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Molecular Tissue Mapping and Real-time Image Guidance for Surgical Resection of Head and Neck Cancers – A Review

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ABSTRACT
Head and neck cancers are the sixth most common cancers worldwide. Surgery is the mainstay of treatment in head and neck oncology. The most challenging part of surgical resection of head and neck cancers is the acquisition of a negative tumor margin free of residual cancers cells. Negative margins are necessary to prevent tumor recurrence, improve prognosis and reduce the associated morbidities of cancer. Novel optical techniques such as Confocal microscopy, Optical Coherence Tomography, Narrow Band Imaging, Fluorescence Imaging, and Raman Spectroscopy have enabled non-invasive, real-time, rapid, intraoperative, and repetitive assessment of tumor margins in association with molecular mapping to enhance surgical resection by identification of both the malignant cells as well as the high-risk zone containing residual tumor cells and the field of cancerization in oral tissues. Although biopsy has been the gold standard of diagnosis, optical imaging modalities prevent loss of tissue and enable examination at the cellular level in areas less accessible by conventional biopsy. They can therefore be used as valuable adjuncts to improve the accuracy of surgical resection in the field of head and neck oncology. However, more research is required to make these techniques commercially available and useful in routine clinical practice.

Keywords: 'Surgical margin' 'Optical imaging' 'Confocal Microscopy' 'Fluorescence' 'Narrow Band Imaging' 'Optical Coherence Tomography' 'Raman Spectroscopy'

I. INTRODUCTION

Head and neck cancers are the sixth most common cancer worldwide with an annual incidence greater than 6,30,000 cases and around 3,50,000 deaths each year. [(1)]Amongst these, Squamous cell carcinoma accounts for 24% of all head and neck cancers and 90% of all oral cancers. [(2)] Despite recent diagnostic and therapeutic advances, the 5-year survival rate for oral cancer has remained less than 50% over the last 50 years. The 5-year recurrence-free survival rate is 80% for stage I oral cancer patients, whereas only 20% of patients with stage IV oral cancer survive after 5 years. [(3)]

The reason for the high mortality and morbidity rates in head and neck cancers is the late diagnosis and hence delayed therapeutic intervention. Although cancers are preceded by several potentially malignant lesions and conditions, the majority of cases are only brought to the attention of the clinician in late stages wherein the disease has significantly progressed in its severity. With the advancement of the disease, the prognosis worsens thereby degrading the overall quality of life and survival of the patients.

Although chemotherapy radiotherapy plays a major role in oncological treatment, surgery continues to be the primary therapeutic option for most cancers. The resection of the entire cancerous tissue while preserving as much of the healthy tissue as possible is a sine qua non in oncological surgery. In this process, it is critical to avoid iatrogenic damage to vital organs and anatomic structures. To achieve this, negative surgical margins need to be established. Setting margins that are too large causes severe functional morbidity, and margins that are too small may leave cancerous tissue behind, as evidenced by frequent positive surgical margins and high local recurrence rates. [(4)] Thus, the extent of tissue resection is determined by the "trade-off" between cancer control and the perioperative, functional, and aesthetic morbidity and mortality of the surgery. Surgical removal aims to obtain a tumor-negative resection margin of at least 5 mm. [(5)] Multiple reports show that tumor-positive resection margins, defined as tumor cells within 1 mm of the resection margin, significantly worsen prognosis. [(6)]

To enable accurate delineation of surgical margins, surgeons usually resort to imaging modalities for anatomic and functional information and histopathological analysis using frozen sections for histopathological information. Currently, preoperative imaging modalities such as Magnetic Resonance Imaging (MRI), Computed Tomography (CT), and Positron Emission Tomography (PET) provide exquisite structural or functional images that highlight the location of

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cancerous tissues. These modalities have facilitated early tumor detection, improved diagnostic accuracy, and helped in better staging and preoperative planning. Unfortunately, these systems are not currently amenable to use in the operating room because of their large hardware footprint, slow image reconstruction, lack of microscopic imaging capability, use of ionizing radiation, prohibitive cost, and specialized operator requirement. [7]

