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ORIGINAL RESEARCH


# Analysis of the Properties of Dental Cements after Exposure to Incubation Media containing *Streptococcus mutans*

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## ABSTRACT

**Aim:** Indirect restorations are increasingly used in dentistry, and the cementation interface is possibly the most critical region of the work. The objective of the present work was to evaluate the influence of exposure to a culture medium containing *S. mutans* on the hardness and solubility of four different cementing agents (zinc phosphate, glass ionomer, glass ionomer modified with resin and resin cement).

**Materials and methods:** Test specimens composed of these cements were exposed for 30 days in a culture medium containing *S. mutans*. After leaching, the test materials were assessed in terms of their solubility (loss of mass) and Knoop (KHN) microhardness. Changes in surface morphology were identified using scanning electron microscopy (SEM).

**Results:** The resin cement showed no significant solubility and its hardness increased following exposure and leaching, while the zinc phosphate cement was the most soluble and its hardness decreased after exposure to the culture medium. SEM analyses identified morphological alterations on the surfaces of the test materials that were compatible with the solubility results.

**Conclusion:** It is concluded that resinous cements perform better than water-based cements when exposed to acidic conditions.

**Clinical significance:** The effects of acids from *Streptococcus mutans* can interfere with the efficiency and properties of some cements used for fixation of indirect restorations, exposed to the buccal environment.

**Keywords:** *Streptococcus mutans*, Luting agents, Physical properties.

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## INTRODUCTION

One of the most important pathologies associated with disequilibria in the buccal biota is dental caries, characterized by a series of complex chemical and microbiological reactions that result in a dynamic demineralization of the hard tissues of the teeth (enamel and dentine), alternating with periods of remineralization. Many investigations have demonstrated an association between dental caries and the presence of the gram-positive bacterium *Streptococcus mutans*. This microorganism is considered to be one of the main etiological agents of dental caries in humans and contributes to the formation and accumulation of bacterial plaque (biofilm) on the tooth surface. The formation of this biofilm is a prerequisite for the development of primary and recurring dental caries. Since it is acidogenic, *S. mutans* alters the buccal environment, especially the saliva, which becomes more acidic.[1,2]

In the relationship between a prosthetic device and the dental structure, it is the intermediate cementing agent that is the most vulnerable part of the system. The attachment interface is commonly located in the cervical region of the tooth, where problems involving marginal adaptation and periodontal health can be exacerbated. The influence of oral bacteria on the properties of cementing agents depends on the type of cement used, since different cements have different structural characteristics. This needs to be considered by the dentist when selecting the material to be used, since it is critical to the durability of the restoration work.[3,4]

Previous studies have investigated the ability of luting agents to withstand solubilization and the action of microorganisms, which could reduce the longevity of

indirect restoration work in the oral cavity.[2,5] It has been shown that acids present in aqueous media favor the solubilization of dental cements based on ceramic materials, while resinous cements are virtually insoluble in the buccal environment.[6]

The objective of the present work was to assess, *in vitro*, the effect of exposure to a culture medium containing *S. mutans* on the hardness and solubility of four cementing agents used to affix prosthetic devices. The cements used were: Glass ionomer (Vidrion C, supplied by SS White); glass ionomer modified with resin (Vitremer, 3M ESPE); resinous cement (Dual Cement, Vigodent); and zinc phosphate (SS White). Scanning electron microscopy (SEM) analyses were performed to examine the morphological characteristics of the surface of each material.

## MATERIALS AND METHODS

### Bacterial Strain

The ATCC 25175 strain of *S. mutans* was purchased from the Fundação André Tosello (Campinas, Brazil). The stock of *S. mutans* samples were stored at –20°C in 40% (v/v) glycerol. The cultures were grown in a candle jar, with weekly subculturing into TSB (see below).

### Cultivation of *S. mutans*

The liquid medium used to culture the bacterium and to check cell viability was either a Tryptic Soy Broth (TSB) (Soybean-Casein Digest Medium) or Tryptic Soy Agar (TSA) (Soybean-Casein Digest Agar), obtained from Difco (Sparks, MD, USA). The medium was prepared according to the manufacturer's instructions and autoclaved at 121°C for 15 minutes prior to use.

All the cultures were grown in a candle jar, under an anaerobic atmosphere obtained after lighting a candle inside the jar to consume part of the oxygen. A 15 ml of the stock culture was initially inoculated into Falcon tubes (15 ml) containing 4 ml of previously autoclaved TSB medium and incubated for 24 hours at 37°C. After this period, the cells were submitted to three additional 12-hour culture periods (these experiments used 15 µl of inoculum in 4 ml of sterile TSB).

