AN INTEGRATED PCR-EC MICRODEVICE FOR SIMULTANEOUS DNA AMPLIFICATION AND DETECTION

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In this work, we report the development of an integrated microdevice for simultaneous target DNA amplification by polymerase chain reaction (PCR) and sequence-specific PCR product detection by electroanalytical techniques. The integration of PCR microreactor and electrochemical (EC) detection system is very promising for the realization of a portable DNA analyzer. Inherent to target amplification of PCR and signal amplification capability of electrochemical transduction schemes, this microchip has great potentials for the sensitive detection of target DNA sequence.

As shown in Fig. 1, the microchip has platinum temperature sensors and heaters patterned on top of the reaction chamber (volume of 8 μL) for real-time temperature monitoring and control. The bottom glass covering the reaction chamber forms the electrochemical detection system. It encompasses a gold working electrode, platinum counter and reference electrodes.

There are three major steps in our assay protocol. First, asymmetric PCR is employed to generate single-strand rich target amplicon, obviating the need to denature the amplicon prior to the hybridization detection. Second, the amplicon is hybridized with DNA capture probe (oligonucleotide complementary to the target sequence), which is immobilized on the gold working electrode surface, for sequence-specific detection of the PCR product. Finally, hybridization indicator is introduced and electrochemically quantified.

Hybridization indicators used in this study include redox-active hybrid intercalator and gold nanoparticle. Fig. 2 shows the electrochemical response of an intercalator (that preferentially binds to double-stranded DNA) in the PCR-EC microchip for the sequence-specific amplification and detection of target gene. Besides, gold nanoparticle combined with signal amplification by silver deposition is utilized as a novel scheme for highly sensitive electrochemical transduction of the hybridization process. The implementation of the gold-silver approach in the microchip will be presented.

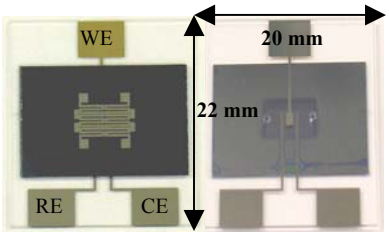

Fig. 1 Photographs showing the top (left) and bottom (right) views of the PCR-EC microchip.

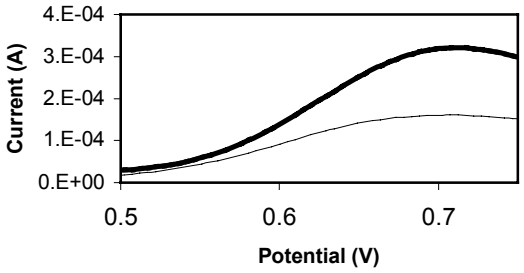

Fig. 2 Linear sweep voltammogram showing oxidation current of 100 μM Hoechst 33258 (supporting electrolyte of 100 mM NaCl/10 mM Na phosphate, pH 7.0) in the PCR-EC microchip. The thicker curve represents a sample containing the template while the thinner one does not.