# IN VITRO INVESTIGATION OF THE POTENTIAL OF 1940 NM THULIUM FIBRE LASER AS A SURGICAL TOOL FOR ORAL SOFT TISSUE

by

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# ABSTRACT

## IN VITRO INVESTIGATION OF THE POTENTIAL OF 1940 NM THULIUM FIBRE LASER AS A SURGICAL TOOL FOR ORAL SOFT TISSUE

Lasers of different wavelengths are being used in oral surgery for making incisions and excisions with minimal bleeding and pain. Thulium fibre laser, with its 1940 nm emission, is well absorbed by water, making it a promising tool for oral soft tissue surgery. This study was conducted to investigate the potential of Tm: fibre laser as an incisional and excisional oral surgical tool.

Both 1940 nm Tm: fibre and 980 nm diode laser were used on ovine tongues in this study for comparative purposes. Both lasers were applied in contact to the tissue, which was completely submerged in saline solution, via a 600 $\mu$mm fibre. The incisions were made by moving the fibre tip manually at different speeds (0.5, 0.75 and 1 mm/s) and with making single, three or five passes, using three different power settings (2.5,3 and 3.5W for Tm:fibre and 12,14 and 16W for diode laser). The samples were stained with H&E for microscopic evaluation of depth of ablation and extent of coagulation, and ablation efficiencies were calculated.

Deeper ablations, as well as larger coagulations were obtained with both lasers by using higher power settings. However, making more passes at constant power yielded deeper ablations without significantly larger coagulation zones. Furthermore, increasing the speed caused shallower ablation and narrowed coagulation zones. Microscopically, a narrow vacuolization and a large coagulation zone were observed for 1940 nm Tm: fibre laser, whereas the coagulation zone produced by 980 nm diode laser was larger and no vacuolization was evident.

**Keywords:** Laser surgery, fibre laser, oral surgery, thulium.

# ÖZET

## 1940 NM TULYUM FİBER LASERİN ORAL DOKU İÇİN CERRAHİ ARAÇ OLARAK İN VİTRO İNCELENMESİ

Farklı dalgaboylarında laserler, en az kanama ve acı ile kesik açma ve kesip çıkarma amaçlarıyla oral cerrahide kullanılmaktadır. 1940 nm dalgaboyuyla Tulyum fiber laser, su tarafından iyi soğurulduğundan oral yumuşak doku cerrahisi için gelecek vaat eden bir araçtır. Bu çalışma tulyum fiber laserin bir oral cerrahi araç olarak kesik açma ve keserek çıkarma potansiyelinin incelenmesi için yapılmıştır.

Karşılaştırma yapabilmek amacıyla bu deneyde hem 1940 nm tulyum fiber laser hem de 980 nm diyot laser koyun dili üzerinde kullanılmıştır. Her iki laser de tamamen salin çözeltisine daldırılmış dokuya 600 $\mu$mlik bir fiber aracılığıyla kontakt olarak uygulanmıştır. Kesikler fiberin ucu farklı hızlarda (0.5, 0.75 ve 1 mm/s) hareket ettirilerek, üç farklı güçte (Tm: fiber için 2.5,3 and 3.5W ve diyot için 12,14 and 16W) ve tek, üç veya beş geçiş yapılarak açılmıştır. Örnekler, mikroskopik değerlendirmede ablasyon derinliği ve koagülasyon genişliğinin ölçülmesi ve ablasyon veriminin hesaplanması için H&E ile boyanmıştır.

Daha yüksek güç kullanılarak daha derin ablasyonla birlikte daha geniş koagülasyon elde edilmiştir. Lakin sabit güçte daha çok geçiş yapmak daha geniş koagülasyona sebebiyet vermeden daha derin ablasyon oluşturmuştur. Bunun yanında, hızı arttırmak daha sığ ablasyon ve daha dar koagülasyona sebep olmuştur. Mikroskopik olarak, 1940 nm tulyum fiber laser için dar bir kofullaşma ve geniş bir koagulasyon alanı gözlemlenirken, 980 nm diyot laserin oluşturduğu koagülasyonun daha geniş olduğu ve kofullaşmanın bulunmadığı görülmüştür.

**Anahtar Sözcükler:** laser cerrahi, fiber laser, oral cerrahi, tulyum.

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| | |
|---|---|
| $\mu_a$ | absorption coefficient |
| $^{\circ}C$ | Degree Celsius |

# LIST OF ABBREVIATIONS

| | |
|---|---|
| $ANOVA$ | Analysis of Variance |
| $cm^{-1}$ | Wavenumber |
| $CO_2$ | Carbon dioxide |
| $CW$ | Continuous Wave |
| $Er:YAG$ | Erbium-doped Yttrium Aluminium Garnet laser |
| $Er,Cr:YSGG$ | Erbium, Chromium doped Yttrium Scandium Gallium Garnet laser |
| $H\&E$ | Haematoxylin and Eosin stain |
| $Hb$ | Haemoglobin |
| $HbO_2$ | Oxyhaemoglobin |
| $He$ | Helium |
| $KTP$ | Potassium titanyl phosphate |
| $LASER$ | Light Amplification by Stimulated Emission of Radiation |
| $LITT$ | Laser-Induced Interstitial Thermotherapy |
| $mm$ | millimeter |
| $\mu$m | micrometer |
| $\mu$s | microsecond |
| $mm/s$ | millimeter per second |
| $mW$ | milliwatt |
| $N_2$ | Nitrogen gas |
| $Nd:YAG$ | Neodymium-doped Yttrium Aluminum Garnet laser |
| $NIR$ | Near Infrared |
| $nm$ | nanometer |
| $Tm:fibre$ | Thulium fibre laser |
| $UV$ | Ultraviolet |
| $W$ | Watt |

# 1. INTRODUCTION

## 1.1 Motivation and Objectives

Oral surgery is a branch of dentistry which deals with the diagnosis and treatment of various diseases, injuries and defects involving the oral cavity. The conditions that require surgical treatment in this region may involve soft (lips, cheeks, gingiva) and hard (bones, teeth) tissues. Such conditions include; teeth extraction, management of traumatic injuries, salivary gland diseases, detection and management of oral cancer and precancerous lesions [7].

On soft tissues, surgical operations are generally carried out for aesthetic, functional or curative reasons on lesions that may be due to acquired, pathological, congenital or developmental factors such as fibroma, mucocele, gingival hyperplasia, leukoplakia and squamous cell carcinoma [8, 9]. Generally, short surgical techniques, called minor oral surgical procedures, applied on superficial tissues under local anaesthesia are sufficient for management of aforementioned lesions. These procedures include excisional and incisional biopsies, frenectomy, gingivectomy, gingivoplasty, vestibuloplasty and leukoplakia removal and constitute a major part of dentistry since the majority of patients need some type of minor surgery during the course of their dental management [10, 11].

Conventionally, these operations are carried out using scalpels and scissors to incise or excise tissue, haemostats to control bleeding, toothed and non-toothed forceps to grasp tissues and arrest bleeding vessels, and sutures and needles to bring the wound edges together. Ancillary instrumentation is often required such as suction apparatus for removing blood, saliva and debris and cautery for controlling bleeding [12]. Undoubtedly, the most critical drawback of conventional surgery is haemorrhage, which needs to be controlled by occluding the vessel or by coagulating the bleeding points [13]. Management of haemorrhage is not only essential for the well-being of the

patient but also required to provide a clean surgical field for the surgeon. Thanks to the advances in technology, bloodless surgery is made possible by utilizing cryosurgery, electrosurgery or laser surgery techniques.

The only common point being the bloodless tissue destruction, these techniques utilize different mechanisms to achieve their goal. In cryosurgery, controlled destruction of tissues by freezing with liquid nitrogen or nitrous oxide is managed although it is reported as painful during the freezing and thawing period, may cause headache and oedema [14]. Electrosurgery, on the other hand, uses electrodes to cut and coagulate tissue however, the heat produced induces pain which requires the use of anaesthetics and the unpleasant odour of burning flesh is unavoidable [15]. Several different lasers are being used to overcome these drawbacks, with new wavelengths being researched for better performance and new models are made available for dentists.

Laser technology had been the subject of various biomedical studies almost as soon as it was demonstrated and quite a few investigations had been conducted since its invention to examine the possible uses as medical instruments. The first dental laser, developed in 1989 by American Dental Technologies, was a Nd: YAG laser emitting at 1064 nm which was deemed suitable for procedures involving soft tissues although low-powered [16]. The number of wavelengths available for soft tissue surgery increased remarkably since then, including $CO_2$, Nd: YAG, argon, Er: YAG, Er,Cr: YSGG, KTP and various diode wavelengths. The effects of each of these lasers on soft tissue had been investigated and used to determine the suitability of each laser for various procedures. Thulium fibre laser is chosen for this study due to its advantages as a fibre laser over other types of dental lasers such as solid state or gas lasers. Fibre lasers are compact and easy to manufacture since the cavity does not require the alignment of mirrors. Furthermore, the flexibility of optical fibres used allows a much greater freedom in construction while the large surface area permits faster heat dissipation providing a thermal advantage. Also, fibre lasers are inherently single mode therefore can be easily coupled to other optical fibres without the need for beam shaping optics [17]. However, laser surgery possesses two most important drawbacks, one being the possible extensive collateral thermal damage, the other being the risk of carbonization.

Both these disadvantages can be overcome with choosing appropriate wavelength and laser parameters.

The motivation of the proposed study is to examine the incisional and coagulational properties of previously untested 1940 nm Thulium fibre laser on oral soft tissues, while comparing these properties to widely used 980 nm diode laser. This study aims the following:

1. Diminishing the collateral thermal damage during incision, and eliminating carbonization,
2. Determining the suitable laser parameters for desired coagulation and ablation effects.

## 1.2 Scope of the Thesis

The coagulation and vaporization properties of previously untested Thulium fibre laser on oral soft tissues was tested against the widely used 980 nm diode laser in this study. 1940 nm Thulium fibre laser was preferred due to its physical advantages such as compact size and fibre delivery, as well as the benefits of the wavelength due to the absorption characteristics of soft tissue.

In the incision studies, the effects of power, fibre tip velocity and the number of passes on the extent of coagulation and ablation efficiency were inspected. The ablation and coagulation areas for the incisions were measured after tissue processing.

## 1.3 Outline

Part 2 of this thesis builds the necessary background information about the soft tissues of the oral cavity, anatomically and histologically, as well as laser- tissue

interactions and the use of lasers in oral soft tissue surgery. The information about the materials used in the experiment together with the methods utilized for the study can be found in Part 3. Part 4 is comprised of the findings and results of the study, whereas these results and their implications are discussed in Part 5. Lastly, Part 6 presents the conclusion and possible further works of the proposed study.

# 2. BACKGROUND

## 2.1 The Soft Tissues of Oral Cavity

Oral cavity is the beginning of the digestive system and can be studied in five parts as seen on Figure 2.1 : lips, cheeks, gingiva, floor of mouth and the palate. The whole of oral cavity is lined with oral mucous membrane which is divided into three subtypes based on the functional adaptation as masticatory (covering gingiva and hard palate), lining (covering cheek, floor of mouth and soft palate) and specialized (covering the dorsum of tongue). Oral mucous membrane consists of two layers of epithelium, which is avascular therefore it grows on underlying vascular connective tissue, termed lamina propria, and covers the submucosa, in which glands, blood vessels and nerves are present. The epithelium of oral mucosa is stratified squamous type and may be keratinized or nonkeratinized [18].

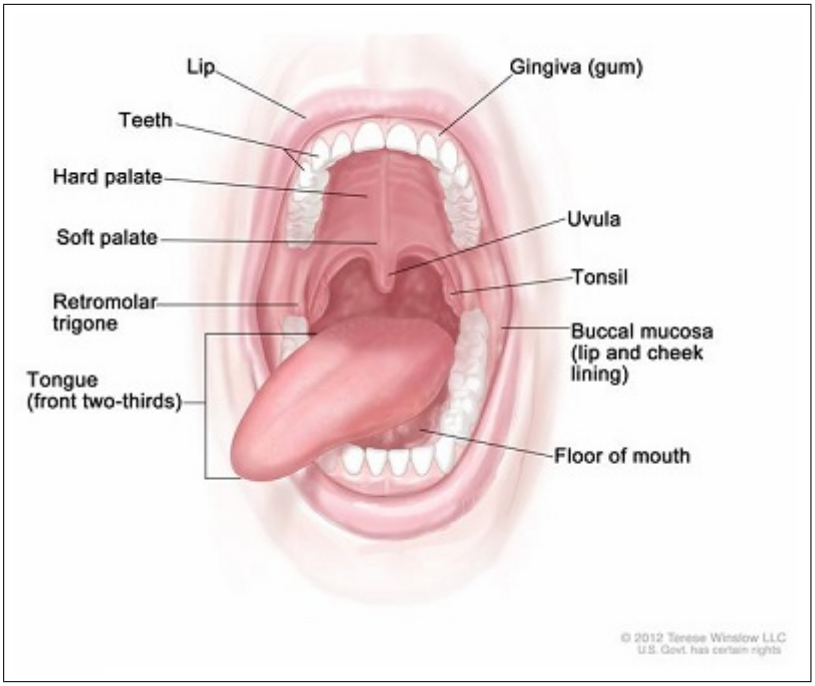


**Figure 2.1** The oral cavity [1].

The lips are the junction between the skin of the face and mucosa of the oral cavity, the zone called vermillion due to its red-purple colour. The inner surface (labial

mucosa) is covered with thick, non-keratinized epithelium and submucosa contains many minor salivary glands. The mucosa is bound to the underlying muscle with dense connective tissue [8].

Cheeks are the lateral borders of the oral cavity, extending anteriorly from the labial commissure (corner of lips), posteriorly to the ramus of the mandible (ascending part of lower jaw under each ear) and limited between maxillary and mandibular vestibules (space between lips and teeth). The lining mucosa is stratified squamous epithelium which contains small salivary glands. A hyperkeratinized line, called linea alba, whose position is related to the occlusal plane, can be observed [19].

Gingiva (gums) is the part of oral mucosa which surrounds the teeth at where the root and crown join. It is coral pink in colour and is covered by keratinized epithelium. Healthy gingiva provides support and protection to teeth and has an important role in proper speech (phonetics) [20].

