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Development of Enzymatic Fumarate Biosensor

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Fumarate is an important metabolite in the energy producing Krebs cycle (tricarboxylic acid). Pluchino et al. have demonstrated the link between levels of metabolites in the Krebs cycle, in particular fumarate, and the progression of Multiple Sclerosis (MS) in the cerebrospinal fluid (CSF) in murine models[1]. Levels fumarate are significantly correlated to the extent of inflammation in the nervous system and the overall neurological disability over time. Therefore, the continuous measuring of fumarate in the CSF presents the opportunity to monitor the state of neurological disability. The development of an implantable fumarate biosensor could have a positive impact on the screening, diagnosing and monitoring of neurodegenerative diseases.

Fumarate biosensors have been demonstrated using bacterium [2], [3] and enzymes[4] as the bioreceptor. These, however, do not demonstrate suitable limit-of-detections for physiological levels, or their designs are unsuitable for in-vivo measurements. We propose a biosensor which can sensitively and selectively detect fumarate at human physiological levels (~40-200 μ M) with a design suitable for potential future in-vivo use.

We have designed a two electrode enzymatic microwire design as shown in Figure 1. Reactions of fumarase are hydrating and do not produce measurable electrical output. Therefore, a two electrode approach using cascading reactions is used. One electrode is coated with fumarase, which catalyses the conversion of fumarate to L-malate, and malate dehydrogenase (MDH), which catalyses the conversion of L-malate to oxaloacetate in a redox reaction producing a measurable electrochemical response. The second sensor contains just MDH. The levels of fumarate can be calculated by the difference between the readings of the two electrodes. A 50 μ m W/Au microwire is contained within a silica tube. One side is coupled to a gold coated pin for electrical connection whilst the wire protrudes 2mm the other end onto which the biorecognition element was immobilised. A layer of polymethylene green is electropolymerised onto the wire which acts as an electrocatalyst for the regeneration of NAD⁺. NAD, which is a coenzyme required in the conversion of malate to oxaloacetate, and Nafion, which acts to immobilise the enzyme and as a protective membrane, are also included in the enzyme mixture.

Initial in-vitro testing of the device has carried out in phosphate buffer solution (PBS) (pH 7.4) at 700mV versus Ag/AgCl. Sensors are placed into the PBS and initial baseline readings are taken after an equilibrium period in which the current stabilises. The concentration of the target analyte is altered by consecutive additions of fumarate in the solution (10 μ M, 25 μ M, 50 μ M, 100 μ M, 200 μ M, 400 μ M). Examples of this test can be seen in Figures 2. This demonstrates a limit-of-detection of 25 μ M and a linear range up to 200 μ M. This covers the human physiological range in which we are interested in.

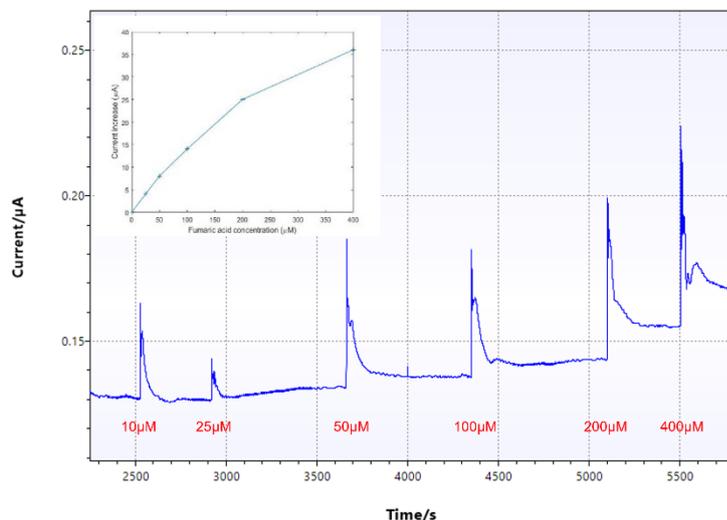
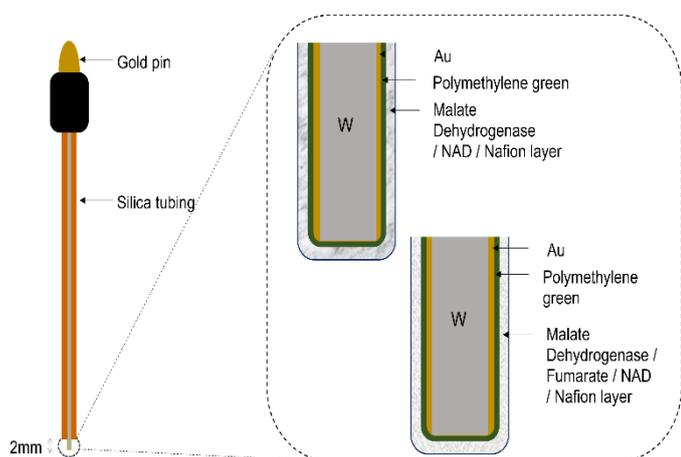
Future work will initially concentrate on the extension of in-vitro testing. Further measurements will be performed to improve the robustness of the results. Optimisation of the sensor design will be carried out by changing the levels of the enzymes, coenzymes and membrane. Selectivity testing will be carried out with

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interfering compounds added to artificial CSF. Following successful testing in in-vitro solutions, we will progress to validation of the biosensors in ex-vivo and in-vivo experimentation of murine models.

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