OpenNano 20 (2024) 100214

Contents lists available at ScienceDirect

OpenNano

journal homepage: www.elsevier.com/locate/onano

Review article

Transformative approaches for siRNA detection

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ARTICLE INFO

Keywords:
Cancer
siRNA
Analytical chemistry
Optical and electrochemical detection
Precision medicine

ABSTRACT

Small interfering RNA (siRNA) is essential for the process of gene silencing, especially for cancer. Despite its considerable promise, siRNA faces challenges due to stability issues of formulation and undesirable off-target side effects. In order to address these difficulties, it is essential to carefully monitor the levels of siRNA. The existing point-of-care (POC) systems cannot precisely and efficiently detect or monitor siRNA levels. In light of these challenges, this review gives the prospects of siRNA detection by proposing a novel hypothesis of existing electrical and optical-based detection of DNA/RNA with the POC platform. This hypothesis offers an interesting novel perspective to potentially fill the existing gaps, in detecting siRNA. By utilising these technologies, there is high potential to develop a proof-of-concept system that will not only overcome the existing challenges, but it will also allow effective and precise monitoring of siRNA, in real-world healthcare environments. In summary, the prospects for siRNA in the realm of POC platforms are quite encouraging, since it allows precise and effective monitoring.

1. Introduction

Gene therapy is a technique used to target genes associated with different diseases as a treatment alternatives [1]. Through the use of small interfering RNA (siRNA), microRNA (miRNA) and antisense oligonucleotides (ASOs) gene therapy can inhibit gene expressions. Plasmid DNA, messenger RNA (mRNA), small activating RNA (saRNA), ASOs, and the CRISPR/Cas systems play an important role in improving gene functions to address diseases resulting from mutations [2]. Various types of RNA such, as messenger RNA (mRNA) antisense oligonucleotides (ASO), and small interfering RNA (siRNA) play a role in controlling gene functions and impacting disease symptoms, through different pathways. An overview of these processes is summarised in Table 1. mRNA, ASO, and siRNA play a vital role in therapeutics by providing distinct capacities to regulate genes in a specific manner. SiRNA is a very important tool in cancer therapy because it uses RNA interference (RNAi) to specifically mute or "knock down" gene expression [3]. siRNA has gained considerable importance in the field of treatment for cancer, specifically in the field of gene therapy. Each of these molecules, whether they are single-stranded or double-stranded, has unique properties and functions that contribute to their effectiveness [4]. siRNA induces RNA interference, effectively silencing genes associated with diseases [5].

A recently published report showed that market demand for RNAi therapeutics has surged to $1.11 billion in 2023, with projections indicating of 14.9 % compound annual growth rate (CAGR). It will drive the market to $4.28 billion by 2033. Notably, siRNA therapy

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https://doi.org/10.1016/j.onano.2024.100214
Received 11 August 2024; Received in revised form 25 August 2024; Accepted 26 August 2024
Available online 30 August 2024

holds the highest market share of 64 % [6]. The growing demand for siRNA technologies underscores their recognized value in medicine, particularly in oncology. Although it is in demand, effective delivery into cells remains challenging due to its degradation before reaching the target area, which can lead to adverse effects and increased mortality rates [7]. To effectively improve outcomes in cancer patients, it is crucial to monitor siRNA closely and identify any issues. To date, imaging [8] and molecular [9] technologies are well-known and widely accepted to monitor the delivery, absorption, and chemical processing of siRNA. Although these techniques are effective it is time-consuming and requires sophisticated equipment, which increases costs and delays treatment decisions, especially in developing countries. However, real-time monitoring of siRNA delivery can counterbalance these limitations ensuring effective targeting and providing crucial information on dosing and timing.

To counterbalance the current gap in siRNA detection, this review explores the potential of integrating established DNA/RNA-based detection methods with lab-on-a-chip (LOC) and point-of-care (POC) technologies. By leveraging the high sensitivity, selectivity, accuracy, and reliability of LOC and POC devices, we can overcome the limitations in siRNA monitoring. Developing a proof-of-concept system using these advanced (DNA/RNA) detection technologies could not only fill existing gaps but also enable accurate and dependable siRNA monitoring in practical healthcare settings, particularly in resource-limited regions. Eventually, the prospective of siRNA detection is influenced by the advancements and improvements in POC platforms, which are crucial for achieving precise and efficient monitoring in clinical practice.

