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Understanding the spatial and topographic characteristics of enamel white spot lesions for targeted remineralization

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A R T I C L E I N F O

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A B S T R A C T

Introduction: White spot lesions are opaque, chalky white or yellowish discolorations on the surface of teeth that result from the demineralization of the tooth structure. Many methods, including SEM, XRD, and FTIR spectroscopy, are crucial for identifying and evaluating enamel white spot lesions. It is imperative to have a thorough grasp of the morphology, crystallographic structure, mineral composition, and chemical changes associated with enamel white spot lesions.

Method: In vitro lesions were meticulously obtained by immersing extracted teeth in hydrochloric acid for a week and drying them using artificial caries. Characterization investigations were conducted with utmost precision and thoroughness using FTIR, XRD, and SEM, ensuring the reliability and validity of the results.

Results: FTIR analysis revealed the existence of calcium oxide and hydroxyapatite, and SEM examination assisted in identifying differences in surface shape. The enamel's crystalline nature was revealed via XRD investigation.

Conclusion: White spot lesions are associated with the development of deep caries. Advanced imaging strategies are needed for additional validation.

1. Introduction

Dental caries is one of the most prevalent kinds of dental lesions. A chalky, opaque white or yellowish discoloration on the tooth's surface, typically found around the gum line or the tooth's biting edge, is called a "white spot lesion (WSL)." It denotes a noticeable shift in the tooth enamel's color or texture brought on by the demineralization of the tooth structure. This lesion can be caused by poor dental hygiene, overeating sugar, or snacking frequently, but it is most commonly observed in patients receiving orthodontic treatment.[1]

WSL typically develops near the brackets in orthodontic patients because food tends to get stuck there. Microbial colonization and the patient's behaviors may contribute to the development of these lesions if they practice poor oral hygiene. They are usually only apparent after treatment.[2] WSL can be classified as either carious or non-carious. Carious WSL manifests as a non-cavitated carious lesion on the tooth's buccal surface, encircling the bracket. Non-carious lesions are smooth and shiny, while they appear rough and opaque.[3]

WSL can develop in two stages: subsurface lesions brought on by mineral loss and enamel surface softening due to mineral loss.[4] If treatment is not received, these lesions may turn into cavities, which may cause discomfort, infection, and even tooth loss. Compared to adults, adolescents are more susceptible to developing WSL.[5] The length of orthodontic therapy is a significant factor in developing WSL. Gorelick L established four categories to describe WSL: 1) no WSL, 2) mild WSL, 3) severe WSL, and 4) cavitation combined with WSL.[6] It was discovered that the canines (48.1 %) had the highest frequency of WSL in the human dentition, followed by the central incisors (32.3 %), lateral central incisors, and first premolars (31.6 percent). WSL was less prevalent in molars (27.2 %) and second premolars (8.9 percent).[7]

WSL has been studied using various methods, including confocal laser scanning microscopy, microradiography, indentation backscattered SEM, and scanning electron microscopy (SEM). Nevertheless, these techniques only offered a momentary view of the lesion

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progression at that specific moment and were detrimental to the neighboring tooth structures.[8] Non-destructive techniques have been studied and found to be more sensitive than conventional techniques, but they also have a higher false-positive diagnosis rate. These techniques include quantitative light-induced fluorescence, DIAGNOdent, optical coherence tomography, and X-ray Micro-Tomography.[9,10] Enamel crystal breakdown and subsurface demineralization holes occur between the enamel rods during the creation of WSL. Therefore, Fourier transform infrared (FTIR), energy dispersive X-ray, and SEM are utilized to analyze the stages of WSL formation to better understand the microscopic features of what happens during WSL creation and devise appropriate treatments.

