Original Article

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Diagnostic evaluation of initial enamel lesions using DIAGNOdent, color difference, and micro-CT

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Objectives: This study aimed to evaluate the relationship between lesion depth of the initial stages of enamel demineralization measured by a laser fluorescence device (DIAGNOdent), and high-resolution Micro-CT imaging, and to assess color differences (ΔE) as potential indicators of demineralization depth.
Materials and Methods: Thirteen human premolars were prepared and subjected to a controlled remineralization and demineralization regimen to simulate initial enamel lesions. Following a series of immersion cycles, specimens were analyzed for DIAGNOdent values, ΔE, and lesion depth through Micro-CT imaging.
Results: The results demonstrated a significant positive correlation between DIAGNOdent readings and lesion depth from micro-CT scan ($p<0.01$, $r = 0.79$), as well as between ΔE values and lesion depth ($p<0.01$, $r = 0.79$). Thus, both DIAGNOdent and ΔE analysis proved effective in assessing enamel demineralization.
Conclusions: This approach reveals the utility of combining advanced diagnostic tools to improve the understanding of early enamel demineralization and underscores the necessity for early detection and intervention in clinical practice to mitigate the progression of dental caries.

Keywords: color difference, depth of lesion, DIAGNOdent, Initial enamel lesion, Micro-CT

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Introduction

The initial stage of enamel lesions is an early manifestation of dental caries and a significant concern in preventive dentistry. These lesions represent the beginning stages of demineralization within the enamel structure, often manifesting as subtle changes in translucency rather than over visual signs, such as white spots caused by the loss of mineral content, including calcium and phosphate [1]. Initial enamel lesions can result from various factors, with poor oral hygiene being a predominant cause, leading to localized mineral loss and structural weakening. At this stage, the lesion is often reversible with appropriate interventions (e.g., fluoride or remineralizing agents) [2].

If demineralization continues at the initial stage, the initial enamel lesion will progress to the early stage. Ongoing demineralization leads to more distinct and potentially larger white spot lesions on the enamel surface. Although the enamel remains intact, the underlying mineral content is diminished, making the tooth more susceptible to caries progression [3]. With interventions such as fluoride treatments, these lesions can still remineralize, or areas of lost minerals can potentially be restored [4].

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If preventive treatments are not applied at the initial stage, the lesion may become more pronounced, potentially turning yellow or brown as deeper enamel layers become involved, turning to moderate caries [3]. This stage may involve cavitation, where small pits or defects begin to appear on the enamel surface, indicating that remineralization is less likely to succeed without interventions such as micro-abrasion or restorations [2]. Without treatment, moderate carious lesions can progress deeper into the tooth structure. At this stage, known as advanced caries, the enamel may exhibit clear cavitation, resulting in the loss of part of the enamel structure. Once cavitation occurs, more invasive dental treatments, such as restorations, may be necessary [5].

Early detection and accurate diagnosis of these initial enamel lesions are critical for halting their progression and facilitating non-invasive remineralization strategies. Early intervention is crucial for effectively managing initial enamel lesions [2]. However, diagnosing these lesions at the initial stages poses considerable challenges, particularly in determining their depth [6]. The depth of the lesion plays a crucial role in guiding treatment decisions and predicting outcomes, as superficial lesions may respond to remineralization therapies, while more advanced lesions could necessitate additional interventions [7]. Among the diagnostic technologies, DIAGNOdent, a laser fluorescence device, has shown promising results in detecting moderate and advanced enamel lesions with cavitation [8]. By measuring fluorescence within the enamel structure, DIAGNOdent offers a sensitive and reliable method for identifying and quantifying early demineralization, even when clinical signs are minimal [9].

As previously mentioned, enamel lesions exhibit noticeable color changes. SpectroShade Micro is a digital color analysis device that integrates a digital camera and an LED spectrophotometer with an internal computer and proprietary analytical software [10]. This method outperforms traditional human evaluation in terms of accuracy and reproducibility [10]. SpectroShade and DIAGNOdent are practical for clinical applications, providing numerical data that is easy to analyze.

