Effect of Light Curing Modes on the Color Stability of a Nanohybrid Composite Immersed in Different Beverages

Gunce Ozan PhD¹; Hande Sar Sancakli DDS²; Murat Tiryaki PhD¹; Isil Bayrak PhD³
1. Department of Restorative Dentistry, Faculty of Dentistry, Istanbul University, Turkey.
2. Associate Professor, Department of Restorative Dentistry, Faculty of Dentistry, Istanbul University, Turkey.
3. Private clinic, Dentist.

Correspondence to: Dr. Gunce Ozan - gunce.saygi@istanbul.edu.tr

Received: 1-VII-2019                        Accepted: 12-VIII-2019  Published Online First: 21-VIII-2019

ABSTRACT

Aim: Sufficient polymerization remains as crucial for composites to prevent discoloration. Both LEDs and halogen units are used for polymerization and different curing modes are improved to overcome inadequate polymerization. This in vitro study investigated staining susceptibility of a nano-hybrid resin composite light-cured in different modes and immersed in different staining media. Methods: Disc-shaped specimens were prepared from nano-hybrid resin composite (Filtek Z550) and light-cured according to following modes: Halojen (GI), LED standard (GII), LED pulse (GIII) or LED ramp (GIV). Half of the specimens of each group (n=7) were stored in one of the staining media (red wine or coffee) for 10 min/day during experimental period. Measurements were performed using spectrophotometer according to CIEL*a*b system at baseline and on 7, 28 and 56 days. Colour differences (∆E) between groups were submitted to statistical analysis. Results: Regarding 7-day evaluation, colour change values of specimens immersed in coffee revealed no remarkable difference among curing modes (p>0.05); whereas specimens in GIV were significantly less stained compared to specimens in GII and GIII when immersed in red wine. Regarding 56-days of evaluation, specimens of GIV showed statistically significant colour change in red wine compared to other groups. However specimens in GI revealed the least staining after 56-days of coffee immersion, and this result was statistically different from LED groups. Conclusion: This study suggest that light-curing mode influences the staining susceptibility of the tested composite. Composites polymerized with halogen unit showed lower staining than all LED modes. Despite novel light sources, halogen units may still used reliably at clinics.

KEYWORDS

Composite resin; LED units; Halogen units; Light curing modes; Color stability; Polymerization.
RESUMEN

Objetivo: La polimerización suficiente sigue siendo crucial para que los compuestos eviten la decoloración. Tanto los LED como las unidades halógenas se utilizan para la polimerización y se mejoran los diferentes modos de curado para superar la polimerización inadecuada. Este estudio in vitro investigó la susceptibilidad a la tinción de un compuesto de resina nano-híbrida curado a la luz en diferentes modos y se sumergió en diferentes medios de tinción. Métodos: Las muestras en forma de disco se prepararon a partir de un compuesto de resina nano-híbrida (Filtek Z550) y se curaron con luz de acuerdo con los siguientes modos: Halogen (GI), estándar de LED (GII), pulso de LED (GIII) o rampa de LED (GIV). La mitad de las muestras de cada grupo (n = 7) se almacenaron en uno de los medios de tinción (vino tinto o café) durante 10 minutos / día durante el período experimental. Las mediciones se realizaron utilizando un espectrofotómetro de acuerdo con el sistema CIEL * a * b en la línea de base y en 7, 28 y 56 días. Las diferencias de color (∆E) entre los grupos se sometieron a análisis estadístico. Resultados: Con respecto a la evaluación de 7 días, los valores de cambio de color de las muestras sumergidas en café no revelaron diferencias notables entre los modos de curado (p> 0.05); mientras que las muestras en GIV se tiñeron significativamente menos en comparación con las muestras en GII y GIII cuando se sumergieron en vino tinto. Con respecto a los 56 días de evaluación, las muestras de GIV mostraron un cambio de color estadísticamente significativo en el vino tinto en comparación con otros grupos. Sin embargo, las muestras en GI revelaron la menor tinción después de 56 días de inmersión en el café, y este resultado fue estadísticamente diferente de los grupos LED. Conclusión: este estudio sugiere que el modo de fotopolimerización influye en la susceptibilidad de tinción del material compuesto probado. Los compuestos polimerizados con una unidad halógena mostraron una tinción más baja que todos los modos LED. A pesar de las nuevas fuentes de luz, las unidades halógenas todavía pueden usarse de manera confiable en las clínicas.

