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Artificial intelligence versus surface microhardness testing in evaluating fluoride remineralization: An *in vitro* study

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Abstract:

Surface microhardness testing is widely used to quantify enamel remineralization, but it is destructive and cannot easily provide rapid image-based prediction of fluoride response. Therefore, it is of interest to compare the artificial intelligence-based image analysis with Vickers surface microhardness testing for evaluating fluoride-mediated remineralization of artificial enamel lesions. One hundred and twenty enamel blocks were demineralized and allocated to five groups (n=24 each): fluoride varnish, fluoride dentifrice slurry, nano-hydroxyapatite, CPP-ACP and control, followed by pH cycling for 14 days. Fluoride varnish produced the highest microhardness recovery ($72.8 \pm 8.9\%$), followed by fluoride dentifrice slurry ($61.4 \pm 7.6\%$) and the artificial intelligence model predicted microhardness recovery with $R^2=0.89$, MAE=6.3 VHN and classification accuracy of 88.6%. Thus, artificial intelligence-based image analysis showed strong agreement with surface microhardness testing and may provide a rapid non-destructive adjunct for evaluating enamel remineralization.

Keywords: Artificial intelligence (AI), surface microhardness, fluoride, remineralization, enamel, vickers hardness, dental caries

Background:

Early enamel demineralization is a reversible stage of dental caries and accurate assessment of remineralization is important for evaluating fluoride-based preventive strategies [1]. Fluoride promotes mineral gain by enhancing remineralization and reducing demineralization, but laboratory evidence depends heavily on the method used to measure surface recovery [2, 3]. Surface microhardness testing is widely accepted for quantifying mineral changes in enamel blocks, yet it is partly destructive, technique-sensitive and limited to selected indentation points [4]. Optical and fluorescence-based methods can monitor changes without repeated indentation, but image interpretation may be influenced by lighting, surface hydration, lesion depth and operator selection of the region of interest [5, 6]. Artificial intelligence may be able to interpret standardized enamel images and estimate mineral recovery by learning texture, brightness and surface-change patterns associated with fluoride exposure [7]. Such models could complement conventional hardness testing. Deep-learning systems have been explored for caries detection and dental image interpretation, but fewer studies have validated image-based artificial intelligence directly against surface microhardness during remineralization experiments [8]. Therefore, it is of interest to compare the artificial intelligence-based image analysis with surface

microhardness testing for evaluating fluoride-mediated enamel remineralization.

Materials and Methods:**Study design and setting:**

This *in vitro* experimental study used extracted sound human premolars collected after orthodontic extraction. Enamel slabs were prepared, polished and embedded in acrylic resin with exposed buccal enamel windows.

Sample size and grouping:

One hundred twenty enamel specimens were randomly allocated to five groups of 24 each: artificial saliva control, fluoride dentifrice slurry, fluoride varnish, nano-hydroxyapatite and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP).

Demineralization and remineralization protocol:

Baseline surface microhardness was recorded before lesion creation. Specimens were immersed in a demineralizing solution for 96 hours, followed by a 14-day pH cycling regimen with assigned remineralizing agents. Solutions were refreshed daily.

Surface microhardness testing:

Vickers microhardness readings were obtained at baseline, after demineralization and after the 14-day remineralization period using a standardized load and dwell time. Three indentations per specimen were averaged to reduce local variation.

Image acquisition and artificial intelligence analysis:

Standardized polarized-light and white-light enamel images were captured using fixed magnification, distance and illumination. Image patches were used to train a convolutional neural network (CNN) to classify remineralization status and estimate microhardness recovery. In parallel, engineered image features describing opacity, fluorescence, texture and intensity were used to train a random forest regressor. Predictions from the CNN and random forest models were then combined in an ensemble model and a fluorescence-only model was retained as an additional comparator.

Outcome variables:

The primary outcome was percentage surface microhardness recovery. Secondary outcomes were artificial intelligence-predicted recovery, classification accuracy for high versus low remineralization, sensitivity, specificity and correlation between predicted and measured values.

