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Serotonin (5-HT) released by activated white blood cells in a biological fuel cell provide a potential energy source for electricity generation

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Abstract – **Previous studies by our group have demonstrated the ability of white blood cells to generate small electrical currents, on the order of 1 - 3μA/cm^2, when placed at the anode compartment of a proton exchange membrane (PEM) biological fuel cell. In this research study, an electrochemical technique is used to further investigate the electron transfer ability of activated white blood cells at interfacing electrodes in an attempt to elucidate the mechanism of electron transfer in the original biological fuel cell experiments. Cyclic voltammograms were obtained for human white blood cells using a three-electrode system. The working and counter electrodes were made from carbon felt and platinum, respectively, while the reference was a saturated calomel electrode (SCE). Oxidation peaks were observed at an average potential of 363 mV vs. SCE for the PMA/ionomycin activated white blood cells in glucose solution. However a corresponding reduction peak was not observed, suggesting irreversibility of the redox reaction. The cyclic voltammograms recorded for the white blood cells bear very close similarities to those of the neurotransmitter serotonin (5-HT). Serotonin released by white blood cells into the extracellular environment may be irreversibly oxidized at the working electrode in the cyclic voltammetry experiments and at the PEM biological fuel cell anode in our earlier electrochemical cell studies.**

I. INTRODUCTION

Presently, there are few options for supplying power to implantable medical devices. Current methods include transcutaneous energy delivery through radiofrequency (RF) links [1] or involve the use of batteries that are either implanted with the device or placed externally, transmitting power through the skin. Other power sources have been investigated, including devices for transcutaneous energy transmission using optical methods [2] and ultrasound [3], piezoelectric devices [4], and nuclear batteries [5].

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Our research group is interested in investigating the possibility of utilizing the body's own biochemical energy stores to generate electricity in-vivo for low power implantable diagnostic and therapeutic device applications. Cyclic voltammetry is an electrochemical technique widely used for the analysis of chemical reactions. It can often reveal important thermodynamic and kinetic information about chemical processes (oxidation and reduction reactions) that occur in solution or at the electrode surface. In this study, the oxidation-reduction activity of isolated human white blood cells (WBCs) is investigated. Previous studies by our group have revealed that small electrical currents, on the order of 1 to 3 μA/cm^2, can be generated from an electrochemical cell (proton exchange membrane biological fuel cell) containing WBCs suspended in phosphate-buffered saline (PBS) solution at the anode compartment and a potassium ferricyanide solution at the cathode compartment [6]. A proton exchange membrane (PEM) was used to separate the anode and the cathode solutions. These studies followed prior investigations of microbial fuel cells (MFC) by other research groups. In studies, such as those by Rabaey et al. [9], electron transfer from glucose to an electrode occurred either through membrane-bound cellular components or through excreted redox mediators. In the Rabaey et al. MFC study, high electron transfer efficiencies were attained (81%) for a mixed bacterial cell culture. We hypothesized that electrons could similarly be transferred between WBCs and the anode electrode of a biological fuel cell through one or both of two possible mechanisms: (i) directly, through membrane bound electron transport proteins such as those associated with the plasma membrane-bound NADPH oxidase complex or (ii) indirectly, through the excretion of chemical mediators or metabolic products from the intracellular environment into the extracellular space. Cyclic voltammetry was used in an attempt to elucidate the mechanism of electron transfer between the WBCs and interfacing electrode surfaces.

II. METHOD

A. Materials and Apparatus

The electrochemical studies were performed using the Gamry Potentiostat, FAS2/Femtostat under control of the Gamry Framework Software from Gamry Instruments. The Gamry Software is installed on a Dell Dimension 2400 Desktop Computer. A three-electrode setup was used, where carbon felt and platinum served as the working and counter electrodes, respectively, while a saturated calomel electrode (SCE) served as the reference. Phorbol-12-myristate-13-

acetate and calcium ionomycin were used to activate the white blood cells. White blood cell activation occurs as a result of non-specific Protein Kinase C (PKC) pathways.