PALABRAS CLAVE

Resina compuesta; Unidades LED; Unidades halógenas; Modos de fotopolimerización; Estabilidad del color; Polimerización.

INTRODUCTION

Resin-based composites are the most preferred direct restorative materials with distinguished esthetic appearances and excellent polishing abilities due to improved nanoparticles in their compositions. Among their advantageous properties, ideal polymerization is a crucial factor for substantial clinical performance. Besides their multi-incremental manipulation capability, inadequate polymerization of light-cured composite materials causes undesirable clinical situations such as yellowish discolorations, pulpal irritations, post-operative sensitivity, or even failures of restorations (1-3). Therefore, efficient polymerization is a major necessity in the application procedure to overcome problems.

One of the most common reasons for replacement of composite restorations is that the esthetics are affected by discolorations of the resin-based materials (4). Therefore, the color stability of composite restorations is a necessity and the color is expected to remain stable throughout the functional lifetime. Both intrinsic and extrinsic factors cause discoloration of resin composites (1-4,5). External discolorations result from adsorption and absorption of water-soluble substances through the resin matrix. Certain dietary habits such as
drinking coffee, tea, cola, red wine, or whiskey or oral habits like tobacco chewing could stain composites to varying degrees (6-13). Besides that, permanent internal discolorations could be related to resin material consistency. The resin matrix, the interface between the matrix and the fillers, the type and amount of fillers, and polymer quality have a considerable influence on discoloration (14). Due to inadequate polymerization, the matrix could easily absorb colorants of beverages and water and thus subsurface staining would be revealed, as well.

Polymerization efficiency is mandatory to achieve higher degrees of conversion leading to smaller amounts of residual monomers, which are responsible for the color deterioration (14). However, ideal polymerization relies on both the materials’ characteristics and the effectiveness of the light-curing units. There are numerous factors related to the ability of curing units to supply adequate polymerization, such as light intensity, wavelength, exposure duration, size, and location of the tip of the source (15-16). Although conventional quartz tungsten halogen (QTH) units are being used for the polymerization of composite materials, many different types of light sources have also been applied, including plasma arc, argon laser, and light-emitting diodes (LEDs) (16). QTH units are used continuously for a long time with their wavelengths of 400-500 nm with output from 400 to 800 mW/cm², which is suitable for a photoinitiator mainly found in composites (17). Nevertheless, they have certain limitations due to the drop in irradiance and heat production. The bulb and the filter of the QTH units should be calibrated regularly in order to prevent lack of polymerization (16-19). On the other hand, LED sources with a narrow blue light spectrum have been employed as satisfactory units for composite resin polymerization due to their definite light-emitting spectrum only regulated for maximum absorption of camphorquinone (468 nm) (20). “Poly wave” LED units, which emit both violet (410 nm) and blue (470 nm) light, were introduced to markets for affecting both camphorquinone and specific initiators such as Ivocerin (17). Moreover, the emission spectrum of LEDs could be advantageous in order to minimalize the formation of byproducts that alter the color of resin composites (21). LED sources convert electricity into light in a more efficient way, enabling their use for many hours without extra filters or outputs producing less heat (22).

Numerous studies have compared LEDs and QTH units with different emission spectra; however, a relevant relation between the polymerization efficiency and the color stability of composites has not been found yet (21). Furthermore, different curing modes such as ramp and pulse have recently emerged to improve polymerization by affecting polymer composition, degree of conversion, and number of cross-links (20). Moreover, some authors have indicated that curing modes reduce polymerization contraction forces and stress (16,23). It could be beneficial to select not only the appropriate light devices but also different curing modalities for planned restorative treatments.

