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Recent advances in bioactive materials: Future perspectives and opportunities in oral cancer biosensing

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ABSTRACT

Bioactive materials and biosensing technologies are emerging as pivotal tools in the early detection and management of oral cancer, a disease characterized by high morbidity and mortality rates. Recent advancements in nanotechnology have facilitated the development of innovative biosensors that utilize bioactive materials for non-invasive diagnostics, particularly through salivary analysis. These biosensors, including electrochemical, optical, and molecular types, target specific biomarkers such as DNA, RNA, and proteins associated with oral cancer. For instance, metal oxide nanoparticles and gold nanoparticles have shown promise in enhancing the sensitivity and specificity of these diagnostic tools. The integration of these nanomaterials allows for real-time monitoring of biomarker levels in saliva, providing a rapid and accurate means of detecting oral cancer at its nascent stages. Furthermore, the utilization of biosensors can circumvent the limitations of traditional biopsy methods, which are often invasive and time-consuming. By focusing on salivary diagnostics, researchers aim to develop point-of-care testing devices that can be used in various settings, thus improving accessibility to early screening for at-risk populations. This innovative approach not only enhances diagnostic accuracy but also holds potential for personalized treatment strategies by enabling continuous monitoring of disease progression and response to therapy. As research continues to evolve, the combination of bioactive materials with advanced biosensing technologies promises to revolutionize oral cancer diagnostics, ultimately leading to improved patient outcomes through earlier intervention and tailored therapeutic approaches.

1. Introduction

Oral cancer significantly reduces productivity in developing nations due to premature mortality rates [1,2]. This aggressive cancer primarily targets oral epithelial cells, presents a risk of metastasis, and can lead to fatal outcomes [3]. Visual examination and biopsy are the primary

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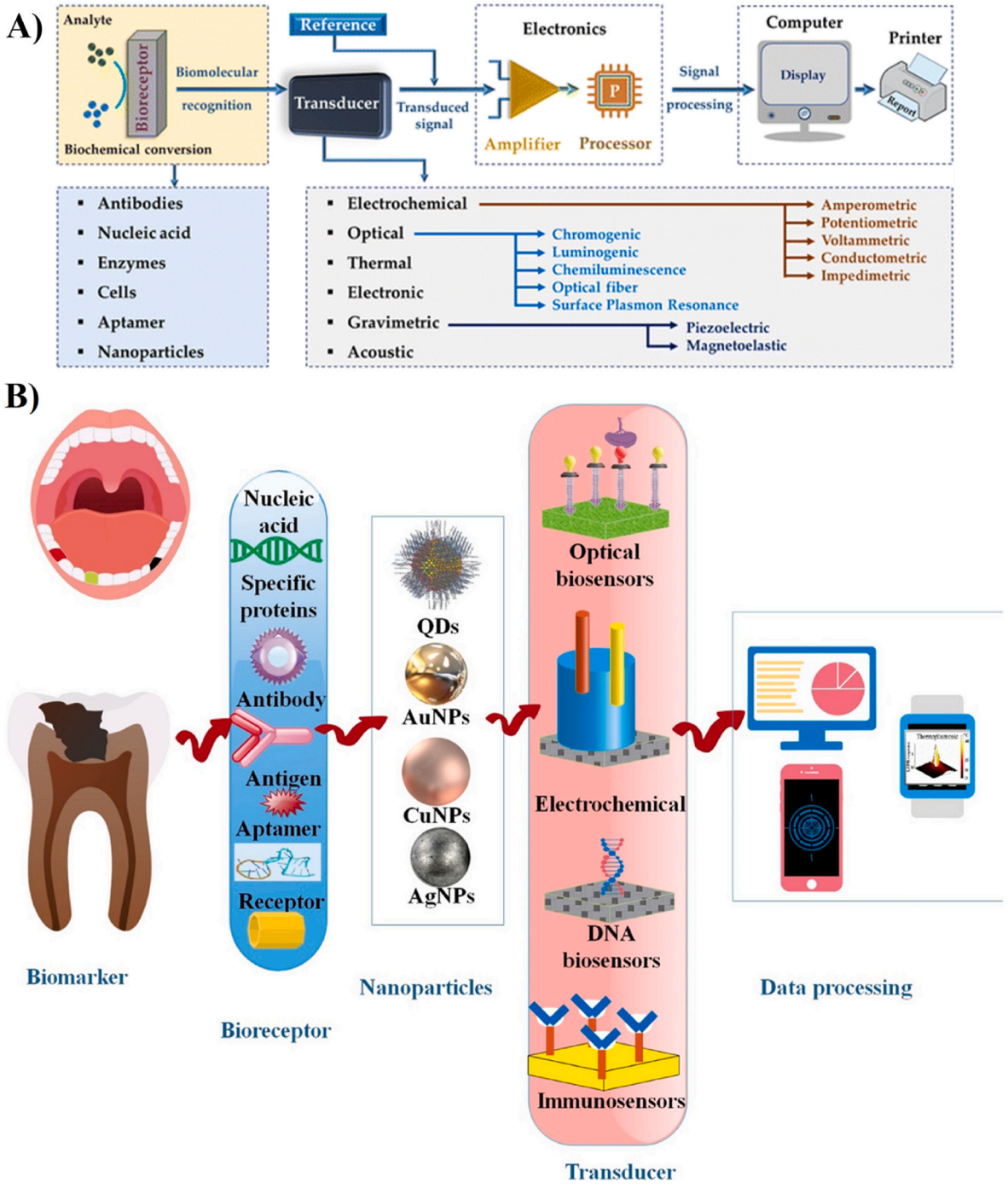


Fig. 1. A) Schematic illustration of a biosensing device, which is comprised of a bioreceptor, transducer, and amplifier. Reprinted with permission from Ref. [18]. **B)** Development of nanomaterials based on biosensors for oral cancer detection. Reprinted with permission from Ref. [19].

methods used to diagnose oral cancer and premalignant lesions, such as erythroplakia and leukoplakia. Currently, diagnosing oral cancer relies on invasive procedures like tissue biopsy of the affected area, supplemented by non-invasive imaging techniques. These imaging methods, however, can be both time-intensive and expensive [4,5]. Time-consuming and costly diagnostic procedures often cannot effectively distinguish between normal and tumor tissue, potentially delaying the initiation of treatment [6,7]. Conventional techniques for detecting oral squamous cell carcinomas from healthy oral mucosa have been used either separately or alongside supplementary investigative strategies. However, these methods tend to be expensive, labor-intensive, invasive, time-consuming, and dependent on the expertise of the investigator. Because of the low levels of biomarkers detected in exfoliated cells, tissue samples, and various biological fluids such as saliva, semen, blood, and urine, the disease may go undiagnosed until a specific diagnostic test is performed. In healthy individuals, these biomarkers are generally found in minimal quantities; however, their concentrations increase as the disease advances. Biosensors are instruments that consist of a receptor-transducer combination, utilizing biological materials to interact with specific analytes [8]. These devices deliver quantitative or semi-quantitative information via a biological recognition component. The utilization of biosensors is rapidly increasing within the medical field, where they serve as diagnostic tools for various applications, including the monitoring hazardous metabolites,

Table 1
An overview of oral cancer diagnosis by biosensors.

Transducer	Nanomaterial	Detection	Sample	limit of detection	Response time	Ref.
Optical	Rhod-NO_2	Fluorescence	Serum	0.6 ng/mL	30 min	[33]
	Tungsten (VI) oxide	Field Effect Transistor (FET)	Artificial saliva	1.26 pg mL^{-1}	30 min	[34]
	Au–Ag hollow nanoparticles (Au–Ag HNPs)	SERS	Saliva	6.51 aM	10 min	[35]
Electrochemical	Silver molybdate nanoparticles	Differential pulse voltammetry (DPV)	Saliva	90 pg mL^{-1}	10 min	[36]
	Graphite	Cyclic voltammetry	Serum and saliva	829.5 pg mL^{-1}	15 min	[37]
	Fluorine doped tin oxide	EIS	Saliva & serum	6 fg mL^{-1}	60 min	[38]
Photoelectrochemical	Titanium dioxide/Indium sulfide	Anthocyanin (ACN)-sensitized poly(indole-5-carboxylic acid) (P5ICA) nanofibers	Real serum	3.3 pg mL^{-1}	60 min	[39]
	Silver nanoclusters; Graphene oxide	photoelectrochemical	Saliva	33 fM	40 min	[40]
	silver nanowires/fullerene-Congo red; Single-walled carbon nanotubes/platinum nanowires	Photoelectrochemical	Saliva	0.57 μM	____	[41]

cholesterol testing, measuring vitamins and other nutrients, identification of infections, and glucose monitoring [9–12]. Biosensors are instruments that convert a biological component, such as DNA, RNA, or protein, into an electrical signal that can be measured and analyzed for the detection of a specific biological analyte [13]. The prefix "bio" denotes the sensor's capability to identify biological entities, including microorganisms, antibodies, proteins, DNA, and RNA. A biosensor is primarily composed of three essential elements: a biological recognition element, a transducer, and a signal processing system (Fig. 1A) [14]. Also, nanomaterials have become highly regarded as potential

