Photodynamic Inactivation of Microbial Pathogens: Medical and Environmental Applications

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Chapter 13

Antimicrobial Photodynamic Therapy (aPDT) for Oral Infections

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13.1. Introduction

The mouth provides a large number of diverse surfaces, such as soft shedding tissues of the buccal mucosa and hard non-shedding surfaces of the teeth, on which a wide variety of complex biofilms are able to form. Dental plaque is the term commonly used for the biofilm formed on teeth; however, the term plaque has now been extended to encompass biofilms on all the oral surfaces. These biofilms consist of a complex microbial community embedded in a matrix of polymers of bacterial and salivary origin. They are inherently more resistant to antibiotics, antimicrobials and antifungal agents which make the use of novel antimicrobials, such as antimicrobial Photodynamic Therapy (aPDT) to be discussed in this chapter, all the more important.

Small alterations in an environment can lead to ecological shifts and subsequent population changes in these complex biofilms and in certain specific cases this may predispose to a more “pathogenic” microbial community. This is the basis of the “ecological plaque hypothesis”,¹ which can be used to understand the microbial aspects of a range of oral infections.

13.2. Caries

Dental caries can be defined as the localized demineralization of the tooth tissue by various acids produced by bacterial fermentation of dietary carbohydrates. Treatment of the disease involves removal of the damaged tooth tissue and its replacement with a restorative material. This disease is arguably the most common, chronic infectious disease in humans. Caries can be split into two categories: coronal (crown) caries and root surface caries.

13.2.1. Coronal Caries

Coronal carries can occur on all surfaces of the crown where the supragingival plaque biofilm is allowed to develop and mature. However, it is most commonly associated with pits and fissures and approximal sites. A major question has been which specific bacteria, if any, are involved in the progression of disease. The “ecological plaque hypothesis” suggests that a shift in the microbiota is brought about by increases in the amount and frequency of dietary fermentable carbohydrates. These substrates are fermented by the bacteria in the supragingival plaque biofilm leading to the production of acid end products like lactic acid. This acid production serves to lower the local pH and favour a shift in the biofilm population to acid-tolerant bacteria, for example, mutans streptococci. In addition to being aciduric, mutans streptococci are also highly acidogenic and can produce enough acid to lower the pH to levels that are inhibitory to many other bacteria within the biofilm (pH 4.5). The production of lactic acid is fundamental to the pathogenesis of mutans streptococci. It has been shown that mutant strains of *Streptococcus rattus*
(a caries causing species in rats and hamsters) that lack lactate dehydrogenase activity fail to demineralize teeth in an animal model despite colonization and plaque formation. Lactobacilli are usually found in supragingival plaque biofilms in low numbers; conversely, however, they have been shown to be present in elevated proportions in established caries lesions and have therefore been associated with the progression of the lesion rather than its initiation. Non-mutans streptococci are thought rarely to be involved with the disease progression of carious lesions although they usually outnumber mutans streptococci. However, they have been shown to produce acid in an acidic environment.

13.2.2. Root Surface Caries

Root surface caries, as the name implies, occur on root cementum or dentine and again are caused by a microbial biofilm. The disease is secondary to gingival recession since in a healthy mouth cementum and dentine are not exposed to the microbiota and are therefore unavailable for colonization. Gingival recession can be caused by a number of factors, for example old age, mechanical injury or periodontal treatment regimens. In industrialized countries the proportion of the population over 65 years of age is increasing, additionally the percentage of these remaining dentate is also increasing. A survey carried out by Steele et al. showed, amongst other things, that in southern England 67% of patients over 60 years old were dentate compared to an equivalent cohort in 1962 which showed only 15% to be dentate.

The microbiological nature of the associated plaque biofilm is different from that associated with crown caries even though it is technically still supragingival plaque. The lesion has been shown to have a definite progression since changes in its clinical appearance are observed over time. Briefly, as the gingiva recedes new cementum/dentine is exposed and, in susceptible hosts, a lesion may start to form. The appearance of the lesion is described as “soft” and consists of a highly demineralized lesion replete with bacteria. Surrounding this, a further demineralized area is apparent and infiltrating bacteria can be observed; this lesion is actively carious. The progression of the lesion leads to a change in appearance and is categorized as “leathery”. This is an intermediate stage and consists of a re-mineralized surface overlaying a heterogeneous mix of bacteria, de-mineralized tissue and re-mineralized tissue. A further progression is to a “hard” lesion; these are fully re-mineralized and inactive with respect to caries. The microbiology of this biofilm has been the subject of numerous investigations over the years; however, only within the last 10–20 years have the problems associated with sampling of the infected underlying dentine, and not the overlying biofilm, been identified and addressed. In their study Beighton and Lynch showed that the bacterial composition of the carious dentine biofilm associated with “soft” lesions consisted of significantly more lactobacilli and Gram-positive pleomorphic rods but, conversely, significantly fewer streptococci compared to the overlying plaque biofilm. Additionally, an increased number and/or proportion of S. mutans in “soft” lesions was observed
compared to “hard” lesions or sound surfaces. *Actinomyces* spp. have historically been associated with root surface caries; however, studies by Brailsford *et al.*\(^{10,11}\) showed *A. naeslundii* was not associated with active carious lesions and that *A. israelii* and *A. gerencseriae* predominated.

It is thought that the lesion occurs as a function of accumulation and subsequent stagnation of a plaque biofilm at the gingival margin. The nature of the microbiota is poorly understood but there seems to be no one species responsible for disease initiation and progression. What may be important is the presence of particular strains of a defined but heterogeneous group of bacteria that are particularly suited to that environment. Indeed, Sansone *et al.*\(^{6}\) showed that the acidogenic and aciduric microbiota associated with a carious lesion is far more diverse (approx. 25 taxa) than the corresponding acidogenic and aciduric microbiota associated with sound root surfaces (approx. 8 taxa).

### 13.3. Periodontal Diseases

Periodontal disease defines a broad group of diseases affecting the periodontal tissues, the commonest of which are inflammatory processes of the gingiva and tissues attaching the tooth. These diseases are usually associated with microbial infection due to accumulation of a plaque biofilm and calculus.

#### 13.3.1. Gingivitis

The bacteria and their extracellular products present within the plaque biofilm on the surfaces of teeth at the gum margin can cause inflammation. This is termed gingivitis and is the most common of the periodontal diseases usually brought about by poor oral hygiene. It can be defined as “a non-specific inflammatory process of the gingiva (gum) without destruction of the supporting tissues”. A complex range of gingival diseases are recognized and have been classified into two main groups with 12 headings and numerous subheadings.\(^{12}\) This disease is usually reversible and on removal of the biofilm (*i.e.* a return to good oral hygiene) the tissues revert to a healthy clinical state. It is likely that the entire human population suffers to some extent from this disease. The microbiota associated with dental plaque-induced gingivitis is different from that associated with health. In addition to an increase in biofilm mass (for example due to poor oral hygiene) the composition shifts from one dominated by streptococci\(^{13}\) to one where *Actinomyces* spp. are dominant.\(^{14,15}\)

#### 13.3.2. Periodontitis

Periodontitis refers to a group of more advanced and related diseases within the broad heading of periodontal disease. It can be defined as “an apical extension of gingival inflammation to involve the tissues supporting the tooth (periodontal ligament and bone)”. The destruction of the fibre attachment results in a
periodontal pocket. There are 48 specific periodontitis disease categories now recognized.\textsuperscript{12}

By far the most common is chronic periodontitis, which is the major cause of tooth loss in the adult population. The disease is mediated by the microbiota forming the plaque biofilm on the tooth surface. Additionally, and as a consequence of the immune response elicited by the bacteria, further destruction may occur due to the host inflammatory response.