The floor of the mouth is the horseshoe shaped region above the mylohyoid muscle which stretches between the anterior portions of the mandible and is occupied by the tongue. Lingual frenum extends from the midline to the inferior surface of the tongue. The tongue is composed of several muscle systems and is covered with specialized mucosa, rough in texture due to the presence of many papillae. The connective tissue between the mucosa and the underlying muscles contain many small salivary glands [21].

The last part, the palate forms the roof of the mouth and is divided to two; the immovable hard palate which lies anteriorly and the movable soft palate lying posteriorly. The hard palate is covered with keratinized mucosa and is firmly bound to the underlying bone. Soft palate, on the other hand, is darker red in colour and seals the oral cavity posteriorly and uvula is placed in the midline at the free edge of soft palate. Palatal mucosa, like the mucosa of cheeks, contains numerous salivary glands [22].

## 2.2 Oral Soft Tissue Surgery

### 2.2.1 Lesions

There are several dental procedures that necessitate surgery of the oral soft tissues such as soft tissue injuries, recontouring and grafting, in which the target tissue can be gingiva, tongue, lips or cheeks. The main soft tissue procedures can be listed as; oral biopsy, ankyloglossia, frenectomy, gingivectomy and gingivoplasty, removal of lesions and removal of leukoplakia which collectively constitute a major part of dentistry since majority of patients need some type of minor surgery during the their dental management.

Biopsy is the sampling of a lesion to examine it microscopically in order to establish a diagnosis. It is required for every pathological condition with a non-characteristic clinical picture. Biopsies can be either incisional, where only a representative part of the lesion is sampled, or excisional, where the entire lesion is excised for examination. Incisional biopsy is always preferred for lesions considered malignant in order to prevent the possibility of healing and obscuring of the original lesion that may result from excisional technique. Excisional biopsy is a combined approach for diagnostic and treatment purposes for unsuspicious lesions [23].

Generally, a frenulum is a fold of mucous membrane that connects two parts. In oral cavity, two types of frenulum are present, lingual and labial. Labial frenula run from the gums to the lips whereas lingual frenulum connects the floor of the mouth to the underside of the tongue in the midline [24]. Shorter or thicker than usual frenula present several problems both for gingiva and tongue, the most prominent one being ankyloglossia (tongue-tie).

Ankyloglossia is a congenital developmental condition in which the lingual frenulum is either too short, or attaches too near to the tip of the tongue. Normally, the tip of the tongue should reach the anterior palate during functional movements yet in case of ankyloglossia it fails to reach that limit. It may cause difficulty in swallowing

food and maintaining oral hygiene, and speech disorders. In severe cases of ankyloglossia, surgical removal of lingual frenulum (frenectomy) can be done to free the tongue. Labial frenectomy, on the other hand, is mostly done during orthodontic treatment to assist with closing a front tooth gap or to improve oral hygiene [25].

Gingivectomy/gingivoplasty is the reshaping of the gingiva via excision of the soft tissue wall of the periodontal pocket (gums). The indications of the procedure can be gingival hyperplasia (enlargement of gums due to medication or genetic factors), to create aesthetic gingival form, to eliminate soft tissue craters resulting from disease or to create clinical crown length for endodontic purposes [26].

Lesions that cannot be classified as any other lesion are called leukoplakia and are characterized histopathologically by hyperkeratinized epithelial thickening that is mostly white in colour. The origin is mostly unknown yet a causal relationship with smoking is established. The surgical removal of leukoplakia is required when malignant transformation is suspected which occurs in approximately 10% of cases [27].

### 2.2.2 Armamentarium

The basic armamentarium for soft tissue procedures is comprised of scalpels, haemostats, toothed and non-toothed forceps, scissors, sutures, needles and electrosurgical unit although there have been significant advances in instrumentation in the last two decades and the list has been expanded to include surgical lasers as well [12].

Oral surgical instruments are manufactured using corrosion resistant steel and many frequently used ones are available as sterile disposable items. The most commonly used instrument is undoubtedly the straight handled scalpel with a suitable blade which is used for all mucosal incisions. Shaping and trimming of tissues are generally done with pointed or blunt scissors. The main drawback of using scalpels and scissors for incision and excisions is bleeding. Especially for highly vascularized tissues the management of haemorrhage is an important problem. The management of

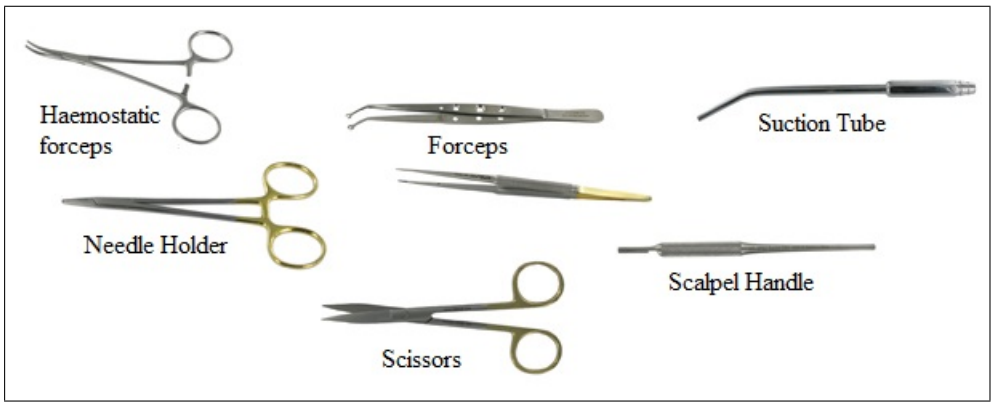


**Figure 2.2** Elements of surgical tray for soft tissue surgery [2].

haemorrhage can be done by applying pressure with gauze, occluding the bleeding vessel via the use of haemostats or coagulating the bleeding points by surgical diathermy [13, 28].

Electrosurgery is a bloodless option for soft tissue procedures compared to conventional scalpel cutting. This method utilizes alternating electric current energy at high frequency, concentrated at tiny electrodes and can be used to produces localized changes in tissues (limited to 2-3 cell layers) that can be cut and coagulate. The main advantage of electrosurgery is easily achieved haemostasis along with rapid procedure and sterile wound [29]. However, it requires anaesthesia almost always. Furthermore, the thermal damage at the excisional margins may delay surgical wound healing and may interfere with the histological examination. There also is the risk of excessively scarring associated with the use of disproportionate application of electrical energy [30].

Another alternative for conventional surgery is cryosurgery, which can be defined as the controlled destruction of undesirable tissues by freezing. Liquid nitrogen is the most effective cryogen that can be used to freeze tissues clinically. While cryosurgery has the advantage of lesion removal without bleeding, unpleasant burning flesh odour or scar formation and has excellent cosmetic results, it also has some disadvantages such as leaving no tissue for histopathological analysis (therefore an incisional biopsy is required for suspicious lesions before cryosurgical treatment), depigmentation in the treated

area, pain during freezing and thawing period, headache and delayed haemorrhage from the wound site [14, 31].

Although conventional surgery performed with scalpel is the most widespread approach in soft tissue procedures, new equipment and techniques are constantly being developed to eliminate drawbacks such as haemorrhage and pain and to facilitate operations. Laser is one the alternative instruments developed to overcome the disadvantages of conventional surgery. Laser technology had been the subject of biomedical research from the very beginning and nowadays, several types of lasers are available for surgical purposes.

## 2.3 Soft Tissue Laser Interactions

### 2.3.1 What is LASER?

The word LASER is an acronym for "Light Amplification by Stimulated Emission of Radiation" which is used to denote a device that makes use of processes 'stimulated emission' and 'optical feedback' to amplify light [32].

The simplified design of a solid state laser is illustrated in Figure 2.3. The light produced by a laser has three characteristic properties: monochromaticity, coherence (both temporal and spatial) and directionality making lasers excellent tools for special purposes such as cutting, welding and selective absorption [33].

Monochromaticity of laser light can be demonstrated by the very narrow laser spectral lines, which are graphs of laser intensity versus laser wavelength, and can simply be explained as the colour of laser light being highly pure due to the exclusion of any other wavelength. The origin of monochromaticity is the atomic transition, which produces the output, whose energy corresponds to a single precise wavelength. The origin of coherence property of laser light, on the other hand, is the stimulated emission mechanism, which is also responsible for the amplification of light in a laser system.

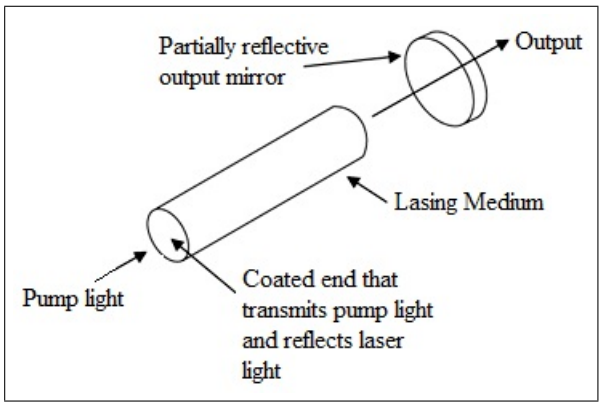


**Figure 2.3** Simplified solid state laser design.

When a passing photon stimulates a transition from a higher energy level to a lower one, another photon is emitted which is in phase with the previous stimulating photon. If an avalanche of photons is produced by the same manner, they will possess the same fixed phase relationship with each other, hence having the property, coherence. Lastly, directionality is a characteristic simply provided by the architecture of the resonant cavity which ensures that only waves travelling along the optical axis are sustained and waves even slightly off axis are not reflected by both mirrors therefore, will be lost. The only light beam exiting the resonant cavity will have to be formed by waves travelling exactly perpendicular to the boundary mirrors, since only such waves have a chance of being reflected back and forth between those mirrors which provides a longer path for amplification [34, 35].

Characterization of lasers can be done either according to the physical state of the active medium; such as gas, liquid and solid state, or according to the wavelength of output beam; such as infrared, visible, ultraviolet and x-ray [33].

These properties of the laser beam are utilized in surgical lasers to provide the surgeon with a multipurpose tool that can cut, ablate, coagulate and stop bleeding. The intense, highly directional beam can be used to incise and excise as well as for reshaping and resurfacing purposes. These effects on tissues are the result of laser-tissue interaction mechanisms which can be studied in five sections: photochemical interaction, thermal interaction, photoablation, plasma induced ablation and photodistruption.

### 2.3.2 Laser-Tissue Interactions

Interactions of light with matter can be studied in two levels; molecular level, which produces absorption, emission (spontaneous and stimulated) and Raman scattering, and bulk matter, which produces absorption, refraction, reflection and scattering.

Since biological tissues are bulk media, light propagation would produce absorption, scattering, refraction and reflection as illustrated in Figure 2.4. Among these interactions, scattering is the most pronounced since tissues are highly scattering turbid media, whose turbidity is caused by its very heterogeneous structure of macromolecules, cell organelles and water. Scattering, as well as absorption, are responsible for the loss of intensity as the light propagates through a tissue [3]. However, absorption is the most important interaction in determining the type and effect of a laser-tissue interaction.

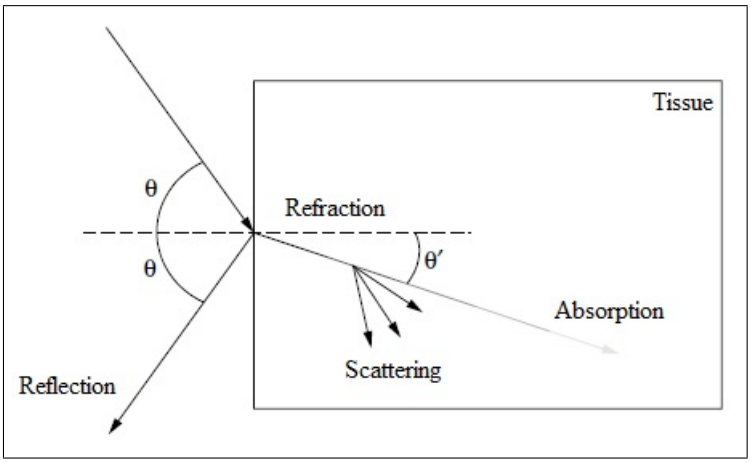


**Figure 2.4** Light-tissue interactions [3].

The type of interaction that takes place upon exposure is not only determined by optical tissue properties but also the laser beam parameters. The tissue properties consist of coefficients of scattering, reflection and absorption, which collectively decide the total transmission of the tissue at a certain wavelength. These transmission values can be reinterpreted as absorption graphics which in turn can be used to select the most appropriate wavelength for the desired tissue penetration and effect.

On the laser side of the interaction, the parameters used to define the type of

interaction are, duration of exposure, wavelength, energy density and intensity of the beam. Among these, duration is the most noteworthy parameter since the effects can be generally divided into thermal effects for pulses longer than 1 $\mu$s and non- thermal for pulses shorter than 1 $\mu$s. Wavelength is the second important parameter which determines how deep the laser penetrates. On the other hand, energy density is a parameter used only to provide a certain condition for the occurrence of an effect, a certain threshold defining the border between plasma-induced ablation and photodistruption [6].

The effect most frequently encountered in oral surgical procedures is thermal since the lasers used are chiefly continuous wave or have pulse widths longer than 1 $\mu$s and also the wavelengths are in the infrared region.

Thermal laser-tissue interactions can be studied in molecular level in which light incident on a molecule can result in radiative (fluorescence) or non-radiative (thermal, photochemical) processes upon absorption. The absorption of photons induces a transition of electrons from the ground electronic level to an excited state, which is energetically unstable. The electrons will struggle to establish their initial state by losing energy by either a radiative process, which is the reemission of the energy of photon in the form of fluorescence, or a non-radiative processes, which can result in either a chemical change via inter or intra molecular energy transfer such as isomerization or ionization, or a thermal change [36].

