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Abstract

Optical biosensors are gaining popularity owing to their portability, miniaturization, no requirement for additional attachments and rapid responsiveness. These features render them suitable for various applications including at-home diagnostics, pharmacology, and continuous molecular monitoring. The integration of functionalized low-dimensional nanomaterials (zero-dimensional (0D), 1D, 2D, and 3D) has redirected focus towards the design, fabrication and optimization of optical biosensors. This review summarizes the fundamental mechanisms underlying optical biosensing. The key mechanisms include localized surface plasmon resonance (LSPR), photoluminescence (PL), surface enhancement Raman scattering (SERS), nanozyme-based colorimetric strategies, chemiluminescence, bioluminescence and

electrochemiluminescence. The advantages of various low-dimensional nanomaterials for different types of optical biosensors are presented. This comparison emphasizes their potential superiority in targeted biosensing applications. Therefore, promoting optical biosensing techniques and recent developments in advanced biosensing strategies for biomedical research and biopharmaceutical applications is necessary to establish their future directions.

Keywords: Optical biosensors; Low-dimensional nanomaterials; Fundamental mechanisms for biosensing; At-home diagnostics; Continuous molecular monitoring; Biopharmaceutical applications

1. Introduction

The field of sensing technology is essential for advancing diagnostics and molecular analysis, facilitating innovations with profound impacts on human health. One of the earliest examples of sensing technology is litmus paper for pH detection, which introduced chemical indicators as reliable tools for simple testing methods [1]. Research soon shifted focus to biological molecules, making enzymes central to the development of sensors. In 1962, scientists pioneered a glucose quantification method by combining an electrochemical oxygen sensor with glucose oxidase, paving the way for optical biosensors based on biological recognition molecules [2,3]. For decades, electrochemical sensing methods have remained dominant in the market due to their versatility and commercial adaptability for point-of-care (POC) testing [4]. However, recent advancements, such as miniature optics and automated microfluidics, have transformed optical sensors into practical, commercially viable devices. While electrochemical sensors hold a large market share primarily for glucose monitoring, optical biosensors have emerged as one of the fastest-growing segments, fueled by the expanding demand for lateral flow assays that extend far beyond pregnancy tests [5,6]. In general, an optical biosensor integrates a receptor and transducer to translate biochemical information into measurable signals with high sensitivity and specificity [7]. Compared to electrochemical sensors, optical nanosensors stand out in biomedical and biopharmaceutical monitoring due to their simplicity, portability, and rapid responsiveness [8]. The increase in miniaturized optical equipment, particularly complementary metal-oxide-semiconductor cameras in smartphones, has redefined optical biosensing. Handheld optical readers reduce costs and streamline detection, whereas smartphone cameras serve as signal readouts that link intensity readings to 3D information through digital holography [9]. The rapid advancements in miniature microscopes and real-time diagnostic capabilities are guiding the future of optical

sensing.

Over the past decade, breakthroughs in nanomaterials combined with advanced bio-recognition techniques have significantly propelled optical biosensing. Nanomaterials, when conjugated to target ligands, enhance the sensitivity at the nanoscale by acting as signal generators or detectors [10]. Nanomaterials have unique advantages, including high reactivity, biological permeability, and multiplexing capabilities. Their properties, such as ultra-small size, high surface area-to-volume ratio, adjustable shape, intense signals, photosensitivity, and biocompatibility, allow for effective operation within the quantum-confined optical domain. Examples include noble metal nanoparticles (NPs), nanorods (NRs), quantum dots (QDs), up-conversion nanocrystals, and porous framework-based nanomaterials [11−13]. Their unprecedented controllability, stability and multifunctionality have opened a new era in chemical and biological sensing, enabling rapid on-site detection with portable and cost-effective solutions. This review summarizes the fundamental mechanisms and key design principles for the assembly of optical sensors, with a focus on advanced nanomaterials that possess unique and essential properties. Additionally, the use of optical sensing in various biomedical and biopharmaceutical applications, such as *in vitro* diagnostics (IVDs), therapeutic drug monitoring, bioimaging, targeted therapy, and continuous drug monitoring using wearable or implantable devices, are summarized. The current limitations of these technologies are also discussed.

2. General principles of designing an optical molecular sensing platform for biomedical applications

An optical biosensor comprises two main components: a recognition system and a transducer platform. The recognition system determines the sensing performance characteristics, including sensitivity and specificity. The transducer platform converts the recognized events into optical signals. To achieve accurate sensing, two universal design strategies are summarized: the employment of reliable molecular recognition elements, and optimization of transducer platforms. Optical nanosensors can be divided into two primary categories based on the interaction mechanisms between the recognition element and targets: affinity and metabolism sensors [14,15]. Affinity sensors operate through binding interactions between a target and a receptor. Examples of these interactions include antigen-antibody binding, complementary DNA hybridization, and aptamer-based probes. The transducer platform generates optical signals in response to these complex formations. However, despite the prevalence of affinity sensors in the market, their progress is often limited by challenges such as

inadequate recovery performance, as exemplified by disposable test strips. In contrast, metabolic or kinetic sensors, such as traditional glucose biosensors, use molecular recognition based on enzymatic reactions. These sensors facilitate the chemical conversion of the analytes into relevant visual products. Consequently, the content of the target analytes can be quantitatively determined using transducer platforms. These sensors offer several advantages, including reusability and portability, as the active enzymes demonstrate prolonged stability. In this section, the mechanisms and principles for constructing optical sensing platforms are introduced, considering the varying transduction of biorecognition events (Fig. 1). Moreover, recent developments in optical sensors for biomedical applications are also discussed.

2.1 Localized surface plasmon resonance (LSPR) for POC tests

Surface plasmon resonance (SPR) is a phenomenon in which conduction electrons on a metal surface are excited to oscillate collectively, coupled with electromagnetic waves due to light irradiation. This technique is effective for investigating the specific interaction modes between biomolecules, providing vital data such as kinetic parameters and affinity constants in a label-free and real-time manner [16]. In contrast to the SPR observed on individual substrates like thin metal films at the microscale, the plasmonic effect resulting from illuminating light on metal NPs at the nanoscale is referred to as LSPR. Noble metal NPs, particularly those composed of gold and silver, are electron-rich metals. They present extinction coefficients that are approximately 1000 times higher than those of most the organic dyes, owing to the LSPR effect. This electron-rich characteristic enhances their interaction with electromagnetic waves, leading to the effective excitation of surface plasmons. The formation of strong electromagnetic fields causes a Gaussian-like peak in the visible frequency range and a corresponding resonance occurs. The intensity and position of the LSPR peak substantially depend on several factors, including the size, shape, composition, surrounding dielectric medium and the distance between adjacent nanostructures [17]. Therefore, target recognition processes can be conveniently identified by monitoring the shifts in the absorption band and corresponding color changes.

Typically, there are two LSPR sensing strategies based on the readout signals. The first strategy involves LSPR shift-based sensors that quantify recognition events based on spectral changes. The second strategy involves colorimetric sensors, which allow visual evaluation with the naked eye due to substantial absorption band shifts resulting from LSPR variation [17]. Spherical noble metal particles less than 50 nm in diameter exhibited a single resonance frequency. Consequently, conventional LSPR-based

colorimetric platforms employ aggregation or disassembly mechanisms because interparticle plasmon coupling results in absorption band shifts of approximately 200 nm [18]. However, intensity or monochromatic changes limit the colorimetric qualification of the naked eye. In addition, non-response aggregation caused by environmental instability depresses the specificity and sensitivity. In response to these challenges, numerous plasmonic multicolorimetric sensors have been designed and proposed to achieve polychromatic transduction by adoption of the "non-aggregation" strategy. Considering the key factors influencing LSPR properties, the methods to achieve the most visible absorption spectrum coverage of LSPR variation resulting in a rainbow-like color change, can be summarized into two patterns: etching and growth-based mechanisms. Among the notable nanomaterials used in plasmonic multicolorimetric sensors are gold NRs (AuNRs), which are one-dimensional (1D) anisotropic NPs possessing at least two LSPR peaks. This property makes them unique for transducing morphological changes into a visible color change since the longitudinal band is particularly susceptible to their aspect ratio [19]. Herein, diverse noble metal nanomaterial-based etching and growth strategies have been proposed and applied to the assembly of multicolorimetric biosensors, especially for at-home blood and POC tests [20]. Proteins and antigens serve as important biomarkers for early diagnosis and prompt control of communicable and noncommunicable diseases, especially malignant tumors, acute cardiac dysfunction and viral infections. In a conventional sandwich immunoassay system, a catalase-labeled antibody recognizes the target protein and subsequently translates the sensing reaction into a colorimetric readout. Unlike the monochromatic changes mediated by catalytic substrates, such as AuNR-etching-based multicolor immunoassays display vast vivid rainbow-like changes, allowing semi-quantitative visual detection [21]. Similar to AuNRs, Ag-shelled Au@Ag NRs (AuNBP@Ag) exhibit marked optical properties during etching process. A multicolor sensor for squamous cell carcinoma antigen (SCCA) detection was developed based on catalase-mediated etching of AuNBP@Ag. The formation of a primary antibody-antigen-secondary antibody-sandwiched structure stimulates the catalysis of H_2O_2 to generate ·OH, resulting in unusual longitudinal LSPR peak movements of the etched AuNBP@Ag NRs. This colorimetric sensor achieved a linear detection of SCCA from 2.5 to 105 ng/mL, with a corresponding limit of detection (LOD) of 0.85 ng/mL with a spectrometer (Fig. 2A) [22]. Likewise, heteroepitaxial growth-mediated core-shell nanostructure formation also causes LSPR peak transitions and colorimetric variations. A facile and rapid enzymatic reaction-guided metallization colorimetric assay was developed based on Ag-deposition on the surface of AuNRs, achieving accurate measurement of β-galactosidase activity with a LOD of 128 pM.

Combined with phage lysis, this assay was further optimized to detect infectious bacterial pathogens [23]. To improve the performance of LSPR sensors, Park and co-workers [24] demonstrated a binary mixture biosensing platform by integrating AuNRs and magnetically Pt@Ni nanorings to regulate plasmonic nanomaterials with an external magnetic field. This approach overcame the limitations of conventional designs such as core-shell NPs or multisegmented hybrid nanocomposites. Owing to its advantages including rapidity, accessibility, cost-effectiveness, portability, and simplicity, the lateral flow immunoassay (LFIA) is currently considered among the leading platforms for POC testing, particularly for at-home infection diagnosis and assessment. Chen and co-workers [25] achieved the ultrasensitive optical detection of hepatitis C virus antibodies in serum using a LFIA test strip. This was accomplished following the surface functionalization of a colloidal plasmonic core@magnetic shell nanocomposite by the co-assembly of Fe_3O_4 NPs and gold NPs (AuNPs) into polymer nanobeads. This enhanced plasmonic signal transducer provided at least a fourfold improvement in sensitivity. In addition, a dual-mode LFIA platform was also developed that utilizes the distinct optical properties of the Au-shelled AgNPs. This platform achieved simultaneous colorimetric and surface enhancement Raman scattering (SERS) detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgG with the LOD of 10^{-7} mg/mL and 0.22 pg/mL in serum, respectively [26].

