European Journal of Surgical Oncology 47 (2021) 2220–2232

Contents lists available at ScienceDirect

European Journal of Surgical Oncology

journal homepage: www.ejso.com

Assessment of the deep resection margin during oral cancer surgery: A systematic review

S.G. Brouwer de Koning [a, *, 1], A.W.M.A. Schaeffers [a, 1], W. Schats [b], M.W.M. van den Brekel [a], T.J.M. Ruers [c, d], M.B. Karakullukcu [a]

[a] Department of Head and Neck Surgery and Oncology, Antoni van Leeuwenhoek, Netherlands Cancer Institute, Amsterdam, the Netherlands
[b] Scientific Information Service, Antoni van Leeuwenhoek, Netherlands Cancer Institute, Amsterdam, the Netherlands
[c] Department of Surgical Oncology, Antoni van Leeuwenhoek, Netherlands Cancer Institute, Amsterdam, the Netherlands
[d] Faculty of Science and Technology, University of Twente, Enschede, the Netherlands

ARTICLE INFO

Article history:
Received 26 February 2021
Accepted 13 April 2021
Available online 17 April 2021

Keywords:
Intra-operative margin assessment
Deep resection margin
Oral squamous cell carcinoma

ABSTRACT

The main challenge for radical resection in oral cancer surgery is to obtain adequate resection margins. Especially the deep margin, which can only be estimated based on palpation during surgery, is often reported inadequate. To increase the percentage of radical resections, there is a need for a quick, easy, minimal invasive method, which assesses the deep resection margin without interrupting or prolonging surgery. This systematic review provides an overview of technologies that are currently being studied with the aim of fulfilling this demand.

A literature search was conducted through the databases Medline, Embase and the Cochrane Library. A total of 62 studies were included. The results were categorized according to the type of technique: 'Frozen Section Analysis', 'Fluorescence', 'Optical Imaging', 'Conventional imaging techniques', and 'Cytological assessment'. This systematic review gives for each technique an overview of the reported performance (accuracy, sensitivity, specificity, positive predictive value, negative predictive value, or a different outcome measure), acquisition time, and sampling depth.

At the moment, the most prevailing technique remains frozen section analysis. In the search for other assessment methods to evaluate the deep resection margin, some technologies are very promising for future use when effectiveness has been shown in larger trials, e.g., fluorescence (real-time, sampling depth up to 6 mm) or optical techniques such as hyperspectral imaging (real-time, sampling depth few mm) for microscopic margin assessment and ultrasound (less than 10 min, sampling depth several cm) for assessment on a macroscopic scale.

© 2021 Elsevier Ltd, BASO ~ The Association for Cancer Surgery, and the European Society of Surgical Oncology. All rights reserved.

Introduction

For patients with early-stage and resectable advanced-stage oral cancer surgery is generally standard of care [1]. Primarily, the goal is to obtain adequate resection margins, since inadequate margins are associated with a higher risk of recurrence and worse prognosis [2].

There is no consensus on what constitutes an adequate resection margin: a recent survey among members of the American Head and Neck Society (AHNS) showed that 56.5% of the respondents define a clear margin as >5 mm [3]. Other definitions used were 3 mm, 2 mm, >1 mm, no ink on tumor on microscopic evaluation or 1–1.5 cm gross margin. The optimal definition of a clear margin in association with local recurrence or overall survival has been evaluated extensively [2,4–14]. However, it is not possible to use the current literature for robust scientific evidence since the large heterogeneity among the different studies [15,16]. The most commonly used guidelines are defined by The Royal College of Pathologists and the National Comprehensive Cancer Network (NCCN). Both guidelines agree on the definition of an adequate margin, i.e., more than 5 mm of healthy tissue between tumor cells and the resection border. However, a positive margin is defined as tumor cells at the resection margin by the Royal College of

* Corresponding author. Antoni van Leeuwenhoek, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, the Netherlands.
E-mail address: s.brouwerdekoning@nki.nl (S.G. Brouwer de Koning).
[1] Equal contribution.

https://doi.org/10.1016/j.ejso.2021.04.016
0748-7983/© 2021 Elsevier Ltd, BASO ~ The Association for Cancer Surgery, and the European Society of Surgical Oncology. All rights reserved.

Pathologist, while a positive margin can involve tumor cells within the first millimeter according to the NCCN [12,17,18]. The definitive status of the resection margin is determined by the histopathologist, several days after surgery. In case positive margins are reported, adjuvant treatment is required, e.g., subsequent surgery, radiotherapy or chemoradiotherapy [1,12,19–24].

During surgery, estimating the extent of tumor growth into tissue is thought to be the main challenge for a radical resection. The superficial pattern of tumor growth in oral squamous cell carcinoma (OSCC) allows a good estimation of the mucosal margin. However, the deep margin can only be estimated based on palpation and information on tumor thickness obtained by preoperative imaging. Due to this limited intra-operative feedback on tumor margins, resections are inadequate in 30%–85% of the procedures [25]. To reduce the number of inadequate resections, there is a need for technologies that can provide information on the status of the margin during surgery. With intra-operative margin assessment, the resected specimen (specimen-driven) or the tumor bed (patient-driven) is examined and the surgeon is informed on whether the margins are sufficient during the initial surgery. In case inadequate margins are found, the surgeon extends the resection directly when feasible, thereby often preventing the necessity of adjuvant postoperative treatment and possibly improving prognosis [12,23]. Hence, intra-operative margin assessment is useful in pursuing adequate resection margins and decision-making during and after surgery.

Recently a systematic review focused on intraoperative margin assessment was published, emphasizing the need for more studies to improve accuracy of techniques to reduce positive margins [26]. However, no distinction between mucosal and deep margins was made. Technologies for intra-operative margin assessment have to distinguish healthy tissue from tumor tissue. Healthy mucosal tissue differs from healthy tissue that is found at the deep margin, and therefore requires a different approach. The focus of intra-operative margin assessment should be on the deep margin for two reasons: Woolgar et al. showed that the deep margin was involved in 87% of the tissues with inadequate margins, and Weijers et al. found that there was no significant difference in recurrence rate between close and clear mucosal margins, suggesting that the deep margin is more important than the mucosal margin [22,27].

The aim of this systematic review is to provide an overview of all intra-operative techniques that are available or under development to assess the deep tumor resection margin in patients with OSCC.

Methods

A literature search was conducted through the databases Medline, Embase and the Cochrane Library, on the August 28, 2020 using a combination of indexed search terms and free text terms: 'margins of excision' OR 'depth of invasion' OR 'invasion depth' OR 'deep resection margin' OR 'deep resection' AND 'Head and neck neoplasms' OR 'Mouth neoplasms' AND 'Intraoperative period'.

The study selection was conducted by two researchers who independently screened titles and abstracts for a relevant contribution to this review. Studies were included that examined OSCC, assessed the surgical margin during surgery for immediate feedback on the status of the margin, evaluated the deep resection margin rather than the mucosal margin, were human studies, and were scholarly journal articles with full texts available. Based on the title and abstract, studies were excluded that evaluated phantoms and animals, cancers other than head and neck, technologies that were not intended for intra-operative use and when the outcome measure was not meeting the purpose of this review. Full texts were evaluated on the following exclusion criteria: when the focus of the article was to evaluate the status of the resection margin as a prognostic predictor, the outcome of the intraoperative assessment of the surgical margin was not compared with a verification method, transoral robotic surgery (TORS) was used, the study population consisted of less than three patients, only mucosal/superficial margins were evaluated, the technology was used for preoperative diagnosis instead of intraoperative assessment, or the study was focused on the presence of specific genes to predict tumor recurrence. Furthermore, the authors believed that studies before the year 1999 could be excluded, because relatively old techniques have been improved and repeatedly studied since. In addition, references of included articles were screened on eligibility for inclusion. Fig. 1 shows the process for study selection.

