An *in vivo* study of the effect of an extended single exposure of Helium-Neon (632.8 nm) laser on collagen concentration in healing incisional wounds.

by

Ian William Ross, B.Sc., D.D.S.

Division of Oral and Maxillofacial Surgery
Faculty of Dentistry
McGill University
Montreal, Quebec

June 1990

A thesis submitted to the
Faculty of Graduate Studies and Research
in partial fulfillment of the degree of
Master of Science

Table of Contents

Acknowledgements

I would like to express my deepest gratitude to Dr. T.W. Head for his guidance and the patience he afforded me during the processes involved in the completion of this thesis.

I would like to thank Dr. M. Lalli for providing the laboratory facilities, and Mr. J. Khan for his assistance during the animal experimentation. In addition, I would like to thank Dr. K.C. Bentley and the Department of Oral and Maxillofacial Surgery for the use of the Soft Laser 632.

My sincerest appreciation is extended to the Shriner's Hospital and, in particular, Dr. P. Roughley and Mr. E. Wan for the analysis of the tissue samples. For guidance in statistical analysis of the experimental data I would like to thank Dr. M. Abrahamowicz. I would like to to also thank Miss Fiona Lees for typing this thesis, her ability to decipher illegible handwriting is nothing short of miraculous.

A special thank you goes to my wife Nadine, my daughters Elyse and Margaux, and the children's nanny, Mrs. Louise Hill, for their support, understanding and sacrifices of their time that allowed for the completion of this manuscript.

This thesis is dedicated to my mother, Mrs. Alena Ross, whose battle with Multiple Sclerosis has been a source of inspiration and strength not only during the preparation of this thesis but in life in general.

Abstract

The effect of a single exposure of He-Ne laser on collagen content was studied *in vivo* using laboratory animals. A standardized 1.5 cm full thickness skin incision was made on the dorsum of 24 Sprague-Dawley rats. The rats were divided into six equal size groups, three groups functioning as controls and three groups functioning as experimental. The experimental animals were irradiated with He-Ne laser four days post-incision for 16 minutes to deliver a total dose of 5.99 J/cm^2. On days 6, 10 and 14 post-incision, control and experimental animals had the healing wounds excised for analysis of collagen content by assaying spectrophotometrically the hydroxyproline content within the tissue samples.

The results of the hydroxyproline content were analyzed using independent-groups t-test and analysis of variance. It was demonstrated that even though the mean values of hydroxyproline within the experimental groups were higher than the respective control groups, no statistical significance could be attributed to this observation.

Resumé

L'effet d'un simple exposition du laser de type Hélium-Néon sur le contenu de collagène à été étudié *in vivo* en utilisant des animaux de laboratoire. Une incision standardisée de l'ordre de 1.5 cm d'épaisseur a eté pratiquée sur la surface dorsale de vingt-quatre rats de type Sprague-Dawley. Les rats furent répartis en six groupes de taille égale, trois d'entres eux servant de groupes de contrôles et les trois restants de groupes expérimentaux. Les animaux expérimentaux furent irradiés avec le laser Hélium-Néon quatre jours suivant l'incision pour une durée de 16 minutes, livrant ainsi une dose totale de 5.99 J/cm^2. Au cours des journées 6, 10 et 14 suivant l'incision, les animaux des groupes contrôles et expérimentaux ont subit une biopsie au site de l'incision afin de déterminer le contenu de collagène et de quantifier spectrophotométriquement le contenu de l'hydroxyproline à l'intérieur des échantillons de tissu.

Les résultats du contenu de l'hydroxyproline furent analysés en utilisant des groupes indépendants test-t et des analyses de variance. Il a eté démontré que même si les valeurs moyennes de l'hydroxyproline parmi les groupes expérimentaux étaient plus élevées que celles des groupes respectifs de contrôle, aucune statistique signifiante peut être attribuée à cette observation.

Introduction

Low energy laser (He-Ne 632.8 nm) has been demonstrated to have many biostimulative effects. To the surgeon the effects of greatest interest are those which demonstrate beneficial properties to the wound healing process. Some of these include: increased rate of migration of fibroblasts[12], increased wound strength[25,28,56,57] and increased collagen synthesis.[1-2,31,36]

It has been demonstrated in animals that single[50] and multiple exposures[25,31,57] of He-Ne laser can increase wound strength during the first few weeks of healing. With time the non-irradiated wounds will ultimately equal those which received laser exposures without the advantage of early increases in tensile strength.

The formation, deposition and organization of collagen fibers are key factors in wound repair. Some authors have suggested that increased wound strength may be related to increased collagen synthesis in wounds exposed to He-Ne laser.[50] Recently, it has been demonstrated that wound strength increases occur before elevated levels of collagen content are appreciated within the wound following repeated He-Ne laser exposure.[31] This suggests that some other process may be occurring to account for the increases in wound strength.

In vitro and *in vivo* studies have demonstrated that multiple exposures of He-Ne laser can cause increased collagen synthesis.[1-3,31] For this to be clinically

practical, it would be advantageous if the same effect could be demonstrated with a single exposure of He-Ne laser. To date, no study has investigated the effect of an extended single exposure of He-Ne laser on collagen content within a healing incision. The purpose of this study was to determine the collagen content of primarily closed, full thickness skin incisions in rats which had been treated with an extended single dose of He-Ne laser.

Literature Review

Lasers

The word "laser" is an acronym standing for "light amplification by stimulated emission of radiation". In order to appreciate fully lasers and their biostimulatory properties, an understanding of the mechanics of electromagnetic radiation is necessary.

The electromagnetic spectrum consists of a wide range of radiation traveling at the speed of light and characterized by its wavelength.[33] The waves comprising the spectrum are oscillating sinusoidal propagations of electric and magnetic energy and its wavelength is defined as the distance between two successive peaks on the wave form.[8] The frequency of oscillation as determined by the wavelength is the only difference between the shortest and longest waves in the spectrum.[8] The shortest waves are the gamma waves while the longest are the radiowaves. Electromagnetic radiation within the visible light region has an accepted unit of wavelength, the nanometer (10^{-9} meter).[8]

Electromagnetic radiation possesses not only the property of waves but, as well, that of particles. The radiation is due to the transitions within the electron configuration of the atoms.[8] Light is a continuous stream of distinct parcels of energy "photons" which are emitted by atoms as they drop from an excited state (E_1) to its normal energy level (E_0). The energy of each photon can be expressed by Planck's relationship. This is represented by

$E = hc/\lambda$, where h is Planck's constant (6.63×10^{-34} joules [j] per second), c is the speed of light (3×10^{10} cm^2 per second), and λ is the wavelength.[33]

The electrons of an atom can exist at a limited number of energy levels. When the atom is stimulated, an outer shell electron usually moves to an excited state (E_1), from its normal ground state (E_0). The more energy absorbed by the atoms in the medium the greater the number of electrons in E_1. The time period in E_1 is short, usually 10^{-9} seconds or less, and is defined as the flucrescent lifetime. When the majority of the atoms in the medium are in the excited state, this is referred to as a population inversion.[8] Certain atoms have fluorescent lifetimes when in the excited state that are relatively longer. These are known as metastable states. Only atoms possessing metastable states are useful as active media in lasers. As the electrons fall from a metastable state to its next lower energy level, a packet of energy, a photon is emitted. The energy of the photon equals the energy difference between the metastable state and the next lower energy levels (E_1-E_0). The wavelength of the photon can be determined by Planck's relationship $\lambda = hc/E_1 - E_0$. The drop in energy levels is called radiative transition and the emission of photons by the means of spontaneous emissions or fluorescence However, in most atoms, electrons at higher energy levels return to their ground state via several competing pathways. These pathways may have several photon emitting transitions;

therefore photons of different energy, and thus different wavelengths, could be produced by a single type of excited atom returning to ground state.[8]

In addition to spontaneous emission there exists stimulated emission. Preceding stimulated emission, a process known as stimulated absorption occurs whereby an atom absorbs a photon with energy equal to that amount necessary to excite an electron from E_0 to E_1. The photons which cause this can be from either an electric current or from spontaneous emission.[33] Now, if the atom which has undergone stimulated absorption, collides with a photon which has energy equal to the difference between the electron's excited and ground states, a return to the ground state can be achieved with the release of a photon. The raw photon released has the same wavelength, phase and direction as the photon which caused its release. Therefore a single photon can cause a chain reaction, resulting in the emission of photons of identical characteristics.[23] This is defined as stimulated emission. In order for stimulated emission to occur a high degree of population inversion must be present.[8] The rationale for this is that when the upper energy level states are more populated than the lower energy level states, the transition probability for stimulated emission overcomes the probability for spontaneous emission.[8]

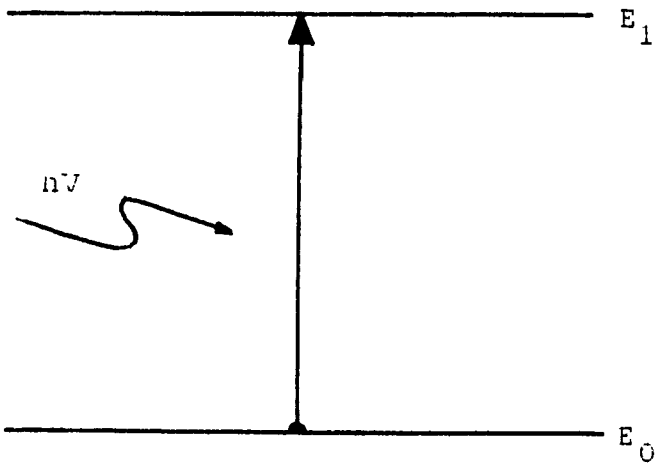
In order for population inversion to occur energy must be supplied to the active medium. This can be obtained by

introducing external energy sources to the system. The application of the energy is a process referred to as pumping. The energy can be derived from high intensity light. The process of optical pumping is very inefficient as only a small portion of light can be used to excite the atoms. Several other methods, including ionization and neutral atom discharge, use electric current to achieve population inversion. Energy from chemical reactions should theoretially also be able to create population inversions, but systems trying to use this method have not been successful to date.[9,12,50]

The processes of spontaneous emission, stimulated absorption and stimulated emission are shown in diagrams (Fig.1,2,3).[9]

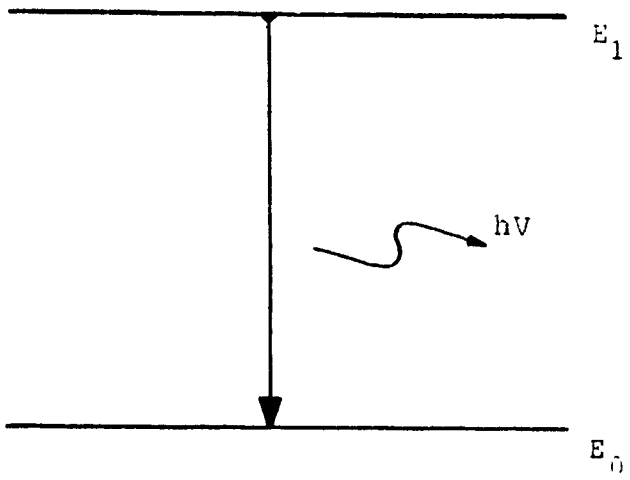
All lasers consist of three basic elements: a laser medium, be it solid, liquid or gas; a source of energy to excite the medium; and a system of mirrors to feedback light through the laser medium.[48] A resonance chamber, with a specific length to produce the desired wavelength laser will house these elements.[9,48]

In order for a laser light to be obtained, the medium must be able to achieve population inversion and a metastable state, as well as sufficient energy available to predictably achieve this.[8] Within the resonance cavity the mirrors are placed at either end. The distance between these mirrors is carefully determined and measured to produce resonance for the particular wavelength of the laser



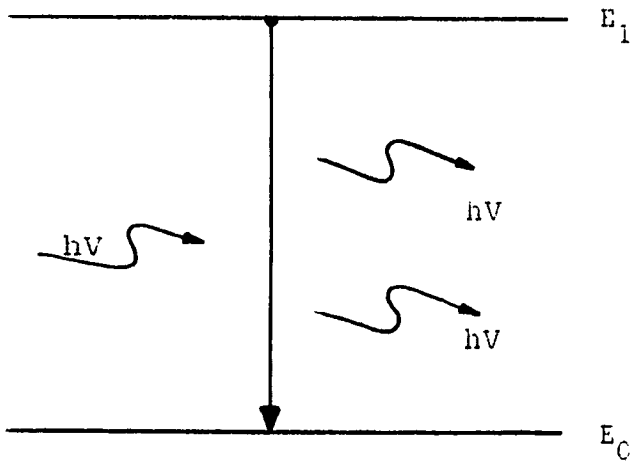
Absorption

Figure 1: Energy level diagram indicating absorption process. Atom originally in ground state E_0. Incident photon of energy $hV = E_1 - E_0$ impinges on atom. The photon energy is absorbed causing atom to raise to excited state E_1.



Spontaneous Emission

Figure 2: Energy level diagram indicating spontaneous emission process. Atom originally in excited state E_1. Due to normal short lifetime of excited state, atom spontaneously falls to ground state E_0. Energy is released of the magnitude $hV = E_1 - E_0$.



Stimulated Emission

Figure 3: Energy level diagram indicating stimulated emission process. Atom originally in excited state E_1. Incident photon of energy $hV = E_1 - E_0$ impinges on atom causing (stimulating) atom to fall to ground state E_0. As a consequence, two photons of identical coherence (phase) relationships are released.

medium.[32] Resonance is defined as the effect produced when the natural or inherent frequency of an oscillating system is greatly amplified by reinforcing vibrations at the same or nearly the same frequency.[15] The excited medium emits photons in random directions. Only those photons travelling parallel to the axis of the resonance chamber are reflected back along the same axis, thus passing repeatedly through the medium. Should one of the photons travelling down the axis of the resonance chamber collide with an excited atom, it will produce two photons which have the same wavelength, direction and phase. As these photons strike other excited atoms a cascade of photons are produced travelling along the axis of the resonance chamber.[12,50] The mirrors at either end of the chamber redirect the photons back along the axis amplifying the light wave and producing resonance.[33] The basic laser system is illustrated in Figure 4.[9]

Photons are emitted in random directions. Those photons which are not travelling parallel to the long axis of the resonance cavity will escape from it, whereas those travelling in a direction parallel to the laser's axis will become greatly amplified.[33] The amplification is achieved by the mirrors, which redirect the photons along the axis producing resonance. The mirror at one end of the resonance cavity is partially reflective, while the other is totally reflective. One to twenty percent of the photons travelling along the axis will escape through the partially reflective surface. This produces the laser beam.[4]

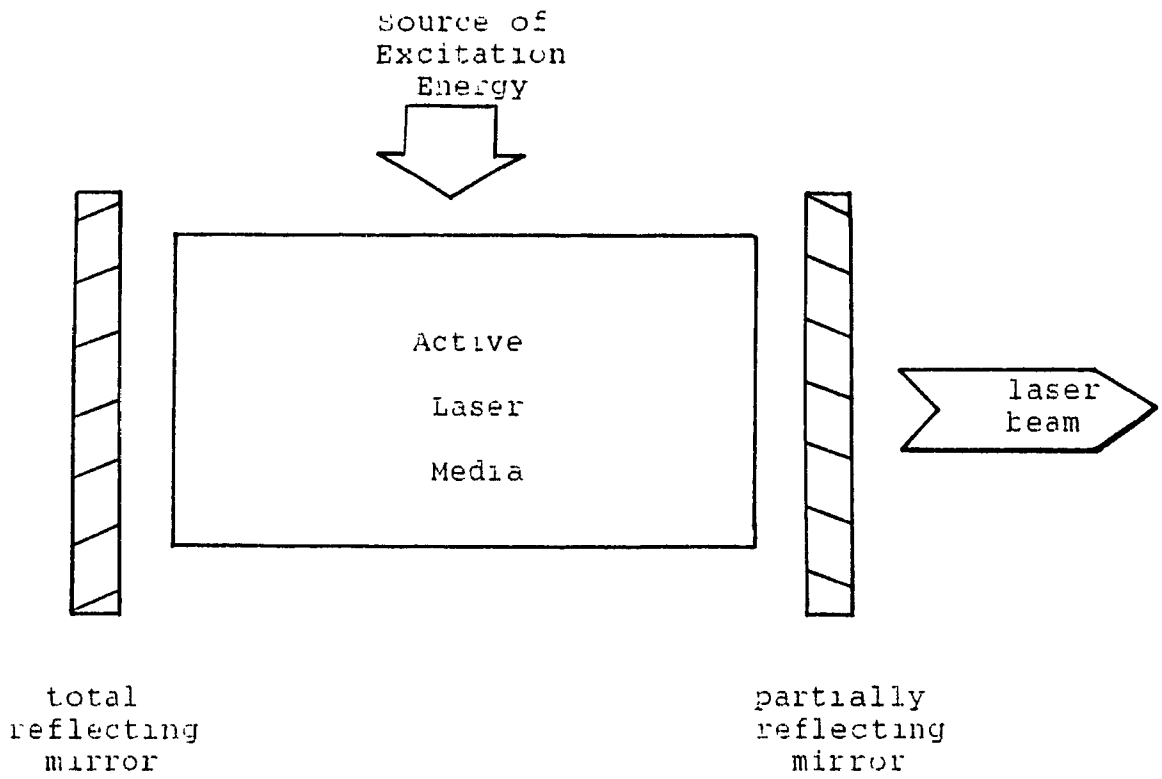


Figure 4: Basic laser system.