2.2 Fluorescence for biomedical diagnostics

Fluorescence is known as an established and dominant optical technique in biomedical diagnostics and real-time monitoring, even offering single-molecule detectable sensitivity and facilitating the real-time visualization of changes. When a fluorophore absorbs radiation at a different energy level, it emits fluorescent light at a specific wavelength. This process creates a wavelength difference referred to as the Stokes shift. In brief, there are two types of photoluminescence (PL) mechanisms: down-conversion and up-conversion. Fluorescent nanoprobes designed by both these processes have long been used in fluorescence-based optical biosensors [27]**.** Among these, NP-based fluorescent sensors have emerged as prominent devices with several distinct advantages, such as excellent photostability and biocompatibility, high emission rates, large Stokes shifts and facile surface tailorability. Nevertheless, in traditional fluorescence-based optical biosensors, the intensity-based signal output can be inaccurate due to inconsistencies in instrumental parameters, background light scattering from sample matrices, and inadequate washout of nanomaterials. Recently, ratiometric fluorescent nanoprobes employing dual-emission strategies have gained

considerable attention in biosensing applications, achieving greater discrimination convenience, higher sensitivity, and improved imaging contrast. The primary principles for designing ratiometric fluorescence-based biosensors can be classified into three categories. First, the close combination (at the nanoscale) of multiple fluorescent dyes with different emissions accomplishes dual-emission via Förster resonance energy transfer (FRET). These systems are defined as “two-dye-embedded NPs”. The second strategy involves choosing one nonluminous NP as a nanocarrier to conjugate two fluorescent dyes, where one fluorescent dye serves as a reference and the other responds to the target, referred to as “NP-dye nanoconjugates”. The final strategy strategy involves the use of single or hybrid NPs with intrinsic dual emissions. The former type of NP simplifies the ratiometric sensing design, whereas the latter retains the advantages of each element and enhances the operability for systematic integration [28]. To achieve a high visual resolution limited by traditional intensity-based color changes, a ratiometric fluorescence LFIA was proposed to detect heart-type fatty acid binding protein. This detection can be identified visually without aids or quantified using a smartphone. The combination of fluorescent silica nanospheres containing AuNPs, red-light-emitting CdSe/CdS/ZnS QDs as signaling reporters, and green-light-emitting CdZnSe/CdS/ZnS QDs as capture probes led to a target-induced color change from green to red. With the assistance of custom smartphone-dependent equipment, the detection limit was achieved at 0.21 ng/mL (Fig. 2B) [29]. However, a significant obstacle in achieving sufficient fluorescent signal quantity for the detection of various targets lies in energy transfer processes. An efficient and feasible strategy for avoiding FRET involves integrating tetrapod CdSe/CdS QDs with large Stokes shifts and a narrow full width at half maximum in conjunction with intrinsic CdSe/ZnS QDs. A conceptual 7 × 7-1 barcoding matrix and a 3D barcode library of 144 identifiable barcodes were constructed. These barcodes exhibited remarked multiplex sensing behavior for five common allergens towards specific IgE antibodies in the corresponding samples with a detection limit of 0.01–0.02 IU/mL [30]. In addition, a fluorescent visualization monitoring aptasensor based on a proximity-enhanced mechanism was demonstrated for efficient and multiple analysis of human epidermal growth factor receptor dimers on cell surfaces. The aptamer recognized protein targets and discriminated the expression states of homodimers and heterodimers by modulating the distance between two DNA-template Ag nanoclusters (AgNCs), resulting in the output of ratiometric signals [31]. Moreover, hybrid NPs with integrated plasmonic-fluorescent properties exhibited excellent performance in glucose sensing. In order to determinate the glucose and cholesterol levels in biological samples, photoluminescent biodots (Ser-Hist dots) and plasmonic AgNPs were combined to enable both fluorescent

and colorimetric sensing. Under ultraviolet (UV) irradiation, Ag ions were anchored onto the biodots, leading to the spontaneous formation of AgNPs. This process displayed the plasmonic characteristics of the AgNPs and significantly quenched the signal intensity of biodots. Subsequently, the enzymatic oxidation of glucose or cholesterol resulted in an increase in H_2O_2 content, which stimulated the AgNP etching reaction, causing naked-eye distinguishable color changes and a simultaneous recovery of the biodots' fluorescent signal [32].

2.3 SERS spectroscopy for single-molecule analysis and circulating tumor cells (CTCs) detection

SERS spectroscopy is another nanoscale plasmonic phenomenon that enables the identification of the fingerprints of adsorbed targets and tracking alterations in surface chemistry [33]. The discovery of amplification and enhancement phenomena makes SERS competitive in optical biosensing, along with its remarked properties such as extremely high sensitivity, intrinsic structural specificity and commendable experimental simplicity [34]. Notably, the enhancement of Raman scattering is closely related to the plasmon intensity, which is highly dependent on the gap distance. NPs such as Au nanowire (AuNW) vesicles provide large-volume "hot spots" and sharp tips as well as abundant gaps, which considerably intensify the signal [35]. The incorporation of revolutionary advancements in spectroscopic instrumentation, along with recent noteworthy developments in nanofabrication tools, has transformed SERS into feasible commercial biosensors rather than merely laboratory products. These techniques include lab-on-a-chip systems, endoscopic imaging, and microfluidics chips [36].

Direct and indirect approaches are the two most commonly employed schemes for SERS sensing. Direct SERS-based sensing represents the intrinsic SERS spectrum of a target in a straightforward manner, whereas indirect SERS strategies utilize the intensity and spectral profiles of Raman reporters to reflect alterations in recognition events [37]. Notably, significant advancements in nanopore-based DNA sequencing techniques have inspired technological renovations in fast and reliable single-molecule protein sequencing and structural analysis. However, the identification of 20 different amino acids, particularly non-aromatic amino acid residues, hinders further development in this field. Apart from the electrical readout method, optical detection methods such as SERS have shown potential in this field. By trapping sub-monolayer molecules adsorbed on Au nanostars (AuNSs) within an Au nanohole to form single strong hot spots, De Angelis and co-workers [38] firstly demonstrated distinct SERS spectroscopy

with superior properties and distinguished 10 types of amino acids at the single-molecule level. Moreover, analysis of protein structures and conformational dynamics at physiological concentrations remains challenging. Dai et al. [39] developed a method that combines optical tweezers with Raman spectroscopy, utilizing two AgNP-coated silica microbeads to create tunable hotspots. This approach fulfilled ultrasensitive monitoring of flowing proteins in their native states and conformations.

In addition, SERS-based sensors have demonstrated potential for ultrasensitive CTCs sensing. Enumeration and analysis of the early stages of cancer have emerged as significant clinical platforms for cancer diagnosis and prognosis, *in vivo* drug resistance testing, and individualized treatments. However, the primary obstacle remains the highly effective and selective enrichment of CTCs from circulating blood. Consequently, several novel approaches have emerged for CTC detection, including immunomagnetic methods, size-based filtration techniques, and DNA-driven nanomaterial self-assemblies. Notably, the employment of NPs with different asymmetric shapes facilitates CTCs monitoring without the need for an enrichment process in biological samples. For example, three SERS-active NPs (AuNPs, AuNRs, and AuNSs) were functionalized with a Raman reporter molecule, reductive bovine serum albumin (BSA) and folic acid. This design achieved strong SERS signal output, high sensitivity, and excellent catching specificity, resulting in an enhanced LOD of 1 cell/mL [40]. Besides, SERS-based technologies have extraordinary potential for separating and analyzing the diverse molecular characteristics of CTCs in real-time. Cui and co-workers [41] reported a size-based microfluidic cell isolation assisted on-chip SERS strategy for real-time monitoring of cell membrane proteins and malignancy classification. A SERS vector was constructed by integrating spherical AuNPs, a thin silver shell, Raman reporters, and DNA aptamers. Additionally, three spectrally orthogonal SERS-based aptamer nanovectors systems were assembled, achieving a good classification performance with high sensitivity and accuracy for various cancer cells. Real-time monitoring of the phenotypic evolution of CTCs during therapeutic processes is important for treatment management. Tsao et al. [42] proposed a multiple CTC surface markers characterization strategy based on antibody-conjugated and Raman reporter-coated AuNPs multiplex SERS nanotags. This approach allowed simultaneous profiling and evolutional monitoring of CTCs during melanoma treatment (Fig. 2C) [42]. This sensor exhibited exceptional sensitivity for CTC detection by identifying 10 cells in 10 mL of blood without the need for isolation. It also responds accurately to tumor cell populations during targeted treatment. Using this strategy, different CTC signatures of underlying clinical significance in drug-resistant clones

were observed.

2.4 Nanozyme for biologically related molecules detection

In 2007, the identification of Fe_3O_4 NPs exhibiting peroxidase-like activities simulated the emergence of numerous enzyme mimics especially “nanozymes” [43]. Nanomaterials have presented extraordinary properties in catalyzing peroxidase substrates such as 3,3′,5,5′-tetramethylbenzidine (TMB), diazoaminobenzene, and *o*-phenylenediamine, leading a detectable color changes due to the oxidization of the substrates in the presence of H_2O_2. These properties have been exploited for the optical sensing of glucose, xanthine and cholesterol [44−46]. As one of the global health issues, diabetes affects a considerable portion of the population, and its management heavily relies on continuous monitoring of blood glucose levels with high accuracy. Currently, optical enzymatic sensors based on glucose oxidation are commonly used to track body glucose levels [47]. Inspired by natural enzymes, nanozymes are generally applied as a potential alternatives because of their cost-effectiveness, stability, and mass-producibility. To overcome challenges related to low diffusion efficiency and unstable intermediates, integrated nanozymes composed of low-dimensional nanomaterials have been widely developed. An innovative hybrid nanosheet was fabricated for the colorimetric measurement of glucose, achieving a detection limit reaching 8.5 mM. This biomimetic catalyst consists of ultrasmall AuNPs and 2D metalloporphyrinic MOF nanosheets, in which the AuNPs serve as artificial glucose oxidase, while the metalloporphyrinic MOF nanosheets function as nanozymes. These hybrid nanosheets were prepared using TCPP(M) (TCPP = tetrakis(4-carboxyphenyl)porphyrin, M = Fe, Co) as ligands and $Cu_2(COO)_4$ paddle-wheel clusters as metal nodes with further growth of AuNPs, and exhibited potential for use as novel artificial enzymes in bioassays and nanomedicines [48]. Moreover, AuNPs were further modified with cyclodextrin, resulting in the construction of macrocycle-AuNP hybrid nanomaterials. Regularly arranged 1D and 2D architectures provide versatile nanoplatforms for detection, self-assembly, and sequential catalysis. Notably, a cascade reaction for glucose sensing was successfully achieved since the proposed nanomaterials demonstrated distinct catalytic activities comparable to those of glucose oxidase and horseradish peroxidase [49]. Han et al. [50] also demonstrated BSA-directed MnO_2 nanoflakes exhibiting cascade enzyme-like properties for colorimetric detection of glucose. The single nanozyme simultaneously oxidized glucose and allowed the optical measurement of H_2O_2 levels (Fig. 2D) [50].

