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REVIEW

Recent Advances in Nano-Bio-Sensing Fabrication Technology for the Detection of Oral Cancer

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Abstract

Nanotechnology-based miniaturized devices have been a breakthrough in the pre-clinical and clinical research areas, e.g. drug delivery, personalized medicine. They have revolutionized the discovery and development of biomarker-based diagnostic devices for detection of various diseases such as tuberculosis, malaria and cancer. Nanomaterials (NMs) hold tremendous diagnostic potential due to their high surface-to-volume ratio and quantum confinement phenomenon, improving the detection limit of clinically relevant biomolecules in bio-fluids. Thus, they are helpful in the translation of bench-on platform to point-of-care (POC) screening device. The nanomaterial-based biosensor fabrication technology has also simplified and improved oral cancer (OC) or oral squamous cell carcinomas (OSCC) diagnosis. The fabrication of nano-bio sensors involves application specific modifications of NMs. The unique properties functionalized NMs have augmented their application on the nano-biosensing platform for the detection of clinically relevant biomolecules in bio-fluids. Therefore, this article summarizes the recent advancements in the process of fabrication of nano-biosensors for detection of OC.

Keywords Prognosis · Nanobiosensors · Nanomaterials · Oral squamous cell carcinoma · Oral cancer · Point-of-care

Introduction

Biosensors research involves the application of cutting-edge technology for the detection of diseases, more precisely a minimally or completely non-invasive method for early disease diagnostics [1–4]. Nanotechnology-based miniaturized devices have been a breakthrough in the pre-clinical and clinical research areas involving drug delivery, personalized medicine and hold tremendous diagnostic potential, the area now termed 'Nano-diagnostics'. The nanomaterials (NMs) have revolutionized the discovery and development of biomarker-based diagnostic devices for the detection of various diseases such as tuberculosis, malaria and cancer [5–7]. Earlier, the conventional techniques and tools were expensive, time consuming, instrument dependent and required technical skills for execution and analysis. But, the present NMs-based biosensors have emerged as the promising platform for the development of cancer theranostics, i.e. diagnosis and therapy occurring simultaneously. Technically, they are Biological microelectromechanical system (BioMEMS), where the biological moieties are manipulated for detection, analysis, characterization and measuring the activity of target biomolecules for attaining a conclusive deduction, i.e. pathology or functionality. The BioMEMSs have been successful in gaining global popularity due to their ability to detect biomolecules in micro-volumes in the millisecond time frame with high sensitivity [8], thereby providing a base for the development of a micro-total analysis system (μTAS) that acts as complete and compact sample preparation, detection and analysis platform, e.g. GeneXpert MTB/RIF, (Cepheid Inc.) for tuberculosis test from sputum [9]. This progression of technology led to the personalization of

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medicine combining both therapeutic and diagnostics, eventually reducing the overall cost, time and ease of operation.

The biosensor-based devices provide an easier way to detect and quantify the target biomolecule from a given mixture of bio-analytes which can be any micro- or macro-biomolecule (such as nucleic acids, peptides, amino acids and carbohydrate), pathogen and metabolite present in biofluids [10–15]. These biosensors have several combined advantages over conventional techniques such as cost effective, ease of handling, miniaturization and do not require an expert for the analysis of results. Previously, it has been reported that fabricated biosensors had limitations such as low sensitivity, instability, etc. [16]. Therefore, combining advances in bio-sensing with nanotechnology helps in overcoming the above limitations and offers an opportunity to significantly improve prospective strategies towards the detection and diagnosis of OSCC. In this regard, various types of NMs have been used for the fabrication of nanobiosensors such as gold nanoparticles (AuNPs), quantum dots (QDs), dendrimers, metal oxides, carbon-based, nanocomposites, etc. The unique properties of above NMs have improved the biosensor functionality with faster detection, improvised detection limits and better reproducibility [17, 18]. Due to above enhancements, these nanobiosensors require low sample volume and provide high accuracy (error rate is less than 1%), specificity, cost effective, less time consuming, simple procedure (no sample pre-treatment required) and easy to implement the automatic analysis. Hence, it is suggested that the nano-based bio-sensing may be translated to improved patient treatment outcome and care by utilizing it for early detection of OC.

Oral cancer (OC) or oral squamous cell carcinoma (OSCC) is the sixth most prevalent form of cancer across the globe [19]. OSCC affects both the genders but has more predilection towards male. OSCC is a huge burden as its delayed diagnosis leads to poor prognosis of the disease. The five-year survival rate of OSCC patient has been estimated to be around 50% [20]. The pre-dominant variables associated with the onset and progression of OSCC depend on the exposure to smoking, chewing tobacco, areca nut, betel quid, diet habits, viruses (human papillomavirus—HPV, cytomegalovirus—CMV) and occupational exposures [21–25]. All these factors lead to genetic and epigenetic changes causing the onset of OSCC. The oral pathogenesis initiates with a burning sensation in the mouth, some restriction in mouth opening, any red or white lesions in the oral cavity, but histology of the lesion may present dysplastic features. Such conditions are categorized under oral potentially malignant disorders (OPMDs). Gradually physical changes become pronounced with non-healing of oral ulcers, vocal changes and may be considered as indications for the onset of OSCC. This can be life threatening and associated with higher morbidity if not detected early at the very initial OPMD stage and hence early detection is critical for the prognosis of the disease and to improve the survival of the sufferer.

The traditional methods which have been used individually or in combination as adjuvant investigation strategy to differentiate OSCC from normal oral mucosa have been summarized in Table 1. These methods are invasive, time consuming, expensive, highly subjective, labour-intensive and depend on the competency of the investigator. Also, because of the low concentration (ranging from fg/ml to µg/ml) of the biomarkers (such as Interleukin-1: IL-1, Interleukin-6: IL-6, Cytokeratin Fragment-21-1: CYFRA-21-1) in the exfoliated cells, tissue samples or biological fluids (saliva, human blood, semen and urine), the disease might remain undetected unless subjected to the specific diagnostic test. The level of these biomarkers remains low in a healthy person, but alters with the progression of the disease (Table 2). When detected early, the prognosis as well as the survival of the patient tends to improve. This demands urgent attention to the availability of minimally or completely non-invasive, patient friendly diagnostic tools. These tools can precisely and accurately evaluate the level of biomarkers and assist in the accurate diagnosis of OSCC progression. Hence, the early detection of OSCC is prerequisite for the treatment of disease and improvement of human well-being.

The advancements in the field of nanobiosensor-based emerging diagnostics and POC screening devices have paved the way for fast, simple, accurate and robust assays for OC or OSCC detection. This article briefly summarizes the improvements in the fabrication process of nanobiosensors and highlights the recent advances in nano-based sensing platform for detection of OSCC.

Nanobiosensor: A Platform for Detection of Target Bio-Analyte

A biosensor is an analytical device that consists of a bio-recognition probe, transducers and signal processing system which has been coupled to detection assembly (Fig. 1). These can be classified broadly based on bio-recognition probe, detection technique and type of interaction utilized in detection of bio-analytes and named accordingly (Fig. 2). In biosensors, the bio-recognition probe includes a whole cell (bacterial or virus), nucleic acids (DNA, RNA, aptamers) and proteins (natural or engineered antibodies, enzymes, plant proteins) [26–29]. The working principle involves the recognition of the target bio-analytes in the bio-fluid whenever in close proximity with the bio-recognition probe. This recognition is done with the help of biomolecule or a biomimetic material immobilized onto the surface of the substrate and their stability is crucial in regulating the sensitivity, precision, reproducibility, stability and robustness

Table 1 The list of clinical methods to classify benign, pre-malignant and malignant conditions in oral cancer

