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Er,CR:YSGG lasers induce fewer dysplastic-like epithelial artefacts than CO_2 lasers: an in vivo experimental study on oral mucosa

A. González-Mosquera [a,d], J. Seoane [a,*], L. García-Caballero [a,d], P. López-Jornet [b,e], T. García-Caballero [c,d], P. Varela-Centelles [a,d]

[a] *Stomatology Department, School of Medicine and Dentistry, University of Santiago de Compostela, Entrerríos s/n, 15782 Santiago de Compostela, Spain*
[b] *Clínica Odontológica Universitaria, Hospital Morales Meseguer, Avda. Marqués de los Velez, s/n, 30008 Murcia, Spain*
[c] *Pathology Morphological Sciences Department, School of Medicine and Dentistry, University of Santiago de San Francisco s/n, 15782 Santiago de Compostela, Spain*

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Abstract

Our aim was to assess wounds made by lasers (CO_2 and Er,Cr:YSGG) for their epithelial architectural changes and width of damage. We allocated 60 Sprague–Dawley® rats into groups: glossectomy by CO_2 laser at 3 different wattages ($n = 10$ in each); glossectomy by Er,Cr:YSGG laser at two different emissions ($n = 10$ in each), and a control group ($n = 10$). Histological examination assessed both prevalence and site of thermal artefacts for each group. Both lasers (CO_2 and Er,Cr:YSGG) caused the same type of cytological artefacts. The 3 W Er,Cr:YSGG laser produced the fewest cytological artefacts/specimen, and was significantly different from the other experimental groups: 3 W CO_2 laser (95% CI = 0.8 to 1.0); the 6 W CO_2 laser (95% CI = 0.1 to 2.0) and the 10 W CO_2 laser (95% CI = 1.1 to 3.0). CO_2 lasers (3–10 W) generate epithelial damage that can simulate dysplastic changes with cytological atypia that affects mainly the basal and suprabasal layers. Irradiation with Er,CR:YSGG laser (2–4 W) produces significantly fewer cellular artefacts and less epithelial damage, which may be potentially useful for biopsy of oral mucosa.

Keywords: Oral biopsy; Laser; Dysplasia; Artefact

Introduction

Laser systems have been reported to be unreliable for biopsy of oral tissue because of their potential to alter the results of the histopathological evaluation.[1] Despite carbon dioxide (CO_2) lasers having proved better than diode lasers and electrotomes for this purpose,[2,3] their use is compromised by thermal cytological artefacts including vacuolation of the superficial layer, detachment and shredding of keratin, and degeneration of basal cells and separation of them from the lamina propria.[4] Laser-treated margins also simulate cytological atypia (hyperchromatism, pleomorphism and elongation of nuclei, and vacuolar degeneration).[1,2,5] CO_2 lasers generate such damage mainly at the basal and suprabasal layers.[6]

These alterations become a challenge when laser-obtained samples from malignant and dysplastic oral lesions are being assessed, and concern all those who use lasers to biopsy oral tissue.[3] However, the many advantages of lasers (minimal blood loss, seal of lymphatics and nerve endings, and minimal seeding of neoplastic cells)[6] justify further investigations.

* Corresponding author. Tel.: +34 981 563100.
E-mail addresses: antonio.gonzalez@usc.es (A. González-Mosquera), juanmanuel.seoane@usc.es (J. Seoane), lugcaballero@hotmail.com (L. García-Caballero), majornet@um.es (P. López-Jornet), tomas.garcia-caballero@usc.es (T. García-Caballero), pvarelac@infomed.es (P. Varela-Centelles).
[d] Tel.: +34 981 563100.
[e] Tel.: +34 868 888588.

