Low-cost Immobilized Enzyme Glucose Sensor based on Laminar Flow

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Abstract. In modern medicine, glucose plays an important role in our body because glucose is the basic nutrition of human body and we can find important healthy information through it. With the development of medicine, many diseases require glucose of blood tests including genetic diseases. People has known that the production and consumption of glucose of blood in a normal people is in a state of dynamic equilibrium and is maintained at a relatively stable level. Once the level higher or lower than the normal level, human probably have some diseases base on concentration of blood sugar like Diabetes or Hypoglycemia. Many schools or research institutions have tested biofuel glucose sensors based on laminar flow, which has desired effect in electricity generation. So in this experiments, we will find a new way to test glucose of blood by microfluidic fuel cell, and use buffer solution contained glucose to simulate blood. Then we will introduce material of electrodes, enzymes immobilization, and how to make a laminar flow channel. Finally finding the relationship between concentration of glucose and electricity current, which can show the possibility of quick detection in blood sugar. Current research shows the great potential of this cheap disposable glucose sensor, and more phenomena and applications based on laminar flow biofuel cell can be discovered in the future.

1. Introduction
In the twentieth century, with the concept of microfluidic fuel cell was proposed, a new technological door opened to people. From 2002 it was invented, the equipment has been designed to apply for many field[1]. Before this type of cells was invented, people has used traditional fuel cells for many years. As we know, fuel cell is a kind of energy conversion device, which is based on the principle of electrochemistry. It directly converts the chemical energy stored in fuel and oxidant into electrical energy, so the actual process is redox reaction. Electrolyte, cathode, anode, and external circuits make up the battery. Fuel flows into the anode and oxidant enters the cathode. Fuel loses electrons at the anode to generate cations, oxidants gain electrons at the cathode to form anions. Ions can pass through the electrolyte and participate in the reaction. During the reaction, the electrons continuously pass through external circuit, forming electric current[2].

But there is a noticeable problem in cost with electrolyte, which usually is a membrane. One of the reasons why fuel cells have not been widely promoted is that electrolyte membranes are expensive, accounting for more than 40% of the total cost of the battery. In addition, there are other problems with membranes, including problems that membranes can easily lead to electrode deviations and dry. So Harvard University proposed the concept of microfluidic fuel cells in the 21st century. Based on the characteristics of microfluidics, fuel and oxidants naturally stratify in a laminar state without the
use of a proton exchange membrane, as shown in Figure 1. This design overcomes the problems caused by proton exchange membranes such as electrode deviations, and also saves the high cost of proton exchange membranes. The flow channel is generally designed as Y type, which is an innovative development of fuel cell design.

![Figure 1. Structure of Y type microfluidic fuel cells.](image)

Along with the development of biology, those micro equipment has been used in biological technology. One of the research popularity is biochemistry, especially for human, and many researchers has designed microfluidic biofuel cells to different aspects. We will discuss the most popular application of microfluidic biofuel cells in biochemistry, as biosensors. Although the equipment has potential to apply for energy production[3], it suit for detect. When it used as biosensors, it can easily detect specific substance in test fluid.

Glucose sensors is a good choice of the application of microfluidic fuel cells, which account for 85% of all biosensor markets[4]. About glucose sensors, many studies have been experimented[5-7]. In these experiments, researchers always use carbon as the material of electrodes, like carbon plates, carbon nanotubes or graphite, graphene. Some of them are specially treated. The reason why they always choice this material is that carbon has strong adsorption, which can successful immobilize enzyme. Simultaneously, carbon is a good conductor. In summary, it is important that electrodes can fix enzyme and transfer electricity.

We plan to test the possibility of portable and inexpensive disposable human blood glucose concentration sensor, the first point to consider is that this micro sensor must be small to carry and store conveniently. It requires that electrodes must be enough small and cheap. We find a combination of two material can solve the problem and is cheaper than most carbon materials, that is sliver glue and chitosan film. The conductive silver glue is mainly based on the bonding effect of the matrix resin to combine the conductive particles together to form a conductive path to realize the conductive connection of the adhered material. The sliver glue as electrodes to link external electrical analyzer, and chitosan film as a link between enzymes and silver glue electrodes because chitosan has conductivity and adsorption. Enzymes fix on chitosan film and the film adhere to silver glue electrodes by adsorption. Due to the characteristics of multiple pores, good toughness, chitosan has a very strong adsorption force and it also has the presence of amine groups. Figure 2 show structure of chitosan.

