A high-accuracy measurement method of glucose concentration in interstitial fluid based on microdialysis

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A High-accuracy Measurement Method of Glucose Concentration in Interstitial Fluid Based on Microdialysis

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Abstract

A high-accuracy microdialysis method which could provide reference values of glucose concentration in interstitial fluid for accuracy evaluation of non-invasive and minimally invasive continuous glucose monitoring was reported in this study. The parameters of microdialysis process were firstly optimized by testing and analyzing three main factors which impact the microdialysis recovery, including the perfusion rate, temperature, and glucose concentration in the microdialysis probe surrounding area. The precision of the optimized microdialysis method was then determined in a simulation system which was designed and established in this study to simulate continuous glucose concentration variations in human body. Finally, the microdialysis method was tested for in vivo interstitial glucose concentration measurement.

Keywords: Continuous monitoring, Glucose concentration, Interstitial fluid, Microdialysis, Reference value

1. Introduction

Diabetes mellitus is a worldwide disease that seriously jeopardizes human health [1]. At present, there are two types of conventional glucose measuring methods in clinical: venous whole blood measurement and fingertip blood measurement [2]. But both of these two methods are unable to achieve continuous glucose monitoring by performing blood sampling repeatedly due to the trauma of blood sampling process, the risk of infection and the pain for individuals with diabetes. Considering the correlation between the interstitial glucose concentration and the blood glucose concentration, continuous glucose monitoring can be implemented indirectly by detecting the glucose concentration in the interstitial fluid [3]. Compared with conventional glucose detection, continuous glucose monitoring could provide much more information about the glucose levels throughout the day to avoid postprandial hypoglycemia [4]. Furthermore, continuous glucose monitoring plays an important role in diabetes diagnosis and treatment with the closed loop insulin delivery system. As a result, continuous glucose monitoring has the potential to revolutionize the care and treatment of individuals with diabetes.

Two types of continuous glucose monitoring methods including non-invasive method and minimally invasive method are still in researching [5, 6]. Non-invasive method detects the interstitial glucose concentration in the subcutaneous tissue based on the interactions between light and tissue in specific human body parts [7]. Minimally invasive glucose monitoring techniques measure the interstitial glucose concentration in the subcutaneous tissue in real time utilizing microsensors implanted under the skin [8]. So far, companies such as Medtronic, DexCom, and Abbott have launched implantable enzyme electrode sensors that can be used for continuous glucose monitoring [9, 10]. But the implantable enzyme electrode sensor could only work for 3 to 7days [10].

The objective of both non-invasive and minimally invasive continuous glucose monitoring approaches is to measure glucose concentrations in interstitial fluid [11]. However, the standard for continuous glucose monitoring in clinical is still blood glucose concentration at present [12]. Hence, a prediction model should be established to convert the glucose concentration in interstitial fluid to the glucose concentration in blood [13]. There are mainly two factors that affect the accuracy of continuous monitoring the glucose concentrations in blood by non-invasive and minimally invasive continuous glucose monitoring methods: the precision of the detecting technology for glucose concentration measurement in the interstitial fluid and the accuracy of the blood glucose prediction model established according to the measurement of interstitial fluid glucose concentration [14]. Many researchers devote their efforts to establishing the prediction model [12-16]. At present, the glucose concentration in blood is always used to evaluate the accuracy of the glucose concentration in interstitial fluid. Nevertheless, there is not only a time delay [14, 17, 18], but also a discrepancy between glucose concentrations in the interstitial fluid and blood under physiological conditions. It is only an approximate method to evaluate the accuracy of interstitial glucose concentration that measured by non-invasive and minimally invasive continuous glucose monitoring methods using blood glucose concentrations. Therefore, a high-accuracy method to evaluate the accuracy of glucose concentration in interstitial fluid is required.

This paper reports a direct microdialysis-based interstitial glucose concentration measurement method to evaluate the accuracy of measurement in interstitial fluid glucose concentration for the non-invasive and minimally invasive continuous glucose monitoring. By inserting a microdialysis probe into the subcutaneous tissue and extracting

interstitial fluid from the human body, interstitial glucose concentrations can be measured in vivo. With this approach, interstitial glucose concentrations could be accurately measured, thereby providing reference values for assessing the accuracy of non-invasive and minimally invasive glucose monitoring results.

2. Microdialysis-based interstitial glucose concentration measurement approach

The basic principle of measuring actual interstitial glucose concentrations via microdialysis involves monitoring the interstitial glucose concentration in vivo by inserting dialysis tubing under the skin and extracting glucose molecules in the interstitial fluid. The monitoring process can be described in detail as follows and is illustrated in figure 1. When the microdialysis probe is inserted into the subcutaneous tissue and perfusion is implemented under non-equilibrium conditions, glucose molecules will diffuse in the adverse concentration gradient direction, penetrate the semipermeable membrane at the probe's tail, enter the dialysis tubing, and move out continuously along with the perfusate flowing through the microdialysis probe. By monitoring the dialysate, the interstitial glucose concentration can be obtained. This detection approach has significant advantages, such as the high continuity, the small required sampling quantity, and the minimal injury.