Studies were categorized into different groups according to the type of technology that was used for intra-operative margin evaluation: 'Frozen Section Analysis', 'Fluorescence', 'Optical Imaging', 'Conventional imaging techniques', and 'Cytological assessment'. Data extracted from the included studies were as follows: (1) study methodology, (2) margin assessment technology, (3) whether margins were assessed on the remaining defect after tumor removal, or at the resection surface of the specimen, or if the tumor was evaluated in situ, (4) verification method, (5) definition of positive margin, (6) sample size, (7) tumor site, (8) accuracy of the technology, or sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), or a different outcome measure, (9) acquisition time, and (10) sampling depth.

Results

Frozen section analysis

With frozen section analysis (FSA), the surgeon and the pathologist collaborate to provide a rapid intraoperative evaluation of the surgical margin. The freshly resected tissue is transported to the pathology department, frozen in a cryostat machine, thinly sliced with a razor, affixed to a glass slide and dipped into fixatives and tissue stains for immediate interpretation [28]. The diagnostic performance of this methodology has been widely studied in both retrospective and prospective studies (Table 1). Frozen sections were obtained from both the remaining defect after tumor excision, as well as from the resected specimen itself, and the diagnosis that was the result of the FSA was verified with the final histopathological outcome. Number of patients that were included by the studies ranged from 20 to 435. FSA is mainly applicable for soft tissue specimen; the high density of bone makes routine FSA of cortical bony margins difficult. Few groups have presented methods for bone margin FSA resulting in sensitivities and specificities of 77–88.9% and 90–100%, respectively [29,30]. Despite the high accuracies achieved with FSA, the technique is subject to false negatives due to the complexity of some surgical specimens. With one frozen section, only a small fraction of the specimen can be evaluated, and the time needed to evaluate one frozen section is 15–30 min.

Fluorescence

More than 90% of head and neck tumors express the epidermal growth factor receptor (EGFR), offering a cancer-specific target for contrast agents, like panitumumab or cetuximab. These antibodies can be conjugated with a near-infrared fluorescent dye (e.g. IRDye800CW, indocyanine green) for intra-operative tumor detection [31]. The advantage of panitumumab over cetuximab is the higher binding affinity and improved safety profile [32]. Acquisition times vary between real time and several minutes (Table 2). In addition, near-infrared fluorescence can penetrate through approximately 5–6 mm tissue, making this a promising

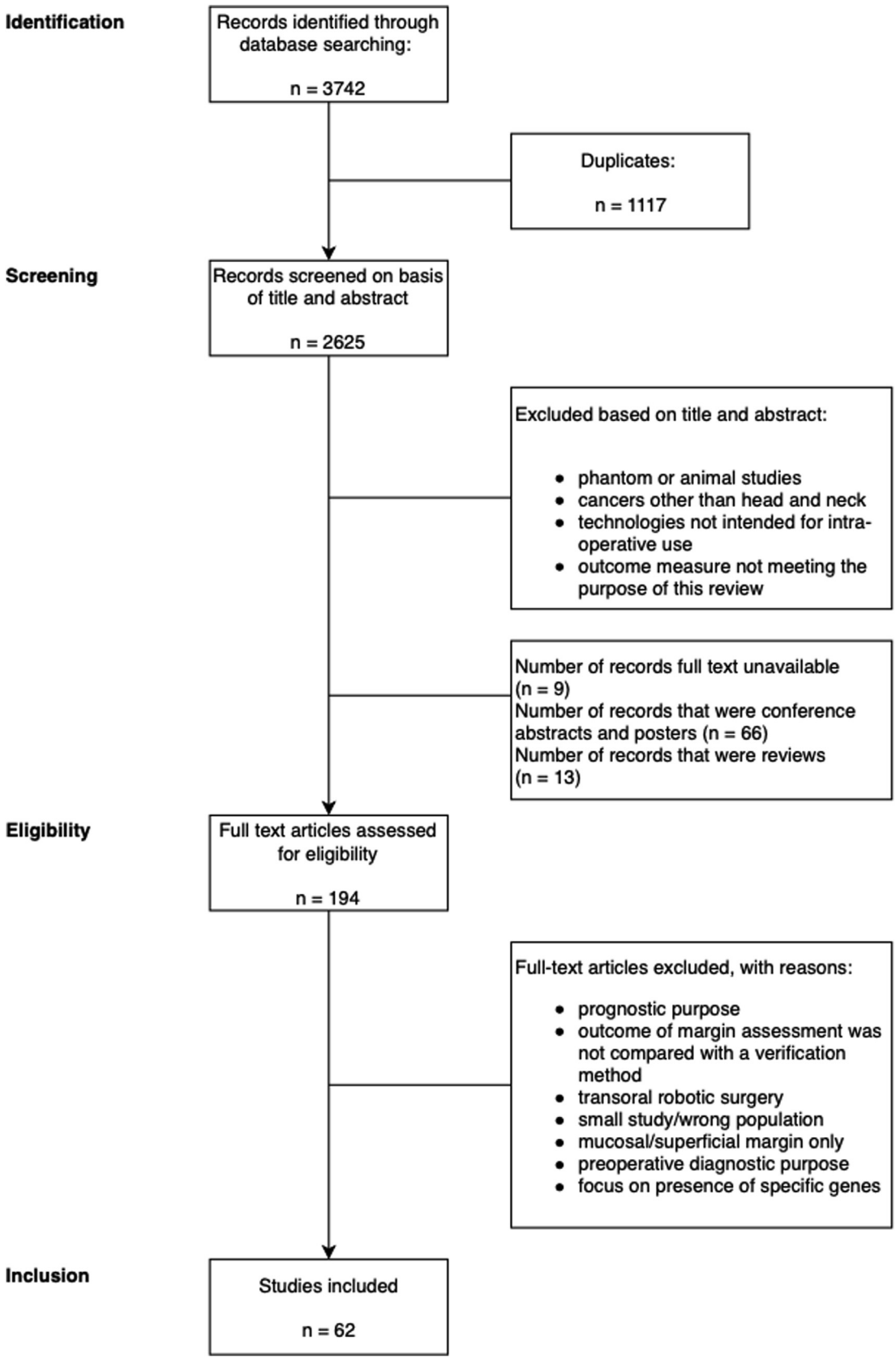

Fig. 1. Flow diagram of selection strategy.

Table 1
Included studies reporting on frozen section analysis for intra-operative margin assessment.

Frozen section															
Author, year	Study methodology	Margin assessment technology	Specimen/defect driven/in situ	Verification method	Optimal margin (mm)	Sample size (number of patients)	Tumor site	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)	Acquisition time	Sampling depth	Other outcome measures/remarks
Abbas, 2017 [81]	Retrospective	FS	defect	histology	10	77	variable: oral soft tissue	72.7	95.3	90.9	66.6	93.9			
Amit, 2015 [73]	Prospective	FS	specimen and defect	histology	5	71	variable: oral soft tissue	91 vs 22	93 vs 100						FP: 9% vs 0%; FN: 17% vs 44%
De Visscher, 2002 [82]	Prospective	FS	specimen	histology	3	72	lip						20 min		8 of the 9 patients who had a positive margin on FS was confirmed by histopathology; FP: 1.4%
DiNardo, 2000 [77]	Retrospective	FS	defect	histology	5	80	variable: oral soft tissue	34.3	100	71.3	100	66.2	15 min		
Du, 2016 [83]	Retrospective	FS	specimen	histology	5	253	variable: oral soft tissue	78	97	93	89	94			
Gooris, 2003 [84]	Retrospective	FS	unknown	histology	5	131	lip			99					
Layfield, 2018 [85]	Retrospective	FS	specimen	histology		288	variable: oral soft tissue	88.9	98.6		93.3	97.6			
Moe, 2019 [86]	Prospective	FS	specimen	histology		30	variable: oral soft tissue	90.9	100	96.8	100	95.2			Correlation coefficient FS and histopathology: >0.95
Mair, 2017 [87]	Retrospective	FS vs GE	specimen (FS) vs in situ (GE)	histology	5	435	variable: oral soft tissue	45.45 vs 61.9	98.8 vs 88.3	92.9 vs 83.7	93.5 vs 91.6	83.3 vs 53.1			
Nayanar, 2019 [88]	Retrospective	FS	specimen	histology	no tumor at margin	265	variable: oral soft tissue	82.05	96.46				20 min		
Oxford, 2006 [29]	Retrospective	FS	unknown	histology		25	mandible and maxilla	88.9	100					superficial	
Pandey, 2010 [89]	Retrospective	FS	specimen	histology	5	104	unknown	78.57	99.55	98.32					
Ribeiro, 2003 [90]	Retrospective	FS	specimen	histology	10	82	variable: oral soft tissue	92.8	99.8						99.5% concordance
Sharma, 2008 [91]	Prospective	FS	specimen	histology		47	variable: oral soft tissue	72	99.4	96.74	94.7	96			FP: 0.59; FN: 28%
Tirelli, 2019 [92]	Prospective	FS	defect	histology	3	42	variable: oral soft tissue	93.6	96.8		90.7	96.8			
Varvares, 2015 [10]	Retrospective	FS	specimen vs defect	histology	5	91 vs 8	variable: oral soft tissue								Agreement FS and histopathology: 95%
Wysluch, 2010 [30]	Prospective	FS	specimen	histology	10	20	mandible	77	90				30 min		

