Smart Nanomaterials Technology

Amit Kumar Mandal
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Functionalized Smart Nanomaterials for Point-of-Care Testing

Springer

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Nanotechnology is a rapidly growing scientific field and has attracted a great interest over the last few years because of its abundant applications in different fields like biology, physics and chemistry. This science deals with the production of minute particles called nanomaterials having dimensions between 1 and 100 nm which may serve as building blocks for various physical and biological systems. On the other hand, there is the class of smart materials where the material that can stimuli by external factors and results a new kind of functional properties. The combination of these two classes forms a new class of smart nanomaterials, which produces unique functional material properties and a great opportunity to larger span of application. Smart nanomaterials have been employed by researchers to use it effectively in agricultural production, soil improvement, disease management, energy and environment, medical science, pharmaceuticals, engineering, food, animal husbandry and forestry sectors.

This book series in Smart Nanomaterials Technology aims to comprehensively cover topics in the fabrication, synthesis and application of these materials for applications in the following fields:

- Energy Systems—Renewable energy, energy storage (supercapacitors and electrochemical cells), hydrogen storage, photocatalytic water splitting for hydrogen production
- Biomedical—controlled release of drugs, treatment of various diseases, biosensors,
- Agricultural—agricultural production, soil improvement, disease management, animal feed, egg, milk and meat production/processing,
- Forestry—wood preservation, protection, disease management
- Environment—wastewater treatment, separation of hazardous contaminants from wastewater, indoor air filters

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Preface

In recent era, nanomaterials are one of the most astonishing makings of human advancement due to their exceptional potential. The nanomaterials are well-known to possess superb thermal, optical, electrical, robust mechanical strength, and catalytic properties. Tools or smart point-of-care devices prepared using such nanoparticles are rummage-sale to identify detrimental substances or contaminants of the environment including their applications on human biomedical health. This book intends to provide the classifications and applications of smart nanomaterials and their modifications as biosensors provide an overview of the newest research on nanosized materials responding to various stimuli, including their up-to-date application in the biomedical field. Various chapters in this book focused on the overview of the current nanomaterials, their synthesis via both green and chemical routes, their properties, and their characterization. In other chapters, researchers made an attempt to comprehend nanoinformatics in the context of nanomedicine and the development of smart nanomaterials, its application in drug delivery, lateral flow assay, CRISPR point-of-care testing devices, in developing diagnostic microdevices, magnetic-nanosensors, nanodevices for pathogens detection, detection of environmental contamination, therapeutics for neurological disorders and its detection, its application in cell and tissue fabrication, and so on. Accordingly, the book in hand is an effort made to meet knowledge necessities on such aspects. It comprises 13 chapters, and the wide coverage of diverse aspects of the subject reflects quite well in the table of contents. This book is primarily intended for undergraduates, postgrdauates, and researchers working in the field of nanosciences and smart materials. We express our sincere thanks to the contributors who have shared their ideas and contributed chapters to this book. We shall be happy to receive comments and criticism, if any, from subject experts and readers of this published book.

Raiganj, India — Amit Kumar Mandal
Raiganj, India — Suvankar Ghorai
Wolaita, Ethiopia — Azamal Husen

About This Book

In recent era, nanomaterials are one of the most astonishing makings of human advancement due to their exceptional potential. The nanomaterials are well-known to possess superb thermal, optical, electrical, robust mechanical strength, and catalytic properties. Tools or smart point-of-care devices prepared using such nanoparticles are rummage-sale to identify detrimental substances or contaminants of the environment including their applications on human biomedical health. This book intends to provide the classifications and applications of smart nanomaterials and their modifications as biosensors provide an overview of the newest research on nanosized materials responding to various stimuli, including their up-to-date application in the biomedical field. Various chapters in this book focused on the overview of the current nanomaterials, their synthesis via both green and chemical routes, their properties, and their characterization. In other chapters, researchers made an attempt to comprehend nanoinformatics in the context of nanomedicine and the development of smart nanomaterials, its application in drug delivery, lateral flow assay, CRISPR point-of-care testing devices, in developing diagnostic microdevices, magnetic-nanosensors, nanodevices for pathogens detection, detection of environmental contamination, therapeutics for neurological disorders and its detection, its application in cell and tissue fabrication, and so on.

Contents

Overview of the Current Nano-Materials, Synthesis, Properties and Characterization 1
Zeynep Cimen, Esma Mutlutürk, Busra Cetin-Ersen, Tugba Gencoglu-Katmerlikaya, Sena Kardelen Dinc, Nalan Oya San Keskin, Esma Sari, Aydan Dag, and Gokcen Birlik Demirel

Nanoinformatics Applied to Smart Nanomaterials 31
Lívia V. Luchi, Valentina Nieto Marín, Maria L. R. Macedo, Octávio L. Franco, and Marlon H. Cardoso

Nanomaterials in Lateral Flow Assay 49
Arash Mohammadinejad, Ghazaleh Aleyaghoob, and Yavuz Nuri Ertas

Aptamer-Based Lateral Flow Assay as a Smart Point-of-Care Devices 83
Melis Canbay, Ahmet Turan Keskintas, and Sevde Altuntas

CRISPR-Based Point-of-Care Testing (POCT) Devices for Detection of Opportunistic Pathogens 97
Joydeep Chakraborty and Hironmoy Sarkar

Microfluidic Chips as Point-of-Care Testing for Develop Diagnostic Microdevices 115
Cagla Celik, Guven Akcay, Nilay Ildız, and Ismail Ocsoy

Magnetic-Nanosensor-Based Diagnostic Chips: An Overview 129
Zozan Guleken

Functionalized Smart Nanomaterials for Point-of-Care Testing 139
Arunima Lala, Hiranmoy Kotal, and Saikat Kumar Jana

Nanodevices for Food-Borne Pathogens and Toxin Detection 161
Merve Bacanli

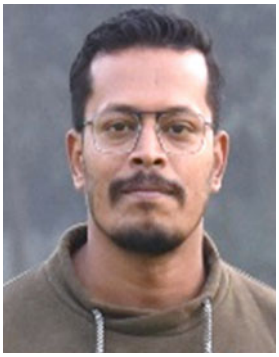
Nanomaterials for the Rapid Identification of Agriculturally Important Plant Pathogens 179
Zehra Karaagac and Ismail Ocsoy

Emerging Role of Nanotechnology-Based Devices for Detection of Environmental Contaminants 199
Deepyaman Das, Dipraj Chakraborty, and Suman Barman

Nanotechnology-Based Point-of-Care Diagnostics and Therapeutics for Neurological Disorders 211
Debayan Banik and Rama Ranjan Bhattacharjee

Functional Biosensors in Cell and Tissue Fabrication for Smart Life-Sciences Applications 235
Guven Akcay, Cagla Celik, Nilay Ildız, and Ismail Ocsoy

About the Editors



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Overview of the Current Nano-Materials, Synthesis, Properties and Characterization

Zeynep Cimen, Esma Mutlutürk, Busra Cetin-Ersen, Tugba Gencoglu-Katmerlikaya, Sena Kardelen Dinc, Nalan Oya San Keskin, Esma Sari, Aydan Dag, and Gokcen Birlik Demirel

Abstract Point of care testing (PoCT) systems, which enable diagnosis and treatment at or close to the care site, play a crucial role in the control of epidemics and other types of infectious diseases that are spread throughout the world due to their advantages such as short turnaround times, portability, reusability, efficiency, ease of use, and low cost. In particular, nanomaterial-based PoCT systems are widely used due to their excellent chemical and physical properties that allow high analytical performance and simplify the detection process. Therefore, recently, many different types of nanomaterials have been used to develop nanomaterial-based PoCT devices in various platforms. Various kinds of nanomaterials such as metal-based nanoparticles, quantum dots, nanoshells, nanotubes, metal–organic frameworks (MOFs) nanogels, nanofibers, and flexible hybrid composites are used to provide detection, signal generation, transduction, and amplification in PoCT devices. In this context,

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the synthesis methods and controlled physical/chemical properties of these nanomaterials are crucial points to improve the performance of the PoCT devices. In this chapter, we will highlight the synthesis and development strategies of nanomaterials currently used in different PoCT devices, along with existing challenges and future prospects.

Keywords Metal NPs · Quantum dots · Nanofibers · Nanogels · Nanoshells · Nanotubes · Metal–organic frameworks (MOFs) · Flexible hybrid composites

1 Introduction

Point-of-care (PoC) devices provide fast, on-site, and cost-effective alternatives to traditional laboratory applications that require long analysis times and expensive equipment [113]. Over the last decade, PoC products have been developed as real-time diagnostic products for use outside the laboratory and in laboratories with limited resources. Therefore, PoC technologies are gaining increasing importance in preventing and controlling the spread of diseases [113]. In this context, the design and the detection type of the sensor platforms are very important. Various types of sensor platforms, which have different readout modalities are being developed, including piezoelectric, magnetic, thermal, electrochemical, optical, and colorimetric detection [80]. A sensor platform can be defined as an analytical device and be selectively produced against a particular disease. Practically, a sensor platform operates on the principle that a target analyte can be specifically detected by chemical reactions or biological recognition, resulting in a specific signal that can be measured by different methods. Recently, the use of various nanomaterials has come to the fore to obtain sensitive, reproducible, and precise signals from sensors [84]. This is because nanomaterials have excellent physical and chemical properties compared to their bulk form, such as biocompatibility, large surface area, and specific catalytic activity. These unique properties of nanomaterials make them excellent candidates for the development of detection probes [24, 84]. For this purpose, various nanomaterials such as gold, silver, and polymer nanoparticles, quantum dots, hybrid nanocomposites, and carbon-based nanomaterials with different sizes, shapes, and compositions have been used to develop a PoC testing platform.

In this chapter, the synthesis of different types of nanomaterials in PoC systems and their application in diagnostics will be reviewed.

2 Metal-Based Nanoparticles

Metal-based nanomaterials are an important milestone in the advancement of nanoscience, which is currently an advanced research area [99, 109]. The development of metal nanoparticles, which first started with the controlled synthesis of

gold nanoparticles, continued with the synthesis and efforts to elucidate the properties of other metal-based nanomaterials [44]. The unique physical, optical, and chemical properties and functionalities of metal nanostructures, which are largely dependent on sizes, shapes/facets, compositions, and architectures, have attracted the massive attention of researchers in science [32, 35, 90, 99]. Although research with metal-based nanowires and nanoclusters has recently been included in the literature, nanoparticles are still the most commonly used metal-based nanomaterials in point-of-care (PoC) systems [3, 84]. An impressive work involving AgNPs was reported by Yuan et al. [129]. They designed a Tyndall effect-inspired assay (TEA) to detect creatine in human urine by taking advantage of colloidal Cit-AgNPs. The citrate-capped AgNPs with a weak Tyndall effect (TE) signal aggregated after being added to creatine and formed a hydrogen bonding network with creatine tautomers under alkaline conditions, resulting in a significant increase in TE signal that was generated and quantified using a smartphone and a portable laser pointer, respectively. The increase in TE signal that can be seen with the unaided eye is directly proportional to the creatinine concentration in the sample. Additionally, this portable quantitative detection platform may be employed by incorporating it into a smartphone. This metal nanomaterials-based point-of-care test system, which is performed without the use of any sophisticated equipment, has a detection limit of ~50 nM for creatinine that is at least 90 times lower than even the most sensitive conventional colorimetric methods.

Fu et al. designed a PoC test system that will perform simultaneous and visual detection of three different analytes with gold nanoparticles (AuNPs) integrated with three different aptamers, should be cited as an example of the application of these [35]. Aptamers prevent the aggregation of AuNPs in a high-salt environment. This is because aptamers interact with gold nanoparticles, and interfere with the interaction of high salt and AuNPs, thereby preventing their aggregation. Aptamers are stripped from the AuNPs when analytes are present because of the greater interaction between aptamers and the analytes. The color of the solution changed dramatically after AuNP aggregation in the high salt condition, allowing for analyte detection with the naked eye. Three analytes were determined simultaneously and visually at the detection limit of 53 nM, 130 nM, and 11 nM, respectively, with a single sensor using a multi-aptamer. This study resulted in the development of a simultaneous and visible multi-component detection platform, which was also successful with blood and urine samples. In another example proposed by Al-Kassawneh et al., the glucose level in human saliva was colorimetrically determined using a gold nanoparticles tablet (AuNPs-pTab) prepared by encapsulating AuNPs with pulluan, a natural, biodegradable ligand used as both a reducing agent and a capture agent, as a simple point-of-care (POC) test kit [6]. To detect glucose, a detection limit of (LoD) 28.7 μM was performed in buffer solution; however, in artificial and real human saliva samples, LoD values 38.2 and 163.04 μM, respectively, were reached. These tablet sensors, developed by this study, which pioneered the use of the reactive encapsulation technique for glucose detection, will significantly contribute to the design of PoC devices that are ready to use and have the potential for OnSpot colorimetric testing for various diseases. In another study that should be mentioned in this

context, homometallic and heterometallic nanohybrids were synthesized by in situ fabrication of AuNPs, AgNPs, and their plasmonic hybrids using sericin protein as a reducing and capturing agent [15]. A surface plasmon-coupled emission (SPCE) application for mobile phones was used to accomplish attomolar level detection of mefenamic acid using the versatile, polarized, and enhanced fluorescent emission of these nanohybrids.

One of the most crucial points of this study is that it will shed light on the design of point-of-care diagnostic tools that can be developed with green nanotechnology without the need for the use of hazardous chemicals and solvents for different applications in the future.

3 Quantum Dots

In recent years, quantum dots (QDs) have received a lot of attention due to their unique optoelectronic properties such as strong absorption, size-dependent photoluminescent emission, high quantum yield (QY), and high optical stability [57, 91]. Different types of QDs have functional properties in a variety of fields, including sensing, optoelectronic devices, biomedicine, and point-of-care (PoC) systems [4, 17]. Although it is stated in many sources in the literature that it should have a size distribution below 10 nm, in fact, the sizes of QDs can reach up to about 30 nm [29, 104]. For a nanometer-sized crystal to be considered a QD, the quantum confinement effect rather than size must be observed. For this, the physical dimensions of nanometer-scale colloidal semiconductor crystals must be smaller than the Bohr radius exciton [104]. Dimensions of QDs generally vary depending on the material from which they are synthesized [4, 111]. The materials used for its preparation are also used to identify the types of QDs [115]. Conventional quantum dots were first prepared as core nanocrystals by combining the III–V, II–VI, and IV–VI groups of the periodic table [4, 111, 115]. Then, with the synthesis of QDs carried out in the form of core–shell nanocrystals, which prevents the leaching of metal ions in the core, the quantum efficiency was increased up to almost 75% [57]. In addition to the high quantum efficiency obtained in this way, it has become the best fluorophore candidate for many applications with its advantages such as extremely broad and intense absorption spectra allowing single wavelength excitation, narrow, symmetric, and size dependent fluorescence spectra, superior photostability and enabling flexibility in excitation [29, 104]. The features that overshadow all these excellent optoelectronic properties are harsh synthesis reaction conditions, complex surface passivation procedure, and especially cytotoxicity created by toxic precursors [17, 111]. Although traditional QDs can be prepared with many different combinations of III–V, II–VI, and IV–VI groups, cadmium-based QDs are the most preferred [115]. However, because of their capacity to attach to thiol groups on essential components in mitochondria and inflict sufficient stress and damage to result in appreciable cell death, cadmium ions were discovered to be the main cause of cytotoxicity of QDs [29]. Their sustained practical application is hampered by this toxicity [115]. For this

reason, researchers have searched for environmentally friendly, biocompatible QDs and have launched them as cadmium-free QDs [58]. Carbon QDs (CQDs), which are obtained from the element carbon, which has been frequently used since the 19th century, were obtained in 2004 by preparative electrophoresis during the purification of single-walled carbon nanotubes [4]. Graphene quantum dots (GQDs) were discovered shortly after the discovery of CQDs, especially due to the combined use of carbon and graphene in electrochemistry applications. The low toxicities of both CQDs and GQDs, as well as the carboxylic acid moieties of GQDs, have allowed these structures to increase in water solubility and allow for biological modification, allowing them to show superiority among other QDs [58]. Another is the next generation Ag_2Se and Ag_2S QDs, which are designated as near infrared QDs class and have been used in biological imaging applications [57]. In addition, although it has only recently been discovered in zero-dimensional black phosphorus QDs synthesized by chemical methods, it has been used in bioimaging, fluorescence sensing, optoelectronic, and flexible devices [4, 111]. The most important features of these structures are small size, high brightness, quick radiation transition rate, good light stability, low biological toxicity and customizable emission spectrum, high quantum yield easily functionalized, and strong biocompatibility, respectively [111]. Finally, in this context, MXene dots should also be mentioned. These quantum dots, which are promising candidates in many fields such as bioimaging, biomedical, and biosensor, are striking with the advantage of a large number of functional groups on their surfaces [4]. The synthesis of QDs is shaped according to their classification [57]. Synthesis of traditional Cd-based QDs was first performed by pyrolysis of organometallic and chalcogen precursors, but since the hydrophobicity of QDs synthesized by this method significantly reduces both their water solubility and biocompatibility, further modification is needed after synthesis. Therefore, researchers focused on the synthesis of hydrophilic QDs. For this, applied to the use of stabilizers such as 3-mercaptopropionic acid (MPA), 2-mercapto ethylamine acid (MA), thioglycolic acid (TGA), and L-cysteine in the aqueous synthetic procedure [57]. Also, microwave-assisted green synthesis, which relies on environmentally sensitive microwave irritation, is one of the popular methods of choice for the preparation of such QDs [48].

Two synthesis approaches have generally been proposed for Carbon QDs and GQDs, which are called natural-based QDs (NQDs). In these methods, which are launched as top-down and bottom-up, top-down is based on the decomposition or exfoliation of large carbon structures or large graphene sheets, while bottom-up is based on the creation of GQDs and carbon QDs from small precursors with solution chemistry methods. Top-down techniques include hydrothermal cutting, solvothermal cutting, electrochemical cutting, nanolithography, microwave-assisted cutting, nanotomy-assisted exfoliation, and ultrasonic shearing; bottom-up techniques like stepwise organic synthesis and cage opening of fullerenes fall under this category as well [48, 91]. There are only a few synthesis techniques recommended for other semiconducting quantum dots. This sort of QDs are often synthesized using several different techniques, including solution-phase-based methods,

microemulsion synthesis, thermally induced disproportionation of solid hydrogen silsesquioxane in a reducing atmosphere, and others [57].

In the characterization of QDs, parameters such as optical properties, size, and morphology are examined with advanced devices. While UV-VIS and photoluminescence spectroscopy are preferred for optical characterization, devices such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS), and X-ray diffraction (XRD) are used for size distribution. It has been reported that the tools used for morphological and structural characterization are X-ray photoemission spectroscopy (XPS), nuclear magnetic resonance spectroscopy (NMR), Rutherford backscattering spectrometry (RBS), atomic force microscopy (AFM), field emission scanning electron microscopy (FESEM), and Fourier transform infrared spectroscopy (FT-IR) [4, 29].

In recent years, the use of fluorescent probes in PoC testing systems has offered great advantages in terms of the accuracy of the detection system and the simplicity of the readout system. In addition, the use of fluorescent materials such as quantum dots in PoC systems is cheaper and easier to convert into disposable chips than other methods, so it is more suitable for real-time use in the field [21]. Therefore, as exemplified below, the use of QDs in PoC systems has become quite common in recent years. A study emphasizing the use of quantum dots in point-of-care applications has been reported by Zhang and Shi(Jingfei) [134]. The antibiotic tetracycline (TC), which is now a severe hazard to both public health and the environment, was used in this study to create a coordination complex with the ion Eu^{3+}, from which a faint luminescence was produced. Then, by transferring energy from MoS_2 QDs with a strong fluorescence property to the Eu-TC coordination molecule, the initial fluorescence intensity was significantly increased. In the study, TC between 10 nM and 60 μM was determined with a detection limit of 2 nM with MoS2 QDs used as both the indicator and enhancer of the ratiometric probe. Additionally, color recognition software was used to adapt this sensor to the smartphone-based portable platform, and visual quantitative detection was carried out sensitively, quickly, and in real time with a detection limit of 0.05 μM.

Another study, which has been reported to design even more efficient PoC testing systems with quantum dots, has been brought to the literature by Sun et al. [98]. Four metal ions were monitored within the scope of this study, on-site, user-friendly, real time, selective manner utilizing a paper-based analytical instrument constructed using S quantum dots. The research is based on the idea that S quantum dots provide a different visual signal with each ion, particularly green for Fe^{3+}, brown for Co^{2+}, bright yellow for Cd^{2+}, and precipitate for Pb^{2+}. This consists of three layers such as isolation, reaction, and base, and it has several spots where the assay will take place. The images obtained after interacting with metal ions were used for on-site and visual determination with the help of a smartphone-based platform and color recognition software. In this ion-responsive platform, a smart strategy was created by integrating multiresponsive blocks into S dots, allowing multiple logic operations (i.e., yes, not, and, inhibit, and nor) for determination. Finally, with this quadruple analyte responsive platform, Fe^{3+}, Co^{2+}, Cd^{2+}, and Pb^{2+} ions were not only determined at

the detection limit of 0.59, 0.47, 0.82, and 0.53 μM, respectively, but also a point-of-care testing (PoCT) system that could be created for various analytes was successfully introduced to the literature.

4 Metal–Organic Frameworks (MOFs)

MOFs are hybrid materials consisting of a large surface area, low density, and highly porous inorganic and organic units. In the last decade, different types of hybrid MOFs with a wide range of uses have been reported based on polymer, metal oxide, carbon, metal nanoparticle, and biomolecule [86]. In addition, MOF-PoC test platforms used in different sensor-based diagnostic and detection applications have been reported recently. The organic units that makeup MOFs are anions such as carboxylate, cyano compounds, imidazole derivative polyamines, phosphonate, sulfonate, and heterocyclic compounds. Metal ions or clusters called secondary structural units (SBUs) form the inorganic units of MOFs [86]. The solvo(hydro)thermal method is generally used for the synthesis of MOFs (Fig. 1). The synthesis is carried out using an autoclave at high temperatures and pressure above the boiling point of the solvent. Under solvothermal conditions, starting reagents can undergo unexpected chemical transformations that can lead to the formation of new ligands. Therefore, optimum reaction conditions must be provided [16]. In addition to these methods, other alternative synthetic methods such as diffusion, mechanochemical, electrochemical, microwave, and ultrasonication methods have been developed in recent years [10, 86].

In the diffusion method, solvent/solvent mixtures with a low boiling point are mostly preferred and the reacting species are transported slowly in the presence of the solvent. Thus, crystal growth and nucleation occur over time at the interface point [89]. Electrochemical synthesis of MOFs can occur in a maximum of 2 h at ambient temperature and pressure. The metal ion is added to the reaction mixture containing organic ligands and electrolytes by anodic dissolution. The method has advantages such as high efficiency, low energy consumption, and the absence of counter ions. In this way, it allows the controlled synthesis of MOFs. Many MOFs prepared by electrochemical methods such as ZIF-8, MOF-5, HKUST-1, MIL-100(Al), MIL-53(Al),

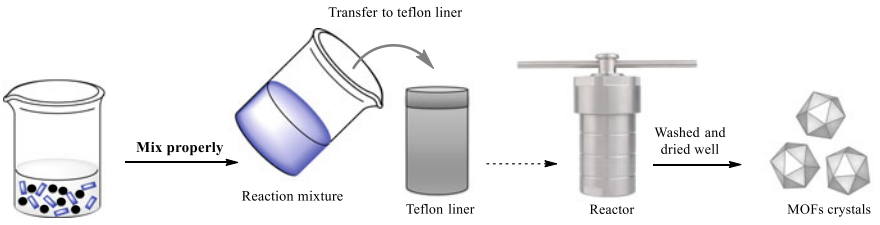

Fig. 1 Schematic presentation of the hydrothermal synthesis route of MOFs

and NH_2-MIL-53(Al) have been developed in the literature [7, 37]. Mechanochemical synthesis of MOFs occurs by solid-state organic reaction with less or no solvents. Furthermore, this method allows for large-scale production of MOFs in shorter reaction times and lower temperature conditions compared to diffusion and solvothermal methods [34]. MOF synthesis by ultrasonication is based on the chemical transformation of molecules under high-energy ultrasonic radiation (20 kHz–10 MHz). Compared with other techniques, it provides simple operating conditions, high efficiency, easy controllability, and short reaction time [10]. MOFs are characterized by several methods such as X-ray diffraction, single crystal X-ray diffraction, scanning electron microscopy (SEM), inductively coupled plasma optical emission spectroscopy (ICP-OES), thermal gravimetric analysis (TGA), nuclear magnetic resonance (NMR) spectroscopy, and Brunauer–Emmett–Teller (BET) analysis [43]. Recent studies show that MOFs have been the focus of researchers for POC tests in many different usage areas with their superior properties [12]. Zhang et al. reported a fluorescent lanthanide-based MOF (L-MOF-enzyme) composition to detect glucose in serum and urine [135]. The composite was prepared by an immobilization between Eu^{3+}@UMOF and glucose oxidase (GOx). Herein, glucose is oxidized by GOx and the H_2O_2 produced can quench the fluorescence of Eu^{3+}@UMOF. The fluorescent intensity of Eu^{3+}@UMOF corresponds to the glucose concentration (CGlu). In the system integrated with the detector, CGlu has three different concentration ranges (0.1–10 μM, 10–10 mM, and >10 mM). Three different outputs; (L(low), M(medium), and H(high)) corresponding to these three concentrations can be determined with the naked eye. The prepared detector provides the detection of glucose levels in the urine with high selectivity and sensitivity. It allows on-site diagnosis without going to the hospital for complex examinations, especially for diabetics. Chen et al. reported a high-sensitivity PoC test fluorescent nanosensor for tetracycline [22]. Tetracycline is an antibiotic frequently used in medicine against bacterial infections. However, its misuse leads to tetracycline residues in animal foods and affects human health. The probe (FL:LZIF-8-Cit-Eu) was prepared by encapsulating fluorescein (FL) in 1-histidine modified ZIF-8 (LZIF-8) and chelating it with the citrate complex. When FL:LZIF-8-Cit-Eu is exposed to tetracycline, a characteristic Eu^{3+} sensitive fluorescence is formed as a result of the coordination between Eu^{3+} and tetracycline. The results demonstrate fast (<20 s), high selectivity, and high sensitivity (LOD = 5.99 nM) PoC detection of tetracycline. Yan et al., on the other hand, prepared a lanthanide-based MOF platform Eu(TATB) for the detection of Sulphamethazine (SMZ), which are another frequently used antibiotic in medicine [123]. Nanoscale Eu(TATB) is prepared by the microemulsion method and has a stable red luminescence in an aqueous solution. In addition, it was embedded in the prepared lanthanide-based MOF filter paper and integrated into the smartphone imaging system. Thus, a paper-based MOF-PoC test system was designed to monitor SMZ.

5 Carbon Nanotubes

Since they have excellent electrochemical properties, physical–chemical stability, mechanical strength, and a large surface area, carbon-based nanomaterials like carbon nanotubes (CNT) are highly in request when creating point-of-care diagnostic tools that can diagnose and treat illnesses quickly, sensitivity, and affordably. CNT are obtained by sp^2 hybridization of graphite in the form of hollow cylindrical tubes with a high surface-to-volume ratio. Based on the number of walls, CNT are classified into three groups such as single-walled nanotubes (SWNT), double-walled nanotubes (DWNT), and multiwalled nanotubes (MWNT). CNT are generally used for bioimaging endowing superior optical properties and assisting easy incorporation of contrast agents such as fluorescent probes, radionuclides, and organic/inorganic nanomaterials with a high ratio for MRI (magnetic resonance imaging), CT (computed tomography), PET (positron emission tomography), and SPECT (single-photon emission computed tomography), etc. CNT serve as an important option for health care platforms, but their hydrophobic nature is one of the major obstacles to the use of CNT in sensing applications, drug delivery, photothermal therapy, and other applications.

The structure and individual properties of CNT have been demonstrated to be precisely dependent on the synthesis methods [87]. Many different methods have been introduced with the studies on high purity production demand, synthesis at low temperatures, and increasing production capacity, and classification has been made as synthesis from solid carbon and gaseous carbon, inspired by the states of materials applied in production. In this part, most typical synthesis strategies are discussed in detail; all of which have been extensively studied. One of the first techniques for creating carbon nanotubes was the arc discharge method. The procedure involves creating a space between two graphite rods, one of which serves as the anode and the other as the cathode, causing an arc to form, and using a direct current to make nanotubes. In the arc discharge process, the nanotubes produced by bombarding a target made of pure graphite are multiwalled, but the nanotubes produced by bombarding a target made of catalysts like Co, Ni, Fe, or Y are single-walled. The anode particle's core contains the catalysts. MWCNT are produced by this method with high crystallinity. Transition metal catalysts must be used in this method for the formation and growth of SWCNT. In theory, the arc discharge approach and the laser evaporation method are comparable. This method uses a laser source rather than an electric discharge to generate a high temperature on a carbon target. Although the laser evaporation method is suitable for producing higher quality SWCNT with higher mechanical strength than the arc discharge method, it is not preferred because of its high cost and low production capacity. For the last two decades, carbon fibers and their filaments have been produced using the chemical vapor deposition method of hydrocarbons in combination with a metal catalyst. The chemical vapor deposition method has several advantages compared to previous synthesis methods. It is a simpler and more economical technique as production takes place at lower temperatures and pressures. The most common techniques for analyzing the general

morphology of CNT samples include electron microscopy, atomic force microscopy (AFM), nuclear magnetic resonance (NMR), electromagnetic spectroscopy, XRD diffraction, and light scattering techniques. In fact, IR, NMR, and Raman spectroscopy are used to confirm the presence of functional groups on CNTs. CNT offer attractive PoC biosensing applications due to having remarkable electro-chemical properties. The recognition process of CNTs principally relies on various enzymatic processes that produce electroactive species for the detection of metabolites, protein biomarkers, and ions [49, 138]. Additionally, recognition elements may be aptamers, antibodies, oligonucleotides (DNA or RNA), ligands, and whole cells. Many research articles have assessed the development of CNT-based biosensors for RNA detection identifying overexpressed microRNA (miRNA)-specific patterns in cancer diagnosis. For example, an electrochemical microRNA (miRNA) nanosensor, which uses CNT and electroactive polymer films, has a low detection limit of ca. 8 fM and has been used for the detection of human prostate metastatic cancer cells [101]. Similarly, Topkaya et al. reported early, label-free electrochemical detection of prostate cancer [77]. As seen in Fig. 2, another work SWCNT-based antibody conjugated optical nanobiosensor has been used for prostate cancer biomarker urokinase plasminogen activator (uPA) detection via surface-enhanced Raman spectroscopy (SERS) in whole serum, plasma, and blood [116].

For the sensitive detection of carcinoma antigen-125, zinc oxide-fabricated MWCNT nanowire immunosensors have been prepared by simple and low-cost electrospinning techniques [81]. Increased sensing performance has been found for BSA functionalized MWCNT-ZnO nanowire immunosensor with an excellent limit of detection (0.00113 U/mL) (Fig. 3).

In another study, a sensor was designed to detect hybridization processes of small DNA and RNA oligonucleotides in vivo with a label-free approach that converts carbon nanotube photoluminescence into spectral changes. The mechanism of action was determined by dielectric, electrostatic factors, and molecular dynamics simulations. They showed that the sensor facilitates multiple sensing using various nanotube chirality and monitoring concurrently of toehold-mediated DNA-strand displacement, which results in signal response reversal. It has also been demonstrated by in vivo optical experiments by implanting that the designed sensor is extremely resistant to non-specific interactions with biological molecules and allows direct detection from serum and urine [41].

SWCNT field-effect transistor biosensors are known for offering the highest level of sensitivity [8]. However, it also provides high selectivity and distinguishes the real signal from other signals in an uncontrolled environment. In a study, they demonstrated the successful integration of a new peptide aptamer with SWCNT field-effect transistors for the specific and sensitive recognition of Cathepsin E, one of the cancer biomarkers. SWCNT were prepared via the CVD method. It is then integrated into a SWCNT field-effect transistor device. The constructed sensors were found to exhibit high selectivity at low concentrations in not only phosphate-buffered saline (2.3 pM), but also human serum (0.23 nM). In conclusion, it has been shown that SWCNT FET sensors modified with peptide aptamer can be used as a remarkable platform for PoCT applications [102].

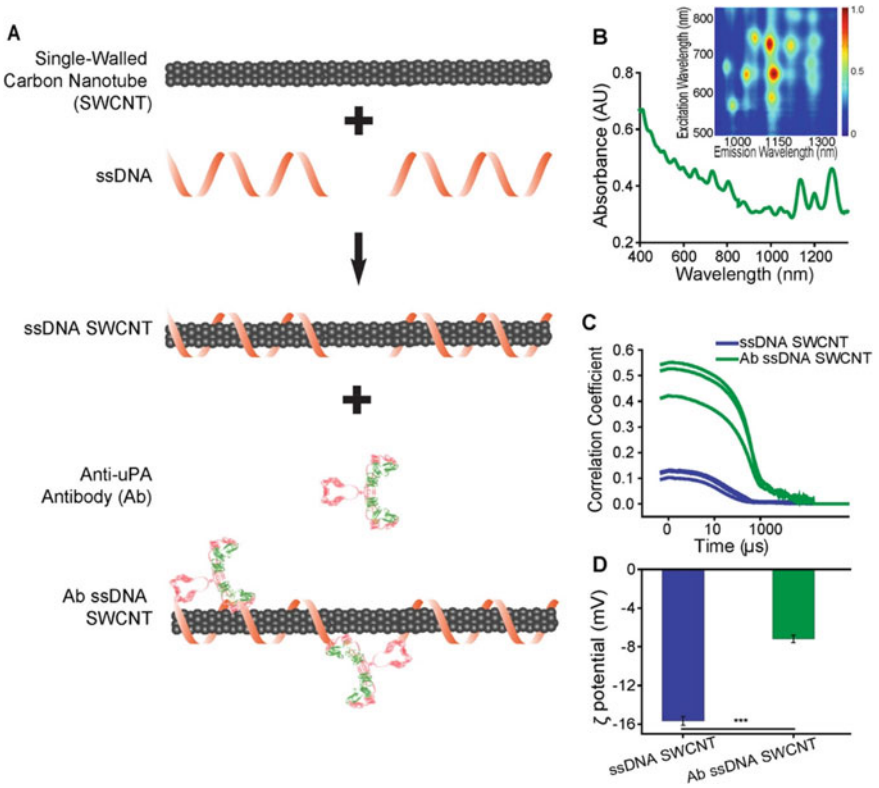

Fig. 2 Schematic representation of the synthesis and characterization of Anti-uPA-DNA-SWCNT nanobiosensor. Adopted from Williams et al. [116]

6 Nanoshells

Nanoshells are defined as a class of nanoparticles with a dielectric core of 10–300 nm in size, covered with an ultrathin metal shell [47]. Nanoshells have great tunable optical properties and these optical properties can be tunable depending on their size and making them good candidates for PoCT. There are different methodologies to obtain nanoshells, which use dielectric cores as templates to grow metal shells on their surfaces. Also, several synthetic approaches can be used to fabricate hollow nanoshells.

Zhou et al. first synthesized metal nanoshells with an inner dielectric Au_2S core surrounded by a gold shell in 1994 [11]. A gold nanoshell refers to a filled or hollow core surrounded by a spherical layer of gold. According to the gold shell thickness and nanoparticle size, the optical characteristics of gold nanoshells can be tuned for biomedical applications. Different core types can be used to fabricate gold nanoshells. Silica is often used as a core material, because of its superior properties for the fabrication of gold nanoshells by several approaches including

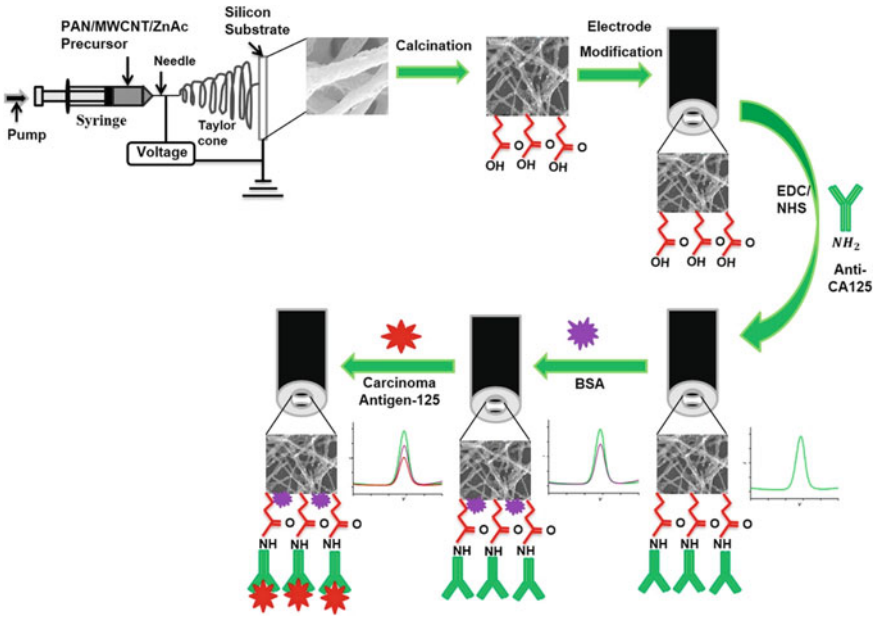

Fig. 3 Schematic representation of the one-step biofunctionalization electrospun MWCNT nanowire immunosensor for the detection of carcinoma antigen-125. Adopted from Paul et al. [81]

surfactant-assisted method, deposition–precipitation method, sonochemical gold seeding method, sandwiched gold seeded shell synthesis, and one-pot synthesis method. Surfactant-assisted seeding method involves using surfactants such as 3-aminopropyltriethoxysilane (APTES), which is a linker to provide NH_2 groups on silica nanoparticles. The amine-functionalized silica nanoparticles could link to gold when a gold colloidal mixture was added [114].

Deposition–precipitation (DP) is a method generally used to form directly gold seeds on a silica core (Fig. 4) [50]. Subsequently, the surface of the silica nanoparticles is decorated with APTES. These amine-functionalized silica nanoparticles are seeded with gold hydroxide nanoparticles. To synthesize gold nanoshells by the DP method, $HAuCl_4$ is hydrolyzed by adding NaOH to give a yellowish gold hydroxide solution. Silica nanoparticles were then added, and the orange-brown colored solution included $Au(OH)_3$ nanoparticles loaded onto silica nanoparticles. A basic gold hydroxide solution (K-gold) and sodium borohydride were added to $Au(OH)_3$ seeded silica nanoparticles to grow nanoshells by reduction of gold. The color of the solution can be red, purple, or green depending on the shell thickness [82].

Another most used core material of nanoshell systems is polymeric nanoparticles. There are several approaches to fabricating gold nanoshells on a polymer core including solvent-assisted method, combined swelling heteroaggregation method, gold colloid seeding method, and gold ion seeding method. In the solvent-assisted

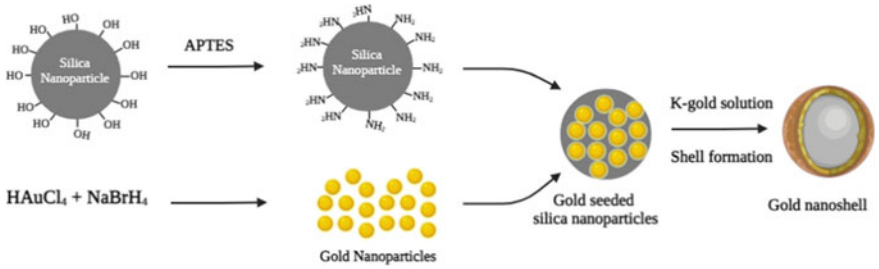

Fig. 4 Synthesis of gold nanoshells on silica core by DP method. Created with BioRender

method, the polymer as a core material is immersed in a solvent that contains gold salt. When the polymer swells, gold ions can permeate into the polymer core to form a gold shell on the polymer core [133].

The gold colloid seeding method involves the formation of gold nanoshells that cover the polymer core by electrostatic interactions or covalent bonds when adding gold colloid solution which forms gold seeds on the surface [63]. By using low metal concentration and controlling reaction conditions, the reduction of gold ions led to the formation of nanoshells on an unfunctionalized polymer surface in the gold ion seeding method. Different reaction conditions such as pH, reducing agent concentration, and temperature affect the morphological characteristics of nanoparticles [13].

Hollow gold nanoshells can be fabricated by using a silica core to synthesize gold nanoshells as described and then using hydrogen fluoride (HF) to remove the silica core. Other methods including sacrificial template method, template galvanic replacement method, electrochemical synthesis method can be also used to prepare hollow gold nanoshells. The sacrificial template method requires two steps for the fabrication of hollow gold nanoshells. First, cobalt nanoparticles which act as a template for the gold nanosphere formed in the presence of sodium borohydride. Then sodium borohydride is removed before adding $HAuCl_4$ solution. After gold nanoshells are formed, air exposure causes oxidation of the residual cobalt, leading to the formation of a hollow gold nanoshell [2]. Also, silver nanoparticles act as a template for the fabrication of hollow gold nanoshells by using the template galvanic replacement method. Redox reaction between Au^{3+} and $Ag^{(0)}$ induced gold shell formation and after the pitting process, hollow nanoshells are formed [62].

Nanoshells can be characterized by advanced devices in terms of parameters such as optical properties, size, morphology, and composition. UV-visible spectroscopy is commonly used to characterize superior optical properties of nanoshells [5]. The ratio of shell thickness and overall diameter of nanoparticles affects the optical characteristics of nanoshells. It is possible to determine the size of the nanoparticles with different characterization tools such as transmission electron microscopy (TEM), scanning electron microscopy (SEM) [69], dynamic light spectroscopy (DLS) [79], and X-ray diffraction (XRD) [9]. Moreover, SEM and TEM provide information about the morphology of nanoparticles such as crystallinity and lattice structure.

Also, the stability and aggregation of nanoparticles can be determined by DLS. XRD is used to determine not only the size and morphological characteristics but also the composition of the nanoparticle. X-ray photoemission spectroscopy (XPS) and energy-dispersive X-ray spectroscopy (EDX) are other methods to evaluate the composition of nanoshells [117]. Nanoshells with unique properties are import for PoCT in terms of having the capability to conjugate antibodies and other biological molecules in immunoassays. PoCT has several advantages such as simplicity, user-friendliness, time-saving, or low cost, and recent studies reveal that nanoshells have been the focus of researchers for PoCT in many areas with their unique properties. Several practical portable analytical platforms have been used for the detection and/or diagnosis via PoCT such as lateral flow assay (LFA). When samples flow through the strip, the analytes interact with recognition molecules and then signals are captured by another recognition molecule (Fig. 5). Commercial LFA platforms can be used for the PoCT of antigens, disease biomarkers, hormones, or microorganisms. For example, Huang et al. developed colloidal gold test strips with Pt nanoshells as a quantitative PoCT method. In their work, myoglobin which is an early biomarker of acute myocardial infarction was used as a model analyte and a pressure-based method was developed to measure potentially the number of various analytes. Colloidal gold combines with the Ag precursor and hydroquinone to generate an Ag shell on the surface. After that, a Pt precursor and ascorbic acid can coat the Ag shell with Pt. After Pt coated nanoshells produce catalytic gas from the decomposing H_2O_2. The amount of Pt nanoshells on the test line correlates with increased pressure due to gas output [45].

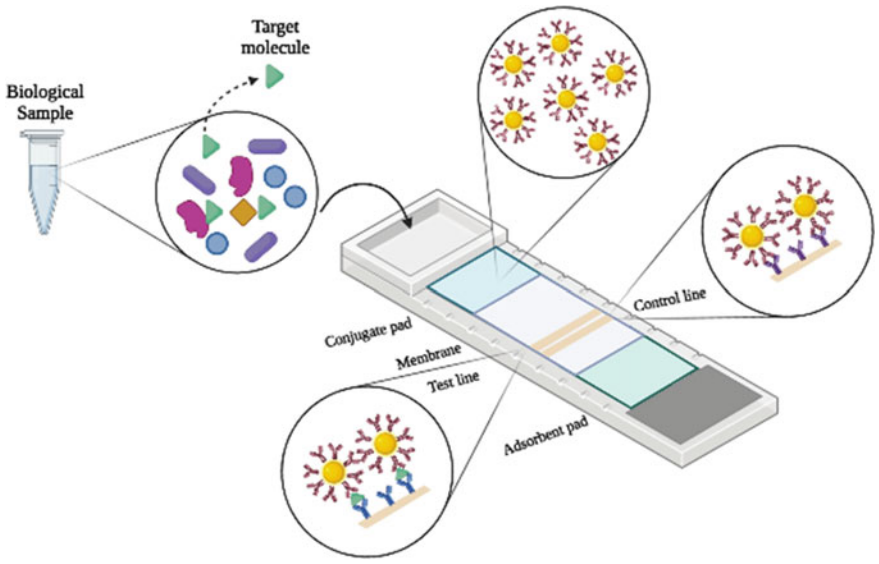

Fig. 5 The schematic representation of LFA strip decorated with antibody-conjugated gold nanoshells. Created with BioRender

In another work, Srinivasan et al. showed how to use gold nanoshells as a tag for LFA with a remarkable increase in the signal without the need for any additional signal amplification steps for the detection of prostate-specific antigen (PSA). They fabricated the gold nanoshells conjugated with anti-PSA antibodies to target PSA obtained from blood serum samples. This work declares that the portable quantitative PSA screening test has the potential to guide patient care, minimize therapeutic turnaround times, and improve clinical care in areas without diagnostic labs or automated immunoassay systems [96]. Similarly, nanoshell-based PoCT has a great potential to recognize microorganisms with high sensitivity. A different example of gold nanoshells-based LFA was developed for the detection of Chagas disease. Chagas multiantigen conjugated to gold nanoshells recognize circulating human anti-Chagas antibodies with high sensitivity and specificity and it is comparable to commercial methods [70]. For the diagnosis of the tuberculosis, dot blot immunoassay was developed to identify tuberculosis-specific CFP-16 antigen from the clinical urine samples by using the formation of copper nanoshells on the gold nanoparticles' surfaces, which can be quickly observed with naked eye [85]. In a recent study, polyhedral nanoshells were developed as paper strips to detect bovine viral diarrhea virus observably with the naked eye by increasing the signal transmission. By using a new bovine viral diarrhea virus (BVDV) recognizing peptides and designing a copper polyhedral nanoshell on the surface of gold nanoparticles, a dot-blot technique for the rapid diagnosis of BVDV was developed. The copper polyhedral nanoshell served as the quantitative diagnostic of the virus and contributed to the distinctive performance of the peptide-based optical biosensor in detecting the target by enhancing the appearance of the pink dot [52]. The colorimetric assay platforms can be used as a reliable detection kit for point-of-care testing. Although the colorimetric test platforms for dissolved hydrogen sulfide were commonly used, they still have mostly low sensitivity. The creation of effective signal amplification techniques is one potential solution to this problem. Last, of all, nanoshells can benefits such as detection, signal generation, and amplification of signals to produce novel PoCT systems, which make possible diagnosis and treatment at the care-site.

7 Nanogels

Hydrogels have been investigated in many applications due to their flexibility, biocompatibility, softness, and high tensile strength [46]. Nanogels (NGs) are nanosized and three-dimensional hydrogels with a particle size between 20 and 200 nm. Physical or chemical cross-interaction between polymer networks leads to permanent nanogels. 3D-dimensional nanogels have features such as adjustable size, swelling ability, flexibility, and large surface area [67]. These properties of the nanogels are adjustable, thus allowing biomedical applications [36, 78]. Traditional laboratory methods used to diagnose pathologies have good selectivity and sensitivity, but they require more time, cost, and staff. The point-of-care testing provides faster and earlier detection of pathologies. Solution-based colorimetric nanosystems and

surface plasmon resonance biosensing are the most commonly used for PoCT [71]. Nano or microgels are of great use in therapeutic and diagnostic applications due to their ability to swell in aqueous solution, ensuring non-specific cell or protein absorption [27, 83]. Nanogels can be defined as chemically or physically cross-linked nanosized polymer networks. Cross-linking of polymer chains ensures a three-dimensional network and high water absorption capacity without dissolution [95]. Generally, nanogels can be prepared in three different ways.

(i) *Physical method*

In this method, physical interactions are reached between polymer chains. These interactions occur between supramolecular contructs without covalent bonding. Compared to other methods, Van der Waals, ionic, hydrophobic–hydrophilic, hydrogen bonds are the driving force for the synthesis of cross-linked networks without additional cross-linking agents. Physical cross-linked nanogels are less stable than chemically cross-linked nanogels [68].

(ii) *Polymerization of monomers*

Polymerization of monomers is an appropriate way for the synthesis of nanogels. The polymerization method works through polymerization of monomer in the presence of initiator, catalyst, and cross-linking agent. Emulsion polymerization, controlled living radical polymerization, and click chemistry are widely used for polymerization of monomers [103]. The emulsion polymerization leads to polymerization of reactive monomer polymers in an aqueous suspension or water-nano emulsion phase [54]. Controlled living radical polymerization method affords the synthesize of well-defined nanogels with high polymer molecular weight, different compositions, and dimensions [68]. Nitroxide-mediated polymerization (NMP), atom transfer radical polymerization (ATRP), and reversible addition-fragmentation chain-transfer (RAFT) polymerization methods are known as SI-CRP methods [76]. Click chemistry is a simple and efficient method and includes copper-catalyzed reactions, copper-free click reactions, and pseudo-click reactions.

(iii) *Cross-linking of polymers*

Covalent cross-linking is widely used for coupling polymer chains to form a gel network [42, 53]. Click chemistry, disulphide-based cross-linking, and amino group-containing cross-linking are methods of cross-linking of polymers. It is possible to produce very different types of functional nanogels using this technique [75].

7.1 Classification of Nanogels

Nanogels can be classified on their behavior as non-responsive and stimuli-responsive nanogels. Stimuli-responsive nanogels change their structural properties in response to internal or exogenous stimuli, including light, pH, temperature, ultrasound, and

magnetic field [33, 72]. These sensitive nanogels are often called "smart" materials. Stimuli-responsive nanogels mostly synthesize from cross-linking of desired monomers. For example, temperature-triggered nanogels tend to swell and deswell at a particular temperature [40, 106]. In this system, external heat ensures remote control and the thermoresponsive nanogels promise controlled and targeted drug delivery. The thermoresponsive polymers such as Poly(N-isopropylacrylamide), poly(amino carbonate), urethane, and polvinylcaprolactam utilized for preparation of stimuli responsive nanogels. pH-responsive nanogels are sensitive to acidic or basic conditions. Hyaluronic acid, alginate, chitosan are natural polymers used for the synthesis of pH-responsive nanogels [20]. Dinh et al. designed pH-responsive coiled-coil peptide-cross-linked hyaluronic acid nanogels (HA-cNGs) for cytochrome C (CC) protein delivery. The HA-cNGs loaded with CC showed a rapid release under mild acidic conditions [28]. In light-responsive systems, photoresponsive molecules are encapsulated in a nanogel. Azobenzenes and spiropbenzopyrans are commonly used in these nanogels' fabrication [1, 14].

The morphology of nanogels is the main point that gives information about the particle size, structure, and shape. Electron microscopes and optical microscopes are mainly used for morphological analysis. Electron microscopes ensure better resolution for imagining smaller nano and microgels. Scanning electron microscope (SEM) and transmission electron microscope (TEM) are widely used to observe the nanogel structure. Dynamic light scattering (DLS) is also the preferred method for measuring size distributions and average sizes in liquids. Charge on nanogels and the effect of cross-linker quantity can be determined by DLS analysis [94, 108]. Wu et al. reported the synthesis of carboxymethyl chitosan-nisin nanogels. TEM analysis was carried out for morphological properties determination. TEM images show that nanogels are spherical in shape and the average size is 45 ± 5.62 nm. Compared to the sizes observed by DLS, the TEM results are smaller because the nanogels are swollen in solution. The presence of functional group carboxymethyl chitosan was confirmed with FT-IR spectroscopy [118]. Determination of swelling properties is crucial for the characterization of nanogels. The swelling degree depends on the structure of the nanogel and the environmental parameters (pH, temperature, etc.) [75]. DLS measurement can be used to calculate the swelling ratio. For this aim, particles that swelled at different times, salinities, and temperatures are measured. The average diameter obtained from DLS substituted in different equations and swelling capacity can be calculated [108]. In addition, the swelling ratio can be determined based on the change in mass between dry particles and swollen particles by substituting the corresponding equation [56]. The monitoring of glucose concentration is very important for diabetes patients. The point-of-care tests promising painless, low-cost, and fast detection of glucose concentration [64, 105]. Li et al. reported glucose-sensitive poly (N-isopropylacrylamide)/poly (acrylic acid) (PNIPAM/PAA) (IPN-BAC) interpenetrating nanogels on colloidal photonic crystals (CPs). IPN-BAC nanogels cross-linked with N,N′-Bis(acryloyl)cystamine (BAC) and encapsulated by glucose-sensitive NIPAM/4-Vinylphenylboronic Acid (VPBA) copolymer shell [59]. The PNIPAM nanogels were synthesized by emulsion polymerization.

PNIPAM/PAA IPN nanogels were then synthesized in situ polymerization of acrylamide within the PNIPAM network. The particle sizes and chemical compositions of the nanogels were determined using DLS and X-ray photoelectron spectroscopy analysis, respectively. The glucose-sensitive core-shell nanogel showed a color change from blue to green depending on glucose concentration [59]. Sharmila and Shankaran fabricated a hydrogel-based nanoplasmonic colorimetric food sensor probe for the detection of melamine (MA) with a detection limit of $\times 10^{-7}$ M. They showed colorimetric sensing of MA in plasmonic nanomaterials (AuNPs) in solution and hydrogel phases. The AuNPs incorporated plasmonic hydrogels were prepared by the chemical and physical cross-linking of cellulose acetate on citrate and β-cyclodextrin (βCD) stabilized on gold nanomaterials. Both AuNPs in solution and hydrogel phases show similar selectivity and sensitivity [93]. Another research group developed localized surface plasmon resonance (LSPR) based poly(N-isopropylacrylamide-co-methacrylic acid) (PNM) on silica gold nanoshells (AuNS@PNM) biosensor [25]. The nanoshells have concentration-dependent red shifts in the LSPR wavelength of AuNS@PNM. PNM nanogels were synthesized on core-gold nanoshells (AuNSs) using the precipitation polymerization method. Nanogel-modified AuNSs were dialyzed at room temperature and centrifuged to remove unbond PNM nanogels. The TEM images of AuNS@PNM showed that a flower-like architecture had been achieved. In this design, PNM hydrogels can change their refractive index as a result of protein binding. The AuNS@PNM composite exhibits the detection of changes in the concentration of lysozyme and lactoferrin. Figure 6 shows that the shift is small at low protein concentrations. The concentration-dependent shift of the LSPR wavelength can be easily measured with a portable spectrometer [25].

8 Nanofibers

Nanofibers are ultra-fine webs of solid fibers with a small pore size, a small diameter, and a high surface area [61]. Lowering fiber diameters to the nanoscale can cause a significant increment of specific surface area to 1000 m^2/g. Nanofiber that has a comparatively small volume can comprise plenty of dense nanofibers. The high surface area provides a remarkable ability to attach or release functional groups, adsorbed molecules, ions, and various types of nanometer-scale particles [61].

Electrospinning has attracted a lot of attention from scientists working in the area of creating ultrathin fibers among other technologies such as phase separation [38], template synthesis [74], chemical vapor deposition [97], and sol–gel method [66]. Electrospinning is a reproducible technique that provides flexible operation and, as a result of its simplicity mainly used. Nano-sized fibrous materials can easily made using electrospinning technology with high efficiency and flexibility in a short time [18]. It offers various chances for shape, chemical composition, structural, and function-based fiber customization. These controllability characteristics endow the nanofiber material with several outstanding attributes that can meet the demands of different industries [110]. A high-voltage power supply, a syringe-driven spinneret

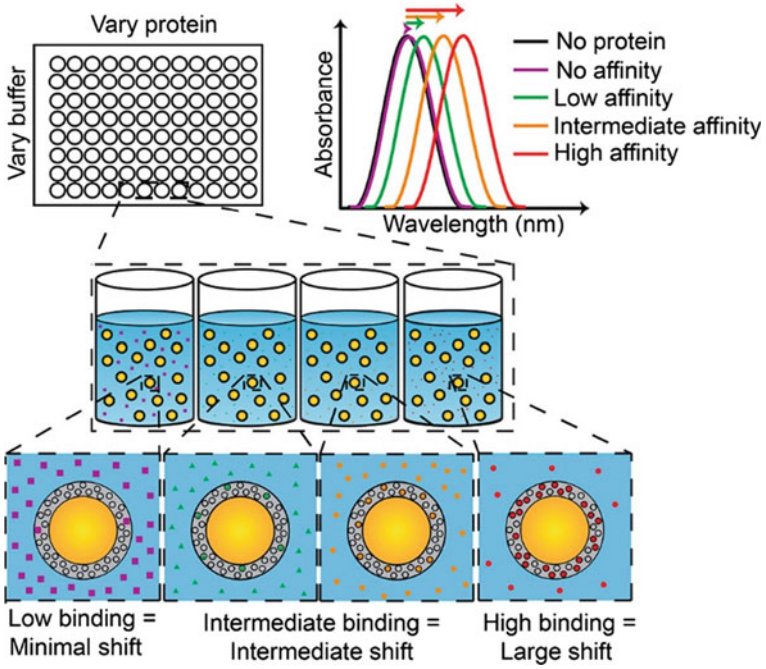

Fig. 6 Schematic diagram of LSPR-based biosensor. Adapted from [25]

connected to a pump and a grounded collector are the main components of electrospinning systems (Fig. 7). The electrospinning process, which is based on the theory of electrostatics, is the use of electrostatic repulsion forces in a strong electric field to produce nanofibers. While the solution to be electrospun is in the syringe, a strong electric field is created between the syringe nozzle and the collector. Due to the potential difference between the nozzle and the collector, the solution droplet at the nozzle acquires a cone-shaped distortion as the solution is ejected. The polymer mixture is pulled into fibers under high pressure during the electrospinning process and then deposited on the collector to create a web of randomly or aligned fibers [19].