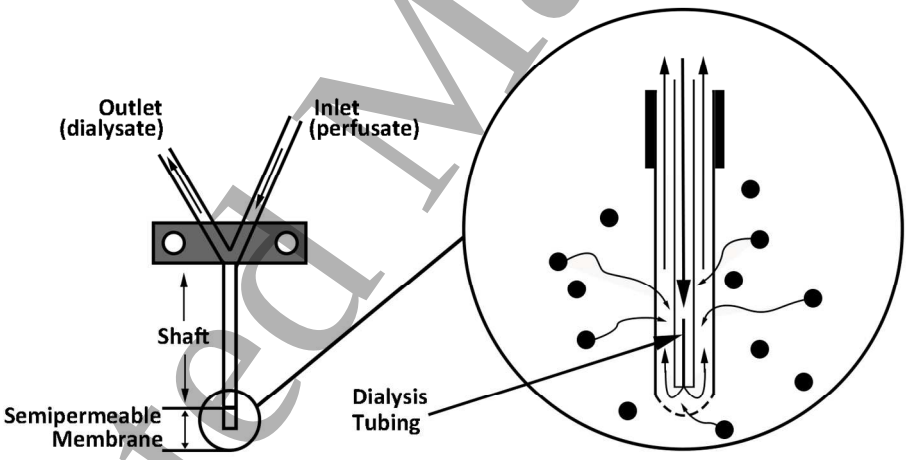

Figure 1. Microdialysis working principle

Microdialysis is a passive diffusion process, which can be described using Fick's first law [19]:

$$J = -DS\frac{dC}{dx} \qquad (1)$$

where J is the number of designated molecules that penetrate through the semipermeable membrane per unit time, D is the diffusion coefficient, S is the surface area of the semipermeable membrane in the microdialysis probe, C is the concentration of the designate molecules, and $J = -DS\frac{dC}{dx}$ is the concentration gradient through the semipermeable membrane. Here, the negative sign indicates that the molecules diffuse in the direction opposite to the concentration gradient.

Because the sampling process is conducted under non-equilibrium conditions in microdialysis, the glucose concentration of the dialysate is less than that in the interstitial fluid around the probe [20]. The ratio between the former and the latter glucose concentration is known as the recovery R [21, 22], which can be expressed as

$$R = \frac{C_{out}}{C_q} \quad (2)$$

where C_{out} and C_q are the glucose concentrations of the dialysate and the interstitial fluid around the probe (units: mmol/L), respectively.

The recovery model developed by Jacobson et al [23] was adopted in this study to describe the correlation between the microdialysis recovery and the surface area of the semipermeable membrane in the microdialysis probe, as well as the effect of perfusion rate on the recovery:

$$R = \frac{C_{out}}{C_q} = 1 - \exp(\frac{-k_m S}{Q}) \quad (3)$$

where k_m is the mass transfer coefficient of the molecules, S is the surface area of the semipermeable membrane in the microdialysis probe, and Q is the perfusion rate.

3. Influencing factors of microdialysis recovery

In order to test and analyze the influencing factors of microdialysis recovery, an experimental system for in vitro microdialysis was constructed. And the effects of major factors, such as the perfusion rate, the glucose concentration, and the temperature, on the microdialysis recovery were tested.

3.1 Experimental system for in vitro microdialysis

The experimental system for in vitro microdialysis consisted of a microsyringe pump for perfusate (0.9% NaCl solution) injection, a microdialysis probe, a constant-temperature water bath for temperature adjustment of the glucose solution, and a miniature glucometer (FreeStyle FREEDOM, Abbott, USA) for glucose concentration detection in the dialysate. The minimal volume required for the glucometer is 0.3μL which can be acquired by microdialysis in our experiments. A CMA20 microdialysis probe (CMA Microdialysis, Sweden) with a molecular weight cutoff of 20kD, a membrane length of 10mm, and a membrane diameter of 0.5mm was adopted.

The experimental system for in vitro microdialysis is shown in figure 2. In the microdialysis experiment, perfusate was injected into the microdialysis probe by the microsyringe pump. And at the probe tail, glucose molecules in the sample solution penetrated the semipermeable membrane and entered the dialysis tubing. Then the dialysate flowed out of the probe for glucose concentration measurement with a glucometer. In the experimental system, the microdialysis recovery was tested with different perfusion rate, temperature, and glucose solution to investigate their relationships.

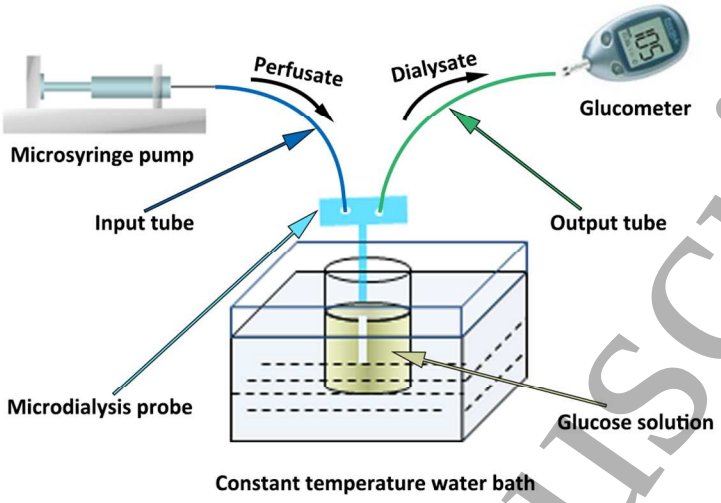

Figure 2. Experimental system for in vitro microdialysis

3.2 Impact of perfusion rate on microdialysis recovery

To test the impact of perfusion rate, the temperature of water bath and the glucose concentration of sample solution were set at 26□ and 5.3mmol/L, respectively. The perfusion rate was changed from 0.3 to 3.0μL/min. Due to the volume of the probe's output tube was 6μL, microdialysis equilibrium was achieved after 20, 12, 6, 3, and 2min when perfusion rate was set at 0.3, 0.5, 1.0, 2.0 and 3.0μL/min, respectively. The dialysate was collected every 5min for measurement after microdialysis equilibrium was reached.