More and more studies has proved chitosan has capacity of adsorption[8], some research groups even use it to deal with wastewater[9]. There are many related researches on biomedical materials. At present, it is considered to have good biocompatibility and no biological toxicity. More importantly, silver glue and chitosan are very common substances, and their prices are quite cheap, much cheaper than some carbon nano tubes. In addition, chitosan could immobilize enzymes through strong physical adsorption and chemical bonds with enzymes through glutaraldehyde. The chemical bond is that aldehyde group of glutaraldehyde and amine group of the chitosan and the enzyme happen
condensation reaction under alkaline conditions to crosslink, this process is immobilizing electrode [10]. There is reaction equation: C=O + R-NH₂→C=N-R + H₂O.

**Figure 2.** Structure of chitosan, it is a deacetylated product of chitin.

In the choice of catalyst, glucose oxidase has been confirmed the excellent ability of glucose oxidation and laccase will be used in oxygen reduction[11]. As early as 2011, glucose biofuel cells using glucose oxidase and laccase as catalysts were tested in France to verify their feasibility[12]. The following is reaction:

**Anode reaction:**

\[ \beta-D-glucose + \text{Enzyme}_\text{ox} \rightarrow \delta-\text{gluconolacton} + \text{Enzyme}_\text{red} + 2H^+ + 2e^- \]

**Cathode reaction:**

\[ O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \]

In this experiment, we plan to fix glucose oxidase in the anode and laccase in the cathode. The liquid flow in this microfluidic biofuel cell is laminar. The anode solution contains glucose molecules and buffer solution (simulating blood), and the cathode solution is pure buffer solution. The anode and cathode will undergo glucose oxidation and oxygen reduction to generate electricity. Then using an electrochemical analyzer to detect electrical signals under different conditions.

2. **Material and method**

2.1. **construction of electrode and microfluidic channel**

The cathode and anode electrodes are manufactured on a 0.1 mm-thick plastic sheet according to the shape in the Figure.3, and the battery lead plate is completed by the manufacturer. Electrode thickness is about 12-15 μm. Figure.3 shows the electrode shape in detail.

**Figure 3.** Shape of the electrodes.

A Y shaped microchannel is designed on the plastic sheet containing the electrode to fit the electrode size[3]. As shown in the Figure.4, the design of the total channel width is 2 mm, which is consistent with the width between the outermost ends of the electrodes on both sides, and the length is about 40 mm, and then the two electrodes are connected to the external circuit outward. Inside the flow channel, the exposed portions of the two electrodes are rectangular planes with a width of 0.5 mm and a length of 20 mm. However, since the thickness of this electrode is too small when it is compared to the height of the flow channel in actual conditions, its height can be ignored. Cross section height of channel is 1mm. The angle between the two tributaries is about 45 °, the width of the branch channel is 1 mm, and the length is about 10 mm. The starting position of the electrode (the electrode is closest to the junction of the branch channels) is placed 1cm behind the junction.
Figure 4. Design of Y type channel and cross section of total channel, the height h of the internal flow channel can be changed to adjust the flow velocity. In this experiment, h is set to a fixed value. (h=1 mm).

Figure 5. Glucose biosensor consists of two parts, the upper part is the flow channel part and the lower part is the electrode part. The liquid flows into the ends of the two channels respectively.

The combination of channel plate and electrode plate manufactured according to the design as shown in the Figure 5, the upper part is the flow channel part, and the lower part is the electrode part. After the enzyme is immobilized on the electrode, the plastic sheet containing the electrode is aligned with the flow channel part, and combining them by adhesive to form a complete sensor. The production process of channel plate is as follows: firstly, preparing the fast-drying fiber adhesives EP-2F 1A (referred to as A) and EP-2F 1B (referred to as B), then mixing A and B in a mass ratio of 2:1, and pour into the mold. After exhausting air with a small needle, let it stand for half an hour to cure the model. Finally, remove the solidified channel model from the mold, and clean the stains on the surface.

2.2. Preparation of immobilized enzymes
We made immobilized enzyme through the following process: firstly, we prepare raw materials: Glucose oxidase, Laccase, Glutaraldehyde, Chitosan, Acetic acid, Pure water, Sodium hydroxide solution (1 mol/L). Then is solution preparation, 0.02 g of glucose oxidase and 0.02 g of laccase were dissolved in distilled water to prepare enzymes solution respectively. Chitosan and acetic acid were mixed and stirred for 30 minutes to prepare a chitosan salt colloid solution. Finally, we have 5 steps to immobilize enzymes: I. Put Chitosan salt colloid solution into beaker and add pure water to make 9 g/ml Chitosan solution. II. Add 1 ml Glutaraldehyde and mix them for 3 h. III. Add 10 ml (2 mg/ml) solution of enzymes, and wait the mixture to become colloid. IV. Wait the mixture to absorb about 10
h in 15 °C. V. Coat the mixture on electrodes and dry it for 30 min in 35 °C. Cross-linking reaction happen at step II and step III[13]. Finally, the finished electrode is shown in Fig.6(a).

