Critical Review
Periodontics

New tendencies in non-surgical periodontal therapy

Alex Nogueira HAAS[a]
Flavia FURLANETO[b]
Eduardo José GAIO[a]
Sabrina Carvalho GOMES[a]
Daniela Bazan PALIOTO[b]
Rogerio Moraes CASTILHO[c]
Mariano SANZ[d]
Michel Reis MESSORA[b]

[a]Universidade Federal do Rio Grande do Sul – UFRGS, School of Dentistry, Department of Periodontology, Porto Alegre, RS, Brazil.

[b]Universidade de São Paulo – USP, School of Dentistry of Ribeirão Preto, Department of Oral Surgery and Periodontology, Ribeirão Preto, SP, Brazil.

[c]Michigan University, School of Dentistry, Department of Periodontics and Oral Medicine, Ann Arbor, MI, USA.

[d]Complutense University of Madrid, Etiology and Therapy of Periodontal and Peri-implant Diseases Research Group, Madrid, Spain.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

Corresponding Author:
Alex Nogueira Haas
E-mail: alexnhaas@gmail.com

https://doi.org/10.1590/1807-3107bor-2021.vol35.0095

Submitted: March 7, 2021
Accepted for publication: March 31, 2021
Last revision: April 13, 2021

Abstract: The aim of this review was to update the evidence of new approaches to non-surgical therapy (NSPT) in the treatment of periodontitis. Preclinical and clinical studies addressing the benefits of adjunctive antimicrobial photodynamic therapy, probiotics, prebiotics/synbiotics, statins, pro-resolving mediators, omega-6 and -3, ozone, and epigenetic therapy were scrutinized and discussed. Currently, the outcomes of these nine new approaches, when compared with subgingival debridement alone, did not demonstrate a significant added clinical benefit. However, some of these new alternative interventions may have the potential to improve the outcomes of NSPT alone. Future evidence based on randomized controlled clinical trials would help clinicians and patients in the selection of different adjunctive therapies.

Keywords: Fatty Acids, Omega-3; Periodontal Debridement; Probiotics; Ozone; Epigenomics.

Introduction

Periodontal therapy comprises a broad range of interventions applied in a stepwise approach with the aim of controlling the infection and arresting the inflammation.[1] The first step of periodontal therapy includes the control of supragingival biofilm, both by the patient and the professional, as well as the control of those proven risk factors in the etiopathogenesis of periodontal diseases.[2,3] For patients diagnosed with gingivitis, this step of therapy should be enough to arrest gingival inflammation once biofilm accumulation has been removed. For patients diagnosed with periodontitis, the first step is a prerequisite before implementing the second step, based on the removal of subgingival biofilm and calculus, which is the basic mode of periodontal therapy. Subgingival instrumentation may include adjunctive local/systemic antimicrobial or anti-inflammatory medications.

A variety of endpoints have been evaluated in periodontal literature to assess the efficacy of these two steps of treatment, most frequently average reductions in probing pocket depth (PPD) and gains in clinical attachment level (CAL). More recently, there has been a concern to define more appropriate endpoints that should be achieved after periodontal therapy.[4] For instance, in the clinical practice guideline from the European Federation of Periodontology (EFP),[1] a clear definition of the desired outcome after treatment of Stage I-II periodontitis has been established.[5] After the completion of periodontal therapy, a stable periodontitis patient is

defined by gingival health on a reduced periodontium (bleeding on probing in < 10% of the sites; shallow probing depths of 4 mm or less and no 4 mm sites with bleeding on probing). When these criteria are met after the completion of periodontal treatment, but bleeding on probing is present at > 10% of the sites, then the patient is diagnosed as a stable periodontitis patient with gingival inflammation.

If the aims of periodontal therapy have not been achieved with this sequence of steps 1 and 2, the step 3 of periodontal therapy needs to be implemented. It consists of either repeated subgingival instrumentation or other periodontal surgical interventions. Finally, the results of step 3 should be reevaluated for the accomplishment of the therapy endpoints and, if these endpoints are achieved, a strict maintenance program should be implemented.[6,7,8]

Non-surgical periodontal therapy (NSPT) is the term used to generally describe subgingival mechanical instrumentation performed during the second step of treatment. Standard NSPT, mainly performed by means of scaling and root planing (SRP), is the gold-standard treatment for Stage I-III periodontitis.[1] Nevertheless, some sites and/or patients may present poor response to standard NSPT. This may be related to microbial factors, when this mode of therapy is not capable of converting the dysbiotic infectious process to a homeostatic/commensal balance, probably due to residual subgingival biofilm in the periodontal pocket after SRP[9] and/or tissue invasion by periodontopathic bateria,[10] or the maintenance of a non-resolving chronic inflammatory response in spite of the subgingival debridement. Therefore, there is a continuous search for adjunctive therapies that can improve the outcomes of subgingival instrumentation alone. Thus, the purpose of this narrative review was to evaluate the evidence from clinical or preclinical studies of new therapeutic approaches used as adjuncts to NSPT.

Antimicrobial photodynamic therapy (aPDT)

The major aim of aPDT is to eradicate or reduce microorganisms. This is achieved when a non-toxic light-sensitive "photosensitizer", excited by a visible light or near infrared with the appropriate wavelength, stimulates the formation of free radicals of singlet oxygen that will act as toxic agents to the bacteria. Toluidine blue O and methylene blue are the most used photosensitizers in Dentistry, although others have been studied. Both have shown to be effective for periodontal pathogen control in planktonic bacteria and in biofilms.[11,12,13] The gold-standard light for applying aPDT in the treatment of periodontitis is the low level laser (LLLT), which has shown favorable healing conditions.[14] LLLT has a potency of 30–100 MW, wavelength of 630–904 nm, and minimum thermal heat. Its efficacy depends on the amount of absorbed light. When appropriate, low intensity laser can help restore the cell balance, especially because of its influence in the inflammatory process *per se*.[15]

One of the major advantages of aPDT is that in the clinical treatment of local infections it might be less injurious to indigenous/commensal biofilms than systemic antibiotic therapy.[16] The use of aPDT was first evaluated in a split-mouth randomized controlled trial (RCT) as a sole therapy, compared with SRP in the treatment of aggressive periodontitis patients.[17] Although the clinical outcomes and biomarker levels in gingival crevicular fluid (TNF-α and RANK-L)[18] were similar 3 months after therapy, the microbiological outcomes (reduction of red complex pathogens) were more favorable in the SRP group.[19] These findings indicated that aPDT should not be considered as a single method to eliminate subgingival biofilm, but should be implemented as adjunctive to SRP.

Several preclinical and clinical studies have evaluated aPDT as an adjunctive method to SRP[17-25] with the goal of penetrating the soft tissues, thus reaching the periodontal pathogens that might have infiltrated into the periodontium.[22] Moreover, the low intensity laser is capable of favoring tissue repair by improving cell behavior and collagen synthesis.[26]

A recent systematic review with meta-analysis has summarized the findings of RCTs assessing the clinical outcomes achieved with aPDT.[27] A total of 17 trials were included and analyzed separately for participants with chronic (n = 13) and aggressive (n = 4) periodontitis. Six additional studies tested aPDT in residual sites after maintenance therapy. In general,

the achieved additional benefits did not lead to a significant effect compared to standard NSPT. In agreement with the conclusions of this systematic review, the EFP Clinical Practice Guidelines for the treatment of Stage I-III periodontitis do not recommend aPDT in a single application (wavelength 660–670 nm or 800–900 nm) in association with SRP for patients with periodontitis based on this available evidence.[1]

The frequency of application of aPDT also deserves some discussion, since there are indications that it may lead to different clinical outcomes. It has been demonstrated that multiple sessions of aPDT bring additional benefits. The first study evaluating the multiple episodes of aPDT in adjunctive to concomitant NSPT[25] found greater probing depth (PD) reduction and more clinical attachment gain 6 months after NSPT alone. Another split-mouth RCT[28] demonstrated that repeated aPDT applications (baseline, 2, 7 and 14 days) adjunctive to SRP were more effective to treat initially deep pockets (≥ 7 mm) in aggressive periodontitis than SRP alone 90 days after the therapies.

Other aspects still need investigation, for example, the specific patient populations that may benefit more from aPDT. It has been hypothesized that patients with systemic conditions with impaired healing or patients with uncontrolled diabetes may benefit from this adjunctive therapy. One study found greater reduction in HbA1c after 3 months with the association of aPDT with SRP, and IL1-β levels were also lower for the association.[29] Smokers would also represent a target population to be treated with aPDT; however, the adjunctive effect of aPDT has failed to improve clinical parameters in such patients. Also, aPDT associated with SRP did not alter periodontal biofilm composition in a positive way, either when applied in single[30] or multiple sessions.[31] Nevertheless, this is only the beginning of studies in this area of aPDT.

In summary, there is evidence that aPDT, as an adjunct to SRP and when applied in multiple applications, can improve microbiological and immunological outcomes. This application could be of interest in patients with systemic comorbidities. The advent of new LED lights and/or nano-photosensitizers may better target the pathogenic biofilm, possibly leading to more significant improvements and, consequently, further encouraging new studies to assess the use of these technologies.

Probiotics

Current concepts of etiopathogenesis of periodontal diseases consider the existence not only of specific periodontopathogens, but of a synergistic and dysbiotic microbial community.[32] Microbial dysbiosis leads to non-resolving inflammations due to alterations in the host's immunoinflammatory response. Therefore, new therapies seeking to regain the microbiome ecological balance are currently being investigated and appear to be more closely aligned with current concepts aiming to restore a healthy oral microbiome and periodontal health.[33]

In a proof of concept study, Teughels et al.[345] introduced *Streptococcus* species within periodontal pockets after SRP in dogs. This adjunctive therapy reduced the periodontal tissue inflammation and modified the microbial composition of the treated sites, reducing and delaying their recolonization by periodontopathogens. The study introduced the concept of guided recolonization of periodontal pockets by using oral health-compatible bacteria as a treatment strategy for periodontitis. Therefore, host microbiota modulation and direct interaction with the immune system are the basic mechanisms that can explain the beneficial effects of probiotics on periodontal health.

