**CO₂ and diode laser for excisional biopsies of oral mucosal lesions**

A pilot study evaluating clinical and histopathological parameters

**Key words:** CO₂ laser, diode laser, benign intraoral lesions, biopsies, thermal side effects

**Summary**  
**Purpose:** The present pilot study evaluates the histopathological characteristics and suitability of CO₂ and diode lasers for performing excisional biopsies in the buccal mucosa with special emphasis on the extent of the thermal damage zone created.

**Patients and Methods:** 15 patients agreed to undergo surgical removal of their fibrous hyperplasias with a laser. These patients were randomly assigned to one diode or two CO₂ laser groups. The CO₂ laser was used in a continuous wave mode (cw) with a power of 5 W (Watts), and in a pulsed char-free mode (cf). Power settings for the diode laser were 5.12 W in a pulsed mode. The thermal damage zone of the three lasers and intraoperative and postoperative complications were assessed and compared.

**Results:** The collateral thermal damage zone on the borders of the excisional biopsies was significantly smaller with the CO₂ laser for both settings tested compared to the diode laser regarding values in μm or histopathological index scores. The only intraoperative complication encountered was bleeding, which had to be controlled with electrocauterization. No postoperative complications occurred in any of the three groups.

**Conclusions:** The CO₂ laser seems to be appropriate for excisional biopsies of benign oral mucosal lesions. The CO₂ laser offers clear advantages in terms of smaller thermal damage zones over the diode laser. More study participants are needed to demonstrate potential differences between the two different CO₂ laser settings tested.

**Introduction**

After the development of the first laser, a ruby laser, in 1960 by Theodore H. Maiman (Maiman 1960), medical and dental researchers soon began to study different types of lasers for extra- and intraoral surgical procedures. Because of its affinity for water-based tissues, the carbon dioxide (CO₂) laser has become a favorite instrument of oral surgeons for treatment of pathologic conditions of the oral mucosa (Sulewski 2000, Bornstein et al. 2003a). The CO₂ laser has been recommended to treat benign oral lesions, such as fibromas, papillomas, hemangiomas, gingival hyperplasias with different causes (idiopathic or due to side effects of medications), aphthous ulcers, mucosal frenula or tongue ties (ankyloglossia), as well as premalignant lesions such as oral leukoplakias (Strauss 2000, Bornstein et al. 2003b, Suter & Bornstein 2009). Some reports on the use of the CO₂ laser also support the possibility of treating malignant oral diseases in early stages (for example T₁N₀ carcinomas) with excisional biopsies (Duncavage & Ossoff 1986, Strauss 2000). A study demonstrated dissemination of cancer cells into the blood circulation upon incisional biopsies with the scalpel, resulting in an increased risk of me-
tastasis (Kusukawa et al. 2000). Here, the CO2 laser, with its sealing effect on vessels smaller than 500 μm in diameter, could be an advantage and therefore even prevent occult micrometastasis.

More recent reports have also mentioned the diode laser with wavelengths ranging from 810 to 980 nm in a continuous or pulsed mode as a possible instrument for soft tissue surgery in the oral cavity (Goharkhay et al. 1999, Saleh & Saafan 2007). Based on the photothermal effect of the CO2 and diode lasers, lesions of the oral mucosa are removed with an excision technique or ablation/vaporization procedures (Van Hillegersberg 1997). The major advantages mentioned in the literature for the use of CO2 and diode lasers are minimal postoperative swelling and scarring, improved wound healing, and decreased postoperative pain (Strauss 2000, Bornstein et al. 2005b).

Additionally, both lasers are reported to have some advantages over a scalpel in soft tissue surgery. Unlike the scalpel, the laser instantly disinfects the surgical wound, and due to its hemostatic effect affords largely bloodless surgery, allowing a non-contact type of operative procedure and therefore no mechanical trauma to the tissue (Pick & Pecaro 1987).

