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İSTANBUL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES IN
SCIENCE AND ENGINEERING**



M.Sc. THESIS

**INSPECTION OF HEALING PERIOD QUALITY OF NIR LASERS
WELDED RAT SKIN TISSUE BY FT-IR SPECTROSCOPY**

Zhin Tahseen SALEH

Department of Biomedical Engineering

Biomedical Engineering Programme

M. Sc. Student transferred from Fatih University which has been closed

SUPERVISOR

Asst. Prof. Dr. Haşim Özgür ABAKOĞLU

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FATİH UNIVERSITY

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MASTER THESIS DEFENSE REPORT

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YAKIN KUZULALI LAZERLER İLE İLA PATILMIŞ BİR AN
DEZİ DOKUSUNUN İYİLEŞME SÜRECİ KALİTESİNİN FT-IR SPECTROSKOPİ İLE İNCELENİ

Thesis Title (English):

INSPECTION OF HEALING PERIOD QUALITY OF N
LASERS WELDED RAT SKIN TISSUE BY FT-IR SPECTROSCOPY

Supervisor : Yrd. Doç. Dr. Haşim Özgür TABAKOĞLU

Jury Member: Assist. Prof. Dr. Murat TÜMER

Jury Member: Assist. Prof. Dr. Saime AKDEMİR AKAR

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ABBREVIATIONS

PGs ; Proteoglycans

NIRS :Near-Infrared& Raman Spectroscopies

EELS : Electron Energy Loss Spectroscopy

DTG : Deuterated Tri Glycine Sulphate

MCT : Mercury Cadmium Telluride



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SUMMARY

INSPECTION OF HEALING PERIOD QUALITY OF NIR LASERS WELDED RAT SKIN TISSUE BY FT-IR SPECTROSCOPY

Zhin Tahseen Saleh

Biomedical Engineering Programme

MSc.Thesis

Advisor: Asst. Prof. Haşim Özgür TABAKOĞLU

Surgical incision is a kind of wound that was created by experts and relatively higher healing rate compared to any other kind of wound. Incisions has been created in compliance with skin pattern of body part which is naturally has its own characteristic texture. Incisions have been closed by different ways such as stapling, suturing, chemical sticks in order to minimize the healing period. Laser skin welding is a very promising method of wound closure, although has not applied in clinics yet. Quality of skin welding has been tried to be determined by various methods like tensile testing, histological examination and other macro inspections. FT-IR spectroscopy is a relatively new method for determining important tissue markers in biomedical researches and has a potential to follow up wound healing period.

In this study FT-IR measurements were taken from incisions treated with Near Infrared (NIR) laser applications, namely; 980 nm, 1064 nm and DUAL mode. Irradiation parameters were: 1 Watt, 10 seconds and with modulation (500 ms on-off time: 5J energy was delivered to each incision). FTIR measurements were taken from samples on 1, 4, 7, 14 and 21 post-operative days of healing period. Absorbance intensities of Collagen (1246 cm^{-1}), Keratin (1544 cm^{-1}), Proteoglycan (1643 cm^{-1}), and Poly-Lysine (3278 cm^{-1}) were considered to be important tissue markers indicating quality of healing period. Origin 6.0 software was used to figure out and to draw raw data from measurements.

According to comparative results, 980 nm treatment's 21-Day healing process has better progression compared to 1064 nm and dual treatments' one. For every type of treatment, tissue markers have returned to their normal values at the end of the healing period.

Keywords: FT-IR spectroscopy, pulsed mode, Dual wavelength, Collagen, Keratin, poly-lysine, proteoglycan, NIR lasers.

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ÖZET

YAKIN KIZILALTI LAZERLER İLE KAPATILMIŞ SIÇAN DERİ DOKUSUNUN İYİLEŞME SÜRECİ KALİTESİNİN FT-IR SPEKTROSKOPİSİ İLE İNCELENMESİ

Zhin Tahseen Saleh

Biyomedikal Mühendisliği Programı

Yüksek Lisans

Danışman: Yrd. Doç. Dr.Haşim Özgür TABAKOĞLU

Cerrahi kesiler bir çeşit yaradır, fakat en önemli özelliği bir uzman tarafından yapılmış olması ve diğer çeşit yaralara kıyasla hızlı bir iyileşme sürecinin olmasıdır. Bunun sebebi cerrahi olarak deri dokusunda oluşturulmuş bir kesinin vücudun o bölgesindeki desene uyumlu olarak yapılmasıdır. Bu tip kesiler iyileşme sürecini hızlandırmak adına çeşitli yöntemler ile örneğin zımbalama, dikiş ve kimyasal yapıştırıcılar ile kapatılmaktadır. Lazer ile doku kaynağı gelecek vaat eden bir yara kapama yöntemi olmasına rağmen henüz klinikte kullanım aşamasına geçememiştir. Yara iyileşmesinin takibi çeşitli yöntemlerle yapılmaktadır. Bunlar; mekanik çekme testleri, histolojik analizler ve makro gözlemlerden ibarettir. FTIR spektroskopisi ise göreceli olarak yeni sayılabilecek bir yöntem olup biyomedikal araştırmalarda özellikle önemli doku belirteçlerinin gözlemlenmesinde kullanılmaktadır ve yara iyileşmesinin takibi için de potansiyel bir kullanım alanına sahiptir.

Bu çalışmada, yakın kızılaltı lazerler (980nm 1064nm ve DUAL mode) ile kapatılmış kesilerden FTIR ölçümleri alınmıştır. Kesiler 1 Watt gücündeki lazerlerin 10 saniye ve kipli olarak (500 ms açık-kapalı) uygulaması ile kapatılmışlardır. Her bir kesi için 5 jüllük bir enerji aktarımı söz konusudur. Yara iyileşmesinin takibi için 1, 4, 7, 14, ve 21. günlerde deri dokusundan FTIR ölçümleri alınmıştır. Kolajen (1246 cm⁻¹), Keratin (1544 cm⁻¹), Proteoglycan (1643 cm⁻¹), ve Poly-Lysine (3278 cm⁻¹) soğrulma değerlerinin iyileşme sürecinin kalitesinin tayininde önemli belirteçler olacağı düşünülmüştür. Elde edilen tüm veriler Origin 6.0 yazılımında işlenerek grafikler elde edilmiştir. Karşılaştırmalı sonuçlar 980 nm ile tedavi edilen kesilerin 21 günlük periyot içerisinde en makul iyileşme süreci izleyen yaralar olduğunu göstermiştir. 21 gün sonunda bahsi geçen doku belirteçlerinin FTIR değerleri her bir yöntem için sağlıklı deri dokusundaki değerler ile yaklaşık olarak aynıdır.

Anahtar kelimeler:FT-IR spektroskopisi, atımlı mod, çift dalga boyu, kolajen, keratin, proteoglikan, poly-lysine, yakın kızılaltı lazerler.

FATİH ÜNİVERSİTESİ -BIYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

CHAPTER 1

INTRODUCTION

1.1 The Properties of Light, Lasers, and Matter

1.1.1 Properties of Light

Light is known as electromagnetic radiation and an electromagnetic spectrum contains all possible waves over a continuum of frequencies and energies. The spectrum spans from radio radiation which has a wavelength $>10^9$ Å ($1 \text{ Å} = 1 \times 10^{-10}$ m) and a frequency $<3 \times 10^9$ Hz to gamma ray radiation which has a wavelength <0.1 Å and a frequency $>3 \times 10^{19}$ Hz. Also, the theory of wave-particle duality allows us to assume that photons and electrons follow the same general principles. However, a few distinctions must be noted. Electrons must obey Fermi statistics while a photon must obey Bose statistics. In Fermi statistics, two electrons in the same system cannot have the same physical properties (i.e., quantum numbers). In Bose statistics there is no restriction so photons can occur in large numbers, having the same energy, and momentum, such as in a pulse of laser light [1].

1.1.2 Properties of Lasers

Laser has certain unique properties namely high monochromaticity, collimation and coherent. In the amplifying medium, an atom or molecule is excited and emits spontaneously a photon as it returns to its lower energy state. As these excited-state atoms release their photons, the photons will interact with other excited atoms to stimulate the release of more photons of the same wavelength, energy and phase. Atoms in the ground state can also absorb photons, and since more electrons are in low energy states than in upper states, the probability of net absorption is larger than a net amplification. For more photons to be produced than removed, more atoms must be in an excited state, thereby creating a population inversion. This is obtained by the energy

source or pump delivering energy to a multiple energy level roster (generally three-level or four-level) and taking advantage of different decay rates. The amplifying medium will therefore determine the wavelength of the laser radiation. The optical cavity consists of precisely aligned mirrors to direct the photons in phase, through the laser medium to realize amplification as well as to provide directionality and increased intensity with high coherence. The laser beam profile will depend on the geometry of the mirrors, their separation, and the optical cavity construction. Therefore, different wavelengths can be emitted from the laser by using different laser systems and mediums. As noted above, a laser produces a highly monochromatic, coherent collimated beam [2].

The beam is of a single wavelength or frequency (with some finite linewidth) which is determined by the amplification medium. The laser beam is also considered one of the most coherent forms of light. Coherence defines the phase correlation between different emitted waves at different times and locations. No other light source can generate a beam with precise directionality and minimum angular spread as a laser.

Another unique feature of lasers is that they are capable of producing short bursts of highly intense light. Pulsed wave lasers emit a pulse or multiple pulses at a selected interval. The excitation energy can also be stored and released suddenly by operating the laser in a Q-switch mode or locking mode. Very short pulses (picosecond or femtosecond) are possible with lasers, but in principle an infinitely short pulse of finite energy will contain all wavelengths with the same power; hence an increase in bandwidth or a reduction in the monochromatic characteristic of the laser light can occur with ultra short pulses. The current research will predominately utilize Q-switched lasers with pulse widths in the ns time scale regime, and wavelengths generally in the UV and visible wavelength regime [3].

1.1.3 Properties of Matter

Anything that has mass and occupies space is defined as matter. Properties of matter result from its relationship with mass and space. Because of mass, all matter has inertia and weight and because it occupies space, all matter has volume and impenetrability because two objects cannot occupy the same space simultaneously. On other hand properties of matter depend on internal

structure. Such properties include ductility, elasticity, hardness, malleability, porosity (ability to permit another substance to flow through it) and tenacity (resistance to pulled apart). Matter is observed in 3 different state ;matter in the solid state has both a definite volume and a definite shape (its volume and shape do not change), matter in liquid state has a definite volume but no a definite shape (is able to form in to the shape of container); matter in the gaseous state has neither a definite volume nor definite shape (has no fixed shape and volume). Now that a basic introduction to light has been presented, the properties of matter will now be briefly reviewed in the context of laser-material interactions. A specific group of atoms within a molecule that is mainly responsible for reactions within the molecule is called a functional group. Relevant to biological tissue, all organic compounds have a functional group of hydrocarbons. Hydrocarbons are divided into two main classes based on the structure and are known as aliphatic and aromatic [4].