The potential use of nanofibers to develop biosensors has been investigated. Miniaturization of designed platforms can also be facilitated by nanofibers. Different physical surface modification methods (layer by layer, atomic deposition), chemical methods (oxidation, cross-linking, hydrolysis, grafting), and thermal methods (heat press, calcination) are utilized to improve nanofiber-based biosensors characteristics [92]. As a result of their interconnectivity properties and large surface area-to-volume ratio, electrospun nanofibers are excellent materials for immobilization. Strong electrophilic functional groups on the nanofiber are employed by indirect immobilization techniques which are generally straightforward. Between the nanofiber and the biomolecule, cross-linkers act as an intermediary. While some

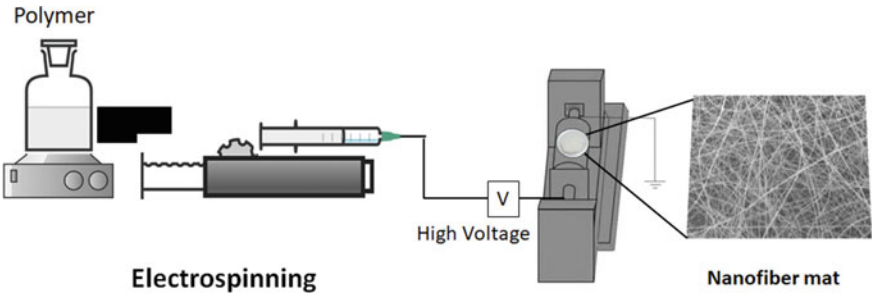

Fig. 7 Electrospinning process for the production of nanofibers

cross-linkers can be found in the final product, others expedite the reactions. For this purpose, the electrospun nanofibers with excellent properties and diverse functions can be modified by physical/chemical methods. Thus, they are potential materials to be employed by point-of-care (POC) biosensors and microfluidic-based analytical systems. Nanofibers have been investigated as ultrasensitive biosensors for POC cancer diagnosis, circulating tumor cell detection in cancer patients, malaria diagnosis, urea, glucose, cholesterol, bacteria detection, etc. Because high surface area property gives nanofibers the ability of ultrasensitive detection by providing them with binding sites in large quantities. More bioreceptors could be located on the surface or the inside of nanofibrous, thus increasing sensitivity [61]. In colorimetric POC detection applications, enzymes should be immobilized on the nanofibrous mat frequently with an adsorption technique [112]. Then, the nanofiber mats were dried usually at 25 °C and used in colorimetric detection with the color gradient scale. In another example, an anti-CAP monoclonal antibody immobilized onto the surface of poly(vinyl-co-ethylene) nanofibers to establish chloramphenicol (CAP) and use for the colorimetric biosensor for CAP [136].

Dhawane et al. [26] fabricated a POC, visual detection kit using chitosan nanofibers via electrospinning for the detection of cholesterol. In this study, uniform chitosan nanofibers (60–90 nm diameter) free of beads, were obtained. Interaction between the enzymes and the chitosan nanofibrous mat was important for the enzyme loading. For this reason, the electrospinning was performed to produce three nanofibrous mats with different thicknesses (6, 12, and 18 h electrospinning time) to find maximum enzyme loading. After 6 h immobilization time, a higher enzyme-loaded (3.8 U/mL) nanofiber was obtained. The nanofibrous mat was, therefore, used for the detection of cholesterol. It was based on a colorimetric method. Results showed that a color scale was developed when nanofiber mats loaded with reacting enzyme contact different concentrations of cholesterol (50–300 mg/dL). As a result, a simple method can be incorporated into a POC strip for cholesterol detection. Li et al., [60] produced electrospun polyethylenimine/poly(vinyl alcohol) (PEI/PVA) nanofibrous films decorated with Au nanorods (NRs) are signal output elements for the multicolor visual POCT of PSA proteins. The proposed aptasensing strategy provided PSA quantifying and semiquantifying in Au-NRs colloidal solution. Au-NRs/PEI/

PVA electrospun nanofibrous films displayed good accuracy, low detection limit, broad linearity, POCT characteristic, and satisfactory reproducibility. Considerably, this method can demonstrate a clear semiquantitative visual effect near the 4.0 and 10.0 ng/mL PSA concentration cutoffs. This has been used as a measure of the incidence of prostate cancer. In one study, electrospun nanofibrous membranes with magnetic nanoparticles have been developed and optimized for rapid and sensitive electrochemical detection of the pathogen bacterium: *E. coli* O157:H7. The biosensor showed linear detection of five different cell concentrations from 10^1 to 10^4 CFU/mL in 8 min. Results of the study show that the application of the low-cost and rapid biosensor can be extended to other organisms in field tests [65]. Overall, the trend and prospects are indications of possibilities to promote the implementation of nanofiber and nanofiber-miniaturized system hybrid for the next generation of diagnostic platforms point-of-care testing.

9 Flexible Hybrid Composites

Flexible nanomaterials have received increased research interest with the development of science and technology [131]. Recently, scientists have demonstrated to flexibility and stretchability of various nanomaterials at both macro and micro scales and focused on novel techniques for the design and synthesis of flexible nanomaterials. Flexible nanomaterials are smart materials that can be deformable, bendable structures, and have the ability to return to their original shape. Especially, combination of flexible nanomaterials with polymers provides great flexibility [23]. The unique properties of flexible nanomaterials allow fabrication of new generations of flexible and wearable electronics. Flexible and wearable electronics can be attached to human skin and biological tissue to enable the monitoring of biological signals. Tracking biological signals generated by the human body provides health assessment and points of diagnosis diseases [120]. In order to collect accurate information from the human body, sensors as the main component of flexible and wearable electronics are required to be flexible and have ideal stretchability [132]. Currently, electronic sensors are generally constructed with rigid materials such as metal or semiconductor. The mechanical properties of these materials are not suitable for the human body as well their lack of flexibility and sensitivity are not appropriate for healthcare monitoring [130]. Recently, considerable efforts have been dedicated to the development of flexible and wearable electronics with good mechanical deformability, stretchability, sensitivity, and comfortable wear for healthcare monitoring [39]. In the field of flexible wearable electronics, sensors are composed of a substrate, a conductive filler, sensing elements, and encapsulation materials [31]. The selection of material and design strategy are important factors in the fabrication of flexible and stretchable sensors [88]. In a flexible and stretchable sensor, component materials need to be designed with good mechanical strength and electronic properties. The flexibility, stretchability, and conductivity properties of materials used in the flexible wearable sensor are the main important criteria to obtain high-performance sensing systems.

Moreover, flexible sensing systems are required to maintain their performance and mechanical stability under mechanical stress. The flexible wearable sensors generally fabricated by using flexible substrate and nanomaterials. Among the flexible substrates polymeric materials including polydimethylsiloxane (PDMS), ecoflex, polyimide (PI), polyethylene terephthalate (PET), polyurethane (PU), and rubber are generally used for the fabrication of flexible sensors due to their intrinsic flexible properties [51]. Flexible and wearable electronics can also be integrated papers, membranes, patches, and so on for point-of-care testing [100]. Flexible carbon-based nanomaterials (graphene, carbon nanotubes (CNTs), nanosheets, nanowires, and nanoparticles) are widely used in flexible sensor fabrication as conducting nanofillers [55].

There are two strategies to obtain flexible and stretchable sensors. The first one is to exploit intrinsically flexible/stretchable materials, and the other is to use a structural design strategy. To fabricate flexible and wearable sensors, a variety of nanomaterials have been utilized such as carbon-based nanomaterials and metallic nanomaterials. Intrinsic stretchability/flexibility in the sensor platform is achieved by integrating these conductive fillers into the flexible materials [126]. This stretchable and flexible sensor system can be developed by using different synthesis methods such as mixing elastomeric substrate with conductive fillers, surface coating, deposition, and printing processes [119]. These methods are conducted to the distribution of conductive fillers homogenously in the elastomeric materials. The ultimate goal is to obtain flexible and stretchable composite sensors in whole processes. Moreover, materials designed with appropriate geometries such as serpentine, percolating network, kirigami, and wave/wrinkle enable the transforming nonstretchable materials into the fabrication of stretchable structure materials [30, 137]. For example, the stretchable wavy structure can be obtained by depositing or transferring metallic materials [119, 127].

Evaluating the performance of the fabricated flexible sensor system is also important to obtaining reliable healthcare monitoring systems. In order to fabricate a flexible and wearable sensor system with high performance, some parameters such as sensitivity, linearity, hysteresis, response time, and stability should be taken into consideration. Electromechanical characterization is generally carried out to determine the suitability of these parameters for flexible and wearable sensors [51]. Different characterization methods such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD) analysis, Fourier transform infrared (FTIR) spectrometer, and atomic force microscopy (AFM) are also exploited to display photographs of materials' nanostructure, structure sensing activity, and sensing mechanism [128]. In addition to the characterization and synthesis methods of the flexible and wearable sensors mentioned above, the fabrication of them depending on the application area is an important consideration.

Point-of-care testing which provides patient-centered diagnosis and real-time health monitoring has become a boasting desire recently [73]. At this point, flexible and wearable sensors stand out as a cornerstone for point-of-care testing. These flexible and wearable sensors that can be integrated into the human body enable real-time monitoring and recording of human physiological and biological vital signals for disease diagnosis and health status. These signals produced by the human body

such as mechanical, electrical, and biological signals can be related to health indicators [107, 120]. Flexible and wearable sensors can be designed to measure at a variety of health indicators such as body motions, body temperature, pulse rate, electrocardiograms (ECGs), blood pressure, breathing rate, and so on [121].

Yang et al. [125] prepared a novel flexible Ag/CNTs-PDMS composite film for the early diagnosis of Parkinson's disease. This flexible sensor was prepared in three stages. Firstly, CNT film was transferred to the PDMS substrate and transformed the CNT into a wrinkled structure. Secondly, Ag film was deposited upon the wrinkled CNT film by ion sputtering method. Lastly, they assembled the flexible and wearable sensor. Yamamoto et al. [122] developed integrated simple flexible sensor systems sensitive to both electrocardiogram (ECG) signal and skin temperature to monitor health condition change based on ECG signal and dehydration and heat stroke applications. This flexible sensor was developed by using printing technology on a flexible PET film and CNT was used as a conductive filler. Breath sensors that can be attached to human skin are useful flexible materials for the diagnosis of diseases including breathlessness, bronchial asthma, and sleep apnea. Yan et al. [124] developed a flexible $AgVO_3$-nanowires breath sensor by merging the $AgVO_3$ NWs with Au-interdigitated electrode on a PI substrate. This sensor system highly sensitive to humidity air shows the resistance change when the human subject exhales and inhales. This flexible sensor system possesses consistency and repeatability to monitor human breath with different respiratory rates, flexible sensor system could offer opportunities early for diagnosing and treating breath related diseases. These potential development of flexible and wearable sensor systems in the fields of point care testing system may provide achieving personalized early diagnosis diseases in the near future.

10 Conclusion and Future Aspects

In this chapter, we have discussed the synthesis and characterization of current nanomaterials and their application in nanomaterial-based biosensors for point-of-care diagnostics. Nanomaterial-based POC test platforms have excellent and promising potential in current clinical diagnostics. However, there are still some obstacles that need to be overcomed, such as high cost, limitations in large-scale applications, relatively low reproducibility, and so forth. The direction of current studies is to develop nanomaterial-based POC testing platforms that overcome these obstacles and provide more effective, reproducible, accurate, and efficient results. We believe that in the very near future, various nanomaterial-based POCT devices will be developed and used in clinical trials.

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Nanoinformatics Applied to Smart Nanomaterials

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Abstract Nanotechnology advances have enabled the development of many nanomaterials. Nevertheless, these nano-sized particles may present some limitations related to their therapeutic properties. To overcome these obstacles, the study and development of smart nanomaterials have grown exponentially. Smart nanomaterials can respond to environmental stimuli and, therefore, could be applied as biosensors, antimicrobials, bioimaging, and drug delivery. Above all, drug delivery systems have shown promising results when it comes to transporting one or more therapeutic agents to their target sites. Considering nanomaterials' importance in the biomedical field, a new research area, called nanoinformatics, has emerged. Nanoinformatics is defined as an artificial intelligence applied to nanomedicine, representing a very promising biotechnological strategy to fine-tune smart nanomaterials. Additionally, nanoinformatics uses the available computational tools to build new approaches for the design of safer and more efficient smart nanomaterials for the successful delivery of diverse classes of therapeutics. In this chapter, we will discuss how nanoinformatics has been applied to smart nanomaterials development.

Keywords Nanoinformatics · Smart nanomaterials · Stimuli · Drug delivery

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1 Introduction

Nanotechnology is defined as atomically precise technology, applied at nanometric scales, which range from 1 to 100 nm. It can utilize functional single atoms to submicrometer molecules to form chemical, physical, and biological systems that provide a specific performance. Thus, through controlled and careful manipulation, it is possible to produce functional nanoscale materials with unique properties [66]. These nanoproperties occur due to the quantum effect of the atoms and molecules that constitute a nanoscale system, generating physical and electrical reactions that are controlled by phenomena that also occur in nanometric dimensions. In this context, scientists and engineers have worked together to understand and design nanometric structures to obtain and control desired properties [93].

Due to its many interesting features, nanotechnology has a wide application in different areas, including industry, environmental safety, health, and agriculture by using nanoparticles, nanocapsules, nanotubes, nanospheres, nanochips, and other devices [36–41, 74]. However, nanotechnology operations stand out in the medical field, being used in genetic material sequencing, biosensors, bioimaging, diagnosis, and drug delivery for point-of-care treatments [83]. Thus, nanomedicine has emerged as the specific concept of nanotechnology application for biomedical purposes, aiming to treat or prevent human diseases. Moreover, the nanomedicine research area has led to the development of nanotherapeutics and nanomaterials that exhibit great advantages in clinical results when compared to conventional small drugs, due to their low toxicity, higher bioavailability, and improved pharmacokinetics and therapeutic effect [105].

The nanomaterial's properties mainly depend on its size, shape, and composition. Therefore, it is possible to control its properties by manipulating these features during nanomaterial synthesis, which can be performed with biological, physical, or chemical approaches [49]. To be considered a nanomaterial, at least one of the dimensions must be smaller than 100 nm, which leads to a dimensional classification [9]. Zero-dimensional nanomaterials have all their dimensions at the nanoscale, such as quantum dots and spherical or cubic nanomaterials. One-dimensional nanomaterials have only one non-nanoscale dimension, including metallic filaments, nanotubes, and nanofibers. Two-dimensional nanomaterials have two non-nanoscale dimensions, including nanoplates, thin films, and nanocoating. Finally, three-dimensional materials have various dimensions higher than 100 nm, such as fibers, polycrystals, and nanotubes [79]. Additionally, nanomaterials can also be categorized based on their chemical composition, being classified mainly as carbonaceous, metallic, dendrimers, and composites [44].

Among the applications of nanomaterials in nanomedicine, their use for drug delivery stands out, aiming to release a drug at the target site for a specific treatment. To improve this method, it is possible that the nanoparticles only release the drug at the target site when they receive some stimulus from the environment, which can be physicochemical, biological, thermal, or electrical stimuli [49]. This responsiveness seems to be very important, since both conventional drugs and common

nanotherapeutics, when administered to the human body, can fail in the balance between protecting healthy cells and eliminating pathological cells. Thus, the ability to respond to endogenous or exogenous stimuli classifies these therapeutics as smart nanomaterials, which can perform more accurate and effective treatments with less nonspecific toxicity [105].

Smart nanomaterials are nanoparticulate systems that exhibit shape, color, texture, density, stiffness, and toughness variations in response to specific stimuli, such as changes in temperature, pressure, electric and magnetic fields, chemical concentration, moisture, ionic strength, pH, and stress [82]. Their selective action on specific tissues enables greater therapeutic potential and higher immunological tolerance, reducing side effects in treatments for infectious diseases or cancer [88]. Therefore, smart nanomaterials have been properly used for numerous nanomedical applications, including drug delivery, imaging, tissue engineering, and disease diagnostics [3, 84], as shown in Table 1. Their high performance in nanomedicine research, diagnosis, and therapy has driven large investments in this nanotechnology field. Accordingly, different types of smart nanomaterials (Fig. 1) have been synthesized, presenting wide variations in their material compositions, formats, and physicochemical properties that make each one suitable for specific applications [67].

It is worth noting that, when administered to biological systems, even small variations in smart nanomaterials' properties can induce drastic modifications in their primary biomedical function, in addition to creating toxicity profiles for the organism. Therefore, before the clinical use of smart nanomaterials, it is crucial to ensure they are safe enough and maintain their expected functions and properties throughout their life cycle in the human body [100]. With this purpose, the nanoinformatics research field has emerged, for understanding the interaction mechanisms between smart nanomaterials and the biological system more profoundly, integrating the use of computational tools for the management of data intrinsic to the nanomaterial, including its structure and properties, along with system data [90]. Nanoinformatics is applied not only for simulating interactions but is also widely used for structural, chemical, and behavioral prediction of nanomaterials, managing raw data collected

Table 1 An overview of smart nanomaterials and their nanomedicine applications

Smart nanomaterial type	Properties/functionalities	Application	References
Metallic nanomaterial	Magnetic hyperthermia, kinetic magnetic activation	Antimicrobial, drug delivery, bioimaging	[22] [4]
Liposomes	Encapsulate the drug	Drug delivery	[3]
Dendrimers	Well-defined structure, high surface area	Drug delivery	[46]
Quantum dots	Optical and electronic properties, high loading capacity	Bioimaging, diagnostics, drug delivery	[91] [45]
Carbon nanotubes	Strong structure, high surface area	Tissue engineering, drug delivery	[107] [84]

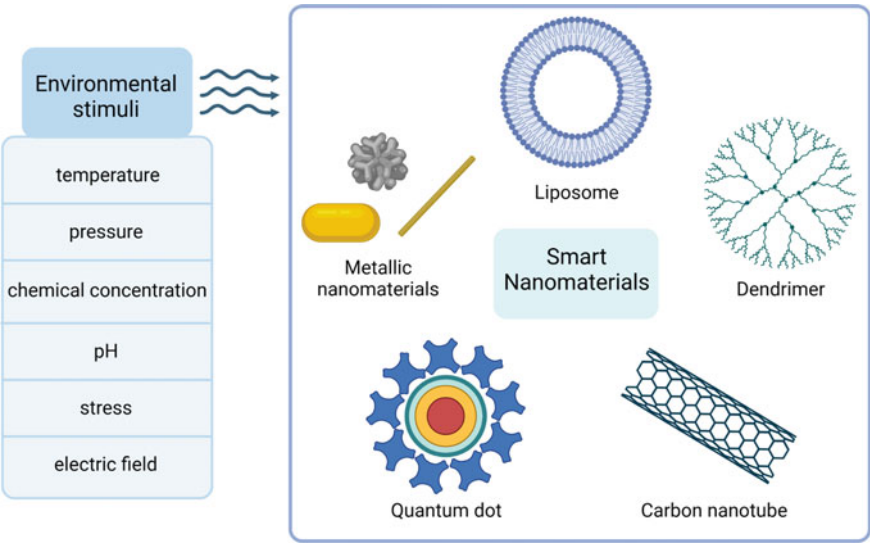

Fig. 1 Illustrative representation of the smart nanomaterials mentioned in the main text and some of the stimuli they can be responsive to

about them, and analyzing their biomedical application data. Thus, the use of nanoinformatics accelerates the research and design of new smart nanomaterials, enabling their faster inclusion in clinical practice [21].

In this context, this chapter presents the computational tools used in nanoinformatics and examines how they can be applied in the context of developing smart nanomaterials, aiming at the design of increasingly technological and safe nanomedical approaches.

2 An Overview of Nanoinformatics in the Context of Nanomedicine and the Development of Smart Nanomaterials

The advent of smart nanomaterials was achieved due to advances in nanotechnology, a growing field of study that is receiving increasing attention and investment. It is mainly based on the research and application of extremely small structures, in nanometric dimensions. Although nanotechnologies can be applied in several areas of knowledge, their use in human health, called nanomedicine, stands out for its promising results [14]. The nanomedicine field operates by implementing products capable of overcoming the barriers of conventional medicine, enabling higher treatment specificity, and avoiding adverse effects. Among the advantages of nanomedicine, there is the production of drugs based on a generic platform, from

which their functional requirements can be modeled, improved, or replaced. This means that it is not necessary to completely reconsider the medical structure every time one of its functions needs to be changed to achieve a better performance [32].

Associated with this medical improvement, the development of computational methods, among other technological advances, has led to the generation of extremely important data for application in the field of science and health. Thus, several informatics methods can be adapted to bioinformatics and used, for example, to discover new drugs and understand the behavior of cell receptors and protein interactions, besides other nanomedicine approaches [14]. The dynamic nature of smart nanomaterials, due to their responsiveness to external stimuli, emphasizes the need to predict a behavior accurately. Therefore, to ensure their safe administration, it is imperative to obtain a detailed description of the possible biological system's stimuli and the response mechanism performed by the nanomaterials [33].

Aiming for that, with nanoinformatics computational analysis, it is possible to obtain and model physical, chemical, and mathematical parameters that guide the formulation of these nanomaterials [96]. Furthermore, this growing field of computational research uses and adapts the available in silico tools and methods, based on artificial intelligence, to predict the behavior and trajectory in vivo of smart nanomaterials, after being administered for therapeutic purposes [2]. Based on these analyzes, new strategies can be proposed to improve the current nanomaterial performance, increasing application properties such as biocompatibility and therapeutic efficacy [65].

More specifically, to design smart nanomaterials for drug delivery, nanoinformatics is applied to evaluate the complex chemical interactions formed between the nanomaterial and the medicine that will be carried through the human body. Subsequently, the release interactions between the drug and the nanomaterial must also be evaluated, as well as the morphology modifications that may occur in the nanomaterial structure. Additionally, the adsorption between the nanomaterial with membranes and surfaces must be modulated to ensure that the nanomaterial trajectory will not be interrupted by biological barriers and that the drug will be delivered to the target [28].

Among the computational tools applied to accurate smart nanomaterials design, there is machine learning. This approach consists of a broad set of algorithms and mathematical models capable of making specific predictions. It is based on empirical data previously supplied to the computer, which begins to identify patterns in the analyzed information [95]. Machine learning is widely used in nanoinformatics for the description, characterization, and risk assessment of smart nanomaterials. Among machine learning techniques, supervised learning is used to correlate the characteristics of nanomaterials with their respective biological responses. Unsupervised learning, by contrast, identifies data patterns that have not yet been classified, based on its acquired knowledge [81].

In general, to design a specific new smart nanomaterial, with the required properties, another nanomaterial already characterized and available in databases is used as

a parameter. Thus, the initial model must be a raw nanomaterial, with fixed parameters, such as structural, mechanical, chemical, electric, magnetic, and thermal properties [95]. These features are used as input into the system to train the computer's knowledge. Aiming at some specific application, the function of nanoinformatics is to predict the new properties of a nanomaterial related to possible changes in its structure or composition [98].

Machine learning operates in the prediction of new nanomaterials for drug delivery mainly through algorithms for the evaluation of different models to identify the one that presents the best performance. First, it is necessary to train the system with a set of raw experimental data already obtained and available in databases. After being trained, it can be used to predict the nanomaterial candidates' properties of interest, performing statistical correlations to define the most appropriate one for a specific application. Furthermore, by knowing the atoms or structures most relevant for the nanomaterial properties, it is possible to modulate specific parameters to improve the model [12].

Additionally, with increasing computational advances, deep learning has emerged as a more advanced prediction method (Fig. 2). It uses a mathematical model called an artificial neural network, which consists of a set of complex nonlinear functions that have structural and functional similarities with the brain neuron network [12]. This algorithm is composed of many interconnected nodes, like artificial neurons, which are organized in many layers. The nodes of the first layer contain the input data about a given problem, such as already obtained properties of a nanomaterial. This information passes through the entire system of nodes, through the hidden layers, until the last layer of nodes has the desired output data [16]. Therefore, using deep learning, a system of artificial neural networks can, for instance, also be used to predict the bioactivity and the release pattern of a smart nanomaterial into a specific environment [96].

3 The Application of Smart Nanomaterials for Drug Delivery

Currently, one of the major focuses of research in the pharmaceutical area is the improvement of drug delivery [50]. This is due to multiple factors, including toxicity problems, instability, and low bioavailability of the currently available drugs [6, 35, 47, 61, 86]. Some examples are the drugs used for chemotherapy or antibiotics. Chemotherapeutics are some of the most cytotoxic drugs, which present multiple side effects once administered. However, these drugs are highly effective in counteracting the growth of cancer cells, making their use very important in the treatment of cancer. Because of this, it is vital to develop vehicles that encapsulate these drugs, so that they can be transported specifically to the desired tissues, avoiding their early degradation and their side effects on other healthy tissues in the body [104].

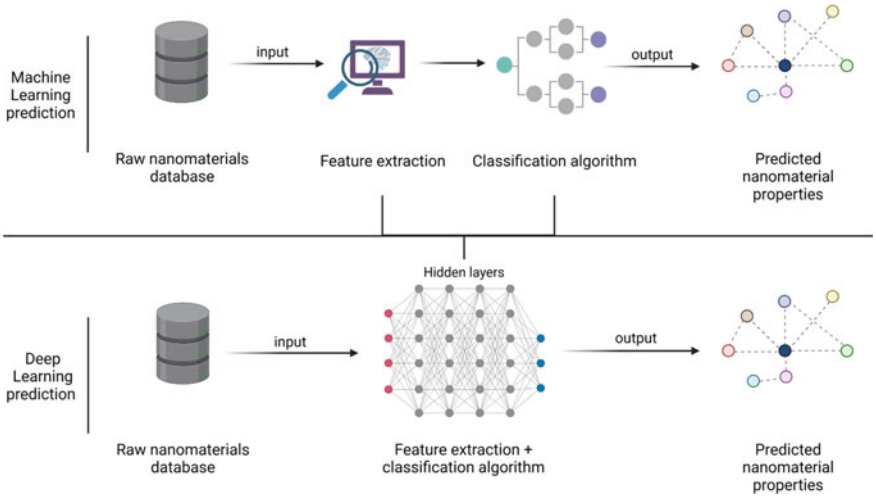

Fig. 2 Prediction of smart nanomaterials' properties using deep and machine learning. By using the machine learning prediction method, the raw data input goes first through the feature extraction and then through the classification algorithm, to generate the final output. In contrast, using the deep learning prediction method, the feature extraction and the classification algorithm are condensed into just one step, performed by the artificial neural network

Besides, antibiotics present another major challenge, due to two aspects. First, there is great concern about the development of antibiotic resistance due to its misuse, leading to a constant and urgent search for new molecules with antimicrobial properties. Second, as in the case of chemotherapeutics, antimicrobials are highly cytotoxic molecules too, but they are also often highly sensitive to degradation, which rapidly decreases their bioavailability [101]. Therefore, the development of nanocarriers is a critical aspect to improve the smart delivery of antimicrobials, avoiding their degradation, and improving their specificity, thus preventing damage to healthy tissues.

However, although it seems like a simple idea, the development of nano-vehicles for drug delivery enhancement presents major challenges. Among them is the design of such vehicles, which must be thought of as molecules with high area/volume ratios, to increase the reactivity and free energy of their surfaces, as well as their ability to adsorb drugs [56]. To achieve this, the vehicles must be very small in size, to increase the Van der Waals forces of attraction, as well as being made of porous materials (in the case of synthetic particles), which also increases their contact surface [57, 59]. However, these vehicles must also be designed in such a way as to avoid attraction between them, thus preventing their agglomeration and improving their circulation and absorption.

Another feature of utmost importance in the design of such nanocarriers is their chemical stability. This is because a critical point must be found at which the vehicle is stable enough to reach the tissues of interest before degrading, but also capable of

controlled release of the drugs it carries to improve drug dosing. This last characteristic is very important for the delivery of chemotherapeutics, for example, since due to their high cytotoxicity, the dosage must be done in a controlled manner. Another major challenge posed by the development of these nano-formulations is the design of particles that, contrary to the drugs they carry, should have low cytotoxicity. This is because the aim is to mitigate the adverse effects of the drugs once they are administered. Therefore, the material from which the particles are made plays a key role in this aspect, as normally non-natural structures that are used as raw material tend to exhibit high cytotoxicity [62].

In summary, the vehicles to improve drug delivery should be (i) small particles that are easily absorbed by the cells, with (ii) high surface/volume ratio, (iii) low reactivity among themselves, but with (iv) high drug absorption capacity, with (v) good stability that allows the tissue of interest to be reached and with (vi) high specificity and that also have (vii) high capacity to release the drugs in a controlled manner and that once the effect is finished, and (viii) present high biodegradability. Finally, the use of nanoinformatics strategies can enhance all these features, as discussed in detail in the next topic.

4 Design of Smart Nanomaterials for Drug Delivery Using Nanoinformatics

Artificial intelligence fields can be applied to nanoinformatics specifically for the safe development of smart nanomaterials for drug delivery. These approaches include molecular dynamics, molecular docking, quantitative structure–activity relationship (QSAR), quantitative structure–property relationship (QSPR), and quantitative structure–toxicity relationship (QSTR) [89].

Molecular dynamics is a computational method used in nanoinformatics to analyze, in a given time interval, the behavior and structural changes of smart nanomaterials when exposed to different conditions and environments [63]. To prepare and run a molecular dynamics simulation, the GROMACS software package is used, which also has analysis tools implemented to evaluate the molecular interactions at the end of the dynamics and to examine the biological relevance of the obtained results [43]. The simulation can predict the behavior and trajectory of each atom in the molecular system during a defined period of time, based on the interatomic interactions that occur between them [70]. Therefore, molecular dynamics simulations can characterize relevant aspects for drug delivery, such as the nanomaterial's behavior and stability in contact with biological tissues, the interactions that occur between the nanomaterial and the drug during its loading, and the binding affinity to the target cells in the release moment [18].

Molecular docking is another nanoinformatics approach used for developing smart nanomaterials [29]. Among the types of software used for docking simulations, AutoDock Vina stands out for applying a knowledge-based scoring function

with Monte Carlo computational algorithms, obtaining higher prediction accuracy. Also, this type of software adopts the Broyden–Fletcher–Goldfarb–Shanno (BFGS) method, an algorithm used for model nonlinear optimization [69]. In the drug delivery context, this approach is applied to simulate the interactions between the nanomaterial, the carried drug, and the target cell. To evaluate these binding affinities, several energy values are obtained during the docking simulation, including electrostatic energy, free binding energy, intramolecular energy, desolvation energy, inhibition constant, and interactive surface [29]. Knowing these predicted energy values, it is possible, for instance, to make specific adjustments to obtain lower free energy at the binding site between the nanomaterial and the drug, aiming at greater stability and efficiency during loading [92].

Also applied in nanoinformatics, QSAR aims to predict the biological and toxicological effects of nanomaterials before their synthesis [90]. Considering the relationship between the structural conformation of a nanomaterial and its functional performance, QSAR uses the available structural information of nanomaterials already synthesized to predict the new nanomaterial bioactivity. Thus, it is possible to design nanomaterials ensuring their expected behavior for a successful application for drug delivery [77]. In the same context, QSPR performs the prediction of the physical, chemical, and biological properties of smart nanomaterials. For instance, QSPR can be used to predict drug solubility, an important parameter for drug release, based on some nanomaterial structural features such as molar refractivity, topological surface area, and McGowan volume [23]. Finally, QSTR is capable of computationally predicting the ecotoxicity and cytotoxicity of nanomaterials under different experimental biological conditions [26]. This toxicity evaluation is one of the most important steps to ensure the nanomaterial can be safely administered for drug delivery in a nanomedical treatment, without causing any adverse effects to the organism [48].

5 The Existing Smart Nanomaterials for Drug Delivery

Due to the nanoinformatics approaches mentioned above, a wide and varied number of smart nanomaterials could be safely developed to act in drug delivery, bringing great progress to the nanomedicine field. To date, drug carriers can be classified into several types according to different characteristics. Some characteristics used for classification are size, shape, and physicochemical properties, among others. The type of material from which the particles are made is one of the most important aspects of particle classification. Thus, roughly speaking, we can say that there are two large groups, “organic” and “inorganic” nanoparticles [17]. This characterization is important when classifying vehicles, since the material they are made of is key to understanding the type of drugs they can carry, as well as the type of treatment for which they might be most effective (Table 2).

Within the large group of organic nanoparticles are those made of lipids such as liposomes, or extracellular vesicles. As mentioned above, liposomes are spherical vesicles composed of a lipid bilayer with water-soluble and lipid-soluble regions, with

Table 2 An overview of the most representative examples of organic and inorganic nanoparticles containing various types of drugs for different biological applications

Drug type	Nanoparticle	Application	References
Vancomycin—glycopeptide	Poly (lactic-co-glycolic) acid	Bone transplantation	[103]
	Polycaprolactone	Antifungal therapeutic Bone implantation	[25] [53]
	Apoptotic bodies (Evs)	Intracellular macrophages infection	[15]
	Liposomes	Biodistribution	[102]
L9—AMP	Silver	Antibiotic and synergism	[24]
Polymyxin—cyclic nonribosomal polypeptide	Silver		[51]
	Liposomes	Permeation	[5]
P13—AMP	Silver	Antibiotic	[31]
Synthetic peptides	Poly (lactic-co-glycolic) acid		[58]
	Liposomes		[52]
LL-37—AMP	Carbon nanotubes	Antibiotic	[73]
Doxorubicin	Poly (lactic-co-glycolic) acid	Cancer	[20]
	Liposomes		
	Polymeric	Alzheimer's inducer	
Simvastatin	Poly (lactic-co-glycolic) acid	Atherosclerosis treatment	[42]
Docetaxel	Chitosan	Cancer	[94]
Epirubicin	Poly (lactic-co-glycolic) acid	Cancer	[27]
Daptomycin— lipopeptide	Gold	Antibiotic	[108]
HHC36—AMP	Carbon nanotubes	Cellular differentiation	[99]
PEP—AMP	Gold	Tissue regeneration, signaling	[72]
Indolicidin—AMP	Silver	Toxicity reduction	[30]
Esc(1–21)—AMP	Gold	Antibiofilm	[19]

an aqueous solution core. Further, extracellular vesicles (EVs) could be considered liposomes, but unlike these, which can be synthetic, EVs are released naturally by cells. Therefore, EVs tend to be more complex structures because they contain a greater diversity of membrane lipids and proteins, as well as molecules from the cell of origin. These types of vehicles have the advantage of greater biocompatibility with tissues, which is very attractive when very small molecules or molecules sensitive to degradation and with high cytotoxicity are to be transported [56]. One of the most famous treatments developed so far for the treatment of cancer is doxorubicin (Doxil), a chemotherapeutic nano-encapsulated in liposomes, created by Johnson & Johnson's Ben Venue laboratories [13]. This drug is an antibiotic that, when encapsulated in liposomes, drastically decreases its cardiotoxicity and increases its specificity. This is also very successful in treating ovarian cancer, myeloma, and HIV-related tumors such as Kaposi's sarcoma.

Other types of organic vesicles are those made of synthetic and natural polymers, such as poly (lactic-co-glycolic) acid (PLGA) and elastin-like polypeptides, respectively [60, 64]. These are highly biocompatible materials, but also very stable and compatible with different types of drugs. Some diseases such as Alzheimer's disease are an important focus for the application of improved drug delivery systems because the blood–brain barrier (BBB) is an additional obstacle that prevents the passage of molecules into the brain. The use of both polymeric and lipidic nanoparticles has been shown to have the capability to overtake BBB endothelial cells to deliver drugs directly into the central nervous system [34]. The number and variety of drugs that have been encapsulated in vehicles to test their ability to overcome the BBB are very large, such as Doxil, Dalagrin, Amitriptyline, Kyotorphin, and Tubocurarine, among others [78]. Many of the drugs used today as chemotherapeutics are hydrophobic, which makes vehicles made from hydrophobic polymers such as PLGA a good choice for use as encapsulating particles.

Regarding the encapsulation of water-soluble drugs, some studies have found low-loading effectiveness in vehicles with hydrophilic lipid cores, such as liposomes or EVs [76]. By contrast, other studies have found high percentages of encapsulation (40–60%) of this same type of water-soluble drug in EVs [15]. Examples of water-soluble drugs are antimicrobial drugs such as vancomycin for the treatment of infections by gram-positive bacteria, and antimicrobial peptides (AMPs), which are generally amphipathic molecules. Both types of antimicrobials have been successfully loaded onto different types of organic nanoparticles, from liposomes and EVs to PLGA-based vehicles [1, 8, 11, 54, 75, 80, 85, 87, 97].

Another important aspect when designing smart nanomaterials is the shape they adopt in solution. It has been seen that the shape of the nanocarriers is a determinant of the correct circulation once they are administered. For example, rod-shaped particles tend to have a better orientation [62]. Therefore, depending on the target tissue or cells for treatment, it may be more or less advantageous to design particles with different shapes. One of the biggest hurdles in designing smart particles is how to avoid elimination by phagocytes, especially macrophages of the immune system. It has been seen that most nanoparticles end up in the spleen and liver after they

are administered, due to recognition and elimination by macrophages found in these organs [10, 55].

Typically, the aim is to prevent the particles from being recognized by the immune system so that the charged particles can successfully reach the target site and thus release the drug. For this, it is important to modify the size and shape of these vehicles, because their nanoscale sizes and more elongated shapes prevent recognition by macrophages. Other options are the design of more complex particles, which are recognized by the immune system as its own, by integrating CD47 molecules on their surface or by encapsulating both drugs and complete vehicles in red blood cells [7, 68, 106]. However, in the case of the treatment of intracellular macrophage infections, the objective is the opposite of most treatments. In these cases, the aim is to increase the rate of recognition and phagocytosis of the immune system cells, which is why the design of spherical particles is more advantageous.

The other group is inorganic nanoparticles, which can be magnetic particles of nickel, cobalt, iron, magnetite, and FePt alloys or those made of metals such as silver and gold [71]. One of the major advantages of this type of particle over organic particles is their greater stability and high cellular uptake rate, which makes them very attractive as drug delivery systems. Some of the most common inorganic vehicles used for drug encapsulation are carbon nanotubes and nanospheres, which have hydrophilic properties. However, the use of inorganic nanoparticles for drug delivery enhancement is not as advanced compared to organic vehicles. Even so, given all the smart nanomaterials for drug delivery mentioned above, it is notable that this area of study has received research investments, which should continue, considering the potential application in several diseases that still affect people in modern times.

6 Conclusion

Advances in the nanomedical field have been very important in obtaining nanotherapeutics with increasingly better clinical results, achieving higher therapeutic potential and fewer side effects to the body. This technological progress in nanomedical treatments has mainly been made possible with nanoinformatics, which implemented computational tools in the nanomedicine field. Thus, based on artificial intelligence, the various nanoinformatic approaches have enabled the development of stimulus-responsive nanotherapeutics, called smart nanomaterials, which can be applied in increasingly specialized treatments. Among them, we can highlight smart nanomaterials designed for targeted drug delivery, which carry medicines to specific body tissues more accurately. With in silico approaches, it is possible to make an accurate prediction of the nanomaterial's properties and to understand the mechanism of action it will have in the organism, ensuring a safer and more effective treatment. Thus, research in the nanoinformatics area should be greatly encouraged, due to its high potential for developing and improving nanomedical procedures.

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Nanomaterials in Lateral Flow Assay

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Abstract Point-of-care devices have garnered the interest of scientists in recent years due to their capacity for on-site, bedside, and in-home surveillance in many fields of the medical, biological, pharmaceutical, and food sciences and industries. These devices can be categorized primarily as either portable or stationary. Due to their simple downsizing, mobility, low cost, and low power consumption, portable devices have attracted a great deal of interest. Recently, lateral flow assays have gained popularity as a portable platform due to the simplicity of strip design and the ability to detect with the naked eye. As inseparable components of lateral flow assay, nanomaterials have played a prominent role in enhancing sensitivity due to their large surface area, ease of functionalization, and tunable physical and chemical characteristics based on size, shape, and composition. The conventional lateral flow approach is an immunoassay in which gold nanoparticles with a unique plasmonic surface property show a red color on the test and control lines to enable qualified detection. This approach, with its quantitative limitations and limited sensitivity, is essential for the introduction of novel nanoparticles. Numerous nanoparticles, including quantum dots, carbon nanotubes, magnetic nanoparticles, nanoenzymes, surface-enhanced Raman scattering nanotags, upconversion nanoparticles, and time-resolved fluorescence nanoparticles, have been utilized in the design of lateral flow assays to date. This chapter focuses mostly on the characteristics of various nanoparticles combined with lateral flow assay and associated transduction method for readout of signals produced by nanoparticles, as well as a critical analysis of the resulting approaches.

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Keywords Point-of-care · Lateral flow assay · Nanomaterial · Biosensor · Immunoassay

1 Introduction

Point-of-care (POC) devices have attracted unprecedented attention in recent years due to their exceptional significance in the self-testing of biological, food, and pharmaceutical samples, among others, with the benefits of high speed, low cost, sensitivity, on-site, and user-friendly detection. The majority of the success of point-of-care (POC) devices may be attributed to the limitations of established procedures such as liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), real-time polymerase chain reaction (qPCR), and enzyme-linked immunosorbent assay (ELISA) [1]. These approaches mostly suffer from the disadvantages of a lengthy procedure, a necessity for a high level of skill, and costly equipment, which might limit their use [2]. Therefore, scientists have a significant interest in the development and introduction of rapid reactions, simple procedures, inexpensive and individual-centered detection technologies.

Lateral flow assay (LFA), a paper-based approach, may significantly assist the objectives of POC technology for the advancement of home testing. This method enables the qualitative and quantitative detection of a wide range of targets, including proteins, antibodies, nucleic acids, whole cells, toxicants, drugs, etc., on a simple, low-cost platform with a negligible sample volume [3]. The standard structure of the LFA strip (4–6 mm × 6–7 cm) includes a sample pad, conjugate pad, detection pad (nitrocellulose membrane), adsorbent pad, and backing pad. These components are composed of cellulose, glass fiber, nitrocellulose membrane, cellulose, and polystyrene**,** respectively [4]. Following the assembly of the sample pad, conjugate pad, detection pad, and adsorbent pad on the backing pad, an appropriate amount of reporter particle-conjugated bioreceptor (antibody, aptamer, or DNA) can be deposited on the conjugated pad for the subsequent operation. The loading of sample onto the sample pad results in the formation of a complex between the target and reporter particle-conjugated bioreceptor, followed by the target's movement toward the adsorbent pad. During the passage of nitrocellulose membrane, the target complex interacts with the detection zone on the membrane including the test line (T-line) and control line (C-line) formed by dispensing bioreceptor of the target (antibody, aptamer, and DNA) and anti-immunoglubin (or complementary nucleic acid strand) on the nitrocellulose membrane, respectively [1]. Depending on the nature of the reporter particle, in the presence and absence of the target, characteristic lines can appear on the T-line or C-line that can be recognized with the naked eye or an instrument.

Despite the numerous advantages of LFA, its application may be limited by several drawbacks. Possibility of nonspecific interactions with the sample matrix in the nitrocellulose pores and saturation of detection zones (T-line and C-line) at high concentrations of analyte, leading to false responses, are downsides of LFA. The

solution to these issues is sample dilution, which may result in decreased sensitivity. Thus, there has been a need for signal amplification, which may be accomplished by combining nanotechnology with LFA technology [5].

Nanomaterials with high surface area, stability, conductivity, and simple functionalization can enhance the detection systems' sensitivity, specificity, reproducibility, repeatability, accuracy, and dependability [6]. The expanding use of nanomaterials in biosensor production has led to the development of portable, miniaturized transduction platforms [7]. Biosensors based on nanomaterials offer ultrasensitive, fast, and concurrent multiple detection of targets, early stage disease diagnosis and on-time therapy, and little sample consumption [8–10]. The development of nanotechnology can facilitate the construction of POC devices, such as LFA or microfluidic devices, that offer tailored molecular detection in several domains, such as food safety monitoring, diagnostic medicine, etc. Due to the significant dependence of LFA development on nanotechnology, this chapter focuses on the nanomaterials used in the design of LFAs. In addition to describing characteristics and critical topics, detection methods and transduction systems are classified based on the nanomaterials' application.

2 Nanomaterials

In recent years, nanomaterials, particularly metallic nanoparticles (NPs), have been widely used in the design of biosensors and POC devices due to their impressive properties, which include a higher surface area to volume ratio ($>10^7$:1) with small size (1–100 nm) compared to macro-sized particles, and inimitable chemical, physical, optical, magnetic, and electrical properties that enable the integration of different transducers with the LFA method [11, 12]. Due to the enhancement of LFA's potential for both quantification and qualification purposes, it has become necessary to use nanoparticles as labels with LFA. In addition to increasing the required sensitivity for quantification detections, this technology can provide signals that can be read by a variety of transducer systems. As depicted in Fig. 1, numerous nanoparticles have been utilized in the implementation of LFA platforms, including gold nanoparticles (AuNPs), which are widely used as label nanoparticles in colorimetry, quantum dots (QDs), carbon nanotubes (CNTs), magnetic nanoparticles (MNPs), nanoenzymes, surface-enhanced Raman scattering (SERS)-nanotags, upconversion nanoparticles (UCNPs), etc. [4, 13]. Table 1 offers an overview of the different nanoparticles and related transduction processes used for LFA signal reading.

2.1 AuNPs

AuNPs have several benefits that increase their applicability in a broad range of disciplines, including healthcare, engineering, the sciences, etc. Scientists are interested

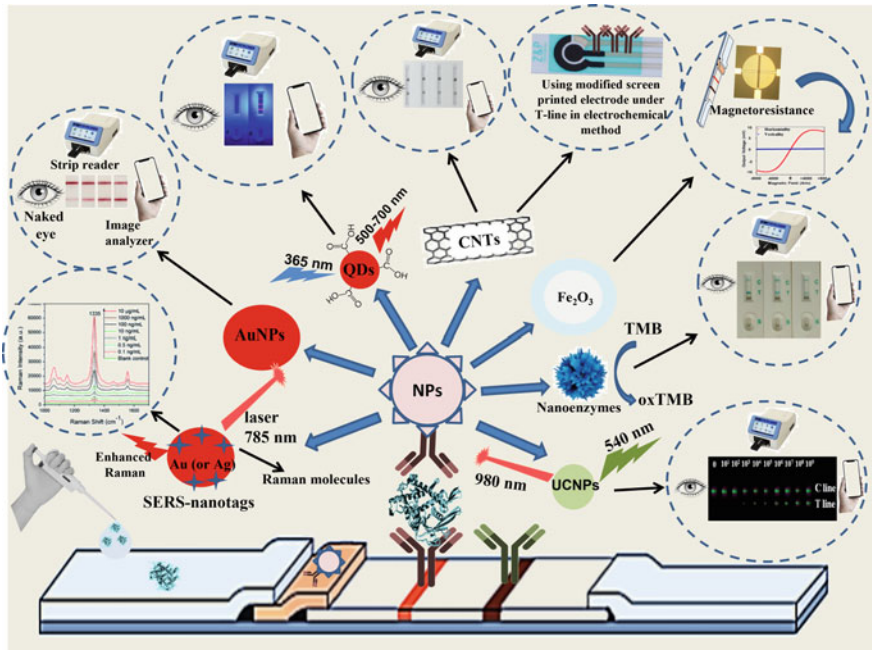

Fig. 1 Application nanoparticles in LFA and possible transduction systems

in the possibility of AuNPs being used in the design of biosensors for diagnosis and therapy, among other uses. These advantages include high safety, redox capability, catalytic behavior, substantial biocompatibility, great conductivity, a high surface-to-volume ratio, surface plasmon resonance (SPR), simple detection of its red color with the naked eye, and simple bioconjugation by antibodies, oligonucleotides, and proteins [51]. Due to the aforementioned characteristics, AuNPs have been predominantly utilized as colorimetric markers for LFAs. In addition, additional approaches, such as electrochemical, SPR, etc., can be combined with LFAs due to the physical and chemical characteristics of AuNPs.

Optical biosensors capable of visual detection of targets with an inexpensive and simple transducer that generates a signal proportional to the concentration of the target. This signal can represent quantifiable changes in the properties of light, such as its intensity, refraction index, and resonance frequency. Nanomaterials can generate light or its variations by transferring electrons between energy levels, resulting in diverse ways such as fluorescence, absorption, colorimetric, luminescence, refractometry, and SPR [52]. Due to the great sensitivity and ease of transduction of signals, which can be conducted with the naked eye, optical detection of LFA signals has been the most often used method in the literature [53]. Due to the straightforward appearance of color on the T-line or C-line, the colorimetric readout has garnered a great deal of attention for inclusion with LFAs, resulting in greater compatibility with the goals of POC devices. Due to the special characteristics of SPR, the application of AuNPs in LFAs has been extensively documented.

Table 1 Summarized data of studies designed LFA based on various NPs

NPs	Target	Detection process	Transduction
AuNPs	Troponin I	Binding of AuNP (40 nm) to AuNP (10 nm)-antibody	Colorimetric
AuNPs	C-reactive protein (CRP)	Automatic sensitivity enhancement using $KAuCl_4$ and $NH_2OH \cdot HCl$ in the presence of AuNP-antibody	Colorimetric
AuNPs	Human immunodeficiency virus type 1 (HIV-1) target nucleic acid	Aggregation of oligonucleotide-conjugated AuNPs on the AuNP-antibody	Colorimetric
AuNPs	Embryonic antigen (CEA) as tumor biomarker Procalcitonin, (PCT) as bacterial infection biomarker	Catalytic activity of horseradish peroxidase (HRP) on the AuNP-antibody for luminol and H_2O_2	Colorimetric (GLFT) & chemiluminescence (C-mode GLFT)
AuNPs	Ochratoxin A	Deposition of silver on the AuNP-antibody using hydroquinone	Colorimetric
AuNPs	Zearalenone (ZEN)	Oxidative polymerization of dopamine on the AuNPs	Colorimetric
AuNPs	Dengue NS1 Protein	Conjugation of ferrocene-antibody on AuNPs-labeled antibody	Electrochemical

(continued)

Table 1 (continued)

NPs	Target	Detection process	Transduction
AuNPs	8-hydroxy-2′-deoxyguanosine (8-OHdG)	1-Evaluation of color of AuNPs-antibody on the T-line (8-OHdG-antibody/antigen) 2-Evaluation of current reduction by capturing free AuNPs-antibody or AuNPs-antibody/8-OHdG on C-line (8-OHdG-antibody)	Colorimetric & electrochemical
AuNPs	Hepatitis B virus (HBV) DNA	Automatic assay by migration of Au^{3+} and binding DNA hybridization on T-line resulted in AuNPs formation	Electrochemical
AuNPs	C-reactive protein (CRP)	Oxidation of $Ru(bpy)_3^{2+}$-labeled AuNPs and tripropylamine (TPA)	Electrochemiluminescent
CNTs	Methamphetamine (MET)	MWCNTs-labeled antibody/antigen on the T-line	Colorimetric
CNTs	Squamous cell carcinoma antigen (SCCA)	Cotton thread immunochromatographic using CNTs@ AuNPs-antibody	Colorimetric
Amorphous carbon nanoparticles (ACNPs)	*Fusarium* mycotoxins (Zearalenone, T-2 Toxin, Deoxynivale)	ACNPs-labeled antibody/antigen captured on T-line	Colorimetric
Carbon dots (CDs)	Zearalenone (ZEN)	Quenching the CD-ovalbumin by AgNPs-antibody	Fluorimetric

(continued)

Table 1 (continued)

NPs	Target	Detection process	Transduction
CNTs	Chlorpyrifos oxon (CPO)	Difference between activation of acetylcholinesterase (AChE) and CPO-AChE on CNT-modified Screen-Printed Electrode (SPE) T-line on	Electrochemical
Graphite-like carbon nitride (g-C3N4)	17β-estradiol (E2)	g-C_3N_4@AuNPs—antibody/antigen on the T-line	Colorimetric
CdTe quantum dots (QDs)	HIV-DNA	Displacement amplification technology between hairpin H1-strand, H2-CdTe QDs, and HIV-DNA	Fluorimetric
Core/shell CdSe/ZnS QDs	Fumonisin mycotoxins	Quenching the QDs@antigen by AgNPs or AuNPs-labeled antibody	Fluorimetric
ZnSe/CdSe core capped with CdS/$Cd_xZn_{1-x}S$/ZnS multishell	Human hepatitis B surface Antigen	QD-labeled antibody/antigen captured on the T-line	Fluorimetric
$CuInZn_xS_{2+x}$ ($x = 1$) capped by ZnS// ZnS	CRP	QD-labeled antibody/antigen captured on the T-line	Fluorimetric
Silica particles porous loaded CdSe/ CdS/ZnS QDs	CRP	QD-labeled antibody/antigen captured on the T-line	Fluorimetric
Quantum dot nanobead (QB)	Aflatoxin B_1 (AFB_1) and zearalenone (ZEN)	QB-labeled antibody/antigen captured on the T-line	Fluorimetric
Fe_2O_3–AuNPs Magnetic nanoparticles (MnGMs)	Aflatoxin B2 (AFB2)	MnGMs-labeled antibody/antigen captured on the C-line	Colorimetric
Fe_2O_3–SiO_2	Hepatitis B surface antigen (HBsAg)	Fe_2O_3@SiO_2-labeled antibody/ antigen captured on the T-line	Magnetometric

(continued)

Table 1 (continued)

NPs	Target	Detection process	Transduction
Streptavidin-modified magnetic nanoparticles (MNP-SA)	*L. monocytogenes* cells	Magnetically enrichment of *L. monocytogenes* through streptavidin and biotin interaction followed by extraction of DNA and detection by AuNP-probe	Colorimetric
Magnetic beads-protein G	Troponin I (cTnI)	Magnetic beads@protein G@antibody/antigen captured on the T-line	Magnetometric
Magnetic beads-COOH	Neuron-specific enolase (NSE) and carcinoembryonic antigen (CEA)	Magnetic beads- antibody/antigen captured on the T-line	Magnetometric
AuNPs@ platinum	Human prostate-specific antigen (PSA)	AuNPs@platinum-antibody/antigen captured on the T-line followed by addition TMB/H_2O_2	Colorimetric
AuNPs@ platinum nanowire	Rabbit IgG	AuNPs@ platinum-antibody/antigen captured on the T-line followed by addition of 3-amino-9-ethyl-carbazole (AEC)/ H_2O_2	Colorimetric
AuNPs@ platinum nanowire	p24 (biomarker of HIV)	AuNPs@ platinum@antibody/ antigen captured on the T-line followed by addition 4-Chloro-1-naphthol/ 3,3′-Diaminobenzidine, tetrahydrochloride (CN/DAB) /H_2O_2	Colorimetric

(continued)

Table 1 (continued)

NPs	Target	Detection process	Transduction
Palladium-platinum (Pd–Pt) nanoparticles	*Escherichia coli* O157:H7	Pd–Pt-labeled anibody/antigen captured on the T-line followed by addition TMB/H_2O_2	Colorimetric
$NaYF_4$:Yb, Tm@ $NaYF_4$ @Ca^{2+} upconversion nanoparticles (UPC	*Avian influenza virus (AIV)*	$NaYF_4$:Yb, Tm@ $NaYF_4$ @Ca^{2+}-labeled antibody/antigen captured on the T-line	Fluorimetric
Europium-chelate	Phospholipase A2 receptor (anti-PLA2R-IgG)	Europium-chelate-antibody/antigen captured on the capture antibody on the surface of well	Fluorimetric
Reporter-labeled hollow gold nanospheres (HGNs)	Staphylococcal enterotoxin B (SEB)	HGNs-labeled antibody/antigen captured on the T-line	SERS
Flower-like gold-silver core-shell bimetallic nanoparticles (AuNF@Ag)	β-adrenergic agonist brombuterol (BB)	AuNF@Ag-labeled antibody/antigen captured on the C-line	SERS
Au@Ag NPs @ two layers of Raman dye 5,5′-dithiobis-(2-nitrobenzoic acid) (Au@DTNB@Ag@DTNB)	Human IgM	Au@DTNB@Ag@DTNB-antibody/antigen captured on the T-line	SERS
Au nanorod (AuNR)@Raman tags@Au	cTnI	Au nanorod (AuNR)@Raman tags@Au-antibody/antigen captured on the T-line	SERS

(continued)

Table 1 (continued)

NPs	Linear range	LOD	Real sample	Time (min)	References
AuNPs	0.10–14.27 ng/mL	0.01 ng/mL	Serum samples of patients with myocardial infarction	10	[14]
AuNPs	0.1 − 5 μg/mL	0.001 μg/mL	Human serum samples	15	[15]
AuNPs	0.1–25 nM	0.1 nM	N.M	20	[16]
AuNPs	GLFT:CEA 10–200 ng/mL PCT 10–10^3 pg/mL C-mode GLFT:CEA 5–200 ng/mL PCT 10–10^4 pg/mL	GLFT:CEA 0.17 ng/mL PCT 10 pg/mL C-mode GLFT:CEA 0.0017 ng/mL PCT 0.05 pg/mL	Whole blood	GLFT: 15 C-mode GLFT:30	[17]
AuNPs	1–20 μg/L	0.9 μg/L	Wines and grape must samples	20	[18]
AuNPs	0.01–50 ng/mL	7.4 pg/mL	Maize	30	[19]
AuNPs	1–25 ng/mL	0.5 ng/mL	N.M	15	[20]
AuNPs	1–200 ng/mL	Colorimetric: 5.76 ng/mL Electrochemical: 8.85 ng/mL	Urine	10	[21]
AuNPs	10 pM–2 μM	7.23 pM	Human serum sample	7	[22]
AuNPs	0.01 − 1000 ng/mL	4.6 pg/mL	Serum	15	[23]
CNTs	62.5–1500 ng/mL	N.M	Human serum, urine, and saliva	30	[24]
CNTs	5–500 ng/mL	3.03 ng/mL	Human serum	20	[25]

(continued)

Table 1 (continued)

NPs	Linear range	LOD	Real sample	Time (min)	References
Amorphous carbon nanoparticles (ACNPs)	Deoxynivalenol 18.75–600 μg/kg T-2 Toxin 9.375–300 μg/kg Zearalenone 0.938–30 μg/kg	Deoxynivalenol 20 μg/kg T-2 Toxin 13 μg/kg Zearalenone 1 μg/kg	Maize	8	[26]
Carbon dots (CDs)	N.M	1–2.5 μg/kg	Cereal samples and their products	5	[27]
CNTs	1.0−200 nM	N.M	Human red blood cells (RBCs) sample	2	[28]
Graphite-like carbon nitride (g-C3N4)	0.25–10 ng/mL	0.5 ng/mL	Food samples including fish, prawn, pork, and chicken	10	[29]
CdTe quantum dots (QDs)	1 pM–10 nM	0.76 pM	Human serum	15	[30]
Core/shell CdSe/ZnS QDs	N.M	62.5 μg/kg	Maize flour samples	15	[31]
ZnSe/CdSe core capped with CdS/$Cd_xZn_{1-x}S$/ZnS multishell	N.M	0.05 ng/mL	N.M	20	[32]
$CuInZn_xS_{2+x}$ ($x = 1$) capped by ZnS//ZnS	0–800 ng/mL	5.8 ng/mL	N.M	3	[33]
Silica particles porous loaded CdSe/CdS/ZnS QDs	0–6.25 ng/mL	0.036 mg/L	Blood samples	15	[34]
Quantum dot nanobead (QB)	2–300 pg/mL	AFB_1 1.65 pg/ mL ZEN 59.15 pg/ mL	Maize extracts	15	[35]

(continued)

Table 1 (continued)

NPs	Linear range	LOD	Real sample	Time (min)	References
Fe_2O_3–AuNPs Magnetic nanoparticles (MnGMs)	N.M	0.9 ng/ml	Peanut, hazelnut, pistacia, and almond	15	[36]
Fe_2O_3–SiO_2	N.M	0.1 pg/mL	Clinical sera specimens	N.M	[37]
Streptavidin-modified magnetic nanoparticles (MNP-SA)	N.M	3.5×10^4 CFU/g	Lettuce samples	1	[38]
Magnetic beads-protein G	0.01–12.5 ng/mL	0.01 ng/mL	N.M	N.M	[39]
Magnetic beads-COOH	0–100 ng/mL	NSE ng/mL 0.094 CEA 0.045 ng/mL	Serum sample	30	[40]
AuNPs@ platinum	10–200 pg/mL	3.1 pg/mL	Plasma	5	[41]
AuNPs@ platinum nanowire	0.05–10 ng/mL	5 pg/mL	Plasma	5	[42]
AuNPs@ platinum nanowire	0.2–10 ng/mL	0.8 pg/mL	Plasma	20	[43]
Palladium-platinum (Pd–Pt) nanoparticles	$1 \times 10^3 - 1 \times 10^6$ cfu/mL	1×10^3 cfu/mL	Milk	25	[44]
$NaYF_4$:Yb, Tm@ $NaYF_4$ @Ca^{2+} upconversion nanoparticles (UPC)	LPAI H5N2 $10^{0.5}$ to 10^4 EID_{50} (50% egg infective dose)/mL HPAI H5N6 $10^{2.5}$–10^5 EID_{50}/mL	LPAI H5N2 10^2 EID_{50}/mL HPAI H5N6 $10^{3.5}$ EID_{50}/mL	Oropharyngeal and cloacal swabs	20	[45]
Europium-chelate	0.03–340 mg/L	0.03 mg/L	Human serum	5	[46]

(continued)

Table 1 (continued)

NPs	Linear range	LOD	Real sample	Time (min)	References
Reporter-labeled hollow gold nanospheres (HGNs)	0.1 pg/mL–1,000 ng/mL	0.001 ng/mL	N.M	6	[47]
Flower-like gold-silver core-shell bimetallic nanoparticles (AuNF@Ag)	N.M	0.5 pg/mL	Swine meat and urine samples	15	[48]
Au@Ag NPs @ two layers of Raman dye 5,5′-dithiobis-(2-nitrobenzoic acid) (Au@DTNB@Ag@DTNB)	0.1 ng/mL–10 μg/mL	0.1 ng/mL	*Mycoplasma pneumoniae* (MP)-specific IgM serum	N.M	[49]
Au nanorod (AuNR)@Raman tags@Au	0.1–100 ng/mL	0.1 ng/mL	Serum	10	[50]

N.M.: Not mentioned

In a straightforward and basic design of LFAs, antibody-conjugated AuNPs were utilized as the detection agent, resulting in sandwich immunocomplex formation and naked-eye detection by capturing antibody-immobilized sites on the nitrocellulose membrane [54]. In addition, colorimetric signals may be measured by using a strip analyzer or intensity image analyzer software. In this simple LFA design, the sensitivity may be enhanced by optimizing the size distribution of the AuNPs. A number of studies have revealed that the diameter of AuNPs significantly affects the sensitivity of AuNPs-based lateral flow immunoassays (LFIA) [14, 55]. In an enhanced design of LFA strips, two conjugation pads containing two distinct sizes of antibody-conjugated AuNPs have been implanted in order to increase sensitivity. The larger AuNPs can be attached to bovine serum albumin (BSA)-antibody, whereas the smaller antibody-conjugated AuNPs are inhibited by BSA [14]. The formation of a complex via BSA-antibody interaction on the T-line leads to the improvement of color and sensitivity by AuNPs with a greater size. Nylated ssDNA can also be used as a connection between AuNPs-streptavidin and AuNPs-labeled antibodies on the T-line or C-line [56]. The in situ increase of AuNPs size (Au deposition) on the T-line or C-line is an additional technique for sensitivity amplification. In the presence of hydroxylamine hydrochloride, the catalytic effect of AuNPs on the reduction of Au^{3+} ions ($KAuCl_4$) to bulk metal resulted in an increase in the size and sensitivity of AuNPs (Fig. 2) [15, 57, 58].