Statistical analysis:

One-way ANOVA and Tukey tests compared remineralization among groups. Pearson correlation, intraclass correlation coefficient, mean absolute error and AUC were used to assess artificial intelligence performance. A p value <0.05 was considered statistically significant. Tooth specimens were sectioned from the middle third of the buccal surface to reduce anatomical variation. The exposed window was standardized with acid-resistant nail varnish so that demineralization and remineralization occurred only within the testing area. For image model training, each enamel window was divided into non-overlapping patches. Patches with glare, incomplete focus, or edge artifact were excluded before training. The test set contained images from specimens not used during model development. The microhardness indenter was calibrated with a reference block before every testing session. Indentations were placed at least 100 micro-meters apart and away from the window margin to avoid overlap and edge effects. All images were captured after blot drying for a fixed duration. Specimens

were not desiccated because excessive drying can exaggerate opacity and alter optical appearance. The same exposure settings were locked for all images within a testing session. Specimens were coded so that the examiner performing microhardness testing was blinded to the treatment group. Image files were renamed before model training so that group labels were not visible during pre-processing. The artificial intelligence dataset was augmented by controlled rotation and brightness adjustment within a narrow range. Augmentation was used only on the training set and not on the final test images.

Results:

All treatment groups showed higher remineralization than the control group. Fluoride varnish produced the greatest surface microhardness recovery, followed by fluoride dentifrice slurry, nano-hydroxyapatite and CPP-ACP. AI-derived opacity and fluorescence features correlated strongly with microhardness recovery. The model performed best for distinguishing high-response specimens from low-response specimens. The random forest regressor produced slightly lower error than the convolutional neural network, while the combined ensemble produced the best overall performance. After demineralization, all groups showed comparable reductions in Vickers hardness, confirming baseline lesion standardization. After pH cycling, fluoride varnish produced the highest recovery, followed by fluoride dentifrice slurry, nano-hydroxyapatite, CPP-ACP and control. The image-derived lesion opacity score decreased as microhardness recovery increased. The ensemble model showed the best balance between low prediction error and high classification accuracy for identifying specimens with strong remineralization response. Specimens with the highest microhardness recovery also showed smoother surface texture and lower opacity scores on image analysis. Control specimens retained irregular opacity and low hardness values after pH cycling, indicating limited spontaneous mineral recovery. The model was most accurate in distinguishing fluoride varnish from control specimens, while more overlap was observed between fluoride dentifrice, nano-hydroxyapatite and CPP-ACP groups. Baseline and post-demineralization microhardness values are summarized in **Table 1**; 14-day remineralization outcomes are presented in **Table 2**; and AI model agreement metrics are shown in **Table 3**.

Table 1: Baseline and post-demineralization microhardness values

Group	n	Baseline VHN	Post-demineralization VHN	p value
Fluoride varnish	24	328.5 ± 21.8	142.4 ± 18.7	0.791
Fluoride dentifrice	24	331.2 ± 20.6	144.8 ± 17.9	
Nano-hydroxyapatite	24	329.8 ± 22.1	141.6 ± 18.4	
CPP-ACP	24	326.9 ± 21.5	143.1 ± 19.2	
Control	24	330.4 ± 20.9	142.9 ± 18.6	

Table 2: Remineralization outcomes after 14-day pH cycling

Group	Final VHN	Microhardness recovery (%)	AI lesion-opacity score	p value
Fluoride varnish	278.1 ± 26.4	72.8 ± 8.9	0.82 ± 0.07	<0.001
Fluoride dentifrice	259.2 ± 24.7	61.4 ± 7.6	0.75 ± 0.08	
Nano-hydroxyapatite	239.6 ± 23.1	51.8 ± 7.4	0.66 ± 0.10	
CPP-ACP	228.5 ± 22.6	46.2 ± 6.9	0.61 ± 0.09	
Control	164.7 ± 20.3	11.7 ± 4.8	0.32 ± 0.11	

Table 3: AI model agreement with surface microhardness testing

Model	R ²	MAE (VHN)	ICC	Accuracy for high response (%)
CNN image model	0.85	7.8	0.86	85.2
Random forest image features	0.87	6.9	0.88	86.9
Ensemble model	0.89	6.3	0.91	88.6
Fluorescence-only model	0.79	9.4	0.80	80.7

Discussion:

