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ORIGINAL ARTICLE

Effectiveness of autofluorescence to identify suspicious oral lesions—a prospective, blinded clinical trial

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Abstract Regular screening through white light inspection of the entire oral mucosa is the most important examination method to identify precancerous lesions and early oral carcinoma. Additionally, the physiologic autofluorescence of the oral mucosa has been described as a novel screening method for the detection of mucosal lesions that are not visible by white light. This study aimed to evaluate the sensitivity and specificity of the autofluorescence examination. Seventy-eight patients were examined in this study. All of them suffered from suspicious oral mucosal lesions. Two different investigation methods were applied: the standard examination by white light and an examination by a novel light source of 400 nm that evoked a green light emission (>500 nm) in normal mucosa. It was proposed that malignant oral mucosal lesions show different autofluorescence characteristics than the green autofluorescence of healthy mucosa. Red autofluorescence indicated SCC with a sensitivity of 20% and a specificity of 98%. The results showed that dysplasia and carcinoma could be identified with a sensitivity of 96% and a specificity of 18% by using the autofluorescence method. The sensitivity decreased according to the grade of mucosal keratosis and was influenced by the localisation of the lesion. In conclusion, benign as well as malignant oral lesions could not be distinguished by a diminished autofluorescence signal. A red autofluorescence signal, however, could indicate cancerous processes of the oral mucosa.

Keywords Autofluorescence · Prevention · Minimal invasive · Oral cancer · Diagnostic · Clinical trial

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Introduction

Cancer located in the mouth or oropharynx concerns 300,000 patients worldwide [1]. The prognosis decreases with advanced cancer stage [2–4], and the therapy of advanced cancer often leads to social stigmatization, speech handicap, and nutrition problems [5–8]. Therefore, early diagnosis of oral carcinoma is crucial for the patient's benefit. In the past, several minimally invasive diagnostic methods for early diagnosis of oral precancerous or malignant lesions have been published [9–17]. These techniques are based on visual as well as cytological principles. Examples include fluorescence or toluidine blue staining and methods for differential diagnosis such as the brush biopsy and consecutive image cytometry, immune cytology, or gene expression analysis [12, 14, 15, 18–20]. The autofluorescence technique used in this clinical trial is a new, commercially available screening instrument to detect suspicious oral lesions.

Physiologically, the oral mucosa shows a characteristic autofluorescence signal of >500 nm if excited by light of 400 nm [21]. Treatment by florescent chemicals is not necessary. Squamous cell carcinomas (SCCs), however, are supposed to be characterized by a different autofluorescence signal [22, 23]. These observations have been obtained by several studies and different wavelengths [21, 22, 24]. Svistun et al. achieved the best sensitivity and specificity for distinguishing cancer or dysplasia from normal mucosa at an excitation wavelength of 400 or 440 nm and a fluorescence observation at 530 nm, as done in the presented study [25]. They analyzed several regions of three resected carcinoma and one dysplasia using white light, autofluorescence, and incision biopsy, followed by a subsequent histopathologic analysis. They found a sensitivity of 100% and a specificity of 83% for the detection of cancer. Lane et al. examined 50 oral lesions to evaluate the accuracy of the autofluorescence in distinguishing SCC and carcinoma in situ from normal mucosa. They reported a significant correlation of malignant lesions with a lower intensity autofluorescence signal [26].

The differential diagnosis of inflammatory diseases such as lichen planus, severe periodontitis, or posttraumatic inflammation was not addressed in these studies.

Since the autofluorescence extinction of the oral mucosa served as a screening instrument to detect invisible lesions, there currently just exist data on the sensitivity. The potency to differentiate benign and malignant lesions has not been evaluated. The clinician, however, needs an examination instrument that supports the clinical diagnostics and the decision on how to treat a detected lesion. Therefore, data on autofluorescence specificity are urgently needed, but not available. This study aims to evaluate the effectiveness of the autofluorescence investigation and the capability to differentiate between suspicious and benign oral lesions, dysplasia, and SCC.

