2022-07-10

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10.1002/wnan.1830
WIREs Nanomedicine and Nanobiotechnology
Wiley

Nano-diagnostics as an emerging platform for oral cancer detection: Current and emerging trends

Article category- Diagnostic tools > Biosensing > Diagnostic nanodevices

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Abstract

Globally, oral cancer kills an estimated 150000 individuals per year, with 300000 new cases being diagnosed annually. The high incidence rate of oral cancer among the South-Asian and American populations is majorly due to overuse of tobacco, alcohol, and poor dental hygiene. Additionally, socio-economic issues and lack of general awareness delay the primary screening of the disease. Availability of early screening techniques for oral cancer can help in carving out a niche for accurate disease prognosis and also its prevention. However, conventional diagnostic approaches and therapeutics are still far from optimal. Thus, enhancing the analytical performance of diagnostic platforms in terms of specificity and precision can help in understanding the disease progression paradigm. Fabrication of efficient nanoprobes that are sensitive, non-invasive, cost-effective, and less labor-intensive can reduce the global cancer burden. Recent advances in optical, electrochemical, and spectroscopy-based nano biosensors that employ noble and superparamagnetic nanoparticles, have been proven to be extremely efficient. Further, these sensitive nanoprobes can also be employed for predicting disease relapse after chemotherapy, when the majority of the biomarker load is eliminated. Herein, we provide the readers with a brief summary of conventional and new-age oral cancer detection techniques. A comprehensive understanding of the inherent challenges associated with conventional oral cancer detection techniques is discussed. We also elaborate on how nanoparticles have shown tremendous promise and effectiveness in radically transforming the approach toward oral cancer detection.

Keywords: Oral cancer detection; Conventional detection; Nanoparticles; Non-invasive; Nano-biosensors; POCTs

Introduction

Oral and oropharyngeal cancer is precisely categorized as malignant neoplasm in the lips, oral cavity, and oropharynx region (ICD 10: C00-C14). Geographic and environmental variables have an important influence in defining the associated risk factors. Southeast Asian countries account for nearly 40% of the global oral cancer burden, where the causative risk factors include consumption of areca nuts, tobacco, and smoking. Meanwhile, HPV-16 infection has been linked to oropharyngeal cancer (OPC) in the younger age group in the US (Gillison et al., 2012; Warnakulasuriya, 2009). Societal elements and individual lifestyle choices also contribute significantly to the growing cases of oral cancer (OC). Government screening programs are designed to work at different levels with a defined framework to prevent the progression of the disease to an invasive stage. Screening sub-mucosal and mucosal abnormalities often referred to as oral potentially malignant lesions (OPMLs) at an early stage can effectively improve the quality of life with a 40-50% survival rate (Coletta et al., 2020). However, a combination of factors such as lack of awareness, unavailability of efficient pharmaceutical adjuncts, and healthcare practitioners' subjective interpretations can result in late-stage presentation with serious complications (Mehrotra and Gupta, 2011). Fig. 1 represents a schematic theme of several determinants involved during the progression of oral oncogenesis.

For a definitive diagnosis of oral malignancy, clinicians still rely on the gold-standard biopsy and histopathological examinations (Sylvie-Louise Avon and Klieb, 2012). This procedure in general is expensive, invasive, and requires visiting clinics for multiple consultations. It is worth mentioning that during the COVID-19 pandemic nearly 14 million individuals in England alone missed their oral examination at desired clinics due to the state-imposed restrictions (Westgarth, 2020). Such circumstances exacerbate co-morbidities and cause massive backlogs, putting a strain on an already overburdened healthcare system. Screening aids that provide real-time analysis at the patient’s site with a minimal turnaround time are the need of the hour. With the advent of nanotechnology, engineered nanoparticles (NPs) have gained substantial attention as biosensors (Bogart et al., 2014). These engineered NPs act as transducers that are vital in the development of any nanobiosensor. Unique physicochemical properties of these NPs such as large surface-area to volume ratio enhance their catalytic and absorbance activity as well as enables easy immobilization of critical biorecognition elements. The use of nanomaterials may improve the biosensor performance including increased

sensitivities and low limit-of-detection of several orders of magnitudes (Malhotra et al, 2018). Further, metal NPs (gold, palladium, and aluminum) alone and in combination with other classes of nanostructures have shown excellent biocompatibility (Malekzad et al, 2017). Skillful labeling of gold, magnetic NPs, and quantum dots have facilitated the development of sensitive optical and electrochemical immunoassays (Masud et al., 2019a; Perfézou et al., 2012). Additionally, nanoarray-based platforms that can sense cancer cell surfaces, tissues, and biofluids are being considered for personalized diagnostic systems (Le et al., 2014). NPs-based Point-of-Care (POC) diagnostic aids such as LFA-strips have demonstrated significant potential towards detecting viral markers and are most widely used for the detection of pregnancy at a lower cost and a short period (Butler et al., 2001; Martiskainen et al., 2021). Needless to say, these facile nanodiagnostic adjuncts can become the cornerstone in deciding the appropriate treatment strategy and indicating disease prognosis.

The aim of the current review is to understand the vital aspects of several diagnostic adjuncts for the detection of oral malignancy. The review presents the status quo of both conventional screening aids as well as the upcoming nano-based technologies. Also, discussed briefly are the new age user-friendly diagnostic innovations and patents for early detection of oral carcinoma. The inherent factors hindering the clinical translation of nanodiagnostic adjuncts have also been critically analyzed. The review aims to help the readers gather information about methodological modifications from a diagnostic point of view. In the future, the knowledge would help the scientific community to better manage the disease.

2. Oral malignancy and conventional detection techniques

Presenting a discrete screening algorithm in the case of OC is difficult as the procedures depend on the patient's history, as well as accessible screening aids. However, at most established clinics and hospitals a patient's workup starts with analyzing the case history followed by a physical examination of the oral cavity. Biomarker profiling and locating tumors using imaging techniques remain essential before proceeding with biopsy. Fig. 2 represents a schematic diagram of the flow chart involved in the screening of OC.

Government-organized sporadic camps that are aimed at screening pre-cancerous lesions often rely on visual examination and low-cost light-based screening aids. A district-level cancer control program namely “ASWAS” in Kerala, India was solely based on a visual examination (Philip et al., 2018). Another study from Uttar Pradesh, India utilized Magnivisualizer for initial screening and cyto-brush analysis for further validation of OC (Parashari et al., 2014). Commercial screening aids that use the principle of chemiluminescence (ViziLite), tissue autofluorescence (Velscope), and/or multispectral fluorescence (IDENTAFI 3000) for visual discrimination between dysplasia and healthy mucosa are frequently used in different clinical settings (Cicciù et al., 2019; Macey et al., 2015). Several of these index tests have shown to have limited applicability as they often present false negative or false positive results (Macey et al., 2015). To rule out such ‘risk of Bias” judgments, additional biomarker analyses are performed. Bottom-up proteomics and top-down proteomics strategies, as well as powerful mass spectrometry, are used to detect proteins that serve as biomarkers in OC. Currently, cancer antigen 125 (CA 125), tissue polypeptide antigen (TPA), interleukin-6 (IL-6), Cytokeratin fragment 21-1 (CYFRA 21-1), tumor necrosis factor-alpha (TNF-alpha), Mac- 2, and telomerase are considered as promising protein biomarkers in the case of early detection of oral cancer (Zhang et al., 2009). According to (Huang et al., 1999) P53 mutations were found in salivary DNA in 62.5% of patients with oral cancer. Identification of epigenetic modifications such as hypermethylated promoters of tumor suppressor gene (TSG) in oral rinses has been shown to assist in monitoring and diagnosis of OSCC (Liyanage et al., 2019). For the fabrication of more practical detection systems, a clear idea about the relative occurrence of possible prognostic, diagnostic and post-operative markers in the different biological fluids can play a crucial role in understanding the progression of the disease. Whole saliva can act as an excellent auxiliary tool in such cases as the mode of sampling is non-invasive, and cost-effective and further saliva can be easily transported and stored. Concomitant increase (400%) in the levels of CA-125 and CYFRA 21-1 protein markers in saliva of patients with OSCC has been reported previously(Nagler et al., 2006). It was observed that the values of CYFRA 21-1 were ~3 folds higher in pre-malignant cases as compared to the healthy individuals(Rajkumar et al., 2015). Though the CA-125 level increases significantly in patients with OSCC, It has lower levels of expression at the early stages of oral cancer(Geng et al., 2013). From these findings, it can be suggested that CYFRA-21-1 can be demarcated as a good diagnostic marker for early detection of OSCC as compared to CA-125. Another combinatorial salivary marker analysis study considered matrix metalloprotease 9 (MMP-9) and 8- hydroxy-2´-

deoxyguanosine (8-OHdG) in patients with OSCC. The representative biomarkers analysis in unstimulated whole saliva indicated that MMP-9 could be employed as both a universal OSCC screening marker as well as a prognostic marker (over a period of 9 months). However, the levels of 8-OHdG, a pivotal marker for measuring the effect of endogenous oxidative DNA damage did not show positive correlation and authors suggest further research to be done to clarify the contradicting differences (Shin et al., 2021). Liquid biopsy based detection of circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) in biological fluids has gained enormous attention in the recent years (Soda et al., 2019). ctDNA being majorly released into the blood stream may reach saliva via passive diffusion or active transport. Recent studies have reported the presence of ctDNA in 95% of the patients enrolled at late stages of OSCC. Patients with clinical reoccurrence of OSCC post-surgery also showed elevated levels of ctDNA suggesting that salivary ctDNA can be a valuable biomarker for oral cancer follow-up and surveillance(Cristaldi et al., 2019). .

