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Hybridization-induced suppression of coffee ring effect for nucleic acid detection

Yuanhang Li[a,1], Zichen Zhao[b,1], Miu Ling Lam[c,d], Wei Liu[b], Pak Piu Yeung[b], Ching-Chang Chieng[b], Ting-Hsuan Chen[b,c,d,*]

[a] Department of Electronic Engineering, City University of Hong Kong, Hong Kong Special Administrative Region
[b] Department of Mechanical and Biomedical Engineering, City University of Hong Kong, Hong Kong Special Administrative Region
[c] School of Creative Media, City University of Hong Kong, Hong Kong Special Administrative Region
[d] Centre for Robotics and Automation, City University of Hong Kong, Hong Kong Special Administrative Region

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ABSTRACT

We demonstrate a method for the detection of nucleic acid using hybridization-induced suppression of the coffee ring effect. When a sessile droplet is pinned on a solid surface, evaporation induces an outward capillary flow that moves suspended particles toward the periphery of the droplet and leaves a ring-shape structure. Compared with spherical particles that are carried to the contact line and form rings, non-spherical particles tend to adhere to each other at the air–water interface where they gain enhanced resistance to the capillary flow and suppress the coffee ring effect. Here, we used suspended microspheres surface-functionalized with single-strand oligonucleotide probes that were complementary to a target DNA. The present target DNA hybridized with the oligonucleotide probe and connected with multiple microspheres, leading to the generation of non-spherical particle agglomerates that resist the capillary flow and form a more uniform deposition of particles after evaporation. Video microscopy and numerical simulation showed that the suppression is because non-spherical particle agglomerates distorted the meniscus surrounding them and exerted a long-range capillary attraction that enhanced the fluidic resistance. Eventually, the microscale hybridization events were translated into the change of coffee ring patterns at macroscale. Using coffee rings as the readout, proof-of-principle studies showed effective sensitivity at low concentrations of 10–100 nM with a wide dynamic range from 10^{-5} M to 10^{-8} M, and high specificity that can distinguish the sequence with a single mismatched nucleotide. Owing to the simplicity of the operation and visual readout without the need of a special detector, our approach demonstrates immense potential for the inexpensive and convenient detection of nucleic acid in at resources-limited settings.

1. Introduction

The detection of nucleic acid is of great interest in broad applications such as diseases diagnostics [1–3], identification of pathogens [4–6], and monitoring environmental safety [7]. For example, seven signature mRNAs elevated in saliva were reported to be an effective biomarker for oral cancer [1]. When analyzing pathogenicity, chromosomal markers with species-specific sequences can be also used to differentiate pathogenic strains [4–6], which is important for food safety and homeland security when faced with bioterrorism threats. To date, the gold standard for detecting nucleic acid has been the polymerase chain reaction (PCR). However, PCR requires sophisticated sample preparation, expensive laboratory instrumentation, and fully trained operators, which are unavailable in many situations, such as local clinics or sites for collecting environmental samples. Accordingly, there is a strong demand for platforms that are simple, inexpensive, and user-friendly. Toward this end, miniaturized formats have been adopted in platforms such as microfluidic-based PCR [5,8], electrochemistry based microfluidic sensors [9,10], and colorimetric assays using nanoparticles [2,11–15] or quantum dots [4,16]. However, cumbersome components (pumps, valves, current meter, or power supplier) and complicated setups are unavoidable in most systems. Efforts are still needed to simplify the detection method further.

The coffee ring effect is a natural phenomenon frequently observed in daily life. When a sessile drop containing suspended substances is pinned on a solid surface, a ring-shaped structure

* Corresponding author. Tel.: +852 3442 4114; fax: +852 3442 0172.
E-mail address: thchen@cityu.edu.hk (T.-H. Chen).
[1] These authors contributed equally to this work.

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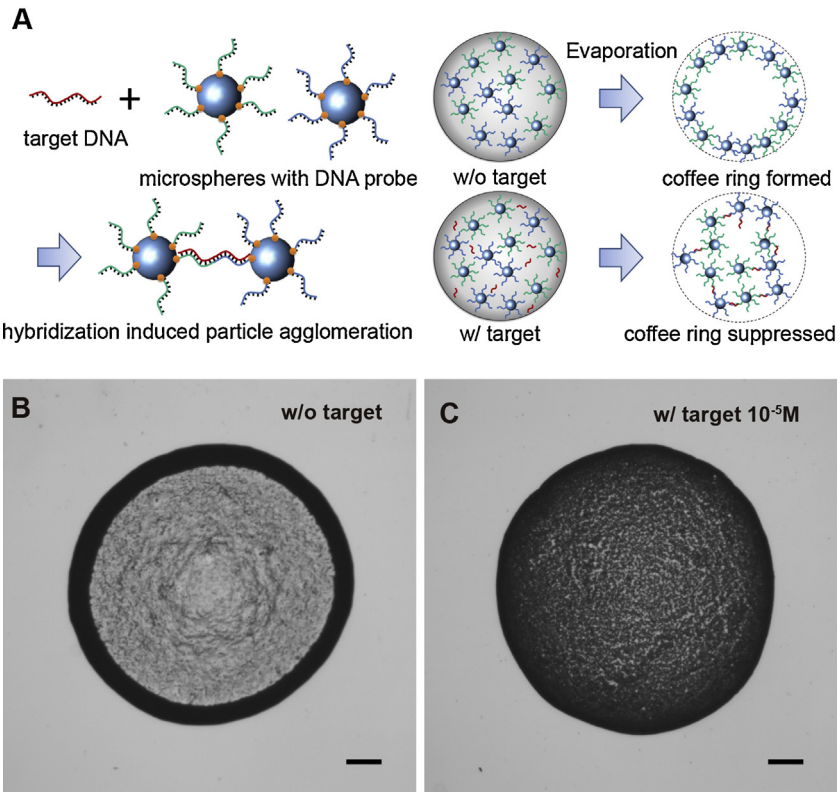

