chemosensors

Review

Can Nano Yield Big Insights? Oligonucleotide-Based Biosensors in Early Diagnosis of Gastric Cancer

Alexandra E. Avanu [1], Alexandra M. Ciubotariu [1] and Gianina Dodi [2,*]

[1] Faculty of Medicine, Grigore T. Popa University of Medicine and Pharmacy of Iasi, 700115 Iasi, Romania; alexandra-elisabeta-avanu@students.umfiasi.ro (A.E.A.); alexandra.ciubotariu99@gmail.com (A.M.C.)
[2] Faculty of Medical Bioengineering, Grigore T. Popa University of Medicine and Pharmacy of Iasi, 700454 Iasi, Romania
* Correspondence: gianina.dodi@umfiasi.ro

Abstract: Gastric cancer (GC) remains a significant global health challenge, with late-stage diagnosis impacting treatment options and decreased survival rates. To address this, there has been a growing interest in the development of innovative screening and diagnostic methods. Over the past 20 years, nanobiosensors have undergone multiple iterations and unveiled remarkable features that pledge to reshape patient care. Despite the excitement over the plethora of ground-breaking advancements for cancer detection, use-ready samples and streamlined healthcare information monitoring and usage, this technology is still awaiting entry into clinical trials, urging a closer gaze within the medical community. Oligonucleotide-based biosensors, leveraging DNA or RNA's long-term storage of information, offer great specificity and sensitivity, as described throughout this paper. Consequently, this renders them as an ideal choice for revolutionizing GC diagnosis and facilitating early intervention. The aim of this review is to provide an overview of this cutting-edge, invaluable technology and its limitations across various aspects.

Keywords: biosensors; nanotechnology; DNA; RNA; point-of-care testing; early cancer detection

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1. Introduction

According to the Globocan database, gastric cancer (GC) stands as a global health problem, with around 1 million new cases diagnosed annually, resulting in almost 700,000 deaths in 2022 and ranking fifth worldwide in terms of mortality rates in both sexes and all ages [1]. The regions most affected are Asia, with a staggering percentage of over 70% and Europe, with around 14.5%, in both incidence and fatalities [1–3].

The aggressive nature of GC, coupled with its poor prognosis, underscores the critical importance of early detection for effective intervention [1]. It is crucial to highlight that with early-stage treatment, survival rates can reach an impressive 92.6% [4,5]. In Japan, early GC (EGC) diagnosis achieves 50%, yielding a 90% 5-year survival rate [6]. On the flip side, for cases at an advanced stage, it ranges from only 10 to 30% [4,5] and it is associated, more often than not, with severe complications [4]. Global mortality rates have shown minor decreases in the last 4 decades [7,8]. For example, in regions like North America and Latin American countries, mortality rates decreased by around 2% annually, between 1980 and 2011. In Europe, the decline was at 3% in the same time span. Because of the lower global incidence, GC screening (by X-rays, endoscopies, etc.) only takes place in affluent Asian countries, more specifically, in Japan and Korea. However, the global burden of GC is substantial, with a 2018 study, across 31 European countries, estimating its costs at around EUR 5 billion, originating from healthcare spending and productivity losses [9,10], emphasizing the need for informed decisions to enhance cancer care.

Traditional and current diagnostic practices are upper gastrointestinal (UGI) endoscopy followed by biopsy sampling and histopathological examination [2,11], with 9.4% of cancers missed, as demonstrated by a meta-analysis by Pimenta-Melo et al. (2016)

involving over 69,000 patients [12,13]. Some of the reasons are a detectable tumor size requirement of ≥5 mm [14], younger age, gender and the gastric pathologies trio, atrophy, adenoma or ulcer [12,13]. Recent advancements in biomedical sciences have led to the development of many tumor marker determination methods. Immunoassay techniques, such as radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), became cornerstones in clinical quantitative tumor marker detection [15]. Despite their prevalence, supported by 95% to 99% accuracy [2,16], these methods are hindered by time-consuming processes, high cost and a dependency on sophisticated instrumentation and skilled personnel [11,17]. Furthermore, protein biomarkers detected in such tests often lack adequate specificity [4] and sensitivity [18].

To increase reliability, it was proposed that multiple biomarkers be used [6]. For instance, Wang et al. (2022) considered the most frequent tumor protein biomarkers used in the clinic, the carcinoembryonic antigen (CEA), the carbohydrate antigen 19-9 (CA19-9) and the tumor-associated glycoprotein 72-4 (CA72-4), and showed a combined specificity of 89% and a sensitivity of 67% [18], while still requiring ample resources for analysis [6].

Another significant focus is on Helicobacter pylori (H. pylori), a well-known carcinogen. Studies showed that its eradication reduces the risk of GC [19]. The issues yet to be addressed are the accurate identification of screening and eradication target populations and antibiotic overuse and resistance, among others. Other biomarkers in use are serum pepsinogens (PGs), which unfortunately identify only gastric precancerous lesions and not GC [6], death-ligand 1 (PD-L1) with only 62% sensitivity and 73% specificity [20] and low prevalence cluster of differentiation 44 variant 9 (CD44-v9), which does not serve as a prognostic biomarker in advanced GC, except in the early stages [21].

In the quest of surmounting these impediments, the preclinical sector turned to nanotechnology, a field with significant breakthroughs in the past decade, allowing the integration of diverse diagnostic modalities into a unified platform. There is evidence that sensitivity could increase 20 times more when compared to ELISA [22,23]. On one hand, in conjunction with a variety of analytical methods, it has propelled the creation of multifunctional nanocarriers-based biological sensors, which will be our focus in this paper and on the other hand there are chemical sensors, or chemosensors for short and together they have the collective goal of optimizing their performance for low-cost, portable, on-site clinical setting diagnosis [24]. Their superior effectiveness is attributed to the specific combination of analytes, bioactive substances (biomarkers or biocomponents) and transducers, converting biological responses into electrical signals [11]. They exhibit compelling viability that is further fortified by accessible samples or analytes, such as patient's saliva, urine, serum, plasma and more, as shown in Figure 1, thereby expediting real-time monitoring capabilities. This has given the cancer research community a beacon of hope for improved diagnostic accuracy and timely intervention.

The development of portable and miniaturized devices suitable for point-of-care applications that allow on-site testing, reducing the need for centralized laboratories and enabling faster decision-making in medical or field settings, in an affordable fashion, is therefore required.

Although nanotechnology has not yet been deployed clinically for cancer diagnosis, it is already on the market in a variety of medical tests and screens, including home pregnancy tests that use gold nanoparticles [25], blood sugar and cholesterol level tracking, infectious disease identification and other additional applications [26].

Nanobiosensors are, amongst others, DNA- and RNA-based. As we examine through this review, these demonstrate high sensitivity and specificity in the early detection of GC biomarkers. DNA is an excellent building block for making nanomaterials, but most nanobiosensors use DNA as a recognition element and different other nanomaterials as transducing components [27]. In contrast, RNA nanotechnology uses single-stranded oligonucleotides and is considered by some, the gold standard for preventing various malignant tumors through focused diagnosis [28].

Overall, this review contributes to a nuanced understanding of the current state of DNA and RNA biosensors using nanotechnology. Also, via a critical analysis of the existing literature, examining both experimental and the few clinical trials available, we intend to shed light on the specific challenges and shortcomings that hinder their effective application in GC diagnosis.