Additionally, size increase can occur through the formation of aggregation. Bioconjugation of AuNPs with complementary oligonucleotide chains results in the formation of AuNP aggregates [16, 59]. In this design, one set of AuNPs is associated with the amplification probe, while another group is associated with the complementing and detecting probes. Amplification and complementary probes hybridize to generate AuNP aggregates that are caught on the T-line and C-line.

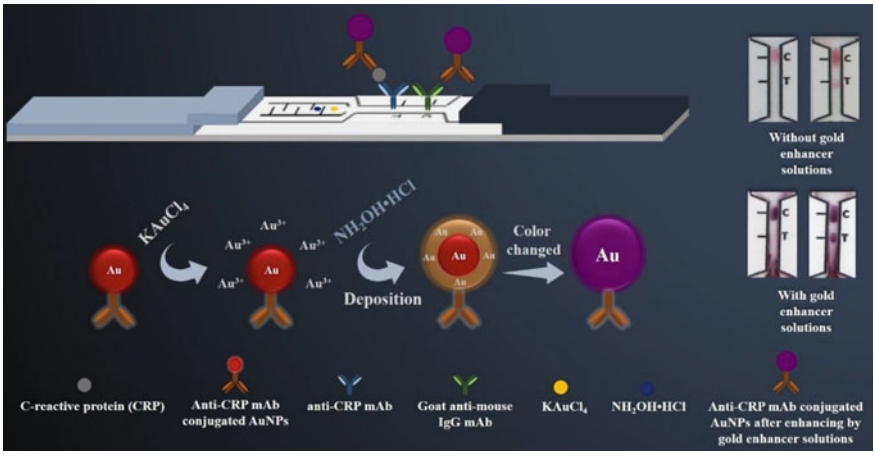

Fig. 2 Automatic sensitivity enhancement using $KAuCl_4$ and $NH_2OH \cdot HCl$. Reprinted from [15], with permission from Elsevier

Incorporation of chemiluminescence with colorimetric method is another strategy for enhancement of sensitivity which is done by modification of AuNPs with horseradish peroxidase (HRP) and antibody which enable chemiluminescence and colorimetric by reaction of luminol [17] and chromogenic agents including TMB (3,3,5,5-tetramethylbenzidine), AEC (3-amino-9-ethylcarbazole) [60, 61]. Moreover, some nanoparticles such as platinum (Pt) nanowires on AuNPs, can successfully replicate the enzymatic actions on the chromogenic agents, leading to an increase in sensitivity and color on the T-line and C-line [42]. In another strategy, after the formation of red color on the T-line and C-line, silver deposition on the gold nanoparticles lead to the formation of black color resulting in enhancement of sensitivity [18]. In a similar manner, polymeric materials such as polydopamine (PDA) can be polymerized onto the AuNPs, which has the benefits of high color intensity and sensitivity, rapid strip detection, and biocompatibility [19]. In order to improve the sensitivity, the decoration of nanosheets or nanoparticles with AuNPs such as graphite-like carbon nitride (g-C_3N_4) with high surface area, was also performed [29, 62].

Integration of strips with a screen-printed electrode (SPE) covered with bioreceptors on the working electrode portion permits LFA with electrochemical readout. In this configuration, AuNPs may transport redox markers such as ferrocene [20]. Occasionally, simple electrochemical LFA strips may be constructed without the addition of redox-active spices. Current may be lowered in this design by trapping AuNPs-antibody conjugates on the detecting zone, which is the working electrode [21]. In another design, Srisomwat et al. synergically used the advantage of automation, delaying architecture, and electrochemical-based LFA [22]. In this design, following the migration of hepatitis B virus (HBV) DNA down to the T-line and capture by the DNA strand on the T-line, Au^{3+} ions are delivered through a baffle barrier with a delayed rate and captured on the hybridized DNA strands via electrostatic and coordination interactions with the phosphate backbone. Subsequently, an anodic stripping voltammetry (ASWV) test was conducted, and the synthesis of AuNPs resulted in the development of a signal owing to the decrease of Au^0.

In addition to some advantages of AuNPs such as excellent electrochemical behavior, high surface area, and considerable biocompatibility, due to their small size and faster migration, single-step electrochemiluminescence (ECL) procedure with mixing tripropylamine as the ECL coreactant with the sample solution can be performed. Benefiting from this, labeling AuNPs with $Ru(bpy)_3^{2+}$ enables the formation of sandwich immunocomplexes at the T-line, which generates an ECL signal in the presence of a $Ru(bpy)_3^{2+}$/tripropylamine (TPA) system (Fig. 3) [23].

2.1.1 Critical Note

Although LFIA strips with naked eye readout have been developed most frequently as the most popular POC device in the diagnosis process, their application may be limited by disadvantages such as qualitative detection, low sensitivity, instability of antibodies, and possible aggregation of AuNPs in serum matrices. Some improvements have been made to improve the sensitivity in an effort to resolve the issues.

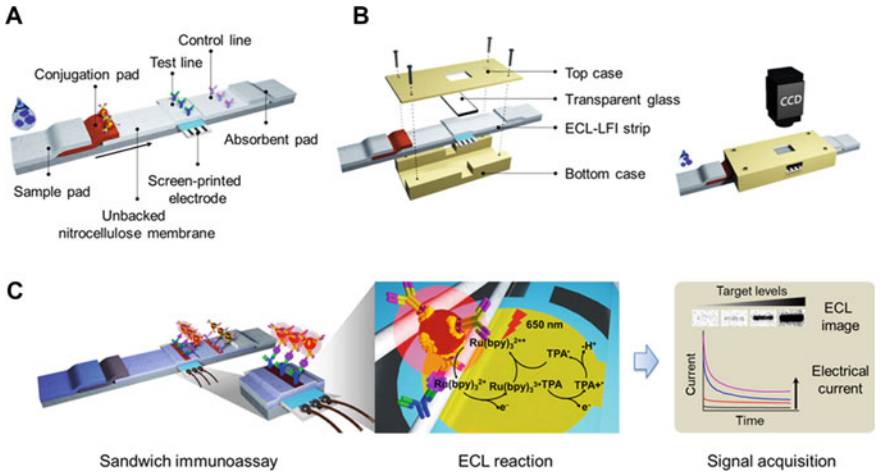

Fig. 3 Application $Ru(bpy)_3^{2+}$-AuNP-Ab for ECL-based LFA: **a** LFA strips structure; **b** assembling the ECL equipment on LFA strip; **c** mechanism of signal producing. Reprinted from [23], with permission from The American Chemical Society

Several amplification strategies, such as deposition of Au, application of two different sizes of AuNPs, and application of AuNPs aggregates, may appear more complicated than the conventional simple LFA method, but they can be executed in a single stage with greater sensitivity than the conventional method.

Another proposed amplification technique involves surrounding AuNPs with HRP in order to perform a chemiluminescence readout. Despite its high sensitivity, this technique may be limited by a time-consuming procedure, the need for a specific reaction temperature (37 °C), and the instability of enzymes. Therefore, alternatives to enzymes such as Pt nanoparticles are advantageous [63]. Although other techniques that increase the color and visibility of T-line and C-line using silver deposition [18] and polymeric materials [19] can be a valuable alternative to the time-consuming enzymatic technique, LFA production can be made affordable by reducing the number of antibodies required. Distribution of AuNPs on a large surface area increases the signal and sensitivity, but the formation of a large nanocomposite reduces the flow rate along the strip and lengthens the testing time, which may limit the performance of this strategy and the use of membranes with small pore sizes that increase sensitivity.

The incorporation of electrochemical approach with LFA has biocompatibility, affordability, compactness, and downsizing capabilities that are more suitable for POC objectives than optical techniques. This idea is implementable on a miniaturized platform containing a small potentiostat, allowing for quick and on-site detection. Due to their exceptional electrochemical properties, AuNPs may be useful in the development of LFAs based on the electrochemical method. However, certain designs, such as ECL-based LFA, can separate LFA technology from POC objectives. Although this technology combines the benefits of AuNPs with ECL in a synergistic

manner, the installation of certain equipment, such as a charge-coupled device (CCD) camera, may raise the cost of the system and restrict its applicability. In addition, for one-step performance and automation of the detection method on the strip, a wax-printing technique is used to create a baffle or zigzag delayed channel. In this method, merging in the non-delayed flow adjusts the transmission of an enhancement reagent such as Au^{3+} to the detecting zone via delayed and non-delayed channels [15, 22]. This design has a high sensitivity and a low LOD, but its complexity and the oxidation of the Au ions may restrict its use.

2.2 Carbon-Based Nanomaterials

Recently, carbon-based nanoparticles such as carbon nanotubes (CNTs), graphene oxide (GO), and carbon dots (CDs) have been widely used in the design of LFA strips due to their high contrast and dark color, low cost, high safety, simple functionalization, portability, and excellent optical and electrochemical properties. The aforementioned benefits are in accordance with the fabrication of POC systems that aim for immediate, inexpensive, and on-site detection of biological targets such as viruses, proteins, DNA, etc., on a compact and portable platform [64]. Compared to AuNPs, CNTs have a greater surface area with high binding sites that are easily functionalized by bioreceptors, resulting in an increased sensitivity. In addition, because of the high contrast between black and white of CTNs, semi-quantification detection may be performed with the naked eye or quantification detection can be conducted during image processing using gray pixels [24]. In a research using CNTs-labeled antibodies for methamphetamine detection, the sensitivity was determined to be 10 times higher than AuNPs-labeled antibodies [24]. CNTs can also be adorned with AuNPs for the immobilization of antibodies, which combines the benefits of both materials [25]. G-C3N4, a two-dimensional (2D) nanomaterial with chemical inertness, a large surface area, and an inexpensive manufacturing technique, is a strong choice for transporting AuNPs (Fig. 4) [29]. Amorphous carbon nanoparticles (ANPs) are unusual nanomaterials with a size greater than 100 nm and a variety of single- and multi-walled CNTs (MWCNTs). ANPs have benefits such as greater sensitivity creation in comparison to AuNPs, non-toxicity, exceptional stability, simple functioning and conjugation, and excellent contrast in comparison to bright backdrops due to their deep black color. The aforementioned characteristics render ANPs appropriate for use as label antibodies in the manufacture of LFA strips [26]. CDs, as carbon-based nanomaterials, are zero-dimensional nanoparticles that possess biocompatibility, low toxicity, inertness, and photostability. These functions employ CDs commonly in drug delivery [65], imaging [66], biosensor [67], and photocatalyst [68]. Thus, the application of CDs as labels of antibodies may be utilized for LFA technique [69]. In this way, hybridization of CDs with other nanoparticles such as SiO_2 might be a useful label for LFA, since it results in great sensitivity for the LFA approach due to its high stability and considerable fluorescence intensity [69].

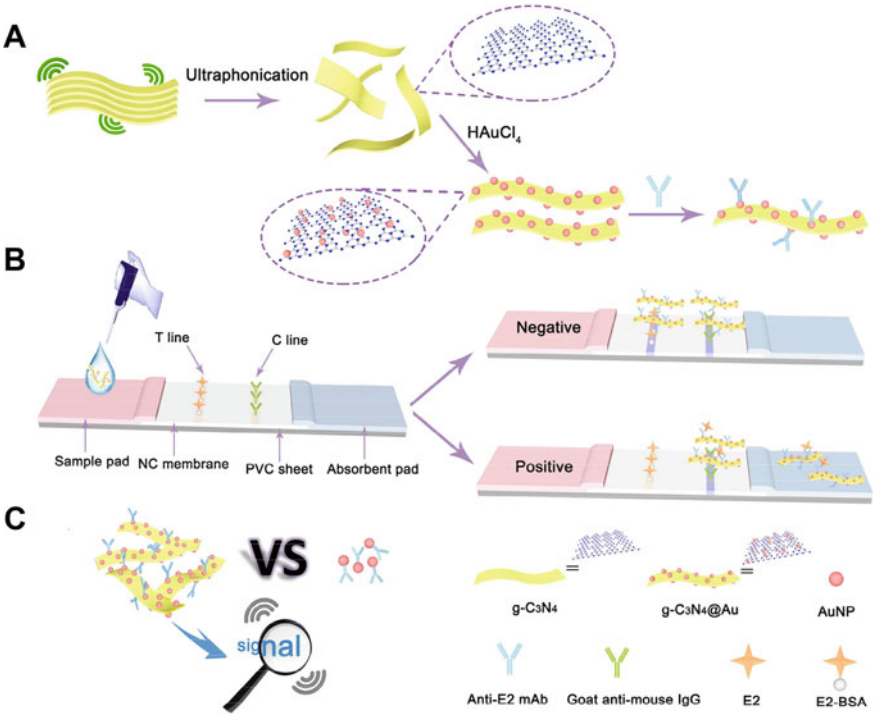

Fig. 4 Application of g-C_3N_4 in LFA: **a** synthesis protocol; **b** detection procedure; **c** enhancement of signal. Reprinted from [29], with permission from Elsevier

Benefiting from the FRET effect of CDs fluorescence intensity with certain quencher nanoparticles, such as silver nanoparticles (AgNPs), substantial absorption at the CDs fluorescence emission wavelength may occur. Li et al. constructed a strip in this manner by immobilizing a combination of zearalenone-ovalbumin and CD-ovalbumin on the T-line and zearalenone-ovalbumin on the C-line. In this configuration, AgNPs-anti-zearalenone served as the acceptor (quencher) while CD-ovalbumin served as the donor [27]. In addition to enhancing the optical characteristics of CNTs, their high conductivity also allows them to be employed as the working electrode. Zue et al. developed a concept for LFA employing CNT paper on the C-line and Ag/AgCl ink-painted copper paper as the reference/counter electrode, followed by lamination of the strip. In this approach, BSA–8-hydroxyguanosine on the T-line collected AuNP–anti–8-hydroxyguanosine conjugates. The AuNP–anti–8-hydroxyguanosine/8-hydroxyguanosine complexes then moved across the T-line and were caught by anti-Mouse IgG on the C-line, resulting in the detection of the antigen [21]. CNT-modified screen-printed carbon electrode (SPE) that is mounted under the T-line using a magnet is a second design option [28]. This structure was designed to

evaluate the enzymatic activity of acetylcholinesterase (AChE) for acetylthiocholine (ATCh) by comparing deactivated AChE to active AChE trapped by anti-AChE on the T-line.

2.2.1 Critical Note

Due to the insolubility of CNT in sample buffers and the slow migration of CNT in the pores of nitrocellulose, some pre-treatment such as oxidation or surfactant binding must be performed before CNTs may be linked to multiple bioreceptors [24]. In addition, CNT modification of the electrode is heavily dependent on organic solvents for effective dispersion. For LFA strips, the suggested nanoparticles with high solubility, such as CDs, and the benefits of easy functionalization, simple synthesis, high safety, low cost, and high quantum yield in solid and aqueous phases are viable candidates. Some developments, such as the inclusion of CDs with other nanoparticles such as SiO_2, result in the non-uniformity of this kind of nanoparticles due to the co-hydrolysis of CDs and tetraethyl orthosilicate (TEOS) [69]. Although designing FRET-based LFA strips enables on-site screening of targets in complex matrices, such as zearalenone in cereal samples and relevant products, the qualitative, semi-quantitative, and probable interferences of matrices may limit the scope of this method's use [27].

2.3 Quantum Dots (QDs)

QDs as semiconductor nanoparticles are typically a mix of elemental groups III–V and II–VI. Due to the quantum confinement of electrons and holes in these nanoparticles, continuous molecular band energy is converted to discrete energy levels, resulting in the potential emission of fluorescence upon excitation and electron-hole recombination. QDs with advantages of controllable size-depended emission, high specific surface area, high fluorescence intensity, long lifetime, high binding sites, wide absorption region, low photodegradation, and photo-bleaching, have been widely used for designing biosensors in comparison with other organic commercial dyes [70]. Due to the aforementioned characteristics, QDs have attracted considerable interest for the creation of LFA strips to detect proteins [71], viruses [72], pharmaceutical materials [73], and nucleic acid [74]. Although QDs indicate a high level of sensitivity to the LFA system, it is possible to process some technologies to increase sensitivity. Due to the formation of hydrophilic QDs in the presence of thiolated acids, an abundance of carboxylic acids can coat the surface, facilitating the immobilization of amino-terminated aptamers and DNAs. Thus, CdTe QDs were combined with strand displacement amplification method for HIV-DNA detection. This approach conducted by hybridization of hairpin H1-strand as a trigger, with HIV-DNA leading to unraveling the hairpin structure of H1. Thus, the remaining H1 strand can hybridize with the CdTe hairpin H2 strand that has been tagged with

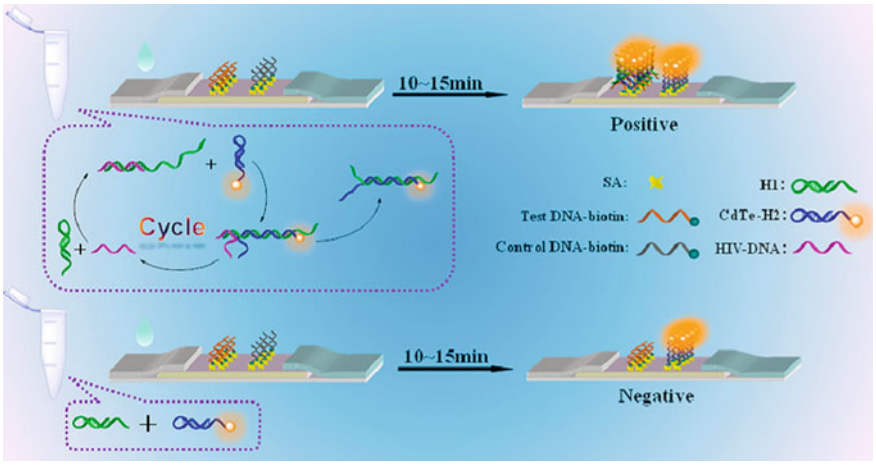

Fig. 5 Strand displacement amplification-based LFA for detection of HIV-DNA. Reprinted from [30], with permission from Elsevier

QDs, followed by the release of HIV-DNA. As seen in Fig. 5, this circle may be repeated and an amplification sample can be deposited onto the sample pad. Finally, H1–H2-CdTe QDs hybridization was captured on the T-line by the unhybridized H2 strand sequence [30].

The employment of core/shell QDs is advised for enhancing the sensitivity of QDs-based LAF techniques. So, CdSe/ZnS QDs are extensively employed in the creation of biosensors, particularly, LFA strips [31]. In this method, core/shell QDs may be constructed via multishell strategy to increase the quantum yield and sensitivity of LFA technology. By preventing exciton leakage, the surrounding ZnSe/CdSe core with a CdS/Cd_xZn_{1-x}S/ZnS multishell may significantly increase the quantum yield to 70% [32]. $CuInZn_xS_{2+x}$ ($x = 1$) as a cadmium-free core is capped by ZnS/ZnS as a thick shell, which is synthesized during two independent shell growth processes for the purpose of multishell development in LFA. This structure can provide a 77% quantum yield [33]. In order to generate cadmium-free and environmentally acceptable QDs, InP/ZnS core/shell QDs were encased in a silica shell for LFA design [75]. QD/SiO_2 nanoparticles including dendritic and porous silica particles with densely loaded CdSe/CdS/ZnS QDs were proposed for creating LFA strips [34]. Compared to typical sandwich-type nanospheres, in which a layer of fluorophores surrounding the silica core, this shape significantly increases the surface area for adsorption of QDs and makes homogenous dispersion of QDs throughout the silicon sphere practical. The accumulation of QDs in each unity led to outstanding optical properties, colloidal stability, and simple biofunctionalization of the suggested nanoparticle. The inclusion of beforementioned properties with LFA strips led to the establishment of a powerful platform for the detection of C-reaction protein (CRP) in complicated biological samples [34]. For enhancement of the sensitivity, adsorption of QDs on the surface of biocompatible nanobeads with large surface area is another approach.

Shao et al. produced nanobeads (Fig. 6) using sodium dodecyl sulfonate (SDS) and poly (maleicanhydride-alt-1-octadecene) (PMAO) for this purpose, which were subsequently coated with CdSe/ZnS QDs [35].

The readout signals from QDs-based LFA can be performed by UV light followed by an assessment of intensities using ImageJ application or by fluorescence strip reader. Signaling from QDs-based LFA can be accomplished by directly emitted fluorescence intensity of QDs-labeled bioreceptors or measurement of QDs intensity quenching. LFA strips can be produced by quenching emitting antigen-linked QDs on the T-line with antibody-linked AgNPs or AuNPs utilizing an inner filter and fluorescence resonance energy transfer (FRET), respectively [31]. Also, a nanocomposite of quantum dots (Biotin-QDs) and MnO_2 nanosheets, which results in the

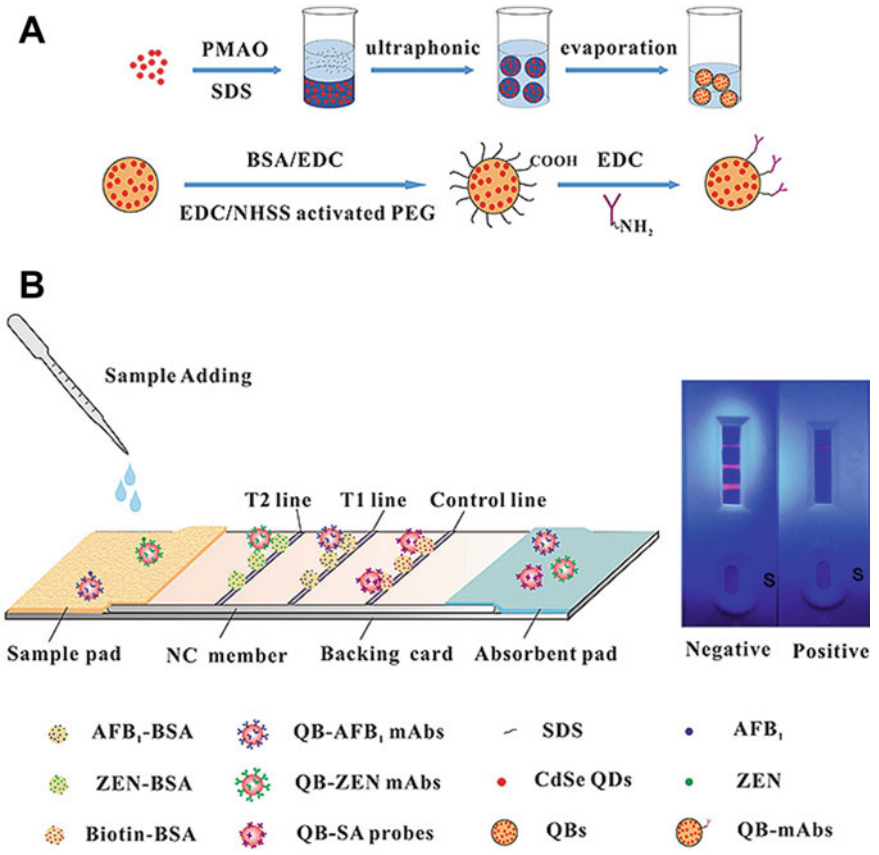

Fig. 6 Application quantum dot nanobeads (QBs) for multiplexed-LFA detection of aflatoxin B1 (AFB1) and zearalenone (ZEN); **a** synthesis protocol; **b** detection process. Reprinted from Analytica Chimica Acta, Vol. 1025, Shao et al., Quantum dot nanobead-based multiplexed immunochromatographic assay for simultaneous detection of aflatoxin B1 and zearalenone, Pages 163–171, Copyright 2018, with permission from Elsevier [35]

quenching of QDs, can be used as a decoration for QDs. MnO_2 nanosheets are degraded in the presence of glutathione (GSH), allowing Biotin-QDs to be collected on a streptavidin-containing T-line [76].

2.3.1 Critical Note

Compared to typical AuNPs, QDs have a greater specific surface area, acceptable biocompatibility, increased sensitivity, quicker strip migration, and simple storage conditions. Furthermore, the simple binding of QDs to nucleic acid strands enables the performance of displacement amplification technology, which introduced a potent method that, in comparison to conventional methods such as loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and polymerase chain reaction (PCR), is easier to operate, does not require expensive biological material, and does not necessitate expert knowledge [30]. Due to the application of the synthesis technique in an organic phase, the stability and quantum yield of core/shell QDs might be diminished after transfer to a biological aqueous environment, hence affecting the sensitivity of the LFA system [33]. In addition, QDs-based LFAs are hampered by issues such as high toxicity of heavy metal elements, limited stability, aggregation in biological samples, and a quenching effect in the presence of biomolecules.

2.4 Magnetic Nanoparticles (MNPs)

Typically, these nanoparticles are produced using iron oxide nanoparticles (Fe_3O_4, γ-Fe_2O_3) as a foundation core, which is then coated with additional nanoparticles and bioreceptors are immobilized. This property enables the construction of core/shell structures for the application of LFA, such as Fe_2O_3 nanoparticles containing gold [36], SiO_2 [37], streptavidin [38], and protein G [39]. In addition, because of their large surface area and simple carboxyl group functionalization, Fe_2O_3 nanoparticles can be directly linked to antibodies [40] or other bioreceptors. The primary flaw of conventional MNPs is aggregation during migration along strips, which slows down the detection process and reduces sensitivity owing to weaker antigen–antibody interactions. Super-paramagnetic nanoparticles (SPMNPs) with a larger surface area and no hysteresis have been shown to be an effective solution to this issue. SPMNPs are ascribed to MNPs less than 20 nm in size. Furthermore, MNPs having a size between 30 and 100 nm are paramagnetic [1]. Wang et al. demonstrated the size of SPMNPs has a substantial impact on the detection time [77]. Different magnetometers, such as resonant coil [78], magnetoresistance [79], and planar coils [80], can read the signal of MNPs. Magnetometers are installed above the detecting zone of the strips for this purpose, as the existence of an external magnetic field is crucial for the emergence of the magnetization effect of MNPs [81]. Due to the obvious brown color of Fe_2O_3 or their coating by AuNPs, T-line and C-line can be read with the naked eye (Fig. 7) [36,

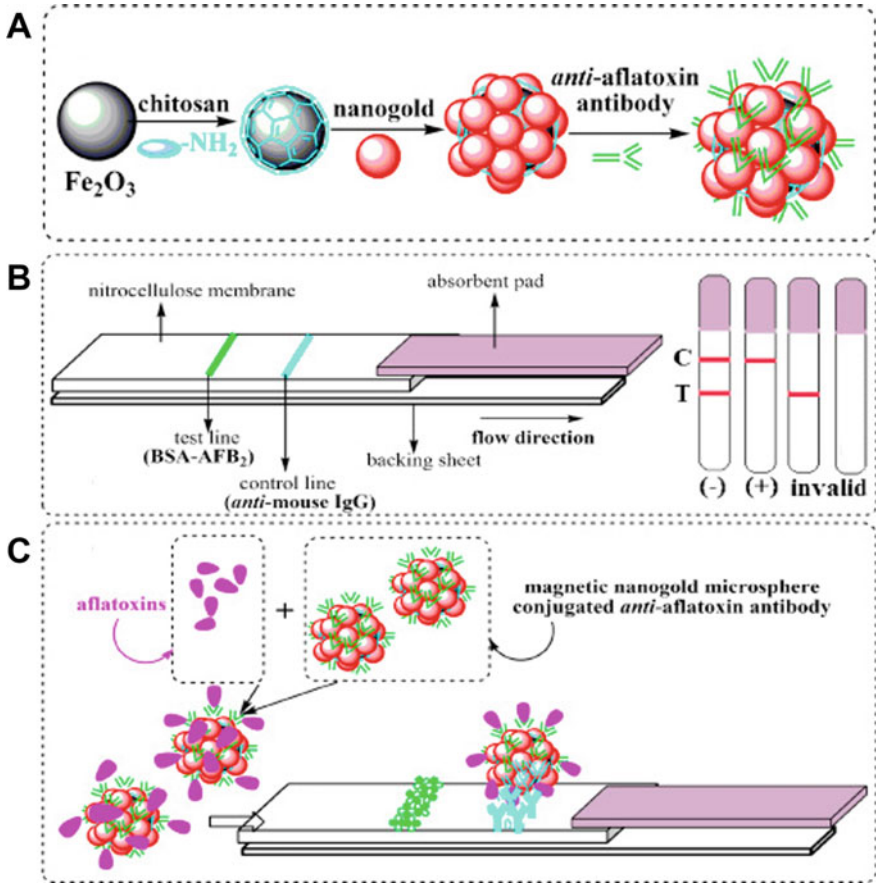

Fig. 7 Application of Fe_2O_3–AuNPs-antibody for detection of aflatoxin B_2. Reprinted from [36], with permission from Elsevier

39]. Incorporation of Fe_2O_3 nanoparticles with LFA can be allocated to the sample preparation prior to its placement on the sample pad, extending their use beyond signal creation. Li et al. implemented magnetic enrichment of *L. monocytogenes* cells from lettuce by streptavidin-biotin interaction, DNA extraction, and detection by AuNP-probe on LFA strip [38].

2.4.1 Critical Note

In comparison to fluorophore nanoparticles, MNPs have the benefits of reduced background, the need for inexpensive, compact, and compact magnetometers, and make on-site and downsized POC detection possible. Furthermore, movement along the strips is navigable utilizing an external magnetic field. Due to limited solubility

and dispersion in water, the use of these nanoparticles may be challenging despite the fact that these characteristics might provide the MNPs-based LFA more credit than other types of LFAs that need expensive and large equipment. In addition, size-dependent issues can be attributed to aggregation and positive errors caused by MNPs of greater sizes, or to poor magnetic signal and low sensitivity caused by MNPs of lower sizes [77]. Another difficulty is the two-step growth of the output signal with time, which may be regulated by the kinetics of immunoreaction [82].

2.5 Nanoenzymes

As a result of the incorporation of natural enzymes with LFA, the signal strength of the redox reaction of chromogenic substrates-H_2O_2 system on the detecting zone may have increased, resulting in visible color. In addition to colorimetry, LFA can also contain chemiluminescence owing to the benefits of nanoenzymes. This technique is typically carried out by trapping horseradish peroxidase (HRP)-labeled antibody on the T-line or C-line, followed by the addition of chromogenic substrates such as TMB (3,30,5,50-tetramethylbenzidine) and ABTS (20-azino-bis(3-ethylbenzothiazole-6-sulfonic acid) [52, 83]. Additionally, G-quadruplexe-hemin DNAzyme has enzymatic effects, but it has been utilized less frequently for LFA. This process often has a number of drawbacks, including particular requirements such as temperature (37 °C), buffer, and expense. Recently, nanoenzymes have attracted the interest of scientists for the creation of LFAs due to their remarkable properties, which include enzyme function mimicry, low cost, ease of manufacture, and increased stability [84]. In this manner, nanoenzymes having peroxidase activity, such as nanoparticles based on platinum (Pt), have been widely utilized in the construction of LFAs. The regular inclusion of nanoenzymes with LFAs has advanced based on the coating of AuNPs with Pt layer [41] or Pt nanowires [42], porous Pt layer [43], which boosts the plasmonic color of AuNPs by adding chromogenic substrate to the detecting zone (Fig. 8). In certain instances, palladium-platinum (Pd–Pt) nanoparticles were produced for LFA design [44].

2.5.1 Critical Note

Application of nanoenzymes in LFA has benefits such as low cost, high stability, and simple preparation, but detection requires the addition of chromogenic substrate to the detecting zone to generate a readable signal, which makes the procedure tedious and time-consuming. Although certain innovations, such as automating the technique by coating and drying the strips with chromogenic substrate, can speed up the detection, constructing the strips with additional channels for the separate migration of chromogenic substrate in the sample solution might complicate the LFA procedure [85].

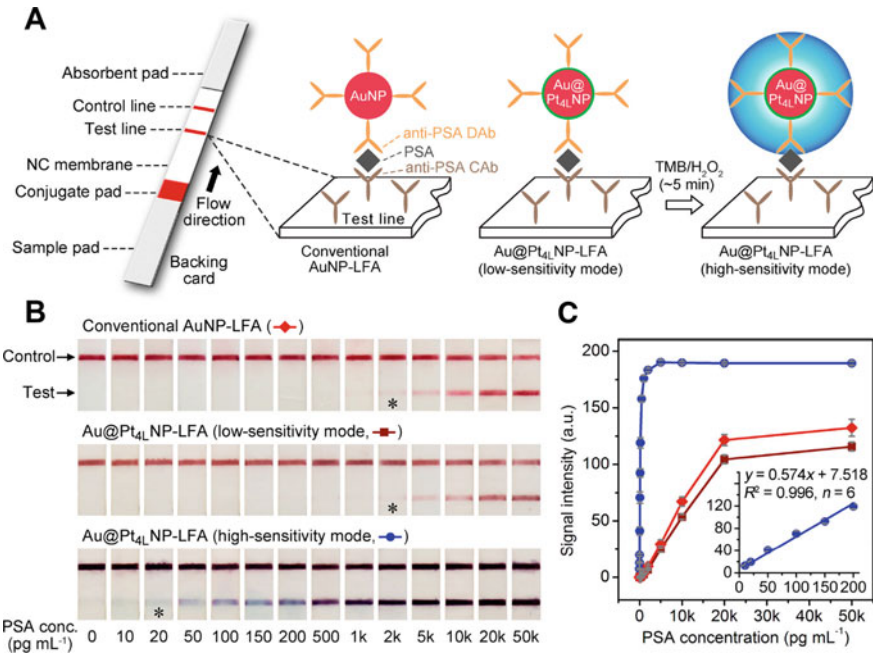

Fig. 8 Catalytic effect of Au@Pt-antibody for TMB/H_2O_2 and enhancement of sensitivity. The sign of (*) shows LOD. Reprinted from [41], with permission from The American Chemical Society

2.6 Other Nanoparticles

Nanoparticles have been widely utilized in the structuring of LFA strips. However, there have been other key nanoparticles that have been less integrated with LFA and have synergistically shared their properties with LFA to permit the high sensitivity and accuracy necessary for POC techniques. These nanoparticles are mentioned and briefly described in the next section. Upconversion nanoparticles (UCNPs) capable of photon upconversion are created by doping transition metals with actinides and lanthanides derived from rare earths. UCNPs are able to absorb a large number of photons from the low-energy near-infrared (NIR) region and convert them into a single photon from the high-energy ultraviolet-visible range (UV-Vis). Scientists are more interested in the application of UCNPs in nanomedicine, biosensors, and in vivo imaging than QDs due to their narrow and high-intensity emission, reduced toxicity, anti-Stokes shifts, high cellular uptake, low background, and strong optical penetration in tissue [86]. $NaYF_4$ double-doped with Yb and Er has been the most often included UCNPs with LFA ($NaYF_4$: Yb, Er). In this system, the matrix with the lowest phonon energy is $NaYF_4$. Also, Yb^{3+} is able to absorb an infrared photon in the host lattice, which is then transmitted to the non-radiative form of Er^{3+}, which transforms it into visible emission [87]. Moreover, in order to enhance the intensity and sensitivity of $NaYF_4$ UCNPs, several modifications might be made [45]. Doping

the Ca^{2+} ions in the shell of $NaYF_4$:Yb, Tm@$NaYF_4$ core/shell UCNPs in this manner can enhance the NIR emission via excitation in the NIR region. This event can occur as a result of lattice destruction followed by the formation of an asymmetric structure driven by the displacement of Y^{3+} with Ca^{2+}, resulting in a highly sensitive electron transition (Fig. 9).

Time-resolved fluorescence nanoparticles (TRFNPs) are fluorescent lanthanide (mostly Europium (III)) chelates nanoparticles with a hydrophobic shell that must be modified with biofunctional groups [88]. With their extended lifetime, chemical stability, large Stokes shift, and broad excitation spectrum, these nanoparticles

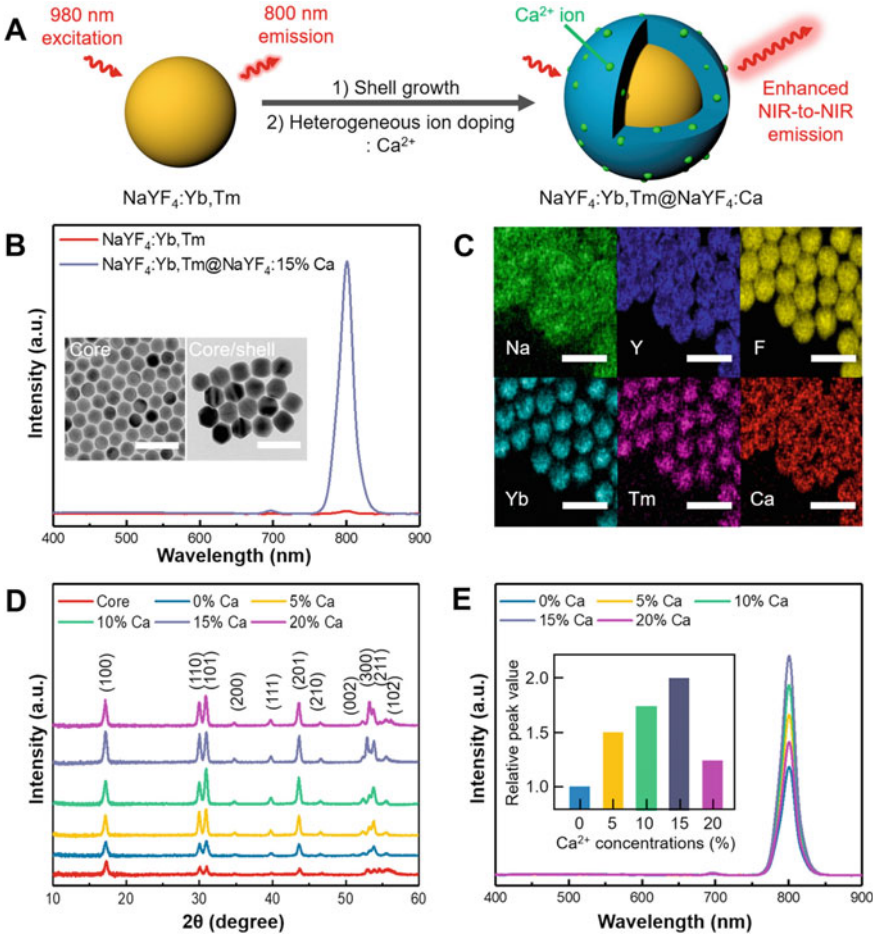

Fig. 9 NIR-to-NIR $NaYF_4$:Yb, Tm@$NaYF_4$@ Ca^{2+} UCNPs: **a** schematic illustration of synthesis; **b** enhancement of fluorescence intensity by $NaYF_4$ shell and Ca^{2+} dopant; **c** elemental mapping; **d** XRD spectra; **e** effect of different amount of Ca^{2+} dopant on the fluorescence intensity. Reprinted from [45], with permission from Elsevier

significantly minimize interferences in biological and complex matrices with transient background. Therefore, TRFNPs may be suitable for integration with LFA [46]. Surface-enhanced Raman scattering (SERS) nanotags are plasmonic metal nanostructures, such as gold and silver, that enable the detection of targets adsorbed on their surface via Raman signal enhancement resulting from electromagnetic field amplification via localized surface plasmon resonance (LSPR) by hot spot effect [89]. This occurrence can be attributed to the increase of the electromagnetic field caused by plasmonic phenomena (Stock, Rayleigh, Anti-Stocks) that lead to SERS in nanoscale gaps between nanostructures [52]. Increased Raman intensity at a constant Raman shift is used for detection (cm^{-1}). In this method, He–Ne laser (365 nm) or Raman (diode) laser (785 nm) is often utilized as the excitation source, and a holographic notch filter is employed to remove the Rayleigh line from the Raman data [47, 50]. Several nanoparticles, including hollow gold nanospheres [47], Au nanoflower @ Ag core/shell [48], Au@Ag nanoparticles [49], Au nanorod (AuNR)@ Au core/shell, have been combined with LFA in this manner [50]. Raman molecules such as malachite green isothiocyanate (MGITC) [47], 4-mercaptobenzoic acid (MBA) [48], 1,4-nitrobenzenthiole (NBT) [50] and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) [49] have been embedded or adsorbed in nanoparticles in order to produce Raman intensity. The fabrication of Au@Ag nanoparticles with dual-layer DTNB is depicted in Fig. 10.

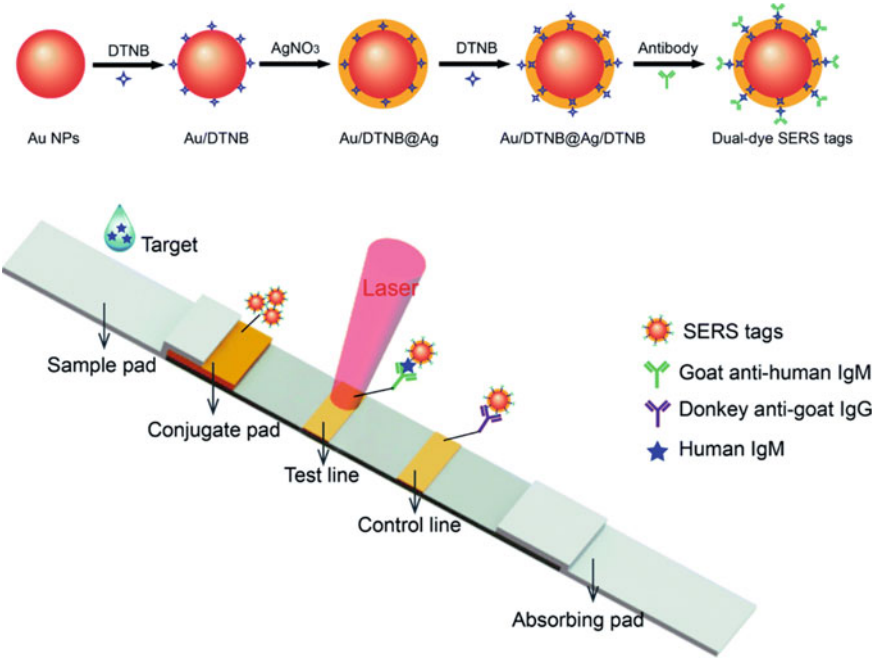

Fig. 10 Preparation and application of Au@DTNB@Ag@DTNB in LFA. Adapted from [49], in accordance with the Creative Commons Attribution 3.0 Unported Licence (CC BY)

2.6.1 Critical Note

The aforementioned nanoparticles serve an indisputable effect in reducing interferences from the background. The UCPs and SERS-nanotags with anti-Stokes or Stokes shifts induced by NIR excitation and UV-Vis or NIR emission significantly reduce the autofluorescence of UV-Vis region-absorbent biomolecules. Also, TRFNPs with extended lifetime fluorescence relative to interferences' short-lived fluorescence may be combined with LFA. Despite these advantages, their applicability may be limited by some downsides. The creation of UCNPs necessitated the use of inert gas (N_2, Ar) or vacuum, which are costly conditions that are available in all laboratories. Due to the poor solubility of UCNPs, migration along strips may also be challenging. Therefore, alteration and surface functionalization are essential. For capturing the signal of TRFNPs over their lifetime, time-resolving techniques, which are not standard on all spectrometers and are costly, are required. Despite the fact that SERS-nanotags improve the limited sensitivity caused by weak signals in the NIR window [90], this technology requires the use of costly commercial Raman molecules in nanoparticles. In contrast, even though the SERS approach attempts to minimize interferences by the use of NIR lasers and stock spectra, some background signal interferences from nanotags may still be present. In order to further limit interferences, the synthesis of very homogeneous nanoparticles may thereby complicate the synthesis technique.

3 Conclusion

The primary objective of point-of-care (POC) devices is to provide rapid, cost-effective, and accurate diagnosis of targets in a variety of domains, including medical, criminal, clinical, and industrial, in order to avoid and forecast potential issues and aid in prompt treatment. One of the most intriguing elements of POC devices is the development of in-home, patient-centered screening and healthcare diagnostics. As an accessible and simple-to-prepare POC gadget, LFA has become a popular diagnostic tool. Nanomaterials, which are an integral part of the LFA methodology, have played a crucial role in the effective design and execution of this method to increase its sensitivity. Nanomaterials have unique benefits, such as adjustable physical and chemical properties based on size, shape, and composition and simple functionalization using bioreceptors combined with LFA. According to the findings given in Table 1, production, modification, and bioconjugation of nanoparticles well-incorporated with LFA strips for the detection of diverse targets include biomarkers, viruses, microorganisms, DNA, mycotoxins, etc. As demonstrated in Table 1, the detection of targets has occurred in less than 30 min, suggesting an adequate rate of diagnosis due to the absence of aggregation, the rapid migration through membrane pores, and the successful interpretation of the signal created by nanoparticles. AuNPs have been the most prevalent and popular nanoparticles that enable naked-eye detection of T-line or C-line. Unfortunately, the typical LFA based on naked-eye qualifying detection has a

limited detection. In addition, certain image analysis software is essential for quantifying data, which makes the measurement challenging. So, to increase sensitivity, the integration of alternative transduction systems with LFA using other nanoparticles has been encouraged. In this context, CNTs with vibrant colors and a strong black-on-white contrast may be suitable for inclusion with LFA. Due to the poor dispersity and hydrophilicity of the migration buffer, however, CNTs are not commonly included with commercially available LFA. In addition, the use of carbon-based nanoparticles for electrochemically-based LFA is in great demand for SPE, making this technology extremely costly. On the other hand, despite the fact that the use of other nanoparticles benefits from the high sensitivity of QDs, the miniaturized magnetometer for MNPs, and the significant reduction of interference by UCNPs and SERS-nanotags, the readout of signals requires the integration of costly equipment with LFA. This issue prohibits personalized detection and in-home use of LFA, which contradicts the objectives of the POC approach. The catalytic impact of nanoenzymes on substrates, accompanied by an increase in sensitivity and a reduction in LOD (10^3 times) [41], can be a valuable alternative to the usual LFA approach for detection with the naked eye. In addition, the design of delayed canals for the automation of LFA may be suitable for commercialization [15].

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Aptamer-Based Lateral Flow Assay as a Smart Point-of-Care Devices

Melis Canbay, Ahmet Turan Keskintas, and Sevde Altuntas

Abstract Point-of-care (POC) devices have become more crucial in recent years. POC provides easy, quick, and low-cost on-site diagnostics and detection without the need of any complex tool or well-trained person. One of the common POC devices is lateral flow assay (LFA). LFA is a paper-based technique that contains different compartments that can detect the target of interest in a sample rapidly. Aptamer-based LFA is currently developing further due to its advantages compared to other types of LFA. For instance, it has the potential to replace antibody-based LFA because of its disadvantages. Moreover, two different major formats of Aptamer-based LFA are sandwich and competitive. The result that they give is interpreted in a different way. Herein, we will discuss aptamer-based LFA as smart POC devices, its difference between antibody-based LFA, development of aptamers for it, its different compartments and formats, interpretation of its results, and lastly its applications in diagnostics and other application areas.

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1 Introduction

In recent years with the increasing number of populations, the importance of smart point-of-care (POC) devices has increased. Developing POC devices provides faster, cheaper, and easy-to-use on-site detection by the important improvements in biosensor technologies [38]. POC devices give opportunities to do POC diagnostics with no need of special laboratory equipment or a trained professional [9, 21]. Lateral flow assay (LFA) is one of the POC techniques, which is paper-based that can identify the targeted substances found in a sample and provide the results in a very short amount of time.

The sensitivity and selectivity of the LFA assays are determined by recognition elements, which are essential parts of an LFA, and antibodies were preferred ligands for years as a recognition element [33]. As a result, the most used type of LFA is antibody-based LFA in general; however, antibodies have several drawbacks. Antibodies have limitations because of their synthesis through the in vivo approach [10]. For instance, they have high batch-to-batch variations, cross-reactivity, limited shelf life, and severe immunogenicity [10]. Also, small molecules are still difficult for antibodies to detect. Additionally, because of their protein origin, antibodies display several drawbacks like irreversible denaturation, which is induced by temperature and the problematic introductions of modifications [33]. Therefore, scientists defined the aptamer in 1990 due to challenges caused by antibodies [33].

Aptamers are single-stranded deoxyribonucleic acid (ssDNA) or ribonucleic acid (RNA), which are synthetic oligonucleotide molecules that have ability to bind different types of molecules with ranging sizes by high affinity and specificity [9, 13, 15, 25, 42, 44]. For example, they can bind small molecules, viruses, proteins to even an entire cell. Moreover, its high affinity and specificity is a result of its single-stranded structure, which allows it to fold into exclusive conformations like secondary or tertiary [13]. Similarly, higher sensitivity and specificity of aptamers came from their higher resistance to rough chemicals, extreme ionizing environments, pH, and organic solvents [11]. Aptamers are highly stable and unlike antibodies in case of denaturation they can recover to their original conformation which makes them satisfactorily flexible for adapting to various assay formats [9].

Differently from antibody production, aptamers are developed in vitro using a process known as "systematic evolution of ligands by exponential enrichment" (SELEX) (Fig. 1), which aims to the finding DNA or RNA aptamer sequences from a randomized oligonucleotide library that is capable of recognizing the target of interest [5, 15, 44]. Consequently, aptamers can be produced for any target with such a wide variety [10, 44]. Another significant fact is that production of antibodies, which can bind low molecular weight compounds is not favorable but the development of aptamers that have the ability to bind low molecular weight compounds

is possible with the aid of the SELEX process [44]. Lastly, a comparison between features of antibody and aptamer in the perspective of LFA can be seen in Table 1.

The Aptamer-based LFA has two separate major formats: sandwich and competitive, which will be discussed further in the text. Besides, there are some aptamer-antibody-hybrid LFA too, which are called hybrid LFA [34]. Also, the analysis and interpretation of their result varies and will be explained separately. Moreover, aptamer-based LFAs have several different application areas from diagnostics to detections from chemicals to biomarkers which will be also explained later.

There are three elements in a standard LFA which are the recognition, reaction and signal transduction elements which are built in a paper strip containing five components [44]. The sample pad, conjugate pad, membrane, absorbent pad, and backing plate are the five standard components of the LFA [9, 44]. Basic demonstration of

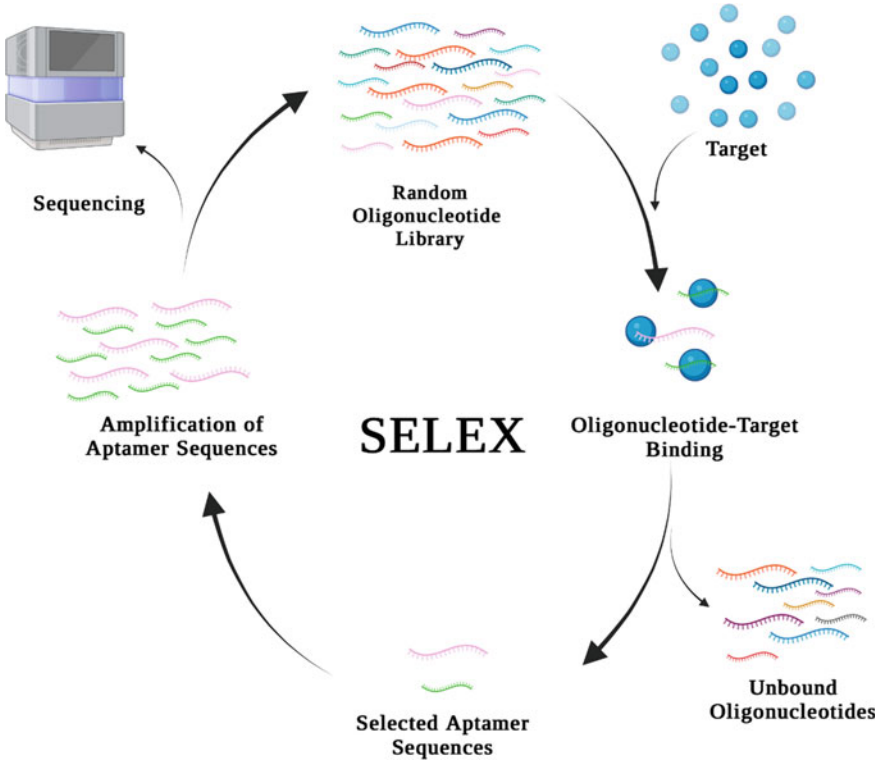

Fig. 1 SELEX process. SELEX is the method that enables finding specific aptamer sequences (ssDNA and RNA) from random oligonucleotide library, which can bind the target of interest. First, the target and oligonucleotide library are put in the same place in vitro which is incubated. The target and aptamer binding occur in this part. Later, it is washed to eliminate the unbound sequences. Then, the target is eluted from the mixture. So, only selected aptamer sequences will go under the amplification process. Amplification process is performed by RT-PCR technique. After that, sequencing is done for the identification of that specific different aptamer sequences

Table 1 A comparison between features of antibody and aptamer in the perspective of LFA. Adapted from [5, 9, 23, 29, 33, 51]

Features	Antibody	Aptamer
Development	In vivo Contamination is possible	In vitro Contamination is not a problem
Production time	Tedious (Weeks to months)	Effective through chemical synthesis (Days to weeks)
Cost	High	Low
Target	Limited	No limitation
Shelf life	Short Needs cold storage	Long Does not need any special storage state
Stability	Low Susceptible to changes in pH and temperature Aggregation is very likely	High Tolerance to changes in pH and temperature No or little aggregation
Modification	Challenging High-cost	Easy Low-cost
Batch-to-batch variation	High Varied	Low (Negligible) Uniform
Reusability	Poor	Good
Reproducibility	Low	High
Immobilization	Challenging	Easy

standard components of the LFA can be seen in Fig. 2. On the backing plate, all other compartments are assembled in place with proper overlapping.

