NANO-DIAGNOSTICS IN ORAL SQUAMOUS CELL CARCINOMA

Abstract

Oral squamous cell carcinoma (OSCC) accounts forabout 90% of oral cancers. The development of OSCC is a multifactorial and multistage process. Most often, oral potentially malignant disorders (OPMDs)precede OSCC development, undergoing a malignant transformation if left unaddressed. Thus, early identification of OPMDs and OSCC is extremely crucial for disease prognosis and patient survival. The invasive nature of biopsy makes it extremely uncomfortable and anxious for the patient. The diagnostic imaging techniques can provide cancer diagnosis in real-time, but their sensitivity for detecting small, early intraepithelial lesions is questionable. Nanotechnology has numerous potential applications in OSCC diagnosis and monitoring. Several nanoparticles like quantum dots, magnetic nanoparticles, metallic nanoparticles, up-conversion nanoparticles, carbon nanotubes, nanospheres, nanorods, nanosheets, nanoprisms, and nanostars have been employed for the early and accurate detection of OSCCs. In this chapter, we will discuss the nanoparticles, and the various imaging and biochemical detection modalities that have been used for the diagnosis of OSCC.

Keywords: quantum dots, nanoparticles, nano-diagnostics, carbon nanotubes, oral cancer

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I. INTRODUCTION

Worldwide statistics suggest that oral cancer is the sixth most prevalent cancer, with reportedly 377,713 new cases and 177,757 deaths in the year 2020 alone (1). The average 5-year survival rate is 83.7%, which varies by stage of the tumor. Reports also point out that almost 70% of cases are diagnosed at an advanced stage, lowering the 5-year survival rate to 64.2% (2). Oral squamous cell carcinoma (OSCC) accounts for about 90% of oral cancers. OSCC is a malignancy of oral epithelial cells, can penetrate the oral mucosal and hard tissues, and can show regional and distant metastases. The tongue is the most commonly affected site, having a poor prognosis (3).

The development of OSCC is a multifactorial and multistage process. Most often, oral potentially malignant disorders (OPMDs) precede OSCC development, undergoing a malignant transformation if left unaddressed(4). Thus, early identification of OPMDs and OSCC is extremely crucial for disease prognosis and patient survival.

Currently, the gold standard diagnostic procedure availableto ascertain OPMDs and OSCC diagnosis is tissue biopsy and its histopathological examination (5). However, the invasive nature of biopsy makes it extremely uncomfortable and anxious for the patient (6). Diagnostic imaging techniques are routinely employed as adjective diagnostic aids to histopathology as they are non-invasive (7). Although these modalities can provide cancer diagnosis in real-time, their sensitivity for detecting small, early intraepithelial lesions is questionable (8).

Several painless diagnostic strategies like vital staining, chemiluminescence, and autofluorescence have been employed for imaging OMPDs and OSCCs (9). However, these non-invasive imaging modalities are subjective and highly dependent on the investigator's experience (10). Thus, newerdiagnostic methods ought to be reviewed to precisely forecast the malignant transformation potential of OPMDs, specifically identify OSCC based on molecular targeting, provide nano-scale ultrasensitive diagnosticsspecify the extent of intraoperative surgical margins, and keep an eye on the survival and prognosis post-OSCC management.

