Laser Photonic Energy Delivery in Clinical Dentistry: Scrutiny of Parameter Variables.

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Abstract.

Laser use in the disciplines of clinical dentistry, oral surgery and oral medicine have developed during a period of over 30 years. During that time, from a rudimentary base of non-awareness of the scientific processes involved or scope of possible application, the knowledge and understanding of lasers has grown and benefitted from structured research, postgraduate courses and qualifications. Both the sophistication and scope of use of laser photonic energy has increased significantly, to the extent that lasers can be incorporated to benefit almost all areas of patient-centred clinical dentistry. Research into laser-assisted therapies has reinforced the importance of "light dose" in order to maximise the benefits of applied coherent, monochromatic light energy to a given procedure, while minimising the risk of conversion of excessive energy into collateral thermal damage.

This thesis by concurrent publication provides a thorough examination of both ablative surgical laser-tissue interaction and sub-ablative photobiomodulation. The importance of achieving accurate light-dose levels of irradiation of oral hard and soft tissues remains fundamental to delivery of appropriate laser therapy whilst minimising the risk of collateral damage to adjacent, non-target oral hard and soft tissues. As demonstrated through published systematic reviews, it is significant to note the absence of full operating parametry in many peer-reviewed published studies; such omission compromises the opportunity to achieve accurate laser applications that are chosen as adjunctive to a clinical procedure. In addition, the consequence of poorly or inaccurately calibrated optic fibre photonic delivery, as the method of choice with visible and near infra-red wavelength "diode" lasers, may compromise the intended outcome of a clinical procedure; taken as a component of a poorly designed study, such errors may distort the development of laser-assisted therapy through inaccurate data acquisition and

interpretation arising out of calibration errors. Once receiving peer-reviewed publication, any reference incorporating laser use helps define the expansion of knowledge and use for the clinician; there is a consequent responsibility to ensure that published studies that directly affect clinical application of laser use are fully detailed, to avoid subsequent misinterpretation and maltreatment.

A further area of concern remains the persistence in attribution of post-surgical healing phenomena, by way of photobiomodulation to those laser wavelengths solely within the "optical window" range of 650 – 1350 nanometers. Both clinical and underlying biochemical data support the concept of "uneventful healing" with all laser wavelengths in dentistry, and exploration of key biochemical processes provide testimony to both photo- and possible thermo-biomodulation actions that follow laser irradiation.

Through examination of the many potential errors that may affect post-irradiation outcome, it is concluded that the risk would appear of greater significance with lower, photobiomodulation (PBM) fluences. The small difference between benefit and non-benefit with PBM, either as a stand-alone therapy or adjunctive to a surgical laser procedure, defines a perspective that underlines the balance of this thesis.

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Declaration. This thesis represents original work of the author, in terms of research, analysis and presentation. Where peer-reviewed publications are included, they represent a majority contribution of 75% by the author as first author, and where publications are co-authored, each contributor has signed a declaration to support the nature of collaboration.

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I would like to express my sincere thanks to everyone who provided me with support and encouragement during the course of this project. Their patience, endurance and perseverance are much appreciated.

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My group of co-authors are recognised for their expert contribution and collegiate approach to investigate the subject matter of each published paper. Respected friends and dear colleagues, Dr Eugenia Anagnostaki, Dr Marianna Chala, Dr Mark Cronshaw, Professor Roy George, Dr Valina Mylona and Professor Laurie Walsh, join Professor Grootveld and Professor Lynch, in having sustained and contributed to my quest for evidence and objectivity in our series of collaborated studies.

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I would like to thank my family; my son Matthew, daughters Sara and Lucy for having the belief that the "old man" still had ambition. Above all, to my dear wife Penny, who faithfully supported me and inspired my quest to square the challenging personal circle of understanding predictable laser-tissue interaction over 30-plus years.

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Publications

Journal Articles Included in this Thesis

Paper #1: Parker S, Anagnostaki E, Mylona V, Cronshaw M, Lynch E, Grootveld M. (2020). Current Concepts of Laser–Oral Tissue Interaction. *Dentistry Journal,* 8, 61. doi:10.3390/dj8030061.

Paper #2: Parker, S., Cronshaw, M., & Grootveld, M. (2022). Photobiomodulation Delivery Parameters in Dentistry: An Evidence-Based Approach. *Photobiomodulation, Photomedicine and Laser Surgery,* 40(1), 42–50. doi.org/10.1089/photob.2021.0116.

Paper #3: Parker, S., Cronshaw, M., Grootveld, M., George, R., Anagnostaki, E., Mylona, V., Chala, M. and Walsh, L. (2022). The influence of delivery power losses and full operating parametry on the effectiveness of diode visible–near infra-red (445–1064 nm) laser therapy in dentistry—a multi-centre investigation. *Lasers in Medical Science.* doi.org/10.1007/s10103-021-03491-y

Paper #4: Parker S, Anagnostaki E, Mylona V, Cronshaw M, Lynch E, Grootveld M. (2020). Systematic review of post-surgical laser-assisted oral soft tissue outcomes using surgical wavelengths outside the 650–1350nm optical window. *Photobiomodulation, Photomedicine and Laser Surgery* 38(10), pp.591–606. doi:10.1089/photob.2020.4847.

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the lower right first and second premolar. Convention would suggest that excision should be accompanied by scarification of the lesion bed in order to reduce the potential for recurrence. Top right: original lesion; bottom right: surgical excision using Nd:YAG 1064nm laser, using the following parameters: 250mj pp / 15 Hz / Av. Power 3.75 Watts and included laser irradiation of the crestal ridge tissues in contact mode. Page 72

Introduction Figure #5: Top: post operative review at 16 days – complaints of pain and gingival bleeding, together with appearance of early bone sequestrum. This was caused by direct thermal damage caused by excess laser power and ill-considered laser wavelength. Bottom (left) and (right): review at five months and 10 years indicating fortunately no long-term tissue damage or dental function. Page 73

Introduction Figure #6: Provisional diagnosis of localised gingival margin disruption at lower left central incisor. Possible cause due to high insertion of lower labial frenum; initial treatment suggested to release the pull of the frenum to allow reassessment of the gingival condition. Top left: presenting condition. Bottom right: use of Nd:YAG 1064nm laser, using the following parameters: 250mj pp / 20 Hz / Av. Power 4.0 Watt., owing to the fibrous nature of the tissue and relatively low tissue pigmentation. Page 74

Introduction Figure #7: Top: Follow up at one week, following severe pain and bad taste. Bottom: one week later. Page 75

the lack of lateral spread from the tips. Note the Gaussian distribution and the relative distribution of energy from high within the surface layers to very low at subsurface levels. Sources are 650 nm calibrated lasers at 200mW output power, beam diameter at source 3.7 mm, in close contact to the target tissue. Page 111

Paper #II Figure #5(B): Three-dimensional render of the beam profile of two parallel optic beams 2.5 cm apart applied to a 3mm thickness porcine muscle tissue sample. Images are offered as a guide to the reader to demonstrate the lack of lateral spread from the tips. Note the Gaussian distribution and the relative distribution of energy from high within the surface layers to very low at subsurface levels. Sources are 650 nm calibrated lasers at 200mW output power, beam diameter at source 3.7 mm, in close contact to the target tissue. Page 111

Chapter 3: Contributory errors arising from optic fibre power losses.

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Paper #III: The influence of delivery power losses and full operating parametry on the effectiveness of diode visible–near infra-red (445–1064 nm) laser therapy in dentistry—a multi-centre investigation.

Paper #III Figure #1 a: Diagrammatic representations of least square mean (LSM) values ± 95% confidence intervals (CIs) for the % deviations from power settings found a between-devices employed. Page 143

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Notation

μ_a	Absorption Coefficient
µm	Micron 10^{-6} metres
µSec	Microsec 10^{-6} Seconds
$^1O_2$	Singlet Oxygen
ADP	Adenosine DiPhosphate
ANCOVA	Analysis Of COVAriance
ANOVA	ANalysis Of Variance

ANSI	American National Standards Institute
AP-1	Activating Protein-1
aPDT	Antimicrobial PhotoDynamic Therapy
ATP	Adenosine TriPhosphate
c	Speed of Light 300 x 10^6 msec $^{-1}$
CCO (CoX)	Cytochrome C Oxydase
CHA	Carbonated HydroxyApatite
CO_2	Carbon Dioxide
CoQ	Coenzyme Q
CT	Connective Tissue
CW	Continuous Wave
DNA	DeoxyriboNucleic Acid
e	Euler's number
EM	Electromagnetic Spectrum
ENT	Ear, Nose and Throat
Er,Cr:YSGG	Erbium Chromium Yttrium Scandium Gallium Garnet
Er:YAG	Erbium Yttrium Aluminium Garnet
ETC	Electron Transport Chain
eV	Electron Volt

FIR	Far Infra Red
Fluence	Radiant exposure / Energy density (Jcm^{-1})
FRP	Free Running Pulsed
GaAs	Gallium Arsenide (diode)
GaN	Gallium Nitride (diode)
h	Planck's Constant = 6.626068 × 10-34 m2 kg / s
HA	HydroxyApatite
Hz	Hertz (frequency)
IEC	International Electrotechnical Committee
IL-1	Interleukin 1
InGaAlP	Indium Gallium Aluminium Phosphide (diode)
InGaAsP	Indium Gallium Arsenide Phosphide (diode)
InGaN	Indium Gallium Nitride (diode)
Irradiance	Radiant power / Power density (Wcm^{-1})
J	Joule (energy)
KTP	Potassium Titanyl Phosphate
LASER	Light Amplification by the Stimulated Emission of Radiation
LSM	Least Mean Square

MASCC/ISOO	Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology
MASER	Microwave Amplification by the Stimulated Emission of Radiation
MeSH	Medical Subject Heading
MIR	Mid Infra Red
mj	Millijoule
mjpp	Millijoule per pulse
ml	Millilitre 10^{-3} litre
mm	Millimetre
mRNA	Messenger RiboNucleic Acid
MRONJ	Medication Related OsteoNecrosis of the Jaws
mW	milliWatt (power)
NAD(H)	Nicotinamide Adenine Dinucleotide (NAD) + hydrogen (H)
Nd:YAP	Neodymium Yttrium Aluminium Perovskite
NF-kB	Nuclear Factor Kappa B
NIR	Near Infra Red
NM	Nanometer 10^{-9} metres
NO	Nitric Oxide

OM	Oral Mucositis
OTM	Orthodontic Tooth Movement
PBM	PhotoBioModulation
PBMT	PhotoBioModulation Therapy
PGE2	Prostaglandin G2
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
q-PBM	Quasi-PhotoBioModulation
RCT	Randomised Clinical Trial
RNA	RiboNucleic Acid
ROB	Risk Of Bias
ROS	Reactive Oxygen Species
SDH	Succinate DeHydrogenase
SEM	Scanning Electron Micrograph
SMA	SubMiniature Assembly (fibre connector)
TGF-β1	Transforming Growth Factor β1
TMJ	TemporoMandibular Joint
TMJDS	TemporoMandibular Joint Dysfunction Syndrome
TRP	Transient Receptor Potential

W	Watt (power)
βFGF	Basic Fibroblast Growth Factor
$\boldsymbol{\pi}$	Pi (3.14159)
$\boldsymbol{\pi r^2}$	Area of a circle
$\mathbf{V} = \frac{4}{3}\ \boldsymbol{\pi r^3}$	Volume of irradiated tissue
$\boldsymbol{\lambda}$	Wavelength (lambda)

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Preface to the Thesis by Concurrent Publication Format

This thesis is presented by the route of concurrent publication, whereby published research apers that represent an interconnected approach to the study's core research questions are substituted for conventional thesis chapters. The papers are presented in the form in which they were published, and thus the formatting styles specific to each journal is maintained. The thesis is supported by a theoretical and critical narrative to contextualise the research papers and discuss the overall findings.

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Chapter 1: Introduction

- Overview of the development of laser technology and wavelengths that have been utilised in delivery of adjunctive therapy in dentistry.
- Exploration of laser operating parameters and how they may influence prospective laser-tissue interaction
- Challenges posed by the anisotropic nature of oral tissue consequent to irradiation and interaction with coherent light.
- Range of interactive outcomes of laser photonic energy and application specific to therapies within clinical dentistry.
- Overview of laser-tissue interaction as published in Paper #1: Parker S, Anagnostaki E, Mylona V, Cronshaw M, Lynch E, Grootveld M. (2020). Current Concepts of Laser–Oral Tissue Interaction. *Dentistry Journal*, 8, 61; doi:10.3390/dj8030061.
- Collectively, the combination of applied laser photothermolysis, adjunctive PBM effects and stand-alone PBM therapies has allowed the scope and value of laser-assisted clinical dentistry to attain considerable application in modern clinical dentistry.

1.1 Background to laser use as pertaining to clinical dentistry

The rather simplistic definition and understanding of light as a source of energy relates to those wavelengths of the electromagnetic (EM) spectrum that may be detected by the human retina; this however, fails to recognise those shorter, adjacent wavelengths outside the narrow range of approximately 350 – 750 nanometers - nm ("violet blue" to "red" visible), and longer, Near - , Mid - , and Far - Infra-red (750nm – 10,600nm) wavelengths; it also fails to acknowledge that intrinsically all wavelengths within the electromagnetic

spectrum are determined by mathematic proportionality – direct or indirect relationships in terms of energy, wavelength and frequency. Additionally, the sinusoidal waveform propagation of electromagnetic energy ("light waves") as a stream of photons, travels through space at a constant speed of 299.8 x 10^6 metres per second. Through his published *Treatise on Electricity and Magnetism* (1873) James Clark Maxwell showed that light was a form of electromagnetic radiation [1, 2], and much of the understanding drew upon the theoretical and practical experiments of Michael Faraday. Following naturally from Maxwell's equations for electromagnetic behaviour, Max Planck postulated [3] that EM energy was emitted in the form of discrete packets – so-called "quanta", and from this, the basic relationship between emission energy and wavelength emerged is expressed as:

$$E = \frac{hc}{\lambda}$$

Where c = 300 x 10^6 msec $^{-1}$

h (Planck's Constant) = 6.626068 × 10^{-34} m^2 kg / s represents the proportionality constant between the energy (E) of a photon and the frequency (ν) of its associated electromagnetic wave.

In developing concepts of how a photoelectric effect was caused by absorption of an appropriate and correct quanta of light, Albert Einstein theorised the possibility of stimulated emission in which a target atom, already excited through a light quantum collision would accept a further quantum; from this would emerge two quanta, identical in both energy and phase [4]. Einstein further showed mathematically why the energy of photons were dependent only on the frequency of the incident light [5]. From this work emerged an acceptance of wave-particle duality in explaining light (photonic) emission.

This correlation provided reassurance when, with further fundamental theory the early practical applications of light and quantum theory manifested; firstly, the MASER (Microwave Amplification by the Stimulated Emission of Radiation), and later the LASER (Light Amplification by the Stimulated Emission of Radiation), as manipulation of basic EM energy, wavelength and frequency. The first laser was developed in 1960 and attributed to Theodore H. Maiman at Hughes Research Laboratories in Malibu California, using a solid-state "active medium" crystal of ruby (chromium-ion doped aluminium oxide – Al_2O_3:Cr), and building on laser prototype projects of Arthur Schawlow and Charles Townes [6, 7].

Laser photonic energy is unique in that two prime properties – monochromaticity (single wavelength) and wave coherence – result from the amplified stimulated emission of photons produced within the excited active medium.

With an appropriate level of external excitation (the so-called "pumping mechanism"), photons produced by the active medium are reflected between co-axial reflective surfaces (Fabry–Pérot interferometer), and with a sufficient level of gain, the beam passes through the proximal mirror and is delivered to the target oral tissue *(Figure #1*). Even with efficient gain, the process of such active media gain is hampered by approximately 97% energy loss as heat, and correspondingly the system must be protected through a suitable cooling system [8].

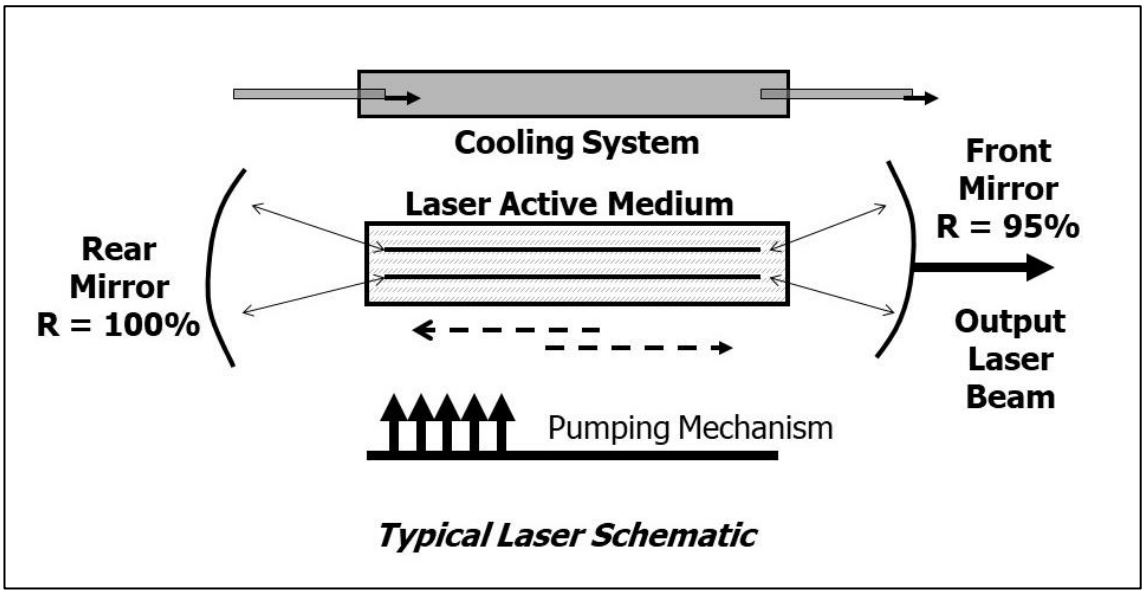

Figure #1. Schematic of a laser system employing a laser cavity, active medium, reflective mirrors (Fabry–Pérot interferometer) and appropriate external energy source (pumping mechanism). Source: Parker S. British Dental Journal. (2007) Jan 13;202(1):21-31. doi: 10.1038/bdj.2006.113. [5]

Amongst several laser pioneers, working independently, 1962 saw the development of semiconductor "diode" lasers; Robert N. Hall (General Electric Laboratory, Schenectady, New York), and Marshall Nathan (IBM Watson Research Center) produced prototype homojunction diode platforms based on gallium arsenic GaAs, and Nick Holonyak Jr (GE's Syracuse, New York) incorporated phosphorus to create a visible red GaAsP semiconductor laser (*Figure #2*) [9 - 11]. Although extremely temperature dependent, such technology was further developed by workers such as Herbert Kroemer (Varian Central Research Laboratory) and Zhores Alferov (Ioffe Physics Institute) in 1963, who produced a more stable room temperature GaAs heterojunction diode laser, incorporating a band gap between the III and V-valency atomic groups [12, 13].

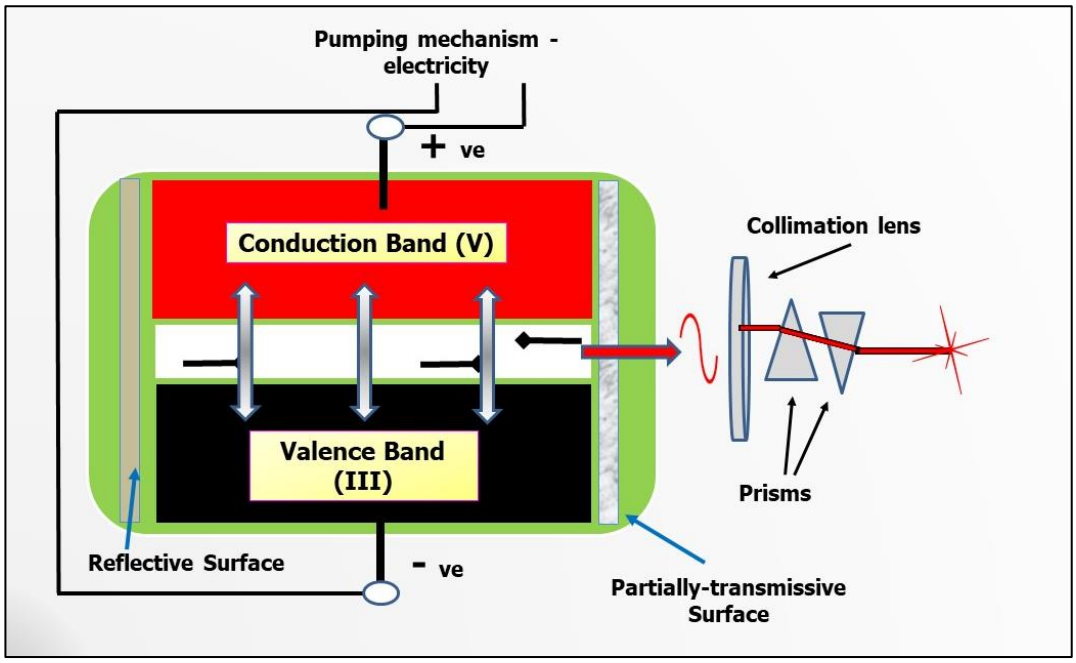

Figure #2. Schematic of a semiconductor (heterojunction diode) laser system employing Group III (eg. Gallium) and Group V (eg. Arsenic) atoms. Population inversion (laser gain) occurs through either applying coatings to each end of the semiconductor crystal or by internal reflection within the crystal boundary (Fabry-Pėrot resonator). Source: S. Parker

Of relevance to laser use in dentistry, a third type of laser was developed during the mid-1960s, in which the development of a carbon dioxide / helium laser (CO_2) occurred at Bell Laboratories through the research of Kumar Patel. Progress in achieving acceptable levels of gain was through concentrating on vibrational transitions of gas molecules [14]. Further progress was made by Eugene Watson, who set up Coherent Radiation Laboratories in 1965, to build commercial CO_2 lasers, utilising a gas mixture of CO_2, nitrogen, helium, and traces of noble gas [15].

1.2 The first "dental" lasers

The first laser designed and manufactured for use in clinical dentistry appeared in 1989; it was the product of a six-year working collaboration of two brothers Dr Terry and Dr William Myers, the former a dentist and the latter an ophthalmic surgeon [16]. Drawing upon the high infra-red fluorescent potential of the element neodymium, formulated as

the di-valent ion within a glass / optically pure crystal medium, the emission wavelength of the Neodymium Yttrium Aluminium Garnet (Nd:YAG) active medium at 1064nm together with basic free-running pulsed emission, defined a narrow range of absorption in predominately pigmented soft tissue elements; in a possible attempt to enhance its appeal to the clinician, it was also claimed to be effective in vaporising pigmented carious dentine, hence suggestive of positive tooth cavity instrumentation as well as delivering induced tooth analgesia [17 - 23].

What was clear was the limited prospect of fulfilling the commercial advantage claimed by the developers of the Nd:YAG 1064nm laser, as a replacement for the use of a rotary dental drill. The rudimentary operating parameters allowed thermal rise to predominate when oral hard and soft tissue were irradiated [24]. When attempts to vaporise accessible dental caries were made, a high potential existed for direct collateral damage to surrounding dental tissue, together with indirect thermal conduction to the dental pulp [25]. Since the original "dental" laser in 1989 was a derivative of a model used in ophthalmology, similar lasers such as the argon-ion (488 / 515nm) and carbon dioxide (10,600nm) lasers evolved to a position of adjunctive dental application in the early 1990s. With its multi-wavelength emission spectrum, the argon ion laser "blue" wavelength of 488nm could be utilised in claimed profound curing of dental composite restoratives and the argon ion "green" wavelength of 514.5nm was abundantly absorbed in oral pigmented tissue and haemoglobin [26]. Both the instrument cost and development of its technology (including emergent semiconductor laser alternatives and non-laser dental curing lights) contributed to the relatively low market penetration of this laser. By comparison, the carbon dioxide laser has remained a choice to the clinician; from its early introduction as an adjunctive surgical soft tissue laser [27 – 29], and despite the potential

thermal risk posed by the 10,600nm continuous wave emission [30], this has remained the subject of positive benefit in studies relating to laser assisted oral soft tissue surgery [31 – 34]. A problem with early CO_2 models was the rudimentary deca-second gating or inherent continuous wave emission mode, which when coupled with the high absorption of this wavelength in tissue water, rapidly would lead to irradiated tissue desiccation and formation of a pre-carbonisation layer, termed eschar. Persistent irradiation of this debris would lead to surface overheating of the carbonised layer.; certainly, with those early laser models, regular removal of the eschar with damp gauze was mandated to enable soft tissue ablation to proceed. Additionally, the development of a 9,300nm mode-locked co-axial water laser version of the CO_2 "family" has allowed the high absorption of this wavelength by the phosphate radical of carbonated hydroxyapatite in enamel and hydroxyapatite in dentine and bone, to be a precursor to the efficient spallation of oral and dental hard tissues [35].

The introduction of commercially available alternative wavelengths saw the emergence of the 810nm GaAs diode laser in 1992, along with gas-medium CO_2 lasers, mostly derived from ENT and dermatological platforms. The commercial availability of an "all-tissue" erbium YAG (Er:YAG) laser in 1992, drawing upon research from 1975, indicated an emission wavelength of 2940nm from activated Er^{3+} ions distributed within a YAG crystal active medium. This wavelength has high absorption in water, i.e. 20,000 times greater than that with the Nd:YAG 1064nm system [36]. Together with an inherent free-running micro-second pulsed emission and co-axial water spray, this represented the first laser platform for use in hard dental and oral osseous tissue ablation [37, 38].

Over time, the breadth of EM wavelengths utilised as commercially available lasers in clinical dentistry has increased to encompass diode 455nm ("blue"), 532nm ("green"), a

series of 635nm – 680nm “red” visible wavelengths, through near infra-red diode and solid state (NIR – 805nm – 1340nm), mid infra-red solid state (MIR – 2780nm – 2940nm) and far infra-red gas (FIR 9,300nm – 10,600nm) laser wavelength choices. Along with such variety of choice, research and clinician understanding profited in both extent and sophistication but retained a fundamentally narrow belief that specific absorption of each individual laser wavelength by targeted oral tissue defined the scope of beneficial laser-tissue interaction.

1.3 Basic concepts of laser – tissue interaction

Human oral and peri-oral tissue is anisotropic, in terms of its structural components and consequent behaviour of applied photonic energy in terms of how it may interact – incident photonic energy being reflected, scattered, absorbed or transmitted through the tissue, depending on the wavelength applied [39]; apart from the basic grouping of soft tissue versues hard dental and osseous tissue, there exist varying tissue elements, capable of absorbing incident laser photonic energy. Absorption theory draws upon the Beer-Lambert law, which considers the influence of the absorption coefficient on the intensity of light, after passing through oral tissue [40]. Commonly cited chromophores and absorptive molecular groups found within structural components of oral tissue may be water, collagenous proteins, melanin, oxy- and deoxy-haemoglobin, together with hard tissue hydroxyapatite and carbonated hydroxyapatite. Research has explored the value of absorption coefficient (μ_a) for each of these tissue elements, and the corresponding scatter coefficient (μ_s) of gross tissue of which each is a component, relative to each laser wavelength [41, 42].

Within the discipline of clinical dentistry, the application of therapeutic laser photonic irradiation may undergo a mixture of physical interaction, according to the incident

wavelength, power density, together with the anisotropic nature of the target tissue [43]. Non-interaction may result in transmission of the beam, or owing to the degree of density or structure, some refraction of the beam. Both effects may negate the expectation of any photonic effects and may also create a potential for onward, non-target tissue exposure and collateral damage.

Positive laser-tissue interactions may arise from photonic scatter or absorption; scatter may be attributable to non-linear photon travel within the tissue, resulting in possible photon energy attenuation with distance, or alternative stochastic positive energy effects through intra-tissue photon collision. Together, these phenomena contribute to a degree of scatter effect described as “speckling”. Additionally, with the predominance of high scatter coefficient relative to visible and NIR laser wavelengths, a degree of back-scatter may occur, and this may compromise the accuracy of laser emission power values.

Fundamental laser-tissue interaction relates to a concept of a “lock and key”, where photon energy is closely matched to the capacity of the target tissue element or chemical group (atom or functional group in which the electronic transition responsible for a given spectral band is approximately situated) that is capable of selective light absorption. The resulting energised state may reflect incident sub-ablative power levels, and effect photobiomodulation or may be of sufficient energy to reach a level of phase change, leading to molecular and tissue dissociation and consequent structural dislocation.

With any laser emission, there are key parameters; these may be viewed in *Table #A* and summarised as:

a/ intrinsic, defined as the laser basic values

b/ adjustable, subject to control panel facility, proposed therapy requirements and combined maximum output limits

c/ calculated – whereby computed values may be derived as the product of one or more adjustable parameter(s)

Intrinsic Parameters	Adjustable Parameters	Calculated Parameters
Laser model / active medium	Average power (Watts W)	Beam spot diameter at tissue
Emission wavelength λ (nm)	Pulse repetition rate (Hertz Hz) FRP laser	Beam spot area (cm^2)
Emission mode: Continuous wave (CW), gated-CW, Free-running pulsed (FRP)	Energy per pulse (mj / mjpp) (FRP laser)	Fluence: (Radiant exposure / Energy density per unit area (Joules J/sq.cm)
Delivery system: optic fibre / waveguide / articulated arm	CW gating - Emission cycle (gated-CW) %	Irradiation: (Power density / Radiant power per unit area (Watts/sq.cm)
Optical fibre diameter (µm / mm)	Delivery "spot size" (tip diameter - µm / mm)	Length of photonic exposure (secs)
Gaussian or Flat-top beam X-section	"Contact / non-contact" mode	Total energy delivered (Joules)
Beam divergence (angle deg.)	Tip-to-tissue distance (mm)	
	Coaxial water (ml / min)	
	Coaxial air (ml / min)	

Table #1. Laser operating parameters. Each laser will have intrinsic parameters, with a choice of control panel interaction to allow adjustable parameters. The product of two or more parameters will provide calculated parameters to confer specificity of actual photonic "dose".

Apart from these fixed and adjustable laser parameters, target oral tissue by virtue of its composition and structure will exhibit optical tissue and physical tissue parameters, relative to the incident laser wavelength. Subject to some positive interaction of photonic energy with the target tissue, these parameters will – consistent with power density phenomena - give rise to absorption and thermal phenomena. With acceptance of these fundamental characteristics, individual patients may exhibit differing racial or ethnic soft

tissue pigmentation levels. In many cases, such pigmentation may not only affect skin colour, but may extend intraorally to create areas of predominantly melanin deposits, which are viewed unfavourably among certain nationalities. For these, it would impact significantly to accept and adopt “average” laser operating parameters for gingival surgery when using laser wavelengths that are highly absorbed in melanin and similar pigmented proteins; in these situations, it would be appropriate to reduce average power values in order to avoid thermal damage.

The other general aspect of gingival surgical management using laser photonic energy is the gingival biotype, commonly graded as “thin”, “thick” and “normal” in terms of tissue depth. Care would normally be advised to recognise that visible and NIR wavelengths being detrimental to underlying osseous tissue and dental structures; lower laser operating parameters together with thermal relaxation measures to avoid overheating and direct or conductive thermoclines.

The scope for optimising laser interaction is best served through absorption of incident photonic energy. The photonic energy of most laser wavelengths in clinical dentistry is insufficient to achieve the dissociation energy of covalent bonds of most proteinaceous structural elements and certainly, the ionic bonds within the hydroxyapatite crystal lattice structure [44]. In consequence, the success in developing laser-tissue interaction as adjunctives to surgery and structural change, lies in the successive stream of photons within an emission beam. Most commonly and fundamental to ablative tissue effects, the conversion of incident photonic energy to thermal energy will result in incremental temperature rises; dependent upon the applied photonic dose and exposure time, an ascending thermal effect from tissue warming, protein denaturation, water vaporisation and soft tissue ablation may be observed, with onward carbonisation through the

application of excess laser irradiation. This process is termed photothermolysis [43], and when applied to hard oral tissue may result in an explosive dislocation and fragmentation of such tissue (bone, enamel, dentine, together with root cementum, dental calculus and dental caries). With erbium (approximately 3.0μm wavelength) laser irradiation of these structures, the interstitial water component is rapidly vaporised and the resultant increase in vapour pressure leading to structural fragmentation, is termed spallation [45]. The closely allied delivery at 9.3μm, albeit through micro-gated CW emission, results in the melting of mineral content within the target hard tissue, which in terms of the ablation outcome is not clinically dissimilar to that achieved with the shorter mid-IR wavelengths.

Another albeit limited interaction is laser photochemistry; historically, the 488nm argon ion laser wavelength brought benefit to the potential for photocuring of dental composite resin restoratives where the high absorption of this blue wavelength in camphorquinone serves as the catalytic reagent of these materials [46]. Over time, the development of much less expensive and convenient curing light technology has made such laser application obsolete. Laser photochemistry effects may be seen in tooth bleaching using higher photonic energy wavelengths such as the 532nm "green" wavelength [47]. In contrast, the development of photochemical activation of applied photosensitisers has created a significant contribution to antibacterial photodynamic therapy – aPDT. Throughout the applied wavelength range of 400 – 800nm, the use of chemical mediators which, when irradiated with low power photonic energy may induce pathogen inactivation and cell death, has proven to significantly benefit areas of periodontal and peri-implantitis treatments [48, 49].

Sub-ablative laser-tissue effects using low intensity light sources, whereby therapeutic irradiation results in the manipulation of cellular behaviour, is termed

photobiomodulation (PBM). As an applied clinical therapy, PBM may be delivered as a stand-alone modality in the adjunctive treatment of certain pathologies, as well as a co-therapy benefit of ablative laser surgery [50, 51]. The PBM benefits of the latter are observable at some distance from the site of photothermal tissue ablation, where photon energy density is reduced through scatter and tissue temperature rise is consequentially reduced to a sub-ablative level.

Paper #I provides an evidence-based exploration of current concepts of laser oral tissue interaction.

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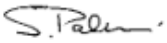
1.4 Paper 1: Current Concepts of Laser Oral Tissue Interaction.

Statement of Co-authorship

We declare that the co-author contributions are correct, and that Steven Parker was primarily responsible for producing the first draft and at least 75 % of the final content of the following paper.

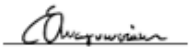
(i) Steven Parker (Candidate) conceived and designed the paper, collected and interpreted data, compiled and wrote the manuscript.

Signed:  Name: Dr Steven Parker

(ii) Mark Cronshaw (co-author) provided text input and data contribution.

Signed:  Name: Dr Mark Cronshaw

(iii) Eugenia Anagnostaki (co-author) provided editorial review and data contribution.

Signed:  Name: Dr Eugenia Anagnostaki

(iv) Valina Mylona (co-author) provided text input and data contribution.

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(v) Edward Lynch (co-author / Second Supervisor) supervised and provided editorial review.

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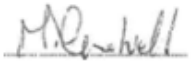
(vi) Martin Grootveld (First Supervisor) supervised and assisted in conception and design of the paper, provided data analysis and editing the manuscript.

Signed:  Name: Prof. Martin Grootveld

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Current Concepts of Laser–Oral Tissue Interaction

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Abstract

Fundamental to the adjunctive use of laser photonic energy for delivering therapy and tissue management, is the ability of the incident energy to be absorbed by target tissues. The aim of this review is to examine the differential performance of the separate components of oral hard and soft tissues when exposed to laser photonic irradiance of variable wavelengths and power values. Through an examination of peer-reviewed published data and materials, the interaction of laser photonic energy and target tissues are explored in detail. Varying laser wavelength emissions relative to anatomical structures explores the ability to optimise laser–tissue interactions, and also identifies possible risk scenarios as they apply to adjacent non-target structures. The concepts and practical aspects of laser photonic energy interactions with target oral tissues are clearly demonstrated. Emphasis was placed on optimising the minimum level of laser power delivery to achieve a desired tissue effect, whilst minimising the risk or outcome of collateral tissue damage.

Keywords: dentistry; laser; laser–tissue interaction; optical properties of tissues; photobiomodulation; photothermolysis

1. Introduction

The oral cavity is a complex environment, both structurally and functionally, where hard and soft tissues exist in close proximity. Additionally, all oral tissue surfaces are subject to contact with bacteria- and bacterial catabolite-laden saliva. Oral tissues can be subjected to laser treatment exposure, but the biophysics governing laser–tissue interactions demand a knowledge of all factors involved in the delivery of this modality.

Laser photonic energy has the unique properties of spatial and temporal coherence of wave propagation, together with the emission monochromaticity derived through a single wavelength value. In consequence, with appropriate delivery parameters, it is possible to deliver selective laser–tissue interactions, and to maximise the light dose effectiveness [1].

Depending on the level of incident energy, the consequent transfer to another form can occur, such as thermal energy, fluorescence, sound emission and the promotion of chemical pathways within tissue cellular environments. These interactions are summarised in *Figure #1*.

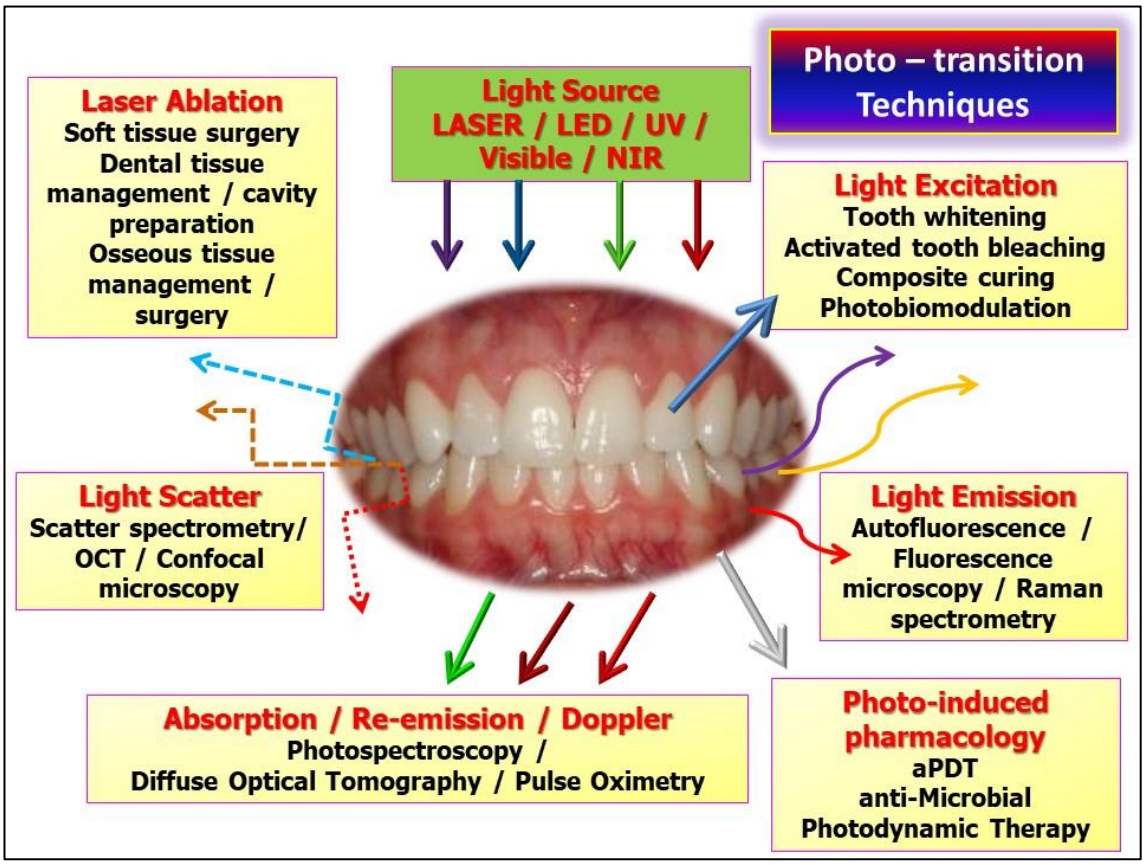

Figure #1. An overview of the manipulation of incident photonic energy, such as laser light as an adjunct to screening, diagnostic and therapeutic clinical activity. Key: LED—light emitting diode, UV—ultra-violet, NIR—near infra-red, with multi-wavelength representation as vertical incident arrows. OCT—optical coherence tomography, aPDT—antimicrobial photodynamic therapy. Re-emission, as direct beam

or scattered irradiance is represented by complete and dotted arrows, either straight or non-linear.

The ability to predict the degree of laser–tissue interaction experienced may pose some difficulties for the clinician, notably through limited target access, choice of laser wavelength, and the amount of power required to achieve desired changes in the target tissue. As the prime factor governing interaction, the absorption coefficient of any tissue element is a function of the degree of energy attenuation of a chosen incident laser wavelength; within the wide range of laser wavelengths available in dentistry, absorption coefficient curves as demonstrated in *Figure #2* and *Figure #3* are an expression of the relative performance of each tissular element across the spectrum of incident photonic irradiation.

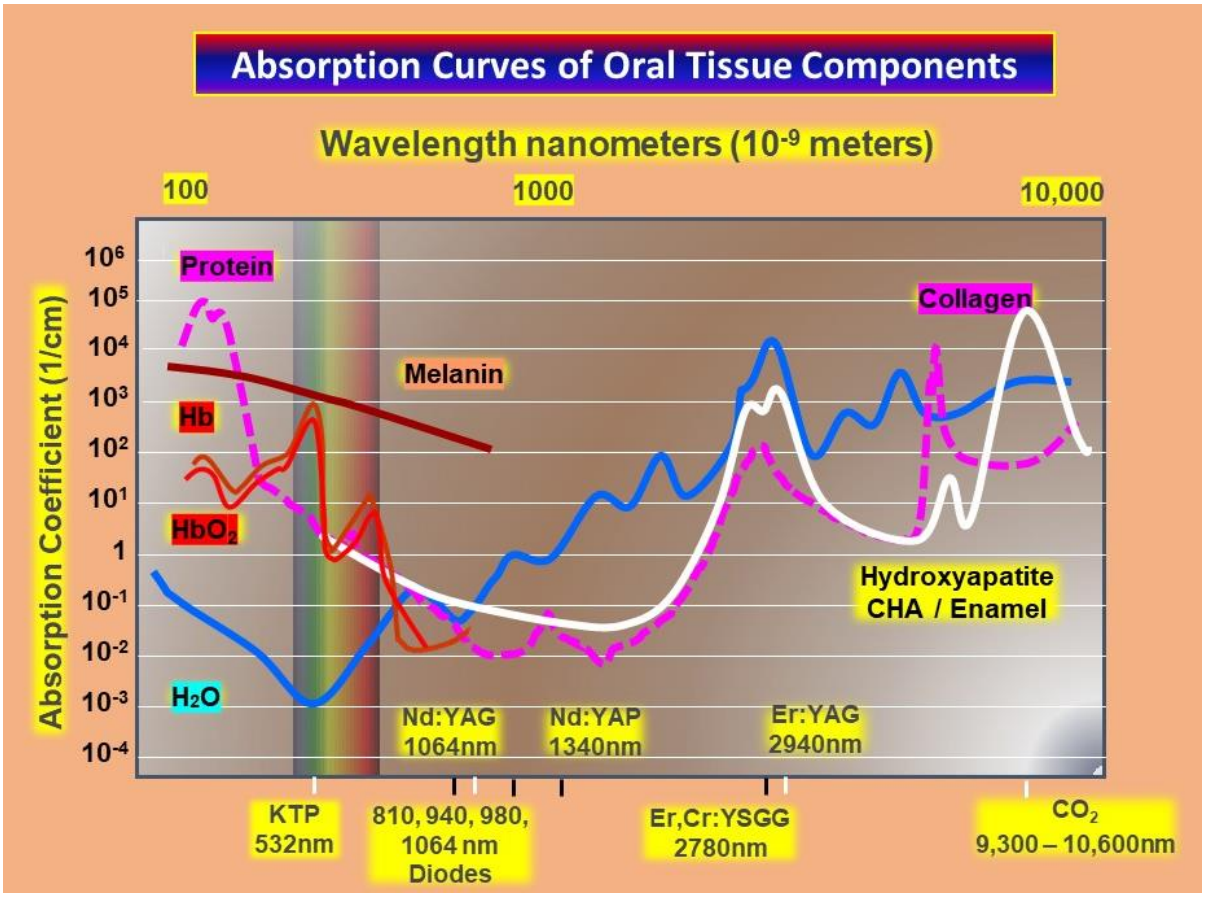

Figure #2. Absorption spectral profiles of major dental/oral structural elements and chromophores associated with soft tissue management. Absorbance is shown relative to

wavelength of irradiation. The depth of penetration is shown as 1/absorbance. Original data and graphics: Parker S. Data source: Parker, S; et al. Laser Essentials for the Dental Practitioner: Foundation Knowledge—Construction, Modes of Operation and Safety. EC Dental Science 2019, 18.9, 2020–2027 [2].

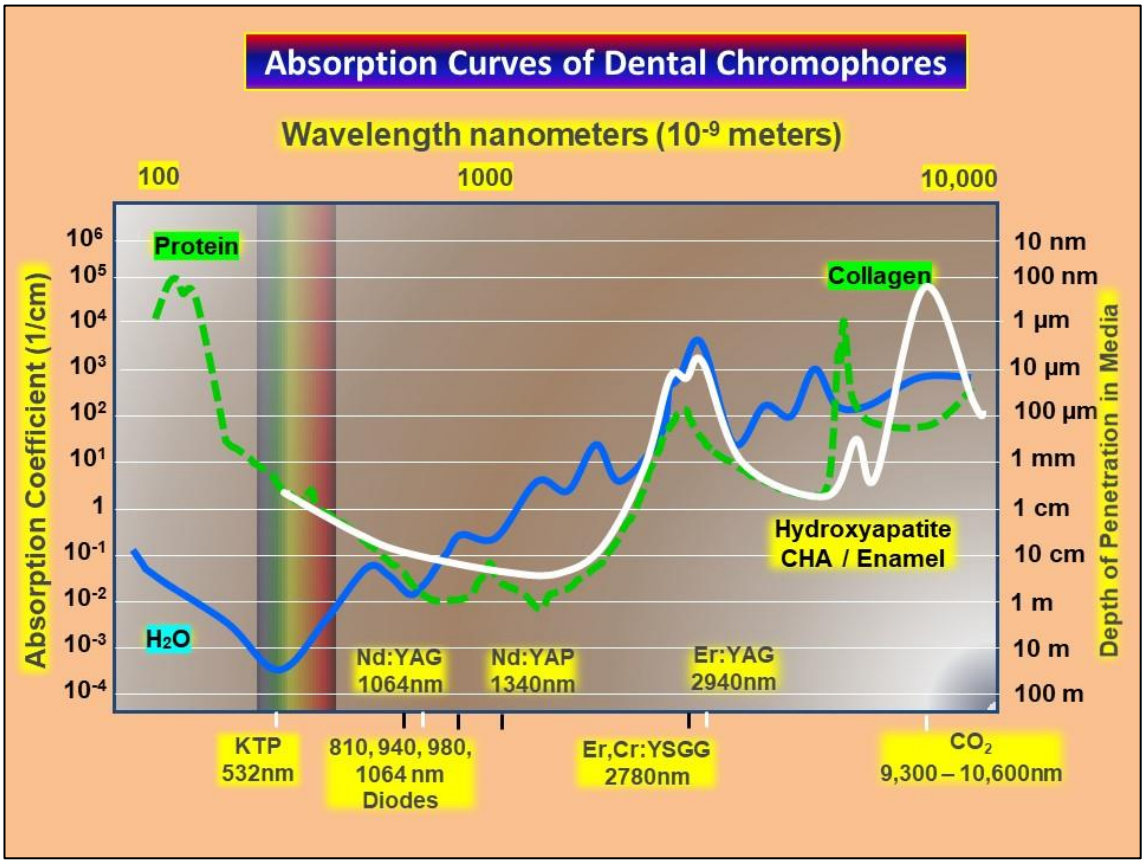

Figure #3. Absorption spectral profiles curves of major tissue elements associated with bone and dental hard tissue management. Absorbance is shown relative to wavelength of irradiation. Key: water (blue), protein/collagen (green), hydroxyapatite—HA and carbonated HA (white). Original data and graphics: Parker S. Data source: Parker, S; et al. Laser Essentials for the Dental Practitioner: Foundation Knowledge—Construction, Modes of Operation and Safety. EC Dental Science 2019, 18.9, 2020–2027 [2].

Within tissue elements, there may be molecules or molecular species termed chromophores. A chromophore is defined as "a chemical group (molecule or molecular species) that absorbs light, or at least shows some absorbance in the visible region of the electromagnetic spectrum and so imparts colour to the molecule".

Each chromophore has molecular structure and the structure, atomic configuration and inter-atomic binding energy at body temperature may define a “ground state” [3]. In achieving ablative laser surgery in soft or hard tissue clinical dentistry, the effect of absorption of incident energy is the conversion to thermal energy and consequent tissue ablation through thermal rise—a process known as photothermolysis. Assuming that absorption can occur, the relationship between the level of incident photonic energy, the density of such energy within an irradiated area, and the exposure time (instantaneous and/or over a sustained or interrupted period), may enable the clinician to influence the type of laser–tissue interaction. By reducing the exposure time and consequent interaction to micro-seconds and on to femto-seconds, successively higher peak power densities above 10^{6-10} W/cm^2 can be obtained. At such powerful levels, the intensity of energy is so great that electromagnetic fields (plasma) develop around the interaction and are sufficient to markedly degrade target molecules, a process known as photoplasmolysis [4].

Conversely, very low irradiance over extended time periods may give rise to energised biochemical pathways associated with tissue health and reparative capabilities, and this represents the core of understanding of photobiomodulation (PBM). *Figure #4* provides a graphic representation of the relationship between power density and exposure time [5].

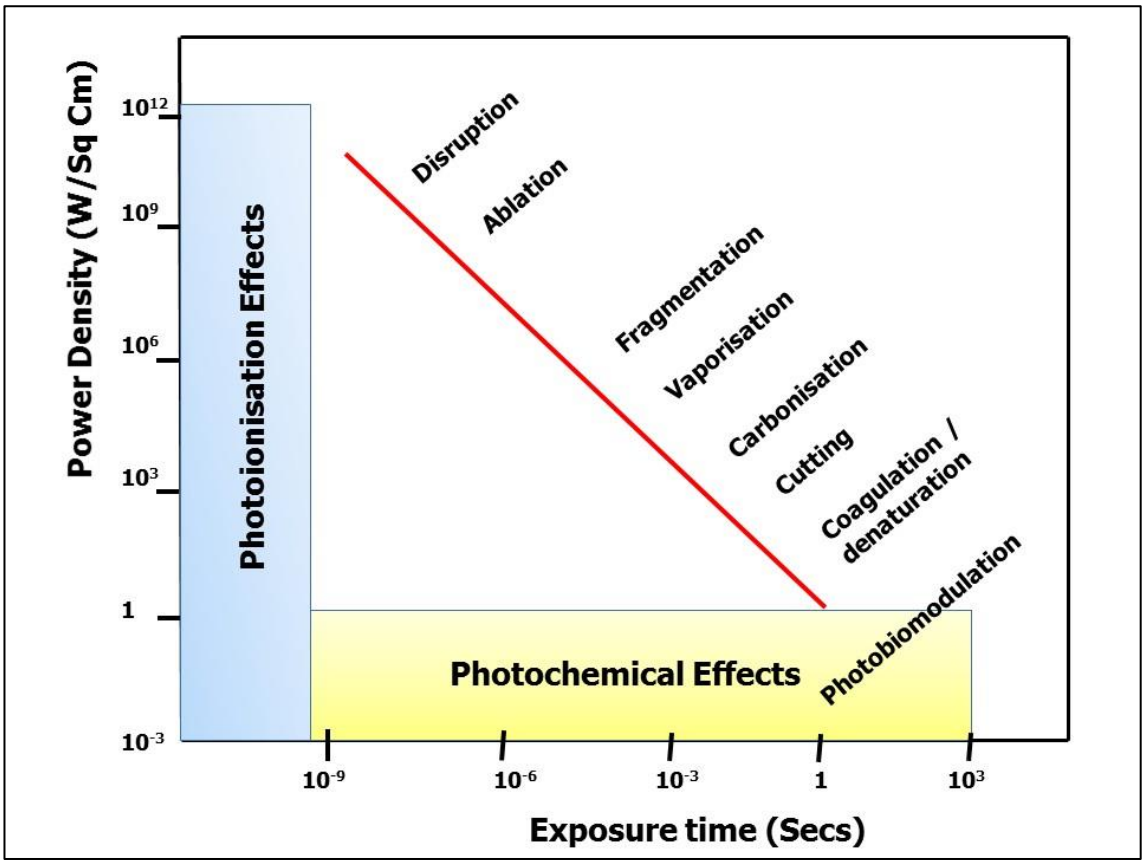

Figure #4. Relationship between incident photonic power density and exposure time (seconds). Changes in the two components of laser–tissue interactions—power density (irradiance) and exposure time, may affect the interaction level. Original graphics: Parker S. Data source: Boulnois, J-L. Laser in Medical Science 1986, (1), 47–66 [5].

2. Laser Photonic Energy

Laser photonic energy is derived through the emission of photons from an energised source. The photon (electromagnetic) energy is derived from the parent stimulated atomic source within the active medium, and its propagation within a stream of photons provides an identical emission waveform (coherence); each photon within the stream has an identical energy value (monochromatic or single wavelength). The relationship between common laser wavelengths within the electromagnetic spectrum is represented in *Figure #5.*

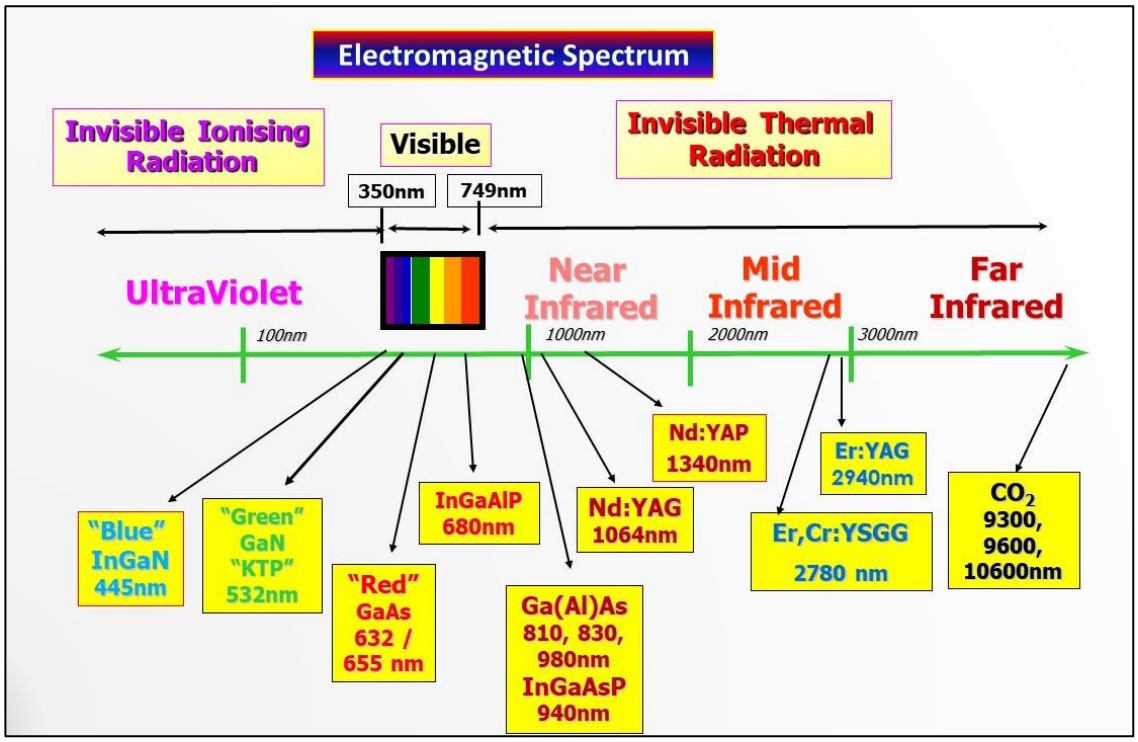

Figure #5. Laser wavelengths in common use in clinical dentistry, arranged according to wavelength in nanometers (10^{-9} m), from blue-visible to the far infra-red regions.

The energy of emitted photons is expressed in Joules (J), or a derived equivalence, eV (a unit of energy equal to the work done on an electron in accelerating it through a potential difference of one volt), where 1 eV = 1.602×10^{-19} J. Since photonic energy and wavelength (λ) have an inverse proportional relationship, photons emitted from different active medium sources will have differing energy values. A basic expression of this relationship is demonstrated in *equation (1)*, in which h = Plank's constant, c = speed of light and E = photon energy in eV.

$$\lambda = hc/E \qquad (1)$$

It is possible to evaluate energy-equivalent values for the laser wavelengths commonly used in dentistry, for example, photons of wavelength 1240 nm (near-infra-red region) equate to an eV value of 1.0, whereas an eV value of 2.0 is represented by a wavelength of 621 nm (visible-red region); 0.13 eV corresponds to 9600 nm [6].

Table #1 provides an overview of laser wavelengths commonly used in dentistry with corresponding photonic energy values:

(eV)	Laser	λ (nm)
2.4	KTP	532
2.0	He-Ne	633
1.6	Diode	810
1.2	Nd:YAG	1064
0.4	Er:YAG	2940
0.1	CO_2	10600

Table #1. Commonly used laser wavelengths associated with dental treatment. Photonic energy and wavelength are inversely related. With ascending numerical values of wavelength, the corresponding photonic energy (expressed as electron volt—eV) is reduced.

A simplistic view of one of the many graphic representations of the inter-relationships between target tissue elements, incident laser wavelengths and relative absorption potential, would suggest that laser photonic energy is capable of ablative interaction with target tissue elements [7]. If external energy is applied, vibrational and spin phenomena are induced—initially reversible to the ground state, but with increased applied energy, a point may be reached where molecular disruption is sufficient to overcome the forces binding atoms or macromolecules together [8]. Incident photonic energy may induce a thermal rise (energy conversion), and examples of the effects of ascending energy levels above an "ablation" threshold include protein dissociation (ca. 60 °C) and water vaporisation. True photonic ablation of a target molecule therefore represents incident energy sufficiently intense to break interatomic binding forces, and this is termed the

dissociation energy. *Table #2.* Provides examples of commonly found tissue element molecules and the dissociation energy value required to break the interatomic bond.

Dissociation energy of selected chemical bonds*	
Type of Bond	**Dissociation energy (eV)**
C=O	**7.1**
C=C	**6.4**
O-H	**4.8**
N-H	**4.1**
C-C	**3.6**
C-N	**3.0**
C-S	**2.7**
Fe-OH	**0.35**
HA Lattice	**310**

Table #2. Dissociation energy, expressed in eV values, required to break the bonds (covalent, coordinate, etc.) that bind atoms within molecules. Examples represent component molecules within tissue water and biofluids, and also ionic forces within the crystal lattice of hydroxyapatite. Data reproduced with thanks from Mó, O; Yáñez, M, et al. (2005). Journal of Physical Chemistry A. 109(19), 4359–4365 [3].

When comparing data in *Table 1 and Table 2*, almost none of the popular laser photonic energies in dentistry are capable of direct intra-molecular bond cleavage, and one may conclude that dental lasers cannot ablate target oral tissues using empirical photonic energies. Such a belief is magnified when the binding (ionic) lattice energies of crystalline carbonated hydroxyapatite are exposed to the mid-IR laser wavelengths (Er,Cr:YSGG, Er:YAG); indeed, the photonic energy values may be considered very small when compared to that required for the dissociation of hard dental tissue [9].

Although individual photons possess insufficient energy to cleave target molecules, even the briefest of laser emissions will deliver millions of individual photons, and with each successive photon absorbed, the gradual build-up of transferred energy causes increasing target molecular vibrations, up to a point where sufficiently high-power density (i.e., energy density within an ultra-short time duration) and consequent thermal rises, drives molecular fragmentation and structural ablation.

Molecular disruption is not the objective when considering the many sub-ablative applications of laser photonic energy within PBM, diagnostic and para-chemical antimicrobial applications. With this group of applications, the modulation of target structural molecules or biochemical pathways remains the objective within a general enhancement of cellular and intercellular activities, with consequent benefits being derived through the healing, analgesic and anti-inflammatory applications of sub-ablative laser irradiation.

When laser photonic energy is delivered and interacts with a tissue medium, three possible pathways exist to account for what arises from the delivered light energy:

(1) The commonest pathway when light is absorbed by living tissue is internal conversion from incident photonic to thermal energy. In surgical laser use, the resulting thermal rise is near-instantaneous and considerable, and rapidly leads to conductive thermal energy into surrounding tissue. With oral soft tissues and visible/near-IR laser wavelengths, the absorption by tissue chromophores gives rise to protein denaturation and secondary vaporisation of interstitial water. Through this, a visible ablation and vaporisation of target tissue occurs [10].

With longer mid- and far-IR laser wavelengths, the prime molecule affected by irradiation in both soft and hard oral tissues is highly abundant water. Ablation of tissue is achieved through the near-instantaneous vaporisation of interstitial water, leading to 'explosive' fragmentation of tissue structure. With hard osseous/dental tissue, this interaction can be quite dramatic [10].

(2) Laser photonic energy values below target tissue ablation levels may result in fluorescence. Fluorescence is a luminescence (re-emission) of light in which the absorption of a photon by a molecule triggers the emission of another photon with a longer wavelength from that molecule. This action provides the basis for optical scanning techniques employed for caries detection in enamel and dentine, and also tomographic techniques utilised in the scanning of soft tissues for neoplastic change.

