35th Annual International Conference of the IEEE EMBS
Osaka, Japan, 3 - 7 July, 2013

Measurement of Subcutaneous Biological Substances Using Thin Metal Needle with Micro Flow Channel

N. Tsuruoka, K. Ishii, T. Matsunaga, R. Nagatomi, and Y. Haga

Abstract— **Concentrations of biological substances are useful as indicators of physiological and pathological states. In order to monitor biological substances in daily life, we developed a minimally invasive needle type device with which biological substances are extracted through a microperfusion system inserted under the skin. The perfusion needle has a flow channel with perforated membrane through which biological substances from subepidermal tissue are extracted. The efficacy of the device was examined by measuring lactate concentration of exercising mice. Lactate was successfully collected from the back skin of the mice running on a treadmill using a fabricated microperfusion needle. Lactate concentration of perfused solution correlated with blood lactate concentration.**

I. INTRODUCTION

Quantification of biological substances in blood samples is a widely used routine in clinics and hospitals as well as in medical checkup occasions, in order to assess health conditions. Blood collection procedure, however, may accompany unfavorable incidences such as pain or accidental infection. Non-invasive and minimally invasive measurements of biological substance were developed as an alternative to blood sampling. For example, concentrations of biological substances in the sweat have been to some extent successful but only for limited substances. Measuring concentration of biological substances in subcutaneous tissue fluid is one of the minimally invasive methods in focus.

An enzymatic electrode needle type sensor has recently been commercialized for glucose monitoring [1]. This sensor utlilizes needle type enzymatic electrode.. Glucose concentration of subcutaneous tissue fluid can be obtained by inserting a minimally invasive needle for more than a day.. Furthermore, this method enables measurement of substances other than glucose, such as lactate, and uric acid that can be quantitated using enzymatic reaction [1, 2]. This sensor, however, involves an insertion of an enzyme coated needle sensor in the subcutaneous tissue. The protein enzyme may not only elicit allergic reaction but also generate highly invasive hydrogen oxide once released into the tissue. Therefore, avoiding protein enzyme introduction in the tissue is a better strategy.

Microperfusion is a sampling technique for measuring concentrations of biological substances in the extracellular fluid. It separates biological substances from extracellular fluid by controlling the mass transfer rate of biological substances. Since microperfusion carries biological substances out of the body, biological substance sensors like enzymatic electrode can be placed at out of the skin. Thus, this method is safer than insertion of needle type enzymatic electrode.

In a recent study, we developed a microperfusion needle that has enough rigidity for inserting through epidermis [3]. In previous works, flat needle [4] and cylindrical soft probe [5] were developed for collecting biological substances. However, they could not be inserted through epidermis because of insufficient rigidity. Our device has enough rigidity for inserting through the skin by using acupuncture needles. A flow channel with a perfusion membrane was fabricated on the surface of a thin steel acupuncture needle (outer diameter= 200 μm). This steel acupuncture needle of around 200 μm in diameter can be safely inserted in the subepidermal tissue without pain or bleeding.

The purpose of this study was to confirm whether the fabricated microperfusion needle can effectively collect biological substances from subcutaneous tissue. In this study, the concentration of lactate, one of the indicators of exercise performance was measured. Continuous monitoring of lactate concentration may effectively indicate the onset of anaerobic metabolism, by which one may effectively adjust his training volume and intensity. In this paper, an animal experiment using mice was performed, and lactate was successfully collected from subcutaneous tissue using this needle.

II. MATERIALS AND METHODS

A. Animals

Five male C57BL/6 mice were purchased from Nihon Clea (Tokyo, Japan), were kept at the Institute for Animal Experimentation, Tohoku University Graduate School of Medicine. The purchased mice were allowed to acclimatize to the facility for at least 1 week before the experiment, and they were used at 10-13 weeks of age. Five mice were housed together in a cage (30 cm × 25 cm × 17.5 cm) and were allowed free access to food and water. Animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The Committee for Animal Experimentation of the Graduate School of Medicine, Tohoku University, approved the experimental procedures describe herein.

