

The Effect of Different Polishing Methods and Dentifrices on Resin Composite: An In-Vitro Study
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Abstract

Background: Discoloration of resin composite restorations is a common concern in dentistry. It can be influenced by various factors, such as the composition of the composite material, surface roughness, and exposure to staining agents like tea. Polishing systems and dentifrices are commonly used to maintain the esthetic appearance of resin composite restorations and prevent or minimize discoloration. Therefore, the aim of this was to evaluate the effect of different polishing systems and dentifrices on discoloration of resin composite after immersion in tea staining solution followed by mechanical brushing.

Methods: 42 Resin composite discs with 3mm thickness and 10mm diameter were prepared. Specimens were divided into three equal main groups, (n=14) according to polishing system used (Mylar strip, Sof-Lex, PoGo). The specimens were fully immersed in equal amounts of Black tea staining solution and incubated at 37°C for 7-days. Each group was further subdivided in two subgroups according to the type of dentifrice used with mechanical brushing (N=7). Spectrophotometer was used to measure the color change after polishing, after staining and after brushing. The collected data was tabulated and statistically analyzed using two-way ANOVA.

Results: Color change between groups was significantly difference after staining. The highest value was found with Sof-lex (2.95±0.18), followed by PoGo (2.75±0.17), while the lowest value was found with Mylar strip (2.41±0.15). Rapid white (5.20±0.45) had significantly higher color change than Colgate (4.83±0.44) (p=0.010).

Conclusions: PoGo produced better surface quality in terms of color stability than Sof-Lex, whitening dentifrice was effective on removing Black tea surface stains than non-whitening.

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1 Introduction

Resin composites are the most commonly used esthetic restorative material in dentistry due to their capacity to easily reproduce tooth-like appearance and mimic tooth color that makes them well accepted by the patients¹. Recently, Nanotechnology was introduced to the field of dental materials supporting the continuous development in resin composites leading to increased clinical performance in terms of both physical and esthetic outcome².

Nanocomposites are one of the most recently developed composites nowadays that made up of nanoscale particles (20–75 nm), which are composed of approximately uniform nanometric particles and create nanoclusters as secondarily formed fillers^{3,4} that have returns such as lower polymerization shrinkage, favored optical behavior, improved mechanical properties, better color stability, excellent polish ability and higher wear resistance⁵. Surface

characteristics and color stability have an impact on the clinical outcome of esthetic restorations⁴.

Conversely, discoloration caused by continuous exposure to daily diet tints continues to be a challenge, resulting in unsatisfactory color match, patient discomfort and need for repair^{6,7}.

Finishing and polishing techniques have a significant impact on the resistance of tooth-colored restorative materials to discoloration⁸. A high polished surface improves the esthetics and longevity of restorations by reducing plaque accumulation, gingival irritation, surface discoloration and secondary caries⁹.

For many years, resin composite restorations were polished with a set of very flexible polyurethane-based finishing and polishing discs covered with aluminum oxide. More recently Diamond polishers and silicone synthetic rubbers have been launched, giving composites a high gloss and reducing clinical time spent on the repair.

They are referred by manufacturers as "one-step" polishing systems since they may be used to create a high shine, contouring, finishing and polishing processes with a single tool. This polishing idea satisfies the clinical requirement for producing a smooth surface in the shortest period of time³. However, there are disagreements on how well these systems perform in vitro.

Even though tooth brushing seems to be the most widely known dental homecare method used to reduce plaque accumulation, periodontal diseases and has an important role to maintain good oral hygiene, it also has the tendency to affect the surface of resin-based restorations with its continuing action^{9,10}.

Dentifrices are sold as cosmetic products that can be used without the consultation of the dentist¹¹. The market offers a wide range of these products. Lately, whitening toothpastes have gained popularity as a treatment for extrinsic stains¹². The majority of whitening dentifrices contain the same main functional components which serves a distinct purpose¹³.

Consequently, there is a great need to detect the ability of different polishing systems on stain susceptibility of resin composite and the effect of brushing with whitening dentifrice on composite's stains removal.

