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Understanding the association between the presence of occlusal fissure calculus and caries.

By

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A THESIS

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Abstract

The pits and fissures of the tooth's occlusal surface are the most vulnerable region of the tooth, accounting for over 80 percent of caries while making up only 13 percent of the overall tooth surface. However, it has also been shown that bacterial contents of the fissures may progressively mineralize. To determine by an *in vitro* study if an inverse relationship exists between calculus and caries within the pits and fissures of the teeth. One molar tooth was selected and sectioned through the buccal-lingual plane using a SCIFAB Series 1000 Deluxe Hard Tissue Microtome (Scientific Fabrications, Littleton, CO, USA). Photomicrographs were taken with an Olympus DP71, 12.5 megapixel cooled CCD camera mounted to a stereomicroscope (Leica S8APO). Scanning electron microscopy (EMITECH SC7620 Mini Sputter Coater and Hitachi TM3000 Tabletop Microscope) was used to assess the presence of calculus in the fissure. Varying amounts of calculus and caries were found in the fissure system. There appeared to be a possible inverse relationship between the amount of calculus and the presence of caries. Calculus can be found in carious fissures and may indicate an inverse relationship to active caries. However, without serial examinations over time it is not possible to determine the relationship between caries activity and fissure calculus. Further work is needed.

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I. Introduction

A. Hypothesis

Over the last century dentistry has evolved from purely restorative practices to adopt more preventative strategies (Thylstrup, Chironga, Carvalho, & Ekstrand, 1989). Accordingly, dental caries in developed countries are on the decline (Horowitz, 2004). Increased access to care, fluoride, and more effective oral hygiene products are all contributing factors (Bamzahim, Shi, & Angmar-Mansson, 2002; Dye et al., 2007). However, approximately 20 percent of children between ages 2 and 4 have detectable caries and by the age of 17 approximately 80 percent will have had at least one cavitated lesion. Additionally, more than two-thirds of middle-aged adults have lost at least one permanent tooth to irreversible damage from dental caries (Horowitz, 2004). This is especially disconcerting considering oral health is essential to an individual's overall health and well-being, as previously recognized by the U.S. Surgeon General (Dye et al., 2007). Amidst all the recent advances in the dental field, the conventional methods for the detection and diagnosis of dental caries are still visual, tactile, and radiographic techniques, which have remained relatively unchanged for the better half of a century (Stookey & Gonzalez-Cabezas, 2001). Unfortunately, these methods are inadequate for clinically recognizing the initial stages of non-cavitated lesions, when the damage is still reversible (Horowitz, 2004). General dental practitioners (GDPs) regard the tooth's occlusal surface to be highly vulnerable to caries (Thylstrup, Chironga, Carvalho, & Ekstrand, 1989). The pits and fissures are the most susceptible region of the tooth accounting for over 80 percent of caries in young permanent teeth while making up only 13 percent of the overall tooth surface (Feigal & Donly, 2006). While caries are prevalent in the pits and fissures it has also been shown that under the right conditions bacterial contents of the fissures may progressively mineralize (Galil & Gwinnett, 1975). The present study was designed to test the hypothesis that a negative correlation exists between the presence of occlusal fissure calculus and the adjacent development of caries. This mineralization within the pits and fissures of posterior teeth may act as a natural sealant that will protect the tooth from caries originating in the pits and fissures of the tooth. If this relationship is found to be true it could have clinical implications in the management of occlusal caries, as well as influence future caries research.

B. Caries, demineralization, and remineralization

In the quest to discovering new methods of diagnosis it is important to evaluate what is currently known about caries. Dental caries is best described as a transmissible bacterial disease characterized by the demineralization of the hard structures of the tooth (Horowitz, 2004). Dental caries is known to be a continuum beginning with the initial loss of calcium and phosphate, compromising the crystal structure of the tooth and ending with cavitation. Caries progression can be arrested and reversed at any stage by the process of remineralization. Whether demineralization or remineralization takes place at any given time is primarily influenced by the pH levels in the oral cavity. A low pH corresponds to demineralization while a high pH corresponds to remineralization (Featherstone, 2008). Factors that contribute to changes in pH levels include the composition of the plaque biofilm, dietary intake of carbohydrates, fluoride exposure, and salivary flow (Dahlen et al., 2010; Featherstone, 2008).

