THE EFFECTS OF 940 NM DIODE LASER ON MORPHOLOGY AND TEMPERATURE OF HUMAN EXTRACTED TEETH

by

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LIST OF ABBREVIATIONS

μm	Micrometre
CO_2	Carbon dioxide lasers
CP	Carbamide peroxide
DI	Direct Innervation
EDTA	Ethylenediaminetetraacetic acid
Er:YAG	Erbium-doped yttrium aluminium garnet laser, erbium YAG laser
GaAlAs	Gallium/aluminium/arsenide diode lasers
HEMA	Hydroxyethyl methacrylate
He-Ne	Helium–neon laser
HP	Hydrogen peroxide
kV	Kilovolt
LED	Light-emitting diode
NaF	Sodium fluoride
Nd:YAG	Neodymium-doped yttrium aluminium garnet
NIR	Near infrared
NM	Nanometer
^{o}C	Degree celsius
OR	Odontoblast Receptor
sec	Seconds
SEM	Scanning electron microscope
V	Volt
W	Watt

KESAN LASER DIOD 940 NM TERHADAP MORFOLOGI DAN SUHU GIGI MANUSIA YANG DIEKSTRAK

ABSTRAK

Gigi sensitif adalah satu perkara biasa dalam bidang pergigian yang boleh menyebabkan halangan besar kepada doktor gigi dan pesakit, disebabkan rasa sakit yang tajam dalam masa yang singkat, kesan daripada tindak balas stimulasi ke atas dentin yang terdedah. Tujuan penyiasatan ini adalah untuk menilai keberkesanan laser diod 940 nm sebagai modaliti rawatan untuk kehiperpekaan dentin. Laser dijangka menyebabkan perubahan ultrastruktur dentin seperti menyempitkan dan menutup tubula dentin yang luas. Parameter yang paling selamat untuk digunakan pada tisu pergigian untuk mencetuskan lebur lapisan dentin tanpa merosakkan tisu pulpa dengan haba akan ditakrifkan. 7 batang gigi premolar atas dengan dua akar yang bebas karies telah dikumpulkan. Gigi-gigi ini telah dicabut untuk tujuan ortodontik atau penyakit periodontal. Permukaan dentin diradiasi dengan laser diod 940 nm pada tetapan kuasa output berbeza: 1.5 W, 2.0 W dan 2.5 W, secara berterusan selama 10 saat. Setiap gigi dibahagikan kepada 4 kumpulan: Kumpulan A (kawalan), Kumpulan B (1.5 W), Kumpulan C (2.0 W) dan Kumpulan D (2.5 W). Penyinaran laser diod pada 2.0 W dan 2.5 W menyebabkan penyempitan atau penyegelan tubular dentin secara efektif tanpa memprovokasi pengkarbonasi atau sebarang kemusnahan pada permukaan dentin. Sampel yang telah disinari dengan 1.5 W mempamerkan sebahagian kecil daripada tubulus dentin. Suhu pulpa meningkat semasa penyinaran, dalam lingkungan 2.6°C hingga 7.3°C. Peningkatan suhu bagi semua sampel adalah ketara secara statistik (p-value <0.001). Dari kajian ini, diperhatikan bahawa penyinaran laser diod 940 nm pada tetapan 2.0 W dan 2.5 W, selama 10 saat dalam mod yang berterusan, dapat menutup

tubul dentin. Di samping itu, parameter ini berada dalam lingkungan yang selamat seperti yang dinilai oleh morfologi dan perubahan suhu sampel, yang tidak akan menyebabkan kesan berbahaya kepada tisu pulpa, dan berkesan dalam rawatan kehiperpekaan dentin.

THE EFFECTS OF 940 NM DIODE LASER ON MORPHOLOGY AND TEMPERATURE OF HUMAN EXTRACTED TEETH

ABSTRACT

Tooth sensitivity is a totally common dental condition that could cause a huge obstacle for dentist and patient, due to the short, sharp pain arising as a response to stimulation applied on uncovered dentine. The aim of this investigation is to evaluate the effectiveness of 940 nm diode laser as a treatment modality for dentine hypersensitivity. The laser is expected to cause ultrastructural changes of dentine such as narrowing and plugging the wide dentinal tubules. The safest parameters to be used on the dental tissues to provoke the melting of dentine layer without damaging the pulp tissue by heat will be defined. Seven sound, caries free upper premolar teeth with two roots were collected. The teeth were extracted for orthodontic purpose or periodontal disease. Dentine surface were irradiated with a 940 nm diode laser at different output power settings: 1.5 W, 2.0 W and 2.5 W, continuously for 10 seconds. Each tooth had been divided into 4 groups: Group A (control), Group B (1.5 W), Group C (2.0 W) and Group D (2.5 W). Diode laser irradiations at 2.0 W and 2.5 W led to effectively narrowing or sealing of dentinal tubules without provoking carbonisation or any destruction on the dentine surface. Samples that had been irradiated with 1.5 W showed partially narrowing of the dentinal tubules. The pulp temperature increased during irradiation, within a range of 2.6°C to 7.3°C. The temperature increment for all samples was statistically significant (p-value < 0.001). From this study, it is observed that irradiation of 940 nm diode laser at 2.0 W and 2.5 W settings, for 10 seconds in a continuous mode, is able to seal the dentinal tubules. Additionally, these parameters are within the safe range as assessed by the morphology and changes in temperature of the samples, which will not cause a harmful effect to the pulp tissues, and could be effective in the treatment of dentinal hypersensitivity.

