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An In Vitro Histological Study of Human Skin Wound Soldering Using 980 nm Diode Laser in Continuous versus Pulsed Modes.

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Abstract: Background: Laser skin wound soldering offers many distinct advantages over conventional closure and laser welding techniques. Objective : to compare the histological effects of human skin wound soldering using 50 % human albumin solder and compound charcoal photosensitiser with 980 nm diode laser acting in various modes of action and parameters. Study Design/Materials and Methods: In this *in vitro* experimental study , Multiple 3-4 cm long full thickness incisions in a specimen of human skin were soldered using a 4 mm spot diameter beam of 980 nm diode laser(at different laser parameters and modes of action) with 50 % human albumin solder mixed with the compound charcoal at 5 % W/V concentration .After obtaining a successful wound soldering , the wound edge were excised and then studied histologically. Results: Although a single pulse per shot , spot by spot soldering technique has resulted in a weaker soldering of the wound experimentally, yet it was less tissue harmful than the continuous mode laser, inspite that the later had resulted in a stronger wound soldering. A strong wound closure didn't happen using repetitive pulsed laser mode at any of the tested parameters or action modes. Conclusion: A single spot by spot , pulsed 980 nm laser wound soldering is less tissue harmful than the continuous mode laser soldering although it has resulted in a weaker soldered wound initial tensile strength.

Introduction

Despite the numerous advantages of laser tissue soldering, the collateral thermal injury and the low tensile strength at the soldering site has prevented its regular clinical use. To avoid all that, laser soldering at variable laser parameters and modes of action are being tested . The photosensetizer absorb the incident laser beam at certain wavelength and converts the energy to heat in the tissue and the solder, raising the temperature of the connective tissue matrix and the solder both of which then undergoes thermal changes that lead to the joining of the wound edges together (Bassam2009 ,Niemz 1996).
The 980-nm diode laser has been chosen in this study because it has the advantage of emitting a laser that has a relatively high absorption peak by skin and solder water while less for melanin ensuring an even deeper penetration of laser energy of the skin (A. Murat 2006) .
This *ex vivo* study carried on human skin for the first time aim to evaluate the effectiveness of 980nm diode laser in laser wound soldering at various parameters and modes of action and to determine the histological effects of these on the wound edges and the surrounding tissues.

Materials and Methods

All experiments have been conducted at the Institute of Laser for Postgraduate Studies “Biomedical department” at Baghdad University /Baghdad /Iraq in the period from 1 /7/ 2010 to

1/3/2011.Human skin, harvested from the surgically excised paniculus of a 45 years old woman who have undergone an Abdominoplasty procedure at Al Kadhimyah teaching Hospital, was used. A 980 nm diode laser device (VELAS 60, China) was used. The tip of the optical fiber delivering the output laser of this device was kept about 2 mm above the target site during all applications and the spot size of the laser beam has been fixed at 4 mm in diameter. Variable Laser settings and parameters of this laser device were used in this work. The compound charcoal has been used as a photosensitiser at a concentration of 5 % w/v with 50 % human albumin solution solder. The albumin sensitizer mixture was then stored at 4 Celsius until use. Multiple full thickness 3-4 cm length incisions were made in the human skin sample then 50 % human albumin solution with 5 % W/V charcoal photosensitiser mixture was added to the wound using a syringe and cannula . After wound edges approximation by the dental tweezers, any excess of the solder was wiped away by a cloth or a blunt metal in order to leave just a thin layer of the solder inside the wound and along its edges .Upon laser beam irradiation of the soldered filled wound ,photo bleaching then coagulation and solidification of the solder and photo sensitizer mixture was noted thereby holding the wound edges approximated together then letting them to cool down back to same surrounding tissue temperature after which the strength of the successfully soldered wound was tested by a gentle traction force applied on one wound edge. The criteria for a successfully soldered wound was a full thickness (depth) wound filling by a laser solidified solder, i.e. transmural wound soldering, that make wound edges inseparable despite application of a reasonable traction force to them. Those successfully soldered wounds then have been excised and the samples were kept in 10 % formaldehyde solution then sent for histopathological studies using Heamatoxillin Eiosein a staining method to confirm the soldering process and exclude collateral thermal damage of the wound edges. Indicators of thermal injury to the epidermis were looked for in histological sections as nuclei shrinkage and cytoplasmic vacuolization, multiple basal lacunae formation, partial basal layer separation and complete epidermal ablation. The presence or absence of tissue carbonization was also searched for as a sign of severe thermal tissue injury. Any successful laser welding or soldering experiment was repeated 3 times at same laser parameters setting to confirm the reproducibility of the result.

