PEG BONDED FLUORESCENT-HYDROGEL FIBERS WITH LESS INFLAMMATION FOR LONG-TERM SUBCUTANEOUS GLUCOSE MONITORING

Y. J. Heo[1,2*], M. Takahashi[2,3], H. Shibata[2,3], T. Okitsu[1,2], T. Kawanishi[2,3] and S. Takeuchi[1,2]

[1]Institute of Industrial Science, The University of Tokyo, JAPAN,
[2]Life BEANS Center, JAPAN and [3]TERUMO Co., JAPAN

ABSTRACT

We present polyethylene glycol (PEG) bonded poly-acrylamide (PAM) hydrogel fibers with glucose-responsive monomer to monitor blood glucose concentration in vivo for over 4 months. To realize long-term subcutaneous glucose monitoring, we employed fiber-structure and PEG-bonded PAM hydrogel. The fiber-shaped sensors can be injected to subcutaneous tissues, remain in an implantation site and be easily removed after use. PEG can enhance biocompatibility, resulting in maintaining the functionality of implanted sensors in vivo for a long-period. The implanted PEG-bonded PAM fibers showed less inflammation, glowed through the skin and continuously responded to blood glucose concentration, thereby promising practical, long-term subcutaneous glucose monitoring.

KEYWORDS: glucose-responsive fluorescence, transdermal detection, biocompatible interface, long-term *in vivo* glucose monitoring

INTRODUCTION

Subcutaneous glucose monitoring facilitates intensive control of blood glucose concentration for diabetic patients and effectively prevents diabetic complications [1]. Fluorescence-based sensors hold great potential of minimally-invasive, long-term subcutaneous glucose monitoring due to wireless transmission and long-lasting activity in vivo [2]. Long-term subcutaneous glucose monitoring can reduce frequency of sensor implantation and replacement, thereby bringing the technology to practical use. However, fluorescence-based sensors (primarily fluorescent microbeads) have yet realized the full potential of long-term subcutaneous glucose monitoring because the sensors cannot stay in the implantation site and respond to blood glucose concentration for a long-period.

