The Efficacy of Adjunctive Antimicrobial Photodynamic Therapy for Residual Pockets in Previously Surgically Treated Teeth: A Randomized Clinical Trial

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Abstract

Purpose. The aim of this study was to assess the clinical effects of antimicrobial photodynamic therapy (aPDT) delivered as an adjunct to maintenance therapy, specifically in patients with residual pockets at previously surgically treated teeth and compare it with patients receiving mechanical debridement only.

Methods and Materials. Twenty-three patients on a regular maintenance schedule who had previously received periodontal surgical care were randomly assigned to receive aPDT or a placebo at their first debridement appointment. The teeth selected had to have at least one site with bleeding on probing and a probing depth of ≥5mm. One week later, patients received the same treatment according to their group allocation. These teeth were simultaneously reassessed at their three- and six-month maintenance appointments. The primary outcome measure was bleeding on probing (BOP) and secondary outcome measures were plaque index (PI), probing depth (PD), and clinical attachment level (CAL).

Results. There were statistically significant (SS) improvements for both groups in mean BOP, PD, and CAL. Individual sites in both groups showed SS improvements in BOP, PD, and CAL as well. When comparing the two groups directly, there was a SS increased mean PD reduction and CAL gain in the adjunctive aPDT treatment over debridement only at 6 months. There were no significant differences between the groups for BOP and PI comparisons.

Conclusion. Adjunctive aPDT significantly improves PD and CAL at previously surgically treated teeth in maintenance care, when compared to mechanical debridement alone.

Acknowledgments

First and foremost, I would like to extend my greatest appreciation to my research advisory committee, Dr. Anastasia Cholakis, Dr. Reem Atout, and Dr. Veronique Benhamou for assisting me through the program and this research over the last three years. I will be forever grateful for all of their insight, expertise, and advice that can only come from the experience acquired within their careers as talented clinicians and instructors of periodontics. I am extremely proud to have collaborated with such accomplished professionals and thankful for their approachability throughout the process.

Dr. Dan Price, who joined our clinic in my final year as the Program Director, was a wealth of knowledge and real-world clinical experience which indirectly helped this project but directly made me a better clinician. I can honestly say that his approach to teaching and life has made a valuable impression on me.

I am indebted to Loring Chuchmach, the lone statistical services consultant, who showed me nothing but patience and kindness from the beginning of our project. He not only expertly helped me navigate the final data set but did it in a time of isolation where his communication skills shone through, despite the obvious obstacles.

Lastly, this would not be possible without the wonderful staff at the Graduate Periodontics Clinic. I want to thank Matilde Kostiw, Christy Janis, Shelley McGavock, Celia Duarte-Trozzo, Danielle Gagliardi, and especially Michelle Dick for all of their time and contributions to this trial. Their commitment to the residents' education is obvious to all of us. Each day was made a little better and a lot easier in your company.

Thank you all.

Dedication

To Ana Elvira Sanchez Muñoz. *Mi Abuela.*

A woman who devoted her entire life to her children and her grandchildren.

Despite hardships of her own, she always made sure everyone was well taken care of and everyone knew that they were loved. She was a completely selfless person whose genuine happiness stemmed from her family and her faith. She would often join my siblings and I around the kitchen table whenever we were visiting and joking, even with her limited English, just to be able to laugh together.

She also knew how to motivate. Early on, she instilled the importance of education in us and reiterated its significance throughout our lives. Her support and belief in me were some of the main reasons I was inspired to return to graduate studies. My acceptance into the program made her incredibly proud and I can only imagine how massive her smile would've been when I complete my Masters. I miss her the most in moments like these.

Te amo Abue.

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Background

Periodontitis is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus.[1] Its primary feature is loss of periodontal tissue support due to inflammation, which can be treated by thorough subgingival debridement of the affected sites.[2-8] However complete elimination of subgingival deposits using closed procedures is difficult and inflammation may persist.[9] The percent of calculus-free surfaces decreases as initial probing depths increase, particularly in nonsurgical therapy.[10, 11] Therefore, clinicians may choose to repeat the treatment in the context of a surgical intervention to reflect the soft tissues for better access, especially in deep initial probing depths (>6mm).[3, 12-17] This decision should be aided by a variety of factors such as residual calculus in complex root anatomy,[13, 18-20] furcation involvement,[21-23] and critical probing depth.[24] Surgical treatment provides better short and long-term pocket depth reductions, clinical attachment gain, and tooth retention in patients with advanced periodontal disease.[17, 25] However, a limited number of residual pockets may still persist due to various reasons[26] which may be patient-, defect-, or therapist-related.[27] Factors can include plaque control,[28, 29] maintenance recall,[30] smoking,[31, 42] number of residual defect walls and overall defect depth,[32, 33] or the experience of a clinician.[34]

Residual pockets carry the risk of continuous presence of periodontal pathogens[35] and may be repopulated leading to periodontal disease.[36-39] Despite meticulous scaling, root planing, and personal hygiene, bacterially invaded dentinal tubules and lacunae can act as reservoirs from which recolonization of treated root surfaces occurs.[40] No eradication of bacteria is to be expected in deep residual pockets.[41] Residual pocket probing depths were found to be a risk

factor for disease progression, and when combined with bleeding on probing, were a risk for tooth loss.[34, 42] As evidence corroborates that a residual site is associated with tooth loss in the long term, the aim of periodontal treatment should be closure or elimination of sites with ≥5mm probing depths.[27] Ideally, maintenance therapy for residual pockets needs to be efficient but also harmless after repeated application. Repetitive instrumentation with metal instruments removes a substantial amount of tooth substance over time,[43-46] may cause gingival recession,[47, 48] or hypersensitivity of teeth to thermal and physical stimuli.[49]

Various alternative and adjunctive antimicrobial regimens have been tested to overcome this issue, such as pocket irrigation, local and systemic antibiotics. Subgingival irrigation is controversial because even though some synergistic effects have been shown, improvements were minimal.[50] Locally delivered antimicrobial adjuncts also only showed modest improvements such as 0.1 – 0.5mm of additional probing depth reduction.[51] In contrast, there is sufficient evidence to support adjunctive systemic antibiotics for significantly improved CAL gain, PD reduction, and decreased BOP[52-54] with a variety of prescriptions. However, the repeated use of antibiotics to treat residual pockets during maintenance care is not advisable as there is concern that their repeated administration may contribute to the development of antimicrobial resistance.[55] Patients and clinicians should also be well aware of other potential adverse effects of systemic antibiotic usage including gastrointestinal disturbances, allergic reactions, pseudomembranous colitis, and multiple drug interactions.[56] Thus, there is a need to evaluate new protocols that are safe and effective for maintenance, without any adverse effects on host tissues.

First introduced[57] and subsequently used in medical therapy, the concept of antimicrobial photodynamic therapy (aPDT) inactivates or destroys cells, microorganisms, or molecules. It has mainly been used for cancers as an alternative to chemotherapy or radiation but has other applications in dermatology, ophthalmology, gastroenterology, cardiology, neonatology, or for the elimination of viruses.[58] Treatment with a diode laser has a bactericidal, detoxifying effect and may therefore be applied as an adjunct to conventional mechanical instrumentation.[59] The combination of the diode laser, with a wavelength between 655 and 980 nm, and a photosensitizer, a nontoxic dye capable of absorbing light of a specific wavelength and transforming it into useful energy,[60] leads to the production of lethal cytotoxic agents that can selectively destroy cells.[2] Its mechanism is based on the illumination of the photosensitizer which is converted from the ground state to the triplet state, thus leading to the generation of a cytotoxic species, usually singlet oxygen, which interacts with the surrounding molecules and cells. As singlet oxygen cannot migrate further than 0.02 microns, it only has a local effect and does not damage distant cells or organs.[61] During the process, free oxygen radicals are produced, which react and cause damage to membranes, mitochondria, and DNA, resulting in the death of the microorganisms.[62-64] The concept gained traction and support with successful results from in-vitro studies[65-69] demonstrating a significant reduction in the numbers and viability of both aerobic and anaerobic bacteria as well as decreased inflammation. Additionally, animal in-vivo studies[70-72] also showed improvements with aPDT, providing further rationale for clinical use.

There is a growing body of evidence examining the clinical effectiveness of aPDT when used as an adjunct to conventional non-surgical treatment of periodontitis patients.[73] In the context of (the formerly recognized) aggressive periodontitis, significant intragroup improvements for

clinical attachment level (CAL), probing depth (PD), and bleeding on probing (BOP) were found after adjunctive aPDT.[74-76] Additionally, superior PD reduction, CAL gain, and BOP reduction compared to debridement alone were found in some studies.[77, 78]

For the treatment of chronic periodontitis, significant improvements with adjunctive aPDT over debridement alone were made with BOP,[79-81] CAL, and PD[79, 82, 83] with no adverse effects. Furthermore, superior microbiological reductions of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *Prevotella nigrescens*, and *T. forsythia* were reported compared to SRP alone.[84] Specifically, in residual sites after active periodontal therapy and during maintenance, significant clinical (CAL gain and reduction of PD and BOP) and microbiological benefits were also found compared to debridement alone.[85-87] Additional benefits of augmented anti-inflammatory IL-4 and reduced proinflammatory IL-1β and IL-6 levels were even found when aPDT was used as a monotherapy.[88] Furthermore, significantly reduced C-reactive protein levels have been found when the adjunctive aPDT was repeated a week later,[89] importantly implicating a decrease in overall systemic inflammation.

Periodontal health should be achieved in the least invasive and most cost-effective manner possible, particularly when considering that a patient should ideally commit to lifelong regular visits for maintenance of therapeutic outcomes.[90] Therefore, adjunctive aPDT becomes a particularly attractive option in patients that continue to have residual pockets despite surgical treatment. The importance of the mechanical removal of subgingival plaque[91] with the added benefit of reducing gram-negative anaerobic organisms[92] is a reasonable therapeutic step towards achieving periodontal health. The purpose of this study was to assess the clinical effects of aPDT

delivered as an adjunct to maintenance therapy with mechanical debridement in patients with residual pockets at previously surgically treated teeth. Since the absence of BOP has high predictive value indicating periodontal stability,[93, 94] this parameter became our primary outcome measure. The hypothesis was that this adjunctive treatment will produce decreased BOP, which will subsequently also result in reduced probing depth, increased attachment gain, and decreased plaque at these residual pocket sites.

Materials and Methods

Patient Selection

All selected participants were systemically healthy adults in a supportive periodontal therapy program and patients at the University of Manitoba Graduate Periodontics Clinic. They had completed active periodontal therapy, including surgical treatment, with at least one surgically treated site that had a residual pocket probing depth of ≥ 5mm with bleeding on probing. Exclusion criteria included uncontrolled diabetes mellitus, cancer, HIV, bone metabolic diseases or disorders that compromise wound healing, radiation or immunosuppressive therapy, pregnancy or lactation, administration of systemic antibiotics taken within the last three months, confirmed or suspected allergy / hypersensitivity to methylene blue, or restrictions that may preclude normal oral hygiene procedures. Once identified as meeting the inclusion criteria, patients at the clinic were asked to voluntarily participate in the study. Interested subjects were required to read and sign the informed consent approved, as part of the ethics submission, by the University of Manitoba Research Ethics Board prior to final acceptance into the trial.

Treatment Protocol

Generally, each participant had a full mouth periodontal charting completed prior to baseline measurements as part of their maintenance program. Once selected the patient saw two clinicians: the examiner (the clinic hygienist) and the operator (the periodontal resident). The examiner recorded the data (BOP, PI, PD, CAL) of the entire dentition, including the selected tooth, and provided initial hygiene treatment. Immediately afterwards, the operator delivered the test or control treatment which was repeated a week later. The treatment assignments were concealed from the patient and the examiner. The operator was unaware of the previously

recorded data except the pocket depth measurements and was not involved in the post-treatment evaluations (scheduled at 3, 6, and 12 months during the hygiene appointments). At 12 months, a full mouth re-evaluation was completed by the examiner as part of the patient's maintenance program.

Specifically, in the first visit, the examiner recorded the BOP, PI, PD, and CAL at all six sites in the entire dentition, including the involved teeth. Thorough SRP was performed under local anesthesia as needed using periodontal curettes (Gracey, Hu-Friedy, USA) and an ultrasonic device (Piezo, Ultradent, USA). Once completed, the operator took over. The patients were randomly assigned to test or control groups by a computer-generated table. The protocol was either: A, the laser is activated during treatments with methylene blue; or B, the laser is never activated during treatments with saline solution. Note that methylene blue possesses antibacterial activity without additional light exposure[95] and therefore would not have been an adequate control. Antimicrobial photodynamic therapy was carried out in the residual pockets using the Periowave system (Ondine Biomedical Inc, Vancouver, Canada). The photosensitizing agent was methylene blue. Approximately 0.2 mL of the solution was applied to each pocket with a blunt-ended side-port irrigator. The site was illuminated for 60 seconds to activate the agent using a disposable, light-diffusing tip that is introduced into the pocket attached to the diode laser (wavelength = 650 - 675 nm, 160 mW of output power). The control treatment consisted of the same procedure, except that the photosensitizer was replaced with saline solution and the light-diffusing tip was kept in the pocket for 60 seconds without activating the laser. Each patient was then sent home with the same oral hygiene instructions and home care package that included a toothbrush, toothpaste, and floss. The second session was scheduled after 1 week. The operator

applied the photosensitizer or solution and activated the laser according to protocol A or B. The examiner maintained the patients on a 3-month hygiene schedule and reassessed the participants at 3, 6, and 12 months after the treatment as well as reinforced the oral hygiene instructions at each visit. Medical history changes and all adverse events are recorded. Clinical parameters were measured the same way as at baseline at all time points. These measurements were calibrated prior to the start of the study.

Results

Overall, 23/24 patients, consisting of 14 men and 9 women, completed their 6-month re-evaluations. One patient had to be removed because they were not compliant with their maintenance appointment schedule. Three subjects were smokers, which was too few to perform any subgroup comparisons. Six patients completed 12-month re-evaluations, but the rest of the recall appointments had to be cancelled and the research team had to stop collecting data due to the COVID-19 pandemic. It was ultimately decided to only use the 6-month data for statistical analysis. Therefore, the data of 11 control patients and their selected tooth (a total of 66 sites) and 12 test patients and their selected tooth (a total of 72 sites) was included in the study. There were no adverse events recorded throughout the study.

Examining individual sites, there were statistically significant (SS; $p<0.05$) BOP reductions in both groups at the 3- and 6-month evaluations, compared to baseline values. At 3 months, there were significant reductions at the MB, ML, and DL sites of the control group and the DB site for the treatment group's teeth. At 6 months, there were significant reductions at the MB and ML sites of the control group and the DB, ML, and DL sites of the treatment group's teeth [Table 1]. Evaluating for the presence of plaque, there were only significant reductions in the control group at the DB and ML sites at 6 months compared to baseline. No other time intervals from either group had significant reductions [Table 2].

Table 1. Intragroup summary comparisons of site-specific BOP over time

BOP Site	Control	Treatment
SSBOP_DB	.33 (p=.846)	8.33 (p=.016)
SSBOP_B	3.00 (p=.223)	2.67 (p=.264)
SSBOP_MB	8.33 (p=.016)	4.00 (p=.135)
SSBOP_DL	7.00 (p=.03)	13.56 (.001)
SSBOP_L	2.00 (p=.368)	3.71 (p=.156)
SSBOP_ML	10.75 (p=.005)	6.75 (p=.034)

(Cochran's Q, significance)

Following this table are frequencies of each time point, test details, and post-hoc pairwise tests for any significant (p<=.05) test statistics shown above.

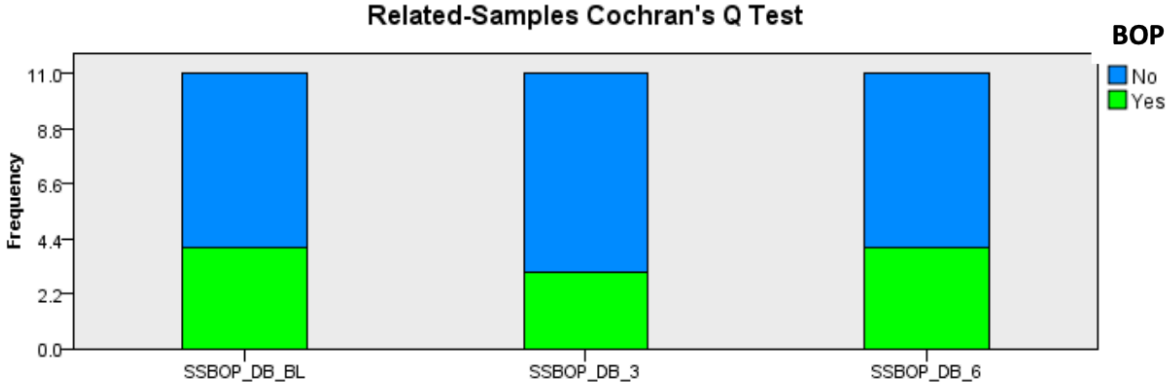
SSBOP_DB

Frequencies

Exp_Group		Value 0	Value 1
0 Control	SSBOP_DB_BL	7	4
	SSBOP_DB_3	8	3
	SSBOP_DB_6	7	4
1 Treatment	SSBOP_DB_BL	5	7
	SSBOP_DB_3	10	2
	SSBOP_DB_6	10	2

Control - SSBOP_DB

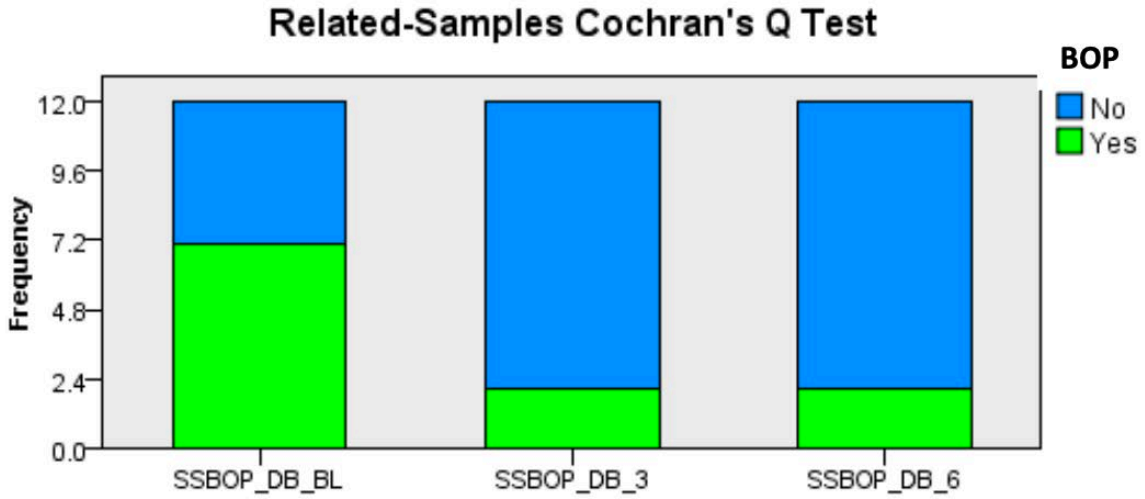
Related-Samples Cochran's Q Test



Total N	11
Test Statistic	.333
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.846

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

Treatment - **SSBOP_DB**



Total N	12
Test Statistic	8.333
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.016

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSBOP_DB_BL-SSBOP_DB_3	-.417	.167	-2.500	.012	.037
SSBOP_DB_BL-SSBOP_DB_6	-.417	.167	-2.500	.012	.037
SSBOP_DB_3-SSBOP_DB_6	.000	.167	.000	1.000	1.000

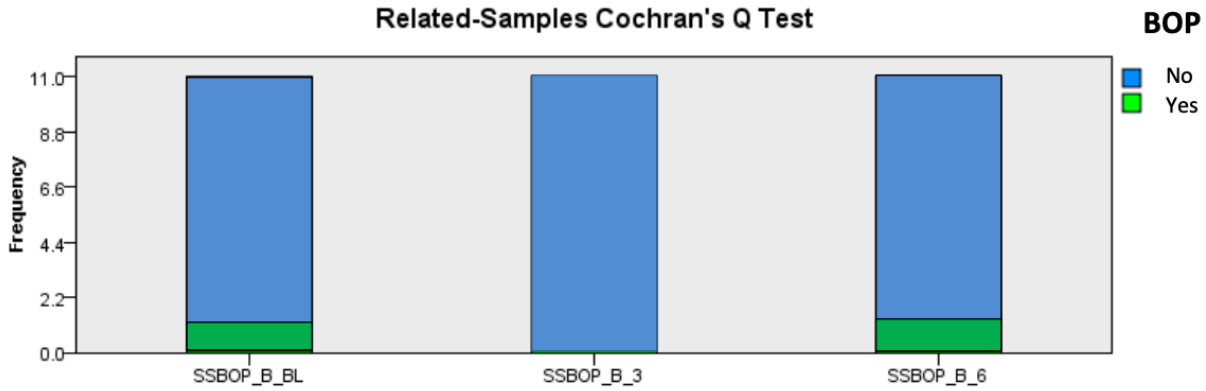
Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

SSBOP_B

Frequencies

Exp_Group		Value 0	Value 1
0 Control	SSBOP_B_BL	10	1
	SSBOP_B_3	11	0
	SSBOP_B_6	9	2
1 Treatment	SSBOP_B_BL	10	2
	SSBOP_B_3	12	0
	SSBOP_B_6	10	2