The laser beam emitted has the properties of monochromaticity and coherence.[33] Monochromaticity means light is formed by one wavelength. Since, when electrons fall from the metastable to ground state, several photon-releasing electron transitions are possible, the laser is not absolutely monochromatic.[33] The unwanted transitions can be eliminated by setting the length of the resonance cavity or if this doesn't achieve the desired result, a filter or prism can be used to suppress these wavelengths to produce a monochromatic beam.[48]

The coherence of the laser beam refers to the constancy or predictability of temporal and spatial variations in the wavelengths of radiation. Temporal coherence means that the wavelength of the laser will not change with time.[8,33] Spatial coherence means that if a plane were drawn perpendicular to the beam, a wave in the centre of the beam would be in the same phase as a wave at the periphery of the beam.[8,33] Figure 5 illustrates the concepts of temporal and spatial coherence.[9]

The coherence of a laser beam is not perfect, and spread of the beam occurs due to diffraction.[8,33] The temporal coherence is a measure of the length of time the beam emitted will remain monochromatic and is calculated by $T = 1/h\nu$. T is the coherence time and $h\nu$ is the frequency spectrum width. The distance travelled by the beam during the coherence time is referred to as coherence length. This is calculated by $L = c\,T$. As the coherent beam exits the

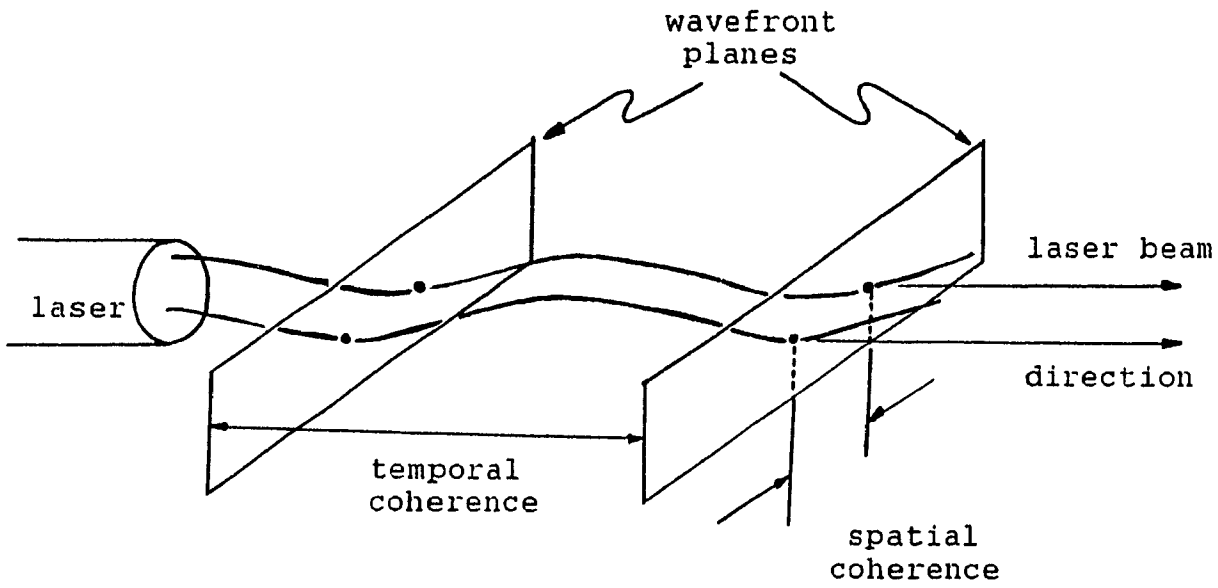


Figure 5: Simplified scheme of coherence factors of a laser beam. A constant time relationship between two wavefronts defines the temporal coherence and constant relationship across any wavefront defined the spatial coherence.

resonance cavity, the beam will immediately start to spread and, as previously mentioned, primarily by diffraction. Beams which spread only as a function of diffraction are known as diffraction limited beams.[8] The distance the beam will maintain parallelism is a measure of spatial coherence. This is calculated by the equation $L = D^2/2.44\lambda$, where D is the diameter of the beam as it exits the resonance cavity and λ is the wavelength of the beam.[23]

The power of a laser relates to the rate at which energy is produced. The energy of a laser relates to its ability to do work. These two properties are measured in units of watts and joules respectively, and one watt equals one joule per second. The intensity of the laser is related to the power of the laser beam and the beam's cross-sectional area. Intensity is expressed as watts/cm^2.[30]

The volume of the active laser material or the density within the laser medium is proportional to the energy output. Solid laser media are typically 3×10^{19} atoms/cm^3 and gas lasers 3×10^{16} to 10^{18}/cm^3. Due to the activation of atoms necessary to induce a laser beam, the initial output power is not proportional to the input. Once the threshold level achieved, a linear relationship exists between the two.

The duration of the beam is dependent upon the characteristics of the laser medium and the laser producing device. Lasers can be continuous wave, pulsed or Q-switched. Q-switch refers to a system where the quality

factor (Q factor) of the reflectance within the resonance chamber can be switched from low reflectance to high.[8] This prevents laser oscillations for a brief instant while in the low Q mode. During this period an overpopulation in the metastable state occurs, therefore storing an enormous potential power. When turned to high Q mode, stimulated emission will rapidly occur producing an intense beam.[8] Continuous wave is an uninterrupted beam, and pulsed beams are achieved by interrupting a continous wave such that a number of pulses are emitted per second.

Helium-Neon Lasers

Helium-Neon (He-Ne) laser is a gas laser system consisting of 85% helium and 15% neon. This combination will allow for stimulated emission to occur from over forty distinct transitions.[32] The original He-Ne lasers utilized a transition wavelength of 1152.3 nM which is within the infrared spectrum. Subsequently, another transition has been selected within the red visible light spectrum: 632.8 nM. This is the wavelength of He-Ne laser most frequently used today.[8]

The mechanism for He-Ne laser generation requires that, via a direct electric current, helium atoms are pumped from a ground state to excited state. The energy within the He atom population becomes coupled with the Ne atoms. This occurs when a metastable He atom collides with a ground state Ne atom, resulting in stimulated absorption.[33] This can be expressed by the equation $He(3s) + Ne \rightarrow Ne(2s) + He$.

Since direct stimulation of neon from ground to excited states is not easily possible, population inversion must occur between the neon and helium metastable states. The 3s metastable state is within 0.15 eV of the 2s metastable state of neon. The energy transitions between the 2s levels and the 2p levels of neon cause photon emissions. The most intense line of radiation being at 1152.3 nM.

Conversely, the 632.8 nM wavelength of laser is achieved by first exciting the helium atoms to a 2s metastable state, then transferring this energy to neon to have a population inversion to a 3s metastable state. The transition between 3s and 2p levels of neon will result in the generation of photons with a wavelength of 632.8 nM. This is represented by Figure 6.[12]

The coherence length of a He-Ne laser ranges from 1000 meters to 10^5 km. The coherence of the laser beam has been related by some authors to provide the biostimulative effect of He-Ne radiation.[30]

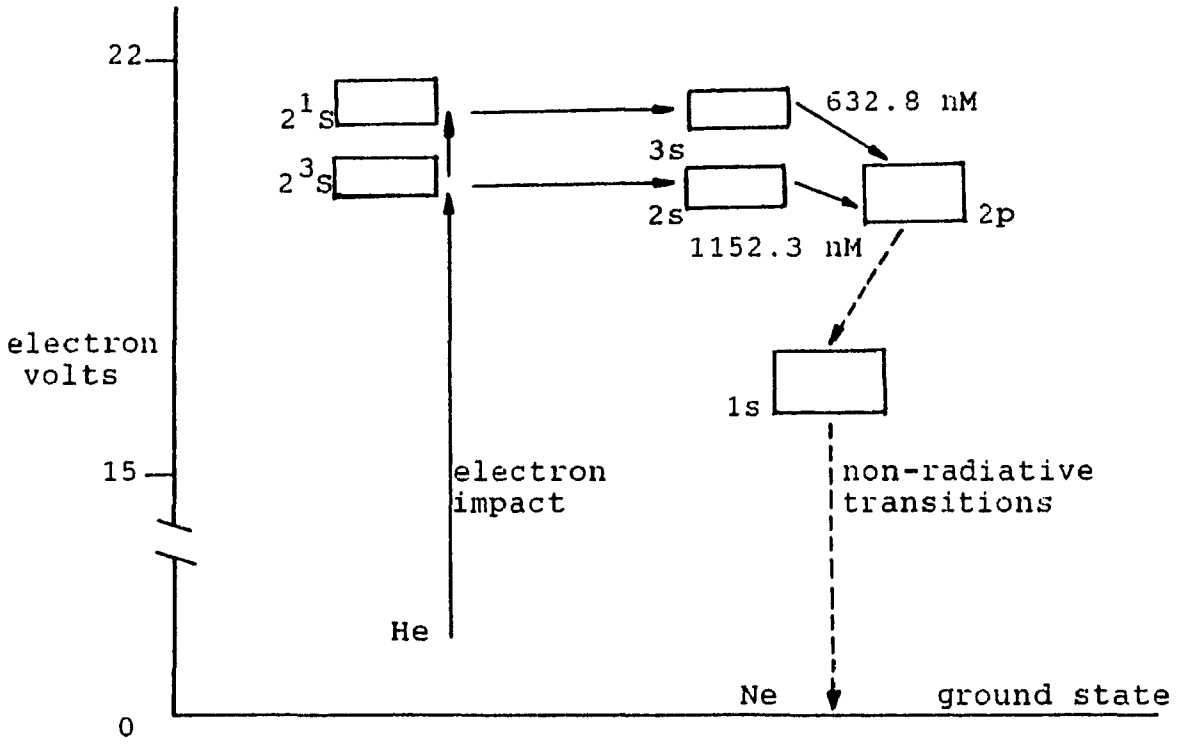


Figure 6: The energy schemes in helium and neon responsible for stimulated emission at 632.8 nM and 1152.3 nM.

Interaction of Laser on Living Systems

When laser is directed at tissues, biological changes occur These changes are related to primarily two basic forms of interactions with living matter: thermal reactions and photochemical reactions.[10,16]

Thermal reactions occur as a consequence of photons penetrating tissue and transferring their energy to the cell. The cells through multiple absorption processes transform the laser energy into heat. The degree to which this reaction occurs depends upon the type of laser, its energy and power output.[11] Some lasers, such as the CO_2 laser, have been developed with specific attention to the thermal reaction and in particular its ability to incise or cauterize tissues. The energy absorption of laser in the visible light range by tissues varies with the tissues intrinsic optical characteristics.[11] Tissues are not homogeneous structures. Thus, the varying optical densities encountered will have varying absorption of laser energy. The intensity of thermal reactions of lasers in tissues is dependent upon a number of variables:

1. the absorption, reflection, and transmission of the tissues at the laser wavelength;
2. the power density of the laser beam;
3. the speed of incision or time of exposure;
4. the volume and the flow velocity of local blood vessels;
5. the degree of tension at the area of incision when cutting is used.[11]

Dependent upon the thermal damage initiated by the laser exposure, one of three possible processes may occur. These are immediate death, delayed death and transient alteration of the functional state and repair. When the latter is encountered the repair can result in either full functional or partial recovery.[16] The for a particular tissue reacts to laser is also dependent on biological parameters as well. These include:

1. the degree of cell differentiation;
2. the mitotic rate;
3. the state of metabolism;
4. the temperature;
5. the reactions of surrounding cells.[16]

In general, the greater the degree of mitotic rate the less time is available for DNA repair which make these cells more sensitive to the thermal effects of laser. The cells with a greater degree of differentiation such as muscle cells or nerve cells tend towards a state of decreased functional competence after exposure. The cell biologists term this dedifferentiation. The higher the level of metabolic demands a cellular structure requires for maintenance of cellular integrity, th emore sensitive it will be to thermal effects. However, those cells that are in close approximation to blood vessels have a better chance of survival. This is related to greater access to substrates for cellular repair and better rates of elimination of toxic products.

When the intensity of a particular laser is below a level to induce thermal reactions, photochemical reactions may occur. Again, some laser of low power output have been specifically developed for this applications such as the 'soft' lasers He-Ne and Ga-Ar. These can induce a number of cellular reactions which will be dealt with in detail in subsequent sections of this thesis. In general, the biostimulative effects of laser on living systems have been attributed to the photochemical reactions which occur.

The basis of photochemical reactions is the transferance of photon energy to molecules within the tissue. This results in activation of molecules resulting in chemical reactions. The mechanisms by which the photochemical reactions induce changes is not yet understood in its detail.[28] The interaction of laser light with a living cell is illustrated in Figure 7.[16]

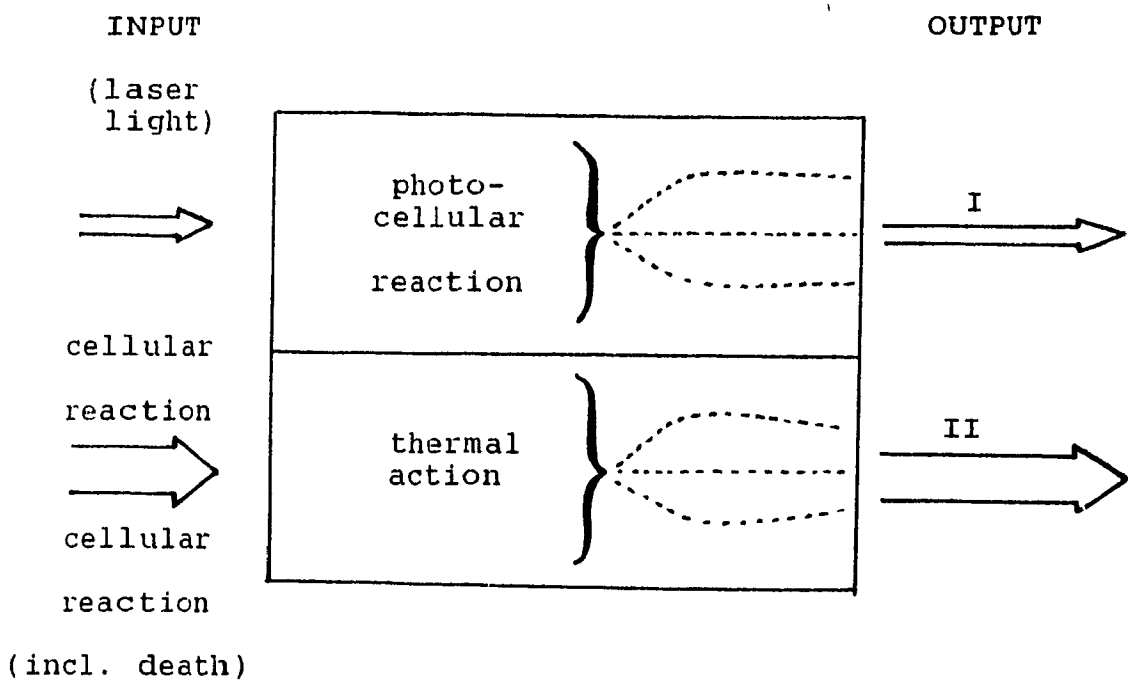


Figure 7: Black box model for the interaction of laser light with a living cell.