1.2 The Interaction of Light and Matter

1.2.1 Light and Matter Interactions on a Bulk Level

When compared to the wavelength of the incident light, matter is considered bulk matter if the tissue dimension is much larger than the wavelength of light. The wave nature of light leads to two important properties of the interaction of light with matter on a bulk level: refraction and reflection. The absorption and scattering of light are two other properties which are important in this type of interaction but the wave model of light cannot explain these phenomena or the effects which absorbed light produces. Therefore, light interacts with bulk matter in three key ways: absorption, reflection-refraction, and scattering. These properties are determined by the sum of the average of the molecular properties that correspond to the bulk matter. The interaction can be described as a bulk medium subjected to an external electric field that responds by becoming electronically polarized. The amount of light absorbed by bulk material is independent of the incident light intensity provided saturation does not occur, and dependent upon the amount of absorbing material through which the light passes [3].

Reflection and refraction are generally dependent upon the material and the incident wavelength. In bulk matter, reflection occurs by re-radiation (i.e., scattering) from the surface upon which it

was incident. Within the plane of incidence, the wave normal of the reflected beam, incident beam, and the reflecting surface normal must all satisfy the laws of reflection. where the index of refraction describes the optical response of a material with respect to propagation of light through it. The amount of refraction of light also depends upon the index of refraction which determines the phase as well as the velocity of propagation and strongly depends on wavelength in regions of high absorption only. Refraction usually occurs when two mediums of different indices of refraction are within the same reflection surface resulting in a change in speed of the light wave. The change in speed of the light wave results in a change of angle of propagation when entering from one medium to another. Reflection and refraction are governed by Fresnel's law and Snell's law, respectively, and can be difficult to measure in a complex medium like tissue due to simultaneous absorption and scattering [5].

Rayleigh scattering scatters in the forward and backward direction at the same rate and depends on λ^{-4} , where λ is the radiation wavelength. Rayleigh scattering will result in blue light scattering more efficiently than red light, as based on scattering alone. In general, the longer the wavelength of light, the deeper it will penetrate into bulk matter [3].

One type of inelastic scattering is Brillouin scattering. Brillouin scattering occurs by inducing inhomogeneity of the refractive index from an acoustic wave interfering with bulk matter. This type of scattering will result in the frequency of the scattered photons shifting up or down.

Another type of inelastic scattering is Raman scattering. The difference in energy between incident Raman scattering radiation is accounted for as energy either added to or removed from vibrational modes of the solid matrix [3,5].

1.2.2 Light and Matter Interactions on a Molecular Level

The stability of molecules can help explain the nature of the reactions that the molecules may undergo. Energy transfer and excited state formation requires one or more molecules interacting, therefore, a minimum molecular size is required and is known as a dimer. A dimer in an excited state is called an excimer (i.e., excited dimer). The interaction of two different molecules with each other, when one is in an excited state is known as an excited state complex or exciplex. On a molecular level, Einstein's quantum theory of radiation is typically used to explain the

interaction between radiation and matter. Stimulated absorption, spontaneous emission, and spontaneous absorption are the three processes that explain this interaction [6].

Absorption of light results in an excitation of certain vibrations or electronic states in the molecules of the absorbing material. Like bulk matter, on the molecular level, the ability to absorb radiation is dependent upon the material composition, the wavelength of radiation, the material properties and the energy state.

A chromophore is a molecular unit where an electron being excited is primarily located. Radiative and non radiative processes are considered photophysical processes which do not lead to an overall chemical change. Excited molecules can also directly produce new products or forms of free radicals or excited molecules, which can lead to physical or chemical changes [5].

The natural state of most molecules is in the ground state and involves paired electrons, that, have a total spin of $S=0$. Excitation of paired electrons from molecules whose ground states are singlet will result in the electrons staying paired and excited in a singlet state S, or the electrons become unpaired and excited in a triplet state T.

1.2.3 Light and Tissue Interactions

The cellular, extracellular, and bulk properties of tissue will produce diverse processes during the interaction of light. Tissue, like other matter, will interact with light through absorption, scattering, reflection and transmission; resulting in radioactive and nonradioactive processes. Important properties of tissue itself include the structure, water content, blood circulation, thermal conductivity, heat capacity, density, and the presence of melanin, other fluorophores, and chromophores. The inhomogeneity of different samples of biological tissue makes it difficult to correlate its optical properties [2].

Absorption of photons by tissue is typically dominated by proteins, DNA, water, melanin, and hemoglobin absorbing the incident photons, these components are known as chromophores. A chromophore will absorb energy and become electronically excited. For tissue, this vibrational and translational energy is typically converted to thermal energy or heat. The longer the

wavelength, the deeper it will penetrate the tissue up to a wavelength of about 1300 nm. At wavelengths above 1300 nm, penetration is only superficial because of the absorption coefficient of tissue water.

Raman scattering of light by tissue will produce excitation of the molecular vibrations and may be significant in tissue. Scattering within tissue will also affect the absorption of light by the tissue. In most tissues, absorption and scattering processes will take place simultaneously, and will occur with varying ratios [7].

1.2.4 The Interaction of Lasers and Bulk Matter

The destructive interaction of lasers with matter is facilitated by acceleration of free electrons. The energy carried by the free electron within the material, for a time interval that depends on the material and laser settings, will originally induce a primary thermalisation effect. For such light and matter interactions, a free electron is produced by the simultaneous absorption of photons or by the kinetic energy of an impacting free electron to exceed the band gap energy. For the interaction of lasers with matter, the exceeded band gap energy requirement is replaced by the effective ionization potential requirement since the oscillation energy of the electron due to the laser's electric field needs to be accounted for. At least one free seed electron produced by multi-photon ionization or by impact ionization is required to start a cascade ionization effect. Photoionization for different laser field strengths and frequencies can result in multi-photon ionization. In impact ionization, the kinetic energy of the impacting electrons is used to overcome the effective ionization potential requirement. The excess energy that remains after the impact of electrons is distributed among the electrons involved in the collision. This results in the electrons needing to gain less energy to reach the critical level [2]. However, under confined stress conditions where heating and stretching are both occurring distinction becomes difficult therefore the term optical breakdown is used. Also, if the laser pulse duration is in the nanosecond range or shorter and has an extremely high spatial density of photons an optical breakdown will result. During optical breakdown, temperatures of several thousand Kelvin can

be reached. Optical breakdown can include bubble formation, plasma luminescence, and ionized regions of extremely high electron density [8].

Material removal via volumetric processes is known as ablation. Ablation processes can remove molecular clusters, molecular fragments, and single molecules as well as cracks the chemical bonds.

1.3 The Interaction of Lasers and Tissue

Laser tissue interaction depends on properties of the tissue and the parameters of the laser. The wavelength dependent penetration depth of laser light into tissue determinate heat flow and thickness of the zone of necrosis The optical properties of the tissue that effect this interaction are reflection and refraction, scattering, and absorption. Laser beams can be used to open, seal, cut, vaporize, coagulate, cauterize, rupture and weld human tissue. Biophysical or biochemical processes can also be monitored it real time using very short pulses of laser light. However, once the beam enters the tissue, scattering occurs resulting in the rapid loss of collimation of the beam. This will result in a loss of the initial directionality of the laser beam. The fundamental principles, depending upon the interaction time and the effective power density of the interaction of lasers with tissue. Photomechanical processes can be achieved with short, high powered laser pulses. Interaction types that occur adjacently cannot always be strictly separated[10]

1.3.1 PhotochemicalEffects

Photochemical effects will occur at long interaction times, from seconds to continuous wave, and at low power densities, typically 1 W/cm². Light is absorbed by the tissue with no primary heating of the tissue and will induce chemical effects and reactions the tissue. Almost all biological relevant photochemical reactions are dependent upon the generation of reactive oxygen species [10] .

1.3.2 Photothermal Effects

The temperature rise within tissue is associated with the photon energy converting to kinetic energy through a combination of non-radioactive processes and vibrational relaxations. Thermal effects will result, in most biomolecules, since a large number of accessible vibrational states are available to facilitate absorption as well as a large number of channels are available for thermal decay and deactivation. Many lasers used for surgical applications fall within this category of converting laser light into thermal energy. When tissue is irradiated by a laser beam, heat is generated inside the tissue and the absorptivity of the tissue can change. In the example of the cornea exposed to UV radiation, this change is associated with both the formation of radicals (whose absorption cross section exceeds that of the original macromolecule links) and the development of light scattering as result of water in the cornea starting to boil. If heat moves faster than molecules can diffuse, thermal energy can be deposited into high absorbance regions through short laser pulses, and this is known as selective heating [9,10].

The temperature that tissue reaches locally during and after exposure to a laser determines the location and extent of thermal effects with the tissue. Heat transport within the tissue is associated with the thermal tissue properties, and losses of heat during laser-tissue interactions also need to be considered. For most laser application the loss of heat is associated with conduction. Lowering water content will generally result in a decrease in heat conductivity and heat capacity [10].

These effects include coagulation, vaporization, ablation, and carbonization of tissue. Inflammation, cellular injury and cellular enzyme deactivation can occur to tissue as the temperature is raised by ~5-10 °C. Tissue generally, can reach up to a temperature of ~45 °C before irreversible damage occurs if exposure time is not limited. Older tissue has a higher density of cross-linking; therefore, higher temperatures are required to undergo these transitions. Further heating of tissue to ~45-50 °C will result in molecules within the tissue entering into an energy activated state and transitioning into an irreversible damaged state [9].

1.3.2.1 Coagulation of Tissue

Tissue will begin to coagulate and denaturalize as temperatures approach ~50-60 °C. At this temperature range, the helical structure of the chains in collagen transform into a denatured structure that is randomly coiled. Most protein molecules will lose their ability to function in the cell and also become denaturalized. In thermal denaturation, both the magnitude and duration of thermal exposure can be interchanged to coagulate tissue. Either high temperatures at low heating times or long heating times at low temperatures will produce the thermal energy for tissue temperature to become ~50-60 °C. It is exactly the denaturation of protein structures that determines the morphology of laser cuts. Successive laser pulses will result in the coagulated zone deepening within the tissue.

Heating of tissue beyond 60 °C will result in a disintegration of collagen where the coagulated tissue becomes necrotic, hydrogen bond breaking and retraction will occur. Above 70 °C denaturation of DNA occurs and at temperature higher than 80°C equilibrium of chemical concentration is destroyed and membrane permeability is drastically increased.

