Dentin Destruction by Bacteria and Fungi in Carious Teeth

Garcia L[1,2], Yu J[3], Dickson JR[4], Sulyanto RM[1,2]

[1] Department of Dentistry, Boston Children's Hospital, Boston, Massachusetts
[2] Harvard School of Dental Medicine, Boston, Massachusetts
[3] Harvard John A. Paulson School of Engineering and Applied Sciences, Boston, Massachusetts
[4] Mass General Brigham, Boston, Massachusetts

Objective

1. To develop a method to detect collagen denaturation and cleavage in dentin.
2. To examine the spatial relationship between altered collagen and localization of bacteria and fungi within carious dentin.

Background

- Dental caries is the most common chronic disease of childhood and severe forms can cause pain and life-threatening systemic infections.[1, 2]
- Serious consequences of dental caries result when microbes access the pulp through dentin tubules.[3, 4] However, microbial invasion of dentin is largely unstudied and methods to study this hard tissue are underdeveloped.
- Dentin has a different composition than enamel with a greater organic content and a lower mineral content, as well as a unique structure with a network of microchannels that communicate directly with the dental pulp.[5, 6]
- Recent work suggests that the ratio of fungus to bacteria is higher in carious dentin compared to surface biofilms. Additionally, unlike that of polymicrobial surface biofilm, dentin caries has a unique microbial spatial biology with single tubules harboring predominantly fungus or bacteria which may be driving dissolution of tooth structure from within the tubules.[7]
- The function of bacteria and fungus in dentin caries and their ability to advance the lesion front, a key pathogenic process, has not been investigated.

Methods

Subject/Tooth Recruitment: Carious (n=3) and non-carious (n=3) primary teeth were collected from healthy children ages 2-10 years.

Histological Processing:

1. Fixation of teeth in 10% neutral-buffered formalin
2. Decalcification of teeth using Christensen's buffer (formic acid) at room temperature for 14 days
3. Paraffin-embedding
4. Sectioning at 5 µm thickness

Immunohistochemistry and Fluorescent Labeling:

- Collagen hybridizing peptide (CHP) (F-CHP; 3Helix, Cat#: FLU60) detecting denatured collagen
- Anti-collagen type 1 cleavage site antibody (rabbit polyclonal; immunoGlobe, Cat# 0217-025) detecting cleaved collagen
- Anti-lipoteichoic acid (LTA) antibody (mouse monoclonal; Thermo Fisher Scientific, Cat# MA1-7402) detecting gram positive bacteria
- Wheat germ agglutinin (WGA) (Alexa Fluor Plus 405 conjugate; Thermo Fisher Scientific, Cat# W11261) detecting fungi

Confocal Raman Microscopy:

- Localization of microbes and detection of altered collagen matrix
- Spectral data analyzed using multivariate curve resolution

Results

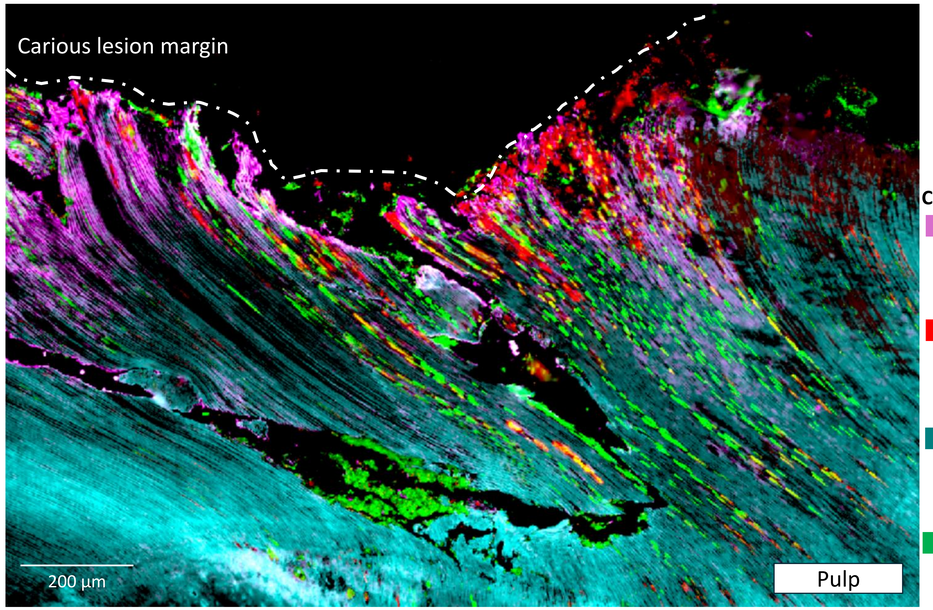

Figure 1. Immunofluorescence microscopy.
Collagen cleavage was concentrated along the outer dentin while collagen denaturation was predominantly localized to the inner dentin.
Regions of collagen denaturation colocalized with regions colonized by fungi, and regions of collagen cleavage colocalized with regions colonized by both fungi and gram-positive bacteria.