The aim of this study is to evaluate the staining susceptibility of a nanohybrid resin composite immersed in two different beverages (red wine and coffee) and polymerized with either various curing modes of LED sources (standard, pulse, ramp) or with QTH (as a control) for different immersion periods. With this methodology, not only will the coloring effect of beverages be evaluated, but the impact of different curing modes of LED and QTH sources on color stability will also be investigated. The hypothesis set was that (1) different curing modes, (2) types of beverages, and (3) immersion periods would all affect the color change of the nanohybrid resin composite.
MATERIALS AND METHODS

Fifty-six disc-shaped specimens (8 mm in diameter and 2 mm in thickness) were prepared from a nanohybrid resin composite (Filtek Z550; 3M ESPE, Seefeld, Germany) using a custom-made stainless steel mold. The resin material was inserted into the mold standing on a glass plate with a Mylar strip in one increment. Subsequently, the top surface of the resin-filled mold was covered with another polyester strip and a glass plate was placed onto it. Constant pressure (weight of 1 kg) was applied to the glass plate for 15 seconds to remove the excess resin from the specimen’s surface, thus obtaining a flat specimen surface without bubble formation. Following removal of the weight and the glass plate, the resin material was polymerized with either a QTH unit (Hilux; Benlioğlu Dental, Ankara, Turkey) or LED unit (SmartLite Max; Dentsply, York, PA, USA) according to the assigned group design. The QTH unit was used as a control (GI) and the LED units were used as experimental groups according to the following modes (n=14): LED standard (GII), LED pulse (GIII), or LED ramp (GIV) (Table 1).

The light-curing unit tip was positioned perpendicular to specimens’ surfaces and the distance between the tip and specimen was standardized using a glass microscope slide (1 mm in thickness). After removal of the specimens from the molds, samples were scored at the bottom surface of each with a scalpel. All specimens were stored in distilled water at 37±1 °C for 24 hours for completion of polymerization. The top surface of each specimen was polished with flexible aluminum oxide discs (Sof-Lex; 3M ESPE, Seefeld, Germany) under running water for 15 seconds for each step. Polishing was performed by the same operator in order to eliminate operator-dependent variability and Sof-Lex discs were renewed after the third use. Half of the specimens of each group (n=7) were stored in one of the staining media (red wine or coffee) for 10 min/day during the experimental period. A non-sweetened black coffee solution of 30 mL was prepared using a 3:10 powder mass ratio. The drinks were used at room temperature in order to standardize consumption. The composition of the composite material used and the properties of beverages tested in the study are shown in Tables 2 and 3.

The specimens were kept immersed in distilled water at 37±1 °C in the interval between cycles. The immersion regimen was followed for 56 days. Color measurements were performed against a white background using a spectrophotometer (Vita Easyshade; Vita Zahnfabrik, Bad Säckingen, Germany) according to the CIE L*a*b* system at the end of one week, 28 days, and 56 days. Before measurements, the top surface of each specimen was blotted dry using tissue paper and the contact guide of the spectrophotometer was positioned on the center of the specimen’s surface. Three consecutive readings were obtained for each specimen and then averaged. The color differences (ΔE) were calculated using the following equation:

\[ \Delta E: \sqrt{((L_0 - L_i)^2 + (a_0 - a_i)^2 + (b_0 - b_i)^2)} \]

Here, \( \Delta E \) is color change, \( L \) is luminance reflectance, \( a \) is red-green color coordinate, \( b \) is yellow-blue color coordinate, \( 0 \) represents baseline, and \( i \) represents after treatment.
Table 1. Light-curing units and modes used in the study.

<table>
<thead>
<tr>
<th>Light-Curing Unit</th>
<th>Light Source</th>
<th>Power Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hilux</td>
<td>Halogen</td>
<td>480-530 mW/cm²</td>
</tr>
<tr>
<td>SmartLite Max</td>
<td>LED</td>
<td></td>
</tr>
</tbody>
</table>

- Standard mode: The light output is at a steady 1200 mW/cm².
- Pulse mode: The light output is pulsed 10 times a second, giving a net effect of half the power output as the continuous mode.
- Ramp mode: There is a steady increase of the output from 0 to 1200 mW/cm² within 4 seconds and then it remains constant.

Table 2. The composition of the nanohybrid resin composite used in the study.

<table>
<thead>
<tr>
<th>Nanohybrid Resin Composite</th>
<th>Brand</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Filtek Z550, 3M ESPE</td>
<td>Bis-GMA, UDMA, TEGDMA, BisEMA, PEGDMA, 20 nm silica, 1-10 µm zirconia/silica particles</td>
</tr>
</tbody>
</table>

Table 3. Properties of the tested beverages.