Heat production, being the most dominant result of absorption, occurs when an increase in the vibrational or rotational level of the absorbing molecule takes place. The temperature rise in the molecule upon exposure to light is directly proportional to the total energy absorbed provided that the absorbed optical energy does not leave the area via diffusion (which means that the radiation is either continuous wave or the pulse duration is shorter than the thermal relaxation time of the molecule). Therefore, in order to selectively heat a particular target (soft tissue in this case) three conditions must be satisfied: specific wavelength of light that will be absorbed by the target, continuous wave or pulse duration longer than thermal relaxation time and radiant

exposure sufficient to produce the desired temperature rise. Often, the pulse duration and radiant exposure of the laser can be manually controlled therefore it is the wavelength of the laser light that needs to be decided according to the nature of the desired effect [37].

In order for a thermal interaction to take place upon laser light exposure, the photons of the incident beam must be absorbed by the target tissue. The molecules responsible for the absorption process in the tissue are called chromophores. Technically, a chromophore is 'the part of a molecular entity in which the electronic transition responsible for a given spectral band is approximately localized' [38], which can be rephrased as 'the part that is responsible for the colour of a molecule'. However, in terms of laser-tissue interactions, a chromophore is a molecule such as water, protein or pigment, which absorbs photons [39]. Every chromophore has its own characteristic absorption spectrum which can also act as its fingerprint. The occurrence and concentration of chromophores depend of the type of the tissue thus, vary throughout the body. Haemoglobin, water, melanin, hydroxyapatite, protein and lipids are the most frequently encountered chromosomes in the body therefore are called main chromosomes.

Haemoglobin has two different absorption spectra depending on whether it is rich in oxygen or not. Oxyhaemoglobin, oxygenated haemoglobin, is rich in oxygen and has three absorption peaks at 418, 542 and 577 nm deoxyhaemoglobin [40] on the other hand, has no oxygen molecule bonded, therefore possesses a different spectrum with only two absorption peaks at 430 and 555 nm. Melanin, which is responsible for the colouring of skin, has no absorption peak but absorbs majorly visible light. It also shows Rayleigh scattering behaviour and thus can be regarded as scattering particles. Likewise, adipose tissue containing most of the lipids is considered as scattering tissue as well however it also has its own characteristic absorption spectrum. In addition, proteins and vitamins can also be regarded as chromophores with their absorption peaks located in the UV range of the spectrum [41].

Water, which is the most abundant molecular content of human soft tissue

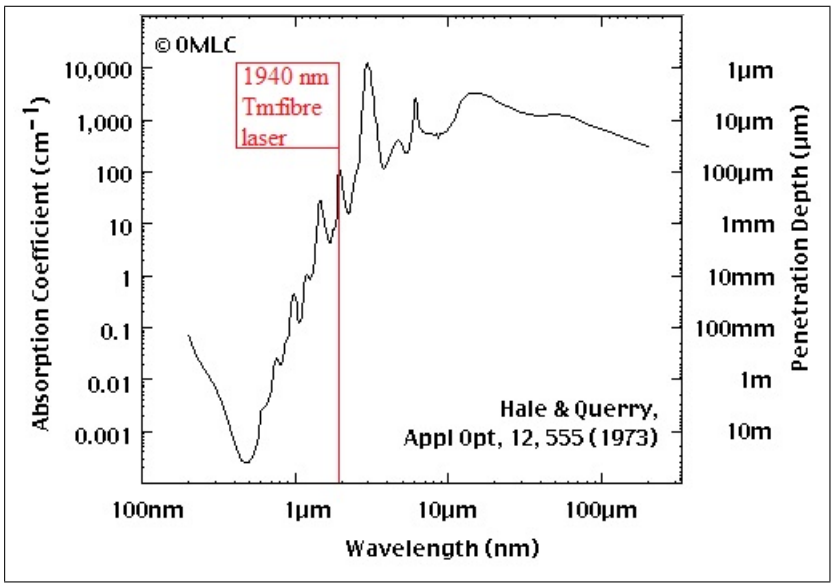


**Figure 2.5** Absorption spectrum of water [4].

(about 65%) [42], is almost completely transparent at visible wavelengths however it becomes a chromophore in the NIR range. As depicted in Figure 2.5 the first absorption peak for water is located at 973 nm which has the lowest absorption coefficient ($\mu_a$) amongst the peaks (0.45 $cm^{-1}$), followed by the peaks at 1440 and 1940 nm. The highest absorption coefficient is observed at the peak located at 2940 nm with $\mu_a$= 12694 $cm^{-1}$. The last absorption peak is observed at 6100 nm [43].

Due to the distinct absorption peaks of various chromophores present in human soft tissue it is possible to selectively target molecules for the desired effect. The chromophore of choice is determined by the nature of the lesion e.g., haemoglobin for telangiectasia and melanin for hyperpigmentation. For excisional and incisional purposes, haemoglobin or water can be targeted depending on the amount of perfusion. In the case of incident light exceeding 1400 nm in wavelength, water becomes the main chromophore due to scattering in tissue becoming insignificant compared to absorption [40, 43].

The absorption spectra of chromophores commonly encountered in soft tissues are shown in Figure 2.6.

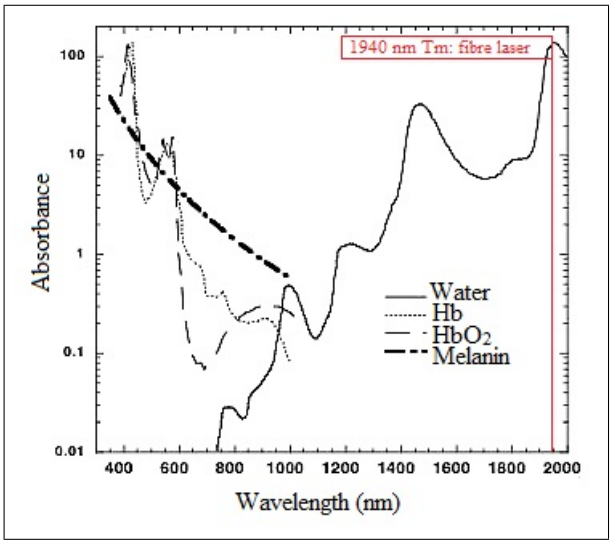


**Figure 2.6** Common chromophores for soft tissue and their absorbance spectra. ($Hb$: Haemoglobin, $HbO_2$: Oxyhaemoglobin) [5].

Photons absorbed by the target chromophore result in a local temperature rise, which in turn provides thermal tissue effects. These effects consist of hyperthermia, coagulation, vaporization and carbonization. Among these, coagulation and vaporization are utilized to necrotize, cut or ablate tissue and therefore are aimed for, whereas carbonization should always be avoided since it reduces visibility during surgery and prevents laser beam from reaching deeper into the tissue by absorbing all the energy [6, 44].

These thermal effects are measureable and are based on the tissue temperature. When tissue is heated starting from normal body temperature ($37°C$) no change is observed until $45°C$ at which point conformational change in molecular structure occurs. However, this change is reversible if the exposure time is carefully controlled and does not exceed several minutes. Tissue necrosis due to thermal effects depends not only on the temperature achieved within the tissue but also the duration which the tissue needs to withstand that temperature. Generally, irreversible damage to the tissue starts to form at $60°C$ lasting for at least six seconds [6, 45].

For temperatures around $60°C$ coagulation is the dominant tissue effect which is the denaturation of proteins and collagen. This denaturation process causes tissue

shrinkage which is responsible for the sealing of small vessels hence it can be said that coagulation is responsible for the haemostatic efficiency of laser and the depth of coagulation determines the size of vessels that may be sealed. Coagulation can be observed macroscopically as the paling of the tissue and microscopically as vacuolization and protein denaturation. It may also be useful to kill malignant tissue, a process on which treatments such as LITT (Laser-induced interstitial thermotherapy) is based [46, 47].

Coagulation gives way to vaporization when a certain threshold in power density is exceeded. Increasing the temperature to $100°C$, to the boiling point of water, water content of the tissue starts to vaporize and thermal decomposition occurs. Vaporized tissue contents increase in volume, forming gas bubbles and causing mechanical ruptures. This effect is utilized to cut and ablate tissue. Further increase in tissue temperature occurs when all water molecules are vaporized and around $150°C$ carbonization takes place. This state is obvious by the blackening of adjacent tissue and escape of smoke [45]-[48].

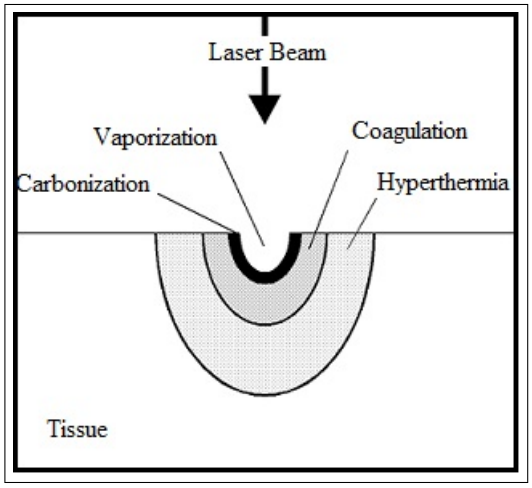


**Figure 2.7** The thermal effects with their location in tissue upon laser beam impact [6].

The location of thermal effects inside the tissue is shown in Figure 2.7. Typically, several thermal processes occur depending on the temperature gradient produced in the tissue although most of the time, only one type is aimed for. The collateral damage caused by the unwanted thermal effects should ideally be minimized by optimizing the

exposure energy and duration as well as the exposure volume [6].

## 2.4 Lasers in Oral Surgery

### 2.4.1 Advantages and Disadvantages of Laser Use in Oral Soft Tissue Surgery

Conventional surgical techniques involve the extensive use of scalpels and scissors to incise and excise for the treatment of various oral lesions which may result in excessive bleeding, especially on highly perfused tissues such as the tongue. Therefore, additional techniques and instruments are required to stop bleeding. The management of haemorrhage can be done by applying pressure with gauze, occluding the bleeding vessel via the use of haemostats or coagulating the bleeding points by surgical diathermy [12, 13]. Haemostasis is important not only to prevent further blood loss but also to provide a clean surgical field for the surgeon. Suction apparatus with the help of a suction tip or cannula is utilized to remove blood, flushing solution, debris and secretions from the surgical field to provide an unobstructed view for the surgeon [7].

Bloodless surgical field has been made possible with the introduction of laser technology to oral surgery. Lasers provide haemostatic surgery by sealing blood vessels of smaller diameter with the help of coagulation which provides a clearer view of surgical field, therefore more precise surgery in shorter time span can be carried out. Similarly, lymphatics are also sealed, reducing oedema and swelling as well as decreasing the seeding of malignant cells [48, 49]. Surgery with diathermy is considered to be as bloodless as laser surgery however; there is considerable collateral thermal damage to the healthy tissue which results in severe pain and oedema. On the other hand, the thermal damage caused by lasers can be controlled by adjusting the parameters and choosing appropriate wavelength [50].

The use lasers for surgical purposes also eliminates the need for suturing since

laser wounds heal with low levels of discomfort and relatively little scaring which means that they can be left to heal by secondary intention, giving a good functional result and satisfactory mobility of soft tissue [48, 51, 52]. Laser resection, contrary to conventional surgical techniques, also induces a tissue reaction, precursor to re-epithelisation, which makes the use of flaps to fill the defects unnecessary, further decreasing the duration of oral cavity cancer operations [53].

Sterilization is another important aspect of surgery, inadequate sterilisation of surgical instruments is known to be resulted in surgical site infection outbreaks [54]. These infections can be further prevented by the use of lasers as incising tools since the incisions opened by thermal lasers are self-sterilising which eliminates the need for antibiotics as well [48, 55].

Nevertheless, surgical lasers have their own disadvantages, the most prominent being the risk of thermal alteration in healthy tissue and carbonization [51]. A scalpel in a trained hand, beyond any doubt, causes no collateral damage to the surrounding healthy tissue, yet lasers can necrotize healthy tissue as well. This risk can be prevented altogether, or minimized by choosing a laser of appropriate wavelength and optimum parameters for the desired effect, which requires the surgeon to have special training and familiarity with the equipment. An extension of this argument states that laser use on biopsies prevents exact histological assessment of the lesion due to the thermal artefacts it causes on the borderlines. However, there have been studies showing that no interference to histopathological diagnosis was present when appropriate laser parameters were used [56, 57].

### 2.4.2 Laser in Oral Soft Tissue Surgery

The ruby laser developed by Maiman in 1960, had been the subject of various biomedical researches almost as soon as it was demonstrated and several investigations had been conducted since then to examine the possible uses of lasers as medical instruments.

**Table 2.1**
Surgical Lasers: Advantages and Disadvantages.

| Advantages | Disadvantages |
|---|---|
| Haemostatic, lasers seal blood vessels | Risk of carbonization and collateral damage |
| Clear surgical view due to lack of bleeding, reduced surgery time | Possible risk of preventing histological assessment |
| No suture needed, wound closure by secondary intention | Non-contact modes lacks haptic feedback, requires practice to get familiar with |
| Less postoperative pain | - |
| Minimal scarring | - |
| Sterile incisions | - |

The first intraoral soft tissue studies were conducted using the ruby laser which was followed by $CO_2$ laser with its ability to ablate soft tissue causing minimal haemorrhage. Studies featuring Nd:YAG laser with its excellent haemostatic abilities shortly followed. Yet, it took almost thirty years for the first dental laser to be developed. DLase 300, produced by American Dental Technologies in 1989, was an Nd:YAG laser emitting at 1064 nm which was deemed suitable for procedures involving soft tissues although low-powered [16, 58].

The number of wavelengths available for soft tissue surgery increased remarkably since the introduction of ruby laser into medicine, including $CO_2$, Nd:YAG, argon, Er:YAG, Er,Cr:YSGG, KTP and various diode wavelengths. The effects of each of these lasers on soft tissue had been investigated and used to determine the suitability of each laser for various procedures. These applications include; gingivectomy/gingivoplasty, excision of tumors/lesions, incion/excision biopsies, frenectomy, leukoplakia treatment and control of bleeding [58].

