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Effect of Gastric Acid on the Surface Properties of Different Composite Resin Restorative Materials: Scanning Electron Microscope (SEM) Evaluation

Gastrik Asitin Farklı Kompozit Rezin Restoratif Materyallerin Yüzey Özelliklerine Etkisi: Taramalı Elektron Mikroskobu (SEM) Değerlendirmesi

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ABSTRACT

Objective: The aim of this study was to evaluate the effect of gastric acid on different resin-based composites with surface microhardness, surface roughness and scanning electron microscopy (SEM).

Methods: Three different composite resin restorative materials (Clearfil Majesty ES-2{Kuraray, Tokyo, Japan}, Beautifil II {Shofu, Ratingen, Germany}, Group Beautifil II LS {Shofu, Ratingen, Germany}) were used. Vickers microhardness and surface roughness measurements were evaluated at baseline, after 7 and 14 days of soaking in gastric acid. SEM images were obtained to examine the effects of gastric acid on the surface properties of the composites.

Results: When the difference in the microhardness values of the composite resins was compared, the time-dependent change in all composites was found to be statistically significant. The most surface roughness and hardness changes occurred in Beautifil II group (*P:0.000; P<.05*). According to SEM images, Beautifil II group was most affected by gastric acid, while Clearfil Majesty group was least affected.

Conclusion: In vitro conditions gastric acid increased the surface roughness of different composites while decreasing their microhardness. As a result, if these restorative materials are to be preferred in patients with reflux, they should be checked frequently. In the presence of an uncontrollable situation, the use of these restorative materials can be limited.

Keywords: Gastric acid, microhardness, surface roughness, SEM

ÖZ

Amaç: Bu çalışmanın amacı, farklı rezin esaslı kompozitlerin üzerine gastrik asitin etkisinin yüzey mikrosertliği, yüzey pürüzlülüğü ve taramalı elektron mikroskobu (SEM) ile değerlendirmektir.

Yöntemler: Üç farklı kompozit rezin restoratif materyal (Clearfil Majesty ES-2{Kuraray, Tokyo, Japonya}, Beautifil II {Shofu, Ratingen, Almanya}, Group Beautifil II LS {Shofu, Ratingen, Almanya}) kullanılmıştır. Vickers mikrosertliği ve yüzey pürüzlülüğü ölçümleri başlangıçta, gastrik asitde 7 ve 14 gün bekletildikten sonra değerlendirilmiştir. Kompozitlerin yüzey özelliklerindeki gastrik asitin etkilerini incelemek için SEM görüntüleri elde edildi.

Bulgular: Kompozit rezinlerin mikrosertlik değerlerindeki fark karşılaştırıldığında, tüm kompozitlerde zamana bağlı değişim istatistiksel olarak anlamlı bulunmuştur. En çok yüzey pürüzlülüğü ve sertlik değişimi Beautifil II grubunda gerçekleşmiştir (*P:,000; P <,05*). SEM görüntülerine göre Beautifil II grubu gastrik asitten en çok etkilenen grup iken, Clearfil Majesty grubu en az etkilenmiştir.

Sonuç: İn vitro koşullarda gastrik asit, farklı kompozitlerin yüzey pürüzlülüğünü arttırırken mikrosertliğini azaltmıştır. Bu verilere göre; reflü hastalarında bu restoratif materyaller tercih edilecekse sıklıkla kontrol edilmelidir. Kontrol edilemeyen bir durumun varlığında bu restoratif materyallerin kullanımı sınırlandırılabilir.

Anahtar Kelimeler: Gastrik asit, mikrosertlik, yüzey pürüzlülüğü, SEM

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INTRODUCTION

Dental erosion (DE) is the chemically irreversible loss of enamel and cement without bacteria.[1] Dental erosion is one of the important issues in dentistry, due to its high prevalence affecting 30% to 50% of primary teeth and 20% to 45% of permanent teeth.[2] DE can be etiologically 'external' and 'internal'. External etiological causes; Some medications such as food intake, chewable vitamin C tablets, antidepressants, asthma medications, and acid action through occupational factors.[3] A lifestyle with a more fruit-containing diet, increased intake of sports drinks and especially fruit juices are among the major exogenous causes of DE today.[4] Internal causes include recurrent vomiting and eating disorders, and gastroesophageal reflux disease, which is defined as acidic stomach contents reaching the oral cavity.[5] In a study, it was determined that DE is 8.5 times more common in individuals with eating disorders.[6] Gastroesophageal reflux disease is defined as decreased involuntary muscle relaxation of the esophageal sphincter, which causes stomach acid to travel up the esophagus and into the oral cavity. Continuous return of stomach contents to the oropharynx is one of the risk factors for DE.[7]