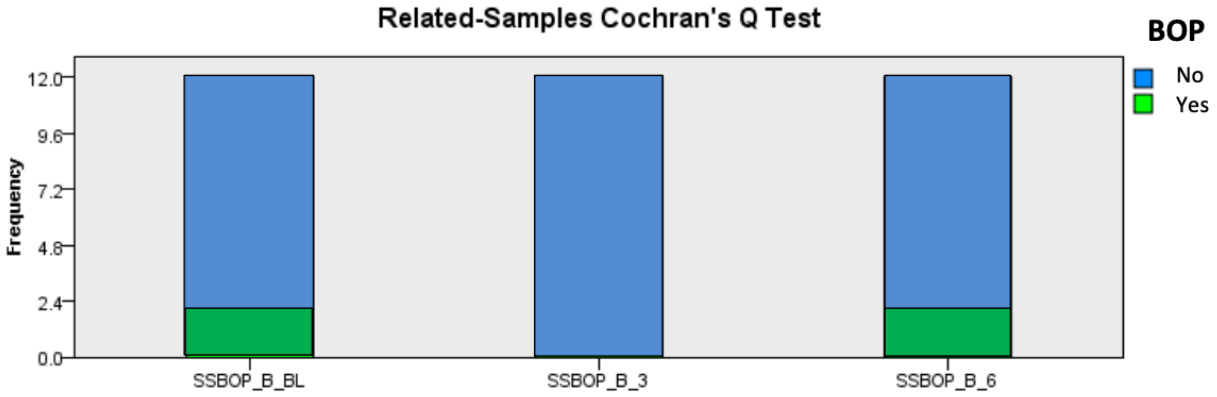
Control - SSBOP_B



Total N	11
Test Statistic	3.000
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.223

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

Treatment - SSBOP_B



Total N	12
Test Statistic	2.667
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.264

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

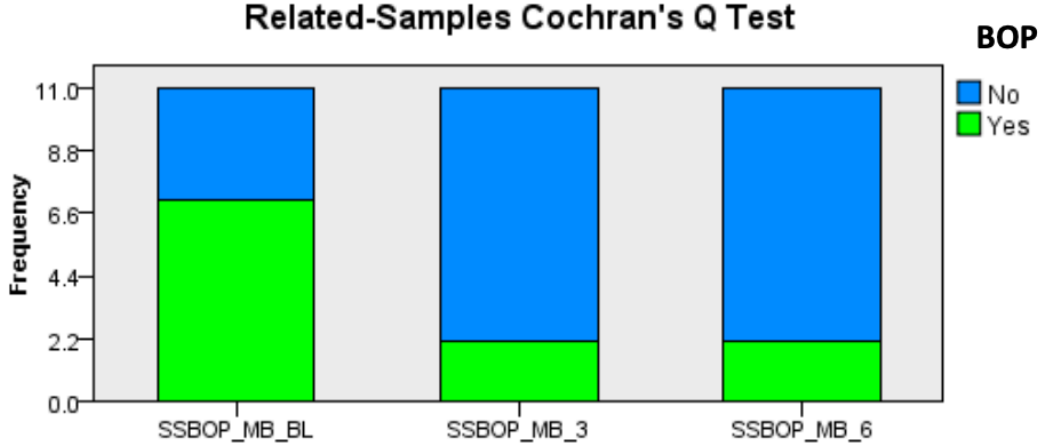
SSBOP_MB

Frequencies

Exp_Group		Value	
		0	1
0 Control	SSBOP_MB_BL	4	7
	SSBOP_MB_3	9	2
	SSBOP_MB_6	9	2
1 Treatment	SSBOP_MB_BL	7	5
	SSBOP_MB_3	11	1
	SSBOP_MB_6	9	3

Control - SSBOP_MB

Related-Samples Cochran's Q Test

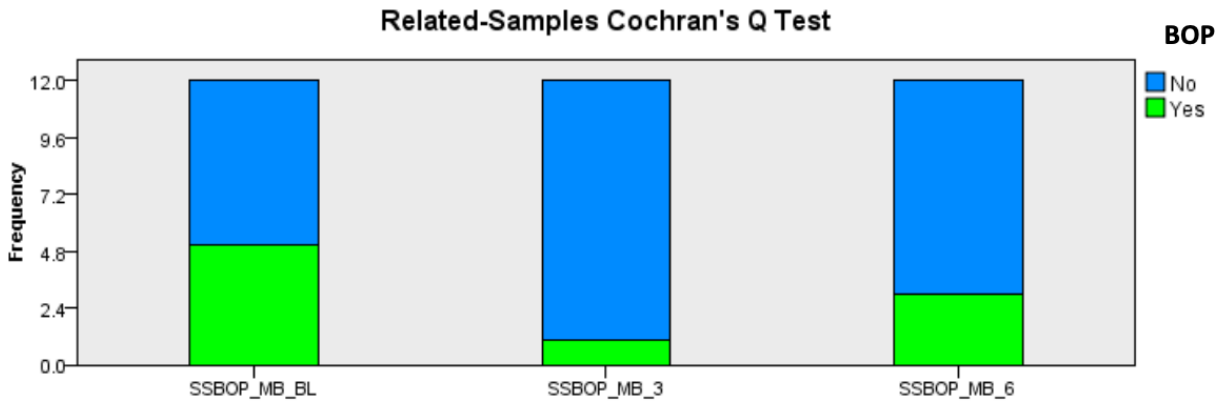


Total N	11
Test Statistic	8.333
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.016

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSBOP_MB_BL-SSBOP_MB_3	-.455	.182	-2.500	.012	.037
SSBOP_MB_BL-SSBOP_MB_6	-.455	.182	-2.500	.012	.037
SSBOP_MB_3-SSBOP_MB_6	.000	.182	.000	1.000	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Treatment - SSBOP_MB



Total N	12
Test Statistic	4.000
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.135

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

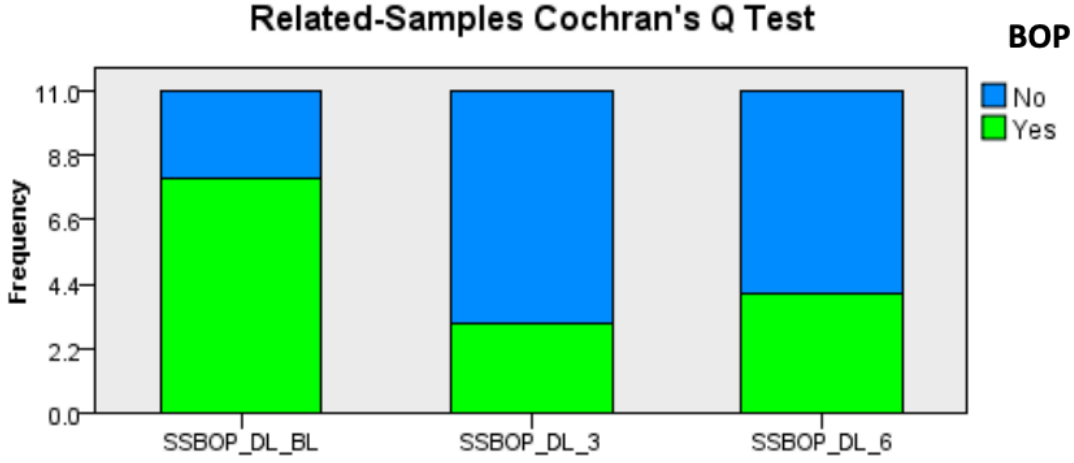
SSBOP_DL

Frequencies

Exp_Group		Value 0	Value 1
0 Control	SSBOP_DL_BL	3	8
	SSBOP_DL_3	8	3
	SSBOP_DL_6	7	4
1 Treatment	SSBOP_DL_BL	3	9
	SSBOP_DL_3	8	4
	SSBOP_DL_6	12	0

Control - SSBOP_DL

Related-Samples Cochran's Q Test

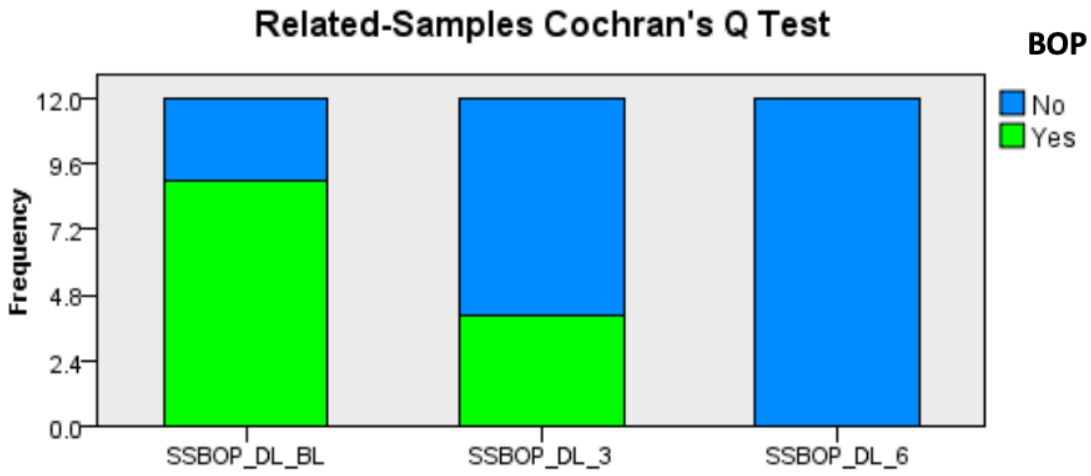


Total N	11
Test Statistic	7.000
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.030

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSBOP_DL_BL-SSBOP_DL_6	-.364	.182	-2.000	.046	.137
SSBOP_DL_BL-SSBOP_DL_3	-.455	.182	-2.500	.012	.037
SSBOP_DL_6-SSBOP_DL_3	.091	.182	.500	.617	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Treatment - SSBOP_DL



Total N	12
Test Statistic	13.556
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.001

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSBOP_DL_BL-SSBOP_DL_3	-.417	.204	-2.041	.041	.124
SSBOP_DL_BL-SSBOP_DL_6	-.750	.204	-3.674	.000	.001
SSBOP_DL_3-SSBOP_DL_6	-.333	.204	-1.633	.102	.307

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

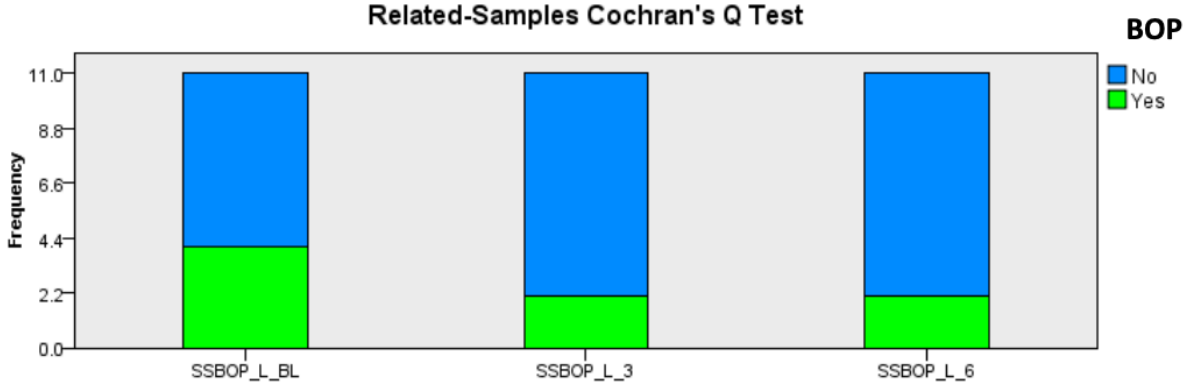
SSBOP_L

Frequencies

Exp_Group		Value 0	Value 1
0 Control	SSBOP_L_BL	7	4
	SSBOP_L_3	9	2
	SSBOP_L_6	9	2
1 Treatment	SSBOP_L_BL	7	5
	SSBOP_L_3	10	2
	SSBOP_L_6	11	1

Control - SSBOP_L

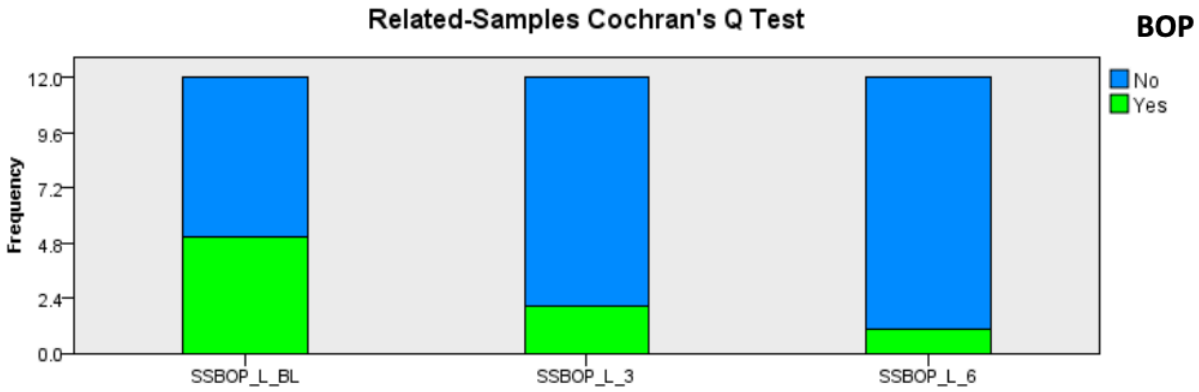
Related-Samples Cochran's Q Test



Total N	11
Test Statistic	2.000
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.368

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

Treatment - SSBOP_L



Total N	12
Test Statistic	3.714
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.156

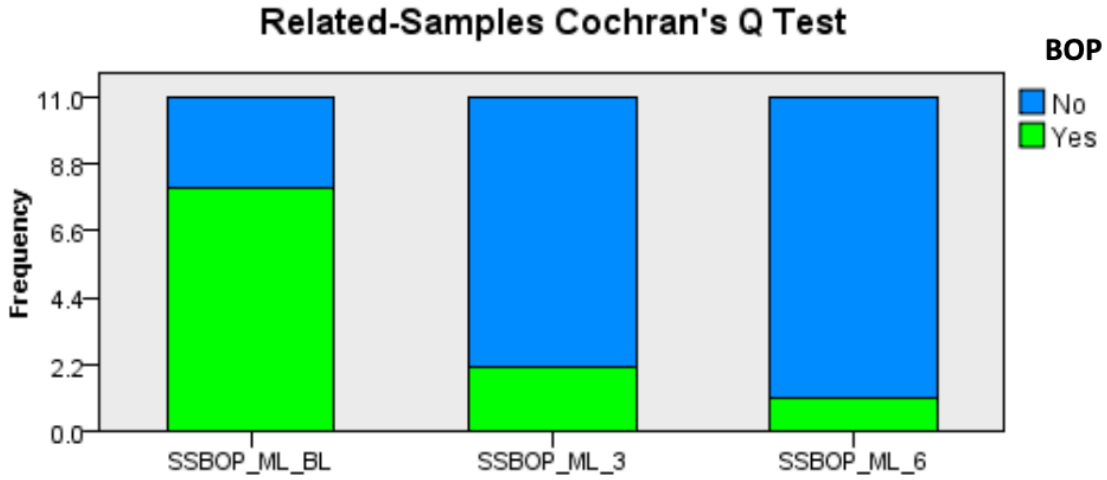
1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

SSBOP_ML

Frequencies

Exp_Group		Value 0	Value 1
0 Control	SSBOP_ML_BL	3	8
	SSBOP_ML_3	9	2
	SSBOP_ML_6	10	1
1 Treatment	SSBOP_ML_BL	4	8
	SSBOP_ML_3	7	5
	SSBOP_ML_6	10	2

Control - SSBOP_ML

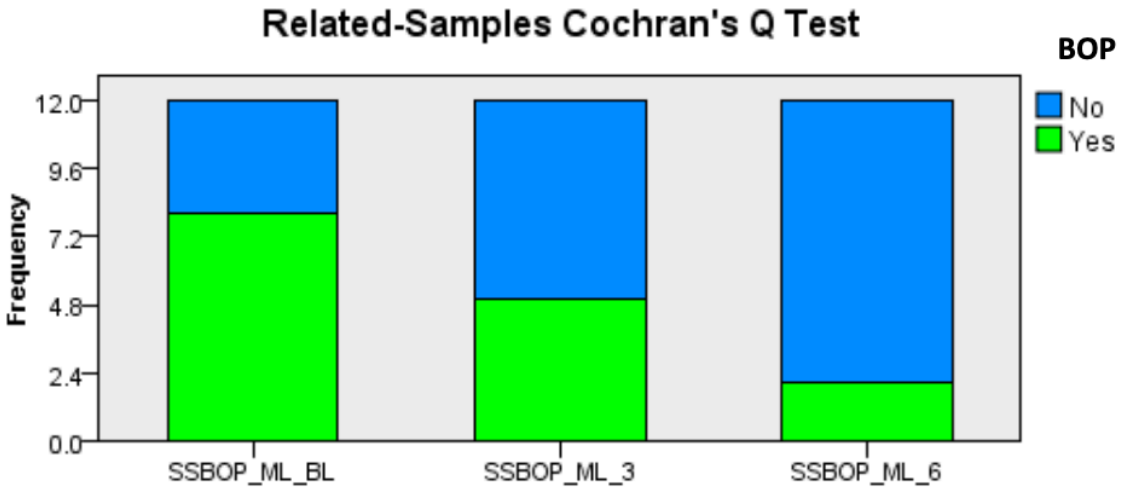


Total N	11
Test Statistic	10.750
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.005

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSBOP_ML_BL-SSBOP_ML_3	-.545	.210	-2.598	.009	.028
SSBOP_ML_BL-SSBOP_ML_6	-.636	.210	-3.031	.002	.007
SSBOP_ML_3-SSBOP_ML_6	-.091	.210	-.433	.665	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Treatment - SSBOP_ML



Total N	12
Test Statistic	6.750
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.034

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSBOP_ML_BL-SSBOP_ML_3	-.250	.192	-1.299	.194	.582
SSBOP_ML_BL-SSBOP_ML_6	-.500	.192	-2.598	.009	.028
SSBOP_ML_3-SSBOP_ML_6	-.250	.192	-1.299	.194	.582

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 2. Intragroup summary comparisons of site-specific PI over time

PI Site	Control	Treatment
SSPI_DB	6.33 (p=.04)	4.00 (p=.14)
SSPI_B	5.20 (p=.07)	1.00 (p=.61)
SSPI_MB	4.67 (p=.10)	3.71 (p=.16)
SSPI_DL	0.75 (p=.69)	5.56 (p=.06)
SSPI_L	3.00 (p=.22)	0.00 (p=1.00)
SSPI_ML	7.71 (p=.02)	0.75 (p=.69)

(Cochran's Q, significance)

Following this table are frequencies of each time point, test details, and post-hoc pairwise tests for any significant (p<=.05) test statistics shown above.