2. siRNA: properties, applications, and overcoming challenges in cancers

siRNA is a powerful double-stranded RNA molecule, typically 20-25 base pairs in length, that plays a critical role in gene regulation [10]. Through the process of RNA interference (RNAi) siRNA can effectively silence genes by attaching to their mRNA and initiating its breakdown, which in turn stops the production of harmful proteins [11]. siRNA has emerged as a weapon in the fight, against cancer allowing for targeted suppression of genes [12]. When siRNA enters in cell, it combines with the RNA-induced silencing complex (RISC). After siRNA unwinds it leaves behind a strand called the guide strand which attaches to the RISC complex [13]. This guide strand directs RISC to the complementary mRNA molecule, leading to its cutting and eventual degradation effectively silencing gene expression [14]. siRNA shows potential, in treating cancer by targeting and inhibiting genes related to cancer development. Its use has proven effective in combating cancers such, as liver, pancreatic c, and leukemia. siRNA treatments target genes like MYC oncogene in hepatocellular carcinoma [15,16], K-RAS in pancreatic cancer [17,18], and FLT3 in acute myeloid leukemia [19,20]. These special therapies have shown success, in slowing the advancement of cancer and enhancing treatment results. The application of siRNA-based delivery systems for cancer treatment shows significant potential due to its unique characteristics and capabilities, as illustrated in Fig. 1a.

However, the successful translation of siRNA from bench to bedside depends on overcoming several biological [23-25] and technical challenges, including its delivery, stability, cellular uptake limitations [26-28], endosomal entrapment [29,30], and the complexities of the tumor microenvironment [31–33]. One of the primary challenges in the clinical application of siRNA is the inability to accurately monitor its presence and activity within the body. In the realm of siRNA treatments obstacles have arisen such as off-target effects and restricted pharmacokinetic characteristics. There is a chance that these obstacles could jeopardize the health and treatment effectiveness. These important concerns highlight the factors to keep in mind for the advancement of RNAi-based treatments, for cancer as shown in Fig. 1b.

To tackle these obstacles using detection techniques enables healthcare professionals to precisely monitor siRNA levels, ensuring proper dosage regimens and minimizing side effects. This system keeps an eye, on patients, around the clock allowing doctors to develop treatment strategies that optimize the treatment regimen and reduce any side effects. Furthermore, consistent observation of siRNA plays a role, in advancing the effectiveness and precision of treatments. Essentially integrating the detection and monitoring of siRNA treatments not only addresses obstacles but also establishes a foundation, for customized healthcare

Table 1
The key difference between mRNA, siRNA, and ASOs.

Characteristics features	mRNA	siRNA	ASOs
Length	1000s of nucleotides	20-25 nucleotides	15-25 nucleotides
Structure	Single-stranded RNA molecule with a 5′-cap and a 3′-polyadenylate tail	Double-stranded RNA molecule with a 2′-O-methyl group on the ribose sugar of each nucleotide	Single-stranded oligonucleotide with a 5′-phosphate group and a 3′-hydroxyl group
Function	Carries genetic information from DNA to the ribosome	Gene silencing	Modulates gene expression
Mechanism of action	Translation	Degradation	Inhibition
Target	Ribosome	mRNA	mRNA
Cellular Uptake	Difficult	Efficient	Efficient
Targeting	Protein expression	Specific gene silencing	Various gene targets
Stability	Relatively unstable and degraded within minutes to hours in cells	More stable than mRNA and can last for days to weeks in cells	The most stable of the three and can last for months to years in cells