Demineralization requires the presence of an oral biofilm (Kidd & Fejerskov, 2004). Oral biofilms are complex aggregations of microorganism that colonize in the mouth and form dental plaque on the exposed surfaces of the tooth (Hiyari & Bennett, 2011). Cariogenic bacteria in this biofilm metabolize carbohydrates into organic acids effectively lowering the pH of the oral cavity and increasing the susceptibility of the teeth to caries (Hiyari & Bennett, 2011). These acids, namely acetic, formic, lactic, and propionic acid, dissolve the calcium and phosphate minerals on susceptible sites of enamel and dentin creating an early subsurface lesion currently undetectable clinically. If left untreated, this non-cavitated lesion may progress to cavitation (Dahlen et al., 2010; Featherstone, 2008). While cavitation is often the end result of caries, regular removal of the biofilm is shown to arrest caries progression and is sometimes adequate to initiate the tooth's repair process, known as remineralization (Kidd & Fejerskov, 2004).

Remineralization is the body's natural repair process and is the direct inverse of demineralization (Duckworth & Huntington, 2005). During remineralization, the tooth absorbs calcium and phosphate, primarily from saliva, along with fluoride, if it is available in the oral environment (White, 1997). These minerals diffuse into the enamel to repair and build on the existing crystal remnants of the tooth. The crystal restructuring renders the repaired site less soluble than the original site, thus less susceptible to future caries attack (Featherstone, 2008). This process can also involve the concomitant mineralization of plaque into dental calculus. The development of caries is the result of demineralization; however, the deposition of calculus is a mineralization process in and of itself. Considering the direct opposition of the two processes it is reasonable to suspect an inverse relationship between caries and calculus (Duckworth & Huntington, 2005).

C. Pit and fissure morphology and mineralization

Pits and fissure morphology and their contents must be explored in order to sufficiently address the vulnerability of occlusal surfaces to caries. Pits and fissures are sites on the tooth where adjacent cusps do not fuse and instead form a groove or a pit on the tooth's surface (Rohr, Makinson, & Burrow, 1991). Fissures vary in width and depth and have been shown to be preferential sites for the development of caries (Galil & Gwinnett, 1975; Rohr, Makinson, & Burrow, 1991; Theilade, Larson, & Karring, 1973). However, it has also been shown that under the right circumstances mineralization can occur in these fissures over time (Galil & Gwinnett, 1975). The addition of calcium phosphate to the oral environment facilitates precipitation of calcium phosphate by dental plaque, effectively mineralizing the bacteria located in the fissure (Clarke & Fanning, 1971; Clarke & Fanning, 1973). Once this occurs, it is reasonable to suspect that the occlusal fissures of the tooth are less susceptible to caries.

II. Materials & Methods

The integrity of this study has been approved by the Creighton University Institutional Review Board (IRB), protocol 11-16269.

A. Tooth collection, selection, staining, and sectioning

Teeth were collected from the oral surgery department of the Creighton University School of Dentistry. The teeth were stored in 1g/L thymol aqueous solution (Cat No. T0501, Sigma-Aldrich, St. Louis, MO). Tooth selection was based on the visual appearance of staining and/or calculus in the occlusal pits and fissures of posterior teeth. Multiple teeth were examined and one molar was ultimately selected for further study.

After selection, a plaque disclosing agent (Young Dental 2-Tone™ Disclosing Solution) was applied to the occlusal surface to visually identify plaque versus calculus. The tooth was then sectioned through the buccal-lingual plane using a SCIFAB Series 1000 Deluxe Hard Tissue Microtome (Scientific Fabrications, Littleton, CO, USA). A total of 8 cuts were made resulting in 16 tooth sections.

