

Research Article

# Effect of Tooth Brushing on Enamel Surface Roughness and Brightness After 25% Hydrogen Peroxide (HP) Whitening: An *In-Vitro* Study

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## Abstract

**Objectives:** The aim of this study is to investigate if tooth brushing has an effect on surface roughness and dental brightness after whitening.

**Materials and Methods:** Five healthy random premolars were used repeatedly to minimize inter-sample variability. They were firstly prepared following ISO 28399 guidelines and thereafter they were whitened using an office product containing 25% Hydrogen Peroxide (HP). Afterward, they were subjected to four sessions of brushing. Both Roughness Ra and Rz were measured at each stage of the study using a mechanical contact profilometer. Similarly, luminance was estimated by recording the voltage 'reflected' from the teeth using an experimental setup that we designed.

**Results:** Surface Roughness Ra remained stable with no significant differences among the groups ( $p > 0.05$ ). However, Rz values generally increased with significant difference among different groups ( $p < 0.05$ ). The generated voltage increased after teeth whitening, remained stable after the brushing sessions and decreased slightly after one month of brushing, with significant differences only between the control group and the other groups ( $p < 0.05$ ).

**Conclusion:** Tooth brushing after whitening increased the enamel surface roughness. This is likely due to abrasion and microstructural deformations. However, brightness did not evaluate as we expected. It remained stable potentially because of dehydration or optical saturation. This emphasizes the need for further investigations under clinically relevant conditions.

**Keywords:** Hydrogen Peroxide; Luminance; Roughness; Tooth Whitening; Tooth Brushing

## Introduction

Nowadays, a brilliant smile is commonly perceived as a sign of good health and self-confidence. However, over time, the natural radiance of teeth can diminish due to various factors such as smoking or eating habits. Products we consume contain pigmented agents called chromogens that

can accumulate either inside the teeth or on their surface resulting, respectively, in intrinsic and extrinsic discoloration<sup>1</sup>. Because of this, many people turn to teeth whitening.

There are several types of teeth whitening, mainly divided into two broad categories: in-office whitening performed by clinicians and at-home whitening using over-the-counter products. Regardless of the method, the whitening effect is ultimately attributed to the Hydrogen Peroxide (HP) [1]. After applying the product, HP decomposes into unstable free radicals that, due to their low molecular weight, penetrate the tooth and break the double bonds of the chromogens. We talk about an oxidation reaction [2]. The effectiveness of teeth whitening depends on several factors. The most frequently studied ones included the PH level of the product which is linked to the release of free radicals, the concentration of HP that impacts the durability of the whitening and the treatment duration through its effect on enamel crystals and their ability to disperse light [3-5].

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Whitening treatments, although effective, can have adverse effects on dental hard tissues. Many studies proved that these products lead to a decrease in enamel micro-hardness and an increase in enamel roughness, compromising its structural integrity [6]. As porous surfaces are more susceptible to retaining stains, dental professionals highly recommend consistent tooth brushing to avoid pigment accumulation and maintain the achieved results from whitening procedures [7]. Thus, it seems interesting to investigate the impact of tooth brushing on surface roughness and dental brightness after whitening.

