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Article

Amplification-Free and Label-Free Multiplexed Profiling of Extracellular Vesicle-Derived MicroRNA via Micropore Sensing Based on PNA-Functionalized Hydrogel Barcodes

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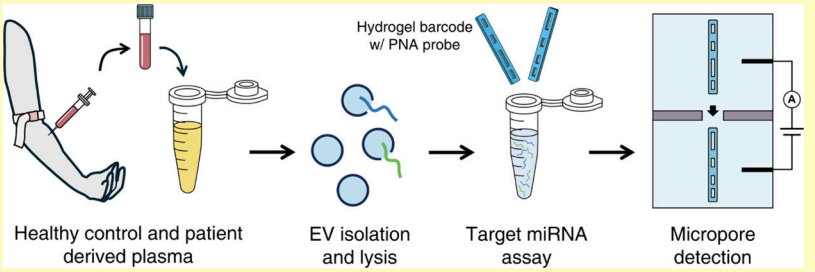
ABSTRACT: Extracellular vesicle (EV)-derived microRNA (miRNA) is a promising biomarker for various diseases, including cancer. However, the current EV-miRNA detection technologies, such as RT-qPCR and microarray, have depended on the complex amplification and labeling processes, which are not preferred for constructing an on-site diagnosis system. Herein, we present an EV-miRNA detection platform utilizing micropore sensing based on peptide nucleic acid (PNA)-functionalized hydrogel barcodes. Based on the low background signal and high affinity to the miRNA of the PNA probes, the breast cancer-related miRNAs (miR-21 and let-7a) can be detected with femtomolar sensitivities (481 and 551 fM) without any amplification and labeling steps by penetrating the target-captured barcodes into the pore and analyzing the electrical signal. By designing the geometrical codes of the particles, the multiplexed detection of miR-21 and let-7a can be implemented with high specificity and practically applicable recovery rates. Finally, we validate the clinical potential of the presented assay by differentiating the expression patterns of the plasma EV-derived miR-21 and let-7a between the breast cancer patients and healthy donors.



KEYWORDS: *extracellular vesicle, microRNA, biosensing, hydrogel, micropore*

MicroRNA (miRNA) is a gene-regulatory RNA fragment normally composed of 18–22 nucleotides.[1] The miRNA has been known to regulate the gene expression process by inhibiting translation and promoting the decomposition of mRNA.[2] Recently, the miRNA has been regarded as a powerful biomarker in diagnostic fields attributed to the deep relevance to the occurrence and development of diverse severe and/or chronic diseases such as cancer,[3]Alzheimer's disease,[4] and rheumatoid arthritis.[5] Especially, detecting the miRNA in extracellular vesicles (EVs), the nanovesicle involved in cell-cell communication, is a cutting-edge liquid biopsy tool since the miRNA in EVs has superior stability against degradation by nucleases in the biological environment.[3,6−8] This excellent stability of EV-miRNA is highly important in the miRNA-based liquid biopsy, enabling reliable and reproducible analysis. Furthermore, considering that a large amount of miRNA is concentrated in EVs,[9] the miRNA detection from the extracted EVs can lead to sensitive and specific analysis instead of detecting miRNA from complex media containing numerous molecules and cell debris.

The representative miRNA sensing technique is a reverse transcription-quantitative polymerase chain reaction (RT-qPCR).[10,11] Based on its excellent sensitivity and specificity, RT-qPCR has been used as a gold-standard miRNA detection technology in research and clinical fields. However, the RT-qPCR has inherent limitations for the miRNA sensing. The major limitation of the RT-qPCR is the requirement for reverse transcription of miRNA into complementary DNA (cDNA), which involves the addition of nucleotides to the miRNA for primer binding. This process makes the overall assay time-consuming and labor-intensive.[12] Furthermore, the entailment of the sophisti- cated primer design and spectral overlapping in the fluorescence-based encoding in multiplex RT-qPCR constrains the simultaneous analysis of multiple miRNAs.[10] This limited multiplexing capability of the RT-qPCR limits the throughput and accuracy of clinical analysis.

As an alternative to RT-qPCR, the microarray technique can be utilized to avoid cumbersome miRNA pretreatment and enhance multiplexing capability.[10,13] The microarray technique

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