

The effect of simulated food liquids on the surface structure and solubility of various esthetic restorations

DAbdurrahman Yalçın¹, DElif Pınar Bakır², DŞeyhmus Bakır²

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ABSTRACT

Aims: The aim of this study is to investigate the surface roughness and solubility of restorative materials when exposed to foodstuffs in the oral environment using simulated food liquids as defined by the Food and Drug Administration.

Methods: In this study, a total of four esthetic restorative materials were used: one universal compomer (Dyract XP, Dentsply), one conventional microhybrid composite (FiltekTM Z250, 3M ESPE), one nanofilled, and one high-viscosity glass ionomer cement (ChemFil Rock, Dentsply). A total of 160 samples, each 8 mm in diameter and 2 mm in thickness, were prepared using molds. The initial weights of the samples were recorded in micrograms using a precision balance to determine solubility values. Initial surface roughness values were measured using an atomic force microscope device. The samples were immersed in four different simulated food liquids (ethanol, heptane, citric acid, and distilled water) for a period of 7 days. After removal from the solutions, the samples were desiccated to a constant weight, and the second set of weights was recorded. Subsequently, the second surface roughness values were measured

Results: Among the materials immersed in the simulated food solutions, ChemFil Rock exhibited the highest solubility and increase in surface roughness. Citric acid was found to be the solution that caused the highest increase in surface roughness values and solubility for this material (p<0.005). It was observed that Dyract XP was more affected by heptane solution, while Filtek Z-250 and G-aenial anterior materials were more affected by ethanol.

Conclusion: All the restorative materials used in our study were found to be affected by simulated food liquids to varying degrees in terms of surface roughness and solubility.

Keywords: Esthetic restorative materials, simulated food liquids, surface roughness, solubility

INTRODUCTION

With the growing importance of aesthetic applications in dentistry, there is increasing interest in restorative materials that mimic the natural structure of teeth. Composite resins, which offer a variety of color options, ease of use, and many advantages, are the most preferred esthetic restorative materials. Glass ionomer cements (GIC), which can chemically bond to dental tissues and have anticariogenic properties, high-viscosity glass ionomer cements (HVGIC) with improved compressive strength and wear resistance, and polyacid-modified composite resins (compomers), frequently preferred especially for pediatric primary teeth, are also commonly used restorative materials.

Restorative materials used in the oral cavity are exposed to various chemical substances and mechanical forces over time. As a result, these materials may exhibit surface roughness, bulk discoloration, or chemical dissolution, leading to compromised marginal integrity. Mechanical forces can cause cracks and fractures in the material, while exposure to

chemical substances can result in aging, degradation of the surface structure, and dissolution.² Studies have shown that immersing restorative materials in simulated food solutions can produce adverse effects similar to those observed in the oral environment over the long term, such as microleakage, discoloration, surface wear and roughness, and reduced surface hardness. Among the solutions most commonly used in studies and defined by the FDA are heptane, ethanol, citric acid, and distilled water. Ethanol simulates carbohydrate-containing foods,³ heptane simulates vegetable and animal fats,^{4,5} citric acid simulates acids found in beverages or foods or acids resulting from food fermentation, and distilled water simulates the environment created by saliva and water in the oral cavity.⁶

The increase in surface roughness resulting from the abrasion of restorative materials leads to greater plaque accumulation over time. The fermentative products within the plaque contribute to further dissolution of the

Corresponding Author: Abdurrahman Yalçın, arahmanylcn@gmail.com



¹Department of Restorative Dentistry, Faculty of Dentistry, Batman University, Batman, Turkiye