Channels:

- **Pink: Anti-Col type I (collagen cleavage)** Wavelength of excitation: 647 nm
- **Red: Anti-LTA (gram positive bacteria)** Wavelength of excitation: 555 nm
- **Teal: CHP (collagen denaturation)** Wavelength of excitation: 488 nm
- **Green: WGA (fungi)** Wavelength of excitation: 405 nm

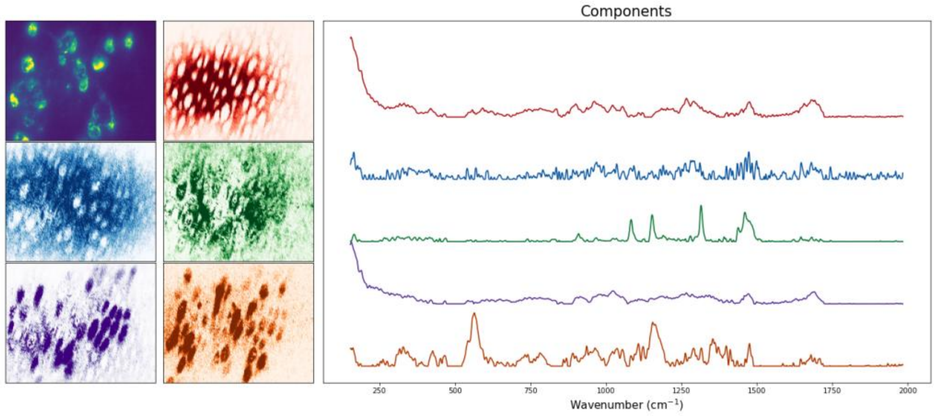

Figure 2. Confocal Raman microscopy.
Confocal microscopy of a carious tooth sample with S. mutans antibody (top left panel). Raman spectroscopy identified five distinct spectral signatures corresponding to bacteria, fungi, and structural states of the collagen matrix.

Conclusion

This study establishes the feasibility of an integrated histologic and multimodal microscopic approach to detect collagen degradation in dental tissues and provides insight into how microbial activity contributes to dentinal extracellular matrix breakdown during caries progression.

References

1. World Health Organization. Ending childhood dental caries: WHO implementation manual. 2019. Available from: https://www.who.int/publications/i/item/9789240000056
2. Early Childhood Caries: Prevalence, Risk Factors, and Prevention. Front Pediatr. 2017; 5: 157. PMID: 28770188
3. Policy on Early Childhood Caries (ECC): Classifications, Consequences, and Preventive Strategies. Pediatr Dent. 2017; 39: 59–61. PMID: 29179321
4. Reeves R, Stanley HR. The relationship of bacterial penetration and pulpal pathosis in carious teeth. Oral Surg Oral Med Oral Pathol. 1966 Jul;22(1):59-65. doi: 10.1016/0030-4220(66)90143-5. PMID: 5220026.
5. Love RM, Jenkinson HF. Invasion of dentinal tubules by oral bacteria. Crit Rev Oral Biol Med. 2002; 13: 171–183. https://doi.org/10.1177/154411130201300207 PMID: 12097359
6. Yoshihara K, Nagaoka N, Nakamura A, Hara T, Hayakawa S, Yoshida Y, Van Meerbeek B. Three-dimensional observation and analysis of remineralization in dentinal caries lesions. Sci Rep. 2020; 10(1):4387. doi: 10.1038/s41598-020-61111-1 PMID: 32152430.
7. Sulyanto RM, Beall CJ, Ha K, Montesano J, Juang J, Dickson JR, et al. (2024) Fungi and bacteria occupy distinct spatial niches within carious dentin. PLoS Pathog 20(5): e1011865. https://doi.org/10.1371/journal.ppat.1011865