The relationship between the perfusion rate and the microdialysis recovery is shown in figure 3. Each data point is the average of 10 measured values. Evidently, the microdialysis recovery continuously and exponentially decreased as the perfusion rate increased. Higher perfusion rate led to lower glucose concentration of the dialysate, which sets stringent demands on the sensors for high-precision glucose detection. However, the dialysate was collected slowly, causing a delay in the interstitial fluid monitoring process at low perfusion rates. Therefore, a perfusion rate of 1.0μL/min was adopted in this study to obtain enough samples for glucose detection in short time period while ensuring sufficiently high recovery values.

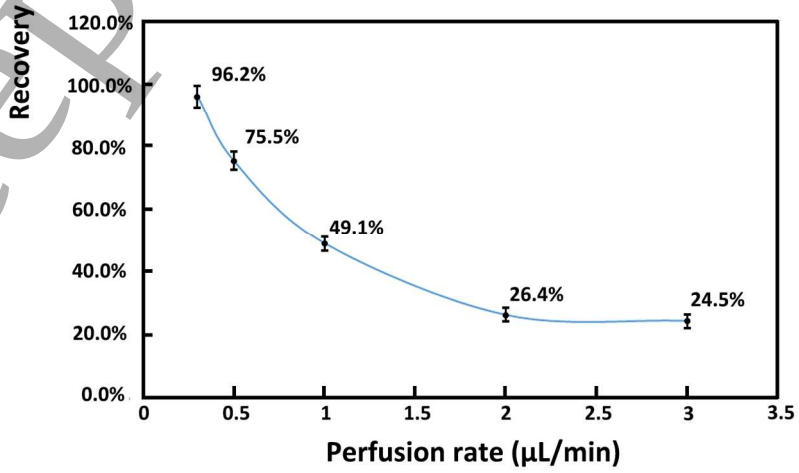

Figure 3. Impact of perfusion rate on microdialysis recovery

3.3 Impact of glucose concentration on microdialysis recovery

To test the impact of glucose concentration solution, the perfusion rate and the temperature were set at 1.0μL/min and 26□, respectively. Four water solutions with different glucose concentrations were utilized for the experiments. The dialysate was collected every 5min for glucose detection after microdialysis equilibrium.

The average microdialysis recovery, which was tested 10 times for each glucose solution, was 48.4%, 49.1%, 47.6%, and 47.1% when the glucose concentration was adopted as 3.1, 5.3, 8.2, and 10.4mmol/L, respectively. And the total average microdialysis recovery was 48.1%, and the standard deviation was 0.9%. A Student's t test was carried out on the four different glucose concentrations. The results show that there are no significance differences at the 95% confidence level for the tested glucose concentrations between 3.1mmol/L and 10.4mmol/L at a constant perfusion rate and temperature. Therefore, it is feasible to apply microdialysis to monitor glucose in solution or tissue with time-varying concentrations.

3.4 Impact of temperature on microdialysis recovery

The impact of temperature on microdialysis recovery was investigated using a constant-temperature water bath. In the experiment, the perfusion rate and the concentration of glucose solution were set at 1.0μL/min and 5.3mmol/L, respectively. The temperature was changed from 25 to 60□. Every time the temperature of water bath was changed, microdialysis was only conducted after the temperature of water bath and glucose solution was stabilized. At each temperature, the measurements were performed three times. The correlation between the recovery and the temperature is shown in figure 4.

Figure 4 reveals that the microdialysis recovery increased rapidly with increasing temperature and there was a linear correlation between them. Thus, the temperature should remain constant in the microdialysis-based interstitial glucose concentration detection process to obtain steady recovery and realize effective correction.

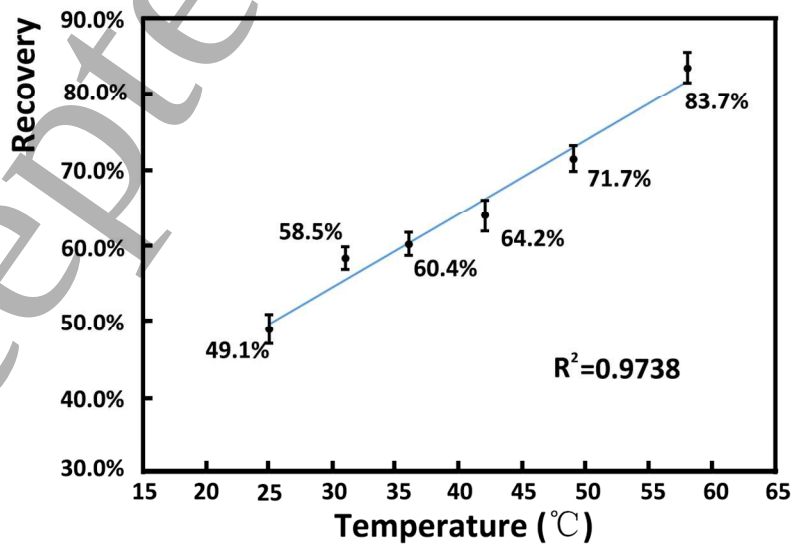

Figure 4. Impact of temperature on microdialysis recovery

4. Simulation of glucose concentration variations in vivo

The glucose concentrations in the interstitial fluid vary continuously due to the normal physiological activities of the human body. In order to simulate the in vivo continuous glucose concentration variations in the interstitial fluid, an approach to get continuous variable glucose concentrations was developed and verified in an experimental system. And glucose monitoring experiment using the microdialysis method was performed in this simulation system to provide the good foundation for animal testing of microdialysis-based interstitial glucose concentration monitoring.

