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# Laser/Light Application in Dental Procedures

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### Abstract

The oral cavity is a relatively confined anatomical region, with a close approximation of hard and soft tissue structures. The comparatively delicate nature of each tissue structure places demands on surgical techniques using lasers and the maximum power parameters used.

The predictable application of any laser wavelength currently commercially-available may be viewed as an expression of incident power and the targeting of a predominant chromophore in the exposed tissue. By way of exploring laser use in dentistry, this may be summarised as surgical management of oral hard tissue and soft tissue, non-surgical application and antibacterial applications.

Laser use in clinical dentistry has spanned 25 years. Although early laser use in general surgery (notably Carbon Dioxide, wavelength 10.6  $\mu$ m) was applied to soft tissue surgical procedures in the mouth, the first true dental laser was a Neodymium YAG (1.064  $\mu$ m) which was launched in 1989. From there the

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63 Walton Park, Harrogate HG1 5LT, North Yorkshire, UK e-mail: thewholetooth@easynet.co.uk next 5 years witnessed the emergence of other major wavelengths, notably the two Erbium wavelengths (Er:YAG and Er,Cr:YSGG) and Diode group of semiconductor based technology. Latterly, the most recent 5–10 years has seen an emergence of technical developments to optimise the interaction of chosen laser photonic emission with target oral tissue; this manipulation of energy and time parameters shows may herald even greater application of lasers in dentistry.

The current commercial developments in wavelength application in dentistry have resulted in lasers whose emissions span the visible, near-, mid- and far-infra-red portions of the electromagnetic spectrum.

Non-surgical, low level laser applications include photo-biomodulation, diagnostics, photo-activated anti-bacterial processes, laser tooth whitening and laser-scanning of tooth cavity preparations.

#### Keywords

Laser dentistry · Oral soft tissue · Oral hard tissue · Photothermolysis · Photobiomodulation · Spallation · Antibacterial photodynamic therapy

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## Perceptions of Laser Use in Dentistry

There is a general paradox surrounding the use of lasers in dentistry; for the majority of patients, dental treatment only extends to the treatment of dental caries ("tooth decay") and, latterly the possibility of cosmetic improvement of teeth including veneers and tooth whitening. The greatest disincentive to dental treatment of this nature has been a perception (or reality) of pain associated with the procedure. Any treatment modality that could address this aspect would be eagerly accepted and the overall marketing concept of the word "laser" only serves to endorse the potential appeal of this instrument in delivering a (supposed) quick, painless and "high-tech" resolution of their dental condition.

For the dentist, the historical approach to the treatment of dental disease and associated procedures has been to use rotary instruments at high speed to remove carious tissue and develop tooth cavities that would be retentive for silver-amalgam restorative material. In addition, the scope of practice of most dentists would extend to treatment of associated soft tissue structures in the mouth, where a majority of procedures would be carried out with a scalpel. Although acknowledgement exists as to the need to control bleeding and postoperative sepsis in soft tissue surgery and tooth damage such as heat, micro-cracking and gross tissue destruction in using a drill, there remains a dogma (not least underlined through undergraduate teaching) that such consequences remained but incidental to conventional instrumentation that could deliver rapid treatment procedures.

In addition, the move away from gross tooth tissue removal and amalgam restoratives, towards a minimalistic, more interceptive and preventative approach to caries treatment has led to a dramatic growth in the use of micro-retentive, bonded nonmetallic restorative materials that address the early carious lesion. The wish to employ adjunctive soft tissue management procedures during tooth tissue surgery places a great need for haemostatic incisions that are not subject to bacterial contamination and additional emerging treatment of periodontal, endodontic and implant-related structures, all constitute needs that can be addressed through laser technology. As such, the complexity and demands of oral tissue treatment has led to the development of laser wavelengths and machines that specifically address the treatment whilst minimising collateral damage. Far from the "nomadic" intrusion of lasers in general into dentistry, the speciality has justified the production of procedure-specific lasers. With such refinement has come responsibility and an ever-growing shift from anecdote and single case presentation, to evidence-based and statistically-robust investigation. For example, a simple search of peer-review

## Dynamics of Laser Light in Dental Procedures

papers using keywords "laser" and "dentistry", provides almost 10,000 individual studies.

The preferred laser-tissue interaction is effected through the maximal absorption of incident laser light by a predominant tissue (or other structural element) chromophore; in surgical use, such absorption leads to the conversion of photonic energy into thermal energy which, if controlled leads to predictable target change with minimal collateral, non-target damage. The complexity of most oral and dental tissue, together with the close approximation of hard and soft tissue elements, places great demands on the clinician to observe care in the selection of laser wavelength, of sufficient incident power to ablate tissue but not to cause adjacent damage. Optimally, the laser wavelength should be one that is maximally absorbed by the predominant tissue chromophore (Fig. 4.1).

Within the short space of time that lasers have been used in dentistry, there has been a greater understanding that, apart from the absorption/ chromophore phenomenon there exists the effect of incident energy dynamics in relation to a desired target tissue effect.

Reference to Fig. 4.2 provides a simple schematic to the pathway of photonic energy absorption leading to irreversible dissociation of a target chromophore. In an ideal surgical (ablative) laser-tissue interaction, this process would predominate; however, often the wider implica-





Fig. 4.2 Fibroma of traumatic origin, lateral border of the tongue

tion of (secondary) conductive thermal events provides a greater understanding of true photothermolysis, that combines a pure consequence of laser photonic irradiation with the outcome of hyperthemic tissue effects.

The early surgical lasers of the 1960s—for example the CO<sub>2</sub> laser, emitted laser energy at 10,600 nm (10<sup>-9</sup> m) which would be preferentially absorbed by water present in target tissue. The continuous wave emission mode led to unremitting thermal effects in the tissue, which potentiated the prospect of conductive heat damage to adjacent structures. With no thermal relaxation, the greater the incident laser power, the greater the rise in temperature. With the introduction of gated- and free-running-pulsed laser delivery, the thermal relaxation potential became greater and higher peak power values could be achieved, whilst retaining a low average power.

Laser photonic energy is a form of electromagnetic energy and the concept of both energy transfer and conversion belies the dynamics of laser-tissue interaction. Figure 4.3a provides a schematic of how, within a "pulse" of laser emission, the extent and value of energy delivered can be appreciated and measured. As may be seen in Fig. 4.3b, if the wave-trace of the pulse has equal rise and fall gradients, it is representative of a time period during which the photonic energy is transferred to the target tissue; in terms of photothermal exchange, this ideal represents an equal amount of tissue heating and thermal relaxation. The total time period if seen in terms of thermal change, represents a "footprint" of energy delivery and capacity of the target tissue to cool.