KEYWORDS: Dentinal tubules, dentine hypersensitivity, 940 nm diode laser.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Tooth sensitivity is a totally common medical and clinical condition that could cause large challenge for dental practitioners and patients (Bartold, 2006). Nowadays, there is an increased awareness that dentine hypersensitivity is a major issue that should be considered, from both diagnostic angle and a management control angle, for example, periodontal diseases management and caries prevention to enhance the oral health (Haywood, 2002).

Management of dentinal hypersensitivity aims to decrease and eliminate pain or discomfort. Through narrowing and occluding the widely open dentinal tubules and try to decrease and block the conducting of impulses which transport through nerves (Cummins, 2009).

With the introduction of laser to the conservative dentistry field, endodontic treatment field was totally filled with varieties of innovation methods that improve the chances for a successful outcome. Laser is considered to be an effective and easy tool for disinfecting the root canal and debriding the canal debris, also to overcome the problems such as inability to reach the full depth of the canal by using disinfecting solutions and the classical tools (Marchesan et al., 2008; Takwda, 1998).

The first application of laser in dentistry as problem solving of dentine hypersensitivity was introduced by Matsumoto et al. (1985) as cited by (Kimura et al., 2000b). Later, many more researchers had successfully investigated the application of various types laser on dentine hypersensitivity such as low output power lasers (helium–neon laser He-Ne and gallium/aluminium/arsenide (GaAlAs) diode lasers), middle output power lasers (neodymium-doped yttrium aluminium garnet Nd:YAG and Carbon dioxide CO_2 lasers) and erbium-doped yttrium aluminium garnet laser, erbium YAG laser (Er:YAG) (Kimura et al., 2000a; Umana et al., 2013; Senda et al., 1985;Zhang et al., 1998; Birang et al., 2007).

Previous *in vitro* studies demonstrated that radiation of dentine surface by diode laser could melt the normal dentine surface by occluding and narrowing the exposed dentinal tubule orifices without making surface cracks (Liu et al., 2013; Umana et al., 2013; Al-khafaji et al., 2017).

Therefore, this study is aimed to evaluate the morphological and thermological changes on dentine surface of extracted teeth after 940 nm diode laser irradiation and looking at the effect on human teeth desensitisation.

1.2 Problem statement

There is a shortage in the number of studies that has investigated the effect of utilising low output power 940 nm diode laser in the plugin of dentinal tubules. Thus, this study evaluated and investigated the effect of using 940 nm diode laser with different parameters (1.5 W, 2.0 W, 2.5 W) on the dentinal tubules, and studied the most safe parameter to decrease dentine hypersensitivity for long term *in vitro*.

1.3 Objectives

1.3.1 General objective

To study the effects of 940 nm diode laser on the morphology and pulpal temperature of human extracted teeth.

1.3.2 Specific objectives

1. To assess the temperature changes in the pulp chamber following diode laser irradiation with different output powers of 1.5 W, 2.0 W, 2.5 W.
2. To evaluate the surface topography of dentinal tubules after radiating with different output powers of diode laser (1.5 W, 2.0 W, 2.5 W) by using scanning electron microscope (SEM).

1.4 Research hypotheses

1.4.1 Alternative hypothesis

1. There is a change in morphology of the dentinal tubules under SEM after radiating the surface using 940 nm diode laser with different parameters (1.5 W, 2.0 W, 2.5 W).
2. There is a rise in the pulp temperature during the radiation with diode laser 940 nm compared to room temperature.

1.4.2 Null hypothesis

1. There is no change in morphology of the dentinal tubules under SEM after radiating the surface using 940 nm diode laser with different parameters (1.5 W, 2.0 W, 2.5 W).

2. There is no change in the pulp temperature during the radiation with diode laser 940 nm compared to room temperature.

CHAPTER 2

LITERATURE REVIEW

2.1 Dentine hypersensitivity

2.1.1 Definition

In 1982, dentine hypersensitivity was thought as an enigma because it was poorly understood (Johnson et al., 1982; Dababneh et al., 1999). The in depth evolution of scientific understanding of this condition was noticed through the past thirty years but the definition of dentine hypersensitivity was officially approved in 2003 with minimum changes by Canadian Advisory Board on Dentine (Hypersensitivity, 2003).