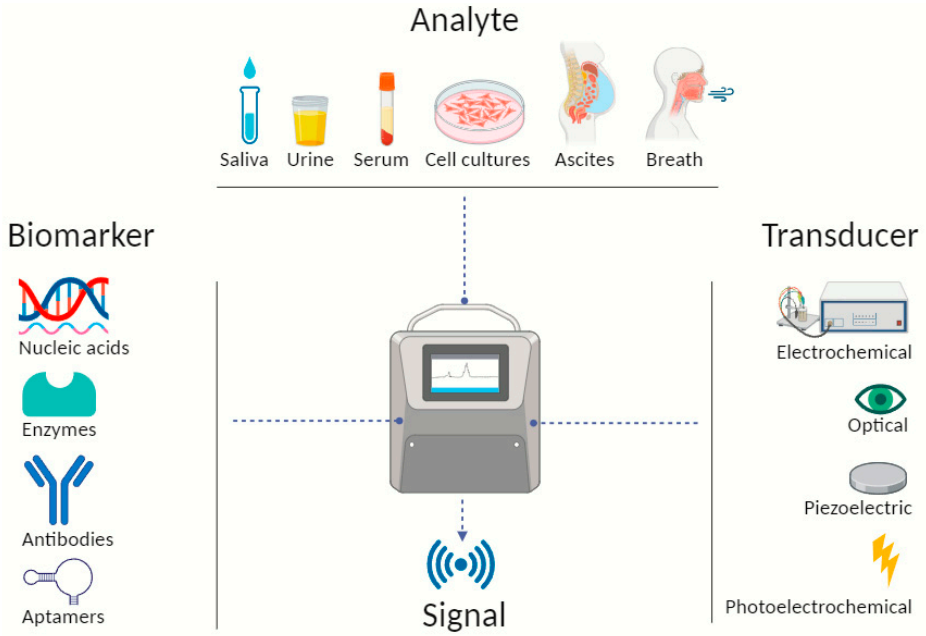

Figure 1. Illustrative scheme describing typical biosensor components detecting and identifying biomarkers from different types of analytes. Created with BioRender.com.

2. Methods

Studies focusing on nanobiosensors, oligonucleotides and GC were retrieved from Scopus, PubMed, Google Scholar, Web of Science, ScienceDirect, and the relevant ones from 2013 to 2023 were selected according to the inclusion criteria, as detailed in Figure 2. Two independent reviewers meticulously examined the selected articles, extracting pertinent information up to December 2023. Exclusion decisions for studies with uncertain eligibility were deferred until after a comprehensive review of the full text. A total of 19 studies were included. The number of yearly publications showed a rise from 2020 to 2023, with Asian countries being the predominant contributors. The data extracted focused on the sensing platform, transducer type, biomarker detected, type of GC sample, limit of detection and other key elements of every included biosensor. Synthesizing diverse perspectives, we identified key trends and assessed both theoretical frameworks and empirical evidence. Our focus was to meaningfully contribute to the ongoing scholarly conversation, enhancing our knowledge, finding missing pieces and highlighting areas of consensus.

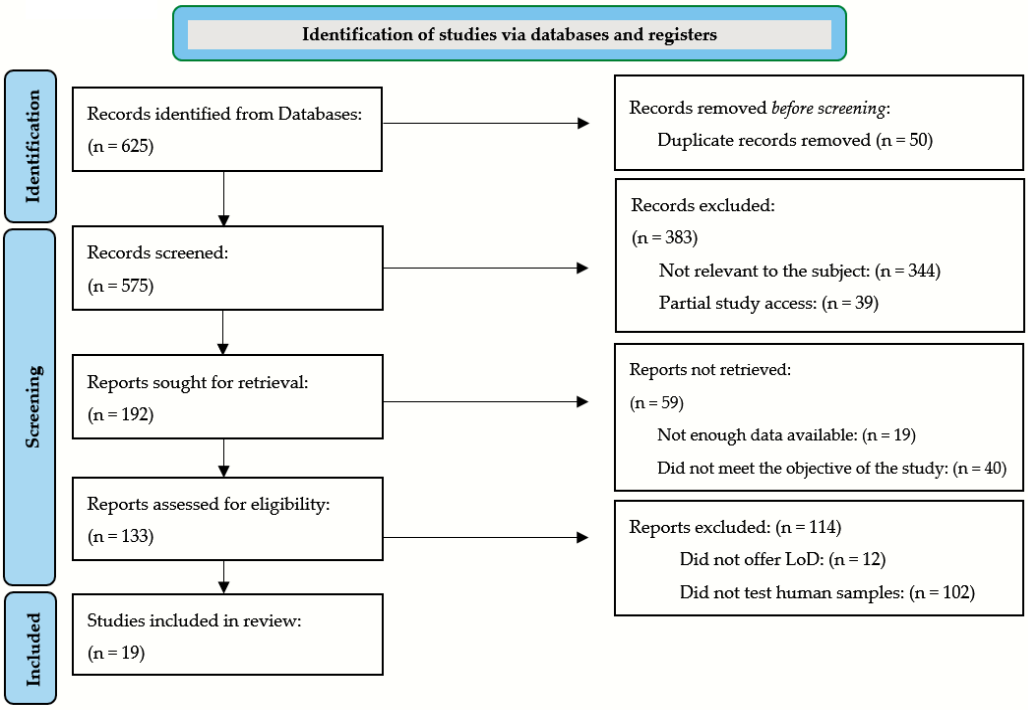

Figure 2. Literature search selection. PRISMA flow diagram.

3. Nanobiosensors—Sensing Mechanism and Attributes

According to their signal detection, sensors can be divided into physical, chemical, thermal and biological types [29]. The pioneers of biological sensors (or biosensors) were Clark and Lyons, who, in 1962, developed the first enzyme-based glucose sensor later upgraded by Updike and Hicks [22,30]. They paved the way for devices that transform the concentration of specific and diverse biomarkers, such as proteins, DNA, RNA, antibodies or microorganisms, into an analytical signal with the help of a transducer [22,31,32], as shown in Figure 1. The latter one converts the recognition signal events into a quantifiable format of electrical signals.

Nanoscience, defined as the study and manipulation of matter on an atomic and molecular scale, has revolutionized various fields, particularly medical diagnostics [33]. Biosensors using nanotechnology offer several key advantages, which have now become standard, and are poised to stimulate screening programs and increase diagnosis accuracy. Bhalla et al. (2020) and Umapathy et al. (2022) mention in each of their works, in-depth and commendable biosensor attributes [22,34], which can be categorized in four broad attribute clusters: precision sensing, swift on-chip validation, sustainable design and integrated autonomy and networking.

Most notably, nanobiosensors demonstrate high specificity, sensitivity and accuracy through customized configurations [22,35] and ready-to-use samples, for point-of-care testing [22], which facilitate early diagnosis and management. This is particularly crucial for the early diagnosis of GC, which ranks as the fifth leading cause of death from malignant diseases worldwide, as mentioned before, and is often detected at advanced stages [36]. Despite the promising capabilities of new technology, such as the use of AI in endoscopic GC detection, with a sensitivity of 86% and a specificity of 93% [37], a significant amount of time elapses before patients seek medical attention. As Car et al. (2016) argue, alignment among three entities is necessary during the diagnostic procedure: the provider, the patient and the health system [38].

The youthful demographic stands to gain significantly from the widespread adoption of nanobiosensors, due to their attributes, particularly since existing screening programs for GC target adults aged 45 and above [39]. Although there have been slight declines in the incidence of GC, these are not ubiquitous, such as the case of the United States, so

the burden of the disease persists. A powerful example of this argument is the case of three young people who eventually presented at an advanced stage of GC after an average duration of 2.1 years, experiencing chronic pain and postprandial vomiting [40]. Compared to older individuals, young adults are less likely to have a primary care physician due to the allure of immediate alternatives in their fast-paced lives, such as their smartphones, walk-in clinics [41] or pharmacies [42]. Soomers et al. (2021) found that among 341 young adults, more than 3 months passed from the onset of symptoms to the first medical consultation in 21% of cancer cases of different etiologies [43].

As for the general population, a study from Sri Lanka involving 145 patients with gastric adenocarcinoma showed that 60% presented at an advanced radiological stage, while 75.1% were diagnosed at an advanced clinical stage [44]. We argue that facilitating early diagnosis in both settings with high numbers of GC cases and also settings where screening programs face practical constraints due to low GC prevalence could be effectively achieved through the utilization of sensitive, handheld, and cost-effective nanobiosensors with a rapid response time. Such technology could prove beneficial for all ages and all populations regardless of income levels, enabling usage in walk-in clinics, homes, remote areas, or locations with limited resources [45].

It is important also to consider that developing high-specificity tailored probes requires a dedicated team and takes months or more [34]. Nonetheless, the early diagnosis of any disease not only preserves more lives but also mitigates treatment expenses [45].

In order to create a disposable, cost-effective sensor system, the optimal strategy involves a modular approach, which entails designing components made from affordable and readily available materials in a separate manner [34]. The components should possess user-friendly characteristics and a minimum lifespan of one month for convenient and durable use.

