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Gold Nanoparticles in Biosensors: A Systematic Review of Synthesis Methods, Electrode Modification, and Bioconjugation for Enhanced Detection

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ABSTRACT

Biosensors are devices that can measure and quantify biomarkers specialized for infectious diseases. Nanoparticles are extensively utilized in biosensors due to their ability to achieve low detection limits. However, biosensor performance can be significantly impacted by challenges such as slow electron transfer kinetics and limited surface area for biomolecule immobilization. Gold nanoparticles (AuNPs) are a type of plasmonic nanoparticle with exceptional optical and physical properties, making them promising for biomedical and analytical applications. This review summarizes common AuNP synthesis methods, their integration into biosensors, and their impact on the biosensors' performance. A literature survey covering the period from 2020 to 2024 was conducted using the Scopus database to examine the synthesis and application of AuNPs in biosensors. A systematic review of 20 studies was performed following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). The review identified nine articles related to the conjugation of AuNPs with bioreceptors. Additionally, studies focusing on AuNPs synthesis methods and electrode modifications were identified, with two papers that addressed both synthesis and bioconjugation. The findings suggest a need for future research to explore alternatives, such as biological approaches to AuNP synthesis, and to further investigate modifications of various electrode surfaces with AuNPs.

Keywords: *Gold nanoparticles, Electrode Modification, Bioconjugates, Biosensors, AuNPs, PRISMA*

1. INTRODUCTION

Biosensing technology has advanced significantly since Clark and Lyons pioneered the use of glucose oxidase (GOD) for electrochemical glucose detection in 1962 (Wang et al., 2022). However, the performance of biosensors is often hindered by challenges such as slow electron transfer kinetics and a limited surface area for biomolecule immobilization (Siciliano et al., 2024). Advancements in nanotechnology have created new opportunities for incorporating nanoparticles into bio diagnostics (Hegde et al., 2022). Nanoparticles are extensively utilized in biosensors due to their ability to achieve low detection limits (Ramesh et al., 2023). Gold is particularly valued in sensing technologies for its unique properties, including rapid electron transfer for enhanced electrical conductivity, which allows for high sensitivity (Ilyani Zulhaimi et al., 2023). Among various metal and non-metal nanoparticles, gold nanoparticles (AuNPs) stand out for their excellent thermal and optical properties, as well as their strong binding stability with biomolecules (Gupta & Ghrera, 2021). The use of gold nanoparticles for probe or analyte conjugation has been shown to significantly boost the performance of detection systems, enabling more effective target validation (You et al., 2019).

Gold nanoparticles have been synthesized using various methods since the 1950s. The early approach reported by Turkevich utilized sodium citrate as a reducing agent (Dong et al., 2020; Turkevich et al., 1951). Since then, various other reducing agents such as gallic acid (Farzaneh et al., 2024), hydrogen peroxide (Vaklev et al., 2007; Q. Li et al., 2012; (Z. Liu et al., 2021), and hydrazine (A. E. F. Oliveira et al., 2023) have been employed. In 1994, Brust and Schiffrin introduced a two-phase method for gold nanoparticle synthesis (Mehravani et al., 2021). However, many of the reducing agents used, such as citric acid, sodium borohydride, and certain surfactants, have been found to be potentially toxic or hazardous (Mobed et al., 2022). To address these concerns, more recent "green" synthesis methods have emerged, using plant extracts, microorganisms, and enzymes as reducing and stabilizing agents (Mehravani et al., 2021).

AuNPs can be synthesized using two distinct strategies: "top-down" and "bottom-up" approaches. The top-down method starts with bulk material, which is broken down into nanoparticles through techniques like laser ablation or ion sputtering (Pišlová et al., 2020). In contrast, the bottom-up method builds nanoparticles from atomic or molecular precursors. Both approaches rely on various physical or chemical treatments to produce nanosized particles (Ielo et al., 2021). Specific top-down techniques include laser ablation, ion sputtering, and UV/IR irradiation, while bottom-up approaches involve the reduction Au^{3+} to Au^0. Based on these strategies, the synthesis of AuNPs can be further categorized into chemical, physical, biological and ultrasound-assisted methods (Dheyab et al., 2022).

2. REVIEW METHOD

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure rigor and transparency in the review process (Page et al., 2021).