Dentine hypersensitivity can be described as short, sharp pain arising as a response to stimulation applied on an uncovered dentine typically evaporative, thermal, osmotic, tactile, or chemical, and which is difficult to be classified to any other dental disease or defect (Addy, 2002), which is also described clinically as an extreme response to a non-noxious stimulation and fulfil all the requirements to be categorised as a true pain syndrome (Hypersensitivity, 2003).

The other common terms used for describing dentine hypersensitivity are dentinal sensitivity, cervical hypersensitivity/sensitivity, root hypersensitivity/sensitivity, and cemental hypersensitivity/sensitivity (Addy, 2002).

The dentine becomes uncovered in a hypersensitive tooth due to many factors. For example, denuded or removed cementum or enamel layer which normally protects the dentine surface as a result of abrasion, attrition or erosion activities. Also, the exposure of dentine could happen as a result of an insufficient enamel layer due to development anomaly in some individuals. In general, dentinal hypersensitivity is considered a multifactorial condition, caused by the earlier mentioned factors (Bartold, 2006). The anatomical structure of a tooth is shown in Figure 2.1 below.

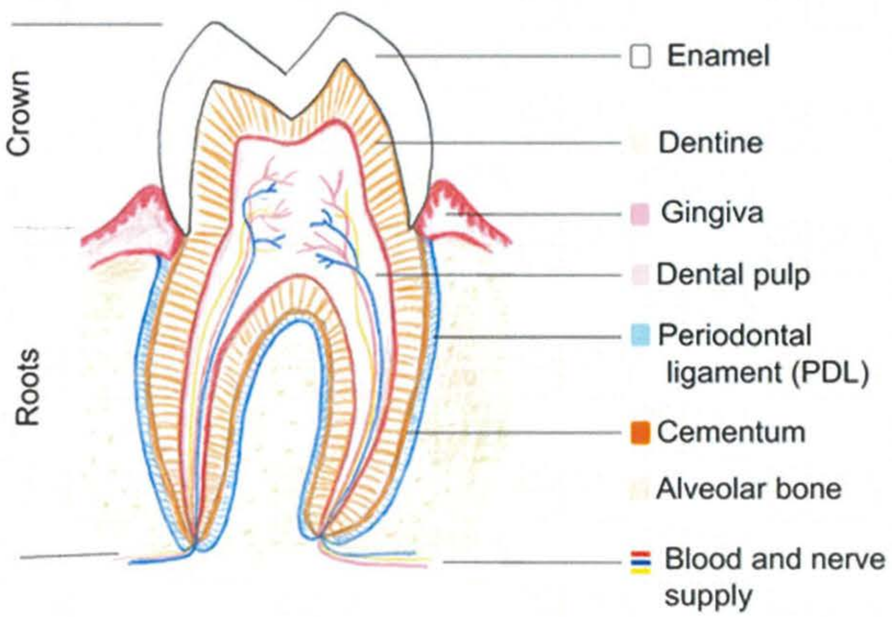

Figure 2.1 The anatomical structure of a tooth (infodentis, Sharpe, 2016)

2.1.2 Aetiology

Enamel loss may happen as a consequence of single or summed factors like attrition, erosion, abrasion, and abfraction. Attrition is defined as wear on tooth surface when teeth are directly contacting each other in occlusal function and mastication process, also involve the parafunctional habits, such as bruxism, that leads to an extreme non-physiological wear and the sensitivity can increase noticeably (Trushkowsky & Oquendo, 2011).

Abrasion can be defined as the wearing of tooth structure caused by hard objects like toothbrush, leading to gingival recession where the exposure of hard root tissue can cause pain (Haywood, 2002).

On the other hand, erosion is defined as losing of tooth structure caused by extrinsic or intrinsic acids. Intrinsic acid is due to exposure of gastric acid from regurgitation, vomiting or gastroesophogeal reflux disease leading to dissolution of teeth (Bueno et al., 2010; Kuchta & Szymańska, 2014).

2.1.3 Epidemiological aspects of dentine hypersensitivity

The prevalence of dentine hypersensitivity in adult population is ranged from 3% to 98% world wide. This wide range could be referred to the variation in the selection criteria forgetting the sample for the study and the differences in diagnostic methods or time borders (Splieth & Tachou, 2013).

In general, a higher incidence of dentine hypersensitivity has been reported in males than females which may reflect their overall worse oral hygiene awareness and health care (Oyama & Matsumoto, 1991). The peak age period was reported between 30–40 years old and the prevalence of erosion lesions due to acid seems to be more within younger age adults compared to older patients, especially after periodontal treatment process and excessive tooth brushing activity (Splieth & Tachou, 2013).

A study done in the United Kingdom revealed that the regions most commonly affected by dentinal hypersensitivity were the buccal and cervical regions of the permanent teeth and there was an indication that the canine, pre-molar, and incisor teeth were more frequently affected than the molar teeth (Addy, 2002; Addy et al., 1987).

