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Electrokinetic stringency control in self-assembled monolayer-based biosensors for multiplex urinary tract infection diagnosis

Tingting Liu, MS[a], Mandy L.Y. Sin, PhD[a,b], Jeff D. Pyne, BS[a], Vincent Gau, PhD[c], Joseph C. Liao, MD[b,d], Pak Kin Wong, PhD[a,*]

[a]*Department of Aerospace and Mechanical Engineering, University of Arizona, Tucson, AZ, USA*
[b]*Department of Urology, Stanford University, Stanford, CA, USA*
[c]*GeneFluidics Inc., Irwindale, CA, USA*
[d]*Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, USA*

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Abstract

Rapid detection of bacterial pathogens is critical toward judicious management of infectious diseases. Herein, we demonstrate an in situ electrokinetic stringency control approach for a self-assembled monolayer-based electrochemical biosensor toward urinary tract infection diagnosis. The in situ electrokinetic stringency control technique generates Joule heating induced temperature rise and electrothermal fluid motion directly on the sensor to improve its performance for detecting bacterial 16S rRNA, a phylogenetic biomarker. The dependence of the hybridization efficiency reveals that in situ electrokinetic stringency control is capable of discriminating single-base mismatches. With electrokinetic stringency control, the background noise due to the matrix effects of clinical urine samples can be reduced by 60%. The applicability of the system is demonstrated by multiplex detection of three uropathogenic clinical isolates with similar 16S rRNA sequences. The results demonstrate that electrokinetic stringency control can significantly improve the signal-to-noise ratio of the biosensor for multiplex urinary tract infection diagnosis.

From the Clinical Editor: Urinary tract infections remain a significant cause of mortality and morbidity as secondary conditions often related to chronic diseases or to immunosuppression. Rapid and sensitive identification of the causative organisms is critical in the appropriate management of this condition. These investigators demonstrate an in situ electrokinetic stringency control approach for a self-assembled monolayer-based electrochemical biosensor toward urinary tract infection diagnosis, establishing that such an approach significantly improves the biosensor's signal-to-noise ratio.

Key words: Urinary tract infection; Stringency control; Multiplex detection; Matrix effects; Self-assembled monolayers

Infectious diseases caused by bacterial pathogens are common causes of patient morbidity and a major global healthcare challenge.[1] For instance, urinary tract infection (UTI), the most common bacterial infection of any organ system, is a major cause of health care expenditures, accounting for millions of office visits and hospital admissions every year in America.[2,3] To properly manage infectious diseases, patient samples, such as urine and blood, are delivered to clinical microbiology laboratories for pathogen identification. A major limitation of the standard culture-based diagnostic approach, however, is that at least two to three days is required from specimen collection to result reporting. The long delay is a result of sample transport to the clinical microbiology laboratory and overnight culture. Without direct information about the pathogens, antibiotic treatments are typically chosen based on the worst-case scenario assumption, even in situations where bacteria pathogens are ultimately found not to be the culprit. Therefore, a biosensor that allows rapid identification of the pathogens at the point of care will be tremendously beneficial to the management and treatment of infectious diseases.

Recently, numerous molecular and nanoengineered pathogen detection strategies have been developed.[4,5] Self-assembled monolayer (SAM)-based electrochemical biosensors, in particular, have received a lot of attention in the point-of-care diagnostics community.[6–11] Electrochemical nucleic acid biosensors have several advantages over other detection methods. These advantages include cost-effectiveness, excellent

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*Corresponding author: Aerospace and Mechanical Engineering, University of Arizona, Tucson, AZ 85721, USA.

E-mail address: pak@email.arizona.edu (P.K. Wong).

