Improvement of disfiguring skin conditions by laser therapy
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TIMED EXPOSURE 10600 NM CO₂ LASER DRILLING:
A NOVEL APPROACH FOR SMALL DERMAL TUMORS
ABSTRACT

Background: benign dermal tumors often are removed by (electro-) surgery. When tumors are multiple, ablative laser surgery is an alternative treatment. Results depend largely on the physicians surgical skills. Carbon dioxide (CO₂) lasers with timed exposure, deliver a fixed number of pulses in a preset interval of a fraction of a second. These laser settings may diminish variation of outcomes and facilitate standardization. Objective: to compare shapes and sizes of lesions created by either conventional or timed exposure CO₂ laser drilling in freshly excised human skin.

Methods: five samples of freshly excised human skin were exposed to conventional CO₂ laser drilling and timed exposure CO₂ laser drilling with six different settings. Histological changes of the skin were compared in H&E stained slides, and depth, width and ablated surface area of the lesions were all measured using Leica Qwin software.

Results: the standard deviation of the ablated surface area in all lesions created by timed exposure CO₂ laser drilling was seven times smaller than after conventional CO₂ laser drilling. The lesions were less variable in shape and size and had even borders without irregularities.

Conclusion: timed exposure CO₂ laser drilling is characterized by a low variation in lesion size and shape and offers standardization of treatment and comparison of results. This makes it a favorable treatment over conventional CO₂ laser drilling in treating multiple benign dermal tumors that are comparable in size.
INTRODUCTION

The presence of benign but multiple dermal tumors can have a large impact on a patient’s quality of life. The treatment of choice for these tumors is (electro-) surgery, but also cryotherapy and laser surgery have been proposed as treatment options for improving the appearance of benign dermal tumors.\textsuperscript{1-4} Especially in patients with a large number of dermal tumors, laser ablation is a practical alternative for conventional surgery. By drilling holes with either the 2940 nm erbium:yttrium aluminum garnet (Er:YAG) laser or the 10600 nm carbon dioxide (CO\textsubscript{2}) laser, dermal tumors like fibrofolliculomas, eruptive vellushair cysts, syringomas, trichoepitheliomas, milia and steatocystoma multiplex have been treated, all with varying results.\textsuperscript{5-11} The conventional drilling technique lacks standardization and results depend largely on the physicians’ surgical skills. Also, there is the risk of scarring when inadequately used. The conventional laser technique is handheld and delivered pulse by pulse. Timed exposure settings limit light emission to a preset interval of a fraction of a second. Using a pulsed mode, a fixed number of pulses is delivered by the laser in that time. A short exposure time minimizes motion artifacts. This method facilitates the standardization of the laser-skin interaction by the assumption of creating uniform, reproducible columns of ablation independent of the treating physician. The aim of this study was to compare lesion shapes and sizes after both conventional CO\textsubscript{2} laser drilling and timed exposure CO\textsubscript{2} laser drilling in freshly excised human skin.

METHODS

Laser procedure

Freshly excised human skin samples were obtained from the Department of Plastic Surgery at the Academic Medical Centre (AMC) Amsterdam after informed consent from the patient. Within 30 minutes after either mammoplasty or abdominoplasty, skin samples were collected and transported in an expanded polystyrene box to minimize temperature loss. In our research department, excess subcutaneous fat was removed and to prevent dehydration, skin samples were placed between two saline (0.9% NaCl) soaked 10x10 cm gauze pads on a digital warming plate. When skin samples had reached a temperature of 37°C (±0.5) at the level of the subcutis, measured through an incision, upper gauze pads were removed and irradiation with the 10600 nm Ultrapulse CO\textsubscript{2} laser (Ultrapulse Encore, Lumenis, Santa Clara, CA, USA) was started. With the timed exposure drilling technique, six lesions were created. Using a 1mm spot size, the laser was set to deliver 16, 21 and 26 pulses of 100 mJ respectively, at a fixed timed exposure of 100ms. Using a 2 mm spot size, 31, 61 and 91 pulses of 100 mJ were delivered. With the conventional drilling technique, according to the manufacturer’s guidelines, the 0.2 mm spot size was used, to create a lesion comparable in size to the 2 mm spot lesions. Each of the seven drilling settings was used on each skin sample by the same researcher. Laser settings are shown in detail in Figure 1.
**Histological analysis**

Directly after irradiation, 5mm biopsies were taken from the treated areas, as well as a control biopsy from a non-treated area. The biopsies were fixed in 4% buffered formalin and paraffin embedded. Between five to eight 3µm sections were cut with a 100µm distance and mounted on silane coated slides. All slides were stained with hematoxylin and eosin (H&E) using the Sakura Tissue Tek Stainer and Coverslipper (Sakura Finetek Europe B.V., Alphen aan den Rijn, The Netherlands). By comparing all slides macroscopically, a selection of the optimal slides, with the deepest lesion, was made per modality. Images of these selected slides were taken and lesion dimensions were measured using a Leica DM LB microscope (magnification 50x), a DC200 camera, and Leica Qwin Software (Leica Microsystems, Wetzlar, Germany).

**Statistical analysis**

All measurements were inserted into a Microsoft Office Excel 2003 (Microsoft Corp, Redmond, WA, USA) database. Results are presented as means with standard deviations.

