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Acoustofluidics-based enzymatic constant determination by rapid and stable in situ mixing

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ABSTRACT

Microfluidic mixings create considerable opportunities for microscale exploration of biomacromolecule function and local reaction kinetics. However, a robust and efficient way for localized mixing performance is not yet well established. In this study, we describe a simple and reliable method for rapid, stable, and controllable in situ mixing using a single-layer acoustofluidic system with a microneedle. We developed the single microstructure for region-confined mixing, which can be controlled dynamically by intentional acoustic activation. Various acoustofluidic impacts including driving frequency, microstructure design, driving voltage, and flow control on homogeneous mixing were systematically investigated. The microneedle-based device allows a fast (~ 85 ms) mixing operation. Robust and high reusability of acoustofluidic system was experimentally demonstrated with up to 200 times of repetitive mixing and 100 min of continuous mixing. Furthermore, the proof-of-concept applications including real-time characterization of fluorescein quenching and β-glucuronidase-catalyzed hydrolysis of 4-methyl-umbelliferyl-β-D-glucuronide were successfully accomplished in the in situ mixing platform. We believe this microfluidic system could be suitable for investigation of different instantaneous chemical/biochemical events associated with various molecular interactions and reactions. Also, the acoustofluidic approach is potentially valuable to the development of mixing-based integrated microfluidic systems for applications in molecular science and life science.

1. Introduction

Microfluidics is becoming an increasingly useful platform for chemist and biologist owing to its excellent performance in spatiotemporal control, precise manipulation and real-time monitoring of microobjects and fluidic conditions at the microscale level [1,2]. Over the past several decades, microfluidic technologies have presented diverse applications associated to on-chip separation [3], culture-based cell screening [4,5], protein analysis [6], and chemical synthesis [7]. In microfluidic systems, the fluid flow is typically laminar (Reynolds numbers $< 10^2$). The properties of fluid in microchannel are mainly controlled by viscous forces rather than inertial forces [8]. Consequently, fluid mixing dominated by molecular diffusion in the channel is generally poor. Rapid and homogeneous mixing methods being critical in chemical/biochemical and medical/clinical events for a wide variety of microfluidic applications such as nanomaterial synthesis, enzymatic catalysis and biomacromolecule folding, are required [9].

Up to now, various microfluidic systems have been developed to address the mixing issue in a passive or active manner. For passive mixing, the function of microfluidic devices is chiefly based on molecular diffusion or chaotic advection [10]. The modification of channel geometries acts to enhance stretching, folding and breaking of the flow, enlarge fluid interface, and then promote mass transfer. Passive mixers have advantages including simple device fabrication, low cost, and easy handling without auxiliary equipment [11]. However, these methods usually demand a long mixing route (over tens of millimeters) and typically lack the flexibilities of mixing control. Active strategies in microfluidics that enable fast, dynamic, and efficient mixing manipulation are necessary.

Many scholars have reported multiple series of excellent methods for controllable mixing using different types of external assistance based on electrical, magnetic, acoustic, and optical energies [12,13]. In particular, acoustofluidic systems have gained increasing attention in various applications due to their non-invasive feature, simple

