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Ultrafast glucose-responsive, high loading capacity erythrocyte to self-regulate the release of insulin

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ABSTRACT

Insulin (INS) delivery system that can mimic normal insulin secretion to maintain the blood glucose level (BGL) in the normal range is an ideal treatment for diabetes. However, most of the existing closed-loop INS delivery systems respond slowly to the changes in BGL, resulting in a time lag between the abnormal BGL and the release of INS, which is not suitable for practical application. In this study, glucose oxidase (GOx)-modified erythrocytes are used as INS carriers (GOx-INS-ER) that can rapidly self-regulate the release of INS upon the changes in BGL. In this system, glucose can be broken down into gluconic acid and hydrogen peroxide by GOx-INS-ER, and the latter will rupture the erythrocyte membrane to release INS within minutes. A pulsatile release of INS can be achieved upon the changes in the glucose concentration. This GOx-INS-ER enables diabetic rats to overcome hyperglycemia within 1 h, and a single injection of this GOx-INS-ER into the STZ-induced diabetic rats can maintain the BGL in the normal range up to 9 days.

Statement of Significance

Diabetes mellitus has been a major public health threatener with global prevalence. Although, glucose-responsive carriers that can release insulin (INS) in a closed loop have been explored greatly in recent years, their sluggish glucose-responsive property and low INS-loading content greatly restrict their practical application [ACS Nano, 2013, 7, 4194].
In this work, we reported INS-loaded erythrocytes featuring ultrafast glucose-responsive property and high INS loading content, which could release INS in a closed loop. These GOX-INS-ERs could respond to the changes in glucose level within several minutes and self-regulate the release of INS for a long time. Single injection of GOX-INS-ER can overcome hyperglycemia in diabetic mice within 1 h and maintain the baseline level of glucose up to 9 days. We think our method may provide a robust way to potentiate diabetes treatment.

1. Introduction

Diabetes mellitus, characterized by the abnormal high level of blood glucose, has been a major public health risk with global prevalence. Currently, direct injection of insulin (INS) into the subcutaneous tissue is the standard treatment for patients with type 1 and some with type 2 diabetes [1]. Owing to the short half-life of insulin in blood, the patient should receive multiple injections of INS with careful dosing required to ensure normoglycemia. Achievement of normoglycemia can be complicated by poor patient compliance, and regular injections introduce the potential risk of long-term organ damage from hyperglycemia or brain damage or death by hypoglycemia [2,3]. Although controlled release systems of INS, such as multilayer films [4–7] and continuous insulin delivery through pumps [8], can deposit INS subcutaneously for a long time, they still face the problems in releasing INS on-demand, accelerating the penetration of INS into circulation, and prolonging the half-life of INS in the blood [4,9]. Recently,

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closed-loop INS delivery systems, which can self-regulate the release of INS upon changes in blood glucose level (BGL), have been designed for diabetes treatment [4]. These systems are usually composed of glucose oxidase (GOx) enzyme and pH-sensitive polymers [5–7]. GOx can convert glucose to gluconic acid and hydrogen peroxide (H_2O_2), thus activating the release of insulin [8]. Unfortunately, most of the previously reported closed-loop INS delivery systems suffered a slow glucose-responsive rate to changes in BGL and are not suitable for practical application [9]. Therefore, an optimal INS delivery system should be rapidly responsive to glucose, biocompatible, have high loading capacity, and have prolonged circulation of non-bioavailable insulin with on-demand release of INS occurring rapidly.

Erythrocytes have long been used as carrier for INS because of their high biocompatibility, long life span, and large internal capacity [10,11]. Through a ligand exchange strategy, a rapidly glucose-responsive insulin delivery system based on erythrocytes was developed [12]. However, the INS loading capacity of this system is not satisfactory owing to the limited INS binding sites on the surface of the erythrocyte. In this study, we demonstrated an erythrocyte-based INS delivery system (GOx-INS-ER) that showed a high INS loading content and could rapidly respond to the elevation in BGL to self-regulate the release of INS to be used for diabetes treatment (Scheme 1). In this system, biotin-modified GOx (biotin-GOx) was tightly attached to the surface of the INS-loaded erythrocytes (INS-ERs) to form GOx-INS-ERs through the biotin–protein interaction [13,14] (Scheme 1A). When GOx-INS-ERs are injected into the blood at normal BGL, they are in the "off" state, work as normal erythrocytes in the blood, and INS is maintained inside the circulation system for a long time. When the BGL rises, GOx will catalyze the conversion of glucose to gluconic acid and H_2O_2 as shown in reaction (1) [8].

$$\text{glucose} + O_2 + H_2O \rightarrow \text{gluconic acid} + H_2O_2 \quad (1)$$