The term "probiotics" was introduced by Lilly and Stillwell in 1965.[35] The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have defined probiotics as "living microorganisms that, when administered in appropriate amounts, confer health benefits to the host".[36] Most of currently used probiotics are lactic acid bacteria of the *Lactobacillus* and *Bifidobacterium* genera, although fungi, species of *Bacillus, Clostridium, Propionibacterium* and Gram-negative bacteria like *Escherichia coli* have also been used as probiotics.[37] It is well documented that lactic acid-producing bacteria have antimicrobial effects on several periodontopathogens.[38-43] A recent *in vitro* study has shown that probiotics alter biofilm

formation and the transcription of *Porphyromonas gingivalis* virulence-associated genes.[44] Probiotic microorganisms provide nutrients, help the host to digest food, and compete for space and nutrients with potential pathogens. Also, they participate in the host's immunoinflammatory response, since they can induce the secretion of antimicrobial peptides or anti-inflammatory molecules through interaction with various cell populations.[45-48] Some clinical and preclinical studies analyzing saliva, gingival crevicular fluid, and gingival biopsies have shown that the ingestion of probiotics can interfere with the levels of several pro- and anti-inflammatory markers.[48-55]

Although the scope of this article is NSPT for periodontitis, probiotics have been mainly evaluated for the treatment of gingivitis, either in experimental gingivitis models[56-59] or in patients with established gingivitis.[60-62] A recent systematic review with meta-analysis[63] including 10 RCTs has summarized the results of using *L. reuteri* as a probiotic. It has shown that, in half of the included studies,[61,63-66] clinical parameters of inflammation significantly improved when compared with the control group, whereas in five studies comparable results in clinical inflammatory parameters were reported between test and control groups.[58,62,67-69] The meta-analysis, however, did not demonstrate a statistically significant difference between probiotic and placebo groups in clinical periodontal inflammatory parameters, although the results are very heterogeneous and prevent a fair evaluation of the results.

In regard to the adjunctive application of probiotics for the treatment of periodontitis, a recent systematic review assessed the impact of probiotics on clinical, microbial, and immunological outcomes when used as adjuncts to NSPT.[70] Ten RCTs were included, with all patients receiving NSPT with probiotics or placebo administration or SRP alone. Both 3- and 12-month data have shown significant benefits in the use of probiotics, with greater magnitude at 12 months in pooled estimates of PPD reductions. The results have shown a significant benefit in probiotics in both PPD and CAL when baseline mean PPD values were ≥ 5 mm. There was no significant difference in periodontal pathogen levels between groups at 3 months. Immunological data were not sufficient for quantitative analysis.

Significant inter-individual human microbiome variability mediated by factors such as age, diet, antibiotic usage, food supplements, underlying medical conditions, and patterns of circadian activity can impact the effects of probiotics.[71,72] In humans, marked person-, strain- and gut region-specific mucosal probiotic colonization patterns clustered individuals into those "permissive" or "resistant" to mucosal probiotic colonization. Importantly, these distinct colonization states had different impacts on probiotics-associated changes in the gut microbial community structure and host transcriptome.[71] Regarding the effects of probiotics on periodontal diseases, it is also mandatory to analyze the influence of the vehicle by which the probiotic strain is administered in its therapeutic potential and oral colonization, as well as its survival in the oral cavity. Additionally, more studies are required to evaluate modes of application, different therapeutic regimens, and the persistence of probiotic microorganisms in the oral cavity after discontinuation of probiotic therapy. This will provide further evidence to support the use of probiotics for NSPT. Meanwhile, this therapeutic alternative is not yet indicated in daily clinical practice.

Prebiotics and synbiotics

Prebiotics are substrates, either naturally present in certain foods or synthetically produced, which are selectively used by beneficial microorganisms colonizing the host. These substrates are intended to multiply and/or become activated metabolically to beneficially alter the composition of the host microbiota in the intestine or elsewhere in the organism.[73,74,75]

The concept of using prebiotics involves three assumptions: a substance (substrate), a mechanism, and a beneficial effect.[73] Oligosaccharide carbohydrates inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), and polydextrose are among the most studied prebiotics.[76] Several health benefits have been attributed to their application: relief of poor digestion of lactose, increased resistance to bacterial infection, and improved immune response.

The main mechanism of action of prebiotics is through the selective stimulation of the growth of beneficial bacteria in the host. Some of these microorganisms have specific enzymes that can hydrolyze prebiotic oligosaccharides, which results in the proliferation of these beneficial bacteria.[77] This selective action prevents changes in the microbial composition associated with diseases, suppressing the growth of pathogenic species.[75,78] Furthermore, prebiotics can offer protection against pathogens through direct interactions.[79] Oligosaccharide derivatives contain sugars that are specific receptors for epithelial cells. Therefore, these receptors become unavailable for the adhesion of pathogenic bacteria, which will lose their ability to colonize and will be eliminated.[80,81]

The fermentation of prebiotics in the large intestine leads to the production of short-chain fatty acids.[82] These molecules increase the intestinal absorption of calcium, reducing intestinal pH, and promote the development of intestinal villi, leading to changes in the intestinal microbiota.[83] In addition, there is evidence that some prebiotics have direct effects on the host immune system, regardless of their effects on resident bacterial populations.[80,84] These effects include stimulation of IL-10 and interferon-γ expression, increased IgA secretion, modulation of inflammatory responses to pathogens, and stabilization of the intestinal mucosal barrier.[80]

The ability of certain prebiotics to increase the growth of resident commensal intestinal bacteria, particularly bifidobacteria and lactobacilli, is well documented.[80,85] It is expected that prebiotics can cause changes in any host microbial ecosystem.[79] There is evidence in the medical literature that prebiotics may be useful in preventing several diseases, like atherosclerosis, osteopenia and/or osteoporosis, and other bone pathologies,[83,86] but only recently has the oral cavity been suggested as a relevant target for the application of this approach.[87] In Periodontics, the first studies identified potential prebiotics that could stimulate beneficial bacteria and suppress the growth of pathogenic species *in vitro*.[75,87] Two compounds, named beta-methyl-D-galactoside and N-acetyl-D-mannosamine, were able to modify two-species biofilm communities to a predominantly beneficial composition.[75] In another experiment, using multispecies biofilms, N-acetyl-D-mannosamine led to a biofilm composition with more than 97% beneficial microorganisms.[87]

The pioneering study to evaluate the effects of a prebiotic on periodontitis was carried out with β-glucans fiber from *Saccharomyces cerevisiae*, administered to rats that underwent diabetes and periodontitis inductions.[81] It demonstrated that prebiotic therapy led to reduction of alveolar bone loss and serum levels of TNF-α, and improved pancreatic β-cell function and intestinal morphology.[81] Another preclinical study, which evaluated the effects of the mannanoligosaccharide prebiotic in animals with experimental periodontitis, demonstrated that the therapy led to a reduction in periodontal destruction and levels of TNF-α and IL-1β, and an increase in TGF-β. Also, it is important to highlight that animals with experimental periodontitis have been shown to present changes in intestinal morphology, pointing to a possible interrelation between oral and intestinal ecosystems.[53,88,89] In the preclinical study with the mannanoligosaccharide prebiotic, the animals treated have shown intestinal morphology more similar to that of animals without disease, which demonstrated the protective role of prebiotics in the intestinal environment under conditions of oral dysbiosis.[88] When the results of this study are compared to other preclinical studies with probiotics, similar beneficial effects are found. However, to date, there are no clinical studies evaluating the effects of prebiotics in the management of periodontitis.

Since oligosaccharides that act as prebiotics can also improve the ability of probiotic strains to adhere to intestinal cells and mucin, it has been suggested that the use of prebiotics with probiotics, that is, synbiotic compounds, may be a potential tool to increase the numbers of probiotic bacteria and the time they remain in the body. This could even reduce the period of administration of the probiotic therapy in some cases.[90,91] A randomized placebo-controlled clinical study evaluated the effects of a synbiotic containing multispecies probiotics and FOS prebiotic as an adjunct to SRP in patients with periodontitis and type 2 diabetes mellitus.[92] The group that received

the synbiotic presented better results than patients in the control group in relation to CAL gain, plaque, bleeding on probing, total antioxidant capacity, glutathione peroxidase, and also in the reduction of IL-1β and malondialdehyde.

In this context, prebiotic or synbiotic therapies can be considered potential adjunct strategies for the prevention and/or treatment of periodontal diseases. The concept of prebiotic was only recently introduced in oral health, and knowledge about its possible effects is still in its "childhood stage" in Dentistry.[87] It is necessary to perform studies to assess the effects of prebiotics and synbiotics on periodontal diseases, as well as on the modulation of oral and intestinal microbiomes, in order to help maintain and enhance the benefits provided by the beneficial microbiota to the host.

Statins

Statins are inhibitors of 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMG-CoA reductase) and act directly in the inhibition of cholesterol biosynthesis.[93] The mechanism of action of statins is through their anti-inflammatory, anticoagulant, and antioxidant effects.[94] In addition, statins inhibit the hepatic synthesis of apolipoprotein B100 and decrease the synthesis and secretion of triglyceride-rich lipoproteins.[95,96] Furthermore, it has been proposed that statins have pleiotropic properties, which leads to improved endothelial cell function and modulation of the inflammatory response.[97] Statins constitute an important group of drugs used in the treatment of several conditions, such as hypercholesterolemia, and are aimed to decrease the risk of developing cardiovascular diseases.[98] There are several types of statins, such as atorvastatin, ezetimibe (usually administered in combination with another drug), fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin.[99]

The application of statins in the treatment of periodontitis has been evaluated by local application in the periodontal pockets. Preclinical studies have evaluated the effects of statins (simvastatin, rosuvastatin, atorvastatin) in animals subjected to periodontitis induction. In general, it was observed that the treatment led to reduction in bone resorption, reductions in pro-inflammatory markers and inflammatory infiltrate, as well as to an increase in anti-inflammatory mediators and antioxidant substances.[100-104]

Clinical investigations have assessed the association of several types of statins with SRP in the treatment of periodontitis.[8,105,106] A recent systematic review with meta-analysis included 12 studies that applied 1.2% atorvastatin, simvastatin, or rosuvastatin gel subgingivally as an adjunct to non-surgical periodontal therapy. Considering intrabony defects, there was an overall mean difference of 2.25 mm in PPD reduction and 2.19 mm in CAL gain at 9 months between the use of statin gels and placebo. Moreover, statin gels provided greater reductions in the mean intrabony/furcation defect depth than placebo at 6 months.[107] However, very high heterogeneity was found, putting into question the efficacy and predictability of statins as adjuncts to SRP. Other systematic reviews with meta-analyses corroborate these findings.[108,109]

Considering the data available so far, it is not possible to conclude which type of statin is the most effective.[107,109] Two studies concluded that rosuvastatin gel is more effective than atorvastatin gel in the reduction of PD in both intrabony and class II furcation defects.[110,111] One study did not find any difference in PD improvements when locally applied atorvastatin and simvastatin gel were compared.[113] Nevertheless, a systematic review with meta-analysis and meta-regression concluded that simvastatin was the only statin to provide significant improvements in all parameters analyzed (PPD reduction, CAL gain, resolution of intrabony defects), when compared with placebo.[108]

The use of statins in the management of periodontitis can be considered a potential adjunctive approach. In addition, statins have low cost, are widely accessible and do not appear to pose risks of complications or allergic/adverse reactions. Nevertheless, it is important to consider that the same research group performed all the clinical trials using locally applied statins until the present moment. Therefore, additional controlled and randomized clinical studies performed by other research centers, including greater numbers of

patients and longer follow-up times, are needed to corroborate the current findings and determine the best protocol (dose, duration, and type of statin) to be used in periodontal treatment.

