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ORIGINAL ARTICLE

INFLUENCE OF DIFFERENT OPACITIES AND LAYERING TECHNIQUE OF NANOTECHNOLOGY COMPOSITE RESINS REGARDING THE WAVELENGTH AND FLUORESCENCE INTENSITY: *IN VITRO* STUDY

Influencia de diferentes opacidades y la técnica de estratificación de resinas compuestas de nanotecnología en la longitud de onda e intensidad de fluorescencia: estudio *in vitro*

Carla Portocarrero-Flores,[1] Thais Alcandré,[1] Alberth Correa-Medina.[1]

1. Universidad Científica del Sur. Escuela de Estomatología. Lima, Perú.

ABSTRACT

Objective: To evaluate the influence of opacity and the layering technique on the fluorescence of different composite resins.

Materials and Methods: Two opacities (enamel and dentin) and the layering technique (enamel + dentin) of the composite resins: Filtek® Z350 and Palfique LX5 were evaluated in vitro. Composite resin discs were fabricated using a preformed matrix of 10 mm diameter and 0.5 mm thick for the single opacity groups and 10 mm thick for the layering technique groups, using 2 layers of 0.5 mm thickness of each opacity (n = 5). Specimens were analyzed using the Raman spectroscopy method. Data were analyzed using the Kruskall-wallis and Mann-Whitney U tests.

Results: When evaluating the intensity of fluorescence, no statistically significant difference was found when comparing the layering technique and enamel opacity ($p^2 > 0.05$) and an increase in the dentin opacity value for both brands of composite resin. Regarding wavelength, no statistically significant difference was found when comparing the layering technique with enamel opacity and dentin opacity for both Filtek® Z350 and Palfique LX5® composite resins ($p2 > 0.05$).

Conclusions: The fluorescence intensity of the layering technique is similar to enamel opacity for both composite resins. Likewise, the wavelength of the layering technique is similar to the enamel opacity and dentin opacity for both brands.

Keywords: *Composite resins; Fluorescence; Spectrum analysis; Spectrum analysis, Raman; In vitro techniques; Nanotechnology.*

RESUMEN

Objetive: Evaluar la influencia de la opacidad y de la técnica de estratificación en la fluorescencia de diferentes resinas compuestas.

Materiales y Métodos: Se evaluó in vitro 2 opacidades (Esmalte y Dentina) y la técnica de estratificación (Esmalte + Dentina) de las resinas compuestas: Filtek® Z350 y Palfique LX5. Se fabricaron discos de resina compuesta, utilizando una matriz preformada de 10 mm de diámetro y 0,5 mm de grosor para los grupos de opacidad única y 10 mm de grosor para los grupos de técnica estratificada, utilizando 2 capas de 0,5 mm de cada opacidad (n = 5). Los especímenes se analizaron mediante el método de Espectroscopía Raman. Los datos se analizaron utilizando la prueba de Kruskall-wallis y Prueba U de Mann Whitney.

Resultado: Al evaluar la intensidad de fluorescencia no se encontró diferencia estadísticamente significativa entre los pares: Técnica estratificada *versus* Opacidad Esmalte para ambas marcas de resina compuesta Filtek® Z350 y para Palfique LX5® ($p2 > 0,05$). Para longitud de onda no se encontró diferencia estadísticamente significativa entre los pares: Técnica estratificada *versus* Opacidad Esmalte y Técnica estratificada VS Opacidad Dentina para ambas resinas compuesta Filtek® Z350 y Palfique LX5® ($p^2 > 0,05$).

Conclusión: La intensidad de fluorescencia de la técnica estratificada es similar a la opacidad Esmalte para ambas resinas compuestas. De igual manera la longitud de onda de la técnica estratificada es similar a la opacidad Esmalte y opacidad Dentina para ambas marcas.

