Original Research

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Evaluation of the influence of enamel thickness on the effectiveness of tooth whitening: an *in vitro* study

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Abstract

Introduction. While tooth whitening is widely used to manage dental discoloration, results can vary substantially across teeth and protocols. In this in vitro study, we assessed whether enamel thickness across tooth regions influences whitening efficacy under different peroxide-based protocols.

Methods. Ninety extracted human premolars were stained and allocated to three protocols (n=30 each): 10% (10 h), 20% (4 h), and 35% (1 h). Enamel thickness was measured on CBCT at the cervical, middle, and occlusal thirds. Color was recorded with a spectrophotometer using one acquisition before whitening and one after whitening; three regional L*a*b* values were extracted from each image. Color change was expressed as CIEDE2000 (ΔE00). Because three regions were analyzed per tooth, the primary analysis used a linear mixed-effects model (LMM) with a random intercept for tooth (ΔE00 ~ Group×Region + (1|Tooth)). Enamel thickness was added as a covariate in a secondary LMM. Robustness was assessed by GEE with robust standard errors and a tooth-level sensitivity analysis (mean ΔE00 per tooth).

Results. ΔE00 differed significantly across protocols in the LMM: compared with 35%, ΔE00 was higher for 20% ($p=0.003$) and 10% ($p<0.001$). Region was not significant ($p>0.05$) and the interaction was not supported overall; a localized 10%×occlusal effect was borderline in LMM ($p\approx0.055$) and significant in GEE ($p\approx0.024$). Baseline color differed between groups and was therefore included a priori as a covariate; adjustment for baseline L* did not alter the main conclusions. After adjustment, enamel thickness was not associated with ΔE00 ($p\approx0.933$). Exposure-dose (concentration × time, % h) showed a positive trend with ΔE00 ($r=0.401$, $p<0.001$; $R^2=0.161$). Only 0.37% of measurements showed ΔE00 ≤ 1.8.

Conclusion. Whitening efficacy was primarily governed by the protocol (cumulative exposure) rather than enamel thickness. Regional modulation was modest, and enamel thickness alone was not predictive.

Keywords: tooth bleaching, enamel thickness, spectrophotometry, CIEDE2000, mixed-effects model

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Introduction

Tooth whitening is a conservative and aesthetic treatment for tooth discoloration, aiming to reduce dental chromatic alterations by modifying the organic chromophores responsible for tooth color, resulting in a lighter appearance [1,2]. Peroxide-based agents are the main active components used in whitening procedures and act through oxidative reactions that alter the light absorption properties of these chromophores [2,3].

Tooth color is strongly influenced by dentin, while enamel, due to its translucency, mainly modulates brightness and overall color perception [4,5]. Consequently, enamel-related characteristics have been considered potential determinants of the final whitening outcome. Previous in-vitro studies suggested that enamel thickness and anatomical region may affect bleaching response, with thinner regions sometimes showing larger color changes [6,7]. However, reported results remain heterogeneous due to differences in experimental design, tooth type, bleaching protocol, and color-difference metric [6–9].

In addition to anatomical factors, bleaching outcomes depend on protocol parameters, notably peroxide concentration and application time. Prolonged exposure to lower concentrations may produce color changes comparable to, or greater than, higher concentrations applied for shorter durations [10–12]. Therefore, the interaction between enamel thickness and protocol parameters warrants objective evaluation using standardized regional measurements.

This study evaluated the influence of regional enamel thickness on whitening efficacy using CBCT-based thickness measurements and spectrophotometric color assessment at three buccal regions before and after whitening. The following null hypotheses were tested:

– Whitening produces comparable color changes across regions

– Different protocols produce comparable changes within each region

– There is no association between enamel thickness and whitening-induced color change.

Methods

Study design

This in-vitro study investigated the association between regional enamel thickness and tooth whitening response using three peroxide-based protocols.

Specimens

Ninety intact human premolars extracted for orthodontic reasons were included. Teeth exhibited intact enamel with no caries, cracks, fractures, abrasions, discolorations, or restorations.

