Dental Research Journal

Original Article

Effect of *Streptococcus mutans* on the flexural strength of resin-based restorative materials

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ABSTRACT

Background: There are a limited number of studies about the effects of microbial aging on the mechanical properties of restorative materials. Therefore, this study aimed to evaluate the effect of simulated aging with *Streptococcus mutans* on the flexural strength of different resin-based materials.
Materials and Methods: This experimental study was performed on the blocks of different types of restorative materials including composite resin, giomer, and a resin-modified glass ionomer (RMGI). Moreover, three types of aging, such as 30-day storage in distilled water, *S. mutans*, and germ-free culture medium, were used in this study. The three-point bending flexural strength of the specimens before and after aging was measured according to the International Organization for Standardization-4049 standard. Data were analyzed by two-way ANOVA and *post hoc* Tukey's tests. A $P < 0.05$ was considered statistically significant.
Results: Results showed that the 30-day aging with the *S. mutans* significantly reduced the flexural strength of all three types of materials ($P = 0.00$). In all restorative materials, storage in a bacteria-free culture medium acted the same as distilled water, and there was no significant difference between these two solutions in terms of the flexural strength of the material, compared to the before-aging strength ($P > 0.05$). Furthermore, no significant difference was observed between *S. mutans*-based aging and distilled water aging regarding RMGI ($P = 0.75$).
Conclusion: It can be concluded that aging by *S. mutans* reduced the flexural strength in all three restorative materials.

Key Words: Aging, flexural strength, *Streptococcus mutans*

Received: 23-Aug-2020
Revised: 27-Sep-2020
Accepted: 19-Apr-2021
Published: 21-Oct-2021

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INTRODUCTION

The complex nature of the oral environment provides several challenges to the restorative materials due to the presence of ions, enzymes, bacteria, pH, and temperature fluctuations. These factors can increase wear, surface roughness, color changes, and microleakage of restorations followed by a reduction in the mechanical properties.[1] The ability of restorative material to withstand these challenges is an important factor in their successful clinical performance. On the other hand, secondary caries and fractures are still the two main causes of restoration failure in the oral environment.[2-4] Despite the preventive measures, dental caries still occur and are among the most common chronic diseases[5] affecting a high percentage of the population.[6]

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www.ncbi.nlm.nih.gov/pmc/journals/1480

How to cite this article: Haghi HV, Peeri-Dogaheh H, Fazlalizadeh S, Abazari M, Mohammadhosseini R. Effect of *Streptococcus mutans* on the flexural strength of resin-based restorative materials. Dent Res J 2021;18:90.

Dental plaque is the major cause of dental caries, and *Streptococcus mutans* is one of the most important bacteria in cariogenic dental plaque. The virulence of these bacteria is mainly due to its high binding ability to biologic and restorative surfaces, as well as its acidogenic and aciduric nature.[7] The acid produced by these bacteria can not only demineralize the tooth structures but also is capable of destroying the resin-based restorative materials.[8] It has been shown that the tendency to form microbial plaque by these bacteria on the restoration surfaces is different among the restorative materials, and the resin-based materials show the greater tendency probably due to the release of un-polymerized resin monomers from these materials, which could accelerate the growth of these bacteria.[9]

Today, composite resins are widely used as restorative materials due to their esthetic and the ability of adhesion to tooth structure.[10,11] In addition to composite resins, other restorative materials that have resin components are also used to restore tooth defects. One of them is a group of resin-based materials called giomers. These light-cure materials have prereacted fillers, which have a similar composition to glass ionomer particles and are added to the resin matrix. These materials have simultaneously the advantages of composite resins' strength and esthetics, as well as glass ionomeric characteristics such as fluoride release and fluoride recharging.[12] Another group of dental materials having resins is resin-modified glass ionomers (RMGI), which are made by adding a small number of resin monomers to conventional glass ionomers. Therefore, they offer the improvement of optical properties, resistance to moisture, and initial better strength along with the benefits of adhesion to the tooth structure and the release of fluoride of conventional glass ionomers.[13]

Various studies have examined the effect of microbial plaque and *S. mutans* on the surface properties of restorative materials that showed some negative effects on the surface roughness and surface morphology of restorative materials;[7,9] however, few investigations have evaluated the effect of these microorganisms on the mechanical properties of restorative materials. Therefore, this study aimed to investigate the effect of microbial aging caused by *S. mutans* on the flexural strength of three groups of resin-containing restorative materials including composite resin, giomer, and RMGI. Moreover, it was attempted to compare their effects with that of the water-based aging. The null hypothesis in this study was the lack of significant changes in the flexural strength of these three types of materials after 30 days of aging with *S. mutans*.