2.5 Chemiluminescence and bioluminescence for in vivo sensing and imaging

In vivo bioimaging techniques possess outstanding capabilities for probing active biological specimens and revealing cell damage or dysfunction associated with serious diseases. Chemiluminescence refers to the emission of light resulting from chemiexcitation during a chemical reaction [51]. Unlike fluorescence-based techniques, which are hindered by fluorescence quenching and poor tissue penetration, chemiluminescence-base sensors eliminate the need for external light excitation and reduce background autofluorescence interference. This results in higher sensitivity detection and deeper tissue imaging with an extremely high signal-to-noise ratio. As an application, a noninvasive strategy for detecting both exogenous and endogenous hypochlorite was developed using a glow-type chemiluminescent probe. This probe was further applied to investigate the physiological and pathological roles of hypochlorite *in vivo* [52]. In contrast to direct chemiluminescence processes, indirect chemiluminescence methods, such as chemiluminescence resonance energy transfer (CRET), involve an energy transfer process from an excited state intermediate to adjacent fluorescent molecules or nanomaterials, which then trigger light emission. Recently, improved nanomaterials, including QDs, metal/metal oxide NPs, mesoporous silica nanomaterials and metal-organic frameworks (MOFs) have been introduced to optimize chemiluminescence sensing and imaging using the CRET strategy [53]. A novel all-in-one CRET-based chemiluminescence photodynamic therapy platform was developed by combining hemoglobin and luminol with MOFs. The porphyrinic photosensitizing linker TCPP, was well dispersed in the framework nanophotosensitizer with the co-encapsulation of luminol and hemoglobin. Upon exposure to elevated levels of H_2O_2 in the tumor microenvironment, luminol generated blue chemiluminescence. Subsequently, the emitted light is absorbed by the TCPP components within the MOF NPs *via* the CRET mechanism, resulting in luminescence emission. The excited-state energy is then transferred to oxygen molecules bound by hemoglobin, leading to the production of cytotoxic reactive oxygen species (ROS). This platform allows simultaneous *in situ* imaging and therapy simultaneously through H_2O_2-activted CRET and subsequent oxygen self-supply (Fig. 2E) [54].

Bioluminescence was initially explored as a natural light-emitting phenomenon in cells or animals in 1667. It has since evolved into an advanced autofluorescence-free optical imaging technique for highly sensitive visualization that relies on the catalytic reaction between luciferase enzymes and a small-molecule luciferin analog to form an excited-state species [55]. However, the light emitted by some natural luciferins, such as coelenterazine, is intensively absorbed by blood or tissue, which presents limitations for *in vivo* imaging [56]. Thus, bioluminescence resonance energy transfer (BRET)

nanoconjugates have been developed as effective solutions to overcome these challenges. Fluorescent nanomaterials, such as QDs or quantum rods (QRs), are perfectly competent to function as BRET acceptors because of their excellent brightness, large Stokes shift and narrow/tunable emission. Notably, the covalent conjugation of NanoLuc luciferase to silver sulfide QDs generates near-infrared II (NIR-II) photons, shifting bioluminescence based on the reaction with luciferin substrate and a single-step BRET process. This innovation has enabled deep tumor *in vivo* imaging with considerably excellent signal-to-noise ratios and sensitivity while remaining nontoxic for NIR-II emission [57]. Moreover, recent findings indicate that BRET ratios are highly responsive to aspect ratios when utilizing QRs as acceptors. The QR-luciferase nanoconjugates featuring short rods exhibiting long-wavelength emissions display the highest efficiency [58]. The integration of BRET and FRET processes represents another effective strategy for enhancing the tissue penetration and spatial resolution of luminol-based biomarker-targeted imaging. Accurate *in vivo* evaluation of the inflammation-related biomarker, heme-containing enzyme myeloperoxidase, is essential for the early diagnosis and surveillance of the progression in inflammatory disorders, including Alzheimer's disease and cancer. A lipid nanobubble fabricated by Liu et al. [59] incorporated two tandem lipophilic dyes to redshift luminol-emitted blue light to NIR region by an integrated transversion of BRET-FRET. This approach achieved bioluminescence/ultrasound dual-modal enhanced myeloperoxidase-dependent inflammation imaging within a breast cancer animal model (Fig. 2F) [59].

2.6 Electrochemiluminescence for bioanalytical detection

Electrochemiluminescence is an attractive signal generation strategy that involves the conversion of electrical energy into radiative energy without requiring external light sources. This occurs when the species generated at the electrodes emit light as a result of undergoing energy-intensive electron-transfer processes that lead to the formation of excited states. This method merges the principles of electrochemistry and spectroscopy, capitalizing on the benefits of both disciplines. Electrochemiluminescence technology offers considerable advantages for bioanalytical detection, including miniaturization, low background noise, cost-effectiveness, high sensitivity, and rapid response. Electrochemiluminescence is typically generated via two primary mechanisms: annihilation and coreactant pathways. Compared with the annihilation pathway, the coreactant pathway exhibits improved stability against radical ions in aqueous environments, higher electrochemiluminescence intensity, and broader applicability, enhancing its suitability for practical use. Regardless of the different

electrochemiluminescence mechanisms, most electrochemiluminescence processes typically involve four main stages: redox reactions at the electrode surface, homogeneous chemical reactions in solution, formation of excited-state species, and emission of light [60]. Five primary types of electrochemiluminescence sensing strategies are categorized. Compared with the most traditional approach involving direct use of an electrochemiluminescence emitter as a signal label, the prevalent method utilizes the spatial hindrance or resistance effects resulting from bio-recognition events. A convenient strategy is to leverage the interactions between analytes and electrochemiluminescence substances like luminophores or excited-state electrochemiluminescence molecules, while the fourth approach focuses on the interaction between analytes and coreactants. The most sophisticated method involves modulating electrochemiluminescence light emission, predominantly through an emerging technique known as electrochemiluminescence resonance energy transfer (ECL-RET). This approach capitalizes on the intrinsic sensitivity of ECL-RET at the nanoscale and the diverse potential of nanomaterials as donors or acceptors. Consequently, this innovative strategy offers substantial opportunities for enhancing electrochemiluminescence sensing applications by utilizing the distinctive characteristics of nanomaterials. In addition to employing a target-specific molecular recognition functionalized working electrode, which is similar to most electrochemical assays, the applied potential in electrochemiluminescence systems generates luminescent signals by exciting the electrochemiluminescence probe. Han and Guo [61] demonstrated a hydrogel-based system incorporating fluorescent Au/Ag NCs to eliminate nonspecific interferences and physical damage to the electrochemiluminescence sensing interfaces. This approach achieved a detection limit of 8.7×10^{-6} M for glutathione sensing in serum (Fig. 2G) [61]. In this study, the fluorescent Au/Ag NCs served as highly effective electrochemiluminescence probes in a hydrophilic hydrogel matrix. These NCs underwent electro-oxidation near the electrode surface to form Au/Ag NC^{+} species. The subsequent reaction with reductive radicals (produced by the electro-oxidation and deprotonation of the co-reagent) led to the formation of an excited state in the NCs, thereby inducing light emission. Recent years have also witnessed advancements in electrochemiluminescence-based biosensors for rapid and high-throughput drug monitoring related to physiological functions. An enzyme-functionalized single microbead-based imaging strategy was developed for the determination of lecithin by utilizing a luminol derivative and H_2O_2. The resulting electrochemiluminescence signal was captured and imaged using a camera, which enabled precise quantitative analysis with a LOD of 0.05 mM. Despite the heterogeneity observed in individual gold microbeads, their luminescence adheres

to statistical consistency [62].

3. Key nanomaterials for optical sensing in biomedical and biopharmaceutical research

Materials characterized by particle sizes reduced to the nanoscale regime exhibit significantly enhanced mechanical properties and have become promising candidates for high-performance sensors components. Fig. 3 provides a comprehensive comparison of key nanomaterials for optical biosensors, categorized into 0D, 1D, 2D, and 3D nanomaterials. 1D materials are related to the structures with two of the three dimensions below 100 nm, whereas 2D materials exhibit a sheet-like architecture with a horizontal dimension exceeding 100 nm [63,64]. Similar to their 0D counterparts such as NPs and metal/carbon compound QDs, 2D nanostructures, including nanosheets, transition metal dichalcogenides (TMD) and transition metal carbides/carbonitrides (MXenes), also exhibit exceptional optical properties as fluorophores, nanoquenchers or nanozymes [65,66]. Moreover, the unique geometrical characteristics of 1D nanostructures, such as NRs, make them particularly desirable for multicolorimetric sensing [22]. In addition, 3D nanomaterials, especially porous framework-based nanomaterials and nanodiamonds, hold great potential in multiple applications, including drug delivery, gas storage and extraction, photonics, and catalysis [65,67].

