Nanozyme-based colorimetric biosensor with a systemic quantification algorithm for noninvasive glucose monitoring

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Abstract

Diabetes mellitus accompanies an abnormally high glucose level in the bloodstream. Early diagnosis and proper glycemic management of blood glucose are essential to prevent further progression and complications. Biosensor-based colorimetric detection has progressed and shown potential in portable and inexpensive daily assessment of glucose levels because of its simplicity, low-cost, and convenient operation without sophisticated instrumentation. Colorimetric glucose biosensors commonly use natural enzymes that recognize glucose and chromophores that detect enzymatic reaction products. However, many natural enzymes have inherent defects, limiting their extensive application. Recently, nanozyme-based colorimetric detection has drawn attention due to its merits including high sensitivity, stability under strict reaction conditions, flexible structural design with low-cost materials, and adjustable catalytic activities. This review discusses various nanozyme materials, colorimetric analytic methods and mechanisms, recent machine learning based analytic methods, quantification systems, applications and future directions for monitoring and managing diabetes.

Key words: nanozyme, colorimetric analytic methods, glucose, colorimetric biosensor

1. Introduction

Diabetes mellitus is a significant cause of death and a chronic disorder affecting more than 422 million people worldwide [1]. Chronically elevated blood glucose can cause retinopathy, cardiovascular diseases, neuropathy, blindness, and a high risk of congenital disability [2], causing 1.6 million deaths every year according to the World Health Organization (WHO) [3]. Therefore, early diagnosis and proper glycemic management are crucial to prevent further progression and complications in diabetic patients. Millions of diabetic patients rely on self-monitoring of blood glucose, but the current approaches have vast opportunities for improvement. In general, to manage blood glucose in diabetic patients, blood should be collected using a finger-prick method several times every day [4, 5].
While direct monitoring of blood glucose is the most accurate measurement [6-8], it can create mental trauma for patients as well as infections and fingertip inflammation due to the requirement of several measurements a day, which is a major issue in terms of patient compliance with glycemic methods. Therefore, the demand for technology development to monitor blood glucose using noninvasive strategies has increased. Nanomaterial-based invasive and noninvasive glucose monitoring methods developed in the last 15 years are summarized in Figure 1. Advances in nanotechnology have led to the development of ultra-sensitive and high-performance platforms, including colorimetric, fluorometric, chemiluminescent, surface-enhanced Raman scattering, and electrochemical biosensors [9]. In addition, nanomaterial-based glucose monitoring biosensors have expanded from using blood to utilizing bodily fluids including sweat, tears, urine, saliva, and interstitial fluid (ISF).

Conventionally, the basic principle of detecting glucose is based on the glucose oxidase (GO) enzyme, which recognizes glucose as a substrate and initiates a biochemical enzymatic reaction. Therefore, since the reactivity of this enzyme has a significant influence on the sensitivity and reliability of the biosensor, much research is being conducted to improve the performance of the GO enzyme using protein engineering technology. Since the term ‘Nanozyme’ was first used in 2004, research and development using various functional nanomaterials that mimic the function of the GO enzyme have been conducted [10]. The definition of a nanozyme has been solidified into an enzyme-mimicking nanomaterial that demonstrates intrinsic peroxidase-like activity [11, 12]. Functional nanomaterials as artificial enzymes (nanozymes) show several remarkable advantages, including simple and excellent tunable catalytic activity, controllable synthesis protocols, ultrahigh environmental stability, ease of modification, low cost, and large-scale production [13, 14]. These properties greatly facilitate automation of multiple processes and high-speed integration of separation and detection procedures, saving time and reducing preparation steps. Recently, a few types of inorganic nanoparticles such as nanocarbon materials (carbon nanotubes and graphene oxide) [15], polymer-coated nanoparticles [16, 17], and nanocomposites [17], have been spotlighted for their ability to catalyze chemical reaction as enzyme-mimics that can be utilized for bio-detection. These nanozymes have been applied as chemical sensors and biosensors for colorimetric detection of pH, temperature, ions, reductive small molecules, \( \text{H}_2\text{O}_2 \), glucose, viruses, bacteria, cancer cells, and pesticides [18]. In addition, invasive and noninvasive biosensors using these nanozymes are being developed to monitor glucose. Over the last decade, colorimetric-based biosensors with nanozymes have expanded due to several advantages including high efficiency, high versatility, low-cost, and high stability.

Nanozyme-based biosensors mainly detect color in a qualitative manner [19, 20]. Therefore, for quantitative measurement of glucose concentration, many algorithms have been developed in different color spaces (RGB, CMYK, HSB/HSL, CIE XYZ, \( \text{L}^*\text{a}^*\text{b}^* \), and YUV models) to analyze color variations. In addition, since color values can be affected by the measurement area of the device or the surrounding environment, algorithms including supervised learning based convolutional neural networks (CNN), artificial neural network (ANN), and support vector machine (SVM), polynomial regression, statistical learning-based color reconstruction, and color checker-based digital image reconstruction are being developed to improve these errors [21].

This review highlights important advances in colorimetric-based glucose detection utilizing nanozymes, various mechanisms, colorimetric analysis methods, and their applications in biosensors. This review also discusses sensor performance in whole blood, saliva, urine, tears, and interstitial fluid. Finally, we address future development requirements of biosensing nanozymes for wearable glucose sensors.

2. Invasive and noninvasive/minimally invasive glucose detection

Various enzyme-based glucose sensors have been developed for bodily fluids including whole blood, urine, saliva, tear, and interstitial fluid (ISF) [22, 23]. The finger-pricking whole blood test is the most widely used method for fast glucose monitoring because blood possesses a high biomolecule concentration [6]. However, since this method requires pricking a finger to extract a small amount of blood for analysis up to 8 times a day, procedural discomfort decreases patient compliance. In addition, there is a high risk of complications such as infection and fibrosis. Therefore, implantable biosensors and microdialysis-type devices that can continuously monitor blood glucose have been developed [6]. However, implantable glucose sensors pose several challenges, including short lifespan, susceptibility to infection, and poor biocompatibility. Therefore, alternative methods have been explored for noninvasive glucose measurements using body fluids [24].
Several studies have shown that glucose concentrations in body fluids such as ISF, tears, saliva, and sweat correlate with that of whole blood [13]. Therefore, many studies have focused on these body fluids to develop noninvasive biosensors for glucose monitoring. To this end, advances in nanotechnology have made it an attractive and alternative sample medium for noninvasive continuous blood monitoring. Table 1 compares the key aspects of the most studied nanomaterials for various physiological measures, including biomarkers, representative concentrations, and their advantages and disadvantages. However, various considerations are required to protect the quality and accuracy of glucose concentration measurements in body fluids. One of the main considerations is that glucose concentration in these body fluids is lower than in whole blood ($2–40 \times 10^{-3}$ M in blood vs. $0.008–1.77 \times 10^{-3}$ M in body fluids).
10⁻³ M in saliva, 0.01–1.11 × 10⁻³ M in sweat, 0.05–5 × 10⁻³ M in tears, and 1.99–22.2 × 10⁻³ M in ISF) [13, 25]. Therefore, an optimized design and analysis method for monitoring systems specialized for each body fluid method should be developed.

The sampling method and physicochemical properties of each body fluid are also important considerations. For example, ISF fills the spaces between most of the body’s cells and provides a significant portion of the body’s liquid environment [13]. ISF has potential for medical diagnosis because it is very similar to whole blood plasma in terms of composition. Subcutaneous injection of a needle is required to monitor ISF, which can be inconvenient for prospective users [13]. Saliva is a complex mixture of 99.5% water and 0.5% electrolytes (glycoproteins, lipase, mucin, amylase, glucose, and antimicrobial enzymes) [26]. Analytes can be easily collected by spitting, but saliva contains many impurities, making it difficult to isolate the intrinsic glucose in the fluid. In tears, they can be excreted from the body as an extracellular fluid containing mucin, lipids, glucose, water, lysozyme, lactoferrin, lipocalin, lacritin, urea, sodium, immunoglobulins, and potassium [25, 26]. Tear glucose concentration has been shown to be highly correlated with blood glucose, with relatively little interference by impurities. However, biosensors require high sensitivity, selectivity, and low limit of detection (LOD) to monitor tear glucose concentration. Lastly, sweat is composed of water, ammonia, urea, salts, and glucose. The glucose level in sweat shows a high correlation with that in whole blood [27]. Sweat is easily accessible, but it is complicated by impurities and other components and requires exercise for collection.

All of these approaches using body fluids for blood glucose monitoring have unresolved hurdles as their respective fluid-based glucose measurements have not been strongly correlated with plasma glucose concentration or clinical evidence. However, painless and noninvasive methods using each body fluid-based glucose measurement are promising as they allow faster and more convenient monitoring of glucose concentrations in diabetes patients. Therefore, for the finding high correlation and clinical evidence, a system capable of requiring more accurate measurement is needed, and many clinical trials are required.