The biofilm present in the gingival crevice and later in the periodontal pocket is extremely diverse with up to 100 culturable species from a single pocket.\textsuperscript{16} Since such a diverse microbiota is present, trying to identify the particular species responsible for disease initiation and progression is a very complex and difficult undertaking. The most plausible explanation for the aetiology is again supplied by the "ecological plaque hypothesis". Briefly, the primary inflammation events due to a large biofilm mass at the gingival margin increase the flow of gingival crevicular fluid (GCF; a tissue exudate that bathes the periodontal tissues) thus changing the local environment and allowing proteolytic and anaerobic species to predominate. GCF is present in small quantities in health but in much larger volumes in diseased sites and contains a wide range of complex molecules derived from a number of sources \textit{e.g.} serum, connective tissue and epithelium.\textsuperscript{17} In addition, polymorphonuclear neutrophilic granulocytes and monocytes are present in GCF in the pocket.

The World Workshop on Clinical Periodontology (American Academy of Periodontology Consensus report 1996) has designated three species as etiologic agents of periodontitis in a susceptible host, namely \textit{Actinobacillus actinomycetemcomitans}, \textit{Porphyromonas gingivalis} and \textit{Tannerella forsythensis}.\textsuperscript{18} The findings from the majority of other microbiology studies are based on data derived from the cultivable microbiota. However, it has been estimated that only 50\% of the oral microbiota is cultivable.\textsuperscript{19,20} More recently molecular techniques have been used to detect and identify the uncultivable portion of this highly diverse biofilm.\textsuperscript{21,22} The aetiology of periodontal disease is further complicated by a range of predisposing factors. The susceptibility of the host has been shown to be important. A specific genotype of the polymorphic interleukin-1 (a pro-inflammatory cytokine and key regulator of the host responses to microbial infection and a major modulator of extracellular matrix catabolism and bone resorption) gene cluster is associated with severity of periodontitis.\textsuperscript{23} There is also evidence to suggest that smoking is a significant risk factor for periodontal disease-associated tooth loss.\textsuperscript{24} Additionally diabetes mellitus is a major risk factor for chronic periodontitis and the more severe and rapidly progressing forms.\textsuperscript{25,26}

As discussed previously, because of the relationship between the presence of bacterial biofilms and caries and periodontal diseases the treatment regimes are essentially based on mechanical removal of these biofilms. However, many individuals are unable or unmotivated to perform tooth brushing with the regularity and efficiency necessary, so that in recent decades there has been an increased interest in the possibility to replace or complement mechanical therapy with the use of antiseptic agents or antibiotics. However, given the
significant increase in the emergence of species resistant to traditional antimicrobial agents and the inherent resistance of biofilms, it has increased the scientific interest in developing alternative antimicrobial therapies that can act as adjuncts in the control of plaque, thus assisting in maintaining the oral health of individuals.

13.3.3. Phototherapy

The therapeutic use of light phototherapy is a term that includes all treatments that use non-ionizing radiation for therapeutic purposes inducing reactions in the human body that may cause benefits to patients. This process can occur when we are exposed to natural light, UVA or UVB. Historically, this treatment has been recommended for several purposes, with applications in the treatment of winter depression in the regulation of circadian rhythm and sleep disorders. Sometimes it is necessary to use a photosensitive drug or photosensitizer with the use of light to achieve specific effects. This process is known as photochemotherapy. In such cases, the drug is activated by light reacting with specific cells that may be causing the disease. A good example of this therapy includes the use of a UV lamp to treat newborns with jaundice where light triggers the release of a chemical reaction that allows the body to eliminate the yellow pigment (photosensitizer) characteristic of the disease.\textsuperscript{27}

Since the advent of laser lights in the past century, the possibilities of the therapeutic use of light have expanded in all areas of science. According to their interaction with the irradiated tissue, lasers can be classified into two major groups: surgical lasers, or lasers operating at high power, and non-surgical lasers, also known as therapeutic or lasers that operate at low power.\textsuperscript{28} Recently, light emitting diodes (LEDs) have been used in medicine as an alternative to the use of conventional lasers. LEDs are semiconductor devices that emit light when biased properly in the visible or invisible range. Although both lasers and LEDs produce monochromatic lights, LEDs do not show good control and coherence, resulting in emission bands that are wider and reduced cost of therapy.

Regarding the effect of the lights in the ability to kill bacteria, we know that this effect is highly influenced by the type of light used. The antimicrobial action of lasers operating at high power is well established\textsuperscript{29} in periodontal therapy, root canal treatment\textsuperscript{30,31} and sterilization of instruments and implants of titanium.\textsuperscript{32} In general these lasers require high doses of energy and reach the surface irradiated with high temperatures promoting sterilization by local thermal action.

The low-power lasers, in turn, are meant to restore the biological balance by improving the conditions of cellular tissue vitality, for example, by modulating anti-inflammatory responses. These effects are generated by photochemical and photoelectric properties of aPDT rather than a thermal action. In the specific case of aPDT, the antimicrobial effect can be achieved once the photosensitizing drug is activated by a low-intensity light (laser or LED) passing to
an excited state and generates substances that can damage and ultimately kill the treated cell.

13.4. Antimicrobial Photodynamic Therapy

aPDT is a technique that can potentially reach harmful cells without affecting normal tissues of the host. This therapy was initially designed for the treatment of cancer, based on the observation that some non-toxic molecules, such as photosensitizers, accumulate primarily in malignant cells. Using this technique, the photosensitizer is initially administered to the patient systemically, and by itself does not cause damage to healthy tissues. However, when a light (usually a laser or LED) is applied to tissues containing the drug, the drug is activated and tissues are destroyed rapidly, in precisely the places where the radiation is beamed. Thus, careful application of the light beam can guarantee that the therapy reaches the diseased tissues selectively.

Thus, photosensitizers are molecules that have the special property of absorbing light energy and use that energy to promote chemical reactions in cells and tissues undergoing photodynamic therapy. To this end, the photosensitizer should be able to absorb light at a wavelength of light emitted by the equipment used. In this process, there is only an adverse effect since some drugs may produce skin sensitivity, which involves the removal of the patient from any light source during the period in which the drug is active in the human body. The effectiveness of therapy combines two principles: the preferential accumulation of photosensitizer in the target cells with a precise irradiation of light, which allows localized and selective use of aPDT.

13.4.1. Antimicrobial Action of Photodynamic Therapy

The rapid emergence of resistance among pathogenic bacteria has led to a growing interest in the scientific community in finding alternatives to antimicrobial therapy which make the emergence of resistant species unlikely. Examples of such therapies include the use of bacteriophages, antimicrobial peptides and photodynamic therapy. Studies evaluating the susceptibility of photodynamic therapy on normal and resistant strains found that both strains are susceptible to photodynamic therapy when the appropriate combination of photosensitizer and light is used.

The ability of a component to absorb incident light does not necessarily mean that it can act as a photosensitizer. To be clinically effective, the photosensitizer must have absorption peaks near the wavelength of light used and must not show toxic effects to the host.

