Two possible in vitro alternatives to evaluate the effect of gastric acid on resin-based composites

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Abstract
Background: Objective: To compare two in-vitro protocols to study the effect of simulated gastric acid on the mechanical properties of resin based composites (RBCs).

Material and Methods: Three RBC FILTEK Supreme XTE (FS), BRILLIANT EverGlow (BE), GrandioSo (GS) were used. They were randomly divided into a control group (CG) and two groups exposed to simulated gastric acid: a 6-month daily protocol (DG) and an accelerated 90-min protocol (AG). Vickers microhardness (VH) and flexural strength were evaluated at baseline and six months. Statistical analysis was performed using repeated measures ANOVA tests for VH and three-way for flexural strength data (α=0.05).

Results: Daily exposure in the CG and DG groups caused a reduction in VH values and flexural strength (p<0.05). The majority of values in the AG remained stable, after an exposure of 90 min; FS (p=0.118) and GS (p=0.729) in VH and FS (p=0.377), BE (p=0.692) and GS (p=0.672) in flexural strength.

Conclusions: Daily exposure during 6 months caused significant changes in the VH values and flexural strength of the RBCs. The acid-accelerated protocol did not cause the same magnitude of change in VH values and flexural strength seen at six months of daily exposure.

Key words: Gastric acid, hardness, composite resins, flexural strength, dental materials.

Introduction
During life, teeth are exposed to a series of physical and chemical attacks that act together and contribute to the wear of the dental structure. Dental erosion is one of the main causes of dental wear. The low pH and erosive capacity of gastric acid (intrinsic) are significantly greater than those of acids from diet or medications (extrinsic), so the level of destruction is usually more severe (1,2). According to recent epidemiological data, dental erosion in adult patients with gastroesophageal reflux disease (GERD) has a prevalence between 24% and 32.5%. (3) Today, given the awareness of the need to preserve the greatest amount of tooth structure, even more so in those dentitions that already show signs of wear, it is crucial to select the appropriate restorative material. (4) Due to improvements in adhesive materials (5-8), it has become
possible to rehabilitate eroded teeth in a less invasive way using direct restorations with RBCs (9). However, these materials inevitably undergo aging, which is influenced by the dynamic and complex environment of the oral cavity (10,11). The degradation of RBCs is a somewhat complex phenomenon that involves mechanisms such as the hydrolysis of both the polymeric network and the silane bonds between the fillers and the matrix, causing the plasticization of the polymeric matrix and therefore the elution of filler particles as well as components that have not reacted (12). This phenomenon results in a decrease in some physical and mechanical properties of the material, such as hardness and flexural strength (13,14). RBCs are hydrophobic but contain hydrophilic monomers (12). The presence of these monomers in different proportions would explain why RBCs cannot be considered inert materials in aqueous media (15) and exhibit even worse properties in circumstances with low pH, as in the case of gastric acid pH 1.5 - 3.0 (15,16).

Although the best environment to evaluate the performance of a material will always be the oral cavity, in vitro tests are useful and are widely used to observe the behavior of materials under different circumstances and provide valuable information for future clinical research (17). Unfortunately, the heterogeneity of experimental designs with respect to intrinsic erosion (2,13,18-22) makes it difficult to compare results and safely extrapolate to the clinical environment (23,24).

Given the lack of consistent evidence in studies analyzing erosion and to observe whether acidic pH values of gastric origin alter the properties of RBCs, the present study aims to evaluate the possible effect of acidic pH values on the mechanical properties, i.e., Vickers hardness (VH) and flexural strength, of RBCs exposed to two types of protocols (daily or accelerated) that simulate endogenous erosion for six months.

**Material and Methods**

The present study examined three RBCs: FILTEK Supreme XTE (FS; 3M ESPE - St Paul, Minnesota, USA), BRILLIANT EverGlow (BE; Coltene/Whaledent AG - Altstatten, Switzerland) and GrandioSo (GS; Voco GmbH - Cuxhaven, Germany) (Table 1).

