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Cell surface-camouflaged graphene oxide immunosensor for identifying immune reactions

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ABSTRACT

Monitoring the intensity of immune reactions is very useful to diagnose the fate of transplanted cells. For directly monitoring immune reactions after pancreatic islet cell transplantation, here graphene oxide (GO) quencher was chemically conjugated with fluorescence dye via Granzyme B (GrB)-specific peptide, so called to GO-based immunosensor. GO-based immunosensor was stably immobilized onto surface of living cells without damage of viability and functionality. The cell-surface camouflaged GO-based immunosensor could detect GrB (>2 unit/ml) secreted from immune cells, thereby dequenching fluorescence. Collectively, we anticipated that the GO-based immunosensor could be utilized to monitor cell graft rejection after cell therapy *in vivo*.

Introduction

The immunosensor has been evolved from food quality into clinical diagnosis [1], and it plays key roles to identify unknown immune response of rare disease or investigate target material in immune reactions [2]. Especially, development of novel immunosensor for highly sensitive, selective, and rapid immune detection is extremely important in organ and cell transplantation (cell therapy). Even though immunosuppression regimen was vitally used in cell transplantation field to immunologically protect the transplanted cells, excessive mediations can result in several adverse effects such as hypertension and peptic ulcers [3]. Inversely, insufficient immunosuppression regimen cannot act as inhibitor to suppress immune reactions. Therefore, visualization of immune reactions may help in determination of proper dose of immunosuppressive drugs.

Graphene oxide (GO), which is generally obtained by oxidation of graphite in a mixture of oxidizing agent, has recently displayed advantageous potential to use biosensing platform due to its unique physical, chemical, and mechanical properties [4]. Among the various properties of GO, it is a representative of super quencher and it can apply the fluorescence resonance energy transfer (FRET) that is recognized as a sensitive and reliable analytical technique [5]. For example, GO have been demonstrated as FRET acceptor for usefulness of fluorescence quenching properties by using DNA base pairs [6] and it has been also applied for cellular imaging and drug delivery by using photoluminescent GO nanosheets [7,8]. Moreover, it has been used as a nanoprobe for intracellular imaging by using fluorescein-PEG-GO complex [9] and specific dye labeled peptide cleavage has been used in specific substrate recognition by fluorescence recovery from GO in cytosol [10,11]. However, FRET-based specific substrate detection by using quenching property of GO in cell surface has not yet been much studied.

In this study, we newly fabricated GO-based cell surface immunosensor, which was chemical conjugation of GO and fluorescein isothiocyanate (FITC) via linker peptide (Fig. 1). This linker peptide (sequence: HALNNIETD) was Granzyme B-specific as shown in Fig. 2. Therefore, the conjugated FITC was quenched by GO nanoparticle without any immune reaction, whereas it was dissociated from GO nanoparticle through proteolysis of linker peptide by Granzyme B (GrB) enzyme. Therefore the dissociated FITC could show fluorescence signal, demonstrating that the immune reactions were stimulated and the GrB was released from

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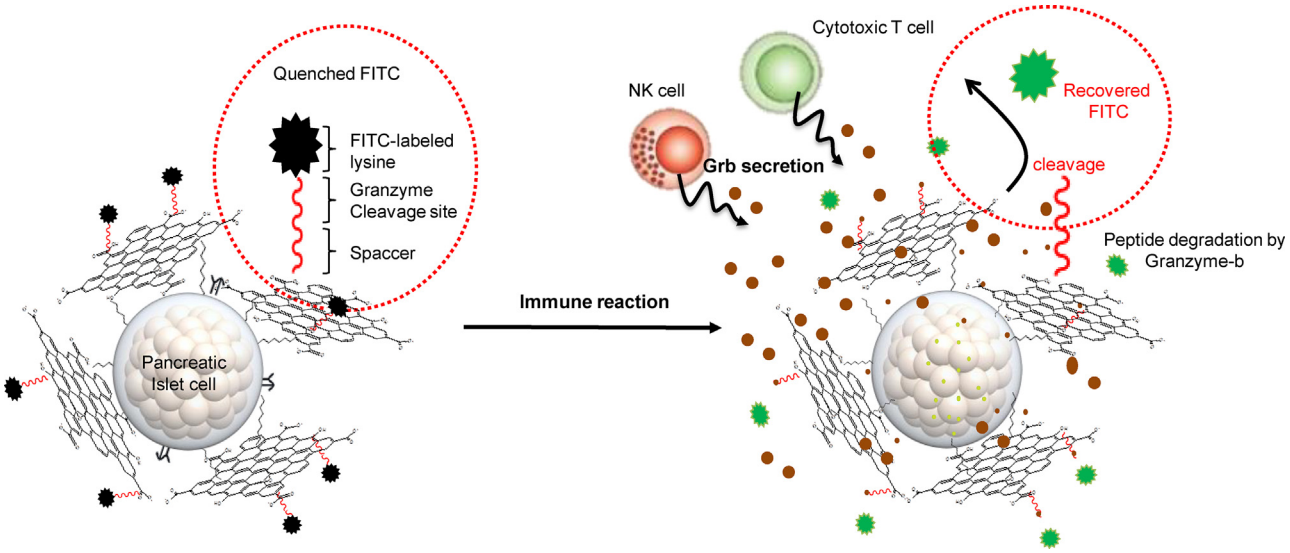

Fig. 1. Scheme of graphene oxide (GO)-based immunosensor. It was chemically immobilized onto cellular membrane of pancreatic islet cells.

