



Effect of *Streptococcus mutans* on surface-topography, microhardness, and mechanical properties of contemporary resin composites

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Abstract

Objective: Dental caries is the most prevalent disease globally, and *Streptococcus mutans* (*S. mutans*) is a common associated oral bacteria. Additionally, *S. mutans* possess esterase activity capable of degrading resin composites (RC). However, the effect of degradation on the physical-mechanical properties of the RC has not been extensively studied. We evaluated the flexure strength (FS), the diametral tensile strength (DTS), the modulus of elasticity (ME), and the microhardness of three contemporary RC to establish if *S. mutans* could affect them.

Methods: One hundred thirty-eight bar-shaped and 276 disc-shaped specimens were fabricated with Enamel Plus HRi, IPS Empress Direct, and Clearfil AP-X, and physical-mechanical testing was done after been incubated during 30 and 60 days in culture media with or without *S. mutans*. Also, a scanning electron microscope was used to identify surface changes.

Results: None of the tested RC were affected in their mechanical properties (FS, ME, and DTS). However, Clearfil AP-X and Enamel Plus HRI showed eroded surfaces and a decreased microhardness after 30 and 60 days *S. mutans* incubation. IPS Empress Direct presented the lowest values in all the tests, but its physical-mechanical features and surface were not affected by bacteria's exposure.

Conclusions: Exposure to *S. mutans* could affect some contemporary RC; however, the effect seems superficial since its mechanical features were not affected.

Keywords

Resin composite, *Streptococcus mutans*, flexural strength, modulus of elasticity, diametral tensile strength, microhardness

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Introduction

Dental caries is the most prevalent disease in the world, and restorative dental materials are essential during its treatment.¹ The main goal of restorative dental material is to replace the esthetic, biologic, and functional properties of a tooth, principally by being resistant to masticatory forces. For this, it is expected that the material has good physical-mechanical properties, like an adequate modulus of elasticity (ME), flexural strength (FS), diametral tensile strength (DTS), and microhardness.^{2,3} The most common way to evaluate and compare restorative dental materials is by physical-mechanical testing.⁴

Resin composites (RC) are the most popular restorative dental materials, mainly because of the high esthetic demands from patients.⁵ RC are formulated with a silane coupling agent that connects inorganic fillers with an organic matrix. Inorganic filler particles are commonly ceramic oxides, quartz, or glass, while the organic matrix can comprise several monomers, such as 2,2-bis[*p*-(2'-hydroxy-3'-methacryloxypropoxy)phenyl]-propane (BisGMA), triethylglycoldimethacrylate (TEGDMA), dimethylaminoethylmethacrylate (DMAEMA), and several additives (photoinitiators-camphoroquinone, stabilizers, and inhibitors).⁶

It is well known that RC possesses short longevity compared to classical materials like the amalgam; this is principally attributed to polymerization shrinkage and failure in adhesion, but also degradation.⁷⁻¹⁰ At first, the wear of RC in the oral environment was attributed entirely to mechanical function.^{11,12} Subsequently, RC wear was related to chemical degradation, and several investigations have shown the contribution of oral enzymes in their chemical breakdown.^{8,13-15} Additionally, it is well known that oral enzyme sources include salivary glands, gingival epithelium, inflammatory responses, and bacteria.^{14,16-19} Hundreds of bacterial species (e.g. *Streptococcus*) are present in the oral cavity; some of them have a crucial role in the development of dental caries and possess a considerable affinity to RC in addition to the ability to produce acids and esterases.²⁰⁻²² It has been reported that depending on the strain, *Streptococcus mutans* (*S. mutans*) possess (in greater or lesser degree) esterase activity capable of degrading RC²³ and changing its surface topography.²⁴ The mechanism is not yet fully understood, but the breakdown of monomers like BisGMA and TEGDMA by the action of enzymes like cholesterol esterase (CE) and pseudocholesterase (PCE) has been shown.¹⁴

A great variety of RC are available for clinical use. Manufacturers claim improvements every year in handling, color stability, biocompatibility, physical-mechanical properties, and longevity. These improvements are mainly associated with modifications in their composition. The use of different fillers and monomers, their combinations, and proportions would result in distinct features and rates of degradation. However, the degradation impact on

the physical-mechanical properties of contemporary RC has not been extensively studied. It is essential to evaluate the possible effect of degradation on its physical-mechanical properties. Then, the objective of this investigation was to measure the ME, FS, DTS, and microhardness of three contemporary RC (Enamel Plus HRi, IPS Empress Direct, and Clearfil AP-X) to establish if degradation by exposure to *S. mutans* could affect these properties and to what extent. The hypothesis tested was that exposure to *S. mutans* can modify the mechanical properties (ME, FS, DTS), microhardness, and the surface of the tested RC.

Methods

Sample preparation

Three contemporary RC brands with distinct formulations were studied (Table 1). Two stainless-steel molds were fabricated. The first had dimensions of 25 mm in length, 2 mm in width, and 2 mm in height (for ME and FS tests) according to ISO standard 4049/2000.²⁵ The second had dimensions of 6 mm in diameter and 4 mm in height (for DTS, microhardness tests, and Scanning Electron Microscope (SEM) observation) according to the ANSI/ADA specification No. 27.²⁶

One hundred thirty-eight bar-shaped specimens (46 of each RC brand) and 276 disc-shaped specimens (92 of each RC brand) were manufactured. Each material was inserted and packed inside the molds. Filled molds were then compressed between two glass slides, and finger pressure was applied to extrude the excess and achieve a uniform surface. The material was light-cured, bar-shaped specimens with three consecutive 10 s exposures points by side, producing a partial overlapping. The disc-shaped specimens had one exposure on each side of 1000 mW/cm² light intensity for 20 s with a Valo cordless LED curing unit (Ultradent Products, South Jordan, Utah, USA). The specimens were extracted from the molds and polished using 1000 and 1200-grit abrasive papers and Sof-Lex discs (3M ESPE, Dental Products, St. Paul, Minneapolis, USA). Specimen dimensions were verified with a digital caliper (Digimatic caliper, Mitutoyo Corp., Tokyo, Japan). All specimens were made in sterile conditions at room temperature (23°C ± 2°C). After each specimen was manufactured, they were immediately settled in sterile distilled water for 1 h, air-dried, and subjected to ultraviolet light for 10 min inside a laminar flow hood before the incubation with or without *S. mutans*.