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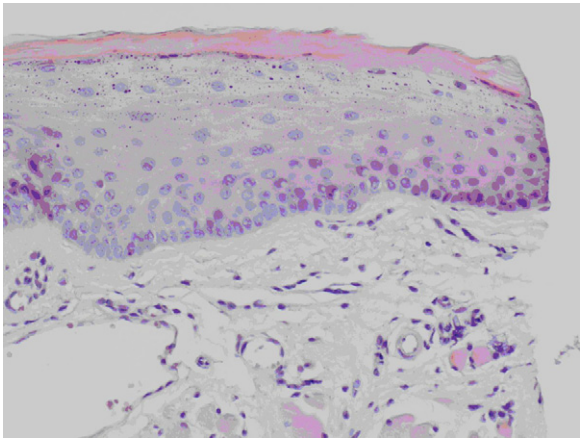

Fig. 1. Margin of a sample obtained with a conventional scalpel showing a regular edge and no artefacts (haematoxylin and eosin, original magnification 40×).

Most reports have used the extension of the hyalinised or coagulated tissue adjacent to the irradiated edge as the outcome measure,[3] and only occasionally considered cytological artefacts,[1] or dysplastic-like changes,[5] at the incision. Only a few authors have described the types of lasers that they considered were adequate to biopsy tissues without rendering the histological diagnosis difficult.[1,3]

Er,Cr:YSGG (erbium, chromium doped yttrium scandium gallium garnet) lasers are thought to induce few cellular artefacts,[3,7] but we know of no studies that have compared the effects of different lasers on the oral epithelium in terms of cytological atypia or dysplastic-like artefacts.

The aims of this study were therefore to examine wounds caused by CO_2 and Er,Cr:YSGG lasers in terms of cytological and epithelial architectural changes, and to assess the width of the thermal damage lateral to the incision.

Material and methods

Sixty Sprague–Dawley® rats that weighed about 250 g were randomly allocated to six groups: 3 groups ($n = 10$ in each) had a glossectomy by a CO_2 laser at different wattages (3, 6, and 10 W); 2 groups ($n = 10$ each) were treated with an Er,Cr:YSGG laser with two different wattages (2 and 4 W); and a control group ($n = 10$) had a glossectomy with a number 15 blade (B/Braun, Aesculap AG, Germany) (Fig. 1).

The CO_2 laser (20 W, Pierre Rolland, Satelec SA, Spain) was used at 3, 6, and 10 W continuous power with a 1.25 mm spot-size straight probe. The energy was applied for 5 s at an approximate velocity of tissue irradiation of 0.88 mm/s.

The Er,Cr:YSGG laser (Waterlase, Biolase Technology, Inc., CA, USA) worked at 2780 nm wavelength (output power range: 0.1–8 W; pulse repetition rate: 10–50 Hz in pulsed mode) and the water/air cooling spray was set at a water/air proportion of 30%/10%. The laser was used at the 3 C preset (recommended for soft tissue incisions), mode S, with 20 pulses/s using a handpiece with a sapphire tip and an optic fibre 600 μm in diameter.

A single surgeon did all the procedures, and directed the laser beam perpendicular to the dorsum of the tongue while stabilising the specimen with a non-toothed Adson forceps applied to the tip of the tongue. The animals were killed immediately afterwards by an overdose of anaesthetic according to the protocols of the EU. The study was approved by the hospital's Ethics Committee.

Specimens were immediately fixed in 10% formalin-buffered saline for 24 h. A single pathologist longitudinally cut all specimens with a new disposable scalpel for each section. Samples were prepared in 4 μm sections, stained with haematoxylin and eosin, and processed by the same technician.

The specimens were coded and studied by two pathologists who were unaware of the source of a specimen until a consensus had been reached for each case. All specimens were examined using an Optiphot-2 microscope (Nikon, Tokyo, Japan) equipped with an eyepiece with a millimetre-calibrated graticule (Graticules Town Bridge, Kent, UK).

