Fungi Invade Carious Dentin

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Background

- Dental caries is the most common chronic disease among children despite being largely preventable.[1] In 2017, 530 million children were reported to have untreated primary tooth caries in the Global Burden of Disease study.
- The role of the fungal mycobiome in oral health and disease is not well understood.
- *Candida* spp. presence in carious lesions is inconsistently identified, but colonization is found of oral mucosa, saliva, and plaque biofilm.[2]
- Little is known regarding the fungal colonization of mineralized tissues, such as dentin.
  - Previous studies have reported a direct relationship between the *Candida* carriage rate and caries severity, the presence of fungal organisms in root-surface caries, and the penetration of dentin by *C. albicans in vitro*.[2,3,4]
  - Others have reported no correlation between *Candida* and caries.[5]

Objectives

- Elucidate the spatial distribution and composition of fungi and bacteria in carious dentin using next-generation sequencing, histology, and microscopy methods.
- Gain a deeper insight into the oral mycobiome and its involvement in dental caries.

Methods

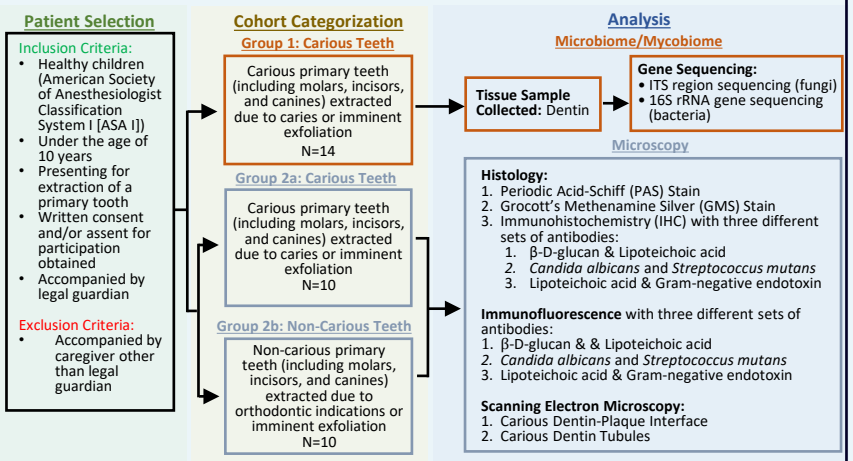

Figure 1. Outline of the study design.

References

1. Benjamin RM. Oral Health: The Silent Epidemic. *Public Health Rep*. 2010;125(2):158-159.
2. Maijala, M., Rautemaa, R., Järvensivu, A., Richardson, M., Salo, T. and Tjäderhane, L. (2007), Candida albicans does not invade carious human dentine. Oral Diseases, 13: 279-284. https://doi.org/10.1111/j.1601-0825.2006.01279.x
3. Nyvad B, Fejerskov O. An Ultrastructural Study of Bacterial Invasion and Tissue Breakdown in Human Experimental Root-Surface Caries. 2016;69(5)
4. Waltimo TM, Ørstavik D, Sirén EK, Haapasalo MP. In vitro yeast infection of human dentin. J Endod. 2000 Apr;26(4):207-9. doi: 10.1097/00004770-200004000-00002. PMID: 11199719.
5. Peretz B, Mazor Y, Dagon N, Bar-Ness Greenstein R. Candida, mutans streptococci, oral hygiene and caries in children. J Clin Pediatr Dent. 2011 Winter;36(2):185-8. doi: 10.17796/jcpd.36.2.f1m4283501374t22. PMID: 22524082.

Results

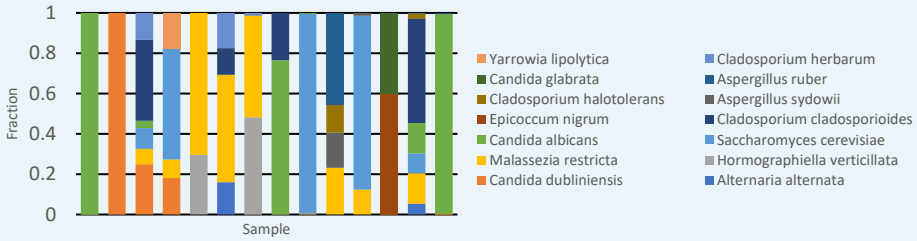

Figure 2. Relative abundance of fungal species with over one percent abundance in 14 carious dentin samples.

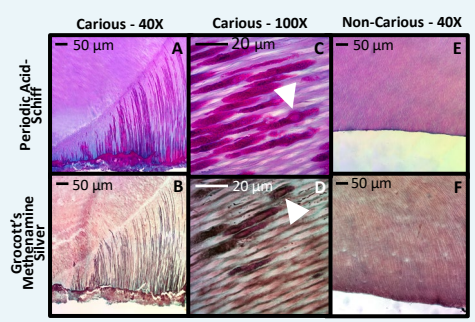

Figure 3. Light microscopy of carious and non-carious dentin using PAS and GMS-stained sections.