**RESULTS**

A total of five different skin samples, Fitzpatrick skin types II-V, was collected directly after either mammoplasty or abdominoplasty. All were irradiated with the 7 different settings of the CO\textsubscript{2} laser, resulting in 35 different lesions. Histological images of each modality after H&E staining are shown in Figure 1. All ablated areas had very regular borders in contrast to those created with conventional CO\textsubscript{2} laser drilling. We noticed a U- or V-shape in lesions created with timed exposure settings that was comparable in each of the 5 different skin samples. With the conventional drilling however, none of the five different lesions were comparable in shape to one another.

The variations in the amount of ablated tissue in the five different skin samples are indicated in Figure 2 for each modality individually. We found a large difference in the ablated surface areas created with all modalities of timed exposure CO\textsubscript{2} drilling compared to conventional CO\textsubscript{2} drilling. The mean ablated surface area for the timed exposure CO\textsubscript{2} laser drilling lesions is $52 \times 10^4 \mu m^2$ (with a mean SD ± $9 \times 10^4 \mu m^2$) and the mean ablated surface area for the conventional CO\textsubscript{2} laser drilling is $145 \times 10^4 \mu m^2$ (with a SD ± $60 \times 10^4 \mu m^2$). The standard deviation is seven times smaller for the timed exposure CO\textsubscript{2} drilling lesions.

In Figure 3 the relation between increasing dose and the depth and diameter of lesions resulting from timed exposure CO\textsubscript{2} laser drilling is presented. With the 1mm spot, there is a partial linear dose-response relation with regard to lesion depth. A mean depth was reached of 902, 1225 and 1238 µm respectively, with increasing number of pulses. The width of the lesion hardly increases with increasing dose. With the 2mm spot, there is a complete linear relation between increasing dose and the depth of the lesions. A mean depth was reached of 561, 1008 and 1645 µm respectively, with increasing number of pulses. Again, the width of the lesion hardly corresponds with the increasing dose.
DISCUSSION

For the past decades, ablative lasers have been used to treat a variety of benign dermal tumors. The conventional drilling technique, using several consecutive ablative pulses, has been proposed to reach the deeper dermis thus removing the entire lesion. However, clinical results are often disappointing because of post-inflammatory pigmentary changes, scarring, or due to a high recurrence rate.\textsuperscript{5,6,9,12}

There is a very small therapeutic window between aggressive ablative laser treatment with a high risk of scarring, and cautious treatment with a high risk of recurrence. Due to intra- and interphysician treatment differences, the variation between lesions created with conventional laser drilling, although performed with the same settings, is too high for reproducible results.

This is the first study to investigate the possibility of standardization of ablative laser drilling by using timed exposure, thus making it a reproducible therapeutic option for skin disorders with multiple benign dermal tumors. We found that the standard deviation of the ablated surface area after timed exposure CO\textsubscript{2} laser drilling is seven...
times smaller than after conventional laser drilling. Also, the lesions are much more comparable in shape and size and have even borders with minor irregularities. This makes lesions after timed exposure laser drilling more comparable to one another, even when different physicians perform the treatment. Therefore, we propose timed exposure CO\textsubscript{2} laser drilling as a promising option to standardize the treatment of multiple small dermal tumors of comparable size.

A linear dose-response relation between the energy and the depth of the lesion was expected but only partially found. Also, we did not find a difference in diameter between the two spot sizes on a histological level. These outcomes can be caused by measurement errors. There was only one observer to perform all measurements. The mismatch between spot size and lesion diameter can be caused by post-laser contraction, when shrinkage occurs due to skin heating, formalin fixation, and post-biopsy collapse. All of these post-laser effects may be more pronounced when higher energy levels are used. Another suggestion for the mismatch between spot size and lesion diameter are the beam profile and the settings used. A Gaussian beam profile as present in the used CO\textsubscript{2} laser will create smaller lesions than the actual laser beam, especially with lower energy density as used with the 2 mm spot The fact that we only used H&E staining can also attribute to measurement errors. Viability assays like LDH (lactate dehydrogenase) might better differentiate between viable and necrotic tissue.\textsuperscript{13}

Other limitations to this study are the small study population, and the fact that we used ex vivo skin, which may respond differently to laser irradiation than in vivo skin. However, Hantash et al. reported a strong agreement between lesions created in ex vivo skin and in vivo skin. We used an ex vivo skin model comparable to that of Hantash’s ex vivo study.\textsuperscript{13,14} A final limitation is that we assessed variance of test spots between different

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{ablation_area.png}
\caption{The mean variations in ablated area of five different skin samples are presented (as standard deviation) for each modality individually; timed exposure settings with 16, 21, 26, 31, 61 and 91 pulses respectively (numbers 1-6), and the conventional drilling technique (number 7).}
\end{figure}
Figure 3. Depth and width of lesions (ablated tissue alone, or in combination with necrotic tissue) when using the CO$_2$ laser drilling technique with timed exposure settings, at increasing energy levels.

tissue samples but not within the same tissue sample. Consequently variation may be overestimated in our study due to unquantifiable differences in substrate.
The benefit of timed exposure settings in CO$_2$ laser therapy is the creation of uniform lesions in a fraction of a second, making outcomes highly reproducible even between users. Moreover, once the optimal settings for a certain condition and location are established, they can be used for other patients as well. Multiple lesions not exceeding the size of 2-3 mm seem best suited for this technique. Except visual removal of the lesion, there are no useful immediate clinical endpoints for the laser drilling technique. We recommend to always perform a trial treatment on a few lesions with 2 or 3 different laser settings.
Summarizing, timed exposure CO$_2$ laser drilling offers the prospect to treat deeper located benign dermal tumors with a low variation in lesion size and shape, thus
promoting standardization of treatment and comparison of results. Future clinical research is necessary to determine the appropriate settings for all different indications.

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