

The Effect of Preheating on Fissure Sealant Viscosity

Ön Isıtmanın Fissür Örtücü Viskozitesine Etkisi

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ABSTRACT

Objective: Fissure sealant application is used for the prevention of caries. The viscosity of the fissure sealant directly affects the retention of the material to the tooth. Heating resin-containing materials before polymerization decrease the viscosity of the material. The purpose of this study was to examine the fluidity levels of four different fissure sealants containing resin with different filling ratios at different temperatures before polymerization and to evaluate the obtained data by comparing them.

Methods: The study of four different fissure sealant materials with resin content with different filler ratios (0%, 30%, 55%, and 70%) were heated at different temperatures (4°C, 23°C, 39°C, and 55°C) before polymerization. With the fluidity measurement between the two glasses, 20 fluidity measurement values were obtained at each temperature value, and a total of 320 measurements were made. The data were statistically evaluated with a two-way robust ANOVA analysis.

Results: The study it was determined that the fluidity values of the fissure sealants increased with the heating process before polymerization. The best result in terms of fluidity in the fissure sealants without filler was obtained at a temperature of at least 23°C. In the fissure sealants containing 30% and 55% filler, there was a significant difference in fluidity values when the temperature difference was large.

Conclusion: It is thought that the filling ratio and temperature affect the fluidity in fissure sealants and that the appropriate temperature should be selected by considering the filling ratios to obtain the best result in terms of fluidity.

Keywords: fluidity, viscosity, preheating, fissure sealant, the heating device.

ÖZ

Amaç: Çürüklerin önlenmesi için fissür örtücü uygulaması kullanılmaktadır. Fissür örtücünün viskozitesi materyalin dişe retansiyonunu doğrudan etkilemektedir. Rezin içerikli materyallerin polimerizasyon öncesi ısıtılması materyalin viskozitesini azaltmaktadır. Bu çalışmanın amacı; farklı doldurucu oranlarına sahip rezin içerikli dört farklı fissür örtücünün polimerizasyon öncesi farklı sıcaklıklarda olması sağlanarak, akışkanlık düzeylerinin in vitro koşullarda incelenmesi ve elde edilen verilerin karşılaştırılarak değerlendirilmesidir.

Yöntemler: Çalışmada, farklı doldurucu oranlarına sahip (%0, %30, %55 ve %70) rezin içerikli dört farklı fissür örtücü materyali polimerizasyon öncesi farklı sıcaklıklarda (4°C, 23°C, 39°C ve 55°C) ısıtıldı. İki cam arasında akışkanlık ölçümü ile, her bir sıcaklık değerinde 20'şer akışkanlık ölçüm değeri elde edilerek toplam 320 ölçüm yapıldı. Veriler iki yönlü robust ANOVA analiziyle istatistiksel olarak değerlendirildi.

Bulgular: Çalışmada polimerizasyon öncesi ısıtma işlemiyle fissür örtücülerin akışkanlık değerlerinde artış olduğu, doldurucu içermeyen fissür örtücülerde akışkanlık açısından en iyi sonucun en az 23°C sıcaklıkta elde edildiği, %30 ve %55 doldurucu içeren fissür örtücülerde ise sıcaklık dereceleri arasındaki fark fazla olduğunda akışkanlık değerlerinde anlamlı fark gösterdiği saptandı.

Sonuç: Fissür örtücülerde doldurucu oranı ve sıcaklığın akışkanlık üzerinde etkili olduğu, akışkanlık açısından en iyi sonucun elde edilebilmesi için doldurucu oranları dikkate alınarak uygun sıcaklığın seçilmesi gerektiği düşünülmektedir.

