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Fluorescence based characterization of early oral squamous cell carcinoma using the Visually Enhanced Light Scope technique

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ABSTRACT

Objectives: Several diagnostic tools have been developed to assess benign and potentially malignant disorders of soft tissues. In this study, we aimed to assess the value of the VELscope® (Visually Enhanced Light Scope) imaging device as a technical tool to investigate malignant lesions of the oral cavity.
Material and methods: In this retrospective study we analyzed the photographs of 90 patients who suffered from malignant oral soft tissue lesions or carcinoma in situ (CIS) from 2008 to 2014 in the Clinic of Oral and Maxillofacial Surgery of LMU in Munich.
Results: In 85.6% of the cases fluorescence quenching/loss could be detected. The average value for the colour red shows a significant difference in pathologic and physiologic tissues ($p = 0.007$) with a higher median for pathologic tissues. For the colours green and blue our measurements show significantly higher values in the healthy tissue ($p < 0.001$.). The shade of red showed significantly higher values for pathologic tissues when compared to all three colours ($p < 0.001$). Furthermore, the shades of green and blue showed significantly lower values in the pathologic tissue ($p < 0.001$).
Conclusion: In the near future, VELscope® could help to a greater extent than visual observation alone in identifying the margins of tumor resections. VELscope® still lacks the ability to identify the overall risk level of oral lesions.

1. Introduction

Oral cancer is the eleventh most common cancer worldwide according to the World Health Organization (Petersen et al., 2005). It accounts for nearly 3% of all cancer cases globally, with an incidence estimated at 274,000 new cases per year (IARC monographs on the evaluation of carcinogenic risks to humans 2012; Parkin, 2001). Oral squamous cell carcinoma (OSCC) accounts for 90% of these oral cancers (Silverman, 1998). OSCC may arise from oral potentially malignant disorders (OPMDs) such as leukoplakia, erythroplakia, or lichen planus (Warnakulasuriya et al., 2007). The most common treatment for OSCC is surgery (Forastiere et al., 2002). Early detection of premalignant lesions therefore offers a great potential benefit to patients. The differentiation of neoplastic tissue alterations from non-dysplastic epithelium represents a major concern of all surgical disciplines.

Recently, several diagnostic tools have been developed to assess benign and potentially malignant disorders. In particular, the use of autofluorescence imaging has gained interest in clinical practice for non-invasive and repeatable imaging of the oral mucosa. Autofluorescence is based on the excitation of different and specific endogenous fluorochromes in oral epithelium and submucosa (Awan et al., 2011). When viewed through a filter and irradiated, all fluorochromes emit light of the green spectral range with wavelengths between 375 and 440 nm (Betz et al., 2002). The VELscope® (Visually Enhanced Light Scope) device, available since June 2006, is a handheld system which uses blue light excitation between 400

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and 460 nm to cause tissue autofluorescence. The VELscope® is a certified medical device (No 41315446) approved by the American Dental Association for the detection of mucosal tissue abnormalities (CDT D0431). In the literature, there have been several studies displaying increased sensitivity and for malignant lesions by the combined use of autofluorescence and clinical examination of the oral cavity (Betz et al., 2002; Rana et al., 2012).

Under VELscope® exposure, normal tissue will fluoresce light green due to endogenous fluorochromes (Kulapaditharom and Boonkitticharoen, 2001). Dysplastic changes of epithelium cause loss of physiologic autofluorescence and the tissue appears dark (Betz et al., 2002; Lane et al., 2006). Loss of autofluorescence has been correlated with disease progression and has already been implemented in other tissue screening procedures such as cervical cancer (Richards-Kortum and Sevick-Muraca, 1996). In this study, we aimed to assess the value of VELscope® imaging as a technical device to determine the reliability of fluorescence loss in histologically proven OSCC.

2. Material and methods

2.1. Patients and data collection

In this retrospective study we analysed the photographs of 90 patients who suffered from malignant oral soft tissue lesions or carcinoma in situ (CIS) from 2008 to 2014 in the Clinic of Oral and Maxillofacial Surgery of LMU in Munich. The study was approved by the Ethical Committee of LMU Munich on the 7th of April 2014, and is listed under UE Nr. 042-14. Inclusion criteria were: age >18 and histologically verified CIS, squamous cell carcinoma, or adenocarcinoma.