To properly analyze the depth and loco-regional invasion of the tumor, cross-sectional imaging is required. Scanning techniques like computed tomography (CT), and positron emission tomography (PET), and magnetic resonance imaging (MRI) provide information about resectability and also help a clinician plan a proper treatment strategy (Waech et al., 2021) (Arya et al., 2012). Clinical staging at this point is possible. However, for a definitive tumor nodes metastasis (TNM) staging biopsy is a mandate. Visual inspection of the gross tissue and tissue staining lets a skilled technician asses the deformities in the cellular architecture of the patient. A detailed analysis of the frequently used medical adjuncts in clinical settings is summarized in initial screening, biomarker assay, advanced optical imaging followed by biopsy and histopathological assay table 1.

Conventional screening methods such as MRI, CT, ultrasound, biopsy, and other techniques as listed in table 1 have inherent shortcomings which limit their usability. While the low-cost technologies compromise the sensitivity, the scanning techniques are relatively costly. On the other hand, the biopsy is invasive, requires skilled labor and significant time. In developing countries, where available resources are scarce, portable adjuncts that facilitate self-examination can be highly effective for detecting oral malignancies at an initial stage. Additionally, a good understanding of different biomarkers may help in the development of LFA-based detection formats. Such diagnostic kits can be used throughout the continuum of care in oncology.

172 **Table 1.** Conventional Screening aids for the detection of oral carcinoma

Screening Adjunct	Principle	Biomarker	Pros	Cons	Ref.
VELscope (400-430 nm)	Loss in tissue autofluorescence due to disruption of intact collagen, elastin, and increased vascularity	Oral mucosa	Non-invasive,cost-effective	Low-sensitivity, false-positive reporting	(Cicciù et al., 2019)
ViziLite (light 430, 540,580 nm)	Illumination with Chemiluminescent light for assessing tissue reflectance	Oral mucosa	Non-invasive, cost-effective, convenient	Low-sensitivity, false-positive reporting	(Mehrotra and Gupta, 2011)
IDENTAFI (405 and 575 nm)	Illumination with green-amber light for capturing both tissue autofluorescence and reflectance, for observing vascularity	Oral mucosa	Non-invasive, relatively high sensitivity (82-87%)	The risk of bias judgment needs further validation	(Messadi, 2013)
Vital staining	Toluidine blue staining of the lesion and microscopic examination	Oral tissue	Inexpensive, non-invasive	False-positive report, low sensitivity	(Missmann et al., 2006)
ELISA	The presence and quantity of antigens binding is detected by	Saliva, serum, blood	Sensitive, can detect low levels of the biomarker	Laborious requires skilled expertise	(Prasad and McCullough, 2013)

	Specific antigen-antibodies reaction				
HPLC	Column chromatography based identification of specific markers	Biofluids containing serum proteins	Quantitative measurement, sensitive	Expensive requires technician for result interpretation	(Reddy et al., 2012)
PCR and PCR-RFLP	DNA amplification, genetic mapping using restriction enzyme sites	Serum and saliva (can indicate HPV infection and genetic polymorphism	Reproducible, sensitive	Tedious sample preparation, expensive, and requires expertise	(Herrero et al., 2003; Sreelekha et al., 2001)
DNA microarray	Measurement of multiple tissue-specific gene expression based on complementary sequence binding	Oral tissue	Sensitive, moderately costly	Tedious instrument handling	(Ziober et al., 2008b)
Mass-Spectroscopy	Measurement of the mass-to-charge ratio of ions based on the intensity	Saliva based metabolic proteins	Details about molecular profiling, sensitive	Expensive instrumentation, not easily accessible	(Hu et al., 2007)

MRI	Use of magnetic fields, and radio waves for imaging the suspected lesion	Tumours located in soft tissue	Can accurately detect the status of invasion, and depth of tumor	Expensive, complicated instrumentation, cannot image patients with metal implants	(Ziober et al., 2008b)
PET	Imaging-based on the usage of a radioactive isotope	Tumors located in head and neck	Can facilitate early detection by a non-invasive mode	Lowered accuracy due to short decay time of the radioactive substance, expensive	(Civantos et al., 2003)
CT	Ionizing radiation, X-ray coupled with an electronic detector for imaging of tissues	Tumors located in soft tissue	Moderately less expensive gives an idea about the spread of carcinoma	Exposure to radiation and contrast agents	(Handschel et al., 2012)
Ultrasound	Usage of high-frequency sound waves	Head and neck	Moderately expensive, non-invasive	Biased interpretation, cannot report vascularization	(Lodder et al., 2011)

Brush biopsy	Use of cytobrush for collection of oral cells followed by microscopic examination	Oral cells	Non-invasive, easy clinical procedure, cost-effective	Not highly sensitive, sampling error and contamination	(Gupta et al., 2014)
Incisional biopsy	Microscopic examination following removal of a small section of the lesion using a scalpel	Oral tissue	Sensitive, accurate analysis can be performed	Invasive, patient discomfort, requires a skilled clinician	(Kusukawa et al., 2000)
Fine-needle biopsy	Fine-needle aspiration followed by microscopic examination	Oral cells	Low cost	Chances of bleeding and infection, false-negative reporting	(Flach et al., 2013)
Immunohistochemistry	Immunochemical staining for identification of antigen-antibody interaction	Oral tissue	Widely used method for validation	Chances of human error, tedious sample preparation	(Lenouvel et al., 2021)

3. New-age products and Patents for oral cancer diagnosis

Integration of technology from several trans-disciplinary fields has introduced several simplified diagnostic aids and kits for early detection of OC. For instance, artificial intelligence (AI)-based technologies that analyze enormous datasets have shown considerable improvement in predicting the

178 occurrence of OC. Reports suggest that the accuracy of prediction analysis is better as compared to
179 the conventional cox- and logistic regression analysis methods (Khanagar et al., 2021). Other products
180 include visualizing aids, salivary diagnostic kits, and real-time imaging tools. A few of these new-age
181 commercial products and patents are discussed below.

182 Modernized visualizing screening aid that uses the principle of autofluorescence has been developed
183 to overcome the shortcomings associated with light-based detection systems (LBDS). GOCCLES®
184 (Glasses for Oral Cancer – Curing Light Exposed – Screening) fabricated with filters are reported to be
185 a cost-effective alternative for VELscope that can aid in visualizing oral lesions via dental curing light
186 (Moro et al., 2015). However, the limitations of the study include a small cohort study (sample size-61)
187 non-randomized multicenter trial on patients at high risk for OSCC. Additionally, inter-observer
188 variability leads to the subjective interpretation of the data. Further stages of clinical trial need to be
189 conducted to accurately define the diagnostic performance of the system.

190 OralScan, a handheld optical imaging multimodal device developed by Sascan Meditech, Kerala, India
191 is reported to be highly efficient in detecting early lesions and preventing unwarranted biopsies (DST,
192 2020). Another, Bengaluru-based startup in collaboration with AIIMS Delhi and Bhubaneswar
193 developed a mobile app that analyses oral lesions with the help of Artificial intelligence, making the
194 overall diagnosis easily accessible and affordable (BerryCare, 2020). It is interesting to note that few of
195 these innovations involve the direct usage of different components of artificial intelligence for presenting
196 their data to the user. Though there is a booming growth of AI, especially in terms of its association with
197 a cancer diagnosis and precision medicine, certain drawbacks such as ambiguous AI algorithms can
198 lead to erroneous data generation. Other concerns regarding patient data privacy and security breaches
199 limit the clinical implementations of AI on large scale (Sunarti et al., 2021)

200 Multiple large trial studies have proved SalliMark™ OSCC an efficient salivary biomarker diagnostic kit.
201 The non-invasive kit uses bio-chemistry analytics for the early detection of OSCC. Analysis of specific
202 biomarkers expressed during the early stages of OSCC is used for better risk stratification
203 (SalliMark™OSCC, 2021). The procedures for saliva collection and shipping of the sample via pre-paid
204 services may not be suitable for individuals with socio-economic challenges. Further, unfortunate
205 logistical constraints may render the sample unusable.

Cook, Watson, and Festy from King's College London, have patented an imaging apparatus that uses only a green wavelength of light for imaging vascular tissue with better contrast. Angiogenesis and/or neovascularization commonly occurring during oral lesion formation could be monitored with higher accuracy due to the light absorption characteristics of hemoglobin (Hb) (US8364222B2, 2013). However, the technique involves performing surgery on the patient making the overall process invasive and causing inconvenience to the patient. The procedure also would require a highly-skilled technician and advanced medical setups.

