Evaluation of Carbon Dioxide (CO₂) Laser Incision Characteristics in A Viable Human Skin

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Abstract
Throughout the last decades, cutaneous laser surgery has gained popularity among surgeons. This kind of surgery based on the principles of selective photo-thermolysis theory. CO₂ laser selectively target water-containing tissue, applying controlled tissue vaporization. The tissue ablation and the zone of residual thermal damage is accompanied with a deeper tissue coagulation. A piece of viable intact human skin was taken. Multiple CO₂ laser incisions were carried out with different laser parameters prior to be totally excised, then a histopathological examination were done for these skin samples. Slides were examined under light microscope to evaluate thermal enjury zones, wound edges, and cutaneous blood vessels status. Histopathological examination showed that the three thermal zones were chenged wherever the laser parameter changed.

Keywords: Carbon Dioxide (CO₂) Laser, photodamage, thermal depth, skin tissue.


Introduction
Laser is a device used to amplify light into very intense beam of almost single wavelength by the stimulated emission of radiation [1]. Incisional CO₂ laser surgery was first performed some four decades ago, but eventually fell from favor because of thermal injury, resulting in a wound that had decreased tensile strength and in many cases healed with unacceptable appearance. The re-emergence of CO₂ laser surgery in the early 1990s, that is attributed to the development of a newer generation of lasers that can produce soft tissue incisions with a reduced amount of thermal damage at wound edges [2]. The CO₂ laser is suggested to be the first laser that produces a well-controlled, localized, precise thermal injury that can offer both incisional and ablative properties for surgical applications [3].

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CO₂-Laser-Tissue Interaction:
Light interacts with matter by the processes of absorption, transmission, reflection, refraction, and scattering. Absorption of light by the irradiated material results directly in an increase in the energy content of the material. If the absorbed photon of laser light is not re-emitted or a photon with less energy than the absorbed one is re-emitted, some energy remains, which is usually converted into thermal motion or heat in the absorbing material [4]. The optical reflection determines the proportion of the beam, which will effectively penetrate the tissue. However, for wavelengths longer than visible range, the reflection tends to diminish considerably. The thermal action, depending on the degree and the duration of heating, this can be summarized into three main actions, Hyperthermia: it signifies a moderate increase in the temperature, by a few Celsius degrees (C°), which consequently can lead to temperatures of 41°C to 44°C for a few dozen of minutes, causing retarded cell death due to the damage caused to the enzymatic processes. It is a difficult process to control, and is therefore not widely used in practice, Coagulation: is an irreversible necrosis without immediate destruction of the tissue. The temperature reach 50-100°C for duration of around one second causes desiccation, whitening, and retraction of the tissues by denaturing the proteins and collagen, Volatilization (Vaporization): it is loss of substance. Various tissue constituents disappear as smoke at a temperature of over 100°C, in a relatively short time, around one tenth of a second. [5,6]. Since the CO₂ laser is highly absorbed by water, a major component of soft biological tissue, that vaporizes resulting in cell burst, so disrupting the individual cell result a controlled, localized, and precise thermal injury, that can offer both incisional and ablative properties. The CO₂ laser has a relatively short penetration depth, that creates a precise irradiation zone, while the large pulse width interacts thermally with the tissue [7]. In 1983, Anderson and Parrish described the theory of selective photo-thermolysis, which is the process of controlling three variables (wavelength, pulse duration, and fluence). This theory revolutionized laser therapy explaining a method of producing localized tissue damage [8]

Materials & Methods
Histopathological evaluation of certain CO₂ laser incisions in a viable human skin samples for the purposes of CO₂ laser evaluation as a cutaneous surgical tool.

Materials
Laser Device, Viable Human Skin Sample, ordinary surgical set, and formalin solution 10%.

Laser Device (CO₂ laser system):
Technical specification: The device (BLITZ 50 SV) is equipped with a Carbon Dioxide (CO₂) laser source, emitting an infrared beam and a Helium: Neon (He: Ne) laser source, emitting a visible red beam. The He: Ne beam is coaxial with the infrared beam and therefore it is used as an aiming beam.
Emission specification: These two laser sources have the following emission specifications:
CO₂ laser source emission specifications
- Wavelength: 10.6 µm
- Output beam diameter: 6 mm
- Output power level in CW mode: 1 W to 50 W step 1W
Output peak power: 80 W max
Max pulse length: 500 ms
Frequencies in PW mode: 1Hz – 100 Hz

