# LASER PHOTONS AND PHARMACOLOGICAL TREATMENTS IN WOUND HEALING

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**The exploitation of photobiology in medicine has been of great interest to mankind. There is a growing interest in the use of lasers for treatment purposes because of the photochemical alterations induced in biomolecules by light energy. In this paper we present our data on laser biostimulation, the combination of pharmacological treatments Solcoseryl™ (SS) and Polygen™ (PG) with light therapy using *in-vitro* and *in-vivo* models. *In-vitro* experiments indicate the ability of laser photons and pharmacological agents SS or PG to augment or abate the cloning efficiency of various cell lines. *In-vivo* studies focused on the dosimetry of various laser wavelengths and the use of wound healing drugs and 632.8nm laser in wound healing. The application of pharmacological treatments combined with laser therapy reveals the utility of light-drug treatment combinations. Given the ever-increasing cost of medical care, the burden incurred on patients, caregivers and society, this line of research fulfills the increasing need to develop treatment methods that enhance wound healing, especially in situations involving resistance to healing.**

*Key Words: Laser Therapy, wound healing, growth factors.*

## Introduction

During the last decade, a growing interest in Photomedicine has emerged from the creative use of lasers, giving rise to the field of "Laser Biostimulation" or "Low Level Laser Therapy". This form of therapy is now widely used for the treatment of a variety of conditions, including wound healing, reduction of edema and pain of various etiologies.

Wound healing photons and its dosimetric parameters have been studied extensively in our laboratory using various lasers: HeCd (442nm), Argon (488-514nm), HeNe (632.8nm), Krypton (670nm), GaAlAs (780nm and 830nm) and $CO_2$ (10,600nm).(1-4) Our studies suggest that HeNe 632.8nm is the best laser for biostimulation and that laser acceleration of wound healing cannot be attributed to laser skin transmission.(5)

We determined the effect of 632.8nm laser *in-vitro* and *in-vivo* with SS, a calf hemodialysate and PG, a cocktail of growth factors and growth hormone (GH). Our *in-vitro* studies support the use of combined laser+SS (Al-Watban et al., in press) and laser+PG treatments in a nutrient deficient medium.(6) Our *in-vivo* studies on normal rats have shown that laser+SS or laser+PG minimally accelerate wound healing when compared to the single effect of 632.8nm laser (Al-Watban et al, in press). As a follow-up to our previous studies, we now present a report on a study in which we used an impaired wound healing model to further test the efficacy of laser+drug therapy.

The need to promote wound healing, especially during a state of impairment, is imperative due to the ever-increasing cost of medical care and the burden imposed on patients, health care services and society, in general.

## Materials and Methods

*In-vivo Experiments*

*Animals:* A number of male Sprague-Dawley rats were used in this study. The animals were originally imported from Charles River Co., UK in 1984. Now they are bred and provided by the Animal Facility of King Faisal Specialist Hospital & Research Center,Riyadh, Saudi Arabia.

*Oval-Full-Thickness Wound Infliction:* Rats were anesthetized with 50mg/kg ketamine and 20mg/kg xylocaine. The gluteus maximus region of each rat was shaved, followed by application of a hair removal lotion to remove residual hair, and to minimize reflection losses. Thereafter, the skin was disinfected with 70% isopropyl alcohol. Oval full-thickness wounds measuring 0.39 $cm^2$ were then created with a scalpel.

*Laser Systems:* The study on laser acceleration of oval full-thickness wound closure was carried out using HeCd

442nm (Liconix 4240NB), Argon laser 488-514nm (Coherent 800), He-Ne laser 632.8nm (Spectra-Physics 127), Krypton 670nm (American Laser Corp.), GaAlAs lasers 786nm and 829nm (Navital GALA System) and $CO_2$ laser, 10,600nm (Coherent System 451). The laser beam was delivered through a system of reflectors for HeCd, Krypton and $CO_2$ lasers, fiber optics for the Argon laser, and expanders for He-Ne laser and GaAlAs lasers. The output power was measured continuously using Liconix 45PM, Molectron MAX5200, Coherent 210 power meters and Laser Guide integrating sphere power meters. Different incident doses were used. The term "incident dose" differs from actual dose, since actual dose is incident dose minus skin beam reflection and plexiglass cage reflection. In the $CO_2$ laser experiment, where photons could not transmit through the plexiglass, a hole was created where the beam and wound area could be aligned during treatment. The treatment schedule of three-times per week was used to compare the effects of the different laser wavelengths in wound healing. The spot size in the infrared laser was observed and localized with the help of a Find-R-Scope (FJW optical systems, Inc.). All spot sizes were calculated as a circular beam, apart from the diode lasers that were calculated as ellipse with the use of a digital planimeter (Planix 7P).