Palabras Clave: *Resinas compuestas; Fluorescencia; Análisis espectral; Espectrometría Raman; Técnicas in vitro; Nanotecnología.*

CORRESPONDING AUTHOR: Carla Portocarrero-Flores. Universidad Científica del Sur. Escuela de Estomatología. Chorrillos 15067, Lima, Perú. E-mail: carladaniela380@hotmail.com

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INTRODUCTION

When restoring teeth, particularly in the anterior region, achieving natural mimicry relies heavily on specific parameters such as value, tone, chroma, opalescence, and fluorescence. Among these, fluorescence holds particular importance for successful restoration outcomes. It occurs naturally in human teeth, contributing to their appearance of whiteness and lightness under daylight and blueness under ultraviolet light.[1]

Fluorescence occurs when a surface absorbs ultraviolet light, causing the electrons of the material to temporarily transition to a higher energy state. Within less than 1 second of ac-tivation (10-8 seconds or 1 nanosecond), these excited electrons release the accumulated energy by emitting photons with a longer wavelength, falling within the visible spectrum perceivable by the human eye.[2] This release of photons is depicted through a fluorescence emission spectrum, composed of the color of these photons (wavelength) and the quantity or concentration of photons released (fluorescence intensity).[3]

Fluorescence in human teeth relies heavily on the organic component, predominantly colla-gen fibers containing photosensitive amino acids like hydroxypyridium and tryptophan. Upon interaction with ultraviolet light, these components emit fluorescence.[4] It is noteworthy that dentin and enamel exhibit differences in fluorescence; dentin usually displays greater fluorescence than enamel due to its higher organic content.[5]

This distinction results in internal luminescence, giving natural teeth a vital appearance. However, replicating this phenomenon with restorative materials poses a challenge for clinicians.[6] One of the most used restorative materials is composite resin, valued for its mechanical, physical, and aesthetic properties. These include a wide range of colors and opacities, facilitating the stratification of opacities (stratified or multi-layer technique) to closely reproduce the natural tone of the tooth.[7]

Among the advancements in the aesthetic properties of composite resins is the addition of artificial luminophores, such as ytterbium, terbium, europium, and other rare earths, to the inorganic matrix. Incorporating these elements in specific concentrations is expected to provide fluorescence similar to that of a natural tooth. However, it is important to note that the necessary concentrations may vary between manufacturers and/or different opacities.[8,9]

Fluorescence of composite resins has been extensively studied in dentistry, with thickness, type, and brand influencing the final fluorescence values in composite resin restorations.[9,10] However, there is limited understanding of how combinations of opacities, whether through selecting a single tone or applying multiple tones, impact fluorescence. Therefore, it is crucial to understand how clinical decisions regarding opacity and layering technique affect the ultima-te fluorescence of composite resin restorations.

There is no standardized method to study fluorescence, as it can be analyzed qualitatively through fluorescent cameras, UV light flashlights, or computer programs, and quantitatively using a spectrometer, more commonly known as a spectrophotometer. This instrument produces, disperses, and measures light, whether UV or visible, through a monochromator that filters and records the data.

Therefore, the present study aims to evaluate the influence of opacity and stratification technique on the fluorescence of different resin systems using a spectrometer. The hypothesis is that there is a difference in the fluorescence of different opacities and the stratification technique of the composite resins: Nano-particulate (Filtek® Z350 - 3M ESPE) and Supra-nano filled (Palfique LX5® – TOKUYAMA). (Table 1).

MATERIALS AND METHODS

The present research was an experimental *in vitro* study, where the opacities of enamel, dentin (single tone), and the stratified technique of both opacities (multiple tones) of two composite resins were evaluated: Filtek® Z350 - 3M and Palfique Lx5® - TOKUYAMA (Table 2).

Fluorescence was assessed using two dimensions: wavelength and fluorescence intensity, employing the Raman spectroscopy method. The sample size consisted of 5 composite resin discs for each group, ensuring a statistical power of 80% and a confidence level of 95%. This research was registered with the Institutional Research Ethics Committee of the Universidad Científica del Sur (CIEI-CIENTÍFICA) under registration number 231-2020-POS8.