He: Ne laser source emission specifications
- Wavelength: 632.8 nm
- Output beam diameter: 0.61mm
- Output power level: 5.0 mw

Surgical characteristics (articulated arm): The articulated arm is an optical system, which conveys laser radiation. The mechanical accuracy of the articulated arm allows the CO\textsubscript{2} laser beam to travel inside it and along its axis however the arm is oriented. Articulated arm characteristics:
- Maneuverability: 7 axes
- Transmission efficiency: 85%
- Operating radius Surgical probes: 2” (2 inches focal length lens) 4” (4 inches focal length lens) 5” (5 inches focal length lens)

Operative characteristics
The CO\textsubscript{2} laser source can operate either in continuous wave -CW- or in pulsed wave -PW. Operative characteristics:
- Aiming beam (He: Ne): Continuously visible
- Emission modes: Continuous (CW), pulsed (PW)
- Shutter on CO\textsubscript{2} laser beam path: Electromechanical controlled by footswitch.
- Shutter control mode: Continuous or timed

Methods
Spot size calibration: The laser spot size can be calculated mathematically by using the equation: [1].

\[ D = \frac{4f\lambda}{\pi Dm} \]  
Where: \(D\) = spot size, \(f\) = lens focal length, \(\lambda\) = Laser wavelength, \(Dm\) = laser beam diameter.

The estimation of the actual spot size for each probe was done by the use of wooden tongue depressor, which is irradiated by the laser, and then the resulted effect of the laser on the wood is measured under light microscope.

Output power calibration: By using an infrared power meter, calibration of the output power of the CO\textsubscript{2} laser system is done prior to the use of the device.

Nine samples of viable intact human skin were taken from a twenty years old female. The samples were taken from the upper breast area, from a skin that will be sacrificed. About 5cm in diameter circular skin area was divided into nine section pieces, then a laser incision was done in each piece with a different parameters, as shown in table 1.
Table 1: Different laser parameters used in the study.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Power/W</th>
<th>Frequency/Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>50</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>100</td>
</tr>
</tbody>
</table>

The piece of skin dissected and removed; then nine samples were prepared and kept in 10% formalin solution. The samples were prepared as slides, for histopathological examination. The slides were examined under light microscope to evaluate the thermal injury zones, wound edges, and blood vessels status adjacent to the laser cuts.

**Results**

Laser Device: calibration of the output power indicates that the actual delivered output power is about 80% from the power presented on the LCD (Liquid Crystal Display) of the device, as shown in table 2.

Table 2: Results of the power output calibration

<table>
<thead>
<tr>
<th>LCD/W</th>
<th>Power meter/W</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
</tr>
<tr>
<td>3</td>
<td>2.4</td>
</tr>
<tr>
<td>4</td>
<td>3.2</td>
</tr>
<tr>
<td>5</td>
<td>4.0</td>
</tr>
<tr>
<td>6</td>
<td>4.8</td>
</tr>
<tr>
<td>7</td>
<td>5.6</td>
</tr>
<tr>
<td>8</td>
<td>6.4</td>
</tr>
</tbody>
</table>

The laser device is supplied with three different surgical end probes, each with different focal length lens, which result different spot sizes, as shown in table 3. The actual measured spot size of each probe was larger than the mathematically measured spot size.

Table 3: Spot size of each surgical end probe, (measured mathematically)

<table>
<thead>
<tr>
<th>Probe</th>
<th>Focal length/mm</th>
<th>Spot size/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>2&quot;</td>
<td>50.8</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Skin samples: CO$_2$ laser incisions with different parameters, as shown in table 4, were examined under light microscope, exploring the thermal damage in the tissue. Three thermal injury zones were seen in the laser wound as described and shown in figure 1.