Another research group patented a technique using a complex compound consisting of a fluorescent and radioactive marker that is selectively taken up by pre-malignant and malignant cells. In the case of oral malignancy cyto-brush is the suggested method of cell collection. The exfoliated cells with the compound can be examined further via fluorescence microscope or flow cytometry and also by PET and single-photon emission computerized tomography SPECT (US6750037B2, 2004). The work patented by the research group represented a minimally invasive detection system that limited the patient trauma and is also inexpensive. However, the use of cytobrush for the collection of cells may induce sampling error issues and the use of PET-based screening may suffer from lowered accuracy due to the short decay time of the radioactive substance

224 **Table 2.** Products and patents for early screening of oral cancer

Name	Company/patents	Collaborators/Inventors	Working Principle	Ref.
GOCCLES®	Pierrel S.p.A., (owner of the rights on the GOCCLES medical device)	NA	The device is equipped with dental curing lights: Elipar S10 3M ESPE, Led. B Carlo de Giorgi and Optilux 501 Kerr Corporation for illumination of the oral mucosa	(Moro et al., 2015)
OralScan (handheld device)	Sascan Meditech	Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram	Diffuse Reflectance, Tissue autofluorescence, cloud-based machine learning, and application of oxygenated hemoglobin (HbO_2) absorption maps for biopsy guidance	(DST, 2020)
BerryCare (mobile app)	Atom360	AIIMS Delhi and Bhubaneswar	Screening and segmenting oral cancer via AI-based analysis of images of the cancerous lesion	(BerryCare, 2020)
SalliMark™ OSCC (Salivary kit)	PeriRX, Broomal, Pensylvania	NA	Salivary biomarker analysis for risk assessment of OSCC	(SalliMark™OSCC, 2021)

Imaging apparatus	US8364222 B2 (Patent)	Richard James Cook Timothy Frederick Watson Frederic Festy	Selective green wavelength based epi-illumination endoscopic imaging apparatus to image vascular tissues	(US8364 222B2, 2013)
The fluorescent and radioactive Detection technique	US6750037 B2 (Patent)	Edwin L. Adair Jeffrey L. Adair	Screening method based on uptake of a complexed compound containing fluorescent molecule (5-ALA, protoporphyrin IX, tetrakis carboxy-phenyl porphine (TCPP), hematoporphyrine derivative, photofrin, and photofrin II) and radioisotope ^{64}Cu or ^{67}Cu	(US6750 037B2, 2004)

4. Nano-biosensors: A step towards advanced detection platforms

Nanotechnology is redefining health care strategies and is predicted to have a significant impact on both the therapeutic and diagnostic sectors in the coming years. Nano biosensors are nano-scale analytical frameworks that incorporate nano-conjugated biological entities as a transducer for the identification of specific bio-chemical, or physical analytes in minuscule quantities (Huang et al., 2021). Nanodiagnostics is being developed as potentially transformative tools for the rapid, convenient, and cost-effective detection of multiple forms of cancer (colorectal, liver, lung, breast, cervical, prostate, Leukaemia, and oral) (Ali et al., 2021). Over the last 15 years, researchers have shown tremendous interest in nano biosensors for the early detection of oral cancer. This is evidenced by the substantial rise in the number of publications as shown in Pubmed (Fig. 3). The details of different nanotechnology-based techniques used for the detection of oral carcinoma are summarized in Table 3. The following

section will address the details of these relevant nano-based oral cancer screening methods that aid in the early diagnosis and help clinicians monitor the different phases of oral malignancy.

4.1 Surface-enhanced Raman scattering (SERS)

SERS is a well-known spectroscopic technique with an ultrasensitive spatial resolution that trumps other traditional techniques. Raman spectroscopy works on the principle of inelastic scattering, in which molecules in tissues interact with the incident photons and scatter at a different wavelength (Moore et al., 2018). The Raman spectrum is a 'biochemical fingerprint' of the molecules present in the sample tissue, which are depicted as molecule-specific bands. For efficient SERS-based detection, free-electron metals such as Ag, Au, and Cu are taken into consideration. A two-step process generates a Raman signal. The first step involves incident local field enhancement (by the excited surface plasmon resonance (SPR) of the plasmonic nanoparticle) leading to the polarization of the adsorbed analyte molecule and transformation of far-field to near field frequency. The second subsequent step involves the plasmonic nanostructures working as a transmitting optical antenna wherein Raman shifted frequencies are transmitted from near field to far-field where the detector is located. The overall phenomenon of plasmon-enhanced Raman scattering (PERS) demonstrated by Ag and Au nanomaterials with nano—gaps, tips, holes, voids, grooves, bumps, and ridges can be considered as excellent SERS-active substrates. Apart from using noble metal nanoparticles as SERS-substrate for the cancer diagnosis, a new class of active SERS-substrate composed of metal oxides has been emerging recently wherein, the selective SERS enhancement of metal oxides originates from photon-induced charge transfer (PICT) process based on Herzberg–Teller selection rule. Apart from the electromagnetic enhancement mechanism for noble nanoparticles, the remarkable SERS activity, in these metal oxide NPs is due to the chemical enhancement mechanism. The underlying principle for efficient electron transition involves the development of a stable charge-transfer complex due to metastable electronic states and high electrostatic potential, (Lin et al., 2021). (Fig. 4i) Analytical techniques based on SERS can be classified as label-free or label-mediated. Direct label-free detection of analytes, requires that the analyte be SERS-active and that its environment be devoid of interfering molecules to maintain high specificity (Blanco-Formoso and Alvarez-Puebla, 2020). The latter involves indirectly detecting the analyte by functionalizing the SERS active molecule as a label or a reporter molecule on the capturing moiety and detecting the reporter's SERS signal as a surrogate measure of the analyte (Zhang et al., 2019) (Fig. 4ii).

(Girish et al., 2014) developed hierarchical TiO_2 nanostructures (needular, bipyramidal, and leaf-like) and further incorporated Raman active 30 nm silver nanoparticles (AgNPs). The fabricated SERS catheter could distinguish between normal and malignant tissues in oral cancer patients, SERS spectra were obtained by the quick absorption of cryosection onto the SERS substrate. It was observed that the nano-leaf-like structure with numerous 20-150 nm adjacent parallel surfaces could potentially accommodate a higher number of AgNPs as compared to nano-needular and bipyramidal structures. The AgNPs in the TiO_2 nano-leaf acted as efficient inter-structure hot-spots and the overall nanostructure demonstrated the highest enhancement factor of $\sim 10^6$. Intense spectral peaks obtained at 645, 680, 794, 825, 1189, 1326, 1585, and 1618 cm^{-1} corresponded to the tumour spectra whereas, normal tissues with intact collagen from connective tissue displayed peaks at 870 and 950 cm^{-1}. Principal component analysis (PCA) was performed to classify normal and malignant tissues with 97.24 % accuracy within 25-30 minutes for each patient.

(Connolly et al., 2016) used silicon-coated silver nanopillar substrates (SERStrates^{TM}) procured from SILMECO (Denmark) for recording SERS signal of salivary and oral cells extracted from patients with OPC. The manufacturer used a patented technique for the fabrication of the SERS substrate that did not involve lithography and complex chemistry. SERS spectra were recorded upon the addition of 20 ul thawed saliva onto the substrate. Distinct spectral signatures (1224, 1275, 1409, and 1417 cm^{-1}) were obtained which reflected molecular and cellular changes associated with oral malignancy. An additional pilot study was conducted wherein desquamated oral cells from OSCC patients were used. Patients with cancer demonstrated intense peaks at 1347 and 1543 cm^{-1}. The diagnostic accuracy of saliva and oral cells was reported to be 73% and 60%, respectively, using principal component analysis (PCA) and linear discriminant analysis (LDA). The study established the potential of SERS as a non-invasive, label-free technique for detecting OC.

In a recent study, (Liu et al., 2021) developed a plasmonic Ag nanocube (AgNC)-based SERS detection platform that used the principle of nicking endonuclease assisted signal amplification (NESA). AgNCs with sharp edges and a strong electromagnetic field generated superior-quality SERS hot spots, enhancing the SERS signal by a factor of 10^8. NESA provides the advantage of recycling the target analyte such as DNA sequences and micro-RNA which improves the assay sensitivity via signal amplification. In this study, the authors, combined heated Au electrodes with NESA for the identification of OC-specific target DNA (tDNA) sequences. The SERS signal was generated by hybridizing the SERS

tag (AgNCs/sDNA/4-MBA) on the heated Au electrode which contained the capture DNA (cDNA). In presence of tDNA, a complex structure containing the SERS nanotag and cDNA/tDNA duplex was formed which could be cleaved by Nt.BstNBI enzyme. This resulted in a decrease in the SERS signal which was proportionally related to the tDNA concentration. The nano biosensor demonstrated excellent sensitivity over a broad linear range of 10 fM –1 nM and a 1 h cleavage time. High selectivity of the proposed sensor in terms of single- double and complete base mismatch at 1586 cm^{-1} were 10.2%, 7.1%. and 2.8% respectively. This shows the application potential of the sensor for early clinical detection of oral cancer (Fig. 5).

(Fălămaș et al., 2020) investigated the amplification of salivary distinctive Raman bands utilizing label-free, gold nanoparticles mixed with saliva from smoking and non-smoking volunteers and OC patients. SERS hotspots were formed as a result of the clustering of AuNPs during the air-drying of saliva samples on glass slides. Using PCA, oral cancer saliva could be distinguished from healthy patients based on multiple SERS signals ascribed primarily to amino acids and proteins. The study revealed that the individuals with cancer had a greater overall level of the 2126 cm^{-1} band area allocated to thiocyanate C–N stretching vibrations.

A few key points that need to be addressed while utilizing SERS-based sensors for cancer diagnostics include the surface geometry of the nanoparticle and intimate contact between detecting analyte and plasmonic SERS substrate. Though reshaping of NP enhances the SERS activity, reliable reproduction of such particles at a large scale is practically challenging. Another major problem frequently encountered is the fouling of the SERS substrate in presence of undiluted biological fluids which leads to lower signal intensity and reduces the reusability of the substrate. Extensive sample pre-treatment is thus required which makes the detection process cumbersome and less economical. Introduction of stealth-modification by utilizing zwitterionic molecule and/or nanoshearing mechanisms can enhance the anti-fouling properties. The use of L-cysteine has been reported to decrease the BSA-mediated fouling of hybrid SERS substrate (graphene-oxide supported star-like gold nanoparticles) by 80.53%(Panikar et al., 2018). Another report suggests the use of tri-methyl amine N-oxide (TMAO) derived zwitterionic polymers that aim at improving the hydration and stability of gold chips (Li et al., 2019). For SERS-based microfluidic systems, another approach for the generation of anti-fouling SERS-substrate can be nanoshearing that essentially incorporates the tunable alternating current electrohydrodynamic force (ac-EHD). The developed method enhances the diffusion of biomarkers and

SERS-nanoprobes resulting in increased fluid flux and minimized non-specific attachment (Panikar et al., 2021). However, both these techniques have their limitations and need further optimization to be implemented as a standard procedure in the development of active SERS substrate.