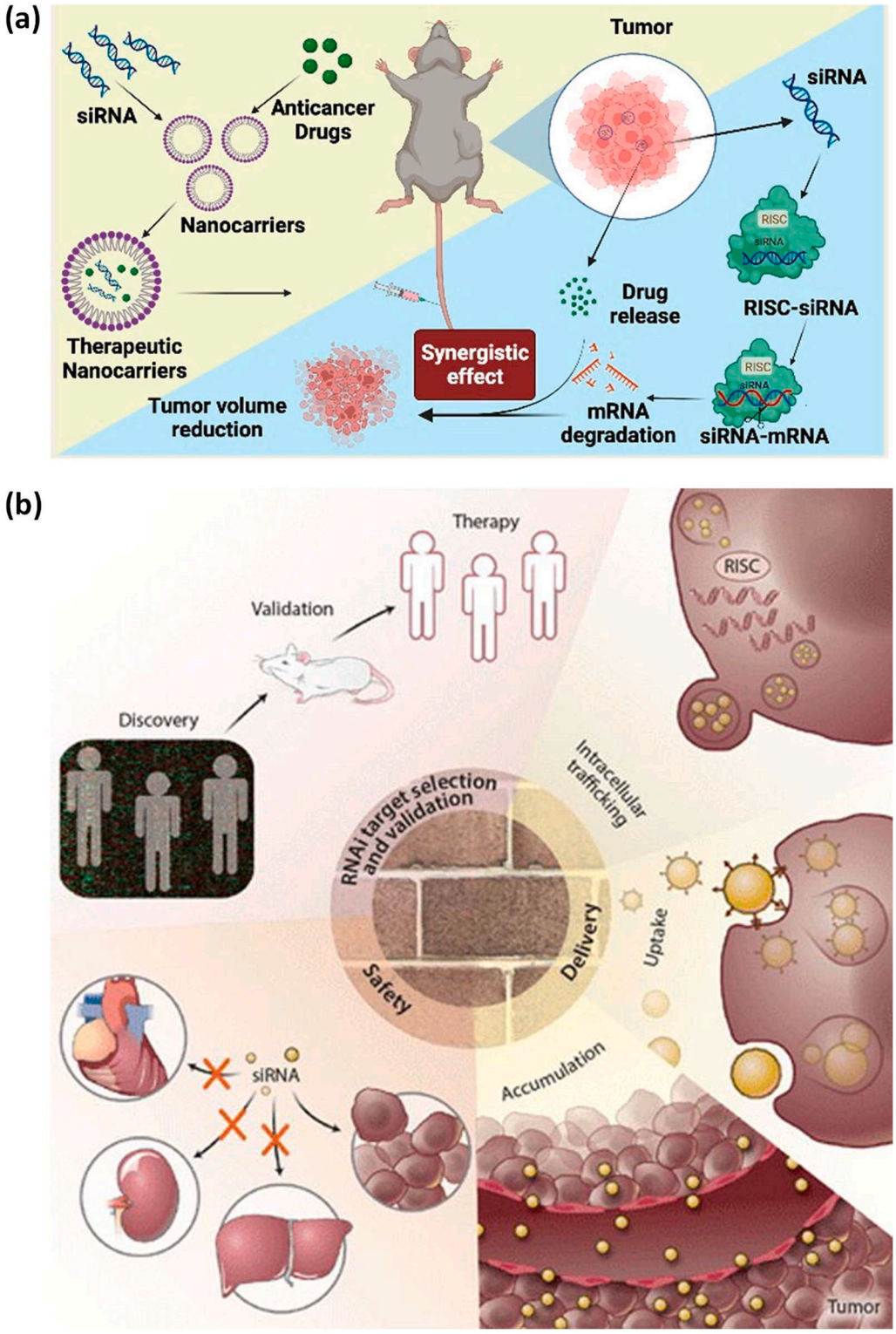

Fig. 1. : (a) Role of siRNA in cancer [21]; (b) Overcoming obstacles. There are multiple steps at which RNAi therapeutic development can hit a wall: delivery (accumulation, uptake, and intracellular trafficking), safety, and siRNA target selection and validation. Shown are critical issues to consider when designing effective RNAi-based cancer therapeutics. Sector sizes suggest degrees of difficulty in breaking the barriers [22].

3. Standard analytical techniques for siRNA detection

Analytical techniques in cancer treatment could revolutionize our approach towards therapies by improving our understanding and refining the delivery of siRNA, within the body through advancements in formulation. Analysing siRNA in the formulation is crucial for ensuring the effectiveness, durability, and safety of siRNA-based therapies. Understanding the characteristics, stability, and effectiveness of siRNA formulations is essential for enhancing therapeutic outcomes. Researchers gain valuable insights into siRNA formulation characteristics through methods that optimize delivery systems and formulation parameters. Techniques like dynamic light scattering (DLS) allow researchers to evaluate particle size and shape, aiding in the creation of reliable delivery systems [26, 34–37]. It is crucial to accurately measure the encapsulation of siRNA to ensure its effectiveness and prevent premature degradation. In the realm of cancer treatments, it is crucial to target cells while administering siRNA to silence genes and yield favorable therapeutic results. Flow cytometry [38] and confocal microscopy [39] offer information on the uptake and intracellular distribution of siRNA within cells, which helps in developing tailored delivery systems, for siRNA. Further exploration, into the mechanisms of drug distribution in the body offers knowledge of siRNA distribution in specific target areas [40]. It's essential to grasp this knowledge when assessing the safety and effectiveness of treatments. It also helps in developing delivery methods to reduce side effects and enhance treatment results.

