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Determining the Difference Between Active and Inactive Caries White Spot Lesions Using Sodium Iodide

By

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A THESIS

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Abstract

Identifying the differences between active and inactive white spot caries lesions is presently a challenging task in clinical dentistry. White spot lesions are normally associated with demineralization that has happened (inactive) or is happening (active) to the tooth at a given time. In inactive white spot lesions, the demineralization is at rest and the infectious carious process is stopped. It is important to be able to differentiate an inactive lesion from an active one because inactive lesions can be treated without surgical methods. Avoiding unnecessary repair will preserve the natural structure of the tooth and increase its longevity. We have tested the ability of a concentrated, 11 molar Sodium Iodide (NaI) solution as a radiographic contrast agent to differentiate between active and inactive caries white spot lesions. The hypothesis is that concentrated NaI solution is likely to penetrate into the tooth structure of active, but not inactive, lesions, thereby providing a visual method for distinguishing the activity states. To test this hypothesis, two teeth (one human maxillary 1st molar and one maxillary 3rd molar) with visible white spot lesions were collected for analysis. Photomicrography, radiography and Scanning Electron Microscopy (SEM) assessments were used to study these specimens. Consistent with our hypothesis, radiographic images revealed a high penetration of NaI solution through the enamel of the 1st molar, which had an active white spot lesion, as confirmed by photomicrography and SEM analysis. However, minimal to no NaI penetration was observed through the enamel of the 3rd molar, which was confirmed to have an inactive white spot lesion. While this study is preliminary, new
methods to identify active carious lesions have the potential to improve the management of caries and reduce unnecessary restorative measures.
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I. Introduction

The widespread use of fluoride has lead to a general decrease in the prevalence of dental caries detection. This new age which has implemented fluoride has made the detection of dental caries more challenging to clinicians. (Fejerskov 2004). This new challenge in caries detection is due to the fact that fluoride slows the progression and allows for potential regression of the dental caries.

In recent years, radiography has been the most trusted diagnostic method of dental caries detection (Silva Neto et al. 2008). However, with the recent widespread use of fluoride, this trusted method of caries detection has lead to several cases of mistaken diagnosis. The use of fluoride allows the teeth to remineralize and is very beneficial. Unfortunately, the constant demineralizing and remineralizing of the tooth makes the detection of caries much more difficult through radiography (Silva Neto et al, 2008). The current radiography method has been unable to detect dental caries progression and led to false negative results. A recent study showed the problem with radiography by testing its specificity vs. sensitivity (Bader et al. 2001). Specificity refers to the ability to see where on the tooth surface they are looking. Sensitivity refers to the ability to see the contrast between two different densities on the tooth surface. This resulted in more false negatives being diagnosed in the presence of disease than false positives diagnosed in the absence of disease (Bader et al. 2001). These false negatives allow the caries to continue to progress deeper and deeper into the tooth structure, which can cause more serious problems to the integrity of the tooth.
A. Enamel and Caries

Enamel, Dentin and Cementum are the three dental hard tissues formed by specialized cellular and biochemical pathways. Enamel is the outermost layer of the tooth and usually the first dental tissue damaged by dental caries (Seow 2013). Dental caries progressively work their way into the structure of the tooth (Seow 2013). Dental Enamel is the hardest tissue in the body made up of 98% mineral (hydroxyapatite crystals) and less than 2% organic matrix and water (Seow 2013). Enamel formation begins in utero and requires four stages: enamel organ, presecretory stage, secretory stage and maturation stage. The enamel organ forms an inner enamel epithelium that differentiates into Ameloblasts (Bartlett 2013). Ameloblasts are the differentiated cells that produce enamel during development. These ameloblasts lay down their proteins in layers called Perikymata. Under a microscope, Perikymata look like layers of lines surrounding the tooth surface (Figure 4). These lines start from the cusp of the tooth and continue down to the cemento-enamel junction. However, once the original structure of the tooth is damaged by demineralization, the perikymata can no longer be seen.