4.2 Colorimetric nanobiosensor

Colorimetric detection is one of the most widely used optical detection techniques because of its easy operation, visible radiation, and rapid reading. Free electrons are naturally oscillating in metallic nanoparticles due to their confinement in a finite region. Absorption happens when metallic NPs are irradiated with a frequency that equals the inherent natural frequency of the oscillating electrons. It results in non-propagating oscillations known as the localized surface plasmon resonance (LSPR) phenomenon (Iarossi et al., 2018). The principle of the colorimetric assays depend on LSPR effect manifested by several metallic nanoparticles. Colorimetric assays typically proceed via a two-step method wherein, the first step includes specific recognition of the target analyte with the plasmonic metal NPs followed by the conversion of these specific recognition events into measurable optical signals in the visible range. Such interactions can either promote aggregation of the metal NPs or induce morphological alterations leading to dramatic spectral shifts (blue shift/red shift)(Kailasa et al., 2018). Typically, metal NPs (Ag, Au, Cu, and Pt) exhibit SPR behavior. Easily-tunable physicochemical properties and biocompatibility make AuNPs excellent nano sensing probes (Fig. 6i) (Akshaya et al., 2020). Over the years, this technique has been used to detect several cancers such as leukemia, oral cancer, lung cancer, exosomes from ovarian cancer, and tumor-specific CD8+ T Cells (Im et al., 2014; Ribaut et al., 2016; Soler et al., 2018; Valsecchi et al., 2016).

(El-Sayed et al., 2005) conjugated AuNPs (35 nm) with monoclonal anti-epidermal growth factor receptor (anti-EGFR) for the diagnosis of malignant oral epithelial cell lines (HOC 313 clone 8 and HSC 3). The homogenous and specific binding of the EGFR expressed by the cancer cell lines to the Ab-coated AuNPs increased the λmax at 545 nm. The absorption maximum for normal HaCaT (normal keratinocyte cell line) which was at 0.01 increased to 0.06 and 0.07 in the case of HOC 313 clone 8 and HSC 3 suggesting an increase in binding efficiency by 600 and 700% respectively. The overall study illustrates the potential application of plasmonic AuNPs for efficient differentiation between cancerous and non-cancerous cells by deploying a simple student microscope for SPR scattering-based imaging as well as with SPR-absorption spectroscopy.

(Wang et al., 2013) demonstrated a label-free OC detection method using rose bengal (RB) conjugated- gold nanorods (GNRs). The RB dye in the nanoprobe with specificity for oral cancer cells (CAL-27 and Tca8113) induces end-to-end assembly of the GNRs leading to aggregation and significant shift in the LSPR of the GNRs. The quantitative sensing range for the cancer cells based on the LSPR shift was 2.2 x 10^3 to 30.3 x10^3 cells mL^{-1} with a detection limit of 2000 cells ml^{-1}. Further, the NIR-absorption of the GNRs probe facilitates optical absorption imaging. The enhancement in NIR-absorption intensity with an increase in the number of cancer cells can be visualized following irradiation with a LED light source. A linear correlation was obtained between the absorption intensity of the nanoprobes and the concentration of cancer cells in the range of 10 and 50 x 10^3 cells/mL. The study highlights the potential application of the conjugated probe as a sensitive NIR- optical biodiagnostic tool.

It has been previously reported that the enzyme PKC α which initiates differentiation and proliferation of malignant cells is hyperactivated in several cases of oral cancer (ZHANG et al., 2005). (Kang et al., 2010) devised a colorimetric technique for cancer detection employing the interaction between anionic citrate-functionalized AuNPs (~20 nm) and cationic protein kinase C (PKC) α - specific peptide with +5 charge resulting in blue color aggregate formation via a non-crosslinking pathway (Fig. 6ii). However, the presence of the enzyme PKC α (highly activated in cancer cells) induced phosphorylation of PKC α -specific peptide and reduces its cationic charge to +3 and declined the aggregation propensity of AuNPs resulting in the formation of red coloured dispersed AuNPs. The visual colour changes of the GNPs dispersion following the addition of cell lysates (A546, B16 melanoma, HeLa and CHO) indicated the concentration of the enzyme PKC α in the range 0 - 0.2 μg/mL. The resultant phosphorylation ratio mediated by the enzyme was further validated using Matrix-Assisted Laser Desorption - Ionisation-Time of Flight Mass (MALDI-TOF-MS) spectrometry and western blotting experiments.

Though colorimetric-based disease detection is considered most simple, fast, and effective, few technical drawbacks limit their application for the sensitive determination of disease biomarkers. Quantitative determination of analyte concentration via visual color change can be subjective and thus reduces the overall accuracy of the detection system. Apart from this, uncontrollable and self-aggregation of the nanomaterial in a complex sample matrix may generate false-positive data along with high background signals (Krishnan, 2022). Recent developments include the incorporation of 2D materials such as graphene, transition metal oxides, and MXenes along with nanoparticles. These 2D

materials can be used as templates for efficient conjugation for the fabrication of hybrid nanomaterials. Further, they also possess a higher amount of analyte binding sites and enzyme-mimicking activity(Zhu et al., 2021). These modifications can enhance the sensitivity and selectivity of the analytical system and can be employed for the detection of several OC markers in the future.

4.3 Fluorescent Nanobiosensor

Fluorescence is an optical phenomenon that occurs when photons are absorbed at one wavelength and emitted at another. Vibrational relaxation and solvent reorganization result in Stokes shift. Following the absorption of light, the excited electron can be subjected to several photophysical events (radiative emission, nonradiative emission, vibrational relaxation, intersystem crossing, and internal conversion) finally, returning to the ground state, while releasing energy in the form of photons at higher wavelengths. (Fig. 7) Quantum dots (QDs), polymer dots (PDs), and upconversion nanoparticles (UCNPs) are the most frequently used fluorescent nanoprobes for cancer diagnostic applications (Berezin and Achilefu, 2010). Conventional fluorophores underperformed for biosensing applications due to their shorter fluorescence lifetimes, low coefficients of molar absorption, limited absorption spectra, and sensitivity to photodegradation, resulting in a substantially low signal-to-noise ratio during bioassays. Fluorescent nano-bio sensors have proven to be a highly sensitive and selective optical sensing aid for the on-site recognition of biomolecules in a dynamically active living cell (Bhardwaj et al., 2017).

Currently, quantum dots (QD) are being used for *In vitro* and *In vivo* molecular and cellular imaging of OSCC. QDs have been shown to exhibit a low nonspecific binding, high fluorescence intensity, and resistance to photobleaching when used for *In vitro* imaging. In this regard, water-soluble thioglycolic acid stabilized CdTe QDs and peptide-conjugated QD800 have been used for immunofluorescent labeling of different human OC cells such as Tca8113 and BcaCD885 (Li et al., 2006; Yang et al., 2010; Zhao et al., 2011). These fluorescent nanoprobes with higher tissue penetration ability can promote early determination of OC as compared to conventional immunoassays using organic fluorescent dyes. Further (Zhu et al., 2017) developed a nanoconjugated probe consisting of Ag_2Se QDs coupled with cetuximab for targeted imaging and therapy of orthotopic tongue cancer. The developed nanomaterial with NIR-emission at 900 nm significantly decreased tumour growth and enhanced the survival rate of

415 the nude mice (57.1%). This work suggests the potential applicability of QDs-based nanotheranostics
416 for OC.

417 Carbon-based nanomaterials such as CQDs have gained notable attention over the years for their
418 exceptional non-blinking photoluminescence and biocompatible property. Recently(Sri et al., 2018)
419 used novel zwitterionic CQDs (2-5 nm) for imaging CAL-27 cells with a high quantum yield of ~80%.
420 The zwitterionic property provides a better advantage over negative and positive CQDs which are
421 known to have lower fluorescent quantum yield and biocompatibility respectively. Interestingly the
422 research group reported the low toxicity even at exceptionally high concentrations of (1600 μg mL^{-1})
423 CQDs used in the experimental procedure. Another study demonstrated the use of UCNPs for Förster
424 resonance energy transfer (FRET)- based detection of MMP-2 (matrix metalloprotease-2) in OC cells
425 (CAL-27). The UCNP@p-QD nanoprobe consisted of SiO_2-coated UCNPs and were further conjugated
426 with $CuInS_2$/ZnS core/ shell QDs via MMP-2 sensitive peptide. The mechanism of detection was based
427 on MMP-2 mediated digestion of the peptide which released the bound QDs decreasing the
428 fluorescence emission intensity at 600 nm and simultaneously increasing the fluorescence emission
429 intensity from the UCNPs (475 nm). The detection of MMP-2 could be performed on a broad range of
430 10^6-1 pg mL^{-1} (Chan et al., 2018).

431 Song and colleagues, developed a 3D network of carbon nanotubes (3DN-CNTs) on a Silicon pillar
432 substrate to detect the oral cancer biomarker cytokeratin-19 antigen (CYFRA 21-1) in clinical samples
433 (Fig. 8). To ensure structural stability, the template was coated with aluminum oxide (Al_2O_3) and then
434 modified with an amino silane reagent to generate a SAM for antibody immobilization. After incubation
435 of 3DN-CNTs with the target analyte, the enhanced fluorescence signal generated from Alexa 558-
436 tagged detector Ab was used for the quantitative detection of CYFRA 21- 1 in the concentration range
437 of 0.1- 10^3 ng mL^{-1} with a detection limit of 0.5 ng mL^{-1}. The fabricated sensor system had 20 times
438 higher sensitivity as compared to the conventional sandwich-type ELISA. It was suggested that 3DN-
439 CNTs provided a larger surface area and higher number of binding sites for capture-Ab binding as well
440 as the hierarchical architecture of 3DN-CNTs eased the accessibility of biomolecules through the
441 ordered pathways of 3DN-CNTs templates. (Song et al., 2018).