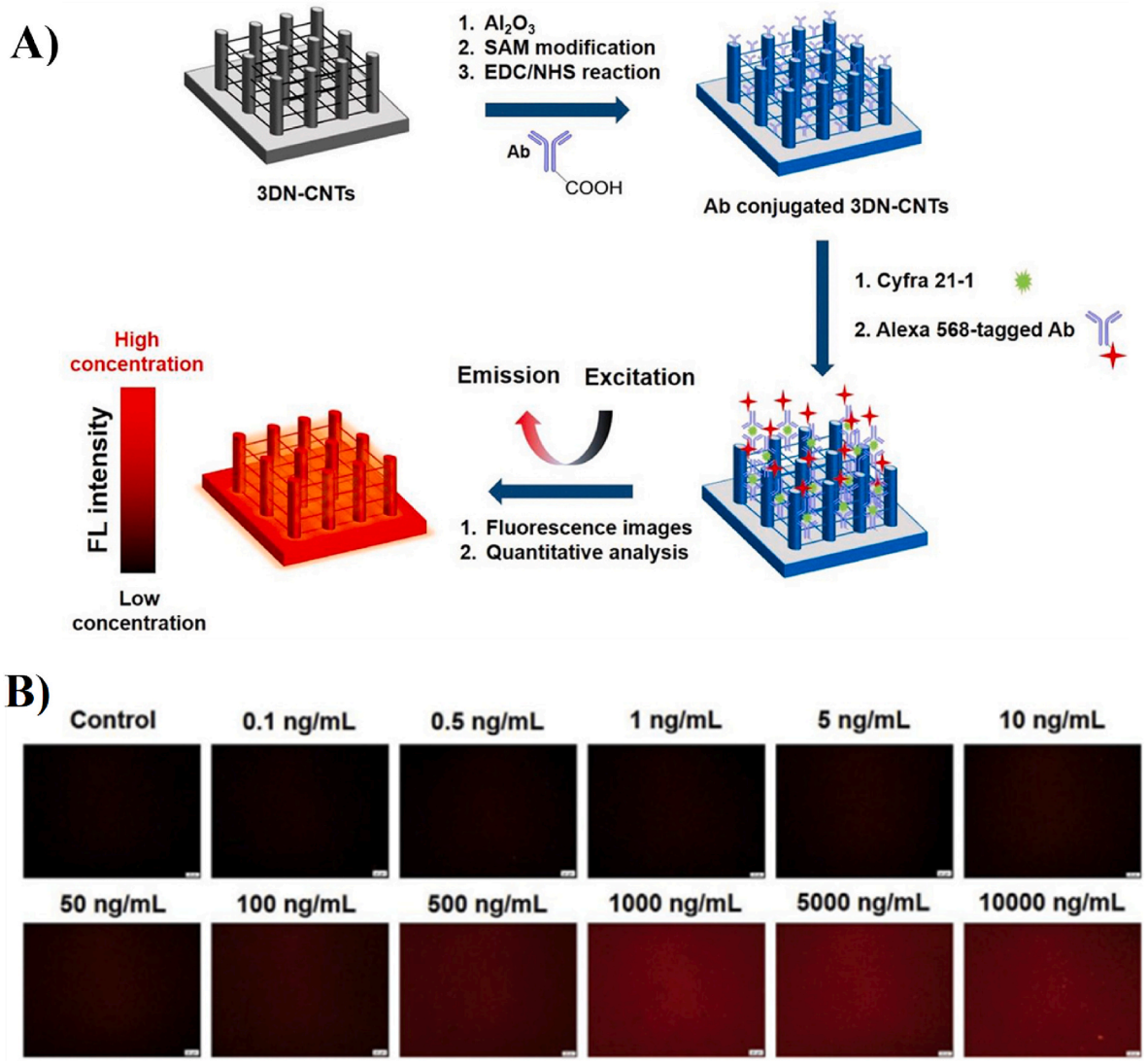


Fig. 2. A) Diagram showing the 3DN-CNTs sensor setup for detecting Cyfra 21-1. B) 3DN-CNTs sensor performance at various Cyfra 21-1 concentrations. Reprinted with permission from Ref. [32] Copyright © 2018 Elsevier B.V. All rights reserved. License Number: 5897361280288.

candidates for advancing biosensors aimed at detecting oral cancer due to their distinctive physicochemical characteristics. These include a high surface-to-volume ratio, tunable surface chemistry, and the ability to conduct electrons efficiently. These properties significantly improve the sensitivity and specificity of biosensors, enabling them to identify even trace amounts of cancer biomarkers in biological fluids such as saliva. Salivary diagnostics using nanomaterial-based biosensors offer a non-invasive alternative that is particularly advantageous for patients with restricted mouth opening or those who require frequent monitoring. Various types of nanoparticles are utilized in these biosensors, including gold nanoparticles, carbon nanotubes, and metal oxide nanoparticles. Gold nanoparticles are particularly favored due to their excellent optical properties and biocompatibility. They can be modified with particular ligands or antibodies to identify cancer-specific biomarkers, including the carcinoembryonic antigen (CEA) and epidermal growth factor receptor (EGFR). This functionalization allows for the precise detection of cancer cells through techniques like surface plasmon resonance (SPR) or fluorescence-based assays (Fig. 1B) [15–17]. The aim of this review article is to evaluate recent advances in bioactive materials, prospects and future opportunities in oral cancer bioassays.

2. Current trends in biosensor technologies for oral cancer

A biosensor is an analytical device that integrates a biologically active element with an appropriate physical transducer to generate a quantifiable signal that corresponds directly to the concentration of chemical substances present in a sample [20]. The intricate and quantitative characteristics of biodegradation stem from the strong interplay between biological detection components, which include enzymes, nucleic acids, and antibodies, and transducer types such as optical, electrochemical, and piezoelectric devices [21]. Quantum dots (QDs), Metal nanoparticles (MNPs), and carbon nanotubes are prominent nanomaterials used in the development of nanosensors, which significantly increase their sensitivity and accuracy [22]. Due to their heightened sensitivity, rapid response times, and practical applications, these technologies can be integrated with lab-on-a-chip systems, resulting in advanced point-of-care (POC) analytical platforms. Their unique characteristics render them effective tools for detecting a diverse range of analytes, such as biomarkers, drugs, proteins, viruses, bacteria, and microbes [23]. Antibody-linked MNPs have been employed for the immunomagnetic separation of proteins, viruses, and nucleic acids. Factors such as the specific capping agents, surface modifications, temperature, and timing of polymer addition can influence the morphology and magnetic characteristics of MNPs. This versatility facilitates the incorporation of functional groups that enable the attachment of different ligands, including proteins, nucleic acids, and antibodies, which are essential for identifying and quantifying target substances [24,25]. Researchers and scientists are actively designing and developing biosensors specifically for the detection of cancer biomarkers, facilitating early cancer diagnosis. In particular, biosensors have shown promise in effectively identifying oral cancer at early stages. Studies have demonstrated the efficacy of protein, RNA, and DNA biosensors in detecting oral cancer, providing valuable insights that support non-invasive detection methods for this disease (Table 1) [26]. The application of biosensors for analyzing protein biomarkers is increasingly recognized as an effective and affordable approach for creating POC devices [27]. Electrochemical biosensors have been utilized for the detection of cancer markers, showcasing their potential in clinical diagnostics [28]. Surface plasmon resonance (SPR) sensors using surface plasmon spectroscopy are increasingly used for label-free detection of cancer biomarkers [29]. Additionally, piezoelectric biosensors have been employed for cancer marker detection due to their high sensitivity, lightweight design, and low power consumption [30]. Optical biosensors are widely used for the detection of biomarkers due to their remarkable specificity and sensitivity, rapid response times, cost-effectiveness, and compact design [31]. Song and colleagues created a fluorescence-based immunosensor. This sensor uses a three-dimensional platform of carbon nanotubes on a silicon pillar substrate (3DN-CNTs). Its goal is to detect oral squamous cell carcinoma (OSCC) in saliva. To enhance the structural stability of the biosensing system, they applied an aluminum oxide coating to the 3DN-CNTs. This biosensor improves accessibility and sensitivity for detecting the Cytokeratin-19 fragment (Cyfra 21-1). It does this by increasing the intensity of the immobilized antibody, benefiting from the high surface area of the 3DN-CNTs. To evaluate the clinical applicability of the 3DN-CNTs sensor, researchers compared it with a commercially available electrochemiluminescence detection system used in hospitals. The results showed a strong linear association. This suggests that the Cyfra 21-1 biomarker in clinical fluids is a promising marker for diagnosing OSCC accurately. Fig. 2A displays the fluorescence-based immunoassay using 3DN-CNTs for quantitative analysis of Cyfra 21-1. Fig. 2B shows that fluorescence intensity significantly increased with higher concentrations of Cyfra 21-1, ranging from 1 to 1000 ng/mL [32].

3. Nanomaterials and their role in biosensing applications

Biosensors designed for cancer detection have recently gained significant attention. A primary challenge hindering the rapid advancement of these biodevices is the complexity associated with cancer, which encompasses a wide range of diseases. To address this, innovative proteomic and genomic molecular tools are being utilized to develop “molecular signatures” and to create comprehensive tumor profiles. Today, oncologists mainly depend on selective biomarkers and histological analysis of tumors for cancer diagnosis and treatment planning. Key signatures include post-translational protein modifications, gene expression changes, genetic and epigenetic markers, and protein profiles. Molecular signatures hold significant promise for advancing biosensor technologies in cancer diagnosis. Biosensors have significant potential to advance molecular diagnostics and pave the way for improved diagnostic methods for cancer patients [42–45]. Also, nanomaterials are characterized as materials that exhibit at least one dimension within the range of 1–100 nm [46]. These materials possess unique characteristics, such as a low density, a high surface-to-volume ratio, and an extensive surface area that enhances their reactivity. They also exhibit thermal and chemical stability, excellent electrical conductivity, and porosity. Furthermore, these materials are recognized for their ability to amplify signals and their compatibility with biological systems [47–49]. These features make them highly effective for biosensing system development. Consequently, they improve the specificity, sensitivity, and response time of biosensors by improving the efficiency of biomolecule immobilization [50,51]. Recent progress in biosensor research has encouraged scientists to delve deeper into nanotechnology. Nanomaterials play a crucial role in the analytical elements of biosensors, leading to substantial reductions in detection limits, as well as marked improvements in both specificity and sensitivity. This has notably advanced the ability to detect individual molecules effectively [52]. Numerous types of nanomaterials are being increasingly employed in the development of biosensors for detecting and measuring biochemical reactions. These include various carbon-based materials such as carbon nanotubes and graphene; metals, such as platinum, gold, and silver; and metal oxides like titanium oxide, zinc oxide, and zirconium oxide [53–56]. Metal nanoparticles have demonstrated significant potential as immobilization matrices for biosensors. Their conductive properties and large surface area enhance the capacity for loading receptor molecules, which is crucial for improving biosensor performance [43,50]. Gold has proven to be an effective nanomaterial for developing metal-based biosensors, owing to its remarkable stability, capacity to create self-assembled monolayers with thiol-functionalized groups, and excellent electrical conductivity [57]. Research teams have emphasized the progress of electrochemical biosensors that use gold for detecting oral cancer. These advancements mark a significant step forward in cancer diagnosis technology. For

