

Background

Fungi Invade Carious Dentin

Ha KD^{1,2}, Montesano JM², Juang JM², Dickson JR³, Ho SP⁴, Beall CJ⁵, Griffen AL⁵, Sulvanto RM^{1,2}



Results

¹Boston Children's Hospital, ²Harvard School of Dental Medicine, ³Massachusetts General Hospital, Boston, MA; ⁴University of San Francisco, San Francisco, CA; and ⁵The Ohio State University College of Dentistry, Columbus, OH

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 0.8 Yarrowia lipolytica Cladosporium herbarum The role of the fungal mycobiome in oral health and disease is not well understood. Candida glabrata Aspergillus ruber Candida spp. presence in carious lesions is inconsistently identified, but colonization is found of oral 0 Cladosporium halotolerans Aspergillus sydowii mucosa, saliva, and plaque biofilm.² Epicoccum nigrum Cladosporium cladosporioides Little is known regarding the fungal colonization of mineralized tissues, such as dentin-Candida albicans Saccharomyces cerevisiae • Previous studies have reported a direct relationship between the Candida carriage rate and caries Malassezia restricta Hormographiella verticillata 02 severity, the presence of fungal organisms in root-surface caries, and the penetration of dentin by C. Candida dubliniensis Alternaria alternata albicans in vitro,^{2,3,4} Others have reported no correlation between Candida and caries.⁵ Sample Figure 2. Relative abundance of fungal species with over one percent abundance in 14 carious dentin samples Objectives Lipoteichoic acid β-D-glucan & S. mutans & Elucidate the spatial distribution and composition of fungi and bacteria in carious dentin using next-Carious - 100X Non-Carious - 40X Carious - 40X Linoteichoic Acid C. albicans & Endotoxin generation sequencing, histology, and microscopy methods. Gain a deeper insight into the oral mycobiome and its involvement in dental caries. Periodic A Methods **Patient Selection Cohort Categorization** Analysis Microbiome/Mycobiome Group 1: Carious Teeth lusion Criteria: Healthy children Gene Sequencing: Carious primary teeth (American Society • ITS region sequencing (fungi) including molars, incisors, of Anesthesiologis Tissue Sample 16S rRNA gene sequencing and canines) extracted Classification Collected: Dentin (bacteria due to caries or imminent System I [ASA I]) exfoliation 2 E E E Under the age of Microscopy N=14 10 vears Presenting for Histology: Group 2a: Carious Teeth extraction of a 1. Periodic Acid-Schiff (PAS) Stain primary tooth Carious primary teeth Grocott's Methenamine Silver (GMS) Stain Figure 4. Immunohistochemistry (IHC) of carious dentin stained Figure 3. Light microscopy of carious and non-carious dentin using Written consent including molars, incisors. 3. Immunohistochemistry (IHC) with three different with different antibody pairs: B-D-glucan (brown) and lipoteichoic PAS and GMS-stained sections and/or assent for sets of antibodies acid (pink); S. mutans (pink) and C. albicans (brown); and against and canines) extracted participation B-D-glucan & Lipoteichoic acid due to caries or imminent gram-positive lipoteichoic acid (pink) and gram-negative endotoxin obtained Candida albicans and Streptococcus mutans (brown) exfoliation Accompanied by Lipoteichoic acid & Gram-negative endotoxin N=10 legal guardian Immunofluorescence with three different sets of Exclusion Criteria: Group 2b: Non-Carious Teeth antihodies Accompanied by Figure 5. 1. β-D-glucan & & Lipoteichoic acid Non-carious primary Immunofluorescence of caregiver other 2. Candida albicans and Streptococcus mutans teeth (including molars, carious dentin stained with than legal 3. Lipoteichoic acid & Gram-negative endotoxin incisors, and canines) antibodies against guardian extracted due to lipoteichoic acid (red) and B Scanning Electron Microscopy: rthodontic indications o D-glucan (green) at 120x 1. Carious Dentin-Plaque Interface imminent exfoliation magnification 2. Carious Dentin Tubules N=10 Figure 1. Outline of the study design

Cross-Sectional

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. Feierskov O. An Ultrastructural Study of Bacterial Invasion and Tissue Breakdown in Human Experimental Root-Surface Caries. 2016;69(5)
- 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.
- Peretz B, Mazor Y, Dagon N, Bar-Ness Greenstein R. Candida, mutans streptococci, oral hygiene and caries in 5. children. J Clin Pediatr Dent. 2011 Winter;36(2):185-8. doi: 10.17796/jcpd.36.2.f1m4283501374t22. PMID: 22524082.





Longitudina

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



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 nextgeneration 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.