Staining and allocation

Teeth were disinfected in a thymol-based solution and inspected (×4 magnification). Specimens were stained by immersion in a standardized coloring solution for 4 days (coffee, tea, and turmeric in water, boiled for 5 minutes). Teeth were numbered (1–90) and randomly allocated to three equal groups (n=30) and stored in distilled water.

CBCT imaging and enamel thickness measurement

Teeth were mounted in arch-shaped models and stabilized to standardize CBCT acquisition. Enamel thickness was measured using Blue Sky Plan software on oblique coronal reconstructions aligned with the long axis of each tooth. Thickness was recorded on the buccal surface at three predefined thirds: cervical, middle, and occlusal. Measurements were repeated twice one week apart by a single calibrated operator; mean differences between duplicates were <0.1 mm.

Whitening protocol

Approximately a 1mm layer of carbamide peroxide gel was applied to the enamel surface. Protocols were: Group H, 35% for 1 h; Group M, 20% for 4 h; and Group P, 10% for 10 h. Gel was removed, specimens were rinsed, dried, and stored in distilled water for 24 h rehydration prior to post-whitening measurements.

Color measurement

Color measurements were performed using a calibrated dental spectrophotometer. One color acquisition was obtained before whitening and one after whitening for each specimen. Measurements were standardized by positioning the spectrophotometer perpendicular to the buccal surface, and color data were extracted at three predefined regions (cervical, middle, and occlusal thirds) corresponding to the enamel thickness measurement sites. For each acquisition, regional CIE L*a*b* values were recorded against a neutral background under controlled lighting conditions. Whitening-induced color change was calculated using the CIEDE2000 color-difference formula (ΔE00), which is recommended for dental applications due to its improved perceptual uniformity and closer correlation with human visual perception.

Statistical analysis

Because three regional measurements were obtained from each tooth, the data had a repeated-measure structure. Therefore, the primary statistical analysis was performed using a linear mixed-effects model (LMM) with tooth included as a random intercept to account for within-tooth correlation. The fixed effects included bleaching protocol, region, and their interaction. Enamel thickness was subsequently introduced as a covariate in a secondary LMM to evaluate its association with whitening-induced color change. Robustness of the findings was assessed using generalized estimating equations (GEE) with robust standard errors, as well as a tooth-level sensitivity analysis based on mean ΔE00 values per tooth. Statistical significance was set at $p < 0.05$.

Results

Three regional measurements were obtained per tooth (n=270 observations from n=90 teeth). Descriptive values for enamel thickness and ΔE00 are provided in tables I–II.

Primary analysis (LMM with random intercept for tooth) confirmed a significant group effect: relative to the 35% protocol, ΔE00 was higher for 20% (p=0.003) and 10% (p<0.001). Region was not significant (middle vs cervical p=0.657; occlusal vs cervical p=0.363). The interaction was not supported overall; the 10%×occlusal term was borderline (p=0.055). Between-tooth variance (random intercept) was 2.58, indicating appreciable heterogeneity and within-tooth correlation.

Thickness-adjusted model: after including enamel thickness as a covariate, thickness was not associated with ΔE00 (p=0.933), while the group effect remained significant.

Robustness: GEE with robust standard errors yielded consistent inference for the main group effect (20% vs 35% p<0.001; 10% vs 35% p<0.001), and identified a significant 10%×occlusal interaction (p=0.024). Tooth-level sensitivity analysis (mean ΔE00 per tooth, n=90) confirmed a persistent group effect (ANOVA p=3.21e-07).

Clinical relevance: only 0.37% of all measurements were ≤ ΔE00 1.8.

Exposure-dose: defining dose as concentration × time, (%·h ; 35, 80, and 100 for the 35%, 20%, and 10% protocols, respectively), ΔE00 showed a positive trend with increasing cumulative exposure ($r = 0.401$, $p < 0.001$; $R^2 = 0.161$). Given that only three discrete exposure levels were tested, this analysis is reported descriptively.

Baseline color: mixed-effects models revealed significant baseline differences between protocol groups, particularly between the P and H protocols for L*, a*, and b* (all p<0.001 for P vs H). Therefore, baseline L* was included a priori as a covariate in the primary model. Adjustment for baseline color did not materially alter the main conclusions regarding protocol effects or the absence of an enamel thickness effect. After adjustment for baseline L*, the 10% and 20% protocols were statistically comparable (10% vs 20%: p(Holm)=0.213), and both remained higher than 35% (20% vs 35%: p(Holm)<0.001; 10% vs 35%: p(Holm)=0.017).

