

## **Salivary Biosensors in Oral Cancer Detection: A Balanced Overview**

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### **Abstract**

A transformative phase in health care is under way with significant leap in clinical research, biosensing technologies, nanoparticles, embedded electronics, cloud-distributed software and services, and artificial intelligence. This current scenario describes the relevance of the term digital health and digital health technologies among the health professionals. Despite of these recent breakthroughs in early detection and diagnosis of oral cancer, it still possesses a major threat especially in developing countries like India with 60,000 new cases reported annually. Early detection and early

treatment has become the motto for cancer diagnosis and the applications of biosensors in this aspect is limitless theoretically. Oral biosensors are basically receptor–transducer devices that uses biological material in the saliva to interact with an analyte embedded with the device and deliver quantitative or semi-quantitative information utilizing a biological recognition element, processed and transferred to any smart devices. They offer real time biomarker monitoring, minimal biomolecule concentration along with high sensitivity and specificity, thus reducing the possibility of false positive and false negative in testing process. This would

in turn enable the opportunity to share clinical data with clinicians could enable better predictions based on innovative algorithms based on big data ultimately better diagnosis, prognosis, and treatment for the patient. This narrative reviews the developments and advancements in different saliva based biosensors used in the early detection of oral cancer. We also explain the methods used by biosensors to target different types of biomarkers and focused on biosensors that function at the molecular level.

**Keywords:** Oral biosensors, Oral cancer, Salivary biomarkers, Microfluidics

### **Introduction**

A transformative phase in health care is under way with significant leap in clinical research, biosensing technologies, nanoparticles, embedded electronics, cloud-distributed software and services, and artificial intelligence. This current scenario describes the relevance of the term digital health and digital health technologies among the health professionals. According to the World Health Organization (WHO), digital health technologies can be defined as: “the field of knowledge and practice associated with the development and use of digital technologies to improve health. Digital health expands the concept of eHealth to include digital consumers, with a wider range of smart and connected devices. It also encompasses other uses of digital technologies for health such as the Internet of Things, advanced computing, big data analytics, artificial intelligence including machine learning, and robotics”<sup>1</sup>. In this context the purpose for a WHO global strategy on Digital Health 2020-2025 focuses to promote healthy lives and wellbeing for everyone, everywhere, at all ages<sup>2</sup>.

Despite of these recent breakthroughs in early detection and diagnosis OC still possess a major threat especially

in developing countries like India. OC accounts for approximately 5% of all cancers globally, while in India it is about 40% of all types of cancer. It is estimated that in India nearly 60,000 new cases of OC are reported annually and over 5 people die every hour everyday indicating the disease is highly fatal in nature<sup>(3)</sup>. According to the data retrieved from most relevant organizations and institutions, in India cancers in lip and oral cavity is ranks first in top three leading cancer with respect to number of cases and number of deaths. In males, OC is the most common cancer with a reported incidence of 1,07,812 cases that constitutes about 15.6% of male population. In females, it is the fourth most common cancer with 35,947 new cases reported that constitutes 5% of the female population. Considering both sexes, OC still ranks second position with a reported new cases of 1,43,759 and 10.2% of the total population<sup>(4)</sup>.

Growth of malignant tissue in the oral cavity is recognized as OC, which usually affects the tongue, floor of the mouth, cheek, gingiva, lips, or palate. The most persistent signal of OC is non-healing sore in the mouth. In early stages more of anatomical symptoms like white or red spots or lesions in the oral mucosa occurs whereas in advanced stages functional symptom pain, persistent sore throat and difficulty eating or swallowing are reported<sup>(5,6)</sup>. The oral symptoms usually begin with a burning sensation in the mouth along with mild restriction in mouth opening and possibly with a white or red lesion that histologically may show dysplastic characteristics. Such precancerous lesions and conditions are categorized under oral potentially malignant disorders (OPMDs). Early identification plays a critical role in disease progression, morbidity and mortality<sup>5-11</sup>.

### **Need For Early Detection of Oral Cancer**

Even though occurring in one of the most accessible anatomical sites of the body, OC can be easily and quickly detected and treated, it still represent a major problem to humankind due to the increasing frequency, as well as serious consequences. Studies reported an average time delay of 9 months from the onset of symptoms to diagnosis, out of which 7 months is attributed to delay in medical pathways. The key reason is need for biopsy to confirm the diagnosis. Most of the population lives in rural areas where access to advanced healthcare infrastructures are limited and these patients do not have access to pathological service and expertise. In such areas it is often referred to larger centers which may be hundreds of miles away. In addition to that it takes around 2 days for a biopsy report<sup>13,14</sup>. All these gets added on to suspected patient's physical and mental hardship to establish a diagnosis. Hence a need for early diagnosis and initiation of treatment is a prerequisite for all cancer suspects<sup>15</sup>.