²Department of Restorative Dentistry, Faculty of Dentistry, Dicle University, Diyarbakır, Turkiye

restoration and cause secondary caries due to the disruption of the tooth-restoration interface. Additionally, accumulated plaque can eventually lead to calculus formation, causing periodontal problems. Furthermore, restorations below the gum line are continuously exposed to gingival crevicular fluid, which results in water absorption, increased solubility, and marginal discoloration. Determining the solubility and roughness values of restorative materials against simulated food liquids is crucial for understanding their behavior in the oral environment. Solubility, in particular, is an important parameter for predicting the longevity of restorations and provides insight into their compatibility with biological structures in relation to individuals' dietary habits. 8-10

The aim of our study is to examine the changes in surface roughness and solubility values of four different tooth-colored restorative materials used in dentistry when exposed to different simulated food liquids without any mechanical forces. Our null hypotheses are: Esthetic restorative materials can maintain surface roughness when exposed to food substances. Esthetic restorative materials do not dissolve in simulated food liquids.

METHODS

In this study, four different tooth-colored restorative materials were used: a universal compomer resin, Dyract XP (Dentsply); a conventional microhybrid-based composite resin, FiltekTM Z250 (3M ESPE); a nanohybrid composite resin, G-Aenial Anterior (GC); and a high-viscosity glass ionomer cement, ChemFill Rock (Dentsply) (Table 1). Ethics committee approval is not required for this study. All procedures were carried out in accordance with the ethical rules and the principles.

The solutions used in our study were simulated food liquids defined by the FDA: ethanol (Teksoll 96% ethyl alcohol+2-propanol, Tekkim Chemical Industry Trade. Ltd. Sty.), heptane (Tekkim Chemical Industry Trade. Ltd. Sty.), and 10% citric acid (Norateks) solutions, along with distilled water (deionized water) as the control group.

Sample Preparation

In our study, all samples were prepared by a single operator to ensure stabilization. Restorative materials were placed into plastic molds with a depth of 2 mm and a diameter of 8 mm using an oral applicator, covered with a transparent strip, and polymerized with a light-curing device. The top and bottom surfaces of each sample were polymerized for 20 seconds each using a Light Emitting Diode (LED) light device (Woodpecker Led-G, China). The top surface of each sample underwent polishing and finishing using Sof-lex disks impregnated with aluminum oxide (3M ESPE, St. Paul, USA). During the polishing process, each sample was polished for 20 seconds per disk, moving from the coarse to the fine-grit disk, and a new disk was used for each sample. The samples were rinsed with water for 10 seconds and air-dried for 5 seconds to remove any debris. After finishing and polishing, all samples were cleaned using an ultrasonic device for 15 minutes.

Calculation of Water Solubility and Determination of Surface Roughness

To determine the water solubility levels of the samples, the standard formula specified in ISO 4049:2009a was used. To prevent mixing of test samples, pre-numbered experimental samples were placed in a desiccator containing silica gel (EN025, Nüve, Turkiye). All samples were kept in the desiccator at 37°C in an oven (Mikrotest mst 55 oven, Turkiye) for 22 hours and then maintained at 24°C for 2 hours. The dry weights of the samples were measured using a precision balance with an accuracy of 0.0001 g (Precisa XB 220A, Zurich, Switzerland). The process was repeated 24 hours later to determine the final weights of the samples, ensuring that the sample weights did not change by more than 0.1 mg. The initial weights of all samples with weight loss of less than 0.1 mg (stabilized weights) were recorded in micrograms (µg). The diameter and thickness of each stabilized sample were measured with a caliper (Jensen JP-1 jku 010, Germany) and their averages were calculated. Thus, the diameter in mm² and the average thickness in mm³ for each sample were determined ($V=\pi r^2h$).