B. White blood cell isolation

This study was approved by the Institutional Review Board for Human Subject Research of the University of Pittsburgh. Human white blood cells were isolated from approximately 10mL of whole human blood using a Ficoll-Paque™ density gradient. Briefly, whole blood was gently added, using a pipette, to an equivalent volume of the Ficoll-Paque™ solution to obtain two clearly defined layers. After centrifugation at 2000 rpm for 20 minutes, four clearly defined layers can be discerned for the gradient – red blood cells at the bottom, followed by a larger volume of the Ficoll solution, then a thin layer of white blood cells (consisting primarily of B and T lymphocytes) and finally a larger volume of blood plasma. The thin white layer of B and T lymphocytes was carefully recovered using a pipette. The cells are washed twice in phosphate-buffered saline (PBS) solution (pH 7.4) by centrifugation at 1600 rpm for 15 minutes and finally the cells were resuspended in 15 mL of the PBS solution. A cell count was then performed using a light microscope and hematocytometer to determine the final cell density in the PBS solution.

C. Cyclic voltammetry

A three-electrode electrochemical setup was employed for the cyclic voltammetry study. The system consisted of a working electrode, which is the site of charge transfer (where the reaction under investigation occurs), and a counter electrode, which serves to complete the circuit. The third electrode, the reference, functions just as its name implies. It is used as a reference for measurements of potential recorded at the working electrode. The reference electrode is designed to have a very high input impedance and as a result, remains unsusceptible to changes in its potential due to the affects of polarization, as only very small currents can flow through. The fact that the reference potential remains relatively constant means that accurate measurements of potential at the working electrode can be made. The majority of the current that flows within the three-electrode electrochemical cells flows between the working and counter electrodes and can be simultaneously measured along with potential. Cyclic voltammetry provides plots reflecting the relationship between current (i) and potential (E). The resultant i-E plot for a redox species in solution has a characteristic "duck" shape, consisting of an oxidation and reduction peak. The potential that corresponds to the middle of the two peaks is usually taken as the midpoint potential, E_m. Using the Gamry interfacial software, parameters such as the voltage scan range, scan rate, maximum current and electrode surface area can be specified. In this study, the white blood cells suspended in PBS solution were scanned within a potential range of -0.5V to 1.2V at a scan rate of 100mV/s. The total working volume was 15mL. The white blood cells were activated by addition of 1 µL each of phorbol-12-myristate-13-acetate (PMA) and ionomycin per 1mL of the total working volume. The peak currents and peak potentials were acquired from the cyclic voltammograms using MATLAB®.

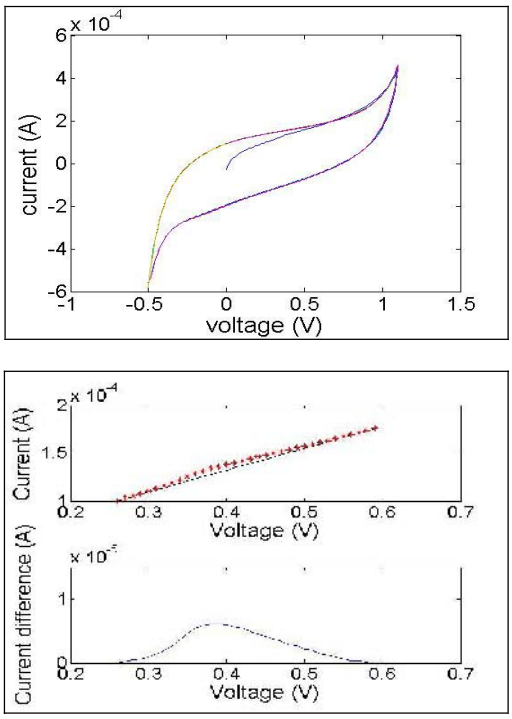

Figure 1: Top panel: Cyclic voltammogram of activated white blood cells (Sample 1) in PBS solution. No glucose was added to the PBS solution. Bottom panel: A peak current of 6 µA was observed relative to a linearly fitted baseline between 0.25 and 0.6V. Peak observed at 0.37V.

III. RESULTS

As mentioned earlier, the primary goal of this study was to investigate whether white blood cells are capable of transferring electrons to an electrode surface either directly through membrane-bound proteins in the cell membrane, or indirectly through chemical mediators released into the extracellular space. Four WBC samples were obtained for the study. Oxidation peaks were observed at an average potential of about 363 mV vs. SCE (Table 1) for activated white blood cells with glucose (1mg/ml). Reduction peaks, however, were not observed for any of the samples scanned. Figures 1 and 2 show examples of the cyclic voltammetry current-potential (i-E) curves obtained for the WBCs. Sample 1 was unique in that peaks were observed both in the presence and absence of dissolved glucose in the PBS solution. For all other WBC samples, oxidation peaks were only observed for PMA activated cells in glucose solution. It was noted that for Sample 1 the peak current (Ip) increased after adding 15mg of glucose from 6 µA (Figure 1) to 12 µA (Figure 2). Cyclic voltammetry scans of PBS only and glucose in PBS revealed neither oxidation nor reduction peaks (results not shown). Oxidation peaks were evident only in the presence of the activated white blood cells at the electrodes.