Wound Healing and the Production of Collagen

The healing or repair process of a wound begins as soon as tissue is traumatized. This occurs in essentially the same fashion be it the repair of a small surgically-induced incision or an injury creating a significant tissue defect. The trauma initiates a series of events within the tissue. Immediately following the injury, hemorrhage occurs filling the defect. Upon coagulation of the hemorrhage, a fibrin clot forms containing many of blood's cellular components. Blood vessels in the region thrombose, contract and ultimately disappear from the injury site. Subsequently, cells of various types invade the clot to revascularize, remove cellular debris and repair via synthesis, the connective tissue elements.[49] The repair process passes through three non-distinct phases termed inflammatory, proliferative and reorganization phases.[53]

The inflammatory phase occurs immediately within the small vessels in the region of the injury. A marked vasoconstriction occurs actually occluding the vessels in portions of the injured tissue. This response is, however, short, lasting approximately five to ten minutes. This is followed by a period of active vasodilation, where all elements of the local vasculature appear to be dilating.[49] The vasodilation appears to be initially a histamine-mediated event. The source of the histamine is primarily mast cells. Histamine is also found within human platelets; however, this low concentration is not believed to play a

significant role in this vasodilation process.[53] The action of histamine lasts for approximately thirty minutes. At this point the local sources of histamines are depleted and other permeability factors will maintain the vasodilatory response in the injured region.

The other permeability factors are prostaglandins, especially the prostaglandin E series, serotonin and kinins. Serotonin acts on the venules to promote swelling and rounding of endothelial cells in an action similar to histamine, resulting in separation of the endothelial cells. Kinins also act in a similar fashion, but because of its rapid destruction by plasma and tissue proteases, it appears unlikely it plays any role in the late vascular response. The prostaglandins appear to play the dominant role and are produced within the injured tissues. Prostaglandins have multiple effects but its role in the maintenance of the vascular response appears to be involved with the displacement of membrane bound Ca^{++} altering the membrane reactions and cellular permeability.[49]

Concurrently, intra-vascular events are occurring. Leukocytes and, to a lesser degree, erythrocytes and platelets begin to adhere to the endothelial lining. The process occurs predominantly in the venules. This acts to plug capillaries, but since platelet-fibrin thrombi do not form until later, this process can still be reversed.[49] In addition, changes are occurring within the endothelial lining of these vessels creating leakage of fluids and

macromolecules into the extravascular tissue. The exudate observed consists largely of polymorphonuclear leukocytes, macrophages, fibrin and free extracellular organelles from the disrupted inflammatory cells.[13,49,52,53] The fluid leakage occurs first. This is plasma and is not initially associated with movement of the intravascular cells into the surrounding tissue. Electron microscopy has demonstrated physical separation of the endothelial lining cells exposing the underlying basement membrane to the luminal contents. The leukocytes which have become "sticky" and adherent to the endothelial lining now, by a process of diapedesis force their way through the basement membrane. This phenomenon involves active motion, and is not fully understood.[49] The holes created by the process now allow the passive movement of erythrocytes through the basement membrane. As the inflammatory exudate matures, polymorphonuclear leukocytes die and are lysed, the exudate assumes the character of pus.[49]

These initial changes are referred to as an acute inflammatory process and lasts for approximately three days. After this point the inflammatory process becomes more chronic in nature. This is marked by a change in the predominant cells from granulocytes to mononuclear cells or monocytes. These cells differentiate into macrophages and become actively phagocytic.[17,49] Their role is to function as scavengers of those materials within the injured tissue that are not readily solubilized by enzymes released by

granulocytes. Some of the monocytes coalesce and become multinucleated giant cells whereas others will differentiate into histiocytes, and in some instances epithelioid cells.[49]

Shortly after the injury to the tissue is sustained, undifferentiated mesenchymal cells peripheral to the injured site differentiate into migratory fibroblasts.[49,52,54] This marks the beginning of the proliferative phase of reparative process. These fibroblasts move into the injured site as the macrophages remove the necrotic tissue and foreign bodies. The fibroblasts require the presence of macrophages to attract them to the injury site and promote their maturation and maximal collagen synthesis.[6,49]

Fibrinogen is found in large concentrations in inflammatory exudate and is converted enzymatically into fibrin which is laid down as a network in the injured site. This network acts as a scaffold for the migrating fibroblasts. Although fibrin acts as a scaffold for fibroblast migration. the large quantities found in injury sites can inhibit migration not only of fibroblasts but of epithelial cells as well. The migrating fibroblsts are closely followed by new capillary in-growth. The endothelial cells of the capillaries contain plasminogen activators which cause fibrinolysis of the fibrin network. This process occurs coincidentally with collagen deposition. In uncomplicated simple wounds, debris is usually removed by the third to fifth day and by that time fibroblasts and capillaries are invading the entire wound area.[49] Capillary

proliferation continues until about the eighth day post-injury. The number of fibroblasts and capillaries in the wound begins to decrease after this time until a steady state of cell numbers is reached at about the fourteenth day.[53]

Fibroblasts not only have migrated but have also proliferated. Initially, they manufacture and secrete elements of ground substance including protein-polysaccharides and various glycoproteins. At approximately the fourth or fifth day post-wounding, collagen synthesis commences and the proliferative stage begins.[49]

During this proliferative phase, the fibroblast cell population doesn't change appreciably. Collagen, the primary synthetic product, is laid down at a rapid rate and epithelium moves across the surface of the granulating wound. The proliferating epithelial cells secrete collagenase which is important in controlling the collagen content of the wound.[49] As fibroblasts come in contact with the epithelium cells, they too become induced to synthesize collagenase. This marks the beginning of the remodelling phase.

The proliferative phase lasts approximately two to four weeks. This depends upon the size and location of the wound. As this phase terminates, the new capillaries which had proliferated into the wound start to regress and diminish in numbers from the wound site. The glycoprotein and mucopolysaccharide content decreases concurrently as

does the number of synthetically active fibroblasts. Eventually the collagen synthesis decreases to a point to balance the rate of collagen destruction.[49,53] Once this is achieved the reorganization or maturation phases is then fully underway.

During the maturation of the scar tissue, new collagen fibers are being laid down and others are being digested and removed. Ultimately, the fibers which remain in the scar are those oriented along the lines of tension. Some interweaving of fibers takes place which greatly enhances the tensile strength of the wound edge. The arrangement of the fibers within the dermis of an injured site is quite different from the surrounding uninjured dermis. With age the scar remodels. Fibers and fiber bundles become more condensed and packed, which aids in positioning individual collagen polymer chains so that intermolecular covalent bonding and cross-linking can take place. This is achieved by the loss of water and mucopolysaccharides from the wound. The cross-linking and interweaving of collagen fibers make the scar resistant to stretch. This is further demonstrated in wound strength studies of ruptures of incision lines where it has been demonstrated that five days post-incision, rupture occurs precisely at the location of the original incision. At days 14 to 21 rupture almost always occurs lateral to the line of incision, perhaps 2 to 3 mm on either side of the central scar in a line differentiating the wound from normal tissue.[49]

The type of collagen laid down will also vary during the healing process. Initially, the collagen within the scar resembles type III or embryonic collagen with large concentrations of hydroxylysine. During the maturation process the Type III collagen is replaced by Type I collagen demonstrated by an increased concentration dihydroxylysino-norleucine cross-linking.[49] This suggests that when a fibroblast is first activated, by modulation from undifferentiated mesenchymal cells, the genes responsible for Type III collagen synthesis are activated.[49] As maturation occurs this gene is suppressed and activation of the gene responsible for Type I collagen occurs. Failure of this suppression of genes responsible for Type III collagen can result in hypertrophic scar formation.[39]

In summary, the healing of the normal incisional wound is divided into three phases: inflammatory, proliferative and maturation. The inflammatory reaction prepares the wound for subsequent healing by removal of bacteria, cellular debris and necrotic tissue. Concurrent mobilization of fibroblasts, epithelial cells and accumulation of proteins and glycoproteins occur. This leads to the formation of fibrin and a framework for further fibroblast migration. During the proliferative phase, which begins at about day six, collagen synthesis and the deposition of mucopolysaccharides and glycoproteins is initiated. The collagen is first secreted in monomeric form but subsequently is formed into fibers. At this point a

rapid increase in wound strength is exhibited. At approximately day fifteen, increases in collagen concentration in the wound ceases. However, a steady gain in tensile strength is noted. This is due to two factors: one, intramolecular and intermolecular cross-linking of collagen fibers, and two, remodelling by dissolution and reformation of collagen fibers to give a stronger more efficient weave.[49] There is no sharp demarcation between the end of the proliferative phase and the beginning of maturation. In actuality a significant amount of overlap is present.[49] The maturation process may require six months to a year to complete.

In order to appreciate the means by which laser can effect collagen synthesis, one has to understand the mechanism by which collagen synthesis occurs. At the time of injury, it is thought that a metabolite or a diffusable extracellular material blocks a normally present repressor substance within the fibroblast.[49] This allows for the synthesis, within the nucleus, of messenger ribonucleic acid (mRNA) from a deoxyribonucleic acid molecule (DNA) template. The DNA template provides a nucleotide sequence to the mRNA to act as a blue-print for the synthesis of a particular type of protein, in this case collagen being the end product. The template is comprised of a particular sequence of purine and pyrimidine nucleotides which will specify the amino acid sequence of the collagen. The mRNA within the cytoplasm becomes associated with a ribosome on the surface

of the rough endoplasmic reticulum. This ribosome also contains protein and an RNA. It is here that the mRNA nucleotide sequence is translated into an amino acid sequence by selecting a specific transfer RNA (tRNA) with its intrinsic amino acid sequence corresponding to the sequence of nucleotides in the mRNA. The tRNA contains the nucleotide sequences specifying one particular amino acid. The synthesis of the polypeptide chain (pre-procollagen) is initiated at the amino terminal end and proceeds in a stepwise fashion toward the carboxyl terminal end. Once the terminal nucleotide sequence of the mRNA has been translated, the desired polypeptide chain is detached rom the ribosome into the cisternae of the rough endoplasmic reticulum and then from here tc the Golgi apparatus for glycosylation of the hydroxylysine residues. These polypeptide chains are the precursor of collagen, procollagen and specifically alpha chains. The transfer of procollagen from rER to Golgi apparatus is via small transfer vesicles which bud off the rER cisterna and fuse to the golgi apparatus. Within the Golgi apparatus the flexible alpha chains are unraveled and are arranged into triple helices characteristic of procollagen.[14,60] These rigid parallel threads aggregate into relatively thick rods. As the rod is brought to the surface by the Golgi apparatus, the amino-terminal and caroxyl-terminal propeptides to each alpha chain are cleaved off enzymatically. This is performed by extracellular proteases and the procollagen is

converted to tropocollagen which will spontaneously be arranged into collagen fibrils.[34,46] Thus, procollagen is secreted onto the surface of the fibroblast where the procollagen molecules will be trimmed down to size, becoming tropocollagen molecules that will in turn be arranged into collagen fibers.[14,46,60]

Research into the Biostimulative Effects of Low Energy Laser Radiation

The majority of the initial research into the biostimulative effects of low energy laser radiation (soft laser) had been developed by the Eastern Bloc countries, in particular the Soviet Union and Hungary. Mester's work in the late 60's initiated investigations into the biostimulative effects of soft laser. Subsequently, a large body of literature has been written in a wide range of specialty pursuits: dermatology, orthopedics and stomatology. In the last decade researchers in Europe, the United States and Japan have started investigating biomedical applications of soft laser.

Mester's work spanned the time of the late sixties to his death in 1984. During this period he reported extensively on the biostimulative effects of soft laser sources. His initial investigations were using ruby laser 694.3 nm.[38,39] In his first reported experiment he investigated the effect of different amounts of exposure of laser on leukocytes ability to phagocytose bacteria.[39] One group exposed to 4 J/cm^2 showed significant impairment of this ability to phagocytose when compared to the control; whereas the second group exposed to .05 J/cm^2 showed a significant increase in phagocytosis when compared to the control. A second experiment investigated the effects of low energy laser on Ehrlich ascites tumor cells *in vitro* when injected into mice.[38] He radiated Ehrlich ascites

tumor cells with .2-1.0 J/cm^2 for 5 milliseconds prior to injection into five mice. A second set of five mice acted as the control and were injected with non-irradiated Ehrlich ascite tumor cells. At 15 days post-injection the radiated tumor cell group had significant weight gains as well as increase in the volume of ascites and number of tumor cells when compared to the control group. From this he concluded that low dose laser radiation stimulates Ehrlich ascites tumor cells.