1.4 Laser Tissue Welding

There is some different type of tissues such as nerve, vessel, skin, gastrointestinal and genitourinary tract, spleen, liver, nerves, reporting the use of laser for wound approximation and closure. The results showed benefits of improving immediate tensile strength and fluid tight seal. The laser-assisted wound approximation can be divided into 2 techniques namely: laser tissue welding which is direct application of precise laser energy to the site to be welded and laser tissue soldering which is using a substrate such as protein-based fluid and dye to absorb the laser energy [11].

Regarding the study in oral mucosa, there was an in vivo study comparing immediate tensile strength between laser welding and suture of incisions on the pig tongues. Although this study showed that CO2 laser welding could produce equivalent or stronger tensile strength than the suture, the immediate tensile strength which was an expected result for promotion of healing in the laser welding group was less than the suture group. Owing to this potential area, it was worth exploring the benefit of combined laser tissue welding and suture to improve the immediate

tensile strength in oral mucosa. The strength of the wounds in the tissue blocks repaired by suture alone with the combined CO2 laser welding and suture[11,12]. There are some benefits of laser tissue like reduce operation time [13,14] reduce skill [15]reduce bleeding [16] Also using laser tissue welding leads to heal faster[15,17,18] Laser welding tissue also produce complete closures, and is benefit for watertight anastomosis intraoperative of vascular[19] gastrointestinal tract [20], and genitourinary repairs [21]. This type of anastomosis are benefits for neural repairs. Laser tissue welding also has some disadvantage like:

1-The low strength resulting anastomosis which leads to anastomotic aneurysm or rupture of the repair.

2- Thermal tissue damage by direct laser heating and heat transfer to tissue which cause irreversible damage of the welding.

Collagen; is one of the most important type of protein in the human body,its found in the skin, muscles, bones, and tendons. Collagens are formed about 20% of the large arterier such as aorta and 50%of peripheral nerves,and about80%of skin [22] collagen also foundin the peripheral nerves and different layers of blood vessels.

Keratin; is ascleroprotein substance which is found in the hair, nails, dead outer skin, horn, claws. Keratin is afibrous structure of protein which protects epithelial cells from damage or stress. It makes the main structural component of outer layer of skin, also of nails and deep skin[23].

Proteoglycans: Proteo means protein,glycan means sugar,aproteoglycan is along polysaccharide chain attached to aprotein and produced in endoplasmic reticulum, PGS are protein molecules found in the space between cells, also it is found in protective organ like throat, any group of PG found in connective tissue and formed of subunits of glycolsaminoglycans linked to aprotein core,and hydrated PGs forms fluid of mucus and the matrix of connective tissue called also mucopolysaccharide [24].

While Poly-lysine refers to several types of lysinehomopolymers, it may differ from each other in terms of stereochemistry and link position.

1.5 Spectroscopic Methods

Spectroscopy techniques can provide a large amount of molecular structure information rapidly. Since the absorption and emission of atoms and molecules must occur between well-defined energy levels, every element has a unique emission spectrum. Each spectroscopic method can be distinguished by how it interacts with the sample material, the energy used and the component it determines. The following sections describe in detail the spectroscopic methods of vibrational and electronic schemes.

Infrared (IR), Raman, Ultraviolet (UV), Visible (VIS), and Fluorescence spectroscopy have all been proposed for biochemical and clinical analysis for in vivo screenings of diseased, (thereby avoiding a biopsy), population screening, early diagnosis, prognosis, monitoring of therapy and many others. Therefore, these techniques will be covered in more detail [25].

1.5.1 Vibrational Spectroscopy (VS)

VS is used for characterization, reaction monitoring, identification, structure elucidation, quality assurance and quality control. Vibration spectroscopy covers infrared spectroscopies (IR), near-infrared (NIRS), and Raman spectroscopies (RS). They are non-destructive and non-invasive tools that give information about the molecular composition, structure, and interaction within a sample. There are 2 techniques that are used for vibration studies of molecules on surface:

a) IR spectroscopies (some forms, e.g. RAIRS, MIR)

b) Electron Energy Loss Spectroscopy (EELS)

Raman and Infrared (IR) spectroscopy are techniques that exploit the normal vibrational modes that occur naturally and predictably within molecules. The photon energies associated with this region of the electromagnetic spectrum can cause vibrational excitation, but are generally too small to result in electronic excitation.

1.5.1.1 Infrared(IR) Spectroscopy

Energy of molecule = Electronic energy +Vibrational energy = Rotational energy. IR spectra mainly used in structure elucidation to determine the functional groups. Infrared spectroscopy is concerned with the study of absorption of infrared radiation, which causes vibrational transition in the molecule. Hence IR spectroscopy also known as (Vibrational spectroscopy) [26] .

1.5.1.2 Dispersive Infrared Spectrometers

This infrared spectrometer was first used in the field of obtaining IR absorbance spectrum. However, this technology was no more in use after the introduction of grating instruments during 1960s that was cheaper and of better quality. The basic components in this spectrometer include the source, the monochromator and the detector device. This separates the components of polychromatic radiation based on their wavelength [27].

This energy then moves to the detector through the sample. The entire sample spectrum can be obtained by the adjustment of a component within the monochromator and allowing the different wavelength to pass through the exit slit at a time. However, the instrument became less popular after the 1950s because of its defects like time consumption, sample overheating and damage due to repeated sample irradiation. Later, these limitations were overcome by the use of advanced instrument called Fourier- transform infrared spectrometer. Diffraction grating is replaced by an interferometer in the Fourier Transform spectrometer.

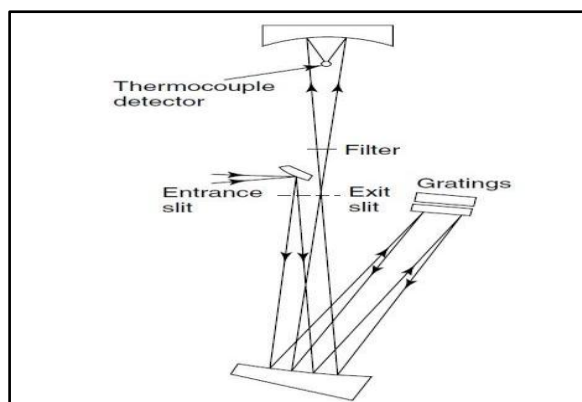


Figure 1.1 Schematic diagram showing the optical path of a double beam infrared spectrometer with a grating monochromator [28].

1.5.1.3 Fourier Transform Infrared Spectroscopy (FTIR)

The computer technology has helped to improve spectrum quality and data time turnover greatly. In addition, this technique has made the study of biological molecules rapidly in a easy way. It acquires the IR absorbance spectrum by the combined use of interferometer and the mathematical processes of Fourier transformation. It has many benefits as it requires very little volume of sample for analysis, less expenses to operate, doesn't heat sample and the resulting spectra can be received within short period of time.

FT-IR spectroscopy with the use of the interferometer achieves the production of an IR radiation signal across all wavelengths. The basic components of this instrument include source, the interferometer, sample and detector.

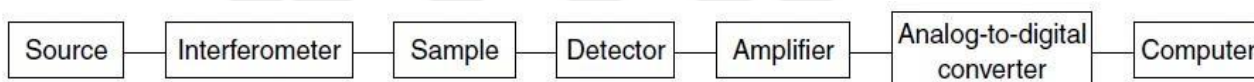


Figure 1.2: Basic components of an FTIR instrument [29].

The analysis process of FT-IR involves the following procedure:

1.5.1.3.1 The Source: The black body source usually Globar or Nerst is used for the mid infrared region. It emits the infrared energy. IR-radiation broadband sources are the commonly used ones; they emit infrared radiation from far infrared to visible range. Their temperature is generally higher than 12000K (Figure 1.3).

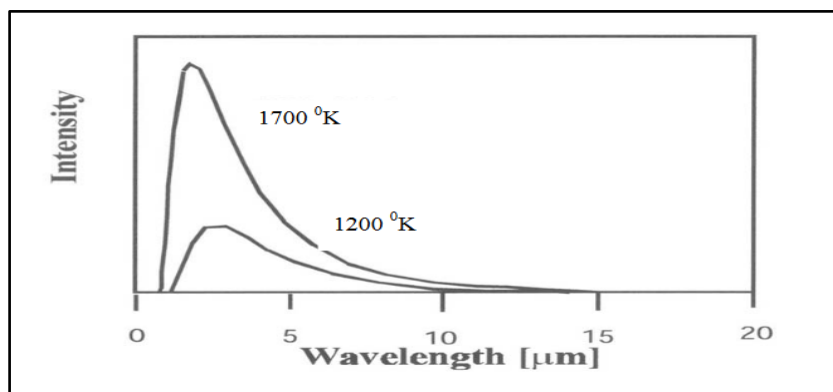


Fig 1.3: The emission intensity of blackbody radiation [28]

Furthermore, the area under the curve increases quickly as the temperature increases. They endure more mechanical exertion than Nernst Glowers. Globars' intensity emission spectrum peaks at $1.92\mu\text{m}$, the emission intensity decreases remarkable at lower wavelengths, and the spectrum intensity is 75% of blackbody radiation. Globars' are inexpensive, but they may not provide enough infrared intensity for some applications [28,29].

1.5.1.3.2 The Sample:The emitted beam enters the sample compartment and according to the need of analysis, this light is transmitted by the surface of the samples. Then the specific frequencies of energy that the sample possesses are absorbed here.

1.5.1.3.3 The Detector:Finally, the beam gets through the detector, which is designed for final measurement of the special interferogram signal. For the mid infrared region, two detectors are commonly used. First type is deuterated triglycine sulphate (DTGS) pyroelectric detector that is heat resistant and placed in an alkali halide window. Second type is mercury cadmium telluride (MCT) photoconductive detectors

The infrared detector is a device measuring the infrared energy of the source, which has gone through the spectrometer. Thermal detectors (thermocouples, bolometer and pyroelectric (DTGS)) and quantum detectors (MCT) are the two basic types used in the IR spectroscopy, and MCT detector for Microscopy. The wavenumber range and the sensitivity depend on the choice of the detector. In this work we used the sensitive MCT detector to obtain a good quality of spectra [29].

1.5.2 Raman Spectroscopy

In 1928, the Indian physicist c.v. Raman discovered that visible wavelength of a small fraction of the radiation scattered by certain molecules differs from that of the incident beam. Furthermore, he noted that the change (shifts) in frequency depend upon the chemical structure of the molecules responsible for the scattering[30].