Detection category	Method	Sample	Sensitivity (%)	Specificity (%)	Advantages	Limitations	References
Biopsy	Incisional biopsy Excisional biopsy Punch biopsy	Tissue	–	–	Gold standard	Invasive method Painful Time consuming Diagnosis depends on quality of specimen and pathologist's subjective judgment	[115]
Cyto-pathological technique	Oral brush test Liquid-based cytology Laser capture microdissection	Oral trans-epithelial cellular samples	55–83.1 88.8 –	100 100 –	Non/minimally invasive, low cost, easy to perform, basic microscopy setup and dyes are required	False-positive results Labour-intensive Diagnosis depends on quality of specimen and pathologist's subjective judgment	[116, 117]
Vital staining	Toluidine blue staining Methylene blue Rose Bengal Lugol's iodine	Intra-oral examination	38–100 90–91.4 90–100 87.5–94.7	9–100 66.6–69 73.7–89.09 83.8–84.2	Low cost Simple Light microscope, cheap dyes and reagents are required	Low specificity due to false-positive results Not considered as a substitute for biopsy Dissimilarity in approaches ranging from one time rinse, double time rinse, oral mucosal "painting" and uncertainty of using or defining pale staining which is subjective	[118–128]
Chemiluminescence and fluorescence-based techniques	ViziLite ViziLite Plus Microlux/DL Orascoptic Tissue fluorescence imaging (VELscope)	Intra-oral examination—oral cavity is rinsed with solutions and examined with handheld light source	71–100 77.3 77.8–94.3 – 30–100	0–84.6 27.8 70.7–99.6 – 15–100	Minimally/non-invasive Very fast Provides information about metabolic and structural abnormalities, amenable to high throughput screening (HTS), amenable to point-of-care device-based detection	Less specific Increased cost Clinical utility needs to be investigated only used to identify lesions that may have been overlooked with a conventional oral examination and not for determining whether a lesion is precancerous or cancerous	[41, 128–135]

Table 1 (continued)

Detection category	Method	Sample	Sensitivity (%)	Specificity (%)	Advantages	Limitations	References
Laser and vibrational spectroscopy-based techniques	Raman spectroscopy	Intra-oral examination	100	77	Sensitive Specific Minimally/non-invasive Sample preparation does not involve intensive skills Amenable to point-of-care device-based detection	Highly subjective Time consuming Examines very less area at a time High cost of instrumentation Extensive process Lack of spatial information and multifaceted algorithms to discern various categories of tissues	[130, 135–144]
	Elastic scattering spectroscopy		72.98	68–75			
	Diffuse reflectance spectroscopy		76–100	76–97			
	Narrow-band imaging		84.62	94.56			
	Optical coherence tomography		85	78			
	Confocal laser endomicroscopy		80	100			
	Confocal reflectance microscopy		73	88			
Molecular and immunohistochemical analysis	Gene alterations Epigenetic changes Viral genome studies Proliferation index and AgNOR analysis Immuno-histochemical identification of markers	Tissue	–	–	Sensitive Specific Amenable to HTS Minimally invasive	Detection methods is limited as the biomarkers with low concentrations in the tissue samples or body fluids may not be detected	[145–148]
Radiographic	Magnetic resonance imaging Computed tomography Positron emission tomography Cone beam computed tomography	Intra-oral examination	–	–	Non-invasive No sample preparation is required High accuracy for locating the recurrent tumour	Sensitivity for detecting small Earlier intra-epithelial lesions are insufficient High cost of instrumentation Radiation hazard	[130, 149, 150]

Table 2 Secretion range of some biomarkers in healthy individuals and OSCC patients

Biomarker	Salivary concentration		Serum concentration		References
	Healthy	Cancerous	Healthy	Cancerous	
IL-1	173.2 ± 66.9 pcg/ml	454.4 ± 215.8 pcg/ml	–	–	[151]
IL-1β	354 ± 61.51 58 pg/ml	906 ± 62.21 58 pg/ml	–	–	[152]
IL-6	1.4 ± 0.9 pcg/ml and 16 ± 3.91 pg/ml	88.2 ± 43.2 pcg/ml and 129 ± 66.59 pg/ml	≤ 6 pg/ml and 3 ± 0.23 pg/ml	≥ 20 pg/ml and 3 ± 0.58 pg/ml	[71, 151–153]
IL-8	1580.7 ± 789.0 pcg/ml and 250 58 pg/ml	3154.1 ± 1023.2 pcg/ml and 720 58 pg/ml	107.5 58 pg/ml	168.1 58 pg/ml	[151, 153, 154]
TNF α	3.0 ± 1.9 pcg/ml and 38 ± 3.23 58 pg/ml	28.9 ± 14.6 pcg/ml and 34 ± 21.58 58 pg/ml	8 ± 1.34 pg/ml	5 ± 2.51 pg/ml	[151, 152]
ET-1	1.16 ± 0.29 58 pg/ml	4.37 ± 1.35 58 pg/ml	2.221 + 0.497 pg/ml	4.063 + 1.002 pg/ml	[155, 156]
Osteopontin	35.1 ng/ml	39.23 ng/ml	–	–	[153]
CYFRA-21-1	3.06 ± 0.25 ng/ml	17.46 ± 1.46 ng/ml	1.07 ± 0.073 ng/ml	5.14 ± 0.38 ng/ml	[44, 157]

of the biosensor. The interaction of target bio-analyte with the immobilized probe results in the generation of electrical/fluorometric/luminometric/colorimetric signals based on the presence or absence of target bio-analyte in bio-fluid. The transducer captures the signals, identifies the variation in any of the parameter (electric current, potential, mass, temperature, impedance, intensity, etc.) and finally converts them into other quantifiable form, i.e. conversion of generated energy by specific interaction into measurable form [30]. The generated signals are amplified and processed into readable output (Fig. 1).

Nanobiosensors and Fabrication Strategies: An Emerging Tool for Detection of OSCC

Advancements in the field of nanotechnology provide the required thrust to the development of nanobiosensor technology. The parameters such as linearity, specificity, sensitivity, shelf life, detection limit, response time and reproducibility require individual/singular optimization and determine the performance of a nanobiosensor [31]. The NMs-based scaffolds have significantly enhanced the above parameters as compared to bulk counterparts due to increased relative surface area and quantum confinement effect. Also, NMs possess a very high surface area-to-volume ratio, which leads to enhanced chemical reactivity and stability. Due to their large surface-to-volume ratio, these scaffolds can accommodate multiple ligands thus enhancing the binding affinity, increasing the selectivity and reducing the detection limit of the fabricated nano-bio-sensing platform. However, the quantum confinement effect restricts the motion of randomly moving electrons in specific energy level with the decrease in particle size until reaching a nanorange. This confinement results in an increase in the bandgap and decrease in wavelength and this band shift is important in controlling the materials' properties. Therefore, quantum confinement results in development of unique optical, electrical and magnetic properties which help in amplifying the signal intensity thereby increasing the sensitivity of the fabricated biosensing platform. Therefore, the combination of NMs with transducing unit acts as an interfacial layer in between the bio-recognition probe and detection assembly and drastically enhances the signal intensity. Along with the major NMs' properties, their shape (nanospheres, nanotubes, nanowires, etc.) and size also play an important role in controlling their behaviour [32–36]. It has been reported that reduction in the particle size of NMs result in improving the bio-sensing behaviour [37]. The broad range of NMs ranging from metallic, bi-metallic, non-metallic, QDs, electromagnetic metamaterials, polymeric, up-conversion NMs (UCNMs), etc. has been explored in the development of nanobiosensors and their unique properties have enabled faster detection and reproducibility than conventional detection system [17, 18, 38].