*Pharmacological treatments:* Solcoseryl™ (Solco-Basle, Switzerland) is a biologically and chemically standardized protein-free calf hemodialysate. SS jelly and ointment tubes containing 20gm protein free dialysate were used according to the manufacturers instructions. Polygen™ (BMHT, Inc., Lenexa, Kansas) is an animal protein extract derived from an agricultural product that contains a blend of growth factors (GF), growth hormones (GH) and trace elements as active ingredients. PG cream containing 5% of the active ingredients was applied daily until the rat wound was completely healed.

*Combined He-Ne laser and Polygen™ or Solcoseryl™ Treatment:* Wound healing drug SS or PG was applied daily according to manufacturer's instruction. Laser exposures were performed before SS or PG treatment on each laser treatment day.

*Data Analysis:* The average number and standard deviation of days required for 80% wound healing and the percentage of wound healing acceleration referred to as accelerated reduction of healing days and wound size were calculated. The accelerated reduction of healing days relative to the control was calculated as follows:

$$\% \text{ Acc D} = [1 - TD/CD] \times 100$$

*TD and CD are the days required for 80% wound healing on the treated (TD) and control (CD) animals, respectively.*

The percentage of wound healing acceleration in wound size reduction was calculated as follows:

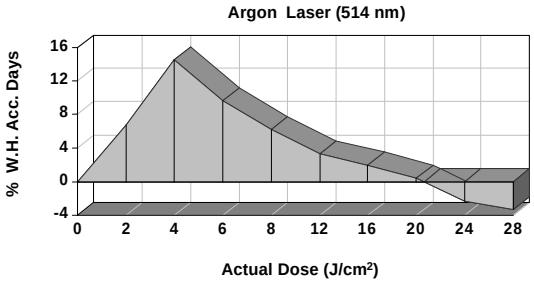
$$\% \text{ Acc S} = [1 - TA/CA] \times 100$$

*TA and CA are the wound areas on the treated (TA) and control (CA) animals when 80% wound healing was reached.*

### *In-Vitro Biostimulation Experiments*

*Effect of HeNe Laser (632.8nm) on Various Cell Lines:* Mouse embryonic fibroblasts (3T3, CCL-226), Chinese hamster ovary (CHO), mouse fibrosarcoma (RIF-1) and adenocarcinoma cells (EMT-6) were grown to semi-confluency using minimum essential medium (MEM, Sigma) supplemented with 100 units Penicillin (Sigma), 100 mg Streptomycin (Sigma), 2mM glutamine (Sigma) and 10% fetal bovine serum (FBS, Sigma) on a T-25 flask. Cells were trypsinized, counted and seeded at 400 cells/dish on a 35mm culture dish. Reduced serum concentration of 5% FBS was used in the succeeding experiments.

Figure 1. Wound Healing: Dose Dependent Stimulation & Inhibition

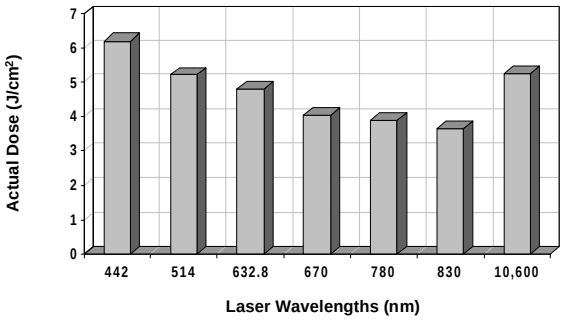



*He-Ne Laser Stimulatory Dose Determination:* A 35mW continuous wave HeNe laser (Spectra Physics 127) with a beam profile at TEM00 mode was configured using a beam expander, a mirror and a 10X objective to deliver a laser beam within the MAX/e intensity point, to cover a spot of 4 cm diameter with a power density of 1.25mW/cm². The output power was measured before, during and after exposure times using Molectron™ MAX5200 and Metrologic™ power meters calibrated at 632.8 nm. Daily exposure times of 16, 32, 48, 64, 80, 96, 112, 128, 144 and 160 seconds, given for three consecutive days following an overnight culture incubation, were done to deliver a radiant exposure of 60, 120, 180, 240, 300, 360, 420, 480, 540 and 600mJ/cm², respectively. Four 35mm-culture dishes seeded with 400 cells were used for each laser dose and control.

*Solcoseryl™ and Polygen™ Stimulatory Concentration Determination:* SS ampoule containing 40mg/ml protein free dialysate or PG powder was dissolved in 5% FBS-

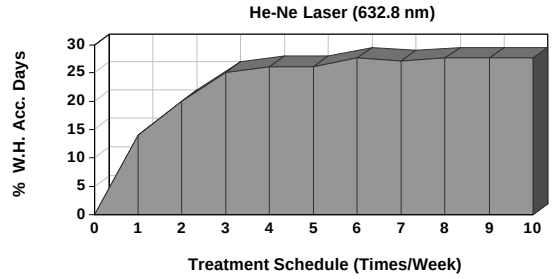
MEM to obtain a 5mg/ml stock concentration. SS or PG was added to give 6, 12, 25, 50, 75, 100, and 125 mg/ml final concentration. Two ml. volumes of SS or PG in 5% FBS-MEM was dispensed in each well of a 12-well cluster plate seeded with 200 cells/well. Plates were incubated undisturbed at 37°C, 5% $CO_2$ for ten days. Quadruplicate cultures were used for each group; treatment and control.