Nanotechnology is the manipulation of matter on the molecular and atomic level, with a length of less than 100 nanometers. In Greek, 'nano' means 'dwarf' or 'very little' and amounts to one thousand millionths of a meter. Dr. Richard P Feynman introduced the concept of nanotechnology in 1959, but it was later popularized by Dr. K. Eric Drexler (11). Norio Taniguchi of Tokyo Science University coined the word nanotechnology and described it as "the processing, separation, consolidation, and deformation of materials by one atom or molecule." (12). Nanomedicine is a widely researched topic of nanotechnology that enhances the prospect of specifically tailored cancer therapy. Nanotechnology also has potential applications in OSCC diagnosis and monitoring (13). In this chapter, we will discuss the nanoparticles, and the various imaging and biochemical detection modalities that have been used for the diagnosis of OSCC.
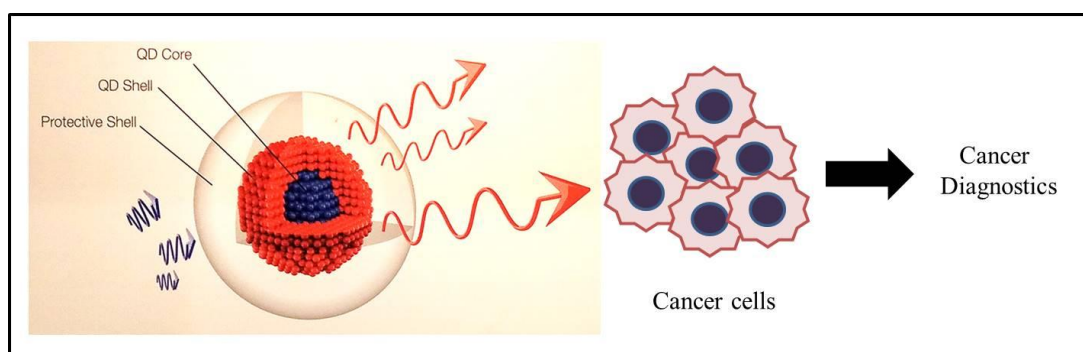
II. NANOPARTICLES EMPLOYED IN OSCC DIAGNOSIS

The various nanoparticles that have been employed for the diagnosis of OSCC have been summarized in figure 1.
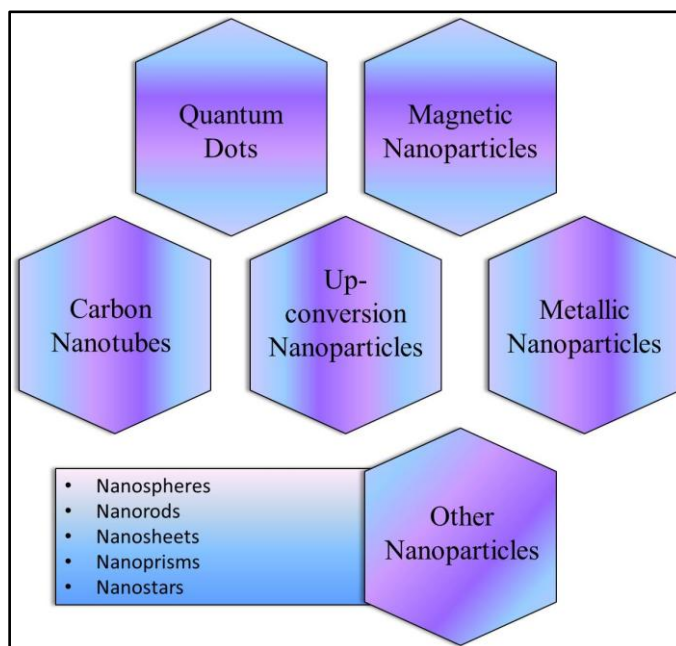
Figure 1: Nanoparticles Used in Diagnosis of OSCC

1. **Quantum Dots:** Quantum Dots (QDs) are fluorescent nanoparticles measuring less than 100 nm. They are made up of a semiconductor core encased within a protective shell. They have diverse electro-optical properties like increased quantum yield, enhanced fluorescence lifespan, good coefficient of absorption, increased brightness, outstanding stability, and a narrow band of emission ranging from visible to infrared. Owing to these features, numerous, highly sensitive and specific modern-day diagnostic probes have been developed using ODs (14). Figure 2 illustrates the structure and function of QDs in OSCC diagnosis.