2.1.4 Mechanisms of dentine sensitivity

It has been stated in a number of previous study that the development of dentine hypersensitivity happened in two phases: phase one (lesion localisation) and phase two (lesion initiation)(Dababneh et al., 1999). Lesion localisation occurs when the dentine loses its protective coverage, leading to exposure to the external environment. This includes loosing of enamel coverage by abrasion, abfraction, erosion, attrition or a combination of these factors with differing proportional effects and the lesion initiation by opening of the dentinal tubulise (Dababneh et al., 1999).

There are three theories available to explain how this hypersensitivity mechanism occurs; Direct Innervation (DI) Theory, Odontoblast Receptor (OR) Theory and Fluid Movement/Hydrodynamic Theory (Walters, 2005; Bartold, 2006).

Earlier study suggested that in the direct innervation theory, nerve endings extend to the dentino-enamel junction by penetrating the dentine . Direct mechanical stimulation of the nerves will start an action potential. Lacking of the evidence that outer dentin, which is the most sensitive region, is innervated lead to shortcomings of this theory (Bartold, 2006).

On odontoblast receptor theory the odontoblasts act as receptors by themselves and convey the signal to a nerve terminal. Study have shown that odontoblasts are matrix forming cells and hence they are not considered to be excitable cells, and no synapses have been demonstrated between odontoblasts and nerve terminals (Pashley, 1996).

The Hydrodynamic Theory was developed in the 1960's, relying on two decades of a continuous research and it was widely agreeable as the main cause of tooth sensitivity . This theory explained that fast shifts in both direction of the fluids inside dentinal tubules after application of a stimulus, would lead to stimulation of sensory nerves impulses in the pulp area and inner dentine region of the tooth (West et al., 2013; Brännström, 1986).

Basically, certain stimuli can cause changes in pressure among the dentine fluids which can then stimulate the intradental nerves response. The pulpal nerves response is proportional to the rate of fluid flow and pressure. With increasing dose of stimuli, such as cold, which cause fluid movement outward from the pulp leads to production of faster and higher responses from pulpal nerves than those, such as heat, which produce an inward response (Matthews & Vongsavan, 1994).

Based on hydrodynamic theory, we can explain that there is a rapid and higher response to cold stimulation compared to the slower and dull response to heat stimulation. Berman (1985), also explained this reaction as the dentinal tubules fluid has coefficient of thermal expansion which is about ten times than that of the walls of dentinal tubules. So, applying heat to dentine surface will lead to expansion of the fluid, while cold will result in contraction of the fluid, both stimuli will lead to an excitation of the 'mechanoreceptor'. The mechanisms of these three different theories are shown in Figure 2.2 below.

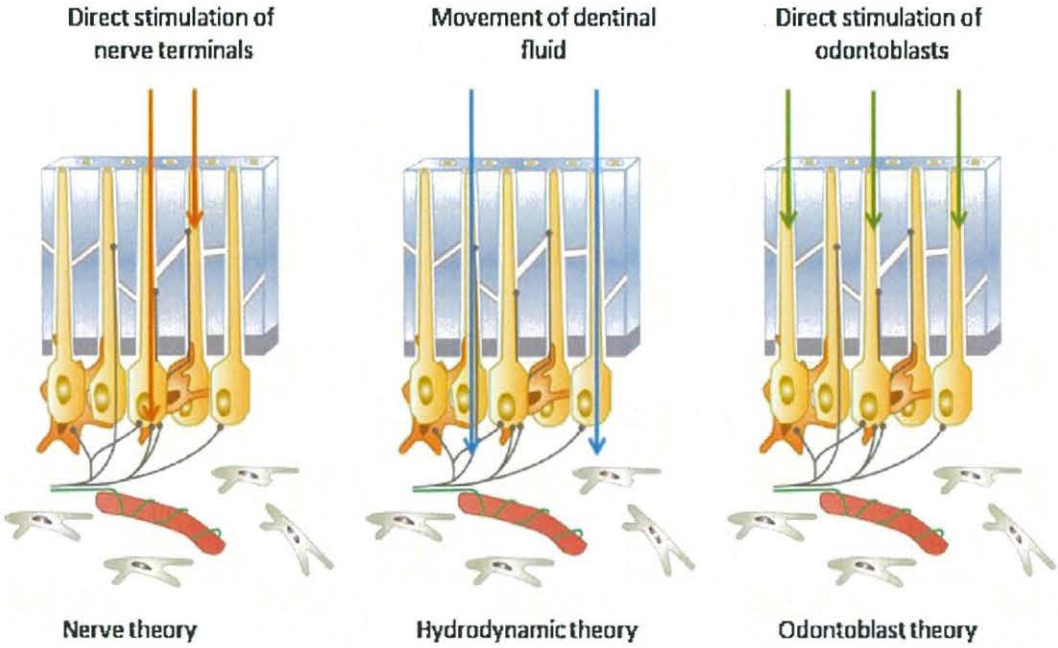

Figure 2.2 Mechanism of dentine hypersensitivity based on different theories (Solé-Magdalena et al., 2017)