Anahtar Sözcükler: akışkanlık, viskozite, ön ısıtma, fissür örtücü, ısıtma cihazı

INTRODUCTION

Dental caries is an infectious disease that is common in all age groups.¹ It occurs when the acid released as a result of the usage of fermentable carbohydrates by microorganisms destroys the calcified tissues of the tooth.² The types of caries are categorized based on their location and include pit-fissure caries, root surface caries, and flat surface caries. Pit-fissure caries are the most common types of caries lesions.³

Although dental caries are largely preventable, it remains a common disease across the globe. One of the various protective applications for the prevention of dental caries is fissure sealant applications.⁴ Fissure sealants prevent tooth decay by closing the pits and fissures of the teeth and preventing the accumulation of bacteria and bacterial nutrients in the area.⁵ Retention is the most important factor in the successful application of fissure sealants. Retention directly affects the adaptation and adhesion of the fissure sealant

to the tooth surface, thus facilitating its success and longevity.⁶ The viscosity of fissure sealants is another factor that affects the retention of the sealant to the tooth. The viscosity of the fissure sealant directly affects the adhesion of the material to the tooth.⁷ Studies have reported that the lower the viscosity value, the better the penetration of the tooth into the fissures and the more effective the retention.⁸ The filling ratio of fissure sealants containing resin affects the viscosity of the material. Fissure sealants with a low filling ratio have lower viscosity and higher fluidity.⁵

Temperature is another factor affecting viscosity. Increasing the temperature of resin-containing materials increases the mobility of free radicals and decreases the viscosity of the material. The decrease in viscosity allows the material to better penetrate the tooth, resulting in higher retention.⁹ Fissure sealants can more effectively penetrate the pits and fissures on the tooth surface when the viscosity value is reduced. In this way, the fissure sealant can successfully penetrate the enamel surface and the formation of caries in the tooth can be prevented.¹⁰

Most of the research on the effectiveness and success of fissure sealants addressed retention and microleakage, but studies evaluating the viscosity or fluidity of fissure sealants and heating fissure sealants are very limited. In light of this information, the present study aimed to examine the viscosity levels of four different fissure sealants containing resin with different filling ratios at different temperatures before polymerization and to evaluate the obtained data by comparing them.

METHODS

In the study, four different fissure sealants containing resin with different filler ratios (0%, 30%, 55%, 70%) were used (Table 1). Each type of fissure sealant was evaluated by heating at four different temperatures (4°C, 23°C, 39°C, and 55°C) before polymerization. According to the power analysis performed before the study, the number of measurement values required for each temperature of each fissure sealant was suggested as 6, with 95% test power (1-β), 95% confidence (1-α), f = 1.045 effect size. In this study, 20 fluidity measurement values were obtained for each of the four types of fissure sealants used at each temperature value; thus, a total of 320 measurements were obtained. The fissure sealants were stored in a refrigerator for 48 hours to reach a temperature of 4°C. To reach 23°C room temperature, the fissure sealants were kept at room temperature for 48 hours. A composite heating device (Micerium, S.p.a., Avegno GE, Italy) was used to heat the fissure sealants to 39°C and 55°C.

For each measurement, 0.05 ml of fissure sealant was placed on the glass layer. To ensure standardization in all measurements, 20 insulin syringes for each temperature of each fissure sealant were prepared with 0.05 ml of fissure sealant in each syringe. The insulin syringes were placed in the heating device. After each type of fissure sealant in the prepared insulin syringes reached the desired temperature, it was placed in the middle of a pre-prepared glass layer (100mmx100mmx 4mm) without decreasing its temperature. Then, a second glass layer weighing 100 g was placed over the fissure sealant. Afterwards, another 500 g weight was placed on the glass and a total of 600 g of weight was applied on the fissure sealant for 300 seconds. Then, the 500 g weight on the glass was removed. For a single measurement value, the diameter of the fissure sealant spread between two layers of glass was measured using a digital caliper from three different points in millimeters, and the average of these three measurement values was calculated and recorded.