Figure 2: Structure and Function of QDs in OSCC Diagnosis

2. **Magnetic Nanoparticles:** Magnetic nanoparticles (MNPs) consist of a magnetic core covered by shell. Under saturation conditions, all MNPs align along the external magnetic field that is applied. They exhibit paramagnetic and ferromagnetic behaviour, which can be altered by changing their structure or composition. They also demonstrate increased cellular attachment, excellent hematological stability, and multiplexed attachment without aggregation, making MNPs extremely effective for the detection of circulating tumor cells (CTCs) in blood (15). The structure of MNPs has been illustrated in figure 3.
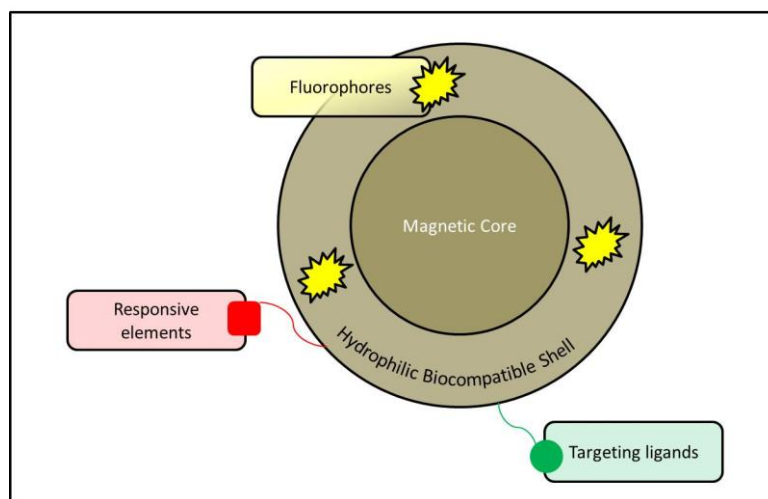

Figure 3: Structure of Magnetic Nanoparticle

3. **Carbon Nanotubes:** Carbon nanotubes (CNTs) are uni-dimensional nanoparticles because electrons only propagate down the nanotube's axis. They are made of single or several graphene sheets arranged in the form of a hollow cylinder. Owing to their powerful carbon bonds and cylindrical structure, CNTs exhibit exceptional mechanical, chemical, and electrical properties. Since their conductivity may be modulated by changes in chemical binding and structure, CNTs are a great choice for the creation of nano-biosensors. CNTs are constructed of graphite sheets, with increased surface area and electrical conduction, enabling electronic detection of CTCs through real-time electrical impedance sensing (16). Figure 4 depicts the structure of CNTs.
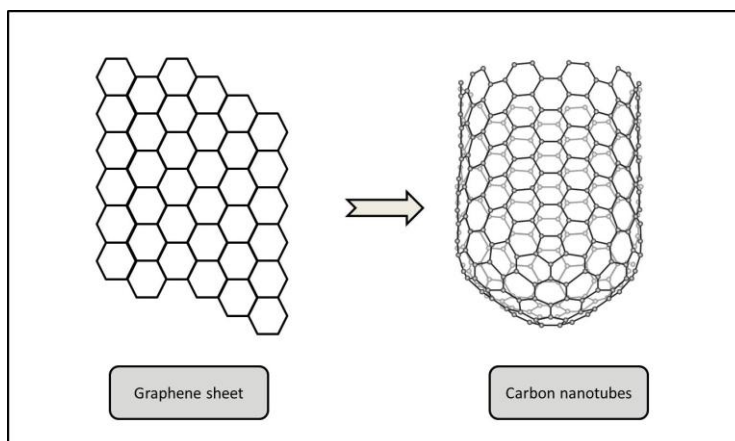
Figure 4: Structure of Carbon Nanotubes