The specimens for VH analysis were made in a cylindrical stainless steel mold (Ø 10 x 1.5 ± 0.05 mm (Smile Line USA Inc., Colorado, USA). For the flexural strength tests, a custom mold was used, and samples of 12 x 2 x 2 ± 0.01 mm were obtained (25). The RBCs were packed inside each mold and polymerized for 40 seconds on each side (800 mW/cm²) with a polymerization lamp (Valo, Ultradent Products Inc., South Jordan, UT, USA) in a polymerization chamber (VISIO BETA vario, 3M/ESPE, Seefeld, Germany) for 7 min. All samples were polished under cooling with abrasive discs of silicon carbide P500, P1200, P2400 and P4000 (LaboPol-1, Struers, Willich, Germany). After polishing, the thickness of each specimen was confirmed with a digital caliper (Coolant Proof Micrometer IP65, Mitutoyo Corporation, Kanagawa, Japan), and the specimen was radiographically examined (RXDC Extend, MyRay, Bicocca, Italy) to rule out the presence of internal defects introduced during preparation. Finally, the specimens were cleaned in an ultrasonicator for 10 min.

Table 2 explains the two protocols performed to simulate endogenous erosion for six months. A total of 210 samples per RBC were used and were assigned by simple random sampling (www.random.org) to seven groups (n = 30). Three groups were used for VH analysis: a control group (CG, distilled water), a daily protocol group (DG, 12% HCl solution) and an accelerated protocol group (AG, 12% HCl solution and 15% dextrose solution) for six months. The specimens for VH analysis were made in a cylindrical stainless steel mold (Ø 10 x 1.5 ± 0.05 mm (Smile Line USA Inc., Colorado, USA). For the flexural strength tests, a custom mold was used, and samples of 12 x 2 x 2 ± 0.01 mm were obtained (25). The RBCs were packed inside each mold and polymerized for 40 seconds on each side (800 mW/cm²) with a polymerization lamp (Valo, Ultradent Products Inc., South Jordan, UT, USA) in a polymerization chamber (VISIO BETA vario, 3M/ESPE, Seefeld, Germany) for 7 min. All samples were polished under cooling with abrasive discs of silicon carbide P500, P1200, P2400 and P4000 (LaboPol-1, Struers, Willich, Germany). After polishing, the thickness of each specimen was confirmed with a digital caliper (Coolant Proof Micrometer IP65, Mitutoyo Corporation, Kanagawa, Japan), and the specimen was radiographically examined (RXDC Extend, MyRay, Bicocca, Italy) to rule out the presence of internal defects introduced during preparation. Finally, the specimens were cleaned in an ultrasonicator for 10 min.

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six months with simulated gastric acid) and an accelerated protocol group (AG, 90 min with simulated gastric acid). Each specimen was measured at two times, T1 (baseline) and T2 (six months). Four groups were set for flexural strength analyses: the initial group (BG), a control group (CG, six months with distilled water), a daily protocol group (DG, six months with simulated gastric acid) and an accelerated protocol group (AG, 90 min with simulated gastric acid).

Microhardness (n = 30/group) was evaluated in a Vickers diamond microindenter (HMV-2 microhardness tester, Shimadzu Corp., Kyoto, Japan). A load of 980.7 mN for 15 s was used as the measurement parameter stipulated by ISO 6507-1: 2018. (26) The mean value of VH for each specimen was calculated as the average of five measurements at least 1 mm apart. At the beginning, the VH of all the specimens was measured (T1), and after six months or 90 min depending on the protocol (T2), the same samples were used to provide a control over time.

Flexural strength (n = 30/group) was determined with a three-point universal test kit (Model 4502, Instron Corp., Canton, Mass., USA). A 5 kN load cell with a crosshead speed of 0.75 ± 0.25 mm/min was used, according to the ISO 4049/2009 standard (26). The distance between the supports (span distance) was set at 10 mm for flexural strength analysis (25). From the maximum recorded load, the uniaxial flexural strength was calculated as α=3Pl/(2bh²), where P is the maximum load exerted on the sample (in Newtons), l is the distance between the supports (10 mm), b is the sample width (mm), and h is the sample height. Before testing, the initial specimens were submerged in water for 24 hours as indicated by ISO 4049/2009.

Statistical analysis was performed with SPSS software (Version 20, SPSS Inc., Chicago, IL, USA). Using the Kolmogorov–Smirnov test, it was found that the distributions of VH and flexural strength values were adjusted to normal, so a parametric approach was used. Repeated-measures analysis of variance (ANOVA) with interactions between RBC and group was performed for the comparison of VH results. For the study of flexural strength (independent samples), three-way multifactorial ANOVA was performed, with RBC, group and time as factors. For multiple comparisons, the Bonferroni test was applied to adequately control for type I statistical error. The reference significance level was 5% (α = 0.05).