### Preparation of the Test Specimens

Standardized specimens, in the form of disks with a diameter of 5 mm and a thickness of 2 mm, were fabricated with the aid of molds cut from flexible polymer dental anesthetic tubes with the aid of a mechanical lathe. A total of 56 devices were produced, 40 for use in the tests (n = 10) and 16 for the control groups (n = 4), using the four cements described above. The cements were prepared according to the manufacturers' instructions. The flexible polymer mould was positioned on a strip of polyester and filled with cement. Another strip of polyester was placed on top of the cement. In order to ensure that the upper and lower surfaces were smooth and flat, a glass slide was then placed on top of the assembly, followed by a cylindrical steel weight (235 gm) to provide a standardized pressure.

In the case of the test specimens composed of resinous cement and resin-modified glass ionomer, after filling the mould the material was photopolymerized by applying blue light to each surface for a period of 40 seconds. The equipment used (Radii LED curing light, SDI Ltd) provided a power density of around 1000 W/cm$^2$.

### Manipulation of the Test Specimens

The test specimens were removed from the polymer moulds by making a lateral cut in the mould with a scalpel blade. They were then examined with a stereoscopic magnifying glass (×40 magnification) to check that the surfaces were smooth and free from pores or other defects. Defective specimens were discarded and new examples were manufactured. All specimens were stored in distilled water at 37°C for 24 hours to promote hydration, and then randomly separated into different groups. Each test specimen was exposed for 30 days in the culture medium containing *S. mutans*, with daily changes of the medium. The pH of the cultures was measured immediately after removal of the test specimens. The control group was immersed in distilled water.

### Evaluation of the Solubility of the Specimens by Loss of Mass after Leaching

After exposure to the culture medium for 30 days the specimens were leached with distilled water using a triplex syringe operated at a pressure of 45 psi. The syringe was positioned 5 cm from the surface of the specimen, at an angle of 45°, and the water jet was operated for 20 seconds. After leaching, the devices were placed on an absorbent paper towel to remove the excess water and then weighed to determine the extent of solubilization. They were then stored individually in receptacles containing silica gel to prevent dampness.

A 6-digit balance was used in the weight loss experiments. The initial weights of the specimens were determined following hydration for 24 hours in distilled water, immediately after lightly drying the surfaces with a paper towel.

### Preparation of the Specimens for Scanning Electron Microscopy (SEM) Analysis

The specimens were replicated by constructing moulds of their surfaces using a hydrophilic polyvinylsiloxane silicone (Adsil, Vigodent) and then filling the moulds with an epoxy resin (Epoxicure, Buehler Ltd). Replicates were needed because the preparation process and analysis under vacuum of the specimens could (especially in the case of those materials that contained water) cause dehydration and cracking, which would reduce the quality of the micrographs obtained. After replication of the surfaces, the replicate specimens were coated with gold in a metallizer operated at 50 mA for 180 seconds and then analyzed using SEM.

### Preparation of the Specimens for Microhardness Analysis

The Knoop microhardness tests were performed using a Model HMV-2 microhardness meter (Shimadzu Corp). Two test specimens were randomly selected from the control group and four specimens from each of the experimental groups. The measurements were made at three equidistant points on one of the plane surfaces of each specimen, using loadings of either 100 gm (for the resin-modified glass ionomer and resin cements) or 25 gm (for the zinc phosphate and glass ionomer cements). In both cases, the loadings were maintained for 30 seconds.

## RESULTS

### Weight Change Experiments

The results of the weight change experiments are provided in Table 1. The resin cement showed a significant weight gain after 30 days of exposure followed by leaching, while the weight of the glass ionomer cement remained stable. The zinc phosphate and resin-modified ionomer cements exhibited significant weight losses.

### Knoop Microhardness

The water-based cements (glass ionomer and zinc phosphate) showed a reduction in Knoop microhardness after exposure to the medium for 30 days, while the microhardness of the resin-modified glass ionomer and resin cements increased significantly over the same time period (Table 2).