Figure 2. Wound Healing: Optimal Stimulative Doses of Various Lasers




*Combined Laser + PG or Laser + SS Stimulation Determination:* The stimulatory concentrations of 6 and 12mg/ml SS or PG and 180 mJ/cm² He-Ne laser cumulative doses determined from previous experiments were used. Dishes with 400 CHO cells/dish were used. Quadruplicate cultures were done for each of the following groups, namely: control, laser, SS or PG 6mg/ml, SS or PG 12mg/ml, laser+SS or PG 6mg/ml and laser+SS or PG 12mg/ml. After overnight incubation at 37°C, 5% $CO_2$, He-Ne laser dose of 60mJ/cm² was given for three consecutive days to the laser groups. Similar manipulations were done on the non-laser group, including the control. Incubation of cultures was continued undisturbed for 7 days. After a total of ten days incubation, cultures were washed twice with phosphate buffered saline, fixed with absolute ethanol for 15 min, aspirated and dried at room temperature. Alcohol-fixed air-dried colonies were stained with crystal violet for one minute, washed twice with tap water, then air dried.

Figure 3. Wound Healing: Treatment Schedule Dependent



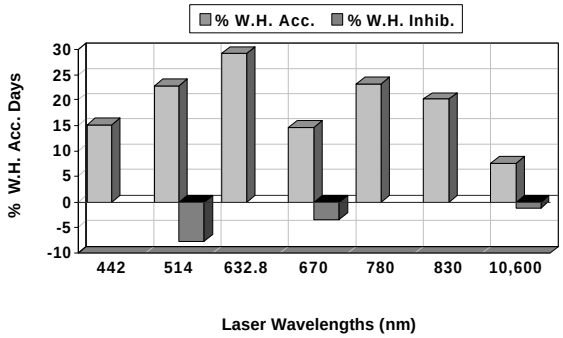

*Colony Count and Data Analysis:* Colonies were counted manually under a stereomicroscope (Bausch & Lomb) using a Manostat pen colony counter. Colonies with less than 50 cells were excluded. No correction for cell multiplicity was done. Colonies counted were expressed as cloning efficiency (CE). Average CE and standard error was determined. Difference in CE from the control was expressed as percent of control.

## Results

*Laser Enhancement of Oval Full-Thickness Wound Closure*

We investigated the influence of various laser wavelengths (442nm, 514nm, 632nm, 670nm, 780nm, 830nm and 10,600nm) on the healing of oval-full-thickness wounds in Sprague-Dawley rats. The effect of laser exposure was dose dependent (Fig. 1) giving the effects of stimulation (2-16 J/cm²), zero bio-activation (20 J/cm²) and inhibition (24-28 J/cm²). Using the above-mentioned wavelengths with their calculated optimum actual doses (Fig. 2) and treatment schedule of three times per week (Fig. 3), the acceleration of wound closure was from 7.7% to 29% in healing days (Fig. 4). HeNe 632.8nm gave the best acceleration in healing days (29 %) (Figs. 4 & 5). For clinical application, actual dose was computed by deducting the plexiglas cage reflection and skin reflection from the incident dose, except for the $CO_2$ laser where plexiglas reflection was not subtracted due to the direct laser photon application (Fig. 6). This study have shown wavelength dependency, treatment schedule dependency and dose dependency of photons in wound healing. The comparison of the acceleration of wound healing *in-vivo*, fibroblast absorption *in-vitro*, skin absorption and transmission using the various lasers is presented to show the relationship of the effect of the various wavelengths *in-vitro* and *in-vivo* (Fig. 7). The relationship of skin transmission and absorption are inversely proportional. Fibroblast absorption and wound healing acceleration is maximal at 632.8nm, indicating that the acceleration of wound healing is not attributed to laser skin transmission. Figure 8 shows the non-proportionality of the incident power density of the various lasers used in our *in vivo* wound healing experiments. HeNe 632.8nm with an incident power density of 10.53mW/cm² gave the optimum wound healing acceleration indicating the non-dose rate dependency of laser photons in enhancing wound healing.

