FABRICATION OF BIOCOMPATIBLE FLUORESCENT HYDROGEL FOR IMPLANTABLE CONTINUOUS GLUCOSE MONITORING DEVICE

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ABSTRACT

We developed the glucose-responsive florescent tetra-PEG gel based on diboronic acid for implantable continuous glucose monitoring. The fluorescent dye having diamine unit was immobilized into tetra-PEG gel formed by mixture of tetraamine-terminated-PEG (TAPEG) and tetra-NHS-glutarate-terminated-PEG (TNPEG). When the glucose concentration increased from 0 mg•dL^{-1} to 1,000 mg•dL^{-1}, the fluorescence intensity of the gel also become higher. In vivo glucose monitoring was conducted using implantable wireless fluorescent monitoring device equipped with the tetra-PEG gel in the back of rat. We have successfully detected the glucose concertation of interstitial fluid continuously. By combining the tetra-PEG gel with small and implantable florescent monitoring device, continuous glucose monitoring (CGM) system using fluorescent hydrogel can be a powerful approach for practical in vivo.

INTRODUCTION

Continuous Glucose Monitoring

Continuous glucose monitoring (CGM) provides information about shifting blood glucose levels and facilitates the making of optimal treatment decisions for the diabetic patient. The current method for in vivo glucose monitoring is based on implanted enzymatic electrode and this type of devices are commercially available. However, the development of long-term usable CGM device for more efficient diabetes treatment faces limitation of the sensor lifetime. Therefore, new approaches to glucose sensing is actively explored and fluorescence-based systems are receiving increasing attention. We reported the glucose-responsive fluorescent hydrogel that consisted of polyacrylamide and glucose responsive fluorescent dye maintains long-lasting responsivity in vitro and in vivo due to enzyme-free and reversible reaction [1,2]. As time goes on, the polyacrylamide hydrogel is lead to the foreign body reaction in vivo, which encapsulates the hydrogel and prevents its potential accuracy with the surrounding tissue.

Tetra-PEG gel

PEG is one of the widely used synthetic polymers for implantation due to its intrinsic low-protein adsorption properties, minimal inflammatory profile and history of safe in vivo use, ease in incorporating various functionalities, and commercial availability of reagents [3]. We used PEG-based hydrogel for immobilizing the glucose-responsive dye consist of 4-arm PEG with NHS glutarate end groups (TAPEG) and 4-arm PEG with amine end groups (TNPEG) as it is called Tetra-PEG gel.

Here, we present the development of the biocompatible glucose-responsive hydrogel disc based on tetra-PEG gel. These fluorescent tetra-PEG gel disc maintained glucose responsiveness and transmitted fluorescent signals in vitro and in vivo. We achieved in vivo continuous glucose monitoring using the implantable fluorescent monitoring device equipped with the tetra-PEG gel.

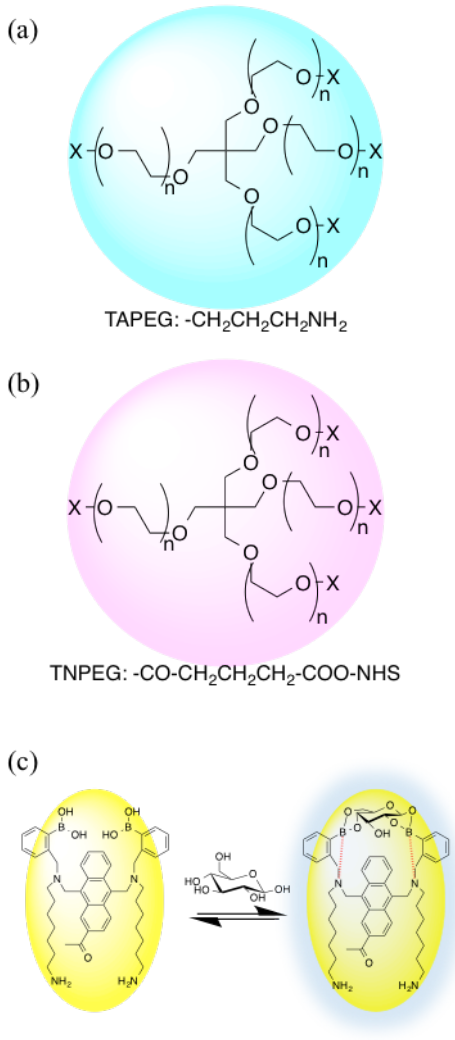

Figure 1: Materials of Glucose-responsible florescent hydrogel biocompatible hydrogel (a)Tetraamine-terminated -PEG (TAPEG). (b) Tetra-NHS-glutarate-terminated-PEG (TNPEG). (c) Glucose-responsible fluorescence dye. Fluorescence intensity of the dye changes depending on the existence of a glucose molecule.