3.1 0D nanomaterials for IVDs and therapeutic drug monitoring

3.1.1 Noble metal nanomaterials

Precise *in vitro* optical diagnostic and therapeutic drug monitoring techniques have substantial value in medical profession and at-home healthcare to make clinical diagnosis rapid, straightforward, easy and less painful. These advancements have also guided personalized medicine, continuous monitoring and targeted therapies in their early stages. Typically, currently available IVD systems are developed to precisely detect individual biological circulating targets, including proteins, nucleic acids, whole cells, metabolites and drug molecules. These targets provide real-time feedback of patients' physiological and pathological conditions. The integration of numerous low-dimensional nanomaterials with superior features has propelled the development of IVDs into a new era towards precision medicine. Currently, 0D materials being extensively explored primarily include noble metal NPs/NCs, metal oxide NPs, semiconductor QDs, nanocarbons and up-conversion NPs. These materials have promising prospects for IVDs and drug monitoring, owing to their diverse synthesis

methods and multiple quantum properties. Noble metal NPs, including Au, Ag, and Cu exhibit attractive LSPR properties and their performance is significantly influenced by their shape and size. The aggregation mechanism of large metal spherical particles below 50 nm such as AuNPs and AgNPs is typically utilized in monochrome colorimetric sensing platforms, where the formation of nearby plasmon couplings leads to noticeable color changes [68]. Besides, noble metal NPs particularly AuNPs have been regarded as promising candidates for SERS biosensing platforms, enhancing the Raman scattering capacity of the absorbed species and enabling spectroscopic identification, even at the single-molecule level. Based on this sensing principle, a sandwich-type SERS biosensor was prepared to detect miR-141 with ultra-high sensitivity at the femtomolar level using a multiple-signal magnification scheme. In particular, giant AuNWs achieved initial signal enlargement by the advantage of large-volume hot spots, while silver stain, signaling molecule R6G and hybridization chain reaction (HCR) as well as the Fe_3O_4@AuNPs capture units further facilitated the sensing performance [35]. A CRISPR-Cas12a-assisted SERS sensor was also established for the ultrasensitive diagnosis of various viral nucleic acids, including those of the hepatitis B virus, human papillomavirus 16 (HPV-16), and HPV-18. In this platform, a nanoarray was functionalized with graphene oxide (GO) and trianglar Au nanoflowers. The Raman probe-integrated AuNPs significantly enhanced the output signals, achieving a sensitive LOD of 1 aM [69]. The development of therapeutic drug monitoring techniques has laid the foundation for an era of “personalized medicine”, enabling the customization of treatments based on individual genetics, lifestyle, and environmental influences. Continuous drug monitoring provides real-time feedback, facilitating timely adjustments to treatment dosages, particularly for medication with substantial side effects, such as doxorubicin. To achieve rapid and cost-effective detection, Quarta et al. [70] developed a plasmonic biosensor utilizing gold nanoislands partially embedded in a glass substrate and subsequently coated with Al_2O_3 to form Au/Al_2O_3 core-shell structures. The LSPR-based detection of doxorubicin relied on a concentration-dependent red-shift peak, achieving LODs of 1 nM in water and 16 nM in both bovine and human serum. By modifying the nanoislands with Al_2O_3, SERS measurements exhibited enhanced sensitivity due to the electronic and energy interactions between adjacent Au nanoislands and between doxorubicin molecules and gold. This method achieved a low LODs of 100 pM in water and 1 nM in fetal bovine serum. Au@Ag core-shell NPs also generated abundant 3D hotspots on the superhydrophobic self-assembled AgNP film, even with the interference from the complex plasma matrix. The proposed silver-film-assisted 3D SERS sensor achieved the measurement of crystal violet at concentrations as low as 10^{-13} M and facilitated

the pharmacokinetics investigation of mitoxantrone and methylene blue following intravenous injection in mice through blood collection [71]. In addition to blood and serum samples, the dermal interstitial fluid shows substantial potential for *in vivo* drug monitoring. A SERS-based microneedle array was developed by integrating core-satellite organized Au@Ag NPs and hydrogel-coated microneedles, enabling the non-invasive and *in situ* analysis of methylene blue and mitoxantrone. Furthermore, results from a drug-injected mouse model demonstrated that the mitoxantrone level within the skin's interstitial fluid was significantly lower than that in the blood, whereas the concentrations of mitoxantrone were comparable [72]. Aminoglycosides, which belong to a category of antibiotics, are frequently used in addressing infections resulting from gram-negative bacteria, such as tuberculosis. Owing to their negative side effects, it is imperative for drug monitoring to ensure safe and effective individualized dosing regimens. A high-throughput biosensor capable of dual detection of aminoglycoside antibiotics using both LSPR and SERS was developed on a single platform, leveraging the aggregation phenomenon of AuNPs induced by surface charge interactions with antibiotics. Although this sensing approach has demonstrated the feasibility of detecting tobramycin, the use of NPs in physiological fluids can lead to inaccurate measurements owing to the formation of a protein corona, resulting in poor repeatability and reduced accuracy. Therefore, essential modifications are frequently necessary to prevent the self-aggregation of 0D metal NPs, thereby limiting their practicality and availability [73]. The SERS-based platform also demonstrated superior capabilities for the real-time monitoring of both the depth of penetration and drug release kinetics of NIR light-responsive nanomedicine *in vivo*. The temperature increase of the waxberry-like AuNPs triggered the release of curcumin, leading to a corresponding decrease in the SERS intensity. This reduction in SERS intensity also accurately reflected the penetration depth of the nanomedicine within the tumor [74].

When the size of metallic particles is smaller than that of the electron mean path, these particles exhibit weak fluorescence emission called NCs. Notably, NCs with ultra-small sizes (<2 nm) present distinct crystal structures and exhibit fluorescence properties, whereas the previous unique LSPR phenomenon disappears. There are two typical strategies for preparing metal NCs, i.e., the reduction of metal precursors and the etching of larger nanomaterials with the assistance of robust stabilizers. The novel intrinsic dual-emissions optical properties of these NCs further expand their biomedical and biopharmaceutical applications in optical sensing, biolabeling, and bioimaging. Hairpin DNA-templated AgNCs represent a novel type of chameleon nanomaterial with two emission peaks. The intensities of these peaks can be inversely regulated through

the hybridization of target DNA on the loop segment, which increases the distance between the two emitters. Based on this strategy, our research group reported a dual-emitter DNA-AgNCs-based ratiometric fluorescent sensor for single-nucleotide polymorphisms (SNPs) discrimination with high accuracy and sensitivity. This optical sensing platform identified various types of SNPs by introducing a peptide nucleic acid clamp and an isothermal amplification reaction. The mismatch of the nucleic acid base pair led to a color shift from red to green, which was easily distinguished by the naked eye [75]. Specifically, the discrimination capacity of this sensor was sufficiently presented through vivid color changes due to the enlargement of the Ag emitter pair, resulting in a substantial reduction in red emission and the subsequent appearance of green emission under UV light stimulation. Moreover, noble metal-based nanomaterials are also promising candidates for therapeutic drug delivery. A multifunctional theranostic nanosystem based on nanocarrier AuNCs was developed to deliver platinum (IV) drugs, featuring NIR-II imaging capability and intracellular glutathione scavenging activity. Inhibition of tumor growth was further validated using two malignant tumor models (Fig. 4A) [76]. However, the sensitivity of NCs to varying sensing conditions, such as pH and temperature, and their tendency to form non-reversible aggregates, complicate their functionalization and storage [77]. Moreover, the controlled preparation of NCs remains poorly understood, as most methods have been developed through trial and error. Therefore, a more comprehensive insight into the diverse aspects of NC synthesis and their interactions with target molecules will advance the rational design of materials. On the other hand, many noble metal nanomaterials, including Au, Ag, Pt, Pd, and their multimetallic NPs, exhibit peroxidase-mimicking properties and have been extensively applied in IVD. Li et al. [78] synthesized an innovative Cu@Zr nanozyme using a self-assembly process that simultaneously displayed attractive enzymatic and fluorescence properties. The cascade catalytic reaction induced by Cu@Zr led to the production of a red quinoneimine, facilitating the colorimetric detection of urease, which was further enhanced by urea hydrolysis. Meanwhile, the fluorescence signals of Cu@Zr gradually decreased due to the accumulation of red quinoneimine, achieving a swab-based dual-mode sensor for real-time identification of urease with the assistance of a smartphone. Active thiol-containing stabilizers, such as *N*-acetyl-L-cysteine (NAC), possess a high affinity for metal ions and improve the catalytic performance of noble-metal nanozymes. A NAC-PtNCs functionalized sensing platform was proposed to quantitative identification of heparin by catalyzing TMB substrate to produce detectable signals, achieving a LOD of 2×10^{-3} μg/mL in heparin spiked human serum samples [79]. This biosensor facilitates the provision of information and guidance regarding medication

dosage for both intraoperative and postoperative clinical procedures. Additionally, it helps prevent serious complications arising from excessive dosage or prolonged use, such as hemorrhage, thrombocytopenia and osteoporosis.

3.1.2 Metal oxide nanomaterials

To avoid the excessive costs associated with high-priced noble metals, researchers have begun to exploit the value of cost-effective and accessible oxide materials such as Cu_2O, Fe_2O_3/Fe_3O_4, Co_3O_4, CeO_2, and MnO_2 [80]. Iron oxide-based magnetic NPs possess distinct intrinsic peroxidase-mimicking properties and that were first reported in 2007. Since then, iron oxide-based peroxidase mimics have been extensively investigated, including Fe_3O_4, Fe_2O_3, doped ferrites and relative compositions. A nanozyme synthesized by integrating $CoFe_2O_4$ with porous carbon demonstrated superior peroxidase mimetic activity compared with that of each individual component, and was employed to detect glucose and glutathione [81]. Additionally, copper containing oxide NPs, such as Cu_2O/polypyrrole composites and molybdenum trioxide NPs, have been widely established as oxidase mimics, whereas Co_3O_4 and ZrO_2 primarily exhibit catalase-mimicking activities at high pH [82,83]. In addition, a variety of oxide nanostructures with different morphologies, including octahedral Ag_2O, ZnO nanosheets, TiO_2 nanostructures, Cu_2O superstructures, SnO_2 NPs, $W_{18}O_{19}$ NWs, and Ta_2O_5 NRs, have garnered substantial interest because of their remarkable characteristics as catalysts and SERS-active probes [84−86]. The excellent biocompatibility, spectral stability, and sensitivity of these oxide nanostructures make them promising candidates for cancer cell diagnosis and imaging. To address the substantial challenge posed by the extreme rarity of CTCs in peripheral blood, an octahedral Ag_2O NPs-based sensing platform was developed. This platform exhibited notable SERS with an ultra-high enhancement factor (EF) of 1.98×10^6 for 4-mercaptopyridine molecules. The synergistic effects of the surface defect-enhanced photo-induced charge transfer and robust vibrational coupling resonance within the Ag_2O-molecule SERS complex significantly amplified the molecular Raman scattering cross-section. After modification with folic acid via an amide bond linkage, this bioprobe achieved a LOD of 1 cell/mL for CTCs in blood samples [84]. In addition, antibody-fabricated crystalline core-shell structured black TiO_2 NPs with positive SERS activity were synthesized to develop a bioanalytical strategy for targeting and monitoring MCF-7 cells with drug resistance (Fig. 4B) [85]. Owing to the highly efficient exciton transfer within the crystalline core and the band bending at the interface of the crystal-amorphous heterojunction, sufficient photoinduced charges were generated, enabling efficient exciton dissociation and charge transfer. This led to the accumulation of photoinduced charges in the amorphous shell, thereby facilitating efficient interfacial photoinduced charge transfer between the substrate and target molecules. Furthermore, this platform was particularly advantageous because of its additional photothermal therapeutic properties under 808 nm laser irradiation, and

black TiO_2 NPs could be also considered as an effective nano-drug. Thus, intelligent theragnostic nanosystems that integrate diagnostic and therapeutic functions have emerged as techniques for early intervention and effective control of cancer. Overall, the biocompatibility, versatility and resource sufficiency of metal oxides have significantly accelerated their progression in IVDs and therapeutic drug monitoring. However, when developed as an integrated diagnostic and therapeutic nano-drug, the biological toxicity and metabolic characteristics of metal oxide nanomaterials require comprehensive investigation.