1.6 Motivation

In one hand, this thesis aims to show little investigations about FT-IR spectroscopic techniques in the study of biological tissues welding. The advantage of such a technology would be to change the conventional clipping, suturing. Stapling with NIR welding may reduce tissue damage. The basic or fundamental of this technique is to be shorten the wound healing time; additionally to replace with suture materials, which are most often sources of wound infection if not thoroughly sterilized.

1.6.1 Purpose of Thesis

This thesis presents technology as a non-destructive inspection of wound healing period of laser welded skin tissue by using FTIR technique. The study will assist in evaluating deposition of important molecules or proteins for wound healing such as collagen, keratin, proteoglycans and poly-lysine at the incision site by using FTIR.

1.6.2 Hypothesis:

Low temperature laser tissue welding is a technique used to provide immediate sealing of wounds; the cut is irradiated with near-infrared laser operated at low power densities which induces a localized heating of the tissue. Among three different applications, closure of dual laser irradiation is supposed to be the most successful one.

CHAPTER 2

2. Material and methods

2.1 Skin Tissue Samples:

Laser welded skin tissue samples were taken from the previous experiments [31]. The samples were classified as 980 nm, 1064 nm, and Dual (980 nm and 1064 nm) welded. FTIR measurements were done for every group as described below.

2.2 Laser Parameters

Laser parameters is given table (TABLE 2.1) below.

Table 2.1: Parameters applied during the welding application of different lasers.

Application Parameters	Power (W)	Duration of application (s)	Energy (J)	On-Off time (s) or Pulsing
980 nm	1 W	10	5	0.5
1064 nm	1 W	10	5	0.5
DUAL	1 W	10	5	0.5

2.3 FT-IR Measurement Procedure:

Fourier Transform Infrared (FTIR) is a vibrational spectroscopic technique that can be used in biological studies, can also be used in clinical investigations related to malignancy and cancer detection. FTIR spectroscopies are potential tools for noninvasive optical tissue diagnosis.

Nicolet 6700 FT-IR spectrometer was used to record FT-IR spectra .IR spectrum was recorded on samples dispersed in KBr pellets in the range of 400- 4000 Cm^{-1}

Step of operation manuals of NXR FT-IR:

If you are using the NXR FT-IR Spectroscopy equipment follow the steps below:

- Start the personnel computer.
- Start the FT-IR Spectroscopy.
- Open the Ominic Application program.
- Adjust the parameters in the Expset up. Interface.
- Place your Sample on top the disc like plate and adjust the pin until the optical pinhead glass touches the sample
- Click on / Press the sample point button.
- Save the file as both CSV & SPA file.
- Sterilize the surface of the Sample plate.

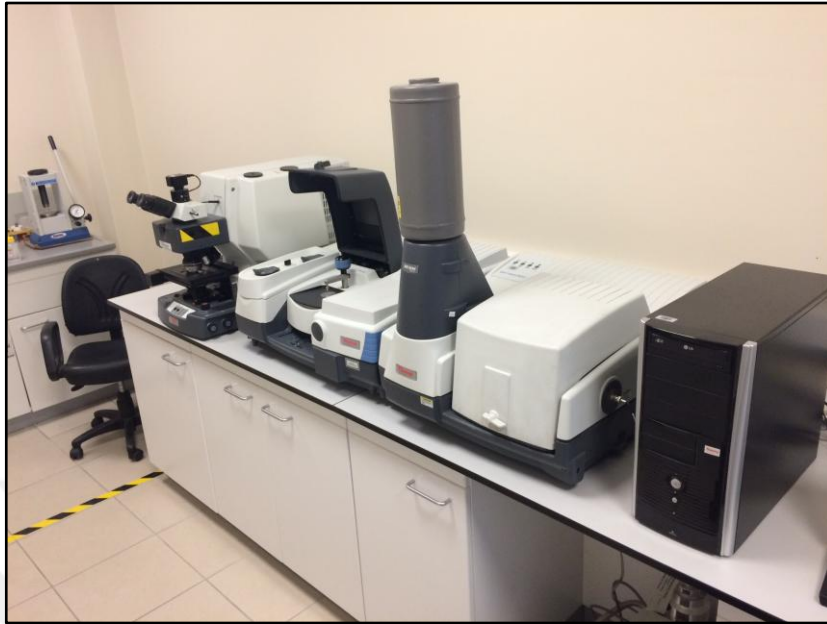


Fig.2.1 NXR FT-IR Spectrophotometry Thermo Nicolet Nexus 670

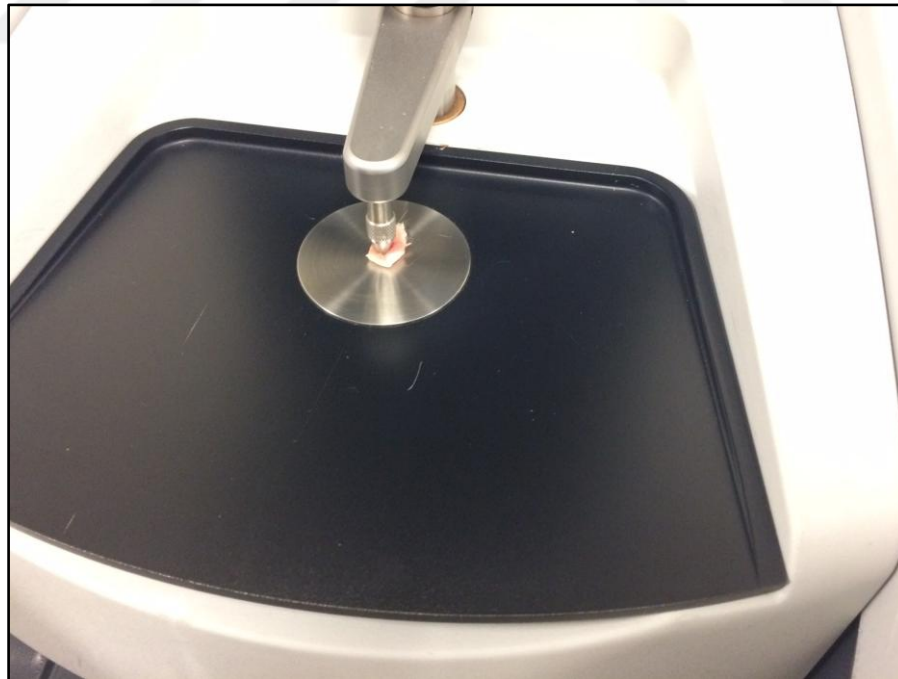


Figure 2.2 Placement of skin tissue under the tip of the measurement unit of the device.

2.4 Origin program procedure:

1. By using FTIR instrument, triplicate of data for samples treated with 980 nm, 1064 nm and Dual (980 + 1064) nm were obtained.
2. Data in SPA form was converted to TEXT form by using software 'Origin' version 6.0.
3. Mean of 3 measurements (triplicates) were taken for each application, namely 980, 1064 and DUAL. They were classified as first, fourth, seven, fourteen and twenty-one days in order to compare healing periods of each laser treatment.
4. Graphs showing the differences among laser applications and healing periods were drawn. (Figure 2.3) is given below
5. In the graphics important peak values for healing were labeled such as 1246, 1544, 1643, and 3278 cm^{-1} . These peaks numbers correspond to collagen, keratin, proteoglycan, and poly-lysine respectively. (Figure 2.4) is given below

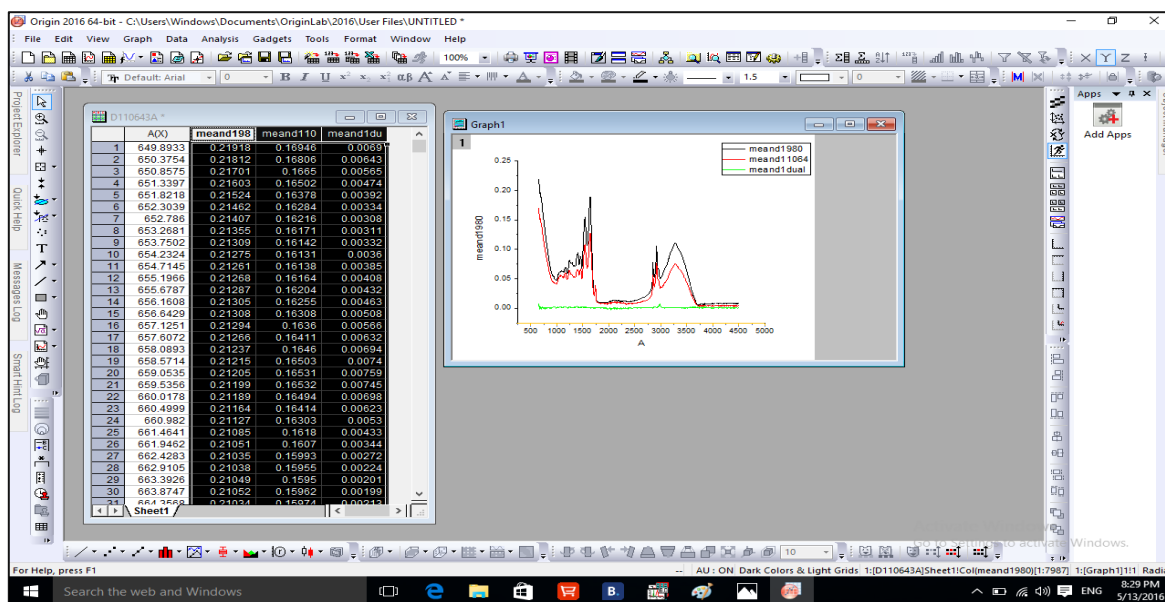


Figure 2.3: Screenshot from software Origin 6.0. Comparison of the three types of treatments' result (Day-1) on the same graph was shown.