While performing LFA, a sample is added to the sample pad, and it is directed to the conjugate pad [4]. It contains a recognition agent conjugated reporter molecules

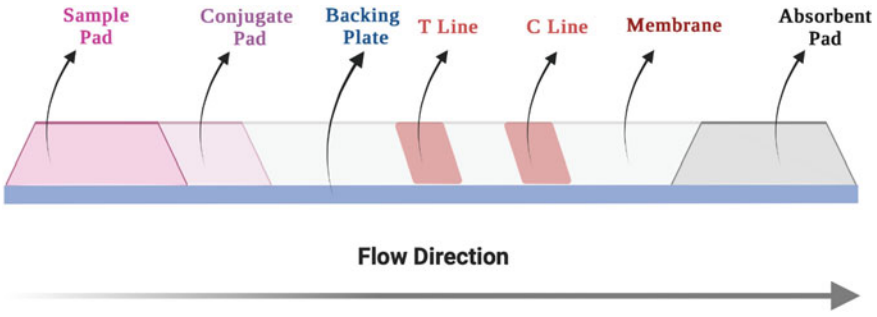

Fig. 2 Standard compartments of lateral flow assay (LFA). LFA consists of a sample pad, conjugate pad, membrane, absorbent pad, and backing plate which are shown in the figure. Additionally, the T-line (test line) and the C-line (control line) are specified

which are capable of hydrating quickly and discharge recognition agent [44]. If there is a target in the sample, a new complex is formed which includes a target, recognition agent, and reporter that will go toward the test and a control line found in the membrane [44]. For aptamer-based LFA, the control line contains a control aptamer. Besides, the test line contains detection aptamer or nothing which will differ in distinct formats of aptamer-based LFA [10]. Also, the absorbent pad makes sure the flow is from the sample pad to the membrane with no sample backflow [10].

Hence, we will cover different formats of aptamer-based LFA, the various interpretation of results, and its applications in diagnostic and other applications areas in this chapter.

2 Aptamers in Developing LFA

As mentioned in the introduction, aptamers can recognize several different substances with different sizes. In developing LFA devices, the use of aptamers is increasing as a recognition agent. Furthermore, aptamers are more advantageous when compared to antibodies for LFA and they can be used for substances that antibodies cannot be developed. For example, because no antibody could be found for the fungicide malachite green, its detection was dependent on expensive HPLC and liquid chromatography-mass spectrometry (LC–MS) assays. However, the development of a malachite green aptamer in 1999 enabled the introduction of an aptamer-based LFA for simplified malachite green residue detection [37].

Similarly, aptamer-based LFAs have been developed for the detection of non-immunogenic targets such as different substances like apple stem pitting virus (ASPV) and other compounds like organophosphorus pesticides. The broad application of aptamers makes them particularly valuable compared to antibodies [43].

3 Flexible LFA Design

The flexible LFA design is a testing method that can detect various target molecules by using materials of different shapes and sizes [44]. Aptamers that specifically bind to the target molecule are used in LFA tests and combined with a marker to identify the molecule's presence as it moves along the strip [31]. This adaptable design can be modified to suit various testing situations, and its portability enables rapid results.

To illustrate, the flexible LFA design used for COVID-19 diagnosis uses an aptamer that specifically binds to the Spike protein on the COVID-19 virus's surface [30]. During testing, a sample (like saliva or nasal discharge) is placed at one end of the LFA strip, and a section marked with the COVID-19 aptamer is present at the strip's beginning [30]. As the aptamer moves along the strip, it binds to Spike proteins

in the sample, and a marker substance indicates the presence of the protein-aptamer complex, resulting in a visible line at the strip's end [30]. This design enables quick and precise COVID-19 testing.

4 Multiplex LFA

The simultaneous evaluation of numerous analytes must be done to ensure efficient and accurate recognition of targets in a short amount of time. Simultaneous and rapid detection of different targets can be achieved at the same time with multiplex FLA.

There are three main types of multiplex LFAs: those that detect many targets on a single strip, those that detect several targets on different strips, and, more recently, those that combine a microarray and an LFA in a single device [29]. Moreover, in comparison to uniplex, multiplexing provides several advantages, such as faster evaluation due to the ability to identify many targets at once, identification of co-infections in the case of infectious diseases, higher sensitivity, and lower test costs [29].

5 Different Formats of Aptamer-Based LFA

Prior to the invention of aptamers, antibody-based LFA was already in use in clinical practice. Given the similarity between aptamers and antibodies in recognizing targets based on tertiary structure, the information obtained from designing antibody-based biosensors could be beneficial in developing aptamer-based LFA [44]. Over the past few decades, several approaches to aptamer-based LFA have been introduced, such as sandwich formats, competitive formats, and other novel techniques that rely on the structural and functional properties of aptamers, as outlined in the following section [32].

5.1 Sandwich Aptamer-Based LFA

5.1.1 Sandwich Aptamer-Based LFA Using Dual Aptamer

The dual-aptamer sandwich aptamer-based LFA provides more precise and selective results than the single-aptamer methods [28, 48]. Initially, the target molecule is trapped by a capture aptamer, and then a second aptamer binds to another area of the target molecule, resulting in a sandwich-like structure [41]. The specific binding between the two aptamers, which bind to distinct regions of the target molecule, helps eliminate false positive results and generate more precise results [44]. This technique is advantageous for identifying low-molecular-weight analytes.

5.1.2 Sandwich Aptamer-Based LFA Using a Combination of Aptamer and Antibody

The aptamer-antibody sandwich LFA method, which utilizes both antibodies and aptamers, offers several advantages over traditional sandwich LFAs that use only one type of molecule [42]. The technique involves capturing the target molecule using a primary capture antibody, which is immobilized on the conjugate pad [42]. Then, a detection aptamer specific to a different region of the target molecule is added to form a sandwich structure, and a reporter molecule conjugated with the detection aptamer generates a signal upon binding to the target molecule [32]. The sandwich complex is then captured by a secondary detection antibody on the test line, resulting in a color change.

Compared to conventional sandwich LFAs, the aptamer-antibody sandwich LFA method provides higher sensitivity and specificity due to aptamers' higher specificity and affinity to target molecules and antibodies' better stability and longer shelf life [44]. The method has been successfully applied to the detection of HIV-1 p24 antigen, where HIV-1 p24 antigens are loaded onto the sample pad, and a primary capture antibody with high specificity is immobilized on the conjugate pad. This method is widely used in clinical settings for the detection of HIV-1 p24 antigen, offering high sensitivity, specificity, and long shelf life [24].

5.1.3 Sandwich Aptamer-Based LFA Using Split Aptamers

The sandwich aptamer-based LFA is a diagnostic test that utilizes aptamers as recognition elements to detect specific molecules in a sample. In this assay, two different aptamers bind to different regions of the target molecule, with one serving as the capture aptamer and the other as the detection aptamer [15]. To enhance the sensitivity and specificity of the assay, split aptamers have been developed. Split aptamers are short DNA or RNA sequences that bind to specific regions of the target molecule but only form a complete aptamer upon hybridization [15]. In sandwich aptamer-based LFA using split aptamers, both the capture and detection aptamers are split into two fragments, and each fragment is attached to different locations on the lateral flow strip [32]. When the target molecule is present in the sample, it binds to the split aptamers, bringing the two halves of the capture aptamer together and forming a complete aptamer that can capture the detection aptamer [41]. This results in a sandwich complex and a signal at the test line.

The use of split aptamers in sandwich aptamer-based LFA has several advantages. Split aptamers allow for better control over the orientation and spacing of the aptamers on the lateral flow strip, which can improve the efficiency of target molecule capture [44]. Split aptamers also reduce the chance of non-specific binding by requiring both halves to bind to the target molecule before forming a complete aptamer [41]. Additionally, split aptamers can be easily modified to target different regions of the target molecule, enabling the development of highly specific and sensitive assays.

Sandwich aptamer-based LFA using split aptamers is a promising approach for the development of highly sensitive and specific diagnostic tests for various targets, such as small molecules, proteins, and even whole cells [8, 45]. The use of split aptamers provides an efficient and reliable method for target detection, making it a valuable tool for various diagnostic applications.

A smart point-of-care device that employs sandwich aptamer-based LFA with split aptamers for detecting the influenza virus has been developed [35]. This device, called the smart flu chip, integrates microfluidics, microheaters, and a smartphone app for quick and accurate diagnosis [35]. The split aptamers-based Sandwich Aptamer-based LFA was employed to detect two strains of influenza virus, H1N1 and H3N2, with high sensitivity and specificity [35]. The capture aptamers were divided into two fragments and were attached to separate locations on the lateral flow strip. Similarly, the detection aptamers were also split into two fragments and labeled with gold nanoparticles. When the target virus was present in the sample, it bound to the split aptamers, joining the two halves of the capture aptamer and creating a complete aptamer that could capture the gold-labeled detection aptamer [35]. This resulted in a sandwich complex and a signal at the test line, which was detected by a smartphone camera. The device also contains microheaters to regulate the reaction temperature, increasing the accuracy and effectiveness of the assay. The smartphone app analyzes the test results and provides real-time data on the existence and concentration of the target virus. This method provides a quick and precise technique for influenza virus detection, making it a valuable tool for point-of-care diagnosis and disease surveillance.

5.2 Competitive Aptamer-Based LFA

The competitive aptamer-based LFA is a diagnostic test that uses aptamers to detect specific molecules in a sample. In this test, a labeled aptamer competes with the sample analyte for binding to a limited amount of immobilized capture aptamer on a lateral flow strip [12]. The strip has a detection zone coated with capture molecules, and when the labeled aptamer binds to the capture aptamer on the strip, it generates a signal that can be read visually or with a portable reader [21]. The signal detected is inversely proportional to the concentration of the analyte in the sample, allowing the presence and amount of the target analyte in the sample to be determined by comparing the signal obtained from the sample with a known concentration of the analyte. Aptamer-based LFAs offer several advantages over traditional antibody-based assays, such as stability, low cost, and ease of development, and have been successfully used for detecting a variety of targets in various sample types [21, 44].

The competition between the target molecule and the complementary sequence for aptamer recognition is a dynamic process that depends on multiple factors and can be finely tuned to achieve optimal sensitivity and selectivity in a competitive assay [31].

Competitive aptamer-based LFA is a type of diagnostic test that uses aptamers as the recognition element to detect specific molecules in a sample. In a competitive aptamer-based LFA, a labeled aptamer competes with the sample analyte for binding to a limited amount of immobilized capture aptamer on a lateral flow strip [31]. The strip contains a detection zone coated with capture molecules, and when the labeled aptamer binds to the capture aptamer on the strip, it generates a signal that can be read visually or with a portable reader [21]. The level of signal detected is inversely proportional to the concentration of analyte in the sample. Thus, by comparing the signal obtained from the sample with the signal obtained from a known concentration of analyte, the presence and amount of the target analyte in the sample can be determined [44]. Aptamer-based LFAs have several advantages over traditional antibody-based assays, such as stability, low cost, and ease of development. They have been used for the detection of a wide range of targets, including small molecules, proteins, and even whole cells, in various sample types, such as blood, urine, and saliva [8].

6 Interpretation of the Results

The interpretation of the results varies for sandwich aptamer-based LFA and competitive aptamer-based LFA. If both lines (Test and Control lines) can be seen on sandwich aptamer-based LFA, the test result is positive confirming that the target of interest is present in the sample. Also, if only the C line is visible, the target of interest is not found in the sample in sandwich aptamer-based LFA which gives a negative test result. However, differently than sandwich aptamer-based LFA, the presence of both lines implies a negative test result in competitive aptamer-based LFA indicating that the target of interest is not present in the sample. In addition, if only the C line can be seen in competitive aptamer-based LFA, it gives a positive test result indicating that the target of interest is present in the sample. Besides, not having any lines or only having the T-line makes the test invalid. The basic demonstration of the aptamer-based LFA results for sandwich aptamer-based LFA, competitive aptamer-based LFA, and invalid test can be found in Fig. 3.

7 Applications of Aptamer-Based LFA

As previously mentioned in the text, numerous advantages of aptamer-based LFA enable its usage on a very broad range of different substances.

Aptamers can be effectively used in both diagnostic and therapeutic applications for microorganisms. For instance, label-free aptamer-based LFA is developed for detection of *Listeria monocytogenes* [40]. Also, another bacteria *Escherichia coli* O157:H7 can be identified in a sample [47]. Moreover, nervous necrosis virus [19] and avian influenza H5N2 whole virus particles can be detected by using aptamer-based LFA [14, 19].

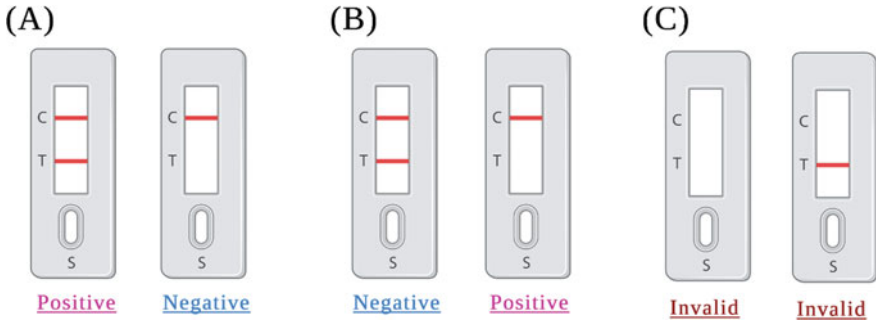

Fig. 3 Basic demonstration of aptamer-based LFA results. **a** Positive and negative results of sandwich aptamer-based LFA. **b** Positive and negative results of competitive aptamer-based LFA. **c** Invalid results of aptamer-based LFA

Most highlighted feature of aptamer is being able to detect small molecules that antibodies cannot do. For example, by using CRISPR/Cas12a-mediated aptamer lateral flow assay, detection of ATP can be done [17]. Also, ampicillin is another small molecule, and label-free ampicillin detection is possible by applying cross-recognition with aptamer and C-reactive protein [12].

Aptamer-based LFA can be developed for several chemicals. There are various examples for the detection of toxic molecules in a sample by aptamer-based LFAs. For instance, acetamiprid [22], patulin [39], zearalenone [46] and paraquat residue [20] are some of them. Furthermore, anticoagulant drug dabigatran etexilate, which is a chemical can be identified in the blood sample by aptamer-based LFA [1]. In addition, antibiotics in different samples can also be detected through aptamer-based LFA. Detection of kanamycin [18] and oxytetracycline [3] antibiotics are the two recent examples of it.

Some hormones also can be identified by aptamer-based LFA. For example, cortisol in sweat [7] and salivary [6] can be detected in the sample. Progesterone hormone is another example that can be identified through aptamer-based LFA [2].

Several protein biomarkers can be detected by aptamer-based LFA. Rapid and easy detection of biomarkers is very crucial. For example, in breast cancers, overexpression of HER2 is observed, and it can be detected by aptamer-based LFA [27]. With hybrid LFA, CXCL9 can be detected as a biomarker for antibody-mediated rejection (AMR) after kidney transplantation [34]. Additionally, antibody-free detection of biomarkers of SARS-CoV-2 with aptamer-based LFA is possible [50]. For multiplexed rapid detection of SARS-CoV-2 wild-type and SARS-CoV-2 omicron variant is done using aptamer-based LFA [49]. An acute inflammatory protein called C-reactive protein (CRP) is produced up to 1,000 times more often in infected or inflamed areas [36]. An aptamer-based LFA is developed for the detection of CRP [26]. For early diagnosis of periodontal disease, ODAM detection can be done by aptamer-based LFA [16].

Finally, the aptamer-based LFA can be used in many different areas from diagnostic to detection and can detect a variety of substances from proteins to cells.

8 Conclusion

As smart POC devices, LFAs are becoming increasingly more important and widespread due to increasing population and developing world. The need for easy, rapid, and low-cost diagnostic and detection methods growth. For instance, POC devices such as LFA, which were commercially available, are used for the detection of SARS-CoV-2 in the COVID-19 pandemic instead of PCR. Even though PCR is a gold standard, it requires well-trained people and special laboratory equipment.

In LFA, there are different recognition elements which are mentioned previously in the text. Usually, antibodies are frequently used for it. However, because of the drawbacks of antibodies, aptamer-based LFA are developed. Although antibodies are preferred more in general, using aptamers in LFA is more advantageous. For example, aptamers can be utilized for LFA with no target limitation, long shelf life, low-cost production, in vitro and rapid development, high stability, high reproducibility, and negligible batch-to-batch variations. With the benefits of aptamers in LFA, its detection range for different substances is very broad.

Antibody-based LFAs are commercially available, however, aptamer-based LFAs are still not, besides all studies done. Thus, with all those advantages, it needs to be commercialized and utilized as a smart POC device. To conclude, the aptamer-based LFA can be further developed and combined with new breakthrough technologies and methods for better diagnostic and detection.

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CRISPR-Based Point-of-Care Testing (POCT) Devices for Detection of Opportunistic Pathogens

Joydeep Chakraborty and Hironmoy Sarkar

Abstract Pathogens are microorganisms that can cause disease in humans or animals. There are many microorganisms that in general do not infect or act as commensals, but in a situation of compromised immunity of the host, these microorganisms cause diseases. These types of pathogens are called opportunistic pathogens; they are mainly bacteria or fungi, but sometimes, protozoa and viruses can also behave like opportunistic ones. Many times routine detection methods even with PCR or isothermal techniques are time-intensive and require sophisticated instruments, and highly proficient personnel, which makes this type of method not very useful in point-of-care treatment (POCT). A new emerging technology based on Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated nuclease (Cas) sensing (CRISPR-Cas) system is a powerful tool for anything from genome editing to disease-causing pathogen identification. This specific and sensitive system has a high potential to become one of the leading detection systems for POCT. This chapter summarizes the various methods of detection based on CRISPR-Cas system on different opportunistic pathogens.

Keywords CRISPR-Cas based detection system · Detection of opportunistic pathogens: bacteria · Protozoa · Fungus · Virus · Point-of-care testing (POCT) devices

1 Introduction

An opportunistic infection arises when pathogens like bacteria, fungi, protozoa, or viruses take advantage of unavailable previous opportunities. Numerous circumstances, including a compromised immune system, cancer treatment, altered microbiome, or violated integumentary barriers like penetrating trauma [63] might lead to

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these opportunities. In healthy hosts with a robust immune system, the majority of these opportunistic infections do not always cause sickness, but in certain situations, may function as commensals until the immune system's homeostasis is broken [64]. Commensal bacteria like *S. epidermidis*, *S. pneumoniae Corynebacterium spp.* are among the most prevalent opportunistic pathogens [27]. Furthermore, there are a few opportunistic infections that cause mild to moderate disease in healthy individuals and can cause severe diseases in those with compromised immune systems [30]. Infectious illnesses account for approximately 22% of all human fatalities, causing widespread public anguish and significant economic loss [39]. The quick identification and surveillance of infectious diseases now depend heavily on fast molecular detection. This can be useful to prevent further transmission and better management of treatment [6]. Point-of-care testing (POCT) will help in making decisions more rapidly, improving service effectiveness, and cutting costs in areas with scarce resources [46]. A wide range of tests for identifying signature regions of nucleic acid from pathogens has arisen [29], including methods based on PCR/qPCR, isothermal amplification, and next-generation sequencing [42, 54, 79]. However, these procedures are time-consuming, expensive, limited specificity, and need specialized, large, and expensive equipment with high-level technical competence making them incompatible with fast point-of-care testing thus prohibiting their widespread usage at POCT [67]. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated nuclease (Cas) sensing (CRISPR-Cas) is potentially a new nucleic acid detection technology [11]. Cas as an effector protein is employed in this approach and has extremely specific target sequence-recognition elements that are paired with a variety of ways for read-out (Fig. 1) for on-site POCT [66].

Bacteria and archaea naturally possess CRISPR-Cas systems, which serve as an adaptive defense mechanism for them against invasive foreign nucleic acids. [2, 23]. Bacterial CRISPR-Cas detects and destroys foreign genomic material mostly from viruses, which can be harmful to them [57]. These systems are typically directed by guide RNA (gRNA) which can also be termed CRISPR RNA (crRNA), that identifies the target and leads the effector proteins (Cas proteins) to identify and break foreign DNA sequence [24]. This mechanism functions in three stages: adaptation or spacer acquisition, crRNA processing, and interference [8]. The spacer acquisition step involves excising a very short segment of the DNA or RNA, called a protospacer, from the foreign nucleic acid during the first invasion. The protospacer is situated just a short distance upstream of a sequence called protospacer adjacent motif or PAM. Then the protospacer gets inserted into the CRISPR array or bacterial genome as a new spacer (Fig. 2) of the bacterial genomic region [18, 43]. crRNA biogenesis is the second step, which involves transcribing pre-CRISPR RNA (pre-crRNA) from the CRISPR array and cleaving it into small mature crRNAs using specific endoribonucleases [47]. Each crRNA has a complementary region of a spacer sequence from the CRISPR array. In the third phase, interference entails cleaving foreign DNA or RNA (in the subsequent invasion) that has a protospacer that has complementary base pairs with the spacer sequence in the crRNA [13]. crRNAs identify and bind with the protospacer region of foreign DNA or RNA to make a complex. That results in the cleavage of the complex by Cas nuclease [38].

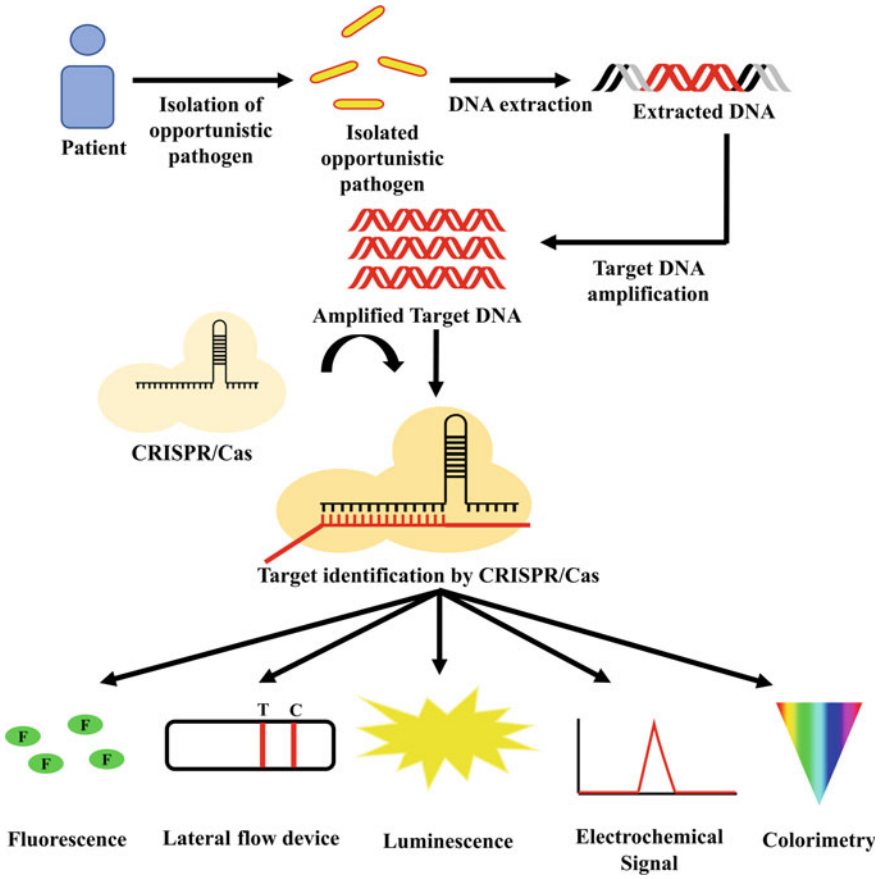

Fig. 1 Isolation and detection of opportunistic pathogens by CRISPR-Cas system. After the opportunistic pathogen was isolated from the patients, a specific target gene was isolated and amplified. The CRISPR-Cas system was then employed to detect different target genes by means of a variety of detection techniques, including fluorescence, lateral flow strips, luminescence, electrochemical signal, and colorimetry

Beyond the biological impact of these reprogrammable enzymatic technologies, CRISPR-Cas has sparked interest among scientists in several research fields where selectivity or specificity is essential to the operation, such as genome editing and the critical creation of innovative biosensing devices [77]. CRISPR-Cas systems have therefore been swiftly adapted for the construction of biosensors and biosensing systems including POCT devices for the detection of various pathogens. A POCT device based on CRISPR-Cas for opportunistic pathogens, which is neglected for many a time, will be an immense help for managing the disease and diagnostics to already overburdened health care system. This review will summarize the detection of different opportunistic pathogens like bacteria, fungi, protozoa, and viruses by CRISPR-based point-of-care testing (POCT) devices.

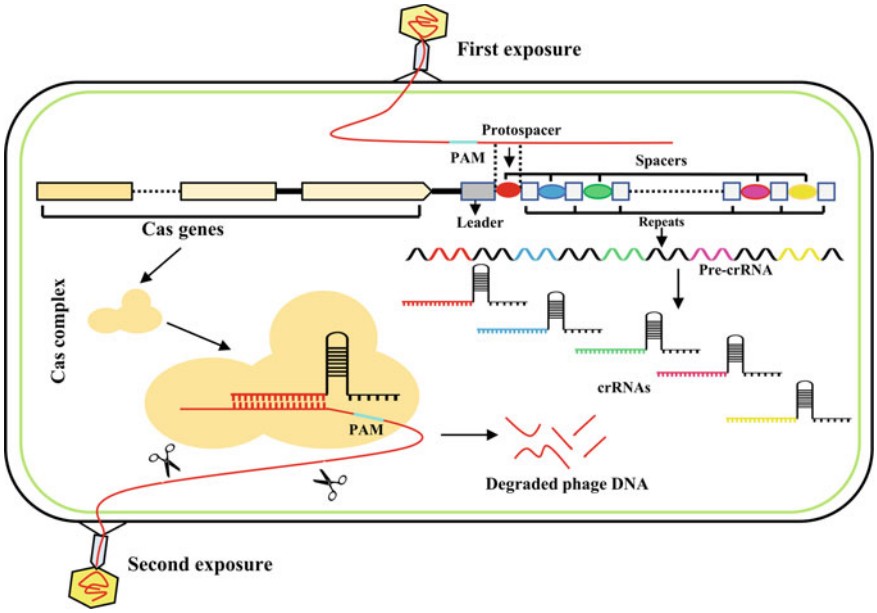

Fig. 2 The bacterial CRISPR-Cas system is composed of short repeats separated by spacers and surrounded by Cas genes. After the entry of a phage genome into the bacterial cell (First exposure), a protospacer, adjacent to PAM, is introduced as a new spacer in the bacterial CRISPR array. Pre-crRNAs are produced by transcription, and processed to produce mature crRNAs. Cas proteins and crRNAs made the effector complexes which act as guides (antisense) searching for the phage DNA, allowing the phage genome (Second exposure) to be degraded by the CRISPR-Cas system

2 CRISPR-Based POCT Detection on Opportunistic Bacteria

Opportunistic bacterial infection involves a large spectrum of microorganisms that can induce an extensive range of diseases. Multiple CRISPR-Cas POC systems are being developed for the majority of well-known opportunistic bacterial pathogens, including *M. tuberculosis, S. aureus, P. aeruginosa, S. enteritidis, S. typhimurium, and S. pyogenes* [8, 55, 62] (Table 1). The majority of targets are bacterial nucleic acid sequences [1, 28], while others utilized non-nucleic acid targets based on aptamers [58] and antibodies [7]. Nucleic acid detections mainly happen through PCR or isothermal methods of amplification. The amplification approach is not only time intensive but also risks airborne interference, therefore amplification-free techniques are becoming more prevalent nowadays. To increase sensitivity, the CRISPR-Cas systems are paired with target amplification systems [26]. To maximize the detection signal, most bacterial studies relied on the trans-cleavage capabilities of Cas enzymes to cleave reporter probes non-specifically [10]. Cas enzymes, Cas12a, Cas13a, and Cas9 are used for bacterial detection. Among the different detection methods, fluorescence sensing is the most widely used detection method. It has

advantages like specific background-free detection, which significantly reduces the background noise when contrasted to other optical technologies [37]. Electrochemical biosensors, lateral flow biosensors, and others such as gel electrophoresis, and colorimetric assays were found to be less regularly used in our study. Turnaround times were observed to range from 30 min [21] to 4 h [72, 73, 80]. The amplification-free techniques employing the dCas9 enzyme were credited with the quick turnaround time. CRISPR-depended DNA-FISH technology for Methicillin-resistant *S. aureus* (MRSA) can be detected with the quickest turnaround time of 30 min [21]. The limit of detection (LoD) of the CRISPR-Cas assays was assessed using three distinct concentrations: CFU per mL [1], copies per mL [53] and molarity [28]. Meanwhile, the vast majority of the research provided the limit of detection (LoD) in the form of CFU/mL, with eight studies reporting an LoD of 1 CFU/mL (Table 1).

3 Detection of Opportunistic Protozoa by CRISPR-Based POCT

A prominent intestinal protozoan parasite of zoonotic origin is *Cryptosporidium parvum* [51] and it is also a well-known opportunistic pathogen [14] that may induce cryptosporidiosis in animals and humans globally. It has been established that, after *Rotavirus*, cryptosporidiosis is the second leading cause of child fatalities [52, 61]. Prolonged cryptosporidiosis is seen in immuno-compromised people, though the illness is often self-limiting [50]. One of the most common varieties of *C. parvum* is the IId subtype family (SF) [68]. Some systems based on CRISPR-Cas were created with great reprogrammability, responsiveness, and precision. Integrated recombinase polymerase amplification (RPA) based Cas12a trans-cleavage system (termed ReCTC) was developed by researchers [76]. This technique may detect as low as a single copy of a cloned 60-kDa glycoprotein (GP60) gene of a *C. parvum* in a clinical fecal sample. For the detection of the output signals, it is possible to use a lateral flow strip (LFS) and the unaided eye to detect fluorescence under blue light for on-site identification. This ReCTC-based diagnostic method has demonstrated no cross-reaction with the other subtype families of *C. parvum* or any other prevalent intestinal protozoa [76]. This work developed a new and novel technique for the detection of *C. parvum* without the use of experienced personnel or costly instruments.

The researchers described an extremely sensitive CRISPR-Cas12a-powered immune-sensing technique for *Cryptosporidium* detection that combines antibody-based identification with CRISPR-Cas12a-based fluorescence signal amplification via an antibody-DNA conjugate [32]. This method detects complete *C. parvum* oocysts with a detection scale of a minimum of 6.25 to a maximum of 1600 oocysts/mL and the highest responsiveness of one oocyst per sample. This study demonstrates the use of a novel CRISPR-Cas-based biosensing technology for significant assessment of water potability and detecting entire pathogens.

Table 1 List of CRISPR-Cas systems used for the detection of different opportunistic pathogens like bacteria, fungi, protozoa, and virus

No	CRISPR-Cas enzymes	Methods	Bacteria	Trans cutting activity	Reporter probes	Amplification	Detection methods	Required instruments	Targets	Assay time (min)	LOD/detection range	References
1	Cas12a	CRISPR-MTB	*M. tuberculosis H37Ra*	✓	ssDNA	RPA	F	qPCR machine	IS6110	90	50 CFU/mL	[1]
2	AacCas12b	TB-QUICK	*M. tuberculosis H37Ra*	✓	ssDNA	LAMP	F	Real-time PCR	IS6111	120	1.3 copy/μl	[53]
3	LbCas12a	NR	*M. tuberculosis*	✓	ssDNA	RPA	F	Real-time PCR	IS1081	240	4.48 fmol/L	[74]
4	LbaCas12a	NR	*M. tuberculosis H37Rv*	✓	ssDNA	RPA	Gel electrophoresis	No	IS6110	40	1 copy/μL	[32]
5	FnCas12a	NR	*M. abscessus*	✓	ssDNA	PCR	F	Thermal cycler & fluorescence reader	rpoB & erm (41)	~ 240	NR	[72]
6	dCas9	NR	*S. aureus, A. baumannii* & *K. pneumoniae*	×	Raman reporter: MB	NO	SERS	Spectrometer	spa, pgi & uge	60	14.1 fM, 9.7 fM & 8.1 fM	[28]
7	LbCas12a	NR	*S. aureus*	✓	ssDNA	PCR	Elementary OR AND INHIBIT logic gates	Microplate reader	*femA*	120	10^3 CFU/mL	[49]
8	Cas12a	E-Si CRISPR	*S. aureus* (MRSA)	✓	ssDNA	No	EB	SPGE & impedance analyzer	*mecA*	90	3.5 fM	[62]
9	dCas9	DNA-FISH	*S. aureus* (MRSA)	×	SYBR Green I	No	F	Fluorescence spectrophotometer	*mecA*	30	10 CFU/mL	[21]

(continued)

Table 1 (continued)

No	CRISPR-Cas enzymes	Methods	Bacteria	Trans cutting activity	Reporter probes	Amplification	Detection methods	Required instruments	Targets	Assay time (min)	LOD/detection range	References
10	LwCas13a	CCB detection	*S. aureus*	✓	ssRNA	PCR & reverse transcription	F	Microplate reader	*nuc*	240	1 CFU/mL	[80]
11	Cas12a	NR	*S. aureus* (MRSA)	✓	ssDNA	RCA	F	Fluorescence spectroscopy	Aptamers (protein A & PBP2a)	~75	NR	[74]
12	LbaCas12a	NR	*E. coli* & *S. aureus*	✓	ssDNA	PCR	EB	Thermocycler & impedance analyser	mdh & nuc	90	3 nM	[4]
13	LbCas12a	RPACas12a-FS	*E. coli, L. monocytogenes, S. aureus* & *V. parahaemolyticus*	✓	ssDNA	RPA	F	Handelled fluorometer	Genomic DNA	45	10 copies (*E. coli, L. monocytogenes, S. aureus*) & 100 copies (*V. parahaemolyticus*)	[36]
14	LbaCas12a	CIA	*P. aeruginosa*	✓	ssDNA	LAMP	LFB	No	Acetyl transferase	50	1 CFU/mL	[44]
15	Cas12f(a)	Cas-TSPE	*E. coli, E. typhi, P. aeruginosa, S. aureus, S. pyogenes* & *E. faecalis*	✓	ssDNA	PCR	F	Fluorescence reader	Variable regions (V3) of 16S rRNA	~210	1 CFU/mL (*S. pyogenes*)	[59]
16	LbuCas13a	APC-Cas	*S. enteritidis*	✓	ssRNA	Reverse transcription & SDA	F	Real-time PCR	Aptamer (SE-3) for whole *S. enteritidis*	140	1 CFU	[58]

(continued)

Table 1 (continued)

No	CRISPR-Cas enzymes	Methods	Bacteria	Trans cutting activity	Reporter probes	Amplification	Detection methods	Required instruments	Targets	Assay time (min)	LOD/detection range	References
17	LbaCas12a	BCA–RPA–Cas12a	*S. typhimurium*	✓	ssDNA	RPA	F	Blue light	Antibodies for *S. typhimurium*	60	1 CFU/mL	[7]
18	Cas12a	NR	*S. typhimurium*	✓	ssDNA	LAMP	F	Milk warmer & UV lamp	gDNA	60	800 CFU/mL	[71]
19	Cas9 Cis (dsDNA)	Cas9nAR	*S. typhimurium*	×	SYBR Green I	Cas9nAR	F	Fluorescence reader	*invA*	60	1 copy/10μL	[69]
20	LbCas12a	NR	*Salmonella spp.*	TMB-H_2O_2 reaction (Blue to yellow color change)	Gquadruplex hemin (DNAzyme)	RPA	Colorimetric analysis	Smartphone (Color Picker APP)	*invA*	180	1 CFU/mL	[75]
21	Cas9 Cis (dsDNA)	NR	*S. typhimurium* & *E. coli*	×	ssDNA	Cas9nAR	LFB	Test strip reader	UidA & invA	180	100 CFU/mL	[70]
22	LwCas13a	PCF detection	*Salmonella spp.*	✓	ssRNA	PCR & reverse transcription	F	Fluorescence reader & Thermal cycler	*invA*	120	10 CFU/mL	[17]
23	LbCas12a	NR	*Salmonella spp.*	✓	ssDNA	PCR	F	NIR irradiator & colorimeter	*invA*	90	1 CFU/mL	[40]

(continued)

Table 1 (continued)

No	CRISPR-Cas enzymes	Methods	Bacteria	Trans cutting activity	Reporter probes	Amplification	Detection methods	Required instruments	Targets	Assay time (min)	LOD/detection range	References
24	Cas14a1	CMP	*S. pyogenes & E. typhi*	✓	ssDNA	Reverse-transcription & APCR	F	Fluorescence plate reader	16S rRNA gene V3 hypervariable region	NR	10^3 & 10^4 CFU/mL	[19]
25	FnCas12a	ReCTC	*C. parvum IId SF*	✓	ssDNA	Nested PCR	F	LFS	GP60	~60	10 oocysts per gram (OPG)	[76]
26	CRISPR-Cas12a	NR	*C. parvum*	✓	ssDNA	No	F	Fluorescence plate reader	Whole cell	~150	6.25–1600 oocysts/mL	[34]
27	LbCas12a	RPA-CRISPR-Cas12a	*T. gondii*	✓	ssDNA	RPA	F	Fluorometer or lateral-flow strip	B1	~60	3.3 copies/μL	[31]
28	Cas12a	RAA-Cas12a-Tg	*Toxoplasma gondii*	✓	dsDNA	RAA	F	Fluorescence reader	529 bp-RE	~60	1 fM	[41]
29	LwCas13a	RAA-Cas13a-LFD	*T. gondii*	✓	ssRNA	RAA	F	LFD (Naked eye/F)	530 bp-RE	120	1×10^{-6} ng/μL	[78]
30	LwCas13a	NR	*A. fumigatus*	✓	ssRNA	RPA	F	Fluorescence detector	Internal transcribed spacer (ITS) region	NR	3 copies/L	[35]

(continued)

Table 1 (continued)

No	CRISPR-Cas enzymes	Methods	Bacteria	Trans cutting activity	Reporter probes	Amplification	Detection methods	Required instruments	Targets	Assay time (min)	LOD/detection range	References
31	Cas12a	μReaCH-PAD	*Candida or Aspergillus*	✓	ssDNA	RAA	Microfluidic chip (paper based)	Visual readout	18 s rRNA fragments	110	10 CFU/mL (Visual),4.90 and 4.13 CFU/mL (Calculated)	[22]
32	LwaCas13a	CRISPR–Cas13 SHERLOCK	*CMV*	✓	ssRNA	RPA	F	Fluorescence reader	Conserved region in the CMV UL54 gene	60	0.6 aM	[25]

A common opportunistic illness brought on by *Toxoplasma gondii* puts human health in peril and has a significant negative economic impact on the animal industry. *T. gondii* is an obligate intracellular parasitic protozoan that infects a variety of vertebrate hosts, including humans. *T. gondii*, an Apicomplexa phylum member, causes toxoplasmosis, an opportunistic infection in humans with compromised immune systems [16], as well as congenital illness in infected babies [20]. Researchers used RPA and a CRISPR-Cas12a system to create a compact one-tube detection of *T. gondii* [31]. *T. gondii* may be effectively extracted from low-concentration samples using a microfiber filter device made of glass. The designed RPA-CRISPR-Cas12a system is highly selective for the *T. gondii* B1 gene. A fluorometer or lateral-flow strip can be used to analyze the visual signal. The detection limit was reported to be 3.3 copies/L. This technique may be used to quickly detect *T. gondii* in extremely contaminated landfill leachate.

In another work, a novel technique called RAA-Cas12a-Tg was designed by the combined employment of recombinase-aided amplification (RAA) with CRISPR-Cas12a [40]. The 529 bp repeat element was considered as the target element for this system in order to identify *T. gondii* oocysts. A portable fluorescence reader may be used to detect signal output. This system's sensitivity was reported to be as low as 1 fM with good specificity. This system effectively detected *T. gondii* in less than one hour while being more responsive than the conventional PCR system-based technologies.

To identify the *T. gondii* 529 bp repeat element, researchers created a quick visual detection technique that combined recombinase-aided amplification (RAA) followed by a lateral flow dipstick (LFD) device with CRISPR-Cas13a fluorescence (RAA-Cas13a-LFD) [78]. The RAACas13a-LFD test was carried out within 2 h in an incubation block at 37 °C, and the result may be observed with the naked eye using LFD. The limit of detection of the RAA-Cas13a-LFD was 1×10^{-6} ng/μL and there was no observed cross-reactivity seen with human blood DNA or other significant parasites. All of the aforementioned systems listed above demonstrated rapidity, sturdiness, and on-site capabilities for detecting nucleic acids, making them viable tools for potential deployments in far-off places (Table 1).

4 Detection by CRISPR-Based POCT on Opportunistic Fungus

Invasive fungus (IF) is now a major concern for human health [5]. *Aspergillus fumigatus* represents the most prevalent *Aspergillus* species isolated in people, and it is associated with Invasive Aspergillosis (IA) [12]. In immunocompromised individuals, it is also an opportunistic human pathogen that can cause potentially fatal invasive infections and is linked to severe asthma and sinusitis [48]. Culture-based diagnostic tests of invasive fungus, which is among the most generally used medical screening approach, has drawbacks such as lengthy and complex operation, and the

requirement for experienced employees, which can postpone the detection of the concerned infection. The sensitivity of the lower respiratory tract fungal cultures in diagnosing invasive pulmonary aspergillosis (IPA) is likewise relatively poor [60]. As a result, the adoption of CRISPR-Cas-based technology as an efficient and accurate diagnostic tool for infectious illnesses shows considerable potential. However, there isn't a lot of pertinent information available right now on the diagnostic strategy of an *A. fumigatus* infection using CRISPR-Cas technology.

Zhengtu Li devised an extremely sensitive and specific approach for the consistent and quick recognition of *A. fumigatus* utilizing the CRISPR-Cas13a system [35]. A conserved internal transcribed spacer region or ITS of *A. fumigatus* was employed in this work to develop crRNA and a specific RPA primer sequence conjugated with T7 promoter for the CRISPR assay. The CRISPR assay included an RPA step followed by a Cas13a detection phase. During the detection phase, the final reaction was continuously kept at 37 °C in order to observe for an increased fluorescence signal. This technique's sensitivity was reported to be 3 copies/L.

In another work, Di Huang et al. described the development of a microfluidic ruler-readout and CRISPR Cas12a-re-joined hydrogel-integrated paper-based analytical device (ReaCH-PAD) for visual and quantifiable point-of-care detection of invasive fungus (IF) [22]. As a target, conserved 18 s rRNA fragments from *Candida* or *Aspergillus* were integrated with PAM sites for Cas12a recognition. This device used a CRISPR Cas12a employed target identification system, a DNA hydrogel coupled to an enzymatic cascade for the amplification of the detection signal, and microfluidic chips (paper-based) for visual output. *Aspergillus* and *Candida* can be detected visually by unassisted eyes using ReaCH-PAD at concentrations as low as 10 CFU/mL. The calculated limit of detection for 1 mL samples was 4.90 and 4.13 CFU/mL, respectively. When compared to qRT-PCR, quantitative detection values on a scale of 10–10^4 CFU/mL may be achieved with excellent selectivity and precision (Table 1).

5 Detection of Virus by CRISPR-Based POCT

A major global cause of morbidity and a serious threat to humanity today is viral infections. Although numerous viral diagnostic techniques and antiviral treatments have been created over time, certain viral illnesses are still difficult to treat [65]. CRISPR-based diagnostic techniques have attracted the most interest in the field of viral infection [3]. There have been several reports on field deployable CRISPR-based diagnostic platforms for viruses, including SHERLOCK, which can detect Zika virus (ZIKV) and dengue virus (DENV) [45], DETECTR, which has been used to identify different HPV strains [9], and HOLMES, which can detect viruses (DNA and RNA) and also has great power of strain differentiation with tremendous sensitivity [33] but there are very few reports regarding the detection of opportunistic viral infections. The most prevalent viral opportunistic infections in people with acquired immunodeficiency virus syndrome (AIDS) are caused by Cytomegalovirus

(CMV) and BK polyomavirus (BKV). Clinical CMV illness has been identified in up to 40% of people with advanced HIV infection [15, 56]. These are two prevalent opportunistic viruses that are extremely important for kidney transplant recipients and other immunologically compromised individuals. Michael M. Kaminski created a CRISPR-based diagnostic method for Cytomegalovirus (CMV) and BK polyomavirus (BKV) [25]. They obtained DNA from the infected patients to screen for active infections of BKV and CMV. Following that, a modified SHERLOCK methodology was used to identify BKV and CMV. Highly conserved sections of the BKV and CMV genome (conserved area in the UL54 gene) were amplified utilizing isothermal recombinase polymerase amplification (RPA). Then LwaCas13a was directed to the target sequence using a crRNA corresponding to 28 nucleotides of the RPA amplified product. When the target was detected, Cas13 was activated, resulting in the collateral cleavage of an oligonucleotide. Fluorescence signals can then be detected, which is directly correlated with the initial load of the concerned target pathogen (Table 1).

6 Conclusion

Nowadays, opportunistic infections are a major concern for human health. They are caused by a diverse spectrum of pathogens and can result in a wide variety of diseases. Both extensive public distress and considerable economic loss are being brought on by it. Fast molecular detection has become essential for locating and tracking these diseases, as well as providing up-to-date disease information to speed up treatment. In the current developing landscape of opportunistic infections, the CRISPR-Cas-based pathogen recognition technologies are a very potent and sophisticated approach with high precision and responsiveness and could be of significant importance in early diagnosis. In this study, we discovered several methods for detecting opportunistic infections caused by bacteria, fungi, parasites, or viruses that were established based on CRISPR-Cas-mediated systems. The devices demonstrated a high level of specificity and sensitivity. They are portable and produce visible results in a very short period, making them suitable for use in potentially field-deployable POCT devices.

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Microfluidic Chips as Point-of-Care Testing for Develop Diagnostic Microdevices

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Abstract Rapid, specific, and reliable diagnostic tests in portable, easy-to-apply systems are of great importance for medical diagnosis, especially in emergencies such as pandemic outbreaks or in environments where resources are scarce. Point-of-care testing platforms are ideal for these purposes, providing fast and timely accurate results. Interest in laboratory-on-a-chip devices has grown rapidly in recent years. Innovative microfluidic devices that have gone through the technology development process have demonstrated the potential to perform unimaginable analyzes using traditional techniques. Advances in the microfluidics chip field have sparked innovative upheavals in various biomedical fields, such as single-cell detection, diagnostic methods, and micro- and nano-size-product manufacturing. Microfluidic chips currently play an important role in multiple biological technologies. Microfluidics have been shown to offer a number of benefits over existing conventional methods, thanks to improved controllability and precision. In this chapter, the authors discussed how point-of-care tests, developed by the integration of numerous nanomaterials into microfluidic chips, play an active role in the diagnosis and diagnosis of many diseases and their potential biomedical applications.

Keywords Microfluidic chips · Point-of-care tests · Diagnosis · Nanomaterials

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1 Introduction

Early detection of infectious diseases plays a critical role in the correct and effective treatment of diseases. The COVID-19 pandemic has shown that it is very important to detect the infectious agent early at the beginning of the infection in order to quickly control the spread of the disease. Early diagnosis and timely treatment are critical not only for infection but also for other types of cancer and serious diseases. Early diagnosis can increase response rates to treatment and reduce treatment costs. Among the tests performed for this purpose, serology, virology, and imaging techniques are among the most preferred medical diagnostic techniques [66].

Serological methods are based on the detection of changes in protein biomarkers related to diseases in the human body. Virological methods consist of techniques for detecting viruses that infect the body, while imaging methods are used to test for structural changes in organs, tissues, and structures in the human body. Expensive test devices used for analysis are available in research laboratories in clinics and require expert personnel due to the complexity of the analysis stages of these methods. Because of the highly costly, long-lasting, labor-intensive nature of detection methods, they may not be sufficiently applicable techniques for the early diagnosis of diseases [75].

Lack of adequate medical facilities delays disease detection, especially for people in underdeveloped countries. Routine medical tests are also not possible for patients living in this region In developing countries such as South-East Asia and Africa, medical facilities such as hospitals, which should be widespread, are very inadequate. Therefore, it is very difficult for countries with inadequate medical resources and health systems to combat bacterial or viral diseases [34]. If a pathogent is present and spread in these regions, the infectious agent may not be detected and isolated in time. In such cases, the infection carries the risk of spreading epidemically. The COVID-19 pandemic is a good example of this issue. The infection agent in a single region has shown a serious spread that can affect all countries of the world as a result of international travel [14].

In countries with inadequate health systems, there are no techniques that enable sensitive rapid detection of diseases in the clinic. Even in rural areas of developed countries, disease prevention services and health screening can be very inadequate [60]. Therefore, it is of great importance to produce inexpensive, portable diagnostic equipment for home self-testing or field testing. When such devices are developed, the workload of existing health systems in underdeveloped regions can be reduced, early diagnosis can be made, and overuse of medical resources can be reduced. Microfluidic technologies have been developed and fabricated in recent years to solve these problems and offer innovative perspectives. The chips developed within microfluidic technologies are small in size, require few reagents, and easy to carry to diagnose the disease. The biggest advantage of microfluidic chips is that they perform detection in a short time compared to conventional detection devices and kits. For this reason, microfluidic chips are technological innovations that can provide fast and effective results in situations where the health system and medical resources are

insufficient, such as in underdeveloped regions. Microfluidic technologies are used in the fields of laboratory tests [57, 65], medical diagnosis [3], and cell analysis due to their small size, short process requirements and portability. The development of microfluidic chips has also contributed significantly to the advancement of point-of-care (POC) test technologies. POC test (POCT) is an analytical test that offers medical diagnosis to patients even in limited and under-resourced healthcare systems [54]. The development and application of POCTs are promising for countries in developing regions in need of medical diagnostic tests [44].

This book chapter includes studies on the necessity and development of technologies for the rapid diagnosis of diseases at an early stage that can be offered to the service of underdeveloped countries. Firstly, we will describe microfluidic chip technologies and introduce their types and advantages. Then, we will then introduce microfluidic POC tests for the early detection of various diseases and describe their current applications.

2 Microfluidic Tests

2.1 Introduction

Microfluidic device is a portable, analyte capture and identification system that can perform sample detection steps. In these innovative systems, reagents and samples are used in very low quantities. In addition, the efficiency and analysis speed of the system is very high. Furthermore, the analysis process can be automated to eliminate human error. Devices developed with microfluidic technology are frequently preferred in physics, biology, chemistry, biomedical sciences, and engineering. Devices manufactured with microfluidic technology are known as portable devices. Because the analytic process is carried out in small-sized devices that are completely portable [64]. The use of small amounts of reagents for analysis in microfluidic devices offers advantages for under-resourced regions in harsh conditions. The small size and small amount of reagent consumption significantly reduces the cost of the analysis [25]. POCTs are portable devices that enable analysis and detection in various regions and for various purposes outside of clinical laboratories [55].

POCT technology can be used not only for humans but also for the detection of animal diseases. In a study, Pascual-Garrigos and coworkers developed the loop-mediated isothermal amplification (LAMP) assay. The purpose of the test is early stage diagnosis of respiratory system diseases [56].

Microfluidic technology has made a significant contribution to the application of POC tests in disease diagnostic systems. Microfluidic devices provide fast results and high sensitivity. Therefore, microfluidic devices integrated with POC tests are the cheapest and easily portable devices that can be preferred to provide rapid and sensitive detection [62].

2.2 Benefits of Microfluidic Tests in Clinic Laboratory

Identifying biomarkers plays a critical role in disease diagnosis. The most preferred method for diagnosis is the ELISA test. The ELISA assay produces measurable signals in the presence of a sufficient amount of analyte. ELISA test uses different enzymes such as horseradish oxidase, alkaline enzyme, and β-galactosidase. Substrates are used to react with the enzymes and produce colorimetric results [20]. ELISA is the most preferred technique for detecting protein-based molecules. Because it is very sensitive in detecting the presence of protein. Detect proteins as well as many pathogens, including viruses and bacteria [70]. ELISA has been used to identify and quantify many viruses such as coronavirus [1, 6], Zika virus [52], dengue virus [49], influenza virus [59]. Conventional ELISA needs to be improved to provide the high sensitivity required for protein identification and quantification [29].

Mass spectrometry (MS) can detect different biomarkers such as proteins [2]. Integration of microfluidic chips into the MS (μchip-MS) has the potential to meet the needs of clinicians. Thus, new methods may be revealed in monitoring stages of diseases other than infectious diseases including cancer, diabetes, and other chronic diseases [26, 50, 58]. The combination of MS and a microfluidic chip platform offers an innovative perspective for microfluidic chips. Compared to conventional immunoassay tests and techniques, μchip-MS has been shown to have a higher specificity and sensitivity in analysis, and also provides faster results with less labour. Surface plasmon resonance (SPR) is another optical detection method. SPR detection method is used in virus detection and detection of cancer biomarkers due to its many reasons for preference such as high accuracy, low production cost, and sensitivity [5, 47]. Liu et al. used microfluidic technologies and nanoparticles together to detect the target protein by the SPR technique. Since the results were better than the method with SPR alone [40], it shows that combining microfluidic technologies with existing technologies will lead to advantageous results.

Many traditional methods have started to be integrated with microfluidic POC devices. Especially for patients in underdeveloped countries, these integrated devices can detect proteins in the serum and provide excellent analysis results [38, 39].

2.3 Equipment Varieties for Microfluidic Tests

The basic components of the first microfluidic system developed were chemical etching technology and photolithography [68]. Subsequently, microfluidic devices made of polydimethylsiloxane (PDMS) materials were also developed and today, most of the microfluidic chips are made of PDMS polymer [46].

In recent years, paper-based technologies and three-dimensional (3D) printing methods has been used to reduce production cost [9, 77]. These materials can be used for POC analysis, especially in undeveloped regions. One of the best examples of combining POC technologies with microfluidics is mobile sensors where microfluidic systems are integrated into smartphone applications [79].

2.3.1 Microfluidic Devices Produced by 3D Printing Techniques

The development of 3D printers has directly influenced the advancement of microfluidics [7]. These devices work automatically and are not dependent on a person, thus eliminating the need for human resources required to produce PDMS-based microfluidics [8]. Moreover, 3D printing techniques provide serious high potential and prototypes can be developed with this technique. Rapid production can increase the efficiency and frequency of experiments and thus, accelerate the commercialization of experimental methods [15, 71].

Microfluidic POCT chips produced with 3D printers have been used in experimental studies. Song and coworkers develop a new approach for sensitive detection of viruses in the platform developed with microfluidic technology. The box prepared for detection was produced by 3D printing technology and only a saliva sample was used to detect the Zika virus [63]. Furthermore, Kadimisetty and coworkers designed a low-cost microfluidic POC test based on the nucleic acid amplification method for the diagnosis of infection [32].

Thanks to three-dimensional printing technology, the commercialization of microfluidic POCT techniques has gained great speed. With 3D printing technology, it has become very easy to rapidly manufacture and produce microfluidic equipment. Therefore, 3D-printed microfluidic POC tests offer a serious advantage for use in undeveloped countries.

2.3.2 Analyzing Microfluidic Chips with Smartphones

Since the early 21st century, the continuous development of microelectronics has resulted in the production of smartphones. Smartphones have the potential to be an alternative to computers for data collection and processing in underdeveloped countries [24, 74, 76]. As a result of these developments, a new generation of mobile sensing techniques has emerged by combining smartphones with microfluidic devices. Combinations of microfluidic devices and smartphones are very useful for regions with inadequate and weak healthcare systems.

Researchers have developed a paper-based microfluidic test to detect Zika virus through reverse transcription cycle-mediated isothermal amplification (RT-LAMP) technique. In this study, ZIKV RNA triggers a color change in the microfluidic system and the results can be obtained within minutes and can be analysis with a smartphone [31]. In another study, Jalal and coworkers fabricated a microfluidic chip consisting of polycarbonate (PC) plastic material and reagent paper to detect chemical molecules in human urine. Using a smartphone, the resulting colorimetric results are recorded with the phone camera [30]. The systems in which microfluidic chip and smartphones are integrated are easy to use. It gives reliable results without requiring specialized personnel. These integrated systems are a promising technology.

3 Microfluidic Tests for POC Diagnosis of Infectious Diseases

3.1 Introduction

Coronavirus diseases affects countries all over the world, causing widespread deaths and straining national economies [35]. Early diagnosis of COVID-19 and timely and correct treatment of patients is one of the most important measures to be taken. Accurate and rapid detection is critical in the fight against infection. In this direction, ELISA, RT-PCR, colloidal gold immunochromatographic assay are among serological methods [16]. However, ELISA and RT-PCR may limit their use in less developed countries. Consequently, POCTs offer an innovative approach in the production of inexpensive and rapid tests for respiratory system diseases.

Compared to conventional tests, microfluidic devices can measure biomarkers and antibodies accurately and sensitively. Microfluidic technologies can be integrated with other conventional methods used to provide efficient test results. Microfluidic chips therefore have promising potential for the detection of SARS-CoV-2.

3.2 Applications for Diagnosis of Infectious Diseases

Immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies are revealed and play an active role in the defense against viral disease agents. Therefore, the progression and treatment of infectious diseases are determined by the detection of antibodies. In a study, Lin and colleagues developed a diagnostic kit for COVID-19 diagnosis by integrating a diagnostic microchip and a POC test that can detect IgM, IgG, and other biomarkers with portable fluorescent detectors [39].

However, microfluidic devices have shortcomings in diagnosis of COVID-19, such as requiring a long incubation time, and further development is needed for rapid detection of SARS-CoV-2 antibodies [67].

Although nasopharyngeal specimens are often preferred for diagnosis of COVID-19, human saliva can also be usable as a test sample [69]. Patients have the advantage of being able to collect their own samples and there are studies showing that saliva samples are also suitable for detection. Wang and coworkers developed a POC test using RT-LAMP technique for diagnosis of COVID-19 in human saliva. This test provides colorimetric results, although the assay only requires a heat source. Due to this feature, it can be used in countries with limited healthcare [73].

4 Microfluidic Tests for POC Diagnosis of CVDs

4.1 Introduction

As reported by the World Health Organization, CVDs cause 17.9 million deaths worldwide each year [28]. High CVD rates also show that health services in developing regions are very inadequate compared to developed regions [4].