In such cases, photodynamic therapy involves the localized application of drug and light on the infected area as opposed to its application for cancer treatment, where the drug is usually administered systemically. aPDT aims to achieve a sufficiently high antimicrobial effect on the causative agent of
infection by selective action of the photosensitizer and light on the target organisms, avoiding damage to healthy tissues of the host. During this process, cellular photosensitive components pass to an excited state when exposed to light of a wavelength that is further characterized by the passage of electrons to higher energy levels. In this excited state, the photosensitizer can interact with molecular oxygen initiating the formation of highly reactive singlet oxygen (photo processes Type II) or interact with other molecules as electron acceptors resulting in the production of hydroxyl radicals and other organic molecules (photo processes Type I).36 The products of these photochemical reactions can then damage the essential components of cells or irreversibly alter their metabolic activity, resulting in bacterial death.37

Among the factors that may influence the success of the antimicrobial effect by aPDT are those derived from photobiological factors and those derived from irradiated bacterial cells. Among the factors are photosensitizer concentration, the time of pre-irradiation, the wavelength of light, the power of the device, the irradiation time and the density of energy used. Among the bacterial factors that may interfere with the process are those related to the physiological characteristics of the bacterial cell, such as the growth phase, whether they are growing as biofilms and whether multiple species are involved.

13.5. aPDT Against Cariogenic Bacteria

A large number of studies have shown that photodynamic therapy is effective in reducing the numbers of bacteria in broth culture.38,39 However, the bacteria that cause caries are more often found in the oral cavity organized in biofilms on the surface of the teeth and surrounding tissues. This intrinsic resistance shown by biofilms appears to be related to the presence of matrix polysaccharides, changes in cell wall composition, slower growth rate as well as the expression of genes for resistance to antimicrobial agents.40

Currently, there are relatively few groups that evaluate the antimicrobial potential of aPDT on biofilms. Previously, Wilson et al.41 showed that the combination of aluminium disulfonated phthalocyanine (AlPcS2) and a laser diode emitting at a wavelength of 660 nm was effective in significantly reducing the numbers of S. sanguinis grown as biofilms. In a study involving multispecies biofilms, Wood et al.42 showed a high mortality rate (results were obtained by transmission electron microscopy and confocal laser microscopy and no quantitative analysis was performed) when bacterial biofilms in situ were treated with a cationic photosensitizer and exposed to white light of 400 W for 30 min. In 2002, O’Neill et al.43 showed a significant antimicrobial effect was obtained in oral biofilms grown on nitrocellulose discs when they were sensitized with toluidine blue and then irradiated with red light with an energy density of 81.9 J cm⁻². In 2005, Zanin et al.44 found that photodynamic therapy was effective to reduce up to 99% of the viable population of S. mutans grown in vitro and sampled at various times (3, 7 and 10 days).
Comparative studies evaluating the antimicrobial effect of aPDT using conventional lasers and LED lights have been performed on biofilms of *S. mutans* grown in an "artificial mouth" biofilm model (Constant Depth Film Fermentor\(^\text{45}\)). The model enabled the simulation of the formation of acquired pellicle on hydroxyapatite discs, while the biofilms of *S. mutans* were grown in an environment continually bathed in artificial saliva. Furthermore, the choice of this model enabled precise control of the frequency distribution of sucrose, temperature, atmosphere and thickness of biofilm. The results demonstrated a significant reduction in the counts of bacteria in biofilms of *S. mutans* although there was no significant difference in antimicrobial effect promoted when a Helium-Neon (HeNe) laser or an LED light were used. Interestingly, confocal laser microscopy showed that most bacterial killing by aPDT occurred predominantly in the outer layers of the biofilm, which may have been caused by the difficulty of the photosensitizer to penetrate.\(^\text{44}\) Such issues could be overcome by the choice of photosensitizers with greater ability to penetrate cationic biofilms as well as by irradiation of the biofilms internally through the use of a fibre optic.

Since the photodynamic therapy can be effectively employed to reduce the microorganisms in the biofilm, thereby helping to prevent the onset of caries lesions, another very interesting application is the use of photodynamic therapy directly on dental caries. Although further research must be performed, some studies have observed that *S. mutans* can be killed in a position very similar to those found in a carious lesion. Burns *et al.\(^\text{46}\)* observed reductions in the number of viable *S. mutans* when they were embedded in a collagen matrix with varying degrees of demineralization.

The use of photodynamic therapy for the disinfection of caries could be beneficial for reducing the amount of dental tissue removed during cavity preparation and promoting an effective decontamination of the treated area before the sealing of the lesion. The treatment might become less traumatic for the patient and faster for the dentist besides improving the prognosis by decreasing the need for dental tissue during cavity preparation.

13.6. aPDT and Periodontal Disease – *in vitro* Studies

Several *in vitro* studies have demonstrated that aPDT using different light sources and photosensitizers can eradicate periopathogenic bacterial strains *in vitro*. Wilson was the first to demonstrate effective killing of pathogens including *P. gingivalis*, *Fusobacterium nucleatum* and *A. actinomycetemcomitans* in homogenous planktonic cultures\(^\text{35}\) and planktonic human subgingival plaque samples\(^\text{47}\) using low-power laser light and dyes such as toluidine blue, methylene blue and crystal violet. Importantly, these studies showed that irradiation in the absence of photosensitizer had no detectable effect, nor did the photosensitizer by itself have a bactericidal effect. Furthermore, two later studies published by this group\(^\text{48,49}\) examined aPDT dosimetry and mechanism of action during lethal photosensitization of *P. gingivalis*. In particular, they
tested photosensitizer concentration, light dose (varied by changing exposure time), pre-incubation time with photosensitizer, presence or absence of serum, pH and bacterial growth phase in an attempt to optimize the antibacterial effect. An energy dose of 4.4 joules with 25 μg ml\(^{-1}\) photosensitizer provided the greatest effect, and efficacy was found to decrease with higher photosensitizer concentrations. Wilson’s group has also demonstrated that these and other strains are susceptible when they comprise a biofilm.\(^{50}\)

Subsequent to the original published work by the Wilson group, several others have demonstrated the efficacy of aPDT against oral microbes. Indeed, when the susceptibility of the three “key” periodontal pathogens grown as biofilms \textit{in vitro} has been investigated the reductions have been in the order of 99.9%.\(^{51}\) Pfizter \textit{et al.}\(^{52}\) used several experimental photosensitizers and light doses in excess of 20 J cm\(^{-2}\) to completely eradicate cultures of \textit{P. gingivalis} and \textit{F. nucleatum}. Furthermore, Chan and Lai\(^{53}\) showed that Gram-negative anaerobes were susceptible to methylene blue mediated aPDT, with \textit{F. nucleatum} and \textit{A. actinomycetemcomitans} being reduced by about 95% compared to the non-treated control. Another group,\(^{54}\) using a porphyrin-derivative photosensitizer, reported a light dose-dependent > 6 log\(_{10}\) reduction in viable count of \textit{P. gingivalis}. Prates \textit{et al.}\(^{55}\) were able to achieve 2–3 log\(_{10}\) reductions from control in planktonic cultures of \textit{A. actinomycetemcomitans} with malachite green and energy doses of 6–9 J cm\(^{-2}\). Another group\(^{56}\) evaluated the effectiveness of aPDT \textit{in vitro} on biofilms comprised primarily of the oral bacteria \textit{Actinomyces israelii}, \textit{F. nucleatum}, \textit{P. gingivalis} and \textit{Prevotella intermedia}. In this study extracted human teeth were inoculated for biofilm growth, and it was found that up to 80% eradication could be achieved after treatment with methylene blue and activation with 30 J cm\(^{-2}\) laser light. Matevski \textit{et al.}\(^{57}\) observed a light dose dependent killing of \textit{P. gingivalis} and several other periopathogens in the presence of serum and blood using a helium-neon laser and the photosensitizer toluidine blue O. Studies such as this, which incorporate serum and proteins into an \textit{in vitro} test model, are important for predicting the overall efficacy of aPDT in an oral \textit{in vivo} environment. Other \textit{in vitro} antimicrobial PDT models have utilized sub- or supragingival mixed plaque samples isolated from human subjects, as opposed to heterogeneous cultures grown in the laboratory. For example, Qin \textit{et al.}\(^{58}\) used such a model to demonstrate that aPDT consisting of 1 mg ml\(^{-1}\) toluidine blue O (TBO) and an energy dose of 12 J cm\(^{-2}\) was effective in eliminating supragingival plaque cultures. Furthermore, they showed that efficacy increased as power density and energy dose were increased, but decreased as photosensitizer concentration was increased (due to interstitial absorbance of light by bulk sensitizer molecules).