B. Characteristics of healthy and diseased enamel

On average, healthy enamel consists of about 93% mineral content, 5.5% water and 2% organic content (Barbosa de Sousa at el. 2013). Conversely, in a previous study, Barbosa de Sousa (2013) showed the surface layer of carious enamel decreases, causing the rest of the tooth composition to change as well. His
findings consisted of about 70% mineral content, 11% water and 17% organic content. Which is consistent with a decrease in mineral content and an increase in water and organic content. In a healthy tooth the enamel will be hard and smooth. Conversely, diseased or cavitated enamel tends to be soft and rough (Audio-Gold and Tomar 2005).

Carious lesions are the initial step of enamel degradation. This caries process begins with the formation of tightly adherent bacteria or biofilms on the tooth surface (Kidd and Fejerskov, 2004). Formation of the white spot, normally associated with carious lesions can vary in time, but can be as short as two weeks, as observed by Kidd and Fejerskov (2004). In Kidd and Fejerskov’s study, after two weeks of undisturbed biofilm on the tooth surface, enamel changes were visible clinically after the sample teeth were air-dried. The white spot caries were now visible. After three to four weeks enamel changes could be seen without air-drying the teeth. Structurally the teeth had complete dissolution of the perikymata overlappings and marked dissolution of the healthy enamel structure (Kidd and Fejerskov, 2004).

The white spot lesions represent a demineralization event has occurred. In Staley’s article (2007) he says the histology of the active vs. the inactive regions differ because of the different states of their surface layers. The active white lesion will have a porous surface layer with active demineralization taking place. The inactive lesion will have a smooth surface because the surface layer of the tooth has been remineralized. The remineralized surface layer prevents any further demineralization (Staley 2007).
C. Demineralization and Remineralization

Carious lesions are constantly in a state of demineralization and remineralization (Kidd and Fejerskov, 2004). Demineralization is caused by an undersaturation in enamel hydroxyapatite crystals in the enamel. Remineralization of the enamel surface is from the formation of fluorapatite on the enamel surface caused by supersaturation. (Kidd and Fejerskov, 2004). A shift in equilibrium and reprecipitation of minerals at the site of the enamel surface is caused by a gradual return of enamel fluids to supersaturation. Although the surface layer of the lesion may become hard and shiny some interior opacity still remains (Artun and Thylstrup, 1989).

When mineral loss is stopped or reversed toward mineral gain the lesion is considered inactive (Kidd and Fejerskov, 2004). On the other hand, if acid is still diffusing through the enamel pores and mineral loss is continuing the carious lesion is active. The active carious lesions will have surface erosion and visible enamel pores underneath the surface layer (Kidd and Fejerskov 2004). Most carious lesions are always moving from active to inactive states. This constantly changing state of the carious lesions makes it difficult for clinicians to diagnose them.

D. Limitations of current methods for caries detection
The main problem with the current methods of caries detection is that the initial phase of enamel demineralization cannot be detected (Silva Neto et al. 2008). In a clinical examination of the interproximal tooth surface, the examiner is unable to detect carious white spot lesions due to lack of physical access. The depth of a bitewing radiographic lucency can be used as an approximate guide to the likely cavitation state of an interproximal surface. In a population with slowly progressing caries only 32% of teeth were reported cavitated with an outer one-third dentin lucency and 72% in middle-third dentin (Hintze H, Wenzel A, Danielsen, et al. Reliability of visual examination, fibre-optic transillumination, and bitewing radiography, and reproducibility of direct visual examination following tooth separation for the identification of cavitated carious lesions in contacting approximal surfaces. Caries Research 1998;32(3):204-209). However, faster progressing lesions can cavitate earlier leading to problems in deciding to restore teeth as radiographs do not reveal the cavitation state. Prior to cavitation, knowledge of the activity state of white spot lesions would be very helpful for dentists to decide how to best manage caries. A static white spot needs no further care but an actively progressing lesion needs fluoride (Marinho VC, Higgins JP, Logan S, Sheiham A, Fluoride mouth rinses for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2003;(3):CD002284. Review. PMID: 12917928). The ability for dentists to use a test that would provide the activity state of non-cavitated lesions would provide important information to better manage caries. Recent studies have investigated the potential use of contrast substances as a mean to improve caries detection and diagnosis. Wilkinson’s work tested the
efficiency of concentrated 9 molar Sodium Iodide solution to provide radiographic contrast when applied to the surface of teeth at different stages of carious lesions. His work was the first to experimentally demonstrate a direct correlation between Sodium Iodide absorption by the tooth and the progression of carious lesions (Wilkinson, 2012).

