Periowave™ Eradicates *P. gingivalis* from Dental Implants Without Altering Surface Chemistry

Program #942

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Introduction

Photodynamic Disinfection (PDD) has a proven record of antimicrobial activity against a wide variety of pathogens in both planktonic and biofilm *in vitro* models. This process uses light energy of an appropriate wavelength to activate a photosensitive molecule. Upon being raised to a short-lived excited energy state, the photosensitive molecule can act in one of two ways. In a type I reaction, the photosensitizer passes its energy directly to the substrate/target. By contrast, a type II reaction involves the excited state photosensitizer transferring energy to molecular oxygen, resulting in the production of damaging reactive oxygen species. These reactions are responsible for the bactericidal action of PDD via lipid peroxidation and irreversible membrane damage to the microbial cell. This highly potent, localized kill process represents a potential alternative to antibiotic treatments for common bacterial infections, including bacterially mediated conditions in the oral cavity.

Periowave™ is a PDD system currently marketed in Canada and the European Union for the treatment of adult chronic periodontitis. Employed adjunctively with scaling and root planing (SRP), this system has been shown to significantly positively affect clinical outcomes. Given that peri-implantitis is a condition that is also associated with pathogenic sub-gingival colonization/infection, Periowave™ may also prove to be effective in this application. Dental implants are manufactured with complex surface chemistries and architectures in order to facilitate biocompatibility and osseo-integration. Thus, it is important that an antimicrobial treatment for peri-implantitis be effective at killing bacteria present on and around the implant without altering the surface chemistry of the implant itself. This study evaluated the effects of the Periowave™ PDD system on the surface chemistry of three commonly used dental implant screws. In addition, we assessed the efficacy of the system for eradication of *Porphyromonas gingivalis* biofilms grown on the surface of dental implant screws.

Methods & Materials

Titanium dental implant screws from three major manufacturers (designated as A, B, and C) were exposed to activated and non-activated Periowave™. These implants were subsequently examined using Electron Spectroscopy for Surface Analysis (ESCA) and high resolution carbon scan to determine whether the treatment significantly altered surface chemistry. Values were expressed in mean atomic percentage of replicate readings with standard deviation. One way ANOVA was used to determine statistical significance of any differences between experimental and control conditions. Next, homogenous biofilms of *P. gingivalis* were grown anaerobically for 72 hours on dental implant screws (n=12) suspended vertically into 12-well tissue culture plates containing $7.5X10^6$ CFU/ml inoculum. After a 30 second rinse to remove free-floating organisms, treatment condition implants were placed in Periowave™ solution for 30 seconds, followed by circumferential illumination for 60 seconds using three Periowave™ laser handpieces and light guide tips (220mW, 670nm) placed at 120 degree angles around the implant screw (**Figure 1**). The exposure/illumination procedure was repeated for a total of two 60 second PDD treatments per implant. Surviving biofilm organisms were recovered from implant surfaces by vortexing and sonicating in PBS/0.5% Tween-80, and subsequently plated onto Brucella Blood Agar supplemented with Hemin and Vitamin K. Colonies were counted after 7 days under anaerobic growth conditions. Controls consisted of no treatment, light-only, and photosensitizer-only conditions.

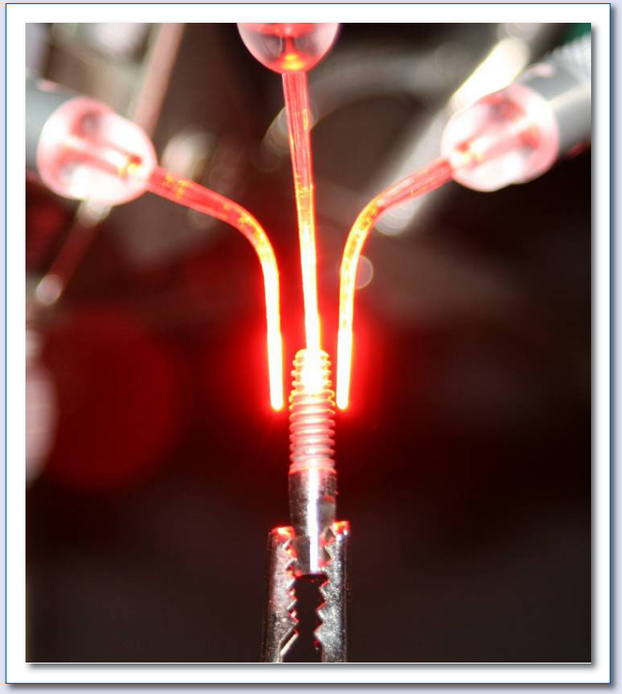

Figure 1: PDD test apparatus used for illumination of dental implants.