Soft tissue laser treatment demands efficient tissue ablation together with (mostly) optimal haemostasis; both effects can be achieved through a temperature rise of between 60 and 100 °C. With a shorter wavelength laser (500–1400 nm) the pro-



Fig. 4.3 Nd: YAG (1064 nm) laser used to resect the lesion (320 µm fibre/contact/200mj pp./10 Hz/Av. Power 3.0 W)

tein denaturation of pigmented tissue components occurs, whereas with longer (2000–11,000 nm) wavelengths, tissue ablation occurs through water vaporisation. To deliver free-running-pulsed laser energy in a micro-second symmetrical peak-wave configuration with shorter wavelengths, the tissue effect is desirous in achieving ablation and haemostasis. Similar wave configuration with longer wavelengths achieves the vaporisation of water, but insufficient conductive thermal exchange occurs to achieve haemostasis. In these cases, it is preferable to use a laser light waveform that has a rapid energy rise and a slower decline to allow diffusion of energy into the tissue (Fig. 4.3c).

With hard tissue ablation, the demands of energy transfer are much more significant, if unwanted effects of thermal cracking, carbonisation and pulpal damage are to be avoided. Here, the need is to deliver a waveform that has a rapid high-energy rise with an almost-instantaneous fall (Fig 4.3d).

The predominant laser-tissue effect in hard tissue ablation is dislocation of structure through interstitial water vaporisation, although the future development of micro-second "pulsed"  $CO_2$  (predominately 9300 nm) lasers already provides great potential for the true ablation of the mineral component of tooth tissue. To this extent and beyond, nano-and femto-second photonic delivery mechanisms provide opportunity to capture near-plasmolytic power density effects of disparate laser wavelengths applied to host tissue and effecting predictable ablation without reference to target chromophores.

#### Use of Lasers with Oral Soft Tissue

The use of a laser, as with more conventional instruments, demands of the clinician basic surgical skills which should remain paramount. Knowledge of the anatomical site, sound diagnostic skills, appreciation of the desired postsurgical outcome and functional needs should be combined with a thorough understanding of the patient's dental and medical history. Where appropriate, the nature of any pathology should be assessed prior to surgical intervention and referral protocols for specialist care should apply, if necessary.

In an otherwise healthy individual, the biological mechanisms that allow healing to take place will always follow the same pathways, irrespective of whether tissue injury is due to a scalpel, thermal, chemical or traumatic cause [1]. Consolidation of the wound-blood clotting and plasma retention, elimination of bacterial contamination and aspects of the inflammatory response—is followed by an in-growth of epithelial and endothelial cell types, which then proceeds to a maturation of wound healing over time. Any potential for scar tissue formation can be affected by the type of tissue, the cause of the wound, presence of tissue mediators and growth factors, whether healing is by primary or secondary intention and, occasionally, racial type [2, 3]. In an ideal situation, the post-surgical healing will be such as to restore stability, form and function to the tissue. In oral soft tissue surgery, where appropriate, the aesthetic appearance of the tissue will be maintained or, as is often the desired outcome improved with regard to dental restorations.

All currently-available laser wavelengths can be used in soft tissue surgical procedures [4, 5]. The structure of oral epithelium—an outer epithelial layer overlying deeper endothelial elements, offers a predominant water chromophore superficially which will absorb longer wavelengths, whereas deeper structures containing pigmented protein—melanin and haemoglobin will absorb shorter wavelengths.

With laser use in surgical soft tissue procedures, assuming correct laser wavelength per tissue site and appropriate power parameters, the healing of oral soft tissue is often termed 'uneventful' [6, 7]. Often, if not always, the need for dressings or sutures is avoided. Irrespective of the wavelength, all soft tissue healing will be by secondary intention. Of note, however, is the phenomenon of lack of postincisional contamination by bacteria, due to a possible reduction in bacteria during tissue ablation [8], but certainly through the protective layer of coagulum of plasma and blood products—a tenacious film that allows early healing to take place underneath [9, 10]. Additionally, studies with longer wavelengths show that there is a lack of fibroblast alignment associated with the incision line and consequent reduced tissue shrinkage through scarring [11]. Such findings are often borne out in the clinical setting (Figs. 4.4, 4.5, 4.6, and 4.7).

The objective of correct ('safe') laser energy per surgical site shall be to use a minimum



**Fig. 4.4** Immediate post-treatment. The site shows a central area of ablation, surrounded by a zone o reversible oedema. Haemostasis is good



Fig. 4.5 Healing at 3 weeks



Fig. 4.6 Denture granuloma lingual aspect of edentulous lower ridge



**Fig. 4.7** Diode (810 nm) laser used to resect excess tissue (320 µm fibre/contact/CW Av. Power 1.0 W)

level commensurate with the desired effect. Insufficient laser energy levels may only warm the tissue and not initiate tissue ablation, whereas excessive levels can lead to carbonisation and possible deep collateral thermal damage. Carbon, whether present as a build-up on a fibre tip or tissue surface, absorbs all light wavelengths and quickly over-heats. This becomes a source of secondary thermal energy and acts as a 'branding iron', leading to conductive thermal damage [12]. For most intra-oral minor surgical procedures, irrespective of the wavelength chosen an average laser power setting should be in the range of 1–3 W. This is based on personal experience and recommended levels found in manufacturers' user manuals.

Care should always be given to the delicate nature of oral soft tissue and the close anatomical association of bone and/or dental structures. Deep penetration of shorter wavelengths, especially in the absence of a water spray or in continuous-wave or gated CW emission modes may cause overheating and carbonisation of hard tissue. Longer wavelengths used without water spray or scant regard to thermal relaxation can similarly affect adjacent structures. The Erbium group of wavelengths (Er:YAG-2940 nm, Er,Cr:YSGG-2780 nm) are commonly used with a co-axial water spray and a short-pulse emission mode, which poses little danger of overheating. Nonetheless, unwanted ablation of hard tissue may occur with inadvertent or careless use in close association with such structures.

In certain areas of the mouth (sub-glossal, lingual aspect of the retromolar area and the mental foramen in the lower jaw and incisive foramen in the upper jaw), vital structures (submandibular duct and nerves respective to foramina) lie very close to the surface and care must be observed when using lasers in these areas [13].

