

Needle biosensor for continuous blood glucose monitoring in flatfish

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Cultured flatfish are bred in aquafarms in a high-density environment. The resulting poor water quality and various physical stresses may lead to mass outbreaks of infectious disease. Variations of blood glucose levels may represent stress in fish (1, 2). Thus, measurement of blood glucose levels is very important for managing fish health. Hemanalysis methods have been developed for cultured fish, and enzyme sensor systems have been developed for the determination of glucose. Our aim is to develop a method of continuously monitoring blood glucose levels in the flatfish *Paralichthys olivaceus*. As previous study, an enzyme sensor, comprising a needle-type hollow container, an immobilized enzyme membrane, and a fiber optic probe coated with ruthenium complexes that produce fluorescence depending on the dissolved oxygen content, was designed for measuring blood glucose levels in fish (3). This system provides rapid and convenient measurements of blood glucose levels. However, measuring blood components in real-time is difficult because sensor output decreases over time due to blood coagulation and protein coalescing on the sensor. In this study, we hypothesized that glucose levels in the fluid-filled inner eyeball sclera (EISF) reflect blood glucose levels. Thus, we designed a novel enzyme sensor system to implant into EISF for estimating blood glucose continuously in fish.

A correlation between blood and EISF glucose levels was investigated. The fish were netted from the preserve and anesthetized by bath exposure. Blood was then sampled from the caudal vein using a heparinized syringe fitted with a needle. EISF samples were obtained from the ISF of the eyeball sclera using syringe fitted with a needle. Glucose levels in these samples were determined using an enzymatic colorimetric method. For continuous EISF glucose monitoring in flatfish, a needle-type enzyme sensor was prepared. A working electrode was made using a 15-mm length of Teflon-coated platinum iridium wire. The Teflon was stripped at one end to expose 1.0 mm of the Pt-Ir wire as a sensing cavity. Copper wire was wrapped around the Teflon coated surface. Ag/AgCl paste was used as a reference electrode/counter electrode. The tip of the wire was sealed with epoxy resin leaving a 0.7-mm long sensing cavity. The working electrode dipped in the nafion dispersion solution and enzyme solution which consists of BSA, glucose oxidase (GOD), phosphate buffer solution. GOD was immobilized by vaporized glutaraldehyde. The sensor was inserted into the ISF of the eyeball sclera for sensor implantation. A 650-mV potential (vs. Ag/AgCl) was applied by the potentiostat to the Pt-Ir working electrode for the amperometric glucose measurement. Continuous blood glucose monitoring was performed under light anesthesia to monitor blood glucose levels.

We investigated the correlation between blood and

EISF glucose levels (Fig. 1). A good correlation was observed between blood and EISF glucose levels ($y = 3.2652 + 0.77279x$, $R = 0.8456$, $n = 19$). It was clear that a variation of blood glucose levels reflected in EISF glucose levels. Using prepared needle-type glucose sensor, the continuous glucose monitoring was carried out by implanting the sensor into eye site. Figure 2 shows Continuous blood glucose monitoring under light anesthesia. The sensor output current was stable in 60 minutes. The study confirms the glucose levels increase with sensor output current over time. Thus, blood glucose change was reflected in EISF continuously. It was possible to monitor for 120 min. When blood glucose levels were estimated by one point calibration method (4), a good correlation was observed between the sensor estimated glucose levels and actual blood glucose levels. ($y = 0.2835 + 0.8588x$, $R = 0.8171$)

REFERENCES

1. J.W. Hur, I.S. Park, Y.J. Chang, *Ichthyol. Res.*, 54, 32-37 (2007)
2. H. Ishioka, *Bull. Nansei Natl. Fish. Res. Inst.*, 16, 63-71 (1984)
3. H. Endo, Y. Yonemori, K. Musiya, M. Maita, T. Shibuya, H. Ren, T. Hayashi, K. Mitsubayashi, *Anal. Chim. Acta*, 573-574, 117-124 (2006)
4. C. Choleau, J. C. Klein, G. Reach, B. Aussedat, V. Demaria-Pesce, G. S. Wilson, R. Gifford, W.K. Ward, *Biosens. Bioelectron.*, 17, 647-654 (2002)

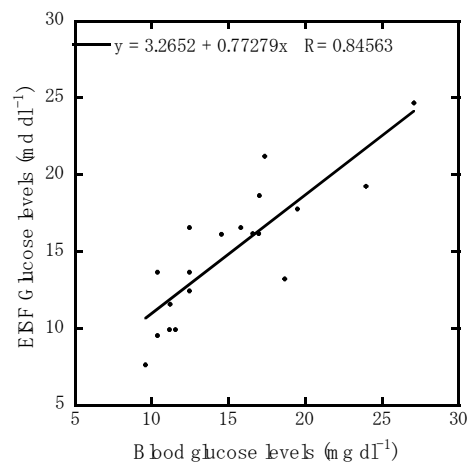


Fig. 1 Correlation between blood and EISF glucose levels (n=19)

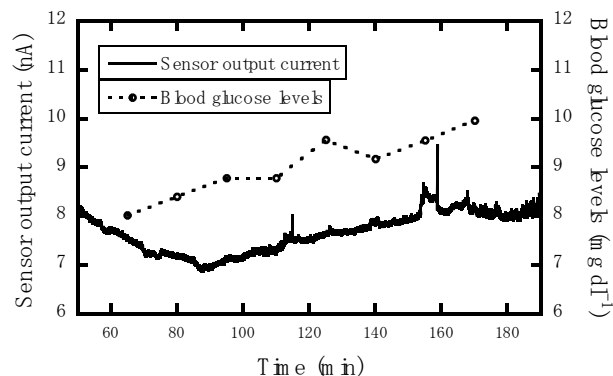


Fig. 2 Continuous blood glucose monitoring in the flatfish.