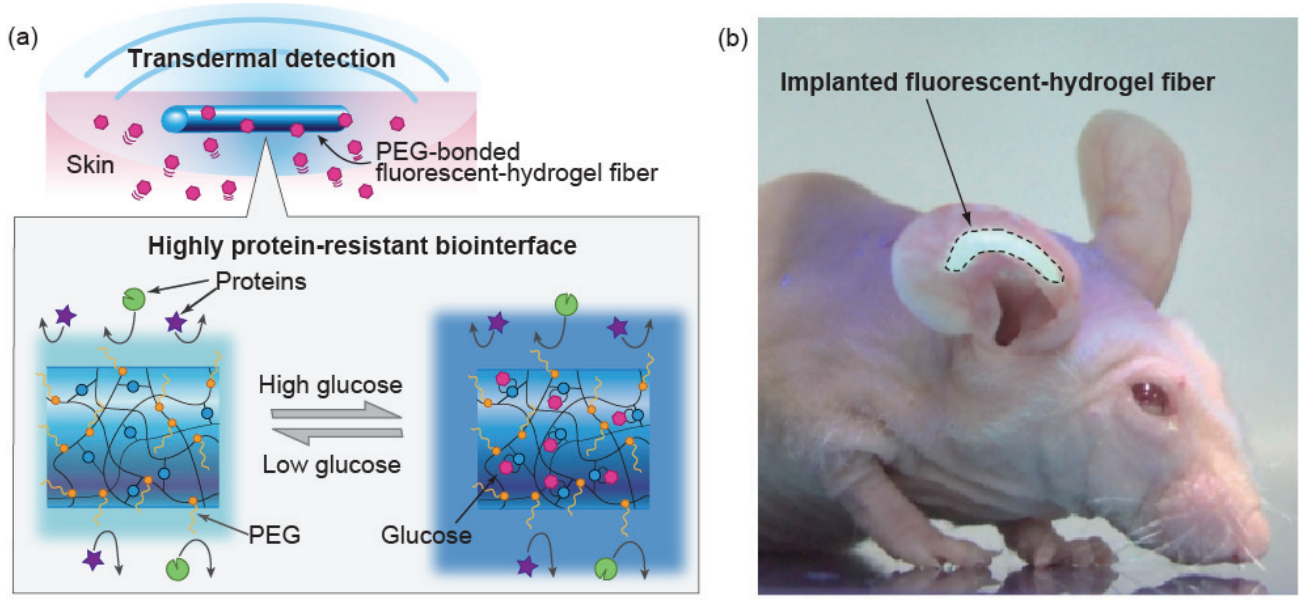

Figure 1: (a) Conceptual illustration of the PEG-bonded fluorescent-hydrogel fiber for long-term subcutaneous glucose monitoring. The fluorescent signal in response to blood glucose concentrations transmits across the skin, thereby detecting blood glucose concentration in a minimally-invasive manner. Fiber-shape can stay in the implantation site for a long-period and PEG-bonded PAM has highly biocompatible interface with tissues; the PEG-bonded PAM hydrogel is highly resistant to protein adsorption, thereby reducing inflammation. As a result, the PEG-bonded fluorescent-hydrogel fiber can be applied to long-term in vivo glucose monitoring. (b) Implanted fluorescent-hydrogel fiber in mouse ear. The mouse ear is excited by ultraviolet light. The fluorescence intensity is sufficiently high to be detected through the skin.

Here, we present PEG-bonded fluorescent-hydrogel fibers for long-term subcutaneous glucose monitoring (Fig. 1). Our approach is two: sensor structure and material. First, we employed fiber-structure. Fiber-structure provides increased contact area with subcutaneous tissue; the increased contact area decreases the mobility of the subcutaneous implants. Second, we employed PEG-bonded PAM fibers to enhance biocompatibility. PEG is well-known material that reduces inflammation [3]. As a result, PEG facilitates the functionality of implanted sensors for a long-period.

EXPERIMENTAL

We immobilized the glucose-responsive monomer [4] in hydrogel fibers that had been obtained by polymerizing a pre-gel solution with acrylamide monomer (15%), Acryl-PEG (5%), polymerization initiator and accelerator in polyolefin microcapillaries of 1,000 μm in diameter (Fig. 2a). The fluorescent image showed that the glucose-responsive monomer was immobilized within the hydrogel fibers having a diameter of 956±9 μm (Fig. 2b).

To test the effect of PEG on biocompatibility of the fibers, we implanted PEG-bonded PAM hydrogel fibers into 4 mice and PAM hydrogel fibers into another 4 mice. We evaluated inflammation based on the color change (reddening), swelling, and scabbing of the ears. If there is reddening, then the reddening scores 1. Similarly, if there are swelling and scabbing, swelling and scabbing score 1, respectively.

We demonstrated the fluorescence intensity of the implanted fibers in response to blood glucose concentration of mice by glucose challenge. We injected glucose to temporarily elevate glucose concentrations within the hyperglycemic range (over 300 $mg{\cdot}dL^{-1}$) and insulin to decrease glucose concentrations within the euglycemic and hypoglycemic ranges (under 140 $mg{\cdot}dL^{-1}$). Blood glucose concentrations were measured with a blood glucose monitoring sensor (Accu-Chek, Roche) using a blood sample from the snipped tail. The fluorescence intensity was estimated from fluorescent images of mouse ears.

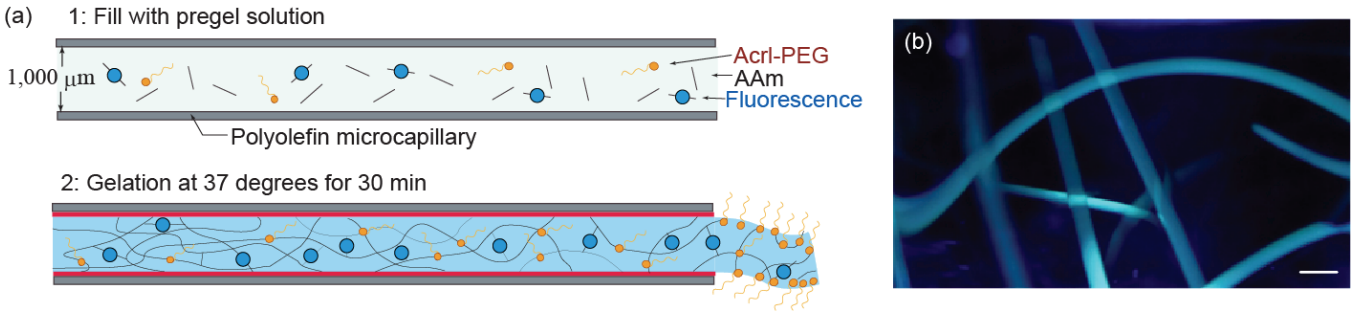

Figure 2: (a) Fabrication process of the PEG-bonded fluorescent-hydrogel fiber in a microcapillary. (b) Fabricated fluorescent-hydrogel fibers. The fibers emitted fluorescence of 488 nm excited by ultraviolet light. Scale bar indicates 2 mm.