Frozen section analysis is an invasive procedure, requires expensive pathology facilities and it does not provide real-time tissue diagnostics in the operating room. [8] Histopathology, the gold standard in the establishment of tumor diagnosis, renders its verdict after the patient has left the operating room. These cases generally necessitate repeat surgery, which is not only expensive but has limited success because of the difficulty in seeing microscopic tumors or diffuse cells. Additionally, scar tissue formation also perturbs the surgical planes, making it more difficult for the surgeon to identify the remnant tumor tissue. Some studies suggested that surgery is a major perturbing factor for metastasis development in lab animals and breast cancer and that the neovasculature spawned after surgery may promote metastatic tumor growth. These studies underline the added importance of complete tumor removal in the first surgery. [9]

Optical imaging modalities have provided a breakthrough in the world of oncology by providing non-invasive, painless, rapid, and real-time intraoperative high-resolution assessment of tumor margins without loss of tissue. These advantages allow repetitive sampling, prevent functional morbidities that result from tissue loss, reduce recall rates for oncological surgery and prevent residual tumors from proliferating because of the perturbations from surgery. [10,11,12]

Local recurrence is defined as the occurrence of another carcinoma less than 2 cm from the site of the initial resected carcinoma within 3 years. [13] Recurrence involves the concept of "field cancerization" first proposed by Slaughter and colleagues whereby after extensive histologic examination of oral cancers, the investigators summarized several observations:

1. Oral and oropharyngeal cancer develops in multifocal regions of premalignant cells
2. Atypical tissue surrounds the tumor
3. Oral and oropharyngeal cancer is composed of multiple, independent lesions
4. The persistence of abnormal tissues gives rise to local recurrence. Oral cancer has the highest risk for the development of second primary tumors ('field cancerization phenomenon') of any cancer. [14]

Field cancerization refers to the lateral spread of tumors due to progressive transformation of cells adjacent to the tumor rather than the spread and invasion of adjacent epithelium by the preexisting tumor. [15] The cells adjacent to cancerous tissue may also undergo dysplastic changes leading to second primary tumors and tumor recurrence leading to significant morbidity and mortality.

A field of preneoplastic cells adjacent to the gross tumor share a similar molecular fingerprint but develop into a malignancy after subsequent separate genomic events. [16] Histologically benign tissue adjacent to premalignant lesions has genomic alterations which may not result in morphologic changes, thus histologic examination alone may be inadequate in accurately defining the extent of disease. The inclusion of molecular characteristics into surgical margin analysis may yield a more sensitive and accurate assessment of the cells by enabling the detection of the "canceled" field that cannot be histologically defined, and therefore allow a better understanding of disease progression and the basis of tumor recurrence. [17,18]

Precise detection of OSCC on-site during surgery is particularly important to eradicate these residual cancer cells. Hence, the use of optical imaging techniques in conjunction with molecular tissue mapping could enhance the accuracy of surgical margin establishment to achieve precise tumor resection and thereby reduce the risk of recurrence and improve the prognosis of oncological surgery. Thus, the present article is a review of these real-time optical image guidance techniques used in conjunction with molecular tissue mapping in the field of head and neck oncology.

Molecular Tissue Mapping:

The acquisition of genetic instability is an essential step during carcinogenesis. In most tumors, including oral squamous cell carcinomas (OSCCs), such a genomic change results in numerical and structural chromosome alterations. Although the molecular basis of this chromosomal instability is as yet unknown, it has been suggested that mutations in one or more mitotic checkpoint genes cause abnormal cell division, leading to an abnormal chromosome constitution. One of the candidate genes is p53, being one of the most frequently altered tumor suppressor genes known to date. Several studies have suggested that loss of normal p53 function leads to destabilization of the

genome and facilitates the development of DNA aneuploidy. This would suggest that mutations of p53, which can be detected immunocytochemically because of its stabilization, precede the acquisition of chromosomal alterations. It has been shown that both p53 immunoreactivity and numerical chromosome alterations can be detected in head and neck carcinogenesis. From a clinical point of view, these markers of genetic instability may be useful for detecting the presence of minimal residual disease. [19,20,21,22]