442 (Li et al., 2020) recently developed non-invasive nano-graphene oxide (NGO)-based
443 immunofluorescent probe (NGO-BBN-AF750) consisting of graphene oxide nanoclusters functionalized

with AF750-6Ahx-Sta-BBN GRPR-specific peptides via hydrogen and π–π bonds (Fig. 6c). Taking the advantage of the fact that oral cancer cells overexpress gastrin-releasing peptide receptor (GRPR), they reported ~ 96% cellular uptake of the nanocluster in HSC-3 cells within 60 min. When compared to normal oral mucous tissues, the malignant tissues exhibited a high-intensity fluorescent signal (2.5-fold increase), indicating a high affinity and specificity for HSC-3 cells. These findings indicate that nano-GO with a higher amount of functional group (carboxyl, hydroxyl, and oxygen), and enhanced permeability and retention (EPR) effect can be fine-tuned for suitable biosensing applications.

(Wu et al., 2021) recently published a study focusing on the detection of exosomes in saliva as a potential biomarker associated with OC, among other malignancies. They fabricated a fluorescent nano biosensor that incorporates magnetic and fluorescence bio-probes (MFBPs) coated with QDs for signal amplification. Aptamer functionalized magnetic microspheres were designed for the specific binding of CD63 proteins on exosomes. Under experimentally optimized conditions, 0.25 mg mL^{-1} of the synthesized nanoprobe interacted with the target sample for 30 min and promoted the reshaping of aptamers and release of QDs-conjugated DNA concatemers as a fluorescent signal. The one-step signal amplification procedure was based on the concept of “one exosome numerous QDs”. The analytical attributes of the sensor included an exosome detection range of 500 - 10^5 particles/μL and a detection limit of 500 particles/μL. The intra-and inter-assay variability was reported to be 3.09% and 5.43% respectively.

Over the years, molecular imprinting technology has gained enormous attention. The development of molecular imprinted fluorescent nanoparticle (MIFN) sensors has shown promising results in the detection of cancer protein markers such as Interleukin-2 (IL-2) in serum (Piloto et al., 2020). The process involves the incorporation of fluorescent nanoparticles such as QDs, CQDs, and/or UCNPs as a fluorescent source into MIP specific recognition unit. The hybrid system fabricated via the sol-gel method, free-radical polymerization, and reverse microemulsion are reported to have high sensitivity, selectivity, and stability. However, several parameters such as the amount of monomer/crosslinker that can affect the MIP layer thickness and subsequently the luminescence efficiency of the fluorescent probe needs to be carefully optimized to successfully implement these techniques for OC detection. (Wang et al., 2020)

4.4 Electrochemical nano-biosensor

Electrochemical nano biosensors are deemed promising due to their simplicity, rapidity, and cost-effectiveness, as well as their potential to be easily coupled with electronics for mass production (Lin et al., 2020). Three integrated constituents are required to design an electrochemical biosensor system: (i) a recognition element that interacts with the analyte; (ii) a signal transducer that converts the analyte-biomolecular layer interaction into a measurable signal; and (iii) an electronic data management system (Fig. 9) (Topkaya et al., 2016). Various NPs such as CNTs, graphene oxide, zinc oxide, AuNPs, AgNPsr, zirconium oxide, etc. are incorporated in electrochemical nano biosensors to improve electron transfer efficiency between the electrodes and analyte. It improves the immunosensor's function by increasing its sensitivity and stability, enabling ultrasensitive detection of multiple OC biomarkers (Hasanzadeh et al., 2017). Numerous electro-analytical techniques are used to quantify these signals, including voltammetry, amperometry, field-effect transistors, impedimetric methods, electrochemiluminescent assays, and electrical approaches based on nanochannel/nanopore structures (Naresh and Lee, 2021).

For the label-free identification of salivary IL-8, an electrochemical immunosensor was devised using an Indium tin oxide (ITO) electrode modified with AuNPs/r-GO nanocomposite. The AuNPs here work as a conduction pocket for smooth charge diffusion between the reaction mixtures to the surface of the electrode. The π-conjugated r-GO facilitates better channeling of electrons as compared to GO. The AuNPs/r-GO electrode has the highest redox current due to the synergistic role of AuNPs and r-GO in the electron transfer process. 1 ng/mL of the analyte was incubated with Anti-IL8/AuNPs-rGO/ITO nanoprobe and quantified using Differential Pulse Voltammetry (DPV). The fabricated nanosensor demonstrated a high degree of specificity, sensitivity, and rapid detection within 9 minutes (Verma et al., 2017). The analytical performance of the fabricated sensor in terms of recovery was 94.15% for the IL-8 spiked saliva sample. Remarkable is the data concerning the long-term stability, with a 94.3% performance retainment after three months and a 91.8% stability after four months of dry storage.

Ding and colleagues reported the first use of vertically aligned carbon nanotube arrays (VANTA) arranged in 2D interdigitated electrodes (IDEs) for the electrochemical sensing of CIP2A- an oral cancer-related oncoprotein. The high height: width aspect ratio (3:1) VANTA incorporated IDEs utilized here, possessed unique light absorption, chemical inertness, and electrical conductive properties along

with high surface area. Ab was immobilized on VANTAs, and samples were analyzed using electrochemical impedance spectroscopy (EIS). The fabricated VANTA-IDE sensor had lower sensing ranges (5-400 pg ml^{-1} in PBS and 1-100 pg ml^{-1} in saliva) as compared to the commercial ELISA Kit. and also minimal interference in the presence of a high concentration of BSA. It must be noted that the nature of the interaction between the antigen and the immobilized Ab on the VANTA varied across different reaction matrices. Lower dissociation constant (K_D) values 13 pg ml^{-1} obtained from Hill equation in case of saliva as compared to PBS indicated a positive cooperative mode of Ag-Ab binding on the sensing platform. Overall a detection limit of 0.24 pg/mL was obtained in saliva specimen within a short response time of 35 minutes (Ding et al., 2018).

Silver molybdate (β-Ag_2MoO_4) nanoparticles on ITO-coated glass substrate have recently been utilized for the label-free detection of salivary IL-8 (Fig. 10). Ab was covalently attached to the immunoelectrode surface via EDC-NHS bioconjugation chemistry. The Ag_2MoO_4/ITO electrode provided an optimal microenvironment for different biomolecular interactions required for the sensing of IL-8. The higher Ag valences and greater O-Ag-O bond length provided a biocompatible environment for the immobilization of IL-8 Ab on the AgO sites. DPV method was used to assess the IL-8 antigen's sensing response at a wide range of concentrations (1 fg mL^{-1} to 40 ng mL^{-1}) within a response time of 10 minutes, the BSA/anti-IL-8/-Ag_2MoO_4/ITO immunosensor was able to detect IL-8 with a detection limit of 90 pg/mL. Additionally, the fabricated electrodes demonstrated the same DPV measurements over a period of 4 weeks when stored at 4 ^{o}C. (Pachauri et al., 2020).

(Zhang et al., 2015) used a top-down lithographic approach to fabricate a monocrystalline silicon nanowire (SiNW) field-effect transistor (FET) sensor device for the label-free, multiplexed detection of two common oral cancer biomarkers, IL-8 and TNF-α (Fig. 7c). The high surface-to-volume ratio of the SiNW enables efficient biomolecular interaction with high affinity, this, in turn, enhances the sensitivity of the analytical system. The Ab of both the biomarkers were immobilized on the SiNW array surfaces and allowed to interact with a specific Ag Considering the different isoelectric points of the detecting analytes, utilization of low ionic strength 1x PBS (pH-8.5) as the measuring buffer took into consideration the Debye-screening length and resulted in accumulation of carrier on positively charged n-doped SiNW in case of IL-8 binding and depletion of SiNW bulk for TNF-α binding. The alterations in the resistance of the FET biosensor resulting in a signal generation were recorded both before and after

target binding. The LOD for both biomarkers in was reported to10 fg ml^{-1} in buffer and 100 fg ml^{-1} in artificial saliva

(Tiwari et al., 2017) reported easy synthesis of lanthanum oxide $La(OH)_3$ NPs by co-precipitation method. The enhanced electron-transfer ability and availability of a large number of binding sites for biomolecules exhibited by $La(OH)_3$ make them suitable for electrochemical sensing applications. The study suggests the use of non-toxic L-cysteine as the capping agent instead of the most frequently used 3-aminopropyl triethoxysilane (APTES) or olylamine (Fig. 11). The detection of CYFRA 21-1 in the range of (0.001-10 ng mL^{-1}) was performed at an optimized pH of 7.0. Higher and lower pH denatured the bound antibody due to their interaction with OH^- and H^+ ions. The decrease in the peak current upon binding of the target analyte with increasing concentration was due to the formation of an electrically insulating Ag-ab complex that hindered the electron transfer between the BSA/anti-Cyfra-21-1/Cys-La(OH)3/ITO immuno electrode and the electrolyte species $[Fe(CN)_6]^{3-/4-}$ present in the reaction buffer. The fabricated sensor exhibited a low detection of 0.001 ng mL^{-1} and the response time was 5 min. Additionally, the immune electrodes had a shelf-life of 13 weeks when stored at 4 ^oC.