'Safe' soft tissue cleavage, avoiding the potential of collateral thermal damage is related to correct wavelength/tissue assessment, minimum laser power values to achieve tissue cleavage and thermal relaxation measures to prevent heat build-up. In some cases, it is advisable to pre-cool the tissue with gauze soaked in icewater, although the close approximation of high-speed suction will assist in providing external tissue cooling. Shorter laser wavelengths (KTP-532 nm; Diode-810, 940, 980 nm; Nd: YAG, 1064 nm) transverse the epithelium and can penetrate 2-6 mm into the tissue, whereas longer wavelengths have minimal penetration [14]. Shorter, visible and near-infrared wavelengths are readily absorbed by pigmented tissue. This can be used advantageously in the treatment of small lesions, especially those of possible traumatic origin [15-18](Figs. 4.8, 4.9, 4.10, and 4.11).



Fig. 4.8 Immediate post-treatment



Fig. 4.9 Healing at 3 weeks. Denture border adapted to new periphery



Fig. 4.10 Combined gingivoplasty/full veneer crowns. Pre-treatment



Fig. 4.11 Gingivoplasty. Erbium YAG (2940 nm) laser (400  $\mu$ m tip/non-contact/150 mj pp./10 Hz/+H<sub>2</sub>O/Av. Power 1.5 W)

With longer wavelengths (Er,Cr:YSGG—2780 nm; Er:YAG—2940 nm;  $CO_2$ —10,600 nm) the risk of deep penetration is minimised and surgical incisions can be deemed less potentially damaging [5].

Probably, the most common procedures include the use of lasers in a range of gingival adaptation procedures, both to allow hard dental tissue restoration and to provide access to crown and tooth cavity margins during restorative procedures. Another large area of clinical activity is the resection of excess gingival tissue (gingivoplasty) associated with the cosmetic enhancement of the appearance of the teeth, either as a stand-alone procedure or in connection with the provision of laminate veneers or crowns. Care should be exercised to avoid violation of the biologic width and to preserve a sufficient width of attached gingiva [19–24] (Figs. 4.12, 4.13, 4.14 and 4.15).

The facility to combine soft tissue management with hard tissue treatment is a major benefit of laser use, when compared to more conventional therapy. This represents considerable advantage to the clinician and the patient management is deemed less complicated, as appointments can be condensed and sutures and dressing packs avoided. Very often, a tooth fracture, otherwise committed to



Fig. 4.12 Immediate post-treatment. Associated upper labial frenectomy using  $CO_2$  laser



**Fig. 4.15** Diode (810 nm) laser used to resect excess tissue (320 µm fibre/contact/CW Av. Power 1.5 W)



Fig. 4.13 Completed case at 12 months



Fig. 4.14 Mucocele lower lip

extraction, can be treated and restored successfully, resulting in many more years of function. In the surgical adjunct to orthodontics, from gingival hyperplasia associated with orthodontic appliances to the exposure of unerupted teeth, the use of laser wavelengths can often enable simple procedures to be carried out without subjecting the child patient to additional anxiety. Both short and long wavelengths can be used, taking care not to cause damage to the underlying tooth or bone, relative to wavelength employed [25–27]. The control of bleeding will allow the placement of bonded orthodontic brackets without undue risk of failure.

The range of benign pathology affecting the muco-periosteal tissue of the dento-alveolar complex includes the following: epulis, giant cell granuloma, inflammatory and drug-induced gingival hyperplasia and cosmetic melanin removal. In addition, pathology affecting non-keratinised gingival tissue and all other accessible soft tissue structures may be commonly-seen within general dental practice; this would include the removal of fibromata, mucocoele, small haemangiomata, denture granulomata, labial and lingual frenectomies and treatment of non-erosive lichen planus and mucocytosis [28].

The aetiology of the lesion should be assessed, together with an understanding of the tissue composition, which will assist in wavelength choice and power parameters used. As with a scalpel, the tissue should be placed under tension, if possible to allow accurate and easier cleavage. In most cases, the laser hand-piece tip is held in close approximation to, and just out of contact with, the tissue surface. In this way the laser energy is allowed to effect the incision and minimise the build-up of debris on the laser delivery tip (Figs. 4.16, 4.17, 4.18 and 4.19).

With simple hyperplasia, wherever possible the laser beam should be directed into the discard tissue and excision completed in a careful and deliberate manner. With a more pedunculated epulis, it should be possible to aid excision by placing the lesion under tension. Aetiological factors should obviously be addressed to prevent recurrence. The use of lasers to treat drug-induced gingival hyperplasia can be of great assistance where either the general medical condition merits a simple surgical procedure, or the underlying blood-picture is compromised.



Fig. 4.16 Immediate post-treatment



Fig. 4.17 Healing at 1 month



**Fig. 4.18** Scanning electron micrograph of human enamel exposed to Er:YAG 2940 nm laser energy. Note the fragmented margin and absence of thermal cracking. Magnification ×500



**Fig. 4.19** Absorption coefficients of carbonated hydroxyapatite verses laser wavelength. The absorption peaks represent component radicals of the molecule (hydroxyl, free-water, carbonate, phosphate). The dotted line represents the absorption of laser energy in whole water Where aesthetics are compromised by melanin patches on the attached gingiva, often seen in Asian and African ethnic groups, an alternative approach to a 'dermabrasion' technique is to use laser energy. Most current wavelengths have been advocated, citing either selective pigment ablation with short wavelengths or superficial layer ablation of the tissue with longer wavelengths [29, 30]. The correct use of the selected laser results in little or no discomfort or inflammation compared to removal using rotary instrumentation [31, 32].

### Use of Lasers with Oral Hard Tissue

The cutting of dental hard tissue during restorative procedures presents considerable demands on the ability to selectively remove diseased carious tissue, obtain outline and retention form and maintain the integrity of supporting tooth tissue without structural weakening. In addition, the requirement to preserve healthy tissue and prevent further breakdown of the restoration places the choice of instrumentation and clinical technique as prime factors for the dental surgeon. Laser use, when compared to conventional rotary instrumentation may offer the following advantages:

- Precise ablation of hard tissue
- Selective ablation of diseased tissue over healthy tissue
- · Ability to utilise micro-retentive cavity design
- Less risk of pulpal injury
- Less risk of thermal and mechanical cracking of tooth structure
- Reduction in bacterial contamination of the tooth cavity
- Less discomfort for the patient

The prime chromophore in current laser application with hard tissue is water; the absorption peak at around 3.0  $\mu$ m wavelength identifies the Er:YAG (2940 nm) and Er,Cr:YSGG (2780 nm), collectively "Erbium" laser wavelengths as the lasers of choice.

