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Clinical effects of adjunctive therapeutic devices in non-surgical treatment of periodontitis patients

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Chapter I

INTRODUCTION

Periodontitis is a chronic multifactorial disease that involves a cascade of inflammatory reactions induced by bacterial subgingival colonization. The disease is characterized by periodontal tissue destruction and alveolar bone resorption (Hajishengallis et al., 2011). Periodontitis is a global burden that has not changed appreciably in terms of prevalence or incidence worldwide in the past 10 years (Kassebaum et al., 2014). Moreover, it is reportedly the main cause of tooth loss in adults (Eke et al., 2015). A recent systematic review (Frencken et al., 2017) reported that severe periodontitis was the sixth most prevalent condition in 2010; it affected 743 million people (10.8%) among individuals aged 15–99 years worldwide, with similar incidences in men and women.

Untreated or inadequately treated periodontitis leads to the loss of tooth-supporting tissues and teeth. Furthermore, periodontal infections are associated with a range of systemic diseases that lead to premature death, including diabetes (Sanz et al., 2018), cardiovascular diseases (Sanz et al., 2019), and adverse pregnancy outcomes (Joint EFP/AAP Workshop, 2013).

Active periodontal therapy (i.e., standard treatment consisting of oral hygiene instructions, combined with biofilm and calculus removal, with or without adjunctive antimicrobials) is provided to patients with periodontitis to establish conditions that allow these patients to maintain dentition without further periodontal breakdown. Specifically, this therapy aims to prevent further episodes of periodontitis, reduce/eliminate gingival inflammation, reduce/eliminate deepened pockets, and regain periodontal attachment of the tooth. These outcomes can ultimately prevent loss of teeth and corresponding dental functions (Pihlstrom, 1992).

PERIODONTITIS: DIAGNOSTIC PARAMETERS

A new classification system was introduced in 2017 (Journal of Periodontology 2017, volume 89) following the World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions, which was jointly organized by the American Academy of Periodontology and the European Federation of Periodontology to create a consensus knowledge basis for a global classification approach.

The new classification system introduces the concept of "staging" (e.g., stages I, II, III, and IV) and supports a multidimensional view of periodontitis that incorporates severity, periodontitis-related tooth loss, and management of the patient's periodontal and overall oral rehabilitation needs. The "grading" parameter (e.g., grades A, B, and C) indicates additional biological dimensions of the disease, including history-based and/or anticipated rate of periodontitis progression, presence and control of risk factors, and potential impacts of periodontitis on general health.

During a periodontal visit, the clinician records specific parameters: clinical attachment level (CAL) ,probing depth (PD) and bleeding on probing (BOP). In conjunction with a proper radiological evaluation, a patient is diagnosed with a periodontal condition involving a specific stage and grade (e.g., periodontitis stage II, grade C). The stage typically does not regress or become less severe; however, sites may demonstrate further clinical attachment loss, radiographic bone loss, and deeper probing depths during maintenance, which may merit a higher stage. Grading indicates the likelihood of post-treatment disease progression. The designations signify slow or no progression (A), moderate progression (B), and rapid progression (C). Only one grade is assigned to a particular patient, based on either direct or indirect evidence regarding the rate of disease progression and risk of future progression. A patient with a low rate of progression does not begin to exhibit more rapid progression if the risk modifiers are controlled.

Evidence regarding the effectiveness of periodontal treatment should focus on patient-centered outcomes (e.g., functional dentition (tooth loss) or patient satisfaction/quality of life); however, few experimental studies have investigated these "true" endpoints. To measure tooth retention in a meaningful manner, long-term studies are needed, with follow-up durations of > 12 months post-treatment. Few studies have reported oral-health-related quality of life; the responsiveness of these tools is unclear with respect to general treatment, compared with its original application in cross-sectional epidemiological studies (Loos & Needleman, 2020).

"Surrogate" outcomes (e.g., CAL, PD and BOP) are commonly used to assess the destruction and inflamation of periodontal structures. They are important clinical indicators for both periodontal disease diagnosis and monitoring treatment success. CAL is measured with a graduated probe (UNC 15) from an anatomical reference point–the cementoenamel junction–to the bottom of the pocket. It usually is regarded as the reference parameter when evaluating periodontal condition and treatment outcomes. CAL is informative for subsequent tooth loss (Hujoel et al., 1999) and has been associated with short-term oral-health-related quality of life (Shanbhag et al., 2012); it has not been extensively investigated for long-term outcomes. PD is measured from the gingival margin to the bottom of the pocket. This dimension is affected by gingival recession or edema due to inflammation. Shallow residual periodontal pockets are presumably unfavorable ecological niches for a dysbiotic biofilm.

If bleeding occures when probing a site, or within 10-15, after removing the probe, a BOP positive score is given indicating an active periodontal disease (Lang et al., 1990): full-mouth BOP score reports the percentage of BOP posiive sites, probing 6 sites per tooth. Recent evidence suggests that, following active periodontal therapy, the achievement of shallow periodontal pockets (≤ 4 mm) with no bleeding on probing (BOP) in patients with full-mouth bleeding scores < 30% confers the greatest likelihood of stable periodontal health and the lowest risk of tooth loss (Loos & Needleman, 2020).

NON-SURGICAL TREATMENT OF PERIODONTITIS

Clinicians are challenged daily to manage varying extent and severity of periodontitis. The treatment sequence should be pre-established, with a stepwise approach that should be incremental, depending on the disease stage, and include distinct interventions.

The European Federation of Periodontology recently developed a three-step (S3) clinical practice guideline (CPG) for the treatment of Stage I-III periodontitis (Sanz et al., 2020). The objective of step one (S1-CPG) initial treatment is to induce a behavioral change by motivating the patient to undertake successful removal of supragingival dental biofilm, using powered or manual toothbrushes and interproximal brushes when indicated. It also involves promoting risk factor control, especially in terms of smoking cessation and diabetes compensation (Ramseier et al., 2020). This treatment phase should be performed in all patients with periodontitis, irrespective of their disease stage, and should be re-evaluated frequently.

The second step (S2-CPG) of treatment comprises cause-related therapy, which intends to

control (reduce/eliminate) the subgingival biofilm and calculus by non-surgical therapy, thereby restoring tissue health around the root surface.

Mechanical scaling and root planing (SRP), which consists of hand or electronic instrumentation of the affected sites, is currently the preferred method for non-surgical treatment of periodontitis (Cobb et al., 2002; Aimettj et al., 2014). Clinical outcomes are reportedly similar with respect to the use of hand instruments or ultrasonic scalers during mechanical debridement, when delivered quadrant-wise in several visits or involving full-mouth instrumentation (within 24 h) (Suvan et al., 2019). When compared with no treatment, SRP treatment resulted in a 0.49-mm mean gain in CAL, with a moderate overall level of confidence in the evidence (Smiley et al., 2015 (1)). Although SRP produces substantial clinical improvements, the complete elimination of bacterial deposits can be difficult to accomplish. Mechanical therapy alone does not induce a statistically significant microbiome shift (Hagenfeld et al., 2018).

In the areas non-responding adequately to S1 and S2-CPG treatments, the third step (S3-CPG) should be considered, which comprises interventions such as repeated subgingival instrumentation, with or without adjunctive therapies, or surgical approach (resective/regenerative periodontal surgery).

LIMITATIONS OF SUPRA- AND SUB-GINGIVAL PROFESSIONAL MECHANICAL PLAQUE REMOVAL

Despite clinical improvements, supra- and sub-gingival professional mechanical plaque removal (PMPR) alone may fail to fully eliminate pathogenic bacteria, especially from reservoirs in the soft tissue, root cement, and dentinal tubules (Doungudomdacha et al., 2001; Socransky & Haffajee, 2002; Umeda et al., 2004), as well as areas that are inaccessible to periodontal instruments (e.g., deep pockets, furcation areas, and root depressions) (Matia et al., 1986; Adriaens et al., 1988). In these “hard to reach” anatomical defects, treatment may result in a moderate and temporary shift in the composition of the microbial flora (Sherman et al., 1990; Cobb, 1996). However, periodontopathic bacteria can persist, leading to early recolonization from other affected areas (Sbordone et al., 1990; Haffajee et al., 2007).

To overcome these limitations of conventional mechanical therapy, several adjunctive protocols have been developed.

ADJUNCTIVE THERAPIES TO PMPR

After active therapy with non-surgical instrumentation (using manual or power-driven devices), residual pockets may persist. Long-term clinical studies have shown that BOP-positive pockets with PD $\geqslant$ 6 mm indicate a risk of disease recurrence and greater likelihood of extraction/tooth loss (Matuliene et al., 2008). Usually, these sites are indicated for additional corrective surgical therapy (i.e., third phase of periodontal treatment) that aims to reduce probing depths through a resective or regenerative approach. Although a surgical approach achieves a greater PD reduction in deep (> 6 mm) and moderately deep (4–6 mm) pockets, a comparable or greater CAL gain can currently be obtained in these sites through non-surgical subgingival debridement (Sanz-Sanchez et al., 2020; Suave et al., 2020). Ideally, the least invasive treatment with the greatest biological cost-effectiveness ratio should be used to restore periodontal health. Thus, current research has focused on the use of adjunctive treatments in combination with standard SRP, which may enhance its effectiveness. These procedures include the following:

- use of antibiotics or antimicrobials/disinfectants (Paddmanabhan, 2013);
- use of host-modulating agents (e.g., silver sulfadiazine (SSD), statins, probiotics, non-steroidal anti-inflammatory drugs (NSAIDs), polyunsaturated fatty acids (PUFAs), and metformin);
- use of physical or chemical agents, such as light, lasers, and/or low abrasive powders (Rajesh et al., 2011).

Overall, the mean added beneficial effects of adjunctive treatments, compared with SRP alone, have been demonstrated in terms of PD (+ 0.376 mm) and CAL (+ 0.207 mm), but not BOP score (Ramanauskaite et al., 2020).

Systemic antimicrobials

Systemic antimicrobial therapy can be used as an adjunct to mechanical therapy in patients with periodontitis, especially who do not respond to mechanical treatment, patients with acute or severe periodontal infection, and patients who are systemically compromised (Barca et al., 2015). In the past, it was common to focus on the presence or absence of bacteria comprising the ‘‘red complex’’ (*Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*), which were implicated in disease (Miller et al., 2010). Reduction of these pathogens after mechanical therapy has been improved by adjunctive systemic antibiotics (e.g., amoxicillin and metronidazole); notably, these therapies can facilitate complete elimination of *Aggregatibacter actinomycetemcomitans* (Socransky et al., 2013; Keestra et al., 2015).

Compared with SRP alone, SRP plus systemic antimicrobials resulted in a 0.35-mm mean gain in CAL, with a limited range of efficacy depending on the active substance: amoxicillin/metronidazole combined (+ 0.39 mm); metronidazole alone (+ 0.18 mm); azithromycin (+ 0.29 mm); moxifloxacin (+ 0.31 mm); and tetracycline (+ 0.34 mm). The overall level of confidence in the evidence was moderate (Smiley et al., 2015 (1)).

Reference laboratories can identify periodontal pathogens and their antibiotic susceptibilities, but dentists have used empirical antibiotic regimens for many years to treat periodontitis by eliminating bacteria that cannot be reached by topical antimicrobial treatment, despite the absence of strong clinical evidence regarding their efficacy (Harks et al., 2015) or the presence of questionable clinical relevance (Berezow et al., 2011; Eickholz et al., 2016). This indiscriminate use of antibiotics has led to global concerns regarding antimicrobial resistance. Their overuse promotes the development of resistant strains of bacteria, which are problematic worldwide. Moreover, antibiotics may have other undesirable side effects. As a class of drugs, they cause allergic reactions in some people. Other adverse events (AEs) commonly reported include rash, diarrhea, abdominal pain, nausea, and vomiting; notably, their rates of occurrence are often minimal (Haffajee et al., 2007; Predeep et al., 2011; Sampaio et al., 2011; Preus et al., 2013; Miranda et al., 2014).

Although properly prescribed antibiotics can aid in the resolution of periodontitis, the widespread use of antibiotics carries risks of inducing antibiotic resistance in important medical pathogens. Greater information regarding the composition of the periodontal microbiome will presumably lead to improved identification of patients who may benefit from adjunctive antibiotic therapy. In general, this class of drugs should not be used routinely, and the use of a combination of amoxicillin and metronidazole may be considered for specific patient categories (e.g., young adults with generalized periodontitis Stage III), for short-term (i.e., less than 21 days) use only (Sanz et al., 2020). Importantly, lower doses may be acceptable over a longer period (Smiley et al., 2015 (2)).

Locally delivered antimicrobials

Locally administered antimicrobial agents are preferred because they are associated with fewer systemic side effects, compared with systemic antimicrobials. However, the procedures to administer these agents inside the periodontal pocket are limited by lack of retention and inability to achieve adequate inhibitory concentrations in the gingival crevicular fluid (Joshi et al., 2016). Multiple local antimicrobial agents have been proposed as adjuvants for the management of periodontal diseases (Kinane, 2000).

Chlorhexidine

Chlorhexidine (CHX) is a bis-biguanide molecule that comprises two (p-chlorophenyl) guanide units linked by a hexamethylene bridge. It is a potent anti-infective and antibacterial mouth-rinse agent, which is used for prophylaxis and therapy with respect to periodontal disease (James et al., 2017).

A CHX chip is a resorbable chip with 2.5 mg of CHX embedded in a cross-linked hydrolyzed gelatin matrix. When subgingivally delivered into deep periodontal pockets, the chip releases a controlled amount of CHX with simultaneous biodegradation over a 1-week period providing a CHX concentration of < 125 mg/mL to the gingival sulcular fluid (Soskolne et al., 1998). CHX chips are more effective than other forms of CHX, such as mouth rinse (Jagadish et al., 2013), irrigation, or gel (Lecic et al., 2016). However, several systematic reviews regarding adjunctive use of the CHX chip with SRP failed to confirm the chip's treatment effectiveness because of inadequate numbers of included studies (Cosyn et al., 2006), heterogeneity in terms of recorded outcome parameters (Matesanz-Perez et al., 2013), or variations in terms of study population, disease severity, SRP quality, and operator experience (Ma et al., 2020). The most recent results indicate that, compared with SRP alone, SRP plus CHX chip treatment resulted in a 0.40-mm mean gain in CAL (95% confidence interval (CI), 0.24–0.56 mm), with a moderate overall level of confidence in the evidence (Smiley et al., 2015 (1)).

According to a Food and Drug Administration safety report and the manufacturer's prescribing information, the most frequently observed AEs were toothache, upper respiratory tract infection, and headache. Oral pain or sensitivity may occur during the first week (or later) after SRP and chip placement, but its severity is typically mild to moderate and it resolves within days. Serious allergic reactions have occurred with dental products containing CHX (Smiley et al., 2015). These results and the safety of CHX chips suggest that the CHX chip may serve as a useful adjuvant to non-surgical periodontal therapy. Although the combination of CHX and SRP cannot be recommended as a preferred treatment, it may be widely considered (Sanz et al., 2020).

The use of CHX mouth rinse as an adjunctive treatment to standard SRP resulted in a slightly greater PD reduction, compared with SRP alone, but had a negligible effect on CAL (da Costa et al., 2017). Potential tooth staining due to prolonged use of CHX mouth rinse may require further mechanical plaque control before initiation of CHX as a temporary adjunct to subgingival therapy.

Doxycycline hyclate gel (DH) gel

DH gel has been approved by the US Food and Drug Administration as a stand-alone product (i.e., for use without SRP). There is no statistically significant difference in outcomes between the use of DH gel alone and SRP alone (Garret et al., 1999; Garret et al., 2000). However, compared with SRP alone, SRP plus DH gel resulted in a 0.64-mm mean gain in CAL (95% CI, 0.00–1.28 mm); notably, the overall level of confidence in the evidence was low (Smiley et al., 2015 (1)).

The package insert lists several potential AEs with DH use including headache, gingival discomfort (e.g., pain or soreness), toothache, periodontal problems (e.g., abscess, exudate, infection, drainage, extreme mobility, and/or suppuration), thermal tooth sensitivity, and/or sore mouth. Furthermore, 1.6% of participants in a DH (Atridox, CollaGenex Pharmaceuticals) clinical trial of more than 1,400 participants reported "unspecified essential hypertension," although no association of oral DH use with essential hypertension has been identified.

Minocycline microspheres

Frequently, tetracyclines and tetracycline analogs are used as local antibiotics because of their actions against bacterial growth and matrix metalloproteinase activities. The US Food and Drug Administration approved the use of minocycline microspheres for periodontal treatment on the basis of their beneficial adjunctive effect on PD, compared with SRP alone. No statistically significant additional clinical improvement has been demonstrated in terms of CAL or BOP (Tabenski et al., 2017), especially in deep periodontal pockets (PD ≥ 6 mm). The mean gain in CAL was 0.24 mm (95% CI, −0.06 to 0.55 mm) and the overall level of confidence in the evidence was low (Smiley et al., 2015 (1)). Notably, the antimicrobial effect of minocycline microspheres as an adjunctive treatment to standard SRP in current smokers has been demonstrated by significant reductions in the numbers and proportions of periodontal pathogens, compared with pretreatment levels (Grossi et al., 2001).

Generally, no AEs have been reported by investigators. Although infrequent, the most common AEs included headache, dental infection, increased periodontitis, tooth sensitivity, tooth caries, dental pain, gingivitis, and stomatitis. No clinically significant changes in vital signs or oral hard or soft tissues were noted in these studies (Smiley et al., 2015 (2)).

Host-modulating agents

Inflammation has a central role in periodontitis progression. Thus, the aim of mechanical removal of biofilm is to eliminate the cause of inflammation. Pharmacological or bioactive agents that serve as adjuncts to standard SRP may facilitate disease resolution. The pool of host-modulating agents has expanded in recent years; their effects have been documented in long-term clinical trials. In particular, a significant PD improvement in infrabony defects was recorded following systemic treatment with sub-antimicrobial-dose doxycycline, and following the use of locally delivered statin gels. Notably, an insignificant benefit in terms of PD reduction can be associated with the use of probiotics, depending on the bacterial strain. Data regarding the use of locally applied metformin gel and 1% alendronate gel are inconclusive but encouraging. In contrast, the roles of other local modulators (e.g., aloe vera, green tea, and gingko biloba), essential oil mouth rinses/irrigation, systemic administration of omega-3 PUFAs, micronutrients, bisphosphonates, and NSAIDs remain under investigation (Donos et al., 2019).

Sub-antimicrobial-dose doxycycline (SSD)

SDD is considered a host-modulating agent. Specifically, it inhibits host collagen-degrading enzymes (Reddy et al., 2003; Gu et al., 2012). For patients with periodontitis, clinicians may consider systemic SDD (20 mg twice daily) for 3–9 months as an adjunct to SRP, with a small net benefit expected. Compared with SRP alone, SRP plus SDD resulted in a 0.35-mm mean gain in CAL (95% CI, 0.15–0.56 mm), with a moderate overall level of confidence in the evidence (Smiley et al., 2015 (1)).

Investigators have reported that SDD is well tolerated, such that participants have described few or no AEs (Caton et al., 2000; Deo et al., 2010; Emingil et al., 2011). According to the package insert, the most frequent AEs that occurred during clinical trials were headache, common cold, flu symptoms, and toothache; however, the potential for AEs from SDD is negligible (Smiley et al., 2015 (2)) and limited to the onset of mild dizziness and tachycardia (Haffajee et al., 2007).

Although SDD is regarded as a highly effective adjunctive therapy to standard SRP (Bonito et al., 2005; Matesanz-Pérez et al., 2013; Donos et al., 2019), a recent meta-analysis (Trombelli et al., 2020) showed that SSD does not produce a greater clinical effect on periodontal conditions during supportive periodontal therapy. Concerns related to the potential risk of AEs and the challenge of patient compliance when taking a systemic medication for

several months have contributed to the current recommendation not to use SSD as an adjunctive treatment to standard SRP (Sanz et al., 2020).

Statins

Statins are inhibitors of the 3-hydroxy-3-methylglutaryl coenzyme A reductase, which is an important enzyme related to cholesterol synthesis. The use of statins is an established therapy for hyperlipidemia and arteriosclerosis, and comprises the primary and secondary method for prevention of coronary artery diseases, mainly due to the reduction of low-density lipoprotein cholesterol levels. Systemically administered atorvastatin and rosuvastatin showed additional benefits in reducing PD; simvastatin also improved CAL (Muniz et al., 2018). The use of locally applied 1.2% rosuvastatin gel in the periodontal pocket was effective in reducing PD in infrabony defects (Pradeep et al., 2016) and class II furcation (Garg & Pradeep, 2017); furthermore, the use of 1.2% atorvastatin and 1.2% simvastatin gels led to minor PD changes (Martande et al., 2017). Local statins appear to be safe overall, well-tolerated, and not associated with AEs or complications. Thus, they can be considered for implementation in clinical practice (Donos et al., 2019), but are not currently recommended (Sanz et al., 2020).

Probiotics

Probiotics may provide potential benefits in the clinical management of periodontitis. Several clinical trials have investigated the effects of probiotics as an adjunct to SRP. However, heterogeneity among these studies and their methodologies (e.g., dosage, duration, probiotic strains, and patient characteristics) complicates broad conclusions. Previous systematic reviews concluded that probiotics are safe and effective adjunctive therapies for standard SRP, such that they provided early clinical benefit and good short-term efficacy (3 months) in terms of PD, with mean gains of 0.18 mm in moderate pockets and 0.67 mm in deeper sites (Martin-Cabezas et al., 2016). They may also reduce the need for surgical intervention and the use of antibiotics (Matsubara et al., 2016). However, there are minimal data concerning their long-term impacts on clinical, microbiological, and immunological outcomes. There is weak evidence that probiotic adjuncts to nonsurgical therapy may confer clinical improvements in periodontal health for up to 1 year; greater benefits may be expected in patients with deeper pockets (Ho et al., 2020).

Importantly, the use of probiotics does not lead to a statistically significant and consistent

composition shift toward a healthy subgingival microbiome (Gruner et al., 2016). This implies a possible "washout" effect after the cessation of probiotic activity, which may lead to pathogenic biofilm recolonization and loss of host inflammatory modulation in the periodontal pocket niche.

Studies of gastrointestinal probiotics have provided extensive evidence, and their use is well recognized in empirical therapy (Parker et al., 2018). The host-microbiome interface, largely recognized as an individualized community, exhibits varying extents of colonization resistance in different individuals (Zmora et al., 2018). This type of resistance indicates a need for personalized approaches. Thus, the use of probiotics as an adjunctive treatment to SRP is not recommended (Sanz et al., 2020).

Physical and chemical agents

Non-surgical use of lasers

Several types of lasers, categorized primarily by the wavelength of the emitted light, are used non-surgically as adjunctive treatments with SRP. These lasers include diode; carbon dioxide (CO_2); neodymium-doped: yttrium aluminum garnet (Nd:YAG); erbium-doped: yttrium aluminum garnet (Er:YAG); and erbium, chromium-doped: yttrium, scandium, gallium, garnet (Er,Cr:YSGG) types. Their wavelengths range from 635 to 10600 nm. The mean CAL gains reported with the use of an adjunctive laser range from 0.41 mm (Nd:YAG laser) (Neill and Melloning, 1997) to 0.18 mm (Er:YAG) (Smiley et al., 2015). The overall level of confidence in the evidence is low; only occasional AEs were reported during the trials (Lopes et al., 2010; Rotundo et al., 2010; Kelbauskiene et al., 2011).

Unlike other instruments, lasers have no defined or accepted protocols for standard usage. Because operators determine their own protocols in terms of various parameters (power intensity and density, power, spot size, energy, repetition rate, tip size, pulsing versus continuous mode, mean energy loss, and time of application), the potential for AEs involving the tooth and patient is higher than when using other local delivery systems. Thus, the use of lasers is not recommended as a standard adjunctive therapy to SRP (Sanz et al., 2020).

