REVIEW ARTICLE

Efficiency of Nanomaterials for Electrochemical Diagnostics based Point-of-Care Detection of Non-Invasive Oral Cancer Biomarkers

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Oral tumours are the sixth most incessant infection with high mortality and morbidity rates in human beings and they pose a serious threat worldwide owing to their soaring case-fatality rate and metastatic characteristics of spreading to other parts of the body. Nanomaterials as of late have become indispensable components for biosensor platforms due to their fantastic mechanical, electronic, and optical properties. Specific emphasis is laid in this review on electrochemical biosensors working at the molecular levels, which can be classified into mainly three groups i.e., DNA biosensors, RNA biosensors, and protein biosensors as indicated by the type of the analytes. The carbon-based and non-carbon-based nanomaterials utilizing electrochemical procedures for recognizing oral cancer biomarkers are also reviewed. An extensive review has been made to cover ongoing advancements in the field of nanomaterials based as electrochemical biosensors. This study mostly sums up the significant electrochemical methods, the ongoing advancements of electrochemical technique-based biosensor frameworks for the discovery of oral cancer biomarkers. This effort aims to provide the reader with a concise view of new advances in areas on oral cancer biomarkers for electrochemical signal amplification and the innovative electroanalytical techniques which have been utilized in the miniaturization and integration of the sensors.

Introduction

Cancer disease is a major public medical issue in many other parts of the world. Cancer is a complex disease depicted by the abnormal growth of cells achieved by a couple of epigenetic changes inciting uncontrolled extension, partition, and assault to near tissues, which further metastasize to undeniable objections or organs, making basic morbidity and mortality [**1**]. Cancer is among the second most happening sicknesses around the world after cardiovascular disease [**2**]. Cancer growth is an infection where cell partition takes place wildly, bringing about the development of tumours. Further, the cells from tumours can metastasize to different organs through veins, spreading the infections to other body parts [**3**]. Cancer incidence is increasing worldwide for all cancers with 19.3 million estimated case incidents in 2020, which would be estimated to increase to 30.2 million new cases in 2040, whereas the mortalities were 9.96 million in 2020, which would increase to 16.3 million new deaths in 2040 [**4**].

More than 100 types of cancers are reported such as lung, oral, breast, ovarian, colorectal, etc., out of which oral cancers get attention due to the increase of patients rapidly. Oral cancer (OC) is the 6th most occurring disease globally and approximately 3.0 million new patients are diagnosed with oral cancer annually, which resulted in over 1.4 million mortalities globally [**5**]. OC, a widespread ailment, has become a hindrance throughout the years because of its huge bleakness and death rates [**6**]. In the current scenario, OC is today considered as one of the principal causes of deaths with an increasing distribution located in developing countries [**7**]. The WHO reported that Cancer incidence is increasing worldwide, particularly for lip and oral cavity cancer with 0.37 million estimated case incidents in 2020, which would increase to .553 million new cases in 2040, whereas mortality 0.178 million in 2020 to 0.263 million new deaths in 2040. The oral cancer incidence and mortality statistics worldwide are presented in **Fig. 1**. The hostile growth rate of OC is increasing continuously due to the consumption of tobacco and its related products like smokeless tobacco, betel-quid chewing, excessive alcohol, filthy oral care, having viral diseases, nutritional deficiency, mechanical trauma, and infection with Candida spp. etc., which have human papillomavirus that causes dangerous effects to it [**8,9**].

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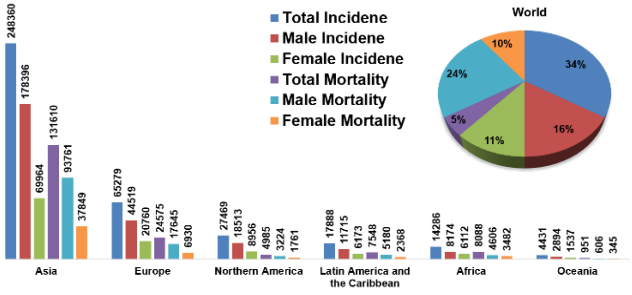

Fig. 1. Oral cancer incidence and mortality statistics worldwide.

Usually, the early-stage oral cancers have no symptoms, and hence being disregarded at the primary stage, resulting in a high death rate for those who are heavily exposed to using tobacco and drinking alcohol [**10**]. Across various sections of society, betel-quid chewing is the main cause of oral cancer reportedly [**11**]. Usually, oral cancers are very antagonistic. Epithelial cells are mainly affected in oral cancer, may enlarge metastasis, and even lead to death [**12**]. Oral squamous cell carcinomas (OSCC) are a dangerous factor for over 90% of all oral cancers [**13**]. The mucosa of the tongue, the floor of the mouth, buccal, alveolar, and the hard palate mainly invaded by these cancers, and the most commonly reported subsite is the tongue with a poor prognosis [**14**]. Many OC patients are often smokers, tobacco chewers. In a Swedish case-control study, a dose of 11-20 cigarettes/day was identified as a strong risk factor. Smokeless tobacco separately or in combination with areca nut, betel nut is both independent risk factors for oral cancer [**9,15,16**].

Over the past years, there is the development of various painless diagnostic plans of action occurred. For detection of possible hostile abrasion, many non-invasive visual tools like toluidine blue (TB), chemiluminescence (Vizi-Lite), and autofluorescence (VELscope) have been used independently or in combination with some supplementary tests [**17-21**]. For OC, the usage of radiographic imaging techniques such as magnetic resonance imaging (MRI), computed tomography (CT), cone-beam computed tomography (CBCT), and positron emission tomography (PET) are usually being done [**22,23**]. The demarcation between harmful abrasion and normal oral mucosa has been detected by changes in reflected returned optical signals which are usually recorded by most commonly used optical diagnostic assays viz., elastic scattering spectroscopy, Raman spectroscopy, diffuse reflectance spectroscopy, narrow-band imaging, and confocal reflectance microscopy [**24**].

Despite these modalities having many advantages like accurate and authentic outcomes, they have some disadvantages like expensive, amount of sample, trained personnel, sensitivity, etc., making them not so user-friendly. Besides, biosensor progression is important for addressing the ability of biomarkers towards the examination of the primary stage of oral cancer as well as these types of tumours [**25**]. For providing quick diagnosis, integrated detection systems like biosensor technology and smartphone-based applications are significant [**26**]. For example, for POC diagnosis of thrombin protein, a smartphone-based EIS biosensor system has been developed [**27**]. The detection of body fluid biomarker is done by quick, simple, and sensitive immunoassay methods which have greater importance in the clinical investigations in different types of OC. The optical and electrochemical biosensors are significantly important for the investigation of biomarkers because they are highly sensible, easy to fabricate and operate and have the potential for miniaturization [**11**]. Moreover, lock and key mechanisms followed by immunosensors are highly selective for binding of an antibody and an antigen [**28**].

In recent times, though the current standard of performing diagnosis in oral pathology is related to incisional biopsy with histology, this method is painful for patients and involves a delay in the diagnosis, although histology has been fully done [**7**]. The analysis of body fluids offers the possibility to shift the detection of cancer to an earlier stage. Recent results have shown both cell-free mRNAs and proteins in saliva present diagnostic values for oral cancer and other systemic diseases [**29**]. Due to the late detection of OC in the last stage, it leads to the possibility of the least ability to cure or almost negative and has only about 20% of survival rate [**30**]. Thus, the development of electrochemical biosensors for the investigation of OC at the primary level is crucial, which has been discussed in detail in this article. The emphasis is laid on the important electrochemical techniques like cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) to assess the OC biomarkers (such as IL-6, IL-8, Cyfra-21-1, CD 59 and CIP2A, etc.) present in saliva, using non-invasive manner. This review focuses on recent advances in point-of-care (POC) cancer diagnostics for efficient treatment along with the key challenges, opportunities, and future scope of these technologies for clinical translation.

A brief overview of saliva-based oral cancer biomarkers

A biomarker can be defined as a characteristic that is an objectively measured and evaluated indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention [**31**]. Biomarkers can be used in different clinical applications, it can be used to determine disease risk, differentiate malignant and non-malignant tumours, the type of malignancy, and as a tool to detect the effectiveness of therapy given during the treatment [**32**]. Biologic markers have become essential in guiding the treatment of many cancers, such as prostate and ovarian cancers. Identification of these markers saves from radiation exposure and conserves time and money [**33**]. Several oral cancers related biomarkers are found in plasma and blood samples, but salivary biomarkers are gaining much attention because saliva can be collected from the sites very close to the oral tumours which makes saliva a more specific and sensitive tool [**34,35**]. Saliva is such a valuable biological fluid that can be used as a biomarker for various diagnostic applications. It contains a group of analytes such as proteins, mRNA, and DNA [**36**]. Saliva samples are easy to handle, easy to store, do not clot. Also, the saliva collection process

does not hurt and does not need any supervision from health workers [**37**]. **Fig. 2** reveals the consistent enhancement in the total number of research publications in the field of oral cancer biomarkers with progressing years.

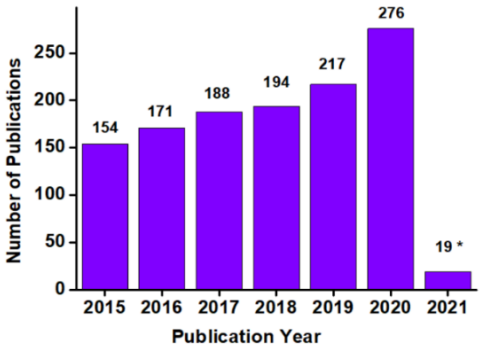

Fig. 2. The number of articles published on oral cancer biomarkers annually. Data were obtained from "Web of Science" with "oral cancer biomarkers" entered as "Subject" in the search box (Last access date: 22.02.2021).

Salivary diagnostics is a dynamic and emerging field using nanotechnology and atomic diagnostics to help in the conclusion of oral and foundational infections and thereby utilizing the salivary biomarkers for disease identification. Different types of biomarkers can be used for oral cancer diagnosis. Mainly, salivary biomarkers are divided into three types, viz., salivary DNA-based biomarkers, salivary RNA-base biomarkers, and salivary protein-based biomarkers [**38,39**].

Overview of saliva-based biomarkers

Cytokeratin (CK) is a part of intermediate filament proteins. The CK cells keep the memory of the origin of malignant cells and this unique property makes it a prominent biomarker. It is part of cytokeratin 19, with a molecular weight of 40,000d. The studies revealed that at the time of cell death, Cyfra-21-1 gets released into the bloodstream and it corresponds to the mass of the tumour. Cyfra-21-1 is found in large amounts of salivary secretion. In healthy individuals not showing any symptoms of malignancy, the Cyfra-21-1 values were found 3.8 ng/mL. On the other hand, in the individuals suffering from cancer, it was found to be17.46 ± 1.46 ng/mL, which is a significantly large value. Based on Cyfra-21-1 values, one can easily distinguish the normal person from the infected one [**40**]. Cyfra-21-1 can be used in diagnosis, prognosis, and it also works as an early indicator of response to chemotherapy [**41**]. Various reports have shown that Cyfra-21-1 salivary biomarker can be used for the early diagnosis of different cancers. Furthermore, TNF-α is another biomarker having a long chain of peptides that include 157 amino acids to form a polypeptide. The TNF-α is secreted by macrophages [**42,43**]. The TNF-α plays an important role in inflammatory processes. Normally, The TNF-α is present in the bloodstream, and its level gets increased when inflammation occurs in the body. This increased level of TNF-α is diagnosed in the case of inflammatory diseases and makes TNF-α an important biomarker [**44,45**]. A 10-fold level of TNF-α is found in patients suffering from oral cancer than normal individuals [**46**]. This value is significantly large and can easily distinguish between oral cancer patients and normal ones.