The chemical composition, mineral content, carbonate substitutions, and protein modifications are all detected by FTIR spectroscopy. This method advances our knowledge of the mechanisms underlying enamel demineralization and aids in describing the lesions and analyzing remineralization therapy. The appearance, structure, and composition of these lesions are all revealed by SEM examination. It facilitates the observation of changes in the surface texture of the enamel, such as surface imperfections, porosities, and roughness, and it assists with high-resolution surface morphology studies. SEM helps track the progression of lesions known as enamel white spots over time and evaluate the effectiveness of dental restorations or remineralization procedures. The demineralization-induced alterations in the enamel structure are examined using X-ray diffraction (XRD). This method offers information on the crystallographic structure, mineral phases, degree of mineralization, and response to therapy, which significantly aids in assessing enamel white spot lesions. XRD aids in defining lesions, understanding their development, and evaluating the efficacy of enamel remineralization therapies. By integrating SEM, XRD, and FTIR spectroscopy, this research offers a holistic approach to WSL evaluation, enabling clinicians to make informed decisions regarding diagnosis and treatment planning. The novelty of this study lies in its innovative methodology, which leverages advanced analytical techniques to deepen our understanding of enamel white spot lesions and improve clinical management strategies.

Integrating these advanced diagnostic and analytical techniques into clinical practice has the potential to revolutionize the early detection and management of WSL, particularly in orthodontic patients undergoing prolonged treatment. By accurately assessing lesion severity and monitoring progression, clinicians can implement targeted interventions, such as remineralization therapy or dental restorations, to prevent further enamel deterioration and maintain oral health. This research offers hope for improved patient outcomes and enhanced clinical management strategies in the long term.

Our research uses various methods, such as SEM, XRD, and FTIR spectroscopy, to detect and evaluate enamel WSL.

2. Materials and methods

2.1. Tooth sample selection

Ten tooth samples were meticulously selected from the Saveetha Dental College tooth repository for this investigation. The sample size of ten teeth was chosen based on prior power calculations derived from the previous article[9] to ensure adequate statistical power and reliability of the results. Both premolars and molars were included in the selection to ensure a comprehensive representation of different tooth types within the oral cavity. Premolars and molars were specifically chosen due to their crucial roles in mastication and their susceptibility to periodontal diseases and dental caries, making them ideal candidates for studying dental health and disease dynamics. The selection criteria ensured that

Fig. 1. Methodology A) Selection of teeth; B) Immersion in 10 % HCl; C) Sectioning of test teeth; D) Sectioned tooth; E) FTIR machine; F) SEM machine; G) XRD machine.

the teeth exhibited no cavities, anomalies, or other dental issues, as depicted in Fig. 1. This careful selection enhances the validity and accuracy of the study's findings by ensuring that the samples were in optimal condition.

A prior power analysis was conducted to determine the required sample size using the following parameters: one-tailed *t*-test, an effect size (dz) of 20, an alpha error probability (α) of 0.05, and a desired power (1-β) of 0.8. The output from this calculation indicated a non-centrality parameter (δ) of 28.284271, a critical t-value of 6.313752, degrees of freedom (df) of 1, a total sample size of 2, and an actual power of 0.999990. Although these calculations suggested that a sample size of 2 would achieve extremely high power, a larger sample size of ten teeth was chosen to ensure a robust analysis and account for potential variability. This decision was informed by previous reference articles recommending a more substantial sample size for greater reliability and validity of results.

2.2. Creation of artificial white spot lesions

To induce artificial white spot lesions, the artificial caries approach was employed:

Submersion in Hydrochloric Acid: The selected teeth were immersed in a diluted solution of hydrochloric acid (concentration between 0.1 % and 1 %) for one week.

Daily Monitoring and Solution Replacement: The hydrochloric acid solution was replaced daily, and changes in the teeth were observed throughout the week.

2.3. Preparation of tooth samples

Following the creation of artificial white spot lesions:

Drying: The teeth were dried for 24 h in a controlled environment to ensure complete dryness.

Sectioning: The dried teeth were sectioned to provide a smooth surface for subsequent analysis.

2.4. Control group

A second set of tooth samples, which were not submerged in hydrochloric acid, was prepared:

Selection and Sectioning: Similar to the first set, these teeth were sectioned to create a smooth surface.

2.5. Characterization studies

Both sets of teeth (acid-treated and control) were subjected to various characterization studies, including XRD, FTIR, and SEM examinations.

2.5.1. Fourier-transform infrared spectroscopy (FTIR)

- Equipment: Alpha II Bruker type spectrometer.
- Wave Number Range: 4000 to 500 cm^{-1}.
- Purpose: To identify the functional groups present in the enamel.