4.1 Simulation system configuration

The experimental system used to simulate continuous glucose concentration variations in vivo is shown in figure 5. This simulation system consisted of two peristaltic pumps, a mixing chamber and a three-way valve. The peristaltic pumps were used to extract the glucose solution and buffer solution separately into the mixing chamber. The completely mixed fluid was output through the delivery tube. The glucose concentration of the mixed fluid can be varied by adjusting the pumping rates of the peristaltic pumps, while holding the sum of their pumping rates constant to prevent the microdialysis recovery from being impacted by variations of the flow rate of the mixed fluid. The three-way valve was employed to switch the flow direction of the mixed fluid and thereby enabled independent glucose monitoring of the dialysate and pre-dialysis mixed fluid.

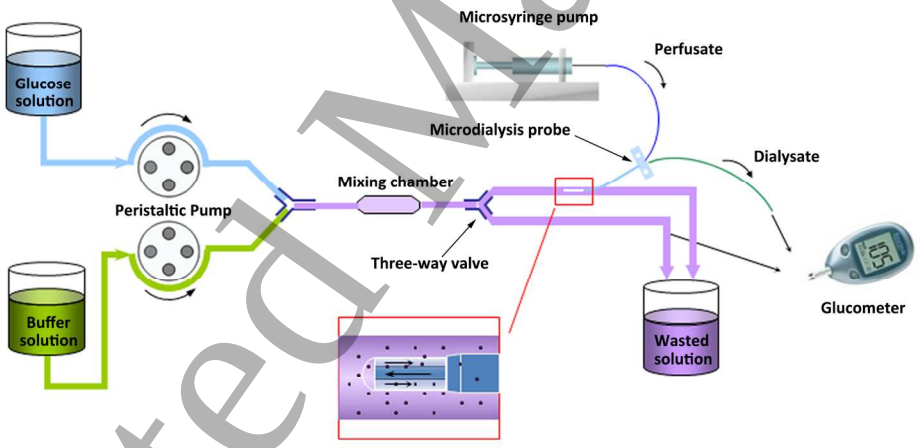

Figure 5. Continuous glucose concentration variation simulation system

4.2 Variation of glucose concentration in the simulation system

The buffer solution (0.9% NaCl) and glucose solution (10.3mmol/L) were mixed in different ratios with a constant total flow rate of 3mL/min. The pumping rates of the peristaltic pumps were adjusted to set the glucose concentration of the mixed fluid in the range of 0mmol/L to 10.3mmol/L.

In the preparation stage, the buffer solution and glucose solution were injected at the flow rates of 2000μL/min and 1000μL/min, respectively. After the glucose concentration of mixed fluid became stable, the flow rates of buffer solution and glucose solution were both changed to 1500μL/min, and this moment was marked as time zero. Subsequently, the mixed fluid was sampled and tested with a glucometer after 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, and 10min. The flow rates of buffer solution and glucose solution were respectively changed to 500μL/min and 2500μL/min at 10min, 750μL/min and 2250μL/min at 20min, and 1750μL/min and 1250μL/min at 30min. Each time

when the flow rates were changed, the mixed fluid was sampled at the times mentioned above for glucose concentration detection. The measured results are presented in figure 6. The glucose concentration of mixed fluid changed rapidly when the flow rates were changed. And 2min later, the glucose concentration reached a steady state with fluctuations less than 2.9%.

