Application Note

Sorting of water-in-oil emulsions with bacteria using a fluorescent nucleic acid probe

Collaboration with Prof. Naohiro Noda (AIST, Biomedical Research Institute)

Introduction

Uniformly-sized water-in-oil emulsion droplets generated in a highly efficient manner by droplet microfluidics-based systems allow each droplet to act as reactors. These small volume reactors have been used in applications such as cultivation of bacteria, droplet digital PCR, high-throughput screening and analysis of enzymatic kinetics. In particular, encapsulating single bacterium into individual droplets prevents the growth competition between fast- and slow-growing species. This is useful for cultivating rare and slow-growing bacteria separately from the bacterial mixture that contains vast amount of fast-growing bacteria. However, after the process of encapsulating bacteria into droplets, isolating droplets containing bacteria from those that do not contain bacteria remains technically challenging, resulting in only a very limited number of successful cultivation of environmental microorganisms in the literature. To overcome this challenge, we developed a simple and reliable selective sorting method that allows bacteria containing droplets to be isolated on our microfluidic chip cell sorter, On-chip Sort. Bacteria containing droplets are sorted based on the fluorescence produced as a result of the interaction between bacteria and fluorescence resonance energy transfer (FRET)-based RNA probe encapsulated inside the droplets. This method is named Fluorescent Nucleic Acid Probe in droplets for Sorting bacteria (FNAP-sort) (Ota, Y., Saito, K. *et al.* PLoS ONE 14(4): e0214533. Under the licence of Attribution 4.0 International (CC BY 4.0) https://creativecommons.org/licenses/by/4.0/).

Methods

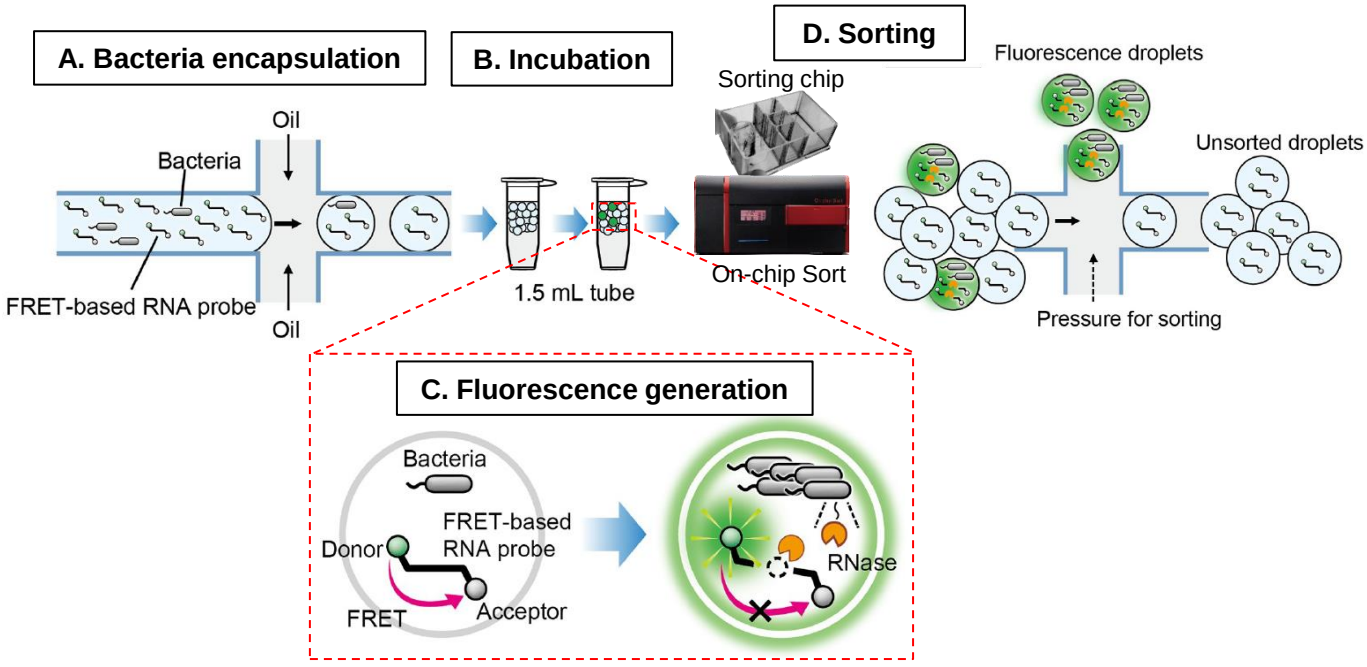

Fig. 1. Workflow of FNAP-sort (Ota, Y., Saito, K. *et al.* PLoS ONE 14(4): e0214533. Under the licence of Attribution 4.0 International (CC BY 4.0) https://creativecommons.org/licenses/by/4.0/).