![Figure 6](image.png)

**Figure 6.** (a) Structure of immobilized enzymes. (b) Preparation of cathode solution and anode solution.

### 2.3. Preparation of channel solution

We stimulate blood with buffer solution containing glucose, and then prepare solution with different glucose concentration to stimulate different blood glucose levels. According to medical research, under normal circumstances, the body will transfer starchy food into glucose, as the body's fuel. Fasting blood glucose concentration in normal people is 90 mg/dL. Insulin is a hormone made by the pancreas that helps glucose enter the cells and provides heat. Diabetic patients cannot make enough insulin in the pancreas, glucose cannot get enough into the cells, blood glucose concentration will rise, and diabetes will form[14]. Usually, fasting blood glucose concentrations above 100 mg/dL are pre-diabetes and need to be vigilant. If it is higher than 126 mg/dL, it is likely to be diabetes. Hypoglycemia is that the blood sugar drops too low to supply the energy needed by the body's cells for normal physiological activities. Severe hypoglycemia can be fatal, causing permanent brain damage and sequelae, such as memory loss and poor learning ability. Therefore, the dangers of hypoglycemia cannot be ignored[15]. Generally, hypoglycemia occurs when the fasting blood glucose concentration is lower than 70 mg/dL. If it is lower than 50 mg/dL, symptoms of hypoglycemia may occur.

In summary, the fasting blood glucose concentration of 50 mg/dL represents the dividing line between mild hypoglycemia and severe hypoglycemia. Hypoglycemia is below 70 mg/dL, while 70-100 mg/dL is the normal range, and 90 mg/dL is the most common fasting blood glucose concentration. More than 100 mg/dL can be considered pre-diabetes, and more than 126 mg/dL can be determined to have diabetes. Base on this, we call find there are 5 different boundaries, 50 mg/dL, 70 mg/dL, 90 mg/dL, 100mg/dL, 126mg/dL. Glucose and buffer solution (pH=7) were used to make the above five glucose solutions with different concentrations as the anode solution. The cathode solution is just buffer solution (pH=7) , as shown at Figure.6(b).
3. Analysis and discussion.

3.1. Calculation of input liquid

Generally, the pipeline Reynolds number $Re < 2100$ is a laminar flow state, $Re > 4000$ is a turbulent flow state, and $Re$ is a transition state when it is $2100 \sim 4000$. In order to guarantee the realization of laminar microfluidic biofuel cells, the Reynolds coefficient needs to be controlled below 2100. According to the Reynolds number calculation formula: $Re = \frac{\rho v d}{\mu}$, this calculation formula is suitable for pipes which have circular cross section, but the pipe of this experiment has rectangular cross section. If we want to calculate Reynolds number of a tube which has rectangle cross section, the size of the tube must be determined by the equivalent diameter ($d$). The equivalent diameter is equal to four times the hydraulic radius. For pipes of any cross-sectional shape, the hydraulic radius is equal to the ratio of the area stamped to the circumference of the pipe. Therefore, for rectangular pipes of length $A$ and $B$, respectively, the equivalent diameter of the pipe with an arbitrary cross-sectional shape can be calculated by four times the cross-sectional area and the ratio of the section circumference. It is calculated according to the formula: $d = \frac{2AB}{A+B}$. From the cross-sectional area of the flow channel, it can be known that $A = 0.2$ cm, $B = 0.1$ cm, and according to calculation, $d = 0.133$ cm. Since the solution in this experiment contains only a very small amount of glucose, its density and viscosity coefficient are replaced by pure water data (The solution temperature is 20 °C). Therefore, through the Reynolds number calculation formula, the maximum flow velocity that the flow channel can be calculated while maintaining laminar flow (the Reynolds number is 2100 at this time), the maximum flow velocity is 157.89 cm/s. And maximum flow is 189.47 mL/min calculated by combining the flow channel area. Since this flow is a combined flow, the input flow at the two inputs is the same, so the input speed at each input does not exceed 94.735 mL/min, which can guarantee laminar flow in the channel. In this experiment, it is planned that the anode of the product consumes blood, so it cannot be input too fast. It is planned to test 1 mL blood about 10 minutes for this sensor. So set the input speed to 0.1 mL/min. This value is far below the limit, so it is theoretically laminar.