3. Glucose monitoring techniques

Developing a practical glucose biosensor with high reliability and sensitivity must be a top consideration for the biosensor industry. Various noninvasive glucose biosensors have been developed including optical, electrochemical, transdermal, colorimetric assay, luminescent detection, magnetic signal detection, and microwave approaches [28]. Table 2 shows the advantages and disadvantages of LOD, nanomaterials, and techniques for glucose detection with different methods and the current research status using body fluids. Optical methods of near-infrared reflectance spectroscopy (NIRS) [29, 30], surface plasmon resonance interferometry [31], photoacoustic spectroscopy [32], polarized optical rotation [33], Raman spectroscopy [34], fluorescence [35] and optical coherence tomography (OCT) [36] are illustrated in Figure 2, 3.
Table 2. Summary of the glucose detection methods, nanomaterials, limits of detection (LODs), advantages, and disadvantages

| Technique                      | Sample              | Nanomaterials | LOD                  | Advantages                                    | Disadvantages                                                                 | Ref  |
|--------------------------------|---------------------|---------------|----------------------|-----------------------------------------------|-------------------------------------------------------------------------------|------|
| Optical                        | Infrared spectroscopy | Glucose solution | CuNPs, quantum dots (QDs), CuBDC | 4.1 μM, 1.2 mM | Low scattering, low-cost materials, high penetration | Not portable, requires high hardware sensitivity, stability, and scanning pressure | [29, 30, 141] |
|                               | Raman spectroscopy   | Saliva, urine  | AuNPs onto Cuxtetra(arylxyrylphenyl) porphyrin chloride (Fe(III)) | 3.9 μM | Sharpen spectra, less sensitive to temperature changes | Not portable, high cost, instability of laser wavelength, low SNR | [34, 142] |
|                               | Fluorescence         | Glucose solution | Boronic acid functionalized CNPs, Carbon-based nanozyme CuAA | 1.56 μM, 10 μM | Highly sensitive, less damage to the body | Not portable, high cost, scattering phenomena can affect accuracy | [35, 143] |
|                               | Surface plasmon      | Glucose solution | Gold in TiO2, coated in Pd, Gold nanoparticles | 10 mg/dL, 25 μM | Rapid detection, real-time monitoring | Not portable, high cost, limited to high molecular weight biomolecules glucose, requires complex setup | [31, 144] |
|                               | Optical coherence    | Blood sample  | Silica gold nanoparticles | 5.78 mM, 0.015-0.045 mg/dL | High resolution, high penetration | Sensitive to individual movement, affected by temperature | [36, 145] |
|                               | Photoacoustic        | Skin, Blood | AuNPs | 0.035 - 0.098 μg/mL | High detection rate, high SNR | Sensitive to changes in temperature and pressure, vulnerable to motion artifacts | [32, 146] |
|                               | Optical Polarimetry  | Tear, skin, glucose solution | Gold nanoparticles | 125.4, 151.1 mg/dL | High resolution, easy to be miniaturized | Sensitive to temperature and motion changes, not portable, long lag time < 30 min | [33] |
| Transdermal                    | Glucose solution     | Gold nanoparticles | Gold nanoparticles | 0.9 μM, 0.02-0.05 mg/dL | Differentiates between extracellular and intracellular fluids | Requires a long processing time | [145, 147] |
|                               | Reverse iontophoresis | Glucose solution | Gold nanoparticles | 0.01 μM, 8.5 μM | Biocompatibility, easy handling | Skin irritation, inaccurate and long-term measurement | [38, 148] |
| Electrochemical                | Enzymatic detection | Tear, saliva, sweat, glucose solution | Gold nanoparticles SiO2, ZnO, Silver, WSNFs | 0.1 μM, 3.7 μM | Real-time monitoring, high detection range sensitivity, and low LOD | Requires electrodes, toxicity, vulnerable to temperature and motion change | [149-151] |
|                               | Amperometry          | Glucose solution | Gold nanoparticles | 0.024 nM/L, 4 μM | Easily commercialized, high accuracy by multiple sensors | Sensor error from drift, calibration error and delays | [152, 153] |
| Other                          | Colorimetric assay   | Tear, saliva, sweat | Nanoparticles | 1.25 ng/mL, 0.1 nM/g | Rapid, widely used, reliable performance, portable and convenient, easy to manipulate, cost-effectiveness | Low accuracy, toxicity, limited by battery life and storage | [58, 154] |
|                               | Luminescent          | Tear, saliva, sweat, H2O2 | ZnO, Lanthanide-doped Nanoparticles and MOFs and CPs | 5 ng/dL, 20 pg/mL, 0.1 μM | High accuracy and sensitivity, widely used | Not portable, need specific excitation source, high autofluorescence background, complicated operation | [155, 156] |
|                               | Magnetic signal      | Tear, saliva, sweat | MoS2/Fe3O4 magnetic nanoparticles (MNPs) | 100 nM, 0.5 ng/mL, 8.6 nM | High accuracy and sensitivity, detect entire magnetic interference, portable, convenient, high accuracy | Requires an ultrasensitive magnetic sensor, vulnerable to external magnetic interference, high cost | [157-159] |
|                               | Microwave            | Glucose solution | CuO nanoparticles | 0.2 - 10 μM, 36-454 mg/dL | High accuracy and sensitivity | High cost, toxicity, sensitive to temperature and motion artifacts | [160, 161] |

Optical methods measure the glucose concentration from body fluids and indirectly calculate the blood glucose concentration using correlations between glucose concentrations in whole blood and body fluids.

### 3.1. Near-infrared reflectance spectroscopy (NIRS) and Raman spectroscopy

Arnold et al. reported noninvasive glucose monitoring in diabetic patients utilizing the visible and near-infrared (NIR) spectral regions [37, 38]. A spectrophotometer can measure reflected/transmitted light intensities as a function of concentration, absorption coefficient, and sample thickness as well as evaluate the effect of scattered light in body fluids (Figure 2A). However, the internal structure of human tissues is complex, and spectral information of numerous materials or substances can interfere with the accuracy of the results. Therefore, NIRS methods require processing algorithms to extract the glucose concentration from multiple parameters such as refractive index, angle information, and information of the illumination light source. To improve this issues, deep neural network (DNN) model and efficient regression models has been designed for prediction of serum glucose concentration from healthy subjects, prediabetic, and diabetic condition [39]. These DNN make more high accuracy serum...
glucose prediction in comparison with other NIR measurement with detection range of 80 - 420 mg/dL. However, since the training data and validation sets were relatively small and it can only detect prediabetic and diabetic condition, it is necessary to collect more clinical sample for the training and validation purpose for the detection of hypoglycemic ranges.

Raman spectroscopy is a non-destructive chemical analysis technique with sharp spectral peaks that provide direct chemical variations of the glucose molecule. Raman scattering is an inelastic scattering involving the target molecule’s vibrational energy changes from interaction with incident photons. This wavelength change, called the Raman shift, can represent the glucose difference between the initial and final vibration states (Figure 2B) [37]. Raman spectroscopy is performed at low frequencies at the end of the NIR band and detects fundamental vibrations of atomic groups, leading to more accurate identification. However, compared with other optical methods, Raman spectroscopy suffers from weak Raman signals, extremely challenging calibration in turbid samples, and strong background noise of the surrounding environments. Thus, current efforts are underway to resolve these issues in a wide range of techniques including photon migration theory and multivariate calibration (MVC) analysis for tissue modulation [40]. However, to extract meaningful information, chemometric algorithms which is mathematical, statistical, and computational methods are required for interpreting analytical data. For this aspect, recently the combination of machine learning and surface enhanced Raman Spectroscopy (SERS), and convolutional neural networks (CNN) composed hidden layers are introduced and enhanced the Raman scattering. In 2022, Wang et al. introduced the Gramian angular field (GAF-CNN) which can convert into blood glucose concentration (output) with 1-D Raman spectral data (input) for prediction of glucose concentration. They showed the high performance with error of prediction (REMS = 0.065) and coefficient of prediction (0.999). These algorithms might be good option to predict of glucose concentration with Raman signal [41, 42].

3.2. Fluorescence spectroscopy

Fluorescence spectroscopy uses specialized molecules called fluorophores that absorb the energy of an excitation photon at a specific wavelength and then emit fluorescence, causing a wavelength difference known as the Stroke’s shift [43]. In glucose detection, fluorophores like intermediate molecules can bind directly to glucose and alter fluorescence [43]. Fluorescent glucose sensing molecules may be configured to increase or decrease fluorescence at baseline depending on ambient glucose concentration. A variety of glucose-sensing fluorophore, ranging from inorganic (e.g. synthetic carbon nanotube materials and quantum dots) to organic (e.g. enzyme, glucose binding proteins (GBPs), and boronic acid derivatives), have been introduced over the decade [44]. Quantum dots (QDs), nanometer-sized semiconductor crystal, can be designed to fluoresce at any wavelength depending on the crystal size and material used. There are three types of organic molecules that generally recognize glucose: (1) enzymes, (2) GBP, and (3) boronic acid derivatives. It consists of a fluorescent glucose sensor that can be integrated with a fluorophore to reversibly bind glucose. These organic molecules have the advantage of being able to monitor a wide spectral range from ultraviolet (380 nm) to near infrared (880 nm) depending on the immobilized fluorophore. Hitomi et al. reported fluorescence resonant energy transfer (FRET) as shown in Figure 2C, which provides excellent sensitivity for detecting low glucose concentrations [45]. Fluorescence biosensors have advantages of high specificity, sensitivity, and a wide detection range, allowing measurement of analyte concentration based on fluorescence intensity and decay time due to the characteristic emission spectra of specific fluorophores. Therefore, in vitro test projects and experiments commonly use fluorescence biosensors. However, fluorescence biosensors have limitations, including potential toxicity issues, susceptibility to interference due to pH changes and oxygen concentrations, short lifespan of the fluorophore, photostability issues, loss of recognition capability, and a low signal-to-noise ratio. In addition, conventional statistical algorithms are often limited by low accuracy under low illumination conditions, long computation times, and incorrect initial assumptions of decay parameter [46]. Therefore, machine learning-based simple training architectures of artificial neural network (ANN) or convolutional neural network (CNN) have been employed to improve the visualization, less computational time, and detect low fluorescent signal. In 2022, Chen et al. used a Wasserstein GAN-based algorithm in which the generator (G) is trained to generate a high-photon-count fluorescence decay histogram using a low-photon-count input. The GAN training algorithm with rectified linear unit (ReLU) activation was up to 2,800 times faster than the gold standard estimation and showed more accurate analysis of low-photon-count histograms. Since these algorithms have not yet been applied to fluorescence-based glucose detection, better neural network architecture with appropriate activation functions will eventually
improve the issues.