(3) The third pathway is broadly termed photochemistry [11]. In view of the energy of the photons involved, covalent bonds primarily remain unbroken. However, the energy may be sufficient for a first excited singlet state to be formed, and this can then undergo intersystem crossing to the long-lived triplet state of the scavenger. Through energy transfer, adjacent diatomic interstitial tissue oxygen (O_2) molecules may undergo redox interconversions to form reactive oxygen species (ROS, such as superoxide anion) or singlet oxygen ($^1O_2$). Singlet oxygen is an ultra-short-lived product of the parent molecule that can cause cell apoptosis through oxidative stress. Such actions can be commonly encountered in photodynamic therapies, where an intermediary chemical—photosensitiser—is employed to direct energy transfer to target bacterial or alternative cellular sites [12,13].

Electron transfer reactions are highly important in the host cell mitochondrial membrane, where the consequence of photon irradiation may be increased production of ATP [14]. An additional photochemical pathway that can occur after the absorption of a red or NIR photon within a host cell is the dissociation of a co-ordinated ligand from its metal ion coordination centre as an active site within an enzyme. One well known candidate for this pathway is the complexation of nitric oxide ($NO^{\bullet}$) to the iron-and copper ion-containing redox centres in unit IV of the mitochondrial respiratory chain, otherwise known as cytochrome c oxidase. Such an action may induce an increase in intracellular pH and generate ATP, and has been cited, as has been the release of increments of molecularly bound $NO^{\bullet}$ and intracellular ROS, as basic photobiomodulation cellular theory for the actions of low-level lasers.

3. Laser–Tissue Interaction: Oral Soft Tissue

Although diverse in structural components, oral soft tissue is a mixture of endothelial collagenous tissue with blood and lymph vessels, muscle, site-specific mucous and salivary gland tissues, nerves and other anatomical structures. All oral soft tissue appending to the lips, buccal cavity and oropharynx is surrounded by epithelial tissue—termed the oral mucous membrane (of varying levels of keratinisation) in order to protect structures against regular exposure to physical wear (gingiva, hard palate and dorsum of the tongue covered with keratinised epithelium), or other sites where abrasion is somewhat lower (lips, oral vestibule, ventral tongue and floor of mouth and soft palate). Laser-sensitive biomolecules include tissue water, proteins, chromophoric molecules and the haem prosthetic group of blood haemoglobin species.

In clinical dentistry, laser applications may involve visible and near-IR wavelengths (absorbed by chromophores, haemoglobin and other proteins), such as the diverse “diode”

group (wavelength range 405nm–1064 nm), mid-infra-red lasers (erbium, chromium: YSGG 2780 nm and erbium: YAG 2940 nm), and the far IR CO_2 wavelength range of 9,300nm–10,600 nm that can target tissue water. The difference in target laser light scavengers as a function of wavelength will affect both the degree of tissue penetration and essential ablation effect; water, being a constituent of all living matter, will give rise to a surface-mediated ”V-shaped” interaction with longer wavelengths, whereas chromophore and protein interactions that are predominant with shorter wavelengths will result in a wider, crater-shaped area of ablation—*Figure #6.*

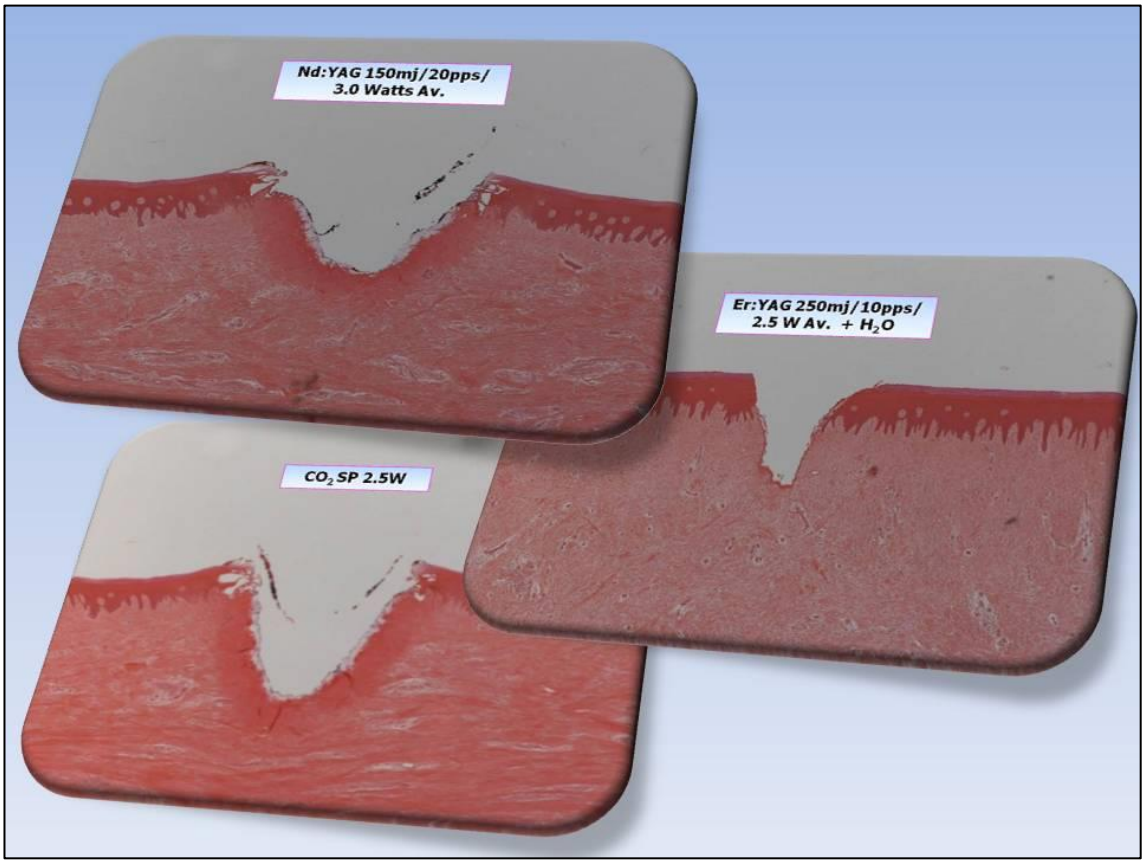

Figure #6. Comparative light micrographs of laser interaction with porcine oral soft tissue. Top: Nd:YAG 1064 nm (an example of the shorter wavelengths employed), causes a wider, crater-shaped area of ablation, with some areas of thermal conduction. Right: Longer wavelengths such as FRP Er:YAG 2940 nm create a sharper “V” shaped incision, whereas Bottom: CO_2 10,600 nm, being a gated CW emission, results in some features of the other two—surface configuration ascribable to absorption in water, but some thermal spread arising from a comparative lack of thermal relaxation.

The power delivery (emission mode) of the early dental lasers was either continuous wave or, in the case of Nd:YAG 1064 nm, micro-pulsed. The disadvantage of the continuous wave delivery was the absence of thermal relaxation, which is required to prevent the gradual and damaging build-up of thermal energy into tissue surrounding the target site. Current technology has allowed the development of chopped or "gated" delivery of an inherent continuous wave, allowing millisecond (ms) and some microsecond (μs) bursts of energy. This has helped to refine the overall "average" power delivery. Irrespective of the emission mode, for oral soft tissue surgery to involve gingiva, oral mucous membrane and associated mucous gland tissue, frenal tissue and other benign soft tissue pathologies, there is an adequate opportunity to deliver predictable laser surgery with mean power ranges of 0.8–2.0 W; indeed, the use of considerably higher average power values to ablate or incise tissue may result in collateral tissue damage. A possible exception may be the use of non-contact irradiation of superficial venous haemangiomata, where greater (> 5 W) mean power levels can be employed [15].

The benefits of laser-mediated soft tissue surgery are claimed to be haemostasis and incisional sterility, with the reduced/absent requirement for sutures. Within the applications referenced above, the power values would be sufficient to seal blood and lymphatic vessels of < 0.5 mm diameter [16], and within such limitations, incisional haemostasis offers a near-ubiquitous advantage of visible and near-IR laser use. Additionally, the use of the erbium family and carbon dioxide laser wavelengths, albeit without co-axial water spray, may induce a significant thermal rise within the surgical site sufficient to also seal small vessels and thus avoid dressings or sutures.

Strict sterility of the surgical site is an ideal requirement, but contamination by the normal oral flora may be considered unavoidable. The incisional temperature will exceed 100 °C,

and even approach 150–200 °C, and hence would provide a prior level of sterility. An improved representation would be a “significant pathogen reduction”. Of considerable advantage, however, is the formation of a denatured plasma and collagen matrix—a so-called “coagulum” [17], that provides initial sealing of the wound and gradually softens through absorption of saliva throughout a 72–96 hour period before becoming detached, leaving early endothelial and epithelial cellular ingrowth to initiate healing. Additionally, a feature often observed with laser-mediated soft tissue incisions is the absence of scarring [18], although healing will always proceed via secondary intention in view of tissue volume loss during surgery, and the non-apposition of cut edges that would normally occur with sutures.

3.1. “Uneventful” Healing—Photobiomodulation

Of significance to both post-surgical tissue healing, and the sub-ablative application of laser photonic energy, is the phenomenon of photobiomodulation (PBM). Photobiology is the scientific study of the interactions of light (non-ionising radiation) and living organisms [19]. Examples of photobiological processes in living cells include photosynthesis, bioluminescence, and indirectly, circadian rhythms.

PBM therapy (PBMT) through the application of photonic energy at specific wavelengths works on the principle of inducing a biological response through energy transfer. Such non-ablative photonic energy delivered into tissues modulates biological processes within that tissue, and within the biological system of which that tissue is a component part. It is a source of some debate, however, which concerns the thermal rise in irradiated cells or tissue, bearing in mind that because of absorption, photonic energy will impart increased target molecular activity. However, within a correct incident dose, PBM has no appreciable thermal effects in irradiated tissue [20].

At a cellular level, the application of PBM and absorption biomolecules, and, where relevant, chromophores such as cytochrome c oxidase, has been suggested to promote modifications in mitochondrial activity—essentially through a shift in cellular metabolism towards an aerobic glycolytic cycle and an increase in the manufacture and extracellular release of $NO^{\bullet}$. Increased cellular activity arising from an optimisation of ATP production and the associated release of ROS together may promote a combination of effects, including activated transcription factors affecting RNA and DNA synthesis, a process positively impacting on cellular repair and healing. Indirect effects including nitrogen oxide liberation through electron transport chain activity will lead to increased local vessel dilation, and increased oxygen availability and cell permeability. Overall, an increase in mitosis and altered cellular autophagy is observed [21].

Tissue-dependant extracellular effects of PBM may include the selective activation of anti-inflammatory cytokine pathways, resulting in the enhanced resolution of acute and chronic inflammation [22], an optimisation of the consequent production of regenerative products such as collagen and bone [23,24,25,26], improvements in lymphatic drainage, and an increase in the availability of O_2 to tissues consequent to vasodilatation and the ability to induce analgesia [27].

Non-surgical pathologies that may respond to PBM therapy include TMJDS, trigeminal neuralgia, oral mucositis, myofacial pain syndrome, herpetic lesions and post-herpetic neuralgia, as well as post-surgery pain management, and also pain associated with various low-grade dental dysaesthesia and that experienced during active orthodontic treatments. Relevant reference sources relevant to the specific dosimetry required, together with the specifics of laser and hand-piece delivery, should be sourced for further detail [28,29,30,31,32,33], although the prescribed dose for many of these conditions in terms

of fluence or energy density is between 2–10 Joules/cm^2, with an appropriate increase in applied "skin dose" to account for deep tissue photon scatter when treating sub-surface structures and conditions [34]. PBM is often cited as the "hidden assistant" during laser soft tissue surgery, in view of photon scatter and power density reductions, and consequent tissue cooling at increasing distances from the surgical site will produce sub-ablative PBM effects. This is considered a significant aspect of the lack of complications following laser soft tissue surgery within the oral cavity. In summary, investigations of the positive effects of PBM have been suggested to suppress inflammatory responses, induce analgesic mechanisms and promote healing [35,36]. *Figure #7* demonstrates the haemostatic control, lack of inflammatory response and uneventful healing in laser-mediated minor oral soft tissue surgery.

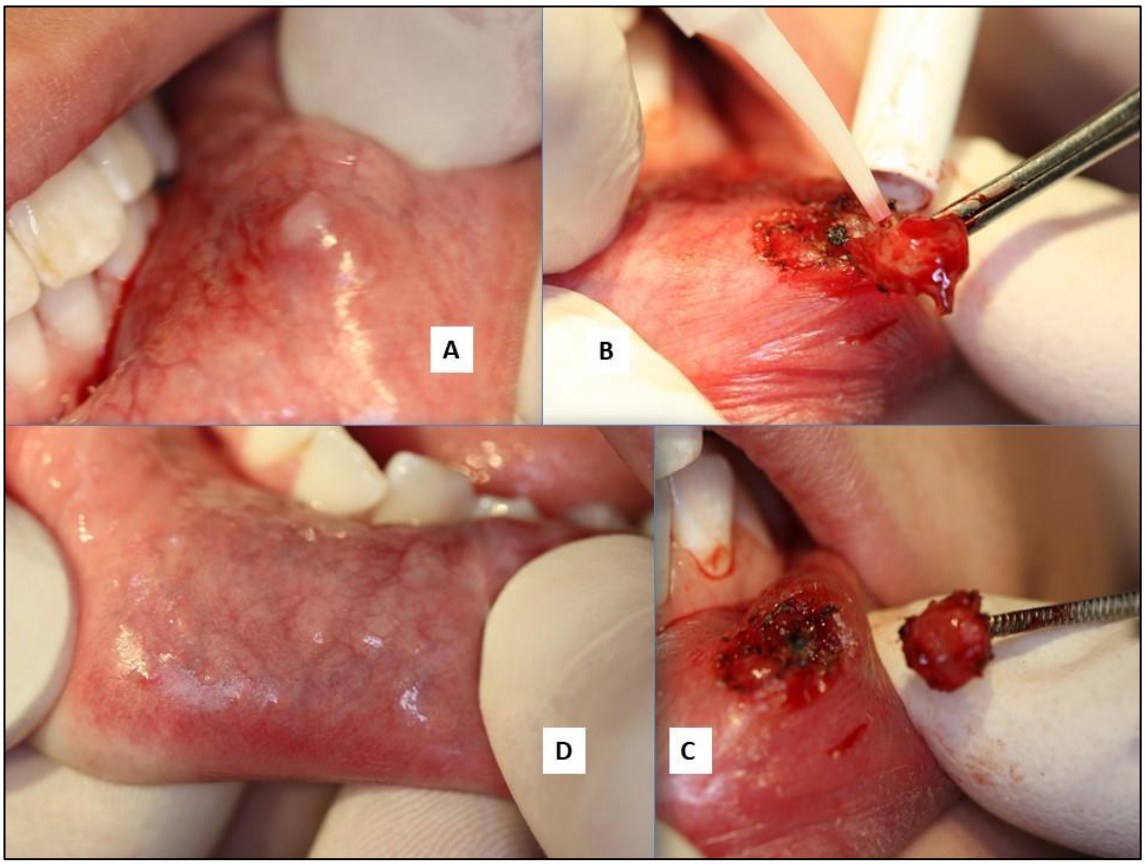

Figure #7. Soft tissue surgery. A mucocele excision, lower lip. The laser used was a diode 980 nm 1.25W CW Fluence 12.2 J/cm2. Time taken: two minutes with pauses. A—pre-op, B—excision with haemostasis, C—immediate post-op, D—healing at one month.

3.2. Laser-Mediated Diagnostics

A number of biological structures have the ability to strongly fluorescence, in which an incident photon of known wavelength is absorbed by the target tissue, then loses a small amount of energy and is re-emitted at a longer wavelength, that may indeed be detectable, either visually or through suitable apparatus [37].

A number of such fluorophores are found in both soft tissue and hard tissue, blood and blood products, and bacterial plaque and calculus. Accordingly, the application of suitable incident wavelengths may enable the clinician to establish not only the presence, but also the relevance of healthy, unhealthy or unwanted molecular components. This can aid the diagnosis of dental caries, dental calculus and periopathic bacterial plaque, soft tissue health status, and dysplastic or neoplastic changes; additionally, it may give rise to false-negative re-emission in composite restoration material, or alternatively form the basis of photosensitiser-mediated antimicrobial photodynamic therapy.

A summary of common fluorophores, the incident light wavelength required for them, and the consequent re-emission at longer wavelengths is provided in *Table #3*.

Fluorophore	Excitation nm	Fluorescence Peak	Comments
Tryptophan	275	350	Protein
Collagen	335	390	Connective tissue (CT)
Elastin	360	410	CT
Keratin	370	505	Surface analysis
Porphyrins	405, 630	590, 625, 635, 705	Cell mitochondria / metallo-, copro-, proto-porphyrins
Healthy enamel	405	533	
Caries	405, 488, 655	580 - 700	
Inorganic composites / GI	655	Fluorescence > 700 nm giving rise to false positives.	
Calculus / plaque	405, 630	Fluorescence peaks assoc. with porphyrins giving rise to false positives.	

Table #3. Common natural and manufactured fluorophores that may be met within clinical dentistry. "Overlapping" excitation—for example, that found in porphyrins as an original component of blood haemoglobin but also a by-product contaminant of dental plaque, calculus, and caries, and also tooth discolourations. Source: Original graphics S. Parker. Data reproduced with thanks: Kim, A; Roy, M; Dadani, FN; Wilson, BC. (2010). Topographic mapping of subsurface fluorescent structures in tissue using multiwavelength excitation. Journal of Biomedical Optics, 15(6), 066026 [37].

Additional examples of changes in light frequency, scatter and transmission phenomena may be observed in diagnostic techniques such as doppler flowmetry, diffuse reflectance spectroscopy, optical coherence tomography and Raman spectroscopy [38,39,40,41]. In general, these adjunctive techniques are based upon incident versus re-emission light performance parametry, as applied against known background control healthy human tissue data.

3.3. Laser–Tissue Interactions: Dental and Oral Hard Tissues

The mineral hydroxyapatite is common to dentine and bone, whereas enamel is composed of carbonated hydroxyapatite. Both forms of the mineral are crystalline structures with strong, resistant ionic bonding within the molecule. Dental hard tissue—enamel and dentine—are composite tissues of varying amounts of mineral, protein, and water. Dentine, of similar composition to bone, has 45–47% mineral, 20–22% water and 33% protein, whereas enamel has a much higher mineral content (85%), 12% water and 3% protein (mostly found as an inter-prismatic boundary material) [42].

Water, either as an interstitial medium or as a source of acidic hydrogen ions (H^+) and alkaline hydroxide ions (OH^-), is readily susceptible to vaporisation when applying radiant erbium family wavelengths, and the substantial volumetric change of vaporisation creates a pressure and temperature change that is sufficient to dislocate the crystalline structure and cause a micro-explosive ablation at the point of application—a process known as spallation. The 9,300 nm CO_2 wavelength, although inherently a CW emission, has a tailored micro-gated delivery; hence, there is both some absorption within the water component, but also some targeting of the phosphate and hydrogen phosphate anions of the parent mineral molecule. The surgical management of both dental and osseous tissue requires accuracy and thermal containment to avoid unwanted collateral damage. Indeed, the employment of appropriate incident photonic power and laser wavelength with adjunctive water cooling and thermal relaxation will allow for the predictable and selective removal of diseased tissue features. *Figure #8 and Figure #9.*

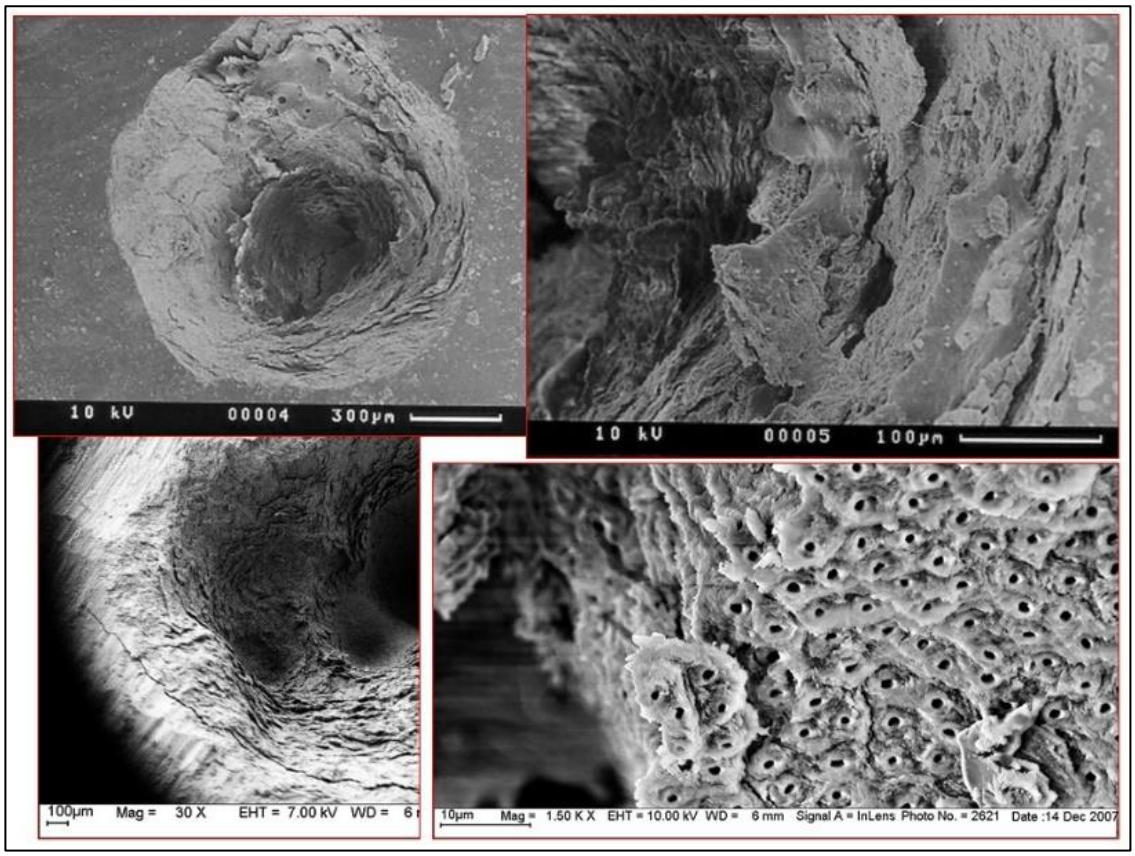

Figure #8. Scanning Electron Microscope (SEM) micrographs of laser-mediated enamel and dentine ablation. Top left and right: the resultant enamel surface is rugged, fragmented and capable of accepting a resin-based composite restoration once any unstable fragments have been removed. Bottom left and right: dentine is rendered smear layer-free, with an intact and stable cut surface.

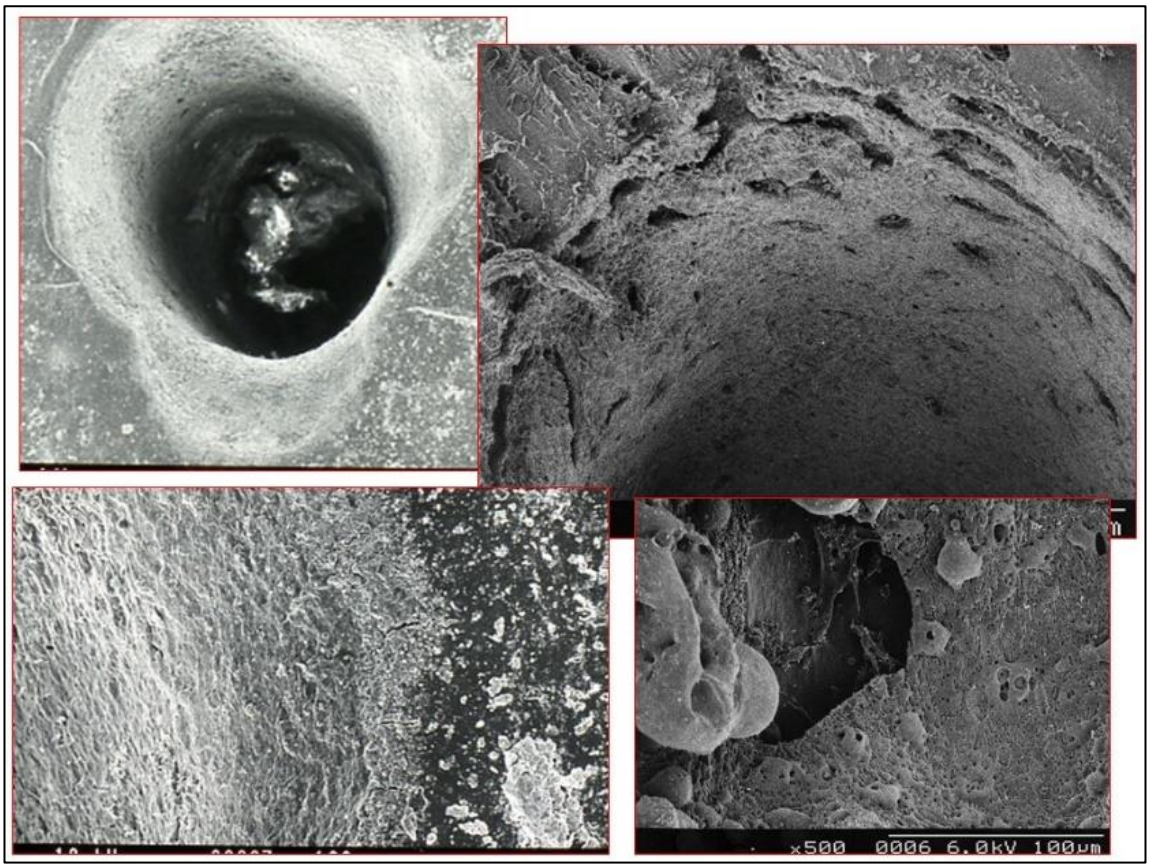

Figure #9. SEM micrograph of laser mediated ablation of bone. Top left, right and bottom left: the use of an erbium laser system enables an accurate and clean ablation without evidence of charring or thermal cracking. Bottom right: this is in contrast to the use of a Nd:YAG system (bottom right), which can result in extensive heat-damage, and melting of the parent hydroxyapatite structure.

In addition, considerable investigations to address and verify the (often anecdotal) claim of "painless" laser-mediated tooth cavity preparation have been performed. Using visual analogue scoring and randomised clinical trials, a quantification of outcome has helped to define the prospect of anaesthesia-free cavity preparation [43].

Both erbium and CO_2 lasers demand co-axial water spray during use to disperse the products of ablation, and to provide adjunctive cooling of the target hard tissue *Figure #10*.

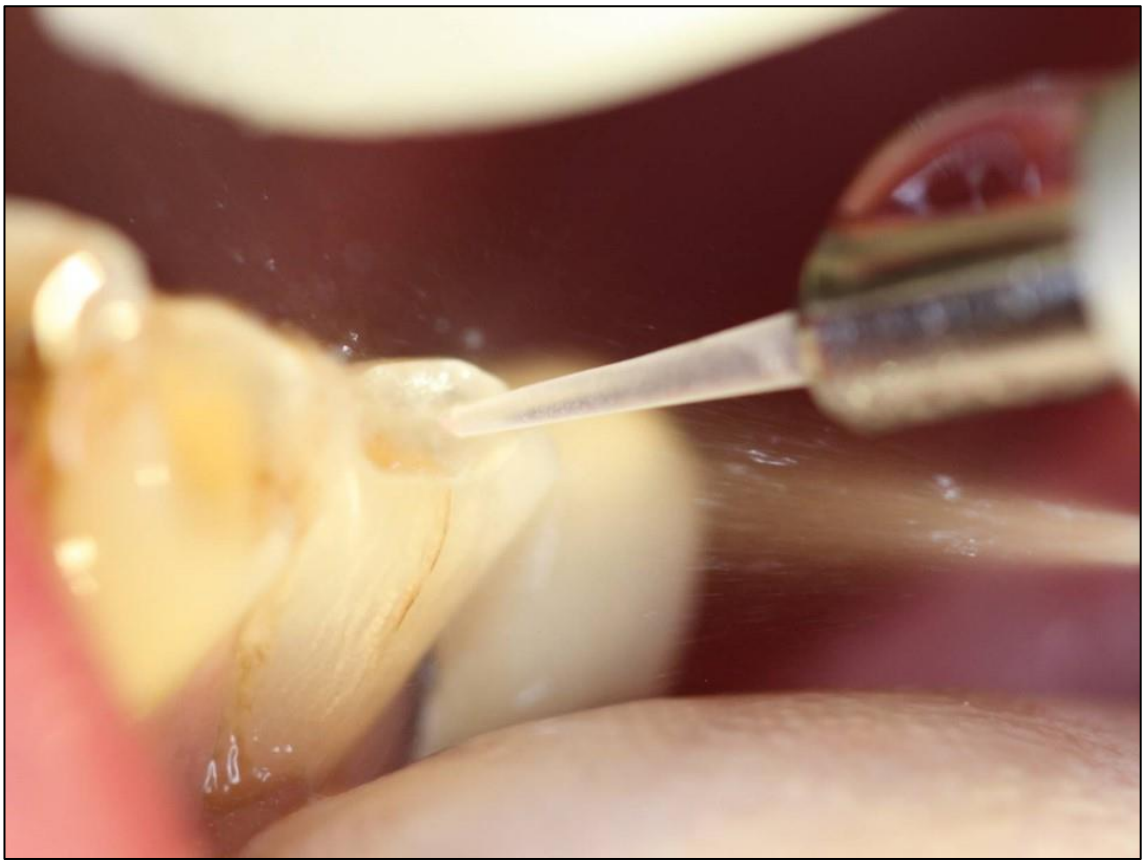

Figure #10. Use of the er YAG laser in tooth cavity preparation. Note the co-axial water spray to aid dispersal of ablation debris and cool the target hard tissue site.

4. Operating Parametry of Laser–Tissue Interactions

Of practical interest to the clinician in using laser photonic energy, the following factors will each and collectively affect the absorption of laser light by a chosen target tissue [44]:

- Laser wavelength
- Laser emission mode
- Tissue composition
- Tissue thickness
- Surface wetness arising from water or saliva
- Incident angle of the laser beam
- Exposure time.

- Contact versus non-contact modes employed between laser delivery tip and tissue.
- Thermal relaxation factors—Exogenous (water spray, tissue precooling, high-speed suction, pulsing/gating laser emission) and endogenous (tissue type and density, blood supply).

The consequence of such appreciation is to enable the chosen laser therapy to be used, and to avoid the disadvantage of excessive and possibly deleterious thermal increases. Whether the intended use of a laser is diagnostics, sub-ablative PBM or supra-ablative target tissue manipulation, three essential elements are required for careful consideration: (1) the correct or appropriate laser wavelength, and (2) the correct or appropriate light delivery power density and appropriate thermal relaxation process. However, the parameters selected will represent the mainstays of competency on the part of clinicians. Failure to observe such protocols may result in unwanted and damaging collateral thermal rises, but also a change in the optical properties of the target tissue that may indeed alter the optimal laser–tissue interaction desired. This is summarised in *Figure 11*.

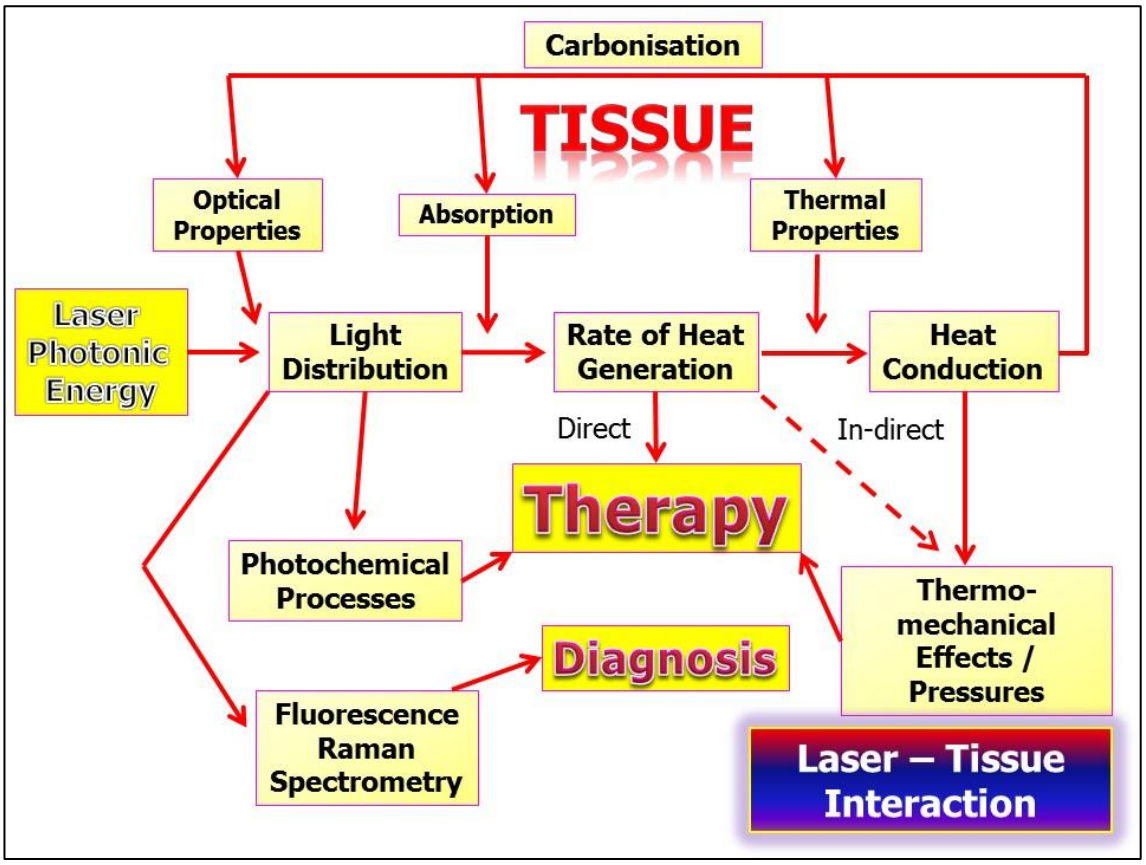

Figure #11. Algorithm depicting the multi-level interaction of varying incident photonic energy as applied to target tissues of quantifiable optical properties. Of prime concern is the thermal containment of laser–tissue interactions. Excessive application of incident power may cause overheating and carbonisation, leading to aberrant interactive effects.

Concern continues to be expressed over the lack of complete and comprehensive laser operating parameters in published literature to enable the clinician to use laser therapy to optimise a desired outcome [45].

5. Conclusions

Exposing oral and dental tissues to photonic energy has enabled a transformation of how both the assessment of oral health and the modality for treating disease can be effectively achieved. Of key importance to laser–tissue interactions within a predicted therapy "envelope" are demands for the clinician to understand the varying compositions of host tissues, and how these may be managed and manipulated using laser energy without

unwanted damaging effects. Laser–tissue interactions may be sometimes inconsistent due to tissue anisotropy and may continue to pose some difficulty for the dental clinician; however, the development of many laser delivery instruments, amounting to a facility to produce laser photonic energy at several wavelengths between the visible and far-IR areas of the electromagnetic spectrum, continue to address many of these inconsistencies.

The current paper outlines the basis of laser–tissue interactions and presents how individual laser wavelengths of varying operating parameters may be applied to interact with target oral and dental tissues.

Author Contributions

Conceptualization, S.P.; Methodology, E.A., V.M. and M.C.; Writing original draft manuscript, S.P.; Review and editing, M.G., E.A., V.M. and E.L.; Project administration, S.P. All authors have read and agreed to the published version of the manuscript.

Funding

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Conflicts of Interest

The authors declare no conflict of interest.

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1.5 Clinical challenge of laser-tissue interaction

An overview of laser integration to clinical dentistry has been given; specific essentials of laser-tissue interactions have been discussed, as they might apply to hard and soft oral and dental tissue, using surgical ablative and sub-ablative photonic fluences and their effects. Additional brief reference is offered relating to the use of photonic energy in relation to photochemical effects in tooth whitening and photodynamic processes.

Of fundamental concern must be the effects of laser operational parametry errors, which may result in possible failure through insufficient laser irradiation; of greater concern is where excessive parameters are employed and collateral, essentially thermal collateral damage ensues [52]. Insufficient light dose as a part of prescribed therapy may be a source of some frustration during photothermal laser surgery but it is however, of much greater significance in relation to the precise delivery of sub-ablative fluence associated with photobiomodulation; this latter aspect will be covered in greater detail later in this thesis.

1.6 Laser surgical "overdose" – oral soft tissue

As discussed in Paper I [43], several factors contribute – individually and collectively – to an ideal outcome of laser soft tissue surgery.

- Laser wavelength
- Laser emission mode
- Tissue composition
- Tissue thickness
- Surface wetness arising from water or saliva
- Incident angle of the laser beam
- Exposure time.

- Contact *versus* non-contact modes employed between the laser delivery tip and tissue.
- Thermal relaxation factors—Exogenous (water spray, tissue precooling, high-speed suction, pulsing/gating laser emission) and endogenous (tissue type and density, blood supply).

Based upon the majority of laser operating protocols issued by manufacturers, a range of average power values to achieve oral soft tissue surgical management is recommended as 0.8Watts – 2.5Watts. Early diode lasers operated with a continuous wave (CW) emission, with a rudimentary optional 50% "gating" mechanism; with improvements in technology, this has been greatly modifed and improved, with a microsecond gating and kilohertz emission train, such that the resulting incision may be both smooth and with inherent degrees of thermal relaxation. This provides greater control of laser incision and ablation. With the Nd:YAG, Er,Cr:YSGG and Er:YAG solid state lasers, the free-running pulse (FRP) delivery has benefitted from improvements that have reduced the pulse width to values as low as 60 microsec, to reduce the "thermal footprint" of photonic delivery. The carbon dioxide (CO_2) laser at 10,600nm CW emission was highly absorbed in water and thermal side-effects through surface desiccation of tissue rendered it necessary to remove the build-up of charred tissue (*Figure #3*). Again, with improvements and development in technology, micro-second gating has improved the thermal containment and the emergence of an all-tissue 9,300nm micro-gated emission laser with a coupled coaxial water spray offers still greater flexibility in soft tissue surgical management.

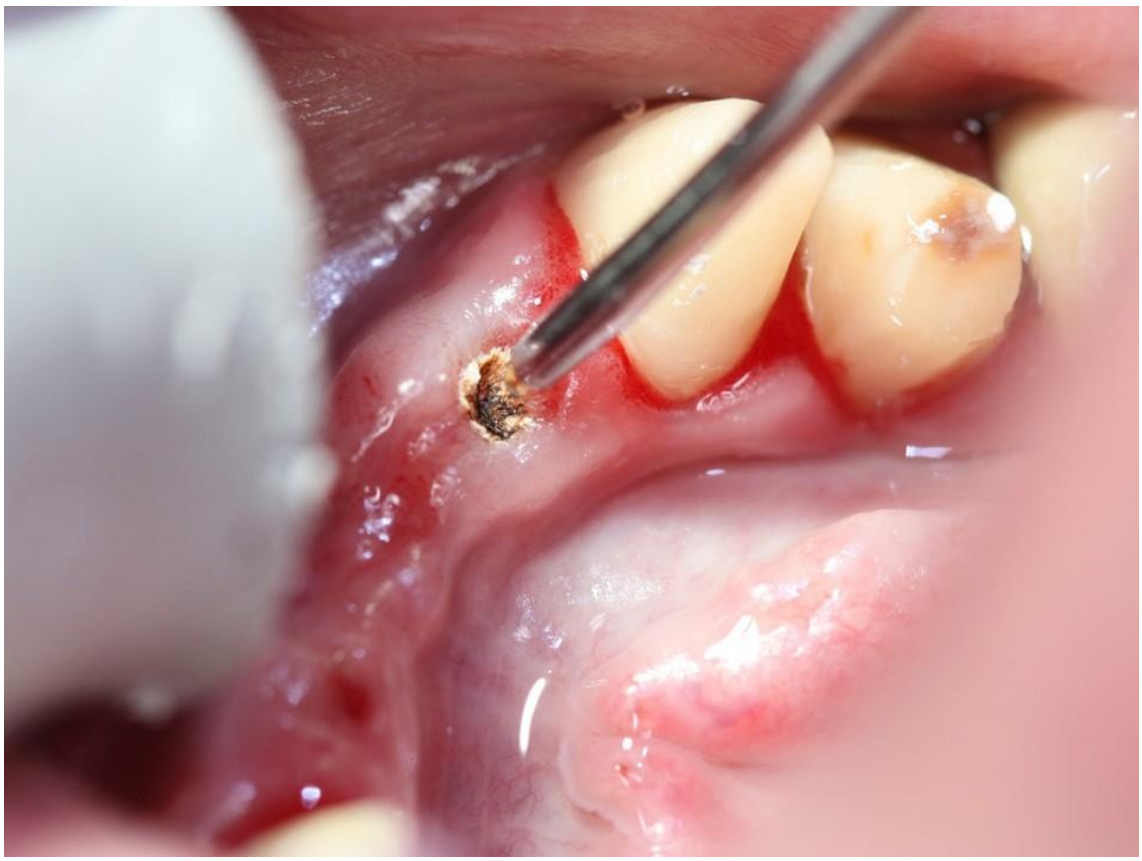

Figure #3. The use of the CW CO_2 10,600nm laser to ablate oral gingiva. Rapid ablation may be compromised by tissue desiccation and potential for thermal damage.

The prime advantages of laser-assisted oral soft tissue surgery may be listed as:

- Incisional haemostasis – small diameter muco-epithelial venules, arterioles and capillaries may be sealed through vessel wall protein and blood protein / plasma denaturation and coagulation.
- Significant pathogen reduction – a tenacious wound coagulum may act as a physical barrier to early bacterial contamination
- Harmonisation of soft and hard tissue management
- Uneventful healing phenomena – Photobiomodulation.

Whether through tissue trauma or surgery, early response may be compromised by an innate inflammatory reaction, as well as a bacteriogenic inflammatory reaction. Laser-induced wound surface coagulum, together with laser-induced PBM effects, may reduce

inflammation, pain and promote uneventful early healing. Ref [53]. A graphical summary of how these benefits may be inter-related is offered in *Integrated Discussion III Figure #2, page 157: "Circle of suffering".*

Predictable progress in any laser-assisted oral soft tissue surgery procedure is the result of photothermolysis; assuming absorption and the application of photonic energy. In terms of photonic energy, its density per "spot size" application, which over a period of time, will initiate an ascending thermal gradient [54]. Tissue warming in itself may predispose to PBM effects, but above 50 ^{0}C, the temperature rise will initially cause structural tissue proteinaceous material to denature, causing a coagulum of collagenous elements and products of blood and plasma products released through the laser incision or ablation.. At 100 ^{0}C, water vaporisation and gradual tissue desiccation will eventually predispose to carbonisation at approximately 200 ^{0}C. Carbonised tissue debris, adherent to the underlying tissue bed will preferentially absorb incident photonic energy and the temperature rise may exceed several hundred degrees, leading to conductive collateral thermal damage. Such significant deleterious outcomes may give rise to post-operative pain, swelling and tissue derangement, together delaying early healing and promoting local tissue disfigurement (Figures #4 - #8).

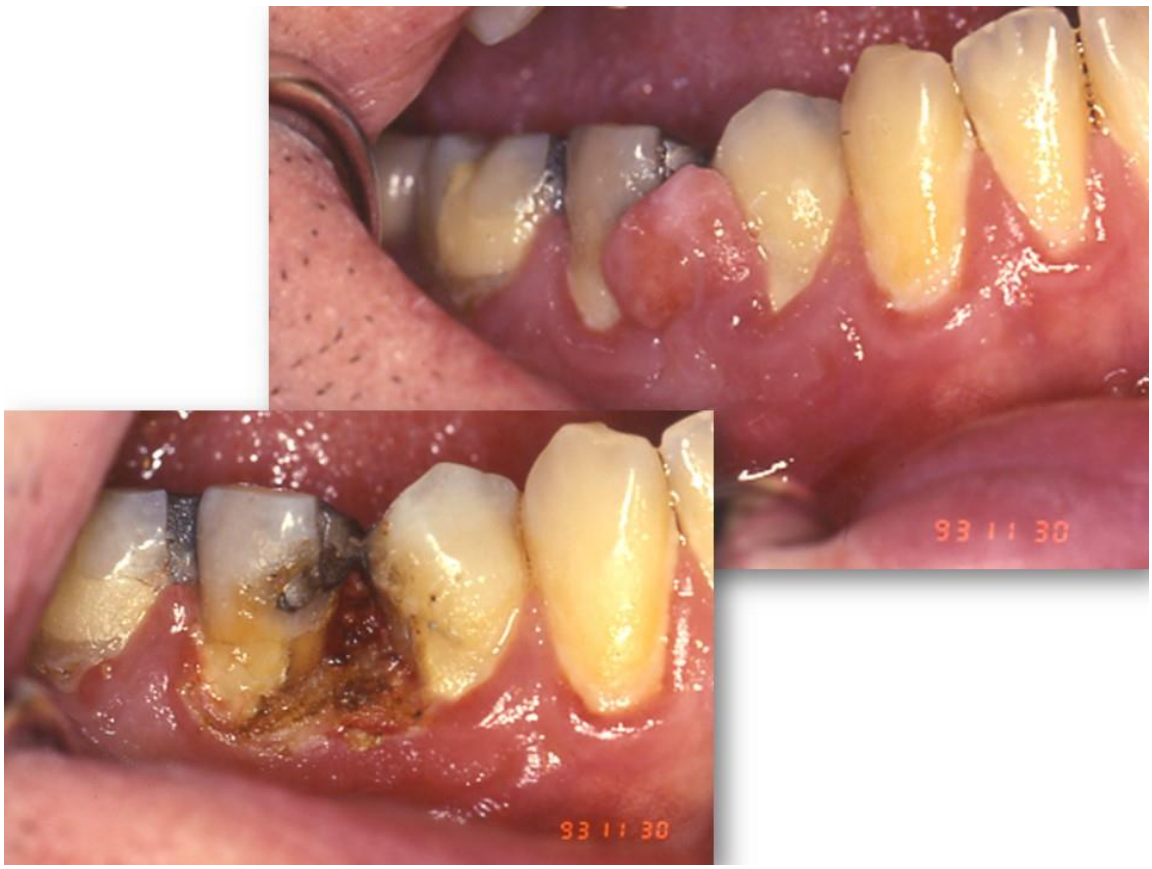

Figure #4 Provisional diagnosis of a giant cell epulis associated with gingival irritation from an open tooth contact between the lower right first and second premolar. Convention would suggest that excision should be accompanied by scarification of the lesion bed in order to reduce the potential for recurrence. Top right: original lesion; bottom right: surgical excision using a Nd:YAG 1064nm laser, with the following parameters: 250mj pp / 15 Hz / Average Power 3.75 Watts and included laser irradiation of the crestal ridge tissues in contact mode.

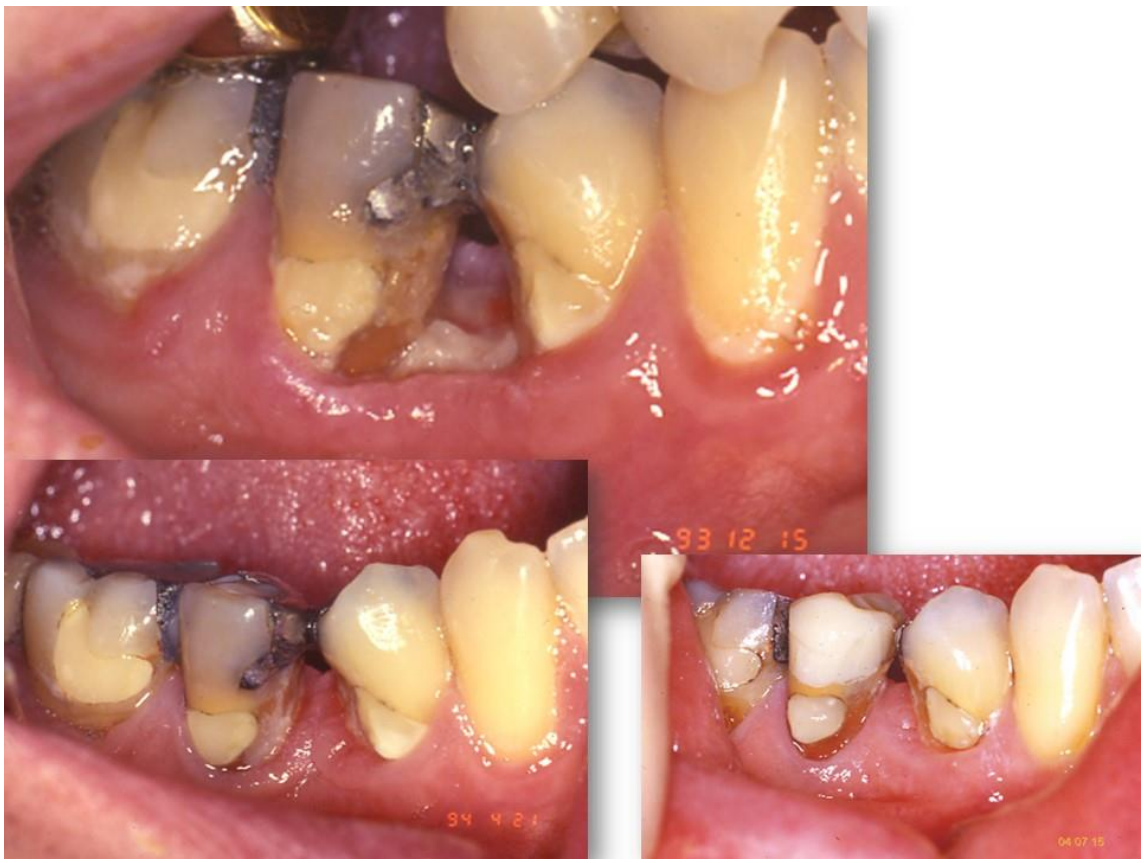

Figure #5. Top: Post operative review at 16 days – complaints of pain and gingival bleeding, together with the appearance of early bone sequestrum. This was caused by direct thermal damage induced by excess laser power and an ill-considered laser wavelength. Bottom (left) and (right): Review at five months and 10 years, fortunately indicating no long-term tissue damage or impaired dental function.

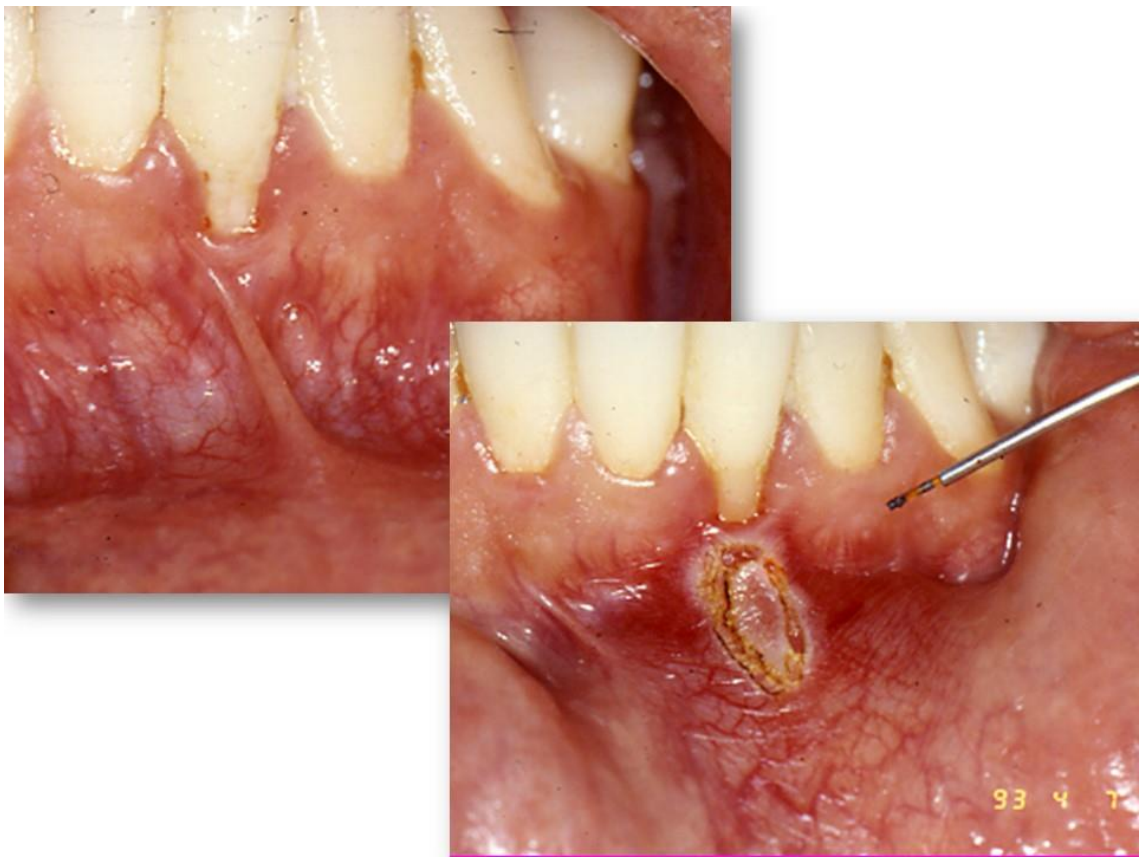

Figure #6. Provisional diagnosis of localised gingival margin disruption at lower left central incisor. Its possible cause is ascribable to the high insertion of lower labial frenum; the initial treatment is suggested to release the 'pull' of the frenum to allow reassessment of the gingival condition. Top left: Presenting condition. Bottom right: use of Nd:YAG 1064 nm laser, using the following parameters: 250 mj pp /20 Hz/Average Power 4.0 Watts in view of the fibrous nature of the tissue and relatively low tissue pigmentation level.

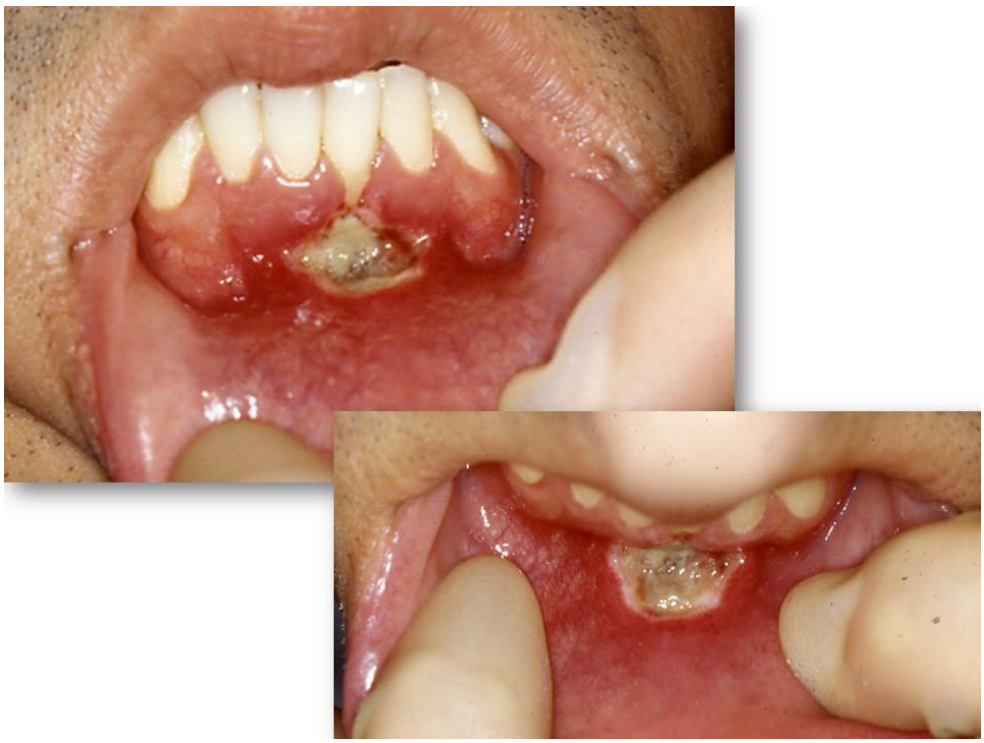

Figure #7. Top: Follow up view of the post-surgical site at one week, following severe pain and bad taste. Bottom: one week later. Absence of resolution and gross tissue damage, ascribable to a combination of poor choice of laser wavelength, excess power parameters and inadvertent poor surgical technique of direct contact towards the alveolus, disruption of the periosteum and contact with underlying alveolus.

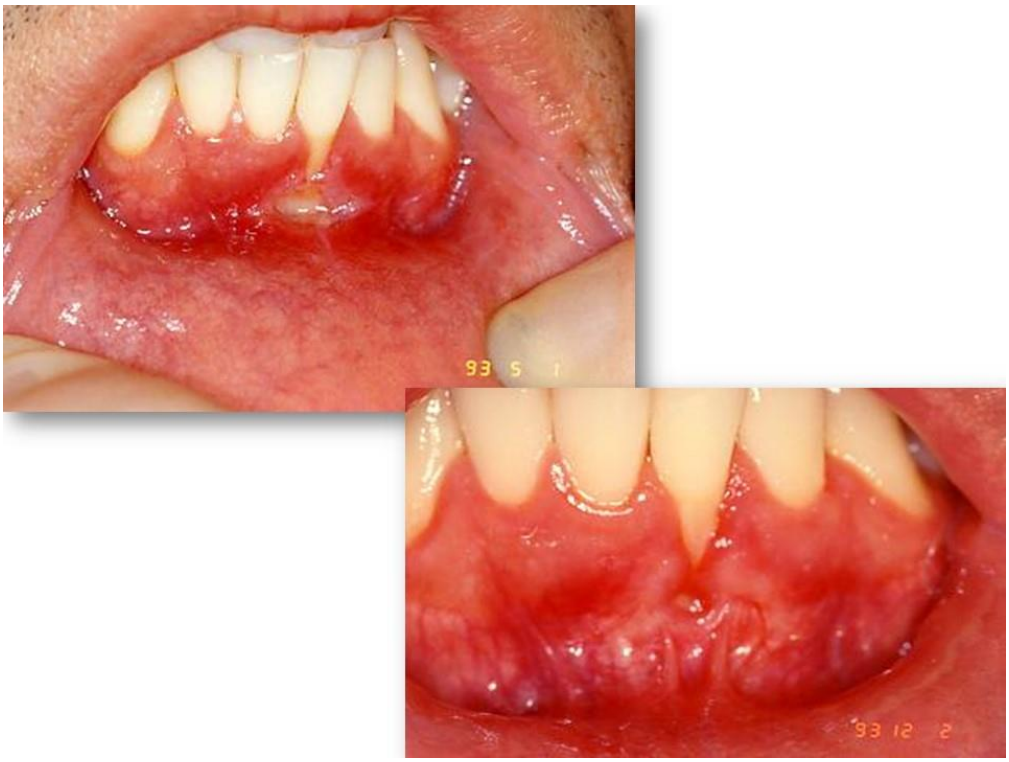

Figure # 8. Top: Review of failed frenectomy at two months. Significant gingival dehiscence with loss of attached gingival tissue and exposure of underlying bone. Tooth vitality remained throughout. Bottom: At 9 months, the decision was made to onward refer to receive corrective periodontal surgery through a laterally repositioned flap procedure.

1.7 *Laser surgical "overdose" – oral hard tissue*

The development of mid-infrared wavelength FRP lasers (Er,Cr:YSGG 2780nm and Er:YAG 2940nm devices) for use in dentistry during the mid-1990s, provided a much-needed opportunity to extend the repertoire of adjunctive laser therapy to hard dental and oral osseous tissue surgical management. The early experimental work of Keller and Hibst in 1989 [38], led to several laser models capable of so-called "all tissue" surgery, in view of the high absorption of both wavelengths in the target tissue water component. The average power output of these early models offered a range of 6 – 12 Watts, with one capable of 15 Watts. Given the fundamental photothermolytic spallation of the interstitial water component as the basis of hard tissue fragmentation in tooth cavity preparation and osseous resection, the comparatively long pulse width emission of 250 - 1000µsec not only resulted in moderately low peak pulse values, but also allowed the development of an extended thermal "footprint". Although the use of coaxial water spray with these lasers allowed debris to be removed, and also contributed to some thermal relaxation during emission, within deeper cavity situations, the risk of thermal "stall -out" was ever-present [55]. Overall, the efficacy of these early lasers rendered their comparison to rotary hard-tissue instrumentation as often mediocre, and allowed a temptation to adopt higher delivery power values in order to overcome the less-than-optimal performance observed.

The risk associated with excessive thermal rise may be seen in *Figures #9 and #10.* Gross thermal cracking in enamel, with also evidence of reformed amorphous (non-prismatic) enamel carbonated hydroxyapatite, indicated temperature levels of 1100^0C [56].