Salivary α-amylase is an enzyme that digests the starch by hydrolysis of its -1,4 glucan linkages and converts it into maltose and dextrin. The change in the level of sAA is seen in the cases of stress, emotions, and fatigue and therefore used as a potential biomarker. The advantage of sAA is that it is in direct contact with the tumour in case of oral cancer and hence is a more effective biomarker. Furthermore, saliva is preferred over other biological fluids as its collection process is easy, less stressful and it is also doesn't require any sophisticated equipment and skilled personnel for sampling [**47,48**]. The CD59 glycoprotein is a single membrane complement regulatory protein (mCRP) responsible for inhibiting the membrane attack complex (MAC) [**49**]. The CD59 is vastly present on almost all the cells of the host and thus restricts the assembly of MAC but can be hijacked by some tumour cells and escape the primary defence mechanism and complement-dependent cytotoxicity by anticancer antibodies [**50,51**]. A key contribution to the development of cancer is ORAOV 1 which is over-expressed in many solid tumours. But the cellular role of ORAOV 1 is unknown. The yeast orthologue of this protein is encoded by the hitherto uncharacterized essential gene, YNL260c. Expression of ORAOV 1 restores viability to yeast cells lacking YNL260c. Under non-permissive conditions, conditional mutants of YNL260c are defective in the maturation of the 60S ribosomal subunit, whereas maturation of the 40S subunit is unaffected. The initiation of translation is also abrogated when the YNL260c function is lost. The YNL260c is indispensable for life in oxygen but is nonessential under anaerobic conditions. Consequently, the toxic effects of aerobic metabolism on the biogenesis and function of the ribosome are alleviated by YNL260c. Hence, YNL260c is renamed as LTO1 that is required for biogenesis of the large ribosomal subunit and initiation of translation in oxygen [**52**].

The hypoxia-inducible factor (HIF-1α) acts as a master regulator of oxygen homeostasis. The oxygen-dependent hydroxylation of HIF-1α is tightly regulated by the prolyl hydroxylase domain-containing three proteins viz., PHD1, PHD2, and PHD3. The prolyl hydroxylation enables the recruitment of the von Hippel-Lindau (VHL) protein, leading to ubiquitination and degradation of HIF-1α by the proteasomes. Apart from this, prolyl hydroxylation and phosphorylation of HIF-1α are central post-translational modifications, which regulate its stability under hypoxic conditions as well as normoxic conditions [**53**]. Hypoxia-Inducible Factor (HIF)-1α is a dimeric protein complex involved in maintaining the water levels in the body and plays an important role in regulating hypoxia. HIF-1α can prevent cancerous cells to spread by inhibiting them [**54**]. CIP2A, an oncoprotein is associated with various types of cancer like oral, breast, urogenital, and many myeloma cancers. The CIP2A promotes malignant cell growth and suppresses the expression of PP2A protein. CIP2A expression is found more in oral cancers than other types of cancer and can be effectively used as a biomarker for oral

cancer diagnosis [**55,56**]. Interleukins are produced by inflammatory cells like macrophages, B cells, monocytes, dendritic cells, T-cells, etc. Interleukins can be used as signalling molecules. Interleukins range from 1-40, some of them play a major role in biomarker analysis. IL-1α is 159 amino acids long polypeptide. The IL-1α plays a major role in the maintenance of the immune system, regulation of the inflammatory process, haematopoiesis, and nociceptive neurotransmission [**57,58**]. The IL-1α originated from the activated macrophages, neutrophils, and epithelial and endothelial cells [**59**]. The IL-1α was detected was175-1000 pg/mL in saliva sample whereas in serum it was found 0-137 pg/mL, and this elevated value of IL-1α in saliva, makes IL-1α a potential salivary biomarker [**60**]. The IL-1β originated from the macrophages and other cells like mucosa epithelial cells, acinar and ductal cells of the salivary gland. The IL-1β plays a major role in the defence mechanism and regulation of immune responses. It is used as a biomarker in the human lung, colon, breast, oral carcinoma, and skin melanoma. The IL-1β value doesn't show much difference in serum samples, but the elevated value can be easily identified in saliva samples [**61**]. The IL-8 originated from the macrophages, lymphocyte, epithelial and endothelial cells. Studies show that a 3-fold concentration of IL-8 was found in saliva samples of cancerous patients than non-cancerous individuals. This increased value of IL-8 can be diagnosed for the early detection of cancer. The IL-8 would be an important biomarker in oral cancer detection [**62**]. The details of OC biomarkers, their properties, and functions are detailed in **Table 1**.

Table 1. Table of oral cancer (OC) biomarkers their properties and functions.

S.N.	Oral cancer biomarker	Properties	Function/Uses	Ref.
A.	**Protein biomarker**			
1	IL-1β	Elevated levels in OC patients	Immune and inflammatory response modulator	[63,64]
2	IL-8	Elevated levels in OC patients	Periimplantitis diagnosis	[65]
3	MMP 2	Highly invasive and metastatic	Regulation of vascularization and metastasis	[66]
4	MMP 8	Elevated levels in OC patients	Very useful salivary biomarker for the diagnosis of PD and PD severity	[67]
5	MMP 9	Highly invasive and metastatic	Associated with the progression of dysplasia to cancer through enhancement of susceptibility to angiogenesis.	[68, 69]
6	MMP 11	Highly invasive and metastatic	Poor prognosis	[66]
7	TGF-β	Unaltered levels	Uncertain diagnosis and prognosis value	[70,71]
8	CD44	Indicates high diagnostic power	Diagnostic and prognostic value	[72]
9	Defensin-1	Increase expression Profile in saliva	High discriminatory, enabling early detection	[73,74]
10	IL-6	Proangiogenic, proinflammatory cytokines	Early diagnosis and prognostic value	[75]
11	TNF-α	Proangiogenic, proinflammatory cytokines	Stimulation of cell proliferation, inhibition of apoptosis, and secretion of proinflammatory cytokines through transcriptional activation of NF-kB	[76]
12	Salivary α amylase	Potential biomarker	Digest the starch by hydrolysis its -1,4 glucan linkages and convert it into maltose and dextrin.	[77]
13	Catalase		Diagnosis of OSCC	[78]
14	IL-1α	Regulate cellular signalling, Proangiogenic, proinflammatory cytokines	Inflammation and angiogenesis	[79]
15	Cyfra-21-1, TPA, CA125	Significantly over expressed in the saliva	An early indicator of response to chemotherapy	[80]
16	M2BP	Increase expression Profile in saliva	Modulation of cell-cell and cell-matrix interactions	[37]
17	S100A12		Differentially abundant in OSCC and healthy control subjects	[81]
18	CD59	Significantly over expressed in the saliva	Activation of T cells.	[82]
19	Profilin		Differentially abundant in OSCC and healthy control subjects	
20	MRP14		Recruitment, adhesion, and migration of leukocytes. Induction of cytokine and chemokine secretion.	
21	Involucrin		Differentially abundant in OSCC and healthy control subjects	
B.	**DNA biomarker**			
22	VEGF, B-cell lymphoma-2, Claudin 4, Yes-associated protein 1, MET proto-oncogene, Receptor tyrosine kinase	Genomic biomarkers	Used to predict radio-resistance in OSCC tissue	[32]
23	LDH	Increase expression Profile in saliva	Production of lactate from pyruvate under anaerobic conditions is the key feature of cancer cells	
24	p53	DNA damage is to arrest the cell cycle and initiate apoptosis	Loss of heterozygosity (LOH)	[83]
25	3p, 9q, 13q, 17p	Early stages of oral carcinogenesis		[84]

26	p27, p63, p73		Allelic loss on chromosomes 9p	[85]
27	Ki67	Elevated levels in saliva	Cell proliferation and metastasis	[86]
28	8-OHdG	Decreased levels in saliva		
29	Cyclic D1	Amplification and overexpression	Poor prognosis	[87]
30	ECAD, RARβ, FHIT, p15, TIMP3, APC		Aberrant methylation	[88]
31	p16, MGMT, DBC1, CDKN2A, MGMT, GSTP1	Salivary rinses obtained from OSCC patients	Methylation	[89]
32	EDN1	G-protein-coupled receptor	Promote tumorigenesis	[37]
33	CCND1	Increase expression Profile in saliva	Cell proliferation and metastasis.	
34	DAPK1	Programmed cell death	Hypermethylated	
35	DCC	Commonly reported in the saliva of OSCC patients	Receptor for netrin required for axon Guidance	[90]
36	KIF1A		Protein kinase involved in apoptosis and DNA damage response	[37]
37	MINT31		Calcium channel regulator	
38	p16INK2A		A receptor of sonic hedgehog	[88]
39	RASSF-1α		Induced growth inhibition along the RAS-activated signalling pathway	
40	TIMP3		T-cell antigen receptor, recognition of foreign antigens	[91]
41	LINE-1		Hypomethylation	
42	TIMP1	Salivary rinses obtained from OSCC patients	PD prognosis (advanced PD)	[92]
C. RNA biomarkers				
43	miRNA-125a	Increase expression Profile in saliva	Inhibits cell proliferation	[37]
44	miRNA-200a		Tumour suppression and early metastasis	
45	miRNA-145		Decreased proliferation, or promoted apoptosis by targeting K-RAS, c- Myc and DFF45	
46	miRNA-31		Enhance cell proliferation, promotes metastasis, monitoring or detecting residual or recurrent tumour.	[93]
47	miRNA-184		Inducing proliferation and inhibiting apoptosis by targeting c-Myc	
C (i) Highly upregulated mRNA				
48	IL-8	Increase expression Profile in saliva	Tumour angiogenesis, cell adhesion, immunity, and cell cycle arrest	[94]
C (ii) Moderately upregulated mRNA				
49	H3F3A	Responsible for the structural integrity of chromosomal nucleosome; acts as a proliferative marker for oral cancer.	Cell Proliferation	[67, 94]
50	IL-1β	Salivary Levels Increased	Takes part in signal transduction, proliferation, inflammation, and apoptosis OR Diagnosis and progression (inflammatory modulation, severity-bone resorption, generalized PD and PD severity)	
51	S100P	Differentially abundant in OSCC and healthy control subjects	Cell cycle regulation and differentiation	
C (iii) Low upregulated mRNA				
52	miRNA-21	Increase expression Profile in saliva	Tumorigenesis and invasiveness through the Wnt/β-catenin pathway, by targeting DKK2	[37, 95]
53	DUSP1	Increase expression Profile in saliva	Role in protein modification; signal transduction and oxidative stress	[94]
54	OAZ1		Takes Part in Polyamine Biosynthesis	
55	SAT		Takes part in enzyme and transferase activity	
C (iv) Other miRNAs				
56	miRNA-181b	Directly joined with intensified severity	Diagnostic and prognostic	[37, 94]
57	miRNA-181c	Salivary level decrease	Tumour Suppressor	
58	miRNA-708	Proposed to denote early events in oral carcinogenesis	Induce carcinogenicity by down-regulating Caspase-2 level.	
59	miRNA-139-5p	Salivary level decrease	Cell proliferation, apoptosis, lymph node involvement and metastasis, metastatic potential of the tumours, a poor prognosis	
60	miRNA-197	Salivary level Increase	Cell proliferation	
61	miRNA-10b	Increase expression Profile in saliva		
62	miRNA-136	Decrease expression Profile in saliva	Tumour-suppression and promotes apoptosis.	[37, 95]
63	miRNA-126		Tumour suppression	
64	miRNA-7	Increase expression Profile in saliva	Involved in the process of metastasis	
65	miRNA-24		Enhances proliferation and reduces apoptosis	

Nanomaterials and their importance for oral cancer detection

Nanomaterials possess various excellent properties like low density, large surface area, high reactivity, high electrical conductivity, high solubility, thermal and chemical stability, size, etc. due to which they are gaining more attention in biosensing application and are extensively used as composites in various fields [**96,97**].