The purpose of the present pilot study was to evaluate the histopathological characteristics and suitability of CO2 and diode lasers for performing excisional biopsies of similar lesions of the oral mucosa. Therefore, primary outcome parameters were the visible collateral thermal effects produced by both lasers in the histological specimens. Secondary parameters evaluated intra- and postoperative complications encountered with both laser types.

Materials and Methods

Patient Selection

Fifteen patients with similar intraoral pathological lesions were consecutively enrolled in the present pilot study. All patients were referred by their private dental practitioner from the Bernese region. The study group comprised seven men and eight women with a mean age of 48.7 years (range 36 to 69 years). All lesions were fibrous hyperplasias located in the buccal mucosa (Fig. 1A, Fig. 2A, Fig. 3A). The patients were randomly allocated to three different groups comprising three different treatment modalities. The treatment modality used to excise the lesion (CO2 laser in a continuous or pulsed mode or diode laser in a pulsed mode) was decided upon with a precalculated computer model (www.randomization.com).

An initial clinical examination consisting of a questionnaire covering past medical and dental history, and a thorough extra- and intraoral examination were performed on all patients. The collected data were evaluated and a clinical diagnosis for the type of mucosal lesion was established. All patients were informed of the advantages and disadvantages of laser surgery and offered an alternative approach with the scalpel. All patients had to sign an informed consent before commencement of the study. The examination and data collection were performed following the guidelines of the World Medical Association Declaration of Helsinki (version 2002; www.wma.net/e/policy/b3.html).

Laser Surgery

Two different class IV lasers were used for the surgeries: a CO2 laser (Spectra DENTA Surgical Carbon Dioxide Laser, MAX Engineering Ltd., Gyeonggi-Do, Korea) with a wavelength of 10.6 μm, an articulated mirror arm, and straight handpieces through which the laser beam is applied to the oral soft tissue in non-contact treatment mode, and a diode laser (lexcision class II, lexion AG, Radolzell, Germany), with a wavelength of 810 nm, fibers with a diameter of 400 μm, and straight handpieces through which the laser beam is applied to the oral soft tissue in contact type treatment mode. All procedures with the laser were performed in a separate operating room, the entry to which was clearly marked with a warning sign.

The CO2 laser was used with a spot size of 0.2 mm in two different modes: in one group in continuous wave mode (cw) with a power of 5 W (A), and in the second group in a char-free mode (cf) with a frequency of 140 Hz, a pulse duration of 400 μsec and pulse energy of 33 mJ (power of 4.62 W; B). Power settings for the diode laser were 5.12 W in pulsed mode (pulse-width of 10 μs, 20,000 pulses per second, 25 W per pulse for all lesions removed surgically; C).

The patients, the surgeon, and the operative staff wore safety glasses throughout the procedures. A wet wooden tongue blade was placed behind the target to minimize possible tissue damage by aberrant laser beams. The surgeries for exophytic or superficial soft tissue lesions were all carried out with a local anesthetic containing articaine (Ultracain D S 4%, Aventis Pharma AG, Zurich, Switzerland). Soft tissue removed from the oral cavity was sent to the pathologist for histological evaluation and analysis of the collateral thermal damage zone in the tissue (Fig. 1B, Fig. 2B, Fig. 3B).

All wounds in the oral mucosa were left to open granulation and secondary epithelialization; therefore, no sutures were required. An adhesive wound paste (Solcoseryl Dental Adhesive Paste, Valeant Pharmaceuticals Switzerland GmbH, Birsfelden, Switzerland) containing the topical anesthetic polidocanol (hydroxypolyethoxydodecan) was given to protect the wound initially, assist the wound healing process, and ease pain and discomfort. The follow-up visits were scheduled two weeks and one month after surgery. Intra- and postoperative complications were noted and compared for both types of lasers used.