### 3.3. Optical polarimetry (OP)

As another optical method, Cameron et al. developed optical polarimetry (OP) to noninvasively measure blood glucose concentration [47]. They used a polarized beam of light to illuminate a glucose solution and measured the rotation angle of the incident light’s polarized plane (Figure 2D) because glucose is an optically active substance and can rotate the plane of the polarized beam [37]. The polarization orientation plane will be changed by the deflection angle from the original incident direction depending on the glucose concentration. The total rotation is proportional to the optical path length, concentration of the analyte, temperature, and wavelength of the laser beam (400-780 nm). Optical polarimetry takes advantage of the easy miniaturization of optical components because it only uses molecules that can rotate the plane of polarized light [48]. However, the relatively low measurement accuracy is challenging issues, mainly due to the presence of other active molecules, high degree of scattering in skin tissue, varying corneal birefringence, and high deflection angle. To improve these issues, a number of geometric and physical model have been proposed to explain physical process, overcoming is difficult because extracting the sample itself is untidy, rendering unpopular sampling methods [49].
3.4. Optical coherence tomography (OCT) and photoacoustic spectroscopy (PAS)

Optical coherence tomography (OCT) has been developed to measure glucose concentration more accurately for high-resolution imaging, providing depth-oriented tomography with two- or three-dimensional images using low-coherence interferometry (Figure 2E). OCT typically employs the NIR range of light and can be used to observe internal biological tissues at depths of 1-2 mm with a spatial resolution of 10-15 microns. OCT was proposed to detect blood glucose from specific skin or in vitro samples and has distinct advantages including continuously monitoring blood glucose concentration with a high signal-to-noise ratio, high resolution, and high noninvasive penetration depth. However, OCT is sensitive to motion artifacts such as skin temperature change, pH, and humidity, leading to low measurement accuracy. In addition, variation of the scattering coefficient by physiological compound is a key factor restricting its development. Therefore, a photoacoustic spectroscopy (PAS) technique was reported by Tanaka et al. [50]. The basic concept of PAS is shown in Figure 3A. The technology of PAS uses ultrasound waves and short laser pulses with wavelengths that are absorbed to produce microscopic-scale localized heating. The localized heating causes a volumetric expansion of the medium, generating an ultrasound wave that can be detected by an acoustic biosensor to measure variations of blood glucose concentration. Even though it can improve the depth and increase detection reliability on the micrometer scale [51], and has low detection sensitivity in the range of physiological glucose concentrations, PAS of the detection sensitivity is still unsatisfactory for clinically approved glucose monitoring. In 2018, Sim et al. developed in vivo microscopic PAS for noninvasive glucose monitoring. They used two laser sources (glucose absorbing and insensitive region detection wavelengths) to improve the system’s overall signal-to-noise ratio (SNR) [52]. In 2022, Abdulrahman et al. introduced a photoacoustic spectroscopy using machine learning to detect glucose level in skin sample with 40, 200 dataset and enhancing the sensitivity ± 25 mg/dL [53]. Even though this study required to demonstrate the feasibility of in vivo, measurement of glucose concentration can be further enhanced with different classification models.

3.5. Luminescent detection

Luminescent detection-based assays have emerged with high glucose detection sensitivity based on fluorescence intensity to determine glucose variations in biochemistry and immunoreaction applications. Fluorescence emission requires excitation energy, usually at a shorter wavelength (Figure 3B). Luminescent detection is performed to capture the fluorescent intensity from luminescent nanoparticles such as quantum dots (QDs) [54], dye-doped nanoparticles [55] and up-converting nanoparticles [56]. Similar to colorimetric detection, luminescent detection can be carried out on a handheld device that is cost-effective and easy to manage compared to bench-top apparatuses. In 2008, Faulstich et al. reported a pocket-size device that contains an illumination source and filters. The end-user inserts the test strip, activates the excitation light source, and monitors the test result with their naked eye [57]. In 2013, to obtain more quantitative results, Kozma et al. developed a handheld fluorescent microarray reader consisting of a filter, CCD camera, laser diode (λ=635 nm) with a collimator, and a prism. The total measurement time was less than 20 s [58]. In 2017, Zhang et al. introduced a more rapid system with an analysis range of 1-100 ng/mL [59]. Conventional imaging setup required high quality of sCMOS or CCD, however, with the rapid development of sensing capabilities, the smartphone can capture luminescence image or fluorescence signal with expansion of attachments and software applications. Nonetheless, luminescent detection requires sophisticated optical components to result in high sensitivity.

4. Colorimetric assay

Colorimetric detection utilizes specific indicators (nanozyme or nanomaterials) that change color when interacting with molecules of interest. The color information is recorded with a complementary metal-oxide-semiconductor (CMOS) or charge-coupled device (CCD) divided into two-dimensional grids, known as pixels. The sensor converts the photons into electrons in each pixel. The electrical signal is processed by an image processor to produce final images in JPEG, PNG, TIFF, and BMP formats, as illustrated in Figure 3C-D. Digital cameras, scanners, and smartphones are now widely used to measure color changes and have emerged as suitable alternatives for colorimetric analysis. Among them, current smartphones are prominent as they are equivalent to a microcomputer with high-capacity internal memories and are equipped with high-resolution cameras and wirelessly communicate with other devices [60]. Significant advances in smartphones make them useful for colorimetric assays, including fluorometric or spectroscopy applications.
In the algorithms aspects, conventional colorimetric analysis is started to track the region of interest (ROI), extracting the true color in color space of RGB, CMYK, HSB/HSI, CIE XYZ, L*a*b*, and YUV models. Each acquired intensity was directly plotted with prepared glucose concentrations, generating a calibration curve. This curve fitting was used to estimate sample glucose concentrations. However, colorimetric analysis is highly affected by ambient light conditions and camera optics. To address this issue, advanced algorithms such as machine learning based deep neuronal networks were proposed in the quantitative glucose evaluation process with automated decision-making and self-learning from the data.

### 4.1. Color space

RGB, CMYK, HSB/HSI, CIE XYZ, CIELAB, and YUV models are commonly used color spaces. The advantages and disadvantages of the color spaces are compared in Table 3. Primary RGB (red, green, and blue) or CMYK (cyan, magenta, yellow, and black) are the most used color spaces. In the RGB color space, each color is assigned to orthogonal coordinate axes in 3D space. The RGB color space ranges from 0 to 255 (8-bit format) or 0 to 1 (fractional format). The RGB color space is commonly used in industry, such as the Bayer filter used in CMOS sensors and many digital products (e.g. smartphones, webcams, flatbed scanners, digital cameras). Utilization of nanozyme in colorimetric-based glucose biosensors can typically use the RGB color space due to its simplicity. In 2022, Firdaus et al. developed smartphone application called glucose analyzer and built the Android Studio platform (DIC-Smartphone), which can achieve a detection limit of 0.043 μM [61]. Although these algorithms can precisely extract RGB color values for calculation of glucose concentration, the RGB color space sometime omits a much smaller number of colors, indicating the limitations of the perceptual space.

On the other hand, the CMYK color space, mainly used for color printing, can be applied to paper-based glucose detection. Each color creates a range of colors from 0 to 100%. All colors are created from combination of different CMYK amounts. In 2018, Wilson et al. introduced a paper-based microfluidic device for glucose analysis employing
artificial neural networks. They used 4-channel CMYK color data to demonstrate the effectiveness of ANN fitting and classification algorithms [62]. Typically, the RGB colors from the images recorded by the camera were converted to the CMYK color space using Photoshop (Adobe, Inc., USA). However, since RGB can produce much more vivid colors than CMYK, a lot of data can be lost during this conversion.

Table 3. Comparison of color spaces for analysis of colorimetric detection

| Color space | Color mixing | Primary parameters | Advantages | Disadvantages |
|-------------|--------------|--------------------|------------|---------------|
| RGB         | Additive     | Red, Green, Blue   | Convenient for image acquisition and display | Non-uniform illumination, colors is not linear |
| HSV/HIS     | Additive     | Hue, Saturation, Value, Hue | Based on human color perception; robust before non-uniform illumination, the chromaticity is decoupled from the intensity | Non-removable singularities |
| CIE         | Additive     | L*, a*, b*, L'*, u**, v* | Efficient in measuring small color differences, chromaticity is decoupled from the intensity | Singularity problems, non-linear transformation |
| CMYK        | Subtractive  | Cyan, Magenta, Yellow, and Black | Commonly used for production printer color | Since it is a subtractive model, the components are pigments or inks |
| YUV, YIQ    | Additive     | Y (luminance), U (blue chroma), V (red chroma) | Efficient coding for TV signal display | The color range is restricted, difficult to recreate image display |

The International Commission on Illumination (ICI) established CIE XYZ in 1931, the first system for scientifically defining light or additive colors. It is widely accepted as an international standard method for defining color. In CIE XYZ, chromaticity is defined by X and Z, and luminance is defined by Y. The calculated CIE XYZ coordinates are not the same as the original RGB values. The CIE XYZ color space is not as easily represented as the RGB color cube, but it is very similar to the RGB color space with noticeable color distortion. Spectrophotometers and digital color analytical instruments with a CCD or sCMOS sensor typically use the CIE XYZ color space because they can provide reflected or transmitted light from samples. In 2021, Samira et al. introduced a smartphone-based colorimetric sensing system for the measurement of glucose concentration in urine samples. They mapped image color acquired in the CIE chromaticity space, compared them with the reference color, and increased sensitivity with commercial reference charts [63]. However, it is not easy to compare two colors due to the non-uniform color distribution.