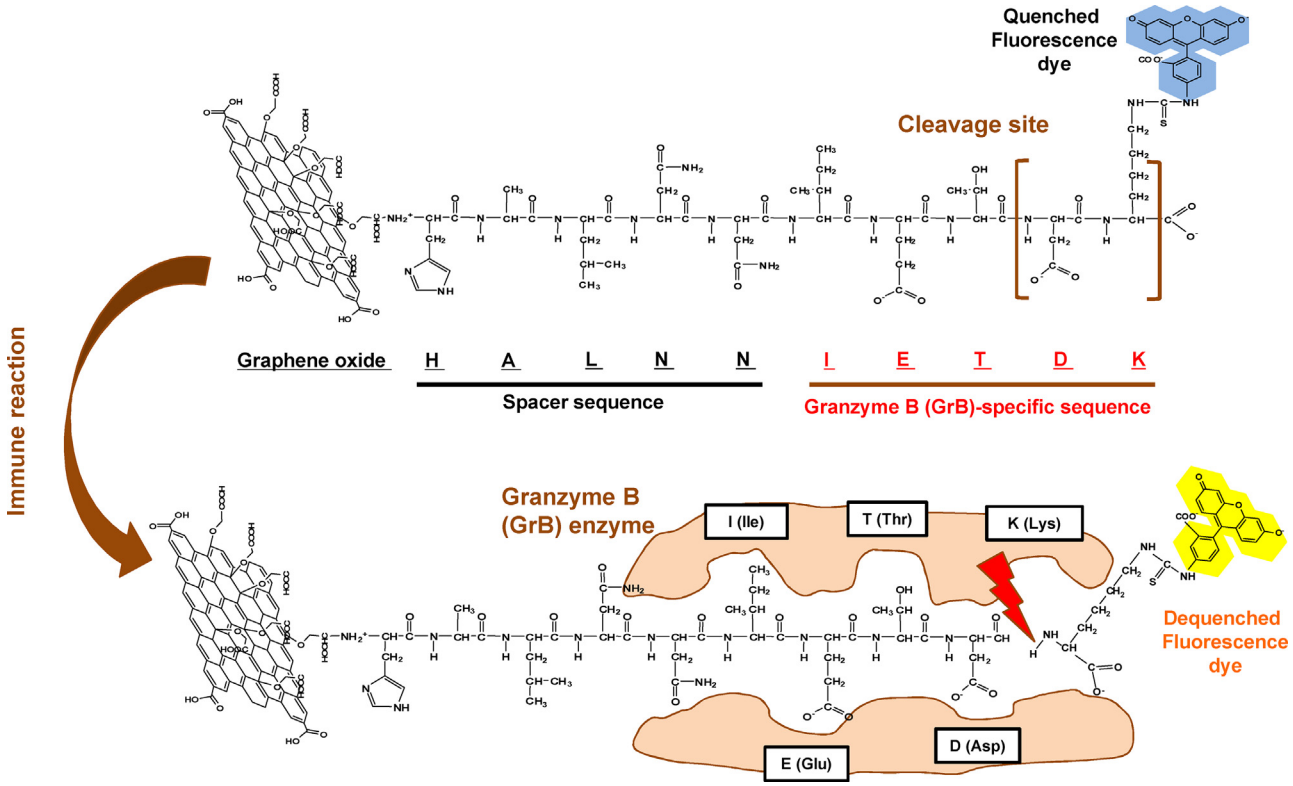

Fig. 2. The chemical structure of GO-based immunosensor. Granzyme B (GrB) enzyme recognized the degradation sequence between Asp(D) and Lys(K) amino acid, thereby dequenching the fluorescence dye.

the cytotoxic lymphocytes (CTLs) and natural killer cells (NK cells). Consequently, when immune substance existed via fluorescence signal, immune reactions could be monitored after pancreatic islet cell transplantation. However, there were many safety concerns of GO that has potential for cellular toxicity [12]. To overcome this limitation, we worked out a design as chemical immobilization of GO-based immunosensor onto cell surface instead of intracellular delivery of GO particles (Fig. 1). In this study, therefore, we newly synthesized the GO-based immunosensor and evaluated its feasibility for monitoring the immune reactions.

Experimental

Preparation of GO-based immunosensor

GO-based immunosensor was synthesized as shown in Fig. 3. Graphene oxide purchased from grapheme laboratories (Calverton, NY, USA) was synthesized using modified Hummers' method. Graphene oxide (GO; 20 mg) was dispersed in distilled water (20 ml) and ultrasonicated for 1 h. The dispersed GO in aqueous solution was obtained by centrifuge (4000 rpm, 10 min). To

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