Preparation of resin discs

Resin discs of 10 mm diameter and 0.5 mm thickness were made for single tones and 1 mm thick for multiple tones. A silicone matrix was made with the corresponding measurements for each group. The composite resin was inserted using a Teflon spatula, in 1 increment of 0.5 mm for single tone and in 2 increments of 0.5 mm for multiple tones, each increment being of different opacity, first Dentin and then Enamel. Dentin A2 and Enamel A1 were used for the Filtek® Z350 - 3M ESPE system.

The OPA2 opacity was used for the Palfique LX5® – TOKUYAMA resinous system, because they have a different classification of tone and shades, which would be equivalent to Dentin A2 and for Enamel opacity A1. It was polymerized with an LED light lamp (Bluephase G2 - Ivoclar Vivadent) for 20 seconds.

To standardize the samples, the thickness and diameter of the discs were checked by placing them in a metal matrix with exact measurements of 10 mm in diameter and 0.5 mm and 1 mm in thickness. Any excess material was removed using a 3M Soflex disc of various grains at low speed intermittently for 15 to 20 seconds until precise adaptation was achieved. The samples were then polished using goat hair brush and universal polishing paste (Ivoclar Vivadent). Discs that exhibited bubbles, fissures, or fractures were excluded from the study.

The samples were stored in plastic containers labeled with a code corresponding to the brand of each composite resin. Additionally, the samples were exposed to the same 632.8 nm laser in a dark room, against a black background, and photographed with a digital camera (Nikon D5200) from a distance of 10 cm. The camera was set to manual mode with an aperture of F3.4, a shutter speed of 1/80, and an ISO of 125, ensuring standardized settings for each photograph (Figure 1).

Fluorescence analysis

The Renishaw inVia system was used to obtain fluorescence values. This advanced Raman spec-troscopy system integrates a high-resolution Raman spectrometer with a confocal microscope. This configuration enabled the use of an excitation laser in the ultraviolet range to induce fluorescence in the sample.

Moreover, the high-resolution spectrometer facilitated the acquisition of precise spectral data, allowing for peak identification and detailed interpretation of sample vibrations. Specialized software provided by Renishaw simplified the efficient acquisition, processing, and interpretation of Raman data. To conduct the analysis, the spectrometer was calibrated using a 632.8 nm laser and a Silicon (Si) reference sample. For the analysis of the resin discs, an excitation wavelength of 325 nm was selected, and appropriate filters were applied to minimize Raman light during fluorescence detection.

This approach allowed the acquisition of an emission spectrum ranging from 400 to 800 nm, recording both the spectrum and the fluorescence intensity peak value in arbitrary units, as well as the fluorescence wavelength in nanometers.
Specialized personnel were trained to ensure the proper collection and interpretation of results provided by the spectrometer using the ORIGIN PRO-8.0 program. This software also facilitated the acquisition of fluorescence emission spectra for each group, (Figure 2 and Figure 3).