Statistical Analysis

Data analysis were done with the R Project version 2021.09.0 (A language and environment for statistical computing. R Foundation for

Statistical Computing, Vienna, Austria). The conformity to the normal distribution was examined using the Shapiro Wilk test. A two-way robust ANOVA test was performed using the WRS2 package for 2-way comparison of the non-normally distributed fluidity values according to group and degree. The quantitative data values were given by finding the mean ± standard deviation value and the median (minimum - maximum) value. The significance level of the analysis was taken as $P < .05$.

RESULTS

A statistically significant difference was observed between the median fluidity values of each fissure sealant group at four different temperatures (Table 2). The highest fluidity median value was found in the group without filler and the lowest fluidity median value was found in the group containing 55% filler. However, no statistically significant difference was observed between the group containing 55% filler and the group containing 70% filler (Table 3).

No significant difference was observed between the fluidity values of the group without filler at 23°C, 39°C, and 55°C. However, a significant difference was found between the fluidity values at 4°C and the fluidity values at the other temperatures (Table 4).

Table 1. Fissure sealants used in the study and their chemical contents

Trade name	Chemical content	Manufacturer	Filler ratio
Teethmate F1 (Resin-containing)	TEGDMA, HEMA, MDP-F, colloidal silica, camphoroquinone, methacryloyl-fluoride-methyl methacrylate copolymer, accelerators, initiator, pigment and dye, hydrophobic dimethacrylates	Kuraray, Osaka, Japan	0%
Dyract seal (Compomer-containing)	DGDMA, hydrated silicon dioxide, strontium alumino-fluoro-silicate glass, phosphoric acid modified methacrylate resin ammonium salt, camphoroquinone, carboxylic acid modified macromonomer, ethyl-4-dimethyl amino benzoate, BHT, titanium dioxide	Dentsply, Konstanz, Germany	30%
Fissured fx (Resin-containing)	Bis-GMA, TEGDMA, UDMA, Bis-EMA, inorganic and glass ionomer filler (maximum 10 μm), 2% NaF	Voco, Cuxhaven, Germany	55%
Grandio Seal (Resin-containing)	Bis-GMA, TEGDMA, Inorganic Nanohybrid Fillers	Voco, Cuxhaven, Germany	70%

Table 2. Comparison of the fissure sealant and fluidity values according to temperature degree

	Test Statistic	P^a
Fissure sealant	925.3141	.001
Degree	444.7368	.001
Fissure sealant * Degree	214.657	.001

^a Robust 2-way ANOVA

Table 3. Median values of the fluidity values of the fissure sealants at four different temperatures

Fissure Sealant	Mean ± S. Deviation	Median (Min. - Max.)
Without Filler Fissure Sealant	57.9 ± 6.0	58.7 ^c (45.1 – 68.2)
Fissure Sealant Containing 30% Filler	45.1 ± 2.4	45.5 ^b (40.1 – 49.3)
Fissure Sealant Containing 55% Filler	42.7 ± 4.4	41.3 ^a (36.2 – 50.2)
Fissure Sealant Containing 70% Filler	42.1 ± 2.0	42.4 ^a (36.8 – 47.1)

^{a-c} There is no difference between the main effects with the same letter ($P < .05$).

Table 4. The flowability values of the fissure sealants evaluated at different temperatures