Antimicrobial photodynamic therapy (aPDT)

aPDT can be carried out using a laser light of appropriate wavelength, in the presence of oxygen, to activate a photosensitizer (e.g., different concentrations of methylene blue, toluidine blue O, phenothiazine chloride, or indocyanine green). The activated photosensitizer binds to

bacterial cells and reacts with molecular oxygen, resulting in the formation of reactive oxygen species (ROS) that damage bacterial cell components and cause cell lysis (Jori et al., 2006).
The ROS half-life is very short (i.e., nanoseconds to microseconds), such that these molecules cannot widely interact with surrounding host molecules and cause side effects (Konan et al., 2002). Because of their simple molecular form, the possibility of bacterial resistance to ROS is very low (Wilson, 1993). Non-activated photosensitizers themselves reportedly have no bactericidal effects on periodontal pathogens (Chan & Lai, 2003).
In the past decade, aPDT with a diode laser (aPDT–DL) has been used as an adjunctive to mechanical debridement. This approach demonstrated notable clinical and bactericidal effects, including a biostimulatory effect that caused more rapid periodontal healing after diode laser application (Prates et al., 2011). A literature analysis highlighted heterogeneity in performing this treatment (regarding dosimetry and laser settings) and inconsistent outcomes (Salvi et al., 2019). Studies have shown improvements in all clinical periodontal parameters, especially reduction of BOP (Andersen et al., 2007; Braun et al., 2008; Christodoulides et al., 2008; Takasaki et al., 2009; Ge et al., 2011; Berakdar et al., 2012; Tabenski et al., 2017) and a significant reduction in the proportion of sites with periodontal pathogenic bacteria (Theodoro et al., 2012). Compared with SRP alone, SRP plus aPDT–DL resulted in a 0.53-mm mean gain in CAL, with a moderate overall level of confidence in the evidence (Smiley et al., 2015(1)).
In contrast, some studies failed to show additional benefits of aPDT–DL on clinical periodontal parameters and subgingival microbial flora (Socransky & Haffajee, 2002; Umeda et al., 2004). Similarly, the results of a recent meta-analysis imply that aPDT–DL as an adjunct to SRP in patients with chronic periodontitis provides only a modest clinical improvement (Trombelli et al., 2020), without substantial microbiological changes (Sgolastra et al., 2013). Compared with SRP alone, SRP plus aPDT plus non-diode laser (wavelength 808–980 nm) resulted in a 0.21-mm mean gain in CAL, although the overall level of confidence in the evidence was low. Investigators reported no AEs such as discomfort, burning sensation, dentin hypersensitivity, or pain related to aPDT plus non-diode laser irradiation (Saglam et al., 2014; Ustun et al., 2014). Investigators in several studies reported no AEs with uneventful post-treatment healing, no pain, no burning sensation, and no discomfort (Christodoulides et al., 2008; Chondros et al., 2009; Betsy et al., 2014). Nevertheless, the use of aPDT, regardless of wavelength (660–670 nm or 800–900 nm), cannot be recommended as a routine adjunctive therapy over standard SRP (Sanz et al., 2020).

Fluorescence photobiomodulation therapy

This treatment was developed as a therapy for diseases such as localized microbial infections, chronic ulcers, tumors and premalignant diseases, periodontitis, and other oral lesions (Babilas et al., 2010). Upon irradiation with light of an appropriate wavelength, a nontoxic drug known as a photosensitizer agent (applied and maintained in the target tissue) undergoes transition from a low-energy "ground state" to a higher-energy condition. This sensitizer can react with biomolecules or molecular oxygen to produce free radicals, radical ions, or singlet oxygen; these cytotoxic species can cause oxidation of cellular constituents such as plasma membranes and DNA, thereby resulting in cell death. Microorganisms such as bacteria, fungi, viruses, and protozoa can be killed by singlet oxygen species. This treatment is effective against antibiotic-sensitive and antibiotic-resistant microorganisms; importantly, repeated applications do not result in bacterial resistance (Gursoy et al., 2013). Furthermore, viral infections (e.g., herpes simplex) can be successfully treated with this therapy (Takasaki et al., 2009).

In recent years, visible light (specifically blue light, wavelength 400–500 nm) has been studied for its antimicrobial and antibiofilm effects. It is currently used in fluorescence photobiomodulation therapy. The visible blue light is absorbed by photoacceptors in pigmented and non-pigmented mammalian cells, activating signaling cascades and downstream mechanisms that lead to the modulation of cellular processes and beneficial effects in inflammation conditions. Limited and contrasting results have been reported regarding the cellular effects of blue-light-induced signaling (Garza et al., 2018). Bacteria have reportedly been killed by exciting large amounts of intracellular porphyrins, which release ROS with antibacterial effects (Serrage et al., 2019; Li et al., 2020).

Several clinical trials for treatment of acne showed positive effects of this wavelength (Dai et al., 2012; Wheeland et al., 2012). In dentistry, it may reduce dental plaque and aid in the treatment and prevention of caries, gingivitis, periodontitis, peri-implantitis, and endodontic diseases (Gursoy et al., 2013). However, most authors have not recorded and reported their parameters appropriately; therefore, optimal therapeutic settings remain unclear. Currently, a considerable number of studies and clinical investigations are underway for the determination of optimal combinations of photosensitizers, light sources, and treatment parameters for various diseases.

AIR POLISHING IN THE CONTEXT OF PERIODONTITIS TREATMENT

Traditionally, calcified and non-calcified bacterial deposits (i.e., calculus and biofilm) are removed from root surfaces by scraping with a steel curette, or by using a steel tip activated by

sonic or ultrasonic oscillation. Treatment with Gracey curettes and ultrasonic scalers, however, may be associated with undesirable side effects, such as root substance removal, discomfort, and pain during treatment. Experimental trials showed comparable histological results in dogs and improved clinical parameters in humans with either complete removal of the root cementum or following root surface polishing only (Nyman et al., 1988; Petersilka et al., 2003b; Petersilka et al., 2011). These findings imply an effective procedure that can remove subgingival biofilm and calculus without invasive impacts on the teeth and periodontal tissues. In recent decades, scientific interest has focused on developing alternative and painless periodontal treatment approaches that can remove bacterial deposits with a jet of compressed air containing an abrasive powder. This procedure is known as "air-polishing;" it has been suggested as an alternative approach to standard SRP. The first powder used for biofilm removal was sodium bicarbonate with particles of up to 250 μm. Nevertheless, despite particle size reduction to 40 μm, sodium bicarbonate remains a high-level abrasive material, which can damage soft and hard tissues, as well as restorative materials (Janiszewska-Olszowska et al., 2020). Concerns regarding these effects of sodium bicarbonate led to the development of low abrasive powders: glycine (glycine powder air-polish (GPAP)) was introduced in 2003. Glycine is a water-soluble, non-essential amino acid, with a naturally sweet taste. It produces very fine, round soft particles (diameter, 25 μm). Biopsy studies have shown that GPAP causes less damage to gingival tissues, compared with manual or ultrasonic SRP, and compared with air-polishing with sodium hydrogen carbonate (Petersilka et al., 2003a; Sekino et al., 2020). Moreover, GPAP does not cause major damage to restorative materials, such as hybrid composite and glass ionomers (Barns et al., 2014). The supragingival use of GPAP effectively removes biofilm on the root surface when the water/powder jet is oriented towards the sulcus. In sites with a clinical PD of 4 mm, a median debridement depth of 2 mm can achieve approximately 60% root surface decontamination; in deeper pockets, the efficacy of root debridement decreases to approximately 40% (Flemmig et al., 2007).

Currently, it is possible to use a nozzle, first described by Moene et al. (2010), to apply the water/powder jet directly into the periodontal pocket. GPAP is a rapid and reliable mode of debridement, which causes very limited damage to the root surface (Petersilka, 2011) and imparts a high level of comfort for both the patient and the operator (Hägi et al., 2015). However, it has shown no superiority in terms of clinical outcome with respect to CAL, PD, and BOP outcomes (Wennstrom et al., 2011; Petersilka et al., 2020). Repeated subgingival use of GPAP has comparable efficiency with conventional oscillating scalers or curettes for standard SRP,

with respect to flat surfaces (Petersilka et al., 2020). Moreover, subgingival debridement with GPAP should be scheduled at intervals of 2–3 months to ensure a minimal bacterial count (Sekino et al., 2020).

Ten years after the introduction of GPAP, an erythritol-based powder was introduced on the market (erythritol powder air-polish (EPAP)). Erythritol is a polyol that serves as an artificial non-cariogenic sweetener: it is non-toxic, chemically neutral, and highly water-soluble. Its mechanical properties (e.g., hardness, particle size and abrasiveness) are comparable with the characteristics of glycine (Hägi et al., 2013). Erythritol has been reported to reduce the prevalence and bioactivity of bacteria associated with periodontal disease (e.g., various *Streptococcus* species and *Prevotella intermedia*) (Hashino et al., 2010; Söderling et al., 2020). Ecological analysis has indicated that the presence of erythritol significantly affects bacterial species composition, inducing a shift from periodontitis- and gingivitis-related species toward an ecology dominated by known "early colonizers" (Janus et al., 2017). However, microbiological changes following the clinical use of subgingival EPAP treatment remain controversial. Compared with manual instrumentation, EPAP ensures biofilm removal with tissue damage comparable with the damage caused by GPAP (Janiszewska-Olszowska et al., 2020); it also promotes fibroblast reattachment (Hägi et al., 2015). The use of EPAP during periodontal surgery is regarded as a valuable minimally invasive adjunct following manual or ultrasonic calculus removal. It may also serve as an important alternative to these methods for calculus-free root surfaces (Cosgarea et al., 2020).

Despite their safe, minimally invasive, and effective approach for biofilm removal, air-polishing procedures alone are insufficient for patients with calculus deposits when they involve low-abrasive glycine powder (Bühler et al., 2016; Caygur et al., 2017) or sodium bicarbonate (Horning et al., 1987). Calculus must be removed using manual/ultrasonic conventional therapy; therefore, GPAP and EPAP have been proposed as an adjunctive treatment to standard SRP. Recent studies demonstrate that the addition of GPAP to full-mouth SRP may have minimal benefits for reducing gingival and systemic inflammation, as well as bacterial concentrations (Zhang et al., 2020). Results regarding the use of EPAP as an adjunctive treatment to standard SRP alone are encouraging, because it can reduce the number of residual periodontal pockets with PD $\geq$ 5 mm (Jentsch et al., 2020).

REFERENCES

Adriaens PA, De Boever JA, Loesche WJ (1988) Bacterial invasion in root cementum and radicular dentin of periodontally diseased teeth in humans. A reservoir of periodontopathic bacteria. J Periodontol 59:222– 230

Aimettj M. Nonsurgical periodontal treatment. Int J Esthet Dent 2014;9(2):251-67.

Andersen R, Loebel N, Hammond D, Wilson M. (2007): Treatment of periodontal disease by photodisinfection compared to scaling and root planing. The Journal of Clinical Dentistry, 18: 34-38.

Babilas P, Schreml S, Landthaler M, Szeimies RM (2010) Photodynamic therapy in dermatology: state-of-the-art. Photodermatol Photoimmunol Photomed 26:118–132.

Barca E, Cifcibasi E, Cintan S. Adjunctive use of antibiotics in periodontal therapy. J Istanb Univ Fac Dent 2015;49(3):55-62.

Barnes CM, Covey D, Watanabe H, Simetich B, Schulte JR, Chen H. An in vitro comparison of the effects of various air polishing powders on enamel and selected esthetic restorative materials. J Clin Dent. 2014;25(4):76-87. PMID: 26054183.

Berakdar, M., Callaway, A., Eddin, M. F., Ross, A., & Willershausen, B. (2012). Comparison between scaling-root-planing (SRP) and SRP/ photodynamic therapy: Six-month study. Head & Face Medicine, 8, 12. https://doi.org/10.1186/1746-160X-8-12

Berezow AB, Darveau RP. Microbial shift and periodontitis. Periodontol 2000. 2011;55: 36–47. pmid:21134227

Betsy J, Prasanth CS, Baiju KV, Prasanthila J, Subhash N. Efficacy of antimicrobial photodynamic therapy in the management of chronic periodontitis: a randomized controlled clinical trial. J Clin Periodontol. 2014; 41(6):573-581.

Bonito AJ, Lux L, Lohr KN. Impact of local adjuncts to scaling and root planing in periodontal disease therapy: a systematic review. J Periodontol. 2005 Aug;76(8):1227-36. doi: 10.1902/jop.2005.76.8.1227. Erratum in: J Periodontol. 2006 Feb;77(2):326. Erratum in: J Periodontol. 2006 Feb;77(2):326-327. PMID: 16101353.

Braun, A., Dehn, C., Krause, F., & Jepsen, S. (2008). Short-term clinical effects of adjunctive antimicrobial photodynamic therapy in periodontal treatment: A randomized clinical trial. Journal of Clinical Periodontology, 35, 877–884. https://doi.org/10.1111/j.1600-051X.2008.01303.x

Breininger DR, O'Leary TJ, Blumenshine RV (1987) Comparative effectiveness of ultrasonic and hand scaling for the removal of subgingival plaque and calculus. J Periodontol 58:9– 18

Bühler J, Amato M, Weiger R, Walter C. A systematic review on the patient perception of periodontal treatment using air polishing devices. Int J Dent Hyg. 2016 Feb;14(1):4-14. doi: 10.1111/idh.12119. Epub 2015 Jan 23. PMID: 25619863.

Caton JG, Ciancio SG, Blieden TM, et al. Treatment with subantimicrobial dose doxycycline improves the efficacy of scaling and root planing in patients with adult periodontitis. J Periodontol. 2000;71(4):521-532.

Caygur A, Albaba MR, Berberoglu A, Yilmaz HG. Efficacy of glycine powder air-polishing combined with scaling and root planing in the treatment of periodontitis and halitosis: A randomised clinical study. J Int Med Res. 2017 Jun;45(3):1168-1174. doi: 10.1177/0300060517705540. Epub 2017 Apr 20. PMID: 28425827; PMCID: PMC5536418.

Chan Y, Lai CH (2003) Bactericidal effects of different laser wave-lengths on periodontopathic germs in photodynamic therapy. Lasers Med Sci 18:51– 55

Chondros P, Nikolidakis D, Christodoulides N, et al. Photodynamic therapy as adjunct to non-surgical periodontal treatment in patients on periodontal maintenance: a randomized controlled clinical trial. Lasers Med Sci. 2009;24(5):681-688.

Christodoulides N, Nikolidakis D, Chondros P, et al. Photodynamic therapy as an adjunct to non-surgical periodontal treatment: a randomized, controlled clinical trial. J Periodontol. 2008;79(9):1638-1644.

Cobb CM. Clinical signifi cance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planing. J Clin Periodontol 2002;29(suppl 2):6-16.

Cosgarea R, Jepsen S, Fimmers R, Bodea A, Eick S, Sculean A. Clinical outcomes following periodontal surgery and root surface decontamination by erythritol-based air polishing. A randomized, controlled, clinical pilot study. Clin Oral Investig. 2020 Aug 24. doi: 10.1007/s00784-020-03533-9. Epub ahead of print. PMID: 32839833.

Cosyn J, Wyn I. A systematic review on the effects of the chlorhexidine chip when used as an adjunct to scaling and root planing in the treatment of chronic periodontitis. J Periodontol. 2006;77:257–64.

da Costa, L., Amaral, C., Barbirato, D. D. S., Leao, A. T. T., & Fogacci, M. F. (2017). Chlorhexidine mouthwash as an adjunct tomechanical therapy in chronic periodontitis: A meta-analysis. Journal of the American Dental Association, 148(5), 308–318. https://doi.org/10.1016/j.adaj.2017.01.021

Deo V, Gupta S, Bhongade ML, Jaiswal R. Evaluation of subantimicrobial dose doxycycline as an adjunct to scaling and root planing in chronic periodontitis patients with diabetes: a randomized, placebo-controlled clinical trial. J Contemp Dent Pract. 2010;11(3):009-016.

Donos, N., Calciolari, E., Brusselaers, N., Goldoni, M., Bostanci, N., & Belibasakis, G. N. (2019). The adjunctive use of host modulators in non-surgical periodontal therapy. A systematic review of randomized, placebo-controlled clinical studies. Journal of Clinical Periodontology, 47(Suppl 22), 116–238. https://doi.org/10.1111/jcpe.13232

Doungudomdacha S, Rawlinson A, Walsh TF, Douglas CW (2001) Effect of non-surgical periodontal treatment on clinical parameters and the numbers of Porphyromonas gingivalis, Prevotella intermedia and Actinobacillus actinomycetemcomitans at adult periodontitis sites. J Clin Periodontol 28:437– 445

Eickholz P, Nickles K, Koch R, Harks I, Hoffmann T, Kim T-S, et al. Is furcation class involvement affected by adjunctive systemic amoxicillin plus metronidazole? A clinical trial's exploratory subanalysis. J Clin Periodontol. 2016; pmid:27393928

Eke PI, Wei L, Borgnakke WS, Thornton-Evans G, Zhang X, Lu H, McGuire LC, Genco RJ. Periodontitis prevalence in adults ≥65years of age, in the USA. Periodontol 2000. 2016 Oct;72(1):76-95. doi: 10.1111/prd.12145. PMID: 27501492.

Emingil G, Gurkan A, Atilla G, Kantarci A. Subantimicrobial-dose doxycycline and cytokine-chemokine levels in gingival crevicular fluid. J Periodontol. 2011;82(3):452-461.

Flemmig TF, Hetzel M, Topoll H, Gerss J, Haeberlein I, Petersilka G. Subgingival debridement efficacy of glycine powder air polishing. J Periodontol. 2007 Jun;78(6):1002-10. doi: 10.1902/jop.2007.060420. PMID: 17539712.

Frencken JE, Sharma P, Stenhouse L, Green D, Laverty D, Dietrich T. Global epidemiology of dental caries and severe periodontitis - a comprehensive review. J Clin Periodontol. 2017 Mar;44 Suppl 18:S94-S105. doi: 10.1111/jcpe.12677. PMID: 28266116

Garg, S., & Pradeep, A. R. (2017). 1.2% Rosuvastatin and 1.2% atorvastatin gel local drug delivery and redelivery in the treatment of class II furcation defects: A randomized controlled clinical trial. Journal of Periodontology, 88, 259–265. https://doi.org/10.1902/jop.2016.160399

Garrett S, Johnson L, Drisko CH, et al. Two multi-center studies evaluating locally delivered doxycycline hyclate, placebo control, oral hygiene, and scaling and root planing in the treatment of periodontitis. J Periodontol. 1999;70(5):490-503. doi:10.1902/jop.1999.70.5.490,

Garrett, S., Adams, D.F., Bogle, G., Donly, K., Hastings Drisko, C., Hallmon, W.W., Brady Hancock, E., Hanes, P., Hawley, C.E., Johnson, L., Kiger, R., Killoy, W., Mellonig, J.T., Raab, F.J., Ryder, M., Stoller, N., Polson, A., Wang, H.-L., Wolinsky, L.E., Yukna, R.A., Harrold, C.Q., Hill, M., Johnson, V.B. and Lee Southard, G. (2000), The Effect of Locally Delivered Controlled-Release Doxycycline or Scaling and Root Planing on Periodontal Maintenance Patients Over 9 Months. Journal of Periodontology, 71: 22-30. doi:10.1902/jop.2000.71.1.22

Garza ZCF, Born M, Hilbers PAJ, van Riel NAW, Liebmann J. Visible Blue Light Therapy: Molecular Mechanisms and Therapeutic Opportunities. Curr Med Chem. 2018;25(40):5564-5577. doi: 10.2174/0929867324666170727112206. PMID: 28748760.

Ge, L., Shu, R., Li, Y., Li, C., Luo, L., Song, Z., Xie, Y., & Liu, D. (2011). Adjunctive effect of photodynamic therapy to scaling and root planing in the treatment of chronic periodontitis. Photomedicine and Laser Surgery, 29, 33–37. https://doi.org/10.1089/pho.2009.2727

Graziani F, Karapetsa D, Alonso B, Herrera D. Nonsurgical and surgical treatment of periodontitis: how many options for one disease? Periodontol 2000. 2017 Oct;75(1):152-188. doi: 10.1111/prd.12201. PMID: 28758300.

Grossi SG, Goodson JM, Gunsolley JC, Otomo-Corgel J, Bland PS, Doherty F, Comiskey J. Mechanical therapy with adjunctive minocycline microspheres reduces red-complex bacteria in smokers. J Periodontol. 2007 Sep;78(9):1741-50. doi: 10.1902/jop.2007.070118. PMID: 17760544.

Gruner D, Paris S, Schwendicke F. Probiotics for managing caries and periodontitis: systematic review and meta-analysis. J Dent 2016;48:16-25.

Gu Y, Walker C, Ryan ME, Payne JB, Golub LM. Non-antibacterial tetracycline formulations: clinical applications in dentistry and medicine. J Oral Microbiol. 2012;4:10.3402/jom.v4i0.19227. doi:10.3402/jom.v4i0.19227

Gursoy H, Ozcakir-Tomruk C, Tanalp J, Yilmaz S. Photodynamic therapy in dentistry: a literature review. Clin Oral Investig. 2013 May;17(4):1113-25. doi: 10.1007/s00784-012-0845-7. Epub 2012 Sep 27. PMID: 23015026.

Gursoy, H., Ozcakir–Tomruk, C., Tanalp, J., and Yilmaz, S. (2013). Photodynamic therapy in dentistry: a literature review. Clin. Oral Investig. 17, 1113–1125.

Haffajee AD, Torresyap G, Socransky SS. Clinical changes following four different periodontal therapies for the treatment of chronic periodontitis: 1-year results. J Clin Periodontol. 2007;34(3):243-253.

Hagenfeld D, Koch R, Jünemann S, Prior K, Harks I, Eickholz P, Hoffmann T, Kim TS, Kocher T, Meyle J, Kaner D, Schlagenhauf U, Ehmke B, Harmsen D Do we treat our patients or rather periodontal microbes with adjunctive antibiotics in periodontal therapy? A 16S rDNA microbial community analysis. PloS one, 13(4):2018: e0195534

Hägi TT, Hofmänner P, Eick S, Donnet M, Salvi GE, Sculean A, Ramseier CA (2015) The effects of erythritol air-polishing powder on microbiologic and clinical outcomes during supportive periodontal therapy: six-month results of a randomized controlled clinical trial. Quintessence Int 46:31–41. https://doi.org/10.3290/j.qi.a32817

Hägi TT, Hofmänner P, Salvi GE, Ramseier CA, Sculean A (2013) Clinical outcomes following subgingival application of a novel erythritol powder by means of air polishing in supportive periodontal therapy: a randomized, controlled clinical study. Quintessence Int 44:753–761. https://doi.org/10.3290/j.qi.a30606

Hajishengallis G, Liang S, Payne MA, et al. Low-abundance biofilm species orchestrates infl ammatory periodontal disease through the commensal microbiota and complement. Cell Host Microbe 2011;10(5):497-506.

Harks I, Koch R, Eickholz P, Hoffmann T, Kim T-S, Kocher T, et al. Is progression of periodontitis relevantly influenced by systemic antibiotics? A clinical randomized trial. J Clin Periodontol. 2015;42: 832–842. pmid:26250060

Hashino E, Kuboniwa M, Alghamdi SA, Yamaguchi M, Yamamoto R, Cho H, Amano A. Erythritol alters microstructure and metabolomic profiles of biofilm composed of Streptococcus gordonii and Porphyromonas gingivalis. Mol Oral Microbiol. 2013 Dec;28(6):435-51. doi: 10.1111/omi.12037. Epub 2013 Jul 29. PMID: 23890177.

Herrera, D., Matesanz, P., Martin, C., Oud, V., Feres, M., & Teughels, W. (2020). Adjunctive effect of locally delivered antimicrobials in periodontitis therapy: A systematic review and meta-analysis. Journal of Clinical Periodontology, 47(Suppl 22), 239–256. https://doi.org/10.1111/jcpe.13230

Ho S, Acharya A, Sidharthan S, Li K, Leung W, McGrath C, Pelekos G. A Systematic Review and Meta-analysis of Clinical, Immunological, and Microbiological Shift in Periodontitis After Nonsurgical Periodontal Therapy With Adjunctive Use of Probiotics. Journal of Evidence-Based Dental Practice, 20(1):2020; 1-14.

Horning GM, Cobb CM, Killoy WJ. Effect of an air-powder abrasive system on root surfaces in periodontal surgery.J Clin Periodontol 1987;14:213–220.

Hou GL, Tsai CC (1989) Clinical observations of the effects of nonsurgical periodontal therapy on human periodontal disease. II. Ultrasonic scaling and root planing for 6 months. Gaoxiong Yi Xue Ke Xue Za Zhi 5:72– 86

Hujoel PP, Löe H, Anerud A, Boysen H, Leroux BG. The informativeness of attachment loss on tooth mortality. J Periodontol. 1999 Jan;70(1):44-8. doi: 10.1902/jop.1999.70.1.44. PMID: 10052769.