Pro-resolving mediators

Specialized pro-resolving mediators (SMP) are endogenously biosynthesized chemical mediators released during acute inflammation that are both pro-resolving and anti-inflammatory. Omega-3 polyunsaturated fatty acids (PUFA)—eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in particular—are the precursors of these molecules, namely lipoxins, resolvins, protectins, and maresins.[113,14,115] The mechanisms underlying the mode of action of such mediators comprise a complex cascade of events during the acute phases of inflammation, and these mediators are potent agonists that control their duration and magnitude. In general, they act as receptor agonists, controlling the resolution of the inflammation and promoting healing.[116]

When compared to conventional anti-inflammatory drugs, these synthetically developed molecules may have the advantage of controlling inflammation through positive agonist/receptor mediated cellular signals without eliciting any side-effects. Their application, therefore, aims to enhance the off-signal rather than inhibit the on-signal.[113,114,116]

Evidence for the application of pro-resolving mediators in the treatment of periodontitis is still in preclinical phases,[117] and two SMPs have been investigated in animal models: resolvin E1 (RvE1) and benzo-lipoxin A4 (bLXA4). Their activity on the periodontal tissues in these experimental *in vivo* investigations up to 2017 has been recently summarized in a systematic review.[118] A total of 6 studies[119-124] from a single research group were reported to have applied pro-resolving mediators locally in periodontal defects. Major findings revealed that these mediators lead to bone gain and consequently bone regeneration by reducing the inflammatory cell infiltration and osteoclastic activity. Although these mediators do not have a direct effect on the microbiota, the resulting regulation of the inflammation shifted the composition of the microbiota, probably through changes in the local environment secondary to the inflammation resolution.[118] Between 2017 and 2020, other preclinical studies confirmed previous findings and provided new insights on their biological mechanisms[126-128] In summary, these preclinical studies have shown promising results that need to be confirmed with clinical studies.

Omega-3 and -6

Fatty acids (FA) mainly act as an essential source of energy and as precursors of inflammatory and anti-inflammatory signaling molecules.[128] Long-chain FAs (LCFAs) comprise those with more than 12 carbons and can be sub-grouped into saturated, mono-saturated, or polyunsaturated (PUFAs). The first carbon double bond position denominates n-6 or n-3 PUFAs, or Omega-6 and Omega-3 respectively.[129]

Different chemical routes of Omega-6 and Omega-3 may drive the cellular inflammatory response. Omega-6 (linoleic acid) cascade originates arachidonic acid (ARA).[128] After being incorporated in the phospholipid membrane of mammalians' cells, ARA acts as a substrate to cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 enzymes that originate the so-called eicosanoids. Eicosanoids [prostaglandins (PGs), thromboxanes (Tr), and leukotrienes (LTs)] are responsible for a pro-inflammatory condition and linked to several systemic chronic conditions, such as rheumatoid arthritis, atherosclerotic plaque rupture, critical illness, sepsis,[128,130,131,132] and periodontal diseases. On the other hand, Omega-3 (α-linolenic acid) originates eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids that, in turn, produce resolvins, which possess anti-inflammatory effects, as described above.

The effect of Omega-3 supplements on the treatment of periodontal diseases was first investigated using the experimental gingivitis model, and a tendency to improve the inflammatory condition was reported.[133,134] In regard to periodontitis, cross-sectional data have shown an association between dietary intake of Omega-3 and Omega-6 and periodontal health.[135] Additionally, there is evidence that Omega-3 intake may improve clinical signs of periodontitis

in interventional studies. A systematic review of the use of Omega-3 supplements has concluded that, depending on the duration and dosage of the supplementation, periodontal disease progression might be reduced. Higher plasma concentrations of EPA and DHA correlated with CAL gain, less severe periodontitis, and a higher number of teeth.[136] More recently, one RCT[138] and a meta-analysis[138] concluded that the intake of Omega-3 as an adjunct to periodontal therapy reduced pocket depth and increased CAL gain.

Altogether, these results show that Omega-3 has the potential benefit of reducing periodontal disease progression and improving NSPT outcomes. However, clinical studies are still needed and should evaluate the ideal dosage of Omega-3, duration and origin of supplementation (diet or capsules), side effects, and a better description of periodontal outcomes.

Ozone therapy

Ozone, also known as triatomic oxygen and trioxygen, is a highly oxidative gas found in the atmosphere. Based on its alleged healing and antimicrobial properties, it has been used in Dentistry for various indications, such as treating early caries, ulcerations, herpetic lesions, and disinfecting root canals. Additionally, the application of ozone as an adjunctive treatment has been suggested as a new strategy in the management of periodontitis.[139] Its *in vitro* mechanism of action is based on the production of free radicals leading to an acute and controlled oxidative stress in human cells, resulting in the modulation of antioxidant response, oxygen metabolism, and cellular energy, which will foster positive biological responses.[140] Moreover, ozone's high solubility and instability warrant its entire absorption and prevent toxicity in the tissues.[141] Its effects would not lead do antimicrobial resistance. In addition to the ozone action in bacteria, viruses, and fungi, there is *in vitro* evidence of action against biofilms.[142,143]

Ozone therapy has been administrated in different ways, but applications in gas and aqueous formulations have been the most common. There are studies that tested ozone as ozonized/ozonated water,[139,144] gaseous ozone,[145,146] and ozonized oil.[147] Despite its promising *in vitro* evidence, the clinical application of ozone in the treatment of periodontal diseases has not achieved a minimum level of efficacy. In general, most clinical trials did not show statistically significant differences in plaque index, gingival index, PPD, and CAL when any kind of ozone therapy was used as an adjunct to NSPT as compared to NSPT alone. In addition, the impact of ozone application on microbiologic and biochemical outcomes was limited.[144,145,148] These limited outcomes may be due to its high volatility and instability, resulting in low substantivity and activity when applied clinically. Additionally, the low quality of studies may have also impacted the clinical outcomes. These findings were summarized in a recent systematic review with meta-analysis of randomized clinical trials, which concluded that there is no scientific support for the use of ozone therapy for NSPT.[149]

Epigenetic therapy

Epigenetics has been defined as the change in gene expression that is not encoded in the DNA sequence, and includes chemical changes in the DNA and its related proteins (called histones), which leads to the remodeling of chromatin and the resulting activation/inactivation of the targeted gene.[150] Epigenetic mechanisms dynamically regulate gene functions (activation or inactivation) and can change in response to different stimuli.[151] For example, systemic (smoking and diabetes mellitus) and local influences (bacteria and their virulence factors) can lead to changes in the epigenetic status of cells. These events may induce changes in immunological responses and thus contribute to the pathogenesis of chronic inflammatory diseases, such as periodontitis.[152] In spite of the early stage of studies about epigenetics in periodontitis, the recent accumulation of evidence has suggested the possible etiopathogenic role of epigenetic changes in the initiation and progression of periodontitis. It has been reported that the levels of multiple cytokine-encoding genes and inflammatory response-related genes may be regulated epigenetically.[153]

Although several epigenetic changes have been described in periodontitis, there is little information on the resolution of these changes after periodontal therapy. In addition, the possible effect of adjunctive treatments using epigenetic drugs (known as Epidrugs) is currently unknown. Only *in vitro* and preclinical *in vivo* studies are available assessing the effect of these molecules on gingival tissues.[154,155,156]

DNA methylation

DNA methylation is a regular epigenetic modification in nuclear cells, characterized by the addition of methyl groups in cytosines within cytosine-guanosine (CpG) dinucleotides, regulated by different DNA methyltransferases. DNA methylation related to disease can occur by hypermethylation or hypomethylation as a result of elevating or suppressing the expression of specific genes.[157] Several studies have investigated DNA methylation of inflammatory cytokines such as IL-6, -8 and TNF-α in different forms of periodontitis.[156,158-161] In addition, other inflammation-related genes were analyzed in periodontitis, such as Toll-like receptors (TLRs)[163] and Interferon gamma (IFN-γ).[163]

It is noteworthy that studies have not tested interventions aiming to change DNA methylation; however, it has been demonstrated that periodontal treatment may alter DNA methylation. For instance, Asa'ad et al.[156] demonstrated that periodontal therapy was able to redefine the DNA methylation status of the inflammatory gene for COX-2 in patients with periodontitis, even though DNA methylation levels for TNF-α and IFN-γ remained similar in the experimental groups. Andia et al.[164] found no change in methylation levels between groups after three months of periodontal therapy. However, variations in methylation status between groups were not assessed at the beginning of the study. As a whole, these findings suggest that periodontal therapy can influence epigenetic changes.

Histone acetylation

Histones can be acetylated or deacetylated on the tails of amino acids in chromatin. While histone acetylation (addition of acetyl groups) is regulated by histone acetyltransferases (HATs), histone deacetylation (removal of acetyl groups) is regulated by histone deacetylases (HDACs). Unlike DNA methylation, which is associated with gene expression, histone acetylation is associated with gene transcription (activation or repression).[165]

Different histone deacetylase inhibitors have been investigated as a potential treatment for bone-related diseases, since they epigenetically regulate the expression of genes associated with osteoclast differentiation, maturation, and activity. In this sense, some histone deacetylase inhibitors have already been approved by the US Food and Drug Administration (FDA), and evidence across therapeutic modalities has shown good results in the treatment of different cancers.[112]

In Periodontics, some promising results in preclinical research have been demonstrated. The early results seem to point to an increase in histone acetylation in induced-periodontitis models. Cantley et al.[166] reported that 1179.4b, a histone deacetylase inhibitor, suppresses bone loss in the induced-periodontitis model by oral inoculation with *Porphyromonas gingivalis*. However, the analysis revealed that 1179.4b reduced bone loss despite having no effect on gingival inflammation. The authors suggested HDACi as a potential therapeutic option for periodontitis in the future. Moreover, HDACi MS-275 has been shown to have an effect on osteoclastogenesis *in vitro* and *in vivo*.[167] MS-275 inhibited osteoclast differentiation of bone marrow-derived macrophages by suppressing RANKL-induced expression, suggesting a potential therapeutic value of HDACi for bone disorders associated with increased bone resorption. Bromodomain and extra-terminal protein (BET) inhibitors have also been studied in alveolar bone loss. BET proteins are regulatory molecules of chromatin that bind to acetylated histones. JQ1, a BET inhibitor, was studied by Meng et al.[168] in an experimental periodontitis model, which demonstrated that JQ1 suppressed the transcription of inflammatory cytokines activated by LPS and osteoclast markers promoted by RANKL.