2.1. Search Strategy

A comprehensive literature search was performed using the Scopus database, covering articles published from 2020 to 2024. The search terms used were "gold nanoparticles" AND "synthesis" OR "gold nanoparticles" AND "biosensors" OR "biosensing." This was designed to capture studies focusing on the synthesis and application of gold nanoparticles in biosensor technology (Elsevier, 2024).

The initial search yielded a total of 367 published papers. These records were screened by applying specific inclusion and exclusion criteria to ensure relevance and quality.

2.2. Inclusion and Exclusion Criteria

The following inclusion criteria were applied:

Language: Only articles written in English were included.
Publication Type: Full-length original research articles were considered. Conference papers, review articles, books, and book chapters were excluded.
Access: Only open-access articles were included to ensure that the information is publicly available.
Research Focus: The study must focus on the synthesis of gold nanoparticles, modification of electrodes, or bioconjugation with biosensors. Articles that lacked data on AuNPs size, synthesis method, or limit of detection (LOD) were excluded.

2.3. Screening Process

The screening process was conducted in two stages:

First stage: The initial screening process involved reviewing 367 identified papers. This first stage removed books, book chapters, review articles, conference proceedings, and published papers before 2020. At this point, 249 papers were excluded due to irrelevance or failure to meet the established criteria.

Second stage: The remaining 118 articles underwent a full-text review. During this process, 49 articles were excluded, leaving access to 69 full-text articles. Of these, 49 were further excluded for lacking detailed information on synthesis methods, electrode modification, or biosensor performance data such as LOD. Finally, 20 articles met all the criteria and were included in the final review. Figure 1 gives a detailed description of the review methodology.

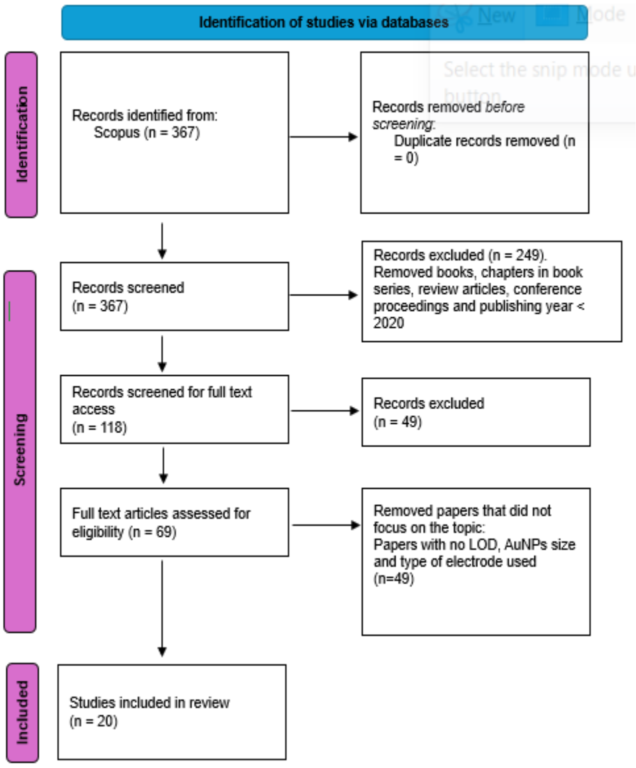

Figure 1. PRISMA flowchart detailing the systematic review process

2.4. Data Extraction, Synthesis and Analysis

The data extraction process involved reviewing each article for specific, relevant information, focusing on the synthesis of gold nanoparticles (AuNPs), electrode modification, and bioconjugation in biosensors. Key parameters extracted included synthesis method, type of electrode used, electrode modification technique, bioreceptors conjugated, and limit of detection (LOD) in the context of biosensor performance. Each of the 20 selected studies was meticulously reviewed to extract, synthesize and analyze data based on the following information:

Synthesis Methods: This included the approach used for nanoparticle synthesis (e.g., chemical, biological, physical) and specific techniques such as the Turkevich method or seed-mediated growth. The review identified that chemical synthesis methods, especially the Turkevich method, were the most commonly used, primarily due to their simplicity (Turkevich et al., 1951). However, concerns over the environmental impact and scalability of these methods were noted. Biological methods, while eco-friendly, face challenges in reproducibility and size control of the nanoparticles (Khan et al., 2022).