The 46 bar-shaped and the 92 disc-shaped specimens of each RC brand were divided into five groups, four of them with 10 specimens and one with six specimens (Figure 1). Groups were named: 24H-DW groups (control groups, *n*=6, incubated in distilled water for 24 h), 30D-sBHI (experimental groups, *n*=10, incubated for 30 days in sterile Brain Heart Infusion (BHI)), 30D-MUT (experimental groups, *n*=10, incubated for 30 days in BHI with *S. mutans*), 60D-sBHI

Table 1. Characteristics of the resin composites used in this study.

Resin composite	Manufacturer	Composition	Shade
Enamel plus HRi (ENA) Lot. 2019009489	Micerium S.p.A, Avegno GE Italy	Resin matrix: UDMA, BisGMA, 1,4-butandiol-dimethacrylate. Fillers: Glass filler, highly dispersed silicon dioxide 53% vol. 75%wt	UD2
IPS Empress Direct Lot. X53229	Ivoclar Vivadent, Schaan, Liechtenstein, Germany	Resin matrix: TEGDMA, BisGMA, UDMA, Bis-EMA. Fillers: Vitro of barium, ytterbium trifluoride, mixed oxides, silicon dioxide. Particle weight: 77.5%–79%	A2
Clearfil AP-X Lot. 6A0015	Kuraray Noritake Dental Inc. Okayama, Japan	Resin matrix: BisGMA; TEGDMA. Fillers: Barium glass, silanated colloidal silica, silanated silica filler (0.02–17 µm, mean 1–3 µm). Filler content weight 85.5%/vol%:71.0	A2D

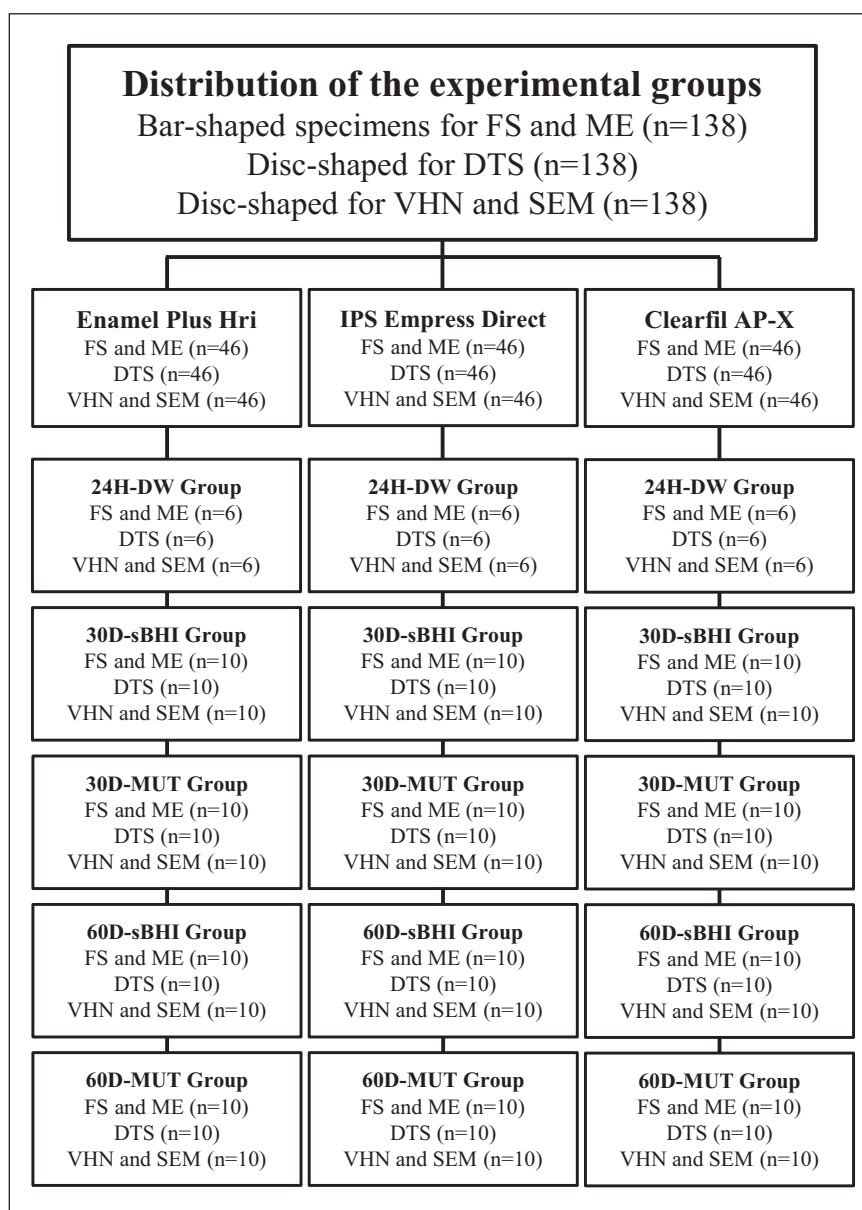


Figure 1. FS (Flexural strength), ME (Modulus of elasticity), DTS (Diametral tensile strength), VHN (Vickers hardness numbers), 24H-DW Group (incubated in distilled water during 24 h), 30D-sBHI (incubated for 30 days in sterile Brain Heart Infusion), 30D-MUT (incubated for 30 days in BHI with *S. mutans*), 60D-sBHI (incubated for 60 days in sterile Brain Heart Infusion), 60D-MUT (incubated for 60 days in Brain Heart Infusion with *S. mutans*).