## Materials and Methods

### Study Design

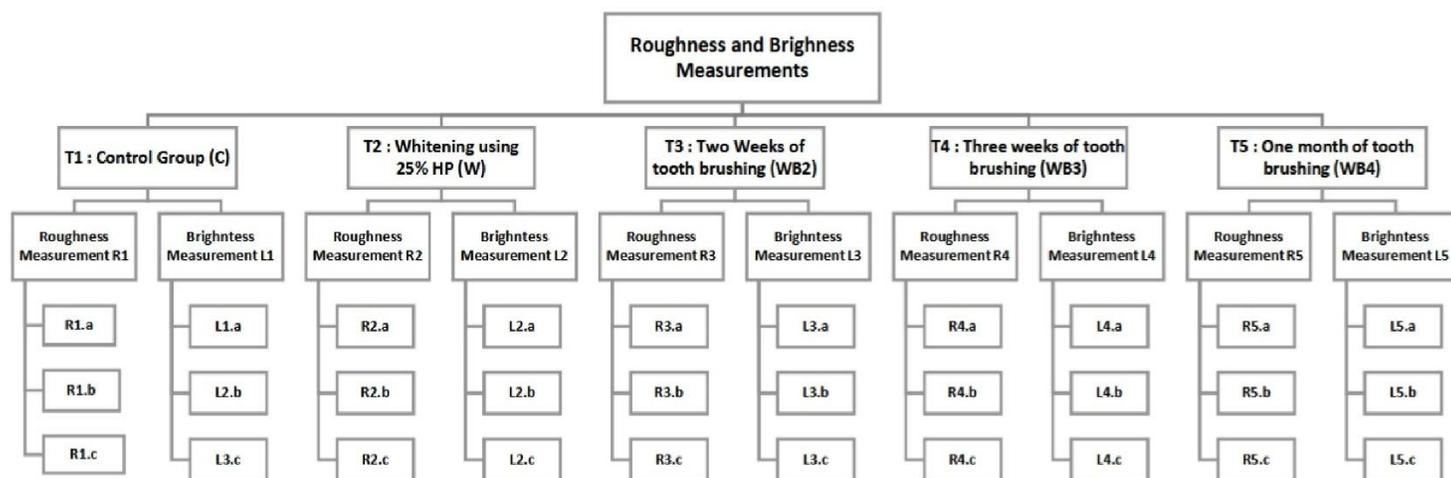
A longitudinal comparative *in-vitro* study was conducted to evaluate how tooth color and surface roughness evolve over time in response to whitening and brushing. Same five extracted human premolars, without caries or visible cracks, were used repeatedly to minimize inter-sample variability. Premolars were chosen due to their relatively flat surface and uniform structure, suitable for reproducible measurements.

### Sample Preparation

The samples were prepared following ISO 28399 (Third Edition 2021-03) guidelines with some modifications made due to existing constraints [8]. The specimens were polished using silicon carbide abrasive papers P1000 under constant water flow, while maintaining a minimum enamel thickness of 1 mm to be tested. The samples, however, were not stored in any solution, as they had already been exposed to ambient air before their collection.

### Protocol Treatment

As illustrated in Fig. 1, the samples were subjected to repeated evaluations of brightness and roughness at different stages of the study. Initial measurements were taken to establish baseline values, serving as the control group T1. Subsequent measurements were then conducted after each experimental condition (T2, T3, T4 and T5) to monitor its specific impact on the enamel. For each measurement point, three values were recorded per sample. This approach was adopted to ensure accuracy, reproducibility and to minimize measurement variability.



**Figure 1:** Schematic representation of different measurements carried out.

The premolars were whitened using cavex bite and white shade correction; an in office product containing 25% of hydrogen peroxide superior. The whitening procedure was performed following the manufacturer's instructions; in accordance with ISO 28399 standards [8]. This specific product was selected because it aligns with current recommendations favoring the use of lower HP concentrations [4].

To replicate the mechanical action of tooth brushing under controlled conditions, an automated brushing apparatus, based on rod-crank mechanism, was custom-built. A brushing force of  $F = 2\text{N}$  was applied using a spring with a stiffness of  $1.6\text{ N/m}$ , ensuring consistent pressure across samples [9]. The motor operated at a fixed speed of  $200\text{ rpm}$ , which corresponds to  $v = \omega \cdot r = 41.9\text{ cm/s}$ . The total brushing time was estimated as  $40\text{ min/week}$  based on typical hygiene oral habits [9].

The brushing was performed in a neutral artificial saliva (ph = 6.8) without any toothpaste in order to isolate the brushing effect. The artificial saliva was prepared using the following components, available in our laboratory: 400 ml of distilled water, 0.2 g of Sodium Chloride (0.05% of NaCl), 0.2 g of Sodium bicarbonate (0.05% of NaHCO<sub>3</sub>), 0.04 g of potassium hydrogen phosphate (0.01% of KH<sub>2</sub>PO<sub>4</sub>), 0.4 g of urea (0.01%) and 0.3 g of potassium chloride (0.075% of KCl) [10].

#### *Roughness Measurement*

The surface roughness (Ra, Rz) measurements were performed using a mechanical contact profilometer (Surface SJ-301, Mitutoyo, Kawasaki, Japan). Measurements were performed in three different directions with a measuring distance of 4 mm and a cut-off value of 0.8 mm.

#### *Lighness Measurement*

To evaluate changes in teeth value after whitening, dentists typically use a shade guide, relying on their eye's sensitivity. In order to be objective and quantify this parameter, we opted, in our study, to assess luminance instead, which refers to the amount of light emitted/reflected by the tooth surface. To this purpose, we developed an experimental setup.

