Fast and sensitive analysis of DNA hybridization in a PDMS micro-fluidic channel using fluorescence resonance energy transfer

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Fluorescence resonance energy transfer has been used to illustrate its applicability to the sensitive detection of DNA hybridization reactions in a PDMS microfluidic channel.

Hybridization analysis of DNA plays an important role in the detection of genetic diseases and gene expression profiling. Today, one of the most popular approaches to DNA hybridization analysis is the use of a microarray chip where probe DNA sequences are immobilized on a solid phase surface and incubated with a mixture of the unknown target DNA.[1] Detection of hybridized sequences generally involves the covalent labelling of target DNA with a fluorescence dye prior to hybridization. The fluorescence is caused by an interaction between a target analyte and an immobilized probe element, and provides valuable information on the presence of a target DNA.[2,3] Although microarray technology provides a cost-effective method for microscale bioassays, it has several drawbacks. First, immobilization schemes using a spotter and an arrayer need to be adopted for the hybridization. Second, a relatively long time (about 1–2 h) is required for complete hybridization because of the diffusion-limited hybridization kinetics. However, a restriction on molecular diffusion near to the surface makes the kinetics of DNA hybridization assays slower than a microarray system.[4,5] These drawbacks make the microarray technique unsuitable for high throughput applications.

To resolve these problems, a quick and accurate DNA analysis technique using microfluidic devices has been developed.[6–9] This microfluidic analysis method does not use an immobilization procedure; instead, it uses a simple syringe pumping system. The laminar flow along the channel can be easily controlled by the channel structure and flow speed.[10,11] By injecting target and probe DNA solutions, it is possible to detect the sequence-specific hybridization of both the probe and target DNA, and the simple operation enables a highly accurate DNA analysis to be performed. Microfluidic devices also overcome the slow hybridization problem[12] caused by the diffusion-limited kinetics on a microarray chip, since the hybridization occurs in solution. As a result, the hybridization time is greatly reduced to less than a few seconds if a properly designed channel to obtain optimized mixing performance is utilized.

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However, a new detection method in a microfluidic device is required for the identification of the change in fluorescence on hybridization, since non-hybridizing fluorescence oligonucleotides cannot be washed out inside the channel. Therefore, we used fluorescence resonance energy transfer (FRET) for this purpose, where two types of DNA oligonucleotides, which share complimentary base sequences, were prepared. Each DNA oligonucleotide was labelled with a different fluorescence dye at the 5′- or 3′-terminus. Here, one oligonucleotide is the fluorescence donor and the other is the acceptor. When the two fluorescent oligonucleotides hybridize to form adjacent sequences in the microfluidic channel, the distance between two fluorophores on the new hybrid becomes close enough for FRET to occur. FRET is caused by an interaction between the donor and acceptor fluorescence dyes located within a distance of <8 nm from each other. The excited state energy of the donor molecule is transferred nonradiatively to the acceptor molecule, and FRET results from a quenching of the donor fluorescence and an enhancement of the acceptor fluorescence intensity.[13,14] By monitoring the change in fluorescence intensity between the donor and acceptor DNA oligonucleotides, it is possible to accurately detect the hybridization process.[15]

In this work, we used FRET in the sensitive detection of DNA hybridization in a solution phase. For this purpose, an alligator teeth-shaped PDMS microfluidic channel was used to obtain efficient mixing between the probe and target DNA oligomers. For an optimum efficiency mixing channel and flow velocity, the quantitative changes in FRET signal for DNA hybridization could be successfully observed. This method does not require either an immobilization procedure or an amplification procedure for DNA analysis. The detection time was also very fast when the microfluidic technique was applied to the analysis of DNA hybridization. These features are suitable for a high throughput bio-analysis method.

The procedure for the fabrication of the alligator teeth-shaped PDMS microfluidic channels for the FRET measurements has been reported elsewhere.[16,17] In brief, the microfluidic channels were fabricated by stacking two PDMS layers that had upper and lower teeth patterns. These layers were produced by pattern replication from mould masters. The layer with the upper teeth pattern was fabricated by compression micro-moulding of a PDMS elastomer. The PDMS pre-polymer was poured into the mould master and compressed with a transparent film and an aluminium disk and allowed to cure for 2 h. The thin upper layer (thickness = 200 μm) was produced by separating it from the mould master. To bond the upper and lower layers, the surfaces of both layers were activated in an oxygen plasma, and then they were aligned using a custom built aligning device. Methanol was

used as the surfactant between both layers. Finally, a cover glass was stacked onto the upper layer.

