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THE EFFECT OF PROBIOTIC MILK IMMERSION ON BULK-FILL COMPOSITE RESIN DISCOLORATION

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ABSTRACT

Backgrounds*: Bulk-fill composite resin is a new type of composite resin that has the ability to absorb polymerization rays up to a depth of 4-5 mm in a single cure because of the modification in its components formation. The main cause of composite resin restorations failure is discoloration. Discoloration may occur due to extrinsic factors such as consuming food or drink which is rich in color and low pH, namely probiotic milk. Consuming probiotic milk for a long time will significantly increase the risk of composite resin discoloration. This significant discoloration eventually causes shorter resistance of restoration in oral environment regarding the decrease in aesthetic value.* ***Objectives:*** *To determine the effect of probiotic milk immersion on bulk-fill composite resin discoloration.* ***Methods:*** *This research was true experimental study with pretest-posttest with control group design that consisted of six groups in total of thirty six samples. Discoloration will be analyzed using digital analysis with the CIELAB method.* ***Results:*** *One-way ANOVA and Post-Hoc Games-Howell tests showed significant differences on bulk-fill composite resin discoloration after immersion in probiotic milk and distilled water (control) for 6, 12 and 18 hours ($p<0,05$). Only in the immersion groups of 6 hours and 12 hours probiotic milk did not have a significant difference on discoloration ($p>0,05$).* ***Conclusion:*** *There is an effect of probiotic milk immersion on bulk-fill composite resin discoloration.*

Keywords : *Bulk-fill composite resin, digital analysis, discoloration, probiotic milk.*

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INTRODUCTION

Restorative materials commonly used in dentistry are amalgam, glass ionomer cement and composite resin. Composite resin is a restoration material that is most often used in dentistry because it has the best esthetics. It is considered to be quite strong, safe and easy to apply and has the most similar mechanical properties to natural teeth compared to other material choices.[1,2] According to Tyas et al. data on one hundred restoration users revealed that 55% of respondents used composite resin restorations, 28% of respondents used amalgam restorations, 15% of respondents used glass ionomer cement and the remaining 2% used polyacid modified composite resin.[3]

In 2010, a new type of composite resin was introduced which has a modification to its manufacturing component, namely bulk-fill composite resin.[4] The manufacturer modifies the initiator and matrix components and reduces the number of particles of inorganic fillers to optimize the polymerization process and increase the penetration depth of light to 4-5 mm in a single cure.[5] This material can be used as direct restoration for anterior and posterior teeth.[6] Bulk-fill composite resin (Tetric N-Ceram Bulk-fill) contains ivocerin which absorbs more light and can increase quantum efficiency so that it can help accelerate polymerization to the bottom of the cavity.[7]

According to Opdam et al, composite resin restorations were only able to last for 5-10 years.[8] The main reasons for failure of composite resin restorations were due to the occurrence of secondary caries, fractures and changes in color restoration.[9] More than 80% of patients were concerned about discoloration of resin restorations the composites they use.[3] Discoloration in composite resins can occur due to intrinsic factors and extrinsic factors. Discoloration due to intrinsic factors occur when the quality of the organic matrix, initiator components and particles of inorganic fillers used are poor. Discoloration due to extrinsic factors occur when someone consumes foods or drinks that are rich in color such as soy sauce, fruit juice, tea, or coffee; smoke; use mouthwash; and consume drinks that have low pH such as carbonated drinks and probiotic milk.[3,10]

Probiotic milk are one type of drink that is consumed by many people every day for health reasons.[11] According to the Yakult Company Profile in 2009, the consumption of this brand of probiotic milk can reach 25 million people every day in twenty seven countries around the world including Indonesia and in the latest data from 2017 to 2018, information is obtained that the amount of beverage consumption has increased to 28 million consumers every day in thirty eight countries around the world. Drinks that contain probiotic bacteria have many advantages when consumed regularly.[12,13]