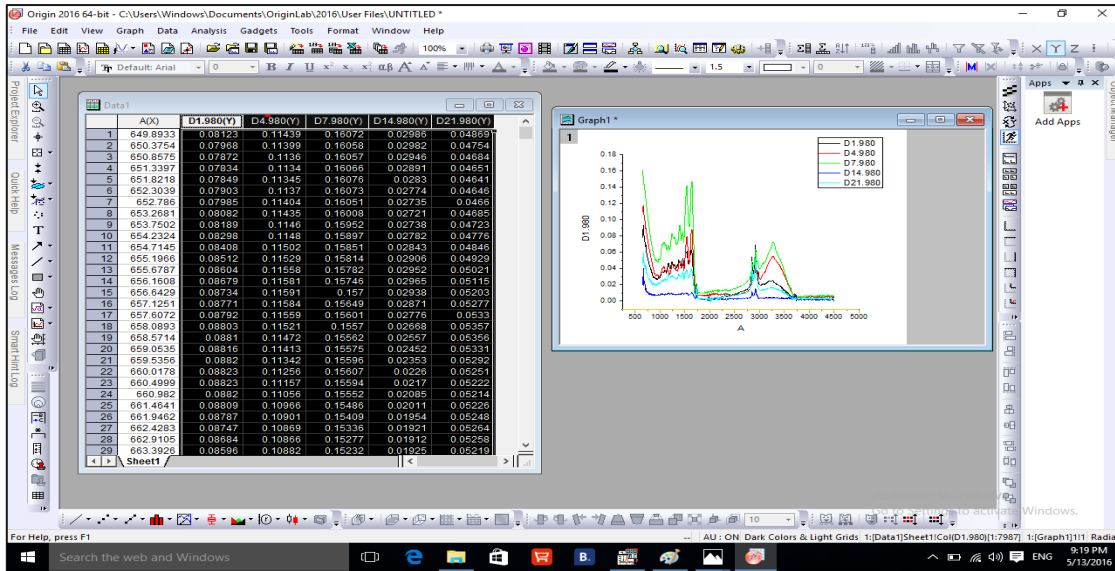


Figure 2.4: Screenshot from software Origin 6.0. Comparison of the specific healing days throughout the healing period was shown on the same graph for one type of the treatment (980 nm).

CHAPTER 3

3.1 RESULTS AND DISCUSSION

In order to characterize the degree of tissue repair at the incision site, the intensity of 1246 cm^{-1} was primarily taken into account. It provides information about collagen deposition and hence, the status of wound healing. It must be also taken into consideration that peaks at 1544 cm^{-1} , 1643 cm^{-1} and 3278 cm^{-1} carry information about keratin, proteoglycan and poly-lysine respectively.

The absorption intensity of all laser groups (980nm, 1064nm and Dual mode nm) for all days were plotted in Figure 3.1, Figure 3.2 and Figure 3.3 respectively and corresponding tables indicating important peak values for collagen, keratin, proteoglycan and poly-lysine are given below each figure (Table 3.1, Table 3.2, Table 3.3).

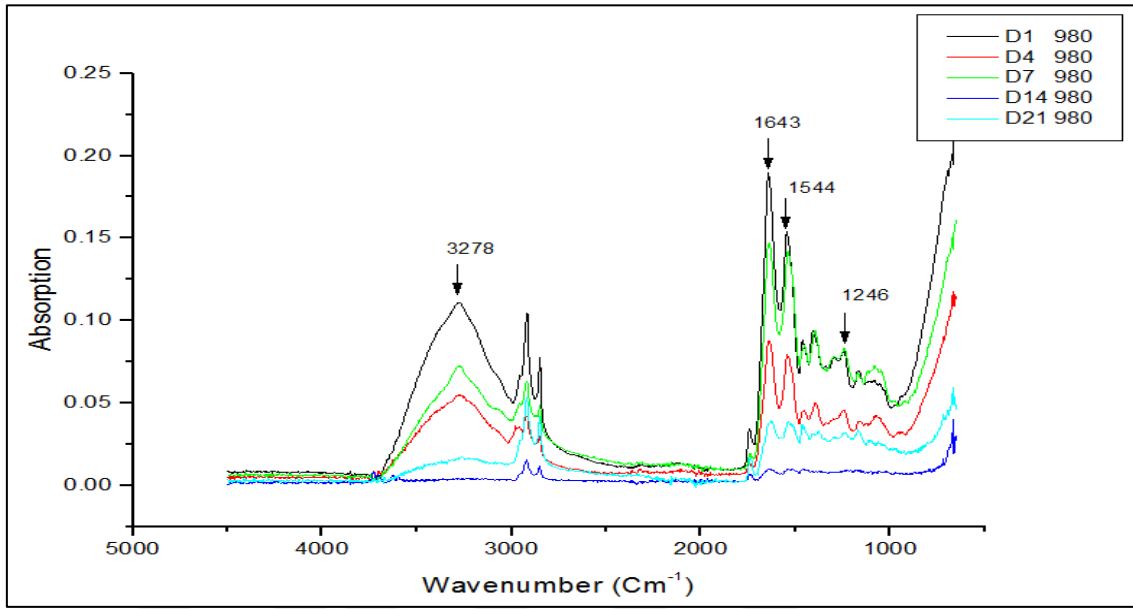


Figure 3.1 FT-IR Spectroscopy chart for specific days during 21-Day healing period of 980 nm welded skin tissue.

Table 3.1 FT-IR Spectroscopy Absorption Intensities for 980nm Welded Skin Tissue

Protein	cm ⁻¹	Control Days	Absorption
Collagen	1246	DAY 1	0,080
		DAY 4	0,048
		DAY 7	0,082
		DAY 14	0,009
		DAY 21	0,033
Keratin	1544	DAY 1	0,154
		DAY 4	0,078
		DAY 7	0,138
		DAY 14	0,010
		DAY 21	0,038
Proteoglycan	1643	DAY 1	0,188
		DAY 4	0,087
		DAY 7	0,146
		DAY 14	0,010
		DAY 21	0,039
Poly-lysine	3278	DAY 1	0,110
		DAY 4	0,054
		DAY 7	0,073
		DAY 14	0,003
		DAY 21	0,017

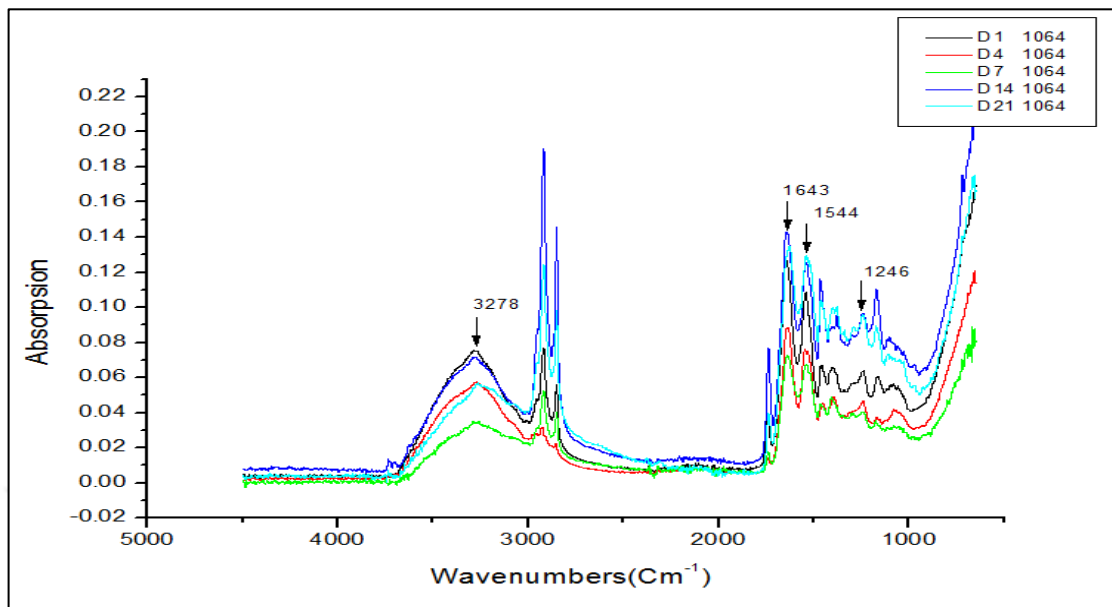


Figure 3.2 FT-IR Spectroscopy chart for specific days during 21-Day healing period of 1064 nm welded skin tissue.

Table 3.2 FT-IR Spectroscopy Absorption Intensities for 1064 nm Welded Skin Tissue

Protein	cm-1	Control Days	Absorption
Collagen	1246	DAY 1	0,063
		DAY 4	0,046
		DAY 7	0,040
		DAY 14	0,097
		DAY 21	0,094
Keratin	1544	DAY 1	0,108
		DAY 4	0,076
		DAY 7	0,067
		DAY 14	0,124
		DAY 21	0,129
Proteoglycan	1643	DAY 1	0,126
		DAY 4	0,088
		DAY 7	0,071
		DAY 14	0,143
		DAY 21	0,134
Poly-lysine	3278	DAY 1	0,076
		DAY 4	0,057
		DAY 7	0,034
		DAY 14	0,071
		DAY 21	0,054

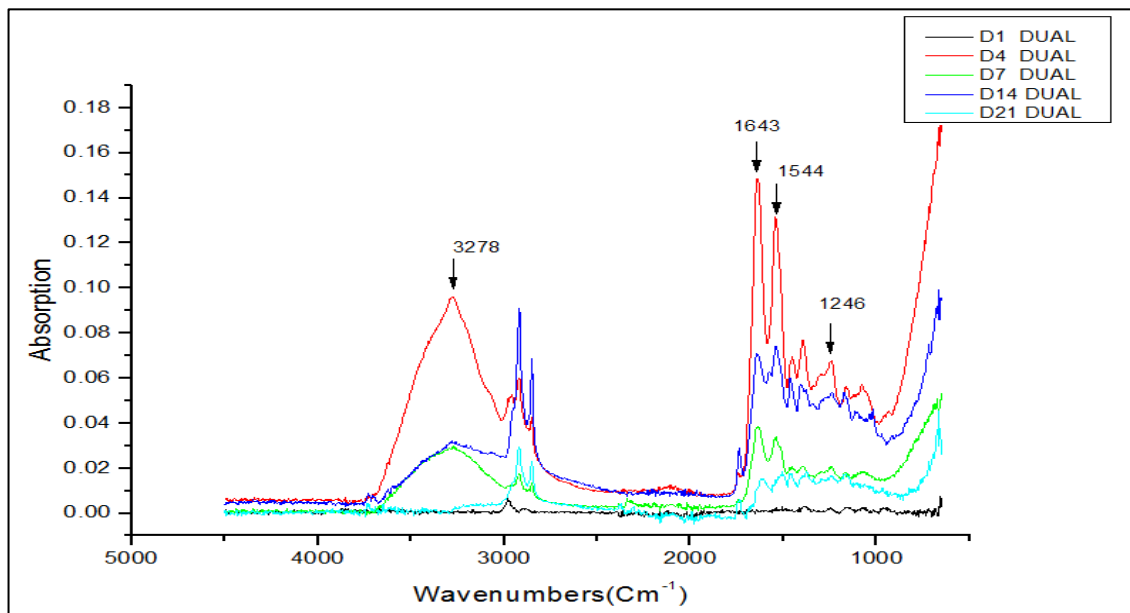


Figure 3.3 FT-IR Spectroscopy chart for specific days during 21-Day healing period of DUAL welded skin tissue.