As suggested by the WHO and the National Institute of Dental and Craniofacial Research(NIDCR), mucosal lesions which persist for two weeks or more after removing possible local irritants should be biopsied, which can be incisional, excisional and incisional biopsies are the most diffused among the two<sup>16,17</sup>. Core-needle aspiration, punch forceps or fine-needle aspiration are recommended for visible lesions, while posteriorly located lesions may require general anesthesia. The drawbacks are biopsy procedure can be extremely stressful, uncomfortable and invasive for the patients; moreover, it involves high costs, time consuming, a high degree of complexity which require clinician training, and can cause infection or damage to nearby tissues. On the contrary, cytological examination of a smear is less invasive but also less sensitive and

specific than biopsy. Modern cytological procedures, such as brush-biopsy and micro biopsy, offered a wealth of opportunities in OC screening. However, still, there is a need for a more accessible version of a test for immediate response<sup>18</sup>. This narrative reviews the developments and advancements in different saliva based biosensors used in the early detection of OSCC. We also explain the methods used by biosensors to target different types of biomarkers and focused on biosensors that function at the molecular level.

### **Biosensors and Oral Cancer**

Personalized medicine and noninvasive diagnosing techniques are gaining wide popularity across the globe. The significant advancement in precision medicine is the integration of biosensors which are receptor–transducer devices that uses biological material to interact with an analyte and deliver quantitative or semi-quantitative information utilizing a biological recognition element. Biosensors are widely used as diagnostic instruments for identifying infections, monitoring and detecting hazardous metabolites, glucose monitoring, cholesterol testing, and vitamin and other nutrient measurements<sup>19,20</sup>. The vision of the future for biosensors even includes chip-scale devices placed on the human body for monitoring vital signs, correcting abnormalities, or even signaling a call for help in an emergency.

Early detection and early treatment has become the motto for cancer diagnosis and the applications of biosensors in this aspect is limitless theoretically. The fact that early cancer detection can improve survival rate and prognosis, necessitates noninvasive, patient friendly diagnostic tools. Because of this researches are carried out worldwide to improvise the practicality of the biosensors in daily life. The term bio refers to the biological element that is been detected. Oral biosensors

are basically receptor–transducer devices that uses biological material in the saliva to interact with an analyte embedded with the device and deliver quantitative or semi-quantitative information utilizing a biological recognition element, processed and transferred to any smart devices<sup>21</sup>.

### **Cornerstones in the Development**

The development of biosensors dates back to 1962 Dr. Leland C. Clark Jr introduced the first enzyme based electrode for glucose detection, marking the birth of modern biosensors<sup>22</sup>. Since then the biosensor technology has rapidly evolved, expanding into various fields including medical diagnostics. In 1970s-1980s exploration of saliva as a diagnostic fluid for biosensors started. Next decade witnessed the electrochemical biosensors that enabled detection of salivary biomarkers in oral health and also introduction of miniaturized sensor components. In early third millennium techniques to incorporate into wearable oral devices like mouth guards, orthodontic appliances have gained popularity. Further the researches are emphasized on the biosensor application to detect cancer biomarkers in saliva. In 2010 nanotechnology experienced a major breakthrough and nanomaterial like gold nanoparticles are graphene enhanced sensor sensitivity and selectivity<sup>23-25</sup>. Further the emergence of optical and piezoelectric biosensors and progress in wireless data transmission in real time monitoring had a great impact in the field of oral biosensors<sup>26</sup>. Recent years with a substantial leap in personalized medicine and point of care devices, development of flexible and compatible biosensors for continuous saliva monitoring and early OC detection is widely accepted. However this technology in dentistry is still developing and with the availability of such devices tests can be rapidly performed within clinical setups.

### **Microfluidics Saliva**

The concept of simple, sensitive and noninvasive techniques for early diagnosis to save more lives gained popularity in the recent COVID-19 pandemics. In addition the real time and portable LOC devices based on saliva as a medium also gained wide popularity. In modern health care systems, saliva is used an ideal specimen since it reflects the physiologic and pathologic state of the body. Studies documented suggests saliva can contain DNA, RNA, exosomes, hormones, mucins, enzymes, secretions from the nasal cavity, serum and blood. It also contains derivatives from the oral ulcers and lesions, microorganisms their products and finally food debris. In addition to being of more convenient, saliva samples does not require any additional action as in blood samples and lower risk of personal contamination urges to move from blood to saliva. Despite of all these the use of saliva based techniques in detection of OC is still needs further refinement and improvement. The usage of saliva as diagnostic and monitoring fluid for OC was extensively studied and pioneered by D.T. Wong and his research group since that moment the field has seen a marked increase in interest among the scientific community<sup>(27,28)</sup>. Prior to any salivary analysis, understanding the human saliva profile is essential to determine if the salivary composition differs due to gender, stimulation, or smoking status. Takeda and co-workers established the composition and concentration of salivary metabolites in a normal human population and how health choices, such as smoking, may affect the metabolic profile<sup>29</sup>. However, there are a number of downsides to using saliva as a diagnostic fluid, the most significant of which is its low specificity and sensitivity. Due to the lack of a consistent quantity of saliva in people, the concentration of analytes might vary substantially depending on when

the sampling/collection method is performed<sup>30</sup>. With the innovations in microfluidics and nanotechnologies, even the lower level of analytes in saliva can be detected, which have enhanced controllability, throughput, reliability and assay speed. Microfluidic based biosensors utilize micro scale fluid flow and nanotechnology principle to detect and quantify biological molecules such as proteins, DNA and cells. They offer real time biomarker monitoring, minimal biomolecule concentration along with high sensitivity and specificity, thus reducing the possibility of false positive and false negative in testing process<sup>31</sup>.