These features depend on the design of the nanostructure and on the materials employed in their fabrication. The available options are nanoparticles, nanorods, nanofibers, nanosheets, nanopillars, nanowires, and so on, made from inorganic, organic, or composite materials [46]. The choice of the nanomaterial is based on the function of the component within the biosensor. For example, inorganic materials (metals and metal oxides) are suitable for electrical biosensor fabrication [47], and organic and polymeric materials are used in other biosensor applications [48], such as nanoscale functionalization with biological components. The most exploited designs for biological sensing applications are the ones based on composite materials, since the biosensor benefits from the characteristics of each component.

In the case of GC nanobiosensors, the utilized nanostructures, according to Tables 1–3, are gold NPs, gold–magnetic NPs and nanospheres, carbon nanotubes, black phosphorus nanosheets, graphene oxide nanocomposite, quantum dots and more, each embedded within a biosensor in order to make them specifically relevant for diagnosis. Visual examples of these can be found in Figure 3. Nanostructure features are described in each specific section based on the biological analyte and relevance to the clinical application.

Thanks to their enhanced spatial confinement potential and the other mentioned qualities, nanostructures outperform larger equivalents in various metrics, with the majority of the processes occurring inside the same platform [49]. Previous drawbacks such as increased size and computation time are now in the past [34]. Nanobiosensors nowadays have a high surface area compared to their volume, (see Figure 4), which makes them more reactive and stable [22]. Their smaller size also allows for the better binding of certain molecules, making them more selective and better for biosensing, mainly at smaller particle sizes.

Theoretically, biosensors generally demonstrate a swift (<1 s) reaction to stimuli. But, subsequent adjustments, such as amplification to enhance weak signals, filtering to remove unwanted noises, adjustments to ensure the signal is compatible with the requirements of the next stage in the system and temperature rectification, etc., might prolong the duration

of the reaction, highlighting the crucial role of optimizing the raw signals received from nanosensors [34].

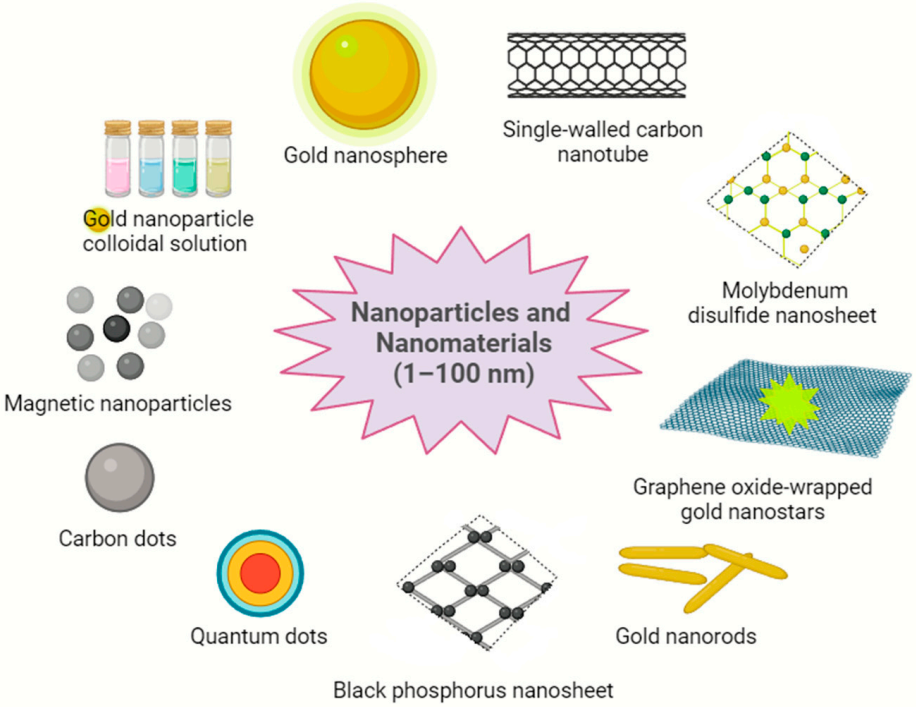

Figure 3. Various types of nanoparticles and nanomaterials with clinical application in GC. Created with BioRender.com.

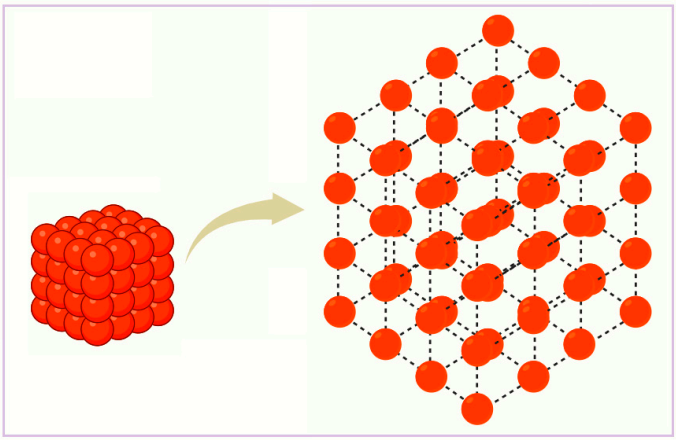

Figure 4. The high surface area of nanoparticles, especially compared to their bulky counterparts, leads to enhanced adsorption of biomolecules. Created with BioRender.com.

Commonly employed signal transduction mechanisms include electrochemical, optical, mass-sensitive (piezoelectric) and photoelectrochemical mechanisms [29], with visual representations of their different types of output signals depicted in Figure 5. For example, electrochemical nanobiosensors, using amperometry as one of the analysis methods, measure the current flow between electrodes due to redox reactions [50]. Among the plethora of electrodes or nanostructured materials, specific types were proven to be particularly effective in the EGC diagnosis. Ma et al. (2020) list carbon nanotubes and graphene, known for their exceptional structural properties at nanoscale [51]. Metals and metal oxides, such as gold, silver and titanium dioxide, compounds formed by combining metals with chalcogen elements like sulfur or selenium, demonstrated also their efficacy. Amperometry aligns very well with the standards mentioned before, such as high sensitivity, specificity,

portability and ease of use [50,52]. The choice of amperometric technique depends on the specific requirements of the biosensing application, including the nature of the analyte, desired sensitivity, limit of detection (LoD) and the kinetics of the electrochemical reactions involved. Among the various techniques available, chronoamperometry, implying the application of a constant potential for a defined period with the ensuing measurement of the resulting current response, is particularly valuable due to its simplicity and sensitivity to changes in analyte concentration over time [53].