Early events associated with genomic instability, including mutations and amplifications, are indicative of loss of heterozygosity (LOH) and may precondition a cell towards tumorigenicity, while still appearing histologically normal. The overall result of these molecular alterations leads to aberrant protein expression and function, which impacts cellular processes that regulate DNA repair, apoptosis, cell cycle progression, and proliferation. LOH at 9p21 chromosomal region associated with CDKN2A gene, which encodes the tumor suppressor protein p16 has been observed in HNSCC. The chromosome region 17p13 is associated with p53 tumor suppressor, which is well-known to be dysregulated in human malignancies of all types. [23,24]

Later genetic events at chromosome regions 11q13, 13q, and 14q31 to 32 have been associated with carcinoma in situ. Amplification in Cyclin D1 gene that promotes cell proliferation, located at 11q13 region and LOH in 13q,27 harboring the tumor suppressor gene BRCA2 (13q13.1) and RB1 (13q14.2), as well as the putative suppressor gene BRCAx (13q21.2–22.1) have been observed in head and neck cancers. [25,26]

The theories of minimal residual cancer and field cancerization can help to explain reasons for tumor recurrence despite negative histologic surgical margins. Molecular tissue mapping can thus be used as a strong prognostic indicator of disease recurrence rather than morphologic analysis alone. Recognition of the molecular alterations could thus increase the accuracy of surgical margin assessment.

Confocal Microscopy:

Confocal microscopy was first described by Marvin Minsky in 1957. However, the in vivo application breakthrough came in 1995, when confocal scanning laser microscopy was used to image human skin in vivo by Rajadhyaksha et al. Since then, this technique has been used in the diagnosis of several malignancies such as Squamous cell carcinoma, Basal Cell Carcinoma, precancers such as Actinic keratitis, pigmented nonmelanocytic lesions such as Seborrheic Keratosis, Dermatofibroma, Pigmented Mammary Paget Disease; melanocytic lesions such as Melanocytic Nevi, Dysplastic Nevi, Melanoma-Superficial Spreading Melanoma, Nodular Melanoma and Nodular Areas of Superficial Spreading Melanoma, Lentigo Maligna Melanoma, Amelanotic Melanoma and in conjunction with minimally invasive therapies like topical imiquimod, Photodynamic Therapy, cryotherapy, etc. It has also been used to guide tissue sampling including fine needle aspiration of cells for molecular analysis. [27]

CFM illustrates the fact that alterations in the functional or molecular properties of tissue can be translated into significant and optically measurable changes in the obtained signals. Reflectance confocal microscopy is based on the illumination of a small region of tissue such as skin or mucosa using a laser beam near the infrared wavelength centered at 830 nm and less than 30 mW to avoid tissue damage; this setup allows examination at a maximum depth of 200-250 mm. The reflected light (reflectance) is sent through a pinhole, which prevents out-of-focus light from reaching a detector so that only light from the in-focus plane (confocal) is detected. In this manner, whole planes of the sample under study are collected by linear scanning of the point source beam, providing thin sections of horizontal tissue in vivo, similar to computerized tomography or MRI. The lens used in RCM is a 30x objective lens of numeric aperture (NA) of 0.9, which provides a lateral resolution of approximately 1 mm and an axial resolution (section thickness) of 3-5 mm. The collected images are transformed into intensity maps (brightness grayscale) that represent the differential reflectance based on the refractive indices of the different structures of the skin or mucosa. Highly reflective structures appear bright/white, while non-reflective structures appear dark. [28,29]

Fluorescent confocal microscopes use He/Ne, Kr, or Ar lasers to illuminate fluorescent samples in a wavelength range of 400-700 nm. Fluorescent dyes such as fluorescein, acriflavine, proflavine, Hypericin, and 5-aminolevulinic (5-ALA) acid are either topically applied or intravenously (IV) injected for in-vivo confocal micro endoscopy. After its rapid diffusion out of blood vessels, the fluorophore stains the interstitial space. [30,31]

Using RCM, the normal epithelium is characterized by regularly spaced cell nuclei with uniform size and shape; moderate dysplasia is

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characterized by an increase in the density of irregularly-spaced nuclei with an occasional irregular nuclear border and severe dysplasia is characterized by its intense nuclear fluorescent staining (hyperchromatic), variable nuclear shape and size (pleomorphism), and an increased density of irregularly-spaced nuclei. Malignancies are recognized as epithelial disarray with densely packed, pleomorphic nuclei exhibiting increased fluorescence intensity. RCM of ex vivo unprocessed tissue can detect neoplastic cells by using 5 % acetic acid and cross-polarized illumination. This technique makes the neoplastic nuclei brighter and the surrounding dermis dark. (32,33,34)