Because of DE, tooth abrasions, sensitivity due to the exposure of the dentin surface owing to abrasion, loss of dental aesthetics and function may be encountered. Tooth surfaces exposed to DE may appear thinner, smoother, brighter and more yellow in color. At the same time, pits may form due to abrasion on the cutting edges and occlusal tubercles.[8] Composite resin restorations age and have degradation due to intermittent or continuous exposure to various chemicals in the oral cavity.[9] Güler et al.[10] Reported that solutions with various pH (Cola, Orange juice, Kefir, Artifical gastric acid and Artifical saliva) affected the surface features of different composite materials.

Today, the demand for composite resins and indirect ceramics has increased due to the increase in aesthetic expectations. Composite resin restorative materials are preferred due to their tooth color, aesthetics and good mechanical properties. Nanohybrid composite resin restorative materials have the lowest organic matrix content, a larger percentage of filler content, and show less polymerization shrinkage.[11]

The content of giomers, which are fluorine-releasing restorative materials, is different from conventional composite resin restorative materials. The combination of composites and glass ionomers where the acid-base reaction takes place is called giomer.[12] In addition to their appropriate aesthetics, they are preferred for their easy polishing and fluoride charging potential. At the same time, these materials have antibacterial effects due to fluoride release.[13]

Restorations made with resin-containing materials may fail in the long and short term for various reasons.[12] The presence of gastric acid in the oral cavity in individuals with reflux may cause various effects on the surface and mechanical properties of resin-containing materials. Studies on this subject are limited[10, 14] and more research is required. The aim of this study was to evaluate the effect of gastric acid on the surface roughness and microhardness of different resin-based materials.

The first null hypothesis is that the surface microhardness values of composite and giomers do not change with exposure to gastric acid simulation. The second null hypothesis is that the roughness values of composite material and giomers do not change with exposure to gastric acid simulation. Finally, the third null hypothesis is that there is a correlation between exposure to gastric acid simulation and surface microhardness and roughness values of two distinct giomers.

METHODS

Ethics committee approval was not obtained as human or animal sources were not used in this study.

Since human resources were not used in this study, informed consent was not obtained.

Preparation of Composite Resin Specimens

In this study, the effect of gastric acid on three distinct composite resin restorative materials with different filler ratio and monomer structure was investigated. The technical properties of the composite materials evaluated in the study are shown in Table 1.

Table 1. Contents of composite resins used in the study

Material	Manufacturer	Composition/ Particle Size	Filling Ratio (%wt-%vol)	Lot Number
CLEARFIL MAJESTY ES-2	Kuraray Medical Inc., Okayama, Japan	Bis-GMA, Silane barium glass filler, 0.37-1.5µm (%Wt)	78%- 40%	320084
BEAUTIFIL II	Shofu Inc., Kyoto, Japan	Bis-GMA, TEGDMA, S-PRG, Fluoroboroaluminosilicate glass, 0.8 µm (%Wt)	83.3%-69**%**	61938
BEAUTIFIL II-LS	Shofu Inc., Kyoto, Japan	Bis-GMA, TEGDMA, S-PRG	83%-68**%**	11925

Bis-GMA: Bisphenol A-Glycidyl Methacrylate; TEGDMA: Triethylene glycol dimethacrylate; S-PRG: Surface pre-reacted glass-ionomer

In the preparation of the composite resin specimens, plexiglass molds with a 5 mm diameter and a 2 mm depth were used. While the composite resin specimens were being prepared, restorative materials were placed in the molds, first the transparent band and then the glass surfaces were positioned with light pressure on both surfaces and polymerized with a LED light curing unit (D-Light Pro, GC, Tokyo, Japan) with a light intensity of 1,200 mW/cm^2 in accordance with the manufacturer's recommendations. The composite resin specimens were finished and polished with polishing discs in order from coarse to fine grained (Super-Snap, Shofu Inc., Kyoto, Japan). After each disc application, it was washed for 10 seconds and dried with light air for 5 seconds.