CVD, also known as cardiovascular system-related diseases including hypo- and hypertension, coronary heart disease, and cerebrovascular disease (stroke) [19]. In recent studies, various biomarkers have been used for the detection of CVDs. Early stage diagnosis of CVD is the main factor that reduces treatment costs and mortality. POC tests have the potential to detect CVD biomarkers rapidly and sensitively [13].

4.2 Applications for Diagnosis of CVDs

There are risks that using a single biomarker to diagnose CVD may lead to misdiagnosis as it may be associated with other diseases. Therefore, it is critical to detect multiple CVD biomarkers at the same time for accurate and sensitive diagnosis. Thus, more reliable, high-specificity results can be obtained. In addition, these techniques reduce the cost and time of analysis [27]. Various platforms have been designed that can detect multiple CVD biomarkers simultaneously [53]. Clinicians advocate the use of these techniques because simultaneous analysis of multiple biomarkers provides more comprehensive and accurate results [18]. Most microfluidic POC tests capable of detecting multiple biomarkers are currently in use [43].

AMI is the most of dangerous diseases. For accurate and timely detection of AMI, multiple biomarkers need to be detected simultaneously [23, 78]. In a study, Li and coworkers developed a 3D printing paper-based microfluidic test (μPAD) that detects numerous biomarkers with three sensing zones. The μPAD can simultaneously measure cTnI, H-FABP, and copeptin using chemiluminescence (CL) emissions. The device has the potential to greatly facilitate early stage AMI diagnosis. Figure 1 shows the design of a 3D μPAD [36].

In another study, Boonkaew and coworkers developed a POC test for the detection of three different CVD biomarkers simultaneously, procalcitonin marker, cTnI, and C-reactive protein. This microfluidic device contains multiple working electrodes and multiple detection sites that can detect different CVD biomarkers in a single human sample [10].

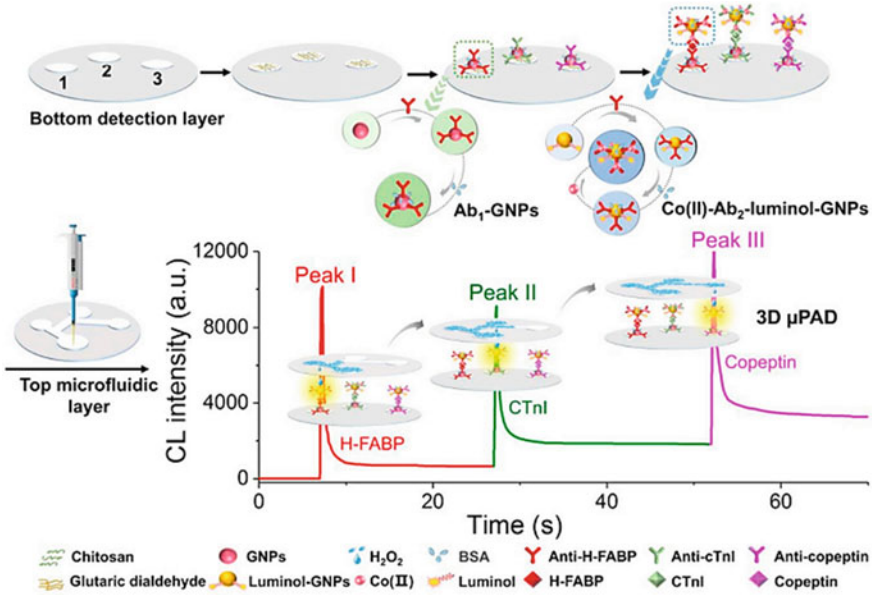

Fig. 1 Schematically illustration for the fabrication of the 3D μPAD for multiplexed CL immunoassay of H-FABP, cTnI and copeptin. Reprinted with permission from [36]. Copyright 2020 Elsevier

5 Microfluidic Tests for POC Diagnosis of Tumors

5.1 Introduction

Cancer diseases are caused by cells that multiply uncontrollably. Cancer disease that is difficult to diagnose, treatment, and follow-up [41]. Cancer is becoming increasingly common and mortality rates are increasing day by day. Cancers of the breast, lung, stomach, and prostate are among the most common varieties of cancer [12]. Symptoms of cancer in the early stage may not be recognized. However, early stage detection of cancer is critical for effective treatment [72]. Current conventional diagnostic techniques for cancer diagnosis such as magnetic resonance imaging, ultrasound tomography are not suitable for routine examinations due to reasons such as cost and radiation exposure. Among the cancer diagnostic methods used in clinics, haematology tests are typically used. Cancer screening with markers found in human serum is widely used to detect cancer at an early stage, reducing patient harm and medical costs [17]. In addition, protein measurements are crucial biomarkers for early stage cancer diagnosis and monitoring disease progression and treatment [48].

5.2 Applications for Diagnosis of Cancer

Research has been made on the effective use of microfluidic devices for cancer detection. Wang and coworkers developed a microfluidic system capable of DNA methylation analysis. The test time takes 3 h including all stages and this technique allows early diagnosis of cancer [72]. CA-125, another cancer biomarker, provides information about the progression of cancer at varying concentrations. Nunna and coworkers developed a POC testing system that combines a biochip with a microfluidic system aiming to measure CA-125 concentration in human serum obtained from a finger prick, similar to glucose measurement in diabetes [51].

Lung cancer (LC) is the leading deaths due to its high mortality rate and significant spread in all over the world [42]. Exosomes have been used as a novel biomarker for early detection and treatment of lung cancer. Yang and coworkers developed a microfluidic device with adjustable membrane pore size to identify biomarkers in human urine samples. This technique is a promising study for the detection of patients with early lung cancer [80]. Microfluidic POC assays can detect different biomarkers in various cancer-related diseases. Prostate cancer (PCa) is one of the most common cancers in men and its diagnosis is very important [21]. Prostate-specific antigen (PSA) is the biomarker of preference in the analysis [22]. In serum samples taken from healthy men, PSA concentration is in the degree of 0–4 ng/mL. People with PSA concentration higher than 4 ng/mL, which is critical in cancer detection. For this reason, if a developed test can detect PSA levels lower than 4 ng/mL in a cost-effective and rapid manner, it will make a significant contribution to the diagnosis of prostate cancer patients [11]. Mandal and coworkers developed a system combination of graphene FETs, dielectrophoresis (DEP), and a microfluidic chip for early detection of prostate cancer [45].

Since cervical cancer is one of the most common cancers in women, early diagnosis is much more important than other cancers [61]. Because cervical cancer that can be detected at an early stage can be completely healed. Karakaya and coworkers developed a microfluidic test that enables early stage diagnosis of cervical cancer by testing the presence of HPV 16 and HPV 18 in less than 40 min [33]. In another study, Lim and coworkers designed a system that integrates exosomatic mRNA sensors and 3D-nanostructured hydrogels into a microfluidic chip. Thanks to this system, exosomal ERBB2 in breast cancer-associated blood can be further detected and the validity of the system in breast cancer diagnosis can be proved [37].

6 Conclusions and Prospects

Medical diagnosis is very difficult in regions with weak health systems and inadequate health infrastructure. Microfluidic POC tests developed for medical diagnostics that can provide a solution to this problem give fast results, are easy to use, and are very low cost.

Microfluidic devices are mostly produced by utilizing paper, PDMS, or 3D printing technology. Integrating smartphones into microfluidic systems is a highly effective solution for POC applications. Although there are microfluidic POC test equipment with different characteristics, low-cost ones are mostly preferred as the main purpose is to be applicable in harsh environments. For this reason, microfluidic POC test components are small in weight and volume and are suitable for fast results.

In clinical laboratories, one of the most important steps in disease detection is the analysis of disease-related biomarkers. Microfluidics technologies can meet the requirements of medical tests in clinical laboratories because they are sensitive, inexpensive, and portable. Microfluidic POC tests have been used effectively in many fields such as CVD detection, detection of infectious diseases, tumor detection. Microfluidic devices can be integrated into many optical and serological techniques. They can detect biomarkers with high sensitivity and accuracy, which are the most critical parameters in disease diagnosis. Integration of existing technologies into microfluidic devices has provided features such as very low detection limit and high specificity.

With microfluidic POC tests, specialized personnel are not required for disease diagnosis and results can be obtained quickly. However, several disadvantages need to be overcome before POCT systems can be used as standard clinical tests. Microfluidic technology does not comply with industrial standards and guidelines. This lack of industry standards can be considered as a barrier to the commercialization of microfluidic devices. After standardization, application to industry can become easier. Thus, the cost and time of the production procedure can be reduced. Finally, for the integration of microfluidic technologies into the industry, investments in biomedical applications should be increased and microfluidic devices should be used more in clinical laboratories.

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Magnetic-Nanosensor-Based Diagnostic Chips: An Overview

Zozan Guleken

Abstract Magnetic nanosensors are showing great potential in detecting and treating numerous illnesses. They represent an effective way to administer drugs and transport contrast agents, making them ideal for use within the body. Furthermore, magnetic nanosensors can also be an external method for removing particular compounds from the bloodstream. This article examines the most recent developments in the field of magnetic nanosensors. It covers how they are created, how they have been made more compatible with biological systems, their clinical uses, and any associated risks. This chapter highlights the current advances in biosensors in nanotechnology, with particular emphasis on magnetic-nanosensor-based diagnostic chip synthesis, factors affecting this process, interaction with biomaterials, and the prospects of magnetic-nanosensor-based diagnostic chips. Nanomaterials' possible dangers and impacts in medical treatments involving magnetic nanosensors are also discussed.

Keywords Biosensors · Magnetic-nanosensors · Diagnostic chips · Biosynthesis

1 Introduction

Biosensors are increasingly used for disease diagnosis because they convert biochemical information into detectable signals [28]. Biosensors usually consist of biological recognition and physicochemical transduction parts, as described in the same article. The biological recognition part of a biosensor can be antibodies, aptamers, or other biomolecules that recognize and interact with target analytes. In contrast, the transduction part converts the recognition event into a measurable signal. The use of diverse biosensors for pathogen detection is gaining popularity due to their ease of use, rapid response time, and cost-effectiveness. As noted in a review by Cui

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et al., integrating nanomaterials into biosensors has shown promise in improving their analytical performance, including sensitivity, selectivity, and analysis speed, thus expanding their potential applications [6].

Additionally, biosensors are becoming increasingly popular as powerful diagnostic tools for various diseases [26]. Analytical devices can convert biochemical information into detectable signals such as optical, electrical, electrochemical, magnetic, or thermal signals. A biosensor typically consists of two parts: the biological recognition part and the physicochemical transduction part. The biological recognition part, such as antibodies and aptamers, recognizes and interacts with the target analytes, while the transduction part converts the recognition event into a measurable physicochemical signal [13].

The use of diverse biosensors for the detection of pathogens is gaining popularity due to their simple operation, fast response, and cost-effectiveness. The utilization of nanomaterials has also shown potential in improving the analytical performance of biosensors in terms of sensitivity, selectivity, and analysis speed, thus expanding the applications of biosensors.

Recent advancements in nanotechnology have paved the way for developing biosensors at the nanoscale level using various nanomaterials. These biosensors have direct interaction and contact with the biomolecules or analytes for which they are intended to be used. As a result, they possess stand-alone properties such as customized magnetic, electrical, and optical properties, enhanced electrical conductivity, high sensitivity, and a low response time compared to traditional biosensors. Therefore, biosensors have gained importance in different bioengineering applications, including drug delivery [22, 40].

For instance [22], developed a three-dimensional porous nickel framework anchored with cross-linked $Ni(OH)_2$ nanosheets, which showed high sensitivity as a nonenzymatic glucose sensor. Similarly [40], developed a nonenzymatic wearable sensor for electrochemical analysis of perspiration glucose using nanomaterials.

Biosensors are emerging as powerful diagnostic tools for various diseases. These analytical devices can convert biochemical information into detectable signals, including optical, electrical, electrochemical, magnetic, or thermal signals [4, 12]. A typical biosensor consists of two parts: the biological recognition part and the physicochemical transduction part. The biological recognition part, such as antibodies and aptamers, interacts with the target analytes, while the transduction part converts the recognition event into a measurable physicochemical signal [2, 23, 25].

Diverse biosensors are being adopted to detect pathogens due to their simple operation, fast response, and cost-effectiveness [19, 33]. The utilization of nanomaterials has also shown the potential to improve the analytical performance of biosensors in terms of sensitivity, selectivity, and analysis speed, thus expanding the applications of biosensors [23, 34].

Magnetic nanoparticles have become an increasingly important class of materials in recent years due to their unique magnetic properties and potential applications in various fields. These nanoparticles typically have dimensions of 1–100 nm and can be synthesized using different physical, chemical, and biological methods.

Physical synthesis methods involve using physical forces such as heat, pressure, or magnetic fields to create the nanoparticles. For example, thermal decomposition, solvothermal synthesis, and high-energy ball milling are all physical methods that can be used to develop magnetic nanoparticles.

Chemical synthesis methods involve the use of chemical reactions to produce nanoparticles. These methods often involve the reduction of metal salts or oxides in the presence of surfactants or other stabilizing agents. Examples of chemical synthesis methods include co-precipitation, thermal decomposition, and hydrothermal synthesis.

Biological synthesis methods involve using living organisms or their products to produce nanoparticles. These methods can be more environmentally friendly and sustainable than traditional chemical methods. Biological synthesis methods can include using bacteria, fungi, plants, or even human cells to produce magnetic nanoparticles, as seen in Fig. 1a. Magnetic nanosensors are an emerging sensing device class that utilizes magnetic nanoparticles to detect and quantify specific analytes with high sensitivity and selectivity. The principles of magnetic nanosensors rely on the unique magnetic properties of nanoparticles and their ability to be functionalized with specific ligands or functional groups.

The synthesis of magnetic nanoparticles involves carefully selecting precursor materials, followed by a series of physical, chemical, or biological steps to create nanoparticles with desired properties such as size, shape, and magnetic behavior. The surface of these nanoparticles can then be coated with a layer of organic or inorganic material to improve their stability and biocompatibility.

In addition to coating, magnetic nanoparticles can be functionalized with linking groups or spacer molecules that facilitate the attachment of specific agents such as

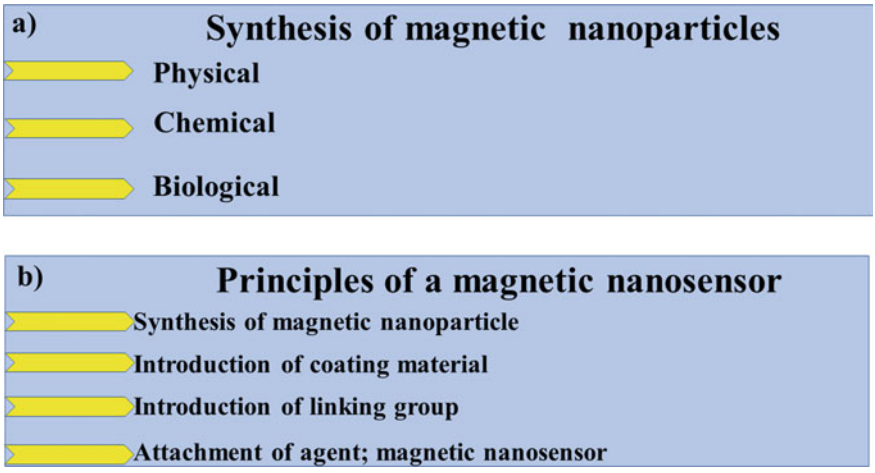

Fig. 1 **a** Types of synthesis of magnetic nanoparticles. **b** The main principles of magnetic nanosensors

ligands or biomolecules. These agents can then bind to specific target analytes in the sample, leading to changes in the magnetic properties of the nanoparticles that can be detected and quantified.

The attachment of these agents to the surface of magnetic nanoparticles forms the basis of magnetic nanosensors. The specificity and sensitivity of these sensors can be improved by carefully selecting the linking group and agent used and optimizing the conditions for the binding reaction.

In all cases, synthesizing magnetic nanoparticles requires careful control of the reaction conditions to achieve the desired particle size, shape, and magnetic properties. Once synthesized, these nanoparticles have many potential applications, including drug delivery, magnetic resonance imaging (MRI), environmental remediation, and data storage. Therefore, the synthesis of magnetic nanoparticles using physical, chemical, and biological methods is an active area of research with significant potential for future development.

Overall, magnetic nanosensors offer a promising avenue for detecting and quantifying a wide range of analytes, with potential applications in fields such as medical diagnostics, environmental monitoring, and food safety. The combination of the unique magnetic properties of nanoparticles and the ability to functionalize them with specific agents provides a powerful tool for developing susceptible and selective sensing devices.

2 Type of Magnetic-Nanosensor-Based Diagnostic Chips

Magnetic-nanosensor-based diagnostic chips have gained significant attention in recent years as a promising technology for the early and accurate detection of various diseases [21, 36]. Integrating magnetic sensors with microfluidic systems has enabled the development of compassionate and specific diagnostic tools to detect biomarkers at low concentrations in biological fluids [24].

Magnetic nanoparticles are used in magnetic nanosensors to specifically bind to biomolecules of interest, which magnetic sensors can then detect [35]. This approach offers several advantages over traditional diagnostic methods, including faster analysis time, reduced sample volume, and increased sensitivity [27].

An antibody or an aptamer can be added to magnetic nanosensors to detect disease markers or biomarkers in bodily fluids. When these nanoparticles bind to the targeted molecules, they generate a detectable signal that can be measured by magnetic sensors [15, 39].

This technology has been applied in diagnosing various diseases, such as cancer, infectious diseases, and cardiovascular diseases.

Magnetic nanosensors eliminate the need for large volumes of bodily fluids and provide faster and more accurate results compared to traditional diagnostic techniques.

As the technology develops, magnetic nanosensors are expected to become even more versatile and widely used in clinical applications, providing clinicians with a powerful tool for early disease detection and personalized medicine.

Magnetic-nanosensor-based diagnostic chips have gained significant attention in recent years for their potential to revolutionize the field of medical diagnostics. These chips integrate magnetic sensors with microfluidic systems to develop highly sensitive and specific diagnostic tools that detect biomarkers at low concentrations in biological fluids [32, 39]. The detection of disease markers or biomarkers in bodily fluids is possible by using magnetic nanoparticles that are functionalized with specific biomolecules, such as antibodies or aptamers [8, 9, 21].

Compared to traditional diagnostic methods, magnetic-nanosensor-based diagnostic chips offer several advantages, including faster analysis time, reduced sample volume, and increased sensitivity [15, 37]. They have been used to diagnose various diseases such as cancer, infectious diseases, and cardiovascular diseases. For instance, in a recent study, magnetic nanosensors were used for the early detection of colorectal cancer, achieving a high sensitivity and specificity [3, 10, 16].

As the technology develops, magnetic nanosensors are expected to become even more versatile and widely used in clinical applications. Magnetic-nanosensor-based diagnostic chips have the potential to provide clinicians with a powerful tool for early disease detection and personalized medicine. Further research and development could enable earlier diagnosis, better disease monitoring, and improved patient outcomes.

Using magnetic nanosensors as diagnostic chips, it is possible to detect biomolecules in complex biological samples faster and more accurately than ever before.

Magnetic nanoparticles are often chosen as the primary magnetic nanosensors for diagnostic chips due to their biocompatibility and ability to selectively bind to target biomolecules. MNPs can also be used for in vivo imaging due to their small size and ability to pass through cell membranes [1, 16].

Magnetic nanowires have shown promise as highly sensitive and specific nanosensors due to their high aspect ratio, which results in a high magnetic moment and sensitivity [6, 31]. MNWs can be integrated into microfluidic channels for rapid and sensitive detection of target biomolecules in biological samples.

Magnetic quantum dots have unique magnetic and optical properties that make them useful for biosensing applications [16, 17]. For sensitive and selective detection of target biomolecules in biological samples, they can be functionalized with biomolecules and integrated into microfluidic channels.

Magnetic microbeads have been widely used in diagnostic chips due to their ease of functionalization and ability to be manipulated by magnetic fields [11]. MMBs can be separated from biological samples using a magnet, making them suitable for point-of-care testing.

In conclusion, magnetic-nanosensor-based diagnostic chips are promising for early and accurate disease detection. They offer several advantages over traditional diagnostic methods and have been utilized to diagnose various diseases with high sensitivity and specificity. As the technology continues to develop, magnetic

nanosensors are expected to become even more versatile and widely used in clinical applications, revolutionizing the field of medical diagnostics.

3 Characterisation Techniques

Characterization techniques are essential for evaluating the performance and sensitivity of magnetic-nanosensor-based diagnostic chips. Various methods have been used to characterize the magnetic nanoparticles and the sensors, including scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), vibrating sample magnetometry (VSM), and magnetic force microscopy (MFM) [5].

SEM and TEM techniques have been used to investigate the size and shape of the magnetic nanoparticles and their distribution within the microfluidic channels. XRD is used to determine the crystal structure and phase of the magnetic nanoparticles. VSM is used to measure the magnetic properties of the nanoparticles, including magnetic moment, coercivity, and remanence [16]. MFM is used to image the magnetic fields of the nanoparticles and their distribution within the microfluidic channels.

Moreover, the electrical and magnetic properties of the magnetic sensors can be evaluated using impedance spectroscopy, AC susceptometry, and Hall effect measurements [29]. These techniques are used to investigate the sensitivity and signal-to-noise ratio of the magnetic sensors.

In summary, characterization techniques are essential for evaluating and optimizing magnetic-nanosensor-based diagnostic chips. These techniques allow researchers to investigate the physical, electrical, and magnetic properties of the magnetic nanoparticles and sensors, leading to the development of more sensitive and specific diagnostic tools.

4 Physical and Chemical Characteristics of Magnetic-Nanosensor-Based Diagnostic Chips

Magnetic nanosensors possess unique physical and chemical properties, making them a promising diagnostic chip platform. Some of the key characteristics of magnetic nanosensors used as diagnostic chips are discussed below:

Magnetic Properties: The strong magnetic properties of magnetic nanosensors make them detectable in low concentrations using magnetic sensors. Nanosensors' magnetic properties depend on their size, shape, and composition. Iron oxide (Fe3O4) and nickel (Ni) are the most commonly used magnetic materials in magnetic nanosensors due to their high magnetic moments and good biocompatibility [35].

Surface Functionalization: The surface of magnetic nanosensors can be functionalized with various molecules to enable their specific binding to biomolecules such as proteins, antibodies, or nucleic acids. The functionalization can be achieved through various chemical reactions such as covalent bonding, electrostatic interaction, or physical adsorption. The surface functionalization is critical for the specificity and sensitivity of the nanosensors in detecting target molecules [4, 7].

Biocompatibility: Magnetic nanosensors used in diagnostic chips must be biocompatible to prevent harmful effects on cells or tissues. Iron oxide-based nanosensors have been shown to exhibit good biocompatibility, low toxicity, and minimal inflammatory response in vivo [35].

Detection Sensitivity: Magnetic nanosensors can detect target molecules in low concentrations, typically in the picomolar range. This high sensitivity is due to the amplification effect of the magnetic signal generated by the nanosensors [11].

In conclusion, magnetic nanosensors have unique physical and chemical properties, making them a promising diagnostic chip platform. Their strong magnetic properties, surface functionalization, biocompatibility, and high detection sensitivity make them a powerful tools for early disease detection and personalized medicine.

5 Application and Impact of Magnetic-Nanosensor-Based Diagnostic Chips

Magnetic nanosensors used as diagnostic chips significantly impact various fields, including medical diagnostics, drug discovery, environmental monitoring, and point-of-care testing.

Disease diagnosis: Magnetic nanosensors have been utilized to diagnose various diseases, including cancer, cardiovascular diseases, and infectious diseases, as mentioned earlier [37, 38]. A study demonstrated the use of magnetic nanosensors for the early detection of pancreatic cancer by detecting a specific biomarker in the blood [19].

Drug discovery: Magnetic nanosensors have been used for drug discovery by screening compounds for their ability to bind to a target molecule. A study demonstrated the use of magnetic nanosensors to identify novel inhibitors of the protein tyrosine phosphatase 1B, a target for treating diabetes and obesity [5, 6].

Environmental monitoring: Magnetic nanosensors have potential applications in environmental monitoring by detecting pollutants and contaminants in water and soil. A study demonstrated the use of magnetic nanosensors for detecting arsenic in water samples with high sensitivity and specificity [18].

Point-of-care testing: Magnetic nanosensors have shown great promise in point-of-care testing by enabling rapid and sensitive detection of biomarkers at the point of

care. A study demonstrated magnetic nanosensors in a handheld device for detecting HIV RNA in plasma samples with high sensitivity and specificity [26, 40].

6 Conclusion

Magnetic nanosensors are highly sensitive and precise tools for disease diagnosis. Using magnetic nanoparticles and surface functionalization agents can specifically target biomolecules for detection. The detection of changes in the magnetic field generated by magnetic nanoparticles can be carried out using a magnetometer or MRI. With further advancements in technology, magnetic nanosensors have great potential for widespread use in diagnosing and treating diseases.

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Functionalized Smart Nanomaterials for Point-of-Care Testing

Arunima Lala, Hiranmoy Kotal, and Saikat Kumar Jana

Abstract Microorganisms especially viruses are one of the major causes of generating serious threats to human health which claims millions of lives annually. Infectious viruses are emerging periodically with their numerous harmful variants such as influenza A virus, Ebola, MERS-CoV-2, most recently SARS-CoV-2(COVID-19), etc. Traditional biochemical and immunological diagnostic methods are limited by sample transportation, processing, high-cost, time-consuming, and expert technicians to operate. Meanwhile, Point-of-Care (POC) devices can be a potential solution to overcome all these drawbacks. These devices provide many benefits in terms of portability, rapidity, low-cost, automation, etc. Nanomaterials of various shapes, size, composition, and physical and chemical properties such as gold nanoparticles, quantum dots, carbon nanomaterials, and hybrid nanocomposites, have been widely used in POC devices to enhance analytical activity and simplify the detection process. In this chapter, we are focusing on various nanomaterials-based POC diagnostic devices for the analysis of viral disease biomarkers. Many more novel, innovative platforms need to come to address the unmet clinical demands. The development of bench-to-bedside and point-of-care devices in recent years has made the term "biosensor" more well known in the scientific community.

Keywords Viral-infections · Biomarkers · Nanomaterials · Biosensors · Nanosensors · Point of care (POC) diagnosis

1 Introduction

Infectious diseases are caused by severe deadly pathogenic microorganisms like bacteria, virus, parasites, and fungi with unknown origin. Infectious diseases can spread rapidly across the global population and pose a life-threatening risk to public

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health in comparison to other diseases [24]. Since 2009, numerous severe life-threatening infectious viruses are emerging periodically with their harmful variants such as Influenza A virus, West African Ebola virus (EBOLA), Middle East respiratory syndrome coronavirus (MERS-CoV), and the recent severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2, named as coronavirus disease (COVID-19)] [26]. Particularly, the outbreak of COVID-19 [61] has caused 350 million of infections and about 5 million of deaths over the past 2 years worldwide. SARS-CoV2 has a higher rate of transmission than SARS-CoV and MERS-CoV [26, 108]. All these infectious diseases are continuously destroying global health, socio-economic conditions, and civilization process of human society. Early-stage detection of infectious viral outbreak is an urgent need to prevent transmission, and contribute effective therapies to save public health. Conventional methods like culturing and microscopy [52] are there for disease detection but they have many limitations like time consuming, microscopy for small size pathogen detection. Biomarkers, the pathogen-specific protein, can be used for diagnosis. Polymerase Chain Reaction (PCR), Western Blotting, Enzyme Linked Immunosorbent Assay (ELISA) [78], Fluorescent Antibody Tests (FAT), and antibody detection, antigen or antibody detection and hemagglutination assay and gene sequencing [70], isothermal amplification techniques [17] and immunochromatography [83] these classical detecting techniques (Fig. 1) have used for accurate diagnosis but these are laboratory-bound, laborious time-consuming process (involving multi-step protocols) and require sophisticated equipment, personnel technicians to handle [62].

Fluorescent [103], chemiluminescent [8], colorimetric [26, 71] and electrochemical [105] based detection techniques are utilized to measure the quantity of viral load. To prevent the transmission of infectious diseases fastest, simple, low-cost detection methods are still in demand. Nowadays, biosensors are more and more useful diagnostic tools for identifying highly contagious infectious diseases [15, 87]. Biosensors are such kinds of devices that can convert biological data to detectable, quantifiable signals like electrochemical, optical, and magnetic signals. Biosensor has two parts—biological signal element and physiochemical transduction part [87]. Signal element includes antibody [60], aptamer [1], and antigen that can bind with target molecule and in transduction part converts the sample into measurable, processable, detectable signal. Depending on transducers, it can be electrochemical, optical, potentiometric, piezoelectric, or thermal [74], as shown in Fig. 2. Biosensors are used in tracking waste, agricultural experiments, forensic testing, and diagnosis of severe diseases. Various biosensor-based detection devices are adopted because of their portability, rapidity, automation, etc. [36, 42, 73]. They have attracted attention because of high sensitivity, rapidity, and low detection limit in real time [42] analysis.

The application of nanomaterials has seen significant increases during the past few decades. Particularly, nanomaterials can enhance the sensitivity, selectivity, analytical performance of biosensor and thus increase the applications of biosensors [19, 20]. The utilization of nanotechnology-based biosensor devices is growing as a promising candidate for detection of infectious diseases [20].

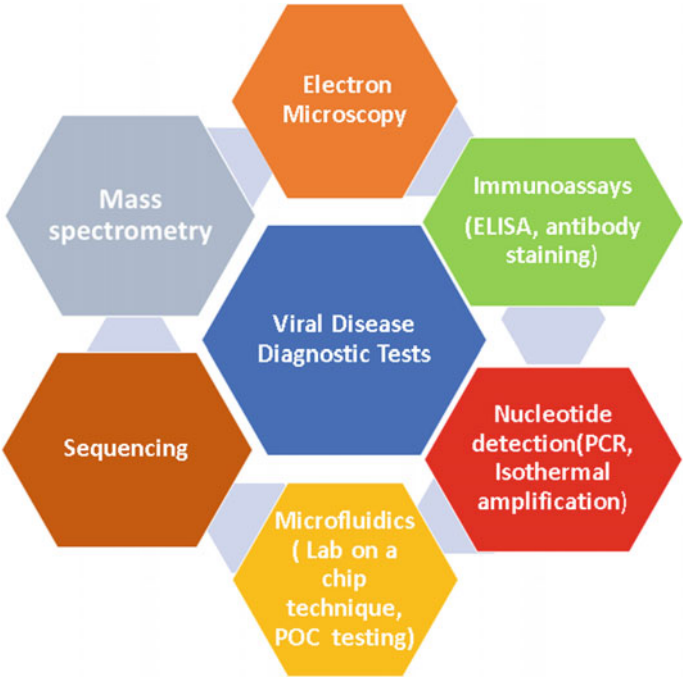

Fig. 1 Available diagnostic tests of viral diseases

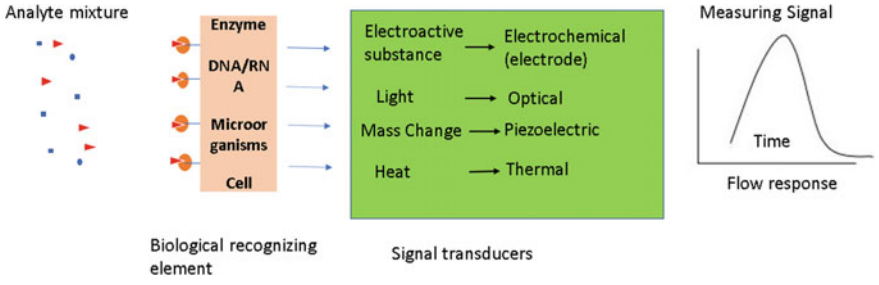

Fig. 2 Schematic diagram of a typical biosensor with all its main components

The tremendous rapid improvement in nanotechnology has imposed a great impact on biosensing. Nanomaterials can be used in specific biosensing system by modulating their physical and chemical properties like morphology, size, surface charge, etc. [45]. Nanomaterials are the synthetic element that ranges within 1–100 nm [29]. It is feasible to improve the sensitivity [7], selectivity, and analytical performance of nanosensors due to the large surface to volume ratio and presence of surface groups of nanomaterials [29, 53]. For example, quantum dots can be used for high

quantum yield in fluorometric detection [2]; gold nanoparticles have considerably higher extinction coefficient than normal dyes in colorimetric detection.

In this book chapter, a variety of nano biosensors are described for detection of viral diseases. Biosensor is compared to traditional diagnostic devices, resource-limited POC devices can be a potential solution for earlier disease detection. Biosensor plays a vital role in constructing POC devices in order to detect the presence or concentration of biomarkers in bodily fluids. POC-based platforms are highly effective, low-cost, user-friendly, rapid, and act in small sample volume. The term "Point of Care" refers to medical diagnostic kit tests carried out close to the location and moment of patient care. Here we will discuss current nanomaterial-based POC testing biosensing devices and how they can improve their effectiveness.

2 Nanomaterials in Disease Diagnosis

Diagnostic tests are a crucial element of any successful approach intended to control new and re-emerging viral diseases and are crucial at every step, from early diagnosis to successful treatment. The development, validation, and implementation of diagnostic tests are challenging, time-consuming processes. The accuracy of nucleic acid amplification test is heavily reliant on taking sample, types, storage, and transportation. False negative results can be obtained if the sample is not taken appropriately or the subject is tested early or lately after viral exposure. To enable rapid screening, new diagnostic platforms that are precise, focused, quick, and simple to use are required. Nowadays research has been shifted to another dynamic diagnosis based on nanomaterials. The efficacy of detection may be improved by NPs' wide surface area, which enables for effective interaction with target analytes. Several nanomaterials have been employed to increase the analytical sensitivity and minimize the detection limits of diagnostic tests. Nanomaterials' unique properties make them appropriate for use in cutting-edge viral detection systems.

2.1 Graphene

The International Union for Pure and Applied Chemistry (IUPAC) defines graphene as a "single carbon layer of graphite structure characterizing its nature by analogy to a polycyclic aromatic hydrocarbon of quasi-infinite dimension" [37]. Graphene is composed of sp2-hybridized carbon atoms and is organized as a thick, single atom, two-dimensional planar nanosheet that mimics a hexagonal or honeycomb structure. It demonstrates a number of exceptional qualities, including a catalytic nature, high surface area, mechanical strength, and conductivity. Due to this, graphene is a very desirable nanomaterial for platforms with sensitive biosensors and fast transistors [76].

In the area of biosensors, graphene and its oxygenated derivatives, such as graphene oxide (GO) and reduced graphene oxide (rGO), are emerging as a significant class of nanomaterials. GO nanosheets are highly hydrophilic due to the oxygenated functional groups they contain, which facilitates chemical functionalization. Inorganic nanoparticles such as metals, metal oxides, semiconducting nanoparticles, quantum dots, organic polymers, and biomolecules can be easily combined with graphene, GO, and rGO nanosheets to produce a variety of graphene-based nanocomposites with improved sensitivity for biosensor applications [55].

Chekin et al. [21] designed porous reduced graphene oxide (prGO)—molybdenum sulphide (MoS2) modified glassy carbon (GC) electrodes for the sensitive and precise detection of the L1-major capsid protein of the human papillomavirus (HPV) [21]. Teymourian et al. [91] described a straightforward electrochemical DNA hybridization biosensor based on Fe_3O_4/r-GO nanocomposite with great sensitivity and specificity to detect HIV [95]. Based on an undecorated graphene oxide (GO) platform, Hu et al. [44] reported a straightforward, label-free approach for DNA hybridization associated with the gene fragment of the HIV-1 pol gene [44].

Using graphene as a sensing platform, Gong et al. [41] developed a straightforward, precise impedimetric DNA biosensor for the sensing of the HIV-1 gene. Torrente-Rodrguez et al. [96] showed the SARS-CoV-2 RapidPlex, which is a portable, wireless, graphene-based electrochemical platform for detecting COVID-19 very quickly [96]. For label-free detection of the H1N1 influenza virus, Singh et al. [87] reported the development of a unique microfluidic chip combined with an RGO-based electrochemical immunosensor. Afsahi et al. [3] created a reasonably priced and transportable graphene-enabled biosensor to identify the Zika virus using an immobilized monoclonal antibody with a high level of specificity [3]. Real-time, quantitative detection of native Zika virus (ZIKV) antigens can be accomplished using Field Effect Biosensing (FEB) and monoclonal antibodies covalently bonded to graphene. For the detection of dengue E protein in blood serum, a label-free immunosensor has been constructed [84]. In another study, Yakoh et al. created an immunosensor based on graphene oxide (GO) that is sensitive and specific for the detection of immunoglobulins produced against SARS-CoV-2 [104]. Beduk and group recently unveiled a miniature electrochemical biosensor based on laser scribed graphene (LSG) with three-dimensional gold nanostructures for the diagnosis of COVID-19 [14].

2.2 Carbon Nanotubes (CNTs)

Biosensor manufacturing heavily relies on carbon-based materials, of which CNT, like graphene, is one allotrope of carbon. Dr. Iijima's discovery of CNTs in 1991 has been going on for 32 years [46], and the pertinent research has been continually broadened and developed over time. A crimping graphene layer forms the cylindrical CNTs. CNTs can be split into single-walled CNTs (SWCNTs) and multi-walled CNTs depending on the number of layers (MWCNTs).

CNTs work efficiently in mechanics. CNTs have an elastic modulus that is equal to that of diamond. Moreover, CNTs have excellent conductivity because they share a sheet structure with graphene. CNTs are also very promising in terms of optical modulation and heat conduction. CNTs also have a variety of benefits, such as their light weight, high specific surface area, chemical stability, superior electrochemical performance, etc., which have the potential to further research in the field of biomolecular detection in medicine [98, 25]. The enormous specific surface area of CNTs offers a variety of reactive sites, facilitating interactions with a variety of biomolecules. For this reason, CNT-based detection systems have gained a lot of attention in recent years [13, 51] with researchers using them all over the world to identify pathogens [43] and viral pathogens [47, 5] Cabral et al. [18] developed a hybrid hyaluronic acid-CNT film-based label-free immunosensor to detect anti-HBc antibodies. The immunosensor responded linearly to anti-HBc up to 6 ng ml^{-1} with a LOD of 0.03 ng ml^{-1} [18]. Pinals and coworkers [80] developed a single-walled carbon nanotube (SWCNT)-based optical sensing method that can find SARS-CoV-2 by S protein recognition [80]. A SWCNT-based semiconductor FET was used by Shao et al. [85] to identify SARS-CoV-2 antigens [85].

2.3 Metal Nanoparticles

Metal NP-based diagnostics are utilized to facilitate in the early diagnosis of infections in humans, especially at the level of a single cell. The rapid detection time, specificity, and sensitivity of metal NPs are important for point-of-care mobile nanodevice development. In order to increase biomolecule detection, several metal NPs, including gold, silver, copper, and cadmium sulfide, have recently been utilized in a wide range of sensors [49].

2.3.1 Gold Nanoparticles (AuNPs)

Because of their excellent sensitivity and selectivity, Au NPs are increasingly being used in biosensors that rely on optical and electrochemical processes. Au NPs-based devices may be used in POC devices in this context to improve trace concentration detection techniques and diagnostics [49]. In order to quickly diagnose SARS-CoV-2, a rapid IgM-IgG mixed antibody kit was developed using a mixture of gold nanoparticles [4]. In a different study, Ma et al. [63] presented an immunological assay based on a label-free electrochemical technique for identifying the hepatitis C virus' core antigen [63]. In this study, an electrochemical immunosensor was constructed combining the synergistic effects of gold nanoparticles, zirconia nanoparticles, and chitosan.

Mashhadizadeh and Talemi [66] successfully created a very accurate and precise hepatitis B DNA biosensor utilizing AuNPs [66]. In this study, mercaptobenzaldehyde was employed for the improved detection of an HBV viral short DNA sequence.

2.3.2 Silver Nanoparticles (AgNPs)

Silver is a special nanoparticle with significant plasmonic characteristics. Due to their distinctive optical properties and band gaps, Ag NPs have been investigated as a potential nanoparticles for the early detection and diagnosis methods in optical biosensors .

In order to facilitate the early diagnosis of HIV, a fluorescent Ag NPs test was designed to detect HIV-1 p24 antigen. It has been demonstrated that the linear detection range is between 10 and 1000 pg mL^{-1} [56]. Cao et al. [20] developed a biosensor that uses the fluorescence activity of silver nanoclusters to detect the target DNA sequence of the (HIV), (HBV), and (HTLV-I) genes.

2.3.3 Copper Nanoparticles (CuNPs)

CuNPs have received a lot of attention because of their enormous potential to replace more costly nanoparticles. Due to small size and high surface-to-volume ratio, CuNPs can interact intimately with viruses and are able to quickly identify them [69].

Using copper nanoparticles, Chen et al. (2010) developed an ultrasensitive electrochemical biosensor for identifying the influenza A virus [22]. With a detection limit as low as fM levels, this biosensor is capable of detecting the single-stranded DNA (ss-DNA) of the influenza A virus. Copper nanoclusters were used by to create a colorimetric biosensing technique. With this particular biosensor, it is likely to recognize the Hepatitis B virus DNA with the naked eye [64].

2.3.4 Cadmium Sulfide (CdS) Nanoparticles

CdS NPs are a strong candidate for photocatalysis because of their favorable bioactivity and good light-driven behavior [49]. For the purpose of diagnosing Hepatitis B surface antigen (HBsAg) via monitoring fluorescence intensity, a strong luminous Polyamidoamine (PAMAM) dendrimers modified CdTe@CdS-based ultrasensitive fluorescence immunosensor was developed [10]. Based on highly stable and photoelectrically active CdS nanorods modified with beta-cyclodextrin (-CD@CdS NRs), a new photoelectrochemical biosensor was developed to identify HIV DNA [35].

3 Nanomaterial-Based Sensors for Diagnosis of Infectious Diseases

Biosensors that merged with nanomaterials have been studied extensively to meet the demand in clinical diagnostic fields [11]. Nanomaterials are well suited for attaching targeted molecules to increase sensitivity because of their high surface-to-volume ratio [93]. The rapid and real time detection of small volume of sample from patients makes it most potential for robust detection of diseases and biomarkers of cancer. These POC-based devices offer sensitivity, selectivity, portability, rapid, and accurate results by using simple blood, serum, sputum, and urine samples from patients. These are advantageous for medical diagnosis, particularly in home, health-care center without using any complex instrument. According to different types of signals, colorimetric, fluorescent, SERS-based, and electrochemical biosensors are described here.

3.1 Colorimetric Biosensors

There is diversified signal readout like electrochemical, colorimetric, and fluorescent. Among them, colorimetric biosensors offer low cost, rapid diagnostic tool that can be monitored by simple color change that is visible in naked eyes. There are many shortcomings of traditional colorimetric sensors but nanomaterials have prominent optical properties that help to process the nanosensors potential for diagnosis of viral diseases. Colorimetric detection strategies play an important role in establishing paper-based POC devices. Nanomaterials can convert the signal from pathogen through unique mechanisms to amplified measurable visual signal. The usage of AuNPs in colorimetric nanosensors has become widespread. Because of the surface plasmon resonance (SPR), visible light is significantly absorbed by AuNPs [29]. For instance, in solution, AuNPs have a red absorption peak at 520 nm [48]. The SPR property of AuNPs depends on a variety of variables, including size [77], shape [28], and interparticle distance. Several AuNP-based colorimetric biosensors have been reported till date. A combined paper-based biosensor and loop-mediated isothermal amplification (LAMP) for nucleic acid detection are proposed [27, 29]. A plasmonic ELISA has been developed to detect disease specific biomarker [77]. Alcohol dehydrogenase enzyme is currently used to measure the colorimetric readout. In this reaction, ethanol and NAD+ are used and then HAucl4 and Au seeds are added (Fig. 3). Acetaldehyde and NADH are produced from ethanol and NAD+ by alcohol dehydrogenase. NADH can reduce the HAucl4 to Au and makes large purple AuNPs from yellow AuNPS seeds. The specific antigen (HBs Ag) and α-fetoprotein (AFP) can be detected by this plasmonic ELISA [29]. $1 \times 10–12$ g/ml is the visible lower concentration of detection limit. Catalase is used in this method [28]. Human immunodeficiency virus type I (HIV-1) capsid antigen p24 can be thus detected with an ultralow limit of detection (LOD) of $1 \times 10-18$ g/mL [29]. Recently, a colorimetric test based

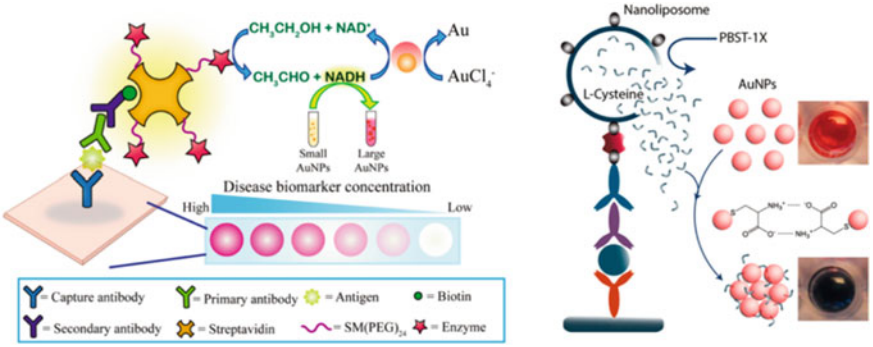

Fig. 3 Colorimetric diagnostic kit, Alcohol dehydrogenase mediated ELISA for the diagnosis of Hepatitis B surface antigen [29, 77]. If the target is present, alcohol dehydrogenase enzyme enlarges the AuNPs and from the color yellow to purple

on AuNPs has been used to detect SARS-CoV-2 nucleic acids with the naked eye [68, 91]. In this study, AuNP had been conjugated with thiol-modified antisense oligonucleotide of SAR-CoV-2. In the presence of RNA target, the AuNP agglomerate turns blue and RNA is cleaved by RNaseH from hybrid and forms precipitate due to AuNP agglomerate [29]. This assay time takes less than 10 min. Silver nanoparticle is also used for colorimetric detection. Multicolor AgNPs are also used for detection of dengue, yellow fever, and Ebola viruses (EBOV) [29, 106]. Nanozymes are nanomaterials with enzymatic activity that have equivalent catalytic activity but most resilient under various circumstances [39]. A unique type of Lateral Flow Assay has been developed by using magnetic nanoparticles for detection of EBOV [29, 33].

3.2 Electrochemical Sensors

Electrochemical sensors monitor changes in charge uniformity on the surface of transducers using impedimetric, potentiometric, or amperometric principles [9, 31, 57]. This kind of sensor has three different kinds of electrodes: a working electrode, a counter electrode, and a reference electrode. In order to increase the analytical performance of sensors, nanomaterials are used in their manufacturing. Nanomaterials have the capacity to increase surface area, electrocatalytic activity, and electron transfer rate. Due to their high conductivity, carbon nanotubes and graphene are frequently used in electrochemical biosensors. Single-type nanomaterials, such as CNTs, AuNPs, or hybrid nanocomposites made of various nanomaterials, such as a mix of CNTs and AuNPs, are also acceptable [92] (Fig. 4). For example, Omidi's group constructed an electrochemical biosensor for detecting PSA utilizing graphene oxide-gold nanostructures, with detection limits of 0.2 and 0.07 ng/mL for total and free prostate specific antigen (PSA), respectively [79]. Layqah and coworkers developed a nanoimmunosensor to detect the spike protein S1 of MERS-CoV2.

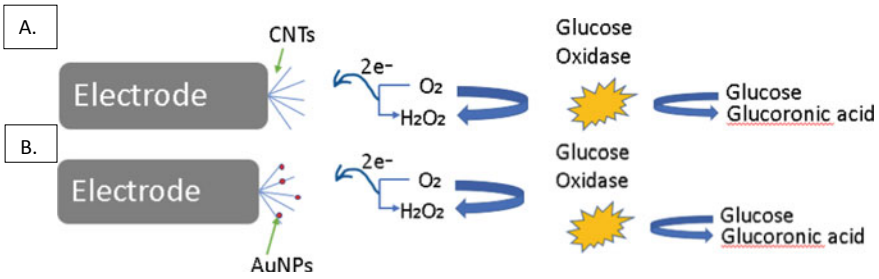

Fig. 4 Schematic diagram of nanomaterial-based Electrochemical Biosensor, **a** single type of nanomaterials (CNTs); **b** Hybrid type of nanocomposite (mixture of CNTs and AuNP) are used

AuNP provides a wider range of biomarker detection in this sensor [58, 32]. This immunosensor exhibits an enhanced sensitivity of 0.4 pg/pg for the detection of the MERS virus at concentrations between 0.001 and 100 ng/mL.

Several types of electrochemical sensors have been developed for detection of dengue NS1 protein [30]. Electrochemical sensors show greater sensitivity in detection of virus than conventional ELISA.

When target nucleic acids bind to DNA or RNA on the surface of electrochemical sensors, the surface of the electrode changes, and a signal is recorded by the speed at which electrons are transferred between the probe and electrode.

Nowadays aptamers are getting more focused on showing more sensitivity with target molecule. Hence, electrochemical sensors are very stable, highly sensitive, and very specific. Using advancements in microelectronics and microelectrode production, electrochemical approaches make it simple to miniaturize immunoassays. In order to sensitive measure of C-reactive protein (CRP) in human serum, Kakabakos et al. developed a disposable screen-printed immunosensor [54]. CRP is a liver-produced acute-phase protein that serves as a helpful biomarker for inflammation and can help predict myocardial infection, peripheral artery disease, stroke, and sudden cardiac death. In this study, a sandwich-type immunoassay employing CRP-capturing antibodies coupled on bismuth citrate-modified screen-printed electrode.

3.3 Optical Sensors

Optical sensors are excellent for detecting multiple target molecules simultaneously. These sensors quantify the optical characteristics of transducers during the interaction between the target molecule and the recognition element [40, 89]. Scientists have developed a unique type of optical sensor to recognize MERS particle. This can be seen in naked eyes, and do not need any experimental equipment. This reaction happens when pyrrolidinyl peptide nucleic acid is present [94]. It shows the aggregation and di-aggregation of silver nanoparticle with target DNA. Another type of optically silicon-coated optical sensor has been created to detect human rhinovirus

by Ostroff and colleagues [75]. Surface Enhanced Raman Scattering (SERS) can also be used for detection of virus based on optical technique of Surface Plasmon Resonance (SPR). This sensor can provide good sensitive results within 30 min.

4 Current POC Devices

One of the essential ways to prevent the outbreak of infectious diseases is through early diagnosis. POC devices, which do not require highly trained employees or expensive machinery, are more effective at fending off infectious diseases in low-resource area [65]. The World Health Organization (WHO) stated certain criteria of POC devices [68, 88] like a. specific, b. sensitive, c. affordable, d. rapid, e. strong, f. big infrastructure-free [29] (Fig. 5) [34].

Pregnancy test strips, and blood glucose meters are the commonly used POCT test kits. By merging the detection technique with nanotechnology, various new POC devices have been reported for early disease diagnosis.

At the beginning, it is known that lateral flow assay is used for detecting only HCG to confirm the woman is pregnant or not. Nowadays this LFA is reported for confirming many target analytes, and biomarkers of infectious diseases. Samples can be different types like blood, urine, sweat, swab, etc. [81]. LFAs are widely used POC devices because of its affordability, quick-response, and robustness. Yet, the broader field is still hampered by its low sensitivity and lack of measurement capability. Numerous attempts have been made to enhance the analytical performance of LFA [50, 97]. The four basic components of traditional LFA are the sample pad, conjugate

Fig. 5 Assured criteria POC test devices according to World Health Organization (WHO) [34]

pad, nitrocellulose membrane, and absorbent pad. The sample pad ensures contact between the liquid sample and the strip. Nanogold-labeled antibodies are already preloaded on the conjugate pad for signal production [38]. The nitrocellulose membrane has two lines drawn on it: the control line and the test line. The control line is used to ensure that the testing is running smoothly while the test line is utilized to detect the target analyte. The liquid sample flows across the strip due to the capillary force, and if the target is available, both lines can be seen on the control and test lines. Only one control line appears when the target molecule is absent. AuNP was used for first-generation lateral flow assay. The label has a color, so that it is visible in naked eyes. The sensitivity of first-generation lateral flow assay was very low. Analytical performance of LFA is enhanced by labeling with various nanoparticles like quantum dots, upconversion nanoparticles [41, 99]. The optical signal is measured by strip readers equipped with specialized optics. Lateral flow tests, commonly used in medical diagnostics, are straightforward, affordable, and typically yield results in 5–20 min. Several POCT devices have been created in recent years by combining LFA with other technologies like loop-mediated isothermal amplification (LAMP), polymerase chain reaction (PCR), and CRISPR [67]. CRISPR-based Cas12 based LFA can detect SARS CoV-2 from paitent's RNA sample within 40 min [100] (Fig. 6).

Microfluidic technology has the capacity to process small amounts of fluid that meet to advantages in different fields [107, 101]. In recent years, the rapid uptake of cellphones with embedded sensors has opened up new opportunities for the POC detection of infectious illnesses [23]. Detection of antibodies against EBOV, a serological POC test using an LFA has been proposed [16].

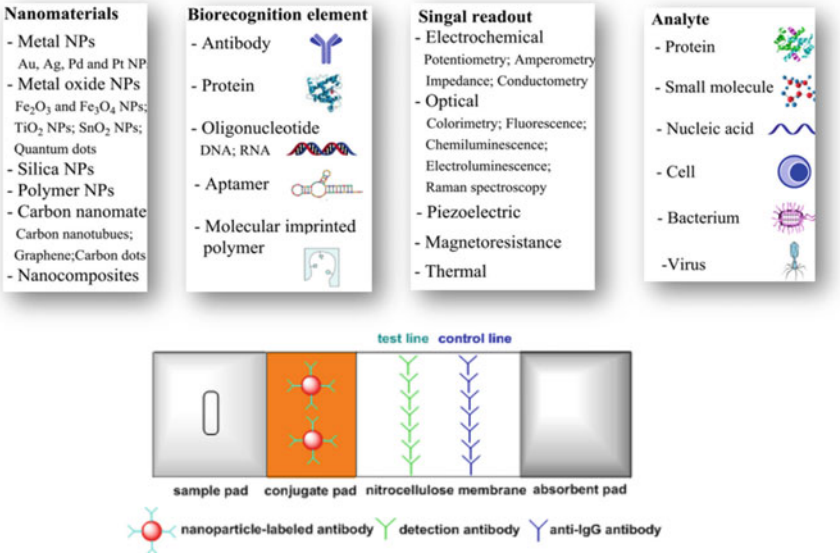

Fig. 6 Schematic diagram of typical lateral flow assay (LFA) [100]

The best method to stop an outbreak is to easily, quickly, and affordably identify infectious pathogen in a sensitive and selective manner. POC is essential to improve clinical outcomes in healthcare administration. POC devices enable untrained staff to diagnose patients quickly and accurately at their homes, critical care units, or medical facilities.

4.1 Fluid Control

One of the most crucial steps to increase the accuracy of this assay is to control the fluid. It is possible to accomplish this by incorporating a number of pumps and valves into the testing equipment. Size and complexity are taken into consideration to handle this instrument. Many kinds of pumps and valves can be incorporated into microfluidic chips under the control of chip readers [86]. McDevitt's team created a portable microfluidic system for measuring protein biomarker utilizing plastic disposable cartridges [27]. There are a number of paper-based microfluidic devices with no pump for fluid control [6]. In these microfluidic devices, a number of nanomaterials including magnetic nanoparticles, quantum dots, UCNPs, CNTs, and graphene oxides, have been used [72]. By using AuNP-based silver attachment, a group of scientists created a smartphone-based point-of-care platform for detecting avian influenza virus [109]. Using quantum dot-barcoded microbeads, Gao et al. created a microfluidic point-of-care system for detection of biomarkers [102]. Microfluidic assays feature exceptional selectivity and sensitivity compared to lateral flow. Microfluidic devices have been used to find many analytes, such as proteins, enzymes, nucleic acids, cancer cells, bacteria, and viruses [82].

5 Challenges and Future Perspectives

There are still certain restrictions even though nanomaterials are often utilized in biosensors successfully to enhance the analytical performance [50]. Identification of the concentration of potential biomarkers of cancers and cardiovascular diseases is far below the detection limit of traditional methods. Therefore, development of sensitive and specific methods is required. Moreover, cerebrospinal fluid (CSF) has comparatively high amounts of numerous brain-derived biomarkers for neurodegenerative disorders, compared to blood, such as neurofilament light and amyloid beta peptide 42. It could be quite invasive to take this sample. Improved sensitivity can detect biomarkers of dangerous diseases directly from blood. Another potential area is the identification of biomarkers in urine or saliva. Saliva and urine may be easily collected and analyzed, helpful for monitoring diseases. Due to the special properties, nanomaterials can be utilized to increase detection sensitivity. Because nanomaterials have a huge surface area, they may be utilized to load several reporter molecules, such as enzymes and fluorophores, to amplify signals. Moreover, the

sensitivity can be increased by combining various ultrasensitive detection methods with nanotechnology, such as digital PCR and single molecule analysis [90].

Nowadays, most assays are only able to detect one analyte at a time. The components of a biological sample are many and include proteins, nucleic acids, and other tiny molecules. All these biological samples work in a simultaneous manner [59]. Multiplex assays can obtain all the data and increase detection technique. When the sample volume is constrained, the assay is extremely helpful in that time. For instance, the collection of CSFS is mainly invasive. Thus, it is highly desirable to simultaneously detect numerous biomarkers in CSF. For multiplex assays, fluorescent nanomaterials of various sizes and shapes and with various emission wavelengths can be used.

Very few nanomaterial-based sensors are available for clinical trials till date. The reproducibility and robustness of nanomaterial-based sensors are the main issues. Low reproducibility depends on the size of nanomaterials which varies from batch to batch. A common problem in most assays is non-specific interaction as well. So, more study is required to grasp the nano manufacturing process and optimize particle aggregation and surface interactions. To ensure that the measurements are accurate and consistent, quality control is necessary for clinical applications.