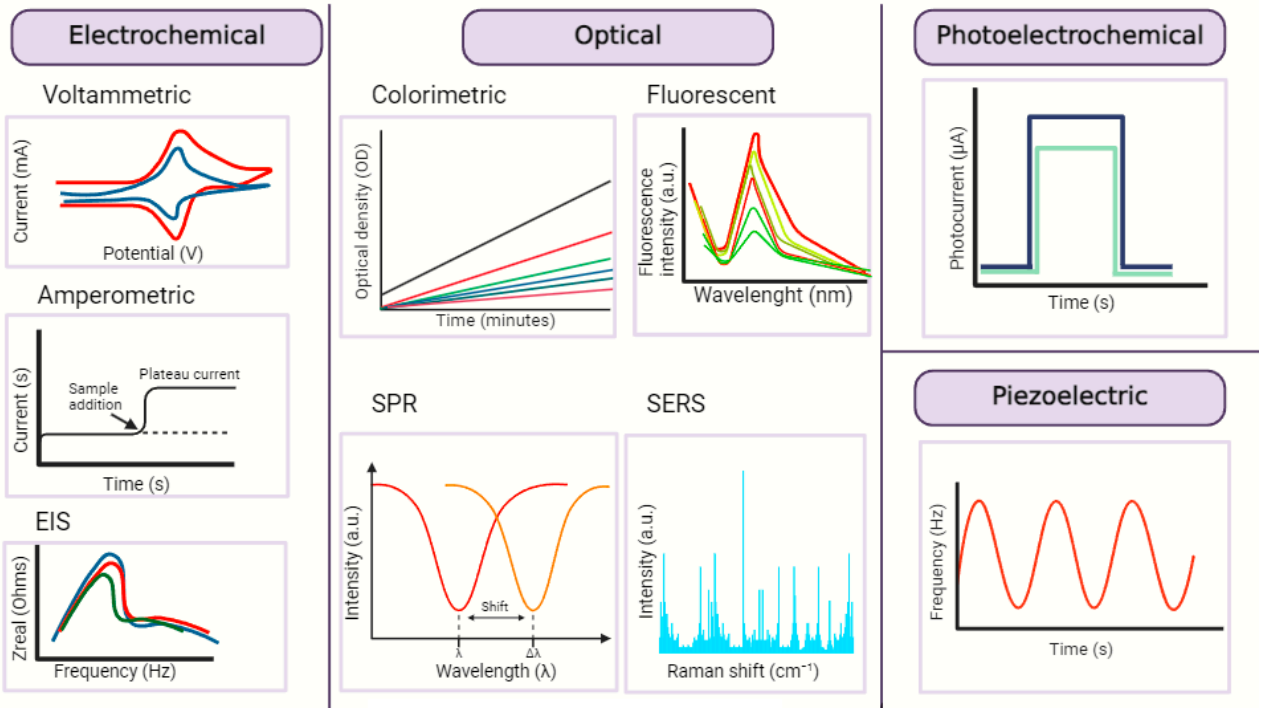

Figure 5. Examples of the typical graphical outputs based on transducer type. Created with BioRender.com.

There are also other variations, such as hybrid photon–electron systems with electrogenerated chemiluminescence (ECL). Photoelectrochemical and piezoelectric methods are beyond the scope of our study as the literature on GC nanodiagnosis that we have reviewed does not encompass these techniques. The constraints associated with nanobiosensors will be discussed in the included studies.

4. DNA Nanobiosensors in GC

Biosensors can be engineered to rapidly recognize and bind specifically to target genes, mutations or pathogenic sequences in real-time [54]. This specificity is achieved through the design of molecular probes or recognition elements that are complementary to the target DNA. This ensures that the biosensor responds only to the presence of the desired DNA sequence, eliminating cross-reactivity. Additionally, many DNA biosensors exhibit high sensitivity, allowing for detection at low concentrations. This is crucial for applications such as early disease diagnosis, where only trace amount of DNA may be present.

DNA biosensors are often compatible with various detection platforms, including optical, electrochemical, and microfluidic systems [29]. This versatility allows for the integration of DNA biosensors into different analytical devices and technologies.

The usual need for amplification techniques such as polymerase chain reaction (PCR), which uses thermal cycling and loop-mediated isothermal amplification (LAMP) that has the requirement of a relatively complex primer design [55], has been replaced with easier and more sensitive techniques leading to lower detection limits, as we demonstrate in the following segment.

In Table 1, we summarize the features of nanobiosensors that detect DNA in human GC samples. We covered details like the operating principle, transducers and their associations with materials like gold nanoparticles, carbon nanotubes and quantum dots. It is important to emphasize that all nanosensors from this table showed rapid response times, high sensitivity, successful optimization and good economical attributes. Therefore, we have exclusively incorporated additional specific characteristics.

Table 1. DNA nanobiosensors for GC.

Sensing Platform	Transducer	Biomarker	Human Sample	LoD	Takeaways	Ref.
High-density "hot spot" AuNPs@SiO array substrate with RCA strategy	Optical (SERS)	M.SssI	Serum	2.51×10^{-4} U mL^{-1}	Simple preparation, high biocompatibility, uniformity, reproducibility, stability	[56]
Polymeric L-arginine and rGO-AuNSs on glass electrode	Electrochemical (CV)	PIK3CA ctDNA		1.0×10^{-20} M	Label-free, desirable stability, wide dynamic response	[57]
SWCN DMEJ with DNA–gold urchin	Electrochemical (IDE)	SOX-17		1 aM	High performance, efficiency, biocompatibility, no cross-reactivity	[58]
Nitrophenyl-functionalized black phosphorus nanosheets and FAM labelling	Optical (fluorescence)	PIK3CA E542K ctDNA	Tumor cell lines	50 fM	Enzyme-free, long-term stability, simple manufacturing process, good discrimination ability of interferences	[59]
Nanoplasmonic, nanogold-linked sorbent assay	Optical (FOPPR and FONLISA)	Methylated SOCS-1	Tumor tissue and cell lines	0.81 fM	PCR- and amplification-free, label- and sequencing-free; superior to PCR and other assays	[60]

Abbreviations: aM—attomolar; GC—gastric cancer; LoD—limit of detection; Ref—references; AuNPs@SiO_2—gold silica nanoparticles; RCA—rolling circle amplification; SERS—surface-enhanced Raman scatting; M.SssI—CpG methyltransferase; rGO-AuNSs—graphene oxide-wrapped gold nanostars; CV—cyclic voltammetry; ctDNA—circulating tumor DNA; SWCN—single-walled carbon nanotube; DMEJ—different dimicroelectrodes junction; IDE—interdigitated electrode; FAM—carboxyfluorescein; fM—femtomolar; FOPPR—fiber optic particle plasmon resonance; FONLISA—fiber optic nanogold-linked immunosorbent assay; PCR—polymerase chain reaction.

According to several studies, irregular changes in the DNA methylation pattern, which normally regulates gene activity and cell differentiation, can serve as a valuable biomarker for EGC detection [61,62]. Hypermethylation contributes to GC and cancers in general, by silencing tumor suppressor genes (TSGs) [63]. Guthula et al. (2022) effectively tackled numerous challenges listed in Table 1 associated with detecting DNA methylation in the frequently inactivated SOCS-1 human genome, a gene linked to cancers such as GC [60]. They created a rapid ($\leq$15 min) PCR-free sensor that exhibited a strong correlation with PCR outcomes and the lowest LoD among amplification-free methods reported previously, affirming the reliability of their approach. Ge et al. (2021) even went a step further back, looking into CpG methyltransferase targeting, which accumulates before proceeding to participate in DNA methylation [56]. Boasting high accuracy, selectivity, and sensitivity, the authors engineered a biosensor that had a particularly low LoD. They employed surface-enhanced Raman scattering (SERS) as the transduction method, surpassing traditional approaches that demand substantial sample quantities. SERS biosensors are developed on the enhancement of Raman scattering signals that occur when molecules are in close proximity to specially designed surfaces featuring nanoscale metallic structures, such as gold or silver nanoparticles [64]. Besides the known addressed limitations of SERS related to substrate preparation, uniformity, external factors and signal fluctuations, the shelf-life

question remains unanswered [65]. Also, some argue that it can be laborious and not easily portable [66].

To improve the detection of target DNA, in 2021, Yu et al. attached a DNA sequence from the SOX-17 gene onto a gold urchin (DNA-GU), linking it to a single-walled carbon nanotube (SWCN)-constructed DMEs junction (DMEJ) [58]. The biosensor demonstrated exceptional sensitivity, detecting DNA concentrations ranging from 1 aM to 10 fM. The successful detection was attributed to the strategic immobilization of the capture molecule, aligning with research showing that higher-density biomolecules enhance sensor performance. Showcasing its selective identification capabilities, the biosensor effectively differentiated target DNA from complementary sequences, including miR-106a, the subject of numerous RNA nanosensors, as we will explore later on.

Introducing another significant biomarker, the circulating tumor DNA (ctDNA) refers to a class of circulating free DNA shed by the tumor cells, desired to provide insights into gastric tumor presence and dynamics through liquid biopsy sampling [67]. Liquid biopsies involve the analysis of blood or other body fluids, eliminating the need for invasive procedures like traditional tissue biopsies [68]. This makes them more suitable for point-of-care testing, which prioritizes quick and minimally invasive diagnostic methods. Rahman et al. (2022) studied the precise diagnosis of GC through hybridization between the capturing DNA probe and PIK3CA gene of ctDNA specimens obtained via liquid biopsy [57]. They chose to combine graphene oxide with a large surface area and star-like shaped gold nanostructures (AuNSs). The material was deposited onto a glass electrode, forming a thin layer of coating, and the outcome showed great promise.