2 Materials and Methods

This study was performed at the department of Conservative Dentistry, faculty of dentistry, modern science and Arts University.

Resin restorative composite material used in this study was Filtek™ Z350XT Universal Restorative Body Shade: A2 Nanofilled composite. Three different methods were used Transparent Mylar strip, One-step PoGo® discs and two-step Sof-Lex™ spiral wheels. Two types of dentifrices were used Rapid white and Colgate and Oral-B Soft-bristled conventional toothbrush used to simulate brushing material's composition, manufacturer and batch no are listed in (Table 1).

2.1 Sample size of Specimens:

A power analysis was designed to have sufficient power to apply a statistical test of the null hypothesis that there is no difference in the color stability and surface roughness of polished and unpolished nanofilled resin composite after staining in tea solution and mechanical brushing with conventional or whitening dentifrices. Based on the results of **MoZZaquatro, Lisandra R., et al (2017)**¹ by accepting an alpha (α) level of 0.05 (5%), a Beta (β) level of 0.20 (20%), i.e. power=80 percent, and an effect size ($f=0.498$), the estimated sample size (n) was a total of (42) samples. That is, 14 samples per group and seven samples per sub-group. G*Power version 3.1.9.4 was used to calculate sample size¹⁴.

Table1. Materials

Material	Product Specifications	Composition		Manufacturer (Batch No.)
		Filler:	Matrix:	
Filtek™ Z350XT Universal Restorative Body Shade: A2	Nanofilled composite	Non-agglomerated nanosilica filler 20 nm, agglomerated zirconia/silica nanocluster 0.6-1.4 µm with primary particle size of 5-20 µm Filler content 59.5-78.5 % by weight	Bisphenol-A-glycol-dimethacrylate, Urethane-dimethacrylate, Tri-ethylene-glycol-dimethacrylate, Polyethylene glycol, dimethacrylate, Bisphenol-A-polyethylene-glycol-diether-dimethacrylate resins	3M ESPE, ST. Paul, MN, USA (NA44144)
Transparent Mylar strip	Transparent rectangle-shaped universal strip.	Flexible Mylar used for contouring and polishing of resin composite restorations (length: 100 mm, width: 10 mm, thickness: 0.02 mm).		Matrix strips, CROSSTEX International, Inc., USA (KSTRIP500)
PoGo® discs Polishing system (One step)	Pre-mounted, single use. Diamond coated micro-polisher (7 µm)	Polymerized UDMA resin, fine diamond powder, and silicon oxide, plastic latch-type mandrel.		Dentsply/Siroa, Milford, Delaware, USA (662010)
Sof-Lex™ spiral wheels	Fine grit (beige) pre-polishing spiral wheel.	Aluminum oxide impregnated in a thermoplastic elastomer (diameter: 12.7 mm, thickness: 0.13 inches).		3M ESPE, St. Paul, MN, USA (NA46599)
	Ultra-fine grit (pink) for final polishing.	Aluminum oxide impregnated in a thermoplastic elastomer (diameter: 12.7 mm, thickness: 0.13 inches).		3M ESPE, St. Paul, MN, USA (NA46599)
Rapid White	(L)893107	Aqua, Hydrated Silica, Glycerin, Sorbitol, Pentasodium Triphosphate, Cocamidopropyl Betaine, PVP, Aroma, Cellulose Gum, Sodium Saccharin, Potassium Acesulfame, Sodium Fluoride, Sodium Hexametaphosphate, Sodium Hydroxide, Sodium Benzoate, Cinnamal, Eugenol, CI 77891. Contains Sodium Fluoride (1100 ppm F)		Rapid White Products U.S.A
Colgate Cavity Protection	210219SA10311	Aqua, Calcium Carbonate, Glycerin, Arginine, Sodium Lauryl Sulfate, Sodium Monofluorophosphate, Cellulose Gum, Aroma, Sodium Bicarbonate, Benzyl Alcohol, Tetrasodium Pyrophosphate, Sodium Saccharin, Sodium Hydroxide, CI 77891, Active Ingredient: Sodium Monofluorophosphate (1450 ppm F)		Colgate Palmolive ARABIA® K.S.A

2.2 Preparation of Specimens:

To standardize specimen dimensions, a unique cylindrical split Teflon mold was made to fabricate 42 resin composite discs with a thickness of 3mm and a diameter of 10mm. The Teflon mold was made up of two pieces that were held together by a custom-made metal ring during the specimen preparation process.

