Examination of periodontal pocket biofilm growth and effects of PDT for perio-infection control

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Abstract #

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."

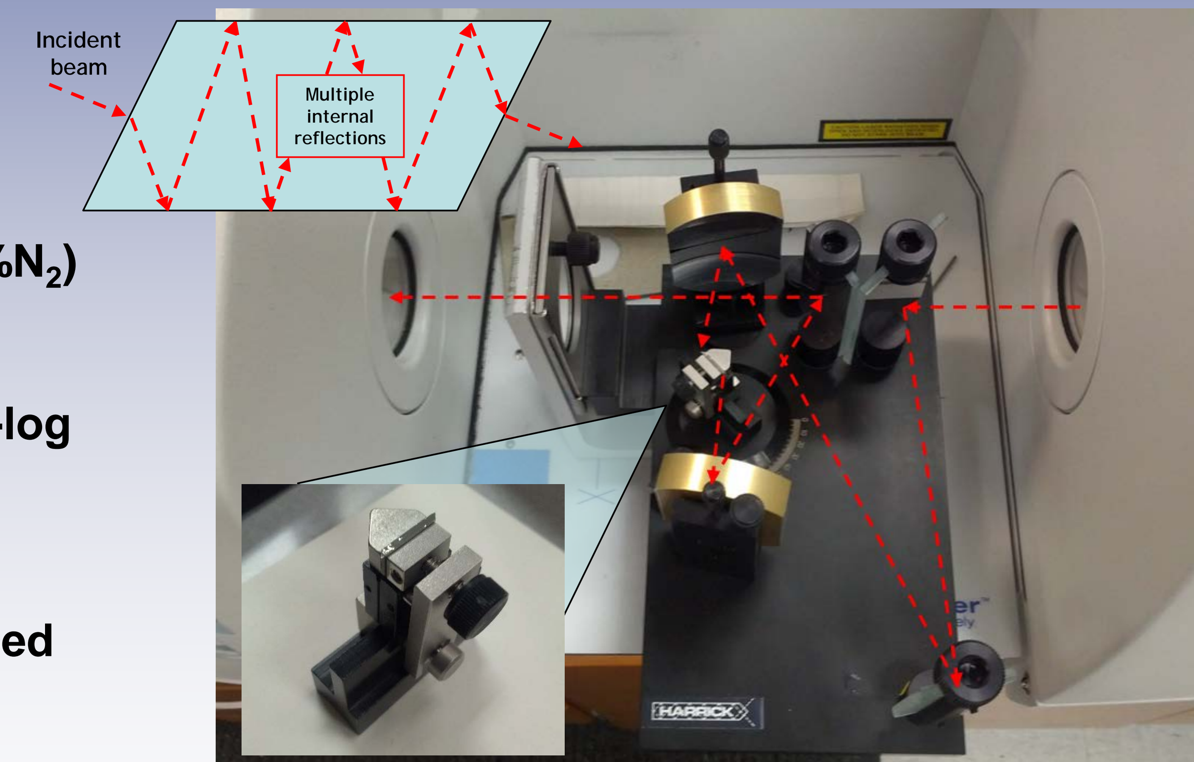
Conclusion: The current data suggest that this method has the potential, in combination with other methodologies, to serve as a reference point for PDT efficacy studies.