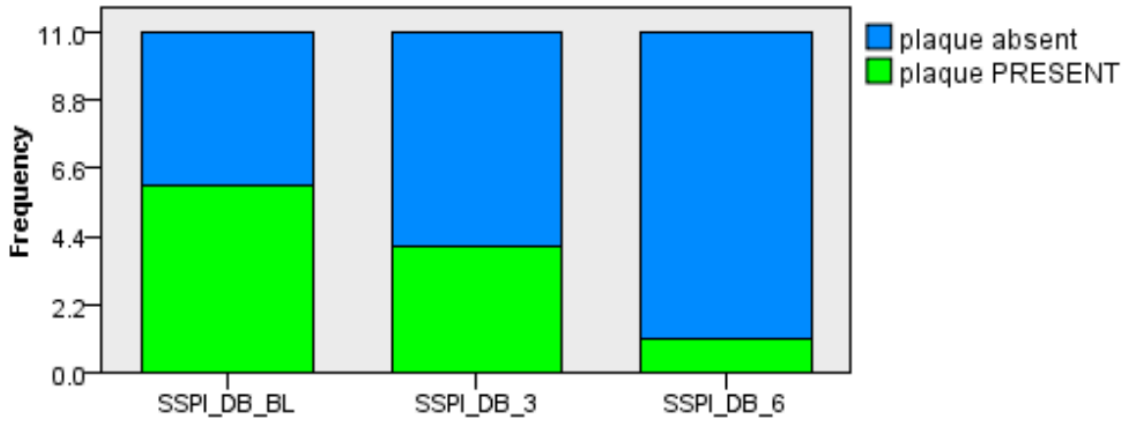
SSPI_DB

Frequencies

Exp_Group		Value 0	Value 1
0 Control	SSPI_DB_BL	5	6
	SSPI_DB_3	7	4
	SSPI_DB_6	10	1
1 Treatment	SSPI_DB_BL	3	9
	SSPI_DB_3	7	5
	SSPI_DB_6	7	5

Control - SSPI_DB

Related-Samples Cochran's Q Test

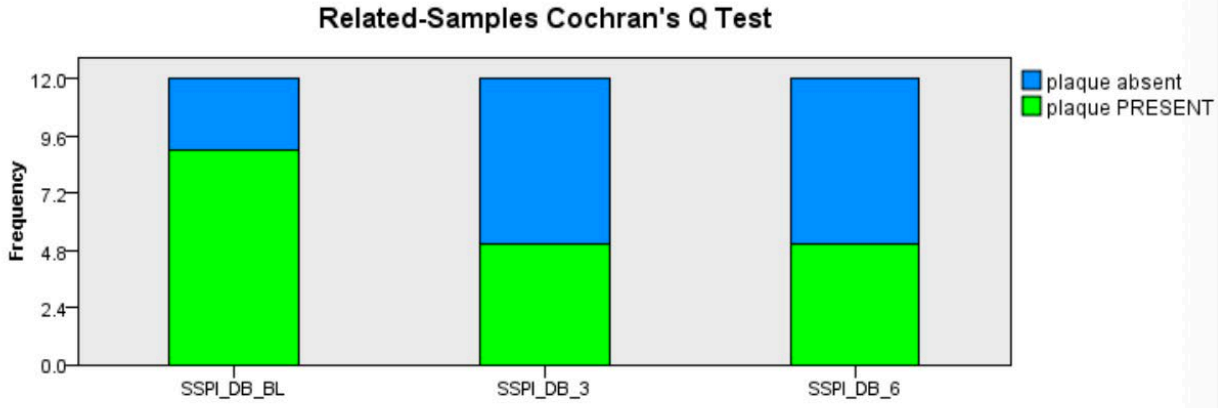


Total N	11
Test Statistic	6.333
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.042

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSPI_DB_BL-SSPI_DB_3	-.182	.182	-1.000	.317	.952
SSPI_DB_BL-SSPI_DB_6	-.455	.182	-2.500	.012	.037
SSPI_DB_3-SSPI_DB_6	-.273	.182	-1.500	.134	.401

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Treatment - SSPI_DB



Total N	12
Test Statistic	4.000
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.135

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

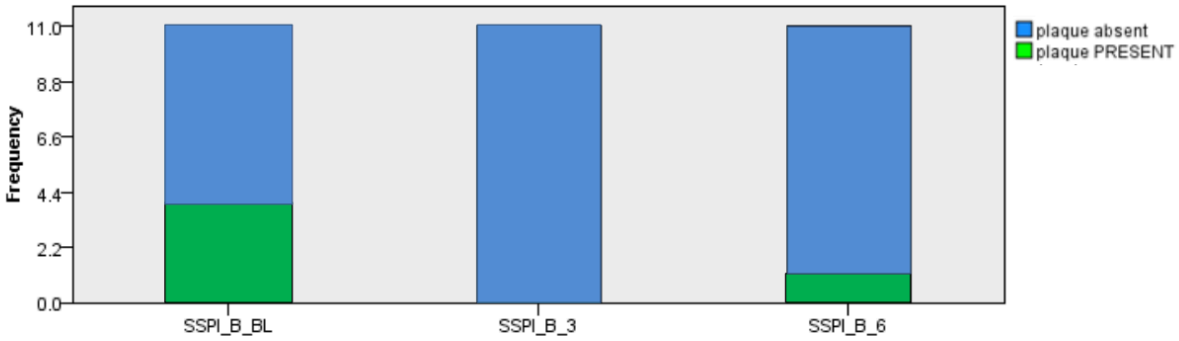
SSPI_B

Frequencies

Exp_Group		Value 0	Value 1
0 Control	SSPI_B_BL	7	4
	SSPI_B_3	11	0
	SSPI_B_6	10	1
1 Treatment	SSPI_B_BL	9	3
	SSPI_B_3	8	4
	SSPI_B_6	9	3

Control - SSPI_B

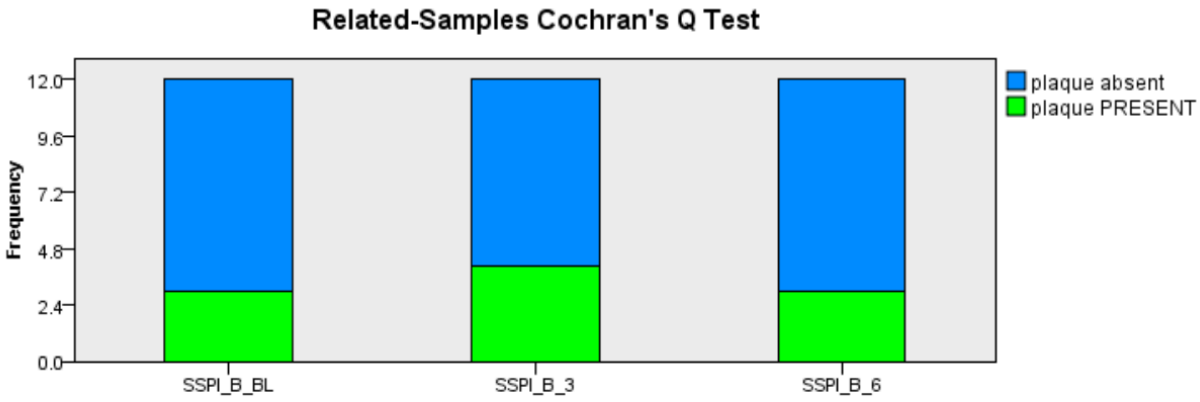
Related-Samples Cochran's Q Test



Total N	11
Test Statistic	5.200
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.074

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

Treatment - SSPI_B



Total N	12
Test Statistic	1.000
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.607

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

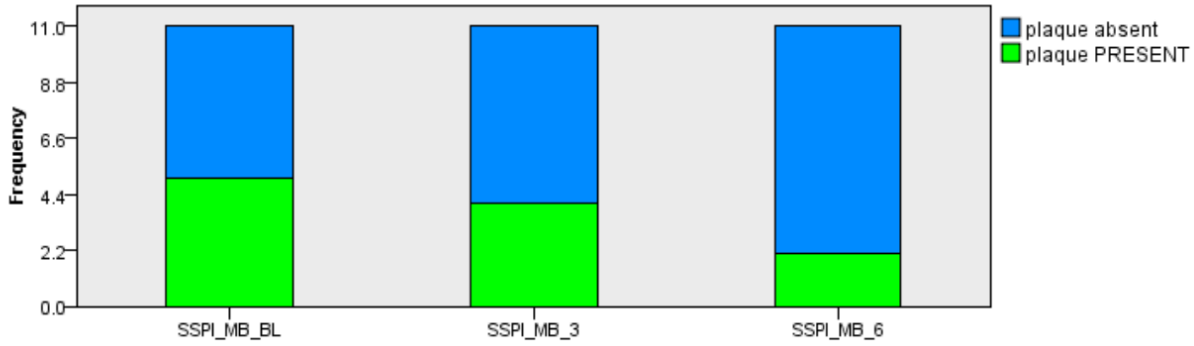
SSPI_MB

Frequencies

Exp_Group		Value 0	Value 1
0 Control	SSPI_MB_BL	6	5
	SSPI_MB_3	7	4
	SSPI_MB_6	9	2
1 Treatment	SSPI_MB_BL	6	6
	SSPI_MB_3	10	2
	SSPI_MB_6	9	3

Control - SSPI_MB

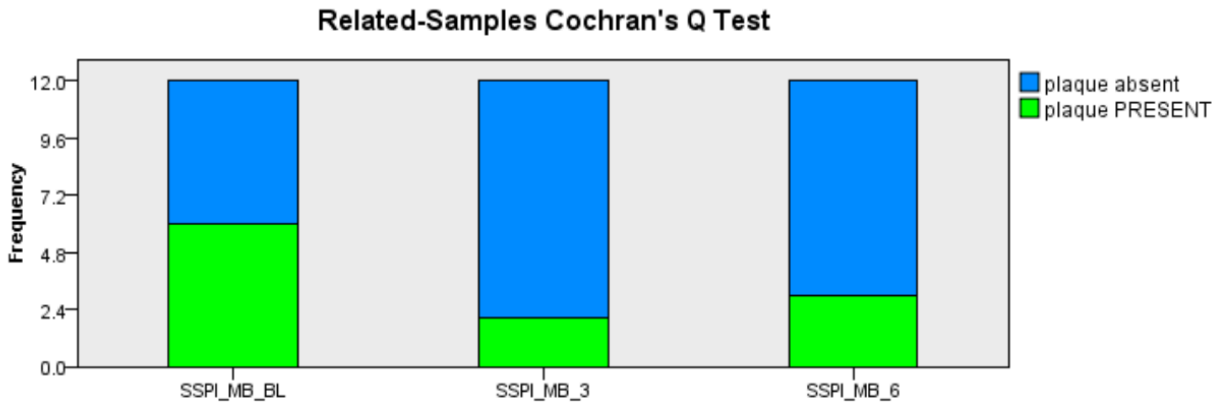
Related-Samples Cochran's Q Test



Total N	11
Test Statistic	4.667
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.097

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

Treatment - SSPI_MB



Total N	12
Test Statistic	3.714
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.156

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

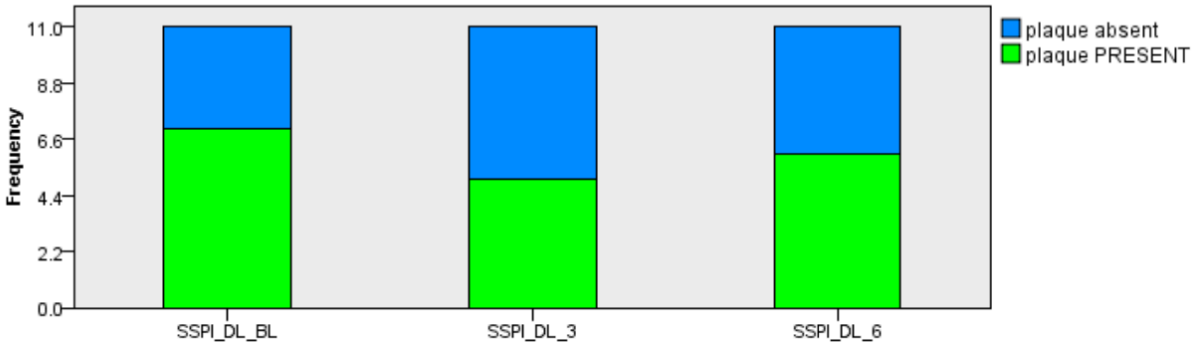
SSPI_DL

Frequencies

Exp_Group		Value 0	Value 1
0 Control	SSPI_DL_BL	4	7
	SSPI_DL_3	6	5
	SSPI_DL_6	5	6
1 Treatment	SSPI_DL_BL	3	9
	SSPI_DL_3	8	4
	SSPI_DL_6	8	4

Control - SSPI_DL

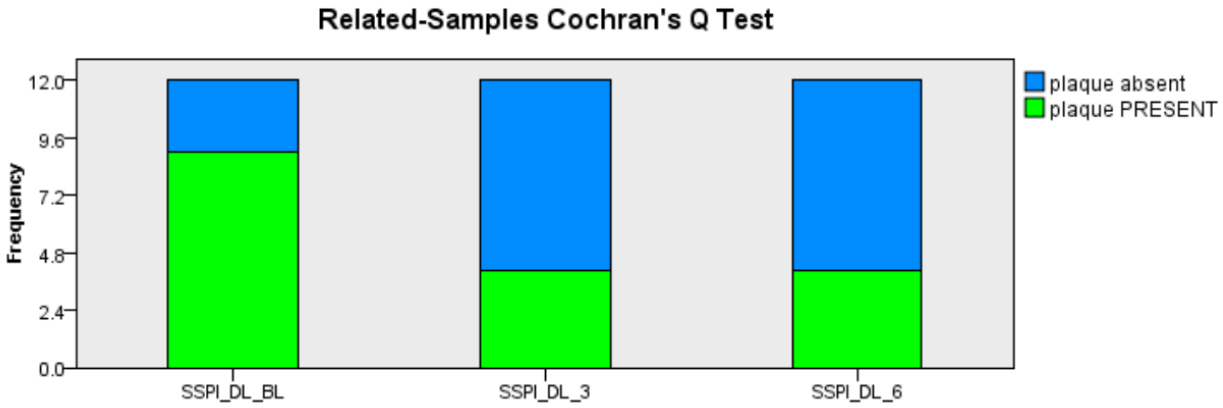
Related-Samples Cochran's Q Test



Total N	11
Test Statistic	.750
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.687

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

Treatment - SSPI_DL



Total N	12
Test Statistic	5.556
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.062

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

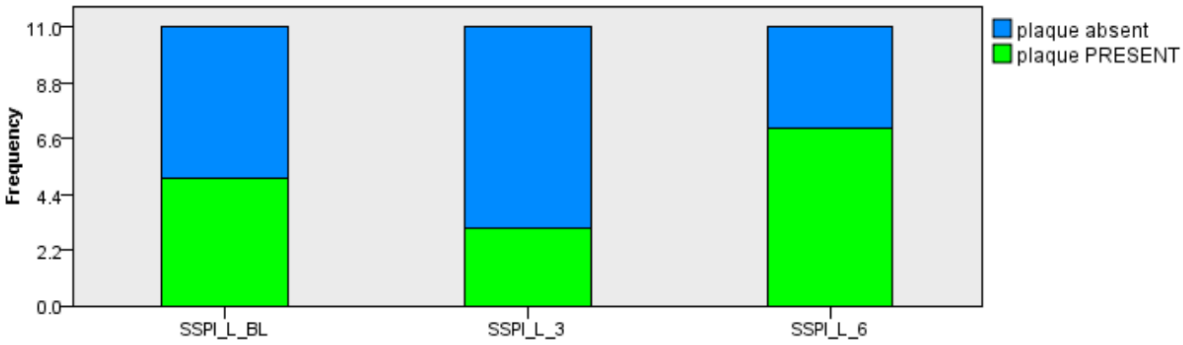
SSPI_L

Frequencies

Exp_Group		Value 0	Value 1
0 Control	SSPI_L_BL	6	5
	SSPI_L_3	8	3
	SSPI_L_6	4	7
1 Treatment	SSPI_L_BL	8	4
	SSPI_L_3	8	4
	SSPI_L_6	8	4

Control - SSPI_L

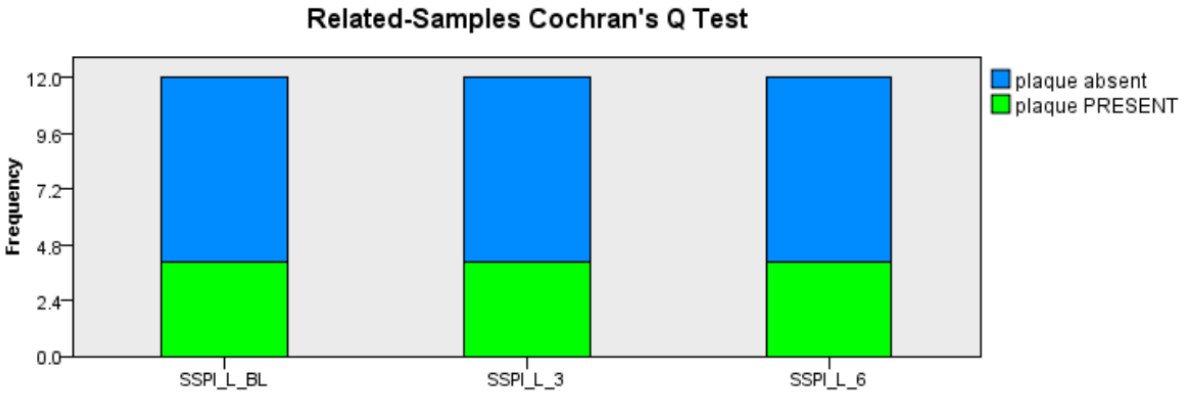
Related-Samples Cochran's Q Test



Total N	11
Test Statistic	3.000
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.223

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

Treatment - SSPI_L



Total N	12
Test Statistic	.000
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	1.000

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

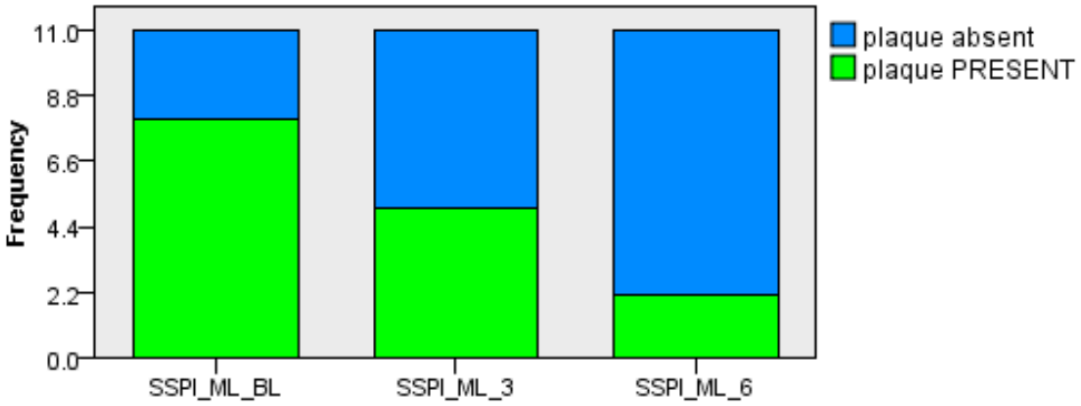
SSPI_ML

Frequencies

Exp_Group		Value 0	Value 1
0 Control	SSPI_ML_BL	3	8
	SSPI_ML_3	6	5
	SSPI_ML_6	9	2
1 Treatment	SSPI_ML_BL	6	6
	SSPI_ML_3	8	4
	SSPI_ML_6	7	5

Control - SSPI_ML

Related-Samples Cochran's Q Test

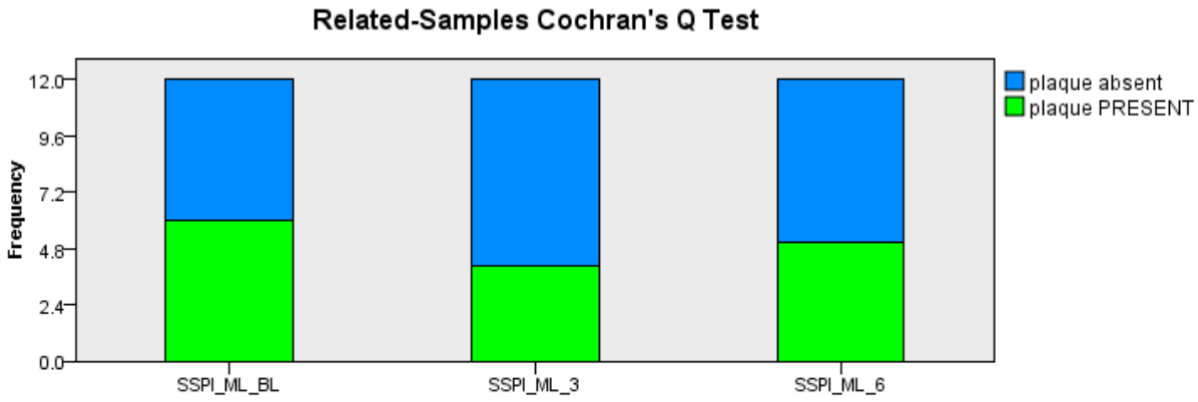


Total N	11
Test Statistic	7.714
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.021

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSPI_ML_BL-SSPI_ML_3	-.273	.196	-1.389	.165	.495
SSPI_ML_BL-SSPI_ML_6	-.545	.196	-2.777	.005	.016
SSPI_ML_3-SSPI_ML_6	-.273	.196	-1.389	.165	.495

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Treatment - SSPI_ML



Total N	12
Test Statistic	.750
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.687

1. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

Comparing the two groups directly across the different sites and time points for bleeding and plaque, the only SS difference was that the aPDT group had more BOP reduction at the DL site 6 months after treatment, compared to the control group [Table 3, Table 4].

Table 3. Comparisons of BOP at specific sites across groups

	Control			Treatment			
BOP Site	% BOP	NO	YES	% BOP	NO	YES	(Sig)
SSBOP_DB_BL	36.4	7	4	58.3	5	7	(.29)[a]
SSBOP_DB_3	27.3	8	3	16.7	10	2	(.64)
SSBOP_DB_6	36.4	7	4	16.7	10	2	(.37)
SSBOP_B_BL	9.1	10	1	16.7	10	2	(1.0)
SSBOP_B_3	0.0	11	0	0.0	12	0	n/a
SSBOP_B_6	18.2	9	2	16.7	10	2	(1.0)
SSBOP_MB_BL	63.6	4	7	41.7	7	5	(.29)[a]
SSBOP_MB_3	18.2	9	2	8.3	11	1	(.59)
SSBOP_MB_6	18.2	9	2	25.0	9	3	(1.0)
SSBOP_DL_BL	72.7	3	8	75.0	3	9	(1.0)
SSBOP_DL_3	27.3	8	3	33.3	8	4	(1.0)
SSBOP_DL_6	36.4	7	4	0.0	12	0	(.04)
SSBOP_L_BL	36.4	7	4	41.7	7	5	(1.0)
SSBOP_L_3	18.2	9	2	16.7	10	2	(1.0)
SSBOP_L_6	18.2	9	2	8.3	11	1	(.59)
SSBOP_ML_BL	72.7	3	8	66.6	4	8	(1.0)
SSBOP_ML_3	18.2	9	2	41.7	7	5	(.37)
SSBOP_ML_6	9.1	10	1	16.7	10	2	(1.0)

Fishers exact significance reported for all with 2 exceptions where [a]Pearson Chi Square significance reported.