Clinically, it is difficult to decide the onset of OPMDs or OSCC based on the presence and absence of a single biomarker. Hence, the strategies involving detection of multiple biomarkers also known as multiplexing are required for better prognosis. This multiplexed detection could be achieved by immobilizing different target-specific bio-recognition probes onto the surface of NMs. The bio-sensing capabilities of nanobiosensors for the detection of single and multiple biomarkers have been successfully studied in a clinical platform for minimal or completely non-invasive detection of OSCC [39, 40]. Even after the detection of OSCC, real-time monitoring is important to support clinical decision making and continuous screening for recurrence, which could be possible with the integration of fluidics-based nano-bio-sensing technology. The collection, identification and characterization of malignant cells in low sample volume have been explored to aid in the diagnosis of cancer stage and monitoring the treatment

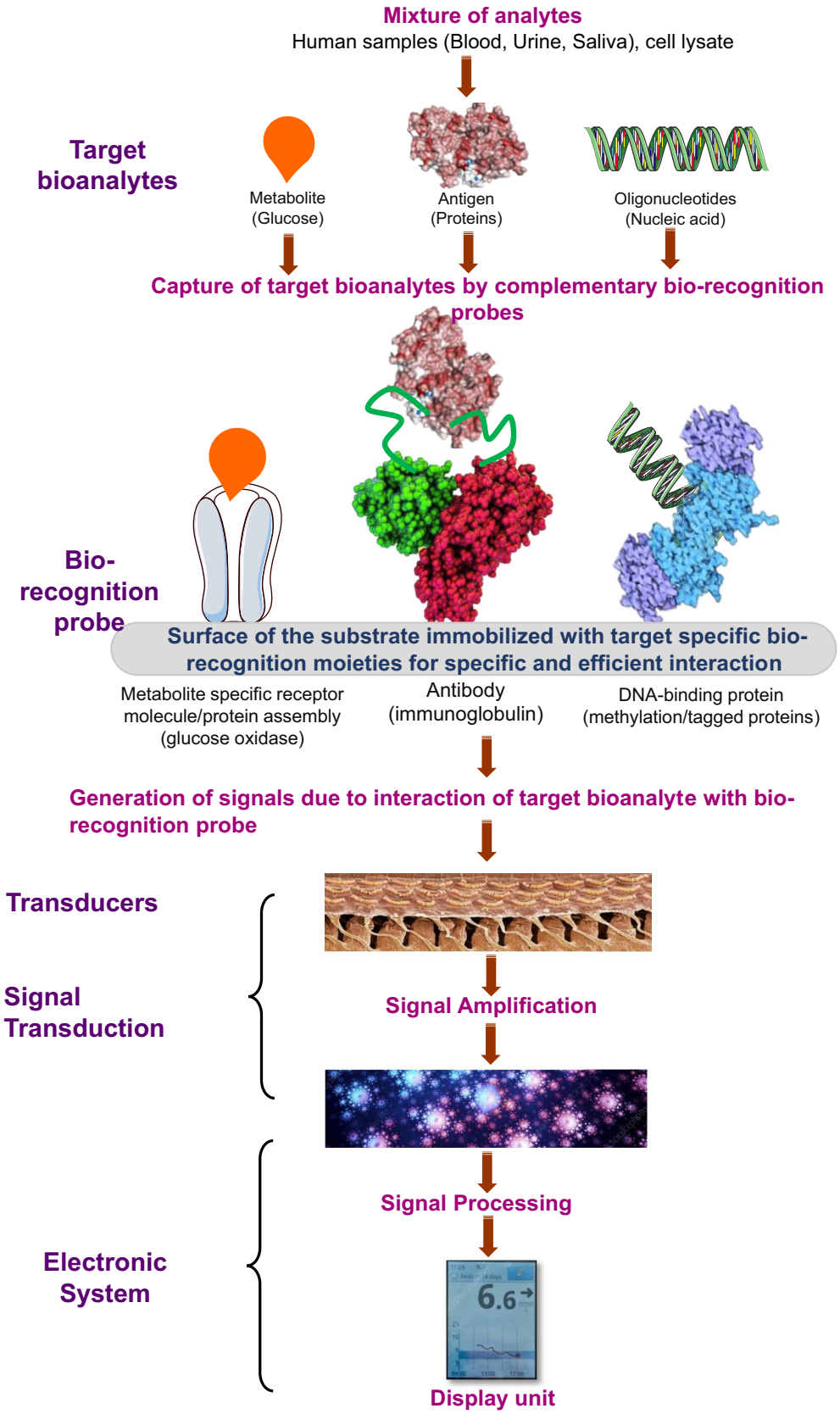

Fig. 1 An illustration of various components of a nanobiosensor with their working principle

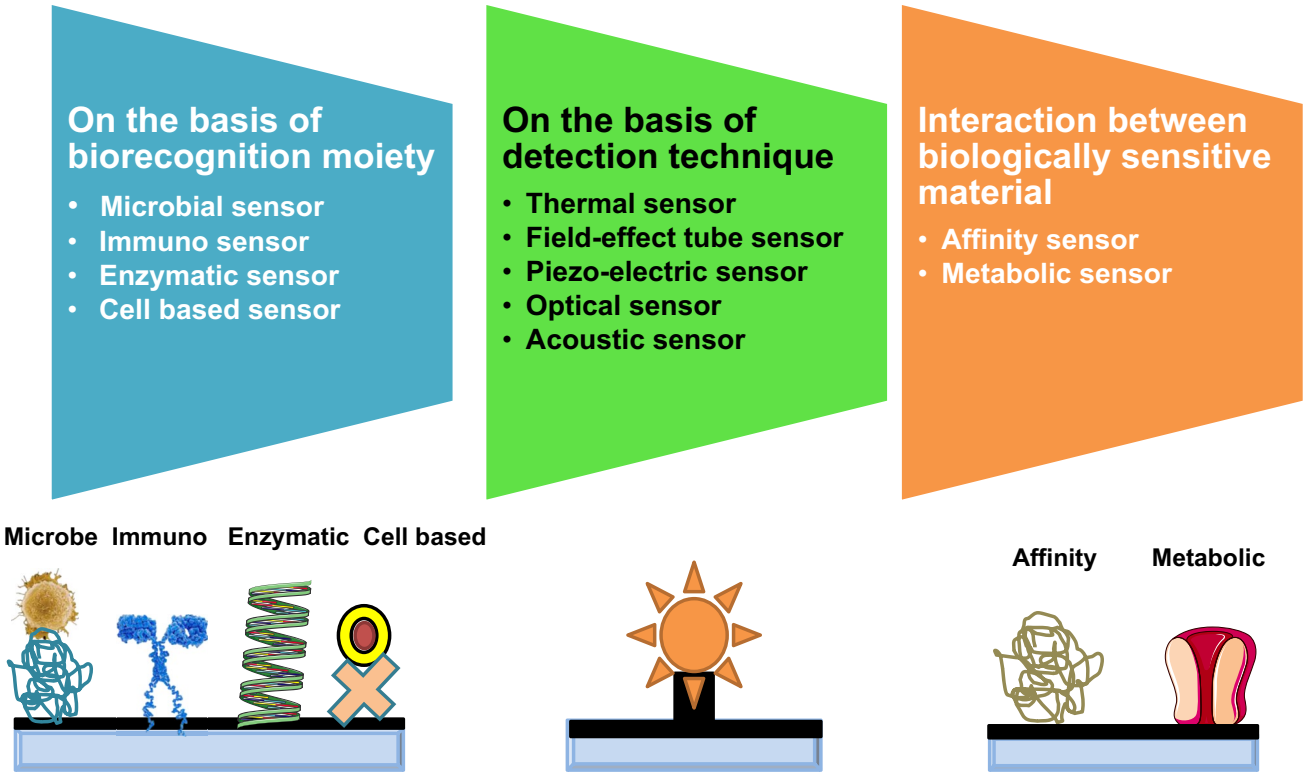

Fig. 2 The broad categorization of the biosensors based on bio-recognition moiety, the detection technique and the type of interaction between biologically sensitive material

progress [41]. Also, the integration of channelization with nano-bio-sensing allows 'nano-fluidic' real-time monitoring of cellular activities like cell adhesion and the proliferation of the OSCC cells. This approach has been explored in a label-free manner using interdigitated microelectrode-based microchip [26]. The integration of nanotechnology with other technical disciplines could improve the current state of POC diagnosis.

Currently, research is focused on the development of 'portable miniaturized device' for the diagnosis of OSCC that can be cost effective for commercialization. It must have the rapid, sensitive and specific for detection without the requirement of human experts. Also, the progress in the field of nanotechnology has opened up a domain of miniaturization to the nanoscale range that might lead to bio-chip technology or a lab-on-a-chip technology which would be the hallmark in the field of nano-based diagnosis of OSCC.

Nanobiosensors: The Fabrication Methods and Techniques

In the following section, the steps of fabrication of biosensing platform with the aid of NMs are discussed briefly. Their efficiency, related parameters and nano-bio-sensing strategies in the detection of OSCC have been summarized and tabulated (Table 3).

