Development and Initial Testing of a Novel Slime Mould Biosensor

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Abstract— **A plurality of whole cell biosensors have been developed using many different cell types. Biosensors incorporate biomolecular components or whole cells to facilitate specific analyte interaction; research documented here presents a novel whole cell biosensor based on the slime mould *Physarum polycephalum* (PP). The electrical response of PP when exposed to multiple chemicals are measured and quantified in terms of amplitude and frequency response. The PP biosensor is capable of detecting the tested chemicals and individually identifying a large number in terms of a specific shift in either oscillation frequency or amplitude.. However, it does exhibit a sensitivity to environmental changes such as light level and temperature which may interfere with the detection of the target analyte but could also be used for wider sensing applications. It is proposed that this novel biosensor is capable of detecting many organic chemicals beyond those presented in this work and that the biosensor may be used for environmental monitoring and toxicity evaluation.**

I. Introduction

Biosensors are transducer systems with a biological component which quantifies input stimuli to a useful output signal for analysis. Cell based biosensors are sensor systems whose sensing elements use either whole cell or cell derived biological components to measure an input variable [1]. Biological transducers take advantage of a biological process whose output can be measured and reliably produces a dynamic response to a given input stimulus; these often include enzymatic, antibody/antigen reactions, genetic expression or metabolic pathway signaling [1]. A large proportion of biosensors are developed for the field of medicine, for example blood glucose monitoring [2], drug/toxicity analysis [3] or metabolic health evaluation [4]; these are often chemical detection biosensors applied to medical monitoring. Other areas of application for cell based biosensors exist such as environment sensing [5–9] and food hygiene evaluation [10]. Currently developed cell based biosensors incorporating biological components include mammalian, bacterial, fungal and viral cells [1,11–13].

Physarum polycephalum is a single cell amoeboid organism of the order Mycetozoa, known colloquially as Slime Mould. It is a large multinucleated yellow cell, capable of growing in excess of 10 centimetres, spanning veins between food sources. These veins are protoplasmic tubes of adaptable membrane filled with flowing cytoplasm; where the spatial arrangement of the tubes is determined by attractant and repellent environmental conditions such as food and light respectively. The method of movement in P. polycephalum is shuttle streaming, whereby actin-myosin filaments in the cell membrane rhythmically contract and relax [14], forcing cytoplasm towards sources of food or other attractants. Food such as oat flakes and decomposing organic matter attract PP as do certain non-food chemicals and warm conditions[15]; PP is repelled strongly by light, and certain chemicals. Shuttle streaming can be detected optically [15] and electrically [16,17], with a fluctuating period of between 60 and 120 seconds; the shuttle streaming frequency increases if attractants are applied and decreases if repellents are applied to the foraging tube [15]. PP can be dried, forming inactive sclerotia, which can be reanimated years later.

VOCs can be present in some laboratories and are given off from many bacteria and other organisms; VOCs present in the environment can indicate chemical or microbial contamination [18,19]. Traditional electronic sensors have been developed which detect VOCs in the local environment [20], often with large power requirements and expensive apparatus.

P. polycephalum has been used to solve spatial problems such as maze solving [21], Voronoi diagrams [22] and shortest path route planning [23]. Recently it has been shown to have a reliable and repeatable electrical response to environmental stimuli [17,24,25] which we have developed further into a biosensor, detailed in this paper.

II. Methods

A. Electrical Measurement of Physarum polycephalum

Physarum polycephalum was cultured on non-nutrient 2% agar gel in 9cm diameter petri dishes (Fisher Scientific, UK), fed daily with organic rolled oat flakes; after a week, the plasmodium culture was transplanted to a fresh agar petri dish to minimise agar contamination. These petri dishes were kept in the dark at room temperature until required.