The aim of present study was to evaluate the relationship between the depth of initial enamel lesions, as measured by DIAGNOdent, and the reference standard provided by Micro-CT imaging. Additionally, the relationship between values measured by DIAGNOdent and lesion depth and between color differences (ΔE) and lesion depth was evaluated to enhance understanding of diagnostic approaches for initial enamel demineralization.

Materials and Methods

This study was approved by the Ethics Committee in Human Research (Faculty of Dentistry/Faculty of Pharmacy, Mahidol University Institutional Review Board; MU-DT/PY-IRB 2025/007.1002

Tooth Sample Collection and Preparation

Thirteen human premolars, caries & crack-free, extracted for orthodontic reasons and stored in a 0.1% thymol solution at pH 7.0, were used in this study. The teeth were sectioned into slabs parallel to the long axis of the tooth, buccolingually at the mesial and distal sides, using a low-speed diamond saw (Isomet™; Buehler, Evanston, IL, USA), achieving two specimens of slabs with a thickness of 3–4 mm per tooth. The enamel surfaces of the specimens underwent polishing protocols using wet silicon carbide abrasive paper

(600, 800, 1000, 1200, 2000, 4000, and 5000 grit: Buehler, Buehler Ltd, Lake Bluff, IL, USA), with 50 strokes each, applying finger pressure to create flat enamel surfaces parallel to the cutting surface. The specimens were then polished with diamond paste ranging from 6 to 0.25 microns (DP-Paste, Struers A/S, Copenhagen, Denmark). The specimens were cleaned for 1 minute in an ultrasonic distilled water bath between each polishing step and for 10 minutes after the final polishing step. The final polished enamel surfaces of the specimens remained within the outer half of the enamel. Specimens will be excluded if exposed dentin was found or the thickness of the specimen was less than 3 mm. Highly polished surfaces obtained after polishing were preferred for subsequent analyses.

The polished specimens were immersed in a remineralizing solution composed of 0.65 g/L KCl, 0.058 g/L $MgCl_2$, 0.165 g/L $CaCl_2$, 0.365 g/L KH_2PO_4, 0.804 g/L K_2HPO_4, 2 g/L sodium carboxymethylcellulose, and 2 ppm sodium fluoride for 1 day. Subsequently, specimens were rinsed with distilled water for 1 minute. All specimens were then coated with nail varnish (Revlon Nail Enamel, Revlon, NY, USA), leaving a 2 x 2 mm window at the polished surface. Finally, the specimens were randomly assigned to five groups based on the number of demineralization cycles, with five specimens in each group (N = 5).

Formation of Artificial Initial Enamel Lesions

Twenty-five specimens were separately immersed in demineralizing solutions in a 24-well plate. The demineralizing solution was prepared by mixing 50 mM acetic acid, 1.5 mM $CaCl_2$, and 0.9 mM KH_2PO_4, with the pH adjusted to 5.0 using 1 M KOH [11]. Each specimen was immersed in 600 µl of solution at 37°C. One cycle of immersion consisted of 3 days of demineralization, with daily renewal of the solution. After each cycle, 5 specimens were removed for analysis using micro-CT and DIAGNOdent at the end of each demineralization cycle. The specimens were categorized into five groups based on their respective cycle durations: Group 1 underwent a total duration of 3 days (equivalent to 1 cycle), Group 2 for 6 days (2 cycles), Group 3 for 9 days (3 cycles), Group 4 for 12 days (4 cycles), and Group 5 for 15 days (5 cycles). After each cycle, the specimens were cleaned with deionized water and dry-stored in a desiccator for 24 hours at 37°C

Specimen Analysis with Micro-CT

The specimens were then scanned using a high-energy micro-CT system (Skyscan 1173, Bruker, Kontich, Belgium) with a scanning layer resolution of 7 µm to ensure precise imaging of lesion characteristics. The reconstructed image sets were imported into 3D visualization software. A 3D image was reconstructed from 505 two-dimensional (2D) images in 16-bit TIFF format with a resolution of 2,240 x 2,240 pixels using Bruker's NRecon software (Bruker, Antwerp, Belgium). Data Viewer was used to define a volume of interest (VOI) centered on the carious lesion (approximately 2.5 mm x 2.5 mm x 2.5 mm) for focused examination. This VOI was subsequently transferred to ImageJ software for detailed analysis. The 2D images were specifically utilized to assess smooth surface lesions, enabling accurate measurement and characterization of lesion depth in microns (µm) and dentin mineral density (g/cm^3).

