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Preliminary Results of In-Vitro Skin Tissue Soldering using Gold Nanoshells and ICG Combination

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***Abstract*—**Laser soldering is based on applying some soldering material (albumin) onto the approximated edges of the cut and heating the solder (and the underlying tissues) by a laser beam. Endogenous and exogenous materials such as indocyanine green (ICG) are often added to solders to enhance light absorption. Gold nanoshells are new materials which have an optical response dictated by the plasmon resonance. The wavelength at which the resonance occurs depends on the core and shell sizes, allowing nanoshells to be tailored for particular applications. The purposes of this study was use combination of ICG and different concentration of gold nanoshells for skin tissue soldering and also to examine the effect of laser soldering parameters on the properties of repaired skin. Two mixtures of albumin solder and different combinations of ICG and gold nanoshells were prepared. A full thickness incision of 2×20 mm^2 was made on the surface and after addition of mixtures it was irradiated by an 810nm diode laser at different power densities. The changes of tensile strength σt due to temperature rise, number of scan (N_s), and scan velocity (V_s) were investigated. The results showed at constant laser power density (I), σt of repaired incisions increases by increasing the concentration of gold nanoshells in solder, N_s and decreasing V_s. It is therefore important to consider the tradeoff between the scan velocity and the surface temperature for achieving an optimum operating condition. In our case this corresponds to σt =1800 gr/cm^2 at I~ 47 Wcm^{-2}, T ~ 85ºC, N_s =10 and V_s=0.3mms^{-1}.

***Keywords*—**Tissue soldering, Gold nanoshells, Indocyanine green, Combination, Tensile strength

I. Introduction

LASER-ASSISTED tissue closure in skin has been investigated in recent decade [1]. Laser soldering is based on applying some soldering material (such as albumin) onto the approximated edges of the cut and heating the solder (and the underlying tissues) by a laser beam. Laser tissue soldering (LTS) using a diode laser and an indocyanine green (ICG)/albumin solder has been shown to be an effective technique in surgical reconstruction by providing a watertight sealant with minimal damage to underlying tissue [2]. Decoste et al [3] and Bass et al [4] have shown that ICG is an effective chromophore for this purpose.

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Gold nanoshells are type of nanoparticles composed of normally silica cores coated with an ultrathin gold layer. They are biologically inert and optically tunable [5]. The optical properties can be tuned by varying the relative size of the core and the thickness of the shell. In particular, these particles can be designed to absorb near infrared light, and when irradiated by a laser, provide an exogenous vehicle to convert optical energy into heat. The interest lies in the unusually large absorption cross-sections, which are much larger than geometric cross-sections, at wavelengths corresponding to surface modes in the particle [6]. Nanoshells are currently being used for a variety of biomedical applications and have been shown to be non toxic and highly biocompatible [7]. For example, in a cancer therapy application, nanoshells have been injected systemically, accumulated within tumors due to vascular permeability, and then used for photo thermal ablation due to their ability to rapidly heat upon exposure to near infrared light [8].

The use of nanoshells has several advantages over ICG. The average diameter of nanoshells is about 100 nm so that reduced diffusion from the site of treatment and concentrating heating at the interface is avoided, which should in effect the minimize damage to surrounding tissue. Hence another advantage of nanoshells is that they are more photo-stable since their absorption properties are determined by their physical structure [9]. Additionally, nanoshells are more strongly absorbing than ICG on a per particle or molecule basis.

Indocyanine green has an absorption cross-section on the order of ~$10^{-2}0m^2$, while nanoshells have absorption cross-section on the order of ~10^{-14} m^2, so nanoshells are approximately a million-fold more effective absorbers [7]. In the previous works we used an 810 nm diode laser with BSA solder containing ICG and gold nanoshells alone [8, 9]. The purpose of this study is to evaluate the effect of combination of ICG and different concentrations of gold nanoshells and laser processing parameters on the quality of repaired skin.

II. Materials And methods

A 40×50 cm2 fresh depilated piece of sheep skin was cut into pieces of 4×5 cm2. After preparation, a full thickness cut of 2×20 mm^2 was made on the skin surface using 11" blades. Protein solder solution was prepared with using 25% BSA (Sigma Chemical Co.) 0.25mg ICG and two different concentrations of gold nanoshells mixed in HPLC grade water. The set up of laser soldering system is shown in Fig.1.