Substrate Selection and Surface Cleaning

The first and foremost step for the fabrication of nanobiosensor is the selection of a suitable substrate. The most popular substrates being used for this purpose are microscopic glass slide, silicon (Si), gold (Au), platinum, indium tin oxide (ITO), etc. [42, 43]. The substrates have

Table 3 A comprehensive list of literature available related to study nanobiosensors for early detection of OSCC

Year	Sample	System	Biomarker	Detection technology	Sensitivity	Linear detection range	Response time	Shelf life/stability	Reference
Proteins as a bio-recognition probe									
2009	Saliva	Biotinylated IL-8 mRNA probe/Au or Biotinylated IL-8 antibody/Au	IL-8 mRNA and IL-8 Protein	Electrochemical sensor	-	IL-8 mRNA = 5 fM to 50 pM. IL-8 = 10 pg/ml to 12, 500 pg/ml	–	–	[112]
2009	Human saliva and serum	αCEA-MWCNT-PEI/SPE	Monoclonal anti-CEA antibody (αCEA)	Electrochemical biosensor, immunosensor	-	1×10^{-12} g/ml	–	–	[54]
2010	Conditioned media from culture cells	SWNT forest combined with multilabel detection strategies	Interleukin-6 (IL-6)	Electrochemical, immunosensor	19.3 nA ml cm^{-2} (pg IL-6)$^{-1}$	0.5 pg/ml	–	–	[71]
2011	Serum	Anti-IL6/GO/glassy carbon and FC-PPN-Ab_2	Interleukin-6 (IL-6)	Electrochemical, immunosensor	-	0.002–20 ng/ml	–	7–30 days	[158]
2011	Serum	Ab_1- GSH-AuNP And Ab_2–MB–HRP	Interleukin-8 (IL-8)	Amperometric sensor	1060 nA ml (fg IL-8)$^{-1}$ cm^{-2}	-	–	–	[73]
2014	Exfoliated cells, cell lines, human tissue	GNRs	–	SERS	–	–	–	–	[159]
2014	Serum, conditioned media	Ab_2-MP-HRP and Ab_1-GSH-AuNPs-PDDA	IL-6, IL-8	Amperometric, microfluidic technology	IL-6 = 5.9 µA ml (fg protein)$^{-1}$ and IL-8 = 6.8 µA ml (fg protein)$^{-1}$	–	–	–	[113]
2011	Serum	Anti-p53/Ag/K9 glass	P53	LSPR based	–	–	–	30 days (vacuum plastic bag) and 7 days (room temperature)	[110]
2015	Saliva	anti-CYFRA-21-1/APTES/ZrO_2 /ITO immunoelectrode	CYFRA-21-1	Cyclic voltammetry, sandwich type immunosensor	2.2 mA mL ng-1	2–16 ng/ml	20 min	42 days	[44]
2015	Serum	Au@MGN-Ab_2	TPA	Electrochemical, sandwich type immunosensor		10^{-5} to 10^{-2} ng/ml	–	30 days	[160]
2015	Artificial saliva	SiNW	IL-8, TNF-α	Field-effect transistor biosensor	–	–	–	–	[39]
2016	Spiked saliva	Anti-CD 59/cys/Au	CD-59	Electrochemical immunosensor	–	mL^{-1}	10 min	95% sensitive up to 35 days	[95]
2016	Human saliva	BSA/anti-CYFRA-21-1/APTES/nHfO_2/ITO	CYFRA-21-1	Immunosensor	9.28 µA ml ng^{-1} cm^{-2}	2–18 ng/ml	15 min	56 days, can be reused up to 30 times	[104]

Table 3 (continued)

Year	Sample	System	Biomarker	Detection technology	Sensitivity	Linear detection range	Response time	Shelf life/stability	Reference
2016	Human saliva	BSA/anti-CYFRA-21-1/serine/nZrO_2/ITO	CYFRA-21-1	Label-free impedimetric immunosensor, electrochemical	0.295 µA ml ng^{-1}	0.01–29 ng/ml	6 min		[76]
2016	Human saliva	BSA/anti-CYFRA-21-1/APTES/ZrO_2–RGO/ITO	CYFRA-21-1 (Protein)	Label-free Immunosensor	0.756 mA ml ng^{-1}	2–22 ng/ml	16 min	56 days	[107]
2016	rhMMP2 (in-vitro)	UCNP-SiO_2@p-Au	MMP2	Fluorescence	–	–	–	–	[38]
2017	Spiked Saliva	PMMA/glass	IL-8, IL-1β and MMP-8 protein	Optical microfluidic biosensor, immunosensor, absorbance-based detection	–	–	30 min	–	[109]
2017	Artificial saliva	BSA/anti-CYFRA-21-1/Cys-La$(OH)_3$/ITO	CYFRA-21-1	Immunosensor	12.044 µA (ng per ml $cm^{-2})^{-1}$	0.001–10.2 ng/ml	5 min	119 days	[75]
2017	Human saliva	Anti-IL8/AuNPs-rGO/ITO	Anti-IL8	electrochemical immunosensor, label-free, DPV	–	500 fg/ml–4 ng/ml	9 min	90 days	[108]
2017	Human saliva and serum	BSA/Anti-TNFα/PS/ITO	TNFα	Electrochemical immunosensor	–	0.01–2 pg/ml	–	Can be reused up to 6 cycles	[29]
2017	CE81T, CE81T-4	PDMS/ITO	–	Dielectrophoretic impedance measurement	–	–	–	–	[161]
2017	OE21, OE21-1, CE81T, and CE81T-4	ZnO/Cu_2O/ITO	–	Photoelectrochemical biosensor/single cell array chips	-	–	0.5 s	–	[162]
2018	Human saliva and serum	BSA/IL-1β /PHA/ITO	interleukin 1β (IL-1β)	Electrochemical, impedimetric immunosensor	–	0.025–3 pg/ml	–	6 cycles, 49 days	[60]
2018	Human saliva and serum	BSA/Anti-IL-8/Polymer composite/ITO-PET	IL-8	label-free impedimetric immunosensor, electrochemical	–	0.01–3 pg/ml	–	5 cycles, 42 days at 4C	[53]
2018	Mouse xenograft tumour model and	UCNP@p-QD	Matrix metalloproteinases 2 (MMP2)	FRET-based biosensor	–	–	–	–	[99]

Table 3 (continued)

Year	Sample	System	Biomarker	Detection technology	Sensitivity	Linear detection range	Response time	Shelf life/stability	Reference
2018	Saliva	BSA/anti-CYFRA-21-1/APTES/nHfO$_2$@RGO/ITO	CYFRA-21-1	Immunosensor, Label-free, electrochemical	18.24 µA ng^{-1} ml ng^{-1}	0–30 ng/ml, LDL 0.16 ng/ml		27 days	[105]
2018	Spiked samples of fresh saliva of normal human's	BSA/Anti-CYFRA-21-1/ncCeO$_2$-RGO/ITO	cytokeratin fragment-21-1(CYFRA-21-1)	Immunobiosensor	14.54 uA ng^{-1} ml ng^{-1} cm^{-2}	0.625 pg/ml–15 ng/ml	-	35 days at 4°C	[106]
2018	Saliva	BSA/Anti-sIgA/AuNPs-PEG/CNF-SPE	sIgA	Label-free-enzyme free, Electrochemical, immunosensor	–	8.71–1000 fg/ml	–	15 days at 4°C	[28]
2018	Saliva	CYFRA 21-1/Al$_2$O$_3$/3DN-CNTs/Si	Cytokeratin-19 antigen (CYFRA 21-1)	Fluorescence-based immunosensor	20 times higher than that of the sandwich ELISA	0.5 ng/ml	–	–	[98]
2018		Bovine serum albumin/anti-CYFRA-21-1/(3-aminopropyl)triethoxysilane/TiO2/indium tin oxide	CYFRA-21-1	Electrochemical impedimetric biosensor, immunosensor	0.573 Ω ml/ng	0–12 ng/ml	-	35 days	[103]
2018	Saliva, tissue	Anti-OPN-HRP/AuNRs and Anti-OPN-HRP/AuNSs	OPN	Absorbance/nano-ELISA	–	Linear detection range 0.31–20 ng/mL	–	–	[18]
2018	Saliva	Anti-CIP2A/CNTs/Al$_2$O$_3$/Si/SiO$_2$	CIP2A	Electrochemical immunosensor	–	1–100 pg/ml	–	–	[43]
2019	Saliva	BSA/anti-CYFRA-21-1/APTES/nY$_2$O$_3$/ITO	CYFRA-21-1	Electrochemical-impedometric electrochemical impedance spectroscopy	226.0 Ω. ml. ng -1	0.01–50 ng/ml	–	35 days	[163]
2019	Saliva	Anti-IL8/ZnO–rGO/ITO	IL-8	Electrochemical immunosensor	12.46 ± 0.82 µA mL/ng	-	10 min	70 days at 4°C	[164]
2020	Saliva	BSA/anti-CYFRA-21-1/APTES/ndZrO$_2$/ITO	CYFRA-21-1	Electrochemical	0.53 µA mL/ng cm^2	0.5–50 ng/ml	–	–	[37]
Nucleic acids as bio-recognition probe									
2011	Saliva	DNA/Au electrode	ORAOV1	Electrochemical, impedance	-	–	–	–	[165]
2013	Saliva and serum	Cp-MB /ITO glass	Single-base mismatch	Microfluidic chip based/fluorescence	–	–	15 min	–	[97]