2.2. Study variables

The parameters which were taken into account for the analysis were age, sex, cigarette or alcohol abuse, tumor location, and tumor classification according to the International Union Against Cancer (UICC) (Sobin et al., 2010), the stage and grade results acquired by PET-CT or CT according to German medical guidelines.

2.3. Analysis of the photographs

The fluorescence analysis was conducted on photographs taken routinely for treatment documentation. For this purpose the models EOS 7D Canon and D1X Nikon were used. In each photograph the oral epithelium lesion and surrounding tissue are depicted. We analyzed the differences of the fluorescence characteristics of different tissue types like masticatory mucosal tissue and epithelium. The camera properties were adjusted for optimizing green autofluorescence of each tissue type. The ISO Nr. was 4000 and shutter speed was 1/100 s, the 11 and 5.6 lens was used.

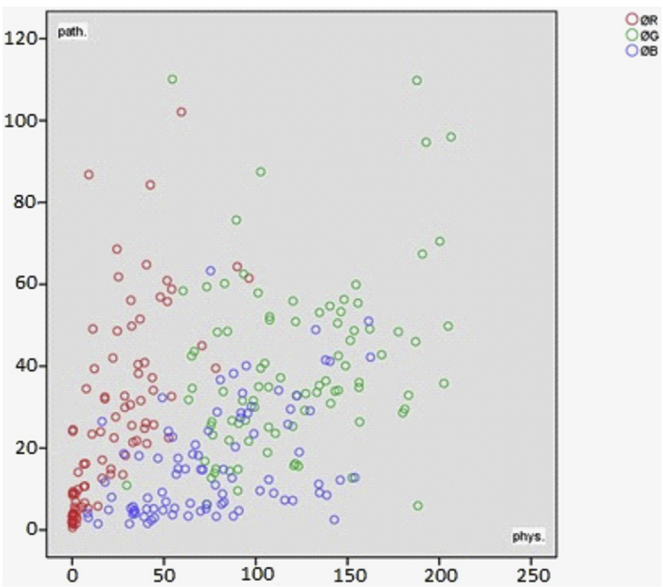
2.4. Measurements

For analysis of our data the RGB System and Adobe Photoshop Elements 10 Editor were used. Photographs acquired during white light inspection showing epithelial lesions suspected for pathologic areas were chosen and compared to the photographs taken with the VELscope®. VELscope® images were obtained at an excitation wave length between 400 and 460 nm. On each photograph, 10 random measurement spots were chosen in the oral lesion and in the surrounding healthy tissue. This technique was described by several groups before (Roblyer et al., 2009; Schwarz et al., 2009; Lane et al., 2006). In Fig. 1 this concept is shown. For each measurement the same tissue type was selected. Then the average values for red, green, and blue were calculated. The result for each colour was added in a triplett. Thus we could calculate the share of each colour of the sum of all three. Furthermore, we calculated the share of each colour to the maximum intensity of 255 (see Table 1). Then the ratio for each colour was calculated in the pathologic and physiologic area.

2.5. Statistics

All statistical analyses were performed with IBM SPSS. The T-test, Wilcoxon Signed Rank Test and Kolmogorov-Smirnov-Test were used. Significance was considered at $p < 0.05$. Additionally the directions of the different values for pathologic and physiologic

Table 1
Scatter plot displaying the distribution of the 3 colours in pathologic and healthy tissues. The colours blue and green are represented in physiological whereas red is displayed in pathological areas more frequently.



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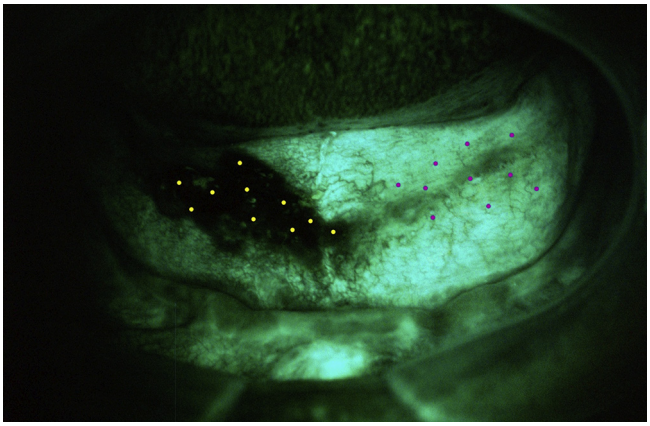