Electrode Modification: Information was extracted regarding how AuNPs were used to modify electrode surfaces, the impact on electron transfer, and any enhancements in biosensor sensitivity. Studies on electrode modification demonstrated that AuNPs significantly improve conductivity and electron transfer rates in biosensors (Janicka et al., 2024). However, the review also highlighted gaps in understanding the long-term stability and reproducibility of AuNP-modified electrodes in practical applications. Composite electrodes, such as those involving copper nanowires and AuNPs, showed promising results but introduced additional complexity in fabrication and consistency (L. Liu et al., 2024).

Bioconjugation: Data was collected on the type of bioreceptors (e.g., antibodies, aptamers) used in conjunction with AuNPs, and how these conjugates influenced biosensor performance, especially in terms of LOD and specificity. The review found that bioconjugation of AuNPs with antibodies, aptamers, and other bioreceptors has led to significant improvements in biosensor sensitivity (Barshilia et al., 2024). Despite these advancements, challenges remain in ensuring stability, specificity, and non-specific binding, especially in complex biological samples (J. P. Oliveira et al., 2019).

2.5. Gold Nanoparticles (AuNPs) Synthesis

The review found that 8 out of the 20 included studies focused on the synthesis of gold nanoparticles. Chemical synthesis was the predominant method, reported in 6 out of these 8 studies. The reviewed literature describes four main chemical approaches for AuNP synthesis. The most widely used is the Turkevich method, first introduced in 1951, which utilizes aqueous citrate solutions to reduce gold (III) compounds like HAuCl4 or NaAuCl4, forming AuNPs (Turkevich et al., 1951; Dheyab et al., 2022). Another prominent chemical approach is the Brust-Schiffrin method, developed in 1994, which employs organic solvents and tetraoctylammonium bromide as a phase transfer agent to synthesize AuNPs (Ielo et al., 2021; Tai et al., 2022). Additional chemical synthesis techniques include seed-mediated growth and polymer-mediated methods. Seed-mediated growth is a common approach for producing rod-shaped AuNPs (Ni et al., 2024). This method involves creating seed particles by reducing gold ions, typically using reducing agents like $NaBH_4$. These seed particles are then introduced into metal ions and a mild reducing agent, such as ascorbic acid, which inhibits further nucleation and promotes the formation of rod-shaped AuNPs (Wei et al., 2021). The shape and configuration of the AuNPs are controlled by the seed particles and the concentration of the reducing agents (Dheyab et al., 2022).

While the chemical methods are well established and valued for their simplicity and ability to produce relatively uniform nanoparticles, they often involve toxic chemicals such as sodium borohydride ($NaBH_4$) and generate hazardous by-products, posing environmental and safety concerns (Kumalasari et al., 2024). This limits their scalability, particularly for biomedical applications, where non-toxic and biocompatible materials are preferred (Sengani et al., 2017). Moreover, while the seed-mediated growth method is effective for producing rod-shaped AuNPs, it lacks consistency in generating uniform nanorods (Amina & Guo, 2020). Variations in size and shape can significantly impact the optical properties and conductivity of the nanoparticles, thereby affecting their overall performance in biosensors (Hammami et al., 2021).

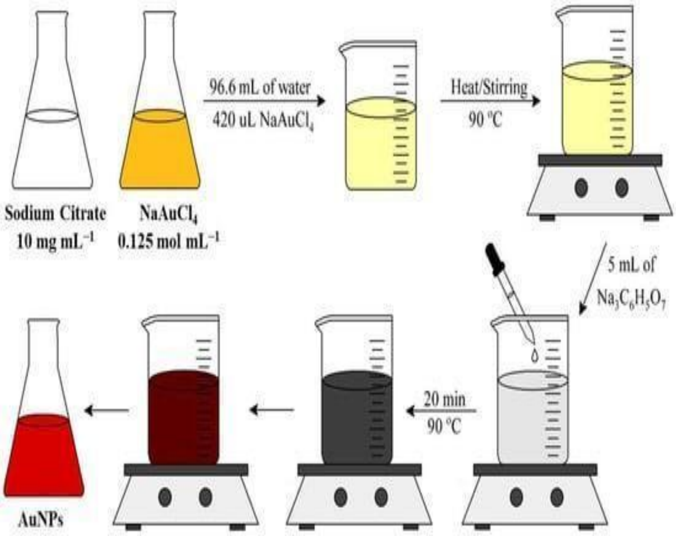

Figure 2. Schematic diagram showing the steps for gold nanoparticles synthesis by chemical reduction method