The histological assessment was made on the ventral mucosa of the tongue and evaluated in terms of epithelial features (dysplastic criteria), namely loss of polarity of the basal cells, presence of more than one layer of basaloid appearance, increased nuclear:cytoplasmatic ratio, drop-shaped rete ridges, irregular epithelial stratification, increased number of mitotic figures, abnormal mitotic figures, presence of mitotic figures in the superficial half of the epithelium, cellular and nuclear polymorphism, nuclear hyperchromatism, enlarged nucleoli, loss of intercellular adherence, and keratinisation of single cell groups in the prickle cell layer.[8] Histological examinations assessed both prevalence and location of thermal artefacts within the epithelium.

Data were analysed by a statistician who was unaware of the design of the study; we used Fisher's exact test to assess the significance of differences between proportions and ANOVA to assess those between means. Probabilities of less than 0.05 were accepted as significant.

Results

Both types of laser (CO_2 and Er,Cr:YSGG) induced the same kinds of artefacts: the presence of fusiform cells with pronounced elongation of nuclei, cellular and nuclear polymorphism, and nuclear hyperchromatism and loss of intercellular adherence, mainly located at basal and suprabasal layers of the lingual epithelium (Fig. 2). Their distribution according to type of laser and wattage is shown in Table 1.

No other cytological or architectural criteria for epithelial dysplasia were found in the samples analysed, nor were there any signs of autolysis or phenomena associated with inadequate fixation of tissue.

Table 1
Distribution of signs of heat damage to oral epithelium by CO_2 and Er,CR:YSGG laser wattages ($n = 10$ in each group). Data are number, or mean (SD).

Histological alterations	3 W CO_2	6 W CO_2	10 W CO_2	2 W Er,CR:YSGG	4 W Er,CR:YSGG
Cellular and nuclear polymorphism	7	8	10	2	3
Nuclear hyperchromatism	10	9	10	1	6
Loss of intercellular adherence	0	1	8	4	2
Width of side thermal damage (μm)	210 (45)	280 (87)	355 (157)	180 (72)	107 (26)
No. of artefacts by specimen	1.7 (0.4)	1.8 (0.7)	2.8 (0.4)	0.7 (0.6)	1.0 (0.9)

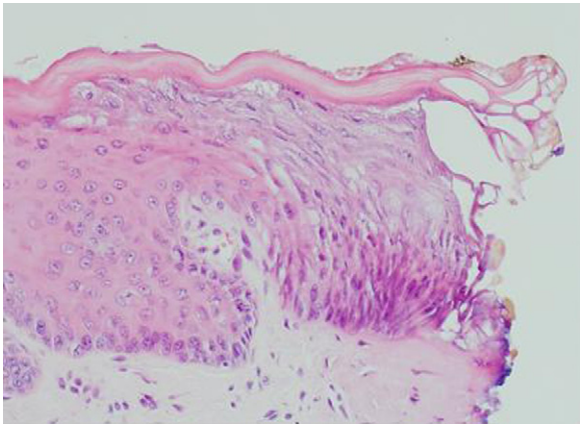

Fig. 2. Sample obtained by CO_2 laser showing an irregular irradiated edge, hyperchromatism, elongation of nuclei, and retraction between the superficial epithelial layer and the layer of keratin (haematoxylin and eosin, original magnification 40×).

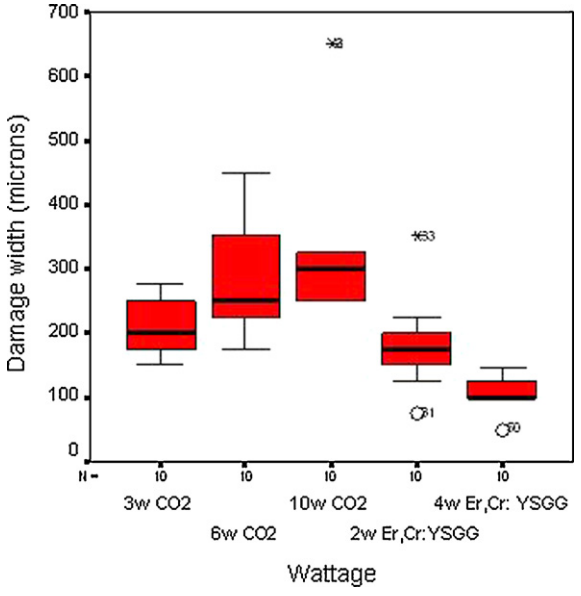

Fig. 3. Box-plot of the distribution of the width of thermal damage caused by different CO_2 and Er,Cr:YSGG laser wattages.