Carbon nanomaterial and its importance

Carbon nanomaterial offers better sensor performance owing to their excellent electrical and mechanical properties, biocompatibility, lower weight, high stability, and high specific surface area. The carbon atom undergoes sp, sp^2, sp^3 hybridizations. Based on the geometry and structure of particles, the nanomaterials can be modified in the form of sheets, tubes, dots, etc. The fullerene, Carbon nanotubes (CNTs), Graphene, Graphene quantum dots (GQDs), and Carbon dots (CDs) are the most commonly and extensively used allotropic modifications of the nanocarbon [**11**]. The 0-dimensional nano diamond, 1-dimensional carbon nanotubes, 2-dimensional graphene nanosheets can be used as nanocomposites. Before using in biosensing application, the surface of the carbon nanomaterials and other nanomaterials undergoes the process of functionalization, in which nanomaterials are modified with functional groups like -NH_2, -COOH, -OH, etc. [**98,99**]. Graphene-based materials are extensively employed carbon-based materials owing to their excellent properties. Metal nanoparticles can be used in integration with graphene oxide and other carbon nanomaterials for a composite which improves the signal amplification and time for measurement which ultimately improves the sensing application and allows the binding of biomolecules without any use of linkers [**100**]. The graphene-based biosensors are leading due to their unique properties, first, it contains certain functional group which eases the binding of biomolecules and secondly the structural patterns on the surface of graphene-based molecules allow the immobilization of biomolecules [**14**]. Like graphene-based nanomaterials, Carbon nanotubes (CNTs) also possess excellent properties like rigidity, strength, elasticity, and high value of aspect ratio. The strength of CNTs is also found 10 to 100-fold high than strong steel [**101**]. Carbon-based nanomaterials are used in electrochemical biosensors for the electrochemical detection of multiple analytes. Subsequently, the incorporation of nanomaterials along with the electrochemical technique improves the sensitivity and selectivity of the fabricated sensors [**11,102**].

Non-carbon nanomaterials and their importance

Recently, nanotechnology has been made a dramatic development in the analysis of oral cancer biomarkers by bringing the novel technology of electrochemical biosensors. Various nanomaterials based on metal/metal oxides have been extensively used in the field of biosensors by researchers owing to their potential optical and electrical characteristics. These properties include modified surface function, strong adsorption ability, high catalytic efficiency, highly active surface reaction, the capability of high charge transfer, electron and phonon confinement, and biocompatibility with a high surface to volume ratio. Furthermore, these properties can be beneficial for immobilization as well as stability of biomolecules on the surface of nanomaterials [**103**]. For instance, the nano-metal oxides (NMOs) can be used for high loading per unit mass of particles of desirable biomolecules. There are several nanomaterials based on metals and metal oxides are reported in the usage of biomarker detection of oral cancer like Mo, Zr, Hf, Au, Ag, or ZnO, ZrO_2, $nHfO_2$, etc. Out of numerous metal oxides, zirconium oxide (ZrO_2), $nHfO_2$ came out as an emerging nanomaterial for the application in biosensor fabrication due to their excellent properties like transfer of a mobile electron, surface basicity, and electrocatalytic ability [**104-106**]. Currently, rare earth metal oxides and hydroxides have gained attention in the field of biosensing technology. Out of several rare earth metal hydroxides, lanthanum hydroxide (La $(OH)_3$) consisting of lanthanum ions [La (III)] have distinctive chemical and physical characteristics, high surface to volume ratio, electrochemical inertia, and chemical coordination ability. The electronic transition within the 4f shell is mainly responsible for electrochemical properties [**107,108**]. Moreover, CeO_2 NPs emerged as a viable nanomaterial for applicability in biosensors. The attachment of CeO_2 NPs with reduced graphene oxide (rGO) are synthesized by a direct self-assembly process and CeO_2 NPs can be distributed onto the basal plane of rGO that improved the dispersion because of synergistic effect. The 2D structure provided by the Vander-Waals interaction between oxygen moiety present in CeO_2 NPs and basal plane or edges of rGO which have an excellent capacity of electron transfer and large surface area. Also, functional groups available onto basal planes or edges of rGO eases the cohering the biomolecules for biosensor application for POC devices. L-Cysteine (Cys) is a naturally occurring proteinogenic amino acid with nontoxicity has -COOH, -NH2, and Sulphur (-S) groups, that are responsible for making it a favourable linker for acquiring stable functionalization of metal oxides with desired biomolecules like antibodies, nucleic acids, etc. [**109**]. Previously, Cys was explored as a capping agent in the research of the various field of environmental and biomedical research applications [**110,111**].

Nanocomposites are also emerging nanomaterials for biosensor applications. Recently, zinc oxide-reduced graphene oxide (ZnO-rGO) nanocomposite was reported which had applicability in transducer matrix for the fabrication of a label-free, non-invasive, and inexpensive immunosensor for the "IL8" oral cancer biomarker detection with high sensitivity, selectivity, and good stability. Furthermore, the ZnO-rGO nanocomposite has high electron-transfer property which allowed the potential detection of IL8 even in saliva. Additionally, Silver molybdate nanoparticles (Ag_2MoO_4 NPs) have gained

attention towards the fabrication of electrochemical POC devices due to their absolute characteristics like good catalytic activity, significant electrochemical behaviour, and high electrochemical conductivity. Although Ag_2MoO_4 NPs have limited exposure in the biomedical applications for electrochemical biosensors especially. Several conducting polymers are utilized as electrode materials because of their unique characteristics. They are not only the electrode materials that are used in sensors and biosensors but, they are usually used in the storage of energy, memory devices, and as electrocatalysts so far [**112, 113**].

Furthermore, conjugated polymers are generally used to alter the surface of working electrodes in electrochemical biosensors [**114,115**]. For the sake of improving the applicability of conjugated polymers in the field of biosensors, a novel method of introducing functional groups like carboxylic acid, amine, sulfonate, epoxy, or thiol groups to the side groups of conjugated polymers are adopted. During biosensor development, immobilization of biorecognition molecules is effectively engaged by these functionalized conjugated polymers [**116**]. Polythiophene polymers have wonderful dependability and are appropriate materials for the development of immunosensors. The epoxy-subbed polythiophene polymer (PThiEpx) is one of the formed polymers, which have a lot of epoxy bunches on its side groups. With its epoxy-groups, the polymer can tie straightforwardly to the NH_2 groups of antibodies [**117**]. In this manner, antibodies tie to the epoxy functionalized substrate employing a two-venture strategy including adsorption and covalent reactions between nucleophilic groups (amino) on antibody and epoxy groups on the surface [**118**]. Polymer P3 is a conducting polymer and builds the sensitivity of immunosensor because of a lot of carboxyl groups on its surface. Subsequently, a lot of biorecognition particles can be immobilized on the electrode surface. Before the immobilization of the biorecognition particle, these carboxyl groups ought to be initiated. For the actuation of carboxyl groups, researchers utilized EDC/NHS chemistry (ITO/polymer P3/NHS/EDC). In this review, we focus on the merits of carbon nanomaterials for the fabrication of biosensor devices that are used as analytical tools for biomarkers detection. The analysis of multiple biomarkers, those associated with cancers or diseases, is of vital importance for early diagnosis of diseases and clinical therapy. **Fig. 3** represents the overview of the working of electrochemical immunosensors for the detection of oral cancer and their advantages, common symptoms of oral cancer, and clinical sampling.

Recent techniques used for the detection of oral cancer biomarkers

Biosensor-based diagnostics have great importance for the screening and diagnosis of cancer biomarkers. Numerous biosensors are reported for the detection of different kinds of OC biomarkers. They are electrochemical, optical, microfluidics, mass-based biosensor [**119,120**]. Furthermore, some other optical imaging platforms such as chemiluminescence, tissue-auto-fluorescence, brush biopsy, optical coherence, and toluidine blue staining are utilized for early-stage screening of oral cancer biomarkers [**121,122**]. Moreover, the major challenges of the early diagnosis are the presence of low quantities of biomarkers in a clinical sample, which is hard to diagnose, and quantify

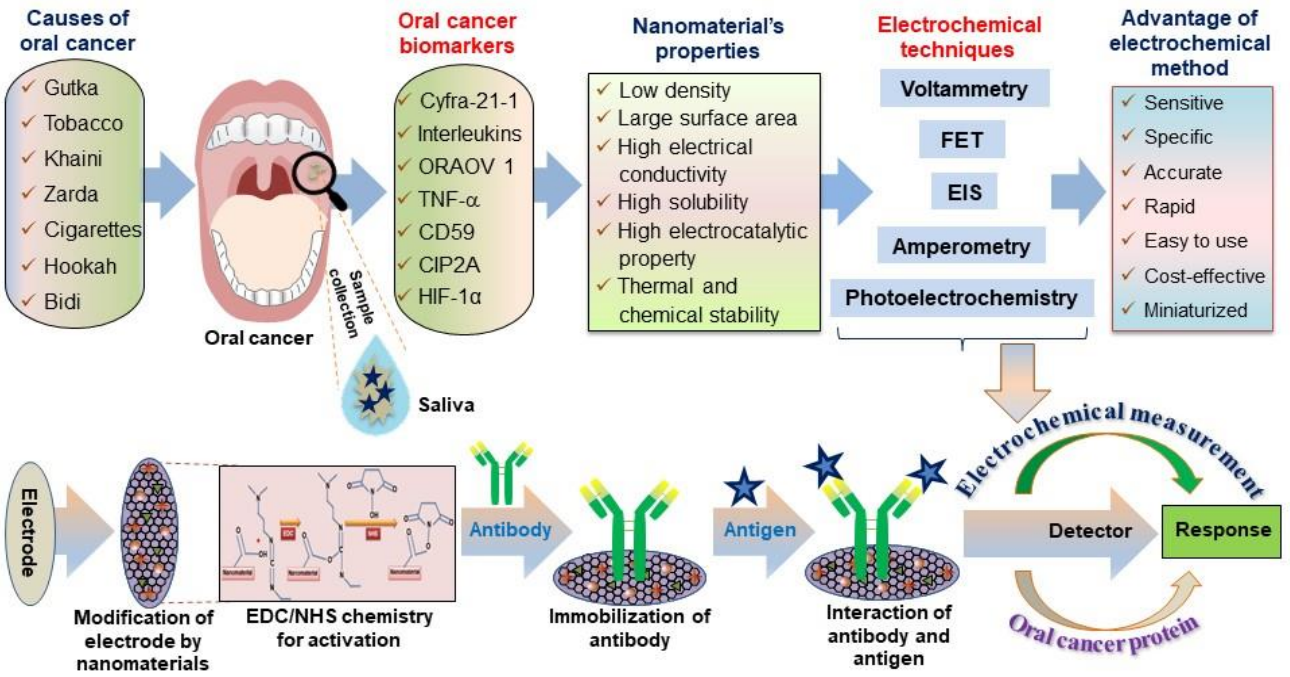

Fig. 3. Conceptualized overview of the electrochemical immunosensor for the detection of oral cancer biomarkers.