Salivary CYFRA 21-1 has been frequently reported to be expressed at high levels in the case of OC. Extensive research has been conducted over the past few years for the sensing of CYFRA-21-1 via electrochemical methods by Kumar and his colleagues. The architectural framework has been slightly modified across the different studies. Different NPs (ZrO_2, $nHfO_2$, and TiO_2) have been electrophoretically deposited onto APTES treated ITO coated glass electrode followed by conjugation with mAb -anti-CYFRA-21-1 and BSA as the blocking agent. (Kumar et al., 2020; Kumar et al., 2018b; Kumar et al., 2016a; Kumar et al., 2015). The measurements were performed in PBS buffer at pH-7 containing $[Fe(CN)_6]^{3-/4-}$ as the electrolyte species. The linear calibration curve for an increase in anodic peak current/ charge transfer resistance vs. different concentrations of CYFRA-21-1 was plotted to further analyze the analytical performance of the respective electrochemical sensors. The authors suggest that the increase in the peak current was due to tyrosine amino acid residues in the CYFRA-21-1 molecules that undergo oxidation and channel a higher number of released electrons. Additionally, conformational alterations following Ag-Ab binding can facilitate the easy transfer of electrons to the electrode. ZrO_2 nanodots were highly efficient as they had a broad CYFRA 21-1 detection range of 0.5-50 ng mL^{-1} with a detection limit of 0.5 ng mL^{-1} as compared to $nHfO_2$ (2-18 ng mL^{-1}; LOD- 0.21 ng mL^{-}

560 ^{1}) and TiO_2 (0 -12 ng mL^{-1}; LOD-0.24 ng mL^{-1}). However, immuno electrode .with nHfO2 showed the
561 highest shelf-life of 8 weeks when stored at 4 ^{o}C.

562 In a separate study, (Malhotra et al., 2010) developed an ultrasensitive Single-Walled Nanotube
563 (SWNT) forest-based immunosensor for the detection of secreted IL-6 from different *in vitro* OC cell
564 preparations. The working mechanism of the fabricated sensor was based on the binding of capture
565 Ab-coated SWNT forests with IL-6 and further interaction with a multi-labeled conjugate system. The
566 conjugated complex consisted of secondary Ab that was attached with 106 HRP per 100 nm of multi-
567 Walled Nanotube (MWNT). The signal generated was measured using rotating disc amperometry at
568 3000 rpm using 1mM hydroquinone as a mediator. The authors suggest that this particular multi-labeling
569 strategy exhibits a 60-fold lower detection limit as compared to treating with Ab2biotin-streptavidin-HRP,
570 providing 14 to 16 HRPs on one Ab. However, the conjugated system had a short shelf life of 10 days
571 and also required 1h of incubation with the target analyte for the generation of a readable signal.

572 From the above studies, it can be understood that modified nanomaterials on the electrochemical
573 sensor surface enhance the interfacial adsorption of the detecting analytes as well as improves the
574 overall electron-transfer kinetics. Modifying the nanoarchitecture in electrochemical sensing platforms
575 can significantly improve the detection of crucial biomarkers such as miRNA for OC diagnosis (Masud
576 et al., 2019b). Different nanoforms of graphene such as nanoribbons, and nanoflower with excellent
577 conductivity and electron-transfer rates have shown significant potential in the development of
578 graphene-based electrochemical biosensors (Ismail et al., 2017). However, the requirement of intricate
579 modifications of the nanoparticles compromises the reproducibility of the sensing system. Nanomaterial
580 aggregation and flaking hamper the stability and shelf-life of the sensors. Considering the fluctuating
581 sensor-to-sensor batch reproducibility, mass production of these sensors is still not a feasible option.
582 These issues have recently been circumvented by the incorporation of sol-gel and ceramics along with
583 nanoparticles(Ferrag and Kerman, 2020). Another major challenge that needs to be addressed for real
584 sample analysis is the use of raw samples which increases the possibilities of non-specific adsorption
585 of unwanted molecules. Extensive sample dilution, to minimize the matrix effect has been a major
586 roadblock against deploying these electrochemical sensors as POC devices.

587 **4.5 Other Nanomaterials based approaches for oral cancer diagnosis**

588 **Optical coherence tomography (OCT)**

OCT uses infrared light to provide cross-sectional architectural images of epithelial layers and basement membranes, making it ideal for early detection of OSCC (Green et al., 2016). It has a resolution of approximately 10 μm, which is better than existing MRI, CT, and ultrasound techniques (Pande et al., 2016; Troutman et al., 2007). AuNPs are effective OCT contrast agents as they are biocompatible and capable of generating LSPR at near-infrared wavelengths. For instance, AuNPs (71 nm) conjugated with EGFR mAb was used as an effective OCT-contrast agent for imaging oral dysplasia in a hamster model. The reflected light from the immobilized mirror and each scattered particle from the specimen generated a signal that was detected by the spectrometer. Microneedles and ultrasound were used to deliver AuNPs which improved OCT penetration and increased the contrast level by approximately 150% (Kim et al., 2009). It must be noted that in the case of OCT, NPs with different anisotropic geometries have been used to increase the imaging contrast. However, specific NPs geometries wherein the plasmon band are close to the central wavelength of the OCT source lead to very strong interaction with the plasmon resonance and enhance the backscattered light. Recently it was observed that branched-AuNPs with three distinct plasmonic peaks are highly efficient as compared to nanorods matching only one central wavelength, or nanospheres that scatter light only if their size is big (bulk effect) (Ponce de León et al., 2012).

Magnetic resonance imaging

MRI has been effective for assessing primary tumors, bone invasion, and delineating tumor margins during surgery. Contrary to conventional MRI contrast agents, Nano-contrast agents can identify unique biomarkers with increased blood circulation half-life, displaying excellent MRI contrast features (Fig 12i). Oral cancers have also been examined using a nano-contrast agent containing a combination of folate-conjugated chitosan and magnetic poly (lactide-coglycolide) (PLGA) NPs.

Asifkhan and colleagues synthesized an MRI contrast agent by combining folate-conjugated chitosan and magnetic poly (lactide-coglycolide) (PLGA) NPs having a magnetization of 35 emu/g. Oral cancer cells with folate receptors showed enhanced nanoparticle uptake by receptor-mediated endocytosis and were measured via mean fluorescence intensity (MFI). The T2 relaxation time was decreased, with an increase in the NPs relaxivity (232.7 mM^{-1} S^{-1}), resulting in high contrast cancer images as compared to commercially available contrast agent ferumoxytol which has a relaxivity of 85 mM^{-1} S^{-1}(Shanavas et al., 2017).

Photoacoustic imaging

Photoacoustic imaging technique generates ultrasonic transients from tissues by employing short laser pulses resulting in transient thermoelastic expansions. The ultrasonic transducer captures photoacoustic waves and converts them into photoacoustic images based on their arrival times. Photoacoustic imaging, in comparison to traditional optical imaging, provides a greater imaging depth and a higher spatial resolution resulting in enhanced imaging (Fig 12ii). Several exogenous contrast agents have been used to enhance the performance of the imaging modality, however, AuNPs are preferred because of their ability to conjugate proteins and generate higher photoacoustic imaging signals (Bayer et al., 2017; Zhang et al., 2016).

(Luke et al., 2014) pioneered the use of ultrasound-guided spectroscopic photoacoustic imaging (sPA) to detect lymph node micrometastases in a metastatic OSCC murine model. The study demonstrated that 40 nm anti-EGFR antibody conjugated, molecularly activated plasmonic nanosensors (MAPS) shifted their λ_{max} towards the NIR region. Due to the molecular interactions between EGFR-targeted MAPS and tumor cells, its injection into the lymph nodes resulted in an increased spectroscopic signal. Furthermore, a single peritumoral injection of MAPS allowed for the rapid detection of micrometastases as tiny as 50 μm, within 30 minutes. The sensitivity and specificity of the developed system were reported to be 100% and 87% respectively. the sPA is considered to be better compared to commercially available radiographic techniques such as PET which has nearly 50% sensitivity. However, the work faces major challenge toward clinical acceptance due to the use of large 40 nm AuNPs that reduces systemic clearance and induces long term physiological toxicity.

Diffusion reflection imaging

In this technique, the white light that enters the tissue is either absorbed or transmitted, the remaining undergoes repeated elastic scattering and becomes diffusely reflected. The reflected light is strongly influenced by cytological and morphological deformities that occur during cancer formation, such as nuclear size and collagen content variation, changes in ECM structure, epithelial thickness, and blood flow (Stephen et al., 2013). It aids in determining surgical margins and can be used to distinguish between normal mucosa, OPMD, and oral cancer (Chen et al., 2015).

(Ankri et al., 2016) attached GNRs (25 x 11 nm) to EGFR mAb and used diffusion reflection imaging to examine the margins of human OSCC specimens. The uptake of the Ab-conjugated GNRs was nearly

21.8 μg for cancer whereas for negative control cells the cellular uptake obtained was 0.2 μg. After 15 minutes of incubation with 50 μl GNR, tissue sections were imaged using the hyperspectral imaging system. Air scanning electron microscopy (air-SEM) visualized that the GNRs-EGFR propagated 1mm between the tumor and the healthy tissue, indicating tumor margins of 1mm. The sensitivity and specificity of the diagnostic mode in terms of differentiating *ex-vivo* positive and negative resected tumour margins as tested on mice-model were observed to be 82 and 75%.

Modified immunoassay

In our previous work, we have developed nano-ELISA for salivary osteopontin (OPN) detection, wherein, multiple antibodies attached to single gold nanorods (GNRs) generated an amplified response in the presence of a low concentration of OPN as compared to classical ELISA. The GNRs-in the nano-ELISA acted as a concentrator of signaling Ab and amplified the signals upon antigen binding (Fig. 13i). The GNR-based nano-ELISA had increased sensitivity and had a detection limit of 0.02 ng mL^{-1}, whereas, for conventional ELISA, it was 0.14 ng mL^{-1}. The same study was also validated using gold nanospheres (AuNS) wherein the detection limit for OPN was 0.03 ng mL^{-1}. The analytical performance of the modified nano-ELISA system validated on OPN-spiked whole and simulated saliva showed good recovery rates of 96.25-98.7%. The study emphasizes the critical necessity of employing different anisotropic nanoparticles with a large surface area that not only enhances the sensitivity but also reduces the wastage of excess unreacted Ab making the sensing system more economical. (Chakraborty et al., 2018).

