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Micropillar-assisted electric field enhancement for high-efficiency inactivation of bacteria

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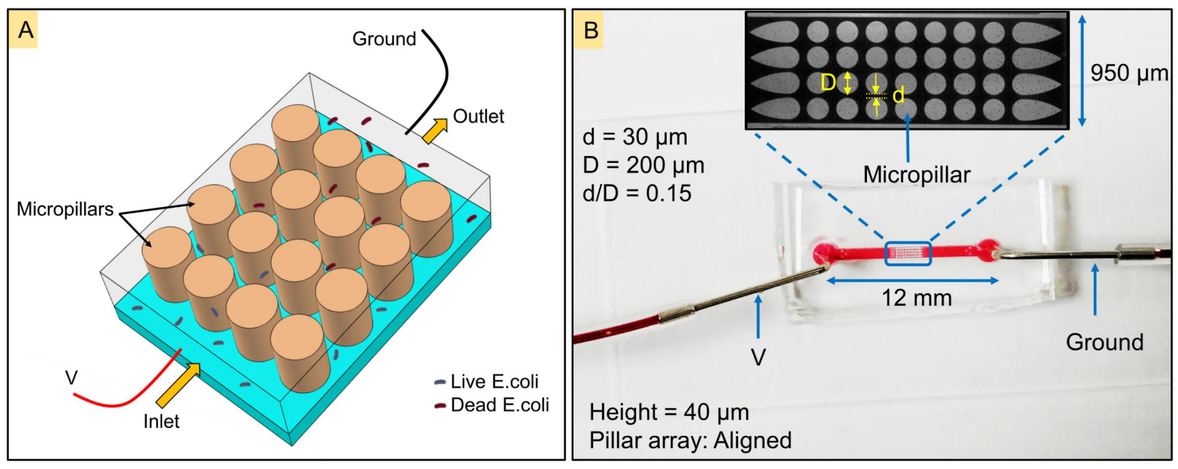
1. Abstract

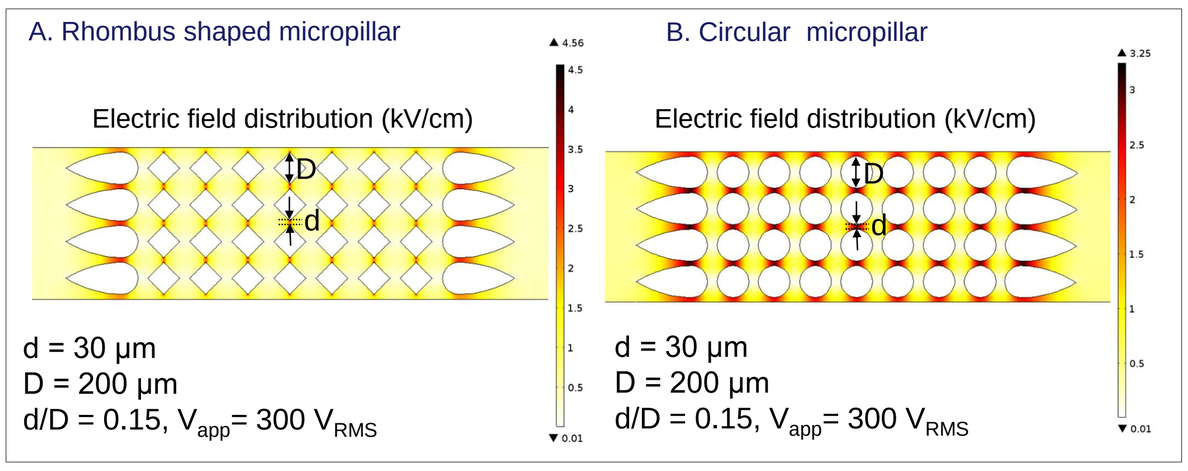
Development of high-efficiency and environment friendly bacterial inactivation methods is of great importance for preventing waterborne diseases which are one of the leading causes of death in the world. Traditional bacterial inactivation techniques have several limitations such as longer treatment time, use of harmful chemicals, formation of toxic byproducts, bacterial regrowth etc. Here, an electroporation-based continuous flow insulator-based AC dielectrophoresis device (iDEP) is developed and that the device achieved high bacterial inactivation performance (>99.9%) within a short exposure time (<1 s). Inactivation performance was evaluated for *Escherichia coli* and *Enterococcus faecalis* under various electric field conditions. More than 4 log removal of bacteria was obtained with an applied voltage of 300 V for the flowrate of 1 mL/hr. Images from scanning electron microscope confirmed the formation of electroporation-induced nano-pore within the cell membrane. The reported method of inactivation does not involve any chemicals and the formation of harmful by-products is also minimized.