Table 4. Comparisons of PI at specific sites across groups

PI Site	Control			Treatment			(Sig)
	% Plaque	NO	YES	% Plaque	NO	YES	
SSPI_DB_BL	54.5	5	6	75.0	3	9	.40
SSPI_DB_3	36.4	7	4	41.7	7	5	1.0
SSPI_DB_6	9.1	10	1	41.7	7	5	.15
SSPI_B_BL	36.4	7	4	25.0	9	3	.67
SSPI_B_3	0.0	11	0	33.0	8	4	.09
SSPI_B_6	9.1	10	1	25.0	9	3	.60
SSPI_MB_BL	45.5	6	5	50.0	6	6	.83[a]
SSPI_MB_3	36.4	7	4	16.7	10	2	.37
SSPI_MB_6	18.2	9	2	25.0	9	3	1.0
SSPI_DL_BL	63.6	4	7	75.0	3	9	.67
SSPI_DL_3	45.5	6	5	33.3	8	4	.68
SSPI_DL_6	54.5	5	6	33.3	8	4	.41
SSPI_L_BL	45.5	6	5	33.3	8	4	.68
SSPI_L_3	27.3	8	3	33.3	8	4	1.0
SSPI_L_6	63.6	4	7	33.3	8	4	.16[a]
SSPI_ML_BL	72.7	3	8	50.0	6	6	.40
SSPI_ML_3	45.5	6	5	33.3	8	4	.68
SSPI_ML_6	18.2	9	2	41.7	7	5	.37

Fishers exact significance reported for all with 2 exceptions where [a]Pearson Chi Square significance reported.

Generally, there was a reduction in mean bleeding on probing and plaque levels from baseline to 3 months and 6 months for both groups. The number of mean control group BOP sites was reduced from 2.91 to 1.09 and 1.36 across 3 and 6 months, respectively. The mean number of test group BOP sites was reduced from 3.00 to 1.17 to 0.83 after 3 and 6 months, respectively. The mean control group plaque was reduced from 3.18 to 1.91 to 1.73 sites and the mean test group plaque was reduced from 3.08 to 1.92 and 2.0 sites after 3 and 6 months, respectively [Table 5]. This translated to SS intragroup reductions of BOP in both groups at 3 and 6 months

compared to baseline [Table 6]. However, although there was a trend towards a meaningful distinction in bleeding at the end of 6 months in favor of the treatment group, there were no SS differences for the mean BOP or plaque values across any time points between the two groups [Figure 1, Table 7; Figure 2, Table 8].

Table 5. Descriptive BOP and PI means within groups

BOP	**Baseline**	**3 Months**	**6 Months**
Control	2.91	1.09	1.36
Treatment	3.0	1.17	0.83
PI			
Control	3.18	1.90	1.72
Treatment	3.08	1.92	2.00

Table 6. Comparison of BOP and PI totals within each experimental group

Group	**BOP**		**PI**	
	Statistic	sig	Statistic	sig
Control	13.24	(p=.001)	8.46	(p=.02)
Treatment	15.59	(p=.000)	4.91	(p=.09)

Friedman's test used.

For significant differences within group, post hoc comparisons follow

Control BOP

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Total BOP at 3 Months-Total BOP at 6 Months	.000	.426	.000	1.000	1.000
Total BOP at 3 Months-Total BOP at Baseline	1.091	.426	2.558	.011	.032
Total BOP at 6 Months-Total BOP at Baseline	1.091	.426	2.558	.011	.032

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Treatment BOP

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Total BOP at 6 Months-Total BOP at 3 Months	.292	.408	.714	.475	1.000
Total BOP at 6 Months-Total BOP at Baseline	1.458	.408	3.572	.000	.001
Total BOP at 3 Months-Total BOP at Baseline	1.167	.408	2.858	.004	.013

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Control PI

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Total PI at 6 Months-Total PI at 3 Months	.091	.426	.213	.831	1.000
Total PI at 6 Months-Total PI at Baseline	1.000	.426	2.345	.019	.057
Total PI at 3 Months-Total PI at Baseline	.909	.426	2.132	.033	.099

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Figure 1. Comparison of mean BOP across time and group

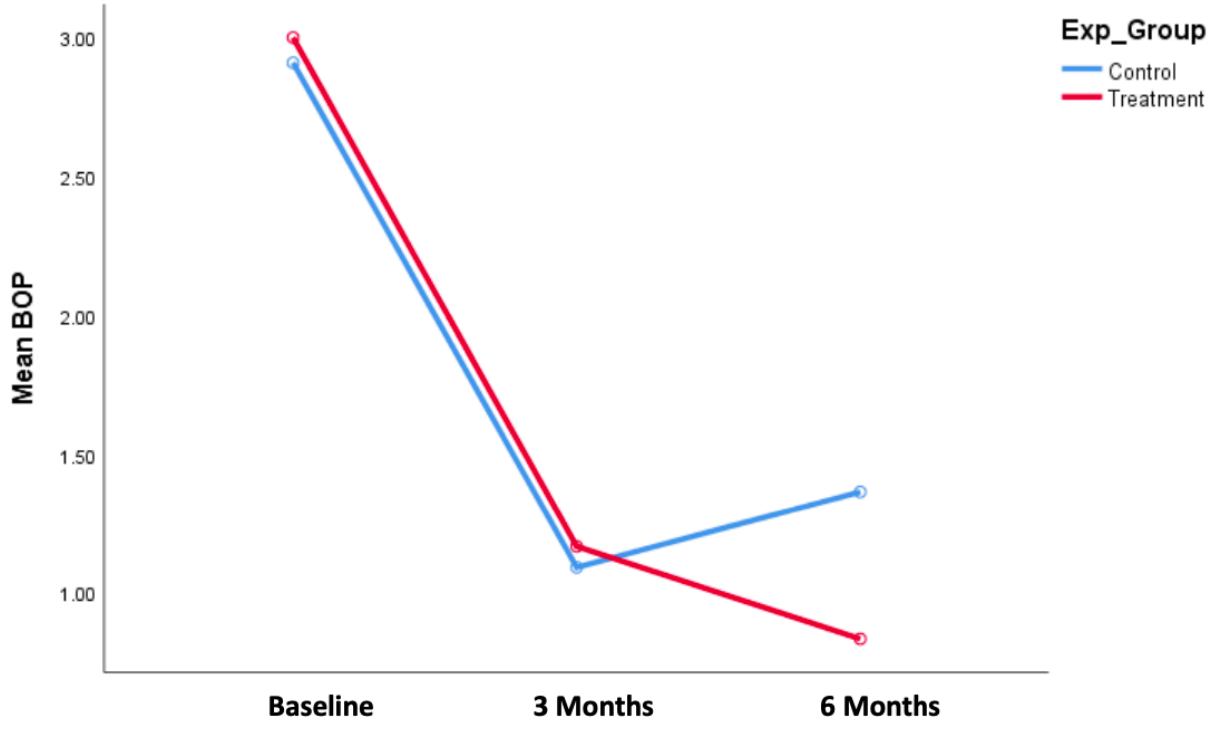


Table 7. Comparison of mean BOP across time and group

BOP Time	Control (mean BOP)	Treatment (mean BOP)	sig
BOP Baseline	2.91	3.00	0.88
BOP 3 Months	1.09	1.17	0.88
BOP 6 Months	1.36	0.83	0.45

Mann Whitney U tests.

Figure 2. Comparison of mean PI across time and group

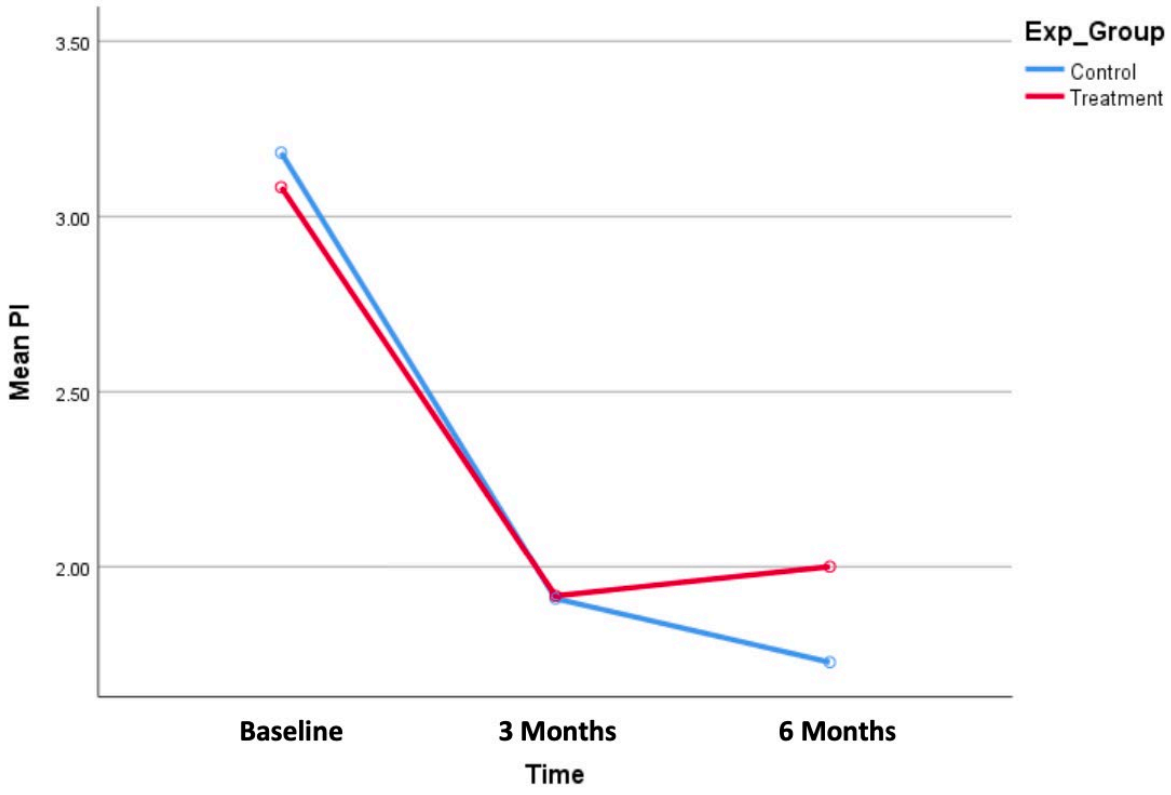


Table 8. Comparison of mean PI across time and group

PI Time	Control (mean PI)	Treatment (mean PI)	sig
PI Baseline	3.18	3.08	.77
PI 3 Months	1.91	1.92	.94
PI 6 Months	1.73	2.0	.80

Mann Whitney U tests.

Examining the PD values at individual sites, there were SS reductions in both groups at the 3-month evaluations. This included the DL and ML sites in the control group and the B, DL, and ML sites in the treatment group, when compared to their baseline values. By 6 months, only the treatment group had comparatively significant reductions, specifically at the DB, DL, L, and ML

sites [Table 9]. Comparing the two groups directly across the different sites and time points, the treatment group had significantly lower mean PD values at 4/6 sites by 6 months (DB [3.00 v 4.27mm], MB [3.08 v 4.09mm], DL [3.67 v 4.64mm], and L [2.50 v 3.27mm] sites) [Table 10].

Table 9. Intragroup summary comparisons of site-specific PD over time

PD Site	Control	Treatment
SSPD_DB	3.72 (p=.16)	10.67 (p=.005)
SSPD_B	4.22 (p=.12)	10.29 (p=.006)
SSPD_MB	2.63 (p=.27)	10.14 (p=.006)
SSPD_DL	12.47 (p=.002)	16.43 (p=.000)
SSPD_L	5.87 (p=.053)	13.00 (p=.002)
SSPD_ML	11.29 (p=.004)	14.76 (p=.001)

(Friedman's test used. ChiSq, sig)

Following this table are post-hoc pairwise tests for any significant (p<=.05) test statistics shown above.

SSPD_DB

Treatment

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSPD_DB_6-SSPD_DB_3	.333	.408	.816	.414	1.000
SSPD_DB_6-SSPD_DB_BL	1.167	.408	2.858	.004	.013
SSPD_DB_3-SSPD_DB_BL	.833	.408	2.041	.041	.124

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

SSPD_B

Treatment

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSPD_B_3-SSPD_B_6	-.208	.408	-.510	.610	1.000
SSPD_B_3-SSPD_B_BL	1.042	.408	2.552	.011	.032
SSPD_B_6-SSPD_B_BL	.833	.408	2.041	.041	.124

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

SSPD_MB

Treatment

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSPD_MB_3-SSPD_MB_6	.000	.408	.000	1.000	1.000
SSPD_MB_3-SSPD_MB_BL	.875	.408	2.143	.032	.096
SSPD_MB_6-SSPD_MB_BL	.875	.408	2.143	.032	.096

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

SSPD_DL

Control

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSPD_DL_3-SSPD_DL_6	-.409	.426	-.959	.337	1.000
SSPD_DL_3-SSPD_DL_BL	1.364	.426	3.198	.001	.004
SSPD_DL_6-SSPD_DL_BL	.955	.426	2.239	.025	.076

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Treatment

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSPD_DL_3-SSPD_DL_6	-.167	.408	-.408	.683	1.000
SSPD_DL_3-SSPD_DL_BL	1.333	.408	3.266	.001	.003
SSPD_DL_6-SSPD_DL_BL	1.167	.408	2.858	.004	.013

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

SSPD_L

Treatment

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSPD_L_6-SSPD_L_3	.458	.408	1.123	.262	.785
SSPD_L_6-SSPD_L_BL	1.292	.408	3.164	.002	.005
SSPD_L_3-SSPD_L_BL	.833	.408	2.041	.041	.124

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

SSPD_ML

Control

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSPD_ML_3-SSPD_ML_6	-.227	.426	-.533	.594	1.000
SSPD_ML_3-SSPD_ML_BL	1.136	.426	2.665	.008	.023
SSPD_ML_6-SSPD_ML_BL	.909	.426	2.132	.033	.099

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Treatment

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSPD_ML_6-SSPD_ML_3	.125	.408	.306	.759	1.000
SSPD_ML_6-SSPD_ML_BL	1.250	.408	3.062	.002	.007
SSPD_ML_3-SSPD_ML_BL	1.125	.408	2.756	.006	.018

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 10. Comparisons of mean PD at specific sites across groups

PD Site	Control		Treatment		
	Mean	Mean Rank	Mean	Mean Rank	(Sig)
SSPD_DB_BL	4.73	12.05	4.75	11.96	.99
SSPD_DB_3	3.82	13.45	3.42	10.67	.33
SSPD_DB_6	4.27	15.32	3.00	8.96	.02
SSPD_B_BL	2.73	10.73	3.08	13.17	.39
SSPD_B_3	2.18	12.50	2.08	11.54	.81
SSPD_B_6	2.45	13.00	2.17	11.08	.44
SSPD_MB_BL	4.64	12.91	4.17	11.17	.53
SSPD_MB_3	3.91	14.50	3.08	9.71	.08
SSPD_MB_6	4.09	14.82	3.08	9.42	.04
SSPD_DL_BL	5.64	12.68	5.50	11.38	.65
SSPD_DL_3	4.18	14.59	3.50	9.63	.08
SSPD_DL_6	4.64	14.95	3.67	9.29	.04
SSPD_L_BL	3.64	12.73	3.75	11.33	.65
SSPD_L_3	2.82	12.14	2.92	11.88	.97
SSPD_L_6	3.27	15.36	2.50	8.92	.01
SSPD_ML_BL	4.82	11.82	4.83	12.17	.92
SSPD_ML_3	3.64	12.82	3.33	11.25	.58
SSPD_ML_6	3.82	13.64	3.25	10.50	.26

Mann Whitney U test used.

Calculating mean PDs, combined across all six sites, shows that both treatment and control groups had SS lower PD values at 3 and 6 months compared to their baseline values [Table 11]. The mean PD decreased from 4.364mm to 3.424mm and 3.758mm at 3 and 6 months, respectively, for the control teeth, while it decreased from 4.347mm to 3.056mm to 2.944mm at 3 and 6 months, respectively, for the treated teeth (a SS difference at 6 months) [Figure 3, Table 12]. Comparing mean PD values between groups shows that the adjunctive aPDT treated teeth had SS more PD reduction compared to SRP alone at 6 months, a difference of 1.403mm vs 0.606mm from their respective baselines [Figure 4, Table 13].

Table 11. Intragroup comparisons of mean PD value over time

Exp_Group	(I) PDTIME	(J) PDTIME	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
0 Control	Baseline	3 months	.939*	.170	.000	.497	1.382
		6 months	.606*	.198	.018	.091	1.121
	3 months	Baseline	-.939*	.170	.000	-1.382	-.497
		6 months	-.333	.142	.085	-.702	.035
	6 months	Baseline	-.606*	.198	.018	-1.121	-.091
		3 months	.333	.142	.085	-.035	.702
1 Treatment	Baseline	3 months	1.292*	.163	.000	.868	1.715
		6 months	1.403*	.189	.000	.910	1.895
	3 months	Baseline	-1.292*	.163	.000	-1.715	-.868
		6 months	.111	.136	1.000	-.241	.464
	6 months	Baseline	-1.403*	.189	.000	-1.895	-.910
		3 months	-.111	.136	1.000	-.464	.241

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Figure 3. Comparison of mean PD value across time and group

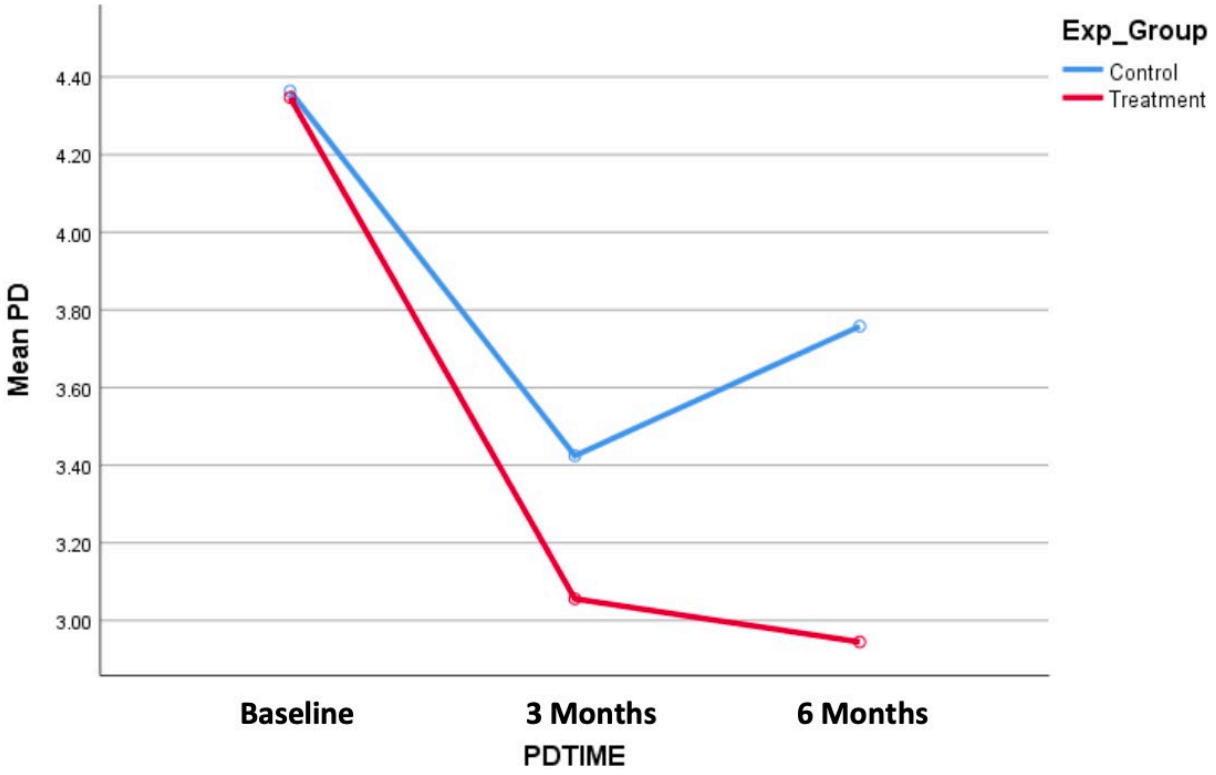


Table 12. Comparison of mean PD value across time and group

PD Time	Control (m, SE)	Treatment (m,SE)	sig
Baseline	4.36 (.230)	4.35 (.220)	p= .959
3 months	3.42 (.119)	3.06 (.114)	p=.036
6 months	3.76 (.182)	2.94 (.174)	p=.004

Figure 4. Comparison of overall mean PD change across time and group

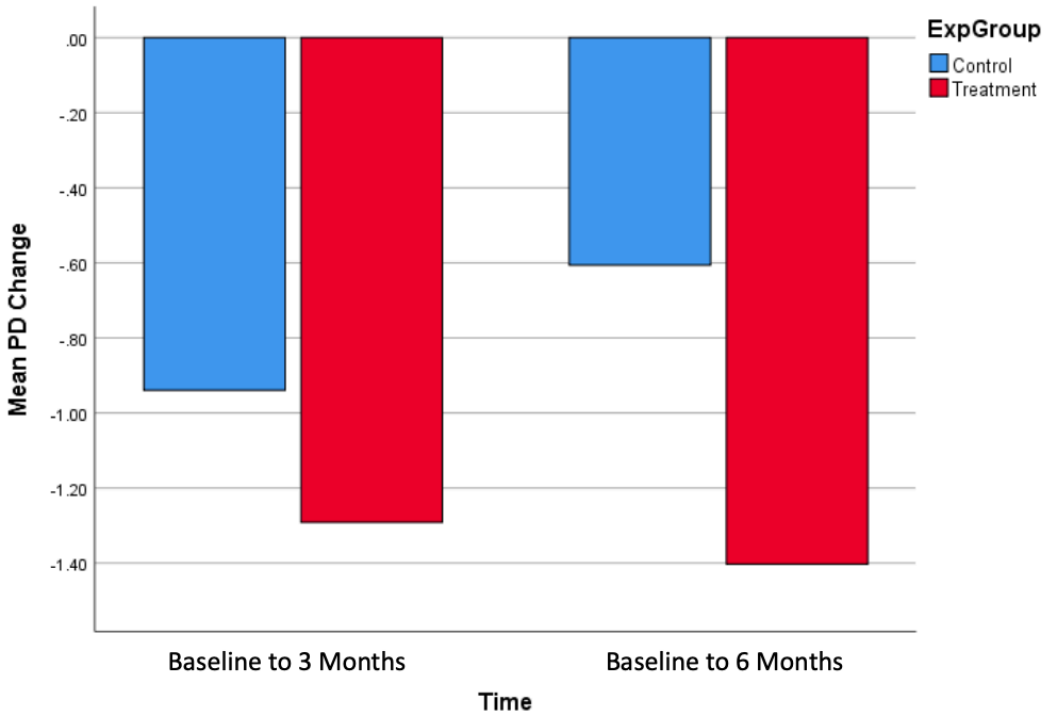


Table 13. Comparison of mean PD change across groups

Comparison	Control	Treatment	Control vs Treatment Change (sig)
PD Baseline to 3 Months	B=4.364 3Month=3.424 **Change= -.939**	B=4.347 3Month=3.056 **Change= -1.292**	-.35 (p=.15)
PD Baseline to 6 Months	B=4.364 6Month=3.758 **Change= -0.606**	B=4.347 6Month=2.944 **Change= -1.403**	-.797 (p=.008)

Examining the CAL values at individual sites, there were SS gains in both groups at the 3-month evaluations, albeit only at the DL site in the control group and the DL and ML sites in the treatment group, when compared to their baseline values. By 6 months, more gains can be seen, specifically at the DL, L, and ML sites in the treatment group and the L site in the control group [Table 14]. Comparing the two groups directly across the different sites and time points, the groups had no SS differences in their mean CAL values at any point [Table 15].