Fig. 1 shows the workflow of FNAP-sort. A single *Escherichia coli* bacterium or bacteria suspended in culture medium with a FRET-based RNA probe was encapsulated into 1-nL water-in-oil emulsion droplets (Figure 1A). Droplets were then transferred to a 1.5 mL tube to allow the encapsulated bacterial cells to grow at 37°C for 1 day (Figure 1B). During incubation, the FRET-based RNA probe does not emit fluorescence by itself; however, in the presence of bacteria, ribonuclease (RNase) secreted by the growing bacteria cleaves the FRET-based RNA probe to generate strong fluorescence inside the droplets (Figure 1C). On-chip Sort with microfluidic chip with 150 μm channel (2D-chip Z1000-w150) was used to isolate the highly fluorescent droplets containing growing bacteria (Figure 1D).

Results

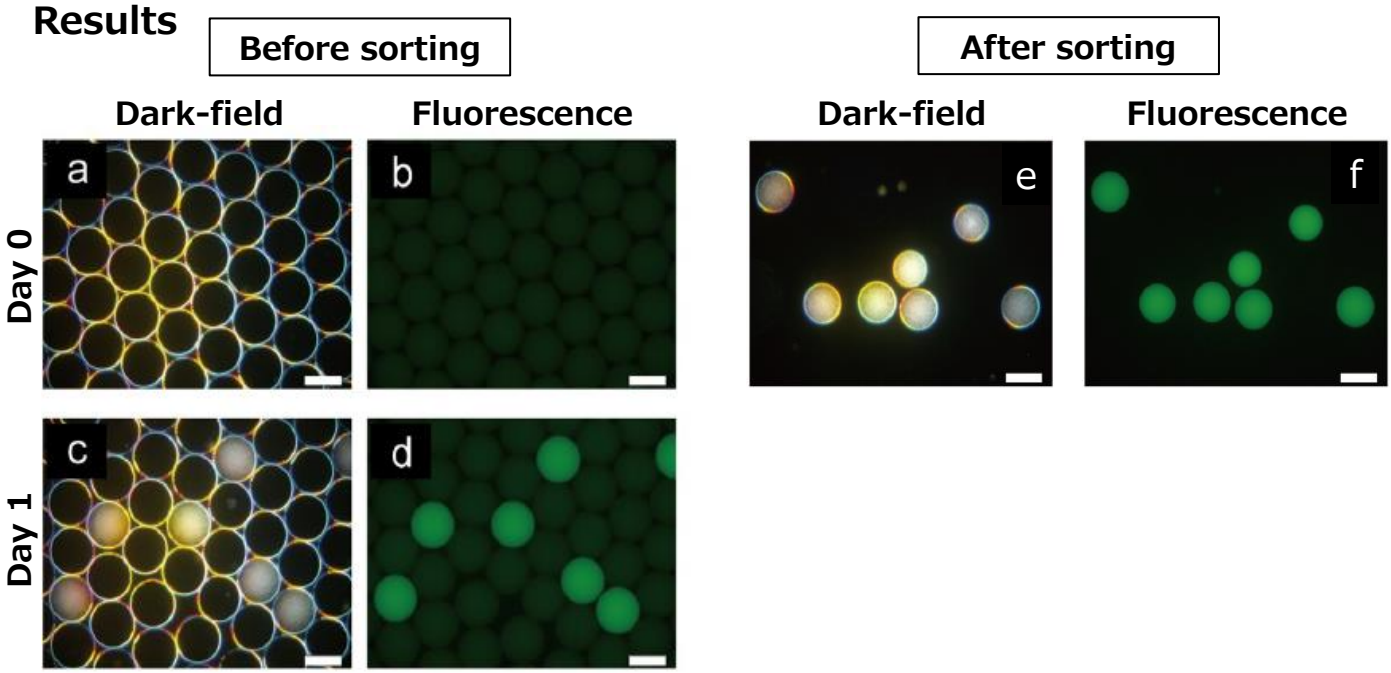

Fig. 2. Dark-field and fluorescence micrographs of droplets containing *E. coli* before (a, b) and after (c, d) 1 day of incubation, and highly fluorescent droplets with growing *E. coli* sorted using On-chip Sort. Scale bars represent 100 μm (Ota, Y., Saito, K. *et al.* PLoS ONE 14(4): e0214533. Under the licence of Attribution 4.0 International (CC BY 4.0) https://creativecommons.org/licenses/by/4.0/).

Droplets containing *E. coli* and FRET-based RNA probe were observed to emit weak fluorescence immediately after being generated (Fig. 2a, b). Droplets containing *E. coli* and FRET-based RNA probe were observed to be brighter after 1 day of incubation at 37°C (Fig. 2 c, d). These results show that the FRET-based RNA probe, when encapsulated with growing bacteria inside the droplets, successfully generated fluorescence observable by dark-field and fluorescence microscopy. Strong fluorescing droplets containing growing *E. coli* after 1 day of incubation were successfully sorted by On-chip Sort (Fig. 2e, f). Therefore, FNAP-sort, the combined use of FRET-based RNA probe for generation of strong fluorescence upon RNA cleavage by RNAses secreted from growing bacteria inside droplets and subsequently sorting the fluorescent droplets with On-chip Sort, could potentially be a useful tool for high-throughput cultivation and isolation of environmental samples in water-in-oil emulsion droplets.

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