Photomicrographs were taken of the occlusal surface with a camera (Olympus DP71, 12.5 megapixel cooled CCD camera) mounted on a stereomicroscope (Leica S8APO) immediately prior to and after being sectioned. Additionally, close up photomicrographs were taken of the fissure of each tooth section. The sections were then returned to the thymol solution.

B. Scanning Electron Microscopy (SEM)

The tooth sections were removed from the thymol solution and allowed to dry for approximately 30 minutes. The section was fixed on a metallic specimen stub and coated with gold and palladium (EMITECH SC7620 Mini Sputter Coater). The sections were then examined with a scanning electron microscope (Hitachi TM3000 Tabletop Microscope). Magnifications of 60x and 15,000x were used to determine the presence of calculus in the tooth sections.

III. Results and Discussion

The present study was designed to allow for testing the hypothesis that a negative correlation exists between the presence of occlusal fissure calculus and the adjacent development of caries in the posterior teeth. After sectioning, we performed a visual examination and determined that calculus was present to varying degrees in the fissure system of the selected tooth. For visual examination we used plaque disclosing agent, light microscopy, and scanning electron microscopy (SEM).

A. Plaque Disclosing Agent

Prior to sectioning, a plaque disclosing agent was applied to visually highlight a difference between plaque and calculus. Plaque appeared to cover the majority of the occlusal surface and this was confirmed after applying the disclosing agent (figure 1). The disclosing agent used is a 2-tone dye, which stains old plaque blue and new plaque red. Based on clinical practice it was expected that the disclosing agent would stain plaque but not calculus. Thus, providing a contrast between the white calculus deposit and the purple plaque (figure 2).

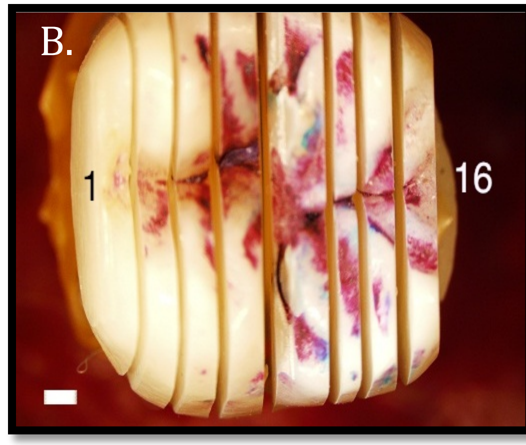

Figure 1: A. Experimental tooth exhibiting staining in the occlusal fissures. B. Same tooth after plaque staining and sectioning through the mesial-buccal plane. *Sections were numbered in sequence from 1 (left) to 16 (right).
Bar = 1mm

B. Light Microscopy

Upon 2.5x magnification it appeared that calculus was present to varying degrees in the fissure system of the tooth. Photomicrographs of each section were taken; however sections that did not contain fissures were not considered. Tooth section 12 appeared to have the most pronounced deposition of calculus.