Figure 4. Wound Healing: Wavelength Dependent



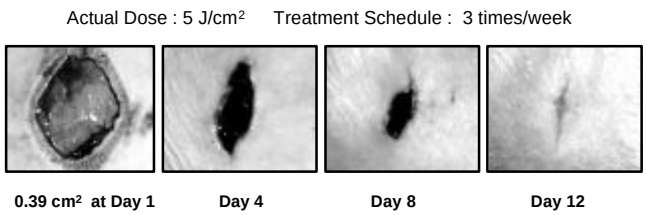
*The Effect of He-Ne Laser (632.8nm) on various Cell Lines*

To be able to determine the effect of HeNe (632.8nm) laser exposure *in-vitro*, mouse embryonic fibroblasts (3T3, CCL-226), Chinese hamster ovary (CHO), mouse fibrosarcoma (RIF-1) and adenocarcinoma cells (EMT-6) were used. Above-mentioned cells were exposed from 60-600 mJ/cm$^2$ cumulative doses. This study demonstrated that 632.8nm photons could stimulate and inhibit non-neoplastic as well as neoplastic cells in 5% FBS-MEM culture conditions (Fig. 9).

*The Effect of Polygen™ or Solcoseryl™ on various cell lines*

The experiments to determine the influence of SS or PG on the cellular proliferation of the various cell lines showed that the modulation of cellular proliferation were drug concentration dependent (Fig. 10 & 11). SS and PG affected the trend of stimulation-zero bio-activation-inhibition on CCL-226 and RIF-1. Stimulation was manifested by CHO and EMT-6, while stimulation and zero bio-activation was seen in the 3T3 cells.

Figure 5. Wound Healing of Oval Full-Thickness Rat Wound During HeNe Laser (632.8nm) Treatment




He-Ne Laser Treated group reached 80 % Wound Healing at 7.72 ( ± 0.1) days, while the Control group at 10.77 ( ± 0.18) days.

He-Ne Treated Wound closure almost completed at day 12,Control 16 days

Figure 6. Laser Treatment and Calculation for Actual Dose

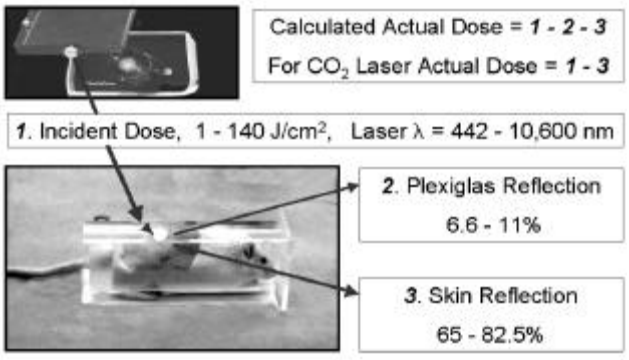



**Figure 7. The Comparison of Wound Acceleration, Fibroblast Absorption, Skin Absorption and Transmission using Various Laser Photons**

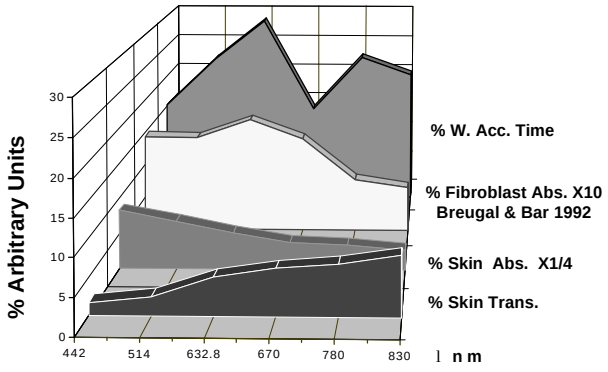



Figure 8. Non-Dose Rate Dependency of Laser Photons in Wound Healing

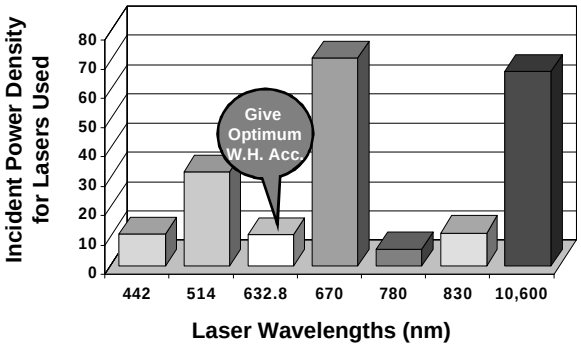



Figure 9. Effect of HeNe Laser (632.8nm) on Various Cells

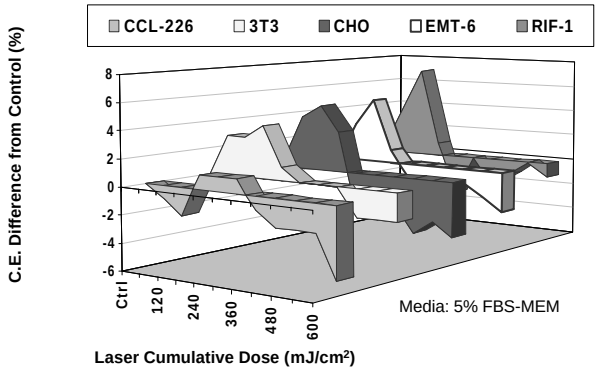
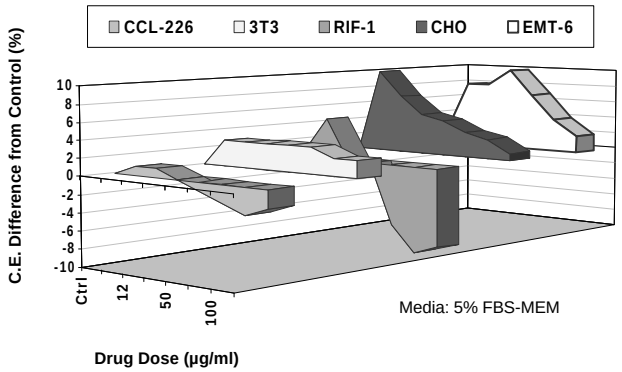