Fig. 1. Figure showing the measurement in the pathologic area (yellow dots) and in the healthy tissue (purple dots). Dots are placed as described above.

measurements were analysed. Concerning the colour distribution in the pathologic and physiologic areas, an ANOVA test was performed. For this purpose 85 of the 90 photographs were taken into consideration for the analysis of the fluorescence measurements. The photographs of 5 patients could not be evaluated. Significance was considered at $p < 0.05$.

3. Results

In this study 57 male and 33 female patients suffered from malignant oral soft tissue lesions or CIS. The mean age was 61, with a range from 30 to 96 years of age. In men, oral carcinoma was diagnosed an average of two years prior to female patients (60.5 years vs. 62.6 years). Of the 90 patients, 52 reported a positive smoking status, 22 reported non-smoking status, and in the remaining 16 cases no information was provided. Most of the tumors were localized within the floor of the mouth and tongue. Infrequent areas of carcinoma occurrence were the maxilla, the alveolar ridge, and the inferior lip.

The pathologically verified diseases present in this study were squamous cell carcinoma (n = 76; 84.4%), primary site recurrence (n = 6; 6.7%), carcinoma in situ (CIS) (n = 7; 7.8%), and adenocarcinoma (n = 1; 1.1%). 35.6% (n = 32) of these presented in stage 1. The grade of histologic differentiation of these tumors was low (G3) in 18.9% (n = 17), moderate (G2) in 55.6% (n = 50), and high (G1) in 12.2% (n = 11).

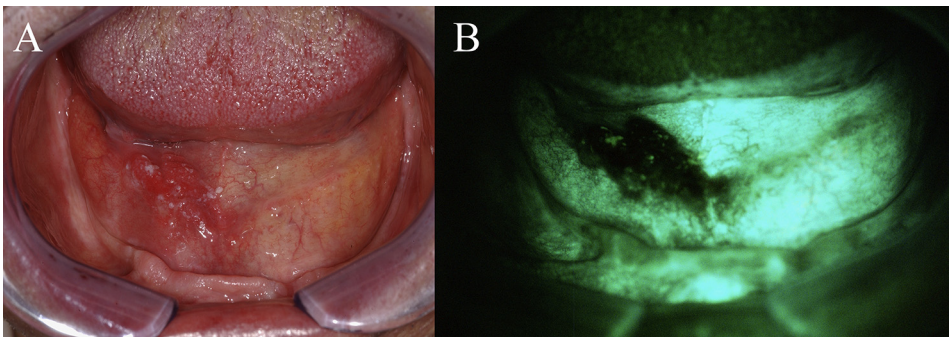

Fig. 2. A) White light image showing a tumor suspect area at the floor of the mouth. B) VELscope image showing a fluorescence loss in the green light spectrum in the tumor suspect area.