Gastric Acid Cycle

The composite resin specimens obtained from composite resin restorative materials were treated for 18 hours in gastric acid solution and 6 hours in deionized water for 14 days. The composite resin specimens kept in gastric acid were washed with distilled water at the end of 18 hours and left in deionized water. The gastric acid solution content was prepared fresh every day in Gazi University Faculty of Dentistry, with Hydrochloric acid (HCl) 0.06 M (Aklar Kimya, Ankara, Turkey) 0.113% deionized water solution and pH 1.2[10]. It was stored for 14 days at 37°C in 100% humidity.

24 hours after preparation of composite resin specimens from composite resin restorative materials, 7. and measurements were made on the 14th day. Measurements were made after the composite resin specimens were washed with distilled water and dried with air-water spray.

Measurement of Surface Hardness

Microhardness values of composite resin specimens were measured with a digital surface microhardness device (HMV-700 Microhardness Tester, Shimadzu, Japan) with Vickers surface hardness test under 490µN load for 15 seconds. Three different measurements were made from different parts of each 24 hours after preparation of composite resin specimens from composite resin restorative materials, and the average value was calculated.

Measurement of Roughness

An area of 100*100 µm^2 was determined with the surface roughness meter (Surftest SJ-301-Mitutoyo, Illinois, USA) and a 0.25 mm line scan was performed across the surface of the composite resin specimens. The profilometer was calibrated with 0.25 mm cut off, 1.25 mm reading length and 0.5 mm/s speed, and the average surface profile was evaluated accordingly. After the preparation of composite resin samples from composite resin restorative materials, three different measurements were made from different parts every 24 hours and the average value was calculated.

The evaluation of Scanning Electron Microscope (SEM) images

SEM images were taken from the composite resin specimens prepared from each group at baseline, 7th and 14th days. The composite resin specimens were gold plated (Leica EM ACE 200, Leica Microsystems, Danaher Corporation, Washington DC, USA) and evaluated by SEM (Hitachi SU5000 FE-SEM,). The entire surface of these composite resin specimens was scanned and photographs were obtained at 10,000x magnification from the areas showing surface structure changes.

Statistical Assessment

These study data were evaluated using the IBM SPSS Statistics 22 program. The compatibility of the data with normal distribution was determined by Kolmogorov-Smirnov and Shapiro Wilks tests. While evaluating the study data, the One Way Anova test was used to compare the data between groups, and the Tukey HDS test was used to determine which group the difference originated from. Analysis of variance in repeated measurements and Bonferroni test as post hoc test were used to compare the data within the group. Significance was evaluated at the *p>.05* level.

RESULTS

Evaluation of Surface Roughness

When the initial roughness levels were evaluated, the post hoc Tukey HSD test was used to determine which group caused the statistically significant difference between the groups. While the initial roughness of the Clearfil Majesty ES-2 composite group was significantly lower than the Beautiful II and Beautiful II LS groups (*P<.05*), there was no statistically significant difference between the Beautiful II and Beautiful II LS groups (*p>.05*) (Table 2).

Table 2. Evaluation of surface roughness (Ra)

Surface Roughness (Ra) Composite Resins	Initial	Day 7	Day 14
Clearfil Majesty ES-2 (Mean±SD)	0.05±0.01[a]	0.27±0.07[a]	0.35±0.09[a]
Beautiful II (Mean±SD)	0,09±0.02[b]	0.33±0.11[a]	0.64±0.11[b]
Beautiful II LS(Mean±SD)	0.08±0.02[b]	0.31±0.10[a]	0.55±0.12[b]
[1]P	0.000*	0.320	0.000*

[1]Oneway ANOVA posthoc Bonferroni Test *P<.05

[a,b]Different letters in the lines indicate the difference between groups.

When the surface roughness levels were evaluated on the 7th day, there was no statistically significant difference between the groups (*P: .032*).

When the 14th day roughness levels were evaluated, a statistically significant difference was found between the groups (*P<.05*) and the 14th day surface roughness of the Clearfil group was found to be significantly lower than the Beautiful II and Beautiful II LS groups (*P<.05*). There was no statistically significant difference between Beautiful II and Beautiful II LS groups in terms of roughness on day 14 (*P>.05*).