Color Difference (ΔE) Analysis

To evaluate the color difference (ΔE) between demineralized and sound enamel areas in a specimen, the Spectroshade Micro was employed 24 hours after the micro-CT scan. Initially, the device (Spectroshade Micro II, MHT, USA) was calibrated according to the

manufacturer's specifications to ensure the accuracy and reliability of measurements. The specimens were positioned in a light-controlled environment with a black background to minimize external variability. The color parameters of both the demineralized and sound enamel areas (L, a, b) were obtained. The ΔE value, representing the perceptible difference in color, was calculated using the formula $\Delta E = [(L_2-L_1)^2+(a_2-a_1)^2+(b_2-b_1)^2]^{1/2}$, where L, a, and b are the respective color dimensions.

Specimen Analysis with DIAGNOdent

A DIAGNOdent device (KaVo, Biberach, Germany) was employed immediately after color difference investigation to analyze whether the results from the device correlated with the depth determined through micro-CT investigation. The calibration process involved positioning the laser fluorescence probe on the calibration standard (C76) as a reference value. Standardized measurements were taken for each tooth by recording a sound spot on the tooth. The flat tip designated by the manufacturer for use on smooth surfaces was then utilized. The laser fluorescence probe was positioned perpendicularly to the surface of each white spot lesion (WSL). Multiple readings were taken across different points of the lesion to ensure consistency and accuracy. Values indicative of lesion depth were recorded for further analysis. To minimize variability, all measurements were conducted by the same operator.

Statistical analysis

Statistical analysis was performed using IBM ® SPSS ® Statistics 23(IBM SPSS Inc., Illinois, USA). A significance level of $\alpha = 0.05$ was applied for all statistical tests. The results from all investigations were calculated and summarized in mean and standard deviation. The normality of distribution was assessed using the Kolmogorov-Smirnov test, while the homogeneity of variance was evaluated through Levene's test. One-way ANOVA was then employed to evaluate the variations among the interventions. Subsequently, Scheffe's multiple comparison was further used for analysis of DIAGNOdent value, and Dunnett T3 multiple comparison were further used for analysis of color difference and depth of lesion.

Pearson's correlation analysis was conducted to assess the relationship between DIAGNOdent values and lesion depth, as well as color difference and lesion depth. The correlation was quantified using Pearson's correlation coefficient (r) and the coefficient of determination (r^2). A regression model and corresponding equation were subsequently developed to characterize the relationship between these parameters

Results

Means and standard deviations of DIAGNOdent value, Color difference (ΔE), mineral density ($g.cm^{-3}$) and depth of lesion (μm) are demonstrated in Table 1.

Data were analyzed for normality with Kolmogorov-Smirnov, revealing non-normal distribution for MD at 15 days and for lesion depth at 3 days, 9 days, and 12 days. The non-homogeneity of variance was found only for ΔE at p=0.01. One-way ANOVA of MD revealed no statistically significant differences among the groups (p=0.08).

One-way ANOVA of DIAGNOdent values revealed a statistically significant difference (p<0.01). Multiple comparisons showed that Groups 4 and 5 had significantly higher DIAGNOdent values (4.80 ± 0.98 and 5.30 ± 2.39, respectively) compared to Groups 1 and 2 (1.00 ± 1.17 and 1.70 ± 0.84, respectively).