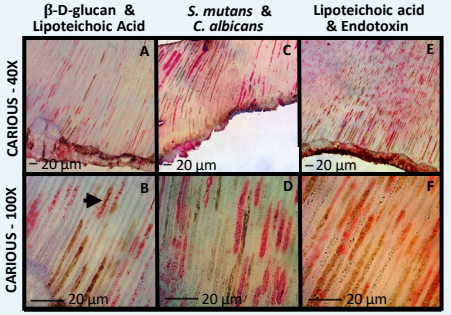

Figure 4. Immunohistochemistry (IHC) of carious dentin stained with different antibody pairs: β-D-glucan (brown) and lipoteichoic acid (pink); *S. mutans* (pink) and *C. albicans* (brown); and against gram-positive lipoteichoic acid (pink) and gram-negative endotoxin (brown).

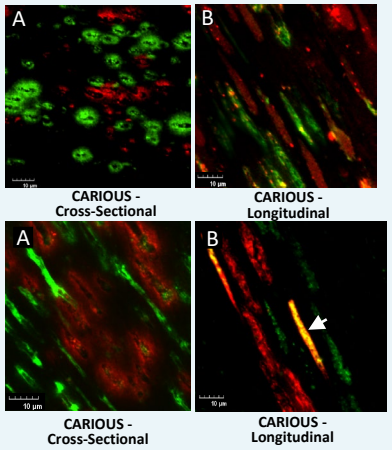

Figure 5. Immunofluorescence of carious dentin stained with antibodies against lipoteichoic acid (red) and β-D-glucan (green) at 120x magnification.

Figure 6. Immunofluorescence of carious dentin stained with antibodies against *S. mutans* (red) and *C. albicans* (green) at 120x magnification.

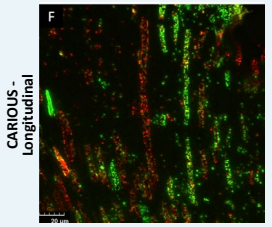

Figure 7. Immunofluorescence of longitudinal carious dentin stained with antibodies against gram-positive lipoteichoic acid (red) and gram-negative endotoxin (green) at 120x magnification.

Results

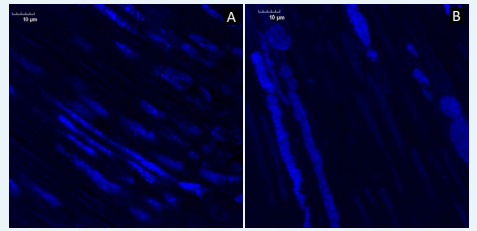

Figure 8: Calcofluor-white florescent stain for cellulose and chitin.

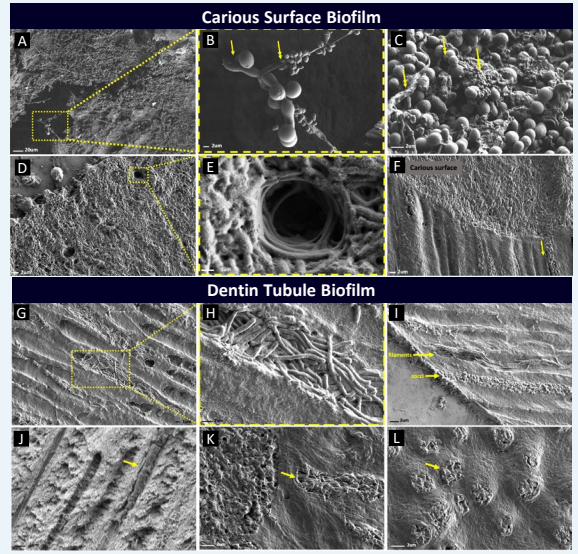

Figure 9: Scanning electron microscopy (SEM) performed on carious surfaces and within dentin tubules.

Conclusions

- Various and diverse bacterial species could be detected in carious dentin by next-generation sequencing, while fungal species were quantitively less diverse.
- Fungi are abundant in carious dentin, particularly *Candida albicans*.
- Fungi tend to invade tubules alone as mono-species biofilms, separate from bacterial species.
- Gram-positive and gram-negative bacteria were found to co-localize within the same dentin tubules, and bacteria tended to colonize with other species of the same morphology within dentin tubules on SEM.
- This study supports the prevalence of fungi within carious dentin and suggests that the oral mycobiome may play a crucial role in caries pathogenesis, which warrants further exploration.