

RAPID CONTINUOUS ELECTRICAL LYSIS OF BACTERIA ON STRUCTURED ELECTRODES PRESERVES RNA INTEGRITY

Mahla Poudineh¹, Reza M. Mohamadi¹, Andrew Sage¹, Laili Mahmoudian¹,
Edward H. Sargent¹, and Shana O. Kelley¹

¹University of Toronto, Canada

ABSTRACT

We present a rapid electrical lysis method using structured gold electrodes in a microfluidic device. This strategy is capable of efficient and high throughput lysis of bacteria to prepare intracellular genomic materials for subsequent analysis. High electrical field at the tips of structured gold electrodes eliminates the requirement of high voltage. We have used AC voltage for lysis, and shown at low frequency; lysis would be electrochemical while at high frequency it occurs due to the electrical field.

KEYWORDS: Structured electrodes, Electrical lysis, RNA Integrity

INTRODUCTION

Released biomarkers are pivotal molecules for the rapid detection and diagnosis of disease. Much effort has been done to make diagnostic devices aim to achieve miniaturized, cost effective and integrated point-of-care devices for rapid medical monitoring [1]. Microfluidic devices are superior candidates for portable and point-of-care diagnostic devices as they are capable of measuring very small volumes and can be integrated with downstream analysis, without the requirement for expert technicians and laboratory facilities [2-3].

One of the essential steps for a point-of-care diagnostic device which could be performed using a microfluidic-based platform is cell lysis with nucleic acid extraction [3]. A number of different on-chip cell lysis strategies have been developed: chemical, thermal, mechanical, electrochemical, and electrical. Hydroxyl generation could be used to rupture fatty cell membrane [4]. The applied electrical field will lead to hydrolysis of H₂O molecules, which in turn generates hydroxyl groups and other reactive radicals such as hypochlorite ion. These reactive agents can degrade biological molecules released from the cell after lysis[5]. Furthermore, it has been shown alkaline environment and by-products of electrolysis at the anode, such as hypochlorous acid, can degrade RNA and negatively affect downstream analysis[6]. Since RNA is an important biomarker for diseases diagnosis, development of techniques to efficiently release RNA from cell lysates is a significant challenge. Herein, we show a new method for purely electrical release of biomarkers, specifically RNA molecule, inline in a microfluidic channel. Central to this new technology is the use of sharp-tipped structured electrodes in lysis, and modulation of the applied voltage at kHz frequencies.

RESULTS AND DISCUSSION

Figure 1 shows the schematic of microfluidic device used for electrical lysis of bacteria. Multiple parallel channels were used to increase the throughput of the device. We prove using both experiment and finite-element modeling that structuring of electrodes could dramatically increase the local electric field concentration necessary for efficient cell wall rupture (Figure 2).

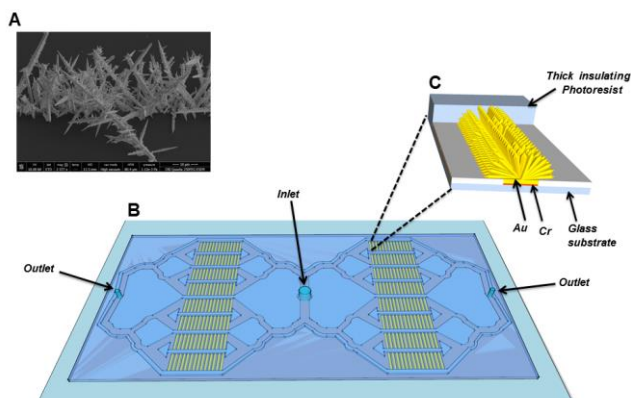


Figure 1: A) Scanning electron microscopy image of electrodeposited structured electrodes. Sharp features of the structure are used for electrical field concentration. B) Schematic of microfluidic device with multiple parallel channels were used to increase the throughput of the device with two outlets. C) Schematic of device cross-section.