Table I. Enamel thickness (mm) by group and region (mean [SD]).

Group	Cervical	Middle	Occlusal
H	0.8 (0.17)	1.44 (0.2)	1.69 (0.26)
M	0.94 (0.2)	1.52 (0.19)	1.84 (0.23)
P	0.9 (0.18)	1.5 (0.22)	1.84 (0.18)

Group H = 35% (1 h), Group M = 20% (4 h), Group P = 10% (10 h).

Table II. Whitening-induced color change (ΔE00) by group and region (mean [SD]).

Region	(H) 35% (1 h)	(M) 20% (4 h)	(P) 10% (10 h)
CERV	7.46 (3.22)	10.16 (3.04)	11.78 (2.51)
MID	7.11 (2.1)	10.02 (2.61)	11.44 (2.75)
OCC	8.19 (2.22)	10.24 (7.23)	10.34 (2.66)

ΔE00 computed using the CIEDE2000 formula from pre- and post-whitening L*a*b* values measured at matched regions.

Table III. Fixed effects from the linear mixed-effects model for ΔE00 (random intercept for tooth).

Term	Estimate	SE	z	P
Intercept (35%, cervical)	7.46	0.64	11.74	<0.001
20% vs 35%	2.69	0.90	3.00	0.003
10% vs 35%	4.32	0.90	4.80	<0.001
Middle vs cervical	−0.35	0.80	−0.44	0.657
Occlusal vs cervical	0.72	0.80	0.91	0.363
20% × middle	0.22	1.13	0.19	0.848
10% × middle	0.01	1.13	0.01	0.992
20% × occlusal	−0.64	1.13	−0.57	0.572
10% × occlusal	−2.17	1.13	−1.92	0.055

Reference levels are 35% protocol and cervical region.

Table IV. Linear mixed-effects model including enamel thickness (random intercept for tooth).

Term	Estimate	SE	z	p
Intercept (35%, cervical)	7.39	1.07	6.89	<0.001
20% vs 35%	2.68	0.91	2.93	0.003
10% vs 35%	4.31	0.91	4.75	<0.001
Middle vs cervical	−0.41	1.05	−0.39	0.696
Occlusal vs cervical	0.64	1.25	0.52	0.605
20% × middle	0.22	1.13	0.20	0.845
10% × middle	0.02	1.13	0.01	0.989
20% × occlusal	−0.64	1.13	−0.57	0.572
10% × occlusal	−2.17	1.13	−1.92	0.055
Enamel thickness (mm)	0.09	1.08	0.08	0.933

Thickness expressed in millimeters. Reference levels are 35% protocol and cervical region.

Thickness non-linearity check: adding a quadratic thickness term did not reveal an association with ΔE00 (both linear and quadratic terms p>0.05), supporting the conclusion that enamel thickness is not predictive under these protocols.

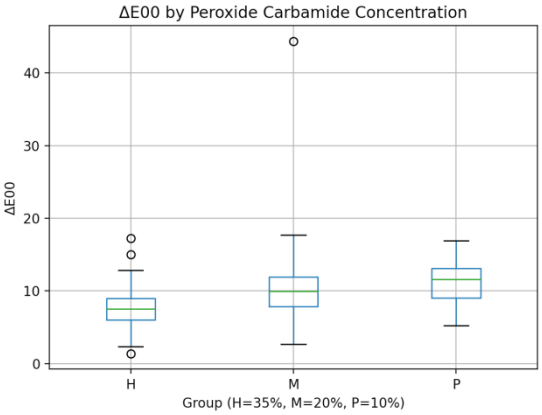

Figure 1. Boxplot of ΔE00 by whitening protocol group.