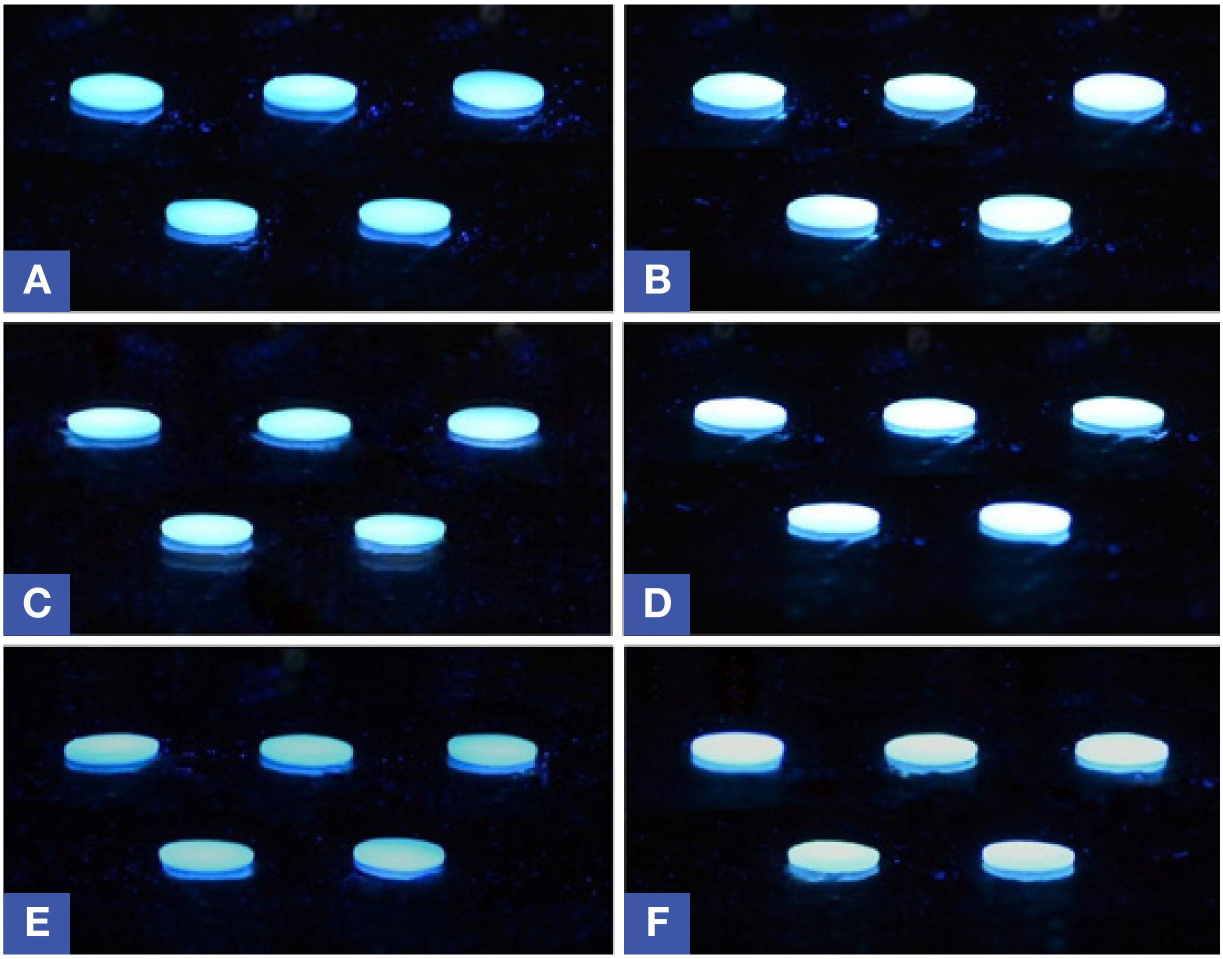
Statistical analysis

The data were analyzed using the SPSS software version 24. The average values for fluorescence intensity and wavelength corresponding to each sample were entered. Non-parametric Kruskal-Wallis tests and pairwise comparisons were conducted using the Mann-Whitney U Test. All statistical tests were performed with a confidence level of 95%.

RESULTS

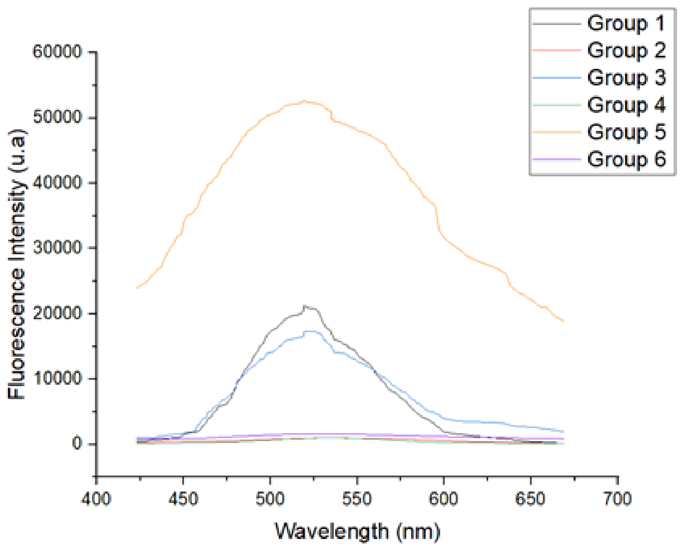
In the present study, fluorescence was evaluated in its two dimensions: wavelength and fluorescence intensity for each study group.