It's important to monitor the effectiveness of siRNA treatments, in cancer care by analyzing and detecting siRNA in the body. Unlike chemotherapy, siRNA therapy specifically targets cancer cells, minimizing the side effects on healthy tissues. To ensure effective treatment, it is important to evaluate the distribution and duration of siRNA in tumors and throughout the body. Detecting siRNA can serve as a biomarker for tracking cancer progression and treatment success. This enables clinicians to assess the impact of treatments and detect early signs of resistance or relapse, enabling quick changes to the treatment plan. Effective cancer treatment demands the monitoring of siRNA delivery and distribution to optimize therapeutic outcomes and minimize side effects. Analytical techniques like polymerase chain reaction (PCR) [40], fluorescence-based assays [41], and imaging technologies [42,43] allow for the sensitive and specific detection of siRNA in biological samples.

3.1. Reverse transcription PCR (RT-PCR)

Analyzing siRNA using RT-PCR is essential for comprehending its influence on the advancement of cancer. RT-PCR is used to investigate siRNA expression in cancer, providing crucial insights into gene regulation and disease progression. This research deepens our understanding of cancer and enhances treatment approaches. Through monitoring of siRNA expression in cells, scientists can pinpoint precise targets for therapeutic intervention. RT-PCR methods are essential for obtaining precise measurements of gene expression, which are fundamental for developing tailored treatments and enhancing cancer therapy [44,45].

This monitoring strategy involves the use of reverse transcriptase, an enzyme that converts siRNA into complementary DNA (cDNA). This change is crucial because it enables the amplification of RNA-based siRNA into PCR-amplified DNA. By using the process of transcriptase siRNA changes into cDNA [46]. This process merges RNA data into a DNA structure, simplifying examination and handling. This transformation allows the use of methods like PCR to amplify and identify the cDNA. Primers, which target specific cDNA regions, ensure correct and effective amplification of the desired genetic content [47]. Different ways to assess PCR outputs involve techniques such, as gel electrophoresis and quantitative methods like qPCR or RT PCR. Specifically designed primers aim at siRNA sequences in cancer cells facilitating DNA replication by attaching to cDNA templates, throughout denaturation annealing, and extension phases. The outcomes can be examined through sequencing, real-time PCR, or gel electrophoresis [47,48]. This approach helps in amplifying and detecting RNA molecules enabling an evaluation of gene silencing outcomes that are fundamental for siRNA-related studies and therapies.

3.2. Imaging techniques

Imaging methods play a role, in cancer studies by identifying siRNAs enabling scientists to see and pinpoint genetic material sequences in cells and tissues [8]. Methods, like fluorescence in situ hybridization (FISH) and optical imaging enable the visualization of siRNAs inside cancer cells providing information, about their absorption, intracellular movement, and distribution. The FISH method accurately identifies nucleic acid sequences in cells or tissues [49]. Labeled nucleic acid probes bind to target sequences through complementary base pairing. Under favorable conditions, single-stranded oligonucleotides anneal to form double-stranded DNA or RNA hybrids. FISH is commonly used for its accuracy and dependability making it a crucial tool, in areas such, as genetics, oncology and microbiology [50]. FISH plays a role, in cancer studies by identifying and showing siRNA sequences in cells [51]. Researchers can directly observe siRNAs, in cancer cells using FISH techniques [52,53]. This visual representation of the process of siRNAs entering and moving within cells is an aspect, of comprehending their role, in cancer progression [54]. In the field of cancer research employing FISH, for siRNA detection contributes to advancing our understanding of RNA interference and plays a role in enhancing the effectiveness of siRNA-based treatments [41,55].