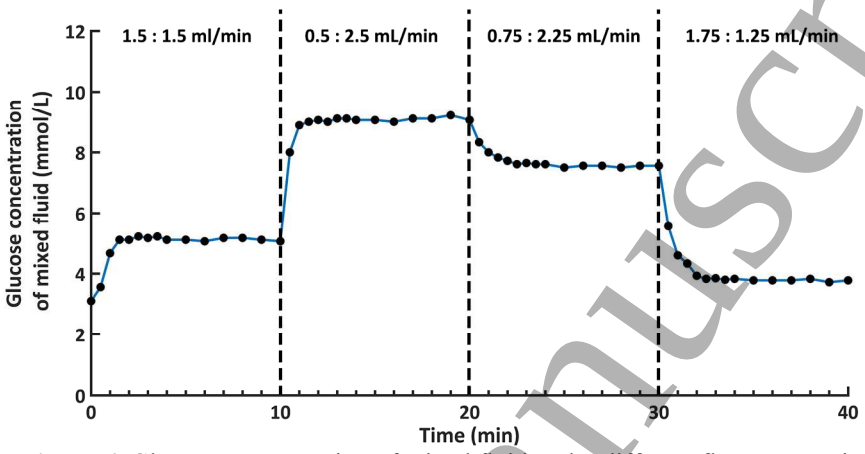

Figure 6. Glucose concentration of mixed fluid under different flow rates ratio

4.3 Continuous glucose monitoring in the simulation system

The microdialysis method developed in this paper was used to monitor the glucose concentration variations in the simulation system. The microdialysis probe was inserted into one of the channels after the three-way valve, and the dialysate was measured with the same glucometer, as shown in figure 5. In the experiment, the perfusion rate and temperature of microdialysis were set at 1.0μL/min and 20□, respectively. As the flow rate of mixed fluid in the simulation system was set at 3mL/min, the glucose concentration of dialysate was detected at 8min (2min for uniformly mixing of the glucose solution and buffer solution, 6min for the dialysate to flow through the output tube of the microdialysis probe) after the pumping rates of peristaltic pumps were adjusted. Then the three-way valve was switched, and the glucose concentration of mixed fluid was detected. The whole process was conducted in the following sequence: pumping rates adjustment, microdialysis sampling and dialysate measurement, three-way valve switching, and mixed fluid testing.

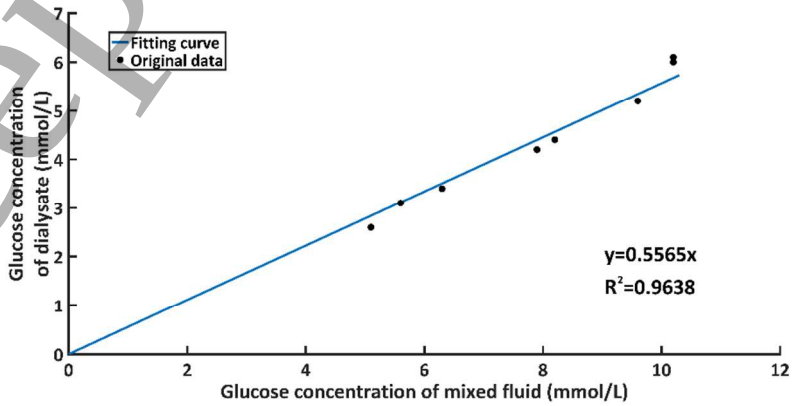

Figure 7. Relation between the glucose concentrations of mixed fluid and dialysate concentrations

The glucose concentration measurement was carried out nine times in the simulation system to get the recovery of

the microdialysis probe. As shown in figure 7, the slope of the fitted line, which was adopted as the microdialysis recovery, was 55.7%, and the uncertainty of the slope ranged from 0.5344 to 0.5787 at the 95% confidence level. Due to the higher water pressure and the stable glucose concentration of the flowing solution, the microdialysis recovery in this experiment was higher than the recovery taken from Figure 4 (about 43%). Then the microdialysis probe was used to continuously monitor the glucose concentration variation in the simulation system. The average recovery was used to calibrate the measurement result of the microdialysis probe. And the calibrated glucose concentrations of the dialysate were compared with the glucose concentrations of mixed fluid (as shown in figure 8). The blue points represent the directly measured glucose concentrations of pre-dialysis mixed fluid, which exhibit two up-down-up sequences. The purple points represent the calibrated glucose concentrations of the dialysate. The curve of calibrated glucose concentration almost exactly coincides with the measured one. 93.8% of the percentage deviations between the calibrated and measured glucose concentrations are less than ±10%. And the average percentage deviation is only 3.7%. The microdialysis method is suitable for interstitial glucose concentration monitoring.

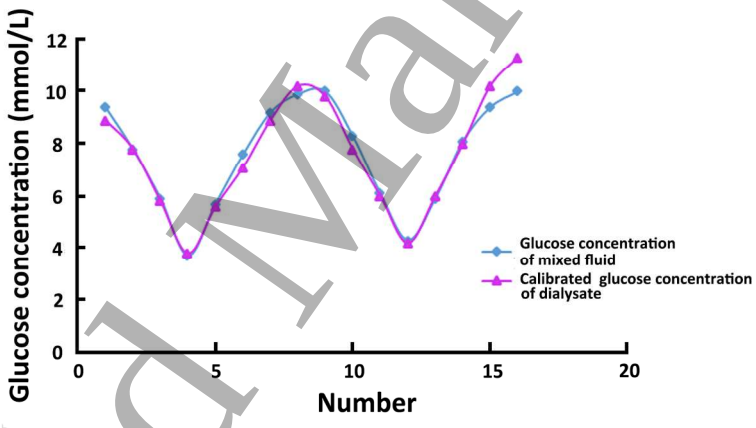

Figure 8. Continuous glucose concentration measurement results

5. In vivo continuous glucose concentration monitoring

After simulation experiment, animal test was performed to assess the microdialysis method in interstitial glucose concentration monitoring. In the animal test, two calibration methods, low-flow-rate method and no-net-flux method, were applied to determine the in vivo microdialysis recovery. Moreover, ultrafiltration was adopted to evaluate the microdialysis measurement results. Considering the size and operability of the experimental subject, an adult rabbit was chosen for the in vivo experiment. In order to keep the animal in a peaceful state throughout the experiment, it was given 3% pentobarbital sodium to induce a certain degree of anesthesia. Moreover, 30U/mg heparin sodium was used as an anticoagulant to protect the implanted microdialysis probe from being blocked by coagulated blood. Ringer's solution (NaCl: 0.85%, KCl: 0.03%, and $CaCl_2$: 0.033%) was employed as the perfusate.

