Fabrication of Microdevices for Thermal Stability Analysis of DNA

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Technologies that rapidly measure the thermal stability of analytes on a cost-efficient platform for high-throughput screening can play a key role in both drug discovery and biomolecular characterization. The melting temperature, T_m, informs on the inherent stability of a biomolecule and can therefore be used to probe stabilizing factors, such as salt concentration or drug molecule binding. Electrochemical measurements on immobilized biomolecules offer a means for analytical characterization on small sample volumes with high sensitivity.[1] The development of an electrochemical platform that couples a planar three-electrode microdevice with an embedded platinum thin film, which can function both as a platinum resistance thermometer (PRT) and a resistive heater is described (Figure 1).[2, 3] Our electrochemical platform is unique in having both localized temperature control and biomolecule monitoring capabilities. Fabrication steps for these devices involved sputtering a serpentine platinum thin film on top of a fused silica wafer. The platinum was insulated with 350 to 1000 µm SiO_2 film and then a set of three surface electrodes was deposited for electrochemical analysis. The microscale platform enables electrochemical characterization of temperature-dependent biomolecular phenomena (using ≤ 10 µL of analyte). Thermal profiles of immobilized DNA secondary structures were measured as were ligand-induced stability changes caused by binding of small molecule drugs to DNA (Figure 2).[3] These experiments showed proof-of-concept for screening libraries of compounds for drug discovery. The design, fabrication, platform assembly, and measurement automation of a two-device array are discussed and shown to be compatible with extension to larger multielement arrays that can facilitate high-throughput screening and characterization.

[1] D. Grieshaber, R. MacKenzie, J. Vörös, and E. Reimhult, Sensors **8**(3), 1400–1458 (2008).
[2] Z. Shen, H. O. Sintim, and S. Semancik, Anal. Chim. Acta **853** 265-270 (2015).
[3] S. M. Robinson, Z. Shen, J. R. Askim, C. B. Montgomery, H. O. Sintim, and S. Semancik, Biosensors **9**(2) (2019).

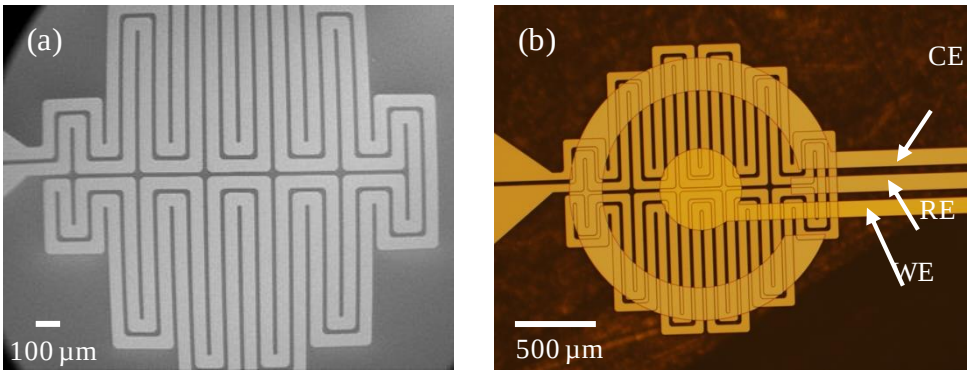

Figure 1: Three-electrode microdevice: (a) SEM micrograph of platinum thin film before insulation. (b) Optical micrograph of completed microdevice with labeled Au working electrode (WE), Pt reference electrode (RE), and Pt counter electrode (CE).[3]

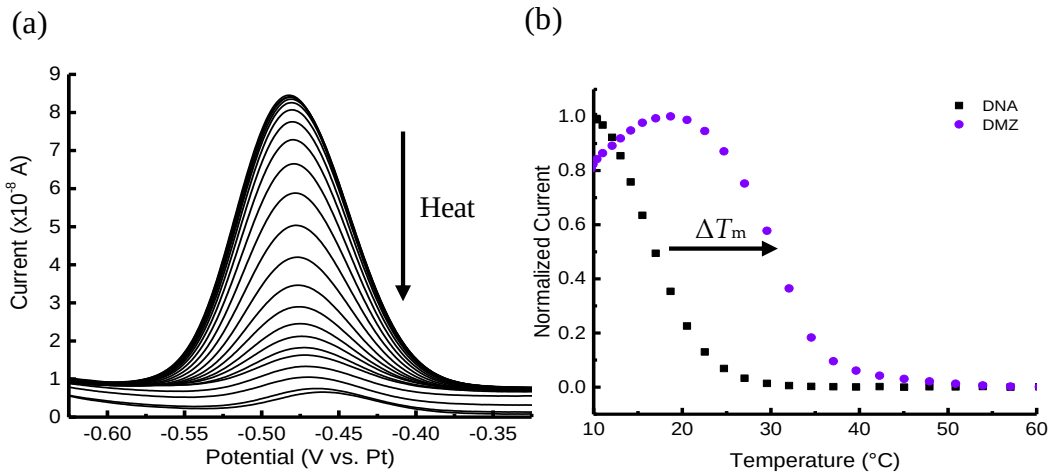

Figure 2: Measurements of Ligand Stabilization of Duplex DNA: (a) Square wave voltammograms of 2 µmol/L methylene blue-labeled cDNA in 10 mmol/L PBS with 100 mmol/L NaCl, pH 7.4. (b) Melting curves (normalized) of duplex in the absence (black) and presence of 13 µmol/L diminazene aceturate (DMZ) (purple).[3]