2.5.2. Scanning electron microscopy (SEM)

- Equipment: JSM-IT800 NANO SEM, a Field Emission Scanning Electron Microscope.
- Additional Feature: Equipped with a JEOL Energy Dispersive X-ray Spectrometer for elemental analysis.
- Purpose: To examine the surface morphology of the enamel.

2.5.3. X-ray diffraction (XRD)

- Equipment: D8 diffractometer family platform.
- Radiation Source: Copper (Cu) with a wavelength of 1.5406 Å.
- Purpose: To analyze the alterations in the enamel's crystalline structure due to the formation of white spot lesions. By employing these methodologies, the aim was to comprehensively characterize the changes induced in the enamel by artificial white spot lesions, providing a detailed understanding of the effects on its structural and compositional properties**.**

3. Result

The result is shown in Figs. 2 and 3.

3.1. FTIR analysis

At 1800 cm^{-1} wavelength, CO_3^{2-} was found to have an absorption peak. The bonding of OH^- occurs at wavelength 1700 cm^{-1}, PO_4^{3-} at wavelength 1500 cm^{-1}, CaO at wavelength 1000 cm^{-1}, and HPO_4^{2-} at wavelength 470 cm^{-1}. Consequently, calcium oxide and hydroxyapatite are absorbed in the 2500 to 500 cm-1 range. In contrast to the usual 2400 cm^{-1}, FTIR shows a shift in CO^2 absorbance at 1800 cm^{-1}. This change in absorption may result from a demineralization-related modification in the bonding connection.

3.2. XRD analysis

Fig. 2C's XRD examination shows a decrease in the primary 211 crystal plane's intensity (JCPDS No. 09–432), which may indicate modifications to the crystallization pattern during demineralization. This decrease in intensity means that the material's crystal structures' orientation and arrangement have changed due to demineralization. Such alterations in the crystalline structure may be ascribed to the elimination of mineral constituents, which could lead to a less ordered or disorganized crystal lattice. These results highlight how demineralization significantly affects the material's structural characteristics and provide insight into the complex relationship between crystalline architecture and mineral content.

3.3. SEM analysis

The mineralized layer showed a notable loss at 1 μm. Surface variations were observed at 5 μm. The worn-off surface and the mineralized surface could be distinguished at 10 μm. Surface alterations, however, were invisible to the unaided eye at 50 μm. Consequently, it becomes necessary to perceive the surface at greater magnification and comprehend the precise surface manifestations arising from different etiological causes of demineralization.

4. Discussion

Dental aesthetics is essential for both visual appeal and self-confidence. However, specific individuals may require functional orthodontic treatment to enhance their look to correct malocclusion, malalignment, or supernumerary diseases. Unfortunately, side effects of orthodontic therapy that can sometimes happen are decalcification and hypo mineralization. As a result, several strategies for handling these risks are being developed.

Our goal is to understand the deeper aspects of white spot lesions so that we can create individualized treatment regimens via remineralization. Plaque accumulation causes opaque patches of decalcified enamel tissue to form, known as white spot lesions. Individuals receiving orthodontic treatment have a higher risk of developing white spot lesions than those who do not because the fixed appliance creates a stagnant space where plaque can build up and cause localized demineralization of the enamel.[11]

Fixed orthodontic appliances raise caries risk by raising dental plaque and lowering its pH. Additionally, they alter the local bacterial flora