5.1 Measurement of in vivo recovery using the low-flow-rate method

Low-flow-rate method which was developed by Jacobson et al was one of the earliest calibration methods in microdialysis application [24]. Due to the diffusion process from the sampling site into the probe is less efficient (more

dilute dialysate being collected) at higher perfusion rates, low-flow-rate method utilizes the relationship between perfusion rate and recovery, where lower perfusion rates yield higher recovery values [25]. In the experiments, the glucose concentration in the interstitial fluid of the rabbit was in the steady state. Perfusion rate was changed from 0.3 to 2.0μL/min. The glucose concentration of the dialysate was measured every 2min from the time point of 5min later than the start time of microdialysis. Due to the time delay of the probe's outlet tube, the average concentration between 21min and 31min was utilized for the perfusion rate of 0.3μL/min, while the average glucose concentration between 13min and 23min was employed for the other perfusion rates. The average glucose concentrations of the dialysate corresponding to different perfusion rates are presented in table 1.

Table 1. Average glucose concentrations of dialysate corresponding to different perfusion rates

Perfusion rate (μL/min)	Glucose concentration of dialysate (mmol/L)
0.3	17.8
0.5	15.5
1.0	10.8
2.0	6.8

According to Eq.(3) and the data in table 1, the fitting curve of dialysate glucose concentration versus perfusion rate was acquired (as shown in figure 9), where the glucose concentration at the perfusion rate of 0μL/min was adopted as the interstitial glucose concentration. In figure 9, the uncertainty of coefficient 18.8063 ranged from 17.89 to 19.72 at the 95% confidence level while the uncertainty of coefficient -0.8705 ranged from -0.9753 to -0.7656 at the 95% confidence level. The interstitial glucose concentration obtained from the fitting curve is 18.8mmol/L. The recovery can be calculated at different perfusion rates using Eq.(3). The recovery was 57.4% at the perfusion rate of 1.0μL/min. The deviation between the recovery in the in vivo experiments and the expected recovery (over 60%) in Figure 4 is caused by the difference between the diffusion coefficient in the probe lumen, dialysis membrane, periprobe environment and that in solution [26, 27]. During the process of continuous glucose monitoring in the interstitial fluid, a certain perfusion rate (such as 1.0μL/min) will be chosen. Then the interstitial glucose concentration can be predicted using the corresponding recovery (such as 57.4%) and the dialysate glucose concentration measured in the continuous microdialysis.

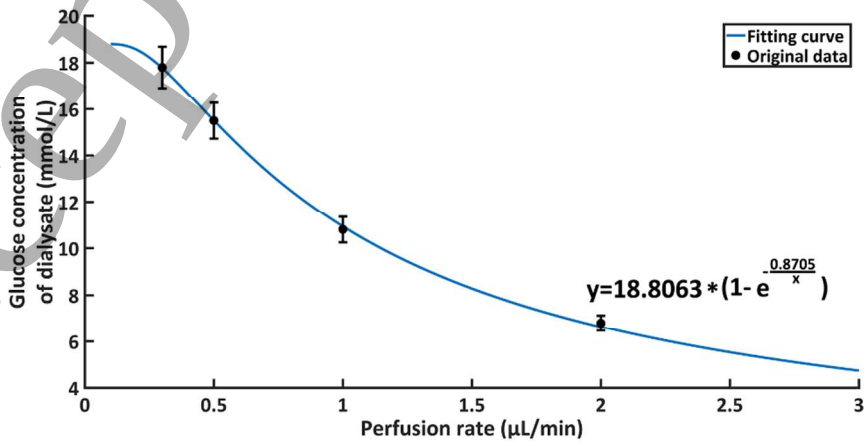

Figure 9. Fitting curve of dialysate glucose concentration versus perfusion rate

5.2 Measurement of in vivo recovery using the no-net-flux method

No-net-flux method is performed by introducing a series of perfusate with different glucose concentrations to the probe surrounding area via a microdialysis probe, and then, collecting and measuring the glucose concentration of the dialysate [24]. In the experiments, glucose solutions with the concentrations of 2.8, 5.4, 8.2, and 10.3mmol/L were adopted as the perfusates for microdialysis with a perfusion rate of 1.0μL/min. The dialysate glucose concentration was measured every 2min from 3min to 21min. For each perfusate, the dialysate glucose concentrations measured between 11min and 21min were recorded and averaged. The average dialysate glucose concentrations and the corresponding glucose concentrations of perfusate are presented in table 2.

Table 2. Average glucose concentrations of dialysate corresponding to different perfusate glucose concentrations (GC)

GC of perfusate (mmol/L)	Average GC of dialysate (mmol/L)
2.8	8.7
5.4	9.4
8.2	10.2
10.3	11.7

The net difference of glucose concentrations between the dialysate and the perfusate was calculated. And the linear fit of the net difference versus perfusate glucose concentration is shown in figure 10. The point at which the line intersects x-axis corresponds to the no-net-flux point and occurs at the perfusate glucose concentration of 12.1mmol/L. That means, the theoretical average interstitial glucose concentration in the testing process is 12.1mmol/L. The slope of the line is -0.6178, suggesting that the average recovery is 61.8%. The uncertainty of slope ranged from -0.9075 to -0.3282 at the 95% confidence level. And the uncertainty of y-intercept term ranged from 5.349 to 9.549 at the 95% confidence level.