Fissure Sealant	Degree	Mean \pm S. Deviation	Median (Min. - Max.)
Without Filler Fissure Sealant	4 °C	50.8 \pm 3.7	50.6 ^G (45.1 – 58.9)
	23 °C	58.9 \pm 4.7	58.8 ^F (52.2 – 68.2)
	39 °C	59.2 \pm 4.9	59.1 ^F (50.0 – 67.1)
	55 °C	62.9 \pm 3.1	64.1 ^F (55.8 – 68.1)
Fissure Sealant Containing 30% Filler	4 °C	42.8 \pm 2.0	42.5 ^{AE} (40.4 – 47.3)
	23 °C	44.4 \pm 2.0	44.3 ^{DE} (40.1 – 48.8)
	39 °C	46.0 \pm 1.6	46.1 ^{BD} (41.3 – 48.4)
	55 °C	47.0 \pm 1.5	47.2 ^B (42.5 – 49.3)
Fissure Sealant Containing 55% Filler	4 °C	38.7 \pm 1.7	38.0 ^C (36.2 – 42.2)
	23 °C	39.2 \pm 1.2	39.4 ^C (37.2 – 40.9)
	39 °C	46.0 \pm 3.0	46.6 ^{BD} (37.2 – 50.2)
	55 °C	46.8 \pm 2.7	47.3 ^{BG} (41.5 – 50.1)
Fissure Sealant Containing 70% Filler	4 °C	41.6 \pm 1.5	41.9 ^A (39.0 – 45.3)
	23 °C	40.8 \pm 2.3	41.8 ^{AC} (36.8 – 44.2)
	39 °C	43.0 \pm 1.3	43.1 ^{AE} (39.8 – 44.9)
	55 °C	43.1 \pm 1.8	42.6 ^{AE} (39.4 – 47.1)

^{A-G} There is no difference between interactions with the same letter ($P < .05$).

Table 5. The flowability values and comparative results at four different temperatures, independent of the fissure sealant groups

Group	Degree	Mean \pm S. Deviation	Median (Min. - Max.)
Total	4 °C	43.5 \pm 5.1	42.2 ^c (36.2 – 58.9)
	23 °C	45.8 \pm 8.3	42.9 ^{bc} (36.8 – 68.2)
	39 °C	48.5 \pm 7.0	46.3 ^{ab} (37.2 – 67.1)
	55 °C	50.0 \pm 8.0	47.2 ^a (39.4 – 68.1)
	Total	46.9 \pm 7.6	45.0 (36.2 – 68.2)

^{a-c} There is no difference between the main effects with the same letter ($P < .05$).

There was no statistically significant difference between the viscosity values of the group containing 30% filler at 4°C and 23°C, 23°C and 39°C, 39°C and 55°C. However, a statistically significant difference was found between the fluidity values at the other temperatures (Table 4).

No statistically significant difference was found between the fluidity values of the group containing 55% filler at 4°C and 23°C and 39°C and 55°C. However, a statistically significant difference was observed between the fluidity values at the other temperatures (Table 4).

There was no statistically significant difference between the fluidity values of the group containing 70% filler at 4°C, 23°C, 39°C, and 55°C (Table 4).

When analyzed independently of the material groups, no statistically significant difference was found between the fluidity values at 4°C and 23°C, 23°C and 39°C, and 39°C and 55°C. However, a statistically significant difference was observed between the fluidity values at 4°C and 39°C, 4°C and 55°C, and 23°C and 55°C (Table 5).

There was no significant difference between the fluidity values of the group containing 30% filler at 4°C and the group containing 70% filler. A significant difference was found between the other groups at 4°C (Table 4).

There was no significant difference between the fluidity values of the group containing 55% filler and the group containing 70% filler at 23°C. A significant difference was found between the other groups at 23°C (Table 4).

There was no significant difference between the fluidity values of the group containing 30% filler and the group containing 55% filler at 39°C and 55°C. A significant difference was found between the other groups at 39°C and 55°C (Table 4).

When the fluidity values were compared regardless of the material groups and temperatures, the highest median fluidity value was obtained at 55°C in the group without filler and the lowest median fluidity value was obtained at 4°C in the group containing 55% filler (Table 4).