Table 3.3 FT-IR Spectroscopy Absorption Intensities for DUAL Welded Skin Tissue

Protein	cm ⁻¹	Control Days	Absorption
Collagen	1246	DAY 1	0,002
		DAY 4	0,067
		DAY 7	0,020
		DAY 14	0,053
		DAY 21	0,016
Keratin	1544	DAY 1	0,002
		DAY 4	0,131
		DAY 7	0,034
		DAY 14	0,074
		DAY 21	0,016
Proteoglycan	1643	DAY 1	0,001
		DAY 4	0,148
		DAY 7	0,038
		DAY 14	0,070
		DAY 21	0,014
Poly-lysine	3278	DAY 1	0,001
		DAY 4	0,095
		DAY 7	0,029
		DAY 14	0,032
		DAY 21	0,003

Degree of tissue repair at the incision site has been determined with daily comparison of treatment methods by following change of tissue markers' (collagen, keratin, proteoglycan and poly-lysine) absorption intensities of FTIR, on the specific healing days. Among the specific healing days of 980 nm treatment, maximum absorption values were observed on DAY-1, while the minimum values were observed on DAY-14. In case of 1064 nm treatment, minimum absorption intensities were recorded on DAY-7, while the minimum intensities were recorded on DAY-14, in general. Finally, in DUAL treatment; minimum absorption intensities were seen on DAY-1, while the maximum intensities were seen on DAY-4. A comparative graph is drawn showing the change of absorption intensities of different types of treatment methods on specific healing days (Figure 3.4), and corresponding table (Table 3.4) showing the specific absorption intensity values on the same days.

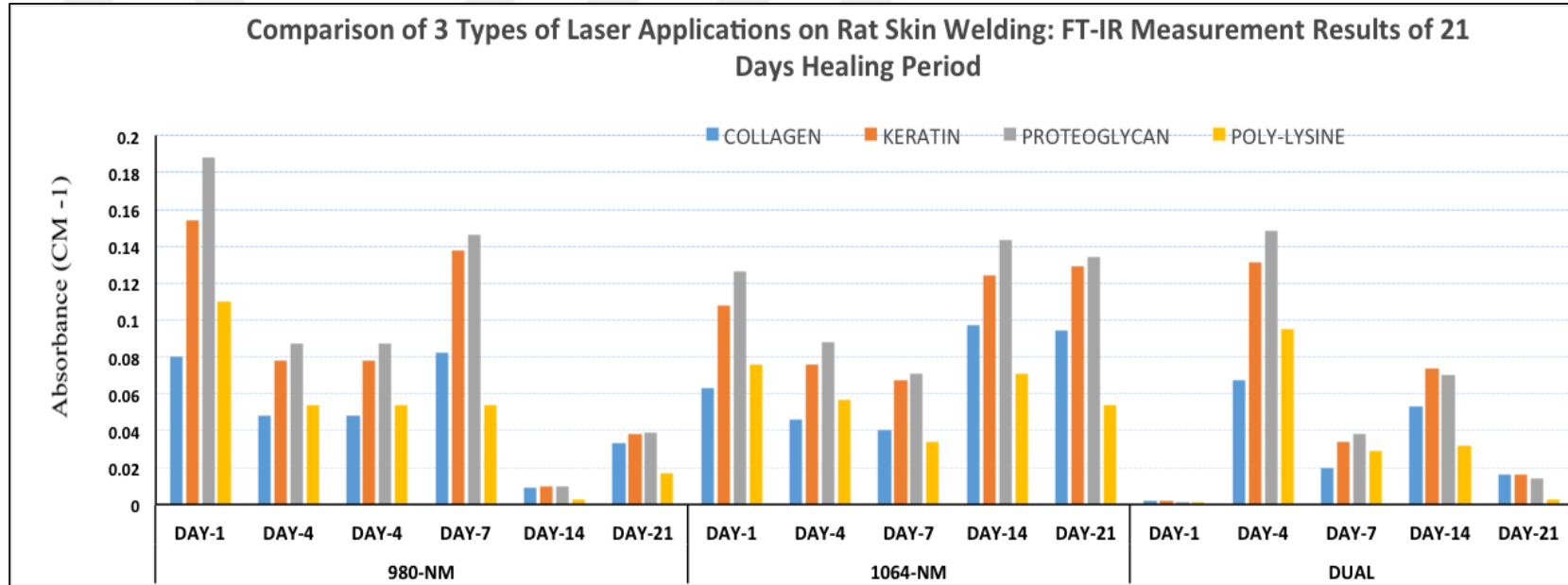


Figure 3.4 FT-IR Spectroscopy absorption intensity comparison of important healing indicators for different laser applications at specific healing days.

Table 3.4 FT-IR Spectroscopy absorption intensity values of important healing indicators for different laser applications at specific healing days.

Proteins	980-NM					1064-NM					DUAL				
	DAY-1	DAY-4	DAY-7	DAY-14	DAY-21	DAY-1	DAY-4	DAY-7	DAY-14	DAY-21	DAY-1	DAY-4	DAY-7	DAY-14	DAY-21
COLLAGEN	0,080	0.048	0.082	0.009	0.033	0.063	0.046	0.04	0.097	0.094	0.002	0.067	0.02	0.053	0.016
KERATIN	0.154	0.078	0.138	0.01	0.038	0.108	0.076	0.067	0.124	0.129	0.002	0.131	0.034	0.074	0.016
PROTEOGLYCAN	0.188	0.087	0.146	0.01	0.039	0.126	0.088	0.071	0.143	0.134	0.001	0.148	0.038	0.07	0.014
POLY-LYSINE	0.11	0.054	0.054	0.003	0.017	0.076	0.057	0.034	0.071	0.054	0.001	0.095	0.029	0.032	0.003

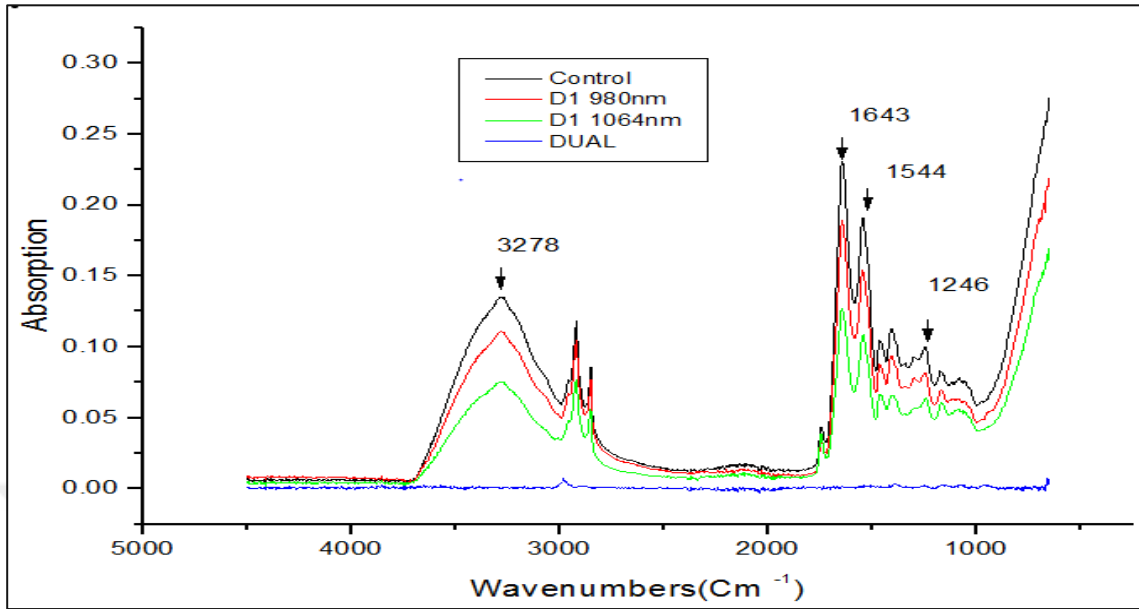


Figure 3.5 Comparison of absorption intensities of FTIR measurements of treatment groups and control group on DAY-1. Control group was the healthy rat skin that was undergone no incision and no treatment.

Table 3.5 Absorption intensities of FTIR measurements of treatment groups and control group on DAY-1.

Protein	Cm ⁻¹	Control Days	Absorption
Collagen	1246	980 NM	0,081
		1064 NM	0,063
		DUAL NM	0,001
		CONTROL	0,099
Keratin	1544	980 NM	0,152
		1064 NM	0,108
		DUAL NM	0,001
		CONTROL	0,191
Proteoglycan	1643	980 NM	0,186
		1064 NM	0,125
		DUAL NM	0,001
		CONTROL	0,228
Poly-lysine	3278	980 NM	0,110
		1064 NM	0,079
		DUAL NM	0,001
		CONTROL	0,138

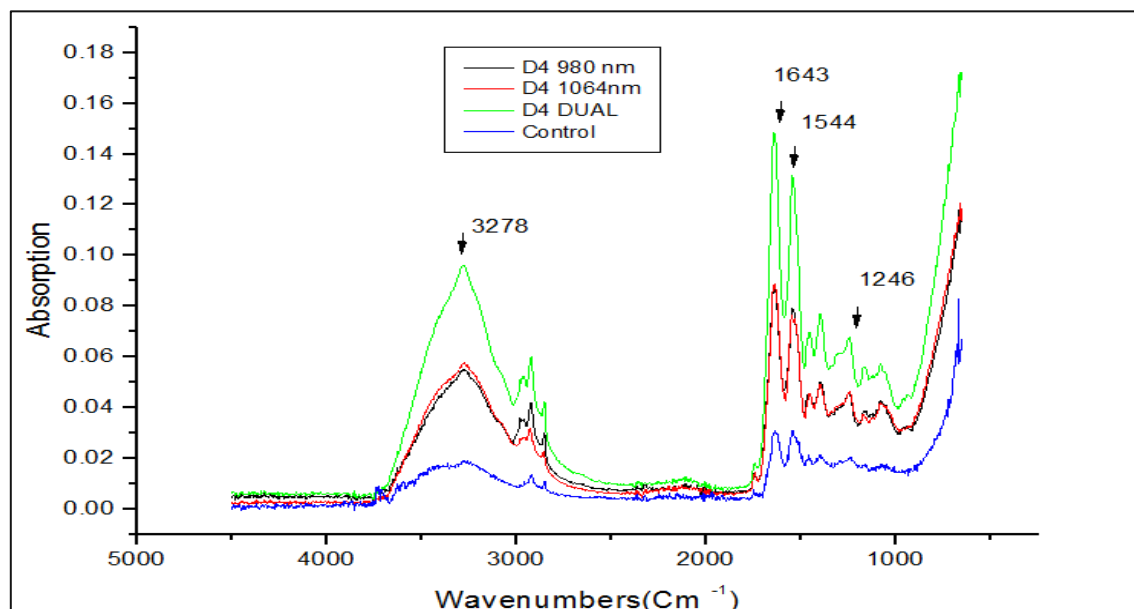


Figure 3.6 Comparison of absorption intensities of FTIR measurements of treatment groups and control group on DAY-4. Control group was the healthy rat skin that was undergone no incision and no treatment.