In another experiment Mester observed that low doses of laser would stimulate hair growth in depilated mice. If, however, these mice were then exposed to higher doses of lasers, the stimulatory effect on hair growth was lost.[38]

When investigating the biostimulative effect of ruby laser (694.3 nm) of varying intensities on third degree wound burns on mice, Mester observed a dose dependent phenomena. The animals had bilateral burns produced on their dorsums at kidney level and functioned as their own control. The mice were then divided into five groups of ten animals and radiated at one burn site with .5, 1.0, 4.0, 5.0 and 10.0 J/cm^2 respectively. The results showed a marked stimulation of healing occurring in the 1.0 J/cm^2 group. The remaining four groups showed either no noticeable effect or non-significant stimulation of healing.[43]

Later, Mester investigated the effect on collagen synthesis in response to low energy laser radiation. This he did by comparing the uptake of radioactive amino acids in

healing incisions in rats. A total dose of 4 J/cm^2 was administered to the surgical site. This was accomplished by the delivery of multiple flashes of radiation ranging from 1-4 J/cm^2 with flash duration of 100 microseconds (i.e. 2 x 2 J/cm^2, 4 x 1 J/cm^2). From this Mester observed a 30-50% increase in collagen production in the laser treated wounds when compared to the control. He hypothesized that this was due to either increased enzymatic activity or greater amounts of enzymes released by the organelles.[37]

Kovacs, a fellow Hungarian and co-worker of Mester, investigated vessel regrowth in rabbit ear chambers. After 18 days the ear chamber exhibited 57% of the surface area of the radiated chamber was revascularized whereas only 8% was noted in the non-radiated group. He theorized that the radiation may be directly activating injured vessels in the region or that macrophages were stimulated to phagocytose faster, thus clearing the way for new vessel ingrowth.[24]

Kovacs and Mester than looked into wound strength, once having demonstrated biostimulative effects on phagocytosis, vessel formation and collagen formation. Incisions 2.5 cm in length were made on the dorsum of 48 experimental and 48 control rats. The wounds were primarily closed and maintained by two skin clips. The experimental animals were radiated twice per day for three minutes using 5 mW He-Ne laser. The experimental and control animals were then sacrificed on days 3, 5, 8 and 12 in groups of 12. After the clips were removed, the tensile strength developed by

the wounds were investigated. Although unable to explain the effect they noted significant increases in breaking strength of the experimental groups sacrificed on the fifth, eighth and twelfth post-operative days.[25]

After exhaustive animal experimentation, human experimentation was initiated by Mester. The purpose of his investigation was to determine whether laser caused a systemic effect or not. It was observed that laser radiation of crural ulcers generated healing not only of the radiated ulcer but as well as non-radiated ulcers elsewhere on the patient. To investigate this, 20 patients received He-Ne laser of 50 mW to their crural ulcers twice a week with 1 J/cm^2. Blood was drawn prior to the first radiation and after each subsequent treatment assessing complement activity, serum protein quantity and quality and the presence of auto-antibodies. The results obtained showed the presence of no auto-antibodies, before or during the laser therapy. However, changes were observed in complement levels and immunoglobulin levels. These observed changes were different between cases which responded to radiation and those which did not. In the group responding to He-Ne, complement levels were normal. However, serum IgM rose significantly after five treatment and then over subsequent treatments fell to just above pre-treatment levels. Conversely, IgG levels fell after five treatments and then increased to above pre-treatment levels. Patients with

resistant ulcers displayed the opposite trend. It was concluded that the success of the laser therapy seemed to be related to normalizing the humoral immune response.[41] Mester followed this with more *in vitro* studies using various laser sources, Argon, Ruby and He-Ne, with a wide range of wavelengths and exposure energies on B and T lymphocytes. He observed direct laser radiation damage on both T and B lymphocytes in proportion to the energy applied to them. Energies greater than 2-5 J had a cell-destructive effect, whereas energies up to 1 J had different effects on different cells. The least destructive wavelengths to lymphocytes were between 488 and 501 nM, and all radiated lymphocytes demonstrated shorter life spans. From this he hypothesized that laser radiation caused suppression of some unknown but undesirable immunoreaction in immune competent cells resulting in stimulated healing.[41,42]

Later research by Mester investigated the effect of He-Ne laser on prostaglandin synthesis. After irradiating skin wounds in rats with 50 mW He-Ne at 1 J/cm^2 he observed elevated prostoglandin PGE_2 and PGF_2 levels in the experimental group four days post-irradiation. After eight days the PGF_2 levels were further elevated whereas the PGE_2 levels had started to decline and had returned to near normal values. He hypothesized that the increased levels of these prostaglandins may be related to the biostimulative effect of He-Ne laser.

Prior to his death, he designed a model to explain laser biostimulation on many different biological systems which is as follows:[36]

> "The electric field strength, of the linearly polarized light, changes the conformation of the lipid bilayer of the cell membrane. It reorders the polar heads of the lipids by electron polarization. As a consequence of this conformational change in the cell membrane, changes in lipid protein connections may occur. These changes in the conformation of the cell membrane may influence every process connected to the cell membrane. This includes energy production of the cell, immunological processes, activation energy of enzymes and active transport. The membrane plays the role of a biological amplifier."[36]

Subsequent experimentation by Kovacs and Kubasova attempted to validate Mester's hypothesis that a change in the lipid bilayer cell membrane was responsible for the biostimulative effect of soft laser radiation. Under the scanning electron microscope no micromorphological changes in the plasma membrane were noted in response to doses of 1 J/cm^2 or 5 J/cm^2 of He-Ne. Functionally, changes were observed. The longer the course of laser radiation, the decreased ability of the fibroblasts to bind lectin and the surface charge become more negative with repeated exposures. Therefore it appears that repeated laser radiation exposures caused functional not micromorphologic changes in the plasma membrane of fibroblasts.[26]

A review of the Soviet literature was written by Mikolai F. Gameleya[7] in Laser Applications in Medicine and Biology. In this a number of applications of soft laser are described. The topics were extensively covered including the following: aphthous ulcers, periodontitis, burns, indolent and trophic ulcers, hypertension, rheumatoid arthritis, endarteritis obliterans, basal cell carcinoma and precancerous skin changes, and bronchial asthma. Although the clinical applications of He-Ne laser were interesting and displayed promising results, the means by which the results were obtained were generally unscientific. Guidelines for dose intensity and duration of therapy are frequently not given, or when given, are confusing. These experiments can, however, under stricter scientific controls provide impetus for further research. When investigating chronic recurrent aphthous ulcers in 60 patients radiated with 20-25 mW He-Ne laser, patients reported after several therapeutic sessions an analgesic effect, and the aphthae apparently healed faster than usual by several days. On subsequent recall one year later, 11 patients had no recurrence and the remaining had less frequent and less severe recurrences than previously experienced. In the treatment of periodontal disease, significant improvements in patients radiated with He-Ne laser in conjunction with traditional treatment modalities (i.e. scaling and curretage) was demonstrated. It is difficult to assess the

validity of the results as no controls were used. As well, no power densities, duration and frequency of radiation were reported.[7]

Burns of varying depths were investigated in 36 patients. These patients were classified on the basis of their injuries into 12 superficial burns, 12 deep burns, 6 controls with superficial burns and 6 controls with deep burns. The burned areas of the experimental groups were irradiated on alternate days with He-Ne laser .1 mW/cm^2 for 5-10 seconds for a total of 10 sessions. They observed in the deep burn patients accelerated re-epithelization and granulation tissue formation after five sessions. Also observed in both experimental groups, were increases in total numbers of neutrophils present in the wound and the rate of phagocytosis was markedly enhanced.[7]

Similar findings were reported in studies which looked at chronic indolent ulcers, ulcers resulting from x-ray radiation, and infected ulcers. Overwhelming positive responses to He-Ne laser were encountered with epithelialization occurring in most cases with a positive side effect of pain relief. No adverse effects of He-Ne laser were observed on clotting system, blood cell counts or kidney function. One study reported erythrocyte sedimentation rate and leukocyte count decreases with erythrocyte increases. In addition, this study demonstrated that bleeding and clotting times decreased and all classes of immunoglobulins except IgM were increased. When

investigating the response of infected wounds to He-Ne laser, it was noted that increases in granulation tissue formation and epithelialization occurred. Also observed were decreases in edema, pus discharge, and fewer remaining bacterial species. In addition, increased numbers and activity of monocytes and macrophages were observed in cytological specimens.[7]

An area of investigation where, in general, limited success was observed the application of He-Ne laser to appropriate acupuncture points in the treatment of hypertension. Several studies were sited with varying degrees of success. In a single study reported on He-Ne laser therapy for bronchial asthma in 21 patients, favorable results were obtained. He-Ne laser of 25 mW was applied to several acupuncture points daily for 40-60 seconds for 10-20 sessions. Patients received 1-3 courses of treatment with intervening periods of 1-2 months. All the patients experienced favorable immediate results with 10-30% improvements in vital capacity and return to normal of inspiratory reserve volumes. No changes were encountered with the expiratory reserve volume and during their reported six month follow-up only two patients with severe bronchial asthma had exacerbations.[7]

When used to treat rheumatoid arthritis the two articles reported had positive results. They reported that nearly all patients had benefits of He-Ne laser applied to

the affected joints. These benefits included decreased discomfort, increased range of motion and increased strength of the affected joint.[7]

Again, using acupuncture points, 23 patients were treated for endartitis obliterans with He-Ne laser. Treatment with He-Ne laser at a power density of 10-12 mW/cm^2 for one to several minutes was continued for twenty days. Patients encountered side effects of mild dizziness and increased pain and cold sensation in affected limbs after several treatments. These symptoms subsequently subsided and the pain associated with intermittent claudications improved.[7]

Soviet researchers have also investigated the application of He-Ne laser in treatment of basal cell carcinomas, dyskeratoses, hyperkeratoses and acanthoses. Favorable results have been obtained in one study which dealt with basal cell carcinomas between one and three centimeters in diameter. Treatments to a maximum of 40 mW were carried out over a 3-4 week period with the laser and a photo sensitizing dye to intensify the light radiation on the lesion. This led to complete disappearance of the tumor and during subsequent follow-up periods, no recurrences were observed.[7]

By the early 1980s, European and Japanese clinical groups started investigating soft laser as an adjunct therapeutic tool. After interesting findings with application of He-Ne laser to acupuncture points as

documented by Soviet literature, several studies appeared in Western literature.[4,21,62] Like their Soviet counterparts, these studies were poorly controlled but again appeared to be beneficial for treatment of head and neck pain. There was observed infrequent side effects reported in one study of nausea and vertigo,[21] similar to those experienced by patients when receiving traditional needle acupuncture therapy. When applied to controlling pain associated with temporomandibular joint dysfunction, limited success could be directly attributed to application of He-Ne laser to appropriate ear points. The patients received comprehensive treatments which included, in addition to laser radiation, splint therapy, relaxation techniques and correction of neck posture.

In recent years, the dental profession has, on the basis of the biostimulative effects of He-Ne laser, embraced the soft laser as an adjunct to conventional therapies. Little scientific data exists to justify the wide range of applications such as: alveolites, oral ulcerations, herpes labialis, periodontal disease, pericoronitis, pulpitis, nausea induced by dental procedures, and as an anti-cariogenic measure.[7,27,29,45,61,63,64] Apparently, this is more the product of good marketing as opposed to sound science.

Positive findings were reported in a case report of application of laser radiation to a fractured pelvis. A He-Ne laser optical conductor had a stainless steel needle

adapted to its end to allow for direct exposure of He-Ne laser to a fracture line. The experimenter made the observation that the biostimulative effects of He-Ne laser stimulated the osseous tissue in the fracture site.[58]

Another area of investigation of soft laser has been in the treatment of venous leg ulcers. These ulcers were treated with daily administrations of He-Ne laser 632.8 nm, 6 days a week at energy densities of 1 J/cm^2 and 4 J/cm^2 in 16 and 17 patients respectively. The control group of 28 received antiseptic local compresses. When the experimental groups were compared to the control no enhancement of healing was observed in the irradiated group. The author therefore concluded that low dosage He-Ne laser irradiation had no advantages over more traditional local therapies.[55]

The animal research in the early 1980's focused around repeating under stricter scientific controls the earlier experiments of Mester. Waidelich and his experiemental group were amongst the first in Western literature to repeat the findings and expand upon them.

Waidelich's initial experiment investigated the effect of He-Ne laser on granulation tissue formation in full thickness skin defects in rats. A standardized skin punch was used and the defect maintained at 15 mm diameter by placing a ring attached at its lower end to the muscle fascia. The wounds were irradiated with He-Ne laser at energy densities of .5. 1.5, 4, 10 and 20 J/cm^2. The duration of exposure was altered such that the power density

was 50 mW/cm^2 per exposure. A control group of equal size had the same defect created, but were not irradiated. The He-Ne laser exposures of the experimental group were performed on a daily basis for ten days whereupon the granulation tissue within the ring was removed and weighed. The laser exposed group demonstrated a statistically significant increase in granulation tissue accumulation, a 13-24% increase in the experimental group was observed over the control.[47] Next, repeating the same experimental design, but substituting in place of laser incoherent light of 4 J/cm^2, a smaller degree of increase in granulation tissue was observed (10%) in the experimental group as compared to the control.[47]

A clinical animal study was undertaken by McKibbins at the Wheatley Farms. The study looked at 90 race horses with bowed tendons, when treated with He-Ne laser. Although no control was incorporated and results were strictly on observation, it was noted that not only did the horses undergo a shorter recovery period and race within similar times and classes post-treatment, but they became healthier without the necessity of operations, drugs and long layoff periods associated with traditional methods of treatment.[35]

Kovacs and his group demonstrated significant increases on breaking strength of wounds when exposed to He-Ne laser Using 96 rats, 48 control and 48 experimental, all animals had 2.5 cm incisions made on their dorsums with primary closure achieved using 2 surgical clips Each experimental

incisional wound was exposed to 5 mW/cm^2 He-Ne laser radiation twice a day for three minutes. Twelve animals were then sacrificed from both the control and experimental groups on days 3, 5, 8 and 12 post-operatively. The surgical clips were removed and the tensile strength of the wounds determined. The breaking strength of the experimental groups was significantly greater than the control groups on days 5, 8 and 12 post-operatively demonstrating 29, 47 and 26 percent increases respectively. Kovacs was unable to explain the mechanism of this phenomenon or why the maximal effect was demonstrated in the eight day group.[25]

Surinchak looked at the effect of low level energy lasers on healing of full thickness skin defects. This was accomplished by conducting four separate studies. The first two studies were conducted to determine the effect of laser radiation on circular full thickness skin wounds that closed primarily by contraction. In study 1 eight rabbits had two skin defects created on their backs, each 16 mm in diameter and 8 cm apart. One of the surgical defects was exposed every third day for 30 min to a total dose of 1.1 J/cm^2 per exposure. The non-exposed wound functioned as the animal's own control. The eschar was removed from both the experimental and control wounds prior to every radiation exposure. The area of the wound was determined by tracing the wound outline on a glass slide and measuring with a polar planimeter. Laser exposure was continued until 80% of

the wound closure was achieved. Surinchak observed no difference in the rate of wound closure between the control and experimental sites.[57]

Using the same experimental format as in the first study, a second study was initiated. This time 26 rabbits were utilized and the total dose per exposure was increased to 2.2 J/cm^2 as well as the frequency to twice daily. Again, no significant difference was observed between the experimental and control wound sites in respect to wound closure.[57]

In his two subsequent studies Surinchak looked at the effect of different laser energies on wound breaking stength at various times post-operatively. In the third study he looked at wound strength in rats. Full thickness skin incisions 6 cm in length were made on the dorsal midline of 38 rats, then closed with surgical staples at 1.5 cm intervals. The animals were then divided into two groups of equal size, one as the experimental group, the other the control. The experimental animals were exposed twice a day for three minutes for 14 days, receiving 2.2 J/cm^2 per exposure. At day 14 the animals were sacrificed, the staples removed and a 1 cm wide by 5 cm long full thickness skin tissue sample was harvested across the incisional line. This was then tested for breaking strength of the wound. This procedure was also performed on the control animals.[57]

To determine if the laser radiation affected wound strength several weeks post-operatively, 37 rats (19

experimental, 18 control) were used in the same fashion as in the 14 day exposure group, but now exposed for 28 days. At day 28 the animals again were sacrificed and tissue samples harvested as previously described. Surinchak observed in the 14 day study a significant increase in breaking strength was observed in the experimental group when compared to the control. The 28 day study animals, however, demonstrated no significant difference between the experimental and control groups.[57]

His fourth study used full thickness skin wounds as in study three. Forty-four rats were used, divided equally into experimental and control groups. This experiment was conducted to see if an increased total radiation exposure affected breaking strength. The experimental animals were exposed for 5 minutes twice a day to a total exposure dose of 4.5 J/cm^2. At day 14 the animals were sacrificed and wound strength investigated as in study 3. Increasing the total radiant exposure demonstrated no statistically significant increase in breaking strength in the experimental group when compared to the control.[57]

Riendeau also looked at the effect of He-Ne laser on the breaking strength of incisional wounds as well as the histological characteristics of the wounds at the light microscopic level. This study used 24 rats divided into four groups of equal size. One group acted as the control with three experimental groups. All animals had 3 cm long dorsalmidline incisions made and coapted with two 5-0 nylon

sutures. On the fourth day post-incision, the rats in experimental groups 1, 2 and 3 had their surgical incisions exposed to He-Ne 632.8 nm laser at a power output of 5.4 mW/cm^2 for 2, 3 and 4 minutes respectively. At day 14 all animals, control and experimental, were re-anaesthetized and breaking strength of the wounds determined after removing the sutures. Tissue biopsies from the incision line along an intact margin were also harvested. The animals were then sacrificed. It was observed that the breaking strength of the radiated experimental groups were significantly higher than the control. Between the experimental groups themselves no significant difference was observed between the two and three minute exposure groups. However, the four minute exposure group showed the greatest increase in breaking strength and was significantly higher than the two or three minute exposures. No observations were made at the light microscopic level which could account for the difference in breaking forces between the control and experimental groups.[50]