MATERIALS AND METHODS

This experimental study was conducted on the resin based restorative material blocks. The restorative materials included a nano-hybrid composite resin (Charisma smart, Kulzer, Germany), a giomer (Beautifil II, Shofu Inc, Japan), and a RMGI (Fuji II LC, GC, Japan). According to the aging method, each material was divided into four subgroups of before aging, distilled water aging, *S. mutans* aging, and storage in a germ-free culture medium. It is worth mentioning that the flexural strength of the samples was measured in this study.

By conducting a pilot study, the mean flexural strength for giomer, resin composite, and RMGI was 105.26, 111.53, and 101.03 MPa, respectively. Considering the variance of 34.4, Type I error of 0.05 and 80% power, the sample size of 6 was selected based on the formula of $n = \frac{\gamma_{g,\alpha,1-\beta}}{\Delta}, \Delta = \frac{\sum(\mu_i - \bar{\mu})^2}{\sigma^2}$

A total of 72 composite blocks were fabricated. Table 1 summarizes the materials and their composition used in this study.

Specimen preparation

Teflon molds with internal dimensions of 25 mm × 2 mm × 2 mm were utilized to prepare study specimens. The mold was placed on a celluloid strip over a glass-slab and fixed. It was then filled with restorative materials, the second piece of celluloid tape was placed on it, and the second slab was pressed to remove excess material. The slab was removed and the material was cured for 40 s in an overlapped manner along the entire length of the specimen using the LEDEX™ WL-070 (Dentmate Technology, Taiwan) light-curing unit with the intensity of 1000 mW/cm^2. Subsequently, the same exposure procedure was

Table 1: Materials used in the study

Material	Composition	Manufacturer
Charisma smart	Bis-GMA, barium aluminum fluoride glass, silicon dioxide	Kulzer GmbH, Hanau, Germany
Beautifil II	Bis-GMA, UDMA, Bis-MPEPP, TEGDMA, fluorosilicate glass	Shofu, Kyoto, Japan
Fuji II lc	HEMA, polyacrylic acid, water, fluoro-aluminum silicate glass	Gc, Tokyo, Japan

repeated on the other side of the specimen. A digital micrometer was employed to measure the dimensions of the prepared specimen (Digimatic, Mitutoyo, Japan), and the specimens with a difference of more than 0.01 mm were excluded from the study. To ensure the complete polymerization of the specimens, they were kept in distilled water over a period of 24-h incubation at 37°C.

Aging conditions

In total, four aging conditions were used in this study as follows:

Before aging

In each group of restorative materials, the flexural strength of six blocks was measured right after 24-h of incubation.

Aging with distilled water

One group was kept in 200 ml of 37°C distilled water for 30 days.

Aging with Streptococcus mutans

In this group, the specimens were immersed for 30 days in 200 ml of brain heart infusion (BHI) culture medium containing *S. mutans*.

Aging with brain heart infusion culture medium

To investigate the possible effects of BHI culture medium on materials' strength, a germ-free culture medium was used with a volume of 200 ml. The solutions were changed daily in all three aging groups.

Bacterial culture conditions

S. mutans (ATCC700610, UA159) were provided from the research laboratory of the Department of Microbiology of Ardabil University of Medical Sciences, Ardabil, Iran, and were incubated aerobically at 37°C and 5% CO_2 in the BHI medium (Sannyo, MCO-191C). The immersion solution containing 10^7 CFU/ml *S. mutans* was prepared daily.

Measurement of flexural strength

The SANTAM STM-150 (Santam, Iran) universal testing machine was used to measure the flexural strength of specimens by a three-point bending test. To calculate the flexural strength, the following equation was used according to ISO-4049 standard:[14]

$$\sigma = \frac{3FL}{2bh^2}$$

Where F represents the maximum load in Newton, L signifies the distance between supports in millimeters, b indicates the specimen width in millimeters, and h presents the specimen thickness in millimeters.