Figure 1. Samples under an ultraviolet light source.



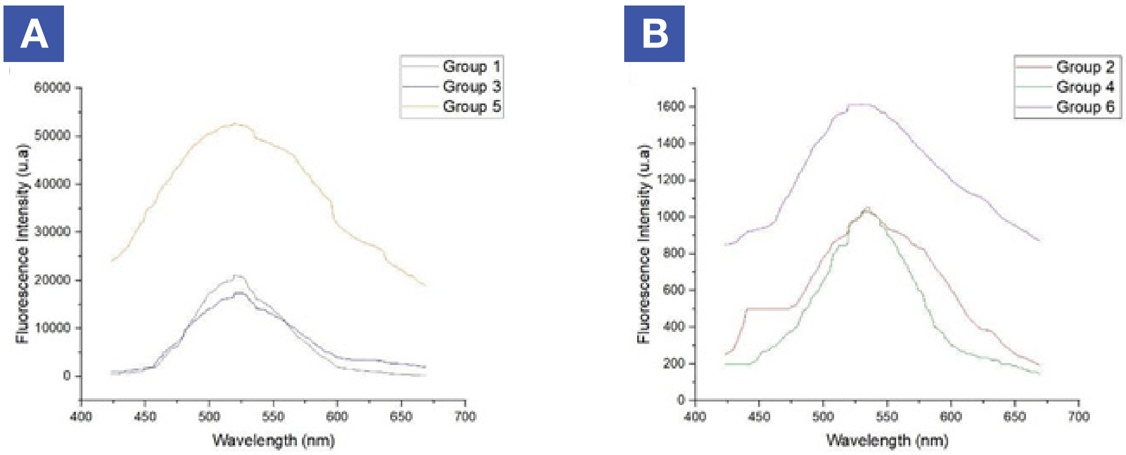
A: Layering technique composite resin disc (enamel + dentin) from Filtek® Z350 - 3M ESPE (G1).
B: Composite resin disc with stratified technique (enamel + dentin) from Palfique LX5® – TOKUYAMA (G2).
C: Enamel opacity composite resin disc from Filtek® Z350 3M ESPE (G3).
D: Enamel opacity composite resin disc from Palfique LX5® – TOKUYAMA (G4).
E: Dentin opacity composite resin disc from Filtek® Z350 - 3M ESPE (G5).
F: Dentin opacity composite resin disc from Palfique LX5® – TOKUYAMA (G6).

Figure 2. Comparison of the fluorescence emission spectra of the different study groups.



GROUP 1: Layering technique (Enamel + Dentin) from Filtek® Z350 - 3M ESPE.
GROUP 2: Stratified technique (Enamel + Dentin) from Palfique LX5® – TOKUYAMA.
GROUP 3: Enamel Opacity from Filtek® Z350 - 3M ESPE.
GROUP 4: Enamel Opacity from Palfique LX5® – TOKUYAMA.
GROUP 5: Dentin Opacity from Filtek® Z350 - 3M ESPE. G6: Dentin Opacity from Palfique LX5® – TOKUYAMA.

Figure 3. Comparison of fluorescence emission spectra according to the brand of composite resin.



A: Fluorescence emission spectrum of the groups from the Filtek® Z350 - 3M ESPE composite resin.
B: Fluorescence emission spectrum from the groups of the Palfique LX5® – TOKUYAMA composite resin. U.A: Arbitrary units.

When evaluating the wavelength, values varied from 519.29 (±0.28) for the dentin opacity group of the Filtek® Z350 (G5) composite resin to 534.92 (±1.28) for the dentin opacity group of Palfique LX5® (G4) composite resin enamel. Regarding the fluorescence intensity dimension, values ranged from 1032.95 (±60.64) for the enamel opacity group of the composite resin (G4) to 52744.74 (±6570.32) for the opacity group of Filtek® Z350 (G5) composite resin dentin. Notably, the latter group exhibited the highest fluorescence intensity among all groups, as shown in Figure 2.