Hunter performed an experiment investigating the skin healing in a porcine model exposed to He-Ne laser. The reason for the porcine model was that the pig is a tight-skinned mammal more analogous to humans than the previous experiments performed on loose-skinned animals. These animals tend to have a very elastic skin and a thin subdermal muscle layer with few deep attachments. These features allowed for rapid wound healing primarily by

contraction. The experiment used two pigs; one received 30, 2 cm split thickness wounds placed in two columns on the dorsum at 2 cm intervals. The second pig had 32 incisions made in a similar fashion. Twenty-seven wounds were chosen at random and irradiated with a He-Ne laser of 64 mW output power 15 seconds per day until wound closure was achieved. This equated to .96 J/cm^2/day. The 35 non-irradiated wounds functioned as controls. In the first pig no differences in wound healing were noted between the experimental and control incisions. However, in the second pig it was observed that the laser treated wounds were significantly smaller between days 6 and 16. Prior to this and subsequently no distinguishable differences were observed between the groups. From this it was concluded that there were no lasting effects of low energy laser therapy in a porcine model on wound healing.[18] A problem with Hunter's experiments is that the animals functioned as their own controls. Many authors have observed possible systemic humoral effects and therefore animals cannot serve as their own controls when using He-Ne laser.[36,40,41,42]

Gretzinger investigated the effect of He-Ne laser on human fibroblast migration *in vitro*. This was accomplished by using six T-25 flasks which were innoculated with fibroblasts and cultured until all flasks had achieved a confluent monolayer of cells. The confluence of the cellular monolayer was disrupted such that a cell-free gap was created. Three of the flasks were radiated once with a

He-Ne laser for a total exposure of .648 J/cm^2. Cell migration was monitored under a microscope by time lapse videotape for eighteen hours.[12] The experimental flasks demonstrated a significant increase in distance of migration at 6, 12 and 18 hours after the creation of the gap when compared to the control. From this it was concluded that He-Ne radiation stimulates the rate of fibroblast migration in vitro.[12]

Mester and Bostra, in separate studies using electron microscopy, observed that He-Ne irradiated tissues demonstrated structural changes within fibroblasts.[5,37] After irradiation, the fibroblasts exposed to laser demonstrated intercellular alterations over non-irradiated. In the irradiated fibroblasts' cytoplasm, considerable hypertrophy of the secretory apparatus occurs. The rough endoplasmic reticulum cisternae take up almost the whole of the cytoplasm and Golgi apparatus can be seen in almost all sections viewed. The mitochondria increase in number as well as size.[5] Within the intercellular matrix abundant aperiodic collagen filaments can be observed. These changes are consistent with earlier studies which found increases in collagen synthesis.[37] It was demonstrated that the production of collagen in laser treated wounds were 30-50% greater than the controls. It was hypothesized that the effect of laser radiation is due to either increased enzymatic activity or to the greater amount of enzymes released by organelles.

Interesting results were obtained by Abergel in fibroblast culture experiments. The cultures were exposed to He-Ne irradiation at different levels of energy by varying the time of exposure from 1-30 minutes and at intervals of once or twice daily on several consecutive days. The results indicated that a single or two consecutive exposures at 24 hour intervals had no effect. However, procollagen production was increased up to four-fold in cultures treated with low energy lasers when subjected to four consecutive exposures at 24 hour intervals.[1] In subsequent in vitro studies Abergel demonstrated more profound results in terms of stimulation of collagen synthesis in human fibroblast cultures. The highest enhancement of 36-fold was observed with an overall average increase of four-fold. The higher enhancements in collagen synthesis were obtained from cultures which initially synthesized procollagen at relatively low levels while lesser increases were noted in cultures which already actively synthesized procollagen. The procollagen production was monitored by the synthesis of ^{3}H-hydroxy-proline following incubation of the cultures with ^{3}H-proline, and DNA replication was assessed by ^{3}H-thymidine incorporation. The He-Ne laser treatment did not affect DNA replication, therefore the procollagen increases could not be explained on the basis of enhanced cell proliferation.[2] It was also shown that the role of the thermal component in laser effect was excluded by demonstrating no changes in culture temperatures during treatment.[3]

Saperia demonstrated elevated Type I and Type III procollagen mRNA levels in cutaneous wounds treated with He-Ne laser.[56] The results suggest that He-Ne laser stimulates wound healing by enhancing procollagen gene expression. In this in vivo study, the Type I procollagen mRNA levels in the He-Ne laser treated group at day 17 demonstrated about a 6.5-fold increase. Lesser increases, which were still statistically significant, were observed at day 28. The Type III procollagen mRNA levels indicated that at day 10, the Type III procollagen mRNA levels were approximately two-fold higher than that in the untreated control wounds. More marked increases were noted at days 17 and 28. A concordance between Type I and Type III procollagen mRNA levels could be noted at days 17 and 28, suggesting a coordinate regulation of the gene expression of these two procollagens.[56]

When He-Ne laser was applied to human embryo fibroblasts at dosages of 1 J/cm^2 via a single exposure, no functional or micromorphological alterations of cell surfaces were observed. However, when irradiating at 24 hour intervals for five consecutive days to a total exposure of 5 J/cm^2, functional as well as surface charge changes on cell membranes were noted. Kubasova believed that these laser induced changes on the cell surface can contribute to the strength of cell to cell contacts.[26]

In a recent article Lyons compared wound strength and collagen synthesis within incisional wounds in mice when

exposed to He-Ne laser.[31] The exposures were of five minutes duration on alternate days to a total dose of 1.22 J/cm^2. The tensile strength of the wounds treated were significantly higher at one and two weeks as compared with non-irradiated wounds. However, no statistically significant difference was found at longer intervals (3, 4 or 8 weeks) post incision. This finding suggests irradiation appears to accelerate the formation of functional scar, but with time no difference between the groups exists. When the collagen concentrations were observed a significant increase was noted at two and four weeks after laser irradiation. The accumulation of collagen was not apparent at one week whereas the tensile strength was markedly improved. This observation suggested that additional factors result in the improved tensile strength. These include increased crosslinking of existing collagen molecules, improved organization of functional collagen fibres, or other processes of extracellular matrix production may be accelerated by He-Ne laser irradiation.[31]

Within the literature a number of studies investigated <u>in vivo</u> the effect of low energy laser radiation on full thickness skin defects.[18-20,22,25,39,43,57] Early reports indicated an accelerated rate of epithelial growth in response to low doses of laser.[39,43] These findings were not without concern as Mester presents the question whether the undoubtedly faster regeneration of the epidermis will create a condition beyond the scope of physiologic repair

which will lead to neoplastic changes. Subsequent literature has not demonstrated neoplastic changes occurring as a consequence of low energy laser stimulation.

Hunter and Surinchak demonstrated acceleration of some aspects of wound healing in the early stages of wound repair. They did not, however, feel that these findings would be clinically significant.[18,57] The laser treated wounds in these articles demonstrated early gains in wound contracture by day 14 and 16, however, beyond this point the wound healing in the experimental and control groups was observed to be equal. The early significant gains in wound healing are similar to those observed in other articles by Ikeuchi and Kana, who demonstrated statistically significant enhancement of wound repair between days 3 and 12 post-operatively.[19,22] Ikeuchi investigated the response of the components of granulation tissue to He-Ne laser irradiation in full thickness skin defects. He found that. (1) the results of tissue hydroxyproline determination showed significantly higher levels by day 5 in the laser treated group as compared to day 14 in the untreated group, suggesting an earlier healing reaction within the laser treated group; (2) when looking at DNA determination. the He-Ne laser treated group were higher by day 3, suggesting earlier protein synthesis; and (3) when looking at the scar tissue histologically, the tissue within the He-Ne laser group had collagen fibers arranged regularly with fewer capillary vessels and fibroblasts, in general a more mature scar than observed in the control.[19]

In summary, the research into biostimulative effects of low energy laser is extensive and diverse. Many of the original published experiments were poorly designed and not readily replicable. This is particularly true of the early Eastern European articles. Over the subsequent years better designed experiments have attempted to delineate the biostimulative effects in both in vivo and in vitro studies.

When looking at the biostimulative dosage of soft laser that will be most efficacious, recurring patterns in the literature occur. In vitro .05-1.0 J/cm^2 radiation dosages[37,41,42] and in vivo 1-4 J/cm^2 radiation dosages[24,25,37,40,41] appear to deliver the most optimum effects of biostimulation. In vitro cell cultures appear to have a greater sensitivity to radiation with negative influences demonstrated to high dosage and repeated exposures of radiation. In vivo, cells appear to tolerate much higher dosages than their in vitro counterparts. This may be directly attributable to the physical modulation and alteration of the laser radiation by overlying cellular structures such as eschar, necrotic tissue, other cells and epidermal appendages. Thus, the deeper cellular structures receive lower levels of radiation.

Mester's contributions to outlining areas of biostimulation of low energy laser are the most comprehensive of any single researcher. Although some experiments were poorly outlined the results justified further investigations into the effects he observed. Some

of Mester's contributions include the observation that low energy laser caused: increased phagocytic activity by leukocytes[39], increased hair growth in depilated animals[38], and the phenomena of dose dependency for optimal biostimulatory effect[38]. As well, he observed changes in levels of prostaglandin synthesis[36], and immunoglobulin and complement levels[41] in response to low energy laser radiation. Of the greatest significance and most germaine to the thesis were his investigations of wound strength[25] and collagen synthesis[37].

Other authors have verified the increases of wound strength in response to exposure of He-Ne laser.[25,31,50,57] In an attempt to explain this finding, histologic investigations both at the light[19,50] and electron[5,37] microscopic level were undertaken. Under the light microscope few histologic changes were evident that would explain the observed increases in breaking strength.[50] Ikeuchi observed changes consistent with a more mature wound at earlier times in the laser exposed animals than in the control animals.[19] Riendeau hypothesized that increases in wound strength may be related to increased collagen formation and recommended further investigation into: (1) the effect of longer single exposures of He-Ne laser on breaking strength· and (2) the changes in collagen content in wounds exposed to He-Ne laser.[50] The electron microscopic studies showed increases in size and quantity of organelles responsible for collagen synthesis.[5,37]

Kubosova, however, felt that changes in cellular surface charge was responsible for the increased breaking strength.[26]

Abergel focused on collagen synthesis in his in vitro studies.[1,2,3] He observed that in order to generate significant increases in collagen synthesis mulitple exposures of He-Ne laser were necessary. The earliest significant increases were seen after four consecutive exposures at 24 hour intervals.[1] Subsequent studies looking at specifically procollagen demonstrated increased levels of procollagen in response to multiple exposures of He-Ne laser.[2,3] The increase in procollagen could not be explained on the basis of enhanced cell proliferation or to a thermal component from the laser exposure.[2,3] Saperia demonstrated elevated Type I and Type III procollagen mRNA levels in cutaneous wounds treated with He-Ne laser. He suggested that low energy laser stimulates wound healing by enhancing procollagen gene expression.[56]

Lyons looked at both collagen synthesis and wound strength in mice exposed to multiple exposures of He-Ne laser.[31] He observed increases in tensile strength of the wounds of the experimental animals initially, with no demonstrable increases in collagen concentration. By the point that collagen concentration increases in the laser exposed animals was evident, significant increases with regards to wound stength were no longer apparent. This suggested two things: one, that He-Ne laser appears to

accelerate scar formation, and two, elevated collagen concentration cannot be entirely responsible for increased wound strength.[31]

In the literature, no one has attempted to demonstrate *in vivo* if an extended single exposure of He-Ne laser would cause an increased level of collagen concentration in a surgical wound. All authors have used short interval[1,2,3,31] (maximum five minutes) and multiple exposures (be it twice a day, once a day or alternate days) to demonstrate their findings. This may be the result of the *in vitro* studies demonstrating no increases in collagen synthesis to a single exposure of He-Ne laser.[1] This thesis will subsequently discuss the effect of a 16 minute single exposure of He-Ne laser (total dose of 5.99 J/cm^2) in incisional wounds closed primarily in rats as it pertains to the collagen concentration levels.

Materials and Methods

In this experiment 24 Sprague-Dawley rats with initial weights ranging from 95 to 110 grams were used. The animals were housed in a laboratory which provided twelve hours of daylight, twelve hours of darkness, and a maintained room temperature of 23° C at all times. The animals' diet consisted of Purina Laboratory Rodent chow and water.

The animals were divided into six groups of equal size consisting of four animals per group. Groups 1, 2 and 3 acted as control groups and Groups 4, 5 and 6 acted as the experimental groups. All animals were prepared for the surgical procedure by intra-peritoneal administration of sodium penthothal (65 mg/cc), 0.06 ml per 100 gms of body weight. After being rendered unconscious, a 5 x 3 cm area on the dorsal surface was shaved using electric clippers. The surgical site was then cleansed using 20% isopropyl alcohol swabs. Under asceptic conditions, a 1.5 cm full thickness skin incision was made in the central region of the prepared area using a number 15 Bard-Parker blade. The skin lateral to the incision was undermined to ensure complete separation of the wound margins. The wounds were then coapted using 5-0 nylon suture material by placing simple interrupted sutures at 5 mm intervals along the surgical incision. This resulted in all animals receiving two interrupted sutures (Plate 1).

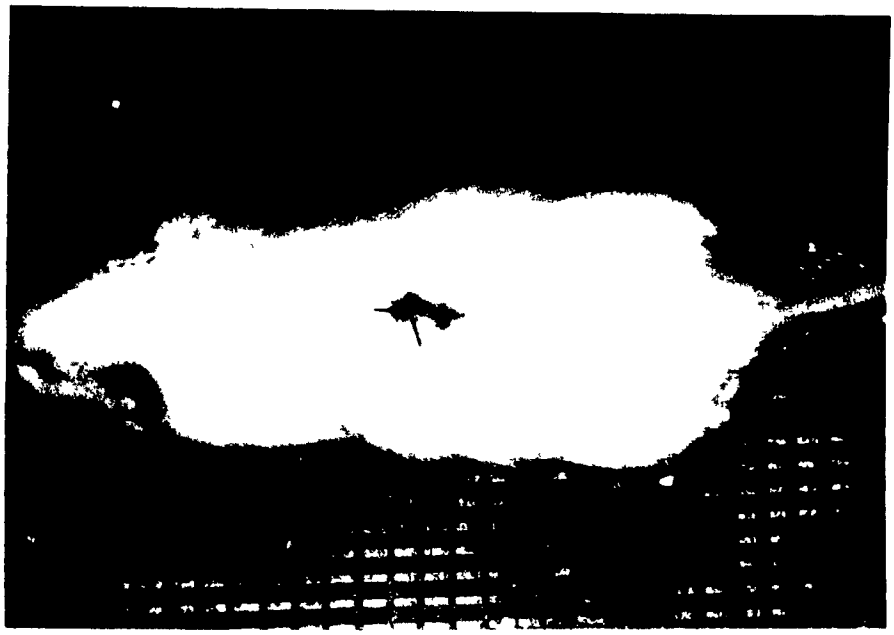


Plate 1

Animal following incision and coaptation of the surgical wound margins.

After the surgical procedures, the animals were released to their cages and allowed to recover from the anaesthetic. On the fourth day post-incision, experimental Groups 4, 5 and 6 were re-anaesthetized and their surgical sites irradiated with He-Ne laser. In this experiment Soft Laser 632 was utilized (Plate 2). This is a helium-neon laser which emits radiation of 632.8 nm with a maximum output power of 10 mW/cm^2. The laser was conducted to the surgical incision via a fiberoptic cord. The laser radiation was delivered in a continous mode at a distance of 1.5 cm from the skin surface such that the surgical incision transected the laser beam. All experimental animals received sixteen minutes of exposure resulting in an energy fluence of 5.99 J/cm^2. Following the laser exposure the animals were returned to their cages and allowed to recover (Plate 3).