CFM has also been applied to head and neck cancer imaging with antibodies or ligands conjugated to fluorescent labels. Biomarkers such as the Epidermal Growth Factor Receptor (EGFR) are over-expressed in HNSCC. Another target that has been proposed for head and neck cancer imaging at the cellular level is avb3 integrin, which is involved in tumor-induced angiogenesis. (35,36)

Optical Coherence Tomography (OCT)

Optical coherence tomography is a non-invasive imaging technology that uses low-coherence interferometry, typically employing near-infrared light from laser sources of relatively long wavelength allowing it to penetrate tissues and to produce cross-sectional images of tissue microstructure. This essentially acts like a low-energy laser-based "optical ultrasound" when evaluating tissue. The technique is similar in principle to ultrasound imaging, but, rather than measuring back-reflected sound echoes from the tissue, it measures the amount of backscattered light. (37)

OCT delivers high resolution because the interferometry used records the optical path length of received photons allowing rejection of most photons that scatter multiple times before detection. Thus, OCT can build up clear 3D images of thick samples by rejecting background signals while collecting light directly reflected from surfaces of interest.

OCT image of a malignancy parallels histopathological status showing variable epithelial thickening with areas of erosion and extensive down growth and invasion into the subepithelial layers, loss of stratification in lower epithelial strata compared with healthy oral mucosa. (38,39)

OCT has been applied in intraoperative margin detection in cutaneous, vulvar, breast, and gastrointestinal malignancy. In head and neck oncology, OCT has been used to examine laryngeal, oral, and esophageal cancers. Hamdoon et al examined the use of OCT for intraoperative margin evaluation in 28 T1-T2 N0M0 oral cavity squamous cell carcinoma patients. They concluded that positive margins could be identified by architectural changes and an increase in epithelial layer thickness with OCT technology. (40)

Narrow Band Imaging

NBI is a novel endoscopic technique that was first used by Gono et al and today is widely used for the examination of the esophageal and pharyngeal mucosa. It has been used extensively in gastrointestinal endoscopy in the identification of Barrett's esophagus, classification of colorectal polyps, and in the identification of atypical dysplastic cells in the colon of patients with ulcerative colitis. Since the oral mucosa and esophagus are both covered by squamous epithelium presenting similar vascular architecture, its use has been expanded to oral and oropharyngeal cancers. (41)

NBI is based on the fact that the depth of penetration of light is dependent on its wavelength. It used special optical filters that narrow the light bandwidth to 440 to 460 nm (blue) and 540 to 560 nm (green), corresponding to the peaks of absorption of hemoglobin, to enhance the visualization of the superficial capillary network and deep submucosal microvasculature respectively. In this way, superficial mucosal lesions that would be missed by standard white light (WL) endoscopy, are better identified given their neoangiogenic pattern, allowing the physician to have additional information to early identify atypical tissues. (42,43,44)

In neoplastic lesions, blood vessels are modified by dilation, meandering, and caliber irregularities distinguishable from healthy oral mucosa. The Intraepithelial Papillary Capillary Loops (IPCLs) classification for oral squamous epithelium was created by dividing the findings into type I (normal mucosa, regular brown dots), type II (IPCL pattern dilatation and crossing), type III (IPCL pattern elongation and meandering), and type IV (IPCL pattern destruction and angiogenesis following a sequence of carcinogenesis progression). Their morphological changes are useful for early diagnosing cancers, determining the depth of invasion and the margin of resection. (45)

Fluorescence Imaging:

Fluorescence is a member of the ubiquitous luminescence family of processes in

which susceptible molecules emit light from electronically excited states created by either a physical (for example, absorption of light), mechanical (friction), or chemical mechanism. Generation of luminescence through excitation of a molecule by ultraviolet or visible light photons is a phenomenon termed photoluminescence, which is formally divided into two categories, fluorescence, and phosphorescence, depending upon the electronic configuration of the excited state and the emission pathway. Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light of longer wavelength after a brief interval whereas phosphorescence occurs like fluorescence, but with a much longer excited-state lifetime. [46]