Physical and ultrasound-assisted techniques have been minimally explored, with only two studies addressing them. Physical methods included UV-induced photochemical synthesis and laser ablation (Tedesco et al., 2020). In the UV-induced photochemical approach, AuNPs are synthesized by leveraging steric hindrance through the capping effects of polymers, dendrimers, or surfactants, which act as soft templates to prevent aggregation (Souza et al., 2023). Conversely, in laser ablation, AuNPs are produced by reducing gold(III) tetrachloroaurate metallic precursors. The reduction is facilitated by the photo-induced effects of a 532 nm laser beam, generating nanoparticles smaller than 5 nm in diameter (Tai et al., 2022). This top-down strategy used aqueous sodium dodecyl sulfate solutions (SDS) as a templating agent, with the researchers examining the influence of SDS concentrations and laser parameters on the synthesized AuNPs (Terracciano et al., 2021; Hassan et al., 2022).

Another method of synthesizing AuNPs not found in this review is the biological or green synthesis approach, which represent a promising environmentally friendly alternative. Gold nanoparticles (AuNPs) can also be synthesized using biological alternatives, with plants and plant extracts being particularly promising due to their availability, low maintenance, and cost-effectiveness (Mikhailova, 2021). Other biological agents such as microbes (Muniyappan et al., 2021), protists, and algae (Oza et al., 2012) can also be utilized in nanoparticle biosynthesis (He et al., 2007). In this approach, a metal salt is combined with a plant extract and incubated at room temperature for a few hours, which facilitates the reduction of the metal salt into nanoparticles (Rajasekar et al., 2020). This method reduces the need for hazardous chemicals and aligns with the growing demand for environmentally friendly manufacturing. However, biological methods have their challenges, including long synthesis time (taking up to hours and days), delicate procedures, and difficulty in controlling nanoparticle size and distribution, which is crucial for biosensor applications (Mikhailova, 2021)

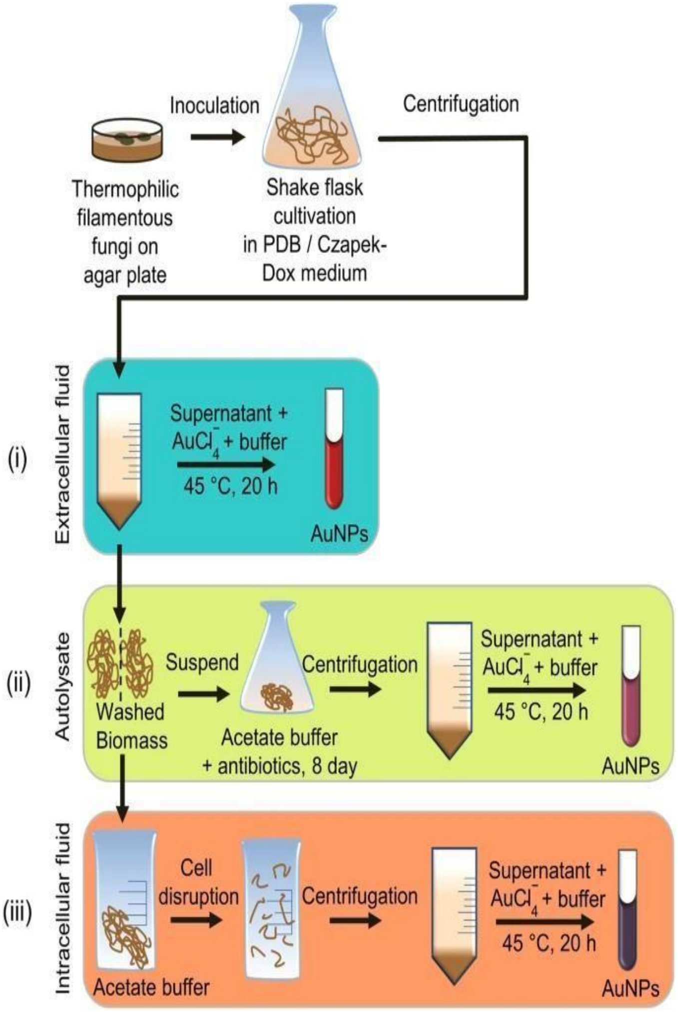

Figure 3. The sketch of the preparation of different extracts from fungi and synthesis of AuNPs by different methods