3.1.3 Semiconductor QDs

Fluorescent QDs are typically made from semiconducting NPs, particularly metal chalcogenide QDs such as CdSe- and CdTe-based QDs which have gained considerable attention owing to their advantageous photonic quantum effects and higher fluorescence quantum yields compared with those of upconversion NPs (UCNPs) [87]. DNA molecules have also demonstrated their potential as precise recognition and nanomaterial self-assembly. By utilizing a CTC-binding aptamer (sgc8c) and HCR, a novel QD-based copolymers were synthesized for trackable magnetic separation of CTCs from blood samples, achieving a high capture efficiency and purity of nearly 80%. The DNA-CdTe/CdS core/shell QDs featured an excellent quantum yield of 41.8%, and these inorganic magneto-fluorescent materials were able to penetrate cells (Fig. 4C) [88]. Similarly, a facile all-nucleic-acid assisted amplification-based QDs reporter was developed for consistent visual and fluorescent detection of A549 lung cancer cells in blood samples. When recognizing mucin 1 or A549 cells, the aptamer is released from the dsDNA probe and triggers catalyzed hairpin assembly-based amplification. Concurrently, the CdTe QDs specifically recognized the unbound Ag^+ from C−Ag^+−C structures to produce visualized fluorescent responses, achieving a LOD of 0.15 fg/mL (mucin 1) and 3 cells/mL (A549 cells) [89]. Among all the DNA-driven nano-assembly-based sensing methods, fluorescence quenching and metal-enhanced fluorescence (MEF) effects have been widely proposed owing to their rapid response times, intense optical activity, and multiplex targeting capabilities. Numerous sensing platforms have been constructed based on the regulated distance between the metallic material and the fluorescent signaling probe, as the fluorescence quenching phenomenon can be manipulated. Nevertheless, when the fluorescent probe is positioned within 10 nm of the noble metal nanostructures, the fluorescence signal is enhanced due to the LSPR of noble metal particles upon exposure to incident light. Based on the MEF effect, precise determination of the prostate cancer marker PCA3 sequence was achieved by regulating the distance between AuNRs and NIR Ag_2S QDs using DNA with various chain lengths

[90]. Some essential amino acids, such as cysteine, play a critical role in maintaining intracellular redox balance to sustain the equilibrium between free thiols and oxidized disulfides. Plasma cysteine levels are strongly associated with various diseases. A rapid-response optical biosensor for cysteine determination was developed based on nitrogen-doped graphene QDs (N-GQDs) with vanadium pentoxide nanosheets, which function as fluorescence turn-off/on nanoprobes. In this system, the V_2O_5 nanosheets act as both fluorescence quenchers and as target recognizers. Specifically, the detection of cysteine initiated the reduction of V_2O_5 to V^{4+} and the release of N-GQDs, leading to a fluorescence response and achieving a LOD of 50 nM with high selectivity [91]. Moreover, immunohistochemistry-based bioimaging techniques are highly suitable for *in situ* single-cell immunoprofiling and can provide comprehensive information about intracellular protein expression owing to the widespread accessibility of specific antibodies and well-established approaches for fluorophore biological conjugation. The combination of QDs and amplification reactions allows for sensitive and multiplexed protein analysis at the single-cell level. Five to ten QD colors can be simultaneously labeled to target antigens using a signal amplification procedure without negatively affecting sample antigenicity [92].

However, the application of flammable and toxic dimethylcadmium has restricted the practicality of QDs. Therefore, extensive efforts have been made to enhance synthesis methods using various stable and safer precursors (e.g., CdO, SeO_2, and $Cd(OOCR)_2$), non-coordinating solvents, and stabilizers [93]. Additionally, passivation and toxicity reduction of fluorescent QDs have been accomplished by coating them with inert shells and performing surface modifications [94]. Notably, when wide-bandgap shells, such as CdS and ZnS, are deposited on the surface to construct core-shell QDs, their luminescence and photostability can be dramatically improved. This coating strategy has also been applied to enhance the fluorescence quantum yields of 1D semiconductor nanostructures, including NRs, NWs, arrows, and tetrapods, leading to unique optical and magnetic properties with diverse applications [95]. Additionally, metal ion doping represents another effective strategy for synthesizing brightly fluorescent QDs with low toxicity, such as Mn-doped ZnS and Cu-doped InZnS particles.

3.1.4 UCNPs

UCNPs, especially hexagonal $NaYF_4$ nanocrystals doped with trivalent lanthanide ions such as Er(III), Yb(III), or Tm(III), offer substantial advantages for fluorescence bioimaging, including minimal interference from autofluorescence, large anti-Stokes

shifts, narrow emission spectra, excellent tissue penetration, and exceptional photostability [96]. UCNPs typically exhibit multiple emission colors, with the peak wavelengths varying according to the type of lanthanide dopant employed, which allows for improved microscopic resolution. The emission process of UCNPs differs from multi-photon processes because it entails the sequential absorption of two or more photons. Three types of upconversion mechanisms exist, i.e., excited state absorption (ESA), energy transfer upconversion (ETU), and photon avalanche. Upconversion nanocrystals generally comprise activators, sensitizers, and a host matrix, in which lanthanide ions (e.g., Er^{3+}, Tm^{3+}, and Ho^{3+}) are usually selected as activators and Yb^{3+} as the sensitizer. Rare-earth fluorides are commonly selected as host materials due to their comparable ionic sizes and chemical properties to lanthanide ions, as well as their low phonon energy and excellent stability [96]. The selective upregulation or downregulation of certain molecules, such as hydrogen sulfide (H_2S) and ROS, significantly affect important physiological processes. For example, metformin overdose can induce H_2S overexpression potentially leading to severe liver damage and toxicity. Consequently, a *Myrica rubra*-like nanoprobe with ratiometric fluorescence properties and orthogonal NIR-II emission was developed to accurately monitor the endogenous H_2S levels in real time and guide optimal oral medication dosing. This nanoprobe utilized a $NaYF_4$:Gd/Yb/Er@$NaYF_4$:Yb@SiO_2 core covered with Ag nanodots and was primarily taken up by the liver, featuring a sulfuration reaction-triggered conversion to a signal unit. The constructed sensing platform detected metformin-induced hepatotoxicity at a highly sensitive level of 0.7 nM and allowed for the ratiometric imaging of the varying degrees of hepatotoxicity *in situ* (Fig. 4D) [97]. The ROS, including O^{2-}, H_2O_2, ·OH, and ClO^- are critical in health and disease, particularly within cellular signaling systems, immune function, and organ injury. To address the drawbacks of inadequate biocompatibility, limited sensitivity, and susceptibility to photo-bleaching in previous ROS probes, Kuang and co-workers [98] fabricated a nanostructure featuring a UCNP core and chiral NiS_xNPs-functionalized zeolitic imidazolate framework-8 (ZIF-8) shell (UCNP@ZIF-NiS_x). This design achieved quantitative and selective monitoring of ROS *in vivo* through the degradation of NiS_x into UCNP@ZIF during the detection process. Particularly, $NaGdF_4$ is extensively employed as a positive contrast medium for MRI imaging. As a result, the optical properties of UCNPs can vary depending on the activators, sensitizers, host materials, as well as their crystal phases, particle sizes, and surface coatings [99]. Furthermore, the random migration of excitation energy from an atom to its neighboring atoms in a 1D atomic chain structure, 2D layer structure and 3D structured crystal sublattice minimizes the depletion of excitation energy and enhances the up-conversion

at the sublattice level. This provides alternative prospects for engineering UCNPs for optical sensing and imaging applications [87]. However, the quantum yield of UCNPs is relatively lower than that of other fluorescent nanomaterials, and the synthesis of highly efficient UCNPs remains a great challenge.

3.2 1D nanomaterials for multicolorimetric and SERS-based sensing

Unlike their 0D counterparts, 1D metal nanostructures, such as AuNRs and AgNWs, have notable optical properties for various applications. AuNRs are widely utilized in optical multicolorimetric sensing and imaging by simply altering the aspect ratio, which tunes their longitudinal LSPR peaks from visible to NIR wavelengths (650−1350 nm), where light penetrates tissues with the greatest efficiency [100]. Compared with AuNRs, AgNRs are also one of the most popular nanomaterials for LSPR shift-based sensing because of their narrower and intense spectra. Additionally, successful control of the aspect ratio of AgNRs causes a linear LSPR shift. Furthermore, controlling the exact shape, as well as formation of shells on the NRs and aggregation of 1D nanostructures, had been employed to adjust their optical characteristics [101]. To date, reports on the colloidal synthesis of 1D metal nanostructures have expanded from 2 (Au and Ag) to 23 different types. However, most of these methods still rely on capping agents for achieving anisotropic growth and precise aspect ratio control [64]. Anisotropic 1D metal nanostructures also serve as a key component in the construction of SERS-based sensors. However, the average EF for most plasmonic NP-based SERS sensors is less than 10^6 due to poor substrate reproducibility and misquoted SERS EF calculations. Depending on the aspect ratio control and analytes, the EF for Ag and Au NRs/NWs can be optimized to exceed 10^7 [102]. Choi and co-workers [103] developed a straightforward fabrication approach for a SERS sensor chip by attaching AuNPs onto vertically aligned zinc oxide NR (ZnO NR) arrays on cellulose paper. This setup achieved an enhanced Raman signal of 1.25×10^7. By utilizing a bio-classification method trained with machine learning and multivariate statistics, the proposed sensor chip could accurately predict and identify various types of prenatal diseases from minute quantities of genuine amniotic fluid with high sensitivity and specificity. Additionally, anisotropic nanomaterials and chiral assemblies possessing intense surface SERS signals are advantageous for identifying microRNAs (miRNAs) in a sequence-specific manner. Arrowhead-shaped AuNR dimers with both parallel and sequential arrangements were selectively modified with DNA and polyethylene glycol. The Raman signals of these dimers were significantly amplified. When the dimers recognized the target miRNAs, they disassembled evenly, resulting in a decrease in

SERS signals, which enabled the efficient identification of target miRNAs and facilitated *in situ* Raman imaging (Fig. 4E) [104].