In the DNA hybridization assay, a pair of interactive fluorophores was attached to the ends of the probe and target DNAs. Here, DNA hybridization can be detected by the change in fluorescence intensity because of the energy transfer between the donor and acceptor fluorophores. If an acceptor fluorophore is brought close to a donor fluorophore, within 2–10 nm, then the intensity of the fluorescence of the acceptor fluorophore increases, whereas the intensity of the donor fluorophore decreases. All the probe and target DNA oligonucleotides were purchased from Bio Basic Inc. (Canada), and used without further purification. The probe DNA (donor) was labelled using TET at the 3′ end. The target DNA (acceptor) had a complementary sequence corresponding to the probe DNA, and was labelled using TAMRA at the 5′ position. The base sequences corresponding to the probe DNA and target DNA were (TET)3′-AAC TTT CAA AAC CGT CAC CA-5′ (TET-P) and (TAMRA)5′-TTG AAA GTT TTG GCA GTG GT-3′ (TAMRA-T), respectively. These DNA oligomers are known to be related to azoospermia. A significant percentage of patients with non-obstructive azoospermia test positive for small deletions in the DNA of the Y chromosome, and those gene sequences are closely associated with the deleted segments.[18] Stock DNA solutions (100 μM) for the FRET measurements were prepared in a PBS buffer solution (pH = 7.4) and stored in a freezer until use. Two DNA oligomer solutions were introduced into the PDMS channel using microsyringes connected *via* tubes to the inlet pipettes. The flow rate was controlled simultaneously using a KD Science microsyringe pump.

An experiment was carried out to establish if it was possible to monitor the solution-based DNA hybridization reaction using our microfluidic system. Fig. 1(a) shows a schematic view of our alligator-teeth shaped PDMS channel and the mixing process of the confluent streams. The probe DNA (TET-P) and target DNA (TAMRA-T) were introduced into the PDMS channel from microsyringes connected *via* tubes to the inlet pipettes. The flow velocity was simultaneously controlled using a KD Science microsyringe pump. The fluorescence emission spectra were measured using a Leica TCS SP confocal fluorescence microscope. The confluent mixing streams were analysed using the two-dimensional confocal fluorescence images in the x–y plane located perpendicular to the optical axis using a 10× water-immersion objective lens. The lateral resolution was estimated to be 1 μm. The laser excitation of the TET and TAMRA occurred at λ = 488 nm, and the emitted fluorescent light was detected between λ = 500 and 680 nm. The image size was 512 × 512 pixels, and the width of each pixel was 0.49 μm. The fluorescence spectra were also measured using the λ-scanning mode of the confocal laser scanning microscope to quantitatively investigate the fluorescence energy transfer through the hybridization process between the two DNA oligomers.

Fig. 1(b) shows the fluorescence spectra measured at seven different points along the channel at a constant flow rate of 1.0 μL min^{-1}. A 1 : 1 molar ratio of donor : acceptor was used, and a complete mixing of both solutions was obtained after passing the saw-shaped channel. The fluorescence peaks located at 536 and 585 nm are the emission bands of the TET-P and TAMRA-T, respectively. The intensity of TET-P decreased with increasing distance along the channel and the intensity of

