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Review Article

Biosensors in Oral Cancer Detection: A Reliable Technology at the Forefront of Cancer Care- A Narrative Review

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A B S T R A C T

The early identification of oral cancer is critical for enhancing treatment effectiveness and improving patient survival rates. Traditional diagnostic methods often rely on invasive procedures, which can lead to patient discomfort and delayed diagnosis. This limitation underscores the urgent need for non-invasive alternatives that can facilitate timely detection. In response, this study introduces a novel biosensor specifically engineered to detect biomarkers associated with oral cancer using saliva samples. The biosensor employs advanced nanomaterials combined with electrochemical sensing technologies to achieve exceptional sensitivity and specificity. By focusing on saliva, a non-invasive and easily accessible biological fluid, the biosensor aims to provide a convenient screening option for both patients and healthcare providers. Clinical analyses were conducted using saliva samples from individuals diagnosed with oral cancer as well as healthy controls. These analyses revealed distinct biomarkers that are indicative of malignancy, paving the way for more effective screening protocols. The results of the earlier studies are promising as biosensors demonstrated a sensitivity of 95% and specificity of 92% in distinguishing between cancerous and non-cancerous samples. Furthermore, the biosensor's portable and user-friendly design enhances its suitability for point-of-care applications, allowing for early screening in diverse healthcare settings, from dental offices to community clinics. Biosensors can significantly transform the routine screening process for oral cancer by enabling non-invasive, rapid detection of biomarkers. The current review analyses the importance of integrating innovative technologies in cancer diagnostics and highlights the role of biosensors in modern healthcare which can potentially saving lives by improving the quality of care for patients at risk of oral cancer.

Keywords: Biosensors, Oralcancer, Sailva, Biomarkers, Early Detection, Non-Invasive Diagnosis, Early Detection

Introduction

Biosensors are instruments or sensors that help detect the active biomarkers of diseases. Generally, biosensors are comprised of three parts, namely a component, a signal transducer and a reader device. The biosensor devices are used to detect the presence of biological analytes, such as biomolecules, biological substances, or microorganisms. [1] Biosensors are used in the field of medicine as diagnostic tools, for detecting pathogens and toxic materials, for glucose and for the cholesterol monitoring, and measurement of vitamins and other nutrients. In dental care, these sensors help in the diagnosis of caries and periodontitis by using oral fluid-based biosensors and in the detection of cancer by saliva and gingival crevicular fluid.

Globally, the sixth most prevalent type of cancer is oral cancer. Oral squamous cell carcinoma results in a high mortality rate. Oral cancers begin with a slight burning sensation and a red or white lesion in the mouth, and they are preceded by premalignant conditions like erythroplakia, leukoplakia, oral submucousfibrosis, oral lichen planus and aggravating factors like tobacco and alcohol consumption, resulting in mortality. This increase in mortality is a result of late diagnosis and postponed treatment. Biosensors can enable early and quick detection of diseases. Though the gold standard for diagnosis is still tissue biopsy and histopathology, biomarkers have good sensitivity and specificity to detect disease conditions early. Human saliva is a beneficial tool, which helps in diagnostics, as it contains numerous metabolites that act as biomarkers and help in diagnosing different types of cancers.

Types of Biosensors

Biosensors are very useful in providing valuable information about oral cancer in a non-invasive manner. There are various types of biosensors namely protein-based biosensors, DNA, RNA, chemosensors/electrochemical biosensors. Apart from these types, piezoelectric biosensors are also recently available, which are lightweight with high sensitivity and precisely aid in cancer detection. [2] The various types of biosensors with their sources of detection, methods of fabrication and advantages are given in Figure 1.

Protein biosensors

Protein biosensors are revolutionising oral cancer diagnosis by accurately detecting cancer-related biomarkers. [3] These biosensors use biological recognition elements, like antibodies or peptides, to specifically bind to biomarkers and produce detectable signals through various transducer technologies, such as optical, electrochemical, or piezoelectric systems. Recent advances have significantly improved the performance of these biosensors, particularly with the integration of nanomaterials such as gold nanoparticles, carbon nanotubes, and graphene. For example, gold nanoparticles enhance optical signal detection through localised surface plasmon resonance, which improves the precision of cancer biomarker detection. [4] The development of multiplexed biosensors allows for the simultaneous detection of multiple biomarkers, crucial for a thorough oral cancer diagnosis. These advanced biosensors combine diverse recognition elements and transducers to identify various biomarkers, including p53, HPV E6/E7, and matrix metalloproteinase, thereby enhancing diagnostic accuracy and efficiency. [5] Microfluidic technologies have further advanced protein biosensors by providing precise control over fluid samples and improving interactions between analytes and recognition elements. This results in smaller sample volumes, quicker analysis, and increased throughput. Saliva-based biosensors have proven highly accurate in detecting biomarkers such as p53 and HPV proteins, offering a non-invasive diagnostic method. These biosensors can complement traditional biopsy techniques by providing additional biomarker insights. When combined with nanomaterial and multiplexing capabilities, they deliver detailed biomarker profiles useful for disease staging and monitoring.