Histopathological Analysis

All biopsy specimens (Fig. 1C, Fig. 2C, Fig. 3C) were fixed in 4% neutral-buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin-eosin for conventional histopathological evaluation. The specimens were all evaluated by the same pathologist (HJA), blinded to the type of laser used. After establishing the histopathological diagnosis of the lesion assessed, the pathologist measured the maximal width of the collateral thermal damage zone in the specimen in μm and classified it using the following index:

- 0 0–100 μm width of collateral thermal damage zone
- 1 100–200 μm
- 2 200–300 μm
- 3 300–400 μm
- 4 400–500 μm
- 5 >500 μm

Statistical Analysis

In addition to descriptive statistics, categorical variables were compared using the Fisher’s exact test and continuous variables using the Kruskal-Wallis rank sum test. Furthermore, the 95% confidence intervals of the mean outcome measures (width and index score of the damage zone) were calculated for each treatment group. The significance level chosen for all statistical tests was p < 0.05. The statistical software package S-Plus Professional (Version 6.2, Insightful Software, Palo Alto, CA, USA) was used for all analyses.
There were no statistically significant differences between treatment groups with respect to gender (p = 0.3007) and age (p = 0.2263). In addition, the diameters of the excised specimens in the different treatment groups were also not significantly different statistically (p = 0.8805).

The histopathological evaluation of the thermal damage zone in the 15 excised specimens demonstrated the widest damage zone for the diode laser (mean value 754.23 μm or mean index score of 5, Tab. I; Fig. 1D, Fig. 2D, Fig. 3D). There was a statistically significant difference with respect to histopathological outcomes (damage zone in μm p = 0.0071 or index scores p = 0.0056) between the three treatment groups. The respective 95% confidence intervals of the mean outcome measures in-

Fig. 1 The preoperative, postoperative, and histopathological aspects of a typical fibrous hyperplasia selected for excision with the CO₂ laser in a cw mode (group A). A) Preoperative aspect; B) Postoperative aspect following laser surgery; C) Excised biopsy specimen before fixation in formalin; D) Histopathological aspect (hematoxylin-eosin stain; * = zone of thermal damage); E) Postoperative aspect one month after excision.

Results

The 15 lesions excised were all located in the buccal mucosa, all clinically and histopathologically diagnosed as fibrous hyperplasias, and had an average diameter of 8.3 mm (range from 5 to 15 mm). During laser surgery of the lesions in all three groups, only one type of intraoperative complication occurred. In the two CO₂ laser groups, venous bleeding occurred which could only be controlled by bipolar electrocauterization (Martin ME 82, Martin Medizin-Technik, Tuttlingen, Germany). During excisional biopsy with the diode laser, arterial bleeding had to be controlled with bipolar electrocauterization and a perivascular suture.
**Fig. 2** The preoperative, postoperative, and histopathological aspects of a typical fibrous hyperplasia selected for excision with the CO₂ laser in a cf mode (group B). A) Preoperative aspect; B) Postoperative aspect following laser surgery; C) Excised biopsy specimen before fixation in formalin; D) Histopathological aspect (hematoxylin-eosin stain; * = zone of thermal damage); E) Postoperative aspect one month after excision.

**Tab. I** Histopathological evaluation of the thermal damage zone in the three different study groups using values in μm or index scores.

<table>
<thead>
<tr>
<th>Exam/Group</th>
<th>CO₂ laser cw</th>
<th>CO₂ laser cf</th>
<th>Diode laser</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Values in μm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>225.46 (61.20–389.72)</td>
<td>233.76 (152.87–314.64)</td>
<td>754.23 (551.04–957.41)</td>
</tr>
<tr>
<td>Maximum</td>
<td>456.3</td>
<td>329.4</td>
<td>999.25</td>
</tr>
<tr>
<td>Minimum</td>
<td>134.2</td>
<td>146.4</td>
<td>587.6</td>
</tr>
<tr>
<td><strong>Index scores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>1.8 (0.18–3.41)</td>
<td>2 (1.12–2.87)</td>
<td>5</td>
</tr>
<tr>
<td>Maximum</td>
<td>4</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Minimum</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

CO₂ laser cw = continuous wave mode (cw) with a power of 5 W; CO₂ laser cf = char-free mode (cf) with a frequency of 140 Hz, a pulse duration of 400 μsec and pulse energy of 33 mJ (power of 4.62 W); diode laser = pulsewidth of 10 μs, 20,000 pulses per second, 25 W per pulse (power of 5.12 W).
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indicating that the two CO2 laser modes were statistically significantly superior to the diode laser mode with respect to the thermal damage zone, and that there was no statistically significant difference between the two CO2 laser groups.

