LANDSCAPING REACTION KINETICS ON A CHIP
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ABSTRACT
We report on a highly integrated microfluidic platform with parallel processes for metering, concentration gradient generation, mixing, and optical detection on a single chip. The characteristics of this automated microfluidic system are not only accurate and for rapid analysis of enzyme reaction kinetics according to the nano/picoliters of reagents, but also provide a spontaneous and vivid three-dimensional output, which provides many advantages, including significant reduction in sample consumption, and greater sensitivity for the biomedical and pharmaceutical applications.

KEYWORDS: Microfluidics, Landscaping, Reaction kinetics, Three dimensional plot

INTRODUCTION
Catalysis is identified as an inherent feature of enzymes in which it increases the rate of a chemical or biological reaction. The kinetic analysis of an enzymatic reaction is essential for understanding and controlling the biological or chemical reaction. The sequential and structural information of enzymes cannot be used for the investigation of real catalytic behaviors and capabilities, hence, new analysis tools are required for the studies of new enzyme investigations.[1, 2] Kinetic plots such as a Michaelis-Menten plot, a Lineweaver-Burk plot and the resultant kinetic parameters are normally used for catalytic evaluation[3]. Instant evaluation of enzyme catalysis in high throughput screening format is one of the most important steps for drug discovery, enzyme discovery and engineering.[4, 5] Recently, miniaturized devices have shown many clear advantages while conducting enzymatic reactions on a chip[6]. These include requirement of low sample consumptions due to the occasional limited availability of enzyme with a very high cost and possibility of integration of sample preparation steps, incubation, mixing, and optical detection.[6-8]

Here, we report on a new microfluidic system, that could generate a three-dimensional plot from forty-five parallel reactions with a single experiment on a chip.

EXPERIMENTAL
With the advantages of total analysis system in a single chip, we developed a highly integrated microfluidic system to accomplish reaction kinetic experiments for generating landcapers. This system is a completely new generation of our previous work[8]. For the device design, two independent parameters—concentrations of two different reagents—were selected. After this, six and seven variables for the reagents were selected to achieve reaction kinetic parameters of the model system. Finally, 42 data points in the X-Y plane were chosen, and three references were added. Figure 1 shows device design, data points, and the landscaping plot. Each processor was designed to be able to accomplish one reaction at a certain concentration of reagents. Hence, one set of six processors can provide reaction kinetic parameters according to various concentration of a reagent at a specific concentration of another reagent. Consequently, with seven sets of six processors, a landscape variation of reaction rate according to change in concentration of two different reagents can be generated with a single experiment on a chip.

Figure 1: Design of the number of processors in order to generate landscaping three dimensional plot for the study of reaction kinetics on a chip: (a) device design, (b) data points in X-Y plane, and (c) an example of landscaped map with yellow and blue dyes.
RESULTS AND DISCUSSION

The geometry of reactors and peristaltic mixing valves was designed based on our previous results of mixing efficiency in an active micromixer. The operation sequence and pressure were optimized by preliminary experiments with food dyes. Blue food dye was loaded into the halves of processors, while red dye was loaded into the other halves. The two different food dyes were then mixed through peristaltic mixing by operating mixing valves. Figure 2 shows mixing phenomena in processors with food dyes. Forty-five processors achieved more than 95% completed mixing below 15 seconds. The variation in mixing efficiencies of the 45 reactors was lower than 5%.

![Figure 2: (a) Mixing phenomena in processors. (b) Mixing efficiencies of 45 processors. Each processor can be accomplished complete mixing in 15 seconds, and 45 processors show constant mixing performance.](image)

To test the functionality of the metering technology, gradients of two different kinds of fluorescent molecules were performed – Texas-red (Sigma) and 5-carboxyfluorescein (5-FAM, Anaspec Inc.) – on the device. The gradient of Texas-red covered the concentration range from 0 to 200 µM, and the concentration of 5-FAM in its gradient started from 0 µM and ended at 5 µM. The Texas-red emits fluorescent light at 585 nm with excitation at 530 nm, whereas excitation and emission wavelengths of 5-FAM are 530 nm and 590 nm. Since only one specific molecule in the final mixture of reagents in processors emits fluorescent light at a given wavelength, we can selectively obtain the amount of target molecule in processors by changing the wavelength. This study therefore scanned a device twice at two particular wavelengths – 530 nm and 590 nm – to monitor the amount of Texas-red and 5-FAM.

Figure 2 shows fluorescent images of processors with loadings of Texas-red and 5-FAM. Figure 3 (a) shows double scanned fluorescent image of the device after performing gradients of Texas-red and 5-FAM. The gradients of 5-FAM and Texas-red are shown as Figure 3 (b) and 3 (c). The landscaped maps from the gradients of fluorescent molecules show relationship between fluorescent intensity and concentration of the fluorescent molecule. The increase of intensity is directly proportional to the concentration of fluorescent molecules, and the linearity of the curve represents the high metering and mixing efficiency of the device.

![Figure 3: (a) Double scanned image of the device after performing the gradients of Texas-red and 5-FAM. The concentration ranges of two reagents are indicated by data points in the plot. (b) The gradient of 5-FAM and landscaped standard curve for the reagent. (c) The gradient of Texas-red and the relationship between intensity and concentration of the reagent.](image)

CONCLUSION

We present new microfluidic system that has a potential to provide with landscaped information about reaction kinetics. The system consists of forty-five processors to perform parallel reactions with forty-five combinations of four reagents out of which the concentration could be formed for two reagents with a single experiment on a chip. We believe that the present work would have great potential to revealing many omic disciplines.
REFERENCES

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