Nanofilled resin Composite was obtained according to the incremental technique, each sample needed two or three increment which were less than 2mm of the nanofilled composite that placed and condensed carefully in the mold against Mylar strip

supported by a microscopic glass slide using a sterile gold-plated instrument, care being taken to avoid any air inclusions or folds in the composite adapted to the Mylar strip then cured for 20s, followed by overfill the mold with uncured composite and another Mylar strip supported by a microscopic glass slide was placed over the last increment of resin composite before curing and gently pressed until touch the mold surface, in order to removing voids and extrude any excess of material ^{2,15}.

2.2.1 Curing of Specimens:

The composite was polymerized in two layers using a light emitting diode (LED) light curing unit [3m ESPE St. Paul, Elipar curing unite, USA (wavelength:

430-480nm, light intensity: 1200 mW/cm²] the first inserted resin composite increment was light-cured for 20 seconds through the Mylar matrix strip and the microscopic glass slide according to manufacturer instructions. Then, the last inserted resin composite increment was light-cured for 20 seconds through the Mylar matrix strip and the microscopic glass slide. On both sides, a Mylar strip and a 1-mm microscopic glass slide were used to standardize the distance between the LED tip and the specimen. After removing of the Mylar strip additional 20 sec light-curing on both sides of the composite disc specimen were done to insure proper curing¹⁵.

2.3 Randomization:

Specimens were randomly distributed using computer generated randomization (www.randomizer.org).

2.4 Grouping of samples

According to the polishing method used, forty-two Nanofilled cured resin composite disk-shaped specimens were allocated into three groups (F0 Mylar strip served as a control group, F1 Sof-Lex spiral wheels, and F2 PoGo) n=14. Then, each group was further divided into two subgroups (D1-rapid white whitening dentifrice and D2-Colgate regular dentifrice) according to the type of denitrifies used with brushing (N=7) for each sub-group individually. The variables to be analyzed, as well as their interactions, are depicted in (Table 2).

Table 2. Variable interactions:

F \ D	D1	D2	Total
F0	F0D1	F0D2	14
F1	F1D1	F1D2	14
F2	F2D1	F2D2	14
Total	21	21	42

2.5 Specimens polishing:

The specimens were reinserted into the Teflon mold with a 1mm prominence, allowing polishing on a tight-fitting base. To guarantee that all polishing was done in the same direction, the metal ring was marked as starting point. In addition, the specimens' surfaces were marked from their side, i.e., next to the non-polished surface to verify that all polishing was done on the same surface^{1,16}.

2.5.1. Polishing with Sof-Lex spiral wheels:

The specimens were polished with a slow-speed micro-motor hand-piece using linear motions. The fine grit

(beige) was applied first at a speed of 20,000 rpm for 20 seconds, followed by the ultra-fine grit at a speed of 20,000 rpm for another 20 seconds for each specimen¹⁶. As manufacturer instruction the Sof-Lex spiral wheels are multipurpose, they were replaced after every five specimens¹.

2.5.2 Polishing with PoGo polisher:

PoGo discs are designed for one-time usage only. Therefore, each specimen was given its own disc. The discs were attached to a slow-speed micro-motor hand-piece and the speed was set at 10,000 rpm for the first 10 seconds with light-hand pressure, followed by a gentle buffing action for 10 seconds at 20,000 rpm¹⁶.

The PoGo polishers were developed to be used without water cooling, which might produce heat with continuous contact therefore they were initially applied with light and intermittent pressure, followed by a gentle buffing motion to enhance the surface gloss. At the end of polishing process, the residual material was removed using an ultrasonic bath at 35°C for three minutes, followed by an absorbent paper drying process¹⁷.