N. Tsuruoka, R. Nagatomi, and Y. Haga are with the graduate school of Biomedical Engineering, Tohoku University, Sendai, Japan (phone: #81-22-795-5251; fax: #81-22-795-5253; e-mail: noriko.tsuruoka@bme.tohoku.ac.jp).

K. Ishii is with the graduate school of engineering, Tohoku University, Sendai, Japan.

T. Matsunaga is with Micro System Integration Center (μSIC), Tohoku University, Sendai, Japan

B. *Microperfusion Needle*

A schematic view of the microperfusion needle is given in figure 1. This needle was fabricated using a non-planer photofabrication process [6]. An acupuncture needle (diameter: 200 μm, Seirin Co., Ltd. Japan) was used as a base material. Polyimide was electrodeposited on the surface of the needle up to 30 μm of thickness, and flow channel (width: 50 μm) was fabricated with laser ablation (wave length: 533 nm). After a flow channel was patterned, Cu was electroplated as a sacrificial layer. A 10 μm thick polyimide layer was electrodeposited on the Cu sacrificial layer and 10 μm-diameter holes on the membrane were ablated with the laser. Finally, the Cu sacrificial layer was etched out by 40 % HNO_3.

Polyethylene tubes (inner diameter: 200 μm, outer diameter: 500 μm, Natsume Seisakusyo co., Ltd. Japan) were connected to the inlet and outlet of the flow channel using a fixture made with silicone. Photograph of this needle is shown in figure 2.

The setup of the perfusion system in for the animal experiment is shown in figure 3. After the back fur was shaved using a fur-removing cream, the mice were anesthetized with Sevoflurane (1,1,1,3,3,3-hexafluoro-2- (fluoromethoxy) propane, SEVOFRANE®, Maruishi Pharmaceutical. co., ltd.). A microperfusion needle was inserted into the back skin and fixed by surgical suture. The end of the inlet tube was connected to a syringe pump and perfused with the dialysate (saline) at a flow late of 5 μl/min. The end of the outlet tube was connected to a syringe and fluid was suctioned at the same volume rate as the inlet syringe pump.

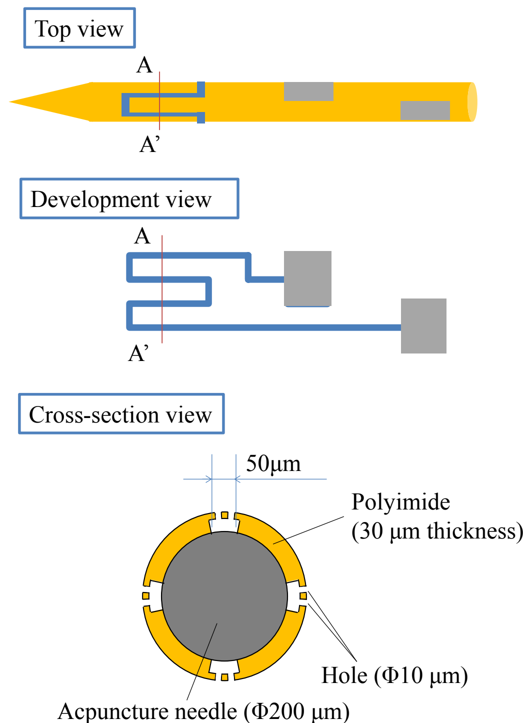


Figure 1: Schematic view of microperfusion needle.

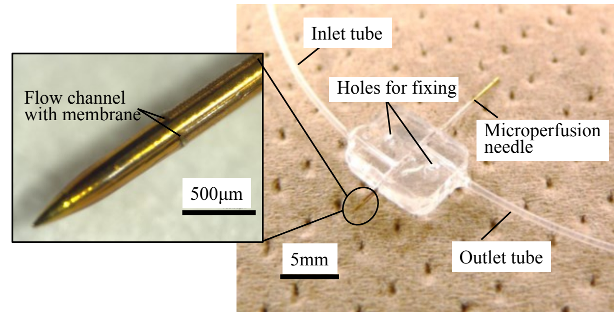


Figure 2: Photograph of fabricated microperfusion needle.

