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Effect of Anti-Halitosis-Mouth Rinses on Surface Properties of Resin Based Restorative Dental Materials

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ABSTRACT

Objectives: This *in-vitro* study aimed to investigate the effect of five different types of mouth rinses used for halitosis on color stability and surface roughness of two types of restorative materials.
Materials and Methods: In this study, a total of 120 disc-shaped samples (10x2mm) were prepared with nano-hybrid resin composite (Filtek Z550) and giomer (Beautifill II) materials. Randomly selected samples were divided into six groups as five different mouth rinses (Listerine Fresh Burst, Listerine Total Care, Colgate Plax, Oderol, Halitosil) and a control group (distilled water). Initial color values were measured by spectrophotometer (Easyshade Compact) according to the CIELAB system, and roughness values (Ra) were measured by contact profilometer (Surtronic 25). All specimens were incubated in mouth rinses at 37°C for 12 hours and measurements were performed in the same procedure. Data were analyzed by using Kruskal-Wallis H and Bonferroni Post Hoc tests with the SPSS 24.0 program at a significance level of 0.05.
Results*:* The results of this study showed that there was not a statistically significant increase in surface roughness values. There was a significant change in CIELAB values in all of the Beautifil II materials after the immersion in mouth rinses. There was a statistically significant difference between the color change values of Beautifil II and Filtek Z550 materials kept in the same mouth rinses. Beautifil II was exhibited color change with values above the clinically acceptable limit (ΔE>3.3).
Conclusions: Color changes occurred in both of the restorative materials kept in different mouth rinses.

Keywords: Halitosis, Mouth Rinses, Resin Composite, Giomer, Surface Roughness.

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Introduction

Halitosis is bad breath from the mouth due to oral or non-oral conditions (Table 1).[1,2] It is usually (80-90%) caused by oral situations such as poor oral hygiene, dental caries, or periodontal disease. The sulfur-containing substrates exist in the different surfaces of the oral cavity, such as the dorsum of the tongue, periodontal pockets, desquamated epithelial cells, serum, or saliva. Gram-negative anaerobic bacteria degrade them, and volatile sulfur compounds are produced.[3-5] Periodontal treatments, mechanical therapies, and mouth rinses are utilizable for controlling chronic halitosis. Antibacterials in mouth rinses target volatile sulfur compound producer bacteria (porphyromonas gingivalis, prevotella intermedia, fusobacterium nucleatum) and reduce their numbers in the oral cavity.[1] Chlorhexidine (CHX), quaternary ammonium compounds (cetylpyridinium chloride (CPC), benzalkonium chloride), triclosan, essential oils, chlorine dioxide, zinc salts, hydrogen peroxide, sodium bicarbonate, amine fluoride/stannous fluoride are antibacterial agents that are used in different concentrations in mouth rinses. Chlorhexidine is a cationic agent that increases bacterial cell membrane permeability. That produces cell lysis and death and thereby reducing VSC production.[6,7] Cetylpyridinium chloride is a cationic quaternary ammonium antiseptic compound that suppresses the expression of specific genes of VSC production in anaerobic periodontal pathogens.[8-10] Triclosan has a broad spectrum of antimicrobial activity against bacteria, especially the gram-negative anaerobic species. Metal ions, such as stannous, mercury, copper and zinc can bind to the sulfur radicals and reduce the expression of the volatile sulfur compounds. Particularly zinc ions in zinc salts have a strong affinity for thiol groups present in the volatile sulfur compounds and converting volatile sulfur compounds to non-volatile sulfides that have low solubility.[7,10-12] In a study comparing the anti-VSC effect of different concentrations of zinc ions (0.1%, 0.3%, 1.0%), chlorhexidine (0.025% and 0.2%) and cetylpyridinium chloride (0.025% and

0.2%); all concentrations of Zn reduced hydrogen sulfide production by 80% during the first hour after use of the solutions. The anti-VSC effect of a 0.2% solution of chlorhexidine increased after the first hour. The anti-VSC effect of 0.2% chlorhexidine after 3 hours was significantly higher than 0.2% cetylpyridinium chloride. In addition, the anti-VSC effect of 0.025% chlorhexidine was significantly higher than the 0.025% cetylpyridinium chloride at the end of all periods. After 3 hours, 0.2% CHX was found to be the most effective agent.[13]