Ioannou I, Dimitriadis N, Papadimitriou K, Sakellari D, Vouros I, Konstantinidis A (2009) Hand instrumentation versus ultrasonic debridement in the treatment of chronic periodontitis: a randomized clinical and microbiological trial. J Clin Periodontol 36:132– 141. doi: 10.1111/j.1600-051X.2008.01347.x

Jagadish Pai BS, Rajan SA, Srinivas M, Padma R, Suragimath G, Walvekar A, et al. Comparison of the efficacy of chlorhexidine varnish and chip in the treatment of chronic periodontitis. Contemp Clin Dent. 2013;4:156–61

James P, Worthington HV, Parnell C, Harding M, Lamont T, Cheung A, et al. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. Cochrane Database Syst Rev. 2017

Janiszewska-Olszowska J, Drozdzik A, Tandecka K, Grocholewicz K. Effect of air-polishing on surface roughness of composite dental restorative material - comparison of three different air-polishing powders. BMC Oral Health. 2020 Jan 30;20(1):30. doi: 10.1186/s12903-020-1007-y. PMID: 32000753; PMCID: PMC6993449.

Janus MM, Volgenant CMC, Brandt BW, Buijs MJ, Keijser BJF, Crielaard W, Zaura E, Krom BP. Effect of erythritol on microbial ecology of in vitro gingivitis biofilms. J Oral Microbiol. 2017 Jun 22;9(1):1337477. doi: 10.1080/20002297.2017.1337477. PMID: 28748040; PMCID: PMC5508376.

Jentsch, Holger & Flechsig, Christian & Kette, Benjamin & Eick, Sigrun. (2020). Adjunctive air-polishing with erythritol in nonsurgical periodontal therapy – a randomized clinical trial. 10.21203/rs.3.rs-47863/v1.

Jori G, Fabris C, Soncin M, Ferro S, Coppellotti O, Dei D, Fantetti L, Chiti G, Roncucci G (2006) Photodynamic therapy in the treatment of microbial infections: Basic principles and perspective applications. Lasers Surg Med 38:468– 481

Joshi D, Garg T, Goyal AK, Rath G. Advanced drug delivery approaches against periodontitis. Drug Deliv. 2016;23:363–77. ,Kalsi R, Vandana KL, Prakash S. Effect of local drug delivery in chronic periodontitis patients: a meta-analysis. J Indian Soc Periodontol. 2011;15:304–9.

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Keestra JAJ, Grosjean I, Coucke W, Quirynen M, Teughels W. Non-surgical periodontal therapy with systemic antibiotics in patients with untreated aggressive periodontitis: a systematic review and meta-analysis. J Periodontal Res. 2015;50: 689–706. pmid:25522248

Kelbauskiene S, Baseviciene N, Goharkhay K, Moritz A, Machiulskiene V. One-year clinical results of Er,Cr:YSGG laser application in addition to scaling and root planing in patients with early to moderate periodontitis. Lasers Med Sci. 2011;26(4):445-452.

Kinane DF. Local antimicrobial therapies in periodontal disease. Ann R Australas Coll Dent Surg. 2000;15:57–60.

Konan YN, Gurny R, Allemann E (2002) State of the art in the delivery of photosensitizers for photodynamic therapy. J Photochem Photobiol 66:89– 106

Lang NP, Adler R, Joss A, Nyman S. Absence of bleeding on probing. An indicator of periodontal stability. J Clin Periodontol. 1990 Nov;17(10):714-21. doi: 10.1111/j.1600-051x.1990.tb01059.x. PMID: 2262585.

Lecic J, Cakic S, Janjic Pavlovic O, Cicmil A, Vukotic O, Petrovic V, et al. Different methods for subgingival application of chlorhexidine in the treatment of patients with chronic periodontitis. Acta Odontol Scand. 2016;74:502–7.

Li H, Sun T, Liu C, Cao Y, Liu X. Photobiomodulation (450 nm) alters the infection of periodontitis bacteria via the ROS/MAPK/mTOR signaling pathway. Free Radic Biol Med. 2020 May 20;152:838-853. doi: 10.1016/j.freeradbiomed.2020.01.184. Epub 2020 Jan 31. PMID: 32014500.

Loos BG, Needleman I. Endpoints of active periodontal therapy. J Clin Periodontol. 2020 Jul;47 Suppl 22(Suppl 22):61-71. doi: 10.1111/jcpe.13253. PMID: 31912527; PMCID: PMC7670400.

Lopes BM, Theodoro LH, Melo RF, Thompson GM, Marcantonio RA. Clinical and microbiologic follow-up evaluations after non-surgical periodontal treatment with erbium:YAG laser and scaling and root planing. J Periodontol. 2010;81(5):682-691.

Ma L., Diao, X. Effect of chlorhexidine chip as an adjunct in non-surgical management of periodontal pockets: a meta-analysis. BMC Oral Health 20, 262 (2020))

Martin-Cabezas R, Davideau JL, Tenenbaum H, Huck O. Clinical effi cacy of probiotics as an adjunctive therapy to non-surgical periodontal treatment of chronic periodontitis: a systematic re-view and meta-analysis. J Clin Periodontol 2016;43(6):520-30.

Martin-Cabezas R, Davideau JL, Tenenbaum H, Huck O. Clinical efficacy of probiotics as an adjunctive therapy to non-surgical periodontal treatment of chronic periodontitis: a systematic review and meta-analysis. J Clin Periodontol. 2016 Jun;43(6):520-30. doi: 10.1111/jcpe.12545. Epub 2016 May 7. PMID: 26970230.

Matesanz-Pérez P, García-Gargallo M, Figuero E, Bascones-Martínez A, Sanz M, Herrera D. A systematic review on the effects of local antimicrobials as adjuncts to subgingival debridement, compared with subgingival debridement alone, in the treatment of chronic periodontitis. J Clin Periodontol. 2013 Mar;40(3):227-41. doi: 10.1111/jcpe.12026. Epub 2013 Jan 16. PMID: 23320860.

Matesanz-Pérez P, García-Gargallo M, Figuero E, Bascones-Martínez A, Sanz M, Herrera D. A systematic review on the effects of local antimicrobials as adjuncts to subgingival debridement, compared with subgingival debridement alone, in the treatment of chronic periodontitis. J Clin Periodontol. 2013;40:227–41.)

Matsubara VH, Bandara HM, Ishikawa KH, Mayer MP, Samaranayake LP. The role of probiotic bacteria in managing periodontal disease: a systematic review. Expert Rev Anti Infect Ther 2016;14(7):643-55.

Matuliene G, Pjetursson BE, Salvi GE, Schmidlin K, Brägger U, Zwahlen M, Lang NP (2008) Influence of residual pockets on progression of periodontitis and tooth loss: results after 11 years of maintenance. J Clin Periodontol 35:685–695. https://doi.org/10.1111/j.1600-051X.2008.01245.x.

Miranda TS, Feres M, Perez-Chaparro PJ, et al. Metronidazole and amoxicillin as adjuncts to scaling and root planing for the treatment of type 2 diabetic subjects with periodontitis: 1-year outcomes of a randomized placebo-controlled clinical trial. J Clin Periodontol. 2014;41(9):890-899.

Moëne R, Décaillet F, Andersen E, Mombelli A. Subgingival plaque removal using a new air-polishing device. J Periodontol. 2010 Jan;81(1):79-88. doi: 10.1902/jop.2009.090394. PMID: 20059420.

Müller N, Moëne R, Cancela JA, Mombelli A. Subgingival air-polishing with erythritol during periodontal maintenance: randomized clinical trial of twelve months. J Clin Periodontol. 2014 Sep;41(9):883-9. doi: 10.1111/jcpe.12289. Epub 2014 Aug 7. PMID: 25041441; PMCID: PMC4287198.

Muniz FWMG, Taminski K, Cavagni J, Celeste RK, Weidlich P, Rösing CK. The effect of statins on periodontal treatment-a systematic review with meta-analyses and meta-regression. Clin Oral Investig. 2018 Mar;22(2):671-687. doi: 10.1007/s00784-018-2354-9. Epub 2018 Feb 2. PMID: 29396642.

Neill ME, Mellonig JT. Clinical efficacy of the Nd:YAG laser for combination periodontitis therapy. Pract Periodontics Aesthet Dent. 1997;9(6 suppl):1-5.

Nyman S, Westfelt E, Sarhed G, Karring T (1988) Role of “diseased” root cementum in healing following treatment of periodontal disease. A clinical study. J Clin Periodontol 15:464-68 http://www.ncbi.nlm.nih.gov/pubmed/3053788.

Paddmanabhan P. Antimicrobials in treatment of periodontal disease -A review. J Med Dent Sci 2013;4(5):19-23.

Parker EA, Roy T, D'Adamo CR, Wieland LS. Probiotics and gastrointestinal conditions: An overview of evidence from the Cochrane Collaboration. Nutrition. 2018 Jan;45:125-134.e11. doi: 10.1016/j.nut.2017.06.024. Epub 2017 Jul 6. PMID: 28870406; PMCID: PMC5683921.

Petersilka G, Koch R, Vomhof A, Joda T, Harks I, Arweiler N, Ehmke B. Retrospective analysis of the long-term effect of subgingival air polishing in supportive periodontal therapy. J Clin Periodontol. 2020 Oct 24. doi: 10.1111/jcpe.13392. Epub ahead of print. PMID: 33098121.

Petersilka GJ (2011) Subgingival air-polishing in the treatment of periodontal biofilm infections. Periodontol 2000 55:124–142. https://doi.org/10.1111/j.1600-0757.2010.00342.x

Petersilka GJ, Steinmann D, Haberlein I, Heinecke A, Flemmig TF (2003b) Subgingival plaque removal in buccal and lingual sites using a novel low abrasive air-polishing powder. J Clin Periodontol 30:328–333. https://doi.org/10.1034/j.1600-051x.2003.00290.x

Petersilka GJ. Subgingival air-polishing in the treatment of periodontal biofilm infections. Periodontol 2000. 2011 Feb;55(1):124-42. doi: 10.1111/j.1600-0757.2010.00342.x. PMID: 21134232.

Petersilka, G. J., Bell, M., Haberlein, I., Mehl, A., Hickel, R. & Flemmig, T. F. (2003a) In vitro evaluation of novel low abrasive air polishing powders. Journal of Clinical Periodontology 30, 9– 13.

Pihlstrom B. Issues in the evaluation of clinical trials of periodontitis: a clinical perspective. J Periodontal Res. 1992 Jul;27(4 Pt 2):433-41. doi: 10.1111/j.1600-0765.1992.tb01710.x. PMID: 1507032.

Pradeep AR, Kathariya R. Clarithromycin, as an adjunct to non surgical periodontal therapy for chronic periodontitis: a double blinded, placebo controlled, randomized clinical trial. Arch Oral Biol. 2011;56(10): 1112-1119.

Pradeep, A. R., Garg, V., Kanoriya, D., & Singhal, S. (2016). 1.2% Rosuvastatin versus 1.2% atorvastatin gel local drug delivery and redelivery in treatment of intrabony defects in chronic periodontitis: A randomized placebo-controlled clinical trial. Journal of Periodontology, 87, 756–762. https://doi.org/10.1902/jop.2016.150706

Prates RA, Yamada AM, Suzuki LC, França CM, Cai S, Mayer MP, Ribeiro AC, Ribeiro MS. Histomorphometric and microbiological assessment of photodynamic therapy as an adjuvant treatment for periodontitis: a short-term evaluation of inflammatory periodontal conditions and bacterial reduction in a rat model. Photomed Laser Surg.

2011 Dec;29(12):835-44. doi: 10.1089/pho.2010.2984. Epub 2011 Sep 14. PMID: 21916615; PMCID: PMC3231969.

Preshaw, P. M. (2018). Host modulation therapy with anti-inflammatory agents. Periodontology 2000, 76, 131–149. https://doi.org/10.1111/prd.12148

Preus HR, Gunleiksrud TM, Sandvik L, Gjermo P, Baelum V. A randomized, double-masked clinical trial comparing four periodontitis treatment strategies: 1-year clinical results. J Periodontol. 2013;84(8):1075-1086.

Rajesh S, Koshi E, Philip K, Mohan A. Antimicrobial photody-namic therapy: an overview. J Indian Soc Periodontol 2011;15(4):323-7.

Ramseier, C. A., Woelber, J. P., Kitzmann, J., Detzen, L., Carra, M. C., & Bouchard, P. (2020). Impact of risk factor control interventions for smoking cessation and promotion of healthy lifestyles in patients with periodontitis: A systematic review. Journal of Clinical Periodontology, 47(Suppl 22), 90–106. https://doi.org/10.1111/jcpe.13240

Reddy MS, Geurs NC, Gunsolley JC. Periodontal host modulation with antiproteinase, anti-inflammatory, and bone-sparing agents. A systematic review. Ann Periodontol. 2003;8(1):12-37. doi:10.1902/annals.2003.8.1.12

Rotundo R, Nieri M, Cairo F, et al. Lack of adjunctive benefit of Er:YAG laser in non-surgical periodontal treatment: a randomized splitmouth clinical trial. J Clin Periodontol. 2010;37(6):526-533.

Saglam M, Kantarci A, Dundar N, Hakki SS. Clinical and biochemical effects of diode laser as an adjunct to nonsurgical treatment of chronic periodontitis: a randomized, controlled clinical trial. Lasers Med Sci. 2014;29(1):37-46.

Sampaio E, Rocha M, Figueiredo LC, et al. Clinical and microbiological effects of azithromycin in the treatment of generalized chronic periodontitis: a randomized placebo-controlled clinical trial. J Clin Periodontol. 2011;38(9):838-846.

Sanz M, Herrera D, Kebschull M, Chapple I, Jepsen S, Beglundh T, Sculean A, Tonetti MS; EFP Workshop Participants and Methodological Consultants. Treatment of stage I-III periodontitis-The EFP S3 level clinical practice guideline. J Clin Periodontol. 2020 Jul;47 Suppl 22(Suppl 22):4-60. doi: 10.1111/jcpe.13290. Erratum in: J Clin Periodontol. 2021 Jan;48(1):163. PMID: 32383274; PMCID: PMC7891343.

Sanz-Sanchez I, Montero E, Citterio F, Romano F, Molina A, Aimetti M (2020) Efficacy of access flap procedures compared to subgingival debridement in the treatment of periodontitis. A systematic review and meta-analysis. J Clin Periodontol 47:282–302. https://doi.org/10.1111/jcpe.1325910.1111/jcpe.13259.

Sbordone L, Ramaglia L, Gulleta E, Iacono Y (1990) Recolonization of the subgingival microflora after scaling and root planning in human periodontitis. J Periodontol 61:579– 584

Sekino S, Ogawa T, Murakashi E, Ito H, Numabe Y. Clinical and microbiological effect of frequent subgingival air polishing on periodontal conditions: a split-mouth

randomized controlled trial. Odontology. 2020 Oct;108(4):688-696. doi: 10.1007/s10266-020-00493-0. Epub 2020 Feb 18. PMID: 32072344.

Serrage H , Heiskanen V , Palin WM , et al. Under the spotlight: mechanisms of photobiomodulation concentrating on blue and green light. Photochem Photobiol Sci. 2019;18(8):1877-1909. doi:10.1039/c9pp00089e

Sgolastra F, Petrucci A, Gatto R, Marzo G, Monaco A. Photodynamic therapy in the treatment of chronic periodontitis: a systematic review and meta-analysis. Lasers Med Sci 2013: 28: 669– 682.

Shanbhag S, Dahiya M, Croucher R. The impact of periodontal therapy on oral health-related quality of life in adults: a systematic review. J Clin Periodontol. 2012 Aug;39(8):725-35. doi: 10.1111/j.1600-051X.2012.01910.x. Epub 2012 Jun 13. PMID: 22694297.

Smiley CJ, Tracy SL, Abt E, et al. (1) Systematic review and meta-analysis on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts. J Am Dent Assoc. 2015;146(7):508-24.e5. doi:10.1016/j.adaj.2015.01.028

Smiley CJ, Tracy SL, Abt E, et al.(2) Evidence-based clinical practice guideline on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts. J Am Dent Assoc. 2015;146(7):525-535.

Socransky SS, Haffajee AD (2002) Dental biofilms: Difficult therapeutic targets. Periodontol 2000:28:12– 55

Socransky SS, Haffajee AD, Teles R, Wennstrom JL, Lindhe J, Bogren A, et al. Effect of periodontal therapy on the subgingival microbiota over a 2-year monitoring period. I. Overall effect and kinetics of change. J Clin Periodontol. 2013;40: 771–780. pmid:23710672

Söderling E, Pienihäkkinen K. Effects of xylitol and erythritol consumption on mutans streptococci and the oral microbiota: a systematic review. Acta Odontol Scand. 2020 Nov;78(8):599-608. doi: 10.1080/00016357.2020.1788721. Epub 2020 Jul 7. PMID: 32633595.

Soskolne WA, Chajek T, Flashner M, Landau I, Stabholtz A, Kolatch B, et al. An in vivo study of the chlorhexidine release profile of the PerioChip in the gingival crevicular fluid, plasma and urine. J Clin Periodontol. 1998;25:1017–21.

Surve SM, Acharya AB, Thakur SL (2015) Efficacy of subgingivally delivered atorvastatin and simvastatin as an adjunct to scaling and root planing. Drug Metabol Personal Ther 30(4):263–269. https://doi.org/10.1515/dmpt-2015-0024

Suvan J, Leira Y, Moreno Sancho FM, Graziani F, Derks J, Tomasi C. Subgingival instrumentation for treatment of periodontitis. A systematic review. J Clin Periodontol. 2020 Jul;47 Suppl 22:155-175. doi: 10.1111/jcpe.13245. PMID: 31889320.

Tabenski, L., Moder, D., Cieplik, F. et al. Antimicrobial photodynamic therapy vs. local minocycline in addition to non-surgical therapy of deep periodontal pockets: a

controlled randomized clinical trial. Clin Oral Invest 21, 2253–2264 (2017). https://doi.org/10.1007/s00784-016-2018-6

Takasaki AA, Aoki A, Mizutani K et al (2009) Application of antimicrobial photodynamic therapy in periodontal and peri-implant diseases. Periodontol 2000 2000(51):109–140

Theodoro, L. H., Silva, S. P., Pires, J. R., Soares, G. H., Pontes, A. E., Zuza, E. P., Spolidorio, D. M., de Toledo, B. E., & Garcia, V. G. (2012). Clinical and microbiological effects of photodynamic therapy associated with nonsurgical periodontal treatment. A 6-month follow-up. Lasers in Medical Science, 27, 687–693. https://doi.org/10.1007/s10103-011-0942-x

Trombelli L, Farina R, Pollard A, Claydon N, Franceschetti G, Khan I, West N. Efficacy of alternative or additional methods to professional mechanical plaque removal during supportive periodontal therapy: A systematic review and meta-analysis. J Clin Periodontol. 2020 Jul;47 Suppl 22:144-154. doi: 10.1111/jcpe.13269. PMID: 32060940.

Umeda M, Takeuchi Y, Noguchi K, Huang Y, Koshy G, Ishikawa I (2004) Effects of nonsurgical periodontal therapy on the microbiota. Periodontol 2000: 36:98– 120

Ustun K, Erciyas K, Sezer U, et al. Clinical and biochemical effects of 810 nm diode laser as an adjunct to periodontal therapy: a randomized split-mouth clinical trial. Photomed Laser Surg. 2014;32(2):61-66.

Van der Weijden GA, Timmerman MF. A systematic review on the clinical efficacy of subgingival debridement in the treatment of chronic periodontitis. J Clin Periodontol. 2002;29 Suppl 3:55-71; discussion 90-1. doi: 10.1034/j.1600-051x.29.s3.3.x. PMID: 12787207.

Wennström JL, Dahlén G, Ramberg P. Subgingival debridement of periodontal pockets by air polishing in comparison with ultrasonic instrumentation during maintenance therapy. J Clin Periodontol. 2011 Sep;38(9):820-7. doi: 10.1111/j.1600-051X.2011.01751.x. Epub 2011 Jul 7. PMID: 21736599.

Wennström JL, Tomasi C, Bertelle A, Dellasega E. Full-mouth ultrasonic debridement versus quadrant scaling and root planing as an initial approach in the treatment of chronic periodontitis. J Clin Periodontol. 2005 Aug;32(8):851-9. doi: 10.1111/j.1600-051X.2005.00776.x. PMID: 15998268.

Wilson M (1993) Photolysis of oral bacteria and its potential use in the treatment of caries and periodontal disease. J Appl Bacteriol 75: 299– 306

Zhang W, Wang W, Chu C, Jing J, Yao NA, Sun Q, Li S. Clinical, inflammatory and microbiological outcomes of full-month scaling with adjunctive glycine powder air-polishing. A randomized trial. J Clin Periodontol. 2020 Nov 10. doi: 10.1111/jcpe.13400. Epub ahead of print. PMID: 33174234.

Zmora N, Zilberman-Schapira G, Suez J, et al. Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. Cell 2018;174(6):1388-1405.e21.

AIM OF THE THESIS

This Ph.D program aimed at evaluating the adjunctive effect of different procedures over standard scaling and root planing (SRP) in the treatment of moderate to severe periodontitis (stage III and IV). Two randomized controlled trials were performed in order to answer specific research/clinical questions:

- What is the adjunctive clinical efficacy of phototerapic protocol (PTP) to standard SRP?
- What is the adjunctive clinical efficacy of sub-gingival air-polishing in the context of EPAP treatment?

Chapter II

No Benefit of an Adjunctive Phototherapy Protocol in Treatment of Periodontitis: a Split-Mouth Randomized Controlled Trial

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Data availability statement:

Data subject to third party restrictions. The data that support the findings of this study are available from Colgate-Palmolive (Europe). Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the authors with the permission of Colgate-Palmolive (Europe).

ABSTRACT

Aim: To assess the efficacy of a commercially-available adjunctive phototherapy protocol ('Perio-1') in treatment of periodontitis.

Materials and Methods: In an examiner-blind, randomized, controlled, split-mouth, multi-centre study, 60 periodontitis patients received root surface debridement (SRP) in sextants either alone (control sextants) or with the adjunctive phototherapy protocol (test sextants). Re-evaluation was performed at 6, 12 and 24 weeks.

Results: No statistically significant differences in mean (± standard deviation) clinical attachment level (CAL) change from baseline to week 24 were observed between test (-1.00±1.16 mm) and control sextants (-0.87±0.79 mm) at sites with probing pocket depths (PDs) ≥ 5 mm ("deep sites") at baseline (p=0.212). Comparisons between test and control sextants for all other parameters (CAL change at all sites, PD change at deep sites/all sites, bleeding on probing, plaque scores), and for all change intervals, failed to identify any statistically significant differences.

Conclusions: The phototherapy protocol did not provide any additional clinical benefits over those achieved by SRP alone. (German Clinical Trials Register DRKS00011229).

CLINICAL RELEVANCE

Scientific rationale for the study

An adjunctive phototherapy protocol ('Perio-1') is available for treatment of periodontitis. However, there is a lack of evidence regarding the clinical efficacy of the protocol.

Principal findings

Sextants treated with root surface debridement (SRP) plus the adjunctive phototherapy protocol did not demonstrate better clinical outcomes compared to sextants treated with SRP alone.

Practical implications

The findings of this research do not support use of the phototherapy protocol in the treatment of periodontitis. It is important that properly designed studies that investigate mechanisms of action and clinical efficacy are performed prior to new technologies being used in patient care.

INTRODUCTION

Periodontitis is a chronic inflammatory disease that results from the complex interplay between the host immune-inflammatory response and the long-term presence of a dysbiotic bacterial biofilm in the subgingival environment. Biofilm control is a key component of therapy, and is routinely achieved via professionally delivered non-surgical therapy that includes root surface debridement (SRP) to remove and reduce biofilm and calculus, together with patient education to improve oral hygiene. Whereas the clinical improvements that can be achieved by SRP are well recognised (Cobb, 2002), it is also known that there are limitations in the effectiveness of the procedure, particularly in deeper pockets. Bacteria may persist within anatomical niches or within the soft tissues, leading to recolonization of the pocket following therapy (Uzel et al., 2011). Accordingly, efforts have been expended to develop local adjuncts to mechanical debridement that may further reduce the bacterial challenge, with the aim to improve outcomes of treatment.