In head and neck oncology, techniques such as autofluorescence, fluorescence visualization loss (VFL), and fluorescence tagged antibodies subjected to fluorescent confocal microscopy have been used for the detection, screening, and delineation of head and neck squamous cell carcinoma. Fluorescence imaging has been used in the diagnosis of pancreatic and colorectal cancers and to map sentinel lymph nodes. [47]

I. **Autofluorescence:**

Autofluorescence is based on the emission of fluorescence by certain cell molecules or external agents that are excited by UV-A (315–400 nm or visible violet⁄ blue (400–450 nm) radiation. These substances are known as fluorophores. Changes in fluorescence reflect a complex interplay of alterations to fluorophores in tissue and structural changes in tissue morphology. [48]

The concept behind the use of autofluorescence in head and neck oncology is that changes in the structure (e.g., hyperkeratosis, hyperchromatic, and increased cellular⁄ nuclear pleomorphism) and metabolism (e.g., concentration of flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD) of the epithelium, as well as changes of the subepithelial stroma (e.g., composition of the collagen matrix and elastin), alter their interaction with light. These epithelial and stromal changes can alter the distribution of tissue fluorophores and, as a consequence, the way they fluoresce after stimulation with UV or visible light or by the application of exogenous contrast agents. [49]

Photodynamic diagnosis (PDD) is a technology that enables the identification of pathological lesions as fluorescent are as excited by the irradiation of light with a specific wavelength after the local or systemic administration of a photosensitizer (PS) or pro-photosensitizer drug. Examples of photosensitizers include Photofrin, Foscan, 5-Aminolevulinic Acid, etc. [50]

Leonard et al. [51] applied PDD to detect neoplasia of the oral cavity, pharynx, hypopharynx, and larynx in 1971. In their study, lesions were visualized as red fluorescence by ultraviolet light (405 nm) after the intravenous application of 0.4% Aminolevulinic acid (ALA) as a photosensitizer. (PS) Following intravenous injection, PSs were incorporated into normal and cancer cells and transported to mitochondria. In normal cells, PSs are promptly metabolized through heme biosynthesis, whereas they accumulate in cancer cells. Malignant lesions were recognized by the emission of red fluorescence, whereas normal cells emit apple green fluorescence. They concluded that photodynamic diagnosis based on autofluorescence could be used in the identification of malignant tissues.

II. **Fluorescently tagged antibodies:**

Clinical trials have suggested that monoclonal antibodies labeled with a fluorescent dye targeting the Epidermal Growth Factor Receptor (EGFR) are observed to be overexpressed in >90% of the patients with OSCC. The Rosenthal et al. group reported extensively on EGFR-targeted fluorescence-guided surgery using cetuximab-IRDye800CW and Panitumumab-IRDye800CW. The agents were administered 2–5 days before surgery. During surgery, the tumor tissue was delineated with an open-field FI camera system which, on being applied at several moments, guided the resection and resulted in adequate malignant tissue removal. Immediately after tumor excision, the margins of the wound bed and the freshly excised specimen were imaged and assessed, which opens up the possibility for an immediate re-resection to ensure complete or radical excision. [52]

III. **Fluorescence visualization loss (FVL)**

Human mucosal tissues exhibit an inherent fluorescence spectrum, which can be used to discriminate malignant from benign tissue due to the loss of fluorescence in dysplastic or malignant tissues. FVL is believed to reflect a decrease in tissue autofluorescence in malignant tissues due to the reduction and breakdown of intrinsic tissue fluorophores such as FAD and collagen cross-links (CCL) owing to the switch to anaerobic respiration (Warburg effect) as part of the increasing metabolic demand of rapidly, uncoordinated and uncontrolled cellular proliferation, tissue remodeling and

angiogenesis associated with neoplastic development. Thus, FVL can identify subclinical high-risk fields with cancerous and precancerous changes in the operating room [53,54]

Loss of fluorescence in the epithelium of malignant tissues has also been used to map the 'field of cancerization' in correlation with specific genetic alterations indicating that fluorescence visualization is superior to conventional clinical examination in the assessment of the size, extent, and distribution of the cancer field.