Table 1. Materials used in the research and their contents								
Materials	Type	Lot No	Content					
Dyract XP (Dentsply, Konstanz, Germany)	Traditional compomer	2103000371	UDMA, TCB, TEGDMA, TMPTMA, camphoroquinone, ethyl-4 (dimethylamino) benzoate, butylated hydroxytoluene (BHT), strontium-alumino-sodiumfluoro-phosphorus silicate glass, strontium fluoride, glass particles (0.8μm), iron oxide and titanium oxide pigments					
Filtek™ Z250 (3M ESPE, St. Pau, MN, ABD)	Composite (microhybrid)	NC45379	Contains bis-GMA, UDMA, bis-EMA, zirconia and silica. The filler ratio by weight is 82% and the filler ratio by volume is 60%.					
G-aenial anterior (GC Corporation Tokyo, Japan)	Composite (nanohybrid)	2012252	Combination of two types of prepolymerized fillers developed in 16-17 µm size. The filler ratio by weight is 76% and the filler ratio by volume is 62%. It does not contain UDMA Dimethacrylate comonomers, strontium, Silica, BisGMA.					
ChemFil Rock Dentsplay Sirona, Kontstantz, Germany*	High viscosity glass ionomer cement	2009000134z	Calcium-aluminiumzinc-fluoro-phosphorus silicate glass, polycarboxylic acid, iron oxide pigments, titanium oxide pigments, tartaric acid, water					

Surface roughness measurements of the prepared samples were performed at the Dicle University Scientific Research and Technology Center (DÜBTAM) using an AFM device (XE-100E atomic force microscopy, Park Systems, South Korea). Prior to conducting surface roughness measurements for each sample, the AFM device was calibrated. Measurements were taken at three different points of 20x20 µm at a speed of 0.2 Hz for each sample. Surface images were obtained at a resolution of 256x256 pixels, and initial surface roughness values were recorded numerically as Ra (nm) values. After recording the initial weights and surface roughness measurements, samples prepared with different esthetic materials were placed in tubes containing various storage solutions and kept in an oven at 37°C for 7 days. On the seventh day, the samples were removed from the solutions and placed in a desiccator for 24 hours to regain their constant mass weights. Subsequently, the solubility values were calculated.

The final surface roughness values of the samples, whose water solubility levels were determined, were measured using the AFM device as previously described and recorded in μm .

Statistical Analysis

The data obtained in our study were analyzed using the licensed IBM SPSS 21 software package. The Shapiro-Wilk test was used to assess the normality of the data distribution due to the sample sizes. When the variables did not follow a normal distribution, the Kruskal-Wallis h test was employed to examine differences between groups. In comparisons involving more than two groups, the Bonferroni-corrected Mann-Whitney U test was used to identify groups with significant differences. For within-group comparisons, the Wilcoxon test was applied when the variables did not follow a normal distribution.

RESULTS

Surface Roughness

In the ethanol group, the surface roughness T1 value of the Dyract XP group was significantly lower than that of the GC and ChemFil Rock groups, while the surface roughness T1 value of the Z-250 group was significantly lower than that of the ChemFil Rock group. In the heptane group, the surface roughness T1 value of the Z-250 group was significantly lower than that of the GC and ChemFil Rock groups, while the surface roughness T1 value of the Dyract XP group was significantly lower than that of the ChemFil Rock group. In the citric acid group, the surface roughness T1 values of the Dyract XP and GC groups were significantly lower than those of the Z-250 and ChemFil Rock groups. In the distilled water group, the surface roughness T1 value of the Dyract XP group was significantly lower than those of the Z-250 and ChemFil Rock groups, while the surface roughness T1 value of the GC group was significantly lower than that of the Z-250 group. In the Dyract XP group, the surface roughness T0 value in the distilled water group was significantly lower than in the ethanol and heptane groups, while the surface roughness T0 value in the citric acid group was significantly lower than in the heptane group. In the Z-250 group, the surface roughness To value in the heptane group was significantly lower than in the citric acid and distilled water groups, while the surface roughness To value in the ethanol group was significantly lower than in the distilled water group. In the GC group, the surface roughness To value in the citric acid group was significantly lower than in the ethanol and heptane groups, while the surface roughness To value in the distilled water group was significantly lower than in the heptane group. In the ChemFil Rock group, the surface roughness To value in the ethanol group was significantly lower than in the heptane and citric acid groups, while the surface roughness To value in the distilled water group was significantly lower than in the citric acid group (Table 2).