Based on Wilkinson’s results, it was hypothesized that an active white spot lesion will absorb 11 molar NaI solution, but not an inactive lesion. This difference should be in part due to the presence of a remineralized (or partially remineralized) surface layer in the inactive white spot lesion. We assume remineralization should prevent NaI penetration in the inactive white spot lesion.

II. Materials and Methods

This study has been approved by IRB and Creighton University School of Dentistry. Two posterior molar teeth from two different adults were obtained from the Oral Surgery Department at Creighton University School of Dentistry. Teeth were visually assessed for carious white spot lesions. Both teeth were identified as having carious white spot lesion with a smooth surface. After extraction the teeth were stored in 1g/L Thymol Aqueous Solution. Photomicrographs were taken using an Olympus DP71, 12.5 megapixel cooled CCD camera mounted to a stereomicroscope (Leica S8APO). Photos were taken at different magnifications (1x, 1.25x, 1.6x, 2.5x, 4x and 5x) to confirm initial visual observations.
A. Radiographs and Radiolucent Dye

Teeth were radiographed using a Planmeca Intra machine, 70 kVp, 8 mA, 2 mm aluminum filtration, 390 mm source to digital intraoral sensor #1. Standardized irradiation geometry was used. XDR radiology (Cyber Medical Imaging, Los Angeles, CA) was used to view the radiographs. The teeth were placed on a wax stage with the white spot facing up so that the sodium iodide could be applied without moving the tooth. A control radiograph was taken of each tooth before the application of the 11 molar Sodium Iodide solution. Moreover, the radiolucent 11 molar Sodium Iodide was applied to the white spots of each tooth, and immediately radiographed (0 seconds). Furthermore, radiographs were then taken at intervals of 30 seconds, 1 minute, 2 minutes, 4 minutes, 8 minutes, 16 minutes and 32 minutes. At the end of each radiograph session, the teeth were washed on distilled water before being stored in 1g/L Thymol Aqueous Solution.

B. Scanning Electron Microscope

Teeth were removed from Thymol and allowed to dry for approximately 15 minutes. A black Sharpie ball point pen was used to draw an arrow to the white spot in order to locate the carious area after sputter coating. The Gold and Palladium from the sputter coater can cover the white spot and make the affected area difficult to see under the Scanning Electron Microscope. For sputter coating, each tooth was carefully placed on a silver stub and coated with Gold and Palladium in an EMITECH SC7620 Mini Sputter Coater. The teeth were then observed using a Scanning Electron Microscope (Hitachi
TM3000 Tabletop Microscope). Various pictures were taken of both the white spot region and a healthy region at magnifications ranging from 60x thru 15,000x.

C. Section and Photomicroscope Teeth

To further confirm enamel demineralization and the presence of active or inactive caries, teeth were sectioned with a diamond saw. To this end, the tooth was held by its roots and the crown of the tooth was slowly guided into the blade, making sure that the blade would bisect the white spot.

The teeth sections were examined under a Leica stereomicroscope. While under the stereomicroscope a ruler was used to measure the depth of the demineralization into the teeth. Microscopic pictures of both teeth were taken various magnifications.