(experimental groups, $n=10$, incubated 60 days in sterile BHI), and 60D-MUT (experimental groups, $n=10$, incubated for 60 days in BHI with *S. mutans*).

Incubation of the specimens

Each group was incubated in individual glass vials containing 8 mL of distilled water, 8 mL of sterile BHI (Becton, Dickinson Sparks, Maryland, USA), or 8 mL of BHI with 50 μ L of an overnight BHI with *S. mutans* GS5 strain. They were incubated for 24h, 30 or 60 days, respectively, at 36°C. The groups with *S. mutans* received an exchange of culture medium every 3 days, 3 mL of old medium was discarded, and 3 mL of fresh sterile medium was added. Growth determination of *S. mutans* was verified by turbidity observation, and samples were cultured in trypticase soy-sucrose bacitracin agar plates once a week, verified with polymerase chain reaction and specific primers at the beginning and end of the experiments. All procedures were done on a laminar flow hood. Once the experimental periods were accomplished, each specimen was rinsed with sterile distilled water, air-dried, and exposed to UV light for 10min before the mechanical tests.

Mechanical tests

A computer-controlled universal testing machine (UTM) (CMS Metrology, Model WDW-5Y, Querétaro, Mexico) was used for the mechanical tests. The FS and ME tests were done with the same bar-shaped specimens and tested by the three-point bend test in concordance to ISO 4049:2000.²⁵ Each specimen was mounted with its edges equidistant from the midline of the UTM. The load was applied at a crosshead speed of 0.5 mm/min until it fractured. Data were collected in Newtons and converted to megapascals (MPa) using the following equation: $FS=3FL/(2BH^2)$, where the maximum load was represented by F, L was the distance between supports (mm), B was the width of the specimen (mm), and H was the height (mm). The ME was determined in gigapascals (GPa) as $ME=FL^3/4BH^3d$, where F was the maximum load (N), L was the distance between supports (mm), B was the width of the specimen (mm), H was the height (mm), and d was the deflexion (mm) corresponding to the F load. DTS was determined in the 138 disc-shaped specimens (46 of each RC brand). They were mounted between the compressive plates of the UTM, and a compressive load was applied at a crosshead speed of 1.0mm/min vertically on the lateral portion of the disc to produce tensile stress perpendicular to the vertical plane. DTS was obtained using $DTS=2P/\pi DL$, where P was the maximum load (N), D was the diameter (mm), and L was the length of the specimen (mm).

Microhardness test

The microhardness test was done with a microhardness tester (CMS Metrology, Model CHV-1, Queretaro,

Mexico). One hundred thirty-eight disc-shaped specimens (different from the DTS test) were used, 46 of each brand. A 2.9-N force was applied using a diamond indenter for 15 s. All measures were generated in Vickers hardness number (VHN). The VHN (kgf/mm^2) was obtained with the following equation: $VHN=1.854(Ld^2)$, where L was the applied load (kgf), and d was the mean diagonal length (mm). This was determined from three indentations at different zones on one side of each specimen.

Scanning electron microscopy (SEM)

Three disc-shaped specimens per group chosen at random after performing the microhardness tests were sonicated (Tuttnauar-Ultrasonic cleaner, Tuttnauer, Israel) for 15 min to remove bacteria or bacterial products and were dried at room temperature (20°C). They were mounted on a holder and coated with 4 nm of carbon. The samples were analyzed using a SEM (Hitachi TM1000, Mito City, Japan) operating at 15 kV. SEM images were obtained at least from three different locations and in different magnifications using a backscattering electrons detector.

Statistical analyses

Results were statistically analyzed with two-way ANOVA combined with a post hoc Tukey-Kramer multiple comparisons test using Graph-Pad InStat, version 3.0 (Graphpad Software, San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

Results

Table 2 shows the ME, FS, DTS, and microhardness means and standard deviation of the three tested RC brands after the experimental conditions. None of the RC brands presented changes when comparing the five experimental conditions in the ME, FS, or DTS tests. However, there were some differences in the three mechanical tests (ME, FS, and DTS) when comparing RC brands. In general, Enamel Plus HRi presented the highest FS, DTS, and microhardness means in each tested condition ($p < 0.05$), while Clearfil AP-X presented the highest ME means ($p < 0.05$).

On the other hand, the Enamel Plus HRi and Clearfil AP-X specimens decreased their microhardness at 30 (71.77–64.23 and 55.66–48.26, respectively) and 60 days (70.25–61.67 and 57.41–47.58, respectively) after being exposed to *S. mutans* ($p < 0.0001$). IPS Empress Direct remained unchanged in its microhardness under all conditions ($p=0.3051$); however, it was the RC with the lowest microhardness means compared to Enamel Plus HRi and Clearfil AP-X ($p < 0.0001$).

Figure 2 shows a representative image of each RC brand in each experimental condition. Eroded surfaces

Table 2. Comparison of the three resin composite brands at each of the experimental conditions.