RESULTS AND DISCUSSION

Fabrication of glucose-responsive tetra-PEG gel disc

We immobilized the glucose-responsive dye in tetra-PEG gel that was synthesized by polycondensation reactions between NHS glutarate end groups (TNPEG) and amino end groups (TAPEG) of 4-arm PEG (Figure. 1a, b). The synthetic 4-arm PEG polymer purchased from NOF

America Corporation, America. Glucose-responsive dye was made to order by NARD Institute, Japan. The glucose-responsive dye comprises of diboronic acid moiety and an anthracene moiety that act as the specific glucose-recognition site and the fluorogenic site, respectively. In the absence of glucose molecules, the fluorescence of the anthracene is quenched. When glucose molecules bind to the diboronic acid, a strong reaction between the nitrogen atom and boron atom. Thus, the fluorescence intensity of anthracene becomes higher (Figure. 1c) [4].

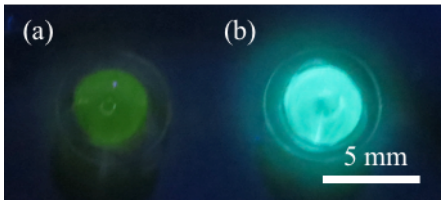

Figure 2: Fluorescent images of tetra-PEG at the glucose concentration of (a)0 mg·dL^{-1}, and (b)1,000 mg·dL^{-1}

The tetra-PEG gel disc was fabricated using PDMS mold. The pre-gel solution was mixture of TAPEG, TNPEG, and the dye in a phosphate buffer. The pre-gel solution was flowed into the silicon mold and then stored at 37 °C. After an hour, the tetra-PEG gel discs were washed with phosphate buffer, for more than 3 hours. We optimized the component of TAPEG and TNPEG in the pre-gel solutions and obtained tetra-PEG gel immobilized glucose-responsive dye.

Glucose responsiveness of the tetra-PEG gel discs

We performed the in vitro glucose responsiveness test using the fabricated tetra-PEG gel disc (Figure. 2). From the fluorescent images for 0 and 1000 mg•dL^{-1} glucose concertation, the glucose responsiveness were confirmed for glucose concertation. When the glucose concentration increased, the fluorescence intensity become higher. The results of glucose response curves were like the conventional polyacrylamide type glucose-responsive hydrogel. The fluorescence intensity of the tetra-PEG discs provided a suitable curve for monitoring blood glucose levels in the physiological range (62.5-500 mg•dL^{-1}) .

In vivo continuous glucose monitoring

We implanted the CGM systems combined by the tetra-PEG gel disc and implantable wireless fluorescent monitoring device into the back of rat (Figure. 3). The devices were separated the implantable fluorescent monitoring unit from a wireless data transfer unit and a rechargeable battery. The implantable unit was integrated of Photo Sensor and UV-LED on the substrate. While the wireless data transfer unit was used TOCOS Wireless Engine. The wireless unit was transmitted the fluorescent intensity data every minute. We purchased six-week-old male Sprague Dawley rats from Japan SLC (Shizuoka, Japan). They had free access to food (CE-2: CLEA Japan, Inc., Tokyo, Japan) and water in an animal room that was maintained at 23 ± 2°C with a 12-h light–dark cycle. For in vivo continuous glucose monitoring, the florescent

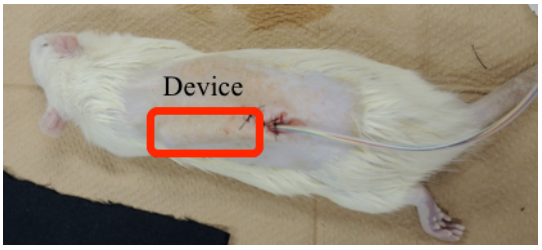

Figure 3: In vivo continuous glucose monitoring

monitoring device equipped with the tetra-PEG gel implanted into the back of rat, and the glucose concertation of interstitial fluid is monitored continuously under anesthesia. The fluorescence intensity continuously corresponds to blood glucose levels of rat, showing efficient glucose monitoring.

CONCLUSION

We developed the biocompatible glucose-responsible florescent tetra-PEG gel based on diboronic acid for implantable continuous glucose monitoring device. The fluorescent dye having diamine unit was immobilized into tetra-PEG gel. We achieved continuous glucose monitoring using this biocompatible glucose-responsible fluorescent hydrogel for the rat.

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