Influence of biofilm matrix components on resistance to photodynamic periodontal disinfection

Rogers, S.C.; *Mang, T.S.; **Andreana, S.; Baier, R.E.

School of Dental Medicine
University at Buffalo The State University of New York

Biomaterials Graduate Program; *Dept. Oral and Maxillofacial Surgery; **Dept. Restorative Dentistry

ABSTRACT

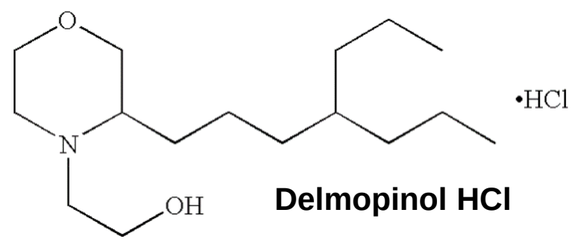
Numerous prior studies have shown that infective microorganisms in biofilm ensembles can significantly resist chemical and antibiotic disinfection by limiting reagent penetration through the biofilms' slime matrixes as well as by microbial phenotypic gene expressions, slow growth in the biofilm state, or microbe-microbe interactions. Recently published literature[1] points to oral bacteria that feature apparent resistance to chlorhexidine, a common bactericidal mouthwash, by means of their protection within dental plaque. The possible protective influence of the biofilm matrix on disinfection effectiveness by PDT[2], Photodynamic Therapy, as clinically practiced for periodontal pocket disinfection, is not yet known. This research employs a non-toxic, biofilm matrix (plaque)-disrupting reagent, delmopinol, before PDT of selected microbes grown in a simulated shear environment followed by assessment of biofilm matrix changes using Multiple Attenuated Internal Reflection Infrared (MAIR-IR) Spectroscopy and remaining bacterial viability by an alamarBlue fluorescence assay. Untreated biofilms and biofilms treated with chlorhexidine, both with and without delmopinol pre-treatment, were the Control specimens for comparison with the PDT experimental group.

RESULTS: Groups treated in combination with the 0.2%(v/v) delmopinol HCl had a statistically significant difference ($p<0.05$) in growth viability compared to both (1) no treatment, and (2) the principal treatment (PDT or CHX) alone. This outcome was confirmed by data collected from both the bacterial viability assay and by MAIR-IR spectroscopic ratio comparisons of selected regions that correspond to biofilm matrix components. Further microscopic evidence of delmopinol's effect on a biofilm's EPS matrix was provided by confocal-IR microscopy.

INTRODUCTION

The aims of this study are:

- **Discern the role of delmopinol HCl in its interaction with the biofilm matrix, and which/how biofilm components are affected**