All produced specimens were stored in three opaque plastic containers, each one had 15 compartments filled with 5 mL of distilled water for each compartment, and kept in an incubator at 37°C for 24 hours for rehydration and polymerization completion¹⁸.

2.6 Initial color measurements:

The Spectrophotometer[≡] was used to take initial color measurements (Figure 1). The color was measured using the CIE L*a*b* system (Commission Internationale de l'Eclairage). This apparatus has ten different colored lamps that are held in a circle and direct light bundle at a 45 ° to record and capture the L*, a*, and b* value of each sample. The lightness synchronize has a rate to axis L* that ranges from zero (black) to 100 (white). In the red-green and yellow-blue axes, the chromaticity axis a* and b* synchronize. A move to red was selected by positive a* values, whereas a shift to green was selected by negative a* values. Furthermore, negative b* values represent the blue color range, whereas positive b* values represent the yellow color range^(5,19).

[≡] Agilent Cary 5000 UV-Vis-NIR, USA



Figure 1. The spectrophotometer Agilent Cary 5000 UV-Vis-NIR, USA.

2.7. Staining of Specimens:

One prefabricated Black tea bag 2g (Lipton, London, United Kingdom) was immersed in 250 ml of boiling distilled water for five minutes to for preparing the staining solution²⁰.

Once baseline measurements were recorded, the specimens in each group were fully immersed in equal amounts of Black tea staining solution. The specimens were then incubated at 37°C for 7 days. After staining period, the specimens were rinsed with distilled water for 5 minutes and slowly dried with a paper towel¹².

2.8 Second Color Measurement:

The second color measurement was evaluated by spectrophotometer for all groups using the same procedure as described above. The second measurement was used as the intervention for each specimen, allowing the color change (ΔE) to be calculated using the following formula: $\Delta E = [(\Delta a)^2 + (\Delta b)^2 + (\Delta L)^2]^{1/2}$, where Δa^* equals ($a^*1 - a^*0$), Δb^* equals ($b^*1 - b^*0$), and ΔL^* equals ($L^*1 - L^*0$). The baseline value represented by 0, and the color value after 7 days of immersion in the staining solutions represented by 1^{12,21}.

2.9 Brushing of specimens with dentifrices:

A custom-made machine was used in order to simulate a brushing mechanism²². The machine is classed as a reciprocating machine as it converts rotational momentum to linear motion at a frequency of two strokes per second. **Figure 2**

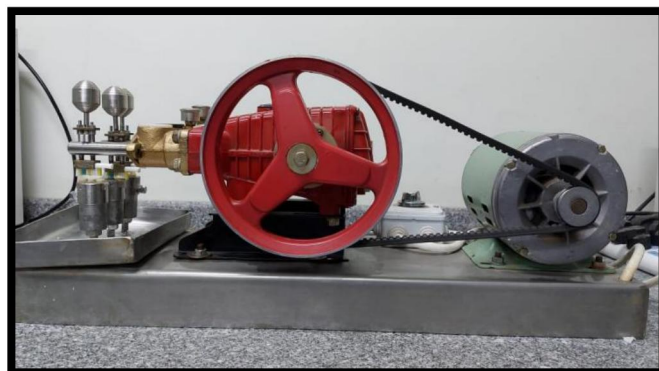


Figure 2. Tooth brushing simulating device

Brushing cycles:

1800 strokes were done in 15 minutes at a rate of 120 strokes per minute using a brushing simulating machine and a toothbrush load of 200 g. Represent three to six months of in-vivo tooth brushing¹. After every 5000 brushing cycles, the toothbrush head was replaced²³. After brushing, all brushed composite specimens were rinsed with air/water spray and dried with absorbent paper. All specimens were placed in an ultrasonic bath at 35°C for three minutes before final color and surface roughness analysis (Ra_i), then dried with an absorbent to ensure clean and debris-free surfaces of the specimens¹⁷.