Many studies evaluating the effects of photodynamic therapy on periodontal bacteria have focused on the therapeutic potential of therapy as well as reducing the viability of the microorganisms. However, studies have shown that aPDT, in addition to having an antimicrobial effect, seems to have some effect on microbial virulence factors. Lipopolysaccharides are one of the most important virulence factors of the bacteria which cause periodontal disease,
having an important role by inducing the host inflammatory response and stimulating the release of cytokines. Komorik et al.\textsuperscript{59} demonstrated that the ability of Gram-negative bacteria to induce cytokine production was reduced after exposure to a HeNe laser in the presence of TBO. Furthermore, aPDT is able to inactivate destructive host cytokines tumour necrosis factor-alpha (TNF-alpha) and interleukin (IL)-1 beta.\textsuperscript{60} Other important virulence factors for the development of periodontal disease are the bacterial enzymes capable of degrading the host tissue. \textit{P. gingivalis} produce proteolytic enzymes that are important mediators of cellular destruction during periodontitis. Packer \textit{et al.}\textsuperscript{61} demonstrated that exposure of \textit{P. gingivalis} photodynamic therapy using the combination of TBO and HeNe laser reduced the proteolytic activity of these enzymes by 50%. Furthermore, aPDT is able to inactivate destructive host cytokines tumour necrosis factor-alpha (TNF-alpha) and interleukin (IL)-1 beta.\textsuperscript{60}

If we consider the clinical use of aPDT in periodontal disease we need to take into account the removal of viable pathogenic microorganisms and reduce the expression of some of its virulence factors. This represents a considerable advantage compared to the use of antibiotics and conventional antisepsics since the latter are capable of decreasing the viability of the bacteria but have no effect on their virulence factors. Therefore, with all of these factors combined, it would be anticipated that by using aPDT there would be a more favourable healing environment.

The possibility of the occurrence of adverse reactions with the use of aPDT has been studied. Animal models have shown that concentrations of photosensitizer and energy densities used to cause damage to microorganisms have minimal effects on host tissues.\textsuperscript{27} Furthermore, Soukos \textit{et al.}\textsuperscript{62} evaluated the effect of chlorin e6 conjugated to pentalysine associated with red light to kill \textit{P. gingivalis} in the presence of epithelial cells. The study showed that it was possible to selectively eliminate \textit{P. gingivalis} without causing significant damage to skin cells.

The use of traditional antimicrobial therapies for the treatment of periodontitis has two obvious disadvantages compared with the use of aPDT. Initially, there is a difficulty in maintaining the antibiotic or antiseptic in high enough concentrations for a period of time sufficient to cause a significant reduction in the viability of bacteria and, secondly, this strategy favours the emergence of resistant bacterial strains.

13.7. Animal Models for Oral aPDT

Animal models have been used extensively by researchers in the field of aPDT to demonstrate both safety \textit{(i.e.} absence of damage to host tissue) and efficacy in the mouth. As discussed previously in the chapter, the aetiology of periodontal disease is very complex and likely is driven by a combination of subgingival microbiota changes and resulting host immunological response.\textsuperscript{16} While \textit{in vitro} studies showing antimicrobial efficacy against implicated
periopathogens are useful to demonstrate proof-of-principle for oral aPDT applications, only studies performed in vivo can accurately predict clinical response. This is because the presence of blood, saliva, interstitial fluid and other factors in the periodontal microenvironment has an impact on aPDT efficacy that would not be observed in less complex in vitro efficacy models.

The use of rodent models for aPDT in the oral cavity provided early evidence that the in vitro efficacy observed against periopathogens carries over to the more complex oral microenvironment. In one of the earlier in vivo reports, investigators evaluated the efficacy of aPDT against the periopathogen Porphyromonas gingivalis using a rat oral model. In this study, maxillary molars of rats were inoculated with P. gingivalis and exposed to up to 48 joules of 630 nm laser light in the presence of the phenothiazinium photosensitizer toluidine blue O (TBO). Immediately following treatment, microbiological specimens were collected using paper point sampling. At three days post treatment animals were sacrificed and jaw samples were excised for histological sectioning and evaluation. In addition, other animals were sacrificed at 90 days post treatment and alveolar bone loss was measured using morphometric and radiographic methods. Results showed that no viable bacteria were detected after application of 1 mg ml\(^{-1}\) TBO in combination with any of the light doses evaluated. At lower concentrations of TBO (0.01 and 0.1 mg ml\(^{-1}\)) and light doses of 6 joules, less bacterial killing (1–2 log\(_{10}\) reduction in viability from control) was observed. Histological examination of tissue samples showed no adverse effects on cell/tissue morphology in the aPDT treated samples.

Several more recent studies using rodent oral aPDT models have been conducted by a Brazilian group. Almeida et al. used a ligature-induced model of periodontal disease in rats, and aPDT treatment in that study consisted of application of the photosensitizer methylene blue followed by activation with laser light. The results of this study showed significantly less bone loss in the aPDT treatment group compared to the control groups at both 5 and 15 days post-treatment. In a subsequent study published by the same group, the investigators focused on evaluating furcation defects in rats resulting from ligature induced periodontal disease. In that study, the aPDT treatment group again exhibited less bone loss compared to the control groups at day 7 and day 15. A third report from the same group used ligatures and chemical induction of diabetes to evaluate the effect of the diabetic state on improvement of periodontal disease in vivo. The results confirmed that aPDT using methylene blue significantly reduced bone loss at all time points in both diabetic and non-diabetic animals. Finally, a recent study by the same group used a rodent model to evaluate the potential to use aPDT for periodontal treatment in immunocompromised individuals. In that study, experimentally immunosuppressed rats were induced to develop periodontal disease and treated with scaling a root planing prior to aPDT consisting of TBO and laser illumination. Results of the study showed significantly less bone loss in animals treated with the full aPDT protocol, as opposed to those treated with SRP or TBO alone. In addition, histological analysis showed healthier periodontal ligament tissue with less evidence of bone resorption in aPDT treated samples. Results were
similar regardless of whether the animal had been previously immunocompromised by chemical injection. While this series of Brazilian studies is important in showing tissue/bone effects after periodontal treatment with aPDT, it should be noted that none directly examined microbiological reductions of periopathogens in response to treatment in an in vivo model of periodontal disease.