and predict the clinical stage [**3,123**]. Among these diagnostic platforms, the electrochemical biosensors for the diagnosis of oral cancer biomarkers have been widely used because these are robust, user-friendly, easy to miniaturize, and offer wider detection limits even when a little volume of analytes are present. The VELscope is a portable device that enables direct visualization of the oral cavity, which is marketed for use in OC screening. Under intense blue excitation light (400-460 nm) normal oral mucosa emits a pale green autofluorescence when viewed through the selective (narrow band) filter incorporated within the instrument [**124**].

Society is facing a serious health burden as the high cost of screening tests makes it out of reach for people of the low socioeconomic group. Early detection of various types of cancer has gained momentum due to the availability of biosensor-based diagnostic technology which are having many advantageous features over traditional diagnostic technologies such as high throughput, non-invasive nature, cost-effectiveness, easily interpretable results, and multiplexing capacity. Despite promising results, certain limitations like high cost, skilled supervision, expert data analysis, and need for the invasive procedure (in biopsy) hampered their usage in real-time diagnosis [**3**].

Electrochemical techniques-based biosensor for detection of oral cancer biomarkers

Electrochemical techniques trigger the measurement for physicochemical properties of the bioreceptor and target analyte to generate a readable signal in the form of electrical current, voltage, resistance, etc. Various forms of electrochemical techniques such as voltammetry (CV, DPV, LSW, SWV), EIS, FET, potentiometry, conductometry, amperometry-based biosensors are employing for the diagnosis of cancer biomarkers. They quantitatively offer analytical statistics against specific biomarkers. An electrochemical biosensor is highly specific, sensitive, and rapid. Also, they are cost-effective, have a nature to miniaturization, and on-site detection hold promising in biosensor application among other biosensors [**125-127**].

Voltammetry biosensors

The voltammetry is related to a category of electro-analytical methods, where, the quantitative information of an analyte is obtained by varying potential and then measuring the resulting current. There are several routes to vary a potential to measure the current and they are categorized as cyclic voltammetry (CV), differential pulse voltammetry (DPV), linear sweep voltammetry (LSV), square wave voltammetry (SWV), and stripping voltammetry (SV) [**128**].

Cyclic voltammetry (CV) biosensors

CV is a powerful analytical technique that provides information on the electrochemical processes of an analyte in a solution. It is widely utilizing for the analysis of standard electrochemical oxidation and reduction potentials. The monitoring of current is done by varying the applied potential at a working electrode in both forward and reverse directions [**129**]. For instance, Kumar *et. al.*, developed a label-free biosensor for the detection of Cyfra-21-1 biomarker in saliva samples. Herein, they constructed the electrode by deposition of APTES and ZrO_2 on the ITO surface, followed by the immobilization of anti-Cyfra-21-1 antibody and BSA. Here, the anti- Cyfra-21-1 achieved the selectivity against Cyfra-21-1 while BSA prevents the non-specific interaction to other biomarkers. This biosensor has high sensitivity and wide linearity of 2.0 - 16.0 ng/mL with a LOD of 0.08 ng/mL. Besides, the biosensor displays satisfactory performance for up to six weeks. Further, they validated this biosensor through the ELISA test [**130**]. In another report, the same group fabricated a CV-based biosensor for detection of Cyfra-21-1 in a saliva sample within 15 minutes. Herein, they constructed an electrode of APTES functionalized hafnium oxide on ITO followed by the immobilization of anti- Cyfra-21-1 and BSA. The biosensor has an excellent detection range of 2.0 - 18.0 ng/mL, and LOD 0.21 ng/mL with high sensitivity. They also revealed that the sensitivity of this sensor is high as compared to the electrode having $nZrO_2$ or $nZrO_2$/rGO alone. Also, the biosensor can be used up to 30 times and it also offers satisfactory performance up to 8 weeks [**131**].

In another study, in which PHA modified ITO electrode was prepared at room temperature, an immunosensor was fabricated by immobilizing anti-IL8 antibodies on PHA modified ITO electrode. The biosensing principle of the immunosensor was based on the specific interaction between anti-IL8 antibody and IL8 antigen. The electrochemical characterization of the PHA modified electrode was performed by recording CV and EIS responses. The results showed a wide detection range from 0.02 pg/mL to 3.0 pg/mL, LOD and limit of quantification (LOQ) were 6 fg/mL (signal to noise of three) and 19 fg/mL (signal to noise of ten), respectively, with good stability of up to 7 weeks [**132**].

Differential pulse voltammetry (DPV) biosensors

In DPV, the potential is scanned with a series of pulses to measure the current, where the current is determined at two points for each pulse before each potential change. The peak height of DPV is directly proportional to the concentration of the analyte [**129**]. For instance, Verma et al. reported gold nanoparticles and reduced graphene oxide-based voltammetric biosensors for the diagnosis of interleukin-8 in saliva samples within nine minutes. Herein, they utilized the AuNPs and rGO for sensor fabrication. Since AuNPs are highly conductive which enhanced the sensitivity of the biosensor along it has long-term stability. However, the oxygen functionality such as hydroxyl, carboxyl present in reduced graphene oxide favours the covalent immobilization of their respective antibodies, anti-IL8 against target IL-8 biomarker. This biosensor is highly specific toward IL-8 and has wide linearity of 500 fg/ML - 4.0 ng/mL with LOD of 72.23 pg/mL. Moreover, the long-

term stability of up to 3 months and reusability make an advantage in biosensor application. The schematic of fabrication of AuNPs-rGO based immunoelectrode for immunosensing application and study of DPV curves show the response studies of the immunosensor towards different concentrations (500 fg/mL - 50 ng/mL) of IL-8 are presented in **Fig. 4** [**133**].

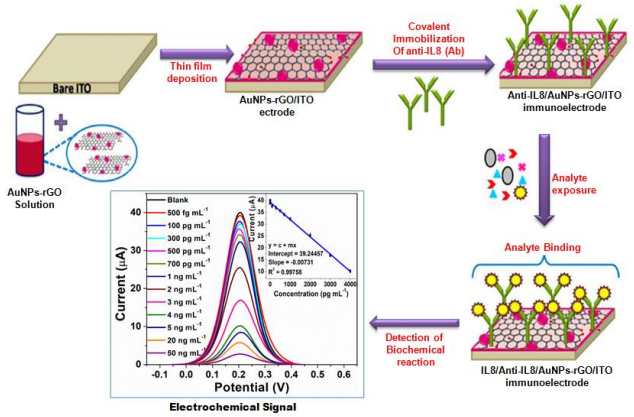

Fig. 4. Schematic of fabrication of AuNPs-rGO based immunoelectrode for immunosensing application. DPV curves show the response studies of the immunosensor towards different concentrations (500 fg/mL - 50 ng/mL) of IL-8. Reprinted with permission from Reference [133].

In another report, Kumar *et. al.*, developed a label-free electrochemical biosensor for the detection of Cyfra-21-1 in a saliva sample. Herein, they constructed an electrode by electrochemical deposition of ZrO_2-rGO on the ITO surface. Further, they were functionalized through APTES to enhance the rate of electron transport of nanocomposite. ZrO_2 nanomaterial possesses a high surface-to-volume ratio, adsorption ability, and electrical properties. Also, the biocompatibility and high oxygen atom make them promising materials in biosensor fabrication. But the easy aggregation nature of ZrO_2 makes them a limiting factor in biosensor fabrication materials. This biosensor is highly sensitive, long linearity of 2.0 - 22.0 ng/mL, LOD 0.122 ng/mL with a long-term stability of up to 8 weeks. Further, they validated the biosensor via ELISA and found their satisfactory performance without losing efficiency. They utilized the APTES for functionalization of nanoparticles that play a role to bridge for immobilization of biomolecule on the sensor surface. But the fumes of APTES have adverse effects on human health which create several chronic diseases [**134**]. To overcome the problem of the hazardous effect of APTES, the same group reported an electrode of serine functionalized ZrO_2 nanocomposites on ITO followed by the immobilization of anti-Cyfra-21-1 for the specific detection of Cyfra-21-1 in saliva specimen with wide linearity of 0.01 - 29.0 ng/mL with LOD of 0.01 ng/mL within six minutes. Serine is a naturally occurring amino acid that contains hydroxyl, carboxyl, and amino functionality that favour the immobilization of antibodies respective to target antigen as well as stabilize the metal oxide nanocomposites. Also, they are non-toxic and biocompatible [**135**]. In another study, Tiwari *et. al.*, fabricated a voltammetric biosensor for the diagnosis of Cyfra-21-1 in a saliva sample. Herein, they constructed the electrode on ITO by electrodeposition of nanocomposites of L-cysteine functionalized lanthanum hydroxide followed by the immobilization of anti-Cyfra-21-1. L-cysteine, which contains functionality such as acid, thiol, and amino group. It acts as a linker and facilitates the enhanced immobilization of biomolecule on the surface. Besides, they are non-hazardous and stabilize the nanocomposites. The constructed electrode is highly sensitive and could efficiently detect the concentration range of 0.001 - 10.2 ng/mL within 5 minutes. The LOD of biosensor was 0.001 ng/mL. They further validated the biosensor through the ELISA test, which revealed that the performance of the biosensor in terms of sensitivity and LOD is better than the "Kinesis DX" a commercially available ELISA test kit [**136**]. Recently, Kumar et al. further utilized hafnium oxide as promising materials in biosensor applications. They synthesized the nanocomposites of APTES functionalized $nHfO_2$ with reduced graphene oxide and deposited them on the electrode surface. This biosensor has wide linearity of 0 - 30.0 ng/mL with a LOD of 0.16 ng/mL for the CYFRA-21-1 in saliva samples. They studied that the Brownian motion caused the agglomeration of HfO_2 nanoparticles which reduces their electrical properties. To overcome these challenges, they synthesized the nanoparticles through a hydrothermal process. After that, they found that it has enhanced 11 times hetero electron transfer (HET) as compared to agglomerated HfO_2 [**137**].