HSB, also known as HSV, represents three components: Hue, Saturation, and Brightness. The HSB color space is a kind of non-linear transformation of RGB defined as the simple addition or subtraction of RGB color. Hue is the color type and can be defined as the length of the illumination spectrum ranging from 0 to 360° (for example, the value of 0° is red and 45° is a shade of orange). Saturation is a color range of high intensity values from 0 to 100%. Here 0% means no color and 100% is the most intense color. Brightness is the visual perception derived by luminance ranging from 0 to 100%, where 0% is colorless (black) and 100% is intense color (white saturated color). The HSL (hue, saturation, lightness) space is similar to that of HSB or HSV. However, the main difference of HSL is that it is symmetric to light and dark. Therefore, HSL usually provides a more accurate color approximation than HSB or HSV. This color space can represent a single parameter (H), avoiding redundant color coordinate information when use in colorimetric-based biosensors. In 2020, Simon et al. developed colorimetric sensor for glucose monitoring using smartphone camera. They used HSL and RGB color space to directly compare the estimated performance and show better results in the HSL models [64]. However, this is not the same as the CIE XYZ color space. Thus, it is also difficult to find significant differences between the two-color spaces [108].

The L*, a*, and b* (CIELAB) spaces are international standard for color measurements adopted by the CIE in 1976. CIELAB consists of a luminance or luminance component (L*) between 0 to 100. The parameter a* is the color change from red to green. The parameter b* is the color change from yellow to blue. Unlike CIE XYZ and HSL, the CIELAB space is perceptually uniform due to slight change in the chromaticity diagram that produce changes perceived by the human eye. Thus, the CIELAB space can extract color differences between two colors, which is relevant for colorimetric-based biosensors. In addition, another advantage of the CIELAB space is that it uses Euclidean distance to determine the amount of color difference between two colors, providing information about color and time function changes for different glucose concentrations based on colorimetric detection via continuous and monotonic color profiles. In 2021, Son et al. introduced a colorimetric biosensor using deep neural network. To address the existing perceptual non-uniformity, they used CIELAB color space and found highly perceptible spectra with CIELAB coordinate values [65]. However, the major disadvantage is the...
requirement of further processing and the number of steps needed to obtain the results.

As the last color space, the YUV model also describes color. The parameter Y is the brightness (luma component) in the range of 0 ~ 100%, the parameter U is the blue luminance of the chrominance component, and the parameter V is the red luminance of the chrominance component. In 2022, Yang et al. developed a biosensor for colorimetric determination of uric acid. For the colorimetric detection, they used algorithm in the YUV color space [66], because the Y value showed good linear relationship in the range of UA concentration. Moreover, the YUV model is suitable for detecting moving objects because YUV simulates human perception of color more closely than does the primary RGB color space model.

4.2. Image processing software and analysis formats

Digital images can be obtained from various devices (e.g., smartphones, webcams, flatbed scanners, digital cameras) with different image qualities. Therefore, to quantitatively monitor the colorimetric variation, the same devices collect images to minimize instrumental errors. Image processing and analysis methods are essential to adopt colorimetric-based biosensors to reduce instrumental error and increase repeatability across devices. Table 4 summarizes colorimetric glucose detection in body fluids. Many acquisition devices are based on the color change used for glucose monitoring. The RGB, CMYK, HSV/HSL, CIE XYZ, CIELAB, and YUV color spaces are usually processed using Matlab, Adobe Photoshop, Image J, Image color Picker processing, and Photometrix software. Matlab, Image J, and Adobe Photoshop are widely used in processing images obtained by digital devices [67]. Several analytical parameters can be calculated to obtain colorimetric differences. In addition, the Lambert-Beer Law (-log (I/I0)) or RGB normalization is used to determine a quantitative correlation of analyte concentration [19], where I0 refers to the background signal intensity (Black), and I refers to the sample signal intensity monitored from chemical or enzymatic reactions [68]. However, CIELAB, CIE XYZ, or HSV are unrelated to color intensity. Thus, the use of images analyzed by different processing programs has been used as a strategy to determine a variety of analytes present in different matrices.

Table 4. Summary of color spaces, image acquisition, and processing for colorimetric glucose analysis using various body fluids

| Color space | Sample | Acquisition device | Acquisition format | Image Processing Software | Ref |
|-------------|--------|--------------------|--------------------|--------------------------|-----|
| HSV         | Serum, urine | Canon Power shot 5S IS digital camera, iPhone 4.0 | JPEG | Image J, Objective C | [162] |
| SRGB        | Glucose solutions | Smartphone | JPEG | Image J, Gray color value filter paper | [89] |
| SRGB        | Urine | Smartphone | JPEG | Android apps | [61] |
| SRGB        | Glucose solutions, human serum | Smartphone | JPEG | Color picker, application n-pads | [163] |
| SRGB        | Glucose solutions, human sweat | iPod Touch | JPEG | Image J | [164] |
| CIELAB      | Artificial sweat, human sweat | Smartphone | JPEG | Image J | [124] |
| CMYK        | Glucose solution | Epson Perfection V600 scanner | JPEG | MATLAB image processing, toolbox, adobe photoshop, Photoshop CS2 | [62] |
| SRGB        | Artificial sweat, human sweat | Smartphone | JPEG | Smartphone based software | [89] |
| SRGB        | Artificial sweat, human sweat | Smartphone | JPEG | Color grab application | [165] |
| CIE-RGB-to-HSV | Human urine | Galaxy A20e | JPEG | C++, Java, API 28 in Android Studio 4.0 | [63] |
| SRGB        | Glucose solutions, human serum, tears | iPhone 6 | JPEG | Image J, Gray value filter paper | [166] |
| RGB, HSL    | Glucose solution | Smartphone | JPEG | Android app | [64] |
| SRGB        | Glucose solutions, artificial urine | Smartphone | JPEG | Urine analysis and android application | [167] |
| SRGB        | Glucose solutions, artificial saliva | iPhone 7 | JPEG | MATLAB | [168] |
| SRGB        | Blood glucose | iPhone 5s | JPEG | Color assist application | [169] |
| SRGB, RGB   | Whole blood Glucose solutions | Sony DSC-HD330, digital camera, Galaxy S5, Tab A, Moto G4 | JPEG | Image J, Avidemux 2.6, Python, Open CV, Android studio | [170] |
| SRGB        | Glucose solutions blood glucose | Xiaomi MI 2SC Scanner (Epson perfection V700), LG Optimus Vu | JPEG | Image J | [171] |
| SRGB, RGB   | Glucose solutions, real samples | Smartphone | JPEG | Image J | [172] |
| HSV         | Glucose solutions, human serum | LG Optimus L5 II | JPEG | Image J | [173] |
| SRGB        | Glucose solutions, human serum | LG Optimus L5 II | JPEG | Image J | [174] |
The image save format also affects the image quality of the color value in colorimetric-based biosensors. The detailed image format characteristics are described in Table 5. JPEG, also called JPG, is widely used to measure colorimetric variation due to their smaller file size, leading to fast image processing. However, the algorithm compresses images when creating JPEG, resulting in quality loss. Therefore, JPEG can lose some information that is not recoverable. Although GIF images undergo a different type of image compression that reduces the file size, they only use 256 colors, leading to poor image quality. PNG and BMP images use different image compression strategies without any quality loss and can be extended up to 24-bit color. Therefore, the images can be saved and analyzed without degradation. In addition, raw TIFF and DNG files are much larger, which means they require more storage capacity and result in a longer transmission time for image processing. However, TIFF and DNG images offer exceptionally detailed and high-quality images. Thus, these formats allow the ability to identify slight differences of color in colorimetric-based biosensors. Recently, smartphones have emerged as attractive capture devices because other devices cannot independently handle the image information and must connect to a computer for colorimetric detection and measurement. Moreover, various smartphone applications exist that can be used to capture digital images, controlling the macro, focal length, brightness, and exposure time. Recent smartphones produce raw image files of DNG, offering numerous advantages for colorimetric-based biosensors.

In general, colorimetric-based biosensors enable quantitative evaluation of glucose concentration with simple image processing without much consideration of errors resulting from illumination from external light sources. However, a proper lighting position, angle, and power control are essential to increase precision and accuracy. Moreover, the focal length...
and exposure time difference are essential factors because they can affect the sensor’s color recognition (CCD or CMOS) [69]. Thus, measuring accurate color values is not trivial and requires specific control. Methods to reduce experimental error in other research fields can be used, such as normalization, training-based color reconstruction, color checker-based digital image reconstruction, auto-tracking, and real-time monitoring algorithms.

4.3. Colorimetric-based glucose detection with nanozyme

Nanomaterials of natural enzymes have attracted enormous attention due to their unique characteristics compared to their molecular and bulk counterparts [70]. However, with the exception of some catalytic RNA molecules, all natural enzymes have some intrinsic disadvantages. Some of the fundamental limitations of natural enzymes include their low stability, storage difficulty, high cost, tedious purification processes, limited application conditions, and specific operating conditions (i.e., narrow substrate, temperature, and pH ranges). For example, degradation upon exposure to various environmental conditions is a risk factor, and natural enzymes are particularly susceptible to digestion by proteases. They also require time-consuming preparation and purification processes, relatively high costs, and specific storage conditions. Therefore, nanomaterial-based artificial mimetic enzymes are receiving considerable attention. Recently, nanomaterials with ‘enzyme-like’ activity that mimic traditional biological catalysts such as catalase, oxidase, and peroxidase have attracted interest for potential applications as artificial enzymes. Several engineered nanoparticles (NPs), called nanozymes, have been used as active substances in bioassay, biosensor, and biomedical fields [71, 72]. These NPs include nanomaterials such as simple metal and metal oxide nanoparticles, metal-organic frameworks (MOF), metal nanoclusters, nanotubes, nanowires, carbon dots as well as quantum dots. These versatile nanomaterials can exhibit enzyme-like catalytic capabilities while overcoming many of the stability limitations and effective range associated with natural enzymes. In addition, the applications of hybrid, synthesis, and stimulus-responsive advanced nanozymes could revolutionize current practices in life sciences and biosensor applications. According to the activity they exhibit, nanozymes are classified into two large families: the oxidoreductase family and the hydrolase family. Nanozymes that are involved in redox catalysis and function similarly to oxidase, peroxidase, catalase, superoxide dismutase, or nitrate reductase are classified in the oxidoreductase family.