Additionally, it is evident that the groups of the Filtek® Z350 composite resin (G1, G3, G5) present higher fluorescence intensity values, differing from groups of the Palfique LX5® composite resin (G2, G4, G6).

Table 3 shows that when comparing the fluorescence intensity values, a statistically significant difference was observed in most pairs, except for the pairs Filtek® Z350 stratified technique (G1) VS Filtek® Z350 enamel opacity (G3) (p^2=0,09), and stratified technique Palfique

LX5® (G2) VS Palfique LX5® enamel opacity (G4) (p^2=0,12), where no statistically significant difference was found. However, a significant difference was observed when comparing it with the dentin opacity groups for both brands, as seen in Figure 3.

Likewise, when comparing the fluorescence wavelength values, it is observed that there is no statistically significant difference between the pairs: G1 *versus* G3 (p^2=0,06), G1 *versus* G5 (p^2=0,08); G2 *versus* G4 (p^2=0,11); G2 *versus* G6 (p^2=0.09). That is, there is no statistically significant difference when comparing the stratified technique with enamel opacity and with dentin opacity for both brands of composite resins, but there is one between enamel versus dentin opacity for both brands.

DISCUSSION

In the present *in vitro* study, six experimental groups were evaluated with the aim of understanding the influence of opacity and stratification technique on the fluorescence of two nanotechnology resin systems: Nano-particulate (Filtek® Z350 - 3M ESPE) and Supra-nanofilled (Palfique LX5® – TOKUYAMA). It is important to note that in this study, fluorescence was evaluated through an emission spectrum analysis in two dimensions: intensity and wavelength. Both features were examined to obtain a comprehensive understanding of the fluorescence phenomena under study.

First, the fluorescence intensity of the study groups was evaluated descriptively. It was observed that the nano-particulate resin (Filtek® Z350) presented the highest numerical values in all its groups: Stratified technique (G1) (21103.52 ± 6060.38), enamel opacity (G3) (17322.66 ± 2480.39), and dentin opacity (G4) (52744.74 ± 6570.32). A higher fluorescence intensity was highlighted in the dentin opacity and a lower one in the enamel opacity (Figure 3). This would indicate that the Filtek® Z350 composite resin, especially in its dentin opacity, presents a higher concentration of fluorophores in its inorganic matrix compared to the other study groups, as there is a linear correlation between the concentration of excited fluorophores or fluorochromes and the fluorescence intensity. In other words, the higher the concentration of fluorophores, the greater the fluorescence intensity.[11]

These findings contrast with previous research, such as that conducted by Kim *et al.,*[8] who concluded, using a digital fluorescence camera based on QLF technology (QLF-D), that the Filtek® Z350 resin exhibited a “darker fluorescence” compared to that of the natural tooth, clinically classifying it as a composite resin with low fluorescence. This discrepancy could be attributed to two specific optical phenomena: fluorescent quenching or self-quenching.

The first one, a process that reduces the fluorescence emitted by a sample, could be linked to the interaction with particles of larger size or opacity, limiting transmittance and hindering the arrival of UV light to all fluorescent particles, resulting in a decrease in the emitted fluorescence.[6,11] The second phenomenon, self-quenching, occurs when identical fluorophores are in close proximity, collide, and cause loss of fluorescence. Despite the high fluorescence intensity, the previous clinical classification of the Filtek® Z350 resin as one with low fluorescence could be explained by the presence of zirconia/silica nanoclusters in its composition, especially in the enamel and dentin tones, comprising approximately 78.5% and 72.5% by weight, respectively.
This content could be related to greater optical

density or opacity, triggering the phenomenon of fluorescent quenching and thus affecting the final fluorescence, supporting previous research suggesting that the greater the opacity of a material, the lower its fluorescence, as noted by Tabatabaei *et al.,*[9] in 2019.