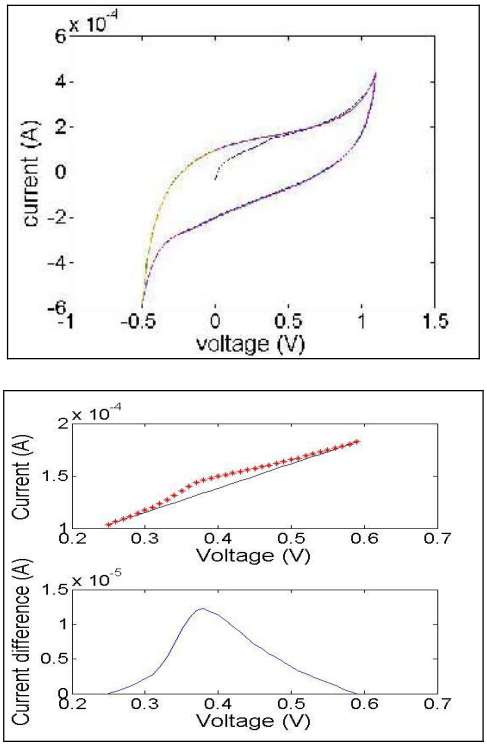

Figure 2: Top panel: Cyclic voltammogram of activated white blood cells (Sample 1) in a PBS solution with 1mg/ml glucose. Bottom panel: A peak current of 12 μA was observed relative to a linear baseline fit between 0.25V and 0.6V.

TABLE I
SUMMARY OF RESULTS

Sample	Cell density (cells/mL)	Peak current density (uA/cm2)	Peak Voltage (mV)
1	1260000	0.300	370
2	940000	0.125	370
3	530000	0.021	360
4	1100000	0.056	350
		Average peak voltage, Ep	363

As expected, a close relationship appears to exist between the cell density and the peak current density. The increased current can be attributed to the greater concentration of the oxidizable species in the bulk solution. Assuming rapid electrode kinetics (reversible reaction) and that mass transport to the electrode surface occurs only by diffusion, a relationship between the peak current and concentration can be established by the following equation [12]:

$$i_p = (2.69\times10^5)n^{3/2}AC_O^{*}D_O^{1/2}v^{1/2} \quad (1)$$

where i_p is the peak current (A), n is the number of electrons transferred, A is the electrode surface area (cm^2), and Co* is the concentration of species O in the bulk solution (mol/cm^3). Table 1 is a summary of the results plotted in Figure 3 for the four WBC samples. For Samples 1, 2 and 3, a very close correlation between cell density and peak current density is observed. The peak current density obtained for Sample 4 was much lower than expected. This could be attributed to a number of factors including the inaccessibility of the cells or their redox components to the electrode surface due to fouling, or as a result of a reduction in the number of viable cells.

An interesting result in these experiments, as noted previously, was that for Sample 1 an increase in the peak current appeared to be associated with the addition of glucose to the PBS solution containing the activated white blood cells (Figures 1 and 2). In an effort to further investigate this event, glucose was added in incremental amounts and the solution scanned to establish a relationship between the quantity of glucose added and the height of the peak current. The results did not reveal any clear relationship (Figure 4). However, it was noted that an increase in the peak current was associated with the addition of about 30mg glucose, in addition to the original 15mg, for Samples 2, 3 and 4.

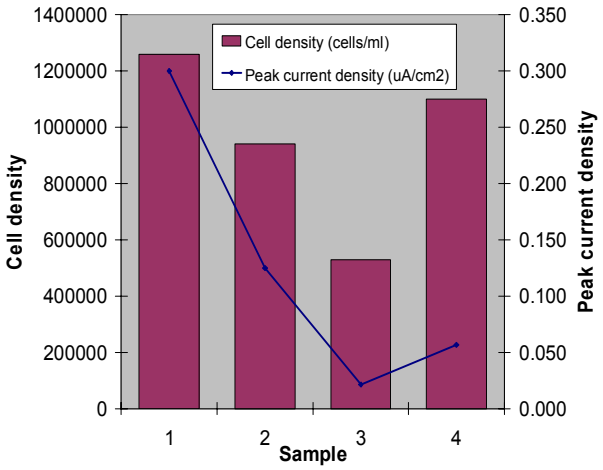

Figure 3: Illustration of relationship between white blood cell density and peak current density. The PMA activated white blood cells were suspended in 1X PBS (pH 7.4) with a glucose concentration of 1mg/mL.