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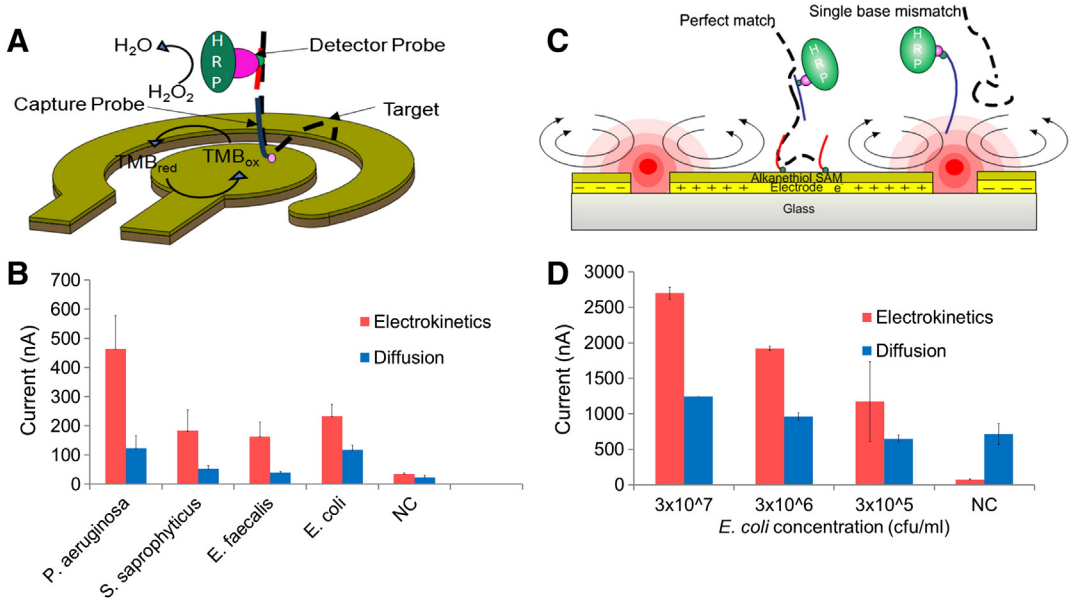

Figure 1. (**A**) Self-assembled monolayer based electrochemical pathogen sensor. (**B**) Non-specific binding is removed by the temperature rise and fluid flow for washing based on electrokinetic control. (**C**) *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, and *Escherichia coli* are detected in the conditions of diffusion and electrokinetics. (**D**) The concentration of *E. coli* was measured with respect to diffusion and electrokinetics. A square wave with voltage of 6 Vpp at 200 kHz was applied for 8 minutes.

sensitivity and specificity, compatibility with micro/nano fabrication technology, and the availability of compact and portable electronic interfaces. For instance, a SAM-based electrochemical biosensor array for detecting species-specific bacterial 16S rRNA has been reported for rapid UTI diagnosis.[12–14] In the "sandwich"-binding scheme of the biosensor, the target 16S rRNA is anchored at the sensor surface by a DNA capture probe and is also hybridized to a DNA detector probe. The detector probe is labeled with a reporter coupled to an oxidoreductase enzyme, which interacts with the substrate and generates a redox signal to be detected by the sensor electrodes (Figure 1, *A*). While electrochemical biosensor is a promising platform for rapid diagnosis of infectious diseases, several technological challenges remain for point-of-care applications. In clinical samples, such as blood and urine, matrix components including metabolites, proteins, and salts can introduce non-specific binding, which reduces the overall sensitivity of the assay and may lead to false positives.[15] Furthermore, bacterial pathogens may share similar nucleic acid sequences that can reduce the diagnostic accuracy of the molecular assay. While it is possible to design highly specific capture and detector probe pairs for each species, multiplex detection with multiple detector probes simultaneously can introduce crosstalk between different sensors.[16–21] The crosstalk may lead to non-specific detection of the bacterial species and can result in an overestimation of the target concentration. Within a clinical context, it is essential to reduce the uncertainty due to non-specific binding and improve the overall reliability of the multiplex pathogen identification assays.

Innovative technologies for stringency control are highly desirable to address the aforementioned issues toward multiplex detection of infectious diseases. Stringency control by adjusting the hybridization temperature and stringency wash has been widely adopted to remove non-specific binding in hybridization assays.[22–26] However, these strategies could be challenging to implement in resource-limited settings. In this study, we exploit alternating current (AC) electrokinetics to generate local heating and fluid motion for in situ electrokinetic stringency control. AC electrokinetics requires only simple electronic interfaces and a low driving voltage, which eliminates the need for a high driving voltage power supply and avoids bubble formation due to electrolysis. These characteristics render AC electrokinetics a promising platform for point-of-care diagnostics.[27]

For stringency control, an applied electric field can create a Joule heating induced temperature elevation, which is a result of the Joule heating as electric current flows through the conductive hybridization buffer.[28] The electrical conductivity of the fluid and the amplitude of the electric field can control the magnitude of the temperature rise. Depending on the electrode configuration, the local heating can also generate a spatial temperature distribution, which induces gradients of conductivity and permittivity. The interaction between these gradients and the electric field can create electrokinetic forces and bulk electrothermal fluid motion. The temperature distribution at equilibrium can be determined by considering the simplified energy equation:[29–32]

$$k\nabla^2 T + \sigma E^2 = 0$$

where T is the temperature of the medium, σ is the conductivity of the medium, k is the thermal diffusivity, and E is the electric field. For a pair of parallel electrodes with a small gap, the temperature rise near the gap of the electrodes has been estimated to be:

$$\Delta T = \sigma V_{rms}^2 / 8k$$