Although the results with histone acetylation inhibitors are promising, there are numerous types of cells present at the site of bone destruction, including inflammatory cells. Thus, inhibition of histone

deacetylases might have the opposite effect on those cells, resulting in increased bone resorption or raised inflammatory reaction. Therefore, further research is required to prove the combined effect of histone deacetylase inhibitors in the treatment of localized bone destruction.

MicroRNAs (miRNAs)

miRNAs are also epigenetic mechanisms that regulate gene expression through post-transcriptional modifications. These molecules describe a group of small non-coding RNAs.[169] MicroRNAs play critical roles in inflammatory responses by modulating cellular processes, such as cell growth, apoptosis, and differentiation. Given this fact, miRNAs are linked to the development of diseases such as rheumatoid arthritis and cancer.[170] In the last years, miRNAs have been investigated in bone-related diseases and bone-remodeling processes due to their importance in osteoclastogenesis, osteogenesis, and osteoclast/osteoblast differentiation. In addition to that, miRNAs have been shown to play a relevant function in the differentiation of periodontal ligament stem cells. An *in vitro* study demonstrated that one type of miRNA stimulated osteogenic differentiation of periodontal ligament stem cells.[171]

miRNAs have also been involved in the control of TLR reaction to bacteria. A recent study[172] reported that infection by three important periodontal pathogens (*Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*) increased the expression levels of miR-146a in mice with experimental periodontitis. The authors also reported that cultured cells simultaneously stimulated with all three periodontal pathogens showed that TNF-α imbalance may be signal for this expression of miRNA.

In summary, our current understanding of the role of epigenetic factors in periodontitis development is still insufficient. However, emerging studies highlight that DNA methylation, histone modifications, and miRNAs are modified during the oral mucosa response to bacteria, environmental factors, and inflammatory processes. Future translational studies are needed to further explore and understand these discoveries and enable the development of highly desirable therapies for periodontal disease based on the regulation of the host immune response.

Concluding remarks

This review addressed the available literature for nine therapeutic approaches with potential benefits over standard NSPT. At the moment, there is a lack of solid and sound evidence to recommend most of these new tendencies in NSPT in daily practice. SRP alone reduces bleeding in up to 63% of the sites and results in 74% of closed pockets (PPD ≤ 4mm and no BOP).[173] Clinical outcomes observed with new therapies approached in this review have not demonstrated minimally acceptable consistency and benefits over those achieved with SRP alone. Consequently, SRP should still be considered the gold-standard treatment for periodontitis. Importantly, evidence is growing, and some of these new tendencies have great potential to improve NSPT outcomes. Future RCTs applying comparable estimates of clinical outcomes are encouraged in order to assist clinicians and patients in their clinical decision about alternative treatments.

References

1. Sanz M, Herrera D, Kebschull M, Chapple I, Jepsen S, Beglundh T, et al. Treatment of stage I-III periodontitis-The EFP S3 level clinical practice guideline. J Clin Periodontol. 2020 Jul;47(S22 Suppl 22):4-60. https://doi.org/10.1111/jcpe.13290
2. Bouchard P, Carra MC, Boillot A, Mora F, Rangé H. Risk factors in periodontology: a conceptual framework. J Clin Periodontol. 2017 Feb;44(2):125-31. https://doi.org/10.1111/jcpe.12650
3. Weijden F, Slot DE. Oral hygiene in the prevention of periodontal diseases: the evidence. Periodontol 2000. 2011 Feb;55(1):104-23. https://doi.org/10.1111/j.1600-0757.2009.00337.x
4. Loos BG, Needleman I. Endpoints of active periodontal therapy. J Clin Periodontol. 2020 Jul;47(S22 Suppl 22):61-71. https://doi.org/10.1111/jcpe.13253

5. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: framework and proposal of a new classification and case definition. J Periodontol. 2018 Jun;89 Suppl 1:S159-72. https://doi.org/10.1002/JPER.18-0006
6. Trombelli L, Franceschetti G, Farina R. Effect of professional mechanical plaque removal performed on a long-term, routine basis in the secondary prevention of periodontitis: a systematic review. J Clin Periodontol. 2015 Apr;42 Suppl 16:S221-36. https://doi.org/10.1111/jcpe.12339
7. Amerio E, Mainas G, Petrova D, Giner Tarrida L, Nart J, Monje A. Compliance with supportive periodontal/peri-implant therapy: a systematic review. J Clin Periodontol. 2020 Jan;47(1):81-100. https://doi.org/10.1111/jcpe.13204
8. Angst PD, Stadler AF, Oppermann RV, Gomes SC. Microbiological outcomes from different periodontal maintenance interventions: a systematic review. Braz Oral Res. 2017 May;31(0):e33. https://doi.org/10.1590/1807-3107bor-2017vol310033
9. Gellin RG, Miller MC, Javed T, Engler WO, Mishkin DJ. The effectiveness of the Titan-S sonic scaler versus curettes in the removal of subgingival calculus: a human surgical evaluation. J Periodontol. 1986 Nov;57(11):672-80. https://doi.org/10.1902/jop.1986.57.11.672
10. Mombelli A. Microbial colonization of the periodontal pocket and its significance for periodontal therapy. Periodontol 2000. 2018 Feb;76(1):85-96. https://doi.org/10.1111/prd.12147
11. Dobson J, Wilson M. Sensitization of oral bacteria in biofilms to killing by light from a low-power laser. Arch Oral Biol. 1992 Nov;37(11):883-7. https://doi.org/10.1016/0003-9969(92)90058-G
12. Chan Y, Lai CH. Bactericidal effects of different laser wavelengths on periodontopathic germs in photodynamic therapy. Lasers Med Sci. 2003;18(1):51-5. https://doi.org/10.1007/s10103-002-0243-5
13. Matevski D, Weersink R, Tenenbaum HC, Wilson B, Ellen RP, Lépine G. Lethal photosensitization of periodontal pathogens by a red-filtered Xenon lamp in vitro. J Periodontal Res. 2003 Aug;38(4):428-35. https://doi.org/10.1034/j.1600-0765.2003.00673.x
14. Braham P, Herron C, Street C, Darveau R. Antimicrobial photodynamic therapy may promote periodontal healing through multiple mechanisms. J Periodontol. 2009 Nov;80(11):1790-8. https://doi.org/10.1902/jop.2009.090214
15. Hosseinpour S, Tunér J, Fekrazad R. Photobiomodulation in oral surgery: a review. Photobiomodul Photomed Laser Surg. 2019 Dec;37(12):814-25. https://doi.org/10.1089/photob.2019.4712
16. Wainwright M. Photodynamic antimicrobial chemotherapy (PACT). J Antimicrob Chemother. 1998 Jul;42(1):13-28. https://doi.org/10.1093/jac/42.1.13
17. Oliveira RR, Schwartz-Filho HO, Novaes Junior AB, Taba Junior M. Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: a preliminary randomized controlled clinical study. J Periodontol. 2007 Jun;78(6):965-73. https://doi.org/10.1902/jop.2007.060494
18. Oliveira RR, Schwartz-Filho HO, Novaes AB, Garlet GP, Souza RF, Taba M, et al. Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: cytokine profile in gingival crevicular fluid, preliminary results. J Periodontol. 2009 Jan;80(1):98-105. https://doi.org/10.1902/jop.2009.070465
19. Novaes Junior AB, Schwartz-Filho HO, Oliveira RR, Feres M, Sato S, Figueiredo LC. Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: microbiological profile. Lasers Med Sci. 2012 Mar;27(2):389-95. https://doi.org/10.1007/s10103-011-0901-6
20. Sarkar S, Wilson M. Lethal photosensitization of bacteria in subgingival plaque from patients with chronic periodontitis. J Periodontal Res. 1993 May;28(3):204-10. https://doi.org/10.1111/j.1600-0765.1993.tb01070.x
21. Bhatti M, MacRobert A, Meghji S, Henderson B, Wilson M. Effect of dosimetric and physiological factors on the lethal photosensitization of Porphyromonas gingivalis in vitro. Photochem Photobiol. 1997 Jun;65(6):1026-31. https://doi.org/10.1111/j.1751-1097.1997.tb07964.x
22. Kömerik N, Nakanishi H, MacRobert AJ, Henderson B, Speight P, Wilson M. In vivo killing of Porphyromonas gingivalis by toluidine blue-mediated photosensitization in an animal model. Antimicrob Agents Chemother. 2003 Mar;47(3):932-40. https://doi.org/10.1128/AAC.47.3.932-940.2003
23. Kömerik N, Wilson M, Poole S. The effect of photodynamic action on two virulence factors of gram-negative bacteria. Photochem Photobiol. 2000 Nov;72(5):676-80. https://doi.org/10.1562/0031-8655(2000)072<0676:TEOPAO>2.0.CO;2
24. Qin YL, Luan XL, Bi LJ, Sheng YQ, Zhou CN, Zhang ZG. Comparison of toluidine blue-mediated photodynamic therapy and conventional scaling treatment for periodontitis in rats. J Periodontal Res. 2008 Apr;43(2):162-7. https://doi.org/10.1111/j.1600-0765.2007.01007.x
25. Lulic M, Leiggener Görög I, Salvi GE, Ramseier CA, Mattheos N, Lang NP. One-year outcomes of repeated adjunctive photodynamic therapy during periodontal maintenance: a proof-of-principle randomized-controlled clinical trial. J Clin Periodontol. 2009 Aug;36(8):661-6. https://doi.org/10.1111/j.1600-051X.2009.01432.x
26. Aykol G, Baser U, Maden I, Kazak Z, Onan U, Tanrikulu-Kucuk S, et al. The effect of low-level laser therapy as an adjunct to non-surgical periodontal treatment. J Periodontol. 2011 Mar;82(3):481-8. https://doi.org/10.1902/jop.2010.100195