### **Components and Working Principle**

Oral biosensor is a completely equipped analytical device that detects and measures the concentration or activity of a chemical molecule in the salivary sample. The basic principle of biosensor is that it transforms a biological entity like DNA, RNA and proteins into an electrical signal, detected and processed to detect a specific biological analyte. It consists of following components: Analyte, Bio receptors, Transducers and Displays. The material of interest that need to be detected is referred as Analyte. The molecule that primarily recognizes the analyte is known as Bio receptors if it is of natural such as DNA, RNA, enzymes, proteins and peptides. Lab derived synthetic receptors like such as molecular imprinted polymers, C-allotropes, nanomaterial or a combination of both natural and synthetic receptors can also be used. This immobilized probes interact with the analyte and signals are generated. It can be heat, pH, light, charge or mass charge etc. Transducer is the component that converts the bio recognition event into a signal which can be measured easily. This process is referred to as signalization. The signal from transduced analyte-bio receptor interaction and prepares it for display on the

device. Display unit constitutes an interface system that generates measurable values or curves easily comprehensible by the user<sup>32, 36</sup>.

### **Types of Biosensors**

Biosensors used for identifying OC are all proven in studies for their efficiency in detection of early stages of cancer, thus forms a reliable noninvasive method in early detection. Based on the components biosensors can be divided as follows:

#### **1. Based on the bio receptor molecule.**

Bioreceptor is the critical component in a biosensor. Early biosensors used naturally occurring recognition elements. Now a day with the advances in technology and synthetic chemistry, many biosensor recognition elements are synthesized in the laboratory to improve the stability and reproducibility of biosensor function.

#### **A. DNA**

In the development and progression of OC in normal oral epithelium mutations like tumor protein p53 (TP53), epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) plays an important role. TP53 mutations can cause loss of normal p53 protein function which is required for cell cycle regulation and death. HER2 overexpression and mutation will lead to development of OC and also contribute to aggressive illness and bad prognosis. EGFR overexpression and mutation activate downstream signaling pathways promoting survival cell proliferation and migration. Oncogene Ras, promotes cell growth and survival and hence its activation plays an important role in the malignant transformation of cells. On the other hand, tumor suppressor genes which serves as a barrier to cancer formation, the loss of function is a hallmark for oral carcinogenesis. In general, cells with genetic mutation or damaged DNA thus evade normal regulatory processes contributing to carcinogenesis and cancer

progression<sup>(37,38)</sup>. The identification DNA sequence and discreteness of sequence is usually identified is done by DNA based diagnostic methods like restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), polymerase chain reaction (PCR) etc., these current procedures are difficult, time consuming and costly. DNA based biosensors is an innovative method that overcome these issues as they are reliable, affordable, sensitive, cost effective and reduces effort and time. The added benefits are ease of manufacturing and operation procedures and the cheap costs, make it an attractive option for the non-invasive early detection of OC in saliva in mass populations<sup>(39,40)</sup>.

### B. RNA

The cancer causing aberrations produces microRNAs (miRNA) following inactivation of the anti-tumor gene, chromosomal degradation, and gene hyper methylation are considered in RNA based biosensors which can detect cancer even when no physical signs are present. miRNAs as a biomarker for early detection and development of OC was discussed in detail by Osan et al. and reported many key role miRNAs including miRNA-21, miRNA-24, miRNA-31, miRNA-145, miRNA-196b etc. miRNA-21 plays a critical role in perineural invasion in turn associated with poor survival rate in OC patients. miRNA-24 is associated with cell proliferation in OC. Similarly, miRNA-31 is an oncogenic factor and is significantly up regulated in saliva in OC patients<sup>(41-44)</sup>.

The current methodologies for the detection of miRNAs include reverse transcription quantitative PCR (RT-qPCR), droplet digital PCR (ddPCR), and next generation sequencing. Nucleic acid amplification test (NAAT) is an alternative that allows rapid detection without thermo cycling. However all these techniques are complex, expensive and time consuming. In contrast

RNA based biosensors facilitate detection with greater precision and hence aligns with the demand of efficient and timely cancer diagnosis.