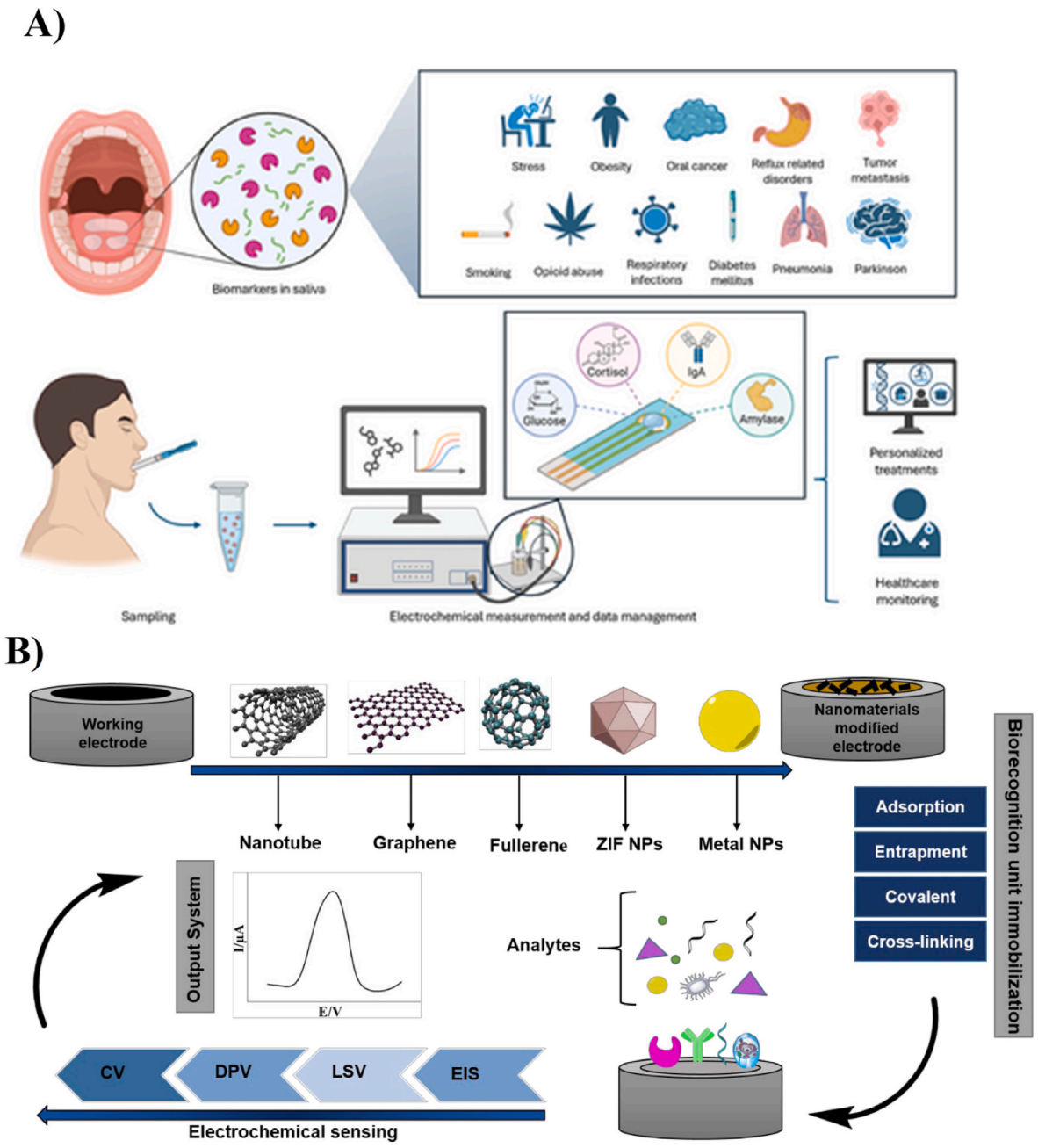


Fig. 3. A) Illustrates various strategies for electrochemical biosensors designed for biomarker detection in saliva. Reprinted with permission from Ref. [62]. B) Schematic of nanoparticle-based biosensors used for analyte detection. Reprinted with permission from Ref. [63] Copyright © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

instance, Mung et al. showed that gold nanoparticles measuring 5 nm, when encapsulated with glutathione (referred to as GSH-AuNP), can effectively identify the IL-8 biomarker present in serum samples from patients diagnosed with oral cancer [58]. Singhal et al. have explored the remarkable potential of nanomaterials in diagnostics, attributing this to their high surface-to-volume ratio and the quantum confinement effect. These characteristics significantly improve the detection limits for clinically relevant biomolecules found in biofluids. Additionally, these nanomaterials can transition from a laboratory-based platform to a POC screening device. The technology for fabricating nanomaterial-based biosensors has notably streamlined and enhanced the diagnostic processes for oral cancer [59]. Li et al. introduced an opal photonic crystal (OPC)-enhanced upconversion fluorescence method for detecting carcinoembryonic antigen (CEA) in saliva using fluorescence techniques. The incorporation of upconversion nanoparticles significantly improves the sensitivity of the biosensor. This approach serves as a valuable tool for early detection and monitoring, allowing patients to perform tests at their residence [60]. Teow et al. explored innovative biosensor techniques for diagnosing oral squamous cell carcinoma (OSCC). They identified biomarkers linked to the progression of OSCC, highlighting that biosensors are engineered to detect these biomarkers, facilitating early cancer diagnosis. Typically, biosensors are enhanced with materials such as carbon-based nanocomposites, metal oxides, gold nanoparticles (AuNPs), dendrimers, quantum dots (QDs), and various other nanomaterials (NMs) [61]. Fig. 3A shows different strategies for electrochemical biosensors designed for biomarker detection in saliva

Table 2
Comparative analysis of detection limits, response time and stability parameters of gold nanoparticles, carbon nanotubes and quantum dots for oral cancer biomarkers.

Type of nanomaterials		Response times	Biomarker	Stability parameters	limit of detection	Linear range	Ref.
Gold nanoparticles	AuNPs@ZIF-8/Cu	Not specified	ORAOV 1	RSD intra-assay: 1.46 %, RSD inter-assay: 1.76 %	63 fM	0.1–104 pM	[64]
	AuNPs-rGO	9 min	IL-8	The reusability and stability up to 3 months	72.73 ± 0.18 pg mL^{-1}	500 fg mL^{-1} to 4 ng mL^{-1}	[65]
Carbon nanotubes	SWCNTs	10 min	IL-6	Stable in storage	0.5 pg mL^{-1} (25 fM)	≤6 pg mL^{-1}	[66]
	DWCNTs	2 h 30 min	IL-1β	The relative standard deviation (RSD) values obtained 0.6 and 2.7 %	0.38 pg mL^{-1}	0.5 and 100 pg mL^{-1}	[67]
Quantum dots	DNA-QDs	30 min	IL-8	Recoveries ranged between 97.20 and 104.4 % and RSDs were all within 5 %	3.36 fg/mL	5 to 5000 fg/mL	[68]
	AuNPs/S-GQD	Not specified	IL-6	The obtained frequency RSD value 1.36 % for at least 40 QCM-based safety sensors	3.33 fg/mL	0.01–2.0 pg mL^{-1}	[69]

Abbreviation: AuNPs@ZIF-8/Cu: Cu^{2+}-doped zeolitic imidazolate frameworks and gold nanoparticle; RSD: The relative standard deviation; IL8: Interleukin-8; AuNPs-rGO: Gold nanoparticles-reduced graphene oxide; IL-6; Interleukin-6; SWCNTs: Single-Walled Carbon Nanotubes; DWCNTs: Duble-walled carbon nanotubes; IL-1β: Interleukin-1β; DNA-QDs: DNA-templated quantum dots; QCM: Quartz crystal microbalance.

Table 3
Various types of electrochemical biosensors used in the diagnosis of oral cancer.