Results

Table 3 shows the mean (SD) VH values under the different study conditions. Figure 1 shows the triple interaction RBC - group - time (p < 0.05). The groups generated different levels of hardness, and the difference depended on the type of RBC and the time of evaluation. After six months, FS resulted in a reduction in mean VH of 20.5% in the DG group, while a change of 11.8% was observed in the CG group, and VH was practically stable in the AG group (+ 1.6%). With BE, the corresponding percent losses were 32% and 14.6%, and a slight gain was also observed for the AG group (+ 3.4%). Finally, with GS, losses of 10.9% and 6.3% and stability for the FS specimens (+ 0.3%) were observed. At six months, all specimens of the CG and DG groups showed significant changes in VH between one another and with respect to the initial values, while the variation in VH in the AG group was not significant for FS (p = 0.118) or GS (p = 0.729).

Table 4 shows the mean (SD) values for flexural strength for all the RBCs studied. The general levels of resistance became more homogeneous between the different RBCs. Specifically, FS and GS exhibited very similar levels but the values were higher than those of BE (p < 0.05). Figure 2 shows that the difference in resistance over time depended on the protocol used (double interaction time
Table 3: Microhardness in Vickers (VH): Mean (SD) for tested RBCs according to period of exposure to the different media.

|              | Control Group | Daily Protocol | Accelerated Protocol |
|--------------|---------------|----------------|----------------------|
|              | CG – T1       | CG – T2        | DG -T1               | DG -T2               | AG - T1       | AG - T2               |
| FS           | 76.48 (2.48) aB | 67.44 (2.94) bB | 75.47 (2.61) aB      | 59.95 (3.31) cB      | 75.17 (2.61) aB | 76.35 (6.67) aB      |
| BE           | 55.50 (3.25) aC | 47.22 (2.18) cC | 56.07 (3.62) aC      | 38.02 (3.05) dC      | 56.9 (2.89) aC | 58.75 (6.32) bC      |
| GS           | 107.96 (1.92) aA | 101.10 (1.37) bA | 107.7 (2.09) aA      | 95.91 (3.15) cA      | 106.5 (2.33) aA | 106.76 (2.14) aA     |

Different capital letter in columns and lowercase letters in the row indicate significant differences (p<0.05)

Fig. 1: Line graphs for the mean values of VH, more intuitive to appreciate the interaction.

Table 4: Mean (SD) values for flexural strength (MPa) according to period of exposure to the different media.

|              | Baseline | Control Group | Gastric Acid Groups |
|--------------|----------|---------------|---------------------|
|              | BG - T1  | CG – T2       | Daily Protocol      | Accelerated Protocol |
| FS           | 109.09 (8.27) aA | 93.73 (13.57) bA | 77.81 (15.16) cA | 106.5 (16.5) aA      |
| BE           | 96.25 (10.0) aB | 77.25 (12.92) bB | 59.7 (15.47) cB    | 97.41 (10.45) aB      |
| GS           | 105.17 (10.58) aA | 88.94 (9.54) bA | 77.18 (11.06) cA | 106.41 (8.68) aA      |

Different capital letter in columns and lowercase letters in the row indicate significant differences (p<0.05)

Fig. 2: Line graphs for the mean values of flexural strength, more intuitive to appreciate the interaction.
Alternative to evaluate acid gastric on composites