Enamel is composed, by volume 85% mineral (predominately carbonated hydroxyapatite) 12%

water and 3% organic proteins. The majority of free water exists within the peri-prismatic protein matrix. Of the major hard tissues, enamel exhibits greatest resistance to laser ablation and this is seen most in healthy, fluoridated, occlusal sites, where ablation rate is approximately 20% of that achieved with a turbine [33–36]. Fluoridated enamel presents a greater resistance, due to the combined effects of a harder fluorapatite mineral and the replacement of the hydroxyl group by fluoride.

Dentine has a higher water content and less mineral density than enamel, being 47% by volume mineral (carbonated hydroxyapatite), 33% protein (mostly collagen) and 20% water. Consequently, ablation rates are faster than for enamel and power parameters can be correspondingly lower. Similar differences occur in the use of Erbium lasers on deciduous tooth tissue [37].

With the Erbium group of lasers the free-running micro-pulse (FRP) emission mode results in rapid and expansive vaporisation of interstitial water and dissociation of the hydroxyl radical in the hydroxyapatite crystal causing an explosive dislocation of the gross structure [33, 38, 39]. Early study into the use of FRP photonic energy at 3.0 µm assumed this dislocation-termed spallation-occurred at normal temperature and pressure [40]. However, the exact mechanism whereby target enamel and dentine may be ablated proposes that absorption of mid-infrared radiation by water within the crystalline complex of carbonated hydroxyapatite (CHA) is a function of temperature and pressure, both of which rise rapidly during an ablative laser pulse train [41]. In some circumstances, the close approximation of laser delivery tip to the tooth surface, the presence of co-axial water in abundance and the prospect of superheated vaporisation may suggest precise ablation at high temperature, with associated cavitation phenomena assisting in the dislocation of crystalling tooth tissue [42, 43] (Fig. 4.20).

The Er,Cr:YSGG laser has a lower absorption coefficient in water than Er:YAG (4000 cm<sup>-1</sup> vs. 13,000 cm<sup>-1</sup> for Er:YAG). When one examines the absorption curve of CHA (enamel), there is a peak, coincident with 2700 nm, representing absorption by the hydroxyl group (OH-) contained in the mineral molecule. It is thought that the simultane-





**Fig. 4.21** Carious cavity buccal margin upper left bicuspid. Caries removed and cavity prepared using the Er:YAG 2940 nm laser (800 μm sapphire tip/contact/350mj pp/10 Hz/Water spray/Av. Power 3.5 W). Insert SEM of margin. Magnification ×1000

ous ablation of this radical, with concomitant rapid heating of the mineral together with some direct vaporisation of whole water in hard tissue, contributes to the explosive dislocation of the target tissue when using this laser [44] (Fig. 4.21).

The 160 nm difference in wavelength measurement between Er,Cr:YSGG and Er:YAG has appreciable effect in terms of the absorption in water; this amounts to a 300% difference between the higher (Er:YAG) absorption and the lower Er,Cr:YSGG [45], but although differences appear in terms of ablation threshold [46], this does not amount to discernable differences in clinical use [47].

Clinically, this is seen as ejection of microfragments of tooth tissue within the laser plume and the change in pressure in the immediately surrounding air results in an audible "popping" sound. In target tissue that has greater water content (caries > dentine > enamel), the popping sound is louder. With experience, this can aid the clinician in selectively ablating carious verses non-carious tissue. Compared to near infra-red wavelengths, the explosive outward effect of Erbium laser energy results in minimal thermal diffusion through the tooth structure. With carious dentine there is a potential in gross caries for the laser beam to quickly pass through the surface layer, thus leading to dehydration in deeper layers. Where gross caries is present it is advisable to use an excavator to remove bulk volume, both to prevent heat damage and to expedite cavity preparation. In addition, compared to bur preparation, there is an absence of smear layer and no alteration in composition or hardness of the cut tooth tissue surface [48]; as



Fig. 4.22 Scanning electron micrograph of porcine bone exposed to Er:YAG 2940 nm laser energy. Note the clean cut margin and absence of thermal cracking. Magnification ×500

such it is advisable to use a dentine protector on open tubules exposed by the ablation process [49] (Fig. 4.22). Co-axial with this laser is a water spray, to aid in dispersing ablation products and to provide cooling of the target site.

With laser-assisted cavity preparation, it is not possible to create sharp cavo-surface line angles and retention form has been addressed through the possibility of micro-retention of composite resin materials [50]. Clinical procedures that may be considered under this heading include fissure sealing, direct composite resin veneers and orthodontic bracket placement.

There is some controversy over the marginal seal and stability of the composite restoration when the cavity has been prepared with a laser. Certainly, the gross appearance of the cavity margin when dried resembles an etch-like appearance, but this is due to the fragmentation of the tooth structure (Fig. 4.23). A majority opinion exists to advocate the additional acid-etch of the cavity margins [51-57], although some studies have suggested that the strength of the seal is less than in conventionally-prepared restorations [58] (Fig. 4.24).

There have been many claims—mostly anecdotal—that in the clinical setting, laser-assisted tooth cavity preparation is painless. Perhaps what is more accurate and borne out through many studies, is the reduced discomfort of laser action, compared to high-speed rotary instrumentation [59–62].



Fig. 4.23 Use of 400 µm hollow tip with Er: YAG laser



Fig. 4.24 Use of laser energy in debridement of the inflamed periodontal pocket. The quartz optic fibre (diameter  $200-320 \ \mu m$ ) allows easy access when using near IR wavelengths

By far the greater application of photonic energy in tooth tissue ablation has been through the Erbium group at approximately 3.0 µm. During the past 10 years, experimental action has given way to clinically-applicable devices whereby ultra-short laser pulses of Near-Infra-Red and Far-Infra-Red irradiation can be used to efficiently ablate tooth tissue. Notable in this regard has been the utilisation of the 9.3 µm wavelength which is highly absorbed by the phosphate radical within the carbonated hydroxyapatite crystal lattice. Hitherto, Carbon dioxide laser wavelength has been emission at 10.6 µm, with preferable absorption in water; the CW or gated CW emission would lead to rapid and destructive thermal rise. The new, filtered, shorter wavelength is delivered at micro-second emission (pulse duration of 10 to 15 µs and repetition rate of 300 Hz) with minimal thermal "foot-print" [63, 64]. Added to this, an ultra-short pulsed laser (Nd:YVO<sub>4</sub>  $\lambda$  = 1064 nm  $\zeta_p$  = 8 ps/500 kHz) has been shown to offer similar ablation efficiency [65–67].

