MICROFLUDIC GEL ARRAYS FOR THE STUDY OF ENZYMATIC ACTIVITY

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ABSTRACT

We present a novel and flexible gel array platform to study enzymatic activity in confined environments. Recent research has highlighted the importance of studying proteins in confined environments that replicate the inside of cells rather than studying proteins in dilute solutions. Our platform allows the punctual and selective introduction of substrates and the quantitative readout of product formation to study the effect of protein confinement on enzymatic activity.

KEYWORDS: Droplet, Array, Hydrogel, Enzyme

INTRODUCTION

The cell cytosol is crowded, with nearly 40% of the cell volume composed of macromolecules. Studies have shown that both the thermodynamic values and enzymatic rates are different in the resulting confined environment from those obtained in dilute solutions [1]. The agarose hydrogel matrix provides a confined environment to study protein structure and function *in vitro*. Conventional technologies such as petri dishes and well plates, limits the number of multiplex experiments that can be performed and the exchange of reagents within the same system. Current gel array devices [2] are difficult to fabricate and not suited for the dynamic measurements of enzymes. We present here a gel array microfluidic device that can overcome these limitations while greatly enhancing the selectivity and control of experiments.

EXPERIMENTAL

PDMS microfluidic chips with channel depth modulations were fabricated using soft lithography. A flow focuser is used to make aqueous droplets, containing 2% agarose and the enzyme horseradish peroxidase (HRP), in fluorinated oil. The gel array is produced by flowing the droplets into a wide channel containing microfabricated wells of increased channel depth. We exploit the property that flattened droplets will expand into areas of decreased confinement to reduce their surface energy. This passive technique, called “rails and anchors” [3], anchors droplets in predetermined positions to quickly produce a droplet array. The preparation of the gel array is performed in an incubator set at 45°C to avoid premature gelation of the agarose. Once the array is created, the device is cooled to room temperature allowing the agarose to solidify.

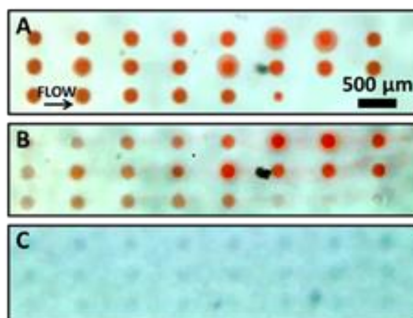

Figure 1: a) *Agarose droplet array containing HRP enzyme and red dye.* ***b)*** *Elution of the red dye when the external oil phase is replaced by buffer solution.* ***c)*** *Production of blue product is observed after the introduction of the enzyme substrates. The blue product elutes from the agarose gel.*

RESULTS AND DISCUSSION

Figure 1a shows agarose gels containing red food coloring surrounded by fluorinated oil. The external oil phase is replaced by buffer (Figure 1b) progressively removing the red food coloring from the agarose gels. HRP catalyzes the oxidation of 2,29-azinobis(3-ethylbenzthiazoline-6-sulphonate) (ABTS) in the presence of hydrogen peroxide to produce a blue product. Enzyme activity within the gel is observed by introducing the substrates into the device. The enzymatic conversion to a blue product can be observed in Figure 1c and 2. The gel array device allows selective and punctual exposure of substrates to confined enzymes.

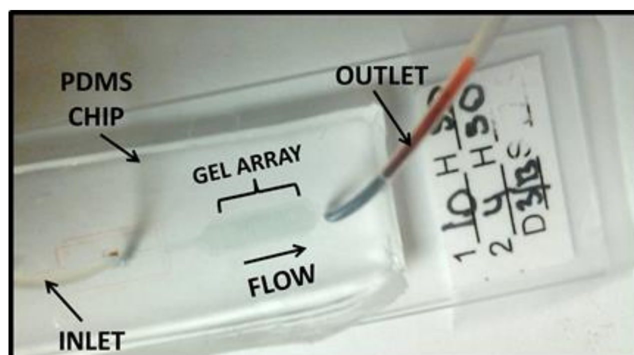

Figure 2*: View of PDMS chip during experiment. The array containing the gel array is stained blue indicating the enzymatic conversion to colored products. The outlet shows clearly the sequentially elution of red dye followed by the blue product.*