Probiotic milk have a relatively low pH of around 3.54.[14] Drinks that have a low pH can trigger a softening of the matrix in composite resins which will affect the integrity of composite resins.[3] Low pH content releases H^+ ions and binds to bonds between matrix, particles filler and coupling agent so that the bond is released and causes composite resin degradation. Bonding between less dense matrix components can increase the resorption of water and dyes which can eventually cause discoloration in composite resins. If the drink of probiotic milk is consumed for a long time, it is feared that this will have an impact on the significant discoloration of the composite resin.[15]

The significant discoloration eventually caused the duration of restoration to be shorter than it should be due to reduced aesthetic factors.[16] According to the Istibsyaroh research in 2018 regarding discoloration of nanofiller composite resins soaked in fermented milk drinks with immersion times of 6 hours, 12 hours and 18 hours found that the longer the time of immersion, the brighter composite resin restoration in color. This is caused by the accumulation of natural white dyes in milk. This causes the color of restoration to be different from the color of the teeth resulting in a restoration that looks ugly and requires replacement.[8] From the above description, it is necessary to do research on the effect of probiotic milk immersion on bulk-fill composite resin discoloration.

MATERIALS AND METHODS

This research has received research ethics permission with No.118 / KEPKG-FKGULM / EC / I / 2019 issued by the Health Research ethics committee Dentistry Faculty of Lambung Mangkurat University. This type of research was true experimental study with a pretest-posttest with control group design. This research consisted of six groups with a total of thirty six samples.

Sample Making Procedure

Composite resin samples was fabricated using molds made from transparent acrylic material with a thickness of 4 mm and 8 mm in diameter in the form of discs.[17] Glass plates were used as the bottom mold base then composite resin material was taken and applied using plastic filling instruments into the molds provided. A condenser is used to condense composite resin on the mold. Celluloid strips and glass slides were placed on top of the sample mold to obtain a smooth sample surface without polishing. Composite resins were illuminated for 10 seconds (according to factory rules) using light curing units of Woodpecker Type I LEDs. The LED end was 1 mm away from the composite resin surface. Composite resin which had been irradiated was removed from the mold.

Test Solution and Control Solution Preparation

A 500 ml beaker glass with six pieces was prepared. Each beaker was given the name of the group using a permanent marker. The test solution in the form of probiotic milk of the Yakult brand of sour milk was included as much as 300 ml each which was measured with a measuring glass and put into a group I, II and III beaker. The control solution in the form of sterile distilled water was put in 300 ml each which was also measured by a measuring glass and put into beaker IV, V and VI.

Immersion and Sample Testing

Artificial saliva solution was inserted into a measuring glass until it reached a volume of 300 ml and was put into a beaker. Samples from each group were taken using tweezers and soaked in an artificial saliva solution that had previously been provided, then the beaker was put into an incubator at 37^0C for 24 hours and measured the length of immersion with the stopwatch. After all the samples have been soaked, one by one the samples were taken with tweezers and placed in an aluminum tray that had been given a sterile gauze bed. The color values of the samples were measured before immersion in drinks of probiotic milk and sterile distilled water by using a digital analyzer detected by using the color component extraction software system of the Commission Internationale de L'Eclairage L*a*b* (CIELAB) and color coordinate values would be obtained L*, a* and b* before treatment. The samples were then immersed in each group, namely in beaker I, II and III for the time of soaking probiotic milk for 6 hours, 12 hours and 18 hours; and in beakers IV, V and VI for distilled water immersion in 6 hours, 12 hours and 18 hours. After that, all the beakers were put in an incubator at 37^0C and soaked according to the specified time. Samples that have been soaked according to the group, then taken one by one from the beaker using tweezers and placed in an aluminum tray that has been given sterile gauze. The sample then measured the color coordinates of L*, a* and b* after treatment using a digital analysis tool with the Commission Internationale de L'Eclairage L*a*b* (CIELAB) color system standard as in the previous measurement.