A group reported a voltametric biosensor for the detection of an IL-8 protein biomarker in a saliva sample, in which, the electrode was constructed by the deposition of zinc oxide-reduced graphene oxide on the ITO surface. Further, they immobilized the anti-IL-8 antibodies to achieve specificity against IL-8. Zinc oxide nanoparticles possess high surface-to-volume area and excellent charge transferability. Also, the biocompatibility nature favours the immobilization of biomolecule on the electrode surface. The biosensor is highly sensitive and efficiently detected the concentration of 100 fg/mL - 5.0 ng/mL with the LOD of 51.53 pg/mL [**138**]. Very recently, Pachauri et al. reported a silver molybdate (Ag_2MoO_4) nanoparticles-based label-free electrochemical biosensor for the detection of IL-8. The linear detection range of the biosensor was 1.0 fg/mL to 40.0 ng/mL with LOD of 90.0 pg/mL for IL-8 in the saliva sample. The Ag_2MoO_4 is a highly electrically conductive material, biocompatible, and has antimicrobial properties that make them a promising material in biosensor application [**139**]. It is also reported that an impedimetric biosensor for the detection of Cyfra-21-1, wherein, they synthesized the nanocomposites of ncCeO2 with reduced graphene oxide. Since, cerium oxide has remarkable properties such as non-toxicity, high electrical conductivity, excellent oxygen transfer capability, minor swelling, high surface area, and biocompatibility make them promising material in biosensors fabrication. Here, the agglomeration of cerium oxide is a challenging task that lowers the application. To resolve this problem, they

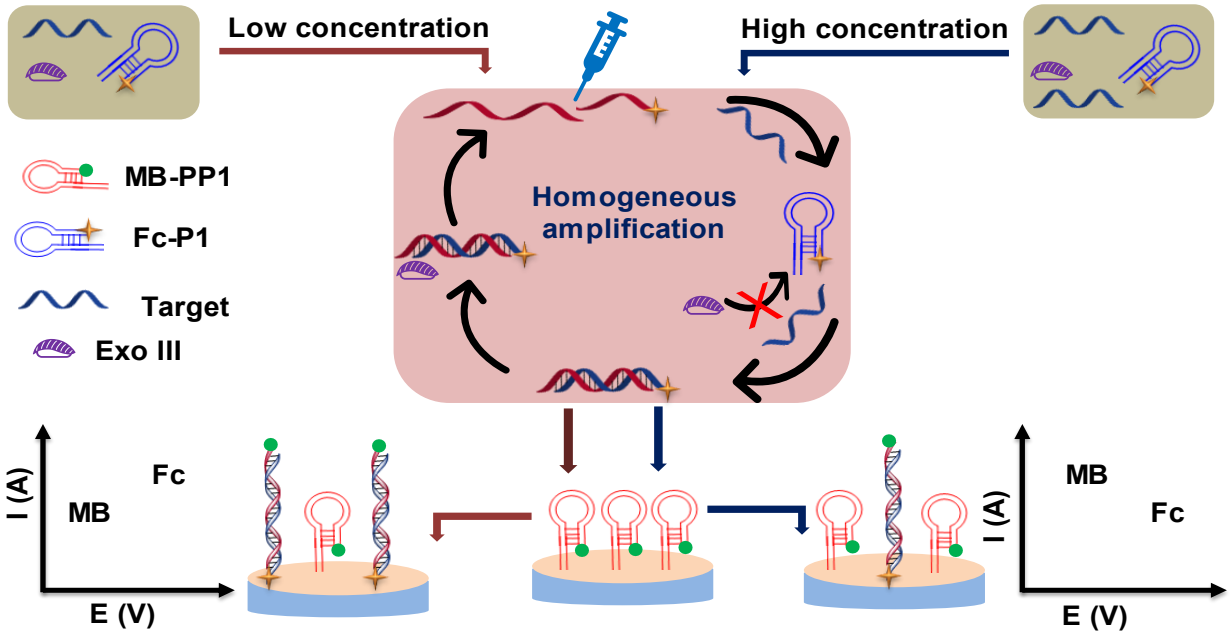

Fig. 5. Schematic illustration of the homogeneous Exo III-assisted target recycling amplification and dual-signal ratiometric electrochemical DNA biosensor.

mixed/functionalized the CeO_2 with rGO. The rGO not only controls the agglomeration of CeO_2 but also enhances the conductivity and provides oxygen functionality which favours the biomolecule immobilization and results in a highly sensitive immunosensor. The biosensor has wide linearity of 0.625 pg/mL - 15.0 ng/mL with LOD of 0.625 pg/mL. They further tested the cross-reactivity of biosensors against glucose, NaCl, mucin 16, IL-8 and found that no remarkable properties [**140**].

Other studies demonstrated a homogeneous immobilization-free, ultra-highly sensitive, and selective electrochemical biosensor for the detection of target DNA species related to ORAOV 1 in saliva. The biosensor has high specificity and can be used to identify a single-base mismatch and single nucleotide polymorphisms (SNPs). The DPV signal displayed a good linear relationship with target DNA concentration in the range from 1-10 pM with a LOD of 0.35 pM [**141**].

Alternating Current Voltammetry (ACV) biosensors

In this process, during ACV an alternating potential is added to the DC potential ramp used for LSV. The AC portion of the total current is measured, and plotted versus the DC potential portion of the potential ramp is recorded [**129**]. Recently, researchers developed the target amplification and one-step triggered dual signal-based ratiometric electrochemical DNA immunosensor for the diagnosis of oral cancer overexpressed 1 (ORAOV 1) DNA in a saliva sample. The initiation of homogeneous exonuclease III-assisted target recycling amplification is initiated, when the target DNA hybridizes with the specifically designed ferrocene-labeled hairpin probe (Fc-P1). This leads to the decrease of the local concentration of Fc-P1. Moreover, the one-step triggered dual-signal ratiometric electrochemical readout was done after the remaining amount of Fc-P1 hybridized with the methylene blue-labeled hairpin probe on the electrode. After ratiometric analysis, the LOD of the biosensor was found to be 12.8 fM for ORAOV 1 [**142**]. The schematic illustration of the homogeneous Exo III-assisted target recycling amplification and dual-signal ratiometric electrochemical DNA biosensor is detailed in **Fig. 5**.

Square wave voltammetry (SWV) biosensors

These electrochemical methods, measure the subsequent current concerning the applied potential. However, in SWV, the current is estimated by the contrast among forward and switch current, while in LSV, the current is measure by the changed potential at a consistent rate through checking. The fast outcome and high affectability make it more beneficial among other electrochemical methods [**143**]. Other studies reported an SWV-based biosensor for the early diagnosis of Cyfra-21-1 in saliva samples. Herein, they synthesized the electrode via self-assembly of cysteamine and glutaraldehyde on gold nanoparticles. The thiol group of cysteamine tightly binds to gold nanoparticles which stabilize the composites as well as favour the biomolecule immobilization and enhances the electron transport rate. However, glutaraldehyde acts as a cross-linker. They examined the target antigen through SWV and calculated that the biosensor has a linearity of 2.5 - 50.0 ng/mL with LOQ of 2.5 ng/mL. The fabrication procedure of electrochemical immunosensor for Cyfra-21-1 detection is shown in **Fig. 6 (A)** and the SWVs responses of the immunosensor for different concentrations of Cyfra-21-1 is represented in **Fig. 6 (B)** [**144**].

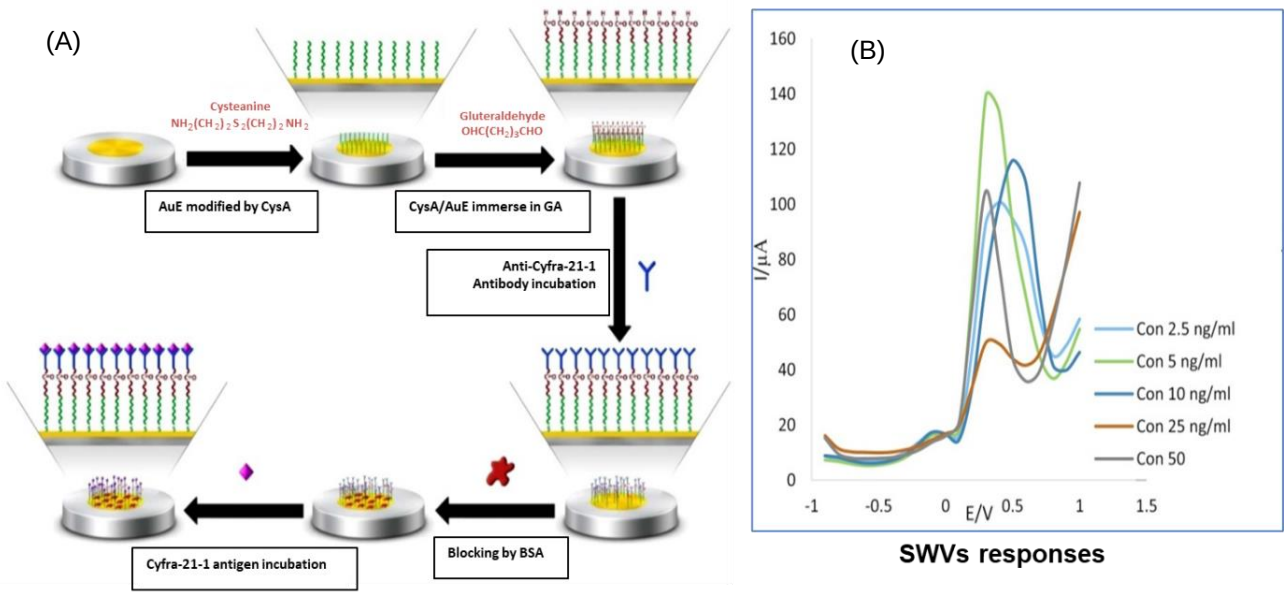

Fig. 6. (A) The fabrication procedure of electrochemical immunosensor for Cyfra-21-1 detection. **(B)** The SWVs responses of the immunosensor for different concentrations of Cyfra-21-1. Reprinted with permission from Reference [144].

Amperometry biosensors

The Amperometry technique continuously measures the resulting current from the oxidation or reduction of electroactive molecules, in which the current response is measured by applying a constant potential to the working electrode [**129**]. For instance, Sanchez-Tirado et al. reported an amperometric biosensor for the diagnosis of two oral cancer biomarkers, IL-1β and TNF-α in serum and saliva samples. Herein, they fabricated a sandwich assay, firstly the 4-carboxyphenyl-functionalized double-walled carbon nanotubes (HOOC-Phe-DWCNTs/SPCEs) were deposited on a dual screen-printed electrode. Further anti-IL-1β and TNFα antibodies immobilized on to modified electrode. A commercial polymer Mix&Go™ was also coated to make the antibodies in oriented form. The developed sensor has a wide linear range of 0.5 - 100 pg/mL with LOD of 0.38 pg/mL for IL-1β, while for TNF-α, it has a linear range of 1.0 - 200 pg/mL with LOD of 0.85 pg/mL in a clinical sample. Besides, this assay detected the biomarkers within 2 h 30 minutes, which is less time-consuming than the ELISA assay [**145**]. In another report, Torrente-Rodríguez et al. simultaneously detected the IL-8 and its messenger RNA (IL-8 mRNA) in an undiluted saliva sample. They constructed the magneto biosensor by carboxylic acid-functionalized magnetic beads followed by the immobilization of hairpin DNA probe on the dual screen-printed electrode. HQ/HRP/H_2O_2 was utilized in the system, which showed the catalytic activity and detected the dual biomarkers. The LOD for IL-8 and IL-8 mRNA was 72.4 pg/mL and 0.21 nM respectively [**146**].