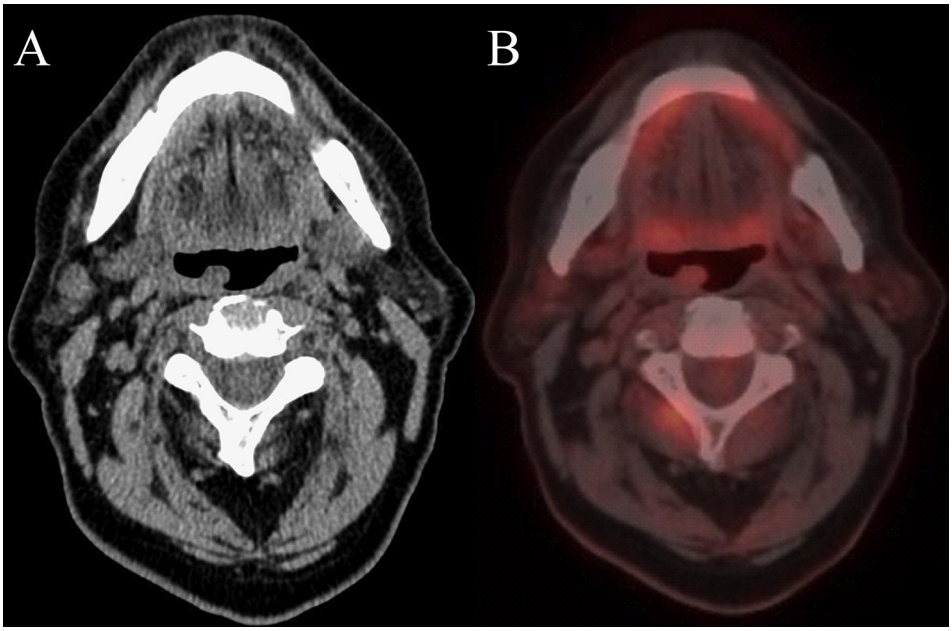

Fig. 3. A) CT scan of the same patient shown in Fig. 2. No soft tissue alterations at the floor of the mouth can be identified. B) PET-CT fusion in the corresponding image and series showing no significant FDG enhancement at the described area.

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In 85.6% (n = 77) of the cases a fluorescence loss could be detected. Comparing the color of tissue fluorescence showed a statistically significant difference between pathologic and healthy corresponding tissue ($p < 0.001$). The average value for the colour red shows a significant difference in pathologic and physiologic tissues ($p = 0.007$) with a higher median for pathologic tissues. For the colours green and blue our measurements show significantly higher values in the healthy tissue ($p < 0.001$.). The shade of red showed significantly higher values for pathologic tissues when compared to all three colours ($p < 0.001$). Furthermore, the shades of green and blue showed significantly lower values in the pathologic tissue ($p < 0.001$).

We then compared the obtained measurements to the stage of disease process. In 76 cases the measurements were compared with a PET and with CT in 77 cases. In 14.5% (n = 11) the PET showed no malignoma suspicious of FDG enhancement. In these cases, the red to green ratios in the pathologic compared to the physiologic tissues was higher than zero, displaying a definite difference in the colour distribution in our study. In 40.3% (n = 31) of the CT scans no tumor could be detected (see Fig. 3).

4. Discussion

In this study we investigated the diagnostic capabilities of VELscope® autofluorescence with histologically verified carcinoma of the oral cavity. This group was homogeneous, lacking confounding autofluorescence factors such as infection, inflammation, or trauma. Our aim was to establish a characteristic colour distribution pattern to differentiate neoplastic and physiologic tissues. Moreover, the various tumor locations of the oral cavity were evaluated for detecting differences between pathologic and physiologic. Additionally, a comparison of the autofluorescence system to a PET and CT scan was conducted, to see if the VELscope® can close the gap in conventional radiation diagnostics concerning the detection of OSCC.

We showed in the present study that there is a quantifiable difference in the red to green ratios of neoplastic and physiologic areas. No significant differences in the colour distributions could be detected in the different localization in the oral cavity. In the cohort we investigated on diagnostic autofluorescence displayed significant difference in the colour distribution between the neoplastic and physiologic area. Not all of the lesions could be visualized or diagnosed using the PET or the CT scan, displaying a potential benefit to VELscope's® sensitivity in early cases of the disease. The patients of the present retrospective study were recruited from the histologically proven cancer or carcinoma in situ. In the past, only Poh et al. investigated a cohort consisting of such a high number of patients (Poh et al., 2006, 2009).

Smoking and alcohol are major risk factors for the development of OSCC (Warnakulasuriya, 2009). Smoking alone increases one's risk up to 8 times, and when combined with alcohol a synergistic effect occurs which can increase one's relative risk from 6.5 to 22.1 (Moreno-Lopez et al., 2000; Lewin et al., 1998). The most common OMPD for SCC is leukoplakia, with an estimated prevalence worldwide of 2% (Petti, 2003).

Although rates of oral cancer are decreasing in most parts of the globe, eastern European countries have actually experienced an increase in cases (Garavello et al., 2010). Furthermore, there is an increasing number of head and neck cancer cases in both the US and Europe related to HPV infections, altering the previous at-risk groups and epidemiology of this disease (Marur et al., 2010; Chaturvedi et al., 2008; Conway et al., 2006). This shift may alter diagnostics and risk factor shifts which could now be assisted by VELscope® and other visual adjuncts (ViziLite®, Microlux TM/DL®, etc) (Nagi et al., 2016).