On day 6 post-incision, control Group 1 and experimental Group 4 were re-anaesthetized, the surgical wounds cleansed, sutures removed, re-shaved and surgical scar band excised (Plate 4 & 5). This was accomplished by making two parallel incisions, 1 mm on either side of the previously placed incision line through the full thickness of the skin. These incisions were released at their ends and the scar bands removed, lysing any subdermal adhesions as necessary. The tissue samples were labelled for identification, wet weights obtained and then stored at -20° C in individually stoppered test tubes awaiting

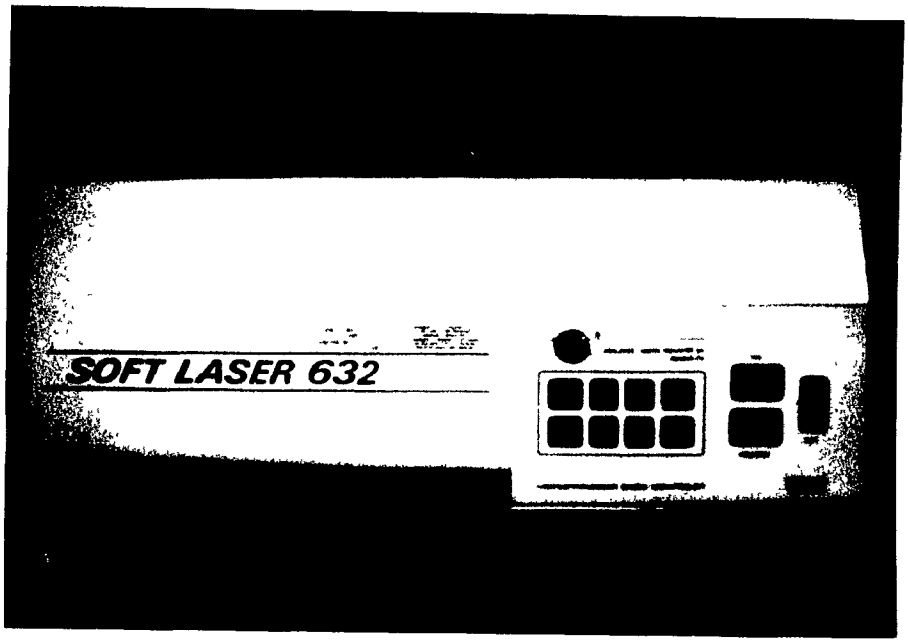


Plate 2

Soft Laser 632 (maximal power output 10 mW/cm^2).

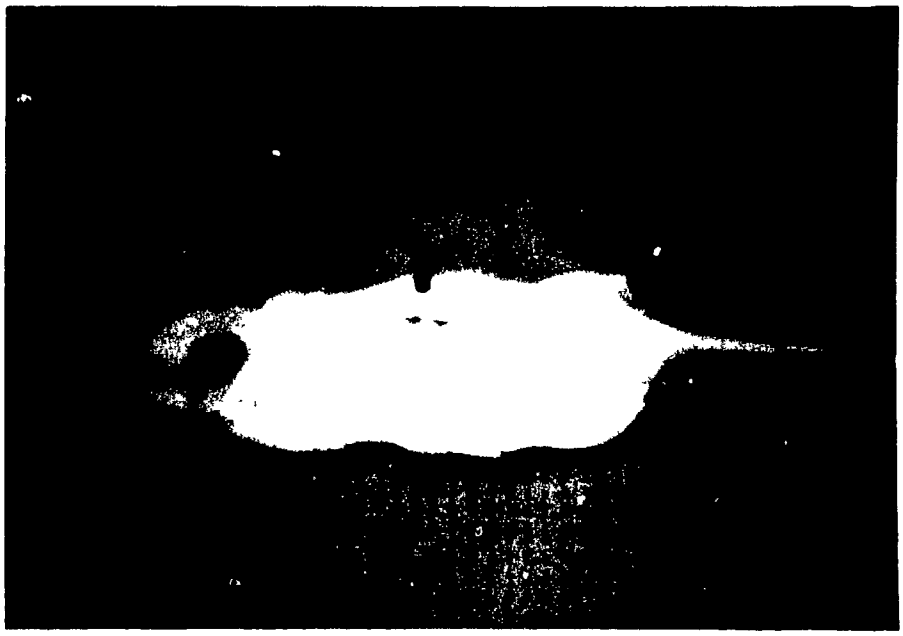


Plate 3

Anaesthetized animal receiving soft laser radiation to the surgical site on day 4 post-incision.

collagen concentration assay. After the harvesting of the scar bands the animals were sacrificed by ether inhalation. Harvesting of tissue was repeated on days 10 and 14 post-incision on Groups 2 and 5 and Groups 3 and 6 respectively (Plates 6,7,8,9).

The tissue samples were then separately hydrolysed in 6 M HCl at 105^{0} C for 20 hours. After being completely hydrolysed and then dried in a speed-vac, the samples were diluted in Na buffer solution of pH 2.2 to 1 µg of tissue residue per 10 µl solution. From this a sample of the solution with hydrolyzed residue was drawn off and placed in an amino acid analyser for spectrophotometric analysis (Plate 10). The amino acid analyser is calibrated to identify and quantify the peak corresponding to hydroxyproline which accounts for 14% of collagen. This allows for direct comparison between the tissue samples' collagen content on the basis of their hydroxyproline concentration as described by Kivirikko [65]

The analysis of the data utilized independent-groups t-test to determine the significance of difference between the control and experimental results. The variance of values within each group were analyzed and the F-values determined. The statistical power of the test was calculated following the method described by Lachin.[66]

Plate 4

Control wound 6 days post-incision.

Plate 5

Experimental wound 6 days post-incision.

Plate 6

Control wound 10 days post-incision.

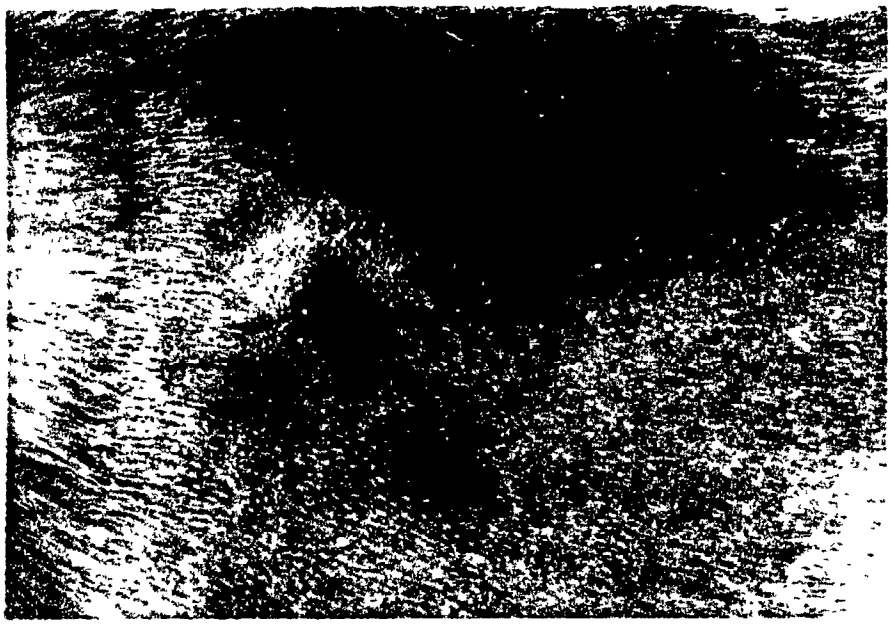


Plate 7

Experimental wound 10 days post-incision.

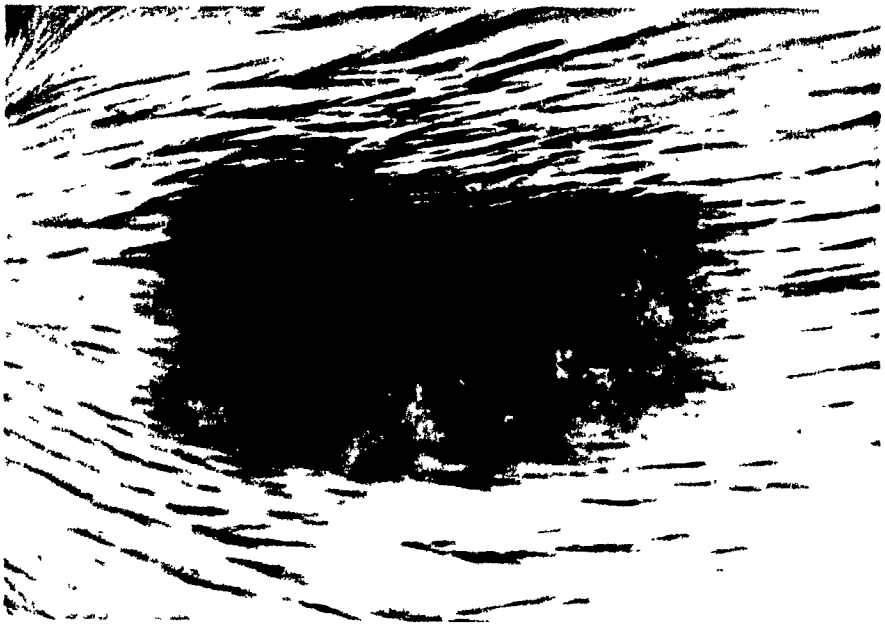


Plate 8

Control wound 14 days post-incision.

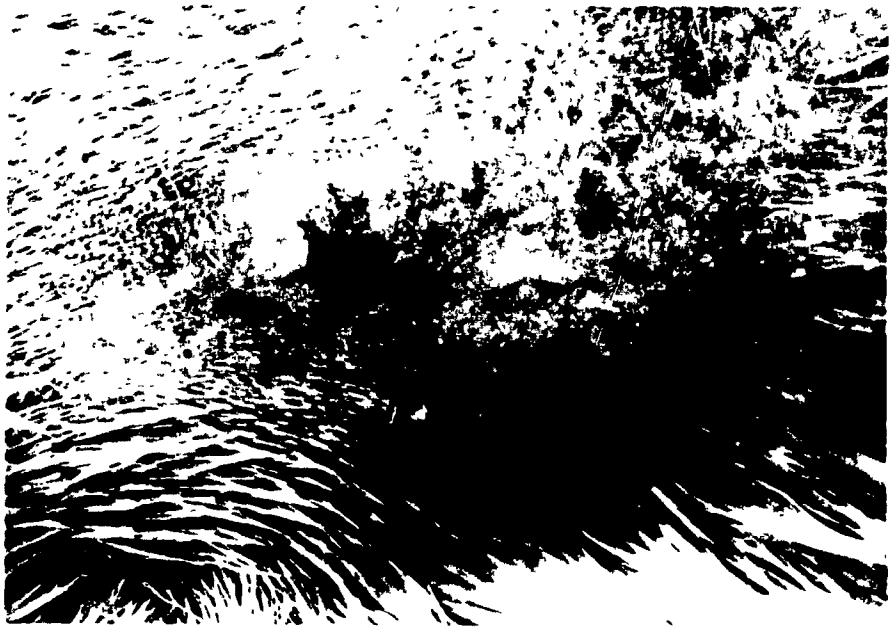


Plate 9

Experimental wound 14 days post-incision

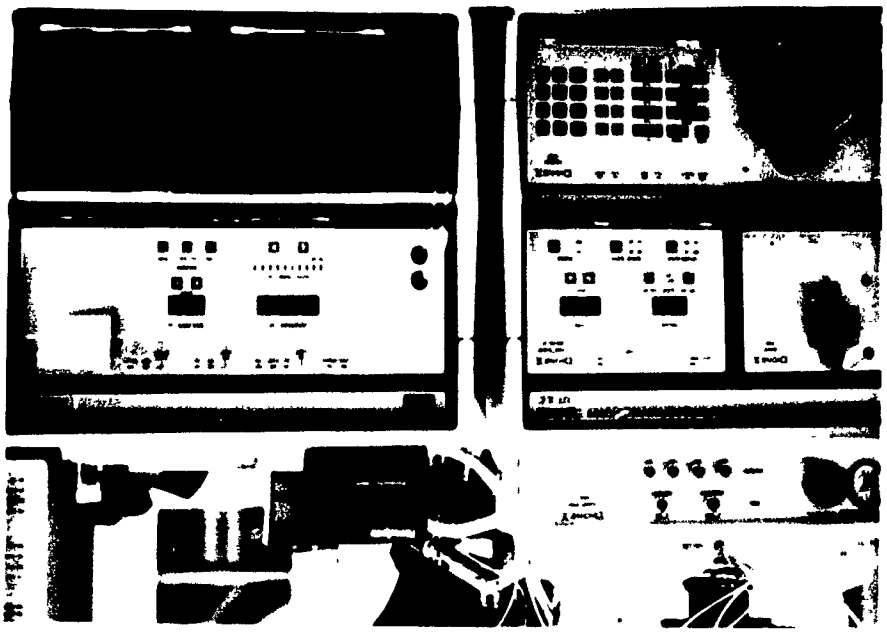


Plate 10

Dionex Amino Acid Analyser.

Results

Collagen Content Assay

The collagen content within the tissue samples was reflected by the amount of hydroxyproline measured. Hydroxyproline accounts for approximately 14% of the components constituting all forms of collagen. The distinctive peak upon spectrophotometric analysis of collagen displayed by hydroxyproline assists in its quantification within a known weight of tissue (Figure 8).

The control Group 1 and experimental Group 4 had the healing incisions excised on day 6. For the experimental group this corresponded to 2 days post-laser exposure. The wet weights (Table 1) of the tissue samples were obtained and hydrolyzing of the samples performed in a uniform manner. The control group had a mean value of hydroxyproline of 6.03 nm with a standard deviation of .522 and a minimum and maximum value of 5.44 nm and 6.70 nm respectively. The experimental group had a mean value of 6.35 nm with a standard deviation of 1.02 and a minimum and maximum value of 5.17 nm and 7.26 nm respectively. The experimental group demonstrated a .32 nm higher mean value than the mean of the corresponding control group (Table 2).