Minimally invasive dentistry is currently the most effective treatment option. Due to the superior aesthetic and mechanical properties of the composites and thanks to the developments in resin composite technology, the preference and use of composites in anterior and posterior teeth are increasing day by day compared to ceramics. Despite all these superior properties, composite restorations may become discolored due to factors; such as lack of oral hygiene, foods, drinks, mouth rinses, which may cause long-term clinical aesthetic failures and shorten the life of the restoration. Color stability in composite materials is related to the chemical properties of the resin matrix and the proportion and properties of the inorganic filler contents. In other words, color change tendency depends on factors such as degree of polymerization, surface characteristics, moisture absorption, diet, and oral hygiene habits.[14,15]

It was reported that the chemical contents and the pH degree of the mouth rinses change the surface properties of the dental restorations. In resin composites, the adsorption of staining agents can cause discoloration due to the increase in the degradation and roughness on the surface.[16-18] The aim of this *in-vitro* study was to evaluate the color and surface roughness changes of recent composite resins when exposed to different anti-halitosis-mouth rinses. The null hypothesis was that there would be statistically significant differences in surface roughness values and color changes values of restorative materials between baseline and after immersion.

Materials and Methods

The properties of the test materials used in this study are shown in Table 2. One hundred twenty disc-shaped specimens of 10 mm in diameter and 2 mm in thickness were prepared in a cylindrical metal mold by the following materials: nano-hybrid resin composite (Filtek Z550, 3M ESPE, St. Paul, MN, USA) and giomer (Beautifill II, Shofu Dental Inc, Kyoto, Japan). A2 shade was chosen as the base color for all materials. The specimens were polymerized with the halogen light-device (Optilux 501, Kerr, West Collins Orange, CA) at a distance of 1 mm for 40 seconds after gently pressing the material between two glass slides to the thickness of 1 mm and a polyester strip (Mylar strip; SS White Co., Philadelphia, PA, USA) for the removing of excess material. All the specimens were incubated in distilled water (Ph 6.55) at 37°C for 24 hours. To mimic clinical conditions and achieve a standardized polished surface 600 (P1200) grit silicon carbide paper (Metaserv SIC Paper, Buehler, Illinois, USA) was applied by polisher machine (Metaserv 250 Grinder/ Polisher, Buehler, Illinois, USA). Silicone finishing polishing discs (Super-Snap Rainbow Technique Kit, Shofu Dental, Kyoto, Japan) were performed for 30 seconds using minimal pressure by a low-speed handpiece at 5000 rpm.

To evaluate surface roughness and the color stability, 60 specimens of each restorative material were randomly divided into six subgroups (n=10) which will be treated with five different types of mouth rinses and distilled water (control group). Baseline surface roughness values (Ra, µm) were measured by using a contact profilometer (Surtronic 25, Taylor-Hobson, Leicester, UK). The cut-off and evaluation lengths of the device were set at 0.25 mm and 1.25 mm, respectively. Three measurements were performed in the center of each sample in different directions and the average value of these three measurements was regarded as the average surface roughness value.[19]

Table 1: Causes of Halitosis1, 2

Oral Conditions	Poor oral hygen, tongue-coating, decreased salivary flow rate (xerostomia), tooth caries, chronic periodontal diseases, oral ulcerations, acute oral infections, oral malignancies, prothetic restorations, bacterial retention areas in oral cavity.
Non- Oral (Systemic) Conditions	-Respiratory system diseases (respiratory tract infections, sinusitis, cleft palate, tonsilloliths, foreign bodies, tonsillitis, lung infections, bronchitis, malignancies), -Gastrointestinal diseases (duodenal obstruction, hypertrophic *pyloric stenosis*, hiatal hernia, gastroesophageal reflux disease, achalasia, *Helicobacter pylori* infection, gastric ulcers), -Hepatic diseases, -Hematological diseases, -Leukemia, -Renal diseases, -Endocrine system disorders (diabetic ketoacidosis) -Menstruation -Metabolic disorders (trimethylaminuria and hypermethioninemia).
Others	Sulfur compounds producer dietary products, drugs, alcohol, tobacco, psychogenic factors (halitophobia).