Table 14. Intragroup summary comparisons of site-specific CAL over time

CAL Site	Control	Treatment
SSCAL_DB	3.21 (p=.20)	10.24 (p=.006)
SSCAL_B	4.20 (p=.12)	6.82 (p=.03)
SSCAL_MB	2.25 (p=.33)	9.66 (p=.008)
SSCAL_DL	12.19 (p=.002)	15.60 (p=.000)
SSCAL_L	5.39 (p=.07)	7.35 (p=.03)
SSCAL_ML	10.79 (p=.005)	14.48 (p=.001)

(Friedman's test used. ChiSq, sig)

Following this table are post-hoc pairwise tests for any significant (p<=.05) test statistics shown above.

SSCAL_DB

Treatment

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSCAL_DB_3-SSCAL_DB_6	-.042	.408	-.102	.919	1.000
SSCAL_DB_3-SSCAL_DB_BL	.958	.408	2.347	.019	.057
SSCAL_DB_6-SSCAL_DB_BL	.917	.408	2.245	.025	.074

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

SSCAL_B

Treatment

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSCAL_B_3-SSCAL_B_6	-.208	.408	-.510	.610	1.000
SSCAL_B_3-SSCAL_B_BL	.917	.408	2.245	.025	.074
SSCAL_B_6-SSCAL_B_BL	.708	.408	1.735	.083	.248

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

SSCAL_MB

Treatment

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSCAL_MB_6-SSCAL_MB_3	.042	.408	.102	.919	1.000
SSCAL_MB_6-SSCAL_MB_BL	.958	.408	2.347	.019	.057
SSCAL_MB_3-SSCAL_MB_BL	.917	.408	2.245	.025	.074

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

SSCAL_DL

Control

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSCAL_DL_3-SSCAL_DL_6	-.409	.426	-.959	.337	1.000
SSCAL_DL_3-SSCAL_DL_BL	1.227	.426	2.878	.004	.012
SSCAL_DL_6-SSCAL_DL_BL	.818	.426	1.919	.055	.165

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Treatment

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSCAL_DL_3-SSCAL_DL_6	-.125	.408	-.306	.759	1.000
SSCAL_DL_3-SSCAL_DL_BL	1.250	.408	3.062	.002	.007
SSCAL_DL_6-SSCAL_DL_BL	1.125	.408	2.756	.006	.018

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

SSCAL_L

Treatment

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSCAL_L_6-SSCAL_L_3	.625	.408	1.531	.126	.377
SSCAL_L_6-SSCAL_L_BL	1.000	.408	2.449	.014	.043
SSCAL_L_3-SSCAL_L_BL	.375	.408	.919	.358	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Treatment

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSCAL_ML_6-SSCAL_ML_3	.167	.408	.408	.683	1.000
SSCAL_ML_6-SSCAL_ML_BL	1.333	.408	3.266	.001	.003
SSCAL_ML_3-SSCAL_ML_BL	1.167	.408	2.858	.004	.013

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

SSCAL_ML

Control

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
SSCAL_ML_6-SSCAL_ML_3	.182	.426	.426	.670	1.000
SSCAL_ML_6-SSCAL_ML_BL	1.045	.426	2.452	.014	.043
SSCAL_ML_3-SSCAL_ML_BL	.864	.426	2.025	.043	.128

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 15. Comparisons of mean CAL at specific sites across groups

CAL Site	Control		Treatment		
	Mean	Mean Rank	Mean	Mean Rank	(Sig)
SSCAL_DB_BL	5.36	(10.27)	6.50	(13.58)	P=.25
SSCAL_DB_3	4.36	(10.64)	5.08	(13.25)	p=.36
SSCAL_DB_6	4.73	(12.09)	4.92	(11.92)	p=.96
SSCAL_B_BL	3.27	(9.09)	4.58	(14.67)	p=.05
SSCAL_B_3	2.64	(9.73)	3.42	(14.08)	p=.12
SSCAL_B_6	2.73	(11.23)	3.50	(12.71)	p=.62
SSCAL_MB_BL	5.09	(11.14)	5.50	(12.79)	p=.59
SSCAL_MB_3	4.27	(11.64)	4.58	(12.33)	p=.83
SSCAL_MB_6	4.64	(12.77)	4.42	(11.29)	p=.62
SSCAL_DL_BL	6.00	(10.95)	6.75	(12.96)	p=.49
SSCAL_DL_3	4.45	(11.86)	4.75	(12.13)	p=.93
SSCAL_DL_6	5.09	(13.14)	4.67	(10.96)	p=.45
SSCAL_L_BL	4.18	(10.45)	5.00	(13.42)	p=.30
SSCAL_L_3	3.18	(9.91)	4.42	(13.92)	p=.16
SSCAL_L_6	3.91	(13.18)	3.50	(10.92)	p=.43
SSCAL_ML_BL	5.00	(9.55)	6.08	(14.25)	p=.10
SSCAL_ML_3	3.91	(11.36)	4.58	(12.58)	p=.68
SSCAL_ML_6	3.91	(11.32)	4.25	(12.63)	p=.67

Mann Whitney U test used.

However, calculating mean overall CAL, combined across all six sites, shows that the two groups had SS lower CAL values at 3 months compared to their baselines. Additionally, the treatment group also had SS lower CAL values at 6 months [Table 16]. The mean CAL value decreased from 4.818mm to 3.803mm and 4.167mm at 3 and 6 months, respectively, for the control teeth, while it decreased from 5.736mm to 4.472mm to 4.208mm at 3 and 6 months, respectively, for the treated teeth [Figure 5, Table 17]. Comparing these mean CAL gains between the groups shows that applying adjunctive aPDT resulted in SS more CAL gain compared to SRP alone by 6 months, by a difference of 1.528mm v 0.652mm [Figure 6, Table

18]. It is interesting to note that the CAL control and treatment groups differed significantly at baseline measurement (p=0.05), despite the PD baseline values having no SS difference.

Table 16. Intragroup comparisons of mean CAL value over time

Exp_Group	(I) CALTIME	(J) CALTIME	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
0 Control	1	2	1.015*	.185	.000	.535	1.496
		3	.652	.291	.108	-.106	1.409
	2	1	-1.015*	.185	.000	-1.496	-.535
		3	-.364	.228	.377	-.957	.230
	3	1	-.652	.291	.108	-1.409	.106
		2	.364	.228	.377	-.230	.957
1 Treatment	1	2	1.264*	.177	.000	.804	1.724
		3	1.528*	.279	.000	.803	2.253
	2	1	-1.264*	.177	.000	-1.724	-.804
		3	.264	.218	.721	-.304	.832
	3	1	-1.528*	.279	.000	-2.253	-.803
		2	-.264	.218	.721	-.832	.304

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Figure 5. Comparison of mean CAL value across time and group

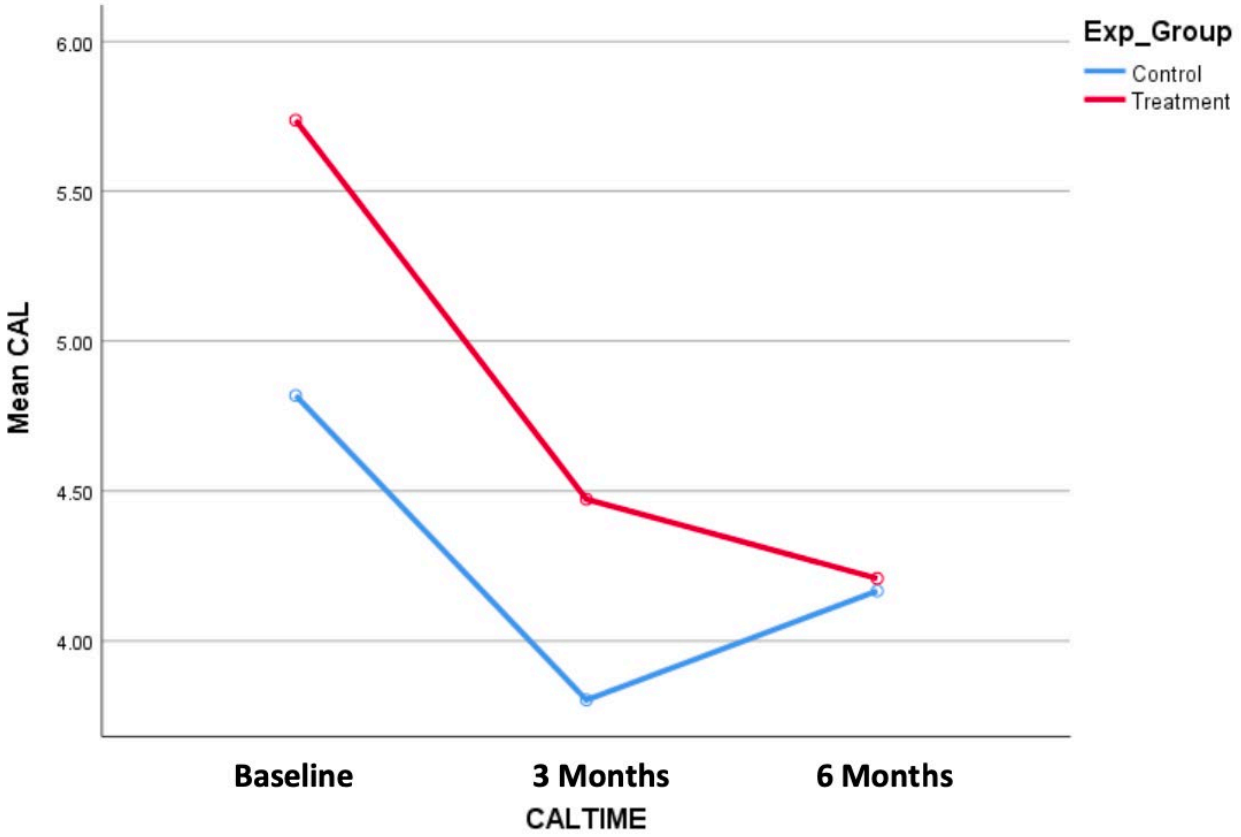


Table 17. Comparison of mean CAL value across time and group

CAL Time	Control (m, SE)	Treatment (m,SE)	sig
Baseline	4.82 (0.32)	5.74 (0.31)	p= .052
3 months	3.80 (0.35)	4.47 (0.32)	p=.178
6 months	4.17 (0.37)	4.21 (0.35)	p=.936

Figure 6. Comparison of overall CAL change across time and group

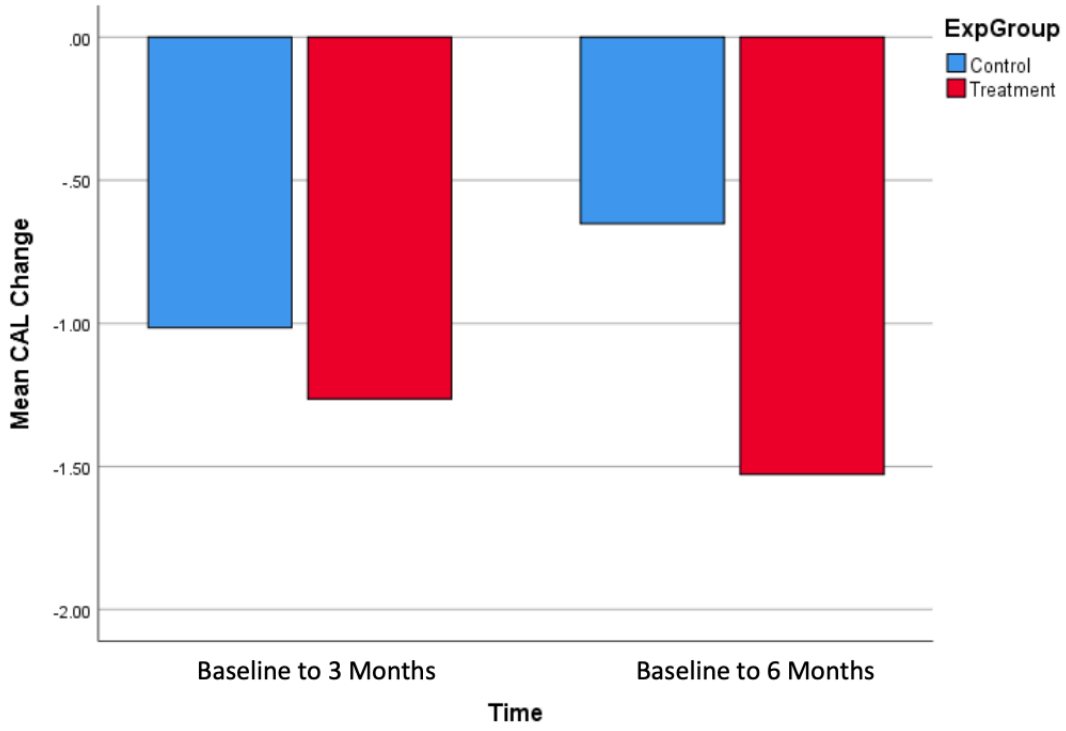


Table 18. Comparison of mean CAL change across groups

Comparison	Control	Treatment	Control vs Treatment Change (sig)
CAL Baseline to 3 Months	B=4.818 3Month=3.803 **Change= -1.015**	B=5.736 3Month=4.472 **Change= -1.264**	-.249 (p=.34)
CAL Baseline to 6 Months	B=4.818 6Month=4.167 **Change= -0.652**	B=5.736 6Month=4.208 **Change= -1.528**	-.876 (p=.041)

Linear mixed model used.

Discussion

The dual presence of bleeding at residual pockets places a greater risk for tooth loss over time,[34,42] therefore, these repeatedly inflamed sites were the focus of our study. Additionally, the absence of bleeding has a high predictive value indicating periodontal stability[93, 94] and consequently the elimination of BOP was our primary outcome.

The results showed that generally, both SRP alone and adjunctive aPDT decreased bleeding at the specified teeth in a significant manner. However, the teeth treated with aPDT mostly did not have statistically significantly more BOP reductions than those that were only given the placebo sterile saline, 6 months after the initial treatment in maintenance care (with the exception of the DL site). Overall, even though there were significant reductions after intervention, there were largely no differences between the treatments that patients received. This is in agreement with other studies that tested adjunctive aPDT as an initial treatment for chronic periodontitis with three[96] and six-month follow-up periods.[84, 97, 98] Balata et al[97] stated that the absence of additional benefits with aPDT in their study could be explained by the significant improvements observed in the control group, including the good plaque control maintained during the entire observation period. They observed that studies showing benefits of the aPDT usually presented poor results of the control treatment when compared to their findings. Our findings at the tested teeth also showed that the control group significantly reduced their plaque at certain sites, whereas there were no such reductions in the treatment group. Furthermore, the patient's personal oral hygiene plays an important role in their periodontal health, as found by Lindhe et al.[14] They reported that at 5 years following periodontal therapy (both surgical and non-surgical), a high frequency of plaque-free tooth surfaces was associated with periodontal health whereas patients with a high

frequency of plaque covered surfaces had greater rates of attachment loss. Therefore, the absence of significant reductions of plaque in the treatment group may have contributed to the lack of additional superior results.

The AAP best evidence review of antimicrobial therapy[73] concluded that aPDT did not promote significant additional improvements in residual sites compared to debridement alone. PD and CAL improvements were not significant in several studies that were reviewed.[87, 99, 100] Furthermore, it states that expert opinion questions its use, evidence is lacking, and the level of certainty is low for use in residual sites after active therapy and during periodontal maintenance. However, note that some of the included studies in their review were using aPDT as a monotherapy,[88, 100] as opposed to an adjunctive treatment. Fontana et al[101] evaluated the in vitro effect of a single PDT episode on plaque bacteria in suspension and under biofilm conditions. They reported a bacterium killing capacity of 63% under suspension conditions and only 32% under biofilm conditions, which suggests the importance of biofilm disturbance in the effectiveness of PDT. In addition, Anderson et al[82] demonstrated that the combined approach of aPDT + SRP showed greater CAL gains when compared to the separate treatments used as monotherapies. This may explain the lack of significant improvement of clinical measures in these included studies for review. Also note that, it is extremely difficult to analyze all "photodynamic therapy" studies in periodontics together as there are so many individual factors in different protocols that are variable. Differences in treatment parameters such as laser light source, power level, exposure time, energy density, type of wave energy (pulsed or continuous), angle and amount of contact between fiber tip and the target tissue, amount of laser tip mobility

while activated, concentration and type of photosensitizer, and number of applications may all account for the diverse results found in different studies and reviews.[73, 102]

In contrast, our findings also showed that adjunctive aPDT resulted in significantly greater mean PD reduction and CAL gain measurements at 6 months (1.41mm v 0.60mm and 1.53 v 0.65mm, respectively) when compared to the control group. Similar positive outcomes have been demonstrated in the literature for initial therapy[79, 82, 83] as well as in residual sites during supportive periodontal therapy,[85, 86] further supporting our results. In the abovementioned maintenance studies,[85, 86] their inclusion criteria consisted of non-smoking patients with single rooted teeth, that had bleeding pockets and ≥5mm probing depths, in their trials. These patients underwent debridement and a single session of aPDT, but additionally were reassessed and had supragingival prophylaxis more often than a regular maintenance schedule (every 7-15 days during the first month, and then monthly until completion of the studies at 3 months). This increased plaque control regimen may have helped with their significant additional PD reduction (2.17 v 1.14 mm;[85] 2.30 v 1.0mm[86]) and CAL gain (1.43 v 0.51mm;[85] 1.30 v 0.30mm[86]). Additionally, there was a reduction in the percentage of sites that had ≥5mm PD with BOP when compared to these sites treated by conventional SRP alone (72.22 v 40%).[85] Moreover, the exclusion of current smokers and multirooted teeth may have improved their response to treatment. Other trials[87, 99] which did not restrict participants based on smoking status or tooth type, found that residual sites in maintenance care did not significantly improve with the addition of aPDT to SRP. Note that when Pihlstrom et al[18] compared molars and non-molar teeth over 6.5 years, they found that periodontal pockets associated with multi-rooted teeth responded less favorably to SRP than pockets at non-molar sites. Additionally, Matuliene et al[42] found that after

11 years of maintenance, 22.9% of non-smokers, 12% of smokers, and 0% of heavy smokers were free from residual pockets, indicating that persisting pockets was related to smoking cigarettes. Therefore, there is literature to support that these factors could have negatively influenced those results. However, despite our study including these patients, a SS improvement of PD reduction and CAL gain was found when adjunctive aPDT was applied to these residual sites.