Among all the materials, the highest statistically significant surface roughness values were observed in the ChemFill Rock material. Compared to the other materials, its T1 values were significantly higher (p<0.05).

The surface topographies of the materials before placing them in different solutions and after seven days of immersion are shown in Figures 1, 2, 3, and 4.

Solubility

In the ethanol, heptane, and distilled water groups, the solubility values of the Dyract XP, Z-250, and GC groups are significantly lower than those of the ChemFil Rock group. In the citric acid group, the solubility value of the GC group is significantly lower than that of the Dyract XP and ChemFil Rock groups; the solubility value of the Z-250 group is significantly lower than that of the ChemFil Rock group. In the Dyract XP group, the solubility value of the distilled water group is significantly lower than that of the ethanol and citric acid groups; the solubility value of the heptane group is significantly lower than that of the citric acid group. In the Z-250 group, the solubility value of the distilled water group is significantly lower than that of the ethanol and citric acid groups. In the GC group, the solubility values of the citric acid and distilled water groups are significantly lower than those of the ethanol and heptane groups. In the ChemFil Rock group, the solubility value of the distilled water group is significantly lower than that of the ethanol and citric acid groups; the solubility value of the heptane group is significantly lower than that of the citric acid group (Table 3).

DISCUSSION

The successful clinical performance of resin-based restorative materials is dependent on their long-term durability against the physical and chemical impacts they encounter. Composite resins, compomers, and glass ionomer cements, as aesthetic restorative materials, are subjected to chemical substances present in saliva, microbial flora, food, and beverages, as well as pH fluctuations and thermal changes resulting from their consumption. The corrosion process, beginning with the leaching of fixed chemicals and water absorption on the resin surface, weakens the bonds between monomers, damages the matrix structure, and thus leads to surface roughness in restorations. Additionally, these effects may result in the separation of the restoration from the tooth tissue due to the dissolution of the filler content. In our study, the surface

Table 2. Surface roughness values and statistical results of the materials used according to the solutions								
			Surface roughness T0	Surface roughness T1	Kruskal Wallis h Test			
Aesthetic Materials	Solutions that mimic foods	n	Mean±SD	Mean±SD	p			
	Ethanol	10	0.026±0.001	0.032±0.001	0.001			
	Heptane	10	0.043±0.002	0.057±0.002				
Dyract XP	Citric acid	10	0.014±0.001	0.029±0.002				
	Distilled water	10	0.012±0.001	0.013±0.001				
	Total	40	0.024±0.013	0.033±0.016				
Z-250	Ethanol	10	0.046 ± 0.002	0.059 ± 0.004	0.001			
	Heptane	10	0.029±0.002	0.037±0.002				
	Citric acid	10	0.069 ± 0.003	0.078 ± 0.003				
	Distilled water	10	0.086±0.003	0.086±0.003				
	Total	Total 40 0.057±0.022 0.065±0.019		0.065±0.019				
G-aenial	Ethanol	10	0.054±0.001	0.062±0.001				
	Heptane	10	0.077±0.001	0.082±0.001				
	Citric acid	10	0.021±0.001	0.029±0.001	0.001			
	Distilled water	10	0.048 ± 0.002	$0.048 {\pm} 0.002$				
	Total	40	0.05±0.02	0.055±0.02				
ChemFill	Ethanol	10	0.035±0.003	0.083±0.008				
	Heptane	10	0.066±0.003	0.097±0.005				
	Citric acid	10	0.093±0.006	8±8	0.001			
	Distilled water		0.062±0.005	0.073±0.005				
	Total	40	0.064±0.021	2.063±3.471				