Material and method

Material

For standard screening of the oral mucosa using white light, the dental chair examination light was used (15V/150W, 64634 HLX OSRAM, Munich, Germany). The light source for autofluorescence excitation (Velscope™, Rocker&Narjes GmbH, Köln) emitted blue light at a wavelength of 400 nm. A dichroic mirror provided coaxial excitation and emission pathway. The autofluorescence was detected at >500 nm by the emission filter, which allowed the green–red fluorescent light to pass and rejected the blue excitation light. Another notch filter divided the fluorescent light spectrum into red and green components.

For documentation and blinded evaluation, the oral lesions were photographed with a digital reflex camera by different light sources (Canon EOS 100 clinical white light documentation, Nikon 50 and ISO 1400 for autofluorescence documentation). To record the intensity of autofluorescence, the camera was directly connected with the fluorescence light source so that the perspective, including refraction and wavelength, matched the examiner's view.

Method

To be included in the study, a mucosal lesion of the oral cavity was required that had been clinically diagnosed as SCC or suspicious epithelial lesions requiring histological evaluation for definitive diagnosis. Patients with clinically healthy mucosa were excluded. The 78 patients participating in the study attended the outpatient clinic of the Oral and Maxillofacial Surgery clinic of the Mainz University Medical Centre and suffered from suspicious oral mucosal lesions. Two different investigation methods were applied: the standard examination by white light and the examination by a 400-nm wavelength light source that is supposed to trigger a green light emission (>500 nm) in normal mucosa. After documentation by digital reflex photography, the suspicious lesion was anesthetized (UDS 1:200.000, Aventis Pharma, Bad Soden, Germany), and a biopsy by incision was performed. Then, the biopsies were fixed with formaldehyde 4.5% (Roti-Histofix, Carl Roth GmbH+ CKG, Karlsruhe, Germany) and processed for light microscopy via paraffin-embedded, haematoxylin–eosin-stained slices. All of these investigations were performed by the same investigator.

The photographs of the standard and autofluorescence examinations were evaluated independently and blindly by two different examiners who categorized the white and the autofluorescence aspect of the lesions. Using white light, the visual aspects of a plain leukoplakia, a verrucous leukoplakia, an erythroplakia, an erythroleukoplakia, an ulcer, a completely fibrin-covered lesion, a partially fibrin-covered ulcer, a partially fibrin-covered erythroleukoplakia, as well as a verrucous, erythematous partially fibrin-covered lesion were distinguished.

The clinical white light examination was conducted by one clinician who specialized in oral oncology. These findings were classified as (1) "abnormal but innocuous" (clinically explainable conditions like inflammation, scar, cheek bite, prosthesis incongruence, etc.), and (2) "suspicious for premalignant or malignant lesions".

The autofluorescence photographs were categorized according to black, dark green, bright green, red, speckled red/black, as well as a speckled green/black aspects (Figs. 1 and 2).

