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INVESTIGATION OF CELL TRAJECTORIES IN CHAOTIC FLOW FIELDS GENERATED BY A HELICAL STATIC MICROMIXER FOR RAPID CROSSLINKING KINETICS

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ABSTRACT

Transcription factor (TF) binding pattern in chromatin provide precise and comprehensive information about the cell state. However, current analysis methods such as chromatin immunoprecipitation (ChIP) does not readily facilitate in-vivo characterization required to study short lived chromatin complexes. Here we develop a microfluidic stop flow system which is capable of mixing bacterial cells with formaldehyde on a sub-second mixing residence time scale enabling us to probe the kinetics of TF bound chromatin intermediates.

KEYWORDS: Chromatin immunoprecipitation, Chaotic mixing, Transcription factor binding.

INTRODUCTION

The location of chromatin regulatory factors associated with DNA along with information about their growth and differentiation has been a major goal of the chromatin field. The process involves crosslinking chromatin fragments with formaldehyde so that the associated DNA can be obtained and assayed via PCR or sequencing [1]. This general approach called chromatic immunoprecipitation (ChIP) is well suited to measure dynamics occurring on a time scale of 20 - 30 minutes. However, to analyze binding kinetics at time scales corresponding to the formation of chromatin complex intermediates (sub second resolution), there is a need for an in-vivo analysis system which incorporates rapid mixing of cells with formaldehyde. Here we develop a microfluidic system with an embedded helical micromixer to perform the equivalent of a stopped flow mixing and quenching analysis. In our model system we study the cell migration trajectories in the flow fields generated by helical micro-mixers and determine the conditions under which chaotic advection can be harnessed to enhance kinetics[2, 3].

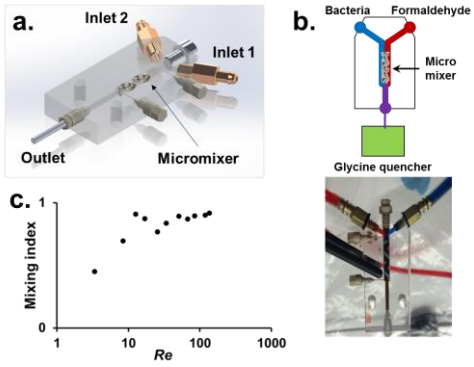

Figure 1: Micromixer chip (a) Y shaped flow chip with up to 3 inlets with an embedded mixer. (b) Schematic demonstrating rapid mixing of bacterial cells with formaldehyde. (c) Mixing efficiency for colored dye mixing over 3 orders of Reynolds number.

THEORY

Due to inherent size scale of micro-scale flows, it is nearly impossible to achieve turbulent flow via inertial instability. Therefore, microfluidic systems have to rely on various active and passive mixing solutions. Our system uses a static mixer comprising of alternating clockwise and counterclockwise 180 º helical twist elements (Figure 1b). This arrangement causes the flow to periodically split and recombine resulting in a "Baker's map" type of transformation where the interfacial area between the two fluid streams doubles after every twist.

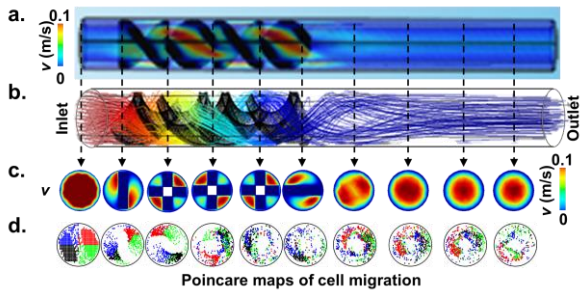

Figure 2: CFD simulation and tracking cell migration. (a) Velocity profile reveals the flow profile around the mixer. (b) Time resolved trajectories of 5 micron cells originating at the inlet. (c) Cross sectional velocity profiles reveals areas of high and low local velocities near the mixer. (d) Poincaré maps of cells originating at the inlet in four distinct islands evolve rapidly at the onset of chaotic advection.

EXPERIMENTAL

The mixer was able to achieve >95% mixing index (Figure 1) at low Reynolds number (mixing index was quantified by injecting red and blue dyes and calculating the standard deviation of red pixel intensity in a region of interest downstream from the mixer).To highlight the spectrum of assessable flow states, we performed computational fluid dynamics (CFD) simulations at for different mixer geometry and channel Reynolds number. Virtual particles mimicking cells were introduced in the flow domain and all relevant hydrodynamic forces (Drag, Saffman lift, pressure gradient and rotational forces) were resolved in a Lagrangian fashion. We also introduced cell mimicking green fluorescent polystyrene particles (5 µm) in flow to visualize the cell mixing with the formaldehyde stream experimentally.

RESULTS AND DISCUSSION

The mixing index remained high at even higher flow rates (Re~100) yielding mixing times on the orders of ~100 ms, sufficient to resolve in-vivo cross linking kinetics. The simulations revealed that a four segmented helical micromixer with alternating twists was sufficient to induce transition from 2D laminar flow trajectories to chaotic advection. This is shown by the disruptions of Kolmogorov-Arnold-Moser (KAM) boundaries in the Poincaré maps (signature of chaos) as the cell trajectories pierce distant areas in the cross section along the channel length (figure 2). Without the mixer, the cells either tend to flow without crossing to over to the formaldehyde stream at low flow rates or hydrodynamically focus away from the channel walls at higher flow rates. Both these unmixed states are lost when the mixer is introduced providing a uniform distribution of beads along the channel cross section (figure 3).

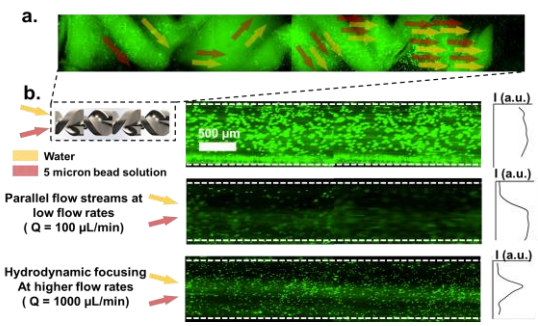

Figure 3: Experimentally visualizing the chaotic cell migration with fluorescent beads. (a) Mixing of bead laden (5 micron microspheres) fluid streams around the helical mixer. (b)Without the mixer water and bead solution remains separated at low flow rates. At higher flow rates, hydrodynamic forces focus the beads along the center. On introducing the micromixer, the chaotic flow field downstream disrupts the hydrodynamic focusing and enables efficient cross flow for rapid mixing.

CONCLUSION

Our computational fluid dynamics model analyzes different flow and geometric properties of helical micro-mixers to generate chaotic advection at laminar condition to enhance chromatic-TF crosslinking kinetics. This approach would allow us to capture chromatin dynamic behavior over a much broader time scale then previously possible.

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