In an in vivo study that did examine microbiological end points after aPDT, Qin et al.\textsuperscript{68} compared the therapeutic efficacy of aPDT to conventional scaling and root planing in a rat model of ligature-induced periodontal disease. Treatment in the aPDT group was administered using 12 J cm\textsuperscript{-2} of 635 nm laser light and 1 mg ml\textsuperscript{-1} TBO, while treatment in the control group consisted of manual debridement followed by cleaning with a topical 0.5% chlorhexidine solution. The authors concluded that aPDT produced similar results to scaling and root planing with approximately 4% bacterial survival in both conditions. In addition, they reported that clinical signs of inflammation were reduced by both treatments with no signs of injury to host tissue. Lin et al.\textsuperscript{69} used a different model in which aPDT was used to treat bacterially infected excision wounds in the oral cavities of rats. Again, the photosensitizer used in this study was TBO and illumination was performed using a 635 nm diode laser system. The authors observed a light dose dependent killing effect whereby 48 joules cm\textsuperscript{-2} produced a 97% kill of human supragingival plaque bacteria inoculated into the wound site. Furthermore, progression of the lesions over time was reduced in the aPDT treatment group vs. non-treated control. The authors concluded that aPDT could serve as a valuable alternative to other chemical disinfectants such as chlorhexidine gluconate for the treatment of oral infections such as periodontal disease, peri-implantitis and endodontic infection.

Several groups have also used large animal models to investigate the effects of antimicrobial PDT in the oral cavity. In a beagle model, in which healthy mouths were inoculated and infected with \textit{P. gingivalis} and \textit{F. nucleatum} and subsequently treated with aPDT,\textsuperscript{70} it was observed that clinical measures of periodontal disease such as inflammation and bleeding on probing were significantly decreased from control. In addition, the number of positively colonized \textit{P. gingivalis} sites was greatly reduced from that of non-treatment control animals. In a related model to ligature-induced periodontal disease, Hayek et al.\textsuperscript{71} compared the effects of aPDT and conventional treatment on microbial reduction in ligature-induced peri-implantitis in dogs. Implants were placed in 18 third premolars in dogs and, following osseointegration and ligature induced peri-implantitis, animals were divided into two treatment groups. One group received conventional treatment of mucoperiosteal flaps to scale implant surfaces followed by irrigation with 0.12% chlorhexidine, while the other received aPDT consisting of mucoperiosteal scaling followed by placement of azulene (final concentration 0.01% w/w) paste photosensitizer and activation with low-power 660 nm laser light. Results showed that \textit{Prevotella} spp., \textit{Fusobacterium} spp. and \textit{S. Beta-haemolyticus} were significantly reduced in both groups, and that the photosensitizer did not stain the implant or the surrounding tissue.
The authors concluded that aPDT treatment was an effective non-invasive alternative to conventional therapy for peri-implantitis. Finally, Shibl et al. also used a dog model of ligature-induced peri-implantitis and exposed infected sites to aPDT using TBO (100 µg ml) and laser light (685 nm for 80 seconds, energy density of 200 J cm⁻²). In block biopsies performed at 5 months post-treatment, this group observed new bone formation in all samples. Unfortunately, as all groups (including controls) in this study underwent aPDT treatment it was difficult to ascertain the relative contribution of aPDT to bone formation and reossseointegration in the mouth.

In addition to the in vivo models designed to show the efficacy of antimicrobial PDT in the oral cavity, there have also been several published reports focusing on safety in animal models. In a 2002 study by Komorik et al., rat buccal mucosal tissue was subjected to varying concentrations of the photosensitizer TBO with subsequent exposure to various power densities of 633 nm laser light. Additionally, control groups consisting of light-alone, photosensitizer-alone and no treatment were performed. After treatment, mucosal tissue was removed and examined histologically while TBO biodistribution was assayed using digital fluorescence imaging. The authors observed that aPDT resulted in no observable necrotic or inflammatory changes in the buccal mucosa. Furthermore, fluorescence imaging showed that TBO penetrated only to the depth of the epithelium, with virtually no fluorescence observed in the underlying connective tissue. These results suggested that TBO-mediated aPDT does not adversely affect buccal mucosa. Another study subjected oral mouse gingival tissue to aPDT using TBO with 635 nm laser light at an energy dose of 60 J cm⁻². Additionally, control groups of light only (140 J cm⁻²), photosensitizer only (2.5 mg ml⁻¹) and no treatment were also performed. Subsequent histological examination showed that aPDT did not alter the gingival tissue structure or gross cellular morphology as compared to untreated controls.

Taken together, the in vivo studies summarized above build upon the body of in vitro evidence supporting safety and efficacy of aPDT in the oral cavity. While several of the large animal studies examined clinically relevant end points such as bleeding and inflammation, none specifically addressed soft tissue reattachment—a necessary outcome for resolution of periodontal disease in humans. The next step was therefore to advance aPDT into controlled clinical studies in order to demonstrate that preclinical results translated to clinical outcome.

13.8. Human Clinical Applications for Oral aPDT

Scaling and root planing (SRP) is currently known as the gold standard for treatment of periodontal disease. This technique has been practised for many years and involves manually removing plaque/biofilm deposits on the tooth below the gum line followed by refinishing of the surface of the root to promote soft tissue reattachment. SRP is well known to result in modest clinical improvement in most patients suffering from mild to severe periodontal disease. However, a recent meta-analysis found that while SRP alone was
somewhat effective in improving clinical outcome, the most effective overall approach was SRP combined with a local antibiotic therapy. Furthermore, Bonito et al. conducted a review of over 40 published studies using local adjuncts to SRP and concluded that adding an antibacterial modality to the treatment of periodontal disease had a measurable positive impact on probing depth (PD) and clinical attachment level (CAL). These reviews suggest that manual removal of bacterial biofilms using SRP does not completely remove all pathogenic organisms, and that combining this with an approach to eradicate the small number of remaining organisms is the best way to create a healthy microenvironment for healing and reattachment. As a result, multiple adjunctive approaches have been evaluated including systemic antibiotics, locally applied antibiotics and topical disinfectants such as chlorhexidine gluconate. In practice, however, growing concerns over antibiotic resistance have made the widespread use of systemic antibiotics impractical. Furthermore, the difficulty in applying topical agents sub-gingivally combined with the propensity for rapid flushing due to excessive secretion of gingival crevicular fluid has limited the effectiveness of most topical adjuncts to SRP.

In recent years lasers have been developed for the thermal removal of sub-gingival plaque and biofilm with varying degrees of reported success. More recently, clinical studies using non-thermal laser systems in the mouth for aPDT have also emerged in the literature. As noted previously in this chapter, but worth reiterating in this context, aPDT eradicates biofilms via an oxygen-mediated photoreaction as opposed to traditional lasers for plaque removal which rely on thermal effects. Importantly, clinical studies of periodontal therapies can focus on microbiological end points (absolute reductions in periopathogenic or total sub-gingival bacterial counts), clinical end points (bleeding on probing, pocket probing depth, attachment level, etc.) or a combination of both. While clinical end points are relatively easily measured by a trained dental professional, studies involving microbiological end points require carefully constructed protocols and assays and can be prone to error during sampling. For these and other reasons relating to regulatory authority requirements, most of the published clinical work to date using aPDT to treat periodontal disease has focused on clinical end points as success indicators.

