surface microhardness values.

Several variables can be used to measure the mechanical properties of Giomers. The results from Silva *et al.* (2021) indicate that Giomers demonstrate superior performance in laboratory tests compared to other materials such as compomers and glass ionomer cement. However, Kukiattrakoon *et al*. (2014) observed that immersion of these materials in acidic media significantly reduced their surface hardness. Additionally, Neves *et al.* (2002); Silva Mara da *et al.* (2019) found that immersion of the material in artificial saliva, combined with brushing simulation, resulted in lower surface hardness compared to conventional composite resin.

When analyzing the data of bacterial growth expressed in Colony-Forming Units (CFU) for each investigated composite resin, it is possible to observe that Shofu Beautifil and Shofu Beautifil Flow composite resins showed reduced values compared to Opus Bulk Fill and Opus Bulk Fill Flow resins. This information indicates that the former has the ability to inhibit bacterial growth. However, no significant difference was found between the bioactive resins, suggesting similar efficacy in preventing the development of microorganisms. The bioactivity of Shofu Beautifil and Shofu Beautifil Flow composite resins is conferred by giomer technology, which is based on the incorporation of Surface Pre-Reacted Glass (S-PRG) particles that allow the release of six types of ions with bioactive properties.

These results are in line with the findings of Oliveira *et al.* (2014); Alqarni *et al.* (2023), which indicate that differences in initial biofilm formation in various composite resins are influenced by differences in their compositions and surface properties. Composite resins with giomer technology demonstrated a lower amount of biofilm accumulation compared to conventional composite resins. Furthermore, in support of the antibacterial action of Giomers, Komalsingsakul *et al.* (2021) observed a significant reduction in the biovolume of S. Mutans compared to conventional composite resins, which supports their antibacterial action. However, different results were found by Feiz *et al*. (2022) where the giomer exhibited reduced antimicrobial activity compared to other groups. According to Gálvez *et al*. (2000); Kim *et al.* (2002), this finding may be attributed to the comparison made with glass ionomer cements, which have inferior mechanical properties that may have contributed to ion release and promoted better antimicrobial action.

The bioactivity of Shofu Beautifil and Shofu Beautifil Flow composite resins is conferred by Giomer technology. This technology is based on the incorporation of glass particles with pre-activated surface (S-PRG), which allow the release of six types of ions with bioactive properties. These materials enable the maintenance and prolongation of dental integrity, as they could neutralize acids, prevent enamel demineralization, inhibit the adhesion and multiplication of plaque bacteria, and recharge and release fluoride in the oral cavity. In this regard, composite resins with Giomer technology demonstrated the ability to inhibit bacterial growth, while Opus Bulk Fill and Opus Bulk Fill Flow resins did not possess this property, indicating the efficacy of bioactive resins in preventing the development of microorganisms.

Conclusion

This study demonstrated that bioactive composite resins incorporating Giomer technology exhibit significantly greater antimicrobial activity than conventional resins, likely due to their ion-releasing capacity. However, these materials exhibit lower surface microhardness compared to conventional composite resins, indicating a trade-off between antimicrobial activity and mechanical strength. These findings highlight the importance of accurate diagnosis and appropriate material selection, favoring bioactivity in high-caries-risk situations and mechanical durability in areas of high occlusal load. Also, further clinical studies are needed to validate these results in long-term applications.

Acknowledgment

Thank you to the publisher for their support in the publication of this research article. We are grateful for the resources and platform provided by the publisher, which have enabled us to share our findings with a wider audience. We appreciate the efforts of the editorial team in reviewing and editing our work, and we are thankful for the opportunity to contribute to the field of research through this publication.

Funding Information

The authors have not received any financial support or funding to report.

Author's Contributions

Bruno Fongaro Dalbosco and Maria Ritha Veiga Colognese: Contributed significantly to the research design, participated in the study development and data collection, and were actively involved in the initial manuscript drafted and writing.