The researcher reported an amperometric biosensor for the diagnosis of oral cancer biomarkers, HIF-1α in saliva samples and successfully applied it for a raw saliva sample. The whole suspension of MBs modified with the sandwich immunocomplexes were pipetted on the SPCEs working electrode surface, which was recently situated in the hand-crafted Teflon packaging with an exemplified neodymium magnet. They have developed the principal electrochemical immunoassay to date for the delicate identification of HIF-1α, utilizing MBs as strong backings and exposed expendable SPCEs as electrochemical transducers. The extraordinary logical exhibition, the simple operation, the expendable SPCEs design, and the possibility to utilize pocket-size electrochemical instrumentation, constitute important advantages for considering the created MBs-based amperometric immunoassay. It is considered an extremely convincing and user-friendly tool for an invasion into the clinical daily practice into versatile and multiplexed POCT gadgets. The strategy works at room temperature and is remarkable for its effortlessness and whole assay time (105 min). Furthermore, it displays a good sensitivity with a LOD of 76 pg/mL [**147**].

Another study describes the amperometry detection of salivary α-amylase (sAA) in human saliva samples using SPCEs. The proposed strategy depended on the roundabout assurance of sAA in an arrangement of two substance responses. Fundamentally, the primary response is the hydrolysis of starch by sAA to create maltose. At that point, the produced decreasing sugar advances the transformation of $[Fe(CN)_6]^{3-}$ into $[Fe(CN)_6]^{4-}$ in a subsequent response. The best electrochemical response was discovered utilizing 5 mmol/L NaOH (pH = 12), reaction time of 20 min, sAA volume of 15 L and 0.5% (w/v) starch. The logical exhibition uncovered a good linear correlation for sAA in a wide focus range (100–1200 U m/L). The accomplished sensitivity and LOD values were 10.7 A/(log U/mL) and 1.1 U/mL, respectively. The analytical performance of the proposed technique for the assurance of sAA levels utilizing SPCEs was studied, keeping consistent with all the optimized conditions. In these analyses, standard solutions

of sAA of various concentrations were utilized to hydrolyse the starch. The response product ($[Fe(CN)_6]^{4-}$) was then observed by chronoamperometric estimations using 0.24 V for 60 s [**148**].

Chronoamperometry (CA) biosensors

Chronoamperometry is an electrochemical technique in which the potential of the working electrode is stepped and the resulting current from faradaic processes occurring at the electrode (caused by the potential step) is monitored as a function of time. Researchers developed a new electrochemical immunosensor dependent on a gold electrode was produced for the identification of the TNF-α antigen (Ag-TNF-α). Gold electrodes were utilized as a transducer for the development of a "sandwich-type" immunosensor dependent on CA investigations. The immunosensor indicated a quick CA reaction, high sensitivity, and selectivity, and was utilized to decide TNF-α in human salivary tests. The CA investigations were then performed utilizing the standard arrangement with various concentrations of Ag-TNF-α in artificial saliva (AS) (1, 5, 10, 15, and 20 pg/mL), with a LOD of 1pg/mL to simulate human saliva [**149**].

Electrochemical Impedance Spectroscopy (EIS) biosensors

The EIS is a label-free technique to determine a variety of analytes, especially biomarkers. This method allows following the changes in capacitance or charge-transfer resistance concerned with the specific interaction between the wanted biomolecule and the biorecognition element on the modified electrode surface [**128**]. For instance, Choudhary et al. demonstrated an impedimetric biosensor for the detection of CD-59 antigen in saliva samples. Herein, they constructed an electrode by the self-assembly monolayer of L-cysteine on gold nanoparticles. The Self-assembly monolayer has advantages to its ease of formation. Also, they have greater stability than the chemically related Langmuir-Blodgett film. The reported biosensor has an efficient range of 1.0 - 1000 fg/mL with LOD of 0.38 fg/mL in the buffer and 1.46 fg/mL in unprocessed saliva samples within 10 minutes. The schematic representation of OC biosensor fabrication methodology and the detection principle is presented in **Fig. 7** [**150**].

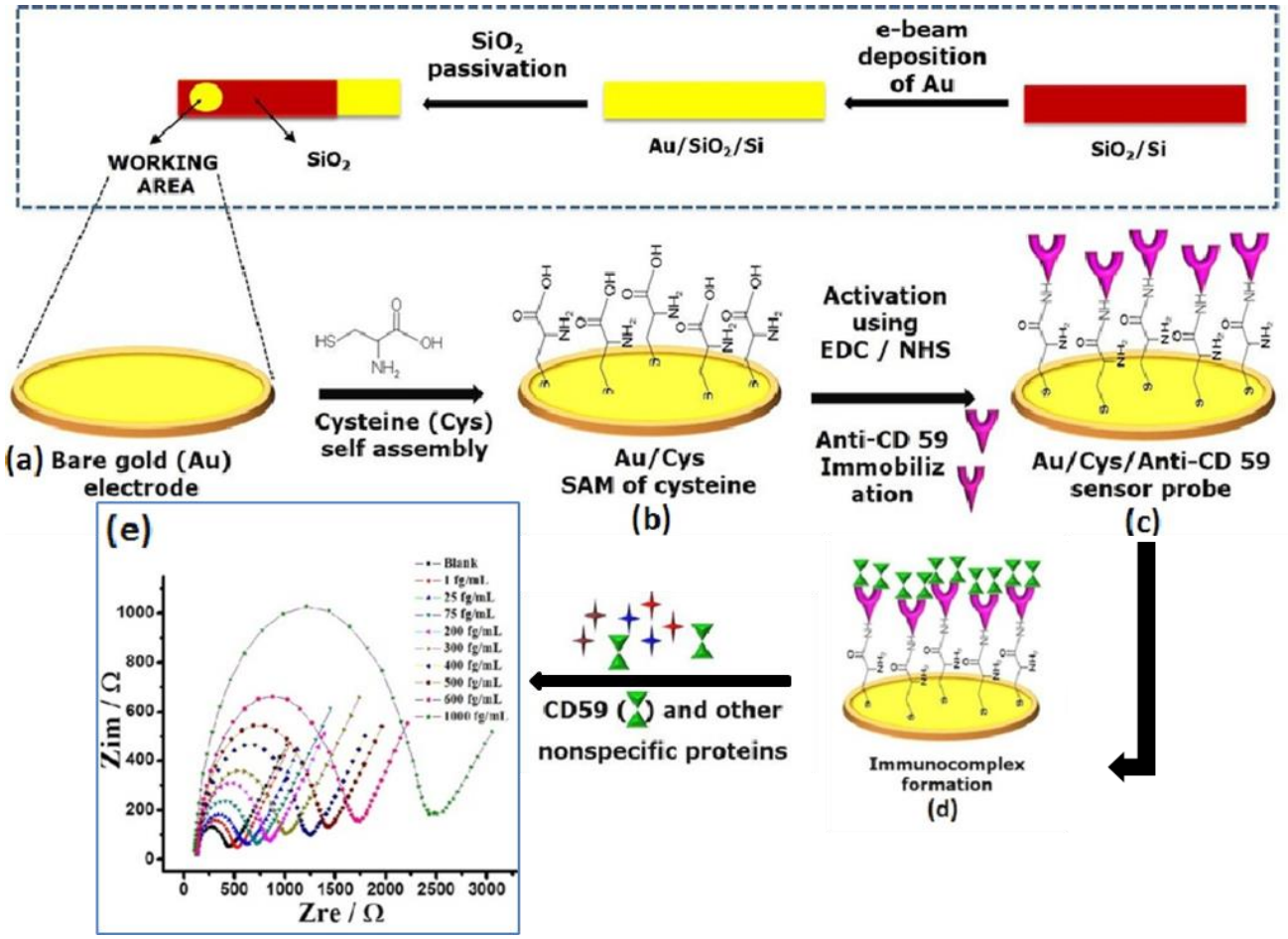

Fig. 7. Schematic representation of OC biosensor fabrication and the detection principle: (a) Passivated planar Au electrode; (b) surface functionalization of Au electrode by L-Cysteine (Cys) to provide carboxyl functional groups; (c) covalent immobilization of anti-CD 59 antibodies onto Au/Cys using EDC-NHS; (d) interaction of Au/Cys/Anti-CD 59 with saliva sample for the detection of CD-59 biomarker and (e) impedimetric response for detection of CD 59. Reprinted with permission from Reference [150].

Others have also reported that an electrode for the detection of Cyfra-21-1 in saliva samples. They synthesized the yttrium oxide (Y_2O_3) through the solvothermal process. This was further functionalized by APTES and electrochemically deposited on to ITO surface. The proposed biosensor has a highly sensitive and linear detection range of 0.01 - 50.0 ng/mL with a LOD of 0.33 ng/mL. The shelf life of the sensor was five weeks. They further validated the biosensor through the ELISA [**151**].

Another investigation fabricated a label-free-impedimetric biosensor for the detection of TNF-α in saliva and serum specimens. Herein, they functionalized the ITO surface by hydroxyl group through the NH_4OH/H_2O_2. Further, they immobilized the polymer, poly (3-thiophene acetic acid) (P3) onto it. Since P3 polymer is rich in acid functionality which favours the immobilization of anti-TNF-α antibody. Therefore, it resulted in the enhancement of the sensitivity of the sensor. The proposed biosensor shows excellent linearity for detection of TNF-α antigen 0.01 - 2.0 pg/mL with LOD of 3.7 fg/mL. They further tested the cross-reactivity with some common drugs, proteins, and other biomarkers and found no such property. Since the fabricated materials are inexpensive, enabling a cost-effective biosensor, it could be a potential alternative for the ELISA test [**152**]. In another report, researchers developed an impedimetric sensor for the detection of CIP2A biomarker in clinical samples. Herein, they immobilized the vertically aligned carbon nanotube on interdigitated electrode through chemical vapor deposition. Further, they immobilized their specific anti-antibodies on the CNT surface to achieve selectivity against CIP2A. They revealed that the oxygen plasma treatment on the surface of CNT results in the enhancement of hydrophilic nature. This increases the immobilization of biomolecule and sensitivity. Impedimetric evaluation of the sensor showed that it has excellent linearity of 5.0 - 400 pg/mL and 1.0 - 100 pg/mL in buffer and saliva respectively. Moreover, the LOD was 4.69 pg/mL in the buffer and 0.24 pg/mL in saliva respectively Since, CIP2A is also found in other cancers, this biosensor would be helpful for the detection of oral cancer as well as other cancers [**153**].