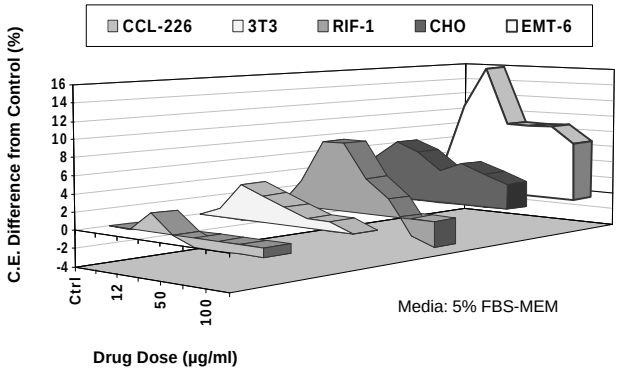

Figure 10. The Effect of Polygen™ on Various Cells



*The Effect of He-Ne Laser (632.8nm) and Pharmacological Treatments In-Vitro*

Studies to determine the effect of combined laser photons with wound healing drugs SS and PG were done *in-vitro.* The optimum laser cumulative dose of 180 mJ/cm$^2$ for the various cells in culture was determined in the previous experiments. Dose response curve for the effect of SS and PG on CHO are presented in figure 12, showing that at doses of 6ug/ml-12ug/ml SS or PG could increase cloning efficiency optimally. Having determined the optimum SS or PG dose, laser+SS or laser+PG experiments were done. The result was that additional stimulation could be achieved using these laser+pharmacological treatment combinations (Fig. 13).

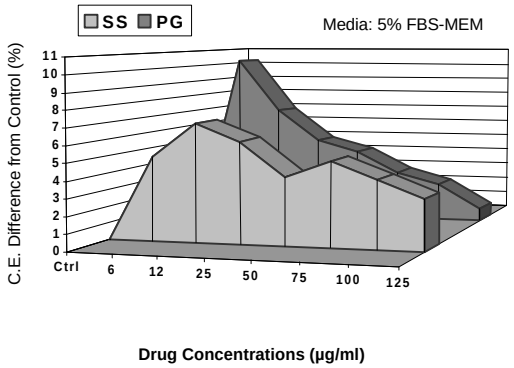
Figure 11. The Effect of Solcoseryl™ on Various Cells




*Wound Healing Efficacy of He-Ne Laser (632.8nm) and Pharmacological Treatments in Normal Rats*

Our *in-vitro* studies suggested the usefulness of laser+SS or laser+PG. A follow-up experiment using normal rats with oval-full-thickness wound and with the following treatments: control, laser, PG, SS, laser+PG and laser+SS (Figure 14) were performed. Comparison of treatment effect was done at 80% wound healing. The average number of days for 80% wound healing are as follows: Control 10.77±0.1 > PG 10.30±0.13 = SS 10.13±0.08 > laser 7.72±0.18 =laser+PG 7.53±0.13 = laser+SS 7.37±0.15. This study shows that all the treatments employed were significantly better than the control, the effect of SS was not significant from PG; solitary laser treatment was five and sevenfold better than SS and PG, respectively. The use of laser+SS or laser+PG gave additional but insignificant acceleration of healing over the laser alone. While our *in-vitro* studies that were done in a nutrient and growth factor deficient culture conditions revealed the utility of laser+SS or laser+PG treatments, the *in-vivo* studies were done in normal rats. A similar study using ischemic and diabetic rat model is recommended, to determine the efficacy of the proposed laser plus pharmacological treatment modality in chronic wounds.

Figure 12. Effect of Polygen™ and Solcoseryl ™ on Chinese Hamster Ovary Cells




## Discussions and Conclusions

Laser therapeutic studies have shown for quite sometime, the dose, wavelength, and treatment schedule dependency of laser treatments relative to the effect one would like to achieve. Our *in-vivo* wound healing study using Argon Laser (514nm) (Fig. 1) shows the actual doses that the desired effect of wound healing acceleration could be attained. Several lasers (Fig. 2) with wavelengths 442nm to 10,600nm were used to determine optimal stimulation doses in the healing of oval full-thickness wounds. HeNe laser (632.8nm) gave the best wound healing acceleration using 5 J/cm$^2$ actual dose and three times per week treatment schedule (Figs. 3 & 4). The actual dose computation was performed because of the manner with which the rat was exposed to photons. A physical barrier, the plexiglass cage and the rat skin, reflect or deflect the beam to a certain degree, that minimizes the amount of photons reaching the target tissue. The computed actual dose is used for clinical applications where direct application of photons is done (Fig. 6).