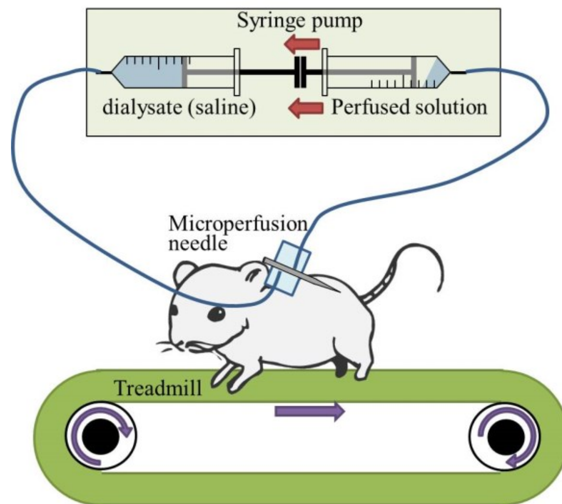


Figure 3: Setup of animal experiment.

C. *Lactate Concentration of Perfused Solution*

Lactate concentration of perfused solutions was measured using a fluorescent stain kit (L-Lactate Assay Kit, Cayman Chemical Company, USA). Fluorometric determination was performed using a fluorescence microplate reader (Fluoroscan Ascent®, Thermo Fisher Scientific K.K.). Duplicate measurement was performed for each sample and the measured result was averaged in order to minimize handling errors.

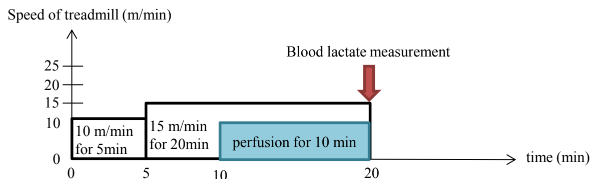
D. *Blood Lactate Measurement*

Blood samples used for the determination of lactate concentration were collected from lateral tail vein. Lactate concentrations of the blood samples were measured using a simplified blood lactate test meter (Lactate Pro, ARKRAY, Inc. Japan).

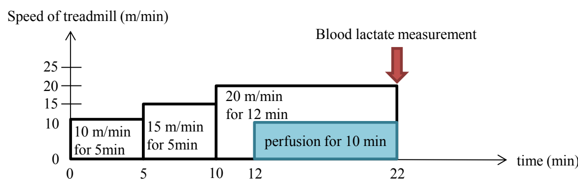
E. *Protocols*

The protocol of this study was shown in figure 4. After the fabricated needle was fixed on mouse's back, mouse was placed on a treadmill (KN-73 RM-5, Natsume Seisakusyo co., Ltd. Japan). Each mouse ran on a separate lane (45 x 12 x 9 cm) of the treadmill at 10 m/min. Running speed was gradually increased by 5 m every 5 min until the target speed.

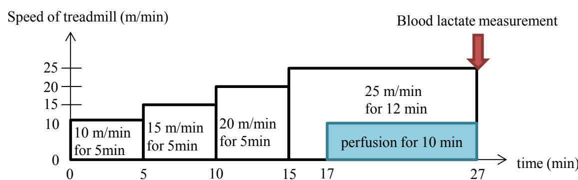
The target speed was maintained until the exhaustion of mice.. Target speeds were 15, 20, and 25 m/min. Nine mice ran at each target speeds. Dialysate (saline) perfusion was started before running to prevent plugging or clotting. Two minutes after fixing the target running speed, perfused saline was collected into the outlet syringe for 10 minutes. After exercise, blood samples were collected, and lactate concentration was measured.



(a) Target speed = 15 m/min



(b) Target speed = 20 m/min



(c) Target speed = 25 m/min

Figure 4: Protocol of animal experiment.

F. Statistics

One-way ANOVA was performed to examine the relationsip between target speed and blood lactate concentration.

Pearson's correlation test was performed to examine the association of lactate concentration of perfused solution and the lactate concentration of blood sample.