Table 1 Means (standard deviations) of DIAGNOdent value (Dv), color difference (ΔE), mineral density (MD) and depth of lesion

	DIAGNOdent value	Color difference (ΔE)	Mineral density ($g.cm^{-3}$)	Depth of lesion (μm)
Group 1: 3 days	1.00 (1.17)[a]	6.34 (2.16)[a]	0.8418 (0.1945)[a]	15.40 (3.13)[a]
Group 2: 6 days	1.70 (0.84)[a]	9.08 (3.77)[a,b]	0.7904 (0.2086)[a]	28.00 (4.95)[b]
Group 3: 9 days	2.90 (1.25)[a,b]	14.72 (1.21)[b]	0.6411 (0.1194)[a]	33.00 (2.83)[b]
Group 4: 12 days	4.80 (0.98)[b]	14.68 (2.06)[b]	0.9058 (0.2742)[a]	44.80 (10.62)[b,c]
Group 5: 15 days	5.30 (2.39)[b]	17.90 (3.92)[b]	1.0076 (0.1382)[a]	63.00 (11.07)[c]

Means and standard deviations with same superscript indicate no statistically difference among columns

For ΔE values, one-way ANOVA showed a significant difference ($p<0.01$). Groups 3, 4, and 5 (14.72 ± 1.21, 14.68 ± 2.06, and 17.90 ± 3.92, respectively) exhibited significantly higher values than Group 1 (6.34 ± 2.16).

Regarding lesion depth, as assessed by micro-CT, one-way ANOVA showed a significant difference ($p<0.01$). Significant differences were observed among all groups, except between Groups 4 and 5 (44.80 ± 10.62 and 63.00 ± 11.07, respectively) and among Groups 2, 3, and 4 (28.00 ± 4.95, 33.00 ± 2.83, and 44.80 ± 10.62, respectively). For MD no significant differences between groups were found. Representative images for lesion depth and color difference are presented in Figure 1.

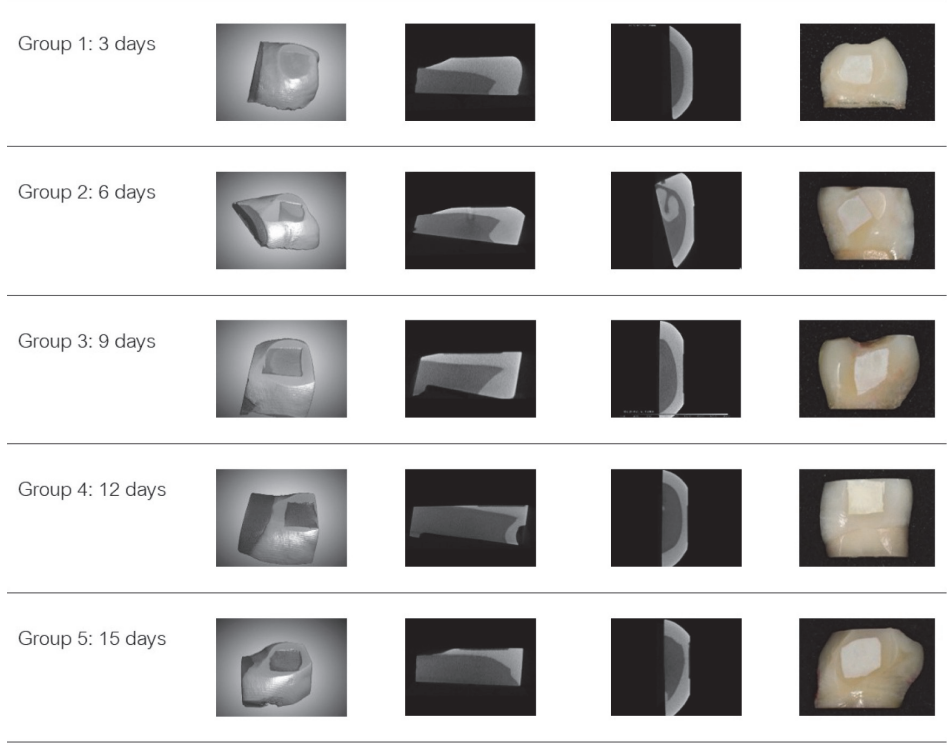

Figure 1 Representative 3D and 2D micro-CT images illustrate the lesion characteristics across different experimental groups.