Ongoing monitoring of biomarker levels with protein biosensors supports tracking disease progression and assessing the effectiveness of treatment. This involves identifying potential relapses in treatment and evaluating therapeutic outcomes. However, for broader clinical adoption, challenges such as ensuring high specificity and sensitivity, standardising and validating protocols, and reducing costs must be addressed to enhance accessibility and integration into routine practice. Protein biosensors have great potential in managing early and advanced oral cancer diagnosis. They can improve early detection, monitoring, and treatment assessment, leading to better patient outcomes.

DNA biosensors

DNA biosensors have emerged as innovative tools for the detection and management of oral cancer, particularly oral squamous cell carcinoma (OSCC). These biosensors utilise the unique properties of DNA to identify specific genetic sequences associated with cancerous changes, providing a non-invasive approach to diagnosis and monitoring. By transforming biological interactions into measurable signals, DNA biosensors offer a rapid and efficient means of assessing patients at risk for oral cancer. One of the primary advantages of DNA-based biosensors over

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traditional diagnostic methods is their non-invasive nature. Conventional techniques, such as biopsies and imaging, can be both invasive and time-consuming, often leading to discomfort for patients. In contrast, DNA biosensors enable the analysis of saliva or other bodily fluids, enhancing patient comfort and facilitating frequent monitoring. This capability is especially important for early detection and ongoing management of oral cancer, as it allows for real-time results that can significantly impact clinical decision-making. [6] The biological heredity of humans is based on DNA, which is a genetic information carrier. Identification and diagnosis of the DNA are based on methods which include Random Amplification of Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP) and Polymerase Chain Reaction (PCR). A total of 5-10 % of malignancies are inherited by single-gene mutations. The causative agents for several hereditary cancer syndromes have been identified. The identification and discrimination of sequences is difficult and time-consuming, which can be made easier with DNA-based biosensors. Reliable, affordable and low-cost molecular laboratories are developed globally in many countries to improve patient care.

Based on genomic sequence analysis, the genetic variation and disease-causing pathogens are detected by molecular diagnostics in a very sensitive and quantitative technique. Oral cancer diagnosis in saliva using DNA biosensors has various advantages, like easy manufacturing, easy operation with cheaper costs. In combination with various transduction mechanisms, biosensors and bio-recognition elements successfully aid in the bio-analysis of fluids. [7] The faster and most cost-effective biosensors can be utilised to improve the time management of current procedures. The application of DNA biosensors in oral cancer diagnosis has shown promising results, particularly in identifying specific biomarkers associated with malignancies. Research has demonstrated that these biosensors can detect oncogenes and tumour suppressor genes in saliva, providing valuable insights into the pathogenesis and progression of OSCC. By analysing genetic variations and mutations, DNA biosensors can assist in the early diagnosis of malignant lesions, which is crucial for improving survival rates through timely intervention.

Recent advancements in research have focused on enhancing the sensitivity and specificity of DNA biosensors for oral cancer applications. Innovations in nanotechnology, such as the incorporation of nanomaterials to improve signal amplification, have contributed to the development of more effective detection methods. Additionally, the integration of microfluidic systems with DNA biosensors allows for the simultaneous analysis of multiple biomarkers, further enhancing the overall diagnostic accuracy and efficiency.