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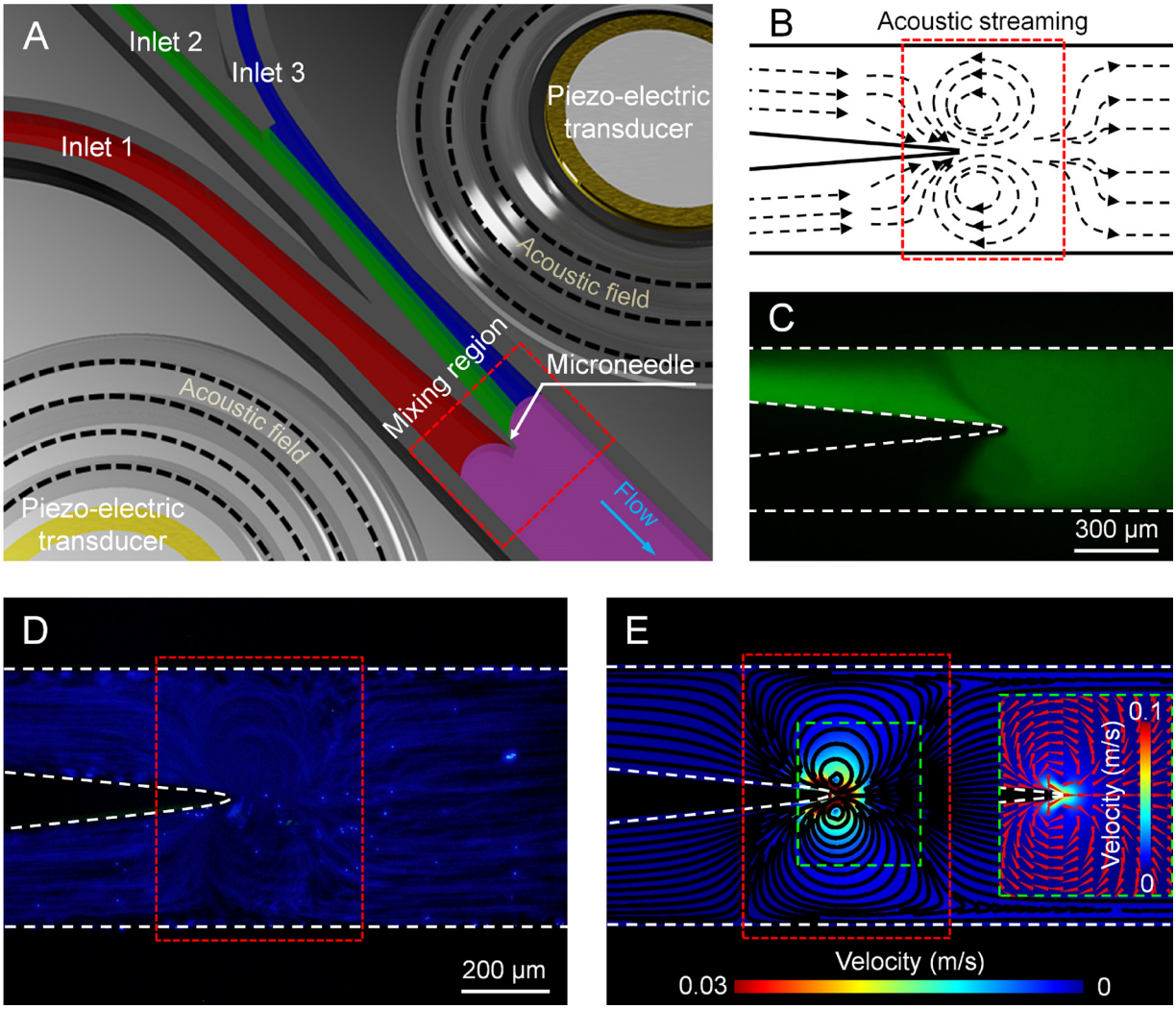

Fig. 1. The microneedle-based acoustofluidic mixing. (A) Schematic of the acoustofluidic mixing device with a microneedle. (B) Acoustic streaming generation around the microneedle tip. (C) Fluorescein mixing in the device. (D) Characterization of the flow field with acoustic streaming using fluorescent microparticles (blue). The transducer driving voltage in C and D is 18 V. (E) Simulation of the flow field with streaming. The red dashed rectangle was the mixing region. The insert corresponded to the green dashed rectangle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

manipulation, and high efficiency [14–16]. In acoustofluidic micromixers, acoustic waves propagate into fluid media and cause pressure fluctuations, leading to the disruption of the original flow field to promote the physical combination of two or more substances [17,18]. Furthermore, the introduction of specific units (e.g., bubbles, membrane, and sharp-edge structures) into the microchannel can improve the mixing performance by generating the acoustic streaming (microstreaming), which creates a remarkable perturbation of the surrounding fluids, extremely facilitating the mixing process [19–22]. In the last ten years, the acoustic mixers have been exploited to investigate enzyme reactions, produce chemical gradients, and enhance DNA hybridization [14,19]. Nevertheless, the popularization of these mixing methods is still restricted by unavoidable obstacles, such as bubble instability, inconvenient bubble localization, complicate multilayer device fabrication and manipulation, as well as poor biocompatibility by heating effect [22,23]. Meanwhile, the previous devices tend to cause large-scale microstreaming production, being actually unsuitable for studying local reaction and being not optimal for in situ biochemical explorations needing a confined mixing route [24]. In addition, to the best of our knowledge, the systematic investigation and demonstration of acoustofluidics for stable and high reusable mixing operations has been less advanced [20,21]. Overall, a rapid, dynamic, and homogeneous way for localized fluid mixing using active acoustic control for a stable and efficient purpose has remained largely out of reach in microfluidic device with uncomplicated configuration.

Here, we describe an active, facile, fast, controllable, and reusable approach for efficient in situ mixing in a single-layer acoustofluidic device with a microneedle. The acoustically activated microneedle vibration caused the double recirculating streaming extremely facilitating mass transportation in microfluidic laminar condition. The region-confined mixing can be accomplished smoothly based on the systematic optimization of various acoustofluidic influences including microstructure design, driving frequency and voltage, and flow condition. We demonstrated that this system with simple device integration and high repeatability allows a stable mixing performance of multiple substances. Moreover, different mixing-based acoustofluidic applications, i.e., real-time studying fluorescence quenching and β-glucuronidase catalysis, were successfully performed in the device.

2. Materials and methods

Detailed information on the materials and methods used can be found in the Supplementary information.