RESULTS AND DISCUSSION

In experimental study, we verified our hypothesis that fiber-shape can stay in the implantation site for longer period compared to microbeads. The implanted fibers remained in the implantation site for a month, while the implanted microbeads dispersed and disappeared from the implantation site. We also tested PEG effects on inflammation and transdermal detection for long-term implants. We implanted the PEG-bonded PAM fibers (n=4) and PAM fibers (n=4) in mouse ears and inspected the mice ears for 4 weeks. As a result, the mouse ears with the PEG-bonded PAM fibers showed less inflammation and fast recovery from inflammation compared to the mouse ears with the PAM fibers (Fig. 3a). The PEG bonded PAM fibers glowed through the skin for an entire 4 weeks, while the PAAm fibers did not (Fig. 3b). Thus, PEG-bonded PAM hydrogel could reduce inflammatory process, resulting in maintaining the functionality of the implanted sensors.

We tested glucose-responsiveness in vivo after implantation in response to an intraperitoneal glucose challenge. The fluorescence intensity of the hydrogel fiber increased as blood glucose concentration increased (Fig. 4a). The hydrogel fiber also responded to blood glucose concentration even after over 4 months (Fig. 4b). The responses of the hydrogel fibers showed time lag of 10 min behind blood glucose concentration. The hydrogel fibers responded to glucose concentration of interstitial fluid (ISF); glucose concentration of ISF lags 10−30 min behind blood glucose concentration. Consequently, the time lag between the response of the implanted fibers and blood glucose concentration was mainly originated from the time lag between glucose concentrations of ISF and blood.

Furthermore, we found that PEG-bonded PAM hydrogel is effective for long-term monitoring. The 3 PEG-bonded PAM fibers among 4 samples responded to blood glucose concentration after over 4 months, whereas a PAM fiber among 4 samples responded to blood glucose concentration after over 4 months. Therefore, we concluded that the PEG-bonded PAM fluorescent-hydrogel fibers could be applied to long-term subcutaneous glucose monitoring with less inflammation, resulting in maintaining the functionality of the implanted fibers. In addition, the fibers could be easily removed from the implantation site, thereby reducing potential side effects from remaining debris of the fibers.

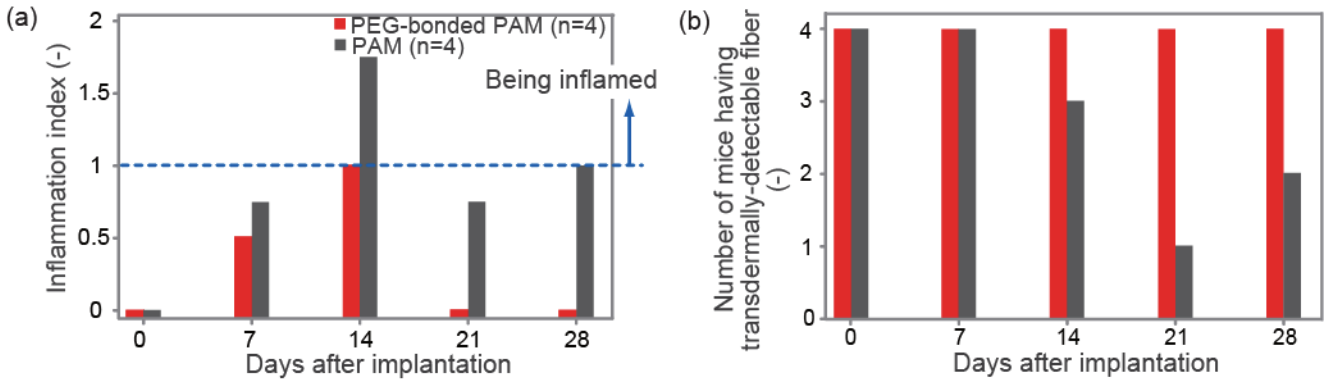

Figure 3: (a) Inflammation of mice ears after fiber implantation. Inflammation The fibers with PEG induced mild inflammation and recovered rapidly. However, the fibers without PEG showed more inflammation and slower recovery compared to the fibers with PEG. (b) Transdermal detection after fiber implantation. The fibers with PEG could glow across the ear skin for an entire 4 weeks, while the fibers without PEG could not.