III. Results and Discussion

A. Sample Selection

In order to establish clinical and diagnostic differences between active and inactive carious white spot lesions, we treated two extracted human teeth (one maxillary first molar and one maxillary third molar) (Figure 1) with a concentrated, 11 Molar, solution of Sodium Iodide. Previous analysis (Wilkinson 2012) supported the efficiency of Sodium
Iodide as a tool for identifying enamel lesions, independently of their stage of development. Sodium Iodide radiolucent properties allow it to be easily seen in radiographs. Wilkinson’s study provided the base to our working hypothesis that sodium iodide penetration would be greater in the enamel of an active but not inactive white spot lesion. Validation of this hypothesis will allow for the development of a novel diagnostic method to identify and treat carious lesions.
Figure 1: Photomicrograph of teeth analyzed. A. White spot of tooth 1 at 1.6x magnification appears dull with a rough surface. B. White spot of tooth 2 at 1.6x magnification appears bright white and has a smooth surface. Bars = 1 mm.

Photomicrographs taken at 1.6x magnification confirmed that white spot lesions were present in both selected teeth, indicating that a demineralization event has occurred at some point in time. The white spot lesion on tooth 1 was visibly larger than the lesion on tooth 2. Additionally, it showed clear characteristics of an active demineralized lesion (Staley 2007), including a dull white color, which was noticeably rough in appearance, due to the porosity on the surface layer of the tooth. Demineralization was further confirmed during analyses of the tooth at higher magnifications. Of note, we did not observe any signs of perikymata, which is normally visible on healthy tooth enamel, in the affected area of tooth 1. In contrast, the white spot on tooth 2 appeared bright white and smooth, indicating remineralization. Once the presence of white spot lesions was confirmed, the selected teeth were processed for radiography.

B. Radiographic Analyses

Control radiographs taken from both teeth before application of Sodium Iodide showed opacities in the enamel where the white spot lesions were located (Figure 2), confirming that enamel demineralization has occurred in those areas.
Figure 2: Control Radiograph of teeth analyzed. A. Tooth 1 has very opaque region in area of white spot lesion. B. Tooth 2 has very slight opacity in the white spot region.

In order to characterize sodium iodide absorption in the teeth over time, sodium iodide was applied to the white spot regions of each tooth and radiographed at different time intervals (i.e., 0 sec, 30 sec, 1 min, 2 min, 4 min, 8 min, 16 min, 32 min). After 32 minutes, the Sodium Iodide clearly penetrates into the enamel surface on tooth 1 (Figure 3A), but little to no penetration was observed on the surface of tooth 2 (Figure 3B). Sodium iodide penetration in tooth 1 began around 2-4 minutes after application (Figure 3A). At 32 minutes, there was significant penetration through the surface and into the enamel (Figure 3B). Conversely, tooth 2 showed no apparent penetration throughout the length of the experiment. Sodium iodide’s quick penetration in tooth 1 is consistent with our previous characterization of the lesion and its active state, as well as its demineralization. The same is true for tooth 2. Lack of sodium iodide penetration in tooth 2 is consistent with an
inactive state of the lesion and probable remineralization. Moreover, the deeper penetration of the contrast into tooth 1 indicates an active carious lesion whereas lack of penetration of sodium iodide in tooth 2 suggests an inactive carious lesion.

**Figure 3:** Temporal assessment of sodium iodide absorption in white spot lesions. Teeth radiographs taken after the application of 11 Molar Sodium Iodide contrast at different time intervals. A. Tooth 1 radiographs showed sodium iodide penetration starting between 2-4 minutes after application and progressing deeper into the enamel over time. B. In contrast, tooth 2 radiographs showed no difference on sodium iodide penetration.
over time. Moreover, a constant area of opacity between the dye and the enamel was overserved at all time intervals.

C. Scanning Electron Microscopy (SEM) Analysis

In order to evaluate the extent of compromised enamel, scanning Electron Microscopy (SEM), was used to analyze the ultra structural aspects of the white spot lesions on both teeth. Under the electron microscope, the demineralized enamel displayed a sponge-like appearance caused by gaps in the tooth surface (Figure 4A, B). Enamel degradation was seen in both teeth to different extents; however it was clearly more conspicuous in tooth 1 compared to tooth 2, where very little demineralization was observed (Figure 4A, B). The sponge-like appearance was attributed to destruction of the tooth rods (Bartlett 2013) which, in turn, may have contributed to the higher penetration of sodium iodide past the surface layer of tooth 1, but not tooth 2.