Table 2: The properties of the test materials.*

a. Mouth Rinses

Product Name	Brand	Contents
Listerine Fresh Burst (LFB) (Ph: 4.88) Alcohol Content: 26%)	Johnson & Johnson Limited, Maidenhead, UK, SL6 3UG. Johnson & Johnson (Ireland) Limited, Airton Road, Tallaght, Dublin 24, Ireland.	[PR-009045], Aqua, Alcohol, Sorbitol, Poloxamer 407, Benzoic Acid, Sodium Saccharin, Eucalyptol, Methyl Salicylate, Aroma, Thymol, Menthol, Sodium Benzoate, CI 47005, CI 42053
Listerine Total Care (LTC) (Ph: 3.43 Alcohol Content: 22%)	Johnson & Johnson Limited, Maidenhead, UK, SL6 3UG. Johnson & Johnson (Ireland) Limited, Airton Road, Tallaght, Dublin 24, Ireland.	[PR-017429], Aqua, Alcohol, Sorbitol, Poloxamer 407, Benzoic Acid, Zinc Chloride, Eucalyptol, Aroma, Sodium Saccharin, Methyl Salicylate, Thymol, Menthol, Sodium Fluoride, Sodium Benzoate, Sucralose, Propylene Alcohol, CI 16035, CI 42090, Contains Sodium Fluoride (220 ppm F)
Colgate Plax (CP) (Ph: 6.05) Alcohol Content: 7.2%)	Colgate-Palmolive Company Limited, Krung Thep Maha Nakhon 10110, Thailand	Aqua, glycerin, Propylene Glycol, Sorbitol, Poloxamer 407, Aroma, Cetylpyridinium Chloride, Potassium Sorbate, Sodium Flouride (225 ppm), Sodium Saccharin, Menthol, Cl 42051.
Oderol (O) (Ph: 5.6 No Alcohol Content)	Helba Ilac Ic Dis San. Tic. A.S. Serifali Mh. Kule Sk. No: 27/5 Umraniye, Istanbul, Türkiye	Chlorhexidine Digluconate 0.025%, Zinc Lactate, Mentha Piperita, Sucralose, Deionized Water.
Halitosil (H) Zn (Pharmol) (Ph: 5.84 No Alcohol Content)	IMK Farma, Tarabya Mh, Aydınevler Bostan Sk. No: 15/1A- 1A Sarıyer, Istanbul, Türkiye.	Aqua, Glycerin, Zinc Chloride, Sodium Chloride, Boric Acid, Potassium Sorbate, Sodium Benzoate, CL 42090

b.Composites

Product Name	Brand	Organic Matrix	Inorganic Fillers	Classification
Filtek Z550	3M ESPE, St.Paul, MN, USA	Bis- GMA, UDMA, Bis-EMA, TEGDMA, PEGDMA	Surface-modified zirconia/silica fillers, non-agglomerated/non-aggregated surface modified silica particles. 81.8%(wt), 68%(vol) (Particle size: 3µm- 20nm)	Nano-hybrid
Beautifil II	Shofu Dental Inc, Kyoto, Japan	Bis-GMA, TEGDMA	S-PRG filler, multi-functional glass, fluoroboraluminosilicate glass. (Particle size: 0.01 µm–4µm) 83.3% (wt), 68.6%(vol)	Giomer

Bis-GMA= bisphenol A glycol dimethacrylate; UDMA= urethane dimethacrylate; PEGDMA= polyethylene glycol di-methacrylate; TEGDMA= triethylene glycol dimethacrylate; Bis-EMA= ethoxylated bisphenol A glycol di-methacrylate; S-PRG= surface reaction type pre-reacted glass-ionomer
*Data obtained from manufacturers.

Baseline color coordinates of specimens were measured with a spectrophotometer (Easyshade Compact, VITA Zahnfabrik, Bad Säckingen, Germany) according to CIELAB system which was introduced by the Commission Internationle d'Éclairage (CIE) in 1976.[28] CIELAB provides representation of a color stimulus by dimensions of lightness, chroma, and hue and produce a three-dimensional color space where the a*(red-green), b*(yellow/blue) axes form one plane to which the L *(White/black) axis is orthogonal. Color changes (ΔE*) were calculated by the followed formulation: [19,20]

$$\Delta E^*_{ab}=[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$$

The spectrophotometer was calibrated using the calibration block according to the manufacturer's instructions. Probe tip for measurement was placed facing and perpendicular to the centers of the sample surfaces and measurement repeated three times for each sample. After initial measurements all specimens were immersed distilled water and mouth rinses at 37°C for 12 hours. Then color and surfaces roughness measurements were performed in the same procedure. All the measurements were obtained by single operator.