Results

The study showed that by :

A: By using *the continues mode* 980 nm laser with 4 mm /sec. back and forth scanning movement, a strong wound soldering was obtained only at the following parameters :

5 Watt power (38.9 Watt/cm^2) for 150 sec. exposure time, 6 Watt power (47.77 Watt/cm^2) for 90 sec. exposure time, 10 Watt power (79.6 Watt/cm^2) for 60 sec. exposure time, 12 Watt power (95.5 Watt/cm^2)for 30 sec. exposure time or 13 Watt power (103.5 Watt/cm^2) for 10 sec. exposure time.

Histologically, a mild and reversible thermal damage was seen in the epidermis. Fortunately, tissue carbonization didn't happen but only with high laser power as high as 20 watt peak powered with 90 second exposure time case (159.23 Watt/cm^2) (figure 1).

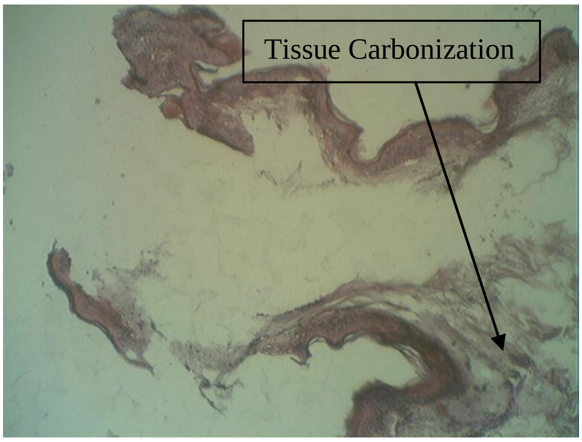
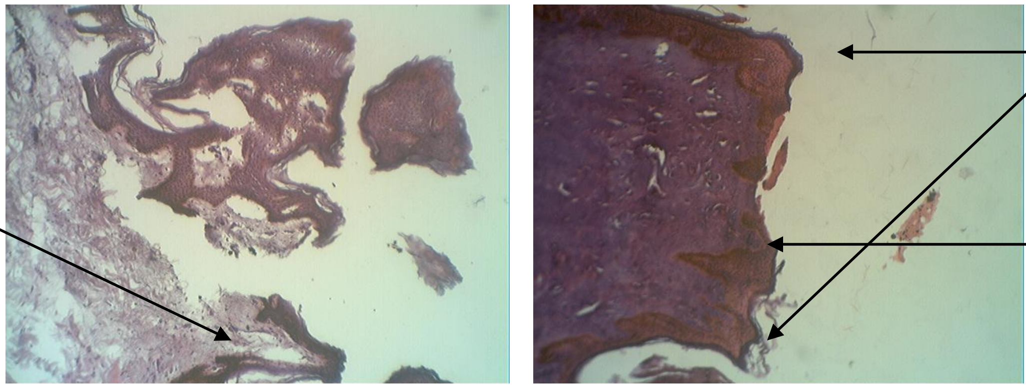


Fig. 1: Carbonization of the epidermal cells at 20 watt peak power 90 sec. exposure (159.23 W/cm^2) lasered soldered wound despite the solder temperature was still less than 60 degree Celsius.

The severity of the epidermal cellular thermal damage has increased when either the number of passes over the solder and wound i.e. the duration of exposure and hence total energy delivered (figure 2) and /or the peak power (figure 3, 4) was increased.