**Table 2:** KNH microhardness before and after 30 days exposure to media containing *S. mutans*. Means followed by different lower case letters in the same line were statistically different at p<0.05

| *Cement* | *Control (no exposure)* | *Experimental (after 30 days exposure to media containing S. mutans)* |
|---|---|---|
| Glass ionomer | 38.71 ± 4.22 a | 23.67 ± 4.10 b |
| Zinc phosphate | 18.43 ± 2.69 a | 7.26 ± 2.16 b |
| Resin-modified glass ionomer | 23.41 ± 2.21 b | 43.36 ± 3.76 a |
| Resin cement | 28.76 ± 1.73 b | 47.12 ± 4.81 a |

### Scanning Electron Microscopy

The SEM results provided support for the findings of the weight change and hardness experiments. The resin and glass ionomer cements (Figs 1A to D) showed no evidence of erosion after exposure, when compared to the control materials. In contrast, the zinc phosphate and resin-modified glass ionomer cements showed obvious erosion after exposure to *S. mutans* culture followed by leaching (Figs 1E to H).

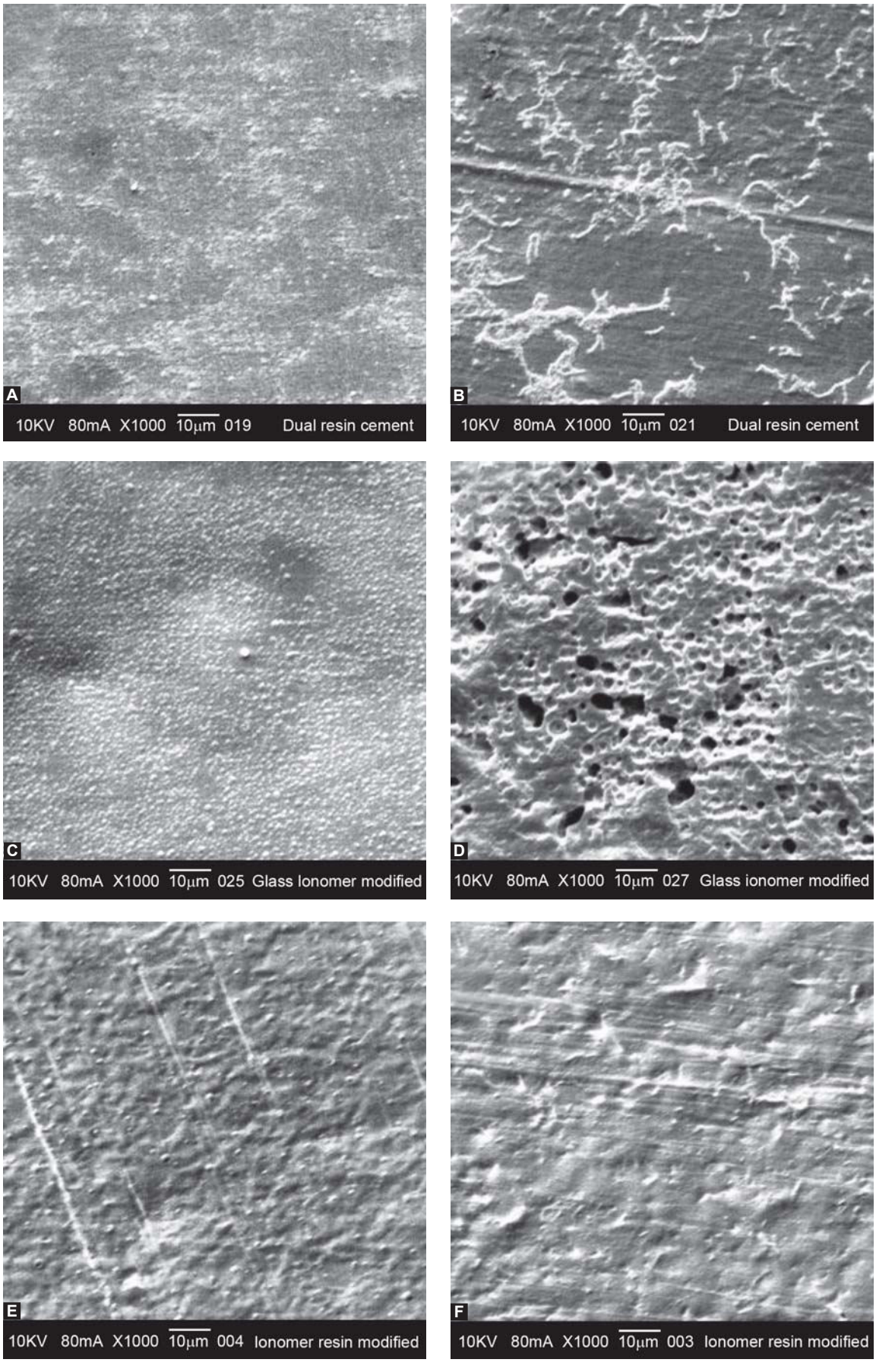
## DISCUSSION

The durability of cemented indirect dental restorations depends above all on the quality of the interface and marginal adaptation. The properties of the cement used are critical. Of the buccal flora, *S. mutans* is the principal producer of acids and is considered to be one of the main etiological agents of dental caries due to its capacity to metabolize sugars, producing the organic acids that cause tooth demineralization.[1,2] The presence of this microorganism can also lead to the erosion of some of the dental materials employed in restorations. Knowledge of the resistance of cementing agents, in terms of their solubility and surface microhardness, is therefore essential to ensure that restoration work is successful.[2,7]