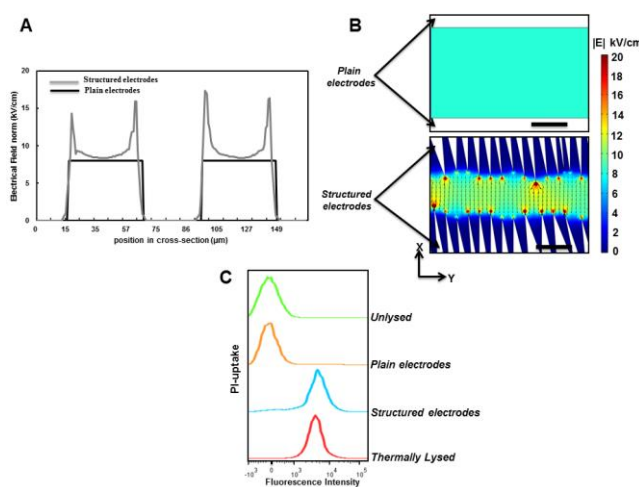


Figure 2: A) Electrical field profile in the center of the each channel for structured and plain electrodes at voltage $V_{pp}=80$ V. B) Electrical field distribution for two adjacent electrodes. High field intensity has been shown in red. C) Flow cytometry measurements of cells that were unlysed, plain or structured electrodes, or thermally lysed. The conditions for electrical lysis are $f=500$ kHz, $V_{pp}=80$ V, and lysis rate= 1600 cell. s^{-1} . Scale bars represent 20 μ m.

Additionally, we indicate that modulation of AC frequency will reduce the electrochemical current production which could lead to RNA degradation. Using RNA Integrity Number (RIN) analysis, it has been shown the new electrical lysis approach offers notably higher RNA quality than electrochemically released analyses. Figure 4 presents lysis efficiency at different frequencies and RIN analysis results for electrical and electrochemical lysis.

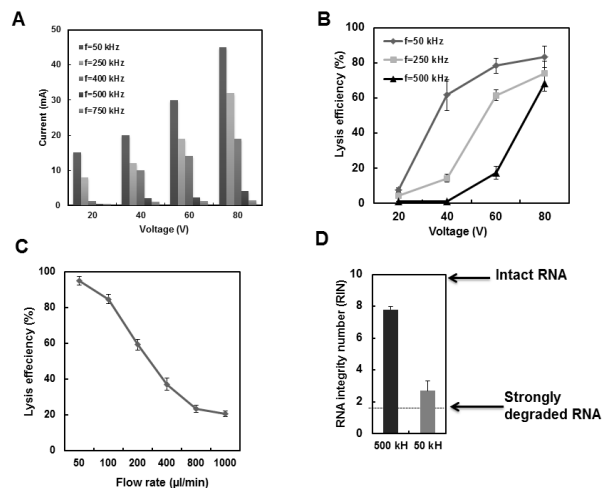


Figure 3: A) Generated current versus voltage at different frequencies (50 kHz, 250 kHz, 400 kHz, 500 kHz and 750 kHz). Reduction in current corresponds to prohibition of electrochemical reactions. B) Lysis efficiency versus voltage at different frequencies. At low frequency and low voltages, lysis mechanism is electrochemical while at high frequency and high voltage, it happens due electrical field. C) Measurements of electrical lysis efficiency at different flow rates. D) RIN measurement results at $f=50$ kHz and $V_{pp}=60$ V (electrochemical lysis) and $f=500$ kHz and $V_{pp}=80$ V (electrical lysis). Values shown represent averages of 6 trials.

CONCLUSION

With this new device platform which could be integrated easily with downstream analysis, we prove that we are able to lyse a clinically-relevant sample volume (100 μ L) of bacteria in under 1 minute, with both high lysis efficiency and high RNA quality.

REFERENCES

- [1] N. L. Rosi and C. a Mirkin, "Nanostructures in biodiagnostics.," *Chem. Rev.*, 105, 4, 2005.
- [2] P. Yager, T. Edwards, E. Fu, K. Helton, K. Nelson, M. R. Tam, and B. H. Weigl, "Microfluidic diagnostic technologies for global public health.," *Nature*, 442, 7101, 2006.
- [3] D. Di Carlo, C. Ionescu-Zanetti, Y. Zhang, P. Hung, and L. P. Lee, "On-chip cell lysis by local hydroxide generation.," *Lab Chip*, 5, 2, 2005.
- [4] J. T. Nevill, R. Cooper, M. Dueck, D. N. Breslauer, and L. P. Lee, "Integrated microfluidic cell culture and lysis on a chip.," *Lab Chip*, 7, 12, 2007.
- [5] H. J. Lee, J.-H. Kim, H. K. Lim, E. C. Cho, N. Huh, C. Ko, J. C. Park, J.-W. Choi, and S. S. Lee, "Electrochemical cell lysis device for DNA extraction.," *Lab Chip*, 10, 5, 2010.
- [6] S. M. McKenna and K. J. Davies, "The inhibition of bacterial growth by hypochlorous acid. Possible role in the bactericidal activity of phagocytes.," *Biochem. J.*, 254, 3, 1988.

CONTACT

* M. Poudineh; mahla.poudineh@mail.utoronto.ca