2.6. Electrode Modification

The incorporation of AuNPs onto the electrode surface using various electrochemical techniques, such as electrodeposition(Wasiewska et al., 2022; Matvieiev et al., 2023), drop casting (Rahman et al., 2022), self-assembly (Janicka et al., 2024), and electrochemical reduction (Jian et al., 2018), has been widely reported as methods to improve the overall performance of gold nanoparticle-based biosensors . Electrochemical biosensors, which use electrodes as transducers, benefit from electrode surface modification with AuNPs due to their exceptional properties that enhance conductivity. Several studies have reported the modification of electrodes for biosensor applications, with 6 out of the 20 reviewed papers demonstrating the use of AuNPs in electrode modification.

Mobed et al. developed a label-free electrochemical immunosensor utilizing disposable ITO electrodes. The electrodes were modified through electrodeposition of AuNPs on to their surfaces. This modification created a suitable substrate, enabling the effective immobilization of biotinylated hyaluronic acid (HA) antibodies. The electrochemical properties of the modified electrodes were observed using Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques. The resulting biosensor was highly selective, exhibited long-term stability with excellent reproducibility. The HA electrochemical immunosensor demonstrated excellent performance, exhibiting a wide dynamic range (0.078 to 160 ng mL^{-1}) and a low limit of quantification (0.078 ng mL^{-1}) in human plasma samples.

In another study, Safarzadeh and Pan developed a sandwich biosensor by modifying a reduced graphene oxide electrode with AuNPs. This modified rGO-AuNPs biosensor was effective in detecting the demethylated MGMT gene, exhibiting a low detection limit of 0.86 pM and a wide linear range from 1 pM to 50 μM (Safarzadeh & Pan, 2022). Similarly, 6 nm AuNPs were deposited on graphite rod electrodes for electrochemical glucose detection. The resulting biosensor demonstrated high sensitivity, good stability, and repeatability, enabling the detection of glucose in serum samples with a wide linear range up to 16.5 mmol L^{-1} (German et al., 2021). Additionally, AuNPs have been shown to enhance conductivity when combined with other nanomaterials for electrode surface modification. In a study by Kusnin and colleagues, the combination of AuNPs and CuNWs on a screen-printed gold electrode resulted in a 2.3-fold increase in the effective surface area compared to the unmodified electrode (Kusnin et al., 2022).

The electrode modification technique significantly influences the performance and conductivity of electrodes. For instance, electrodes modified using the drop-casting method often face limitations in achieving a uniform distribution of nanoparticles on the surface, which can make the electrodes more fragile (Baig et al., 2019). Although the modification of electrodes with AuNPs has significantly improved electron transfer and conductivity in biosensors, there are notable challenges. One issue is the long-term stability of AuNP-modified electrodes, particularly in complex biological environments (Sarakatsanou et al., 2023). Studies like that of Mobed et al. have demonstrated good initial performance, but the reproducibility and longevity of these modifications in real-world applications remain uncertain. Moreover, the size and shape of AuNPs are crucial for maximizing electrode surface area and enhancing conductivity. However, maintaining uniformity in nanoparticle size and shape during synthesis is a persistent challenge, as even slight variations can affect the sensitivity and detection limits of the biosensors (Kumalasari et al., 2024)

While composite materials like CuNWs-AuNPs offer improvements in electrochemical performance, they introduce new complexities (L. Liu et al., 2024). For instance, combining materials can lead to incompatibilities or difficulties in reproducibility, which are not always fully addressed in the studies reviewed (Kusnin et al., 2022). As a result, future research should focus on developing standardized protocols for electrode modification to ensure consistent performance across different biosensor platforms.