Recently, a disposable, label-free impedimetric biosensor was reported for the detection of IL-1α antigen. Herein, the author synthesized the epoxy functionalized polythiophene and immobilized it on to ITO surface. The thiophene is a highly conductive and long-term stable material while the epoxy group favours the direct immobilization of antibody on the sensor surface. The proposed biosensor detected the concentration of IL-1α antigen of 0.01 - 5.5 pg/mL with the LOD of 3.4 fg/mL [**154**]. Similarly, this group reported an impedimetric biosensor for the detection of IL-1β in serum and saliva samples. They constructed an electrode by immobilization of poly (2-thiophen-3-yl-malonic acid) (P3-TMA) on hydroxylated ITO followed by the anti-IL-1β antibody onto it. Which measured the linearity of the sensor as 0.01 – 3.0 pg/mL with LOD of 3.0 fg/mL. The biosensor has good sensitivity, reproducibility, and long-term stability also [**155**]. They have done the electrochemical analysis of IL-1β using CV, EIS, and SFI techniques, in which the wide concentration range of 0.025-3.0 pg/mL was analysed using CV and EIS. Through the impedimetric measurement, they calculated that the biosensor had a LOD of 7.5 fg/mL with a LOQ of 25 fg/mL. Further, they tested the biosensor in serum and saliva samples and found satisfactory performance for the detection of IL-1β [**156**].

Single Frequency Impedance (SFI) biosensors

The single Frequency Impedance (SFI) is an electrochemical impedance spectroscopy strategy where a single frequency is used as an energizing sign rather than a wide recurrence range. With this technique, the association between immunizer and antigen can be checked without any problem. The most well-known use is in impedance-put together sensor improvement and for line sensor frameworks. Instead of EIS, the SFI impedance technique measures electrochemical impedance at one frequency periodically. The estimation is made with a constant DC potential applied to the cell. This technique is suitable, simple, and reasonable for the analysis of clinical and on-field assays [**152,157,158**].

It is also reported that using conducting materials based on single-frequency impedance (SFI) biosensor for detection of IL-8 in serum and saliva samples. Herein, they constructed the electrode surface by immobilization of super P, polyvinylidene fluoride (PVDF), star polymer (SPGMA), and an anti-IL-8 antibody on the ITO surface. Super P is a highly conductive carbon material that enhances electron transferability. However, the star polymer having a branched shape and possesses a bunch of branching arms and they favour the immobilization of anti-IL-8. They monitored the antibody and antigen interaction through Single-Frequency Impedance (SFI) technique. The SFI technique is based on the calculation of impedance at one frequency periodically. The changes at impedance are recorded versus time. The LOD of biosensor was 3.3 fg/mL. They further validated the sensor through ELISA and found remarkable sensitivity and LOD [**159**].

Field-effect transistor (FET) biosensors

The FET-based biosensors observe the change in source-drain channel conductivity arising from the electric field after the binding of molecules. The electrical signals are rapidly sensed via the change in the source-drain voltage-current [**160**]. For instance, Hao et al. reported an aptameric graphene-based field-effect transistor for the detection of salivary biomarker IL-6. Since graphene is an excellent conductive material that enhances the electron flow in the system results in an increase of current. They fabricated the FET sensor surface by a thick layer of HfO_2 on the SiO_2/Si surface. After that, a layer of graphene was deposited followed by the functionalization through the aptameric chain. Moreover, source and drain electrodes were constructed by Cr/Au electrode. The LOD of the biosensor

was 12.0 pM, and detection of biomarkers was observed within seven minutes. Further, they converted this into a smartphone-based portable device that has the potential to employ for on-site detection. Through the portable device, it is easy to monitor and record on the digital platform as a cloud server or Wi-Fi connection [**161**]. The System-level block diagram is shown in **Fig. 8(a)** and schematic illustrations of the aptameric GFET nanosensing system for cytokines detection is depicted in **Fig. 8(b)**.

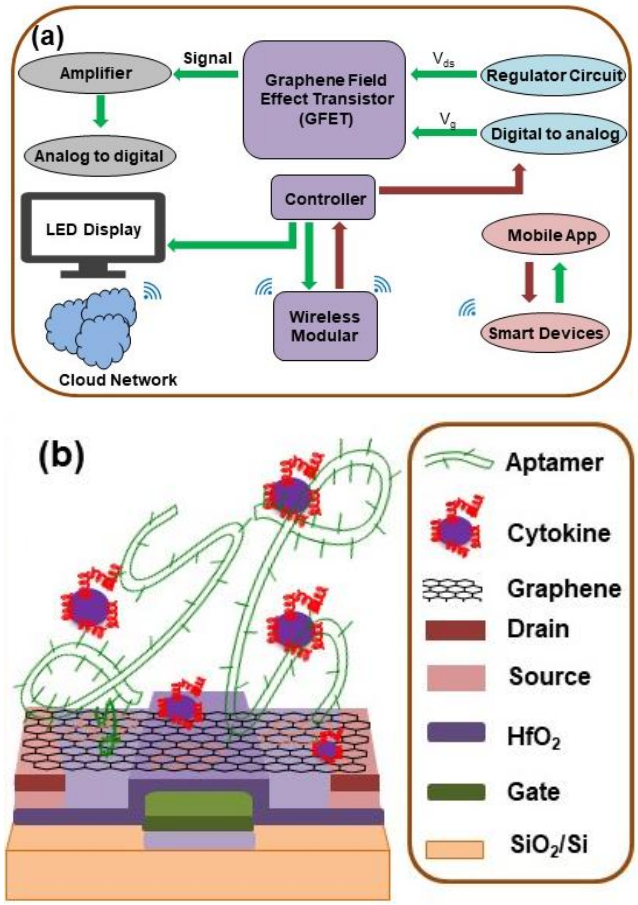

Fig. 8. (a) System-level block diagram of the nanosensing system. **(b)** Schematic of the aptameric GFET with the buried-gate geometry for cytokine detection.

Similarly, Zhang *et. al.*, reported a label-free FET biosensor for the detection of dual biomarkers IL-8 and TNF-α in clinical samples. The electrode of FET was fabricated with silicon nanowires and then immobilized their respective anti-antibodies against the target analyte. The LOD of the FET biosensor was 10.0 fg/mL in PBS solution, while 100 fg/mL in artificial saliva. The high surface to volume ratio and low dimensional silicon nanowires offers the bio affinity and high sensitivity of the sensor [**162**].

Photoelectrochemistry (PEC) biosensors

Photoelectrochemistry (PEC), as a recently arose and promising scientific procedure, has stirred broad interest. Incorporating the light source as excitation segment and electrochemical workstation as a recognition framework, PEC claims more points of interest, for example, positive versatility, low foundation signal, quick reaction, high affectability, and exactness [**163,164**]. Investigation revealed that a photoelectrochemical (PEC) biosensor for the detection of ORAOV1 in saliva samples was also fabricated. They employed the DNA rolling motor based on catalytic hairpin assembly (CHA) amplification strategy for diagnostics application. Since graphene oxide and hemin show excellent photoelectrochemical activity, they are promising materials. In this regard, they are promising materials. Furthermore, the target-triggered catalytic hairpin assembly (CHA) cycling strategy of DNA-templated silver nanoclusters (DNA-Ag NCs) was utilized as signal amplification labels which enhances the photoelectrochemical activity. They estimated that the linearity of the biosensor was 1.0×10^{-15} -1.0×10^{-10} mol/L with LOD of 3.3×10^{-16} mol/L in saliva [**165**].

Various electrochemical-based biosensors available so far for the detection of various biomarkers associated with oral cancer are enlisted in **Table 2**, in which, biorecognition elements, amplification method, assay type, the limit of detection along linear range are also included.

Applicability of biosensors for cancer management

Biosensors play an important role in cancer management, as they are the first line of defence against the spread of cancerous cells. The accurate and early detection of the tumour cells would enable a quick and efficient treatment plan for the patient and even cure the early-stage diseases when diagnosed in time. There is a critical need for the development of reliable, robust, adaptable, sensitive, and specific biosensors that should have the ability to detect the extremely low concentrations of the cancerous cells, diagnose the location, cause, and type of the tumour as early as possible. Primary recognition of biomarkers responsible for oral cancer will probably reduce the mortality rate of the fatal disease. There are significant two fronts that can aid in better cancer management, one being an effective biosensor and simultaneously another being a skilled clinician who can interpret the results with accurate diagnosis and treatment. The advancements in the biosensor techniques would enable early detection, still, at the same time, the clinicians should be able to be knowledgeable about cancer's aetiology and follow the advancements of the detection methods. Since the cancer cells may be premalignant or malignant based on the stage of cancer, and detection needs to be highly sensitive and precise. For the sensitive and specific outcomes, biosensors are the advance modality for the management of cancer disease nowadays. Keeping in mind the sensitivity and selectivity in detection, the continuous evolution of biosensors needs sustainable commercialization of the product to become a meaningful outcome [**166**].

Table 2. List of biosensors and potential Biomarkers associated with Oral Cancer.