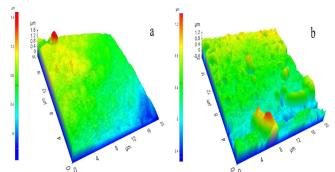


Figure 1. AFM images of Dyract XP before (A) and after (B) immersion in citric acid

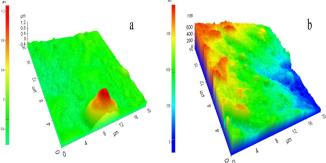


Figure 3. AFM images of Z-250 before (A) and after (B) immersion in heptane $\,$

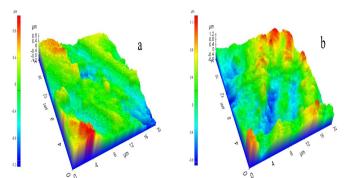


Figure 2. AFM images of ChemFill Rock before (A) and after (B) immersion in heptane

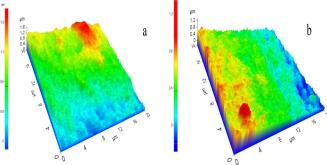


Figure 4. AFM images of G-aenial anterior before and after immersion in ethanol

Table 3. Solubility values a	nd statistical results of the	materials u	sed according to	the solutions			
		Resolution value					Kruskal Wallis h test
		n	Mean	Min	Max	SD	p
Dyract XP	Ethanol	10	5.862	2.98	7.95	1.958	0.001
	Heptane	10	3.974	0.99	5.96	1.754	
	Citric acid	10	17.9	12.93	22.87	3.443	
	Distilled water	10	0.99	0	1.98	0.933	
	Total	40	7.182	0	22.87	6.849	
	Ethanol	10	8.451	3.97	16.91	3.581	0.001
	Heptane	10	4.669	1.98	8.95	1.993	
Z-250	Citric acid	10	7.653	3.97	11.93	2.528	
	Distilled water	10	0.693	0	1.98	0.815	
	Total	40	5.367	0	16.91	3.874	
G-aenial	Ethanol	10	6.261	3.97	7.95	1.331	0.001
	Heptane	10	5.663	0.99	7.95	2.046	
	Citric acid	10	1.188	0	1.98	0.626	
	Distilled water	10	0.099	0	0.99	0.313	
	Total	40	3.303	0	7.95	2.99	
ChemFill	Ethanol	10	33.717	19.89	50.73	9.026	0.001
	Heptane	10	18.199	9.94	27.85	6.516	
	Citric acid	10	599.816	509.3	642.59	45.14	
	Distilled water	10	13.922	7.95	19.89	3.572	
	Total	40	166.413	7.95	642.59	254.51	

roughness increased in the food mimic solutions, leading to the rejection of our first null hypothesis. Furthermore, all materials dissolved in the food mimic liquids, leading to the rejection of our second null hypothesis.

In many in vitro studies simulating the oral environment, various food mimic liquids, such as ethanol, heptane, citric acid, and distilled water, defined by the FDA, have been used to replicate the effects of the complex chemical composition of the oral cavity on different resin-based restorative materials. Resin-based restorative materials exhibit physical changes when exposed to alcohol and other beverages, fruits, and fatty foods, which can also be replicated under in vitro conditions using ethanol, heptane, citric acid, and distilled water. It has been reported that when restorative materials are immersed in food mimic liquids, inorganic fillers can leach from the surface and dissolve, thus altering surface properties. Laboratory studies have demonstrated that restorative materials exposed continuously to these liquids can mimic the expected deterioration in the oral cavity. 13-15

The 75% concentration of ethanol solution, also known as Wu solvent, causes the degradation of the polymer structure of resin-based materials and facilitates diffusion regulation under artificial abrasion conditions. It is widely used in many studies to mimic the accelerated aging of dental restorative materials. Additionally, it leads to the dissolution and separation of monomers such as Bis-GMA and UDMA present

in composite resins. It is generally accepted that the most soluble and sensitive component to dissolution in composite resins is the dilute monomer TEGDMA. Furthermore, it has been reported that monomers such as Bis-GMA and UDMA are also soluble in water. There is information available indicating that each food mimic liquid causes degradation in one of the components of restorative materials. Numerous studies have noted that ethanol-containing foods have an effect on the inorganic matrix of restorative materials. It is also known that inorganic fillers can be degraded by the effect of citric acid. It has been determined that foods containing heptane primarily cause damage to the organic structure of resin-based restorative materials.