Discoloration Measurement

The measurement of the discoloration value was done after the color coordinate values L *, a * and b * before and after the sample treatment were obtained. Each sample was taken two times, namely before and after treatment by an observer. The bulk-fill composite resin samples which will be measured in color coordinates were included in a dark box for the process of taking pictures using a high resolution digital camera (webcam). The sample was placed in the middle while adjusting to the camera. To standardize light conditions when shooting, LED lights were used as light sources. The results of digital images would automatically be saved in JPG format and the discoloration value could be seen using the color component extraction software. Discolorations could be detected by Commission Internationale de L'Eclairage L*a*b* (CIELAB) color system recommended by ADA. The discoloration in each sample was calculated by the standard formula as follows.[8]

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

$\Delta L^* = L^*_o - L^*_t$
$\Delta a^* = a^*_o - a^*_t$
$\Delta b^* = b^*_o - b^*_t$

Description :
ΔE : Discoloration
L* : Brightness coordinate with a scale of 0 (black) to 100 (white).
a* : Cromatic coordinate with a scale of green (a<0) to red (a>0).
b* : Cromatic coordinate with a scale blue (b<0) to yellow (b>0).
L^*_t, a^*_t, b^*_t : Before immersion
L^*_o, a^*_o, b^*_o : After immersion

RESULTS

Table 5.1 Mean and standard deviation of discoloration (ΔE) and color components (ΔL*, Δa*, Δb*) in bulk-fill composite resin.

Groups	ΔL*	Δa*	Δb*	ΔE
I	2,53 ± 0,53	-0,48 ± 0,08	-2,39 ± 0,18	4,15 ± 0,73
II	2,75 ± 0,48	-0,11 ± 0,01	-2,69 ± 0,58	5,25 ± 0,51
III	4,06 ± 0,19	-0,68 ± 0,07	-5,00 ± 0,54	7,42 ± 1,05
IV	0,39 ± 0,04	-0,03 ± 0,005	-0,05 ± 0,001	0,84 ± 0,15
V	0,96 ± 0,002	-0,65 ± 0,08	-0,08 ± 0,01	1,48 ± 0,30
VI	2,41 ± 0,51	-0,29 ± 0,05	-0,12 ± 0,02	2,61 ± 0,53

Table 5.1 shows that the mean discoloration and the mean color component in the bulk-fill composite resin studied. Regarding discoloration (ΔE) based on the duration of probiotic milk immersion, it was found that the highest mean was in the 18-hour immersion group, while the lowest mean group was in the 6-hour immersion group. When compared with their respective control groups, all groups immersed in probiotic milk had higher mean discolorations. On the mean difference in the value of the light-dark coordinate (ΔL*) based on the duration of probiotic milk immersion, it was found that all values were positive (brighter) and the highest mean was in the 18-hour immersion group, while the lowest mean group was in the 6-hour soaking. When compared with their respective control groups,

all groups immersed in probiotic milk had a higher positive ΔL*. Meanwhile, the mean of red-green coordinate values (Δa*) based on the duration of probiotic milk immersion presented in negative result (greener) and the highest mean was in the 18-hour immersion group, while the lowest mean group was in the 12-hour immersion group. When compared with their respective control groups, all groups immersed in probiotic milk had a higher negative Δa* except for the 12-hour immersion group. On the mean difference in the yellow-blue coordinate value (Δb*) based on the duration of probiotic milk immersion, it was found that all mean values were negative (bluer) and the highest mean was in the 18-hour immersion group, while the lowest mean group was in the 6-hour immersion group. When compared with their respective control groups, all groups immersed in probiotic milk had a higher negative Δb*.

Table 5.2 The result of Shapiro-Wilk normality test for discoloration (ΔE) and color component (ΔL*, Δa*, Δb*) mean on bulk-fill composite resin.

Groups	Sig. (p)			
	ΔL*	Δa*	Δb*	ΔE
Group I	0,305	0,583	0,955	0,068
Group II	0,630	0,983	0,231	0,383
Group III	0,113	0,140	0,242	0,613
Group IV	0,601	0,989	0,762	0,279
Group V	0,058	0,269	0,099	0,495
Group VI	0,931	0,140	0,126	0,498

Based on Table 5.2, it was found that the values of ΔE, ΔL*, Δa* and Δb* which were analyzed by the Shapiro-Wilk normality test produced $p > 0.05$ in all groups, which meant that all group data were normally distributed. The data analysis was then followed by a homogeneity test using Levene 's test to find out the group variants that can be seen in table 5.3.