The light source emits radiation, part of which is reflected by the tooth surface and captured by a photovoltaic cell as Irradiance (E, W/m<sup>2</sup>). An aluminum-lined tube was incorporate into the setup to concentrate the light beam towards the tooth and minimize its dispersion. The cell converts this light energy into an electrical voltage (U, V), that is related to the luminance (L, m<sup>2</sup>sr) by the following equations [11,12]:

$$V = \alpha \cdot \ln(E)$$

$$L = \frac{kEd^2}{A\alpha d\epsilon}$$

k is a parameter related to the surface area of both tooth and cell,  $\alpha$  is a factor associated with other parameters such as temperature and the internal resistance of the cell and d is the distance between the tooth and the cell. In our study, several positions of the photovoltaic cell were tested, to determine the optimal placement (d,  $\theta$ ) ensuring maximum capture of reflected light. Thus, the final relationship between voltage and Luminance is expressed as

$$L = \frac{V}{k e \alpha d^2}$$

If we consider that the experimental conditions were maintained consistent (temperature, distance between the tooth and the cell, cell and tooth surfaces), we can conclude that as the voltage increases, luminance also increases, leading to a corresponding rise in perceived brightness [13].

#### *Statistical Analysis*

Statistical analyses were performed using the Real Statistics Resource Pack add-in for Microsoft Excel 2013. The Shapiro-Wilk test was used to assess data's normality. Voltage and Ra were then, analyzed with one-way repeated measures ANOVA. Rz was analyzed using the nonparametric Friedman test. Post-Hoc tests were then used to determine which specific group means are different. Statistical significance was considered for  $p < 0.05$  in all cases.

### **Results**

The evolution of Enamel Surface Roughness after whitening and brushing is illus-trated in Fig. 2. Ra remained relatively stable, with a slight increase observed after two weeks of brushing, followed by as gradual decline. The overall surface morphology was not significantly affected, as confirmed by statistical analysis ( $p > 0.05$ ) (Table 1). In the other hand, there were significant differences regarding Rz values ( $p < 0.05$ ) (Table 1). Mean Rz values have been rising steadily with significant differences between (C Group - WB4 Group), (W Group - WB4 Group) and (WB2 Group - WB3 Group) ( $p < 0.05$ ). The variation of the voltage generated by the photovoltaic cell in response to light reflected from the enamel surface is illustrated in Fig. 3. After whitening, the recorded voltage increased. Over the following weeks of brushing, the voltage remained stable, before decreasing slightly at the fourth week. There were statistically significant differences among the groups as shown in Table 2 ( $p < 0.05$ ). Significant difference was observed between the control Group and the other groups ( $p < 0.05$ ). However, the statistical differences between the rest of the groups were not significant ( $p > 0.05$ ).

	Ra		Rz	
	Mean Value +/- SD	p-value (Repeated Measures ANOVA)	Mean Value +/- SD	p-value (Friedman test)
C Group <sup>a, A</sup>	0.523 +/- 0.1	0,25811356	2.758 +/- 0.52	0,04135206
W Group <sup>b, BF</sup>	0.547 +/- 0.08		2.78 +/- 0.58	
WB2 Group <sup>c, CG</sup>	0.655 +/- 0.2		3.842 +/- 0.76	
WB3 Group <sup>d, DG</sup>	0.544 +/- 0.1		2.762 +/- 0.8	
WB4 Group <sup>e, EF</sup>	0.48 +/- 0.05		3.65 +/- 0.76	