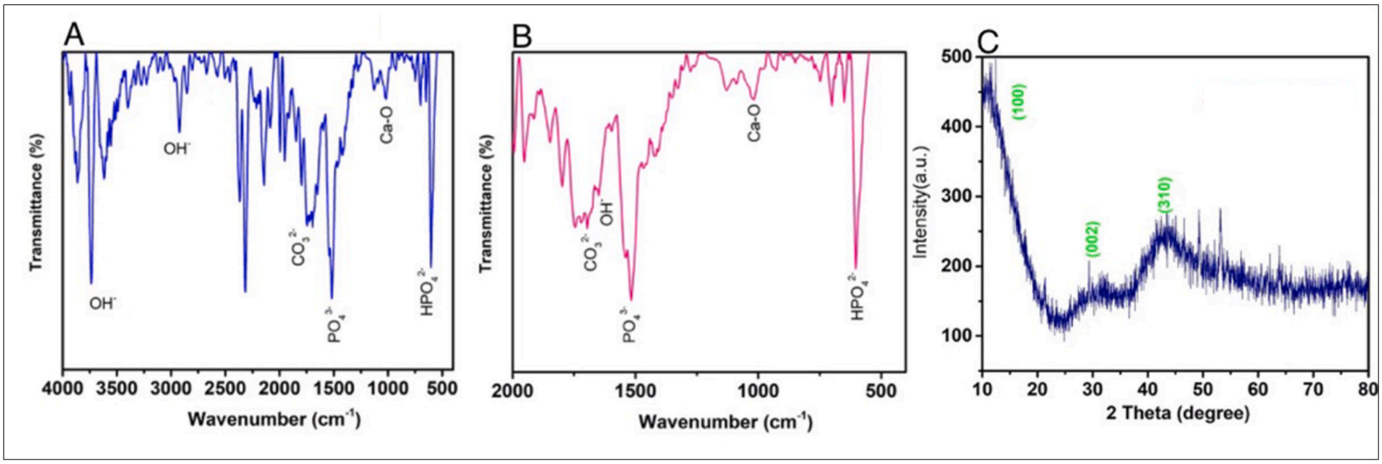

Fig. 2. A) FTIR for hydroxyapatite and Calcium Oxide in the range of 2500-500/cm range B) FTIR shows the absorbance of CO_2 at 500–2000 range C) XRD.

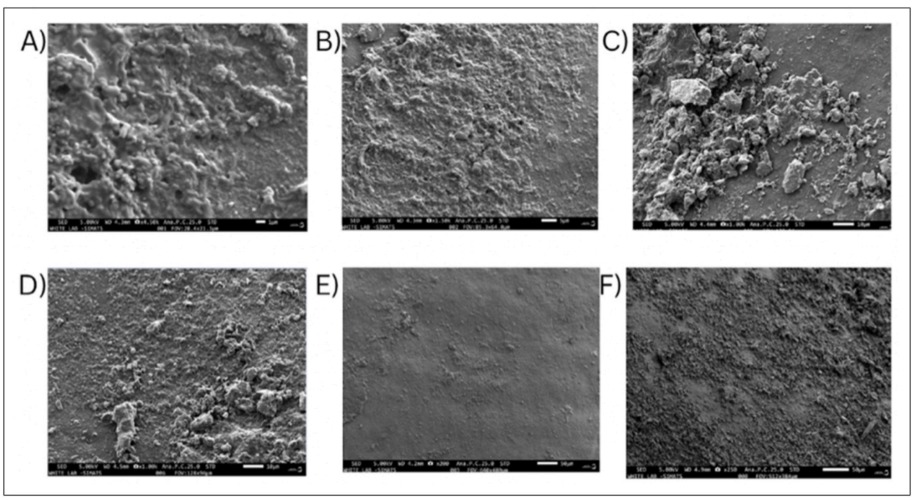

Fig. 3. SEM ANALYSIS- The surface morphology of the enamel of treated teeth at various intensities was obtained. As -A)1 µm, B)5 µm, C) & D)10 µm, E) & F) 50 µm.

and increase the concentrations of acidogenic bacteria such as S. mutans. The first indications of demineralization are white spot lesions, which are brought on by pH shifts caused by these bacteria.[12,13] A study by Eser Tufekci et al. found that after six months, 38 % of patients receiving orthodontic treatment had apparent white spot lesions, and 46 % of the individuals at twelve months.

Numerous articles have explained the development of WSL.[14–16] The prevalent belief is that the primary factors contributing to demineralization include inadequate dental hygiene, plaque accumulation, acidogenic bacteria, and fixed appliances that create plaque retention sites.[14] Research has demonstrated that WSLs frequently impact particular teeth, including the maxillary lateral incisor,[15] maxillary and mandibular first molars,[16,17] canines, and maxillary lateral incisors.[18] WSLs can appear in individuals with poor dental hygiene as soon as one month after bonding; thus, now is the best time to address the WSLs proactively.[19]

