23447 | Biogeography of Oral Biofilm with NAM-FISH

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Background & Aim: Periodontal biofilms are microbial communities in balance with the host. When this balance is disrupted, disease occurs. To better understand disease establishment, it is necessary to study the spatial distribution and interactions of bacteria within the biofilm. *Streptococcus sp.* are the main colonizers of periodontal biofilms. To visualize biofilms, Locked Nucleic Acid (LNA) and 2'-O-Methyl-RNA (2'OMe) probes with confocal laser scanning microscopy (CLSM) enable high-resolution imaging while preserving spatial structure, offering deeper insights. **Methods:** Subgingival and supragingival plaque samples were carefully collected to minimize disruption of the micron structure, fixed in 4% paraformaldehyde, stored in 50% ethanol at -20°C. For FISH, samples were sectioned, hybridized with 20µL of 200nM Streptococcus-specific NAM probe (previously developed by our group) at 60–64ºC and visualized by fluorescence microscopy and CLSM. Sample analysis was complemented by DAPI staining. **Results:** This study showed that the Streptococcus-specific NAM probe was able to identify Streptococcus in all samples collected, consistent with previous research. Our results showed *Streptococcus* and polymicrobial aggregates, forming well-defined structures unveiling intricate bacterial organization. This suggests that the micron structure of the biofilm was preserved during biofilm collection and sample processing, making this method suitable for studying oral biofilm organization and behavior. **Conclusions:** The NAM-FISH technique applied to bacterial plaque samples, is a valuable tool for analyzing of Streptococcus distribution patterns within supra and subgingival biofilms. This approach allowed the precise identification of microorganisms and provided deeper insights into the structural organization and complex interactions within this diverse microbial ecosystem.

Keywords: Oral biofilm, dental plaque, biogeography oral microbiome, fluorescence in situ hybridization, nucleic acid mimics.

References:

[1] MARK WELCH, J. L. et al. Biogeography of a human oral microbiome at the micron scale. Proceedings of the National Academy of Sciences of the United States of America, v. 113, n. 6, p. E791–E800, 2016Guimaraes, N.M., N.F. Azevedo, and C. Almeida, FISH Variants. Methods Mol Biol, 2021.2246: p. 17-33.

[2] AZEVEDO, N. F.; ALMEIDA, C. Fluorescence In-Situ Hybridization (FISH) for Microbial Cells. [s.l.] Humana, 2021.

[3] Barbosa, A., et al., Imaging biofilms using fluorescence in situ hybridization: seeing is believing. Frontiers in Cellular and Infection Microbiology, 2023. 13.

[4] Berger D, Rakhamimova A, Pollack A, Loewy Z. Oral Biofilms: Development, Control, and Analysis. High Throughput. 2018 Aug 31;7(3):24. doi: 10.3390/ht7030024. PMID: 30200379; PMCID: PMC6163956.

[5] Azevedo AS, Fernandes RM, Faria AR, Silvestre OF, Nieder JB, Lou C, et al. Spectral imaging and nucleic acid mimics fluorescence in situ hybridization (SI-NAM-FISH) for multiplex detection of clinical pathogens. Front Microbiol. 2022;13:976639.