Table 3 (continued)

Year	Sample	System	Biomarker	Detection technology	Sensitivity	Linear detection range	Response time	Shelf life/stability	Reference
2013	Oral cancer cell lysates	RB-GNR probes	Protein/DNA from cell lysates	Label, SPR based	–	–	–	–	[166]
2013	Artificail saliva	BSA/Sp-complementary-miRNA/Cp-streptavidin/MB	miRNA	Electrochemical	–	–	–	–	[26]
2014	Cell lines	Polyurethane/glass and functionalized polystyrene-polymer-based nanomembrane	microRNA (miRNA)	Microfluidic	–	–	20 min per sample	8 days	[27]
2015	–	(DNA22)DNA2/MCH/DNA1/Pd-Au/Pd-Au-CS/GCE	DNA probe	Electrochemical	–	10^{-16} to 10^{-10} mol/l	-	–	[111]
2015	Saliva	eMB/ITO with negative charge	ORAOV1	Electrochemical/DPV	–	–	30 min	–	[72]
2016	HeLa cells	probe 1 (MPT NPs/AuNPs/hairpin DNA 1/HT) and probe 2 (TiO_2 NPs/AuNPs/hairpin DNA 2/HT) photoactive bionanoconjugates	ORVOA 1 gene and p53 gene	Photoelectrochemical	–	–	–	–	[40]
2017	Serum	anti-E6-HPV-16 ODN / AuMNPs and anti-dsDNAAbs/CdTe-HWR_QDs	Anti-E6-HPV-16 ODN	Fluorescence-based hybridization assay	–	–	–	–	[96]
2017	Spiked saliva	Anti-IL8/AuNPs-rGO/ITO	Anti-IL8	electrochemical immunosensor, label-free, DPV	–	500 fg/ml to 4 ng/ml	9 min	Stable up to 90 days, reused for four times	[108]
2018	HKESC-1, HKESC-4) and tissue samples	miRNA/Au@NPFe_2O_3NC	miR-107	Electrochemical detection	–	–	–	–	[167]
2020	Saliva	Ag NCs/T7 Exo- (H1-(H2-2)-Au NPs) /MCH/Track/H_2PtCl_6-He-GO/GCE	ORAOV1	Photoelectrochemical	–	1.0×10^{-15} to 1.0×10^{-10} mol/l	–	–	[74]
Proteins and nucleic acids combined as a bio-recognition probe									
2009	Saliva	DNA dendrimer directed polypyrrole (DDPpy) electrodes	Biotinylated human IL-8/IL-1β mab and IL-8 mRNA	Amperometric/electrochemical biosensor	–	Proteins – 100–200 fg/ml mRNA – 10aM	–	–	[52]

Table 3 (continued)

Year	Sample	System	Biomarker	Detection technology	Sensitivity	Linear detection range	Response time	Shelf life/stability	Reference
2016	Saliva	Modified-MBs, SPCEs	IL-8 protein and IL-8 mRNA	Electrochemical biosensor	–	–	–	11 days for IL-8 mRNA and 30 days for IL-8 protein	[114]
Other molecules as bio-recognition probe									
2018	Saliva	Urea/AuNPs/APTMS/glass	Nitrite	SERS chip	–	–	–	–	[42]

a flat surface or are in the form of electrodes that could be further modified according to the sensor applications [42, 44, 45]. After the selection of substrate, the next step is to clean the surface of the substrate properly to remove the impurities which can be done by physical (high power laser) and/or chemical (organic solvents such as acetone, isopropanol) methods (Fig. 3a).

Surface Enhancement

Substrate morphology plays an important role in deciding the specificity and stability of the nanobiosensor which depends on stable immobilization of bio-recognition probe onto the substrate in an active form. Therefore, the selection of substrate morphology is one of the important steps during the fabrication process of nanobiosensors (Fig. 3b).

Surface Enhancement by Topographic Patterning

The substrate considered for the immobilization of the bio-recognition probe can be flat or have some topographical pattern (Fig. 3b-i, b-ii). It has been well documented that the substrate with enhanced topography results in improved capture efficiency and target detection limit [46, 47]. The surface enhancement by topological patterning is accomplished mainly by using lithography (photo and soft) and electrospinning techniques [48–50]. The photo-lithography technique has been used widely for patterning live elements and biomimetic materials upon the surface. However, costly instrumentation, the need for cleanroom facility and the complexity associated with photo-lithography make it unfavourable for bio-sensing application. On the other hand, the soft-lithography technique overcomes these limitations and involves the implication of 'soft' materials, e.g. soft channels fabricated in elastomeric material for surface enhancement by topographical pattern formation. This technique is superior to the photo-lithography in being less expensive and the patterning can be easily achieved over a wide variety of planar and non-planar surfaces [51]. Besides, the simple steps of soft lithography could be performed without complicated protocols and expensive cleanroom environment. However, it has its own limitations, i.e. not favourable for manufacturing multilayered structured and requires clean surrounding and expensive equipment. Therefore, the selection of the lithography technique should be decided based on the requirement of the topographic patterning for best results.

Surface Enhancement by Incorporation of NMs

The NMs have more relative surface area as compared to bulkier counterparts. Therefore, application of NMs onto the surface increases the loading efficiency of the bio-recognition probe thereby increasing the selectivity towards