DISCUSSION

Fissure sealant application is the method most frequently used to prevent caries on the occlusal surfaces of teeth.¹¹ Today, resin-based fissure sealants and glass ionomer-based are most commonly used as pit and fissure sealants. Many studies have reported that resin-based fissure sealants are the most successful fissure sealants.^{5,7} Most of the research on the efficacy and success of fissure sealant materials addressed retention and microleakage. Few studies have evaluated the viscosity or fluidity of fissure sealants. It is thought that the limited number of studies examining the fluidity of fissure sealants may be due to the assumption that less viscous materials show better flow resulting in better penetration into pit and fissure cavities.¹⁰

Viscosity is the resistance of a liquid to flow. This resistance is controlled by the internal frictional forces in the fluid. A liquid with a high viscosity flows slowly. Higher viscosity causes poor adaptation of the sealant to the tooth and incomplete penetration while reducing the retention of the sealant to the tooth. A low-viscosity sealant has a higher potential to spread and more quickly penetrate the tooth surface.¹² The depth of penetration is an important factor that can increase the life, durability, and adaptation of the fissure sealant. The low fluidity of the fissure sealant can lead to a decrease in the flow of the fissure sealant to the base of the fissures, resulting in incomplete penetration depth, especially in the case of narrow and deep fissures, such as IK-type and I-type. Moreover, less penetration depth reduces the retention of the fissure sealant.¹³

The polymerization of resin-containing fissure sealants is carried out with light. For the restoration to be healthier, the degree of polymerization must be high. When the degree of polymerization increases, the amount of residual monomer decreases, and the physical properties of the material increase.¹⁴ If the polymerization of the material is not sufficient, microleakage, discoloration in the restoration, secondary caries formation, and retention problems can be observed. Many factors affect the depth of polymerization, including the power of the light source, the particle size, the color of the resin, and the temperature.^{9,15} Increasing the temperature of resin-containing materials increases the degree of polymerization. The rise in temperature increases the mobility of free radicals; accordingly, the viscosity of the resin decreases and additional polymerization occurs, resulting in better chemical and physical properties of the material.^{9,16} The adaptation of the resin-containing material to the cavity walls increases as the viscosity of the resin-containing materials decreases, and the fluidity increase with the heat application process before polymerization. The increase in the adaptation of the material increases the retention between the tooth and the restoration, thus reducing

secondary caries and post-operative sensitivity. Moreover, heat application before polymerization shortens the amount of time needed for the resin-containing material to harden.^{9,16}

The use of the heating process has increased in popularity in recent years, but the heating process is generally used in dentistry for composite materials and canal filling pastes.¹⁷ To date, few studies have focused on the heating of fissure sealants. In the study conducted by Gorseta et al., it was found that the application of preheating to glass ionomer cement, which can be used as pit and fissure sealants, improves the mechanical properties of the fissure sealants.¹⁸ In another study by Gorseta et al., it was reported that when the fissure sealant containing glass ionomer is heated, microleakage decreases, and marginal adaptation improves.¹⁹ Borges et al.²⁰ reported that fissure sealants heated to 68°C with a preheating process showed better marginal adaptation in comparison to those used at room temperature, and less space was formed between the fissure sealant and the tooth.

In a study by Knight et al., three different composite materials were preheated. That study found that the heating process reduces the film thickness of the material and reduces its viscosity.²¹ In a study in which the composite material was preheated, it was reported that the fluidity of six different restorative composite resins that were heated to 60°C before polymerization increased in comparison to the materials used at 23°C room temperature.¹⁶ In another study, it was reported that preheating of composites increased the microhardness of the samples and decreased their viscosity.²² Marcondes et al.²³ applied the heating process to apply to 10 different composites; they reported that the viscosity of the composites decreased between 47% and 92% with the preheating process. Loumprinis et al.²⁴ reported that preheating causes a 30–82% decrease in the viscosity of composites. Al-Ahdal et al.²⁵ reported that the increase in temperature decreased the viscosity of all the studied composites by 40% to 90%. Davari et al. heated two different composites at 4°C, 23°C, and 37°C to evaluate the micro-tensile bond strength of the composite to dentin; they reported that heating increased the bond to dentin.²⁶ Similar to these results, in our study, when the fluidity values of the four different fissure sealants at four different temperatures were examined, the highest median fluidity value for each fissure sealant was found at 55°C and the lowest median value was found at 4°C. Similar to the studies performed with resin-containing materials, the present study observed that the fluidity values increase with the increase in temperature in fissure sealant materials.