In the Clearfil Majesty group; Statistically significant difference was found when baseline, 7th day and 14th day surface roughness levels were evaluated (*P<.05*). The increases observed on the 7th and 14th days according to the initial surface roughness were statistically significant (*P<.05*). The increase in the roughness of the 14th day compared to the 7th day was also statistically significant (*P<.05*).

In the group Beautiful II; There was a statistically significant difference between the surface roughness levels at baseline, 7th day and 14th day (*P<.05*). The increases observed on the 7th and 14th days according to the initial surface roughness were statistically significant (*P<.05*). The increase in the roughness of the 14th day compared to the 7th day was also statistically significant (*P<.05*).

Beautiful II LS group; There was a statistically significant difference between the surface roughness levels at baseline, 7th day and 14th day (*P<.05*). The increases observed on the 7th and 14th days according to the initial surface roughness were statistically significant (*P<.05*). The increase in the roughness of the 14th day compared to the 7th day was also statistically significant (*P<.05*).

Evaluation of Surface Hardness

When the initial surface hardness levels were evaluated, the post hoc Tukey HSD test was used to determine which group caused the statistically significant difference between the groups. The initial surface hardness of the Clearfil Majesty ES-2 composite group was significantly higher than the Beautiful II and Beautiful II LS groups (*P<.05*). There was no statistically significant difference between the Beautiful II and Beautiful II LS groups in terms of initial surface hardness (*P>.05*).

While the 7th day hardness values of the Clearfil Majesty ES-2 composite group were found to be significantly higher than the Beautiful II and Beautiful II LS groups (*P<.05*), there was no statistically significant difference between the Beautiful II and Beautiful II LS groups in terms of the 7th day surface hardness values (*P>.05*).

The 14th day hardness values of the Clearfil Majesty ES-2 composite group were significantly higher than the Beautiful II and Beautiful II LS groups (*P<.05*). There was no statistically significant difference between Beautiful II and Beautiful II LS groups in terms of surface hardness values on day 14 (*P>.05*).

In all three groups; Statistically significant difference was found between initial, 7th day and 14th day surface hardness values (*P<.05*). While the decreases observed in the 7th and 14th days compared to the initial surface hardness were statistically significant, the decrease in the 14th day surface hardness compared to the 7th day was also statistically significant (*P<.05*).

Scanning Electron Microscope (SEM) Investigations

SEM images were obtained at 10,000x (Figure 1) magnification from the **composite resin specimens** obtained as a result of initial, 7th and 14th day gastric acid exposure of all groups in the study. For all groups; When the surface examinations were examined on the 7th day (Figure 1-B/E/H) in SEM imaging, it was observed that the surface roughness increased compared to the baseline (Figure 1-A/D/G) and pits were formed. Likewise, similar results were seen at day 14 (Figure 1-C/F/I). According to SEM data, Beautifil II group (Figure 1-E/F) was most affected by gastric acid, while Clearfil Majesty ES-2 group (Figure 1-B/C) was least affected.

Table 3. Evaluation of surface hardness (kg/mm²)

Surface Hardness (kg/mm²) Composite Resins	Initial	Day 7	Day 14
Clearfil Majesty ES-2 (Mean±SD)	102.6±6.37[a]	95.92±6.17[a]	92.50±5.33[a]
Beautiful II (Mean±SD)	73.88±2.91[b]	56.52±2.16[b]	33.25±4.73[b]
Beautiful II LS(Mean±SD)	71.65±4.18[b]	59.71±4.14[b]	36.03±7.09[b]
[1]P	0.000*	0.000*	0.000*

[1]Oneway ANOVA posthoc Bonferroni Test *$P<.05$

[a,b]Different letters in the lines indicate the difference between groups.