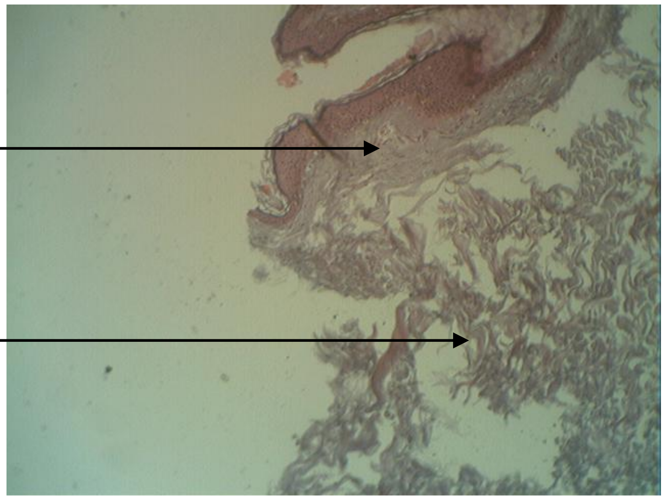
These slides also has revealed that albumin solder did actually intermingle with the native collagen in all successfully transmurally soldered wounds (figure 2 B and figure 5) but not in superficially soldered wounds (figure 2 A).



A
5 W power (38.9W/cm^2) 60 sec. exposure time

B
5 W power (38.9W/cm^2) 150 sec exposure time

Fig. 2: Reversible thermal damage was more severe in longer exposure time soldering case (B) even at same power density irradiation .The solder intermingling with native skin collagen was more perfect in the longer exposure time photograph(B).



8 W (63.7 W/cm^2) 30 sec. exposure

Fig.3 : Minimal thermal damage with weaker soldering is seen in shorter exposure duration although a higher peak power and power density was used.

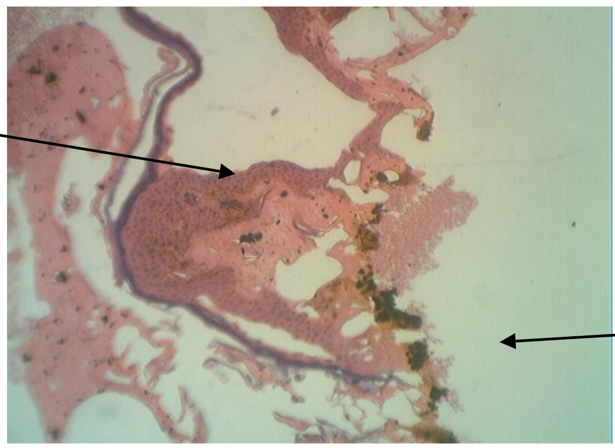
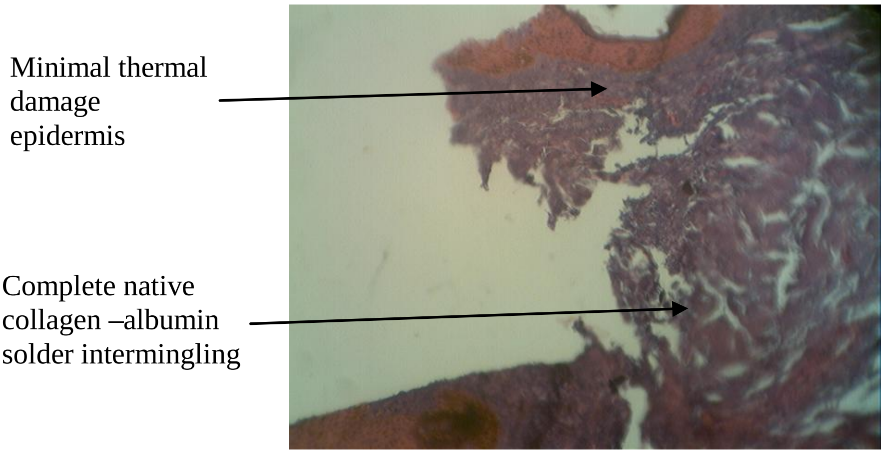


Fig. 4: Thermal damage of epidermis at 13 W peak power (103.5 W/cm^2) 10 sec. exposure soldering showing a big vacuole formation below stratum corneum of epidermis.