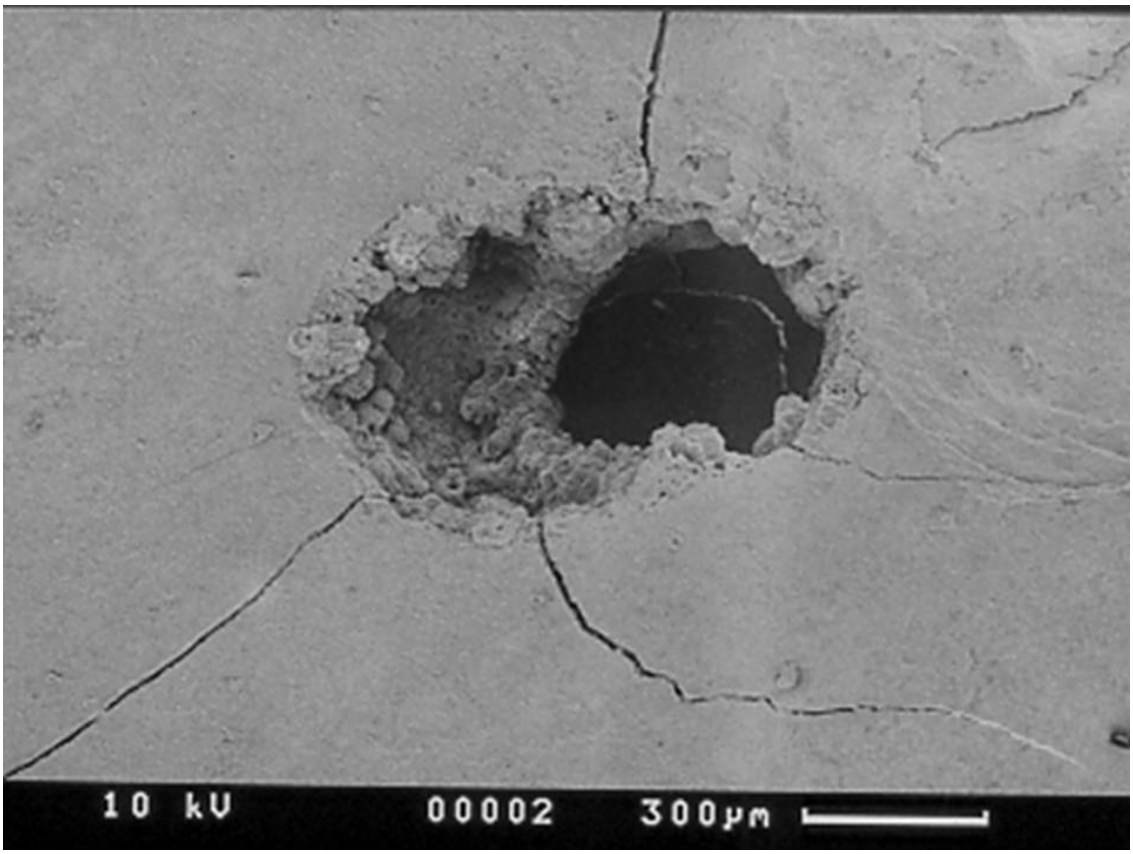

Figure #9. Scanning Electron micrograph (SEM) of human enamel exposed to high thermal deposition FRP laser emission. Gross thermally induced cracking extends from the ablation site (Source ref. [57].

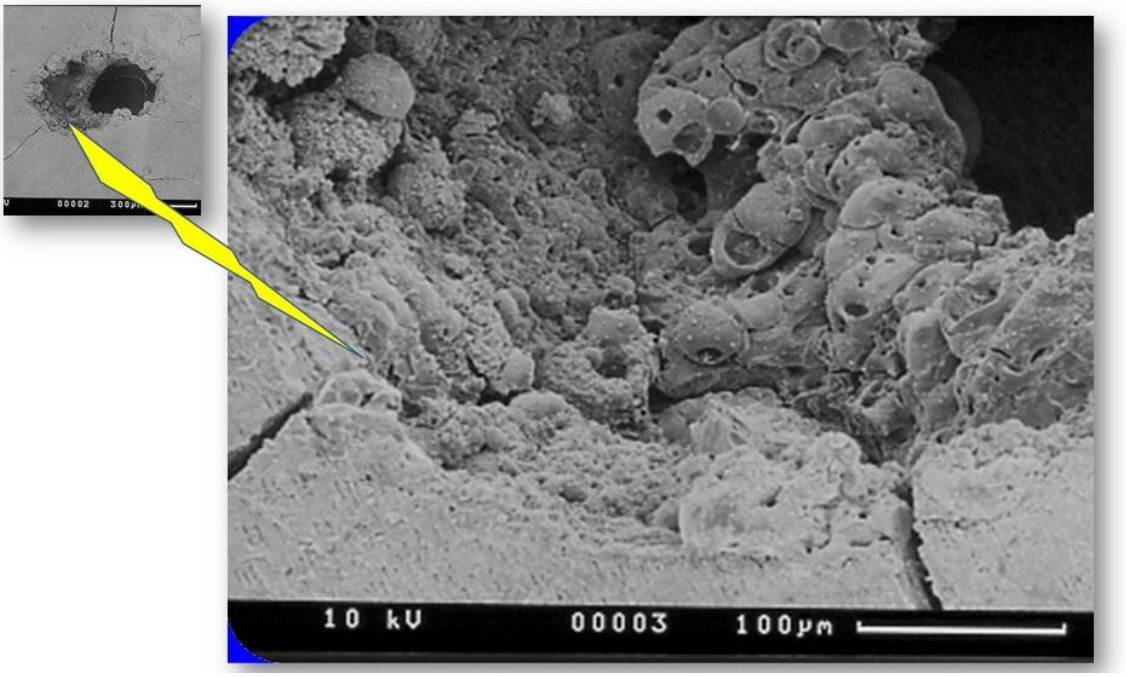

Figure #10. Higher magnification of SEM in figure D. Reformed amorphous (non-prismatic) enamel carbonated hydroxyapatite globules are indicative of thermal exposure at 1100 ^{0}C [Temperature ref. 56, SEM ref. 57].

Of equal significance is the choice of laser emission parameter, relative to its prime chromophore(s) or absorptive tissue element(s). With regard to dental mineral, the crystalline carbonated hydroxyapatite (CHA) may be represented by the general formula:

$$[Ca_{10}(PO_4)_{6-Y}(CO_3)_Z(OH)_2] + H_2O$$

Figure #11 provides a graphical representation of the absorption coefficient of CHA, relative to incident mid-IR and far-IR laser wavelengths. From this, it is possible to identify absorption bands at 2780, 2940 and 9300 nm, coincident with intra- free water and inter-crystalline hydroxyl and phosphate functions [57], and indication of peak absorption of the carbonate group at approximately 7,000 nm [58]. These sharp absorption bands indicate potential for incident photonic energy to be converted to thermal ablative energy within these specific arms of the parent CHA molecule.

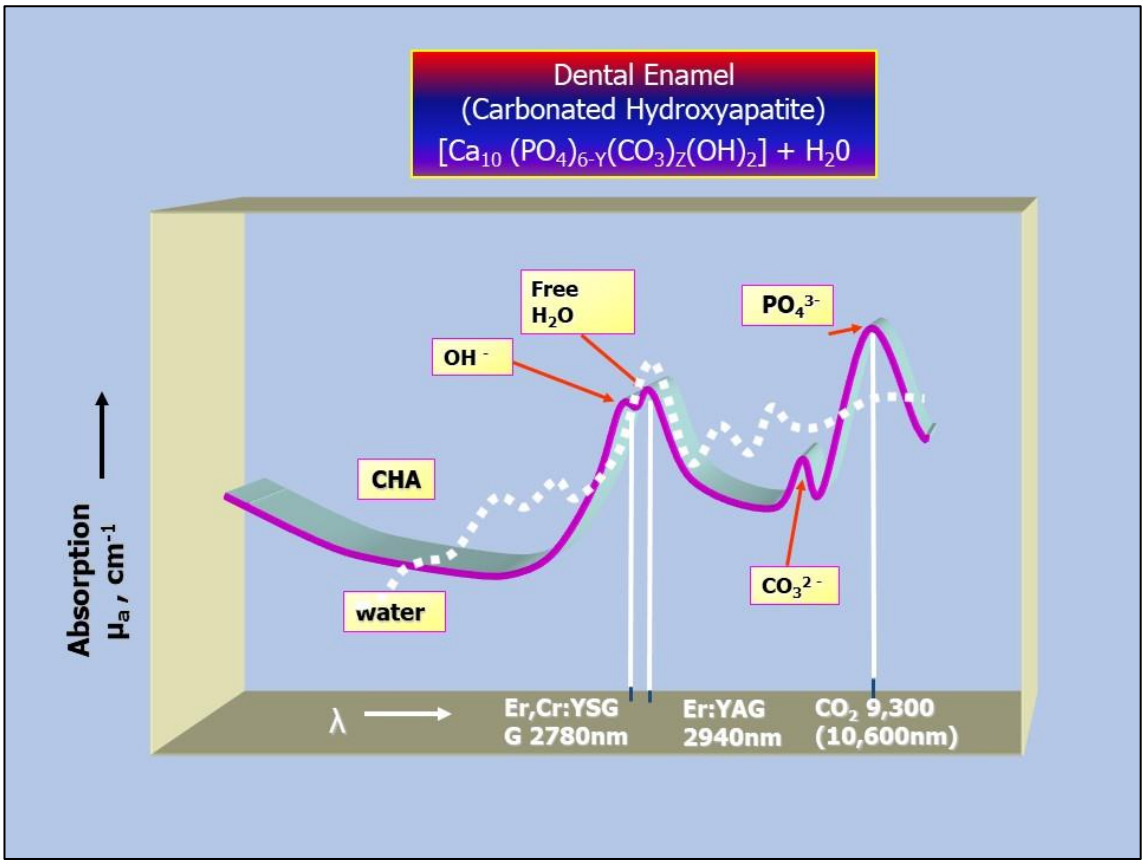

Figure #11. Graphic representation of absorption coefficient of carbonated hydroxyapatite (CHA) relative to mid-IR and far-IR laser wavelengths. Peaks coincident with free water and CHA functions. Source ref [57].

While this may offer intuitive reasoning to support the application of mid-IR erbium lasers, in the absence of any other interactive factors there is argument to support the use of the CO_2 laser wavelength in hard dental tissue. However, the fundamental emission mode of the gas laser remains a continuous wave and as such the thermal build up and carbonisation potential may be rapid and of risk to the vital tooth pulp *Figure #12.*

Recent development has concentrated on utilising the 9,300nm CO_2 emission wavelength [59 - 62], which with its high absorption in the CHA phosphate group, together with microsecond gating of the photonic emission and co-axial air and water, overcame the disadvantage of the soft tissue CO_2 laser and provided an alternative to rotary instrumentation in the treatment of dental decay.

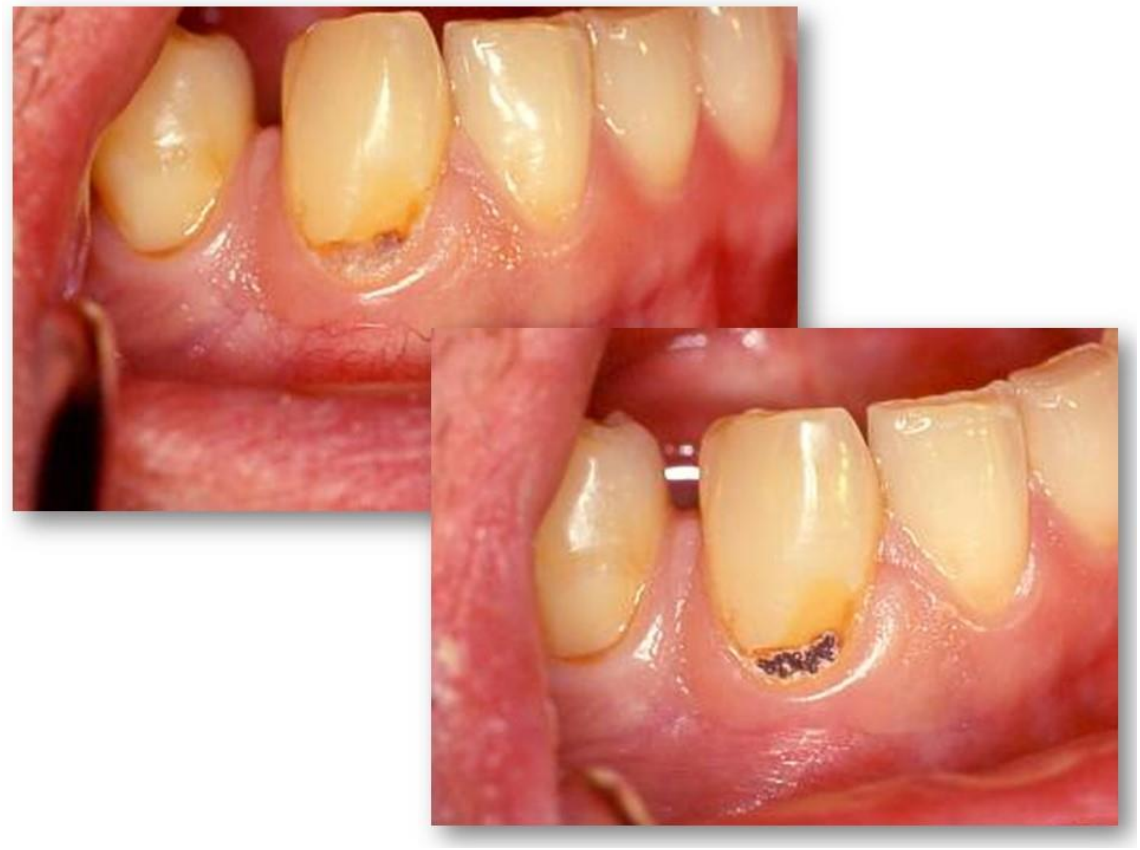

Figure #12. Clinical appearance of rapid carbonisation resulting from irradiation of carious dentine, using the CO_2 10,600nm laser. Although the recognition that water and CHA provided high absorption with this wavelength, the lack of appreciation of full operating parameters such as CW emission, no thermal relaxation and no co-axial water, contributed to the immediate cessation of this laser use in delivering a cavity preparation.

With the development of greater sophistication surrounding micro-gated mode-locked CW emission with the CO_2 laser, and ultra-short FRP pulse width as low as 50µsec associated with both erbium dental lasers [63, 64], laser-assisted hard oral tissue management has greatly improved. The resulting very high peak power but low thermal residue has significantly improved the beneficial outcome of laser-assisted tooth tissue management.

1.8 *Summary*

Several questions may be laid at the development of both laser wavelength availability and operating parametry; these may be summarised as follows:

i/ The quest of early dental laser manufactures to dominate the emerging market has led to claims of laser-tissue interaction that were beyond either the prevailing evidence-base or the output capability of laser technology as represented by the device being promoted. Was this an example of anecdote leading the science, necessitating later investigation to explore and expose the "sales hype"?

ii/ The laser wavelengths represented commercially between 1989 and 1994 may be judged as predominately soft tissue lasers, given the comparatively rudimentary emission delivery options, such as continuous wave power and possible high (30 – 50%) duty cycle values. Despite the research conducted on prototypes demonstrating emerging FRP mid-IR laser capabilities with hard tissue ablation, did the claims of shorter wavelength, photothermal ablation of pigmented dental caries, result in iatrogenic pulpal damage?

Iii/ Control panel values as displayed on early lasers represented the basic values of average power or (where applicable) energy per pulse and pulse frequency (Hz). To what extent did these rather crude parameters, compared to later photo-delivery sophistication contribute to inadvertent collateral thermal damage?

Iv/ This in turn led to much of the early (1990–2005) peer-reviewed published works restricting the record of operating parameters to such rudimentary levels.

v/ It has been demonstrated through both anecdotal case examples and published data, that in many aspects of laser-assisted dental treatment the optimal irradiation range is often very narrow; the risk of either excessive or inadequate "dose" may significantly affect the expected or investigated outcome.

Vi/ From the evaluation of aspects of laser-tissue interaction, it remains the impact of this thesis to examine published data to consider the hypothesis that the combination of incomplete representation of operational values, photonic delivery errors and a perceived concentration on visible red – NIR laser wavelengths, has significantly impacted the many claimed benefits of laser dentistry and distorted opinion as to its efficacy.

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Chapter 2: Laser operating parameters: The impact of incomplete data reporting.

In the preceding section, it has been demonstrated that the application of laser use within all aspects of clinical dentistry can be evaluated through analysis of light biophysics, chemical pathways and visual evidence of outcome.

The optimal pathway to growth in the uptake in laser use, as in many fields of clinical therapy is primarily derived from data and technique reproducibility; empirical published data in giving way to randomised clinical studies, allows a foundation of knowledge to define accepted and even preferred new therapies that utilise laser photonic energy. In this chapter, a background is provided to analyse the potential problems and risks consequent upon inadequate or inaccurate reporting of full laser operating parameters.

- The growth in applied dental laser research has been hampered, to a significant level, by difficulties in reproducing exact laser-tissue interaction effects; it is believed that this may have contributed to the inconsistency in successive studies and investigations. Moreover, this may have been ascribable to either

empirical “dose” errors, or the persistent use of early research as a justification for laser applications, as referenced in previously cited published data.

- Throughout the period of investigations and published articles on all aspects of laser dentistry, a persistence in omitting essential parameters continues; this has distorted evidence-based opinion regarding the benefits of laser use over alternative treatment modalities.
- The concept of “parameter” may draw upon the full operating fixed and variable delivery values, and the consideration of how operating parameters may be affected by delivery losses. “Parameters” will also extend to the variable values of target tissue physical and optical properties, and how they may respond to a given laser “dose”.
- A systematic review of 141 published randomised clinical trials has provided the opportunity to examine the percentage of operating parameters, considered essential to the complete portrayal of materials and methods, and this is offered in Paper II: *“Photobiomodulation Delivery Parameters in Dentistry - An Evidence-Based Approach”.*
- Fundamental measurement of delivery power using a laser power meter, fails to identify value errors that may influence study conclusions; such errors may distort the development of laser-assisted therapy through inaccurate data acquisition and interpretation arising from calibration errors. This is explored with reference to Paper III: *“The influence of delivery power losses and full operating parametry on the effectiveness of diode visible–near infra-red (445nm–1064 nm) laser therapy in dentistry—a multi-centre investigation”.*

2.1 Anecdote vs Data

This consideration offers some insight to the position of lasers in clinical dentistry, as opposed to oral and maxilla-facial surgery, to reflect on the poor applied knowledge within the clinical professional community that accompanied the emergence of early devices – often as cross-over facilities from adjacent medical specialities such as dermatology, ENT, ophthalmology and vascular surgery. Following the initial few years of the 1990s, when successive laser wavelengths were introduced, a brief period ensued to evaluate the fundamentals of surgical applications [65 – 67]. Based largely on the scant awareness of the underlying biophysics of laser-tissue interaction and often drawing more on the "hi-tech" impact of the word "laser" to a large extent, the progress of laser understanding was mainly driven by case reports – often elaborate surgical procedures that demonstrated competent individual prowess but, in general justifying the maxim "the plural of anecdote is not data".

An example of the uncertainty of the success of lasers in dentistry was published in the paper of Zakariasen and Dederich (1991) [68], and summarised in the abstract as follows:

"laser dentistry must be developed through extensive scientific inquiry--as all of our treatment modalities should be. We, as a profession, must insist that such laser development is done properly, not foisted upon us based on anecdotal reports and incomplete research".

Apart from early scepticism, much of it from academia, some scientific investigations emerged. Much of the published articles however, appeared in non-peer-reviewed journals and centred on case reports. Despite this, although early dental lasers had comparatively rudimentary operating parameter choices, their use in predominantly soft tissue procedures gained support, mostly as surgical instruments and their use in

established procedures in minor oral surgery and periodontics [69 – 72]. Many published articles discussed the benefits of laser use in soft tissue, such as incisional haemostasis, pathogen reduction and reduced requirement for sutures, but little was offered to account for claims of "uneventful" healing of the surgical wound.

2.2 Basic operating parameters

In terms of laser operating parameters, most early lasers fell into one of two groups – those with continuous wave (CW) emission, with additional basic deca-second gating, and those solid-state lasers with free-running pulsed (FRP) emission mode, with fixed value pulse width; for those early lasers in dentistry, this amounted to carbon dioxide (CW) and Nd:YAG (FRP), and from 1992 the emergence of examples of semiconductor (diode) lasers with CW emission. *Table #1* provides the rudimentary laser operating parameters that could be chosen by the clinician:

Solid-state FRP laser (Nd:YAG 1064nm)	**CW laser (CO_2 10600nm / GaAS diode 810nm)**	
Energy per pulse – mj/pp	CW	Gated-CW
Pulse frequency – Hertz Hz	Average Power	Emission cycle (deca-second gating – 30%, 50%
Average Power – Watts W (Energy / pulse x Hz)		

Table #1. Early dental laser operating parameters that could be chosen by the clinician and available via the laser control panel.

As may be noted through published peer-reviewed articles to investigate disadvantages and damaging effects of lasers in dental treatment, the potential for misinterpretation or mal-application of operating parameters might be considered to be of some significance [73 – 77]. Despite this negative background, the ability of adjunctive surgical lasers to deliver peri- and post-operative benefits over alternative instruments was maintained and

gradual expansion in both technology and operator choice in determining suitable laser parameters henceforth developed.

As was noted earlier and drawing upon the tabulated results recording the molecular dissociation energies amongst common oral tissue structural components [44] and with the exception of hydroxy function/substituent covalent bonds, it would appear that the majority of red visible, near-IR, mid-IR and far-IR photons have insufficient individual energy to cleave tissue molecules. Notably, the predominance of photothermolysis as the indirect medium of surgical tissue ablation has remained. Although of insufficient individual energy, the bombardment of tissue by multi-billion photons will give rise to gradual target molecular excitement, vibrational and rotational moments and general thermal outcomes of energy transfer, sufficient ultimately to break weak intermolecular bonds. By way of example, the stream of photons of an 810nm laser, delivering 1.0 Joule of energy may be calculated and quantified as follows:

Using the formula

$$E = \frac{hc}{\lambda}$$

Where h (Planck's Constant) = 6.626×10^{-34} m^2 kg / s

$c = 300 \times 10^6$ msec $^{-1}$

and E represents the photonic energy of incident wavelength of 810×10^{-9} metres, to equate to the other component values.

$$E = 6.626 \times 10^{-34} \; x \; \frac{3.0 \; x \; 10^8}{810.0 \; x \; 10^{-9}}$$

$$= 2.958 \; x \; 10^{-19} \; Joules$$

With the laser emission delivering a total energy value of 1.0 Joule, the total number of 810nm photons required will be:

$$1.0\ J\ \ x\ \frac{1\ Photon}{2.958.0\ x\ 10^{-19}}\ \ = 25.41\ x\ 10^{17}\ photons$$

Thus, having determined the number of photons, in order to influence the delivery of such a photon stream, core remaining parameters to be changed would be concentration of energy per unit of exposed target tissue, and also irradiation time period.

With a continuous wave emission, the delivery of 1.0 Joule per second would provide 1.0 Watt of laser power. Over an exposed area of irradiation of 1.0 cm^2, the power density will be 1.0 Wcm^{-2}. Within the absorptive capacity and thermal capacity and diffusivity of the irradiated soft tissue, the conversion of incident photonic energy to thermal rise will be incrementally evident through tissue warming, protein denaturation, water vaporisation and tissue ablation. However, the thermal rise would lead to ultimate carbonisation and collateral damage.

By "gating" the CW emission or choosing a free-running pulsed (FRP) emission laser and influencing the length of active emission per second – from decisecond through millisecond to microsecond (and onwards), the peak power of concentrated active emission stream rises.

With the original 1.0-Watt average power of the CW laser, by choosing the same, delivered via a FRP microsecond emission and frequency of 10 Hertz, the power distribution over time is changed:

Average power 1.0 Watts at 10 Hz (10 pulses per second) = 100 milli-joules per pulse (100 mj/pulse) and is calculated as:

$$100\ x\ 10^{-3} = 10^{-1}\ W$$

$$10\ Hz\ x\ 10^{-1} = 1.0\ W\ Average\ Power$$

Using the same energy per pulse (100 mj and repetition rate (10 Hz), with a FRP laser emission pulse width of 100 microseconds, the average power value of 1.0 Watts gives rise to peak power values per pulse as follows:

Peak Power = Energy per pulse ÷ Pulse width

$$\frac{100.0\ x\ 10^{-3}}{100.0\ x\ 10^{-6}} = 1.0\ x\ 10^{3}$$

Peak power = 1000 Watts

With an exposure area (spot size) of 1.0cm^2, a value for peak power density may be indicative of much more efficient tissue ablation, but with a much-reduced emission time through microsecond and even shorter pulses the potential for conductive thermal spread is correspondingly reduced [78].

With laser hard tissue surgery however, the essential element of efficient ablation is to address the relatively high average power (>3.0 Watts) with ultra-short pulse width delivery, in order to minimise collateral thermal damage, desiccated debris build-up and stall-out interruption of interaction. Early versions of the Er,Cr:YSGG (2780nm) and Er:YAG (2940nm) were either of fixed repetition rate (20Hz with the early 2780nm laser) or moderately long FRP pulse width (200 - 250µsecs), which allowed potentially damaging thermal exchange with hard tissue targets. Anecdotally, the frustrating rate of ablation with such operating parameters prompted the choice of ever-greater average power values, which added to the "vicious circle" of increased thermal damage potential.

In consequence, using a delivery tip (spot size) of 500 µm diameter, average power of 3.5 Watts, 10 Hz repetition and a pulse width of 75 µsec, the resulting peak power density (peak power ÷ πr^2) is calculated as 4,753,427.6 Wcm^{-2} (4,666.67 Wm^{-2}).

2.3 Contemporary laser parameter choice

Thus, by considering an extended number of adjustable parameters, the basic consideration of incident laser wavelength and positive absorption coefficient may be positively extrapolated, initially to appreciate the number of photons relative to energy delivered. By further adjusting the emission mode and area of exposure, the capacity for ablative power may be maximised while minimising the potential for conductive thermal damage [79].

Through development, current soft tissue CW surgical lasers have allowed a selection of emission modes, based upon gating to deliver high frequency, microsecond "pulses" of photonic energy [80]. Common values would suggest a pulse width of 15 - 20 μsecs and an emission frequency of 20 KHz, and the benefit to the clinician is that efficient peak power values are achievable, albeit with a thermal relaxation time of over 50% per emission second [81 - 83]. This provides good incisional efficiency with haemostasis and in large part overcomes damaging carbonisation processes [84, 85].

Hard tissue surgical management has been shown to achieve efficient and comparable ablation rates to that achieved with rotary instrumentation. Microsecond and evolving mode-locked picosecond pulse widths will allow increased suitability, whilst preserving single digit average power values [86 – 89]. Additional parameter manipulation is observed with so-called "all tissue" lasers, where a choice may exist to select the pulse width to maximise benefits with hard tissue verses soft tissue – efficient ablation with predictable thermal deposition where haemostasis is required. Manufacturer's pre-set parameters also assist in maximising the varying combination of laser operating parameters, together with adjustable coaxial air and water for use with the mid-IR erbium lasers and the 9,300nm "all tissue" CO_2 laser [90 – 95].

Through such improvements, together with greater operator awareness of the scope of use for a given laser, the potential for error in all areas of ablative laser-assisted tissue management may be judged to have been reduced.

2.4 Laser operating parameters in photobiomodulation

Photobiomodulation (PBM) may be viewed to be of influence as a by-product of ablative surgical laser use, and along a combination of thermal and scatter gradients [96, 97]. PBM threshold fluence (energy density) values may be triggered through tissue warming and low scatter coefficients, either as single stand-alone or combined phenomena at some distance from the surgical zone of laser ablation. Additionally, PBM therapy may be delivered as a prescribed sub-ablative fluence, in the adjunctive treatment of existing oral, perioral or facial pathology / syndrome.

It is of note that although awareness of PBM had been known from the early research of Endre Mester [98,], early investigations were conducted with the helium-neon (632nm, 50mW CW) and argon (488nm, 100mW Gated 50%) lasers, which developed a fluence of 3 – 4 Jcm^{-2} [99]. A broad range of both cellular and animal and later human studies helped provide a scientific basis to the observed effects of sub-ablative fluences using visible light lasers [99 - 101]. However, such research does not appear to have gained traction in terms of its application in dentistry until the early years of the 1990s with the work of Tiina Karu and others [102 – 106].

2.5 Cellular and "downstream" effects of PBM

The focus of research into the primary intracellular effects of wavelength-specific incident light concerned the activity within the mitochondrial membrane and specifically, the mitochondrial respiratory chain, or more commonly known as the electron transport chain (ETC). The intermembrane space contains several different kinds of electron

carriers: flavin mononucleotide, iron-sulphur proteins, coenzyme Q, heme-containing cytochromes, and copper ions.

Compartmentalised as a series of enzyme sites, the sequence may be listed as follows:

Complex I – NADH ubiquinone oxidoreductase (NADH-CoQ reductase), provides the catalyst for the oxidation of NADH, the reduction of ubiquinone, and the transfer of $4H^+$/NADH across the coupling membrane [107].

$$NADH + H^+ + Q + 4H^+_N \Leftrightarrow NAD^+ + QH_2 + 4H^+_P$$

Complex II - Succinate dehydrogenase (SDH) plays a pivotal role in oxidative metabolism. SDH is a tricarboxylic acid (TCA) cycle intermediate that interacts directly with the ETC and ATP production via oxidative metabolism [108].

Complex III - Cytochrome c reductase (ubiquinol cytochrome c reductase) mediates the transfer of electrons from coenzyme Q10 to cytochrome c, the two mobile electron carriers in the respiratory chain [109].

Complex IV - Cytochrome c oxidase (CCO) acts as an electron acceptor from Complex III. It is a terminal oxidase of the mitochondrial electron transport chain [110].

Complex V - ATP synthase uses the free energy arising from the electrochemical gradient (proton pump) within the ETC, to synthesise ATP from ADP [111].

As a result of this heightened activity, three molecules emerge with direct (primary) effects [112, 113]:

Adenosine Triphosphate ATP – the main energy source for cellular function. Increased levels positively affect cellular activity.

Reactive Oxygen Species ROS – modulation activates transcription factors, positively impacting cellular repair and healing.

Nitric Oxide $NO^{\bullet}$ – a potent vasodilator; release of this species increases circulation, decreases inflammation and enhances transport of oxygen and immune cells throughout tissue, together with promoting further (secondary) activity, such as increased cellular redox potential, increased transcription factors associated with RNA and DNA biosynthesis, increased cellular pH, and membrane permeability to anions (K^{+}, Na^{+}, Ca^{++}).

The promoted increased activity of mitochondrial complex elements may be summarised in *Table #2*, (drawn from Vinesh E. 2017) [114].

PBM effects of mitochondrial Complex I / IV ↑ ROS production	**PBM effects through upregulated cytochrome oxidase activity**	**"Downstream" tissue effects of PBM**
Gene expression Activates AP-1, NF-kB Activates TGF-β1 Antioxidant modulation	↑ATP production Synthesis of DNA, RNA Dissociation of $NO^{\bullet}$ from CoX Fe/Cu ligand redox activity. $NO^{\bullet}$ ↑ keratinocyte proliferation Cell repair / regeneration / autophagy / apoptosis modulation	Stimulation of cytokines and growth factors production Stimulation platelet / coagulation pathways Re-epithelialisation / wound healing promotion Proliferation / degranulation of mast cells Proliferation of oral keratinococytes, fibroblasts Neovascularisation

Table #2 A summary of direct and indirect (intra- and extra-cellular) consequences of PBM irradiation fluence. Data source ref. [114]. Abbreviations: AP-1 Activator Protein 1, NF-kB Nuclear Factor kappa B, TGF-β1 Transforming Growth Factor beta-1, ATP Adenosine Triphosphate, NO Nitric Oxide.

PBM-induced changes in cell activity may vary to reflect the complexity and sophistication of the cell type involved and may also account for the period of irradiation required to induce sustained activity changes. In principle, *Table #3* provides a list of

common oral structural cell lines, together with comments regarding the effects of PBM fluence.

Cell type	Observed effect of applied PBM irradiation
Fibroblasts	Proliferation, maturation, locomotion. Transformation into myofibroblasts Reduced secretion of PGE2 and IL-1 Enhanced secretion of βFGF
Macrophages	Phagocytosis Secretion of fibroblast growth factors Fibrin resorption
Lymphocytes	Activation Enhanced proliferation
Epithelial cells	Motility
Endothelium	Increased granulation tissue Relaxation of vascular smooth muscle
Neural tissue	Reduced synthesis of inflammatory mediators. Maturation and regeneration of axonal growth
Osteoblasts	Enhanced proliferation

Table #3. Summary of effects of applied PBM fluence on common oral tissue structural cell lines. Abbreviations: PGE2 Prostaglandin E2 also known as dinoprostone, IL-1 Interleukin 1, βFGF β Fibroblast Growth Factor.

Through investigation of these specific and general effects of applied PBM, cellular, local tissue and regional complex tissue outcomes have been referenced through peer-reviewed publication [115 – 123].

With regard to this therapy, additional photo-biological principles may be derived through the following laws:

- Grotthus-Draper law, also known as the first law of photochemistry, or principle of photochemical activation, where the incident light must be of appropriate wavelength (λ) to be absorbed by a recipient absorptive molecule.
- Stark-Einstein law, also known as the second law of photochemistry states that for each photon of light absorbed by a chemical system, only one molecule is activated for subsequent reaction i.e., each molecule absorbs one quantum of (incident) light.

- Bunsen-Roscoe law The photo-chemical effect is a function of the product of light intensity and duration of exposure.

The effects of PBM may manifest themselves within the irradiated cell, local tissue and downstream via the circulation and neural pathways – i.e., the autocrine, paracrine and endocrine effects [124].

The choice of laser wavelength centred around the visible red, extending to the near-IR regions [125], where principal tissue chromophores (haemoglobin and melanin), along with intracellular mitochondrial cytochrome c oxidase, were shown to have high absorption at shorter wavelengths, and with high tissue scattering; tissue water has been shown to strongly absorb infrared light at wavelengths > 1100-nm.

Through the expansion of clinical evaluation studies, a number of oral and maxillofacial pathologies have been shown to benefit from laser PBM effects, either as the prime protocol or as a side-effect benefit of laser surgical action [126]:

- Surgical wounds of oral soft tissues
- Oral mucositis associated with cancer & radiotherapy
- Supportive treatment in MRONJ
- TMJ Disorders- pain relief/ healing
- Xerostomia
- Osseointegration & Bone regeneration
- Accelerating healing of damaged neural tissues
- Accelerated tooth movement / pain relief in orthodontics

What is of fundamental relevance to the benefits of laser-induced PBM is the pairing of wavelength and photon fluence. Fundamental cell-directed research would offer

empirical dose-outcome evaluation and current opinion would suggest that the range of fluence values, both associated with healing promotion and analgesia, are narrow and critical, as represented through text and graphics explanations provided in *Chapter IV, Page 211 Figure #13* [123].

2.6 Photonic "dose" in PBM

The photonic energy delivered at a cellular level will be dependent on anatomy and optical properties of the irradiated tissue. The optimal biostimulation fluence has been determined to be between 3-10 J/cm^2 depending on tissue type [127]. It may be safely extrapolated from such investigation that these critical fluence values would apply to the "uneventful healing" phenomenon cited as a significant benefit of surgical laser use in oral tissue management. In a similar fashion, to extend the dose applied beyond positive biostimulation, the inhibitory effects extend to influence and inhibit neural pathways, nociceptor sensitivity and also axonal conduction [123].

"Apple pie recipe"- Notwithstanding the fundamental parameters of laser photonic delivery – wavelength and photon fluence – the extensive list of intrinsic, adjustable and calculated (extrapolated) parameters *(Introduction, Table #1, page 37),* will exert an increasing influence on the reproducibility of the procedure where laser use is a chosen modality. By way of simplicity, to use an internet search engine to obtain a recipe for apple pie: an image of a perfect product defines the outcome, yet the preparation and cooking instructions amount to "apples, pastry, sugar" and "cook in oven"; for a given laser-assisted procedure such as labial frenectomy, similar operational words "fire laser", "obtain haemostasis", "1.0 Watts", would severely impact on any possibility of a controlled or satisfactory outcome.

If one is seeking to ensure a perfectly prepared and cooked apple pie, in much the same manner as if one would seek to carry out an optimal laser-assisted frenal resection, the nearer that the contents of Table #1 were available to apply, then clearly the greater the chances of success.

Choosing the correct "light dose" is difficult. The dose depends on the pathology and symptoms and in particular upon how deep the light will penetrate into the tissue. Additional factors related to light dose may be viewed as:

"Instantaneous" dose

"Cumulative" dose – repetition / timeline

2-Dimensional dose: Fluence – Radiant Exposure (Joules / cm^2) x Time

3-Dimension (volume) dose - Energy Density (Joules / cm^3) x Time

Total energy (Joules) delivered

This concept remains the core fundamental aspect of this doctoral thesis. To date, there appears to be only a scant structured audit of laser operating parameters, with most comments being in the form of editorial or personal comment [128 -131].

The second published paper (Paper II: *Photobiomodulation Delivery Parameters in Dentistry: An Evidence-Based Approach*) included in this thesis by concurrent publication represents an audit of 141 published articles, in order to assess the type and number of optimal operating parameters. Additionally, the influence of optimising clinical outcome relative to the area of tissue exposed to a given laser application, is subjected to statistical analysis to identify the ideal applicator, its area of application and depth of penetration (relative to wavelength) and site of pathology to be treated.

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2.7 Paper II: Photobiomodulation Delivery Parameters in Dentistry: An Evidence-Based Approach

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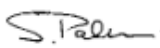
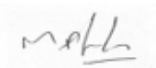
Statement of Co-authorship

We declare that the co-author contributions are correct, and that Steven Parker was primarily responsible for producing the first draft and at least 75 % of the final content of the following paper.

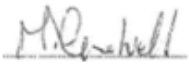
(i) Steven Parker (Candidate) conceived and designed the paper, collected and interpreted data, compiled and wrote the manuscript.

Signed:  Name: Dr Steven Parker

(ii) Mark Cronshaw (co-author) provided text input and data contribution.

Signed:  Name: Dr Mark Cronshaw

(iii) Martin Grootveld (First Supervisor) supervised and assisted in conception and design of the paper, provided data analysis and editing the manuscript.

Signed:  Name: Prof. Martin Grootveld

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Photobiomodulation Delivery Parameters in Dentistry: An Evidence-Based Approach

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Keywords: photobiomodulation, orthodontics, dental hypersensitivity, oral surgery, oral mucositis

Background

Notwithstanding around 50 years of continued research, and with an ascending level of current research submissions, there is at present no agreed methodology and process to adopted treatment protocols. Dosimetry at target tissue level is, however, largely agreed, and more recent proposals have promoted the concept of a multiphasic dose–response to clinical outcomes [1]. Higher dosimetry appears to be the most effective for analgesia, and a lower dose bracket for the growth-promoting and optimal tissue healing benefits associated with tissue regeneration and the resolution of inflammation [2–6]. There is, however, no agreement with respect to wavelengths adopted, spectral emission modes, optical beam spatial profiles, and the optical surface spot size, together with the inter-relationship of these variables to optimize therapy outcome. Further, energy delivery to subsurface targets has proven to be a significant challenge, with the requirement for the operator to deliver a meaningful photonic dose to target through overlying anisotropic multiple layers of variable tissue types [7–9].

However, despite the many difficulties that have been encountered by researchers and clinicians alike, there is considerable interest in harnessing the proven benefits of photobiomodulation (PBM) as a therapy. This has led the authors to consider broadly and as widely as possible within the prior published evidence base factors that can more consistently lead to reduced operator errors and a higher level of research and clinical endeavour outcomes.

For the purpose of this study, the authors considered that a prime objective in maintaining the sustainability of evidence-based data is to mandate a full description of laser operating parameters, both those concerning control panel running parameters and computed data relating to photonic dose. In this regard, the authors conducted an audit of published randomized clinical trial (RCT)-level articles that formed the basis of five recent systematic reviews. Within a total of 141 published articles, a selection of criteria that contributed to a ‘‘risk of bias’’ determination was examined. From this, it is asserted that the high level of absence of some basic values of photonic energy delivery renders cause for concern regarding the scientific rigor of conclusions obtained in such studies.

Methods

Using keywords (Photobiomodulation Parameters, Orthodontics, Dental Hypersensitivity, Oral Surgery, Myofacial Pain Management, and Oral Mucositis), our five recent published systematic reviews identified a series of group sets of articles that are subject to the inclusion and exclusion criteria as recommended in the PRISMA statement [10], and not the least of which, the results of randomized clinical trials [11–15]. Further, a modified risk of bias assessment was adopted [16], where appropriate, in the four most recently published clinical systematic reviews focused on laser-assisted post-surgical tissue outcomes using wavelengths outside the 650nm–1350 nm ‘‘optical

window,'' laser-assisted oral mucositis management, and two articles based on dose parameters and methodology in clinical dental studies.

The latter studies evaluated (1) parameters associated with PBM therapy and benign pathology; (2) tissue healing/PBM therapy applied post-surgically in relation to pain/swelling and early healing; (3) TMJDS and pain/salivary flow with reference to PBM therapy; pain/PBM therapy with tooth hypersensitivity; (4) myofacial pain dysfunction syndrome and PBM therapy related to orthodontic tooth movement (OTM)/rapid maxillary expansion; and (5) pain associated with orthodontic treatment. Where possible, reported delivery parameters were collated and weighted against a five-point scale of clinical success rating outcome versus control from zero (null) in increments of 20% to a top level of five representing an outcome versus control of 80% plus.

A variety of statistical tools have been employed using, where appropriate, ANOVA and ANCOVA, along with multivariate and further univariate analysis. In the oral mucositis study, a partial least-squares regression technique was performed, where the dependent variable was treatment outcome, and the potential explanatory ones were 2 qualitative and 13 quantitative predictor variables to define a log differential of outcome using XLSTAT2016 software. For statistical significance, a Bonferroni-corrected p-value was set as a significance level, which in the case of the oral mucositis study was 0.0033, and in the most recent parameters study, 0.031.

The focus of this report was to consider the percentage value of individual ''risk of bias'' elements recorded in the representative sample of articles focused on PBM applications in dentistry and examined in the five most recent systematic reviews [11–15].

Elements examined were as follows: emission mode; use of a power meter; ‘‘spot size’’ measured; fluence (radiant exposure); irradiance (power density); total energy delivered; ‘‘dose’’ frequency; and PBM outcome significance.

Results

Primary investigation of delivery parameters included an audit of a total of 141 published articles, forming a database within five systematic reviews, all of which were human trials published from 2009 to 2019 [11–15]. Eight risk of bias elements were chosen to provide a range of parameter values that were identified as indicative of a ‘‘best practice’’ approach to inclusiveness, and ease of study reproducibility. The average percentage values for each risk element represent studies where either this element was recorded as part of the study design, or where its value recorded.

Table 1 provides a summary of risk of bias elements.

TABLE 1. TABLE PROVIDES A SUMMARY OF RISK OF BIAS ELEMENTS

		Risk of bias elements by % present							
Article Ref.	*Published RCT number analyzed*	*Emission mode*	*Power meter*	*"Spot size" measured*	*Fluence (radiant exposure)*	*Irradiance (power density)*	*Total energy delivered*	*Dose frequency*	*PBM outcome significance*
11	52	20.4	30.2	73.6	96.3	18.8	52.8	96.3	66.4
12	43	28.5	36.5	81.3	97.2	15.8	83.8	64.9	72.2
13	29	13.7	23.7	69.0	83.8	26.4	69.0	65.3	82.5
14	25	24.5	4.0	40.0	32.2	28.7	38.7	8.7	"Assumed" 84.6
15	17	46.8	22.4	41.2	41.2	23.5	29.5	41.2	88.2
Total	141	Av. 26.78%	Av. 23.36%	Av. 61.02%	Av. 70.14%	Av. 22.64%	Av. 54.74%	Av. 55.28%	Av. 78.78%

All articles were previously subjected to PRISMA and Cochrane Risk of Bias assessment and each of the five systematic review articles restricted the data pool to human randomized controlled clinical trials.
PBM, photobiomodulation; RCT, randomized clinical trial.

Table 1 summary of risk of bias elements.

Discussion

An audit of selected risk of bias elements has been carried out through the analysis of human RCT studies published between 2009 and 2019, and which were contained within a series of five systematic reviews. Many reports demonstrated inconsistencies in reportage with apparent frequent omissions of the use of a power meter to calibrate the optical source, since the majority of studies (>70%) examined failed to record the use of a power meter.

In consequence, for studies that investigate a comparative range of average power values, levels of power loss from a noncalibrated source can render data acquired as severely compromised. In addition, there were many absent details regarding the optical spot size at the target, the technique adopted (such as contact vs. non-contact methods), beam divergence angles, distance to the target from the optical source, size and depth of the target, treatment times, and emission modes (i.e., gated vs. continuous wave). Our later investigation of optical parameters included 38 human studies throughout an 11-year period from 2009, which were grouped according to surface optical spot size into 3 groups of small (16 studies), medium (9 studies), and large (13 studies) [12].

A comprehensive dataset of reported parameters and outcomes was subjected to analysis to identify common threads associated with successful, inconclusive or null outcomes. From these evaluations, it was found that there was a strong association between surface optical spot size and success where a larger optical spot size was employed. This difference was particularly noteworthy in the management of subsurface dental conditions ($p < 0.003$). In our study of oral mucositis, 29 human studies spanning a period of 24 years from 1995 were included [13].

The timing of the PBM therapy was found to be a critical indicator of a high rate of success, with a pre- or contiguous-timing of irradiation to the radio- or chemotherapy being delivered. Again, the application of larger surface spot sizes was identified as a more successful strategy, and a trend was identified associating higher energy applications to improved pain control in the management of extant cancer chemotherapy- or radiotherapy-associated oral ulceration.

A further study investigated the possible PBM effects in wavelengths outside the normal range adopted of 630nm–1350 nm, with the application of strict selection criteria, which included 25 human studies from a period of 10 years up to May 2020 [14]. Clear evidence of PBM effects in both shorter as well as longer wavelengths was identified. Moreover, a 2019 systematic review of OTM and pain control from our group included 17 human clinical trials covering the 5 years from December 2013 to December 2018 [15]. A low level of evidence to support accelerated OTM was identified, with a more moderate degree of verification for pain prevention by the use of PBM at the time of orthodontic intervention.

Scientific progress is marked by the essential requirement for continued re-appraisal as new evidence emerges, together with a willingness to adapt. For better or for worse, this creates the occasion to re-validate given beliefs and at its best allows a favorable progression. Thanks to the good efforts of many researchers and clinicians worldwide, buoyed by an ever growing evidence base, PBM is one of the fastest growing areas of current medical scientific endeavour. Given the absolute need for solutions related to chronic disease inclusive of age-related changes, there is an unprecedented level of interest in the potential clinical gain for PBM in many therapeutic applications.

However, despite the large volume of published studies, it is regrettable that there is continued controversy with respect to predictable and reliable dosing and dose delivery strategies. As identified in our earlier systematic review featuring a report of delivery parameters, there are many aspects of methodology and accurate descriptions sufficient for a consistent level of reproducible research. In view of this, comparisons of results acquired from different studies globally are rendered very complicated. Also, there are core aspects of knowledge related to optical delivery that are essential to determine an appropriate dose for a target of variable dimensions and subsurface levels.

An added consideration is the volumetric effects of energy delivery, whereby by virtue of photon attenuation consequent to optical scatter, there may be dual layers of outcome. Nearest to the optical source, there may be sufficient applied energy to result in tissue inhibition with associated analgesia, whereas at the periphery of the tissue, the energy levels maybe more consistent with photobiostimulatory effects, including the resolution of inflammation. For this reason, caution must be exercised before attempting to extrapolate outcomes based on planar tissue culture research on to animal or indeed human clinical studies.

To avoid confusion to both researchers and clinicians, it is most important to have reproducible measures, and standardization of the approach to treatment requires a mature appreciation of the significance of the dosimetry terminology employed, particularly with respect to subsurface tissue targets. A common expression of dose is fluence or energy density; however, in view of the volumetric nature of the cellular target, and the variable potential rate of energy delivery, these terms are prone to mislead, since the dose is the time accumulated in J/cm^2 at tissue level. As identified by Hadis et al. [17], fluence (radiant exposure) is the correct technical term for J/cm^2, whereas dose must take into

account the optical exposure time element (T). The term energy density is frequently applied in the literature to indicate dose; however, this is a volumetric measure of J/cm^3.

Within such considerations, it may be helpful to reference the depth of penetration of the beam when delivering PBM therapy to subsurface pathology—commonly accepted as a linear measurement, but instead is often a function of tissue depth and scatter coefficient [18]. In essence, to achieve a meaningful dose at subsurface levels, it is essential to compensate for optical tissue attenuation by administering a higher time accumulated surface radiant exposure ($J/cm^2 \cdot T$).

Given the size of the extant literature, it is becoming possible to seek common attributes for successful or failed approaches to dosimetry and delivery processes. The verification that a large optical surface spot size is associated with a considerably higher number of reported positive outcomes is significant. Many of the earlier studies that focused on oral mucositis management, however, have reported favorable results using a small diameter optical probe [19,20].

This is most likely, in our opinion, attributable to the condition nature being a superficial rather than deeper placed pathology. Indeed, the statistical analysis of differences in outcomes in a wider range of oral pathologies between the use of small, medium, and large surface optical spot sizes indicated a highly significant difference between large and medium and small spot sizes in deeper pathologies [13]. Also, there was a pronounced differential between large and medium versus small optical spot sizes in superficial conditions *(Fig. 1).*

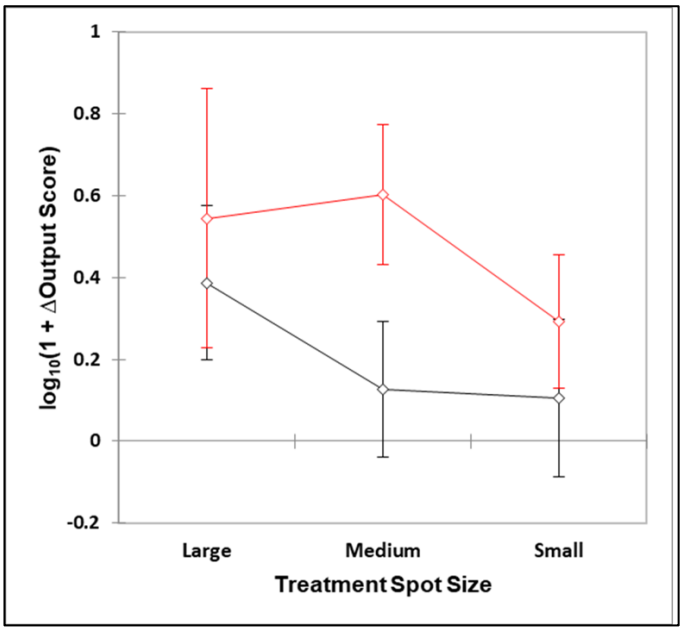


FIG. 1. Plots of mean – 95% confidence intervals for the large, medium, and small spot size treatments at both deep (black) and superficial (red) target sites. Δ Output score represents differences determined at both deep (black) and superficial (red) target sites. Δ Output score represents differences between the mean clinical outcome score variables observed for the laser and control treatment groups, determined between the mean clinical outcome score variables observed for the laser and control, which was positive for all 38 studies reported (reproduced from Ref.12).

In respect of clinical practicalities, the application of multiple points with a small probe takes far longer and requires greater operator skill than using a larger diameter beam. Further, to achieve a meaningful dose at the required depth with a small diameter probe poses many difficulties. In view of the high photon diffusion and optical remission within the tissue occasioned by reflection and optical scattering with red to near-infrared sources, there is a considerable degree of power losses. These occur first of all at the surface with reflection from the skin surface resulting in a 4–7% power loss. At the epidermal-dermal boundary, there is considerable lateral diffusion of the beam with a high photon boundary overlaying a zone of onward progress toward a subsurface target [18].

Beyond this boundary zone, there will be some amplification occasioned by photon collision if a coherent light source (laser) is employed. Onward optical transport of red to

near infra-red (NIR) wavelengths is primarily forward rather than lateral scattering, although there can be significant power losses experienced because of back remission of the photon stream, a process resulting in radiant exitance. In a study of a 660 nm source in gingival tissues, Alvarenga et al. [21] found this to amount to a ca. 50% loss of power at a 5-mm depth from the tissue surface *(Fig. 2).*

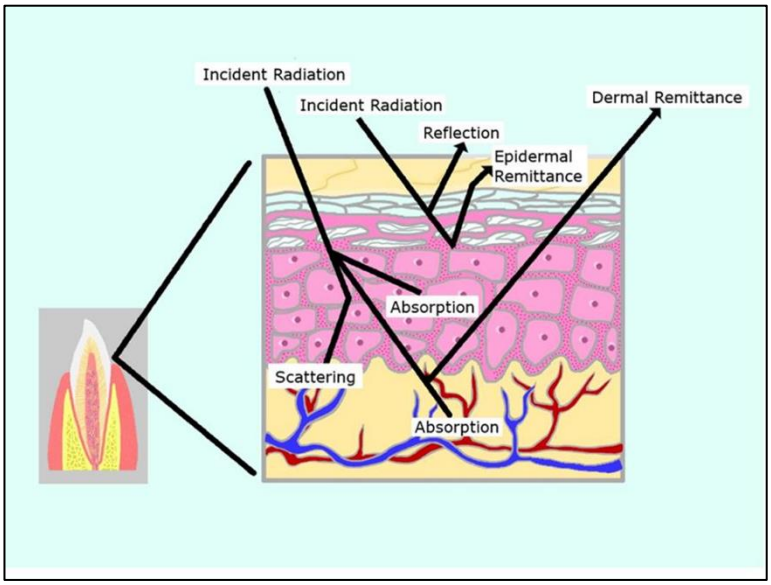


FIG. 2. Outcomes of optical transport at a target tissue give rise to significant energy loss (radiant exitance) (reproduced from Ref.12).

We are currently assessing optical transmission patterns under a wide variety of parameters, and to date have achieved some interesting results. By use of a porcine muscle tissue model of various tissue thicknesses, the transmitted power levels and optical spatial distribution of the system were recorded using an Ophir beam profilometer. The outcome very clearly demonstrated that to achieve dose delivery to the required depth requires a greater volume of tissue exposure than that for identical settings for radiant exposure. Moreover, irradiance with a small optic probe or cluster of probes was unable to penetrate to anywhere near the same volume at depth as a larger probe *(Fig. 3A, B and Fig. 5A, B).*

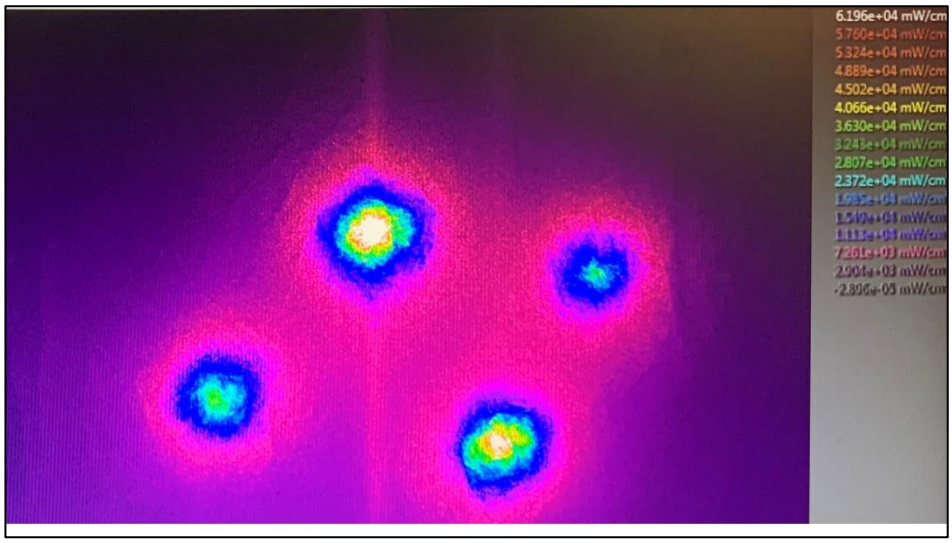
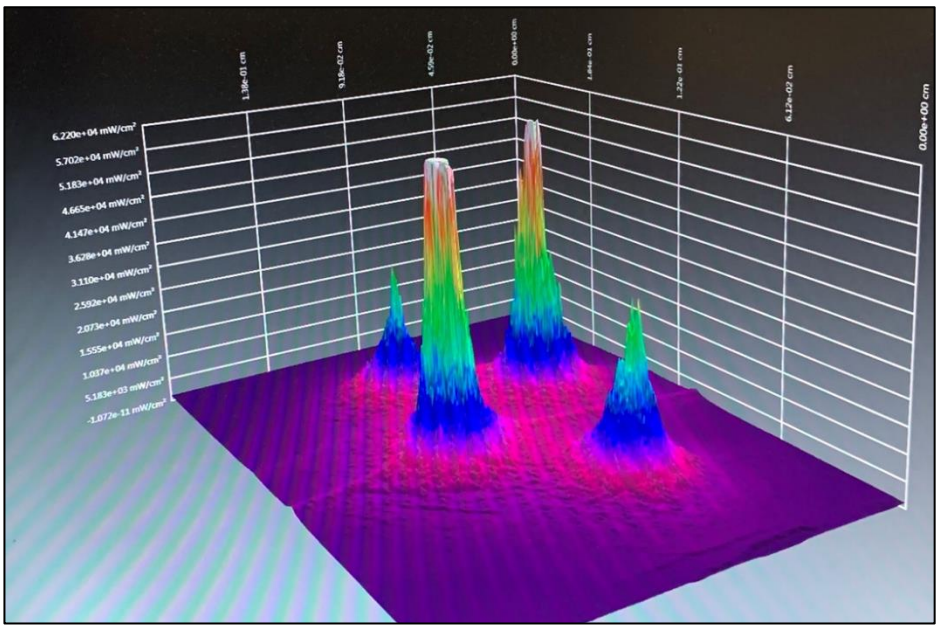


FIG. 3. (A) Planar render of the spatial beam profile of 4 · 3.7mm diameter 650 nm calibrated laser sources 2 cm apart, each set at 200mW output power, applied to a 4mm thickness porcine muscle tissue sample.



(B) Three-dimensional render of the spatial beam profile of 4 · 3.7mm diameter 650 nm calibrated laser sources 2 cm apart, each set at 200mW output power, applied to a 4mm thickness porcine muscle tissue sample.

A further observation is that an optical scanning technique to cover larger areas, especially in subsurface pathologies, is unlikely to succeed with a small sized probe. This is ascribable to the relatively low exposure of the tissue volume to irradiation, by

comparison to the similar application in the scanning mode to a larger diameter applicator *(Fig. 4A, B).*

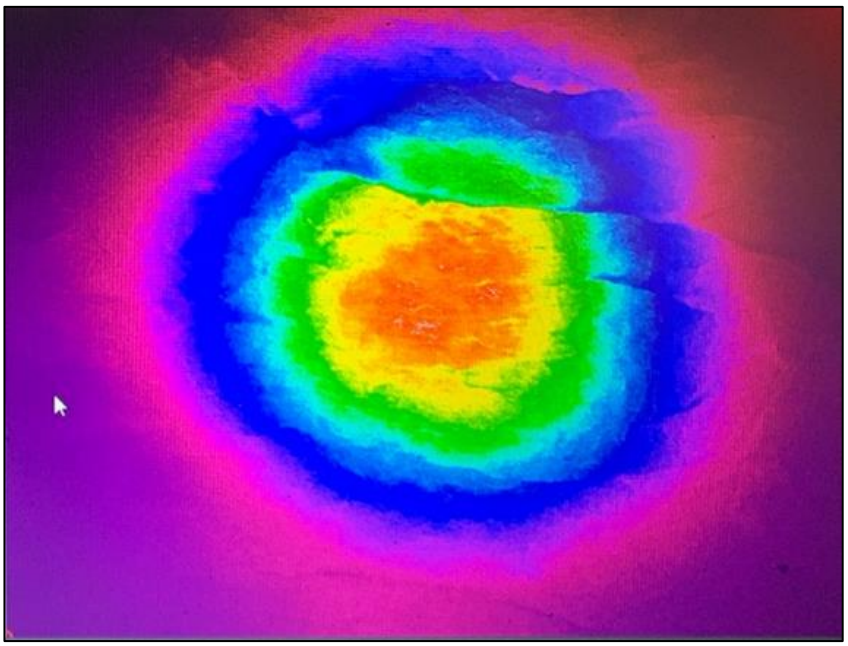


FIG. 4. (A) Planar render of the spatial beam profile of a 13mm diameter 650 nm calibrated single laser source at 550mW power applied to the same tissue sample at 4mm thickness (image from our current research: article in preparation for submission).

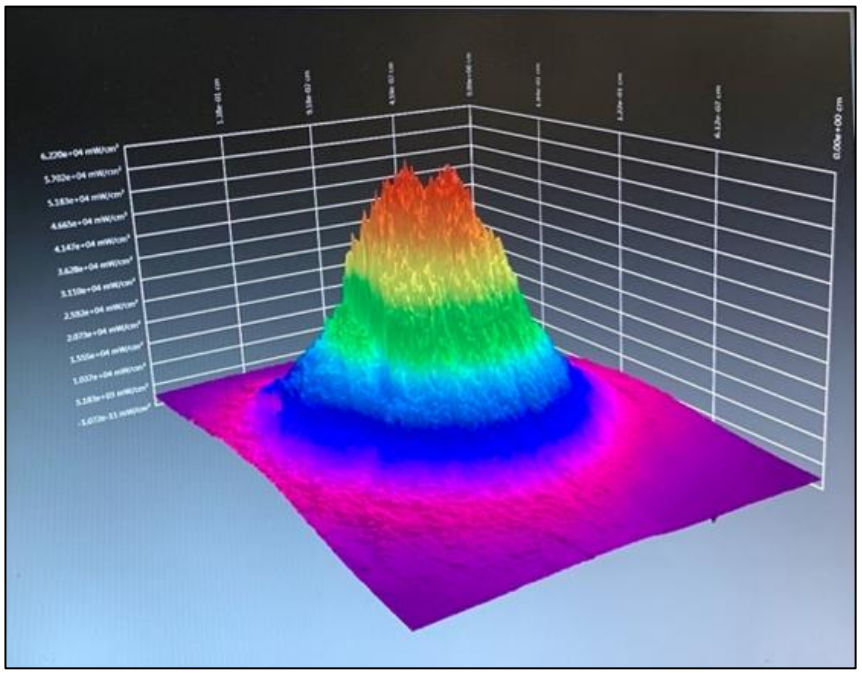


FIG. 4. (B) Three-dimensional render of the spatial beam profile of a 13mm diameter 650 nm calibrated single laser source at 550mW power applied to the same tissue

sample at 4mm thickness (image from our current research: article in preparation for submission).

