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Generating a Multiplex Sensing Platform for the Accurate Diagnosis of Sepsis

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Abstract : Sepsis is a complex and rapidly evolving condition, resulting from uncontrolled prolonged activation of host immune system due to pathogenic insult. The aim of this study is the development of a multiplex electrochemical sensing platform, capable of detecting both pathogen associated and host immune markers to enable the rapid and definitive diagnosis of sepsis. A combination of aptamers and molecular imprinting approaches have been employed to generate sensing systems for lipopolysaccharide (LPS), c-reactive protein (CRP) and procalcitonin (PCT). Gold working electrodes were mechanically polished and electrochemically cleaned with 0.1 M sulphuric acid using cyclic voltammetry (CV). Following activation, a self-assembled monolayer (SAM) was generated, by incubating the electrodes with a thiolated anti-LPS aptamer / dithiodibutiric acid (DTBA) mixture (1:20). 3-aminophenylboronic acid (3-APBA) in combination with the anti-LPS aptamer was used for the development of the hybrid molecularly imprinted sensor (apta-MIP). Aptasensors, targeting PCT and CRP were also fabricated, following the same approach as in the case of LPS, with mercaptohexanol (MCH) replacing DTBA. In the case of the CRP aptasensor, the SAM was formed following incubation of a 1:1 aptamer: MCH mixture. However, in the case of PCT, the SAM was formed with the aptamer itself, with subsequent backfilling with 1 µM MCH. The binding performance of all systems has been evaluated using electrochemical impedance spectroscopy. The apta-MIP's polymer thickness is controlled by varying the number of electropolymerisation cycles. In the ideal number of polymerisation cycles, the polymer must cover the electrode surface and create a binding pocket around LPS and its aptamer binding site. Less polymerisation cycles will create a hybrid system which resembles an aptasensor, while more cycles will be able to cover the complex and demonstrate a bulk polymer-like behaviour. Both aptasensor and apta-MIP were challenged with LPS and compared to conventional imprinted (absence of aptamer from the binding site, polymer formed in presence of LPS) and non-imprinted polymers (NIPS, absence of LPS whilst hybrid polymer is formed). A stable LPS aptasensor, capable of detecting down to 5 pg/ml of LPS was generated. The apparent Kd of the system was estimated at 17 pM, with a Bmax of approximately 50 pM. The aptasensor demonstrated high specificity to LPS. The apta-MIP demonstrated superior recognition properties with a limit of detection of 1 fg/ml and a Bmax of 100 pg/ml. The CRP and PCT aptasensors were both able to detect down to 5 pg/ml. Whilst full binding performance is currently being evaluated, there is none of the sensors demonstrate cross-reactivity towards LPS, CRP or PCT. In conclusion, stable aptasensors capable of detecting LPS, PCT and CRP at low concentrations have been generated. The realisation of a multiplex panel such as described herein, will effectively contribute to the rapid, personalised diagnosis of sepsis.

Keywords : aptamer, electrochemical impedance spectroscopy, molecularly imprinted polymers, sepsis
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