ENHANCED MOBILE HYBRIDIZATION OF GOLD NANOPARTICLES DECORATED WITH OLIGONUCLEOTIDE IN MICROCHANNEL DEVICES

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ABSTRACT

We have designed a microfluidic device that is capable of enhancing passive mixing through the micro-structures, positioned along the channels of the device. We investigated the capability of these structures to promote mobile hybridization of fluorophore-labeled target oligonucleotides to Au-NP probes. This process is monitored with fluorescence through the quenching of the fluorescent signal within the device as the target oligonucleotides become bound to the Au-NP probes. We evaluated the fluorescent intensity of a sample image which was completed in about 7.2 s. Mobile hybridization is thus much more effective than traditional static hybridization limited by molecular diffusion.

KEYWORDS: Micro-structures, Mobile hybridization, Target, Probes

INTRODUCTION

Microfluidic systems integrated modified gold nanoparticles have been implemented for real-time detection [1]. The mixing and binding of modified gold nanoparticles to the target objects in microchannels that dominate the sensitivity and accuracy of the read-out and data interpretation has attracted little research attention. A microfluid in a laminar state typically lacks turbulent fluctuation to accelerate flow mixing. Various active or passive devices as mixer or reaction chamber were thus designed to solve the issue, expecting complete fluid mixing and reaction to be achieved within such small distance and duration [2, 3]. We designed passive devices for mixing and micro-reaction [4] based on the principles of splitting and recombination (SAR) and chaotic advection [5]. Here we propose an efficient microreactor with enhanced mixing and mobile conjugation.

CONCEPT

The objective of the experiment was to analyze the hybridization of a target DNA and modified gold nanoparticles in microchannels as shown in Figure 1. The modified gold nanoparticles flow from one end of the flow channel, one entry; the target and test DNA flow from the other entry. Synthesis of the Au-NP probe results from modifying the surface of gold nanoparticles with a single-strand DNA. The fluorochrome FAM was conjugated on the target DNA. The test DNA was modified with fluorochrome TAMRA.

Figure 1 shows that, at the confluence of the microchannels, target DNA and control DNA have not mixed and bound to the Au-NP probe because the emission spectra clearly contain two separate signals from the fluorescence of each of FAM and TAMRA. After passing through the reaction channel, the target DNA binds to the Au-NP probe causing a significant quenching of the FAM fluorescence by the gold nanoparticles (Au-NP absorb light about 520 nm). Through monitoring the two fluorescent spectral signals, we follow the extent of hybridization between the target DNA and the Au-NP probes, and thus observe the dispersion of the test DNA without hybridization in the microchannels. Using this experimental design, we simultaneously analyzed the hybridization and the mixing of the DNA and nanoparticle probes in the microchannels.

Figure 1: Analytic strategy for mobile hybridization and (a) Schematic illustration of the straight geometry; (b) SAR microreactor geometry and configuration.
EXPERIMENTAL

The Y-shaped microchannel and SAR micro-reactor were made of polydimethylsiloxane, which is highly biocompatible and readily manufactured as shown in Figure 1 (a)(b). One inflow contained fluorophore solutions with a 20-mer adenine sequence of oligonucleotide modified with 5′-FAM (λ<sub>ex</sub> = 495, λ<sub>em</sub> = 521 nm, 1 × 10⁻⁶ M) and a 20-mer cytosine sequence of oligonucleotide modified with 5′-TAMRA (λ<sub>ex</sub> = 560, λ<sub>em</sub> = 583 nm, 1 × 10⁻⁶ M). The other inflow was a buffer solution (phosphate-buffered saline, PBS, pH = 7.4) containing a 20-mer thymine sequence of oligonucleotide modified with Au-NP (13 nm, 1 × 10⁻⁸ M). The average oligonucleotide surface coverage of alkanethiol-modified 20-mer oligonucleotide on Au nanoparticles was 30 pmol cm⁻². For 13-nm Au-NP, this condition corresponds to roughly 100 thiol-bound 20-mer strands per gold particle. All experiments were conducted with the flow driven with pressure generated by a syringe pump. The flow rate was controlled through the setting of the syringe pump (3 mm s⁻¹). The mixing patterns of fluids in the devices were visualized with a confocal microscope (Nikon A1R).