**2.4.2.1 $CO_2$ (Carbon Dioxide) Laser.** $CO_2$ laser, the workhorse of oral and maxillofacial surgery, has an active medium of a mixture of $CO_2$, $N_2$ and He gases and produces a beam of infrared light with principal bands at 9400 and 10600 nm

however it is generally operated at 10600 nm for medical applications which is well absorbed by intra- and extracellular water, making it a well suited tool for soft tissue surgery. This rapid absorption by water results in a minimal penetration depth and fast energy deposition in tissue causing quick heating of target. Cells explode due to almost instantaneous evaporation of water content, creating a zone of tissue vaporization [51, 59]. $CO_2$ laser beam can be used as an excisional/incisional tool by focusing the beam whereas defocused beam serves as a superficial vaporization mode. It can operate in continuous, chopped or superpulsed mode and the power used ranges between 10 to 100 Watts [48].

The emission wavelength produces little coagulation to adjacent tissue therefore causes little collateral thermal damage however, it produces a wide area of altered tissue with vacuolization and carbonization [60]. The major drawback of $CO_2$ laser is the wavelength of the beam which requires articulated arms or hollow core fibres in order to be transmitted which limits its manoeuvrability in confined spaces such as mouth [61]. Furthermore, the beam can be absorbed by the water content of dental hard tissues as well; leading to thermal damage, therefore contact to those tissues must be avoided [62].

The bulkiness of the delivery system and the char layer produced as a result of vaporization notwithstanding, $CO_2$ laser is widely used in oral and maxillofacial surgery to treat a variety of lesions ranging from minor operations to total glossectomies. In one of the earliest studies, on $CO_2$ laser oral surgery, treatment of 25 cases of hemangioma, fibroma or carcinoma via partial or total glossectomies were reported, in which recurrence was observed in only one of the cases, all operations were reported to be bloodless and with minimal post-operative pain [50]. However, the haemostatic effect of $CO_2$ laser was shown to deteriorate when the char layer left on the wound after ablation was removed mechanically, which resulted in oozing of blood [63]. Among the malignant and premalignant lesion that can be treated by laser surgery, leukoplakia draws significant attention due to the difficulty of removing it with conventional techniques. $CO_2$ laser ablation of leukoplakia is a fast and effective technique with reduced recurrence and malignant transformation rate yet in the studies the recurrence rate

varies from 10% [64] to 40% [65], decreasing with increased application power. Laser ablation of oral dysplasia is the recommended treatment to prevent recurrence and post-operative dysfunction that may be encountered by other conventional modalities [66]. Several complications of $CO_2$ laser procedures were reported in the study conducted by Brandon et al., which include normal microbial flora opportunistically colonizing the surgically exposed submucosal layers due to laser beam stripping off its protective layer, contact dermatitis due to overreaction to topical postoperative antibiotics and the possibility of malignant transformation by laser energy [67].

According to the study by Merigo et al., compared to other surgical lasers used for biopsies, specimens obtained using $CO_2$ laser were found to be of higher quality from a histological point of view probably due to the fact that the thermal alteration caused by $CO_2$ laser was shallower than that of 810 and 808nm diode, Nd:YAG and KTP lasers [68, 69].

The $CO_2$ laser is in use for oral surgery for more than thirty years and the results obtained have been consistent when treating soft tissue. Although it requires time and training for the operator to get familiar with the non-contact technique of incising and excising, the haemostatic quality and lack of postoperative pain makes $CO_2$ laser the most widely used laser in oral surgery [58].

**2.4.2.2 Nd:YAG Laser.** Nd:YAG laser is a solid state laser, functioning in the near infrared part of the spectrum at 1064 nm with an active medium of yttrium aluminum garnet crystal doped with neodymium ions. It exhibits maximal penetration due to its low absorption which allows for the coagulation of deep tissues [58].

Nd:YAG laser can be used in contact mode for making incisions and in non-contact mode for haemostasis. Typically, in contact mode, it causes central vaporization with deep peripheral coagulation which facilitates haemostatic cutting; however causes more oedema and post-operational pain when compared to $CO_2$ laser [70]. Higher power has been observed to be leading to larger coagulation zones rather than

increased cutting efficiency, but it increases the depth of ablation [71]. Pulsed Nd:YAG in contact mode has been proved effective in several procedures including gingivectomy, frenectomy and tissue excision by White et al., though slower in making incisions than the scalpel yet more advantageous than $CO_2$ laser due to its fibre delivery method that can easily be manipulated even in tightly confined spaces [72].

Nd:YAG laser has also been used to treat oral leukoplakia successfully by Lalabonova et al., resulting in evaporation of the lesion with no significant pain or discomfort and with smooth postoperative period [73]. It has also been reported as more effective than $CO_2$ laser for removing vascular lesions due to its better coagulation properties [74].

On the other hand, biopsies taken using Nd:YAG laser reveal serious thermal artefacts (the worst case compared to $CO_2$,diode and KTP lasers) in small specimens that might interfere with histopathological diagnosis yet the quality of these incisions can be improved by decreasing the power and increasing the pulse frequency [68, 75]. Also, having no significant water absorption which gives it the ability to penetrate deep into tissues means that care should be taken while using Nd:YAG laser in close proximity to sensitive structures such as the mental nerve since the peripheral thermal damage might have detrimental effects on such structures [76].

**2.4.2.3 Diode Laser.** Diode lasers are electrically pumped semiconductor lasers in which the active medium is formed by a p-n junction of a diode. Medical diode lasers are advantageous due to their compact and portable design and relatively moderate price. Although the wavelength range of diode lasers can be quite wide (from 375 nm to 3330 nm), medical diode lasers are usually in the range of 800- 980 nm which is preferred due to good absorption for haemoglobin which provides good haemostasis [58].

The most widely used diode laser for oral procedures has a wavelength of 980 nm with powers varying from 1 to 12 W. Elanchezhiyan et al has observed it to yield better

results with decreased bleeding and faster healing response than scalpel use in treating ankyloglossia [77]. However, considering a wider set of operations, 980 nm diode laser exhibits delayed healing. Used on management of gingival hyperpigmentation by Mani et al [78], it presented a smooth but delayed healing with no inflammatory reaction which might be the result of high power use causing superficial tissue necrosis that delays healing [79]. It has been observed to provide good coagulation and no pain, discomfort, infection or scarring when used to treat especially vascular lesions [79, 80].

Another widely used wavelength of diode laser is 808 nm which yields an incision better than Nd:YAG with similar coagulative properties [81]. The thermal damage, though tolerable, causes alterations which might interfere with the histopathological diagnosis if the specimen size is not sufficiently large [82]. Furthermore, the incisions made with 808 nm diode laser are slower than scalpel or electrosurgical unit [83].

Generally, diode laser provides good haemostasis and causes less oedema and pain both intra and post operatively however healing is delayed at the initial stage and care should be taken when biopsy specimens are obtained to avoid interference with diagnosis [84].

**2.4.2.4 KTP Laser.** Potassium-titanyl-phosphate (KTP) is actually the name of the frequency doubling nonlinear optical crystal which is commonly used with Nd:YAG laser. KTP crystal, when coupled to an Nd:YAG laser, combine the photons to form new ones with twice the frequency (thus, half the wavelength)at 532 nm which is perceived as visible green light. Light at that frequency is well absorbed by both oxygenated haemoglobin and melanin, making KTP laser a good tool to use on pigmented lesions and vessels [45].

It has been used to perform frenectomies and lesion ablation on 52 patients by Fornaini et al., who observed good results with no oedema or infection, requiring only topical anaesthetics when low power settings are used [85]. When compared to widely used $CO_2$ laser for such operations KTP has found to yield greater incision depth with

greater thermal injury which causes slow healing [86]. Another study comparing $CO_2$ with KTP has been conducted on treating leukoplakia by Lim et al., which states that KTP yield lower recurrence rates probably due to extensive tissue penetration that eliminates deep nests [87]. KTP has also been used to obtain biopsy specimens with minimal peripheral damage when low powers are used. Although thermal damage was evident on the boundaries, it has been reported as not interfering with diagnosis [56].

**2.4.2.5 Erbium (Er:YAG and Er,Cr:YSGG) Lasers.** Er:YAG (erbium doped yttrium-aluminum-garnet) and Er,Cr:YSGG (erbium,chromium doped yttrium-scandium-gallium-garnet) are both solid state lasers that emit wavelengths in the near-infrared zone of the spectrum (2940 nm and 2780 nm, respectively). Although similar in emission wavelength, these lasers have slightly different absorption coefficient, but both are well absorbed by water. The delivery system for both lasers is articulated arm, hollow core fibre or special glass fibre and can be applied both in contact and non-contact mode [58].

Er:YAG laser, when used on tissues with a sufficient water content, causes rapid evaporation of water in the most superficial layer of tissue, leaving little to no peripheral thermal damage, thus making is a good tool for soft tissue operations. It has been used in clinic for several soft tissue operations by Watanabe et al., resulting in smooth operation which lacks carbonized layer commonly seen after $CO_2$ laser application. The only drawback encountered during Er:YAG laser treatment of soft tissue has been reported as the low haemostatic effect compared to other lasers and mild bleeding [88]. Further comparison of Er:YAG to $CO_2$ laser has been done by Zaffe et al., who reported Er:YAG and $CO_2$ laser both as useful tools for excisional and incisional biopsies producing sharp wound edges, with $CO_2$ laser showing a little bit more epithelial damage. The only noted difference has been the low haemostatic effect of Er:YAG laser [89].

Er, Cr:YSGG, likewise, present good water absorption and has been demonstrated as a good tool for biopsies and soft tissue operations in general, due to its shallow penetration in tissues resulting in little peripheral damage [90]. Its major ad-

vantage over $CO_2$ laser is the clean cut of the incisions and excisions, with minimal thermal damage to adjacent tissues and no carbonization layer [91], makes it an excellent tool for obtaining biopsy specimens, eliminating the need to leave safety margins around the lesion [57].

**2.4.2.6 Thulium Fibre Laser.** Operating around the 2 $\mu$m wavelength with silica based fibres, thulium doped fibre lasers fill an important mid-infrared gap which enables minimally invasive surgery in various branches. 1940 nm Tm: fibre laser has great practical importance when soft tissues are being treated due to the high absorption coefficient of water at that wavelength [92].

Several in vitro soft tissue applications of Tm: fibre laser has been studied. The study, conducted by Fried et al., investigated the ablative effect of Tm: fibre laser in canine prostate. The study utilized a 40W, continuous wave laser, used in non-contact mode. The ablated samples, after staining, showed a thin carbonization layer (approximately 50 $\mu$m), followed by a vacuolization layer which denotes either a mechanical effect of an incomplete ablation of tissue, and a thermal coagulation layer extending from 500 $\mu$m to 2000 $\mu$m. The coagulation layer was observed to be larger than that of $CO_2$ laser, which demonstrates that Tm: fibre laser is capable of providing good haemostasis in even highly vascular tissues [93].

Another in vitro testing has been done by Keller et al, using chicken breast, porcine skin and bovine liver as target tissues. 1940 nm Tm: fibre laser was used in continuous wave at powers ranging from 11W to 20W in non-contact mode. Thermal damage zone was measured after staining and was observed to be ranging from 600 $\mu$m to 1 mm in width. The thin carbonization layer followed by vacuolization was also observed in this study [94].

Ablative effects of Tm: fibre laser on ovine brain tissue has been investigated by Tunc et al, in which the samples were irradiated in non-contact mode, both continuous wave and pulsed, at powers ranging from 200 mW to 800 mW and ablation efficiency

was calculated. Continuous wave was observed to yield more thermal effects and more ablation and the diameter of the ablated zone was observed to widen with increasing power [95].

A prospective study on clinical feasibility of Tm: fibre laser has also been conducted by Sroka et al, in which reduction of hyperplastic inferior nasal turbinates was carried out on 11 patients by coagulating the tissues in non-contact mode at 5W. The results were observed to be good with superficial coagulation being very good and fast, no bleeding was observed and no complications were encountered. Significant tissue reduction was attained [96].

# 3. MATERIALS AND METHODS

## 3.1 Sample Preparation

Fresh ovine tongues were used in this study, chosen due to their structural similarity to human tongue, widespread availability and no requirement of special handling. The samples were kept at $4°C$ after slaughter. The samples were washed with and immersed in saline before application to prevent dehydration and to simulate the damp nature of intraoral environment. The dorsal surface of the tongue was used in experiments, and three incisions were made on each tongue.

## 3.2 Experimental Set-up

Two different laser systems were used in this study: -1940 nm Thulium Fibre Laser -980 nm Diode Laser The Tm: fibre laser (TLR-5-1940, IPG Laser GmbH, Germany), emitting at 1940 nm, had a maximum power of 5W, and the output power and exposure time were controlled by a custom-built controller unit (Teknofil, Inc., Istanbul, Turkey) which could be accessed via the LabView interface to set the parameters. The output was provided through a fibre optic cable which was coupled to a 600 $\mu$m fibre via focusing lenses and xyz alignment apparatus. Output of the fibre was checked with an optical powermeter (1918-C, Newport, CA, USA) before every application.

The diode laser system (OPC-D010-980-FCPS, Opto Power Corporation, USA) was composed of diode laser source emitting at 980 nm and a controller unit which is connected to a computer. Laser parameters were set through LabView software interface. A 600 $\mu$m optical fibre was connected to the laser source via a connector, and the output power was measured with the help of the powermeter before every application.

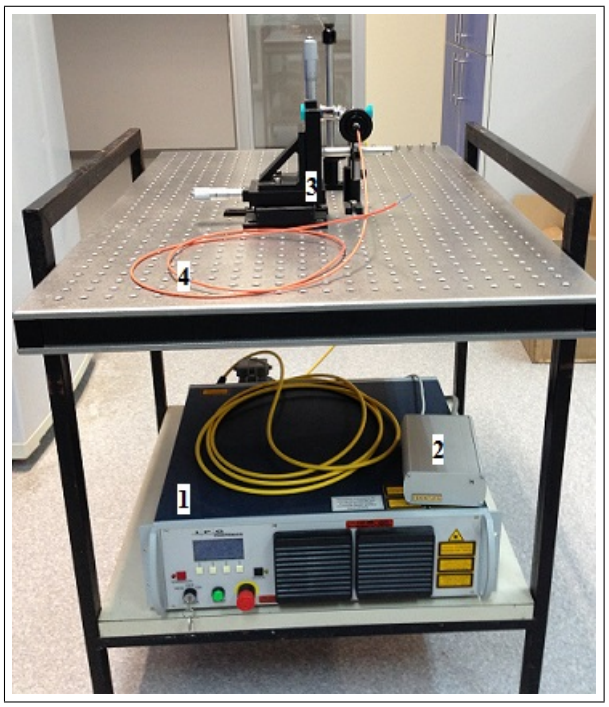


**Figure 3.1** Thulium fibre laser (1) with controller unit (2). The optical components used to couple the output to the 600 $\mu$m optical fibre (4) are located above (3).