2.10 Final color and analysis (Ra_i):

Final color records were obtained using a Spectrophotometer²⁴, and color measurements were collected for all groups using the same method in the initial and second measurements.

2.11. Statistical analysis:

The Shapiro-Wilk and Leven's tests were used to examine numerical data for normality and variance homogeneity. As the data had a parametric distribution and variances were homogeneous across groups, they were expressed as mean and standard deviation (SD) values and analysed using one-way ANOVA followed by Tukey's post hoc test. Within all tests, the significance level was set at $p \leq 0.05$. R statistical analysis software version 4.1.0 for Windows²⁵ was used for the statistical analysis.

3 Results

There was a significant difference among different groups ($p < 0.001$). The highest value was found with Sof-lex (2.95 ± 0.18), followed by PoGo (2.75 ± 0.17), while the lowest value was found with Mylar strip (2.41 ± 0.15). Post hoc pairwise comparisons showed value of different groups to be significantly different from each other ($p < 0.001$).

²⁵R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

Mean and standard deviation (SD) values of color change (ΔE) values for polishing systems at (baseline-staining) were presented in (table 3) & (Figure 3).

Table 3. Mean \pm standard deviation (SD) of color change (ΔE) values for different intervals

Polishing system	Tooth paste	Color change (ΔE) (mean \pm SD)			p-value
		Baseline-Staining	Staining-brushing	Baseline-brushing	
(F0)	D1	2.33 \pm 0.13 ^b	4.45 \pm 0.21 ^a	2.13 \pm 0.16 ^b	<0.001*
	D2	2.49 \pm 0.14 ^b	4.80 \pm 0.24 ^a	2.35 \pm 0.21 ^b	<0.001*
(F1)	D1	2.97 \pm 0.15 ^b	5.38 \pm 0.13 ^a	2.46 \pm 0.19 ^c	<0.001*
	D2	2.94 \pm 0.22 ^b	5.77 \pm 0.14 ^a	2.87 \pm 0.20 ^b	<0.001*
(F2)	D1	2.74 \pm 0.18 ^b	4.65 \pm 0.11 ^a	1.93 \pm 0.19 ^c	<0.001*
	D2	2.76 \pm 0.16 ^b	5.03 \pm 0.14 ^a	2.30 \pm 0.22 ^c	<0.001*

Means with different superscript letters within the same row are statistically significantly different *; significant ($p \leq 0.05$) ns; non-significant ($p > 0.05$)

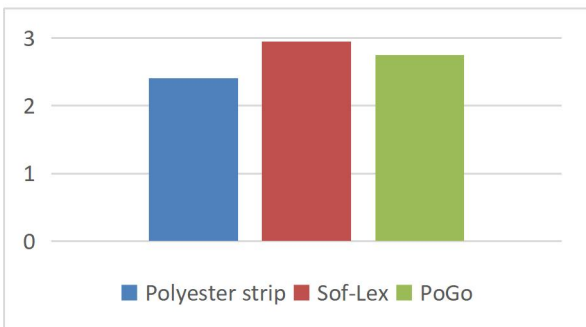


Figure 3. Bar chart showing average color change (ΔE) in different polishing systems at (baseline-staining).

•Staining-brushing: Rapid white (5.20 \pm 0.45) had a significantly higher value than Colgate (4.83 \pm 0.44) ($p=0.010$).

•Baseline-brushing: Rapid white (2.51 \pm 0.33) had a significantly higher value than Colgate (2.17 \pm 0.28) ($p=0.001$). The highest mean value was recorded in group A (AH Plus) (651.19 \pm 61.97), with a significantly lower value recorded in group C (GuttaFlow Bioseal) (556.16 \pm 36.24), followed by group B (Ceraseal) (511.71 \pm 53.5). One Way ANOVA test revealed a significant difference between groups ($p=0.000$). Mean and standard deviation (SD) values of color change (ΔE) values for

dentifrices within time were presented in (table 4) & (Figure 4).