Therefore, according to our results, there is further evidence supporting a potential benefit for aPDT in periodontal treatment. Its unique localized destructive effect on periopathogenic bacteria, even when organized in biofilm,[67] can lower or eliminate bacterial thresholds, to the point where periodontal tissue deterioration may not occur. Aside from its antimicrobial effect, there may also be biological effects, known as photobiomodulation, that can assist in healing.[103] Aoki et al[104] reviewed how the promotion of proliferation and differentiation of periodontal cells is advantageous for early wound healing. According to several in vitro and in vivo studies, activation and proliferation of human gingival fibroblasts, PDL cells, osteoblasts, and mesenchymal stem cells, release of growth factors, as well as the suppression of proinflammatory cytokines that modulate the host response, were enhanced by low-level laser irradiation. Clinical trials furthered this hypothesis by showing improved clinical parameters and inflammatory mediator levels. In their summary, they stated that lasers are expected to help tissues in an inflamed or damaged state enter the healing and regenerative phases rapidly by thorough debridement and decontamination of diseased tissues, and by modulating or activating cell metabolism in the surrounding tissues.

Caution still has to be exercised when interpreting our overall results as this study has several limitations. Even though the original protocol was designed for a one-year follow-up, sufficient data for statistical analysis was only available up to 6 months, making it a shorter-term study. To recruit as many patients as possible, the exclusion criteria did not specify tooth type or smoking status. The specific type of previous periodontal surgery was also not differentiated. Our participant sample pool was heterogenous in this respect of characteristics but did not have enough participants to do meaningful separate analyses of each group. Also, a parallel arm design was chosen, again due to a limited number of available patients. Lastly, trying to compare studies is extremely difficult due to differences in laser treatment parameters such as light source, power level, exposure time, energy density, type of wave energy (pulsed or continuous), angle and amount of contact between fiber tip and the target tissue, amount of laser tip mobility while activated, concentration and type of photosensitizer, and number of applications. The large variability of aPDT aspects in the literature may account for the range of results that have been found. In the future, more stringent grouping and review of identical protocols should be performed, with meta-analyses once the number of studies allow for it. A comparison among the different protocols should be completed to determine which allows for the best results. An ideal standardized aPDT protocol should be established, which can then be further reviewed and analyzed with future recommendations made with stronger evidence.

Conclusion

Within the limits of this randomized clinical trial, it can be concluded that adjunctive antimicrobial photodynamic therapy significantly improves probing depth and clinical attachment level at residual pockets in maintenance care that were previously treated in surgery, when compared to scaling and root planing treatment alone. For residual pockets in previously surgically treated teeth, clinicians may consider this alternative intervention.

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Appendix 1 – Certificate of Final Approval for New Studies

UNIVERSITY OF MANITOBA | Research Ethics and Compliance

Research Ethics - Bannatyne
P126-770 Bannatyne Avenue
Winnipeg, MB
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Fax +204-789-3414

BIOMEDICAL RESEARCH ETHICS BOARD (BREB)
CERTIFICATE OF FINAL APPROVAL FOR NEW STUDIES
Full Board Review

PRINCIPAL INVESTIGATOR:	INSTITUTION/DEPARTMENT:	ETHICS #:
Dr. Javier Cabrales	U of M/Dentristry/Dental Diagnostic and Surgical Sciences/Periodontics	HS22197 (B2018:102)
BREB MEETING DATE:	**APPROVAL DATE:**	**EXPIRY DATE:**
September 24, 2018	December 3, 2018	September 24, 2019
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE:
NA	The efficacy of adjunctive antimicrobial photodynamic therapy for residual pockets In previously surgically treated teeth: a randomized clinical trial
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: NA	

Submission Date(s) of Investigator Documents:	REB Receipt Date(s) of Documents:
August 30 and November 23, 2018	September 4 and November 30, 2018

THE FOLLOWING ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol:		
Protocol including Clarifications as per Letter dated November 23, 2018		30/08/2018
Consent and Assent Form(s):		
Research Participant Information and Consent Form		23/11/2018
Other:		
Research Data Form - Baseline Form		23/11/18
Research Data Form - 3-Month Form		23/11/18
Research Data Form - 6-Month Form		23/11/18
Research Data Form - 12-Month Form		23/11/18
Master List		23/11/18

CERTIFICATION
The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the research study/project named on this ***Certificate of Final Approval*** at the ***full board meeting*** date noted above and was found to be acceptable on ethical grounds for research involving human participants. The study/project and documents listed above was granted final approval by the Chair or Acting Chair, UM BREB.

BREB ATTESTATION
The University of Manitoba (UM) Biomedical Research Board (BREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba.

In respect to clinical trials, the BREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

QUALITY ASSURANCE
The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

CONDITIONS OF APPROVAL:

1. The study is acceptable on scientific and ethical grounds for the ethics of human use only. ***For logistics of performing the study, approval must be sought from the relevant institution(s).***
2. This research study/project is to be conducted by the local principal investigator listed on this certificate of approval.
3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of approval. A Bannatyne Campus Annual Study Status Report** must be submitted to the REB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the BREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form.**
6. Adverse events and unanticipated problems must be reported to the REB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM BREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report**.

Sincerely,

K Nicolle

Lindsay Nicolle, MD, FRCPC
Chair, Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above Human Ethics Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255/ **Fax:** (204) 789-3414

Appendix 2 – Certificate of Annual Approval

UNIVERSITY OF MANITOBA | Research Ethics and Compliance

Research Ethics - Bannatyne
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BIOMEDICAL RESEARCH ETHICS BOARD (BREB)

CERTIFICATE OF ANNUAL APPROVAL

PRINCIPAL INVESTIGATOR: Dr. Javier Cabrales	INSTITUTION/DEPARTMENT: U of M/Dentistry/Dental Diagnostic and Surgical Sciences/Periodontics	ETHICS #: HS22197 (B2018:102)
BREB MEETING DATE (If applicable):	APPROVAL DATE: September 16, 2019	EXPIRY DATE: **September 24, 2020**
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER: NA	PROJECT OR PROTOCOL TITLE: The efficacy of adjunctive antimicrobial photodynamic therapy for residual pockets In previously surgically treated teeth: a randomized clinical trial
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: NA	

Submission Date of Investigator Documents: July 31, 2019	BREB Receipt Date of Documents: July 31, 2019

REVIEW CATEGORY OF ANNUAL REVIEW: Full Board Review ☐ Delegated Review ☒

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name(if applicable)	Version(if applicable)	Date

Annual approval

*Annual approval implies that the most recent **BREB approved** versions of the protocol, Investigator Brochures, advertisements, letters of initial contact or questionnaires, and recruitment methods, etc. are approved.*

Consent and Assent Form(s):

CERTIFICATION

The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the annual study status report for the research study/project named on this ***Certificate of Annual Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. Annual approval was granted by the Chair or Acting Chair, UM BREB, per the response to the conditions of approval outlined during the initial review (full board or delegated) of the annual study status report.

BREB ATTESTATION

The University of Manitoba (UM) Biomedical Research Board (BREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba. In respect to clinical trials, the BREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

QUALITY ASSURANCE
The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

CONFLICT OF INTEREST
Any Principal or Co-Investigators of this study who are members of the UMBREB did not participate in the review or voting of this study.

CONDITIONS OF APPROVAL:

1. The study is acceptable on scientific and ethical grounds for the ethics of human use only. ***For logistics of performing the study, approval must be sought from the relevant institution(s).***
2. This research study/project is to be conducted by the local principal investigator listed on this certificate of approval.
3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of annual approval. A Bannatyne Campus Annual Study Status Report** must be submitted to the REB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the BREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form.**
6. Adverse events and unanticipated problems must be reported to the REB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM BREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report**.

Sincerely,

Lindsay Nicolle, MD, FRCPC
Chair, Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above Human Ethics Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone:** (204) 789-3255/ **Fax:** (204) 789-3414

Appendix 3 – Informed Consent Form

UNIVERSITY OF MANITOBA

GRADUATE PERIODONTICS CLINIC
College of Dentistry, Faculty of Health Sciences,
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FACULTY OF DENTISTRY
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RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

The efficacy of adjunctive antimicrobial photodynamic therapy for residual pockets in previously surgically treated teeth: a randomized clinical trial.

Principal Investigators: Dr. Javier Cabrales, Dr. Anastasia Kelekis-Cholakis
Dental Diagnostic and Surgical Sciences, Periodontics
University of Manitoba
D343-780 Bannatyne Ave

You are being asked to participate in a Clinical Trial (a human research study). Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends, and family before you make your decision. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any words or information that you do not clearly understand.

WHAT IS THE RESEARCH ABOUT?

This Clinical Trial is being conducted to study whether bacteria-targeting photodynamic therapy (the Periowave laser system) is beneficial for recurrent (repeating) pockets that were surgically treated in the past. You are being asked to take part in this study because you are a patient of the Graduate Periodontics Clinic, College of Dentistry, University of Manitoba and have at least one site that has been previously treated with surgery but has a pocket that is still inflamed. A total of 28 participants will take part in this study.

The purpose of this study is to compare the effects of bacteria-targeting photodynamic (laser) therapy in addition to hand scaling and root planing (cleaning) vs hand scaling and root planing (cleaning) alone. This research is being done because currently, there are no studies that have looked into whether photodynamic (laser) therapy is beneficial for repeating pockets in surgically treated periodontal sites. **This study is voluntary. If you decide not to participate in the study or withdraw from the study, your normal dental care will not be affected in any way.**

AM I ELIGIBLE TO PARTICIPATE?

Healthy patients (in other words, not having uncontrolled diabetes mellitus, cancer, HIV, bone metabolic diseases, or disorders that compromise wound healing) of the Graduate Periodontics Clinic, College of Dentistry, University of Manitoba with at least one surgically treated site with a pocket probing depth of ≥5 mm and bleeding on probing are invited to participate in this study. Unfortunately, if you have a confirmed or suspected allergy or hypersensitivity to methylene blue, or you have had systemic antibiotics in the last three months, you will not be allowed to participate.

WHAT WILL I HAVE TO DO?

In this study, each patient will be "randomized" into one of two study groups described below. "Randomized" means that you are put into a group by chance, like flipping a coin. One group will be placed in the "treatment group" where the selected site will be treated with a cleaning and the Periowave laser system. The other group will be placed in a "placebo group" where the selected site will be treated with a cleaning and a false (inactivated) laser treatment. Please note that neither you nor the hygienist will know which group you are in, as part of the study design to avoid bias.

At the first appointment, the dental hygienist will record all the data on all the teeth and provide the cleaning. Afterwards, the resident clinician will provide the activated (Periowave laser) or inactivated laser therapy on the selected tooth, depending on the group you are placed in. The same therapy will be repeated one week later at a second appointment. There will be a liquid that is added to the selected gum pocket, and then a laser is placed inside this pocket for one full minute. You will have freezing in the area being treated, as is routine for this procedure, and will experience no pain during these appointments.

You will also have the site re-evaluated at 3, 6, and 12 months by the dental hygienist at the Graduate Periodontics Clinic as part of your cleanings. You will also receive oral hygiene instructions by the hygienist at each appointment. You will be provided with a home care kit with a new toothbrush, interdental aids, and toothpaste. A full mouth exam is required prior to the start of the treatment and after 12 months and full mouth cleanings are necessary every 3 months.

The researcher may decide to take you off this study if funding is stopped, new information becomes available, or you become medically compromised during the study. You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the study staff and your regular dentist first.

WHAT ARE THE POSSIBLE HARMS OR BENEFITS?

Regular periodontal maintenance (cleaning) is recommended for patients who have received periodontal surgery. This service is being provided regardless of which group you will be placed in. There is no known harm to the photodynamic therapy provided. The additional therapy may or may not provide a benefit to the recurrent pocket.

Minimal side effects may be observed after both therapies. Some temporary side effects may include minor bleeding, sore gums and potential sensitivity to cold or hot. Sometimes localized gum recession may occur as a result of the decrease in inflammation.

PAYMENT AND PARTICIPATION

This is a research study, so you may not personally benefit by participating in this study. However, the last hygiene appointment will be provided at no cost. All your cleaning aids (toothbrushes, toothpaste and interdental aids) will be provided to you at no cost. Eventually, the results of this study may benefit you and future patients by determining whether photodynamic (laser) therapy may be beneficial to repeating pockets of previously treated surgical sites.

WHAT IS THE COST OF THE STUDY?

All clinic and professional fees used in this study fall under the standard clinical protocol and fee guide and will be at no additional cost to you. A full periodontal exam is required prior to treatment and after 12 months, as well as cleanings every 3 months.
You will be responsible for the cost of the cleaning and full exam appointments at the University of Manitoba Graduate Periodontics Clinic. However, you will not be responsible for the photodynamic (laser) therapy.

IS THE STUDY CONFIDENTIAL?

Information gathered in this research study may be published or presented in public forums; however your name and other identifying information will not be used or revealed. Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. All study documents related to you will bear only your assigned patient code/number instead of your name. Only your file marked with your specific code will be kept securely in an office safe at the Graduate Periodontics Clinic. Data based on your clinical measurements will be entered into the computer and transmitted electronically based on your patient code. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Biomedical Research Ethics Board (BREB) at the University of Manitoba.

The University of Manitoba Biomedical Research Ethics Board may review research-related records for quality assurance purposes. If any of your medical/research records need to be copied to any of the above, your name and all identifying information will be removed. No information revealing any personal information such as your name, address or telephone number will leave the Graduate Periodontics Clinic.

WHAT ELSE SHOULD I KNOW?
You have the right to withdraw from the study at any time. The Investigators reserve the right to end your participation for any reason. If the person withdraws, the measurements already taken (if any) will be discarded. You are entitled to know the scientific and technical results at the end of the research project and may request that a copy of any reports be sent to you upon completion of the study.

The data collected from you during this study may be shared in an anonymized (de-identified) form to academic journals for publication purposes. The data may also be stored by the academic journal under an open access policy in which case it may be used by other researchers for further data analysis and research purposes.

WHO CAN I CONTACT FOR MORE INFORMATION?
You are free to ask any questions that you may have about your treatment and your rights as a research participant. If any questions come up during or after the study or if you have a research-related injury, contact:

The Bannatyne Campus Research Ethics Office
University of Manitoba
(204) 789 - 3389

In addition, ClinicalTrials.gov is a website that provides information about federally and privately supported clinical trials. A description of this clinical trial will be available on http://ClinicalTrials.gov.
This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

CONSENT FORM

I have read this consent form. I have had the opportunity to discuss this research study with Dr. Cabrales and his study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statement or implied statements. Any relationship (such as employee, student or family member) I may have with the study team has not affected my decision to participate. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this clinical trial is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of my medical records by The University of Manitoba Biomedical Research Ethics Board.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study. I understand that I can end my participation at any time and for any reason and that this will not affect my care at the Graduate Periodontal Clinic. I agree to participate in the research protocol for "The efficacy of adjunctive antimicrobial photodynamic therapy for residual pockets in previously surgically treated teeth: a randomized clinical trial."

Participant printed name: _____ Date _____
(day/month/year)

Participant signature: _____

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: _____ Date _____
(day/month/year)

Signature: _____

Appendix 4 – Summary of Data

ID	Exp	FMPI_BL	FMPI_F	FMBOP_BL	FMBOP_F	PD(4-5)_BL	PD(4-5)_F	PD(6+)_BL	PD(6+)_F
1	T	51%	7%	16%	10%	43	12 sites	3	0 sites
2	T	9%	20%	3%	1%	12	23 sites	1	0 sites
3	T	23%	7%	10%	3%	30	25 sites	3	0 sites
4	C	28%	17%	18%	1%	42	20 sites	1	0 sites
5	C	19%		40%		29		3	
6	T	63%	69%	26%	1%	23	6 sites	0	0 sites
7	T	2%	4%	9%	5%	19	15 sites	4	0 sites
8	T	43%		16%		3		1	
9	T	13%		13%		6		0	
10	T	42%		20%		28		0	
11	C	80%		21%		68		17	
12	C	17%		7%		19		1	
13	T	4%		2%		5		1	
14	C	17%		9%		10		0	
15	C	32%		24%		23		1	
16	C	52%		20%		47		7	
17	T	54%		49%		17		0	
18	C	56%		48%		38		7	
19	C	1%		1%		9		0	
20	T	19%		31%		16		0	
21	C	49%		23%		50		5	
22	C	36%		32%		23		1	
23	T	45%		19%		47		4	
24	T	1%		15%		18		7	

ID	SSPI_DB_BL	SSPI_DB_3	SSPI_DB_6	SSPI_DB_F	SSPI_B_BL	SSPI_B_3	SSPI_B_6	SSPI_B_F	SSPI_MB_BL
1	1	0	1	0	0	0	0	0	1
2	1	0	0	0	0	0	0	1	0
3	1	0	0	0	0	0	0	0	0
4	1	1	0	0	1	0	0	0	1
5	1	0	0		1	0	0		0
6	1	0	1	1	1	1	0	0	1
7	0	1	1	0	0	0	0	0	0
8	1	1	1		1	1	1		1
9	0	0	0		0	0	0		0
10	1	1	0		1	1	1		1
11	1	1	1		0	0	1		1
12	0	0	0		0	0	0		0
13	0	0	0		0	0	0		0
14	0	0	0		0	0	0		0
15	0	0	0		0	0	0		1
16	1	0	0		1	0	0		0
17	0	1			0	1			1
18	0	1	0		1	0	0		1
19	0	0	0		0	0	0		0
20	1	1	1		0	1	1		1
21	1	1	0		0	0	0		1
22	1	0	0		0	0	0		0
23	1	1	0		0	0	0		0
24	1	0	0		0	0	0		1

ID	SSPI_MB_3	SSPI_MB_6	SSPI_MB_F	SSPI_DL_BL	SSPI_DL_3	SSPI_DL_6	SSPI_DL_F	SSPI_L_BL	SSPI_L_3
1	0	0	0	1	0	1	1	1	1
2	0	0	0	1	0	0	0	0	0
3	0	0	0	1	0	0	0	0	0
4	1	0	1	1	1	0	0	0	0
5	0	0		0	0	0		1	0
6	1	0	1	1	1	1	1	1	1
7	0	1	1	0	1	0	0	0	0
8	0	1		0	0	1		0	0
9	0	0		0	0	0		1	0
10	0	0		1	0	0		0	0
11	1	1		1	1	0		0	0
12	0	0		0	0	1		0	0
13	0	0		1	0	0		1	0
14	0	0		1	1	1		1	1
15	0	0		1	0	1		1	0
16	0	0		0	1	1		1	1
17	1			1	1			1	1
18	1	0		1	0	1		1	0
19	0	0		0	0	0		0	0
20	1	0		1	1	1		0	1
21	1	1		1	1	0		0	1
22	0	0		1	0	1		0	0
23	0	0		1	1	0		0	1
24	0	1		1	0	0		0	0

ID	SSPI_L_6	SSPI_L_F	SSPI_ML_BL	SSPI_ML_3	SSPI_ML_6	SSPI_ML_F	SSPD_DB_BL	SSPD_DB_3	SSPD_DB_6
1	1	1	1	0	1	1	6mm	2mm	3mm
2	0	1	0	1	0	0	3mm	3mm	4mm
3	0	0	1	1	0	0	5mm	4mm	4mm
4	0	0	1	1	0	0	4mm	4mm	3mm
5	0		0	0	0		4mm	3mm	3mm
6	0	1	1	1	1	1	2mm	3mm	2mm
7	0	0	0	0	0	1	4mm	3mm	2mm
8	1		0	0	1		5mm	3mm	2mm
9	1		1	0	1		3mm	3mm	3mm
10	1		1	0	1		5mm	4mm	2mm
11	1		1	1	0		8mm	4mm	6mm
12	1		1	0	1		5mm	4mm	4mm
13	0		1	0	0		3mm	2mm	2mm
14	0		1	1	0		3mm	3mm	4mm
15	1		0	0	0		4mm	4mm	2mm
16	1		1	1	0		6mm	6mm	5mm
17			1	1			3mm	3mm	
18	1		1	0	0		4mm	2mm	5mm
19	0		1	0	0		5mm	4mm	4mm
20	0		0	1	0		5mm	4mm	3mm
21	1		1	1	1		4mm	5mm	6mm
22	1		0	0	0		5mm	3mm	5mm
23	0		0	0	0		7mm	6mm	5mm
24	0		0	0	0		9mm	4mm	4mm

ID	SSPD_DB_F	SSPD_B_BL	SSPD_B_3	SSPD_B_6	SSPD_B_F	SSPD_MB_BL	SSPD_MB_3	SSPD_MB_6	SSPD_MB_F
1	3mm	3mm	2mm	3mm	2mm	3mm	3mm	3mm	3mm
2	4mm	2mm	2mm	3mm	4mm	3mm	4mm	3mm	3mm
3	4mm	3mm	2mm	3mm	2mm	5mm	3mm	3mm	4mm
4	2mm	3mm	2mm	2mm	2mm	5mm	4mm	4mm	2mm
5		3mm	2mm	2mm		7mm	4mm	4mm	
6	2mm	3mm	2mm	2mm	1mm	5mm	3mm	3mm	3mm
7	3mm	4mm	2mm	2mm	2mm	6mm	4mm	3mm	4mm
8		3mm	2mm	2mm		5mm	3mm	3mm	
9		2mm	2mm	2mm		4mm	3mm	4mm	
10		5mm	3mm	3mm		5mm	2mm	3mm	
11		4mm	2mm	2mm		6mm	7mm	7mm	
12		2mm	2mm	2mm		3mm	3mm	3mm	
13		2mm	3mm	1mm		5mm	5mm	5mm	
14		2mm	2mm	3mm		3mm	3mm	3mm	
15		3mm	2mm	3mm		5mm	3mm	5mm	
16		2mm	3mm	2mm		3mm	3mm	2mm	
17		2mm	3mm			5mm	4mm		
18		3mm	3mm	4mm		7mm	5mm	5mm	
19		3mm	2mm	2mm		5mm	4mm	4mm	
20		4mm	2mm	2mm		3mm	2mm	2mm	
21		2mm	2mm	2mm		4mm	3mm	5mm	
22		3mm	2mm	3mm		3mm	4mm	3mm	
23		3mm	2mm	2mm		4mm	3mm	3mm	
24		3mm	1mm	1mm		2mm	2mm	2mm	

