Point-of-Care Testing of microRNAs

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MicroRNAs (miRNAs) are a class of small noncoding RNAs that are approximately 22 nt in length and regulate gene expression post-transcriptionally. miRNAs play a vital role in both physiological and pathological processes and are regarded as promising biomarkers for cancer, cardiovascular diseases, neurodegenerative diseases, and so on. Accurate detection of miRNA expression level in clinical samples is important for miRNA-guided diagnostics. However, the common miRNA detection approaches like RNA sequencing, qRT-PCR, and miRNA microarray are performed in a professional laboratory with complex intermediate steps and are time-consuming and costly, challenging the miRNA-guided diagnostics. Hence, sensitive, highly specific, rapid, and easy-to-use detection of miRNAs is crucial for clinical diagnosis based on miRNAs. With the advantages of being specific, sensitive, efficient, cost-saving, and easy to operate, point-of-care testing (POCT) has been widely used in the detection of miRNAs.

microRNA (miRNA) point-of-care testing (POCT) visual detection portable instruments

1. Introduction

MicroRNAs (miRNAs) are a type of small noncoding RNA with a length of ~21–25 nt that act as regulators of gene expression at the post-transcriptional level [1]. The miRNA genes are transcribed into hairpin-containing pre-miRNA by RNA polymerase III, and the long dsRNA precursors are processed by Drosha and Dicer consecutively [2][3]. The generated small dsRNAs are loaded onto an argonaute family protein (AGO) to form an RNA-induced silencing complex (RISC). After loading, the passenger strand of the miRNA duplex exits to produce a single-stranded mature miRNA, and the mature RISC induces translational repression, mRNA deadenylation, and mRNA decay [4][5]. miRNAs play vital roles in development. miRNAs regulate cellular activities, including cell growth, differentiation, and apoptosis, and aberrant expression of miRNAs promotes the occurrence and development of diseases. In recent decades, miRNAs have been implicated in various human diseases. Hence, many studies have attempted to apply miRNAs to disease diagnosis, and miRNAs show great promise as diagnostic biomarkers, as miRNAs can not only circulate in the human blood in remarkably stable forms, such as exosomes, but they are also widely present in other bio-microenvironments, such as urine, saliva, and cerebrospinal fluid [6][7]. Accurate detection of dysregulated circulating miRNAs in biofluids is important for miRNA-guided diagnostics in a noninvasive fashion. There have been many conventional methods for the quantitative detection of miRNAs, such as northern blot, microarray, RNA-seq and RT-qPCR [8]. Although these traditional methods are relatively highly sensitive and specific, these approaches also have various limitations. For example, northern blotting and real-time PCR are sensitive and specific, but they are also labor-intensive and require specialized equipment. Microarray and RNA-seq are high-throughput methods that allow the simultaneous detection of multiple miRNAs, but they are also

expensive and require complex data analysis, and these approaches for miRNA detection are performed in a professional laboratory, which is challenging for the application of miRNA detection in clinical practice. Therefore, it has driven the development of reliable point-of-care testing (POCT) of miRNAs. Point-of-care testing (POCT) is defined as testing performed near or in the field of a patient, for whom faster results may lead to changes in patient care [9]. Recently, POCT has been applied to the quantitative detection of miRNAs and has made rapid progress. To be more detailed, POCT can provide accurate and ultrasensitive tumor screening results for patients with the advantages of a no-fuss operation, low cost, and rapidity [10][11][12][13]. At the same time, POCT is also suitable for resource-limited areas, or even for self-testing. Previous reviews provided valuable information on the evolution of POCT-detection methods for miRNAs and the applied amplification strategies in POCT for miRNAs [14][15]. The development in detection of multiple miRNAs and the new progress in biosensors, microfluidics, and lateral flow assays (LFAs) for miRNA detection have also been well reviewed [16][17][18].