To facilitate the electrical measurement of protoplasmic streaming and the change in frequency of streaming due to stimuli, a customised petri dish (fig. 1) was used; electrically conductive aluminium tape (Advance Tape AT521, RS Components, UK) was applied as shown, with a 10 mm gap at the centre of the petri dish; 1ml of non-nutrient 2% agar was applied to each aluminium electrode tip, creating hemispherical electrodes. A *P. polycephalum* inoculated oat flake from the culture was placed on one agar hemisphere while a bare oat flake was placed on the other; after a period of several hours to days, a protoplasmic tube would grow, connecting the two agar electrodes with a “PP Wire”. A

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Picolog ADC-24 high resolution data logger (Picotechnology, UK) was connected to the electrodes and the voltage fluctuation associated with shuttle streaming within the protoplasmic tube was recorded. A laptop installed with the associated Picolog Recorder V5.22.8 software was used to record the data at a sampling frequency of 2 Hz. Stimulus was applied near the recently colonised agar (fig 1) and the original electrode was grounded; experiments were performed in the dark due to the light sensitivity of PP.

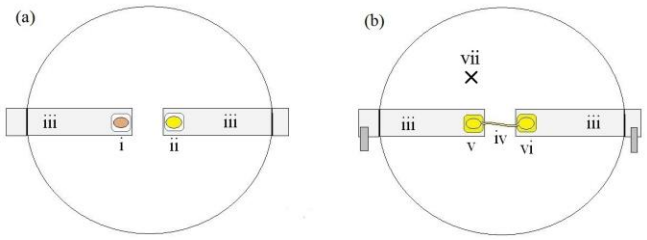

Figure 1. (a) Petri dish set up for stimuli assessment before growth. (b) Petri dish with correct growth of *Physarum polycephalum*, connected for electrical potential recording. (i) Bare oat flake on agar electrode. (ii) Physarum inoculated oat flake on agar electode. (iii) Conductive tape. (iv) Single protoplasmic tube between electrodes between agar electrodes. (v) Positive recording electrode. (vi) Ground reference terminal. (vii) site of chemical stimuli application.

For each measurement, the voltage change due to shuttle streaming was observed until the oscillation reached a stable state and continued for 10 minutes, the stimulus was then applied and a further 10 minutes was recorded in order to create a pre and post stimulus comparison. Percentage frequency change and percentage amplitude change was calculated from this data using the mean frequency and amplitude post and pre stimulation. A control experiment was performed where the pre and post stimulus period was recorded, with no stimulus applied; experiments were repeated 12 times for each stimulus. The control of no change was the same experiment with no chemical added.

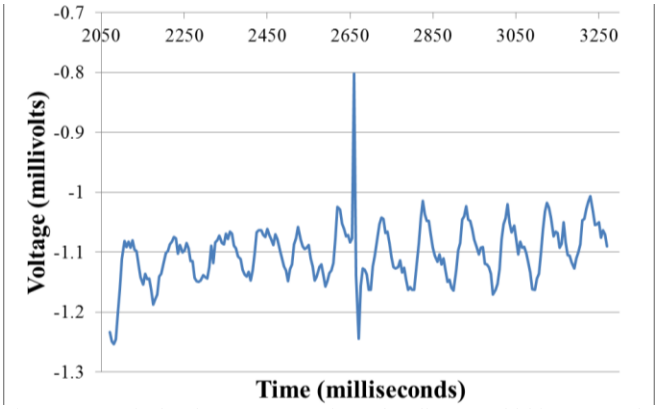

Figure 2. Typical voltage response from the slime mould biosensor. The stimulus was applied at 2650 seconds with a noticable spike caused by vibration; post stimulus there is a visible change in amplitude and frequency.

B. Biosensor Stimuli

Known attractants and repellents of PP [17,26,27] were tested, including food sources and Volatile Organic Chemicals (VOCs). Knowles et al [27] reported that basic carbohydrates, including those present in oat flakes, attracted Physarum. Costello et al [26] previously reported that VOCs Nonanal, Linalool, Benzyl Alcohol and Geraniol repelled PP, while Cis-3-Hexenylacetate produced no chemotaxis effect and S-Limonene Tridecane and Farnesene attracted it. The response to these chemicals, and that of an oat flake, was tested by adding 250µl of the liquid chemical (or 1 rolled oat flake) to the petri-dish, 10mm from the recording electrode. During practical use, the biosensor may by subjected to interference from other environmental stimuli such as light and heat, therefore a chemical stimulus (oat flake) was added while simultaneously exposed to light and heat in separate tests as its chemotaxis properties are well documented [15,21,23,28–30].