3.3 2D and 3D nanomaterials for continuous molecular monitoring and/or targeted therapy

Ultrathin 2D metal nanomaterials typically present crystal structures identical to those of their corresponding bulk materials, with metal atoms closely arranged with neighboring atoms in face-centered cubic (fcc), hexagonal close-packed (hcp), or body-centered cubic (bcc) structures. Specifically, metals such as Au, Ag, Pt, Pd, Rh, and Ir typically crystallize in the standard fcc structure, whereas Ru and Os adopt an hcp phase [64]. Recently, reconfigurable arrangements of AuNPs, including linear assemblies (1D), monolayer films (MFGS) and superstructures with controlled particle spacing and arrangement, have provided new possibilities for optical sensing and catalytic applications [105]. Among these, 2D MFGS exhibit features such as accessible fabrication, strong stability, adjustable electromagnetic enhancement, unique optical absorption and electron transport abilities. The consistent arrangement of NPs in 2D MFGS not only enhances the stability and reliability of SERS detection by coupling hot spots but also enables multicolor displays in hybrid films [106]. Interfacial assembly is one of the primary methods for constructing optimized 2D MFGS due to its time-saving, simple fabrication procedure and accurate regulation of the spatial distribution of AuNPs in the resulting films. Chen and co-workers [107] has promoted an interfacial chemical cross-linking strategy to assemble shrinkable AuNP MFGS and reoriented AuNRs on a Si substrate by increasing their interaction to achieve directional alignment. Moreover, recent studies have reported achievements in environmentally friendly and renewable 2D MFGS through the on-site reduction of AuNPs linked to polymers and the development of novel self-supporting 2D Janus AuNP films [108−110].

In contrast to microfluidic flexible electronics integrated biosensors and chromogenic agent-based wearable technology, optical wearable nanosensors based on 2D and 3D nanomaterials represent an emerging field that are still in their infancy. Undeniably, they offer unparalleled advantages for health monitoring because of their non-invasive nature, portability, and rapid responsiveness [111,112]. Various prototypes have been proposed for monitoring biomarkers on surface and in biofluids by using different smart nanomaterials. Zhou and co-workers [113] developed scalable and washing-reusable SERS membranes and textiles using a template-guided self-assembly method to incorporate AuNPs into highly hydrophobic microwell templates, which were then transferred via UV-curable resist-based micro/nanoimprinting. The proposed

membranes exhibited promising potential for wearable biochemical sensing owing to their robust mechanical properties and the immobilization of UV-resistant AuNP aggregates. Notably, minimally invasive or noninvasive optical glucose sensors address the discomfort and procedural inconveniences associated with blood-based glucose self-testing. Tear fluid has been proposed as an alternative medium for reflecting glucose levels, providing a superior option for continuous *in vivo* and *in situ* testing. Chung and co-workers [114] designed a camera-oriented glucose optical monitoring system using cerium oxide NPs and glucose oxidase embedded in contact lenses. This system detects changes in tear glucose levels through color changes, eliminating the need for complex electronic components. Additionally, with the implementation of an image processing algorithm, the measurement accuracy is enhanced, even in the presence of image blurring, allowing for quantitative fully automatic efficacy to benefit patients with diabetes. Moreover, plasmonic nanostructures with SERS activity also facilitate *in situ* broad-spectrum molecular fingerprint identification at biointerfaces. Jung and co-workers [115] successfully demonstrated unconventional 3D plasmonic nanostructures exhibiting high SERS activity and consistency by employing nanotransfer printing. They further fabricated a SERS contact lens capable of measuring glucose levels in tears, achieving a linear range from 10^{-1} to 10^{-4} M (Fig. 5A) [115]. However, the optimization of the statistical quantification proved challenging owning to the substantial overlap between the primary SERS peaks of glucose and those of the monolayer. Consequently, for the practical implementation of nanomaterial-based SERS contact lenses, it is essential to validate the feasibility of retina-safe laser excitation and offer suitable guidelines. These aspects continue to be key objectives in advancing this technology. Moreover, the inherently weak Raman signals and limited chemical affinity of glucose with typical SERS substrates, such as gold or silver, are challenging. Consequently, SERS-based glucose biosensors often employ linker molecules to enhance sensitivity and specificity. By reorganizing 3D AuNP clusters into a vertical-pillar configuration, the SERS emission shift of mercaptophenylboronic acid was correlated with glucose levels from 0.1 to 30 mM in aqueous humor and blood. Eye measurements using this platform aligned closely with those of commercial sensors, with a difference within 0.5 mM, highlighting its potential for extended continuous monitoring [116]. Recently, a wearable plasmonic sensor capable of recognizing a wide range of analytes was developed using a silver nanocube structure as the SERS-active component. This sensor was used for *in situ* monitoring of trace amounts of drugs in sweat, providing individual drug metabolic profiles and demonstrating its feasibility for universal and sensitive molecular tracking relevant to human health assessment [117]. On the other hand, human skin serves as a sophisticated

sensor that interacts with the surrounding environment and fosters numerous studies, including robotics, electronic (E)-skin development, and human-computer interaction. A wearable electrochemiluminescence-based tactile sensors was designed with visual alarm features to prevent bodily harm from external stimuli by using carbon nanotubes (CNTs) embedded in polydimethylsiloxane as the electrode. Three specific luminophores producing red, green, and blue emissions were integrated into the electrochemiluminescence layer, enabling the continuous visualization of external stimuli by shape, size, and position [118]. Wang et al. [119] demonstrated a smart AuNCs-integrated antibacterial wound dressing capable of real-time monitoring of nanomedicine residues via *in situ* fluorescence, thereby facilitating timely dressing replacement. Taken together, nanomaterial-based optical biosensors for molecular drug or nano-drug monitoring and their beneficial aspects in biopharmaceutical applications especially for targeted therapy is summarized in Table 1 [42,62,70–74,76,79,85,97,119].

Nowadays, research has expanded from graphene to alternative ultrathin 2D nanomaterials, including TMDs, hexagonal boron nitride (h-BN), graphitic carbon nitride (g-C_3N_4), black phosphorus and MXenes, among others [120]. These materials, with unique properties, such as substantial bandgaps, high conductivity, rapid electron transfer, and notable fluorescence, serve as transduction components and substrates for biosensing technologies. To enhance the sensitivity, surface chemistry modifications, such as defect engineering, doping, and creating heterostructures, are common. TMDs, in particular, are the most widely used in optical diagnostics, followed by MXenes, carbon nitrides, and BNs [63]. Some TMD QDs and g-C_3N_4 nanosheets exhibit strong fluorescence, enabling their use as fluorescent markers in protein assays and biomolecule identification [121]. Furthermore, 2D materials function as nanoquenchers, allowing distance-dependent fluorescence quenching tied to recognition events without the influence from donor emission spectra [63,122]. They are also employed in colorimetric systems because of their peroxidase-mimicking activity, with color responses observable by UV-vis spectrometry [123].

In contrast, nanozymes and 2D nanomaterials are prominent in tumor biomarker analyses. A hollow Janus hybrid nanozyme vector with dual-sided Ag−Au nanocages exhibits superior peroxidase-mimicking capabilities and precise targeting owing to its silver gate and DNAzyme nanobrushes. This “all-in-one” vector supports a stringent SERS liquid biopsy platform, achieving miRNA detection at 166 fM sensitivity [124]. Antimonene has demonstrated superior sensitivity compared with that of conventional

2D material graphene by exploring the chemical interactions between single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA). Bao and co-workers [125] designed a SPR sensor for label-free molecular-level quantification and single nucleotide mutation identification, achieving unprecedented sensitivity in clinical nucleic acid detection. First, AuNRs were utilized to conjugate with ssDNA to enhance the SPR signal. Subsequently, the AuNR-ssDNA complex was adsorbed onto the antimonene nanosheets driven by the robust interaction between ssDNA and antimonene. Upon addition of complementary miRNA, the hybridized targets were readily desorbed from the antimonene surface, as dsDNA exhibited a weak binding affinity for antimonene. The miRNA concentration was quantified based on a negative shift in the SPR signal (Fig. 5B) [125]. Recently, composite systems of 2D materials have shown promising theranostic capabilities. For instance, Lin et al. [126] utilized soybean phospholipid-modified Ta_4C_3 nanosheets for dual-mode photoacoustic/computed tomography imaging and hyperthermia in tumors to demonstrate the photothermal efficiency of 4T1 breast cancer cells. Ji et al. [127] constructed a 2D MoS_2-glycoprobe (glycosheet) to control the ROS release from cells with asialoglycoprotein receptors.