2. POCT of miRNAs

Point-of-care testing (POCT) is defined as testing conducted near or at the site of the patient, and rapid testing may improve patient care [9]. POCT can provide accurate and ultrasensitive disease screening results for patients with the advantages of easy operation, low cost, rapidity, and a visual readout [10][11][12][13]. The development and validation of POCT for early screening of a series of clinical diseases holds great significance. Moreover, POCT provides the possibility of medical guidance and disease screening in remote areas. Recently, POCT has been applied to the rapid and quantitative detection of miRNAs and has made rapid progress. Microfluidics, paper-based biosensors, portable instruments, and visual detection play important roles in POCT and are very promising methods for POCT of miRNAs. To date, dozens of specialized strategies of miRNA detection based on microfluidics and paper-based biosensors have been reported. Microfluidics and paper-based biosensors for miRNA detection have been well reviewed [17][18].

2.1. POCT of miRNAs Based on Portable Instruments

To avoid the need for bulky instruments and auxiliary devices to obtain a high-sensitivity quantitative signal output, we urgently need a sensing strategy that is controllable, low in cost, and independent of sophisticated equipment but that can offer automated readouts for disease-related miRNAs. In this section, the researchers introduce the current situation of the application of off-the-shelf instruments in miRNA detection, analyze and evaluate the possibility and feasibility of their application, and predict their future development trend. A summary of reported POCT methods for miRNAs based on portable instruments is presented below (**Table 1**).

Table 1. The detection methods of miRNAs based on portable instruments.

Methods	miRNA	Detection Limit	Samples	Time	Reference
Personal glucose meter	miR-21	0.41 nM/ 1 million cells	synthesized miR-21/A549 cell lysates	<2 h	[19]
	miR-21	10 fM	synthesized miR-21	<2 h	[20]
	miR-21 miRNA205	2.4 pM 1.1 pM	synthesized miR-21 synthesized miRNA205	<3 h	[21]
	miR-21	3.65 nM	synthesized miR-21 clinical serum samples from cancer patients	2 h	[22]
	miR-21	60 pM 3×10^6 cells/mL	synthesized miR-21 MCF-7, A549 and HeLa cell lysates	<3 h	[23]
	miR-21	68.08 fM	synthesized miR-21 urine samples from DIKI mice	1.5 h	[24]
	miRNA-155	0.36 fM	synthesized miRNA-155	>5 h	[25]
	miR-21, miR-335, miR-155, and miR-122	0.325 fmol	synthesized miRNAs extract from HeLa, HepG2, MCF-7, and L02 Cells	6 h	[26]

Methods	miRNA	Detection Limit	Samples	Time	Reference
Thermometer	miR-21	7.8 nM	synthesized miR-21 HeLa cell lysate	Not mentioned	[27]
	miRNA-141	0.5 pM	synthesized miRNA-141	>8 h	[28]
Pressure meter	miR-21	7.6 fM 100 cells	synthesized miR-21 A549, MCF-7, HepG2 and HL-7702 cells	20 min	[29]
	miR-21	10 pM	Serum	0.5 h	[30]
Portable fluorometer	miR-574-5p	2 ng/μL	RNA extract from 5XFAD mice	>3 h	[31]
Capillary force meter	miR-21	10 nM	Human serum	1 h	[32]
	miR-21		MCF-7 cell line	25 min	[33]
Smartphone	miR-133a	0.3 pM	synthesized miR-133a in serum	>5 h	[34]
	miRNA-499, miRNA-133a	10 fM	synthesized miR-133a in serum	13 h	[35]
	let-7a	1.7 fM	synthesized let-7a	2.75 h	[36]

Methods	miRNA	Detection Limit	Samples	Time	Reference
			human serum		
	miR-133a, miR-499	1 fM	Synthesized miRNAs human serum	-	[37]
	miR-21, let-7a	fM	Synthesized miRNAs human serum	<2 h	[38]
	miR-21	1.43 pM	Synthesized miR-21 human serum, urine	0.5 h	[39]
	miR-224	1.6 fM	Synthesized miR-224 human plasma	<4.5 h	[40]
	miR-21	100 fM 500 cells	Synthesized miR-21 MCF-7 and L02 cells	>1 h	[41]

4. Higa, G.S.; de Sousa, E.; Walter, L.T.; Kinjo, E.R.; Resende, R.R.; Kihara, A.H. MicroRNAs in neuronal communication. Mol. Neurobiol. 2014, 49, 1309–1326.

