**ABSTRACT**

Objectives: Photodynamic therapy (PDT) was first used to eliminate microorganisms over 100 years ago, and is known to require an ample oxygen supply. Infective biofilms grown in periodontal pockets are oxygen-starved and difficult to characterize. To evaluate the potential “in-pocket” efficacy of this re-emerging therapy for periodontal infection control, a novel methodological procedure of small-space biofilm growth and characterization must be developed.

Methods: We are now testing the utility of Multiple Attenuated Internal Reflection – Infrared Spectroscopy, using germanium miniature prisms and commercially pure titanium test pieces, anaerobically incubated for 24 hours with Fusobacterium nucleatum to allow for biofilm formation.

Results: Specimens dried and mounted upon the appropriate apparatus confirmed biofilm growth by using MAIR-IR spectroscopy and collecting the spectral data output. Biofilm growth is corroborated using SEM analysis and a colony-forming unit (CFU) count technique termed: “drop spot counting.”

Conclusion: The current data suggest that this method has the potential, in combination with other methodologies, to serve as a reference point for PDT efficacy studies.

**INTRODUCTION**

- Common oral pathogen grown on miniature germanium prisms to characterize growth via Multiple Attenuated Internal Reflection Infrared Spectroscopy (MAIR-IR)
- Specimens dried and either subjected to a photosensitizer(PS) + PDT, or no treatment
- CFU counts conducted to ascertain treatment effectiveness
- SEM images collected to inspect surface for growth

**MATERIALS and METHODS**

- Fusobacterium nucleatum (ATCC 25586) maintained at 37degC in tryptic soy broth (DIFCO) containing:
  - 0.1% L-cysteine + 0.5% yeast extract
  - anaerobic condition (10%CO2; 10%H2; 80%N2)
- Number of bacteria adjusted to OD 0.3-0.4 at 600nm after bacterial growth reached the mid-log phase
- 2ml of bacterial solution applied to 6-well cell culture plates (Costar) with specimens, wrapped with cellophane
- Dried 1 week PDT Tx
- Dried 2 days No PDT Tx
- Colonies of viable bacteria

**RESULTS**

- SEM of Ge-mini

**CONCLUSIONS**

- Between 2 days and 7 days, there is considerable biofilm maturation shown by a substantial increase of “slime layer” (polysaccharides, ~1080cm⁻¹) and the prevalence of multiple hydroxide and hydrocarbon bands
- Although PDT was killing, as suggested by CFU counts, there was not much removal of the biofilm
- Literature supports PDT as an effective antimicrobial therapy in the oral cavity.
- This effectiveness is limited by the very nature of biofilms and their inherent antimicrobial resistance in the form of an exopolymer exudate.
- Despite PDT treatment, this biofilm will likely reestablish itself and continue to thrive

**ACKNOWLEDGMENTS**

Dr. Anne Meyer and the Department of Oral Diagnostic Sciences for complete usage of their laboratories, lab materials and equipment, in addition to unwavering guidance and support. All other supporting authors for their work with the PDT equipment, SEM, CFU methodology, and microbiological contributions.

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