As shown in Figure 4, by modifying the surface of iron oxide nanozyme or glucose oxidase or configuring it in a hybrid form, it is possible to construct a nanozyme capable of performing the function of an oxidase. In this assembly, oxidase activity is crucial as it provides a peroxidase-like nanozyme with hydrogen peroxide to induce a color change or emit light in colorimetric or fluorescent biosensors. These synergistic characteristics have led to ultra-sensitive platforms and high performance, including colorimetric, fluorometric, chemiluminescent, surface-enhanced Raman scattering, and electrochemical biosensors.

The most widely studied nanozymes in these biosensing systems are summarized in Table 6, including metal nanoparticles (NPs), metal oxide NPs, and carbon-based nanomaterials. Various nanomaterials, such as Fe3O4 NPs, Co3O4 NPs, carbon nanotubes, and graphene oxide, have peroxidase mimic activity [73, 74]. In addition, CNPs have been demonstrated to have peroxidase-like activity, which can be applied to design corresponding colorimetric sensing systems [75]. The peroxidase-like activity of iron oxide nanocomposites has been widely used for glucose detection. As peroxidase-mimicking nanozymes can oxidize chromogenic substrates (e.g. TMB, ABTS, and OPD) and produce color in the presence of H2O2, they can directly detect H2O2 or other H2O2-generating substrates (e.g. glucose). In all cases, these materials were combined with GOx, and the synergistic effect of these two enzymes is a key factor in achieving high sensitivity and superior analytical performance in biomolecular detection. The superior activity of these nanozymes facilitated colorimetric assays of H2O2, glucose, and sarcosine. In addition, it is possible to increase the effective catalytic surface area by introducing pores into iron oxide nanoparticles and to increase glucose detection sensitivity by exposing metal ions to the surface.

Recently, supramolecular peptide nanomaterials are attracting attention as candidates for constructing organic nanozymes because peptides and enzymes are composed of amino acids as basic units [76]. These organic nanozymes can be rationally developed through self-assembly of peptides containing key amino acid sequences that participate in the formation of catalytically active sites. Amphiphilic amino acids can serve as building blocks for the supramolecular construction of nanoassemblies with the help of cofactors such as metal ions, and offer the possibility to fabricate nanozymes with minimal biological building blocks [77]. Several organic nanozymes studied so far are inspired by the supramolecular structure and redox principle of horseradish peroxidase (HRP), a typical natural metalloenzyme.
that catalyzes oxidative substrates by H$_2$O$_2$. In this regard, histidine in native HRP plays an important role in participating in oxidation reactions and providing binding sites for coordination interactions with iron in heme. Therefore, inspired by the supramolecular structure of HRP, Geng et al. reported an organic nanozyme by co-assembly of an amphiphilic amino acid (Fmoc-histidine, FH) and a heme derivative (hemin) [78]. The FH/hemin assembly showed flexible nanostructures and morphologies by tuning the molar ratio between FH hemins and optimizing catalytic activity to establish a sensing platform for rapid and sensitive glucose detection.

Figure 4. Nanozyme-based colorimetric biosensor with color classification. (A) Nanozyme based colorimetric detection with naked eye. (B) An illustration of nanozymes through assembly with peroxidase mimics.
Metal-organic frameworks (MOFs) are emerging as important candidates in the field of glucose sensing. MOFs are a class of materials composed of organic ligands and metal nodes. MOF nanozymes exhibit additional properties due to their diverse structures and functions compared to nanozymes based on noble metals, carbon materials, or transition metal compounds. Strong coordination interactions between metal ions and organic ligands allow the formation of unique framework structures with multimodal properties; (1) the porous structure of the MOF provides abundant surfaces and channels for rapid mass transfer; (2) the specific pore size of the MOF is conducive to the loading, adsorption and separation of the target; (3) the presence of metal nodes in MOFs contributes to possible active sites for catalysis; (4) the organic ligands of MOFs provide attractive electrical, optical and thermal properties and abundant functional groups for chemical modification [79]. Mechanically, the enzyme-like catalytic ability of MOFs can be attributed to two aspects: First, MOFs containing metal nodes such as Fe, Ce, Cu, Co or Ni can provide enzyme-mimicking catalytic activity due to the presence of these metal redox pairs. On the other side, the organic ligands of MOFs act as electron mediators, accepting electrons from a substrate and then donating electrons to other substrates, facilitating reactions similar to natural enzymes. Recently, several MOFs have been introduced as promising nanozymes with peroxidase-mimicking activity for glucose detection. In the study of Lin et al., terephthalic acid (TA) was introduced as promising nanozymes with sensitivities of 1792 and 1002 µAm/M/cm², ranges: 1 µM to 0.33 mM and 0.33 to 1.38 mM, with LOD [µM] 0.043 and 0.6, respectively. In the context of colorimetric glucose sensing, glucose oxidase@Cu-hemin metal-organic
frameworks (GOD@Cu-hemin MOFs) with ball-flower structures as bi-enzyme catalysts for glucose detection have been reported by Lin et al. [82]. The absorption intensity of oXTMB increases linearly with increasing glucose concentration from 0.01 to 1.0 mM, with a detection limit of 2.8 μM, which is claimed to be reasonably designed for colorimetric glucose sensors. Another example of MOF for glucose detection was designed to fabricate a nanoassembly by binding an amphiphilic amino acid, a histidine derivative, to a heme derivative containing an iron ion at the center [83]. The iron ion of the heme derivative and the side chain of the histidine derivative interact non-covalently and exhibit peroxidase mimicking properties that can confer glucose sensing ability.

Although electrochemical-based biosensors utilizing nanozymes demonstrate highly selective and sensitive performance, they require sophisticated fabrication, storage capacity, electrodes, and chips for wireless communication. These drawbacks have led researchers to design glucose biosensors that do not require electrodes. It would be very useful if colorimetric biosensors could achieve the same performance as electrochemical biosensors. Therefore, nanozymes in colorimetric-based biosensors are important to achieve cost-effectiveness, high sensitivity, high selectivity, and stable biosensors for diagnosis and management of diabetic patients. Colorimetric glucose detection using nanozymes has the advantage of providing a rapid response (color change) to obtain visual observation (color camera and naked eye) [84]. After the first results are obtained, the concentration and severity of the disease can be quantified using a color camera (CCD or CMOS) or other quantitative measurements to determine the management strategies and treatment options. Additionally, the digital cameras, scanners, and smartphones are now widely used to measure color changes and have emerged as suitable alternatives for colorimetric analysis. Among them, current smartphones are prominent as they are equivalent to a microcomputer with high-capacity internal memories and are equipped with high-resolution cameras and wirelessly communicate with other devices. Significant advances in smartphones make them useful for colorimetric assays, including fluorometric or spectroscopy applications. It might be overcome using smartphone-based glucose detection platforms for widespread use and better sensitivity for self-diagnosis and management of diabetic patients. Also, algorithm software, including machine learning-based detection and polynomial regression, can improve sensitive glucose detection. Therefore, colorimetric-based glucose detection using nanozymes is suitable for self-monitoring of glucose because of its rapid and cost-effectiveness glucose detection.

5. The current colorimetric analytical devices with nanozymes

5.1. Paper-based colorimetric glucose biosensors

A paper-based colorimetric detection with nanozymes consists of the conjugation of sample analyte, detection, and signal amplification. Paper can serve as the base material for biosensing platform with a significantly lower manufacturing cost. This assay platform is promising for glucose monitoring. To this end, nanozymes enable low-cost glucose detection and have been used as high-sensitivity probes for detection and signal amplification [85]. One of the important advantages of using nanozymes is that they can be easily synthesized without expensive chemical and sophisticated instrumentation, reducing the overall manufacturing cost. This allows the application of numerous metals, metal oxides, and MOF nanozymes for inexpensive colorimetric-based biosensors. Ornatska et al. introduced the fabrication of a cerium oxide (CeO2)-based bioactive sensing paper strip to detect H2O2 and glucose concentrations (Figure 5A). With a reproducibility of 4.3%, this paper-based detection can be used for a minimum of 10 cycles without loss of activity, reducing costs in each experimental cycle [86]. The basic concept of this study was to use simple electrostatic adsorption method using functionalization of CeO2 nanoparticles. Glucose oxidation produces higher concentration of H2O2, and the physicochemical properties of CeO2 change with oxidation state, resulting in colorimetric detection of glucose and H2O2. The acquired images were analyzed using Adobe Photoshop software, and the blue color intensity are mainly monitored because blue is the complementary color of yellow/orange. Reusability and the use of cost-effective materials are the main advantages of this study. Another advantage of nanozymes is their high thermal stability and mild storage conditions, which can reduce manufacturing costs. Although there are significant advantages in terms of cost-effectiveness from the materials, the acquired image system is bulky and inconvenient for the end-user.