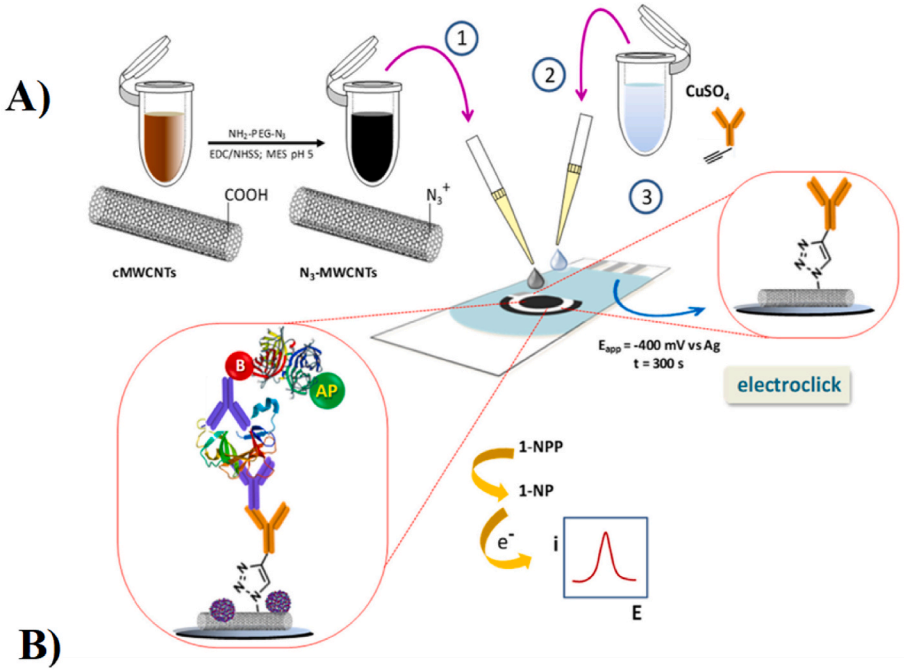
Types of electrochemical biosensors	Biomarker	provenance	pH	Temperature	Buffer composition	Advantage	Detection Limit	Ref.
Molecularly imprinted polymer (MIP)-based electrochemical sensor	Transforming growth factor β1 (TGF-β1)	Saliva	7.4	22 °C	PBS[a]	Large-scale rapid screening in the diagnosis of oral cancer	0.09 ng/mL	[91]
Molybdenum disulfide (MoS_2)-zinc oxide (ZnO) Nanocomposite Mediated Immunosensor	Interleukin-8 (IL8)	Saliva	7.4	25 °C	PBS	Excellent stability, high accuracy sensitivity, repeatability, and reproducibility	0.1 M	[92]
Electrochemical Immunosensor	Cytokeratinfragment 21-1 (CYFRA 21-1)	Saliva	7.4	700 °C	PBS	High reproducibility and good selectivity	0.014 ng/mL	[93]
MoS_2 nanoflower based electrochemical biosensor	Tumour Necrosis Factor-α (TNF-α)	Serum	7.4	4 °C	PBS	High sensitivity, and selectivity	0.202 pg ml^{-1}	[94]

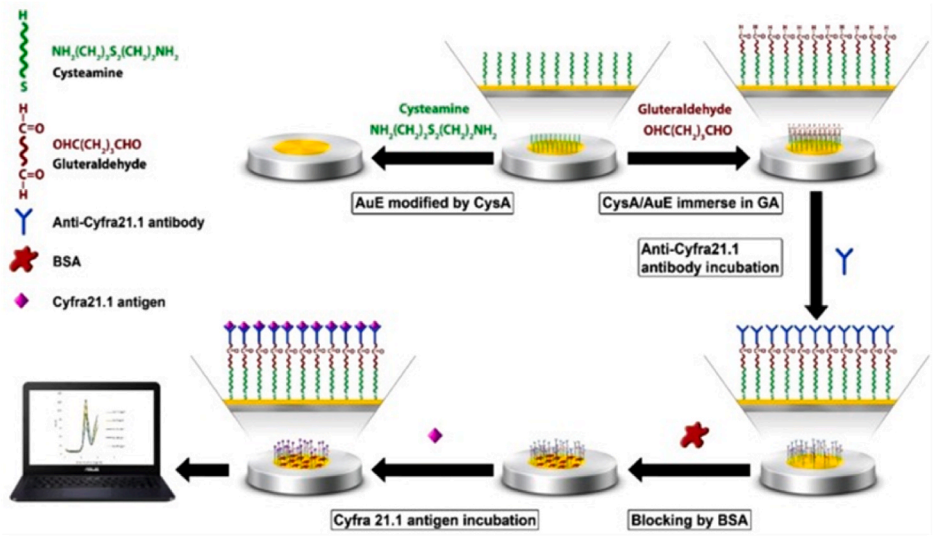
[a] Phosphate-buffered saline (PBS): The composition includes sodium phosphate (as the weak acid/base pair) and sodium chloride to maintain isotonicity with cells.

and also Fig. 3B shows the schematic of nanoparticle-based biosensors used for analyte detection. Table 2 shows a comparative analysis of the detection limits, response time, and stability parameters of gold nanoparticles, carbon nanotubes, and quantum dots for oral cancer biomarkers.

4. Electrochemical vs. optical biosensors: a comparative analysis

Electrochemical biosensors are more widely adopted compared to other biosensor technologies due to their ability to detect a broad range of biomarkers and their compatibility with standard laboratory equipment. Additionally, their potential for miniaturization makes them suitable for integration into portable and wearable devices [70–72]. Integrating electrochemical sensors into compact devices requires adherence to stringent standards for flexibility, convenience, and ease of operation. Creating portable and reliable point-of-care devices that are highly sensitive poses considerable difficulties. Addressing these challenges is crucial for advancing healthcare technology. Researchers have explored numerous strategies to enhance the functionality of sensing electrode surfaces. Approaches such as antibody conjugation, magnetic bead integration, and the use of aptamers have been widely investigated for improving detection capabilities (see Table 3). Additionally, numerous potential biomarkers for oral cancer have been identified by researchers [73]. Electrochemical biosensors are a well-established category of biosensors that primarily utilize solid electrodes. These devices leverage the inherent recognition properties of biomolecules, allowing them to immobilize sensitive biological entities on the electrode's surface. Once the biomolecules are affixed, they can selectively identify the target analytes present in the sample. To facilitate either quantitative or qualitative analysis of these analytes, the solid electrode converts the concentration of the target molecules into measurable electrical signals, such as capacitance, resistance, potential, or current, which serve as response indicators [74]. Electrochemical mechanisms serve as the foundation for most biosensors developed today. These biosensors offer advantages such as high sensitivity and selectivity, ease of miniaturization, user-friendliness, and cost-effectiveness. Due to these benefits, electrochemical biosensors have found widespread application across fields including environmental monitoring, healthcare, food safety, industrial processes, and agriculture [75–77]. Electrochemical detection mechanisms often rely on electrode modification to enhance sensitivity, selectivity, and stability [78,79]. These modifications involve coating the electrode surface with functional materials such as nanoparticles, polymers, self-assembled monolayers, or biomolecules [80,81]. Nanoparticles, like gold or carbon-based materials, increase the surface area and conductivity, while polymers and monolayers enable selective analyte binding [82]. Biomolecule modifications, such as enzymes or antibodies, facilitate specific biological interactions [83,84]. These tailored surfaces improve electron transfer kinetics and reduce interference, optimizing detection performance [85]. Modification techniques include drop-casting, electrodeposition, chemical grafting, and layer-by-layer assembly, ensuring the electrode is suited for specific analytes and operating conditions in various sensing applications [86]. Cho et al. presented the electrochemical biosensors into (1) carbon-based nanomaterials with graphene and carbon nanotubes, and (2) non-carbon-based nanomaterials with organic materials, indium tin oxide, metallic and silica nanoparticles, and nanowire. In view of their sizeable active surface area with an effective electron transfer rate, the carbon allotropes can be applied as an electrode and supporting scaffolds in the biosensors [87]. Guerrero and colleagues recently





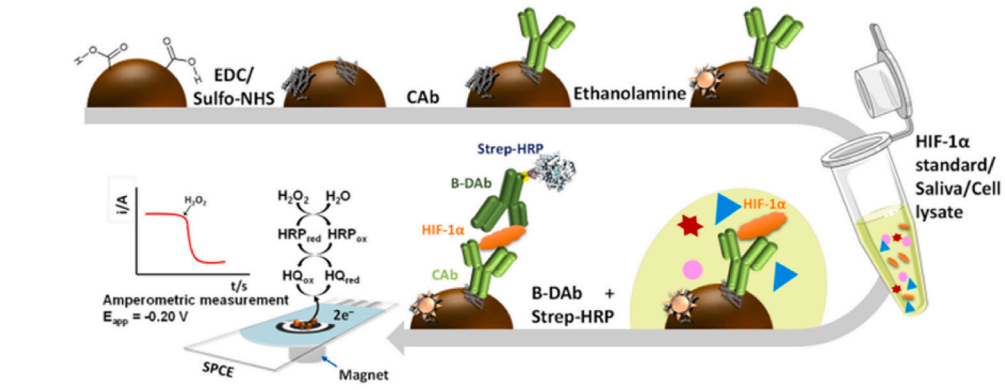


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Fig. 4. Overview of detection of oral cancer biomarkers in saliva by new electrochemical biosensors. a) Designing a sandwich-type immunosensor using differential pulse voltammetry (DPV) to detect interleukin 1β in saliva. Reprinted with permission from Ref. [88] Copyright © 2020 Elsevier B.V. All rights reserved. License Number: 5897380658596. **B)** Development of an immunosensor using a gold electrode modified with glutaraldehyde and cysteamine to detect Cyfra21.1. Reprinted with permission from Ref. [89] Copyright © 2020 The Author(s). Published by Elsevier Masson SAS. **C)** Detection of amperometric hypoxia-inducible factor-1 alpha by sandwich immunoassay method based on magnetic beads. Reprinted with permission from Ref. [90] Copyright © 2019 Elsevier B.V. All rights reserved. License Number: 5897390097510.

Table 4
Classification of optical biosensors utilized in oral cancer detection.