FS = frozen section analysis; GE = general examination.

technique for detection of positive and close margins [33,34]. However, disadvantages of the use of these conjugated antibodies are the intravenous administration that may lead to adverse reactions, the long plasma half-lives (unbound tracers result in non-specific background fluorescence; administration requires additional planning since it needs to be done several days in advance of the surgery), and the relatively high doses required to have sufficient tracers reach the tumor. Therefore, additional research has been performed to activatable fluorescent tracers that can be applied topically, like γ-glutamyl hydroxymethyl rhodamine green (g-Glu-HMRG) and 5-aminolevulinic acid-induced protoporphyrin IX (5-ALA-induced PPIX) [35–37]. These tracers required an incubation period of 10 min and 1–2.5 h respectively, before malignant tissue fluoresced. Also, sampling depth is limited to less than 1 mm.

Focusing on bone resection margins, Nieberler et al. evaluated the use of integrin αvβ6-targeting arginylglycylaspartic acid peptides as a marker for fluorescent cytology [38]. They reported on high diagnostic values and the technique required 40 min to use.

Another type of fluorescence use is fluorescence lifetime imaging, in which endogenous fluorophore lifetime of tissue is probed by illumination with a pulsed, long-wave ultraviolet light source [39]. This technique has been evaluated by Tajudeen et al., in combination with dynamic optical contrast imaging (DOCI) so that the fluorophore lifetime can be mapped over a macroscopic field of view. Significant differences ($p < 0.05$) were found in fluorescence lifetime in different types of tissue and acquisition time was less than 2 min.

Optical techniques

The most studied optical techniques used for intra-operative margin assessment in oral squamous cell carcinoma are Raman spectroscopy (RM), diffuse reflectance spectroscopy (DRS), hyperspectral imaging (HSI), optical coherence tomography (OCT) and narrow band imaging (NBI) (Table 3).

Raman Spectroscopy

Raman spectroscopy (RS) is an optical technique based on inelastic scattering of light by molecules in tissue and therefore provides detailed information about its molecular composition [40]. RS is able to discriminate tumor from healthy tissue by the difference in water concentration in these two tissue types. Barosso et al., Cals et al. and Yu et al. used a different part of the spectrum (2500-4000 cm^{-1}, 400-1800 cm^{-1} and 300-3950 cm^{-1}, respectively) and obtained comparable results in the discrimination of OSCC and healthy tissue in tongue specimen (sensitivity 99%/100%/99%, specificity 92%/78%/94%, respectively) [41–43]. Similar results are also reported for mandibular specimens [40]. The technique can be used directly on tissue because it is non-destructive, and there is no need for reagents or labelling [40]. RS is fast (measurements in the order of 1 s or less, with real-time signal analysis) and can be applied through the use of hand-held fiber-optic probes at any location. However, the sampling area per measurement is in the order of 300–1000 μm, so multiple measurements are needed to evaluate the whole resection surface [40,42,44]. Also, the sampling depth is up to 40–50 μm, which challenges the detection of close margins where tumor cells are present within 5 mm from the resection surface. RS is now built into a needle that can be inserted several millimeters into the tissue as an approach to overcome this limited sampling depth. The published results on this are expected soon (Erasmus Medical Center, The Netherlands, project number: 106467).

Diffuse reflectance spectroscopy

In diffuse reflectance spectroscopy (DRS), diffusely reflected light is measured after illuminating the tissue with a broadband white light source. The reflectance spectrum contains information about the absorption and scattering properties of the illuminated tissue. Differences in these properties allow for tissue characterization, e.g., to discriminate tumor from healthy tissue. A total of 28 tumor specimens of tongue, oropharynx, floor of mouth and cheek were evaluated and a sensitivity and specificity of 89% and 82%, respectively, was reported [45]. The handheld probe has to be positioned directly on the tissue, the technique is non-invasive and does not require the administration of agents. Using DRS, tissue type characterization can be made available real-time. However, the sampling area is limited to a few millimeters, requiring multiple measurements to evaluate a surface. Sampling depth is approximately 1 mm, which will not be enough to detect close margins that have tumor cells within 5 mm from the surface. Also, for intra-operative use, it is required to turn off the light in the operation room, because this will interfere with the technique.

Hyperspectral imaging

The image acquired by hyperspectral imaging (HSI) is constructed of a diffuse reflectance spectrum for each pixel, allowing to evaluate the whole resection surface in one view. Results are reported for the detection of the reflected light in the visual (VIS) part of the wavelength spectrum (400–950 nm) and the near infrared (NIR) part (950–1700 nm) [46–48]. The extension of the spectral range toward the infrared spectrum, where absorption of light by blood is negligible, should make the technology more applicable for use during surgery. Results of two different studies reporting on 14 tongue specimens and 21 tongue, larynx, pharynx and mandible specimens using a VIS HSI camera were comparable in the discriminative power of tumor and healthy tissue (sensitivity of 84% and 81%; specificity of 77% and 80%, respectively) [46,47]. Recently, Halicek et al. reported on a larger study on 102 patients using a deep learning model to detect squamous cell carcinoma with VIS HSI in less than 2 min with a sampling depth of less than 3 mm [48].

A sensitivity of 80% and a specificity of 77% were obtained with the NIR camera on tongue specimens [46].

This technology is non-invasive and does not require the administration of an agent. Image acquisition and tissue type characterization can be achieved within seconds. The field of view is in the order of several centimeters, and the sampling depth of a few millimeters. Challenges are the rough surfaces that create shadows on the imaging field. Also, wet surfaces completely reflect light, creating specular glare. Shadowed and glare pixels do not contain useful information for tissue characterization. Like for DRS, also for HSI darkness is required. It is unknown whether HSI is able to detect small tumor pockets more than 3 mm below the resection surface.

Optical coherence tomography

In optical coherence tomography (OCT), a light beam of a specific wavelength in the near infrared spectrum is projected on the tissue. Tissue type characterization is based on the echo delay time of the reflected light by the different layers of the tissue. With OCT, two-dimensional cross-sectional images can be constructed with a high resolution that is comparable to low resolution histology [49]. Images can be acquired non-invasively, without the need for tissue preparation. Hamdoon et al. evaluated OCT images for (superior, inferior, lateral and medial) margin assessment of 28 freshly resected specimen of the tongue, floor of mouth, buccal mucosa and retromolar trigone [50]. Sensitivity and specificity were 81.5% and 87%, respectively. Maximum image width used was 6 mm, and the resulting image could be on the screen instantly. The major limitation of OCT lies into the sampling depth: a loss of tissue

Table 2
Included studies reporting on fluorescence for intra-operative margin assessment.

