INFLUENCE OF METHYLENE BLUE-MEDIATED PHOTODYNAMIC THERAPY ON THE RESISTANCE TO DETACHMENT OF STREPTOCOCCUS MUTANS BIOFILMS FROM TITANIUM SUBSTRATA

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ABSTRACT

Objectives: Testing the effect of Photodynamic Therapy (PDT) on bioadhesion strength to titanium substrata as a method of cleaning biomaterial surface by examining whether the detachment shear stress would be affected by prior PDT treatment independently from microbial viability

Methods: Biofilms of *Streptococcus mutans* (ATCC strain 27351) were grown with sucrose addition to Brain Heart Infusion media, initiating the biofilms with bacteria of three different ages (phases). One set of biofilm samples received no PDT (controls); another set received methylene-blue-mediated PDT. A water-jet impingement apparatus was used to determine the adhesive strength of biofilms to the titanium substrata. Scanning electron microscopy (SEM) was performed to obtain images of the samples before and after jet impingement. Replicate biofilms prepared on germanium prisms were characterized by MAIR-infrared spectroscopy.

Results: PDT'd microbial biofilms were significantly ($p<0.05$) and differentially delaminated and ultimately removed from their substrata biomaterials by the hydrodynamic forces of water-jet impingement. Control (no PDT) biofilms of varying thicknesses required 144-228 dynes/cm² shear stress to delaminate from titanium, while PDT'd biofilms were removed at 90-140 dynes/cm², depending on water flow rate. However, separation of microbial layers from the exopolymer matrix required only 57-68 dynes/cm² shear stress (controls) and 39-51 dynes/cm² (PDT'd biofilms), again depending on water flow rate. The thicker areas of biofilms had greater susceptibility to detachment by water–jet impingement. MAIR-IR spectra of replicate biofilms and SEM images of control and PDT'd biofilms confirmed these findings. Colony-forming-unit (CFU) counts routinely correlated well with results from a spectrophotometric Alamar Blue (AB) assay, except at long incubation times when the AB reagents showed some autoreduction-induced color changes.

Conclusion: These results are consistent with proposals that methylene-blue-mediated PDT induces oxidative embrittlement and fragmentation of biofilm matrix biopolymers, allowing easier release by hydrodynamic (rinsing) forces.

INTRODUCTION

In dental settings, as well as in other natural systems, plaque-forming microorganisms develop biofilms in which the microbes become protected via their own phenotypic changes and their polymeric exudates from disinfection by washes and antibiotics. Photodynamic Therapy (PDT) is variably effective against these microorganisms, depending on such factors as whether the bacteria are Gram positive or Gram negative, plaque age and thickness, and internal biofilm oxygen concentration.

This investigation applied a novel combination of PDT and water-jet impingement techniques to Streptococcus mutans (ATCC strain 27351)-formed biofilms on commercially pure titanium (cpTi) starting with three different phases (ages) of the bacteria, to examine whether the detachment shear stress –as a signature for the work required for removal of the biofilms- would be affected by prior PDT treatment independently from microbial viability.

MATERIALS and METHODS

Biofilms were grown with sucrose addition to Brain Heart Infusion media on Germanium prisms and cp Ti, producing visible thick films and nearly invisible thin films (within the same piece) having the same numbers of culturable microorganisms, the thicker films having greater susceptibility to detachment by water–jet impingement (Figure 1). Colony-forming-unit (CFU) counts routinely correlated well with results from a spectrophotometric Alamar Blue (AB) assay (Figure 2), except at long incubation times when the AB reagents showed some autoreduction-induced color changes. Use of Methylene Blue (MB) as a photosensitizer (PS) for PDT of biofilms (delivered by Periowave laser system Figure 3) did not interfere with the AB assay but did mask AB reduction spectral changes when employed with planktonic organisms.