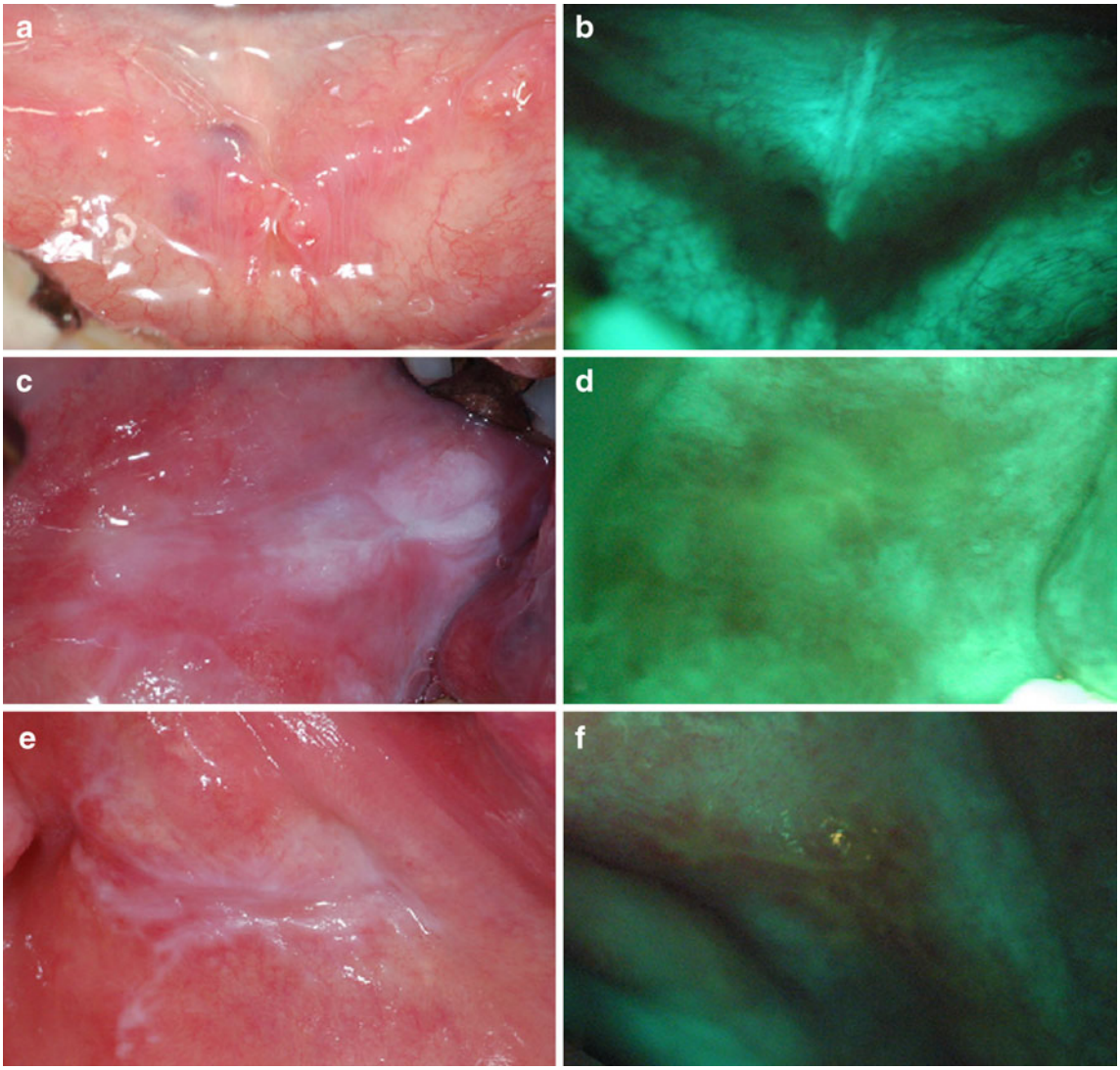

Fig. 1 Examples of fluorescence classification: **a**, **b** autofluoresence extinction (*white light aspect* normal mucosa, *histology* healthy mucosa); **c**, **d** low autofluorescence signal (*white light aspect* leukoplakia, *histology* oral lichen planus); **e**, **f** physiological autofluorescence signal (*white light aspect* leukoplakia, *histology* oral lichen planus)

These visual aspects were matched afterwards with the histopathological diagnoses of the scalpel biopsies. The diagnoses of mucosal hyperkeratosis, dysplasia, lichen planus, inflammation, healthy mucosa, dysplasia, and SCC were distinguished.

The sensitivity, specificity, positive and negative predictive values to diagnose SCC, and dysplasia were calculated depending on two different autofluorescence features:

(1) A low or absent autofluorescence signal (black or dark green aspect), as well as red autofluorescence signal, was evaluated as an indicator for dysplasia or SCC (positive). Also, a speckled, heterotopic aspect of both green and autofluorescence negative or reddish regions indicated a positive finding.

(2) The presence of red mucosal autofluorescence was evaluated as a separate indicator for dysplasia or SCC (positive).

Furthermore, the clinical diagnoses were evaluated by cross table analysis and the sensitivity, specificity, positive, and negative predictive values were calculated as well.

Using a blinded study design, the influence of the different examiners was minimized. The effect of the clinical aspects, as hyperkeratosis or hyperemia, and the localization of the lesion on the autofluorescence characteristics have been demonstrated by cross tables. The sensitivity and specificity were evaluated.

The statistical evaluation was performed using the SPSS software (SPSS 15.0 for Windows, SPSS Inc., Chicago, USA).