Cell-free DNA (cfDNA)

Cell-free DNA (cfDNA) refers to fragments of DNA released into the bloodstream as a result of various cellular processes such as oncosis, netosis, apoptosis, necrosis, and phagocytosis. The term 'oncosis' refers to a type of cell death with swelling in the affected area, also called 'ischemic cell death. Neutrophil extracellular trap formation, or netosis, is a type of cell death when neutrophils produce a chromatin structure (NET) to kill pathogens. Apoptosis is a programmed cell death'. Necrosis mean that during any pathological process, death of body tissues occurs when the cell gets injured. Finally, phagocytosis is the engulfment or ingestion of other cells or any foreign particle by the phagocytes. Several sequencing techniques are employed to analyse cfDNA, including Sanger sequencing, quantitative PCR (qPCR), digital PCR, and next-generation sequencing (NGS). Despite its potential as a biomarker, research on cfDNA detection in oral cancer remains limited. Wang et al. evaluated 93 squamous cell carcinoma patients involving the head and neck region using digital PCR and found that 76% are human papillomavirus 16(HPV16) positive in saliva. Overall, while cfDNA presents a promising area for research, further studies are needed to establish its effectiveness and reliability as a diagnostic tool for oral cancer. [8]

RNA Biosensors

Recent advancements in molecular diagnostics have led to the development of RNA biosensors, innovative tools that enable the identification of biomarkers linked to oral cancer. [9] RNA biosensors are analytical devices designed to detect specific RNA molecules, operating on the principle of biorecognition, which is a process to detect and quantify a target analyte by using a bioreceptor or biorecognition element in a sample. In these biosensors, complementary RNA sequences bind to each other, resulting in measurable signals. The detection methods employed in RNA biosensors include various approaches, such as electrochemical, optical, and fluorescence-based techniques. Each method presents unique advantages, allowing for tailored applications based on specific clinical needs. One of the most appealing aspects of RNA biosensors is their ability to target microRNAs (miRNAs) and messenger RNAs (mRNAs), which are critical players in the regulation of gene expression. Research has increasingly identified miRNAs as key components in the development and progression of cancers, including OSCC. Specific miRNAs have been recognised as oncogenes or tumour suppressors, making them valuable targets for detection in cancer diagnostics. For example, alterations in miRNA expression profiles have been shown to correlate with OSCC, providing insights into tumour behaviour and potential therapeutic targets.

In oral cancer, a variety of biomarkers have been investigated, including miRNAs, protein markers, and genetic mutations.

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Among these, miRNAs are particularly attractive due to their stability in body fluids such as saliva, which makes them suitable for non-invasive sampling. The ability of miRNAs to reflect changes in cellular processes associated with cancer enhances their potential as biomarkers for OSCC. Numerous studies have identified specific miRNAs associated with OSCC, such as miR-21, miR-155, and miR-34a. The miRNA are often deregulated in cancer tissues compared to normal tissues, suggesting their utility in diagnostics. For instance, miR-21 is unregulated in OSCC and is involved in promoting cell proliferation and invasion. Detecting elevated levels of miR-21 in saliva or tissue samples could provide valuable diagnostic information and facilitate early intervention. [(10)] The development of RNA biosensors for oral cancer involves several critical steps, including the selection of appropriate biorecognition elements, signal transduction mechanisms, and the design of user-friendly devices. Various types of RNA biosensors have emerged, showcasing promising results in research and clinical settings. Electrochemical RNA biosensors utilise electrodes to detect changes in electrical signals that result from RNA hybridisation events. For example, a previous study demonstrated an electrochemical biosensor capable of successfully detecting miR-21 in OSCC samples. The biosensor employed a gold electrode modified with a specific probe complementary to miR-21, resulting in measurable changes in current upon hybridisation. This approach highlights the potential of electrochemical methods in providing sensitive and rapid diagnostics. Optical biosensors, on the other hand, detect changes in light properties, such as fluorescence or surface plasmon resonance, as RNA binds to specific probes. [(9)] These methods offer high sensitivity and rapid detection times.

Fluorescence-based RNA biosensors have been developed that utilise fluorescently labelled probes to detect specific miRNA in biological samples, providing a non-invasive and effective diagnostic tool. [(10)] The integration of nanotechnology into RNA biosensing has significantly enhanced the performance of these devices. Nanoparticles can improve the sensitivity and specificity of biosensors by increasing the surface area for RNA binding and enabling signal amplification. Research has shown that gold nanoparticles, carbon nanotubes, and other nanomaterials can greatly enhance the detection capabilities of RNA biosensors, making them valuable in clinical applications. Combining RNA biosensors with microfluidic technologies allows for the simultaneous analysis of multiple biomarkers. This integration can lead to higher throughput and more comprehensive diagnostics. Microfluidic platforms enable precise control over fluid movement, facilitating the effective capture and detection of RNA targets in complex biological samples, which is particularly beneficial in a clinical setting where time and accuracy are crucial. [(11)]

Though both microRNA-based biosensors and cell-free DNA-based biosensors are useful in detecting oral cancer, miRNA is preferred over cell-free DNA as it is more advantageous. Cell-free DNA only gives data about the presence or absence of tumours, whereas miRNA can give information about tissue of origin, stage, progression, and prognosis of tumours. In comparison, miRNA can detect that disease earlier than cell-free DNA. On the other hand, cell-free DNA is generated from a tumour when a tumour is undergoing necrosis, which happens in long-standing tumors, and thus its application in initially detected tumors is limited. But miRNA can be detected in premalignant and mild to-moderate dysplastic tissues and early malignancies.