In 2018, Tran et al. developed a nanocomposite using peroxidase mimicry to detect glucose in human urine using FEOOH and N-doped carbon nanosheets. They demonstrated Fe-CN nanocomposite stability for up to 90 days. Another excellent example of low-cost biosensor fabrication utilizing highly stable
nanozymes was established by Kim et al. (Figure 5B) [87]. Their work utilized plasmonic paper-based gold nanoparticle formation (AuNPs) and detected color change. An image of RGB values acquired from a scanner (1200 dpi) was converted into CIE XYZ in 1931 space. Glucose concentrations were estimated using exponential smoothing curve fitting. They showed a high linear correlation ($R^2 = 0.97$) and similar sensitivity within low glucose concentrations. These studies demonstrate that nanozymes reduce manufacturing cost and provide much higher detection sensitivity compared to natural enzymes. However, despite the cost-effectiveness and facile synthesis of nanozymes, color change measurement requires expensive and bulky UV or visible spectrophotometer equipment, which can be overcome using smartphone-based glucose detection platforms.

Currently, smartphones have become an essential part of our lives and are being used for scientific purposes. Most smartphones have built-in sensors such as Bluetooth, HD (high definition) cameras, USB (universal serial bus) ports, thermometers, microphones, and gyroscopes. These features make smartphones an attractive platform for analytical devices in environmental monitoring and disease surveillance [88]. Li et al. first reported Antimony-doped tin oxide nanoparticles (ATO NPs) loaded on a filter paper mimicking peroxidase-like activity, and combined them with a smartphone to an analyzer that detects $\text{H}_2\text{O}_2$ and glucose [89]. This approach was used to determine glucose in aqueous samples. In addition, for the development of rapid, disposable, cost-effective manufacturing and inexpensive devices, Pinheiro et al. developed a colorimetric paper-based assay for determination of glucose concentration determination [90]. They synthesized gold nanoparticles (AuNPs) by reducing the gold salt precursor to directly measure glucose using a smartphone camera in Figure 5C. Smartphone cameras utilizing nanozymes for colorimetric glucose detection show harness portability, high-speed processing, and high sensitivity. However, the development of digital systems that can interface with mobile sensors in an efficient manner remains a challenge because of the low sensitivity and optical noise of ambient light. In 2021, Balbach et al. reported a smartphone application that estimates colorimetric signals for glucose detection. As shown in Figure 5D, they put in a reference color chart and a CIE-RGB to HSV color space conversion to remove background noise provided by ambient lighting. These efforts have been devoted to the development of smartphone applications for colorimetric detection. Such a system would provide benefits to diabetics who need to constantly monitor their blood glucose levels on a daily basis.

### 5.2. Microfluidic paper-based device for glucose biosensors

The performance of microfluidic paper-based devices (μPADs) has been extensively investigated for monitoring glucose concentrations [91]. μPAD is attractive for the following reasons: first, μPAD is ubiquitous and consists of very inexpensive materials. Second, μPAD is compatible with other chemical, biochemical, and medical applications. Third, μPAD uses capillary forces to transport liquids without external forces. Various two-dimensional (2D) and 3D microfluidic channels have been made on paper, which transport body fluids in pre-designed μPAD pathways, allowing quantitative detection of glucose concentrations [92]. Coltro et al. reported a paper-based colorimetric biosensor for the measurement of surface acetic acid-to-chitosan-modified tear glucose [114]. They measured glucose concentrations in human tears using TMB as a chromogenic reagent (Figure 6A). Images are recorded with office scanner at 600-dpi resolution and converted to RGB color space. Pinheiro et al. also presented the application of chemically produced and tailored AuNPs in μPADs for glucose sensing (Figure 6B). They also used commercial scanner to minimize the effect of ambient light conditions, and a AuNP-based plasmon for colorimetric transformation of paper substrate sand showed an LOD of 1.25 mM at a time of 2 min [93]. Although μPAD is a cost-effective material and commercial scanners can also improve glucose detection sensitivity, the paper may fluoresce under prolonged illumination and interfere with true color detection. Pomili et al. introduced fully integrated all-in-one paper-based device to detect salivary glucose concentrations. They used colloidal 60-nm multibranched AuNP (MGNPs) and read the colorimetric response within 10 min with the naked eye or using a smartphone. Ortiz-Gómez et al. also reported a paper-based microfluidic colorimetric device for measuring glucose in urine and serum based on a Fe-MIL-101 metal-organic framework (MOF) [123]. The assay was based on Fe-MIL-101 MOF to mimic horseradish peroxidase (HRP) immobilized on commercial cellulose paper. Their μPAD allowed for accurate measurement of glucose using a small sample volume (10 μL) with low LOD (2.5-10 μM/L). Images acquired with a smartphone can be processed on the same device as the developed iOS application without a separate attachment.
Figure 5. A paper-based colorimetric glucose biosensor. (A) Paper strip to detect H₂O₂ and glucose utilizing a cerium oxide (CeO₂)-based bioactive biosensor. Adapted with permission from [86], Copyright 2011, American Chemical Society. (B) Chromatic characteristic of plasmonic paper with gold nanoparticles (AuNPs) formation in the CIE XYZ in 1931 color space. Adapted with permission from [87], Copyright 2020, MDPI. (C) Schematic illustration of the ATO-based paper biosensor as peroxidase mimics for colorimetric detection of glucose using smartphone read-out. Adapted with permission from [88], Copyright 2019, Springer. (D) User interface with smartphone readout of glucose urine tests using smartphone app. Adapted with permission from [63], Copyright 2021, Royal Society of Chemistry.

However, accurate measurement requires the system to set certain conditions, including shutter speed, aperture value, focal length, automatic white balance, and the same ambient lighting conditions. Also, the captured image is processed after saving the JPEG, where the raw color information can be lost.
Therefore, in 2021, Mercan et al. reported a portable platform based on a color change in μPAD by implementing machine learning classifier with Linear Discriminant Analysis (LDA), Gradient Boosting Classifier (GBC), and Random Forest (RF) [94]. They used different smartphones to train images captured in seven different lighting conditions. Among the tested and calibrated image sets, TMB (98.24%) with an LOD of 0.8 μM obtained the highest accuracy classification. The platform can automatically find ROI and minimize human error, contributing to user-friendly and accurate measurement of POC systems.

For improved sensitivity, Freitas et al. used mass spectrometry in combination with matrix-assisted laser desorption/ionization (MALDI) and desorption electrospray ionization (DESI) to monitor color gradient-based μPAD (Figure 6C). To understand assay performance such as reproducibility and sensitivity, they used a glucose enzyme assay using potassium iodide (KI) as a chromogen for generating color formation [93]. Although MALDI and DESI imaging techniques have been successfully explored in enzymatic assays for glucose colorimetric detection, the above studies have reusability issues, information loss due to RGB color space conversion, and bulky imaging setups. From these points of view, inexpensive, simple and reliable self-monitoring image acquisition systems and algorithms are highly demanded for POC devices. For the development of POC devices, Wang et al. developed a smartphone-based spectrometer for colorimetric-based glucose biosensors containing aptamer-functionalized AuNP [95]. The smartphone-based spectrometer is integrated with the grid substrate, and it uses the built-in camera and LED flash in the smartphone. They experimentally demonstrated the detection of glucose and human cardiac Troponin I (cTnl) with peptide-functionalized AuNP. A smartphone-based spectrometer with spectral numerical correction enables fast and sensitive real-time glucose monitoring with LOD from 0.2 to 0.47 mM. However, the integrated grating substrate is expensive and still requires additional devices and complex processing.

Meanwhile, to improve sensitivity, Darabdhara et al. fabricated paper strips to exploit the peroxidase and oxidase mimic activity [96]. They prepared bimetallic Cu-Pd NPs to reduce graphitic carbon nitride (g-C3N4), graphene oxide (rGO) and MoS2 sheets with a size of less than 10 nm. They optimized the synthesis of Cu-Pd NPs with the desired shape, size, and oxidation state (Figure 6D). A designed biosensor strip of μPAD measured glucose in serum with a detection limit of 0.29 μM and a detection range from 0.2 to 50 μM [96]. Tian et al. designed a 2D layer of PtS2 integrated with dopamine-functionalyzed hyaluronic acid (HA-DA) hydrogel microspheres for sensing H2O2 using a low-cost ultrasonication-assisted liquid exfoliation method [97]. This biosensor has a greater color change than the PtS2 nanosheets by adding it directly to the glucose solution. Subsequently, a colorimetric biosensor based on PtS2 nanosheets and PtS2@HA-DA microspheres was developed to quantitatively determine glucose concentrations in buffer and human serum, respectively. The PtS2 nanosheet showed linearity in the dynamic range (0.5 to 150 μM) with a low LOD of 0.20 μM. Although a μPAD has been reported using this bifunctional oxidase-peroxide mimicking nanozyme and has provided a low-cost and simple platform for glucose detection, μPADs require reaction time to premix samples prior to the final reaction. From this point of view, inexpensive and metal-free bifunctional nanozymes using earth-abundant elements are highly desirable. Zhang et al. reported metal-free nanozymes of modified graphitic carbon nitride (g-C3N4: GCN) and demonstrated an enzymatic mimic role of this function [98]. They demonstrated dual-functional enzyme-mimicking behaviors that combines the roles of oxidase (GOx) and peroxidase (HRP) using metal-free nanozymes based on modified graphitic carbon nitride (g-C3N4: GCN). In addition, they demonstrated bifunctional cascade catalysis in microfluidics for continuous colorimetric detection of glucose with an LOD of 0.8 μM within 30 s (Figure 6E). However, although this study showed a low LOD in microfluidic device that is sensitive enough for clinical glucose concentration, clinical validation and in vivo test are further required for practical application.