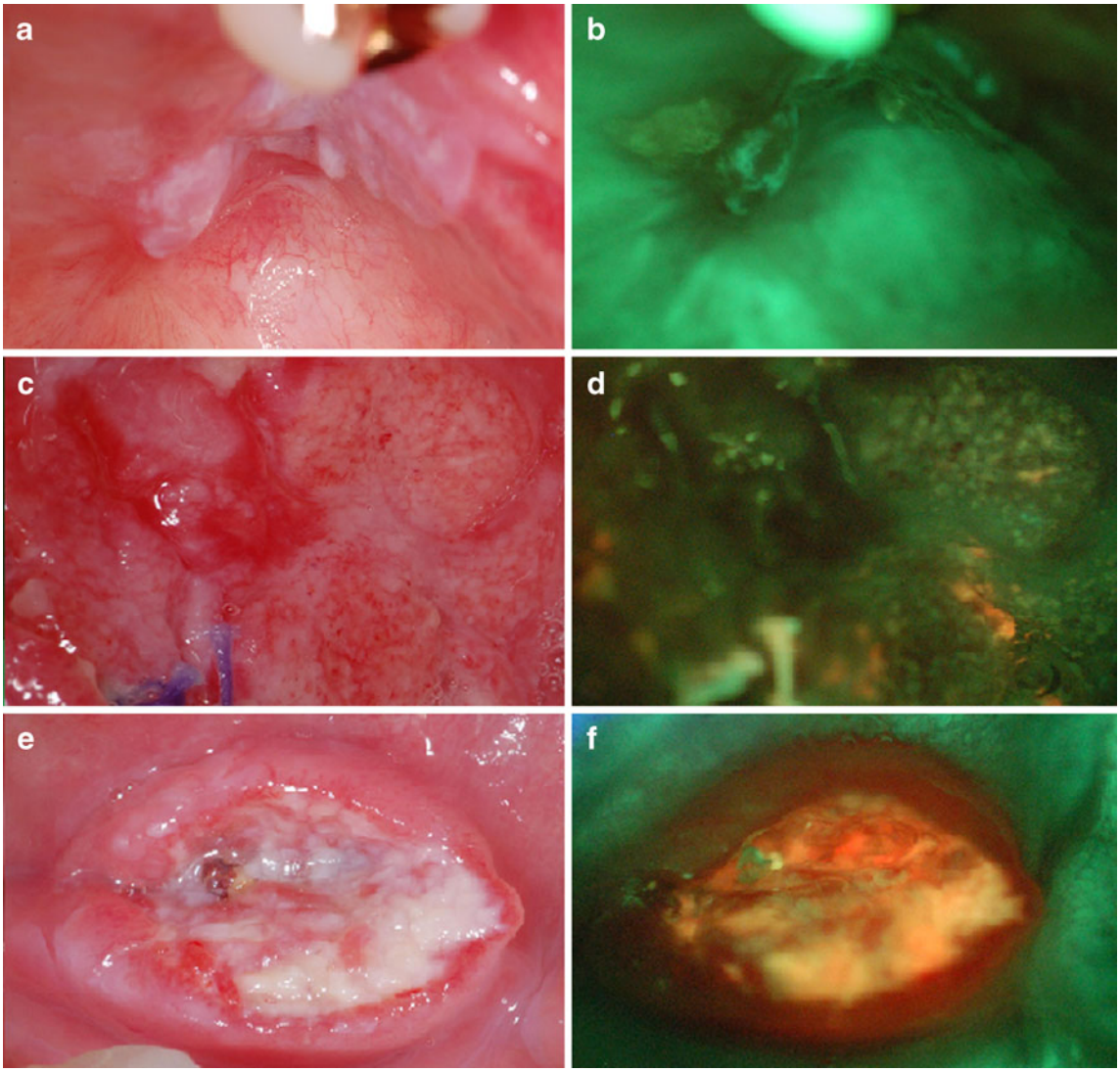

Fig. 2 Examples of fluorescence classification: **a**, **b** speckled green autofluorescence and low autofluorescence signal (*white light aspect* erythroleukoplakia, *histology* dysplasia); **c**, **d** speckled red autofluorescence and low autofluorescence signal (*white light aspect* (verrucous) erythroleukoplakia, *histology* SCC); **e**, **f**: red autofluorescence (*white light aspect* ulcer and fibrin, *histology* SCC)

Results

The 78 patients in this study had an average age of 61.7 years and 59% of them were males.