Statistical analysis

Data were analyzed in SPSS software (version 23, statistical software IBM, USA). Due to the sample size of six in each group, the adjusted Kolmogorov–Smirnov test (Lilliefors test) was used to evaluate the data distribution. Moreover, to compare the flexural strength of materials under different aging conditions, two-way ANOVA and *post hoc* Tukey's test were utilized in this study. A $P < 0.05$ was considered statistically significant.

RESULTS

The Lilliefors test results showed the normal distribution of the data in all groups ($P > 0.05$). Therefore, the two-way ANOVA test was used to compare the flexural strength values in different groups and showed significant differences between material groups ($P < 0.001$) and aging conditions ($P < 0.001$). However, there were no significant differences considering two factor of aging conditions and material types ($P = 044$). Table 2 summarizes the mean and standard deviations of the flexural strength values of the study groups. Furthermore, it demonstrates the results of the *post hoc* Tukey's test to compare the flexural strength values. The results showed that flexural strength values of all three types of materials significantly decreased after *S. mutans* aging condition ($P = 0.001$ for giomer, $P = 0.003$ for RMGI, $P < 0.001$ for composite resin). In addition, the values of flexural strength after *S. mutans* aging were lower than those in distilled water aging in the giomer ($P < 0.001$) and composite ($P = 0.005$) groups; however, they were the same in the RMGI group ($P = 0.784$).

DISCUSSION

In the present study, the effect of microbial aging caused by *S. mutans* was investigated on the flexural strength of resin-containing restorative materials including composite resin, giomer, and RMGI. According to the results, 30-day storage in conjunction with these bacteria significantly reduced the flexural strength of all three types of materials. In the experimental studies, aging by storage in different solutions, such as water,[15-17] is widely used for aging studies of restorative materials. However, this may not be sufficient to investigate the behavior of restorative materials in the oral environment, in which they are subjected to a variety of challenges.[18] One of the

Table 2: Mean and standard deviations of flexural strength values

Restorative materials	Aging conditions			
	Before aging	Aging with distilled water	Aging with *Streptococcus mutans*	Aging with BHI culture medium
Composite	147.26 (11.33) a, A	127.86 (11.48) a, A	89.69 (20.89) b, A	145.32 (11.02) a, A
Giomer	124.13 (8.78) a, A, C	105.71 (20.68) a, A	71.29 (23.88) b, A, C	119.50 (7.17) a, A
Glass ionomer	94.38 (10.33) a, B, C	71.07 (23.58) a, c, B	54.11 (8.79) b, c, B, C	80.56 (18.44) a, c, B

Lowercase letters for each restorative material individually (rows) values denoted with same letters are not significantly different (P>0.05), Uppercase letters for each aging condition individually (column) values denoted with same letters are not significantly different (P>0.05). BHI: Brain heart infusion

challenges is microbial factors that should also be considered in aging studies. There are few studies in the literature regarding the effects of microbial aging on the strength of restorative materials. In 2018, Zhou *et al*. examined the effect of microbial aging on three common restorative materials including composite resin, giomer, and conventional glass ionomer. It should be noted that their results were consistent with the findings of the present study.[6] In 2019, a study was conducted by Algamaiah *et al*. on Bis-GMA-based composite material, ormocer, and an oxirane/acrylate experimental composite which yielded similar results for fracture toughness, except for oxirane/acrylate composite, the toughness of which did not change significantly after biofilm aging. This could be due to the hydrophobicity of the oxirane monomers.[19]

In our study, the resin components of the studied materials included monomers such as UDMA, Bis-GMA, TEGDMA, and HEMA. It has been shown that lactic acid and enzymes such as esterase produced by *S. mutans* can cause hydrolysis and chemical degradation of ester bonds in the resin matrix.[7,9] Therefore, reduction in flexural strength due to streptococcal aging in the composite and giomer groups can be attributed to resin degradation. Moreover, the resin/filler interface is affected by lactic acid. Destruction of interface leads to the separation of the mineral filler particles from the matrix that can further reduce the strength of the material.[6] In the case of the RMGI, which has a lower concentration of resin monomers than the other two groups, the resulted decrease in strength in addition to the reason for resin components' degradation can be due to an increase in mineral dissolution.