An important group of such adjunctive therapies includes those activated by light (i.e. phototherapies), including antimicrobial photodynamic therapy (aPDT) and photobiomodulation (PBM). In periodontal therapy, aPDT involves application of a photosensitising agent into the subgingival environment followed by its activation by laser light of specific wavelength, resulting in the formation of reactive oxygen species that enhance bacterial killing (Chambrone et al., 2018). Systematic reviews on the use of aPDT in periodontal therapy that were conducted as part of the 2019 European Workshop on Periodontology, and which informed the EFP S3-level evidence-based guidelines (Sanz et al., 2020), did not support the use of aPDT at wavelengths of either 660–670 nm or 800–900 nm in patients with periodontitis during either step I-II of therapy (Salvi et al., 2020) or supportive periodontal care (Trombelli et al., 2020). PBM refers to phototherapy in which low energy light is used to elicit a biological response. This has been investigated and employed in several branches of medical practice, and has been previously delivered using either laser or light-emitting diode (LED) light emission. Several possible mechanisms may be involved in therapeutic gains from these therapies, and such gains may be greater in tissues with high turnover, inflammation, oxidative stress or tissue damage (Hamblin, 2017, Hamblin, 2018, Passarella and Karu, 2014). PBM (alone or combined with photodynamic therapy) has been reported to be effective in management of oral mucositis for patients undergoing cancer treatment (Pires Marques et al., 2020) as well as for pain relief following endodontic treatment (Nunes et al., 2020).

Recently, a new phototherapy product for use in periodontal treatment has become commercially available that has built on developments in dermatology (Antoniou et al 2016). This methodology is based on delivery of light absorbing molecules (chromophores), which subsequently are able to act to convert incident light energy such as that from a local light source into emitted fluorescence. This, in turn, is purported to act to modify cellular activity, or at certain wavelengths, to have possible antimicrobial activity. The penetration and activating ability of these agents can potentially be adjusted by selection of differing fluorescence agents, related to fluorescence emission wavelength. Preliminary observations on the combined use of SRP with the phototherapy product showed a clinical benefit in terms of attachment gain and probing depth reduction (Kamma et al., 2015). However, clinical efficacy in a larger-scale RCT has not been reported.

Therefore, we conducted a clinical study that aimed to assess the efficacy of the phototherapy product (as based on manufacturer's recommendations and protocol) when used as an adjunct to SRP in the treatment of periodontitis. The null hypothesis was that there would be no significant differences in change in the primary efficacy variable between sextants treated with the adjunctive phototherapy compared to sextants treated by SRP alone.

MATERIALS AND METHODS

Study outline

This was an examiner-blind, randomized, controlled, split-mouth, multi-centre study conducted at three university research centres: School of Dental Sciences at Newcastle University (UK), Faculty of Dentistry Oral and Craniofacial Sciences of King's College London (UK), and University of Ferrara (Italy). The clinical phases of the study ran from September 2016 (first patient recruited 09.09.2016) to June 2017 (last visit of last patient 30.06.2017). The aim was to assess the effectiveness and safety of the phototherapy protocol when used as an adjunct to SRP in patients with periodontitis. The study was conducted in compliance with the requirements of directive 93/42/EEC, and was registered on the German Clinical Trials Register (https://www.drks.de/drks_web/ ref. DRKS00011229). Ethical approval was obtained from ethics committees in the UK (London Camden and Kings Cross NHS Research Ethics Committee, ref. 16/LO/1259) and in Italy (Comitato Etico Unico della Provincia di Ferrara, ref. CE 160587) prior to commencement. The study was sponsored by Colgate-Palmolive (Europe)

and monitoring and statistical analyzes were performed by a contract research organisation (OPIS Europe).

Study participants

Participants were recruited from hospital clinics at the three study centres. Participants were diagnosed with moderate-to-severe chronic periodontitis according to the classification system in use at the time (Armitage, 1999), corresponding to periodontitis stage 3 or 4, grade B according to the new classification (Tonetti et al., 2018). Inclusion criteria were: (i) signed informed consent, (ii) male or female, aged 18-70 years inclusive, (iii) good general health (e.g. free from systemic diseases such as diabetes, arthritis, HIV, genetic disorders, malignancy, as determined by medical history), (iv) periodontitis with a minimum of two posterior sextants with probing pocket depths (PDs) 5 mm or greater. Posterior sextants were defined as the dentition from the distal of the canine to the distal of the second molar, and containing at least two teeth in contact. Exclusion criteria were: (i) orthodontic appliances, (ii) tumours or other significant pathology of the oral cavity, (iii) PDs of ≥10 mm in test or control sextants, (iv) caries lesions requiring immediate care, (v) participation in any other clinical study within the last 30 days, (vi) pregnant or lactating women (by medical history), (vii) history of allergies to tooth whitening products, H_2O_2, personal care consumer products or their ingredients, (viii) use of locally applied or systemic antibacterial agents or dental prophylaxis in the last 30 days, (ix) use of drugs or products known to induce photosensitivity reactions, (x) any medical condition with known photosensitivity, (xii) skin hypersensitivity.

Test protocol

The test protocol was based on a CE-marked medical device (‘Perio-1’, Klox Technologies, Quebec, Canada) comprising a two-phase photo-converter gel containing an active ingredient chromophore (specific absorption to blue-green light), producing O_2 and singlet oxygen when photo-activated with light at 430 nm. According to the manufacturer’s instructions, the gel was mixed then applied and photo-activated twice: immediately before SRP and then repeated following SRP. The method of application was the same both times, and the gel was applied to all sites in the test sextant. Specifically, the gel was dispensed to the depth of the pockets using a syringe until the pockets were filled and slightly overflowing (Figure 2.1a). It was then photo-activated by inserting a clear plastic probe-like tip attached to the 10 mm diameter tip of a standard dental curing light (Bluephase LED light, Ivoclar Vivadent) to the depth of the pockets

at each tooth in the sextant (Figure 2.1b). This multi-LED light source emits a broadband spectrum of 385-515 nm, with a main peak at 465 nm and a secondary peak at 405 nm. The light was illuminated for 30 seconds buccally, and then 30 seconds lingually/palatally at each tooth, keeping the tip slowly moving so that it occupied approximately 10 seconds at each periodontal site. The power density of the LED light is estimated to be 1,200 mW/cm^2 at a distance of 3-5 mm from the light source with a radiant fluence (or dose) during a single 10 second exposure of $0.3W/cm^2$ or $3J/cm^2$ per site. Any visible gel at the end of photo-activation was removed with gauze, and it was not washed off or washed out of the pockets.

Randomisation, blinding and allocation concealment

The study sextants were two posterior sextants that were allocated randomly to one of the two treatments (test sextant and control sextant, i.e. split mouth design). Stratification was undertaken based on the number of deep sites at baseline (PD ≥5 mm) to ensure similar extent of disease in test and control sextants. Randomisation was undertaken centrally through an electronic system integrated into the electronic case report form (e-CRF) and only occurred when all inclusion/exclusion criteria had been verified and the patient was attending for the treatment visit, to ensure allocation concealment. The study was performed under examiner-blind conditions. Assessing examiners (i.e. clinicians who examined patients and recorded periodontal indices), study monitors, study coordinators, data managers, and statisticians, were unaware of the treatment that was administered in the study sextants. Knowledge of the randomization list was restricted to individuals involved in the application of the study product only. All investigators, treating clinicians and clinical examiners from the three study centres undertook a 2-day joint training exercise in the administration of the phototherapy protocol and the clinical evaluations prior to study commencement. Those clinicians who performed the periodontal treatment and phototherapy procedure took no part in any other study procedures or evaluations.

Study procedures and outcome measures

The study protocol is detailed in Figure 2.2. At visit 1 (baseline), written informed consent was obtained, and demographic and medical history data were collected. Randomisation of sextants occurred at visit 2, immediately prior to SRP. The control sextant was treated by SRP using a combination of ultrasonic and hand instruments under local anaesthesia as indicated clinically. Following this, the test sextant was treated in the same manner, except with the application and

photo-activation of the gel immediately before and immediately after SRP. Thus, the sequence of treatment was: (i) control sextant SRP, (ii) test sextant, first gel application and photo-activation, (iii) test sextant SRP, (iv) test sextant, second gel application and photo-activation. All participants were provided with a standard toothpaste (Colgate Great Regular Flavour fluoride toothpaste containing sodium monofluorophosphate 0.79%, 0.1% w/v fluoride) for use during the study. Participants were asked to continue to use their regular manual or powered toothbrush, and to use interproximal cleaning devices as indicated, but were asked to refrain from using any other adjunctive oral home care products (e.g. mouthrinses). Participants completed a pain diary to record any pain in study sextants (test/control) following the treatment, how long it lasted following recovery of sensation (after anaesthesia had dissipated), and its severity on a 100mm visual analogue scale (VAS, limits marked 'no pain' and 'unbearable pain'). The clinician who provided the treatment at visit 2 indicated the sextants to be considered in the pain diary (e.g. upper right, lower left, but did not specify where gel had been applied), and this was returned by post to the study centre within 24 hours and received by staff who took no part in any clinical assessments to maintain blinding.

General health status, oral soft tissue status, and details of any concomitant therapy or adverse events were recorded at each visit. Periodontal parameters were recorded at 6 sites per tooth at all visits except visit 2. Plaque score was determined following disclosing (Butler Gum Red-Cote) as absent (0) or present (1). PDs and recession were recorded using a manual UNC-15 periodontal probe and bleeding on probing (BOP) was recorded as absent (0) or present (1) following probing at each site. Clinical attachment level (CAL) was calculated from PD and recession. If SRP was also required in regions other than the test and control sextants, this was provided between 1 and 7 days following visit 2. At visits 3 and 4, if supportive periodontal therapy or reinforcement of oral hygiene was required, this was provided following the collection of study data. Following visit 5 (end of study), participants were entered into routine recall for periodontal maintenance or scheduled for any further interventions as indicated clinically. Participants who withdrew from the study prematurely underwent the final examination at the time of study termination.

Statistical analyzes

The primary efficacy variable was change in mean CAL at deep sites (sites with PD ≥5 mm at baseline) from baseline to 24 weeks. A sample size of 50 participants was determined to be

sufficient to detect a clinically relevant difference of 1 mm in the primary efficacy variable, assuming a standard deviation (SD) of 2 mm in both groups, using a two-tailed paired test of the difference between treatment means, significance level α=0.05 and 90% power (Kamma et al., 2015). To allow for drop-outs, the recruitment target was 60 participants.

The unit of analysis was the study sextant, i.e. test sextants treated with SRP plus adjunctive phototherapy, and control sextants treated with SRP only. Analysis was performed comparing test sextants vs. control sextants. The primary efficacy variable (mean CAL change at deep sites) was analyzed by analysis of covariance (ANCOVA), with subject (random factor), treatment (fixed factor), study centre (fixed factor), number of deep sites at baseline ($\leq$4 deep sites or >4 deep sites, fixed effect) and centre-by-treatment interaction (fixed factor) as terms, and baseline mean CAL as covariate in the model. Analysis of the primary efficacy variable (and also mean CAL change at all sites) was conducted for the intent-to-treat (ITT) population using a last observation carried forward (LOCF) approach. The secondary efficacy variables were analyzed using the same ANCOVA model, and included changes in mean plaque scores, PDs (at deep sites and all sites) and %BOP. Analysis of the secondary efficacy endpoints was conducted for the ITT population but did not use a LOCF approach. Normality testing was performed using the Shapiro Wilk test, and in the case of non-normal distribution of the data, the ANCOVA model was applied on rank transformed data. Safety variables were the number of adverse events (AEs) and serious adverse events (SAEs) occurring. Systemic AEs were analyzed at the patient level, while oral cavity AEs were analyzed at the sextant level by treatment group.

Two analysis sets are reported: an ITT population and a safety population. The ITT population (n=60) consisted of each randomized participant in the study who received the study treatment, and from whom at least one post-treatment measurement was available in both study sextants. The safety population consisted of all participants who received the study treatment (n=69).

RESULTS

A total of 81 potential participants were screened, and 69 were randomized to study treatment (safety population). Of these, 68 participants completed the study, with one participant discontinuing the study early due to a SAE. Nine of the 69 randomized participants were excluded from the ITT population because of using systemic antibiotics during the study.

Accordingly, the ITT population consisted of 60 participants (Newcastle: n=24; London: n=19; Ferrara: n=17). Figure 2.3 presents the flow chart of the study.

The mean ± SD age of the ITT population was 51.4±8.4 years, 29 (48.3%) were female, and the racial distribution was white Caucasian: 50 (83%); Black or African American: 2 (3.3%); Asian: 5 (8.3%) and other: 3 (5.0%). 4 participants (8.3%) were cigarette smokers, smoking an average of 11.8 ± 5.4 per day, and one smoked cigars (3 per day). Regarding use of oral hygiene products, 25 (41.7%) used a manual toothbrush and 35 (58.3%) used a powered toothbrush. 56 participants (93.3%) reported routine use of interdental cleaning aids; of these, dental floss was used by 25.0%, interdental brushes by 92.9% and woodsticks by 5.4%.

Table 2.1 presents mean CAL data at deep sites and all sites over the course of the study, and Table 2.2 presents mean CAL change data at deep sites and all sites from visit 1 (baseline) to visit 5 (week 24), using the LOCF approach. With regards to the primary efficacy variable, although mean CAL values were significantly lower at both deep sites and all sites in text and control sextants at visit 5 compared to baseline, there were no statistically significant differences in CAL change between test and control sextants from baseline to week 24 (Table 2, deep sites p=0.212; all sites p=0.255). A subsequent sensitivity analysis performed by means of a mixed-effect ANCOVA model fitted according to the conventional LOCF approach without adjustment for number of deep sites at baseline provided further confirmation of group homogeneity, with no statistically significant differences identified between test and control sextants (p=0.223). An additional per-protocol analysis (which excluded data from any participants who experienced protocol deviations) also failed to identify statistically significant differences between test and control sextants over the course of the study (data not shown). Furthermore, no statistically significant differences in CAL change (at deep sites or all sites) between test and control sextants were identified at the individual treatment centres (data not shown).

Table 2.3 presents mean CAL and PD data (for deep sites and all sites) as well as %BOP and plaque, and Table 2.4 presents change data from visit 1 (baseline) to visit 3 (week 6), visit 4 (week 12) and visit 5 (week 24), not using the LOCF approach. For mean CAL (deep sites and all sites), PD (deep sites and all sites), and %BOP (all sites), statistically significant reductions were observed from baseline to all post-treatment time points (all $p<0.0001$). However,

comparisons between test and control sextants for all parameters and for all change intervals failed to identify any significant differences between test and control (all $p>0.05$). Furthermore, no significant changes in plaque scores were detected between baseline and any post-treatment time point in either test or control sextants, or between test and control sextants at any change interval. Sensitivity analyzes performed without adjustment for number of deep sites at baseline yielded similar outcomes, with no significant differences between test and control sextants identified for any assessed variable (all $p>0.05$). Furthermore, no significant differences (centre effects) in the secondary efficacy variables were identified when analyzing data from the individual treatment centres (data not shown). We also evaluated the number of residual deep sites (PD ≥5 mm) at visit 5, but found no evidence of differences between groups; in test sextants, the mean number of deep sites reduced from 9.2 ± 4.9 (visit 1) to 5.1 ± 4.5 (visit 5), compared to 9.7 ± 5.0 (visit 1) to 5.3 ± 4.3 (visit 5) in control sextants.

Analysis of the pain diaries completed after visit 2 revealed that 19 participants reported pain/discomfort in test sextants, which lasted for 11.1 ± 9.2 hours, compared to 15 participants who reported pain/discomfort in control sextants, which lasted for 9.1 ± 9.7 hours. Mean VAS scores for severity of pain/discomfort were 28.2 ± 23.7 mm in test sextants and 26.5 ± 30.3 mm in control sextants. No significant differences between test and control sextants were evident for either duration or severity of pain/discomfort following treatment.

Analysis of safety outcomes (safety population, n=69) revealed one SAE (myocardial infarction, not considered related to study products or study participation) recorded in one participant who exited the study between visits 3 and 4. The most frequently recorded systemic adverse event was influenza/respiratory tract infections, reported by 7 participants. The most frequently reported oral cavity-related adverse events were procedural pain (e.g. as a result of SRP or other study procedures such as probing, test sextants, n=40; control sextants, n=37), and sensitivity/toothache (test sextants, n=4; control sextants, n=5).

DISCUSSION

In our study, using a split-mouth RCT design, we aimed to evaluate the efficacy of a new commercially-available phototherapy that had shown initial promise in the management of periodontitis, as reported in a EuroPerio abstract (Kamma et al., 2015). Our findings demonstrated no clinical benefit of the adjunctive treatment, whether considering either the

primary efficacy endpoint (mean CAL change at deep sites from baseline to week 24), or the secondary efficacy endpoints (mean CAL change at all sites, mean PD change at deep sites and all sites, mean change in %BOP and plaque scores). We could not identify statistically significant differences between test and control sextants for any clinical parameter, or across any change interval from baseline, whether considering the ITT or per protocol analysis.

Post-treatment CAL and PD changes in test and control sextants were consistent with reports from the literature given the baseline PD values (Cobb, 2002). Furthermore, %BOP scores also reduced following treatment, with levels at week 24 (27-30%) reasonably consistent with those that might be expected following an initial course of non-surgical therapy (Claffey et al., 2004). However, plaque scores remained consistently high throughout the study, with no clear evidence of reductions following treatment and were well above typical target values that might be considered in periodontal therapy. The reason for this finding is not clear, particularly given that the (clinically relevant) PD and BOP reductions were consistent with what would be expected following non-surgical treatment. We consider that the method of plaque scoring (using disclosing solution) in combination with a low threshold for examiners to indicate the presence of plaque at a site may have contributed to the high recorded plaque scores. We did not identify any evidence of a centre effect in relation to the plaque scores, and consider that a plaque index system (e.g. the Silness and Loe Plaque Index) may have provided better sensitivity for detecting changes in plaque levels (Silness and Loe, 1964).

Our primary outcome variable was change in mean CAL. However, we also recognize the current guidance that threshold changes (e.g. CAL gain $\geq$ 2 mm or $\geq$ 3 mm) are preferable to use as they are more clinically relevant (Loos and Needleman, 2020). These authors also stated that the target outcome for periodontal treatment should be resolution of inflammation as manifested by PD and BOP reductions, and that the achievement of shallow pockets ($\leq$ 4 mm) that do not bleed on probing confers the highest chance of periodontal stability. Indeed, a key factor for clinicians when evaluating the outcome of therapy is to assess the number of deep sites (e.g. PDs $\geq$ 5 mm) that remain. Accordingly, we evaluated this outcome, and identified that there was no difference observed between test and control sextants, which demonstrated similar numbers of deep residual sites at visit five (5.1 ± 4.5 and 5.3 ± 4.3 in the test and control sextants, respectively).

Our study has some limitations. Whereas every precaution was taken to ensure examiner blinding, we were not able to blind participants, as this would have involved the use of a placebo gel (which we did not have access to), and/or sham light activation. On the other hand, a positive aspect of our study was the multi-centre approach with a reasonably large participant population. We utilized a split-mouth design, principally in an attempt to reduce the potential for any differences between subjects to influence group comparisons that might have occurred if we had randomized at the patient level. However, we recognize the limitation of this approach in that treatment in the test sextants may potentially carry across to control sextants (Hujoel and DeRouen, 1992), and careful consideration to this matter should be given in future studies with regards to the use of split-mouth versus parallel-group designs.

According to the manufacturer's instructions and protocol for this commercially available product, the adjunctive phototherapy treatment was applied both before and after debridement of the root surfaces as it was supposed to facilitate instrumentation (Kamma et al., 2015). However, this EuroPerio abstract does not provide any detail on what is meant by treatment being facilitated or how this was measured. Our data demonstrated no efficacy of the adjunctive product over SRP in the treatment of periodontitis and accordingly we cannot recommend its use following the specified protocol. Although the intimate mechanism of biostimulation has been proven in other medical fields (Hamblin, 2017, Hamblin, 2018, Passarella and Karu, 2014), the scientific background for periodontal application of the technology that was tested in this research is, to the best of the author's knowledge, unknown, and we are unaware of any publications in the scientific literature that confirm the mechanism of action of the chromophore gel (i.e. fluorescence biostimulation) in the subgingival environment. Many PBM protocols rely on the application of specific light wavelengths, and this may have a major impact on tissue responses. According to the manufacturer's instructions for this adjunctive therapy, we used a dental curing light emitting a broad spectrum of 385 nm - 515 nm, and delivered the light to the depth of the pocket by using a clear plastic probe-like tip. A major issue is the amount of energy that could be converted by the chromophores. In the previously published data on this product (EuroPerio abstract), significant clinical improvements were obtained following application of light at wavelengths 532 nm and 430 nm (Kamma et al., 2015). In our study, activation of the chromophores was considered to require light at wavelength 430 nm, but not 532 nm, and the wavelengths with energy transfer maxima of the dental curing light (i.e., 405 nm and 465 nm) did not correspond to those needed for chromophore activation. In addition, the known power

density of the LED light (that was referred to a distance of 3-5 mm between the light source and the target) may have suffered from a substantial energy loss when transferred by the working tip to the periodontal pocket. Thus, it may be that only a fraction of the energy was used, preventing adequate power density at the tip within the periodontal pocket with the most suitable wavelength distributions to cause chromophore activation, if indeed chromophore activation was occurring.

These aspects raise the question on why the findings of this study should be published. A protocol has been tested in an appropriately designed RCT. Ethical approval was granted for the study, and the product is licensed (with a CE mark in Europe and a Medical Device License in Canada) and commercially-available. Patients and clinicians may become aware of the product and wish to know whether or not its use would be of benefit. Based on the evidence from our study, we cannot support the use of this product (according to the protocol followed), in the treatment of periodontitis. Given that patients have been exposed to an intervention, we consider it important from an ethical perspective that the findings are published so that the clinical community can become aware of the outcomes, and make more informed decisions on whether to use the product according to the protocol suggested by the manufacturer. However, we recognise the fundamental concern that despite a multicentre RCT being conducted, little, if any, biological effect might be expected given that it is not known whether the treatment protocol employed was able to generate the desired outcome (fluorescence biostimulation) in the context of periodontitis treatment. It can be speculated that a different outcome might be seen with a more powerful light source, or if different specific wavelengths of light were delivered to the chromophore *in situ*. The EuroPerio abstract (Kamma et al., 2015) reports only clinical data, and we are unaware of any publications in the scientific literature that document the mechanism of action of the tested combination of chromophore gel and light source in the subgingival environment.

Concerns regarding specific technologies used as adjunctive periodontal therapies have recently been raised in the context of aPDT. Following publication of the systematic review and meta-analysis on the use of aPDT in non-surgical periodontal treatment (Salvi et al., 2020), a subsequent letter to the editor raised concerns about some of the included studies, specifically that they utilised laser wavelengths that did not correspond to the absorption peak of the photosensitizer employed in the study (Damante, 2021). Given that, in order for a photodynamic reaction to occur, the absorption peak of the photosensitizer should be in the same range as the laser wavelength, the author makes the observation that in studies where this was not the case,

then the desired photodynamic reaction would be unlikely to occur, and thus there would be little or no clinical benefit. This can create problems in interpretation of data from multiple studies (e.g. in the context of a meta-analysis) because the systematic review process does not necessarily evaluate all the technical details of included studies (Salvi et al., 2021). Clearly, it is important that future investigations of phototherapy protocols should use light wavelengths that match the absorption peak of the photosensitiser employed (Chapple & Jepsen, 2021).

Accordingly, we consider it important that commercially-driven protocols of new products are carefully evaluated prior to committing to conduct larger scale multi-centre clinical trials. Preliminary studies should provide evidence not only on clinical outcomes, but also mechanisms of action. It is clear that appropriately designed RCTs are essential for reducing the potential bias in the evaluation of new products. The absence of clinical efficacy in our study might have been expected, given the lack of published scientific data regarding any biological effect in the subgingival environment of the combination of chromophore gel and application of light that was used.

CONCLUSION

Within the limitations of our research, we found that the tested adjunctive phototherapy protocol did not provide any additional clinical benefits over those achieved by SRP alone in terms of CAL gain, or PD and BOP reductions. This may have resulted from a lack of biological effect of the tested protocol, and it is important that future studies must be founded on published data that not only demonstrate clinical effect of new products in pilot studies, but also provide robust evidence of mechanism of action.