Forty-one percent of the oral lesions showed red features like the erythroplakia (17%) or the erythroleukoplakia (24%). A white, hyperkeratotic feature like the leukoplakia was found in 21% of the cases. An ulcerous aspect was described in 21% of the cases, and in 17%, a speckled aspect was found, including fibrin-covered lesions.

The histology results identified 14% of the lesions as mucosal hyperkeratosis, 33% as oral lichen planus, 9% as inflammation, 4% as dysplasia, and 39% of the oral lesions as a SCC. In 1% of the cases, normal mucosa was histologically found, although an erythematous aspect has been presented clinically (Table 1).

The accuracy of the clinical diagnosis to identify SCC was evaluated by high sensitivity (97%) and specificity (95.8%) values due to the experience of a specialized examiner (Table 2).

Table 1 Histopathological diagnosis of lesions included

	Frequency	Percent
Mucosal hyperkeratosis	11	14
Lichen planus	26	33
SCC	30	39
Inflammation	7	9
Dysplasia	3	4
Healthy mucosa	1	1
Σ	78	

Table 2 Test characteristics of the clinical diagnosis by white light examination

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
SCC	96.6	95.8	93.5	97.9
SCC/dysplasia	93.8	97.8	96.8	95.7

The blinded autofluorescence analysis revealed complete autofluorescence extinction in 49% (38) cases. In 13% (10) of the lesions, a physiological green autofluorescence was found. Thirty-eight percent (30) of the lesions were characterized by low autofluorescence, red autofluorescence, or a speckled, heterotopic aspect of both green and autofluorescence negative, as well as reddish regions at the same time.

The findings were reproducible by two different investigators in a blinded study design. Following the definition (1) of positive findings, cross table calculations showed a sensitivity of 93% and a specificity of 13–17% in identifying SCC. The positive predictive value (PPV) was calculated at 41%, the negative predictive value (NPV) at 75–80% (Table 3).

Pooling the histopathological findings of dysplasia and SCC, a high sensitivity and a low specificity were also found (sensitivity, 94%; specificity, 13–18%; PPV, 44–46%; NPV, 75–80%; Table 3).

If only the red autofluorescence findings were used to diagnose SCCs, according to definition (2), the sensitivity was 18–21%, the specificity 98%, the PPV 86–88%, and the NPV 62–63% (Table 3). If the histopathological diagnoses of SCC and dysplasia were pooled, they were identified with a low sensitivity and high specificity using red autofluorescence (Table 3).

Taking the results of the clinical and the autofluorescence examinations together, the sensitivity to identify SCCs could not be improved because the hyperkeratotic SCC that was not diagnosed clinically did not show any autofluorescence abnormalities either.

Looking at white light aspects and their autofluorescence signals, 77% of the oral lesions that showed a physiological autofluorescence of green light had a leukoplakia-like aspect. The sensitivity of diagnosing hyperkeratotic SCC correctly was 50% (Table 4).

Table 5 presents the autofluorescence characteristics and their anatomical localization. Particularly, the dorsum of the tongue did not show autofluorescence extinctions, although two SCCs had been diagnosed in this area by means of histology. Another cancerous lesion of this region could be identified by a red autofluorescence pattern.

Discussion

This study evaluated the intensity and quality of the emitted autofluorescence signal of >500 nm after excitation by 400 nm, and included 78 suspicious inflammation lesions, mucosal hyperkeratosis, lichen planus , dysplasia, and SCC. Taking all lesions of a deviated autofluorescence signal as positive for SCC, a sensitivity of 93% and a specificity of 13–17% were found (definition (1)). Evaluating only clinically erythematous features, such as dysplasia, lichenoid lesions, or inflammation, the autofluorescence diagnosis led to a false positive result in 59% of these cases (PPV, 41%; Table 3). Erythematous, benign lesions could, therefore, not be distinguished from SCC by autofluorescence.

For red autofluorescence, the PPV was 84–88 %; the sensitivity to distinguish SCC from all other lesions, however, was only 18–21%, the specificity, 98%, and the NPV, 42–43% (definition (2)). Therefore, lesions showing a red autofluorescence signal should need further clarification via histology, indicated by a high PPV and a high specificity value.

These results suggest that autofluorescence could help to identify any type of pathological oral lesions using lower fluorescence signal, but could not reliably distinguish benign oral lesions from dysplasia or SCC.