Microfluidics

Microfluidic systems frequently referred to as "Lab-on-a-chip," can serve as a miniature automated version of integrated experimental operations on a single device (Ziober et al., 2008a)**.** Currently, this technique is used in a variety of biomedical applications, including disease detection, drug delivery, and drug discovery. Processing minuscule biopsy samples or identifying tumor biomarkers in biological fluids promptly while maintaining high repeatability and consistency boosts its therapeutic potential for cancer management (Fig.13ii). In a recent report, polythiophene was used as bulk heterojunctions for spin-coating of organic photodetectors (OPDs) and incorporation onto microfluidic chips made of poly(methyl methacrylate) (PMMA). The immunogold-silver assay (IGSA) was used to detect various salivary analytes, including MMP-8, IL-1, and IL-8, using the immunoreaction compartment of the

microfluidic chip. The chip's immuno-reaction compartment was oriented with the OPDs to measure the photocurrents from the light absorbed (λ_{650}) by the IGSA. This diagnostic platform was cost-effective and provided multiplexity in the early diagnosis of OC (Dong and Pires, 2017).

Table 3. Nanotechnology-based methods used for the diagnosis of oral carcinoma

Technique	Nano biosensor component	Sample	Biomarker	Range of detection	Limit of Detection/ Sensitivity	Response Time (in min.)	Ref.
SERS	Ag-TiO_2 SERS substrate	OSCC tissue	-	100 uM to 1 nM	1 nM	15–20	(Girish et al., 2014)
	silver-coated silicon nanopillar substrates (SERStrates™)	Saliva	-	-	89%	-	(Connolly et al., 2016)
	Nt.BstNBI/AgNCs /HAuE	Saliva	-	10 fM - 1 nM	3.1 fM	60	(Liu et al., 2021)
	AuNP/DNA probe/MGITC	Saliva	S100P mRNA	Free solution- 1.2–200 nM; Vertical flow chip-, 10–100 mM	Free solution - 1.1 nM ; Vertical flow chip - 10 nM	53	(Han et al., 2019)
Differential pulse voltammetry	anti-CYFRA-21–1/ APTES/ ZrO_2-rGO/ITO	Saliva	Cyfra-21-1	2–22 ng mL^{-1}	0.122 ng mL^{-1}	16	(Kumar et al., 2016b)

	BSA/anti-CYFRA- 21-1/Ser/ $nZrO_2$/ITO	Saliva	Cyfra-21-1	0.01–29 ng mL^{-1}	0.01 ng mL^{-1}	6	(Kumar et al., 2016a)
	BSA/anti-Cyfra-21- 1/Cys-$La(OH)_3$/ITO	Artificial Saliva	Cyfra-21-1	0.001–10.2 ng mL^{-1}	0.001 ng mL^{-1}	5	(Tiwari et al., 2017)
	BSA/anti-CYFRA- 21-1/APTES/ $nHfO_2$@RGO/ITO	Saliva	Cyfra-21-1	0–30 ng mL^{-1}	0.16 ng mL^{-1}	15	(Kumar et al., 2018a)
	Anti-IL-8/AuNPsrGO/ITO	Saliva	IL-8	500 fg mL^{-1}– 4 ng mL^{-1}	72.73 pg mL^{-1}	9	(Verma et al., 2017)
	BSA/Anti-IL-8/β-Ag_2MoO_4/ITO	Saliva	IL-8	1 fg mL^{-1}– 40 ng mL^{-1}	90 pg mL^{-1}	10	(Pachauri et al., 2020)
	Anti-IL8/ZnO–rGO/ITO	Saliva	IL-8	100 fg mL^{-1}– 5 ng mL^{-1}	51.53 pg mL^{-1}	10	(Verma and Singh, 2019)
Square wave voltammetry	Anti-IL 6/GO/GCE	Serum	IL-6	0.002–20 ng mL^{-1}	1 pg mL^{-1}	-	(Li and Yang, 2011)

Amperometry	Anti-IL8/GSH-AuNP/pyrolytic graphite	Serum	IL8	1–500 fg mL^{-1}	1 fg mL^{-1}	-	(Munge et al., 2011)
	8 AuNP array	Serum	IL-6, IL-8, VEGF, and VEGF-C	0-1000 fg mL^{-1}	IL-6, IL-8,-10 and 15 fg mL^{-1} VEGF, and VEGF-C -8 and -60 fg mL^{-1}respectively	50	(Malhotra et al., 2012)
	anti-IL6/SWNTs/graphite	HNSCC cell lines/ Serum	IL-6	0.5–30 pg mL^{-1}	calf serum-0.5 pg mL^{-1}	60	(Malhotra et al., 2010)
Cyclic voltammetry	anti-CYFRA-21–1/ APTES/ZrO_2/ITO	Saliva	Cyfra-21-1	2–16 ng mL^{-1}	0.08 ng mL^{-1}	20	(Kumar et al., 2015)
	BSA/anti-CYFRA-21-1/APTES/ nHfO_2/ITO	Saliva	Cyfra-21-1	2–18 ng mL^{-1}	0.21 ng mL^{-1}	15	(Kumar et al., 2016a)
Electrochemical impedance spectroscopy	BSA/anti-CYFRA-21 –	Saliva	Cyfra-21-1	0–12 ng mL^{-1}	0.24 ng mL^{-1}	35	(Kumar et al., 2018b)

	1/APTES/TiO2/ITO						
	Anti CIP2A/VANTA-IDE	Saliva	CIP2A	1–100 pg mL^{-1}	0.24 pg mL^{-1}	35	(Ding et al., 2018)
Field effect transistor	Array of SiNW sensor	Artificial saliva	IL-8 and TNF-α	1 ng mL^{-1} – 1 fg mL^{-1}	Buffer, saliva -10 and 100 fg mL^{-1} respectively	120	(Zhang et al., 2015)
Fluorescence	Anti-Cyfra-21-1/Al_2O_3-coated 3DN-CNTs	Saliva	Cyfra-21-1	1–1000 ng mL^{-1}	0.5 ng mL^{-1}	30	(Song et al., 2018)
	MFBP coated with QDs	Saliva	CD63	500 to 10^5 particles/μL	500 particles/μL	30	(Wu et al., 2021)
	NGO-BBN-AF750	HSC-3 cell line	GRPR	-	-	40	(Li et al., 2020)
Colorimetric	RB-GNR	OSCC cell line CAL27 and Tca8113	-	2.2 X 10^3 to 30.3 X 10^3 cells/mL	2000 cells/mL	-	(Wang et al., 2013)

	Au NP	-	PKC- α	0-0.2 μg mL^{-1}	-	-	(Kang et al., 2010)
	Au NP	HOC 313 clone 8 and HSC 3 cell lines	-	-	-	-	(El-Sayed et al., 2005)
Modified Immunoassay	Au nanorods; Au nanosphere	Saliva	osteopontin	0.31-20 ng mL^{-1}	AuNRs-0.02 ng mL^{-1}; AuNPs-0.03 ng mL^{-1}	-	(Chakraborty et al., 2018)
Optical coherence tomography (OCT)	AuNP/EGFR	cheek pouch tissues	-	-	-	-	(Kim et al., 2009)
MRI	fol-cht coated magnetic PLGA (fcMagP)	KB oral cancer cells	-	-	-	-	(Shanavas et al., 2017)
Photoacoustic imaging	molecularly activated plasmonic nanosensors (MAPS)	Lymph node tissue	EGFR	-	-	30	(Luke et al., 2014)

Diffusion reflection imaging	AuNRs	OSCC tissue	EGFR	-	-	15	(Ankri et al., 2016)

5. Bench-to-bedside transition of nanosensing platforms: challenges and limitations

Nanotechnology has tremendous promise in the field of oral cancer diagnostics, but so far only a few studies have been translated into commercial prototypes. The biosensing technologies require further fine-tuning to support clinical applications (Singhal et al., 2021). The specificity and sensitivity of a biosensing technique must be evaluated in the form of a receiver operating characteristic (ROC) curve before it can be applied to clinical practices. The efficacy can be represented as an area under the curve (AUC) and a minimum value of 0.8 is essential for successful adoption (Brocklehurst and Speight, 2018). Several bottlenecks such as ethical aspects, low sample requirements, portability, sensitivity, and automation require immediate redressal. This section discusses the challenges involved with each of the nano biosensing methods (Noah and Ndangili, 2019).

The enhancement factors for SERS substrates are not reproducible. Numerous studies have indicated inconsistent results where the same substrate was fabricated and used in multiple laboratories. Furthermore, non-specific binding and interfering species can obscure the SERS signal from the target analyte (Bantz et al., 2011).

In fluorescent nano biosensors, it can be challenging to achieve a lower limit of detection due to the restricted extinction coefficients or quantum yields of organic dyes and a lower ratio of dye-to-reporter molecule labeling (Zhong, 2009).

Colorimetric assays suffer from limited accuracy due to the hindrance caused by the matrix effect of the sample, which makes it difficult to convert minute signals into a colour readout (Yu et al., 2021). Additionally, stability issues due to in high ionic strength of the experimental samples result in non-specific nanoparticle aggregation, triggering associated colour change (Aldewachi et al., 2018).

The primary issue of electrochemical nano biosensors is the miniaturization of the device into a portable system for POC diagnosis (Mani et al., 2021). Other constraints include the complex fabrication

techniques, lack of mechanistic transparency, and stability issues while using single or reusable electrodes (Shandilya et al., 2019).