2.7. Gold Nanoparticles (AuNPs) Bioconjugates

The review underscored the significant role of bioconjugation in enhancing biosensor performance. Eight studies successfully conjugated AuNPs with bioreceptors such as antibodies, aptamers, and proteins, resulting in highly sensitive biosensors. The antibody-gold nanoparticle (Ab-AuNP) conjugates are among the most widely used in biosensing, prompting researchers to explore various methods for attaching antibodies to gold nanoparticles ((L. Zhang et al., 2020; (Sepanlou et al., 2020). In general, the most common and effective strategy for developing gold nanoparticle-based biosensors involves bioconjugating AuNPs with biomolecules such as antibodies, enzymes, or DNA. This approach enhances the biosensor's ability to specifically detect target molecules, making it a key technique in biosensor development (Ziefuss et al., 2022).

Chávez and colleagues proposed a novel biosensor involving the bioconjugation of hemoproteins with anisotropic gold nanoparticles. The bioconjugation enhanced the biosensor's analytical capabilities for detecting H_2O_2, $NaNO_2$, and O_2. The researchers demonstrated that the stable bioconjugation of hemoproteins with AuNTs can increase biosensor sensitivity by enhancing the electron transfer rate constant. The biosensor parameters were comparable to, or even superior to, those reported for similar systems based on other nanomaterials, indicating their potential for the latest developments in sensing devices.

Additionally, P. Li et al. reported the conjugation of aptamers with AuNPs to enable the sensitive and quantitative detection of malathion in tap water, with a detection limit as low as 1.48 μg/L. Furthermore, AuNPs have also been functionalized with oligonucleotides for integration with SPR nanobiosensors to detect SARS-CoV-2. This system was designed to target a region of the N gene, detecting concentrations ranging from 0.1 to 50×10^3 ng/mL, with a limit of detection (LOD) of 1 ng/mL (2.7×10^3 copies per μL), showcasing excellent sensitivity (Tessaro et al., 2022).

Although the use of AuNP-bioconjugates has undoubtedly improved the sensitivity and selectivity of biosensors, significant challenges remain, particularly regarding non-specific binding and bioreceptor stability (Jazayeri et al., 2016; J. P. Oliveira et al., 2019). For example, studies from this review involving antibody-AuNP conjugates have demonstrated high sensitivity in target detection but often do not address the long-term stability of these conjugates in complex biological fluids. Additionally, antibody conjugation can suffer from issues of non-specific adsorption, where the AuNPs bind to unintended targets, thereby reducing biosensor accuracy (L. Zhang et al., 2020). Moreover, while aptamer-AuNP conjugates have shown great promise for their flexibility and specificity, their performance in real-world samples can be limited by environmental factors like pH and temperature (Yang et al., 2023). In many cases, these studies are conducted under controlled laboratory conditions, which do not fully replicate real-world environments.

3. RESULTS AND KEY FINDINGS

In this review, we examined 20 articles on the synthesis and application of AuNPs in biosensing. Various methods and approaches for synthesizing AuNPs were discussed, specifically the "Top-Down" and "Bottom-Up" strategies, which involve chemical, physical, and biological processes. These strategies are further categorized into physical, chemical, and biological synthesis methods. Our findings reveal that the chemical method, particularly the Turkevich approach, is the most widely used, followed by seed-mediated growth. AuNPs were shown to play a crucial role in biosensing technologies. By modifying the surface of electrodes, AuNPs significantly enhance conductivity. Additionally, AuNP bioconjugates have demonstrated improved sensitivity in biosensors.

Eight out of the 20 papers reported the methods used in AuNPs synthesis (shown in table 1). Five articles focused on electrode modification (shown in table 2). And finally, nine articles discussed the bioconjugation of the AuNPs with bioreceptors to enhance the sensitivity of the biosensors (shown in table 3), with two papers intersecting on synthesis and bioconjugation.

Key Findings:

- **Chemical synthesis** remains the most popular method for producing AuNPs, with the Turkevich method dominating due to its simplicity and effectiveness. However, the use of toxic chemicals limits its scalability, and the field must continue exploring green synthesis approaches for more sustainable nanoparticle production.

- **Electrode modification** with AuNPs significantly enhances the conductivity and electron transfer rates, leading to improved biosensor performance. However, challenges remain in achieving consistent nanoparticle size and morphology, which are critical to optimizing electrode properties.

- **Bioconjugation** of AuNPs with bioreceptors such as antibodies and aptamers has shown promising results in enhancing biosensor sensitivity. Despite these advances, issues of stability and uniformity persist, particularly in clinical applications where long-term stability is crucial.