Table 5.3 The result of Levene homogeneity test for discoloration (ΔE) and color component (ΔL*, Δa*, Δb*) mean on bulk-fill composite resin.

Mean	Sig. (p)
ΔL*	0,000
Δa*	0,842
Δb*	0,02
ΔE	0,01

Based on table 5.3, the results of the Levene homogeneity test on the values of ΔE, ΔL* and Δb * have p values < 0.05 indicating that the data variants were not homogeneous. The homogeneity test results at the value Δa * had a value of p> 0.05 which indicates that the data variant was homogeneous. All data were normally distributed even though not all data is homogeneous so that it could be continued with the one way ANOVA parametric test. Data analysis was then followed by a one way ANOVA parametric test with a confidence level of 95% ($p = 0.05$). The results of the one way ANOVA test can be seen in table 5.4.

Table 5.4 The result of one way ANOVA parametric test for discoloration (ΔE) and color component (ΔL*, Δa*, Δb*) mean on bulk-fill composite resin.

Nilai rerata	Sig. (p)
ΔL*	0,07
Δa*	0,39
Δb*	0,001
ΔE	0,0001

Based on table 5.4, the results of one way ANOVA parametric test on the mean ΔL* and Δa* presented a value of p> 0.05 which indicates that there is no significant difference between the treatment groups. The value of ΔL* means that there is no effect in the form of a brighter discoloration than bulk-fill composite resin after being immersed in a probiotic milk drink. The value of Δa* means that there is no effect in the form of a greener discoloration than bulk-fill composite resin after being immersed in a probiotic milk drink. The results of the one way ANOVA test on the mean ΔE and Δb* resulted in a value of $p < 0.05$ which indicates that there are significant differences between treatment groups. For the value of ΔE, it means that there is an effect of probiotic milk immersion on the discoloration of bulk-fill composite resin. For the value of Δb*, it means that there is an influence in the form of a more blue discoloration from bulk-fill composite resin after being immersed in a probiotic milk.

Follow-up tests was performed using the Games-Howell Post Hoc test on mean values ΔE and Δb* to determine groups which represent any significant differences.

Table 5.5 The result of Post Hoc Games-Howell test for discoloration (ΔE) on bulk-fill composite resin.

	K1	K2	K3	K4	K5	K6
K1	-	0,105	0,001*	0,001*	-	-
K2		-	0,019*	-	0,000*	-
K3			-	-	-	0,000*
K4				-	-	-
K5					-	-
K6						-

* : There is a significant difference (p<0,05)

Table 5.5 shows the results of Games-Howell Post Hoc test among treatment groups. All groups that were compared had a significant difference in discoloration values because the value of p <0.05, only group I compared to (immersion in probiotic milk for 6 hours) group II (immersion in probiotic milk for 12 hours) which has a difference in discoloration value that is not meaningful or can be interpreted comparable result in discoloration between these two groups with p value less than 0.05 (p = 0.105).

Table 5.6 The result of Post Hoc Games-Howell test for discoloration (Δb*) on bulk-fill composite resin.

	K1	K2	K3	K4	K5	K6
K1	-	1,000	0,390	0,145	-	-
K2		-	0,783	-	0,555	-
K3			-	-	-	0,028*
K4				-	-	-
K5					-	-
K6						-

* : There is a significant difference (p<0,05)

Table 5.6 shows the results of the Games-Howell Post Hoc test between treatment groups. All groups that have been compared have different yellow-blue coordinate difference values (Δb*) which are not significant because the value of p>0.05. Only group III comparison (immersion in probiotic milk for 18 hours) with group VI (immersion in probiotic milk for 18 hours) has a significant difference in Δb* values because p value is less than 0.05 (p = 0.028).