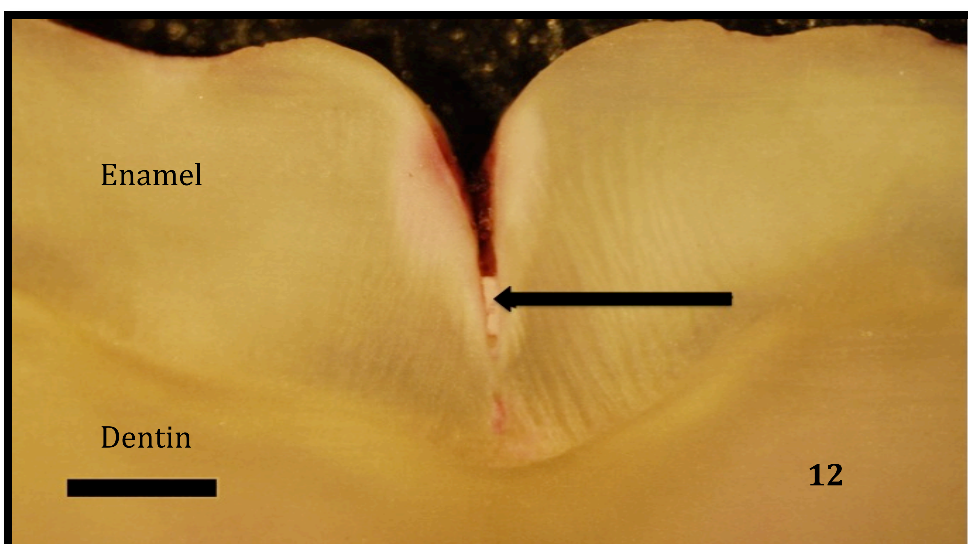

Figure 2: Fissure of tooth section 12. Calculus deposition indicated by the arrow.
Bar = 1 mm

C. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was used to confirm the presence of calculus in the fissure of section 12. The composition of calculus contains two distinct patterns of calcification. Since calculus is mineralized plaque, one of the components is formed by the precipitation of calcified crystals on microorganisms within the plaque matrix. These crystals tend to be spherical and give a spongy appearance with SEM imaging (Lustmann, Lewin-Epstein, & Shteyer, 1976). The calculus in the SEM images in figure 3 is consistent with this characteristic description.

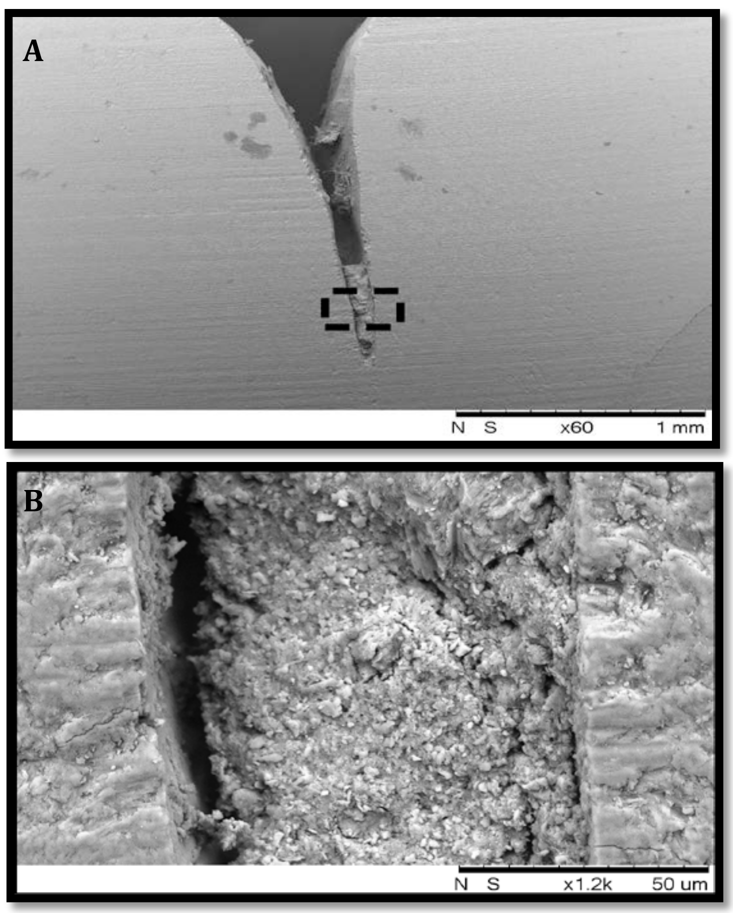

Figure 3: Scanning electron microscopy (SEM) images of fissure observed on section 12. Boxed region in A is shown at higher power in B.
A = 60x, B = 1,500x. Bar A = 1mm, Bar B = 50 μm

D. Calculus vs. Caries

All three methods confirmed the presence of calculus in the fissure of tooth section 12. It is now possible to evaluate the pattern of adjacent caries development in this section and compare it to the tooth sections lacking mineralization in the fissures.