Table 4. Mean \pm standard deviation (SD) of color change (ΔE) values for different Dentifrices within time

Interval	Colgate		Rapid white		p-value	Effect size (Partial eta squared)
	Mean	SD	Mean	SD		
Staining-brushing	4.38	0.44	5.20	0.45	0.010*	0.155
Baseline-brushing	2.17	0.28	2.51	0.33	0.001*	0.235

*: Significant at $P \leq 0.05$

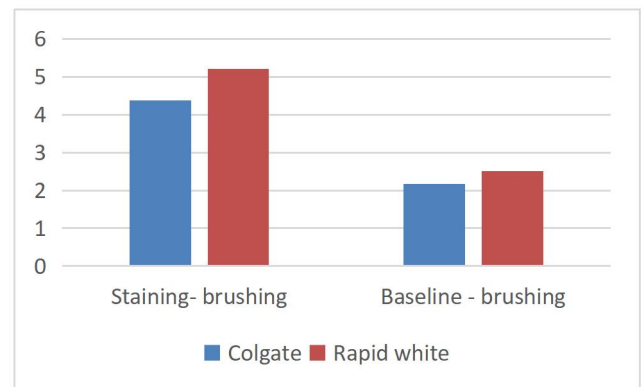


Figure 4. Bar chart showing average color change (ΔE) in different toothpastes within time

4 Discussion

The introduction of nanotechnology to composite led to many advantages. Firstly, an increased filler load due to the smaller particle size and higher surface area, which had contributed to the increase in surface polish and better handling properties. That justify the use of universal nanofilled Filtek Z350 XT in the present study^{3, 16}. To ensure consistency in the comparison, all specimens were prepared from shade A2, which is one of the most commonly used shade in dentistry because to its common in human teeth³. For the specimen standardization a Mylar matrix strip was used in this investigation, even though it is proven efficient in-vitro that might not be the case in-vivo as proximal application might be challenging²⁴. The groups that received no treatment were served as control and they were compared to specimens treated with different polishing systems and that was in accordance with previous studies **Bansal et al, (2019)**²⁵ and **Iris et al, (2016)**²⁶.

Since black tea is one of the most popular drinks in Egypt and across the world, it was used as a staining agent in the current investigation. In order to test the possibility for long-term stain retention, the current investigation used a 7-day immersion time, the 7-day storage time is simulated throughout a seven-month period of black tea consumption^{27, 28}. During the storage

period, the staining solution was replaced every 24 hours with a new one. The specimens were incubated in a dark environment at temperature of 37 °C during exposed to staining solutions to simulate the oral conditions of the patient. In our study discoloration below or above the value (ΔE 3.3) was referred to as acceptable or unsatisfactory, as it was often used in literature regarding in-vitro color assessment of dental composites^{8, 27, 29}. The CIE L*a*b* parameters were calculated by measuring shade changes immediately, after polishing and after immersion in staining solutions using a spectrophotometer. Color change after seven days of immersion in the black tea staining solution showed that all polishing systems demonstrated clinically acceptable alterations. The ΔE detected in all groups after the staining challenge stayed below the threshold of (3.3). Sof-lex had the greatest value, followed by PoGo, and Mylar strip had the lowest. Post hoc pairwise comparisons showed value of different groups to be significantly different from each other ($p < 0.001$). Thus, the null hypothesis tested which stated that, there will be no difference in the color stability between polished (PoGo, Sof-Lex) and unpolished nanofilled resin composite after immersion in staining solution, was rejected. Mylar strip group showed significantly less color change than PoGo group and both was less than Sof-Lex group, this could be due to the defects and scratches caused by the polishing treatment on the materials' surfaces made them more liable to staining. The polishing procedure strained the surface of the nanofilled composite, making it more susceptible to discoloration. During the polishing process, Sof-Lex and PoGo may cause strain in the composite resin surfaces. The dye accumulates more easily when the strain increases the activity of atoms on the surface. The filler particles may be separated from the heat-softened resin when the molecular arrangement of the resin matrix is strained³⁰. These results are in accordance with those previous studies of **Barakah et al, (2014)**³¹ and **Deljoo et al, (2020)**²¹, who reported that the least staining susceptibility of nanofilled composite restorations was created by a well-adapted Mylar strip with the smoothest surface. Alternatively, this results disagreed with **Berger et al, (2013)**³², who reported no significant changes in color stability of Filtek Z-350 nanofilled composite across all polishing systems (Sof-Lex, PoGo) and control groups. This difference might be explained by a change in the staining solution applied (absolute alcohol), which may have a different PH and stainability than black tea. Furthermore, the study used a different approach; the immersion time was 24 hours, followed by a three minute centrifugation at 3000 rpm. The brushing showed to roughen the surface of resin composite-based