Figure 1: Zones of laser thermal injury

<table>
<thead>
<tr>
<th>Slide</th>
<th>Power W</th>
<th>Frequency Hz</th>
<th>Pulse width sec.</th>
<th>Energy/pulse J</th>
<th>Fluence J/cm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.4</td>
<td>25</td>
<td>0.02</td>
<td>0.048</td>
<td>21.05</td>
</tr>
<tr>
<td>2</td>
<td>2.4</td>
<td>50</td>
<td>0.01</td>
<td>0.024</td>
<td>10.52</td>
</tr>
<tr>
<td>3</td>
<td>2.4</td>
<td>100</td>
<td>0.005</td>
<td>0.012</td>
<td>5.26</td>
</tr>
<tr>
<td>4</td>
<td>4.8</td>
<td>25</td>
<td>0.02</td>
<td>0.096</td>
<td>42.10</td>
</tr>
<tr>
<td>5</td>
<td>4.8</td>
<td>50</td>
<td>0.01</td>
<td>0.048</td>
<td>21.05</td>
</tr>
<tr>
<td>6</td>
<td>4.8</td>
<td>100</td>
<td>0.005</td>
<td>0.024</td>
<td>10.52</td>
</tr>
<tr>
<td>7</td>
<td>9.6</td>
<td>25</td>
<td>0.02</td>
<td>0.192</td>
<td>84.21</td>
</tr>
<tr>
<td>8</td>
<td>9.6</td>
<td>50</td>
<td>0.01</td>
<td>0.096</td>
<td>42.10</td>
</tr>
<tr>
<td>9</td>
<td>9.6</td>
<td>100</td>
<td>0.005</td>
<td>0.048</td>
<td>21.05</td>
</tr>
</tbody>
</table>

CO$_2$ laser creates a wound that can be characterized as thermal injury along the wound edges. The extent and type of injury depend on both the power density used and the time that the tissue is exposed to that power. The cutaneous wound that created by laser beam, focused beam mode, produces three zones of thermal injury:

Central zone: represents the actual incision, from which the tissue has been vaporized.

Surrounding the central zone is the thermal damaged tissue zone, which is represented by an area of relatively amorphous tissue, directly adjacent to the wound edges.

Peripheral zone: adjacent to the second zone, the tissue looks like normal tissue, in some areas with mild inflammatory reaction, which results from the elevation of the temperature to a level insufficient to cause tissue thermal damage. On observation, the histological changes showed that the best parameters used to incise the skin with minimal damage to epidermis as shown in slide number 6 [figure 2], where the fluence was 10.52 J/cm$^2$. Although the same fluence was used in sample number 2, more thermal damage to the epidermis had been shown in slide number 2, due to the longer pulse duration (low frequency) used.
[figure 2]. The result of higher fluence used in slides number 4, 7, 8, and 9 indicating more thermal damage to the wound edges (epidermis) and wider coagulate necrosis zone, due to either low frequency used or high energy, as shown in figure 2.

Discussion and Conclusions

During the use of CO$_2$ laser, it seems to be that the vaporization of intracellular water results in a thousand-fold expansion of cells, the production and explosive release of steam causes ablation of the biological tissue and the thermal energy is conducted to the deeper tissue planes produces a zone of permanent heat damage zone evidenced by protein denaturation. The volume or thickness of this zone is directly proportional to the power density of the laser at the superficial lased tissue interface as well as on the duration of laser energy exposure and this is agreed with [9, 10, 11]. The non-target adjacent tissue has radiant thermal energy histological changes consisting of a narrow zone of carbonized tissue, an underlying desiccated tissue necrosis zone, and a peripheral zone of edematous damaged tissue [12,13]. The resultant infolding of the wound edges which was the reaction of the epidermal tissue to the thermal energy of the CO$_2$ laser beam used, might be due to the shrinkage of the wound edges. In conclusion: The more pulse duration or the longer exposure time, the more the lateral thermal tissue damage. This can be avoided by using very short pulse duration (high frequency), with high peak power of the pulse, and a relaxation time more than the pulse duration to overcome the heat conduction to the adjacent tissue of the wound. This is of benefit for the excision or debulking of lesions lying sub-cutaneously where the need for minimal thermal damage to the dermis and epidermis. Since the epidermis is the first layer which face the laser energy, and it contains a high concentration of intracellular water, and the microscopic observation of the tissue, gave the idea that, the majority of tissue vaporization occurs in the first laser exposure over the epidermis, that agreed with Brian S. Biesman, and Jemshed Khan, comments, the characteristic features of CO$_2$ laser cutaneous incision include the infolding of the epidermis and a zone of epidermal thermal injury [2]. The area, which is surrounding the intended target tissue that is irreversibly damaged due to the thermal effect of the laser, gives the character of coagulation necrosis. The longer the laser energy is applied, the wider the zone of coagulation necrosis. The positive effect of this coagulation that is any vessel smaller in diameter than the area of coagulation is coagulated, providing hemostasis during surgery and would lead to the sterility of the wound after surgery. The irradiated tissues constrict against the proximal vasculature and the vessels shrink as a result of the laser energy applied to the collagen composition of their walls, which results in vessels wall shrinkage leading to enhance hemostasis associated with laser surgery.

References:


Figure 2: the histological changes of sample slides that showed the best parameters to incise the skin.