The width of epithelial damage adjacent to carbon dioxide laser incisions, considering the mean (SD) value of all wattages used, was 281.6 (119.7) μm, whereas the Er,CR:YSGG scored 144.0 (65.0) μm. The damage was significantly less in the Er,CR:YSGG laser groups (95% CI 78.6 to 196.7) (Fig. 3).

The proportion of cellular artefacts – cellular and nuclear polymorphism and nuclear hyperchromatism – was also significantly lower for the Er,Cr:YSGG laser groups ($p = 0.0001$) (Fig. 4), as was the number of cytological artefacts/specimen (0.8 (0.8) for the Er,Cr:YSGG laser) the differences between the types of laser being significant (95% CI 0.7 to 1.7).

The extension of the damage to the thermal side at the basal cell layer for the CO_2 low power (3 W) group was 210 (45.9) μm, and for the 6 W group 280.0 (87.2) μm group; the high wattage group (10 W) scored 355.0 (157.5) μm. The one-way ANOVA could identify differences only between the lowest and the highest wattage groups, and the epithelial damage generated at 10 W was significantly higher than that induced at 3 W (95% CI 22.4 to 267.5). Differences in the number of artefacts/specimen between the 6 and 10 W CO_2 laser groups and the 3 W group were also significant. When the experimental groups were considered together, the 3 W Er,Cr:YSGG laser produced the lowest number of cytological artefacts/specimen, which differed considerable from the numbers scored by the other experimental groups: 3 W CO_2 laser (95% CI 0.8 to 1.0); the 6 W CO_2 laser (95% CI 0.1 to 2.0) and the 10 W CO_2 laser (95% CI 1.1 to 3.0).

Discussion

Various animals and human cadaveric material have been used as experimental models for techniques of oral biopsy,[4] and their validity have been proved.[9] This experimental model has already been used in studies to assess thermal damage by CO_2 lasers in oral mucosa and vocal cords.[9] As the effect of lasers on tissues varies according to differences in water content or tissue density,[10] the dorsum of the tongue was not used because of its high keratinisation; we focused on the ventral surface, which is similar to human oral mucosa.[5]

Although diagnosis of invasive squamous cell carcinoma (SCC) is generally straightforward, histological diagnosis of oral premalignant lesions can be challenging. Oral epithelial dysplasia is a relatively common premalignant condition that affects about 2.5–5‰ of the population, and is defined as a precancerous lesion of stratified squamous epithelium that is characterised by cellular atypia and loss of normal maturation and stratification short of carcinoma in situ.[8] The grading of dysplasia depends on the extent of the involvement of the epithelial layers by the dysplastic changes.[11] Oral dysplasia can be diagnosed only histologically, and this process can be subjective and prone to a wide range of interpretation.[11–13]

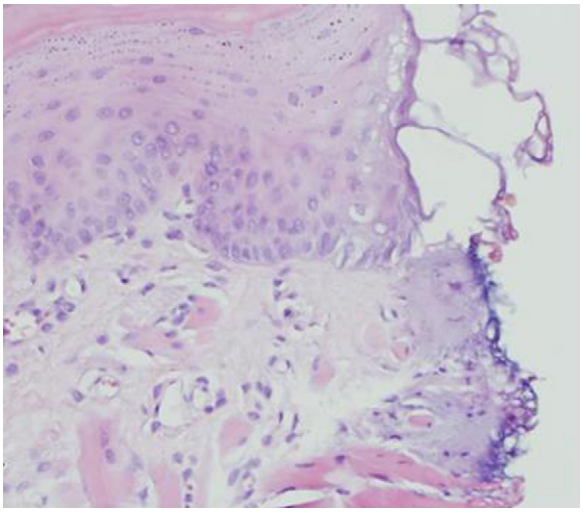

Fig. 4. Minimum artefacts seen in the epithelial cells using an Er,CR:YSGG laser, but the keratin is detached and the collagen shows basophilic changes at the edge of the irradiated area (haematoxylin and eosin, original magnification 40×).