2. Microfluidic device



Above figures depicts (A) schematic of our microfluidic device and (B) PDMS-glass microfluidic device. Microchannel was fabricated using standard soft lithography technique.

3. Numerical simulation: Electric field distribution



Comparison of distribution of electric field (kV/cm) for two microfluidic devices with; (A) rhombus shaped micropillar and (B) circular micropillar. Darker region indicates higher magnitude of electric field.

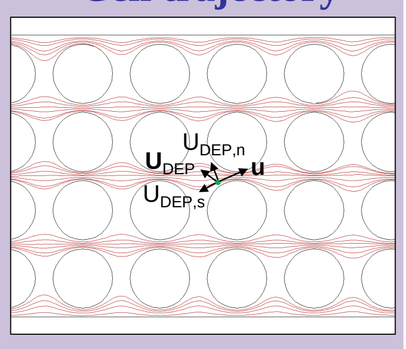
Cell velocity can be obtained as [1]:

$$\boldsymbol{u_p} = \boldsymbol{u} + \frac{f_{CM}\varepsilon_m r^2}{6\mu}\boldsymbol{\nabla E^2}$$

(Cell size)

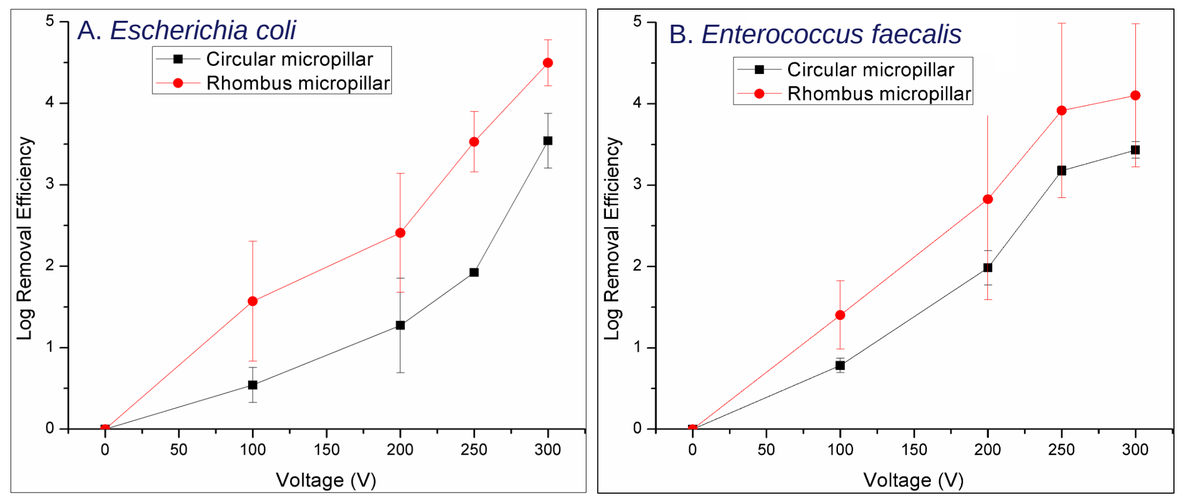
$$f_{CM} = \frac{\varepsilon_c^* - \varepsilon_m^*}{\varepsilon_c^* + 2\varepsilon_m^*} \sim \frac{\sigma_c - \sigma_m}{\sigma_c + 2\sigma_m}$$ (for low frequency AC (<10 kHz) or DC) [2,3].