Porous framework-based nanomaterials, which represent a class of typical 3D nanomaterials, are regarded as highly promising candidates for optical biosensing applications. In particular, MOFs and covalent organic frameworks (COFs) serve as two prominent representatives. The functionalization capabilities, uniform pore structures, and solvent stability of MOFs render them effective for selective optical sensing. Typically, MOFs comprise two primary elements: metal ions or clusters and organic linkers. These components are connected by precisely defined coordination bonds. An extensive variety of metal ions and metal clusters can be employed, while designable organic linkers offer further possibilities for tailoring the structure and physicochemical properties of MOFs. Owing to these versatile characteristics, a diverse array of MOFs have been successfully synthesized, including several well-known structures, such as MOF-5, HKUST-1, MIL-101, and UiO-66 [128]. Moreover, the isoreticular principle, which allows the modification of a structure's size and composition without altering its fundamental topology, is vital for designing and synthesizing MOFs with expanded pore sizes and increased pore volumes. This methodology has contributed to the development of an isoreticular series, such as isoreticular and zirconium-based MOFs, including UiO-66, UiO-67, and UiO-68 [128]. In the field of biosensing, specific pore sizes and functional ligands enable host-guest interactions at atomic level, and their varied optical and catalytic properties allow for

the targeted detection of ions, gases, and biomolecules. Advanced optical techniques are now utilizing MOFs for complex biomarker diagnostics, including volatile organic compounds (VOCs). Qiao et al. [129] established a SERS-based VOC detection technique that enhanced Raman scattering for the early detection of lung malignancies. The ZIF-8 MOF shell layered on gold superparticles slowed the gaseous biomarker flow and optimized aldehyde detection for cancer diagnostics in mixed gases. Unlike inorganic platforms (e.g., QDs and noble metal NPs), MOFs are inherently degradable and biocompatible, offering non-toxic and highly stable sensing options [130]. Research on MOF-based chromism sensors is still in its early stages compared with that of the well-established noble metal-based colorimetric platforms. Typically, color changes in MOF chromic sensors arise through two main mechanisms: alterations in metal coordination geometry and shifts in charge transfer between the ground and excited electronic states [131]. When exposed to water vapor in air, the Cu^{+}-MOF assembled from CuI and 1-benzimidazolyl-3,5-*bis*(4-pyridyl)benzene acted as a sensitive colorimetric transducer that responded to air humidity by replacing the encapsulated organic molecules, producing a yellow-to-reddish-brown color shift corresponding to varying humidity levels [132]. In addition, reactive viologen groups incorporated into MOFs exhibit marked photochromism via charge transfer with electron-rich molecules (e.g., amines) upon light exposure, resulting in a dark-yellow to black color change [131]. In parallel, MOF-based luminescent systems have attracted considerable attention for biomedical and optical sensing applications. Numerous luminescent MOFs have been identified, with luminescence derived from organic ligands, emissive metal ions, guest ions, or fluorescent dyes encapsulated within the MOF pores, as well as from charge-transfer or catalytic activities. Ligands featuring aromatic or extended π-conjugation systems significantly contribute to MOF luminescence, and commonly used metal ions for functionalization include lanthanides, d10 transition metals, and silver clusters. In addition, encapsulating luminescent species such as QDs, metal complexes, organic dyes, and photosensitizers within MOF pores or through self-assembly further enhances MOF luminescence [133]. Recent advancements in MOF technology include the synthesis of a porphyrin-based heterobimetallic 2D MOF (Zn-porphyrin-based Co(II)-MOF) through the self-assembly of ZnTCPP and Co(II) salts in the presence of 2-methylimidazole (MeIm) at ambient temperature. The incorporation of paddle-whee $[Co_2(-CO_2)_4]$ units facilitates electron transfer between the Co(II) ions and oxygen. This configuration enabled exceptional electrochemiluminescence performance, allowing the development of a non-amplified electrochemiluminescence biosensor based on this 2D MOF probe for the sensitive identification of the *RdRp* gene of SARS-CoV-2, demonstrating

considerable potential for accurate viral detection (Fig. 5C) [134]. However, the specific geometric morphologies, limited functionalities, and unsatisfactory performances of pure MOFs restrict their broader applications. A large proportion of MOFs synthesized in deep eutectic solvents employ choline chloride/urea or its derivatives, whereas other combinations remain underexplored. Furthermore, the mechanisms governing surfactant interactions with metal ions and bridging ligands during the reaction process warrant further investigation. Innovations in 2D MOFs, such as conductive MOF nanosheets have also addressed the electrical limitations of MOF-based sensors. In contrast to MOFs, COFs are mainly constructed through covalent bonds with elements such as C, O, N, and B, and they show remarkable thermal and chemical stability. Guo et al. [135] fabricated COFs@MoS_2-Pd composites by incorporating MoS_2 nanosheets onto the surface of MoO_3@COFs microcables through a hydrothermal method, where the MoS_2 nanosheets acted as a support for the subsequent introduction of PdNPs. The incorporation of COF microtubes not only enhanced the electrical conductivity of the materials but also reduced the aggregation of MoS_2 nanosheets, thereby contributing to an enhancement in catalytic performance. The developed hybrids exhibited improved peroxidase-like activity and enabled selective colorimetric biosensing of uric acid. Moreover, COFs have also demonstrated considerable competitiveness in electrochemiluminescence-based biosensing. A COF exhibiting stable and intense electrochemiluminescent properties was prepared through the condensation reaction between perylene-3,4,9,10-tetracarboxylic dianhydride and melamine, functioned as a emitter. Upon the aptamer recognizing the pesticide residue of acetamiprid, the DNA trigger was released, subsequently activating the CRISPR/Cas12a system. This activation resulted in the cleavage of Fc-DNA and the initiation of the electrochemiluminescent signal. Moreover, the well-organized distribution of luminescent units within the COF structure, combined with the pore confinement effect, substantially improved the performance stability and sensitivity of the COF [136].

3.4 Nanocarbons for bioanalysis and bioimaging

Nanocarbons of various dimensions, such as 0D fullerenes and carbon/graphene QDs, 1D CNTs and graphene nanoribbons, 2D graphene and graphene oxides, as well as 3D nanodiamonds, have gained substantial attention for their applications in refractive sensing and visualization because of their distinct characteristics of chemical inertness, size- and wavelength-dependent emission, resistance to photobleaching, broadband optical absorption, enzyme-like characteristics, ease of bioconjugation,

simple and inexpensive synthesis without the need for heavy metal ions or organic solvents, low intrinsic toxicity, good cell permeability, and versatile surface functionalization. To date, in addition to the aforementioned nanomaterials, nanocarbon materials have also been extensively explored as alternative tools for developing next-generation optical biosensors designed for colorimetric/fluorescent detection, chemiluminescence, up-conversion PL, and fluorescence quenching, including FRET and CRET [137]. A comparison of detection performance based on diverse mechanisms and variously scaled nanomaterials for optical sensing is summarized in Table 2 [21−26,29−32,35,38−41,48−50,54.57,59,61,69,75,81,84,88−92,98,104,114−116,124, 125,129,134,135,138−141]. Carbon QDs (CQDs), also known as carbon dots (CDs), are small clusters of carbon atoms with diameters smaller than 10 nm that possess semiconductor-like characteristics and are commonly used as substitutes for QDs in bioimaging. CQDs exhibit strong fluorescence without doping or labeling, with excitation and emission spectra typically extending from UV to red (650 nm) by varying the synthesis conditions (e.g., pH), sizes, morphologies, surface functionalities, and types or amounts of doping. Multi-photon excited CQDs facilitate multiplexing functions, and their fluorescence can be up-conversion or down-conversion [142]. As the basic building blocks of living organisms, cells convey tremendous amounts of genomic and transcriptomic information to sustain regular biochemical processes. Specifically, organelles, including the nucleus, mitochondria, and Golgi apparatus, are closely associated with tumorigenesis, cardiovascular, and neurodegenerative diseases. Thus, the *in situ* monitoring of organelle variations in cells is of great significance for early diagnosis and intervention. Shuang et al. [138] synthesized four types of CDs featuring distinct surface groups and varying degrees of lipophilicity for tunable organelle imaging through various uptake pathways, demonstrating their significant potential for specific organelle-targeting imaging in cell division and 3D reconstruction. Graphene QDs (GQDs), a type of CQD typically derived from graphene and/or graphene oxide, present graphene lattices that resemble the crystal structure of one or several graphene layers. GQDs typically have a diameters that range from 1 to 10 nm and are composed of fewer than 10 graphene layers. Compared with CQDs, GQDs typically exhibit stronger crystallinity and fewer defects owning to their higher proportion of crystalline sp^2 carbon. Both CQDs and GQDs generally possess numerous oxygen-based functional groups for surface modification, of which nitrogen doping is the most frequently used. Green luminescent GQDs have been successfully converted to blue luminescence via surface chemical modification with alkylamines. Similarly, GQDs modified with a combination of polyamidoamine and (3-aminopropyl)-triethoxysilane (APTES) showed an increased fluorescence intensity compared with

that of CQDs modified with APTES or polyamidoamine individually, owning to the presence of multiple nitrogenous and oxygenated groups [142]. Functionalized spherical fullerenes (C_{60}), consisting of graphene in a spherical shape with pentagonal structures, serve as excellent nanomediators through functionalization or conjugation with amplification nanotags, such as AuNPs, to enhance the sensitivity of optical sensors. Increasing interest is focused on fluorescent fullerene NPs that exhibit tunable emission peaks based on their size and surface functional groups for multicolor optical sensing, both *in vitro* and *in vivo* [139]. Similar to CQDs and GQDs, 3D nanodiamonds can emit strong fluorescence owing to intrinsic nitrogen vacancy center defects, which serve as fluorescent emission centers. PL from these defect centers can be excited through both one-photon and two-photon stimulations [143]. The advantages of facile functionalization, nonphotobleaching, nonphotoblinking and strong fluorescence intensity make nanodiamonds ideal for continuous cell tracking, although their rigid and costly preparation procedures limit large-scale use.

Since the unique fluorescence of individual single-walled CNTs (SWCNTs) was first identified in 2002, substantial research has concentrated on exploiting the inherent optical properties of 1D nanocarbons, including SWCNTs and single-walled carbon nanohorns (SWCNHs). The composition of SWCNHs closely resembles that of SWCNTs, but with closed nanotubules forming into conical rather than tubular shapes. SWCNHs generally have diameters below 10 nm and lengths between 10 to 70 nm [137]. The indefinite photostability, single-molecule combination sensitivity, catalytic activity, and natural fluorescence in the near-infrared range provide substantial potential for detection systems in various applications. However, a major challenge in developing CNT-based materials is mitigating their tendency to self-associate in water, forming thick, insoluble, and potentially harmful aggregates. Surface functionalization strategies, including both covalent and non-covalent modifications of the sidewalls, have been adopted to improve dispersibility and biocompatibility. Carboxylated SWCNTs can be covalently fabricated using AuNPs, QDs, and superparamagnetic iron oxide NPs for multifunctional imaging. In SWCNT immunoassays, avidin and biotin functional groups are also widely employed as linkers for additional labels [144]. In contrast to covalent methods, non-covalent wrappings or decorations avoid modifying the extended π-conjugated system and optical properties of SWCNTs while stabilizing them in water, imparting additional attributes, such as molecular recognition. Biopolymers, including DNA, RNA, peptides, and proteins, have considerable advantages for improving SWCNT-based optical sensors for label-free target detection. Moreover, SWCNTs exhibit peroxidase-like properties, which are influenced by pH,

temperature, and H_2O_2 concentration, resembling HRP activity. These properties have been utilized to develop optical biosensing platforms [140,145]. In contrast, SWCNHs, arranged in round clusters resembling dahlia flowers owning to van der Waals forces between exposed ends, ranged in size from 40 to 200 nm. This structure provides a higher surface-to-volume ratio than that of SWCNTs, enabling more efficient functionalization with biomolecules or NPs for fluorescence labeling [146].