5. Vishnoi, A.; Rani, S. MiRNA Biogenesis and Regulation of Diseases: An Overview. Methods Mol. Biol. 2017, 1509, 1–10.

6. Weber, J.A.; Baxter, D.H.; Zhang, S.; Huang, D.Y.; Huang, K.H.; Lee, M.J.; Galas, D.J.; Wang, K. The microRNA spectrum in 12 body fluids. Clin. Chem. 2010, 56, 1733–1741.

7. Mitchell, P.S.; Parkin, R.K.; Kroh, E.M.; Fritz, B.R.; Wyman, S.K.; Pogosova-Agadjanyan, E.L.; Peterson, A.; Noteboom, J.; O'Briant, K.C.; Allen, A.; et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc. Natl. Acad. Sci. USA 2008, 105, 10513–10518.

8. Cheng, Y.; Dong, L.; Zhang, J.; Zhao, Y.; Li, Z. Recent advances in microRNA detection. Analyst 2018, 143, 1758–1774.

2.2. Visual Detection of miRNAs Based on Colorimetry

Visual detection is particularly attractive for POCT because the readout can be read with the naked eye with no need for instruments. In this section, the researchers summarize recent advances in the visual detection of miRNAs, mainly focusing on colorimetric methods. The researchers provide an all-sided discussion of the principles of the methods and rationally evaluate the applicability of these visual detection methods for early diagnosis based on miRNA detection. A summary of the reported POCT for miRNAs based on colorimetric methods is presented below (**Table 2**).

Colorimetric assays provide qualitative or quantitative measurement of targets by measuring color changes with no need for special instruments. Detecting a change in color can be used to determine the presence or absence of a

target or even to determine its amount range. As color changes can be conveniently judged by the naked eye, colorimetric assays have attracted attention for POCT of miRNAs [12].

Table 2. The detection methods of miRNAs based on visual methods.

Methods	miRNA	Detection Limit	Samples	Time	Reference
Colorimetric detection based on Au-NPs	miR-21 miR-155	5 ng μL^{-1}	Plasma	<3 min	[42]
	miR-93 miR-223	-	Human serum	-	[43]
	miR-34a miR-210	50 ng μL^{-1}	Urine	<20 min	[44]
	miR-195	40 fM	Human serum	10 min	[45]
	miR-210-3p	10 pM	Urine	20 min	[46]
	miR-21 miR-155	1 ng μL^{-1}	Multiple cancerous cell lines and primary fibroblast	<10 min	[47]
	miR-21	3 pM	Synthesized miRNA human serum samples	<5 h	[48]

9. Luppa, P.B.; Muller, C.; Schlichtiger, A.; Schlebusch, H. Point-of-care testing (POCT): Current techniques and future perspectives. Trends Anal. Chem. 2011, 30, 887–898.

10. Wei, H.; Peng, Y.; Bai, Z.; Rong, Z.; Wang, S. Duplex-specific nuclease signal amplification-based fluorescent lateral flow assay for the point-of-care detection of microRNAs. Anal. 2021, 146, 558–

21. Gong, S.; Li, J.; Pan, W.; Li, N.; Tang, B. Duplex-Specific Nuclease-Assisted CRISPR-Cas12a Strategy for MicroRNA Detection Using a Personal Glucose Meter. Anal. Chem. 2021, 93, 10719–

Methods	miRNA	Detection Limit	Samples	Time	Reference
	miR-141				
	miR-137	0.5 nM	Plasma	1 min	[49]
	miR-146a	5 nM	Raw cow milk	20 min	[50]
	let-7a	0.13 pM	A549 cells	50 min	[51]
	miR-148a	1.9 nM	Plasma	5 min	[52]
	miR-122	16 pM	Cancerous cell lines	2 h	[53]
	let-7a	3.13 fM	Human serum	1 h	[54]
	miR-203	10 pM	MCF-7 cells	-	[55]
	miR-21	0.23 fM	HeLa, MCF-7, AGS cells	0.5 h	[56]
	let-7a	4.176 aM	Synthesized let-7a	1 h	[57]
	miR-	46 fM	BEL-7404, MDA-MB231,	1 h	[58]