DISCUSSION

The effect of probiotic milk immersion on bulk-fill composite resin discoloration research is resulted in the presence of probiotic milk immersion effect on bulk-fill composite resin color. The mean value of bulk-fill composite resin discoloration soaked in probiotic milk has increased with the length of time immersion conducted. The length of immersion time of composite resin samples carried out in this research was 6 hours, 12 hours and 18 hours, according to the research of Istibsyaroh et al in 2018 which stated that nanofil composite resins undergo discoloration after being immersed in fermented milk drinks during this period. Soaking bulk-fill composite resin for 6 hours is in accordance with the assumption that someone has the habit of consuming 2 bottles of probiotic milk every day in a row in 2.5 years. Soaking bulk-fill composite resin for 12 hours is in accordance with the assumption that a person has the habit of consuming 2 bottles of probiotic milk every day in a row within 5 years. Soaking composite resin type bulk-fill for 18 hours is in accordance with the assumption that a person has a habit of consuming 2 bottles of probiotic milk every consecutive day in seven and half years.[8]

The results of this research are in line with Kaunang et al's 2015 research of the differences in nanofil composite resins discoloration after immersed in factory fruit juices and fresh soaked juices for 3 days, 6 days and 9 days which showed that longer duration of immersion will increase the degree of discoloration. Discolorations occur because of the presence of dyes in the solution that accumulates in the composite resin restoration.[17] The longer the composite resin is exposed to the solution, the more absorption occurs in the matrix. The absorption process can interfere with the silane bond (coupling agent) with the filler material called siloxan bond so that the bond is broken which eventually causes the hydrolysis process to occur. The hydrolysis process is characterized by the release of particles from the composite resin.[19]

Factors that can affect the discoloration of composite resin consist of extrinsic and intrinsic factors. Extrinsic factors that cause discolorations can be in the form of drinking habits that have a low pH and coloring agent.[3,10] The treatment groups I, II, and III used immersion solutions in the form of probiotic milk, while groups IV, V and VI used immersion solutions in the form of distilled water sterile which is useful as a control solution. Probiotic milk have a relatively low pH of around 3.54.[14] Sterile distilled water has a neutral pH of 7.[20]. In the sample group immersed in probiotic milk (groups I, II and III)

have a higher mean discoloration than the soaked sample group in sterile distilled water (groups IV, V and VI). This happens because probiotic milk has a lower pH compared to distilled water. Probiotic milk has a low pH because they contain lactic acid. Acid-containing solutions have high H^+ ions. The H^+ ion will react with a double bond carbon group (C =) in the nanohibride composite resin polymer chain which will damage the polymer bond.[21] The degraded polymer component will cause the filler and dyestuff components to be released so that the composite resin color becomes brighter. This will also cause microcracks or crevices in the composite resin to form so that it will be susceptible to absorption of dyes from the outside which can cause discoloration.[3,22] The results of this research are also in accordance with the research conducted by Aulia et al in 2017 stating that the higher the pH of the solution, the lower the discoloration value produced therefore composite resin soaked in sterile distilled water experienced a lower discoloration.[21] Sterile distilled water has a neutral pH, but can experience slight discoloration due to the natural nature of the composite resin matrix which is hydrophilic. The hydrophilic properties make composite resins easier to absorb fluids from the outside and if the absorption of the liquid occurs continuously it will cause accumulation of fluid which suppresses composite resin components which will eventually form a microcrack or cracks between the components so that it will facilitate the penetration of the dyestuff from outside and the presence of discoloration.[23]

Discolorations can also occur because of the dyestuff content of the solution accumulated on the composite resin surface. The dyestuff components of probiotic milk are white and greenish. White substances are produced by colloidal fat grains, calcium casein and calcium phosphate. Greenish substances are produced by riboflavin dyes derived from animal feed. This study result indicates that treatment groups I, II, and III experience a significant change in color component after the immersion in probiotic milk. This can be observed from the mean value of ΔL* (light-dark), Δa* (red-green) and Δb* (yellow-blue).[16,24]