The presence of calculus in the fissure of tooth section 12 correlates with a decreased penetration of caries directly adjacent to the calculus (figure 2). Conversely, within the same fissure, carious lesions penetrated farther into the enamel adjacent to regions that lack mineralization. We also do not see any caries penetration into the dentin layer of the tooth. The extensive calculus deposition suggests that this fissure was in an environment favorable to remineralization. Moreover, within tooth sections 11 and 13, the two sections directly adjacent to section 12, exhibit a similar decrease in caries penetration into the enamel (figure 4). The close proximity of these sections led us to suggest that they were exposed to a similar remineralizing environment as tooth section 12.

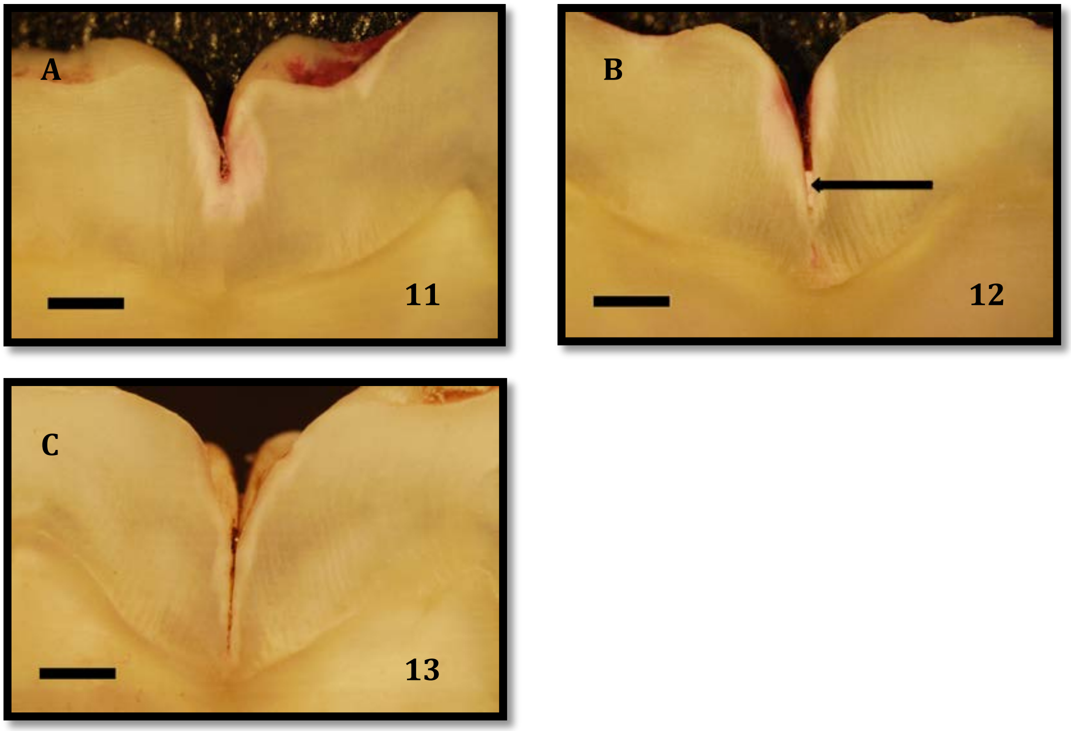

Figure 4: A. Decrease in caries penetration and lack of dentin destruction exhibited in tooth section 11. B. A significant concentration of calculus (arrow) may have contributed to decreased penetration of caries in the enamel, particularly in the area physically adjacent to the calculus' location in tooth section 12. C. Decrease in caries penetration and lack of dentin destruction exhibited in tooth section 13.
Bar = 1mm

Also important to consider are the sections in which the fissures do not contain any or significant amounts of mineralization, figure 5. In these sections the carious lesions have penetrated into the enamel along the entire length of the respective fissures. Of note, in fissure with little or no mineralization, caries have penetrated in a cone shape from the base of the fissure and there are signs of significant dentin destruction. This would suggest that the fissures with no mineralization have been exposed to a carious environment favorable to demineralization.