<table>
<thead>
<tr>
<th>Beverages</th>
<th>pH</th>
<th>Storage Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red wine, Villa Doluca</td>
<td>3.72</td>
<td>Specimens in each group were immersed in containers containing 35 mL of the respective beverages</td>
</tr>
<tr>
<td>(14% alcohol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee, Nestlé-Nescafé Classic</td>
<td>5.24</td>
<td></td>
</tr>
</tbody>
</table>

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS 15 for Windows (SPSS Inc., Chicago, IL, USA). Non-parametric tests were chosen regarding the number and abnormal distribution of the specimens in the present study. Color differences (ΔE) for each immersion medium were submitted to chi-square and Kruskal-Wallis tests at the 0.05 level of significance.

RESULTS

Statistical analyses showed that there were significant differences not only between control and experimental groups but also among different curing modes. Data obtained from a total of 56 specimens further showed that there was a significant difference in color alterations (ΔE) caused by red wine and coffee (p<0.05). Regarding immersion periods of the study, specimens immersed in coffee showed significant color change after 56 days; however, specimens immersed in red wine showed significant change after 28 days. Mean values of color changes in the experimental period are shown in Table 4.

In the 7-day evaluation period, discoloration values of specimens immersed in coffee revealed no remarkable differences among the groups.
(p>0.05), whereas specimens in GIV (LED-ramp mode) were significantly less stained compared to specimens in GII (LED-standard mode) and GIII (LED-pulse mode) when immersed in red wine (p<0.05). Specimens in the QTH group had the least staining overall.

Comparing the 28-day results, specimens immersed in coffee solution and cured with QTH still had the least staining overall and that was statistically different from the standard LED group (p<0.05). There were also significant changes among the LED pulse and ramp modes; however, there was no difference among specimens immersed in red wine.

After 56 days of immersion in red wine, specimens in GII showed the least staining, followed by GI, GIII, and GIV, respectively, and GIV showed higher color change compared to the other groups (p=0.043). However, specimens in GI revealed the least staining after 56 days of coffee immersion, and that was statistically different from all LED groups (p<0.05).

**Table 4.** Mean values of color differences (ΔE) of each group with standard deviations and significances among groups.

<table>
<thead>
<tr>
<th></th>
<th>GI-Control (Halogen) ΔE (±SD)</th>
<th>GII (LED Standard Mode) ΔE (±SD)</th>
<th>GIII (LED Pulse Mode) ΔE (±SD)</th>
<th>GIV (LED Ramp Mode) ΔE (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>7 Days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.89ᵃ⁺¹</td>
<td>1.61ᵃ⁺³</td>
<td>8.39ᵃ⁺¹</td>
<td>2.56ᵃ⁺³</td>
</tr>
<tr>
<td></td>
<td>(±1.69)</td>
<td>(±0.63)</td>
<td>(±2.29)</td>
<td>(±0.67)</td>
</tr>
<tr>
<td><strong>28 Days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.18ᵃ⁺¹</td>
<td>2.67ᵃ⁺³</td>
<td>10.92ᵃ⁺¹</td>
<td>4.34ᵃ⁺³</td>
</tr>
<tr>
<td></td>
<td>(±3.56)</td>
<td>(±0.68)</td>
<td>(±3.73)</td>
<td>(±0.70)</td>
</tr>
<tr>
<td><strong>56 Days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.0ᵃ⁺²</td>
<td>5.74ᵃ⁺⁴</td>
<td>15.71ᵃ⁺²</td>
<td>7.53ᵃ⁺⁴</td>
</tr>
<tr>
<td></td>
<td>(±3.01)</td>
<td>(±0.59)</td>
<td>(±1.12)</td>
<td>(±1.02)</td>
</tr>
</tbody>
</table>

Different superscript letters in the same row show statistical significance between curing modes (p<0.05). Uppercase letters are used for red wine groups and lowercase letters are used for coffee groups. Different superscript numbers in the same column show statistical significance between time periods.