Fluorescence														
Author, year	Study methodology	Margin assessment technology	Specimen/ defect driven/in situ	Verification method	Optimal margin (mm)	Sample size (number of patients)	Tumor site	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Acquisition time	Sampling depth	Other outcome measures/remarks
Gao, 2018 [32]	Prospective	FLUO (panitumumab-IRDye800CW, 800 nm)	specimen	histology	5	21	variable	91	88	80	93	real time	1 mm and 2 mm	
Leunig, 2000 [37]	Prospective	FLUO (5-ALA, 375−440 nm)	specimen and defect	histology		58	tongue and gingiva	99	60	77	98	up to 2.5 h		
Nieberler, 2018 [38]	Prospective	FLUO (integrin αvβ6-targeting RGD peptides)	specimen	cytology		122	mandible and maxilla	100	98.3	92	100	40 min		
Pan, 2020 [93]	Prospective	FLUO (indocyanine green, 785 nm)	specimen, defect and in situ	histology		20	variable: oral soft tissue					real time		Tumor to background ratio *in vivo* 1.56; *in vitro* 1.43
Rosenthal, 2015 [94]	Prospective	FLUO (cetuximab-IRDye800)	specimen, defect and in situ	histology		12	variable: oral soft tissue					real time (video 30 s)		Tumor to background ratio of 5.2
Shimane, 2016 [36]	Prospective	FLUO (g-Glu-HMRG)	specimen and in situ	histology		10	variable: oral soft tissue					10 min		OSCC tissue fluoresced 4 times brighter than normal tissue
Slooter, 2018 [35]	Prospective	FLUO (g-Glu-HMRG, 525 nm)	specimen	histology		15	variable: oral soft tissue	80	87			10 min	>1 mm	
Tajudeen, 2016 [39]	Prospective	FLUO (autofluorescence, 400−500 nm)	specimen	histology		15	variable: oral soft tissue					<2 min		Significant difference between fluorescence lifetime of different tissue types (tumor, muscle, collagen, fat) ($p < 0.05$).
Van Keulen, 2018 [33]	Prospective	FLUO (panitumumab-IRDye800CW, 800 nm)	specimen, defect and in situ	histology	5	14	variable: oral soft and hard tissue					real time	<6.3 mm	Improved surgical decision making in 3 cases (21.4%): identification of a close margin (n = 1) and unanticipated regions of primary disease (n = 2).
Van Keulen, 2019 [95]	Prospective	FLUO (panitumumab-IRDye800CW, 800 nm)	specimen	histology	5	8	variable: oral soft tissue	95	89			7 min	5 mm	To detect tumor within 2 mm of the specimen surface, sensitivity was 100%.
Van Keulen, 2020 [34]	Prospective	FLUO (panitumumab-IRDye800CW, 800 nm)	specimen	histology	5	12	variable: oral soft tissue					2.5 min	5 mm	The highest intensity peak consistently detected the closest margin to the tumor.
Voskuil, 2020 [96]	Prospective	FLUO (cetuximab-800CW, 778−795 nm)	specimen	histology	1	15	variable	100	91			10 min		Fluorescence intensities were significantly higher in tumor tissue compared to normal tissue.
Warram, 2015 [97]	Prospective	FLUO (cetuximab-IRDye800)	specimen	histology		11	variable: oral soft tissue	90.5	78.6	80.9	89.2	real time		

FLUO = fluorescence.

accuracy and definition occurred beyond 2 mm. Recently, De Leeuw et al. evaluated full-field OCT, that is able to produce en-face images with both large fields of view and a μm resolution, but a limited sampling depth of 50 μm. Five minutes are required to acquire and interpret OCT images of one square cm. A sensitivity and specificity of 90% and 87% were found, respectively, from OCT images of 32 specimens.

Narrow Band Imaging

Narrow band imaging (NBI) uses two specific wavelengths of the visible spectrum, that correspond to the absorption peak of hemoglobin, so that the microvascular abnormalities can be visualized. It is mostly used to determine the mucosal margins, however Tirelli et al. evaluated both mucosal and deep margins [51]. Although the technique seemed to achieve a precise definition of the superficial tumor extension, the authors concluded that NBI is ineffective in defining deep margins.

Conventional imaging techniques

Ultrasound

In radiology, ultrasound (US) is used to measure the tumor thickness for diagnostic purposes, indicating that the border of the tumor can be imaged on an US image [52]. Several studies have looked into the use of US for tumor margin assessment as well, both during the resection as well as directly on the resected specimen. US can evaluate the tissue up to several centimeters in depth, depending on the frequency used, it is a cost-effective, non-invasive approach that is widely available. In the largest study, evaluating tongue specimens of 31 patients, the mean (SD) difference between the deep resection margin measured on US and histopathology was 1.1 (0.9), with a Pearson's correlation coefficient of 0.79 ($p < 0.01$) [53].

Songra et al. reported on sensitivity, specificity and correlation coefficient (83%, 63% and 0.0648 respectively) comparing the margin measured on US and histopathology of 14 patients [54]. Margins of five tongue specimens measured on US by Helbig et al. differed 0–4 mm from the margin measured on histopathology [55]. Acquisition time varied between real time and 20 min. The review of Tarabichi et al. encourages to conduct further research using standardized imaging protocols and well-defined patient populations to evaluate the use of US in therapeutic decision making further [56]. Kodama et al. reported on a sampling depth of 2 cm, others only mention a few centimeters (Table 4).

Computed tomography

Ivashchenko et al. verified resection margins of maxillary malignancies by cone-beam computed tomography (CBCT) in six patients [57]. Preoperatively, the intended resection volume was delineated on the diagnostic CT and this was compared to the actual resection that was imaged by a CBCT at the end of the surgery. They found that an intraoperative CBCT is a promising way to assess surgical margins of maxillary tumors. Their method required 10 min intraoperatively, however, an intraoperative sterile cone-beam CT is required in the OR, artefacts from dental fillings hamper accurate image acquisition and this method is limited to the evaluation of bone margins only due to the poor soft tissue contrast on CT.

Specimen radiography

Radiography on mandible specimens can be useful in evaluating the completeness of excision [58,59]. The method is cheap, easy to perform, widely available and requires 20 min. However, convex structures, such as the mandible are difficult to interpret on a two-dimensional plane. The researchers also found a loss of accuracy when images were taken in the anterior-posterior direction, due to compact structure of the cortical bone in the mandible [58]. They encourage further studies to determine whether the technique is able to detect small bone infiltrations in the different sizes and shapes of the specimens.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) was evaluated for resection margin status of tongue specimens with OSCC in two studies: 10 tongue specimens imaged with an ex-vivo 7 Tesla MRI and 10 tongue specimens imaged with a 3 Tesla clinical whole-body MRI [60,61].

The tumor could be recognized on the ex-vivo 7 Tesla MRI when invasion depth >3 mm [60]. The study suggested that it will be difficult to detect small tumors with MRI and the inability to visualize microscopic invasive growth patterns will hamper the prediction of the resection margin. To be feasible for clinical application, the scan time needs to be decreased (total time in this study was 1.5 h), the resolution needs to be increased, and larger study populations have to be evaluated. An MRI would lead to extra costs; however, the authors expect that this would outweigh the costs from subsequent surgeries and additional radiotherapy. The 3 Tesla clinical whole-body MRI was logistically more favorable, and after optimization of the method for an envisioned clinical application, this imaging technology was evaluated for margin identification [61]. However, the identification of margins less than 5 mm was very poor and requires improvement to allow use of MRI for clinical practice.

Image guided surgery

Feichtinger el al. used 3D-navigation based on positron emission tomography/computed tomography (PET/CT) image fusion to evaluate the resection margins during surgery in six patients with maxillary sinus or oral cavity tumors [62]. After setting up the navigation system and ablation of the tumor, the defect was navigated with the pointer and the distance between the resection plane and the 3D image of the tumor image on the PET/CT was measured in every direction. Additional resection was performed when the distance was not sufficient. The technique was evaluated in six patients and inadequate resection margins were confirmed by histopathological examination. This technique requires a navigation system, pre-operative preparation of the virtual tumor model and edentate patients receive screws in the supraorbital region for registration purposes one day before surgery. However, the results on deep margin assessment are promising and larger study populations are necessary to confirm the effectiveness of this technique. However, in soft tissues like the tongue, navigation remains very difficult and this can only be done in tumors in or attached to bony structures.