27. Chambrone L, Wang HL, Romanos GE. Antimicrobial photodynamic therapy for the treatment of periodontitis and peri-implantitis: an American Academy of Periodontology best evidence review. J Periodontol. 2018 07;89(7):783-803. https://doi.org/10.1902/jop.2017.170172
28. Moreira AL, Novaes Junior AB, Grisi MF, Taba Junior M, Souza SL, Palioto DB, et al. Antimicrobial photodynamic therapy as an adjunct to non-surgical treatment of aggressive periodontitis: a split-mouth randomized controlled trial. J Periodontol. 2015 Mar;86(3):376-86. https://doi.org/10.1902/jop.2014.140392
29. Ramos UD, Ayub LG, Reino DM, Grisi MF, Taba Junior M, Souza SL, et al. Antimicrobial photodynamic therapy as an alternative to systemic antibiotics: results from a double-blind, randomized, placebo-controlled, clinical study on type 2 diabetics. J Clin Periodontol. 2016 Feb;43(2):147-55. https://doi.org/10.1111/jcpe.12498
30. Queiroz AC, Suaid FA, Andrade PF, Novaes Junior AB, Taba M Jr, Palioto DB, et al. Antimicrobial photodynamic therapy associated to nonsurgical periodontal treatment in smokers: microbiological results. J Photochem Photobiol B. 2014 Dec;141:170-5. https://doi.org/10.1016/j.jphotobiol.2014.10.017
31. Soares MSM, Borges CA, Invernici MM, Frantz FG, Figueiredo LC, Souza SL, et al. Antimicrobial photodynamic therapy as adjunct to non-surgical periodontal treatment in smokers: a randomized clinical trial. Clin Oral Investig. 2019 Aug;23(8):3173-82. https://doi.org/10.1007/s00784-018-2740-3
32. Hajishengallis G, Lamont RJ. Beyond the red complex and into more complexity: the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. Mol Oral Microbiol. 2012 Dec;27(6):409-19. https://doi.org/10.1111/j.2041-1014.2012.00663.x
33. Willis JR, Gabaldón T. The human oral microbiome in health and disease: from sequences to ecosystems. Microorganisms. 2020 Feb;8(2):E308. https://doi.org/10.3390/microorganisms8020308
34. Teughels W, Newman MG, Coucke W, Haffajee AD, Van Der Mei HC, Haake SK, et al. Guiding periodontal pocket recolonization: a proof of concept. J Dent Res. 2007 Nov;86(11):1078-82. https://doi.org/10.1177/154405910708601111
35. Lilly DM, Stillwell RH. Probiotics: growth-promoting factors produced by microorganisms. Science. 1965 Feb;147(3659):747-8. https://doi.org/10.1126/science.147.3659.74737
36. Food and Agriculture Organization of the United Nations, World Health Organization. Probiotics in food, health and nutritional properties and guidelines for evaluation. Rome: Food and Agriculture Organization of the United Nations; 2006. (FAO food and nutrition report, v 0254-4725).
37. Foligné B, Daniel C, Pot B. Probiotics from research to market: the possibilities, risks and challenges. Curr Opin Microbiol. 2013 Jun;16(3):284-92. https://doi.org/10.1016/j.mib.2013.06.008
38. Grenier D. Antagonistic effect of oral bacteria towards *Treponema denticola*. J Clin Microbiol. 1996 May;34(5):1249-52. https://doi.org/10.1128/JCM.34.5.1249-1252.1996
39. Teanpaisan R, Piwat S, Dahlén G. Inhibitory effect of oral Lactobacillus against oral pathogens. Lett Appl Microbiol. 2011 Oct;53(4):452-9. https://doi.org/10.1111/j.1472-765X.2011.03132.x
40. Zhao JJ, Feng XP, Zhang XL, Le KY. Effect of *Porphyromonas gingivalis* and *Lactobacillus acidophilus* on secretion of IL1B, IL6, and IL8 by gingival epithelial cells. Inflammation. 2012 Aug;35(4):1330-7. https://doi.org/10.1007/s10753-012-9446-5
41. Nissen L, Sgorbati B, Biavati B, Belibasakis GN. *Lactobacillus salivarius* and *L. gasseri* down-regulate *Aggregatibacter actinomycetemcomitans* exotoxins expression. Ann Microbiol. 2014;64(2):611-7. https://doi.org/10.1007/s13213-013-0694-x
42. Khalaf H, Nakka SS, Sandén C, Svärd A, Hultenby K, Scherbak N, et al. Antibacterial effects of Lactobacillus and bacteriocin PLNC8 αβ on the periodontal pathogen *Porphyromonas gingivalis*. BMC Microbiol. 2016 Aug;16(1):188. https://doi.org/10.1186/s12866-016-0810-8
43. Elavarasu S, Suthanthiran T, Thangavelu A, Kanagaraj SS, Mohandas L, Sekar S. Evaluation of efficacy of probiotic (BIFILAC) on *Porphyromonas gingivalis*: *in vitro* study. J Pharm Bioallied Sci. 2016 Oct;8 Suppl 1:S45-7. https://doi.org/10.4103/0975-7406.191966
44. Ishikawa KH, Mita D, Kawamoto D, Nicoli JR, Albuquerque-Souza E, Simionato MRL, et al. Probiotics alter biofilm formation and the transcription of Porphyromonas gingivalis virulence-associated genes. J Oral Microbiol. 2020 Aug;12(1):1805553. https://doi.org/10.1080/20002297.2020.1805553
45. Teitelbaum JE, Walker WA. Nutritional impact of pre- and probiotics as protective gastrointestinal organisms. Annu Rev Nutr. 2002;22(1):107-38. https://doi.org/10.1146/annurev.nutr.22.110901.145412
46. Sartor RB. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. Gastroenterology. 2004 May;126(6):1620-33. https://doi.org/10.1053/j.gastro.2004.03.024
47. Patel RM, Denning PW. Therapeutic use of prebiotics, probiotics, and postbiotics to prevent necrotizing enterocolitis: what is the current evidence? Clin Perinatol. 2013 Mar;40(1):11-25. https://doi.org/10.1016/j.clp.2012.12.002
48. Vuotto C, Longo F, Donelli G. Probiotics to counteract biofilm-associated infections: promising and conflicting data. Int J Oral Sci. 2014 Dec;6(4):189-94. https://doi.org/10.1038/ijos.2014.52

49. Riccia DN, Bizzini F, Perilli MG, Polimeni A, Trinchieri V, Amicosante G, et al. Anti-inflammatory effects of *Lactobacillus brevis* (CD2) on periodontal disease. Oral Dis. 2007 Jul;13(4):376-85. https://doi.org/10.1111/j.1601-0825.2006.01291.x
50. Shimauchi H, Mayanagi G, Nakaya S, Minamibuchi M, Ito Y, Yamaki K, et al. Improvement of periodontal condition by probiotics with *Lactobacillus salivarius* WB21: a randomized, double-blind, placebo-controlled study. J Clin Periodontol. 2008 Oct;35(10):897-905. https://doi.org/10.1111/j.1600-051X.2008.01306.x
51. Szkaradkiewicz AK, Stopa J, Karpiński TM. Effect of oral administration involving a probiotic strain of *Lactobacillus reuteri* on pro-inflammatory cytokine response in patients with chronic periodontitis. Arch Immunol Ther Exp (Warsz). 2014 Dec;62(6):495-500. https://doi.org/10.1007/s00005-014-0277-y
52. Maekawa T, Hajishengallis G. Topical treatment with probiotic *Lactobacillus brevis* CD2 inhibits experimental periodontal inflammation and bone loss. J Periodontal Res. 2014 Dec;49(6):785-91. https://doi.org/10.1111/jre.12164
53. Foureaux RC, Messora MR, Oliveira LF, Napimoga MH, Pereira AN, Ferreira MS, et al. Effects of probiotic therapy on metabolic and inflammatory parameters of rats with ligature-induced periodontitis associated with restraint stress. J Periodontol. 2014 Jul;85(7):975-83. https://doi.org/10.1902/jop.2013.130356
54. Messora MR, Pereira LJ, Foureaux R, Oliveira LF, Sordi CG, Alves AJ, et al. Favourable effects of *Bacillus subtilis* and *Bacillus licheniformis* on experimental periodontitis in rats. Arch Oral Biol. 2016 Jun;66:108-19. https://doi.org/10.1016/j.archoralbio.2016.02.014
55. Oliveira LF, Salvador SL, Silva PH, Furlaneto FA, Figueiredo L, Casarin R, et al. Benefits of *Bifidobacterium animalis* subsp. lactis Probiotic in Experimental Periodontitis. J Periodontol. 2017 Feb;88(2):197-208. https://doi.org/10.1902/jop.2016.160217
56. Staab B, Eick S, Knöfler G, Jentsch H. The influence of a probiotic milk drink on the development of gingivitis: a pilot study. J Clin Periodontol. 2009 Oct;36(10):850-6. https://doi.org/10.1111/j.1600-051X.2009.01459.x
57. Lee JK, Kim SJ, Ko SH, Ouwehand AC, Ma DS. Modulation of the host response by probiotic *Lactobacillus brevis* CD2 in experimental gingivitis. Oral Dis. 2015 Sep;21(6):705-12. https://doi.org/10.1111/odi.12332
58. Hallström H, Lindgren S, Yucel-Lindberg T, Dahlén G, Renvert S, Twetman S. Effect of probiotic lozenges on inflammatory reactions and oral biofilm during experimental gingivitis. Acta Odontol Scand. 2013 May-Jul;71(3-4):828-33. https://doi.org/10.3109/00016357.2012.734406
59. Slawik S, Staufenbiel I, Schilke R, Nicksch S, Weinspach K, Stiesch M, et al. Probiotics affect the clinical inflammatory parameters of experimental gingivitis in humans. Eur J Clin Nutr. 2011 Jul;65(7):857-63. https://doi.org/10.1038/ejcn.2011.45
60. Twetman S, Derawi B, Keller M, Ekstrand K, Yucel-Lindberg T, Stecksen-Blicks C. Short-term effect of chewing gums containing probiotic *Lactobacillus reuteri* on the levels of inflammatory mediators in gingival crevicular fluid. Acta Odontol Scand. 2009;67(1):19-24. https://doi.org/10.1080/00016350802516170
61. Krasse P, Carlsson B, Dahl C, Paulsson A, Nilsson A, Sinkiewicz G. Decreased gum bleeding and reduced gingivitis by the *probiotic Lactobacillus reuteri*. Swed Dent J. 2006;30(2):55-60.
62. Iniesta M, Herrera D, Montero E, Zurbriggen M, Matos AR, Marín MJ, et al. Probiotic effects of orally administered Lactobacillus reuteri-containing tablets on the subgingival and salivary microbiota in patients with gingivitis: a randomized clinical trial. J Clin Periodontol. 2012 Aug;39(8):736-44. https://doi.org/10.1111/j.1600-051X.2012.01914.x
63. Akram Z, Shafqat SS, Aati S, Kujan O, Fawzy A. Clinical efficacy of probiotics in the treatment of gingivitis: a systematic review and meta-analysis. Aust Dent J. 2020 Mar;65(1):12-20. https://doi.org/10.1111/adj.12733
64. Sabatini S, Lauritano D, Candotto V, Silvestre FJ, Nardi GM. Oral probiotics in the management of gingivitis in diabetic patients: a double blinded randomized controlled study. J Biol Regul Homeost Agents. 2017 Apr-Jun;31(2 Suppl 1):197-202.
65. Kuru BE, Laleman I, Yalnızoğlu T, Kuru L, Teughels W. The Influence of a *Bifidobacterium animalis* probiotic on gingival health: a randomized controlled clinical trial. J Periodontol. 2017 Nov;88(11):1115-23. https://doi.org/10.1902/jop.2017.170213
66. Schlagenhauf U, Jakob L, Eigenthaler M, Segerer S, Jockel-Schneider Y, Rehn M. Regular consumption of *Lactobacillus reuteri*-containing lozenges reduces pregnancy gingivitis: an RCT. J Clin Periodontol. 2016 Nov;43(11):948-54. https://doi.org/10.1111/jcpe.12606
67. Montero E, Iniesta M, Rodrigo M, Marín MJ, Figuero E, Herrera D, et al. Clinical and microbiological effects of the adjunctive use of probiotics in the treatment of gingivitis: a randomized controlled clinical trial. J Clin Periodontol. 2017 Jul;44(7):708-16. https://doi.org/10.1111/jcpe.12752
68. Alkaya B, Laleman I, Keceli S, Ozcelik O, Cenk Haytac M, Teughels W. Clinical effects of probiotics containing Bacillus species on gingivitis: a pilot randomized controlled trial. J Periodontal Res. 2017 Jun;52(3):497-504. https://doi.org/10.1111/jre.12415
69. Keller MK, Brandsborg E, Holmstrøm K, Twetman S. Effect of tablets containing probiotic candidate strains on gingival inflammation and composition of the salivary microbiome: a randomised controlled trial. Benef Microbes. 2018 Apr;9(3):487-94. https://doi.org/10.3920/BM2017.0104
70. Ho SN, Acharya A, Sidharthan S, Li KY, Leung WK, McGrath C, et al. A systematic review and meta-analysis of clinical, immunological, and microbiological shift in periodontitis after nonsurgical periodontal therapy with adjunctive use of probiotics. J Evid Based Dent Pract. 2020 Mar;20(1):101397. https://doi.org/10.1016/j.jebdp.2020.101397

71. Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S, et al. Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. Cell. 2018 Sep;174(6):1388-1405.e21. https://doi.org/10.1016/j.cell.2018.08.041
72. Zmora N, Zeevi D, Korem T, Segal E, Elinav E. Taking it personally: personalized utilization of the human microbiome in health and disease. Cell Host Microbe. 2016 Jan;19(1):12-20. https://doi.org/10.1016/j.chom.2015.12.016
73. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol. 2017 Aug;14(8):491-502. https://doi.org/10.1038/nrgastro.2017.75
74. Martinez RC, Bedani R, Saad SM. Scientific evidence for health effects attributed to the consumption of probiotics and prebiotics: an update for current perspectives and future challenges. Br J Nutr. 2015 Dec;114(12):1993-2015. https://doi.org/10.1017/S0007114515003864
75. Slomka V, Hernandez-Sanabria E, Herrero ER, Zaidel L, Bernaerts K, Boon N, et al. Nutritional stimulation of commensal oral bacteria suppresses pathogens: the prebiotic concept. J Clin Periodontol. 2017 Apr;44(4):344-52. https://doi.org/10.1111/jcpe.12700
76. Shokryazdan P, Faseleh Jahromi M, Navidshad B, Liang JB. Effects of prebiotics on immune system and cytokine expression. Med Microbiol Immunol (Berl). 2017 Feb;206(1):1-9. https://doi.org/10.1007/s00430-016-0481-y
77. Looijer-van Langen MA, Dieleman LA. Prebiotics in chronic intestinal inflammation. Inflamm Bowel Dis. 2009 Mar;15(3):454-62. https://doi.org/10.1002/ibd.20737
78. Marsh PD, Moter A, Devine DA. Dental plaque biofilms: communities, conflict and control. Periodontol 2000. 2011 Feb;55(1):16-35. https://doi.org/10.1111/j.1600-0757.2009.00339.x
79. Monteagudo-Mera A, Rastall RA, Gibson GR, Charalampopoulos D, Chatzifragkou A. Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. Appl Microbiol Biotechnol. 2019 Aug;103(16):6463-72. https://doi.org/10.1007/s00253-019-09978-7
80. Devine DA, Marsh PD. Prospects for the development of probiotics and prebiotics for oral applications. J Oral Microbiol. 2009 May;1(1):1949. https://doi.org/10.3402/jom.v1i0.1949
81. Silva LP, Nörnberg JL. Prebióticos na nutrição de não ruminantes. Cienc Rural. 2003;33(5):983-90. https://doi.org/10.1590/S0103-84782003000500029
82. Weaver CM. Diet, gut microbiome, and bone health. Curr Osteoporos Rep. 2015 Apr;13(2):125-30. https://doi.org/10.1007/s11914-015-0257-0
83. Locantore P, Del Gatto V, Gelli S, Paragliola RM, Pontecorvi A. The Interplay between immune system and microbiota in osteoporosis. Mediators Inflamm. 2020 Feb;2020:3686749. https://doi.org/10.1155/2020/3686749
84. Langlands SJ, Hopkins MJ, Coleman N, Cummings JH. Prebiotic carbohydrates modify the mucosa associated microflora of the human large bowel. Gut. 2004 Nov;53(11):1610-6. https://doi.org/10.1136/gut.2003.037580
85. Gibson GR, McCartney AL, Rastall RA. Prebiotics and resistance to gastrointestinal infections. Br J Nutr. 2005 Apr;93(S1 Suppl 1):S31-4. https://doi.org/10.1079/BJN20041343
86. Tiihonen K, Suomalainen T, Tynkkynen S, Rautonen N. Effect of prebiotic supplementation on a probiotic bacteria mixture: comparison between a rat model and clinical trials. Br J Nutr. 2008 Apr;99(4):826-31. https://doi.org/10.1017/S0007114507825141
87. Slomka V, Herrero ER, Boon N, Bernaerts K, Trivedi HM, Daep C, et al. Oral prebiotics and the influence of environmental conditions in vitro. J Periodontol. 2018 Jun;89(6):708-17. https://doi.org/10.1002/JPER.17-0437
88. Levi YL, Novais GS, Dias RB, Andraus RA, Messora MR, Bracco Neto H, et al. Effects of the prebiotic mannan oligosaccharide on the experimental periodontitis in rats. J Clin Periodontol. 2018 Sep;45(9):1078-89. https://doi.org/10.1111/jcpe.12987
89. Messora MR, Oliveira LF, Foureaux RC, Taba Junior M, Zangerônimo MG, Furlaneto FA, et al. Probiotic therapy reduces periodontal tissue destruction and improves the intestinal morphology in rats with ligature-induced periodontitis. J Periodontol. 2013 Dec;84(12):1818-26. https://doi.org/10.1902/jop.2013.120644
90. Celebioglu HU, Olesen SV, Prehn K, Lahtinen SJ, Brix S, Hachem MA, et al. Mucin- and carbohydrate-stimulated adhesion and subproteome changes of the probiotic bacterium Lactobacillus acidophilus NCFM. J Proteomics. 2017 Jun;163:102-10. https://doi.org/10.1016/j.jprot.2017.05.015
91. Liu Z, Liu F, Wang W, Sun C, Gao D, Ma J, et al. Study of the alleviation effects of a combination of Lactobacillus rhamnosus and inulin on mice with colitis. Food Funct. 2020 May;11(5):3823-37. https://doi.org/10.1039/C9FO02992C
92. Bazyar H, Maghsoumi-Norouzabad L, Yarahmadi M, Gholinezhad H, Moradi L, Salehi P, et al. The impacts of synbiotic supplementation on periodontal indices and biomarkers of oxidative stress in type 2 Diabetes Mellitus patients with chronic periodontitis under non-surgical periodontal therapy. a double-blind, placebo-controlled trial. Diabetes Metab Syndr Obes. 2020 Jan;13:19-29. https://doi.org/10.2147/DMSO.S230060
93. Tobert JA. New developments in lipid-lowering therapy: the role of inhibitors of hydroxymethylglutaryl-coenzyme A reductase. Circulation. 1987 Sep;76(3):534-8. https://doi.org/10.1161/01.CIR.76.3.534