FIGURES AND TABLES

Figure 2.1: After mixing according to the manufacturer instruction, the gel was dispensed to the depth of the pockets using a syringe until the pockets were filled and slightly overflowing (a). The gel positioned in the pocket was photo-activated (b) by inserting a clear plastic probe-like tip attached to the 10 mm diameter tip of a dental curing light (Bluephase LED light, Ivoclar Vivadent).

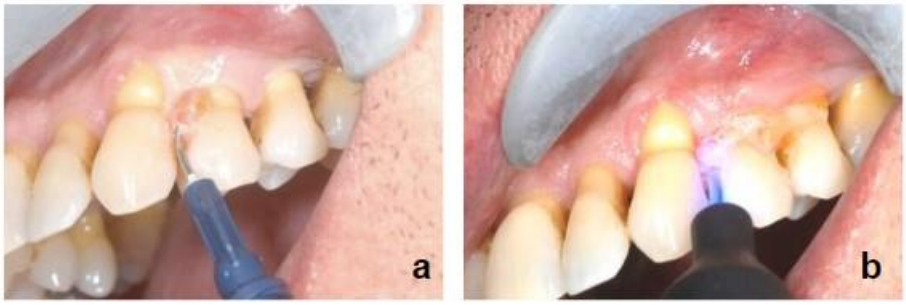

Figure 2.2: Study visit protocol.
V, visit number; SRP, root surface debridement; PTP, phototherapy protocol

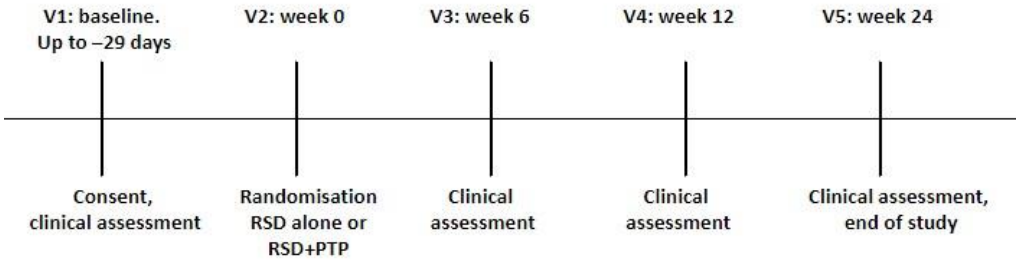

Figure 2.3: Study flow chart.
ITT, intent-to-treat; LOCF, last observation carried forward; SRP, root surface debridement; PTP, phototherapy protocol.

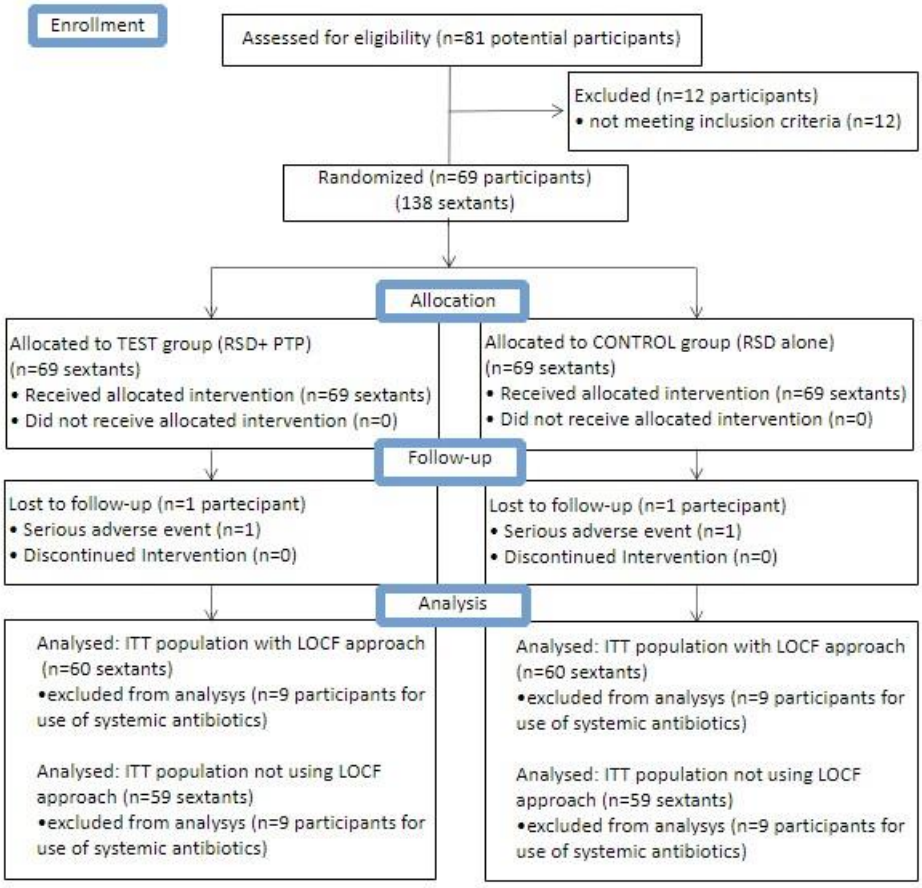

Table 2.1 **Mean CAL values in test and control sextants at each time point**

	Visit 1 (baseline)		Visit 3 (week 6)		Visit 4 (week 12)		Visit 5 (week 24)	
	Test sextants (n=60)	Control sextants (n=60)	Test sextants (n=60)	Control sextants (n=60)	Test sextants (n=60)	Control sextants (n=60)	Test sextants (n=60)	Control sextants (n=60)
CAL deep sites (mm)	6.61 ± 1.00	6.40 ± 0.95	5.69 ± 1.61	5.43 ± 1.23	5.67 ± 1.68	5.49 ± 1.28	5.61 ± 1.68	5.52 ± 1.28
CAL all sites (mm)	4.53 ± 1.22	4.51 ± 1.12	4.22 ± 1.12	4.22 ± 1.07	4.23 ± 1.13	4.32 ± 1.13	4.23 ± 1.13	4.33 ± 1.14

Data presented as mean ± standard deviation for the intent-to-treat (ITT) population according to the last observation carried forward (LOCF) approach. CAL, clinical attachment level.

Table 2.2 **Mean change data for CAL in test and control sextants from visit 1 (baseline) to visit 5 (week 24)**

	Change: visit 1 to visit 5		
	Test sextants (n=60)	Control sextants (n=60)	p value (T vs. C)[†]
CAL change deep sites (mm)	-1.00 ± 1.16*	-0.87 ± 0.79*	0.212
CAL change all sites (mm)	-0.31 ± 0.71*	-0.18 ± 0.69*	0.255

Data presented as mean ± standard deviation for the intent-to-treat (ITT) population according to the last observation carried forward (LOCF) approach. Negative values for CAL change indicate reduction from visit 1 to visit 5 (i.e. CAL gain, clinical improvement). CAL, clinical attachment level.

[†] p value for mixed-effect ANCOVA model for comparison of change data between test (T) versus control (C) sextants.

* statistically significant reduction from baseline within test or control sextants, $p<0.0001$.

Table 2.3 Mean CAL, PD, BOP and plaque values in test and control sextants at each time point

	Visit 1 (baseline)		Visit 3 (week 6)		Visit 4 (week 12)		Visit 5 (week 24)	
	Test sextants (n=60)	Control sextants (n=60)	Test sextants (n=60)	Control sextants (n=60)	Test sextants (n=59)	Control sextants (n=59)	Test sextants (n=59)	Control sextants (n=59)
CAL deep sites (mm)	6.61 ± 1.00	6.40 ± 0.95	5.69 ± 1.61	5.43 ± 1.23	5.70 ± 1.68	5.52 ± 1.27	5.59 ± 1.69	5.43 ± 1.20
CAL all sites (mm)	4.53 ± 1.22	4.51 ± 1.12	4.22 ± 1.12	4.22 ± 1.07	4.25 ± 1.13	4.35 ± 1.11	4.22 ± 1.14	4.30 ± 1.14
PD deep sites (mm)	5.94 ± 0.60	5.82 ± 0.56	4.57 ± 1.02	4.51 ± 0.96	4.62 ± 1.02	4.54 ± 0.90	4.47 ± 1.08	4.41 ± 0.81
PD all sites (mm)	3.89 ± 0.94	3.91 ± 0.84	3.29 ± 0.73	3.34 ± 0.72	3.27 ± 0.76	3.39 ± 0.73	3.22 ± 0.76	3.31 ± 0.76
BOP all sites (%)	51.6 ± 27.3	47.7 ± 27.9	32.0 ± 23.0	31.4 ± 22.3	30.7 ± 22.3	31.3 ± 21.9	29.6 ± 23.3	27.5 ± 23.0
Plaque all sites (%)	63.9 ± 26.2	62.6 ± 24.8	62.6 ± 23.8	64.6 ± 21.6	62.2 ± 21.6	60.0 ± 23.4	61.7 ± 22.4	57.2 ± 23.8

Data presented as mean ± standard deviation for the intent-to-treat (ITT) population, not using the last observation carried forward (LOCF) approach.
CAL, clinical attachment level; PD, probing pocket depth; BOP, bleeding on probing.

Table 2.4 Mean change data for CAL, PD, BOP and plaque in test and control sextants from visit 1 to visits 3, 4 and 5

	Change: visit 1 to visit 3			Change: visit 1 to visit 4			Change: visit 1 to visit 5		
	Test sextants (n=60)	Control sextants (n=60)	p value (T vs. C)†	Test sextants (n=59)	Control sextants (n=59)	p value (T vs. C)†	Test sextants (n=59)	Control sextants (n=59)	p value (T vs. C)†
CAL change deep sites (mm)	-0.92 ± 1.12*	-0.97 ± 0.87*	0.991	-0.93 ± 1.22*	-0.89 ± 0.80*	0.406	-1.01 ± 1.17*	-0.91 ± 0.76*	0.230
CAL change all sites (mm)	-0.31 ± 0.60*	-0.30 ± 0.59*	0.965	-0.31 ± 0.65*	-0.19 ± 0.61*	0.139	-0.32 ± 0.72*	-0.21 ± 0.65*	0.334
PD change deep sites (mm)	-1.37 ± 0.86*	-1.31 ± 0.82*	0.764	-1.33 ± 0.89*	-1.28 ± 0.83*	0.967	-1.46 ± 0.93*	-1.37 ± 0.69*	0.858
PD change all sites (mm)	-0.60 ± 0.55*	-0.56 ± 0.57*	0.619	-0.63 ± 0.57*	-0.53 ± 0.59*	0.195	-0.69 ± 0.56*	-0.61 ± 0.56*	0.481
BOP change all sites (%)	-19.6 ± 20.4*	-16.3 ± 19.6*	0.733	-21.3 ± 22.7*	-17.0 ± 19.0*	0.527	-22.3 ± 21.5*	-20.7 ± 17.9*	0.657
Plaque change all sites (%)	-1.24 ± 18.0	1.97 ± 19.5	0.437	-1.61 ± 21.5	-2.52 ± 20.8	0.451	-2.81 ± 20.1	-5.6 ± 23.2	0.188

Data presented as mean ± standard deviation for the intent-to-treat (ITT) population, not using the last observation carried forward (LOCF) approach. Negative values indicate reductions (i.e. clinical improvement) from visit 1 to subsequent time points (i.e. CAL gain, PD reduction and BOP reduction). CAL, clinical attachment level; PD, probing pocket depth; BOP, bleeding on probing.

† p value for mixed-effect ANCOVA model for comparison of change data between test (T) versus control (C) sextants.

* statistically significant reduction from baseline within test or control sextants, $p<0.0001$.

REFERENCES

Antoniou, C., Dessinioti, C., Sotiriadis, D., Kalokasidis, K., Kontochristopoulos, G., Petridis, A., Rigopoulos, D., Vezina, D. & Nikolis, A. (2016), A multicenter, randomized, split-face clinical trial evaluating the efficacy and safety of chromophore gel-assisted blue light phototherapy for the treatment of acne. International Journal of Dermatology 55, 1321-1328. doi: 10.1111/ijd.13349

Armitage, G. C. (1999) Development of a classification system for periodontal diseases and conditions. Annals of Periodontology 4, 1-6.

Chambrone, L., Wang, H. L., & Romanos, G. E. (2018). Antimicrobial photodynamic therapy for the treatment of periodontitis and peri-implantitis: An American Academy of Periodontology best evidence review. Journal of Periodontology 89, 783–803.

Chapple, I. L. C. & Jepsen, S. (2021). Response to LETTER "Laser parameters in systematic reviews" by Dr. Carla DAMANTE. Journal of Clinical Periodontology, 1–3. https://doi.org/10.1111/jcpe.13425

Claffey, N., Polyzois, I. & Ziaka, P. (2004) An overview of nonsurgical and surgical therapy. Periodontology 2000 36, 35-44.

Cobb, C. M. (2002) Clinical significance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planing. Journal of Clinical Periodontology 29 (Suppl. 2), 6-16.

Damante, C. (2021). Laser parameters in systematic reviews (Letter). Journal of Clinical Periodontology, 1–3. https://doi.org/10.1111/jcpe.13421

Hamblin, M. R. (2017) Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. AIMS Biophysics 4, 337-361. doi:10.3934/biophy.2017.3.337.

Hamblin, M. R. (2018) Mechanisms and mitochondrial redox signaling in photobiomodulation. Photochemistry and Photobiology 94, 199-212. doi:10.1111/php.12864.

Hujoel, P. P. & DeRouen, T. A. (1992) Validity issues in split-mouth trials. Journal of Clinical Periodontology 19, 625-627.

Kamma, J., Loupis, N., Karapetsa, D., Vichos, S. & Piergallini, R. (2015) A new biophotonic approach as an adjunct to scaling and root planing (SRP) in the treatment of chronic periodontitis (ChP). Preliminary results (EuroPerio 8 abstract). Journal of Clinical Periodontology 42, 38 (D023).

Loos, B. G. & Needleman, I. (2020) Endpoints of active periodontal therapy. Journal of Clinical Periodontology 47, 61-71.

Nunes, E. C., Herkrath, F. J., Suzuki, E. H., Gualberto Junior, E. C., Marques, A. A. F. & Sponchiado Junior, E. C. (2020) Comparison of the effect of photobiomodulation therapy and Ibuprofen on postoperative pain after endodontic treatment: randomized, controlled, clinical study. Lasers in Medical Science 35, 971-978. doi:10.1007/s10103-019-02929-8.

Passarella, S. & Karu, T. (2014) Absorption of monochromatic and narrow band radiation in the visible and near IR by both mitochondrial and non-mitochondrial photoacceptors results in photobiomodulation. Journal of Photochemistry and Photobiology B: Biology 140, 344-358. doi:10.1016/j.jphotobiol.2014.07.021.

Pires Marques, E. C., Piccolo Lopes, F., Nascimento, I. C., Morelli, J., Pereira, M. V., Machado Meiken, V. M. & Pinheiro, S. L. (2020) Photobiomodulation and photodynamic therapy for the treatment of oral mucositis in patients with cancer. Photodiagnosis and Photodynamic Therapy 29, 101621. doi:10.1016/j.pdpdt.2019.101621.

Salvi, G. E., Stähli, A., Schmidt, J. C., Ramseier, C. A., Sculean, A., & Walter, C. (2020). Adjunctive laser or antimicrobial photodynamic therapy to non-surgical mechanical instrumentation in patients with untreated periodontitis: a systematic review and metaanalysis. Journal of Clinical Periodontology, 47(Suppl 22), 176–198.

Salvi GE, Stähli A, Schmidt JC, Ramseier CA, Sculean A, Walter C. (2021) Reply letter to the editor. Journal of Clinical Periodontology.00: 1–2. https://doi.org/10.1111/jcpe.13431

Sanz, M., Herrera, D., Kebschull, M. et al; On behalf of the EFP Workshop Participants and Methodological Consultants. (2020) Treatment of stage I–III periodontitis - The EFP S3 level clinical practice guideline. Journal of Clinical Periodontology 47, 4–60. https://doi.org/10.1111/jcpe.13290

Silness, J. & Loe, H. (1964) Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontologica Scandinavica 22, 121-135.

Tonetti, M. S., Greenwell, H. & Kornman, K. S. (2018) Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. Journal of Clinical Periodontology 45 Suppl 20, S149-S161. doi:10.1111/jcpe.12945.

Trombelli, L., Farina, R., Pollard, A., Claydon, N., Franceschetti, G., Khan, I. & West, N. (2020) Efficacy of alternative or additional methods to professional mechanical plaque removal during supportive periodontal therapy: A systematic review and meta-analysis. Journal of Clinical Periodontology 47, 144–154. https://doi.org/10.1111/ jcpe.13269

Uzel, N. G., Teles, F. R., Teles, R. P., Song, X. Q., Torresyap, G., Socransky, S. S. & Haffajee, A. D. (2011) Microbial shifts during dental biofilm re-development in the absence of oral hygiene in periodontal health and disease. Journal of Clinical Periodontology 38, 612-620. doi:10.1111/j.1600-051X.2011.01730.x.

Chapter III

Efficacy of the additional use of subgingival air polishing with erythritol powder in the treatment of periodontitis patients: a randomized controlled clinical trial

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ABSTRACT

Objectives: To date, scarce evidence exists around the application of sub-gingival air-polishing during treatment of severe periodontitis. The aim of this study was to evaluate the benefits of sub-gingival air-polishing during non-surgical treatment of deep bleeding pockets in stage III-IV periodontitis patients.

Materials and Methods: 40 patients with stage III-IV periodontitis were selected, and pockets with probing depth (PD) 5-9 mm and bleeding on probing (BOP) were selected as experimental sites. All patients underwent a full-mouth session of erythritol powder supra-gingival air-polishing and ultrasonic instrumentation. Test group received additional sub-gingival air-polishing at experimental sites.The proportion of experimental sites shifting to PD≤4 mm and no BOP at 3 months (i.e., non-bleeding closed pockets, NBCPs) was regarded as the primary outcome variable.

Results: The proportion of NBCP was comparable between test and control group (47.9% and 44.7%, respectively). Baseline PD of 7-9 mm, multi-rooted teeth and presence of plaque negatively influenced the probability of obtaining NBCP.

Conclusions: The additional application of sub-gingival air-polishiilg does not seem to provide any significant clinicl advantage in achieving closure at moderate to deep bleeding pockets in treatment of stage III-IV periodontitis patients. The study was registered on Clinical Trials.gov (NCT04264624).

Clinical relevance: While air-polishing can play a role in biofilm removal at supra-gingival and shallow sites, ultrasonic root surface debridement alone is stili the choice for initial treatment of deep bleeding periodontal pockets.

INTRODUCTION

With the introduction of low-abrasiveness powders, the range of applications of air-polishing expanded from supra-gingival stain removal only to supra- and sub-gingival biofilm management (Sculean et al, 2013; Cobb et al, 2017). Air-polishing with glycine or erythritol powder seems more effective than manual instrumentation at disrupting biofilm in both shallow and deep pockets (Flemming et al, 2012; Hägi et al, 2015; Petersilka et al, 2003 (a), (b)), whilst safe on the root surface (Bozbay et al, 2018) and soft tissues (Petersilka et al, 2018), and allowing proper attachment of periodontal ligament fibroblasts (Hägi et al.,2015).

Flemmig et al. (2012) elaborated the concept of Full-Mouth Glycine Powder Air-Polishing (FM-GPAP), involving the application of air-polishing followed by ultrasonic or manual removal of visible calculus. They concluded that the technique is more effective than manual SRP in biofilm removal and may result in a beneficial shift of the oral microbiota. When used to instrument pockets utilizing a specifically designed plastic nozzle, air-polishing is proven a suitable option for maintenance therapy of periodontal patients (Muller et al, 2014; Wennström et al, 2011; Hägi et al, 2015) leading to similar clinical and microbiological results as traditional instrumentation (Wennström et al, 2011), but in a more time-efficient and comfortable manner (Muller et al, 2014; Wennström et al, 2011).

To date, only three clinical trials have investigated the additional benefits of sub-gingival air-polishing during treatment of periodontitis (Caygur et al, 2017; Park et al, 2018; Tsang et al, 2018). In these studies, erythritol or glycine powder was applied in selected pockets after ultrasonic and manual scaling and root planing (SRP). No additional clinical benefits could be demonstrated. However, the complete full-mouth air-polishing protocol (Flemming et al, 2012) was not administered, limiting its application to the selected pockets, and after traditional SRP.

The present randomized controlled trial aimed at evaluating the adjunctive clinical effect of sub-gingival air-polishing with erythritol powder in the non-surgical treatment of deep bleeding pockets in stage III-IV periodontitis patients (Tonetti et al, 2018). The hypothesis was that air-polishing could improve biofilm removal in areas of the pocket normally difficult to reach with traditional mechanical instruments, leading to a better clinical outcome. The periodontal treatment was carried on according to the FM-GPAP protocol as outlined by Flemmig et al. (2012) (Flemming et al, 2012) followed by ultrasonic pocket debridement.

MATERIALS AND METHODS

Study design and approval

This multicenter, single (examiner) blinded, parallel-arm randomized controlled clinical trial was conducted at the Section of Periodontics, School of Dentistiy, Department of Surgical Specialties, Radiological Science and Public Health of the University of Brescia, within the ASST Spedali Civili di Brescia, Department of Odontostomatology, and at the Research Centre for the Study of Periodontal and Peri-implant Diseases, University of Ferrara. The protocol was reviewed and approved by the Ethics Committee of the University-Hospital of Brescia (CE: 2971) and the Ethics Committee of Area Vasta Emilia Centrale (protocol number: 83/2018/Disp/Unife) and the study conducted in accordance with the ethical principles of the Declaration of Helsinki. All participants signed written informed consent before the beginning of the study.The study was registered on Clinical Trials.gov (NCT04264624).

Patient selection and allocation

The inclusion criteria were as follows: ≥18 years and ≤75 years of age, good general health, diagnosis of Stage III-IV periodontitis (Tonetti et al, 2018), at least 15 sites with probing depth (PD) 5-9 mm and positive to bleeding on probing (BOP) (Ainamo & Bay, 1975).

The exclusion criteria were as follows: pregnant or lactating patients, current or past (within 3 months of enrolment) medications that may influence periodontal conditions and/or interfere with healing following periodontal treatment (i.e., corticosteroids, calcium channel blockers), non-surgical and/or surgical periodontal debridement within 3 months of enrolment, use of systemically administered antibiotics within 3 months of enrolment, tumors or significant pathology of the soft or hard tissues of the oral cavity, current radiotherapy or chemotherapy, chronic obstructive pulmonary disease and asthma, history of allergy to erythritol, presence of orthodontic appliances.

Randomized patient allocation to either test or control intervention was performed centrally using ad-hoc software (R version 3.6.1, R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org), using a blocked randomization scheme to achieve balanced treatment groups within centres.

Clinical parameters

At baseline (T0) and 3 months after treatment (T1), PD, clinical attachment level (CAL), gingival recession (REC), BOP, and presence of supra-gingival plaque (PlI) were collected by a blinded examiner at 6 sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual) for each tooth present. BOP and PlI were expressed as % of positive sites on total examined sites. Probing measurements were performed using a UNC-15 periodontal probe (Hu Friedy®, Chicago, IL, USA) and rounded at nearest mm. For each patient, all sites that showed PD 5-9 mm and were BOP-positive at T0 were identified as experimental sites.

Interventions

Interventions were performed by the same two experienced calibrated operators (ES and CF), responsible only for treatment delivery to ensure examiner blinding. In all subjects, after identification of the treatment group according to the generated randomization table, the entire dentition received the application of a disclosing agent (Mira-2-Ton®, Hager & Werken, Duisburg, Germany) to guide plaque removal and achieve better biofilm removal (Mensi et al, 2020). Supra- and juxta-gingival areas were air-polished (Airflow Prophylaxis Master, EMS, Nyon, Switzerland) with erythritol +chlorhexidine powder (PLUS powder, EMS, Nyon, Switzerland– Figure 3.1a and 3.1b), followed by ultrasonic instrumentation for calculus removal with dedicated tip (PS tip, Airflow Prophylaxis Master, EMS, Nyon, Switzerland, – Figure 3.1c).

This procedure is commonly known by practitioners with the name of Guided Biofilm Therapy (GBT).