33. Wang, H.; Wang, H.H.; Li, Y.S.; Jiang, C.; Chen, D.S.; Wen, Y.Q.; Li, Z.P. Capillarity self-driven DNA hydrogel sensor for visual quantification of microRNA. Sens. Actuat. B-Chem. 2020, 313,

Methods	miRNA	Detection Limit	Samples	Time	Reference
	221-3p		HeLa, and 22Rv1cells		
	miR-143	1 fM	Synthesized miR-143 Prostate cancer cell lines VCaP, LNCaP, Du145, and PC-3	>1.5 h	[59]
Colorimetric detection based on enzymatic chromogenic reactions	let-7a	7.4 fM	Synthesized let-7a	2.5 h	[60]
	miR-122	0.15 aM	Serum	5 min	[61]
	miR-21	0.2 pM	Serum	50 min	[62]
	miR-21	1 aM	Serum	<4 h	[63]
	Let-7a	34 fM	A549 cells	4 h	[64]
	miR-10b	1 fM	Serum and cell extracts	20 min	[65]
	miR-141	0.48 nM	Serum	>3 h	[66]
	miR-21	1 pM	Serum	150 min	[67]

Determination of certain urinary microRNAs as promising biomarkers in diabetic nephropathy patients using gold nanoparticles. Anal. Biochem. 2020, 609, 113967.

45. Xu, Z.; Zheng, K.; Du, Z.; Xin, J.; Luo, M.; Wang, F. Colorimetric identification of miRNA-195 sequence for diagnosing osteosarcoma. Biotechnol. Appl. Biochem. 2022, 69, 974–980.

Methods	miRNA	Detection Limit	Samples	Time	Reference
	miR-141	0.5 pM	Prostate cancer cells	210 min	[68]
	miR-21	90.3 fM	Serum	<1.5 h	[69]
	miR-21, miR-17	1.7 fM	MCF-7	80 min	[70]
	let-7a	0.1 nM	Serum	3 min	[71]
	miR-21	44.76 fM	Exosome	2 h	[72]
	miR-21, miR-155	0.38 nM	Blood	>1 h	[73]
	miR-21	4.5 nM	MCF-7 and serum	130 min	[74]
	miR-21	5 fM	Plasma sample Cancer cells Tumor tissues	>6.5 h	[75]

amplification reaction and triplex DNA-mediated aggregation of gold nanoparticles for sensitive colorimetric detection of microRNA. Anal. Chim. Acta 2020, 1095, 179–184.

57. Jiang, J.; Zhang, B.; Zhang, C.; Guan, Y. A Novel Design Combining Isothermal Exponential Amplification and Gold-Nanoparticles Visualization for Rapid Detection of miRNAs. Int. J. Mol. Sci. 2018, 19, 3374.

Methods	miRNA	Detection Limit	Samples	Time	Reference
	miR-155	0.6 pM	Plasma	15 min	[76]
	miR-205, miR-944	36.4 fM	Serum	>2 h	[77]
	miR-155	31.8 fM	Serum	1 h	[78]
	miR-223 miR-143	20 pM	Synthesized miR-223 iPSCs and CMs	3.5 h	[79]