*Laser ablation of bone*: Early study into the effect of the Er:YAG laser on bone showed that, as with enamel and dentine ablation, tissue cutting is a thermally induced explosive process [68].

Laser-assisted surgical removal of alveolar bone can form part of a range of treatments including surgical removal of teeth, tooth apical surgery, access to bony pathology and periodontal bone management. Additionally, the development of delivery tips for use with Erbium lasers has prompted osteotomy site preparation for the placement of implants. The use of erbium lasers in dento-alveolar surgery represents a less-traumatic experience for the patient, when compared to the intense vibration of the slow-speed surgical bur—clinically with maxillary alveolar bone, the speed of laser cutting is comparable with that of a bur and slightly slower in the mandible, reflecting the greater cortical bone composition.

The micro-analysis of the cut surface reveals little evidence of thermal damage and any char layer appears to be restricted to a minimal zone of 20–30  $\mu$ m in depth [69, 70] (Fig. 4.25). Studies into the healing of lased bone would support the contention that the reduced physical trauma, reduced heating effects and reduced bacterial



Fig. 4.25 The 320 µm diameter quartz optic fibre



Fig. 4.26 The 200  $\mu$ m diameter quartz optic fibre compared to a ISO # 15 hand file

contamination, together with some claims to an osteogenic potential, lead to uncomplicated healing processes, when compared to conventional use of a surgical bur [71–74].

An advantage of the fine diameter laser tips available allows a more precise removal or remodelling of crestal alveolar bone associated with periodontal pockets or crown lengthening procedures (Fig. 4.26). With a closed flap approach to crown lengthening, the end-cutting nature of the tip is an absolute advantage over rotary instrumentation and has led to a growth in this treatment modality [75–78]; however, there is always the danger of not being able to visualise the target tissue and caution must be exercised in this respect.

## Use of Lasers in Anti-Bacterial Techniques Adjunctive to Dental Surgery: Laser Use in Periodontology, Endodontics, Implantology

Laser Use in Periodontology: There can be no compromise over the employment of thorough and evidence-based therapeutic measures in the dental specialties of periodontology, endodontics and implantology. All aetiological and pre-disposing factors must be evaluated and applied against the presenting condition in order to define the type and scope of therapy. Successful and predictable outcome is mandated in that a correct diagnosis is made and proper treatment implemented to achieve a stable result. Within such protocols, the use of lasers must be viewed as adjunctive.

Periodontal disease is a multi-factoral, predominately chronic inflammatory process whereby bacterial deposits associated with the gingival tissues produce toxins that evoke tissue reaction. Genetic, functional, lifestyle, systemic and local influences will all determine the host tissue response and, consequently, the course and outcome of the disease.

The use of surgical lasers in periodontology can be seen in three areas of treatment: removal of diseased pocket lining epithelium, bactericidal effect of lasers on pocket organisms and the removal of calculus deposits and root surface detoxification. When integrated into a sound approach to pocket reduction, all current dental wavelengths have been advocated for the removal of diseased epithelium [79-83]. Added to the current wavelengths is the recent development of а frequency-doubled (wavelength halved) Nd:YAG laser at 532 nm, termed the KTP laser which has a range of action similar to that of the 810 nm diode. Delivery fibres and tips can be fine enough to facilitate easy access into periodontal pocket (Figs. 4.27 and 4.28). The haemostatic advantage of using laser energy confers a controlling factor that is beneficial to both clinician and patient. Conceptually, in a periodontal pocket that is essentially supra-bony, the removal of



**Fig. 4.27** 320  $\mu$ m diameter quartz optic fibre inserted into a root canal. The red aiming beam shows the extent of light distribution that might be expected with IR laser energy



**Fig. 4.28** Peri-implantitis associated with upper anterior implant fixture. Lesion shows the amount of bone destruction. Soft granulation tissue removed

hyperplastic soft tissue together with a reduction in bacterial strains, renders the post-laser surgical site amenable to healing within normal limits. Where the pocket is infra-bony, a number of procedures have been advocated, including laser-ENAP (excisional new attachment procedure) where the Nd:YAG (1064 nm) laser is used in a non-flap procedure to reduce pocket depths of several millimetres through a succession of treatment appointments [84, 85].

Among the bacteria most implicated in periodontal disease and bone loss are Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis and Bacteroides forsythus. Other bacteria associated with periodontal disease are Treponema denticola, T. sokranskii and Prevotella intermedia. These latter bacteria, together with P. gingivalis, are frequently present at the same sites and are associated with deep periodontal pockets. Most studies reported in the literature focus on the in vitro action of various laser wavelengths on these selected bacterial species. The effectiveness of any laser wavelength is dependent upon the absorption characteristics of the target bacterial structure (water, pigment) being matched by the incident beam. In addition, in vivo the difficulty within the pocket of definable parameters of laser energy dosage, concentration of bacterial colonies and accuracy of exposure, may give rise to some scepticism as to the predictability of this therapy.

Nevertheless, a number of studies have been carried out to support the action of laser energy on various bacterial strains implicated in chronic periodontal disease [86-92]. Short wavelength lasers interact with pigmented strains, whereas longer wavelength laser energy is absorbed by cellular water, leading to fragmentation of cellular structure. Calculus, being a non-uniform mixture of inorganic salts, organic material, bacterial strains and water, can be viewed as a ready absorber of all wavelengths. However, the close association of calculus deposits with tooth and periodontal structures does pose a potential risk of collateral damage. Of the wavelengths investigated, Erbium YAG (2940 nm), Erbium Chromium YSGG (2780 nm) and the experimental frequency-doubled alexandrite (377 nm) have been shown to interact and remove calculus selectively [93–96].