At the follow-up visits two weeks and one month after laser treatment, no postoperative complications were recorded. Pain control after CO2 and diode laser surgery could be performed with the adhesive wound paste alone in all patients, without any additional systemic non-opiate analgesics (Fig. 1E, Fig. 2E, Fig. 3E).

Discussion

In an early study on the use of the CO2 laser in oral and maxillofacial surgery, Pecaro & Garehime (1983) examined the results after CO2 laser treatment of 40 intraoral lesions ranging from tongue ties to hemangiomas. General anesthesia was used in 28 patients. The remaining 12 underwent laser surgery with local anesthesia. Postoperatively, the laser-treated patients were hospitalized for a period of two days or less. Overnight hospitalization was required in patients only when warranted by preexisting medical conditions. Since then, the CO2 laser has become the most commonly used laser in oral and maxillofacial surgery. The development of smaller, portable office-based lasers has allowed even minor routine procedures to be treated with the laser on an out-patient basis outside of hospital operating rooms (Pick 1997). These changes are reflected in the present study, where no patient had to undergo general anesthesia and no sutures were required for any of the procedures.

With a wavelength of 10.6 μm, CO2 laser energy has a high water absorption coefficient and causes vaporization of any

Fig 3 The preoperative, postoperative, and histopathological aspects of a typical fibrous hyperplasia selected for excision with the diode laser (group C).

A) Preoperative aspect; B) Postoperative aspect following laser surgery; C) Excised biopsy specimen before fixation in formalin; D) Histopathological aspect (hematoxylin-eosin stain; * = zone of thermal damage); E) Postoperative aspect one month after excision.
water-rich tissue. Pogrel et al. 1990 studied the thermal tissue changes in five different soft tissues while evaluating 23 excisional biopsy specimens. The widest zone of thermal damage was seen in dense connective tissue (mean width of 96.1 μm) and mucosal epithelium (85.9 μm). Significantly less damage occurred in loose connective tissue (51.1 μm) and salivary glands (41.5 μm). A variable zone of reversible thermal changes (100–500 μm in width) was visible adjacent to the thermal damage zone.

The thermal side effects of the laser can further be controlled and reduced by altering the use of power, pulse duration and pulse repetition rate. In the present study, the CO2 laser was used with power settings of 5 W in a cw mode and 4.62 W using a cf mode with a frequency of 140 Hz, a pulse duration of 400 μsec and pulse energy of 33 mJ. Wilder-Smith et al. 1997 observed that wounds created with a superpulse mode showed a zone of collateral thermal damage one-half to one-third the size of that produced with a continuous mode. Similar results were found at the study in which incisions were made on 150 tissue samples from reduction mammoplasties (Spector et al. 2003). In the present pilot study, these results could not be reproduced, and neither CO2 laser mode demonstrated any statistically significant differences. This could be due to the low number of study participants. Therefore, more patients need to be included in future studies to demonstrate potential differences between the two modes of laser surgery.

A clearly different type of laser is the diode laser, introduced in dentistry and oral surgery in the mid-90s (Harris & Pick 1995). The relatively small size and lower cost of diode lasers have made them attractive to dental practitioners and oral surgeons for use in various indications (Coley 2004, Andreana 2005, Deppe & Horch 2007). Diode lasers have a wavelength between 805 and 980 nm, and, depending on the clinical procedure, are used in a continuous or pulsed mode with contact handpieces.