6 Summary

The chapter emphasized on the effectiveness of nanostructured materials for enhancing the sensitivity of disposable sensors by amplifying the responses to analyte concentration. The size, shape-dependent physical catalytic characteristics of nanomaterials play main role in the development of biosensor. The nanoparticles considerably increase the surface area of electrochemically active sensors. Biosensor components are required to be integrated into a device that allows simultaneously sample and reagent load, and signal detection in an automated form, called lab-on-chip (LOC) [12]. LOC is a device for analysis that can scale down laboratory operations to a chip format up to a few square centimetres. There has been a sharp rise in the development of lab-on-a-chip instruments for clinical diagnostics over the past decades. Modern POC systems provide quick, easily accessible, trustworthy, real time analyzed information from bodily fluids, and wireless data transfer from smart devices to smartphones or other cloud devices. Among several methods, nanosensor-based POC devices provide sensitive, selective, and rapid results for detecting infectious pathogens. These devices require several detection steps, and signal readout equipment. Microfluidic technologies and smartphone devices offer solution to these problems. Microfluidic techniques can simplify the detection steps and smartphones with built-in sensors can function as portable instruments for signal readouts. Many more novel, innovative platforms need to come to address the unmet clinical demands. The development of bench-to-bedside and point-of-care devices in recent years has made the term “biosensor” more well known in the scientific community.

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Nanodevices for Food-Borne Pathogens and Toxin Detection

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Abstract Foodborne pathogens and toxins not only reduce the quality of food, but also generate a serious threat to human health. Therefore, it is very significant to identify these pathogens and toxins before consumption of foods. The traditional methods used for this purpose are time waster and expensive, and the reliability of the results becomes controversial. Nanotechnology is widely used in food science and food technology today. Among the most notable of these applications are nanodevices such as nanosensors. Studies with nanodevices used in the determination of foodborne pathogens and toxins are interesting. Nanodevices used for this purpose have started to be used today in terms of ease of use, allowing selective analysis, giving results in a short time and obtaining reliable results. Within the scope of this book chapter, it is aimed to provide information about foodborne pathogens, toxins, and nanodevices used in the determination of these compounds.

Keywords Toxin · Nanosensor · Aptamer · Food · Pathogen

1 Introduction

Nanotechnology is identified by the NSF's National Nanotechnology Initiative as "the understanding and control of matter, roughly 1–100 nm in size, where unique phenomena enable new applications" [27].

Multidisciplinary nanotechnology presents significant prospects for various applications [2, 3, 6, 7, 29, 30, 32, 48, 89], including the food industry, encompassing areas such as food safety and quality control, as well as the development of novel food additives, and fortifiers [11, 34, 71]. Nanotechnology holds great potential in the production of healthier foods [23, 31, 34]. The prospects for the usage of nanotechnology in food industry can be listed as the improving of sensitive biosensors to detect

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pathogens and toxins in foods and food processing, and food preservation through the immobilization of antimicrobials on nanomaterials for enhanced stability and action [12, 34, 71].

Insufficient adherence to proper food hygiene practices has been identified as a causative factor in the escalation of foodborne disease outbreaks, thereby exerting direct repercussions on public health. World Health Organization (WHO) has reported that a staggering estimated figure of 600 million individuals experience health deterioration as a consequence of consuming food contaminated with pathogens, while the annual death toll from foodborne diseases stands at 42,000 individuals. The mounting concerns surrounding food safety have engendered a sense of urgency, fueling the rapid advancement and implementation of robust food standards. These standards serve as comprehensive frameworks that regulate and govern various facets of the food production and handling processes, aiming to mitigate the risks associated with foodborne illnesses [28].

Food biotoxins encompass a wide range of toxic substances originating from various organisms such as plants, animals, fungi, and others. Their presence in food can result from contamination during processing, storage, and transportation, and the types of biotoxins produced can vary across different countries. Among these biotoxins, mycotoxins, primarily generated by fungi, constitute a significant class of contaminants. Notably, mycotoxins of agricultural and economic importance include aflatoxins, fumonisins, ochratoxins, deoxynivalenol, zearalenone, and other trichothecenes, which pose substantial risks to food safety. The health costs associated with these toxins depend on determinants such as toxin type, level of exposure, and route of entry. Therefore, the identification and detection of food toxins exhibit a significant role in monitoring the risk of food poisoning, enabling timely interventions to ensure public health and safety [28].

In the microbiological sampling process, large numbers of targeted foods must be collected and analyzed in a relatively short time. Because food acts as a transmission mediocre for various foodborne pathogens, there is a need to detect different pathogens in the assessment of the safety of food [16, 80]. Multi-detection has become a new research area because of convenient and high requests, as food can only be offered for sale after all indicators have been adjusted. Multiple detection methods have been established to enable multiple samples to be tested on the same instrument at the same time, resulting in avoiding sample waste, reducing equipment costs, simplifying the operating procedure as well as shortening the testing time [25].

The burgeoning interest in nanotechnology within the food industry is witnessing an upward trajectory. Nanotechnology is being widely utilized across diverse applications in the food industry, including nanoencapsulation for controlled delivery of nutrients and bioactive molecules, the implementation of biosensors for sensitive pathogen detection, and the use of nanocoatings for food composition modification and preservation of fruits and vegetables. These advancements in nanotechnology hold significant promise in enhancing food quality, safety, and functionality. Nanoencapsulation facilitates the protection and targeted release of sensitive compounds, such as vitamins and antioxidants, improving their stability and bioavailability. Biosensors equipped with nanomaterials enable quick, susceptible, and selective

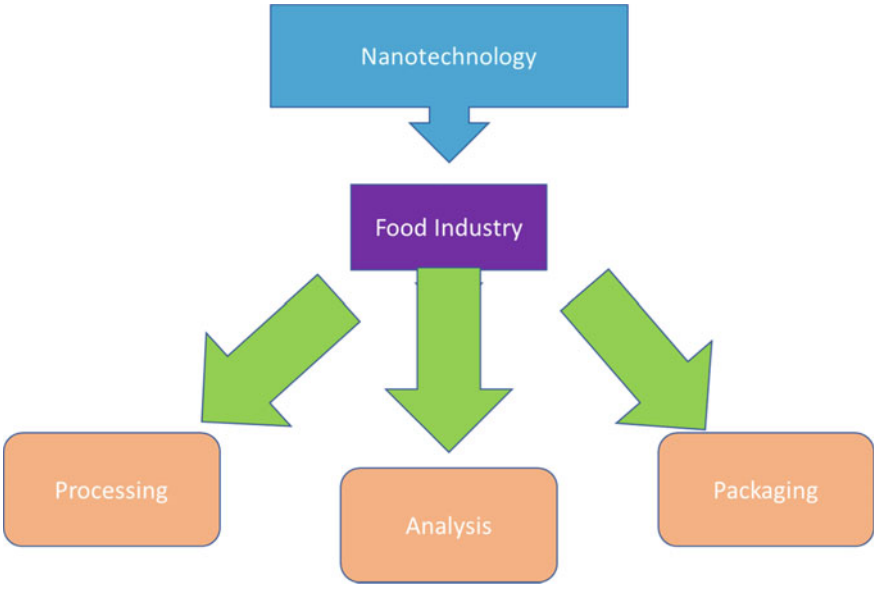

Fig. 1 Nanotechnology in food industry

detection of foodborne pathogens, thereby facilitating early warning systems and prompt interventions. Furthermore, the utilization of nanoscale coatings on food surfaces imparts enhanced protection against microbial contamination and oxidative degradation, thereby stretching shelf life and providing the integrity of fresh produce. The incorporation of nanotechnology-based strategies in the food industry represents a dynamic field of research with potential implications for improving food production, preservation, and overall consumer experience [15] (Fig. 1).

The advancement of biosensors utilizing nanomaterials holds great promise in obviating the reliance on costly or intricate instruments and enabling quick determination of foodborne pathogens through movable or hand-operated devices. The integration of immunonanoparticles into conventional pathogenic biosensors offers an avenue for enhancing pathogen detection capabilities. Moreover, the synergistic combination of immunoanoparticles with enzymatic catalysis in electrochemical immune sensors can lead to swift, efficient, and precise pathogen detection. This amalgamation of nanomaterials, immunonanoparticles, and enzymatic catalysis in biosensors not only enhances sensitivity and selectivity but also contributes to the miniaturization and portability of detection devices. These advancements hold significant potential for revolutionizing pathogen detection in the food industry, providing a practical and accessible solution for rapid and accurate monitoring of food safety in various settings [94].

2 Nanodevices in Food Analysis

Conventional techniques employed for pathogen detection in food samples are associated with various limitations, such as prolonged analysis times, high costs, labor-intensive procedures of sample preparation, and the requirement for skilled persons. The primary traditional assays utilized in food analysis include colony counting methods, immunoassays, and polymerase chain reaction (PCR)-based approaches. Colony counting methods, which involve the enumeration of bacterial colonies on microbiological culture plates, are naturally complicated, time waster, and susceptible to errors. The determination process typically extends from 3 to 9 days, and the confirmation of positive results may require up to 2 weeks. Immunoassays, while providing improved sensitivity and specificity, still necessitate sample preparation steps and exhibit certain limitations in terms of turnaround time and resource requirements. Similarly, PCR-based methods offer enhanced sensitivity and selectivity but are encumbered by the need for specialized equipment, complex protocols, and skilled personnel. Hence, the aforementioned constraints inherent in traditional pathogen detection methods underscore the pressing need for alternative approaches that can overcome these limitations and enable faster, more cost-effective, and basic detection of foodborne pathogens [5].

Immunoassays, including ELISA, lateral flow, and spot blot, have emerged as alternative approaches for pathogen detection by targeting specific pathogen antigens. These assays utilize **monoclonal or polyclonal antibodies** that selectively bind to the desired pathogen, enabling the testing of large sample volumes and on-site pathogen detection. However, immunoassays often exhibit limited sensitivity, necessitating further confirmation through supplementary testing. In contrast, PCR-based methods provide quick and highly selective pathogen detection. Nevertheless, PCR-based methods encounter certain limitations, such as the potential for incorrect results due to DNA polymerase inhibition by components in the food matrix, hindering the amplification of target DNA, as well as the possibility of false positive results arising from the amplification of non-target DNA fragments. These factors underscore the ongoing need for continuous research and development of innovative techniques to overcome the drawbacks associated with traditional methods and increase the sensitivity, specificity, and reliability of pathogen detection in food samples [5].

Innovative and portable biosensors have witnessed significant advancements in recent years, addressing the limitations associated with conventional and molecular detection technologies, as well as other biosensors, particularly in the realm of quantitative detection and screening of pathogens in various areas [33, 83]. Electrochemical platforms have emerged as the most popular biosensors due to their high specificity toward analytes and adaptability for multi-analysis, offering superior analytical accuracy even in complex food matrices with varying compositions, densities, and pH levels. Electrochemical observation of pathogens relies on the utilization of a working electrode modified with specific recognition elements, such as antibodies, aptamers, or DNA probes, ensuring selectivity, sensitivity, and specificity in measurements. Studies emphasize the incorporation of various nanomaterials as enhancers, labeling

agents, or immobilizer supports in electrochemical biosensors, ensuring the overall feasibility of the platform for diagnostics and detection practices. The integration of nanomaterials shows a pivotal role in enhancing the performance and sensitivity of electrochemical biosensors, further advancing their potential in diverse diagnostic and detection scenarios [5].

The determination of foodborne pathogens in products primarily relies on the identification of genetic material of bacteria or the entire bacterial cell using traditional microbiological techniques. These techniques have long been regarded as reliable for pathogen control, but they also exhibit inherent complexity [35]. The utilization of nanotechnology facilitates the implementation of cost-effective nanosensors in food packaging for the determination of diverse pathogens commonly encountered in various products [63].

Nanodevices, characterized as nanoparticles specifically designed to interact with cells and tissues, are engineered to perform precise functions and carry out targeted tasks within biological systems. These nanoscale devices are tailored with specific properties and functionalities that enable them to navigate complex biological environments, interact with cellular components, and execute designated functions with high precision [45].

Nanosensors are sophisticated bioanalytical devices constructed through the integration of diverse nanostructured materials and biological receptors, resulting in an integrated system design. Their significance in the food industry has garnered considerable attention, primarily attributed to their ability to rapidly detect analytes, maintain integrity, exhibit high sensitivity and specificity, and offer cost-effective solutions. Nanosensors have the potential to revolutionize food safety and quality control measures by enabling real-time monitoring and detection of contaminants, pathogens, and various quality indicators in food products. Their nanoscale size and functional properties allow for enhanced surface interactions and signal transduction, facilitating sensitive and selective detection. The utilization of nanosensors in the food industry holds great promise for improving food safety, reducing the risk of foodborne illnesses, and enhancing overall consumer confidence [21]. Nanosensors exhibit exceptional optical and electrical properties, attributed to their conjugation with diverse types of nanomaterials. This incorporation of nanomaterials enables nanosensors to achieve a high surface-to-volume ratio, facilitating enhanced sensing capabilities. The original properties of these nanomaterials, such as their tunable surface chemistry, conductivity, optical properties, and biocompatibility, contribute to the overall performance and functionality of the nanosensors. By exploiting these advantageous properties, nanosensors offer exceptional sensitivity, selectivity, and response toward target analytes in various areas, such as food safety, environmental monitoring, and biomedical diagnostics. The integration of different nanomaterials with nanosensors presents a versatile and promising approach for developing advanced sensing devices with enhanced performance characteristics [14]. Currently, nanosensors are widely employed for the detection of foodborne pathogens, additives, toxins, chemicals, and pesticides in diverse food products [61].

The utilization of various nanomaterials offers the potential to increase the analysis performance of electrochemical sensors through signal amplification and improvement. By incorporating nanomaterials into the electrode, the surface area is increased, leading to enhanced loading capacity and improved bulk transport of reactants. This augmentation in surface area allows for greater sensitivity in detecting target analytes. Also, nanomaterials can serve as carriers for redox probes, facilitating selective detection or enhancing the dynamics of redox changes, thus significantly improving the sensor's readout. The integration of nanomaterials into electrochemical sensors presents a valuable strategy to increase their sensitivity, selectivity, and overall performance, enabling more accurate and reliable detection of analytes of interest [33, 59].

Nanomaterials encompass a diverse range of structures, including quantum dots, carbon dots, nanoparticles (0D), nanotubes, nanowires, nanorods (1D), nanoplates, nanosheets, nanodiscs (2D), and nanoflowers, nanocones, nanoballs (3D) [56]. These nanomaterials have found widespread application in the construction of electrodes for electrochemical biosensors utilized in the determination of foodborne pathogens [66]. Electrochemical sensors and biosensors incorporating novel nanomaterials, such as carbon nanotubes, metallic nanoparticles, and superparamagnetic nanoparticles, are currently employed for the detection of various toxins present in food products [90]. The integration of these nanomaterials into electrochemical biosensors has enabled enhanced sensitivity, selectivity, and performance in detecting and analyzing foodborne contaminants, facilitating improved food safety and quality control measures.

Metal nanoparticles, particularly gold nanoparticles, are commonly favored for integration into electrochemical biosensors designed for the detection of foodborne pathogens. This preference stems from their exceptional conductivity, biocompatibility, and ability to preserve biomolecular activity over extended periods [53]. Quantum dots (QDs), on the other hand, demonstrate significant potential for implementation in compact-sized electrochemical biosensing devices due to their compact size and consistent performance characteristics [13]. In terms of material classification, quantum dots can be categorized as metal QDs, carbon dots (CDs), and graphene quantum dots (GQDs) [5]. The utilization of these nanomaterials, including gold nanoparticles and quantum dots, enables the development of highly sensitive and reliable electrochemical biosensors for the detection of foodborne pathogens, offering improved capabilities in ensuring food safety and quality control.

Examples of nanomaterials used in food analysis are presented in Table 1.

2.1 Metalic and Other Related Nanodevices

Gold nanoparticles (AuNPs) are commonly preferred for integration into electrochemical biosensors targeting foodborne pathogens due to their excellent improved electrical properties, biocompatibility, and long-term preservation of biomolecular activity [84].

Table 1 Examples of nanomaterials used in food analysis

Analysis purpose	Nanomaterial
Pathogen	Single-walled carbon nanotubes (SWCT) Silica nanoparticles Gold nanoparticles Graphen nanoparticles
Toxin	Magnetic nanoparticles Gold nanoparticles Zinc nanoparticles Single-walled carbon nanotubes (SWCT) and multi-walled carbon nanotubes (MWCT)

Metal oxide nanomaterials, which can be fabricated in numerous forms ranging from 0 to 3D, offer a valuable platform for the development of electrochemical biosensors [51]. These nanomaterials possess advantageous properties such as low cost, high biocompatibility, antimicrobial activity, and a broad catalytic domain with notable electrocatalytic activity [97]. Recent investigations have focused on nanocomposite heterostructures, combining different components to exploit their distinct properties and enhance the performance of electrochemical biosensors for the detection of foodborne pathogens. For instance, a comparative study on porous nanocomposites, namely ZrO_2–Ag–G–SiO_2 and In_2O_3–G SiO_2, was conducted for the rapid and high-throughput detection of *Escherichia coli* using cyclic voltammetry, resulting in the creation of a bacteria-identifying nanodevice with enhanced sensitivity [20]. These advancements highlight the potential of metal oxide nanomaterials and nanocomposite heterostructures in the design of efficient electrochemical biosensors for food pathogen detection.

Transition metal oxides, which are readily available in soil, exhibit promising prospects in electrochemical applications, particularly in the development of electrochemical biosensors targeting foodborne pathogens [43]. For example, selective determination of *Salmonella typhimurium* in food has been achieved using a SiO_2@MnO_2 nanocomposite impedance biosensor developed on interdigitated array microelectrodes coupled with immunomagnetic separation [88].

Numerous studies have demonstrated significant enhancements in detection capabilities through the utilization of metal oxide nanoparticles in electrochemical biosensors. For instance, Muniandy et al. engineered a reduced graphene oxide-nano TiO_2 composite aptasensor for the specific detection of *Salmonella enterica*, yielding notable improvements in detection performance [49]. Similarly, Nadzirah et al. employed pure TiO_2 nanoparticles and developed interdigitated electrodes for the high-specificity and reproducible detection of *E. coli* [50]. Additionally, Teng et al. demonstrated that ZnO nanorods exhibited enhanced signal amplification for targeting *E. coli* [77]. Furthermore, Purwidyantri et al. utilized ZnO decorated with Au to fabricate a detection platform for *Staphylococcus epidermidis* based on DNA hybridization, further highlighting the potential of metal oxide nanoparticles in enhancing detection capabilities in electrochemical biosensors [57].

An electrochemical gene sensor based on SnO_2 nanocrystalline quantum dots for the determination of *Vibrio cholerae* using the principle of DNA hybridization was developed by Patel et al. The study demonstrated that SnO_2 nanoparticles provide an appropriate surface for the immobilization of the DNA probe and increased electron transport and enhanced signal readout, providing high long-term stability [54].

The utilization of magnetic iron oxide nanoparticles proved successful in the isolation of *Listeria monocytogenes* DNA, a pathogen commonly associated with dairy products [93]. Surface-enhanced Raman spectroscopy in combination with silver nanosensors has emerged as a highly effective technique for the detection of pathogenic bacteria [19]. Additionally, nanosensors incorporating various nanomaterials have become standard tools for the detection of foodborne pathogens [4].

The utilization of gold nanoparticles (AuNPs) in the improving of electrochemical biosensors has gained increasing attention due to their advantageous properties [44]. Deposition of AuNPs onto gold electrodes leads to a substantial enhancement in the electrode's surface area, enabling enhanced target recognition and improved analytical performance [39]. When immobilized on electrodes, AuNPs enhance surface biocompatibility, facilitate electron transfer between the electrode and immobilized molecules, increase the effective surface area, and enable facile bioconjugation of recognition elements. Raj et al. showed the integration of Au@MoS_2-PANI nanocomposite onto a glassy carbon electrode for the detection of *E. coli*, utilizing the enhanced electrode properties [62]. Additionally, Hassan et al. improved a label-free electrochemical biosensor employing the electrocatalytic properties of AuNPs for the very sensitive and quick detection of *E. coli* O157:H7 in minced meat and water [28].

Despite the widespread use of AuNPs-based electrochemical biosensors, their complex nature presents challenges that restrict their broad application, particularly in complex food matrices. Typically, these biosensors involve multiple steps that require manual intervention by the user during testing, including repetitive washing, sample and reagent loading, which prolongs the analysis time and may lead to inconclusive results. Consequently, there have been endeavors to address these limitations by integrating microfluidic technology with the electrochemical cell, aiming to automate manual interventions. This integration offers the potential for improved efficiency, reduced user involvement, and enhanced reliability in the analysis of food samples [5].

AuNP-based electrochemical biosensors have demonstrated their utility in the detection of viruses, benefiting from the original properties of gold nanoparticles for selective capture and recognition of viral particles [38]. For example, the MERSCoV has been found to contaminate dairy products through electrochemical biosensors containing these nanoparticles [79].

Metal-organic frameworks (MOFs) have emerged as an important advancing class of microporous materials with extensive potential in various applications [87]. While 0D and 1D nanostructures are being developed, they primarily represent 2D or 3D porous architectures assembled through coordination linkages between metal cation salts and polydentate organic ligands [47]. MOFs exhibit remarkable characteristics such as high surface area, pore volume, porosity, surface functionality, and adjustable

structures. Small-scale MOFs combine the unique properties of both MOFs and nanostructures, enabling the design of complex nanocomposites such as NP@MOFs. Notably, 2D MOFs have gained attention for biosensing applications, leveraging their controllable properties and exceptionally high surface area, which are anticipated to surpass the performance of conventional electrochemical sensors.

Varsha and Nageswaran [81]. The controllable tunability of properties and the exceptional surface area of MOFs are anticipated to surpass the capabilities of traditional electrochemical sensors [5].

Carbon materials have prolonged use in electrochemical sensor electrodes [55]. The discovery of new carbon allotropes such as fullerene, carbon nanotubes (CNTs) [55] and graphene [52] has triggered active exploration of their applications in various types of biosensors due to their high electrical properties. Several studies have discussed perspectives of the application of graphene and carbon nanomaterials as electrode materials to improve electrochemical sensors [18], including foodborne pathogen detection [82].

Graphene and carbon nanotube technology has proven to be valuable for the development of movable electrochemical sensors [1, 72]. In the context of food-borne pathogen detection, graphene-based electrochemical sensors offer significant advancements as they can operate directly in biological and food matrices [82]. Cheap carbon materials, such as graphene and carbon nanofibers, possess large surface areas, high electron transfer rates, and excellent catalytic properties, making them vital for the development of miniaturized sensing platforms for point-of-need testing [5].

Graphene derivatives, specifically graphene oxide (GO) and reduced graphene oxide (rGO), have emerged as the preferred materials for electrochemical electrode modification owing to their low-cost scalability and compatibility with integrated device fabrication and processing technologies [24]. In the field of pathogen detection, a rapid and highly sensitive electrochemical invAgene biosensor for the detection of *Salmonella* was developed by employing a polypyrrole-rGO nanocomposite on a glassy carbon electrode [96]. The incorporation of rGO in the electrode material enhances the electrochemical performance, enabling improved sensing capabilities for the targeted pathogen. These findings highlight the potential of graphene derivatives in the development of important electrochemical biosensors for pathogen detection.

Recently, novel methods involving the direct writing of graphene-based electrodes have been implemented for the development of portable sensors [36]. Laser-induced graphene (LIG) is a simple and scalable technology that involves the local heat treatment of polymers such as polyimide, resulting in the formation of porous graphene material [41]. The resulting material possesses the desirable properties of graphene, such as high surface area and electrical conductivity, while also offering a large number of active sites for surface modifications with various receptors [37]. In one study, LIG electrodes modified with polyclonal antibodies demonstrated highly selective detection of *Salmonella enterica serovar typhimurium* [73]. This highlights the potential of LIG-based electrodes as promising platforms for the development of sensitive and specific biosensors for pathogen detection applications.

Carbon nanotubes are classified into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) based on the number of graphene sheets they contain [70]. SWCNT composites have been utilized for the highly sensitive detection of bacterial and viral model strains, such as *E. coli* O157:H7 and bacteriophage T7, respectively [22]. In terms of material modification, MWCNTs have demonstrated advantages over SWCNTs as they are more robust, easier to produce on a larger scale, and more cost-effective, leading to improved sensitivity as reported in several studies. Specifically, MWCNTs deposited on indium tin oxide (ITO) electrodes and modified with aptamers have been employed for the detection of *Salmonella enteritidis* and *S. thyhimuri* [26]. The results underscore the considerable potential of both single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) as versatile platforms with high sensitivity for biosensor development aimed at pathogen detection.

A prominent trend in contemporary electrochemical biosensors for the detection of pathogens and toxins is the utilization of nanocomposites, which combine multiple nanomaterials in electrode design, resulting in notable synergistic effects that enhance detection performance. Furthermore, certain nanomaterials like graphene and metal oxide nanoparticles possess inherent antibacterial properties. The integration of these nanomaterials into biosensors yields multifunctional platforms capable not only of pathogen identification and quantification but also of pathogen elimination, offering a comprehensive approach to pathogen detection and control [5].

2.2 Aptamers

Aptamers found in the 1990s are defined as short, single-stranded nucleic acids with high affinity and selectivity [17]. Aptamers, which can be synthesized with high purity and cheap, increase the repeatability of analysis due to these features. Aptamers can identify and connect to a variety of food contaminants due to functional interactions [65]. Moreover, aptamers also have the potential to be ingenious and adaptive biosensors with three-dimensional conformational changes [28].

Given these features, many aptasensors based on different transduction strategies have been designed to identify food contaminants in the last decade. On the other hand, relatively few aptasensors have been applied to food safety testing. Therefore, it is useful to develop the recognition stability of the aptamer by using the complex food matrix [28].

Aptamers have been developed to detect different types of mycotoxins found in foods [42]. To develop a new electrochemical aptasensor, Yang et al. studied aptamer/NH2 prepared Janus particles with one side belonging to the glassy carbon with peptide bond and one side functionalized with anti-ochratoxin A aptamer for specific detection [95]. In addition, Xu et al. also designed the aptasensor that performs ultra-sensitive detection of *Staphylococcal enterotoxin* B [91].

The ricin and abrin toxins found in castor beans and rosary peas, respectively, act as type 2 ribosome-inactivating proteins, both of which lead to cell death and

go undiagnosed. Detection of these toxins in adulterated food and beverages is very important. In order to identify these toxins, an aptasensor was prepared by combining the C_3N_4–MnO_2 nanolayer with liposome amplification [46]; a specific aptamer has also been isolated for abrin analysis [76].

However, the discovery of aptasensors that detect animal toxins such as tetrodotoxin and saxitoxin has been infrequently reported. Recently, a temperature-assisted fluorescent biosensor has been developed to detect saxitoxin by Cheng et al. The aptamer-saxitoxin complex can trigger a temperature-assisted fluorescence resonance energy transfer method by lowering the melting temperature [9].

2.3 Microfluidic Devices

Microfluidic sensors are a type of nanosensor based on microfluidics together with liposomes and offer advantages in detecting toxic substances in samples even in the microliter [78]. Microfluidic biosensors are frequently used for the detection of bacteria due to their quick analysis, simple use, low cost, easy integration, and field detection advantages [86]. Micropump and microvalve are important components in the improving of microfluidic chips. Recently, new power-independent micropumps [92] and finger-operated micropumps [75] have attracted much attention.

It has been noticed that the chip in the microfluidic devices has micronscale and sometimes nanoscale. Therefore, it has higher surface area and diffusion coefficient and easily transfers heat [99]. For this reason, microfluidics has important advantages like time-consuming effects, preventing contamination, and reducing costs. Integration with microfluidics can provide various advantages to existing sensing techniques [60]. Some studies have been conducted on bacteria detection with microfluidic devices [99].

Due to the complex background of food samples and the low concentration of pathogenic bacteria, signal amplification is regularly required to detect target bacteria using enzymes, fluorescent probes, and other nanomaterials [58].

Due to their various advantages, nanozymes are widely used for bacteria detection [8]. For example, gold@platinum nanocatalysts (Au@PtNCs) have larger specific surface area and more catalytic active sites for the catalytic reaction. In this different study, a power-free microfluidic biosensor with an on-chip micropump and an on-chip microstirrer was developed and Au@PtNC amplification combined with smartphone imaging to achieve rapid, sensitive, and successful on-site detection of *Salmonella*. This obtained biosensor was able to detect *Salmonella typhimurium* bacteria at a concentration as low as 350 CFU/mL in chicken meat and milk samples quickly and significantly reduced the cost of each test.

3 Advantages of Nanodevices on Food Safety

The most important disadvantages of the current methods used in food analysis are the lack of fast and reliable procedures for detecting low amounts of pathogens and toxins [94].

DNA and protein-based detection methods are faster but require at least a few hours to perform these methods. A culture enrichment period ranging from several hours to a day is required for the determination of bacteria contained in a food sample. In the food industry, waiting this long for results can be expensive and inconvenient. In contrast, the complex of bionanomaterial bacterial cells can be detected or confirmed within 3 h without bacterial culture and enrichment [85].

The development of biosensors utilizing nanomaterials holds great promise for overcoming the limitations associated with expensive or complex instruments, enabling the rapid detection of foodborne pathogens in portable or hand-held devices [94].

The small size of nanomaterials allows for their binding to target bacterial cells, leading to significant modifications in their optical, physical, and chemical properties. This property enables nanomaterials to serve as signal transducers or amplifiers, facilitating real-time detection of pathogenic bacteria. Functionalized nanomaterials have been extensively investigated for their integration into biosensors, serving as absorbers and carriers of pathogens. These nanomaterial-based biosensors offer the advantage of rapid detection, enabling the completion of the detection process within a short timeframe [94].

Fluorescent dye-doped nanoparticles have been developed as markers for sensitive bacteria detection due to their positive properties such as high fluorescence quantum yields, photostability, and tunable fluorescent bands [40]. For example, Zhao et al. developed a bioassay method based on fluorescent nanoparticles conjugated with anti-E for the detection of *E. Coli* O57 in minced meat samples [98].

Magnetic nanomaterials are most commonly used to eliminate interference from complex food matrices and to concentrate target cells, which can eliminate the need for time-consuming enrichment through a culture process. While studying for signal amplification with fluorescent nanomaterials, metal and semiconductor nanomaterials have been chosen because of their electronic or optical transduction on biorecognition in the development of biosensors. Bioconjugated nanomaterials have demonstrated advantages over traditional (non-nanomaterial-based) methods for specific pathogen detection in nutrient broth, food products, and biofilms [94].

4 Safety Concerns About Nanodevices

The utilization of diverse nanomaterials in the food industry offers numerous benefits; however, it also presents significant concerns regarding human health, environmental impact, and other ecosystems due to their cytotoxic effects. Recently, heightened

attention has been directed toward the potential risks associated with nanomaterials, including those that lack toxic constituents in their composition but possess inherent hazards associated with their small size and subcellular interactions with organisms [69]. For instance, certain nanoparticles carry the risk of penetrating the skin and inducing toxic effects in humans, animals, and plants alike [67].

To gain insight into the toxicity mechanisms of different nanomaterials on human health and the environment, it is crucial to establish a clear understanding of the various routes through which nanomaterials from the food industry can be exposed and enter the human body. Nanomaterials are intentionally or unintentionally consumed through processed food products via oral, dermal, and pulmonary routes. Oral ingestion serves as the primary pathway for the intake of chemicals, water, and nutrients. Presently, a wide array of nanomaterials are found in various food products, and it is presumed that the gastrointestinal system is directly exposed to these nanomaterials on a daily basis. Nanoparticles ingested orally traverse from the oral cavity to the stomach and subsequently reach the intestines, giving rise to significant health concerns for humans [64].

Different in silico, in vitro and in vivo methods are used to evaluate the toxicity of nanomaterials. Especially in the uptake and biodistribution evaluations of nanostructures, it has been revealed that these structures are located in certain locations in the human body [10].

More research is needed on the cytotoxic effects of nanoparticles used in nanodevices and their potential impact on consumer health and safety and the environment. Potential risks, toxicological issues, and environmental issues must be addressed when acquiring these tools. Relevant rules and regulations will only be put in place to tackle the various applicable safety issues and then govern the entire food industry field [68].

5 Conclusion

In recent years, the field of nanobiotechnology has witnessed significant advancements, leading to the development of highly sensitive and miniaturized nanomaterial-based devices for ensuring food safety and quality. These devices employ various nanomaterials and nanosensors. However, along with these advancements, there is a growing recognition of the potential environmental and health risks associated with the use of nanomaterials and nanodevices in the food industry. Therefore, it is crucial to prioritize research efforts aimed at understanding and mitigating the potential toxic effects linked to the utilization of nanomaterials and nanodevices. Additionally, it is essential to thoroughly investigate and enhance parameters such as accuracy, reproducibility, precision, and specificity to ensure the reliability and validity of nanodevices. Furthermore, optimization of the synthesis procedures for integrated nanomaterials is necessary to establish robust protocols for large-scale production and strict quality control, ensuring the absence of chemical impurities that could compromise the sensing properties or lead to environmental pollution [74].

It is important to choose fast, sensitive, specific, and easily applicable processes that do not cause analyte loss during the detection of pathogens and toxins in foods. For this reason, these features should be considered during the design of the biosensor to be used. It will result in the discovery of effective nanotools to be used in food analysis that will ensure safe food presentation through collaborations and advanced studies between those working in the field of nanotechnology and food technology.

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Nanomaterials for the Rapid Identification of Agriculturally Important Plant Pathogens

Zehra Karaagac and Ismail Ocsoy

Abstract Nanomaterials (NMs) have shown great potential for the rapid identification of agriculturally important plant pathogens. The small size and unique properties of NMs make them well-suited for use in biosensors and diagnostic assays. The NMs have not been only used for detection or identification of plant pathogens, they have the potential to protect crops from different pathogens in order to minimize the reduction in crops production and the monetary losses. The NMs have been utilized in various techniques including microneedle applications, nano-barcoding systems, nano-biosensors, miRNA-based nano diagnosis/array-based nano-sensors, and nano-diagnostic apparatus, for the rapid identification of agricultural-related plant pathogens. NMs, such as gold (Au) NMs and carbon nanotubes (CNTs), have been used in the development of biosensors for the detection of plant viruses and bacteria. These biosensors exploit the unique optical and electrical properties of these NMs to provide highly sensitive and specific responses toward target plant pathogens. Similarly, CNTs have been used for the detection of bacteria that cause plant diseases, such as *Xanthomonas campestris*, which is responsible for black rot disease in cruciferous crops. CNTs have a large surface area and high electrical conductivity, making them ideal for use in electrochemical sensors that can detect the presence of bacterial pathogens in plant samples. In addition to biosensors, NMs have also been used to develop rapid diagnostic assays based on metallic NMs. For example, magnetic NMs have been used to develop rapid diagnostic tests for plant diseases such as late blight, which is caused by the oomycete pathogen *Phytophthora infestans*.

Keywords Nanomaterials · Plant pathogens · Diagnostic methods

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1 Introduction to Nanomaterials

Nanomaterials (NMs) are a type of material obtained by arranging matter at atomic and molecular scales. Nano-sized materials have superior properties and functions compared to bulk materials [122]. Nano-sized structures, It is divided into different classes such as nanotubes, nanoparticles, nanowires, nanorods, and nanofilms [70]. NMs can be synthesized in different chemical structures and morphologies (Fig. 1).

Today, NMs can be synthesized as metal, metal alloy, metal oxides, ceramic and polymer-based or composite structures [130]. Metallic NMs exhibit different physical and chemical properties based upon their surface/volume ratio, low melting point, surface roughness, mechanical properties, magnetic properties, etc., compared to bulk metals. In addition to these features, gold (Au) NMs attract attention with their optical properties [72]. For instance, the 25 nm and 50 nm Au NMs have a specific wine red color and purple color in solution, respectively [74]. This indicates that color of Au NMs solution can be varied by tuning their size. In addition to that wine red color the 25 nm Au NM is observed in their colloidal form, but aggregation of 25 nm Au NM changes it from wine red to purple color. Thus, Au NMs can be integrated in colorimetric sensor design by benefiting from these size-dependent color change properties. This is also true for other metallic NPs [7]. For example, silver NPs are yellow, platinum, iron and palladium NPs are black.

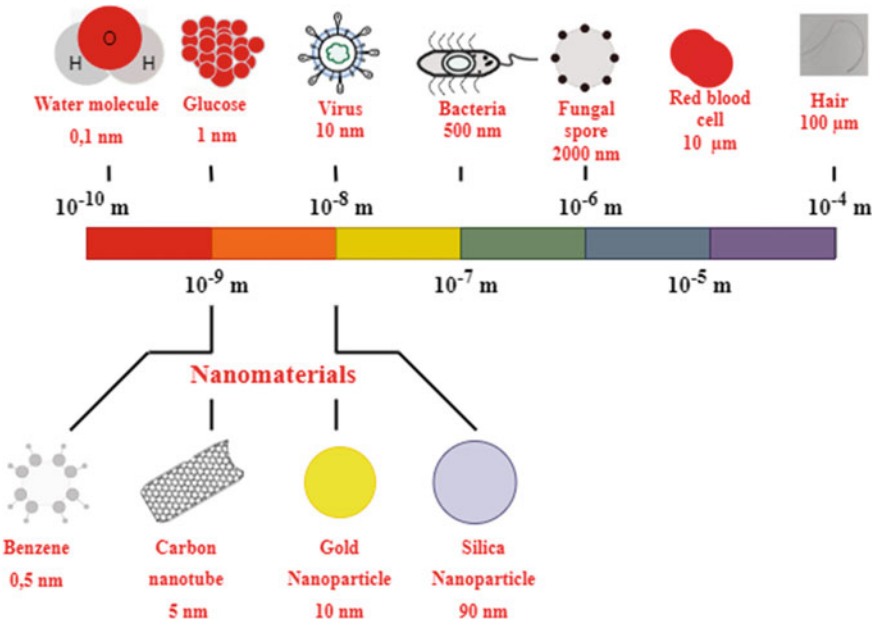

Fig. 1 Nanoscale integration of biomolecules and nanomaterials

In general, the synthesis conditions are known to directly affect the application areas of NMs. Synthesis of NMs with uniform morphology and stable structure is possible in high temperature and organic solvent environment. But this synthesis medium changes the solvent type of NMs. NMs that are insoluble in the aqueous phase quickly coagulate and aggregate in the biological application medium. In addition, organic NMs are toxic to living organisms [6, 42, 83]. Therefore, the production of NMs in an aqueous and non-toxic phase is very important [49].

2 Synthesis Types of Nanomaterials and Characterization

A general synthesis system for colloidal NMs consists of three components. These are: precursors (metal, polymer, nonmetal, organic–inorganic compounds, etc.), reducing agents (such as oleamine, oleic acid, sodium borohydride, sodium citrate), and solvents (organic or inorganic solvents. By heating the reaction medium to a sufficiently high temperature, the precursor molecules are converted into chemically active atomic or molecular species (monomers). The resulting monomers grow with active reducing agents to form NP structures [45]. Then the growth of core structures takes place. In nucleation, the precursor monomer must be at a relatively high temperature for the molecules to reach the upper saturation level. Thus, the reaction begins, followed by a nucleation explosion. These nuclei can grow further using additional monomers with the aid of temperature or other triggering conditions. It is possible to obtain NPs with wide size distribution by adding monomer and varying the amount of reducing agent [60].

There are two basic approaches in the synthesis of NMs. The first of these is the "top-down" approach. It is based on nanoscale material fabrication from bulk material. In general, the size of the material is reduced by physical–mechanical approaches. The other is the "bottom-up" approach. It relies on combining atoms to create nano-sized materials. In the top-down approach, although NMs are produced for industrial applications, problems in size control of NMs have serious disadvantages due to structure and morphology errors [19, 68]. The morphology, chemical structure, and size distribution control of NMs were successfully obtained with a bottom-up approach. However, despite all its advantages, the production efficiency is quite low compared to the top-down approach [11, 16]. Chemical methods such as micro-emulsion/colloidal, solvothermal, thermal separation, and laser methods are commonly used in the bottom-up approach [19, 58, 68]. Widely used methods for NMs synthesis in "bottom-up" approach are chemical and biological methods.

2.1 Chemical Synthesis Nanomaterials

The chemical synthesis method is generally based on the reduction of the precursor material with surfactants at the appropriate environment and temperature with

different reducing agents [103]. In this method, rapid, uniform morphology and narrow size distribution NM synthesis is possible depending on the use of organic solvent, long chain reducing agent and high temperature [88]. Synthesized NMs retain their properties for a long time without degradation or aggregation. With this method, NMs can be synthesized with various functional properties or can be functionalized after synthesis [86]. This flexible synthesis method paves the way for the emergence of many NM designs. Chemical synthesis methods are frequently and effectively used in the literature [108]. Commonly used methods: Microemulsion, co-precipitation, and thermal decomposition are synthesis strategies. The disadvantage of this method is that the synthesized NMs are hydrophobic due to their surface [106]. This condition is toxic to living organisms. Therefore, the use of synthesized NMs in biological applications is limited. There are various applications to prevent this situation [53, 119]. These disadvantages can be overcome by converting the surface ligand structure to the aqueous phase. Ligand exchange methods are obtained by optimizing critical parameters such as the surface structure, adhesion strength, and bond structure of the NP [51]. One of the most basic methods of increasing the solubility of hydrophobic NPs in aqueous solution is based on the placement of new ligands and the removal of the hydrophobic layer from the surface [52, 63]. In the literature, there are main methods known as ligand exchange or phase transfer on NP surfaces [50, 110]. The main ones are as shown in Fig. 2, Ligand modification with amphiphilic micelles, Bifunctional ligand modification, Polymer coating method, and Silica coating method [116].

In a study, the transfer of Au NPs synthesized with oleamine surfactant in the organic phase to the aqueous phase was successfully carried out. The organic ligands on the Au NP surface were cleaned simply and quickly with a special synthesis method, and amino phenyl boronic acid ligands dissolved in the aqueous phase were attached to their place simultaneously. The effectiveness on bacteria was tested with the subsequent application [49] (Fig. 3).

2.2 Green Synthesis Nanomaterials

Synthesis of NMs by biological methods, different biomolecules; Enzymes, peptides, protein groups, nucleic acids (DNA and RNA), plant extracts, standard molecules as well as some microorganisms such as algae, fungi, actinomycetes, bacteria, and viruses can be used as reducing agents [65, 79, 81]. There are many advantages in the application of biological methods. At the beginning of these; less chemical use, cheap, practical, and fast application, and being suitable for high-volume production. In addition, biological synthesis applications do not require the use of high pressure, high energy, temperature, and toxic chemicals [4, 64, 78, 80, 113, 117, 121, 128]. It is possible to examine biological NM synthesis in two parts.

First, in the bio reduction method: it is the use of microorganisms and microorganism enzymes in the reduction of metal ions. With this method, stable and inert materials can be synthesized cheaply and quickly [23, 83, 91, 105]. The other method

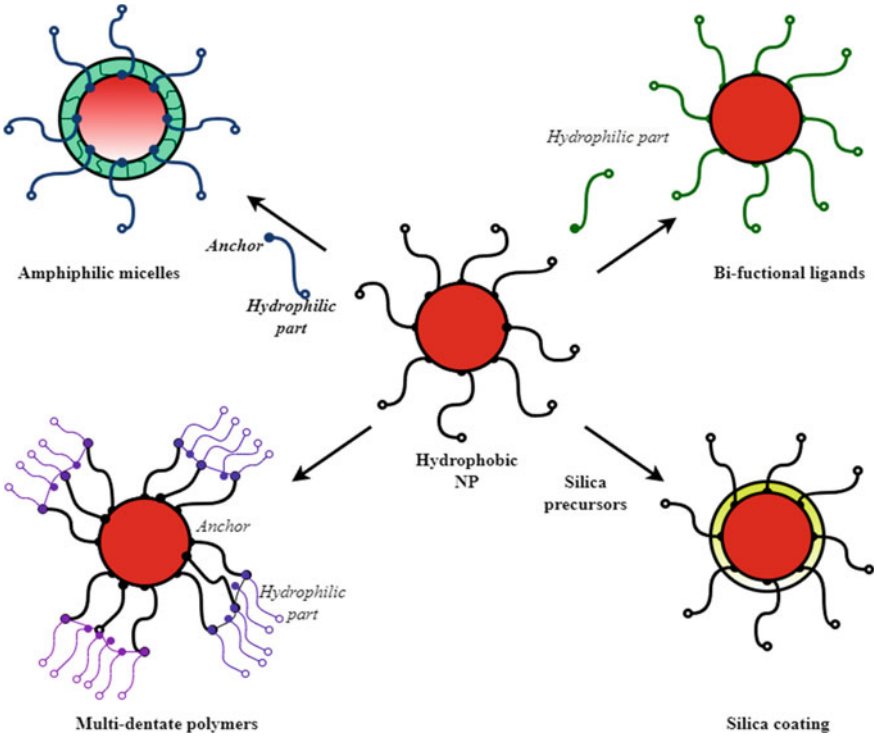

Fig. 2 Phase transfer methods for nanomaterials

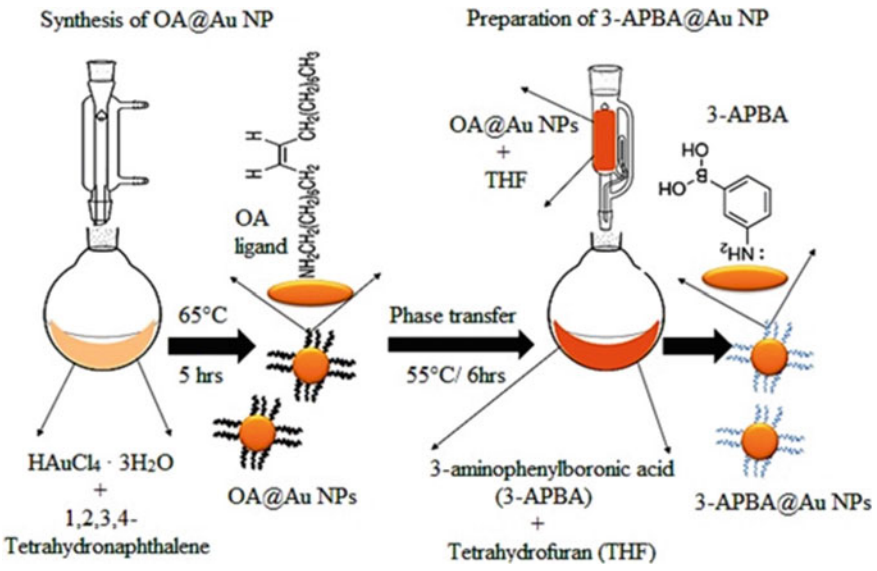

Fig. 3 Ligand exchange of Au NP in organic phase to aqua phase [49]

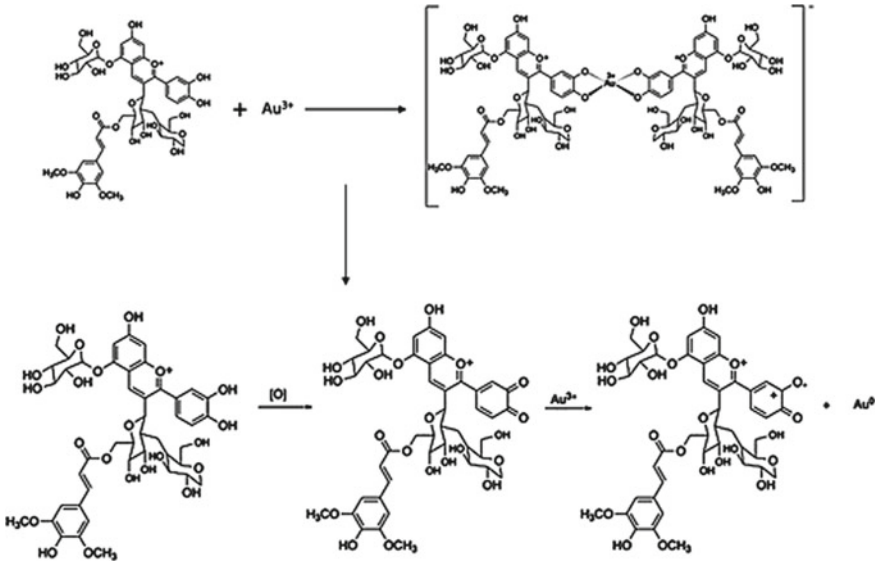

Fig. 4 Synthesis mechanism for Au NP formation containing three following steps: (1) Nucleation for formation of seeds; (2) Growth of seeds; and (3) Formation of Au NPs [23]

is the biosorption method. In this method, it is a NM synthesis method in which stable NPs are obtained by the interaction of metal cations in the solution medium, cell wall, or peptide [24, 56, 114].

As shown in Fig. 4; In a study, red raspberry (*Rubus idaeus*), strawberry (*Fragaria ananassa*), and blackberry (*Rubus fruticosus*) extracts were used for the synthesis of Au NPs. Synthesis of monodisperse, stable, and colloidal Au NPs (Au NPs) with $HAuCl_4 \cdot 3H_2O$ and strawberry extract concentration has been reported. Anthocyanin molecules give preferential coordination reaction with Au ions (Au^{3+}) to form NP seeds (anthocyanin-Au^{3+}), and then catecholamine oxidation results in electron flow from anthocyanins to Au. Thus, NP seeds begin to form for anisotropic growth. Finally, the surface of Au NPs is saturated with anthocyanins and monodisperse, and stable Au NPs are obtained [23].

3 Plant Pathogens

Plant pathogens are one of the most serious threats to agricultural productivity and food security worldwide. The main deformations caused by pathogens in the plant are: leaf spot, crusting, blight, overgrowth, rot, and galls [12, 31]. Plant pathogens can be found in various parts of the plant, especially in xylem and phloem. In particular, bacteria usually settle in intracellular and intercellular spaces and invade parts of the plant such as leaves and roots [5]. Diseases that develop in plants due to bacteria

follow a different course compared to other pathogens. Bacteria can secrete specific enzymes that break down the plant's cell wall. In addition to enzymes, various plant hormones, polysaccharides, and proteinases may also contribute to the formation and development of bacteria [15, 29, 130]. Thus, the environment for the development of diseases in the plant is prepared.

Another type of plant pathogen is fungi. About 8000 species of fungi and oomycetes are known that can reduce yields in agricultural applications [28]. Diseases commonly caused by pathogenic fungi; leaf spot, anthracnose, blight, thrush, gall, powdery mildew, and root rot [46]. Traditional methods for the detection of fungal species, polymerase chain reaction (PCR) [18] and isothermal amplification methods (LAMP) are used. Phytoviruses constitute approximately 50% of known plant pathogens [10]. Viruses can reproduce easily and quickly in natural events (rain, wind, etc.) and insects [30, 34]. Because of all these effects, phytoviruses pose a serious threat all over the world. Phytoviruses contain simple ribonucleic acid (RNA). However, deoxyribonucleic acid (DNA)-based genomes, factors that help isolation (human, environment, animal, etc.), and rapid adaptation processes in the face of changing conditions make it difficult to control phytoviruses [43]. Phytoviruses frequently encountered in food agriculture; Tobacco mosaic virus (TMV) [2], Pepper mild mottle virus (PMMoV) [73], cucumber mosaic virus (CMV) [37], tomato yellow leafroll virus (TYLCV), Papaya Ring Spot Virus [27], Barley yellow mosaic virus (BaYMV), Turnip mosaic virus [38] (Table 1)

4 Nanomaterials as Nano-Weapon for Combating with Plant Pathogens

Plant pathogens are one of the biggest threats to crop productivity and agricultural sustainability. The most widely used method in the fight against pathogens is pesticide application. Today, very effective drugs are used to protect plants [109]. However, pathogens develop resistance to these plants over time. Thus, drug applications lose their effectiveness [48]. As another spraying method, it has been applied to reduce the use of pesticides [57]. However, in this case, only 1–5% of the applied pesticide amount was able to inhibit the target pathogen [47, 87]. In addition, the applications cause contamination of the soil and water, causing the pollution of the environment and the infertility of the soil. Therefore, early, and rapid detection of plant pathogens is critical to protect farmland and increase food productivity. There are many methods in the literature for this purpose [89, 90].

One of these methods is to increase the yield of pesticides and chemical fertilizers applied to plants. It is aimed to increase the activity of chemical fertilizers and pesticides applied for this purpose by reducing them to nano size. Carbon nanotubes, magnetic NPs, Au NPs, silver NPs, and polymeric NPs are the leading nano-sized designs.

Table 1 Plant and plant pathogens examples [14]

Culture	Virus	Fungi	Bacteria	Symptoms
Almond			*X. arboricola pv.Pruni* [67]	Leaf. stem, and trunk injuries. Defoliation and fruit drop
Apple plants			Erwinia amylovora/ pseudomonas syringae pv. syringae [17]	Storage disease in apple with evident moisture formation on the frnit Wilt and blackening twigs, flowers, and leaves Leaf necrosis and systemic vascular wilt
Citrus plants	*Citrus Tristeza* [40]		*Candidatus Liberibacter* [111]	Decline of plants and yellowing of leaves yellowing of shoots, leaf spot decrease in size, and deformity of the fruit
Cucumber plant		*Oidium neolycopersici* [32]	*Pseudomonas syringae pv. Lachrymans*	Chlorosis and white powdery lesions on the leaves. Rapid aging and reduction in the size and quality of the fruit leaves with water-soaked lesions. Necrosis and reduction of photosynthetic capacity
Brassica			*X. campestris* [92]	Leaf necrosis with V-shaped lesions and blackened vascular bundles

(continued)

Table 1 (continued)

Culture	Virus	Fungi	Bacteria	Symptoms
Ginseng		*Alternaria panax Whetz* [118]		Reddish to dark brown elongated lesions
Maize plants		*Arbuscular mycorrhizal* [21]		Change in root mass, length, or architecture.
Orchids	Cymbidium mosaic/ odontoglossumringspot [3]			Leaves and flowers with necrotic chlorotic stains, growth inhibition color break in flow as and spots yellowing on leaves
Pear plants			*Erwinia amylovora* [107]	Wilt and blackening twigs, flowers, and leaves
Pepper plant		*Oidivm neolycopersici* [32]		Chlorosis and white powdery lesions on the leaves Rapid aging and reduction in the size and quality of the fruit
Plantsap samples			*Xanthomonas axonopodis* [36]	Fruit stains, leaf falls and fruit tree decline
Potato	*Leafroll virus* [107]	*Phytophthora infestans* [104, 126]		Tuber is stunted and erect. Rigid, curled leaves. Leaves like brownish-purple oily patches. Leaves with grayish white mycelium rings and spores
Scots pine		*Mycorrhizal colonization* [99]		Change in root mass, length, or architecture
Stone fruit trees	*Plum Pox*			Leaves with stains or chlorotic rings, unblocking of veins. Deformed fruits

(continued)

Table 1 (continued)

Culture	Virus	Fungi	Bacteria	Symptoms
Strawberry		*Rhizopus* sp. *and Aspergillus* sp. *section Nigri/P. cactorum*		Grayish color for Rhizopus and black appearance at Aspergillus infected fruit & Leaf size reduction and decreased productivity
Tabaco	Tobacco mosaic virus [25]	Yellow leaf curl virus [104]		Leaf with chlorine or mosaic with white to light green color
Tomato crops	Yellow leaf curl virus [98]	*Oidium lycopersicum/ Phytophthora infestans* [66]		Infected leaves are small, yellow in color and curve upwards. Leaves, petioles, and stems have lions superficial with white powdery. Desiccation, necrosis, and

Carbon nanotubes help it bind nitrogen from ammonia and release hydrogen ions. Thus, the nitrogen (N), phosphorus (P), and potassium (K) uptake of the plant can be improved [123, 124]. In addition, the fast electron transfer of the carbon element is preferred in bio-nanosensor applications due to the larger length/diameter ratio of the nanotube structure. CNTs are particularly sensitive in the identification of phenolic compounds [9, 125].

Au NPs are frequently used in the diagnosis and treatment of pathogens [44]. Thanks to its optical properties, it is widely used as a targeting agent. Au NPs functionalized with DNA, enzymes, proteins, or other biomolecules play an important role in inhibiting and destroying phytopathogens [47]. DNA-based biosensors are analytical tools used for the detection of sequence-specific DNA in plant pathogens. These tools are widely used due to their rapid production, cost-effectiveness, and sensitive and fast results. The use of AuNPs in DNA biosensor design provides rapid DNA hybridization by immobilization of DNA on the probe electrode and aids in the labeling of pathogens [96]. In one study, Colloidal AuNPs were used to label single-stranded DNA (ssDNA) specific to the bacterium *Acidovorax citrulli*, which causes fruit spots. For this purpose, a Strip-based DNA sensor was designed for the detection of pathogenic bacteria. With the designed biosensor, an analysis system

sensitive enough to detect 4 nm target DNA was obtained [129] In the diagnosis of *Pseudomonas syringae* bacteria, which causes serious damage especially in crop plants, colorimetric detection of pathogenic DNA molecules was achieved by using AuNP-based DNA probes. In this way, when AuNPs specifically targeted to the bacteria of interest were hybridized with the target DNA, the color of the sensor changed from red to purple.

Silver NPs can inhibit plant pathogens quickly and effectively. When the studies were examined, it was seen that effective results were obtained on plant pathogens due to the high antibacterial/antifungal activity of Ag NPs [20]. In the studies carried out for the inhibition of *Cochliobolus sativus* and *Magnaporthe grisea* fungi on *Lolium perenne*, both the ionic form and the nano form of silver were investigated. It has been proven that nano-sized silver has a higher antifungal effect than its ionic form. In another study, the effect of silver NP concentration on pathogens was investigated. It has been determined that the application of 100 ppm Ag NP before and after the disease appears plays an important role in controlling plant diseases [55].

ZnO NPs are metal oxide NPs that are often preferred because of their biocompatibility, high stability, low toxicity, and less cost compared to plasmonic NPs [85]. ZnO NPs were used to combat *Xanthomonas oryzae* pv.oryzae, a highly contagious pathogen. *Paenibacillus polymyxa* bacterial strain was used as reducing agent in NP synthesis [75]. In the study, positive results were obtained in the leaf blight disease seen on the leaves of the rice plant. Aqueous extraction of leaves of Mentha spicata plant was used as reducing agent in green synthesis of ZnO NPs. Spherical NPs in the range of 10–90 nm were synthesized and its anti-viral activity against Tobacco mosaic virus (TMV) were investigated and successful results were obtained [1].