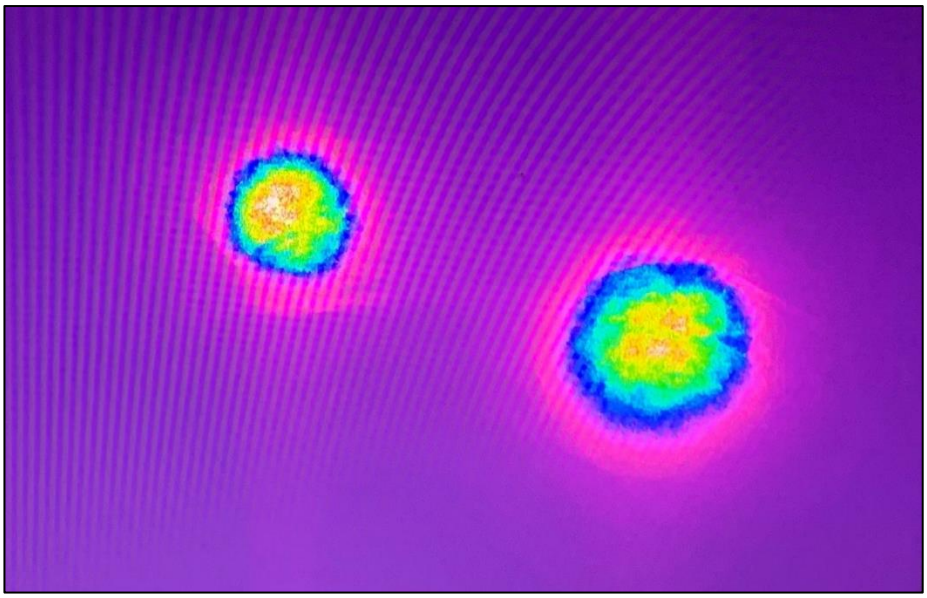


FIG. 5. (A) Planar view render of the beam profile of two parallel optic beams 2.5 cm apart applied to a 3mm thickness porcine muscle tissue sample. Image offered as a guide to the reader to demonstrate the lack of lateral spread from the tips. Note the Gaussian distribution and the relative distribution of energy from high within the surface layers to very low at subsurface levels. Sources are 650 nm calibrated lasers at 200mW output power, beam diameter at source 3.7 mm, in close contact to the target tissue (image from our current research: article in preparation for submission).

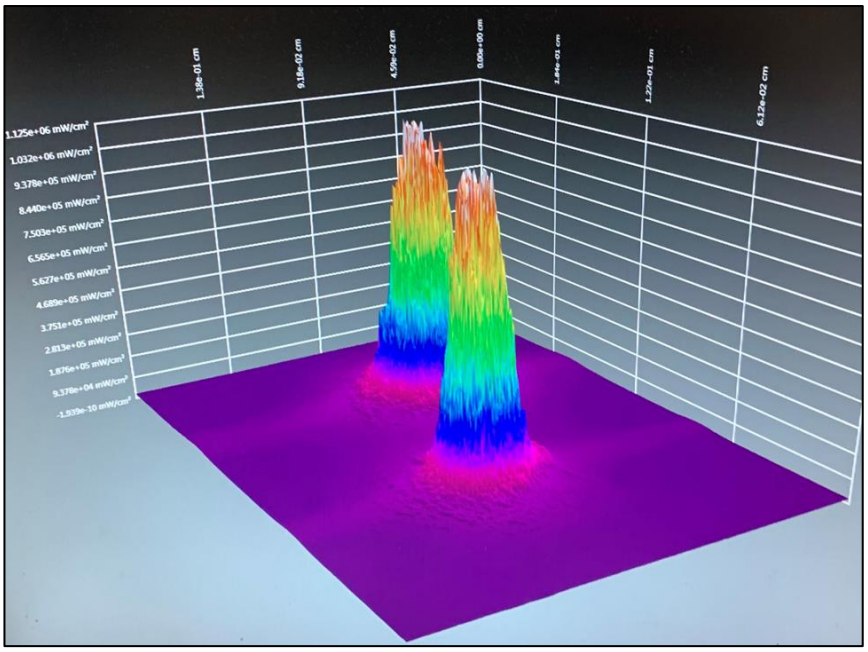


FIG. 5. (B) Three-dimensional render of the beam profile of two parallel optic beams 2.5 cm apart applied to a 3mm thickness porcine muscle tissue sample. Images are offered as a guide to the reader to demonstrate the lack of lateral spread from the tips. Note the Gaussian distribution and the relative distribution of energy from high within the surface layers to very low at subsurface levels. Sources are 650 nm calibrated lasers at 200mW output power, beam diameter at source 3.7 mm, in close contact to the target tissue (image from our current research: article in preparation for submission).

Returning to our recent oral mucositis systematic review, a key finding was that early intervention with PBM before or at the time of the medical intervention by radiotherapy or chemotherapy was the optimal approach to employ [13]. Our statistical analysis provides proof to support the expert consensus statement on oral mucositis from the MASCC/ISOO 2019 systematic review by Zadik et al., and the outcome determination was indeed a statistically robust effect [22].

It is recognized that PBM optimizes cellular metabolism into an aerobic cycle of metabolism, and consequently, there is a reduction of tissue stress-induced apoptosis. PBM works on many planes since there can be a photo-induced mitigation of inflammatory cytokines, plus an improvement in vascular perfusion of tissues, along with an increase in lymphatic drainage. Further, there are many changes in the immune response, which can favour a positive outcome to an intervention such as surgery [2,3].

The concept of pre-conditioning or contiguous conditioning of tissues before a tissue traumatic event, we believe warrants further investigation, since this may be of assistance in many clinical situations [23]. In our recent orthodontic systematic review, we found evidence to support the use of PBM to reduce pain associated with orthodontic treatment [15,24]. In addition, there are a number of small-scale studies that have compared the equivalent or near-equivalent effects of PBM to steroids toward the mitigation of post-surgery edema and trismus following third molar extractions [25,26]. Aside from routine care, we propose that this concept of conditioning may be worthy of examination as a

supportive measure, that is, a prequel to medical and surgical interventions, especially in medically compromised patients.

In respect of modes of action of PBM and dosage, the literature reflects the dominance of the concept that PBM is primarily a function of the selective absorption of photons at complex IV of the mitochondrial electron transport chain, a process increasing the activity of cytochrome c-oxidase (CCO), which results in an increase in ATP production at complex V. It has been proposed that certain wavelengths have a high affinity for CCO, and as such, these represent the optimum wavelengths to use for PBM [27,28].

However, the evidence base does not support this contention, since many wavelengths have been found to give rise to positive photon-induced cellular metabolic changes [14,29,30]. Indeed, our recent systematic review of wavelengths outside of the conventional visible to NIR wavelengths usually applied for PBM therapies found evidence to support biomodulatory effects in wavelengths both shorter and much longer than those that fall into the so called ‘‘optical window’’ for therapy.

Photon energy transduction mechanisms can include many pathways, including photothermal, photoelectrical, photomechanical, photochemical, photofluorescence, and photomagnetic changes [31,32]. There are many potential cellular targets for PBM-related effects, including photo-labile membrane-bound ion gates, as well as other intracellular organelles such as the endoplasmic reticulum. Broader band absorption by dark pigmented bodies such as flavins, porphyrins, and nuclear chromatin, along with Cu/Fe and other transition metal ion protein clusters, may be important factors in PBM-related metabolic changes.

Our published review on the evolutionary and metabolic aspects of mitochondrial regulation explores these issues further [1]. From an expanded review of the non-PBM literature available, there is evidence supporting the requirement to take into account intracellular microthermal inclines, which may be of high significance in the processes associated with laser-induced analgesia. Given the extended non-PBM evidence base, for example, that related to heat stress proteins, the presence of membrane-bound transport ion gates, including photo-labile transient responsive proteins, and the uncoupled protein response to elevated cytoplasmic levels of reactive oxygen species among other pathways of cellular reactivity to stimulus, we proposed that it was appropriate to review all the potential mechanisms of PBM action within the greater context of these reports.

A further recommendation from our 2019 literature review was that it may be time to move away from the binary concept enshrined by the Arndt-Schulz rule [33] to that of a multiphasic event. This premise was based on the observation that PBM is not solely an effect restricted to biostimulation, since there is a dose range beyond that associated with the stimulatory range of 2–8 J/cm^2, which offers benefit in photon-induced analgesia. Our recent extended studies of the literature support the dosimetry of 2–8 J/cm^2 at tissue level for biostimulatory effects, and 10–30 J/cm^2 for PBM associated analgesia.

There are a number of important considerations to take into account before instituting treatment. First of all, there is the size and depth of the target: is it superficial or is it subsurface, and if so, at what depth? The dosimetry we derived from our multiple systematic reviews is the level of energy required at the target level. Visible red to NIR laser sources are subject to a high degree of optical scattering and optical remission resulting in energy losses, such that at 1 cm depth, there remains around 5–10% of the energy applied at the surface [7,18,34]. For example, to achieve a unit dose of 5 J/cm^2 at

1 cm in depth, it would be necessary to supply a minimum of 10x the delivered dose at the surface (50 J/cm^2).

Similarly, to reach 15 J/cm^2 at 1 cm in depth, it would be necessary to administer 150 J/cm^2 to the surface. It is premature to offer a definitive solution at this stage in the evolution of the evidence base, for the management of deep seated pathologies, beyond 1 cm in tissue depth.

However, when delivering higher doses to the surface, there is the potential hazard of an appreciable thermal rise in the superficial tissues overlying the target. To compound this concern, the optical spatial beam profile across the beam is not even since it is a Gaussian beam with an energy in the mid-third of the beam 2–4x higher that of the perimeter. This is particularly pertinent to larger handpiece attachments as to allow for the larger surface optic footprint the overall power output by necessity is higher than it would be for a small probe. For example, with a hand piece with an optic footprint of 3 cm in diameter, the area is ~7 cm^2.

To deliver an average dose of 250 mW/cm^2, a power output setting of 1.75 W would be necessary. With a static beam, the irradiance of the central third of the beam is achieving a peak in excess of 1 W. Given an extended treatment time to achieve an adequate dose at depth, this may overexpose the tissues at the surface, resulting in phototoxicity expressed as a photo-induced dermatitis [35].

To avoid this difficulty and to achieve a more uniform dose delivery, it is advisable to adopt a slow sweeping motion to more evenly distribute the energy and avoid overexposure of the tissues in the center of the beam. Other possible solutions include the adoption of an optically corrected source as in the so-called ‘‘Flat-Top’’ hand piece or to

integrate a thermal camera with a safety system. Our current research also indicates that the choice of wavelength applied is significant both for optical transport of photons to depth as well as the mitigation of superficial layer thermal trauma.

There is considerable scope for continued guided research into PBM dosage and delivery techniques. Indeed, our current work for future publication is investigating many aspects of dose parametry with respect to the choice of wavelength, irradiance, fluence, thermography, and optical spatial beam profiles, particularly with regard to large Gaussian beam devices and optically corrected ‘‘flat-top’’ surface beam sources. Our group is also most interested in further investigating some of the underlying mechanisms of PBM, most especially in the context of wavelengths beyond those within the visible red to NIR regions. We view PBM as an area with considerable clinical applications, and together with the excellent efforts of our colleagues worldwide, we expect considerable progress in this exciting field commensurate with the greater adoption of this safe, nontoxic, and effective therapeutic approach.

Conclusions

Our extended systemized reviews of published literature have allowed the examination of several key laser operating parameters, using an adopted Cochrane-type risk of bias analysis. Our chosen models included orthodontics, along with the management of cancer therapy-related oral mucositis and two further broader studies encompassing many dental-related conditions. Our analyses have provided support for our consensus of the need to re-appraise the concepts associated with PBM mechanisms, as well as added value to our proposal that PBM is a multiphasic event.

Further, we have identified that early interventions with PBM offer an optimal approach toward a successful level of patient care. Our recommendations are that dose delivery is

best served with a large surface spot size and a corresponding higher overall delivery of energy, although at lower levels of radiant exposure and irradiance.

Finally, to effectively and reliably deliver the correct dose requires an appreciation of target size and location, along with an understanding of scientifically correct terminology, together with the added knowledge concerning photon tissue transport mechanics.

Author Disclosure Statement

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2.8 Risks associated with incomplete operating parameters

Taken from the Paper II study, herewith *Table #4* provides tabulated audited representation in Paper II of "Risk of Bias" (ROB) [132] elements, applied across the analysis of five systematic reviews [133 – 137]:

Paper Ref.	Published RCT number analysed	"Risk of Bias" Elements by % present							
		Emission mode	Power meter	"Spot size" measured	Fluence (Radiant Exposure)	Irradiance (W/cm^2)	Total energy delivered	Dose frequency	PBM outcome significance
#1	52	20.4	30.2	73.6	96.3	18.8	52.8	96.3	66.4
#2	43	28.5	36.5	81.3	97.2	15.8	83.8	64.9	72.2
#3	29	13.7	23.7	69.0	83.8	26.4	69.0	65.3	82.5
#4	25	24.5	4.0	40.0	32.2	28.7	38.7	8.7	"Assumed" 84.6
#5	17	46.8	22.4	41.2	41.2	23.5	29.5	41.2	88.2
Total	141	Average 26.78%	Average 23.36%	Average 61.02%	Average 70.14%	Average 22.64%	Average 54.74%	Average 55.28%	Average 78.78%

Table #4. Modified Cochrane Risk of Bias elements of laser operating parameters, their average numerical representation in the collective of five systematic review published papers (Paper Ref. #1 - #5). Collected data over all 141 papers is then averaged to provide a representation of how included each parameter is referenced, running from 22.6 to 78.8%.

For the purpose of Paper II, the ROB elements chosen were:

- Emission mode
- Use of a power meter
- "Spot size" measured
- Fluence (radiant exposure)
- Irradiance (W/cm^2)
- Total energy delivered
- "Dose" frequency
- Significance of PBM outcome

The implication of such findings receives greater significance when the source paper with modest to low operating parameter details is used to promote a clinical procedure. The "apple pie" recipe metaphor has been shown to be pertinent, and the implication for poor treatment outcome when incomplete details of parameters are allowed to persist.

Of greater concern, the low findings of 22.6, 23.3 and 26.7%, relating to the recording of irradiance (power density), the use of a power meter and laser emission mode respectively, may predispose to the clinician who seeks to emulate the paper's findings creating an unintended outcome of tissue damage, postoperative pain or underachieved surgical outcome through the incomplete consideration of operating parameters.

As part of the study audit, analysis was performed regarding the significance of optical "spot" size, in terms of efficient PBM therapy and optimal tissue penetration. To this end, the study provided clear verification that a large optical surface spot size is associated with a considerably higher number of reported positive outcomes, and this was markedly significant; in addition, irradiance with a small optic probe was unable to penetrate to the same depth as a larger probe.

Further cause for concern relates to the influence of gaussian distribution of fluence / irradiation in terms of actual densities at varying points relative to the overall average value of the measurement being made. Since the graphical representation of gaussian distribution allows an average to be determined as $1/e^2$ (e = Euler's number), at the centre of the irradiated area and at the periphery, density values provide high and ever-reducing intensities, respectively, as determined mathematically and accepted through publication [138]. Taken from this study, the centre of the "beam spot" may deliver 40 – 50% higher than the "average"; in consequence, average power values as portrayed in published studies may then offer a distorted picture of optimal laser-tissue interaction, relative to the reported outcome of treatment.

Furthermore, with large, irradiated areas and gaussian X-sectional beam distribution, the effects of fluence / irradiation variation may be exacerbated and compromise the effective

photonic dose. With a nominally small “spot” size, related to the commonly found quartz optic fibre as 300µm diameter, the consequence of such distortion would be negligible ($0.0007cm^2$). However, with a much larger spot size and diameter of 3.0cm, the affected area becomes much greater, i.e. 7.0 cm^2, and errors assume greater significance. Once more, the failure to indicate the diameter of the delivery tip and computed fluence, relative to the average power would indeed have significant implications. As shown in Paper II, there is statistical significance between both beneficial outcome and tissue penetration, when choosing a larger rather than smaller delivery units.

It is pertinent to explore the relative emphasis of parameter recording, since it may be applied to PBM, and compared to a general area of surgical fluences with both soft and hard oral tissues. Key aspects may be listed as:

(i) Differences in delivery tip temperature

(ii) Differences in fluence / dose value

(iii) Differences in delivery spot size

(v) Differences in emission modes

(iv) Influence (PBM) of volumetric energy transfer with deep pathology (<1cm)

(i) Differences in delivery tip temperature: with visible, near-IR and far-IR wavelength laser action, the predominant laser-tissue interaction associated with surgical management of oral soft tissue, is photothermal i.e. incident photonic energy is absorbed by target tissue and converted to thermal energy; as referenced above, key temperature levels are 60 ^{0}C (protein denaturation), 100 ^{0}C (water vaporisation). Longer wavelength far-IR irradiation at 10,600nm, through its high absorption in tissue water leads to ready

desiccation of surface tissue components and eschar; it remains incumbent on the clinician to recognise such potential and remove the desiccated deposit to prevent carbonisation and allow fresh tissue irradiation to progress [139]. Such potential for eschar and carbonisation remains the prime caution associated with this wavelength when applied to oral soft tissue. Albeit the depth of interaction of this wavelength amounts to a depth of penetration at 1.0 mm of oral mucogingival tissue [140], the lateral conductive collateral damage potential of carbonisation remains a challenge to ideal surgical outcome.

With shorter wavelengths, the challenge remains the empirical absorptive capacity of tissue elements relative to wavelength and inherent photonic energy. Tables #1 and #2 (Paper #1) explores the intrinsic incapacity of pure photonic energy relative to wavelength, of the majority of available laser wavelengths. In essence, the individual photonic energy value of the majority of wavelengths from 600 – 1064nm fail to deliver sufficient incident energy to cleave the structural tissue molecular component bonds. Since earlier discussion has related to the very high number of photons and their effect on target oral soft tissues (Chapter I, Page #40), the most efficacious method of enhancing laser – tissue interactions is to "initiate" the quartz optic fibre tip to develop a suitable layer of pigment absorption at the fibre tip, in order to attenuate the majority of incident energy but to also allow a sufficient (approx. 15%) quantity of wavelength-specific photons to enter the target tissue and also provide potential for PBM effects [141 - 143]. Consequently, from such initiation, a "hot tip" is developed to temperatures approaching 1000 ^{0}C, that allows efficient soft tissue ablation; (this should be compares to the use of optic fibre delivery of PBM fluences). As such, a possible 10 – 20% "dose" error with a

hot-tip fibre would be deemed irrelevant, whereas fibre tip temperature does not represent an issue with the delivery of sub-ablative PBM fluences.

(ii) Differences in fluence / dose value: With surgical laser-tissue interactions, the crucial point is the ablation / phase change threshold and corresponding temperature. For example, the delivery of 2.0W CW diode irradiation using a 300 µm diameter optic fibre, sufficient to efficiently ablate oral soft tissue, results in a fluence (energy density) of 332 Jcm^{-2}. A reduction of 25% in delivered power would develop a fluence of 249 Jcm^{-2} and even a 50% reduction to 1.0W delivered power but would still give rise to a fluence of 166 Jcm^{-2}; each value would still allow surgical interactions. Correspondingly, with a desired target PBM fluence of 5 Jcm^{-2}, such a reduction would significantly affect the biostimulatory effects of irradiation.

(iii) Differences in delivery spot size Taking the nominated 2.0W average power delivery but increasing the fibre diameter from the 300µm surgical tip to a 15mm diameter PBM probe, results in a fluence reduction from 332 to 5.6 Jcm^{-2}, and without altering any other laser operating parameter, the laser-tissue interaction is modified from an efficient surgical ablation to optimal PBM fluence.

Additionally, the concept of total energy delivered may give rise to errors, where even with identical power density (irradiance) delivery, but through differing diameter delivery tips - *Table #5*.

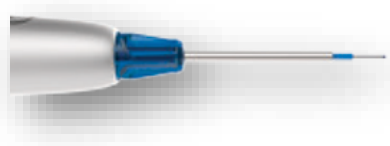
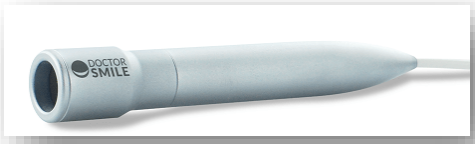
Delivery tip	Same irradiance / power density = 0.4 Wcm^{-2}	Total energy delivered in 10 secs
	Surgical handpiece fibre tip 400µm dia. 0.00126cm^2 area @ 0.5mW power = 0.4 Wcm^{-2}	0.05 Joules
	4 mm glass optical guide 0.125cm^2 area @ 50mW power = 0.4 Wcm^{-2}	0.5 Joules
	8 mm glass optical guide 0.5cm^2 area @ 200mW power = 0.4 Wcm^{-2}	2.0 Joules
	11 mm glass optical guide 1.0cm^2 area @ 400mW power = 0.4 Wcm^{-2}	4.0 Joules

Table #5. Delivery of the same power density / irradiance (0.4 Wcm^{-2}, albeit through delivery tips of differing diameter and consequent spot size. During a period of irradiation of 10 seconds, the total energy delivered will differ radically from 0.05 – 4.0 Joules – a multiplication factor of 80.

(iv) Differences in emission modes To enable direct comparison with relevance to the impact of error in irradiation values, between surgical and PBM therapies, a wavelength range of 500 to 1300nm, visible – near-IR provides some significance in that pigment absorbance coefficient is inversely proportional to wavelength, whereas the scatter coefficient is directly proportional to wavelength. This would have further significance when providing a PBM therapy fluence of an optimal 5 Jcm^{-2} to a chosen cell type at a depth of 10mm, where with an applied wavelength of 810nm, the absorbance

/ scatter effects would reduce the delivered fluence at depth by 90% [145]. Correspondingly, the applied fluence at the tissue surface would need to be approximately 50 Jcm^{-2}, with the possibility of surface overheating and pain for the patient. Changing the emission mode from CW to gated-CW, and certainly developing an average power target but with micro-second gating, would possibly increase the irradiation time, but also significantly influence the thermal relaxation potential of the photonic delivery. However, the study failed to provide substantive support of endorsement of FRP emission mode, partly, since the visible and near-IR (<1064nm) emission of commercial dental lasers remains inherently CW, but moreso in view of the significant peak power values of FRP 1064nm Nd:YAG, there would be a risk of breaching the tissue ablation threshold [145].

With regard to the latter, when considering the use of mid-IR erbium and 9,300nm far-IR carbon dioxide lasers in delivering tissue ablation through the vaporisation of the water component of oral hard dental and osseous tissue, both the vaporisation temperature and pressure may rise considerably, a phenomenon contributing to superheating ablation dynamics [146]; rapid temperature rises (>100 ^{0}C) arises in view of the containment of water and OH- radicals within the structure of the hard tissue hydroxyapatite crystal. In turn, this generates high pressure, and the phase explosion (spallation) may also give rise to structural stresses that add to tissue disruption [147]. Although the ablation mechanism is similar, with identical absorptive tissue water in soft tissue, the fragmentation potential is much less [148]. It remains a similar conclusion with longer wavelengths, i.e. error percentage may exert a less significant effect in delivering surgical supra-ablative coefficient fluences.

(v) Influence (PBM) of volumetric energy transfer with deep pathology (<1cm). When values of energy density / fluence are reported, invariably the reference is at cell

depth within the tissue. As discussed above in *(iv)*, significant energy losses may occur, relative to tissue optical properties, incident laser wavelength and coefficients of absorption and scatter [145]. Published data have been employed to develop a concept of volumetric values to represent the dissemination and influence of delivered energy at depth through the tissue and specifically, the total energy (Joules) deposited during a timed exposure, rendering the computed power density as $\pi r^2 h.t$ [149]. This latter extended value was termed "dose optical exposure time". However, if one considers that at approximately 800nm, the energy loss at a 1.0cm depth may be 90%; however, apart from a component of backscatter that may account for 40% [150], a significant amount of energy may enter the overlying tissue. In those clinical situations where oro-facial pathology at depth is subjected to PBM therapy, the omission of such extended energy deposited values would constitute yet another source of error. This level of error would consequently have relevance in terms of protocol reproducibility but also in those situations where wavelength choice differed, with impacts on absorption and scatter coefficients, together with ease of tissue penetration.

From the above, it is possible to conclude that both full disclosure of all operating parameters together with cognisance of the effect of error percentage, remains of greater significance when delivering PBM therapy compared to surgical ablative laser irradiation approaches.

The hidden implication of poor or incomplete reporting of clinical laser parameters may be illustrated through the following:

A published paper as a review of laser use in periodontology (Cobb, C. 2006) [151] drew upon the inadequate study design, data analysis and poor reproducibility of many

published investigations. Particular examples were provided to cite the work of three groups who had investigated the proposed benefits of diode laser use in bacterial and pocket inflammation reductions. Indeed reasoned debate was offered by the author to draw attention to inconsistencies in these three papers – poor or inadequate data, absence of appropriate statistical analysis, and unsupported extrapolation to define an "accepted" protocol of diode laser use in periodontal therapy. What was of much greater significance was that such was the renowned reputation of the three sets of authors, the unsubstantiated concluded outcome of each paper resonated through referencing in most subsequent published papers related to research in laser periodontics, during a period of almost ten years.

Such is the significance of the paper *"Photobiomodulation Delivery Parameters in Dentistry: An Evidence-Based Approach",* which is in line with other similar systematic reviews and meta-analyses, is that within a far-reaching field of laser-assisted therapy, incomplete or poorly deduced data may have promoted the acceptance of respective, possibly inaccurate conclusions regarding the benefits or otherwise of laser use. Given that each of the 141 studies analysed satisfied a criterion of randomised clinical trial, the implications of negativity assume some credibility. Equally, it is beyond this discussion to recognise possible similar occurrences throughout clinical dentistry.

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Chapter 3: Contributory errors arising from optic fibre power losses.

In *Chapter #2* of this thesis, through the audit of a significant number of randomised clinical studies over a 10-year preceding period of publication, it emerged that, among analysis of many laser operating parameters, less than 25% of papers recorded use of a power meter to define accurate laser power delivery. In that such omission may form the basis of a null hypothesis as to any claim of negative influence on study outcome, a possible analysis of the extent, prevalence and significance of any power delivery errors overlooked through non-use of a power meter, may help to provide counter-evidence.

In *Chapter #3*, the contention is that, having defined the narrow therapeutic laser power range that appears to define intended benefits of clinical application of laser use in *Chapter #1 – Introduction*, would the outcome of an investigation across many laser diode wavelengths and study centres, have relevance? Additionally, would the significance of errors to the intended reproducibility and accuracy of consequent studies that drew upon such published studies, adversely influence the validity of future study conclusions?

3.1 The challenge of optic fibre delivery mechanisms

Having examined the significance of full operating parameters, a further area of potential error in determining optimal "light dose" concerns the potential for power losses along the delivery mechanism of the laser.

The development of early wavelength choices, irrespective of emission mode related to a narrow breadth of wavelength values within the visible – near IR regions of the electromagnetic spectrum ("diode" wavelengths (635 – 980nm) and Nd:YAG 1064nm). The chosen delivery mechanism for these early lasers was quartz optic fibre, taken from

existing success with predecessor lasers in use in sister medical and surgical modalities. As examined in detail in Paper III, a significant determinant factor in light transmissibility of quartz optic fibre is the presences of impurities within the basic silica (SiO_2) crystalline structure. High grade “pure” optic fibre is considerably expensive, and hence the means to maintain cost-effectiveness with diode and Nd:YAG delivery systems exposes a compromise whereby impurities assume a significant presence. *Figure #1* provides a graphical representation of absorption coefficient relative to the laser wavelength of water as the absorptive tissue element. For this reason, those longer laser wavelengths and especially erbium-based lasers around 3.0 µm, and carbon dioxide lasers around 10.0 µm wavelengths, each very highly absorbed in water, fail to progress along such quartz optic fibre materials. Attempts to provide alternative “fibre” delivery conduits, using germanium oxide as an example core material, are themselves compromised through high costs and the disadvantage of increased fibre rigidity. An analysis of alternatives using low / non—OH constituent material for longer (>2,500nm) wavelength delivery provides an illustration of the drawbacks of alternative materials (*Table #6*).

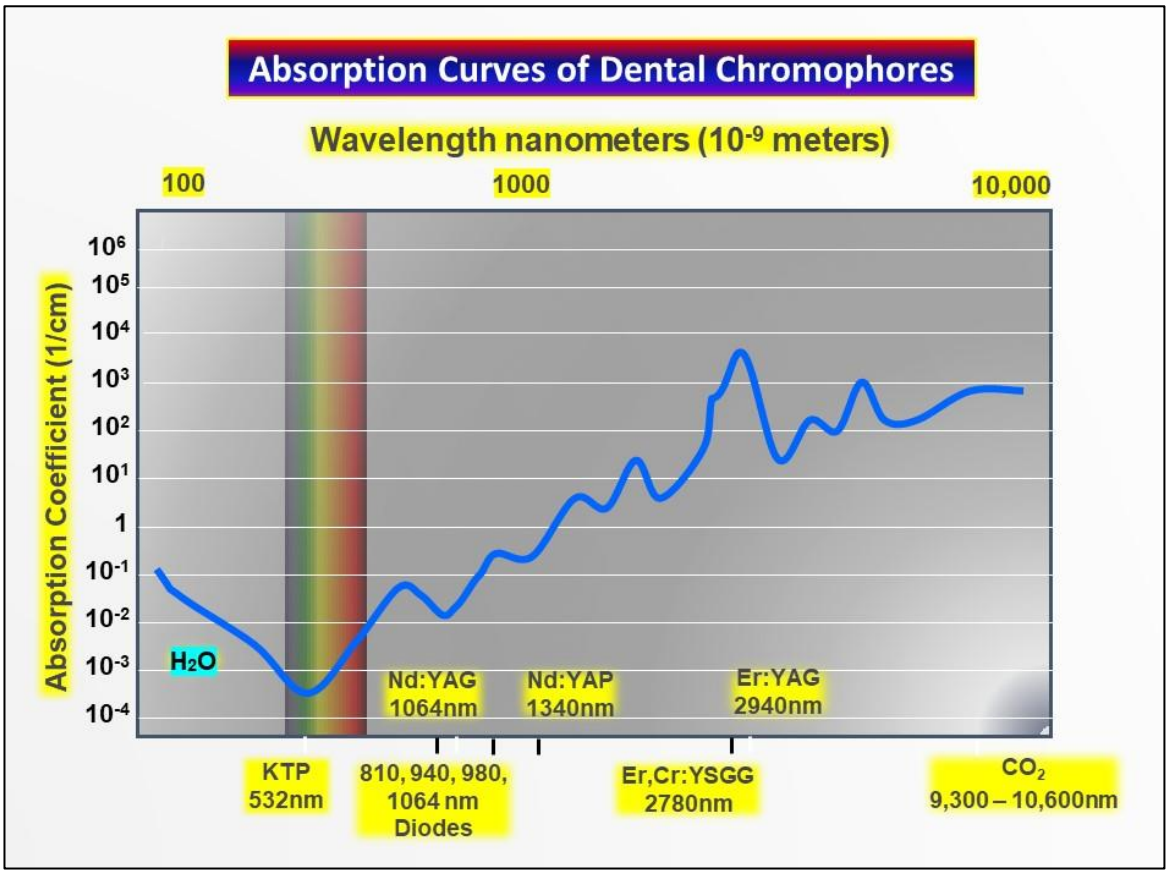

Figure #1. Graphic representation of the relationship of absorption coefficient and wavelength for water.

Material	Germanium Oxide	Zirconium Fluoride	Sapphire	Hollow Silica
Fibre core (microns)	400	380	425	300
Attenuation (dB/m)	0.7	0.1	1.0	2.0
Cost per meter	$550	$450	$500	$330
Advantages	Flexible	Low attenuation	High melting temp.	High power
Drawbacks	Low melting temp.	Hygroscopic, hard	Limited flexibility	Inflexible

Table #6. Illustrative alternatives to quartz optic fibre when considering longer wavelengths. [Ref #152: Fried, N.M. (2001). Potential Applications of the Erbium:YAG Laser in Endourology. Journal of Endourology, 15(9), pp.889–894.]

Another factor in study design to support choice of laser units, was to respect the high uptake in benchtop diode laser units and the common factor of quartz optic fibre delivery

mechanisms. Where possible, the distal end of the fibre was not further compromised by complex end-tips and for each fibre, the proximal SMA (sub-miniature assembly) fibre connector to the parent laser unit was inspected and cleansed of any grease or dust. In this manner, further possible contributing or distorting elements were eliminated.

Drawing upon published research criteria [153] an analytical / observational study design was adopted, drawing upon a multi-centred source of 38 “diode” semiconductor lasers, spanning a wavelength range of 445 – 1064nm.

It is an obligation on the part of the laser dental clinician to ensure that their device, as a nominated Class IV laser receives regular maintenance [154]; it is considered appropriate to adopt a yearly schedule, and this includes a calibration check regarding the accuracy of emission power relative to the control panel value. As referenced in the Materials and Methods section of the study, each laser had been recently calibrated and serviced.

Nonetheless, the investigation produced both significant and, in some cases large discrepancies, when end-fibre emission was measured using a power meter.

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3.2 Paper III: The influence of delivery power losses and full operating parametry on the effectiveness of diode visible–near infra-red (445–1064 nm) laser therapy in dentistry—a multi-centre investigation

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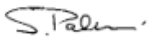
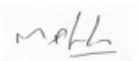
Statement of Co-authorship

We declare that the co-author contributions are correct, and that Steven Parker was primarily responsible for producing the first draft and at least 75 % of the final content of the following paper.

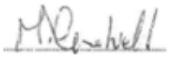
(i) Steven Parker (Candidate) conceived and designed the paper, collected and interpreted data, compiled and wrote the manuscript.

Signed:  Name: Dr Steven Parker

(ii) Mark Cronshaw (co-author) provided text input and data contribution.

Signed:  Name: Dr Mark Cronshaw

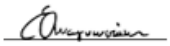
(iii) Martin Grootveld (Supervisor / co-author) supervised and assisted in conception and design of the paper, provided data analysis and editing the manuscript.

Signed:  Name: Prof. Martin Grootveld

(iv) Roy George (co-author) provided data contribution

Signed:  Name: Prof. Roy George

(v) Eugenia Anagnostaki (co-author) provided editorial review and data contribution.

Signed:  Name: Dr Eugenia Anagnostaki

(iv) Valina Mylona (co-author) provided data contribution.

Signed:  Name: Dr Valina Mylona

(vi) Marianna Chala (co-author) provided data contribution

Signed:  Name: Dr Marianna Chala

(vii) Laurence Walsh assisted in conception and design of the paper, provided data analysis and editing the manuscript.

Signed:  Name: Prof. Laurence Walsh

The following paper is an adapted version of the authors' accepted manuscript. The published manuscript is available at https://link.springer.com/article/10.1007/s10103-021-03491-y

ORIGINAL ARTICLE

The influence of delivery power losses and full operating parametry on the effectiveness of diode visible–near infra-red (445nm–1064 nm) laser therapy in dentistry—a multi-centre investigation

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Abstract

The development of protocols for laser-assisted therapy demands strict compliance with comprehensive operating parametry. The purpose of this investigation was to examine the accuracy of correlation between laser control panel and fibre emission power values in a selection of diode dental lasers. Through retrospective analysis using successive systematic review and meta-analysis, it is clear that there is inconsistency in the details, and possible inaccuracies in laser power applied and associated computed data. Through a multi-centre investigation, 38 semi-conductor ("diode") dental laser units were chosen, with emission wavelengths ranging from 445nm to 1064 nm. Each unit had been recently serviced according to manufacturer's recommendations, and delivery fibre assembly checked for patency and correct alignment with the parent laser unit. Subject

to the output capacity of each laser, four average power values were chosen using the laser control panel—100 mW, 500mW, 1.0 W, and 2.0 W. Using a calibrated power meter, the post-fibre emission power value was measured, and a percentage power loss calculated. For each emission, a series of six measurements were made and analysed to investigate sources of power losses along the delivery fibre, and to evaluate the precision of power loss determinations. Statistical analysis of a dataset comprising % deviations from power setting levels was performed using a factorial ANOVA model, and this demonstrated very highly significant differences between devices tested and emission power levels applied ($p < 10^{-142}$ and $< 10^{-52}$ respectively). The devices × emission power interaction effect was also markedly significant ($p < 10^{-66}$), and this confirmed that differences observed in these deviations for each prior power setting parameter were dependent on the device employed for delivery. Power losses were found to be negatively related to power settings applied. Significant differences have emerged to recommend the need to standardize a minimum set of parameters that should form the basis of comparative research into laser–tissue interactions, both in vitro and in vivo.

Keywords: Diode laser · Dentistry · Operating parameters · Power losses

Introduction

Evidence-based laser-assisted clinical therapies have experienced a considerable growth in their use within both general and specialist dental practice. Utilizing a suitable laser wavelength to deliver a predictable clinical outcome would depend on the understanding of the target tissue type, predictable laser–tissue interaction, and technique applied. In order to achieve predictable laser–tissue interaction, whether it be ablative (surgical) or sub-ablative (photobiomodulation), it is imperative that a photonic radiation product of laser power and exposure time should be observed; this is in order to avoid under-

exposure and sub-optimal outcome, or over-exposure with the consequences of collateral damage effects.

The reporting of laser operating parameters in dentistry has been the subject of recent publications; through retrospective analysis using successive systematic review and meta-analysis, it is clear that there is inconsistency in the details, and possible inaccuracies in laser power applied and associated computed data [1–3]. The core element that governs predictable outcome of any laser therapy is the management of photothermal conversion of incident coherent, monochromatic photonic energy, through a combination of dose, exposure time, and thermal relaxation. At sub-ablative doses, thermal rise associated with photobiomodulation (PBM) may be considered minimal. However, the applied dose and attendant thermal rise to render laser surgical tissue management may be considerable [4].

All current Class IIIB and IV laser facilities must conform to IEC and ANSI (and devolved) regulations that govern safe operation. A large group of visible and near infra-red (NIR) wavelengths are based on semi-conductor, so-called diode technologies, with attendant benefits of small machine footprint, and often bench-top size. Many of these devices offer a wide range of emission mode options, from continuous wave (CW) through gated-CW and micro-gated (often termed “pulsed”) emission to allow interactive efficiency, and also contribute to thermal relaxation to control damaging excessive heat buildup. The laser control panel of these devices may offer interactive function to allow the clinician/laser safety supervisor to choose a correct and appropriate photonic stream (“dose”) to enable a desired laser–tissue interaction to be achieved. Key operating parameters represented on the control panel may include average power, emission mode, and a series of pre-set options relative to common clinical applications that are possible

with the laser. The other added benefit of diode lasers is the opportunity to efficiently deliver photonic emission to an intra-oral target tissue using an optic quartz fibre—a lightweight, highly flexible, and non-linear delivery mechanism.

Transmission of light through fibre optics is not 100% efficient, with power losses [4]. Power loss depends on the wavelength of the light, the distance travelled, and the propagating material [4, 5]. Quartz-based optic fibres (quartz sic—silica/silicon dioxide) are relatively inexpensive photo-conduits, but often the transmission suffers due to crystal inclusions and impurities associated with low-cost manufacture [5, 6]. A common finding is the presence of hydroxyl groups, which are highly absorbing at longer mid-IR and far-IR wavelengths, and this restricts its application to shorter NIR and visible wavelengths [4]. Together with crystal inclusions and minor structural faults, such phenomena constitute the possibility of "power losses." Attenuation is defined as reduction in the amplitude and intensity of a signal. Passage of laser energy through fibre optics can suffer from attenuation due to intrinsic or extrinsic causes [5–8].

Intrinsic Intrinsic causes of transmission losses are absorption by the core material and by residual impurities, and by Rayleigh scattering from microscopic inhomogeneities, which are dimensionally smaller than the optical wavelength. Imperfections in the atomic structure induce absorption by the presence of missing molecules or oxygen defects. Silica fibres have low intrinsic absorption at wavelengths from 700nm to 1600 nm, and hence are well suited for transmission of lasers within this range.

Extrinsic Extrinsic causes of attenuation include improper fabrication, geometric effects of fibre design (sharp bends and micro bends), and losses occurring when laser energy is coupled into and out of a fibre optic. Extrinsic absorption may also be caused by impurities in the fibre material, particularly traces of metal impurities, such as iron, nickel,

and chromium. These metal ions can undergo electronic transition from one energy level to another [9].

The beam diameter can be modified by altering the distance from the target or by using larger diameter tips. Stopp et al. [10] reported that where the beam diameter is modified by moving the delivery system further away from the object, the resulting beam will have a decreased intensity, resulting craters with smaller depths and larger diameters. Beam diameter can also be varied by using fibre optic tips of variable size, in combination with dental laser handpieces. The effect is governed not only by the fibre diameter, but also by collimation, i.e., the extent of the beam that can be accepted by the fibre.

Previously published studies into optic fibre laser clinical therapy indicate that power losses may be significant (i.e. > 10%) along "quartz" optical fibre delivery systems.

Indeed, it is accepted that such power losses occur with varying types and quality of optical fibres [11–15]. A range of factors relating to the optical pathway from the laser head to the fibre can result in loss of power. These include problems with beam collimation (i.e. the extent of the beam that can be accepted by the fibre) [10], such that only the central regions of the circular beam from a solid-state laser enter the fibre. Other contributory factors may include sub-optimal optical issues, such as poorly performing anti-reflective coatings on lenses, insufficient convergence in the final lens before the fibre, and using a fibre size that is too small, hence causing loss of the beam periphery due to collimation. A particular challenge occurs with diode lasers since the beam is elliptical. A poor optical design may prevent the central region of the beam entering the fibre, and hence the edges are lost.

When selecting an optical fibre, the proper fibre material must be used, which transmits the laser wavelength in use. Other factors which must be considered before selecting a

fibre include the following: numerical aperture, back reflection, attenuation, packing fraction, minimum bend radius, input/output phenomenon [16].

Surgical diode lasers mostly emit in Transmission Emission Mode 00 (TEM00), which preserves the Gaussian spatial energy distribution generated within the optical resonance space. This is maintained within the coaxial fibre optic cable and becomes apparent in the visible spectrum when larger optical spot sizes are adopted, for example in photobiomodulation therapies. For small diameter surgical fibre optic delivery tips, however, this is not inherently a significant source of power loss, and this type of arrangement is used frequently in optical communications systems where the length of the fibre may extend to many kilometres. However, at the interface of a disposable fibre tip, there may be some significant failures of optic transmission consequent to operator error in tip placement.

Furthermore, very narrow diameter disposable tips may exert a significant attenuation of power consequent to scatter at the internal interface of the tip, plus an increased relative ratio of absorption attributable to impurities in the tip coating. In addition to power losses attributable to quartz optic delivery, other sources of spatial power loss may include irradiation surface backscatter [17–19], concepts of dose that may be affected by laser–tissue interaction [3]—i.e. Gaussian vs. uniform ("Flat Top") fluence/irradiation/spot size, infra-tissue scatter [20–23], along with consequent errors in effective "skin dose" with PBM therapy [24].

Differences in control panel operating power parameters and actual tissue dose may constitute a source of error resulting in inefficient laser–tissue interaction. With significant levels of loss, a proposal gains weight to endorse the use of a calibrated power meter to confirm the actual photonic dose being delivered to target oral tissue sites.

This study aims to investigate the performance of several diode lasers that are commercially available in clinical dentistry, and to evaluate possible power losses within the laser delivery system. The clinical significance of the results acquired is discussed.

Materials and methods

A total of 38 laser units with wavelengths within the visible and near infra-red (NIR) regions of the electromagnetic spectrum were chosen for this study, i.e. 20 different types overall. Laser devices and power meters used were subject to routine maintenance. *Table 1* provides full details regarding the devices evaluated.

For each laser, a lower and higher value of power output was chosen, specifically 100mW/0.5 W average power for photobiomodulation (PBM) units, and 1.0 and 2.0 W average power for surgical units. Following manufacturer's recommendations, the units were assembled and tested, utilizing appropriate laser safety measures of controlled area and appropriate optical density (OD) protective eyewear. Each laser was "charged" through mains connection or battery power-up for 15 min before any measurements were taken, with due regard to over-ride any time-out pre-set period of a given machine. Using the laser control panel, the nominated average power value ($P_{control}$) was selected.

With the delivery fibre/handpiece applied to permit vertical beam application, the beam was targeted onto a power meter and a series of n = 6 replicate readings were taken and their mean values of power determined (P_{fibre}). This was repeated for each laser unit, with the selected power output value indicated above. The mean "between-replicate-within-episode" coefficient of variance (CV) value was 14.8% for a total of n = 106 experimental episodes (i.e. all replicate determinations performed for each one), although more than 50% of these parameters were < 5%, an observation indicating a highly satisfactory level of within-episode precision and reproducibility.

Experimental design and statistical analysis

Deviations in emission power from their expected values in mW were expressed as percentages, negative or positive. These observations (n = 623 in total) were statistically analysed using an analysis of variance (ANOVA)–based experimental design, which was classified as a three-factor system with devices (n = 20), selected wavelengths involved (445nm, 660nm, 808nm, 810nm, 940nm, 970nm and 980nm and 1064 nm), and device emission power level applied (100, 500, 1000, and 2000 milli-Watts mW) being fixed qualitative effects at 20, 8, and 4 levels, respectively. An ANOVA rather and an analysis-of covariance (ANCOVA) design with device emission power as a quantitative predictor variable was employed since the latter requires a linear relationship between emission power loss and that power applied, and that was not the case. Six replicate determinations were made for each treatment-wavelength combination. The mathematical model for this design is shown in *Eq. (1)*, where μ represents the null mean value in the absence of the influence of all sources of variation, and D_i, W_j, P_k, and e_{ijkl} represent the "between-devices," "between wavelengths," "between-device emission power," and fundamental error sources of variation. $DWij$, $DP_{ik,}$ and WP_{jk} represent the devices × wavelengths, devices × device emission power, and wavelength × devise emission power first-order y_{ijkl} represents the monitored response variable, i.e. % deviation in emission power observed for each test performed.

These interaction effects were included so that the differential responses of each devices applied could be explored across the full wavelength and emission power ranges. The probability value p for significance was set to < 0.05. The statistical significance of each ANOVA model factors was determined by comparisons of their variables' least-square mean (LSM) values. LSMs represent the best linear-unbiased estimates of the marginal

means for the experimental design involved, and the post hoc testing of differences between them is independent of the methods used for the coding of categorical predictor variable effects. Hence, these parameters are computed on the basis of the model described by *Eq. (1)*. Post hoc comparisons of individual factor variable LSM values were made using the Bonferroni method. Statistical analysis was performed using XLSTAT2020 software (Addinsoft, New York, NY, USA; www.xlstat.com).

$$y_{ijkl} = \mu + D_i + W_j + P_k + DW_{ij}\, DP_{ik} + WP_{jk} + e_{ijkl} \quad Eq.\ (1)$$

Results

In total, 20 different laser models (38 laser units in total) were examined in this study, representing different versions of commercially available lasers used in dental practice. Each device was annotated by letter, and where model difference, duplicates, or multi-wavelength emission existed, the letter was further annotated by number (A, A1, A2, etc.). The range of emission wavelengths was 445–1064 nm. Where possible, measurements of four chosen emission power values were made, unless the design of the machine limited the range of output. For each laser unit, a series of six measurements were averaged and the percentage loss calculated *(Table 1)*. Where data indicated an end-fibre emission power greater than the control panel value, this figure has a " + " prefix.

Device Code	Wavelength	100mW (% loss measured)	500mW (% loss measured)	1.0W (% loss measured)	2.0W (% loss measured)
A (1)	445	103 (+3)	493 (2)	1.02 (+2)	1.85 (7.5)
A (2)	660	92 (8)	N/A	N/A	N/A
A (2)	660	91.5 (8.5)	N/A	N/A	N/A
A (1)	445	157 (+ 57)	385 (23)	0.767 (23)	1.53 (24)
B	980	92 (8)	464 (7)	0.92 (8)	1.53 (23)
B	980	89 (11)	435 (13)	0.853 (15)	NA
B	980	85 (15)	478 (4)	0.96 (4)	NA
A (2)	660	81 (19)	N/A	N/A	N/A
A (3)	970	N/A	559 (+12)	1.13 (+13)	2.4 (+20)
A (1)	445	N/A	494 (1)	0.981 (2)	1.98 (0)
A (3)	970	191 (+91)	449 (10)	0.859 (14)	1.76 (12)
C	810	90 (10)	418 (8)	0.830 (17)	1.57 (22)
D	810	N/A	N/A	870 (13)	1.67 (15)
E	940	115 (+ 15)	415 (7)	0.873 (13)	1.82 (9)
F	940	70 (30)	470 (6)	0.878 (12)	1.78 (11)
L	1064	75 (25)	428 (14)	0.827 (17)	1.76 (12)
G	940	120 (+20)	459 (8)	0.886 (11)	1.718 (14)
G	940	126 (+26)	498 (0)	0.956 (4)	N/A
G	940	87 (13)	314 (37)	0.598 (40)	1.17 (40)
G	940	102 (+ 2)	374 (25)	0.718 (28)	N/A
J (1)	650	71 (29)	395 (21)	0.798 (20)	1.336 (33)
J (2)	810	140 (+40)	502 (0)	0.929 (7)	1.84 (8)
J (3)	980	99 (1)	459 (8)	0.912 (9)	1.80 (10)
J (4)	1064	130 (+30)	494 (1)	0.937 (6)	1.829 (8.5)
K	810	79 (21)	406 (19)	0.788 (21)	1.671 (17)
K	810	68 (32)	408 (18)	0.809 (19)	N/A
H (1)	810	N/A	N/A	1.405 (+40)	N/A
H (2)	980	N/A	N/A	1.414 (+41)	N/A
H (1)	810	N/A	N/A	N/A	2.59 (+30)
M	904	84 (16)	N/A	N/A	N/A
N (1)	660	110 (+10)	N/A	N/A	N/A
N (2)	808	182 (+82)	N/A	N/A	N/A
D	810	N/A	N/A	1.24 (+24)	2.46 (+23)
H (1)	810	N/A	N/A	1.14 (+14)	2.24 (+12)
H (2)	980	N/A	N/A	1.2 (+2)	2.29 (+15)
H (2)	980	N/A	N/A	1.2 (+2)	2.28 (+14)
E	940	N/A	N/A	2.28 (+14))	1.8 (10)
D	810	N/A	N/A	1.22 (+22)	2.18 (+11)

Table #1. 38 laser unit emissions were measured. Each laser model is annotated by a letter (A, B, C, etc.), and where model difference, duplicates, or multiwavelength

emission existed, the letter was further annotated by number (A1, A2, etc.). Emission power values were measured using a calibrated power meter and power losses calculated as a percentage of the laser control panel value. Where the emission was greater, the percentage is expressed as a positive (+) value.

Statistical analysis

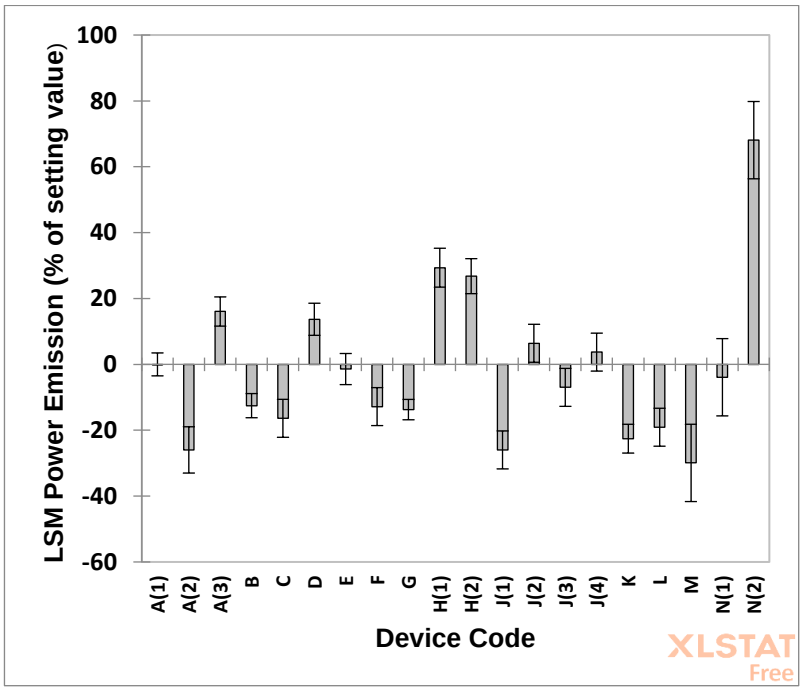
ANOVA revealed that differences observed for the % power deviations were extremely significant "between-devices" and "between-emission powers" ($p = 3.13 \times 10^{-143}$ and 7.96×10^{-53} respectively). Notwithstanding, the first order "devices × emission power" effect was also very highly statistically significant ($p = 1.10 \times 10^{-67}$), and this result provided strong evidence for differential non-additive dependencies of the % power loss values observed on emission power levels for each device explored. For example, the order of LSM power deviation for application of devices J(2) and J(4) markedly differs from that of device E (*Fig. 1c*). Further contrasting non-additive model differences are also clearly visible in this figure. The "between-wavelengths," and devices × wavelengths and wavelength × device emission power first-order interaction effects were all found not to be statistically significant.

From *Fig. 1a*, it should be noted that only six of the device products evaluated had mean LSM deviations not significantly different from zero. However, nine of them exhibited significant power losses (ranging from ca. – 10 to − 30%), and five of them displayed excessive LSM power outage levels (from ca. 10 to as much as 65%).

These results clearly demonstrate that in addition to the critical LSM differences in power deviation between the devices tested, there was a clear dependence of the emission power applied, with LSM values decreasing with increasing power level. Indeed, the low power emission of 100 mW gave rise to a positive deviation from that expected, but the 500, 1000, and 2000 mW power settings all had lower values than those expected, with deviations ranging from − 2.4% at 500 mW to − 8.65% at 2000 mW (*Fig. 1b*). The

negative deviations in LSM values observed at 1000 and 2000 mW were very significantly less than zero, whereas the increase observed at 100 mW (+ 14.9%) was substantially significantly greater than zero.

Figure 1d shows a full plot of observed observation values versus those predicted from the experimental design model shown in Eq. (1), i.e. the y_{ijkl} estimates. Clearly, there was a very acceptable level of agreement between these values.



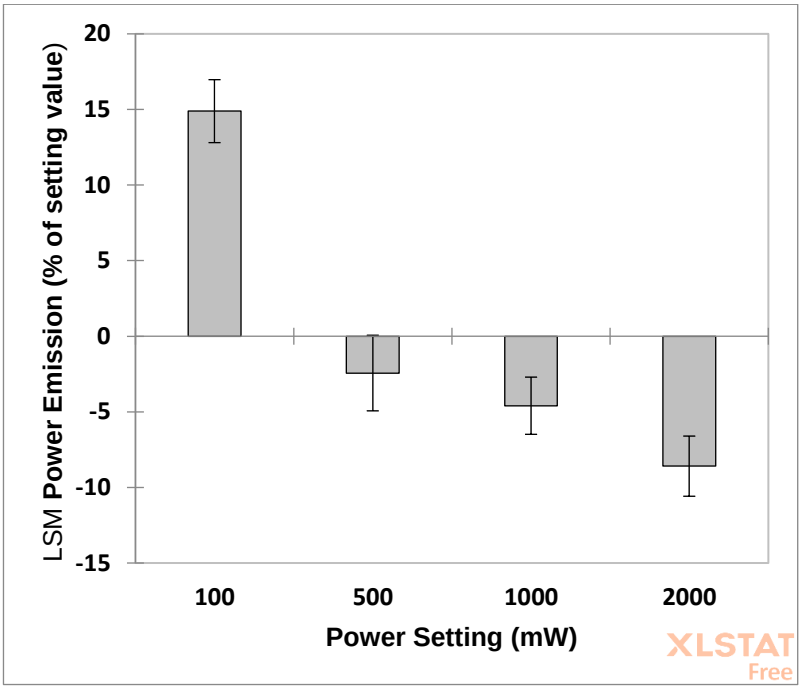
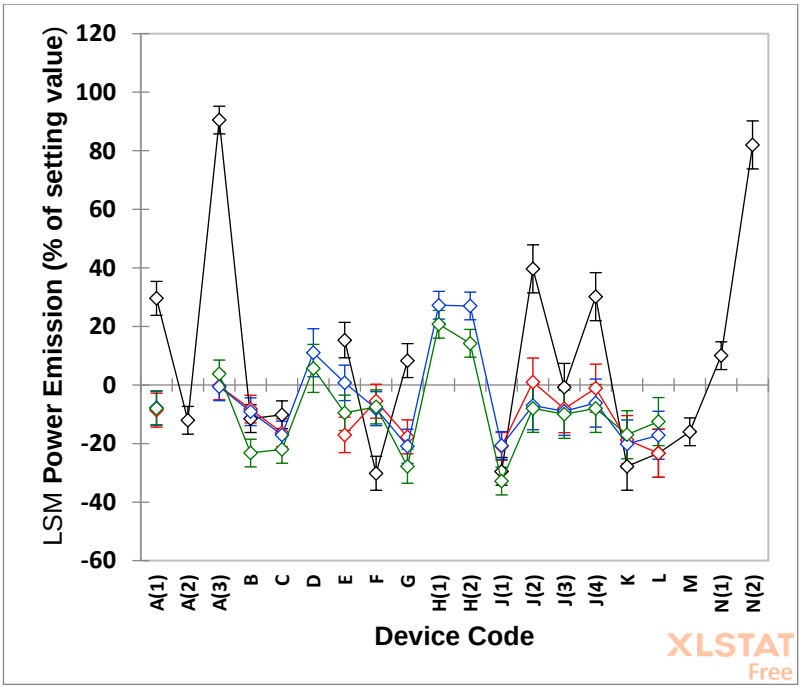


Fig. 1 a, b *Diagrammatic representations of least square mean (LSM) values ± 95% confidence intervals (CIs) for the % deviations from power settings found* ***a*** *between-devices employed and* ***b*** *between their emission power settings, demonstrating very highly significant differences between predictor variables for both of these factors.*



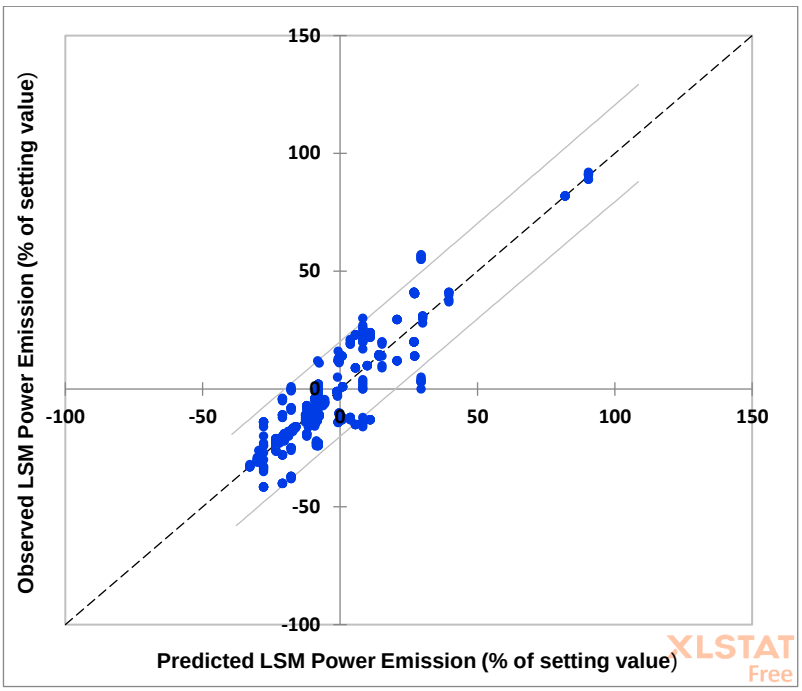


Fig. 1c, d *Corresponding LSM ± 95% CIs plot showing the dependence of deviational responses at each emission power for each of the 20 different devices investigated, which clearly shows sources of the very highly significant device × emission power first-order interaction effect. Black, red, blue, and green lines represent device emission powers of 100, 500, 1000, and 2000 MHz respectively.* ***d*** *Plot of the observed % deviation from measured power output as a function of that predicted from the experimental design model in Eq. (1).*

Discussion

The significance of power losses in fibre delivery diode laser use has been demonstrated by our study. What is of equal significance remains the number of studies where multiple laser active groups are included in the selection of control and active samples. As has been shown in this study, power losses within chosen operating average power values, together with associated factors of backscatter [25] and beam cross-sectional Gaussian distribution [26], can amount to over a 20% loss in actual therapeutic fluence [27]. When multiple laser active groups in any study are separated by average power applied values of < 20% difference, some concern must be given to the significance of the conclusions drawn from such studies.

As suggested in various studies [1, 28–31], there is a need to seek a standardization of operating parameter reporting in manuscripts submitted for peer-review publication. These minimum parameters are listed in *Table #2* as taken from ref. #1.

Laser Operating Parameters		
Wavelength	Diameter of optic probe	Optical spot size at target
Beam spatial profile (Gaussian/Flat top)	Beam divergence angle	Estimated target size & approximate depth
J/cm^2 (radiant exposure) at surface / J/cm^3 for body exposure	Joules delivered	Treatment time
Power output: verified by calibrated power meter	Irradiance W/cm^2 at surface	Treatment frequency
	Technique employed: contact/ non-contact static/ scanning)	

Table #2. Proposed basic laser emission and interactive parameters applied within submission guidelines for peer-reviewed publication

The prime objective in clinical laser-assisted dentistry is to apply a minimum level of photonic energy in order to maximize a desired outcome [32]. Such protocol optimizes outcome to avoid unwanted conductive thermal events and collateral tissue damage. The predictable benefits of laser assisted therapy in dentistry draw upon the manipulation of multiple operating parameters encompassing fixed and machine variables. Collectively, these represent a concept of "light dose" which is a critical factor to achieve a reliable and predictable clinical outcome. The wavelength range that spans the visible and near infra-red (NIR) areas of the electromagnetic spectrum has been the subject of considerable investigation both with regard to surgical applications and in the context of photomedical treatments, including antibacterial photodynamic therapies (aPDT) as well

as the current rise in research activity in the clinical applications of a variety of conditions that may be amenable to photobiomodulation therapies [33–39]. Of prime importance in establishing evidence-based success must be reproducibility of laser parametry.