In one of the first published reports of aPDT for the treatment of periodontal disease in humans, ten patients with aggressive periodontal disease were treated using either methylene blue and laser light or conventional scaling and root planing. This small study utilized a split-mouth design and patients were measured at pre-treatment baseline and 3 months post-treatment for various clinical parameters including bleeding on probing, probing depth, gingival recession and clinical attachment level. The investigators found significant improvements in all measures from baseline to 3 months post-treatment, and no complications or adverse events were noted. The conclusion of the study was that aPDT produced clinical improvements in periodontal disease similar to that using the gold standard of SRP. It should be noted that the split-mouth study design has been criticized in the past because treatment in one quadrant can cause effects on the other side of the mouth (i.e. whole-mouth effect).
In 2009, the same group reported on further results from the same ten-patient study,\(^{86}\) in which samples of gingival crevicular fluid (GCF) were collected from aPDT and SRP treated periodontal pockets and subjected to protein analysis (ELISA) to quantify the cytokines TNF-alpha and RANKL (receptor activator of nuclear factor kappa-B ligand). The results of the study showed that both aPDT and SRP treatments led to significant reductions in TNF-alpha and RANKL in GCF at a period of 90 days post-treatment. The cytokine reductions observed were similar across the two treatment groups. These results show that aPDT using methylene blue and laser activation can lead to significant reductions in host-derived cytokines in the periodontal microenvironment. Since it is known that chronic sub-gingival bacterial colonization leads to immune activation and production of inflammatory cytokines, and that the presence of these cytokines over sustained periods plays a part in the tissue/bone destruction characteristic of periodontal disease, it can be concluded that inactivation of cytokines in the sub-gingival space would at least delay the progression of periodontal disease. The correlation in clinical measures and cytokine profiles across the two studies would seem to support this conclusion.

In another study using the Helbo Photodynamic Systems aPDT system, consisting of methylene blue with red laser light activation, 20 subjects were treated again using a split-mouth study design.\(^{87}\) In this design all patients received conventional SRP before randomization into groups receiving aPDT treatment in two of four quadrants. The clinical parameters measured at baseline, 1 week and 3 months post-treatment were sulcular fluid flow rate and bleeding on probing. In addition, measurements of probing depth, gingival recession and relative attachment level, as well as degree of tooth mobility and furcation involvement, were taken at baseline and 3 months post-treatment. The results of this study showed that at 3 months post-treatment, relative attachment level, probing depth, sulcular fluid flow rate and bleeding on probing improved significantly more in the aPDT group as compared to the SRP-alone group. As a result, the investigators concluded that adjunctive aPDT can improve outcomes seen with SRP in patients with chronic periodontal disease.

In 2007, Andersen et al.\(^{88}\) reported the results of a pilot human study to evaluate oral aPDT using the Periowave™ system, consisting of methylene blue in liquid formulation activated using 670 nm laser light. This randomized controlled trial involved 33 adult subjects presenting with chronic adult periodontal disease. Initially, probing depth measurements were obtained from all teeth (six sites per tooth) and candidate treatment sites with \(\geq 6\) mm depth were identified. In addition, bleeding on probing and clinical attachment level were measured before treatment. Subjects were randomly assigned to three groups: 1) SRP alone, 2) aPDT alone and 3) SRP with aPDT administered adjunctively. Measurements of probing depth, bleeding on probing and clinical attachment level were taken at 3, 6 and 12 weeks following treatment for each group. A total of 622 individual sub-gingival sites were treated as part of this study, and no treatment-related adverse events were reported. In the SRP with aPDT group, a significant improvement in attachment level was seen at 6 and
12 weeks compared to the SRP alone group (approximately 3-fold difference). Bleeding on probing was significantly improved in all three groups; however, the largest improvement was seen in the aPDT-alone group, in which bleeding decreased 71% at 6 weeks and 73% at 12 weeks. In the group treated with SRP and aPDT, there were also large improvements in probing depth, with mean reductions of 1.16 ± 0.39 mm and 1.11 ± 0.53 mm at 6 and 12 weeks, respectively (p < 0.06 for 6 weeks and p < 0.05 for 12 weeks compared to SRP alone). The results of this study showed that the Periowave™ aPDT system was effective when used adjunctively to the current standard therapy for periodontal disease. While this study suggested that aPDT alone showed clinical improvements similar to that of the SRP-alone group, eliminating the manual plaque/biofilm removal step in practice may not be advisable as presumably bacterial recolonization could occur faster on rough surfaces containing unremoved, previously treated, plaque deposits.

In a 2009 study, Chondros et al. examined the clinical and microbiological effects of aPDT adjunctively to SRP in patients already receiving supportive periodontal therapy. In that study, 24 patients received sub-gingival SRP alone or SRP followed by a single treatment of aPDT. Multiple clinical parameters (bleeding on probing, pocket depth, clinical attachment level, full mouth plaque score, gingival recession) as well as microbiological assessments were taken at baseline, 3 and 6 months. It was found that patients receiving adjunctive aPDT exhibited a statistically significant improvement in bleeding on probing compared to SRP-alone at 3 and 6 months post-treatment, although no differences were observed in pocket depth, clinical attachment level and full mouth plaque score. Microbiological results for this study were variable. While both SRP-alone and SRP with adjunctive aPDT groups showed significant reductions from baseline in several common periodontal pathogens, the aPDT group also had significantly lower levels of the bacterial species *Fusobacterium nucleatum* and *Eikenella corrodens* at 3 months as compared to the SRP-alone treatment group.

In the largest clinical study of aPDT in the oral cavity conducted to date, 121 Canadian patients were enrolled in a randomized, prospective, examiner-blinded, parallel-group study evaluating aPDT for the treatment of chronic adult periodontal disease. The results of this study, taking place across three major dental schools and one private clinical practice, have been submitted in full elsewhere for publication but will be summarized as part of this chapter. Subjects diagnosed with chronic periodontal disease, with at least 18 or more fully erupted teeth and at least 4 measurement sites exhibiting probing depth of 6–9 mm in at least two quadrants of the mouth were eligible to participate in this study. Subjects were randomized into two protocol groups: (1) Control Group receiving SRP-alone and (2) Test Group receiving SRP followed by aPDT treatment in qualifying sites. aPDT was carried out using the Periowave™ system, consisting of sub-gingival placement of a methylene blue based photosensitizer formulation followed by 60 second (per site) illumination with 670 nm wavelength laser light. Two different energy doses were used over the course of the study (15 patients received 9 joules per aPDT site and 48 patients
received 13 joules per aPDT site), and the population treated with the higher dose was analysed along with the total data set as well as separately (post-hoc) to determine whether an increase in energy dose influenced clinical efficacy. Two clinical measurement indices (clinical attachment level and probing depth) as well as a scoring evaluation (bleeding on probing) were used as end points, with no microbiological assessment performed as part of the study. For the treatment protocol, SRP treatment was administered over two separate sessions (half mouth per session), and aPDT was carried out during the second visit in the test arm. Clinical outcome was assessed at 6 and 12 weeks after the final treatment visit. In all, measurements were taken from more than 4000 treated periodontal sites over the course of the study. Taking all aPDT treated pockets together (i.e., combining subjects that received 9 and 13 joule energy doses), with a single treatment of aPDT adjunctively to SRP the improvements achieved for clinical attachment level and probing depth at 6 and 12 weeks, while clinically significant, fell just short of statistical significance in terms of superiority to SRP alone ($p = 0.07$ and $p = 0.10$, respectively). However, in the subset of patients ($n = 48$) receiving the higher energy dose (13 joules per site), Periowave™ adjunctive to SRP resulted in highly statistically significant improvements in both attachment level and probing depth compared to SRP alone ($p < 0.0001$ vs. SRP alone for attachment level and probing depth). These results are summarized in Table 13.1. It should also be noted that clinical attachment level improvement for all measured pockets (both treated and untreated) in the aPDT treatment arm showed a statistically significant improvement ($p < 0.01$) over that in the SRP-alone arm. This observation implies a derived benefit from aPDT, most likely via a “whole-mouth effect”, which is a derived benefit throughout the mouth in response to treatment of specific infected sites. This whole-mouth effect extended to probing depth reduction as well; analysis of probing depth reduction for all measured pockets in the aPDT arm demonstrated a statistically significant response ($p < 0.05$) compared to the SRP-alone arm. The results of this controlled multicentre clinical study clearly demonstrated the utility of aPDT administered adjunctively to the current standard of care in a North American population. In post-hoc analysis it was also shown that energy dose is a key variable, and there appears to be a clear relationship between energy applied and magnitude of