Types of electrochemical biosensors	Biomarker	provenance	Advantage	Detection Limit	Ref.
Surface plasmon resonance-based optical fiber biosensor	Malondialdehyde (MDA)	Saliva	These methods present several advantages, particularly in terms of sample preparation and specificity	14 pM	[102]
Optical fiber ball-shaped biosensor	Interleukin 8 (IL-8)	Artificial saliva	Achieve high sensitivity for the target protein and minimal signal alterations for the control protein	0.91 fM	[103]
lateral flow strip biosensor platform (HRCA-strip)	MicroRNA 31-5p (miRNA 31)	Saliva	Portability, rapid analysis, and low cost	3.21 fM	[104]
Plasmonic optical-fiber-based point-of-care test	Macrophage inflammatory protein (MIP)-1α	Saliva	High selectivity and very low limit of detection	2.7 pg mL^{-1}	[105]

introduced an innovative sandwich type electrochemical immunosensor designed to detect interleukin-1β in saliva. This sensor employs an electro-click approach to enhance detection sensitivity and specificity (see Fig. 4A). Their preferred detection method was differential pulse voltammetry (DPV). They used azide-modified multiwalled carbon nanotubes to reinforce disposable screen-printed carbon electrodes. Their study showed that the sandwich immunosensor achieved a significantly lower limit of detection at 5.2 pg ml^{-1} using a secondary labeling strategy for signal amplification and better performance than conventional ELISA kits [88]. Jafari and colleagues have recently created an immunosensor by attaching anti-Cyfra21.1 to a gold electrode modified with glutaraldehyde (GA) and cysteamine (CysA). To evaluate the performance of this biosensor, they utilized square wave voltammetry and compared the results to those obtained from a commercial ELISA test for validation purposes. They found that the detection limit and linear detection range were 2.5 ng/mL and 2.5–50 ng/mL, respectively (Figure 4B) [89]. Munoz-San Martín and their colleagues pioneered the amperometric detection of hypoxia-inducible factor 1 alpha. They accomplished this by utilizing magnetic beads for electrochemical sensing (Fig. 4C). Hypoxia-inducible factor-1 alpha is a crucial biomarker for predicting the outcomes of oral squamous cell carcinoma. Its significance arises from its role in the hypoxic environment of tumors. Based on the results in a rapid assay time of only 105 min, the developed biosensors could achieve a lower limit of detection of 76 pg mL^{-1} [90].

Optical devices provide an innovative approach to salivary analysis, delivering high selectivity and sensitivity without the requirement for labeling. Various non-invasive optical technologies have been developed, including surface-enhanced Raman spectroscopy biosensors, surface plasmon resonance biosensors, photonic crystal biosensors, and fluorescence-based biosensors [95]. One of the types of optical sensors known as colorimetry based on biosensors allows the non-invasive detection of biological fluids using fast and high-quality methods. These sensors typically use microfluidic channels to efficiently transport biofluids to the detection area, where the sensor reads and analyzes the sample [96]. Surface-enhanced Raman spectroscopy (SERS) is the most widely used analytical technique in optical methods due to its remarkable multiplexing capability, simplicity, sensitivity, and adaptability [97]. Nanostructured materials are often utilized to enhance the sensitivity of these devices. Optical signals have distinct advantages over other physical signals such as high superior stability, resistance to external interference, low noise levels, and sensitivity. As a result, optical biosensors have exhibited excellent performance in detecting biological systems and have made significant strides in areas such as food processing control, environmental monitoring, drug development, and clinical diagnostics (see Table 4) [98,99]. In this regard, an increase in device sensitivity occurs when using nanostructured materials. Liu and colleagues amplified the SERS signal using a biosensing platform made of plasmonic silver nanocubes (see Fig. 5A). To detect a DNA sequence associated with oral cancer, they used this endonuclease-assisted signal amplification platform integrated with heated electrodes. The results showed that the DNA biosensor achieved a cleavage time of only 1 h and also showed remarkable sensitivity in the linear range from 10 fM ~1 nm [100]. Wu and colleagues explored the use of salivary exosomes as promising biomarkers for a range of cancers, particularly oral cancer. They created a fluorescent biosensor. This biosensor employs magnetic and fluorescent bio-probes that incorporate quantum dots. This design enhances signal amplification significantly (see Fig. 5B). Specific aptamers are attached to the surfaces of magnetic microspheres. This method allows for the selective capture of CD63 proteins found in exosomes. The binding of exosomes induced shape changes in the aptamers, resulting in the release of QDs-conjugated tethered DNA concatemers, which further amplified the signal. They placed the biosensor to accurately assess the matrix effect in real samples of human saliva and buffer solutions. The results showed that the biosensor has a lower detection limit of 500 particles per microliter of solution [101].

5. Salivary biomarkers: the future of non-invasive diagnostics

Saliva is a clear, watery fluid produced by the salivary glands that is released into the oral cavity. Components of saliva can be transported either by active transport mechanisms or by passive diffusion from the associated blood vessels or the salivary glands. Head and neck cancers rank as the sixth most prevalent cancer globally, with approximately 40 % of these cases occurring in the oral cavity. Saliva contains various biomarkers, some of which can be detected using infrared spectroscopy. Rapid cell proliferation associated with cancer can lead to quick metastasis. In addition to monitoring periodontal diseases (as shown in Table 5) [106,107], saliva has been utilized to detect systemic inflammation and for screening in epidemiological research [108], highlighting its potential as a diagnostic tool. Researchers suggest that specific biomolecules in saliva could serve as biomarkers for cancer, providing a non-invasive option for early detection [109]. Various salivary proteins have been identified in the literature as potential biomarkers for oral cancer due to the direct contact of saliva with oral cancer lesions. These include salivary transferrin, interleukin-8 (IL-8) [110–112], and tumor necrosis factor-alpha [113]. Saliva has several limitations as a diagnostic fluid, with low sensitivity and specificity being among the most significant. Variability in saliva volume among individuals can lead to fluctuating analyte concentrations, which can significantly impact test accuracy depending on the timing and method

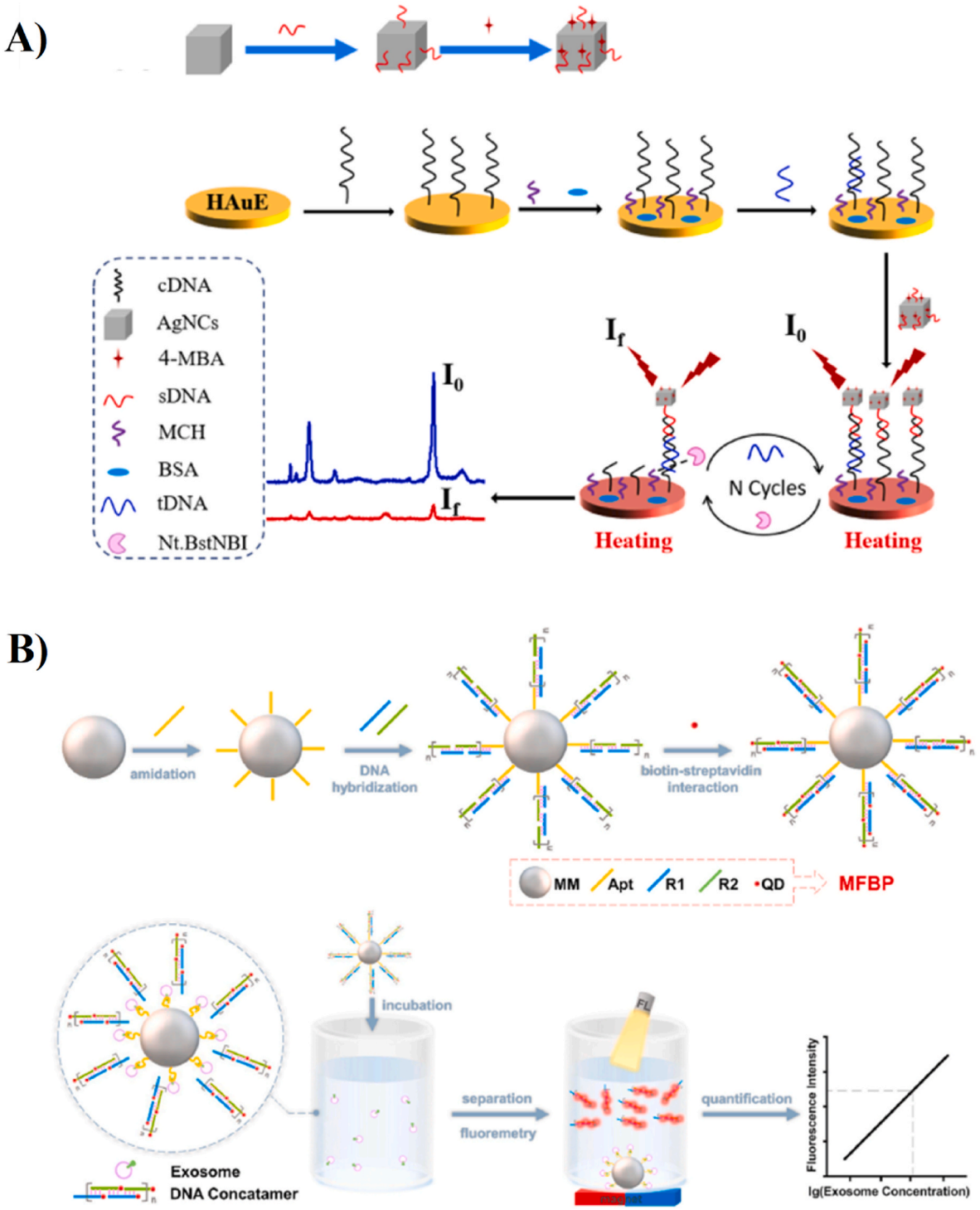

Fig. 5. Visual representation depicting the detection of oral cancer indicators in saliva through the use of sophisticated optical biosensors a) Surface-enhanced Raman scattering (SERS) integrated with a novel optical DNA biosensor. Reprinted with permission from Ref. [100] Copyright © 2021 Elsevier B.V. All rights reserved. License Number: 5897600361300. **B)** The use of fluorescent and magnetic biological probes for non-invasive detection of oral cancer through the detection of salivary exosomes. Reprinted with permission from Ref. [101] Copyright © 2020 Elsevier B.V. All rights reserved. License Number: 5897630892225.

of sample collection [114]. Since saliva biomarkers have demonstrated potential for cancer diagnosis, various biosensor technologies have been explored to identify these indicators. One such development is a surface-immobilized optical protein sensor designed to detect IL-8 protein, which serves as a salivary biomarker for oral cancer [111]. Multiple detection of different salivary biomarkers is possible due to significant efforts towards the development of assays and biosensors. Goldoni et al. explored new developments in graphene-based nano-biosensors. Their research focuses on detecting salivary biomarkers. They present an extensive overview that encapsulates the most recent advancements in graphene-based nanobiosensors and oral bioelectronics aimed at identifying salivary biomarkers. Furthermore, they delve into the intricacies of the structural configurations of graphene electronics, the utility of salivary biomarkers, the efficacy of current sensor technologies, and their potential applications in health monitoring (Fig. 6A) [115]. Wei and his team developed the first multi-electrochemical sensor for

Table 5
Summary of biomarkers observed in oral diseases such as periodontal and dental caries.