5.3. Colorimetric-based wearable glucose biosensors

With digitization of glucose monitoring, wearable systems are a convenient way for diabetes patients. Colorimetric-based wearable biosensors connected to digital cameras and smartphones can allow continuous monitoring by the end-user, while sample collection can be performed painlessly, an essential advantage for improving patient compliance [99]. The development of wearable biosensors requires specific functions, such as soft, thin, and stretchable features [100]. Therefore, the wearable biosensor withstands the physical burden and is in close contact with the body surface, making it convenient to wear and avoid physical perturbation due to skin contact. For example, if the material is hard, stabilization may occur during operation, which can affect measurement errors.

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Different materials could alter the mechanical properties of the wearable glucose biosensors. Standard materials are textiles or fabrics, polymer composites, and papers that can be transformed into tattoos, patches, contact lenses, smartwatches, eyeglasses, and e-skin biosensors to act as sensors with integrated electronic devices [101].

Microneedles (MNs) are introduced in implantable wearable devices to achieve instrument-free glucose detection and continuous blood glucose control. Ensuring the stability and accuracy of implantable wearable biosensors is very important for commercialization. Yang et al. reported a glucose biosensor without a noninvasive instrument [102]. They designed a glucose-biosensing microneedle patch (GBMP) composed of GOx-conjugated MnO2/graphene oxide nanozymes (GOx-MnO2@GO) and swollen methacrylate gelatin (MeGel). GBMP is demonstrated by inserting into the skin to measure glucose concentration in ISF under hyperglycemic conditions to generate gluconic acid and H2O2 due to the enzymatic reaction of GOx and glucose in the body. Color changes are monitored with the naked eye or as RGB value with a smartphone camera. Color intensity is based on the quantitative analysis of glucose concentration and collects 5.1 ± 0.3 μL sweat from skin ISF within 3 min. Sun et al. also introduced an ultrasensitive optical transducer for wireless
glucose monitoring with smartphone (Figure 7A). They developed oxygen-sensitive polymer dots (Pdots) and implanted subcutaneously on the dorsal side of mice for in vivo glucose monitoring [103]. In order to clearly distinguish between normoglycemia and hyperglycemia, the image acquired with a smartphone was processed with an RGB model, and the contrast ratios of red and blue were compared. This design has the advantages of being easy to use, cost effective, high sensitivity, short measurement time, sustainability, and pain-free by detecting the color change of various samples. However, to increase accuracy by reducing measurement noise through advanced diagnostic algorithms such as machine learning and artificial intelligence, huge demand must be met.

In 2019, Choi et al. developed a sweat-based stretchable microfluidic device for the development of a patch-type wearable device [21]. It is the most advanced colorimetric-based biosensor platform for commercialization by optimizing microfluidic designs, chemistry and device layouts to enable precise evaluation. This platform is capable of measuring sweat, sweat rate, sweat temperature, pH, glucose concentration, chloride, and lactate in a physiologically relevant range (Figure 7B). To reduce errors in this work, they adopted a reference marker and measured glucose concentrations of ~0.1 μM. The integrated color reference markers can provide accurate colorimetric estimation of body fluids under various lighting conditions, and it is demonstrated at the different smartphone models in a remote setting. Koh et al. also reported that sweat is harvested and stored from human skin to measure glucose concentration, chloride, sweat rate, lactate, and pH levels [104]. As shown in Figure 7C, tortuous channels within the microfluidic automatically collect sweat and image combined with a smartphone camera for RGB information. This allows the glucose concentration to be quantitatively determined from the RGB values. One distinct advantage of a sweat colorimetric-based wearable biosensor is that the wearer can interpret the qualitative analysis of the analytes in real-time without the need for an external device. However, wider use of the device requires the introduction of uncalibrated sensors associated with colorimetric glucose sensing systems. In addition, in vivo and clinical validation for meaningful medical applications is needed to monitor the association of sweat glucose concentration with blood readings.

Tear-based continuous enzyme sensing of a contact lens type, a wearable device, centers around glucose sensing, and current designs of intraocular nanozyme sensing have been incorporated into polymer matrix-forming contact lenses [105]. In 2011, Yao et al. reported a contact lens sensing platform to measure glucose concentration by applying a GOD/titania sol-gel film immobilized glucose oxidase [106]. They used Nafion® to decrease potential interference from the tear film and demonstrated real-time monitoring with a loop antenna, a wireless communication chip embedded in a polymeric contact lens. This kind of biosensor has fast response time (20 s) and dynamic range (0.1 - 0.6 mM). The 2014 Google glucose-sensing contact lens project was based on this work. In 2017, Kim et al. incorporated silver nanowires and graphene hybrid to improve the stretchability, conductivity, transparency, and contact lens-based biosensor [107]. They designed a graphene/nanowire hybrid source-drain and graphene channel supported by biocompatible parylene substrate. This contact lens-based biosensor was in vivo demonstrated in rabbit and further ex vivo monitored glucose on a bovine eyeball. From this point of view, colorimetric detection using nanozyme is a promising method without requiring electrodes, a bulky system, energy storage capacity or detectors. However, the biosensors in this device are not generally biocompatible.

Moreddu et al. reported a paper-based microfluidic approach with contact lens [108]. The glucose detection biosensor was a lab-made poly-HEMA contact lens combined with paper to detect multiple components, such as pH, ascorbic acid, glucose, proteins, and nitrite ions, in 2 μL of artificial tear in less than 35 s. In addition, they applied a smartphone application for glucose detection, converted to the CIE XYZ in 1931 chromaticity diagram to compare color changes, and used nearest-neighbor color search to return density values corresponding to the corrected color in the plot. These techniques can expand the number of calibration points and improve specificity by combining them within machine learning algorithm. Their paper-based microfluidic contact lens provided high sensitivity (3.9 nM/L) and LOD (1.1 mM/L) with reduced error under various lighting conditions. However, although this biosensor was not associated with toxicity issues, it is not evaluated in animal models in vivo and in vitro to understand body responses.
Recently, Park et al. developed a biocompatible biosensor of a nanoparticle-embedded contact lens (NECL) composed of complexes of cerium oxide nanoparticles (CNPs), glucose oxidase (GOx), and a polyethylene glycol (PEG) [20]. After reacting with tear glucose and \( \text{H}_2\text{O}_2 \), NECLs are oxidized and changed into a yellowish color. A spectrometer was used for quantitative analysis of color change to measure glucose concentration using NECL. A detectable change in the reflection spectrum of NECL was found in relation glucose concentration (Figure 7D) [38]. However, current spectrometers are expensive and require pre- and post-processing steps. Thus, they developed a simple RGB camera and
smartphone-based optical monitoring system utilizing the NECL in animal models and clinical human tears [19]. To reduce the environmental errors and enable continuous monitoring of the NECL in animal models, they developed an image processing algorithm that automatically optimizes the measurement accuracy even when images are obscured by motion artifacts (Figure 7E). This algorithm would be of great benefit to patients by enabling near real-time visualization and measurement of tear glucose concentration. In fact, they conducted blind evaluation of human tears provided by normal person and diabetic patients, and as a result, using the developed system, they were able to accurately discriminate between diabetic patients and normal person with a probability of over 90%. Furthermore, they evaluated the cytotoxicity of NECLs in human umbilical vein endothelial cells (HUVECs) and human corneal epithelial cells (HCECs). The cytotoxicity of NECLs was not noticeable, indicating that no problems occurred during contact lens fabrication.

6. Future direction

Recent research on nanozymes with enzyme-mimicking activity has overwhelmingly increased, expanding the scope of application of colorimetric-based glucose detection. Nanozymes integrated with colorimetric-based glucose biosensors have several advantages over natural enzymes as key functional components for analyte detection, including higher efficiency, greater versatility, lower cost, and higher stability. However, despite these significant advances, some research gaps and hurdles remain at these boundaries, and some work remains to realize the great potential of nanozymes in the development of colorimetric-based biosensors. First, although many nanomaterials have been used to mimic various natural enzymes in terms of material, oxidoreductase mimetics are mainly peroxidase mimics, because of their low-cost, easy storage, rapidity, and high sensitivity. Given the diversity of natural enzymes, additional efforts are needed to develop novel strategies for designing nanozyme with novel catalytic properties and machine learning based algorithms for quantitatively measure of glucose concentration. This increases the flexibility of nanozyme applications in biosensor development and provides affordability for detecting a wider range of analytes. Second, the sensitivity of colorimetric-based biosensors depends mainly on the catalytic efficiency of nanozymes. However, more attention should be paid to rational design of high-performance nanozymes to achieve the desired sensitivity-based colorimetric biosensors. For example, natural enzymes work together as enzyme clusters to provide high catalytic efficiencies within a confined environment for cascade reactions. For better sensitivity, nanozyme assembly is a good option, such as aggregation of other nanozymes or nanozymes with native enzymes. In addition, researchers have introduced several new materials with outstanding performance, such as the metal-doped composite materials or perovskite materials [14, 72, 74]. Perovskite is a ceramic oxide with the molecular formula ABX₃. The A is usually rare earth metal such as lanthanide and the B is a transition metal. Both A and B can be replaced by other metal ions of similar radius to form various compounds. There are studies that perovskite nanocomposites have high oxidase-like catalytic activity by a simple and accurate colorimetric detection method [109]. Third, some studies have used other biosensing mechanisms instead of colorimetric signals for the growth of portable biosensors. However, the lack of portability and the existence of sophisticated and complex instruments limit the versatility of nanozymes using fluorescence or other sensing methods that rely on optical properties. Lastly, it has not been highly reported that studies on the toxicological mechanisms or potential toxicity of nanozymes identified so far. Since most of nanozymes are nanomaterials produced through metal-based bottom-up chemical synthesis, research on the toxicity of nanozymes is very important. For example, a previous study investigated the toxic effects of CeO₂, a type of metal oxide constituting nanozymes [110]. In that study, morphological-dependent cytotoxicity was confirmed by increased serum concentrations of lactate dehydrogenase (LDH) and tumor necrosis factor alpha (TNF-α) in rod-shaped CeO₂ compared to cubic/octahedral CeO₂. As shown in this example, a close toxicity analysis is required because there is a change in toxicity depending on the form, concentration, and degree of oxidation of the nanozyme even with the same component. The integration of nanozymes with wearable, implantable, and collectable biosensors requires careful control of the effective window to avoid potential toxicity, and systematic studies are required to evaluate the toxicity of nanozymes. Although previous reports have highlighted the therapeutic importance of several nanozymes in animal models, translating drugs into clinical applications remains a challenge [111]. In addition, a thorough mechanism and understanding of the experimental phenomena under the practical application of nanozymes limits their rapid development for practical applications. The reason for this is that the relatively low catalytic activity and potential toxicity issues of nanozymes compared to natural enzymes make it very difficult to meet the practical...
application requirements of nanozymes. Therefore, this challenge remains a major hurdle to overcome in nanozyme applications in biosensing and will undoubtedly become an active area for future research. More research may be done in the future, especially to address issues above the processing step, simplify complex physical fabrication, reduce potential toxicity, and combine nanozyme monitoring systems with other technologies that continue to improve.