2.1.5 Clinical treatment of dentine hypersensitivity and diagnosis

For a successful result, the correct and accurate diagnosis of dentine hypersensitivity is critical before receiving the treatment. The diagnosis of the disease starts through taking the medical and dental history of the patient besides the clinical examination. For investigating the medical history, a number of questions should be asked about the time of the starting of dentine hypersensitivity symptoms, the intensity of the pain, the period of time of the pain, and the key players that increase or reduce the severity of the disease (Miglani et al., 2010).

The clinical examination include a number of techniques such as using dental air syringe to create cold air blasts, cold water, and suction from a dental aspirator tip (physical) to assimilate the stimulating factors and to detect exactly the severity of pain the patient experience (Gillam & Orchardson, 2006).

By narrowing and plugging of dentinal tubules, the interruption of the transmission of nerve sensory happened so the dentine hypersensitivity pain can be controlled. Agents used for occlusion of the dentinal tubules will physically plug the uncovered dentinal tubules to prevent any external stimuli from exciting the movement of dentinal fluid and thereby eliminating pain response. These agents can be conveyed by over the counter products that patient can use easily at home or be applied by dentists in the dental office (Cummins, 2009).

It has been stated that mild and responsive dentine touchiness be overseen by less complex agents, otherwise over the counter desensitisers and dental office treatment, be held for more in extreme headstrong cases (Orchardson & Gillam, 2006). There are strategies that should be taken as management for dentine hypersensitivity as shown in Table 2.1 below.

Table 2.1 Management strategy for dentinal hypersensitivity: a "hierarchial model"

General Guidelines	Improve awareness regarding risk factors in aetiology of dentinal hypersensitivity Suggest dietary modifications (avoidance of dietary acids) Recommend appropriate tooth brushing technique
Level 1: Non-invasive intervention (At home)	Fluoride and Potassium nitrate dentifrices Mouth washes/chewing gum
Level 2: Non-invasive intervention (Professional/ In office)	Fluorides (sodium fluoride, stannous fluoride) Oxalates (potassium oxalate, aluminium oxalate) Varnish
Level 3: Invasive Intervention	Dentine Bonding Agents 2-hydroxyethyl methacrylate (HEMA) Lasers Restorative material Periodontal surgery

(Madhavan et al., 2018)

2.1.6 History of laser application in dentistry

Laser has been defined as an acronym source of light amplification by stimulated emission of radiations (Miglani et al., 2010). Stern & Sognnaes (1964) and Goldman et al. (1964) were considered the pioneer in studying the benefits of using the ruby laser in dentistry. Their investigations on laser started on dentine and enamel by studying the ability to use the ruby laser to reduce subsurface demineralisation (Niemz, 1996). Their results showed a successful reduction in the permeability and acid demineralisation, after radiating the enamel with laser (Wilder-Smith et al., 2000).

The initial experiments started by using ruby laser, later on, the dental practitioner began to use other lasers, for example argon (Ar), carbon dioxide (CO_2), neodymium: yttrium- aluminum-garnet (Nd:YAG), and erbium Er:YAG lasers. A study showed the effects of laser on decreasing and eliminating dentine hypersensitivity in the range of 5% - 100% according to the type of laser and therapeutic parameters, the laser's wavelength of beam, also the period of time needed while using of laser (Sgolastra et al., 2011).

With an increase interest in this topic for the last 17 years, many publications about laser therapeutic application for dentine hypersensitivity treatment have been found (Kimura et al., 2000b), that gathered many information regarding its use. However, clinical practitioners' acceptance for this technology remains limited (Kimura et al., 2000b).

Several laser types have been investigated *in vivo*, which showed noticeable thermal effects. However, the inadequate controlled for laser parameters will cause rise in the concerns of the thermal harmful effect on the pulpal tissues (Kimura et al., 2000b).

2.2 Diode laser application in previous studies

The usage of laser system in dentistry started in the middle of 90's. Among the different types of lasers with varied wavelengths, diode laser system started to establish itself rapidly for its small size, and its lowest price compared to the other laser systems besides its good performance in cutting the soft tissues (Pirnat, 2007).

Diode lasers can be utilised for a versatile of dental clinical procedures, as with soft tissue procedures and soft tissue surgery (Goharkhay et al., 1999; Vizo et al., 2016;Kaur et al., 2017). Besides reducing the dental pocket (Saglam et al., 2014; Kreisler et al., 2005), it can also be used for other dental applications such as cutting the hard tissues (teeth), disinfecting root canal system in endodontics (Schoop et al., 2004; Bitter et al., 2017) and assisting in tooth whiting dental procedure (Lopes et al., 2018).