INTRODUCTION

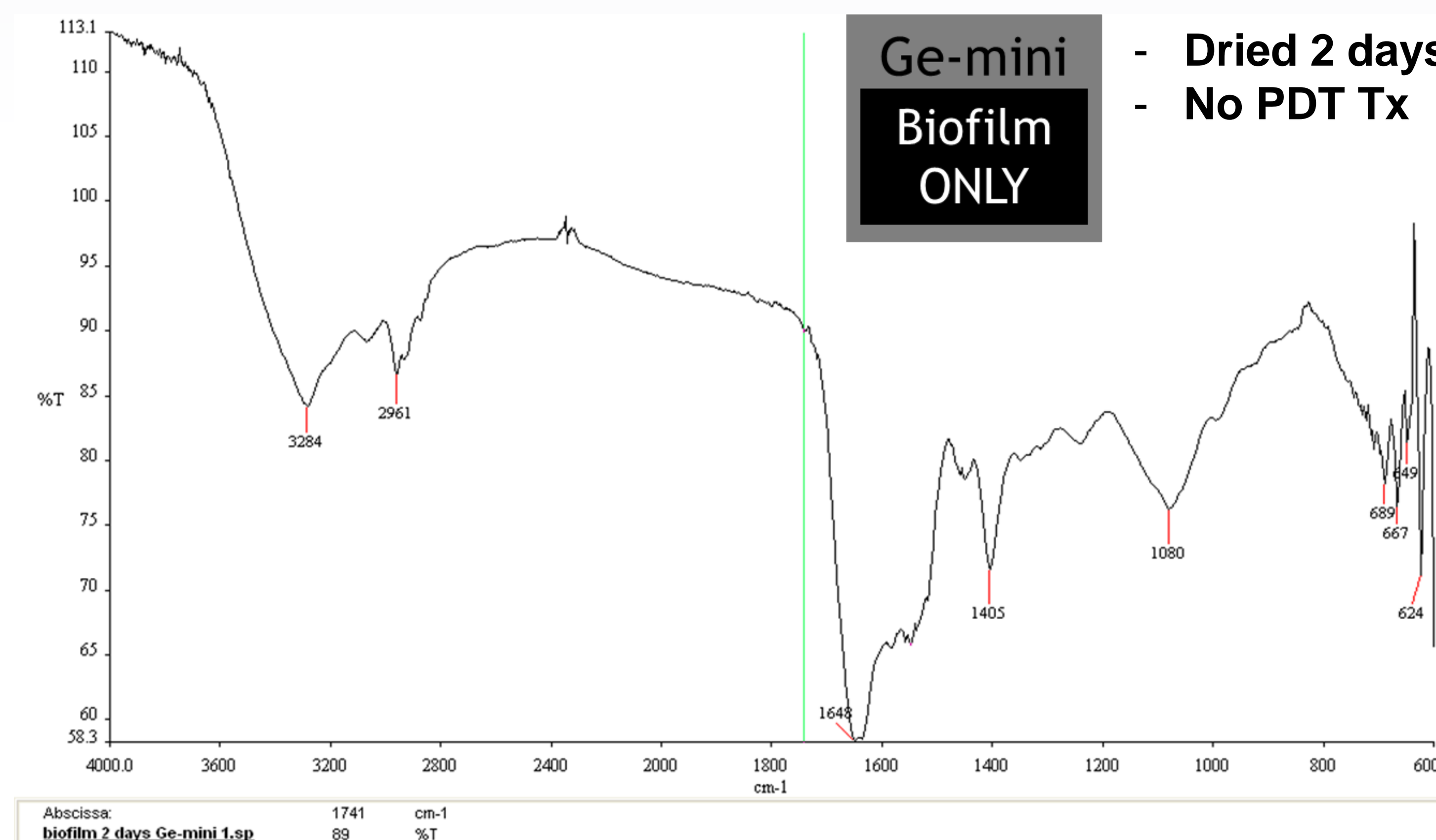
- Common oral pathogen grown on miniature germanium prisms to characterize growth via Multiple Attenuated Internal Reflection Infrared Spectroscopy (MAIR-IR)
- Specimens dried and either subjected to a photosensitizer(PS) + PDT, or no treatment
- CFU counts[b] conducted to ascertain treatment effectiveness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