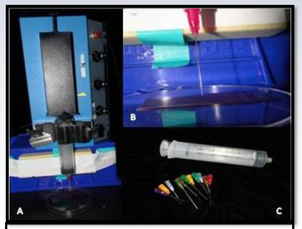


Fig.1: Component of jet impingement; A: complete set, B: the sample laying flat with pre determined distance from the nozzle opening, C: delivering needles with a variety of diameters and lengths and syringes

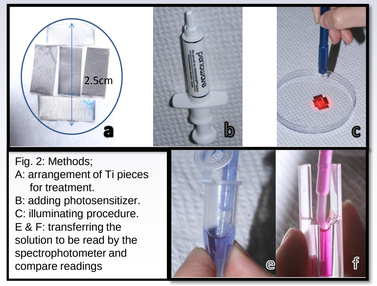


Fig. 2: Methods;
A: arrangement of Ti pieces for treatment.
B: adding photosensitizer.
C: illuminating procedure.
E & F: transferring the solution to be read by the spectrophotometer and compare readings

Fig. 3: Periowave System

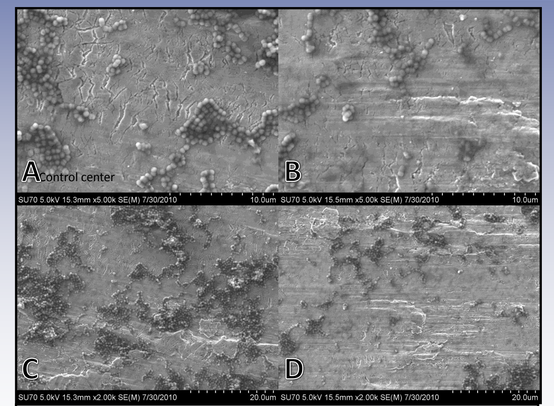
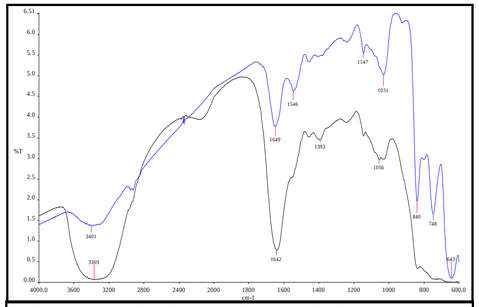


Fig. 5: A and C are SEM image of the central area of the detached circle in a control biofilm with different magnifications. B and D are the center of detached circle in PDT (MB) treated biofilm. Note the difference in bacterial count. Both biofilms were grown under the same conditions by receiving 24 hrs bacteria at the initial adhesion.

RESULTS

In the preliminary testing on a biofilm grown on germanium prisms having surface properties similar to those of cp Ti , Multiple Attenuated Internal Reflection InfraRed spectra results showed differences in film-removal susceptibility for shear stresses as low as 10 dynes/cm², and illustrated the PDT-induced preferential removal of predominantly the polysaccharide biofilm components (Graph 1). After testing on cp Ti, it was discovered in this work that PD-treated microbial biofilms, independently from starting or PS-influenced microorganism viability, were significantly ($p<0.05$) and differentially delaminated and ultimately removed from their substrata biomaterials by the hydrodynamic forces of water-jet impingement (Figure 4). Control biofilms of varying thickness, not receiving PDT treatment, required between 144 and 228 dynes/cm² of shear stress to delaminate from titanium while PDT-treated companion biofilms were removed at 90 to 140 dynes/cm², depending on water flow rate. In comparison, it required only between 57 and 68 dynes/cm² shear stress to separate microbial layers from within the exopolymer matrix of control biofilms, and between 39 and 51 dynes/cm² to delaminate PDT-treated matrix sections of varying thickness biofilms, again depending on water flow rate (Table 1). Scanning Electron Microscopy of Control and PDT-treated biofilms before and after water-jet impingement confirmed these findings (Figure 5).



Graph 1: Treated biofilm: black (lower spectrum) is the treated biofilm; blue (upper spectrum) is the same sample after water impinging. This biofilm was initiated by 48 hrs bacteria.

Fig. 4: Even when central circles and halos are almost equal in size in control (right) and treated biofilms (left), the water jet penetrates deeper through the treated biofilm.

Table 1: Shear stress values for biofilm grown from younger bacteria:

		txhal20	txcen20	con.cen.20	con.hal.20	tx.cen.10	con.cen.10	tx.hal.10	con.hal.10
N	Valid	23	26	26	22	17	12	21	13
	Mean dyne/cm²	51.0	89.4	134.3	67.2	139.4	226.3	38.9	56.8
	Std. Deviation	1.5	13.9	27.5	13.7	70.4	26.5	21.6	17.7

CONCLUSIONS

These results are consistent with proposals that PDT induces oxidative embrittlement and fragmentation of plaque/biofilm matrix biopolymers, allowing easier release by hydrodynamic (rinsing) forces.

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