Another important factor, both for the aesthetic qualities and the lifespan of restorative materials, is the surface quality. A smooth restoration surface minimizes bacterial adhesion and food retention in the oral cavity. Polishing the restoration surface plays a significant role in reducing surface roughness. This not only enhances the aesthetic appearance of the restoration but also extends its lifespan. The ratio and size of fillers in the structure of resin-based materials are crucial factors that increase the polishability of the material's surface. ²¹

Various techniques and devices, such as profilometers and SEM (scanning electron microscopy), are used to measure the surface roughness of test materials. Although AFM and SEM

assessments can show surface properties more clearly, they are more costly than other devices. The most reliable method for evaluating surface roughness is reported to be AFM.^{22,23} The AFM device enables three-dimensional measurements and allows for the scanning of smaller areas, providing the determination of surface roughness across the entire scanned area.²⁴

Although there is no universally established value to consider a restoration surface as smooth, some researchers have indicated that restorations with a surface roughness of less than 0.2 µm are acceptable for oral tissues.²⁵ Jones et al.²⁶ emphasized that surfaces with roughness values above $0.5\,\mu m$ can be detected by patients' tongue tips. The most commonly used parameter in surface roughness evaluations is the Ra (roughness average), with a unit of µm.27 Although it is not considered very reliable in surface roughness evaluations, it is the most frequently used parameter in dental research.²⁸ In our study, AFM (atomic force microscopy) was used to measure the surface roughness of the samples. Although different levels of roughness were observed among the materials, only the ChemFill Rock material exhibited surface roughness greater than 1 µm. While surface roughness was observed in other materials, it was found to be below $0.5 \mu m$, which is below the detectable level by oral tissues.

Many studies on surface roughness have reported that conventional glass ionomer cements exhibit the highest roughness values.²⁹ It is claimed that glass ionomer cements demonstrate lower microhardness and wear resistance compared to composite restorations.30 In their studies examining the surface roughness of various restorative materials, Eick et al.31 reported that the highest roughness values were observed in conventional glass ionomer cement and high-viscosity glass ionomer cement, respectively. Welbury et al.³² reported in their study using compomer and glass ionomer that compomer materials were more successful than glass ionomer cements, attributing this to the superior physicomechanical properties of compomers and their higher wear resistance in the oral environment. In our study, the high-viscosity glass ionomer cement material, ChemFill Rock, exhibited the highest surface roughness and solubility values, yielding results similar to previous studies. Specifically, the ChemFil Rock samples immersed in citric acid showed the highest solubility and surface roughness values. The high surface roughness and solubility values of the high-viscosity glass ionomer cement samples can be attributed to the large size and heterogeneous distribution of the glass particles in the cement. Additionally, the dissolution of the siliceous hydrogel layer can lead to the dissolution of glass particles within the glass ionomer, thus resulting in higher solubility and surface roughness.

There are many studies assessing the effects of food mimic solutions on the surface roughness of restorative materials. In their study, Abdallah et al.³³ concluded that the surface roughness of restorative materials increased with the use of food mimic solutions, examining Equia Forte, activa bioactive composite, Cention-N, and Tetric-N Ceram Bulk Fill. According to Abdallah et al.,³³ Cention-N provided surface resistance comparable to commonly used tooth-colored direct restorative materials against food aging.