materials using a three-body wear process. Brushing for teeth with resin composite restoration can dissolve the softer polymer matrix and exposing the tougher reinforcing particles³³. Tooth-Brushing can also have a roughening impact since the bristles and dentifrices do not abrade the surfaces as uniformly as flat discs or rubber cups do in polishing processes^{17, 34}. Every person brushes each tooth surface for eight seconds every day at a frequency of two strokes per second, for a total of 16 strokes per tooth surface per day¹⁷. As a result, in this present investigation, a total of 1800 strokes (15 minutes) were done at a frequency of 120 strokes per minute by the brushing simulating machine. Represent three to six months of personal teeth brushing^{1, 17}. The bulk of research evaluating dentifrice whitening effects are carried out over a period ranging from two weeks to six months. Whitening dentifrices have recently been offered on the market with a wide variety for the prevention or treatment of discoloration. According to **Heintze et al, (2010)**³⁵ (ISO) advises that the relative dentin abrasiveness (RDA) of toothpaste should not exceed 250, low abrasive dentifrices have RDAs between 0-70, medium between 70-100, and high between 101-150^{36,37}. So, in the current investigation Rapid White high RDA as a whitening dentifrice and Colgate low RDA as a regular (non-whitening) dentifrice were applied to examine their influence on color stability of stained nanofilled composite. Color change ΔE results of dentifrice type between stained and after brushing showed that all groups brushed with Rapid white had a higher statistically significant ΔE value than Colgate ($p = 0.010$). Also (ΔE) among baseline and after brushing showed that all groups brushed with Rapid white had a significantly higher value than Colgate ($p = 0.001$). Thus, the null hypothesis tested which stated that, there will be no difference in color stability of resin composite after mechanical brushing with conventional or whitening dentifrices, was rejected. These findings are broadly consistent with several studies of **Alhotan A et al, (2023)**²⁹, **Amaral et al, (2006)**³⁸, **Chour et al, (2016)**³ and **Roopa et al, (2016)**¹⁰, who revealed that whitening dentifrices with high abrasiveness may represent a greater increase in ΔE than regular non-whitening dentifrices as a result of filler interface degradation and release of filler particles from the resin matrix of stained surface. After the abrasive challenges, the ΔE found in all groups stayed below the (3.3) threshold demonstrating that brushing was unable to affect the organic matrix geometry of the composite to be clinically seen. This is consistent with other in-situ and in-vitro studies, which reported that however there was a decrease in luminosity of composites after brushing with abrasive dentifrices the change was not significant enough to modify the resin color^{36, 39}. Alternatively, the current study results contradict those of

Roselino et al, (2013)⁴⁰ who reported that there was no color change in composite when subjected to brushing with various dentifrices. This difference could be attributed to variations in methodology.

5 Conclusion

- I. PoGo polishing system produced better surface quality in terms of color stability than Sof-Lex polishing system.
- II. Brushing with whitening dentifrice was more effective on removing Black tea surface stains from the resin composite than non-whitening.

Authors' Contributions

Ahmed Sabry Fouda, principal author.
 Prof. Dr. Mahmoud Abdel-Mohsen Mohamed,
 Assistant Professor Ali Abdel-Nabi.
 All authors have read and approved the manuscript.

Conflict of interest

The authors declare that they hold no competing interests.

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