The lactic acid produced by *S. mutans* reduces the pH by 4.5,[20] and the mineral parts of the glass ionomer, such as AL_2, O_3, and CaF_2, react with this acid.[21] It is well known that, in acidic environments, ionomeric materials are more susceptible to degradation than resinous materials.[22] Therefore, despite the release of fluoride, which is expected to reduce microbial metabolism and plaque formation on the glass ionomeric and giomeric materials,[23] the flexural strength of RMGI decreased after microbial aging. This is probably due to the greater sensitivity of glass ionomer to dissolution and degradation in an acidic and hydrophilic environment in this study. Furthermore, some studies suggest that fluoride release of restorative materials is not a dominant or significant factor in plaque control; in addition, the released amount of fluoride is probably not sufficient due to washing out.[24]

In the present study, the effect of aging caused by *S. mutans* was compared with storage in distilled water. A germ-free culture medium was also used in a group to investigate the possible effects of BHI culture medium on flexural strength. In all types of the studied materials, storage in the BHI culture medium acted the same as distilled water, and there was no significant difference between these two conditions in terms of the flexural strength of the material. Therefore, the observed effects in *S. mutans* aging can be attributed to the direct effect of the bacteria.

After making a comparison between the strength of materials in the *S. mutans* and distilled water aging groups, the difference was significant, except for the RMGI material group. It is somewhat consistent with the results of a study performed by Zhou *et al*. In the aforementioned study, the composite resin showed no decrease in strength by retaining in water; however, after microbial aging, the strength decreased, which was in line with the results of our study. Nonetheless, in their study, glass ionomer and giomer showed significant reductions in strength by water aging. This difference in findings can be attributed to the use of RMGI in our study, which had lower solubility and higher moisture resistance, compared to conventional glass ionomer that used in the mentioned study.[6] Furthermore, the giomer used in the study carried out by Zhou *et al*. was a flowable type; therefore, the decrease in strength after water aging can be explained by the lower filler rate. Regarding the effect of water-induced aging on the mechanical properties of composite resin materials, some studies have not shown a change in this regard,[6,25] whereas others reported a significant decrease. It seems that the change in strength is related to the duration of storage, and in 180 days of storage, all composite materials showed a reduced strength.[26,27] It is considered that the sensitivity to aqueous environments varies depending on the composition of the resin and its hydrophilicity.[28,29]

In the case of giomer and RMGI, the release of ions from fillers occurs in aqueous environments. As a result, the filler-matrix interface destroys due to the weakening of the filler surface that leads to a decrease in mechanical strength.[30] This strength reduction in the case of giomer is less than that in the RMGI since the acid-base reaction in the fillers of giomer takes place during the fabrication process. Therefore, the surface of the fillers readily has a modified layer that protects the central particles from the damaging effects of moisture.[30,31] In addition, the presence of a high percentage of fillers and different types of fillers in giomer is associated with more stable mechanical properties.[31]

It is noticeable that, in the RMGI group, although microbial aging can decrease the strength, compared to the initial strength before aging, it was not significantly different from the value of water aging. This finding probably indicates that the reduction in strength due to the *S. mutans* aging in the RMGI is somehow less than that in the other two materials. This finding can be attributed to the small effect of fluoride release.[23]

CONCLUSION

Considering the limitations of the study, it can be concluded that aging caused by *S. mutans* in all three restorative materials reduced flexural strength. In the case of composite resin and giomer, the flexural strength after *S. mutans* aging was significantly different from water aging. Moreover, 30 days of storage in water showed no significant effect on the flexural strength of all three materials.

Acknowledgment

This study was extracted from a research project No#2562 submitted to Ardabil University of Medical Sciences, Ardabil, Iran, and financially supported by the Deputy of Research and Technology. The study protocol was approved by the Ethics Committee of Ardabil University of Medical Sciences, Ardabil, Iran (IR.ARUMS.REC.1398.444).

Financial support and sponsorship

Nil.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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