Delmopinol HCl

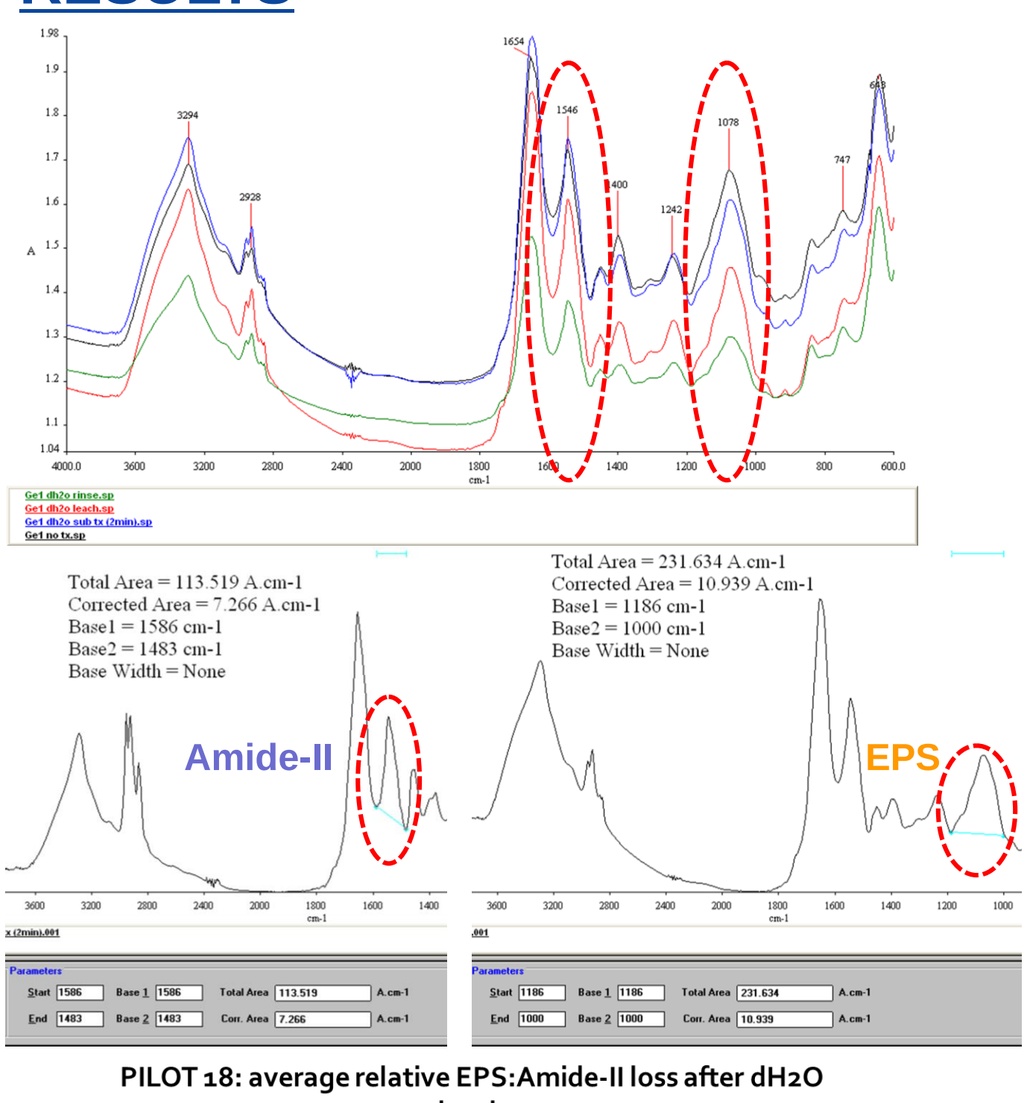
- **Determine if different treatment modalities are more efficacious after delmopinol HCl pre-treatment**

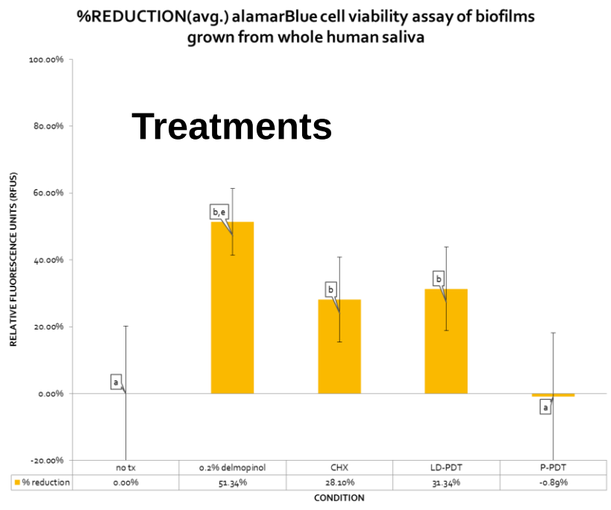
MATERIALS AND METHODS

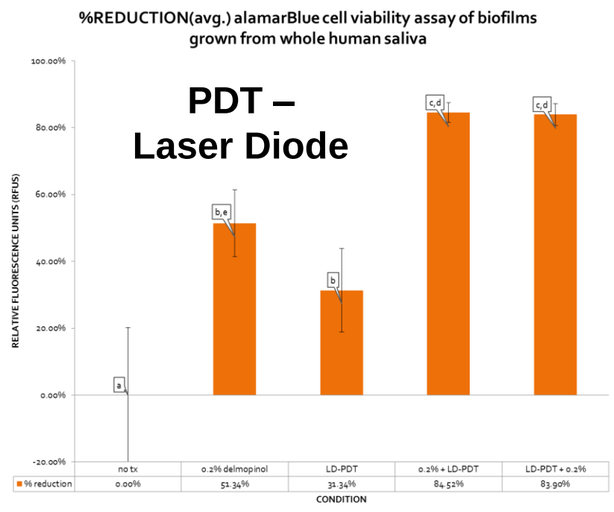
- **Biofilms grown in rotating-well apparatus on polystyrene and germanium**