6 watt (47.77 W/cm^2) 90 sec. exposure time

Fig. 5: Minimal thermal damage with perfect collagen solder intermingling at 6 W peak power (47.77 W/cm^2) 90 sec. exposure time CW laser irradiation .

B: Using *a single pulse per shot, spot by spot technique* with various peak powers and pulse durations, soldering of human skin has proved to be possible only with a relatively much higher energy setting, more than those used in the previous continuous lasering technique. By this soldering technique ,a strong transmural full thickness human wound soldering has happened successfully only at 13 W peak power at 1 second pulse duration (103.5 J/cm^2), 18 W peak power at 0.8 second duration (114.46 J/cm^2) pulses and 20 W peak power at 0.6 second duration (95.54J/cm^2) pulses.

This soldering technique also proved to be less tissue harmful than the continuous laser mode irradiation soldering technique (Figure 6 and 7).

Inspite that intermingling of the collagen to albumin solder was also seen in these histology slide images, it has happened to a lesser extent than in continuous mode laser irradiation.

Again, the severity of thermal damage of epidermis has appeared only with highest peak power settings (figure 8) but they were at much higher peak power than those of continuous mode laser irradiation and the tissue injury was much milder in severity.

Interestingly, those experiments using single pulse per shot soldering, didn't show any transmural wound soldering as strong as that was obtained using the continuous mode laser, since the resulting solder clot was grossly rather inhomogeneous, spotty and not so water tight.

C: Using *the repetitive pulsed mode*, laser has failed to transmurarily solder any wound at the tested laser parameters (Figure 9) although a solder clot has actually formed. Those experimental results were true also at the cellular level. There was no interwining of the native collagen-albumin solder in any applied parameter despite a successful superficial solder clotting may have happened in them as shown in the slide image of figure 10.

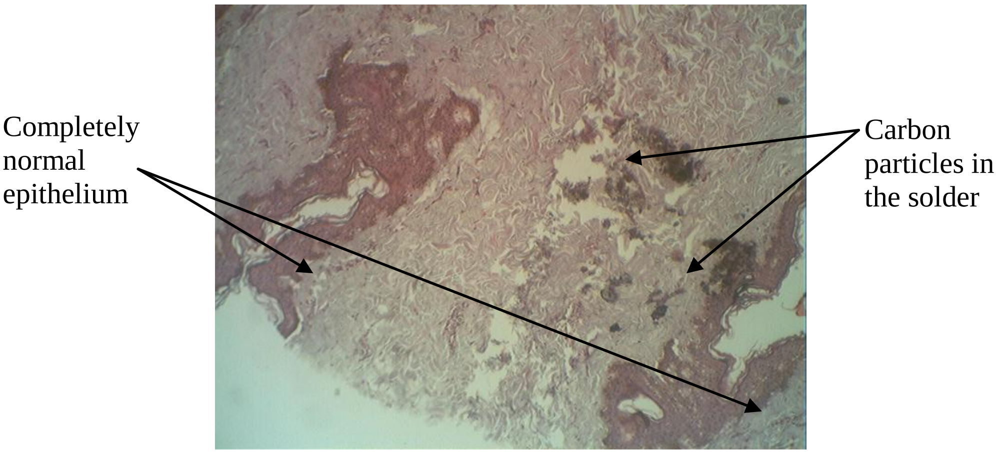


Fig. 6: A successful transmural wound soldering slide image at 13 W 1 second pulse duration (103.5 J/cm^2) single pulse per spot technique. The epithelium looks completely normal.

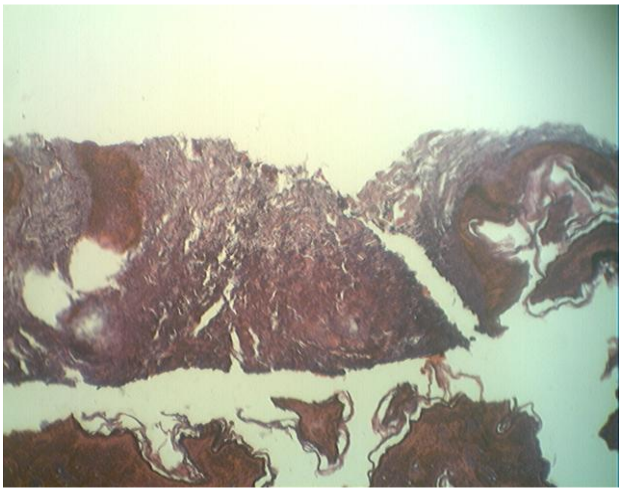


Fig. 7: A successful transmural wound soldering slide image at 18 W 0.8 second pulse duration (114.46 J/cm^2) single pulse per spot technique. The epithelium looks completely normal.