Regarding the investigation under micro-CT as presented in Figure 1, lesion depth exhibits a progressive increase with repeated demineralization cycles, indicating a deepening trend. Lesion shade follows a similar pattern, with increasing opacity and a pronounced white appearance corresponding to greater lesion depth

The correlations between DIAGNOdent values and lesion depth, as well as ΔE and lesion depth, were analyzed. A significant positive correlation was identified for both pairs. DIAGNOdent values and lesion depth, and Pearson's correlation yielded $p<0.01$ and correlation coefficient = 0.812. For analysis of regression, it demonstrated $r^2 = 0.659$ with regressive linear model of Lesion depth = 6.698 (Dv) + 15.809. ΔE values and lesion depth, and Pearson's correlation yielded $p<0.01$ and correlation coefficient = 0.824. For analysis of regression, it demonstrated $r^2 = 0.678$ with regressive linear model of Lesion depth = 2.928 (ΔE) + 0.111. Linear correlation models are presented in Figure 2.

Discussion

The findings of this study provide significant insights into the relationship between initial enamel lesion characteristics and various diagnostic parameters such as color change and DIAGNOdent values, reinforcing the importance of accurate and non-invasive diagnostic tools in preventive and minimally invasive dentistry. The initial enamel lesion involves surface softening, which is undetectable by conventional methods but may be identified using technology-based techniques. The process begins in the most acid-soluble sites on the tooth surface, forming small defects that allow biofilm acids to penetrate and demineralize the subsurface structure. The first visible sign of dental caries appears as white spot lesions, detectable only after drying the enamel. The caries process involves the dissolution of minerals at the enamel surface, such as carbonate and magnesium, followed by calcium and phosphorus. These lesions are categorized into four zones based on porosity: the surface zone, the body of the lesion, the dark zone, and the translucent zone. At this stage, the demineralization has not yet reached the dentin [12].

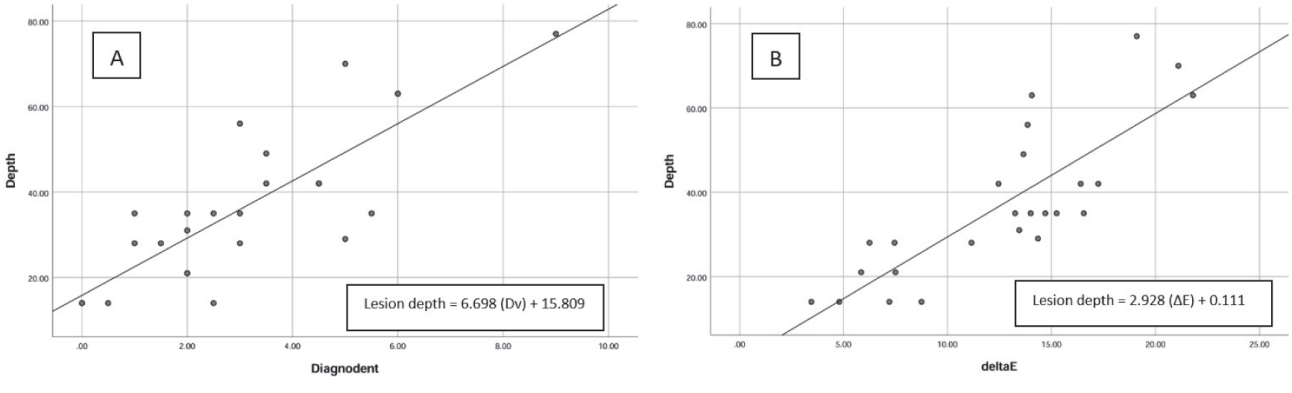

Figure 2 (A) Regression model of DIAGNOdent value (Dv) and Lesion depth in μm with regression equation indicated. (B) Regression model of Color difference (ΔE) and Lesion depth in μm with regression equation indicated.