To date, an earlier study has proven that one of the widest wavelength range used in diode lasers which is available to dentists are mainly near infrared (NIR) laser system light (Pirnat, 2007).

2.3 Overview of technical characteristics of a diode laser

2.3.1 Basic design of a diode laser system

As mentioned earlier, the diode laser system has is more superior compared to other laser systems, due to its small size (8.1" x 0.75", 20.5 cm x 1.9 cm) and light weight (0.22 lbs, 0.10 kg), which occupies only a very little space in the office and great portability due to its low weight. It is a universal device and available with a unique bending fibre tip delivery system (200/300/400 μm) diameter as shown in Figure 2.3 below (Biolase, 2018a).

Figure 2.3 The dental diode laser system (Sirona brand) (Laser, 2018)

Another advantage of diode laser is its lower price which makes it more attractive to the dental professionals, who seek faster and more effective procedures, and who are planning to expand the dental services offered by their clinics. The other benefit of the system is the treatment beam will be ready for use immediately after switching on the system, unlike the other laser systems which usually need minutes to be ready (Biolase, 2018b).

Furthermore, the small amount of power consumed by diode laser system compared to other laser systems, contributes to the protection of the environment and saving the users money. Another important aspect to consider is its widespread use and its reliability, as more than 40 million devices produced annually which are being used in versatile pieces, ranging from laser pointers to DVD-players (Hayamizu et al., 2004; Pirnat, 2007).

2.3.2 Laser emission modes

Lasers have been stated to be emitted either in pulsed mode or continuous mode. This will be linked to the rate of emission of light from laser and the time (Biolase, 2018b). With the prime privilege of a pulsed mode, which will increase the ability of the treated tissue to cool down between each session of pulses. The continuous mode has been considered the more rapid method to cut tissues, but unfortunately building up the heat may lead to collateral harmful effect to the treated tissue and adjacent area. Nowadays, the modern dental diode laser systems can be applied in both continuous and pulsed mode(Al-Khatib & Al-Azzawi, 2015; Pirnat, 2007).

2.3.3 Delivery to the target tissue

Majority of dental diode lasers have flexible unique optic fiber which can be fixed into an appropriate place of the handpiece for easier handling to deliver the therapeutic beam to the targeted tissue (Pirnat, 2007).

2.4 NIR laser light and soft tissue cutting

Using the appropriate parameter range will cause tissue ablation in the central area of application which is surrounded by a zone of irreversible denaturation protein. Also, around the central area, a reactionary, reversible swelling area will develop with the thermal gradient. In the ideal scenario cases, the incision line will be equal to the diameter of emitted beam. Heat building up in the targeted area will disinfect the zone and boundaries area and discourages bacterial contamination of the wound by production of a surface coagulum. It has been noticed that the use of NIR lasers on targeted soft tissue shows nearly no bleeding as a result of both tightening and plugging of small blood vessels through denaturation the tissue protein beside inducing of coagulation factors such as factor VII. Also, the heat build-up allows for the sealing of small lymphatic vessels which results in a reduced oedema, as a consequence. Thus, using suture to close the wound is not necessary due to the surface coagulum (Goharkhay et al., 1999; Fisher et al., 1983; Sarver, 2006).

2.5 NIR laser light and hard tissue cutting

It has been approved that near infrared wavelengths have small absorption in dental hard tissue and may lead to thermal fissures and cracking, and amorphous alteration in the structure of hydroxyapatite crystal. High NIR wavelengths through tooth hard tissue such as enamel and dentine can cause damage to dental pulp tissue as the pulpal temperature increases (Von Fraunhofer & Allen, 1993).

This multifactorial combination leads to an instant vaporisation of water content inside enamel and dentine causing a prominent damage of the tissue, thus using of water sprays while applying laser is needed to disperse ablated tissue and cool down the heat in the target zone (Hoke et al., 1990).

2.6 Clinical application

2.6.1 Diode laser in soft tissue therapy

Different application has been stated in previous studies including the removal of labial and lingual frenums or bladed fibroma, mucocele, denture granulomata, small lesions haemangiomata, and treatment of nonerosive lichen planus, major aphthae ulcer and herpes lesions (Parker, 2007b; Bladowski et al., 2004; Azma & Safavi, 2013; Parker, 2007a).

Laser application should be done after determining clearly the disease lesion with paying attention to any abnormal tissue lesions. This will help getting accurate cleavage while applying diode laser for surgery. The tip of laser handpiece should be placed closely to the desired dental tissue surface to allow the energy of the laser to create an appropriate incision, besides preventing build-up of debris on the handpiece tip, which will lead to thermal damage on the tissue surface. It has been approved that the suitable setting is in the range of 2.0 W – 3.5 W in minor intraoral surgical cases (Parker, 2007a).