Light is a known repellent [31,32] so the response to light was tested using a 34 watt halogen bulb placed 50 cm above the Petri dish, only illuminating the recording electrode. Temperature change affects shuttle streaming frequency[33,34] so heat was applied to the underside of the petri dish below the recording electrode by a 1.4W Peltier Element (RS Components, UK) causing a 10°C increase in temperature at the recording electrode (typically from 20°C to 30°C).. The response of PP to multiple stimuli has not been investigated, so all possible two stimuli combinations of the oat flake food source with light and heat were performed to test sensory combination in PP and to test multiple stimuli interference in a PP biosensor.

III. Results

TABLE I. SUMMARY OF FREQUENCY AND AMPLITUDE CHANGES

Stimulus	Frequency Change (Standard Deviation)	Amplitude Change (Standard deviation)
Control	2.1% (6.9)	2.4% (5.8)
Oat Flake	17.8% (12.6%)	36.5% (21.7%)
White Light	-12.7% (6.5)	-36.1% (17.3)
Heat Only	22.1% (8.8)	63.4% (19.)
S-Limonene	-1.5% (7.5)	-1.7% (27.3)
Cis-3-Hexenyl acetate	-1.3% (21)	-6% (42.6)
Geraniol	0% (10.5)	-26% (22)
Tridecane	17% (36.7)	10.4% (45.7)
Farnesene	25.5% (24.9)	-35.4% (16.6)
Linalool	-32.4% (21.3)	41.4% (31.4)
Benzyl Alcohol	-10.7% (11.4)	25.9% (25.4)
Nonanal	-26.9% (16.4)	-28.2% (0.184)
Heat and Oat	32.4% (10.2)	51.5% (39.5)
Oat and Light	2.2% (11.2)	6.6% (27.7)
Light and Heat	14.8% (15.3)	44% (28.2)

While most chemicals produced a reliable and repeatable response as shown in Table 1, Nonanal and Linalool often ceased all electrical activity, essentially killing the slime mould; the mechanism of this is unknown however more reliable measurements were made by moving the point of chemical addition for these two chemicals to 30 mm from the recording electrode. Table 1 shows the mean and standard deviation from 12 repetitions for both frequency change and amplitude for each chemical or physical stimuli. The simultaneous exposure of Oat flake and Heat produced a frequency response equal to the sum of the individual effects while the amplitude was less than when heat alone was

applied. The exposure of *PP* to an oat flake while illuminated with white light resulted in negligible change in either frequency or amplitude; white light exposure modifies the response of chemical stimuli to the point of invalidating the results. The chemotaxis strength correlates with magnitude of frequency change, with strong attractants producing a large increase and repellents producing a large decrease in frequency. There is no correlation between chemotaxis and amplitude change, however it does offer a method of chemical identification beyond frequency change alone.

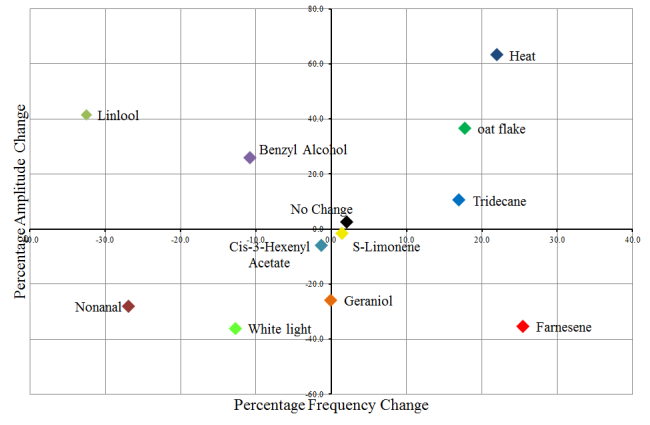

Figure 3. Amplitude and frequency response of the biosensor to stimuli.