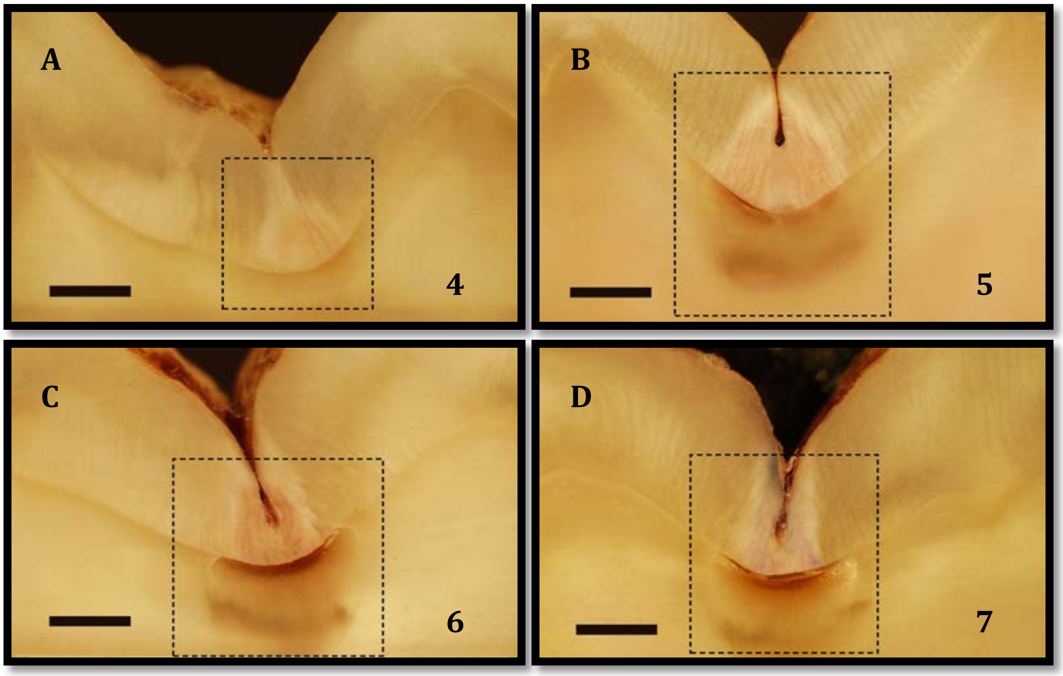

Figure 5: A – D lack mineralization within the occlusal fissures, which appears to be associated with extensive caries penetrating through the enamel layer of the tooth into the dentin. Boxed region contains the carious lesion presenting itself as a white spot extending from the fissure. The boxed region also contains the dentin destruction presenting itself as the brown discoloration beneath the dentinoenamel junction. Bar = 1mm

E. Summary

Overall, varying levels of calculus were identified in the fissures of the tooth sections and an inverse relationship may be present between calculus and caries in these fissures. This data is consistent with our working hypothesis that a negative correlation exists between the presence of occlusal fissure calculus and the adjacent development of caries. However, further examination is needed with an increased sample size and a method to accomplish serial sectioning over a period of time to completely understand this correlation. A larger sample size will help determine the consistency of this phenomenon. While calculus has previously been shown to promote caries resistance, the incidence of such phenomenon in the occlusal fissures of the general population is unknown. Moreover, without serial sectioning over time it is not possible to determine the relationship between caries activity and fissure mineralization. This data would provide a timeline of caries progression versus calculus deposition and allow us to accurately assess the correlation between the two. Given the clinical relevance of this piece of information, continuation of this study is likely to provide much needed knowledge with significant translational potential.

IV. Conclusion

The goal of this study was to explore the relationship between calculus and caries in the occlusal fissures of the posterior teeth. It was proposed that an inverse relationship exists between calculus and caries. We have confirmed that calculus can be present in occlusal fissures and when present appears to be associated with a decreased occurrence of adjacent caries penetration. Conversely, where calculus is not present carious lesions have penetrated deeper into the tooth. This data is consistent with our working hypothesis that a negative correlation exists between the presence of occlusal fissure calculus and the adjacent development of caries. However, a larger sample size as well as a method to determine a timeline of fissure mineralization versus caries progression is necessary to draw statistically significant results. Although preliminary, this research poses new avenues for future examination.

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