The recovery rate (Recovery) was calculated using the following equation.

$$\text{Recovery} = \frac{C_p}{C_b} \quad (1)$$

C_p is the lactate concentration in the perfused solution, C_b is the blood lactate concentration. Recovery rate is presented as mean ± SD.

The JMP® Pro statistical software, version 9.0.2 (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

III. RESULTS

The relationship between the target speed and the lactate concentration of blood is shown in figure 5. There was no correlation between the target speed and the blood lactate concentration.

The relationship beween the blood lactate concentration and the perfused solution is shown in figure 6. Lactate concentration of perfused solution was significantly correlated ($P < 0.05$) with blood lactate concentrations.

The recovery rate of lactate was about 1.41 ± 0.82 % (mean ± SD).

No apparent damage of the fabricated needle was detected after the animal experiment. No apparent bleeding or infection of the back skin of the mice was detected after the experiment.

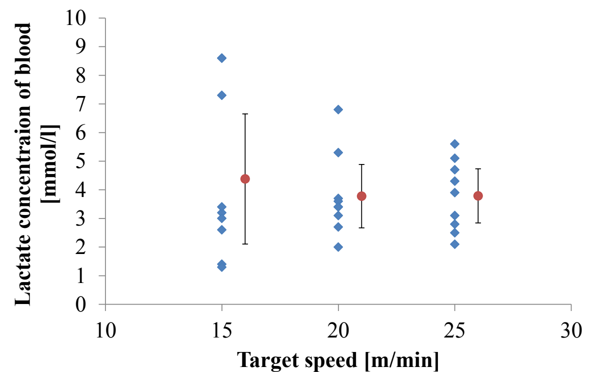


Figure 5: Relation between target speed and lactate concentration of blood. Solid Line is 95 % CI.

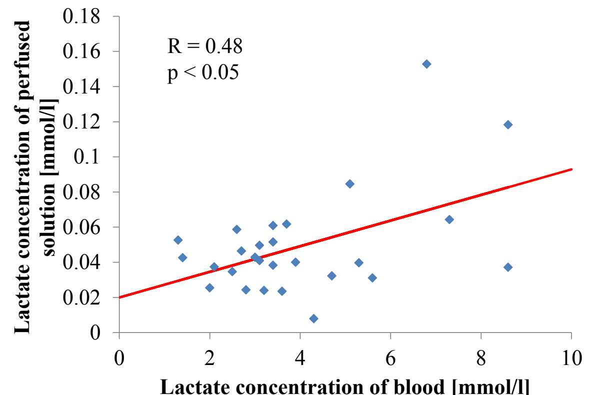


Figure 6: Relation between lactate concentration of blood and perfused solution.

IV. DISCUSSION

There was no correlation between the target speed and blood lactate concentration. Outlier points were found at higher target speeds of 15 m/min and 20 m/min. Since lactate concentration may increase under stress conditions including anesthesia, handling after needle insertion may have elicited stress responses. Since the lag time between needle insertion

and lactate extraction by microperfusion may be long, the increase of subcutaneous lactate was only detected only at later sessions at target speed of 25 m/min. Long running time at 10 m/min (warm-up exercise), for 15 min or more, may have beeb able to minimize the influence of anesthesia during collection of perfused solutions. Without theses outlier points lactate concentration of blood seems to increase as the target speed was increased. Nevertheless, lactate concentration of perfused solution correlated with blood,lactate concentration suggesting that bloodless lactate measurement using the novel microperfusion needle is a valid minimally invasive method to measure biological substances.

Coefficient of correlation (R=0.48) of lactate concentration of blood and perfused solution, however, was not high. There are three possible factors of variance. The first is the long collecting time of perfused solutions. In this experiment, lactate concentration of perfused solution was averaged because accumulated lactate within the collection time was measured (for 10 min). On the other hand, blood sample may only reflect circulating lactate concentration at the sampling point. If lactate concentration was to increase gradually during the collection of perfused solution, averaged lactate concentration in the perfused sample should be lower because of dilution with extracellular fluid collected before exercise and during exercise at lower speed which does not elicit lactate production. Thus, this method may not be appropriate for detecting a quick change of biological substances.

