Parallelized Quantitative Electrochemical Isothermal Amplification

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The human papillomaviruses (HPV) are globally distributed, heterogeneous, small double-stranded DNA that infect epithelial tissues at a variety of anatomic sites.[1] HPV genotypes have different oncogenic capacities and are classified as high and low-risk genotypes.[2] Infection by low-risk types, such as HPV6, HPV11, HPV42, etc., have a negligible risk of malignant progression,[3] whereas as high-risk HPV DNA such as HPV16, HPV18, HPV33 is found to be present in 99.7% cervical cancer worldwide.[4] Cervical cancer, on the global scale, is the third most common cancer-related death in women.[5] Therefore, HPV detection is a crucial step in the early diagnosis of cervical cancer. Whilst PCR is the most commonly used method to detect HPV, applying solid-phase bridge amplification on an electrochemical biosensor can surpass PCR's limitations and increase the sensitivity of the assay by achieving parallelized quantitative detection of multiple targets. We are currently developing a generic platform for the parallelized quantitative electrochemical isothermal amplification of nucleic acids which can be used at the point-of-need. Recombinase Polymerase Amplification (RPA) is an isothermal amplification technique that has several advantages over other isothermal techniques due to its simplicity, sensitivity and rapid amplification at a constant temperature (between 25 and 42°C), without the need for tight temperature control.[6] In solid-phase, at least one primer is linked to a surface and amplification may occur simultaneously both in the liquid and at the solid-phase, whereas in bridge amplification both 5'-primers are immobilised eliminating the primer-dimer problem. In our approach, we exploit the use of ferrocene modified dNTPs and 5'-thiolated primers, which are immobilised on the surface of gold electrodes of an array. The RPA amplicons are measured electrochemically using square wave voltammetry. To date we have optimized the reaction time and temperature for the simultaneous detection of HPV16 and β-globin and the platform will be expanded to the quantitative detection of multiple HPVs.

References

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