Rafael da Silva Vanolli and Poliana Maria de Faveri Cardoso: Contributed to the methodological framework development and were responsible for data acquisition.

Veridiana Camilotti, Carlos Eduardo Misiak Godoy and Julio Katuhide Ueda: Provided overall supervision of the study, contributed to the critical

review and editing of the manuscript, and are responsible for the final approval of the submitted version.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Alqarni, D., Nakajima, M., Tagami, J., Alzahrani, M. S., Sá-Pinto, A. C., Alghamdi, A., Hosaka, K., Alzahrani, F., Alsadon, O. A., Alharbi, R. A., Almalki, S. S., & Alzahrani, A. A. H. (2023). Study of Streptococcus mutans in Early Biofilms at the Surfaces of Various Dental Composite Resins. *Cureus*, *15*(4), e38090. https://doi.org/10.7759/cureus.38090

Feiz, A, Nicoo, M. A., Parastesh, A., Jafari, N., & Sarfaraz, D. (2022). Comparison of antibacterial activity and fluoride release in tooth-colored restorative materials: Resin-modified glass ionomer, zirconomer, giomer, and cention N. *Dental Research Journal*, *19*(1), 104. https://doi.org/10.4103/1735-3327.363534

Gálvez, D. P. C. A., Rosa, O. P. D. S., Costa, B., Silva, S. M. B. D., & Torres, S. A. (2000). Avaliação in vitro da liberação de flúor e atividade antimicrobiana de compômeros restauradores. *Rev Fac Odontol Bauru*,*8*(1/2), 59-64

Kim, K.-H., Ong, J. L., & Okuno, O. (2002). The effect of filler loading and morphology on the mechanical properties of contemporary composites. *The Journal of Prosthetic Dentistry*, *87*(6), 642–649. https://doi.org/10.1067/mpr.2002.125179

Komalsingsakul, A., Srisatjaluk, R. L., & Senawongse, P. (2021). Efeito da escovação na rugosidade da superfície, liberação de flúor e formação de biofilme com diferentes materiais da cor do dente. *Journal of Dental Sciences*, *17*(1), 389–398. https://doi.org/10.1016/j.jds.2021.08.013

Kukiattrakoon, B., Siriporananon, C., Ornprasert, N., Mettasitthikorn, W., Likhitpreeda, S., Waewsanga, S., & Tanthanuch, S. (2014). The effect of different beverages on surface hardness of nanohybrid resin composite and giomer. *Journal of Conservative Dentistry*, *17*(3), 261. https://doi.org/10.4103/0972-0707.131791

Neves, A. D., Discacciati, J. A. C., Oréfice, R. L., & Jansen, W. C. (2002). Correlação entre grau de conversão, microdureza e conteúdo inorgânico em compósitos. *Pesquisa Odontológica Brasileira*, *16*(4), 349–354. https://doi.org/10.1590/s1517-74912002000400012

Oliveira, A. L. B. M. de, Lorenzetti, C. C., Garcia, P. P. N. S., & Giro, E. M. A. (2014). Effect of finishing and polishing on color stability of a nanofilled resin immersed in different media. *Revista de Odontologia Da UNESP*, *43*(5), 338–342. https://doi.org/10.1590/rou.2014.054

Silva Mara da, T., Barbosa Dantas, D. C., Franco, T. T., Franco, L. T., & Rocha Lima Huhtala, M. F. (2019). Surface degradation of composite resins under staining and brushing challenges. *Journal of Dental Sciences*, *14*(1), 87–92. https://doi.org/10.1016/j.jds.2018.11.005

Silva, A. R. J. da, Feitosa, V. G., Souza, A. N. de, Muniz, R. P. D., Ornellas, M. C. A. S., Silva, E. P. L., & Braz, R. (2021). Mechanical properties of composites with bioactive technology Giomer: a literature review. *Research, Society and Development*,*10*(3), e43310313413. https://doi.org/10.33448/rsd-v10i3.13413