S. No.	Techniques	Biomarker	Sample	Electrode	Sensitivity	Response Time	Self-Life	Linear range	Limit of detection	Ref.
1.	CV	Cyfra-21-1	Saliva	APTES/ZrO_2/ITO	2.2 mA mL/ng	20 Min.	6 weeks	2.0 – 16.0 ng/mL	0.08 ng/mL	[130]
2.		Cyfra-21-1	Saliva	APTES/nHfO_2/ITO	9.28 A mL/ng/cm^{+2}	15 Min.	8 weeks	2.0 – 18.0 ng/mL	0.21 ng/mL	[131]
3.		IL-8	Serum and saliva	PHA/ITO	-	-	7 weeks	0.02 – 3.0 pg/mL	6 fg/mL	[132]
4.	DPV	IL-8	Saliva	AuNPs-rGO/ITO	-	9 Min	-	500 fg/mL – 4.0 ng/mL	72.73 pg/mL	[133]
5.		Cyfra-21-1	Saliva	APTES/ZrO_2–rGO/ITO	0.756 mA mL/ng	16 Min.	8 weeks	2.0 – 22.0 ng/mL	0.122 ng/mL	[134]
6.		Cyfra-21-1	Saliva	Serine/nZrO_2/ITO	0.295 µA mL/ng	6 min	45 days	0.01 – 29.0 ng/mL	0.01 ng/mL	[135]
7.		Cyfra-21-1	Saliva	Cys-$La(OH)_3$/ITO	12.044 µA/(ng/mL/cm^{+2})	5 min	-	0.001 - 10.2 ng/mL	0.001 ng/mL	[136]
8.		Cyfra-21-1	Saliva	nHfO_2@rGO	18.24 µA mL/ng			0 to 30.0 ng/mL	0.16 ng/mL	[137]
9.		IL-8	Saliva	ZnO–rGO/ITO	~ 12.46 µA mL/ng	-	-	100 fg/mL – 5.0 ng/mL	~ 51.53 pg/mL	[138]
10.		IL-8	Saliva	Ag_2MoO_4/ITO	7.03 µA/ng mL/cm^{+2}	10 min.	4 weeks	1 fg/mL – 40.0 ng/mL	90.0 pg/mL	[139]
11.		Cyfra-21-1	Saliva	ncCeO_2-rGO/ITO	14.54 µA/ng mL/cm^{+2}	-	-	0.625 pg/mL – 15.0 ng/mL	0.625 pg/mL	[140]
12.		ORAOV 1	Saliva	ITO	-	-	-	1.0 pM – 10.0 pM	0.35 pM	[141]
13.	ACV	ORAOV 1	Artificial saliva	Fc-P1/MCH/MB-PP1/Au	-	-	-	0.02 pM – 2.0 nM	12.8 fM	[142]
14.	SWV	Cyfra-21-1	saliva	CysA-GA/AuE	-	-	-	2.5 – 50.0 ng/mL	LOQ- 2.5 ng/mL	[143]
15.	Amperometry	IL-1β TNF-α	Serum, Saliva	Phe DWCNTs/SPCEs	- -	- -	- -	0.5 – 100 pg/mL 1.0 - 200 pg/mL	0.38 pg/mL 0.85 pg/mL	[145]
16.		IL-8, IL-8 mRNA	Saliva	MB/SPCEs	-	-	-	4000 pg/mL 2.5 nM	72.4 pg/mL 0.21 nM	[146]
17.		HIF-1α	Saliva	MB/SPCEs	-	-	-	-	76 pg/mL	[147]
18.		α-amylase	Saliva	Fe/SPCEs	10.7 A/(log U/mL)	20 min	-	100 - 1200 U/mL	1.1 U/mL	[148]
19.	CA	TNF-α	Saliva	CMA/Gold	-	-	-	1.0-20.0 pg/mL	1.0 pg/mL	[149]
20.	EIS	CD 59	Saliva	Au/Cys/anti-CD 59 immunoelectrode	-	10 Min.	-	1.0 – 1000 fg/mL	0.38 fg/mL	[150]
21.		Cyfra-21-1	Saliva	APTES/nY_2O_3/ITO	226.0 ΩmL/ng	-	5 weeks	0.01–50.0 ng/mL	0.33 ng/mL	[151]
22.		TNF-α	Saliva and serum	P3/ITO	-	-	-	0.01 – 2.0 pg/mL	3.7 fg/mL	[152]
23.		CIP2A	Saliva	VANTAs	-	< 35 minutes	-	1.0 – 100 pg/mL	0.24 pg/mL	[153]
24.		IL-1α	Serum	PThiEpx/ITO	4.099 pg/mL kohm/cm^{+2}	-	-	0.01 - 5.5 pg/mL	3.4 fg/mL	[154]
25.		IL-1β	Serum and saliva	P3-TMA/ITO	-	-	-	0.01-3.0 pg/mL	3.0 fg/mL	[155]
26.		IL-1β	Serum and saliva	PHA/ITO	-	-	-	0.025-3.0 pg/mL	7.5 fg/mL	[156]
27.	SFI	IL-8	Serum and Saliva	Carbon black/SPGMA /ITO	-	-	-	0.01 - 3.0 pg/mL	3.3 fg/mL	[159]
28.	FET	IL-6	Saliva	Graphene	-	<7 min	-	-	12.0 pM	[161]
29.		IL-8 TNF-α	Buffer Saliva	SiNWs	- -	- -	- -	- -	10.0 fg/mL in PBS 100 fg/mL in saliva	[162]
30.	PEC	ORAOV 1	Saliva	GO/hemin	-	-	-	1.0×10^{-15} - 1.0×10^{-10} mol/L	3.3×10^{-16} mol/L	[165]

Futuristic approaches to manage cancer in a personalized manner

Predominantly, the management of oral cancer involves surgery. However, the treatment should be made personalized with a prescription of precision medicines. The personalized approach is based on pre-requisite information of the site of tumour, stage of cancerous growth, and biology of tumour. Even the individual's health conditions, patient history, genomics, and feasibility of treatment need to be taken into consideration. Biosensors would help in the management of cervical nodal metastasis during prognosis [**167**]. To make the point-of-care and personalized medicines sustain in a real-life environment, they need to overcome some of the basic bottlenecks such as cheaper tests, quicker response time, ease to use, reliable, and accurate results. Advancements in biosensors include the adaptation of new analyte detection, alternative and better biomarkers, direct detection of pathogens, for the optimal personalized treatment [**168**].

The clinicians can improve the survival rate and quality of life of cancer struck patients by targeting biomarkers that help in early diagnosis, prognosis, and development of precision treatments. The selection of optimized treatment depends upon specific biomarkers and their effect on the patients. The whole protocol needs optimization by decreasing side-effects, improving survival, adjusting the dose, intensity, and sequence of the treatments. Molecular assays are another significant diagnostic for monitoring suspicious lesions, as they are vital to developing new therapies. Furthermore, healthcare education, oral health hygiene, and awareness among the population demand specific attention to delivering favourable prognosis, early diagnosis, and personalized treatment [**169**]. With the difference in human biology, and variation in effects of the disease the standard treatments are less effective for a larger patient domain. The varied molecular biology of patients, genetics may cause different symptoms and dysfunctions for same disease in the different patients. Therefore, precision and personalized medication will be able to treat a variety of changes for each patient separately. The need for vast data and research is still underway to effectively characterize molecular differences between tumours in patients, which would lead to the formulation of effective drug treatment. Sincere consideration of ethics, regulations, and efficacy of the drug should be taken care of before public use. The associated groups: scientists, biopharmaceutical companies, insurers, clinicians, regulators, and patients towards the personalized approach must have a collaborative effort for necessary advancements in precision medication [**170**].

Challenges

The early-stage diagnosis of oral cancer can be deceptive, malignancy could be misdiagnosed. The difference in origin and cause for oral cancer poses challenges to the clinicians and may cause difficulty in proper diagnosis. Awareness of the new molecular changes along with the new detection methods needs to be studied by experts to effectively test the tumours. Non-invasive sampling has become more appropriate in combination with a variety of tests including vital staining, cytological studies, tissue fluorescence, and other cytochemical and molecular studies. Molecular studies are highly sensitive than other techniques which highlight the possibility of misdiagnosis by clinicians and inappropriate management of malignancy may lead to poor prognosis and lack of treatment [**171**].

Some of the practical problems associated with the spread of oral cancer include lack of awareness among most of the population, lack of knowledge about cancer-causing substances, lack of reliable healthcare systems, lack of early screening and diagnostic centres for the public, lack of initiatives to discontinue the harmful habits, lack of proper follow-up and extended care, lack of medical facilities in low resource countries. These challenges and patient healthcare can be improved by few basic changes from a responsible point of views such as education about the risk factors to the general population, social prevention and individual intervention programs to stop the origin of oral cancer, development of early diagnostic tools, biosensors would be helpful, personalized healthcare would reduce the spread and lead to better referral and treatment systems. Collective efforts are needed to streamline these solutions into a sustainable program for the affected population [**172**].

The clinical advancements and preclinical data are needed for the components of tumour microenvironment, identification of genetic variants, mutations, activated pathways, and networks from the study of omics. Genome-wide analysis through genomics, epigenomics, transcriptomics, proteomics, metabolomics, are based on large-scale datasets, these will enable the characterization of specific genes and help identify candidates for personalized treatment. During the fate of survival through targeted biomarkers and corresponding therapies with the help of Oral squamous cell carcinomas sampling there is a need to design validated tests and studies for long-term usage. Effectively, Liquid biopsy of blood or saliva, is better in diagnosis since it is easily accessible and has circulating tumour cells DNA, RNA, proteins, and biomarkers.

Undetected and delayed late-stage cancers lead to other chronic diseases causing multi-mortality decreasing life-expectancy. Oral cancer is the pool for numerous bacteria which contribute to tumour progression. For example, Prevotella intermedia, Porphyromonas gingivalis, and Fusobacterium nucleatum, bacteria have been associated with oral cancer. Therefore, the study of oral microbes is a growing challenge for researchers, this even might help justify the risk factors associated with unconventional oral cancer in patients without traditional risk factors of oral causing tobacco, alcohol, or any other substances. The therapeutic shortfall of oral cancer depends on the understanding of tumour microenvironment, associated biomarkers, aetiology, and risk factors of oral cancer growth. An interdisciplinary approach is required to

overcome such challenges with the acquisition of mechanism of action, preventive knowledge, detection techniques, practical limitations, with the collective approach of diagnostics, prevention, and therapeutics [**173**].

Conclusion and futuristic outlook

Oral tumours are the sixth most incessant infection with high mortality and morbidity rates in human beings and they pose a serious threat worldwide owing to their soaring case-fatality rate and metastatic characteristics of spreading to other parts of the body. A detailed understanding of the saliva-based oral cancer biomarkers along with their importance has been briefly discussed. Special attention is paid to review the oral cancer biomarkers that are very promising for ultra-sensitive and specific cancer biomarker detection. The carbon-based and non-carbon-based nanomaterials utilizing electrochemical procedures for recognizing oral cancer biomarkers are also reviewed. Carbon nanomaterials and non-carbon nanomaterials became essential elements for biosensor platforms during the last decade due to their various excellent properties like low density, large surface area, high electrical conductivity, high solubility, thermal and chemical stability, etc and it is expected that novel functionalization will expand their application possibilities. Specific emphasis is laid in this review on electrochemical biosensors working at the molecular levels, which can be classified into mainly three groups i.e., DNA biosensors, RNA biosensors, and protein biosensors as indicated by the type of the analytes. Emphasis is laid on cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) to assess the oral cancer present in saliva have been discussed as promising candidates to provide crucial information for developing non-invasive oral cancer diagnosis, using nanomaterials. Besides, these are easy-to-use, cost-effective, reproducible results, disposable, and their nature of miniaturization makes them promising platforms in immunosensor applications. This effort aims to provide the reader with a clear and concise view of new advances in areas on oral cancer biomarkers for electrochemical signal amplification and the novel electroanalytical techniques used in the miniaturization and integration of the sensors.

There are a lot of prospects and possibilities in this field. A large portion of the sensor improvements up to this point have thought about the identification of a single target. However, simultaneous estimation of numerous biomarkers can improve the symptomatic worth because numerous disease biomarkers are indicative of various illnesses. The development of multi-analyte immunosensors is still in its early stage, and future research is expected to move in this direction. Moreover, the improvement of electrochemical biosensors on-chip will be one of the primary non-invasive techniques of oral cancer in a precise manner. SPCEs and 16-array chips offer the system to detect multiple relevant oral cancer biomarkers simultaneously. Complicated sensor assembly processes, expensive materials, possible undesired properties at the nanoscale, and lack of storage stability are some limiting factors that preventing their mass production. Methods for delivering indistinguishable sensor clusters and scaling-up to large-scale manufacturing, just as the combination of biosensors into computerized and scaled-down frameworks are yet to be created. Although carbon nanomaterial and non-carbon nanomaterials-based biosensors research for cancer and disease detection is currently still at the advanced laboratory stage. It has already provided a promise and vision about future disease diagnosis and health monitoring. Further advancement in this field is expected to lead to the improvement of biomarker sensors for routine clinical applications.

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Keywords

Oral cancer, electrochemical biosensor, nanomaterials, biomarkers, non-invasive.

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