In the fluorescence imaging method labeled siRNA molecules are used to monitor their uptake and distribution, within cells. This approach provides sensitivity, enabling the continuous monitoring of gene silencing by siRNA in cancer cells [56]. The Fluorescence Intensity Distribution Analysis (FIDA) polarization system is a technique used to detect siRNA in cells and animals by analyzing the distribution of fluorescence intensity. Through the integration of fluorescence labeling, polarization analysis, and cutting-edge imaging techniques this method enables the sensitive detection of siRNA, within biological systems. This contributes to deepening our comprehension of RNA interference and facilitating the progress of RNA treatments [8,57]. Fluorescence microscopy and confocal

microscopy are commonly used to identify labeled siRNAs or nanoparticles that transport siRNA, in cancer cells. The method helps in understanding of distribution, accumulation, and mechanism of gene silencing [58,59]. Other imaging approaches such as positron emission tomography (PET) and magnetic resonance imaging (MRI) provide comprehensive imaging capabilities for enhancing siRNA delivery for treatment effectiveness [60].

3.3. Other techniques

Immunocytochemistry (ICC) and Immunohistochemistry (IHC) are two widely used methods for protein detection and localization in cells and tissues [61]. Experts use antibodies to precisely identify and visualize proteins or nucleic acids inside cells or tissues, facilitating the understanding of cancer progression and identification of therapy targets [62-65]. Another is, gel electrophoresis is an approach used to separate DNA, RNA, or proteins by their size and charge. In this method, an electric field is applied to a gel matrix causing charged molecules to move through the gel at speeds and arrange themselves by size. This method has played a role, in studies, particularly in identifying siRNA in cancer. It has enhanced our understanding of diseases and expanded treatment options, especially in cancer management. Gel electrophoresis enables researchers to study the siRNAs present in cancer cells or tissues [66,67]. The Northern blotting method is crucial for detecting siRNA and gaining insights into the underlying processes. This method involves the isolation and quantification of RNA, including siRNA, by gel electrophoresis, and then putting it onto a membrane. siRNA detection is accomplished by using radiolabeled or non-nucleic acid probes via molecular hybridization. Streit and colleagues conducted a research study where they used blot analysis to detect gene expression patterns at the RNA level, in cancer cells and tissues of humans specifically focusing on cancer [68].

4. Detecting siRNA: DNA/RNA approach

siRNA detection might be hard due to the absence of precise detection techniques. However, by using inventive approaches, researchers could overcome these challenges and achieve significant improvements in this field. Traditional techniques for detection, which include blotting and RT-PCR, have several limitations, including issues with sensitivity and long time required for processing, which can make them costly. Moreover, these methods often encounter challenges in differentiating between single-stranded and double-stranded structures, hence increasing the difficulty of accurately detecting siRNA. The lack of understanding hinders progress in understanding gene regulation and subsequently in the improvement of treatments. Our proposal is to develop a system that can detect siRNA by using pre-existing platforms specifically developed for single-stranded and double-stranded DNA and RNA. The article presents a platform that addresses existing limits in detection, allowing for a comprehensive investigation of siRNA behavior. This advancement has the potential to enhance gene therapy, disease diagnostics, and medication development. By addressing these limitations, the platform will unlock new possibilities in molecular biology.

Recent progress, in biosensor technologies, shows potential for sensitive detection of siRNA. Based on a literature search in PubMed using the keywords "optical biosensors" and "electrochemical biosensors," approximately 14,637 papers have been published on optical biosensors and 22,120 papers on electrochemical biosensors from 2004 to the present year. The outcome of the findings reflects the trend, that electrochemical biosensors have gained more attention in the scientific community due to their straightforward instrumentation, cost-effectiveness, and versatility. Both biosensors are recognized for their flexibility, in detecting both double-stranded molecules like DNA and RNA. By integrating these two technologies, researchers can develop sensors that provide sensitivity, selectivity, and the ability to detect targets simultaneously. In addition, the advancement of, on-the-spot tools for detecting siRNA could bring about a transformation in healthcare and tailored medical treatments. Yet there are hurdles like standardizing assays dealing with sample intricacies and ensuring the stability of biosensors that must be overcome to unleash the capabilities of these innovations. Moving forward research endeavors must concentrate on refining probe construction enhancing signal amplification techniques and developing bioinformatics resources to support dependable detection of siRNA, in biological settings.