This study has considered the presence and influence of so-called power losses. Inasmuch as the clinician may either select a manufacturer's pre-set power delivery value, or choose an individual average power setting, the photonic delivery may be subject to loss along the length of the delivery optic fibre; at the junction of the fibre trunk to the device (SMA—Sub-Minature Assembly) as well as at the interface with a removable tip, together with highly significant surface and sub-surface tissue attenuation factors,

collectively a degree of care is required of the clinician to appreciate the chosen laser control panel power value and the nett value of delivered photonic energy at tissue level. Additionally, with extended irradiated tissue area ("spot size"), the consequence of the predominant Gaussian spatial distribution of photon energy over the beam area, wherein there is a concentration of energy in the centre of the beam, can give rise to irregularity in optimal applied light dose. With regard to the latter, this may have significance only in cases where a large diameter Gaussian beam is used to deliver PBM therapy, yet with the emergence of research into post surgery/post oncology/myofacial pain syndrome regional facial laser PBM irradiance, such nonuniform values of radiant exposure may have significant effects on the success of such treatment [3].

The significance of photon backscatter—a common feature with the wavelength range considered here—may have influence within tissue surface interaction and with values of backscatter approaching 20% may lead to higher average power delivery [19]. However, the common use of diode lasers within the root canal and periodontal pocket, where laser irradiation is contained within a tissue space, may prevent backscatter power losses and

compound the risk of overdose. Although poorly investigated, the issue of power losses consequent to backscatter may also have influence when dose-dependent antimicrobial photodynamic therapy (aPDT) activation of applied photosensitizers may be compromised.

All in all, whilst outside the remit of this study, it may be reasonable to consider to what extent all the power loss factors have contributed to those study results that evoke equivocal estimation of laser-assisted procedures in dentistry.

The majority of intra-oral soft tissue surgical procedures can be achieved with laser average power delivery of 1–2 Watts [40]. Perhaps with greater regard to clinical significance than power losses in surgery, it is more important to ensure the use of a well initiated diode tip. Failure in achieving the same can result in operator frustration with a slower rate of cut and increased bleeding plus there is the added potential of accidental over-exposure to radiant energy to collateral tissues [41]. There are many commercially available diode lasers in the wavelength range of 445nm and 1064 nm in contemporary dental practice. The literature reflects that considerable interest has been shown to establish the absorption characteristics of oral soft tissue chromophores and absorptive tissue elements with individual laser wavelengths. However, the predominance of optical scatter over the relatively low tissue absorption in the red to near infra-red wavelengths is highly significant in clinical technique [42]. With the exception of higher photonic energy wavelengths at 445–532 nm, efficient photothermal incision and vaporization of oral soft tissue at lower power with reduced collateral tissue damage with most diode lasers, is compromised by relatively insufficient “true” photothermolytic photon energy and requires the addition of a contact hot-tip technique, to enable surgical procedures to be completed [41]. The added appreciation of the nature of the laser–tissue interaction

permits the performance of all of the commercially available lasers between 445 and 1064 nm to differ only slightly in the ability to perform laser-assisted soft tissue surgery.

To avoid confusion to both researchers and clinicians, it is most important to have reproducible measures and to standardize the approach to treatment requires a mature appreciation of the significance of the dosimetry terminology particularly in photobiomodulation therapies directed to sub-surface tissue targets. A common expression of dose is fluence or energy density; however, due to the volumetric nature of the cellular target and the variable potential rate of energy delivery, these terms are prone to mislead as the dose is the time accumulated in Joules/cm^2 at tissue level. As identified by Hadis et al., fluence (radiant exposure) is the correct technical term for J/cm^2 whereas dose must take into account the optical exposure time element [42].

A recent series of systematic reviews that considered the effectiveness of laser-assisted PBM, endodontic, tooth whitening, peri-implant, and photochemical antimicrobial effects [3, 38, 43–45], utilized a risk of bias tool [46] within the study structure. A significant element of omission of materials and method surrounded the use of a power meter to establish the “true” operating fluence and irradiation values. In another study [1], a total of 52 published randomized clinical trials that satisfied examination criteria, failed to record a power meter check on emission fluence in 66% of the studies. Furthermore, given the potential for operator error in the setup of the device with an incorrectly attached SMA trunk fibre coupling or a poorly installed tip, it is prudent to check the power emission prior to each experimental series. With concepts of PBM, a correlation exists between a therapeutic “healing” dose, an “analgesic” dose and any discrepancy between a “skin” or surface dose and “cellular” dose which may be located at tissue depth. Through published studies, ranges of 4–10 J/cm^2 and 10–20 J/cm^2 have been suggested as cellular

level healing and analgesia benefits of therapy [24]. PBM effects as required for deeper tissue therapy are subject to combined heterogenous beam attenuation due to absorption and scatter phenomena, essentially imprecise due to the nature of the irradiated soft tissue but considered to approximate a 90–95% reduction in dose through a penetration depth of 10 mm in the visible red to NIR wavelengths [47]. It is essential for the clinician to estimate such elements of therapy such as the approximate depth and size of the target in determining the cellular and corresponding surface applied skin dose to optimize the potential therapy outcome.

Given the multiple potential causes for error in experimental study designs, it is essential that a check list apply as a necessity to avoid the confusion and wasted effort that may arise. This need has been commented on in previous studies; however, regrettably there is as yet no agreed standard for researchers, editors, and associated peer reviewers to apply. Given the large increase in published papers particularly on PBM, the need for the same is pressing and with respect to the eminent authors who have called for the same we humbly offer our own. It is our belief that the implementation of the same will greatly benefit the further progression of the science which in turn will translate into many patient and clinical operator benefits.

Conclusions

This multi-centre study has involved the power meter measurement of possible power losses in 38 calibrated and serviced diode lasers, between the laser control panel value and that delivered to the target site along a quartz optic fibre. Significant differences have emerged to recommend the need to standardize a minimum set of parameters that should form the basis of comparative research into laser–tissue interaction, both in vitro and in

vivo. Through such data specification, clinical applications of surgical and PBM therapies may define higher levels of confidence and reproducibility.

Author contribution All authors contributed to the study conception and design. Material preparation and data collection were performed by all authors. Data analysis was performed by Martin Grootveld and Steven Parker. The first draft of the manuscript was written by Steven Parker and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declarations

Conflict of interest: The authors declare no competing interests.

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3.3 Summary

Through the publication of Papers II and III, *"Photobiomodulation Delivery Parameters in Dentistry: An Evidence based Approach"* and *"The influence of delivery power losses and full operating parametry on the effectiveness of diode visible–near infra-red (445–1064 nm) laser therapy in dentistry—a multi-centre investigation",* it has been shown that incomplete parameter reporting, delivery system power losses and deeper tissue beam performance may exert major significance regarding "false negative" study outcomes. Additionally, such false negatives may subsequently manifest through the reproduction of the proposed treatment protocol, but with incomplete operating variables.

Collectively, the delivery of therapeutic photonic energy constitutes a "light dose". It has been argued that whereas all laser-tissue interactions are mandated by the application of minimum power values, to effect an optimal outcome, the significance of "dose error",

either through negligent omission of full parametry, or deficiency in accurate calibration, has a greater impact with the precise delivery of PBM fluences.

Through over 30 years of investigation, laser-assisted dental surgery and PBM therapy has moved from anecdote to science! The plural of anecdote are not data, and there is a responsibility on the part of those seeking to prove optimal benefits of laser dentistry, to minimise the risk of reproducibility errors through inadequate parameter recording.

Peer-review studies should be mandated to provide a minimum of standing and computed photonic parametry. With the development of the balance of opinion, relative to evidence and to address the possible recommendations of this discourse, a revision of the full inclusion and value of all measurable elements, when submitting a study or investigation for peer-reviewed evaluation and publication, represents a major duty of care in order to maximise the potential for error-free reproducibility. Table #7 provides further guidance in this respect.

Laser Emission and Interactive Parameters		
Type of study—such as RCT	Sample size/groups/control/randomization	Blinding (single / double blinding)
Laser used—emission wavelength (nm)	Delivery system Fibre, Waveguide, Articulated Arm	Gaussian / ''Flat top'' beam x-section
Emission mode (CW, Gated-CW, FRP)	Power meter used and output calibrated	Mean, median, max / min power (W)
Target tissue / lesion dimensions (mm) / Target tissue depth (mm)	Tip to tissue distance (mm)	Irradiated ''spot'' size (cm^2)
Fluence / Radiant exposure (J/cm^2)	Irradiation / Power density (W/cm^2)	Total energy delivered (J)
Irradiation PBM therapy time (sec)	Irradiation frequency (n x days)	Tip movement / area covered (mm/area)

Table #7. Proposed extended laser emission and interactive parameters applied within submission guidelines for peer-reviewed publication

Overall, the significance of correct parameters and light dose draws upon the following:

- Laser-assisted therapy in dentistry extends to fluoroscopic / spectroscopic diagnostics, photochemistry, soft and hard dental and osseous surgical tissue manipulation, and photobiomodulation (PBM). PBM may be considered as a stand-alone therapy or as part (a "by-product") of surgical phototherapy, through a combination of thermal and light scatter gradients.
- As discussed, there are greater influences of extended light dose errors with PBM, compared to similar errors with surgical laser operating power values.
- Crucial to predictable PBM effects remains the concept of accurate dose, relative to anatomical target site, applied photonic power and tissue optical properties – in all aspects of delivery, including the accurate use of parameter terminology.
- The "optical window" wavelength range extends the concept of dose to a volumetric irradiation, significantly dependent on intra-tissue photon scatter!
- With extended irradiation periods, in order to enable light dose at depth and prevent tissue surface overheating, the concept of total energy delivery may have greater significance, notably when compared to just the energy density.
- With the emergence of large bulk tissue pathologies, the Gaussian distribution of beam profile requires caution to deliver a correct dose.

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Chapter 4: The influence of accurate laser parameters in defining "uneventful" post-surgical healing

The aforementioned summary in *Chapter #3* and associated published papers II and III helps to provide a context of error analysis that may encourage greater discipline and attention to detail as future laser therapy is investigated. To the laser clinician, a prime responsibility rests in a duty of care in delivery of clinical treatment to the patient. Part of such duty of care encompasses minimising peri- and post-surgical complications and this would also apply to subablative laser use in PBM and aPDT.

For any experienced clinician, the library of (mostly anecdotal) laser cases provides a ready catalogue of clinical success where the epithet "uneventful" predominates. As discussed in many areas of this thesis, it is notable that a large portion of applied research into laser use has, in historical and medium-past periods, focussed on the use of those laser wavelengths in the visible red – NIR range. As demonstrated, the essential aspects of uneventful healing following laser irradiation appear to be significant no matter which laser wavelength – from "blue" diode to FIR carbon dioxide – is chosen.

Structured critique of a recommendation towards full and accurate laser power delivery parameters appears to have greatest relevance in those applications where PBM may have either primary relevance, or secondary to photothermal surgical tissue management. Through this philosophy, it is of importance to the laser clinician that with applied knowledge and appreciation of aspects of laser-tissue interaction, relative to the choice of laser wavelength, predictable outcome may prevail. With such awareness, the scope for the clinician to expand laser use would be best-served by well-designed clinical studies that placed comprehensive parameter recording and ease of reproducibility at its core.

- A prime benefit of laser use in soft tissue oral surgery is the lack of peri- and post-operative complications, contributing to a concept of "uneventful" healing.
- The acceptance of the so-called "optical window" to define the breadth of wavelength range associated with delivery of PBM-influenced post-surgical clinical presentation, may benefit from acceptance and greater understanding of the effects of varying incident laser wavelengths.
- Research into laser-tissue interaction that may predispose to "classic" PBM effects has given rise to a casual acceptance of a single optical window.
- Examination of published databases demonstrates the existence of other optical regions that embrace co-existent optical properties.
- Surgical laser use in dentistry, to obviate demonstrable disadvantages of scalpel or other modality-surgery, offers tissue outcomes that may be judged as indicative of a PBM or PBM-type influence. Such outcomes also appear to be associated with laser wavelength choice that lie outside the nominated optical window.
- Contributory biochemical activity may be either photo-induced, or present as a co-existent phenomenon consequent upon low-grade thermal rises. Co-existence of activated tissue factors and co-enzymes span both photobiomodulation and thermobiomodulation.

4.1 "Uneventful" healing associated with clinical dentistry

"Uneventful" healing is the idiomatic term to denote the combination of many of the benefits of laser-assisted oral soft tissue surgery, when compared to scalpel use. Although drawing as much from anecdotes as an evidence-based, for the dental surgeon these benefits may be listed as:

- Incisional haemostasis

- Significant pathogen reduction

Other benefits suggest harmonisation of hard and soft dental / oral surgical management, such as might be found for example, with sub-gingival dental caries, or defining a soft tissue emergence profile with crown and bridge prosthetic provision. Additionally, within such a concept, another benefit – "precision" – is intuitively undemonstrative, considering both the classic irregular edges of the incision but also the minimum width of the incision conforming to the 300 - 500µm minimum diameter of the quartz delivery tip being used to create the laser incision [155]. Certainly, there is scope for error with such an irregular zone of ablation, but perhaps, when faced with the complex linear tracing of for example, a quadrant gingivoplasty procedure, the instrument of choice ceases to be the usual scalpel, favouring the fine styptic quartz fibre optic delivery tip.

Although argument has been developed to endorse a belief that laser output power accuracy is not as crucial as that of PBM irradiation, in terms of treatment error potential, a general maxim of "minimum output – maximum outcome" (MIMO), is offered to encourage the clinician to limit any potential for parameter errors (*See Chapter #3 Figure #4, Page #162)*.

The employment of appropriate operating parameters allows a dual application of ablative fluence to complete the surgical tissue management, with a "trickle down" reduction of fluence that promotes wavelength specific PBM, through thermal and scatter gradients. *Figure #1* provides clinical and graphical illustration of how the thermal and (wavelength specific) photon scatter within soft tissue adjacent to the surgical zone may be envisaged to accommodate these mechanisms. The clinical procedure relates to the surgical removal

of hyperplastic mucogingival tissue associated with an ill-fitting lower full denture, offering peri-operative tissue ablation and a post-surgery image at 14 days.

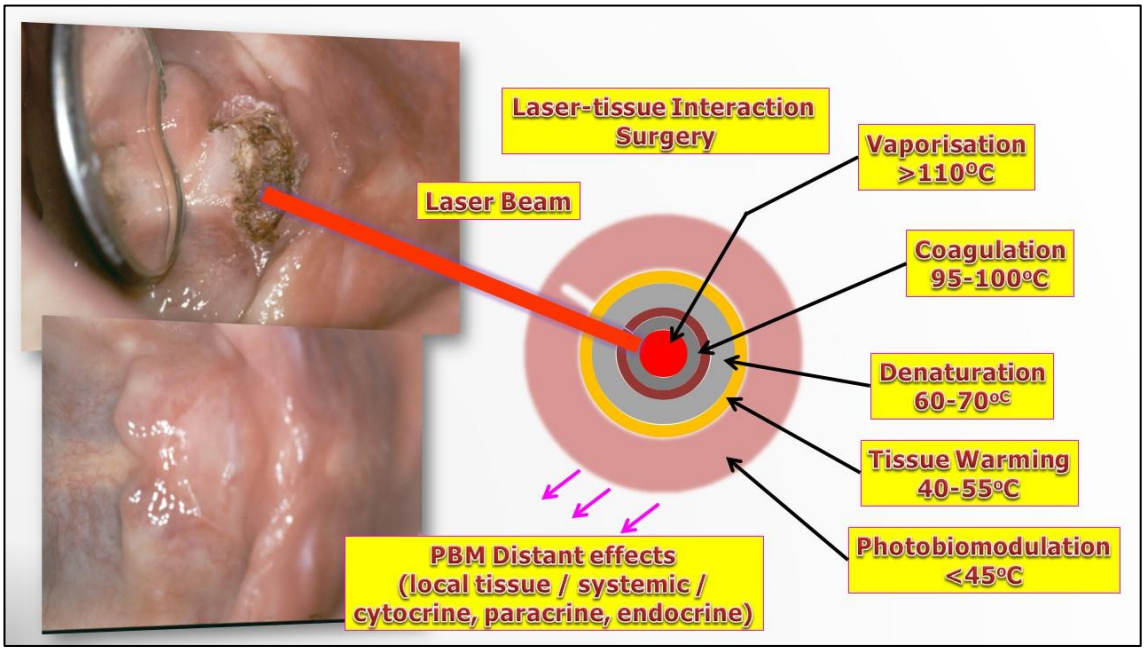

Figure #1. Laser-assisted surgical management provides a zone of ablation, protected by a tenacious coagulum. Peripheral to the wound, temperature reduction arising from photon scatter promotes a zone of photobiomodulation.

4.2 The link between soft tissue oral surgery and PBM effects exerted

The crucial links between the surgical and PBM aspects are photon energy / wavelength and the tenacity of the proteinaceous coagulum; the latter gains significance in terms of the photothermolytic temperature of several hundred degrees which renders incisional sterility and the formation of coagulum, a process providing a physical seal which in turn, prevents secondary post-surgical bacterial contamination. Typically, the surface coagulum associated with mucogingival tissue surgery would remain during an initial 3 days, and will gradually re-hydrate through absorption of hydrous water content in saliva, and will eventually become softened and detached around days #5 - #7. In the event of

early breakdown of the seal, usually through local functional frictional forces, bacterial contamination and consequent toxin release would incite renewed inflammation and early tissue resolution impairment.

As discussed in Paper I, part of the overall effects representing PBM is the reduction in inflammation, achieved through a combination of suppression of pro-inflammatory mediators, and via the promotion of anti-inflammatory agents. Moreover, intra-oral soft tissue surgical procedures would be always compromised by the omnipresent commensal and opportunistic pathogenic intra-oral bacterial load. Tissue incision would allow bacterial contamination, and hence add to the balance of host immune capacity and promoting features of inflammatory reaction, including pain. The link between laser benefits (haemostasis and significant bacterial reduction at the surgical site) and co-existent PBM action are primarily assisted through the surface coagulum, as discussed above. In *Figure #2*, a schematic representation of the interrelationship between laser surgical action and the sub-ablative fluence effects of PBM is shown. The concept of a "circle of suffering" pertains to the challenge of non-laser oral soft tissue surgery, where the tissue disruption, whether through scalpel incision or trauma is linked to a sequence of related bacterial, cellular and biochemical activities.

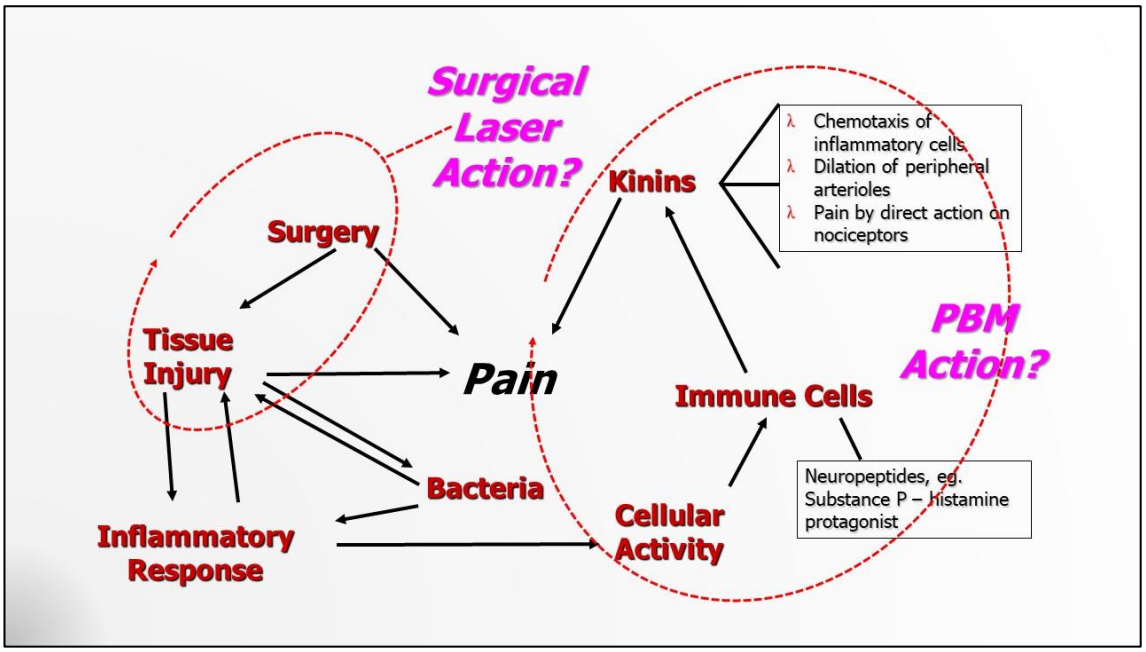

Figure #2. "Circle of Suffering". Tissue injury results in pain and inflammation, the latter exacerbated through bacterial contamination. Laser coagulum provides a physical barrier and photon scatter reduces ablation fluence to a level consistent with PBM promotion. Through this, a physical protection and anti-inflammatory / analgesic action offered by PBM may reduce post-surgical complications.

4.3 Contact vs non-contact mode: fibre "hot tip" initiation

It is important to apply laser operating parameters that are appropriate to achieve the optimal outcome, but also allow the promotion of PBM effects. Correct application of the delivery tip to offer either radiant or conductive incident photonic energy, will be appropriate for the wavelength being used, absorptive capacity of the target tissue in terms of optical properties, and emission mode of beam delivery. Where a contact-mode is chosen, correct consideration of tip conditioning is essentially where visible-red and near IR wavelengths are chosen, and individual photon energy is insufficient to permit efficient surgical cleavage. In these cases, a pre-surgical fibre initiation involves the application of end-coating of an attenuation medium, usually a pigmented compound to restrict onward emission of the laser beam. Consequent upon such application, the fibre

tip becomes hot (*sic* “hot tip”), but which also allows some photon passage (approximately 15%) to promote tissue PBM. Through personal investigation, this has been best achieved through the use of blue articulation paper, momentarily applied to a fresh quartz optic fibre tip. Successive applications are applied with laser output at 300mW, a representation of the individual laser’s lowest control panel output, with the paper in contact before each momentary “fire”.

Once again, the implication of correct laser parameters would apply in this procedure, since the use of greater average power settings merely causes ablation holes in the articulation paper, and failure of fibre initiation.

A simple investigation into the efficiency of fibre initiation, using a power meter to gauge the degree of consequent attenuation (loss of transmission) of the initiated fibre indicated through successive passes, to register approximately 85% attenuation of emitted photonic energy with four applications, was conducted as a short personal study by the author; beyond this number of passes there appeared little improvement *(Figure # 3)*. Fibre initiation allows the development of the colloquial “hot tip”, where the fibre end may become heated to several hundred degrees C. In this manner, the relatively low photonic energy of red-visible and near-IR laser wavelength emissions can be compensated for during in-contact surgical use. Although not of statistical significance or subject to scientific rigour, the anecdotal improvement in cutting efficiency of delivery fibres initiated in this way appears both consistent and conducive to achieving surgical management without the impact of high-power collateral thermal damage, but still allowing photon passage into the target tissue area.

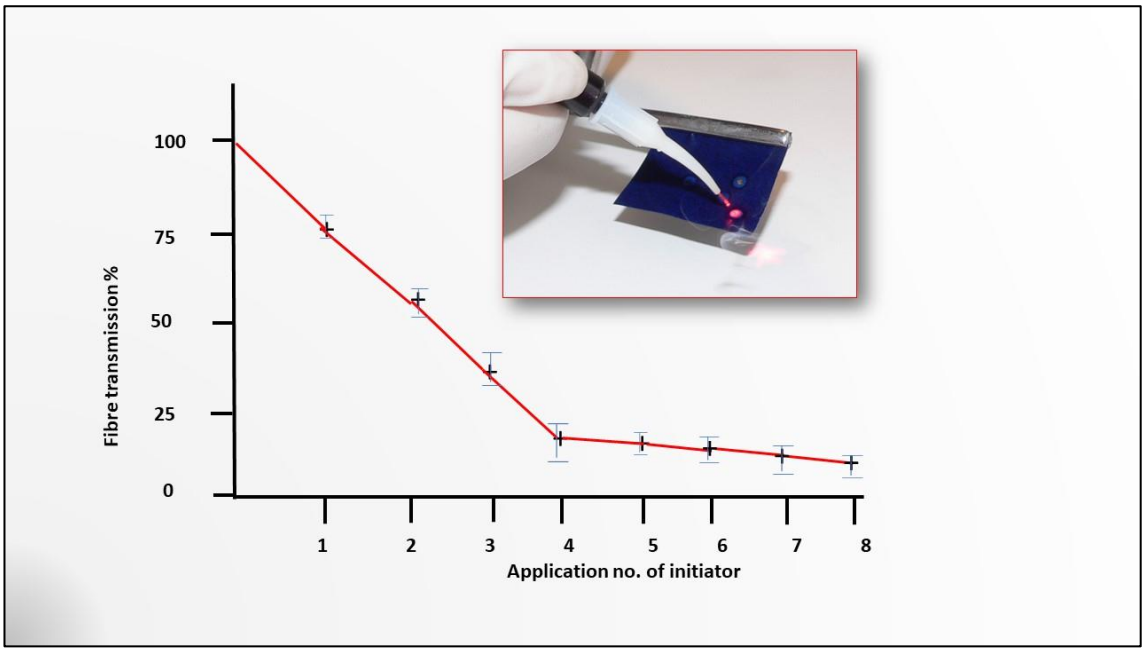

Figure #3. Graphic representation of reduction in fibre transmission by successive application of blue articulating paper to an optic fibre delivery tip, emitting 300mW of average power. From this, the proposed achievement of approximately 85% attenuation occurs after four passes. Source: personal study S. Parker.

Therefore, through evaluation of the proposed surgical tissue management procedure, the constituent target tissue in terms of optical properties and absorptive potential *vs* scatter potential of the structural tissue elements, a suitable laser wavelength may be chosen and adapted for optimal use. Correct laser output parameters draw upon the average power, delivered through the chosen delivery tip, to develop a value of energy and power density (fluence and irradiance) sufficient to produce supra-ablative ablation and vaporisation. Together with mindful adherence to tissue thermal relaxation to prevent overheating, the overall laser and tissue management is consistent with the desired surgical outcome.

Through correct delivery tip manipulation and consistent with a contact *vs* non-contact use (conductive *vs* radiant photothermolysis), a suitable surgical technique may be adopted to maximise the efficiency and benefits of laser photonic therapy. Coexistent with the supra-ablative action, as discussed above, the “hidden helper” of scattered photons and thermal reduction, may trigger PBM promoted effects. In summation, the

immediate post-surgical appearance of an irregular, discoloured and rough tissue surface appears disturbing, yet within 72 hours, the coagulum rehydrates and sloughs, and the underlying early organisation of reparative cellular and extra-cellular matrix would offer expectation of positive healing. Moreover, the effects of PBM within local tissue would promote cellular activity as well as increase local vascularity of arterioles, venules and lymphatics, and this would, in turn, support early tissue regeneration and reduce oedema [156]. In view of the absence of linear tissue stresses of post-surgery suturing, myofibroblast organisation has been shown not to induce scarring [156]. Such clinical events may be only appreciated through visual assessments of controlled tissue reactions to the surgery, reduced elements of tissue inflammation, and post-operative pain. In effect, "the surgery is ugly, but the healing is beautiful" (*Figure #4*).

Figure #4. Surgical removal of "denture hyperplasia" tissue overgrowth, associated with the ill-fitting lingual margin of a full lower denture. The laser used (diode 810nm) employed contact-initiated tip delivery of appropriate "MIMO" (Minimum Input power, Maximum Outcome benefit) operating parameters. Immediate post-operative and early healing clinical appearances were indicative of correct laser parameters and PBM effects.

The extent of laser wavelengths wherein photon scatter predominates and incident light has a range of penetration from 1.0 – 6.0mm, is termed the NIR optical window, in total running from 650 to 1350nm [158, 159]. In view of the predominance of absorption within groups of biological molecules, it may be seen that this range is split into two optical windows; the first NIR window spans 650nm – 950nm and a second from 1100nm – 1350nm, with investigation into a so-called third NIR optical window (1600nm – 1870nm), together with the possibility of a fourth window at approximately 2200nm [160].

It remains coincidental that early research into intracellular PBM effects utilised a red-visible wavelength [161], and that both primary PBM and that supplemental to soft tissue surgery studies centred on laser wavelengths that spanned the first and second NIR optical windows [162, 163]. Notwithstanding the split boundary, it has become commonplace within laser dentistry to combine these two regions into a single optical window, from 650nm – 1350nm. Studies into this region have allowed the specificity of correct operating parameters to prevail and have enabled a choice of surgical fluences, sufficient to achieve optimal tissue management as well as allow potential PBM effects to promote uneventful healing [164 – 166] *(Figure #5)*.

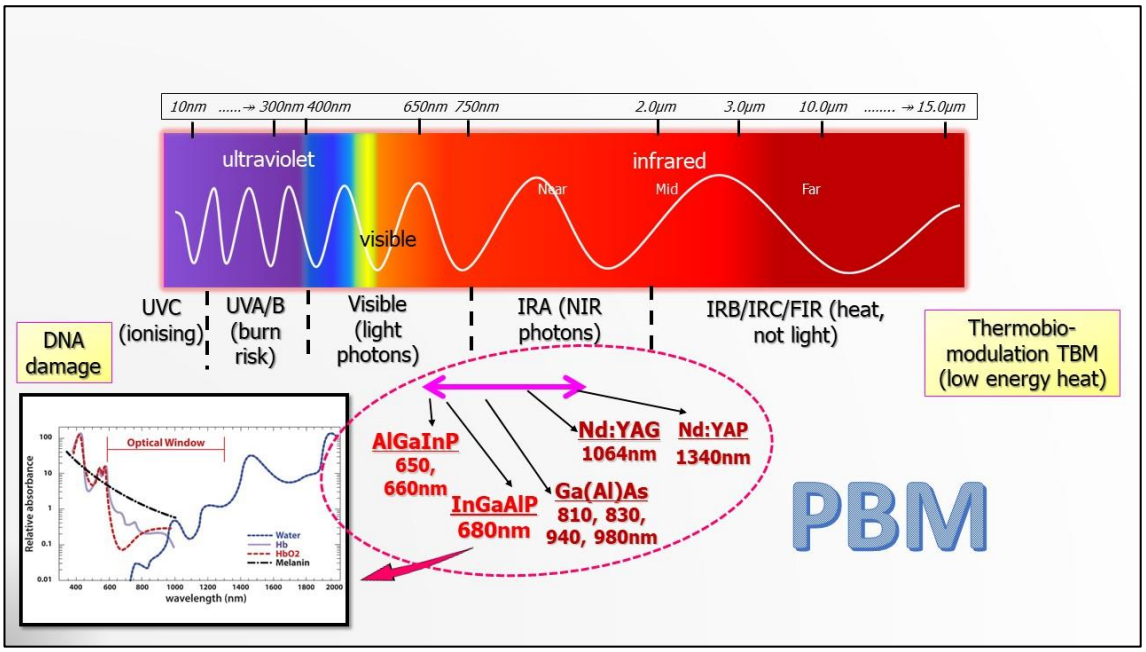

Figure #5. Within published dental laser peer-reviewed articles, the range of wavelengths (650 – 1350nm) has been collectively referred to as the "optical window", at variance with other photobiological disciplines referring to a succession of three optical windows. Within the optical window red-visible and NIR diode and FRP soft tissue lasers offer tissue penetration and a high scatter coefficient predisposes to PBM effects. [Source optical window graph Ref.#161]

In acknowledging the immense amount of research into PBM (estimates suggest an average of 500 published peer-review papers during the past 8 years) [167], by far the greater majority has been with laser wavelengths within the NIR optical window. Additionally, there appears to have been a shift in emphasis from early research into (predominately) soft tissue laser action, from PBM as a secondary event supplemental to a prime surgical procedure protocol; gradually, the breadth of investigations into cytokines and other biochemical markers of PBM activity has led to research into paracrine and endocrine signalling, and also to establish PBM therapy as a protocol of choice for the delivery of non-surgical treatments for a number of oral, head and neck pathologies and conditions. With this shift in emphasis, the discipline of correct laser operating parameters in delivery of appropriate therapeutic fluence at the cellular level, has assumed greater importance and significance.

A level of confusion arises, when the use of laser wavelengths outside the NIR optical window are considered. Sub-ablative, non-focussed far-IR laser photonic irradiation as a post-oncology therapy has been previously investigated [168,169], but with the emergence of mid- and far-IR wavelengths in dentistry, it was shown that healing profiles appeared clinically similar to surgical treatments with shorter wavelengths even when the absorption profile of the incident wavelength was considerably different to NIR wavelengths [170 – 173]. Clinical assessment with many of these early investigations centred on the prime, rather nebulous benefits of haemostasis, pathogen reduction and "uneventful" healing, as applied equally with shorter wavelengths. The benefits extended with longer wavelengths to consider the superficial laser-tissue interaction with cellular and interstitial water, together with the benefits of thermal relaxation offered through FRP emission (erbium laser family) and coaxial air / water. The 10,600nm CO_2 wavelength, as an intrinsic CW emission, would benefit from gating and low duty-cycle emission, together with the frequent removal of desiccated tissue debris that may build up.

In addition, interest was shown on the emerging use of wavelengths shorter than the 650nm lower limit of the NIR optical window. The "green" visible KTP 532nm and "blue" visible 445nm wavelength devices became popular lasers in dentistry, since both were available as bench-top, semiconductor diode lasers, complete with sophisticated emission mode manipulation. A great advantage was suggested to be through the higher photonic energy of the shorter wavelengths, suffice that both radiant and non-initiated fibre delivery was possible in delivering efficient surgical management [174, 175]. A consideration was demanded however, to employ lower surgical fluence values in order to avoid errors and consequent collateral thermal damage.

The question would then arise, to consider the biochemical and cellular response with those laser wavelengths outside the NIR optical window, in order to evaluate the supposed PBM effects, implicit through research to be a preserve of 650 – 1350nm wavelengths yet clinically indistinguishable when shorter and longer wavelength surgery was conducted, and post-operative results compared. This is discussed in the following paper – Paper IV:

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4.4 Paper IV: Systematic Review of Post-Surgical Laser-Assisted Oral Soft Tissue Outcomes Using Surgical Wavelengths Outside the 650nm–1350nm Optical Window

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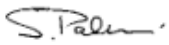
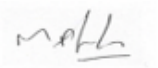
Statement of Co-authorship

We declare that the co-author contributions are correct, and that Steven Parker was primarily responsible for producing the first draft and at least 75 % of the final content of the following paper.

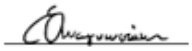
(i) Steven Parker (Candidate) conceived and designed the paper, collected and interpreted data, compiled and wrote the manuscript.

Signed:  Name: Dr Steven Parker

(ii) Mark Cronshaw (co-author) provided text input and data contribution.

Signed:  Name: Dr Mark Cronshaw

(iii) Eugenia Anagnostaki (co-author) provided editorial review and data contribution.

Signed:  Name: Dr Eugenia Anagnostaki

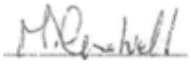
(iv) Valina Mylona (co-author) provided text input and data contribution.

Signed:  Name: Dr Valina Mylona

(v) Edward Lynch (co-author / Second Supervisor) supervised and provided editorial review.

Signed:  Name: Prof. Edward Lynch

(vi) Martin Grootveld (First Supervisor) supervised and assisted in conception and design of the paper, provided data analysis and editing the manuscript.

Signed:  Name: Prof. Martin Grootveld

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Original Research—Review

Systematic Review of Post-Surgical Laser-Assisted Oral Soft Tissue Outcomes Using Surgical Wavelengths Outside the 650nm–1350nm Optical Window

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Abstract

Objective: To explore via systematic review the validation of uneventful post-surgical healing, associated with shorter and longer laser wavelength applications in minor oral surgery procedures.

Methods: From April 28 to May 11, 2020, PubMed, Cochrane Database of Systemic Reviews, and Google Scholar search engines were applied to identify human clinical trials of photobiomodulation (PBM) therapy in clinical dentistry. The searches were carried out with reference to (1) dental laser wavelengths shorter than 650 nm; (2) wavelengths localized within the 2780nm–2940 nm; and (3) the 9300nm–10,600 nm range. Selected articles were further assessed by three independent reviewers for strict compliance with PRISMA guidelines and modified Cochrane Risk of Bias to determine eligibility. **Results:** Using selection filters of randomized clinical trials, moderate/low risk of bias, and the applied period, and following PRISMA guidelines, 25 articles were selected and examined. A risk of bias was completed, where 11 out of 25 publications were classified as low risk of bias, and 14 out of 25 were classified as medium risk status. In total, 6 out of 13 (46% of) studies comparing the examined laser wavelengths with scalpel-based treatment showed positive results, whereas 6 out of 13 (46%) showed no difference, and only 1 out of 13 (7.7%) presented a negative outcome. In addition, 5 out of 6 (83% of) studies comparing the examined laser wavelengths with other diodes (808–980 nm) showed positive results, whereas 1 out of 6 (17%) had negative outcomes.

Conclusions: A detailed and blinded examination of published studies has been undertaken, applying strict criteria to demonstrate research outcome data, which suggests positive or at worst neutral comparatives when a given laser wavelength system is used against an alternative control therapy. As such, substantiated evidence for laser surgery in delivering uneventful healing and analgesic effects, as an expression of a PBM-like (quasi-PBM) influence, has been shown.

Keywords: laser, oral, soft tissue, healing, photobiomodulation, optical window

Introduction

By convention, photobiomodulation (PBM) involves the manipulation of cellular behaviour by using low intensity light sources. Originally referred to as Low Level Laser Therapy [1], this was superseded by the Medical Subject Heading (MeSH) term ‘‘PBM’’ in 2015 [2]. PBM has been developed from a concept to received evidence-based accreditation through considerable research efforts. As a phenomenon, PBM therapy has been found to be beneficial in its effects toward cellular, local tissue, systemic, and biochemical processes, and it can significantly contribute as an adjunct in the resolution of pain and inflammation, together with the treatment of the pathophysiological processes of disease [3]; as such, it may be considered an attribute in achieving the delivery of ‘‘uneventful healing’’ that accompanies post-surgical, oral soft tissue laser therapy in clinical dentistry [4].

During soft tissue laser surgical therapy, attenuation of the incoming photonic energy at deeper and distant areas may be viewed as a function of the incident wavelength relative to the absorption and scattering coefficients and tissue anisotropy, along with other factors, including the temperature of irradiated tissues [5,6]. At a distance from the site

of tissue ablation, and along a combined thermal and scatter gradient, the reduction in applied laser fluence may deliver sub-ablative photonic energy density; with absorption by tissue components, a modulation process ensues, with examples of enhanced cellular activity, increased local vascular and lymphatic circulation, and analgesic effects that, combined, can promote a positive and advantageous healing process. The latter represents a combination of direct suppression of an inflammatory cascade, in addition to the facilitated optimization of conditions that are conducive to cellular repair and regeneration phenomena [7–9].

Through an ‘‘assumed’’ convention, the capacity of a relatively narrow bandwidth of visible and near infra-red (NIR) wavelengths to deliver tissue penetration and photon scatter has been denoted as the ‘‘optical window,’’ with emissions ranging from approx. 650nm to 1350 nm [10–13]. The NIR optical window may be computed from the absorption coefficient spectrum expressed relative to that of target tissue components [14]. The length of the optical pathway may be viewed as a function of multiple factors, including the refractive index of the tissue medium, the angle of incidence, and the wavelength itself, as well as parameters such as irradiance, surface spot size, and optical beam profile.

Hence, some questions remain regarding accountability for the degree of uneventful healing that may accompany surgical procedures when using laser wavelengths that are outside or remote from this optical window bandwidth.

This systematic review sets out to evaluate published articles related to para-surgical, post-surgical, and healing outcomes of dental laser applications whose emission wavelengths are shorter and longer than the optical window (650nm–1350 nm) range.

Prime criteria applied are randomized controlled human studies during a 10-year period that conform to an acceptable risk of bias, and they also represent post-surgical outcomes that have been independently evaluated by three of the review authors.

Materials and Methods

During the period April 28 to May 11, 2020, an electronic literature search was conducted through the search engines PubMed, Cochrane Database of Systematic Reviews, and Google Scholar. The applied keywords and their combinations were as follows:

(CO_2 OR carbon dioxide OR erbium OR Er:YAG OR Er,Cr:YSGG OR green OR KTP OR blue OR 445 OR 450 OR 532 OR visible) AND laser AND (soft tissue OR oral surgery OR lip OR tongue OR buccal mucosa OR granuloma OR gingivectomy OR biopsy OR leukoplakia OR lichen OR ankyloglossia OR frenectomy OR fibroma OR mucocele OR ranula OR gingiva hyperpigmentation OR gingiva depigmentation OR implant recovery OR second stage surgery).

In addition, the following MeSH Terms were employed: surgery, oral; lasers; ankyloglossia; gingiva; tongue; granuloma; ranula; mucocele; mouth mucosa; biopsy; leukoplakia; gingivectomy; fibroma; oral surgical procedures; erbium; lip; hyperpigmentation; carbon dioxide; lichen; and sub-heading: pathology.

From this, it could be arrived at that 8378 articles were first indicated before limitation to clinical, humans, 10 years, and from peer-reviewed journals in the English language only. After application of these selection criteria, this total number was reduced to 282 articles.

After removal of pilot studies, no clinical trials, in vitro studies, other medical fields (e.g., dermatology, general surgery), non-surgical dental fields [e.g., periodontology, endodontics, antimicrobial photodynamic therapy (aPDT)], three independent reviewers

further reduced the total to 58 articles. After removal of duplicates, 46 articles remained thereafter.

These articles were subject to full-text evaluation; studies with small sample sizes (<10 patients), no control group, case series, animal studies, and literature reviews were removed.

From 31 articles, non-randomized controlled trials were excluded, a process further reducing the final number of eligible articles to 25. Within this final number, 4 articles were related to the use of ‘‘blue/green’’ (445nm–532 nm), 12 related to a mid-IR group (2780nm–2940 nm), and 9 articles to a far-IR (9300nm–10,600 nm) group of wavelengths.

Within this number and range, the following inclusion and exclusion criteria were then strictly represented:

Inclusion criteria:

- laser wavelength outside the ‘‘optical window’’ (650nm–1350 nm) used as a light source;
- only randomized controlled clinical trials;
- ≥10 samples/participants per group;
- an applicable ‘‘control’’ group.

Exclusion criteria:

- dermal and/or general medical applications;
- duplicates or studies with the same ethical approval number;

- no control group;
- no randomized controlled clinical trials or pilot studies;
- low sample sizes.

In accordance with the PRISMA statement [15], details of the selection criteria are presented in *Figure. 1*.

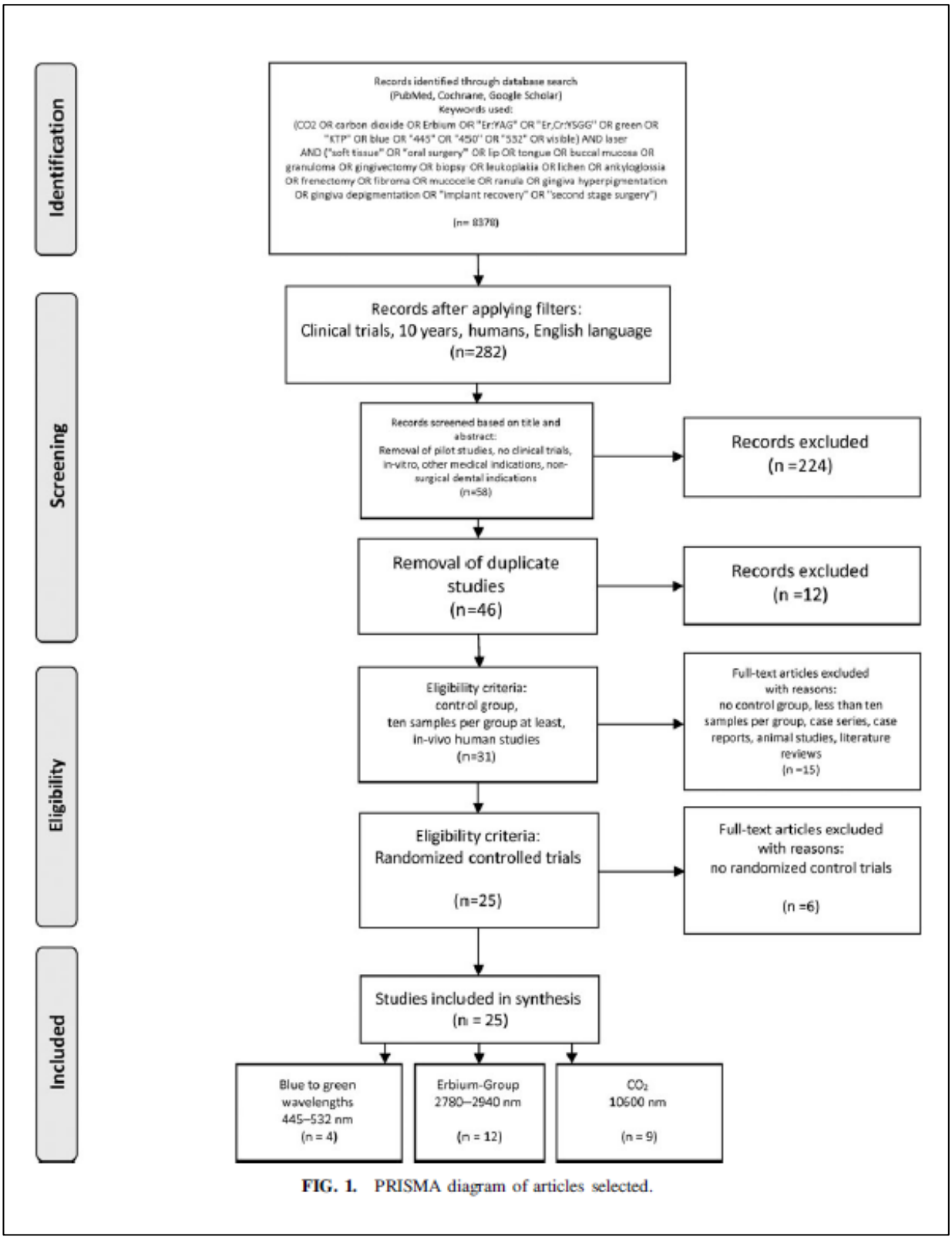

FIG. 1. PRISMA diagram of articles selected.

Figure #1. PRISMA diagram of articles selected.

Data extraction

Having reached an agreement regarding the selection of included articles, the three reviewers involved subsequently and independently extracted data regarding:

- Citation (first author and publication year);
- Test/control groups;
- Type of study/number of patients;
- Aim of study;
- Examined parameters;
- Laser parameters;
- Follow-up;
- Outcomes.

Quality assessment

During data extraction, the reliability and validity of the included articles were further evaluated by assessing their risk of bias adhering to the PRISMA statement [15]. The Cochrane Risk of Bias tool16 was modified according to the requirements of this systematic review.

The risk of bias was determined according to the number of ‘‘yes’’ or ‘‘no’’ responses to the parameters provided next, which were allocated to each study:

- Sufficient randomization?
- Sample size calculation and required sample numbers included?
- Baseline situation similar to that of the test group?

- Existence of blinding?
- Parameters of laser use described appropriately and correctly?
- Power meter used?
- Numerical results available (statistics)?
- No missing outcome data?
- All samples/patients completed the follow-up evaluation?
- Correct interpretation of data acquired?

The classification was performed according to the total number of ‘‘yes’’ answers to the questions just cited. For the current study, the degree of bias was computed according to the score limits provided next:

High risk: 0–4; moderate risk: 5–7; low risk: 8–10.

Results

Primary outcome

The primary goal of this systematic review was to critically appraise the treatment outcome regarding indices of healing and pain after oral soft tissue surgeries performed by laser wavelengths outside the 650–1350 nm optical window selection criterion.

Data presentation

The results related to combined Blue/Green and CO_2 wavelengths are grouped to recognize the similar inherent continuous wave (CW) emission modes. Details of each study are shown in *Tables 1 and 2*.

Table 1. Published Articles Identified from a Review of the Literature Regarding the Healing Outcome Effects

Citations	*Test/control*	*Study type/patients per group*	*Aim of study*	*Examined parameters*	*Laser operating parameters applied/"dose"*	*Follow up*	*Outcome*
Blue-green							
Gobbo et al.[17]	445 nm (1) 970 nm (2) scalpel (3)	RCT/ 39 patients (1) 27 patients (2) 27 patients (3)	Excisional biopsy of benign oral lesions	Pain (VAS) Bleeding Pain killer consumption Biopsy thermal damage (μm)	PP 2 W, t-on 20 ms and t-off 8 ms, AP 1.4 W, 320 μm fiber	30 days	Group 1 compared with both other groups: Pain: sign.diff. at 7 and 14 days ($p<0.05$) Bleeding: sign.diff. only at day 0 ($p<0.05$) Pain killer consumption: sign.diff. only at day 7 ($p<0.05$) Biopsy thermal damage: sign.diff. ($p<0.05$)
Rocca et al.[18]	450 nm (1) 2940 nm (2) 635 nm (3) 808 nm (4)	RCT/ 15 patients (1) 15 patients (2) 15 patients (3) 15 patients (4)	Aphthae management	Recurrent aphthous stomatitis Pain (VAS)	450 nm: AP 0.5 W CW 300 μm fiber, 2 cm distance, scanning mode on area of 1.5 cm diameter, 60 sec irradiance 0.28 W/cm^2, fluence 17 J/cm^2 2940 nm: 50 mJ, 20 Hz, 80 μs pulse, 1.3 mm tip, scanning mode on area of 1 cm diameter, 60 sec fluence 76.43 J/cm^2 no air no water	7 days	450 nm results similar to 808 nm with no significant difference between them. Er:YAG only significantly improved immediately after treatment (compared with earlier)
Romeo et al.[19]	532 nm	RCT 18 patients laser-only/ 31 patients laser+hyaluronic aminoacid compound	Role of laser+hyaluronic aminoacid compound in surgical biopsies wound healing	PHI NRS	1.5 W CW, 300 μm tip. Fluence of 2123 J/cm^2	7 days	PHI: laser+compound significantly better $p=0.0447$ NRS: no significant difference $p=0.77$
Bargiela-Pérez et al.[20]	532 nm (1) 980 nm (2)	RCT 532 nm 10 patients 980 nm 10 patients	Resection of benign hyperplastic lesions of the oral mucosa	NRS (1–5) for pain, scarring, inflammation, and consumption of drugs	532 nm 1.5 W CW, 320 μm fiber. PBM post-op 0.5 W, non-contact	28 days	532 nm: pain significantly worse at day 28 ($p=0.035$) Inflammation significantly worse at day 28 ($p=0.023$) Scarring and drug consumption no difference No significant differences between groups in any parameters for days 0, 1, and 14

(*continued*)

TABLE 1. (CONTINUED)

Citations	*Test/control*	*Study type/patients per group*	*Aim of study*	*Examined parameters*	*Laser operating parameters applied/"dose"*	*Follow up*	*Outcome*
CO_2							
Suter et al.[33]	10,600 nm CW/ 10,600 nm CF	RCT *N*= 60 patients 30 patients CO_2 CW 30 patients CO_2 cf mode. Surgical excision of buccal fibroma lesions	Excisional biopsies of fibrous hyperplasia	Time taken, complications, histopathologic collateral damage zones, pain (VAS), analgesics taken	(i) CW 5 W. 200 μm at 1–2 mm (ii) "Char Free mode" 4.62 W, 140 Hz, 400 μs pulse, 33 mJ, 200 μm spot, 1–2 mm distance	Pain VAS 3 days, 7 days. Histopathologic evaluation. Follow-up 2 weeks, 1 month	Post-operative complications: no significant difference (p 0.55). Histopathologic collateral damage zones no significant difference. No statistically significant difference between the VAS values. Analgesic intake (p= 0.23, not significant). No statistically significant correlation between TDZs and post-op VAS scores
Hegde et al.[34]	10,600/2780 nm/ scalpel	Split-mouth RCT. *N*=35 patients (140 sites: 10,600 nm 35 sites, 2780 nm 35 sites, surgical stripping 70 sites)	Gingival depigmentation	Pain, change in DOPI, Hedin index, and change in area of pigmentation from baseline to 6 months	CO_2 2–4 W CW, non-contact, defocused	1-, 3-, 6-month post-op.	Comparison of microscopic evaluation, VAS, and DOPI No significant differences between groups in re-pigmentation, changes in area of pigmentation, and changes in histologic parameters

Blue/green and CO_2.[17–20,33,34]

AP, average power; CF, char-free mode; CW, continuous wave; DOPI, Dummett oral pigmentation index; NRS, numeric rating scale; PBM, photobiomodulation; PHI, percentage healing index; PP, peak power; RCT, randomized clinical trial; TDZ, thermal damage zone; VAS, visual analogue scale.

Table 2. Published Articles Identified From a Review of the Literature Regarding the Healing Outcome Effects

Citations	*Test/control*	*Study type/patients per group*	*Aim of study*	*Examined parameters*	*Laser operating parameters applied/"dose"*	*Follow-up*	*Outcome*
CO_2							
Monteiro et al.[35]	10,600 nm multi λ lasers, scalpel, electrosur	RCT. $N=130$ patients 27 CO_2 (6 Gps—electroscalpel, cold scalpel, diode, Nd:YAG, Er:YAG and CO_2).	Excision of oral fibrous-epithelial lesions	Changes in histological mix of tissue, thermal extension, and degree of carbonization	4.0 W, 50 mJ, 80 Hz 500 μm spot, focused, Fluence 40.8 J/cm^2 power density 2040.8 W/cm^2	Histological study, no follow-up	CO_2 moderate improvement over scalpel. Most regular incisions among the lasers ($p=0.001$)
Suter et al.[36]	10,600 nm (CW gated/CF pulsed)	RCT. $N=100$ 49 patients CO_2 CW 51 patients CO_2 CF mode. Surgical excision of buccal fibroma lesions	Excision of oral fibrous hyperplasia	Post-op pain (2 weeks) VAS, analgesics, and post-op compl'ns, max. width of collateral thermal damage (mm) in excised tissue	(i) CW 5 W. 200 μm at 1–2 mm. (ii) "Char free mode" 4.62 W, 140 Hz, 400 μs pulse, 33 mJ, 200 μm spot, 1–2 mm distance	6 months. 78 re-attended 22 FTA	No significant differences between groups in post-operative pain VAS, post-operative complications, intraoperative bleeding, or thermal damage. More analgesics intake ($p=0.04$) in CW group More scars with CF ($p=0.03$).
Suter et al.[24]	10,600 nm/erbium YAG/scalpel	RCT $n=75$ lesions. CO_2 25 patients/2940 nm 25 patients/Scalpel 25 patients. Blinding. Sample size calculation Baseline similar	Excisional biopsies of fibrous hyperplasia	Duration, intra-op bleeding, need for bleeding control, post-op pain, post-op complications, need for analgesics, VAS, and TDZ	"Char Free mode" 4.62 W, 140 Hz, 400 μs pulse, 33 mJ, 200 μm spot, 1–2 mm distance	3, 7, 15 days, 6 months	CO_2-group: duration significantly less than scalpel control ($p<0.001$), bleeding sign less than in scalpel group ($p<0.001$). Need for sutures sign lower ($p<0.001$). Intra-op bleeding, Post-op pain, post-op complications, need for analgesics VAS: no significant differences TDZ significantly higher in CO_2 group ($p<0.001$) compared with 2940 nm

(*continued*)

TABLE 2. (CONTINUED)

Citations	Test/control	Study type/patients per group	Aim of study	Examined parameters	Laser operating parameters applied/"dose"	Follow-up	Outcome
López-Jornet et al.[37]	10,600 nm cf cold knife	RCT. 10,600 nm (20 patients) cold knife (28 patients)	Excision of oral leukoplakia	VAS for pain VAS for swelling	5–15 W CW, 15 mm distance, defocused, 5–15 sec	7 days	CO_2 group significant difference in pain and swelling, respectively: 12 h ($p = 0.001$, $p < 0.001$) 1 days ($p = 0.001$, $p = 0.008$) 2 days ($p = 0.003$, $p = 0.007$) 3 days ($p = 0.029$, $p = 0.019$)
Chee et al.[38]	10,600 nm cf scalpel	RCT. 10,600 nm (24 procedures) scalpel (23 procedures)	Excision of oral leukoplakia	Time, blood loss, bipolar cautery use, and intraoperative margins needed	10 W CW	Ongoing	10,600 nm group significantly better in: blood loss and need for bipolar cautery ($p < 0.05$) intraoperative margins ($p = 0.03$) No difference in operation time
Karimi et al.[39]	10,600 nm cf scalpel	RCT split-mouth (19 patients)	Excision of epulis fissuratum	Time, bleeding, vestibular depth, re-epithelialization, and edema	AP 6.2 W, 50 Hz, pulse duration 3 ms, 0.7 mm spot, focused	7, 14 days	10,600 nm group: less surgery time ($p = 0.002$) less bleeding ($p < 0.001$) more vestibular depth (medial $p = 0.076$/ middle $p = 0.018$/ lateral $p = 0.005$) less re-epithelialization at day 7 ($p = 0.021$), no difference at day 14 ($p = 0.18$) edema no difference ($p = 0$)
Agha-Hosseini et al.[40]	10,600 nm cf LLLT (890 + 633 nm)	RCT 10,600 nm (13 patients with 27 lesions) LLLT (15 patients with 30 lesions)	Treatment of oral lichen planus	Lesion size (scaled tongue blade) Pain (VAS) Clinical sign (Thongprasom)	3 W, defocused	2 weeks, and at 1, 2 and 3 months	10,600 nm group significantly worse at all time intervals in all examined parameters ($p < 0.05$)

CO_2: [35–40]
FTA, failed to affend; Gps, groups; LLLT, low level laser therapy.

Information acquired related to the erbium chromium YSGG and erbium YAG (free running pulsed [FRP] emission mode) is shown in *Tables 3 and 4*.

Table 3. Published Articles Identified from a Review of the Literature Regarding the Healing Outcome Effects of Mid-Infra-Red Surgical Laser Use

Citations	*Test/control*	*Study type/patients per group*	*Aim of study*	*Examined parameters*	*Laser operating parameters applied/"dose"*	*Follow up*	*Outcome*
Er,Cr:YSGG Er:YAG							
Giannelli et al.[21]	2940/810 nm	Split-mouth RCT. $n=21$ patients 1 Q 2940 nm/1 Q 810 nm	Gingival depigmentation	Wound healing, bleeding, pain on a scale 0–3 Histology of 13 samples	2940 nm, 100 mJ 10 Hz, 400 μs pulse width, 1.0 W AP, 800 μm tip, in contact, 2.5 mm/s, Fluence 50 J/cm^2	7, 30, 180 days	Pain during treatment $p<0.005$, on day of treatment $p<0.001$, and until day 7 $p<0.001$ significantly higher in 2940 nm group. Healing time and bleeding significantly higher in 2940 nm group, $p<0.001$
Alhabashneh et al.[22]	2940 nm/scalpel	Split-mouth blinded prospective clinical trial 20 patients	Gingival depigmentation	DOPI, HMI, hemostasis, time, pain (NRS), Wound healing (degree of epithelialization), tx time, level of satisfaction	1.0 W AP, 800 μm tip, 5 mm distance, brush stroke movement, 50% air/water	1, 2 weeks, 1, 3, and 6 months	DOPI, HMI, tx time, pain, degree of epithelialization, patient preference, and satisfaction with non-significant differences at any timepoint. Bleeding statistically significant higher in scalpel group $p<0.001$
Ipek et al.[23]	2940 nm/Kirkland knife	Split-mouth RCT 20 patients	Gingival depigmentation	Tissue injury and inflammation by osmotic pressure changes (OP), pain (VAS)	2 W AP, 200 mJ, 10 Hz, 1000 μs pulse. 1300 μm tip at 1 mm. Brush technique	2, 8 h, daily until day 7	Test group significantly better in: VAS 2 h ($p<0.001$), 8 h ($p<0.001$), 1 day ($p<0.05$), 2 days ($p<0.05$) OP: no statistical differences between groups
Suter et al.[24]	2940 nm/CO_2/scalpel	RCT $n=75$ 2940 nm 25 patients/CO_2 25 patients/scalpel 25 patients	Excisional biopsies of fibrous hyperplasias	Duration, intra-op bleeding, need for bleeding control, post-op pain, post-op complications, need for analgesics, VAS, TDZ	7 W AP, 200 mJ, 35 Hz, 297 μs pulse, 400 μm tip, non-contact, Water 22.5 mL/min	3, 7, 15 days	2940 nm-group. Duration significantly less than scalpel control ($p=0.003$), need for electrocauter bleeding control significantly higher in 2940 nm-group ($p<0.001$), Need for sutures sign lower ($p<0.001$). Intra-op bleeding, post-op pain, post-op complications, need for analgesics, VAS: no significant differences. TDZ significantly lower in 2940 nm group ($p<0.001$) compared with CO_2

(*continued*)

TABLE 3. (CONTINUED)

Citations	*Test/control*	*Study type/patients per group*	*Aim of study*	*Examined parameters*	*Laser operating parameters applied/"dose"*	*Follow up*	*Outcome*
Suter et al.[25]	2940 nm/CO_2	RCT 31 patients 2940 nm 16/CO_2 15	Excisional biopsies of fibrous hyperplasias	Duration, intra-op bleeding, need for bleeding control, post-op pain, post-op complications, need for analgesics, VAS, TDZ	7 W AP, 200 mJ, 35 Hz, 297 μs pulse, 400 μm tip, non-contact, water 22.5 mL/min	1, 7, 15 days	Intra-op bleeding significantly higher in 2940 nm group $p=0.007$, need for electrocauterization $p=0.015$, No significant differences in duration of surgery, post-op pain, post-op complications, need for analgesics, VAS. TDZ significantly lower in 2940 nm group ($p<0.0001$)
Broccoletti et al.[26]	2940 nm/scalpel	RCT $n=344$ patients 2940 nm 173 lesions/scalpel 221 lesions	Excision of non-dysplastic white oral lesions	Pain (VAS), duration of surgery, QOL test, Oral Health Impact Profile, number of analgesics	1.5 W AP 150 mJ 10 Hz 500 μs pulse. 900 μm spot, distance 2 mm	3 days, 1 week	2940 nm significant difference in duration of surgery-shorter ($p<0.001$), VAS lower d3 $p=0.005$ QOL better $p=0.038$ Analgesics in lesions >1 cm $p=0.026$
Arduino et al.[27]	2940 nm/scalpel	RCT $n=117$ lesions 2940 nm 59 lesions, scalpel 58 lesions	Excision of non-dysplastic oral leukoplakia	Healing, recurrence	1.5 W AP 150 mJ 10 Hz 500 μs pulse. 900 μm spot	Every 6 months for 5 years	No significant difference between groups
Aras et al.[28]	2940 nm/diode 808 nm	RCT $n=16$ patients 2940 nm 8 patients/808 nm 8 patients	Lingual frenectomy	Pain level and post-surgical discomfort (5-point Likert-type scale) Requirement for local anesthesia	1.0 W AP, 80% air no water	7 days	2940 nm in the first 3 h significantly higher pain $p=0.005$ No significant difference in any other timepoint Post-surgical discomfort: no significant difference in any other timepoint No statistics for requirement for local anesthesia

References.[21–28]
HMI, hedin melanin index; OP, osmotic pressure; QOL, quality of life.