Statistical Analysis

Data were analyzed with the SPSS (IBM SPSS for Windows, Ver.24) program at the significance level of 0.05 (α). The Shapiro-Wilk test ($n < 50$) was used to determine whether the mean values were normally distributed. Non-parametric tests were performed because the measured values of some of the groups of variables were not normally distributed. Kruskal-Wallis H test was used to compare the measurements according to the groups, and Bonferroni Post Hoc (multiple comparison) test was used to determine the different groups that were significant.

Table 3. Means and standart deviations for surface roughness values (Ra in µm) of the tested restorative materials in different mouth rinses.

Tested Materials	Filtek Z550	Beautifil II
Base	0.14(0.15)	0.16(0.01)
After Halitosil	0.16(0.02)	0.17(0.02)
Base	0.16(0.03)	0.15(0.02)
After Oderol	0.17(0.03)	0.17(0.02)
Base	0.15(0.03)	0.15(0.02)
After Colgate Plax	0.17(0.02)	0.17(0.02)
Base	0.17(0.02)	0.16(0.01)
After Listerine Fresh Burst	0.18(0.01)	0.17(0.02)
Base	0.15(0.04)	0.16(0.02)
After Listerine Total Care	0.19(0.05)	0.21(0.03)

No statistical differences between the groups (p>0.05).

Table 4. Means and standart deviations for color changes (ΔE) of the tested restorative materials in different mouth rinses.

Tested Materials	Listerine Total Care (N=10)	Halitosil (N=10)	Oderol (N=10)	Colgate Plax (N=10)	Listerine Fresh Burst (N=10)	Control Group (Distilled Water) (N=10)
Filtek Z550	1.92(1.01)^{aA}	1.30(0.42)^{abA}	1.40(0.93)^{abA}	1.66(0.60)^{abA}	1.84(1.90)^{aA}	0.60(0.20)^{bA}
Beautifil II	6.47*(1.88)^{aB}	5.26*(1.46)^{bB}	5.79*(0.63)^{abB}	4.15*(0.89)^{cB}	6.24*(1.37)^{abB}	0.78(0.29)^{dA}

*Clinically acceptable color change value is below ΔE * = 3.3.; a, b, c: Shows the difference between groups in same row; A, B, C: Shows the difference between groups in same column; (according to Bonferroni Post hoc comparison test, p<0.05).

Results

As a result of the Kruskal-Wallis H analysis, p<0.05 was accepted as significance level. The average surface roughness values obtained before and after immersion in mouth rinses of the composite materials are given in Table 3. Initial average roughness values of composites; Ra(µm)=0.167(±0.01) for Filtek Z550 and Ra(µm)=0.161(±0.01) for Beautifil II. When the surface roughness values obtained before and after immersion in the mouth rinses were compared, no statistically significant difference was found in Filtek Z550 or Beautifil II (p>0.05). Listerine Total Care was produced the highest surface roughness change for the Z550 (ΔRa=0.041µm) and Beautifill II (ΔRa=0.052µm).

Means and standard deviations for color changes (ΔE) of the tested materials are given in Table 4. It was found that the color changes after immersing in the mouth rinses were statistically significant between Filtek Z550 and Beautifil II groups (p<0.001). For Filtek Z550 there was a significant difference between control groups and LTC groups; control groups and LFB groups (p<0.05). For Beautifil II statistically significant differences were found between the control and all the mouth rinse groups (p<0.05). The highest ΔE values were consistently observed in the Beautifil II composite regardless of the mouth rinses used. The lowest color change values for both materials were found in control groups (distilled water). The color change values obtained for Beautifil II were from highest to lowest respectively, Listerine Total Care, Listerine Fresh Burst, Oderol, Halitosil and Colgate Plax. For Filtek Z550 composite; the highest color change values were obtained in Listerin Total Care and Listerin Fresh Burst mouth rinses, respectively.