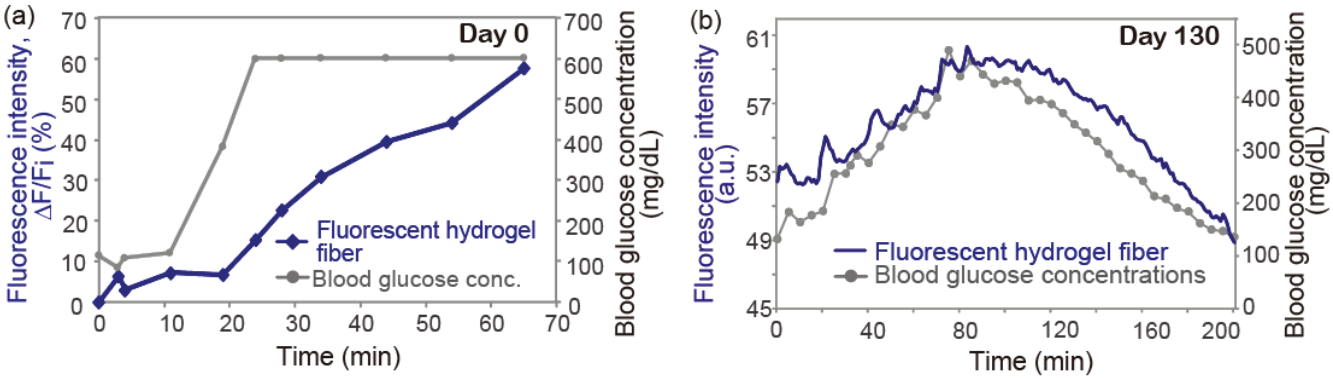

Figure 4: (a) Blood glucose monitoring after implantation. The fluorescence intensity of the hydrogel fiber responded to blood glucose concentrations. (b) Blood glucose monitoring after 130 days from implantation. The fluorescence intensity of the hydrogel fiber continuously changed in response to blood glucose concentrations even after over 4 months.

CONCLUSION

In this study, we have made a breakthrough in long-term in vivo glucose monitoring by employing the fiber structure and highly-biocompatible material (PEG-bonded PAM hydrogel) for the subcutaneous implants. We found that PEG-bonded fluorescent-hydrogel fibers remained in the implantation site, glowed through the skin and continuously responded to blood glucose concentration for over 4 months. Therefore, our methodology can contribute to effective point-of-care for diabetic patients and improve the quality of patients' life.

ACKNOWLEDGEMENTS

This work was supported by the New Energy and Industrial Technology Development Organization (NEDO).

REFERENCES

[1] D. C. Klonoff, "Continuous glucose monigoring: roadmap for 21st century diabetes therapy," *Diabetes Care*, vol. 28, pp. 1231-1239, 2005.

[2] T. Kawanishi, M. A. Romey, P. C. Zhu, M. Z. Holody and S. Shinkai, "A study of boronic acid based fluorescent glucose sensors," *J. Fluoresc.*, vol. 14, pp. 499-512, 2004.

[3] M. Malmsten, K. Emoto and J. M. V. Alstine, "Effect of chain density on inhibition of protein adsorption by poly(ethylene glycol) based coatings," *J. Colloid Interf. Science*, vol. 202, pp. 507-517, 1998

[4] *H. Shibata, *Y. J. Heo, T. Okitsu, Y. Matsunaga, T. Kawanishi and S. Takeuchi, "Injectable hydrogel microbeads for fluorescence-based in vivo continuous glucose monitoring," *Proc Natl Acad Sci USA*, vol. 107, pp. 17894-17898, 2010. *equal contribution.

CONTACT

*Y. J. Heo, tel: +81-3-5452-6650; heo@iis.u-tokyo.ac.jp