The combination of clinical investigation – anatomical location, lymph node inspection, lesion size – along with demographic information – patient age, and sex, HPV status, alcohol and smoking abuse – can aid SCC determination (Troeltzsch et al., 2014). Recognition on OMPD and distinction between neoplastic and physiologic tissue is an additional aid in risk evaluation, diagnosis, and treatment. As Troeltzsch at al showed, not only the early detection of malignant lesions but also the grading and the anatomic location play a major role in the formation of cervical metastasis (Troeltzsch et al., 2016). VELscope® has a limited benefit identifying erythroleukoplakia, lesional risk, or depth (Nagi et al., 2016). However, the VELscope® has previously been shown to benefit identification of OMPDs such as leukoplakia (Nagi et al., 2016). Reichart reported that the prevalence of leukoplakia in Germany alone is 2.3% for men, and 0.9% for women who harbor a higher transformation rate (Reichart, 2000; Shipman et al., 1998).

The average recurrence rate of squamous cell carcinoma is about 25–48%, with a 10–30% recurrence rate at the primary site – displaying the importance of full resection with safety space at time of tumor removal (Tabor et al., 2004; Schwartz et al., 2000; Brennan et al., 1995; Leemans et al., 1994). Mücke et al. reported that local relapse ranges between 24% and 28% within 6–18 months of surgical SCC removal (Mücke et al., 2009), and the five year survival rate is 64% (Howlader et al., 2016). Recurrence of OSCC at primary sites of excision displays the importance of full resection at time of tumor removal. Improving identification of leukoplakia and distinction of neoplastic and physiologic tissues as shown here with VELscope®, may be able to improve early detection of malignant and dysplastic epithelium. The VELscope® sensitivity in detecting malignant lesions ranges from 22% to 100% with a specificity range of 16%–100% depending on the study (Nagi et al., 2016; Sawan and Mashlah, 2015; Trullenque-Eriksson et al., 2009). In support with Poh, we encourage further research on surgical margin detection, as well as the establishment of lesional margins during chemoprevention in an in depth manner for future meta-analysis (Poh et al., 2009).

One of the limitations of the VELscope® technique and the methodology of this study is that only superficial lesions can be detected. Neoplasia like adenocarcinoma which leave the oral mucosa uninfiltrated would not cause any fluorescence alterations. Therefore the indication bandwidth of the VELscope® is small. Its advantage lies in the detection of early stages of OSCC in clinical suspect lesions which cannot be visualized in CT or PET/CT scans because of their minor size (see Fig. 3). Furthermore in advanced stages of head and neck carcinoma in which a clarification of the local in depth extension or lymphogenic metastatic spread is needed, these modalities are superior to the VELscope®.

As another limitation of the study the missing comparison of MRI representing the modality displaying soft tissue lesions more accurately than CT and the VELscope® technique can be mentioned. However due to practical reasons like accessibility and artifact induction in patients with metallic dental restorations, the application of MRI in a clinical setting is limited (Probst et al., 2017).

5. Conclusion

Since early cancerous lesions of the oral cavity often present asymptomatic, accurate detection and differentiation at early stages can have a significant effect on increasing early CIS diagnosis and VELscope® may be a beneficial tool in the evaluation of mucosal lesions when situational context is taken into account. Our results showed a significant difference between neoplastic and physiologic mucosa, supporting VELscope's® use as a non-invasive aid in carcinoma detection and its potential for the identification of lesional margins in the future.

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Conflict of interest

We declare that there are no financial and personal relationships with other people or organisations.