**DISCUSSION**

Color stability constitutes one of the major preliminary factors influencing esthetic outcome and the clinical performance of composite restorations. Color alterations and surface deteriorations observed during clinical longevity could be related to both the material itself and the habitual factors of the individuals. Owing to the fact that ideal light-curing effectiveness has a crucial impact on the mentioned clinical performance, curing devices are desired to offer the required light intensity to maintain ideal polymerization capacity and monomer conversion to overcome those complications. In the present study, not only was the curing effectiveness of different modes of LED sources comparatively evaluated but the color stability of a nanohybrid composite was also investigated in regard to polymerization efficiency. The results revealed that despite its required optimal light properties, such as wavelength and power intensity, the curing modes of the LED unit did not show superior results compared to the QTH unit concerning the indirect effect on color stability. There were statistical differences among curing modes of LED units, as well. The type and
mode of light-curing devices affected the color of the nanohybrid composites so the first hypothesis was accepted. There were also remarkable color changes among both beverages and immersion periods. Thus, the second and third hypotheses of the present study were accepted, as well.

In the present study, in order to eliminate composite-related factors affecting polymerization variability of materials with different filler size, percentage, and monomer type, a nanohybrid composite, Filtek Z550, was selected. In the study of Öztürk-Bozkurt et al., in which the clinical application of Filtek Z550 was investigated, color stability was one of the main criteria and according to the results there were no significant changes over 3 years (24). However, color change according to polymerization variables was not observed in that study. Many studies on the color change of Filtek Z550 further demonstrated that it substantially changed color only after light polymerization (25-28). One of these studies showed that the ∆E value after light polymerization was 3.9 (25), while another yielded a value of 4.39 (28), both of which exceeded the critical value. Thus, with only light-curing variability, the initial color of the material could change visibly, supporting the high importance of the property of light-curing units.

Regarding the results, both LED and QTH groups exhibited discoloration beyond the critical value (∆E of ≥3.3). When different curing devices are taken into consideration, polymerization efficiency is an important factor for color stability of composite resins. Polymerization is affected by individual properties of light sources related to the wavelength of the output, intensity of the light, duration of the exposure, and location of the light (15,29). Specimens were designed as 8-mm-diameter cylinders in accordance with 8-mm-diameter light-curing tips. Although the tips of the sources were entirely in contact with the surfaces of the specimens, the whole surface of the light tip was not beamed. Price et al. (30) confirmed that there is lack of irradiance uniformity on the light guides of various curing units and the external diameter of the tip is not superposed with the active diameter that actually beams. That may cause less polymerized peripheral areas, leading to color changes beyond the clinical threshold.

LED modes and the QTH unit caused different discoloration values for two beverages: red wine and coffee. At the end of the experimental period, there was no statistically significant difference in red wine groups of LED modes and the QTH unit, but specimens cured with the QTH unit did show slightly lower color changes. The QTH unit also showed statistically more resistance to color change in all coffee groups (p<0.05). Despite the high energy generated by LED modes (1200 mW/cm²), groups polymerized with the QTH unit (480-530 mW/cm²) had better color stability. The light energy obtained from the QTH unit is only 1% of all the energy produced; the rest of it is released in the form of heat (14). The reason for the superior results of the QTH unit could be that the aforementioned heat energy produced during the polymerization process may accelerate the movement of monomers to react with each other to produce polymer chains and increase the degree of conversion of carbon-carbon double bonds (21). Supporting this fact, LEDs and a similar-intensity QTH unit showed no statistically different color stability in a 30-day experimental period (31). On the contrary, some studies investigating LEDs found that they had higher color stability than QTH units due to their high power intensity (21,32). It was clarified that the power intensity of light-curing units may change the monomer action; thus, they may affect polymerization and clinical properties indirectly (20). However, regarding the effect of monomer movement and holding a narrow spectrum, the QTH unit supplied better polymerization than LED modes in the present study. That supports the spectrum efficiency of QTH units having a greater effect on composite material polymerization than the power intensity of
LED units. Moreover, the energy distributed by LED units may not be emitted by the whole composite mass, which could be another factor explaining the inferior results obtained for the samples cured with LED units (22).