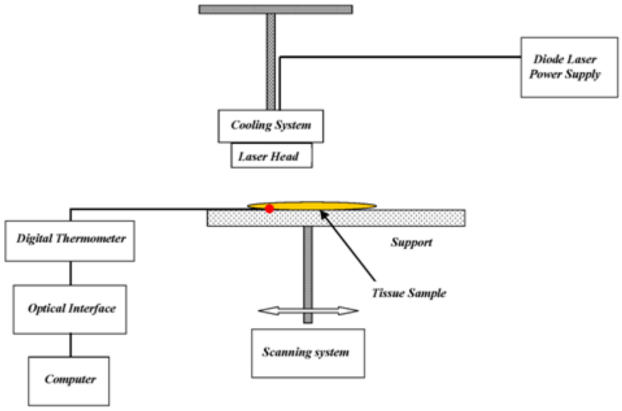

Fig.1 Experimental setup for in-vitro laser soldering of skin. The system includes :(1) 810 nm diode laser, (2) scanning system, (3) digital thermometer, (4) computer

For each combination of ICG and gold nanoshells the solder (50μl) was applied to the cut edges, and the edges were brought into contact with one another and then irradiated with different power densities in the dynamic mode. The temperature rise at the skin during the irradiation was measured with a digital thermometer. The laser beam moved from one place to the next, along the cut line, so that each line slightly overlapped the previous one. Tensile strength measurements were performed to test the integrity of the resultant repairs immediately following the laser procedure using a load machine (Zwick/Roell, HCT 25/400 series). Skin strips were inserted into the head of the loading machine, and tensile strength measurements were carried out with a constant crosshead return speed of 2 mm/min. An automatic data acquisition system was used to collect the data during the tensile test. The tensile strength was calculated by dividing the maximum load at the rupture point (F) by the cross-sectional area of the specimen. For each solder combination and laser parameter the experiment was repeated five times.

For basic histological examination the skin specimen were fixed in a 4% formaldehyde solution and transferred to baths of concentrated ethanol to remove the water. This step was followed by alcohol removal using a hydrophobic clearing agent (such as xylene), at which point the samples were embedded in paraffin. Finally, the specimens were cut into 4-μm slices and stained with haematoxylin eosin (H&E).

III. Results and discussion

Tensile strength of repaired specimens as a function of laser irradiance and the solder combination is shown in Fig.2. As it is seen from the figure 2, not only the value of σ increases with power density but also increases with increasing the concentration of nanoshells in combination.

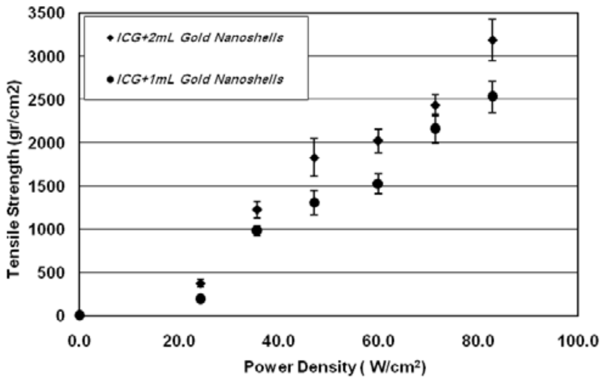

Fig. 2 Variation of tissue tensile strength with laser power densities at different solder combinations

Tissue temperature rise as a function of laser power density for different solder combination is shown in Fig.3, where each peak was observed when the laser beam was coincided with the tip of thermometer.

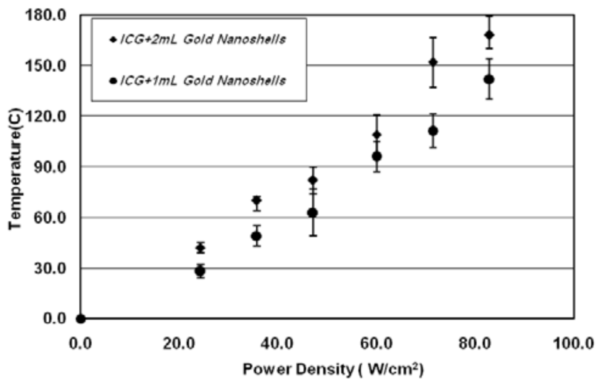

Fig. 3 Tissue temperatures rises as a function of laser power density and solder combination

The effect of number of scans (N_s) on the tensile strength of repaired skin for two different combinations at constant irradiance (83W/cm^2) is illustrated in Fig.4. For the given irradiance, tensile strength increases by increasing the number of scans which may be explained by the fact that not only the depth of thermal denaturation increases with temperature rise, also by overlapping tales of thermal signals before the complete cooling of scanned region.

