

# Advanced Biofuel Cell Composed of Highly Modified Electrodes for Biomedical Applications

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## ABSTRACT

A biofuel cell comprised an anode and cathode modified with highly branched polymers, nano Au sol-gel, and reaction specified bioenzymes was constructed. In this study, lactate dehydrogenase (LDH) and *glutamate dehydrogenase* (GDH) were the enzymes used to modify the anode and cathode respectively for the reaction of lactate and  $\alpha$ -ketoglutarate to generate pyruvate and glutamate, and 0.05 V for the overall reaction. Different combinations of highly branched polymers were used for the construction of the electrodes; both modified anode and cathode were characterized individually for their viability and durability. The nano Au sol-gel functioned as an anchoring system that effectively stabilized the degradation of the enzymes, that in turn enabled the specific redox reactions to generate the current flow. Thus, these nano Au sol-gel modified electrodes make a long lasting enzymatic fuel cell possible. The current fuel cell system could only generate a low power potential of 0.05 V; but all the reactants are naturally present in our body, and hence this fuel cell concept can potentially be used as power source for biomedical devices such as pacemaker and insulin pump.

**Key words:** LDH; GDH; nanoparticle; biofuel cell; PAMAM

## 1 INTRODUCTION

Biochemical fuel cell has been studied for nearly half of a century [1], the recent energy crisis and the development of micro circuitry reiterate the interest of biofuel cell; with the aid of nano technology, biofuel cell has leapfrogged to a new level. In addition to the conventional application of waste conversion to energy recycling concept, the micro and nano technology enable the concept of biofuel cell to directly extract energy from organisms and plants [2, 3].

Our research group has started a biofuel fuel cell initiative, the objective of this initiative is to develop a

biofuel cell system that is non-toxic and biological compatible that can be employed in a natural environment or human body. In this paper, we are developing an enzymatic biofuel cell system that was constructed with electrodes that were enhanced with polymer and nanogold particles sol-gel that can be implanted into our body as power source for biomedical devices such as pacemaker and insulin pump; this cell/battery can be modified to generate electrical current to be utilized at point of generation or stored as battery power. The energy source or reactants in this report were lactate,  $\alpha$ -ketoglutarate, and ammonia which are all existing moieties in our body or as parts of our body fluid, other energy sources development is also undertaking in our laboratory.

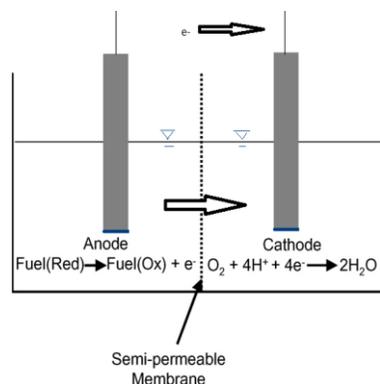
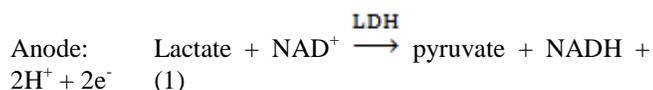


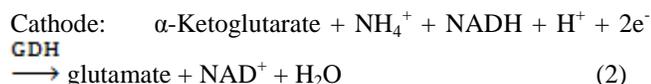
Figure 1 A typical fuel cell with a semipermeable membrane dividing the cell into an anode and a cathode.

Figure 1 shows a typical fuel cell, products from the reactants (fuel oxidants) can migrate to the cathode side (from left to right side) through a semi-permeable membrane to complete the overall fuel cell reactions.

For our biofuel cell system, the anodic half-cell was made by an Au electrode coated with lactate dehydrogenase (LDH) submerged in solution containing lactate and nicotinamide adenine dinucleotide (NAD<sup>+</sup>), thus, for the anodic half reaction:

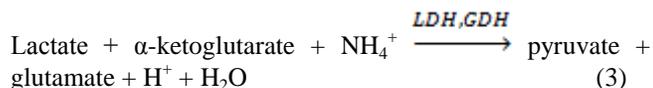


As lactate was oxidized into pyruvate in the anode, the oxidative potential,  $E_0'$ (25 °C), would be 0.19 V. The cathodic half-cell was made by a glassy carbon electrode (GCE) coated with glutamate dehydrogenase (GDH) submerged in solution containing ammonia and reduced nicotinamide adenine dinucleotide (NADH), the cathodic half reaction was:



As  $\alpha$ -ketoglutarate was reduced into glutamate in the cathode, it generated a reductive potential,  $E_0'$ (25 °C), of -0.14 V.

Combining the anode and cathode, the total potential for the fuel cell was 0.05 V. The overall reaction for the biofuel cell thus was [4]:



Since the  $\Delta E_0'$  of Equation (3) is greater than 0, the reaction is spontaneous and would proceed as written from left to right without additional energy input.