**Figure 3.2** 980 nm diode laser.

## 3.3 Experimental Procedure

The experiments were carried out in two parts, first one using the Tm: fibre laser and the second one using the diode laser. Both parts followed the same course. Suitable goggles were worn throughout the procedure to protect eyes from stray radiation. Firstly, the power output was measured with the help of the powermeter to

make sure the output stays at the set power level. The suitable output powers were determined according to the results of the preliminary studies to avoid carbonization. The experiments were carried out only in continuous wave (CW) mode to ensure only the thermal effects of the laser were being observed. The laser was applied on contact to the dorsal surface of the tongue. The fibre was held at an angle of 30 degrees to the surface, and was moved manually, from middle towards the apex, at a constant speed to simulate the conditions encountered in the clinic.

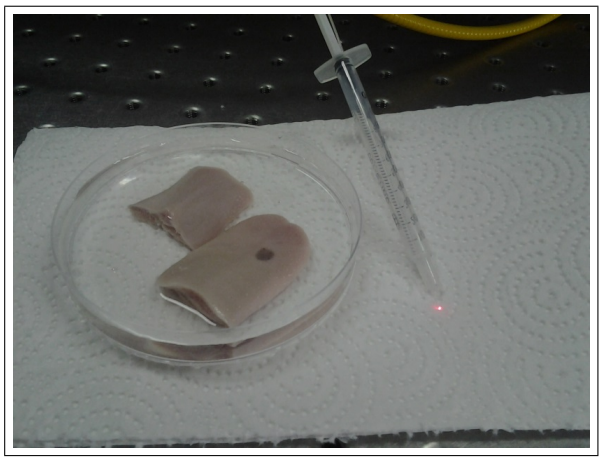

**Figure 3.3** Tongue immersed in saline in petri dish with laser fibre tip.

The incisions were then cut out in rectangular blocks for tissue processing, allowing enough (2-3 mm) unaffected tissue at the margins. The effect of three different parameters on the extent of ablation and coagulation was tested in this experiment with two different laser wavelengths. These are; power, the number of passes and speed of the fibre tip. The number of passes and speed parameters were the same for both lasers used; however, power was changed according to the results of the preliminary studies. This is because the power at which ablation started changed at different wavelengths used. The experimental groups were determined according to these parameters and incisions were divided into 27 different groups for each laser as shown below.

Power was adjusted by using the controller interfaces for both lasers and was verified with the help of the powermeter. The number of passes was increased by making more passes on top of each other with the determined power and velocity. Speed, on the other hand, was determined manually by moving the fibre tip along a

| 1940 nm CW n=8 | | |
|---|---|---|
| **Speed** | **Power** | **Number of Passes** |
| 0,5 mm/s | 2,5 W | Single pass |
| | 3 W | Three passes |
| | 3,5 W | Five passes |
| 0,75 mm/s | 2,5 W | Single pass |
| | 3 W | Three passes |
| | 3,5 W | Five passes |
| 1 mm/s | 2,5 W | Single pass |
| | 3 W | Three passes |
| | 3,5 W | Five passes |

**Figure 3.4** Experimental groups for 1940 nm thulium fibre laser.

| 980 nm CW n=8 | | |
|---|---|---|
| **Speed** | **Power** | **Number of Passes** |
| 0,5 mm/s | 12 W | Single pass |
| | 14 W | Three passes |
| | 16 W | Five passes |
| 0,75 mm/s | 12 W | Single pass |
| | 14 W | Three passes |
| | 16 W | Five passes |
| 1 mm/s | 12 W | Single pass |
| | 14 W | Three passes |
| | 16 W | Five passes |

**Figure 3.5** Experimental groups for 980 nm thulium fibre laser.

determined length for 20 seconds. The pre-set length of incisions were 1 cm (for 0,5 mm/s), 1,5 cm (for 0,75 mm/s) and 2 cm (for 1 mm/s).

## 3.4 Tissue Preparation and Staining

The incision samples were collected immediately after the experiment, and fixed in 10% buffered formalin for 4 days. At the end of the duration, an automatic tissue processor was used (TP 1020, Leica Biosystems Nussloch GmbH, Germany) to prepare the specimens for staining. An 11-hour programme, shown below was used.

**Table 3.1**
Steps of tissue processing.

| | |
|---|---|
| Alcohol 70% | 1 Hour |
| Alcohol 80% | 1 Hour |
| Alcohol 80% | 1 Hour |
| Alcohol 90% | 1 Hour |
| Alcohol 90% | 1 Hour |
| Alcohol 100% | 1 Hour |
| Alcohol 100% | 1 Hour |
| Xylene | 1 Hour |
| Xylene | 1 Hour |
| Paraffin | 1 Hour |
| Paraffin | 1 Hour |

**Table 3.2**
H&E staining procedure.

| | |
|---|---|
| Xylene | 5 minutes |
| Alcohol 100% | 1.5 minutes |
| Alcohol 90% | 1.5 minutes |
| Hematoxylene | 1.5 minutes |
| Alcohol 90% | 1.5 minutes |
| Eosine | 2 minutes |
| Alcohol 90% | 1.5 minutes |
| Alcohol 90% | 1.5 minutes |
| Alcohol 100% | 1.5 minutes |
| Xylene | 1.5 minutes |

The samples were embedded in paraffin blocks with the help of a paraffin em-

bedding module (EG1150 H, Leica Biosystems Nussloch GmbH, Germany) after the completion of processing. Afterwards, 10 $\mu$m sections of the blocks were obtained using a microtome (RM 2255, Leica Biosystems Nussloch GmbH, Germany). The sections were then stained with haematoxylin and eosin following the procedure in Table 3.2.

## 3.5 Data Collection and Analysis

The slides were examined under a light microscope (Eclipse 80i, Nikon Co., Tokyo, Japan) and measurements of ablation depth, total thermal damage on surface as well as ablated and thermally altered areas were performed using the imaging software (NIS Elements-D, Nikon Co, Tokyo, Japan). The areas measured were used to calculate the ablation efficiency, which was demonstrated as an effective concept previously [95]. The presence of statistically meaningful differences in depth, width and efficiency data were investigated using ANOVA test, followed by Tukey test to determine which groups differed significantly. The significance level was set to $p<0.05$.

# 4. RESULTS

Two types of lasers were used in this study. Continuous wave mode was used throughout the experiments to ensure only thermal effects of lasers were being observed. The depth of incisions and total coagulation widths on surface were measured under a light microscope after staining. Ablated area and total thermally altered areas were measured with the help of imaging software. The ablation efficiencies were calculated as the ratio of the ablated area to the total thermally altered area, which provided an insight about how much tissue has been removed in proportion to the amount of thermally damaged tissue. The incisions were divided into three groups according to the speed of the fibre tip, each group containing nine different parameter sets. The statistical analysis was performed using ANOVA test to determine whether any significant differences were present between the groups. Tukey test was used afterwards to find out the statistically significant groups, where $p<0.05$ was set as the threshold value.

## 4.1 Thulium Fibre Laser Application

### 4.1.1 Incision Depth, Coagulation Width and Ablation Efficiency at 0.5 mm/s

The depth of incisions and the total thermally altered area (coagulation width) at the surface for 0.5 mm/s are measured, and shown in Figure 4.1 whereas the ablation efficiency is also plotted on the same graph.

The statistically significant differences between these nine groups are investigated using ANOVA and Tukey test. Significantly different groups at 0.5 mm/s with power variable kept constant are marked with an asterisk in Figure 4.2. Significant differences between different power groups when the number of passes was kept constant

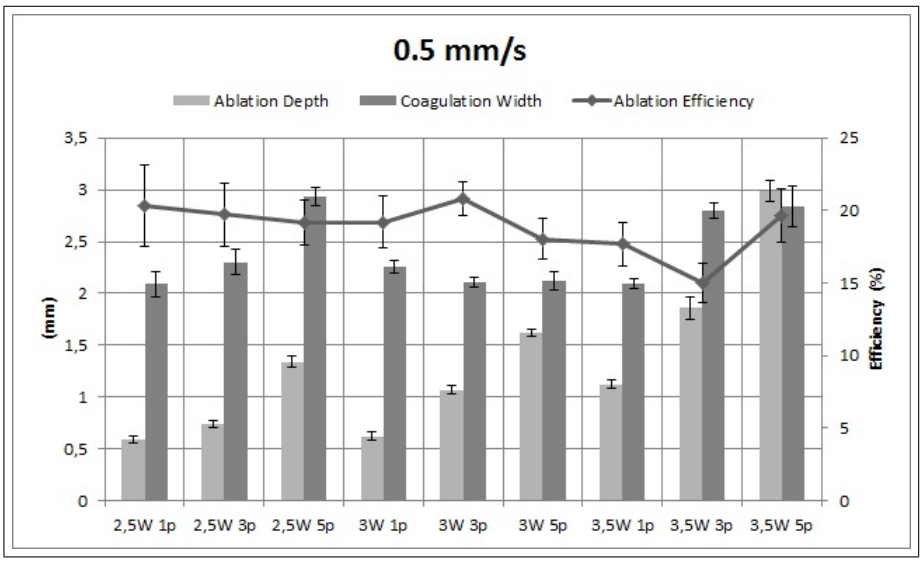


**Figure 4.1** Mean incision depths, coagulation widths and ablation efficiencies for each parameter set at 0.5 mm/s speed (1p:single pass, 3p:three passes, 5p:five passes).

| Power | Number of passes | | | Ablation Depth | | | Coagulation Width | | | Ablation Efficiency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2.5 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | | * | * | | | |
| 3 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | | | | | | |
| 3.5 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | * | | * | | | * |

**Figure 4.2** Significantly different groups for ablation depth, coagulation width and ablation efficiency at 0.5 mm/s when power is kept constant (1p:single pass, 3p:three passes, 5p:five passes).

| Number of Passes | Power | | | Ablation Depth | | | Coagulation Width | | | Ablation Efficiency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Single Pass | 2.5W-3W | 3W-3.5W | 2.5W-3.5W | | * | * | | | | | | |
| Three Passes | 2.5W-3W | 3W-3.5W | 2.5W-3.5W | * | * | * | | * | * | | * | * |
| Five Passes | 2.5W-3W | 3W-3.5W | 2.5W-3.5W | | * | * | * | * | | | | |

**Figure 4.3** Significantly different groups for ablation depth, coagulation width and ablation efficiency at 0.5 mm/s when the number of passes is kept constant.

were shown in Figure 4.3.

### 4.1.2 Incision Depth, Coagulation Width and Ablation Efficiency at 0.75 mm/s

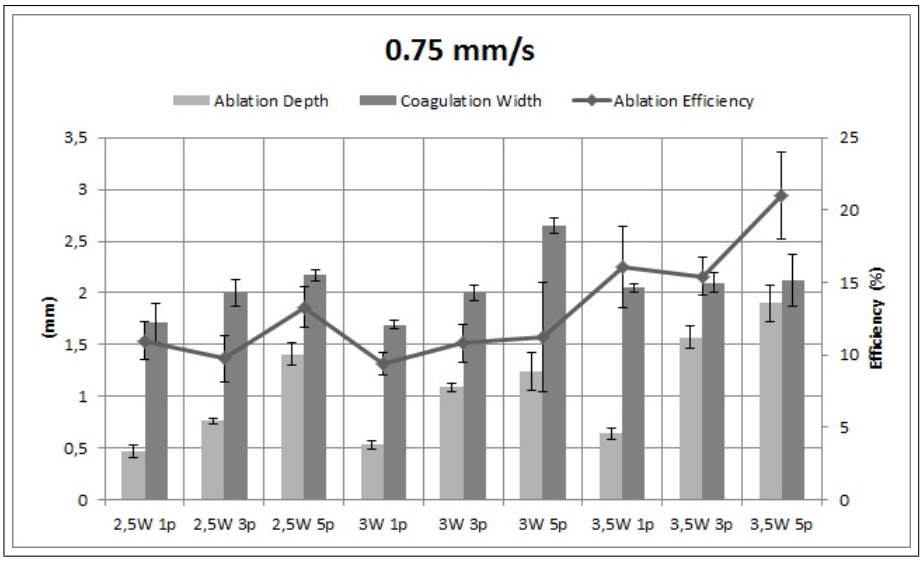


**Figure 4.4** Mean incision depths, coagulation widths and ablation efficiencies for each parameter set at 0.75 mm/s speed (1p:single pass, 3p:three passes, 5p:five passes).

Ablation depth, coagulation width and ablation efficiency data for 0.75 mm/s speed is shown collectively in Figure 4.4.

| Power | Number of passes | | | Ablation Depth | | | Coagulation Width | | | Ablation Efficiency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2.5 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | * | | * | | | |
| 3 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | * | * | * | | | |
| 3.5 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | | | | | * | * |

**Figure 4.5** Significantly different groups for ablation depth, coagulation width and ablation efficiency at 0.75 mm/s when power is kept constant (1p:single pass, 3p:three passes, 5p:five passes).

The results of the statistical analysis are presented in Figure 4.5 for when power is kept constant, and in Figure 4.6 for when the number of passes are kept constant. The significantly different groups are marked with an asterisk.

| Number of Passes | Power | | | Ablation Depth | | | Coagulation Width | | | Ablation Efficiency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Single Pass | 2.5W-3W | 3W-3.5W | 2.5W-3.5W | | | * | | * | * | | * | * |
| Three Passes | 2.5W-3W | 3W-3.5W | 2.5W-3.5W | * | * | * | | | | | * | * |
| Five Passes | 2.5W-3W | 3W-3.5W | 2.5W-3.5W | * | * | * | * | * | | | * | * |

**Figure 4.6** Significantly different groups for ablation depth, coagulation width and ablation efficiency at 0.75 mm/s when the number of passes is kept constant.