Next, we selected and compared sections of the sound regions of tooth 1 and 2. Contrasting with the clear differences observed between the white spot regions, no differences were observed between the sound regions of both teeth. Supporting their sound state, the selected regions in teeth 1 and 2 still exhibited the perikymata, a histological feature of healthy enamel (Figure 5).
**Figure 4:** Scanning Electron Microscope (SEM) pictures of white spot lesions in teeth 1 (A) and 2 (B). A. Extensive enamel surface demineralization showing a sponge-like, porous appearance due to loss of tooth rods in active carious lesion. B. Compared to tooth 1, a smoother looking enamel surface with minimal amounts of demineralization was observed in tooth 2.
Figure 5: Scanning Electron Microscope (SEM) photo of Tooth 2 at 400x magnification. The black lines running across the enamel of the tooth are the perikymata of the tooth. The presence of Perikymata indicates healthy enamel.

D. Tooth Sections

By sectioning the teeth we were able to physically measure the depth of the white spot lesion of each tooth. Visual assessment of the sectioned white spot lesions on tooth #1 and tooth #2 was comparatively similar in depth. In both teeth it expanded from the enamel to the dentinoenamel junction (Figure 6A, B). An artifactual space at the
dentinoenamel junction was observed in both teeth, possibly from excessive drying during experimental processes (Figure 6A, B).

Further analysis of tooth #1 revealed the presence of brown pigmentation of dentin indicating that the demineralization continued deep into the dentin of the tooth. Therefore confirming the presence of an active white spot carious lesion. In contrast, the white spot carious lesion on tooth #2 did not spread beyond the dentinoenamel junction (Figure 6B). While it penetrated through the enamel, it did not appear as dense as the lesion in tooth 1 (Figure 6A). Additionally, the natural white color of the dentin layer of tooth 2 supports our previous observation that the carious lesion did not penetrate into the dentin of the tooth.
**Figure 6:** Section through the enamel-dentin junction (DEJ) of tooth 1 and 2. **A.** White spot lesion of tooth 1 shows penetration into the dentin (arrow), as evidenced by the brown colored stain in the enamel-dentin junction. **B.** Enamel and dentin in tooth 2 display a natural white color (arrow) indicating that the carious lesion did not penetrate into the dentin. Artificial space between enamel and dentin layers are artefactual, generated during teeth sectioning and processing. Bar = 1 mm.
E. Overall Remarks

This study is the first step towards the development of a diagnostic tool that will allow dentists to effectively differentiate between active and inactive carious white spot lesions. The constant physiological changes in the active state of white spot lesions makes it difficult to differentiate one from the other in a clinical setting. The present results support the effectiveness of the radiolucent sodium iodide as a contrast substance to differentiate between active (demineralized) and inactive (remineralized) carious lesions.

While beneficial to the practitioner, the direct beneficiary of this method should be the patient. Correct diagnosis of carious lesions will prevent unnecessary treatment of an otherwise healthy tooth. Consistent with its initial stage and innovative nature, this method still needs improvements, in order to correct some of its current limitations. To this end, the next phase of this study should focus on improving sodium iodide delivery to the patients’ mouth and tooth/teeth of interest, as well as prevention of sodium iodide dilution in the saliva.

Dental restorations result in the loss of the tooth’s natural structure as well as a weakening of the tooth, which puts the tooth at risk for further problems later in life. Overall, the development and application of this novel diagnostic method to identify carious lesions has the potential to move the dental field forward by providing a new tool to facilitate the work of dental professionals when treating a patient’s teeth, as well as preventing unnecessary restorative measures.
IV. Conclusions

This study showed penetration of sodium iodide contrast through the enamel of the active carious lesion over time. Conversely, the inactive white spot carious lesion absorbed minimal to none of the sodium iodide dye. These limited findings provide initial support to the hypothesis that sodium iodide is likely to penetrate into active but not inactive white spot carious lesions. A larger sample size in a clinical setting will be necessary to demonstrate that these findings will occur with patients in clinical practice.
V. References


*Creighton University School of Dentistry.*