Laser Use in Endodontics: In common with laser use in periodontology, the confines and difficult access posed by the root canal present specific challenges in endodontic treatment. Peri-radicular lesions are either primarily or secondarily caused by micro-organisms and conventional treatments suggest the combination of mechanical debridement and chemical anti-bacterial agents [97–101]. In order to address the end-on emission of laser light from the delivery system, modified intra-canal instruments have been developed and experimental devices to produce non-axial laser light propagation have been investigated [102–104]. The fine (200–320  $\mu$ m) diameters of quartz optic fibres used with the range of Diode and Nd:YAG lasers have enabled these wavelengths to be easily used in bacterial decontamination of the root canal [105–109] (Figs. 4.29 and 4.30). Of the current lasers available, the CO<sub>2</sub> wavelength would appear least suc-



Fig. 4.29 Bacterial decontamination of titanium implant surface using the Diode 810 nm laser (320  $\mu$ m fibre/non-contact/1.0 W CW)



**Fig. 4.30** Peri-apical radiographs of the affected implant. Left: the extent of the bone destruction, Right: following surgery and use of bone graft matrix, healing at 3 months

cessful in effecting bacterial decontamination and the effectiveness of laser use appears to depend on fluence values and direct access [110]. In addition, some concern has been expressed that the plume produced during laser action might allow bacterial contamination to spread [111, 112]. As with laser anti-bacterial action in other clinical sites, sub-ablative energy levels should be employed for all wavelengths. Comparative studies on two common bacterial pathogens, Escherichia coli and E. faecalis have shown that the more complex cell wall of the latter (layered murein, lipoprotein and lipopolsaccharide) can reduce the effectiveness of laser action in that the structure is more resistant to the sub-ablative energy levels [113]. Studies undertaken using the erbium group of lasers on E. faecalis have underlined the effectiveness of these lasers in removing dentinal structure and allowing direct action on this bacterium [114].

In recent years, greater emphasis has been shown to recognise and investigate the influence of biofilm structure and physical debridement of the root canal walls [115–118], together with the emergence of multi-wavelength combined procedures using ablative and sub-ablative energy levels. As such, this merging of surgical laser photoonics with low-level aPDT techniques shows promise in maximising adjunctive laser use in endodontic procedures.

Laser Use in Peri-implantitis: The success of osseointegrated root-form titanium dental implants as abutment support for fixed restorative prosthetics has given rise to an explosion in uptake world-wide. During the past 10–15 years, there has emerged a growing awareness of failure in soft tissue (peri-mucositis) and hard tissue support (peri-implantitis) for fixtures [119–123].

Peri-implantitis is recognised as a rapidly progressive failure of osseo-integration [124] in which the production of bacterial toxins precipitates inflammatory change and bone loss [125]. The development of peri-implantitis is not restricted to any one type of implant design or construction [126–128] and is cited as one of the greater causes of implant loss [129, 130].

Peri-mucositis is considered a component of support failure, but one where the inflammatory

signs are limited to the soft tissue cuff and there is no evidence of marginal bone infiltration. Given that the aetiological factors associated with individual sites can be addressed, the therapy directed at resolving the soft tissue condition might be considered similar to that in early marginal periodontitis [131].

When breakdown has involved supporting bone, direct action is indicated to prevent further tissue failure (Figs. 4.31, 4.32 and 4.33). Therapy is viewed through the following stages: establishing and neutralising aetiological factors, debridement, decontamination, re-establishment of biocompatibility, structural matrix as required, with on-going maintenance and review to optimise health and stability. Laser-assisted therapy



**Fig. 4.31** Low-level laser probe in use in the treatment of pulpitis. The laser device is hand-held



**Fig. 4.32** Use of the DiagnDent (Kavo, Germany). The tip is applied to the tooth surface and findings recorded using the analogue score



Fig. 4.33 Non-vital upper left central incisor prior to bleaching

may be seen as appropriate through many of these stages [132].

Laser-assisted debridement: Hyperplastic gingival tissue and intra-bony granulation tissue may be ablated using any of the clinically-available surgical laser wavelengths. Concerns amount to be able to access all aspects of the implant site in order to ablate the diseased tissue without exposing metal or bone to direct photothermal energy. A combination of laser and sharp curette may help to overcome difficulties.

Laser decontamination of implant surface: studies have investigated the use of all laser wavelengths in defining significant pathogen reduction and the ability of laser energy in bacterial decontamination appears to place its use above that of other modalities [133–136].

However, there is less evidence of beneficial use where the implant is coated with a ceramic or hydroxyapatite; this may be mostly due to the micro-complex surface irregularities which have been shown to harbour bacteria and foreign ions in a failing situation [137, 138].

As with periodontal bacterial reduction, emphasis is now placed on investigations of biofilm complexes over planktonic solution of single strains and the significance of bacterial CFU reduction in terms of a log<sub>5</sub> level of difference between modality and control [138, 139].

Additionally, the use of combined therapies (surgical laser plus aPDT) is an emerging concept which attracts investigation [140–142].

## Low-Level Laser Use in Dental Surgery

The use of LLLT in dental patients almost exclusively involves red and near-infrared light. There is a so-called "optical window" of these wavelengths in tissue (approx. 650–1100 nm), where the effective tissue penetration of light is maximised. Principal tissue chromophores (haemoglobin and melanin) have high absorption bands at shorter wavelengths, tissue scattering of light is higher at shorter wavelengths and water strongly absorbs infrared light at wavelengths >1100-nm [143].

Light energy is absorbed within living tissue by cellular photoreceptors, e.g. cytochromophores. The incident electromagnetic energy is converted by cellular mitochondria into ATP (adenosine tri-phosphate) [144]. Consequently, the stimulated increase in ATP production would suggest an increased cellular activity in e.g. fibroblasts involved in tissue healing [145]. In addition, the conversion of some of the incident energy into heat would suggest an increase in local micro-circulation through vasodilation.

According to the first law of thermodynamics, the energy delivered to the tissue must be conserved and three possible pathways exist to account for what happens when low level laser therapy is delivered into tissue.

First pathway-photonic energy is absorbed by a tissue/cellular chromophore and this raises the energy state of the chromophore to an unstable upper level. A chromophore is a molecule (or part of a molecule) which imparts some decided "colour" (absorptive capacity) to the tissue of which it is a component. The consequent first excited singlet state of the chromophore undergoes a transition from a higher to a lower electronic state and the energy of the electronically excited state is given off to vibrational modes of the molecule, i.e. the excitation energy is transformed into heat [146]. Examples of chromophores can be seen in haemoglobin, cytochrome c oxidase (Cox), myoglobin, flavins, and porphyrins [147].