### 4.1.3 Incision Depth, Coagulation Width and Ablation Efficiency at 1 mm/s

Figure 4.7 depicts the depth and width dimension data as well as the ablation efficiency for 1 mm/s speed.

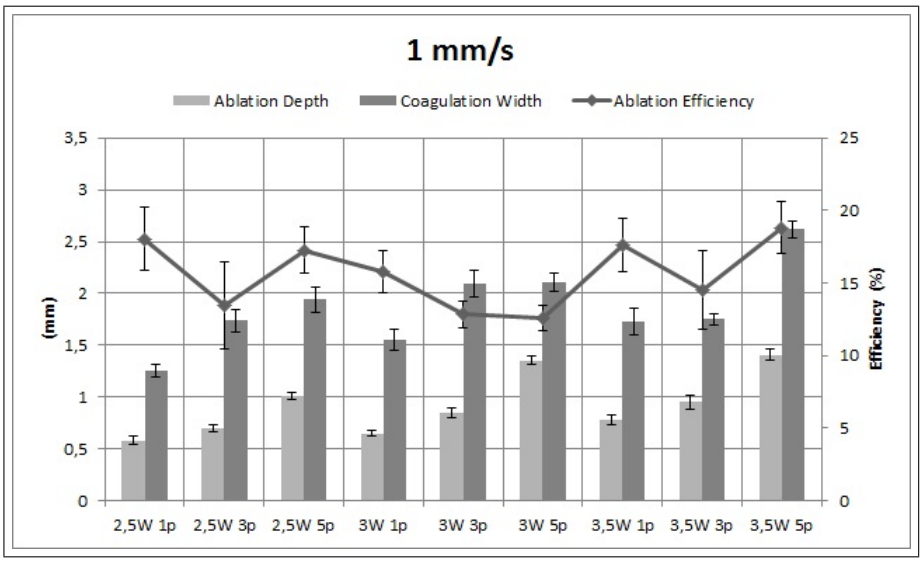


**Figure 4.7** Mean incision depths, coagulation widths and ablation efficiencies for each parameter set at 1 mm/s speed (1p:single pass, 3p:three passes, 5p:five passes).

Significantly different groups amongst these nine are marked with an asterisk in Figure 4.8 which shows the groups arranged according to constant power.

Below, the figure shows the significantly different groups when arrangement is done by keeping the number of passes constant.

| Power | Number of passes | | | Ablation Depth | | | Coagulation Width | | | Ablation Efficiency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2.5 W | 1p-3p | 3p-5p | 1p-5p | | * | * | * | | * | * | * | |
| 3 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | * | | * | | | |
| 3.5 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | | * | * | | * | |

**Figure 4.8** Significantly different groups for ablation depth, coagulation width and ablation efficiency at 1 mm/s when power is kept constant (1p:single pass, 3p:three passes, 5p:five passes).

| Number of Passes | Power | | | Ablation Depth | | | Coagulation Width | | | Ablation Efficiency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Single Pass | 2.5W-3W | 3W-3.5W | 2.5W-3.5W | | | * | * | | * | | | |
| Three Passes | 2.5W-3W | 3W-3.5W | 2.5W-3.5W | * | | * | * | * | | | | |
| Five Passes | 2.5W-3W | 3W-3.5W | 2.5W-3.5W | * | | * | | * | * | * | * | |

**Figure 4.9** Significantly different groups for ablation depth, coagulation width and ablation efficiency at 1 mm/s when the number of passes is kept constant.

## 4.2 Diode Laser Application

### 4.2.1 Incision Depth, Coagulation Width and Ablation Efficiency at 0.5 mm/s

The measured incision depth and coagulation width dimensions as well as the calculated ablation efficiency for 980 nm diode laser at 0.5 mm/s speed are illustrated in Figure 4.10.

These nine groups are statistically analysed to reveal the significantly different groups in means of both dimensions and efficiency. The figure below illustrates the results of this analysis where significantly different groups are marked with an asterisk when the groups are arranged according to constant power.

If the groups are arranged by keeping the number of passes constant, the signif-

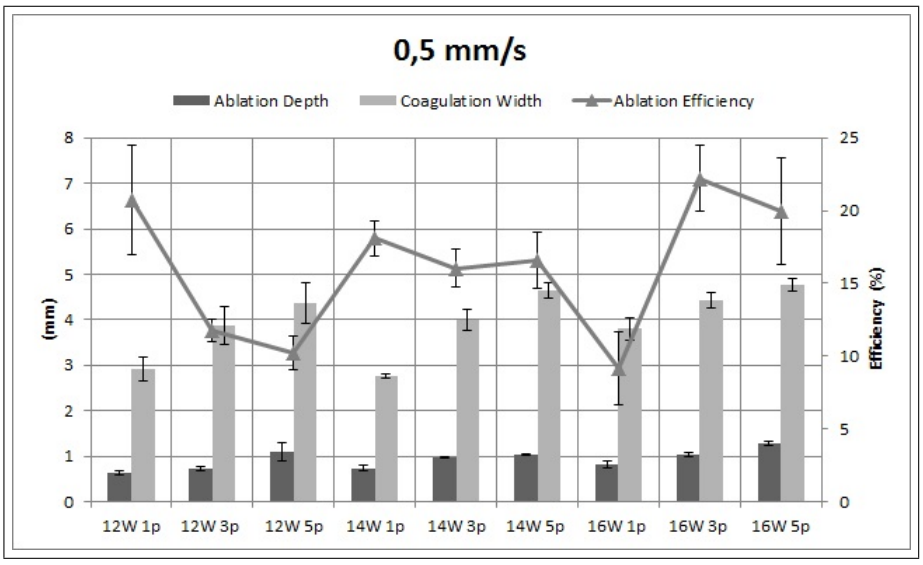


**Figure 4.10** Mean incision depths, coagulation widths and ablation efficiencies for nine different parameters sets at 0.5 mm/s. (1p:single pass, 3p:three passes, 5p:five passes).

| Power | Number of passes | | | Ablation Depth | | | Coagulation Width | | | Ablation Efficiency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 W | 1p-3p | 3p-5p | 1p-5p | | * | * | * | * | * | * | | * |
| 14 W | 1p-3p | 3p-5p | 1p-5p | * | | * | * | * | * | | | |
| 16 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | * | | * | * | | * |

**Figure 4.11** Significantly different groups in means of ablation depth, coagulation width and ablation efficiency when groups are arranged by keeping power constant (1p:single pass, 3p:three passes, 5p:five passes).

icantly different groups can be shown as in the figure below.

| Number of Passes | Power | | | Ablation Depth | | | Coagulation Width | | | Ablation Efficiency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Single Pass | 12W-14W | 14W-16W | 12W-16W | | | * | | * | * | | * | * |
| Three Passes | 12W-14W | 14W-16W | 12W-16W | * | | * | | * | * | * | * | * |
| Five Passes | 12W-14W | 14W-16W | 12W-16W | | * | * | | | | * | | * |

**Figure 4.12** Significantly different groups in means of ablation depth, coagulation width and ablation efficiency when groups are arranged by keeping the number of passes constant.

### 4.2.2 Incision Depth, Coagulation Width and Ablation Efficiency at 0.75 mm/s

The dimensions for the depth of incision and the width of the coagulation zone for nine different parameters set at 0.75 mm/s are shown below whereas the ablation efficiency is also plotted on the same graph.

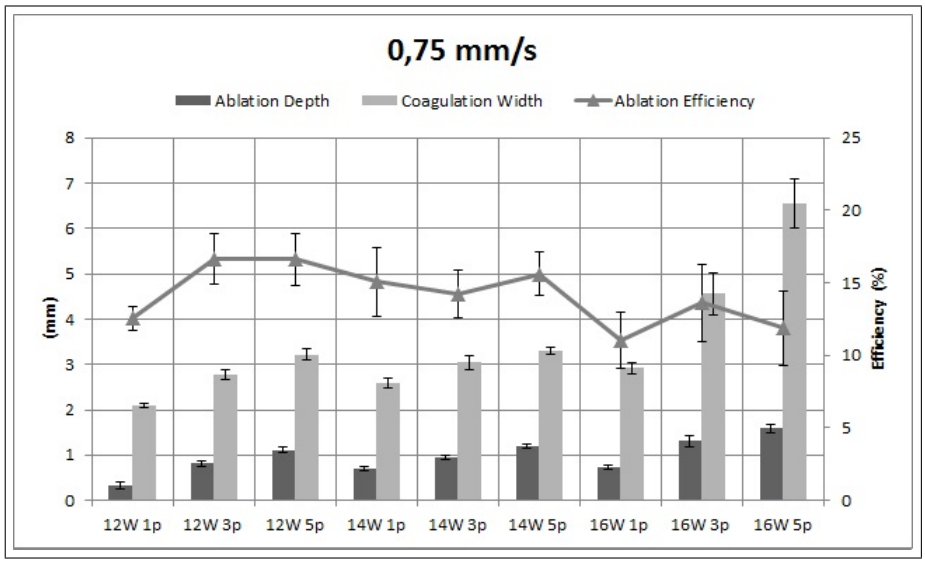


**Figure 4.13** Mean incision depths, coagulation widths and ablation efficiencies for nine different parameters sets at 0.75 mm/s (1p:single pass, 3p:three passes, 5p:five passes).

| Power | Number of passes | | | Ablation Depth | | | Coagulation Width | | | Ablation Efficiency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | * | * | * | * | | * |
| 14 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | * | | * | | | |
| 16 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | * | * | * | | | |

**Figure 4.14** Statistically significant differences between groups (marked with an asterisk) for depth, width and efficiency data when groups are arranged according to constant power (1p:single pass, 3p:three passes, 5p:five passes).

Statistical analysis is performed on these groups to reveal the significantly different groups in means of ablation depth, coagulation width and ablation efficiency, and the results are summarized in the following Figures 4.14 (when power is set constant) and 4.15(when the number of passes is set constant).

| Number of Passes | Power | | | Ablation Depth | | | Coagulation Width | | | Ablation Efficiency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Single Pass | 12W-14W | 14W-16W | 12W-16W | * | | * | * | | * | | * | |
| Three Passes | 12W-14W | 14W-16W | 12W-16W | | * | * | | * | * | | | |
| Five Passes | 12W-14W | 14W-16W | 12W-16W | | * | * | | * | * | | | * |

**Figure 4.15** Statistically significant differences between groups (marked with an asterisk) for depth, width and efficiency data when groups are arranged according to constant number of passes.

### 4.2.3 Incision Depth, Coagulation Width and Ablation Efficiency at 1 mm/s

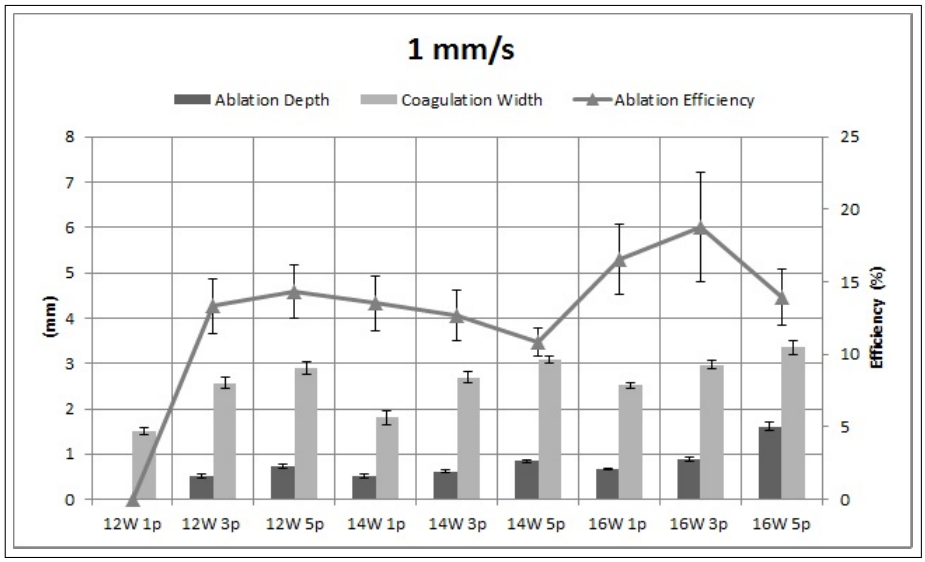


**Figure 4.16** Mean incision depths, coagulation widths and ablation efficiencies for nine different parameters sets at 1 mm/s (1p:single pass, 3p:three passes, 5p:five passes).

Figure 4.16 presents the incision depth and coagulation width data as well as the ablation efficiency for nine parameter sets at 1 mm/s speed.

Statistical analysis is performed on these nine groups to reveal the significantly different groups. In the first figure the groups are arranged according to number of passes while power is kept constant meanwhile the next figure presents groups arranged according to power while the number of passes is kept constant.

| Power | Number of passes | | | Ablation Depth | | | Coagulation Width | | | Ablation Efficiency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | * | | * | * | | * |
| 14 W | 1p-3p | 3p-5p | 1p-5p | | * | * | * | | * | | | |
| 16 W | 1p-3p | 3p-5p | 1p-5p | * | * | * | * | | * | | * | |

**Figure 4.17** Statistically significant differences between groups (marked with an asterisk) for depth, width and efficiency data when groups are arranged according to constant power (1p:single pass, 3p:three passes, 5p:five passes).

| Number of Passes | Power | | | Ablation Depth | | | Coagulation Width | | | Ablation Efficiency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Single Pass | 12W-14W | 14W-16W | 12W-16W | * | * | * | | * | * | * | | * |
| Three Passes | 12W-14W | 14W-16W | 12W-16W | | * | * | | | | | * | * |
| Five Passes | 12W-14W | 14W-16W | 12W-16W | | * | * | | | * | | | |

**Figure 4.18** Statistically significant differences between groups (marked with an asterisk) for depth, width and efficiency data when groups are arranged according to constant number of passes.