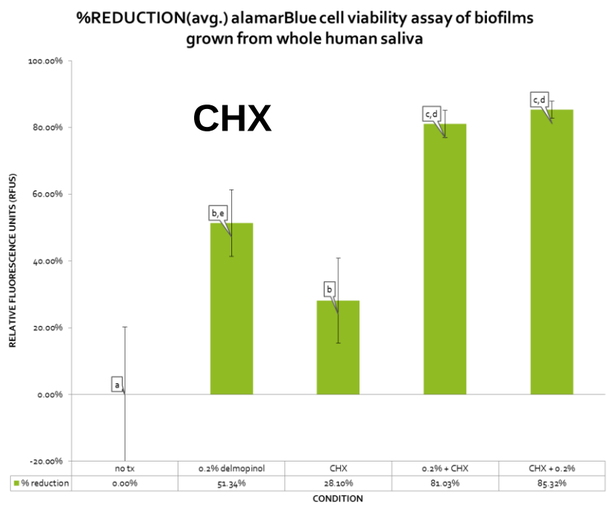
- **Inoculated with unstimulated, whole human saliva, HSIRB#: SIS0680310E**
- **Biofilms treated with a variety of treatments, delmopinol HCl, PDT (Periowave, laser diode), CHX**
- **alamarBlue® assay: bacterial viability**
- **MAIR-IR spectroscopy: covalent bonding chemistries**
- **Confocal-IR microscopy: biofilm visualization**
- **ANOVA+TukeyHSD *post-hoc***

