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# Biofuel Cells: A possible power source for implantable electronic devices

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*Abstract*— **Biofuel cells were designed to investigate electricity production from *Escherichia Coli* and human white blood cells as a preliminary investigation into the possible future use of such fuel cells as power sources for implantable electronic devices. The biofuel cell's function is based on the coupling of glucose oxidation to the reduction of oxygen to water. It might, therefore, be possible to utilize the cellular processes involved in oxidative metabolism to generate electrical energy for numerous medical applications. In the bacteria experiment, we were able to generate small electrical currents, which gradually decreased over a (2) hour measurement period. In the human white blood cell experiment, our biofuel cell attained current outputs, which were smaller in magnitude than values recorded from the microbial biofuel cell.**

## I. Introduction

A major challenge in the development of implantable devices for clinical use is in finding a suitable power source for such devices. The power source should be capable of generating power for prolonged periods of time. Biofuel cells (BFC) provide some promise in achieving this, as their function is based on the coupling of glucose oxidation to the reduction of molecular oxygen to water. Under ideal conditions, the only byproducts of the biofuel cell would be water and carbon dioxide.

Both glucose and oxygen are present in the cells of all eukaryotic organisms, including human beings. It might, therefore, be possible to utilize our body's own resources, to generate enough energy to power numerous devices - including, drug delivery systems, diagnostic tools, devices for biosignal and neural recordings, and human augmentation devices. This paper outlines two simple experiments, performed in order to explore current production in biofuel cells employing microbes and human white blood cells.

Microbial fuel cells (MFC) have been shown to generate electrical currents based on the oxidative metabolism of sugars [1]. The MFCs harness energy from microbes by tapping into their electron transport chain. Bacteriae such as *E. Coli* break down glucose in order to generate adenosine triphosphate (ATP), which is utilized by cells for energy storage. Neutral red or methylene blue can be used as electron mediators (electronophores), to efficiently facilitate the transfer of electrons from the microorganism to the electrode [8]. The exact mechanism by which the transfer of electrons takes place through these electron mediators is not known. However, it is known that they insert themselves into the bacterial membrane and essentially "hijack" the electron transport process of glucose metabolism, chemically reducing nicotinamide adenine dinucleotide ($NAD^+$) to NADH. MFCs have been reported to generate current densities of up to $1.5 mAcm^{-2}$ and power outputs of as much as $3.6 Wm^{-2}$ [7], [10]. Greater efficiency in electron transfer and thus an increase in current generation and power output have been attained by modifying the electrodes [5], [6], by using bacteria with higher metabolic rates, such as *E. Coli* [6], [7], and by using mixed bacterial cultures [2], [7].

White blood cells have recently been shown to be capable of generating electron currents across their extracellular membrane. Electron transport is generally not observed in the plasma membrane of eukaryotic cells, except in phagocyte NADPH oxidase [3], [9]. It has been found that this enzyme is responsible for the generation of the reactive oxygen species, superoxide ($O_2^-$), through electron transfer from NADPH to extracellular oxygen. In a process called *'respiratory burst'*, superoxide is formed, and used as a means by which the blood phagocytes attack and destroy microbes.

Based on this concept, an attempt was made to design a BFC, which utilizes white blood cells as electron donors as opposed to microbial organisms such as E. Coli. A similar principle would be employed to that of the MFC.

## II. Microbial Fuel Cell Experiment

### A. Biofuel Cell Design

A special container was designed and manufactured from plexiglass to be used as the housing case for the BFC. The BFC consisted of two compartments separated by a proton exchange membrane (PEM), Nafion-117 film (Sigma-Aldrich, U.S.), which allows hydrogen ions generated in the anode compartment to be transferred across the membrane into the cathode compartment. Reactions at the anode and cathode are indicated below.

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At the anode:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24e^- + 24H^+ \quad (1)$$

At the cathode:

$$4Fe(CN)_6^{3-} + 4e^- \rightarrow 4Fe(CN)_6^{4-} \quad (2)$$

$$4Fe(CN)_6^{4-} + 4H^+ + O_2 \rightarrow 4Fe(CN)_6^{3-} + 2H_2O \quad (3)$$

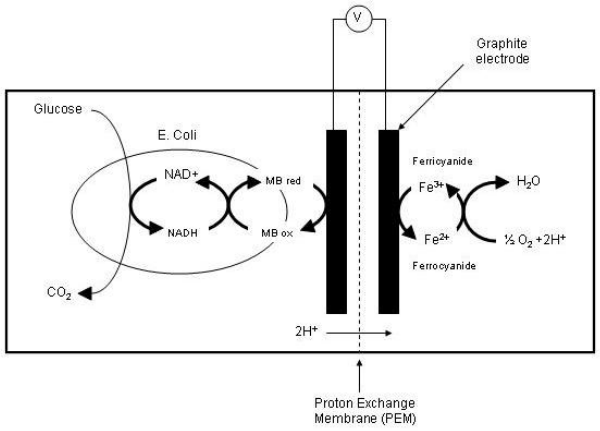


Fig. 1. Illustration of a Microbial Fuel Cell with Methylene blue (MB) as the electron mediator