Table 3.6 Absorption intensities of FTIR measurements of treatment groups and control group on DAY-4.

Protein	cm ⁻¹	Control Days	Absorption
Collagen	1246	980 NM	0,047
		1064 NM	0,047
		DUAL NM	0,068
		CONTROL	0,020
Keratin	1544	980 NM	0,078
		1064 NM	0,077
		DUAL NM	0,129
		CONTROL	0,031
Proteoglycan	1643	980 NM	0,085
		1064 NM	0,088
		DUAL NM	0,148
		CONTROL	0,031
Poly-lysine	3278	980 NM	0,054
		1064 NM	0,057
		DUAL NM	0,095
		CONTROL	0,018

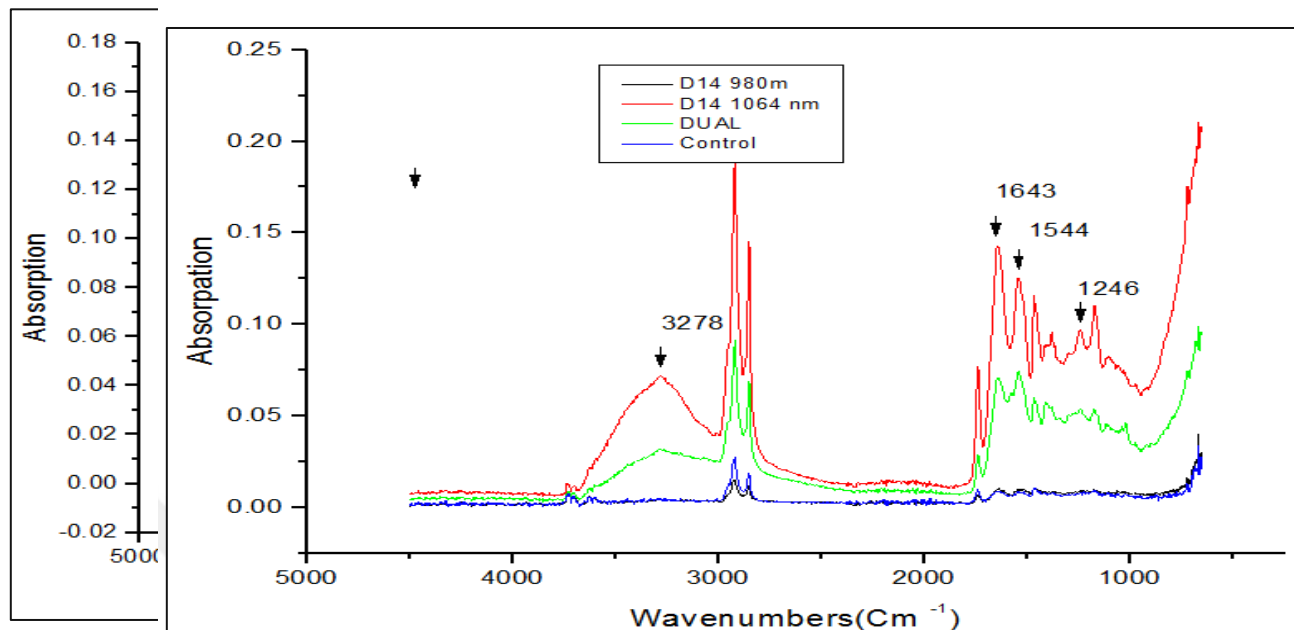


Figure 3.7 Comparison of absorption intensities of FTIR measurements of treatment groups and control group on DAY-7. Control group was the healthy rat skin that was undergone no incision and no treatment.

Table 3.7 Absorption intensities of FTIR measurements of treatment groups and control group on DAY-7.

Protein	cm ⁻¹	Control Days	Absorption
Collagen	1246	980 NM	0,082
		1064 NM	0,040
		DUAL NM	0,020
		CONTROL	0,020
Keratin	1544	980 NM	0,141
		1064 NM	0,067
		DUAL NM	0,033
		CONTROL	0,030
Proteoglycan	1643	980 NM	0,146
		1064 NM	0,072
		DUAL NM	0,038
		CONTROL	0,030
Poly-lysine	3278	980 NM	0,072
		1064 NM	0,035
		DUAL NM	0,029
		CONTROL	0,019

Figure 3.8 Comparison of absorption intensities of FTIR measurements of treatment groups and control group on DAY-14. Control group was the healthy rat skin that was undergone no incision and no treatment.

Table 3.8 Absorption intensities of FTIR measurements of treatment groups and control group on DAY- 14.

Protein	Cm ⁻¹	Control Days	Absorption
Collagen	1246	980 NM	0,010
		1064 NM	0,096
		DUAL NM	0,054
		CONTROL	0,008
Keratin	1544	980 NM	0,010
		1064 NM	0,126
		DUAL NM	0,073
		CONTROL	0,007
Proteoglycan	1643	980 NM	0,010
		1064 NM	0,142
		DUAL NM	0,032
		CONTROL	0,008
Poly-lysine	3278	980 NM	0,004
		1064 NM	0,071
		DUAL NM	0,032
		CONTROL	0,004

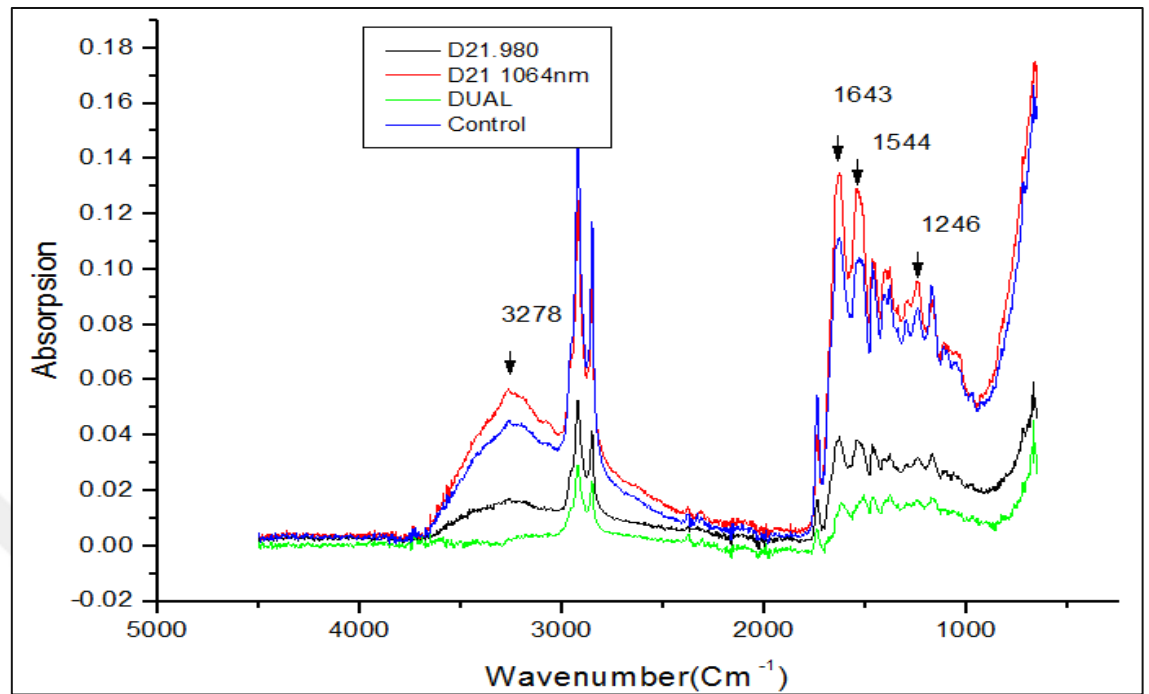


Figure 3.9 Comparison of absorption intensities of FTIR measurements of treatment groups and control group on DAY-21. Control group was the healthy rat skin that was undergone no incision and no treatment.

Table 3.9 Absorption intensities of FTIR measurements of treatment groups and control group on DAY-21.

Protein	Cm ⁻¹	Control Days	Absorption
Collagen	1246	980 NM	0,031
		1064 NM	0,095
		DUAL NM	0,017
		CONTROL	0,085
Keratin	1544	980 NM	0,039
		1064 NM	0,127
		DUAL NM	0,018
		CONTROL	0,101
Proteoglycan	1643	980 NM	0,039
		1064 NM	0,134
		DUAL NM	0,015
		CONTROL	0,111
Poly-lysine	3278	980 NM	0,017
		1064 NM	0,056
		DUAL NM	0,002
		CONTROL	0,043

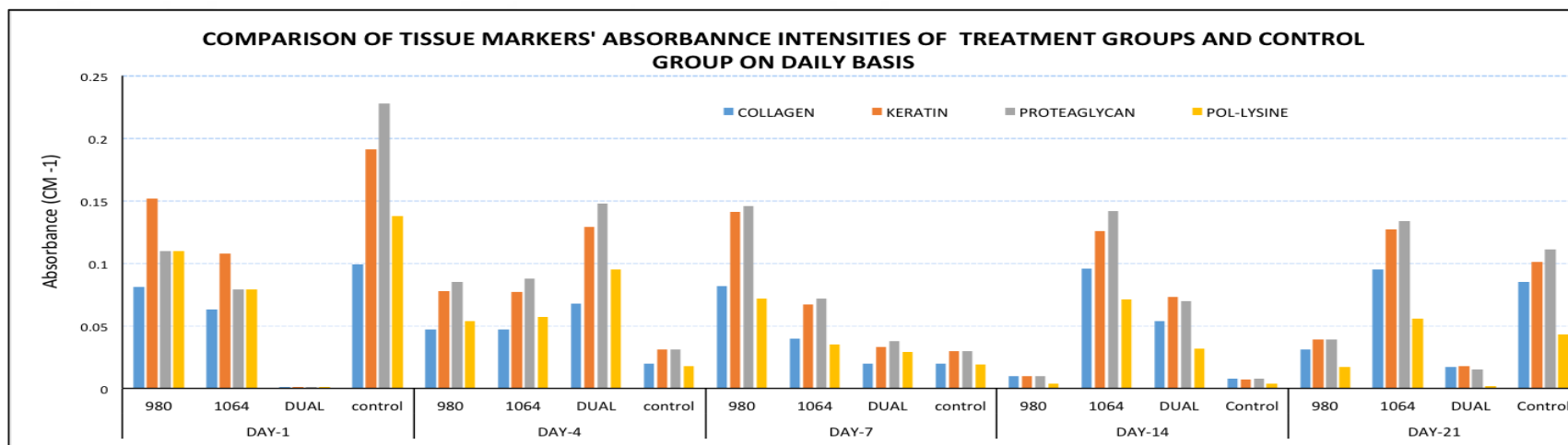


Figure 3.10: Day-by-day comparison of FT-IR spectroscopy absorption intensities for treatment groups and control group. This figure is another type of representation of Figure 3.4.

