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Construction and optimization of an electrochemical genosensor for the detection of *BDNF* gene *Val66Met* polymorphism

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Major depression disorder (MDD) is the most prevalent psychiatric condition worldwide. Currently, antidepressants constitute the main pharmacological treatment for this condition. Despite the growing arsenal of antidepressant medications, almost half of MDD patients have insufficient remission rates during their treatments. A growing body of scientific evidence points to genetic factors as having a crucial role in patients' response to antidepressant treatments. One of these genetic components is the *BDNF* gene, that codes for the BDNF neurotrophin, an important player in neuroplasticity and neurogenesis. In fact, BDNF deficit has been associated with a variety of neuropsychiatric illnesses. Moreover, the non-synonymous polymorphism (SNP) *Val66Met* has been shown to have a role in variations in antidepressant treatment response between different populations. Therefore, genotyping these patients becomes increasingly more important, to better personalize therapeutic options, increasing remission rates and diminishing relapse rates. Current methodologies for patient genotyping involve the polymerase chain reaction (PCR) technique. Although efficacious, some drawbacks such as expensive equipment and personalized staff requirements, as well as extensive experiment times, make this approach difficult to implement in everyday clinician use. Hence, other methodologies that rapidly and precisely genotype patients regarding the *Val66Met* polymorphism are warranted. Biosensors are interesting alternatives in this regard.

In our work, a low-cost electrochemical genosensor capable of distinguishing between both *Val66Met* SNP alleles was developed. DNA target sequences of this polymorphism (one for the Val allele ("TC") and another for the Met allele ("TT")) were designed *in-silico*. The experimental protocol involved 4 steps: the pre-treatment of the gold electrode platform with pure ethanol and ultrapure water; the sensing phase, in which the DNA capture probe and mercaptohexanol (MCH) immobilization was carried out; the sandwich hybridization assay, performed via a 2-step hybridization methodology (homogeneous and heterogeneous hybridization) and the electrochemical detection (chronoamperometry). The resulting sensor utilizes the electrochemical profile of two single stranded DNA probes (capture probe "T" and target probes (TT and TC)), that produce an electrical signal when they hybridize.

Firstly, several experimental conditions (capture probe concentration, MCH concentration and incubation time, homogeneous and heterogeneous hybridization times and antibody concentration and incubation time) were optimized. Afterwards, different concentrations of both DNA target probes were tested, establishing calibration curves with a linear relationship in the 0.1-2.0 nM range. Moreover, the complementary target probe (TT) showed greater electrochemical currents than the non-complementary (TC), as expected. A limit of detection (LOD) of 14.40 pM and 17.06 pm was determined for TT and TC, respectively. Additionally, the validation of the sensor through conventional PCR methodologies is currently underway. To summarize, the sensor revealed itself as a great alternative genotyping tool for the *Val66Met* polymorphism, discriminating accurately between both alleles.

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