Type of oral diseases	Biomarker	The method used	Number of patients	Sample	Ref.
Periodontal	Phosphates	Multiplex magnetic bead panels	8314	saliva	[117]
	Alkaline phosphatase	ALP kit	72	saliva, serum, and gingival crevicular fluid	[118]
	Hypoxia-inducible factor 1α (HIF-1α)	Enzyme-linked immunosorbent assay (ELISA) and real-time PCR (RT-PCR)	48	Serum, plasma, cell culture supernatant	[119]
Dental caries	Ghrelin & Leptin	Multiplex Magnetic Bead Panels on a Luminex 200 Platform	5456	Saliva	[120]
	Carbonic anhydrase VI activity ($CA\ VI_{ACT}$)	Zymography	44	Saliva and biofilm	[121]
	1β, IL-6, IL-8, and IL-10	ELISA	128	Saliva	[122]

identifying salivary biomarkers linked to oral cancer. Their findings demonstrated that detecting both IL-8 mRNA and protein together enhances diagnostic accuracy. This innovative approach significantly improves the potential for early oral cancer diagnosis. In their study, they used an electrochemical (EC) sensor that has 16 integrated gold electrode arrays pre-coated with specific protein and mRNA probes on different electrodes. The detection process uses a sandwich assay. Standard in vitro translated (IVT) RNA and protein are added to saliva samples (Fig. 6B(a)). For the IL-8 mRNA probe, researchers compared complementary IL-8 mRNA with the salivary internal reference gene S100A8 RNA. For the IL-8 protein probe, they compared IL-8 protein with IL-1β protein, another known oral cancer biomarker. The amperometric detection results are shown in Fig. 6B(b). The accompanying bar graphs are also presented in Fig. 6B(c) [116].

6. DNA, RNA and protein salivary-based biosensors for oral cancer

Salivary-based sensors are classified into three major types depending on their biosensing targets: Biosensors for deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein [123]. DNA-based biosensors utilize the principle of complementary base pairing to attain enhanced affinity and specificity in their detection mechanisms. The DNA recognition elements can effectively distinguish between DNA sequences, even in the presence of single-base mismatches [124]. In contrast to proteins, DNA molecules exhibit greater resistance to denaturation [125]. DNA-based biosensors are capable of multiplex detection while achieving a low detection limit [126]. However, the process of deriving DNA ligands through the SELEX procedure to target analytes is notably time-consuming. Furthermore, the SELEX process is labor-intensive, necessitating the involvement of skilled technicians or experts in the field. One significant challenge that DNA-based biosensors encounter is the maintenance of aptamer conformation.At present, the majority of DNA-based biosensors exhibit peak performance only under particular conditions. The conformation of aptamers can be significantly affected by a range of physical and chemical factors, resulting in conformational changes that greatly impact their binding affinity in practical applications [127]. Identifying and discriminating DNA sequences can be time-consuming, associated with low hybridization efficiency, and challenging. To tackle these challenges, DNA sensors have been integrated into high-throughput analysis, leading to substantial reductions in cost, effort, and time. Biosensors, acting as biorecognition elements when paired with various transduction mechanisms, have significantly contributed to the rapid advancement of bioanalysis and related technologies. These characteristics, combined with benefits like simple manufacturing and low operational costs, make them an attractive option for non-invasive oral cancer detection using saliva analysis. This approach offers an efficient solution for early diagnosis in patients [124,128,129]. Stobiecka et al. conducted a comprehensive study on advancements in biosensing systems and biosensors aimed at early cancer detection, specifically targeting the anti-apoptotic protein survivin and its messenger RNA (Sur mRNA). They found that Survivin, a member of the inhibitor of apoptosis proteins (IAP) family, plays a crucial role in regulating the cell cycle, inhibiting programmed cell death (apoptosis), and promoting enhanced cellular proliferation. These functions contribute to uncontrolled cancer growth and metastasis. Based on their results, biosensors for Sur detection in exosomes and circulating tumor cells is a promising alternative for cancer screening [130]. Li et al. developed an electrochemical DNA biosensor that integrates a target-triggered, entropy-driven, nonenzymatic, and isothermal amplification approach with nanocomposites made of gold nanoparticles/zeolitic imidazolate frameworks-8. This innovative design allows for highly sensitive detection of the oral cancer biomarker ORAOV 1 in saliva. Fig. 7A illustrates the entropy-driven catalytic (EDC) amplification mechanism. Specifically, a substrate complex (L/S1/S2) is assembled through the hybridization of S1, S2, and L. Here, L acts as a linker with two toehold regions (toehold 1 and toehold 2), while the fuel strand (F) serves as a complementary molecule to L. Initially, toehold 2 is blocked by S1, preventing the hybridization of F with L. Upon binding of the target (T) to L, a strand displacement reaction mediated by toehold 1 occurs, releasing S1 and exposing toehold 2. The exposed toehold 2 then facilitates the hybridization of L with F, resulting in the release of S2 and the target molecule (T). The re-released target initiates subsequent cycles, generating additional S1, which captures numerous P2-AuNPs@ZIF-8 nanocomposites through a sandwich hybrid structure involving P1, S1, and P2, thereby producing amplified electrochemical signals. Leveraging the EDC amplification strategy, combined with the high electrical conductivity and outstanding electrocatalytic properties of AuNPs@ZIF-8 nanocomposites, an ultra-sensitive, enzyme-free, and label-free electrochemical DNA biosensor has been developed. The biosensor operates effectively across a linear concentration range from 1 fM to 1 nM. It achieves an extraordinarily low limit of detection of 163 aM. The biosensor demonstrates high specificity toward the target biomarker ORAOV 1 [131]. RNA-based biosensors are particularly impactful due to their ability to detect specific RNA transcripts that correlate with pathophysiological changes in oral cancer. Electrochemical bioplatforms have been developed to detect IL-8 mRNA in raw saliva, showcasing their rapid assay time and high fidelity in distinguishing between cancerous and non-cancerous states [31]. The dynamic approach of measuring both RNA and protein biomarkers allows for a more comprehensive understanding of the disease state and progression [132]. During the carcinogenesis process, tumor-derived materials are directly released into both the bloodstream and saliva, leading to the detection of altered microRNA (miRNA) expression in various malignancies [133,134]. This release of miRNAs into the bloodstream and saliva can be attributed to both necrotic cell death and apoptotic, as well as active secretion by living cells [135]. Oral tumors release various biomarkers, including tumor DNA and miRNAs, into saliva. These markers can be found either free or encapsulated within extracellular vesicles like exosomes [136]. Luo and his colleagues were able to create a ratiometric electrochemical biosensor using a nucleic acid-assisted strand displacement mechanism. They found that it was