Light-curing modes are generated in order to reach optimum polymerization by decreasing polymerization stresses. Four curing modes with different power densities were investigated in the present study. The standard mode LED (1200 mW/cm²) had nearly twice the power intensity of the QTH unit (480-530 mW/cm²) and pulse mode LED (600 mW/cm²). The energy starts from 0 to 1200 mW/cm² in the ramp mode LED, and that is why ramp mode could be called a “soft start,” as well. It showed the lowest results among the LED groups after 56 days of the experiment. These inferior results may arise from the lack of double bonds or a decrease in cross-link intensity in ramp mode curing (33). As mentioned above, high power densities may not always correlate with the quality of polymerization. In accordance with our study, Aguiar et al. detected a lower degree of conversion at soft starts, in which the physical properties of composites weaken (27). Even though ramp mode had lower ΔE values in 7 days, it showed a high increase in results at the end of the experimental period, which might be a result of a low rate of post-irradiation polymerization. Pulse mode in the LED unit had results similar to those of the QTH unit in both beverages, which can be explained by the similar power densities of the outputs (~600 mW/cm²).

Considering the two beverages tested in the present study design, specimens immersed in red wine had higher color changes through the whole experimental period. In accordance with our study, Aguiar et al. measured lower ΔE values in coffee groups in comparison to red wine groups (32). It was explained in many studies (34,35) that, due to the extensive penetration of ethanol between monomers, red wine has more influence on composite staining than coffee. It is also obvious that prolonged contact with ethanol initiates organic matrix deterioration more rapidly than storage in water (34). Regarding the alcoholic characteristics of red wine, the plasticizing of the organic matrix of composites, clinically causing wear, softening, severe degradation, and higher staining, is once more supported by the results of this study (36,37). Moreover, the tannins found in red wine were shown to be potential colorants for composite resins in many studies and could be the reason for increased staining (11,38,39). At the end of the immersion period, samples cured with the standard mode LED unit showed higher resistance to red wine staining. That might be due to the higher interaction of the material with the high power intensity (1200 mW/cm²) of the standard mode LED. On the other hand, the composite material itself had the tendency to stain according to its own components. Filtek Z550 has a colored photoinitiator, camphorquinone, which turns transparent when photoactivated. If the initiator remains inactivated, the yellow color itself may cause a darker appearance (14).

Evaluating the monomers of related composites, zirconia and silica-based tiny fillers ranging from 20 nm to 3 µm in a ratio of 81.8% by weight and surrounded by Bis-GMA, TEGDMA, and UDMA mainly constituted the test material content. Due to the strengthened content of the zirconia and silica-based fillers, Z550 was shown to be resistant and stable against physical and mechanical challenges (40). However, the causes of the clinically unacceptable ΔE values of the specimens may be the monomers included or the organic content of the material. Hydrophilic UDMA and TEGDMA may draw water despite the dense polymer chain at different polymerization rates obtained by varying the curing modes in the present study. Moreover, the incompatibility of the material and the disparity of the brand of light-curing units and intense spectrum variability may help to explain the diverse results achieved in the present study. Obviously, further studies are needed to clarify the interrelation between the

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uncured remaining monomers and the content of the beverages in terms of both alcoholic content and the included colorants.

CONCLUSION

The findings of this study suggest that light-curing mode influences the color stability of the tested nanohybrid resin composite.

Polymerization with QTH unit showed lower susceptibility to staining of resin composite than curing modes of LED unit. Among various curing modes, composite samples polymerized with ramp mode showed significant staining of red wine.

Beverages with alcoholic content cause more staining than colorant beverages due to softening resin material, itself. Distinct color alteration is revealed by after 4 weeks. Consuming regular red wine causes earlier color changes of composite restorations than consuming coffee.

It is also obvious that spectrum properties or power intensity is directly related but not completely relevant to the quality of polymerization. Despite the novel light sources with elevated advantages, QTH units still show considerable results on the discoloration of composites in regards to polymerization efficiency.

AUTHOR CONTRIBUTIONS

Dr. Gunce Ozan had done the literature search in the related topic, designed the whole research and done the final review.

Assoc. Prof. Hande Sar Sancakli had corrected the language and edited the assay. She is the guarantor of the study.

Dr. Isil Bayrak had written down the methodology, results and discussion parts.

Dr. Murat Tiryaki and Dr. Gunce Ozan had done the experimental period and written the introduction part of the main text.

CONFLICT OF INTERESTS

All authors declared that there is no conflict of interests.

REFERENCES

27. Paravina R. D., Kimura M., Powers J. M. Evaluation of polymerization dependent...