Cytological assessment

In this review, intraoperative cytological assessment (ICA) covers the range of methodologies that discriminate tumor from healthy tissue on a cytological level from obtaining tissue with scrape, bench or imprint smears that are stained by e.g. hematoxylin and eosin or toluidine (Table 5). All studies verified their results with the final histopathological outcome. Both soft tissue margins and bony resection margins were studied. One study evaluated the surgical defect, the rest of the studies focused on the resected specimen. Table 5 shows the high performance of the methodologies in differentiating between tumor and healthy tissue. The number of patients included by the studies ranged from 15 to 154. All studies included for this review reported on the low costs of ICA, on the fact that no training is required, that the time needed is

Table 3
Included studies reporting on optical techniques for intra-operative margin assessment.

Optical techniques															
Author, year	Study methodology	Margin assessment technology	Specimen/ defect driven/ in situ	Verification method	Optimal margin (mm)	Sample size (number of patients)	Tumor site	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)	Acquisition time	Sampling depth	Other outcome measures/ remarks
Barroso, 2015 [41]	Prospective	RS (2500-4000 cm-1)	specimen	histology		14	tongue	99	92				<30 min		
Barroso, 2018 [40]	Prospective	RS (2500-4000 cm-1)	specimen	histology		22	mandible	95	87	95			<60 min	40 um	
Yu, 2019 [43]	Prospective	RS (300-3950 cm-1)	specimen	histology		12	tongue	99.31	94.44	96.9					
Brouwer de Koning, 2018 [45]	Prospective	DRS (400 −1600 nm)	specimen	histology	5	28	variable: oral soft tissue	89	82	86			Real time	>1 mm	
Cals, 2016 [42]	Prospective	RS (400-1800 cm-1)	specimen	histology	5	10	tongue	100	78	91					
Brouwer De Koning, 2019 [46]	Prospective	HSI (400 −950 nm)	specimen	histology		14	tongue	84	77	82			Real time	few mm	also HSI NIR (950−1700 nm): sensitivity 80%, specificity 77%
Halicek, 2018 [47]	Prospective	HSI (450 −900 nm)	specimen	histology		21	variable: oral soft tissue	81	80	81					
Halicek, 2019 [48]	Prospective	HSI (450 −900 nm)	specimen	histology		102	variable: oral soft tissue						1 min/ image	<3 mm	AUC's upwards of 0.80−0.90
De Leeuw, 2020 [98]	Prospective	OCT	specimen	histology	5	32	variable: oral soft tissue	90	87				5 min/cm^2	50 μm	
Hamdoon, 2016 [50]	Prospective	OCT (1310 nm)	specimen	histology	5	28	variable: oral soft tissue	81.5	87	88	61.5	95	Real time	2 mm	Surgeon 2 achieved accuracy 84%
Tirelli, 2018 [51]	Prospective	NBI (415 nm and 540 nm)	in situ	histology	3	61	variable: oral soft tissue						5 min		Conclusion: NBI only works for mucosal margin, not for deep margin

RS = Raman Spectroscopy; DRS = Diffuse Reflectance Spectroscopy; HSI = Hyperspectral Imaging; OCT = Optical Coherence Tomography; NBI = Narrow Band Imaging.

Table 4
Included studies reporting on conventional imaging techniques for intra-operative margin assessment.

Conventional imaging techniques														
Author, year	Study methodology	Margin assessment technology	Specimen/ defect driven/in situ	Verification method	Optimal margin (mm)	Sample size (number of patients)	Tumor site	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Acquisition time	Sampling depth	Other outcome measures/remarks
Brouwer de Koning, 2020 [53]	Prospective	US, 5 −10 MHz probe	specimen	histology	5	31	tongue					5 min		Mean (SD) deep resection margins measured on US images differed by 1.1 (0.9) mm from those reported by the histopathologist (Pearson's correlation coefficient: 0.79, $p < 0.01$).
Helbig, 2001 [55]	Prospective	US, 8 −12 MHz probe	in situ	histology	2	5	tongue					<10 min		Difference between margin measured on US and histopathology varied between 0 and 4 mm.
Kodama, 2010 [99]	Prospective	US, 7.5 MHz probe	specimen and in situ	histology	10	4	tongue						>2 cm	
Songra, 2006 [54]	Prospective	US, 5 −10 MHz probe	specimen and in situ	histology	5	14	variable: oral soft tissue	83	63	63	83	real time	up to a few cm	Pearson correlation coefficient US and histopathology: 0.648 ($P < 0.01$).
Tarabichi, 2018 [100]	Unclear	US, 7 −15 MHz probe	specimen and in situ	histology	5	12	tongue						several cm	Preliminary results that suggest that ultrasound has the potential to improve our ability to obtain a clear, deep margin based on more objective assessment.
Tominaga, 2007 [101]	Prospective	US, 7.5 MHz probe	specimen	histology	5	3	tongue					>20 min	several cm	Quick and eficient method to confirm surgical clearance.
Ivashchenko, 2019 [57]	Prospective	CT	defect	histology and preoperative planning	10	6	maxilla					<10 min	3D view	Two resections were reported pathologically as less than radical, each of which was detected by intraoperative CT. The mean (SD) distance between the planned and the actual resection was 1.49 (2.78) mm.
Ntomouchtsis, 2013 [58]	Prospective	RADIOGR	specimen	histology	5	16	mandible	100	100			20 min	3D view	
Shan, 2019 [59]	Prospective	RADIOGR	specimen	histology	10	10	mandible					'fast'		
Heidkamp, 2020 [61]	Prospective	MRI	specimen	histology	5	10	tongue	36	92	38	91	<30 min		
Steens, 2017 [60]	Prospective	MRI	specimen	histology		10	tongue					>1,5 h	3D view	Tumor can be recognized on MR when invasion depth >3 mm. Difference between margin measured on MR and histopathology varied between 0.1 and 1.8 mm.
Feichtinger, 2010 [62]	Prospective	nav	defect	histology	5	6	variable: oral soft and hard tissue						3D view	Intraoperative navigation showed an unsafe resection margin in 4 patients. This was confirmed by the histopathological examination.

US = ultrasound; CT = computed tomography; RADIOGR = radiography; MRI = magnetic resonance imaging; nav = navigation.

Table 5
Included studies reporting on cytological assessment for intra-operative margin assessment.

Cytological techniques															
Author, year	Study methodology	Margin assessment technology	Specimen/ defect driven/in situ	Verification method	Optimal margin (mm)	Sample size (number of patients)	Tumor site	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)	Acquisition time	Sampling depth	Other outcome measures/remarks
Junaid, 2013 [63]	Prospective	Staining (toluiniunm chloride)	defect	histology		56	variable: oral soft tissue	100	84.9	85.71	27.2	100	5 min		
Kurita, 2008 [64]	Prospective	Staining (indigo carmine and Congo red)	specimen	histology		15	variable: oral soft tissue						several minutes		No significant difference in the tumor-margin distance between histopathological and digital microscopic examination (Wilcoxon signed-ranks test, $P > 0.63$). The deviation ranged from 0.4 to 4.1 mm with a median absolute difference of 1.7 mm.
Cariati, 2019 [102]	Prospective	Cyto	unclear	histology	no tumor at margin	17	variable	33.3	85.7		33.3	85.7	<35 min		
Namin, 2015 [103]	Retrospective	Cyto	specimen	histology	10	51	mandible and maxilla			100					
Nieberler, 2016 [67]	Prospective	Cyto	specimen	histology	10	102	variable: hard tissue	94.4	97.4	97	85	99.1	20 min	a few mm	
Nieberler, 2020 [69]	Prospective	Cyto	specimen	histology		107	variable: hard tissue	78.6	95.7	93.5	73.3	96.7	<20 min		
Nieberler, 2017 [68]	Prospective	Cell isolation	specimen	histology		154	variable: hard tissue	92.3	100		100	97.4	1 h	1 cm^3	
Ojha, 2018 [65]	Prospective	Staining (Field staining)	specimen	histology		23	variable: oral soft tissue						5 min		100% concordance
Yadav, 2013 [66]	Prospective	Touch imprint	specimen	histology	no tumor at margin	30	variable: oral soft tissue	91.1	74.4	83	97.2	88.6		up to 1 cm	

Cyto = cytological assessment.

limited to only several minutes, cellular details are preserved and that a wider area of the resection margin can be assessed at once [63–66]. However, the main limitation of ICA is the fact that it can only assess the superficial layer of the resection margin and cannot assess whether the tumor is in close proximity to that margin. Nieberler et al. developed a method to isolate cells from up to a cm for evaluation so that even close bone margins could be found [67–69]. However, this increased the processing time significantly.