In this research, all groups obtained positive ΔL*. The mean value of ΔL* was increase which can be interpreted as brighter result in accordance with longer immersion time.[8] All groups obtained negative result in Δa*. The mean value of Δa* was decrease which can be interpreted as greenish result in accordance with longer immersion time, although group II has a value of Δa* higher than group I. This occurs because the content of the green color possessed by probiotic milk varies depending on the feed consumed and the type of milk produced by livestocks. But in the values of Δa*, group I and II are higher than group III so that it can be interpreted that the longer the immersion, the more greenish and reddish composite resin in color. All groups get negative result for Δb* with increase in soaking time. The mean value of Δb* was decrease which can be interpreted that the longer the immersion, the more bluish and yellow the composite color will be. This happens because the dyes contained in the probiotic milk are absorbed by composite resins and the dyes possessed by dissolved composite resins because the immersion solution has a low pH.[3]

Solutions that have a low pH can result in a bond between composite resin matrix and coupling agent that strengthens the link between to-be-damaged matrix and filling material. The matrix and coupling agent bonding that is damaged causes the release of components from the composite resin, namely fillers and dyes. The composite resin color becomes brighter (ΔL* positive) because it absorbs white substances from probiotic milk and composite resin dye components namely ferric oxide (red) and ferric hydroxide (yellow) are released. The composite resin color becomes greenish and the red color decreases (Δa* negative) because it absorbs riboflavin dyes and the red dye component (ferric oxide) is released. The composite resin color becomes bluish and the yellow color decreases (Δb* negative) due to the release of the composite resin yellow substance (ferric hydroxide) .[3,21,24]

This is in accordance with Effendi et al's research in 2014 which stated that the dye component from its immersion solution in the form of soda strawberry flavor and lemon soda caused a discoloration in the nanohibride composite resin. Soaking the composite resin in strawberry soda causes a positive increase in the mean value of Δa* which means that the color of the composite resin becomes redder as the red dye of the solution is absorbed. On the contrary, immersion of composite resin in lemon soda will increase the mean value of ΔL* into positive axis which can be interpreted that the color of composite resin becomes brighter because composite resin dyes in the form of ferric oxide (red) and ferric hydroxide (yellow) are detached. It was found that in groups IV, V

and VI treated with the immersion in sterile distilled water (control solution) experienced a slight change in the mean value of ΔL* (light-dark), Δa* (red-green) and Δb* (yellow-blue). This is consistent with Al-Abdullah et al's research in 2017 which stated that bulk-fill composite resin after immersion in sterile distilled water only experienced a slight change in color due to the absence of the dye component in this solution.[25]

In the results of the statistical analysis of this research, it was found that all treatment groups resulted in a significant difference in discoloration (ΔE) except for 6 hours and 12 hours probiotic milk immersion time. The results of statistical analysis on the mean value of ΔL* produced were also not significantly different in all treatment groups. This result is different from a previous research conducted by Istibsyaroh et al in 2018 which found that there were significant differences in discolorations and color of composite resins to be brighter in all treatment groups.[8]

This happens because there is a difference in the type of composite resin used. In this research bulk-fill composite resin was used, whereas in previous studies using nanofil composite resin. Bulk-fill composite resin has several advantages in the components used. Bulk-fill composite resin uses ivocerin as an initiator booster that can increase and accelerate polymerization so that the resulting in better quality of composite resin.[7] Bulk-fill composite resin possesses a nanohybride type filler. Nanohybrid composite resin has advantages, one of which is good polishing results because it has nano and micro sized filler particles so that the gap between the filler particles becomes smaller and will produce smoother composite resin surface. A smoother composite resin surface will reduce the possibility of dye retention from the solution.[18] Bulk-fill composite resin contains UDMA matrix which has the ability to reduce water absorption because it can reduce the hydrophilic properties of composite resins.[10] Based on the results of this research it can be concluded that there is an effect of probiotic milk immersion on bulk-fill composite resin discoloration.

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