4.1. Optical biosensors

Optical biosensors are advantageous due to their compact size, high sensitivity, and immunity to stray fields. These features enable portable and accurate detection, making them valuable for various applications [69,70]. Hakimian et al. developed an ultrasensitive optical biosensor for early-stage breast cancer diagnosis by detecting microRNA-155 (miR-155). The biosensor utilizes covalent binding between probe DNA and negatively charged gold nanoparticles, followed by electrostatic adsorption of the target miR-155 onto positively charged gold nanoparticles. Hybridization between the two nanoparticle complexes enables quantification of miR-155 content. The biosensor demonstrates specificity for miR-155, including detection of mismatches and differentiation from genomic DNA. Its novelty lies in trapping the label-free target using branched positively charged polyethylenimine, increasing target loading on the nanoparticles' surface. The biosensor achieves highly sensitive miR-155 detection with a low detection limit of 100 aM and a wide linear range from 100 aM to 100 fM [71]. Recently, Kadhim et al reported biosensor-based graphene oxide (GO)-DNA nanohybrid for lung cancer. They presented a DNA-GO nanohybrid biosensor for identifying deletion mutations associated with lung cancer. The GO nanomaterial was synthesized using Hummers' method and confirmed through FT-IR spectrometry, UV-vis spectrometry, and TEM imaging. The biosensor utilizes a FAM-labeled DNA probe and fluorescence spectrometry for mutation detection [72]. Rodríguez Montelongo et al. have developed porous silicon (PSi) biosensors for detecting high-risk human papillomavirus 16 and 18 (HPV16 and HPV18), which are associated with the development of pre-cancerous and cancerous lesions. The biosensors utilize PSi, a biocompatible material with unique optical properties and a porous structure that enables easy surface modification. By attaching

HPV 16 and 18-specific ssDNA oligonucleotides inside PSi pores, the biosensor (PSiMc/HPV-ssDNA) can selectively detect molecular binding through a shift in reflectance spectra. The biosensor demonstrated the ability to discriminate between complementary and non-complementary DNA. These findings suggested that the PSi biosensor, based on reflectance spectra shift, could serve as a practical and portable device for HPV detection. The decision to use a PSi microcavity was influenced by its properties the resonance modes [73].

4.2. Electrochemical biosensor

Electrochemical techniques show promise, in detecting cancer due to their sensitivity, simplicity, and cost-effectiveness [74,75]. By leveraging the characteristics of cancer biomarkers these methods allow for both qualitative analysis [76]. Electrochemical biosensors offer detection of cancer biomarkers, in biological samples fostering early cancer detection personalized therapy, and improved patient outcomes [77-80]. Cimmino and co-workers developed a platform with screen-printed gold electrodes to detect miRNA 21, a marker associated with cancer. By employing a DNA sequence modified with methylene blue and utilizing square wave voltammetry optimization they successfully reached a detection threshold of 2 nM. This innovative technique provides a method, for cancer detection through the analysis of liquid biopsies [81]. Chen et al. developed a highly sensitive electrochemical biosensor using DNA-modified gold-coated magnetic nanoparticles for rapid DNA methylation detection in blood. It distinguishes methylated from unmethylated DNA with a range of 2 aM to 20 nM and a limit of 2 aM, providing results in 35 minutes and aiding in minimally invasive ovarian cancer diagnosis [82]. Zhang et al. developed a sensitive technique for circRNA detection using gold nanoparticle-modified electrodes. Magnetic beads with capture probes isolate circRNA, which is then detected through changes in the methylene blue signal. This method shows high sensitivity with LOD = 1.0 pM and indicating its clinical potential [83].

Advancements, in electrochemical biosensors, show potential for detecting DNA/RNA with sensitivity and efficiency. The DNA/RNA method can be proposed as a platform for detecting siRNA quantitatively. The integration of optical and electrochemical techniques enables the development of hybrid sensing platforms endowed with heightened sensitivity, selectivity, and multiplexing capabilities for siRNA detection. Furthermore, the advent of portable, POC devices holds transformative potential in diagnostics and personalized medicine, offering accessibility and convenience in healthcare delivery. Based on this hypothesis, we presented Table 2 summarizing the analytical features of the described sensing platforms for DNA/RNA detection using optical and electrochemical methods. The tables include details on the LOD, sensitivity, and linear detection range, along with the corresponding references.