The control Group 2 and experimental Group 5 had the healing incisions excised on day 10 post-incision. This represented 6 days post-laser therapy for the experimental group. The mean value of hydroxyproline for the control group was 6.61 nm with a standard deviation of .425, and

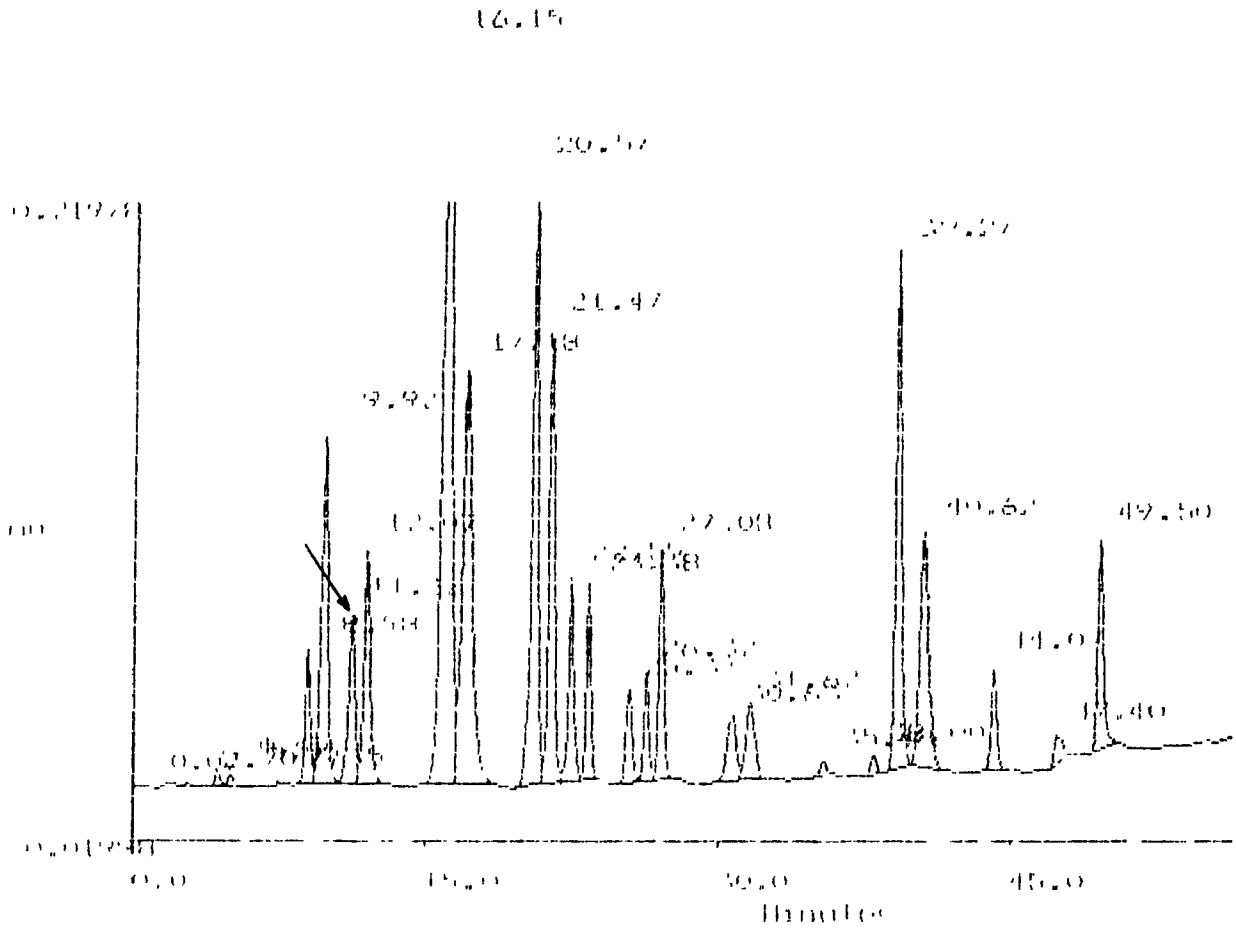


Figure 8: An example of the spectrophotometric analysis of the tissue sample with arrow identifying the peak corresponding to hydroxyproline.

Control Group	Rat	Sample Wet Weight (grams) Day 6	Day 10	Day 14
1	1	.0631		
	2	.0334		
	3	.0409		
	4	.0762		
2	1		.0660	
	2		.0301	
	3		.0693	
	4		.0492	
3	1			.0430
	2			.0658
	3			.0570
	4			.0830
Experimental Group				
4	1	.0722		
	2	.0531		
	3	.0650		
	4	.0656		
5	1		.0712	
	2		.0692	
	3		.0622	
	4		.0836	
6	1			.0522
	2			.0620
	3			.0318
	4			.0539

Table 1: The wet weights of tissue samples harvested.

Control Group	Rat	Mean Values (nanomoles) Day 6	Day 10	Day 14
1	1	6.07		
	2	5.89		
	3	6.70		
	4	5.44		
		6.03*		
2	1		6.05	
	2		6.91	
	3		6.97	
	4		6.52	
			6.61*	
3	1			7.77
	2			7.31
	3			6.89
	4			lost
				7.32*
Experimental Group				
4	1	5.17		
	2	7.15		
	3	5.82		
	4	7.26		
		6.35*		
5	1		8.13	
	2		5.49	
	3		9.00	
	4		7.56	
			7.54*	
6	1			7.57
	2			lost
	3			lost
	4			lost

*mean

Table 2: The mean values of the hydroxyproline content in nanometers. Note: "lost" samples are due to variation in hydrolyzing of tissue samples which rendered them unacceptable for direct comparisons.

minimum and maximum observed values of 6.05 nm and 6.97 nm respectively. The experimental group had a mean value of 7.55 nm with a standard deviation of 1.49, and minimum and maximum value of 5.49 and 9.0 nm respectively. The experimental group again demonstrated a higher mean value when compared to the control by .94 nm. Also noted was a marked increase in range of values of hydroxyproline observed within the experimental Group 5 when compared to the control (Table 2).

The control Group 3 and experimental Group 6 had the healing incisions excised on day 14. This represented 10 days post-laser therapy for the experimental group. Due to a hydrolyzing technique variation, one sample was lost from the control group and three samples from the experimental group. The values obtained were not utilized in the statistical analyses, but did follow a pattern similar to the previously discussed groups. The mean value of hydroxyproline for the control group was 7.32 nm with minimum and maximum values of 6.89 nm and 7.77 nm respectively. The single experimental value was 7.57 nm. The experimental group maintained a marginally higher hydroxyproline content when compared to the control. However, no appreciable increase from the previous experimental Group 5 (Graph 1) was observed

The largest magnitude single increase in mean values of hydroxyproline was observed between experimental Group 4 and 5. An observed increase of 1.19 nm in the mean values was noted.

Statistical Analysis

In order to chose the right version of the test for comparison of means, the equality of variances was tested and F values were:

for Day 6: $t = -0.564$ ($p = 0.6002$)

for Day 10: $t = 1.2019$ ($p = 0.3060$)

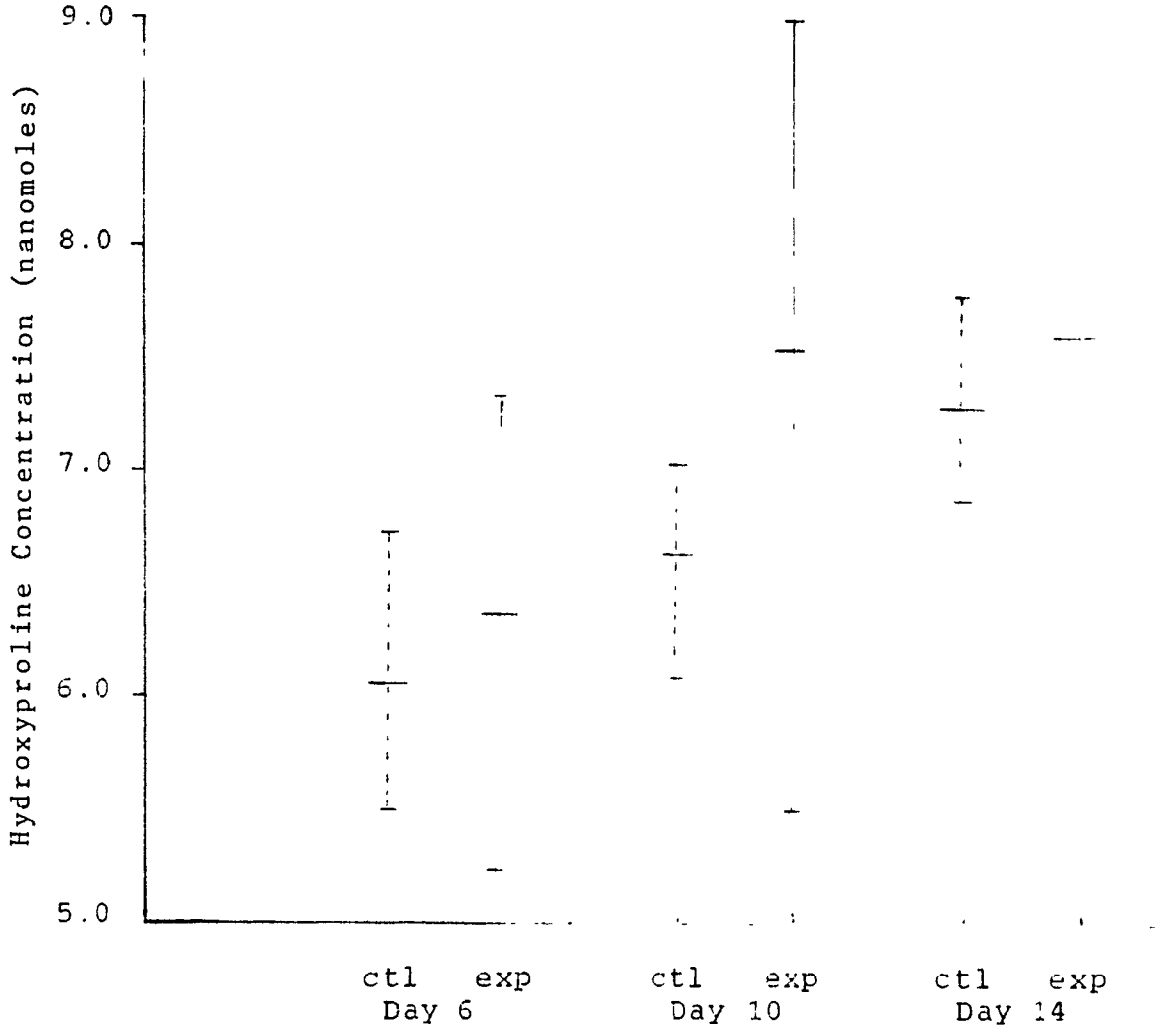
The mean values of the control and experimental groups on days 6 and 10 were compared using independent-groups t-test with the respective variances assumed to be equal as both F values are non-significant. Both t-tests gave non-significant results of.

for Day 6: $F_{3,3} = 3.84$ ($p = 0.2985$)

for Day 10: $F_{3,3} = 12.35$ ($p = 0.0681$)

However, the mean values in both experimental groups are higher than those in the controls. Moreover, the respective F values show that standard deviations in the experimental He-Ne laser groups were substantially higher than those in the controls (three to five times higher on Day 6 and 20 times higher on Day 10). Thus the lack of significance may be partly due to the high variance in the He-Ne group and to the small sample sizes ($N = 4$ in each group).

Therefore, the statistical power of the test was calculated as described by Lachin.[67] The power is interpreted here as the probability of detecting the least clinically important increase of 5% in hydroxyproline content (in comparison with the controls) due to exposure to He-Ne laser by the test of size ($N = 4$) and significance



Graph 1: The mean values of each experimental and control group and the associated range of values for each respective group.

level α = 0.05 (the relative increase of 5% corresponds to 0.3 nm in obsolute terms). For the power calculations the estimated pooled variances for the respective days were used:

$$\bar{s}^2 \text{ (6 days)} = 0.660$$

$$\bar{s}^2 \text{ (10 days)} = 1.203$$

The power calculated based upon the above assumption was about 14% (Z_{β} = 1.09) for Day 6 and 33% (Z_{β} = 0.44) for Day 10. The low statistical power of both tests, when combined with the fact that the means for the laser group were actually higher, indicates that a larger sample size may lead to a significant result.

Discussion

Numerous *in vitro* and *in vivo* studies have demonstrated biostimulative properties of low energy laser on factors related to the healing process. Of particular interest are those studies which have demonstrated increased synthesis of collagen either *in vitro* or *in vivo* as a consequence of He-Ne laser exposure. Mester[37] was the first to describe this finding and subsequent western studies by Abergel[1] and Lyons[31] have supported his findings. However, in each instance where elevated levels of collagen synthesis was observed the cell cultures or animals were exposed to repeated doses of laser radiation over several days. Abergel observed *in vitro* that in order to appreciate a change in collagen content repeated daily exposures were necessary for at least four days.[1] Subsequent *in vivo* studies using exposure doses and intervals similar to Abergel's have demonstrated elevated levels of collagen content within healing wounds. A question left unanswered by those studies is would a single exposure equal to those doses necessary to observe increased collagen content have any effect on collagen content within a healing wound? In terms of a clinical situation, a single exposure would be advantageous to both patient and clinician if it was demonstrated to be beneficial.

The present study was performed to look at the effect of a single extended exposure (16 minutes) dose (5.99 J/cm^2) of He-Ne laser (632.8 nm) on healing primarily closed full thickness skin incisions in rats.

The laser administration was performed four days post-incision on the basis of the fact that, according to Peacock[49] this point marks the beginning of the proliferative phase of wound repair. This phase begins between days three and five and extends to days 14 to 21. Prior to this the inflammatory phase of healing is predominant. Therefore, the assumption is made that if elevated collagen content is the desired biostimulative response to He-Ne laser, exposure to the laser is best initiated when the wound has the appropriate cells in position to do so, i.e. three to five days after injury.

Lyons[31] observed in mice that exposure doses of 1.22 J/cm^2 administered on alternate days demonstrated significant increases in collagen content after two weeks. This would mean that an approximate cumulative dose of 8.5 J/cm^2 was administered to the animals for a total duration of approximately 35 minutes. This would appear to exceed what is considered an optimum biostimulative dosage if it was to be administered as a single exposure.[24,25,37,40,41] The dose for the study was therefore selected to reflect the higher limits of what could be considered biostimulative at 5.99 J/cm^2 in a single exposure and the length of time necessary to obtain this energy fluence was calculated to be 16 minutes.

With no previous studies performed looking at the effect of an extended single exposure of He-Ne laser on collagen content to function as a guide, the times post-

incision at which tissue samples were harvested were selected to reflect a cross-section of points within the proliferative phase of wound repair. Peacock[47] noted that the maximum collagen content within a wound is achieved at approximately day 15. It was believed that the selected days for tissue harvesting of 6 10 and 14 days post-incision would reflect a cross section of the proliferative phase. If a single exposure of He-Ne laser on a wound at the beginning of the proliferative phase of repair was to alter the collagen content within the wound, the selected intervals would be able to demonstrate if the effect was sustained or transient.

From the data obtained within this experiment the mean values of hydroxyproline content within the excised wounds were higher in the experimental animals at all intervals observed when compared to their respective control group (Graph 1). Unfortunately, some samples in control and experimental Groups at day 14 were lost, rendering them unacceptable for statistical analysis. Even though the mean values on Day 6 and 10 reflected elevated level of collagen content (Table 2) within the experimental groups, the increases were determined to be non-significant. This was due to the small sample size (N = 4) and the higher degree of variability within the experimental groups (standard deviations of 1.02 on Day 6 and 1.49 on Day 10).

The response to a single exposure of He-Ne laser appeared to be the most robust on Day 10 post-incision which

equates to Day 6 post-laser exposure. The single Day 14 experimental result demonstrated no further increase in hydroxyproline content over the mean value of the Day 10 experimental group. This observation suggests that the peak collagen content may have been achieved within the Day 10 experimental group thereby demonstrating a more rapid wound healing response to the He-Ne laser when compared to the control. This observation would be consistent with the findings of Hunter[18], Ikeuchi[19], Kana[22] and Surinchak[57].

The higher degree of variability within the values of the experimental groups may be partially a factor of experimental inconsistencies. In terms of the angle of delivery of the laser exposure, an attempt was made to deliver the laser uniformly at right angles to the wound surface. Minor variations existed due to slight alterations in the actual wound placement. As well, some movements of the sedated animals may have occurred altering the field of exposure of the laser and the distance of the wound surface to the laser source.