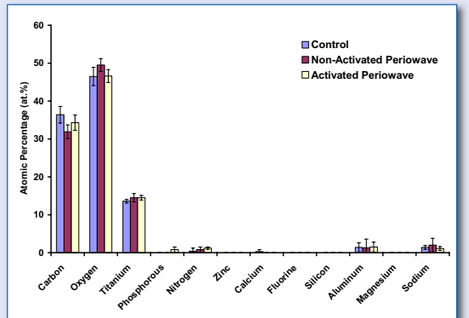

Figure 2: ESCA results for surface chemistry of implant sample A exposed to non-activated and activated Periowave™

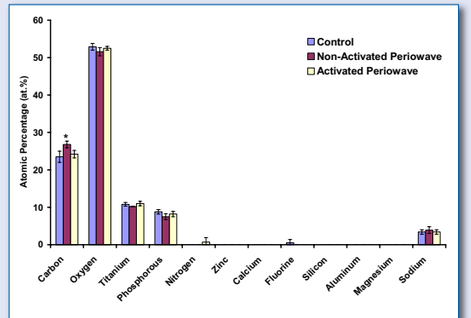

Figure 3: ESCA results for surface chemistry of implant sample B exposed to non-activated and activated Periowave™. Asterisk indicates statistically significant differences ($p<0.05$).

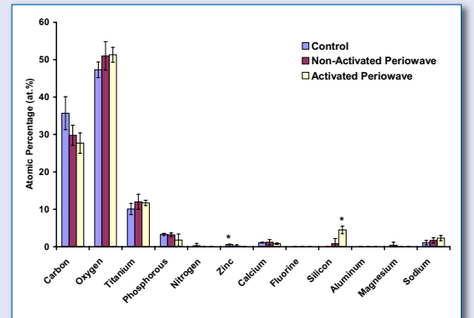

Figure 4: ESCA results for surface chemistry of implant sample C exposed to non-activated and activated Periowave™. Asterisk indicates statistically significant differences ($p<0.05$).

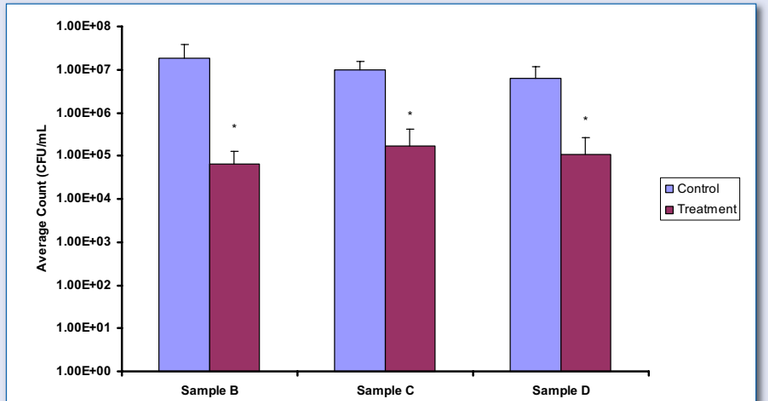

Figure 5: Eradication of *P. gingivalis* biofilms on dental implants using the Periowave™ system. Values are expressed in colony forming units per milliliter (CFU/mL) as an average count with standard deviation. Asterisks indicate statistically significant reduction from corresponding control.

Results

ESCA and carbon scan of implant sample A showed no significant difference in surface elemental composition between experimental and control conditions (**Figure 2**). Implants B and C also showed no significant changes in surface chemistry, with the exception of slightly higher carbon content in the non-activated Periowave condition in Implant Sample B and a slightly higher proportion of silicon in the activated Periowave condition in Implant Sample C (**Figures 3** and **4**).

Treatment with the Periowave™ system significantly reduced *P. gingivalis* biofilm viability on the implants tested (**Figure 5**). Implant samples B and C, both with titanium oxide surface chemistry, showed a >2log$_{10}$ (>99%) reduction of viable organisms from control after Periowave treatment. Implant sample D, with hydroxyapatite surface coating, also showed a >2log10 (>99%) reduction from control. Implant sample A was not tested in this model because a sufficient number of screws for performing all control/treatment conditions were not available. No significant reductions from control were observed in photosensitizer-only and light-only conditions for any of the implant samples (data not shown). Recoveries from untreated control surfaces showed that the brief exposure to aerobic conditions during testing did not contribute to reduced biofilm viability.

Conclusions

This study showed that exposure to Periowave™ did not alter the surface chemistry of three commonly used dental implants. Furthermore, it was demonstrated that established biofilms of *P. gingivalis*, a major pathogen associated with periodontal disease and peri-implantitis, can be effectively eradicated from the surface of dental implant screws using the Periowave™ PDD system.

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