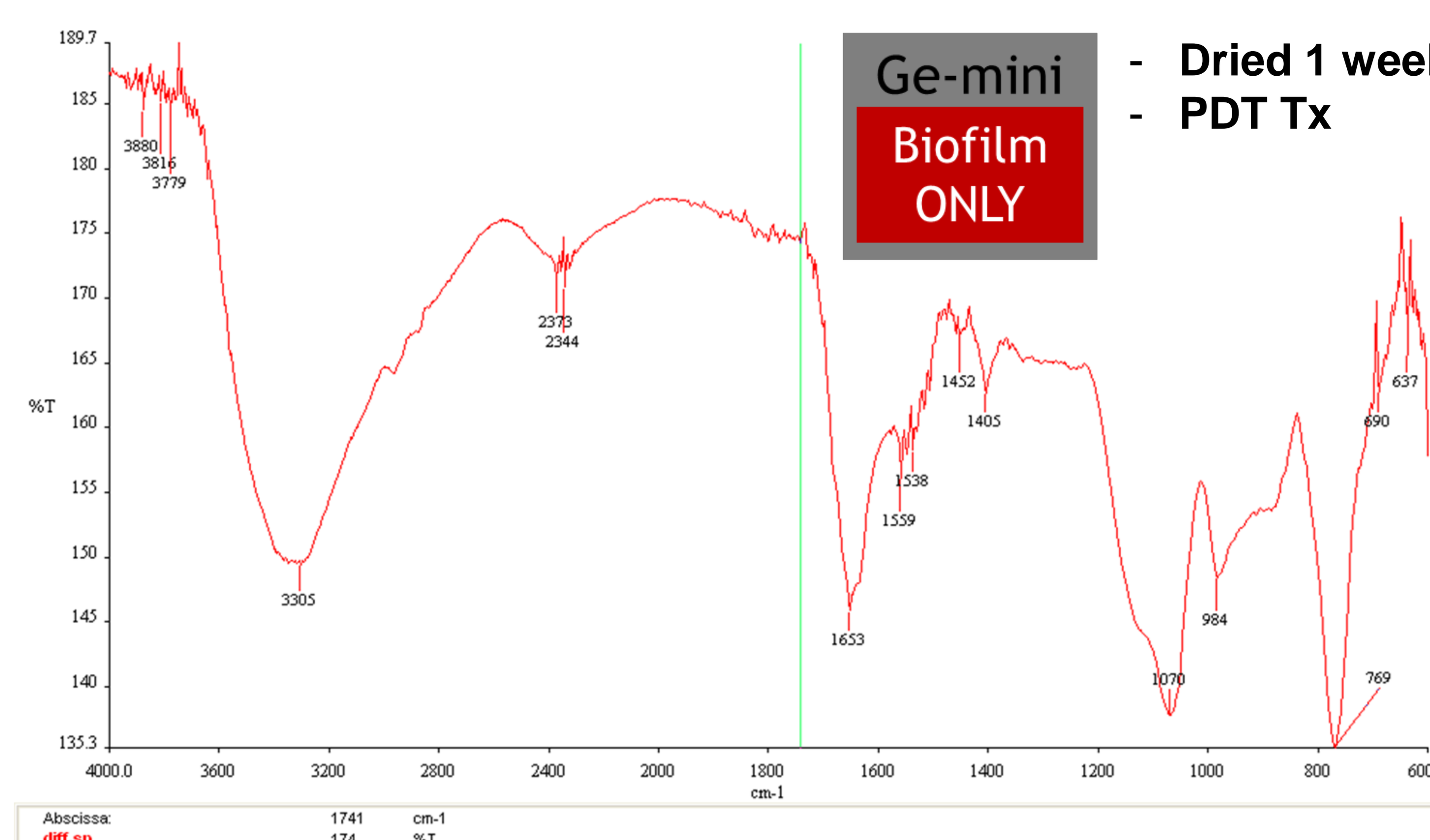
MATERIALS and METHODS

- Fusobacterium nucleatum (ATCC 25586) maintained at 37degC in tryptic soy broth (DIFCO) containing:
  - 0.1% L-cysteine + 0.5% yeast extract
  - anaerobic condition (10%CO_2; 10%H_2; 80%N_2)
- 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

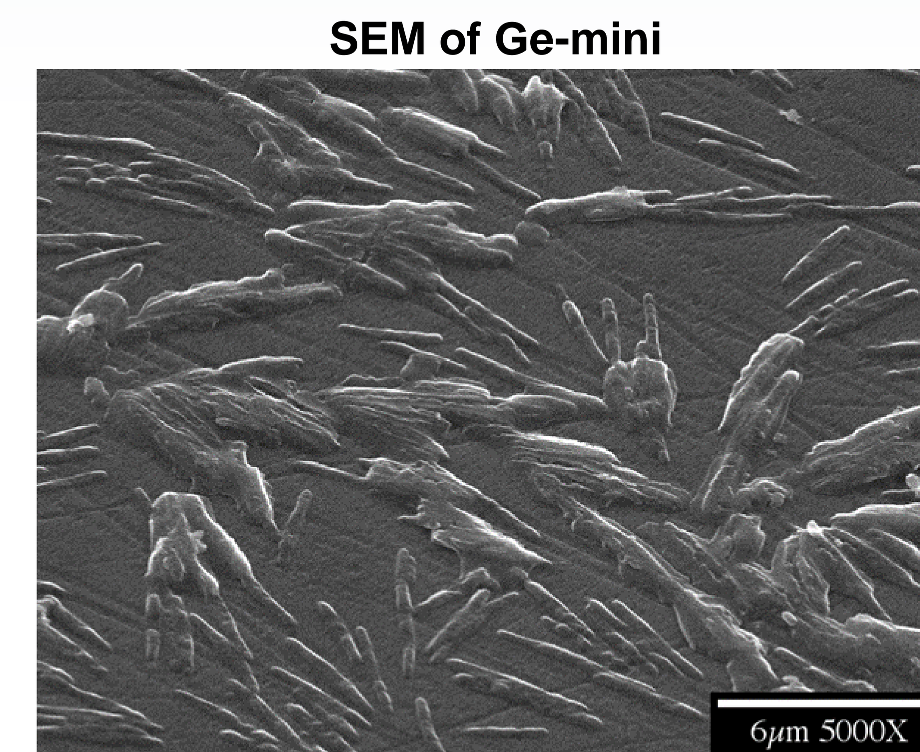


RESULTS





SEM of Ge-mini



Colony Forming Units (viable bacteria)

	specimen	Laser time (sec)	CFU*
1	Ti	untreated	208,000/ml
2	Ge	untreated	192,000/ml
3	Ge	167	90,000/ml
4	Ge	167	10,000/ml
5	Ge	167	330,000/ml
6	Ti	167	100/ml
7	Ti	333	1,670/ml
8	Ti	333	67/ml
9	Ti	667	0
10	Ti	667	0

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[b], there was not much removal of the biofilm
- Literature supports PDT as an effective antimicrobial therapy in the oral cavity[a,d]
- This effectiveness is limited by the very nature of biofilms and their inherent antimicrobial resistance in the form of an exopolymer exudate[c]
- Despite PDT treatment, this biofilm will likely reestablish itself and continue to thrive

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