### B. Method

*1) Bacterial culture:* The method employed here primarily followed that described by Park et al [4], [5], [6]. The bacterial culture (E. Coli, strain HB101) was grown aerobically overnight in 100 mL LB medium (10g/L bacto-tryptone, 5g/L yeast extract, 10g/L NaCl) at $37^0$C with vigorous agitation (250 rpm). The bacteria were harvested by centrifugation at 4000 rpm for 15 minutes using a Sorvall Super T21 maintained at a temperature of $4^0$C. The resting cells were washed twice and suspended in Medium I (100 mM phosphate buffer [pH 7], 10g/L sodium lactate, 5g/L peptone, and 5g/L yeast extract). The optical density of the bacterial suspension was then determined by spectrophotometry and used for the calculation of the cell protein concentration. An optical density of 2.03 was recorded at 660 nm, which corresponds to a cell protein concentration of 2.74 mg/mL.

*2) Biofuel cell experiment:* The biofuel cell case was 10 cm long, 6.2 cm wide and 5 cm high. Each compartment had a volume of 100 mL. The graphite electrodes had dimensions 5.5 cm x 4 cm x 0.64 cm, giving a total surface area of 56.16 $cm^2$. The proton exchange membrane (PEM) had an available surface area of about 9 $cm^2$ exposed to either compartment.

The proton exchange membrane (PEM) and the electrodes were thoroughly cleaned using soap and water. The anode compartment was then filled with 50 mL of bacterial suspension (absorbance of 2.03 at 660nm), 25 mL of 0.2 M phosphate buffer, 1 mL of 55 mM glucose solution, 24 mL of distilled water, and 0.373g methylene blue. The cathode compartment was filled with 50 mL 0.2 M phosphate buffer, 50 mL 0.1 M potassium ferricyanide solution. Measurements of voltage and current were made over a two hour period.

Two control experiments were also conducted in an attempt to establish whether the bacterial cells are the primary contributors to any observed currents. In the first, the bacterial culture was excluded from the biofuel cell preparation. In the second control experiment, both the anode and cathode compartments were completely filled with distilled water. Measurements of voltage and current were made using a multimeter.

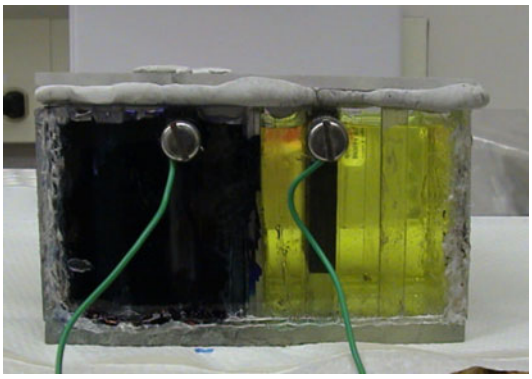

Fig. 2. Biofuel Cell Setup (Front View)

### C. Results

The biofuel cell devised in this experiment generated a small electric current. Measurements of electric potential and current density were measured over a 2 hour period using a multimeter.

The current density and voltage rapidly decreased over the first 20 minutes of the experiment, after which they began to stabilize. Decreasing currents might be attributed to:

- Diffusion of hydrogen ions in the anode compartment, reducing the rate of transfer across the PEM.
- Formation of products which could coat the electrode or the proton exchange membrane.
- Cells dying over time as a result of changes in pH.
- Denaturation of the electron mediator

Excluding bacteria from the biofuel cell preparation resulted in a significantly reduced current and electric potential (by more than one order of magnitude). Neither a current nor potential was observed when distilled water was placed in both the anode and cathode compartments.