64. Li, R.; Liu, Q.; Jin, Y.; Li, B. Sensitive colorimetric determination of microRNA let-7a through rolling circle amplification and a peroxidase-mimicking system composed of trimeric G-triplex and hemin DNAzyme. Mikrochim. Acta 2020, 187, 139.
65. Zhou, T.; Huang, M.; Lin, J.; Huang, R.; Xing, D. High-Fidelity CRISPR/Cas13a trans-Cleavage-Triggered Rolling Circle Amplified DNAzyme for Visual Profiling of MicroRNA. Anal. Chem. 2021, 93, 2038–2044.
66. Park, Y.; Lee, C.Y.; Kang, S.; Kim, H.; Park, K.S.; Park, H.G. Universal, colorimetric microRNA detection strategy based on target-catalyzed toehold-mediated strand displacement reaction. Nanotechnology 2018, 29, 085501.
67. Hosseinzadeh, E.; Ravan, H.; Mohammadi, A.; Pourghadamyari, H. Colorimetric detection of miRNA-21 by DNAzyme-coupled branched DNA constructs. Talanta 2020, 216, 120913.
68. Zhou, W.; Liang, W.; Li, X.; Chai, Y.; Yuan, R.; Xiang, Y. MicroRNA-triggered, cascaded and catalytic self-assembly of functional "DNAzyme ferris wheel" nanostructures for highly sensitive colorimetric detection of cancer cells. Nanoscale 2015, 7, 9055–9061.
69. Yang, X.; Yuan, L.; Xu, Y.; He, B. Target-catalyzed self-assembled spherical G-quadruplex/hemin DNAzymes for highly sensitive colorimetric detection of microRNA in serum. Anal. Chim. Acta 2023, 1247, 340879.

70. Yan, Y.; Shen, B.; Wang, H.; Sun, X.; Cheng, W.; Zhao, H.; Ju, H.; Ding, S. A novel and versatile nanomachine for ultrasensitive and specific detection of microRNAs based on molecular beacon initiated strand displacement amplification coupled with catalytic hairpin assembly with DNAzyme formation. Anal. 2015, 140, 5469–5474.
71. Xu, H.; Yang, F.; Chen, D.; Ye, W.; Xue, G.; Jia, L. Trigging stepwise-strand displacement amplification lights up numerous G-quadruplex for colorimetric signaling of serum microRNAs. iScience 2023, 26, 106331.
72. Xia, Y.; Chen, T.; Zhang, L.; Zhang, X.; Shi, W.; Chen, G.; Chen, W.; Lan, J.; Li, C.; Sun, W.; et al. Colorimetric detection of exosomal microRNA through switching the visible-light-induced oxidase mimic activity of acridone derivate. Biosens. Bioelectron. 2021, 173, 112834.
73. Agahi, M.; Rahaie, M. A novel DNA tweezers-based nanobiosensor for multiple detections of circulating exosomal microRNAs in breast cancer. Anal. Biochem. 2022, 651, 114697.
74. Lan, L.; Wang, R.L.; Liu, L.; Cheng, L. A label-free colorimetric detection of microRNA via G-quadruplex-based signal quenching strategy. Anal. Chim. Acta 2019, 1079, 207–211.
75. Zeng, Y.; Yue, H.; Cao, B.; Li, Y.; Yang, M.; Mao, C. Target-Triggered Formation of Artificial Enzymes on Filamentous Phage for Ultrasensitive Direct Detection of Circulating miRNA Biomarkers in Clinical Samples. Angew. Chem. Int. Ed. Engl. 2022, 61, e202210121.
76. Borghei, Y.S.; Hosseini, M.; Ganjali, M.R. Visual detection of miRNA using peroxidase-like catalytic activity of DNA-CuNCs and methylene blue as indicator. Clin. Chim. Acta Int. J. Clin. Chem. 2018, 483, 119–125.
77. Gong, S.; Wang, X.; Zhou, P.; Pan, W.; Li, N.; Tang, B. AND Logic-Gate-Based CRISPR/Cas12a Biosensing Platform for the Sensitive Colorimetric Detection of Dual miRNAs. Anal. Chem. 2022, 94, 15839–15846.
78. Ying, N.; Sun, T.; Chen, Z.; Song, G.; Qi, B.; Bu, S.; Sun, X.; Wan, J.; Li, Z. Colorimetric detection of microRNA based hybridization chain reaction for signal amplification and enzyme for visualization. Anal. Biochem. 2017, 528, 7–12.
79. Broto, M.; Kaminski, M.M.; Adrianus, C.; Kim, N.; Greensmith, R.; Dissanayake-Perera, S.; Schubert, A.J.; Tan, X.; Kim, H.; Dighe, A.S.; et al. Nanozyme-catalysed CRISPR assay for preamplification-free detection of non-coding RNAs. Nat. Nanotechnol. 2022, 17, 1120–1126.