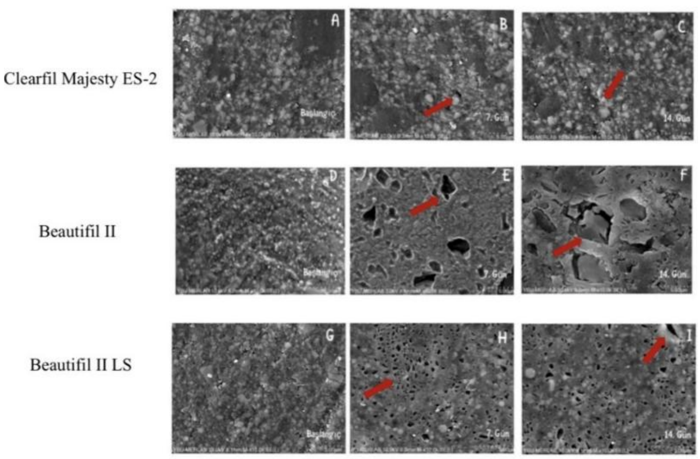

Figure 1. SEM images at 10,000x magnification; (A): Clearfil Majesty ES-2 initial SEM image (B): Clearfil Majesty ES-2 day 7 SEM image (C): Clearfil Majesty ES-2 day 14 SEM image (D): Beautifil II initial SEM image (E): Beautifil II day 7 SEM image (F): Beautifil II day 14 SEM image (G): Beautifil II LS initial SEM image (H): Beautifil II LS day 7 SEM image (I): Beautifil II LS 14th day SEM image

DISCUSSION

When the prevalence of dental erosion resulting in loss of dental hard tissues is examined, gastroesophageal reflux disease (GERD) is seen in 17 percent of patients (range 21-83%).[15] According to studies, 40 percent of the adult population has been reported to have GERD symptoms at some point in their lives.[7] Clinical symptoms may occur as a result of demineralization of dental hard tissues as a result of diseases such as GERD and repeated exposure of the oral cavity to gastric acid.[16] The aim of this study is to examine the effect of pH change, which causes damage to dental hard tissues, on dental restorative materials.

In this study, the effect of a nanohybrid composite resin restorative material and two giomers, claimed to exhibit low polymerization shrinkage (Beautifil II LS), on the roughness and surface microhardness of the materials in a time span of 13 years of gastric acid were tested. Measurements were made at baseline (T0), after 7 days (T1), and after 14 days (T2). SEM images were taken from composite resin specimens at T0, T1 and T2 stages. Exposure to low pH caused a statistically significant increase in roughness and hardness in all three groups. Based on the data obtained from this study, the first and second null hypotheses were rejected. Surface hardness and roughness increased after gastric acid cycle in all three materials compared to baseline. The last null hypothesis that there is a correlation between exposure to gastric acid simulation and microhardness and surface roughness values of two different giomers was also rejected. There is a difference in microhardness and surface roughness values between the two giomers. Changes in all these surface properties can be supported by SEM images.

Different immersion times have been planned in studies examining the effect of different acidity liquids on the mechanical and physical properties of dental restorative materials. Different immersion periods were used in some studies ranging from 1 day to 1 month[17-19], and in some studies from 1 month to 1 year.[20-23] There are studies in the literature reporting that the 14-day test period simulates the intraoral environment of approximately 13 years to evaluate the effect of various beverages on dental restorations.[10,22] In this study; a 7-day and 14-day immersion period (18 hours per day in gastric acid and 6 hours in distilled water) was planned to achieve a reasonable immersion time representative of the intraoral environment.[14] Since the tested composite resin specimens were not exposed to a mechanical force factor, the changes in surface hardness and roughness, which were also observed in SEM images, were caused by a chemical reaction or dissolution that developed depending on the Ph level of gastric acid.

The most commonly used microhardness tests in dentistry in the literatüre are the Knoop and Vickers microhardness tests. Vickers hardness test, which is also accepted as an indicator of the degree of polymerization of composite resin restorative materials, is widely used in the literature.[24, 25] In this study, Vickers microhardness test was preferred. The Vickers microhardness value reflects the resistance of materials to deformation and wear resistance.[26] There are several factors that can affect the hardness of resin-containing restorative materials. The filler size, filler content and resin monomer type are considered within these factors.[27] Surface roughness is generally associated with filler size in restorative materials.[28, 29] In the literature, it has been observed that smoother surfaces are obtained in materials with smaller filler size.[29, 30]

Clearfil Majesty ES-2 is a nanohybrid composite resin containing Bis-GMA. BisGMA is an essential component in the composite resin matrix, but one of its drawbacks is its high viscosity. Strong molecular interactions driven by the H bond are effective in this viscosity.[31] High viscosity weakens the mechanical properties of the composite and reduces its lifetime.[31] This content of the material may explain the increase in surface hardness and roughness in acidic environment. For Clearfil Majesty ES-2 group; When the surface examinations on the 7th day (Figure 1-B) and 14th day (Figure 1-C) were examined in SEM imaging, it was observed that the roughness increased and pits were formed compared to the baseline (Figure 1-A). This finding supports the surface roughness data.