### C. Protein biosensors

Protein biosensors for cancer detection measures components that are thought to be indicators of aberrant biological processes, disease processes, or treatment intermediation responses. They make use of electrochemical transducers that provide a sensitive, fast, and low-cost diagnostic framework for detection. In these biosensors the electrode surfaces are modified with synthetic receptors such as antibodies or aptamers. The interactions between an antibody and an antigen operate similar to a lock-and-key binding mechanism that makes the immunosensors are very selective. The presence of protein biomarkers and their expression level in saliva indicates the disease status<sup>21, 44</sup>. Wei et al. pioneered in the development of multiplexed electrochemical sensors for the measurement of salivary biomarkers interleukin-8 (IL-8) (both mRNA and protein) for precise diagnosis of OC<sup>(45)</sup>. The enzyme-linked immunosorbent test (ELISA) is the gold standard approach for clinical biomarker detection for protein analytes with the limits of detection (LOD) 1–3 pg mL. When it comes to POC diagnostics ELISA is not a first option because of the expense of the test kits and equipment, the length of time it takes to measure, and the difficulty of multiplexing. When compared to standard biosensors, aptamer-based biosensors are label-free and high sensitivity for electrochemical detection. Lin et al created a capacitive aptasensor to track the overexpression of the HER2 in oral malignancies<sup>46</sup>. Anti-HER2 aptamers (ssDNA) are immobilized on a gold microelectrode surface and various doses of HER2 were spiked in diluted human serum. The link between capacitance and the HER2 concentration was finally determined<sup>47,48</sup>. Aptamers were

also used to identify the IL-6 protein in which electrochemical impedance spectroscopy (EIS) was used to develop a nano-aptamer sensor to detect IL-6 in bio fluids and 0.02 pg mL was considered to be the detection limit. Furthermore, after two weeks, the aptasensors had 90% of their original impedance response signal, indicating the sensor's great stability<sup>49</sup>.

Yet another type of immunoassays are protein sensors in which recognized element mostly a primary antibody is immobilized on an electrode surface in a sandwich immunoassay to capture the specific analyte. A secondary antibody coupled with an enzyme, such as horseradish peroxidase (HRP), is added to the solution which binds with the antigen. HRP can convert its substrate to electrochemically active species, allowing it to translate chemical signals into electrochemical signals<sup>50</sup>.

Biosensors are further categorised into the following groups based on the signal transduction mechanism: electrochemical, thermal, optical, and nano biosensors.

### 1. Electrochemical transducers

The signals from the transducer are amplified and displayed by an electrical systems and hence referred to as electrochemical biosensors. It shows a higher rate of implementation than other biosensor technologies and can detect practically any type of biomarker and are simple to combine with typical laboratory bench top equipment. Furthermore, because they are easily downsized, they are likely to be integrated into wearable and portable devices. The integration of electrochemical sensors into compact devices enhances the requirements for comfort, convenience, ease of operation, and flexibility, making the creation of dependable, wearable, and portable POC ultrasensitive devices difficult<sup>51-54</sup>. To functionalize the surface of the sensing electrodes, a variety of methods have been explored including

antibodies, magnetic beads, and aptamers. Cyfra21.1 a fragment of cytokeratin-19 that has been extensively researched in saliva using electrochemical biosensors in detection of OC. Jafari et al. in 2020 fabricated an immunosensor wherein anti-Cyfra21.1 is immobilized on a cysteamine (CysA) and glutaraldehyde (GA) modified gold electrode. The device performance was investigated through square wave voltammetry (SWV) and further validation with ELISA test reported a LOD of 2.5 ng/mL and a linear range between 2.5 and 50 ng/MI<sup>(55)</sup>. This platform thus provides a low-cost, reliable, and robust method to perform non-invasive OC detection. Other biomarkers include interleukin-1, Hypoxia inducible factor-1 alpha (HIF-1), Oral Cancer Overexpressed 1 (ORAOV1), TNF- and IL-8 were extensively studied and incorporated in electrochemical platform for biosensors<sup>26,56,57</sup>.

An innovative research by Aydin et al. resulted in the use of 6-phosphonohexanoic acid (PHA) as a biomolecule immobilization matrix to attach anti-IL-1 $\beta$  for selective binding of the analyte and verified through electrochemical impedance spectroscopy it to be a suitable material for specific, reliable, reproducible, repeatable and stable operation. The impedimetric immunosensor reached a LOD of 7.5 fg/mL in a 0.025–3 pg/mL concentration range<sup>(58)</sup>. Yet another potential biomarker for the prognosis of OSCC is HIF-1 $\alpha$ , involved in tumoral hypoxia. Munoz-San Martìn et al. made use of magnetic beads coated with HIF-1 $\alpha$  in a electrochemical assay designed with a sandwich configuration, exhibited a low LOD of 76 pg/mL<sup>59</sup>.

### 2. Optical transducers:

Optical systems provide a new pathway to perform salivary analysis with high sensitivity, selectivity, and label-free operation. Transducers are optical sensors which includes microfluidic channels that ensures the