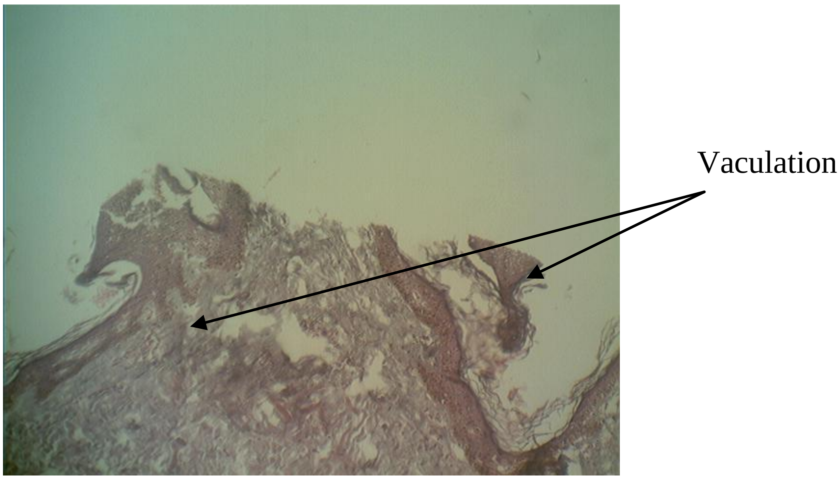


Fig. 8: A successful transmural wound soldering slide image at 20 W 0.6 second pulse duration (95.54J/cm^2) single pulse per spot technique. The epithelium shows mild reversible thermal damage in form of vaculation.

A B C

Fig. 9 : An intact superficial solder clotting (B) at 20 W peak powered 1 sec. pulses for 17 sec. exposure time only (A) .The solder clot (C) was not adherent to the wound margins.

Fig. 10: no collagen –albumin solder intermingling seen at 8 W power 0.5 second pulse duration 0.05 sec. pulse off time parameter despite formation of a relatively strong sold clotting.

Discussion

In this *in vitro* experimental study, the histology of human skin wound soldering was compared with a 980-nm laser system acting in various modes of action for the first time. The aim was to verify whether there is any difference in the soldering achieved with this laser when it's used in continuous, single pulse per spot or in the repetitive pulsed modes and which laser mode will yield the strongest and is less thermally harmful on tissues adjacent to the wound edges.

It's quite inappropriate to directly compare the laser parameters and its histological effects when laser is utilized to achieve laser assisted closure of human skin wounds in this study with the other animal skin based studies since human skin differs a lot from animal skin in its layers thickness, blood flow, thermal and optical properties. There is paucity of literature for studies conducted on real human skin especially using the same laser wavelength adopted in this study.

A quick review of those studies on animal skin revealed an initial delay healing phase that happens in the first few days following the laser wound closure in most of these studies indicating that there was always some laser induced cellular thermal damage or at least inflammation of the skin margins that delays the start of the wound healing process .This then have lead to a temporary decrease in the wound tensile strength in first few days following the laser action on the wound. Several studies have found that this delay can be avoided when real time temperature measurement control systems was adopted in laser assisted wound closure(Andre 2005, Atsumasa 2010, Bleustein CB 2000, Bleustein 2000, Brosh2004, Byrd 2003 ,Capon 2001, Charles 2007, DeCoste 1992, Elaine 1992, Haşim 2006 , Jason 2009, Kirsch 1996, Lars 2010, Lauto 1998, Lauto 1999, Lauto A 1998, Lauto 2004, McNally 1999, McNally 2000, Mia 2001, Massicotte 1998, McNally 1999, Myron 1977, Nathaniel 1999, Nathaniel 2000, Oz MC 1989, Saba 2010, Simhon 2001, Simhon 2004, Simhon 2007, Small 1997, Stadelmann 1988, Tang 2000, Phillips 1999, Poppas 1996, Poppas DP 1996, Wider 1991, Wolfgang 2002, Yoko 2005).