4. **Up-conversion Nanoparticles:** Lanthanide ions make up up-conversion nanoparticles (UCNPs). They convert multiple near-infrared region photons to a single high-energy, visible light region photon. They can be used for highly sensitive imaging of OSCC tissues because of their unique luminous features, and limited background signals and photobleaching (17). Figure 5 illustrates the working principle of UCNPs.
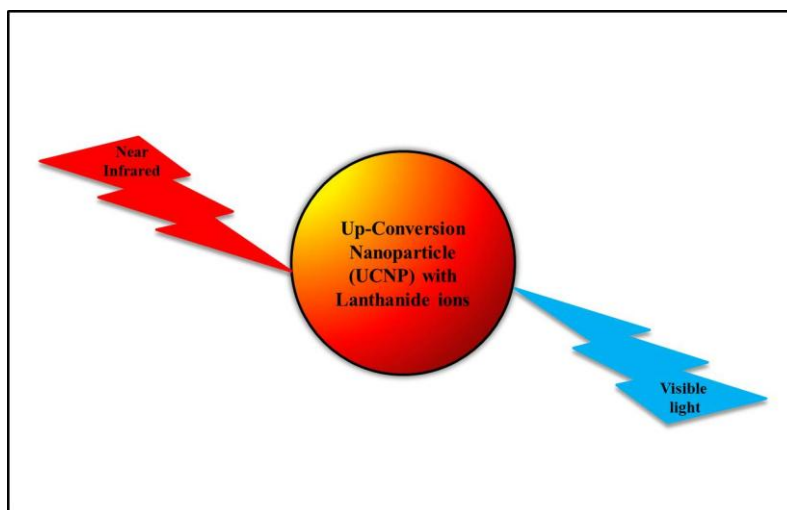

Figure 5: Working Principle of Up-Conversion Nanoparticles

5. **Metallic Nanoparticles:** Metallic NPs contain valence electrons that conjointly vibrate in response to an external electromagnetic field. Silver and gold NPs have excellent biological properties and are chemically stable They are also safe for biological applications like diagnostic imaging of OSCCs (18).

6. **Other Nanomaterials:** The characteristics and sensitivity of nanostructural probes are substantially influenced by their form and size. The most commonly used nanostructure substrate shapes include nanospheres, nanorods, nanosheets, nanoprisms, and nanostars. These nanostructures have increased cellular capture and affinity, allowing them to functionalize targeted ligands for use in detecting OSCC via ligand-antigen interaction (19).

III. NANO-DIAGNOSTICS IN ORAL SQUAMOUS CELL CARCINOMA

The various nanoparticle-based diagnostic modalities for early and accurate diagnosis of OSCC have been summarized in figure 6.
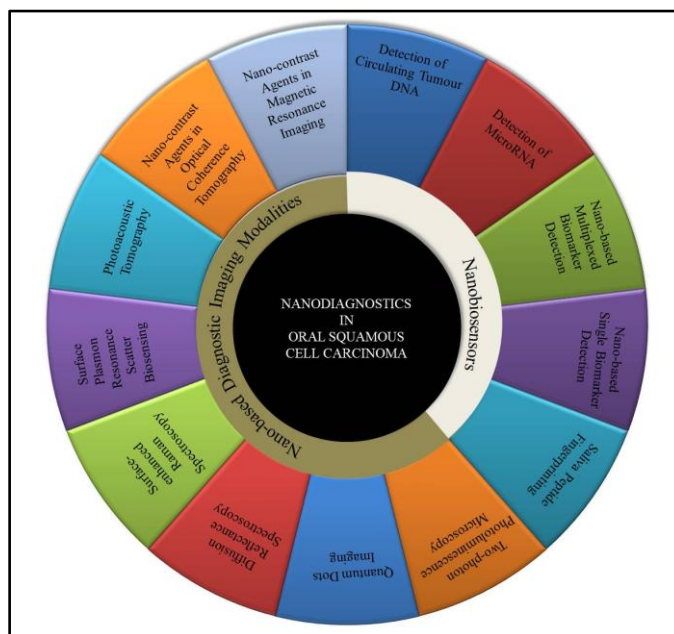
Figure 6: Nanoparticle-Based Diagnostic Modalities for OSCC

