Since their introduction in dental practice, lasers have provided many advantages to dentists and oral surgeons. During the application of lasers on oral soft tissues, light energy is transformed into thermal energy, which in turn heats the target tissue to produce the desired effect. In comparison to the scalpel used in surgical procedures, the laser beam is characterized by sterility and minimum bleeding during the surgical procedures, due to the sealing of blood vessels. The various effects achieved by the temperature elevation during the laser application on the soft tissue are: 1. coagulation and hemostasis; 2. tissue sterilization; 3. tissue sealing; 4. incision and excision; 5. ablation and vaporization.

However, in oral pathology, there are still many controversies among authors on the use of laser in oral surgery. This is because of the peripheral thermal damage that the laser could create, causing several problems for pathologists in the evaluation of the infiltrating potential of neoplastic or dysplastic lesions. The

### Effects of Different Laser Devices on Oral Soft Tissues: In Vitro Experience

Umberto Romeo, Alessandro Del Vecchio, Francesca Ripari, Gaspare Palia, Celeste Chiappafreddo, Gianluca Tenore, Paolo Visca

**a** Assistant Professor, Department of Odontostomatological Science, “Sapienza” University of Rome, Rome, Italy.

**b** Research Fellow, Department of Odontostomatological Science, “Sapienza” University of Rome, Rome, Italy.

**c** Researcher, Department of Odontostomatological Science, “Sapienza” University of Rome, Rome, Italy.

**d** PhD Student, Department of Odontostomatological Science, “Sapienza” University of Rome, Rome, Italy.

**e** Odontologist in Private Practice, Rome, Italy.

**f** Pathologist, Department of Cytology and Cellular Diagnostics, Regina Elena Institute, Rome, Italy.

**Purpose:** Using different lasers with various wavelengths, the main goal of the study was to evaluate the tissue damage in the first peripheral microns of oral soft-tissue specimens, the most important area for the evaluation of infiltrating potential of dysplastic lesions.

**Materials and Methods:** Er,Cr:YSGG, Nd:YAG, and two diode lasers (808 nm and 980 nm) were tested, taking bioptic specimens from pig tongues. The results were compared with a scalpel specimen. All the samples were fixed in formaline, stained with hematoxylin-eosin, and examined with an optical microscope by two pathologists.

**Results:** The 808 nm diode laser in pulsed mode and the Er,Cr:YSGG showed the best results, with less than 1 mm of marginal damage.

**Conclusion:** The results of the study suggest that lasers can be even used in bioptic investigations of dysplastic lesions, extending the edges of the excision to assure a safe histological evaluation, which is fundamental for a correct histological diagnosis.

**Keywords:** biopsy, laser, peri-incisional damage.
aim of this study was to evaluate the histological effects of various lasers on oral soft tissues, and to determine the exact extent of peripheral thermal damage.

MATERIALS AND METHODS

The study was performed in vitro on pig tongues, histologically similar to human oral mucosa. The study was conducted in cooperation with the Department of Cytology and Cellular Diagnostics of the Regina Elena Institute of Rome.

The following lasers were tested:

1) 808 nm diode (Laser Innovation; Rome, Italy)
2) 980 nm diode (Laser Innovation)
3) Nd:YAG (1064 nm, ADT; Corpus Christi, TX, USA)
4) Er,Cr:YSGG (2780 nm, Biolase; San Clemente, CA, USA).

In the first phase of the study, 9 mucosal specimens were taken with each laser, in order to evaluate the three different power settings (Fig 1). All the samples were taken by the same operator, while a second operator placed the various specimens in sterile test tubes labelled alphanumerically (eg, A1, A2, A3...B1, B2, B3 etc) containing a 10% buffered formalin solution. A control specimen (R) was taken with a scalpel. The total number of mucosal samples was 37.

The employed parameters for each laser are summarized in Table 1.

In the second phase of the study, the specimens were embedded in paraffin and stained with hematoxylin-eosin for the histological evaluation, which was performed by two different pathologists.

RESULTS

808-nm diode laser
Group A: All the samples showed wide peripheral damage, with coagulation of collagen and corion; thermal artifacts were present in the epithelial layers that disappeared at an average of 3 mm (Fig 2).

Group B: In this group, there was generally a coagulative necrosis of the subepithelial connective tissue with total destruction of the epithelium for about 1.5 mm of each section.

Group C: In all the samples of the group, the peri-incisional cellular damage was markedly reduced. The edges of the specimens showed 8 to 10 rows of damaged keratinocytes. The damage observed in the underlying connective tissue was clearly reduced (Fig 3).

980-nm diode laser
Group D: The analyzed samples showed epithelial and connective-tissue damage with homogenization of collagen and dermoepithelial detachment.

