RAPID OPTIMIZATION OF BACTERIAL ELECTROPORATION CONDITIONS

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ABSTRACT

We have developed a novel microfluidic platform to determine the minimal electric field threshold for bacterial electroporation. This assay-based technology employs microfluidic channels with an electric field gradient due to the geometrical configurations. The fluorescent dyes used allow for visual identification and quantification of the onset and extent of electroporation. Ultimately, this work provides a simple platform to quickly determine electroporation conditions for bacterial strains for which traditional field assisted genetic transformation has been unsuccessful. Results of this study will broaden the scope of bacteria available for applications in genetic engineering, synthetic biology, and biotechnology.

KEYWORDS: Electropermeabilization, Microorganisms, Inactivation, Microfluidics, Electric Field

INTRODUCTION

Electroporation is a powerful biotechnological tool for enhancing the delivery of exogenous agents to cells with pulsed electric fields (PEFs). Microfluidic devices have been developed for single cell electroporation using low voltages [1, 2]. Flow-through electroporation methods have been developed by Geng *et al.* [3] for delivery of genes into cells and by Wang *et al.* for electrical lysis of bacterial cells [4] using a constriction to amplify the electric field in the microchannel. This paper reports on a rapid assay to visualize and quantify the electric field threshold required for successful field-assisted genetic transformation. Our diagnostic assay differs from that of the group of Lu *et al.* [5] in that our aim is to rapidly quantify the critical field strength required for inducing genetic transformation.

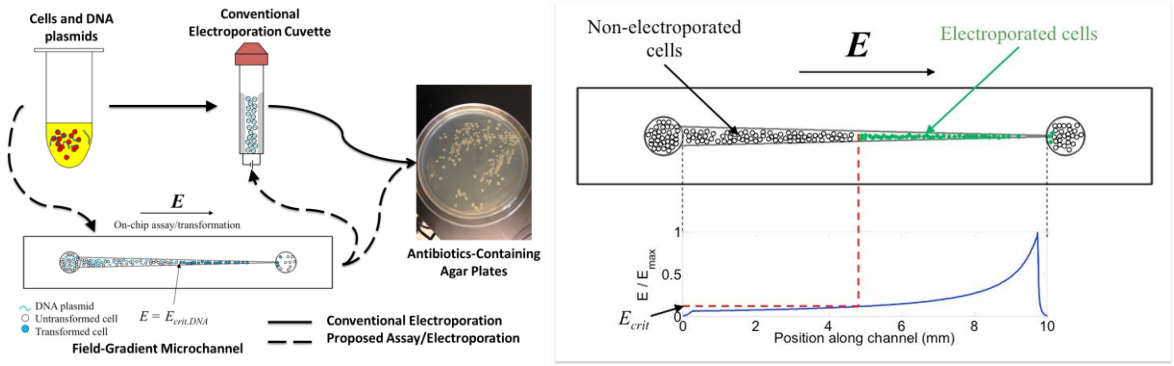

Figure 1: Electroporation assay determine the minimum electric field required for electroporation of a given bacterium. (left) The system consists of a converging microchannel to rapidly assay and optimize electrical conditions for bacterial electroporation. The conventional (solid) and the proposed (dashed) protocols for bacterial transformation are shown with the vision to identify optimal electrical conditions for bacterial electroporation. (right) In this converging microchannel, the electric field magnitude varies with position along the channel as shown above (normalized by the maximum value). Electroporation occurs only in the regions where the local field exceeds the critical threshold for pore formation, which we denote as E_{crit}.

EXPERIMENTAL

The assay works by introducing a mixture of cells in media with a dye that fluoresces when the membrane is compromised. Membrane permeabilization could occur due to exposure to external electric pulses, membrane-damaging chemicals, thermal shock, or other membrane perturbations. We flow the

mixture of cells in media with fluorescent dye, SYTOX® (Life Technologies), which is unable to permeate the cell membrane (Figure 1). We then apply an electric pulse that disrupts the cell envelope and detect SYTOX® dye uptake with very fast response time (~ 20 ms) to detect electroporation, as shown in Figure 1. This procedure is applied to *Escherichia coli* strains K12 and BL21.

The microdevice is fabricated using traditional soft lithography techniques outlined in Figure 2 in which a photomask is designed in AutoCAD 2014 with the desired features. The features are then patterned in a Si wafer using SU-8 lithography. In our microfabrication, the height is set to 15 μm in order to maintain dimensions that are close to the dimensions of the bacteria (~ 2 μm). Once the master stamp is fabricated, we use PDMS Sylgard 184 (Dow Corning) at a 10:1 ratio. Once the PDMS is cured for at least two hours, the geometry is bonded to a glass slide after plasma treatment.