With the nanoencapsulation method of all chemicals used on plants, structures that allow the slow release of active ingredients are being developed [94]. In this context, by changing the solubility ratio of nanocapsule structures in aqueous media, the mixing ratio of active components in water and soil can be controlled. In a study, nano capsule surface design was made using poly(ethylene)glycol polymer. With this structure, a controlled release (CR) is made, allowing the active ingredients to be effective on plant pathogens for a long time. In another study, carbon nanotube-citric acid (MWCNT-g-PCA) combined multilayer capsule design was created. This capsule design was studied on the fungus *Alternaria alternata.* With this design, inhibition of *Alternaria alternata* was achieved much faster and more effectively than in bulk pesticide applications [101].

5 Use of Nanomaterials for Detection and Identification of Plant Pathogens

For rapid plant pathogen detection, many molecular analysis methods have been developed. However, these methods are generally high cost, slow, equipment and operator-requiring applications [61]. In the detection of plant pathogens, on-site and

rapid analysis methods are generally advantageous in terms of application. For this reason, the existence of fast and efficient analysis methods at nano scale is important [22]. There are many studies in the literature for this purpose. Microneedle applications, nano-barcoding systems, nano-biosensors, miRNA-based nano diagnosis/array-based nano-sensors, nano-diagnostic apparatus are recommended for plant pathogen diagnosis [102].

In a study, easy and effective detection of *Phytoptera infestans*, which is common in potatoes and tomatoes, was reported using a polymer-based microneedle patch approach [90]. In another study, a sensor system was reported in which plant pathogens were detected quickly by using microneedle patch application. In the application, the plant leaf was compressed in the sensor and quickly extracted. The obtained DNA and RNA samples were compared with the reference samples to provide the diagnosis. In this sensor application, the diagnosis process can be completed in 30 min in a phone application in coordination with the microneedle patch system [89] (Fig. 5).

Another application developed for the rapid and effective detection of plant pathogens is the nano-barcoding system. This method is extremely efficient for the identification of specific enzyme-free proteins and nucleic acids. Compared to traditional ELISA based applications, it can give extremely sensitive results at low intensity [77]. In a study, it has been shown that the target protein can be quickly taken from the plant and analyzed with oligonucleotide-modified magnetic Au NPs (Au-MNPs). This study shows promise in terms of enabling rapid detection of sample DNA by optimizing other protein targets to the system [35].

Another method used in rapid diagnosis and diagnosis of pathogens is nanosensors. Rapid and early detection of plant pathogens is possible with nano-bio sensors

Fig. 5 An integrated MN-smartphone microneedle amplification platform [89]

designed using especially metallic NPs, carbon nanotubes, and quantum dots [69]. In plant infections, the defects caused by bacterial and fungal microorganisms can be detected early and precautions can be taken with nanosensors containing microfluidics [8]. In a study conducted for this purpose, *Aspergillus Ochraceus* could be detected early with an immunoelectrode system designed with chitosan-doped SiO_2 NP. A sensor that detects zearalenone produced by the fungus *Fusarium Oxysporum* in a fast and sensitive way has been reported. In addition to these, a nanosensor was designed with layered nanocarbon structures to quickly detect zearalenone mycotoxin in corn silages. In another study, an electrochemical immune sensor design that detects Aflatoxin M1 (AFM1) produced by the mold fungus *Aspergillus flavus* at very low concentrations (up to 0.01 ppb) has been reported [41]. In another study, a PCR technique applied together with AuNP-based lateral flow assay (LFA) was proposed for the detection of *P. infestans* fungus in potato plant. In the study, asymmetric PCR technique was applied to obtain a large amount of ssDNA, then the prepared sample was compacted by sandwich hybridization in LFA. In the presence of pathogen DNA, the AuNP probe on the test line of LFA turned red with the accumulation of target DNA on the kit. Afterward, the sensitivity of the kit was investigated by changing the ssDNA density. As a result of the study, it was shown that the hybrid sandwich kit prepared could detect the pathogen at the level of 0.1 pg/L [126].

A fast and portable colorimetric sensor was designed for the detection of *Aspergillus niger* fungus using Au NPs modified with a specific binding peptide ligand (Fig. 6). In this colorimetric sensor, rapid binding of *A. Niger* spores to Au NPs was achieved with peptide ligand. Upon binding of the peptide with spores, Au NPs coagulate, and a color change is observed in the separated supernatant. This design is simultaneously linked to a phone application. At the same time, it gives results up to 50 spores precision in less than 10 min.

The use of microfluidic techniques for the detection of plant pathogens is quite common in the literature [62].

The use of microfluidic devices with other plant pathogen detection systems is possible in device design. In a study, a microfluidic device design used in the diagnosis of viruses is presented. A microfluidic device was designed using an embedded optical fiber system and polydimethylsiloxane polymer. It was then tested using the leaves and flowers of the *Phalaenopsis amabilis* plant. With its RNA isolation and purification feature, this device can detect viruses quickly and instantly [54].

6 Conclusion and Future Remarks

In conclusion, NMs have shown great potential for rapid identification of agriculturally important plant pathogens. Biosensors, microneedle analysis kits, and many more metal oxide NP-based systems have been used to provide fast, simple, and highly sensitive tools to diagnose plant diseases. However, while these techniques show great promise, much work remains to be done to fully realize the potential of NMs for the rapid identification of plant disease pathogens. For example, many

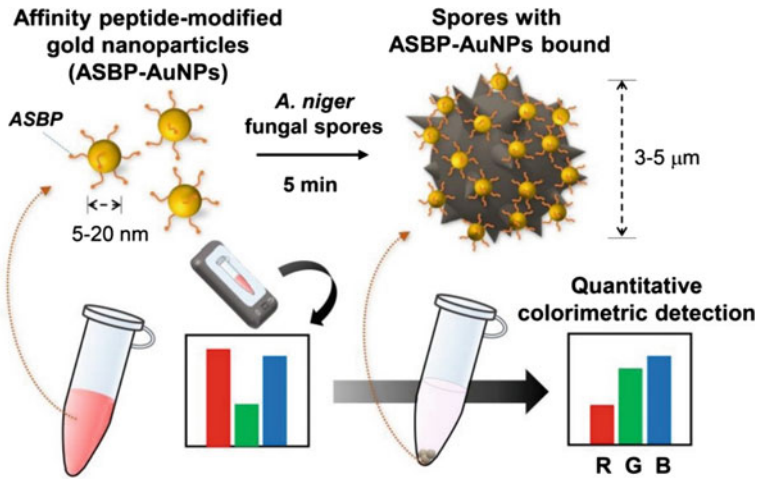

Fig. 6 Detection system and colorimetric sensor of ASBP modified Au NPs (ASBP-AuNPs) via UV/Vis spectroscopy or smartphone-based image analysis [62]

of these techniques are still in the early stages of development and require further refinement and optimization. In addition, there are some challenges that need to be addressed, such as the potential toxicity of NPs and the cost of commercialization.

In the future, we are likely to see continued progress in the development and application of NMs for rapid identification of agriculturally important plant pathogens. As these techniques become more widely adopted, they have the potential to significantly impact the way we detect and control plant diseases, helping to protect crops, reduce crop losses, and improve food security. In addition, the destruction of used diagnostic kits poses a great environmental threat. There is no comprehensive study for the destruction of NPs and quantum dots contained in sensors. On the other hand, considering the one-time use of the kits and the environmental pollution caused by the content, as well as the costs during production, it is thought that it will cause serious economic losses.

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Emerging Role of Nanotechnology-Based Devices for Detection of Environmental Contaminants

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Abstract Nanotechnology has opened up new possibilities for detecting and monitoring environmental pollutants. Nanodevices, which are devices at the nanoscale level, have emerged as a promising tool for pollution detection due to their unique physical and chemical properties. Nanodevices can be designed to detect a wide range of pollutants such as heavy metals, organic compounds, and gases. They offer several advantages over conventional detection methods such as high sensitivity, selectivity, and specificity. Additionally, they are small, portable, and cost-effective, making them ideal for field-based monitoring of pollution. One of the most promising applications of nanodevices for pollution detection is in water quality monitoring. For example, researchers have developed nanodevices that can detect heavy metals in water at very low concentrations. These nanodevices work by binding to the heavy metal ions, producing a measurable electrical signal that indicates the presence and concentration of the pollutant. Nanodevices are also being used to monitor air quality by detecting harmful gases such as nitrogen oxides, carbon monoxide, and sulfur dioxide. These nanodevices are designed to be small and lightweight, making them ideal for integration into portable air monitoring devices. In conclusion, nanodevices are emerging as a powerful tool for pollution detection and monitoring. They offer high sensitivity, selectivity, and specificity, and can be designed to detect a wide range of pollutants. As the technology continues to advance, nanodevices are likely to play an increasingly important role in protecting the environment and human health. In this chapter, we discuss the emerging roles of gold, silver, copper, and titanium nanoparticles-based nanodevices that are being used for pollutant detection.

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1 Introduction

Nanoparticles are tiny particles with sizes ranging from 1 to 100 nm. They can be made from a variety of materials, including metals, semiconductors, and polymers, and can have unique properties that differ from their bulk counterparts [12]. Due to their small size, nanoparticles have a high surface area-to-volume ratio, which makes them highly reactive and useful in a range of applications, from medicine to electronics. Nanoparticles can be synthesized using a variety of methods, including physical, chemical, and biological approaches. Physical methods include milling, laser ablation, and lithography, while chemical methods include sol-gel synthesis, precipitation, and hydrothermal synthesis [24]. Biological methods involve the use of living organisms, such as bacteria or fungi, to produce nanoparticles.

Nanodevices are small devices that can detect and analyze environmental pollutants at a very small scale. These devices can be used to monitor and analyze air, water, and soil pollution, among other things [1]. Nanodevices use nanotechnology to detect and analyze pollutants, and they can provide more accurate and precise results than traditional detection methods. One example of a nanodevice for environmental pollutant detection is a carbon nanotube-based sensor. These sensors can detect pollutants such as carbon monoxide, nitrogen oxides, and volatile organic compounds in the air [32]. Carbon nanotubes are extremely small and have a large surface area, which allows them to interact with pollutants and detect them at very low concentrations. Another example is a nanodevice based on gold nanoparticles. These sensors can detect heavy metals such as lead, mercury, and cadmium in water [28]. The gold nanoparticles are functionalized with specific molecules that can bind to the heavy metals, allowing them to be detected at very low concentrations [35]. Nanodevices have the potential to revolutionize environmental monitoring and pollution control. They can provide more accurate and precise data, and can be used to detect pollutants in real-time. They are also more cost-effective than traditional methods of pollutant detection. However, there are still some challenges to be overcome, such as ensuring the reliability and durability of the devices, and addressing potential environmental and health risks associated with their use.

Also, nanodevices have shown great potential for detecting allergens in food and other environments. One example of a nanodevice for allergen detection is the immunosensor, which uses nanoscale materials to detect specific allergens. Immunosensors work by using antibodies that are attached to a nanomaterial surface [19]. When the allergen comes into contact with the antibody, it binds to the surface and produces a measurable signal, such as a change in electrical conductivity. This signal can be detected and used to identify the presence and concentration of the allergen [19]. Other nanodevices for allergen detection include nanomaterial-based optical sensors, which use changes in light absorption or reflection to detect allergens, and nanomaterial-based electrochemical sensors, which measure changes in electrical current to detect allergens.

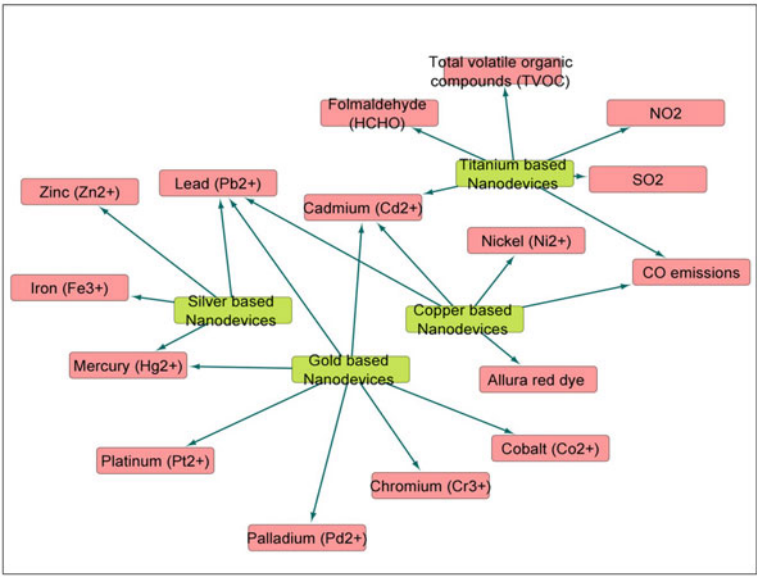

Fig. 1 Pollutant remediation capabilities of Gold, Silver, Copper, and Titanium based nanoparticles. The green nodes represent the nanoparticle-based nanodevices and the pink nodes represent pollutants that they are capable of remediating. Note that three nanoparticle-based nanodevices are capable of remediating lead (Pb^{2+}), two nanoparticle-based nanodevices are capable of remediating cadmium (Cd^{2+}) and two nanoparticle-based nanodevices are capable of reducing CO emissions

Overall, nanoparticles have unique physical and chemical properties that make them attractive for the development of nanodevices for environmental pollutant detection and remediation. Nanoparticles can be designed to selectively bind to specific pollutants and generate a detectable signal in response to their presence. Again, nanodevices have the potential to provide rapid and accurate detection of allergens, which is important for individuals with allergies and for ensuring the safety of food and other products. This chapter discusses various nanodevices based on gold, silver, copper, and titanium that are being used for environmental pollutant detection (Fig. 1).

2 Gold Nanoparticle-Based Nanodevices in Environmental Pollution

The nanoparticles based on gold also called Gold Nanoparticles or GNPs have been heavily used to prepare nanodevices and engrossed in different fields of biology including medical sciences [7]. It has been shown, that these gold-based nanodevices are very useful in detecting environmental pollutants such as heavy metals as they

show the typical surface plasmon resonance and absorption property depending on the shape, dimensions, and intermolecular distance [45]. One such device was shown by Wei Ha et al. who developed an eco-friendly heavy metal detection process that uses GNPs orchestrated with *Xanthocerus sorbifolia* tannin as a color imparting probe. Here the Cr^{3+} detection both in river water and tap water was done by colorimetric method after *Xanthocerus* stabilized GNPs were successfully able to chelate with $Cr^{3+.}$ Subsequently the aggregation of GNPs induced the change in color from red to purple quickly [10]. Similar device was developed to detect Hg^{2+} ions in aquatic environment where citrus fruits such as *Citrus limon* and *Citrus limmeta* were used to prepare gold-based nanoparticles and colorimetric detection technique was employed to search Hg^{2+} in micromolar concentration in water [29]. It has been found that the Hg^{2+} ions can also be detected by colorimetric method using gold nanoparticles functionalized by poly gamma glutamic acid (PGA) [9]. Negatively charged PGA was assembled using an electrostatic self-assembly process on top of positively charged cetyltrimethylammonium bromide (CTAB)-capped GNPs. The color of the solution would evolve from light red to purple blue as the quantity of Hg^{2+} increased. With correlation values of 0.998 and 0.991, respectively, the results demonstrated that the absorbance ratio (A750/A580) was linear with the Hg^{2+} concentration in the range of 0.01–10 μM and from 50 to 300 μM. The determination of Hg^{2+} in tap water and mineral water using this method was effective, with recoveries ranging from 90 to 103% and from 103.53% to 113%, respectively. The suggested approach allows for the quick, inexpensive, and equipment-free analysis of Hg^{2+} in a variety of water samples. The polluting agent Pb^{2+} can also be detected by GNPs. In aqueous solution, Au^{3+} is stabilized and transformed into gold nanoparticles by glutathione (GSH) [23]. These GNPs aggregate in the presence of Pb^{2+} ions in NaCl containing aqueous solution and can be monitored by both colorimetrically and UV-vis spectroscopy [23]. Another device for detecting Pb^{2+} was developed by Karuvath et al., where gallic acid was used to produce GNPs at room temperature [42]. To detect the presence of Pt^{2+}, Pd^{2+}, and Co^{2+} at micromolar concentration peptide-functionalized GNPs have been demonstrated as useful nanodevices [33]. An important biomarker for tracking plant damage caused by heavy metal stress is vitronectin-like proteins (VN), which are found on the surface of plant cells. To track hidden damage to plant cells brought on by cadmium (Cd) or lead (Pb), a live plant cell-based biosensor has been developed [39]. L-cysteine was first changed on a glassy carbon electrode, then anti-IgG-Au antibody, in order to create this sensor. The live plant cells were then modified onto the electrode and treated with the anti-VN. By detecting changes in electrochemical impedance, the sensor operated. In the linear dynamic ranges of 45–210 and 120–360 μmol L^{-1}, respectively, Cd and Pb were identified. Additionally, this biosensor's Cd and Pb detection limits were 18.5 nmol L^{-1} and 25.6 nmol L^{-1}, respectively [39]. Pb^{2+} can also be detected rapidly in soil by producing GNPs strip biosensor functionalized by GR-5 DNAzyme. Here the graphene oxide provides assistance to detect Pb^{2+} ions specifically [37]. In the presence of additional divalent metal ions, the strip biosensor displayed high selectivity toward Pb^{2+}. The obtained recoveries for actual soil samples ranged from 91.5 to 113.1%. Thus, gold nanoparticle-based devices are emerging as technological breakthrough in environmental pollution detection.

3 Silver Nanoparticle-Based Nanodevices in Environmental Pollution

Silver-based nanodevices can be an effective tool for detecting environmental pollution. Nanoparticles of silver have unique optical and electronic properties because they are capable of absorbing and scattering light efficiently [6]. This property can be utilized in a variety of sensing applications. Heavy metal ions are a major source of environmental pollution [5]. Silver nanoparticles can be functionalized with ligands that selectively bind to specific metal ions, allowing for their detection in environmental samples. Based on a linear change in the strength of the surface plasmon resonance absorption, it is shown that polyvinylpyrolidone-modified silver nanoparticles (AgNPs) can detect the concentration of the heavy metal contamination Fe^{3+} ions in water [27]. Another study reported that Hg^{2+} and Cu^{2+} detection in water is possible using various concentrations of AgNPs [22]. In order to identify Hg^{2+} present in water using a colorimetric approach, AgNP was functionalized using 3-mercapto-1, 2-propanediol (MPD). When Hg^{2+} solution was added to MPD-functionalized AgNP (MPD-AgNP), new peak at about 606 nm appeared. The aggregations brought on by MPD-AgNP's detection of the heavy metal ion Hg^{2+} through the dipropionate ion may be the cause of the new peak. Also, neem extract-based AgNPs offer good solution for eradicating heavy metal toxicity. It was reported that at micromolar concentrations, sun-dried neem leaf extract-based AgNPs (ND-AgNPs) selectively sense Hg^{2+} and Pb^{2+} [15]. AgNPs made from neem bark extract demonstrated selective colorimetric sensing of Zn^{2+} and Hg^{2+}. AgNPs made from green tea extract (GT-AgNPs) and mango leaf extract (MF-AgNPs) also demonstrated selective colorimetric detection of Hg^{2+} and Pb^{2+} ions [15]. Hg^{2+}, Pb^{2+}, and Zn^{2+} selective colorimetric sensor characteristics were present in AgNPs made from pepper seed extracts. Importantly, these environmentally friendly synthetic AgNPs were capable of detecting the presence of dangerous metal ions in aqueous solutions throughout a wide pH range (2.0–11), which is a highly desirable property from the standpoint of various water pollution sources.

Silver nanoparticles can be functionalized with biomolecules or polymers that selectively bind to organic pollutants, such as pesticides or hydrocarbons. This can allow for the detection of these pollutants in environmental samples. A sizable portion of water contaminants are organic pollutants. They damage aquatic life and terrestrial life through drinking water when present in water. Pesticides, organic dyes, pharmaceuticals, nitro-aromatics, and mycotoxins are just a few of the several forms of organic pollutants that can be found in the environment. In agricultural production, pesticides are used to lessen crop damage from weeds and pests [44]. Organic dyes, which are utilized in textiles, leather, paints, papers, and plastics, are made up of a generous number of intricate aromatic compounds [43]. Due to their severe toxicity, nonbiodegradability, and potential to change into agents that are carcinogenic, teratogenic, and even mutagenic, pesticides and organic dyes have garnered a lot of attention as environmental pollutants from a worldwide perspective [13]. For the detection of pharmaceuticals, nitro-aromatics [30], pesticides [11], organic dyes

[8], and mycotoxins [17], AgNP-based optical sensors have been described. When compared to optical sensors, electrochemical sensors, such as those based on AgNP, are thought to be more capable of detecting organic contaminants with enough sensitivity and selectivity [40]. They also take less time to set up and take less effort. While different targeted analytes need to be transformed into detectable species for optical sensors, targeted analytes can be detected immediately by electrochemical sensors. Electrochemical sensors can be used for in situ studies since they can directly detect the desired analytes. Electrochemical sensors can also track the evolution of analyte concentration over time.

Silver nanoparticles have been shown to have antimicrobial properties, which can be utilized in the detection of bacteria and viruses in environmental samples. It has been proposed that the lipid-enveloped virus's exterior membrane can be bound by silver nanoparticles (AgNPs) to stop infection [18]. Nevertheless, little is known about how AgNPs interact with viruses. AgNPs have been examined specifically in relation to HIV, where it was shown how the nanoparticles work against viruses as well as how they prevent the spread of HIV-1 infection in human cervix organ culture [18]. Silver nanoparticles can be incorporated into gas sensors to detect air pollutants, such as carbon monoxide [16]. Overall, silver-based nanodevices have the potential to be an effective tool for detecting environmental pollution. However, more research is needed to optimize their performance and develop practical applications for their use.

4 Copper Nanoparticle-Based Nanodevices in Environmental Pollution Detection

Copper-based nanodevices can potentially be used for environmental pollutant remediation. Copper nanoparticles have been shown to have antibacterial properties. The chitosan-copper nanoparticles' exceptional high surface-to-volume ratio allows them to make contact with the *P. aeruginosa* cell membrane through its surface, ultimately killing *P. aeruginosa* [36]. Thus, it can be used to remove pollutants from contaminated water. They can also be used to detect pesticides and dyes. Like, Thiram is essential in preventing many crop diseases from harming fruits and vegetables, but its leftovers have a negative impact on the environment and pose a substantial risk to human health. According to a study, Tween 80-capped copper nanoparticles (Tween 80-CuNPs) are a practical and affordable colorimetric probe for the targeted detection of the pesticides thiram. The CuNPs-based colorimetric probe with a Tween 80 cap demonstrated good selectivity and high sensitivity (LOD around 0.17 M). The maximum residue limit (MRL) set by the governments of the Europe and Vietnam was found to be higher than the thiram limit of detection (LOD) of the proposed sensor [3]. Copper nanoparticles can also be used to remove heavy metals from water by adsorbing them onto their surfaces. The adsorption application of CuO NPs

on the removal of Pb^{2+}, Ni^{2+}, and Cd^{2+} is shown to be dependent on the nanosorbent dosage, the metal ions concentration, pH, and the contact duration, as demonstrated by the green CuO NPs synthesized using mint leaf and orange peel extracts as reducing agents. These metal ions had an affinity for CuO NPs in the order $Pb^{2+} > Ni^{2+} > Cd^{2+}$. For demonstrating wastewater remediation under typical environmental circumstances, the removal efficiency of Pb^{2+}, Ni^{2+}, and Cd^{2+} was determined to be 84.000, 52.50%, and 18,000%, respectively, and attained at pH 6. With CuO NPs-1, the highest adsorption uptakes for Pb^{2+}, Ni^{2+}, and Cd^{2+} were 88.80, 54.90, and 15.60 mg g^{-1} [20]. According to these results, CuO NPs can effectively remove heavy metals from polluted water, and more research into their regeneration and reuse is necessary.

Copper oxide nanowires can be used for the photocatalytic degradation of pollutants. When exposed to light, copper oxide nanowires can break down pollutants, such as organic dyes, into harmless substances. For example, The Allura Red AC (AR) dye, an organic pollutant/food dye, was degraded effectively by porous CuO nanosheets, as demonstrated by a color change from red to colorless and monitored by UV-vis spectrophotometric analysis [25]. Copper-based sensors can also be used to detect pollutants in the environment. For example, copper oxide nanowires can be used to detect nitrogen dioxide, a common air pollutant [38]. Copper-based electrochemical sensors can also be used to detect heavy metals like lead (Pb) in surface water [14]. Lastly, copper-based catalytic converters can be used to reduce the emissions of pollutants from cars and other vehicles. Copper-based catalysts can convert harmful pollutants, such as carbon monoxide and nitrogen oxides, into harmless substances. It has been reported that a copper-based catalytic converter reduces the hydrocarbon and CO emissions from a four-stroke single-cylinder Compression Ignition (CI) engine by 38% and 33%, respectively, at full load [2]. Overall, copper-based nanodevices hold great potential for environmental pollutant remediation, and research in this field is ongoing.

5 Titanium Nanoparticle-Based Nanodevices in Environmental Pollution Detection

Titanium nanodevices have the potential to be used in a variety of environmental applications, including pollution control and remediation. The formation of titanium metal and titanium oxide nanoparticles is just a couple of the many useful features and uses of titanium oxide (TiO_2). Rutile titanium dioxide and anatase titanium dioxide are its two main forms. Their outward appearances are what distinguishes them the most. Rutile titanium dioxide often has a dark red hue while anatase titanium dioxide is colorless. Anatase titanium dioxide has an optically negative spectrum, whereas rutile titanium dioxide has a positive spectrum [41]. Titanium dioxide (TiO_2) is a common material used in water purification due to its ability to break down organic pollutants and harmful microorganisms. When exposed to ultraviolet

light, TiO_2 nanoparticles can produce reactive oxygen species that can oxidize and destroy pollutants. This process is known as photocatalysis and has been shown to be effective in removing a wide range of contaminants from water, including pesticides, dyes, and pharmaceuticals. According to [26], Degussa P-25, a commercially available TiO_2 photocatalyst, contains roughly 25% rutile and 75% anatase form [26]. Numerous researches have applied it as a benchmark for photocatalytic degradation [34]. Furthermore, TiO_2 anatase form, which is more effective than rutile form due to its increased surface area and open structure, was the most extensively employed photocatalyst [4].

Titanium nanodevices can also be used to purify the air. TiO_2 nanoparticles can be coated onto air filters or used as a thin film on surfaces to break down pollutants when exposed to light. This technology can be particularly useful in indoor environments where air quality is a concern, such as hospitals or schools. Accordingly, it was found that Saudi myrtle plants treated with TiO_2, reduced the concentrations of formaldehyde, TVOCs, NO_2, SO_2, and carbon monoxide (CO) from 0.251, 401, 0.032, 0.009, and 0.99 to 0.014, 54,0.0003, 0.003, and 0.01 in air in the fourth day after intervention [31]. Titanium nanodevices can also be used to remediate contaminated soil. Cu and Cd were observed to be eliminated by 88.01% and 70.67%, respectively from soil, upon application of $NTiO_2$-NCh [21]. Overall, the use of titanium nanodevices in environmental pollution control and remediation shows promise and warrants further investigation and development.

6 Conclusion

While nanodevices have shown great potential for detecting pollutants, there are several limitations and shortcomings that need to be addressed before they can be widely used for environmental monitoring. For example, nanodevices can detect very low levels of pollutants, but their sensitivity can be affected by various environmental factors, such as temperature, humidity, and interference from other chemicals. Also, nanodevices can also be prone to false positives or false negatives, as they may not be able to distinguish between similar chemicals or may react to other substances in the environment. Some nanomaterials used in nanodevices might also be sensitive to oxidation, moisture, or temperature, which can affect their stability and accuracy over time. Lastly, developing and producing nanodevices can be expensive, which can limit their accessibility and affordability for widespread use. Overall, while nanodevices hold great promise for detecting pollutants, their limitations and challenges need to be carefully considered and addressed to ensure their successful implementation for environmental monitoring.

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Nanotechnology-Based Point-of-Care Diagnostics and Therapeutics for Neurological Disorders

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Abstract In today's world, there is a growing population of people suffering from neurological disorders. Many inherit such diseases due to hereditary reasons and many due to the very complex environment and societal reasons. One way of fighting this kind of problem is to detect it in very early stages and then start treatment so that the disease cannot mature. There are many techniques in which these neurological disorders can be tracked easily but are not easy to use and available due to constraints in the technology. Nanotechnology has played a key role in providing solutions to such problems, especially point of care devices and non-invasive techniques have recently evolved where nanomaterials and nanotechnology can play a vital role in making sure that accurate detection and analysis can be done without compromising the safety and security of the patients.

Keywords Parkinson's disease · Cerebrovascular disease · Alzheimer's disease · Traumatic brain injury · Nanomaterials · Applications · Uses

1 Introduction

Promising candidates for fluid sensing with better responsivity, selectivity, quick response, high stability, and accuracy but at low cost and easy to tailor made are chemiresistive sensors. These sensors are more reliable and are versatile for diverse uses. In this chapter we are focused on detecting VOCs that are present in the exhale breadth of patients and non-invasive techniques for the detection of diseases like COVID-19, Parkinson's, disease and Alzheimer's disease. Neurological diseases are some of the most common and complex diseases affecting the central nervous system

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(CNS). The blood-cerebrospinal fluid barrier also called blood brain barrier (BBB) regulates the stability of the nervous system and also helps maintain a fragile microenvironment that inhibits the release of therapeutic moieties to the central nervous system and controls diseases in the nervous system. Parkinson's Disease (PD), Cerebrovascular Disease (CVD), Alzheimer's Disease (AD), and Traumatic Brain Injury (TBI) are difficult to cure. Nanoparticle (NP) induced technology provides a platform for the development of specific drug delivery network due to its sustainability. Effective NPs have increased interest in the field of nanomedicine that can successfully cross the BBB and sustain high drug bioavailability in the neural parenchyma. NPs also exist in various forms such as solid lipid nanoparticles (SLNs), quantum dots, polymeric NPs, and liposomes, making it easy to combine with different molecules such as surfactants to deliver the body's needs or medicine.

This nano-delivery process represents a new possibility for the medicament and diagnosis of neurological diseases with less invasiveness. Most plan of actions like efflux mechanisms, including adsorption-mediated transcytosis (AMT) and receptor-mediated transcytosis (RMT), target-specific biological indicators, or lesions, are used as common tactics to change the ability of NPs to cross the BBB. Thereby increasing tissue-specific targets and minimizing adverse side effects. This article provides an understanding of the neurological and CNS drug delivery problems due to the presence of the BBB, presenting an in-depth review of nanoparticle-based theranostic and medicament strategies [1]. Other than diseases like cancer, bacterial infections, heart disease, COPD, and renal failure, neurological disorders (NDs) cause significant numbers of deaths and have high mortality rates across the globe. In clinical settings, NDs are lately detectable and have limited treatment options. NDs are chronic diseases and have poor prognosis. Neurological disorders can cause large number of abnormalities ranging from paralysis, Alzheimer's Disease (AD), epilepsy, brain tumors neuromuscular disorders, etc. Under such a limited scope of clinical treatment available, nano-technology brings a lot of advantages to combat ND. Nanotechnology increases the scope of treatments and helps to overcome the hindrances. In recent biomedical science research, point-of-care diagnostics and therapeutics has evolved massively. Affordable and quick diagnostic approach has been the major concern in point-of-care clinical settings. In today's clinical settings, personalized medicines are playing a crucial role. The 2019 coronavirus (COVID-19) was a global crisis because it is spreading rapidly and is causing many deaths and damages. The number of people infected with the virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which seeds the disease COVID-19, thereby increasing rapidly globally. Patients affected with COVID-19 may build pneumonia, severe acute thoracic and respiratory distress syndrome (ARDS) symptoms, and multi-organ failure. There is an increasing clinical report that immune patterns are linked with infection in infected individual. Decreased peripheral white blood cells like T cell subsets are a specific feature of individual with acute respiratory disease (SARS). In the recovered patients, there was a rapid recovery of peripheral T cell subsets; therefore, peripheral blood T cell count may be a precise diagnostic tool for SARS. Study showed alike phenomenon, in which the immune system was found to be affected during SARS. Another study found that Ebola patients had fewer natural killer (NK)

cells compared to healthy donors. After the onset of Ebola symptoms, proinflammatory cytokines were elevated, while recovered patients had low cytokines. With increasing awareness of the relationship between the immune system's response to COVID-19, the immune system is now recognized as a potential infection marker and is a clinical target for COVID-19 [2–5].

2 Molecular Mechanism of Neurological Disorders

Neurodegenerative diseases are characterized by brain protein aggregates, increased number of neurons, synaptic abnormalities that result in decreased memory, cognition, and motor symptoms. The main factors that cause neurodegenerative diseases are still under research and unknown, but many risk factors have been identified, like genetic mutations of many types of diseases, environmental pollution, and family history. The genetics of neurodegenerative diseases have been determined. Recent advances have been made in research on the use of genes related to neurodegenerative diseases. Several case studies and research have shown that the BBB undergoes pathophysiological changes, leading to less endothelial tight junction working molecules. These are defined as neuroinflammation-induced "collapse" which increases BBB permeability. The phenomenon results in the response to treatment in the brain of patients with neurodegenerative diseases is different from that of the normal brain. The behavior of pathological changes in the blood-cerebrospinal fluid barrier in influencing drug distribution to the brain is still not crystal clear. It should be noted that the neurodegenerative brain enlarges leaving more brain pharmacokinetics than the normal brain [1, 6–8].

3 Molecular Mechanism of Alzheimer's Disease (AD)

The most recurrent cause of dementia is AD. Nearly 70% of dementia diagnoses are due to AD. The biggest factor for having AD is aging. Most patients of the age greater than 60 suffer from AD. The fundamental feature of the neurodegenerative disease Alzheimer's is cognitive loss. Memory impairment is one of the first and most prominent symptoms. Cognitive loss leads to deficits in language as the illness progresses. Further visuospatial orientation and motor function is observed. Multiplex proteins, which are extracellular aggregates of amyloid (Aβ) tend to gather at the cerebral cortex of AD individuals like senile plaques. The amyloid cascade hypothesis was the best-known hypothesis to explain AD pathology that ultimately leads to neuroinflammation, neuronal damage, neurofibrillary tangles, and death. Other effects can be impaired neuronal communication and other pathological manifestations of AD. Conflicting results suggest that accumulation of Aβ plaques may not be the cause of AD development. According to some research, a plaque doesn't form until after neurofibrillary tangles do. Due to contradictory evidence, the main contribution of

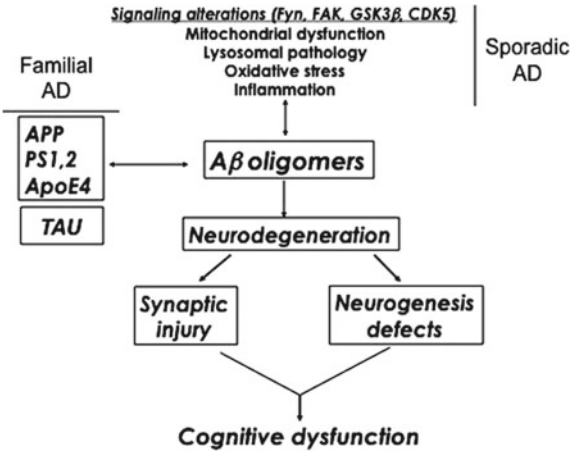

Fig. 1 Mechanisms of neurodegeneration in AD. Aβ dimers, trimers, and oligomers build up as a result of abnormal cellular processes, which hinder neurogenesis and damage synapses [15]. Adopted from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2875049/pdf/ddq160.pdf/?tool=EBI

Aβ in the pathogenesis of AD remains controversial. Further research is needed in the future to determine the mechanisms leading to AD pathogenesis [9–14].

The methods through which APP metabolites such as Aβ monomers and oligomers can result in synaptic degeneration and other types of neurodegenerations are unclear. Possibilities like creation of pore-like structures with channel functions; Circuit hyperexcitability; mitochondrial dysfunction; Connection with lysosomal failure and synaptic plasticity; changes in glutamate receptors and excitotoxicity; neurogenesis and alteration of signaling pathways associated with neuronal cell death. Previous research studies have shown that various expression of molecules is involved in the neurological disorder processes, including glycogen synthase kinase 3β, fyn kinase, and cyclin-dependent kinase. Other signaling pathways include the MAPK family such as JNK and ERK and others such as p21-activated kinase (Fig. 1). Sudden activation of signaling pathways can lead to synapse dysfunction and neurogenesis by promoting cytoskeletal abnormalities, activation of caspase-mediated apoptotic pathways, tau phosphorylation and aggregation, and increasing calcium and calpain changes [15].

4 Present Therapeutic Treatment Strategies for AD

Recently, AD treatment has focused on improving memory by enhancing antioxidants, calcium channel blockers, and cholinergic neurotransmission. More recently, the target has been on minimizing the accumulation of Tau or Aβ. Alternatively to preserve the selected neuronal population and promote synapse creation and neurogenesis. Various methods are presently being pursued to reduce Aβ aggregation by

(i) block the β- or γ-secretase pathway or increase α-secretion Enzyme activity to regulate APP proteolysis.
(ii) anti-aggregation drugs that block oligomers and fibrils,
(iii) reduce APP production (e.g. siRNA),
(iv) increased clearance by lysosomal and proteasomal pathways,
(v) control APP activity by altering cholesterol and lipid metabolism,
(vi) increased clearance of Aβ by activation degradation (e.g. NEP and IDE delivery),
(vii) neurotoxic Aβ oligomers (e.g. G. Fyn kinase, GSK3β and CDK5 inhibitors, and glutamate receptor blockers) and
(viii) increased clearance of Aβ by immunoglobins, ApoE, and other chaperones (such as HSP70).

Neuroprotective tactics include the use of neurotrophic agents (e.g., brain-derived neurotrophic factor, nerve growth factor), calcium channel blockers (e.g., memantine), antioxidants (e.g., curcumin, vitamin E), neuroprotective peptides (e.g., cerebrolysin), and Tau. Tau is also an important target. Recent studies have shown that APP transgenic mice are protected from Aβ toxicity in a Tau-deficient background. Tau has been targeted by minimizing Tau formation or by reducing the phosphorylation of Tau with compounds such as lithium. In addition to conventional administration routes and minor oral techniques, new methods are currently being tested, including vaccines, lifestyle changes to improve neurogenesis, gene therapy, the use of lipid-conjugated compounds, and intrathecal administration [15–19].

5 The Blood Brain Barrier (BBB) as Blood-Cerebrospinal Fluid Barrier

Three cellular components of the brain microvasculature are pericytes (PCs), BBB-endothelial cells, and astrocyte endopods. Close junctions exist between the brain's endothelial cells to form a selective barrier to prevent blood from entering the brain. Astrocyte terminals tightly assemble around the vessel wall and appear to be important for initiation and maintenance of the close junction barrier. Poor functioning of the BBB, such as a bad junction seal can lead to many diseases in the brain, including stroke and neuroinflammatory diseases. The consequences of increased BBB results in permeability in hypoxic-ischemia and inflammatory processes, including HIV-induced dementia, BBB in septic encephalopathy, Alzheimer's disease, and multiple sclerosis [20].

6 Nanotechnology Approaches Break the Blood-Cerebrospinal Fluid Barrier and Deliver Targeted Drugs to the Specific Central Nervous System Site

Nanotechnology deals with materials and equipment that have worked on the scale from a few nanometers to 100 nm. Nanoengineered materials and tools applied in chemistry and medicine, particularly neuroscience, are designed to interact with tissues and cells at the atomic level. The creation of procedures and methods for pharmaceuticals and other tiny molecules to interact with the central nervous system (CNS) is a particularly significant use of nanotechnology. These species involve genes, oligonucleotides, and agents to work differentially to cross the blood-cerebrospinal fluid barrier. BBB isolates and protects CNS structures from the rest of the body and creates a special biochemical and protective surrounding. The CNS structures include brain and spinal cord. Clinically, in many cases, drugs or other small molecules must enter the CNS after systemic administration. The species must travel the BBB. Nanotechnology can be designed to perform many specific functions simultaneously or in a predetermined manner that are important for successful therapy and use of drugs and other molecules in the CNS. Hence nanotechnology has a unique advantage over other complementary technologies and methods. Most studies to cross the BBB with nanotechnology have focused on the delivery of antineoplastic drugs to CNS solid tumors. For example, radiolabeled polyethylene glycol coated cetylcyanoalkanoate nanospheres were tested for their targeting and aggregation abilities in mouse gliosarcoma models. Others achieved good result by encapsulating the antineoplastic drug paclitaxel in poly(lactic-co-glycolic acid) NPs. In vitro experiments with 29 different malignant cells showed 13 times higher target cytotoxicity than the drug alone. Researchers demonstrated that drugs are transported by nanoparticles with high encapsulation efficiency. They used a variety of physical and chemical methods, including multiple spectroscopy and atomic force microscopy techniques. Studies focusing on the delivery of many antineoplastic drugs are important. Many of these drugs are poorly soluble in physiological conditions. Hence these drugs face insufficient intake issues, which can have serious side effects. In another case, several compounds were functionalized on the surface of poly(butyl cyanoacrylate) nanoparticles coated with polysorbate. These included neuropeptides such as enkephalin, the N-methyl-D-aspartate receptor antagonist MRZ 2/576, and the drug doxorubicin. Polysorbate on the surface of nanoparticles adsorbs apolipoproteins B and E and is absorbed by the brain capillary endothelium by receptor-mediated endocytosis cellular uptake. Nanoparticle-functionalized doxorubicin delivery was investigated in mouse glioblastoma models. More importantly, recent studies in a mouse model of glioblastoma showed significant release with minimum toxicity, laying the groundwork for clinical trials. Non-pharmacological molecules, including genes, oligonucleotides other agents should cross the cerebrospinal fluid barrier for therapeutic or diagnostic purposes for any other applications. Lipid NPs functionalized with iron oxide along with microemulsions of coagulated oil nanodroplets

injected into mice, have been shown to cross the BBB and get stored in the brain with long-term kinetics. Iron oxide is a typical superparamagnetic magnetic resonance imaging (MRI) contrast agent. Since iron oxides are insoluble in water, they must be used in the form of modified colloids for medical applications. Usually, coating iron oxide NPs with hydrophilic molecules such as dextran solves problems. Iron oxide could provide a new way to image CNS using MRI by exploiting the efficiency of lipid NPs to cross the BBB [21].

A reliable chemi-resistor containing an immediate sensing layer clubbing the combination of electrodes which became a promising applicant. Furthermore, the advantages are not limited to easy fabrication, can be used in very minimum quantity (in milligram unit), and highly distributed adoption of sensitized materials. Therefore, chemi-resistors gain popularity in certain commercialization.

Chemi-resistors for gas sensing have the following three main processes:

- charge carrier transport unit
- surface reaction unit (including charge transfer), and
- diffusion/molecule capture unit,

Until now, most of these sensors and/or sensor arrays utilize sensing elements that are based on transduction mechanism or single material. Usually, intrinsic sensing activity or additional thermal/photonic energy is employed as the driving force to activate sensing effects of target gases.

Few demerits are:

(i) Due to the high affinity of conductive polymers like polyaniline, polypyrrole, and polythiophene toward volatile organic chemicals and humidity present in the atmosphere, there was a lack of long-term stability and sensitivity of organic chemi-resistors.
(ii) baseline drift, high functioning temperatures (>200 °C), oxidation/decomposition, and fixed selectivity of VOCs in the case of inorganic materials (mainly metal oxide materials, e.g., TiO_2, SnO_2, ZnO, etc.-based chemi-resistors.

An effective solution to such downside is the design and utilization of new gas sensing materials based on hybrid organic–organic, inorganic–organic, and inorganic–inorganic hybrid materials as sensitive transducer possess several advantages compared with the single constituent. Such as:

- To create unique sensing behaviors, it is feasible to combine an essentially limitless continuum of changeable components (interface-dependent factor, surface-dependent factor, and structure-dependent factor). This is due to the inexhaustible supply of hybrid materials (both in the innovative nanostructures and in the intricate constituents).
- Second, hybrid materials could be used to add an increasing number of chemical/physical processes with various improved mechanisms. Through a catalytic interaction with charge transfer, analyte, molecular binding/sieving, charge carrier transport manipulation/construction of heterojunctions, and their combinations,

hybrid materials make it simple to accurately regulate, create, and enhance sensing performance.

7 Hybrid Chemi–Resistive Gas Sensors

Improved sensing properties can be achieved by hybrid materials using one or a combination of the five fundamental hybridizing types. These forms are divided into five sensing-governed factors:

- The first factor is based on a quick charge transfer procedure. Such process is often referred to as electron acceptor or acceptance between guest and additive, carrier withdrawal or donation between the host material (for example CNTs) and reduced graphene oxide (rGO) (classified as an interface dependent factor).
- The second combination relies on catalytic processes. It takes place between analyte gas and decorated catalysts on the surface of the host semi-conductive material using noble metal catalysts, such as Pt, Pd, Au, and Ag.
- The third relies on regulating the charge carrier transport in a conductive/ semi-conductive materials like gold nanoparticles (GNPs)-thiols, like CNT-metallo-supramolecular polymer (MSP), and less with N,N′-diphenyl perylene tetracarboxylic diimide (PTCDI-Ph)/para-sexiphenyl upon reaction with gas analytes.
- The fourth focuses on the building of heterojunctions made of heterogeneous semi-conductive materials such as n–n, p–n, p–p, and p–n–p (classified as an interface-dependent factor).
- The final method depends on semiconductors functionalized with ligands or complexes that selectively bind to sieve gas molecules.

8 Role of Nanomaterial in Detection of VOCs

The nanostructure of electrodes working with nanocarbon modifiers has new attractive properties such as:

- simple physical adsorption through Van der Waals interactions onto electrodes.
- because of their incredibly high conductivity in some directions, they have quick electron transfer kinetics.
- high surface area, which enhances volatile organic molecules adsorption.
- due to their special electrical or plasmonic structure, catalysts are highly selective and adjustable.
- and adjustable surface chemistry for a specific capture probe or analyte species in the direction of the assembly.

9 Role of Carbon Nanomaterials

VOC gas sensors using carbon-based nanomaterials have attracted increasing attention due to their advantages such as low power consumption, miniaturization, and incorporation into portable devices for medical diagnostics. However, it is difficult for weather sensors to select the response to certain VOC biomarkers and ensure that they are unaffected by other factors. Il-Doo Kim et al. reported a sensitive and selective acetone sensor using SnO_2 nanofibers functionalized with rGO nanolayers. Furthermore, the LOD on this acetone sensor is low (100 ppb). The authors believe the sensor could be very fine and select the response to acetone residues in the breath, so that diabetes can be successfully diagnosed. As we all know, the exhaled air is mostly with water molecules, which causes the water vapor effect of the air sensors and affects the detection accuracy of the target VOC analytes. Therefore, the use of carbon-based nanomaterials has been developed to create humidity-sensitive VOC gas sensors to provide accurate detection of target VOC gases in inspired air (Table1). However, it is not easy to obtain good sensors with good moisture resistance, low LOD, and high detection selectivity at the same time. The PVDF-HFP sensor, for instance, was made to be insensitive to humidity, but its detection limit for VOC gases is higher than the amounts of VOC gases in exhaled human breath. The rGO/CuO sensor's resistance value may be resistant to water vapor, but this introduces the issue of high operating temperatures. The effective detection of biomarkers and non-invasive diagnosis of various diseases hence requires additional advancements in the sensing performance of VOC sensors utilizing carbon-based nanomaterials.

Table 1 Examples of chemi-resistive response of original and modified CNTs toward different gases

Material	Gas detection	Detection range	Working temperature
CuxO/multilayer graphene	NO_X	97 ppb–97 ppm	RT
rGO/NiO	NO_2	0.25–60 ppm	RT
ZnO QDs/graphene	HCHO	25–100 ppm	RT
SnO_2/Rgo	H_2S	10–100 ppm	RT
Graphite/polyaniline	NH_3	50–1600 ppm	RT
SnO_2/graphene	CH_4	3.3–100 ppm	150 °C
SnO_2 CQD/MWCNT	H_2S	100–1000 ppm	70 °C
rGO/TiO_2–Nb	CO	50 ppb–50 ppm	380 °C
Fe_3O_4@rGO	NO_2	50 ppb–50 ppm	RT

10 Role of Carbon Quantum Dots

10.1 Gas Sensing with Conducting Carbon Dots

CQDs can be utilized to detect gases and VOCs since they have powerful electronic capabilities in addition to their optical characteristics. A small number of gas molecules is all that is needed to alter the electrical characteristics of the electronic material because nanotubes, nanowires, and nanoparticles are so small. This makes it possible to identify tiny chemical vapors. Small, low-cost sensors that can detect chemicals in the same manner that dogs can detect vapors from explosives or drugs at airports are the goal.

It is now possible to create tiny, low-cost sensors that can quickly recognize chemical vapors, giving rise to a nano-hound that is useful in a variety of ways and doesn't require rest or exercise. Installing sensors throughout the airport or any place where there are any safety concerns would be a logical application to find explosive vapors. These sensors can be used in workplaces where chemicals are used in the production process to capture the release of chemical vapors. Sensors that detect hydrogen gas leaks will be useful in warning of leaks when hydrogen fuel is activated in vehicles or other applications. The technology should also connect air quality monitoring stations to improve air pollution monitoring.

CQDs have not been extensively investigated. Our group reported the electrical conductivity of polymer-passivated CQDs, which was the first to report the electrical conductivity of individual CQDs. They evaluated an individual's processing of objects with a scanning probe (SPM) using the SRI imaging method. The lower energy state responsible for the excitation-induced fluorescence features was demonstrated by the fluorescent excitation spectra of the CQDs. When harmonics surpass zero voltage, as shown by the current-to-voltage (I–V) characteristics of single polystyrene sulfonate stabilized CQDs (PSS-CQDs), the current behaves erratically and rapidly jumps. The band gap of CQDs was calculated using cyclic voltammetry (CV). The I–V data and AFM images of each CQD.

There is still a dearth of information on the conducting qualities of CQDs. These investigations are crucial for gaining a fundamental knowledge of the conducting phenomenon that CQDs have shown, and they also hold the potential to enhance the tunability of conducting nanocomposites.

10.2 Designing of Gas Sensors Using Carbonaceous Nanomaterials

CQDs are usually obtained in powder form, making sensors difficult to manufacture. It should be used as a composite material with polymer materials. Insulating polymers inhibit the electronic properties of CQDs. Therefore, it is often desired to make

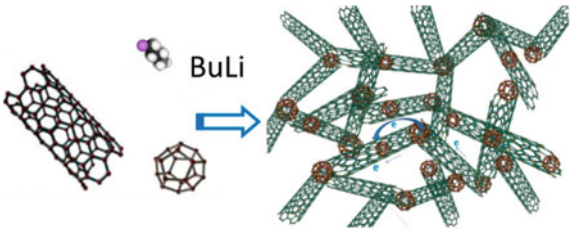

Fig. 2 Reaction between C_{60} BuLi with CNTs for the active sensor. Adopted from https://www.mdpi.com/2227-9040/9/4/66

polymer composites with CQDs. Conductive polymers such as PPy have been utilized to build gas and VOC sensors for a long time.

These components can be employed in sensing applications and have good current–voltage characteristics. These polymers' LUMO levels take in free electrons from many carbon atoms and VOCS, and the HOMO electrons can share the remaining electrons. This molecular interaction causes a change in I–V properties, resulting in hearing loss. The incorporation of nanomaterials into polymer matrices has been shown many times to increase the sensitivity of sensors. Nanomaterials induce active sites in polymers for rapid gas adsorption as they improve surface activity, thus completing the experience of mainstream conducting polymers. The use of carbon nanomaterials in conductive polymers such as C60, CNT, and CQD has shown great improvement in sensor applications (Figs. 2 and 3). The main idea behind these compounds is that carbon nanomaterials are generally hydrophobic, so they have a good affinity for making polymers that are often hydrophobic in nature. In addition, the specific conditions of C60 are discussed, which will help us understand the impact of CQDs on the performance of polymers (Table 2).

10.3 Effect of CQDs on the Electronic Properties of Conducting Polymers

The integration of C_{60} into PPy was said to improve the I–V characteristics in the literature. Carbon in C_{60} has a strained sp^2 structure. The material's structure resembles that of CQDs, which do not contain extended sp^2 domains like those seen in C_{60}, although corresponding parallels may still exist. It was discovered that C_{60}, although being n-type doped, functions as a conducting polymer matrix for p-dopant. In other words, the C_{60} LUMO receives an electron from a polymeric chain, charging it in the process. An additional positively charged polaron in the PPy chain was produced by the ionic state. The doping action was confirmed by the substantially altered absorption spectra and the noticeably subdued fluorescence in the doped PPy.

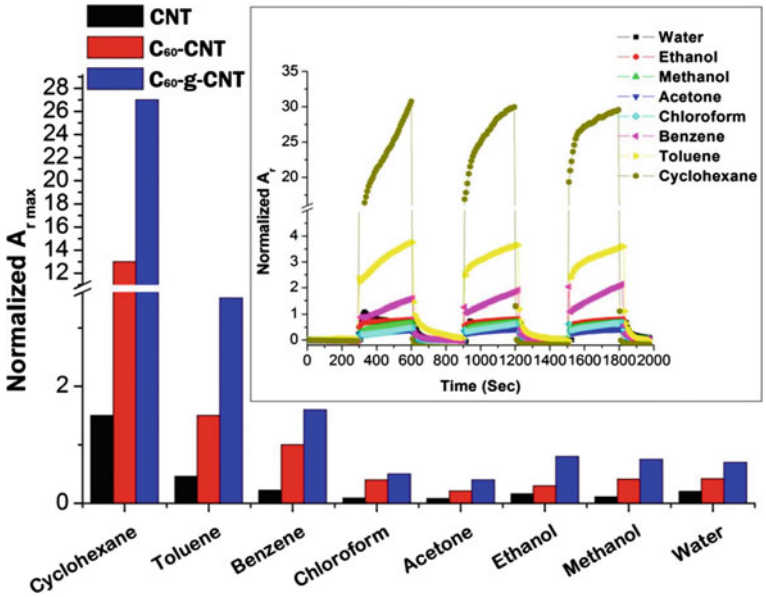

Fig. 3 Detection of VOC in presence of humidity using CNT and C_{60}. Adopted from https://www.mdpi.com/2227-9040/9/4/66

Table 2 Basic resistance values of various CNT nanocomposites

Nature	Process	R_0 (kΩ)
CNT	2 layers of CNT suspension in $CHCl_3$ sprayed	5 ± 2
CNT-g-C_{60}	2 layers of C_{60}-g-CNT suspension in $CHCl_3$ sprayed	8 ± 3
CNT-I-C_{60}	1 layer of CNT suspension in $CHCl_3$ sprayed followed by spray of 1 layer of C_{60} suspension in toluene	4 ± 2
rGO	5 layers of rGO suspension in acetone sprayed	10 ± 4
rGO-g-C_{60}	5 layers of rGO-g-C_{60} suspension in acetone sprayed	10 ± 5

11 VOC Sensor Based on Carbonaceous Nanomaterials

The first carbon quantum dots, known as GQDs, were created from graphite nanoparticles using chemical and physical processes. A flawless graphene sheet won't glow because of the extended-conjugation's lack of electrons. Therefore, much work has gone into changing the graphene network end to end to produce electron gaps and therefore fluorescence emission in graphene. In other words, the big aromatic molecules found in graphene sheets and the isolated conjugated domains are structurally related.

The advantages of composite materials are cost-effectiveness and easy production. In their work (PANI-GQDs), nanocomposites were prepared by in situ electrochemical polymerization of aniline monomers in the presence of GQDs, which showed promising catalytic activity in promoting triiodide reduction. During polymerization, fluorine-doped tin oxide (FTO) coated glass is immersed in a solution of aniline and GQD. DSSCs formed from PANI-GQD nanocomposite electrodes exhibited a power conversion of 1.6%.

The PANI-GQD nanocomposite's electrochemical catalytic activity was greater than that of pure PANI because of the synergistic interaction between PANI and GQD. As a result, DSSC structures based on PANI-GQD electrodes exhibit superior photovoltaic performance than those based on PANI electrodes.

The findings motivated us to look into the electronic characteristics of CQDs in more detail. By integrating polypyrrole (PPy) on the surface of CQDs, or colloidal particles based on stable PPy coated CQDs (CQD-PPy) water-based stable writing pen, this study aims to improve the electrical properties of PSS-CQDs. For the purpose of growing PPy on the CQD surface, a site-specific autocatalytic method was created.

CQD-PPy functions as a semiconductor ink and is highly dispersible in aqueous suspension. Data indicate that electronic polymers are employed as chemicals to stop snack items from going rancid. Variations in the quantity of VOCs released from food can reveal crucial details regarding food contamination. In order to identify and track the organic volatiles produced by rice grains during storage, a polypyrrole (PPy) based oil sensor has been created. The difference in sensitivity with different analytes is evaluated in response to PPy's stable resistance, which is utilized extensively as a chemical resistance sensor. Certain analytes can be detected by polymer-based sensors, however they cannot tell apart analytes that are identical. As a result, PPy changes its resistance when VOC is present, but this change is seen at any stable voltage. Therefore, it is vital to alter PPy's characteristics so that it can recognize and differentiate between various items, which will aid in the identification of VOCs. This can be used to differentiate the flavors of various snacks.

Oxidative rancidity is the term used to describe the foul taste and odor that develops when oils like lard, shortening, vegetable oils, and cooking oils are exposed to oxygen in the air. Fish, chicken, pork, frozen veggies, and powdered milk are just a few examples of products that contain these fats that can cloud the product's oil. These foods' polyunsaturated fatty acid content reacts with oxygen to produce peroxides. Aldehydes, ketones, and other dangerous chemicals can be found in combinations created by the decomposition of peroxide. This product may have a "sour" odor and taste. That's why it occurred to us to examine inks for three important aldehydes that are often found in foods. Hexanal, heptanal, and octanal are three aldehydes that differ in the number of carbon atoms in the main chain.

12 CNT-Based Sensors

Types of design:

1. random networks (RN)
2. field effect transistor (FET)
3. conductive polymer nanocomposites (CPC).

13 Graphene-Based Sensors

Graphene and CNTs have been accepted for the creation of smart electrodes. This is due to the special features observed in graphene-like materials. Hence, graphene may overcome limitations of traditional carbon materials like poor electrical conductivity and mechanical strength. However, due to the limited fabrication methods and high cost of commercial manufacture, graphenes may experience issues [5].