Discussion

Surgery is the first choice of treatment of OSCC and radical tumor resection is crucial for recurrence-free, disease-free and overall survival [2,17]. A range of 30%–85% of the surgeries results in resection margins that are inadequate, in predominantly deep margins [25,27,70]. This shows the need for a technique to evaluate the deep resection margin during surgery. To gain insight into which technologies are being studied for this purpose, 3742 articles were systematically reviewed and 62 articles were included. An overview was provided on the reported performance (accuracy, sensitivity, specificity, positive predictive value, negative predictive value, or a different outcome measure), acquisition time, and sampling depth of each technique.

Margin assessment is challenging, since the accuracy is affected by communication between surgeon and pathologist, accurate tumor localization, technique and type of margin sampling and the influence of tumor cut-through [71]. After resection, the tissue is subject to tissue shrinkage, leading to a smaller margin than the margin that was accounted for during the resection [72]. To be applicable in the operating room, the technique should be fast and easy enough so that the surgical procedure does not need to be extended or interrupted too long. Ideally, the technique should be able to identify deep, mucosal and bone margins simultaneously. A recent survey of Bulbul et al. showed that 86% of the American Head and Neck surgeons are willing to use such a technique to assess margins intraoperatively [3].

FSA is the most commonly used intra-operative margin assessment method: 97% of American Head and Neck surgeons reported to use FSA in current practice [3]. However, FSA has disadvantages concerning the use in bone margins, high rates of false negatives and required time [29,30,73]. Interestingly, overall survival of margin revisions after positive FSA is not equal to initial negative margin resection and does not lead to better local control [74]. Moreover, specimen driven FSA leads to improved sensitivity compared to patient driven FSA, although sampling techniques differed between studies [4,73]. Relocating the sample site after a reported positive margin is challenging after resection: Kerawala et al. showed a mean error of 12 mm for relocating the deep margin [75]. To overcome the relocation issue, Van Lanschot et al. recently proposed a method for accurate relocation of inadequate tumor resection margins in the wound bed: the surgeon places numbered tags on both sides of the resection line in a pair-wise manner, so that after the resection, one tag of each pair remains on the specimen and the corresponding tag remains on the wound bed [76]. Cost-effectiveness analysis has been performed for FSA, showing a cost-benefit ratio of 20:1. However, a reoperation compared to re-resection in case of positive margins on FSA during the initial operation leads to higher expenses [77]. Concluding, FSA is an acceptable, yet not optimal, intra-operative technique.

In the search for other margin assessment methods than FSA, some techniques are very promising for future use after proven effectiveness in larger trials. For example, fluorescence techniques could be useful assessing deep margins to a maximum of 6.3 mm deep and lead to high sensitivities and specificities [33]. Real time assessment, with high sensitivities and specificities is possible using Raman spectroscopy and needle insertion with this technology is promising to reach sufficient sampling depth. Other optical imaging techniques perform accurately but have the same disadvantage regarding sampling depth and the need to dim theater lights [49,50].

Ultrasound is promising, although standardized imaging protocols need to be developed and evaluated on well-defined patient populations [56]. Radiography might work for bone margins, but is difficult to interpret in convex structures [58,59]. Computed tomography (CT) and magnetic resonance imaging (MRI) of the specimen provide encouraging results, but these imaging technologies are challenging for real-time feedback on tumor margins in the operation room itself. Image guided surgery using positron emission tomography/CT showed promising results on deep margin assessment in maxillary tumors but larger study populations are necessary. Cytological assessment is a low-cost, widely available and quick alternative, but margin assessment is limited to the surface of the specimen [67–69].

This review is limited by the inability to equally compare the different techniques directly, because different selection criteria and outcome measures were used in the reviewed studies. Furthermore, only studies on techniques that are feasible for theatre are reviewed, excluding techniques that might be superior in discriminating tumor from healthy tissue in the future, e.g. Jakobsohn et al. showed that gold nanorods could properly differentiate tumor from normal cells *in vitro* with real-time photothermal molecular imaging [78]. Optical molecular imaging utilizing pH responsive peptide combined with fluorescence showed a more intense signal in cancerous than normal tissue [79]. Goldenberg et al. found that a quantitative methylation-specific polymerase chain reaction could intra-operatively detect cancerous cells [80]. However, all of these studies are still in either the pre-clinical phase or not yet feasible for clinical use.

Lastly, the margin discussion still raises the question on how to handle initially positive margins that become negative after re-resection: should patients receive adjuvant treatment as a result of their initially positive margin? There are studies available that show worse local control in the patient group with initial positive margins that were converted into negative margins, when compared to the patients with initially negative margins [4]. Also, the fact that there is no consensus on the optimal margin definition, limits the development of techniques for intra-operative margin evaluation, since the sampling depth is a critical requirement for the technique to meet.

Conclusion

In this review, we systematically analyzed literature on intra-operative deep margin assessment methods for oral squamous cell carcinoma. At the moment, the most prevailing technique remains frozen section analysis. In the search for other assessment methods to evaluate the deep resection margin, some technologies are very promising for future use when effectiveness has been shown in larger trials, e.g., fluorescence (real-time, sampling depth up to 6 mm) or optical techniques such as hyperspectral imaging (real-time, sampling depth few mm) for microscopic margin assessment and ultrasound (less than 10 min, sampling depth several cm) for assessment on a macroscopic scale.

Declaration of competing interest

None declared.