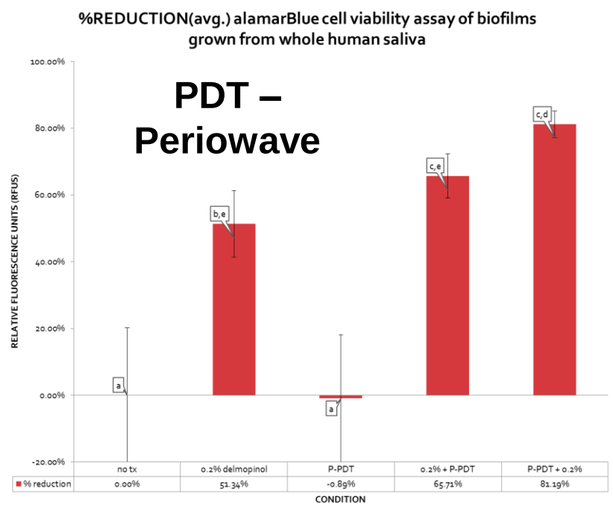
RESULTS

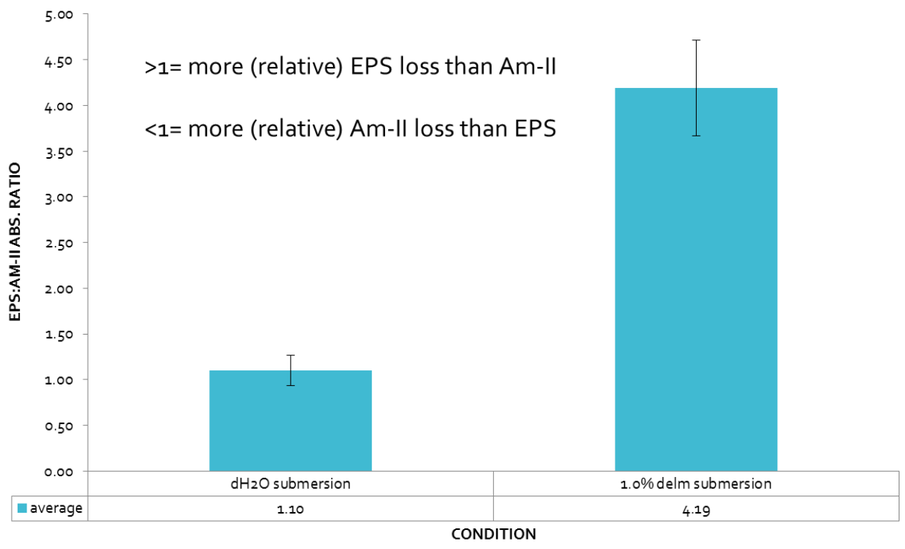




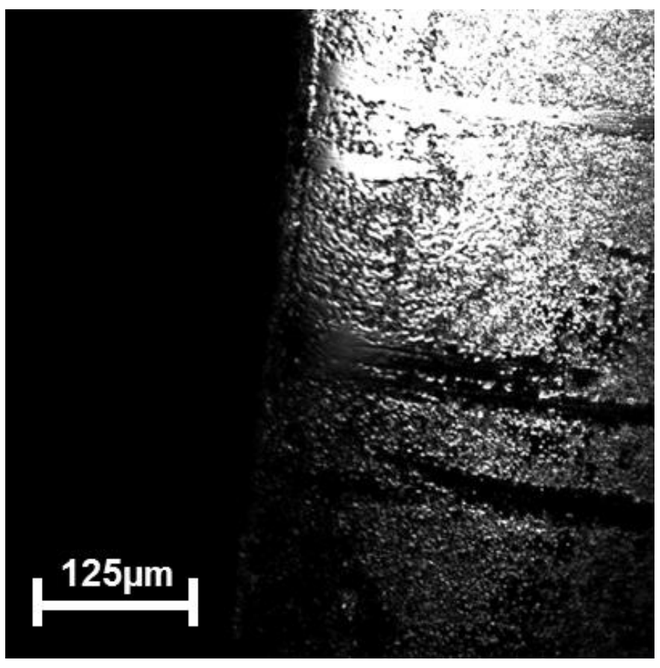




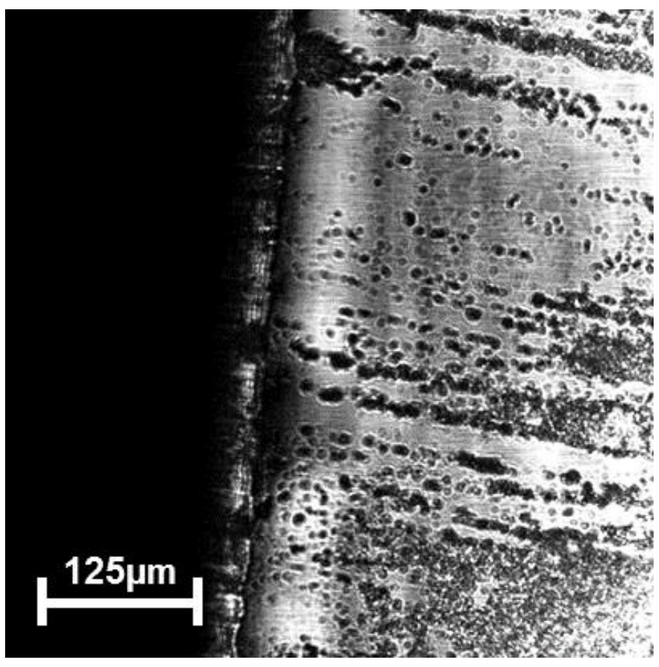




before delmopinol HCl 0.2% (v/v)



after delmopinol HCl 0.2% (v/v)



CONCLUSIONS

Evidence supports:

- **delmopinol HCl preferentially removes or coagulates the biofilm's protective slime matrix, after which the principal treatment has improved access to microorganisms.**
- **Pre-treatment of biofilms with delmopinol HCl improves the efficacy of the principal treatment.**

Tested:

- **PDT (MB-mediated)**
  - **Periowave system (clinical)**
  - **Laser Diode (research)**
- **chlorhexidine gluconate 0.12% (Periogard®)**

REFERENCES

[1]Scannapieco, F. A., J. Yu, K. Raghavendran, A. Vacanti, S. I. Owens, K. Wood, and J. M. Mylotte. "A Randomized Trial of Chlorhexidine Gluconate on Oral Bacterial Pathogens in Mechanically Ventilated Patients." Crit Care 13.4 (2009): R117. Print.

[2]Sgolastra, F., A. Petrucci, M. Severino, F. Graziani, R. Gatto, and A. Monaco. "Adjunctive Photodynamic Therapy to Non-Surgical Treatment of Chronic Periodontitis: A Systematic Review and Meta-Analysis." J Clin Periodontol 40.5 (2013): 514-26. Print.

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