## 2 MATERIALS AND METHODS

### 2.1 Electrodes

Gold electrode had diameter of 0.3 cm and the GCE had diameter of 0.35 cm, they both were purchased from Tianjin Aida Heng Sheng Co, Tianjin, China.

### 2.2. Electrode Preparation

**Anode:** A clean gold electrode was immersed in 0.1 M cysteamine solution for 2 hours in the dark, then it was dipped into colloidal gold nanoparticles for 24 hours, finally the electrode was dipped into LDH solution for 20 hours before it was used for testing. This was a LDH-Au-cysteamine (sol-gel)-modified Au anode [5]. LDH was embedded in the Au sol-gel matrix which also enabled effective electronic transfer.

**Cathode:** Several combinations of polymers (poly(amido amine)) (PAMAM) of 0<sup>th</sup> and 2<sup>nd</sup> generation, and cysteamine) with GDH and nanogold particles were tested for their efficacies to enable the reductive reaction as in (2). A cleaned glassy carbon electrode was first immersed in 0.1 M cysteamine (or PAMAM) solution in darkness. The resulting monolayer-modified electrode was rinsed thoroughly with twice-distilled water and soaked in water. Then, it was dipped into the colloidal gold. The gold colloid-cysteamine-modified cathode was dipped into the l-

glutamate dehydrogenase (GDH) solution (pH 7.4) (or GDH solution containing NADH). In such a way, a GDH (/NADH) gold colloid-cysteamine (or PAMAM)-modified glassy carbon cathode was obtained.

For cathode that both cysteamine and PAMAM were coated, the cleaned glassy carbon electrode was first immersed in cysteamine solution, the resulting electrode was dipped into the PAMAM solution. Then, it was dipped into the colloidal gold. The gold colloid-cysteamine/PAMAM-modified electrode was dipped into the GDH solution (pH 7.4) (or GDH solution containing NADH). In such a way, a GDH (/NADH) gold colloid-cysteamine/PAMAM-modified glassy carbon electrode was obtained

### 2.3. Nanoparticles Solution Preparations

Nanoparticles Au was prepared by reacting HAuCl<sub>4</sub> with citric acid [5].

All chemical reagents used in this study were analytical grade or the highest grade available, water was double deionized distilled water. All the experiments were carried out under deoxygenated condition in 0.1 M phosphate buffer solution. All enzymes and biochemicals were purchased from Sigma-Aldrich Chemical Co, St. Louis, MO, USA.

### 2.4. Detections

Cyclic voltammetry was conducted by using a Gamry 600 Potentiostat. Voltammetric potential was measured against a saturated chloride electrode (SCE).

## 3 RESULTS AND DISCUSSION

### 3.1. Anodic Half-Cell

Figure 2 shows the oxidative responses of the modified Au electrode with nanogold particles, cysteamine and LDH. The characteristic peak of lactate conversion to pyruvate was detected at 250 mV and the accumulative current increased linearly with added lactate instantaneously [6]. It verified that the modified anodic electrode was functioning as expected in Equation (1).

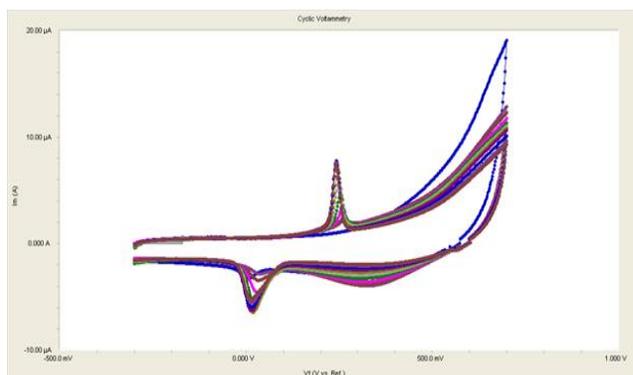


Figure 2. Voltammetric responses of the modified gold electrode. Results show stepwise additions of lactate at  $1 \times 10^{-3}$  M at 250 mV, response of stepwise increase of the current peak was nearly instantaneous indicated effective electron transfer for the electrode.