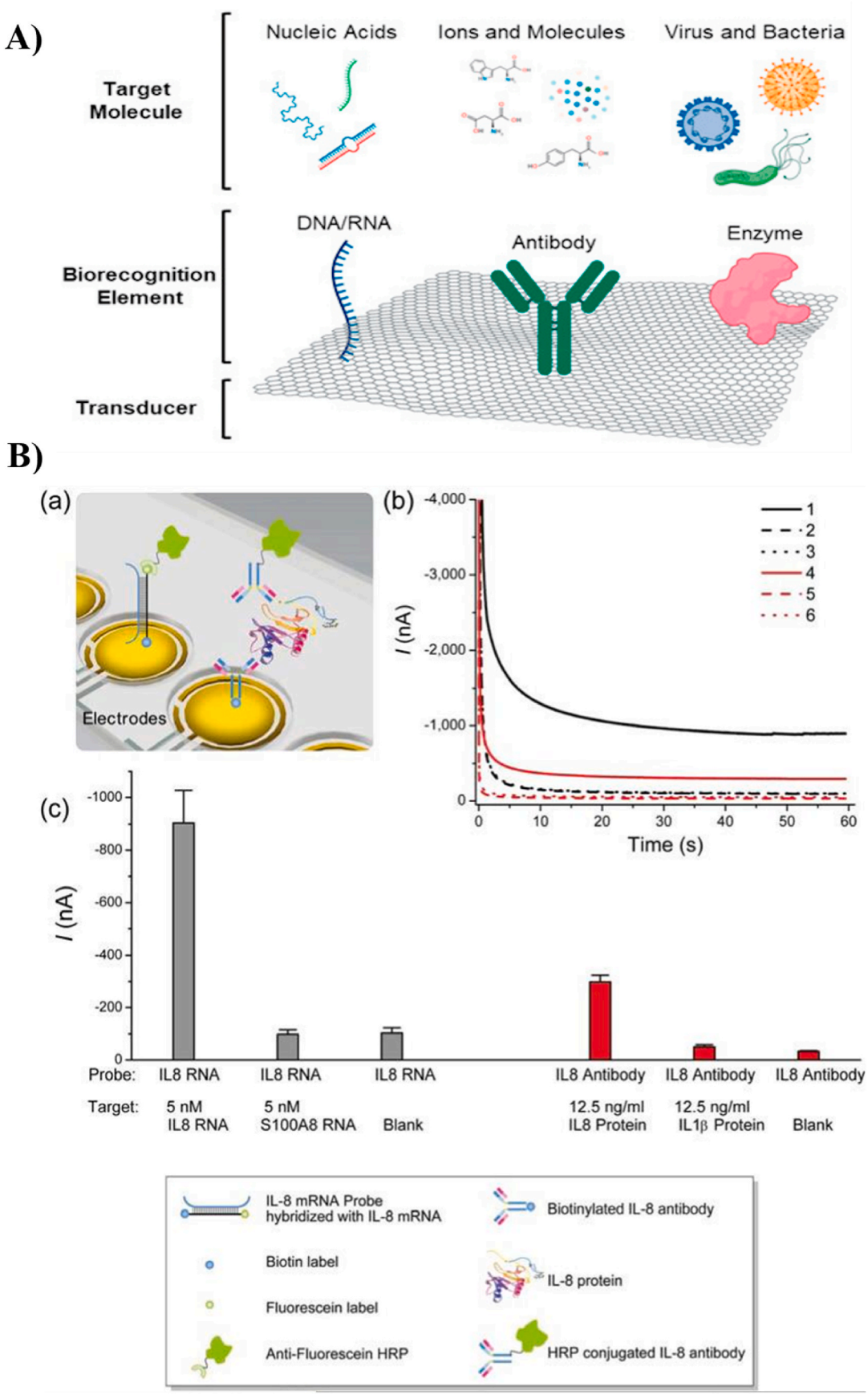

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Fig. 6. A) A conventional saliva-based biosensor is composed of three primary elements: a substrate, which can be either carbon-based or metallic; a biorecognition element, such as probes, enzymes, or antibodies, engineered to identify specific targets like proteins, nucleic acids, or antibodies; and a transducer that converts the binding events into measurable signals. The interactions at the molecular level lead to observable changes, either colorimetric or spectrometric, which can be detected using various technologies. Reprinted with permission from Ref. [115] Copyright © 2020 Elsevier B.V. All rights reserved. License Number: 5897631328854. **B)** Electrochemical (EC) sensor for the detection of multiple salivary biomarkers (a) The figure shows a collection of electrodes. These electrodes identify proteins on the right. They also identify mRNA on the left. (b) It presents the amperometric detection of the IL-8 mRNA probe. This includes (1) 5 nM of IL-8 IVT RNA, (2) 5 nM of S100A8 IVT RNA, and (3) a blank control. It displays the IL-8 protein probe as well. This includes (4) 12.5 ng/ml of IL-8 protein standard, (5) 12.5 ng/ml of IL-1β protein standard, and (6) a blank control. (c) A bar graph illustrates the levels of IL-8 mRNA and IL-8 protein in saliva. It also shows control experiments involving S100A8 and IL-1β. The average values and standard deviations are based on three repeated trials. Reprinted with permission from Ref. [116].

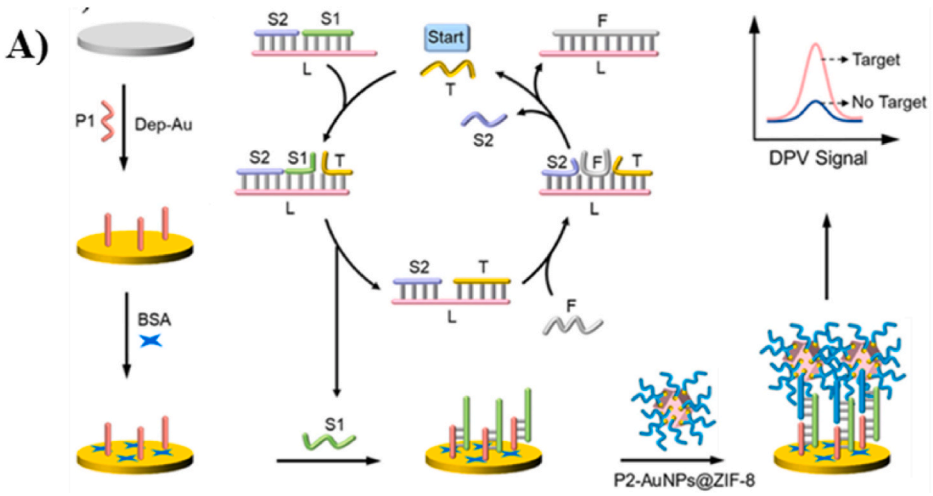
possible to achieve a detection limit of 2.3 fM through enhanced reproducibility for the detection of cancer-associated exosomal miR-21. Their design featured a Y-like structure activated by the presence of miR-21 as a target biomarker, with detection confirmed through differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) [137]. Óscar Rapado-González and colleagues investigated the potential of cell-free microRNAs as biomarkers for oral cancer. In their research, it was found that exosomes containing tumor-derived miRNAs travel to the salivary glands through the bloodstream, leading to changes in salivary secretion due to tumor invasion (Fig. 7B) [138]. Additionally, protein biomarkers for cancer detection can measure components believed to signal abnormal biological processes, disease progression, or responses to treatment interventions [66,124]. Biomarkers are often extracted from biofluids, and their levels typically reflect disease status. Salivary samples are particularly valuable for non-invasive screening of oral cancer, as several tumor markers have been identified in saliva [139,140]. In a separate study, human saliva was used to develop electrochemical magneto-biosensors designed for the detection of the protein IL-8 and mRNA [31]. Dong et al. reviewed advances in electrochemical biosensors based on nanomaterials for protein biomarker detection in saliva. The strategy for utilizing electrochemical methods to detect protein biomarkers in saliva is illustrated in Fig. 7C [141].

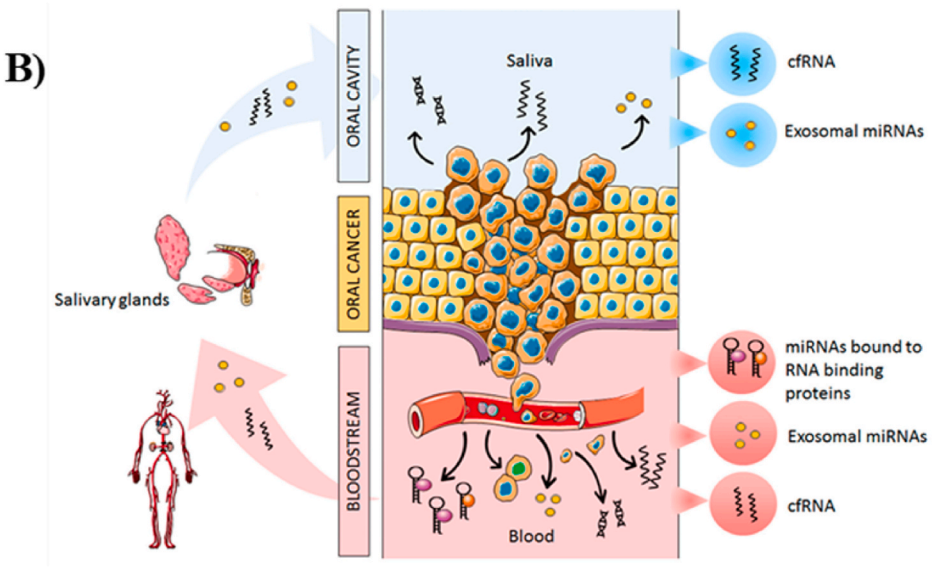
7. Emerging strategies for early detection of oral cancer

Early detection of oral cancer is pivotal for effective treatment and improving patient survival rates, leading to substantial research into innovative diagnostic strategies. Emerging biosensors using saliva have gained traction as a non-invasive method for identifying oral cancer biomarkers, allowing for rapid and accurate diagnosis. Saliva is an accessible biofluid that contains a myriad of molecular markers such as DNA, RNA, and proteins corresponding to neoplastic changes in oral tissues. Utilizing salivary biomarkers, researchers can detect alterations in DNA methylation patterns known to be associated with malignancies, thus facilitating early diagnosis [142]. Furthermore, advancements in nanotechnology have revolutionized diagnostics, particularly through the use of nanomaterials in biosensing platforms. Nanoparticle-based sensors enhance the sensitivity of biomarkers detection, enabling these devices to capture low-concentration cancer markers in saliva [143]. Various techniques, such as electrochemical and optical biosensors, have been developed to leverage these nanomaterials, resulting in improved analysis of salivary biomarkers with minimal sample preparation time. For instance, specific DNA sequences can be designed to hybridize with RNA targets from cancerous cells, enabling precise and sensitive detection of oral cancers. Additionally, the “Omics” technologies are changing the landscape of oral cancer diagnosis. These include genomics, proteomics, and metabolomics. They help detect oral cancer earlier and more effectively. These technologies enable comprehensive profiling of the biomolecular landscape of saliva, revealing unique signatures that can differentiate cancerous from non-cancerous states [144]. Through these approaches, researchers have identified salivary proteins such as matrix metalloproteinases (MMPs) and interleukins that serve as potential biomarkers for early-stage oral cancers [145]. Personalized medicine is also becoming integral to early detection strategies, as these omics approaches facilitate tailored treatment plans based on individual molecular profiles. Integrating artificial intelligence (AI) into diagnostic practices represents another frontier in the early detection of oral cancer. Machine learning algorithms can analyze vast datasets from imaging, biosensor outputs, and biomarker profiles, allowing for the identification of cancerous lesions that are not always evident during visual examinations [146]. Advanced imaging technologies, such as narrow-band imaging (NBI) and fluorescence-based techniques, complement these developments by enhancing visualization of oral mucosa and improving detection rates of dysplastic lesions [147]. The combination of these imaging techniques with biomarker analysis can yield a multi-modal approach that significantly increases the accuracy of early diagnosis [148]. Moreover, mobile health applications that enable remote monitoring of salivary biomarkers are beginning to emerge. These applications focus on real-time data collection and analysis, empowering patients and healthcare providers to track potential indicators of oral cancer more proactively [149].