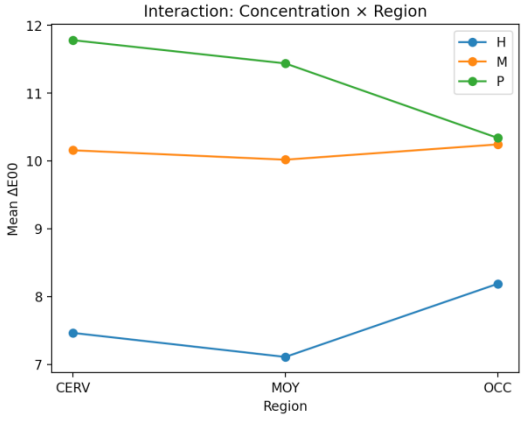

Figure 2. Interaction plot (mean ΔE00) by group and region.

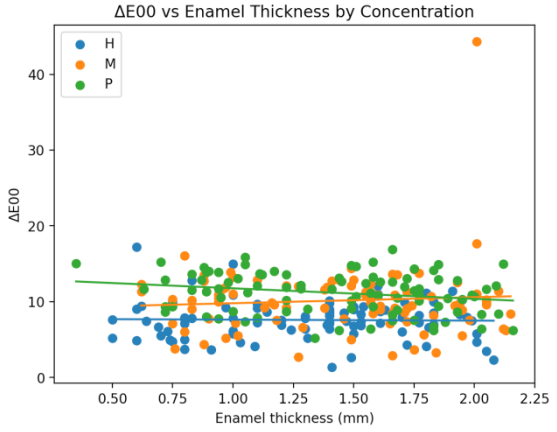

Figure 3. ΔE00 versus enamel thickness with linear regression lines by group.

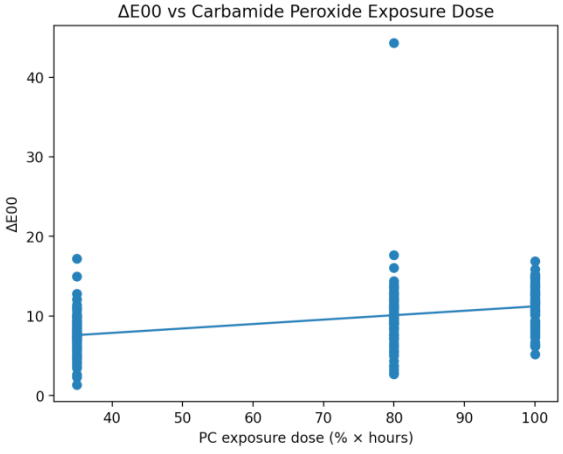

Figure 4. Dose–exposure relationship between ΔE00 and concentration×time (%·h).

Table V. Holm-adjusted protocol contrasts based on estimated marginal means (ΔE00).

Contrast	Estimate	SE	z	p (Holm)
20% vs 35%	2.47	0.62	3.98	<0.001
10% vs 35%	1.68	0.64	2.63	0.017
10% vs 20%	−0.79	0.63	−1.24	0.213

Estimated marginal means averaged over regions and adjusted for baseline L*. Holm correction applied.

Discussion

Enamel thickness as a non-autonomous determinant of whitening efficacy

In the present study, whitening efficacy assessed by ΔE00 was primarily influenced by the bleaching protocol parameters, namely carbamide peroxide concentration and application time, whereas enamel thickness did not emerge as an independent predictor. This finding supports a dose–kinetic model of dental bleaching, in which chromatic changes are governed predominantly by the quantity of oxidizing species delivered over time and their interaction with intrinsic chromophores, rather than by enamel thickness alone.

Previous experimental studies have demonstrated that prolonging bleaching application time significantly enhances color change, even at constant peroxide concentrations, while also affecting enamel mineral composition and surface properties [12]. These observations suggest that the bleaching response is primarily time-dependent and that geometric parameters may exert only a secondary influence when protocol intensity varies substantially.

Furthermore, it is well established that immediate post-bleaching color measurements can be confounded by transient enamel dehydration, leading to an overestimation of whitening effect. Partial color regression during subsequent saliva-mediated rehydration has been reported within 24–72 hours, with optical stabilization occurring up to 7–14 days post-treatment [10,13]. Consequently, enamel thickness alone cannot be expected to act as a robust predictor of ΔE00 under clinically realistic conditions where both protocol intensity and baseline color vary.