Carbonaceous nanomaterials provide tremendous options due to their simplicity of synthesis, enhanced biocompatibility, and flexibility in organic functionalization of their surface. The development of electrochemical sensors has attracted a lot of attention in carbon-based nanomaterials, particularly carbon dots (CQDs) and graphene quantum dots (GQDs) [3].

The primary graphene-based materials utilized in the construction of electrochemical biosensors are depicted in Fig. 4.

GQDs are a type of zero-dimensional (0D) nanostructure that belong to the carbon family. It has similarities with both graphene and CQDs. These nanomaterials exhibit novel features as a result of quantum confinement and edge effects similar to CDs. The situation is similar to 2D graphene sheets when transformed into 0D GQDs. No matter how small the dots are, GQDs have a graphene structure that provides typical characteristics of graphene. Unlike CQDs, less than 10 nm thick and 100 nm in lateral size layers are present in GQDs [6]. Moreover, GQDs are known to be effective electron transporters that can interact with some electroactive species. This

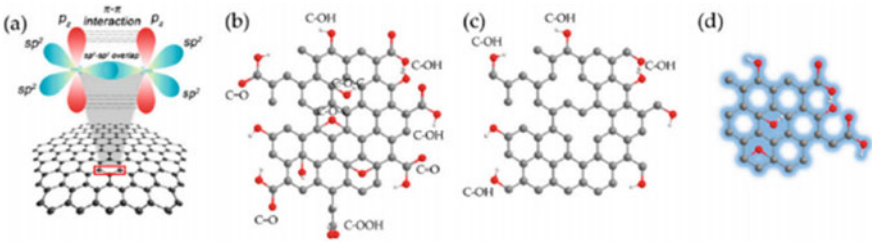

Fig. 4 Structures of graphene-based nanomaterials, including pristine graphene (pure-arranged carbon atoms) with sp^2-hybridized carbon atoms (**a**) and the chemically modified graphene: graphene oxide (GO) (**b**), reduced graphene oxide (rGO) (**c**), and graphene quantum dot (GQD) (**d**). Adopted from https://www.mdpi.com/1422-0067/23/1/22

increases the analyte contact area and the electrochemically active surface area. Since the geometric surface area is a very important parameter in electrochemistry, modification of various substrates with GQDs can increase the rate of electrochemical reaction. GQDs usually contain functional groups, such as hydroxyl, carbonyl, carboxyl or epoxide, at their edges and basal plane that can act as active reaction sites.

14 Techniques for Fabrication of Graphene-Based VOC Sensors

The intrinsic characteristics and nanostructure of the sensing material affect how well VOC sensors operate. The focus has been on the construction and integration of nanostructured materials into VOC sensors to build high-performance VOC sensors. Nanostructured materials have been incorporated into sensors using a variety of methods.

This section covers processes like: drop casting, spin coating, layer-by-layer (LbL) assembly, and spray layer-by-layer (sLbL) assembly.

15 Drop Casting

Nanomaterials are frequently bonded to surfaces through drop casting using solution evaporation. The quality of the graphene film depends on several elements, including the concentration, weight, and heating of the graphene solution. A graphene-polyaniline-based sensor for the detection of ammonia by dripping a solution onto an interdigital transducer (IDT) was developed, according to Wu et al. Cui and co. Graphene sensors coated with Ag are demonstrated by drop casting on gold electrodes. In this experiment, rG-O dispersions were dropped onto hot electrodes and heated to 200 °C before being annealed for an hour. In a micro arc plasma reactor, Ag NPs were physically vapor-deposited onto the surface of rG-O. Hasan and co. Use of ultra-large graphene oxide (UL-G-O) nanocomposites and poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT-PSS) for VOC detection.

16 Spin Coating

Accurate control over the homogeneity and thickness of the active layer is essential for producing reliable and reproducible detection devices. Spin coating is a method in which the active layer can be widely spread over the entire substrate surface

by centrifugal and viscous forces and is commonly used to produce thin films (2–10 μm) on thick as well as flat substrates. Spin coating technology is widely used in semiconductor devices, lithography, magnetic disks, microelectronics industry, and other related fields. The thickness of the film can be controlled by varying the time, fluid viscosity, speed, and solvent evaporation rate. The spin layer can be used to build graphene-based sensors. Fowler et al. reported that the designing and production of sensors for the detection of NH_3, NO_2, and 2,4-dinitrotoluene by spin coating to form a graphene film layer on interlocking electrodes. Dong et al. reported the production of poly(3,4-ethylenedioxythiophene) and r-GO hybrid thin film sensors for VOC detection at the ppm level by spin coating on an interdigitated microelectrode array. The spin coater of PIL-modified r-GO can control the film thickness by varying the spin coater speed. Thin and smooth graphene layers enable PEDOT to grow through gas-phase polymerization, providing high-performance hybrid thin-film sensors.

The spin coating process is characterized by controllable thickness, low power consumption, uniform deposition, and easy installation. However, the spinning process can only be used on flat and small substrates. In addition, centrifugal force during rotation often causes negative angle lines or variations in sensor thickness.

17 Layer-by-Layer Self-assembly

One of the best methods for creating nanomaterial thin films with the correct thickness is layer-by-layer (LbL) assembly. The bonding of nanomaterials in various levels through electrostatic interactions is known as layer-by-layer (LbL) assembly. The process of releasing the movie follows a cycle created to create the movie, in which the steps are set up in a preset order. Its ease of use for spin-plating LbL components, Langmuir-Blodgett deposition, and other processes makes it an appealing option to traditional deposition techniques like numerous materials, including polyelectrolytes, dendrimers, biomaterials, metal nanoparticles, and nanocarbons, can be employed with it. By adjusting the charge type and number of adsorption cycles, it is possible to change the electrochemical behavior and nature of the reaction process. By adjusting the pH, temperature, and concentration of the solution, it is also possible to alter the layer's roughness, thickness, and porosity.

For the manufacture of high-performance chemically resistant vapor sensors, control of the conductive structure, including the junction points in the percolation network, is crucial. Control of nanomaterial structure can be realized by electrostatic layer-by-layer assembly. LbL technology should be used to create multilayer graphene films with different thicknesses and layers. However, a better understanding of the interaction between electrostatic forces and solvency is required to control the final nature of the reaction process. In addition, further studies are required for polyelectrolytes used in LbL processing, since they may affect the stability of the sensors.

18 Spray Coating Layer-by-Layer

Spray-by-Layer (sLbL) was developed by Schlenoff et al. It is considered one of the best methods due to its low cost, ease of coating, and modification. sLbL involves the direct injection of chemicals into the substrate by spraying. sLbL enables high-thickness and repeatability sensor arrays to be mass-produced in a short time, and allows nanomaterials to be used on non-planar and complex 3D substrates that are difficult to obtain with conventional methods such as layers. Control of junctions and nanostructures is important for the development of chemical resistance sensors.

sLbL can also be used to make better connections and nanostructures for ultra-sensitive sensors. Recent research suggests the development of sLbL technology for the development of sensor devices (Dong et al.). Production of graphene quantum well-sensing skin and VOC detection using sLbL technology. The initial resistance of the converter is controlled by adjusting the number of layers.

Their theory holds that changing QRS nanostructures results in biased tunnel conduction, which generates extremely high sensitivity and selectivity. The researchers also discussed the sLbL-based production of composite materials with magnetic Fe_3O_4 nanoparticles formed of rG-O, PEDOT, and poly(ionic liquid). The sensor can distinguish between polar and non-polar VOC molecules with acceptable reversibility at room temperature. To detect both polar and non-polar VOCs, the ssLbL method deposits graphene hybrid composites on interdigitated Pt electrodes. An efficient chemical resistance sensor with core–shell hybrid nanostructures for the detection of lung cancer biomarkers was created with sLbL assembly to intermeshing electrodes (25% Ag/75% Pd orbitals). Nag et al. reported the creation of an ultra-sensitive VOC sensor using sLbL technology for the detection of lung cancer biomarkers (Table 3). The sLbL technology has advantages over spin-coating and LbL mounting techniques, including cheaper sensor manufacturing costs. sLbL provides a lot of benefits and can quickly produce nanostructures.

19 Current Advances in Graphene and Graphene Composites for Chemiresistive VOC Sensors

Nanomaterials have been used to design sensors for VOC detection due to their high contrast and good physical properties for selective detection. Various nanoscale materials have been used for local VOC sensitivity, including metal nanoparticles, nanocarbons, and semiconductor metal oxides [21]. VOC detection is based on the interaction between the analyte molecules and nanomaterials. The coupling of charged VOCs to the sensor surface makes detection possible by changing the carrier density/Fermi energy without interference from the background solvent.

The use of solutions with higher ionic strength is limited due to ion resistance and electrical double-layer formation, so sensors often run dry or solve problems with

Table 3 Various VOCs as cancer biomarkers

VOC type	Representative vapor biomarker	Concentration range in breadth
Alcohols	Methanol Ethanol 1-Propanol	157–344 96–2848 4–13
Aldehydes	Pentanal Heptanal Nonanal	2–7 2–7 2–107
Alkanes	Pentane 4-Methyloctane Cyclohexane	2–18 16–19 0.1–15
Halo hydrocarbons	Chloroform	10
Ketones	Acetone 2-Butanone 3-hydroxy-2-butanone	35–1000 0.002–3 0.002–0.05
Alkenes	Isoprene	41–109
Aromatics	Ethyl benzene Benzene Toluene	1–18 1.1–3.5 1–37

low ionic strength. For these reasons, it is important to build sensors that operate at high ionic strength (100 mM) for real-time monitoring.

The chemical resistant VOC detection technology relies on changes in electrical properties through the interaction of VOC molecules with the detection platform. Various types of nanomaterials have been investigated for chemiresistance method of VOC detection and non-invasive disease detection with SMOs, carbon-based nanomaterials, and hybrid composites have become very important in recent times. Among carbon-based nanomaterials, chemically modified graphene or reduced graphene oxide (r-GO) has been reported for anti-VOC sensors. Most rG-O based sensors have r-GO flakes of different sizes, shapes, and thicknesses resulting in randomly overlapping flakes. Therefore, the electrical properties of rG-O thin film are different and devices made of thin film have different properties from device to device. These sensors have a greater selectivity to distinguish between different alcohols such as ethanol, methanol, and isopropanol.

20 CQD-Based VOC Sensors

CQDs are quasi-spherical nanoparticles less than 10 nm in diameter that exhibit better dispersibility in various solvents, improved biocompatibility, and less cytotoxic. GQDs have been used much more than CQDs in electrochemical biosensing, though both types of carbon quantum dots possess interesting features. GQDs possess advantage over CQDs in the design of nanomaterial-based biosensors because of their

low intrinsic toxicity, their high surface area with larger length-to-diameter ratio (the ratio of length to thickness), chemical inertness, mechanical stiffness, excellent solubility, photoluminescence, thermal conductivities, easier grafting of their surface with receptors, and greater electrical and high stability compared with conventional semiconductor quantum dots. These superior properties are due to the π–π bonds below and above the atomic plane. On the other hand, fluorescent CQD possesses great potential in designing electrical and electronic devices. Recently, there have been several reports on the electronic properties of CQDs and their applications as materials in chemical resistance sensors.

Bhattacharjee et al. developed and tested a new method for the preparation of a stable hydrocolloidal CQD-PPy solution. Stabilizing PPy on CQDs as colloidal particles is a challenge here that can be solved without the use of additional stabilizers or other oxidizing agents. PSS-passivated CQDs act as both stabilizers and additives to stabilize conductive colloidal CQD-PPy formation. The method is simple and scalable. The CQD-PPy suspension can be coagulated to form a stable ink with no other additives. The ink creates a permanent film/print on most substrates with a simple dip coating process. Ink spots on paper and fabric exhibit long-range semiconductor I–V properties. I–V curves were shown to select responses to different flavors of three different snacks. Because the ink material is environmentally friendly, it can be seen as a durable material for flexible electronic devices and sensor applications.

A conjugate material of CQD and NiO is used as an efficient nanomaterial for the detection of methane (CH_4) gas. The conjugate for the detection of CH_4 is effective at 150 °C. The detection mechanism is:

$$CH_4 + 4O^- = 4H_2O + CO_2 + 4e^-$$

Initially, on the surface of CQD-NiO, the adsorption of oxygen takes place by reducing to O^-. This is followed by the generation of a hole accumulation layer (HAL). NiO is highly efficient in capturing O_2 since the edges have a polygonal structure. A hetero-junction potential barrier is formed between the two layers that act as a potential CH_4 gas adsorption site due to the deposition of CQD on NiO. The thickness of the HAL decreases with time along with the increase in the rate of adsorption of gas molecules. Finally, at the completion of the reaction, the electron is transferred from the CQD to NiO followed by a lowering in the hole concentration of NiO. The reactive species such as O^- and CH_4 undergo simultaneous oxidation and reduction reactions that result in an attenuation of the HAL and as enhancement in resistance as well. This is how the methane is detected.

Till date not many reports have been found except the two above mentioned works on CQD as VOC sensor material. From the literature of graphene-based sensors, CQDs can also be designed as potential sensor material for detection of VOCs in exhaled breath related to diseases like PD, AD, or SARS-COVID-19.

21 VOCs Related to Alzheimer's Disease (AD) and Parkinson's Disease (PD) and SARS-COVID-19

The analysis of VOCs provides illness early identification. In reality, breath analysis has been utilized as a diagnostic technique for illnesses like typhoid fever and weak kidneys in Asian nations since ancient times by monitoring frequency. The identification of biomarkers in exhaled breath has drawn attention since Linus Pauling's original study in 1971 and is now a fast expanding field of study. Testing VOC biomarkers frequently involves selective detection of (pre)identified VOCs or cross validation with standard validation. The measurement of changes in the electrical, optical, chemical, and biological aspects of sensory data linked with VOC molecules is the fundamental basis for the detection of VOC biomarkers. The two most prevalent neurodegenerative disorders are Alzheimer's disease (AD) and Parkinson's disease (PD). Today, PD affects more than 1.6% of the world's population, and AD affects more than 26 million individuals globally. Patients, carers, medical professionals, and society are under increasing strain as a result of the rise in neurodegenerative disorders caused by the aging population. Since PD and AD are progressive, neurodegenerative illnesses with notable motor symptoms, there is no chemical diagnostic test for their detection. Identification of volatile organic compound (VOC) patterns in exhaled breath is a novel diagnostic strategy. Breath patterns that are distinctive to a disease could be beneficial as reliable and accessible biomarkers. This strategy is justified by the possibility that, even at the very beginning of the disease, lung-mediated changes in blood chemistry may be partially conveyed to the alveolar exhaled breath.

On the other hand, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is highly contagious, emerged in 2019 and caused serious respiratory infections known as "Covid-19" virus disease, threatening human health and public safety. The testing method for COVID-19 is based on polymerase chain reaction (PCR) technology. PCR provides accuracy and specificity, and the effectiveness of this method is affected by slow delivery, usually within 1 or 2 days after testing. Therefore, rapid tests based on external analysis or ELISA methods are often used as preliminary tests. On the other hand, direct detection of the COVID-19 virus by inhalation can be done routinely using specialized equipment that can collect exhaled breath and concentrate it for a few minutes and use this condensation to remove and track the virus pattern PCR. No amplification was detected. In addition, it has been widely documented for the cellular production of metabolites that lead to the respiration of volatile organic compounds (VOCs) by bacteria. These VOCs can be targeted for respiratory testing and used to measure health without harming the patient (Table 4).

VOCs detected for disease diagnosis in recent years have received a lot of attention due to their lack of diagnosis and movement. Fast and inexpensive diagnostic tools and real-time detection are an added benefit of VOC detection. Compared to other treatment modalities, the lack of VOC analysis studies for the detection of PD biomarkers and the absence of a matrix for the detection of potential biomarkers

Table 4 VOCs as biomarkers and their source. The biomarkers have been detected using GC-MS

Disease	Disease-related VOCs	Sample source
Alzheimer's disease	Styrene	Breath
	1-methyl-2-(1-methylethyl)-benzene	
	4-methyl-octane	
	2,6,10-trimethyl-dodecane	
	3,7-dimethyl-decane	
	Butylated hydroxytoluene	
	2,4-dimethyl-1-heptene	
	2,3-dimethyl-heptane	
	Propyl-benzene	
	2,2,4,6,6-pentamethyl-heptane	
	2,5,6-trimethyl-octane	
	5-ethyl-2-methyl-octane	
	2,6,10,14-tetramethyl-hexadecane	
	3,7-dimethyl-propanoate (E)	
	2,6-octadien-1-ol	
	2,3,5-trimethyl-hexane	
	1-methylethyl-benzene	
	1-methylpropyl-cyclooctane	
	2,2-dimethylpropanoic acid (pivalic acid)	
	2-ethylhexyl tetradecyl ester	
	Oxalic acid	
	2-butyl-1-octanol	
	Dodecane	
	1-chloro-nonadecane	
	3-ethyl-2,2-dimethyl-pentane	
	1,1′-oxybis-octane	
Parkinson's disease	Styrene	Breath
	2,3,6,7-tetramethyl-octane	
	Butylated hydroxytoluene	
	5-ethyl-2-methyl-octane	
	Decamethyl-cyclopentasiloxane	
	Ethylbenzene	
	1-methyl-3-(1-methylethyl)-benzene	
	3,7-dimethyl-decane	
	2,3-dimethyl-heptane	
	5-ethyl-2-methyl-octane	
	2,3,5-trimethyl-hexane	

(continued)

Table 4 (continued)

Disease	Disease-related VOCs	Sample source
	Hexadecane	
COVID-19	Methylpent-2-enal	Breath
	2,4-octadiene	
	1-chloroheptane	
	Nonanal	

are limitations of its practical application. One limitation is the uncertainty of VOC biomarkers because their structure is dependent on genetics and other factors such as changes in environment, age, sex, and metabolism. There are several reports of the use of graphene sensors to detect NO in exhaled breath for PD diagnosis.

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Functional Biosensors in Cell and Tissue Fabrication for Smart Life-Sciences Applications

Guven Akcay, Cagla Celik, Nilay Ildız, and Ismail Ocsoy

Abstract Biosensors are sensitive, selective, and rapid bioanalytical applications for diagnosis in cell and tissue engineering technologies. Compared to the enzyme-linked immunosorbent assay performed by standard methods, biosensors provide many advantages. In particular, biosensors designed through a combination of chemical, biological, and physical methods provide a good strategy for monitoring microbio-physiological signals in real time and in situ. This chapter summarizes the latest developments in innovative biosensor applications for different technologies of biological interest, essentially cell and tissue engineering. The latest studies and innovative approaches to biosensors for the diagnosis and bioimaging of tissue disease modes due to central nervous system, cardiovascular system, and endocrine system disorders are discussed. In addition, various biochip approaches such as cell/tissue-based biosensors, flexible biosensors, and paper-based biochips are mentioned. Finally, we discuss the diagnostic challenges current biosensors face and highlight the future prospects of biosensors for cell/tissue engineering applications.

Keywords Biosensors · Tissue engineering · Diagnosis · Biochips

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1 Introduction

Biosensors are known as the next generation sensing technology that encompasses a variety of technologies and knowledge from many disciplines, including biology, chemistry, and physics. Combining interdisciplinary technologies, biosensors offer a functional analytical platform to analyze the need in environmental, food, public health, microbiology, and biomedical sciences. Biosensors first detect results through optical, chemical, or electrical components and then use receivers, transducers, and imaging systems to convert this detection into a measurable signal [6]. Thanks to components, biosensors can measure signals at very low levels. Thus, they provide fast results with high stability and high sensitivity from a small number or quantity of human samples, without the need for specialized personnel. The first biosensor to be developed for measured glucose with a method based on electrochemical techniques using an electrode containing immobilized glucose oxidase [11] enzyme. With the advances in manufacturing techniques [50] and innovative approaches including nanotechnology, electrochemistry, and photolithography [51], biosensor applications have made incredible progress. Nowadays, the parts of biosensors that act as receptors have been given various functions to detect toxic substances, proteins, cell, and tissue behavior [59, 60]. Especially in clinical studies, biosensors developed to detect molecules are widely used to detect genes, proteins, or cytokines in patient samples. Molecule-labeled biosensors utilize a variety of specific biochemical reactions mediated by DNA and ion channels to detect enzymes, receptors, antigens, and antibodies [7]. In particular, the detection of substances released as a result of cellular reaction in microfluidic biochips is possible by immobilizing antibodies or aptamers specific to the cells used as biomarkers to the biosensor [39, 61]. The main advantage of molecular-based biosensors is that the conjugation of biomarkers with highly selective biomolecules allows the identification of a range of analytes with high sensitivity and selectivity [15]. In order to analyze the targeted molecules, genomic probes with specificity such as antibodies, nucleic acids, enzymes, etc., are integrated onto the surface of the sensor by physical adsorption and chemical grafting methods. Subsequently, the change of piezoelectric, calorimetric, optical, or electrochemical signals from the transducer components is converted into output in the form of electrical signals [12]. In order to detect interleukin-2 (IL-2), Arya and co-workers used 4-fluoro-3-nitrophenyl (FNP) as a coupling agent to bind the IL-2 antibody to a gold electrode surface. Thus, in the developed biosensor, IL-2 was detected by measuring the anti-IL-2-fixed cyclic voltammetry (CV) results resulting from the interactions between antigen and antibody on the gold electrode surface [2]. Furthermore, Chen et al. developed a biosensor consisting of aptamer-based porous silica nanoparticles (MSN) that can provide controlled release [8]. In this sensor, they conjugated the FITC fluorescent dye encapsulated in MSN to DNA by click-chemistry method. After detection of thrombin with DNA aptamers on the MSN, the encapsulated FITC fluorescent dye is revealed from the pores of the MSN, indicating that biosensors can also be controlled depending on a stimulus. Another technique exploits the physical properties of material surfaces to recognize different molecules.

Cell- or tissue-based biosensors, like molecule-based biosensors, offer significant innovations for the detection of cell-associated analytes in situ over the last decade [22, 36, 45]. Such biosensors provide information about the responses of cells and tissues by measuring phenotypic outcomes in cells [18]. Essentially, cell-based biosensors consist of three components: viable cells, bioactive components, and transducers. Thanks to the bioactivity of the components present as substrates, biomarkers released from cells grown on the biosensors can be measured, or cellular polarity resulting from the charge exchange of the cells.

After cells are treated with pharmaceutical or biochemical agents, the changes induced by the agents in the cells can be recorded through physiological parameters such as change in cellular polarity, change in cell membrane permeability, or ligand expression [36]. Molecule-based detection biosensor technologies perform a high selectivity for the analyte molecule to be detected compared to cell-based detection biosensors. However, the short lifetime and the cost of the isolation process of the molecules that will identify the biomarker of interest, such as antibodies, limit the applications of such biosensors. Therefore, cell-based biosensors offer an innovative approach to the diagnosis of diseases with rapid analysis. Biosensor systems are advanced from the cell level to the tissue level to provide more precise results for disease diagnosis. Multicellular cultures and organoids are often used in tissue-based biosensors as they are considered biomimetic structures due to their resemblance to natural tissues. Instead of multicellular cultures or organoids, complex 3D tissue-like structures that provide the functions and properties of natural tissues are being developed by tissue engineering. In recent developments, the production of 3D structures with biological effects in vitro has contributed significantly to tissue-based biosensors, offering potential biosensor strategies to predict, monitor, and diagnose the effects of pharmaceutical agents. The innovative tissue-based biosensors, the so-called organ-on-a-chip platform, is attracting intense interest as the 3D structures developed to mimic the properties of biological tissues. Integrated with a mobile control system, the structures can behave as physiological mechanisms such as blood flow. Another study by Bavli et al., an innovative biosensor, was developed using particle-type oxygen sensors and liver organoid [5]. With this sensor, it is possible to monitor the change in mitochondrial function after the administration of drugs, providing a liver-like response and exciting those working in this field.

In this book chapter, we review the current biosensor progress in the biomedical field, including biosensor fabrication technologies in different biological materials, biosensor types, and their applications in various fields, in order to highlight how and for what purpose biosensors are used in the field of tissue engineering. Thus, we hope to contribute to the knowledge of scientists working in this field to understand the shortcomings and working mechanisms of current biosensors to enable them to realize advanced biomedical applications.

2 Current Technological Approaches for Development of Biosensors

Various sensors such as biochips, paper-based biosensors, nanoparticles, and labeled or label-free biosensors including flexible biosensors are being developed. In recent years, different methods such as computerized numerical control (CNC), photolithography, and casting have been used to develop various biosensors. Biosensors are often produced not only by one of the mentioned methods, but by combining two or more methods. This shows that existing techniques perform a multifunctional activity to design complex or simple sensor mechanisms.

Photolithography is a favored technique for modeling proteins and cell structures in biosensor applications and tissue engineering studies. Photolithography is the method of transferring different patterns on a mask to a glass or silicon surface using UV light [23]. In biosensor design, photolithography allows microelectrodes of different shapes and sizes to be patterned on the surface of biochips using UV light. This enables the fabrication of transducer structures in very small sizes [40].

The developed biochips require model cells to create biomimetic structures. Factors affecting cell binding and cell behavior include pattern shape, size, surface modification, and surface topology. In developed enzyme sensors, DNA sensors, immune sensors, and several biomarkers are modeled on biochips with interlocking electrode arrays [13, 43].

3 Biosensor Development in Tissue Engineering

Tissue engineering, which works in line with engineering sciences and biological principles, carries out studies to develop various tissue structures to restore, repair, or maintain the impaired functions of damaged cells and tissues [31]. In tissue engineering techniques, cell-to-cell and cell-to-material interactions must coexist for optimal physical and cellular signaling to occur. Accordingly, it is required to identify and monitor cellular responses, cellular signals, cell functionality, and behavior. As seen in recent developments, biosensors are widely used in tissue engineering applications. In particular, it has become common to develop tissue engineering systems in microfluidic platforms where biosensors developed to monitor cellular behavior and specific biomolecules in tissues are integrated. These miniaturized systems provide critical information for rapid and real-time prediction of physiological response through electrical, optical, and electrochemical systems [24].

3.1 Biosensors for Cell-Based Applications

3.1.1 Biosensors for Monitoring Cell Polarity Change

Biosensors based on measuring polarity change are used to monitor biochemical reactions occurring at the cellular level. In a study, a polarity-sensitive biosensor that can fluoresce was developed based on the detection of cell apoptosis with images of viable cells [28]. This biosensor was applied to investigate changes in neuronal degeneration process in vivo and in vitro.

3.1.2 Biosensors Developed for Monitoring Cell Behaviors Such as Metabolization, Proliferation of Stem Cells

Biosensors developed for monitoring cellular changes are used in the fields of cellular signal detection, cell behavior, drug toxicity, and disease modeling. Various biosensors have been developed for the transmission of cellular signals such as measurement of cellular metabolic activities, and monitoring the change of charge potential in the cell membrane. Tissue engineering is critical for the evaluation of electrophysiological properties of cardiomyocytes and neuronal cells. Microelectrode arrays (MEAs) have been developed to detect the electrophysiological properties of living cells as a result of their reactions. Chowdhury et al. developed a method that combines MEA with optical mapping to record action potential and contact electrograms simultaneously. Because they thought that the cellular action potential and contact electrogram are linked and should change during arrhythmogenesis [10]. In addition, biomechanical measurements associated with cardiomyocytes to investigate the pathophysiological electro-mechanical coupling were previously performed using different devices [29]. In another study, microbead-based sensors based on fluorescence irradiation were developed to detect hepatocyte growth factor and transforming growth factor-β1 [48]. Various sensors have also been developed to measure signaling molecules released from cells such as nitric oxide (NO) and hydrogen peroxide (H_2O_2) [47, 55]. NO is an important messenger molecule in biological systems and H_2O_2 plays an active role in cellular communication, cell migration, and immunity formation. In Fig. 1, Au nanoclusters and a poly(toluidine blue) modified electrode were combined to detect H_2O_2 and nitric oxide, respectively using microfluidic technology. Similarly, Visser and co-workers preferred inkjet methods to print carbon MEAs on materials that can mimic the extracellular matrix consisting of gelatin, PDMS, and various types of hydrogels to produce soft MEAs [52].

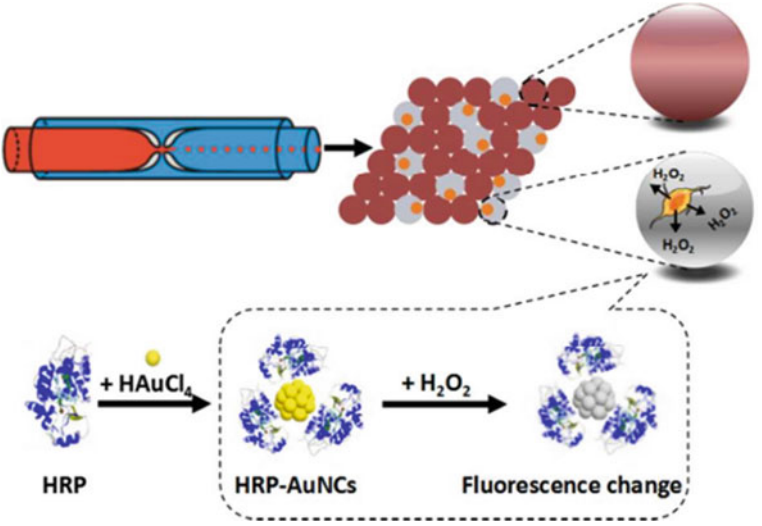

Fig. 1 Detection of H_2O_2 by Au nanoparticles combined microfluidic droplet sensor. Reprinted with permission from [47]. Copyright (2018) ACS publishing

3.2 Strategies of Biosensors to Detect Cell-Released Analytes

3.2.1 Label-Free Biosensors

The determination of the number of proliferating cells, protein quantification, or cellular migration is often preferable to monitoring cellular behavior in a given culture medium. Traditionally, the detection of cell viability and cell number can usually be performed by counting cells under a microscope, measuring DNA content, or detecting viability, such as the MTT assay. However, these techniques are labor-intensive and lengthy procedures. Noninvasive and label-free detection methods, on the other hand, offer advantages in tissue engineering applications and stem cell technology, such as the ability to stain, fix, and fluoresce as desired.

3.2.2 Surface Plasmon Resonance (SPR)-Based Biosensors

Biosensors based on SPR have recently been revealed as a multipurpose biosensor providing the advantages of small sample volumes, live cell analysis, and high throughput. In addition, SPR biosensors enable label-free, real-time analysis with increased sensitivity to the change in refractive index of the analyzed structures [27].

In recent studies, an SPR-based biosensing device has been developed that offers an innovative approach to analyze the osteogenic change of mesenchymal stem cells [30]. Moreover, the SPR-based biosensor has been used to monitor cardiac troponin

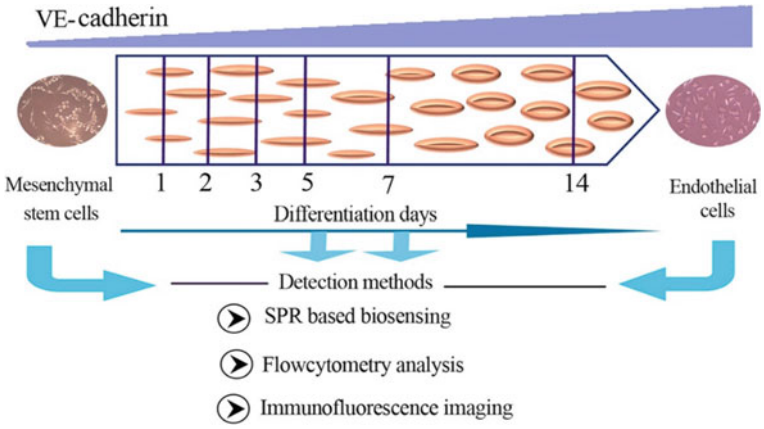

Fig. 2 Schematic illustration of SPR biosensor for detecting vascular endothelial-cadherin expression. Reprinted with permission from [19]. Copyright (2017) ELSEVIER publishing

T and fatty acid binding protein 3, two biomarkers used clinically for the assessment of cardiotoxicity [1].

Furthermore, Fathi and co-workers designed an SPR detection system in Fig. 2 that responds accurately and rapidly to SPR signals by analyzing vascular endothelial-cadherin expression, which can detect endothelial differentiation early [19].

3.3 Biosensor Applications in Various Diseases

3.3.1 Biosensor Applications in Neural Diseases

Biosensors enable the diagnosis of diseases by detecting various signals from tissue. Traditional methods of diagnosing neurological diseases are time-consuming and inconvenient to use. This is because a clinician is needed to check the symptoms of the diseases and in most of diseases, such as Parkinson's disease (PD), there is a risk that about 40% of people may be underestimated in the early stages [44]. Neurological studies are one of the research areas where cell-based biosensors are proving to be important. With MEA technology, it is possible to monitor neuronal circuits, physiological system and abnormalities, and detect malfunctions [46]. The MEA technique offers advantages such as multisite recording, long-term culture, and non-invasive monitoring of the electrophysiological activity of neuronal cells for high-throughput screening [9]. Furthermore, Lourenco and co-workers developed a new multimodal technique for metabolic, electrical, and hemodynamic measurements together with neuron cell activity [38].

Based on the MEA technique, the scientists fabricated an innovative micro-electrode arrays in Fig. 3 that combined neuronal network development under the

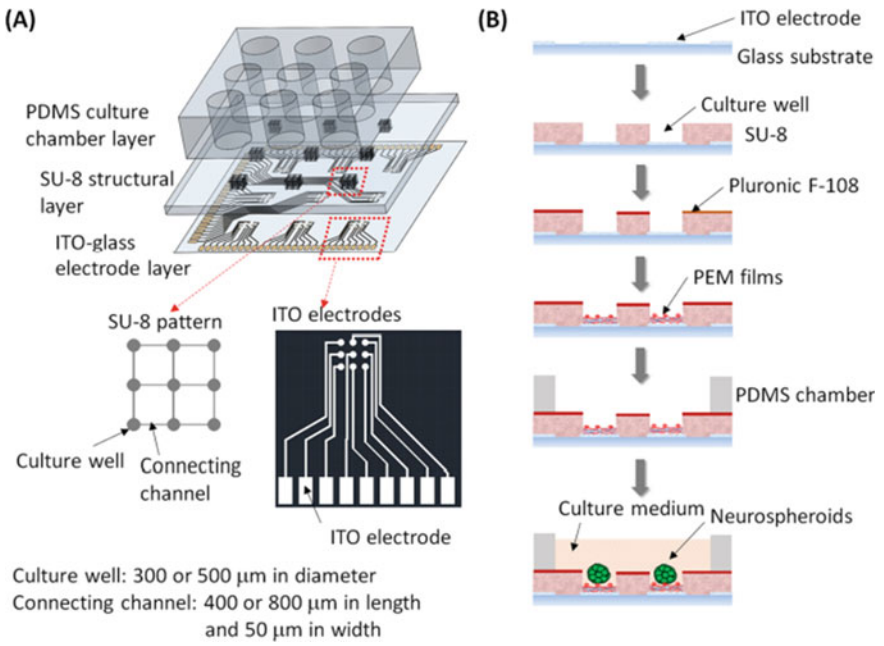

Fig. 3 Schematic illustration of the design of a biochip by using the MEA method. **a** Schematic illustrating the design of the biochip. **b** The process used to fabricate the biochip. Reprinted with permission from [37]. Copyright (2018) ACS publications

guidance of neurite outgrowth ITO-PEM microfluidic system [37]. The biochip in Fig. 3 included 3 different functional layers [37]. It offers a good alternative for on-chip organ development, tissue engineering, drug discovery, disease modeling, and biomaterial testing.

Biochips integrated into biosensors are exciting and make important contributions to related fields of study. In particular, biomimetic chip and biosensor integrated systems make it possible to monitor the electrophysiological properties of cells, early-stage differentiation, stem cell proliferation, neural network formation, and stimulation response. Even micro-sized and ultra-flexible electrocorticography arrays using glassy carbon electrodes have been used to monitor brain activity [53]. For instance, in another study, Xie et al. developed a nanoelectronic probe with microscale pores to solve problems such as mechanical incompatibility and instability of conventional metal and silicon microprobes used for brain recording [57]. Thus, they developed a device with 3D microscale pores decorated with wire-shaped nanoelectrodes. This probe with micropores supports integration with the brain by providing a neuron/probe interface. This makes it possible to record action potentials from the somatosensory cortex in the brain. Live cell-based devices have been used to monitor endothelial barrier function and molecules released from cells [34]. Li and co-workers fabricated a reversible electrode for rapid diagnosis of Alzheimer's disease using graphene oxide nano structures with magnetic properties (Fig. 4) [33].

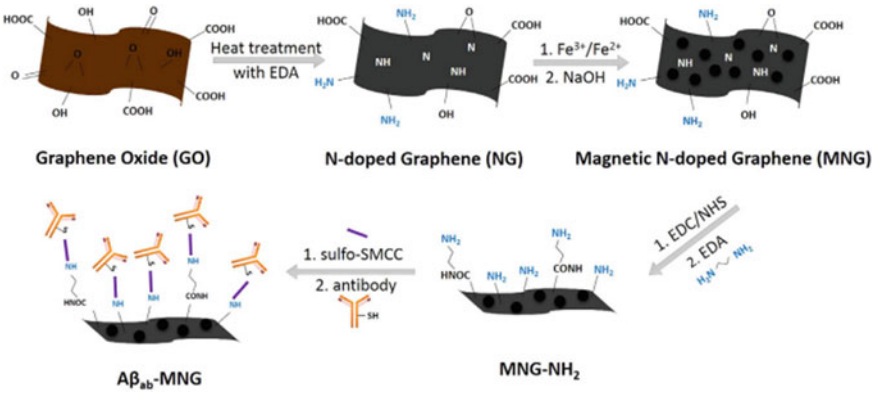

Fig. 4 Schematic illustration of Aβ42-immobilized graphene-based biosensors for diagnosis of Alzheimer's disease. Reprinted with permission from [33]. Copyright (2016) Springer Nature Limited.

They integrated an Alzheimer's disease's biomarker of Amyloid-beta peptide 1–42 (Aβ42), onto a nitrogen-doped graphene (MNG) with magnetic properties. Furthermore, another biosensor that rapidly detects PD was developed by Yang et al. (Fig. 5) [58]. They formed a monolayer (SAM) by attaching a DNA aptamer and an SH-spacer onto the substrate molecule. When the biosensor is treated with PD biomarker (a-synuclein), the DNA aptamer performs a specific attaching to the a-synuclein protein. Subsequently, the changing signals based on optical analysis resulting from the capturing of a-synuclein can be easily identificated under a microscope. When the developed biosensor is compared to conventional Western blot or ELISA methods, these sensors appear to be novel, easily applicable and rapid strategies to facilitate the diagnosis of neurologically based diseases.

3.3.2 Biosensor Applications in Cardiac Diseases

It has been clearly demonstrated that the monitoring and measurement of electrical signals generated in physiological mechanisms in the body is one of the main functions of biosensors. Remarkably, bioelectric activity is often monitored to control the function of cardiac tissues. Bioelectric activity can be generated by cardiomyocytes, which induce changes in the action potential in the membrane of the capillary cells. Thus, due to the alteration of the action potential, cardiac cells can induce a synchronized pumping behavior through organized electrical propagation [16]. Dutta et al. conducted a study showing that the difference in oxygen level measured in tissues significantly interrupts the regular routine of the action potential [17]. Consequently, continued monitoring of electrocardiogram (ECG) data has emerged as a traditional method to detect the cardiac rhythm signal in the diagnosis of cap-related diseases. Lee and co-workers designed a small-size wearable flexible biosensor to easily and continuously monitor heart rhythm signals [32]. With the developed cardiac

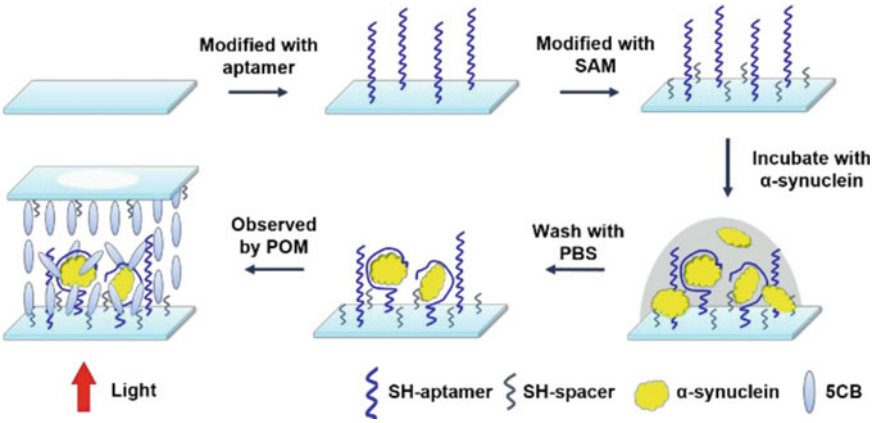

Fig. 5 Schematic illustration of the diagnosis of a PD using PD biomarker (α-synuclein) with a biosensor. Reprinted with permission from [58]. Copyright (2020) Royal Society of Chemistry

biosensor, changes in patients' heart rhythm signals can be monitored directly on their smartphones (Fig. 6). Feiner and colleagues also produced a degradable electronic scaffold as a heart patch. With this flexible product, the spontaneous contraction-relaxation signals of heart cells can be detected. It also provides an external electrical stimulation to control the rhythm of irregularly contracting heart cells [21]. This work, a chip containing a biosensor, also contributes to heart cells that mimic heart-related diseases.

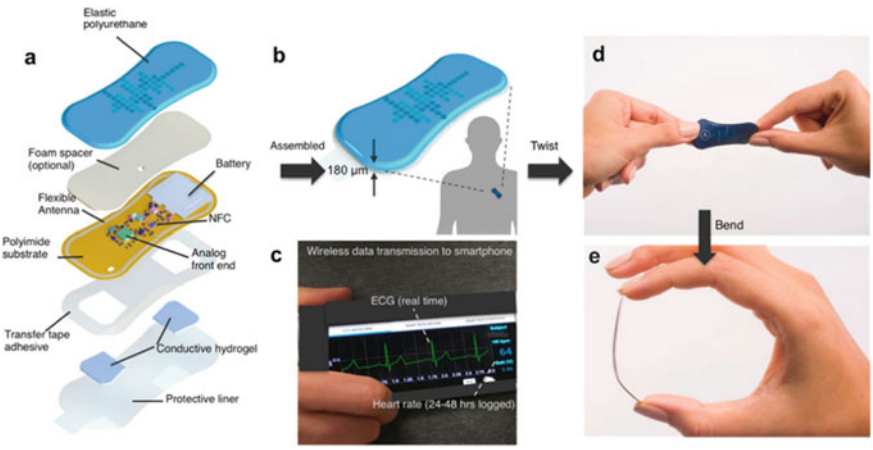

Fig. 6 Schematic images of a flexible, soft cardiac biosensor capable of electrocardiogram (ECG) waveforms and measuring real-time heart rate. Reprinted with permission from [32]. Copyright (2018) Springer Nature Limited.

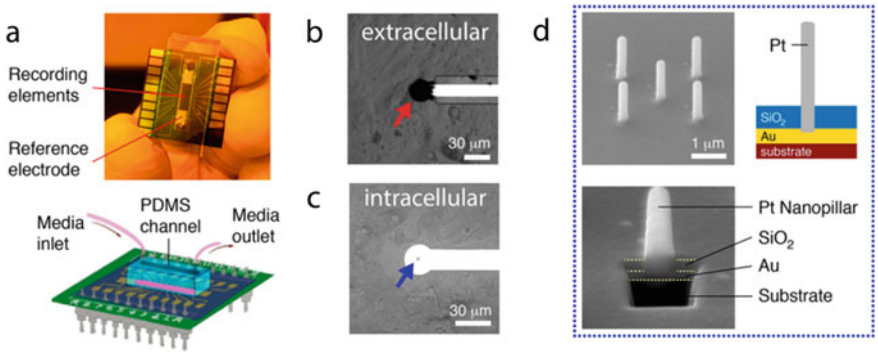

Fig. 7 Schematic illustrations of a heart-on-a-chip with Au electrode and PDMS channels. Reprinted with permission from [35]. Copyright (2020) ACS publications

Liu and co-workers developed a Pt nanostructure array integrated on Au electrode as shown in Fig. 7 [35]. The developed on-chip biosensor makes a significant contribution to explain the effect on the electrophysiological behavior of the heart in hypoxic state.

3.3.3 Biosensor Applications in Cancer Diseases Detection

In the last decade, cancer research has gained great momentum. Traditionally, cancer research has focused on the development of effective therapeutic methods to treat cancer diseases. But in some cases, patients are often diagnosed with cancer at the last stage. Early diagnosis is vital in cancer treatment. For this reason, the most effective treatment period for patients diagnosed at the last stage is over [20]. Therefore, how to diagnose cancer diseases quickly and accurately has recently become a popular topic for cancer research. Various biomimetic cancer models have been designed to investigate the formation, mutation, and metastasis mechanisms of cancer cells. In another study, Kamei et al. produced a microfluidic chip that creates a cancer model by designing a chip combining heart and liver cancer cells [26]. With this model, the effects of drugs in the bloodstream on the migration and metabolism of liver cancer cells can be easily monitored. Biosensors developed for early-stage detection offer a fast and easy strategy for producing biosensors that can diagnose versatile cancers. For instance, Pan et al. designed a chip labeled with two different biomarkers, vascular endothelial growth factor (VEGF) and prostate-specific antigen (PSA), to detect prostate cancer and its tumor cells (Fig. 8) [42]. These biomarkers were designed by immobilizing gold nanorods (GNR) on a silicon chip. Then, biomarkers secreted from cancer cells in the circulatory system were specifically captured by the labeled chip. The binding was proven by the absorbance value obtained from UV-Vis spectrophotometer after one hour of incubation.

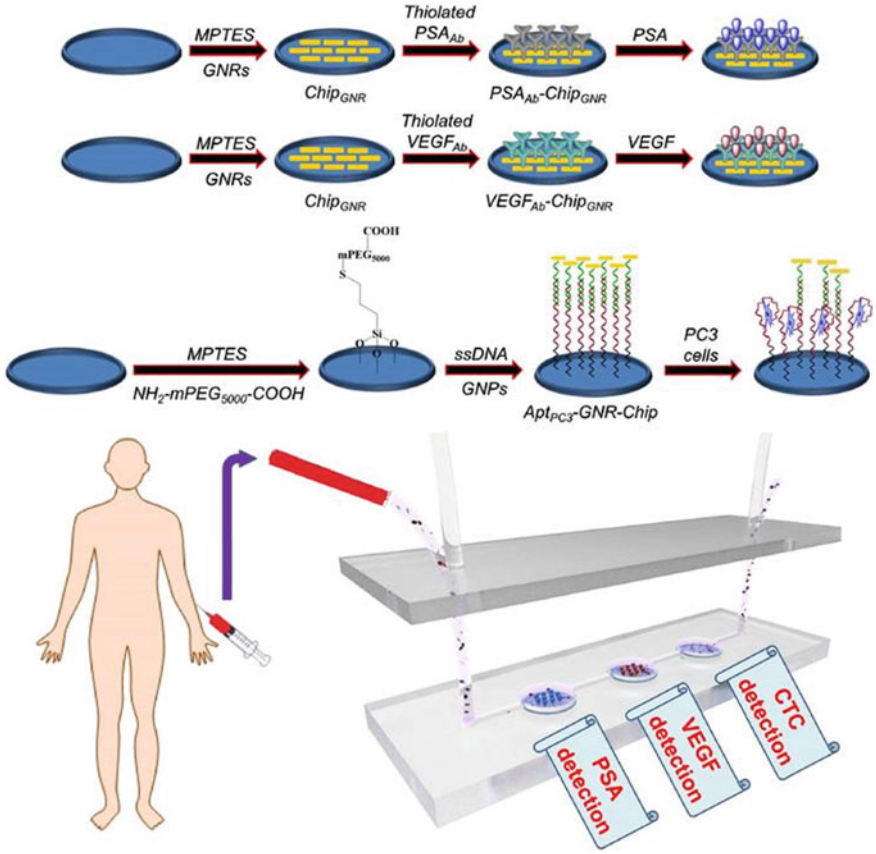

Fig. 8 Schematic representation of a prostate cancer biosensor that captures VEGF and PSA. Reprinted with permission from [42]. Copyright (2017) Ivy Spring International Publisher

In another study, Hu et al. designed a different type of visible signal-generating biosensor that could enable detection. Detection of low-expressed extracellular vesicle (EV)-associated RNA in the early stage of cancer is very difficult. To overcome this challenge, they developed a chip that integrates nanoparticles to capture and detect EV-associated RNA. The cationic polymer nanoparticles triggered the binding of glypican-1 mRNA, a pancreatic cancer biomarker, to EV in serum, producing multiple signal outputs after 30 min of incubation. This technique is an innovative approach to detect pancreatic cancer patients even at an early stage. These studies show that biosensors integrating genomic probes play a critical role in the rapid and early stage diagnosis of cancer diseases (Fig. 9) [25].

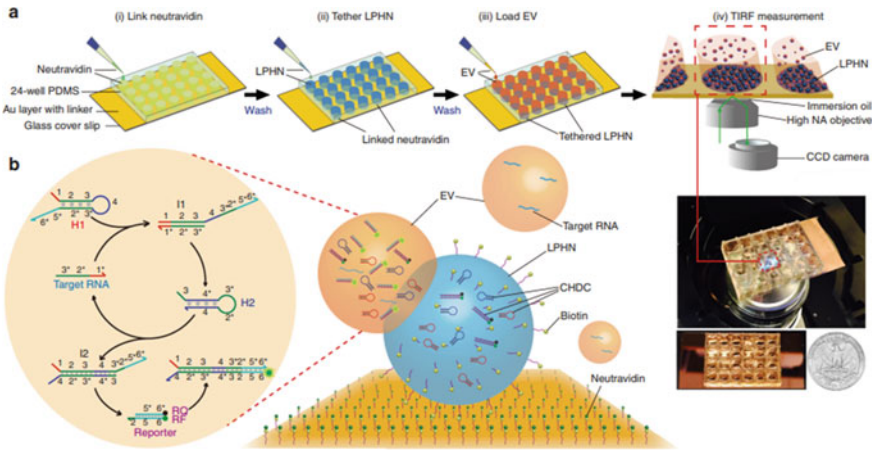

Fig. 9 Schematic illustration of the biochip by linking neutravidin with a tether lipid-polymer hybrid nanoparticle (LPHN) and loading extracellular vesicle (EV) on an Au layer. Reprinted with permission from [25]. Copyright (2017) Springer Nature Limited.

3.4 Biosensor Applications in Bioimaging Technologies

Apart from in vitro applications for disease detection, biosensors are used to improve sensing capability in bioimaging applications as they have a fast and sensitive labeling function. Related to this concept, nanomaterials, which are candidates for bioimaging due to their small size, high surface area, and modifiable properties, are used in biosensor design for various purposes [56]. Various types of nanomaterials, including polymers, silicas, polymers, and carbon dots have been fabricated for different aims, suitable for diagnostic machines such as computer topography imaging (CT imaging), magnetic resonance imaging (MRI), fluorescence microscope images [56]. In a study, they synthesized porous silica nanoparticles encapsulated with a dye that fluoresces in the near infrared (NIR) as a breast cancer cell targeting agent with LS277 [41]. Compared to the case where only LS277 is delivered, the mesoporous silica nanoparticle delivers images with five times more resolution than that used in LS277 alone. Furthermore, Bao et al. used an NIR triggering technique to develop carbon dot-containing nanoparticles [3]. By synthesizing carbon dots with urea, DMSO, citric acid an N, S-doped carbon dot with NIR fluorescence can be produced and rapidly removed from organs such as kidney or liver 24 h after intravenous injection. Interestingly, NIR fluorescence images after injection showed that S, N-doped carbon dots accumulated considerably in tumor tissues after application. This result indicates that S, N-doped carbon dots activate tumor labeling of a compound. Bao and co-workers developed magnetic iron oxide nanoparticles for MRI imaging and Dong et al. developed gold nanoparticles for bioimaging in CT techniques [4, 14]. Both results from their studies confirmed that the nanomaterials function as contrast agents for use in CT and MRI imaging. By adjusting the size of

gold nanoparticles or magnetic iron oxide, the image contrast of the nanoparticles in diverse tissues and organs can be enhanced (Fig. 10). For instance, magnetic iron oxide in 4 nm sizes can enhance T1 image contrasts in MR images. The same is true for CT imaging when the size of gold nanoparticles is 4 nm [4, 14]. Moreover, by decorating the surface of nanoparticles with chemical structures such as folic acid, they can act as tumor contrast agents [4]. Therefore, various types of nanoparticles used in the design of biosensors have potential use as bioimaging agents.

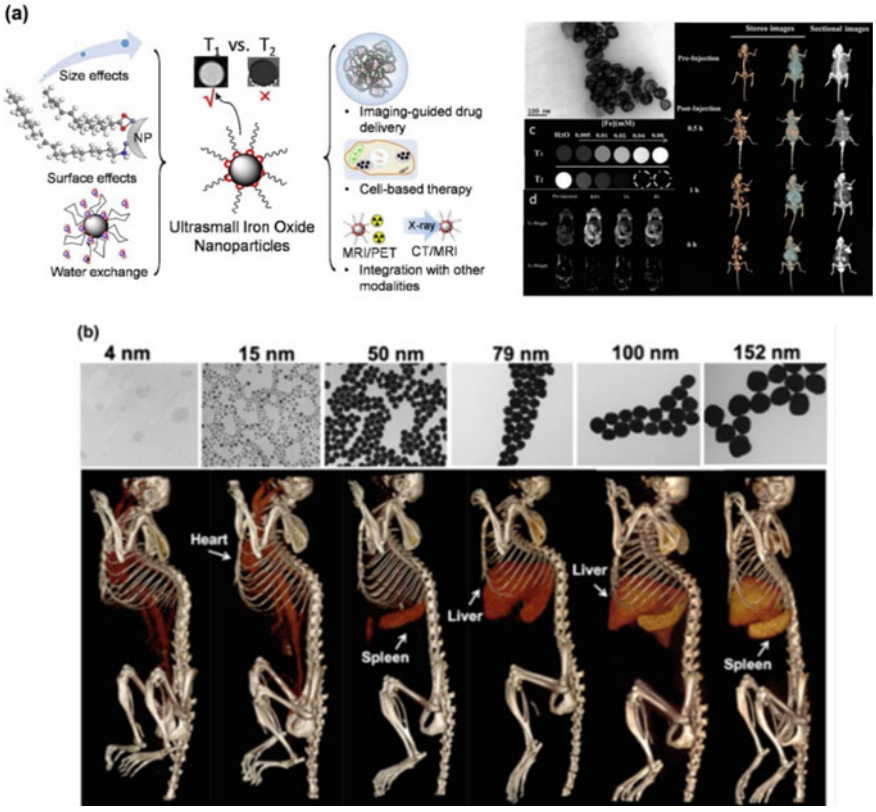

Fig. 10 **a** Schematic illustration of iron oxide nanoparticles as T1 MRI contrast agents and possible integration with CT and PET. Reprinted with permission from 64. Copyright (2018) Royal Society of Chemistry. **b** The TEM images of gold nanoparticles with different particle sizes. The images indicate that controlling the size of nanoparticles effect enhances the image contrast at various organs. Reprinted with permission from [14]. Copyright (2019) Springer Nature Limited.

4 Challenges and Future Perspectives

Next-generation biosensors are promising techniques that provide sensitive, selective, and rapid disease diagnosis for various applications in the field of tissue engineering. Compared to conventional ELISA assays, the developed biosensors offer a good solution for real-time acquisition of physiological signals by combining chemical, physical, and biological technologies. Biosensor systems have made significant progress in the last decade, but there is still a need to improve some of their functions. The biggest challenges of biosensors are their long-term stability and scale-up process. Stability is still a major challenge, especially during conversion to commercial products. Biosensors are mostly developed as prototypes in a research laboratory. For this reason, scale-up technology is very important in the commercialization phase. This is because it requires rapid mass production of good quality biosensors from the laboratory level to the industrial level. It is very difficult and costly to scale up the results obtained in the research laboratory to a commercially viable level on a large scale. Furthermore, the time from the industrial production level to the retail level to reach the user is often longer than expected. To overcome this challenge, some biosensor chips are developed by immobilizing proteins, growth factors, and antigens that can cause a short expiry date.

In general, biosensors suffer from the problem of stability in order to be able to successfully perform the sensor task again in the case of long-term storage. Furthermore, biosensors are advanced systems that allow even extremely weak signals to be recorded from small amounts of samples in a low-noise environment. But in a real clinical setting, there is a complex matrix that cannot be predicted in advance. Therefore, background noise can increase, which can reduce the accuracy of the results. Furthermore, when biosensors are working, they often need to be connected to controllers, imagers, and other equipments. This can make biosensors less desirable to the user as they require complex operation and have user-unfriendly interfaces.

In order to solve the aforementioned problems, techniques with higher sensitivity and faster response should be developed through interdisciplinary studies during the production phase of biosensors. For instance, the development of various nanostructures such as nanorods, nanocages, and nanostars within nanotechnological developments may provide the opportunity to increase the signal and sensitivity of the samples [54]. In addition, cell-based biosensors that can be used in vitro have continuous and real-time monitoring functions, while lightweight biosensors in small sizes, such as paper-based biosensors, offer great convenience for direct observation of results. Lightweight biosensors are more suitable for industrial production and are more prone to the feasibility of scale-up from the prototype stage developed in the laboratory to the industrial stage. Consequently, the current progression of sensors developed for tissue engineering applications is toward the evolution of small-sized, flexible, and relatively lightweight biosensors. To this end, technologies are being developed to facilitate the integration of biosensors into wearable devices.

For instance, tear-based biosensors have been preferred in the development of biosensors integrated into contact lenses. By using tears as a sample, glucose levels can be measured, enabling continuous monitoring of diabetes [49].

Thus, by using body fluids such as saliva, sweat, and tears through wearable biosensors, information about the physiological system can be obtained directly. Furthermore, wearable biosensors integrated with smartphone applications offer an innovative perspective that can be used for real-time continuous monitoring, diagnosis, or disease prognosis. We hope that this book chapter will be informative for scientists producing new technologies in the fields of biology, chemistry, and physics on how to design disease-related biosensors for applications in tissue engineering. Thus, we hope that new potential applications and technologies can be discovered for the active use of biosensors developed through academic research in clinical applications.

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