The property of the autofluorescence technique to detect oral lesions that are difficult to identify by white light has already been demonstrated by Huff et al. and is accepted [27]. Several other studies, however, have claimed that fluorescence analysis is highly sensitive for identifying malignant mucosal lesions in the oral cavity [26, 28]. These excellent test results could be caused by a study population

Table 3 Test results of autofluorescence results for identification of SCC, lesions of SCC or dysplasia by low autofluorescence signal (a) and by red color autofluorescence signal (b) (evaluation range is due to different investigators)

	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
	a	b	a	b	a	b	a	b
SCC	93	20 (18–21)	15 (13–17)	98	41 (40–41)	87 (86–88)	78 (75–80)	63 (62–63)
SCC/dysplasia	94	22 (20–23)	16 (13–18)	98	45 (44–46)	87 (86–88)	77 (75–80)	67 (66–67)

Table 4 Diagnostic effectiveness to identify SCC of hyperkeratinized and reddish aspect

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Hyperkeratosis	50	53	14	87
Erythema	92	0	41	0

of completely obvious malignant or benign findings of SCCs and healthy mucosa. Suspicious inflammation of the oral mucosa or oral lichen planus has not been included. However, to evaluate the clinical relevance of fluorescence analysis, these differential diagnoses have to be investigated.

As done by the presented study, also Jayaprakash et al. investigated the autofluorescence characteristics of oral lesions, identified by white light examination. They reported a sensitivity of 80% to identify cancer by white light examination, which is comparable to the results of our study, showing 96.6%. They conducted a loss of autofluorescence to identify suspicious oral lesions. By this autofluorescence algorithm, a test sensitivity of 93.3% to identify cancer and 96% to identify cancer, as well as high-risk-lesions, were described. If white light examination and autofluorescence examination were taken together, all cancer and high-risk lesions had been identified correctly [29].

Our results, however, could not support the additional diagnostic help of autofluorescence application. No cancerous lesion that was clinically not identified was found by the aid of the autofluorescence technique. The influence of lesion characteristics and lesion localization on autofluorescence characteristics, as well as the red autofluorescence, has not been concerned by Jayaprakash et al. [29].

The strong concordance of physiological green fluorescence and the hyperkeratosis of the lesion support the assumption that hyperkeratotic lesions could elude autofluorescence detection. Concordantly, Betz et al. found lesions easier to detect if they were not verrucous or exophytic [30, 31]. Also, concordantly, these authors found a limited assessment of the dorsum of the tongue [31]. No lesion localized at the dorsum of the tongue showed autofluorescence extinction, although two of these lesions of green autofluorescence turned out to be invasive carcinoma after histological diagnosis (Table 5). Concerning hyperkeratotic oral lesions or lesions localized at the dorsum of the tongue, these results suggest a limited benefit for cancer screening by means of loss of autofluorescence.

The exact mechanisms underlying alteration in epithelial autofluorescence remain unclear. Several fluorophores and chromophores which could absorb the autofluorescence signal, as well as an altered tissue structure, could influence the overall optical signals. Fluorophores that emit light at >500 nm are ceroide and eosinophile Granula, amino acids such as tryptophan, and also NADH and oxidized FAD. These coenzymes of the oxidative phosphorylation and glycolysis are altered in the case of malignant mutation as well as inflammation. An influence of inflammation on autofluorescence signal therefore seems feasible, as shown by this study, although Svistun proposed that inflammation did not influence the autofluorescence characteristics [25]. The source of red autofluorescence could be caused by porphyrin that is a typical product of bacterial metabolism. If this were the case, red autofluorescence would not be an appropriate indicator for early diagnosis of SCC or dysplasia [30]. Other fluorophores such as ceroide, however, could also show red autofluorescence and are also being considered.