In the *continuous laser mode*, this study has revealed an increase in tissue damage upon increasing the power density or number of laser passes over the wound even when the solder temperature were kept below 60 degree Celsius most of the time..This means that increasing the total energy delivered to the region would increase the harmful effect of laser on tissue. A possible explanation of this may be due to loss of the solder heat sink effect with the continuous prolonged laser exposure as the solder and the tissue water was evaporating from the region (Christopher 2001). Insufficient cooling of tissue due to the loss of this heat sink has resulted in the subsequent buildup of temperature in the surrounding regional tissue leading to an increase in thermal tissue damage (Leo 1999).

In *pulsed laser mode ,single pulse per shot ,spot by spot soldering technique* ,the results revealed the need for a much higher laser device energy setting than the continuous laser mode .This is quite explainable since what is important in any soldering technique is to raised the temperature of the tissue and solder water to about 60 degrees celcius in order to achieve the coagulation of the albumin protein and de-coiling then recoiling of tissue native collagen with intermingling of both (Raymond 2005) ,a process that depends on the laser energy absorption by solder and tissue water and hence conversion of it to heat .In pulsed laser mode ,the total amount of laser energy delivered to this volume of water per unit time exposure would certainly be less than that delivered when the same laser using the same power density will act in the continuous mode .Moreover, the solder water will quickly dissipate away the buildup of the local temperature generated at the single pulse impact site .This has then a double action : it will prevent excessive heating up of the solder and tissue minimizing the tissue thermal injury but at the same time, it would require a much higher laser device energy setting in order to achieve the net regional temperature raise necessary to achieve the solder clotting and collage solder intermingling or the device should fire in repetitive pulsed mode rather than single pulse per shot mode.

The thermal damage of the epithelium using this soldering technique was more severe with the shorter pulse duration setting .In other words, longer pulse durations seems less tissue harmful than shorter one (Tianhong 2003).Haşim Özgür et al has found a similar result in their study on live rats (Haşim 2006) .

What was also grossly noticeable is that wound soldering using this technique has yielded a much weaker wound closure which was also not so water tight .The single pulse per shot ,spot by spot soldering seems to cause a very localized raise in solder and tissue temperature ,just enough to cause a local spot soldering ,contrary to the whole wound one piece solder clot that would result from wound closure by the continuous laser mode action .

This finding however contradicts Hashim et al 2006, Capon et al 2001 and A Murat et al 2006 findings in their live rat studies. A possible explanation for this is that their studies were a wound welding with spot overlap rather than a wound soldering one so there was no liquid solder to dissipate the laser deposited heat in the tissue and to cause this spotty low strength soldering wound closure.

Nathaniel M. Fried et al actually also have concluded that continuous laser irradiation actually yields stronger wound closure than spot by spot laser irradiation method .They used a scanned CW laser system .This has provided several advantages over a spot welding technique, including a smoothing out of inhomogeneities in the laser beam profile and a more uniform delivery of radiation along the wound site (Nathaniel 1999 and 2000).

The most sticking result that this study have unroofed is the inability to solder any wound by laser acting in *the repetitive pulse mode* at the tested laser parameters even though coagulation of the solder did actually happen but interestingly, it was not adhesive to wound edges. Obviously, the repetitive pulsed laser photo thermal effect was indeed very localized to the target solder at the tested laser parameters and that there was no enough time for heat diffusion from this target site or solder to the surrounding wound margin tissue to take place that would normally allow the intermingling of the deep dermal layer heat denaturated semi liquid collagen fibers (that actually become a gelatin) with the heated albumin of the solder that upon cooling down again would form a single one strong solid cement like substance that strongly bond the wound edges together(A. Zając 2008).