that the initial presence of solvent in the matrix will only
continuous exposure for 90 min. Egilmez showed a slight increase in flexural strength after con-
tinual values. In contrast, in the present study, BE and GS
strength (13) have been reported with respect to the ini-
However, no significant decreases in VH (16) or flexural
properties decrease continuously until the polymer network
stabilizes upon saturation; normally, RBCs reach satu-
ration in 7-60 days (28). Degradation, which consists
of hydrolysis of the polymeric networks, such as the
silanol bonds between the filler and matrix or the crac-
kling of the polymeric matrix, can be absent or continue
without significant changes until saturation is reached
(13). This phenomenon would explain why hardly any
changes were found in the mechanical properties of the
RBCs when using the accelerated protocol. Despite the
low pH of the simulated gastric acid, it may have been
that 90 min of immersion, although designed to simu-
late six months of erosion, was not enough to cause the
hydrolytic process, which is reflected by the significant
reductions in the mechanical properties of the RBCs. In
addition, the RBCs are composed of polymeric networks
that are highly cross-linked by covalent bonds, which
slows the entry of the solvent into the matrix (15,22).
Recently, the erosive stability of RBCs has been increa-
singly studied following accelerated protocols (13,16)
consisting of continuous exposure durations between
12 hours and five months with different concentrations
of HCl, with the objective of predicting the degradation
of RBCs over several years in patients with erosion.
However, no significant decreases in VH (16) or flexural
strength (13) have been reported with respect to the ini-
tial values. In contrast, in the present study, BE and GS
showed a slight increase in flexural strength after con-
tinuous exposure for 90 min. Egilmez et al. explained
that the initial presence of solvent in the matrix will only
blunt the tips of cracks present in the interior, reducing
stress concentration and propagation. Further prolong-
ing the exposure time is all that is required to observe the
true effect of simulated gastric acid on RBCs (29).
Currently, there is no clear consensus in the literature on
the best method of simulating erosion and the equivalent
exposure time needed to replicate an in vitro model, but
there are certain important variables to consider when
studying erosion (23,24). Although acidic pH has been
reported to have an effect on the mechanical properties
of RBCs after continuous exposure for two or five wee-
ks (13), a clinically appropriate range should not be ex-
ceeded; that is, several short bursts of no more than a
few minutes alternating with cycles of saliva or distilled
water are preferable, since such conditions better reflect
erosive attack in the oral cavity. In this study, two cycles
were used based on an average of two daily meals since
reflux or vomiting events are postprandial (28), and a
period of two minutes, a time also considered by another
author, (24) was selected because the pH of oral fluids
regains neutrality one to three minutes after the presence
of acid.
Six months of storage with daily erosive exposure
allowed the entry of the solvent into the polymeric
networks until saturation was reached, and thus, the
mechanical properties could be correctly evaluated.
The RBCs showed significant reductions in VH and
flexural strength after six months of exposure, as also ob-
served in other studies of erosive resistance (13,20,22).
Higrosopic and hydrolytic effects in the networks of
dental polymers depend on the polarity and typology of
the polymeric matrix, the system of the inorganic fillers
and the solvent (12). The main effect of the solvent is to
reduce the interactions between the chains of the poly-
meric network, causing the plasticization of the matrix
and affecting the integrity and stability of the properties
of RBCs (12,14). The three RBCs studied had almost the
same polymeric matrix composition; however, their in-
organic compositions were different. Therefore, the di-
fferences in VH and flexural strength between the RBCs
could be explained by the size, shape and quantity of
filler particles present in the materials (6,4,28).
Among the RBCs studied, BE showed the highest per-
cent reduction in the mechanical properties tested. Two
possible reasons could be argued: on the one hand, the
low percentage of filling in volume of BE (56 vol% -74
weight%) compared to FS (63.3% vol% -78.5 weight%) and
GS (73 vol% - 89 weight%) could be responsible. RBCs with the highest filler content and a small particle
size positively influence the properties of the material.
The incorporation of a greater amount of filler in the
polymer matrix can reduce the free volume available for
water absorption, in addition to allowing the filler to act
as a protective agent of the matrix, which is vulnerable
to hydrolysis (6). On the other hand, BE is composed of
barium glass powder, which is considered a radiopaque glass. This type of filler has exhibited greater dissolution in water and saline solutions than have fillers containing silica or pure quartz, which are inert in water (28). The absence of radiopaque glass, as well as the higher percentage of filling and therefore lower percentage of matrix, would explain the greater erosive resistance of GS than the other RBCs tested, as seen in a similar study (11).

Exposure to simulated gastric acid caused a marked deterioration in both VH and flexural resistance with respect to the initial values and the control group, consistent with previous studies (20,22). Chemically, gastric acid acts as a powerful plasticizer that accelerates the sorption and solubility processes of RBCs, enabling the erosion of the material (20). Acids provide a sufficient concentration of protonated protons (H+) that catalyze the hydrolysis of ester groups in the matrix. The products resulting from hydrolysis, such as alcohols and carboxylic molecules, accelerate degradation by further reducing the pH within the matrix (12). Acids can also cause erosion on fill surfaces, contributing to the leaching of the fill and leaving a rougher surface (20,22).

Although simulated gastric acid is a significant source of deterioration, time is still an essential factor for observing an effect. This would explain why in previous studies, (20,22) exposure to gastric acid for a short time did not cause significant changes in VH.

A limitation of this study is it was a short-term in vitro study with only three RBCs. It would be highly valuable to validate a protocol to study in vitro erosion of endogenous origin, as well as to study the erosive stability of the properties of materials with different compositions. Reductions in microhardness and flexural strength after six months were evident. The changes were more pronounced in RBCs exposed to simulated gastric acid, with acidic pH accelerating the reduction.

Time is a fundamental factor affecting the mechanical properties of RBCs, regardless of the medium to which they are exposed. The 90 min duration of the accelerated protocol was not enough to cause the same magnitude of changes in VH and flexural strength seen with six months of daily exposure.

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Conflict of interest
Non declared.