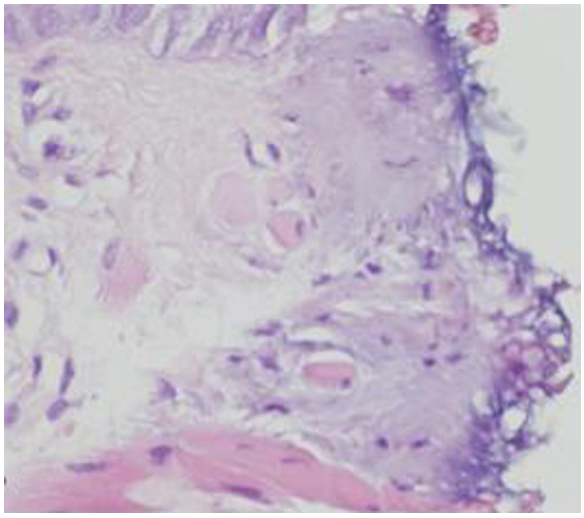

Fig. 5. After irradiation with an Er,CR:YSGG laser, the condensation of the lamina propria at the edge of the sample should eventually avoid bleeding from vessels (haematoxylin and eosin, original magnification 100×).

To diminish these biases, the samples were analysed by two experienced pathologists.

Despite the fact that no cytological or structural changes were found at the margin of the samples obtained with a conventional scalpel, the specimens may be affected by a number of squeeze artefacts such as crush, splits, fragmentation, and pseudocysts resulting from inappropriate handling of the samples.[14,15]

The use of CO_2 lasers in the maxillofacial area has many advantages such as precision, conservative and site-specific minimally invasive surgery, little intraoperative haemorrhage, sterilisation of the surgical area, little postoperative pain, healing with minimal scarring, and reduced postoperative swelling.[16] These advantages have made its use common practice for the management of oral malignant and dysplastic lesions[17] and even for biopsying lesions, in an attempt to minimise the seeding of cells.[6]

The basement membrane and the connective tissue stroma are the main barriers to the migration of tumour cells.[18–20] When these barriers are broken cancer cells can disseminate into the circulation, increasing the risk of metastases. About half the animals who had incisional biopsy specimens taken for the diagnosis of a primary carcinoma developed metastatic spread to the lymph nodes.[21] Neck metastases from stage I or II oral SCC are more common when the diagnosis is made from biopsy specimens taken from the incision,[10,22] as circulating cancer cells have been identified in peripheral blood from patients with stage III disease 15 min after conventional biopsy.[10]

The number of cancer cells in circulation at any time seems to depend not only on the detachment of cells from primary tumours, but also on their accessibility to vascular channels and on the rates at which they are removed from the circulation.[20] Irradiation with both CO_2 and Er,Cr:YSGG lasers at the settings described induced a thermal effect that produced a hyalinised area next to the incision and sealed the vessels in the wound (Fig. 5).[3,7]

However, the thermal cytological artefactual epithelial changes (presence of fusiform cells,[5,6] hyperchromatism,[1,14] polymorphism, and elongation of nuclei[1]), and the loss of intercellular adherence[5] related to the use of carbon dioxide laser, may well be mistaken for epithelial dysplasia in oral biopsy specimens.[1,23] Photocoagulation of proteins may mask or alter surface epitopes, and render some diagnostic immunohistochemical stains less useful.[17] Previous reports have linked some cytological atypias to the use of high wattages in the continuous mode,[1,23] but each one of these cytological atypias has also been found at low wattage (3–4 W) CO_2 lasers on pulsed mode. Our results show hyperchromatism and elongation of nuclei as the most common cytological artefacts in samples obtained using CO_2 lasers.