	24H-DW (n=6)	30D-sBHI (n=10)	60D-sBHI (n=10)	30D-MUT (n=10)	60D-MUT (n=10)	
Modulus of elasticity	X ± SD					p Value
Enamel Plus HRi	4.630 ± 0.47 ^b	4.576 ± 0.61 ^b	4.557 ± 0.70 ^b	4.696 ± 0.30 ^b	4.600 ± 0.71 ^b	0.9885
IPS Empress Direct	4.399 ± 0.19 ^b	4.061 ± 0.29 ^b	4.054 ± 0.35 ^b	4.116 ± 0.64 ^b	4.171 ± 0.31 ^b	0.3339
Clearfil AP-X	5.583 ± 0.55	5.622 ± 0.65	5.539 ± 0.43	5.546 ± 0.66	5.347 ± 0.54	0.8735
p value	<0.0001	<0.0001	<0.0001	<0.0001	0.0005	
Flexure strength	X ± SD					p Value
Enamel Plus HRi	120.2 ± 12.5 ^b	118.8 ± 16.1 ^{a,b}	118.3 ± 18.6 ^{a,b}	122.0 ± 8.17 ^{a,b}	119.5 ± 18.9 ^{a,b}	0.9885
IPS Empress Direct	109.4 ± 4.80	97.45 ± 7.03	97.27 ± 8.54	100.2 ± 15.5	101.5 ± 7.56	0.0588
Clearfil AP-X	98.38 ± 9.70	99.06 ± 11.4	97.61 ± 7.60	97.73 ± 11.6	94.26 ± 9.54	0.8735
p Value	0.0003	0.0015	0.0019	0.0004	0.0013	
Diametral tensile strength	X ± SD					p Value
Enamel Plus HRi	42.74 ± 5.31 ^b	43.08 ± 3.74 ^{a,b}	39.71 ± 5.01 ^b	42.33 ± 3.04 ^b	40.97 ± 3.25 ^b	0.4130
IPS Empress Direct	41.63 ± 4.34 ^b	36.99 ± 5.14	38.62 ± 4.53 ^b	39.15 ± 2.55	38.76 ± 2.73 ^b	0.1994
Clearfil AP-X	36.57 ± 1.47	34.92 ± 5.97	33.27 ± 3.32	36.87 ± 3.30	32.39 ± 4.82	0.0973
p Value	0.0081	0.0060	0.0098	0.0028	0.0002	
Microhardness (VHN)	X ± SD					p Value
Enamel Plus HRi	73.95 ± 2.84 ^{a,b}	71.77 ± 4.69 ^{a,b}	70.25 ± 6.79 ^{a,b}	64.23 ± 4.04 ^{a,b}	61.67 ± 8.74 ^{a,b}	<0.0001
IPS Empress Direct	42.61 ± 3.76 ^b	41.84 ± 3.37 ^b	42.74 ± 1.92 ^b	41.35 ± 1.16 ^b	40.62 ± 0.81 ^b	0.3051
Clearfil AP-X	56.86 ± 6.38	55.66 ± 7.63	57.41 ± 2.85	48.26 ± 2.37	47.58 ± 2.14	<0.0001
p Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

24H-DW: incubated in distilled water during 24 h; 30D-MUT: incubated 30 days in BHI with *S. mutans*; 30D-sBHI: incubated 30 days in sterile Brain Heart Infusion (BHI); 60D-MUT: incubated 60 days in BHI with *S. mutans*; 60D-sBHI: incubated 60 days in sterile BHI; SD: standard deviation; VHN: Vickers hardness number; X: mean.

^aSignificant difference versus IPS Empress Direct, in the same column.

^bSignificant difference versus Clearfil AP-X in the same column. ANOVA and post hoc Tukey-Kramer multiple comparisons test.

were evident in *S. mutans* incubated groups (30D-MUT and 60D-MUT) of Enamel Plus HRi and Clearfil AP-X, mainly due to a decrease in the number of small particles; even in some areas the complete lack of these are evident; therefore, large particles are more easily observed. In Figure 3, a two-and-a-half-dimensional (2.5D) perspective of the same scanning electron microscope images in Figure 2 are shown for better appreciation of surface differences.

Discussion

The quality of a RC is essential for clinical success, and suitable mechanical properties are necessary for supporting occlusal forces during chewing. FS is considered the most representative feature of a RC due to considerable flexural stresses that occur during chewing.^{27,28} ME describes material rigidity,²⁹ a low ME could result in higher deformability, which would result in serious failures.⁶ While the DTS test could reveal different features for brittle materials that are similar with few or no plastic deformation.^{30,31}

Although mechanical tests are not enough to establish a valid prediction of material performance or long-term success, it is hypothesized that stronger materials better distribute the stress, resist fracture and deformation, have stability, longevity, and a higher probability of success.

Then, the evaluation of the physical-mechanical properties of RC are common, but not after simulating a clinical scenario related to degradation. In the present investigation, three contemporary RC were incubated with *S. mutans* GS5 strain and tested through the most common mechanical tests.

Our results showed that the three tested RC brands remained without differences in the ME, FS, and DTS tests regardless of exposure to bacteria; then, the tested hypothesis has to be rejected. This is consistent with the only previous investigation where a mechanical test was done on a laboratory formulated resin incubated with *S. mutans*; the FS neither presented changes.³²

On the other hand, differences between the RC were present; this could be explained because of their distinct composition.^{33,34} It has been reported that the filler concentration strongly influences FS and ME.³⁵ However, their influence is of controversy since some studies have reported that RC with lower filler content (% volume) present low FS,³⁶ while others report a similar FS in RC with different filler volume.³⁷ While a correlation between the ME and the percentage of filler by volume³⁸ or weight³⁹ has been reported. Also, the content, the filler particles' size, and shape influence the RC mechanical performance.³⁹ Regarding the organic matrix, it could also influence mechanical results; it has been reported that