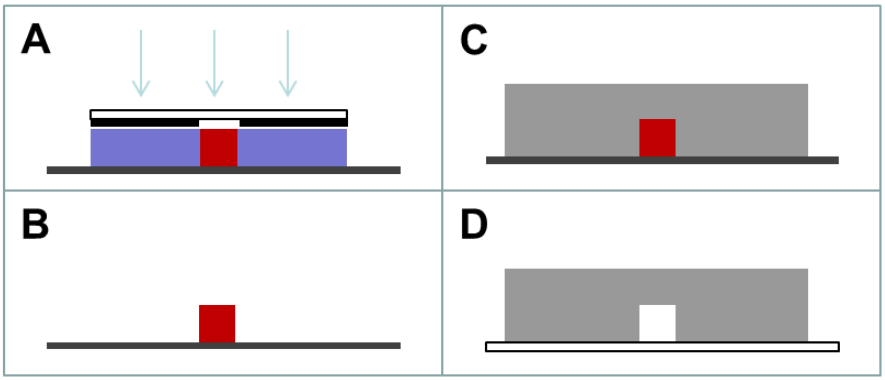

Figure 2: Device fabrication process using soft lithography. A) SU-8 lithography on Si wafer. B) Washing away un-exposed SU-8. C) Molding PDMS using SU-8 master. D) Peeling PDMS off the SU-8 master and bond PDMS to the glass slide.

RESULTS AND DISCUSSION

The fluorescence activity of the cells before, during, and after exposure to the electric pulses is recorded in order to quantify the onset and extent of dye uptake, which can be used as a surrogate for DNA uptake. After the electric field is applied, a region of the channel where the field is sufficiently high fluoresces, indicating electroporation has occurred. Specifically, we simulated the electric field distribution using COMSOL Multiphysics (Burlington, MA) with an applied voltage of 3000 V. Due to the converging configuration of the device, the bacteria are exposed to an amplified electric field as the channel narrows. This assay has been used to determine that the minimum electric field required for electroporation of *E. coli* BL21 is approximately 12 kV/cm (Figure 3), a similar magnitude to the 12.5 kV/cm suggested in the user's manual to the MicroPulser™ electroporation systems (Bio-Rad, Hercules, CA).

CONCLUSION

In summary, we have developed a microfluidic device that can be used to assess bacterial electroporation conditions. The geometry can be optimized to alter the electric field gradient within the channel, which allows for sensitive detection of threshold electric fields for electroporation. The vision of this rapid assay is to be able to use a single chip to provide microbiologists with the tools required to determine the required electric field that will provide the greatest chance of successful genetic transformation. Our work differs from the traditional trial and error approach employed in that we develop an assay for systematically sampling a continuum of electric fields that can be used to probe any type of bacteria. Ultimately this work provides a simple platform to quickly determine electroporation conditions for bacterial strains for which traditional field assisted genetic transformation has been

unsuccessful. Results of this study will broaden the scope of bacteria available for applications in genetic engineering and synthetic biology.

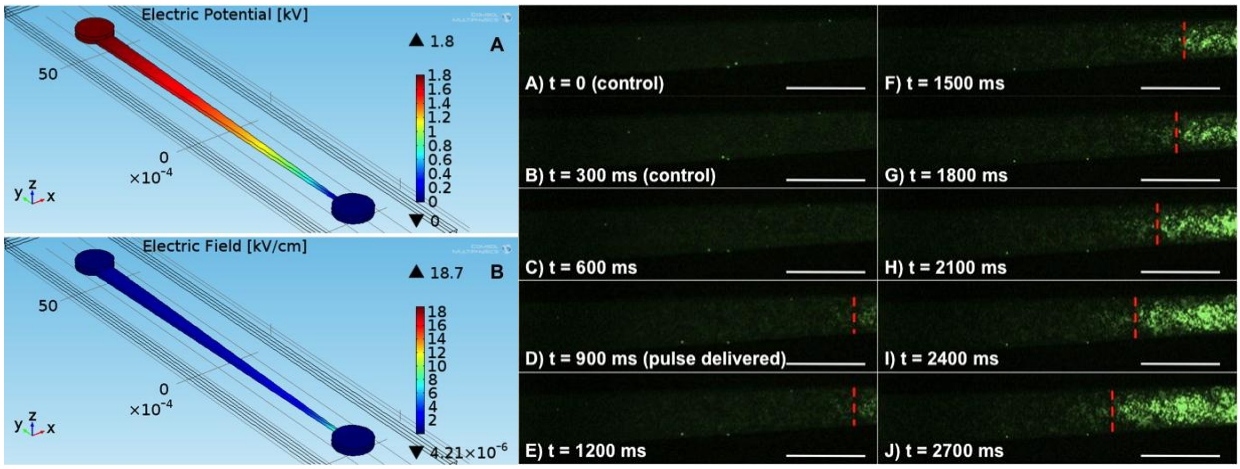

Figure 3: (left) Simulated A) Electric potential and B) electric field distributions. These computational results were computed to determine the E_{crit} required for electroporation and to determine if the electric field is sufficiently high in our device to achieve electroporation of cells. (right) E. coli BL21 bacteria undergoing electroporation in the microdevice. We detected the location at which fluorescence of, e.g., SYTO-X (which is indicative of membrane permeabilization) begins; E_{crit} is then determined as the magnitude of the electric field at this location from the numerical models.

ACKNOWLEDGEMENTS

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