Table 1. Overview of Gold Nanoparticles (AuNPs) Synthesis Methods and Resulting Sizes from Reviewed Studies

Method	Size	References
Turkevich	13 nm	P. Li et al., 2023
Seed-mediated growth	80 nm	Chávez et al., 2023
Brust-Schiffrin	54 nm	Salvo-comino et al., 2020
Turkevich	15nm	Y. Li et al., 2021
Turkevich	9-16 nm	Kusnin et al., 2022
Laser ablation	25 nm	Tedesco et al., 2020
Seed-mediated growth	84 nm	Do et al., 2020
Ultrasound assisted	8-14 nm	Crespo-Rosa et al., 2021

Table 2. Applications of Gold Nanoparticles (AuNPs) in Electrode Modification for Target Detection

Target	Modification technique	Electrode	LOD	References
Hyaluronic acid	Electrodeposition	ITO-PET	0.078 ng mL^{-1}	Mobed et al., 2022
DS-MGMT gene	Electrodeposition	rGO	0.86 PM	Safarzadeh & Pan, 2022
Glucose	Drop casting	Graphite rod	0.070 $mmolL^{-1}$	German et al., 2021
Dopamine	Electrodeposition	SNGC	5.56 µM	Rahman et al., 2022
STEC	Not specified	IDE	100 aM	Wasiewska et al., 2022
Norovirus	Self-assembly	AuE	1×10^{-18} $TCID_{50}$	Janicka et al., 2024

DS-MGMT = Doubles stranded-Methylguanine-DNA-methyltransferase, ITO-PET= Indium tin oxide-polyethylene terephthalate, rGO= Reduced graphene oxide, STEC= Shiga toxinproducing E. coli, SNGC= screening of Sonogel-Carbon

Table 3. Summary of Gold Nanoparticles (AuNPs) Bioconjugates and Their Applications in Biosensing

Target	Biosensor	Conjugate	References
H_2O_2, $NaNO_2$ & O_2	Electrochemical	AuNPs & Hemoproteins	Chávez et al., 2023
Malathion	Colorimetric	AuNPs & aptamers	P. Li et al., 2023
CRP	Optical	AuNPs & antibodies	C. Liu et al., 2020
PCT	Optical	AuNPs & antibodies	Barshilia et al., 2024
Dengue virus cDNA	Optical	AuNP–PSA–rDNA	Jeningsih et al., 2020
Alpha-synuclein	Electrochemical	AuNPs & antibody	X. Zhang et al., 2023
Anti-SARS-CoV-2 IgG	Electrochemical	AuNPs & protein	Ye et al., 2022
SCC-Ag	Electrochemical	AuNPs & antibody	X. Liu et al., 2020

CRP= C-reactive protein, cDNA= complementary deoxyribonucleic acid, PSA= poly (styreneco-acrylic acid), PCT = Procalcitonin, rDNA= ribosomal deoxyribonucleic acid, SCC-Ag= Squamous cell carcinoma antigen

4. DISCUSSION AND CONCLUSION

The use of AuNPs in biosensors has made significant strides in enhancing sensitivity, specificity, and overall performance. This systematic review analyzed 20 studies published between 2020 and 2024, highlighting the most commonly used synthesis methods, the application of AuNPs in electrode modification, and the role of bioconjugation in improving biosensor performance.

In electrode modification, AuNPs improve conductivity, resulting in higher sensitivity. Additionally, conjugating a probe or analyte with AuNPs has been shown to improve detection systems, leading to high performance in target validation. However, more research is needed on green synthesis methods that provide eco-friendly alternatives to chemical synthesis, while offering better control over nanoparticle size and morphology.

Exploring novel bioreceptors, such as DNA, enzymes, and PNAs, could further advance biosensor sensitivity and specificity. Moreover, scalable methods for controlling the size and shape of AuNPs during synthesis are critical for achieving predictable and reproducible electrode performance.

Future studies should aim to improve the stability of AuNP-modified electrodes under prolonged use and harsh environmental conditions. Addressing issues of reproducibility and long-term stability is essential for the real-world application of AuNP-based biosensors, particularly in clinical diagnostics.

In conclusion, AuNPs hold great promise for advancing biosensor technology. However, further research is needed to overcome current limitations and unlock their full potential for commercial and clinical applications.

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