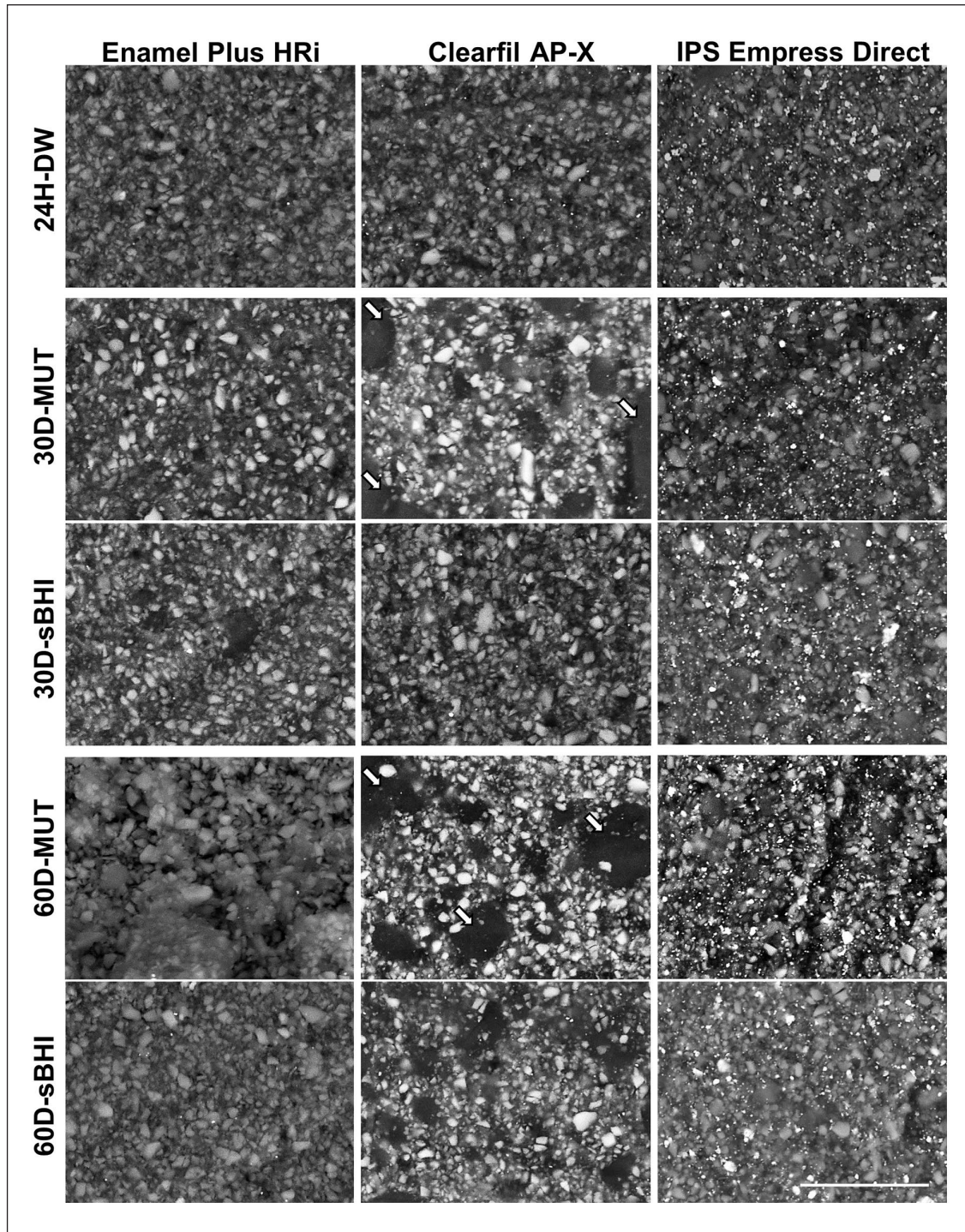


Figure 2. Scanning electron microscope photographs (8000 X) representatives of the surface of each resin composite brand after each experimental condition. The scale bar represents 10 μm and applies to all figures. Note the erosion on the Enamel Plus HRi and Clearfil AP-X bacteria-incubated specimens mainly due to a decrease in the number of small particles, in some areas a complete lack of them (white arrow) and the presence of the larger ones more easily observed.

24H-DW: incubated in distilled water for 24 h; 30D-MUT: incubated 30 days in BHI with *S. mutans*; 30D-sBHI: incubated 30 days in sterile Brain Heart Infusion (BHI); 60D-MUT: incubated 60 days in BHI with *S. mutans*; 60D-sBHI: incubated 60 days in sterile BHI.

BisGMA provides the RC with good mechanical properties.⁴⁰ While the presence of TEGDMA in RC formulations has been associated with a significant increase in

ME and a decrease in FS.⁴¹ This is consistent with our general results since Clearfil AP-X and IPS Empress Direct showed the lowest FS, both specimens have