Cell trajectory



In the above equation, electrokinetic effects (EO and EP) were neglected as only AC field was applied in our study.

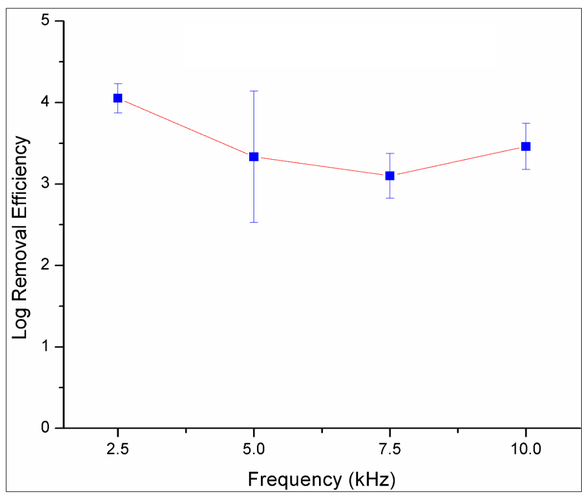
4. Influence of micropillar shape



(A) Comparison of log removal efficiencies for *E. coli* (gram-negative bacteria) for microfluidic devices with rhombus shaped micropillars and circular micropillars

(B) Comparison of log removal efficiencies for *E. faecalis* (gram-positive bacteria) for microfluidic devices with rhombus shaped micropillars and circular micropillars

5. Effect of electrical parameters



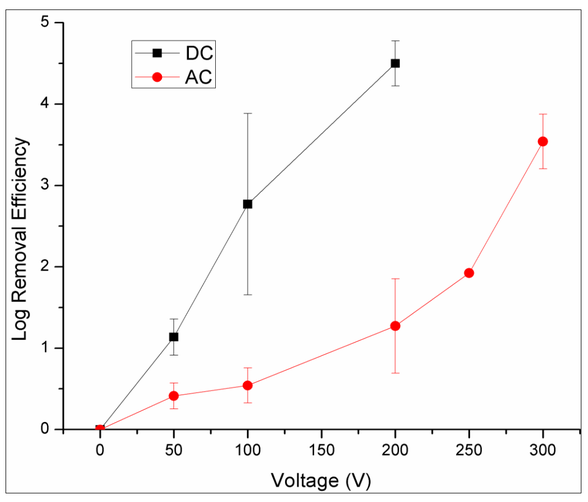
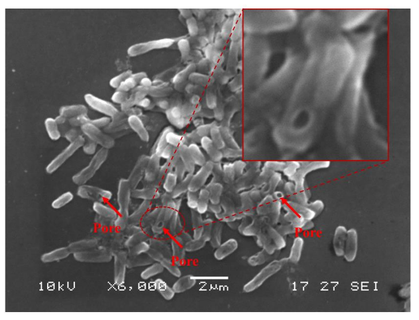


Figure (A) shows the effect of applied AC frequency on log removal efficiency of *E.coli* under an applied AC voltage of 300 V and flow rate being 1 mL/hr.

Figure (B) represents comparison of log removal efficiencies of *E.coli* for various AC and DC voltages. Flow rate is 1 mL/hr.

6. Scanning electron microscopy

Demonstration of nano-pores formation on the cell membrane of *E. coli* cells using scanning electron microscopy (SEM) image.



7. Conclusion

- iDEP device is developed for investigating bacterial inactivation using electroporation technique
- As seen from numerical simulation results, rhombus shaped micropillar are more effective in enhancing electric field. Experiments confirms higher log removal efficiency for rhombus shaped micropillary device.
- Under the same operating conditions, gram-positive bacteria are more resistant than gram-negative bacteria

8. References

[1] Kale, A., Patel, S., Hu, G., Xuan, X., *Electrophoresis* 2013, *34*, 674-683.

[2] Lewpiriyawong, N., Yang, C., Lam, Y. C., *Electrophoresis* 2010, *31*, 2622-2631.

[3] Pudasaini S, Perera A. T. K., Ahmed S. S. U., Ng S. H., Yang, C., *Electrophoresis* 2019.

Acknowledgements

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