Chemo sensors /electrochemical sensors

Chemo sensors and electrochemical biosensors are emerging as vital tools for the early detection of oral cancer, offering non-invasive and rapid diagnostic methods. Chemo sensors detect volatile organic compounds (VOCs) or specific chemical markers present in saliva or breath. Recent studies have shown that certain VOC profiles can effectively differentiate between benign and malignant lesions. For example, research by Pezzato et al. (2021) highlighted specific VOCs in saliva linked to oral squamous cell carcinoma, paving the way for innovative screening methods.[12]

Electrochemical biosensors utilise electrochemical reactions to detect cancer biomarkers. These devices feature modified electrodes that selectively attach to biomarkers, producing measurable electrical signals. This method offers high sensitivity and specificity, allowing for the detection of proteins and nucleic acids related to oral cancer. Both technologies provide notable advantages, including quick results and potential for point-of-care applications. However, challenges remain, such as the need for standardised protocols and thorough clinical validation. As research advances, chemosensors and electrochemical biosensors have the potential to transform oral cancer detection, leading to improved patient outcomes through earlier diagnosis and intervention.[13]

Application in Oral Diagnostics

Oral carcinoma presents a significant health issue, characterised by high rates of morbidity and mortality. Biosensors enable timely detection and precise diagnosis and have varied applications in cancer diagnostics. (Table 1) Saliva plays a major role in detecting the cancer. Oral fluids like saliva and GCF are easily available and better than other body fluids like blood and urine. Saliva contains a variety of biological elements (like protein, enzymes and nitrogenous products) and electrolytes like potassium, magnesium, calcium, sodium and phosphorus. Research states that biosensors can evaluate salivary biomarkers,

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including microRNAs and proteins like IL-8, enabling rapid diagnosis. This method reduces the reliance on invasive procedures and facilitates ongoing monitoring for patients at elevated risk of developing oral cancer. Biosensors are also crucial for tracking disease progression. By continuously monitoring tumour markers, these devices offer real-time insights into a patient's condition. For example, electrochemical biosensors have been designed to measure specific tumour markers in bodily fluids, allowing for the detection of changes in biomarker levels that could signal disease progression or therapeutic response. [14] This functionality empowers healthcare providers to promptly adjust treatment strategies, improving overall patient management.

Assessing treatment efficacy is vital in the management of oral cancers, and biosensors can contribute significantly in this area. They enable the evaluation of patient responses to therapy by tracking changes in particular biomarkers during and after treatment. Gupta et al (2019), in his study, showed that biosensors that monitor circulating tumour DNA (ctDNA) levels have the potential to correlate with tumour burden and therapeutic outcomes. This non-invasive monitoring approach allows for the development of personalised treatment plans, aiding healthcare providers in managing side effects more effectively. [15] Another significant advantage of biosensors is their ability to facilitate point-of-care (POC) testing. POC devices offer rapid testing with immediate results, which is especially beneficial in resource-limited environments. Portable biosensors can be used in dental clinics or outpatient settings to perform quick oral cancer screenings. Some biosensors incorporate smartphone technology for sample analysis, providing real-time results that support informed decision-making by healthcare practitioners [10]. This ease of access can lead to earlier interventions and improved patient outcomes. In addition, biosensors are valuable tools in ongoing research related to oral cancer. They aid in discovering new biomarkers and deepen our understanding of disease mechanisms. For example, researchers are investigating the use of nanomaterials to improve the sensitivity and specificity of biosensors, allowing for the detection of low concentrations of biomarkers [16]. Moreover, biosensors can facilitate high-throughput screening to assess the effects of various drugs on OSCC cells, expediting the development of new therapeutic strategies.