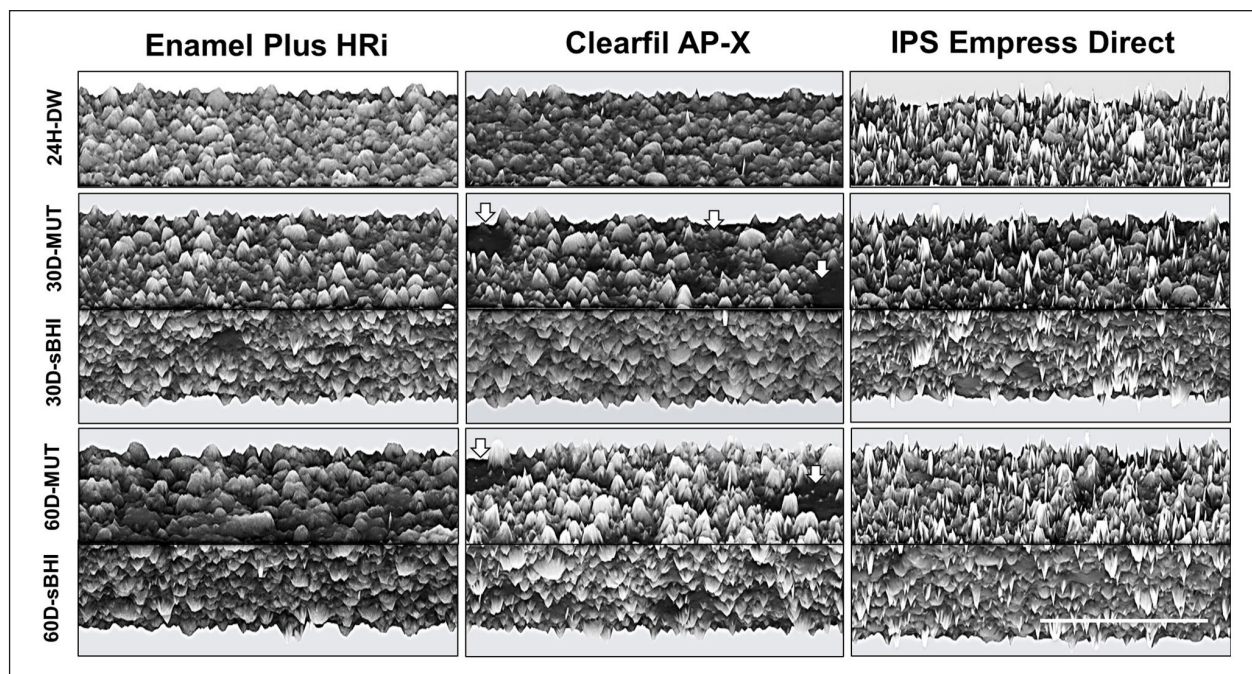


Figure 3. Two-and-a-half-dimensional (2.5D) perspectives of the same scanning electron microscope images (8000 X) of the surface of each resin composite brand after each experimental condition shown in Figure 2. The scale bar represents 10 μm and applies to all images. The 30 and 60 day images of the sBHI groups have rotated 180° and were spliced with the MUT groups to appreciate the surfaces better. Note the erosion on the Enamel Plus HRi and Clearfil AP-X bacteria-incubated specimens mainly due to a decrease in the number of small particles, in some areas a complete lack of them (white arrow) and the presence of the larger ones more easily observed. Processed by the 2.5D tool of imaging software Zen 2011, blue edition (Carl Zeiss MicroImaging GmbH, 1997–2011).

TEGDMA as a component. At the same time, Clearfil AP-X showed the highest ME.

Most of commercial and model RC contain BisGMA and/or TEGDMA, making them susceptible to degradation, mainly attributed to esterases like CE and PCE.^{4,17,18} Modify the proportion of monomers, as well as the design of new ones, is the primary strategy of researchers and manufacturers to avoid or at least reduce RC degradation. Attending to this, other monomers are currently used in the composition of contemporary RC, like urethane dimethacrylate (UDMA), 1,4-butandiol-dimethacrylate (1,4-BDMA), or 2,2-bis(4-(2-Methacryl-oxoethoxy)phenyl) propane (BIS-EMA). However, until now, it is unknown which may be the best combination or their exact proportions. Currently, different RC proposals exist. All are manufactured with different monomers and in different proportions; additionally, they have significant differences in their fillers, particle size, and shape that can be confusing factors to establish the best organic matrix option. While this happens, it is necessary to have as much information about the commercial RC that are already being clinically used, allowing the clinician to make the best choice.

Although in this investigation there were no changes in the physical-mechanical tests, SEM images showed that exposure to *S. mutans* GS5 modifies the surface of two of

the tested RC. This observation agrees with another report which described an increase in roughness on some RC after 35 days of bacterial exposure.⁴² Also; it has been reported that exposure to bacteria increases the roughness in a time-dependent manner³² which also could be seen in our results, the surface of the 60-day bacteria incubated groups is more eroded than the surface of 30-day bacteria incubated groups. However, these observations warrant further investigation to quantify and investigate these changes in more depth.

Differences in the surface after bacterial exposure may be due to the loss of the organic matrix caused by bacteria-enzyme degradation which causes the removal of small particles (inorganic and intrinsically stable), and the exposure of the larger ones, which promote surface erosion.⁴² This is evident when comparing 24H-DW (with a lot of small particles) versus 60D-MUT (with few small but abundant large particles) SEM images of Enamel Plus HRi and Clearfil AP-X in Figure 2.

Regarding microhardness decrease, it seems to be associated with surface erosion. The RC with evident surface modifications after 30 and 60 days of bacteria exposure (Enamel Plus HRi and Clearfil AP-X) decreased microhardness. On the other hand, the IPS Empress Direct did not show convincing surface modifications, neither showed differences in its microhardness. These differences between

RC could be attributed to the fact that IPS Empress Direct was the only one formulated with four different monomers (TEGDMA, BISGMA, UDMA, and BIS-EMA), this combination and their proportion could be blocking the degradation. Until now, there is no information about the possible degradation of UDMA and BIS-EMA by esterases. This association (Surface erosion-Microhardness decrease) could be due to when the organic matrix of the surface is lost, and the small inorganic particles are removed, the large particles remain on the surface, but they are separated by larger areas (which were occupied by the small particles). Therefore, when the indentation is carried out, the tip encounters less resistance on the surface (only that represented by large and separated particles), and a diminished microhardness is registered.