8. Integration of biosensors in clinical practice for oral cancer

The integration of biosensors in clinical practice for oral cancer diagnosis signifies a pivotal advancement in the realm of early detection and personalized medicine. Biosensors, particularly those utilizing saliva as a diagnostic medium, offer a non-invasive alternative to traditional tissue biopsies, facilitating easier and more frequent screening of at-risk populations [150]. The ability to detect specific biomarkers associated with oral cancer, such as tumor necrosis factor-α (TNF-α), through saliva greatly enhances the potential for early diagnosis, which is critical given the often-asymptomatic nature of the disease in its initial stages. Recent developments have led to the creation of highly sensitive electrochemical immunosensors that leverage advanced materials, like reduced graphene oxide hydrogels, to achieve low limits of detection and high specificity even in complex biological fluids [151]. These innovations promise rapid results and can be easily implemented in clinical settings, increasing the likelihood of routine screening and continuous patient monitoring. Moreover, the concept of point-of-care testing is becoming increasingly feasible with biosensor technology, allowing healthcare providers to perform tests at the patient’s location, thereby reducing the time between sample collection and diagnosis. The integration of these technologies is further supported by the rise of personalized medicine, where diagnostic approaches can be tailored based on individual biomarker profiles, enhancing treatment outcomes [152]. However, challenges remain, including the need for regulatory approvals and standardization of testing protocols to ensure reliability across varied clinical environments. Addressing these challenges will be crucial for the widespread adoption of biosensors in oral cancer diagnostics. The advancement of biosensor technologies is poised to revolutionize clinical applications, particularly in the realm of oral cancer. By facilitating earlier detection and better management strategies, these innovations are likely to lead to improved outcomes for patients suffering from this disease [150]. Table 6 shows a specific case study or clinical trial results of the practical application of biosensors in oral cancer with actual sensitivity and specificity values of patient samples.





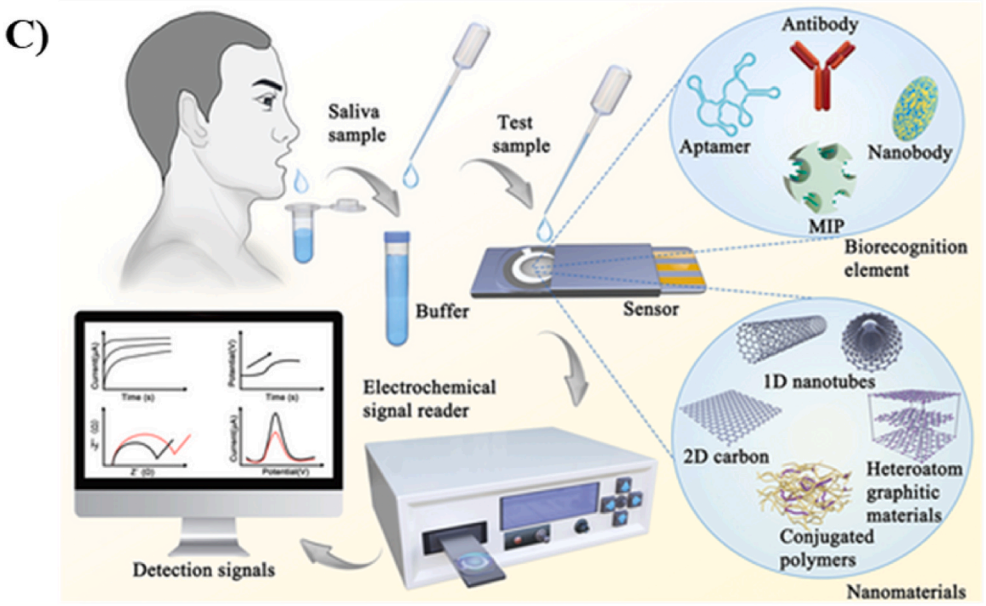


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Fig. 7. A) working principle of electrochemical DNA biosensor for detection of ORAOV 1 based on target-triggered and entropy-driven catalytic amplification strategy integrated with AuNPs@ZIF-8 nanocomposites Reprinted with permission from Ref. [131] Copyright © 2024 Elsevier B.V. All rights reserved. License Number: 5897640936463. B) A diagrammatic representation showing how cell-free miRNAs are released into the blood and saliva as a result of oral cancer (cfRNA, cell-free RNA) Reprinted with permission from Ref. [138]. C) Illustration of electrochemical detection of protein biomarkers in saliva by the nanomaterial-based biosensor Reprinted with permission from Ref. [141].

Table 6
Specific case study or clinical trial results of practical application of biosensors in oral cancer.

Type of diagnosis	Case study	Sample	Biomarker	Sensitivity	Specificity	Ref.
Oral cancer	34	Saliva	_____	100 %	89 %	[153]
Oral squamous cell carcinoma	43	Saliva	P16INK4A/RASSF1A	53.5 %	87.5 %	[154]
Periodontitis	41	Saliva	miR-155	97.14 %	78 %	[155]
			miR-146a	88.57 %	58.54 %	
Periodontal	45	Saliva	Matrix metalloproteinase-8 (MMP-8)	88.6 %	75 %	[156]
Periodontitis	27	Second rinse	Active Metalloproteinase-8	85.2 %	100 %	[157]

9. Future perspectives and opportunities

Future perspectives on oral cancer bioassays hold considerable promise, with technological advances offering new ways to improve early detection, diagnosis, and monitoring. Biosensors, which leverage biological molecules for detecting cancer biomarkers in saliva, blood, or tissues, present a non-invasive and cost-effective tool for oral cancer management. Future perspectives focus on enhancing sensitivity and specificity, enabling detection of cancer at the earliest stages when treatment outcomes are most favorable. Nanotechnology, electrochemical biosensors, and bioinformatics-driven sensors are key areas poised for rapid development. These technologies can potentially integrate with portable devices or point-of-care diagnostics, expanding accessibility to underserved populations and reducing healthcare disparities. However, one of the major challenges is the inherent complexity and heterogeneity of oral cancers, which complicates the identification of universal biomarkers for effective screening. Additionally, traditional diagnostic techniques often lack the sensitivity required to detect early-stage cancers, leading to delayed treatments and poorer prognoses for patients. Socioeconomic factors further hinder access to advanced biosensing technologies, particularly in underserved populations, creating disparities in early detection rates. However, advancements in nanotechnology and biosensing platforms present significant opportunities for innovation. The development of highly sensitive, nanoparticle-based biosensors can enable non-invasive detection of cancer biomarkers from saliva, which not only enhances accuracy but also improves patient compliance with screening protocols. Furthermore, integrating artificial intelligence into biosensing technologies can facilitate better data analysis, leading to improved identification of subtle abnormalities that may indicate malignancies. This integration of AI can also personalize diagnostic approaches, tailoring them to individual patient profiles based on specific biomarker signatures. Additionally, as the field moves towards point-of-care diagnostics, making these technologies more accessible and user-friendly can further bridge the gap in health disparities. The confluence of these evolving technologies and approaches promises to not only address the current challenges but also to foster a future where early detection of oral cancer becomes significantly more effective, ultimately enhancing survival rates and improving patient outcomes overall.

10. Conclusion

Nanomaterials-based biosensors have become a valuable asset for the early detection and diagnosis of oral cancer, owing to their improved sensitivity, specificity, and fast response times. The distinctive physicochemical properties of nanomaterials, including high surface-to-volume ratios, adjustable electrical and optical traits, and the capacity to conjugate with biomolecules, render them exceptionally well-suited for biosensing applications. These biosensors can detect a range of biomarkers associated with oral cancer, including nucleic acids, proteins, and metabolites, at very low concentrations, offering potential for early diagnosis when treatment is most effective. Various types of nanomaterials, such as gold nanoparticles, carbon nanotubes, graphene, and quantum dots, have been integrated into biosensors to enhance their performance. Nanomaterial-based biosensors can be engineered to operate using various mechanisms, including electrochemical, optical, or piezoelectric methods. Each of these mechanisms provides distinct advantages regarding sensitivity and ease of operation. Additionally, these biosensors facilitate point-of-care diagnostics, a vital feature in resource-limited environments where access to advanced medical facilities is often constrained. Despite the significant progress, challenges remain in translating these biosensors from the laboratory to clinical practice. Issues such as large-scale fabrication, stability, reproducibility, and regulatory approval need to be addressed. Additionally, more research is required to validate their efficacy in real-world settings, ensuring their accuracy in diverse patient populations and different stages of oral cancer. In conclusion, nanomaterials-based biosensors hold great potential for revolutionizing oral cancer detection and monitoring. Their ability to provide rapid, sensitive, and non-invasive diagnosis offers a promising path toward improving patient outcomes through early intervention, personalized treatment, and better disease management.

CRediT authorship contribution statement

Yasamin Ghahramani: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Seyedeh Sara Tabibi:** Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Mohammad Mizanur Rahman Khan:** Formal analysis, Investigation, Methodology, Resources, Writing – review & editing. **Alireza Asadi:** Writing – review & editing, Software, Resources, Investigation, Formal analysis. **Elaheh Mohammadi:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis. **Ehsan Khaksar:** Writing – review & editing, Software, Resources, Methodology, Investigation. **Erfan Khaksar:** Writing – review & editing, Software, Resources, Methodology, Investigation. **Masoomeh Yari Kalashgrani:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis. **Mohammed M. Rahman:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Wei-Hung Chiang:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Conceptualization. **Seyyed Mojtaba Mousavi:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

Data will be made available on request.

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