Microfluidic isotachophoretic Fluorescence *In Situ* Hybridization of bacterial cells

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ABSTRACT

Inline fluorescence *in situ* hybridization (FISH) of intact bacteria is demonstrated using microfluidic isotachophoresis (ITP). Cells and FISH probe are focused together, prior to being separated using a spacer ion in a second region containing PVP (7%) as sieving matrix. The hybridization assay could be conducted within 4 minutes.

KEYWORDS: Isotachophoresis, bacteria cells, fluorescence *in situ* hybridization (FISH)

INTRODUCTION

Nucleic acid hybridization has been extensively used for diagnostic and biological sample processing [1] because the specificity allowing for the detection of a single sequence from an entire genome. FISH is widely used in clinical analysis to detect whole cells based on their DNA or RNA sequence using fluorescently labelled hybridisation probes [1]. However, the downfall of FISH is that whole cells need to be fixed and incubated with the probe for at least 30 min before analysis, preventing rapid turn around. Recently, ITP has been demonstrated to increase the nucleic acid hybridisation rate of DNA and RNA in free solution [2], and ionic spacers can be selected to separate the hybridised and free probe [3].

Here, we take a similar approach to the selective labelling of bacterial cells. In order to allow the FISH probe to enter the cells, DMSO was added to the terminating electrolyte to increase the rate at which the probe permeates into the cells. Using a fluorescently labelled hybridisation probe with 27R DNA sequence, ITP was used for on chip FISH of *Escherichia coli (E.coli),* specifically staining *E.coli* in less than 4 min.

EXPERIMENTAL

The DMSO concentration was optimised on a bench top capillary electrophoresis instrument. Cells were suspended in the terminating electrolyte (TE) comprising 5 mM HEPES with different DMSO concentration and the leading electrolyte (LE) was 50 mM Tris HCl (pH 8.0) + 0.5% PVP and 1 μM SYTO 9 (universal nucleic acid dye).

The same electrolyte system was used with a sieving matrix, to separate the fluorescently labelled *E.coli* in-line after FISH from the free probe. A cross-geometry PDMS microchip (channel 50 μm wide and 42 μm deep) was used in combination with an inverted microscope for visualisation.

Universal 27R oligo probe labelled with Cy5 fluorophore was suspended in the LE while *E.coli* was suspended in TE. 1% v/v of DMSO was added into TE with the cells. 2 mM MES was used as a spacer ion and added in the TE to allow separation of free unlabelled probe from the labelled bacteria cells upon entering the sieving matrix. 7% PVP was selected as a sieving matrix and filled at one end of the microchannel.

RESULTS AND DISCUSSION

During ITP, the cells were focused in the ITP band and stained with the SYTO 9. As illustrated in figure 1a, the optimal DMSO concentration in the TE was 2%, allowing for 75% hybridization efficiency. Fur-

ther increases in the DMSO concentration decreased the hybridization possibly due to the interaction with the PVP coating used to supressed the EOF. Figure 1b shows the overlaid isotachopherograms comparing off-line stained SYTO 9 cells with on-line ITP staining in a capillary using 2% DMSO.

A mobility study revealed that MES has a mobility between the free labelled probed and the cell-probe complexes in a 7% of PVP sieving matrix. This means that whilst outside of the sieving matrix the cells and FISH probe co-migrate, they can be separated in the PVP. Figure 2 shows how the ITP-spacer cells assay works.

To ensure that the probe was able to penetrate the cells during ITP, we firstly stained the cells offline with SYTO 9. Excess SYTO 9 was then removed before suspending the cells into the TE consist of 1% DMSO. 1% of DMSO was used because 2% of DMSO shows incompatible to the PDMS chip. A universal oligoprobe (20 bp with Cy5 attached at the 3' end) was then added in the LE solution. Figure 3a shows the microchannel used and figures 3b-d show the zone with cells and free probe, the removal of the probe and the band with stained cells, respectively. Figure 3e is an image of the second ITP band was made stationary by pausing the voltage, demonstrating the ITP has successfully enabled FISH staining of live bacteria, followed by the separation of the free probe from the stained cells within 4 minutes. The green dots are the offline, SYTO 9 labelled *E.coli* cells, while the red-orange colour is the Cy5 oligo probe. As the probe permeates into the offline SYTO 9 stained cells, the *E.coli* fluoresces orange-green. The orange glow in figures 3d and e suggests the presence of free cy5 probe out of the cells. We postulate that this is the excess probe that permeated into cells but leaks out due to low stringency hybridization.