Kedici et al.³⁴ examined the effects of food mimic solutions such as ethanol and citric acid on the surface roughness of singleshade universal composites (Essentia Universal, Omnichroma, and Vittra APS Unique) using FE-SEM. They found that Omnichroma showed the most surface changes when stored in ethanol, while Vittra Unique and Essentia showed the most surface changes when stored in citric acid. The results of our study partly align with these findings.

Compared to other monomers, increasing the TEGDMA content in resin matrix systems has been reported to enhance the material's hydrophilic properties and increase water absorption.¹³ Zhang and Xu reported that the solubility of monomers in organic solvents is higher than in water.35 Furthermore, materials containing UDMA monomer have been noted to be more susceptible to solubility in food mimic solutions compared to Bis-GMA-based materials.³⁶ In our study, the lowest solubility and roughness values were observed in the distilled water control group. This result is consistent with the findings of Zhang and Xu. The Z-250 material, which contains both BisGMA and UDMA monomers, exhibited higher surface roughness and solubility values compared to the G-aenial anterior material, which only contains UDMA. We attribute this to its higher organic monomer content and the presence of different sized inorganic particles in its microhybrid structure.

In our study, although all samples had acceptable initial surface roughness, the Dyract XP compomer material was identified as having the lowest initial surface roughness. This can be explained by the material's lower inorganic content and smaller particles compared to other materials. However, we believe that its higher organic content may have resulted in greater surface roughness in subsequent measurements.

Some studies have indicated that organic solvents cause surface damage to resin-based restorative materials. The literature contains information that the solubility parameters of Bis-GMA and UDMA monomers in composite resin materials are close to the solubility values of 75% ethanol solution. ^{20,15} Ethanol, as an organic solvent, has the potential to cause polymer damage. It can penetrate the resin matrix completely and lead to the release of unreacted monomers. Partial dissolution of the resin matrix causes degradation of the filler-matrix interface. ¹⁵

In a study by Yap et al.,³⁶ they observed an increase in the surface hardness of methacrylate-based composite resins stored in heptane, attributing this result to the reduction of the oxygen inhibition layer by the heptane solution and the prevention of silica filler dissolution. Voltarelli and colleagues, in a similar study, reported that the effects of heptane solution on the surface roughness of composite resins were not statistically significant.³⁷ Eweis and colleagues stated that the heptane solution prevents the separation of silica and other fillers in the materials' structure and does not dissolve in water due to its hydrocarbon structure.³⁸

In our study, however, it was observed that the heptane solution increased the surface roughness and solubility of all materials. ChemFil Rock, a high-viscosity glass ionomer cement, was found to be most susceptible to solubility due to

heptane solution. It was determined that heptane solution had the highest effect on the Dyract XP componer material after citric acid, and on other materials after ethanol and citric acid.

CONCLUSION

Upon evaluating the findings of our study, it was determined that aesthetic restorative materials exhibit an increase in surface roughness when exposed to food mimic solutions. Since the materials used were not subjected to mechanical forces, we can assert that the observed results are solely due to the chemical effects of the solutions. We believe that regular polishing of resin-based restorations' surfaces can enhance their longevity. Moreover, the limitations of our study, including the use of only a limited number of food mimic solutions and the lack of exposure to mechanical forces, prevented the adequate simulation of the oral environment. Therefore, we suggest that future studies could benefit from the use of a broader range of food mimic solutions, subjecting the materials to forces that simulate the oral environment more accurately, and exposing them to temperature variations, which could yield more detailed results. This study is derived from the specialization thesis titled "The Effect of Food Mimic Liquids on the Surface Structure and Solubility of Different Aesthetic Restorations" by Dt. Abdurrahman YALÇIN, under the supervision of Asst. Prof. Dr. Şeyhmus BAKIR.

ETHICAL DECLARATIONS

Ethics Committee Approval

It is not required for this study.

Informed Consent

Participant consent is not required for this study.

Referee Evaluation Process

Externally peer-reviewed.

Conflicts of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, conduct and analysis of the work, and that they have approved the final version.

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