94. Liao JK. Effects of statins on 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition beyond low-density lipoprotein cholesterol. Am J Cardiol. 2005 Sep;96(5 5A):24F-33F. https://doi.org/10.1016/j.amjcard.2005.06.009
95. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. Fundam Clin Pharmacol. 2005 Feb;19(1):117-25. https://doi.org/10.1111/j.1472-8206.2004.00299.x
96. Maron DJ, Fazio S, Linton MF. Current perspectives on statins. Circulation. 2000 Jan;101(2):207-13. https://doi.org/10.1161/01.CIR.101.2.207
97. Liao JK. Beyond lipid lowering: the role of statins in vascular protection. Int J Cardiol. 2002 Nov;86(1):5-18. https://doi.org/10.1016/S0167-5273(02)00195-X
98. Xie W, Huang H, Xiao S, Yang X, Zhang Z. Effect of statin use on cardiovascular events and all-cause mortality in immune-mediated inflammatory diseases: a systematic review and meta-analysis involving 148,722 participants. Pharmacol Res. 2020 Oct;160:105057. https://doi.org/10.1016/j.phrs.2020.105057
99. LiverTox. Clinical and research information on drug-induced liver injury. Bethesda: National Institute of Diabetes and Digestive and Kidney Diseases; 2012.
100. Sousa LH, Linhares EV, Alexandre JT, Lisboa MR, Furlaneto F, Freitas R, et al. Effects of Atorvastatin on periodontitis of rats subjected to glucocorticoid-induced osteoporosis. J Periodontol. 2016 Oct;87(10):1206-16. https://doi.org/10.1902/jop.2016.160075
101. Machado WM, Prestes AP, Costa TP, Mendes RT, Olchanheski LR Jr, Sordi R, et al. The effect of simvastatin on systemic inflammation and endothelial dysfunction induced by periodontitis. J Periodontal Res. 2014 Oct;49(5):634-41. https://doi.org/10.1111/jre.12145
102. Kırzıoğlu FY, Tözüm Bulut M, Doğan B, Fentoğlu Ö, Özmen Ö, Çarsancaklı SA, et al. Anti-inflammatory effect of rosuvastatin decreases alveolar bone loss in experimental periodontitis. J Oral Sci. 2017;59(2):247-55. https://doi.org/10.2334/josnusd.16-0398
103. Özdoğan AI, İlarslan YD, Kösemehmetoğlu K, Akca G, Kutlu HB, Comerdov E, et al. In vivo evaluation of chitosan based local delivery systems for atorvastatin in treatment of periodontitis. Int J Pharm. 2018 Oct;550(1-2):470-6. https://doi.org/10.1016/j.ijpharm.2018.08.058
104. Messora MR, Apolinário Vieira GH, Vanderlei JM, Mariguela VC, Fernandes PG, Palioto DB, et al. Rosuvastatin promotes benefits on induced periodontitis in hypertensive rats. J Periodontal Res. 2017 Aug;52(4):734-44. https://doi.org/10.1111/jre.12442
105. Rao NS, Pradeep AR, Kumari M, Naik SB. Locally delivered 1% metformin gel in the treatment of smokers with chronic periodontitis: a randomized controlled clinical trial. J Periodontol. 2013 Aug;84(8):1165-71. https://doi.org/10.1902/jop.2012.120298
106. Pankaj D, Sahu I, Kurian IG, Pradeep AR. Comparative evaluation of subgingivally delivered 1.2% rosuvastatin and 1% metformin gel in treatment of intrabony defects in chronic periodontitis: a randomized controlled clinical trial. J Periodontol. 2018 Nov;89(11):1318-25. https://doi.org/10.1002/JPER.17-0434
107. Donos N, Calciolari E, Brusselaers N, Goldoni M, Bostanci N, Belibasakis GN. The adjunctive use of host modulators in non-surgical periodontal therapy. A systematic review of randomized, placebo-controlled clinical studies. J Clin Periodontol. 2020 Jul;47(S22 Suppl 22):199-238. https://doi.org/10.1111/jcpe.13232
108. Muniz FW, 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):671-87. https://doi.org/10.1007/s00784-018-2354-9
109. Cao R, Li Q, Chen Y, Yao M, Wu Q, Zhou H. Efficacy of locally-delivered statins adjunct to non-surgical periodontal therapy for chronic periodontitis: a Bayesian network analysis. BMC Oral Health. 2019 Jun;19(1):105. https://doi.org/10.1186/s12903-019-0789-2
110. Pradeep AR, Garg V, Kanoriya D, Singhal S. 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. J Periodontol. 2016 Jul;87(7):756-62. https://doi.org/10.1902/jop.2016.150706
111. Garg S, Pradeep AR. 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. J Periodontol. 2017 Mar;88(3):259-65. https://doi.org/10.1902/jop.2016.160399
112. Eckschlager T, Plch J, Stiborova M, Hrabeta J. Histone Deacetylase Inhibitors as Anticancer Drugs. Int J Mol Sci. 2017 Jul;18(7):E1414. https://doi.org/10.3390/ijms18071414
113. Van Dyke TE. Proresolving lipid mediators: potential for prevention and treatment of periodontitis. J Clin Periodontol. 2011 Mar;38 Suppl 11:119-25. https://doi.org/10.1111/j.1600-051X.2010.01662.x
114. Van Dyke TE. Pro-resolving mediators in the regulation of periodontal disease. Mol Aspects Med. 2017 Dec;58:21-36. https://doi.org/10.1016/j.mam.2017.04.006
115. Balta MG, Loos BG, Nicu EA. Emerging concepts in the resolution of periodontal inflammation: a role for Resolvin E1. Front Immunol. 2017 Dec;8:1682. https://doi.org/10.3389/fimmu.2017.01682
116. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol. 2008 May;8(5):349-61. https://doi.org/10.1038/nri2294
117. Van Dyke TE. Shifting the paradigm from inhibitors of inflammation to resolvers of inflammation in periodontitis. J Periodontol. 2020 Oct;91(S1 Suppl 1):S19-25. https://doi.org/10.1002/JPER.20-0088

118. Osorio Parra MM, Elangovan S, Lee CT. Specialized pro-resolving lipid mediators in experimental periodontitis: a systematic review. Oral Dis. 2019 Jul;25(5):1265-76. https://doi.org/10.1111/odi.12979
119. Hasturk H, Abdallah R, Kantarci A, Nguyen D, Giordano N, Hamilton J, et al. Resolvin E1 (RvE1) attenuates atherosclerotic plaque formation in diet and inflammation-induced atherogenesis. Arterioscler Thromb Vasc Biol. 2015 May;35(5):1123-33. https://doi.org/10.1161/ATVBAHA.115.305324
120. Hasturk H, Kantarci A, Goguet-Surmenian E, Blackwood A, Andry C, Serhan CN, et al. Resolvin E1 regulates inflammation at the cellular and tissue level and restores tissue homeostasis in vivo. J Immunol. 2007 Nov;179(10):7021-9. https://doi.org/10.4049/jimmunol.179.10.7021
121. Hasturk H, Kantarci A, Ohira T, Arita M, Ebrahimi N, Chiang N, et al. RvE1 protects from local inflammation and osteoclast- mediated bone destruction in periodontitis. FASEB J. 2006 Feb;20(2):401-3. https://doi.org/10.1096/fj.05-4724fje
122. Lee CT, Teles R, Kantarci A, Chen T, McCafferty J, Starr JR, et al. Resolvin E1 reverses experimental periodontitis and dysbiosis. J Immunol. 2016 Oct;197(7):2796-806. https://doi.org/10.4049/jimmunol.1600859
123. Serhan CN, Jain A, Marleau S, Clish C, Kantarci A, Behbehani B, et al. Reduced inflammation and tissue damage in transgenic rabbits overexpressing 15-lipoxygenase and endogenous anti-inflammatory lipid mediators. J Immunol. 2003 Dec;171(12):6856-65. https://doi.org/10.4049/jimmunol.171.12.6856
124. Van Dyke TE, Hasturk H, Kantarci A, Freire MO, Nguyen D, Dalli J, et al. Proresolving nanomedicines activate bone regeneration in periodontitis. J Dent Res. 2015 Jan;94(1):148-56. https://doi.org/10.1177/0022034514557331
125. Mizraji G, Heyman O, Van Dyke TE, Wilensky A. Resolvin D2 Restrains Th1 immunity and prevents alveolar bone loss in murine periodontitis. Front Immunol. 2018 Apr;9:785. https://doi.org/10.3389/fimmu.2018.00785
126. El Kholy K, Freire M, Chen T, Van Dyke TE. Resolvin E1 promotes bone preservation under inflammatory conditions. Front Immunol. 2018 Jun;9:1300. https://doi.org/10.3389/fimmu.2018.01300
127. Ferguson B, Bokka NR, Maddipati KR, Ayilavarapu S, Weltman R, Zhu L, et al. Distinct profiles of specialized pro-resolving lipid mediators and corresponding receptor gene expression in periodontal inflammation. Front Immunol. 2020 Jun;11:1307. https://doi.org/10.3389/fimmu.2020.01307
128. Calder PC. Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochem Soc Trans. 2017 Oct;45(5):1105-15. https://doi.org/10.1042/BST20160474
129. Dawson DR 3rd, Branch-Mays G, Gonzalez OA, Ebersole JL. Dietary modulation of the inflammatory cascade. Periodontol 2000. 2014 Feb;64(1):161-97. https://doi.org/10.1111/j.1600-0757.2012.00458.x
130. Arnalich F, Garcia-Palomero E, López J, Jiménez M, Madero R, Renart J, et al. Predictive value of nuclear factor kappaB activity and plasma cytokine levels in patients with sepsis. Infect Immun. 2000 Apr;68(4):1942-5. https://doi.org/10.1128/IAI.68.4.1942-1945.2000
131. Plutzky J. Atherosclerotic plaque rupture: emerging insights and opportunities. Am J Cardiol. 1999 Jul;84(1 1A):15J-20J. https://doi.org/10.1016/S0002-9149(99)00352-5
132. Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim Biophys Acta. 2015 Apr;1851(4):469-84. https://doi.org/10.1016/j.bbalip.2014.08.010
133. Campan P, Planchand PO, Duran D. [Polyunsaturated omega-3 fatty acids in the treatment of experimental human gingivitis]. Bull Group Int Rech Sci Stomatol Odontol. 1996 Feb-Mar;39(1-2):25-31. French.
134. Campan P, Planchand PO, Duran D. Pilot study on n-3 polyunsaturated fatty acids in the treatment of human experimental gingivitis. J Clin Periodontol. 1997 Dec;24(12):907-13. https://doi.org/10.1111/j.1600-051X.1997.tb01210.x
135. Iwasaki M, Taylor GW, Moynihan P, Yoshihara A, Muramatsu K, Watanabe R, et al. Dietary ratio of n-6 to n-3 polyunsaturated fatty acids and periodontal disease in community-based older Japanese: a 3-year follow-up study. Prostaglandins Leukot Essent Fatty Acids. 2011 Aug;85(2):107-12. https://doi.org/10.1016/j.plefa.2011.04.002
136. Azzi DV, Viafara JA, Zangeronimo MG, Lima RR, Marques LS, Pereira LJ. n-3 Ingestion may modulate the severity of periodontal disease? Systematic review. Crit Rev Food Sci Nutr. 2018 Jul;58(11):1937-42. https://doi.org/10.1080/10408398.2017.1278677
137. Stańdo M, Piatek P, Namiecinska M, Lewkowicz P, Lewkowicz N. Omega-3 Polyunsaturated fatty acids EPA and DHA as an ADJUNCT TO NON-SURGICAL TREATMENT OF PERIODONTITIS: A RANDOMIZED CLINICAL TRIAL. Nutrients. 2020 Aug;12(9):E2614. https://doi.org/10.3390/nu12092614
138. Kruse AB, Kowalski CD, Leuthold S, Vach K, Ratka-Krüger P, Woelber JP. What is the impact of the adjunctive use of omega-3 fatty acids in the treatment of periodontitis? A systematic review and meta-analysis. Lipids Health Dis. 2020 May;19(1):100. https://doi.org/10.1186/s12944-020-01267-x
139. Hayakumo S, Arakawa S, Mano Y, Izumi Y. Clinical and microbiological effects of ozone nano-bubble water irrigation as an adjunct to mechanical subgingival debridement in periodontitis patients in a randomized controlled trial. Clin Oral Investig. 2013 Mar;17(2):379-88. https://doi.org/10.1007/s00784-012-0711-7
140. Huth KC, Jakob FM, Saugel B, Cappello C, Paschos E, Hollweck R, et al. Effect of ozone on oral cells compared with established antimicrobials. Eur J Oral Sci. 2006 Oct;114(5):435-40. https://doi.org/10.1111/j.1600-0722.2006.00390.x