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Human rights: We declare that the study was in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

References

Awan KH, Morgan PR, Warnakulasuriya S: Evaluation of an autofluorescence based imaging system (VELscope) in the detection of oral potentially malignant disorders and benign keratoses. Oral Oncol 47(4): 274–277. http://dx.doi.org/10.1016/j.oraloncology, 2011

Betz CS, Stepp H, Janda P, Arbogast S, Grevers G, Baumgartner R, et al: A comparative study of normal inspection, autofluorescence and 5-ALA-induced PPIX fluorescence for oral cancer diagnosis. Int J Cancer 97(2): 245–252, 2002

Brennan JA, Mao L, Hruban RH, Boyle JO, Eby YJ, Koch WM, et al: Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. N Engl J Med 332: 429–435, 1995

Chaturvedi AK, Engels EA, Anderson WF, Gillison ML: Incidence trends for human papillomavirus-related and – unrelated oral squamous cell carcinomas in the United States. J Clin Oncol 26: 612–619, 2008

Conway DI, Stockton DL, Warnakulasuriya KA, Ogden G, Macpherson LM: Incidence of oral and oropharyngeal cancer in United Kingdom (1990–1999)-recent trends and regional variation. Oral Oncol 42: 586–592, 2006

Forastiere A, Koch W, Trotti A, Sidransky D: Head and neck cancer. N Engl J Med 346: 1890–1900, 2002 published erratum appears in N Engl J Med.346:788

Garavello W, Bertuccio P, Levi F, Lucchini F, Bosetti C, Malvezzi M, et al: The oral cancer epidemic in central and eastern Europe. Int J Cancer 127: 160–171, 2010

Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Altekruse SF, et al. (eds), SEER cancer statistics review, 1975-2013. Bethesda, MD: National Cancer Institute, http://seer.cancer.gov/csr/1975_2013/, April 2016

Kulapaditharom B, Boonkitticharoen V: Performance characteristics of fluorescence endoscope in detection of head and neck cancers. Ann Otol Rhinol Laryngol 110(1): 45–52, 2001

Lane PM, Gilhuly T, Whitehead P, Zeng H, Poh CF, Ng S, et al: Simple device for the direct visualization of oral-cavity tissue fluorescence. J Biomed Opt 11: 24006, 2006

Leemans CR, Tiwari R, Nauta JJ, van der Waal I, Snow GB: Recurrence at the primary site in head and neck cancer and the significance of neck lymph node metastases as a prognostic factor. Cancer 73: 187–190, 1994

Lewin F, Norell SE, Johansson H, Gustavsson P, Wennerberg J, Biörklund A, et al: Smoking tobacco, oral snuff, and alcohol in the etiology of squamous cell carcinoma of the head and neck: a population-based case-referent study in Sweden. Cancer 82(7): 1367–1375, 1998

Marur S, D'Souza G, Westra WH, Forastiere AA: HPV-associated head and neck cancer: a virus-related cancer epidemic. Lancet Oncol 11: 781–789, 2010

Moreno-Lopez LA, Esparza-Gomez GC, Gonzalez-Navarro A, Cerero-Lapiedra R, González-Hernández MJ, Domínguez-Rojas V: Risk of oral cancer associated with tobacco smoking, alcohol consumption, and oral hygiene: a case-control study in Madrid, Spain. Oral Oncol 36: 170–174, 2000

Mücke T, Wagenpfeil S, Kesting MR, Hölzle F, Wolff KD: Recurrence interval affects survival after local relapse of oral cancer. Oral Oncol 45(8): 687–691, 2009

Nagi R, Reddy-Kantharaj YB, Rakesh N, Janardhan-Reddy S, Sahu S: Efficacy of light based detection systems for early detection of oral cancer and oral potentially malignant disorders: systematic review. Med Oral Patol Oral Cir Bucal 21(4): e447–e455, 2016

Parkin DM: Global cancer statistics in the year 2000. Lancet Oncol 2(9): 533–543, 2001

Petersen PE: Global data on incidence of oral cancer. World Health Organization, http://www.who.int/oral_health/publications/oral_cancer_brochure.pdf?ua=1, 2005

Petti S: Pooled estimate of world leukoplakia prevalence: a systematic review. Oral Oncol 39(8): 770–780, 2003

Poh CF, Zhang L, Anderson DW, Durham JS, Williams PM, Priddy RW, et al: Fluorescence visualization detection of field alterations in tumor margins of oral cancer patients. Clin Cancer Res 12: 6716–6722, 2006