IV. CONCLUSIONS AND DISCUSSION

The results of our experiments suggest that activated white blood cells release redox active species into solution, which facilitate indirect electron transfer between the cells and the electrode. Based on studies by previous authors, it is likely that the redox species responsible for the oxidation peaks is serotonin (5-HT) [8]. It has been suggested that serotonin is released from certain white blood cells, particularly in response to allergens. In the literature, the oxidation peak for serotonin was normally reported around 330 mV vs. SCE [8, 10]. The absence of a reduction peak was often noted, and has been attributed to the irreversible oxidation of serotonin (5-HT) to 5-hydroxyindoleacetic acid (5-HIAA) [10]. A possible mechanism for electricity

generation in our previous biological fuel cells, incorporating white blood cells, may be that serotonin released by the cells was oxidized at the anode, while oxygen was reduced to water at the cathode (Figure 5).

From these investigations, we were neither able to verify nor disprove that electron transfer occurs directly through membrane-bound components, such as those that make up the NADPH oxidase complex. NADPH oxidase, for example, comprises flavocytochrome b558. Cytochrome b558 has two midpoint potentials, E_m, at -264mV and -233mV [11]. Potentials in the negative potential range were not observed in our cyclic voltammetry experiments. This may reflect the low efficiency in electron transfer between these membrane components and the electrode. Further research is necessary to determine whether it is possible to access electrons mediated by NADPH oxidase, which are derived from the pentose phosphate pathway of glucose metabolism.

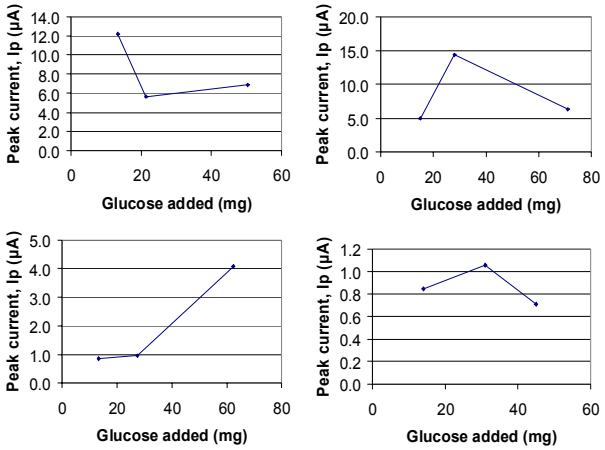

Figure 4: Relationship between D-(+)-glucose added (mg) and peak current, Ip for (a) Sample 1 (top left); (b) Sample 2 (top right); (c) Sample 3 (bottom left); and (d) Sample 4 (bottom right).

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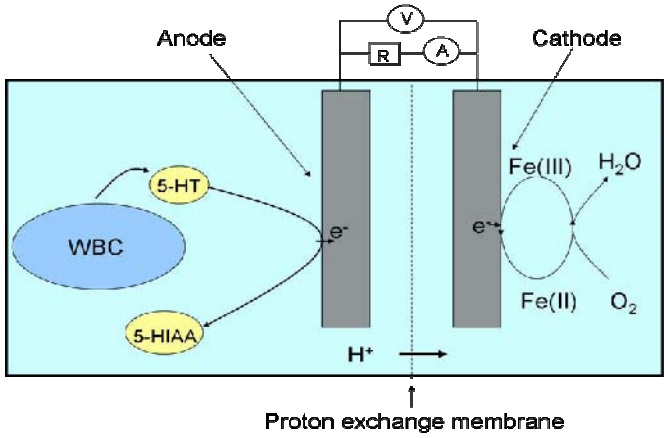

Figure 5: Theoretical mechanism for electricity generation in original biological fuel cell studies by our group. The white blood cells release serotonin (5-HT) which may be followed by oxidation of the neurotransmitter to 5-hydroxyindoleacetic acid (5-HIAA). The objects labeled V, R and A represent in the external circuitry the voltmeter, resistor and ammeter, respectively.

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