Table 5 Cross table of anatomical region and autofluorescence signal

	Autofluorescence						Total
	No signal	Low signal	Red	Green	Speckled: red + no signal	Speckled: no signal + green	
Region							
Cheek	11	9	0	3	2	1	26
Gingival	15	2	1	2	1	1	22
Floor of the mouth	3	0	0	0	4	0	7
Sulcus glossoalv.	2	0	0	0	0	0	2
Tongue lower side	0	0	2	0	0	0	2
Tongue dorsum	0	0	1	2	0	0	3
Palate	3	0	2	1	2	0	8
Arcus palatogloss.	3	0	1	0	0	1	5
Inner lips	1	0	0	2	0	0	3
Total	38	11	7	10	9	3	78

The proposed benefit to detect many invisible, possibly malignant lesions is challenged by the necessity to find a definitive diagnosis of these mucosal lesions. Considering our study results, the autofluorescence does not support the examiner in terms of further therapy decisions because the autofluorescence is not capable to distinguish benign and malignant mucosal lesions. The low test specificity of the autofluorescence screening does not justify an invasive diagnostic effort. In case of clinically unsuspicious oral lesions, minimal invasive methods should be applied then.

Conclusion

With a high sensitivity and NPV, but a low specificity and PPV, oral mucosal lesions could be detected by autofluorescence. The autofluorescence examination, however, is not able to differentiate between benign and malignant oral lesions. Red autofluorescence should be an indication for scalpel biopsy due to a high PPV for cancer.

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Conflict of interest The authors confirm that they don't have any conflict of interest.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P (2005) Global cancer statistics, 2002. CA Cancer J Clin 55:74–108
2. Silverman S Jr (1988) Early diagnosis of oral cancer. Cancer 62:1796–1799
3. Silverman S Jr (2001) Demographics and occurrence of oral and pharyngeal cancers. The outcomes, the trends, the challenge. J Am Dent Assoc 132(Suppl):7S–11S
4. Kujan O, Glenny AM, Oliver RJ, Thakker N, Sloan P (2006) Screening programmes for the early detection and prevention of oral cancer. The cochrane collaboration CD004150
5. Meyer TK, Kuhn JC, Campbell BH, Marbella AM, Myers KB, Layde PM (2004) Speech intelligibility and quality of life in head and neck cancer survivors. Laryngoscope 114:1977–1981
6. Holloway RL, Hellewell JL, Marbella AM, Layde PM, Myers KB, Campbell BH (2005) Psychosocial effects in long-term head and neck cancer survivors. Head Neck 27:281–288
7. Campbell BH, Spinelli K, Marbella AM, Myers KB, Kuhn JC, Layde PM (2004) Aspiration, weight loss, and quality of life in head and neck cancer survivors. Arch Otolaryngol Head Neck Surg 130:1100–1103
8. Kosicki DM, Riva C, Pajarola GF, Burkhardt A, Gratz KW (2007) OralCDx brush biopsy—a tool for early diagnosis of oral squamous cell carcinoma. Schweiz Monatsschr Zahnmed 117: 222–227
9. Böcking A, Striepecke E, Auer H, Füzesi L (1994) Static DNA-cytometry. Biological background, technique and diagnostic interpretation. In: Wied G, Keebler CM, Rosenthal DL, Schenk U, Somrak TM, Vooijs GP (eds) Compendium on quality assurance. Tutorials of Cytology, Chicago, pp 107–128
10. Grote HJ, Friedrichs N, Pomjanski N, Guhde HF, Reich O, Bocking A (2001) Prognostic significance of DNA cytometry in carcinoma of the uterine cervix FIGO stage IB and II. Anal Cell Pathol 23:97–105
11. Scheifele C, Schlechte H, Bethke G, Reichart PA (2002) Detection of TP53-mutations in brush biopsies from oral leukoplakias. Mund Kiefer Gesichtschir 6:410–414
12. Zhang L, Rosin MP (2001) Loss of heterozygosity: a potential tool in management of oral premalignant lesions? J Oral Pathol Med 30:513–520
13. Driemel O, Kosmehl H, Rosenhahn J, Berndt A, Reichert TE, Zardi L, Dahse R (2007) Expression analysis of extracellular matrix components in brush biopsies of oral lesions. Anticancer Res 27:1565–1570