Poh CF, MacAulay CE, Zhang L, Rosin MP: Tracing the "at-risk" oral mucosa field with autofluorescence: steps toward clinical impact. Cancer Prev Res 2: 401–404, 2009

Probst M, Richter V, Weitz J, Kirschke JS, Ganter C, Troeltzsch M, et al: Magnetic resonance imaging of the inferior alveolar nerve with special regard to metal artifact reduction. J Craniomaxillofac Surg 45(4): 558–569. http://dx.doi.org/10.1016/j.jcms.2017.01.009, 2017 Epub 2017 Jan 25

Rana M, Zapf A, Kuehle M, Gellrich NC, Eckardt AM: Clinical evaluation of an autofluorescence diagnostic device for oral cancer detection: a prospective randomized diagnostic study. Eur J Cancer Prev 21(5): 460–466. http://dx.doi.org/10.1097/CEJ.0b013e32834fdb6d, 2012 Sep. PubMed PMID: 22217551

Reichart PA: Oral mucosal lesions in a representative cross-sectional study of aging Germans. Community Dent Oral Epidemiol 28(5): 390–398, 2000

Richards-Kortum R, Sevick-Muraca E: Quantitative optical spectroscopy for tissue diagnosis. Annu Rev Phys Chem 47: 555–606, 1996

Roblyer D, Kurachi C, Stepanek V, Williams MD, El-Naggar AK, Lee JJ, et al: Objective detection and delineation of oral neoplasia using autofluorescence imaging. Cancer Prev Res 2(5): 423–431, 2009

Sawan D, Mashlah A: Evaluation of premalignant and malignant lesions by fluorescent light (VELscope). J Int Soc Prev Community Dent 5(3): 248–254, 2015

Schepman KP, van der Meij EH, Smeele LE, van der Waal I: Malignant transformation of oral leukoplakia: a follow-up study of a hospital-based population of 166 patients with oral leukoplakia from The Netherlands. Oral Oncol 34(4): 270–275, 1998

Schwartz GJ, Mehta RH, Wenig BL, Shaligram C, Portugal LG: Salvage treatment for recurrent squamous cell carcinoma of the oral cavity. Head Neck 22(1): 34–41, 2000

Schwarz RA, Gao W, Weber CR, Kurachi C, Lee JJ, El-Naggar AK, et al: Noninvasive evaluation of oral lesions using depth-sensitive optical spectroscopy. Cancer 115(8): 1669–1679, 2009

Silverman Jr S: Oral cancer, 4th ed. Hamilton, Ontario, Canada: Decker, 1998

Sobin L, Gospodarowicz M, Wittekind C: International union against cancer (UICC) TNM classification of malignant tumors, 7th ed. West Sussex UK: Wiley-Blackwell, 2010

Tabor MP, Brakenhoff RH, Ruijter-Schippers HJ, Kummer JA, Leemans CR, Braakhuis BJ: Genetically altered fields as origin of locally recurrent head and neck cancer: a retrospective study. Clin Cancer Res 10: 3607–3613, 2004

Troeltzsch M, Knösel T, Woodlock T, Troeltzsch M, Pianka A, Probst FA, et al: Are there clinical or pathological parameters of maxillary oral squamous cell carcinoma with an influence on the occurrence of neck node Metastasis? An appraisal of 92 patients. J Oral Maxillofac Surg 74(1): 79–86. http://dx.doi.org/10.1016/j.joms.2015.07.011, 2016

Troeltzsch M, Knösel T, Eichinger C, Probst F, Troeltzsch M, Woodlock T, et al: Clinicopathologic features of oral squamous cell carcinoma: do they vary in different age groups? J Oral Maxillofac Surg 72(7): 1291–1300. http://dx.doi.org/10.1016/j.joms, 2014

Trullenque-Eriksson A, Muñoz-Corcuera M, Campo-Trapero J, Cano-Sánchez J, Bascones-Martínez A: Analysis of new diagnostic methods in suspicious lesions in the oral mucosa. Med Oral Patol Oral Cir Bucal 14(5): E210–E216, 2009

Warnakulasuriya S: Causes of oral cancer–an appraisal of controversies. Br Dent J 207: 471–475, 2009

Warnakulasuriya S, Johnson NW, van der Waal I: Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med 36(10): 575–580, 2007