More attention should be applied when using laser close to anatomical sites to reduce the damaged due to its higher power values (Spencer et al., 1998). As an example, higher values of power settings may cause thermal damage to the underlying periosteum bone (Parker, 2007a). To emit the destructive effect for these anatomical sites, the appropriate power levels of laser should be used, laser beam should be applied parallel and has enough distance away from the underlying bone, and using proper time intervals for radiation to allow sufficient tissue to cool down (Parker, 2007a).

Diode laser has been used more on soft tissue operations such as removing gingival hyperplasia, exposing tooth surface and removing hyperpigmentation. In addition, it is used in teeth restoration and restorative cavity preparation procedures (Parker, 2007a). The laser energy will act as a mean of incision, excision or ablation with no sutures needed, no bleeding and little chance for infection of the wound (Parker, 2007a).

2.6.2 Diode laser in periodontology

Utilising dental diode laser in the periodontal therapy and surgery procedures aimed to eliminate the infected pocket lining epithelium and disinfection of dental pockets. The design of optic fibre systems allows an easy access into the periodontal pocket difficult area, also after scaling and/or root-planning to disinfect the pocket by using the optical fibres (Parker, 2007c).

Diode laser has been shown to have a great effect as bactericidal and can eliminate the inflammation in the periodontal pockets, it has been used as a re-assessment tool after scaling and root planning (Pirnat, 2007). In addition to conventional root planning procedure with scaling, laser usage may improve healing of the periodontal pockets by reducing the number of bacteria that usually causing periodontal disease and bone loss (Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis) (Moritz et al., 1998; Pirnat, 2007).

2.6.3 Diode laser in implantology

In implantology, dental diode laser can be done in the second stage of implant recovery and as a treatment aid of peri-implantitis (Parker, 2007d). In the second stage of implant recovery, care must be taken to avoid the direct contact with the implant body while trying to expose the neck of the implant. Soft tissue cutting leads to good healing outcome and the procedure is more favourable to be performed under topical anaesthesia. The suitable power setting for removing gingival tissue covering the implant cover screw is around 1.0 W – 2.0 W. Besides, the use of diode laser will provide a clear visual access field to the cover screw due to haemostasis, the production of the protective coagulum will aid in healing and reduce pain to the patient (Parker, 2007d).

Peri-implantitis is considered as one of the major causes for implant failure. This condition is not limited or connected to any type of implant design or construction (Martins et al., 2004; Shibli et al., 2003), and is mentioned as a fast progressive failure of osseointegration process (Pirnat, 2007) . Toxins which are released by bacteria may lead to inflammatory alteration and bone loss (Leonhardt et al., 1999).

2.6.4 Diode laser in endodontics

Research has shown that a diode laser can be used to perform curettage of granular tissue besides disinfecting the treated zone (Leonhardt et al., 1999). Previous findings have shown an effective result in root canal treatment by reduction of bacteria which have migrated far into the dentine, thus increasing the success rate in endodontic therapy (Gutknecht et al., 2004), but when comparing diode laser to NIR Nd:YAG laser system, it will show small bactericidal effect towards Enterococcus faecalis (Schoop et al., 2004).

Furthermore, a previous study mentioned that antibacterial effect has been noticed when combining different chemical disinfectants with laser, such as sodium hypochlorite (NaOCl) against Enterococcus faecalis, even in the deeper layers of dentine. The procedure can be done by drying out the root canal with paper points and using files to enlarge the root canal orifice up to ISO 30 (Pirnat, 2007).

2.6.5 Diode laser in teeth bleaching

The whitening technique has been used and documented in the mid-90s and the patients' demand is still growing nowadays as they want to have whiter teeth. This has caused an increase in the public desire for changing the shades of their natural dentition. Bleaching can enhance the shades of pigmented teeth, and is considered the less expensive aesthetic treatment option (Dostalova et al., 2004).

Previous article has been published regarding this topic, mentioning that this procedure is considered to be simple and non-invasive (Sulieman et al., 2006). The common bleaching materials used are hydrogen peroxide (HP) or carbamide peroxide (CP) as active ingredient. The bleaching process works by breaking down of the HP gel to release free radicals that penetrate the tooth pigmented layer and cause the oxidation of stain molecules within the tooth structure. The bleaching agents present in a form of gel, which is easily applied on the dentition surfaces and then initiated with diode laser light, giving a great whitish effect on teeth and thus improve the appearance. As diode laser light also produce heat on the tooth surface, it should be used in an appropriate parameter to ensure the safety of the pulp tissue (Sulieman et al., 2006; Alqahtani, 2014).

A previous study investigated the whitening capability of light source laser and light-emitting diode (LED) when applied on different bleaching agents and found that laser showed superiority than the LED (Wetter et al., 2004). The results covered in both aspects of chroma and luminosity when associated with the whiteness of HP bleaching agent.