Alternatively, the external oil can be retained around the gel array and reagents can be introduced by the fusion of the gel and a droplet containing substrates. Using passive techniques described in [4], pairs of droplets of different content can be placed in the same well (first panel of Figure 3). The droplets are anchored in place despite a continued flow of the external oil. Flowing external oil without the surfactant removes surfactant from the droplet interface and provokes droplets fusion [5] (Figure 3).

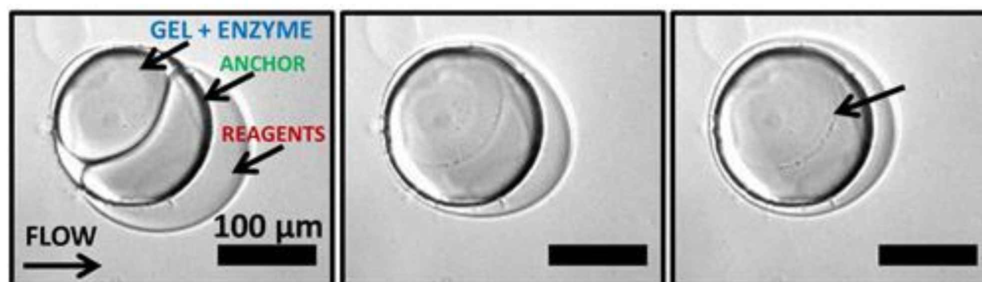

Figure 3: *Image sequence of fusion of droplet of agarose gel containing the HRP enzyme and a droplet containing reaction reagents and substrates. The gel interface is still visible after droplet fusion as indicated by the arrow in the third panel.*

The production of product can be observed in the droplet fusion method by a change in color of the droplet (Figure 4a) and measured as a function of time (Figure 4b). The small scale of the gel allows the quick exchange of reagents and is therefore appropriate for the measurement of enzyme kinetics of confined proteins.

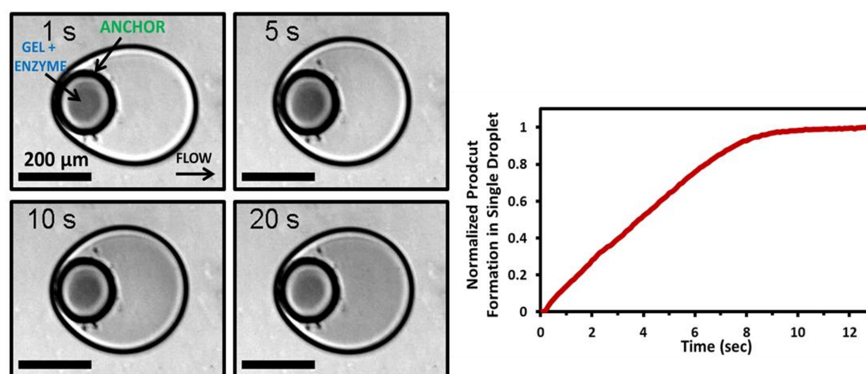

*Figure 4: **a)** Image sequence directly after fusion of gel containing HRP enzyme and the substrate droplet. Product formation is observed by a progressive darkening of the droplet. **b)** Normalized product formation as of function of time for the individual droplet.*

CONCLUSION

The device will allow a larger set of conditions to be explored than previous studies of proteins in gels. Enzyme kinetics of confined droplets can be explored on a time scale not easily accessible by other methods by fusing nanoliter droplets of different contents. The combination of temporal and spatial selectivity makes this a powerful tool to acquire information on how confined environments influence enzyme structure and function.

ACKNOWLEDGEMENTS

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