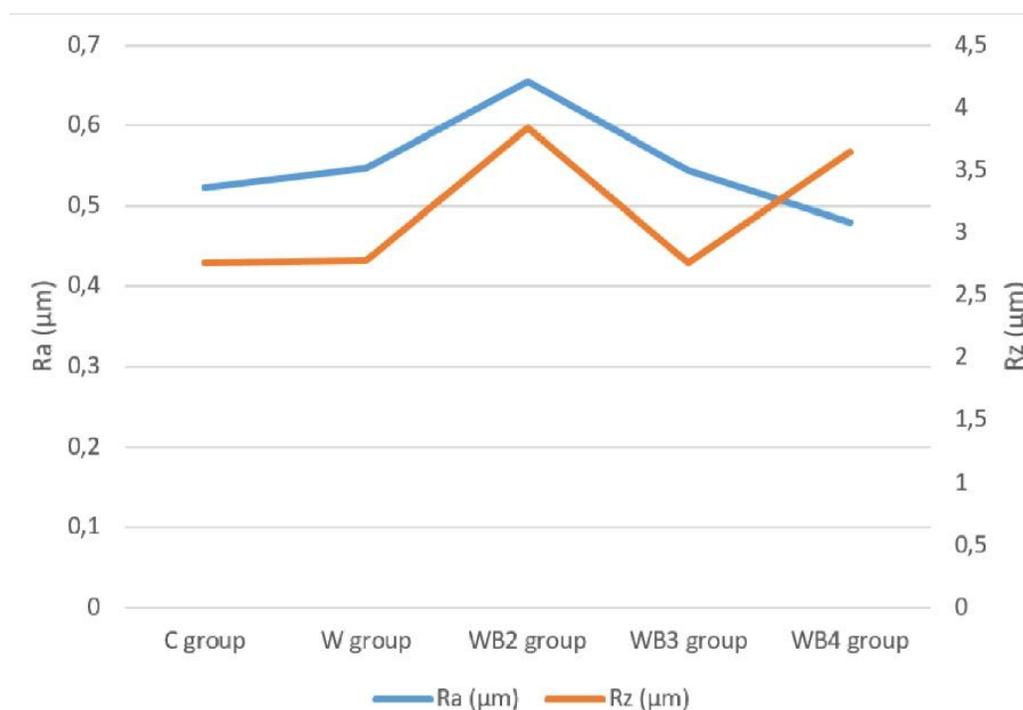
\*Different letters within lines indicate statistically significant differences ( $p < 0.05$ ). Lowercases represent Ra differences, while uppercases represent Rz differences.

**Table 1:** Roughness Ra and Rz outcomes for each study group.

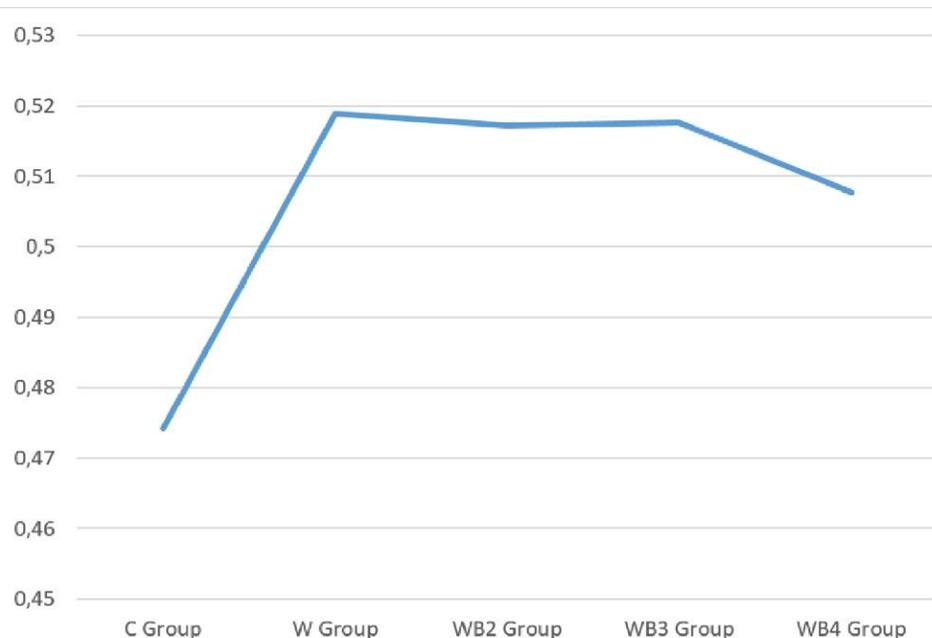
	Recorded Voltage (V)	
	Mean V +/- SD	p-value (Repeated Measures ANOVA)
C Group <sup>abcd</sup>	0.474 +/- 0.004	0,0000047581
W Group <sup>a</sup>	0.52 +/- 0.004	
WB2 Group <sup>b</sup>	0.517 +/- 0.002	
WB3 Group <sup>c</sup>	0.517 +/- 0.008	
WB4 Group <sup>d</sup>	0.507 +/- 0.005	

\*Different letters within lines indicate statistically significant differences ( $p < 0.05$ ).