In contact with an acidic medium, water-based cements are the most soluble and hence the most liable to erosion.[4,8] In the present work the zinc phosphate cement was found to be the most soluble, in terms of absolute weight loss, while the glass ionomer cement (Vidrion C) showed no

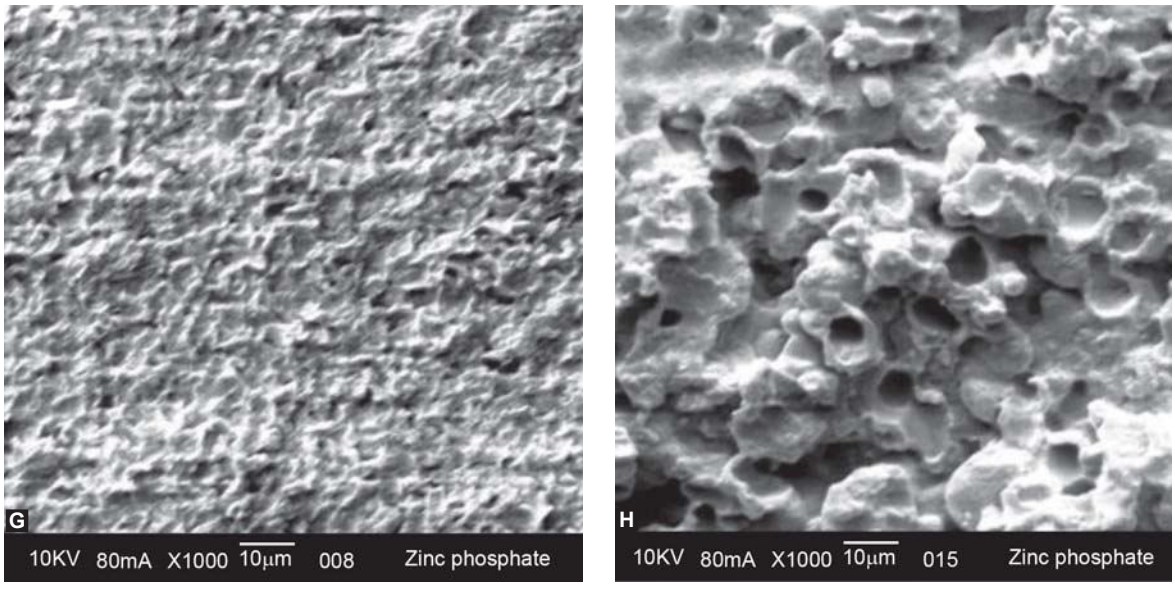
**Table 1:** Weights (gm) of the different specimens (weight ± SD). Means followed by different lower case letters in the same line were statistically different at p<0.05

| *Cement* | *Dry weight* | *Wet weight* | *Weight after exposure and leaching* |
|---|---|---|---|
| Glass ionomer | 0.12360 ± 0.00378 a | 0.123593 ± 0.00365 a | 0.12566 ± 0.00347 a |
| Zinc phosphate | 0.19762 ± 0.00441 a | 0.20232 ± 0.00403 a | 0.18170 ± 0.00515 b |
| Resin-modified glass ionomer | 0.13687 ± 0.00369 a | 0.13758 ± 0.00377 a | 0.13022 ± 0.00416 b |
| Resin cement | 0.12028 ± 0.00462 a | 0.12837 ± 0.00368 b | 0.13794 ± 0.00365 c |


A
10KV 80mA X1000 10μm 019 Dual resin cement
B
10KV 80mA X1000 10μm 021 Dual resin cement
C
10KV 80mA X1000 10μm 025 Glass Ionomer modified
D
10KV 80mA X1000 10μm 027 Glass ionomer modified
E
10KV 80mA X1000 10μm 004 Ionomer resin modified
F
10KV 80mA X1000 10μm 003 Ionomer resin modified