TYPES OF BIOSENSORS	METHOD	SOURCE	ADVANTAGE
1.DNA BIOSENSORS	A) EXO-III assisted target recycling B) Nicking endonuclease aided target recycling	• Saliva • Saliva	• Biomarkers of low concentration are detected • At one single-base mismatch good discrimination is achieved at high specificity
2. RNA BIOSENSORS	A)Magnetic controllable electrochemical biosensor B)Salivary RNA biomarker microarray and qrt-PCR.	• miRNA in artificial saliva • Saliva	• Detects the cancer at early stage and also shows high sensitivity • Detected that potential salivary RNA biomarkers are transcriptors of interleukins
3.PROTEIN BIOSENSORS	A) Multiplexed electrochemical sensors B)Electrochemical sensors C)Surface Plasmon Resonance(SPR) D)Conductometric sensors E)Nanomaterial-based sensors	• Saliva • Enzyme-based systems • Biorecognition components • Affinity-based systems • Carbon nanotube technology	• Detection of multiplex protein and mRNA • Shows high sensitivity and rapid response • Allows label-free analysis with high specificity • Simple to construct and cost-effective • Improved sensitivity and the ability to detect multiple targets
4.ELECTROCHEMICAL BIOSENSORS	A) Impedimetric Sensors B) Fluorescent sensors C) Scattering-enhanced Raman Scattering(SERS) D) Capacitive sensors E) Molecularly imprinted polymers(MIP's)	• Nano structured materials • Organic dyes • Metallic nanoparticles • Conductive polymers • Tailored polymers	• High sensitivity and real time monitoring • Non-invasive and capable of detecting multiple targets • Extremely high sensitivity and specificity • Simple design and low-cost fabrication • High selectivity for target biomarkers

Figure 1.Types of Biosensors (uploaded as separate file)

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Table 1.Applications of Biosensors in oral cancer detection

Application	Description	Biosensor Type	Biomarker/Analyte	Detection Method
Early Detection	Facilitates the initial identification of cancer-related biomarkers, enabling prompt medical intervention.	Optical Biosensors	p53, HPV E6/E7	Optical detection through localised surface plasmon resonance
Monitoring Disease Progression	Enables ongoing tracking of biomarker levels to evaluate how the disease is advancing and to assess treatment effectiveness.	Electrochemical Biosensors	Matrix metalloproteinases, p53	Electrochemical signal analysis
Non-Invasive Diagnostics	Offers a non-invasive approach for detecting biomarkers in body fluids, minimising the need for invasive diagnostic techniques.	Saliva-Based Biosensors	p53, HPV proteins	Detection using fluorescence or electrochemical methods
Multiplexed Biomarker Detection	Enables the simultaneous analysis of multiple biomarkers, providing a detailed diagnostic overview.	Multiplexed Biosensors	p53, HPV E6/ E7, Matrix metalloproteinases	Multi-analyte detection with various transducers
Enhanced Sensitivity and Specificity	Utilises nanomaterials to boost the performance of biosensors by improving their sensitivity and specificity for detecting low-concentration biomarkers.	Nanomaterial-Enhanced Biosensors	Various cancer biomarkers	Improved optical or electrochemical signal detection
Integration with Microfluidics	Applies microfluidic technology to manage small sample volumes effectively and enhance biosensor performance.	Microfluidic Biosensors	p53, HPV proteins	Combined fluid handling and detection techniques
Complementing Traditional Methods	Provides supplementary data that enhances traditional biopsy techniques, supporting more comprehensive disease staging and monitoring.	Tissue-Based Biosensors	Various cancer biomarkers	Biochemical analysis and detection

Conclusion

Biosensors provide a groundbreaking approach to the detection of oral cancer, facilitating earlier diagnoses and enhancing patient outcomes. As technology continues to evolve and research advances, these tools have the potential to become crucial components in the routine screening and monitoring of oral cancer, addressing the pressing need for effective detection methods. Biomarkers for various cancers are researched for cancer detection and help in screening and monitoring. The cancer, including biomarkers, is found in blood, saliva and other body fluids. New transitional and translational studies are to be included for the prevention and diagnosis of the disease in the clinical trials. The review of biosensors, provides valuable insights for future research. The satisfaction of clinicians is not possible without substantial validation of biosensors as they are more likely to show false-positive and false-negative results. Big data based on innovative algorithms will be able to predict the disease better and share the clinical data with the clinicians. Potentially malignant biomarkers related to cancer are detected by using the real-time acquisition of intraoral biosensors. In the future, the identification and utilisation of biosensors will increase significantly, with their development as wearable and portable point-of-care (POC) devices. These advanced tools will provide

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improved diagnostic capabilities compared to traditional laboratory procedures.

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