Studies evaluating the thermal tissue effects of diode lasers are not conclusive. In a histological comparison of four different common medical laser systems (Ho:YAG, Nd:YAG, and two diode lasers with wavelengths of 830 and 940 nm), Nd:YAG and diode lasers in contact application showed low thermal tissue effects in depth, resulting from a high power loss caused by the development of large carbonization zones at the surface of the tissue (Janda et al. 2003). In another experimental study, histological analysis to verify vertical and horizontal tissue damage as well as incision depth and width was performed in the oral mucosa of fresh pig mandibles using a diode laser with a wavelength of 810 nm (Goharkhay et al. 1999). Mean vertical and horizontal damage varied between values under 100 μm, correlating strongly with average powers used, but not with other laser parameters or the tip diameter. The authors concluded that the remarkable cutting ability and the tolerable damage zone clearly showed the diode laser to be very effective and a useful alternative in soft tissue surgery in the oral cavity. In a recent experimental study in rats, diode laser incisions performed at a power of 6 W showed a greater inflammatory reaction and delay in tissue organization at the initial stage compared to conventional scalpel surgical procedures (D’Arcangelo et al. 2007).

The evaluation of the thermal damage zone of the different groups in the present pilot study demonstrated significantly smaller diameters of thermal destruction for the CO2 laser for both settings tested (cf and cw mode) in comparison to the diode laser. Similar results were obtained in a recent histological comparison of skin biopsy specimens collected from dogs with the CO2 laser or a 810-nm diode laser (Rizzo et al. 2004). In that study, results indicated that the CO2 laser caused less thermal injury at the margins of the skin biopsies. The authors therefore recommended the use of a CO2 laser if a surgical laser is used for removal of skin biopsy specimens. Regarding these results, the clinician should be advised to be cautious when using the diode laser in precursor lesions or even initial stages of oral squamous cell carcinomas, where evaluation of the tissue margins is crucial. Although the number of participants in this pilot study was limited, the difference between CO2 laser and diode laser is clear enough that the authors have decided to use only the two CO2 laser settings (cf vs. cw mode) for further evaluation of differences between the parameters.

The literature does not contain many reports about complications during or after laser surgery; often, complications were not even recorded systematically. Horch et al. (1986) reported their experiences after CO2 laser treatment of oral premalignant lesions. During these surgeries, considerable hemorrhages or other intraoperative complications did not occur. In another study of various mucosal lesions removed with the CO2 laser, the patients experienced no complications during surgery of benign lesions or leukoplakias (Frame 1985). In treatment of more widespread malignant lesions, bleeding from arteries was reported that could be arrested with sutures or diathermy, although no exact numbers were given. In a recent case series study of 139 patients with 164 intraoral pathological lesions treated with the CO2 laser, intra- and postoperative complications and the method of pain control during and after surgery were recorded (Bornsstein et al. 2005). Nine intra- and six postoperative complications occurred. In all nine intraoperative cases, minor arterial bleeding had to be controlled with electrocauterization. One arterial (diode laser) and two venous bleedings (CO2 laser) during treatment were the only intraoperative complications observed in the patients of the present pilot study.

The most common complication after laser surgery mentioned in the literature is pain and discomfort (Pick & Pecaro 1987, Frame 1985, Bornstein et al. 2005). Postoperative bleeding is only reported for laser-assisted uvulopalatoplasty (Brandon & Strauss 2004) and was not encountered after treatment of the fibrous hyperplasias in the present study. Otherwise, decreased postoperative swelling and improved tissue healing and scarring are reported. However, in the present study, we did not evaluate these parameters with objective methods including a proper control group (scapel incision), and the number of participants was not high enough to do so. Therefore, the findings are inconclusive in that regard. The observation that laser wounds generally heal with less scar formation than do scalpel incisions is attributed to the minimal degree of wound contraction following laser irradiation as a consequence of the smaller number of myofibroblast present (Fisher et al. 1983, Chomette et al. 1991, Zeinoun et al. 2001).