Table 4. Published Articles Identified from a Review of the Literature Regarding the Healing Outcome Effects of Mid-Infra-Red Surgical Laser Use

Citations	*Test/control*	*Study type/patients per group*	*Aim of study*	*Examined parameters*	*Laser operating parameters applied/"dose"*	*Follow-up*	*Outcome*
Er,Cr:YSGG Er:YAG							
Eroglu et al.[29]	2780 nm/scalpel	RCT $n=44$ cases 2780 nm 22 lesions/scalpel 22 lesions	Excision of epulis fissuratum	Pain (VAS) Total wound surface (Bates–Jensen Wound Assessment Tool), edema, healing erythema, suppuration	Ablation: 2.75 W AP 75 Hz 140 μs pulse Air 20/water 40. 550 μm tip. 1 mm spot, slight contact Hemostasis: 1.5 W AP, 50 Hz, 700 μs pulse, air 20/water 0. 660 μm tip. 1 mm spot	2, 7, 14, and 30 days	
Gholami et al.[30]	2780 nm two settings/scalpel	RCT $n=66$ treated sites 2780 nm setting 1 22 sites/ 2780 nm setting 2 22 sites/ Scalpel 22 sites	Gingival depigmentation	Pain (VAS) Patient satisfaction, Gingival wound healing, relapse (DOPI Hedin indices)	2780 nm setting 1 4.5 W, 50 Hz, 60 μs pulse, 80% air, 17 mL/min water, 800 μm tip in contact, brush strokes 2780 nm setting 2 2.5 W, 50 Hz, 700 μs pulse, 40% air, 9 mL/min water, 800 μm tip in contact, brush strokes	VAS 1 days 7 days Patient satisfaction 7 days Wound healing	No significant difference between groups in pain, patient satisfaction at any timepoint. Healing laser groups better $p=0.05$ at day 1, $p=0.001$ at day 7 30 days no significant difference. Relapse, no significant difference between groups at 12 months
Pié-Sánchez et al. [31]	2780/10,600 nm	RCT 50 patients 2780 nm 25 patients, 10,600 nm 25 patients	Upper lip frenectomy	Surgical bleeding Time required Healing Relapse	1.5 W AP. 20 Hz, 75 mJ, pulse width 140 μs, 12% water and 8% air, spot 0.6 mm, 600 μm tip, straight handpiece. Fluence 26.54 J/cm^2. Mean irradiation time 77 sec	7, 14, and 21 days and 4 months	No statistical analysis 2780 nm: less hemostatic effect than 10,600 nm., longer time required, healing faster. No relapse for any group after 4 months
Tunc et al.[32]	2780/980 nm diode	RCT, X-sectional study, 40 patients 2780 nm 51 implants/ 980 nm 50 implants	Second-stage implant surgery	Surgery time, bleeding, VAS, post-op complications	2.00 W AP, 550 μm tip, 100 Hz, 20 mJ 140 μs pulse, 10% water, and 10% air	3 days. Onward restorative treatment	Overall no statistically significant differences in time, bleeding, VAS, and complications. Surgery (p 0.7). VAS ($p=0.6$), swelling ($p=1.0$)

References.[29–32]

Table 5. Risk of Bias of Articles Selected

Citations	Randomization	Sample size calculation and required number included	Baseline situation similar	Blinding	Parameters of laser use described appropriately and calculations correct	Power-meter used	Numerical results available (statistics)	No missing outcome data	All samples/ patients completed the follow-up	Correct inter-pretation of data	Total score/10
Blue green 4 articles											
Gobbo et al.[17]	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	8
Rocca et al.[18]	Yes	No	Yes	No	Yes	No	No	Yes	Yes	Yes	6
Romeo et al.[19]	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	6
Bargiela-Pérez al.[20]	Yes	No	No	No	No	No	Yes	Yes	Yes	Yes	5
Erbium group 12 articles											
Giannelli et al.[21]	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	8
Alhabashneh et al.[22]	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	7
Ipek et al.[23]	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	Yes	7
Suter et al.[24]	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	9
Suter et al.[25]	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	8
Broccoletti et al.[26]	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	9
Arduino et al.[27]	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	7
Arias et al.[28]	Yes	No	No	No	No	No	Yes	Yes	Yes	Yes	5
Eroglu et al.[29]	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	8
Gholami et al.[30]	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	8
Pié-Sánchez et al.[31]	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes	Yes	7
Tunc et al.[32]	Yes	No	Yes	No	No	No	Yes	Yes	Yes	Yes	6
CO_2 group 9 articles											
Suter et al.[33]	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	8
Hegde et al.[34]	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	7
Monteiro et al.[35]	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	8
Suter et al.[36]	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	8
Suter et al.[24]	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	9
López-Jornet et al.[37]	Yes	No	Yes	No	No	No	Yes	Yes	Yes	Yes	6
Chee et al.[38]	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	7
Karimi et al.[39]	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	No	6
Agha-Hosseini et al.[40]	Yes	No	Yes	No	No	No	Yes	Yes	Yes	Yes	6

Quality assessment presentation

The risk of bias of the included studies is presented in *Table 5.* In consideration of the factors determined by the authors to indicate scientific rigor, the risk was valued as

high risk: 0–4; moderate risk: 5–7; low risk: 8–10.

In total, 11 out of 25 (44% of) articles showed a low risk of bias with the following gradings:

- 9/10 score—3 articles [24,24,26] (Ref.24 in Erbium and CO2 groups)
- 8/10 score—8 articles [17,21,25,29,30,33,35,36]

Overall, 14 out of 25 (56%) articles, respectively, showed a medium risk of bias with the following gradings:

- 7/10 score—6 articles]22,23,27,31,34,38]
- 6/10 score—6 articles [18,19,32,37,39,40]
- 5/10 score—2 articles [20,28]

As far as the risk factor that gained the most common negative answers, the use of a power meter was recorded in only one of the studies, and only five of them performed sample size calculation and included the required number of participants. Regarding the correct and complete description of the laser protocol applied, 15 out of 25 studies had indicated it appropriately.

Analysis of data

Analysis of the studies was performed to evaluate the treatment outcome per each wavelength, with reference to the respective control group:

- Blue (445 nm) compared with:

Diodes (808nm and 970 nm): 2/2 positive [17,18]

- Green (532 nm) compared with:

  Diode 980 nm: 1/1 positive for the first 14 days [19]

  532 nm + hyaluronic acid compound: 1/1 negative in healing, and no difference in pain [20]

- Erbium (2940nm or 2780 nm) compared with:

  Scalpel: 3/7 positive, [24,26,29]

  4/7 no difference [22,23,27,30]

  Diodes (808nm, 810nm and 980 nm):

  2/3 positive, [21,32 1/3] negative [28]

  CO_2 (10,600 nm): 1/2 positive, [24] 1/2 no difference [25]

- CO_2 (10,600 nm) compared with:

  Scalpel: 3/6 positive, [24,37,38]

  2/6 no difference, [34,39]

  1/6 negative [35]

  Low level laser therapy (LLLT) (890nm and 633 nm):

  1/1 negative [40]

  CW versus gated mode: 2/2 no difference [33,36]

In total, 6 out of 13 (46% of) studies comparing laser wavelengths with scalpel treatment showed positive results, whereas 6 out of 13 (46%) reported no difference and only 1 out of 13 (8%) presented negative outcomes.

In addition, 5 out of 6 (83% of) studies comparing laser wavelengths with other diodes (808nm–980 nm) showed positive results, whereas 1 out of 6 (17%) had negative outcomes.

Regarding the laser protocol applied, 10 out of 25 (40%) articles reported an incomplete laser parameter description [19,20,22,27,28,32,34,37,38,40]. The deficiencies concerned the following parameters:

- Average Power delivered: 1/10 articles [38]
- Tip or spot size: 5/10 articles [28,34,37,38,40]
- Gated/pulsed frequency: 3/10 articles [28,34,37]
- Fluence/irradiance (either missing or wrongly calculated): 6/10 articles [20,28,34,37,38,40]
- Total energy delivered: 8/10 articles [20,22,28,32,34,37,38,40]
- Tip-to-tissue distance: 7/10 articles [19,20,27,28,34,38,40]
- Gated/pulse duration: 3/10 articles [19,20,22]
- Irradiation time: 7/10 articles. [27,28,32,34,37,38,40]

Discussion

This systematic review has applied strict evaluation and risk-of-bias criteria to identify the quality and quantity of outcomes from laser-assisted oral soft tissue surgery, and to explore those outcomes that might be interpreted as examples of non-ablated tissue PBM effects.

It was noted that the specifics of laser parametry and energy delivered was unclear in many of the articles examined. Similar deficiencies were found in the selected articles within this investigation, such as power meter used, fluence, irradiation, and total energy delivered. In addition, it was challenging to establish accurate values for the outcomes that were examined. Without a risk of bias and consideration of probability values related

to statistical significance, a meaningful interpretation of results would have been difficult. An earlier article [41] by the authors in this group highlighted the significant disparity in reported parameters within published articles focused on PBM.

However, it is encouraging from the results that 46% of the post-operative observations were positive in relation to laser versus scalpel treatment, and compared with other diode lasers (808–980 nm), this respective percentage was 83%. It may be possible to explore the reasons for such results in terms of laser-tissue interactions as a function of varying wavelengths applied.

Laser (and other light) therapies are effective, and their benefits are based on the principle of inducing a biological response through energy transfer [42]. This applies to both surgical (ablative) therapy and sub-ablative PBM therapy.

The optimal photonic dose for oral soft tissue surgical management (photothermal ablation/incision) must be the subject of recommended settings and power meter confirmation. In addition, with NIR ‘‘hot tip’’ delivery, sufficient attenuation of the beam must be achieved to not only enable the tip temperature but also allow sufficient wavelength-specific photons to enter the tissue to achieve PBM [43]. Through anecdotal investigation, the authors estimate an optimal onward transmission of 15%. Photothermolysis should also represent a measured function of tissue type, laser wavelength relative to chromophore/absorptive tissue bimolecular components, and a deliverance of energy concentration to achieve efficient tissue ablation with minimum risk of collateral thermal damage. Average power, peak power, energy and power densities, exposure time, and, at some distance from the tissue zone of maximum photothermolysis and ablation (along a thermal and scatter gradient), the applied value of

fluence will diminish and tissue temperature will reduce to a level where dose parameters for PBM effects may occur [44]; therapies are usually within the range of 2–10 J/cm^2 for enhanced qualities of healing, with a higher range of 10–30 J/cm^2 for laser-induced analgesia [45].

Within research limited to dental and oral applications, an optical window has been understood to be between 650nm and 1350nm, without doubt founded on important existential phenomena of absorption and scatter effects of these wavelengths within oral soft tissues [46]. The predominance of investigations into laser-assisted surgical procedures with a generic ‘‘diode’’ laser offers an extremely high number of publications that are referenced in the PRISMA diagram related to the >650 nm wavelength range. Clearly, this fails to offer explanations regarding reported similar healing benefits when using laser wavelengths that are both shorter and longer than those represented by the NIR optical window.

With accepted opinion related to the delivery of PBM effects with near IR wavelengths, a question regarding what mechanisms might pertain in promoting positive uneventful healing phenomena when using shorter and longer wavelengths is therefore posed.

Shorter wavelengths of 400nm–600 nm have relatively low optical transmission within tissues. This is a consequence of absorption by chromophoric proteins as well as further colored agents, particularly those containing transition metal ions such as those of iron and copper, and to a lesser extent, manganese. The effects of absorption are dose related and can range from protein denaturation to mild transient hyperthermia [47]. In surgical mode, a small optical spot size is applied typically in contact with the tissues. The high radiant exposure and localized absorption results in tissue destruction by vaporization

and/or coagulation, or at even higher settings carbonization. In the immediate periphery of the zone of destruction, there can be sufficient localized heat to generate collagen contraction, which results in capillary closure. Dependent on operator technique, this can produce a relatively narrow zone of collateral tissue damage with excellent hemostasis. Also, in view of the high localized thermal zone, there is an area of disinfection since most bacteria, viruses, and fungi are sensitive to temperatures elevated above 50^0C. Consequently, beyond the immediate wound edge, there is an interstitial zone of sterile coagulation that acts as what may be termed a ‘‘laser bandage.’’ Beyond this zone of coagulation, however, the vital tissues respond with a classic tissue damage cascade, and as previously noted, there is an activation of the immune system producing an initiation of the cycle of wound damage mitigation, inflammation, primary wound matrix deposition, healing, and finally wound remodelling to the point of regeneration under optimal conditions [48].

Longer wavelengths in the mid-IR range of 2780nm–3000 nm are strongly absorbed by water. Since biological tissues are water rich, these wavelengths permit only a shallow depth of field surgery. If adequate superficial cooling air/water sprays dissipate heat accumulation, the zone of collateral destruction beyond the area of tissue disruption is of the order of up to 50 μm after explosive ablation and spallation. This type of tissue cutting is a differential affair to that associated with either the short or near IR wavelengths that primarily cut via photothermolysis. This phenomenon arises from the use of FRP laser devices with ultra-short pulse durations, along with exceptionally high peak power with the erbium-type laser sources. Since chemical -O-H functions are highly responsive to photo-excitation at these wavelengths, there is a process of phase transition from water to steam within microseconds of exposure. The consequent rapid volumetric change of the

expanded area of superheated vapor within a contained area, therefore, produces an explosive tissue disruption [49,50]. The fragmented target tissues are removed by exposure to an adjunct water spray and high-volume evacuation. Consequently, there is remarkably little heat diffusion to the deeper layers of the collateral tissues. Subject again to good operator technique and the informed use of appropriate parameters, the area of collateral tissue damage may be minimal; moreover, there can again be a highly localized tissue rim that is disinfected and coagulated again with an associated vasoconstriction to support hemostasis. In addition, there can be a localized rim of tissue that is disinfected and coagulated with an associated vasoconstriction to support hemostasis.

Given the reduced optical penetration of both the shorter and the longer wavelengths of applied photonic energy, it is interesting to consider the multiple processes that may explain the mitigation of post-operative pain and swelling. Further, there is some evidence of an enhanced quality of repair; for example, Kesler *et al* [51]. in an animal study identified a sustained increase in platelet-derived growth factor in osseous tissues exposed to an erbium laser wavelength of 2940 nm, and Pourzarandian *et al* [52]. reported that COX-2 gene expression and PGE-2 concentration increased in an output dependent manner by irradiating human fibroblasts (*in vitro*) with an Er:YAG laser system. In addition, a report by Lubart [53] claimed that an Er-YAG laser system dissociates water and generates -OH radicals, possibly via an intermolecular vibrational (V-V) energy transfer in water, which competed with vibrational relaxation and was dependent on the pulse repetition rate and energy density per laser pulse. At low concentrations of such reactive oxygen species (ROS), fibroblast stimulation may cause collagen and extracellular matrix formation. Therefore, ROS formation may be a contributory factor featured in the wound-healing effect of erbium lasers in dentistry.

In consequence, it was the purpose of this review to examine the evidence base to critically evaluate and identify the nature of any added value of clinical laser integration into practice, and also to facilitate the highlighting of any areas worthy of consideration for future research into the mechanisms of PBM. The acronym ‘‘q-PBM’’—that is, quasi-PBM [54]—has been suggested to possibly explain a post-surgery tissue response that mimics that achieved within the ‘‘optical window,’’ and also to question the cellular and biochemical processes that may be stimulated by these longer and shorter wavelengths involved.

In providing surgery for the treatment of oral soft tissue pathology and cosmetic alterations, the dental professional has an obligation to maintain a positive benefit–risk ratio. The use of laser photonic energy of appropriate applied wavelength, dose, and timing has been shown to offer benefits during surgical procedures, and also to the patient during the early healing period. Indeed, there are considerable published data available to support the effectiveness of incisional hemostasis, and the development of a post-surgical surface coagulum [55–60] when using a range of surgical laser wavelengths, although a predominance of investigations into‘‘uneventful’’ healing has centered on the IR wavelengths that fall within the 650nm–1350nm optical window. Significantly, PBM is also considered an attribute to the delivery of ‘‘uneventful healing’’ that accompanies post-surgical laser therapy within clinical dentistry [4]. However, to date, the processes associated with these benefits are not fully understood. As stated earlier, at a distance from the site of tissue ablation, along a combined thermal and scatter gradient, there may be a direct influence of sub-ablative photonic energy density during a modulation process of enhanced cellular activity, increased local vascular and lymphatic circulation, and analgesic effects that, combined, can promote a positive and advantageous healing

process. The latter represents a combination of direct suppression of an inflammatory cascade, in addition to the facilitated optimization of conditions that are conducive to cellular repair and regeneration phenomena [6–8].

With the development of lasers as adjunctive surgical instruments, the benefits of each wavelength range within the series of commercially available dental lasers available have been summarized to represent one or more of the following [61]:

Precision/control of non-linear incisions: relative to width/depth of ablation

Incisional hemostasis: relative to laser wavelength

Selective ablation: relative to tissue composition

Pathogen control: incisional pathogen reduction/wound protection through post-ablation coagulum

Hard/soft tissue treatment harmonization: co-treatment, stage harmonization

Positive healing phenomena

As has been adequately described in the studies evaluated, such terminology is used to describe the para- and post-surgical outcomes of surgical laser-tissue interaction. Critical to photothermolysis, a thermal threshold exists within the tissue, below which the incident photonic energy values are insufficient to initiate structural disruption, a process resulting in warming, and below a sustained threshold of 45.5^{0}C, tissues are not irreparably harmed [62,63], with the potential for beneficial positive changes in cellular structures and biochemical processes [64]. In addition, with the use of red visible and NIR laser wavelengths, photon attenuation with a distance from the site of application will result in diminished irradiance to a point below that required to achieve the ablation threshold of

the tissue, yet it also allows the absorption of energy by cellular structures, resulting in collateral PBM remote from the zone of tissular ablation [65].

With all incident wavelengths, laser-assisted soft tissue surgery (tissue injury) prompts a succession of responses and reactions in the host tissue that may be summarized as the wounding, inflammation, proliferation, and remodelling phases. The primary wound and bleeding prompt the trigger of a coagulation cascade, and activation of the complement system and the kinin cascade [66,67]. The cellular response after wounding begins early, showing considerable changes already at 12–24h post-surgery, and from a clinical viewpoint, healing during the first post-operative days is crucial for the maintenance of wound stability and subsequent successful treatment outcomes [68].

Conclusions

A detailed and blinded examination of published studies has been undertaken, applying strict criteria to demonstrate result data that suggest positive, or at worst neutral comparatives when a given laser wavelength is used against an alternative control therapy. With reference to the number of published articles examined, a significant improvement in addressing risk of bias and in reporting laser operating parameters would enable a wider range of published data to be examined within the strict criteria of this systematic review. As such, a substantiated evidence of laser surgery in delivering uneventful healing and analgesic effects, as an expression of PBM-like (quasi-PBM) influence, has been shown. From this series of investigations, a greater understanding of molecular, cellular, and regional tissue responses to applied energy at these wider wavelength ranges is sought. A summation of investigations of currently commercially available laser wavelengths in dentistry may then allow a more-inclusive meld of PBM effects within the current optical window, together with an adoption of a descriptive q-

PBM to explain similar outcome effects of wider laser wavelength use in oral surgery. In this way, a desired outcome of understanding of soft tissue laser-tissue interaction, across the current therapeutic electromagnetic spectrum, may evolve.

This systematic review supports a belief that through multiple biophysical and host cellular and biochemical responses, oral soft tissues are positively modulated through laser use.

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4.5 Cause for concern?

From the contents of Paper IV there is opportunity to evaluate the implication that across the three groups of laser wavelengths, some interpretation of assessment of "uneventful" healing might be connected with contemporal, but sub-ablative biomodulation. This proved difficult since although consistent with so many systematic reviews, the lack of full operating parameters is both significant and disappointing. Of the 25 peer-reviewed evidence-based RCT papers examined, the following, taken from Paper IV, offer some extent of the data, which is considered essential to the reproducibility of each of 10 papers in developing treatment protocols where parameters were absent:

The deficiencies concerned the following parameters:

- Average Power delivered: 1/10 articles
- Tip or spot size: 5/10 articles
- Gated/pulsed frequency: 3/10 articles
- Fluence/irradiance (either missing or wrongly calculated): 6/10 articles
- Total energy delivered: 8/10 articles
- Tip-to-tissue distance: 7/10 articles
- Gated/pulse duration: 3/10 articles
- Irradiation time: 7/10 articles.

In that the significance of fluence, irradiation time and total energy delivered have already been established, the concept of "apple pie" reproducibility is severely compromised through the extent and identity of such omissions.

This lack of fulsome recording would undoubtedly exert an influence on the clinical methodology being proposed through the study being evaluated, and also would bring doubt regarding the value of conclusions being drawn from each study.

The outcome of surgical laser tissue manipulation will depend on several factors, each of which (or together collectively) may significantly affect the degree of interaction. Tissue composition, vascularity and bulk (thickness) will define optical properties and essential absorptive potential when expressed relative to the incident laser wavelength, but also the capacity to withstand the degree of thermal rise through photothermolysis.

Aspects of variable parameters will be affected by the incident angle of beam and any surface wetness attributable to saliva, which may predispose to beam reflection and non-absorption. Contact *vs* non-contact modes of delivery may affect the accuracy of the laser beam, its power density (W/cm^2), and depth of penetration (λ specific). Contact mode is commonly found with visible / near IR surgical irradiation; disadvantages of this mode of application may include a risk of "hot-tip" effects, and the possibility of direct thermal damage or conductive thermal effects from the build-up and carbonisation of adherent ablation tissue debris. Inexperienced use of a quartz optic fibre in contact with the target tissue may lead to a risk of "scalpel" action.

Laser light emitting from an optic fibre will undergo divergence over distance, through degrees of divergence of 10 – 20%. Laser light emitting from a simple fibre conduit handpiece will demand a close apposition to the target, in order to maximise accuracy. Alternatively, the beam can be focussed to a fixed distance where the handpiece is designed as non-contact beam delivery. Additionally, increased power density within a given spot area will lead to faster ablation, possible deeper penetration (relative to tissue

optical properties / λ), and reduced width of cut; reduced power density conversely provides slower ablation and a reduced level of penetration and may increase the width of cut in view of collateral thermal effects of increased irradiation time.

Excessive delivery of laser photonic power over extended time predisposes to tissue carbonisation; this surface interface will distort laser – tissue interactions and may cause thermal damage, post-operative tissue deformation and pain. Within the concept of photonic density per chosen beam area (spot size) an ascending level of thermally-induced changes may be observed (*Table #1*):

Energy Density (J/cm^2)	**Tissue Effect**	**Approximate Tissue temperature. (^{0}C)**
10	Warming	40
200	Coagulation	60
500	Welding	80
1000	Vaporisation	100
1000-5000	Carbonisation	200
>5000	Rapid cutting	>200 with thermal containment

Table #1. Correlation between applied radiant exposure (energy density) of ascending value with observed effects in oral soft tissue. Ref. Tuchin, V. [176].

As an example, delivery of FRP near-IR irradiation, using a 300µm diameter quartz fibre and pulsed output of 100mj/pulse will deliver 141 J cm^{-2} per pulse. With a repetition rate of 50 Hz, the energy density (radiant exposure) will climb to 7,050 J cm^{-2}, with consequent rapid cutting potential, but also with a significant risk of carbonisation.

Excess thermal rise, achievable with even modest laser energy output but compounded through beam manipulation (as discussed above) may be reduced and mitigated through thermal relaxation measures, as listed in *Table #2*:

Thermal Relaxation Influencing Factors		
Laser Absorption Characteristics	Laser Emission Mode	Duty Cycle
Laser Incident Power (Watts – Joules Sec^{-1})	Laser Power Density (Watts Cm^{-2})	Beam Movement Relative to Tissue Site
Time	Endogenous Coolant (Blood Flow)	Exogenous Coolant (Water, Air, Pre-cooling of tissue)

Table #2. Thermal relaxation. Laser photonic variables that may exert an influence on mitigating excessive thermal rises in irradiated target tissues.

Careful consideration should be given to optimise the cutting dynamics of an applied wavelength to a chosen surgical site, in order to embrace the physical limitations of photonic density and how they might come to influence the achievement of successful surgical management. The extensive number of variables together with the inherent risks associated with the omission of so many of them within published papers, underlines the importance of the subject and the obligation of *"Primum non nocere"* – first do no harm.

The fundamental aspect of this concern is the degree to which correct operating variables contribute to optimal co-existent sub-ablative biomodulation effects.

As discussed, the early investigations and hypotheses centred around the readily available visible-red and NIR laser wavelengths. The primary intracellular and secondary biochemical pathways and gene transcription leading to RNA and DNA activation, drew upon the specific matching of visible-red (~650nm) photonic energy to elements of the mitochondrial membrane electron transport chain, a process initiating the redox elements of ATP, ROS and $NO^{\bullet}$ production. Of course, the two basic points not addressed through this early research remain: specifically the apparent similarity in post-surgical oral soft tissue with emerging short and long wavelength laser use, together with PBM-like activity

with the wide range of dissimilar cell lines within target structural anisotropic and turbid tissue.

The details of mitochondrial ETC have been summarised on page #90 and specific to activity, areas of PBM-promoted effects may be identified. As a result of wavelength specific PBM-dissociated cytochrome activity (complexes IV and V), ATP production is increased, together with the release of $NO^{\bullet}$ through the redox processes at the cytochrome c oxidase (CCO) ligands for its Fe/Cu metal ions. Through these processes, synthesis of RNA and DNA is promoted and $NO^{\bullet}$-induced cell membrane and local vessel dilatation is advanced; local tissue cell repair and regeneration are positively influenced.

PBM dissociated ROS activity influences gene expression, together with stimulated production of activator protein (AP-1), nuclear factor kappa-B (NF-kB) and transforming growth factor beta-1 (TGF-β1). Within the early stages of wound stability and healing, PBM-promoted effects may be observed as a stimulation of cytokines and growth factors, platelet / coagulation pathways, angiogenesis and neovascularisation. Epithelial and endothelial structural cellular stimulation leads to proliferation and degranulation of mast cells, oral keratinococytes and fibroblasts, together with re-epithelialisation and early wound coverage. This is summarised in *Figure #6*. The balance and intensity of effects may reflect the multifactorial influence of tissue optical properties, applied and effective fluence, and the exposure time and frequency of application, together with the wavelength of applied irradiation.

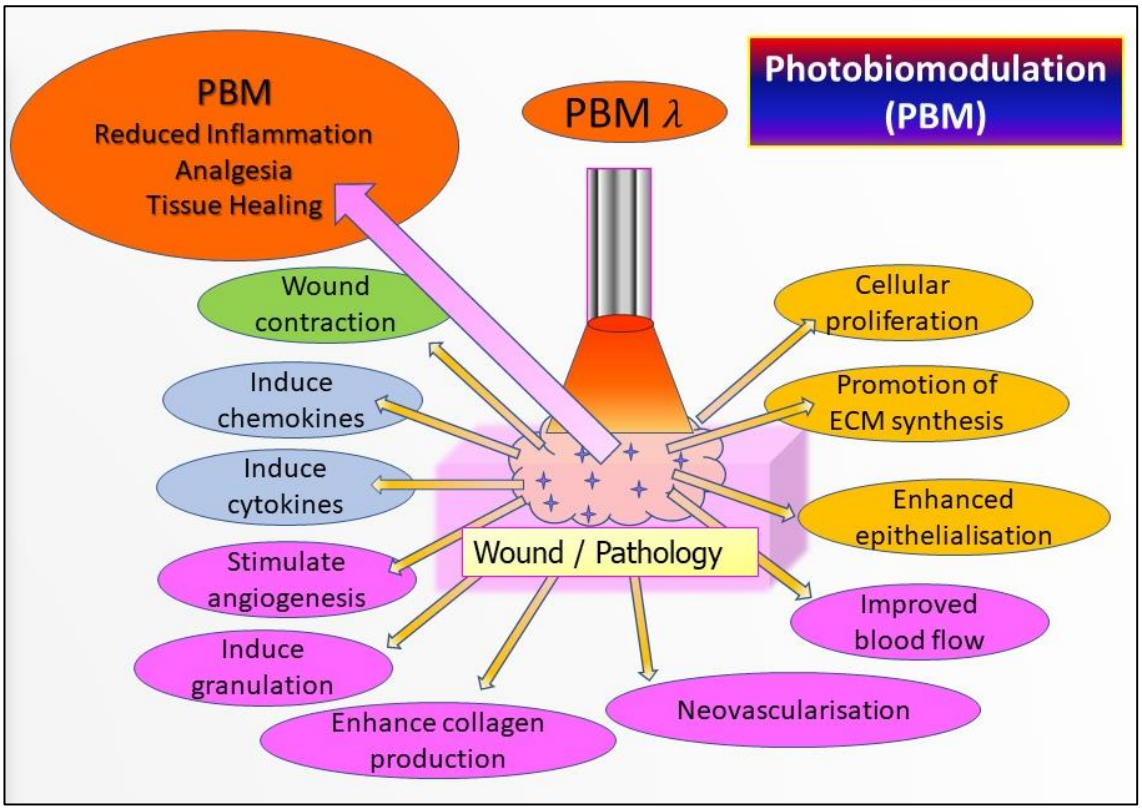


Figure #6. Areas of cellular and local tissue activity associated with PBM effects. Immediate and early medium effects involve epithelial, endothelial and biochemical elements, that combined may result in reduced inflammation, healing pathways and analgesia.

4.6 Wound healing: implications of the correct light dose through clinical outcome

These effects are examples of the complexity of the many laser and host factors and variables. Of prime interest would be the anticipated effect that collectively, photonic "trickle down" PBM effects might exert. *Figure #7* is taken from reference #177 to graphically illustrate tissue cellular response over a short (immediate), mid- and long-term timeline. As part of the specific events and assuming tissue disruption during a surgical procedure on oral soft tissue using a scalpel, the following activities are notable:

Immediate tissue response (immediate – 10 days): bleeding, coagulation, platelet activation, complement activation, host-mediated inflammatory response, bacteria-mediated inflammatory response.

Mid-term tissue response (1 – 30 days): cytokines, macrophages, phagocytosis, granulocytes, fibroplasia, re-epithelialisation, extra-cellular matrix synthesis, collagens, angiogenesis.

Long-term tissue response (10 days – 1 year): Extra-cellular matrix synthesis, degradation and re-modelling, increased tissue tensile strength, cellularity and maturity; functional re-establishment.

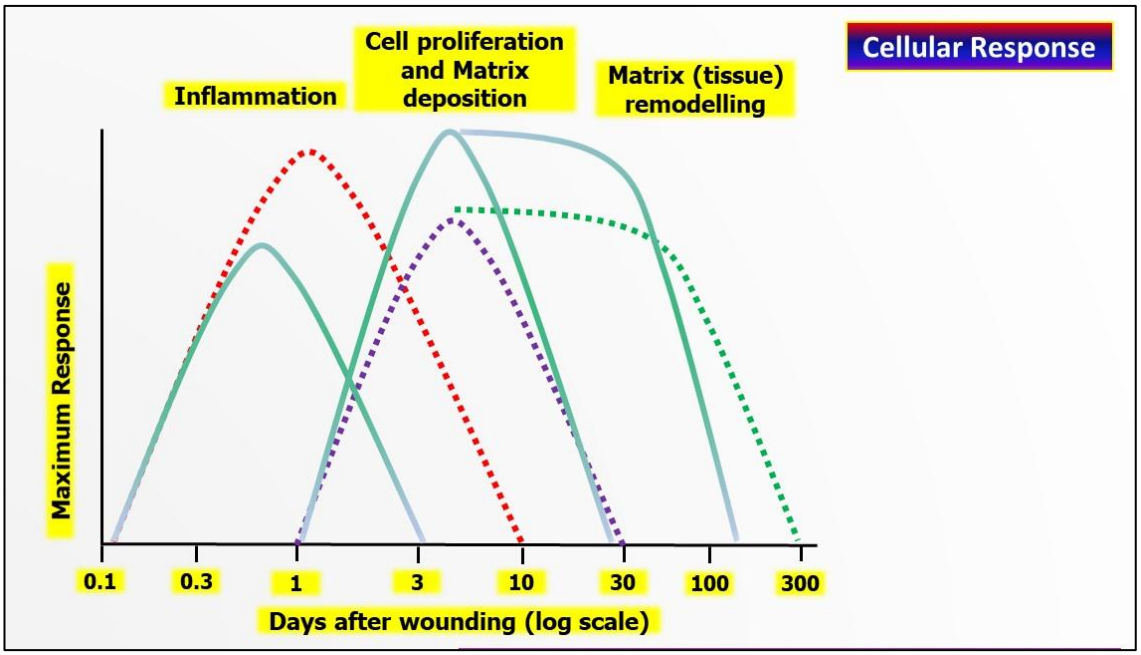


Figure #7. Relationship between scalpel soft tissue incision and host response, compared with the modulating effects of laser surgical application and "trickle down" PBM effects. Original data taken from Ref. #177 with additional data (dotted line) to illustrate PBM effects (Source: S.Parker – personal observation).

Against this complex but well-organised expression of both time and intensity, the influence of applied photonic energy and PBM effects would serve to achieve the following:

Inflammation: the host inflammatory response is subdued, relative to the individual, but together with the incisional pathogen reduction and formation of a laser coagulum and tenacious plasma protein denaturation, the intensity of the inflammatory reaction is reduced and somewhat shortened in duration.

Cell proliferation and matrix deposition: PBM effects on individual cell activity, mitosis and extended apoptosis, together with extra-cellular matrix and local nutrient channels (blood, lymph), would suggest an intensification of the mid-term response, over the same period of time.

Tissue remodelling: with the earlier, more intense cell and local tissue organisation, it may be observed that the long-term maturation is more pronounced and less subject to disruption. Classically, this may be noted as a collective series of events that constitutes "uneventful" healing.

Taken together, the reality remains that for a given surgical event, representing minor oral soft tissue surgical manipulation, irrespective of the laser wavelength chosen, the peri-operative, immediate post-operative and healing phases appear very similar. This may be noted in the examples shown in *Figures #8, 9, and 10*. *Figures #8* and *#9* demonstrate the removal of a benign irritation (traumatic) fibroma from the buccal mucosa, using a selection of laser wavelengths. *In Figure #8,* the wavelength selection is a 445nm InGaN diode laser, and a 810nm GaAs diode laser, and in *Figure #9,* the chosen wavelengths are the 2940nm Er:YAG and the 10,600nm CO_2 lasers. It is of note that both diode lasers and the CO_2 laser have inherently CW emission modes, whereas the erbium laser has a FRP co-axial air / water emission. Notwithstanding, the chosen variable parameters reflect a commonality of average power at 1.5–1.75 Watts.

Figure #8. Surgical excision of buccal fibroma using either a 445nm (left) or 810nm (right) diode laser. Equable average power parameters. Clinical images of the 445nm laser system courtesy of Dr E. Anagnostakis.

With the two diode lasers shown in *Figure #8*, although the photonic energy of the shorter wavelength is higher, with operating parameters reduced slightly to compensate, there is near-identical appearance of the haemostatic incision and creation of tenacious proteinaceous coagulum at the time of surgery. Although the appearance denotes a rough outline (compared to the clean sharp incision line of a scalpel), the early healing and tissue resolution over 10 – 14 days is characterised by a distinct lack of inflammation surrounding the surgical site, together with no evidence of any linear tissue modifications, suggestive of possible later scar formation. This is supported through published data (#33, #157).

With a similar series of clinical images and the same benign soft tissue lesion, the choice of longer wavelengths in *Figure #9*, provides a greater degree of difference, but with the same outcome post-operatively. The FRP erbium laser offers micro-second pulsing and high peak power per pulse; the co-axial water / air system is designed to reduce surface

tissue temperature and facilitate the washing away of debris. The thermal containment of the tissue is correspondingly reduced, resulting in some slight oozing of blood at the time of surgery. In this case, the achievement of a peri-operative coagulum is lost, however, by varying the operating parameters to switch off the coaxial elements, the photothermal consequence of laser action causes some tissue heating to enable the creation of an immediate post-operative tissue coagulum. As with *Figure #8*, the two wavelength choices appear to differ very slightly during the 10 – 14 day period post-operatively.

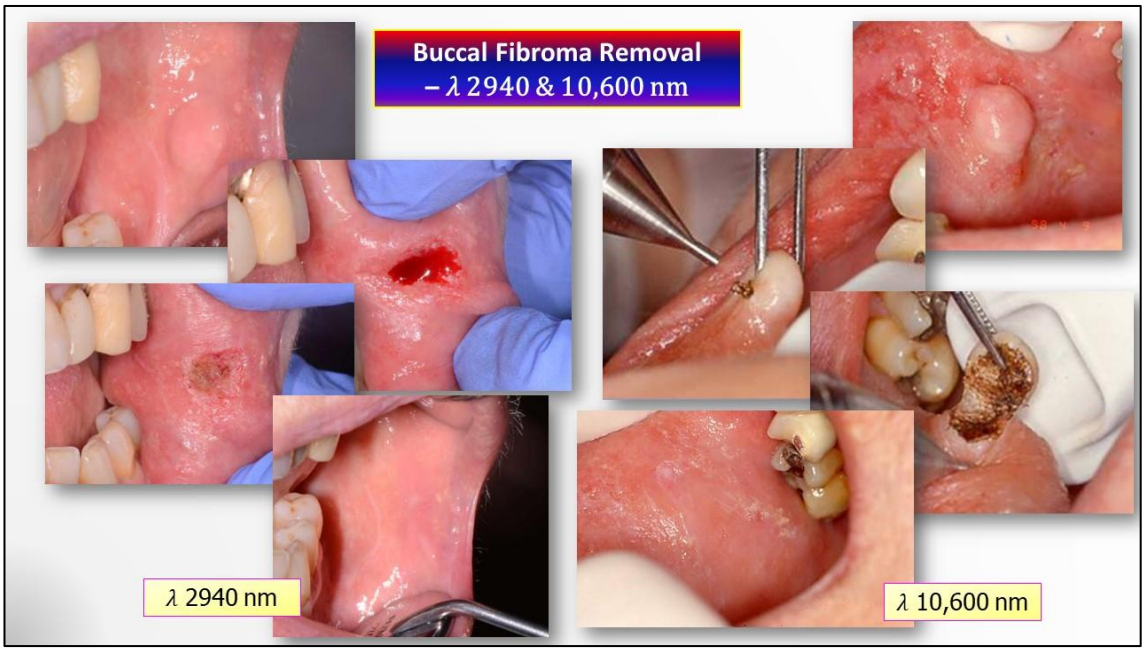


Figure #9. Similar benign lesion removal, using the Er:YAG (2940nm) laser (left) and the 10,600nm CO_2 laser (right). Peri-operative and post-operative appearances in both cases appears to show uneventful outcome.

A similar consistency of outcome appears to span the choice of wavelengths between 810, 2940 and 10,600nm, with a series of second-stage implant recovery / restoration (*Figure #10*). As with the fibroma series, the difference in emission mode variables (CW, gated-CW and FRP) is addressed through adoption of similar average power values. From these treatment series, it appears that similar and consistent outcomes can be achieved irrespective of the laser wavelength choice, provided comparable power variables are

addressed [178, 179]. In addition, the clinical appearance of "uneventful healing" would suggest that although those wavelengths within the so-called "optical window" (cumulative span of first and second NIR optical windows) at 650–1350nm, would draw upon evidence-based adjunctive PBM effects; a similar clinical appearance would suggest some form of tissue biomodulation with those wavelengths either side of the optical window.

There is scant *in vivo* clinical assessment of what cellular and biochemical changes and pathways may be activated or energised with these extraneous wavelength applications; however, there is adequate published data taken from *in vivo* animal studies and *in vitro* cellular irradiation studies, to enable some understanding of the supportive sub-ablative activity that is subject to a general consideration of biomodulation.

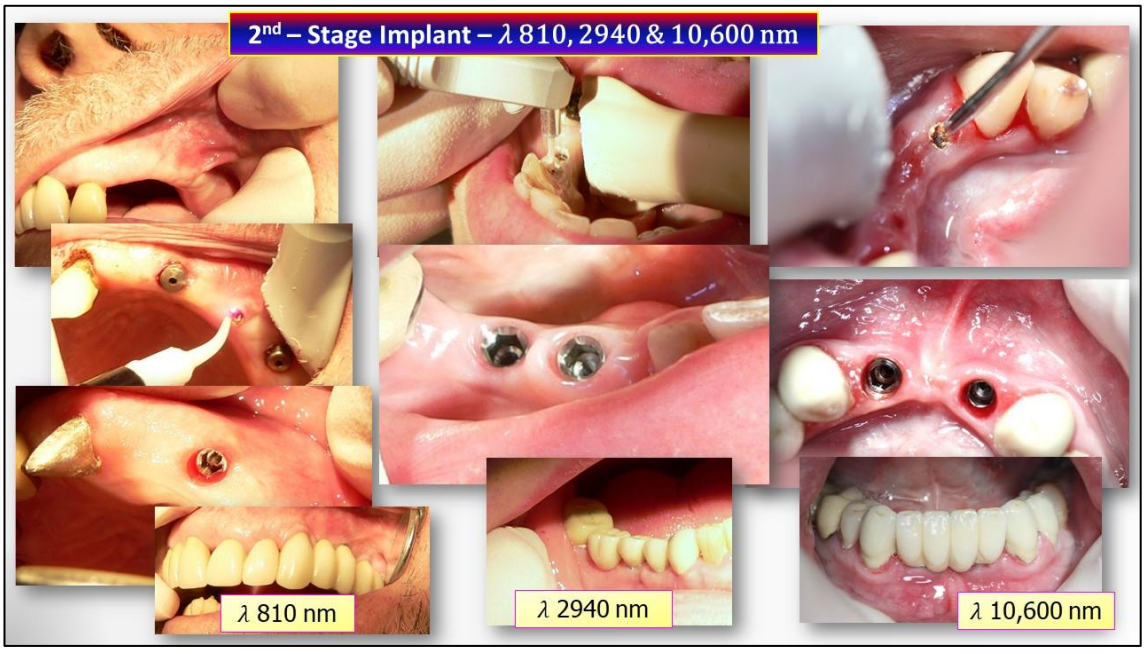


Figure #10. A three-case comparison of laser-assisted soft tissue ablation adjunctive to 2nd stage implant recovery / restoration. The use of the 810 nm GaAs diode laser (left) appears to deliver precise surgical incision and haemostasis, while demonstrating evidence of PBM effects, post-surgery. There is consistent similarity when longer 2940nm erbium YAG and 10,600nm CO_2 laser wavelengths are used.

With those laser wavelengths currently commercially available to dental clinicians, the extraneous choice extends to the 445nm InGaN "blue" and 523nm KTP "green" lasers as shorter wavelengths, together with the 2,780nm Er,Cr:YSGG, 2,940nm Er:YAG and 9,300-10,600nm CO_2 longer wavelengths (*Figure #11*).

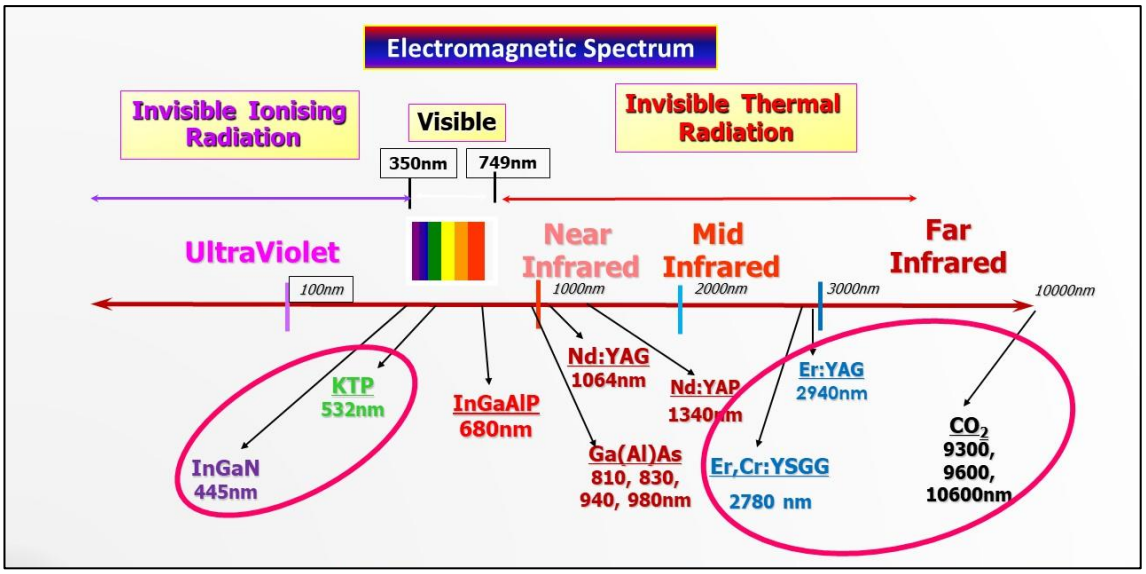


Figure #11. Schematic of the visible / NIR – FIR EM spectrum. Those wavelengths outside the optical window are circled in red.

Research conducted on oxidative mitochondrial respiration provides an established base to consider those wavelengths where CCO-centred absorption is a significant component. Several significant published studies have provided data to illustrate the outcome of cellular and tissue sample irradiation with shorter [180–184] and longer [185–188] wavelengths. In a study by Kim (2011), a number of mediators have been identified within the 650nm–1350nm range, and these have been arranged in *Table #3* [189]; from this, a review of published papers has enabled a cross-reference of common effects that are reported as being associated with either shorter or longer wavelengths. From this, it may be appreciated that the extensive commonality of effects when using all laser wavelengths at the disposal of the dental clinician, whether ascribable to true photobiomodulation, or other thermally-mediated molecular rotational / vibrational

activities. Although the contention that PBM being non-thermally mediated is steadfastly resolute, it is evident from research that some temperature-related effects do indeed exist [181].

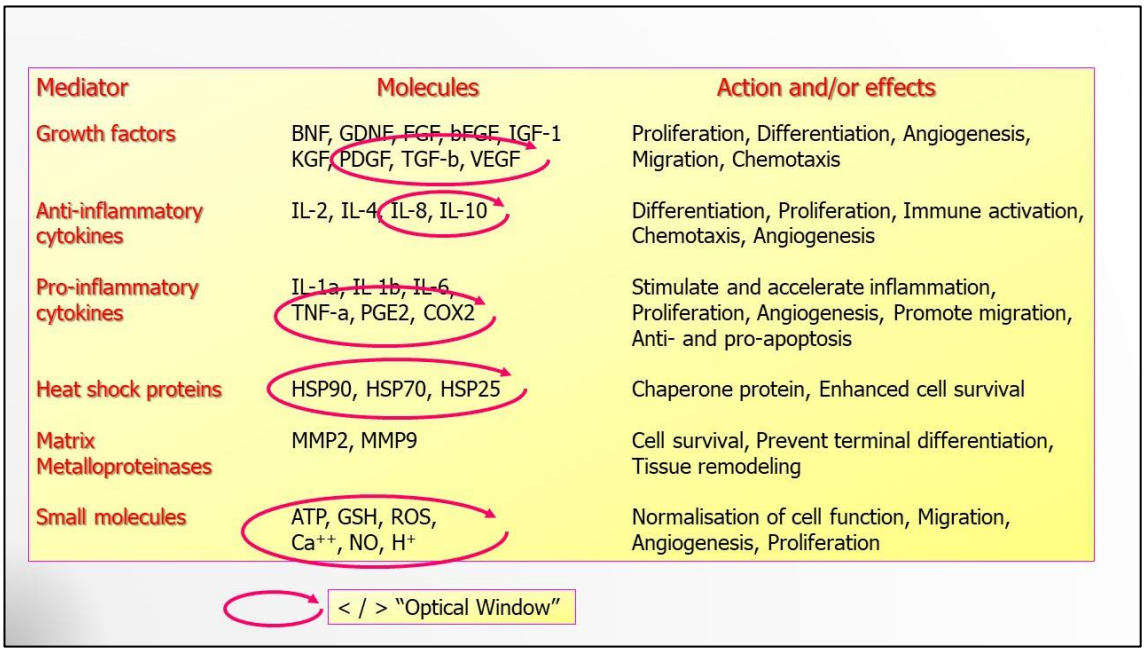


Table #3. Basic data from ref. #189, with cross-referenced evidence of post-irradiation short and long wavelength proliferation of mediators.

Central to this commonality of effects across the visible-blue to far-IR EM spectrum, evidence available suggests increased cellular permeability and short wavelength ROS production, which are linked in secondary gene-signalling. Cytokine activity with growth factor proliferation provides evidence of PBM-like effects in post-surgical wound resolution.

It is without question that both "true" PBM and other modulatory effects are wavelength specific. Across the spectrum, the notable effects that may be considered as contributory can be viewed as follows:

405nm–450nm "Blue" / 532nm "Green": Opsin-related enzyme signalling (OPN-2, 3, 5 in dermal tissue). A downstream effect of opsin stimulation and activation by light are

the transient receptor potential (TRP) channels and in particular TRPV1 (capsaicin receptor). Once stimulated and the channel opened, calcium ions (Ca^{2+}) may enter cells, induce calcium-calmodulin dependent kinase II (CAMKII), as part of cAMP phosphorylation in the nucleus. Blue light also induces significant ROS production in the cell, through the excitation of cryptochromes, notably flavins such as flavin mononucleotide (FMN) – located in Complex I and active in the reduction of molecular oxygen to superoxide ($O_2^{\cdot-}$). Other flavin cytochromes are found in Complex II and may mimic some of the mitochondrial membrane activity observed with red light. Additionally, blue light is also highly absorbed by porphyrins including that present in the heme group of CCO in Complex IV; activation through absorption of blue light photonic energy contributes to the redox transition through the electron transport chain. Blue light irradiation has also been shown to promote the production of heat shock proteins, indicative of the small thermal exchange of incident light to thermal energy. The 532nm "Green" laser wavelength has been demonstrated to excite green-sensitive opsin channels, leading to intracellular calcium ion increase and associated promotion of ROS production. Green light has also been reported to induce epithelial migration and proliferation.

650nm–680nm "Red": The well-researched effects of red light on the mitochondrial membrane space ECT, has allowed an understanding of the staging through the ETC redox phosphorylation of AMP to ATP. Additionally, productions of ROS and $NO^{\bullet}$ are noted, with downstream effects of increase in extracellular vasodilation and membrane permeability, related to the latter. ROS secondary effects include activation of enzyme pathways, notably nuclear factor kappa-B (NF-$\boldsymbol{\kappa}$B), and consequent signalling transcription factor activation.

900nm–1,100nm NIR: Notable effects include activation of heat/light-gated ion channels, with calcium ion levels increasing and onward signalling. It is of note that in view of the ascending level of absorption of this wavelength range in water, the 980 nm wavelength affects temperature-gated calcium ion channels, whilst the 810 nm one largely affects mitochondrial cytochrome c oxidase.

Mid- and Far-Infra-red: The high absorption of these wavelengths in water may contribute to the dissociation of intramolecular target OH^- and ROS production. Additional effects relate to generation of heat shock proteins, along with increased viability and proliferation of epithelial and endothelial cell lines.

These effects are summarised in *Figure #12*.

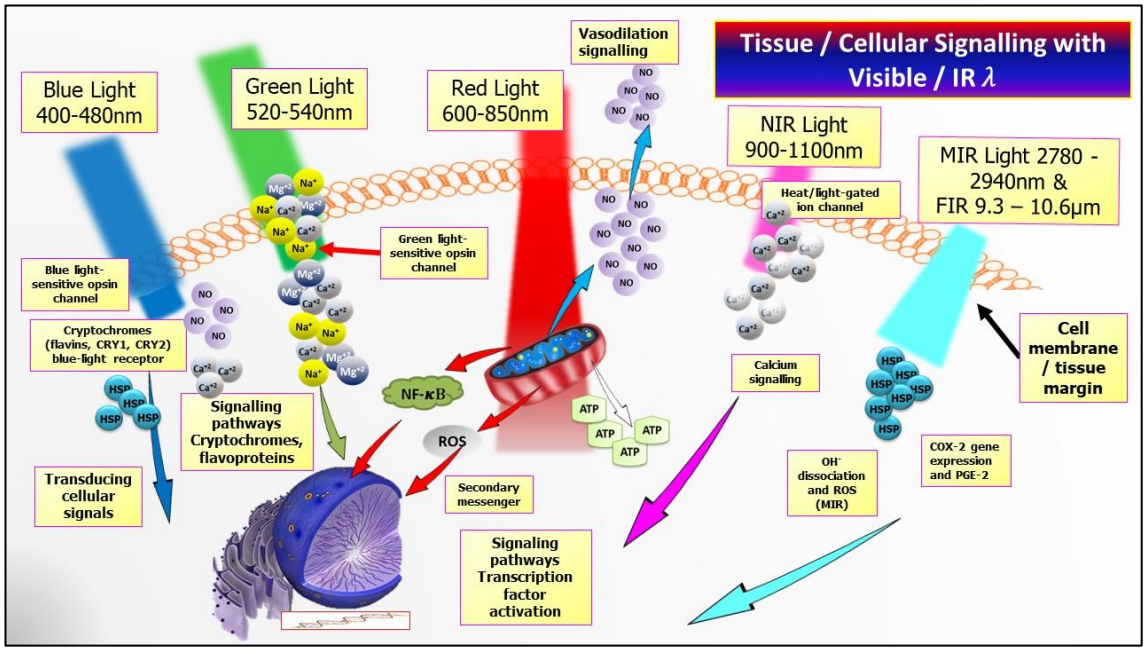


Figure #12. Examples of various dental laser wavelengths and their PBM or PBM-like effects on cellular structure. Graphics S. Parker with respect to Salehpour F, et al. MMol Neurobiol. 2018 Aug;55(8):6601-6636.

The uncertain relationship between sub-ablative laser fluences of shorter visible and longer mid- and far-IR wavelengths rests upon the interpretation of the core laser-tissue

interaction and energy conversion; is the incident photon stream energy absorbed within the target tissue, and does it relate to molecular vibrational and rotational modes of adjusted activity and consequent pathway activation, or is the precursor simply photothermal conversion arising from a rise in thermal energy within the sub-ablative limits of interaction?

Again, it is essential that variables of both wavelength and power/exposure time are respected, since it is evident that any excess applied irradiation would result in heat, not just to the extent that tissue ablation would be the risk, but that a more discrete excess energy and rise in fluence might exceed the bi-phasic peak of active positive benefit, and move towards the influence of a negative and undesired inhibitory effect.

With direct surgical action, the end-tip temperature of the initiated contact optic fibre tip will, of course, reach supra-ablative temperatures of several hundred degrees. In these cases, care would need to be exercised in order to respect the thermal diffusivity of the target tissue relative to the conductive thermal rise, in order to avoid damaging carbonisation. Operating parameter variable management would include possible pre-cooling of the target tissue, use of external cooling (high speed evacuation of laser plume), employment of gated-CW modes (milli- or micro-second gating), or FRP delivery relative to the laser being used and the employment of time intervals and/or removal of any ablative debris/carbonisation from both the fibre tip and the surgical site.

The containment of excessive temperature rises observed with such techniques will enable the thermal (and possible scatter) gradients to reduce the photon/temperature load within a short peri-ablation zone; once the phase limit of either protein denaturation or

water vaporisation had been under-achieved, tissue warming to around 40–45 ºC will serve as an indicator of the biomodulation of host tissue (*as per Figure #1, page 155*).

Where the choice of therapy utilises PBM action directly applied to oral/peri-oral pathology or syndromic symptoms, careful assessment of all variables is mandated. With reference to the systematic review reported by Cronshaw et al. (2019) [123], there has been an evaluation of the original concepts of the biphasic action of ascending levels of stimulation. Originally derived from the concepts of hormesis expressed through the Arndt Schultz study and publication in 1888 [191], which was primarily related to plant response, the positive/inhibitory cycle of dose-to-response relationship has been adapted for the purpose of investigating light dose PBM irradiation of animal and human tissues [162, 192].

Taken from the above paper [123], the dose/response effects are extended into the effects of higher PBM fluences (*Figure #13*):

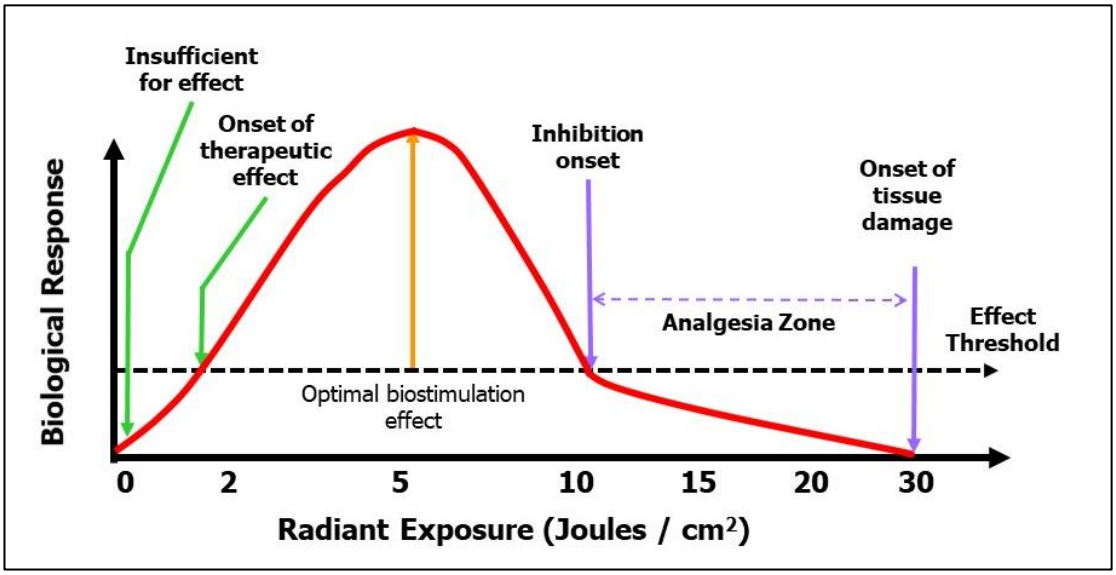


Figure #13. Extended graphic representation of relationship between applied radiant exposure (fluence) and biological response. Copied with permission from Ref: [123]: Cronshaw, M., Parker, S. and Arany, P. (2019). Feeling the Heat: Evolutionary and

The PBM dose regimens represent a multi-phasic biological response, and emphasize the complexity of clinical dosing within a specific therapeutic window. From these extended data, ascending fluence values may be viewed to deliver initial biostimulation, which is beyond a "high-point", further increase, which represents a decline in response towards bioinhibition. It has been concluded that an optimal mean value of applied radiant exposure would be 5 Joules per cm^2; doses in the "red-NIR λ" range, for superficial pathologies, have a range of 2-10 J/cm^2 [119, 193]. At a concept point of 10 J/cm^2, further increase would define levels of bioinhibition, including that associated with neural conduction [194], and a correspondingly annotated "analgesia zone"; as such, dosimetry for analgesia may require 10–30 J/cm^2 at tissue level, with a nominated mean value of 15 J/cm^2.

A further consideration is the delivery of PBM therapy to pathology located at depth. It is fortunate that within the confines of oral and maxillofacial regions, the maximum depth from any surface–skin or intraoral mucosa is in the region of 10 mm (1.0 cm). The applied surface dose must be computed to account for two basic variables, the applied wavelength and optical properties of the overlying tissue. Added to these, are of course the nature of therapy required (biostimulation/analgesia/bioinhibition) [195, 196].

Within the red visible–NIR wavelength range, the approximate attenuation of photonic energy at depth is 10% for each successive 1.0 mm of soft tissue depth. At the outer limits of deep pathology in facial tissues, this would amount to a surface dose correction of up to 90%, to account for losses through tissue thickness. Doses of the NIR λ value for deeper-seated disorders can therefore be higher, i.e. within the 10-50 J/cm^2 range. This

would then require further assessment of applied dose to accommodate extended periods of irradiation at relatively high surface fluence, a process leading to thermal rise and pain. In theses cases, a reduction in the average power delivery may be required, with a corresponding extension in irradiation time in order that the requisite dose at depth is delivered. Failure to acknowledge these factors of parameter manipulation and control may result in both high dose inhibition, or the delivery of insufficient photonic energy to achieve the desired level of biologic response. Additionally, therapy may require successive applications and with such demands. Indeed, PBM treatment is usually repeated either every day, every other day, or extended subject to target cell type, and a course of treatment can last for periods of two weeks or more.