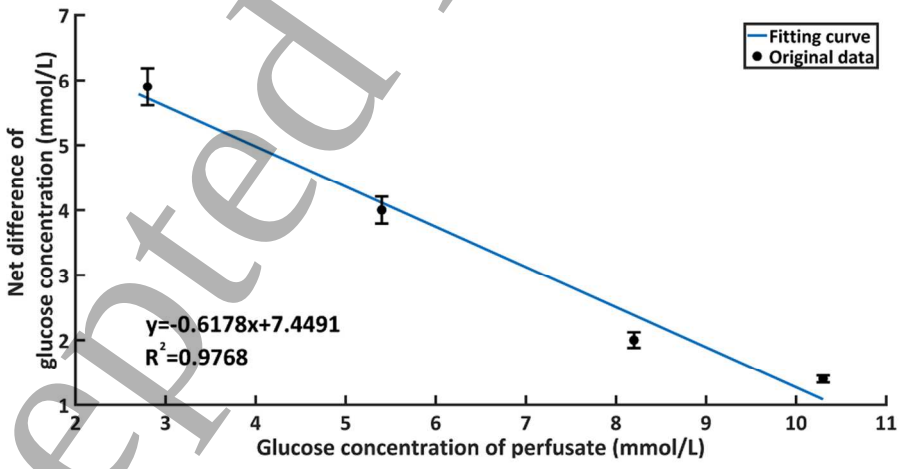

Figure 10. Linear fit of net glucose concentration difference versus perfusate glucose concentration

5.3 Selection of microdialysis calibration method

As described above, two recovery values were obtained using the low-flow-rate and no-net-flux methods. To verify the accuracies of the recovery values measured using these methods, continuous monitoring experiments based on microdialysis were carried out. The two previously obtained recovery values were used to calibrate the measurement results. The calibrated results were compared with the interstitial glucose concentrations detected using ultrafiltration to explore which method could yield more accurate recovery values.

Ultrafiltration is a simple and convenient in vivo sampling technique that can be performed easily and sustainably [28-30]. Ultrafiltration extracts the interstitial fluid by employing the negative pressure provided by a vacuum, which does not dilute the interstitial fluid and therefore can be used to obtain the interstitial glucose concentration directly. However, it is difficult to operate on human body due to the ultrafiltration probe is too long, but rather only in animal testing [31-33]. Hence, ultrafiltration cannot be used in experimental evaluations of non-invasive and minimally invasive blood glucose monitoring techniques in the human body.

In the experiments, Ringer's solution without glucose was adopted as the perfusate with a perfusion rate of 1.0μL/min. The glucose concentrations of dialysate and ultrafiltration-extracted solution were measured every 5min. In the ultrafiltration process, the sample was extracted using a KDS200 microsyringe pump (KDScientific, USA), a 100μL injection syringe (Hamilton, Switzerland) and an uf 3-12 ultrafiltration probe (BASi, USA). To get the negative pressure for ultrafiltration, the pulling speed and pulling distance of microsyringe pump was set at 200μL/min and 100mm, respectively. The measured glucose concentrations of dialysate were calibrated using the two recovery values mentioned above, and compared with the glucose concentrations of ultrafitrate, as shown in table 3.

Table 3 demonstrates that the glucose concentrations calibrated using the low-flow-rate method have smaller errors. Specifically, the errors of glucose concentrations obtained using the low-flow-rate method have a 77.8% probability of falling within ±6%. The negative bias in relative errors for the NNF method may due to the underestimate of the microdialysis recovery which was causing by using lower perfusate glucose concentration than the interstitial glucose concentration in the tests. The fact that the relative errors of glucose concentrations calibrated using the no-net-flux method are larger can potentially be explained by the fact that injecting a series of perfusate with different glucose concentrations is likely to induce changes to the original glucose concentration at the sampling site which causes inconsistency. Thus, low-flow-rate method is chosen as the microdialysis calibration method in this paper.

Table 3. Comparison results of the two microdialysis calibration methods
(GC: Glucose Concentration, LFR: Low-flow-rate, NNF: No-net-flux)

Time (min)	GC of ultrafiltrate (mmol/L)	GC of dialysate (mmol/L)	GC calibrated by LFR method (mmol/L)	Relative error (LFR method)	GC calibrated by NNF method (mmol/L)	Relative error (NNF method)
10	15.8	8.3	14.98	-5.18%	13.43	-15.00%
15	15.1	8.8	15.88	5.20%	14.24	-5.70%
20	16.2	8.7	15.70	-3.06%	14.08	-13.10%
25	15.7	9.3	16.79	6.92%	15.05	-4.15%
30	15.3	9.3	16.79	9.72%	15.05	-1.64%
35	15.2	8.9	16.06	5.69%	14.40	-5.25%
40	16.0	9.2	16.61	3.79%	14.89	-6.96%
45	15.8	8.6	15.52	-1.75%	13.92	-11.92%
50	16.1	9.2	16.61	3.15%	14.89	-7.54%
			Average absolute error	4.94%	Average absolute error	7.92%

5.4 Evaluation of microdialysis-based interstitial glucose concentration monitoring

In order to further verify the feasibility of utilizing the microdialysis method for interstitial glucose concentration monitoring, a microdialysis probe and an ultrafiltration probe were implanted into a rabbit to monitor the variation of

interstitial glucose concentration causing by oral glucose intake and insulin injection (as shown in figure 11). And the accuracy of microdialysis-based interstitial glucose concentration measurement was evaluated by comparing with the measurement results of ultrafiltration.

