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Ratiometric Detection of microRNA Using Hybridization Chain Reaction and Fluorogenic Silver Nanoclusters

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Abstract: miRNA (miR)-155 is a potential biomarker for breast cancers. We aimed at developing a nanosensor for miR-155 detection by integrating hybridization chain reaction (HCR) and silver nanoclusters (AgNCs). HCR serves as an enzyme-free and isothermal amplification method, whereas AgNCs provide a built-in fluorogenic detection probe that could simplify the downstream analysis. The two components were integrated by adding a nucleation sequence of AgNCs to the hairpin of HCR. The working principle was based on the influence of microenvironment towards the hosted AgNCs, whereby unfolding of hairpin upon HCR has manipulated the distance between the hosted AgNCs and cytosine-rich toehold region of hairpin. As such, the dominant emission of AgNCs changed from red to yellow in the absence and presence of miR-155, enabling a ratiometric measurement of miR with high sensitivity. The limit of detection (LOD) of our HCR-AgNCs nanosensor is 1.13 fM in buffered solution. We have also tested the assay in diluted serum samples, with comparable LOD of 1.58 fM obtained. This shows the great promise of our HCR-AgNCs nanosensor for clinical application.

Introduction

MicroRNAs (miRs) are short endogenous non-coding RNAs that regulate post-transcriptional gene expression. Over the years, miRs have been extensively studied for diagnostic and prognostic values as biomarkers, owing to their remarkable stability in human blood and serum, as well as their dysregulated expression on the onset of metabolic conditions.[1] For example, breast cancer patients often report an overexpression of miR-155, as compared to healthy individuals.[2] However, current detection methods for miRs, such as small RNA sequencing, RT-qPCR and miRs microarray, pose a challenge due to inconsistencies in the miRs extraction methods, quality control, stability issues, as well as high labor and operational costs.[3]

Recent studies have been focused on developing biosensors that are cost-effective, fast, simple and with direct-sampling, by utilizing different sensing strategies.[4] These include electrochemical,[4a] surface plasmon resonance,[4b] colorimetric[4c] and fluorescence biosensors.[4d] Fluorescence-based biosensors, in particular, has gained high attention owing to their high sensitivity, selectivity and simple operation.[5] DNA-templated silver nanoclusters (DNA-AgNCs) belong to a class of label-free fluorescence probes with tuneable emission colours.[6] Comprising two to tens of silver atoms, the DNA-AgNCs are smaller and less probe to blinking problem as compared to quantum dots. They are also comparatively brighter and more photostable than organic dyes.[7] While DNA template renders great versatility to the materials, the AgNCs hosted in a DNA template are found to possess more extensive and tunable emission range, in contrast to nanoclusters of other metal types.[8] The DNA-AgNCs are extremely sensitive to their microenvironment, whereby presence of other biomolecules (*e.g.* nucleic acid targets or proteins) may disrupt their fluorescence output.[9] As such, this nanomaterial could serve an excellent signal transducer for detection of biomolecules.[10] For instance, one can apply the DNA-AgNCs in tandem with different amplification strategies to further augment the analytical performance of a biosensor.

Hybridization chain reaction (HCR) is an isothermal amplification method that functions through self-assembly of two or more DNA hairpins in the presence of an initiator or target molecule.[11] A DNA hairpin comprises loop, stem and toehold regions.[12] The HCR is triggered when a target sequence opens the DNA hairpin structure through complementary binding *via* the toehold region. This subsequently exposes the loop region, which then serves as a template for complementary binding with another DNA hairpin. The hybridization process continues until the hairpin supply becomes exhausted, generating long nicked double-helices.[13] The simple mechanism and operation at mild conditions make HCR ideal for application in complex biological samples.[14]

Recently, there have been several studies reported on combining HCR and AgNCs for different bio-applications. These sensors integrate amplification and signal generation into one step, which simplify the sensing process and reduce the overall detection time.[15] Mansourian *et al.* developed a turn-off HCR-AgNCs assay, where a cytosine-rich hairpin loop was employed to cage AgNCs. The nanobiosensor could be applied for direct detection of miR-145 in real clinical samples without the need

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