Discussion

This *in-vitro* study evaluated the effect of anti-halitosis-mouth rinses on color stability and surface roughness of nano-hybrid and giomer restorative materials for a period of 12 hours. It was stated that the immersing time of 12 hours is equivalent to 2 minutes per day/total of 1-year mouth rinse usage.[21,22] The null hypothesis was that there would be statistically significant differences in surface roughness and color changes values of composites between baseline and after immersion, partially accepted. Mouth rinses produced a significant color change but did not cause a statistically significant increase in surface roughness values.

In this study there was no statistically significant difference was found in surface roughness values for Filtek Z550 or Beautifil II materials after immersion. In terms of microbiologically, the acceptable surface roughness limit for restorative materials is considered to be 0.2µm[23] and the surface roughness values obtained in this study did not exceed this limit. The researchers stated that the surface characteristics of the materials affect the optical properties, and the increasing surface roughness changes the color coordinates.[24,25] In our study, similar to the Celik *et al.*[26], although there was no significant change in surface roughness, statistically significant color change occurred in Filtek Z550 samples kept in LTC and LFB, and in all samples of Beautifill II kept in mouth rinses.

Listerine Total Care has the highest alcohol content after LFB and the lowest pH among all. Almeida *et al.*[27] indicated that the low pH of mouth rinses may cause catalysis of ester groups from dimethacrylate monomers in the resin matrix composition. Hydrolysis of these ester groups would result in the release of alcohol and carboxylic acid which may increase the degradation of the resin composite. It was stated that alcohol, which was a dimetacrilate solvent causes plasticization in the resin matrix and therefore softens and accelerates degradation and discoloration.[27,28] This degradation process in the structure of the material also causes an increase in surface roughness.[29] In this study, similar to the Cengiz *et al.*[18] the mouth rinse with the lowest pH (LTC) was produced the highest surface roughness change (ΔRa) for both of the restorative materials.

The color change of materials is one of the factors that shorten the life of the resin composite restorations, and it is a reason for the restoration repair or change, especially in the anterior region. Vichi *et al*.[30] stated that the color change threshold that the human eye can perceive is ΔE * = 1 and the clinically acceptable color change value is below ΔE * = 3.3. In our study, the most color change in Filtek Z550 material occurred in LTC and LFB groups, but it was below the clinically acceptable limit. In Beautifill II, all ΔE * values exceeded the clinically acceptable limit and there was a statistically significant difference between the ΔE* values of Filtek Z550 and Beautifil II, after immersion of mouth rinses. In previous studies investigating color stability, fluoride in materials such as Beautifil II, a giomer, has been reported to significantly increase water absorption and color change (ΔE) values due to its water solubility.[31,32]

Festuccia *et al.*[33] reported that the color stability of resin composite materials was related to monomer conversion degree and water absorption tendency of polymer matrix. Filtek Z550 contains Bis-GMA, UDMA, Bis-EMA, TEGDMA, PEGDMA, and Beautifil II contains Bis-GMA, TEGDMA monomers in the matrix structure. Bis-GMA (bisphenol A-glycerolate dimethacrylate) is a methacrylate with a high viscosity provided by the hydroxyl groups and the aromatic core. [34,35] To reduce the disadvantages of Bis-GMA such as high viscosity, low mobility, and to increase the degree of conversion, it is combined with diluting high mobility bifunctional co-monomers such as TEGDMA (triethylene glycol dimethacrylate), EGDMA (ethylene glycol dimethacrylate), UDMA (urethane dimethacrylate) and BisEMA (ethoxylated bisphenol A dimethacrylate).[34-37] Bis-EMA monomer, which is the ethoxylated form of Bis-GMA, is less hydrophilic and less viscosity due to lack of secondary functional hydroxyl groups. Longer ethylene glycol spacer produces higher flexural strength and higher mobility. Because of it's higher viscosity, Bis-GMA reaches the gelation point earlier in the polymerization reaction, which leads to a lower degree of conversion than Bis-EMA based materials. [37-39] Low viscosity monomer UDMA presents an aliphatic urethane chain that high flexibility, higher flexural strength, elastic modulus, and hardness.[35,37] UDMA monomers were significantly more reactive and has higher conversion rates than Bis-GMA monomers.[40] TEGDMA is a highly flexible monomer, it has a low molecular weight, low viscosity, and high mobility during polymerization and this reason it has great monomer conversion rates. However, due to its hydrophilic properties, it increases the water absorption of the structure, which accelerates degradation, decreases the mechanical properties and negatively affects the color stability.[34,37] In a study Fonseca *et al.*[35] investigated the degree of conversion, water sorption and optical properties of experimental dental composites composed of BisGMA, BisEMA, BisEMA 30, and two UDMA-based monomers, that mixtured with TEGDMA. BisEMA mixed with TEGDMA presented the synergistic effect with TEGDMA and had the best performance in terms of all the parameters tested. Ranking of the conventional base monomers for color stability was BisEMA>UDMA>BisGMA. This informations may explain that the color stability of the Filtek Z550, which contains UDMA, Bis-EMA monomers in the matrix structure, was better than Beautifil II.