delivery of biofluid in the area of where the detector/reader is positioned. Some of the recently developed optical biosensors in detection of OC includes fluorescence-based biosensors, surface-enhanced Raman spectroscopy (SERS) biosensors, photonic crystal biosensors and surface plasmon resonance (SPR) biosensors. Among these SERS is versatile and most frequently used due to its ease of operation and multiplexed capability. Liu et al. developed plasmonic Ag nano-cubes (AgNCs) based platform nicking endonuclease assisted signal amplification (NESA) with heated electrodes to enhance SERS signals for the detection of a DNA sequence related to OC<sup>60</sup>. Song et al. developed a fluorescence based immunosensor in which to detect Cyfra21.1, OC biomarker. A three dimensional network of antibody-functionalized carbon nanotubes is created among these Si pillars and, through the use of a specific tag, fluorescence images are obtained<sup>61</sup>. Yet another breakthrough by Dong and Pires, absorbance based microfluidic biosensor for the multiplexed detection of three salivary biomarkers, IL-8, IL-1 $\beta$  and MMP-8 commonly associated with oral cancer. Organic photodetectors (OPDs), have photoactive area on a glass substrate, and vertically aligned with the microfluidic polymeric channel, featuring chambers with antibody-functionalized gold-silver immunoassays are also validated to prove its utility in clinical practice<sup>62</sup>. a fluorescent biosensor based on magnetic and fluorescent bio-probes (MFBPs) loaded with quantum dots (QDs) for an efficient signal amplification was developed by Wu et al. to detect salivary exosomes associated with OC. Specific aptamers were immobilized on the surface of magnetic microspheres (MMs) to selectively bind CD63 proteins on exosomes. This technique reached a lower LOD of 500 particles per microliter of solution<sup>63</sup>.

### **3. Nano biosensors**

Integration of nanotechnology aids in biosensing methods helps in overcoming the restraints like affordability, simplicity of handling, miniaturisation, and the lack of the need for an expert to analyse the data. In this context, gold nanoparticles (AuNPs), quantum dots (QDs), dendrimers, metal oxides, carbon-based nanocomposites, and other nanomaterials (NMs) have been employed to fabricate nanobiosensors. With these functionality has significantly increased as a result of the unique features like quicker detection, improved detection limits, and enhanced repeatability, require a smaller sample volume and offer high precision, specificity, cost-effectiveness and reduced time and ease of implementation for automatic analysis. Nano-based biosensing for the early identification of OC could lead to enhanced patient treatment outcomes and care. Nanotechnology-based miniaturised devices are a breakthrough in pre-clinical and clinical research fields including medication delivery, customised medicine, and diagnostic potential, a field now known as “nanodiagnostics”.

Upcoming version nanoparticles (UCNPs), which display photon upconversion phenomena when activated by incident near-infrared (NIR) light with emission in the visible region of the electromagnetic spectrum, are other prominent types of NMs in biosensing. It has been reported that biocompatible UCNP composites based on fluorescence resonance energy transfer (FRET) may detect OSCC biomarkers using energy changes in the red and blue wavelength ranges. The expression of matrix metalloproteinase 2 (MMP2) in tumour models and OC tissues could be detected by the emission of blue fluorescence when irradiated<sup>44,64,65</sup>.

## Conclusion

This review provides an overview of the most advanced biosensing strategies that have been developed to perform early-stage diagnosis of OC. The importance of saliva as a readily available and continuously renovated biofluid has been stressed in order to stimulate further research on salivary biomarkers, whose timely detection could improve patients' quality of life and their survival. All these materials and process should be thoroughly studied for its applicability to clinical practice. Well-designed clinical trials on diagnostic, predictive and prognostic performance of saliva biomarkers will complete the path to their application in clinical practice. Wearable intraoral bioelectronic platforms and portable POC devices are expected to see an increasing clinical applicability in the near future, as they offer substantial advantages compared to traditional laboratory equipment and procedures. Given that the concentration of salivary biomarkers is typically lower than that in other biofluids, extremely sensitive biosensors are needed to reliably detect the analyte of interest. Limit of detection and sensitivity matches mostly overcome the limits that can be reached with traditional techniques. The integration of miniaturized wireless communication units within these integrated bioelectronic devices is also a key enabler for the future of smart interconnected systems. This would in turn enable the opportunity to share clinical data with clinicians could enable better predictions based on innovative algorithms based on big data ultimately better diagnosis, prognosis, and treatment for the patient. We expect intraoral biosensors to play a pivotal role in continuous real-time data acquisition for the detection of potentially malignant biomarkers related to oral cancer. Among the various types of malignancies, oral cancer is best suited to be tackled with wearable sensors placed in the oral cavity due to the direct contact

of saliva with premalignant or malignant lesions. Therefore, the aim of this review is that of stimulating research in this direction, while considering the requirements of a translational research that should not only offer interesting new research avenues to pursue inside a laboratory, but also find a sustainable and effective way of developing devices that can have a wide applicability in clinical settings.

## References

1. Yeung AWK, 1,2, Torkamani A , Butte AJ, Glicksberg BS , Schuller B, Rodriguez B, Ting DSW, Bates D, Schaden E, Peng H, Willschke H, van der Laak J, Josip Car J,18, Rahimi K, Celi LA, Banach M, Kletecka-Pulker M, Kimberger O, Eils R, Islam SMS, Wong ST, Wong TY, Gao W, Søren Brunak S, Atanasov AG. The promise of digital healthcare technologies. *Frontier public health*.2023 Sep26;11:1196597.
2. World Health Organization (2021). Global strategy on digital health 2020–2025
3. Indian council of medical research, national centre for disease informatics and research. National cancer registry programme: Consolidated report of population based cancer registries 2020-2022. Bengaluru: ICMR-NCDIR;2024.
4. International agency for research on cancer. GLOBOCAN 2024: Global cancer observatory. Lyon, France: IARC;2024
5. Rivera, C. Essentials of oral cancer. *Int. J. Clin. Exp. Pathol.* 2015, 8, 11884–11894.
6. Montero, P.H.; Patel, S.G. Cancer of the oral cavity. *Surg. Oncol. Clin. N. Am.* 2015, 24, 491–508.
7. Li, Q.; Hu, Y.; Zhou, X.; Liu, S.; Han, Q.; Cheng, L. Role of oral bacteria in the development of oral squamous cell carcinoma. *Cancers* 2020, 12, 2797.