In addition to conventional composite resins, materials with fluorine release in giomer structure are used in restorative dentistry. Giomers exhibit similar aesthetic and physical properties to composite resin restorative materials, superior to other fluorine-releasing conventional glass ionomers and resin modified glass ionomer cements (RMGIC). Many studies in the literature have shown that acid attacks in the oral cavity cause fluoride removal from glass ionomer restorative materials.[32,33] Beautifil II and Beautifil II LS release fluoride from its surface in acidic environment due to its giomer structure. It is thought that fluoride release is the reason for the increase in surface roughness and decrease in microhardness values in these groups after gastric acid exposure.

The giomers have a conventional bis-GMA matrix and contain bioactive glass fillers.[34] S-PRG (surface pre-reacted glass-ionomer) filler particles in giomers have been reported to act as a fluoride reservoir.[34] TEGDMA provides fluidity, flexibility and heterogeneity to the material it is in. The heterogeneity in the matrix allows for the formation of larger micropores between the polymers.[34]

According to the results of the study, the surface roughness of Beautifil II and Beautifil II LS groups, which are in gionomer structure, showed a statistically significant increase. In this case, it is thought that the fluoride content has an effect as well as the TEGDMA content in their structure. Although there is no statistically significant difference in surface roughness between Beautifil II and Beautifil II LS groups, the Beautifil II group has a higher surface roughness than the Beautifil II LS group, and SEM images have shown results that support this. With a particle size of 0.8 µm, Beautifil II group is the group that shows the most variation in surface roughness. Likewise, the fluoride content separated from the surface by the effect of gastric acid also explains the decrease in microhardness. Another reason for the decrease in surface hardness is the softening of bisphenol-A-glycidyl methacrylate (Bis-GMA) based polymers in the structure of composite resins exposed to gastric acid.[35] For Beautifil II and Beautifil II LS groups; When the surface examinations on the 7th day (Figure 1-E/H) and 14th day (Figure 1-F/I) were examined in SEM imaging, it was observed that the roughness increased and pits were formed compared to the baseline (Figure 1-D/G). According to SEM data, while materials with giomer structure were affected more by gastric acid, the most surface change was observed in Beautifil II group (Figure 1-E/F).

In support of this study; In their study, Guler and Unal reported that various acidic liquids, including gastric acid, increase the roughness on the surface of resin-containing materials and cause color change in the restorative material[15]. In the presence of factors affecting erosion such as gastroesophageal reflux in patients, physicians should be careful in the selection of restorative materials to be preferred and patients should be informed about this issue. Limitations of this study include deficiencies in mimicking the complex oral environment and ignoring the effects of temperature change and the buffering effect of saliva. Further research can examine the in vivo effects of gastric acid on different restorative materials with different evaluation methods (Atomic force microscopy (AFM), Knoop surface hardness test). Although the complex does not fully reflect the oral environment in this study, it does confirm the corrosive potential of gastric acid on restorative materials.

CONCLUSION

According to this study, gastric acid was found to be effective on the surface hardness and roughness of restorative materials.

- A decrease in surface hardness was observed in restorative materials after exposure to gastric acid.
- The surface roughness of the restorative materials also increased after gastric acid exposure.
- As a result, the choice of dental material is very important in patients with reflux.
- If the dental materials evaluated in this study are to be preferred, patients should be checked more frequently considering the results of the study.
- The use of these dental materials may be limited in the presence of a situation where case follow-up cannot be performed.

Etik Komite Onayı: Bu çalışmada insanlardan ya da hayvanlardan elde edilen kaynaklar kullanılmadığından etik kurul onayı alınmamıştır.
Hasta Onamı: Bu makale, insan katılımcılar ile yapılan herhangi bir araştırma içermemektedir.
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