References

[1] Colevas AD, et al. NCCN guidelines insights: head and neck cancers, version 1.2018. J Natl Compr Canc Netw 2018;16(5):479–90.
[2] Jain PV, et al. Redefining adequate margins in oral squamous cell carcinoma: outcomes from close and positive margins. Eur Arch Oto-Rhino-Laryngol 2020;277(4):1155–65.
[3] Bulbul MG, et al. Margin practices in oral cavity cancer resections: survey of American head and neck society members. Laryngoscope; 2020.
[4] Buchakjian MR, et al. Association of main specimen and tumor bed margin status with local recurrence and survival in oral cancer surgery. JAMA Otolaryngol Head Neck Surg 2016;142(12):1191–8.
[5] Zanoni DK, et al. A proposal to redefine close surgical margins in squamous cell carcinoma of the oral tongue. JAMA Otolaryngol Head Neck Surg 2017;143(6):555–60.
[6] Lee DY, et al. Survival and recurrence of resectable tongue cancer: resection margin cutoff value by T classification. Head Neck 2018;40(2):283–91.
[7] Tasche KK, et al. Definition of "close margin" in oral cancer surgery and association of margin distance with local recurrence rate. JAMA Otolaryngol Head Neck Surg 2017;143(12):1166–72.
[8] Yamada S, et al. Estimation of the width of free margin with a significant impact on local recurrence in surgical resection of oral squamous cell carcinoma. Int J Oral Maxillofac Surg 2016;45(2):147–52.
[9] Dillon JK, et al. How does the close surgical margin impact recurrence and survival when treating oral squamous cell carcinoma? J Oral Maxillofac Surg 2015;73(6):1182–8.
[10] Varvares MA, et al. Surgical margins and primary site resection in achieving local control in oral cancer resections. Laryngoscope 2015;125(10):2298–307.
[11] Wong LS, et al. Influence of close resection margins on local recurrence and disease-specific survival in oral and oropharyngeal carcinoma. Br J Oral Maxillofac Surg 2012;50(2):102–8.
[12] Nason RW, et al. What is the adequate margin of surgical resection in oral cancer? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107(5):625–9.
[13] Binahmed A, Nason RW, Abdoh AA. The clinical significance of the positive surgical margin in oral cancer. Oral Oncol 2007;43(8):780–4.
[14] Sutton DN, et al. The prognostic implications of the surgical margin in oral squamous cell carcinoma. Int J Oral Maxillofac Surg 2003;32(1):30–4.
[15] Baddour Jr HM, Magliocca KR, Chen AY. The importance of margins in head and neck cancer. J Surg Oncol 2016;113(3):248–55.
[16] Kubik MW, et al. Intraoperative margin assessment in head and neck cancer: a case of misuse and abuse? Head Neck Pathol 2020;14(2):291–302.
[17] NCCN guidelines - head and neck cancers [internet]. 2018. Version 1.2018.
[18] Helliwell T, Woolgar JA. Standards and datasets for reporting cancers. Dataset for histopathology reporting of nodal excisions and neck dissection specimens associated with head and neck carcinomas. London, UK: The Royal College of Pathologists; 2013.
[19] Loree TR, Strong EW. Significance of positive margins in oral cavity squamous carcinoma. Am J Surg 1990:160.
[20] Slootweg PJ, et al. Treatment failure and margin status in head and neck cancer. A critical view on the potential value of molecular pathology. Oral Oncol 2002;38:500–3.
[21] Weijers M, et al. The clinical relevance of epithelial dysplasia in the surgical margins of tongue and floor of mouth squamous cell carcinoma: an analysis of 37 patients. J Oral Pathol Med 2002;31(11–5).
[22] Weijers M, et al. The status of the deep surgical margins in tongue and floor of mouth squamous cell carcinoma and risk of local recurrence; an analysis of 68 patients. Int J Oral Maxillofac Surg 2004;33(2):146–9.
[23] Bernier J, et al. Defining risk levels in locally advanced head and neck cancers: a comparative analysis of concurrent postoperative radiation plus chemotherapy trials of the EORTC (#22931) and RTOG (# 9501). Head Neck 2005;27(10):843–50.
[24] Eckardt A, et al. Recurrent carcinoma of the head and neck: treatment strategies and survival analysis in a 20-year period. Oral Oncol 2004;40(4):427–32.
[25] Smits RW, et al. Resection margins in oral cancer surgery: room for improvement. Head Neck 2016;38(Suppl 1):E2197–203.
[26] Kain JJ, et al. Surgical margins in oral cavity squamous cell carcinoma: current practices and future directions. Laryngoscope 2020;130(1):128–38.
[27] Woolgar JA, Triantafyllou A. A histopathological appraisal of surgical margins in oral and oropharyngeal cancer resection specimens. Oral Oncol 2005;41(10):1034–43.
[28] Black C, et al. Critical evaluation of frozen section margins in head and neck cancer resections. Cancer 2006;107(12):2792–800.
[29] Oxford LE, Ducic Y. Intraoperative evaluation of cortical bony margins with frozen-section analysis. Otolaryngol Head Neck Surg 2006;134(1):138–41.
[30] Wysluch A, et al. Intraoperative evaluation of bony margins with frozen-section analysis and trephine drill extraction technique: a preliminary study. Head Neck 2010;32(11):1473–8.
[31] Lee YJ, et al. Intraoperative fluorescence-guided surgery in head and neck squamous cell carcinoma. Laryngoscope; 2020.
[32] Gao RW, et al. Determination of tumor margins with surgical specimen mapping using near-infrared fluorescence. Cancer Res 2018;78(17):5144–54.
[33] van Keulen S, et al. The clinical application of fluorescence-guided surgery in head and neck cancer. J Nucl Med 2019;60(6):758–63.
[34] van Keulen S, et al. The sentinel margin: intraoperative ex vivo specimen mapping using relative fluorescence intensity. Clin Canc Res 2019;25(15):4656–62.
[35] Slooter MD, et al. Detecting tumour-positive resection margins after oral cancer surgery by spraying a fluorescent tracer activated by gamma-glutamyltranspeptidase. Oral Oncol 2018;78:1–7.
[36] Shimane T, et al. Oral cancer intraoperative detection by topically spraying a gamma-glutamyl transpeptidase-activated fluorescent probe. Oral Oncol 2016;54:e16–8.
[37] Leunig A, et al. Detection of squamous cell carcinoma of the oral cavity by imaging 5-aminolevulinic acid-induced protoporphyrin IX fluorescence. Laryngoscope 2000;110(1):78–83.
[38] Nieberler M, et al. Fluorescence imaging of invasive head and neck carcinoma cells with integrin alphavbeta6-targeting RGD-peptides: an approach to a fluorescence-assisted intraoperative cytological assessment of bony resection margins. Br J Oral Maxillofac Surg 2018;56(10):972–8.
[39] Tajudeen BA, et al. Dynamic optical contrast imaging as a novel modality for rapidly distinguishing head and neck squamous cell carcinoma from surrounding normal tissue. Cancer 2017;123(5):879–86.
[40] Barroso EM, et al. Raman spectroscopy for assessment of bone resection margins in mandibulectomy for oral cavity squamous cell carcinoma. Eur J Canc 2018;92:77–87.
[41] Barroso EM, et al. Discrimination between oral cancer and healthy tissue based on water content determined by Raman spectroscopy. Anal Chem 2015;87(4):2419–26.
[42] Cals FL, et al. Development and validation of Raman spectroscopic classification models to discriminate tongue squamous cell carcinoma from non-tumorous tissue. Oral Oncol 2016;60:41–7.
[43] Yu M, et al. Deep convolutional neural networks for tongue squamous cell carcinoma classification using Raman spectroscopy. Photodiagnosis Photodyn Ther 2019;26:430–5.
[44] Barroso EM, et al. Water concentration analysis by Raman spectroscopy to determine the location of the tumor border in oral cancer surgery. Cancer Res 2016;76(20):5945–53.
[45] Brouwer de Koning SG, et al. Toward complete oral cavity cancer resection using a handheld diffuse reflectance spectroscopy probe. J Biomed Opt 2018;23(12):1–8.
[46] Brouwer de Koning SG, et al. Toward assessment of resection margins using hyperspectral diffuse reflection imaging (400-1,700 nm) during tongue cancer surgery. Laser Surg Med 2020;52(6):496–502.
[47] Halicek M, et al. Optical biopsy of head and neck cancer using hyperspectral imaging and convolutional neural networks. Proc SPIE-Int Soc Opt Eng 2018:10469.
[48] Halicek M, et al. Hyperspectral imaging of head and neck squamous cell carcinoma for cancer margin detection in surgical specimens from 102 patients using deep learning. Cancers 2019;11(9):14.
[49] van Manen L, et al. The clinical usefulness of optical coherence tomography during cancer interventions. J Canc Res Clin Oncol 2018;144(10):1967–90.
[50] Hamdoon Z, et al. Optical coherence tomography in the assessment of oral squamous cell carcinoma resection margins. Photodiagnosis Photodyn Ther 2016;13:211–7.
[51] Tirelli G, et al. Tailored resections in oral and oropharyngeal cancer using narrow band imaging. Am J Otolaryngol 2018;39(2):197–203.
[52] Lodder WL, et al. Tumour thickness in oral cancer using an intra-oral ultrasound probe. Eur Radiol 2011;21(1):98–106.
[53] Brouwer de Koning SG, et al. Ultrasound aids in intraoperative assessment of deep resection margins of squamous cell carcinoma of the tongue. Br J Oral Maxillofac Surg 2020;58(3):285–90.
[54] Songra AK, et al. Observation of tumour thickness and resection margin at surgical excision of primary oral squamous cell carcinoma–assessment by ultrasound. Int J Oral Maxillofac Surg 2006;35(4):324–31.
[55] Helbig M, et al. Intraoperative B-mode endosonography of tongue carcinoma. Head Neck 2001;23(3):233–7.
[56] Tarabichi O, et al. Utility of intraoral ultrasound in managing oral tongue squamous cell carcinoma: systematic review. Laryngoscope 2019;129(3):662–70.
[57] Ivashchenko O, et al. Intraoperative verification of resection margins of maxillary malignancies by cone-beam computed tomography. Br J Oral Maxillofac Surg 2019;57(2):174–81.
[58] Ntomouchtsis A, et al. Pilot study of intraoperative digital imaging with the use of a mammograph for assessment of bone surgical margins in the head and neck region. Clin Radiol 2013;68(3):e136–42.
[59] Shan A, et al. *Intraoperative radiographic Assessment of bone resection margins during mandibulectomy: a case series*. Ear, nose. & Throat Journal; 2019. 145561319888034.
[60] Steens, S., et al., Evaluation of tongue squamous cell carcinoma resection margins using ex vivo MR. vol. 1: p. S24.
[61] Heidkamp J, et al. Assessment of surgical tumor-free resection margins in fresh squamous-cell carcinoma resection specimens of the tongue using a clinical MRI system. Head Neck 2020;42(8):2039–49.
[62] Feichtinger M, et al. Intraoperative control of resection margins in advanced head and neck cancer using a 3D-navigation system based on PET/CT image