Graphene nanosheets, 2D atomic-thin layer materials with zero bandgap (~0.34 nm) as the fundamental unit due to their sp^2 hybridization, have numerous functional characteristics, including high conductivity, intrinsic catalytic activity, low cytotoxicity, and an exceptionally large surface area (up to 2630 m^2/g) with each carbon atom exposed. Since graphene was isolated from bulk graphite, it has been widely studied for applications in highly sensitive fluorescence-quenching biosensors, Raman imaging, and nanozyme-based bioanalysis across multiple fields [147]. The GO nanosheets have been extensively used as highly responsive signal indicators for detecting biomolecules and effective nanocarriers due to uniform size, excellent solubility, biocompatibility, and inherent NIR PL, making them suitable for cellular imaging with minimal background interference [148]. Moreover, numerous carbon-based composites, such as hemin-graphene and PtAuNP-GO, have functioned as peroxidase mimics for H_2O_2, oxidase substrates, ions, nucleic acid, and protein detection [141,147]. Guo et al. [141] synthesized hemin-graphene hybrid nanosheets that exhibit intrinsic peroxidase-like activity. By exploiting the distinct affinities of ssDNA and dsDNA to these nanosheets, a label-free colorimetric detection method for HBV DNA was established, achieving a LOD as low as 2 nM. Moreover, this sensor demonstrated sufficient selectivity to effectively distinguish single-base mismatches.

4. Conclusion

Advancements in functionalized low-dimensional nanomaterials have accelerated the development of optical biosensors, supporting a range of biomedical research and biopharmaceutical applications, including IVDs, POC tools, therapeutic drug monitoring, bioimaging, targeted therapy, and wearable and implantable systems for continuous drug monitoring. This review systematically summarizes the underlying design principles for assembling optical sensors and highlights the specific advantages and properties of smart nanomaterials for each type of biosensor. Notably, nanomaterial-functionalized LSPR-based POC nanosystems offer a compelling approach by converting target recognition into absorption band shifts and color variations readily observable by the unaided eye. Further advances in multicolorimetric

strategies based on etching and growth mechanisms have enabled the semi-quantification of targets, often using noble metal nanomaterials, such as 1D anisotropic AuNRs and Au@Ag nanocomposites. As metal nanomaterials shrink below 2 nm, their unique photoluminescent properties emerge, with the disappearance of the LSPR phenomenon.

Current ratiometric fluorescence platforms have achieved high visual resolution using novel nanomaterials, such as DNA-templated AgNCs, CdSe, and CdTe-based QDs, NIR Ag_2S QDs, CDs/GQDs, lanthanide-doped UCNPs, TMD QDs, and g-C_3N_4 nanosheets. Dual-mode sensing platforms combining colorimetric, fluorescence, or SERS readouts are particularly competitive in IVD and POC applications. SERS spectroscopy is especially powerful, using large-volume "hot spots" of nanomaterials to amplify target signals, facilitating single-molecule analysis and CTCs detection. However, the high cost of noble metals limits the large-scale preparation of these materials, prompting interest in low-cost oxides with considerable stability in nanozymes and SERS platforms for the detection of biomolecules, such as glucose, xanthine, and cholesterol. Meanwhile, chemiluminescence and bioluminescence-based sensors show promising capabilities for probing biological specimens and detecting cell damage or disease through *in vivo* imaging. By eliminating external light excitation with CRET and BRET, these sensors can overcome challenges such as fluorescence quenching and poor tissue penetration. Electrochemiluminescence systems generate luminescent signals via nanomaterial-based electrochemiluminescence probes for biosensing and high-throughput drug monitoring, with further development of optical wearable nanosensors relying on 1D CNT-embedded electrodes. Additionally, 2D MFGS and uniform 3D cross-point plasmonic nanostructures have potential for use in SERS-based wearable biochemical sensors and theranostic applications in continuous drug monitoring and targeted therapy. Altogether, nanomaterial-functionalized optical biosensors are undergoing rapid evolution and extensive growth.

However, several challenges remain unaddressed. Current deficiencies include the need for reliable and accurate POC tools for rapid viral detection, miniaturized sensors for real-time at-home monitoring (such as wearable or implantable nanosensors), and simplified read-out devices that are easily observable without aid. Although the integration of nanomaterials enhances the feasibility and rapidness of their target detection and monitoring, the sensitivity of nanomaterials to varying sensing conditions and tendency to form non-reversible aggregates must be addressed to improve their functionalization and storage stability. Additionally, innovative preparation and

fabrication methods for cost-effective and low-toxicity nanostructures that leverage machine learning remain urgent priorities because the controlled preparation process is still poorly understood, with most methods have been developed through trial and error. For *in vivo* monitoring and integrated theranostic systems, the biological toxicity and metabolic characteristics of nanomaterials also require comprehensive investigation. Future directions in nanomaterial-based optical biosensors should focus on bridging the gap between academic research and commercial applications, addressing biomedical and pharmaceutical complexities by integrating advances in spectroscopic instrumentation with recent developments in nanofabrication technologies.

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Figure captions

Fig. 1. Summary of the fundamental principles for nanomaterial-based optical biosensing. LSPR: localized surface plasmon resonance; SERS: surface enhancement Raman scattering; TMB: 3,3′,5,5′-tetramethylbenzidine; BRET: bioluminescence resonance energy transfer.

Fig. 2. Nanomaterial-enhanced optical biosensors and their biomedical applications: Mechanisms based on varying transduction of biorecognition events. (A) Schematic illustration of the Ag-shelled Au@Ag nanorods (AuNBP@Ag)-based immunosensor and the etching process of the AuNBP@Ag in the absence and in the presence of different concentrations of H_2O_2 [22]. (B) Illustration of ratiometric fluorescent lateral

flow immunoassay (RFLFIA) strip for visual and quantitative detection of heart-type fatty acid binding protein (H-FABP) [29]. (C) Circulating tumor cells (CTCs) detection by incubating with antibody-conjugated and Raman reporter-coated gold nanoparticles (AuNPs) labels (Ab-SERS) and characterization with Raman spectroscopy [42]. (D) Nonenzymatic glucose colorimetric sensing based on MnO_2 nanoflakes (NFs) [50]. (E) Schematic diagram of hierarchically porous porphyrinic metal-organic framework (MOF) to co-encapsulate luminol and hemoglobin for efficient CRET-mediated and oxygen self-supply photodynamic therapy of cancer [54]. (F) Schematic illustration of luminol + 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)−1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate (DiD) nanobubbles for dual-modal imaging of myeloperoxidase (MPO) activity through the bioluminescence resonance energy transfer (BRET), and the following Förster resonance energy transfer (FRET) between DiI and DiD [59]. (G) Schematic illustration of bovine serum albumin (BSA) directed fluorescent Au/Ag alloy nanoclusters (Au/Ag NCs@BSA) hydrogel-based electrochemiluminescence sensing system for glutathione detection [61]. TMB: 3,3′,5,5′-tetramethylbenzidine; LHHP: luminol and hemoglobin co-encapsulated hierarchically porous porphyrinic MOF; GCE: glassy carbon electrode; TEA: triethylamine; Hb: hemoglobin; HSA: human serum protein; GSH: glutathione. Reprinted from Refs. [22,29,42,50,54,59,61] with permission.

Fig. 3. Comparison of key nanomaterials for optical biosensors, classified according to the categories of zero-dimensional (0D), 1D, 2D, and 3D nanomaterials. UCNPs: upconversion nanoparticles; NPs: nanoparticles; QDs: quantum dots; CDs: carbon dots; *h*-BN: hexagonal boron nitride; g-C_3N_4: graphitic carbon nitride.

Fig. 4. Zero-dimensional (0D) and 1D nanomaterials for *in vitro* diagnostics (IVDs) and therapeutic drug monitoring. (A) Synthesis illustration of the platinum (IV) delivered gold nanoclusters (AuNCs-Pt) and the corresponding near-infrared II (NIR-II) tumor imaging capacity penetrates deep tissues, allowing visualization of the platinum transportation [76]. (B) Schematic diagram of the design process for black TiO_2 (B-TiO_2) bioprobe and its application in biological surface enhancement Raman scattering (SERS) imaging and photothermal therapy [85]. (C) Schematic illustration of DNA-templated magnetic nanoparticle-quantum dot (QD)-aptamer copolymers (MQAPs) for magnetic isolation of circulating tumor cells (CTCs) [88]. (D) Schematic design of activatable orthogonal near-infrared II (NIR-II) emitting $NaYF_4$:Gd/Yb/Er@$NaYF_4$:Yb@SiO_2@Ag nanoprobes for hepatotoxicity detection induced by overdose metformin [97]. (E) Scheme of DNA-bridged arrowhead gold

nanorod (NR) dimers with side-by-side and end-to-end motifs for miR-21 detection in living cells [104]. EDC: N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride; NHS: N-hydroxysuccinimide; PDHC: a patient-derived tumor xenograft model of hepatocellular carcinoma; GSH: glutathione; PEG: polyethylene glycol; HCR: hybridization chain reaction; MNP: magnetic nanoparticle (NP); CBA: a DNA aptamer (sgc8c); MQAP: DNA-templated MNP-QD-aptamer copolymers; VC: vitamin C. Reprinted from Refs. [76,85,88,97,104] with permission.

Fig. 5. Two-dimensional (2D) and 3D nanomaterials for continuous molecular monitoring and/or targeted therapy. (A) Schematic procedure for the fabrication of 3D cross-point nanostructures for surface enhancement Raman scattering (SERS) analysis (i) and schematic procedure for the fabrication of SERS contact lens via transfer printing for glucose detection (ii) [115]. (B) Schematic illustration of the strategy employed to detect microRNA (miRNA) hybridization events by assembling the antimonene nanosheets on the surface of Au film [125]. I−IV indicates the assembly of antimonene nanosheets on the Au film surface, the adsorption of gold nanorod (AuNR)-single stranded DNA (ssDNA) onto the antimonene nanosheets, the interaction of miRNA with AuNR-ssDNA on the antimonene surface, and the release of miRNA-bound AuNR-ssDNA from the antimonene nanosheets, respectively. (C) Design and construction of porphyrin-based heterobimetallic 2D metal-organic framework (MOF) [134]. PMMA: poly-methlymethacrylate; Sb: antimonene; TCPP: tetrakis (4-carboxyphenyl)porphine; SBU: secondary building unit. Reprinted from Refs. [115,125,134] with permission.