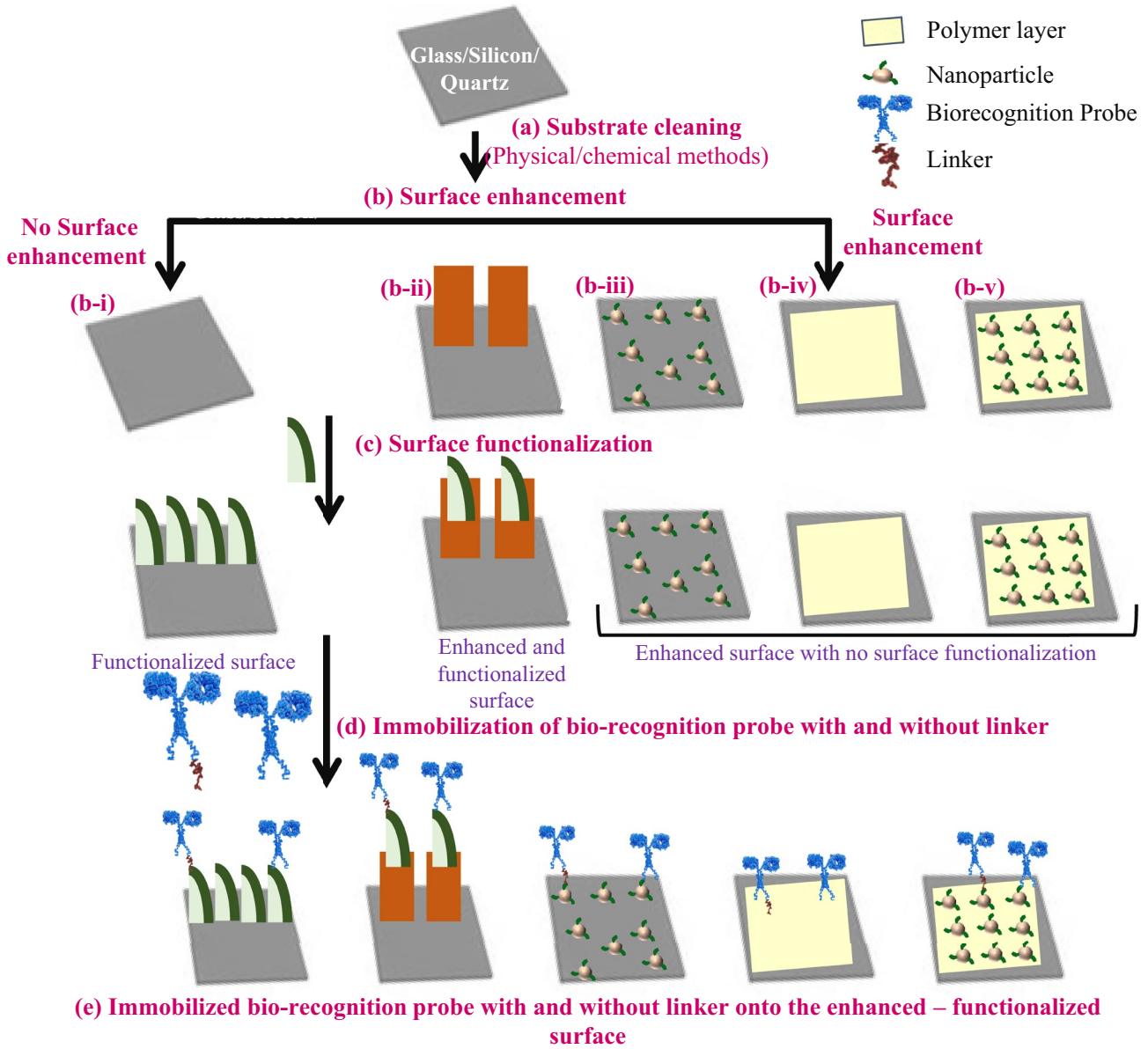

Fig. 3 An illustration of various steps involved in the fabrication of nanobiosensors starting with **a** substrate cleaning: by physical/chemical methods; **b** surface enhancement: **b-i** represents the bare surface means without surface enhancement, **b-ii** topographic patterning: lithography/electrospinning techniques used for patterning which improves the capture efficiency, **b-iii** surface enhancement by incorporation of nanomaterials, **b-iv** surface enhancement by depositing a thin layer of polymer, **b-v** deposition of nanomaterials onto the polymer layer; **c** surface functionalization: functionalized chemically for the sake of increasing affinity and stable interactions; **d** immobilization of bio-recognition probe with or without linker onto the substrate surface; **e** immobilized bio-recognition probe with and without linker onto the enhanced functionalized surface

the target with optimum response time (Fig. 3b-iii). Thus, the selection capability of the conjugated target-specific bio-recognition probe is ideally proportional to the availability of the surface atoms on NMs for conjugation. This enhances the bio-recognition specificity in a heterogeneous population of biomarkers in a bio-fluid, hence resulting in enhanced biomarker-based identification device.

Surface Enhancement by Polymers

This step involves the introduction of thin layer of polymer embedded with or without biodopant onto the surface of the substrate (abiotic or bio-abiotic interface) (Fig. 3b-iv). This process increases the affinity and stability of the immobilized bio-recognition probe. This mechanism involves the

shielding of protein integrity and conformational changes due to surface–protein interaction and enhancing the signal transduction phenomenon [52]. Different types of polymers such as poly(3,4-ethylene dioxythiophene) (PEDOT), polythiophene (PTs), polypyrrole (Ppy), polycarbazole, polyaniline (PANI) have been used independently or in combination with NMs for the formation of conducting layer [52–54] (Fig. 3b-iv, b-v). Also, the introduction of a polymer layer onto the substrate helps in setting of NMs in the polymeric mesh in a controlled uniform manner without any treatment of harsh chemical [55]. This reduces an extra step of functionalization of the surface with strong chemicals for further immobilization of the bio-recognition probe. Also, the structural morphology of the NMs can be tuned with the confinement of polymer layer thereby tuning the physicochemical properties of the deposited NMs which aids in enhancing the sensing parameters [56, 57].

Surface Functionalization

The surface enhancement is followed by surface functionalization (Fig. 3c). It is required for maintaining the optimum density of bio-recognition probe. It also allows a stable interaction between the surface and the immobilized probe. The functionalization strategy is based upon the presence of free functional groups available on the bio-recognition probe for conjugation. This enables the application of the best suited surface functionalization chemistry for establishing a stable interaction. The most successful and common method for surface functionalization is by using organic molecule-based self-assembled monolayers (SAMs) with different end functional groups. SAMs provide an ultrathin organic layer with specific properties on the surface and the controlled thickness [58]. The carboxylate SAMs, organosilane SAMs, thiols SAMs, etc. have been explored on oxide and noble metal surfaces for immobilization of bio-recognition probe for the fabrication of a sensing platform [59, 60]. The organosilanes, organophosphorus acids and their derivatives have been used for the surface modification of metal oxides and monolayer formation. The characteristic properties of both types of monolayers have been compared [61, 62]. Besides, the chemical treatment has also been used for surface functionalization, e.g. hydrolysis of surface for specific time duration creates a hydrophilic surface with dense −OH groups which were further used for sensor fabrication [63, 64].

Hence, the above-mentioned approaches allowed the bio-recognition probes to be immobilized on the substrate more firmly which provides better selectivity and capture efficiency thereby improving the sensitivity of detection towards target bio-analyte. These approaches need further optimization while designing any nano-bio-sensing platform.

Bio-Recognition Probe and Its Immobilization

The next and a major step in the fabrication of any nanobiosensor is the immobilization of the bio-recognition probes on the surface of the substrate (Fig. 3d). This is an important process that governs the specificity, stability and shelf life of the nanobiosensors. The optimum density and orientation of immobilized probes with the exposed active site are an essential pre-requisite for an efficient interaction [65–68]. Other properties that influence the stability of immobilized bio-recognition probes are ionic strength, surface charge, inherent folding interactions and their hydrophobicity or hydrophilicity [69, 70].

The target specificity could be achieved by immobilizing the surface with the bio-analyte specific probes, e.g. complementary sequences of the nucleic acids (DNA/RNA) and proteins (antibodies/enzymes/ antigens) [43, 71–74] with exposed active sites for stable interaction. The optimum surface density of bio-recognition probe is essential to capture the maximum number of target bio-analytes. The density of the bio-recognition probe lower than the required optimal density shall fail to produce the required signal strength. In case of higher than maximum density, the spatial hindrance shall reduce the required signal strength. Therefore, the prospective biomolecule candidate for immobilization must be in the optimum density, proper orientation and retain their conformational integrity for efficient signal production.

An additional linker could be attached to provide a bridge by maintaining the required distance between substrate—bio-recognition probe or substrate—NMs or NMs—bio-recognition probe for maintenance of conformation and steric flexibility and prevent the loss in activity of nanobiosensor (Fig. 3e). Some linkers such as 3-mercaptopropyl triethoxysilane (MPTES) and 3-aminopropyl triethoxysilane (APTES) have been used for the same. It is noteworthy that APTES as linker molecule in between bio-recognition probe and NMs is known as hazardous for human health and limits its usage in biomedical applications. Also, the surface modification of NMs with APTES is tedious and time consuming, limiting its usage in biomedical applications. To overcome the above limitation, non-toxic and biocompatible linkers have been used for surface functionalization of NMs and firm binding of target bio-recognition probe. Some organic amino acid-based linkers like serine (Ser) and cysteine (Cys) are found to be non-toxic, and hence could be used for biosensor-based applications [75, 76]. The hydroxyl group (−OH) in the amino acids provides stable surface functionalization. Further, the presence of carboxylic (−COOH) and amine ($-NH_2$) groups may provide efficient immobilization of target bio-recognition probe onto the modified surface of the NMs. The surface modification of NMs with non-hazardous amino acids allows fabrication of nano-biosensing platform as well as fabrication of highly sensitive,

biocompatible implantable bio-chip. These types of linkers have been used for the fabrication of biocompatible nano-bio-sensing platform for OSCC biomarker (CYFRA-21-1) detection and found to be stable for 45 days [76] with a response time of five to six minutes only. The fabricated immunosensor was found to be better in terms of sensitivity and detection limit when compared with formerly reported biosensors and commercially available ELISA kit (Kinesis DX) for detection of this particular CYFRA-21-1 biomarker [75].