Fig. 5 indicates that, at a constant I, an increase in the scan velocity cause the corresponding value of σ to decrease due to a smaller temperature rise.

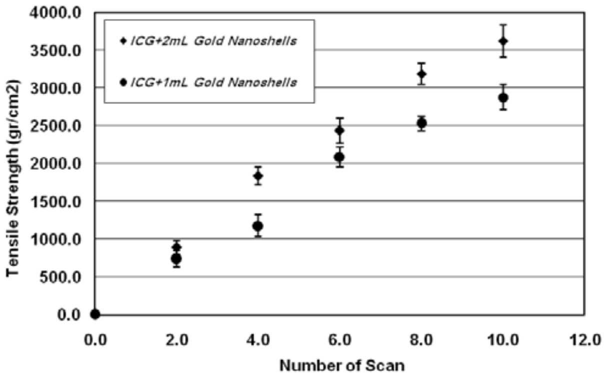

Fig.4 Effect of number of scans and nanoshells concentration on tissue tensile strength at a given power density.

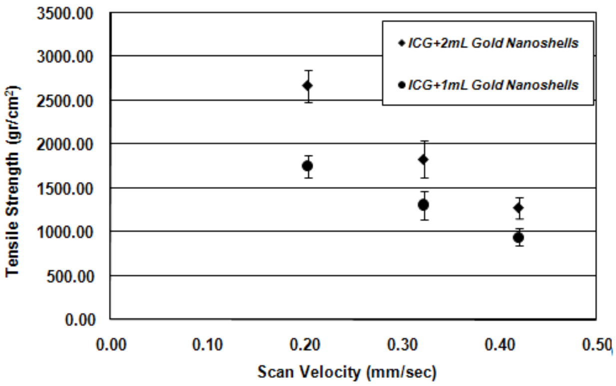

Fig.5 Effect of laser scans velocity on tissue tensile strength at a given power density.

Histological images of laser soldered incisions by 47 Wcm^{-2} and is illustrated in Figure 6. A very mild burn was observed on the right margin of the bonded wound, represented by a basophilic stain (H&E, original magnification × 10).

Our results indicate that laser power density is the most important parameter affecting solder temperature during the process of laser tissue soldering. While varying gold nanoshells concentration within the solder resulted in few changes in the temperature profile, it may be manipulated to better reflect the completion of heating of the solder varying laser power density, however, affected both the rate of rise in temperature of the solder as well as the maximal temperature reached during laser activation. The findings of this study show that temperatures recorded in the beneath of the skin can reach up to 80°C. Increasing tensile strength with power density has a same trend in compare with nanoshells and ICG alone but in each power density the tensile strength in the case of ICG + GNs is higher .For example the tensile strengths of repaired skin at power destiny of 47 Wcm^{-2} are 1190±90, 1422±120 and 1830 ± 215 $grcm^{-2}$ for ICG, gold nanoshells and ICG + GNs respectively.

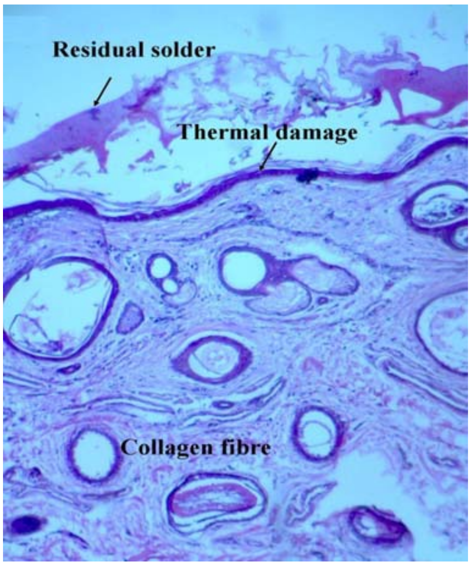

Fig.6 Histological images of laser soldered incisions at 47 W/cm^2.

Studies by DeCoste et al. [13] using ICG dye alone showed that at temperatures above 60°C a significant release of ICG and a steep increase in the temperature. This presumably resulted from the release of ICG from collagen as the molecule reached its fibrillar melting transition temperature. In a study by Kirsch et al. [14], temperatures during laser activation with an ICG/albumin solder were determined within the solder and underlying dermis over 1 minute of activation. Their results showed temperature within the solder reached 101 °C, while temperature in the superficial and deep skin was 70 and 65°C, respectively. Fung et al [15] results showed that wounds repaired at higher temperatures (85 and 95°C) were observed to have lower mea wound strength than wounds repaired at lower temperatures (65 and 75°C) .