Several *in vivo* nano-contrast agents are commonly used for sentinel lymph node biopsy, tumor contour imaging, and, enhanced visualization of lesions (Kircher et al., 2003; Kobayashi et al., 2004). It has been reported previously that the contrasting efficiency of MRI nanoprobes such as that of superparamagnetic iron oxide NPs (SPIONs) is affected by the biofouling of the NPs surface due to protein corona formation (Amiri et al., 2013, Gao et al, 2016). Anti-biofouling coatings such as polyethylene glycol (PEG), zwitterions, and fluorinated coatings can suppress the unwanted protein corona formation and enhance the targeting ability of the nanoprobes (Sanchez-Cano et al., 2020). Additionally, arrays of nano-cantilevers, tubes, and wires are emerging as promising tools for the detection of multiple serum markers. A Low-signal-to-noise ratio due to interference from serum matrix limits the applicability of such nano sensing platforms (Ferrari, 2005).

Therefore, a prospective nano biosensor for early diagnosis and monitoring of OC is still in its infancy. Significant research is required to improve them and expand their application from the bench- to-bedside.

6. Conclusion and future work

Diagnostics, along with vaccines and therapeutics, form the essential and often overlooked "third imperative" of reducing any disease burden. Early diagnosis regarding oral carcinoma, can be a real game-changer as it critically determines a patient's quality of life. In this article, we have attempted to cover different diagnostic innovations and methods that are aimed at screening oral carcinoma. Achieving better and more feasible nanosensors that are portable, reusable, and most importantly non-invasive can be highly beneficial for OC detection. Technical modifications of the current nanosensing methods and other future research advances that can change the current oral cancer diagnosis scenario are discussed below (Fig. 14).

- The development of new techniques such as bioplasmonic paper using advanced biomimetic nanoparticles can be easily deployed in resource-limited settings. Gold nanorattles with higher refractive index sensitivity as compared to other anisotropic gold nanostructures can form efficient biodiagnostic platforms (Hu et al., 2019). However mechanical instability of paper,

unidirectional flow of the analyte, and water adsorbing property hinder the transitioning of such systems into efficient lateral flow assays and microfluidic paper-based analytical devices (μPADs). The use of synthetic polymers, such as polymer polydimethylsiloxane (PDMS), conducting polymers (CPs), and molecularly imprinted polymers (MIPs) can solve such issues and can be beneficial in the development of highly sensitive systems for OC diagnosis.

- Nanoparticle-based bio barcode assay wherein magnetic beads and AuNPs are used for ultrasensitive detection of target analyte using minimal sample volume, is a classic example of nanogold mediated diagnostic technology. Magnetic bead ensures analyte separation and barcode DNA conjugated AuNPs serve the purpose of signal amplification in such sensing systems. The use of bimetallic nanomaterials that possess unique peroxidase-like activity that can catalyze sensitive “turn on” fluorescence signals in presence of the target molecule can simplify the overall detection strategy. (Lin et al., 2017)
- Another major technical obstacle is the lower abundance of critical biomarkers during the early stages of OSCC. Lower availability of ctDNA, miRNA, ctRNA, long non-coding RNA (lnRNA), and exosomes in saliva samples have been reported previously (Soda et al., 2019). High-quality pre-isolation and amplification techniques are mandatory prerequisites (Lousada-Fernandez et al., 2018). In this regard, integrated systems that perform sample enrichment and sensing can be highly beneficial in terms of saving time, cost and improving the analytical performance of the nanosensors (Gorgannezhad et al., 2018). Multiplexed systems such as PCR/SERS assays wherein, magnetic beads mediated enrichment of the analyte is performed followed by imaging via SERS nanotag can effectively increase the sensitivity of the detection by 10 folds as compared to commercially available PCR (Wee et al., 2016). Another SERS-based recent advancement that promotes high-throughput analysis is the development of SERS-fluorescence joint spectral encoded (SFJSE) methods. This technique improves the sample encoding capacity by employing a multi-layered nanoprobe containing a magnetic core (for analyte enrichment), metallic nanoparticle (SERS generator), and quantum dots (fluorescence signal)(Wang et al., 2014). Such modified systems can be well adapted for sensitive detection of several diagnostic, prognostic and intra/post-operative biomarkers related to OC.

- Wearable sensors have revolutionized the concept of daily health monitoring and play a crucial role in disease prediction and treatment. Wireless Graphene/silk nanobiosensors have previously shown promising results for remote monitoring of pathogens in saliva(Mannoor et al., 2012). Typical architectural modifications by using similar components can be applied for the fabrication of a mouth-guard type sensing device for recording different types of salivary OC markers.
- Amongst the recent emerging trends is the introduction of AI-based specific behavioral changes for monitoring the health status of an individual. Such detection mechanism works on the core principle of machine learning with advanced AI, nudge theory for behavioral alterations, and social impact bonds (SIB)(Misawa et al., 2020). The advanced method can be utilized for the early prediction of OC in large heterogeneous populations.

Further, saliva-based POC adjuncts are gaining tremendous momentum in recent years. The development of a proper biomarker panel exclusively for oral malignancy can promote the fabrication of several multiplexed bioassay systems. It can be suggested that with a better understanding of the concepts of machine learning and artificial intelligence, as well as their applications in data analysis, precise and accurate determination of oral malignancy at early stages will be possible in near future.

Acknowledgment

We would like to acknowledge the financial support from the CSIR-SRF fellowship grant number (09/844(0101)/2020-EMR-I)

Author Contributions

Debolina Chakraborty and Debayan Ghosh: Conceptualization; writing-original draft; writing-review and editing. **Sanjit Kumar:** Writing-review and editing. **David Jenkins**: Writing-review and editing. **Natarajan Chandrasekaran:** Editing, supervision. **Amitava Mukherjee:** Conceptualization, writing-review, editing, and supervision.

Conflicts of interest

There are no conflicts of interest to declare

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Figure legends

Fig. 1. Schematic representation of the causative factors, stages of disease progression, and government program design for oral malignancy

Fig. 2. (i) Schematic representation of patient's workup for screening oral carcinogenesis. (ii) VELscope VX model, Reproduced from Cicciù, M.,Early diagnosis on oral and potentially oral malignant lesions: a systematic review on the VELscope® fluorescence method. Dentistry journal 7, 93, 2019, MDPI (iii) The use of Identafi optical system on a suspicious lesion showing loss of fluorescence and increase vascularity. (A) Picture of a non-healing ulcer on the right lateral border of the tongue of a 62-year-old male taken with the white reflectance (regular light) Identafi 3000 DentalEZ optical device. (B) Application of the Identafi DentalEZ device with violet fluorescent light shows dark area (loss of auto-fluorescence) in suspicious areas. (C) Application of the Identafi DentalEZ with the green amber reflectance light show increase vascularity in the suspicious areas. A biopsy taken from this area showed a moderately differentiated squamous cell carcinoma. Reproduced from D.V. Messadi, Diagnostic aids for detection of oral precancerous conditions, Int. J. Oral Sci. 5 (2013) 59, Springer Nature.

Fig. 3. The number of PubMed records for terms "nanoparticles for oral cancer detection" from 2007 to 2021

Fig. 4. (i) Represents the schematic illustration of the A) Collective oscillation of the free electrons after illuminating the nanoparticles with the monochromatic light with resonance wavelength, B) The role of electromagnetic and chemical enhancement factor in SERS. Reprinted from (Panikar et al., 2021) with permission from Elsevier (ii). SERS-based analysis using a label-free SERS approach (left) or SERS tags (right). In label-free SERS, the spectroscopic signal results from analyte adsorption onto the SERS substrate, whereas in SERS tags-based specific recognition assays, the spectroscopic signal results from the reporter molecules on the SERS tags (Zhang et al., 2019).

Fig. 5. Schematic diagram of the proposed SERS biosensor for DNA detection. Reprinted from (Liu et al., 2021) with permission from Elsevier

Fig. 6. (i) Physical and chemical properties of AuNPs and schematic illustration of AuNPs (aggregation/dispersion) colorimetric based detection systems. The unique properties of AuNPs, such

as their high absorption coefficient, scattering flux, luminescence and conductivity, as well as their ability to enhance electromagnetic fields, quench (or enhance) fluorescence and catalyse reactions, provide numerous possibilities to exploit these particles for sensing and quantification purposes. Reprinted with permission from (Aldewachi et al., 2018). Royal Society of Chemistry. (ii) Schematic of the GNP-based assay for cancer diagnosis. This system is based on the non-crosslinking aggregation mechanism and a cationic PKC-α- specific substrate peptide is used as a coagulant of citrate-coated GNPs with anionic surface charges. Reprinted from (Kang et al., 2010) with permission from Elsevier.

Fig. 7. Jablonski diagram and a timescale of photophysical processes for organic molecules.(Berezin and Achilefu, 2010)

Fig. 8. Schematic illustration of the 3DN-CNTs sensor for Cyfra 21-1 detection. Reprinted from (Song et al., 2018) with permission from Elsevier.

Fig. 9. Schematic representation of nanoparticle-based electrochemical biosensor

Fig. 10. Synthesis of β-Ag2MoO4 NPs and immunoelectrode fabrication. Reprinted from (Pachauri et al., 2020) with permission from Elsevier.

Fig. 11. Tentative procedure for synthesis of Cys-La(OH)3 NPs and fabrication of BSA/anti-Cyfra-21-1/Cys- La(OH)3/ITO immunoelectrode. Reprinted from (Tiwari et al., 2017) with permission from Elsevier.

Fig. 12. (i) Schematic representation of PLGA nanoparticle contrast agents for advanced MRI detection of oral cancer (ii) Schema representing the various components and working methodology of nanomaterial based photoacoustic imaging of oral cancer tissues

Fig. 13. (i) Amplified signal generation via nano-ELISA method for detection of oral cancer specific biomarker (ii) Graphical representation of nanosensor-based microfluidic approach for sensitive detection of oral cancer biomarker

Fig. 14. Pictorial diagram suggesting the possible futuristic developments in the field of oral cancer sensing.