In patients allocated to test intervention, experimental sites received sub-gingival biofilm removal with erythritol + chlorhexidine powder delivered via a specifically designed nozzle (Perioflow, EMS, Nyon, Switzerland;– Figure 3.2a) followed by sub-gingival ultrasonic instrunentation (Figure 3.2b). In patients allocated to control intervention, experimental sites received subg-ingival ultrasonic instrumentation only.

At the end of the session, the patients received oral hygiene instructions on manual toothbrushing and use of interdental cleaning devices.

In Figure 3.3, it is shown an example of pre and post-treatment outcome.

Endpoints and Statistical Analysis

At 3 months (Tl) after treatment, experimental sites were categorized as: non-bleeding closed pockets (NBCP): PD ≤4mm and BOP-negative; bleeding closed pockets (BCP): PD

≤ 4mm and BOP-positive; non-bleeding residual pockets (NBRP): PD ≥5mm and BOP-negative; bleeding residual pockets (BRP): PD ≥ 5mm and BOP-positive.

The proportion of experimental sites that shifted to NBCP at T1 was the primary outcome variable. 3-month values for CAL, PD, BOP and PlI were the secondary outcome variables. Sample size was estimated via Monte Carlo simulation. According to a previous study from Wennström et al. (2005) (Wennström et al, 2005), we can expect 58% of periodontal pockets to be closed at 3 months after treatment with ultrasonic debridement. Given that the endpoint of the present study closed pockets with no bleeding, and given that Wennström et al. (2005) reported a BOP score of 48% at 3 months. the authors assumed a proportion of NBCP at T1 of 40% in the control group.
Being the present study the first applying the test protocol a 1.7 odds-ratio of test group versus control was arbitrarily set, based on evidence around air-polishing efficacy in sub-gingival plaque removal (Petersilka et al, 2003 (a), (b)) and reduction of the red complex periodontal bacteria (Reinhardt et al, 2019). We used a fixed number of probed sites for every subject (N=120, i.e. 6 sites for at least 20 teeth) and assumed a patient variance of 0.3. We simulated 1000 realizations of the event (PD< 5 mm and BOP-negative) at T1 using a binomial distribution and then modelled the simulated data using a GLMM logistic model with Treatment (test vs control) as fixed effect and a single random component (patient intercept). The power is estimated as the proportion of simulations where the treatment effect was significant at the chosen 5% significant level. A sample size of 18 patients per group allowed for a power of at least 80%. Assuming a 10% attrition, we estimated a total sample size of 40 patients, equally randomized to the two treatment groups.
Data were described using standard summary statistics such as mean and standard deviations for quantitative variables and proportions for categorical variables. The overall percentage of NBCP were modelled using a logistic model fitted using Generalized Estimation Equations (GEE) with subject as clustering factor and binomial family, using a once-versus-all approach (i.e. estimating the percentage of one category versus all the others combined).
The probability to achieve at site level an NBCP at T1 was modelled using a logistic regression fitted using Generalized Linear Mixed Model (GLMM); this allowed to model the odds of pocket closure at site level accounting for within-subject measurements clustering.
Secondary continuous outcomes were modelled using longitudinal analysis of covariance (ANCOVA) (Twistk et al, 2018) models fitted by Linear Mixed Model (LMM), adjusting for

baseline values (baseline measurements added as covariate) based on Gaussian error distribution. All (G)LMM models accounted for a 2-level hierarchical structure (clustering level: patient and tooth within patient). Both PD and CAL were transformed on log scale prior to modelling. PlI was modelled using a beta regression (Ferrari et al, 2004) while BOP was modelled using logistic regression.

Results were expressed as estimates and conesponding 95% confidence intervals. A significant level of 5% was assumed for all the comparisons, and all analyses were performed using R (version 3.6.1 or higher) and Stata (version 15).

RESULTS

A total of 40 patients (20 for each centre) were allocated to either test (n= 20) and control (n= 20) group. During the study, 2 patients in each group were excluded due to failure to attend to the appointments (n= 2) and need for antibiotic treatment due to other unrelated health issues (n= 2). Demographic data and baseline clinical parameters of the study population who completed the study are presented in Table 3.1a. Groups were comparable for all considered variables. Table 3.1b describes the site characteristics as recorded full-mouth and at experimental sites, respectively.

The distribution of the experimental sites into the 4 categories at 3 months (Tl) is illustrated in Table 3.2. 47.9% and 44.7% of experimental sites shifted to NBCP at T1 in test and control group, respectively, with no significant inter-group difference ($p = 0.64$). The average overall pocket closure (NBCP +BCP) was 80% for test group and 77.6% for control group.

Analysis of the patient- and site-level factors influencing the probability to achieve a NBCP at Tl is displayed in Table 3.3. For continuous predictors, such as %of experimental sites, the OR corresponds to variation in probability as a consequence of a one-unit increase in the predictor values: an OR> 1 indicates that when the variable grows the probability of NBCP increases and an OR< 1 indicates that when the variable grows the probability of NBCP decreases. Test treatment seemed to influence positively the probability to achieve NBCP (OR 1.23 (O.70 – 2.16)), but failed to reach statistical significance ($p = 0.476$). Factors positively affecting primary outcome were: treatment at BS centre (OR 0.27 (0.15 -0.49)), absence of plaque at T1 (OR 0.66 (0.51 -0.84)) and single-rooted tooth (OR 0.66 (0.51 – 0.86)) ($p < 0{,}001$). Smoking status did not significantly influence the outcome ($p = 0.701$). For both treatments, the higher the PD at T0, the

lower the probability to get NCBP at Tl. Test treatment seemed to perform slightly better than control especially at shallower sites (Figure 3.1).

A further analysis investigating the impact of different combinations of treatment, type of tooth and baseline PD on the probability of obtaining NBCP at Tl is shown in Table 3.4. Test treatment seemed to give a higher probability of pocket closure at sites with initial PD of 5-6 mm at multi-rooted teeth compared to control (56.4% vs 40.8%). Table 3.5 shows the comparison of estimated periodontal parameters at Tl adjusted for baseline levels. No significant difference was found between the two treatment groups.

No adverse effects with probable or certain association with experimental treatment were registered during the study or reported by the patients.

DISCUSSION

This study was performed to evaluate the clinical outcome of the adjunctive use of subgingival air-polishing with erythritol powder at deep bleeding pockets in the treatment of stage III-IV periodontitis patients.

The periodontal treatment was carried out in both groups via full-mouth air-polishing, followed by ultrasonic calculus removal and pocket debridement. Full-mouth air-polishing and ultrasonic pocket debridement can be considered an alternative option to traditional SRP for biofilm and calculus management (Flemming et al, 2012; Hägi et al, 2015Bozbay et al, 2018; Hägi et al, 2015; Twisk et al, 2018), and are the chosen techniques in the centres where the study took place. The Test and Control groups differed only in the additional application of a sub-gingival nozzle for air-polishing, intending to highlight the possible benefits of this adjunctive measure.

The outcome analysis revolved around the 3-month clinical healing of baseline BOP-positive sites with PD≥5mm (experimental sites). The results were comparable in test and control group, failing to prove any significant difference between treatment protocols.

To date, three other clinical trials have investigated the effects of subgingival air-polishing during treatment of periodontal disease. However, the difference in study design and the lack of details about the selected experimental sites make comparison difficult. Tsang et al. (2018) utilized glycine powder air-polishing subsequently to ultrasonic and manual SRP ("within one week"); Park et al. (2018) (Park et al, 2018) perfomed supra-gingival calculus removal first, followed by SRP of all periodontal pockets with Gracey curettes and application of erytluitol + chlorhexidine powder in the test group; finally, Caygur et al. applied glycine powder in pockets after manual and ultrasonic SRP. While these studies failed to prove any additional

clinical effect over standard SRP, Tsang et al. (2018) observed a reduced volume of crevicular fluid, indicating a lower level of subclinical gingival inflammation, and Park et al (2018) detected a lower relative level of *P. gingivalis,* probably due to the reported antimicrobial effects of the powder (Hashino et al, 2014).

In our study design, the two treatment groups differed only for sub-gingival air-polishing at experimental sites, whereas both groups received full-mouth supra- and juxta -gingival air-polishing and ultrasonic debridement. In the clinical protocol, the application of sub-gingival ultrasonic instrumentation is performed as last operative step to avoid the occurrence of air emphysema. Based on our findings, air-polishing seems to provide no additional clinical benefit over ultrasonic instrumentation in moderate to deep bleeding pockets. It may be speculated that, although proven effective for dental biofilm removal in both in vitro (Hägi et al., 2015) and clinical (Petersilka et al, 2003 (a), (b)) studies, the clinical effect of sub-gingival air-polishing (in terms of pocket closure and elimination of periodontal inflammation) could have been masked by the well-established effectiveness of sub-gingival ultrasonic instrumentation, particularly when perfomed with thin, dedicated tips (Wennström et al, 2005). In our study, post-treatment changes in sub-gingival microbiome were not assessed. A previous study (Hägi et al, 2015) has shown a beneficial shift in the sub-gingival microflora at 6 months following sub-gingival air-polishing with erythritol powder (EPAP). Whether and to what extent the impact of EPAP on sub-gingival periopathogens may translate in improved clinical outcomes at observation intervals longer than 3 months needs be fully understood.

Both treatments led to pocket closure and elimination of BOP (NBCP) in more than 40% of the experimental sites, plus an additional ~32% of sites reaching PD≤4mm and persistent BOP (BCP) (Table 3.2). The overall pocket closure, defined by PD reduction to ≤4mm regardless of the BOP status (NBCP +BCP), was 80% for test group and 77.6% for control group. These results can be considered positive when compared to the data available in literature. In a study by Wennström et al. (2005), 41 patients with chronic periodontitis underwent either full-mouth ultrasonic debridement (Fm-UD) or quadrant SRP (Q-SRP). At the 3-month re-evaluation, the percentage of pocket closure for the Q-SRP group was 66. Therefore, the outcome suggests that full-mouth air-polishing followed by ultrasonic removal of calculus and pocket debridement can be a suitable option for periodontal treatment. A multilevel analysis showed that a PD of 7-9 mm, location of the pocket at a multi-rooted tooth and presence of plaque at the site reduce the probability of obtaining a NBCP. These findings are in agreement with the results reported by Tomasi et al. (2007). Conversely, in the present study, the smoking status did not seem to affect the outcome negatively. The

magnitude of treatment effect in terms of pocket closure observed in both groups may have masked the detrimental effect of smoking on non-surgical treatment of deep pockets as reported in baseline PD: the deeper the pocket, the less probable the shift to NBCP, with test protocol performing slightly (but not significantly) better at shallow pockets. A further statistical analysis was performed on single- or multi-rooted with different initial PD. Test protocol seems to increase the chance of obtaining a NBCP at multi-rooted teeth with sites with 5-6 mm PD compared to control (56.4% vs 40.8%). Nevertheless, further clinical trials with larger sample size are required to draw any conclusion.

Possible limitations of the present study are the small sample size and the patients' allocation in the study groups at the beginning of the intervention rather than just before the subgingival treatment.

CONCLUSIONS

The additional application of subgingival air-polishing does not seem to provide any significant additional advantage in achieving closure at moderate to deep bleeding pockets during treatment of stage III-IV periodontitis patients.

COMPLIANCE \VITH ETHICAL STANDARDS

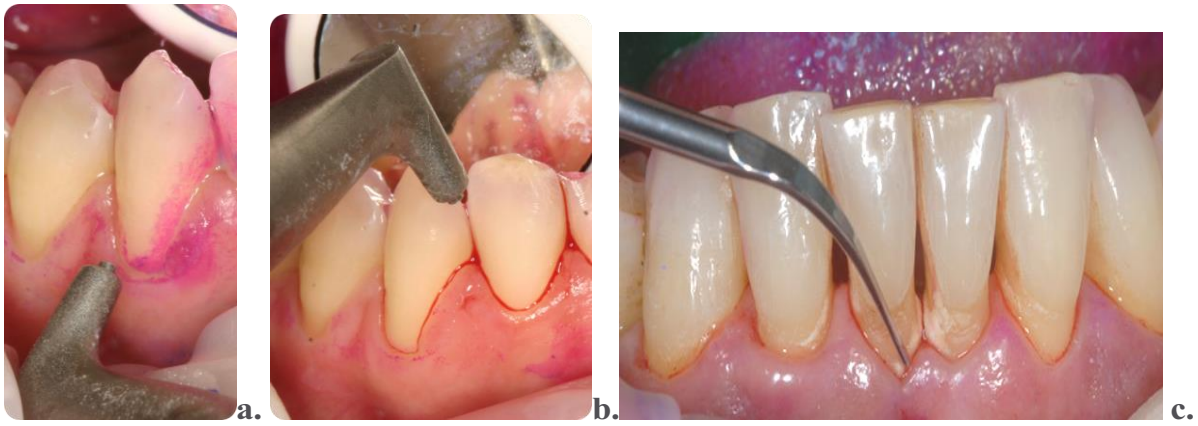
Conflict of Interest: M. Mensi reports grants, personal fees and non-financial support from E.M.S. – Electro Medical Systems, E. Scotti reports grants, personal fees and non-financial support from E.M.S. - Electro Medical Systems, A. Sordillo reports personal fees from E.M.S. -Electro Medicai Systems, S. Calza declares that he has no conflict of interest, M.E. Guamelli declares that she has no conflict of interest, C. Fabbri declares that she has no conflict of interest, R. Farina declares that he has no conflict of interest, L. Trombelli reports grants and non-financial support from E.M.S. - Electro Medical Systems. Funding: The work was supported by the E.M.S. – Electro Medicai Systems.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of the University-Hospital of Brescia (CE: 2971) and the Ethics Committee of Area Vasta Emilia Centrale (protocol number: 83/2018/Disp/Unife) and with the 1964 Helsinki declaration and its later amendments.

Informed consent: Informed consent was obtained from all individual participants included in the study.

FIGURES AND TABLES

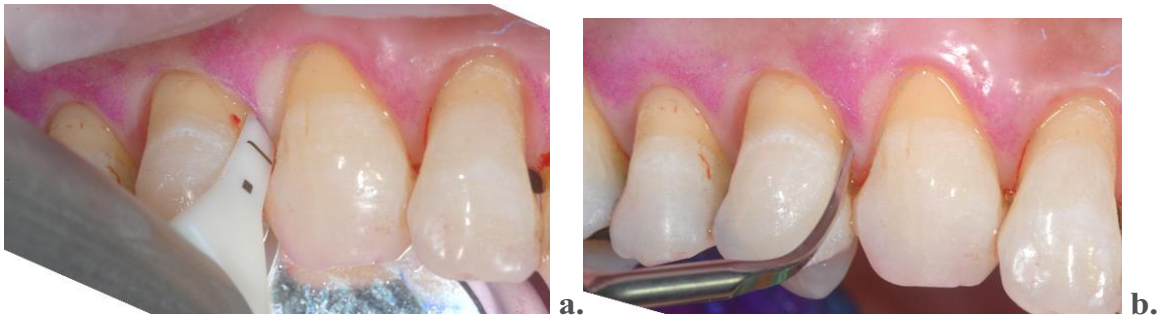
FIGURE 3.1



a. **b.** **c.**

a.supra-gingival biofilm removal with EPAP approach (Airflow Prophylaxis Master, EMS, Nyon, Switzerland); b. Intrasulcular biofilm removal with EPAP; c.supra-gingival scaling with ultrasonic device (PS tip, Airflow Prophylaxis Master, EMS, Nyon, Switzerland).

FIGURE 3.2

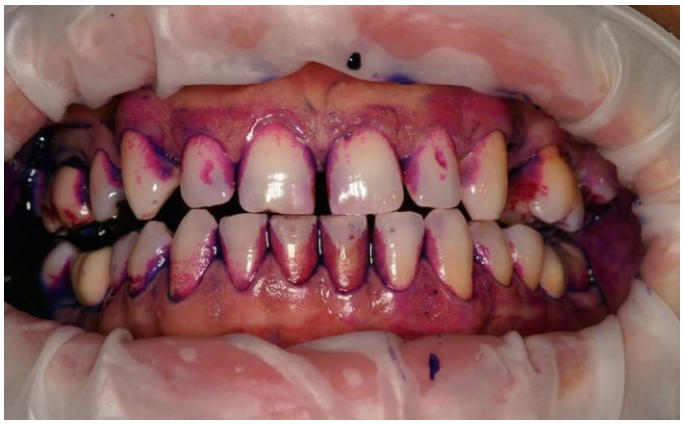


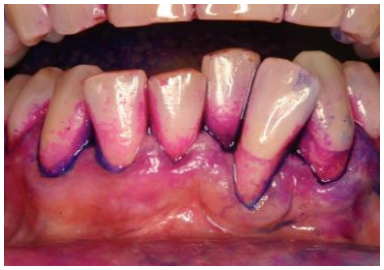
a. **b.**

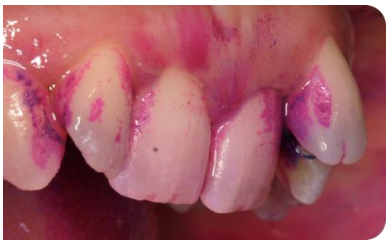
a.sub-gingival biofilm removal with a specific nozzle (Perioflow, EMS, Nyon, Switzerland) designed nozzle to deliver EPAP into the periodontal pocket ; b. sub-gingival scaling with ultrasonic device (PS tip, Airflow Prophylaxis Master, EMS, Nyon, Switzerland)

FIGURE 3.3

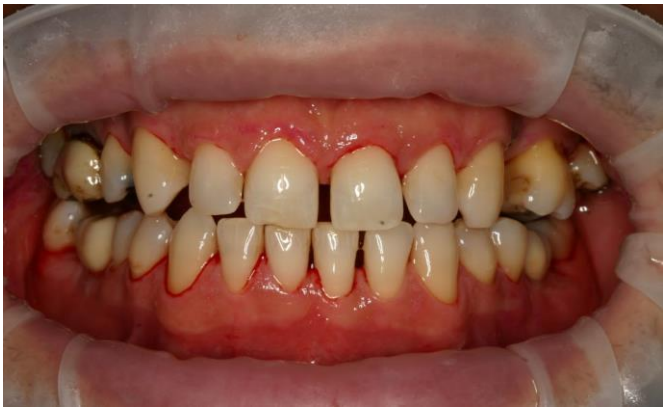
Pre-treatment.

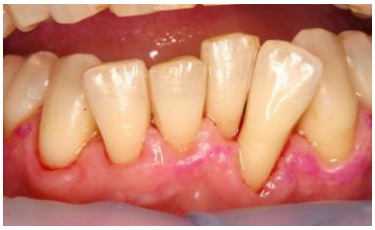


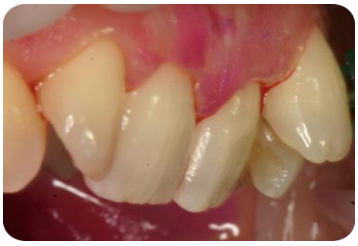




Post-treatment.







According to study protocol, the entire dentition received the application of a disclosing agent (Mira-2-Ton®, Hager & Werken, Duisburg, Germany) to guide plaque removal and achieve better biofilm removal prior to treatment. This procedure is commonly known by practitioners with the name of Guided Biofilm Therapy (GBT).

Table 3.1a. Patients characteristics in test and control group.

	Control (N=18)	**Test (N=18)**
Number of elements		
Mean (SD)	24.98 (3.16)	24.94 (2.41)
Median (Q1, Q3)	26.00 (23.00, 27.75)	25.50 (23.25, 26.75)
Smoker	3 (16.7%)	4 (22.2%)
Male	7 (38.9%)	11 (61.1%)
Age		
Mean (SD)	48.44 (9.31)	52.06 (10.17)
Median (Q1, Q3)	49.50 (42.50, 54.25)	53.00 (46.25, 60.00)
N° anaesthetic doses (1.8mL)		
Mean (SD)	1.28 (2.40)	1.67 (2.83)
Median (Q1, Q3)	0.00 (0.00, 0.75)	0.00 (0.00, 3.00)
BOP index (%)		
Mean (SD)	84.08 (16.91)	83.88 (14.28)
Median (Q1, Q3)	86.01 (69.58, 100.00)	86.51 (71.90, 98.61)
Plaque index (%)		
Mean (SD)	81.17 (21.36)	92.53 (7.63)
Median (Q1, Q3)	84.66 (75.22, 96.34)	93.29 (87.45, 100.00)
Number of experimental sites		
Mean (SD)	59.89 (17.61)	49.56 (16.96)
Median (Q1, Q3)	60.00 (45.50, 76.25)	45.50 (38.00, 58.25)

BOP: Bleeding on Probing

Table 3.1b. Sites characteristics in treatment and control group - all sites.

	Control (N=2688)	Test (N=2694)
PD (mm)		
Mean (SD)	4.16 (2.14)	3.89 (1.97)
Median (Q1, Q3)	4.00 (2.00, 6.00)	4.00 (2.00, 5.00)
Recession (mm)		
Mean (SD)	0.60 (1.24)	0.58 (1.16)
Median (Q1, Q3)	0.00 (0.00, 1.00)	0.00 (0.00, 1.00)
CAL (mm)		
Mean (SD)	4.76 (2.51)	4.47 (2.19)
Median (Q1, Q3)	4.00 (3.00, 6.00)	4.00 (3.00, 6.00)
Number of experimental sites	1078 (40.1%)	892 (33.1%)
PD (cat)		
<5 mm	1548 (57.6%)	1736 (64.4%)
5-6 mm	783 (29.1%)	707 (26.2%)
7-9 mm	315 (11.7%)	216 (8.0%)
>9 mm	42 (1.6%)	35 (1.3%)

PD: Probing Depth, CAL: Clinical Attachment Level

Table 3.1c. Sites characteristics in treatment and control group - experimental sites only.

	Control (N=1078)	**Test (N=892)**
PPD (mm)		
Mean (SD)	6.04 (1.12)	5.85 (1.06)
Median (Q1, Q3)	6.00 (5.00, 7.00)	5.00 (5.00, 6.00)
Range	5.00 - 9.00	5.00 - 9.00
Recession (mm)		
Mean (SD)	0.66 (1.28)	0.48 (1.00)
Median (Q1, Q3)	0.00 (0.00, 1.00)	0.00 (0.00, 0.00)
Range	0.00 - 10.00	0.00 - 6.00
CAL (mm)		
Mean (SD)	6.70 (1.72)	6.33 (1.49)
Median (Q1, Q3)	6.00 (5.00, 8.00)	6.00 (5.00, 7.00)
Range	5.00 - 19.00	5.00 - 13.00
Plaque		
No	125 (11.6%)	14 (1.6%)
Yes	953 (88.4%)	878 (98.4%)
PD (cat)		
5-6 mm	763 (70.8%)	679 (76.1%)
7-9 mm	315 (29.2%)	213 (23.9%)
Type of tooth		
Monorooted	625 (58.0%)	508 (57.0%)
Multirooted	453 (42.0%)	384 (43.0%)

PD: Pocket Probing Depth, CAL: Clinical Attachment Level, BOP: Bleeding on Probing, PI: Plaque Index.

Table 3.2. Distribution of experimental sites at T1 according to PD (closed/residual pocket) and BOP (+/-).

	CTRL	**TEST**	**OR (CI 95%)**	***p-value***
Closed Pocket BOP- (NBCP)	44.7% *(36.0%; 53.7%)*	47.9% *(38.1%; 57.8%)*	1.14 *(0.66; 1.95)*	0.64
Closed Pocket BOP+ (BCP)	32.9% *(26.1%; 40.6%)*	32.1% *(24.9%; 40.2%)*	0.96 *(0.59; 1.56)*	0.87

Residual Pocket BOP– (NBRP)	6.9% *(4.1%; 11.2%)*	4.6% *(3.0%; 7.0%)*	0.65 *(0.32; 1.32)*	0.24
Residual Pocket BOP+ (BRP)	15.5% *(9.8%; 23.7%)*	15.5% *(10.6%; 22.1%)*	1.00 *(0.50; 1.98)*	1

OR: Odds Ratio

Table 3.3. Patient-level and site-level factors influencing the probability of obtaining Non-Bleeding Closed Pocket (NBCP) at experimental sites at T1.