The viscosity of a resin-containing material is affected by both organic and inorganic compounds. The type and amount of each monomer used are directly responsible for the viscosity of the organic matrix. The amount, shape, and size of the filler particles in resin materials also directly affect the viscosity.²⁷ It is thought that a fissure sealant that does not contain filler will penetrate deeper into the enamel surface and provide better adhesion.⁵

In our study, there was no significant difference between the fluidity values at 23°C, 39°C, and 55°C in the group without filler; however, a significant difference was found between the fluidity values at 4°C and 23°C and 39°C and 55°C. Thus, the fluidity value of the group without filler is significantly lower at 4°C than at the other temperatures, but there was no significant increase in the fluidity value of the material at temperatures higher than 23°C. Our study results suggest that instead of using filler-free fissure sealants at a temperature of 4°C, bringing them to a temperature of at least 23°C would be the best way to use them in terms of fluidity of the material. Similar to the results of our study, Papacchini et al.²⁸ compared the bond strengths of three different composites heated before polymerization at 4°C, 23°C, and 37°C and reported that the temperature had a significant effect on the bond strength by reducing the viscosity. The bond strength of two different

composites increased significantly when the temperature was increased from 4°C to 23°C, but no significant difference was found between 23°C and 37°C.²⁸

In our study, when comparing the groups with fillers at different temperatures, there was no significant difference between the fluidity values of the group containing 30% filler at 4°C and 23°C, 23°C and 39°C, and 39°C and 55°C when the temperature difference increased, a significant difference was found between the fluidity values between 4°C and 39°C, 4°C and 55°C, and 23°C and 55°C. There was no statistically significant difference between the fluidity values of the group containing 55% filler when compared at 4°C and 23°C and at 39°C and 55°C. However, a significant difference was found between the fluidity values at other temperatures. These results suggest that in order to create a significant difference in the fluidity values between the two temperatures of the 30% filler group and the 55% filler group, the difference between the temperatures should be great. It has been reported that the fluidity values of fissure sealants increase as the temperature difference increases. Choudhary et al.²⁹ evaluated the viscosity and adaptation rates of two different composites at 23°C, 37°C, and 54°C. They observed better adaptation and less total void space formation at 54°C in comparison to 23°C and 37°C, which is similar to our study, and no significant difference was found between 23°C and 37°C. In the study by Dionysopoulo, the film thickness of the material was evaluated by heating the composite material at 23°C, 54°C, and 60°C. They reported that there was no significant difference in film thickness between the groups heated to 54°C and 60°C.³⁰ In the study by Davari et al.,²⁶ two different composites were heated at 4°C, 23°C, and 37°C, and the microtensile bond strength of the composite was evaluated; there was no significant difference between the microtensile bond strengths in the dentin of the two composites at 4°C and 23°C. The viscosity of conventional and flowable composites was evaluated by Loumprinis et al. at five different temperatures (23°C, 30°C, 37°C, 45°C, and 54°C). In that study, a significant difference was found between 23°C and 54°C for all composite types, but no significant difference was found between all the materials at the other temperature values.²⁴ These results support the finding in our study that the difference between the temperatures should be large in order to observe a significant difference in the fluidity values of the groups containing 30% and 55% filler.