## 4.3 Comparison of Incisions Made with Thulium Fibre Laser and 980 nm Diode Laser

The incisions made with different parameter sets with two different lasers are compared in this section. These incisions are grouped considering the highest and lowest values of the depth of the incision, the total coagulation width on the surface and the efficiency of ablation; and the values obtained by two different lasers are compared. Statistical analysis is performed using ANOVA followed by Tukey test on the sets of whole data of ablation efficiencies, ablation depths and coagulation widths regardless of the laser used. Groups corresponding to the highest and lowest values of depth, width and efficiency are then compared using the analysis.

| | | 1940 nm Thulium Fibre Laser | 980 nm Diode Laser | Significantly Different? |
|---|---|---|---|---|
| Ablation Depth | High | 2.99 mm<br>0.5 mm/s 3.5W 5p | 1.61 mm<br>1 mm/s 16W 5p | Yes |
| | Low | 0.47 mm<br>0.75 mm/s 2.5W 1p | 0.33mm<br>0.75 mm/s 12W 1p | No |
| Coagulation Width | High | 2.93 mm<br>0.5 mm/s 2.5W 5p | 6.55 mm<br>0.75 mm/s 16W 5p | Yes |
| | Low | 1.26 mm<br>1 mm/s 2.5W 1p | 1.51 mm<br>1 mm/s 12W 1p | No |
| Ablation Efficiency | High | 20.79%<br>0.5 mm/s 3W 3p | 22.19%<br>0.5 mm/s 16W 3p | Yes |
| | Low | 9.36%<br>0.75 mm/s 3W 1p | 9.17%<br>0.5 mm/s 16W 1p | Yes |

**Figure 4.19** Comparison of incisions made with Tm: fibre and 980 nm diode laser in means of ablation depth, coagulation width and ablation efficiency (1p:single pass, 3p:three passes, 5p:five passes).

## 4.4 Histology of Incisions Made with Thulium Fibre Laser and 980 nm Diode Laser

Section are obtained from each incision after tissue processing and stained with H&E for better visualization of thermal damage under light microscope. These sections are mostly used for measurements of depth and width data, as well as areas of ablation and total damage for calculation of ablation efficiency. However, these sections also provide other information concerning the nature of the incisions.

Figure 4.20 depicts the effect of speed on the incisions at constant power and number of passes (3W, single pass), made with 1940 nm Tm: fibre laser. It can be observed that the depth of incision decreases and the coagulation zone shrinks as the speed increases. While 0.5 mm/s and 0.75 mm/s enables the incision to reach underneath the mucosal layer, at 1 mm/s the incision can be seen as confined in the mucosa.

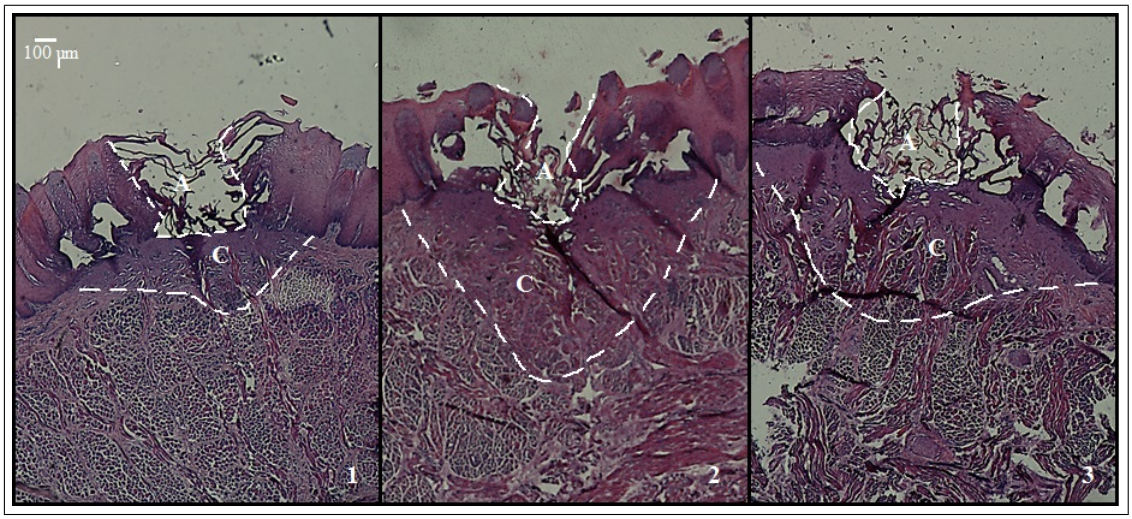


**Figure 4.20** The effect of speed on incisions made with 1940 nm, 3W single pass (40X). Samples stained with H&E. A: Ablation area C: Coagulation Area 1) 1 mm/s 2) 0.75 mm/s 3) 0.5 mm/s.

The same can be said for incisions made with 980 nm diode laser (Figure 4.21). An increase in speed results in shallower ablations. Furthermore, slower ablations are observed to yield cleaner incisions.

On the other hand, the effect of the number of passes on the depth of ablation can be observed in Figure 4.22 for Tm: fibre laser and in Figure 4.23 for 980 nm diode laser. In both cases, an increase in ablation depth, as well as in coagulation width is evident with increasing number of passes. The shape of the incisions also changes from U-shape to V-shape as the depth increases.

Finally, the effect of power on the incisions made with different lasers is illustrated below, in Figure 4.24 for 1940 nm Tm: fibre laser, and in Figure 4.25 for 980 nm diode laser. As expected, incisions run deeper with increasing power for both lasers; however with higher power comes higher risk of carbonization, which can be observed as the thin black lining inside the incision in the rightmost part of the figures.

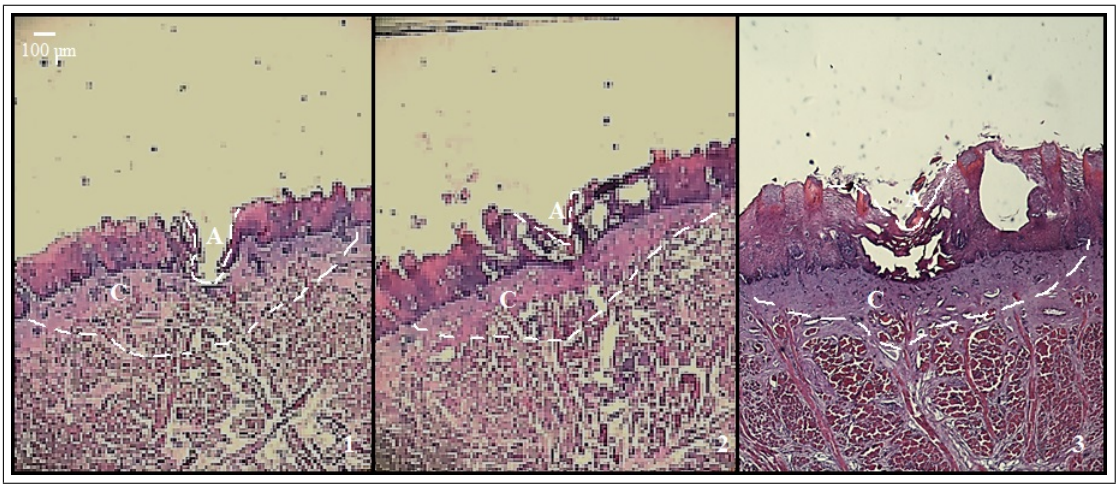


**Figure 4.21** The effect of speed on incisions made with 980 nm, 16W single pass (40X). Samples stained with H&E. A: Ablation area C: Coagulation Area 1) 0.5 mm/s 2) 0.75 mm/s 3) 1 mm/s.

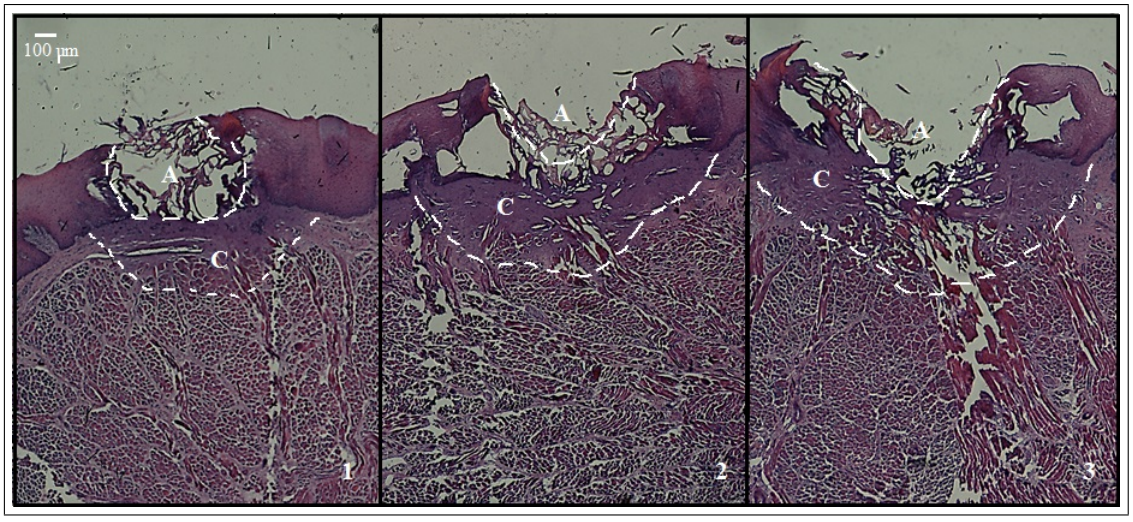


**Figure 4.22** The effect of number of passes on incisions made with 1940 nm at 2.5W with increasing number of passes (40X). Samples stained with H&E. A: Ablation area C: Coagulation Area 1) single pass 2) three passes 3) five passes.

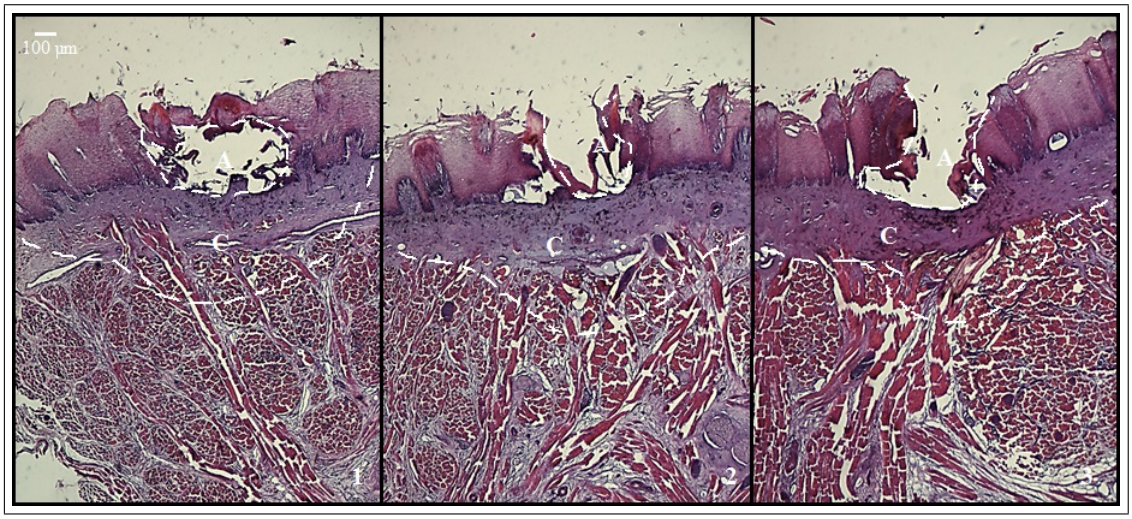


**Figure 4.23** The effect of number of passes on incisions made with 980 nm at 14W with increasing number of passes (40X). Samples stained with H&E. A: Ablation area C: Coagulation Area 1) single pass 2) three passes 3) five passes.

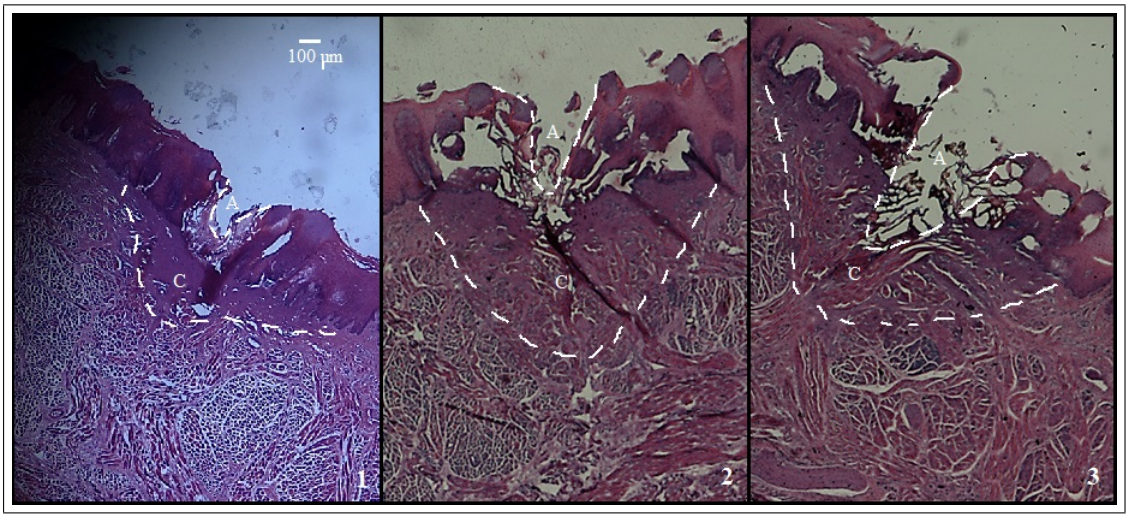


**Figure 4.24** The effect of power on incisions made with 1940 nm at 0.75 mm/s single pass with increasing power (40X). Samples stained with H&E. A: Ablation area C: Coagulation Area 1) 2.5 W 2) 3 W 3) 3.5 W.