## III. Leukocyte Biofuel Cell Experiment

### A. Method

Human white blood cells (neutrophils and mononucleated cells) were purified using standard Ficoll gradient. After being washed in phosphate-buffered saline (PBS) once, the cells were resuspended in PBS. The suspension was placed in the

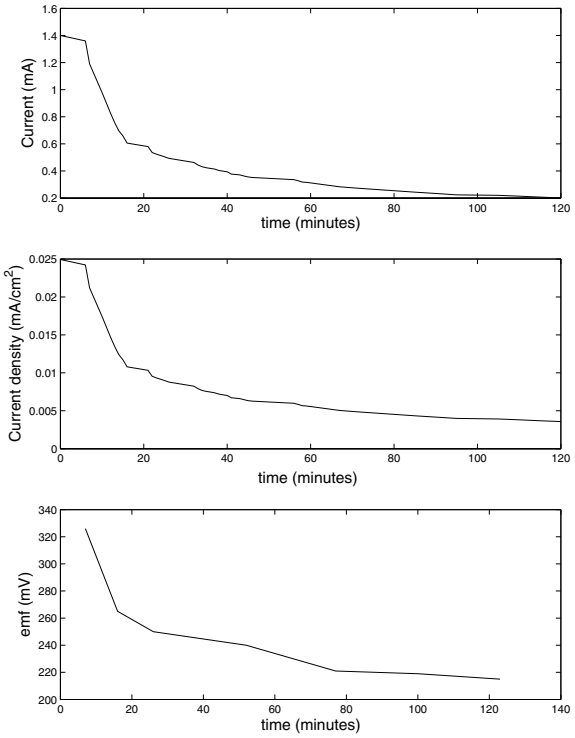


Fig. 3. Measurements of current, current density and electromotive force acquired from the microbial fuel cell

anode compartment of a biofuel cell container (purchased from the National Center of Biotechnology Education, University of Reading, U.K.). The cathode solution was made from 5 mL of 0.1 M potassium ferricyanide and 5 mL PBS (pH 7.4). Phorbol-12-myristate-13-acetate (PMA) and calcium ionomycin were used to activate NADPH oxidase. 10 $\mu$L each of PMA (5 ng/mL) and ionomycin (500 ng/mL) were transferred by micropipette to the anode compartment containing the white blood cells.

The effects of the antioxidant N-acetyl-L-cysteine (NAC) and a non-ionic detergent, Igepal, were investigated, in an effort to gain some insight into the mechanism by which electrons are transfered from the cells to the electrode. One would expect the current produced by the cells to decrease in the presence of both of these agents, if it is assumed that either superoxide or NADPH oxidase plays a direct role in the electron transfer process. NAC should reduce or extinguish the effect of the superoxide, while Igepal should disrupt the cellular membrane, essentially destroying the functional NADPH oxidase enzyme complex.

### *B. Results*

Currents were observed for the biofuel cell employing human white blood cells, when placed across a 100$\Omega$ resistor. The current densities are shown in Figure 5. Addition of NAC did not seem to have a very great effect on the current output. In fact, a slight increase in the current was observed. However,

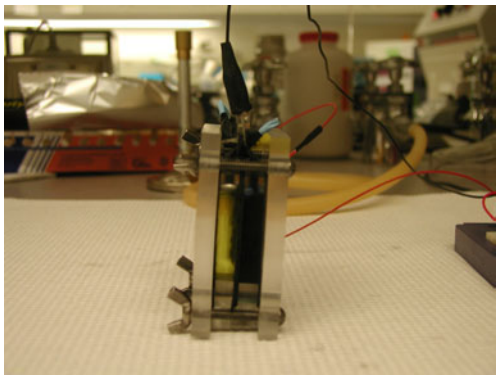

Fig. 4. Leukocyte biofuel cell experimental setup. The apparatus was assembled from parts acquired in a toolkit from the National Center of Biotechnology Education (NCBE).

upon addition of the non-ionic detergent, Igepal, a decrease in the current was observed, followed by an increase in current. Sizeable currents were observed in our study before addition of the PMA and ionomcyin for activation and also in the absence of the white blood cells. This suggests that other factors might be contributing to the observed currents.