The second is the relationship between perfusion rate and diffusion rate of lactate in the subcutaneous extracellular fluid., Collection effeciency of lactate dependends on the balance between the diffusion speed of lactate ion and the perfusion speed. The present data suggest the necessity of further adjustment of perfusion rate to optimize the sample collection..

The third is the error of blood collecting. If collected blood volume from mouse's tail was not sufficient for lactate concentration measurement by simplified blood lactate test meter, measurement result of blood lactate concentration will be lower value than actual value.

The recovery rate of lactate was about 1.41 %. Ideal recovery rate is 100 % to measure small changes in lactate concentration. However, if the sensitivity of biological substance sensor is high, recovery rate of 1.41 % may be sufficient. It was shown that lactate concentration of subepidermal tissue fluid is similar to that of the blood sample [7]. Faster flow rate exceeding the diffusion rate may, compromise the recovery rate of lactate.

There was no damage in the microperfusion needle and mouse skin of the insertion site. This shows our device can be used in safety even under heavy exercise.

V. Conclusion

Minimally invasive microperfusion needle was fabricated to measure concentration of biological substances in subepidermal tissue. Lactate was collected using this method, and compared to blood lactate concentration. There was correlation between blood lactate concentration and lactate concentration of perfused solution. This result shows the possibility of bloodless measurement of biological substance using our developed needle. In the future, we are planning to perform continuous monitoring of biological substances using our fabricated needle combined with on-line biological substance sensor. In addition, we are planning to measure other biological substance, and optimize perfusion method (flow rate, length of flow channel, and so on). Finally, we will develop wearable system by miniaturization of whole microperfusion system including on-line biological substance sensor.

Acknowledgment

This work was supported by Program for Revitalization Promotion (A-STEP; Seeds actualization type: 241FS0116) from the Japan Science and Technology Agency (JST).

References

[1] T. M. Gross, B. W. Bode, D. Einhorn, D. M. Kayne, J. H. reed, N. H. White, and J. J. Mastrototaro, "Performance Evaluation of the MiniMed® Continuous Glucose Monitoring System During Patient Home Use," *Diabetes Thechnol. Ther*, vol. 2, 2000, pp. 49-56.

[2] Q. Yang, P. Atanasov, and E. Wilkins, "An Integrated Needle-Type Biosensor for Intravascular Glucose and Lactate Monitoring," *Electroanalysis*, vol. 10, No. 11, 1998, pp. 752-757.

[3] N. Tsuruoka, K. Ishii, T. Matsunaga, R. Nagatomi, and Y. Haga, "Development of Minimally Invasive Microdialysis Needle for Continuous Monitoring of Biological Substances," · *The 25th International Conference on Micro Electro Mechanical Systems* , Paris, FRANCE, 29 January - 2 February, 2012.

[4] J. D. Zahn, D. Trebotich, D. Liepmann, "Microdialysis Microneedles for Continuous Medical Monitoring," *Microdevices*, vol. 7, 2005, pp. 59-69.

[5] M. Ellemerer, L. Schaupp, Z. Trajanoski, G. Jobst, I. Moser, G. Urban, F. Skrabal, P. Wach, "Continuous measurement of subcutaneous lactate concentration during exercise by combining open-flow microperfusion and thin-film lactate sensors," *Biosens. Bioelectron*, vol. 13, 1998, pp. 1007-1013.

[6] S. Goto, T. Matsunaga, J. J. Chen, W. Makishi, M. Esashi, Y. Haga, "Fabrication Techniques for Multilayer Metalization and Patterning, and Surface Mounting of Components on Cylindrical Substrates for Tube-Shaped Micro-Tools," *in Proceedings of 2006 International Conference on Microtechnologies in Medicine and Biology*, Okinawa, May, 2006, pp. 297-300.

[7] N. Ito, T. Matsumoto, H. Fujiwara, Y. Matsumoto, S. Kayashima, T. Arai, M. Kikuchi, I. Karube, "Transcutaneous lactate monitoring based on a micro-planar amperometric biosensor," *Analytica Chimica Acta* , Vol. 312, 1995, pp. 323-328.