Determinant	**Odds Ratio (OR) * (CI 95%)**	***p-value***
TEST / CONTROL	1.23 (0.70 – 2.16)	0.476
Centre (FE / BS)	0.27 (0.15 – 0.49)	**<0.001***
PD at baseline (7-9 / 5-6 mm)	0.30 (0.23 – 0.40)	**<0.001***
Age (years)	0.99 (0.96 – 1.02)	0.38
Gender (M / F)	1.53 (0.88 – 2.67)	0.131
Smoke (Yes / No)	0.87 (0.43 – 1.77)	0.701
Multi / Single-rooted	0.66 (0.51 – 0.86)	**0.002***
Plaque (Yes / No)	0.66 (0.51 – 0.84)	**0.001***
% Experimental sites at baseline	0.99 (0.97 – 1.01)	0.504

PD: Probing Depth, FE: Ferrara, BS: Brescia

* OR> 1 indicates an increased probability of NBCP at the numerator, whereas OR< 1 indicates an increased probability of NBCP at the denominator.

Table 3.4. Probability of probability of obtaining Non-bleeding Closed Pocket (NBCP) at T1 at experimental site based on initial Probing Depth and type of tooth (Single/Multi-rooted).

Treatment	Single/Multi-rooted	PD (mm)	Probability (%)
TEST	Single	5-6	59.4
	Multi	5-6	56.4
CONTROL	Single	5-6	53.6
	Multi	5-6	40.8
TEST	Single	7-9	28.6
	Multi	7-9	19.7
CONTROL	Single	7-9	34.8
	Multi	7-9	17.8

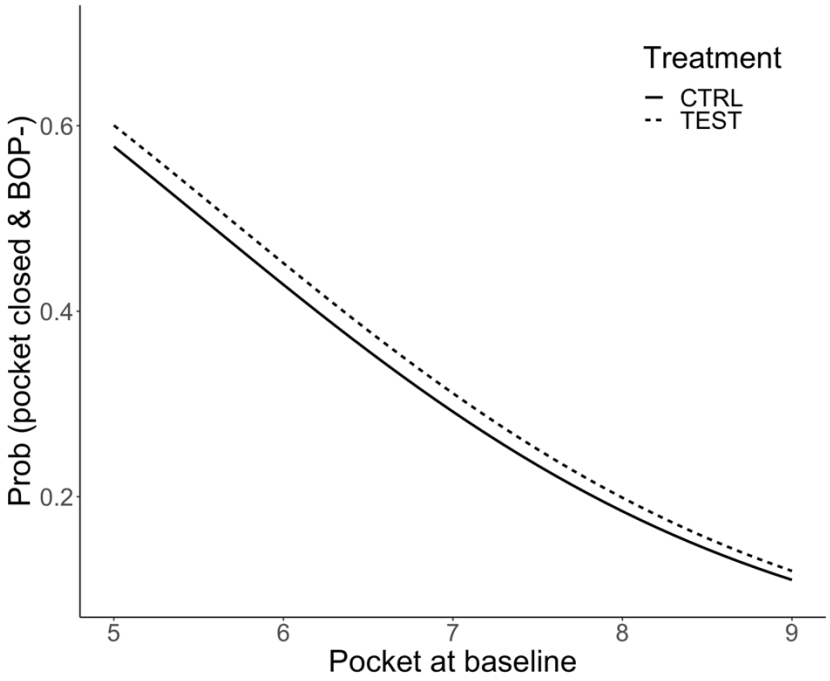
PD: Probing Depth

Table 3.5. Estimated periodontal parameters at T1. Probing Depth (PD), Clinical Attachment Level (CAL) and Bleeding on Probing (BOP) and Plaque Index (PI) values at T1 were estimated via analysis of covariance (ANCOVA) and adjusted on the mean baseline values. PD, CAL, BOP and PII values at T1 were estimated via analysis of covariance (ANCOVA) adjusted on the mean baseline values. PD and CAL were modelled on a log scale; therefore, between-treatment effect (T1 estimates in TEST compared to T1 estimate in Control) is reported as a ratio. BOP and PII were modelled on logit scale (log odds); therefore estimate is an Odds Ratio (OR).

Variable	T0	T1|CONTROL	T1|TEST	Between-treatment effect ratio* (TEST/CONTROL)	p-value
PD (mm)	5.86	3.24 (2.88 ; 3.65)	3.29 (2.92 ; 3.70)	1.01 (0.86 ; 1.20)	0.875
CAL (mm)	6.35	3.79 (3.36 ; 4.29)	3.73 (3.30 ; 4.21)	0.98 (0.83 ; 1.17)	0.839
BOP (%)	100	48 (37; 60)	47 (35 ; 58)	0.93 (0.48; 1.81)	0.834
PI (%)	86	40 (31 ; 50)	46 (36; 56)	1.25 (0.71; 2.22)	0.439

*OR for BOP, PI

Graphic 3.1: Probability of obtaining a Non-bleeding Closed Pocket (NBCP) at T1 at experimental sites per each treatment, based on the initial Probing Depth.



REFERENCES

Ainamo J, Bay I (1975) Problems and proposals for recording gingivitis and plaque. Int Dent J 25:229–235

Bozbay E, Dominici F, Gokbuget AY et al (2018) Preservation of root cementum: a comparative evaluation of power-driven versus hand instruments. Int J Dent Hyg 16:202–209. https://doi.org/10.1111/idh.12249

Caygur A, AlbabaMR, Berberoglu A, Yilmaz HG (2017) Efficacy of glycine powder air-polishing combined with scaling and root planing in the treatment of periodontitis and halitosis:Arandomisedclinical study. J Int Med Res 45(3):1168–1174. https://doi.org/10.

Cobb CM, Daubert DM, Davis K et al (2017) Consensus conference findings on supragingival and subgingival air polishing.Compend Contin EducDent 38:e1–e4

Ferrari SLP, Cribari-Neto F (2004) Beta Regression for Modeling Rates and Proportions. J Appl Stat 31(7):799–815

Flemmig TF, Arushanov D, Daubert D, Rothen M, Mueller G,Leroux BG (2012) Randomized controlled trial assessing efficacy and safety of glycine powder air polishing in moderate-to-deep periodontal pockets. J Periodontol 83:444–452. https://doi.org/10.1902/jop.2011.110367

Hägi TT, Hofmänner P, Eick S et al (2015) The effects of erythritol air-polishing powder on microbiologic and clinical outcomes during supportive periodontal therapy: Six-month results of a randomized controlled clinical trial. Quintessence Int 46:31–41. https://doi.org/10.3290/j.qi.a32817

Hägi TT,Klemensberger S, Bereiter R et al (2015)A biofilm pocket model to evaluate different non- surgical periodontal treatment modalities in terms of biofilm removal and reformation, surface alterations and attachment of periodontal ligament fibroblasts. PloS ONE 10:1–17. https://doi.org/10.1371/journal.pone.0131056

Hashino E, Kuboniwa M, Alghamdi SA et al (2014) Erythritol alters microstructure and metabolomic profiles of biofilm composed of Streptococcus gordonii and Porphyromonas gingivalis. Mol Oral Microbiol 28:435–451. https://doi.org/10.1111/omi.12037

Mensi M, Scotti E, Sordillo A, Agosti R, Calza S (2020) Plaque disclosing agent as a guide for professional biofilm removal: a randomized controlled clinical trial. (published online ahead of print, 2020 Apr 29). Int J Dent Hyg. https://doi.org/10.1111/idh.12442

Müller N, Moëne R, Cancela JA, Mombelli A (2014) Subgingival air-polishing with erythritol during periodontal maintenance: Randomized clinical trial of twelve months. J Clin Periodontol 41:883–889

Park EJ, Kwon EY, Kim HJ, Lee JY, Choi J, Joo JY (2018) Clinical and microbiological effects of the supplementary use of an erythritol powder air-polishing device in non-surgical periodontal therapy: a randomized clinical trial. J Periodontal Implant 48:295–304. https://doi.org/10.5051/jpis.2018.48.5.295

Petersilka G, Heckel R, Koch R, Ehmke B, Arweiler N (2018) Evaluation of an ex vivo porcine model to investigate the effect of low abrasive airpolishing. Clin Oral Investig 22:2669–2673. https://doi.org/10.1007/s00784-018-2536-5

Petersilka GJ, Steinmann D, Häberlein I, Heinecke A, Flemmig TF(2003) Subgingival plaque removal in buccal and lingual sites using a novel low abrasive air-polishing powder. J Clin Periodontol 30:328–333

Petersilka GJ, Tunkel J, Barakos K, Heinecke A, Häberlein I, Flemmig TF (2003) Subgingival plaque removal at interdental sites using a low-abrasive air polishing powder. J Periodontol 74:307–311

Preshaw PM, Lauffart B, Zak E, Jeffcoat MK, Barton I, HeasmanPA (1999) progression and treatment of chronic adult periodontitis. J Periodontol 70(10):1209–1220

Reinhardt B, Klocke A, Neering SH, Selbach S, Peters U, Flemmig TF, Beikler T (2019) Microbiological dynamics of red complex bacteria following full-mouth air polishing in periodontally healthy subjects-a randomized clinical pilot study. Clin Oral Investig 23(10):3905–3914. https://doi.org/10.1007/s00784-019-02821-3

Sculean A, Bastendorf KD, Becker C et al (2013) A paradigm shift in mechanical biofilm management? Subgingival air polishing: a new way to improve mechanical biofilm management in the dental practice. Quintessence Int 44:475–477. https://doi.org/10.3290/j.qi.a29615.

Tomasi C, Leyland AH,Wennström JL (2007) Factors influencing the outcome of non-surgical periodontal treatment: a multilevel approach. J Clin Periodontol 34:682–690. https://doi.org/10.1111/j.1600-051X.2007.01111.x

Tonetti MS, Greenwell H, Kornman KS (2018) Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Periodontol 89(Suppl 1):S159–S172. https://doi.org/10.1002/JPER.18-0006

Tsang YC, Corbet EF, Jin LJ (2018) Subgingival glycine powder air- polishing as an additional approach to nonsurgical periodontal therapy in subjects with untreated chronic periodontitis. J Periodontal Res 53:440–445. https://doi.org/10.1111/jre.12532

Twisk J, Bosman L, Hoekstra T, Rijnhart J, Welten M, HeymansM (2018) Different Ways to Estimate Treatment Effects in Randomised Controlled Trials. Contemp Clin Trials Commun 10(June):80–85

Wennström JL, Dahlén G, Ramberg P (2011) Subgingival debridement of periodontal pockets by air polishing in comparison to ultrasonic instrumentation during maintenance therapy. J Clin Periodontol 38:820–827. https://doi.org/10.1111/j.1600-051X.2011.01751.x

Wennström JL, Tomasi C, Bertelle A, Dellasega E (2005) Full mouth ultrasonic debridement versus quadrant scaling and root planing as an initial approach in the treatment of chronic periodontitis. J Clin Periodontol 32:851–859. https://doi.org/10.1111/j.1600-051X.2005.00776.x

Zuabi O, Machtei EE, Ben-Aryeh H, Ardekian L, Peled M, Laufer D (1999) The effect of smoking and periodontal treatment on salivary composition in patients with established periodontitis. J Periodontol 70(10):1240–1246

Chapter IV

GENERAL DISCUSSION

Supra- and sub-gingival PMPR is the preferred method for non-surgical treatment of periodontitis. The debridement of "hard to reach" anatomical defects (e.g., furcations, root depressions, and deep pockets) is arduous due to the increased difficulty in accessing these sites. Importantly, without the use of ultra-fine ultrasonic tips and/or mini-curettes, non-surgical root surface debridement results in extensive residual calculus, especially when performed by less experienced clinicians (Nibali & Calciolari, 2018). Although a surgical approach achieves a greater PD reduction in deep (> 6 mm) and moderately deep (4–6 mm) defects, a comparable or greater gain in CAL can be obtained with non-surgical subgingival debridement in similar sites when performed by skilled clinicians (Sanz-Sanchez et al., 2020). Economic analysis indicates that the use of a new additional technology involves considerable expenses (asset and upkeep costs) and time must be invested in operator training. These limitations must be considered in the context of potential superior clinical improvement and/or a higher degree of comfort for both patient and operator.

Recent reviews (Smiley et al., 2015 (1), (2); John et al., 2017) have reported that SRP as a monotherapy yields a moderate CAL gain (mean, 0.49 mm), often with no post-treatment AEs. Various adjunctive procedures to standard SRP have been proposed, comprising both systemic and locally applied treatments. These contribute to an additional mean CAL gain of 0.32 mm (range, 0.2–0.6 mm) within 6–12 months after treatment. This "small adjunctive effect" with known modifiers of treatment outcome favorably compares with the negative effect of smoking on CAL gain both after non-surgical (Labriola et al., 2005) and surgical periodontal therapy (Kotsakis et al., 2015) (0.11 mm and 0.35 mm, respectively). Nevertheless, the majority of these treatments cannot currently be recommended as routine adjunctive therapies (Sanz et al., 2020). In the randomized controlled trials (RCTs) described in Chapters II and III, the adjunctive effects of two procedures in the treatment of moderate to severe periodontitis (stages III and IV) were compared with standard SRP to address specific research/clinical questions.

What is the adjunctive clinical efficacy of the proposed phototherapy protocol (PTP) to standard SRP?

The proposed PTP protocol did not provide any additional clinical benefits over those achieved by SRP performed with ultrasonic device alone.

Nevertheless, as stated in Chapter II, the tested procedure resulted in a clinical improvement after the follow-up period of 6 months (Appendix I for visual representation). The use of PTP associated with SRP resulted in a significant mean CAL gain at the 6-month follow-up of 0.31 ± 0.79 mm ($p < 0.0001$). Furthermore, the use of the tested adjunct resulted in a significant change in PD mean values ($p < 0.0001$), which decreased at each intermediate post-treatment visit, from baseline (3.89 ± 0.94 mm) to 1 month (3.29 ± 0.73 mm), 3 months (3.27 ± 0.76 mm), and 6 months (3.22 ± 0.76 mm). The respective changes were 0.60 ± 0.55 mm, 0.63 ± 0.72 mm, and 0.69 ± 0.56 mm. BOP scores steadily decreased after treatment, reaching a maximum of 22.3 ± 22.5% at the 6-month follow-up.

The mean pocket depth reduction following non-surgical periodontal therapy may vary from 1.29 mm (for defects with initial PD 5–6 mm) to 2.2 mm (for deeper sites); the mean CAL gain is 0.5–2 mm (Van der Weijden et al., 2002; Smiley et al., 2015 (1)). The results reported in both Chapters II and III indicate that initial periodontitis severity is a key aspect in determining the outcomes of SRP alone or with adjuncts. As indicated in the tables in Chapter II, changes recorded in sites with initial PD ≥ 5 mm after treatment with SRP alone and SRP with the aid of PTP were reported for the "deep sites" subgroup. Considering only these deep pockets (initial PD ≥ 5 mm), PD significantly improved ($p < 0.0001$) after SRP associated with PTP: it decreased from a baseline value of 5.94 ± 0.60 mm to 4.57 ± 1.02 mm at 1 month, 4.51 ± 0.96 mm at 3 months, and 4.47 ± 1.08 mm after 6 months. The mean overall gain was 1.46 ± 0.93 mm. Furthermore, CAL values improved with a mean gain of 1.01 ± 1.17 mm at 6 months. However, PD and CAL values evaluated in deep sites treated with SRP alone showed a similar post-treatment course. Comparing the number of "deep sites" over time, SRP with adjunctive PTP led to a reduction of 42.38% at 3 months, comparable with the results obtained using SRP alone (40.16%).

The achievement of shallow pockets (PD ≤ 4 mm) with resolved inflammation (i.e., absence of BOP) confers the greatest likelihood of periodontal stability. This condition can be achieved in pockets with PD < 5 mm, but is less predictable in sites with deep intrabony defects and greater

initial pocket depth (Van der Weijden et al., 2002; Tomasi et al., 2007; Suvan et al, 2020). The RCTs described in Chapter II demonstrated that SRP alone and SRP combined with PTP achieved similar results in terms of the post-treatment number of sites with PD ≤ 4 mm and absence of BOP. Single-rooted teeth and teeth with no furcation involvement usually respond more robustly to non-surgical treatment and are more easily maintained over time (Tomasi et al., 2007; Graziani et al., 2017). Treatment outcomes of SRP combined with PTP on multi-rooted teeth were comparable with those obtained using SRP that involved ultrasonic treatment alone.

When considering any intervention, the clinician and patient must balance potential benefits with potential AEs. In the absence of substantial CAL gain differences across treatments, more emphasis can be placed on other treatment characteristics for clinical decision-making. Any type of root planing, including hand and ultrasonic instrumentation, carries the risk of post-procedural discomfort and/or root sensitivity; these AEs are generally expected. In the study described in Chapter II, patients received a split-mouth treatment with two sextants randomly assigned to receive either SRP alone or SRP associated with PTP treatment; after recovery from local anesthesia, they reported equal and limited discomfort in both areas.

While recruiting for the RCTs described in Chapters II and III, patients with diabetes were excluded and no results can be reported for this population. However, smokers were included and their statuses (current or previous smoking habit) were recorded for further analysis. In the study described in Chapter II, only 8.4% of participants were smokers; this limited number of patients did not allow a proper population sub-analysis.

The tested PTP aimed to achieve further pocket decontamination by the use of a chromophore gel activated by a luminous wavelength. The absorption range of the chromophore gel is specific (430 nm) and the PTP used a standard curing light emitting a wider spectrum (385–515 nm). The 430-nm excitation wavelength of the gel was included, while the introduced energy of the outer wavelengths (385–429 nm and 431–515 nm) remained unused, thereby reducing the potential activation energy. Moreover, if the light energy emitted by the lamp unit is not well distributed across the tip, the right amount of energy is limited to specific areas. The irradiance is the flux of radiant energy per unit area. The effect of proper irradiance is noticeable in restorative dentistry, when a curing lamp with a standard power unit and an excessively large diameter tip may have insufficient irradiance to cure the composite efficiently. In the RCT proposed in Chapter II, some tip areas provided adequate irradiance to activate the gel, while others did not. The manufacturer provided small light tips to convey the light into the

periodontal pockets, thus resolving the irradiance issue because the lamp total power achieved a consistent distribution on this small tip diameter. Notably, the standard curing lamp provided was considerably maneuverable with respect to its dimensions. Moreover, the tip used was sufficiently thin to reach the bottom of the defects, especially in inflamed areas with looser attachment. The use of a maneuverable curing light with a small tip (e.g., a fiber-like attachment) and a specific wavelength could be a focus of further research regarding this chromophore gel.

What is the adjunctive clinical efficacy of subgingival air-polishing in the context of EPAP treatment?

The additional use of subgingival air-polishing does not provide any significant clinical advantage when compared to the use of ultrasonic device alone in the treatment of periodontitis.

However, in Chapter III, the trial showed how subgingival EPAP associated with SRP performed using an ultrasonic device led to a significant improvement ($p < 0.0001$) in the participants' periodontal condition (Appendix II for visual representation). PD values (unpublished data) showed a reduction from an initial mean of 3.90 ± 2.00 mm to 2.50 ± 1.40 mm at the 3-month post-treatment evaluation; furthermore, CAL values decreased from 4.50 ± 2.20 mm to 3.10 ± 1.90 mm. However, subgingival debridement performed using an ultrasonic device alone resulted in comparable outcomes. In deep sites, SRP with adjunct subgingival EPAP resulted in a mean PD improvement of 2.28 mm, similar to the result of SRP alone (2.37 mm). The use of EPAP thus tended to provide a slightly better outcome in defects with an initial PD of 5–6 mm, but no additional benefit over SRP alone was evident in moderate to deep (PD 7–9 mm) pockets with BOP. Comparing the number of deep sites over time, standard SRP performed using an ultrasonic device combined with subgingival EPAP achieved a significant reduction in the number of deep sites by 80% at 3 months (compared with 66% in published literature). However, SRP alone (i.e., the control group in the study described in Chapter III) led to an inferior result (77.6%). Subgingival adjunctive treatment with EPAP resulted in a slightly superior result when compared with SRP alone (+ 2.4%), but the differences were not statistically significant when compared with the results obtained with SRP + PTP (+ 2.2%).

The RCTs described in Chapter III demonstrated that SRP alone or SRP associated with EPAP achieved similar results in terms of the post-treatment pocket-closure rate. The use of subgingival EPAP combined with SRP on multi-rooted teeth with moderate defects (5–6 mm)

enhanced the likelihood of obtaining a PD ≤ 4 mm without BOP. Recent studies reported that subgingival debridement with low-abrasive powders should be scheduled at 2–3-month intervals to maintain the minimal bacterial count (Sekino et al., 2020). However, a tendency for furcation deterioration has been observed with repeated use of these devices (Petersilka et al., 2020). Thus, it may be necessary to use conventional modalities of debridement (e.g., standard ultrasonic tips) in these sites. In the trial reported in Chapter III, 19.4% of participants were smokers; there was no statistically significant difference between groups treated with SRP alone and those treated with adjunctive subgingival EPAP administration. Thus, a smoking habit may not influence SRP + EPAP treatment outcomes.

The design of the study reported in Chapter III did not regard the use of local anesthesia prior to treatment as mandatory; thus, anesthesia was performed in accordance with each patient's needs. When a dose of anesthetic was defined as the administration of 1.8 mL of mepivacaine (one standard cartridge), it was necessary to use two doses over the entire population (1.28 for 18 patients in the control group; 1.67 for patients in the test group). The use of the ultrasonic device provided, alone or in association with EPAP, was well tolerated by the participants.

The design of the nozzle used to introduce EPAP into the periodontal pockets is innovative. It contains two lateral ejection sites for water and erythritol powder to decontaminate the tooth surface and the pocket epithelium; it contains an ejection site on the tip (ejecting only water) to remove all debris and minimize the risk of tissue damage. However, its dimensions do not allow easy access to periodontal pockets with tight tissue attachment, limiting the action of the powder in deeper areas of those defects. Repeated subgingival EPAP alone reduces the number of pockets in a manner similar to standard debridement performed with ultrasonic devices alone (Wennström et al., 2011; Müller et al., 2014). However, its use as an adjunctive treatment to standard SRP has limited additional effects. Notably, erythritol reportedly induces a microbial shift from periodontitis- and gingivitis-related species toward a biofilm comprised mostly of early colonizers (Janus et al., 2017). This implies that the use of EPAP, introducing erythritol both supragingivally and subgingivally, may suppresses maturation of oral biofilms and promote a healthy microbiome. However, the composition of the pocket microbiome after treatment with EPAP remains controversial and requires further research.

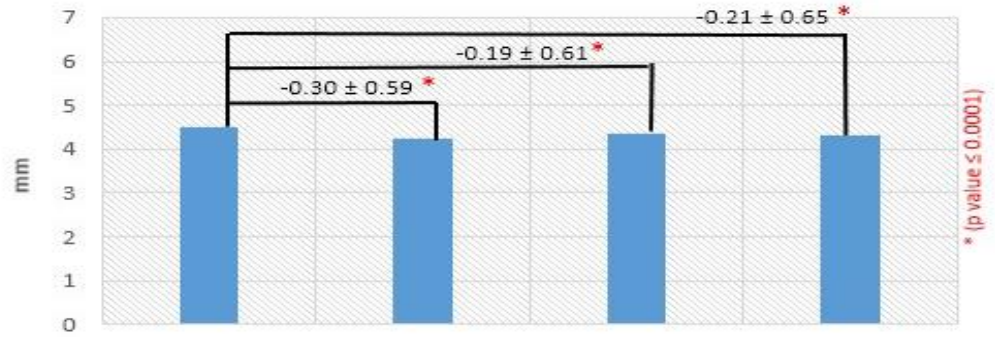
CONCLUDING REMARKS

The general purpose of the studies included in this Ph.D. activity was to evaluate the effectiveness of distinct adjunctive therapies for standard SRP in the treatment of periodontitis. Specifically, the efficacy of a PTP and the use of subgingival air-polishing with EPAP. On the basis of the evidence in these trials, the following conclusions can be drawn:

- The effects of standard SRP performed using an ultrasonic device associated with PTP or subgingival EPAP are limited, especially in sites with PD ≥ 7 mm and in multi-rooted teeth.
- At the 3-month follow-up, subgingival EPAP combined with SRP led to PD ≤ 4 mm in 80% of sites that had exhibited initial PD of 5–9 mm, but no significant difference with the use of SRP alone was found.
- In patients who are smokers, SRP associated with subgingival EPAP can provide benefits in shallow and moderate pockets (PD 1–5 mm) that are comparable to the benefits for non-smoking patients.