Remineralization was attempted using low supersaturation calcium phosphate solutions. Still, it was found that the rate of remineralization was slow and less effective on the acid-etched enamel surface of humans. Artificial WSL was prepared on bovine enamel surfaces using lactate buffer containing methyl hydroxy diphosphonate ions.[20] Over the years, researchers have examined a variety of therapy approaches. Using 10 % carbamide peroxide and CPP-ACP paste on the enamel is one of these methods; these methods, in turn, decreased color variations, preserved the microhardness of the enamel, and demonstrated mineral gain, respectively.[21] According to ICDAS, there were differences in the degree of mineralization between mild and severe WSLs. Fluoride toothpaste was an explored treatment, but it didn't work effectively. On the other hand, remineralization was aided by the combination of fluoride varnish and F toothpaste, as well as CPP-ACP and F toothpaste.[22] Throughout 5–12 years, the WSLs progressively disappear, and saliva is ineffective as a remineralizing agent to restore deep lesions' structural and aesthetic integrity. As a result, additional research must be done, and the deeper sections of the WSLs must be restored more quickly to improve structural and aesthetic strengthening.[23]

Patients who exhibit traumatic hypo mineralization sometimes react favorably to icon resin infiltration. The resin infiltration approach, which tries to prevent the formation of recently established carious lesions, is the most effective method.[24] Since the size of the intervals between enamel crystals causes yellowing, resin with an enamel-like refractive index was created, and the study's 12-month results showed that it was effective.[25]

Our research has uncovered significant structural changes in enamel resulting from the demineralization process associated with S. mutans bacteria. Through applying FTIR, XRD, and Nano SEM analyses, we have acquired valuable insights into the composition and morphology of the affected tooth sections. The FTIR analysis has revealed alterations in absorption patterns, indicating modifications in bonding connections due to demineralization. While Jing Zhang et al.[26] utilized FTIR to investigate the remineralization effect in white spot lesions (WSL), our study focused on comprehending the demineralization effects, which could lay the groundwork for improving remineralization processes.

XRD intensity decreased, indicating that the material's crystal structures' orientation and arrangement had changed due to demineralization. Ren J et al. used XRD to investigate remineralization.[27] Our study is the first to test demineralization. Nano SEM showed alteration in crystal structure up to 50 nm depth. However, further investigation using nano-CT and AFM can offer a more detailed understanding of the molecular-level alterations.

The implications of our findings extend beyond the immediate results. The comprehensive characterization of enamel demineralization can guide the development of more effective remineralization treatments and preventive measures. Understanding the changes in enamel composition and structure can lead to targeted therapies that precisely address the areas most affected by demineralization. Furthermore, our methodologies can be extrapolated to future studies exploring other forms of enamel degradation or assessing the efficacy of different remineralization agents.

Moreover, our research underscores the importance of advanced diagnostic tools in dental research and clinical practice. By integrating sophisticated techniques such as FTIR, XRD, and Nano SEM, dental professionals can enhance their ability to diagnose and monitor the progression of enamel lesions, thereby facilitating the development of more personalized and effective treatment plans.

In future research, it would be advantageous to investigate the long-term effects of various remineralization agents on demineralized enamel and the potential for reversing the observed structural changes. Additionally, broadening the scope of the study to encompass a larger sample size and diverse types of enamel lesions could yield a more comprehensive understanding of the demineralization and remineralization processes.

5. Conclusion

Understanding these molecular-level changes is crucial to developing targeted treatment methods for white spot lesions. By gaining insights into the specific alterations at the molecular level, researchers and dental professionals can explore more effective and precise treatment options to address these lesions. Moreover, this comprehensive approach will contribute to developing preventive strategies to inhibit the progression of white spot lesions and preserve enamel integrity.

Ethical clearance

As it is an invitro study, no ethical clearance was required. Also, we have selected only extracted teeth from the tooth repository.

Patient's/Guardian's consent

Not applicable as we have selected tooth from the repository.

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Contributions of author

Devanshi.Rajesh.Chhabria - Conceptualization, Methodology, Draft Preparation.

Ramya Ramadoss – Conceptualization, Methodology, validation, Data curation, Visualization and Supervision.

Hema Shree. K - Methodology, validation, Data curation, Visualization, Draft Preparation.

Sandhya.Sundar – Methodology, Investigation, Data curation.

Suganya.Panneer Selvam - Methodology, Investigation, Data Curation.

Pratibha Ramani - Methodology, Software, Investigation.

Declaration of competing interest

There is no conflict of interest among the authors.

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