8. Panarese, I.; Aquino, G.; Ronchi, A.; Longo, F.; Montella, M.; Cozzolino, I.; Roccuzzo, G.; Colella, G.; Caraglia, M.; Franco, R. Oral and oropharyngeal squamous cell carcinoma: Prognostic and predictive parameters in the etiopathogenetic route. *Expert Rev. Anticancer Ther.* 2019, 19, 105–119.
9. Kumar, M.; Nanavati, R.; Modi, T.G.; Dobariya, C. Oral cancer: Etiology and risk factors: A review. *J. Cancer Res. Ther.* 2016, 12, 458–463.
10. Sasahira, T.; Kirita, T. Hallmarks of cancer-related newly prognostic factors of oral squamous cell carcinoma. *Int. J. Mol. Sci.* 2018, 19, 2413.
11. Bagan, J.; Sarrion, G.; Jimenez, Y. Oral cancer: Clinical features. *Oral Oncol.* 2010, 46, 414–417.
12. Neville, B.; Damm, D.; Allen, C.; Bouquot, J. *Oral and Maxillofacial Pathology*, 3rd ed.; Saunders: St. Louis, MO, USA, 2008; p. 984
13. Qu LG, Brand NR, Chao A, Ilbawi AM. Interventions addressing to delayed cancer Diagnosis in low and middle income countries: a systematic review. *Oncologist.* 2020 Sep;25(9):e1382-e1395.
14. Al-Azri MH. Delay in cancer diagnosis: causes and possible solutions. *Oman Med J.* 2016 Sep;31(5):325-326
15. Sujir N, Ahmed J, Pai K, Denny C, Shenoy N. Challenges in early diagnosis of oral cancer: case series. *Acta StomatolCroat.* 2019 Jun;53(2):174-180.
16. Abati, S.; Bramati, C.; Bondi, S.; Lissoni, A.; Trimarchi, M. Oral cancer and precancer: A narrative review on the relevance of early diagnosis. *Int. J. Environ. Res. Public Health* 2020, 17, 9160.
17. Chakraborty, D.; Natarajan, C.; Mukherjee, A. Advances in oral cancer detection. *Adv. Clin. Chem.* 2019, 91, 181–200.
18. Khurshid, Z.; Zafar, M.S.; Khan, R.S.; Najeeb, S.; Slowey, P.D.; Rehman, I.U. Role of salivary biomarkers in oral cancer detection. *Adv. Clin. Chem.* 2018, 86, 23–70.
19. Turner, A.P.; Karube, I.; Wilson, G.S. *Biosensors Fundamentals and Applications*; Oxford University Press: Oxford, UK, 1987.
20. Kokbas, U.; Kaynn, L.; Tuli, A. Biosensors and their medical applications. *Arch. Med. Rev. J.* 2013, 22, 499–513
21. Goldoni, R.; Scolaro, A.; Boccalari, E.; Dolci, C.; Scarano, A.; Inchingolo, F.; Ravazzani, P.; Muti, P.; Tartaglia, G. Malignancies and Biosensors: A Focus on Oral Cancer Detection through Salivary Biomarkers. *Biosensors* 2021, 11, 396
22. Clark, L.C., Jr.; Lyons, C. Electrode systems for continuous monitoring in cardiovascular surgery. *Ann. N. Y. Acad. Sci.* 1962, 102, 29–45.
23. Ilkhani, H.; Sarparast, M.; Noori, A.; Bathaie, S.Z.; Mousavi, M.F. Electrochemical aptamer/antibody based sandwich immunosensor for the detection of EGFR, a cancer biomarker, using gold nanoparticles as a signaling probe. *Biosens. Bioelectron.* 2015, 74, 491–497
24. Kumar, S.; Sharma, J.G.; Maji, S.; Malhotra, B.D. Nanostructured zirconia decorated reduced graphene oxide based efficient biosensing platform for non-invasive oral cancer detection. *Biosens. Bioelectron.* 2016, 78, 497–504
25. Goldoni, R.; Farronato, M.; Connelly, S.T.; Tartaglia, G.M.; Yeo, W.-H. Recent advances in graphene-based nanobiosensors for salivary biomarker detection. *Biosens. Bioelectron.* 2021, 171, 112723
26. Tan, W.; Sabet, L.; Li, Y.; Yu, T.; Klokkevold, P.R.; Wong, D.T.; Ho, C.-M. Optical protein sensor for