In our study, no statistically significant difference was observed between the fluidity values of the group containing 70% filler at 4°C, 23°C, 39°C, and 55°C. This result demonstrates that there is no significant difference in the fluidity value when the heating process is applied to fissure sealants with a high filling ratio. When all the results were evaluated, as the filling ratio of the fissure sealant increased, the effect of the heating process on the fluidity of the material decreased. This result suggests that the structure that causes the change of fluidity by heating the resin-containing materials is the organic matrix structure, not the filler content of the material. Blalock et al. evaluated two composite materials with similar filler contents (77% and 60%) heated to 54°C; they reported that there is a more than two-fold difference between the film thicknesses of the two materials brought to the desired temperature.³¹ The heating process does not directly affect the ceramic-containing inorganic particle. The heating process before polymerization increases the fluidity of the material by affecting the ease of movement of the filler particle in the heated resin matrix, so the organic matrix structure of the resin materials can affect the fluidity. Blalock et al.³¹ explained that the difference in film thickness between these two materials with close filler contents is very high due to the difference in the organic matrix structure of the materials. Lee et al.³² investigated the viscosity changes by increasing the temperature according to the organic matrix composition of resin-containing materials. When the temperature was increased from 25°C to 35°C, the viscosity decreased

significantly and the material with an organic matrix structure with high Bis-pheno A glycidyl metakrilat (Bis-GMA) content became more fluid as the temperature increased in comparison to the material with high Trietilen Glikol Dimetakrilat (TEGDMA) content. That study shows that the organic matrix structure is also effective in changing the fluidity value of resin-containing materials with a temperature increase.³²

In our study, when the fluidity values were compared regardless of the material groups and the different temperatures, the highest fluidity median value was obtained at 55°C in the group without filler and the lowest fluidity median value was found at 4°C in the group containing 55% filler. Since the organic matrix ratio was higher in the filler-free group than in the other filler-containing groups, the highest fluidity value was reached at 55°C, that is, at the highest temperature. When the filling ratio of the fissure sealant is the highest, the organic matrix ratio is the lowest and the effect of the heating process on the fluidity value decreases. Supporting this, the lowest fluidity value was found in the group containing high filler: 55% at 4°C. Loumprinis et al. measured the fluidity values of composites at five different temperatures, and the highest fluidity value was measured in the composite with the lowest filler ratio and at the highest temperature of 54°C.²⁴ Marcondes et al.²³ evaluated the viscosity of 10 restorative resin composites by heating them to 39°C and 68°C; it was determined that the lowest viscosity value was found at 68°C and in the group with the lowest filler ratio. In these studies, similar to our study, it was reported that when the filling ratio of the material decreased and the heating process was applied, the fluidity decreased.

CONCLUSION

As a result of our study, it is thought that the filler ratio and temperature have an effect on the fluidity of fissure sealants. Thus, to ensure best fluidity, the appropriate temperature should be selected by considering the filler ratios. Since there are very few studies in the literature on this subject, the findings of this study need to be supported by in vitro and in vivo studies.

Etik Komite Onayı: Bu çalışma in vitro materyal değerlendirmesi olduğundan Etik Kurul Onayı gerektirmemektedir.

Hasta Onamı: İn vitro çalışma olduğundan onam formu alınmamıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir – EHB; Tasarım - EHB; Denetim - EHB; Kaynaklar -İŞÇ; Malzemeler -İŞÇ; Veri Toplanması ve/veya İşlemesi -İŞÇ; Analiz ve/veya Yorum - İŞÇ; Literatür Taraması – EHB, İŞÇ; Makaleyi Yazan - İŞÇ; Eleştirel İnceleme - EHB; Diğer –EHB, İŞÇ.

Çıkar Çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.

Finansal Destek: Hibe bilgisi ve diğer mali destek kaynakları mevcut değildir.

Ethics Committee Approval: This study is an in vitro material evaluation and therefore does not require an Ethics Committee

Informed Consent: Since it was an in vitro study, no consent form was taken

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - EHB; Design - EHB; Supervision - EHB; Resources -ISC; Materials - ISC; Data Collection and/or Processing - ISC; Analysis and/or Interpretation - ISC; Literature Search – EHB, İŞÇ; Writing Manuscript - ISC; Critical Review - EHB; Other –EHB, İŞÇ

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