Furthermore, when comparing the fluorescence intensity values, no statistically significant differences were found between the stratified technique (G1) and enamel opacity (G3) groups of the Filtek® Z350 composite resin (p^2=0,09), nor between the stratified technique (G2) and enamel opacity (G4) of the palfique LX5® composite resin (p^2=0,12).
However, statistically significant differences were observed between the dentin opacity groups of their respective composite resins. This suggests that the fluorescence intensity is directly related to the last or most superficial layer, in this case enamel opacity, which aligns with some previous studies such as Catelan *et al.,*[11] which suggest that, in composite resins, fluorescence is mainly influenced by the last layer of the material since it could reduce or block the fluorescence of the composite resins. This could be explained by the same fluorescence quenching phenomenon.

Regarding the fluorescence wavelength dimension, it was observed that the Palfique LX5® composite resin presented higher numerical values in all its groups: stratified technique (G2) (534.80 ± 7.81 nm), enamel opacity (G4) (534.92 ± 1.89 nm) and dentin opacity (G6) (529.69 ± 1.70 nm); compared to the groups composed of Filtek® Z350 resin: Layering technique (G1) (519 ± 0.75 nm), enamel opacity (G3) (520.46 ± 0.51 nm), and dentin opacity (G5) (519.29 ± 0.28 nm). This suggests a tint or shade closer to cyan-greenish (540 nm) in Palfique LX5® and closer to a bluish tint (500 nm) in Filtek® Z350, (Figure 1).

On the other hand, when comparing the fluorescence wavelength between the study groups, it was found that there is no statistically significant difference in the pairs of the Filtek® Z350 composite resin: stratified technique (G1) vs enamel opacity (G3) (p^2=0,06) and stratified technique (G1) *versus* dentin opacity (G5) (p^2=0,08) and for the Palfique LX5® composite resin between the pairs: stratified technique (G2) *versus* enamel opacity (G4) (p^2=0,11) and stratified technique (G2) *versus* dentin opacity (G6) (p^2=0,09) This indicates that the study groups that present the same composite resin exhibit the same fluorescence shade, which may be due to the manufacturer of a brand of composite resin could use the same fluorophore in its different opacities, since a specific fluorophore fluoresces at a certain wavelength.

However, they would not use the same concentration of these fluorophores in their different opacities, as the fluorescence intensity is what varies the most between the opacities studied. This coincides with Volpato *et al.,*[12] who mentions that in order to achieve different optical properties with composite resins such as enamel and dentin opacities, manufacturers must make different compositions, varying the pigments used to determine the different opacities, which can alter the final fluorescence.[13] This last point is difficult to verify due to the lack of information provided by manufacturers on the concentration and composition of fluorescent additives in their composite resins.[14,15]
The study analyzed fluorescence quantitatively, though comparison with prior research was challenging due to the lack of a standardized method. Nevertheless, it provided insights into how fluorescence in composite resins is influenced by various opacities and layering techniques, common in clinical practice.

CONCLUSION

Within the limitations of this study, fluorescence was found to be influenced by both the opacities and brands of the composite resins. The impact of the stratified technique was prominent, particularly in its association with the most superficial layer.

This was evident in the lack of significant differences observed when comparing the stratified technique with Enamel opacity, whereas differences were noted with Dentin opacity in both brands. Additionally, distinct fluorescence shades were identified among the evaluated brands.

These findings highlight the importance of considering these factors when selecting composite resins in clinical practice and raise questions that could be addressed in future research regarding the relationship between fluorescence and its clinical perception in these materials.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

ETHICS APPROVAL

Study approved by the Institutional Research Ethics Committee of the Universidad Científica del Sur (CIEI-CIENTÍFICA) under registration number 231-2020-POS8

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AUTHORS' CONTRIBUTIONS

Carla Portocarrero-Flores: Conceptualization; data curation; formal analysis; funding acquisition, investigation; methodology, visualization; writing – original draft; writing – review and editing.
Thais Alcandré : Conceptualization, methodology; project administration; resources.
Alberth Correa-Medina: Conceptualization, Supervision.

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ORCID

Carla Portocarrero-Flores
0000-0002-9866-3624
Thais Alcandré
0009-0009-0712-072X
Alberth Correa-Medina
0000-0002-2853-2231

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