**Figs 1A to F**

*Contd.*




**Figs 1A to H:** SEM analyses of the specimens before (A, C, E and G) and after (B, D, F and H) exposure to media containing *S. mutans* and leaching. (A and B) resin cement; (C and D) glass ionomer; (E and F) resin-modified glass ionomer; (G and H) zinc phosphate

weight change after exposure. There was a significant change in the weight of the resin cement (Dual Cement) after exposure; however, in this case it was a weight gain rather than a weight loss. This can be explained by the insolubility of this cement in the incubation medium, together with the fact that resinous materials have the capacity to absorb water.[9] The resin-modified glass ionomer cement (Vitremer) showed significant weight loss after exposure and leaching, albeit to a lesser extent than that found for the zinc phosphate cement. This could be related to the occurrence of acid/base reactions during the curing process, with the amount of resin present in the material being insufficient either to render it insoluble or to permit any increase in mass.

Glass ionomers exhibit antimicrobial activity, due to the release of fluorine.[10] This was confirmed by other research,[11] who measured the halo of bacterial growth inhibition in a solid medium. However, in the present work, a liquid medium was used, so that there was no spatial restriction on microbial growth with bacteria able to populate the entire volume of the liquid. The hydrogen cations responsible for the solubilization of the surfaces of ceramic materials were also dispersed throughout the entire volume. The microorganism studied is acid-forming,[12] as demonstrated by the measured decrease of pH during the incubations. Since the glass ionomer cement contained fluorine, it was conceivable that the fluoride ions dispersed throughout the liquid medium might be able to retard bacterial growth. However, if this had occurred, the pH of the medium would not have reduced to the same extent as that observed in the presence of the other materials. The fact that the same pH behavior was observed for all four materials indicated that the presence of the glass ionomer did not adversely affect bacterial growth. The presence of fluorine in cements reduces bacterial adhesion, hence inhibiting the development of the microoganisms.[1,13,14] It is for this reason that dentists have tended to use these materials, especially glass ionomers.

Yanikoglu and Yesil[12] reported that cements placed in a pH 7 medium were stable with respect to solubility, while those immersed in an acidic medium showed increased solubility. Zinc phosphate was the most soluble of the cements tested. It was found that prolonged storage in an aqueous medium could also cause solubilization. This was confirmed in the present work, since the control specimens also exhibited surface erosion, even in the absence of acidity (although the solubility increased at lower pH). Similar findings were described by Eisenburger et al.[5]

Resin-based cements can increase in weight after installation due to the incorporation of water molecules into the polymer.[3,9,15] The time after curing of the material influences its solubility, since the polymerization process is progressive, with increasing cross-bonding providing greater polymer stability.[16] The material therefore becomes increasingly less soluble with time. This was demonstrated in the present work, since the resinous cement increased in mass following immersion in aqueous media. The increased microhardness with time of the resinous and resin-modified glass ionomer cements could also be explained by the fact that the polymers formed during the polymerization process incorporate water molecules by means of hydrogen bridges,[6,14,15] altering the composition of the solid substances formed.

The increased microhardness of the resin-containing cements following immersion in aqueous media was in contrast to the behavior shown by the zinc phosphate and glass ionomer cements, for which the microhardness decreased, probably due to increased porosity.[17] When the probe of the microhardness meter contacted the porous surface it penetrated the microcavities produced by erosion. No microcavities were observed on the surfaces of the resin-containing cements, in agreement with Eisenburger et al,[5] who found that such materials are insoluble. Reduced hardness of glass ionomer cements was also reported by Bayindir and Yildiz.[14]

The SEM images provided support for the absence of any solubilization of the resinous cement (Dual Cement), since no surface pores were present. It was also observed that bacterial remnants, in the form of necklace-shaped filaments (Fig. 1B), remained attached to the plane surface after leaching of the specimens, indicating that the water jet was not sufficiently powerful to remove all of the microbial material. The presence of these chains of cocci was probably related to the insolubility of the resinous material and the fact that no sections of the surface were removed during the leaching process. Furthermore, adhesion is greater on resin-based materials due to their higher surface energy, making it more difficult to dislodge bacterial growths.

## CONCLUSION

The results showed that the Knoop microhardness of water-based cementing agents diminished following immersion in a culture medium containing *S. mutans*, while resin-based cements (especially resinous cement) provided the best performance in terms of microhardness. Using weight change experiments, it was found that zinc phosphate cement was the most soluble in the culture medium, and that glass ionomer cement was the most stable in terms of weight change. From a clinical perspective, considering the parameters investigated and the ubiquitous presence of *S. mutans* in the buccal environment, it appears that resin-based cements may be the best option for use in indirect dental restoration work.

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