1. **Nano-Based Diagnsotic Imaging Modalities:**

- **Nano-Contrast Agents in Magnetic Resonance Imaging:** Numerous nanoparticles have been used as nano-contrast agents for magnetic resonance imaging of OSCC patients (20). Nano-contrast agents can identify distinct cell surface markers, resulting in improved MRI contrast qualities, and also have a longer half-life (21). A combination of folate-chitosan shell and magnetic poly-lactide-co-glycolide (PLGA) nanoparticle core have shown reduced overall relaxation time, increased relaxivity, and an enhanced contrast. Meanwhile, it has also shown increased uptake and enhanced cytotoxicity in OSCC KB cell lines (22). Gadolinium-coated amorphous titanium dioxide nanoparticles have also exhibited prolonged longitudinal relaxivity, enhanced contrast along with excellent biocompatibility on OSCC cell lines (23).

- **Nano-Contrast Agents in Optical Coherence Tomography:** Optical Coherence Tomography (OCT) uses backscattered or back-reflected light for obtaining high-resolution cross-sectional tomographic images of the internal structure of biological materials and systems. It generates cross-sectional images of sub-surface tissues like epithelium and basal lamina with a penetration depth of approximately 2 mm, thereby enabling early diagnosis of OMPDs and OSCCs (24). Although the resolution of OCT is better than CT, MRI, and ultrasound, the imaging contrast between healthy and neoplastic tissues remains insufficient (25). Surface plasmon resonant gold nanoparticles can improve the contrast of OCT images. To improve the transport of gold nanoparticles across biological barriers, a multimodal system of antibody-conjugated PEGylated gold nanoparticles by microneedle and ultrasound has been developed. This multimodal delivery system can improve OCT penetration depth and increase the OCT image contrast in OSCC tissues (26).

- **Photoacoustic Tomography:** Photoacoustic tomography (PAT) works by conversion of absorbed optical energy into acoustic energy. PAT can produce high-resolution images because acoustic waves exhibit much lesser scatter in tissues than optical waves. Although various exogenous contrast agents have improved the contrast of photoacoustic imaging, gold nanoparticles exhibit enhanced bio-conjugation and produce stronger photoacoustic imaging signals (27). In metastatic mouse models of OSCC, ultrasound-guided spectroscopic photoacoustic imaging of molecularly activated plasmonicnanosensors (MAPS) have been used to detect lymph node micrometastases. It has been found that, after particular interactions with nodal metastatic cells, MAPS targeting EGFR alter their optical absorption spectrum to the red-near-infrared region, allowing PAT to identify them.. These findings offer a non-invasive alternative to sentinel lymph node biopsy analysis after resection of OSCC tissues (28).

- **Surface Plasmon Resonance Scatter Biosensing:** The collective oscillation of conduction electrons in noble metals creates surface plasmon waves. Surface Plasmon Resonance (SPR) scatter biosensing has evolved into one of the most potent and versatile medical diagnostic technologies. Owing to their ease of fabrication and bioconjugation, unique optical properties, and excellent stability, gold nanoparticles have been extensively used in SPR scatter biosensing (29). When SPR images and absorption spectra are analyzed and compared between OSCC cell lines and noncancerous cell lines, it has been found that the gold nanoparticles conjugated with anti-EGFR antibody bind homogeneously and specifically to the OSCC cell surface with greater affinity compared to the noncancerous cells, with a sharper absorption band (30). These findings imply that SPR absorption spectroscopy using antibody-coated gold nanoparticles may be effective in fabricating biosensors for the early detection of OPMDs and OSCCs.