ID	SSPD_DL_BL	SSPD_DL_3	SSPD_DL_6	SSPD_DL_F	SSPD_L_BL	SSPD_L_3	SSPD_L_6	SSPD_L_F	SSPD_ML_BL
1	6mm	3mm	3mm	5mm	6mm	5mm	3mm	5mm	5mm
2	6mm	3mm	4mm	5mm	3mm	2mm	2mm	2mm	4mm
3	6mm	3mm	4mm	3mm	4mm	2mm	3mm	4mm	6mm
4	5mm	4mm	4mm	5mm	4mm	3mm	3mm	2mm	5mm
5	4mm	5mm	4mm		4mm	5mm	4mm		5mm
6	4mm	3mm	3mm	2mm	3mm	3mm	2mm	1mm	5mm
7	4mm	3mm	4mm	3mm	3mm	2mm	2mm	3mm	6mm
8	8mm	4mm	6mm		8mm	6mm	7mm		8mm
9	3mm	3mm	3mm		2mm	2mm	2mm		5mm
10	4mm	4mm	2mm		2mm	3mm	2mm		3mm
11	7mm	4mm	5mm		4mm	2mm	3mm		7mm
12	6mm	5mm	4mm		3mm	2mm	3mm		5mm
13	3mm	2mm	2mm		3mm	2mm	1mm		6mm
14	5mm	4mm	5mm		3mm	2mm	3mm		4mm
15	6mm	4mm	4mm		2mm	2mm	2mm		4mm
16	7mm	5mm	6mm		4mm	3mm	3mm		4mm
17	5mm	3mm			2mm	3mm			4mm
18	6mm	4mm	5mm		4mm	4mm	5mm		5mm
19	4mm	3mm	3mm		3mm	2mm	2mm		5mm
20	5mm	4mm	4mm		3mm	2mm	2mm		4mm
21	6mm	5mm	6mm		6mm	3mm	3mm		5mm
22	6mm	3mm	5mm		3mm	3mm	5mm		4mm
23	9mm	5mm	5mm		3mm	3mm	2mm		4mm
24	8mm	5mm	4mm		5mm	3mm	2mm		2mm

ID	SSPD_ML_3	SSPD_ML_6	SSPD_ML_F	SSBOP_DB_BL	SSBOP_DB_3	SSBOP_DB_6	SSBOP_DB_F
1	4mm	3mm	4mm	Y	N	Y	N
2	5mm	3mm	3mm	Y	N	N	N
3	3mm	4mm	5mm	Y	N	N	N
4	4mm	4mm	4mm	N	N	N	N
5	4mm	4mm		N	N	N	
6	3mm	3mm	3mm	N	N	N	N
7	4mm	3mm	4mm	N	N	N	N
8	4mm	4mm		N	N	N	
9	3mm	5mm		N	N	N	
10	2mm	2mm		Y	N	N	
11	6mm	6mm		Y	N	Y	
12	3mm	3mm		N	N	N	
13	5mm	5mm		N	N	N	
14	4mm	4mm		N	N	N	
15	2mm	3mm		Y	Y	N	
16	2mm	2mm		Y	N	Y	
17	3mm			Y	Y		
18	5mm	4mm		N	Y	Y	
19	2mm	2mm		N	N	N	
20	2mm	2mm		Y	Y	Y	
21	4mm	5mm		N	Y	Y	
22	4mm	5mm		Y	N	N	
23	3mm	3mm		Y	N	N	
24	2mm	2mm		Y	Y	N	

ID	SSBOP_B_BL	SSBOP_B_3	SSBOP_B_6	SSBOP_B_F	SSBOP_MB_BL	SSBOP_MB_3
1	N	N	N	N	N	N
2	N	N	N	N	N	N
3	N	N	N	N	N	N
4	N	N	N	N	Y	N
5	N	N	N		Y	N
6	N	N	N	N	Y	N
7	N	N	Y	N	Y	N
8	N	N	N		Y	N
9	N	N	N		N	N
10	Y	N	Y		Y	N
11	Y	N	Y		Y	Y
12	N	N	N		N	N
13	N	N	N		Y	Y
14	N	N	N		N	N
15	N	N	N		Y	N
16	N	N	N		N	N
17	Y	N			Y	Y
18	N	N	Y		Y	Y
19	N	N	N		Y	N
20	Y	N	N		N	N
21	N	N	N		N	N
22	N	N	N		Y	N
23	N	N	N		N	N
24	N	N	N		N	N

ID	SSBOP_MB_6	SSBOP_MB_F	SSBOP_DL_BL	SSBOP_DL_3	SSBOP_DL_6	SSBOP_DL_F
1	N	N	Y	N	N	Y
2	N	N	Y	N	N	N
3	Y	N	Y	Y	N	Y
4	N	N	N	N	N	N
5	N		Y	Y	Y	
6	Y	N	Y	N	N	N
7	N	Y	N	N	N	N
8	N		Y	N	N	
9	N		N	N	N	
10	Y		Y	Y	N	
11	Y		N	N	N	
12	N		Y	Y	N	
13	N		N	N	N	
14	N		Y	N	N	
15	N		Y	N	N	
16	N		Y	N	Y	
17			Y	Y		
18	N		Y	N	N	
19	N		N	N	N	
20	N		Y	Y	N	
21	N		Y	Y	Y	
22	Y		Y	N	Y	
23	N		Y	N	N	
24	N		Y	Y	N	

ID	SSBOP_L_BL	SSBOP_L_3	SSBOP_L_6	SSBOP_L_F	SSBOP_ML_BL	SSBOP_ML_3
1	Y	N	N	Y	N	N
2	N	N	N	N	N	Y
3	N	N	N	Y	Y	Y
4	N	N	N	N	Y	N
5	Y	Y	Y		Y	Y
6	Y	N	N	N	Y	N
7	N	N	N	Y	Y	N
8	Y	N	N		Y	N
9	Y	N	Y		Y	N
10	N	Y	N		Y	Y
11	N	Y	N		N	Y
12	N	N	N		N	N
13	N	N	N		Y	Y
14	Y	N	N		Y	N
15	N	N	N		Y	N
16	N	N	N		N	N
17	N	N			Y	Y
18	Y	N	N		Y	N
19	N	N	N		Y	N
20	Y	N	N		N	N
21	N	N	N		Y	N
22	Y	N	Y		Y	N
23	N	Y	N		Y	Y
24	N	N	N		N	N

ID	SSBOP_ML_6	SSBOP_ML_F	SSCAL_DB_BL	SSCAL_DB_3	SSCAL_DB_6	SSCAL_DB_F	SSCAL_B_BL
1	N	Y	6mm	2mm	3mm	3mm	3mm
2	N	N	9mm	8mm	9mm	7mm	6mm
3	N	Y	6mm	4mm	4mm	5mm	5mm
4	N	N	5mm	5mm	4mm	3mm	4mm
5	Y		4mm	4mm	3mm		3mm
6	N	N	5mm	5mm	5mm	5mm	7mm
7	N	Y	4mm	6mm	6mm	3mm	4mm
8	N		7mm	4mm	4mm		4mm
9	Y		5mm	5mm	5mm		3mm
10	N		9mm	8mm	8mm		8mm
11	N		8mm	4mm	6mm		4mm
12	N		6mm	5mm	4mm		3mm
13	N		3mm	2mm	2mm		2mm
14	N		3mm	3mm	4mm		2mm
15	N		6mm	6mm	4mm		5mm
16	N		8mm	7mm	6mm		2mm
17			6mm	5mm			6mm
18	N		4mm	2mm	5mm		3mm
19	N		5mm	4mm	4mm		3mm
20	N		5mm	4mm	4mm		4mm
21	N		4mm	5mm	6mm		4mm
22	N		6mm	3mm	6mm		3mm
23	Y		8mm	7mm	5mm		4mm
24	N		11mm	6mm	4mm		5mm

ID	SSCAL_B_3	SSCAL_B_6	SSCAL_B_F	SSCAL_MB_BL	SSCAL_MB_3	SSCAL_MB_6	SSCAL_MB_F	SSCAL_DL_BL
1	2mm	3mm	2mm	3mm	3mm	3mm	4mm	6mm
2	5mm	7mm	7mm	5mm	9mm	9mm	6mm	9mm
3	2mm	3mm	4mm	5mm	3mm	3mm	4mm	6mm
4	3mm	3mm	3mm	6mm	5mm	5mm	3mm	5mm
5	2mm	2mm		7mm	3mm	4mm		4mm
6	5mm	5mm	4mm	8mm	6mm	6mm	6mm	7mm
7	3mm	2mm	2mm	6mm	3mm	2mm	4mm	4mm
8	4mm	5mm		5mm	4mm	4mm		8mm
9	3mm	3mm		6mm	5mm	6mm		4mm
10	6mm	8mm		7mm	4mm	7mm		10mm
11	2mm	2mm		6mm	7mm	7mm		7mm
12	3mm	2mm		3mm	3mm	4mm		6mm
13	3mm	1mm		7mm	6mm	6mm		3mm
14	2mm	3mm		4mm	3mm	3mm		5mm
15	5mm	5mm		7mm	5mm	8mm		7mm
16	3mm	2mm		3mm	3mm	2mm		9mm
17	7mm			8mm	6mm			9mm
18	3mm	4mm		7mm	5mm	5mm		6mm
19	2mm	2mm		5mm	4mm	4mm		4mm
20	2mm	2mm		3mm	2mm	2mm		5mm
21	2mm	2mm		4mm	5mm	5mm		6mm
22	2mm	3mm		4mm	4mm	4mm		7mm
23	3mm	2mm		7mm	6mm	3mm		10mm
24	3mm	1mm		4mm	4mm	2mm		9mm

ID	SSCAL_DL_3	SSCAL_DL_6	SSCAL_DL_F	SSCAL_L_BL	SSCAL_L_3	SSCAL_L_6	SSCAL_L_F	SSCAL_ML_BL
1	3mm	3mm	5mm	6mm	5mm	3mm	5mm	5mm
2	6mm	8mm	7mm	6mm	5mm	6mm	5mm	7mm
3	3mm	5mm	3mm	4mm	4mm	5mm	4mm	6mm
4	4mm	4mm	5mm	5mm	4mm	4mm	3mm	5mm
5	4mm	4mm		4mm	5mm	4mm		5mm
6	5mm	6mm	4mm	5mm	6mm	4mm	3mm	7mm
7	4mm	4mm	3mm	6mm	2mm	2mm	6mm	6mm
8	4mm	6mm		8mm	7mm	7mm		8mm
9	4mm	4mm		2mm	3mm	2mm		6mm
10	10mm	4mm		8mm	9mm	5mm		8mm
11	4mm	5mm		4mm	2mm	3mm		7mm
12	5mm	4mm		4mm	3mm	4mm		6mm
13	2mm	2mm		2mm	2mm	1mm		8mm
14	4mm	5mm		3mm	2mm	3mm		4mm
15	5mm	7mm		3mm	4mm	4mm		5mm
16	7mm	6mm		6mm	2mm	3mm		4mm
17	6mm			6mm	6mm			8mm
18	4mm	5mm		4mm	4mm	5mm		5mm
19	3mm	3mm		3mm	2mm	2mm		5mm
20	4mm	4mm		3mm	2mm	2mm		4mm
21	6mm	6mm		6mm	4mm	4mm		5mm
22	3mm	7mm		4mm	3mm	7mm		4mm
23	6mm	6mm		4mm	4mm	3mm		4mm
24	6mm	4mm		6mm	4mm	2mm		4mm

ID	SSCAL_ML_3	SSCAL_ML_6	SSCAL_ML_F
1	4mm	3mm	4mm
2	9mm	6mm	6mm
3	3mm	5mm	5mm
4	4mm	4mm	4mm
5	5mm	4mm	
6	6mm	5mm	4mm
7	3mm	3mm	4mm
8	4mm	4mm	
9	4mm	6mm	
10	7mm	4mm	
11	6mm	6mm	
12	3mm	4mm	
13	7mm	7mm	
14	4mm	4mm	
15	3mm	5mm	
16	2mm	2mm	
17	7mm		
18	5mm	4mm	
19	2mm	2mm	
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23	3mm	4mm	
24	3mm	2mm	

Appendix 5 – Journal Article

TITLE PAGE

The Efficacy of Adjunctive Antimicrobial Photodynamic Therapy for Residual Pockets in Previously Surgically Treated Teeth: A Randomized Clinical Trial

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Photodynamic Therapy for Residual Pockets After Surgery

Adjunctive antimicrobial photodynamic therapy significantly improves PD reduction and CAL gain at previously surgically treated teeth in maintenance care, when compared to mechanical debridement alone.

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ABSTRACT

Background. Residual pockets are a risk for periodontal disease progression and may persist even after surgical treatment. The aim of this study was to assess the clinical effects of adjunctive antimicrobial photodynamic therapy (aPDT) delivered as an adjunct to maintenance therapy, specifically in patients with residual pockets at previously surgically treated teeth.

Methods and Materials. Twenty-three patients on a regular maintenance schedule who had previously received periodontal surgical care were randomly assigned to receive aPDT or a placebo at their first debridement appointment. The teeth selected had to have at least one site with bleeding on probing and a probing depth of ≥5mm. One week later, patients received the same treatment according to their group allocation. These teeth were simultaneously reassessed at their three- and six-month maintenance appointments. The primary outcome measure was bleeding on probing (BOP) and secondary outcome measures were plaque index (PI), probing depth (PD), and clinical attachment level (CAL).

Results. There were statistically significant (SS) improvements for both groups in mean BOP, PD, and CAL. Individual sites in both groups showed SS improvements in BOP, PD, and CAL as well. When comparing the two groups directly, there was a SS increased mean PD reduction and CAL gain in the adjunctive aPDT treatment over debridement only at 6 months. There were no significant differences between the groups for BOP and PI comparisons.

Conclusion. Adjunctive aPDT significantly improves PD and CAL at previously surgically treated teeth in maintenance care, when compared to mechanical debridement alone.

Key Words: photodynamic therapy; chronic periodontitis; residual pocket; periodontal surgery

BACKGROUND

Periodontitis is a chronic multifactorial inflammatory disease characterized by progressive destruction of the tooth-supporting apparatus.[1] Its primary feature is loss of periodontal tissue support due to inflammation, which can be treated by thorough subgingival debridement of the affected sites.[2, 3] However complete elimination of subgingival deposits using closed procedures is difficult and inflammation may persist.[4] Therefore, clinicians may choose to repeat the treatment surgically for better access, particularly in deep initial probing depths. [3, 5-10] Surgical treatment generally provides better short and long-term PD reductions, CAL gain, and tooth retention in patients with advanced periodontal disease.[10, 11] However, a limited number of residual pockets may still persist for a variety of reasons[12] which may be patient-, defect-, or therapist-related.[13]

Residual pockets carry a continuous presence of periodontal pathogens.[14] Residual pocket probing depths were found to be a risk factor for disease progression, and when combined with bleeding on probing, were a risk for tooth loss.[15,16] As evidence corroborates that a residual site is associated with tooth loss in the long term, the aim of periodontal treatment should be closure or elimination of sites with ≥5mm probing depths.[13]

Various alternative and adjunctive antimicrobial regimens have been tested to overcome this issue, such as pocket irrigation, local and systemic antibiotics. Subgingival irrigation is controversial because even though some synergistic effects have been shown, improvements were minimal.[17] Locally delivered antimicrobial adjuncts also only showed modest improvements such as 0.1 – 0.5mm of additional probing depth reduction.[18] In contrast, there is sufficient evidence to support adjunctive systemic antibiotics for significantly improved CAL gain, PD reduction, and decreased BOP[19-21] with a variety of prescriptions. However, the

repeated use of antibiotics to treat residual pockets during maintenance care is not advisable as there is concern that their repeated administration may contribute to the development of antimicrobial resistance.[22] Patients and clinicians should also be well aware of other potential adverse effects of systemic antibiotic usage including gastrointestinal disturbances, allergic reactions, pseudomembranous colitis, and multiple drug interactions.[23] Thus, there is a need to evaluate new protocols that are safe and effective for maintenance, without any adverse effects on host tissues.

One possible alternative is antimicrobial photodynamic therapy (aPDT). First applied in medicine, aPDT is the combination of a diode laser and a nontoxic dye (or photosensitizer) capable of absorbing light and transforming it into lethal cytotoxic agents that can selectively destroy cells.[2, 24] Its mechanism is based on the illumination of the photosensitizer which is converted from the ground state to the triplet state, thus leading to the generation of a cytotoxic species, usually singlet oxygen, which interacts with the surrounding molecules and cells. As singlet oxygen cannot migrate further than 0.02 microns, it only has a local effect and does not damage distant cells or organs.[25] During the process, free oxygen radicals are produced, which react and cause damage to membranes, mitochondria, and DNA, resulting in the death of the microorganisms.[26-28]

There is a growing body of evidence examining the clinical efficacy of aPDT when used as an adjunct to conventional non-surgical treatment of aggressive and chronic periodontitis patients.[29] Specifically, in residual sites after active periodontal therapy & during maintenance, significant clinical and microbiological benefits were also found compared to debridement alone.[30-32] Additionally, improvements in the host response at a local and systemic level have been reported.[33, 34] The importance of the removal of subgingival plaque with the added benefit

of reducing gram-negative anaerobic microorganisms & an exaggerated host response is a reasonable therapeutic step towards achieving periodontal health. Therefore, adjunctive aPDT becomes a particularly attractive option in patients that continue to have residual pockets, even after surgical treatment.

The purpose of this study was to assess the clinical effects of aPDT delivered as an adjunct to maintenance therapy, specifically in patients with residual pockets at previously surgically treated teeth and compare it with patients receiving mechanical debridement only. Since the absence of BOP has high predictive value indicating periodontal stability,[35, 36] this parameter was our primary outcome measure. Secondary measures included PI, PD, and CAL. The hypothesis was that this adjunctive treatment would produce decreased BOP, reduced probing depth, increased attachment gain, and decreased plaque at these residual pocket sites.

MATERIALS AND METHODS

All selected participants were systemically healthy adults in a supportive periodontal therapy program and patients at the University of Manitoba Graduate Periodontics Clinic. They had completed active periodontal therapy, including surgical treatment, with at least one surgically treated site that had a residual pocket probing depth of ≥ 5mm with bleeding on probing. Exclusion criteria included uncontrolled diabetes mellitus, cancer, HIV, bone metabolic diseases or disorders that compromise wound healing, radiation or immunosuppressive therapy, pregnancy or lactation, administration of systemic antibiotics taken within the last three months, confirmed or suspected allergy / hypersensitivity to methylene blue, or restrictions that may preclude normal oral hygiene procedures. Once identified as meeting the inclusion criteria, patients at the clinic were asked to voluntarily participate in the study. Interested subjects were

required to read and sign the informed consent approved, as part of the ethics submission, by the University of Manitoba Research Ethics Board prior to final acceptance into the trial.

Each participant had a full mouth periodontal charting completed prior to baseline measurements as part of their maintenance program. Once selected the patient saw two clinicians: the examiner (the clinic hygienist) and the operator (the periodontal resident). The examiner recorded the data of the entire dentition, including the selected tooth, and provided initial hygiene treatment. Immediately afterwards, the operator delivered the test or control treatment which was repeated a week later. The treatment assignments were concealed from the patient and the examiner. The operator was unaware of the previously recorded data except the pocket depth measurements and was not involved in the post-treatment evaluations (scheduled at 3, 6, and 12 months during the hygiene appointments). At 12 months, a full mouth re-evaluation was completed by the examiner as part of the patient's maintenance program.

Specifically, in the first visit, the examiner recorded the BOP, PI, PD, and CAL at all six sites in the entire dentition, including the involved teeth. Thorough SRP was performed under local anesthesia as needed using periodontal curettes (Gracey, Hu-Friedy, USA) and an ultrasonic device (Piezo, Ultradent, USA). Once completed, the operator took over. The patients were randomly assigned to test or control groups by a computer-generated table. The protocol was either: A, the laser is activated during treatments with methylene blue; or B, the laser is never activated during treatments with saline solution. Note that methylene blue possesses antibacterial activity without additional light exposure[37] and therefore would not have been an adequate control. Antimicrobial photodynamic therapy was carried out in the residual pockets using the Periowave system (Ondine Biomedical Inc, Vancouver, Canada). The photosensitizing agent was methylene blue. Approximately 0.2 mL of the solution was applied to each pocket with a blunt-

ended side-port irrigator. The site was illuminated for 60 seconds to activate the agent using a disposable, light-diffusing tip that is introduced into the pocket attached to the diode laser (wavelength = 650 - 675 nm, 160 mW of output power). The control treatment consisted of the same procedure, except that the photosensitizer was replaced with saline solution and the light-diffusing tip was kept in the pocket for 60 seconds without activating the laser. Each patient was then sent home with the same oral hygiene instructions and home care package that included a toothbrush, toothpaste, and floss. The second session was scheduled after 1 week. The operator applied the photosensitizer or solution and activated the laser according to protocol A or B. The examiner maintained the patients on a 3-month hygiene schedule and reassessed the participants at 3, 6, and 12 months after the treatment as well as reinforced the oral hygiene instructions at each visit. Medical history changes and all adverse events are recorded. Clinical parameters were measured the same way as at baseline at all time points. These measurements were calibrated prior to the start of the study.