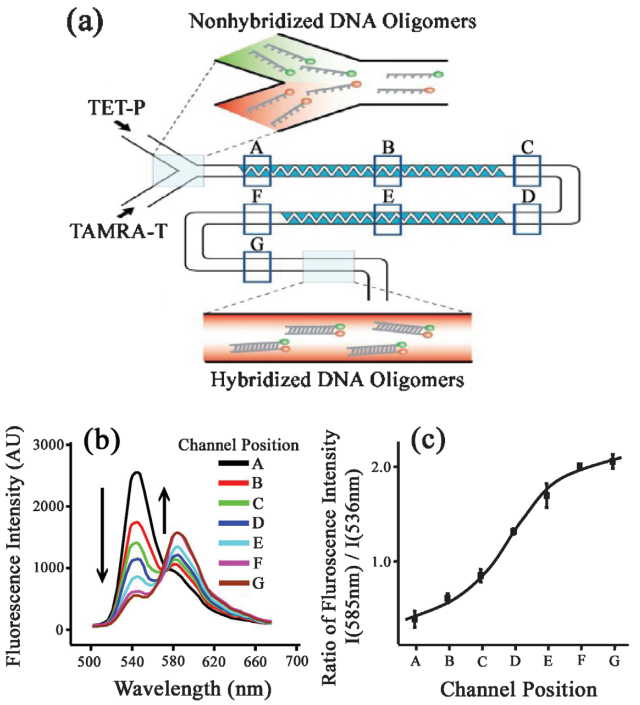

Fig. 1 (a) Schematic illustration of a PDMS microfluidic channel for the FRET detection of DNA hybridization. The seven blue boxes denote the FRET measurement areas. (b) The corresponding fluorescence spectra for each channel position. (c) A calibration curve showing the relationship between the ratio of the fluorescence intensity at 585 nm and at 536 nm in the spectra with channel distance. The flow rate was 1 μL min^{-1}.

TAMRA-T increased. This means that the fluorescence energy transfer between the donor TET-P and the acceptor TAMRA-T successfully occurred on DNA hybridization. Fig. 1(c) shows a calibration curve indicating the relative fluorescence intensity at 585 and 536 nm with distance along the channel. This result shows that DNA hybridization between the donor and acceptor fluorophores increases steadily with increasing distance along the channel. In addition, similar fluorescence band intensities in the channel at Points F and G indicate that the hybridization reaction was almost complete just after the confluent streams passed through the alligator teeth-shaped saw channel. This three-dimensional PDMS channel shows a high mixing efficiency, since a strong chaotic advection is developed by the simultaneous vertical and transverse dispersion of the confluent streams.

Fig. 2(a) shows the fluorescence emission spectra of different molar ratios of donor and acceptor DNA oligonucleotides in a microfluidic channel. The molar ratio of TAMRA-T : TET-P was changed from 0.1 to 1.0. Fig. 2(b) shows the corresponding calibration curve, which illustrates the relationship between the ratio of the fluorescence intensity in the spectra and the molar ratio of the DNA hybrids. Each fluorescence spectrum was measured in the channel at Point G. The FRET efficiency increased with increasing concentration of TAMRA-T.

DNA hybridization analysis using microfluidic technology overcomes many of the drawbacks of microarray chips, such as the long hybridization time and inconvenient immobilization procedures. In a microfluidic device, however, a new detection method is required, since nonhybridizing fluorescence oligonucleotides cannot be washed out inside the channel. In our work,

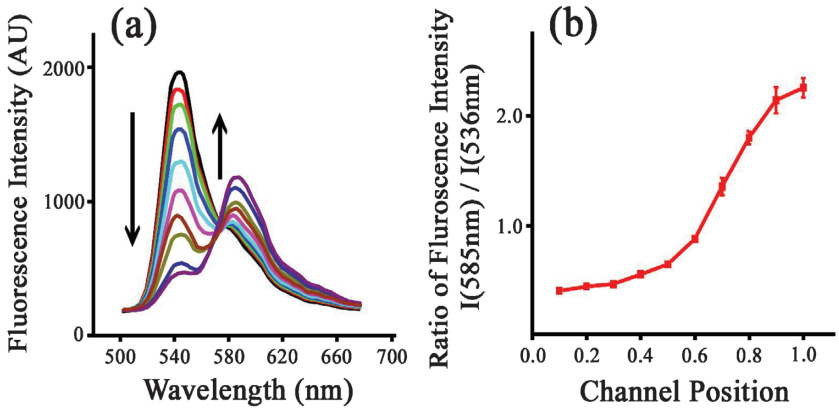

Fig. 2 (a) Changes in fluorescence spectra on DNA hybridization for different molar ratios of TAMRA-T : TET-P = 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0. Each spectrum was measured in the channel at Point G. (b) A calibration curve showing the relationship between the ratio of the fluorescence intensity and the molar ratio. The flow rate was 1 μL min^{-1}.

detection using FRET successfully resolved this problem. Compared to the previously reported work on DNA analysis using microfluidic devices, both the detection sensitivity and the quantitative measurement capability have been greatly improved. However, this technique is inconvenient for the quantitative detection of unknown target DNA samples, since both the probe and the target DNA strands need to be labelled with a suitable fluorescence dye. To resolve this problem, we are now working on target DNA detection using a molecular beacon as probe DNA in a microfluidic channel. In this case, the target DNA does not have to be labelled, since the fluorescence dye or the dye/quencher is attached to the ends of the probe DNA. We expect this analytical technique using FRET to be successfully applied to DNA analysis in a microfluidic channel.

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