For the same biomarker, Huang et al. (2020) developed a biosensor that leveraged nitrophenyl-functionalized black phosphorus nanosheets (NP-BPs) [59]. It was constructed on the foundation of surface-modified BPs, discriminated well between different DNA structures. Practical experiments revealed exceptional sensitivity, detecting ctDNA concentrations as low as 50 fM, with a broad linear detection range of 50 fM to 80 picomolar (pM). The biosensor's clinical application was demonstrated by successfully detecting ctDNA in clinical serum samples, presenting a LoD of 0.5 nanomolar. Furthermore, the biosensor's performance was enhanced by combining it with conventional magnetic extraction, achieving a lower detection limit of 50 fM. While the assay offered advantages like a 15 min speed and simplicity, its sensitivity fell short compared to that of PCR.

The previous study and others have explored different methods for synthesizing nanocomposites, such as graphene/metal oxide, graphite electrodes plus various metals and metal oxides. These cannot be applied directly in detecting ctDNA in serum samples due to the interference from the strong nonspecific absorption of serum proteins. In this context, Ma et al. (2020) presented a one-step strategy for preparing zinc-based nanohybrids with tunable structures [51]. The proposed approach involved the use of conducting polypyrrole (PPy) as a heating source under microwave irradiation for PIK3CA gene detection. Additionally, the nanocomposites showed a reliable performance in distinguishing mismatches in DNA, highlighting their applicability in detecting genetic variations associated with GC.

Cao et al. (2022) present another notable example, albeit in the preclinical stage and conducted on mice serum, proposing the detection of the PIK3CA gene in ctDNA [66]. They created a microfluidic chip for SERS, pursuing PIK3CA E542K and TP53 genes detection. The removal of enzymes as catalysts which are usually used in amplification strategies like RCA led to the combination of two enzyme-free signal amplification strategies, namely the catalytic hairpin assembly (CHA) and hybridization chain reaction (HCR) in order to overcome insufficient signal gain and sensitivity [66]. The study conducted stands out in the realm of microfluidic methods, achieving an exceptionally favorable LoD in the aM range, with reported values of 1.26 aM and 2.04 aM, respectively, and a detection speed of 13 min.

However, in 2022, Dang et al. affirmed that ctDNA's practical value in the clinical setting is yet to be established [69]. Its absence, emphasize the authors, cannot definitively

rule out GC or other types of cancer. In 2023, Bittla et al. (2023) also sternly concluded in a systematic review that despite expectations and efforts, ctDNA has not demonstrated its usefulness in cancer detection but could be used in the future only as a predictor [70].

These diverse biosensing approaches demonstrate both progress and challenges in the quest for effective and reliable diagnostic tools for GC. Future research should focus on addressing remaining challenges, such as shelf life or limitations of specific nanomaterials.

5. RNA Nanobiosensors in GC

Alterations to typical characteristics of normal cells, such as to microRNAs (miRs), are considered RNA-based cancer biomarkers [71]. Although critical to cell physiology, miRs, small non-coding RNAs, act as molecular signatures for cancer detection and are linked to cancer stage, tumor size and cell proliferation. These molecules can persist in a detectable and consistent manner, making them reliable biomarkers [72]. Various methods, including electrochemical methods, optical methods or the combination of the two, using nanotechnology have been explored for GC detection. Conventional techniques like PCR or Northern blot, while capable of identifying RNA biomarkers, have limitations and lack sensitivity [73]. Table 2 provides an overview of the most recent nanobiosensors detecting RNA, highlighting their key features in EGC diagnosis. The same principle of adding only distinct and supplementary characteristics was again consistently applied. Having said that, each sensor exhibited swift detection times, elevated sensitivity and appreciable cost-effectiveness.

Table 2. RNA nanobiosensors in GC.

Sensing Platform	Transducer	Biomarker	Human Sample	LoD	Takeaways	Ref.
Blackberry-like magnetic DNA/FMMA nanospheres on gold stir-bar using CHA-HCR and RAFT amplification	Electrochemical (V)	miR-106a	Serum	0.68 aM	Enzyme-free, simple nanomaterials, acceptable storage stability, RNA extraction-free, sample pretreatment-free technique, high recovery	[74]
Gold–magnetic NPs single-strand (ss) probe 1 (P1)	Electrochemical (EIS, CV, DPV)	miR-106a	Serum	0.3 fM	Great performance, stability, simplicity, reproducibility, agreeable storage stability	[75]
AuNPs and CdSe@CdS QDs-contained magnetic nanocomposites labels with polythiophene/rGO-modified carbon electrodes	Electrochemical (CV, DPV)	miR-106a let-7a	Plasma	0.06 fM (miR-106a) 0.02 fM (let-7a)	Multiplexing, good recovery, reproducibility, appropriate storage stability	[76]
AgNRs array coated by the mF-MoS2 NSs, dual mode detection assay	Optical (SERS) and electrochemical (SWV)	miR-106a	Serum	67.44 fM 248.01 fM	In situ, stability, reliability, reproducibility, minimal interference	[77]
Perovskite–graphene oxide nanocomposite on an electrode, genosensing assay	Electrochemical (chronoamper-ometry)	miR-21	Cell lines	2.94 fM	Label-free, reproducibility, reusability, stability, versatility, robustness	[78]
Ratiometric strategy using CDs with triple function and FAM-labeled ssDNA	Optical (fluorescence)	miR-21	Plasma	1 pM	Reproducibility, reliability, simplicity, strong anti-interference ability, excellent performance	[79]

Table 2. *Cont.*

Sensing Platform	Transducer	Biomarker	Human Sample	LoD	Takeaways	Ref.
Two-stage cyclic enzymatic amplification with T4 RNA ligase 2 and T7 exonuclease and AuNPs	Electrochemical (DPV)	miR-21	Serum	0.36 fM	Convenience, reproducibility, excellent performance, stability	[80]
MXene-derivative QDs (Mo2TiC2 QDs) and SnS2 nanosheets/lipid bilayer	Electrochemical and optical (voltammetry and fluorescence)	miR-27a-3p	Ascites	1 fM	Reproducibility, low background noise, wide dynamic range, good stability, minimal interference	[81]
"Hot spot" bismuth nano-nest/Ti3CN QD- SPC-ECL	Electrochemical and optical (voltammetry and fluorescence)	miR-421	Ascites	0.3 fM	Improved luminescence and catalytic activity, stability, controllability	[82]
Dual-response–single-amplification nanomachine	Optical (fluorescence)	miR-5585-5p & PLS3 mRNA	Serum	1.19 fM (miR-5585-5p) 16.37 fM (PLS3)	Enzyme-free, extraction-free, high recovery, great performance	[83]
CPs/AuNP-AuE with DSN	Electrochemical (chronoamperometry and CV)	miR-100	Serum	100 aM	Enzyme-free, reliability, controllability, effectiveness	[84]

Abbreviations: FMMA—ferrocenylmethyl methacrylate; CHA-HCR—catalyzed hairpin assembly—hybridization chain reaction; RAFT—reversible addition fragmentation transfer; V—voltammetry; miR—microRNA; NPs—nanoparticles; EIS—electrochemical impedance spectroscopy; CV—cyclic voltammetry; DPV—differential pulse voltammetry; AuNPs—gold nanoparticles; QDs—quantum dots; rGO—reduced graphene oxide; AgNRs—Ag nanorods; mF-MoS2—multi-functionalized molybdenum disulfide nanosheet; NSs—nanostars; SERS—surface enhanced Raman scattering; SWV—square wave voltammetry; CDs—carbon dots; FAM—carboxyfluorescein; ssDNA—single-stranded DNA; Mo2TiC2—molybdenum titanium carbide; SnS2—tin sulfide; Ti3CN—titanium carbonitride; SPC-ECL—surface plasmon coupling electrochemiluminescence; CPs—capture probes; AuE—Au electrode; DSN—duplex-specific nuclease.