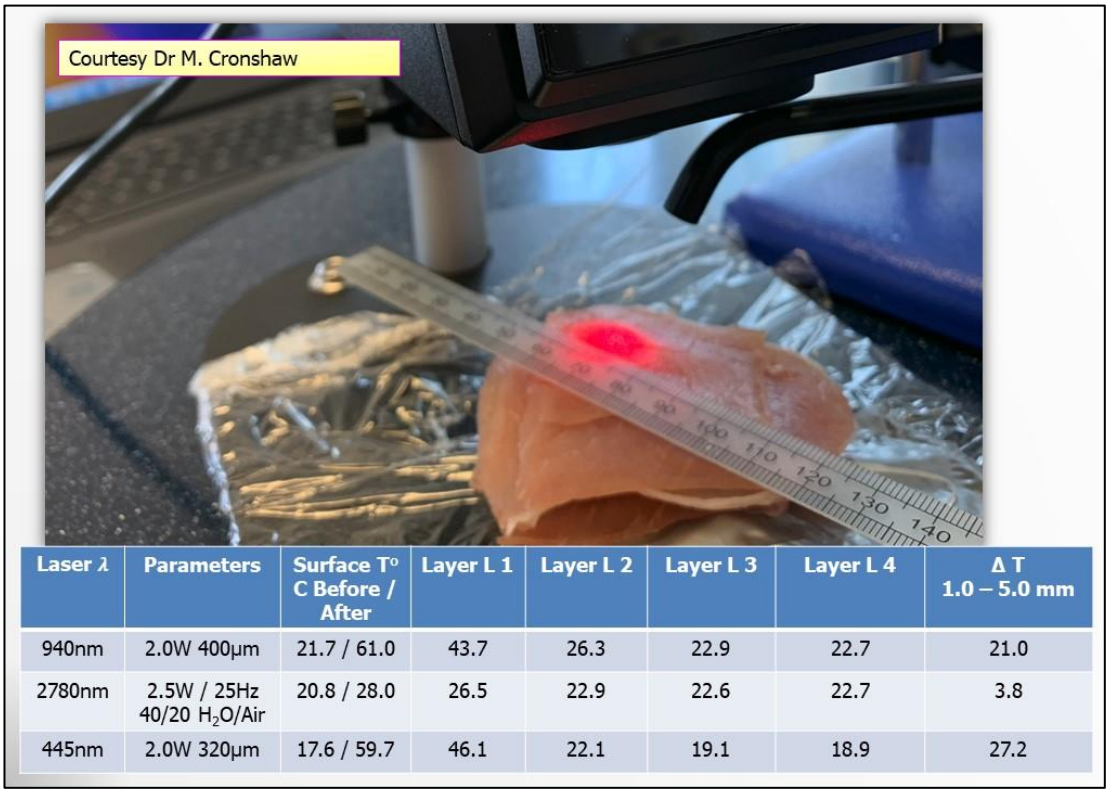
A final aspect of light-dose variable parameters may be observed relative to the size and distribution of the irradiated area associated with an indicated PBM therapy. As indicated in *Paper II* (page #96), the beam (spot) area of the delivery tip/applicator would influence several aspects of successful PBM effects, including the accuracy of lesion scanning, delivery of PBM at depth, and treatment time. As such, the choice of using a 300 μm diameter quartz optic fibre would have an acceptable indication for those lesions/pathologies of less than 1.0 cm^2; even then, the use of such fibre delivery, held at a distance of 10 mm above the target tissue, and assuming a beam divergence angle of 12^o, would only cover a "spot" size of approximately 0.5 cm^2. If the applicator tip is enlarged to a 10 mm diameter tip and held in near-contact with the tissue surface, the irradiation spot size would increase to 78 cm^2. Assuming appropriate demands of the lesion size, it is significant to choose an appropriate delivery tip size, in order to optimise correct PBM fluence and treatment time.

In connection with exposure time and at-depth delivery, the total energy delivered has some significance. Assuming the fluence value loss is through attenuation and not primarily beam scatter or transmission, the irradiated volume of tissue will accommodate the full value of delivered photonic energy; since the prime laser-tissue conversion as photothermal, it is possible that tissue temperatures may rise significantly. Additionally, at those more superficial tissue layers overlying the target lesion, such may be the applied fluence that bioinhibitory effects may prevail and influence the post-treatment period accordingly.

Fortunately, current dental laser therapy has given rise to choices of applicator tips and devices, to enable larger irradiation areas to be correctly treated.

The variation in laser-tissue interaction using sub-ablative fluences reflects a variation in absorption coefficients of individual tissue components with variable applied laser wavelengths; scatter coefficient values, inversely proportional to (specifically) near-IR and visible red wavelengths will indeed affect the degree of energy conversion. As discussed at length, the variation of evidence of "true" PBM has been explored, compared to an appreciation of some thermal rise. The amount of thermal rise appears critical and within an assumed "biological" range of approximately 6.0 ^{o}C above the 37^{0}C body temperature to avoid irreversible change [197]. This technique has been commonly adopted in areas of dermatology, where non-photonic, pre-heating of tissue has been shown to assist in post-surgical tissue conditioning and early healing [198 – 200]. A key aspect of such adjunctive application of thermal conditioning, sufficient to raise the temperature by a few degrees, is to create in 'stressed' tissue the "molecular chaperones" or heat shock proteins (HSP). As noted in Figure #28, several HSP molecules have been identified with response-linked cell stresses, including heat, radiation and trauma (HS90,

HS70, HS25). In a recent bench-top exercise, as part of a future publication (Cronshaw M, Parker S), a selection of dental laser wavelengths were examined for the delivery of appropriate PBM fluences to porcine muscle *in vitro*, arranged in 1.0 mm thickness layers to represent 5.0 mm overall tissue thickness. Thermographic analysis has shown that, whereas the acknowledged NIR 940 nm photothermal wavelength led to considerable tissue thermal rise to depth, even the 2,780 nm Er,Cr:YSGG wavelength, delivering similar average power with coaxial air/water, resulted in some conductive thermal rise of a few =^{o}C. This exercise is represented in *Figure #14, and Table #4*:



Laser λ	Parameters	Surface T° C Before / After	Layer L 1	Layer L 2	Layer L 3	Layer L 4	Δ T 1.0 – 5.0 mm
940nm	2.0W 400µm	21.7 / 61.0	43.7	26.3	22.9	22.7	21.0
2780nm	2.5W / 25Hz 40/20 H_2O/Air	20.8 / 28.0	26.5	22.9	22.6	22.7	3.8
445nm	2.0W 320µm	17.6 / 59.7	46.1	22.1	19.1	18.9	27.2

Figure #14. Image of bench-top exercise using thermographic camera to record various applied laser wavelengths and thermal rises observed at in vitro tissue thicknesses of 1.0–5.0 mm (courtesy of Dr M. Cronshaw). Within Figure #14 - Table #4. Reproduction of readings of thermal rise (deg. C), per applied wavelengths – 445nm, 940nm, 2780nm) and per tissue thickness. (Data courtesy of Dr M. Cronshaw).

Although this exercise awaits the deduction of statistical significance, it may be observed that although considered a surface-interactive laser wavelength with oral soft tissue, the

erbium 2,780 nm wavelength delivers only a slight thermal rise, consistent with a heat shock protein threshold. Although subject to further investigation, such events may help explain the promotion of clinical post-surgical appearance with such longer wavelengths, consistent with a description of “uneventful healing” and supplemental PBM-like, thermobiomodulation.

4.7 Summary

- Discussion of this area of investigation is offered through Paper IV: Parker S, Anagnostaki E, Mylona V, Cronshaw M, Lynch E, Grootveld M. (2020). Systematic Review of Post-Surgical Laser-Assisted Oral Soft Tissue Outcomes Using Surgical Wavelengths Outside the 650nm-1350 nm Optical Window. *Photobiomodul Photomed Laser Surg.* 38(10):591- 606. doi:10.1089/photob.2020.4847.
- There has been scant investigation into this area of laser oral surgery, other than generalised reference to post-operative pain, healing and inflammation, together with patient acceptance. With analysis of a risk of bias tool, some extrapolation has been possible together with statistically significant conclusions regarding the extent of laser-induced tissue photo- and thermos-biomodulation.
- The exploration of possible contributing factors of sub-ablative modulation with shorter and longer dental laser wavelengths, would indicate varying biochemical activity to allow an understanding of uneventful healing with post-surgical soft tissue with these lasers.
- A common characteristic of many RCT studies to research further the phenomenon of laser-assisted uneventful healing, has highlighted the lack of availability of full laser operating parameters.

- Significantly, this contributes to imprecise replication of treatment modality and protocol, with an attendant risk of patient harm.

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Chapter 5: Future Challenge and Conclusions

5.1 Future challenges

The research into laser-adjunctive benefits in both clinical surgical and non-surgical dentistry has developed to embrace improvements in specificity and scientific rigour. There may be limits to the simplistic extrapolation of research based on animal and *in-vitro* studies to the human model, and progress should be through quality studies, to provide challenge and evidence-base to the implicit attraction of anecdote in defining the benefits of laser use.

The enormous challenge is where there is either ethical or study design constraints to examine laser use as a clinical monotherapy. Often, any investigation of laser use over alternative therapy combines not only the limits of ethical committee overview, but also suffers the potential risk of choice of cohort groups and multi-variable biotype, host response, and the specificity of treatment protocols. Additionally, within those studies that explore optimal light dosage relative to a clinical need being met through laser use, the significance of active group operating parameters remains paramount.

A perception arises of a casual acceptance of laser use offering benefits that supplant those multifactorial essential contributing parameters that have been explored through this thesis. A clinical investigation may be undertaken without the necessary study design

and range of data acquisition in able to enable robust statistical analyses and significance determination.

As a result, the evolution of scientifically-robust investigations into laser use in clinical dentistry has suffered – mostly through poor reproducibility or, more fundamentally, poor or inadequate data arising from the incomplete recording of operating parameters; as witnessed through Paper #II, the additional significance of power losses along the delivery fibres associated with visible and near-IR lasers adds a further dimension of data non-reliability.

In summary, incomplete materials and methods and the specific lack of record of the use of a power meter to confirm the delivered laser photonic dose (relative to the control panel values) are very important considerations. It is proposed that these deficiencies have contributed to a distorted evaluation of lasers as an evidence-based adjunctive instrument.

There has been a significant increase in recent peer-reviewed investigations, to acknowledge the contribution of photobiomodulation in the primary resolution of pathology or as a therapeutic partner in the delivery of uneventful healing following tissue surgery. In many instances, previous opinion, often extending to dogma as to the essential surgical benefits of laser use, has been re-evaluated as being substantially influenced through PBM-mediated tissue pathways. Not only has the underlying emphasis of this thesis been directed to the significant influence of PBM, but also the increased risk of light dose error when considering the comparatively narrow range of delivered fluence, when compared to that associated with surgical laser power delivery.

Furthermore, and of peripheral consideration, when compared to the remit of this thesis, recent awareness of the significance of laser PBM in delivering analgesia relative to head

and neck pathology and post-surgery has been suggested to address the growing incidence of opioid drug dependency. A scale of the challenge has been highlighted in a recent published article [201] to examine population uptakes of prescribed analgesics. Given that regulatory bodies in many countries are considering the possible alternative benefits of PBM phototherapy over drug medication, the immense significance of correct and extensive laser operating parameters must be recognised.

Through the evolution of peer-reviewed evidence-based research into PBM, the defining early principles of mostly mitochondrial and other intracellular effects received worthy prominence. However, the gradual emergence of the wide and far-reaching downstream effects of PBM has helped to provide an increased scientific base to the influence of wavelength-specific photonic energy in host tissue response. In the discussion following *Paper IV* (also shown in the *Discussion II Table #3, page 205),* drawing upon reference #189, a wide range of bioactive mediators have been shown to be associated with photonic irradiation. As shown through a personal literature search, these mediators have been analysed as being affected by laser wavelengths outside the optical window of 650–1100nm. Such findings provide a strong argument regarding contributions to combined post-surgical clinical outcomes – so-called uneventful healing – that may be seen with 445nm–650nm and >2780nm wavelength dental laser use. Notable to photothermal temperature rise of even a few degrees, the generation of heat-shock proteins may have significance. Further research would be justified to examine a contributing thermobiomodulation, as a basis for the tissue response seen with those wavelengths outside the optical window range. Such research is of vital importance to the clinician in seeking an optimal laser wavelength for a given procedure. An attempt has been made in Paper IV in order to accommodate this breadth of concept, to explain through “quasi-

PBM", the collective positive outcomes of irradiation with shorter and longer laser wavelengths. What may supersede this line of research would appear to examine an overlap of "true" PBM effects (650nm–1350nm) with those associated with thermobiomodulation.

5.2 Conclusion

This thesis has explored parameter variables associated with the delivery of laser photonic energy in dentistry. It has enabled three major areas of application to be explored:

a/ Laser-tissue interaction, both sub-ablative diagnostic and photobiomodulation as well as supra-ablative surgical – essentially photothermolytic – action. Paper I – *"Current Concepts of Laser–Oral Tissue Interaction"*, explored the early inappropriate range of parameter flexibility, but how contemporary laser devices enable much more flexibility in choosing correct power parameters. In addition, the paper considered the range of laser-tissue phenomena, relative to variable laser wavelengths and photonic energy delivery.

b/ The wider scope for photonic energy delivery errors, including the choice of appropriate parameters consistent with the expected outcome, power losses through delivery system faults, over-treating through excessive fluences and the consequences of collateral laser-induced thermal damage.

Through the publication of Papers II and III, *"Photobiomodulation Delivery Parameters in Dentistry: An Evidence based Approach"* and *"The influence of delivery power losses and full operating parametry on the effectiveness of diode visible–near infra-red (445nm–1064 nm) laser therapy in dentistry - a multi-centre investigation",* it has been shown that incomplete parameter reporting, delivery system power losses, and deeper tissue beam performance may exert significance in "false negative" study outcomes.

c/ The influence of peri-surgical, predominantly thermal gradient, but also wavelength-specific scatter gradient, on surgical laser action, with a concomitant tissue biomodulation, together may give rise to "uneventful healing" i.e., the effects are consistent with concepts of PBM. Crucial to predictable PBM effects remains the concept of accurate dose, relative to anatomical target site, applied photonic power, and tissue optical properties – in all aspects of delivery, including the accurate use of parameter terminology

PBM may be considered as a "stand-alone" therapy, or as part of a surgical phototherapy, through a combination of thermal and light scatter gradients, and it is appropriate to consider that the same philosophy applies within surgical ablative laser fluences, albeit with wider margins of accommodating error potential.

Collectively, the delivery of therapeutic photonic energy constitutes a measurable light dose. In conclusion, this thesis has demonstrated four important points:

- All laser-tissue interaction is mandated by the application of minimum power values applied to effect an optimal outcome;
- The significance of "dose error", which through negligent omission of full parametry, or deficiency in accurate calibration, significantly interferes with and compromises the desired dental treatment outcome. The clinical surgical cases above have demonstrated how excessive thermal damage to fragile oral tissue can occur; similarly, a dose error occurring during PBM therapy could result in no expected benefit and potential inhibition.
- "Optical window" wavelength range, consistent with those visible-red and near infra-red surgical and PBM emissions, through their deeper tissue penetration,

extends the concept of dose to a volumetric irradiation and which is significantly dependent on intra-tissue photon scatter!

- With the emergence of large bulk tissue pathologies, the Gaussian distribution of beam profile requires caution to deliver a correct dose.

It is beholden on the clinical dental professional, irrespective of treatment or instrumentation, to observe *"Primum non nocere"* as a basic duty of care to those patients undergoing dental treatment. As defined through International Electrotechnical Commission regulations, Class IIIB and Class IV lasers are those that are potentially capable of exerting some damaging outcomes, and there is therefore a responsibility to apply and interpret the appropriate choice of operating parameters to minimise any risk of harm. To a considerable extent, a level of ignorance existed amongst the early adopters of laser use in clinical dentistry, not least because of effects arising from the application of inappropriate, often excessive "light doses". However, much of these deficiencies have now been resolved through research and the publication of peer-reviewed studies, although it remains of considerable significance that many of the intricate and inter-related variables featured in laser photonic deliveries are either not reported or ignored.

It is therefore hoped that through this thesis, a greater awareness of the multi-faceted aspects of potential error and consequent negligent iatrogenic damage will be acknowledged, with an improved understanding of the influence of all measurable values of laser delivery may emerge, along with their many variances.

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Reference List for Thesis and Published Papers

Thesis References (Omitting references associated with Papers #1 - #4 – see associated lists).

1/ Maxwell, J C. (1865). A dynamical theory of the electromagnetic field. *Philosophical Transactions of the Royal Society* VIII, pp 155459–155512. doi. 10.1098/rstl.1865.0008.

2/ Maxwell, J C. (1868). On a method of making a direct comparison of electrostatic with electro-magnetic force; with a note on the electromagnetic theory of light. *Philosophical Transactions of the Royal Society* XXVI, pp 158643–158657. doi.10.1098/rstl.1868.0027

3/ Planck, M. (1901). On the law of distribution of energy in the normal spectrum. *Annalen der physik*, 4(553), p.1. doi.org/10.1016/B978-0-08-012102-4.50013-9

4/ Einstein, A. (1905). Uber einen die Erzeugung und Verwandlung des Lichtes betreffenden heuristischen Gesichtspunkt. (sic: On a Heuristic Point of View about the Creation and Conversion of Light) *Annalen der Physik* 17, pp132-148. doi.org/10.1002/andp.19053220607

5/ Einstein, A. (1917). Zur quantentheorie der strahlung (sic: Of the quantum theory of wavelength). *Physiolishe Z*, 18, pp 121-128

6/ Maiman, T H. (1960). Stimulated Optical Radiation in Ruby. *Nature,* Aug, 187(4736), pp 493–94. doi:10.1038/187493a0.

7/ Schawlow, A L,. Townes, C H. (1958). Infrared and optical masers. *Physical Review*, Dec,112 (6–15), pp 1940–1949. doi:10.1103/PhysRev.112.1940.

8/ Parker, S. (2007). Verifiable CPD paper: introduction, history of lasers and laser light production. *British Dental Journal*. Jan 13, 202(1), pp 21-31. doi: 10.1038/bdj.2006.113.

9/ Hall, RN., Fenner, GE., Kingsley, JD., Soltys, TJ., Carlson, RO. (1962). Coherent light emission from GaAs junctions. *Physical Review Letters,* Nov 1,9(9), pp 366. doi.org/10.1103/PhysRevLett.9.366 0031-9007

10/ Nathan, MI., Dumke, WP., Burns, G., Dill Jr, FH., Lasher, G. (1962). Stimulated emission of radiation from GaAs p-n junctions. *Applied Physics Letters,* Nov1,1(3), pp 62-64. doi.org/10.1063/1.1777371 0003-6951

11/ Holonyak Jr, N., Bevacqua, SF. (1962). Coherent (visible) light emission from Ga (As1− x P x) junctions. *Applied Physics Letters,* Dec 1,1(4), pp 82-3. doi.10.1063/1.17537060003-6951

12/ Kroemer, H,. (1963). A proposed class of hetero-junction injection lasers. *Proceedings of the Institute of Electrical and Electronics Engineers,* Dec,51(12), pp 1782-1783.

13/ Alferov, ZI., Andreev, VM., Korolkov, VI., Tretyako, DN., Tuchkevi, VM. (1968). High-voltage pn junctions in Ga_xAl_{1-x} As crystals. *Soviet Physics Semiconductors-USSR,* Jan 1,1(10)pp 1313.

14/ Patel, C K., Tien, P., McFee, J. (1965). CW high-power CO_2–N_2–He laser. *Applied Physics Letters* 7(11), pp 290–292. doi.10.1063/1.1754264

15/ Watson, E. (2006). Founder looks back as Coherent marks 40 years in business. *Laser Focus World,* 42(8), pp 11–12.

16/ Myers, TD., Myers, WD., Stone, RM. (1989). First soft tissue study utilizing a pulsed Nd:YAG dental laser. *Northwest Dentistry,* Mar-Apr,68(2), pp 14-7.

17/ Myers, TD., McDaniel, JD. (1991). The pulsed Nd:YAG dental laser: review of clinical applications. *Journal of the Californian Dental Association,* Nov;19(11), pp 25-30.

18/ Parkins, FM., Miller, RL., Furnish, GM., O'Toole, TJ. (1991). A preliminary report: YAG laser treatment in pediatric dentistry. *Journal of the Californian Dental Association,* Nov,19(11), pp43-4, 46-8, 50.

19/ Whitters, CJ., Hall, A., Creanor, SL., Moseley, H., Gilmour, WH., Strang, R., Saunders, WP., Orchardson, R. (1995). A clinical study of pulsed Nd: YAG laser-induced pulpal analgesia. *Journal of Dentistry,* Jun,23(3), pp 145-50.

20/ Abdel-Aziem, F. (1994). Clinical evaluation of pulsed ND:YAG dental laser applied on oral soft tissues. *Egyptian Dental Journal,* Oct,40(4), pp 863-70.

21/ White, JM., Goodis, HE., Setcos, JC., Eakle, S., Hulscher, BE., Rose, CL. (1993). Effects of pulsed Nd:YAG laser energy on human teeth: a three-year follow-up study. *Journal of the American Dental Association,* Jul,124(7), pp 45-51. doi:10.14219/jada.archive.1993.0273.

22/ Wigdor, HA., Walsh, JT Jr., Featherstone, JD., Visuri, SR., Fried, D., Waldvogel, JL. (1995). Lasers in dentistry. *Lasers in Surgery and Medicine*, 16(2), pp 103-33.

23/ Wigdor, H., Abt, E., Ashrafi, S., Walsh, JT Jr. (1993). The effect of lasers on dental hard tissues. *Journal of the American Dental Association,* Feb,124(2), pp 65-70.

24/ Frentzen, M., Koort, HJ. (1990). Lasers in dentistry: new possibilities with advancing laser technology? *International Dental Journal,* Dec,40(6), pp323-32.

25/ von Fraunhofer, JA., Allen, DJ. (1993).Thermal effects associated with the Nd:YAG dental laser. *Angle Orthodontics*, Winter,63(4), pp 299-303. doi: 10.1043/0003-3219(1993)063<0299:TEAWTY>2.0.CO;2.

26/ Kelsey, WP 3rd., Blankenau, RJ., Powell, GL. (1991). Application of the argon laser to dentistry. *Lasers in Surgery and Medicine,* 11(6), pp 495-498.

27/ Salman, RA. (1991). Carbon dioxide laser surgery. *Special Care Dentist,* Sep-Oct,11(5), pp 211-212. doi: 10.1111/j.1754-4505.1991.tb01734.x.

28/ Midda, M. (1992). Lasers in periodontics. *Periodontal Clinical Investigations,* Spring,14(1), pp 14-20. PMID: 1392501

29/ Dunsche, A., Fleiner, B., Terheyden, H., Hoffmeister, B. (1995). Use of carbon dioxide laser in the therapy of benign oral soft-tissue lesions. *Advances in Otorhinolaryngology,* 49, pp 136-139. doi: 10.1159/000424357.

30/ Powell, GL., Whisenant, BK., Morton, TH. (1990). Carbon dioxide laser oral safety parameters for teeth. *Lasers in Surgery and Medicine,* 10(4), pp 389-392.

31/ Garg, N., Verma, S., Chadha, M., Rastogi, P. (2015). Use of carbon dioxide laser in oral soft tissue procedures. *National Journal of Maxillofacial Surgery,* Jan-Jun,6(1), pp 84-88.

32/ Huang, Z., Wang, Y., Liang, Q., Zhang, L., Zhang, D., Chen, W. (2015). The application of a carbon dioxide laser in the treatment of superficial oral mucosal lesions. *Journal of Craniofacial Surgery,* May,26(3),e277-9.

33/ Hanna, R., Parker, S. (2016). The advantages of carbon dioxide laser applications in paediatric oral surgery. A prospective cohort study. *Lasers in Medical Science,* Nov,31(8), pp 1527-1536.

34/ Saibene, AM., Rosso, C., Castellarin, P., Vultaggio, F., Pipolo, C., Maccari, A., Ferrari, D., Abati, S., Felisati, G. (2019). Managing Benign and Malignant Oral Lesions with Carbon Dioxide Laser: Indications, Techniques, and Outcomes for Outpatient Surgery. *Surgery Journal of New York,* Aug 5,5(3),e69-e75.

35/ Xue, VW., Zhao, IS., Yin, IX., Niu, JY., Lo, ECM., Chu, CH.. (2021). Effects of 9,300 nm Carbon Dioxide Laser on Dental Hard Tissue: A Concise Review. *Clinical Cosmetic Investigations in Dentistry*, Apr 30,13, pp 155-161.

36/ Walsh, JT Jr., Cummings, JP. (1994). Effect of the dynamic optical properties of water on midinfrared laser ablation. *Lasers in Surgery and Medicine,* 15(3), pp 295-305.

37/ Mercer, C. (1996). Lasers in dentistry: a review. Part 1. *Dental Update,* Mar,23(2), pp 74-80. PMID: 8948198

38/ Hibst, R., Keller U. (1989). Experimental studies of the application of the Er:YAG laser on dental hard substances: I. Measurement of the ablation rate. *Lasers in Surgery and Medicine,* 9(4), pp 338-344.

39/ Peramo, A., Marcelo, CL., Feinberg, SE. (2012). Tissue engineering of lips and mucocutaneous junctions: in vitro development of tissue engineered constructs of oral mucosa and skin for lip reconstruction. *Tissue Engineering - Part C: Methods*, Apr,18(4), pp 273-282.

40/ Maikala, R. (2010). Modified Beer's Law – historical perspectives and relevance in near-infrared monitoring of optical properties of human tissue. *International Journal of Industrial Ergonomics,* 40(2), pp 125-134.

41/ Feder, I., Duadi, H., Fixler, D. (2020). Single wavelength measurements of absorption coefficients based on iso-pathlength point. *Biomedical Optical Express*, Sep 22,11(10), pp 5760-5771.

42/ Mourant, JR., Freyer, JP., Hielscher, AH., Eick, AA., Shen, D., Johnson, TM. (1998). Mechanisms of light scattering from biological cells relevant to noninvasive optical-tissue diagnostics. *Applied Optics* Jun 1,37(16), pp 3586-3593.

43/ Parker, S., Anagnostaki, E., Mylona, V., Cronshaw, M., Lynch, E., Grootveld, M. (2020). Current Concepts of Laser–Oral Tissue Interaction. *Dentistry Journal*, 8, 61;

44/ Mó, O., Yáñez, M., Eckert-Maksić, M., Maksić, Z.B., Alkorta, I. and Elguero, J. (2005). Periodic Trends in Bond Dissociation Energies. A Theoretical Study. *The Journal of Physical Chemistry A*, 109(19), pp.4359–4365
doi: 10.1021/jp050857o.

45/ Mir, M., Gutknecht, N., Poprawe, R., Vanweersch, L. and Lampert, F. (2008). Visualising the procedures in the influence of water on the ablation of dental hard

tissue with erbium:yttrium–aluminium–garnet and erbium, chromium:yttrium–scandium–gallium-garnet laser pulses. *Lasers in Medical Science*, 24(3), pp.365–374.

46/ Powell, GL., Blankenau, RJ,. (1996). Effects of argon laser curing on dentin shear bond strengths. *Journal of Clinical Laser Medicine & Surgery*. 14(3), pp 111-113.

47/ Zhang, C., Wang, X., Kinoshita, J.-I., Zhao, B., Toko, T., Kimura, Y. and Matsumoto, K. (2007). Effects of KTP Laser Irradiation, Diode Laser, and LED on Tooth Bleaching: A Comparative Study. *Photomedicine and Laser Surgery*, 25(2), pp.91–95.

48/ Mylona, V., Anagnostaki, E., Parker, S., Cronshaw, M., Lynch, E. and Grootveld, M. (2020). Laser-Assisted aPDT Protocols in Randomized Controlled Clinical Trials in Dentistry: A Systematic Review. *Dentistry Journal*, 8(3), p.107.

49/ Świder, K., Dominiak, M., Grzech-Leśniak, K. and Matys, J. (2019). Effect of Different Laser Wavelengths on Periodontopathogens in Peri-Implantitis: A Review of In Vivo Studies. *Microorganisms*, 7(7), p.189.

50/ Anders, J.J., Lanzafame, R.J. and Arany, P.R. (2015). Low-Level Light/Laser Therapy Versus Photobiomodulation Therapy. *Photomedicine and Laser Surgery*, [online] 33(4), pp.183–184. doi: 10.1089/pho.2015.9848.

51/ Cronshaw, M., Parker, S., Anagnostaki, E., Mylona, V., Lynch, E. and Grootveld, M. (2020). Photobiomodulation Dose Parameters in Dentistry: A Systematic Review and Meta-Analysis. *Dentistry Journal*, 8(4), p.114.

52/ Tuncer, I., Özçakır-Tomruk, C., Şencift, K. and Çöloğlu, S. (2010). Comparison of Conventional Surgery and CO_2 Laser on Intraoral Soft Tissue Pathologies and Evaluation of the Collateral Thermal Damage. *Photomedicine and Laser Surgery*, 28(1), pp.75–79.

53/ Parker, S. (2007). Low-level laser use in dentistry. *British Dental Journal*, 202(3), pp.131–138. doi: 10.1038/bdj.2007.75.

54/ Parker, S. (2007). Lasers and soft tissue: "loose" soft tissue surgery. *British Dental Journal*, 202(4), pp.185–191. doi: 10.1038/bdj.2007.128.

55/ Hibst, R., 2002. Lasers for Caries Removal and Cavity Preparation: State of the Art and Future Directions. *Journal of Oral Laser Applications*, 2(4).

56/ Chemicalbook.com. Available at: *https://www.chemicalbook.com/ChemicalProductProperty_EN_CB2131094.htm#:~:text=Hydroxyapatite%20Properties,1100%20%C2%B0C(lit.)*

57/ Parker, S. (2007). Surgical lasers and hard dental tissue. *British Dental Journal*, 202(8), pp.445–454.

58/ Seka, W.D., Featherstone, J.D., Fried, D., Visuri, S.R. and Walsh, J.T., 1996, April. Laser ablation of dental hard tissue: from explosive ablation to plasma-mediated ablation. *In Lasers in dentistry II* (Vol. 2672, pp. 144-158). SPIE - Society of Photo-Optical Instrumentation Engineers - the International Society for Optical Engineering.

59/ Assa, S., Meyer, S. and Fried, D. (2008). Ablation of dental hard tissues with a microsecond pulsed carbon dioxide laser operating at 9.3-μm with an integrated

scanner. *Proceedings of SPIE - Society of Photo-Optical Instrumentation Engineers - the International Society for Optical Engineering*, 6843, 684308.

60/ Fried, D., Zuerlein, M.J., Le, C.Q. and Featherstone, J.D.B. (2002). Thermal and chemical modification of dentin by 9-11-μm CO_2 laser pulses of 5-100-μs duration. *Lasers in Surgery and Medicine*, 31(4), pp.275–282.

61/ Nguyen, D., Chang, K., Hedayatollahnajafi, S., Staninec, M., Chan, K., Lee, R. and Fried, D. (2011). High-speed scanning ablation of dental hard tissues with a λ = 9.3 μm CO_2 laser: adhesion, mechanical strength, heat accumulation, and peripheral thermal damage. *Journal of Biomedical Optics*, 16(7), p.071410.

62/ Chan, K.H. and Fried, D. (2018). Selective ablation of dental caries using coaxial CO_2(9.3-μm) and near-IR (1880-nm) lasers. *Lasers in Surgery and Medicine*, 51(2), pp.176–184.

63/ Lukač, M., Marincek, M. and Grad, L., 2004. Super VSP Er: YAG Pulses for Fast and Precise Cavity Preparation. *Journal of Oral Laser Applications*, 4(3).

64/ Trevelin, L.T., Marques, M.M., Aranha, A.C.C., Arana-Chavez, V.E. and Matos, A.B. (2015). Effect of super short pulse Er:YAG laser on human dentin-Scanning electron microscopy analysis. *Microscopy Research and Technique*, 78(6), pp.472–478.

65/ Carruth, J.A.S. (1991). Lasers in Oral Surgery. *Journal of Clinical Laser Medicine & Surgery*, 9(5), pp.379–380.

66/ Frentzen, M., Koort, HJ. (1990). Lasers in dentistry: new possibilities with advancing laser technology? *International Dental Journal*. 40(6), pp.323-32.

67/ Wigdor, H.A., Walsh, J.T., Featherstone, J.D.B., Visuri, S.R., Fried, D. and Waldvogel, J.L. (1995). Lasers in dentistry. *Lasers in Surgery and Medicine*, 16(2), pp.103–133.

68/ Zakariasen, KL., Dederich, DN. (1991). Dental lasers and science. *Journal of the Canadian Dental Association.* 57(7), pp.570-3.

69/ Miller, PD Jr. (1993). Periodontal plastic surgery. *Current Opinion in Periodontology*. Pp.136-143.

70/ Pick, RM., Powell, GL. (1993). Laser in dentistry. Soft-tissue procedures. *Dental Clinics of North America*. 37(2), pp.281-296.

71/ White, J.M., Goodis, H.E. and Rose, C.L. (1991). Use of the pulsed Nd:YAG laser for intraoral soft tissue surgery. *Lasers in Surgery and Medicine*, 11(5), pp.455–461.

72/ Arcoria, C.J., Steele, R.E., Vitasek, B.A. and Wagner, M.J. (1992). Effects of coaxial CO2/Nd:YAG irradiation on periodontal wound healing. *Lasers in Surgery and Medicine*, [online] 12(4), pp.401–409.

73/ Cobb, C.M. (1997). Lasers in periodontics: use and abuse. *Compendium of Continuing Education in Dentistry*, 18(9), pp.847-852, pp.854-855, pp.858-8599; quiz pp.860.

74/ Morlock, B.J., Pippin, D.J., Cobb, C.M., Killoy, W.J. and Rapley, J.W. (1992). The Effect of Nd:YAG Laser Exposure on Root Surfaces When Used as an Adjunct to Root Planing: An In Vitro Study. *Journal of Periodontology*, 63(7), pp.637–641.

75/ Gelskey, S.C., White, J.M., Gelskey, D.E. and Kremers, W. (1998). Vapor emissions resulting from Nd:YAG laser interaction with tooth structure. *Dental Materials*, 14(6), pp.453–457.

76/ Tucker, D., Cobb, C.M., Rapley, J.W., Killoy, W.J. (1996). Morphologic changes following in vitro CO_2 laser treatment of calculus-ladened root surfaces. *Lasers in Surgery and Medicine,* 18(2), pp.150-156.

77/ Judy, M.M., Matthews, J.L., Aronoff, B.L. and Hults, D.F. (1993). Soft tissue studies with 805 nm diode laser radiation: Thermal effects with contact tips and comparison with effects of 1064 nm Nd:YAG laser radiation. *Lasers in Surgery and Medicine,* 13(5), pp.528–536.

78/ Li, W., Zheng, J., Zhang, Y., Yuan, F. and Lyu, P. (2018). Temperature and depth evaluation of the in vitro effects of femtosecond laser on oral soft tissue, with or without air-cooling. *Lasers in Medical Science,* 34(4), pp.649–658.

79/ Walsh, J.T., Flotte, T.J. and Deutsch, T.F. (1989). Er:YAG laser ablation of tissue: Effect of pulse duration and tissue type on thermal damage. *Lasers in Surgery and Medicine,* 9(4), pp.314–326.

80/ Chi, M., Müller, A., Hansen, A.K., Jensen, O.B., Petersen, P.M. and Sumpf, B. (2020). Microsecond pulse-mode operation of a micro-integrated high-power external-cavity tapered diode laser at 808 nm. *Applied Optics*, 59(26), p.7836.

81/ Braun, A., Kettner, M., Berthold, M., Wenzler, J.-S., Heymann, P.G.B. and Frankenberger, R. (2018). Efficiency of soft tissue incision with a novel 445-nm semiconductor laser. *Lasers in Medical Science,* [online] 33(1), pp.27–33.

82/ Hanke, A., Fimmers, R., Frentzen, M. and Meister, J. (2021). Quantitative determination of cut efficiency during soft tissue surgery using diode lasers in the wavelength range between 400 and 1500 nm. *Lasers in Medical Science*, 36(8), pp.1633–1647.

83/ Fornaini, C., Fekrazad, R., Rocca, J.-P., Zhang, S. and Merigo, E. (2021). Use of Blue and Blue-Violet Lasers in Dentistry: A Narrative Review. *Lasers in Medical Science,* 12(1), pp.e31–e31.

84/ Beer, F., Körpert, W., Passow, H., Steidler, A., Meinl, A., Buchmair, A.G. and Moritz, A. (2011). Reduction of collateral thermal impact of diode laser irradiation on soft tissue due to modified application parameters. *Lasers in Medical Science,* 27(5), pp.917–921.

85/ Sivaprasad, S., Elagouz, M., McHugh, D., Shona, O. and Dorin, G. (2010). Micropulsed Diode Laser Therapy: Evolution and Clinical Applications. *Survey of Ophthalmology*, 55(6), pp.516–530.

86/ Shinkai, K., Takada, M., Kawashima, S., Suzuki, M. and Suzuki, S. (2018). Effects of the percentage of air/water in spray on the efficiency of tooth ablation with erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser irradiation. *Lasers in Medical Science,* 34(1), pp.99–105.

87/ Olivi, G., Angiero, F., Benedicenti, S., Iaria, G., Signore, A. and Kaitsas, V. (2009). Use of the erbium, chromium:yttrium–scandium–gallium–garnet laser on human enamel tissues. Influence of the air–water spray on the laser–tissue interaction: scanning electron microscope evaluations. *Lasers in Medical Science,* 25(6), pp.793–797.

88/ Colucci, V., Lucisano Botelho do Amaral, F., Pécora, J.D., Palma-Dibb, R.G. and Milori Corona, S.A. (2008). Water flow on erbium:yttrium–aluminum–garnet laser irradiation: effects on dental tissues. *Lasers in Medical Science*, 24(5), pp.811–818.

89/ Geminiani, A., Caton, J.G. and Romanos, G.E. (2011). Temperature Increase During CO_2 and Er: YAG Irradiation on Implant Surfaces. *Implant Dentistry*, p.1.

90/ Sant'Anna, EF., Araújo, MTS., Nojima, LI., Cunha, ACD., Silveira, BLD., Marquezan, M. (2017). High-intensity laser application in Orthodontics. *The Dental Press Journal of Orthodontics*, Nov-Dec,22(6), pp 99-109.

91/ Olivi, G., Caprioglio, C., Olivi, M., Genovese, MD. (2017). Paediatric laser dentistry. Part 2: Hard tissue laser applications. *European Journal of Paediatric Dentistry*, Jun,18(2), pp 163-166.

92/ Beloica, M., Vulicevic, Z., Mandinic, Z., Radovic, I., Jovicic, O., Carevic, M. and Tekic, J. (2014). Hard dental tissue minimal-invasive preparation using contemporary polymer rotating instruments and laser. *Srpski Arhiv Za Celokupno Lekarstvo (Serbian Archives of Medicine)*, 142(5-6), pp.365–370.

93/ Mohammadi, Z., Shalavi, S., Kinoshita, J.-I., Jafarzadeh, H. and Giardino, L. (2017). Lasers in Apicoectomy: A Brief Review. *The Journal of Contemporary Dental Practice*, 18(2), pp.170–173.

94/ Froum, S.H., Cantor-Balan, R., Kerbage, C. and Froum, S.J. (2019). Thermal Testing of Titanium Implants and the Surrounding Ex-Vivo Tissue Irradiated With 9.3um CO_2 Laser. *Implant Dentistry*, 28(5), pp.463–471.

95/ Altayeb, W., Arnabat-Dominguez, J., Low, S., Abdullah, A. and Romanos, G. (2022). Laser-Assisted Esthetic Crown Lengthening: Open-Flap Versus Flapless. *The International Journal of Periodontics & Restorative Dentistry*, 42(1), pp.53–62.

96/ Parrish, J.A., Deutsch, T.F., (1984). Laser photomedicine *IEEE Journal of Quantum Electronics*, QE-20, pp.1386-1396.

97/ Karu, T.I., (1996). Mechanisms of interaction of monochromatic visible light with cells. In Effects of low-power light on biological systems. *International Society for Optics and Photonics,* Jan,Vol. 2630, pp. 2-9.

98/ Mester, E., Juhasz, J., Varga, P. and Karika, G., (1969). Contribution to the clinical application of lasers. *Lyon Chirurgical,* 65(3), pp.335-345.

99/ Mester, E., Szende, B., Tota, J.G . (1967). Effect of laser on hair growth of mice. *Kiserletes Orvostudomany*, 19, pp 628–631.

100/ Mester, E., Nagylucskay, S., Waidelich, W., Tisza, S., Greguss, P., Haina, D. and Mester, A., (1978). Effects of direct laser radiation on human lymphocytes (transl). *Archives of Dermatological Research*, 263(3), pp.241-245.

101/ Mester, E., Ludany, G., Frenyo, V., Sellyei, M., Szende, B., Gyenes, G., Ihász, M., Kiss, A.F., Döklen, A. and Tota, G.F., (1971). Experimental and clinical observations with laser. *Panminerva Medica*, 13, p.538.

102/ Karu, T. (1989). Photobiology of Low-power Laser Effects. *Health Physics*, 56(5), pp.691–704.

103/ van Breugel, H.H.F.I. and Bär, P.R.D. (1992). Power density and exposure time of He-Ne laser irradiation are more important than total energy dose in photo-biomodulation of human fibroblasts in vitro. *Lasers in Surgery and Medicine*, 12(5), pp.528–537.

104/ Hrnjak, M., Kuljić-Kapulica, N., Budisin, A., Giser, A. (1995). Stimulatory effect of low-power density He-Ne laser radiation on human fibroblasts in vitro. *Vojnosanitetski Pregled,* 52(6), pp.539-46. PMID: 8644477

105/ Conlan, M.J., Rapley, J.W. and Cobb, C.M. (1996). Biostimulation of wound healing by low-energy laser irradiation A review. *Journal of Clinical Periodontology*, 23(5), pp.492– 496.

106/ Neiburger, EJ. (1999). Rapid healing of gingival incisions by the helium-neon diode laser. *Journal of the Massachusetts Dental Society,* 48(1), pp.8-13, 40.

107/ Yagi, T., (1993). The bacterial energy-transducing NADH-quinone oxidoreductases. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1141(1), pp.1-17.

108/ Rustin, P., Munnich, A. and Rötig, A. (2002). Succinate dehydrogenase and human diseases: new insights into a well-known enzyme. *European Journal of Human Genetics*, [online] 10(5), pp.289–291.

109/ Cardol, P., Figueroa, F., Remacle, C., Franzén, L.-G. and González-Halphen, D. (2009). Oxidative Phosphorylation. *The Chlamydomonas Sourcebook*, pp.469–502.

110/ Hildebrandt, P., Lecomte, S. (1999). Biochemical Applications of Raman Spectroscopy, in *Encyclopedia of Spectroscopy and Spectrometry (Second Edition), eds Lindon, J. Academic Press, Oxford*. Pp.118–127.

111/ Pelley, J.W. (2007). Citric Acid Cycle, Electron Transport Chain, and Oxidative Phosphorylation. *Elsevier's Integrated Biochemistry*, pp.55–63.

112/ Karu, T. (1989). Laser biostimulation: a photobiological phenomenon. *Journal of Photochemistry and Photobiology*, 3, pp.638-640.

113/ Quirk, B.J., Whelan, H.T., (2020). What lies at the heart of photobiomodulation: light, cytochrome c oxidase, and nitric oxide—review of the evidence. *Photobiomodulation, Photomedicine and Laser Surgery*, 38(9), pp.527-530.

114/ Vinesh, E., Jeyapriya, S., M, Kumar, M S., Arunachalam, M. (2017). Photobiomodulation and oral wound healing. *Indian Journal of Multidisciplinary Dentistry* 7(7), pp.129-134

115/ Kim, K., Lee, J., Jang, H., Park, S., Na, J., Myung, J., Kim, M.-J., Jang, W.-S., Lee, S.-J., Kim, H., Myung, H., Kang, J. and Shim, S. (2019). Photobiomodulation Enhances the Angiogenic Effect of Mesenchymal Stem Cells to Mitigate Radiation-Induced Enteropathy. *International Journal of Molecular Sciences*, 20(5), p.1131.

116/ Heidari, M., Paknejad, M., Jamali, R., Nokhbatolfoghahaei, H., Fekrazad, R. and Moslemi, N. (2017). Effect of laser photobiomodulation on wound healing and postoperative pain following free gingival graft: A split-mouth triple-blind randomized controlled clinical trial. *Journal of Photochemistry and Photobiology B: Biology*, 172, pp.109–114.

117/ Yucesoy, T., Kutuk, N., Canpolat, D.G. and Alkan, A. (2017). Comparison of Ozone and Photo-Biomodulation Therapies on Mental Nerve Injury in Rats. *Journal of Oral and Maxillofacial Surgery*, 75(11), pp.2323–2332.

118/ Cronshaw, M., Parker, S., Anagnostaki, E., Mylona, V., Lynch, E. and Grootveld, M. (2020). Photobiomodulation and Oral Mucositis: A Systematic Review. *Dentistry Journal*, [online] 8(3), p.87.

119/ Arany, P.R. (2016). Craniofacial Wound Healing with Photobiomodulation Therapy. *Journal of Dental Research*, 95(9), pp.977–984.

120/ Hamblin, M. (2017). Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophysics*, 4(3), pp.337–361.

121/ Epstein, J.B., Raber-Durlacher, J.E., Lill, M., Linhares, Y.P.L., Chang, J., Barasch, A., Slief, R.I.C., Geuke, M., Zecha, J.A.E.M., Milstein, D.M.J. and Tzachanis, D. (2016). Photobiomodulation therapy in the management of chronic oral graft-versus-host disease. *Supportive Care in Cancer*, 25(2), pp.357–364.

122/ Chow, R.T. and Armati, P.J. (2016). Photobiomodulation: Implications for Anesthesia and Pain Relief. *Photomedicine and Laser Surgery*, [online] 34(12), pp.599–609.

123/ Cronshaw, M., Parker, S. and Arany, P. (2019). Feeling the Heat: Evolutionary and Microbial Basis for the Analgesic Mechanisms of Photobiomodulation Therapy. *Photobiomodulation, Photomedicine and Laser Surgery*, 37(9), pp.517–526.

124/ Tse, L.H. and Wong, Y.H. (2019). GPCRs in Autocrine and Paracrine Regulations. *Frontiers in Endocrinology*, 10. Art. 428. Pp.1 -11.

125/ Karu, TI., Afanas'eva, NI. (1995). Cytochrome c oxidase as the primary photoacceptor upon laser exposure of cultured cells to visible and near IR-range light. *Doklady Akademii nauk SSSR* 1995;342:693-5.

126/ Carroll, J.D., Milward, M.R., Cooper, P.R., Hadis, M. and Palin, W.M. (2014). Developments in low level light therapy (LLLT) for dentistry. *Dental Materials*, 30(5), pp.465–475.

127/ Engel, K.W., Khan, I. and Arany, P.R. (2016). Cell lineage responses to photobiomodulation therapy. *Journal of Biophotonics*, 9(11-12), pp.1148–1156.

128/ Knappe, V., Frank, F. and Rohde, E. (2004). Principles of Lasers and Biophotonic Effects. *Photomedicine and Laser Surgery*, 22(5), pp.411–417.

129/ Tunér, J. and Jenkins, P.A. (2016). Parameter Reproducibility in Photobiomodulation. *Photomedicine and Laser Surgery*, 34(3), pp.91–92.

130/ Tunér, J. (2018). Photobiomodulation: How Many Sessions and How Often? *Photomedicine and Laser Surgery*, 36(2), pp.59–60.

131/ Lanzafame, R. (2020). Light Dosing and Tissue Penetration: It Is Complicated. *Photobiomodulation, Photomedicine and Laser Surgery*, 38(7), pp.393–394.

132/ Higgins J, Savovic´ J, Page M, Elbers R, Sterne J. Assessing risk of bias in a randomized trial. In: *Cochrane Handbook for Systematic Reviews of Interventions, 2nd ed. J Higgins, J Thomas, J Chandler, M Cumpston, T Li, M*

Page, V Welch (eds.). Chichester, United Kingdom: John Wiley & Sons, 2019; pp. 205–228.

133/ Parker, S., Cronshaw, M., Anagnostaki, E., Bordin-Aykroyd, S.R. and Lynch, E. (2019). Systematic Review of Delivery Parameters Used in Dental Photobiomodulation Therapy. *Photobiomodulation, Photomedicine and Laser Surgery*, Dec;37(12):784-797.

134/ Cronshaw, M., Parker, S., Anagnostaki, E., Mylona, V., Lynch, E. and Grootveld, M. (2020). Photobiomodulation Dose Parameters in Dentistry: A Systematic Review and Meta-Analysis. *Dentistry Journal*, 8(4), p.114.

135/ Cronshaw, M., Parker, S., Anagnostaki, E., Mylona, V., Lynch, E. and Grootveld, M. (2020). Photobiomodulation and Oral Mucositis: A Systematic Review. *Dentistry Journal*, [online] 8(3), p.87.

136/ Parker, S., Anagnostaki, E., Mylona, V., Cronshaw, M., Lynch, E. and Grootveld, M. (2020). Systematic Review of Post-Surgical Laser-Assisted Oral Soft Tissue Outcomes Using Surgical Wavelengths Outside the 650–1350 nm Optical Window. *Photobiomodulation, Photomedicine and Laser Surgery*, 38(10), pp.591–606.

137/ Cronshaw, M., Parker, S., Anagnostaki, E. and Lynch, E. (2019). Systematic Review of Orthodontic Treatment Management with Photobiomodulation Therapy. *Photobiomodulation, Photomedicine and Laser Surgery*, 37(12), 862–868.

138/ Brahme, A., Lax, I. and Andreo, P. (1981). Electron Beam Dose Planning Using Discrete Gaussian Beams: Mathematical background. *Acta Radiologica: Oncology*, 20(2), pp.147–158.

139/ Tuncer, I., Özçakır-Tomruk, C., Şencift, K. and Çöloğlu, S. (2010). Comparison of Conventional Surgery and CO_2 Laser on Intraoral Soft Tissue Pathologies and Evaluation of the Collateral Thermal Damage. *Photomedicine and Laser Surgery*, 28(1), pp.75–79.

140/ White, J.M., Chaudhry, S.I., Kudler, J.J., Sekandari, N., Schoelch, M.L., Silverman, S. (1998). Nd:YAG and CO_2 Laser Therapy of Oral Mucosal Lesions. *Journal of Clinical Laser Medicine & Surgery*, 16(6), pp.299–304.

141/ Hanke, A., Fimmers, R., Frentzen, M. and Meister, J. (2021). Quantitative determination of cut efficiency during soft tissue surgery using diode lasers in the wavelength range between 400 and 1500 nm. *Lasers in Medical Science*, 36(8), pp.1633–1647.

142/ Belikov, A.V. and Skrypnik, A.V. (2018). Soft tissue cutting efficiency by 980 nm laser with carbon-, erbium-, and titanium-doped optothermal fiber converters. *Lasers in Surgery and Medicine*, 51(2), pp.185–200.

143/ Romanos, G.E., Sacks, D., Montanaro, N., Delgado-Ruiz, R., Calvo-Guirado, J.L. and Javed, F. (2018). Effect of Initiators on Thermal Changes in Soft Tissues Using a Diode Laser. *Photomedicine and Laser Surgery*, 36(7), pp.386–390.

144/ Romanos GE. (2013). Diode laser soft-tissue surgery: advancements aimed at consistent cutting, improved clinical outcomes. *Compendium of Continuing Education in Dentistry*, 34(10), pp.752-7577.

145/ Hashmi, J.T., Huang, Y.-Y., Sharma, S.K., Kurup, D.B., De Taboada, L., Carroll, J.D. and Hamblin, M.R. (2010). Effect of pulsing in low-level light therapy. *Lasers in Surgery and Medicine*, 42(6), pp.450–466.

146/ Walsh, J.T. and Cummings, J.P. (1994). Effect of the dynamic optical properties of water on midinfrared laser ablation. *Lasers in Surgery and Medicine*, 15(3), pp.295–305.

147/ Vogel, A., Venugopalan, V. (2003). Mechanisms of pulsed laser ablation of biological tissues. *Chemical Reviews*,103(2):577-644.

148/ Kaufmann, R., Hibst, R. (1996). Pulsed Erbium:YAG laser ablation in cutaneous surgery. *Lasers in Surgery and Medicine*, 1996;19(3):324-30.

149/ Hadis, M.A., Zainal, S.A., Holder, M.J., Carroll, J.D., Cooper, P.R., Milward, M.R. and Palin, W.M. (2016). The dark art of light measurement: accurate radiometry for low-level light therapy. *Lasers in Medical Science*, 31(4), pp.789–809.

150/ Srinivasan, R. and Singh, M. (2003). Laser backscattering and transillumination imaging of human tissues and their equivalent phantoms. *IEEE Transactions on Biomedical Engineering*, 50(6), pp.724–730.

151/ Cobb, C.M. (2006). Lasers in Periodontics: A Review of the Literature. *Journal of Periodontology*, 77(4), pp.545–564.

152/ Fried, N.M. (2001). Potential Applications of the Erbium:YAG Laser in Endourology. *Journal of Endourology*, 15(9), pp.889–894.

153/ Ranganathan, P., & Aggarwal, R. (2018). Study designs: Part 1 - An overview and classification. *Perspectives in clinical research*, 9(4), 184–186.

154/ Parker, S. (2007). Laser regulation and safety in general dental practice. *British Dental Journal,* 202(9), pp.523–532.

155/ Parker, S. (2007). Laser-tissue interaction. *British Dental Journal,* 202(2), pp.73–81.

156/ Aoki, A., Mizutani, K., Schwarz, F., Sculean, A., Yukna, R.A., Takasaki, A.A., Romanos, G.E., Taniguchi, Y., Sasaki, K.M., Zeredo, J.L., Koshy, G., Coluzzi, D.J., White, J.M., Abiko, Y., Ishikawa, I. and Izumi, Y. (2015). Periodontal and peri-implant wound healing following laser therapy. *Periodontology 2000*, [online] 68(1), pp.217–269.

157/ Frame, J.W. (1985). Carbon dioxide laser surgery for benign oral lesions. *British Dental Journal,* 158(4), pp.125–128.

158/ Smith, A.M., Mancini, M.C. and Nie, S. (2009). Second window for in vivo imaging. *Nature Nanotechnology*, 4(11), pp.710–711.

159/ NIR optical window - Wang LV, Wu H. (2007) Absorption and its biological origins. *In: Biomedical optics, Principles and Imaging. Hoboken, NJ: Wiley,* Ch. 1.3, pp. 5.

160/ Sordillo, L.A., Pu, Y., Pratavieira, S., Budansky, Y. and Alfano, R.R. (2014). Deep optical imaging of tissue using the second and third near-infrared spectral windows. *Journal of Biomedical Optics*, 19(5), p.056004.

161/ Karu, T.I. and Kolyakov, S.F. (2005). Exact action spectra for cellular responses relevant to phototherapy. *Photomedicine and Laser Surgery*, [online] 23(4), pp.355–361.

162/ Huang, Y.-Y., Chen, A.C.-H. ., Carroll, J.D. and Hamblin, M.R. (2009). Biphasic Dose Response in *Low Level Light Therapy*. *Dose-Response*, [online] 7(4), p.dose-response.0.

163/ Chung, H., Dai, T., Sharma, S.K., Huang, Y.-Y., Carroll, J.D. and Hamblin, M.R. (2011). The Nuts and Bolts of Low-level Laser (Light) Therapy. *Annals of Biomedical Engineering*, 40(2), pp.516–533.

164/ Hamblin, M. (2017). Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophysics*, 4(3), pp.337–361.

165/ George, S., Hamblin, M.R. and Abrahamse, H. (2018). Effect of red light and near infrared laser on the generation of reactive oxygen species in primary dermal fibroblasts. *Journal of Photochemistry and Photobiology B: Biology*, 188, pp.60–68.

166/ Flores Luna, G.L., de Andrade, A.L.M., Brassolatti, P., Bossini, P.S., Anibal, F. de F., Parizotto, N.A. and Leal, Â.M. de O. (2020). Biphasic Dose/Response of Photobiomodulation Therapy on Culture of Human Fibroblasts. *Photobiomodulation, Photomedicine and Laser Surgery*, 38(7), pp.413–418.

167/ Hamblin, M., Ferraresi, C., Huang, Y-Y., Freitas de Freitas, L., Carroll, J. (2018). Low-level light therapy : photobiomodulation. *SPIE Press, Series: Tutorial texts in optical engineering* ; volume TT 115. Ch. 1.7, pp.13. ISBN 9781510614161.

168/ Murty, G.E., Diver, J.P. and Bradley, P.J. (1993). Carcinoma in Situ of the Glottis: Radiotherapy or Excision Biopsy? *Annals of Otology, Rhinology & Laryngology*, 102(8), pp.592–595.

169/ Egawa, S., Ishioka, K., & Kawada, Y. (1984). Trials of combined radiation and hyperthermia with various heating modalities in cancer therapy. *Radiation Medicine*, 2(4), 260–264.

170/ Jin, J. Y., Lee, S. H., & Yoon, H. J. (2010). A comparative study of wound healing following incision with a scalpel, diode laser or Er,Cr:YSGG laser in guinea pig oral mucosa: A histological and immunohistochemical analysis. *Acta Odontologica Scandinavica*, 68(4), 232–238

171/ Gajendran, P., Selvaganesh, S., Nesappan, T. and Prabhu, A. (2021). Comparison of clinical efficacy of diode laser and erbium, chromium: Yttrium, scandium, gallium, and garnet for implant stage 2 recovery procedure – A randomized control clinical study. *Journal of Indian Society of Periodontology*, 25(4), p.335.

172/ Sindel, A., Dereci, O., Hatipoglu, M., Ozalp, O., Dereci, O.N., Kocabalkan, B. and Ozturk, A. (2019). Evaluation of temperature rise following the application of diode and ErCr:Ysgg lasers: an ex vivo study. *European Oral Research*, 52(3), pp.131–136.

173/ Ryu, S.-W., Lee, S.-H. and Yoon, H.-J. (2011). A comparative histological and immunohistochemical study of wound healing following incision with a scalpel, CO_2 laser or Er,Cr:YSGG laser in the Guinea pig oral mucosa. *Acta Odontologica Scandinavica*, 70(6), pp.448–454.

174/ Palaia, G., Pergolini, D., D'Alessandro, L., Carletti, R., Del Vecchio, A., Tenore, G., Di Gioia, C.R.T. and Romeo, U. (2020). Histological Effects of an Innovative 445 Nm Blue Laser During Oral Soft Tissue Biopsy. *International Journal of Environmental Research and Public Health*, 17(8), p.2651.

175/ Romeo, U., Russo, C., Palaia, G., Lo Giudice, R., Del Vecchio, A., Visca, P., Migliau, G. and De Biase, A. (2014). Biopsy of Different Oral Soft Tissues Lesions by KTP and Diode Laser: Histological Evaluation. *The Scientific World Journal*, 2014, pp.1–6.

176/ Tuchin, V.V. (1997). Light propagation in tissues with controlled optical properties. *Journal of Biomedical Optics*, 2(4), p.401.

177/ Enoch, S., Price, PE,. (2004). Cellular, molecular and biochemical differences in the pathophysiology of healing between acute wounds, chronic wounds, and wounds in the aged. *World Wide Wounds*. Available from http://www.worldwidewounds.com//2004/august/Enoch/Pathophysiology-Of-Healing.html.

178/ Bargiela-Pérez, P., González-Merchan, J., Díaz-Sanchez, R., Serrera-Figallo, M. A., Volland, G., Joergens, M., Gutiérrez-Perez, J. L., & Torres-Lagares, D. (2018). Prospective study of the 532 nm laser (KTP) versus diode laser 980 nm in the resection of hyperplastic lesions of the oral cavity. *Medicina Oral, Patologia Oral, Cirugia Bucal* 23(1), e78–e85.

179/ Suter, V. G., Altermatt, H. J., Sendi, P., Mettraux, G., & Bornstein, M. M. (2010). CO_2 and diode laser for excisional biopsies of oral mucosal lesions. A pilot study

evaluating clinical and histopathological parameters. *Schweizer Monatsschrift fur Zahnmedizin* 120(8), 664–671.

180/ Serrage, H., Heiskanen, V., Palin, W.M., Cooper, P.R., Milward, M.R., Hadis, M. and Hamblin, M.R. (2019). Under the spotlight: mechanisms of photobiomodulation concentrating on blue and green light. *Photochemical & Photobiological Sciences*, 18(8), pp.1877–1909.

181/ Hamblin, M.R. and Liebert, A. (2022). Photobiomodulation Therapy Mechanisms Beyond Cytochrome c Oxidase. *Photobiomodulation, Photomedicine and Laser Surgery*, 40(2), pp.75–77.

182/ Kushibiki, T., Hirasawa, T., Okawa, S., & Ishihara, M. (2013). Blue laser irradiation generates intracellular reactive oxygen species in various types of cells. *Photomedicine and Laser Surgery*, 31(3), 95–104.

183/ Bouly, J. P., Schleicher, E., Dionisio-Sese, M., Vandenbussche, F., Van Der Straeten, D., Bakrim, N., Meier, S., Batschauer, A., Galland, P., Bittl, R., & Ahmad, M. (2007). Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. *The Journal of Biological Chemistry*, 282(13), 9383–9391.

184/ Day, R. M., & Suzuki, Y. J. (2006). Cell proliferation, reactive oxygen and cellular glutathione. *Dose-response : a publication of International Hormesis Society*, 3(3), 425–442.