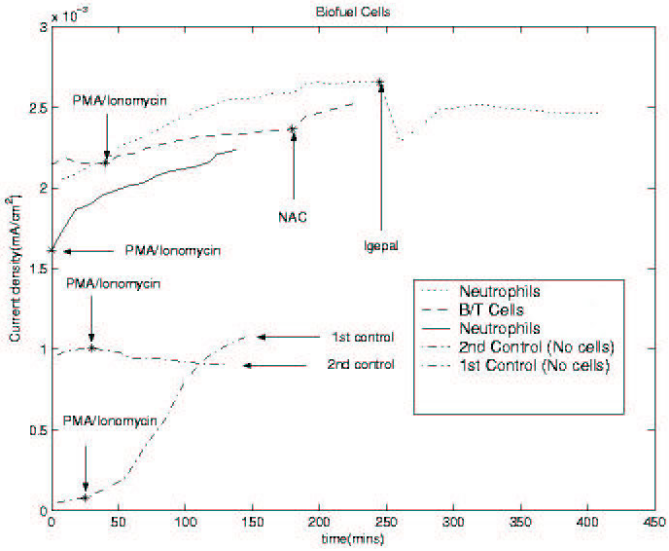


Fig. 5. Current densities acquired from the leukocyte biofuel.

## IV. Conclusions

Biofuel cells incorporating E. Coli as the bacteria and methylene blue as an electron mediator do generate electricity. A maximum electric potential of 0.326V was recorded which decreased to 0.215V by the end of the experimental period. Current densities decreased from 24.9 $\mu Acm^{-2}$ to 3.58 $\mu Acm^{-2}$ within the two hour period. The current densities

recorded are similar to values recently reported in the literature.

Our experiments seem to suggest that white blood cells, specifically neutrophils and mononucleated cells, generate electrical currents when integrated into biofuel cells. The neutrophils seemed to be able to generate fairly stable currents, normally between 1.4 $\mu$Acm$^{-2}$ and 2.7 $\mu$Acm$^{-2}$. The ionic detergent, Igepal, decreased currents, though not completely, suggesting that the white blood cells might play a role in the observed currents generated by the biofuel cell. However, before addition of the PMA and ionomycin, as well as in the absence of cells, sizeable currents were still observed. It is, therefore, reasonable to conclude that there may be other factors contributing to the observed currents.

## V. Discussion

The results show that there is an electrical potential and current generation associated with the bacterial oxidation of glucose. The experiment described above was very basic in its set-up. The E. Coli was grown under aerobic conditions and the anode compartment maintained an oxygen atmosphere. The presence of oxygen at the anode compartment would significantly affect the current output. Molecular oxygen is the last electron acceptor in the electron transport chain. Its presence at the anode would mean that a siginificant number of electrons would be transferred to oxgyen, as opposed to being transferred to methylene blue molecules and subsequently to the electrode. One would, therefore, expect a reduced current output because of the limited availability of electrons under these conditions for transfer to the electrode. Higher currents would be expected under anaerobic conditions.

A rapid decrease in both current and voltage (on the order of mV for the potential and $\mu$A for current), followed by an equilibrium state was observed for the biofuel cell described. This might have been a result of how measurements were made, where the biofuel cell was directly connected to the multimeter without including a load (resistance). The change in the values of electric potential might have been the result of polarization of the electrodes. A definitive reason for this observation has not been ascertained.

The leukcoyte-based biofuel cells described demonstrated rather stable current outputs over the two hour period of measurement. The exact mechanism by which the cells transfer electrons to the electrode is not known. There is the possibility that electron transfer might occur via the superoxide as a mediator. Generation of superoxide would be followed by the oxygen radical giving up its electron to the anode, thereby regenerating molecular oxygen. The oxygen could subsequently be reused by NADPH oxidase to produce more superoxide.

The possibility also exists that the NADPH oxidase could directly transfer the electrons to the electrodes, without employing a mediator. Further investigation into the mechanisms by which such an electron transfer takes place needs to be performed.

The antioxidant NAC was used to determine whether superoxide plays a role in the current generation. Upon addition of NAC, there was a slight increase in current. At present, this observation cannot be explained. The fact that the expected decrease was not observed implies that oxygen radicals might not play a role in the electron transfer.

When the non-ionic detergent, Igepal, was added, a significant decrease in the current was observed. However, the current was not completely extinguished. This decrease in current seems to suggest that the white blood cells do play a role in the currents generated by the biofuel cell. The exact mechanism, however, remains undefined. Igepal would disrupt the cellular membrane, rendering membrane bound proteins dysfunctional. As a result, one would expect the currents to decrease, based on the theory that NADPH oxidase is responsible for transfering electrons to the graphite electrode.

Some important questions have been raised as a result of the previously described experiment and remain to be answered. What is contributing to the currents observed in the absence of white blood cells at the anode? To what extent are these currents affecting electron transfer from the white blood cells to the electrode? Further research needs to be performed to investigate these observed phenomena.

## VI. Acknowledgments

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