In terms of colorimetric analysis, naked eye detection is the simplest detection method. However, visual perception varies from person to person and depends on ambient conditions such as lighting. Therefore, in order to accurately and quantitatively measure the colorimetric change, a simple scanner or a digital camera that can accurately measure the colorimetric change is required. The advantage of digital devices is that no skilled manpower is required, high-resolution images can be generated, and color change can be quantitatively measured. Many colorimetric-based studies using smartphones to monitor glucose concentration have been heavily introduced due to their convenience and portability. However, there are several issues that need to be addressed using smartphones. First, images acquired from smartphone cameras are easily distorted and compressed, resulting in low correlation with real-world signals. The result is low repeatability, low accuracy, and low sensitivity in colorimetric-based glucose detection. These limitations are caused by ambient or environmental conditions, including varying lighting conditions, shooting distance, angle of the acquired image, and motion artifacts such as breathing and subtle movements. Second, different smartphone models have different RGB responses, so that different colors can be obtained from images processed by different smartphones. To reduce interference and increase accuracy, color correction methods using polynomial or linear regression algorithms using a color checker have been developed as basic information for color intensity [112]. Accurate correction is performed by processing the acquired images through image normalization and white balance, brightness correction, saturation correction, and color transformation steps. Finally, accurate evaluation algorithms have been developed to improve the detection performance of in vivo models. Nowadays the application of machine learning in nanozyme will increase rapidly in the next few years, especially in material sciences and related fields as shown in Table 7. Because machine learning is great option, a subfield of artificial intelligence (AI) that uses statistics to improve accuracy techniques. It provides computer models with the ability to learn from a dataset, allowing models to perform specific tasks without explicit programming.

| Learning type | Purpose | Image format | Machine learning model | Software | Training data | Color space | Sample | Ref |
|---------------|---------|--------------|------------------------|----------|--------------|------------|--------|-----|
| Supervised learning | Classification | RAW | Least-Squares Support-Vector Machine (LS-SVM) | MATLAB | 385 images | RGB, HSV, LAB | Hydrogen peroxide | [208] |
| Supervised learning | Classification | JPEG | Linear Discriminant analysis (LDA), Gradient Boosting Classifier (GBC), Random forest RF | Python, MATLAB | 224 images | RGB, HSV, LAB | Artificial saliva | [94] |
| Supervised learning | Classification | JPEG | LDA, SVM, ANN | MATLAB, Python, Android studio | - | RGB, HSV, YUV, Lab | Alcoholic solution | [209] |
| Supervised learning | Classification | JPEG | Ensemble bagging classifier (EBC) | Matlab, Android studio | 616 images | RGB, HSV, YUV, LAB | Artificial saliva | [210] |
| Supervised learning | Classification | JPEG | Convolutional neural network (CNN) | MATLAB | 1600 images | RGB | Glucose solution | [211] |
| Supervised learning | Classification | Spectrum | Support vector machine-radial basis function (SVM-RBF) | - | - | - | Glucose solution | [212] |
| Supervised learning | Classification | JPEG | Multi-Layer Perceptron (MLP), Residual Network (ResNet), CNN | - | 490 images | RGB | C-reactive protein (CRP) prepared PH solution | [212] |
| Supervised learning | Classification | JPEG, RAW | LS-SVM | MATLAB | 450 images | RGB | Urine | [213] |
| Supervised learning | Classification | JPEG, Spectrum | Faster region-based (CNN) | - | 1500 images | RGB | Artificial urine | [102] |
| Supervised learning | Classification | RAW | Artificial neural networks (ANNs) | MATLAB | 160 and 54 data points | CMYK | Serum glucose | [62] |
| Supervised learning | Classification | NIR Spectrum | Deep neuronal network (DNN) | - | 1024 dataset | - | Glucose solution | [39] |
| Supervised learning | Classification | Spectrum | Multi-Channel -CNN | - | - | - | Glucose solution | [214] |
Artificial neural network (ANN) or convolutional neural network (CNN), and support vector machine (SVM) are very powerful ML models that can learn continuous functions using linear or non-linear systems with or without hidden layers. They show fast computation and easy implementation because they have fascinating features of learning [113]. They only require input-output variables and identification of the relationship between processing parameters. Although they can adapt to real-time operations and calculate the fast result, they have little application in biosensors for colorimetric-based glucose detection. Additionally, the optical hyperspectral image (HSI) can provide more detailed color information, because it covers narrow spectral bands in the visible, NIR, and IR range instead of assigning primary colors (Red, Green, and Blue). Therefore, more detailed information can be achieved from colorimetric detection than the primary colors [114]. However, spectrophotometers are bulky and expensive, making it challenging to apply glucose monitoring in various conditions. Recently low-cost hyperspectral imaging with smartphone techniques has been introduced [115]. This is statistical learning-based image processing algorithms to be included in machine learning. They can convert RGB into hyperspectral image by training between actual measurements of spectral reflectance obtained from a hyperspectral camera or handheld spectrometer and RGB image captured by smartphone. Obviously, the reconstructed hyperspectral images computed on the smartphone allow for accurate glucose detection and are very useful. However, since the nanozyme-based colorimetric biosensor has not yet been integrated as a standard, additional work is required to apply clinical purpose, and it should be possible to calibrate the smartphone image obtained through it. Therefore, we argue that developing nanozymes with different enzymatic activities and developing various algorithms that can accurately detect color values are excellent options for improving the accuracy of colorimetric-based glucose sensing.

7. Conclusion

The success of colorimetric-based biosensors utilizing nanozymes for glucose detection in diabetic patients requires high accuracy, user-friendly device portability, biocompatibility, and ease-of-use in image acquisition. Collectively, the challenges faced in design of colorimetric-based glucose biosensor applications are development of nanozymes. Rather than hindering future research, essential information collected from the current state-of-the-research is introduced along with detailed analyses to suggest future research directions.

Abbreviations

ISF: interstitial fluid; LOD: limit of detection; NIRS: near-infrared reflectance spectroscopy; OCT: optical coherence tomography; NIR: near-infrared; MVC: multivariate calibration; GBPs: glucose binding proteins; QDs: quantum dots; FRET: fluorescence resonant energy transfer; OP: optical polarimetry; PAS: photoacoustic spectroscopy; SNR: signal-to-noise ratio; RI: reverse iontophoresis; GOx: glucose oxidase; EIS: electrical impedance spectroscopy; CMOS: complementary metal-oxide-semiconductor; CCD: charge couple device; NPs: nanoparticles; AuNPs: gold nanoparticles; RGB: red, green, and blue; CMYK: cyan, magenta, yellow, and black; HSB: hue, saturation, and brightness; CeO2: cerium oxide; μPADs: microfluidic paper-based devices; cTnl: cardiac troponin I; MOF: metal-organic framework; HRP: horseradish peroxidase; TMB: tetramethylbenzidine; AuNPs: gold nanoparticles; CMOS: complementary metal-oxide-semiconductor; GBM: glucose-biosensing microneedle patch; GOx-MnO2@GO: GOx-conjugated MnO2/graphene oxide nanozymes; GOx-MnO2@GO: GOx-conjugated MnO2/graphene oxide nanozymes; MeGel: methacrylate gelatin; CNPs: cerium oxide nanoparticles; NECI: nanoparticle-embedded contact lens; PEG: polyethylene glycol; HUVECs: human umbilical vein endothelial cells; HCECs: human corneal epithelial cells; LS-SVM: Least-Squares Support-Vector Machine; LDA: Linear Discriminant analysis; GBC: Gradient Boosting Classifier; RF: Random forest; EBC: Ensemble bagging classifier; SVM-RBF: Support vector machine-radial basis function; CNN: Convolutional neural network; MLP: Multi-Layer Perceptron; ResNet: Residual Network; CuBDC: copper 1,4-benzenedicarboxylate, copper-doped carbon-based nanozyme; PBNS: Prussian blue nanoparticles; WSNF: nanohybrid fiber.

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Author contributions

H.J.J., E.C., and D.Y.L. collected the related papers and drafted the manuscript. H.J.J. and H.S.K. prepared the figures and tables. H.J.J., H.S.K., E.C., and D.Y.L.
revised the manuscript. E.C. and D.Y.L. conceived the review article and provided supervision. All authors read and approved the final manuscript.

Competing Interests

The authors have declared that no competing interest exists.

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