Conclusions

The CO2 and diode lasers can be used as alternative instruments for excisional biopsies of oral soft tissue lesions. Intra- and postoperative complications are rare, with minor bleeding being the only complication observed during our surgeries. The thermal damage zone of the excised specimens created by the CO2 laser was significantly less pronounced than with the diode laser. Therefore, the CO2 laser is more appropriate than
the diode laser for intraoral excision of premalignant or ma-
alignant lesions where the margins of the removed specimens
must be histopathologically evaluated. Further studies with a
higher number of patients are needed to evaluate the differ-
ences between the char-free and the continuous wave mode of
the CO2 laser used.

Acknowledgments
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Dental Association (SSO grant number 235-08).

Zusammenfassung
Ziel der Studie: Die vorliegende Pilotstudie untersucht die kli-
nische Eignung und die histopathologischen Eigenschaften
des CO2- und Diodenlaser bei Exzisionsbiopsien von Mund-
schleimhautläsionen. Der Schwerpunkt der Untersuchung liegt
in der Bestimmung der thermischen Schädigungszonen.

Patienten und Methoden: 15 Patienten willigen zur laserchiru-
gischen Entfernung ihrer fibroïsen Hyperplasien im Planum
bukkale ein. Die Patienten wurden zufällig einer der drei fol-
genden Gruppen zugeteilt: Exzision mit dem Diodenlaser (25 W,
Pulslänge von 10 μs, 20000 Pulse pro Sekunde) oder mit dem
CO2-Laser (Dauerstrichbetrieb bei 5 W oder Pulsbetrieb bei einer
Frequenz von 140 Hz, einer Pulsdauer von 400 μsec und einer
Energie von 33 mJ). Die thermische Schädigungszonen, sowie
die intraoperativen und postoperativen Komplikationen wur-
den ausgewertet und verglichen.

Resultate: Die thermische Schädigungszone am Rande der
Exzisionspräparate (Messwerte in μm und histopathologischer
Index) war bei beiden CO2-Laser-Gruppen signifikant geringer
als bei der Diodenlaser-Gruppe. Die einzigen aufgetretenen
intraoperativen Komplikationen waren Blutungen, welche
jeweils mit dem Bipolar-Elektrokoagulator gestillt werden konnten.
Postoperative Komplikationen wurden keine festgestellt.

Schlussfolgerungen: Der CO2-Laser eignet sich zur Exzision von
gutartigen intraoralen Schleimhautläsionen und ist durch seine
geringe thermische Schädigungszonen dem Diodenlaser überle-
gen. Um mögliche Unterschiede zwischen den zwei Betrieb-
arten des CO2-Lasers aufzeigen, ist eine größere Probanden-
zahl nötig.

Résumé
But: L’étude-pilote présente analyse l’aptitude clinique et his-
topathologique du laser au CO2 et du laser diode pour l’exci-
sion de lésions de la muqueuse buccale. L’accent de l’étude
est mis sur l’analyse et la détermination de la zone de dommage
thermique.

Patients et méthode: 15 patients ont donné leur accord pour
l’exérèse du laser de leur hyperplasie fibreuse située au niveau de
la muqueuse buccale. Les patients ont été répartis par rando-
misation soit dans le groupe d’exclusion au laser CO2 (25 W,
durée de pulsation de 10 μs, 20000 pulsations par seconde),
soit dans l’un des deux groupes d’exclusion au laser au CO2
(mode continu de 5 W ou mode pulsé avec une fréquence de
140 Hz, une durée de pulsation de 400 μsec et une énergie de
33 mJ).

Résultats: Les zones de dommage collatérales au bord du tissu
excisé étaient significativement plus petites (mesures en μm et
index histopathologique) après l’exérèse avec le laser au CO2
qu’avec le laser diode. Les seules complications intra-opéra-
toires survenues dans les trois groupes furent des saignements
qui ont été stoppés avec l’électrocoagulé. Aucune complication
postopéraoire n’est survenue.

Conclusion: Le laser au CO2 convient à l’exérèse de tumeurs
bénignes intraorales. Grâce à sa zone de dommage thermique
plus petite, le laser au CO2 a des avantages par rapport au laser
diode. Un plus grand nombre de participants serait néces-
saires pour démontrer les différences entre les deux modes de
fonctionnement du laser au CO2.

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