2.6.6 Diode laser system for treating dentine hypersensitivity

Madhavan et al. (2018), Kirpa Johar et al. (2011) stated that diode laser plays an effective role as analgesic tool by blocking and reducing the nerve transmission and prevent the depolarisation of C afferent fibres. Findings from a clinical study (Corona et al., 2003), it was concluded that low-level Gallium-Aluminum-Arsenide (GaAlAs) laser and sodium fluoride (NaF) varnish could decreased cervical dentine hypersensitivity in the severe cases of dentine hypersensitivity.

Recent articles published regarding the role of diode laser *in vitro* with different power output range from 810 nm - 980 nm proved the effectiveness of these wavelengths to seal and narrow the exposed dentinal tubules with minimum harm or cracks on the dentine surface (Umana et al., 2013; Al-khafaji et al., 2017).

A clinical study performed in 2012 demonstrated that GaAlAs laser has proven to have a very high ability to reduce pain caused by dentine hypersensitivity solely or with an addition of sodium fluoride (NaF) gel (Umberto et al., 2012).

CHAPTER 3

MATERIAL AND METHODS

3.1 Study design

This is an *in vitro* study investigating the effect of 940 nm diode laser on the dentinal tubules of extracted permanent teeth. The temperature changes in the pulp while radiating the teeth were also observed. The morphological changes of the dentinal tubules were assessed under SEM. The approval to conduct the study has been obtained from Human Research Ethics Committee (HREC) of Universiti Sains Malaysia (Appendix 1).

3.2 Sample size calculation

The sample size was calculated using the PS software (Dupont & Plummer, 1998), based on comparing two mean formula. To study the effect of laser parameters on the dentinal tubules and temperatures, the power was set at 80%, the alpha (α) at 0.05, the significant temperature changes at 0.8, and standard deviation at 0.4 (Umana et al., 2013). Therefore, a total of 4 teeth (N=4) were required for each group in this study, which would give a total of 16 samples (n=16) for four groups. Considering the dropout rate, we decided to use 7 teeth (N=7, n=28).

3.3 Criteria for teeth selection

3.3.1 Inclusion criteria

1. Sound permanent premolar teeth.
2. Caries free premolar teeth.
3. Extracted teeth due to orthodontic purpose or periodontal disease.

3.3.2 Exclusion criteria

1. Premolar teeth with caries or crack.

2. Premolar teeth with restorations.

3.4 Material

3.4.1 Teeth selection

Seven (7) permanent upper first premolar teeth (Figure 3.4.1) were used in this experiment due to the anatomical structure of two roots that would assist in the sectioning step. Furthermore, the teeth had enough amount of dentine surface needed for the experiment.

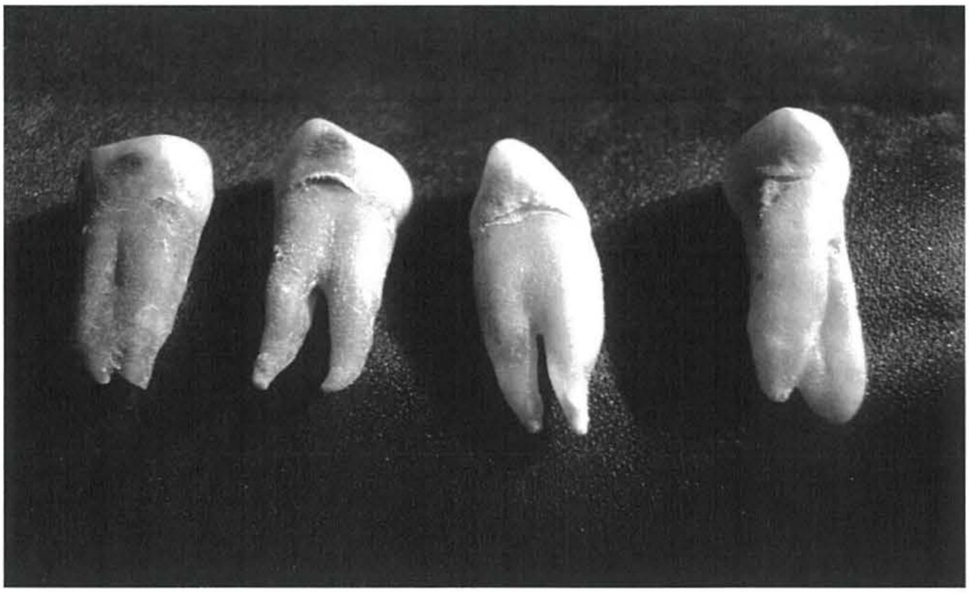

Figure 3.4.1 An overview of the permanent upper premolar teeth used in the experiment (G. Richard Scott, 2017)

3.4.2 Ilase diode laser

An ilase diode laser (Biolase, USA) (Figure 3.4.2) was used in this study to evaluate the effect of different wavelengths on the prepared tooth morphology and to measure the temperature changes. This laser produces 940 nm of wavelength. The 940