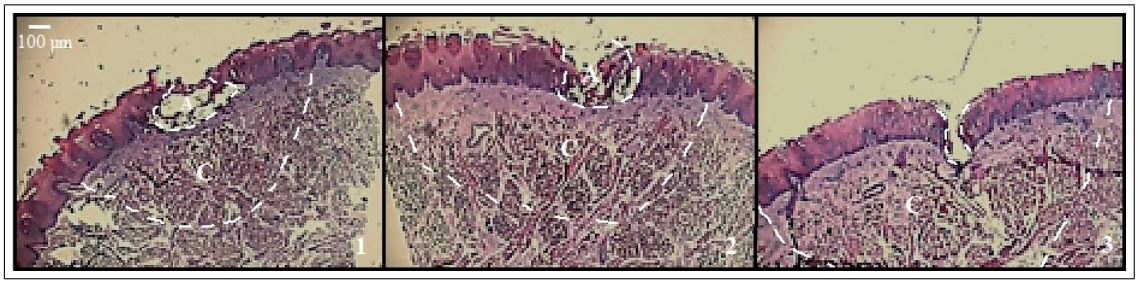


**Figure 4.25** The effect of power on incisions made with 980 nm at 0.5 mm/s single pass with increasing power (40X). Samples stained with H&E. A: Ablation area C: Coagulation Area 1) 12 W 2) 14 W 3) 16 W.

# 5. DISCUSSION

The use of lasers for soft tissue procedures became popular in recent years due to the advantages over other techniques such as scalpel or electrosurgical unit use. Good haemostatic and sterilization properties made them especially preferable for oral procedures. Several different lasers have been developed for oral soft tissue operations since the production of the first dental laser. However, optimum laser wavelength and parameters for specific procedures are still being investigated. This study was conducted to investigate the use of 1940 nm Tm: fibre laser as an oral surgical tool, as compared 980 nm diode laser which is widely used in dental practice. For this purpose, incisions were made with three different speeds and powers for each laser with single, three or five passes on target tissue.

Research on laser systems used as oral surgical tools are plenty, case reports on the use of $CO_2$ laser comprises a large part [50, 53, 89],[63]-[69]. Other case reports about the use of oral surgical lasers involve diode laser [77, 79, 80, 82, 83], Nd:YAG [72]-[74], KTP [56, 85, 87] and erbium lasers [88, 89, 91, 57]. However, on Tm: fibre laser case reports are scarce [96] and rarely on the topic of oral surgery. In vitro studies about Tm: fibre laser are also present, although target tissues are different, such as; urinary system (canine bladder and prostate) [93], porcine skin and chicken muscle [94] and ovine brain [95].

Tm: fibre laser and 980 nm diode laser were used on ovine tongues as an incisional tool in this study. 980 nm diode laser has a widespread use in oral surgery, with a wide range of applications that includes incisions, excisions and coagulations. The power setting that is reported to be used in case reports range from 1.1W to 25W according to the lesion and the target tissue [69, 79, 80, 83]. In this study, three different powers (12W, 14W and 16W) were used considering the results of preliminary experiments. For Tm: fibre laser, power settings used in existing in vitro studies range from as low as 0.2 W for brain [95] to 40 W for urinary tissues [93]. Three different

powers (2.5W, 3W and 3.5W) were used in this study for ovine tongue.

This study utilized a 600 $\mu$m fibre held in contact with the target tissue to incise. Contact use of fibres are advantageous since it provides tactile feedback to the operator and also causes little vapour to form while decreasing the reflection of laser beam from sample surface [44]. Whether the use would be in contact or non-contact is generally determined by the type of the laser and the desired effect. While $CO_2$ lasers are mostly used non-contact [52, 69], Nd:YAG lasers are used in contact [52, 71, 74]. For KTP and diode lasers both contact and non-contact use are utilized [79, 80]. In this study, contact use was exercised for both lasers. In this study, the speed of the fibre tip was adjusted manually by changing the length of the incision while the radiation time was kept constant to simulate the conditions in the clinic. In vitro studies in the literature that involves non-stationary ablation utilize either the manual approach, where the incisions were made by an operator at a fixed rate of movement [71]; or the motorized approach, where the fibre was attached to a motorized device that moves the fibre over a fixed length [81].

## 5.1 The Effect of Power

It was observed that increasing the power applied at a fixed number of passes and speed, generally increased the depth of incision. For 1940 nm Tm: fibre laser, at 0.5 mm/s, the increase in the depth of incisions were statistically significant for all number of passes. However, at 0.75 mm/s, increasing the power only resulted in significantly deeper incisions for three and five passes, and not for single pass groups. At 1 mm/s, increasing the power from 2.5W to 3 or 3.5W increased the depth significantly.

For 980 nm diode laser, the incisions made with 16 W were significantly deeper than those made with 12 W and 14 W at 0.5 mm/s and 0.75 mm/s. When speed was increased to 1 mm/s, the depth was significantly shallower at 12 W whereas increasing power from 14 W to 16 W did not yield any significant increase in depth.

Although not always significant, increasing power had always resulted in an increased depth of ablation as seen in figures 4.1, 4.5 and 4.9 for Tm: fibre laser and, 4.13, 4.17, 4.21 for 980 nm diode laser. This positive correlation between ablation depth and power had been established for other oral surgical lasers as well, such as Nd: YAG [71], diode [81], $CO_2$ and KTP [86] lasers.

Considering the coagulation width (total thermal damage on surface) for Tm: fibre laser, a general increase by increasing power had been observed. At 0.5 mm/s, increasing power yielded significantly larger coagulation zones except with single pass. However, at 0.75 mm/s only 3.5W produced significantly larger coagulation. At 1 mm/s 2.5W produced significantly narrower coagulation at single pass, whereas it was significantly larger at 3.5W five passes.

980 nm at 0.5 mm/s, on the other hand, produced significantly larger coagulation zones with increasing power with single and three passes, however, at five passes, increasing the power did not yield any significant difference in coagulation width. This limited width of coagulation zone at five passes might be due to the speed used or the tissue type. At 0.75 mm/s, increasing the power to 16W produced significantly larger coagulation zones than 12W and 14W. Increasing the speed to 1 mm/s, power affected the coagulation width significantly only at single pass, whereas at three and five passes, increasing power resulted in no significant change in width.

High power settings were observed to result in larger thermal damage areas with other lasers as well. For $CO_2$ laser, the horizontal thermal damage was found to be correlating strongly with average powers [69], whereas for diode laser, biopsy specimens obtained with lower power exhibit smaller thermal injuries [82]. Nd: YAG laser was found to be causing serious thermal damage to the biopsy specimens yet these injuries were not statistically significant when different power settings were considered [75]. Although ablation efficiency seems to change with power for Tm: Fibre laser in Figures 4.2, 4.6 and 4.10; these changes are not significant except at 0.5 mm/s where 3.5W three passes produced significantly lower efficiency and 0.75 mm/s where increasing the power to 3.5W increased the efficiency significantly. For diode laser, ablation efficiency

increased significantly with increasing power at 0.5 mm/s with three and five passes. At 1 mm/s, efficiency significantly increased when power increased to 16W at three passes. Histologically, the incisions made with lowest power used for both lasers with a single pass had observed to be confined to the mucosal layer and no carbonization had been evident. These shallow incisions with low powers might be useful for operation in which only the outermost tissue layers are desired to be affected since low power minimizes the collateral thermal damage. Furthermore, incisions made with Tm: fibre laser exhibit a vacuolization zone even at 3.5W around the ablation crater, whereas with 980 nm diode laser, cleaner cuts were obtained with increasing power.

## 5.2 The Effect of the Number of Passes

The number of passes made to form an incision has similar effects to power, the incision depth increases with increasing number of passes when speed and power are kept constant. This increase was significant for both Tm: fibre and 980 nm diode laser at all three speeds used.

On the other hand, the increase in the coagulation zone was not always significant. For 980 nm diode laser, the coagulation width increased significantly with increasing number of passes up to 1 mm/s speed. At 1 mm/s, thermal damage caused by three and five passes did not differ significantly. Likewise, coagulation width formed with Tm: fibre laser at 1 mm/s did not differ significantly when three or five passes were done. However, only at 0.75 mm/s with 3W power coagulation width observed to increase significantly with increasing number of passes.

Ablation efficiency for Tm: fibre laser did not change significantly with increasing number of passes at 0.5 and 0.75 mm/s speed. At 1 mm/s, however, a significant decrease in ablation efficiency was observed at three passes, which then increased significantly when five passes were made. For 980 nm diode laser, on the other hand, no significant change was observed in ablation efficiency when the number of passes increased from three to five, at all speeds.

These results, together with the significant increase in ablation depth as the number of passes increases, leads to the idea of making more passes when in need of a deeper ablation without causing further damage to the adjacent tissue. This method would provide better confinement of thermal damage than simply increasing the power since increased power causes an increase in both the ablation depth and the coagulation width [58]. Furthermore, adjusting the depth of incision by doing more passes might prove useful in lesion where layer by layer removal of tissue is desired since each pass would ablate only a certain thickness.

## 5.3 The Effect of Speed

Faster motion of the fibre tip is expected to decrease the depth of the incision as well as the lateral thermal damage since it decreases the total energy exposure to the tissue [58].

The decrease in ablation depth with increasing speed had been observed for both Tm: fibre and 980 nm diode lasers. However, for Tm: fibre, this decrease was not significant until power was increased to 3W and three passes were made. From that point to 3.5W power, three passes were reached; 0.5 mm/s speed produced significantly deeper incisions. At 3.5W three and five passes however, ablation depths did not differ significantly with increased speed.

For 980 nm diode laser, although 12W single pass did not yield significantly deeper ablation with decreasing speed, 0.5 mm/s speed always produced significantly shallower ablations.

Coagulation widths also decreased with increasing speed. This decrease was significant for Tm: fibre laser, except for 3W three passes group. For 980 nm diode laser, this decrease was significant for all speeds at 12W single pass and 16W five passes groups. In other groups, 0.5 mm/s produced significantly larger coagulation zones.

Ablation efficiency for Tm: Fibre laser was significantly lower for 0.75 mm/s groups until 3.5W power was reached. At 3.5W, the speed did not have a significant effect on ablation efficiency for any number of passes. For diode laser, 12W single pass group exhibited a significant decrease in ablation efficiency with increasing power, whereas 14W three passes group showed no significant change associated with speed.

These results obtained for Tm: fibre laser on soft tissue correlates with the limited studies available on the use of Tm: fibre laser on different soft tissues. The zones of damage, described by Fried et al. as a thin carbonization, followed by vacuolization and a large thermal coagulation were observed [93]. Coagulation zone was narrower in this study, most probably due to the fact that power setting used was completely different (25W on canine urinary tissues, as compared to 3.5W on ovine tongue). Another study conducted by Keller et al., described the same zones yet the ablations were done at 0.5 cm/s with power changing from 10 to 20 W [94]. However, with the power as low as utilized in this study, such an increase in fibre speed would be impractical as no incision would be done at higher speed.

Comparing the two different laser used, it can be said that Tm: fibre laser causes less collateral thermal damage than 980 nm diode laser. For example, the deepest ablations were made with Tm: fibre laser at 3.5W, 0.5 mm/s speed with five passes, which is 2.99 mm with a total thermal damage of 2.84 mm. For 980 nm diode laser this value is 1.61 mm at 16W, 1 mm/s speed with five passes and it causes a total thermal damage of 3.35 mm. Therefore, 980 nm diode laser would inflict more thermal damage for a fixed depth of incision.

However, the limitations of the experimental design should be kept in mind before an exact conclusion is drawn. These experiments had been carried out in vitro; therefore no blood flow was present. Since the output of 980 nm diode laser is well absorbed also by haemoglobin in blood, well perfused tissues such as tongue is expected to interact slightly differently in in vivo experiments. These differences might include deeper ablations and higher ablation efficiencies.

Furthermore, the presence of blood flow would also acts as a cooler mechanism for both lasers, further decreasing the coagulation width. The ablation efficiencies of both lasers can be improved by applying higher powers. However, this might also cause larger coagulation zones, and even carbonization. Another method of improving efficiency might be with the application of specialized fibre tips that would tightly focus the beam on the tissue surface for increased energy density. However, this method would also reduce the coagulation area which might possibly interfere with the haemostatic effect of the lasers.

In order to prevent collateral thermal damage, all three of the parameters presented in this study might be changed. Deeper ablations can be achieved with either increasing the power, the number of passes or by decreasing the speed of the fibre tip. Out of these options, changing the speed is mostly related to the discomfort the patient might feel during the procedure. Moving the fibre quicker would decrease the total energy exposure and therefore eliminates discomfort [58]. Hence, in order to achieve deeper ablations the operator would either increase power of make multiple passes. Either of these options have their own advantages and disadvantages. Higher power would indeed result in deeper ablations; however a greater risk of lateral damage and carbonization would arise. Making multiple passes minimizes the risk of excessive thermal damage, yet it would increase the operation time.

# 6. CONCLUSION

The incisional effect of 1940 nm Tm: fibre laser on oral soft tissues with different parameter sets (speed, power and number of passes) were investigated in this study and the incisions made with Tm: fibre and 980 nm diode laser were compared.

In means of ablation depth, Tm: fibre laser was observed to provide deeper incisions with the given parameters. The depth of incisions increased when higher power was applied to the tissue. Increasing the number of passes or decreasing the speed of the fibre tip was also observed to increase the incision depth for both lasers.

In means of coagulation width, 980 nm diode laser exhibited the largest coagulation zone. While this wide zone would be useful in providing haemostasis, especially in highly perfused tissues such as tongue, it also presented a wide collateral thermal damage zone, which would delay the healing process and harm the healthy tissue. The coagulation zone of Tm: fibre laser was narrower than 980 nm diode laser, however it was still large enough to provide better haemostasis than $CO_2$ laser. The width of coagulation was observed to increase significantly with increasing power; however making more passes did not yield a significant increase as the number of passes increased from three to five.

In means of ablation efficiency, 980 nm diode laser provided the highest efficiency. However, the change in ablation efficiency between different parameters sets for Tm: fibre laser was found to be lesser than the change between different groups for diode laser.

1940 nm Thulium fibre laser has an output frequency that is well absorbed by water, therefore its tissue penetration is shallower than 980 nm diode laser yet deeper than $CO_2$ laser. Therefore, Tm: fibre laser is capable of making deep incisions while producing a coagulation zone wide enough to provide haemostasis but still tolerable

as a thermal damage zone. 980 nm diode laser, on the other hand, is well absorbed by haemoglobin as well, therefore provides good haemostasis yet produces a large thermal damage zone. In conclusion, Tm: fibre laser is a promising tool for oral soft tissue surgery, that is capable of clean incisions and coagulation zones large enough to provide haemostasis, yet not too extensive to impede healing.

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