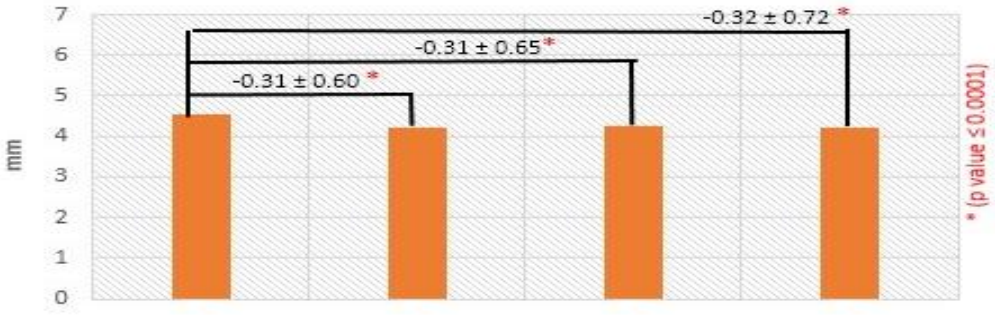
APPENDIX I

SRP ALONE
mean CAL values (mm) – ALL sites



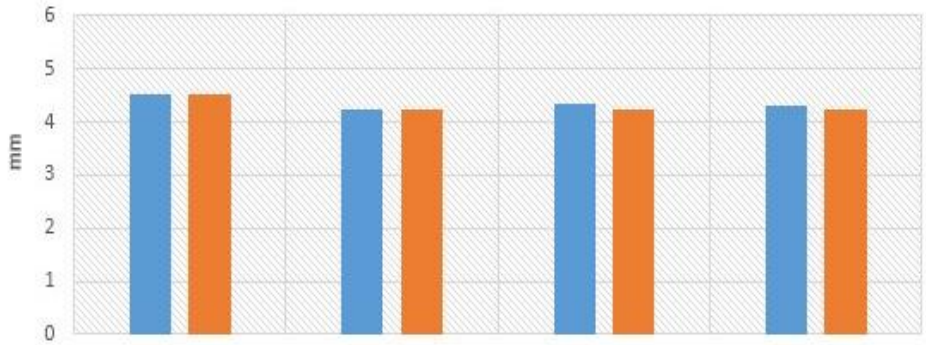
	Baseline		+6 weeks		+12 weeks		+24 weeks	
	mm	St dev	mm	St dev	mm	St dev	mm	St dev
SRP	4.51	1.12	4.22	1.07	4.35	1.11	4.3	1.14

SRP + PTP
mean CAL values (mm) – ALL sites



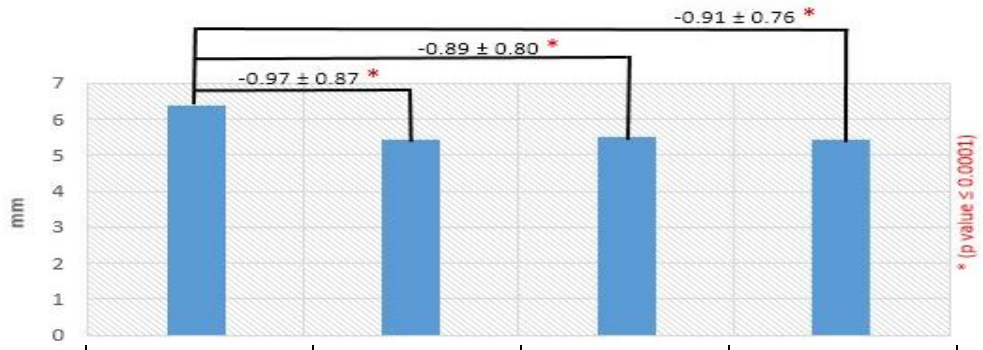
	Baseline		+6 weeks		+12 weeks		+24 weeks	
	mm	St dev	mm	St dev	mm	St dev	mm	St dev
SRP + PTP	4.53	1.22	4.22	1.12	4.25	1.13	4.22	1.14

SRP ALONE vs SRP + PTP
mean CAL value (mm) – ALL sites



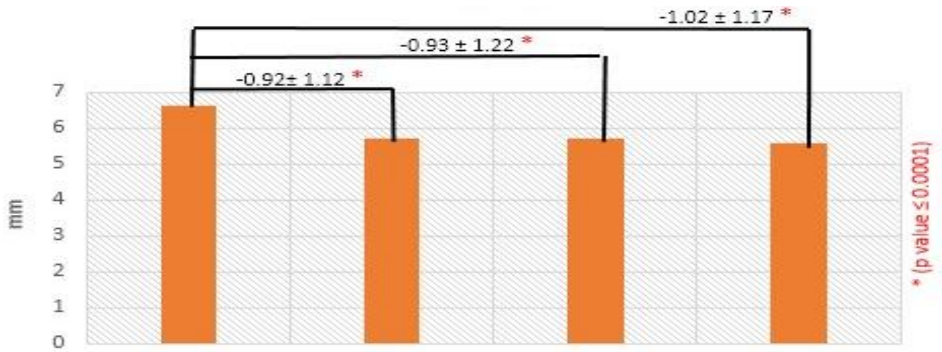
	Baseline		+6 weeks		+12 weeks		+24 weeks	
	mm	St dev	mm	St dev	mm	St dev	mm	St dev
SRP	4.51	1.12	4.22	1.07	4.35	1.11	4.30	1.14
SRP + PTP	4.53	1.22	4.22	1.12	4.25	1.13	4.22	1.14

SRP ALONE
mean CAL values (mm) – DEEP sites



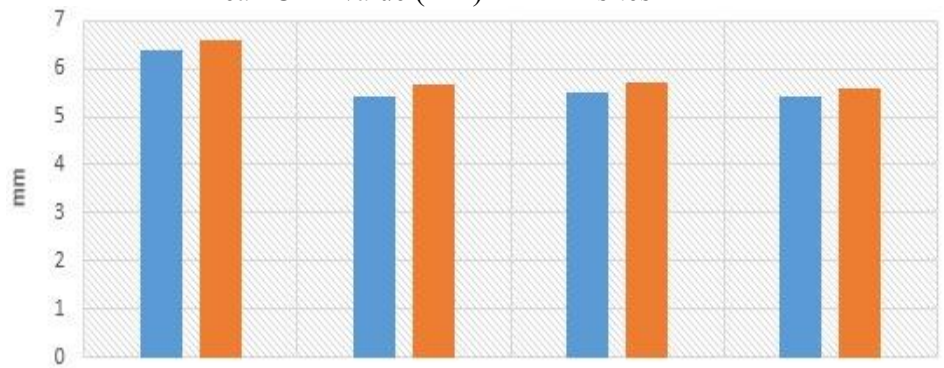
	Baseline		+6 weeks		+12 weeks		+24 weeks	
	mm	St dev	mm	St dev	mm	St dev	mm	St dev
SRP	6.40	0.95	5.43	1.23	5.52	1.27	5.43	1.20

SRP + PTP
mean CAL values (mm) – DEEP sites



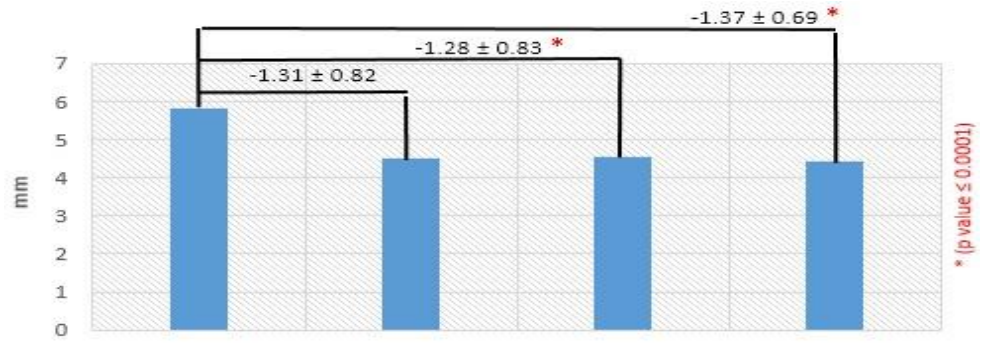
	Baseline		+6 weeks		+12 weeks		+24 weeks	
	mm	St dev	mm	St dev	mm	St dev	mm	St dev
SRP + PTP	6.61	1.00	5.69	1.61	5.70	1.83	5.59	1.69

SRP ALONE vs SRP + PTP
mean CAL value (mm) – DEEP sites



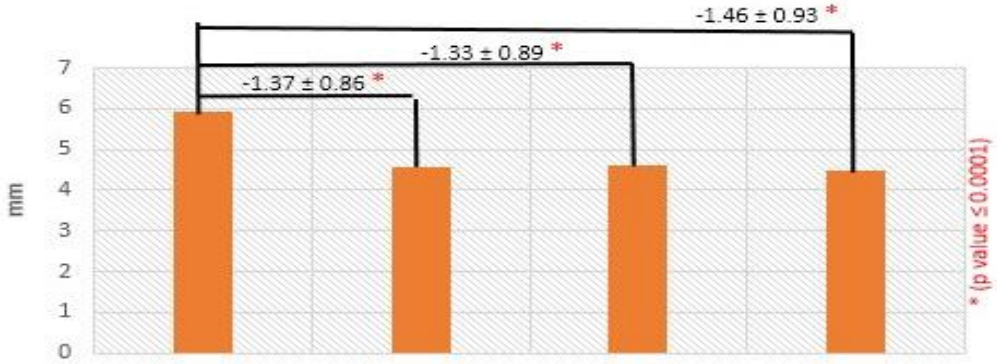
	Baseline		+6 weeks		+12 weeks		+24 weeks	
	mm	St dev	mm	St dev	mm	St dev	mm	St dev
SRP	6.40	0.95	5.43	1.23	5.52	1.27	5.43	1.20
SRP + PTP	6.61	1.00	5.69	1.61	5.70	1.83	5.59	1.69

SRP ALONE
mean PD values (mm) – DEEP sites



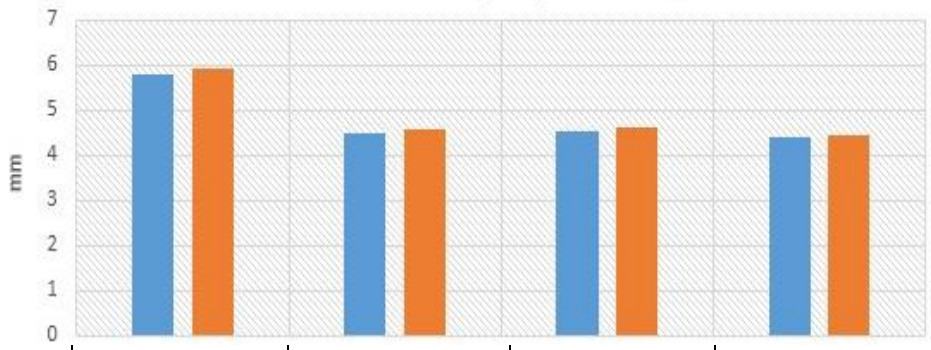
Pd deep	Baseline		+6 weeks		+12 weeks		+24 weeks	
	mm	St dev	mm	St dev	mm	St dev	mm	St dev
SRP	5.82	0.56	4.51	0.96	4.54	0.90	4.41	0.81

SRP + PTP
mean PD values (mm) – DEEP sites



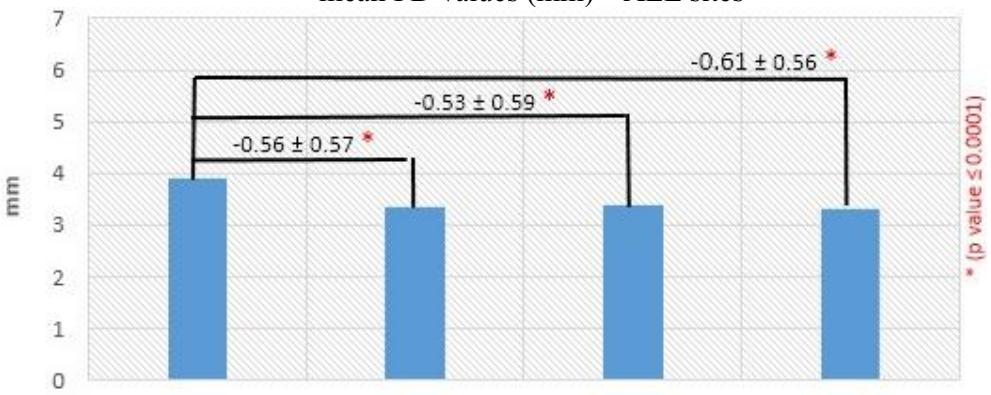
	Baseline		+6 weeks		+12 weeks		+24 weeks	
	mm	St dev	mm	St dev	mm	St dev	mm	St dev
SRP + PTP	5.94	0.6	4.57	1.3	4.62	1.02	4.47	1.08

SRP ALONE vs SRP + PTP
mean PD value (mm) – DEEP sites



	Baseline		+6 weeks		+12 weeks		+24 weeks	
	mm	St dev	mm	St dev	mm	St dev	mm	St dev
SRP	5.82	0.56	4.51	0.96	4.54	0.9	4.41	0.81
SRP + PTP	5.94	0.60	4.57	1.30	4.62	1.02	4.47	1.08

SRP ALONE
mean PD values (mm) – ALL sites

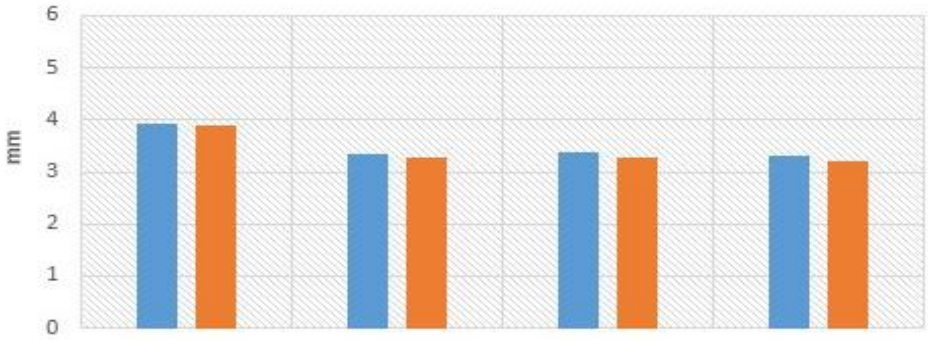


	Baseline		+6 weeks		+12 weeks		+24 weeks	
	mm	St dev	mm	St dev	mm	St dev	mm	St dev
SRP	3.91	0.84	3.34	0.72	3.39	0.73	3.31	0.76

SRP ALONE
mean PD values (mm) – ALL sites

	Baseline		+6 weeks		+12 weeks		+24 weeks	
	mm	St dev	mm	St dev	mm	St dev	mm	St dev
SRP + PTP	3.89	0.94	3.29	0.73	3.27	0.76	3.22	0.76

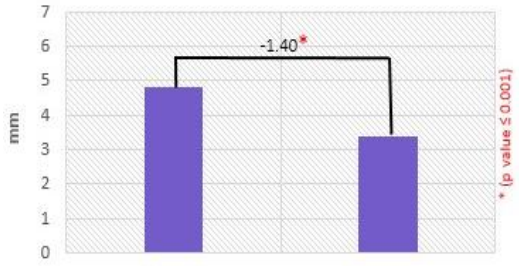
SRP ALONE vs SRP + PTP
mean PD values (mm) – ALL sites



	Baseline		+6 weeks		+12 weeks		+24 weeks	
	mm	St dev	mm	St dev	mm	St dev	mm	St dev
SRP	3.91	0.84	3.34	0.72	3.39	0.73	3.31	0.76
SRP + PTP	3.89	0.94	3.29	0.73	3.27	0.76	3.22	0.76

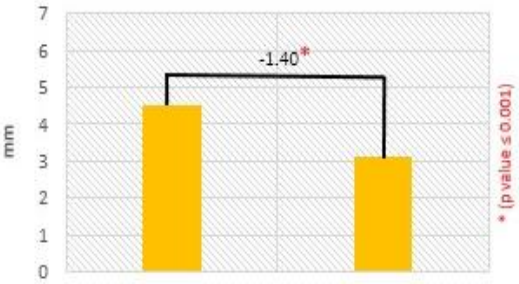
APPENDIX II

SRP ALONE
mean CAL values (mm) – ALL sites



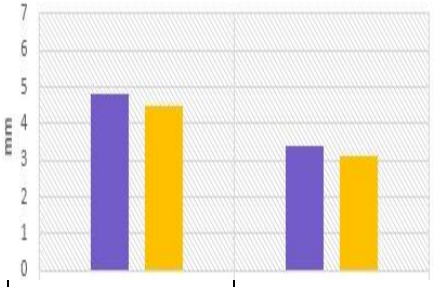
	Baseline		+12 weeks	
	mm	St dev	mm	St dev
SRP	4.80	2.50	3.40	2.10

SRP + EPAP
mean CAL values (mm) – ALL sites

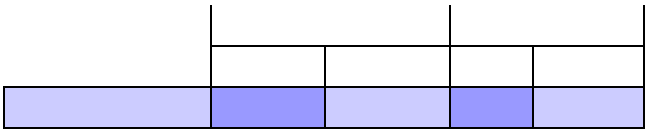


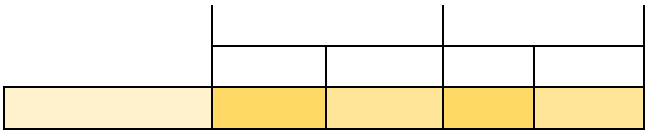
	Baseline		+12 weeks	
	mm	St dev	mm	St dev
SRP + EPAP	4.50	2.20	3.10	1.90

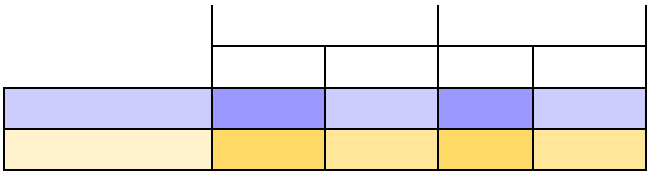
SRP ALONE vs SRP + EPAP
mean CAL values (mm) – ALL sites



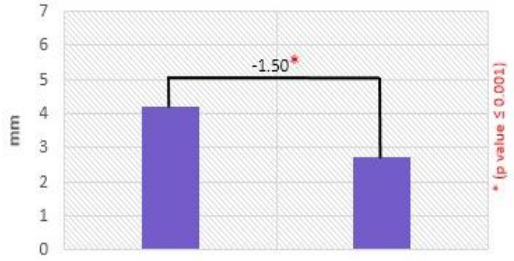
	Baseline		+12 weeks	
	mm	St dev	mm	St dev
SRP	4.80	2.50	3.40	2.10
SRP + EPAP	4.50	2.20	3.10	1.90





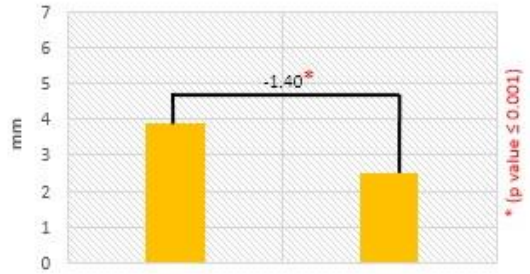


SRP ALONE
mean PD values (mm) – ALL sites



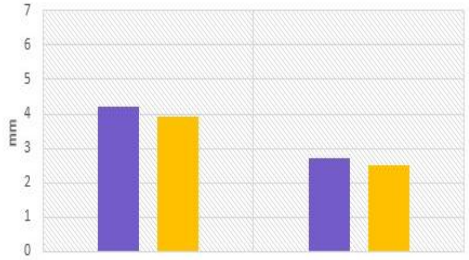
	Baseline		+12 weeks	
	mm	St dev	mm	St dev
SRP	4.20	2.10	2.70	1.50

SRP + EPAP
mean PD values (mm) – ALL sites



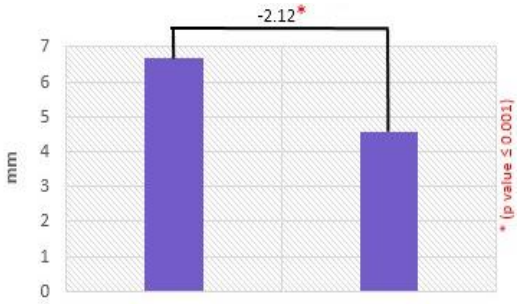
	Baseline		+12 weeks	
	mm	St dev	mm	St dev
SRP + EPAP	3.90	2.00	2.50	1.40

SRP ALONE vs SRP + EPAP
mean PD values (mm) – ALL sites



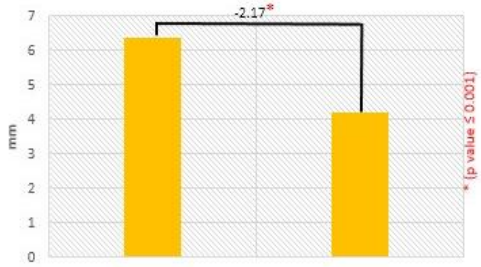
	Baseline		+12 weeks	
	mm	St dev	mm	St dev
SRP	4.20	2.10	2.70	1.50
SRP + EPAP	3.90	2.00	2.50	1.40

SRP ALONE
mean CAL values (mm) – DEEP sites



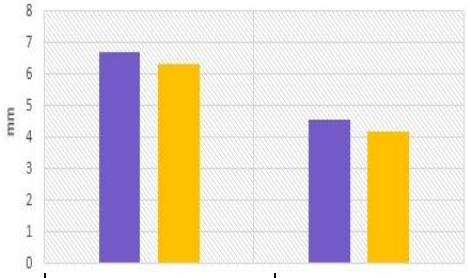
	Baseline		+12 weeks	
	mm	St dev	mm	St dev
SRP	6.70	1.72	4.58	2.22

SRP + EPAP
mean CAL values (mm) – DEEP sites



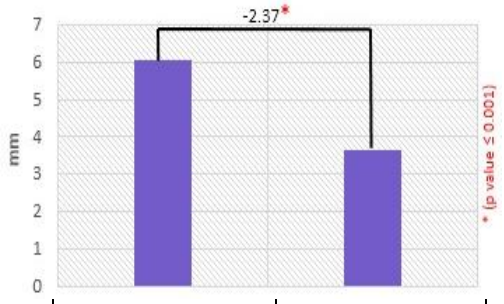
	Baseline		12 weeks	
	mm	St dev	mm	St dev
SRP + EPAP	6.33	1.49	4.16	1.83

SRP ALONE vs SRP + EPAP
mean CAL values (mm) – DEEP sites



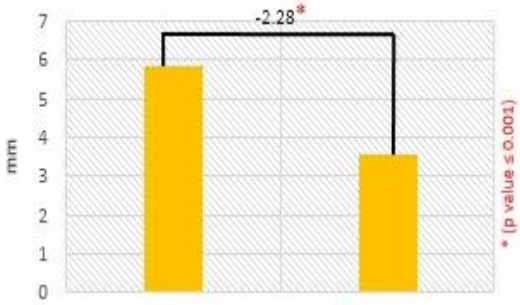
	Baseline		+12 weeks	
	mm	St dev	mm	St dev
SRP	6.70	1.72	4.58	2.22
SRP + EPAP	6.33	1.49	4.16	1.83

SRP ALONE
mean PD values (mm) – DEEP sites



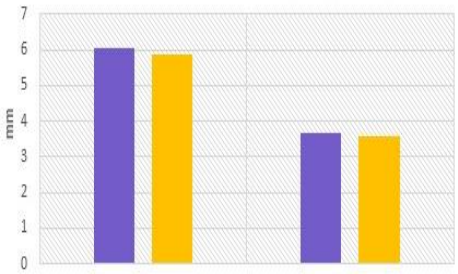
	Baseline		+12 weeks	
	mm	St dev	mm	St dev
SRP	6.04	1.12	3.67	1.54

SRP + EPAP
mean PD values (mm) – DEEP sites



	Baseline		+12 weeks	
	mm	St dev	mm	St dev
SRP + EPAP	5.85	1.06	3.57	1.30

SRP ALONE vs SRP + EPAP
mean PD values (mm) – DEEP sites



	Baseline		+12 weeks	
	mm	St dev	mm	St dev
SRP	6.04	1.12	3.67	1.54
SRP + EPAP	5.85	1.06	3.57	1.30