Wound strength studies[25,28,50,56,57] have demonstrated increases in wound strength in response to He-Ne laser during the first fourteen days of wound healing. Riendeau[50] suggested this may be a consequence of elevated collagen content within a wound. Lyons[31], however, in a subsequent study found that in response to multiple exposures of He-Ne laser, collagen content was increased after the elevated wound strength was no longer significantly different from

the control animals. He concluded that the wound strength increases may be due to something other than increased collagen content within the wound. Although wound strength was not directly addressed in this study, if the observed trend of increased hydroxyproline content in the laser exposed animals is indeed correct, collagen formation and content may be playing a greater role in early increases in wound strength than suggested by Lyons [31]

While demonstrating a trend of elevation in hydroxyproline content within healing wounds, the present data is not statistically significant. This may be due at least in part to the small size. The findings do, however, support the need for repeating the experiment utilizing larger group sizes where there may indeed be significant differences observed. This would be a finding not previously appreciated in the literature and suggest broader applications of single exposure He-Ne laser as a therapeutic tool in wound repair.

Summary and Conclusions

This experiment studied the effect of an extended single exposure dose of He-Ne laser (632.8 nm) on healing, primarily closed full thickness skin incisions on the dorsum of young Sprague-Dawley rats. The wounds were radiated with He-Ne laser of a 5.99 J/cm^2 dose over a 16-minute period, four days after the initial full thickness skin incision was made. The wounds were excised on days 6, 10 and 14 post-operatively and collagen content assayed by measuring spectrophotometrically the hydroxyproline content within the excised wounds.

The mean values of hydroxyproline in all laser exposed groups were observed to be greater than those in the control animals. However, no statistical significance could be attributed to this finding due to (1) the small sample size involved (N = 4), and (2) the high degree of variability within the results obtained in the experimental animals.

Therefore no conclusion can be drawn upon the effect of a single exposure of He-Ne laser on collagen content within a healing wound. It does, however, suggest that an effect may exist and indicates that the experiment should be repeated with larger groups sizes in an attempt to demonstrate statistical significance.

References

1. Abergel R P. Lam, T.S , Merken, C A., Dwyer P M and Uitto, J. Low energy lasers stimulate collagen production in human skin fibroblasts. Clin Res 32.16A, 1984.

2. Abergel, R.P . Lam, T.S . Meeker, C.A. Castil, C J , Dwyer, R.M and Uitto J. Biostimulation of procollagen production by low energy lasers in human skin fibroblast cultures. Abstract submitted to 45th Annual Meeting of the Society for Investigative Dermatology, Inc., Washington D.C. May, 1984

3. Abergel, R.P., Meeker, C A., Lam, T.S., Dwyer, P.M Levavog, M.A. and Uitto, J. Control of connective tissue metabolism by lasers: Recent developments and future prospects. Dermatologic Surgery. 11 6, 1984

4. Barnes, J.F Electronic acupuncture and cold laser therapy as adjuncts to pain treatment. J. Craniomandibular Practice, 2.149, 1984.

5. Bosatra, M. and Jucci, A. In vitro fibroblast and dermis fibroblast activation by laser irradiation at low-energy. Dermatologica 168:157-162, 1984.

6. Clark, R., Stone, R.D., Leung, D.Y.K., Silver, I.A., Hohn, D. and Hunt, T.K. Role of macrophages in woudn healing. Surgical Forum 27:17, 1976.

7. Gamaleya, N.F. Laser biomedical research in the U.S.S.R. In: Laser Applications in Medicine and Biology Vol. 3, pp. 110-126. New York: Plenum Press, 1977.

8. Goldman, L. and Rockwell, R.J. Jr. Characteristics of laser radiation. In: Lasers in Medicine, pp. 83-107. New York Science Publishers Inc., 1971.

9 Goldman, L. and Rockwell, R.J. Jr. The history and physics of laser emission. In: Lasers in Medicine, pp 26-37. New York: Science Publishers Inc., 1971.

10 Goldman, L. Biomedical Aspects of the Laser, pp. 57-65. New York: Springer-Verlag, 1967.

11 Goldman, L. (Editor). The Biomedical Laser: Technology and Clinical Applications, pp. 7-11. New York. Springer-Verlag, 1981.

12. Gretzinger H A An in vitro study of the effect of He-Ne laser on human fibroblast migration. Thesis submitted to the Faculty of Graduate Studies and Research, McGill University. Montreal 1985.

13 Guyton, A C. Textbook of Medical Physiology. Philadelphia: W.B. Saunders Company, 1936.

14. Ham, A.W. and Cormack, D.H. Histology, 8th Edition. Toronto: J.E. Lippincourt, 1979.

15 Hensgt, W R (Ed.) Stedman's Medical Dictionary 24th Edition. Baltimore: Wialliams and Wilkins, 1982.

16. Hillenkamp, F., Pratesi, R. and Sacchi, C. Lasers in Biology and Medicine, pp. 69-75. New York: Plenum Press, 1979.

17. Hunt, T.K. (Editor). Wound Healing and Wound Infection, pp. 1-26. New York: Appelton-Century-Crofts, 1980.

18. Hunter J., Leonard, L., Wilson, R., Snider, G. and Dixon, J. Effects on low energy laser on wound healing in a porcine model. Lasers in Surgery and Medicine, 3(4):285, 1984.

19. Ikeuchi, S., Ohsaka, F , Asanami, S. and Nomoto, T Effects of low power He-Ne laser on the healing of full thickness skin defects. In Lasers in Dentistry, pp. 89. New York: Elsevier Science Publishers B V., 1989.

20 Jongsma, F., Bogaard, A., van Gemert, M. and Hulsbergen-Henning, J. Is closure of open skin wounds in rats accelerated by argon laser exposure? Lasers in Surgery and Medicine 3:75, 1983.

21. Kamikawa K. Development of laser acupuncture systems and their clinical applications. In New Frontiers in Laser Medicine and Surgery (A. Kazuniko, Ed.), pp. 498. Princeton: Excerpta Medica, 1983.

22. Kana, J.S., Hutschenreiter, G., Naina, D and Waidelich, W. Effect of low power density laser radiation on healing of open skin wounds in rats. Arch. Surg. 116:293, 1981

23. Koozikanani, L. Deby, D.P , Drutchoff, A. and Giftan, M. Measurements of the laser output. Proc. I.R.E., 50:207, 1962.

24. Kovacs. I.B., Mester, E and Gorag, P. Laser-induced stimulation of the vascularization of the healing wound - An ear chamber experiment. Experimentia, 30:341, 1974.

25. Kovacs, I.B., Mester, E. and Gorag, P. Stimulation of wound healing with laser beam in the rat. Experimentia 30.1275, 1974.

26. Kubasova, T., Kovacs, L., Somosy, Z., Unk, P. and Kokai, A. Biological effects of He-Ne laser: Investigations on functional and micromorphological alterations of cell membranes in vitro. Lasers in Surgery and Medicine, 4.381, 1984.

27. Kunin, A. Use of the He-Ne laser in treating periodontal diseases. Stomatologia (Mosk) 62:26, 1983.

28. Lasers and Optical Radiation. Geneva: World Health Organization, pp. 53-58, 1982.

29. Laufer, G and Haber, S. Numerical analyses of the thermochemical tooth damage induced by laser radiation. J. Biomech Eng. 107:234, 1985.

30. Lederer, H., Skonbil, K., Denk, R. and Waidelich, W. Influence of light on human immunocompetent cells in vitro. In: Optcelectrics in Medicine - Proceedings of the 5th International Congress Laser 81 (Waidelich, W., Ed.), pp. 170-184. New York: Springer-Verlag, 1982.

31. Lyons, R.F., Abergel, R.P., White. R.O., Dwyer, R.M., Castel, J.C. and Uitto, J. Biostimulation of wound healing in vivo by Helium-Neon laser. Annals of Plastic Surgery 18(1):47, 1987.

32. Mann, D.P. Basic aspects of laser operation. In: Proceedings of the First Annual Conference on

Histological Effects of Laser Radiation (Libaries, M.S. & Carle, K.M., eds.). Federation Proceedings, Suppl 14-15 8, Jan.-Feb., 1965

33. Mainster, M.A. Ophthalmic laser surgery principles, technology and technique. In: Symposium of the Laser in Ophthalmology and Glaucoma Update (Transactions of New Orleans Academy of Ophthalmology). Toronto: C V Mosby, 1985.

34. McGilvery, R.W. and Goldstein, G.W. (Editors). Biochemistry - A Functional Approach. Philadelphia. W.E. Saunders, 1987.

35. McKibbins, L.S. An evaluation of the effects of low energy laser on soft tissues in horses In: New Frontiers in Laser Medicine and Surgery (Kazuhiko, A. Ed.). pp. 509-515. Princeton: Excerpta Medica.

36. Mester, E., Hazg. L., Fenyo, M., Kertesz, I., Toth, N., Jaranyi, Z. and Toth, J. The biostimulating effect of laser beam. In: Optoelectronics in Medicine - Proceedings of the 5th International Congress Laser 81 (W. Waidelich Ed.), pp. 146-152. New York: Springer-Verlag, 1982

37. Mester, E. and Jaszsagi-Nagy, E. The effect of laser radiation on wound healing and collagen synthesis. Studia biophysica 35:227-230, 1973

38. Mester, E., Ludary, G., Vajda, G. and Toto, J. Uber die wirkung von laser-sthales auf die bacteriumphagocytose des leukocytes. Acta Biol. Med. German 21:317, 1968.

38. Mester, E., Ludanj, G., Vajdo, J., Razgha, A., Kareka, J. and Toto, G. Uber die wirkung von laser-strahlen auf die bakterin phagozytose des leukozcyten. Acta Biol. Med. Germ., 21:317, 1968.

39. Mester. E., Ludanj, G., Selyei, M., Szende, B. and Toto, G. The stimulatory effect of low power laser rays on biological systems. Laser Review, 1:3, 1968.

40. Mester, E., Mester, A. and Toth, J. Biostimulative effect of laser beams. In: New Frontiers in Laser Medicine and Surgery (A. Kazuhiko, Ed.), pp. 481-490. Princeton: Excerpta Medica, 1983.

41. Mester, E., Nagylucskay, S., Tisza, S. and Mester, A. New investigations into the effect of laser beams on the healing of wounds: Immunological aspects. Z. Exper. Chirurg., 10:301, 1977.

42. Mester, E., Nagylucskay, S., Tisza, S. and Mester, A. Stimulation of wound healing by means of laser rays. Acta Chir. Acad. Sci. Hung., 19:163, 1978.

43. Mester, E. and Spirg, T. Effect of laser rays on wound healing. ,Am. J. Surg. 122:532-535, 1971.

43. Mester, E., Spirj, T., Szende, B. and Toto, J. Effect of laser rays on wound healing. Am. J. Surg., 122:532, 1971.

44. Mester, E., Szende, B. and Gartrer, P. Die wirkung der laser-strahlen auf den haarwuchs der maus. Radiobiol. Radioth. 9:621, 1968.

45. Merritt, R. and Burns, J.C. In: Letters to the Editor, Oral Surg. Oral Path. Oral Radiology, 67:271, 1989.

46. Murray, R K., Branner, D.K., Mayes, P.A. and Rodwell, V.W. (Editors). Harper's Biochemistry 21st Ed. Norwalk, Connecticut: Appleton & Lange, 1988.

47. Naina, D., Brunner, R., Landthaler M , Braun-Falco, O and Waidelich, W. Animal experiments on light-induced wound healing. In: Optoelectronics in Medicine, Proceedings of the 5th International Congress Laser 81 (Waidelich, W., Ed), pp. 164-169. New York Springer Verlag, 1982.

48 Patek, K. Gas lasers. In: Lasers, pp. 108-112. Cleveland: CRC Press, 1967.

49. Peacock, E.E. Wound Repair 3rd Edition, pp. 1-186 Philadelphia: W.B. Saunders Company, 1984.

50. Riendeau, F. An in vivo study of the effect of Helium-Neon laser on the breaking force and histological characteristics. Master of Science Thesis, McGill University, Montreal, 1987.

51. Ross, G. (Editor). Essentials of Human Physiology, pp. 162. Chicago: Year Book Medical Publishers, Inc., 1972.

52. Ross, R. and Benditt, E.P. Wound healing and collagen formation. J. Biophys. Biochem. Cytol. 2:677, 1961.

53. Ross, R. The fibroblast and wound repair. Biol. Rev. 43:51, 1968.

54. Ross, R., Everett, N.B. and Tyler, R. Wound healing and collagen formation. J. Cell. Biol. 44:645, 1970.

55. Santoianni, P., Monfucola, G., Martellotta, D. and Ayala, F. Inadequate effect of Helium-neon laser on venous leg ulcers. Photodermatol. 1(5):245, 1984.

56. Saperia, D., Glassberg, E., Lyons, R.F., Abergel, R.P., Baneux, P., Castel, J.C., Dwyer, R.M. and Uitto, J. Demonstration of elevated type I and type III procollagen mRNA levels in cutaneous wounds treated with Helium-Neon lasers. Biochemical and Biophysical Research, 138(3):1123, 1984.

57. Surinchak, J.S., Alago, M.L., Bellamy, R.F , Stuck, B.E. and Belkin, M. Effect of low-energy lasers on the healing of full-thickness skin defects. Lasers in Surgery and Medicine, 2:267, 1983.

58. Trelles, M. The biostimulatory effect of He-Ne laser beams for osseous regeneration. In: Optoelectronics in Medicine - Proceedings of the 5th International Congress on Lasers (W. Waidelich, Ed.), pp. 153. New York: Springer Verlag, 1982.

59. VanWinkle, W., Hastings, J.C., Barker, E. and Wohland, W. Role of fibroblasts in controlling rate and extent of repair in wounds in various tissues. In: Biology of Fibroblasts (E. Kulonen, Ed.), pp. 559. London: Academic Press, 1973.

60. Weinstock, M. and Leblond, C.P. Synthesis, migration and release of precursor collagen by odontoblasts as visualized by radioautography after ^{3}H-proline administration. J. Cell. Biol. 60.92, 1974.

61. Wilder-Smith, P. The soft laser: Therapeutic tool or popular placebo? Oral Surg. Oral Path. Oral Radiology, 66:654, 1988.

62. Wu, W. Recent advances in laser puncture. In: New Frontiers in Laser Medicine and Surgery (A. Kazuhiko, Ed.), pp. 401. Princeton: Excerpta Medica, 1983.

63. Yamada, K. Study on caries prevention by laser irritation in pediatric dentistry. Shoni Shikagaku Zasshi, 23:575, 1985.

64. Zhou, Yu-Cheng. An advanced clinical trial with laser acupuncture anesthesia for minor operations in the oromaxillofacial region. Laser Sug. Med. 4:297, 1984.

65. Kivirikko, K.I., Laitinen, O. and Prockop, D.J. Modification of a specific assay for hydroxyproline in urine. Analytical Biochemistry 19:249-255, 1967.

66. Lachin, J. Introduction to sample size determination and power analysis for clinical trials. Controlled Clinical Trials 2:93-113, 1981

60. Weinstock, M. and Leblond, C.P. Synthesis, migration and release of precursor collagen by odontoblasts as visualized by radioautography after ^{3}H-proline administration. J. Cell. Biol. 60 92, 1974.

61. Wilder-Smith, P. The soft laser: Therapeutic tool or popular placebo? Oral Surg. Oral Path. Oral Radiology, 66:654, 1988.

62. Wu, W. Recent advances in laser puncture. In: New Frontiers in Laser Medicine and Surgery (A. Kazuhiko, Ed.), pp. 491. Princeton: Excerpta Medica, 1983.

63. Yamada, K. Study on caries prevention by laser irritation in pediatric dentistry. Shoni Shikagaku Zasshi, 23:575, 1985.

64. Zhou, Yo-Cheng. An advanced clinical trial with laser acupuncture anesthesia for minor operations in the oromaxillofacial region. Laser Sug. Med. 4:297, 1984.

65. Kivirikko, K.I., Laitinen, O. and Prockop, D.J. Modification of a specific assay for hydroxyproline in urine. Analytical Biochemistry 19:249-255, 1967.

66. Lachin, J. Introduction to sample size determination and power analysis for clinical trials. Controlled Clinical Trials 2:93-113, 1981