The selection of method for the immobilization of bio-molecule depends upon the physicochemical properties of both the partners. The immobilization mechanism involves various covalent and non-covalent interactions. The interactions could be categorized as electrostatic, antigen–antibody, hydrophobic, hydrophilic interactions, physical adsorption and covalent binding for immobilizing bio-recognition probe onto the modified surface of the substrate, etc. [77].

The electrostatic interaction is a fast, simple, single and reversible method of immobilization. These interactions are favourable when the orientation of bioactive molecule is not considered [78, 79] and has been applied in the fabrication of optical and electrochemical biosensors [80–82]. The methods employing hydrophobic interactions are also simple, fast and direct methods for immobilization of lipophilic probes [83–85]. The antigen–antibody interactions have been successfully applied for immobilization of bioactive molecule, e.g. streptavidin–biotin, neutravidin–biotin or avidin–biotin interactions [86, 87]. The covalent interaction-based methods namely click chemistry (cycloaddition of alkyne and azide) and carbodiimide crosslinker chemistry [1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride-mediated attachment] have been applied for immobilization procedure due to their better bond stability and high binding strength [88–92].

Every technique has certain limitation due to which the interaction in between substrate—bio-recognition probe or substrate—NMs or NMs—bio-recognition probe fails to provide the desired stability and shelf life to the nanobiosensor. In this context, the application of passive trapping and covalent binding techniques was limited by the loss of stability of immobilized proteins during continuous use, thus affecting the functional stability [93, 94]. Due to this limitation, various research groups have adopted self-assembly approach onto the functionalized ITO/gold or polymer-coated surface that does not require any chemical treatment [60, 95].

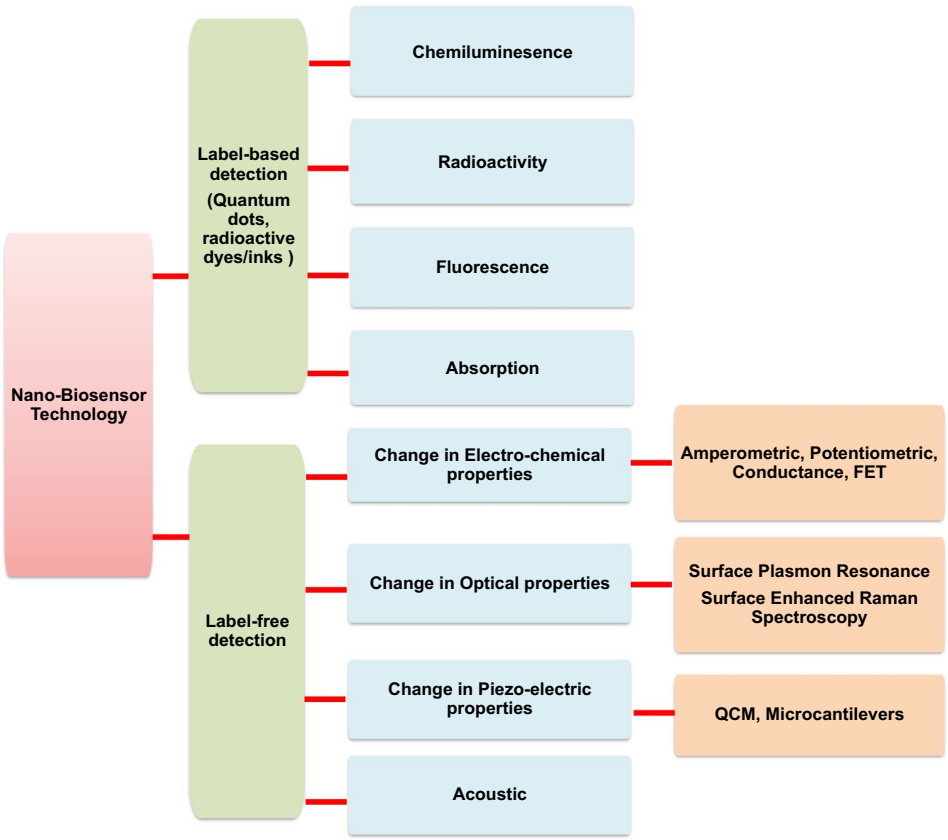

Fig. 4 Schematic representation of labelled and label-free bio-molecular transduction methodologies in the fabrication of a nanobiosensor. *FET* field-effect transistor; *QCM* quartz crystal microbalance

Role of Nano-Bio-Sensing Technology in OSCC Diagnostics

The interaction of target bio-analytes with bio-recognition probe results in electrical/fluorometric/colorimetric signals which are processed into measurable units with the help of transducer and the electronic system (Fig. 1). This signal intensity of nanobiosensor has been greatly enhanced with the use of NMs which has been broadly categorized under labelled and label-free phenomenon (Fig. 4).

Nanobiosensor Technology Based Upon Labelling Phenomenon

The labelling of a bio-recognition element with enzymes, fluorescent dyes, radioactive isotopes or inks is based upon the principles of *chemiluminescence, fluorescence, radioactivity* and *absorption*. Among all, the fluorescence-based nano-bio-sensing platform has been employed for the detection of HPV, single-base mismatch, biomarkers such as matrix metalloproteinases (MMPs). The MMPs have a regulatory role in extracellular matrix remodelling and interaction with CYFRA-21-1 antigen. Fluorescence-based nanoconstruct was developed for detection of specific HPV-DNA in which QDs were used as a fluorophore for the generation of signals. The specificity of the fabricated system was evaluated by using HIV, influenza, HPV and HBV-specific oligonucleotides and it was found to be highly sensitive for the detection of HPV-16 E6 in clinical samples from the patients diagnosed with head and neck cancer (HNC) [96]. While for detection of single-base mismatch in saliva and serum samples of OSCC patients, a sensitive and ultra-specific microfluidic chip-based fluorescence DNA biosensor was developed which has screening time of 15 min per sample [97]. Further, a fluorescence-based immunosensor was fabricated for detection of CYFRA-21-1 biomarker in clinical saliva samples, using a three-dimensional network of carbon nanotubes on Si pillar substrate (3DN-CNTs/Si) and Alexa-fluor® 568-tagged antibodies against CYFRA-21-1. The introduction of 3DN-CNTs resulted in enhancing the sensitivity of designed biosensor by approximately twenty times when compared with a conventional sandwich ELISA system [98]. Another class of NMs which have been gained importance for bio-sensing is upconversion nanoparticles (UCNPs) [38, 99] that exhibit photon upconversion phenomenon, excited by the incident of near-infrared (NIR) light with emission in the visible region of the electromagnetic spectrum. These UCNPs are doped, rare earth element, optically active NPs, possess higher chemical stability, deep penetration power and are highly resistant to photo-bleaching. Upon irradiation with NIR light, these NPs are excited by sequential absorption of multiple low-energy photons (longer wavelength) and lead to the emission of a single high-energy photon (shorter wavelength) through long-lived intermediate energy states [100]. The efficiency of UCNPs could also be adjusted by the dopant concentration. It has been reported that the fluorescence resonance energy transfer (FRET)-based biocompatible UCNP-composites can detect OSCC biomarkers utilizing energy shifts in red and blue wavelength range. These UCNP-composites have potential to detect matrix metalloproteinase 2 (MMP2) expression in tumor models and OSCC tissues. When irradiated, the designed nanocomposite emits FRET-induced red fluorescence but in the presence of MMP2, it emits blue fluorescence [99], thus providing their potential for further exploration in clinical diagnosis in OSCC.