<table>
<thead>
<tr>
<th>Table 13.1</th>
<th>Clinical outcomes in patients ($n = 106$) receiving SRP alone or SRP with adjunctive aPDT using a 13 joule energy dose. Values are reported as change in clinical attachment level or probing depth in millimetres from a pre-treatment baseline measurement.</th>
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<tr>
<td></td>
<td>Clinical attachment level</td>
</tr>
<tr>
<td></td>
<td>SRP-Alone</td>
</tr>
<tr>
<td>Number of pockets</td>
<td>2349</td>
</tr>
<tr>
<td>Week 6 change (mm)</td>
<td>$-0.50 + 0.05$</td>
</tr>
<tr>
<td>Week 12 change (mm)</td>
<td>$-0.54 + 0.05$</td>
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$^*$ indicates significance at $p < 0.0001$ vs. SRP alone for global treatment effect (week 6 and 12 results combined).
response that should be considered carefully in the application of aPDT in a clinical setting.

Since the concept of using aPDT to treat oral infections was first contemplated by Wilson and others in the early 1990s, several reviews have been published worldwide detailing the potential of this application. The majority of these reviews have concluded that aPDT will emerge as an important treatment modality in oral/dental applications, with the caveat that further clinical evidence is needed to optimize the technology and drive adoption by clinicians. As noted in a more recent systematic review by Azarpazhooh et al., to date the success of aPDT in the clinic for the adjunctive treatment of periodontal disease has been variable. Reasons for this variability, while likely in part due to small sample sizes of studies published to date, can be theorized from preclinical studies that preceded this clinical work. As demonstrated in several preclinical aPDT studies, the importance of delivering an optimal activating light energy dose cannot be understated. While it is difficult to pinpoint a precise power density or energy dose threshold for clinical use using preclinical results, it is clear that antibacterial efficacy drops off dramatically when insufficient light is delivered to the site of treatment. Importantly, insufficient light dose can also occur with the use of non-optimized photosensitizer concentrations, as very high concentrations of dyes such as methylene blue and TBO lead to extreme interstitial light absorption, effectively “shielding” the bacterial biofilm from the photoreaction. These considerations further accentuate the need to optimize not only the light source/energy dose, but also the photosensitizer formulation used in clinical application.

13.9. Case Study 1 – Adjunctive aPDT for Severe Aggressive Adult Periodontitis

13.9.1. Background

Patient #1 was a healthy 28-year-old male with no familial history of systemic diseases. The patient was not taking any medications at the time of presentation, and did not have a history of smoking. A thorough medical check-up including extensive blood work revealed no significant findings. The patient did report a history of early tooth loss in his immediate family, and was subsequently diagnosed with severe generalized aggressive periodontitis. Because of the severity and presentation of the condition, this patient was deemed to be a suitable candidate for aPDT treatment in addition to standard non-surgical debridement (SRP).

13.9.2. Treatment

All examinations and treatments were performed at the Department of Periodontics at McGill University in Montreal, Canada. After a complete initial periodontal examination, non-surgical SRP was performed under local
anaesthesia over the course of three appointments. aPDT was administered after SRP at each appointment using the Periowave™ system, consisting of sub-gingival application of a liquid methylene blue-based photosensitizer formulation followed by 60 second illumination from a non-thermal 670 nm diode laser. The full treatment schedule was as follows: at appointment #1 SRP and aPDT were performed in quadrants 1 and 4, at appointment #2 SRP and aPDT were performed in quadrants 2 and 3 with aPDT repeated in quadrants 1 and 4 and at appointment #3 aPDT was repeated in quadrants 2 and 3. The time between each of these appointments was 2 to 3 weeks. Teeth 34 and 35 were extracted during appointment #2, and tooth 11 exfoliated spontaneously prior to therapy. The patient was seen every three months for maintenance recall therapy where supra- and sub-gingival scaling was performed and oral hygiene instructions re-enforced. Photographs and panoramic radiographs were taken prior to therapy and at 1 year post-treatment. The patient did not take any local or systemic antibiotics over the course of the treatment and follow-up periods.

13.9.3. Results

Figure 13.1 shows a photograph of the patient’s upper and lower dentition prior to therapy. Note the inflammation of the gingival tissues and the heavy calculus deposits. Probing depths >6 mm and bleeding on probing were generalized. All the teeth had a class II or higher mobility. Figure 13.2 shows the panoramic radiographic presentation of the case pre-operatively. Note the extensive generalized alveolar bone loss. Figure 13.3 shows the same photographic view taken at a 1-year post-treatment follow-up visit. Figure 13.4 depicts the corresponding panoramic radiograph at 1 year post-treatment. The probing depths were reduced to <4 mm, and minimal bleeding on probing was

Figure 13.1  Dental photograph of Patient #1 taken prior to debridement and aPDT.
Figure 13.2  Panoramic radiograph of Patient #1 taken prior to debridement and aPDT.

Figure 13.3  Dental photograph of Patient #1 taken at one year post-treatment. Treatment consisted of three appointments to conduct full mouth debridement with two rounds of adjunctive aPDT.

Figure 13.4  Panoramic radiograph of Patient #1 taken at one year post-treatment.
observed. In addition, the tooth mobility was significantly reduced. Of special note was the minimal amount of gingival recession observed. Figure 13.5 illustrates the probing depth and osseous improvements seen in one of Patient #1's treated molar sites at 1 year follow-up post-initiation of treatment.

13.9.4. Conclusions

The results seen in this patient suggest that the combination of standard SRP and adjunctive aPDT are effective in restoring healthy gingival tissue, reducing probing depth and promoting bone regeneration in adult individuals with aggressive periodontitis. The clinical improvements seen in this patient following treatment are well in excess of what would normally be expected with standard SRP/debridement techniques.

13.10. Case Study 2 – Osseous Changes after Adjunctive aPDT Treatment for Aggressive Periodontitis

13.10.1. Background

Patient #2 was a 12-year-old boy presenting to the clinic with severe aggressive periodontitis. The patient was a non-smoker, was not on any medications and had no significant medical history otherwise.

13.10.2. Treatment

All examinations and treatments were performed at the Department of Periodontics at McGill University in Montreal, Canada. After a complete initial periodontal examination, non-surgical SRP was performed under local
anaesthesia over the course of three appointments. aPDT was administered after SRP at each appointment using the Periowave™ system, consisting of sub-gingival application of a liquid methylene blue-based photosensitizer formulation followed by 60 second illumination from a non-thermal 670 nm diode laser. The teeth exhibiting deep pockets (>7 mm) were treated at each probing site for 60 seconds or more buccally and lingually. Figures 13.6 and 13.7 show representative photographs of placement of photosensitizer and illumination in a single site, respectively. The full treatment schedule was as follows: at appointment #1 SRP and aPDT were performed in quadrants 1 and 4, at appointment #2 SRP and aPDT were performed in quadrants 2 and 3 with

Figure 13.6  Photograph of methylene blue photosensitizer formulation being placed subgingivally during aPDT treatment.