Group E: The laser thermal effect was generally very extended and the corion was damaged for more than 1.5 mm; the epithelium also was always damaged for more than 1 mm (Fig 4).
Group F: In samples “F1-3”, the peripheral damage was larger than in the previous specimens, with wide dermoepithelial detachment and corion homogenization.

Nd:YAG laser

Group G: Nd:YAG proved to be the most aggressive device. In samples “G1-3”, a wide detachment of the epithelium from the underlying connective tissue was observed; even the basal layer showed considerable damage.

Group H: The epithelium always showed severe damage, being removed for at least 1.5 mm. The damage was deeply extended, involving the subepithelial connective tissue.

Group I: This group showed the most severely damaged specimens, always presenting large detachment of the epithelium from the corion. In general, the whole epithelial surface was widely compromised and, in one specimen, less than 0.5 cm of the specimen was clearly interpretable (Fig 5).

Table 1  Irradiation parameters in the different experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Laser (WL)</th>
<th>Fiber</th>
<th>Power</th>
<th>Frequency/energy</th>
<th>%Air/H₂O</th>
<th>Fluence (Ed)</th>
<th>Irradiance(Pd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>808nm</td>
<td>320µ</td>
<td>2W</td>
<td>Cw</td>
<td>-</td>
<td>2.4 kJ/cm²</td>
<td>2.4 kW/cm²</td>
</tr>
<tr>
<td>B</td>
<td>808nm</td>
<td>320µ</td>
<td>1.5W</td>
<td>Cw</td>
<td>-</td>
<td>1.8 kJ/cm²</td>
<td>1.8 kW/cm²</td>
</tr>
<tr>
<td>C</td>
<td>808nm</td>
<td>320µ</td>
<td>2W</td>
<td>Ton 100ms-Off 100ms</td>
<td>-</td>
<td>248 J/cm²</td>
<td>2.4 kW/cm²</td>
</tr>
<tr>
<td>D</td>
<td>980nm</td>
<td>320µ</td>
<td>2W</td>
<td>Cw</td>
<td>-</td>
<td>2.4 kJ/cm²</td>
<td>2.4 kW/cm²</td>
</tr>
<tr>
<td>E</td>
<td>980nm</td>
<td>320µ</td>
<td>1.5W</td>
<td>Cw</td>
<td>-</td>
<td>1.8 kJ/cm²</td>
<td>1.8 kW/cm²</td>
</tr>
<tr>
<td>F</td>
<td>980nm</td>
<td>320µ</td>
<td>2W</td>
<td>Ton 100ms-Off 100ms</td>
<td>-</td>
<td>248 J/cm²</td>
<td>2.4 kW/cm²</td>
</tr>
<tr>
<td>G</td>
<td>1064nm</td>
<td>400µ</td>
<td>4.8W</td>
<td>40Hz/120mJ</td>
<td>-</td>
<td>95.5 J/cm²</td>
<td>3.8 kW/cm²</td>
</tr>
<tr>
<td>H</td>
<td>1064nm</td>
<td>400µ</td>
<td>6W</td>
<td>50Hz/120mJ</td>
<td>-</td>
<td>95.5 J/cm²</td>
<td>4.7 kW/cm²</td>
</tr>
<tr>
<td>I</td>
<td>1064nm</td>
<td>400µ</td>
<td>5.4W</td>
<td>90Hz/60mJ</td>
<td>-</td>
<td>47.7 J/cm²</td>
<td>4.3 kW/cm²</td>
</tr>
<tr>
<td>L</td>
<td>2780nm</td>
<td>600µ</td>
<td>2.5W</td>
<td>20Hz</td>
<td>11/07</td>
<td>44.2 J/cm²</td>
<td>884 W/cm²</td>
</tr>
<tr>
<td>M</td>
<td>2780nm</td>
<td>600µ</td>
<td>2W</td>
<td>20Hz</td>
<td>11/10</td>
<td>35 J/cm²</td>
<td>707 W/cm²</td>
</tr>
<tr>
<td>N</td>
<td>2780nm</td>
<td>600µ</td>
<td>3W</td>
<td>20Hz</td>
<td>11/15</td>
<td>53 J/cm²</td>
<td>1 kW/cm²</td>
</tr>
</tbody>
</table>

WL (Wavelength), Ed (Energy density), Pd (Power density).

Fig 3  Sample C (808-nm diode laser) showed reduced peripheral thermal damage (HE staining; original magnification 25X).

Fig 4  Specimen E (980-nm diode laser) exhibited serious peripheral artifacts at 1.5 W cw (HE staining; original magnification 10X).
Er,Cr:YSGG laser

Group L: In samples “L1-3” the epithelial surface showed a degeneration of keratinocytes for a thickness of about 1 mm.