This result does not concur with a previous investigation that reported an increase in surface roughness but no difference in the RC microhardness.²⁴ Several factors could be involved in this discrepancy, one of the most important is the difference in composition, filler size, and loading of the tested RC since correlations of volume,⁴³ mass fraction of filler,⁴⁴ and hardness have been reported. Another discrepancy could be the bacteria strain chosen in both experiments. While in this investigation, we used *S. mutans* GS5 strain (serotype c) which has high esterase activity,²³ they experimented with *S. mutans* strain (ATCC 27351) with no information of esterase activity, probably being minor. These and other variables could influence the results of their investigation in such a way that the degradation of their RC was able to be observed on the surface, but to a lesser degree that is not enough to be registered in the microhardness test. Probably a bacterial exposure for a longer time and the use of a strain with higher enzymatic activity could increase degradation and consequently affect microhardness. This same could be applied in the case of IPS Empress Direct; a longer exposure time could at some point affect it in such a way that its microhardness decreases.

There is an imperious need for more investigation on RC to improve the reduction or blockage of the degradative effect of enzymes. Although this investigation did not simulate the complex mechanisms involved in the complete degradation process that could occur in the mouth, excluding factors like the thermal and other mechanisms of degradation and the chewing process; it is reasonable to state that the degradation observed in two of the RC could be maximized in a clinical situation because once the action of enzymes softens a layer of the RC, the chewing forces will easily expose a new surface layer⁴⁵ and the chemical attack can continue. Furthermore, it is well known that surface roughness plays an essential role in biofilm formation^{46,47} promoting microbial adhesion and plaque retention,⁴⁸ allowing more bacteria to attach and colonize the surface, increasing the degradation and promoting more erosion. All these will undoubtedly influence the restoration longevity.

Conclusions

It was demonstrated that *S. mutans* GS5 strain eroded the surface of the Clearfil AP-X and Enamel Plus HRI RC and decreased their microhardness after 30 and 60 days. However, the softening effect was superficial since the mechanical properties (FS, ME, and DTS) were not affected. IPS Empress Direct RC presented the lowest values in all the tests, but its properties were not affected after exposure to GS5 *S. mutans* strain.

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Author contributions

AMRM: Methodology, Investigation, Writing-Original Draft. DCOV: Methodology, Investigation, Visualization. MVG: Methodology, Formal Analysis, Visualization. LFEC: Formal Analysis, Investigation, Data Curation. MCAA: Conceptualization, Validation, Visualization. JECR: Investigation, Visualization, Methodology. RADP: Supervision, Conceptualization, Formal Analysis, Methodology, Writing-review, Data Curation, and editing. All authors reviewed and approved the final version of the manuscript.

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References

1. Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B, Murray CJ and Marcenes W. Global burden of untreated caries: a systematic review and metaregression. *J Dent Res* 2015; 94: 650–658.
2. Della Bona A, Benetti P, Borba M and Cecchetti D. Flexural and diametral tensile strength of composite resins. *Braz Oral Res* 2008; 22: 84–89.