- **Surface-Enhanced Raman Spectroscopy:** Raman spectroscopy is an imaging modality that relies on the interaction of light with matter. Because these vibrations are coupled with certain chemical bonds, they produce unique, fingerprint-like Raman spectra. The OMPDs and OSCC can be delineated by using the inelastic light scattering. Normal tissues have homogeneous signals, whereas malignant cells have heterogeneous signals, reflecting alterations in their chemical and structural make-up. In Surface-enhanced Raman Spectroscopy (SERS), gold nanoparticles amplify the Raman scattering intensity of molecules adsorbed on its surface (31). Small, spherical, SERS-active, and NIR-sensitive gold nanoparticles with exceptionally narrow intra-nanogap architectures have been used for imaging of OSCC cells. These NPs selectively target the cell organelles and get distributed intracellularly, thereby producing Raman images with a higher resolution (32).

- **Diffusion Reflectance Spectroscopy:** Diffusion reflectance spectroscopy (DRS) is a simple, safe, and economical optical diagnostic technology capable of imaging tissues using low radiation with high penetration depths (33). In DRS, a fraction of the incident white light is absorbed or transmitted by the tissue. Meanwhile, the remainder light gets reflected diffusely by multiple elastic scattering. The cytological and morphological changes during carcinogenesis have a significant impact on the reflected light, thereby enabling DRS to differentiate normal mucosa, OPMDs, and

OSCCs (34). DRS can distinguish OSCC from OMPDs with a sensitivity of 98.5% and specificity of 96.0%, while it can distinguish OPMDs from healthy mucosa with a sensitivity of 95.0% and specificity of 100.0% (34).

EGFR-conjugated gold nanorods can evaluate the surgical margins of OSCC specimens by DRS. The nanorods in tissues are visualized using air scanning electron microscopy, which spread to maximum of 1 mm between the healthy tissue and the tumor. DRS with a resolution of 1 mm is subsequently performed, indicating that the tumor edge is within a distance of 4-5 mm. It has also been found that the reflectance intensity increases with an increase in dysplastic changes (35). Thus, DRS can be used as a potential diagnostic tool for detecting residual tumors intraoperatively in real-time, and for large-scale screening of OPMDs and OSCCs.

- **Quantum Dots Imaging:** QDs possess high fluorescent intensity, specific binding and are resistant to photobleaching during in-vitro imaging of OSCC cell lines (36). Near-infrared QDs can penetrate the tissues deeper, are safe to use and possess an emission wavelength rage of 700-900 nm (37). Researchers have also reported that EGFR-conjugated QDs can provide high-resolution images of OSCCs (38).

- **Two-photon Photoluminescence Microscopy:** Nanomaterialsdemonstrating two-photon photoluminescence (TPL) are highly attractive asnono-contrast agents for diagnostic imaging of OSCCs. Gold nanorods produce extremely bright TPL signals, which are several times brighter than typical fluorophores (39). Thus, TPL microscopy is an extremely appealing choice with sufficiant imaging depths to view the epithelium and the connective tissue of oral mucosa. Gold nanorods have found to be forty times brighter than the surrounding tissues using TPL microscopy. Intravital imaging also demonstrates 3D microvasculature and aberrant vessels using much lower incident powers in dysplastic tissues compared to healthy tissues. Thus, gold nanorods can be used as high-contrast imaging agents to visualize in vivo aspects of carcinogenesis using TPL microscopy for real-time diagnosis of OSCC (40).

2. **Nano-Biosensors:** The use of nanoparticles in biosensors and the fabrication of diagnostic devices have received a lot of attention. Owing to their nanoscale dimension, the designed nanostructured materials have an expanding surface-to-volume ratio, high mechanical strength, enhanced electrical conductivity, catalytic activity, and biocompatibility (41). Strategies for the detection and diagnosis of OSCC are greatly improved by combining biosensing and advancements in nanotechnology to help overcome limitations. Gold nanoparticles, quantum dots, dendrimers, nanocomposites, and other nanomaterials have been employed for the fabrication of nanosensors (42).