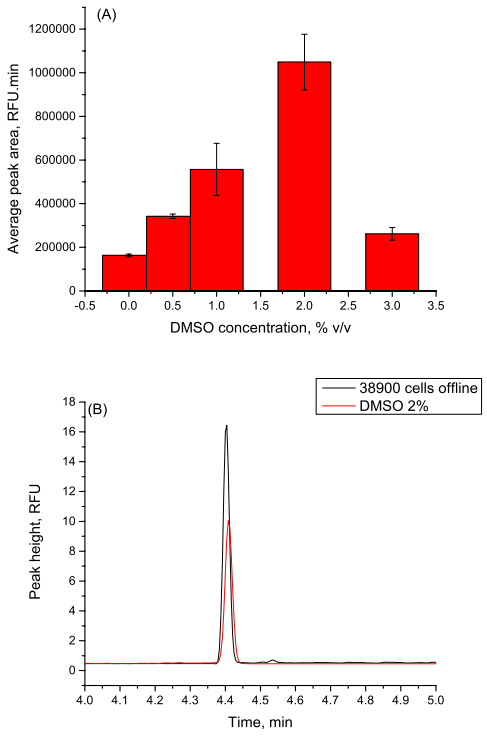

Figure 1: *(a) Histogram of the effect of the DMSO concentration on peak area. (b) Overlaid isotachopherograms of cells offline stained offline (black) or online in presence of 2% DMSO (red) with SYTO 9.*

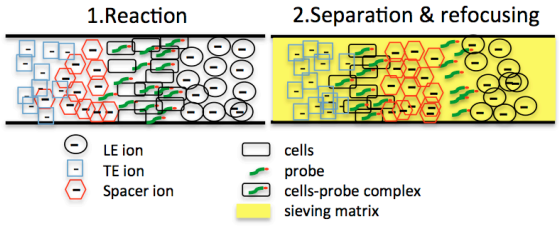

Figure 2: *Schematic of ITP-based FISH assay. 1.The bacterial cells focus between LE and TE, allowing for interaction with the probe, which comes out of the LE due to its lower mobility. In free solution, the spacer ion has a lower mobility than the bacteria cells because of its lower mobility. 2. Upon entering the sieving matrix (7%PVP), the cell-probe complexes are slowed down, enabling the spacer ions to overtake them . The excess fluorescent probe is faster than the spacer, hence focuses between the LE and spacer leaving the cell-probe complexes cells between the spacer and the TE.*

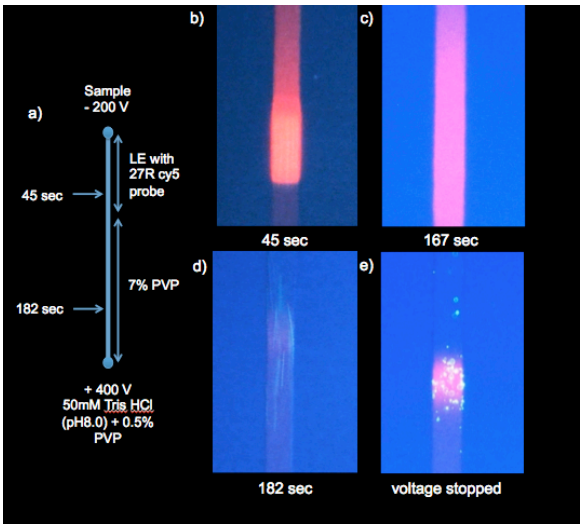

Figure 3: *Schematic of the chip with position of snapshots of ITP of cells at different time b-e. e is the snapshot at the same position of c and d when voltage is stopped.*

CONCLUSION

We report the use of isotachophoresis for rapid inline fluorescence in situ hybridization of live bacterial cells in less than 4 minutes. The introduction of spacer ions and sieving matrix with this ITP method enables the online separation of stained cells from the free probe.

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