141. Cardile V, Jiang X, Russo A, Casella F, Renis M, Bindoni M. Effects of ozone on some biological activities of cells in vitro. Cell Biol Toxicol. 1995 Feb;11(1):11-21. https://doi.org/10.1007/BF00769988
142. Arita M, Nagayoshi M, Fukuizumi T, Okinaga T, Masumi S, Morikawa M, et al. Microbicidal efficacy of ozonated water against *Candida albicans* adhering to acrylic denture plates. Oral Microbiol Immunol. 2005 Aug;20(4):206-10. https://doi.org/10.1111/j.1399-302X.2005.00213.x
143. Kim JG, Yousef AE, Dave S. Application of ozone for enhancing the microbiological safety and quality of foods: a review. J Food Prot. 1999 Sep;62(9):1071-87. https://doi.org/10.4315/0362-028X-62.9.1071
144. Al Habashneh R, Alsalman W, Khader Y. Ozone as an adjunct to conventional nonsurgical therapy in chronic periodontitis: a randomized controlled clinical trial. J Periodontal Res. 2015 Feb;50(1):37-43. https://doi.org/10.1111/jre.12177
145. Skurska A, Pietruska MD, Paniczko-Dрężek A, Dolińska E, Zelazowska-Rutkowska B, Zak J, et al. Evaluation of the influence of ozonotherapy on the clinical parameters and MMP levels in patients with chronic and aggressive periodontitis. Adv Med Sci. 2010;55(2):297-307. https://doi.org/10.2478/v10039-010-0048-x
146. Yılmaz S, Algan S, Gursoy H, Noyan U, Kuru BE, Kadir T. Evaluation of the clinical and antimicrobial effects of the Er:YAG laser or topical gaseous ozone as adjuncts to initial periodontal therapy. Photomed Laser Surg. 2013 Jun;31(6):293-8. https://doi.org/10.1089/pho.2012.3379
147. Patel PV, Patel A, Kumar S, Holmes JC. Effect of subgingival application of topical ozonated olive oil in the treatment of chronic periodontitis: a randomized, controlled, double blind, clinical and microbiological study. Minerva Stomatol. 2012 Sep;61(9):381-98.
148. Nicolini AC, Rotta ID, Langa GP, Friedrich SA, Arroyo-Bonilla DA, Wagner MC, et al. Efficacy of ozonated water mouthwash on early plaque formation and gingival inflammation: a randomized controlled crossover clinical trial. Clin Oral Investig. 2021 Mar;25(3):1337-44. https://doi.org/10.1007/s00784-020-03441-y
149. Moraschini V, Kischinhevsky IC, Calasans-Maia MD, Shibli JA, Sartoretto SC, Figueredo CM, et al. Ineffectiveness of ozone therapy in nonsurgical periodontal treatment: a systematic review and metaanalysis of randomized clinical trials. Clin Oral Investig. 2020 Jun;24(6):1877-88. https://doi.org/10.1007/s00784-020-03289-2
150. Bird A. DNA methylation patterns and epigenetic memory. Genes Dev. 2002 Jan;16(1):6-21. https://doi.org/10.1101/gad.947102
151. Kurushima Y, Tsai PC, Castillo-Fernandez J, Couto Alves A, El-Sayed Moustafa JS, Le Roy C, et al. Epigenetic findings in periodontitis in UK twins: a cross-sectional study. Clin Epigenetics. 2019 Feb;11(1):27. https://doi.org/10.1186/s13148-019-0614-4
152. Ari G, Cherukuri S, Namasivayam A. Epigenetics and periodontitis: a contemporary review. J Clin Diagn Res. 2016 Nov;10(11):ZE07-09. https://doi.org/10.7860/JCDR/2016/21025.8864
153. Luo Y, Peng X, Duan D, Liu C, Xu X, Zhou X. Epigenetic regulations in the pathogenesis of periodontitis. Curr Stem Cell Res Ther. 2018;13(2):144-50. https://doi.org/10.2174/1574888X12666170718161740
154. Palioto DB, Finoti LS, Kinane DF, Benakanakere M. Epigenetic and inflammatory events in experimental periodontitis following systemic microbial challenge. J Clin Periodontol. 2019 Aug;46(8):819-29. https://doi.org/10.1111/jcpe.13151
155. Martins MD, Jiao Y, Larsson L, Almeida LO, Garaicoa-Pazmino C, Le JM, et al. Epigenetic modifications of histones in periodontal disease. J Dent Res. 2016 Feb;95(2):215-22. https://doi.org/10.1177/0022034515611876
156. Asa'ad F, Bollati V, Pagni G, Castilho RM, Rossi E, Pomingi F, et al. Evaluation of DNA methylation of inflammatory genes following treatment of chronic periodontitis: a pilot case-control study. J Clin Periodontol. 2017 Sep;44(9):905-14. https://doi.org/10.1111/jcpe.12783
157. Grummt I, Pikaard CS. Epigenetic silencing of RNA polymerase I transcription. Nat Rev Mol Cell Biol. 2003 Aug;4(8):641-9. https://doi.org/10.1038/nrm1171
158. Andia DC, Oliveira NF, Casarin RC, Casati MZ, Line SR, Souza AP. DNA methylation status of the IL8 gene promoter in aggressive periodontitis. J Periodontol. 2010 Sep;81(9):1336-41. https://doi.org/10.1902/jop.2010.100082
159. Oliveira NF, Damm GR, Andia DC, Salmon C, Nociti FH Jr, Line SR, et al. DNA methylation status of the IL8 gene promoter in oral cells of smokers and non-smokers with chronic periodontitis. J Clin Periodontol. 2009 Sep;36(9):719-25. https://doi.org/10.1111/j.1600-051X.2009.01446.x
160. Kobayashi T, Ishida K, Yoshie H. Increased expression of interleukin-6 (IL-6) gene transcript in relation to IL-6 promoter hypomethylation in gingival tissue from patients with chronic periodontitis. Arch Oral Biol. 2016 Sep;69:89-94. https://doi.org/10.1016/j.archoralbio.2016.05.018
161. Kojima A, Kobayashi T, Ito S, Murasawa A, Nakazono K, Yoshie H. Tumor necrosis factor-alpha gene promoter methylation in Japanese adults with chronic periodontitis and rheumatoid arthritis. J Periodontal Res. 2016 Jun;51(3):350-8. https://doi.org/10.1111/jre.12314
162. Amormino SAF, Arão TC, Saraiva AM, Gomez RS, Dutra WO, Costa JE, et al. Hypermethylation and low transcription of TLR2 gene in chronic periodontitis. Hum Immunol. 2013 Sep;74(9):1231-6. https://doi.org/10.1016/j.humimm.2013.04.037
163. Viana MB, Cardoso FP, Diniz MG, Costa FO, Costa JE, Gomez RS, et al. Methylation pattern of IFN-γ and IL-10 genes in periodontal tissues. Immunobiology. 2011 Aug;216(8):936-41. https://doi.org/10.1016/j.imbio.2011.01.006

164. Andia DC, Planello AC, Portinho D, Silva RA, Salmon CR, Sallum EA, et al. DNA methylation analysis of SOCS1, SOCS3, and LINE-1 in microdissected gingival tissue. Clin Oral Investig. 2015 Dec;19(9):2337-44. https://doi.org/10.1007/s00784-015-1460-1
165. Turner BM. Cellular memory and the histone code. Cell. 2002 Nov;111(3):285-91. https://doi.org/10.1016/S0092-8674(02)01080-2
166. Cantley MD, Bartold PM, Marino V, Fairlie DP, Le GT, Lucke AJ, et al. Histone deacetylase inhibitors and periodontal bone loss. J Periodontal Res. 2011 Dec;46(6):697-703. https://doi.org/10.1111/j.1600-0765.2011.01392.x
167. Cantley MD, Fairlie DP, Bartold PM, Rainsford KD, Le GT, Lucke AJ, et al. Inhibitors of histone deacetylases in class I and class II suppress human osteoclasts in vitro. J Cell Physiol. 2011 Dec;226(12):3233-41. https://doi.org/10.1002/jcp.22684
168. Meng S, Zhang L, Tang Y, Tu Q, Zheng L, Yu L, et al. BET Inhibitor JQ1 blocks inflammation and bone destruction. J Dent Res. 2014 Jul;93(7):657-62. https://doi.org/10.1177/0022034514534261
169. Maqbool R, Ul Hussain M. MicroRNAs and human diseases: diagnostic and therapeutic potential. Cell Tissue Res. 2014 Oct;358(1):1-15. https://doi.org/10.1007/s00441-013-1787-3
170. Sonkoly E, Pivarcsi A. Advances in microRNAs: implications for immunity and inflammatory diseases. J Cell Mol Med. 2009 Jan;13(1):24-38. https://doi.org/10.1111/j.1582-4934.2008.00534.x
171. Hung PS, Chen FC, Kuang SH, Kao SY, Lin SC, Chang KW. miR-146a induces differentiation of periodontal ligament cells. J Dent Res. 2010 Mar;89(3):252-7. https://doi.org/10.1177/0022034509357411
172. Nahid MA, Rivera M, Lucas A, Chan EK, Kesavalu L. Polymicrobial infection with periodontal pathogens specifically enhances microRNA miR-146a in ApoE-/- mice during experimental periodontal disease. Infect Immun. 2011 Apr;79(4):1597-605. https://doi.org/10.1128/IAI.01062-10
173. 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(S22 Suppl 22):155-75. https://doi.org/10.1111/jcpe.13245