Wound healing is a complex biologic and biochemical process that commences right after tissue injury. Low oxygen tension and the formation of platelet plug highlights the coagulation phase. Macrophages, polymorphnuclear neutrophils and lymphocytes appear during the inflammatory phase for debris and infection control, and the secretion of growth factors for fibroplasia, angiogenesis and re-epithelialization. The responsiveness of the cellular components of wound healing to photon stimulation has been studied.(7-10) The increase in cellular energy and tissue oxygenation, enhanced micro-circulation and synthesis of specialized signaling proteins, such as growth factors, have been shown to be influenced by photons — the reason why wound healing is accelerated.

Figure 13. Effect of Combined Laser and Polygen™ or Solcoseryl™ Treatment on Chinese Hamster Ovary Cells

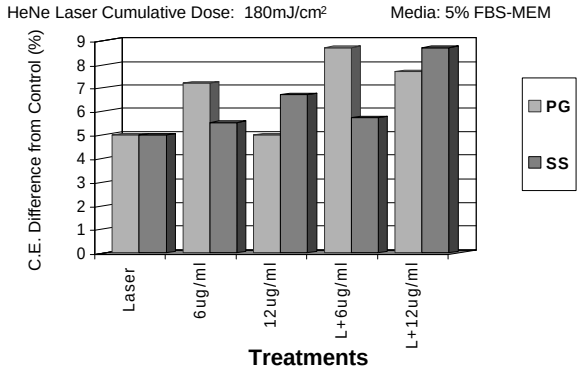
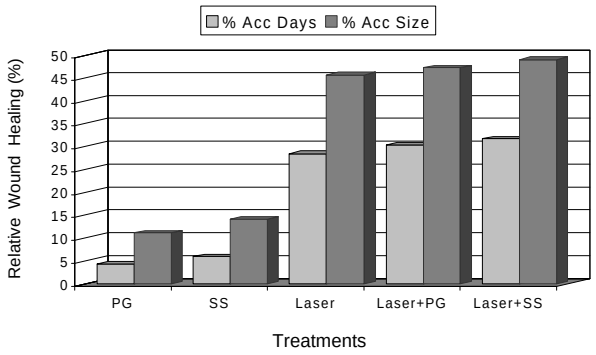



Figure 14. Effect of Combined Laser and Polygen™ or Solcoseryl™ on Oval Full-Thickness Wounds in Normal Rats




The *in-vitro* study using HeNe laser (632.8nm) 60-600 mJ/cm² cumulative doses with various cell lines were done to determine the effect of laser on cloning efficiency (Fig. 9). All the cell lines exhibited the trend of stimulation, zero bio-activation and inhibition. However, the levels with which they are stimulated, zero bio-activated and inhibited, differ, implying that different photo-biochemical pathways are in play even with similar photon and energy doses. The optimum stimulatory dose was determined at 180 mJ/cm². All cell lines were inhibited at 600 mJ/cm². The optimum stimulatory dose of 180mJ/cm² obtained compare favorably with Nara, et al.(11) and van Breugel and Bar.(12) Karu has demonstrated that low-power laser irradiation preferentially stimulate resting cells rather than the proliferating ones.(13) When growth factors are withdrawn from dependent cell as in the reduction of FBS concentration from 10% to 5%, metabolism is disrupted and shifts a portion of the actively proliferating cell population to rest. As discussed by Vander Heiden, et al, disturbance in ATP/ADP exchange across mitochondrial membranes occur resulting to an accumulation of stored metabolic energy in the mitochondrial intermembrane space in the form of creatine phosphate. If outer membrane impermeability persists, the disruption of mitochondrial homeostasis culminates in the loss of outer mitochondrial membrane integrity, cytochrome-c redistribution and apoptosis that could be averted by prompt re-introduction of growth factors.(14) The ability of He-Ne laser to revert resting cell to proliferate in low serum cultures may be due partly to its influence on micro-circulation and cytokine production.

Laser doses above the optimum stimulatory dose have also been shown to effect inhibition. The mechanisms underlying this effect are not yet fully understood. One possible mechanism could be that excessive production of reactive oxygen species (ROS) and the product of its dismutation, hydrogen peroxide ($H_20_2$) lead to oxidative stress, triggering the release of cytochrome-coactivating the caspase cascade, resulting in a shutdown of mitochondrial energy production, necrosis and apoptosis,(15, 16) and hence, inhibition.