Reconciling the present findings with thickness-dependent peroxide diffusion studies

Although enamel thickness did not significantly influence ΔE00 in our model, numerous studies have demonstrated that tooth anatomy plays a critical role in hydrogen peroxide diffusion toward the pulp chamber.

Recent investigations on human teeth have shown that thinner enamel–dentin complexes allow greater trans-tissue peroxide penetration following in-office bleaching, resulting in higher intrapulpal peroxide concentrations and increased risk of postoperative sensitivity [14]. Similarly, Esteves et al. demonstrated that anatomical differences among tooth groups significantly influence peroxide diffusion, with penetration inversely proportional to hard-tissue thickness [15].

De Oliveira Duque et al. further reported that variations in enamel and dentin thickness affect not only esthetic outcomes but also cytotoxic responses in pulp-derived cells, emphasizing that tissue thickness is a major determinant of biological exposure, rather than a linear predictor of visible color change [7].

Taken together, these data suggest a dissociation between optical efficacy (ΔE00) and biological permeability (peroxide diffusion and pulp response). This distinction explains why enamel thickness may strongly influence pulp safety and sensitivity without necessarily producing proportional changes in whitening magnitude.

Optical considerations: why ΔE00 does not systematically track enamel thickness

From an optical perspective, dentin is the primary contributor to tooth color, while enamel functions mainly as a translucent modulating layer [4,5]. As a result, teeth with different enamel thicknesses may exhibit similar ΔE00 values if dentin chromophore oxidation is comparable.

Additionally, enamel microstructural variability including prism orientation, mineral density, and porosity has been shown to affect peroxide diffusion and light scattering independently of thickness [6]. These factors may outweigh simple geometric effects in intact human teeth, particularly when compared with simplified models using enamel slabs or bovine specimens.

This helps explain why thickness-dependent effects are more consistently observed in highly controlled in-vitro models, whereas studies using whole human teeth frequently report weaker or inconsistent correlations.

Measurement robustness and clinical interpretation of ΔE00

The use of ΔE00 is considered the most perceptually uniform color difference formula in dentistry and is closely aligned with clinically relevant perceptibility and acceptability thresholds [16]. However, recent multicenter studies have highlighted significant inter-instrument variability among dental spectrophotometers, underscoring the importance of strict calibration and standardized acquisition protocols [17].

By using a single calibrated spectrophotometer

and a uniform measurement protocol, the present study minimizes measurement bias and strengthens internal validity. Nevertheless, comparisons across studies remain limited by methodological heterogeneity, which may partially account for discrepancies in reported enamel thickness effects.

Clinical implications

Within the limits of the tested protocols, the present findings indicate that whitening efficacy is predominantly governed by protocol-related dose kinetics. Enamel thickness alone does not reliably predict ΔE00. Tissue thickness nevertheless remains clinically relevant for peroxide diffusion, pulp exposure, and sensitivity risk.

This distinction is clinically important. While increasing peroxide concentration or application time may overcome geometric barriers to color change, such strategies simultaneously increase biological exposure, particularly in teeth with thin enamel–dentin complexes. Consequently, protocol selection should prioritize anatomical risk assessment and pulp safety, rather than relying on enamel thickness as a predictor of esthetic outcome.

Contribution to the current body of knowledge

This study contributes to the growing evidence that dental bleaching outcomes are primarily dose-dependent rather than geometry-driven, while reinforcing existing literature demonstrating that enamel and dentin thickness critically modulate peroxide diffusion and biological effects.

By clearly separating esthetic efficacy from biological permeability, the present findings help reconcile previously conflicting reports and support a more nuanced understanding of bleaching biomechanics: enamel thickness influences safety margins more than whitening magnitude.

Data availability

An anonymized per-tooth per-region dataset (enamel thickness, pre- and post-whitening L*a*b* values, and ΔE00) is provided as Supplementary material (CSV).

Conclusions

Within the limitations of this in-vitro study, whitening efficacy was primarily governed by the bleaching protocol, particularly cumulative exposure (concentration × time), rather than enamel thickness. Regional modulation was modest, and enamel thickness alone was not predictive once the repeated-measure structure was appropriately accounted for. These conclusions were consistent across complementary analytical approaches, including mixed-effects modeling, generalized estimating equations, and tooth-level sensitivity analyses.

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