185/ Lin, T., Yu, C.-C., Liu, C.-M., Hsieh, P.-L., Liao, Y.-W., Yu, C.-H. and Chen, C.-J. (2021). Er:YAG laser promotes proliferation and wound healing capacity of

human periodontal ligament fibroblasts through Galectin-7 induction. *Journal of the Formosan Medical Association*, 120(1), pp.388–394.

186/ Ogita, M., Tsuchida, S., Aoki, A., Satoh, M., Kado, S., Sawabe, M., Nanbara, H., Kobayashi, H., Takeuchi, Y., Mizutani, K., Sasaki, Y., Nomura, F. and Izumi, Y. (2014). Increased cell proliferation and differential protein expression induced by low-level Er:YAG laser irradiation in human gingival fibroblasts: proteomic analysis. *Lasers in Medical Science*, 30(7), pp.1855–1866.

187/ Talebi-Ardakani, M. R., Torshabi, M., Karami, E., Arbabi, E., & Rezaei Esfahrood, Z. (2016). In Vitro Study of Er:YAG and Er, Cr:YSGG Laser Irradiation on Human Gingival Fibroblast Cell Line. *Acta Medica Iranica*, 54(4), 251–255.

188/ Ohsugi, Y., Niimi, H., Shimohira, T., Hatasa, M., Katagiri, S., Aoki, A. and Iwata, T. (2020). In Vitro Cytological Responses against Laser Photobiomodulation for Periodontal Regeneration. *International Journal of Molecular Sciences*, 21(23), p.9002.

189/ Adapted from: Kim WS, Calderhead RG. Is light-emitting diode phototherapy (LED-LLLT) really effective? *Laser Therapy* 2011;20:205–15.

190/ Wang, Y., Huang, Y.-Y., Wang, Y., Lyu, P. and Hamblin, M.R. (2017). Photobiomodulation of human adipose-derived stem cells using 810nm and 980nm lasers operates via different mechanisms of action. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1861(2), pp.441–449.

191/ Schultz, H. (1888) Uber hefegifte (About yeast poisons). *Pflugers Archive* 42, 517–541.

192/ Lubart, R., Lavi, R., Friedmann, H. and Rochkind, S. (2006). Photochemistry and Photobiology of Light Absorption by Living Cells. *Photomedicine and Laser Surgery*, 24(2), pp.179–185.

193/ Khan, I., Tang, E., Arany, P. (2015). Molecular pathway of near infrared laser phototoxicity involves ATF-4 orchestrated ER stress. *Science Reports*, 5, pp 10581.

194/ Isolan, CP., de Azevedo Kinalski, M., de Andrade Lea˜o, OA., et al. (2021). Photobiomodulation therapy reduces postoperative pain after third molar extractions: a randomized clinical trial. *Medicina Oral, Patologia Oral, Cirugia Bucal*, pp e341.

195/ Hadis, M., Zainal, SA., Holder, MJ., et al. (2016). The dark art of light measurement: accurate radiometry for low-level light therapy. *Lasers in Medical Science*, 31, pp 789–809.

196/ Melnik, I., Steiner, R., Kienle, A. (1993). Light penetration in human skin: in-vivo measurements using isotropic detector. *Proceedings of SPIE - Society of Photo-Optical Instrumentation Engineers*, 1881, 146313.

197/ Sommer, AP. (2019). Revisiting the photon/cell interaction mechanism in low-level light therapy. *Photobiomodulation, Photomedicine and Laser Surgery*, 37, pp 336–341.

198/ Ikeda, T., Tayefeh, F., Sessler, D. I., Kurz, A., Plattner, O., Petschnigg, B., Hopf, H. W., & West, J. (1998). Local radiant heating increases subcutaneous oxygen tension. *American Journal of Surgery*, 175(1), 33–37.

199/ Hughes, M. A., Tang, C., & Cherry, G. W. (2003). Effect of intermittent radiant warming on proliferation of human dermal endothelial cells in vitro. *Journal of Wound Care*, 12(4), 135–137.

200/ Xia, Z., Sato, A., Hughes, M. A., & Cherry, G. W. (2000). Stimulation of fibroblast growth in vitro by intermittent radiant warming. *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society*, 8(2), 138–144

201/ The Lancet (2022). Managing the opioid crisis in North America and beyond. *The Lancet*, 399(10324), p.495.

_____ // _____

Reference List for Published Papers

(i) **Parker, S., Cronshaw, M., Anagnostaki, E., Mylona, V., Lynch, E. and Grootveld, M. (2020). Current Concepts of Laser–Oral Tissue Interaction. *Dentistry Journal*, 8(3), p.61.**

1/ Parker, S. (2017).: Laser-Tissue Interaction, in *Lasers in Dentistry - Current Concepts. Eds Coluzzi, D., Parker, S., Springer Nature: Cham, Switzerland*, Chapter 3, pp. 29–53.

2/ Parker, S., Cronshaw, M., Anagnostaki, E., Lynch, E. (2019). Laser Essentials for the Dental Practitioner: Foundation Knowledge-Construction, Modes of Operation and Safety. *EC Dental. Science.* 18, pp 2020–2027.

3/ Mó, O., Yáñez, M., Eckert-Maksić, M., Maksić, Z.B., Alkorta, I., Elguero, J. (2005). Periodic trends in bond dissociation energies. A theoretical study. *The Journal of Physical Chemistry A*109, pp 4359–4365.

4/ Fisher J. C. (1992). Photons, physiatrics, and physicians: a practical guide to understanding laser light interaction with living tissue, part I. *Journal of Clinical Laser Medicine and Surgery*, 10(6), 419–426.

5/ Boulnois, J.-L. (1986). Photophysical processes in recent medical laser developments: A review. *Lasers in Medical Science*, 1, pp 47–66.

6/ Mooney, J., Kambhampati, P. (2013). Get the Basics Right: Jacobian Conversion of Wavelength and Energy Scales for Quantitative Analysis of Emission Spectra. *The Journal of Physical Chemistry Letters*, 4, pp 3316–3318.

7/ Sutherland J. C. (2002). Biological effects of polychromatic light. *Photochemistry and Photobiology*, 76(2), pp 164–170.

8/ Knappe, V., Frank, F., & Rohde, E. (2004). Principles of lasers and biophotonic effects. *Photomedicine and Laser Surgery*, 22(5), pp 411–417.

9/ Zhang, D., & Tamilselvan, A. (2007). Lattice energy and mechanical stiffness of hydroxyapatite. Journal of materials science. *Materials in Medicine*, 18(1), 79–87.

10/ Parker S. (2007). Verifiable CPD paper: laser-tissue interaction. *British Dental Journal*, 202(2), 73–81.

11/ Karu T. (1999). Primary and secondary mechanisms of action of visible to near-IR radiation on cells. *J. Photochemistry and Photobiology. B, Biology*, 49(1), pp 1–17.

12/ Robertson, C. A., Evans, D. H., & Abrahamse, H. (2009). Photodynamic therapy (PDT): a short review on cellular mechanisms and cancer research applications for PDT. *J. Photochemistry and Photobiology. B, Biology*, 96(1), 1–8.

13/ Maisch T. (2009). A new strategy to destroy antibiotic resistant microorganisms: antimicrobial photodynamic treatment. *Mini Reviews in Medicinal Chemistry*, 9(8), 974–983.

14/ Hamblin, M.R., Demidova, T.N. (2006). Mechanisms of low level light therapy in *Mechanisms for Low-Light Therapy*; *Hamblin, M.R., Waynant, R.W., Anders, J., Eds.* Proc. SPIE 6140; SPIE-The International Society for Optical Engineering: Bellingham, WA, USA, pp. 614001.

15/ Crippa, R., Fruili, S., Parker, S., Angiero, F., Benedicenti, S., Berne, E. (2013). Management of intraoral venous malformations using laser-assisted forced dehydration induced photocoagulation. *IL Dentista Moderna* 9, pp 80–97.

16/ Jain, K., Gorisch, W. (1979). Repair of small blood vessels with the Neodymium-YAG laser: A preliminary report. *Surgery*, 85, pp 684–688.

17/ Suragimath, G., Lohana, M. H., & Varma, S. (2016). A Split Mouth Randomized Clinical Comparative Study to Evaluate the Efficacy of Gingival Depigmentation Procedure Using Conventional Scalpel Technique or Diode Laser. *Journal of Lasers in Medical Sciences*, 7(4), pp 227–232.

18/ Zaffe, D., Vitale, M. C., Martignone, A., Scarpelli, F., & Botticelli, A. R. (2004). Morphological, histochemical, and immunocytochemical study of CO_2 and Er:YAG laser effect on oral soft tissues. *Photomedicine and Laser Surgery*, 22(3), pp 185–189.

19/ Smith, K. (1991). The Photobiological Basis of Low Level Laser Radiation Therapy. *Laser Therapy,* 36, pp 19–24.

20/ Chung, H., Dai, T., Sharma, S. K., Huang, Y. Y., Carroll, J. D., & Hamblin, M. R. (2012). The nuts and bolts of low-level laser (light) therapy. *Annals of Biomedical Engineering*, 40(2), pp 516–533.

21/ Karu, T. (2014). Cellular and molecular mechanisms of photobiomodulation (low level laser therapy). *IEEE Journal of Selected Topics in Quantum Electronics*, 20, pp 143–148.

22/ Hamblin M. R. (2017). Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophysics*, 4(3), pp 337–361.

23/ Amaroli, A., Ravera, S., Baldini, F., Benedicenti, S., Panfoli, I., & Vergani, L. (2019). Photobiomodulation with 808-nm diode laser light promotes wound healing of human endothelial cells through increased reactive oxygen species production stimulating mitochondrial oxidative phosphorylation. *Lasers in Medical Science*, *34*(3), pp 495–504.

24/ Kim, K., Lee, J., Jang, H., Park, S., Na, J., Myung, J. K., Kim, M. J., Jang, W. S., Lee, S. J., Kim, H., Myung, H., Kang, J., & Shim, S. (2019). Photobiomodulation Enhances the Angiogenic Effect of Mesenchymal Stem Cells to Mitigate

Radiation-Induced Enteropathy. *International Journal of Molecular Sciences*, *20*(5), pp 1131.

25/ Heidari, M., Paknejad, M., Jamali, R., Nokhbatolfoghahaei, H., Fekrazad, R., & Moslemi, N. (2017). Effect of laser photobiomodulation on wound healing and postoperative pain following free gingival graft: A split-mouth triple-blind randomized controlled clinical trial. *Journal of Photochemistry and Photobiology. B, Biology*, *172*, pp 109–114.

26/ Arany P. R. (2016). Craniofacial Wound Healing with Photobiomodulation Therapy: New Insights and Current Challenges. *Journal of Dental Research*, *95*(9), pp 977–984.

27/ Chow, R. T., & Armati, P. J. (2016). Photobiomodulation: Implications for Anesthesia and Pain Relief. *Photomedicine and Laser Surgery*, 34(12), pp 599–609.

28/ Sugaya, N. N., Silva, É. F., Kato, I. T., Prates, R., Gallo, C. B., & Pellegrini, V. D. (2016). Low Intensity laser therapy in patients with burning mouth syndrome: a randomized, placebo-controlled study. *Brazilian Oral Research*, *30*(1), e108.

29/ Dias, S. B., Fonseca, M. V., Dos Santos, N. C., Mathias, I. F., Martinho, F. C., Junior, M. S., Jardini, M. A., & Santamaria, M. P. (2015). Effect of GaAlAs low-level laser therapy on the healing of human palate mucosa after connective tissue graft harvesting: randomized clinical trial. *Lasers in Medical Science*, *30*(6), pp 1695–1702.

30/ Arbabi-Kalati, F., Arbabi-Kalati, F., & Moridi, T. (2013). Evaluation of the effect of low level laser on prevention of chemotherapy-induced mucositis. *Acta medica Iranica*, *51*(3), pp 157–162.

31/ Shirani, A. M., Gutknecht, N., Taghizadeh, M., & Mir, M. (2009). Low-level laser therapy and myofacial pain dysfunction syndrome: a randomized controlled clinical trial. *Lasers in Medical Science*, 24(5), pp 715–720.

32/ Caccianiga, G., Paiusco, A., Perillo, L., Nucera, R., Pinsino, A., Maddalone, M., Cordasco, G., & Lo Giudice, A. (2017). Does Low-Level Laser Therapy Enhance the Efficiency of Orthodontic Dental Alignment? Results from a Randomized Pilot Study. *Photomedicine and Laser Surgery*, *35*(8), pp 421–426.

33/ Farias, R. D., Closs, L. Q., & Miguens, S. A., Jr (2016). Evaluation of the use of low-level laser therapy in pain control in orthodontic patients: A randomized split-mouth clinical trial. *The Angle Orthodontist*, 86(2), pp 193–198.

34/ Cronshaw, M., Parker, S., & Arany, P. (2019). Feeling the Heat: Evolutionary and Microbial Basis for the Analgesic Mechanisms of Photobiomodulation Therapy. *Photobiomodulation, Photomedicine and Laser Surgery*, *37*(9), pp 517–526.

35/ Vinesh, E., Jeyapriya, S.M., Kumar, M.S., Arunachalam, M. (2017). Photobiomodulation and oral wound healing. *Indian Journal of Multidisciplinary Dentistry*, 7, pp 129–134

36/ Hode, L., Tuner, J. (2014). *Laser Phototherapy*; Prima Books in Sweded AB: Grangesberg, Sweden, pp. 92–94.

37/ Kim, A., Roy, M., Dadani, F. N., & Wilson, B. C. (2010). Topographic mapping of subsurface fluorescent structures in tissue using multiwavelength excitation. *Journal of Biomedical Optics*, *15*(6), 066026.

38/ Rajan, V., Varghese, B., van Leeuwen, T. G., & Steenbergen, W. (2009). Review of methodological developments in laser Doppler flowmetry. *Lasers in Medical Science*, *24*(2), pp 269–283.

39/ Jayanthi, J. L., Nisha, G. U., Manju, S., Philip, E. K., Jeemon, P., Baiju, K. V., Beena, V. T., & Subhash, N. (2011). Diffuse reflectance spectroscopy: diagnostic accuracy of a non-invasive screening technique for early detection of malignant changes in the oral cavity. *British Medical Journal Open*, 1(1), e000071.

40/ Eom, J., Ahn, J., Eom, J., Park, A. (2018). Wide field of view optical coherence tomography for structural and functional diagnoses in dentistry. *Journal of Biomedical Optics,* 23, pp 1–8.

41/ Torres, C. P., Miranda Gomes-Silva, J., Menezes-Oliveira, M., Silva Soares, L. E., Palma-Dibb, R. G., & Borsatto, M. C. (2018). FT-Raman spectroscopy, µ-EDXRF spectrometry, and microhardness analysis of the dentin of primary and permanent teeth. *Microscopy research and technique*, *81*(5), pp 509–514.

42/ Driessens, F. C., van Dijk, J. W., & Borggreven, J. M. (1978). Biological calcium phosphates and their role in the physiology of bone and dental tissues I. Composition and solubility of calcium phosphates. *Calcified Tissue Research*, *26*(2), pp 127–137.

43/ Poli, R., Parker, S. (2015). Achieving dental analgesia with the erbium chromium yttrium scandium gallium garnet laser (2780nm): A protocol for painless conservative treatment. *Photomedicine and Laser Surgery*, 33, pp 1–8.

44/ Dederich D. N. (1993). Laser/tissue interaction: what happens to laser light when it strikes tissue?. *Journal of the American Dental Association (1939)*, *124*(2), pp 57–61.

45/ Parker, S., Cronshaw, M., Anagnostaki, E., Bordin-Aykroyd, S. R., & Lynch, E. (2019). Systematic Review of Delivery Parameters Used in Dental Photobiomodulation Therapy. *Photobiomodulation, Photomedicine and Laser Surgery*, *37*(12), pp 784–797.

_____ // _____

(ii) **Parker, S., Cronshaw, M. and Grootveld, M. (2022). Photobiomodulation Delivery Parameters in Dentistry: An Evidence-Based Approach. *Photobiomodul, Photomed Laser Surg*, 40(1), pp 42-50.**

1/ Parker, S., Cronshaw, M., Anagnostaki, E., Bordin-Aykroyd, S. R., & Lynch, E. (2019). Systematic Review of Delivery Parameters Used in Dental Photobiomodulation Therapy. *Photobiomodulation, Photomedicine and Laser Surgery*, *37*(12), pp 784–797.

2/ Cronshaw, M., Parker, S., Anagnostaki, E., & Lynch, E. (2019). Systematic Review of Orthodontic Treatment Management with Photobiomodulation Therapy. *Photobiomodulation, Photomedicine and Laser Surgery*, *37*(12), pp 862–868.

3/ Cronshaw, M., Parker, S., Anagnostaki, E., Mylona, V., Lynch, E., & Grootveld, M. (2020). Photobiomodulation Dose Parameters in Dentistry: A Systematic Review and Meta-Analysis. *Dentistry Journal*, *8*(4), pp 114.

4/ Parker, S., Cronshaw, M., Anagnostaki, E., Mylona, V., Lynch, E., & Grootveld, M. (2020). Current Concepts of Laser-Oral Tissue Interaction. *Dentistry Journal*, *8*(3), pp 61.

5/ Pedrotti, FL., Pedrotti, LM., Pedrotti, LS. (2007) Fiber Optics. *In: Introduction to optics pages. 3rd ed. Upper Saddle River, N.J: Pearson Prentice Hall* pp 243–260

6/ Papadopoulos, I. N., Farahi, S., Moser, C., & Psaltis, D. (2012). Focusing and scanning light through a multimode optical fiber using digital phase conjugation. *Optics Express*, *20*(10), pp 10583–10590.

7/ Sakr, H., Chen, Y., Jasion, G.T., Bradley, T.D., Hayes, J.R., Mulvad, H.C.H., Davidson, I.A., Numkam Fokoua, E. and Poletti, F. (2020). Hollow core optical fibres with comparable attenuation to silica fibres between 600 and 1100 nm. *Nature Communications*, [online] 11(1), p.6030.

8/ Gao, Y., Feng, G., Liu, Y., Zhou, S. and Zhu, S. (2013). The Loss of Optical Fiber with Pure Quartz Core and Fluorine—Doped Glass Cladding. *Optics and Photonics Journal*, 03(01), pp.117–121.

9/ Werzinger, S., Bunge, CA. (2015) Statistical analysis of intrinsic and extrinsic coupling losses for step-index polymer optical fibers. *Optics Express* 23(17):22318–29.

10/ Stopp, S., Svejdar, D., Deppe, H., Lueth, TC. (2007) A new method for optimized laser treatment by laser focus navigation and distance visualization. *Conference*

Proceedings of the IEEE Engineering in Medicine and Biology Society, pp 1738–1741.

11/ Allardice, JT., Abulafi, AM., Dean, R. et al. (1993) Light delivery systems for adjunctive intraoperative photodynamic therapy. *Lasers in Medical Science*, 8, pp 1–14.

12/ Verdaasdonk, R., van Swol, C. (1997) Laser light delivery systems for medical applications. *Physics in Medicine and Biology*, 42:869.

13/ Wei Shi, Y., Ito, K., Matsuura, Y., Miyagi, M. (2005) Multiwavelength laser light transmission of hollow optical fiber from the visible to the mid-infrared *Optics Letters*, 30, pp 2867–2869

14/ Beck, T., Reng, NK. (1993) Richter fiber type and quality dictate beam delivery characteristics. *Laser Focus World* 29, pp 111–115

15/ Hunter, B., Leong, K., Miller, C., Golden, J., Glesias, R., Laverty, P. (1996) Selecting a high-power fiber-optic laser beam delivery system. *The International Congress on Applications of Lasers & Electro-Optics*, E173-E182.

16/ Ballato, J., Hawkins, T., Foy, P., et al (2008) Silicon optical fiber. *Optics Express* 16(23), pp 18675–83.

17/ Baranyai, L. (2020) Laser induced diffuse reflectance imaging-Monte Carlo simulation of backscattering measured on the surface. *Methods X*. 7, 100958.

18/ Jacques, SL. (1998) Light distributions from point, line and plane sources for photochemical reactions and fluorescence in turbid biological tissues. *Photochemistry and Photobiology*, 67(1), pp 23–32.

19/ Tuchin, V. (2015) Optical properties of tissues with strong (multiple) scattering. *In Tissue optics, 3rd edn.* SPIE Press Bellingham, Wa, USA, pp 3–18.

20/ Hode, T., Duncan, D., Kirkpatrick, S. et al (2009) The importance of coherence in phototherapy. *Proceedings of SPIE - The International Society for Optical Engineering*, 7165–716507.

21/ Steiner, R. (2011) Laser-tissue interaction. *Laser and IPL Technology in Dermatology and Aesthetic Medicine Springer-Verlag* pp 23–39.

22/ Karu, T. (1999) Primary and secondary mechanisms of action of visible to near-IR radiation on cells. *Journal of Photochemistry and Photobiology B* 49, pp 1–17.

23/ Alvarenga, L., Ribeiro, M., Kato, I. (2018) Evaluation of red light scattering in gingival tissue-in vivo study. *Photodiagnosis and Photodynamic Therapy* 23, pp 32–34

24/ Cronshaw, M., Parker, S., Arany, P. (2019) Feeling the heat: evolutionary and microbial basis for the analgesic mechanisms of photobiomodulation therapy. *Photobiomodulation, Photomedicine and Laser Surgery*, 37(9), pp 517–526.

25/ Lister, T., Wright, P., Chappell, P. (2012) Optical properties of human skin. *Journal of Biomedical Optics,* 17(9), 090901.

26/ Dasgupta, A., Wahed, A. (2014) *Clinical chemistry, immunology and laboratory quality control Elsevier,* pp. 47–49.

27/ Michel, AP., Liakat, S., Bors, K., Gmachl, CF. (2013) In vivo measurement of mid-infrared light scattering from human skin. *Biomedical Optics Express*, 4(4), pp 520–30.

28/ Enwemeka, CS. (2008) Standard parameters in laser phototherapy. *Photomedicine and Laser Surgery*, 26, 5, pp 411.

29/ Anders, JJ., Lanzafame, RJ., Arany, PR. (2015) Low-level light/laser therapy versus photobiomodulation therapy. *Photomedicine and Laser Surgery*, 33(4), pp 183–4.

30/ Lanzafame, R. (2020) Light dosing and tissue penetration: it is complicated. *Photobiomodulation, Photomedicine and Laser Surgery*, 38, pp 7393–7394.

31/ Fekrazad, R., Arany, P. (2019) Photobiomodulation therapy in clinical dentistry. *Photobiomodulation, Photomedicine and Laser Surgery*, 37(12), pp 737–738.

32/ Parker, S. (2007) Laser tissue interaction. *Br Dent J* 202(2), pp 73–81.

33/ Gadzhula, N., Shinkaruk-Dykovytska, M., Cherepakha, O., Goray, M., Horlenko, I. (2020) Efficiency of using the diode laser in the treatment of periodontal inflammatory diseases. *Wiadomosci Lekarskie*, 73(5), pp 841–845.

34/ Mulder-van Staden, S., Holmes, H., Hille, J. (2020) In vivo investigation of diode laser application on red complex bacteria in non-surgical periodontal therapy: a split-mouth randomised control trial. *Scientific Reports*, 4(10), 1–21311.

35/ Chiang, C., Hsieh, O., Tai, W., Chen, Y., Chang, P. (2020) Clinical outcomes of adjunctive indocyanine green-diode lasers therapy for treating refractory periodontitis: a randomized controlled trial with in vitro assessment. *Journal of the Formosan Medical Association* 119(2), pp 652–659.

36/ Albaker, A., ArRejaie, A., Alrabiah, M., Abduljabbar, T. (2018) Effect of photodynamic and laser therapy in the treatment of peri-implant mucositis: a systematic review. *Photodiagnosis and Photodynamic Therapy*, 21, pp 147–152

37/ Borzabadi-Farahani, A. (2017) The adjunctive soft-tissue diode laser in orthodontics. *Compendium of Continuing Education in Dentistry*, 38(eBook 5),e18-e31

38/ Anagnostaki, E., Mylona, V., Parker, S., Lynch, E., Grootveld, M. (2020) Systematic review on the role of lasers in endodontic therapy: valuable adjunct treatment? *Dentistry Journal*, 8, pp 63.

39/ Kulkarni, S., Meer, M., George, R. (2020) The effect of photobiomodulation on human dental pulp-derived stem cells: systematic review. *Lasers in Medical Science,* 35(9), pp 1889–1897.

40/ Pang, P., Andreana, S., Aoki, A., Coluzzi, D., Obeidi, A., Olivi, G., Parker, S., Rechmann, P., Sulewski, J., Sweeney, C., Swick, M., Yung, F. (2010) Laser energy in oral soft tissue applications. *Journal of Laser Dentistry,* 18(3), pp123–131.

41/ Romanos, GE. (2013) Diode laser soft-tissue surgery: advancements aimed at consistent cutting, improved clinical outcomes. *Compendium of Continuing Education in Dentistry,* 34, pp 10752–10757.

42/ Hadis, M., Zainal, SA., Holder, MJ., Carroll, JD., Cooper, PR., Milward, MR., Palin, WM. (2016) The dark art of light measurement: accurate radiometry for low-level light therapy. *Lasers in Medical Science,* 31, pp 789–809.

43/ Anagnostaki, E., Mylona, V., Kosma, K., Parker, S., Chala, M., Cronshaw, M., Dimitriou, V., Tatarakis, M., Papadogiannis, N., Lynch, E., Grootveld, M. (2020) A spectrophotometric study on light attenuation properties of dental bleaching gels: potential relevance to irradiation parameters. *Dentistry Journal*, 8, pp 137.

44/ Chala, M., Anagnostaki, E., Mylona, V., Chalas, A., Parker, S., Lynch, E. (2020) Adjunctive use of lasers in peri-implant mucositis and periimplantitis treatment: a systematic review. *Dentistry Journal*, 8, pp 63–68.

45/ Mylona, V., Anagnostaki, E., Parker, S., Cronshaw, M., Lynch, E., Grootveld, M. (2020) Laser-assisted aPDT protocols in randomized controlled clinical trials in dentistry: a systematic review. *Dentistry Journal*, 8, pp107.

46/ Higgins, J., Savović, J., Page, M., Elbers, R., Sterne, J. (2019) Assessing risk of bias in a randomized trial. *In: Higgins J, Thomas J, Chandler J, Cumpston M, Li T, Page M, Welch V (eds) Cochrane handbook for systematic reviews of interventions, 2nd edn. John Wiley & Sons, Chichester, United Kingdom*, pp 205–228.

47/ Alvarenga, LH., Ribeiro, MS., Kato, IT., Núñez, SC., Prates, RA. (2018) Evaluation of red light scattering in gingival tissue-in vivo study. *Photodiagnosis and Photodynamic Therapy*, 23, pp 32–34.

_____ // _____

(iii) Parker, S., Cronshaw, M., Grootveld, M., George, R., Anagnostaki, E., Mylona, V., Chala, M., & Walsh, L. (2022). The influence of delivery power losses and full operating parametry on the effectiveness of diode visible-near infra-red (445-1064 nm) laser therapy in dentistry-a multi-centre investigation. *Lasers Med Sci*. 2022 Jan 14. doi: 10.1007/s10103-021-03491-y. Online ahead of print.

1/ Smith, K.C., 1991. The photobiological basis of low level laser radiation therapy. *Laser Therapy*, *3*(1), pp.19-24.

2/ Medical Subject Heading (MeSH). U.S. National Library of Medicine 2015.

3/ Hamblin M. R. (2017). Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophysics*, *4*(3), 337–361.

4/ Parker S. (2007). Lasers and soft tissue: 'loose' soft tissue surgery. *British Dental Journal*, *202*(4), 185–191.

5/ Steiner R. (2011). Laser-tissue interactions. *In: Raulin C, Karsai S. Laser and IPL Technology in Dermatology and Aesthetic Medicine.* New York: Springer, Ch.2 pp. 23–36.

6/ Tuchin VV. (2015). *Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis.* Bellingham, WA: SPIE—The International Society for Optical Engineering.

7/ Karu T. (1996). Mechanisms of interaction of monochromatic visible light with cells. *In: Proceedings of SPIE 2639. Bellingham, WA: SPIE—The International Society for Optical Engineering*, pp. 2–9.

8/ Parrish, JA., Deutsch, TF. (1984). Laser photomedicine. *IEEE Journal of Quantum Electronics* QE-20, pp 1386–1396.

9/ Kudoh, T., 1989. Effects of 830 nm gallium aluminium arsenide diode laser radiation on rat saphenous nerve sodiumpotassium-adenosine triphosphatase activity: a possible pain attenuation mechanism examined. *Laser Therapy*, *1*, pp.63- 67.

10/ Sordillo, L. A., Pu, Y., Pratavieira, S., Budansky, Y., & Alfano, R. R. (2014). Deep optical imaging of tissue using the second and third near-infrared spectral windows. *Journal of Biomedical Optics*, *19*(5), 056004

11/ Huang, Y. Y., Chen, A. C., Carroll, J. D., & Hamblin, M. R. (2009). Biphasic dose response in low level light therapy. *Dose-response : a publication of International Hormesis Society*, *7*(4), 358–383.

12/ Anderson, R. R., & Parrish, J. A. (1981). The optics of human skin. *The Journal of Investigative Dermatology*, *77*(1), 13–19.

13/ Curcio, J.A. and Petty, C.C., 1951. The near infrared absorption spectrum of liquid water. *Journal of the Optical Society of America*, *41*(5), pp.302-304.

14/ Wang LV, Wu H. (2007). Absorption and its biological origins. *In: Biomedical optics, Principles and Imaging. Hoboken, NJ: Wiley*, Ch. 1.3, pp. 5.

15/ Moher, D., Liberati, A., Tetzlaff, J., Altman, DG., Group, TP. (2009). Preferred reporting items for systematic reviews and metaanalyses: the PRISMA statement (reprinted from annals of internal medicine). *Public Library of Science,* 6, e1000097.

16/ Higgins, J. P., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., Savovic, J., Schulz, K. F., Weeks, L., Sterne, J. A., Cochrane Bias Methods Group, & Cochrane Statistical Methods Group (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *British Medical Journal (Clinical Research ed.), 343*, d5928.

17/ Gobbo, M., Bussani, R., Perinetti, G., Rupel, K., Bevilaqua, L., Ottaviani, G., & Biasotto, M. (2017). Blue diode laser versus traditional infrared diode laser and quantic molecular resonance scalpel: clinical and histological findings after excisional biopsy of benign oral lesions. *Journal of Biomedical Optics*, *22*(12), 121602.

18/ Rocca, J. P., Zhao, M., Fornaini, C., Tan, L., Zhao, Z., & Merigo, E. (2018). Effect of laser irradiation on aphthae pain management: A four different wavelengths comparison. *Journal of Photochemistry and Photobiology. B, Biology*, *189*, 1–4.

19/ Romeo, U., Libotte, F., Palaia, G., Galanakis, A., Gaimari, G., Tenore, G., Del Vecchio, A., & Polimeni, A. (2014). Oral soft tissue wound healing after laser surgery with or without a pool of amino acids and sodium hyaluronate: a randomized clinical study. *Photomedicine and Laser Surgery*, *32*(1), 10–16.

20/ Bargiela-Pérez, P., González-Merchan, J., Díaz-Sanchez, R., Serrera-Figallo, M. A., Volland, G., Joergens, M., Gutiérrez-Perez, J. L., & Torres-Lagares, D. (2018). Prospective study of the 532 nm laser (KTP) versus diode laser 980 nm in the resection of hyperplastic lesions of the oral cavity. *Medicina Oral, Patologia Oral y Cirugia Bucal*, *23*(1), e78–e85.

21/ Giannelli, M., Formigli, L., & Bani, D. (2014). Comparative evaluation of photoablative efficacy of erbium: yttrium-aluminium-garnet and diode laser for the treatment of gingival hyperpigmentation. A randomized split-mouth clinical trial. *Journal of Periodontology*, *85*(4), 554–561.

22/ Lama Ashour, B., 2018. Gingival depigmentation using Er: YAG laser and scalpel technique: A six-month prospective clinical study. *Quintessence International*, *49*, pp.113-122.

23/ Ipek, H., Kirtiloglu, T., Diraman, E., & Acikgoz, G. (2019). A comparison of gingival depigmentation by Er:YAG laser and Kirkland knife: osmotic pressure and visual analog scale. *Journal of Cosmetic and Laser Therapy: official publication of the European Society for Laser Dermatology*, *21*(4), 209–212

24/ Suter, V., Altermatt, H. J., & Bornstein, M. M. (2020). A randomized controlled trial comparing surgical excisional biopsies using CO_2 laser, Er:YAG laser and scalpel. *International Journal of Oral and Maxillofacial Surgery*, *49*(1), 99–106.

25/ Suter, V., Altermatt, H. J., & Bornstein, M. M. (2017). A randomized controlled clinical and histopathological trial comparing excisional biopsies of oral fibrous hyperplasias using CO_2 and Er:YAG laser. *Lasers in Medical Science*, *32*(3), 573–581.

26/ Broccoletti, R., Cafaro, A., Gambino, A., Romagnoli, E., & Arduino, P. G. (2015). Er:YAG Laser Versus Cold Knife Excision in the Treatment of Nondysplastic Oral Lesions: A Randomized Comparative Study for the Postoperative Period. *Photomedicine and Laser Surgery*, *33*(12), 604–609.

27/ Arduino, P. G., Cafaro, A., Cabras, M., Gambino, A., & Broccoletti, R. (2018). Treatment Outcome of Oral Leukoplakia with Er:YAG Laser: A 5-Year Follow-Up Prospective Comparative Study. *Photomedicine and Laser Surgery*, *36*(12), 631–633.

28/ Aras, M. H., Göregen, M., Güngörmüş, M., & Akgül, H. M. (2010). Comparison of diode laser and Er:YAG lasers in the treatment of ankyloglossia. *Photomedicine and Laser Surgery*, *28*(2), 173–177.

29/ Eroglu, C. N., Tunç, S. K., & Elasan, S. (2015). Removal of epulis fissuratum by Er,Cr:YSGG laser in comparison with the conventional method. *Photomedicine and Laser Surgery*, *33*(11), 533–539.

30/ Gholami, L., Moghaddam, S. A., Rigi Ladiz, M. A., Molai Manesh, Z., Hashemzehi, H., Fallah, A., & Gutknecht, N. (2018). Comparison of gingival

depigmentation with Er,Cr:YSGG laser and surgical stripping, a 12-month follow-up. *Lasers in Medical Science, 33*(8), 1647–1656.

31/ Pié-Sánchez, J., España-Tost, A.J., Arnabat-Domínguez, J. and Gay-Escoda, C., 2012. Comparative study of upper lip frenectomy with the CO2 laser versus the Er, Cr: YSGG laser. *Medicina Oral, Patologia Oral y Cirugia Bucal*, *17*(2), p.e228.

32/ Tunc, S.K., Yayli, N.Z., Talmac, A.C., Feslihan, E. and Akbal, D., 2019. Clinical comparison of the use of Er, Cr: YSGG and diode lasers in second stage implant surgery. *Saudi Medical Journal*, *40*(5), p.490.

33/ Suter, V.G., Altermatt, H.J., Dietrich, T., Reichart, P.A. and Bornstein, M.M., 2012. Does a pulsed mode offer advantages over a continuous wave mode for excisional biopsies performed using a carbon dioxide laser?. *Journal of Oral and Maxillofacial Surgery*, *70*(8), pp.1781-1788.

34/ Hegde, R., Padhye, A., Sumanth, S., Jain, A.S. and Thukral, N., 2013. Comparison of surgical stripping; erbium-doped: yttrium, aluminum, and garnet laser; and carbon dioxide laser techniques for gingival depigmentation: a clinical and histologic study. *Journal of Periodontology*, *84*(6), pp.738-748.

35/ Monteiro, L., Delgado, M.L., Garcês, F., Machado, M., Ferreira, F., Martins, M., Salazar, F. and Pacheco, J.J., 2019. A histological evaluation of the surgical margins from human oral fibrous-epithelial lesions excised with CO_2 laser, Diode laser, Er: YAG laser, Nd: YAG laser, electrosurgical scalpel and cold scalpel. *Medicina Oral, Patologia Oral y Cirugia Bucal*, *24*(2), p.e271.

36/ Suter, V.G., Altermatt, H.J., Dietrich, T., Warnakulasuriya, S. and Bornstein, M.M., 2014. Pulsed versus continuous wave CO2 laser excisions of 100 oral fibrous hyperplasias: a randomized controlled clinical and histopathological study. *Lasers in Surgery and Medicine*, *46*(5), pp.396-404.

37/ López-Jornet, P. and Camacho-Alonso, F., 2013. Comparison of pain and swelling after removal of oral leukoplakia with CO2 laser and cold knife: a randomized clinical trial. *Medicina Oral, Patologia Oral y Cirugia Bucal*, *18*(1), p.e38.

38/ Chee, M. and Sasaki, C., 2013. Carbon dioxide laser fiber for the excision of oral leukoplakia. *Annals of Otology, Rhinology & Laryngology*, *122*(9), pp.547-549.

39/ Karimi, A., Sobouti, F., Torabi, S., Bakhshandehfard, A., Amirian, A., Shariati, M., Morshedi, E., & Barati, M. (2016). Comparison of Carbon Dioxide Laser With Surgical Blade for Removal of Epulis Fissuratum. A Randomized Clinical Trial. *Journal of Lasers in Medical Sciences*, *7*(3), 201–204.

40/ Agha-Hosseini, F., Moslemi, E. and Mirzaii-Dizgah, I., 2012. Comparative evaluation of low-level laser and CO2 laser in treatment of patients with oral lichen planus. *International Journal of Oral and Maxillofacial Surgery*, *41*(10), pp.1265-1269.

41/ Parker, S., Cronshaw, M., Anagnostaki, E. and Lynch, E., 2019. Parameters for photobiomodulation therapy in clinical dentistry: a systematic review. *Photobiomodulation, Photomedicine and Laser Surgery*, *37*, pp.784-797.

42/ Hamblin, M., Ferraresi, C., Huang, Y., Freitas, L., Carroll, J. (2018). Chapter 1: Photobiomodulation. *In: Low-Level Light Therapy, 1st ed. Bellingham, WA: SPIE Press*, pp. 1.

43/ Romanos, G.E., Sacks, D., Montanaro, N., Delgado-Ruiz, R., Calvo-Guirado, J.L. and Javed, F., 2018. Effect of initiators on thermal changes in soft tissues using a diode laser. *Photomedicine and Laser Surgery*, *36*(7), pp.386-390.

44/ Wehner, M., Betz, P. and Aden, M., 2019. Influence of laser wavelength and beam profile on the coagulation depth in a soft tissue phantom model. *Lasers in Medical Science, 34*(2), pp.335-341.

45/ Cronshaw, M., Parker, S., & Arany, P. (2019). Feeling the Heat: Evolutionary and Microbial Basis for the Analgesic Mechanisms of Photobiomodulation Therapy. *Photobiomodulation, Photomedicine and Laser Surgery*, *37*(9), 517–526.

46/ Sordillo, D.C., Sordillo, L.A., Sordillo, P.P., Shi, L. and Alfano, R.R., 2017. Short wavelength infrared optical windows for evaluation of benign and malignant tissues. *Journal of Biomedical Optics*, *22*(4), p.045002.

47/ Serrage, H., Heiskanen, V., Palin, W.M., Cooper, P.R., Milward, M.R., Hadis, M. and Hamblin, M.R., 2019. Under the spotlight: mechanisms of photobiomodulation concentrating on blue and green light. *Photochemical and Photobiological Sciences*, *18*(8), pp.1877-1909.

48/ Chung, H., Dai, T., Sharma, S.K., Huang, Y.Y., Carroll, J.D. and Hamblin, M.R., 2012. The nuts and bolts of low-level laser (light) therapy. *Annals of Biomedical Engineering*, *40*(2), pp.516-533.

49/ Rizoiu, I.M., Eversole, L.R. and Kimmel, A.I., 1996. Effects of an erbium, chromium: yttrium, scandium, gallium, garnet laser on mucocutanous soft tissues. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *82*(4), pp.386-395.

50/ Palmieri, B., Capone, S. and Rottigni, V., 2011. Er: YAG laser: tissue interaction and histomorphological characterization. *La Clinica Terapeutica*, *162*(5), pp.473-486.

51/ Kesler, G., Shvero, D. K., Tov, Y. S., & Romanos, G. (2011). Platelet derived growth factor secretion and bone healing after Er:YAG laser bone irradiation. *The Journal of Oral Implantology*, *37 Spec No*, 195–204.

52/ Pourzarandian, A., Watanabe, H., Ruwanpura, S.M.P.M., Aoki, A., Noguchi, K. and Ishikawa, I., 2005. Er: YAG laser irradiation increases prostaglandin E2 production via the induction of cyclooxygenase-2 mRNA in human gingival fibroblasts. *Journal of Periodontal Research*, *40*(2), pp.182-186.

53/ Lubart, R., Kesler, G., Lavie, R. and Friedmann, H., 2005. Er: YAG laser promotes gingival wound repair by photo-dissociating water molecules. *Photomedicine and Laser Therapy*, *23*(4), pp.369-372.

54/ Q-PBM Trademark No. UK00003419626 2019. UK Patent (Intellectual Property) Office. Available at: www.gov.uk.po (Last accessed 2020).

55/ Shang, J., Gong, K., Xu, D. P., Sun, L. W., & Qu, W. D. (2020). The Nd:YAG Laser or Combined with Er:YAG Laser Therapy for Oral Venous Lakes. *Photobiomodulation, Photomedicine and Laser Surgery*, *38*(4), 244–248.

56/ Protásio, A.C.R., Galvão, E.L. and Falci, S.G.M., 2019. Laser techniques or scalpel incision for labial frenectomy: a meta-analysis. *Journal of Maxillofacial and Oral Surgery*, *18*(4), pp.490-499.

57/ Uraz, A., Çetiner, F. D., Cula, S., Guler, B., & Oztoprak, S. (2018). Patient perceptions and clinical efficacy of labial frenectomies using diode laser versus conventional techniques. *Journal of Stomatology, Oral and Maxillofacial Surgery*, *119*(3), 182–186.

58/ Tenore, G., Palaia, G., Mohsen, A., Ambrogiano, S., Gioia, C., Dominiak, M., & Romeo, U. (2019). Could the super-pulsed CO2 laser be used for oral excisional biopsies?. *Advances in Clinical and Experimental Medicine: official organ Wroclaw Medical University*, *28*(11), 1513–1517.

59/ Andreadis, D., Lazaridi, I., Anagnostou, E., Poulopoulos, A., Panta, P., & Patil, S. (2019). Diode laser assisted excision of a gingival pyogenic granuloma: A case report. *Clinics and Practice*, *9*(3), 1179.

60/ Fekrazad, R., Nokhbatolfoghahaei, H., Khoei, F. and Kalhori, K.A., 2014. Pyogenic granuloma: surgical treatment with Er: YAG laser. *Journal of Lasers in Medical Sciences*, *5*(4), p.199.

61/ Parker, S. (2011). Laser–tissue interaction and its application in clinical dentistry. *International Journal of Laser Dentistry* 2011;1:1–8.

62/ Letokhov, V.S., 1991. Effects of transient local heating of spatially and spectrally heterogeneous biotissue by short laser pulses. *Il Nuovo Cimento D*, *13*(7), pp.939-948.

63/ Lepock, J.R., 2003. Cellular effects of hyperthermia: relevance to the minimum dose for thermal damage. *International Journal of Hyperthermia*, *19*(3), pp.252-266.

64/ Chen, L., Arbieva, Z.H., Guo, S., Marucha, P.T., Mustoe, T.A. and DiPietro, L.A., 2010. Positional differences in the wound transcriptome of skin and oral mucosa. *BMC Genomics*, *11*(1), pp.1-15.

65/ Karu, T., 1999. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. *Journal of Photochemistry and Photobiology B: Biology*, *49*(1), pp.1-17.

66/ Nagarajan, V.K. and Yu, B., 2016. Monitoring of tissue optical properties during thermal coagulation of ex vivo tissues. *Lasers in Surgery and Medicine*, *48*(7), pp.686-694.

67/ Wong, M.E., Hollinger, J.O. and Pinero, G.J., 1996. Integrated processes responsible for soft tissue healing. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *82*(5), pp.475-492.

68/ Ausprunk, D.H. and Folkman, J., 1977. Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. *Microvascular Research*, *14*(1), pp.53-65.

_____ // _____

(iv) Parker, S., Anagnostaki, E., Mylona, V., Cronshaw, M., Lynch, E. and Grootveld, M. (2020). Systematic Review of Post-Surgical Laser-Assisted

Oral Soft Tissue Outcomes Using Surgical Wavelengths Outside the 650–1350 nm Optical Window. *Photobiomodulation, Photomedicine and Laser Surgery*, 38(10), pp.591–606.

1/ Cronshaw, M., Parker, S., & Arany, P. (2019). Feeling the Heat: Evolutionary and Microbial Basis for the Analgesic Mechanisms of Photobiomodulation Therapy. *Photobiomodulation, Photomedicine and Laser Surgery*, *37*(9), pp 517–526.

2/ Hamblin M. R. (2017). Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophysics*, *4*(3), pp 337–361.

3/ Hahm, E., Kulhari, S., Arany P. Targeting the pain, inflammation and immune (PII) axis: plausible rationale for LLLT. *Photon Lasers Med* 2012;1, pp 241–254.

4/ Chung, H., Dai, T., Sharma, S. K., Huang, Y. Y., Carroll, J. D., & Hamblin, M. R. (2012). The nuts and bolts of low-level laser (light) therapy. *Annals of Biomedical Engineering*, *40*(2), pp 516–533.

5/ Romagnoli, E., Cafaro, A. (2017). PBM. Theoretical and applied concepts of adjunctive use of LLLT/PBM within clinical dentistry. *In: Lasers in Dentistry—Current Concepts. D Coluzzi, S Parker (eds.). New York: Springer*, pp. 132–155.

6/ Kate, R. J., Rubatt, S., Enwemeka, C. S., & Huddleston, W. E. (2018). Optimal Laser Phototherapy Parameters for Pain Relief. *Photomedicine and Laser Surgery*, *36*(7), pp 354–362.

7/ Bashkatov, AN., Genina, EA., Kochubey, VI., Tuchin, VV. (2005). Optical properties of human skin, subcutaneous and mucous tissues in the wavelength range from 400 to 2000nm. *Journal of Physics D: Applied Physics*, 38, pp 2543.

8/ Steiner, R. (2011). Laser-tissue interactions. *In: Laser and IPL Technology in Dermatology and Aesthetic Medicine. C Raulin, S Karsai (eds)*. *New York: Springer*, pp. 23–36.

9/ Tuchin, VV. (2015). *Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis.* Bellingham, WA: SPIE.

10/ Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & PRISMA Group (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *The Public Library of Science Medicine*, *6*(7), e1000097.

11/ Parker, S., Cronshaw, M., Anagnostaki, E., Bordin-Aykroyd, S. R., & Lynch, E. (2019). Systematic Review of Delivery Parameters Used in Dental Photobiomodulation Therapy. *Photobiomodulation, Photomedicine and Laser Surgery*, *37*(12), pp 784–797.

12/ Cronshaw, M., Parker, S., Anagnostaki, E., Mylona, V., Lynch, E., & Grootveld, M. (2020). Photobiomodulation Dose Parameters in Dentistry: A Systematic Review and Meta-Analysis. *Dentistry Journal*, *8*(4), pp 114.

13/ Cronshaw, M., Parker, S., Anagnostaki, E., Mylona, V., Lynch, E., & Grootveld, M. (2020). Photobiomodulation and Oral Mucositis: A Systematic Review. *Dentistry Journal*, *8*(3), pp 87.

14/ Parker, S., Anagnostaki, E., Mylona, V., Cronshaw, M., Lynch, E., & Grootveld, M. (2020). Systematic Review of Post-Surgical Laser-Assisted Oral Soft Tissue Outcomes Using Surgical Wavelengths Outside the 650-1350 nm Optical Window. *Photobiomodulation, Photomedicine and Laser Surgery*, *38*(10), pp 591–606.

15/ Cronshaw, M., Parker, S., Anagnostaki, E., Lynch, E. (2019). Systematic review of orthodontic treatment management with photobiomodulation therapy. *Photobiomodulation, Photomedicine and Laser Surgery*, pp 862–868.

16/ Higgins, J., Savovic´, J., Page, M., Elbers, R., Sterne, J. (2019). Assessing risk of bias in a randomized trial. *In: Cochrane Handbook for Systematic Reviews of Interventions, 2nd ed. J Higgins, J Thomas, J Chandler, M Cumpston, T Li, M Page, V Welch (eds.). Chichester, United Kingdom: John Wiley & Sons*, pp. 205–228.

17/ Hadis, M. A., Zainal, S. A., Holder, M. J., Carroll, J. D., Cooper, P. R., Milward, M. R., & Palin, W. M. (2016). The dark art of light measurement: accurate radiometry for low-level light therapy. *Lasers in Medical Science*, *31*(4), pp 789–809.

18/ Melnik, I., Steiner, R., Kienle, (1993A. Light penetration in human skin: in-vivo measurements using isotropic detector. *Proceedings of SPIE - The International Society for Optical Engineering* 1881: 146313.

19/ Bjordal, J. M., Bensadoun, R. J., Tunèr, J., Frigo, L., Gjerde, K., & Lopes-Martins, R. A. (2011). A systematic review with meta-analysis of the effect of low-level laser therapy (LLLT) in cancer therapy-induced oral mucositis. *Supportive care*

in cancer : official journal of the Multinational Association of Supportive Care in Cancer, *19*(8), pp 1069–1077.

20/ Zecha, J. A., Raber-Durlacher, J. E., Nair, R. G., Epstein, J. B., Elad, S., Hamblin, M. R., Barasch, A., Migliorati, C. A., Milstein, D. M., Genot, M. T., Lansaat, L., van der Brink, R., Arnabat-Dominguez, J., van der Molen, L., Jacobi, I., van Diessen, J., de Lange, J., Smeele, L. E., Schubert, M. M., & Bensadoun, R. J. (2016). Low-level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: part 2: proposed applications and treatment protocols. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*, *24*(6), pp 2793–2805.

21/ Alvarenga, L. H., Ribeiro, M. S., Kato, I. T., Núñez, S. C., & Prates, R. A. (2018). Evaluation of red light scattering in gingival tissue - in vivo study. *Photodiagnosis and Photodynamic Therapy*, *23*, pp 32–34.

22/ Zadik, Y., Arany, P. R., Fregnani, E. R., Bossi, P., Antunes, H. S., Bensadoun, R. J., Gueiros, L. A., Majorana, A., Nair, R. G., Ranna, V., Tissing, W., Vaddi, A., Lubart, R., Migliorati, C. A., Lalla, R. V., Cheng, K., Elad, S., & Mucositis Study Group of the Multinational Association of Supportive Care in Cancer/International Society of Oral Oncology (MASCC/ISOO) (2019). Systematic review of photobiomodulation for the management of oral mucositis in cancer patients and clinical practice guidelines. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*, *27*(10), pp 3969–3983.

23/ Agrawal, T., Gupta, G. K., Rai, V., Carroll, J. D., & Hamblin, M. R. (2014). Pre-conditioning with low-level laser (light) therapy: light before the storm. *Dose-response : a publication of International Hormesis Society*, *12*(4), pp 619–649.

24/ Sonesson, M., De Geer, E., Subraian, J., Petre´n, S. (2017). Efficacy of low-level laser therapy in accelerating tooth movement, preventing relapse and managing acute pain during orthodontic treatment in humans: a systematic review. *Biomed Central Oral Health,* 17, pp1–2.

25/ Aras, M. H., & Güngörmüş, M. (2009). The effect of low-level laser therapy on trismus and facial swelling following surgical extraction of a lower third molar. *Photomedicine and Laser Surgery*, *27*(1), pp 21–24.

26/ Isolan, C., Kinalski, M. D., Leão, O. A., Post, L. K., Isolan, T. M., & Dos Santos, M. B. (2021). Photobiomodulation therapy reduces postoperative pain after third molar extractions: A randomized clinical trial. *Medicina Oral, Patologia Oral y Cirugia Bucal*, *26*(3), e341–e348.

27/ Karu, T. (1996). Mechanisms of interaction of monochromatic visible light with cells. *Proceedings of SPIE - The International Society for Optical Engineering*, 2639, pp 2–9.

28/ Karu T. (1999). Primary and secondary mechanisms of action of visible to near-IR radiation on cells. *Journal of Photochemistry and Photobiology. B, Biology*, *49*(1), pp 1–17.

29/ Serrage, H., , Heiskanen, V., , Palin, W. M., , Cooper, P. R., , Milward, M. R., , Hadis, M., , & Hamblin, M. R., (2019). Under the spotlight: mechanisms of photobiomodulation concentrating on blue and green light. *Photochemical &*

photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology, 18(8), pp 1877–1909.

30/ Sommer A. P. (2019). Revisiting the Photon/Cell Interaction Mechanism in Low-Level Light Therapy. *Photobiomodulation, Photomedicine and Laser Surgery, 37*(6), pp 336–341.

31/ Niemz, MH. (2007). *Laser Tissue Interaction, 3rd ed. Berlin, Germany: Springer*.

32/ Vladimirov, YA., Osipov, AN., Klebanov, GI. (2004). Photobiological principles of therapeutic applications of laser radiation. *Biochemistry (Moscow)* 69, pp103–113.

33/ Schultz, H. Skin toxins. (1888). *Pflueger's archive for the entire physiology of humans and animals,* 42, pp 517–541.

34/ Jacques S. L. (2013). Optical properties of biological tissues: a review. *Physics in Medicine and Biology, 58*(11), R37–R61.

35/ Khan, I., Tang, E., & Arany, P. (2015). Molecular pathway of near-infrared laser phototoxicity involves ATF-4 orchestrated ER stress. *Scientific reports*, *5*, 10581.

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Appendix

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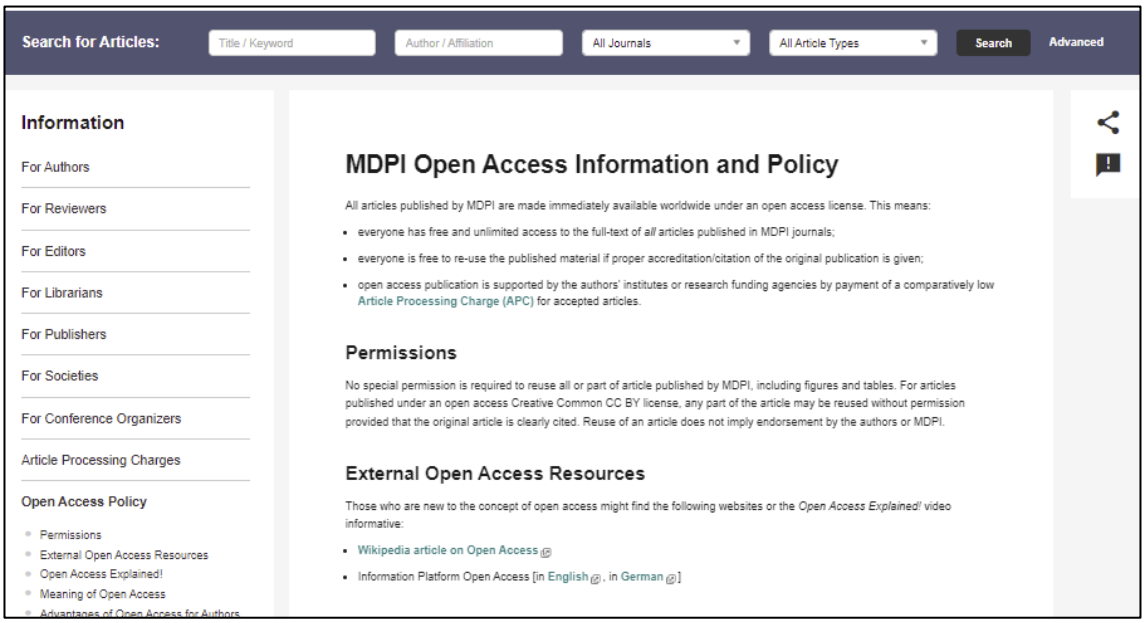
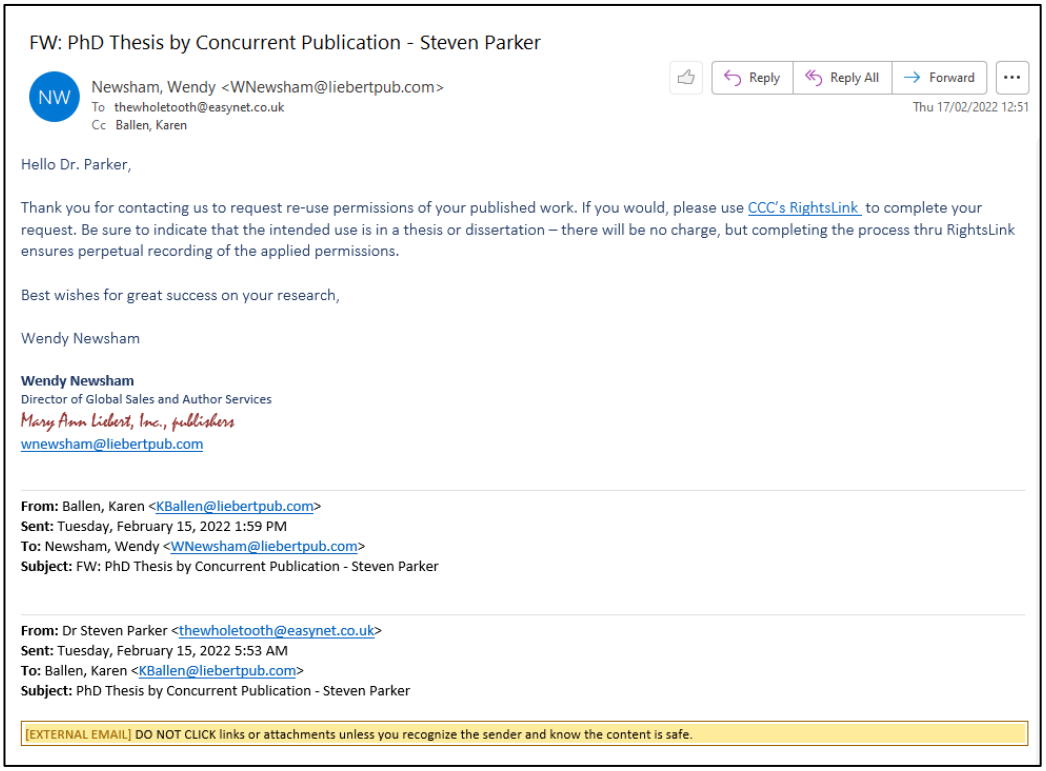
Paper #1: Parker S, Anagnostaki E, Mylona V, Cronshaw M, Lynch E, Grootveld M. (2020). Current Concepts of Laser–Oral Tissue Interaction. *Dentistry Journal*, 8, 61; doi:10.3390/dj8030061.



Paper #2: Parker S, Anagnostaki E, Mylona V, Cronshaw M, Lynch E, Grootveld M. (2020). Systematic review of post-surgical laser-assisted oral soft tissue outcomes using surgical wavelengths outside the 650nm–1350nm optical window. *Photobiomodulation, Photomedicine and Laser Surgery*, 38(10), pp.591–606. doi:10.1089/photob.2020.4847.

Paper #3: Parker, S., Cronshaw, M., & Grootveld, M. (2022). Photobiomodulation Delivery Parameters in Dentistry: An Evidence-Based Approach. *Photobiomodulation, Photomedicine and Laser Surgery*, 40(1), 42–50. doi.org/10.1089/photob.2021.0116



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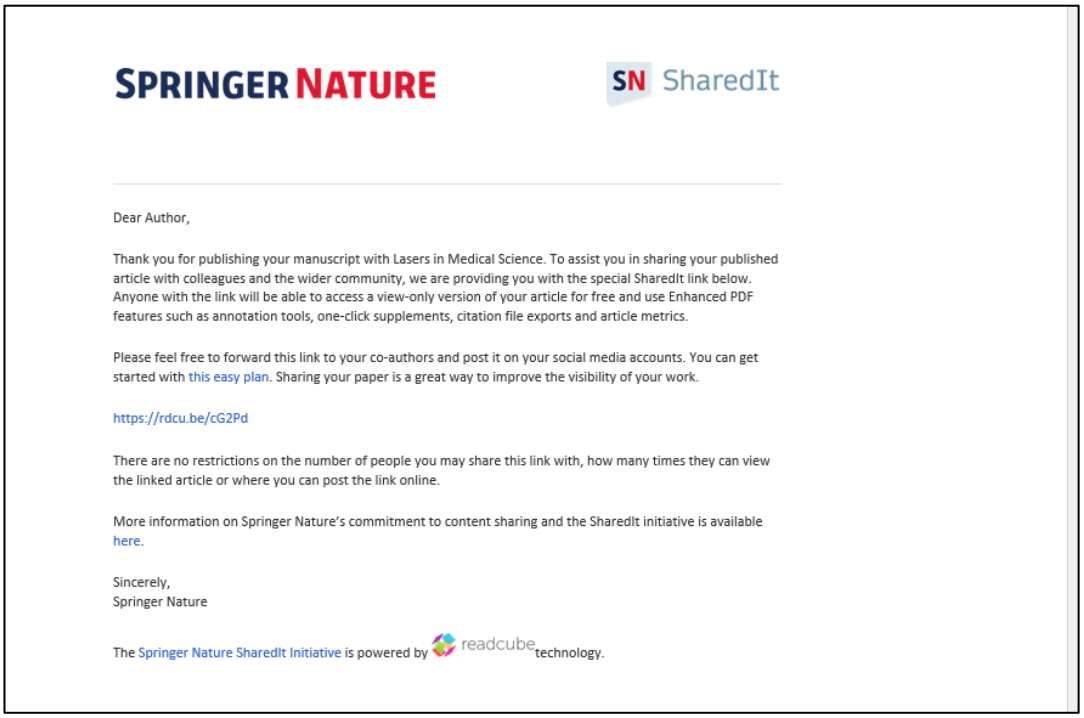

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