The effect of number of scan on tensile strength of repaired skin is also studied. At eight scans and power density of 83Wcm^{-2}, the tensile strength of soldered skin with ICG alone is 1920±80 $grcm^{-2}$, which at the same condition this value is 2407±124 and 3189±139 $grcm^{-2}$ for gold nanoshells and combination of ICG and gold nanoshells respectively. Also with combination of these light absorbers we can achieve the acceptable tensile strength in compare with previous studies with lower number of scans. For example using four scans the tensile strength reached to 1840±122 $grcm^{-2}$ which is comparable to tensile strength of soldered skin with ICG using eight scans. This finding can be important for clinical applications if less operation time to be considered.

As it is show in Fig.5, by decreasing the scan velocity the tensile strength of repaired skin increased for different concentration of gold nanoshells in combination of solder. For a constant scan velocity, for example 0.3 $mmsec^{-1}$, tensile strength of skin soldered with ICG, gold nanoshells and combination of ICG and gold nanoshells was 1350±120, 1422±160 and 1830±210 $grcm^{-2}$ respectively. The latter value is same as the tensile strength achieved by Vs of 0.2$mmsec^{-1}$

for ICG alone. Table 1 represents a comparison between LTS results.

TABLE I
COMPARISON OF LTS RESULTS WITH DIFFERENT SOLDER AND LASER PARAMETERS. COLUMN (1) IS OBTAINED FROM REF. 11 AND COLUMN 2 FROM REF. 12.

	ICG	GNs	ICG+GNs
I (47Wcm^{-2})	1075±90	1422±120	1830±210
I (60Wcm^{-2})	1400±120	1610±110	2022±140
I (83Wcm^{-2})	1920±110	2407±162	3189±240
Ns=4	630±80	930±80	1180±122
Ns=8	1300±75	1860±95	2530±90
Vs=0.2mms^{-1}	1800±140	2120±190	2670±180
Vs=0.3mms^{-1}	1350±190	1422±160	1830±210
Vs=0.4mms^{-1}	1080±90	1200±125	1280±120

REFERENCES

[1] M.Gulsoy, Z.Dereli , H.Tabakogh: Laser Med Sci., 2 (2006) 5
[2] M.McNally, B.S.Sorg , E.K.Chan , A.J.Welch , Lasers Surg Med., 26(2000) 346.
[3] S.D. DeCoste, W. Farinelli, T. Flotte, Lasers Surg Med., 12 (1992) 25.
[4] L.S. Bass , Lasers Surg Med., 17, (1995)315
[5] M. Hu, J.Y. Chen and Z.Y. Li: Chem. Soc. Rev. 35, (2006) 1084.
[6] J.B. Jackson, S.L. Westcott, L.R. Hirsch, J.L.West ,N.J. Halas: Appl. Phys. Lett. 82, (2003) 257.
[7] J.M. Stern, J.Stanfield ,W. Kabbani , J Urol., 179, (2008) 748.
[8] Y.Wang, W.Qian, Y.Tan , Biosens Bioelectron .,23 (2008) 1166.
[9] J. Malicka, I. Gryczynski,C.D. Geddes , J.R. Lakowicz: J. Biomed. Opt. 8(3), (2003) 472.
[10] L.R.Hirsch, R.J. Stafford, J.A. Bankson, Proc. Natl.Acad .Sci. USA,. 100 (2003) 13549.
[11] M.E.Khosroshahi, M.S.. Nourbakhsh, S. Saremi, F. Tabatabaee F, Laser Med Sci., 25,(2010) 207.
[12] M.S.Nourbakhsh,M.E.Khosroshahi, WASET Conf., (2010) Rome, Italy
[13] S.D. DeCoste, W. Farinelli, T. Flotte , R.R. Anderson, : Lasers Surg. Med. 12, (1992) 25.
[14] A.J. Kirsch, J.W. Duckett, H.M. Snyder, D.A. Canning, D.W. Harshaw, Urology 50, (1997) 263.
[15] L.C.Fung, G.C.Mingin, M.Massicotte, D.Felsen , D.P. Poppas: Lasers Surg. Med. 25, (1999) 285.