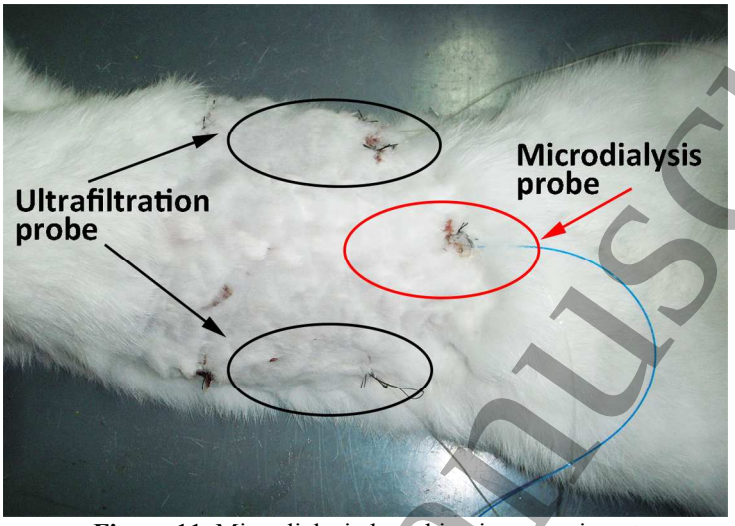

Figure 11. Microdialysis-based in vivo experiment

At the beginning of the experiment, microdialysis was conducted with the perfusion rate of 1.0μL/min. At the same time, the interstitial fluid was extracted using ultrafiltration. Glucose concentration measurements were performed every 10min. After 100min, the rabbit in fasting state was fed with 3g/kg of glucose. Then the rabbit was injected with 1U/kg of insulin after 200min. The glucose concentrations of dialysate were calibrated using the in vivo recovery obtained via the low-flow-rate method to generate an interstitial glucose concentration calibration curve, which was compared with the interstitial glucose concentration curve acquired by ultrafiltration, as shown in figure 12.

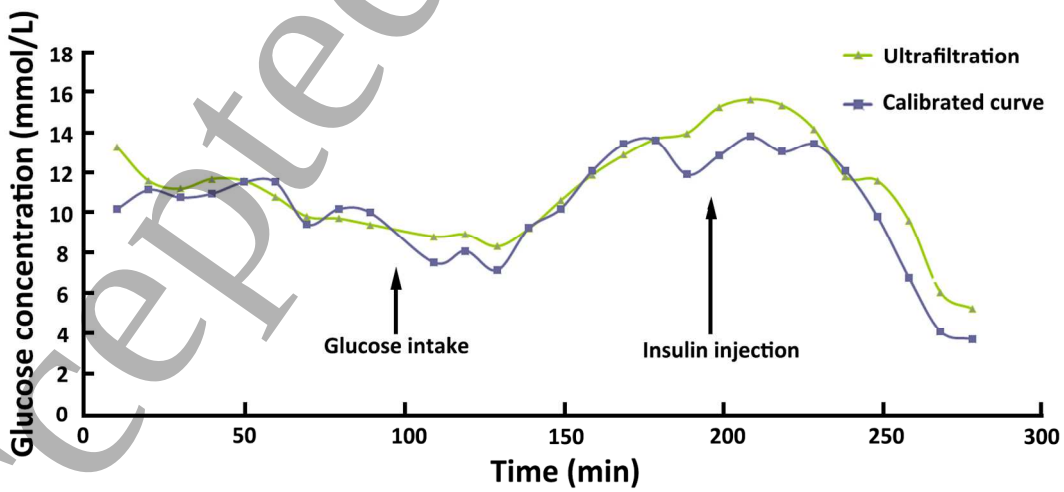

Figure 12. Interstitial glucose concentration curve

Figure 12 demonstrates that the calibrated microdialysis curve agrees closely with the ultrafiltration curve. 85.2% of the deviations between the calibrated glucose concentrations and the actual interstitial glucose concentrations fall within ±16%. And the standard error was obtained by the following formula

$$SE=\sqrt{\frac{\sum(x-y)^2}{N}} \qquad (4)$$

where x and y were the interstitial glucose concentrations acquired by ultrafiltration and calibration, respectively. And N was the total measurement times. The standard error was 1.4mmol/L. And the average percentage deviation is 10.4%. The calibrated glucose concentrations well represent the actual interstitial glucose concentrations. The accuracy of the microdialysis method proposed in this paper for in vivo interstitial glucose concentration monitoring was validated. This method is able to provide reference values of glucose concentration in interstitial fluid for accuracy evaluation of non-invasive and minimally invasive continuous glucose monitoring.

6. Conclusions

To address the worldwide challenge of developing a high-accuracy interstitial glucose concentration measurement method to provide reference values for accuracy evaluation of continuous glucose monitoring system based on interstitial glucose detection, the microdialysis-based method was reported in this paper. After parameter optimization of the microdialysis method, in vitro tests were carried out in the simulation system of glucose concentration variations in human body. 93.8% of the deviations between the calibrated glucose concentrations of dialysate and directly measured glucose concentrations are less than ±10%. And the average deviation is only 3.7%. Furthermore, the microdialysis-based interstitial glucose concentration measurements were performed on a rabbit. And the glucose monitoring results were evaluated by comparing with the ultrafiltration measurement results. The results indicate that the calibrated glucose concentrations and the actual interstitial glucose concentrations are in a good consistency. Therefore, the high-accuracy microdialysis method can provide reference values for evaluating the non-invasive and minimally invasive continuous glucose monitoring system. The accuracy evaluation of continuous glucose monitoring system by using the microdialysis-based interstitial glucose concentration measurement method is still ongoing.

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