MiR-106a, a member of the miR-17 family, recognized as an oncogene in GC cells, exhibits a direct association with the occurrence of tumor metastasis [85]. This molecular behavior, coupled with its detectability in liquid biopsies, positions miR-106a as a compelling biomarker for biosensors. In 2016, Daneshpour et al. pioneered a nanobiosensor featuring double-probe sandwich architecture that incorporates gold–magnetic NPs [75]. This sensor demonstrated exceptional precision, sensitivity and selectivity in detecting miR-106a, showcasing prolonged stability for over 7 weeks. Building on this success, in 2018, Daneshpour et al. introduced a novel biosensing technology with multiplexing capabilities for the simultaneous detection of miR-106a and let-7a, both associated with GC [76]. The advanced multiplexed biosensing platform utilized a modified screen-printed carbon electrode (SPCE) with polythiophene (PTh), a conducting polymer and reduced graphene oxide (rGO). The procedure occurred at room temperature in physiological pH conditions. The method's sensitivity was evaluated, revealing a low detection limit of 0.06 fM for miR-106a and 0.02 fM for let-7a. The combination of PTh and rGO layers on the SPCE surface aimed to enhance the conductivity and stability of the electrode, which was vital for improving the performance of the biosensing platform. In the same year, Park et al., introduced on-chip colorimetric biosensing for the early detection of the same biomarker [86]. The platform was based on the plasmon coupling of hybridized AuNPs showing high specificity and sensitivity. Two years after, Shafiee et al. (2020) leveraged the unique properties of DNA, such as molecular programmability and nanoscale controllability, which led to the creation of a complementary DNA strand for miR-106a [87].

DNA, renowned for its organic ligand characteristics, proved to be an excellent choice, being widely acknowledged as a fundamental building block for novel nanomaterials [27]. In both cases, despite the improved capabilities, this analysis seemed to necessitate more time than the optimal duration expected for an ideal biosensor. Nevertheless, Radfar et al. addressed this challenge in 2022 by employing a combination of CHA-HCR and RAFT polymerization for signal amplification [74]. This innovative approach significantly enhanced the sensitivity of miR-106a detection and obtained a LoD of 0.68 aM. Importantly, the authors successfully tackled the issue of shelf-life, with 94.3% of the oxidation peak current being retained after 6 weeks. Through an innovative dual transducing mode, Zhai et al. (2022) used multi-functionalized molybdenum disulfide nanosheet (mF-MoS2 NS) probes and SERS-active Ag nanorods (AgNRs) array electrode, to build an miR-106-detecting biosensor with superior reproducibility and higher sensitivity [77]. Limitations, such as using different instruments for the synchronous multimodal analysis, were successfully addressed. Samples were obtained via liquid biopsies, of which we are now familiar with.

Upregulated across various cancers, miR-21 acts as an oncogenic microRNA influencing multiple TSGs, and given its frequent upregulation in GC, it could serve as a potential diagnostic biomarker for GC [88]. In 2016, Li et al. employed T4 RNA ligase 2, an enzyme proficient in catalyzing the ligation of both inter- and intramolecular RNA molecules [80]. This enzyme was utilized to initiate a specific ligation reaction based on the target RNA sequence. Additionally, T7 exonuclease, known for degrading single-stranded DNA in a 5′ to 3′ direction, was employed to instigate and propel the cyclic amplification of the target RNA. Through this two-stage cyclic enzymatic amplification method (CEAM), the researchers successfully detected miRNA-21 at a low concentration of 0.36 fM, showcasing exceptional specificity. Notably, the introduction of mismatched non-complementary RNAs did not induce noticeable signal changes, affirming the success of this nanosensor. Similarly, in the biosensor designed by Wang et al. (2020) the ratiometric fluorescence strategy, along with T7 exonuclease-mediated cyclic enzymatic amplification, was employed to enhance the precision and accuracy of the detection process [79]. The use of carbon dots (CDs) and 6-carboxyfluorescein (FAM) as labels contributed to the ratiometric fluorescence approach. The results demonstrated good correlation with quantitative reverse transcription polymerase chain reaction (qRT-PCR), and notably, in healthy patients, the expression of miR-21 was significantly lower.

Another crucial oncogenic miRNA in GC was put through tests by Li et al. [81]. They utilized molybdenum titanium carbide quantum dots (Mo2TiC2 QDs) in an electrochemiluminescence (ECL) biosensor to detect GC marker miR-27a-3p. The biosensor incorporated SnS2 nanosheets and a lipid bilayer, enhancing QD luminosity and stability. The synergistic system achieved a wide miRNA-27a-3p detection range (1 fM to 10 nM) with a LoD at 1 fM.

Li et al. (2023) introduced a novel surface plasmon-coupled electrochemiluminescence (SPC-ECL) biosensor, combining Ti3CN QDs with enhanced luminescence and a specially designed bismuth nano-nest structure with strong localized surface plasmon resonance (LSPR) effects [82]. The biosensor successfully quantified miRNA-421 in a concentration range of 1 fM to 10 nM and demonstrated clinical applicability using ascites samples from GC patients. Of course, minimally invasive or non-invasive sampling approaches are preferred.

Multiplexing is pivotal for biosensors as it enables the simultaneous detection of multiple analytes, enhancing efficiency and providing comprehensive diagnostic information [89]. Zhang et al. (2023) introduced an innovative dual-target responsive fluorescent nanomachine for the simultaneous detection of miR-5585-5p and PLS3 mRNA [83]. Guided by advanced techniques such as next-generation sequencing, the nanomachine operated without the need for RNA extraction or PCR, ensuring simplicity. Having achieved ultrasensitive detection at the femtomolar level, the nanomachine outperformed the clinical biomarker CA 72-4, demonstrating superior diagnostic capabilities.

Lastly, in developing a biosensor (CPs/AuNP-AuE) for miR-100 detection, a gold nanoparticle (AuNP)-modified Au electrode (AuE) with DNA capture probes (CPs) was

crafted, demonstrating enhanced electrical conductivity and an increased electrode area [84]. Differential pulse voltammetry (DPV) analysis confirmed the biosensor's efficacy in detecting miR-100, exhibiting a linear response within a concentration range of 100 aM to 10 pM. The biosensor's specificity was underscored by its ability to distinguish a one base-pair mistake in miR-100, and reproducibility was confirmed. When applied clinically, the biosensor revealed 100% specificity and 90% sensitivity in distinguishing miR-100 content in GC patient serum, surpassing the performance of quantitative RT-PCR.

These technologies collectively contribute to enhanced miRNA detection, painting a comprehensive picture of cellular activity and fostering improved diagnostic capabilities for EGC.

6. Exosomes-Based Nanobiosensors in GC

Exosomes are small extracellular vesicles, ranging in size from 30 to 150 nm with a crucial role in intercellular communication by transporting various bioactive molecules, including proteins, lipids and nucleic acids, between cells [90]. These molecules often reflect the molecular signature of the cell from which the exosome originated. As a result, exosomes can serve as carriers of specific biomarkers associated with various diseases, such as cancer. They are often released by cells early in the progression of diseases, sometimes even before clinical symptoms appear, and can be retrieved from various minimally invasive biological fluids, such as blood, urine, and saliva, making them potential indicators for early disease detection [91]. Their stability throughout the disease makes them reliable biomarkers. However, challenges like low concentrations or lengthy and complex analysis methods, which would be impractical for screening programs and resource-limited environments, have encouraged scientist to look for new solutions.

In the nanobiosensors field, exosomes can detect specific biomarkers associated with GC, such as miRs and CDs, as described in Table 3. As they carry abundant tumor-indicative information, they are considered important in liquid biopsies and have gained significant ground in early tumor nanodiagnosis due to their minimally invasive nature.

Table 3. Nanobiosensors detecting GC exosomes.

Sensing Platform	Transducer	Biomarker	Human Sample	LoD	Takeaways	Ref.
MoS2 QDs-MXene heterostructure and AuNPs@biomimetic lipid layer	Electrochemical and optical (V and fluorescence)	Exosomal miR-135b	Ascites	10 fM	Versatility, reproducibility, reliability, low background noise, high accuracy; large surface area, excellent flexibility and superior conductivity of substrates, excellent antifouling property	[92]
"Hot spot" AuNSs-decorated MoS2 nanocomposite (MoS2-AuNSs) aptasensors	Optical (SERS)	CD63 of exosomes	Serum	17 particles μL^{-1}	Reliability, reproducibility, good stability long term, excellent Raman enhancement effect and generability in bioanalysis	[14]

Abbreviations: MoS2—molybdenum disulfide; AuNPs—gold nanoparticles; V—voltammetry; miR—microRNA; fM—femtomolar; AuNSs—gold nanostars; SERS—surface-enhanced Raman spectroscopy; CD—cluster of differentiation.