3. Wang L, D'Alpino PH, Lopes LG and Pereira JC. Mechanical properties of dental restorative materials: relative contribution of laboratory tests. *J Appl Oral Sci* 2003; 11: 162–167.
4. Santerre JP, Shajii L and Leung BW. Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. *Crit Rev Oral Biol Med* 2001; 12: 136–151.
5. Sadowsky SJ. An overview of treatment considerations for esthetic restorations: a review of the literature. *J Prosthet Dent* 2006; 96: 433–442.
6. Moldovan M, Balazsi R, Soanca A, et al. Evaluation of the degree of conversion, residual monomers and mechanical properties of some light-cured dental resin composites. *Materials* 2019; 12: 2109.
7. Kermanshahi S, Santerre JP, Cvitkovitch DG and Finer Y. Biodegradation of resin-dentin interfaces increases bacterial microleakage. *J Dent Res* 2010; 89: 996–1001.
8. Delaviz Y, Finer Y and Santerre JP. Biodegradation of resin composites and adhesives by oral bacteria and saliva: a rationale for new material designs that consider the clinical environment and treatment challenges. *Dent Mater* 2014; 30: 16–32.
9. Khalichi P, Singh J, Cvitkovitch DG and Santerre JP. The influence of triethylene glycol derived from dental composite resins on the regulation of *Streptococcus mutans* gene expression. *Biomaterials* 2009; 30: 452–459.
10. Bernardo M, Luis H, Martin MD, et al. Survival and reasons for failure of amalgam versus composite posterior restorations placed in a randomized clinical trial. *J Am Dent Assoc* 2007; 138: 775–783.
11. Truong VT and Tyas MJ. Prediction of in vivo wear in posterior composite resins: a fracture mechanics approach. *Dent Mater* 1988; 4: 318–327.
12. Taylor DF, Bayne SC, Sturdevant JR and Wilder AD. Comparison of direct and indirect methods for analyzing wear of posterior composite restorations. *Dent Mater* 1989; 5: 157–160.
13. Finer Y, Jaffer F and Santerre JP. Mutual influence of cholesterol esterase and pseudocholinesterase on the biodegradation of dental composites. *Biomaterials* 2004; 25: 1787–1793.
14. Finer Y and Santerre JP. Biodegradation of a dental composite by esterases: dependence on enzyme concentration and specificity. *J Biomater Sci Polym Ed* 2003; 14: 837–849.
15. Finer Y and Santerre JP. The influence of resin chemistry on a dental composite's biodegradation. *J Biomed Mater Res* 2004; 69A: 233–246.
16. Lindqvist L, Nord C-E and Söder PO. Origin of esterases in human whole saliva. *Enzyme* 1977; 22: 166–175.
17. Santerre JP, Shajii L and Tsang H. Biodegradation of commercial dental composites by cholesterol esterase. *J Dent Res* 1999; 78: 1459–1468.
18. Leung BWH. Role of surfactants in the biodegradation of commercial Bis-GMA based resins by esterases. *J Dent Res* 1997; 76: 321.
19. Gu B, Kaneko T, Zaw SYM, et al. Macrophage populations show an M1-to-M2 transition in an experimental model of coronal pulp tissue engineering with mesenchymal stem cells. *Int Endod J* 2019; 52: 504–514.
20. Svanberg M, Mjör IA and Orstavik D. Mutans streptococci in plaque from margins of amalgam, composite, and glass-ionomer restorations. *J Dent Res* 1990; 69: 861–864.
21. He J, Söderling E, Österblad M, Vallittu PK and Lassila LV. Synthesis of methacrylate monomers with antibacterial effects against *S. mutans*. *Molecules* 2011; 16: 9755–9763.
22. Zhu H, Liu M, Sumbly P and Lei B. The secreted esterase of group a streptococcus is important for invasive skin infection and dissemination in mice. *Infect Immun* 2009; 77: 5225–5232.
23. Bourbia M, Ma D, Cvitkovitch DG, Santerre JP and Finer Y. Cariogenic bacteria degrade dental resin composites and adhesives. *J Dent Res* 2013; 92: 989–994.
24. Beyth N, Bahir R, Matalon S, Domb AJ and Weiss EI. *Streptococcus mutans* biofilm changes surface-topography of resin composites. *Dent Mater* 2008; 24: 732–736.
25. International Organization for Standardization. *ISO 4049/2000 – dentistry – polymer-based filling, restorative and luting materials*. Geneva: ISO, 2000.
26. American National Standard. American Dental Association Specification n. 27 for Resin-based filling materials. 1993.
27. Hahnel S, Henrich A, Bürgers R, Handel G and Rosentritt M. Investigation of mechanical properties of modern dental composites after artificial aging for one year. *Oper Dent* 2010; 35: 412–419.
28. Sideridou ID, Karabela MM and Bikiaris DN. Aging studies of light cured dimethacrylate-based dental resins and a resin composite in water or ethanol/water. *Dent Mater* 2007; 23: 1142–1149.
29. Rodrigues Junior SA, Zanchi CH, Carvalho RV and Demarco FF. Flexural strength and modulus of elasticity of different types of resin-based composites. *Braz Oral Res* 2007; 21(1): 16–21.
30. Anusavice KJ. *Phillips' science of dental materials*. St Louis: WB Saunders, 2003, p.596.
31. Palin WM, Fleming GJ, Burke FJ, Marquis PM and Randall RC. The reliability in flexural strength testing of a novel dental composite. *J Dent* 2003; 31: 549–557.
32. Gregson KS, Shih H and Gregory RL. The impact of three strains of oral bacteria on the surface and mechanical properties of a dental resin material. *Clin Oral Investig* 2012; 16: 1095–1103.
33. Bonilla ED, Mardirossian G and Caputo AA. Fracture toughness of posterior resin composites. *Quintessence Int* 2001; 32: 206–210.
34. Adabo GL, dos Santos Cruz CA, Fonseca RG and Vaz LG. The volumetric fraction of inorganic particles and the flexural strength of composites for posterior teeth. *J Dent* 2003; 31: 353–359.
35. Willems G, Lambrechts P, Braem M, et al. Composite resins in the 21st century. *Quintessence Int* 1993; 24: 641–658.
36. Kim K-H, Ong JL and Okuno O. The effect of filler loading and morphology on the mechanical properties of contemporary composites. *J Prosthet Dent* 2002; 87: 642–649.
37. Jiang L, Chen CR, Jin DC, et al. Changes in mechanical properties of seven light-cured composite resins after thermal cycling. *J South Med Univ* 2011; 31: 1957–1962.
38. Ikejima I, Nomoto R and McCabe JF. Shear punch strength and flexural strength of model composites with varying filler volume fraction, particle size and silanation. *Dent Mater* 2003; 19: 206–211.

39. Sabbagh J, Vreven J and Leloup G. Dynamic and static moduli of elasticity of resin-based materials. *Dent Mater* 2002; 18: 64–71.
40. Yap AU and Teoh SH. Comparison of flexural properties of composite restoratives using the ISO and mini-flexural tests. *J Oral Rehabil* 2003; 30: 171–177.
41. Asmussen E and Peutzfeldt A. Influence of UEDMA, BisGMA and TEGDMA on selected mechanical properties of experimental resin composites. *Dent Mater* 1998; 14(1): 51–56.
42. Willershausen B, Callaway A, Ernst CP and Stender E. The influence of oral bacteria on the surfaces of resin-based dental restorative materials – an in vitro study. *Int Dent J* 1999; 49: 231–239.
43. Chung KH and Greener EH. Correlation between degree of conversion, filler concentration and mechanical properties of posterior composite resins. *J Oral Rehabil* 1990; 17: 487–494.
44. Hosseinalipour M, Javadpour J, Rezaie H, Dadras T and Hayati AN. Investigation of mechanical properties of experimental Bis-GMA/TEGDMA dental composite resins containing various mass fractions of silica nanoparticles. *J Prosthodont* 2010; 19: 112–117.
45. Larsen IB, Freund M and Munksgaard EC. Change in surface hardness of BisGMA/TEGDMA polymer due to enzymatic action. *J Dent Res* 1992; 71: 1851–1853.
46. Hahnel S, Leyer A, Rosentritt M, Handel G and Bürgers R. Surface properties and in vitro streptococcus mutans adhesion to self-etching adhesives. *J Adhes Dent* 2009; 11: 263–269.
47. Park JW, Song CW, Jung JH, Ahn SJ and Ferracane JL. The effects of surface roughness of composite resin on biofilm formation of Streptococcus mutans in the presence of saliva. *Oper Dent* 2012; 37: 532–539.
48. Tanner J, Carlén A, Söderling E and Vallittu PK. Adsorption of parotid saliva proteins and adhesion of Streptococcus mutans ATCC 21752 to dental fiber-reinforced composites. *J Biomed Mater Res B Appl Biomater* 2003; 66B: 391–398.