- detecting cancer markers in saliva. *Biosens. Bioelectron.* 2008, 24, 266–271.
27. Wong, D. Salivary diagnostics. *Oper. Dent.* 2012, 37, 562–570.
28. Wong, D.T. Salivary diagnostics powered by nanotechnologies, proteomics and genomics. *J. Am. Dent. Assoc.* 2006, 137, 313–321.
29. Takeda, I.; Stretch, C.; Barnaby, P.; Bhatnager, K.; Rankin, K.; Fu, H.; Weljie, A.; Jha, N.; Slupsky, C. Understanding the human salivary metabolome. *NMR Biomed.* 2009, 22, 577–584.
30. Kaczor-Urbanowicz, K.E.; Martin Carreras-Presas, C.; Aro, K.; Tu, M.; Garcia-Godoy, F.; Wong, D.T. Saliva diagnostics - Current views and directions. *Exp. Biol. Med.* 2017, 242, 459–472
31. Kumari M, Gupta V, Kumar N, Arun RK. Microfluidics based nanobiosensors for healthcare monitoring. *Mol biotechnol.* 2024 Mar;66(3):378-401.
32. Bhalla N, Jolly P, Formisano N, Estrela P. Introduction to biosensors. *Essays Biochem.* 2016 Jun 30;60(1):1-8.
33. Naresh V, Lee N. A review on biosensors and recent development of nanostructured materials-Enabled biosensors. *Sensors (Basel).* 2021 Feb5;21(4):1109.
34. Arnold, M.A.; Meyerhoff, M.E. Recent advances in the development and analytical applications of biosensing probes. *Crit. Rev. Anal. Chem* 1988, 20, 149–196.
35. Bohunicky, B.; Mousa, S.A. Biosensors: The new wave in cancer diagnosis. *Nanotechnol. Sci. Appl.* 2011, 4, 1–10.
36. Perumal, V.; Hashim, U. Advances in Biosensors: Principle, architecture and applications. *J. Appl. Biomed.* 2014, 12, 1–15
37. Ali J, Sabiha B, Jan HU, Haider SA, Khan AA, Ali SS. Genetic etiology of oral cancer. *Oral Oncol.* 2017 Jul;70:23-28.
38. Riedel, F.; Zaiss, I.; Herzog, D.; Götte, K.; Naim, R.; Hörmann, K. Serum levels of interleukin-6 in patients with primary head and neck squamous cell carcinoma. *Anticancer Res.* 2005, 25, 2761–2766
39. Ilkhani, H.; Sarparast, M.; Noori, A.; Bathaie, S.Z.; Mousavi, M.F. Electrochemical aptamer/antibody based sandwich immunosensor for the detection of EGFR, a cancer biomarker, using gold nanoparticles as a signaling probe. *Biosens. Bioelectron.* 2015, 74, 491–497.
40. Tan, Y.; Wei, X.; Zhao, M.; Qiu, B.; Guo, L.; Lin, Z.; Yang, H.H. Ultrasensitive homogeneous electrochemical biosensor for DNA species related to oral cancer based on nicking endonuclease assisted target recycling amplification. *Anal. Chem.* 2015, 87, 9204–9208.
41. Cheng YS, Jordan L, Rees T, Chen HS, Oxford L, Brinkmann O, Wong D. Levels of potential oral cancer salivary mRNA biomarkers in oral cancer patients in remission and oral lichen planus patients. *Clin Oral Investig.* 2014 Apr;18(3):985-93
42. Wang J, Lv N, Lu X, Yuan R, Chen Z, Yu J. Diagnostic and therapeutic role of microRNAs in oral cancer. *Oncol Rep.* 2021 Jan;45(1):58-64
43. Osan C, Chira S, Nutu A M, Braicu C, Bacuit M, Korban SS, Berinndan-Neagoe I. the connection between MicroRNAs and oral cancer pathogenesis: emerging biomarkers in oral cancer management. *Genes(Basel).* 2021 Dec 15;12(12):1989.
44. Umopathy, V.R.; Natarajan, P.M.; Swamikannu, B.; Moses, J.; Jones, S.; Chandran, M.P.; Anbumozhi, M.K. Emerging Biosensors for Oral Cancer