Rosenthal et al [55] have reported a dose-escalation study with cetuximab-IRDye800 in 12 patients undergoing resection of head and neck squamous cell carcinoma. The authors reported that fluorescence imaging with an intraoperative, wide-field device successfully differentiated tumor from normal tissue during resection with an average tumor-to-background ratio of 5.2 in the highest dose range with sub-millimeter resolution. This work demonstrated the first clinical use of fluorescence-guided imaging and the identification of head and neck cancer. They concluded that monoclonal antibodies when conjugated to fluorescent dyes, can identify subclinical tumors, which translates into improved outcomes in oncologic surgery.

Poh et al [56] published a retrospective observational study including 246 patients undergoing surgery for high-grade dysplasia or oral carcinoma. 154 patients underwent surgery with fluorescent visualization of intraoperative tumor margins, with 92 undergoing traditional surgery serving as controls. The fluorescent system was the VELscope system. Patients were followed after surgery and patients who underwent surgery with fluorescence visualization had a dramatic reduction in the 3-year local recurrence rate. This publication has validated the concept that fluorescence imaging-guided margin delineation can result in improved oncologic outcomes.

In another study by Poh et al [57] 20 patients with early (T0-T2) stage oral cancer underwent intraoperative autofluorescence visualization with a handheld device (VELscope), which showed that loss of fluorescence visualization (FVL) significantly correlated with high-grade dysplasia ($P < 0.0001$). FVL was identified in 20 out of 20 tumors and, in 19 of 20 tumors, FVL extended beyond the clinically apparent lesion. Furthermore, 35 of 36 margin biopsies in the FVL field demonstrated histologic dysplasia/cancer and/or genetic alterations known to have associated molecular risk. Only 4 of the 36 (11%) FVL margin biopsies were not dysplastic; however, 3 of those showed LOH with subsequent molecular assessment. They concluded that the use of FVL significantly correlates with histopathological findings and can be used for tumor assessment in head and neck oncology.

Leunig et al. [58] type of porphyrin accumulation in the neoplastic and surrounding healthy tissue of 58 patients with a suspected oral carcinoma by measuring emission spectra of 5-ALA-induced PPIX fluorescence. After topical application of 0.4% 5-ALA and incubation for 1 to 2.5 hours, all patients with malignancy exhibited higher intensities of red fluorescence compared with the surrounding normal tissue. An evaluation of the biopsy specimens resulted in a specificity of 60% and a sensitivity of 99%. As a fluorescent marker, PPIX could represent a possible new diagnostic tool to detect early malignant and secondary lesions in the oral cavity. They concluded that 5-ALA-induced PPIX fluorescence is promising as a useful intraoperative tool for determining adequate surgical margins of resection of oral cancers.

Raman Spectroscopy

Raman spectroscopy is a spectroscopic technique that uses the property of inelastic scattering, or Raman scattering, of monochromatic light in a variety of visible, NIR, or NUV wavelengths. The light interacts with the tissue samples and resultant spectrums are obtained. An alteration in the biochemical composition of tissues causes changes in the molecular vibrations leading to spectral differences in those tissues. These spectral characteristics provide fingerprint information, characteristic of the constituent chemical bonds, unique to each sample. [59]

The Raman Effect was first discovered by Professor Raman of Calcutta University for which he was awarded the Nobel prize in 1930. This effect is based on light's interaction with matter; as photons are directed towards target matter, most will pass through unchanged. However, some photons will come into contact with molecules in the matter. Most of these photons will interact with the molecules of the substance, exciting the particles to a partial quantum state, with the emission of a photon at the same frequency as the incident photon. This process is known as elastic scattering. A smaller number of these (approximately 1 in 106 to 1 in 108) photons will undergo a process called Raman or inelastic scattering. The photon is discharged from the material or 'scattered' at a differing wavelength than the incident photon and it is this wavelength shift that is recorded in Raman spectroscopy. [60]

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The fingerprint region (700 cm−1 to 1800 cm−1) in biological tissues is rich in proteins, nucleic acids, amino acids, carbohydrates, and lipids. Normal tissue spectrums exhibit lipid-dominated peaks while the malignant tissue peaks are protein-dominated. Singh et al. give two explanations for protein dominance in the pathological tissue. Firstly, there is a loss of architectural arrangement of different layers in the pathological tissue. Therefore, the lipid characteristics are reduced if the content of different layers is mixed. Secondly, the number of surface and receptor proteins, antigens, antibodies, and enzymes are high in cells of pathological tissue, which is assumed to give rise to protein-dominated spectra. (61)