RESULTS

Overall, 23/24 patients, consisting of 14 men and 9 women, completed their 6-month re-evaluations. Three subjects were smokers, which was too few to perform any subgroup comparisons. Six patients completed 12-month re-evaluations, but the rest of the recall appointments had to be cancelled and the research team had to stop collecting data due to the COVID-19 pandemic. It was ultimately decided to only use the 6-month data for statistical analysis. Therefore, the data of 11 control patients and their selected tooth (total of 66 sites) and 12 test patients and their selected tooth (total of 72 sites) was included in the final project. There were no significant adverse events recorded throughout the study.

Examining individual sites, there were statistically significant (SS; $p<0.05$) BOP reductions in both groups at the 3- and 6-month evaluations, compared to baseline values. At 3 months, there were significant reductions at the MB, ML, and DL sites of the control group and the DB site for the treatment group's teeth. At 6 months, there were significant reductions at the MB and ML sites of the control group and the DB, ML, and DL sites of the treatment group's teeth [Supplementary Table 1 – Cochran's Q, significance]. Evaluating for the presence of plaque, there were only significant reductions in the control group at the DB and ML sites at 6 months compared to baseline. No other time intervals from either group had significant reductions [Supplementary Table 2]. Comparing the two groups directly across the different sites and time points for bleeding and plaque, the only SS difference was that the aPDT group had more BOP reduction at the DL site 6 months after treatment, compared to the control group [Table 1 – Fishers exact significance; Pearson Chi Square Test (x2), Supplementary Table 3].

Generally, after combining all six sites together, there was a reduction in mean bleeding on probing and plaque levels from baseline to 3 months and 6 months for both groups. The number of mean control group BOP sites was reduced from 2.91 to 1.09 and 1.36 across 3 and 6 months, respectively. The mean number of test group BOP sites was reduced from 3.00 to 1.17 to 0.83 after 3 and 6 months, respectively. The mean control group plaque was reduced from 3.18 to 1.91 to 1.73 sites and the mean test group plaque was reduced from 3.08 to 1.92 and 2.0 sites after 3 and 6 months, respectively. This translated to SS intragroup reductions of BOP in both groups at 3 and 6 months compared to baseline [Supplementary Table 4 – Friedman's test]. However, although there was a trend towards a meaningful distinction in bleeding at the end of 6 months in favor of the treatment group, there were no SS differences for the mean BOP or plaque

values across any time points between the two groups [Figure 1 (+Table 7); Figure 2 (+Table 8) – Mann Whitney U tests].

Examining the PD values at individual sites, there were SS reductions in both groups at the 3-month evaluations. This included the DL and ML sites in the control group and the B, DL, and ML sites in the treatment group, when compared to their baseline values. By 6 months, only the treatment group had comparatively significant reductions, specifically at the DB, DL, L, and ML sites [Supplementary Table 5 – Friedman's test; ChiSq sig]. Comparing the two groups directly across the different sites and time points, the treatment group had significantly lower mean PD values at 4/6 sites by 6 months (DB [3.00 v 4.27mm], MB [3.08 v 4.09mm], DL [3.67 v 4.64mm], and L [2.50 v 3.27mm] sites) [Table 2 – Mann Whitney U tests].

Calculating mean PDs, combined across all six sites, shows that both treatment and control groups had SS lower PD values at 3 and 6 months compared to their baseline values [Supplementary Table 6 – repeated measures ANOVA post hoc comparisons]. The mean PD decreased from 4.364mm to 3.424mm and 3.758mm at 3 and 6 months, respectively, for the control teeth, while it decreased from 4.347mm to 3.056mm to 2.944mm at 3 and 6 months, respectively, for the treated teeth (a SS difference at 6 months) [Supplementary Figure 1 (+Table 12) – repeated measures ANOVA test post hoc]. Comparing mean PD values between groups shows that the adjunctive aPDT treated teeth had SS more PD reduction compared to SRP alone at 6 months, a difference of 1.403mm vs 0.606mm from their respective baselines [Figure 3 (+Table 13) – Linear Mixed Model test].

Examining the CAL values at individual sites, there were SS gains in both groups at the 3-month evaluations, albeit only at the DL site in the control group and the DL and ML sites in the treatment group, when compared to their baseline values. By 6 months, more gains can be

seen, specifically at the DL, L, and ML sites in the treatment group and the L site in the control group [Supplementary Table 7 – Friedman's test, ChiSq sig]. Comparing the two groups directly across the different sites and time points, the groups had no SS differences in their mean CAL values at any point [Supplementary Table 8 – Mann Whitney U test].

However, calculating mean overall CAL, combined across all six sites, shows that the two groups had SS lower CAL values at 3 months compared to their baselines. Additionally, the treatment group also had SS lower CAL values at 6 months [Supplementary Table 9 – repeated measures ANOVA post hoc comparisons]. The mean CAL value decreased from 4.818mm to 3.803mm and 4.167mm at 3 and 6 months, respectively, for the control teeth, while it decreased from 5.736mm to 4.472mm to 4.208mm at 3 and 6 months, respectively, for the treated teeth [Supplementary Figure 2 (+Table 17) – repeated measures ANOVA post hoc]. Comparing these mean CAL gains between the groups shows that applying adjunctive aPDT resulted in SS more CAL gain compared to SRP alone by 6 months, by a difference of 1.528mm v 0.652mm [Figure 4 (+Table 18) – Linear Mixed Model test]. It is interesting to note that the CAL control and treatment groups differed significantly at baseline measurement ($p=0.05$), despite the PD baseline values having no SS difference.

DISCUSSION

The dual presence of bleeding at residual pockets places a greater risk for tooth loss over time,[15, 16] therefore, these repeatedly inflamed sites were the focus of our study. Additionally, the absence of bleeding has a high predictive value indicating periodontal stability[35, 36] and consequently the elimination of BOP was our primary outcome.

The results showed that generally, both SRP alone and adjunctive aPDT decreased bleeding at the specified teeth in a significant manner. However, the teeth treated with aPDT mostly did not have statistically significantly more BOP reductions than those that were only given the placebo sterile saline, 6 months after the initial treatment in maintenance care (with the exception of the DL site). Overall, even though there were significant reductions after intervention, there were largely no differences between the treatments that patients received. This is in agreement with other studies that tested adjunctive aPDT as an initial treatment for chronic periodontitis with 6-month follow-up periods.[38-40] Balata et al[38] stated that the absence of additional benefits with aPDT in their study could be explained by the significant improvements observed in the control group, including the good plaque control maintained during the entire observation period. They observed that studies showing benefits of the aPDT usually presented poor results of the control treatment when compared to their findings. Our findings at the tested teeth also showed that the control group significantly reduced their plaque at certain sites, whereas there were no such reductions in the treatment group. Furthermore, the patient's personal oral hygiene plays an important role in their periodontal health, as found by Lindhe et al.[7] They reported that at 5 years following periodontal therapy (both surgical and non-surgical), a high frequency of plaque-free tooth surfaces was associated with periodontal health whereas patients with a high frequency of plaque covered surfaces had greater rates of attachment loss. Therefore, the absence of significant reductions of plaque in the treatment group may have contributed to the lack of additional superior results.

The AAP best evidence review of antimicrobial therapy[29] concluded that aPDT did not promote significant additional improvements in residual sites compared to debridement alone. PD and CAL improvements were not significant in several studies that were reviewed.[32, 41, 42]

Furthermore, it states that expert opinion questions its use, evidence is lacking, and the level of certainty is low for use in residual sites after active therapy and during periodontal maintenance. However, note that some of the included studies in their review were using aPDT as a monotherapy,[33, 42] as opposed to an adjunctive treatment. Fontana et al[43] evaluated the in vitro effect of a single PDT episode on plaque bacteria in suspension and under biofilm conditions. They reported a bacterium killing capacity of 63% under suspension conditions and only 32% under biofilm conditions, which suggests the importance of biofilm disturbance in the effectiveness of PDT. In addition, Anderson et al[44] demonstrated that the combined approach of aPDT + SRP showed greater CAL gains when compared to the separate treatments used as monotherapies. This may explain the lack of significant improvement of clinical measures in these included studies for review. Also note that, it is extremely difficult to analyze all "photodynamic therapy" studies in periodontics together as there are so many individual factors in different protocols that are variable. Differences in treatment parameters such as laser light source, power level, exposure time, energy density, type of wave energy (pulsed or continuous), angle and amount of contact between fiber tip and the target tissue, amount of laser tip mobility while activated, concentration and type of photosensitizer, and number of applications may all account for the diverse results found in different studies and reviews.[29, 45]

In contrast, our findings also showed that adjunctive aPDT resulted in significantly greater mean PD reduction and CAL gain measurements at 6 months (1.41mm v 0.60mm and 1.53 v 0.65mm, respectively) when compared to the control group. Similar positive outcomes have been demonstrated in the literature for initial therapy[44, 46, 47] as well as in residual sites during supportive periodontal therapy,[30, 31] further supporting our results. In the abovementioned maintenance studies,[30, 31] their inclusion criteria consisted of non-smoking patients with single

rooted teeth, that had bleeding pockets and ≥5mm probing depths, in their trials. These patients underwent debridement and a single session of aPDT, but additionally were reassessed and had supragingival prophylaxis more often than a regular maintenance schedule (every 7-15 days during the first month, and then monthly until completion of the studies at 3 months). This increased plaque control regimen may have helped with their significant additional PD reduction (2.17 vs 1.14 mm;[30] 2.30 vs 1.0mm[31]) and CAL gain (1.43 vs 0.51mm;[30] 1.30 v 0.30mm[31]). Additionally, there was a reduction in the percentage of sites that had ≥5mm PD with BOP when compared to these sites treated by conventional SRP alone (72.22 v 40%).[30] Moreover, the exclusion of current smokers and multirooted teeth may have improved their response to treatment. Other trials[32, 41] which did not restrict participants based on smoking status or tooth type, found that residual sites in maintenance care did not significantly improve with the addition of aPDT to SRP. Note that when Pihlstrom et al[48] compared molars and non-molar teeth over 6.5 years, they found that periodontal pockets associated with multi-rooted teeth responded less favorably to SRP than pockets at non-molar sites. Additionally, Matuliene et al[16] found that after 11 years of maintenance, 22.9% of non-smokers, 12% of smokers, and 0% of heavy smokers were free from residual pockets, indicating that persisting pockets was related to smoking cigarettes. Therefore, there is literature to support that these factors could have negatively influenced those results. However, despite our study including these patients, a SS improvement of PD reduction and CAL gain was found when adjunctive aPDT was applied to these residual sites.

Caution still has to be exercised when interpreting our overall results as this study has several limitations. Even though the original protocol was designed for a one-year follow-up, sufficient data for statistical analysis was only available up to 6 months, making it a shorter-term study. To

recruit as many patients as possible, the exclusion criteria did not specify tooth type or smoking status. The specific type of previous periodontal surgery was also not differentiated. Our participant sample pool was heterogenous in this respect of characteristics but did not have enough participants to do meaningful separate analyses of each group. Also, a parallel arm design was chosen, again due to a limited number of available patients. Lastly, as mentioned previously, trying to compare studies is extremely difficult due to differences in laser treatment parameters. The large variability of aPDT aspects in the literature may account for the range of results that have been found.

In the future, more stringent grouping and review of identical protocols should be performed, with meta-analyses once the number of studies allow for it. A comparison among the different protocols should be completed to determine which allows for the best results. An ideal standardized aPDT protocol should be established, which can then be further reviewed and analyzed with future recommendations made with stronger evidence.

CONCLUSION

Within the limits of this randomized clinical trial, it can be concluded that adjunctive antimicrobial photodynamic therapy significantly improves probing depth and clinical attachment level at residual pockets in maintenance care that were previously treated in surgery, when compared to scaling and root planing treatment alone. For residual pockets in previously surgically treated teeth, clinicians may consider this alternative intervention.

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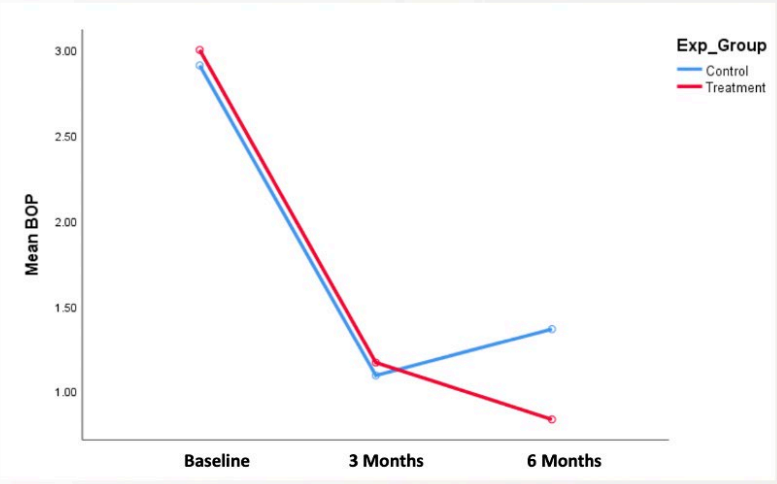
Figures

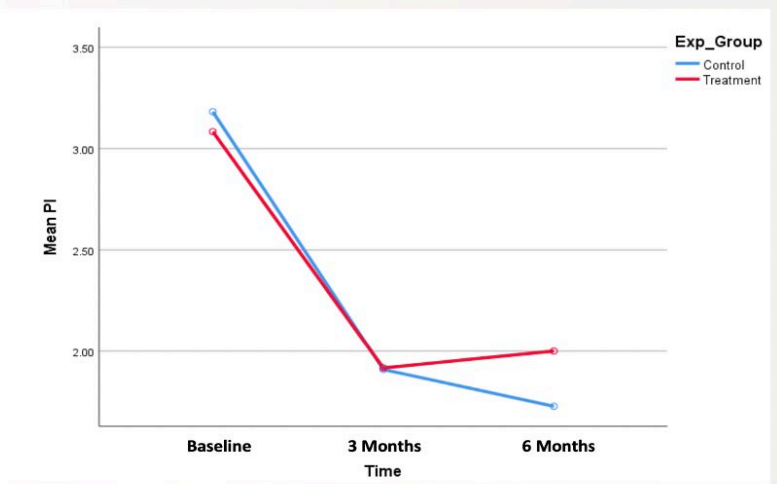
Figure 1. Comparison of mean BOP across time and group



BOP Time	Control (mean BOP)	Treatment (mean BOP)	sig
BOP Baseline	2.91	3.00	0.88
BOP 3 Months	1.09	1.17	0.88
BOP 6 Months	1.36	0.83	0.45

Mann Whitney U tests.

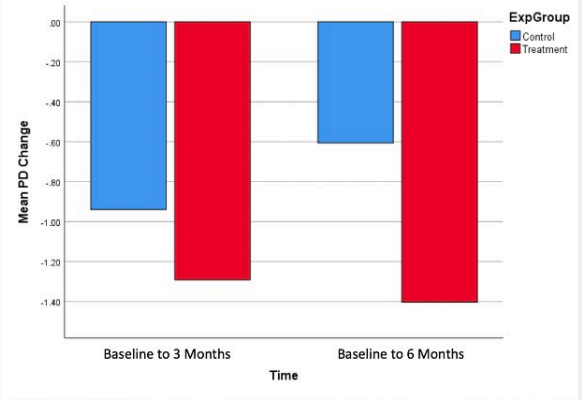
Figure 2. Comparison of mean PI across time and group



PI Time	Control (mean PI)	Treatment (mean PI)	sig
PI Baseline	3.18	3.08	.77
PI 3 Months	1.91	1.92	.94
PI 6 Months	1.73	2.0	.80

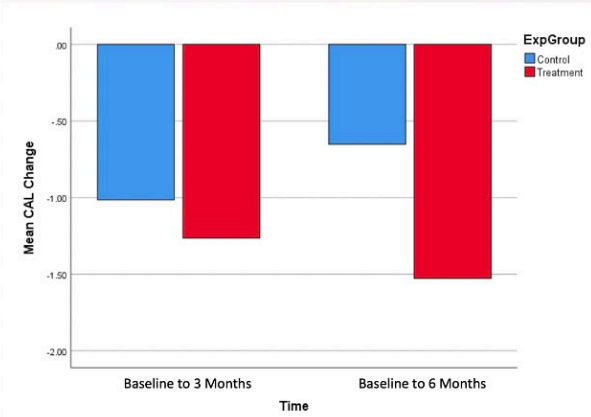
Mann Whitney U tests.

Figure 3. Comparison of overall mean PD change across time and group



Comparison	Control	Treatment	Control vs Treatment Change (sig)
PD Baseline to 3 Months	B=4.364 3Month=3.424 **Change= -.939**	B=4.347 3Month=3.056 **Change= -1.292**	-.35 (p=.15)
PD Baseline to 6 Months	B=4.364 6Month=3.758 **Change= -0.606**	B=4.347 6Month=2.944 **Change= -1.403**	-.797 (p=.008)

Figure 4. Comparison of overall mean CAL change across time and group



Comparison	Control	Treatment	Control vs Treatment Change (sig)
CAL Baseline to 3 Months	B=4.818 3Month=3.803 **Change= -1.015**	B=5.736 3Month=4.472 **Change= -1.264**	-.249 (p=.34)
CAL Baseline to 6 Months	B=4.818 6Month=4.167 **Change= -0.652**	B=5.736 6Month=4.208 **Change= -1.528**	-.876 (p=.041)

Tables

Table 1. Comparisons of BOP at specific sites across groups

BOP Site	Control % BOP	Control NO	Control YES	Treatment % BOP	Treatment NO	Treatment YES	(Sig)
SSBOP_DB_BL	36.4	7	4	58.3	5	7	(.29)[a]
SSBOP_DB_3	27.3	8	3	16.7	10	2	(.64)
SSBOP_DB_6	36.4	7	4	16.7	10	2	(.37)
SSBOP_B_BL	9.1	10	1	16.7	10	2	(1.0)
SSBOP_B_3	0.0	11	0	0.0	12	0	n/a
SSBOP_B_6	18.2	9	2	16.7	10	2	(1.0)
SSBOP_MB_BL	63.6	4	7	41.7	7	5	(.29)[a]
SSBOP_MB_3	18.2	9	2	8.3	11	1	(.59)
SSBOP_MB_6	18.2	9	2	25.0	9	3	(1.0)
SSBOP_DL_BL	72.7	3	8	75.0	3	9	(1.0)
SSBOP_DL_3	27.3	8	3	33.3	8	4	(1.0)
SSBOP_DL_6	36.4	7	4	0.0	12	0	(.04)
SSBOP_L_BL	36.4	7	4	41.7	7	5	(1.0)
SSBOP_L_3	18.2	9	2	16.7	10	2	(1.0)
SSBOP_L_6	18.2	9	2	8.3	11	1	(.59)
SSBOP_ML_BL	72.7	3	8	66.6	4	8	(1.0)
SSBOP_ML_3	18.2	9	2	41.7	7	5	(.37)
SSBOP_ML_6	9.1	10	1	16.7	10	2	(1.0)

Fishers exact significance reported for all with 2 exceptions where [a]Pearson Chi Square significance reported.

Table 2. Comparisons of mean PD at specific sites across groups

PD Site	Control Mean	Control Mean Rank	Treatment Mean	Treatment Mean Rank	(Sig)
SSPD_DB_BL	4.73	12.05	4.75	11.96	.99
SSPD_DB_3	3.82	13.45	3.42	10.67	.33
SSPD_DB_6	4.27	15.32	3.00	8.96	.02
SSPD_B_BL	2.73	10.73	3.08	13.17	.39
SSPD_B_3	2.18	12.50	2.08	11.54	.81
SSPD_B_6	2.45	13.00	2.17	11.08	.44
SSPD_MB_BL	4.64	12.91	4.17	11.17	.53
SSPD_MB_3	3.91	14.50	3.08	9.71	.08
SSPD_MB_6	4.09	14.82	3.08	9.42	.04
SSPD_DL_BL	5.64	12.68	5.50	11.38	.65
SSPD_DL_3	4.18	14.59	3.50	9.63	.08
SSPD_DL_6	4.64	14.95	3.67	9.29	.04
SSPD_L_BL	3.64	12.73	3.75	11.33	.65
SSPD_L_3	2.82	12.14	2.92	11.88	.97
SSPD_L_6	3.27	15.36	2.50	8.92	.01
SSPD_ML_BL	4.82	11.82	4.83	12.17	.92
SSPD_ML_3	3.64	12.82	3.33	11.25	.58
SSPD_ML_6	3.82	13.64	3.25	10.50	.26

Mann Whitney U test used.

Appendix 6 – Journal Receipt Conformation

To be attached after submission.