This study found that fluoride-treated specimens showed significantly higher microhardness recovery than artificial saliva controls and artificial intelligence image analysis showed strong agreement with surface microhardness measurements [9]. The superior recovery observed with high-fluoride varnish is expected because prolonged fluoride availability supports formation of calcium fluoride-like reservoirs and enhances mineral deposition during pH cycling [10]. The image model performed well because remineralized enamel develops visual changes in surface texture, opacity and light scattering. These patterns may be subtle to human observers but detectable when thousands of standardized image features are evaluated together [11]. Surface microhardness remains a valuable reference method because it directly measures resistance to indentation, which relates to mineral content and enamel surface integrity. Artificial intelligence should therefore be viewed as an adjunctive screening tool rather than an independent replacement [12]. The findings have practical value for laboratory cariology. Non-destructive image-based prediction could reduce the number of specimens required for repeated testing and allow longitudinal monitoring of the same enamel surface without additional indentation damage [13]. The study was limited by artificial lesion creation, controlled imaging and short remineralization duration. Natural lesions vary in porosity, depth, organic content and plaque exposure, which may affect image-based prediction. Future research should include transverse microradiography, micro-CT mineral density, longer pH cycling and external image datasets. Clinical translation would require careful standardization of moisture control, illumination and tooth-surface orientation. The baseline similarity after lesion formation strengthens interpretation because treatment differences were unlikely to be caused by uneven initial demineralization. This is important in enamel studies, where natural structural variation can otherwise obscure treatment effects. The non-destructive nature of image-based analysis is the main potential advantage. A specimen can be followed across multiple time points without creating additional indentation marks that may alter surface behavior or interfere with later optical evaluation. Before clinical use, the model must be tested on natural white-spot lesions where plaque history, enamel maturation, surface staining and lesion depth vary. Laboratory accuracy is a necessary first step, but clinical images will require more robust preprocessing and calibration. The image model may be useful for ranking remineralization treatments, particularly in early-phase product evaluation. It can identify promising groups that deserve confirmatory testing with more detailed mineral-density methods. The approach also reduces specimen damage. Traditional microhardness testing leaves permanent marks, whereas repeated imaging can follow

the same lesion over time and allow a more complete time-course assessment of remineralization. Clinical application would require strict control of plaque, stains, saliva and illumination. In the mouth, enamel surfaces are curved and wet, so future algorithms must handle reflections and non-uniform surfaces before they can support chairside remineralization monitoring. The overlap between active treatment groups reflects the biological complexity of remineralization. Different agents may improve hardness through related mineral pathways, making exact group discrimination harder than separating active treatment from no treatment. The strongest use of the model may therefore be quantifying response rather than naming the product used. A clinical tool would need to estimate whether a lesion is improving, stable, or worsening over time. The present approach also encourages standardized photography in preventive dentistry research. If image acquisition varies widely, algorithmic interpretation becomes unreliable regardless of model architecture. The artificial intelligence model was not trained to replace the microhardness machine but to estimate its outcome from standardized surface images. This distinction is important because algorithmic results remain meaningful only when anchored to a validated physical reference method. The study supports the idea that remineralization assessment should be multimodal. Surface microhardness, optical imaging and possibly mineral-density imaging each describe a different part of the lesion-repair process. The fluoride varnish group showed the strongest improvement, but the model was also able to identify partial recovery in other active groups. This is important because preventive agents may not all act with the same speed or depth of mineral deposition. Image analysis may be especially useful in longitudinal experiments where the same enamel specimen is evaluated repeatedly. Tracking a lesion through demineralization and recovery may provide more information than comparing separate specimens at only one endpoint. The method could also reduce examiner variability. Manual selection of a region of interest can influence results, while a trained algorithm can apply the same segmentation rules consistently across large image sets. The present model remains dependent on controlled laboratory photography. Before chairside translation, it should be tested under different cameras, light sources, tooth curvatures and moisture levels to confirm robustness. For preventive dentistry research, the approach may accelerate early screening. Products showing weak image-based recovery can be deprioritized, while promising agents can proceed to more detailed mineral analysis and clinical trials. Mechanistically, the fluoride response observed here is consistent with established evidence describing fluoride-enhanced remineralization and calcium-fluoride-like mineral reservoirs on enamel surfaces [14]. A 2024 *in vitro* study by

Rahman and Diab similarly used Vickers microhardness to show recovery of artificial white-spot lesions after remineralizing interventions, supporting microhardness as a suitable reference outcome for comparative enamel studies [15]. Recent evidence also supports the potential of AI-assisted caries image analysis while emphasizing the need for careful validation: a 2024 umbrella review reported promising performance across imaging modalities [16] and a 2025 study showed high diagnostic performance of deep-learning models using intraoral photographs, including early white-spot lesions [17]. A 2026 umbrella review further concluded that AI should presently function as decision support rather than as a stand-alone diagnostic method until stronger prospective and external validation is available [18].

Conclusion:

Fluoride varnish produced the greatest enamel microhardness recovery in this *in vitro* pH-cycling model. Artificial intelligence-based image analysis showed strong correlation and agreement with Vickers surface microhardness testing and accurately classified high remineralization responses. Thus, this approach is a rapid, non-destructive adjunct for laboratory screening of remineralizing agents.

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