**Table 2:** Voltage outcomes for each study group.



**Figure 2:** Roughness Ra and Rz evolution (μm).



**Figure 3:** Voltage evolution (V).

### Discussion

One of the findings of this study indicate that brushing did not significantly modify the average surface roughness Ra after whitening, but it led to an increase in the Rz parameter, representing the most marked peaks and valleys of the enamel surface. Tooth brushing over a one-month period revealed a nonlinear progression of enamel surface degradation. Rz increased notably after two weeks of brushing, decreased slightly at the third week and then increased again by the fourth week. This suggests that even if the overall surface remains relatively homogenous, deeper or more pronounced localized irregularities appear after brushing.

The peroxide contained in the whitening gels causes morphological changes in enamel surface; it increases pores and reduces the mineral content of the enamel, causing deformations in both enamel prism and interprism areas [14-16]. Those irregularities can mechanically result in an increase in Rz.

In the other hand, several studies have demonstrated that toothbrushing can significantly alter enamel surface topography. Bhola et al., 2023 (17) reported that different tooth-brush designs exert varying abrasive forces. Swain M, specified that manual toothbrushes tend to create more surface scratches [18]. Mohan P, added that soft-bristle brushes, as the one used in this study, result in the greatest abrasive wear [19]. Their findings support the general concept that mechanical brushing contributes to enamel surface degradation, especially when enamel is already compromised.

Another significant difference between WB2 group and WB3 group was observed. The decrease in Rz at the third week, may reflect a mechanical smoothing effect or a transient surface compensation due to uniform abrasion. Similar findings have been reported in literature, where repeated abrasion or a uniform mechanical action can smooth out surface irregularities, resulting in temporary lower roughness values [20]. However, the subsequent increase at the fourth week suggests that this effect is not sustained. Given that the difference between these last two groups was not statistically significant, these fluctuations should be interpreted with caution.

The second notable finding of this study concerns the change in teeth brightness, indirectly assessed through the voltage response of the photovoltaic cell to the light reflected by the enamel surface. After whitening procedure, the voltage increased, suggesting a significant enhancement in surface brightness. Besides, statistically significant difference was observed between the C Group and the other groups. Conversely, no significant differences were observed across W, WB2, WB3 and WB4 Groups. Subsequent brushing cycles did not result in notable changes in voltage, indicating that mechanical brushing had negligible influence on the optical properties of enamel surface, particularly luminance.

These results were not aligned with our expectations, as we anticipated that the volt-age recorded would increase, particularly after the first and the last brushing sessions (WB2-WB4), since surface roughness had increased at both time points. Many previous studies, such those by Joiner A, have reported a positive correlation between surface roughness and light diffusion [21].

Furthermore, on the microscopic scale, R. Ekaterina, reported that the in-crease in surface roughness disrupts the enamel's crystal uniformity, leading to greater light scattering and diffuse reflection [22]. Vargas-Koudriavtsev T, added that the application of low-concentration HP for a long time cause a greater diminution in enamel crystal size relative to short-term use of high-concentration HP [23]. This reduction enhances light scattering at the enamel surface, since the size of enamel crystals are inversely proportional to teeth brightness [24].

The apparent divergence can likely be explained by intrinsic optical phenomena such as optical saturation: a threshold beyond which no measurable changes in lightness are detected, even though surface transformations may still occur [25]. Thus, the lack of further significant measurable change in voltage after brushing may be because the enamel had already attained its maximum whitening effect after the first treatment (W Group). Otherwise, the impact was not sufficient to reach under our experimental conditions.

Thus, several limitations should be taken into account. The premolars used were limited and randomly selected, making it impossible to account for previous factors such as dental age, gender or any past treatments. Additionally, tooth were not stored in saliva and brushed for continuous 40 minutes, which does not reflect typical oral hygiene practices. Moreover, *in-vitro* studies do not fully replicate clinical conditions, which limits the applicability of the results. All that reduces the clinical relevance of the results. Given these limitations, further studies, particularly *in vivo* studies, with a larger sample size, using advanced techniques are needed. This could offer more details about the microstructural changes and its impact on the optical behavior.

### Conclusion

Post-Whitening tooth brushing increased enamel roughness, probably, due to abrasion and deformations in both prisms and interprism areas. Yet, brightness remained stable, possibly due to dehydration or optical saturation. These results suggest complex interactions between surface changes and optical behavior and emphasize the need for further investigations under clinically relevant conditions.

### Conflict of Interest

There are no conflicts of interest that may have influenced the research, authorship or publication of the article.

### Financial Disclosure

The Whitening product used in this study was provided free of charge by 'Mia Dent' for the purposes of this study. No additional funding or services were received.

### Ethical Statement

This project was exempt from IRB review as it did not qualify as human subject research under federal regulations.

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