Filtek Z550 is a nanohybrid resin composite with surface-modified zirconia/silica fillers in its structure[19]. Beautifil II is a giomer material that provides fluoride-releasing through the S-PRG fillers (surface pre-reacted glass filler particles) it contains. The water absorption properties of resin-based restorative materials affect the amount of coloring agents entering the resin matrix and the color change values[16]. Gonluol *et al*.[32] (2015) also stated in their study that Beautifill II has higher solubility and water absorption properties than Filtek Z550 and other composite types. In addition, in the same study[32], similar to our study, a statistically significantly more color change occurred in Beautifil II than in Filtek Z550. On the other hand, Park *et al.*[31](2007) stated that in materials with fluoride release, the place of the filler dissolved from the surface is covered with water, and this causes hole formation and softening on the surface. However, no significant difference was found in the surface roughness values in our study, and the microhardness parameter was not tested either. For these reasons, it is necessary to carry out additional studies examining the color change, surface roughness and microhardness values together.

Chlorhexidine, zinc lactate, zinc chloride, stannous fluoride, essential oils (eucalyptol, menthol and methyl salicylate) and cetylpyridinium chloride are the most commonly used anti-microbial agents in anti-halitosis effective mouth rinses.[8,41] Chlorhexidine is a broad spectrum and most preferred cationic agent, but it has side effects such as calculus formation, altered taste perception, and yellow-brown extrinsic staining.[13,42] These discolorations are formed by processes such as protein denaturation, which leads to the formation of metal sulfide, or the Maillard reaction, which creates melanoid substances that cause brown staining.[41] Like Chlorhexidine, cetylpyridinium chloride is a broad-spectrum cationic agent with side effects such as ulceration, burning sensation and discoloration.[13] The most important side effect of zinc lactate or zinc chloride is that it's metallic taste. Stannous fluoride has a discoloration feature due to the Sn it contains.[8,13]

In this study, the most color change was expected from Oderol that containing chlorhexidine digluconate 0.025% and Colgate Plax that containing cetylpyridinium chloride. But the mouth rinses that produced the statistical significant color change in Filtek Z550 material were LTC and LFB. And the mouth rinses that produced the highest color change in Beautifil II material were Listerine Total

Care, Listerine Fresh Burst, Oderol, Halitosil and Colgate plax respectively. This could be explained by lower pH value and higher alcohol content than other mouth rinses. In previous studies [43,44], it was stated that mouthwashes containing alcohol showed better results in wound healing and plaque control. In our study, the chosen alcohol-containing mouthwashes are the most widely used mouthwashes for the relief of halitosis in the market. But Aydın *et al.*[45] stated that alcohol is not necessary in halitosis mouth rinses as it dries the oral mucosa and exacerbates bad breath and has no effect on sulfur gases. Almeida *et al.*[27] stated that alcohol-free mouth rinses presented a similar effectiveness on plaque control and gingival inflammation reduction compared to mouth rinses containing alcohol.

Conclusions

In this study, immersion of mouth rinses for 12h had no significant effect on the surface roughness values. Immersion of Listerine Total Care and Listerine Fresh Burst mouth rinses had a significant effect on the color changes in the Beautifil II and Filtek Z550 specimens. Beautifil II showed higher discolorations than Filtek Z550. Clinicians should consider the effects of pH degrees and alcohol contents of mouth rinses used for halitosis on restorative materials.

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Conflicts of Interest Statement

The authors declare no conflict of interest in this study.

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