*Second pathway—Fluorescence*. Fluorescence is a luminescence in which the molecular absorp-

tion of a photon triggers the emission of another photon with a longer wavelength. The energy difference between the absorbed and emitted photons ends up as molecular vibrations or heat.

*Third pathway—Photochemistry*. The level of irradiation and energy of the photons involved is insufficient to cause covalent bonds to be broken. The energy however is sufficient for a first excited singlet state to be formed and this can undergo intersystem crossing to the longer-lived triplet state of the chromophore. This allows reactions to occur, such as energy transfer to ground state molecular oxygen to form the reactive singlet oxygen (ROS).

Alternatively the chromophore triplet state may undergo electron transfer (reduction) to form the radical anion that can then transfer an electron to oxygen to form superoxide. A third photochemistry pathway that can occur after the absorption of a photon is the stimulation of the mitochondrial respiratory chain.

In summary, the absorption of photonic energy by cell mitochondria and associated chromophores leads to biochemical pathway changes (positive and negative), cell and transcription signalling, from which examples of enhanced tissue repair and healing may be seen.

*First pathway effects—photobiomodulation*: There is growing evidence that LLLT works well on a range of oral pathologies; these include improvements in:

Reported effects of LLLT photo-biomodulation in clinical dentistry may include the following [148–153]:

- Dentine hypersensitivity
- Post-extraction socket/post-trauma sites, drug-induced/X-Ray-induced osteonecrosis
- · Bone density, Orthodontic tooth movement
- Viral infections: herpes labialis, herpes simplex
- Neuropathy: trigeminal neuralgia, paraesthesia, Bell's palsy
- · Apthous ulceration
- Osseointegration of implants
- TMJDS, Trismus
- Post-oncology: mucositis, dermatitis, postsurgery healing

The stimulatory effects of LLLT include the following [154–157]:

- Proliferation of macrophages
- · Proliferation of lymphocytes
- Proliferation of fibroblasts
- Proliferation of endothelial cells
- Proliferation of keratinocytes
- Increased cell respiration/ATP synthesis
- · Release of growth factors and other cytokines
- Transformation of fibroblasts into myofibroblasts
- Collagen synthesis.

In addition, there is evidence to support the analgesic effects of LLLT, through an enhanced synthesis of endorphins and bradykinins, decreased c-fibre activity and an altered pain threshold. Therapeutic analgesic effects may also occur, through the release of serotonin and ace-tylcholine centrally and histamine and prosta-glandins peripherally [158, 159].

Photobiomodulation (PBM) is the manipulation of cellular behaviour using low intensity light sources. Laser therapy (application of photonic energy at specific wavelengths) works on the principle of inducing a biological response through energy transfer. Photonic energy delivered into the tissue modulates biological processes within that tissue and within the biological system of which that tissue is a part (Fig. 4.34).

PBM has no appreciable thermal effect in irradiated tissue.



Fig. 4.34 Tooth appearance after laser tooth whitening

*Second pathway effects—fluorescence*: Fluorescent- and photodynamic-diagnosis may provide screening facility or part of a hierarchical series of tissue investigation.

Suspect lesions of the oral mucosa must be subjected to biopsy and other investigations. Auto-fluorescence imaging may give good results for the distinction of lesions from normal mucosa, although it is inappropriate to place auto-fluorescence investigation in any role other than as an adjunctive scanning technique.

If possible, auto-fluorescence spectroscopy could be used to find the optimal, most dysplastic location for biopsy. Unfortunately, the literature shows that auto-fluorescence is not specific enough for this purpose.

The use of fluorescence in caries detection was first suggested more than a century ago, but received greater significance with introduction of laser technology into dentistry. Wavelengths used are commonly between 405 and 670 nm. In the 1980s, a clinically applicable visual detection method, focussing on the natural green fluorescence of tooth tissue was developed [160, 161]. The technique used a 488 nm excitation wavelength from an argon-ion laser to discriminate bright green fluorescing of healthy tooth tissue from poorly fluorescing carious lesions. The technique was developed further in the early 1990s into what is now known as quantitative light-induced fluorescence (QLF), where the digitisation of fluorescence images is used to quantify the measure of mineral loss [162].

Around that time, a red fluorescence method emerged. The red fluorescence, excited either using long UV (350–410 nm) or red (550– 670 nm) wavelengths, was observed in advanced caries as well as plaque and calculus on teeth. As opposed to the green fluorescence loss observed in caries, a substantial red fluorescence occurs between 650 and 800 nm in caries lesions and this is much brighter than that found with sound enamel or dentine [163, 164]. The first commercially available unit using a red laser was manufactured by Kavo (Kavo GmbH) in 1998, with an emission wavelength of 655 nm (Fig. 4.35).

Dental caries is multifactorial in aetiology. Diagnosis and treatment should be respectful of



**Fig. 4.35** Application of methylene blue solution as photosensitiser. The application follows initial pocket debridement

aetiology, lesion site and 3-D extent. Detection methods include tactile, radiographic, chemical and illuminescence and photo-diagnostic fluoroscopy [165] techniques. Studies appear to suggest that combination techniques offer greater accuracy.

Laser irradiation promotes differential fluorescence of tooth tissue/caries/plaque/calculus. Fluorescence is a product of Laser wavelength (photonic energy). Narrow waveband (non-coherent) irradiation may allow differential spectrometry.

Laser fluorescence may be a useful adjunct in the detection of early enamel caries. The level of energy used in this application poses little risk to the patient and offers potential benefits [166].

Optical Coherence Tomography [167] (OCT) is a technique for obtaining sub-surface images of translucent or opaque materials at a resolution equivalent to a low-power microscope.

Conceptually it is equivalent to an 'optical ultrasound', imaging reflections from within tissue to provide cross-sectional images [168, 169].

Not all methods accurately detect early lesions, and false positives and false negatives may occur. Detecting early lesions in combination with assessing activity status is essential for establishing the prognosis and threshold required for preventive intervention [170].

*Third pathway effects—photochemistry*: The clinical application of laser-initiated photochemical actions include tooth whitening, scanning techniques and photodynamic antimicrobial chemotherapy.