Nanobiosensor Technology Based Upon the Label-Free Phenomenon

The techniques for labelling of bio-recognition probes with enzymes, fluorescent dyes, radioactive isotopes or inks have limitations of requiring skilled supervision and costly technical support. Also, the size of these labelling agents causes steric hindrance during molecular interaction and reduces or inhibits the functional activity of the bio-recognition probe [101]. Owing to the above reasons, the label-free technology has gained popularity over labelling technique since the past decade. The label-free technology is based upon electrochemical detection method, surface plasmon resonance (SPR), mass spectrometry, acoustic, field-effect transistor (FET), etc. In all these technologies, the physio-chemical properties of bio-analytes and NMs are used as a detection target [102] and, hence, the label-free technology allows the reduction in the cost of fabrication, restricts complex processes involved in labelling of the bio-recognition probe and maintains the integrity of the bio-recognition probe.

The combination of metallic–non-metallic and their nano-oxide-based transducer matrix attributes to quick response, high efficiency and stability for the label-free detection of OSCC biomarkers. In this regard, some nano-metal oxides (NMOs) such as zirconium oxide (ZrO_2), zinc oxide (ZnO), hafnium oxide (HfO_2), iron oxide (Fe_2O_3), titanium oxide (TiO_2), magnesium oxide (MgO), etc. have been explored for label-free detection of OSCC biomarkers [44, 103]. It has been reported that nanostructured HfO_2-based label-free nanobiosensor for non-invasive detection of OSCC from human saliva samples was more sensitive than fluorescence-based biosensor [98] with a lower detection limit of 0.21 ng/ml [104]. Besides, the nanostructured HfO_2/ZrO_2-based label-free immune-electrodes have a response time of 15–20 min with shelf life of 6–8 weeks [44, 104]. Its another

advantage is that the same electrode can be used up to 30 times [104]. Overall, the above facts signify the efficiency, stability and recyclability of the label-free nanobiosensors that allow more precise and quick quantitation.

However, the formation of aggregates is a limitation associated with the usage of NMOs. It has been reported that ZrO_2 NPs and HfO_2 NMs form aggregates in solution. This aggregation results in the formation of large clusters, having low surface-to-volume ratio, thus influencing the biosensing parameters. The above limitation was subdued by restricting the Brownian motion of NMOs and providing a supporting matrix which has good conductivity and high surface area. In this regard, chemically reduced graphene oxide (RGO) has been observed as a promising substrate which provides uniform distribution of NMOs. The reason behind is the presence of rich defects and electro-active sites which might obstruct the agglomeration of nano-sized molecules. Additionally, the electrochemical properties could be enhanced by the presence of reduced chemical groups which facilitate charge transfer kinetics. With the enhancement of electrochemical properties, there might be an increase in the covalent and non-covalent sites for surface functionalization to facilitate loading of biomolecules and provide uniform conducting surface. Thus, integration of RGO with NMOs resolved the issues of dispersivity and results in enhancing conductivity and stability, provides better catalytic behaviour and improved charge transfer kinetics synergistically [105–107]. A similar effect was reported with metallic NMs and RGO [108].

Optical nanobiosensors have gained popularity as a label-free detection technique. In optical nanobiosensors, the optical fibres allow detection of bio-analytes based upon the phenomenon of light scattering or its absorption. The optical nanobiosensor may include both catalytic and affinity reaction for the measurement of target bio-analytes in the bio-fluid. These reactions alter the light absorbance due to change in the refractive index between the surfaces of the two media with different density. A novel optical microfluidic nanobiosensor has been fabricated for absorbance-based multiplexed detection of clinically relevant salivary protein biomarkers such as IL-8, IL-1β and MMP-8 protein [109]. The fabricated biosensor has highly sensitive organic photodetectors (OPDs) and has a response time of 30 min. The use of noble metal NMs allows the detection based on localized SPR (LSPR), a sensitive and material-specific measurement technique. Based on the LSPR phenomenon, nanobiosensors have been fabricated using triangular silver (Ag) NMs for detection of serum p53 protein level in HNC patients. A significant difference in LSPR shifts (Δλmax) in these patients was observed when compared with healthy control individuals [110]. Despite, the simplicity, specificity and accuracy of the optical technology, its major limitation lies in the conversion of the optical-based nano-bio-sensing platform into POC device. On the contrary, the electrochemical-based sensors have been popular in POC device for detection of OSCC due to their lower space constraint, i.e. the ability to perform miniaturized, low-cost, simple and easy operation. Depending upon the working principle, the electrochemical biosensors can employ amperometric, potentiometric and impedimetric transducers that convert the captured information into a quantifiable signal. Both nucleic acid- and protein-based electrochemical nanobiosensors have been explored for the detection of OSCC using different types of NMs. The efficiency of palladium-Au alloy nanocrystals as bi-metallic NMs exhibits improved electrocatalytic activity from their mono-metallic components [111]. Further, carbon nanotube forest electrodes have also been used for the fabrication of an ultrasensitive electrochemical immunosensor with multilabel amplification strategies for the detection of single biomarker in biological samples [71]. In another study, a sensitive and specific electrochemical sensor was designed for simultaneous detection of two salivary biomarkers [interleukin-8 (IL-8) mRNA and IL-8 protein] in OSCC patients [112]. Besides, a modular microfluidic system with PDMS chambers was designed for multiplying and was found to facilitate faster detection of two protein marker, interleukin-6 (IL-6) and interleukin-8 (IL-8) in serum samples [113]. Another, strategy involves the use of functionalized magnetic beads for the multiplexed detection of IL-8 protein and IL-8 mRNA in human saliva samples and the performance of the fabricated platform was compared with the commercially available ELISA kit. The magnetobiosensor is easy to operate and provides a similar LOD but in less time duration which showed its capability to translate to POC device [114]. It was observed that the label-free technology significantly reduced the cost of fabrication, restricts complex steps of labelling and conserves the integrity of bio-recognition moiety.

Challenges and Future Perspectives

The discovery of NMs for bio-sensing technology has been instrumental in miniaturization of biosensors. As a result, fast, accurate, specific and robust lab-on-a-chip devices for detection of various target bio-analytes such as nucleic acids, proteins and metabolites were designed and fabricated. The incorporation of NMs in biosensors provide an increased surface area for immobilization of bio-recognition probe and hence efficient interaction occurs, thus enhancing specificity and sensitivity towards low concentration detection. Also, the transduction mechanisms have been improved considerably with the use of NMs and their composites. But, the limitation associated with the usage of NMs should be considered while fabricating any nano-bio-sensing platform.

The functionalization of nanostructured materials with non-hazardous amino acid opens a new domain in the

fabrication of nano-bio-sensing platform as well as in the fabrication of highly sensitive, biocompatible implantable bio-chip. The application of conduction polymers in increasing the efficiency of nano-bio-sensing has to be explored intensively and might also provide an advantage of fabricating lightweight flexible devices. Also, the strategy of deposition of conducting polymer (by 3D printing, lithography, spin coating, dip coating, etc.) onto the surface of a substrate and its effect on the sensitivity and specificity of a biosensor is another domain of research that requires further exploration.

Despite all these technological breakthroughs, the biosensing technologies require further fine-tuning to support clinical applications, i.e. development of more efficient and robust POC devices. Several challenges require immediate redressal such as early detection of OPMDs and OSCCs for timely therapeutic intervention in OC patients. As observed that the expression of a single biomarker cannot resolve the identification of OC predisposition, hence the application of multiplexing, i.e. incorporation of expression profile/signature of multiple biomarkers, has been recommended for accurate prognosis. The development of nanobiosensors based upon multiplexed detection is looked upon by oncology practitioners around the world.

Although the introduction of NMs has opened a new domain of development of nanobiosensors for quick, easy, smarter, user friendly and cost-effective detection of disease, the ethical bottlenecks and the issues related to transfer of this technology from a laboratory to clinical trials result in delayed commercialization. Therefore, a potential biosensor capable of detection of OPMDs and OC at an early stage is still in its infancy.

Summary and Conclusion

In this article, we have attempted to summarize the fabrication process and progress in the detection of OC biomarkers (proteins and nucleic acids) aimed at real diagnostic applications. Recent advances in detection of OSCC through nano-bio-sensing platform provide a brief curtain raiser of the upcoming technologies for detection of OPMDs and OC. Further research may lead this knowledge and use of nanobiosensors in translating bench-on platform to the point-of-care device, which could be a wearable biosensor or implanted into the human body itself. This quest may be unfolded by any innovative breakthrough in NMs through its nano-bio-sensing technology.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this manuscript.

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