Guo et al. created, in 2023, an innovative ECL biosensor that incorporated an MoS2 QDs-MXene heterogenous structure and excellent physicochemical properties such as a large surface-to-volume ratio and great optical features of the QDs with a AuNPs@biomimetic lipid layer [92].

Another example of a biosensing technology for the detection of exosomes is the one introduced by Pan et al., (2022), where a novel SERS nanoprobe (MoS2-AuNSs) was

used to detect CD63, a representative GC exosome surface marker [14]. A 6-carboxyl-X-rhodamine (red fluorescent dye used for labelling oligonucleotides)-labelled aptamer (ROX-Apt) was used as the recognition element and was immobilized on MoS2-AuNSs, a composite material made up of molybdenum disulfide (MoS2) and gold nanospheres, providing SERS signals. The ultralow LoD aptasensor was versatile enough to detect exosomes derived from various GC cell lines. This suggests that the sensor's performance is robust and applicable across different sources of exosomes.

7. Bench to Clinic: Trials

To the best of our knowledge, there are only three clinical trials focusing on the diagnosis of GC using nanosensors tailored for biomarkers and no clinical trials using oligonucleotides. We will go into detail with each of them in order to stimulate further research in clinical settings. The vast majority of the clinical trials found by the authors explored endoscopic, laparoscopic, sentinel lymph node mapping and microscopical methods [93–104].

The first mention of nanobiosensors used in GC diagnosis was in 2011 within a proof-of-concept pilot of a nanomaterial-based breath test for GC [2,105]. Then, their conclusions were compounded by the following clinical trial (DGLES) carried out in 2016 [105,106]. Both aimed to differentiate between malignant and benign gastric lesions in patients with similar symptoms.

Firstly, they enrolled 130 patients, 37 with GC and 93 with non-oncologic gastric pathologies [2,105]. They employed a specialized array consisting of 14 chemical nanosensors. These nanosensors were designed with layers of AuNPs, each coated with 11 distinct organic ligands. Additionally, the array incorporated layers of SWCN, each capped with four organic overlayers. This intricate design aimed to enhance the sensitivity and specificity of the nanosensors. Through the definition of exclusive criteria, they finally selected five volatile organic compounds (VOCs) suitable as biomarkers, notably, 2-propenenitrile (a Class 2B carcinogen), furfural and 6-methyl-5-hepten-2-one. After collecting two samples (alveolar breath) from each volunteer, into a gas sampling bag (one-step process), 25% of the tests experienced unbiased testing. The complete data set, including the remaining 75%, underwent analysis using both nanosensors and gas chromatography coupled with mass spectrometry (GC-MS), an expensive, long and complicated analysis but with reliable output. The results correlated well with the standard diagnosis methods (endoscopy and biopsy), as rendered in Table 4 with limited inaccuracy, facilitating the confirmation or ruling out of the diseases. The metrics clearly demonstrated a great capability to distinguish between GC and non-malignant gastric conditions, as well as effectively staging early and late GC, as illustrated in Table 5. Consistent stability was achieved across multiple discriminant factor analysis (DFA) models. DFA is a method of classifying unknown data through statistical techniques that involve evaluating and distinguishing among multiple groups or classes based on relevant variables [107]. Significantly, these models showed insensitivity to tobacco or alcohol consumption and *H. pylori* infection, reinforcing their reliability [2,105]. Negligible humidity had minimal impact, which is crucial for optimal breath analysis.

Table 4. DGLES clinical trial [105]—diagnostic test evaluation metrics.

	GC—Non-Malignant Gastric Conditions		**Early-Stage GC vs. Late-Stage GC**	**GC vs. OLGIM III-IV**
	[2]	[106]	[2]	[106]
Accuracy	90%	92%	91%	90%
Sensitivity	89%	73%	89%	93%
Specificity	90%	98%	94%	80%

Abbreviations: GC—gastric cancer; OLGIM—operative link on gastric intestinal metaplasia.

Table 5. DGLES clinical trial [105]—concentration profiles of compounds in different patient conditions.

Trial	Number of Patients	Compound	LoD (ppb)	Less Severe Condition Concentration Range (ppb)	Gastric Ulcer Concentration Range (ppb)	GC Concentration Rage (ppb)
[2]	130	**2-propene-nitrile**	**1.34**	**2.62 ± 0.57**	**3.65 ± 1.06**	**4.24 ± 1.28**
		furfural	1.37	1.88 ± 0.18	2.09 ± 0.17	2.32 ± 0.22
		6-methyl-5-	1.88	4.12 ± 0.98	6.03 ± 1.50	6.05 ± 1.18
[106]	484	**2-propene-nitrile**	**1.3**	**7.5 ± 6.2**	**6.1 ± 1.1**	**13.2 ± 13.7**
		hexadecane	2.3	4.2 ± 4.0	3.0 ± 2.1	10.7 ± 12.3
		1,2,3-trimethylbenzene	2.7	11.6 ±7.7	12.0 ±7.8	20.0 ± 13.6
		2-butanone	2.9	90 ± 43.1	89.5 ±83.0	68.3 ± 49.0

Summary of the concentrations of the most relevant VOCs acquired from the exhaled breath in the initial study conducted by Xu et al. [2] and the subsequent clinical trial led by Amal et al. [106]. Abbreviations: LoD—limit of detection; ppb—parts per billion; GC—gastric cancer.

The challenges highlighted in the pilot encompassed several aspects:

- Limited sample size;
- Lack of an independent sample set for blind validation before building DFA models;
- The nanomaterial-based sensors are typically more sensitive to certain classes of VOCs and less sensitive to other classes;
- Absence of histology data;
- Exclusion criteria included medication for gastric upset (common in this population)
- The origin of other VOCs cannot yet be easily understood;
- Cautious interpretation, particularly for the VOCs in room air samples below the limit of quantification.

Considering these factors, a more nuanced interpretation and robust model verification were evidently required. Despite its limited scale, the encouraging findings from the pilot study have prompted the initiation of a larger multicenter clinical trial.

In the subsequent clinical trial 484 individuals participated, comprising 99 with GC and 385 healthy volunteers [105,106]. Each sensor was composed of AuNPs and SWCNTs, covered with different organic films (ligands). Added to the previous sampling methodology was the biopsy for histologic diagnosis, with 70% of samples designated for DFA models, while the remaining 30% constituted the blinded analysis. After GC-MS analysis, both cancer patients and individuals at elevated risk exhibited distinct breathprint compositions. This time, eight VOCs were short-listed: furfural, 2-propene-nitrile, 2-butoxy-ethanol, hexadecane, 4-methyl octane, 2-butanone, 1,2,3-trimethylbenzene and α-methyl-styrene. Patients at high risk were catalogued as OLGIM (operative link on gastric intestinal metaplasia) stages III–IV. They were accurately distinguished from GC patients, but at lower stages of metaplasia, the results were not very convincing.

As a rule of thumb, excellent nanosensors are characterized by good pattern recognition methods [108]. In this case, seven of the VOCs were in a much lower concentration in the OLGIM group than in GC, which differentiated with high accuracy, specificity and sensitivity, but individually, only 1,2,3-trimethylbenzene significantly differentiated between OLGIM staging, and only 2-propene-nitrile, hexadecane, 1,2,3-trimethylbenzene, 2-butanone differed between the OLGIM and GC groups [105]. The levels of these VOCs, which showed statistical significance, are outlined in Table 5. We highlighted the values of 2-propen-nitrile, which was pertinent in both studies.

The authors could not evade certain challenges. Restricting chemotherapy or radiation therapy before enrolment narrowed down the pool of subjects who could benefit from these biosensors [2,105,106]. Other challenges included the exclusion of individuals with

peptic ulcer disease and confirmed dysplasia in the stomach mucosa due to small sample sizes and lower accuracy in OLGIM 0 and OLGIM I-II stages.