- Detection and Diagnosis—A Review Unravelling Their Role in Past and Present Advancements in the Field of Early Diagnosis. *Biosensors* 2022, 12, 498.
45. Wei, F.; Liao, W.; Xu, Z.; Yang, Y.; Wong, D.T.; Ho, C.M. Bio/Abiotic interface constructed from nanoscale DNA dendrimer and conducting polymer for ultrasensitive biomolecular diagnosis. *Small* 2009, 5, 1784–1790.
46. Lin, T.E.; Chen, W.H.; Shiang, Y.C.; Huang, C.C.; Chang, H.T. Colorimetric detection of platelet-derived growth factors through competitive interactions between proteins and functional gold nanoparticles. *Biosens. Bioelectron.* 2011, 29, 204–209.
47. Slamon, D.J.; Godolphin, W.; Jones, L.A.; Holt, J.A.; Wong, S.G.; Keith, D.E.; Levin, W.J.; Stuart, S.G.; Udove, J.; Ullrich, A.; et al. Studies of the HER-2/Neu proto-oncogene in human breast and ovarian cancer. *Science* 1989, 244, 707–712.
48. Qureshi, A.; Gurbuz, Y.; Niazi, J.H. Label-free capacitance based aptasensor platform for the detection of HER2/ErbB2 cancer biomarker in serum. *Sens. Actuators B Chem.* 2015, 220, 1145–1151.
49. Malhotra, R.; Patel, V.; Vaqué, J.P.; Gutkind, J.S.; Rusling, J.F. Ultrasensitive electrochemical immunosensor for oral cancer biomarker IL-6 using carbon nanotube forest electrodes and multilabel amplification. *Anal. Chem.* 2010, 82, 3118–3123.
50. Zhang, Y.; Chen, R.; Xu, L.; Ning, Y.; Xie, S.; Zhang, G.J. Silicon nanowire biosensor for highly sensitive and multiplexed detection of oral squamous cell carcinoma biomarkers in saliva. *Anal. Sci.* 2015, 31, 73–78.
51. Bandodkar, A.J.; Wang, J. Non-invasive wearable electrochemical sensors: A review. *Trends Biotechnol.* 2014, 32, 363–371.
52. Windmiller, J.R.; Wang, J. Wearable electrochemical sensors and biosensors: A review. *Electroanalysis* 2013, 25, 29–46.
53. Bandodkar, A.J.; Jia, W.; Wang, J. Tattoo-based wearable electrochemical devices: A review. *Electroanalysis* 2015, 27, 562–572.
54. Tu, J.; Torrente-Rodríguez, R.M.; Wang, M.; Gao, W. The era of digital health: A review of portable and wearable affinity biosensors. *Adv. Funct. Mater.* 2020, 30, 1906713.
55. Jafari, M.; Hasanzadeh, M. Non-invasive bioassay of cytokeratin Fragment 21.1 (Cyfra 21.1) protein in human saliva samples using immunoreaction method: An efficient platform for early-stage diagnosis of oral cancer based on biomedicine. *Biomed. Pharmacother.* 2020, 131, 110671.
56. Aydın, E.B.; Aydın, M.; Sezginürk, M.K. A highly sensitive immunosensor based on ITO thin films covered by a new semiconductive conjugated polymer for the determination of TNF $\alpha$  in human saliva and serum samples. *Biosens. Bioelectron.* 2017, 97, 169–176.
57. Verma, S.; Singh, A.; Shukla, A.; Kaswan, J.; Arora, K.; Ramirez-Vick, J.; Singh, P.; Singh, S.P. Anti-IL8/AuNPs-rGO/ITO as an immunosensing platform for noninvasive electrochemical detection of oral cancer. *ACS Appl. Mater. Interfaces* 2017, 9, 27462–27474.
58. Aydın, E.B.; Sezginürk, M.K. A disposable and ultrasensitive ITO based biosensor modified by 6-phosphonohexanoic acid for electrochemical sensing of IL-1 $\beta$  in human serum and saliva. *Anal. Chim. Acta* 2018, 1039, 41–50.

59. Muñoz-San Martín, C.; Gamella, M.; Pedrero, M.; Montero-Calle, A.; Barderas, R.; Campuzano, S.; Pingarrón, J.M. Magnetic beads based electrochemical immunosensing of HIF-1 $\alpha$ , a biomarker of tumoral hypoxia. *Sens. Actuators B Chem.* 2020, using electro-click chemistry. Application to the detection of IL-1 $\beta$  cytokine in saliva. *Bioelectrochemistry* 2020, 133, 107484.
60. Liu, Y.; Wu, S.-H.; Du, X.-Y.; Sun, J.-J. Plasmonic Ag nanocube enhanced SERS biosensor for sensitive detection of oral cancer DNA based on nicking endonuclease signal amplification and heated electrode. *Sens. Actuators B Chem.* 2021, 338, 129854.
61. Song, C.K.; Oh, E.; Kang, M.S.; Shin, B.S.; Han, S.Y.; Jung, M.; Lee, E.S.; Yoon, S.-Y.; Sung, M.M.; Ng, W.B.; et al. Fluorescence-based immunosensor using three-dimensional CNT network structure for sensitive and reproducible detection of oral squamous cell carcinoma biomarker. *Anal. Chim. Acta* 2018, 1027, 101–108
62. Dong, T.; Pires, N.M.M. Immunodetection of salivary biomarkers by an optical microfluidic biosensor with polyethyleniminemodified polythiophene-C70 organic photodetectors. *Biosens. Bioelectron.* 2017, 94, 321–327.
63. Wu, M.; Chen, Z.; Xie, Q.; Xiao, B.; Zhou, G.; Chen, G.; Bian, Z. One-step quantification of salivary exosomes based on combined aptamer recognition and quantum dot signal amplification. *Biosens. Bioelectron.* 2021, 171, 112733\
64. Chan, Y.-C.; Chan, M.-H.; Chen, C.-W.; Liu, R.-S.; Hsiao, M.; Tsai, D.P. Near-infrared-activated fluorescence resonance energy transfer-based nanocomposite to sense MMP2-overexpressing oral cancer cells. *ACS Omega* 2018, 3, 1627–1634.
65. Guerrero, S.; Agüí, L.; Yáñez-Sedeño, P.; Pingarrón, J.M. Design of electrochemical immunosensors