Figure 13.7  Photograph of subgingival illumination using the Periowave™ aPDT system during aPDT treatment.
aPDT repeated in quadrants 1 and 4 and at appointment #3 aPDT was repeated in quadrants 2 and 3. After treatment, the patient was seen every three months for maintenance recall therapy where supra- and sub-gingival scaling was performed and oral hygiene instructions re-enforced. Radiographs were taken prior to initiation of treatment and at 6 months post-treatment. The patient did not take any local or systemic antibiotics over the course of the treatment and follow-up periods.

13.10.3. Results

In Figure 13.8, the radiographs taken from Patient #2 prior to therapy show extensive bone loss around all first molars. Figure 13.9 shows the radiographs from the same angle taken at 6 months post-SRP with adjunctive aPDT treatment. Note the regeneration of the bone within the defects.

13.10.4. Conclusions

Patient #2 exhibited severe periodontitis with extensive bone loss, and was at risk to lose several teeth at a young age. However, with the combination of non-surgical debridement therapy and adjunctive aPDT significant long-term clinical improvements were seen in both soft and hard tissue. Importantly, bone regeneration as shown by radiographs before and after treatment was pronounced, and certainly in excess of what would normally be anticipated after

Figure 13.8  Radiographs from Patient #2 taken prior to debridement and adjunctive aPDT treatment.
standard SRP therapy. This result supports the use of aPDT as an adjunct to non-surgical debridement for the treatment of aggressive periodontitis.

13.11. Other Clinical Applications for Oral aPDT

While the majority of preclinical and clinical oral aPDT work to date has focused on periodontal disease, there are several other microbiologically mediated oral conditions that aPDT may be useful for. As mentioned previously in the chapter, root caries are localized hard tissue demineralization caused by supragingival bacterial biofilm processes. However, to date no human clinical studies have been reported for the use of aPDT to prevent or treat carious lesions. Similarly, peri-implantitis is a condition occurring when bacterial pathogens infect the soft tissue around dental implants. This can rapidly lead to tissue/bone breakdown and eventually will result in loss of the implant itself. Peri-implantitis is known to be caused primarily by the same Gram-negative pathogens that mediate periodontal disease subgingivally, so it is reasonable to conclude that aPDT could be used for this application. While large animal (dog) studies have been conducted using aPDT to treat ligature-induced peri-implantitis, no clinical trials have been published to date in this area. Given the similarities in the aetiology of peri-implantitis and periodontal disease, it seems reasonable to predict that aPDT could be successfully used for this application.
Endodontic infections lead to necrosis of soft tissue within the tooth structure, and elimination of this infection is another potential application for aPDT. This level of infection almost always requires mechanical removal of infected tissue followed by sealing of the empty space created within the root; however, reinfection can result due to inadequate removal of persistent microorganisms. The major species of bacteria that cause endodontic infections are often different from those that are commonly associated with periodontal disease, and include *Enterococcus faecalis* along with various species of *Firmicutes, Bacteroidetes, Actinobacteria* and *Proteobacteria*. Several groups have published reports of photodynamic eradication of these bacterial strains in extracted tooth models. Soukos *et al.* demonstrated that methylene blue activated by high doses of red light eliminated 97% of *E. faecalis* biofilm inside experimentally infected root canals of extracted teeth. Several years later the same group measured the effectiveness of aPDT *in vitro* on root canal biofilms comprised of the oral bacteria *A. israelii, F. nucleatum, P. gingivalis* and *P. intermedia*. In that study, scanning electron microscopy (SEM) and viability assessments post-treatment showed 80% reductions in bacterial counts after treatment with methylene blue and red light. Garcez *et al.* also used an extracted tooth model infected with engineered bioluminescent strains of *P. mirabilis* and *P. aeruginosa* to evaluate the potential for aPDT to treat endodontic infections. In that study, the combination of conventional endodontic treatment and aPDT led to >98% reductions in bacterial viability and delayed re-growth within the root canal. Finally, another study showed that the optimization of excipients/solvents used to formulate methylene blue led to enhanced penetration of dentinal tubules and greater antibacterial efficacy in infected extracted tooth root canals. To the author's knowledge, no clinical studies evaluating aPDT for endodontic infections have been undertaken to date. The relatively rare incidence of endodontic therapy failures, coupled with the long follow-up periods required, complicate the design of a clinical study in this area, although the promising preclinical results reported suggest utility in this application.

Finally, oral candidiasis represents the most common fungal infection in humans. Pathogenesis of this condition is not fully understood, but it is believed sometimes to result from a bacterial disturbance/imbalance allowing unchecked growth of the fungal species on previously occupied oral tissues. Several preclinical studies have shown that aPDT eradicates *Candida sp.* in lab models, and that this likely occurs via membrane damage and permeabilization. Specific methods of photosensitizer delivery in the mouth have even been proposed for this application, but no clinical studies have been reported to date using aPDT to treat oral candidiasis.

### 13.12. Conclusions and Future Directions

The phenothiazinium photosensitizers methylene blue and TBO have been the most widely used to date for oral antimicrobial applications. While there has
been recent work examining the mechanism of antibacterial action for activated phenothiaziniums,\textsuperscript{110,111} future studies should address the contribution of type I and type II photoreactions at a biochemical level with attention to variability between pathogenic strains. Moreover, other phenotypic strain-specific characteristics such as matrix secretion, production of singlet oxygen scavengers and propensity for biofilm formation may influence the efficacy of aPDT \textit{in vivo}. In addition, the significance of differences in the mechanism of action of aPDT acting on Gram-positive and Gram-negative organisms should be examined \textit{in vivo} with an eye towards selectivity to periodontal pathogens. Finally, light dosimetry is another parameter that has been addressed in several preclinical studies, but needs to be studied more closely in future clinical work. So far, clinical evidence suggests that there is a threshold energy dose required to achieve significant clinical outcomes in the treatment of periodontal disease. Most importantly, as a rule any aPDT systems under development should be extensively optimized in \textit{in vitro} and animal models with respect to matching of photosensitizer (type, concentration, formulation) and light source (type, power output, wavelength) before being subjected to the rigours of clinical trials in humans. As with any emerging technology, premature human testing and/or commercialization of non-optimized systems poses a serious threat to the acceptance of aPDT for oral indications and to eventual adoption of the technology into clinical practice.

Taken together, the preclinical and clinical data reported to date convincingly support the use of aPDT in the oral cavity from both a safety and an efficacy standpoint. The vast majority of published work to date focuses on aPDT for the treatment of periodontal disease; however, there are several other indications for which the technology may be useful. In addition to a growing body of literature supporting aPDT efficacy in the treatment of periodontal diseases, the impressive safety profile for this technology should also be noted. Specifically, none of the published clinical reports to date have noted significant adverse events or safety concerns related to oral aPDT treatment. This is to be expected, as the treatment is topical in nature and the photosensitizers used clinically to date have well-known properties and safety profiles. It is anticipated that continued development of aPDT applications for the oral cavity will underscore both the clinical utility and “patient-friendly” nature of the technology.

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