Ironically, the undesirable heat diffusion away from the laser intended target site which is responsible for all undesired collateral thermal damage in most clinical laser applications become a necessity in laser assisted wound closure process. The way of solder and wound edge heating obtained by the laser acting in the repetitive mode is not synonymous with continuous mode action even when maximum pulse duration an repetition rate was used . Temel Bilic et al achieved rat wound closure using a continuous and repetitive pulsed mode laser irradiation at 200 ms on 200 ms off pulses, each mode for 5 second exposure from a 1980 nm Thulium laser. The results also showed a weaker wound closure though less thermal tissue damage signs in repetitive pulsed mode than in the continuous mode even at same power setting (Temel 2009).

Christopher et al also has successfully soldered wounds using this laser repetitive modality. That study has found that the power density is the most important factor in deciding whether thermal tissue damage is to happen or not (Christopher 2001).

To sum up , in order to achieve a strong transmural wound soldering then laser acting in continuous mode at some ideal parameters would guarantee this to happen but ironically, at same time, it may cause some thermal tissue damage and delays wound healing process . A low energy setting with a long exposure time is thus recommended; otherwise, temperature-controlled laser systems with constant surface temperature feedback are highly advisable.

Use of laser in pulsed mode in spot by spot soldering ,on the other hand ,though would be less harmful to the wound margins but would not result in a wound closure neither as strong as nor as water tightly sealed as that obtained by the continuous mode lasers. Even in this approach, a relatively higher energy setting and long pulse durations are required and thus it would appear as if laser is actually acting in an interrupted continuous mode heating fashion rather than a homogenous heating of the entire dermal skin layer or the solder as when it takes place in an actual continuous mode laser irradiation.

Laser soldering using the repetitive pulsed mode was not successful neither experimentally nor histologically at the tested laser parameters and further future studies with different laser parameters are thus recommended.

A real time temperature controlled laser systems are highly advisable to be used in them for more accurate results.

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دراسة نسيجية خارج الجسم الحي للصاق جروح جلد الانسان باستخدام ليزر اشباه الموصلات 980 نانومتر بنمط عمل مستمر مقارنة بنمط عمل نبضي .

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الخلاصة: المقدمة : الصاق الجروح بمساعدة الليزر ذو مزايا وفوائد عديدة مقارنة باغلاق الجروح بالطرق التقليدية أواللحام بالليزر. الغاية:دراسه التاثير النسيجي للصاق جروح جلد الانسان باستخدام خليط من لاصق 50% البومين بشري و كاربون مركب بمساعدة ليزر أشباه الموصلات 980 نانومتر باستخدام انماط عمل وطاقات ليزر مختلفة. المواد وطرائق العمل: لقد استخدم في هذه الدراسة خارج الجسم الحي عينات من جلد الانسان حيث تم استحداث عدة جروح طولية قطعية بطول 3-4 سم كاملة العمق خلال عينة الجلد هذه ثم تمت محاولة غلقها باللصق بتعريضها لليزر اشباه الموصلات بطول موجي 980 نانو متر وقطر البقعة 4 ملم باستخدام طاقات وأنماط عمل ليزر مختلفة مع اضافة 50% ألبومين بشري يحتوي على 5 % كربون مركب كعامل متحسس للضوء.لقد تمت مقارنة وتحليل النتائج مختبريا ونسيجيا. النتائج: ان لصاق الجروح بالليزر تكون اقل ضررا على الانسجه عندما يستخدم نمط عمل نبضي بطريقة نقطية منفرده ولكنها اضعف من حيث المتانه مقارنة باللصق بالليزر عندما يعمل الليزر بنمط عمل مستمر . لم ينجح اللصق بالليزر عندما يعمل بطريقة النبض المتكرر . ألأستنتاجات: أن لصق جروج جلد الانسان باستخدام الليزر تكون اقل ضررا على الانسجه ولكنها اقل متانة ايضا عندما يعمل الليزر بنمط عمل نبضي بطريقة لصق نقطية مقارنة بانماط العمل الاخرى.هناك الحاجة لإجراء المزيد من الدراسات المستقبلية قبل الاستخدام والتطبيق الفعلي ألسريري لهذه الطريقة الجديدة .