The third clinical trial that we found is a 2020 interventional study at Nouvelle Clinique Bonnefron in collaboration with University Hospital, Montpellier, that delved into olfactory nanosensors examining exhaled air in a GC, Alzheimer, urologic cancer and pulmonary cancer cohort [109]. The exclusion criteria were deliberately lenient, aligning with the possible nature of a future screening program. The study evaluated the positive and negative predictive values of the electronic nose over 18 months. No additional data are currently available at the moment of reading, at 4th of November 2023.

8. Discussion—The Other Side of the Coin

At the outset of this review, our intention was particularly to illustrate the trajectory of a successful DNA/RNA biosensor or any other sensor employed in cancer detection beyond the pre-clinical realm in order to stimulate collaborative initiatives among scientists, technologists and therapists to prompt a more exhaustive exploration of nanotechnology-based approaches for GC diagnosis and propose future research trends. Such examples proved elusive.

A decade ago, the prevailing optimism centered on the belief that biosensors would herald a revolution in cancer diagnosis. For example, 9 years ago, Krishnamoorthy's review paper [110] represented the emerging requirements set by the clinical and industrial end-users for nanostructured sensors, such as ultrahigh sensitivity, specificity, quick response times with the capability for continuous monitoring, fabrication extendible to high-throughput platforms for desired substrates/configurations, flexible, stretchable formats, multifunctionality, modularity and, not least, biocompatibility. At that time, the plasmonic sensors, using LSPR, metal-enhanced fluorescence (MEF) and SERS, were considered to assemble the rigorous quality control specifications and satisfied simultaneously the clinical and market criteria. Those nanosensors that either exploited tumor-specific molecular information presented by exosomes to detect cancer [111], or the viral load measurements towards the detection of HIV subtypes [112], type 1 diabetes with a performance comparable to radio immunoassays [113], or tumor-targeting SERS-active nanoparticles as highly sensitive image contrast agents for the early-stage detection of colorectal cancers [114] demonstrated high sensitivity, specificity, quick response times and low sample volumes, multiplexing possibilities, and the potential to be tailored-shaped. Since the industry end-users' needs referred to wearable devices (either as textiles, mobile devices, patches), implantable sensors, and portable, POC devices, the R&D sector followed the direction of plasmonic sensors into the desired configurations in order to deliver customized solutions for diagnosis and therapy, monitoring therapeutic outcomes and predictive tools to model the efficacy of therapies.

Taking the above-mentioned setup for nanosensors and the content of this review paper regarding the progressive trend of the diagnostic devices in GC, one can observe that the challenges have now shifted to a different direction, but with the same outcome, namely regarding the patient. Acknowledging the progress made, it is evident that strides have been taken for the application of sensors in cancer diagnosis, including GC.

This phenomenon is propelled by factors such as the increasing occurrence of chronic diseases, progress in nanotechnology, a growing need for point-of-care testing, the integration of various technologies into one device and a boosted focus on personalized medicine [115]. Reportedly, the market for these systems is valued in the tens of billions of dollars.

However, it is crucial to acknowledge the current absence of well-defined protocols and extensive large-scale clinical trials. The lack of standardized procedures and comprehensive validation efforts raises questions about the anticipated seamless integration of biosensors into routine clinical practices within the initially envisioned timeframe, a milestone that would have been expected by now.

There are a few answers that start with one of the crucial objectives of transitioning from experimental setups to point-of-care testing conducted on-site [116]. This shift requires the capability of multiplexing, maintaining the consistently high accuracy and reliability associated with laboratory-based analysis, upholding rigorous standards. Another notable challenge stems from the intricate design, potentially hindering their unified integration into routine clinical workflows. Furthermore, environmental factors, such as variations in sample matrices and conditions, jeopardize the reproducibility and reliability of these sensors in real-world clinical settings. Additionally, the influence of genetic factors introduces an additional layer of complexity as variations could impact the performance and accuracy.

This nuanced perspective underscores the importance of ongoing research efforts to capitalize on the advancements achieved and bridge the existing gap between optimistic aspirations and the imperative need for comprehensive guidelines. This approach is essential to fully unlock the potential for revolutionizing early cancer diagnosis and beyond.

9. Conclusions

In this review, we focused on advancements in DNA and RNA nanotechnology specifically related to GC diagnosis. The exploration of novel nanotechnology-based tools for cancer detection has garnered significant attention in recent years. Oligonucleotide-based nanobiosensors demonstrated notable improvements in sensitivity and selectivity over the years when compared to conventional diagnostic methods, contributing to the improved chances of survival for cancer patients. It is our assessment that this innovation could boast accessibility, reliability, off-site/remote data collection, the transitivity of data through standardization, centralized integration and management that also would enable real-time monitoring.

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Abbreviations

AgNRs—Silver nanorods; AI—Artificial intelligence; aM—Attomolar; Au@SiO2 —Gold silicon oxide; AuE—Au electrode; AuNPs—Gold nanoparticles; AuNPs@SiO2—Gold silica nanoparticles; AuNSs—Gold nanostars; CA19-9—Carbohydrate antigen 19-9; CA72-4—Tumor-associated glycoprotein 72-4; CD—Cluster of differentiation; CD44-v9—Cluster of differentiation 44 variant 9; CDs—Carbon dots; CEA—Carcinoembryonic antigen; CHA—Catalytic hairpin assembly; CHA–HCR—Catalytic hairpin assembly–hybridization chain reaction; CPs—Capture probes; CSDA—Circular strand displacement amplification; ctDNA—Circulating tumor DNA; CV—Cyclic voltammetry; DFA—Discriminant factor analysis; DMEJ—Different dimicroelectrodes junction; DPV—Differential pulse voltammetry; dsDNA—Double-stranded DNA; DSN—Duplex-specific nuclease; ECL—Electrogenerated chemiluminescence; EIS—Electrochemical impedance spectroscopy; ELISA—Enzyme-linked immunosorbent assay; EGC—Early gastric cancer; FAM —Carboxyfluorescein; fM—Femtomolar; FMMA—Ferrocene methyl methacrylate; FONLISA—Fiber optic nanogold-linked immunosorbent assay;

FOPPR—Fiber optic particle plasmon resonance; GC—Gastric cancer; GCE—Glassy carbon electrode; GC-MS—Gas chromatography coupled with mass spectrometry; H. pylori—Helicobacter pylori; HCR—Hybridization chain reaction; HRP—Horseradish peroxidase; IDE—Interdigitated electrode; LAMP—Loop-mediated isothermal amplification; LoD—Limit of detection; LSPR—Localized surface plasmon resonance; M.SssI—CpG methyltransferase; MEF—Metal-enhanced fluorescence; mF-MoS2—Multi-functionalized molybdenum disulfide nanosheet; miR—microRNA; Mo2TiC2—Molybdenum titanium carbide; MOF—Metal organic framework; MoS2—Molybdenum disulfide; NP-BPs—Black phosphorus nanosheets; NSs—Nanostars; OLGIM—Operative link on gastric intestinal metaplasia; PCR—Polymerase chain reaction; PD-L1—Programmed death-ligand 1; PGs—Pepsinogens; PLS3—Plastin-3; pM—Picomolar; POC—Point-of-care; ppb—Parts per billion; Ppy—Polypyrrole; PTh—Polythiophene; QDs—Quantum dots; qRT-PCR—Quantitative reverse transcription polymerase chain reaction; R&D—Research and development; RAFT—Reversible addition fragmentation transfer; RCA—Rolling circle amplification; Ref—References; rGO—Reduced graphene oxide; rGO-AuNSs—Graphene oxide-wrapped gold nanostars; RIA—Radioimmunoassay; ROX—Apt-6-carboxy-X-rhodamine aptamer; RT-PCR Reverse transcription polymerase chain reaction; SERS—Surface-enhanced Raman spectroscopy; SnS2—Tin sulfide; SPC—Surface plasmon coupling; SPC-ECL—Surface plasmon coupling electrochemiluminescence; SPCE—Screen-printed carbon electrode; SPR—Surface plasmon resonance; ssDNA—Single-stranded DNA; SWCNTs—Single-wall carbon nanotubes; SWV—Square wave voltammetry; Ti3CN—Titanium carbonitride; TiO2—Titanium dioxide; TSG—Tumor suppressor gene; UGI—Upper gastrointestinal; VOCs—Volatile organic compounds.

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