IV. Discussion

A. Biosensor reliability

The response of PP to individual chemical, optical and temperature stimuli appears to be somewhat repeatable; it is possible to differentiate between several different chemical, optical and temperature stimuli using electrical measurement of a PP protoplasmic tube after 20 minutes. Most chemicals showed a change in frequency or amplitude; the percentage frequency change was positive for strong attractant stimuli and negative for strong repellents, appearing fairly linear in correlation with the attractant or repellent strength. Frequency response to an oat flake was a large and positive increase suggesting it increases shuttle streaming thereby increasing growth towards the food source.

Stimuli interference from light and heat is demonstrated; however given a dark and relatively stable temperature, the response to chemical stimulation appears accurate, however due to the large standard deviation of response, some chemicals may not be individually identifiable in every instance. Although each chemical addition is repeated 12 times, there would be no averaging in a practical biosensor; this is a current limitation of the PP biosensor.

Fluctuation in temperature causes a similar change in frequency and amplitude of shuttle streaming voltage; this is demonstrated when measuring the oat flake and heat. There may be an instance where a chemical is misidentified due to the additional effect of temperature increase. The most susceptible measurements to temperature interference are those whose amplitude and frequency shift are minimal; a small change in temperature may cause misidentification of a chemical due to artifactual changes due to heating or cooling. Temperature rises in practical biosensors are unlikely to be subject to such rapid and marked changes in temperature therefore the magnitude of change is likely to be far smaller than that reported in table 1. Due to the similarity in the response of S-Limonene and Cis-3-Benzyl Alcohol with the control, it may be difficult to determine in an individual instance, if there is either chemical present; the large standard deviations of response from the two chemicals may mean they are likely to be confused with the control given a single measurement.

The authors have maintained an operational life of 8 days for the slime mould biosensor; this is limited by drying of agar electrodes and lack of viable nutrients for the cell. Humidity control and a constant food source is likely to increase the operational life as a culture of plasmodium can be maintained for months provided the agar maintains sufficient water and contamination with other microbes does not occur. PP biosensors can be manufactured and then immobilized as a sclerotia for years, after which the biosensor can be reanimated and be operational, producing a very long shelf life; often a limit for cell biosensors[12].

Olfactory biosensors [35] can detect a variety of chemicals, mimicking olfactory epithelium; it is proposed that the PP chemical biosensor is a novel and reliable addition to olfactory biosensors with advantages of long shelf-life, large number of growth substrates and quick response.

B. Biosensor applications

This article indicates the potential of a Biosensor based on a protoplasmic tube of the slime mould *P. polycephalum*; VOCs can be detected in the air and individually be identified based on the voltage response of the organism. Simplifications and automation could provide a sensitive environmental sensor capable of detecting VOCs and other chemicals. Some VOCs are toxic therefore toxicity testing could be performed using this biosensor, ensuring precise detection of these chemicals in the environment. This sensor is designed to be single use, therefore it would not be important if the chemical was so toxic that it killed PP. If there were a class of compounds which was toxic to both humans and PP, it would not matter that chemicals within the class could be individually identified, just that the presence of any could be identified.

It is possible that *P. polycephalum* is capable of detecting a plurality of other chemicals, therefore the limits of this biosensor are currently unknown; further testing will determine the scope of applications for this novel biosensor. A pesticide sensitive biosensor has value in agriculture; Linalool[36], Farnesene[37] and Geraniol[38] are commonly used insecticides or insect repellents, use of these chemicals is permitted in the production of organic food, however as they are biologically derived there is little investigation as to how long they persist in the environment or on food items. These chemicals are detected by the slime mould biosensor meaning detailed investigation into natural pesticides and their longevity on food can be performed.

V. Conclusion

PP can grow on a variety of substrates such as plastics, paper, agar, aluminium, glass, fiberglass, copper circuit tracks, silicone gel [39] and possibly more untested surfaces; for this reason it is ideal for an integrated biosensor. Bacterial and fungal cell biosensors typically have to be immobilized or in suspension and may not grow on a wide variety of substrates[1,12], limiting the cell-transducer interface.

The PP biosensor can be improved by increasing the repeatability of the response so that chemicals can be individually identified. Investigating more chemicals and highlighting those toxic to both PP and humans is important, as a PP biosensor has the potential ability to detect small amounts of toxic chemicals, useful in high risk environments.

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