Fig. 1. Detection of target DNA using the coffee ring effect. (A) Schematics of the sensing mechanism. With microspheres functionalized with to $P1_{rpoB}$ (blue) or $P2_{rpoB}$ (green) that have a complementary sequence with the target DNA, rpoB (red), in juxtaposition, the target DNA rpoB can bridge two microspheres together (left). After evaporation, the droplet with no target DNA forms a perfect coffee ring at its edge, whereas the droplet with target DNA deposits the microspheres more uniformly (right). (B) Without target DNA, rpoB, the microspheres form a coffee ring. (C) When target DNA is present at a concentration of 10^{-5} M, the microspheres are uniformly deposited after evaporation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

is formed after evaporation. The effect is driven by the maximum evaporative flux that occurs around the periphery of the sessile drop and the pinning effect that prevents receding of the contact line [17–19]. As a result, a radial, outward capillary flow is established to replenish the increased removal of solvent at the periphery. This phenomenon concentrates the suspended particles at the rim, eventually leaving a ring-shape structure after drying [17,18]. The coffee ring effect has gained increased attention due to its intriguing physics [17,18,20] and applications for paint manufacture, ink jet printing [21], and particle assembly [22]. In many cases, the coffee ring effect is undesired because it causes a non-uniform deposition of entities. Extensive attempts have been made to eliminate the coffee ring effect using the Marangoni effect [23], capillary repulsion [24], addition of surfactant [25], and control of the droplet size [20]. In contrast, earlier studies were also made to take advantage on the self-driven capillary flow for handling biological entities, such as chromatographic separation [26], protein-based diagnoses [27–30], and preparation of biological fluids [31].

Recently, reports have shown that the formation of the coffee ring is dependent on the shape of the suspended particles [32]. Compared with spherical particles that form perfect rings, anisotropic ellipsoids tend to stay at the air–water interface, where they experience strong particle–particle attraction and form a loosely packed structure that has higher resistance to the primary capillary flow. Consequently, rather than being concentrated at the periphery of the droplet, non-spherical ellipsoids become uniformly deposited after drying, showing their ability to suppress the coffee ring effect. Importantly, this shape-dependent suppression can be visualized without delicate environmental controls (humidity, temperature, or external pump). However, studies that exploit this phenomenon for biomedical detection have not been reported.

Here, we describe a method for the detection of nucleic acid using hybridization-induced suppression of the coffee ring effect. Our working principle is based on the phenomenon that non-spherical particles have higher resistance to the coffee ring formation, the schematics of which are shown in Fig. 1A. Suspended microspheres were surface-functionalized with single-strand DNA probes that can recognize a target DNA. Two types of probes, P1 (blue) and P2 (green), were designed to hybridize in juxtaposition with one target DNA (red). Using microspheres functionalized with either P1 or P2, the present target DNA hybridized simultaneously with both P1 and P2, leading to the generation of non-spherical particle agglomerates through inter-particle cross-linking. Thus, when the aspect ratio (α = length/width) of the suspended particles significantly increases (from $\alpha = 1$ for mono-dispersed microspheres to $\alpha > 1$ for particle agglomerates), the particle agglomerates can be deemed non-spherical to resist the capillary flow and to suppress the coffee ring formation.

In this proof-of-concept study, we establish a method of detection that is effectively sensitive at low concentrations of 10–100 nM, with a wide dynamic range from 10^{-5} M to 10^{-8} M, and sufficiently specific to distinguish sequences with a single mismatched nucleotide. In addition, the mechanistic insight underlying the suppression of the coffee ring effect was also elucidated by video microscopy and numerical simulations. In the present study, we show how the coffee ring effect was exploited to amplify the microscale binding events into changes of pattern formation at macroscale without cumbersome instrumentations, thus offering a simple, inexpensive, and user-friend interface for detection of nucleic acid.