RESULTS AND DISCUSSION

Reaction of the samples in microchannels

The solution containing FAM-tagged target DNA and test DNA with TAMRA flows through one entry of the channel and the Au-NP probe flows through the other entry. We compared the effect of mixing and reaction of samples in a straight channel having no structure with those in the reaction channel proposed in this work.

The behavior of test DNA in a straight channel, as shown in images 1 to 22 in Figure 2 (left), reveals that the fluid mixes slowly as the primary driving force of the fluid mixture in such a channel, based mainly on diffusion between molecules, is generally inefficient. Concurrently with the test DNA gradually diffusing and mixing from upstream to downstream, the concentration of fluorescer decreases through dilution, and the fluorescent intensity correspondingly diminishes. For the target DNA, the results indicate that the reaction is similar to the mixing of test DNA; only little target DNA hence hybridized to the Au-NP probe, and the fluorescent intensity decreased only slightly, similarly to diffusive mixing.

For the newly designed reaction channel, the mixing of test DNA in the flow channel differs from that of the straight channel in that the structural design significantly promoted the rapid mixing of the test DNA so that a uniform state at every stage (images 6 - 22, as shown in Figure 2 (right)) was already achieved. For the target DNA, every image showed satisfactory uniformity, but the intensity of the image gradually decreased (darkened) from upstream to downstream. In addition to the mixing between fluids in the process, the intensity of target DNA fluorescence decreased because of quenching by Au-NP upon hybridization of target DNA to the probes.

Quantification of mixture and reaction

To quantify the sample reaction and mixing distance in a flow channel, we analyzed the captured images with 12-bit gray-level intensity. With data processing of the sampled images, we calculated the average intensity of all pixels. The normalized intensity (I') is defined as

\[ I' = \frac{I - I_{\text{min}}}{I_{\text{max}} - I_{\text{min}}} \]  

in which I<sub>max</sub> and I<sub>min</sub> are the maximum and minimum intensities, respectively, of pixels from all sampled images; \( \bar{I} \) is the average intensity of a sampled image. As all curves converge to a common value downstream, the region downstream from the hybridization or mixing reaction is stable. The continuous flow presents a dynamic equilibrium, and the completion of the hybridization and the mixing is adjustable with the convergence value (minimum value) of the curve. The required distance and duration of the reaction and mixture thus become defined.

For the newly designed reaction channel, the test DNA attained complete mixing rapidly and presented a homogenous distribution of fluorescent intensity in images 6 to 22. The fluid hence achieved completely homogenous mixing before the first corner. As deduced from the curve in Figure 3, the mixing distance of the fluid in the reaction channel is less than 21.8
mm. For the target DNA, the effect of the structure of the flow channel makes DNA not only mix rapidly with the other flow, but also accelerates and enhances the efficiency of hybridization. When target DNA moves from upstream to downstream, the fluorescent intensity is similar to that of the complete mixing at the first corner. The fluorescence tends to become quenched further downstream, indicating that the target DNA reacts rapidly and continuously with the Au-NP probes. The fluorescent intensity in a steady state implies that the hybridization of the target DNA and the Au-NP probe has attained saturation. The duration of hybridization is about 7.2 s from the estimated period of retention. For the hybridization to be completed in the straight channel takes 15 min. According to the traditional static hybridization, the hybridization occurs through diffusion of the driving fluid; an overnight period is typically required to improve the hybridization. According to the concept of our microfluidic system, mobile hybridization occurs between samples, thus enhancing the speed and efficiency of hybridization by means of the channel microstructure design.

**CONCLUSION**

We have demonstrated the concept mobile hybridization by verifying the conjugation of DNA with modified gold nanoparticles in a designed microreactor. In the hybridization experiment, the samples and probes flow through Y-shaped channels simultaneously for a dynamic mobile hybridization in the microreactor. The effect of the structure in the microreactor enables the reaction to attain saturation in only 7.2 s, a duration much less than for traditional static hybridization. In medical tests, one can diagnose the result in a flow channel in real time. In our experiment, the mixing and hybridization of target and test DNA with the Au-NP probes are monitored concurrently in the microreactor. The results show that the microreactor presents a length of travel to achieve complete hybridization near the length required for only homogenous mixing, but the required duration is much smaller than that required for traditional static hybridization systems that typically require a few hours. The practice of mobile hybridization in a sample is significantly improved through the structural design of the microreactor presented here.

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