14. Koch FP, Kaemmerer PW, Biesterfeld S, Wagner W (2008) Benefit of diverse techniques to diagnose early carcinoma. J Cranio-Maxillo Facial Surg 36:S185
15. Toyoshima T, Koch F, Kaemmerer P, Vairaktaris E, Al-Nawas B, Wagner W (2009) Expression of cytokeratin 17 mRNA in oral squamous cell carcinoma cells obtained by brush biopsy: preliminary results. J Oral Pathol Med 38:530–534
16. Toyoshima T, Koch F, Kaemmerer P, Wagner W (2008) K17 in oral squamous cell carcinoma cells by brush biopsy. J Cranio-Maxillo Facial Surg 36:38
17. Guneri P, Epstein JB, Ergun S, Boyacioglu H (2010) Toluidine blue color perception in identification of oral mucosal lesions. Clin Oral Investig (in press)
18. Driemel O, Dahse R, Hakim SG, Tsioutsias T, Pistner H, Reichert TE, Kosmehl H (2007) Laminin-5 immunocytochemistry: a new tool for identifying dysplastic cells in oral brush biopsies. Cytopathology 18:348–355
19. Remmerbach TW, Mathes SN, Weidenbach H, Hemprich A, Bocking A (2004) Noninvasive brush biopsy as an innovative tool for early detection of oral carcinomas. Mund Kiefer Gesichtschir 8:229–236
20. Krauss E, Rauthe S, Gattenlohner S, Reuther T, Kochel M, Kriegebaum U, Kubler AC, Muller-Richter UD (2010) MAGE-A antigens in lesions of the oral mucosa. Clin Oral Investig. doi:10.1007/s00784-010-0387-9
21. Richards-Kortum R, Sevick-Muraca E (1996) Quantitative optical spectroscopy for tissue diagnosis. Annu Rev Phys Chem 47:555–606
22. de Veld DC, Skurichina M, Witjes MJ, Duin RP, Sterenborg HJ, Roodenburg JL (2005) Autofluorescence and diffuse reflectance spectroscopy for oral oncology. Lasers Surg Med 36:356–364
23. Poh CF, Ng SP, Williams PM, Zhang L, Laronde DM, Lane P, Macaulay C, Rosin MP (2007) Direct fluorescence visualization of clinically occult high-risk oral premalignant disease using a simple hand-held device. Head Neck 29:71–76
24. Ganesan S, Sacks PG, Yang Y, Katz A, Al-Rawi M, Savage HE, Schantz SP, Alfano RR (1998) Native fluorescence spectroscopy of normal and malignant epithelial cells. Cancer Biochem Biophys 16:365–373
25. Svistun E, Alizadeh-Naderi R, El-Naggar A, Jacob R, Gillenwater A, Richards-Kortum R (2004) Vision enhancement system for detection of oral cavity neoplasia based on autofluorescence. Head Neck 26:205–215
26. Lane PM, Gilhuly T, Whitehead P, Zeng H, Poh CF, Ng S, Williams PM, Zhang L, Rosin MP, MacAulay CE (2006) Simple device for the direct visualization of oral-cavity tissue fluorescence. J Biomed Opt 11:024006
27. Huff K, Stark PC, Solomon LW (2009) Sensitivity of direct tissue fluorescence visualization in screening for oral premalignant lesions in general practice. Gen Dent 57:34–38

28. Poh CF, Zhang L, Anderson DW, Durham JS, Williams PM, Priddy RW, Berean KW, Ng S, Tseng OL, MacAulay C, Rosin MP (2006) Fluorescence visualization detection of field alterations in tumor margins of oral cancer patients. Clin Cancer Res 12:6716–6722
29. Jayaprakash V, Sullivan M, Merzianu M, Rigual NR, Loree TR, Popat SR, Moysich KB, Ramananda S, Johnson T, Marshall JR, Hutson AD, Mang TS, Wilson BC, Gill SR, Frustino J, Bogaards A, Reid ME (2009) Autofluorescence-guided surveillance for oral cancer. Cancer Prev Res (Phila Pa) 2:966–974
30. Betz CS, Mehlmann M, Rick K, Stepp H, Grevers G, Baumgartner R, Leunig A (1999) Autofluorescence imaging and spectroscopy of normal and malignant mucosa in patients with head and neck cancer. Lasers Surg Med 25:323–334
31. Betz CS, Stepp H, Janda P, Arbogast S, Grevers G, Baumgartner R, Leunig A (2002) A comparative study of normal inspection, autofluorescence and 5-ALA-induced PPIX fluorescence for oral cancer diagnosis. Int J Cancer 97:245–252