fusion. J Cranio-Maxillo-Fac Surg 2010;38(8):589–94.
[63] Junaid M, et al. Toluidine blue: yet another low cost method for screening oral cavity tumour margins in third world countries. J Pakistan Med Assoc 2013;63(7):835–7.
[64] Kurita H, et al. Accuracy of intraoperative tissue staining in delineating deep surgical margins in oral carcinoma surgery. Oral Oncol 2008;44(10):935–40.
[65] Ojha SS, et al. Role of field staining in the cytological assessment of intra-operative surgical specimens. Acta Cytol 2018;62(5–6):327–32.
[66] Yadav GS, et al. Intraoperative imprint evaluation of surgical margins in oral squamous cell carcinoma. Acta Cytol 2013;57(1):75–83.
[67] Nieberler M, et al. Clinical impact of intraoperative cytological assessment of bone resection margins in patients with head and neck carcinoma. Ann Surg Oncol 2016;23(11):3579–86.
[68] Nieberler M, et al. Intraoperative cell isolation for a cytological assessment of bone resection margins in patients with head and neck cancer. Br J Oral Maxillofac Surg 2017;55(5):510–6.
[69] Nieberler M, et al. Defining secure surgical bone margins in head and neck squamous cell carcinomas: the diagnostic impact of intraoperative cytological assessment of bone resection margins compared with preoperative imaging. Oral Oncol 2020;102:104579.
[70] Lawaetz M, Homoe P. Risk factors for and consequences of inadequate surgical margins in oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol 2014;118(6):642–6.
[71] Williams MD. Determining adequate margins in head and neck cancers: practice and continued challenges. Curr Oncol Rep 2016;18(9):54.
[72] El-Fol HA, et al. Significance of post-resection tissue shrinkage on surgical margins of oral squamous cell carcinoma. J Cranio-Maxillo-Fac Surg 2015;43(4):475–82.
[73] Amit M, et al. Improving the rate of negative margins after surgery for oral cavity squamous cell carcinoma: a prospective randomized controlled study. Head Neck 2016;38(Suppl 1):E1803–9.
[74] Bulbul MG, et al. Does clearance of positive margins improve local control in oral cavity cancer? A meta-analysis. Otolaryngol Head Neck Surg 2019;161(2):235–44.
[75] Kerawala C, Ong KW. Relocating the site of frozen sections – is there room for improvement? Head Neck 2000;23:230–2.
[76] van Lanschot CGF, et al. Relocation of inadequate resection margins in the wound bed during oral cavity oncological surgery: a feasibility study. Head Neck 2019;41(7):2159–66.
[77] DiNardo LJ, et al. Accuracy, utility, and cost of frozen section margins in head and neck cancer surgery. Laryngoscope 2000;110(10 Pt 1):1773–6.
[78] Jakobsohn K, et al. Towards real-time detection of tumor margins using photothermal imaging of immune-targeted gold nanoparticles. Int J Nanomed 2012;7:4707–13.
[79] Loja MN, et al. Optical molecular imaging detects changes in extracellular pH with the development of head and neck cancer. Int J Canc 2013;132(7):1613–23.
[80] Goldenberg D, et al. Intraoperative molecular margin analysis in head and neck cancer. Arch Otolaryngol Head Neck Surg 2004;130(1):39–44.
[81] Abbas SA, et al. Accuracy of frozen sections in oral cancer resections, an experience of a tertiary care hospital. J Pakistan Med Assoc 2017;67(5):806–9.
[82] de Visscher JG, et al. Surgical margins for resection of squamous cell carcinoma of the lower lip. Int J Oral Maxillofac Surg 2002;31(2):154–7.
[83] Du E, et al. Refining the utility and role of Frozen section in head and neck squamous cell carcinoma resection. Laryngoscope 2016;126(8):1768–75.
[84] Gooris PJ, et al. Frozen section examination of the margins for resection of squamous cell carcinoma of the lower lip. J Oral Maxillofac Surg 2003;61(8):890–4. discussion 895-7.
[85] Layfield EM, et al. Frozen section evaluation of margin status in primary squamous cell carcinomas of the head and neck: a correlation study of frozen section and final diagnoses. Head Neck Pathol 2018;12(2):175–80.
[86] Moe, J., et al., Intraoperative depth of invasion is accurate in early-stage oral cavity squamous cell carcinoma.
[87] Mair M, et al. Intraoperative gross examination vs frozen section for achievement of adequate margin in oral cancer surgery. Oral Surg Oral Med Oral Pathol Oral Radiol 2017;123(5):544–9.
[88] Nayanar SK, et al. Frozen section evaluation in head and neck oncosurgery: an initial experience in a tertiary cancer center. Turk Patoloji Derg 2019;35(1):46–51.
[89] Pandey S, Bhamra S, Singh A. Accuracy of intraoperative frozen section in assessing margins in oral cancers: a tertiary care hospital based study. Indian J Pathol Oncol 2020;7(3):415–9.
[90] Ribeiro NFF, et al. De frozen sections help achieve adequate surgical margins in the resection of oral carcinoma? J Oral Maxillofac Surg 2003;32(2):152–8.
[91] Sharma SM, et al. Accuracy of intraoperative frozensection in assessing margins in oral cancer resection. J Maxillofac Oral Surg 2009;8(4):357–61.
[92] Tirelli G, et al. Frozen sections and complete resection in oral cancer surgery. Oral Dis 2019;25(5):1309–17.
[93] Pan J, et al. Real-time surveillance of surgical margins via ICG-based near-infrared fluorescence imaging in patients with OSCC. World J Surg Oncol 2020;18(1):96.
[94] Rosenthal EL, et al. Safety and tumor specificity of cetuximab-IRDye800 for surgical navigation in head and neck cancer. Clin Canc Res 2015;21(16):3658–66.
[95] van Keulen S, et al. Rapid, non-invasive fluorescence margin assessment: optical specimen mapping in oral squamous cell carcinoma. Oral Oncol 2019;88:58–65.
[96] Voskuil FJ, et al. Fluorescence-guided imaging for resection margin evaluation in head and neck cancer patients using cetuximab-800CW: a quantitative dose-escalation study. Theranostics 2020;10(9):3994–4005.
[97] Warram JM, et al. A ratiometric threshold for determining presence of cancer during fluorescence-guided surgery. J Surg Oncol 2015;112(1):2–8.
[98] De Leeuw F, et al. Value of full-field optical coherence tomography imaging for the histological assessment of head and neck cancer. Laser Surg Med 2020;18:18.
[99] Kodama M, et al. Ultrasonography for intraoperative determination of tumor thickness and resection margin in tongue carcinomas. J Oral Maxillofac Surg 2010;68(8):1746–52.
[100] Tarabichi O, et al. Intraoperative ultrasound in oral tongue cancer resection: feasibility study and early outcomes. Otolaryngol Head Neck Surg 2018;158(4):645–8.
[101] Tominaga K, et al. Intraoperative surgical clearance confirmation of tongue carcinomas using ultrasound. Dentomaxillofacial Radiol 2007;36(7):409–11.
[102] Cariati P, et al. Intraoperative cytological examination of bone medullary. A useful technique to predict the extension of bone invasion in segmental mandibulectomy. Am J Otolaryngol 2019;40(5):743–6.
[103] Namin AW, et al. Efficacy of bone marrow cytologic evaluations in detecting occult cancellous invasion. Laryngoscope 2015;125(5):E173–9.