CRISPR-Cas12a Technology for the Empowered Electrochemical Readout of HPV18 Nucleic Acid

E. Pargoletti[1,2], Z. Lotfibakalani[1], G. Burgio[3], A. Newman[3], D. R. Nisbet[4], G. Cappelletti[2] and A. Tricoli[1,5]

[1] *Faculty of Engineering, School of Biomedical Engineering, University of Sydney, Camperdown, 2006, NSW, Sydney, Australia*
(eleonora.pargoletti@sydney.edu.au; eleonora.pargoletti@unimi.it)
[2] *Department of Chemistry, University of Milan, Golgi 19, 20133, Milan, Italy*
[3] *John Curtin School of Medical Research, ANU College of Health and Medicine, Australian National University, 2601, Canberra, Australia*
[4] *The Graeme Clark Institute, The University of Melbourne, Melbourne, Parkville, VIC, 3010, Australia*
[5] *Research School of Chemistry, Australian National University, 2601, Canberra, Australia*

Human papillomaviruses (HPVs) are a group of circular double-strand DNA (dsDNA) viruses that can be sexually transmitted [1]. Particularly, high-risk HPVs can cause several cancers and, among them, HPV16 and HPV18 are the two most virulent and common subtypes, provoking about 70% of invasive cervical cancers worldwide[1]. Thus, HPV early-stage diagnosis is fundamental and various methods have been developed so far among which quantitative polymerase chain reaction (qPCR) is the most common approach. However, it's cumbersome and it requires a relatively long assay time. Hence, very recently, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-based diagnostic technologies have attracted great interest in the biosensing community [2]. With the guiding of the CRISPR-associated RNA (crRNA), the CRISPR-Cas system can be activated by the DNA target, resulting in the cis-cleavage-initiated trans-cleavage effect on another nonspecific DNA probe, attached to an electrode surface. As such, an electrochemical readout is gained, which can be very accurate, easy to fabricate, sensitive, portable and time efficient.

In this context, herein, we developed an innovative CRISPR-Cas12a biosensor for the detection of HPV18, based on screen printed electrodes (SPE). Specifically, different gold surfaces (commercial gold SPE [3], Au nanoparticles deposited onto carbon SPE by electrodeposition or by an innovative method based on flame spray pyrolysis [4]) were investigated to assess the highest active platform to attach nonspecific ssDNA. After this optimization, methylene blue redox probe was electrostatically attached to DNA to be able to follow the DNA trans-cleavage by CRISPR-Cas12a system using electrochemical measurements, *i.e.* pulsed voltammetries, as square wave voltammetry (SWV), and electrochemical impedance spectroscopy (EIS). From preliminary results and after having optimized the non-specific ssDNA, CRISPR-Cas12a, and target dsDNA concentrations, a sub-nanomolar sensitivity was reached, showing also good system stability and reliability. Moreover, the engineered platforms exhibited the potential of being used as point-of-care devices, paving the way for their future use towards accurate diagnosis of other viral infectious diseases.

References

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