A comparison of the *in-vitro* with *in-vivo* laser doses shows a close relationship. The optimum stimulatory dose *in-vitro* of 60 mJ/cm² given three times for three consecutive days with a cumulative dose of 180 mJ/cm² (Fig. 9) and *in-vivo* optimum actual dose of 5 J/cm² given three times/week until the wound is fully healed (Fig. 2) were compared. The relationship of the *in-vitro* and *in-vivo* optimum doses could be determined if we look at how the cells were exposed to photons. Four hundred cells seeded in 3.5-cm diameter dish (area of 9.6cm²) allow single cells to attach to the plate with ample space for colony expansion. This means that the dose of 60 mJ/cm² is treating only a single cell layer. The *in-vivo* actual dose of 5 J/cm² is used to treat the wound bed and its margins with the laser photons penetrating at least 1mm (1000mm) deep into the skin. Assuming the diameter of cells to be from 10mm-15mm, then at 1mm depth we have 67 to 100 layers of cells. Multiplying the *in-vitro* stimulatory dose of 60 mJ/cm2 by 67 and 100 would give actual doses of 4,020 mJ/cm² and 6,000 mJ/cm² (4 J/cm² and 6 J/cm²), respectively, which when averaged, corresponds to our *in-vivo* actual dose of 5 J/cm². *In-vitro* the cells were inhibited at 300 mJ/cm² to 600 mJ/cm² (Fig. 6) and *in-vivo* at 24 J/cm² to 28J/cm² (Fig. 1). When similar analysis is applied, *in-vitro* inhibitory doses multiplied by 67 and 100 gives 20,100 mJ/cm² and 60,000 mJ/cm² (20.1 J/cm² and 60 J/cm²), respectively, showing that the *in-vitro* and *in-vivo* laser dosimetry correlated very well in our experiments.

A comparison of the acceleration of wound healing *in-vivo*, fibroblast absorption *in-vitro*, skin absorption and transmission using the various lasers was done to show the relationship of the effect of the various wavelengths *in-vitro* and *in-vivo* (Fig. 7). The relationship of skin transmission and absorption are inversely proportional. That as, the laser wavelength increases from 442nm to 830nm, the percentage of skin transmission increases as skin absorption

decreases. Fibroblast absorption and wound healing acceleration is maximal at 632.8nm. These observations indicate the importance of fibroblast absorption and that laser wound healing acceleration is not dependent on laser skin penetration, but on the photo-biochemical effect that follow after the absorption of photons. Figure 8 shows the non-proportionality of the incident power density of the various lasers used in our in vivo wound healing experiments. HeNe 632.8nm with an incident power density of 10.53mW/cm$^2$ gave the optimum wound healing acceleration indicating the non-dose rate dependency of laser photons in wound healing acceleration

The drug response curves of SS and PG on the various cell lines (Fig. 10 & 11) have shown that the modulation of cellular proliferation was dose dependent and that the degree of response vary with each cell line. In this experiment CHO was most responsive to SS and PG in terms of stimulation when compared to the other non-neoplastic cells, 3T3 and CCL-226. RIF-1 and EMT-6, both neoplastic, responded differently. RIF-1 was inhibited by PG from 75-125mg/ml concentrations, and by SS at 100-125mg/ml, while EMT-6 was stimulated from 6-125mg /ml.

Based on the result of the HeNe (632.8nm) laser, SS and PG dose response on the various cell lines, CHO cell was chosen for the determination of the *in-vitro* efficacy of laser+SS or PG. The dose response curves of CHO with SS and PG is presented to show their optimum stimulatory concentrations (Fig. 12). The combined laser+SS or PG treatment reveals that additional increase in the cloning efficiency of CHO cells could be achieved (Fig. 13). SS has been shown to enhance cellular proliferation via its ability to increase oxygen consumption, ATP synthesis, micro-circulation and its growth factor-like activity.(17, 18) PG, a cocktail of growth factors such as GH, EGF, FGF TGF and PDGF, have been shown to increase cellular proliferation.

The combination of laser+SS showed additional increase in cellular proliferation. Although laser and SS may trigger increased ATP synthesis, enhanced cell respiration and micro-circulation, the mechanisms by which these cellular processes are initiated differ. With SS the classical cellular biochemical mechanism of electron transfer is enhanced, whereas with laser the photochemical and photophysical mechanisms utilizing the wave particle dualism of electron and the importance of radiation energy during the process are suggested.(19) The photochemical absorption of radiation by chromophores in the mitochondria leads to the generation of extra electrochemical potential, a short term primary response, that produces a change in reduction-oxidation state of the respiratory chain.(20, 21) The light quanta acts only as a trigger for cellular metabolic regulation and the photo signal transduction from the cytoplasm to the nucleus is not specific for light signals but includes the normal way of controlling cell proliferation.(22) The amino acids, nucleosides and glycosphingolipids supplied exogenously by SS, aside from being a source of substrate for protein and nucleic acid synthesis, improve oxidative phosphorylation and consequently increases the available ATP.