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*Tooth whitening*: Differing treatment modalities have been developed to address the phenomenal growth in demand for tooth whitening. Originally, the Argon 488 nm laser wavelength was marketed to provide intense photonic energy to assist the action of hydrogen peroxide on stained enamel and dentine, but the cost of the unit together with the safety requirements led to its decline in use [171]. Other techniques emerged, ranging from the use of LED and plasma-arc lights to home-use kits, using a preformed custom tray system.

The present resurgence in laser-assisted tooth whitening has been the development of a diodebased KTP (Potassium Titanyl Phosphate) 532 nm laser. This laser interacts with bleaching gel containing carbamide peroxide in a photoactivated way, as opposed to the longer (Diode 810 nm,  $CO_2$  10,600 nm) wavelengths, which act in a photothermal way to provide heat to the gel and consequently accelerate the chemical reaction [172] (Fig. 4.36).

With the KTP laser technique a red gel, containing Rhodamine B and hydrogen peroxide is applied to the tooth and exposed to the laser energy. The Rhodamine B molecule has its maximal absorption at 539 nm. When this dye is exposed to 532 nm light, it absorbs photons of energy with subsequent electron transition to the singlet excited state. The molecule may then undergo reactions with molecular oxygen, result-



**Fig. 4.36** Methylene blue photosensitiser exposed to diffuse laser photonic energy ( $\lambda = 670 \text{ nm}/200 \text{ mW}/60 \text{ s per site}$ )

ing in the production of hydroxyl radicals, superoxide ions, peroxides, labile singlet oxygen, or reactive oxygen species. In this way, the interaction between the KTP laser energy and the dye is a photochemical process [173].

A portion of the KTP laser energy aborbed into the Rhodamine B dye is also transferred from the excited molecule into the bleaching gel in the form of thermal energy. This transfer results in controlled heating of the gel and not the tooth, minimising the possibility of thermal damage to the pulp. This superficial heating of the gel accelerates the breakdown of hydrogen peroxide, which furter boosts the overall yield of perhydroxyl radicals over a given time [173].

Apart from extrinsic staining due to lifestyle factors, a common source of intrinsic staining is due to the administration of tetracycline antibiotics during tooth formation. Such staining has been shown to be resistant to chemical bleaching agents that produce oxidising radicals, whereas the tetracycline molecule can be photo-oxidised with the 532 nm laser [173].

Scanning and spectrometry: The development of laser-based measuring devices (e.g. the confocal micrometer), utilising beam-splitting of a low-energy laser and an optical detector, has enabled accurate replication of the morphology of dental and oral structures and materials used in restorative dentistry. The earliest use of laser scanning was in the field of orthodontics and facial development to provide 3D imaging and recording of pre- and post-treatment of deformities [174–176]. Scanned data was linked to computer software using CAD (computer-assisted design). This concept has been expanded during the last decade, to enable the scanning of restorative cavities prior to the production of cast or milled indirect restorations and the recording of oral and facial swellings [177, 178].

The development of laser Doppler flowmetry into applications in dentistry has allowed detailed analysis of pulpal and gingival blood flow, to assist in treatment planning [179–181].

An additional associated use of laser light in oral medicine is through Raman spectroscopy. A Raman spectrum represents the scattering of incident laser light by molecular or crystal vibrations. Such vibration is quite sensitive to the molecular composition of samples being investigated, and areas of research include the in vitro and in vivo study of disease processes such as cancer, atherosclerosis and bone disease. With regard to the latter, Raman spectroscopic analysis in vivo of mineral and matrix changes has been shown to be useful in mapping early changes in bone tissue [182].

*Photo-activated antimicrobial chemotherapy*: The concept of light-activated drug-therapy is well-established in medicine in the form of photodynamic therapy. Photo-activated antimicrobial chemotherapy is a development over and above the conventional use of chemicals to achieve bacterial decontamination in aspects of periodontal and restorative dentistry. Acronyms to describe this therapy abound and consensus has tended to adopt the use of antimicrobial photodynamic therapy—aPDT. Currently, topical application of a photosensitizer on infected tissues and subsequent illumination seems to be the most promising feature of antimicrobial photodynamic therapy [183].

The technique involves the application of a suitable chemical-a photosensitiser-to the treatment site. A photosensitiser is a chemical compound that readily undergoes photo-excitation when exposed to laser irradiation and then transfers its energy to other molecules. Host tissue oxygen, when in close proximity will undergo intersystem crossing to form oxygen radicals  $(O_2^-)$  and other free-radicals  $(H_2O_2 \text{ and } OH^-)$ . Additionally, the production of reactive oxygen species (ROS)-electronically excited and highly reactive state of oxygen, known as singlet oxygen  $({}^{1}O_{2})$  which can interact with a large number of biological substrates inducing oxidative damage on the cell membrane and cell wall [184]. These destructive reactions will kill cells through apoptosis or necrosis.

A number of photosensitisers have been used to investigate aPDT. Each photosensitiser has a unique absorption peak corresponding to applied laser wavelength. Examples of commerciallyavailable dyes are as follows:

Circumin (Yellow)—430 nm Methylene Blue—660 nm Radachlorin (chlorophyll derivative)—660 nm Toluidine Blue—680 nm Indocyanine Green—810 nm

And these have been shown to exert statistically-significant bacteriocidal effects on a range of periopathic bacterial species [185–187].

Photosensitiser triplet state molecules excited by  $\lambda > 850$  nm have insufficient energy to induce ROS in adjacent tissue O<sub>2</sub> [188]. The peak absorption with Indocyanine green at 810 nm has given rise to belief that it's action is not purely photodynamic, but is predominately photothermal [189, 190].

Investigations in the early 1990s, notably by Wilson and Pearson at the Eastman Dental Institute, London, determined the susceptibility to aPDT of Streptococcus mutans when the organism was present in a collagen matrix-an environment similar to that which would exist within a carious tooth [191]. If bacterial contamination of the prepared cavity could be rendered sterile, the hypothesis suggested that the potential for recurrent caries might be significantly reduced. The concept has also been expanded to consider a more-interceptive treatment of demineralised, but otherwise intact enamel surfaces, where bacterial elimination and fluoride therapy might prevent development of a more significant carious cavity [192]. Recent in vitro and in vivo studies into the use of PAD in endodontics [193, 194] have demonstrated the effectiveness of this therapy against a number of anaerobic bacterial strains associated with endodontic infections (Fusobacterium nucleatum, Peptostreptococcus micros, Prevotella intermedia and Streptococcus intermedius). In addition, PAD has been shown to be effective against *Enterococcus faecalis* [195].

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