### 3.2. Cathodic Half-Cell

Figure 3 is the voltammetric responses of the PAMAM-Au-GDH modified cathodic half-cell measured at 0.7 V, the electrode gave about 0.32  $\mu$ A. Figure 4 is the voltammetric responses of a GCE modified with cysteamine-Au-GDH/NADH, his half-cell gave about 6.2  $\mu$ A. Considering the electrode surface area of 0.0962  $\text{cm}^2$  (diameter of 0.35 cm) and reactants consumption of 0.3 mM, thus, the current density ( $\text{A}/\text{cm}^2$ ) would be 64.4  $\mu\text{A}/\text{cm}^2$  and the current generated from the reaction was 20.7 mA/M. Therefore the latter electrode was a more efficient electrode for the reaction system. It was observed that with the different combinations of cysteamine and PAMAMs, polymers with the most branched structure would support the sol-gel nanogold structure with the highest efficiency to anchor the embedded enzymes (LDH and GDH, for examples) for the enzymatic conversions [6]. In addition, this sol-gel structure on the electrodes could stabilize the enzymes that normally would easily decompose rapidly once they are in diluted solution, which was the case in our electrode preparation. Our anode and cathode performed repeatedly consistently as they were freshly prepared even after they were stored submerged in distilled water at 4  $^{\circ}\text{C}$  for 2 weeks. It should be noted that a stable and cost effective enzymatic system is very important for the realization of using enzymatic biofuel cell for practical applications.

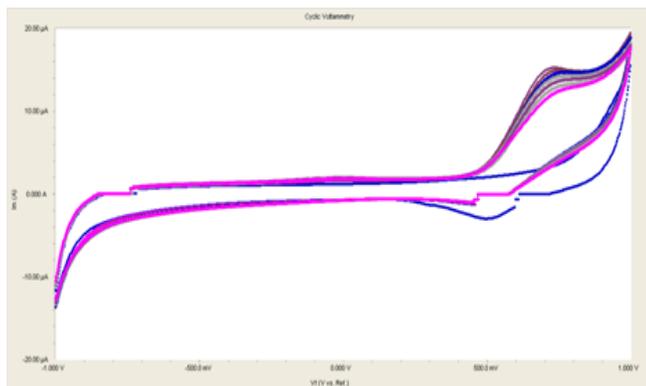


Figure 3 Voltammetric responses of the modified glassy carbon electrodes modified with cysteamine/PAMAM\_0-Au-GDH.

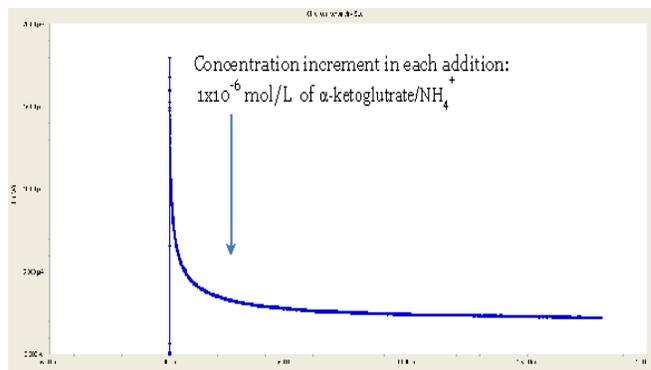


Figure 4. Reductive responses of a GCE modified with cysteamine-Au-GDH/NADH. At 0.71V, total time 1800 s; add  $\alpha$ -ketoglutarate from 100 to 1800 s, each addition portions of  $\alpha$ -ketoglutarate/ $\text{NH}_4^+$  was  $1 \times 10^{-7}$  mol/L every 60 s.

This biofuel cell system would generate a relative low potential (0.05V), but the reactants are all readily available from our body which make it closer to reality for the system to be implanted into our body as power source for biomedical devices. In our laboratory, we continue to experiment new systems that generate higher power outputs.

### 3.3. pH Effect

The biofuel cell, behaves similarly as many other biological systems, is affected by pH. Figure 5 demonstrates how pH would affect the reaction potential and current with variations of pH.

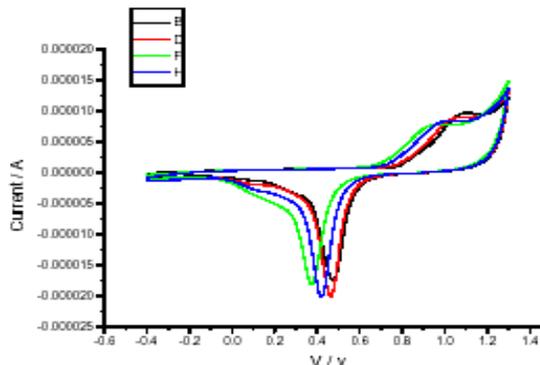


Figure 5. Higher pH shifted the reaction potential to lower voltage requirement for the GCE. Green: 7.8; blue:7.5; red: 7.0; black: 6.5.

## 4 CONCLUSIONS

An enzymatic biofuel cell system consisted of anode and cathode modified with enzymes, highly branched polymers, and Au nanoparticles sol-gel was constructed and characterized. This enzymatic biofuel cell concept can be used to generate power for various biomedical and

environmental applications, depending on the available energy sources. This demonstrative biofuel cell system generates a moderate power potential, new enzymatic systems should be further explored. Other factors to be considered in future development include control of current output and pH stability.

## 5 ACKNOWLEDGMENTS

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