The observed positive correlation between DIAGNOdent values and lesion depth, as well as ΔE values and lesion depth, shows the utility of both laser fluorescence and color difference analysis in assessing demineralization progression. Specifically, the significant Pearson's correlation coefficient for DIAGNOdent values = 0.812, $p<0.01$, $r^2 = 0.659$ and Pearson's correlation coefficient for ΔE values = 0.824, $p<0.01$, $r^2 = 0.678$ highlights the potential of DIAGNOdent values and ΔE as surrogate measures for lesion depth in initial enamel lesions. However, no significant differences in mineral density (MD) were observed among the experimental groups. This outcome may be attributed to the relatively short duration of demineralization, as the minimal lesion depth over the 3–15 days period may not have been sufficient to reveal measurable changes in MD (13).

The progressive increase in DIAGNOdent value and ΔE values with the duration of demineralization cycles aligns with the expectation that enamel demineralization alters the enamel porosity and optical properties of the enamel surface. This is consistent with a previous study suggesting that the increased porosity and structural changes in demineralized enamel lead to greater light scattering and, consequently, perceptible color differences [14].

The results also confirm the capability of DIAGNOdent as a reliable tool for quantifying the extent of enamel demineralization. The significant increase in DIAGNOdent values with longer demineralization cycles and the regression equation (Lesion depth = 6.698 (Dv) + 15.809) allows for the estimation of lesion depth based on fluorescence readings. However, the variability in DIAGNOdent readings, particularly in later groups, suggests that while the tool is highly sensitive, it may be influenced by factors such as lesion morphology or probe positioning, warranting standardization in clinical applications [8, 15].

The depth measurements obtained via micro-CT serve as the gold standard for lesion characterization in this study [16]. The significant differences in lesion depth across groups, except for between Groups 1 and 2, Groups 3 and 4, and Groups 4 and 5, affirm the precision and resolution of micro-CT in detecting early enamel changes. Additionally, the regression model for ΔE values (Lesion depth = 2.928 (ΔE) + 0.111) provides an alternative quantitative approach for estimating lesion depth based on colorimetric parameters.

This study's integrative approach, employing micro-CT, DIAGNOdent, and ΔE analysis, offers a comprehensive evaluation of initial enamel lesions, even though there is no significant change in mineral density of the enamel. Notably, the values of DIAGNOdent in this study are all lower than the diagnosing threshold from the manufacturer (the manufacturer's diagnosing threshold starting at 14). Findings demonstrate that combining advanced diagnostic modalities can enhance the understanding of lesion progression and guide tailored preventive or therapeutic interventions. Clinically, this reinforces the utility of DIAGNOdent as a diagnostic adjunct, enabling early detection of initial enamel lesions before irreversible damage occurs. The ability to estimate lesion depth using fluorescence readings or colorimetric parameters allows clinicians to tailor their preventive and restorative strategies, reducing the need for aggressive intervention. However, certain limitations warrant consideration. For example, the in vitro nature of the study may not fully replicate clinical conditions, and the polished enamel surfaces, although necessary for standardized analysis, may influence the natural behavior of lesion formation and detection. Also, with the statistical limitation of the study, the power of the test for DIAGNOdent, color difference, mineral density, and lesion depth at n = 5 was determined to be 70%, 98%, 32%, and 99%, respectively. These findings indicate that careful interpretation is required, particularly for DIAGNOdent values and mineral density. To enhance the reliability of the results, further investigations should be conducted with a larger sample size.

Future research should address these limitations by validating the findings in vivo and exploring the long-term efficacy of diagnostic and remineralization strategies informed by such methodologies.

Conclusion

In conclusion, the strong correlations observed between lesion depth, DIAGNOdent values, and ΔE highlight the complementary roles of laser fluorescence and colorimetric analysis in diagnosing initial enamel lesions. The findings lead to new and practical diagnostic methods that use optical and fluorescence measurements, which could help with early detection and management of initial enamel lesions.

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