Group M: The damage was slightly increased and a dermoepithelial detachment of approximately 2.5 mm was always observed.

Group N: In samples “N1-3” the marginal damage was extremely reduced. In fact, laser effects were visible about in 1 mm both in epithelium and in connective tissue (Fig 6).

In all the specimens taken with the Er,Cr:YSGG laser, a wide inclusion of water into keratinocytes was observed, due to the hydrokinetic properties of the device. However, this did not create any problem for the correct diagnosis.

Scalpel

In the scalpel specimen (Group R), as expected, no artifacts either in connective or epithelial tissue were present (Fig 7).

DISCUSSION

The interaction of laser energy with target tissue is mainly determined by two non operator-dependent factors: the specific wavelength of the laser and the optical properties of the target tissues.6-8 Power density, energy density, pulse repetition rate, pulse duration and the mode of energy transferring to the tissue are dictated by the clinician. Combinations of these factors make it possible to control the optimal response for clinical application, specifically for oral biopsies.

For a clear histological diagnosis, it is very important that the whole biopic specimen is intact and clearly interpretable. In particular, in suspected neoplastic or dysplastic lesions, the peripheral cellular layers are of extreme value for the evaluation of the infiltrating potential of the lesion.9

There are two different kinds of biopsies; the incisional biopsy, performed by taking one or more parts of a lesion, and the excisional biopsy, performed by the excision of the whole lesion. In oral soft-tissue pathology, laser devices provide important advantages, especially in the treatment of certain lesions. However, they have also fueled great controversy in the analysis of
suspected dysplastic or neoplastic lesions, provoking doubt about laser’s suitability for taking biopsies.\textsuperscript{10}

Thermal damage is always present in a tissue treated with lasers; it is caused by the photothermal effect of laser devices on soft tissues. At the point of incidence of the laser beam, an increase of temperature of over 100°C is induced, with vaporization of the tissue. Around this area, the thermal increase exceeds 50°C, creating an area of coagulative necrosis. In surrounding areas, the thermal damage is reversible since the thermal increase is less than 50°C. The damage extent is related to both the wavelength and the parameters of utilization of laser devices.\textsuperscript{1,2}

Reduced peripheral thermal damage is of fundamental importance in oral pathology, since in neoplastic or dysplastic pathologies, the invasion of surrounding tissues is one of the most important parameters in diagnosis and prognosis of the pathology itself. In fact, it is well known that the partial excision of a dysplastic lesion creates the basis for worsening of dysplastic degree.\textsuperscript{3}

This study showed that the best results were obtained with the 808-nm diode laser device in pulsed wave mode, and with Er,Cr:YSGG laser at higher power, which created peripheral damage for less than 1 mm.

A deeper thermal effect in the connective tissue was observed with the Nd:YAG laser. Although Nd:YAG laser worked with a lower fluence than diode laser in continuous wave mode, this side effect can be explained by a different coefficient of absorption on the soft tissues of all examined wavelengths.

However, it is important to remember that these results were obtained in vitro and from healthy mucosa; the extent of thermal damage could be larger in pathological tissues due to the presence of pathological signs, such as reduced cellular adhesion, inflammation, vascularization.

According to our experience, we think that it is necessary to differentiate the lesions of oral soft tissues into two groups according to the dysplastic potential. In clinically non-suspicous lesions (eg, fibroma, angioma, etc) laser is fundamental in the resolution of the pathology. In contrast, in suspected dysplastic or neoplastic lesions (precancerous lesions, carcinoma, melanoma, etc), the peripheral thermal damage creates risks of failing to recognize the real extent of the lesion.

\textbf{CONCLUSIONS}

This study showed that all the tested laser devices function well enough for a clear histological diagnosis. However, peripheral thermal damage was visible in all the specimens studied. The results must be followed by further evaluations in vivo, to understand if the pathological alterations of the tissues could increase the extent of the damage. These results do not induce us to forbid the treatment of suspicious lesions with laser devices, but prompt us to recommend that in suspicious dysplastic or neoplastic lesions, the bioptic incision should be larger\textsuperscript{4} than a traditional scalpel incision to prevent laser thermal effects from creating tissue artifacts, which critically jeopardize a certain diagnosis.

Modern technologies provide fundamental advantages in oral surgical practice, making several treatments easier and safer; however, these positive properties cannot replace the knowledge and the ability of the oral surgeon.

\textbf{REFERENCES}


\textbf{Contact address:} Prof. U. Romeo, Department of Odon-tostomatological Science, Umberto I Polyclinic, Viale Regina Elena 287/A, 00161 Rome, Italy. Tel: +39-333-413-4697, Fax: +39-064-423-0811. e-mail: umberto.romeo@uniroma1.it