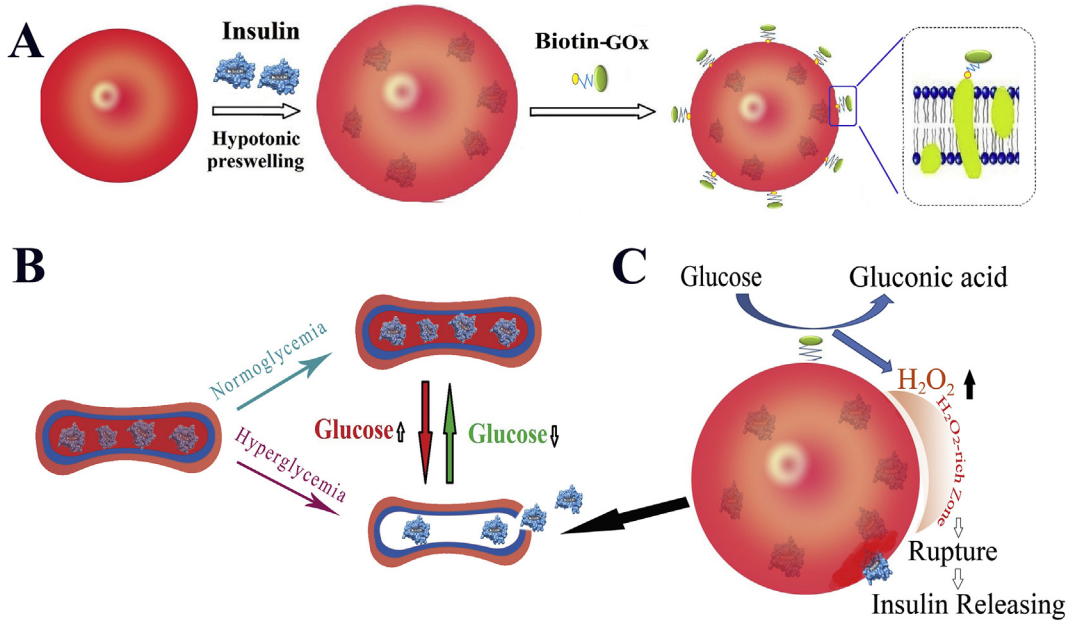
Because all the GOx are docked on the surface of erythrocytes, most of the produced H_2O_2 is near the surface of the erythrocyte, resulting in a high local concentration of H_2O_2. The increased local concentration of H_2O_2 thus will rupture the membrane of erythrocytes rapidly to form pores in the bilayer lipid membrane of the erythrocyte, which turns "on" the GOx-INS-ER system to release INS (Scheme 1C) [15,16]. The released INS reduces the BGL to the normal range, at which the production of H_2O_2 is greatly suppressed. Consequently, the broken bilayer lipid membrane of the erythrocyte will be restored to close the pores, which will stop the release of INS (Scheme 1B). This cycle will be repeated depending on the BGL, which results in a glucose-responsive, self-regulated INS-releasing system. On the contrary, the generated H_2O_2 will finally diffuse into the circulation system and be quickly decomposed into water and oxygen to minimize the side effect of H_2O_2 against normal erythrocytes and organs. We anticipate that this system can maintain the blood glucose homeostasis precisely for a long time to reduce the complications in patients with diabetes.

2. Experimental section

2.1. Materials and animals

Recombinant cow insulin (INS, potency: 27 U/mg), streptozotocin (STZ) and biotin were purchased from Bomei Biotechnology (Hefei, China). GOx was purchased from Tokyo Chemical Industry (Tokyo, Japan). Dialysis bags (molecular cut-off, 14 kDa and 10 kDa) were purchased from Green Bird Science & Technology Company (Shanghai, China). FITC-INS (fluorescein isothiocyanate) was provided by Zhongke Chenyu Biotechnology (Beijing, China). All other reagents were of analytical grade and used without further purification.

The Experimental Animal Center of Nantong University provided the male Sprague-Dawley (SD) rats (275–300 g) and BALB/c mice (18–22 g). All animals were housed in individual cages under constant temperature (24 ± 2 °C), humidity, and light (12-h light/dark cycle). All animal experiments were performed according to the relevant laws and institutional guidelines inspected by the Division of Comparative Medicine at Nantong University (Protocol No.: 20160225-001).



Scheme 1. (A) Synthesis of INS-loaded GOx-modified erythrocyte (GOx-INS-ER) and (B) closed-loop release of INS. Biotinylated GOx was docked on the surface of erythrocyte working as a glucose-activating switch. GOx catalyzed the oxidation of glucose to generate H_2O_2. (C) The high local concentration of H_2O_2 subsequently ruptured the membrane of erythrocyte to release the INS.

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