The combination of laser+PG also showed additional increase in cellular proliferation. ATP and its metabolites(23, 24) have been reported to act synergistically with peptide growth factors PDGF, EGF, TGF alpha, TGF beta and IGF-1 in the enhancement of several events that are important for cell proliferation such as: DNA synthesis, protein synthesis, increased cell number, cell-cycle progression and tyrosine phosphorylation. The intracellular or extra-cellular deficiency of high-energy phosphate or its metabolites, therefore, may hamper susceptibility to peptide growth factor stimulation. The increased availability of ATP through laser irradiation enhances the effect of peptide growth factors in various biochemical reactions that take place in the cell, producing enhanced cellular proliferation.

Our study of laser with pharmacological treatments showned additional wound healing acceleration in the normal rat (Fig. 14). HeNe laser (632.8nm) was employed using the computed actual dose of 5 J/cm$^2$. SS or PG was used following the manufacturer's suggested doses. This study showed the efficacy of HeNe laser, SS, PG and laser+wound healing drug combinations in the enhancement of wound healing in oval full thickness wounds in normal rats. The average number of days for 80% wound healing was used for comparison. Laser, SS and PG treatment significantly exhibited a decrease in the number of healing days when compared to controls ($p<0.005$), with laser giving the least number of healing days. SS and PG treatment, while showing a significant decrease in the number of healing days compared to controls, were less effective than the laser. SS affects wound healing through its enhancement of micro-circulation, cellular respiration, ATP synthesis and exhibition of a growth factor-like activity. SS active ingredients also serve as a source of nutrients and substrate for nucleic acid and protein synthesis. PG, a blend of GF and GH influence wound healing through the activation of various growth factor receptors and protein kinases leading to increased cellular proliferation.

Controls differed significantly from combined laser and pharmacologic treatments ($p<0.001$), while laser vs laser+pharmacologic treatments gave non-significant differences. Wounds in normal rats have been demonstrated

by Yu, et al. to express EGF, PDGF, basic-FGF and TGF-b in early skin wound healing following acute inflammation. (25) The presence of these GF in the wound were detected after six hours and peaked during the first day. The effect of adding exogenous GF through PG may have been masked partially by the presence of endogenous GF in the wound.

SS serves to increase micro-circulation, oxygen consumption, ATP synthesis and source of nutrients. SS effect on micro-circulation produces endogenous substrates, aside from the exogenous substrates it provides, that are needed for repair and regeneration. The laser effect on micro-circulation, electron transport chain that results in the production of molecular $O_2$ for cellular respiration and ATP synthesis gives similar effect as SS while the mechanisms in the initiation of the cascade of reactions differ. The former being photochemical and the latter, biochemical.

The presence of endogenous GF in the normal rat wound, its synergy with the increased amount of ATP produced through laser irradiation, the laser effect on micro-circulation enhancing the mobility of endogenous substrates for better tissue oxygenation and nutrition, were contributing factors to the outstanding efficacy of the laser as a single treatment; congruent only to the effects of combined laser+SS or laser+PG.

This study has demonstrated the following: a) that each of the treatment parameters, SS, PG, laser, laser+SS and laser+PG enhance wound healing significantly; b) that the wound healing drugs, SS and PG, were equally effective; c) that laser, laser+SS and laser+PG were five and seven-fold better compared to the sole effect of SS and PG, respectively; d) that laser, laser+SS and laser+PG were equally effective; and e) that laser treatment is the best single modality for enhancing rat wound healing in an unimpaired healing conditions. While our *in-vitro* studies proposed the usefulness of laser+PG and laser+SS in a GF starved and nutrient deficient culture conditions, our *in-vivo* study was done in a normal wound-healing model.

Various researchers have shown the efficacy of photons in wound healing. Others have tried to elucidate the mechanisms regarding its effect. The question as to how photons are incorporated or absorbed by the tissue, the biochemical processes that are initiated when it is absorbed by photo-acceptor molecules or cellular organelles, etc., are areas that need some attention. But due to the complexity of the photochemical and biochemical processes involved and the expense that goes with it, no single group of investigators have done a thorough investigation on the effects of photons at the molecular level, including how anti-oncogenes and proto-oncogenes are regulated during light treatments. The control of cancer and the acceleration of wound healing are very much related, in the sense that when one wants to control proliferation of cancer cells, the inhibitory dose of photons could be used. And when one wants to accelerate wound healing, the dose of photons triggering the mechanism of cancer cell proliferation are employed, except that cell proliferation is controlled and undergoes differentiation and maturation.

Pilot studies using multi-wavelength LED treatment in oval full-thickness wound and first degree burns in normal and impaired wound healing models are ongoing. Its goals are to determine the efficacy of multi-wavelength LED therapy and to further ascertain the efficacy of light+drug combination therapy in an impaired-wound-healing condition. Studies on light dosimetry and its synergy with pharmacological agents are directed toward optimal enhancement of wound healing especially during impaired healing conditions, to reduce the suffering of those afflicted, as well as their families, who are deprived of financial resources due to the staggering cost of medical care.

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