5. Future prospects and conclusion

The ongoing demand for precise and accurate monitoring in siRNA-based cancer research highlights the critical importance of future advancements in siRNA detection. siRNA has become an important tool for silencing genes showing potential, in cancer management. Traditional methods for siRNA detection often rely on labor-intensive techniques with limited sensitivity and specificity. While cutting-edge technologies like sequencing, single-molecule imaging, and droplet digital PCR have shown potential, there is still room for improvement in enhancing their ability to accurately measure siRNA levels [93]. Improving the precision of siRNA detection could aid in studying their impact and potentially reveal targets, for cancer treatment. Furthermore, there are forms in which siRNAs can be found potentially impacting their effectiveness. Understanding these differences is crucial, for grasping their roles in developing treatment methods accurately [94,95]. With the progress made in detecting siRNA, there are still opportunities, for further innovation and improvement.

Detecting siRNA presents challenges that necessitate improvements in sensitivity, precision, and effectiveness. Advancements, in the fields of biology, bioinformatics, and nanotechnology are expected to enhance the effectiveness and flexibility of detection methods, in the future. By integrating DNA/RNA-based optical and electrical technologies, with advancements could lead to the development of portable devices, for detecting siRNA, which could greatly impact diagnostic practices. These portable devices could revolutionize the monitoring of siRNA, in a cost-efficient manner. This advancement has the potential to support personalized

Table 2
Optical and electrochemical techniques for detection of DNA/RNA.

Optical detection methods for DNA/RNA					
Sensor type	Target	LOD	Sensitivity	Linear detection rang	Reference
Thiolated probe/aunp and PEI-aunp/target	miRNA	100 aM	superiority in hybridization	100 aM to 100 fM	[71]
DNA-modified gold nanoparticle	DNA	300 nM	moderate	0–100 pmol	[84]
AuNPs	MiRNA-205	3.8 pM	moderate	3.8 pM to 10 nM	[85]
Fluorescence quenching of graphene oxide (GO)	MiRNA-126	3.0 fM	high sensitivity	0.02 to 100 pM	[86]
multicomponent AuNP-based nanoprobe	DNA	25 pM	high sensitivity	25 pM– 1.0 nM	[87]
Electrochemical detection methods for DNA/RNA					
Zinc Finger Protein Specific to DNA–RNA Hybrids	miRNA-21	2 fM	high sensitivity	2 fM to 1 nM	[88]
Graphene-Composite Electrodes	miR-29b-1 and miR-141	5 fM	moderate	1 fM to 1 nM	[89]
MXene/Pt/C nanocomposite	DNA	0.4 aM	high sensitivity	1 aM–100 nM	[90]
RNA-triggered Cu^{2+} reduction method	miRNA	33.2 zM	high sensitivity	1 aM - 10 nM	[91]
Au nanoporous electrode array	viral RNA	~1 fM	high sensitivity	100 pM to 1 fM	[92]

treatments and interventions, for cancer providing needed relief to patients.

Emerging DNA/RNA approaches provide fascinating possibilities for developing siRNA detection devices with vast diagnostic and therapeutic uses. Additionally, exciting developments are happening in the fields of artificial intelligence (AI) and machine learning (ML), particularly in the areas of healthcare, ethical AI, and natural language processing (NLP). These developments are expected to result in remarkable discoveries in automated diagnosis, human-computer interaction, and tailored treatment, which will change the way healthcare is provided and raise important ethical issues.

This review emphasizes the bright outlook for POC or LOC-based premature cancer detection and siRNA-based therapeutic monitoring, which can prevent drug resistance and unintended therapy delivery to non-targeted sites. These exciting advancements have the potential to greatly propel science and technology forward, bringing about a positive change in society and fostering fairness.

CRediT authorship contribution statement

Sima Singh: Writing – review & editing, Writing – original draft. **Ada Raucci:** Writing – review & editing, Data curation. **Wanda Cimmino:** Writing – review & editing. **Antonella Miglione:** Writing – review & editing. **Panagiota M Kalligosfyri:** Writing – review & editing, Writing – original draft. **Stefano Cinti:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We acknowledge the grant CN00000041, “National Center for Gene Therapy and Drugs based on RNA Technology” (concession number 1035 of June 17, 2022 - PNRR MUR - M4C2 - Investment 1.4 Call “National Centers,” financed by the EU-NextGenerationEU), project code CUP E63C22000940007. S.C. acknowledges funding from AIRC under the MFAG 2022 (ID 27586) project. This project also received funding from the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 101110684 (SMART - Sima Singh).

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