Although scarce morphological changes in the areas adjacent to Er,Cr:YSGG laser incisions[3] or even their absence[7] have been described, our results show the same kind of cytological alterations for both CO_2 and Er,Cr:YSGG lasers, though in significantly lower proportions for the latter (Table 1).

Signs of dysplasia are commonly seen in the epithelium adjacent to oral carcinomas, and the presence of mild to moderate dysplasia at the margins of excised oral SCC carries an appreciable risk of recurrence.[24] Previous reports have described a high proportion of mild to moderate dysplasia at the margins of oral dysplastic lesions resected with CO_2 lasers.[17] Occasionally, the histological edge of the primary tumour is ill-defined because multiple foci of invasion are present against a background of carcinoma in situ or dysplasia.[18] In these circumstances, lack of awareness of these pseudodysplastic artefacts may well generate overtreatment and erroneous therapeutic approaches.

In cases with mild dysplasia, cytological and architectural changes are confined to the lower third of the epithelium and may reach up to two-thirds in cases of moderate dysplasia.[8] Our data have shown cytological atypias (artefacts) that affect mainly the basal and suprabasal layers, but no sample depicted artefactual drop-shaped rete ridges, irregular epithelial stratification, increased numbers of mitotic figures, the presence of mitotic figures in the superficial half of the epithelium, or keratinisation of single cell groups in the prickle cell layer. Obviously the presence of any of the criteria for dysplasia would contribute to the elimination of the diagnosis of dysplasia-like artefacts linked to use of a laser.

Even though the dissemination of energy to the side in the CO_2 laser-generated wounds is low,[25] the size of the damaged area essentially depends upon the wave length of the laser and the density of the energy applied, which in turn increases with time and spot size. Thermal damage can be reduced by using the smallest spot size.[8] Oral epithelium shows necrosis lateral to CO_2 laser incisions in a range of widths, from 70 to 750 μm.[5,9,16]

Our results fit within this range, and may justify the need for including an additional amount of healthy marginal tissue beyond the expected extension of the epithelial thermal damage (at least 1 mm around the lesion). The epithelial damage caused by the Er,CR:YSGG laser was minor.

In summary, we have shown quantitative and qualitative advantages of the use of the Er,Cr:YSGG laser within the explored wattages that would make it better than CO_2 lasers for maintaining safe and readable cut margins to permit histological visualisation with minimal artefacts.