- **Saliva Peptide Fingerprinting:** For salivary proteomics analysis, the saliva peptide fingerprint method is a helpful tool that can forecast possible biomarkers beneficial for OSCC diagnosis. Matrix-assisted Laser-desorption Ionization–time-of-flight–Mass spectrometry is a powerful diagnostic tool that can detect and analyze salivary proteins with adequate resolution and sensitivity. One of the more promising materials for separating beads is magnetic beads made of nanoparticles. Interestingly, there have been substantial differences in expression levels of fifty proteins amongpatients

with OSCC patients and healthy volunteers in the reported studies. This provides a novel non-invasive, high-throughput method for screening of OSCC biomarkers (43).

- **Nano-Based Single Biomarker Detection:** Nano-based single biomarker detection approach can also be effective in OSCC diagnosis. Total internal reflection fluorescence microscopy (TIRFM) can be used to detect TNF-α utilizing a gold protein chip technique, thereby enabling ultra-sensitive detection of OSCC (44). Field emission scanning electron microscopy (FESEM), and atomic force microscopy (AFM) with high resolution can also be used to exhibit the substructure details of solitary human saliva exosomes, and their reversible nature of mechanical deformations in OSCC (45).

- **Nano-Based Multiplexed Biomarker Detection:** In certain cases, a single OSCC biomarker may not suffice for reliable diagnosis. False positive and negative results arising due to single biomarker detection can be reduced by multiplexed biomarker detection. In this method, an entirebiomarker panel can be analysed simultaneouslyusing an ultrasensitive electrochemical microfluidic array. An abundance of nanobiosensors and labeled magnetic nanobeads are present in the microfluidic device. The protein panel has demonstrated a sensitivity and specificity of 89% and 98% respectively. This research offers a simple, economical tool for accurately diagnosing OSCC (46). In another study, nano-ultra-performance liquid chromatography (nano-UPLC) ion-mobility mass spectrometry has been used to analyze proteins and biomarkers in OSCC cell lines. Approximately 952 proteins in total have been identified. A high-throughput method for protein quantification and comparison amongst various samples is possible by this method (47).

- **Detection of Circulating Tumor DNA:** Circulating tumor DNAs (ctDNAs) are tumor-derived DNA fragments that circulate in the bloodstream. ctDNA can be used to identify cancer-related genetic mutations. The detection of ctDNAabnormalities can assist OSCC diagnosis, prior to the clinical signs and symptoms (48). Ultrabright SERS nanorattle-based sandwich assay has been used to detect the cytokeratin-14 (CK14) gene for head and neck SCC micrometastases in the lymph nodes. Synthetic CK14 target sequences have been identified from a negative control by creating capture probes and reporter probes that are specific to the target sequence (49). This will offer a distinctive, affordable, quick diagnostic alternative to provide a point-of-care diagnosis for OSCC and HNSCC patients with cervical lymphadenopathy.

- **Detection of Micro RNA:** Micro-RNAs (miRNAs) binds to mRNA and prevent its translation. Dysregulation in miRNAs leads to disruption of normal cellular function, ultimately leading to cancer development (50). For the colorimetric measurement of OSCC-associated microRNA 31-5p (miRNA 31), a lateral flow strip biosensor has been fabricated using the cascade nucleic acid amplification technology. These miRNA strips can enable the formation of portable biosensor devices for real-time diagnosis of OSCC with excellent sensitivity and specificity (51).

IV. CONCLUSION

Nanotechnology has developed novel methods for OSCC diagnosis in recent years. Nanoparticles are a promising diagnostic tool because of their performance characteristics - including function-specific morphology, better half-life, biocompatibility, and improved cell surface affinity. Further, their properties can be modulated by altering the materials, methods, or surface chemistry used in their formation. The use of nanoparticles in the oral cavity has led to non-invasive, accurate and real-time diagnosis of OSCC and precise identification of surgical margins, thereby improving the prognosis and overall survival of OSCC patients.

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