![Figure 7. Laminar flow under a magnifying glass.](image)

In order to facilitate observation, a transparent plastic sheet was used in the test experiment instead of the plastic sheet containing the electrode. For clarity, we used water and blue ink to instead of the anode and cathode solution respectively in Figure 7. There is a very clear and obvious laminar flow between the two liquids.

3.2. Sensor duration

Duration is an important indicator for sensors, because stability is a basic requirement for instrument operation. In this experiment, the relationship between the current and time was tested using an electrical analyzer at a room temperature of 20 °C, and the results are shown in the Figure 8.
Figure 8. Image of sensor current over time. Glucose concentration of anode solution in this experiment: 90 mg/dL.

Before 150s, the current drops rapidly. It is observed that when the electrode is fixed, a chitosan film is first covered, and then glutaraldehyde is used to react with the chitosan film and the amino group on the enzyme to connect them. Due to the porous nature of the chitosan membrane, some enzymes are absorbed into the membrane and cross-linked with it, but some of them are not immobilized. Therefore, when there is fluid in the flow channel, the enzymes that have not been fixed will be quickly washed away, resulting in a short-term decrease in current. In the later period, although the current is about to stabilize, a slight drop can be observed. This is because with the washing of the liquid, the enzyme is continuously lost. The last sudden drop is presumably because the enzyme activity has been lost.

3.3. Detection of simulated blood glucose concentration

In our plan, the blood glucose concentration in 5 cases will be used as the cut-off standard, and the measurement standard and measurement duration of each type are the same. These five cases are 50, 70, 90, 100, and 126 mg/dL. Each group of data is tested for 10 minutes. There are two causes for this: 1. It is planned to use only 1 mL blood for the patient to perform a quick test. So the injection at a rate of 0.1 mL/min can just maintain 10min; 2. It can be proved in the battery duration that the battery duration is about 20min, of which the relatively stable current from 150s to about 1200s, and the current from 150s to 600s is stable. At the same time, the influence of current fluctuations that appear later is also excluded. The specific data is shown in the Figure 9(a). In the image, we can see that, except for the time from the beginning to 150s, the time from 150s to 600s, the current is basically stable.

Figure 9 (a) 10-minute current test with 5 different glucose solutions as anode solutions. (b) The relationship between the current obtained from the average of the five reliable parts in the graph (a) and the glucose concentration.
The data of 150 seconds to 600 seconds of each concentration were selected and averaged to obtain 5 averages, each average corresponding to a blood glucose concentration value. List 5 points in the figure, and find a linear equation that can express its law. as the Figure 9(b) shows. This line is fitted with a linear function, the purpose is to find the linear relationship between the average value of the measured current and the glucose concentration of the test solution. We used statistics to perform linear regression analysis on the data, and R² = 0.99311. R² is the ratio of the sum of the squares of the regression and the sum of the squares of the total dispersion, and represents the proportion of the sum of the squares of the total dispersion that can be explained by the sum of the squares of the regression. The closer this ratio is to 1, the more accurate the model. So this fitting line fits the data well. After the patient's blood glucose sample sensing current is obtained, it can directly deduce to the corresponding blood glucose concentration in this congruent relationship, so as to quickly determine some basic diseases.

4. Conclusion
It can be seen from this experiment that the method of adsorbing to the electrode by the strong adsorption force of the chitosan membrane and then cross-linking the enzyme by glutaraldehyde is feasible. In addition, cost of this sensor is very low and it is able to maintain high reliability. When testing the current, generation of current is relatively stable and the fluctuation is small. Compared with the experiments of other researchers, the sensor in this experiment has a shorter continuous working time [16], but it is designed to test the human blood glucose level for a short time, so the goal is reached. The next step is to experiment with a more advanced process to extend operating time. The calculated relationship between the glucose concentration and the average current, it can be judged that this tester can be used to quickly measure the glucose concentration.

The above is obtained from this experiment, but this experiment still needs to be improved. The first is that chitosan does have weak conductivity, but this undoubtedly affects the conduction of current. In some experiments, noble metal particles are added to chitosan to increase conductivity, but this can increase costs significantly [17, 18]. Secondly, the components of the real human blood environment will not only include buffers and glucose, but also trace metals. Some of these metal ions may promote the catalytic ability of the enzyme, and some may inhibit the enzyme activity [19-21]. These are also the parts that need to be tested in the future. Finally, because the flow channel is Y shaped, when the two liquids meet, there will be an interlayer diffusion phenomenon [22]. This phenomenon is also needed to research in the future.

5. Reference

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