Table 3.10 Daily FTIR Spectroscopy absorption intensity for treatment groups and control group.

TISSUE MARKERS	DAY-1				DAY-4				DAY-7				DAY-14				DAY-21			
	980	1064	DUAL	Control	980	1064	DUAL	Control	980	1064	DUAL	Control	980	1064	DUAL	Control	980	1064	DUAL	Control
COLLAGEN	0,08	0,06	0,001	0,099	0,047	0,05	0,068	0,02	0,08	0,04	0,02	0,02	0,01	0,096	0,05	0,008	0,031	0,095	0,017	0,085
KERATIN	0,15	0,11	0,001	0,019	0,078	0,08	0,129	0,031	0,14	0,067	0,033	0,03	0,01	0,126	0,07	0,007	0,039	0,127	0,018	0,101
PROTEAGLYCAN	0,11	0,08	0,001	0,228	0,085	0,09	0,148	0,031	0,15	0,072	0,038	0,03	0,01	0,142	0,07	0,008	0,039	0,134	0,015	0,111
POLY-LYSINE	0,11	0,08	0,001	0,138	0,054	0,06	0,095	0,018	0,07	0,035	0,029	0,019	0,004	0,071	0,03	0,004	0,017	0,056	0,002	0,043

Daily comparisons have been performed in order to see differences clearly among the treatment groups and differences between treatment groups and control group on that specific date. Detailed daily analyses are given below:

DAY-1: 980 nm welded skin tissue markers' intensities was slightly higher than that of 1064 nm welded skin. Among the treatment groups' the minimum intensities were observed for DUAL treatment, as well as compared to control, they were also lower.

DAY-4: Similar absorbance values were observed for the 980 nm and 1064 nm treatments. DUAL treatment intensities increased and reached the maximum values for 21-day healing period. Minimum values were observed for the control group compared to treatment groups.

DAY-7: Maximum absorbance intensities for tissue markers were observed for 980 nm treatment compared to other treatment groups and control group. Intensities for Dual treatment and control group were almost same. Marker intensities for 1064 nm treatment were higher than control and dual treatment groups.

DAY-14: Absorbance intensities for 980 nm and control group were almost same. Markers for 1064 nm treatment have the maximum values among all groups. Intensities for dual treatment were higher than that of control and 980 nm treatment groups.

DAY-21: 1064 nm and control group has the higher intensities compared to 980 nm and dual treatments.

This representation (Figure 3.10) gives a rough estimation about the healing process of treatment groups.

In order to better understand the period, it is appropriate to take ratio of treatment groups over control group for each specific day (Figure 3.11).

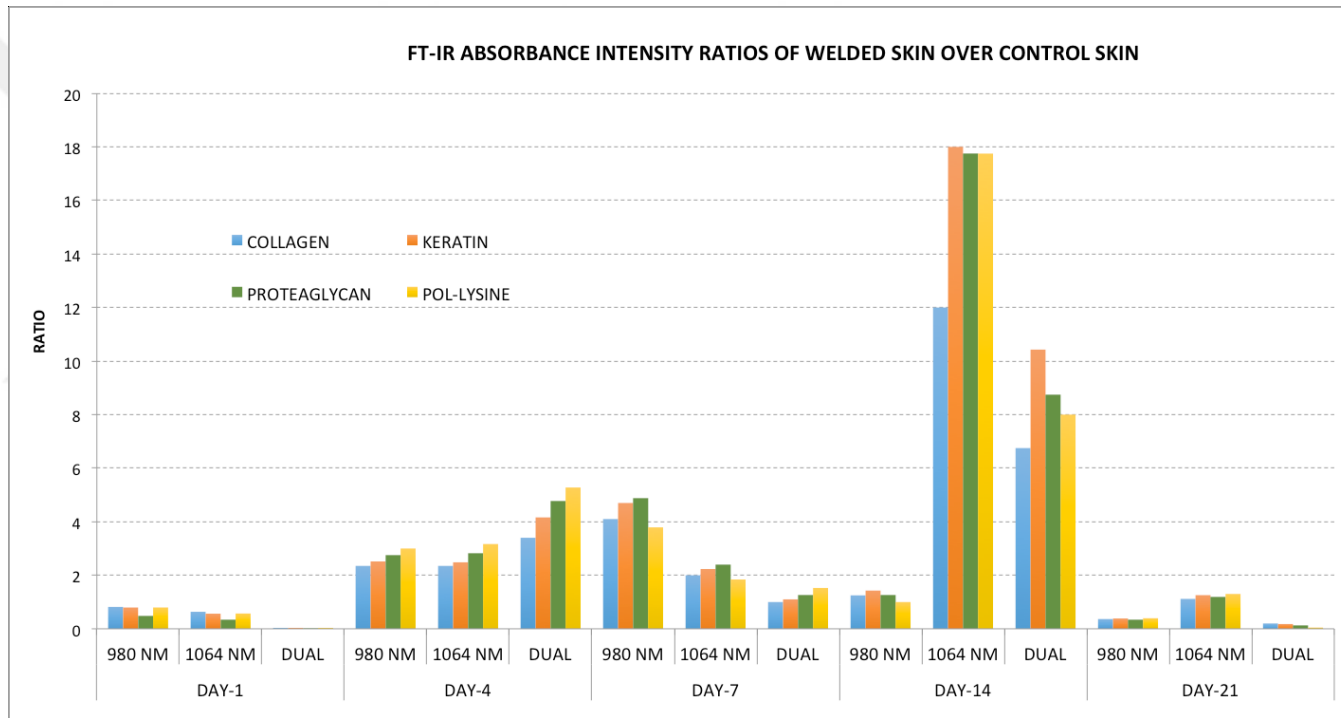


Figure 3.11: Intensity ratios of important tissue markers of treatment groups over control groups was calculated and represented in this figure.

Table 3.11: Intensity ratio values of important tissue markers of treatment groups over control groups

Tissue Markers	DAY-1			DAY-4			DAY-7			DAY-14			DAY-21		
	980 NM	1064 NM	DUAL	980 NM	1064 NM	DUAL	980 NM	1064 NM	DUAL	980 NM	1064 NM	DUAL	980 NM	1064 NM	DUAL
COLLAGEN	0,81	0,63	0,01	2,35	2,35	3,4	4,1	2	1	1,25	12	6,75	0,36	1,11	0,2
KERATIN	0,79	0,56	0,005	2,51	2,48	4,16	4,7	2,23	1,1	1,44	18	10,43	0,38	1,25	0,17
PROTEAGLYCAN	0,48	0,34	0,004	2,74	2,83	4,77	4,86	2,4	1,26	1,25	17,75	8,75	0,35	1,20	0,13
POLY-LYSINE	0,79	0,57	0,007	3	3,16	5,27	3,78	1,84	1,57	1	17,75	8	0,39	1,30	0,04

Absorbance value of treated skin / absorbance value of normal (healthy) skin was calculated for each specific day and represented in Figure 3.11. Daily analysis is given below.

DAY-1: 980 nm and 1064 nm treatment group intensities were found to be quite similar. Healthy skin has higher content of tissue markers compared to all treatment groups on Day-1. Tissue markers for dual treatment were almost close to zero intensity. This means that there was no cell synthesis of these markers.

DAY-4: Tissue marker synthesis was doubled compared to normal skin. Healing has already started. 980 nm and 1064 nm follow the same pattern; DUAL treatment was higher than that of other treatment groups.

DAY-7: 1064 nm and DUAL treatment markers have started to decrease while 980 nm treatment markers was increased compared to DAY-4.

DAY-14: Considerably higher tissue marker synthesis was observed for 1064 nm and DUAL treatment groups for all specific healing days. This could be the consequence of that deeper tissue penetration of these two laser wavelength might better stimulate the synthesis of corresponding tissue markers. This could be also related with the photothermal tissue damage given during the lasers application.

DAY-21: Ratios were observed to decrease back to their normal values. Especially 1064 nm treatment values were very similar with control skin.

3.2 CONCLUSION

Laser tissue welding showed to be a very promising method of wound closure, especially when scar formation is undesirable. The intensity absorption of the 1247 cm⁻¹ band to the 3278 cm⁻¹ band showed to be a good marker for collagen, keratin, proteoglycan and polylysine deposition and for measuring the extent of the healing process. The ratio of the treatments showed that, the 980 nm laser treatment has the regular 21-day healing pattern while 1064 nm and DUAL treatment have abnormalities on DAY-14. For the first week of healing period, DUAL treatment showed better healing compared to other treatment methods.

For the future study, histological examination of the treatment groups could be performed in order to match the healing period sections with FT-IR results.

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CURRICULUM VITAE

PERSONAL SUMMARY

Possessing a comprehensive knowledge of Biomedical Engineering projects to required specifications, focusing on business, safety, reliability, quality and sustainability. Also seeking an academic career that will lead me towards the pursuit of knowledge to the highest possible level,

PERSONAL INFORMATION

Name: zhin tahseen salieh

Date of birth: 2.8.1982

Nationality: IRAQ

Gender: Female

Email: zhin.salih@yahoo.com

Address: Arbil zaniary

ACADEMIC QUALIFICATIONS

Primary School:

BSc Genetics and Bioengineering:

MSc Biomedical Engineering:

WORK EXPERIENCE

Master's Thesis

Title: Molecular evaluation of skin after Laser Tissue Welding (LTW) using FT-IR Spectroscopy in vivo.

Supervisor:

Description: Laser Tissue Welding using 980nm and 1064nm laser, to evaluate collagen deposition and thermal effect on the skin using FT-IR spectroscopy.

KEY SKILLS AND COMPETENCIES

- Able to explain technical ideas clearly.
- Experience of preparing projects reports.
- Knowledge of Microsoft word
- Excellent working with a computer windows/OS X.
- Knowledge of Raman and FT-IR spectroscopy.
- Self-motivated with a positive and friendly attitude.
- Providing expert technical advice.
- Giving PowerPoint presentations.
- Management skills

LANGUAGES

- Arabic
- English
- Kurdish

HOBBIES AND INTEREST