References

1. Eversole LR. Laser artifacts and diagnostic biopsy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1997;**83**:639–40.
2. Matsumoto K, Suzuki H, Usami Y, Hattori M, Komoro T. Histological evaluation of artifacts in tongue tissue produced by the CO_2 laser and the electrotome. *Photomed Laser Surg* 2008;**26**:573–7.
3. Cercadillo-Ibarguren I, España-Tost A, Arnabat-Domínguez J, Valmaseda-Castellón E, Berini-Aytés L, Gay-Escoda C. Histologic evaluation of thermal damage produced on soft tissues by CO_2, Er,Cr:YSGG and diode lasers. *Med Oral Patol Oral Cir Bucal* 2010;**15**:e912–8.
4. Horch HH, Gerlach KL, Schaefer HE. CO_2 laser surgery of oral premalignant lesions. *Int J Oral Maxillofac Surg* 1986;**15**:19–24.
5. Seoane J, Caballero TG, Urizar JMA, Almagro M, Mosquera AG, Varela-Centelles P. Pseudodysplastic epithelial artefacts associated with oral mucosa CO_2 laser excision: an assessment of margin status. *Int J Oral Maxillofac Surg* 2010;**39**:783–7.
6. Klein DR. The use of the carbon dioxide laser in plastic surgery. *South Med J* 1977;**70**:429–31.
7. Rizoiu IM, Eversole LR, Kimmel AI. Effects of an erbium, chromium: yttrium, scandium, gallium, garnet laser on mucocutaneous soft tissues. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1996;**82**: 386–95.
8. Pindborg JJ, Reichart PA, Smith CJ, Van der Waal, editors. *Histological typing of cancer and precancer of the oral mucosa. WHO International histological classification of tumours*. 2nd ed. Berlin: Springer; 1997. p. 25–6.
9. Fleiner B, Plath T. Histological evaluation of leukoplakia following CO_2 laser excision. A clinical and experimental study. *Adv Otorhinolaryngol* 1995;**49**:125–9.
10. Kusukawa J, Suefuji Y, Ryu F, Noguchi R, Iwamoto O, Kameyama T. Dissemination of cancer cells into circulation occurs by incisional biopsy of oral squamous cell carcinoma. *J Oral Pathol Med* 2000;**29**:303–7.
11. Poh CF, Ng S, Berean KW, Williams PM, Rosin MP, Zhang L. Biopsy and histopathologic diagnosis of oral premalignant and malignant lesions. *J Can Dent Assoc* 2008;**74**:283–8.
12. Lingen MW, Pinto A, Mendes RA, Franchini R, Czerninski R, Tilakaratne WM, et al. Genetics/epigenetics of oral premalignancy: current status and future research. *Oral Dis* 2011;**17**(Suppl. 1):7–22.
13. Warnakulasuriya S. Histological grading of oral epithelial dysplasia revisited. *J Pathol* 2001;**194**:294–7.
14. Ficarra G, McClintock B, Hansen LS. Artefacts created during oral biopsy procedures. *J Craniomaxillofac Surg* 1987;**15**:34–7.
15. Seoane J, Varela-Centelles P, Ramirez JR, Romero MA, De La Cruz A. Artefacts produced by suture traction during incisional biopsy of oral lesions. *Clin Otolaryngol Allied Sci* 2002;**27**:549–53.
16. Wilder-Smith P, Arrastia AA, Liaw L, Berns M. Incision properties and thermal effects of three CO_2 lasers in soft tissue. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995;**79**:685–91.
17. Jerjes W, Upile T, Hamdoon Z, Mosse CA, Akram S, Hopper C. Prospective evaluation of outcome after transoral CO_2 laser resection of T1/T2 oral squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011;**112**:180–7.
18. Woolgar JA, Triantafyllou A. A histopathological appraisal of surgical margins in oral and oropharyngeal cancer resection specimens. *Oral Oncol* 2005;**41**:1034–43.
19. Santos NR, Aciole GT, Marchionni AM, Soares LG, dos Santos JN, Pinheiro AL. A feasible procedure in dental practice: the treatment of oral dysplastic hyperkeratotic lesions of the oral cavity with the CO_2 laser. *Photomed Laser Surg* 2010;**28**(Suppl. 2):S121–6.
20. Weiss H. Metastases caused by fine needle puncture? *Ultraschall Med* 1989;**10**:147–51 [in German].
21. Safour IM, Wood NK, Tsiklakis K, Doemling DB, Joseph G. Incisional biopsy and seeding in hamster cheek pouch carcinoma. *J Dent Res* 1984;**63**:1116–20.
22. Ohtake K, Shingaki S, Nakajima T. Effects of incision and irradiation on regional lymph node metastasis in carcinoma of the hamster tongue. *Oral Surg Oral Med Oral Pathol* 1990;**70**:62–9.
23. Convissar RA. Laser biopsy artifact. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1997;**84**:458.
24. Thomson PJ, Wylie J. Interventional laser surgery: an effective surgical and diagnostic tool in oral precancer management. *Int J Oral Maxillofac Surg* 2002;**31**:145–53.
25. Pogrel MA, McCracken KJ, Daniels TE. Histologic evaluation of the width of soft tissue necrosis adjacent to carbon dioxide laser incisions. *Oral Surg Oral Med Oral Pathol* 1990;**70**:564–8.