Raman spectroscopy has been extensively used in the early detection of gastrointestinal malignancies and for the intraoperative assessment of breast cancer margins. In HNSCC, Jiabin Xia et al (62) observed Raman spectra significant difference in the intensities of bands at 739, 846, 993, 1101, 1236, 1299,1452,1577,1650, 1708, and 1770 cm–1 in of carcinomatous tissues. These spectral changes are attributed to the difference in biochemical composition in normal tissues and carcinoma. The peaks at 993 and 1236 cm−1 indicated the presence of phenylalanine, tyrosine, and tryptophan, which are typical proteinaceous components prevalent in cancerous tissues. They concluded that Raman spectroscopy can be used to distinguish carcinoma from normal tissues.

Ming-JerJeng et al (63) observed that the Raman spectral peaks at 1004, 1156, 1339, 1450, 1523, and 1656 cm−1 dominated the spectra of normal tissues, whereas peaks at 754, 1064, 1168, and 1220 cm−1 dominated those of malignant tissue samples. The peak at 1004 cm−1 is attributed to the symmetric ring breathing mode of phenylalanine, an amino acid observed in protein-enriched malignant tissue spectra. A sharp and intense peak at 1155,1156 cm−1 arises from the proteins and was dominated by the protein signal in the tumor tissues. The peak at1220∼1240 cm−1 is associated with =CH bending in lipids. The high peak at 1449∼50 cm−1 is associated with CH2 bending in proteins. The peaks at 1339 cm−1 in the tumor spectrum were associated with the adenine feature of nucleic acid. The peak at 1518∼1524 cm−1 was observed and is associated with the beta-carotene or porphyrin feature and was obtained in both normal and tumor samples but showed lower intensity in normal tissues. The peak at 1650∼1655 cm−1 is a characteristic of proteins in the alpha-helix structure of amide I, which yields a strong signal in the spectral of tumor tissues. In normal tissues, the small peak at 1655 cm−1 is generated by the C = C bond in lipids or phospholipids, and not amide I. Normal tissues yielded a small peak at 1123 cm−1, which is attributable to the C − C skeletal stretch in lipids, while tumor tissues had a (C − N) stretching mode of protein. Tumor tissues yielded a high peak at 750 cm−1 and a small peek at 823 cm−1 due to the Tryptophan and Tyrosine in protein, respectively. Normal tissues yielded Raman peaks at 754, 1064, 1168, and 1220–84 cm−1 (= CH bending) that are associated with the lipid. They concluded that tumors can be distinguished from normal tissues by Raman spectroscopy.

Barroso et al (64) performed Raman spectroscopy on 14 patients who underwent tongue resection for squamous cell carcinoma. He reported that the water content values from squamous cell carcinoma measurements were significantly higher than from surrounding healthy tissue. They concluded that Raman spectroscopy could be used to discriminate between normal and malignant oral tissues.

II. CONCLUSION:

To maximize survival, reduce recurrence, and improve the quality of life for patients undergoing oncologic head and neck surgery, negative margins are paramount. Optical imaging techniques have been a breakthrough and a true boon in the field of head and neck oncology. They provide a non-invasive, real-time, and rapid assessment of surgical margins of head and neck cancers. These techniques enable intraoperative and repetitive sampling of tissues without loss of tissue thus preventing the health and esthetic ramifications associated with invasive procedures. These novel imaging modalities can be used in conjunction with molecular tissue mapping as they have tapped the myriad of genetic mutations in HNSCC to enhance the accuracy and sensitivity of tumor margin assessment and contribute towards improved localization of biopsy sites. By identification of residual tumor cells and the field of cancerization, surgical resection would enable removal of these tissues and thus prevent recurrence and morbidity associated with head and neck cancers. This would improve the overall quality of life of patients. Literature suggests that they can be used as helpful adjuncts in the delineation of surgical margins for resection of head and neck cancers. However, as with any young technology, some drawbacks do exist and need to be overcome. More clinical trials are required to translate these promising optical techniques to routine clinics and make them fully

operable commercially to truly unleash and expand their potential in the diagnosis, therapeutic decision making, and molecular assessment for image-guided surgery.

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