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A New Bio-Inorganic Nanocomposite Membrane for Glucose-Modulated Release of Insulin

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ABSTRACT

This work focuses on the development of a new bio-inorganic nanocomposite glucose-responsive membrane to be applied as a single self-regulated platform for insulin delivery. Crosslinked bovine serum albumin (BSA)-based membranes were prepared containing impregnated pH-responsive poly(N-isopropyl acrylamide-co-methacrylic acid) nanoparticles (hydrogel NPs), glucose oxidase (GOx), catalase (CAT), with or without MnO_2 NPs. The membrane acts as a glucose sensor and insulin release attenuator. In this system glucose is oxidized by GOx to produce gluconic acid, which regulates the permeability of the membrane to insulin. CAT and/or MnO_2 NPs are introduced into the membrane in order to quench unwanted H_2O_2 produced by GOx turnover cycles, which can cause inactivation of GOx and toxicity. The glucose-modulated insulin release through the membrane is determined by alternating glucose concentration between 100 – 400 mg/dL (normal and hyperglycemic levels, respectively). The results show that the combination of CAT and MnO_2 NPs in the membrane formulation leads to better efficiency in quenching the H_2O_2 and better long-term stability of GOx than using either alone. Very small amounts of insulin permeate though the membrane at the normal blood glucose level while a four-fold increase in the release rate is observed when glucose concentration is raised to a hyperglycemic level. The release rate of insulin drops when the glucose level is reduced to a normal value. These results demonstrate the self-regulated capability of the system.

INTRODUCTION

Diabetes mellitus is a major public health problem that affects more 246 million people worldwide. It is a disorder in glucose regulation, characterized by the accumulation of glucose in the blood due to the inability of the pancreas to secrete insulin or the body incapability to respond to insulin. The conventional way of controlling glycemia in insulin-dependent diabetic patients is the frequent self-administration of insulin injections, which often results in hypoglycemia along with bad patient compliance [1, 2]. A more effective approach to delivering insulin in direct response to blood glucose levels mimicking a healthy human pancreas is thus highly desirable [3]. For this propose, smart nanomaterials have been investigated for glucose-modulated insulin delivery [3, 4]. Glucose-responsive nanocomposite membranes, for example, are able to control the permeation of insulin as a direct response to glucose concentration [8-7]. Our approach to producing glucose-responsive membranes involves the incorporation of pH-

sensitive hydrogel NPs and the enzyme GOx into a polymeric matrix [5 - 7]. The membrane acts as a glucose sensor by the action of GOx, which catalyzes the oxidation of glucose to gluconic acid. This reaction creates a low pH microenvironment in the membrane, causing the hydrogel NPs to shrink. As a result, an interconnected porous framework is formed and the insulin permeation across the membrane is increased. In this system, CAT is normally co-immobilized with GOx in order to quench the H_2O_2 produced during GOx turn over cycles and replenish one half of the O_2 required for glucose oxidation. Still, several factors can lead to GOx inactivation and consequently to the failure of regulated insulin release. Besides the accumulation of hydrogen peroxide which leads to deleterious effects on GOx activity, the hydrophobic nature of the polymeric matrices utilized until now and the harsh conditions applied for the preparation of membranes can also contribute to GOx inactivation.

In order to improve GOx stability and optimize the membrane's response to glucose concentration a hydrophilic membrane matrix, an effective H_2O_2 scavenger and mild conditions for membrane preparation are highly desirable. Herein, we propose the application of a new bio-inorganic hybrid material composed of crosslinked BSA and MnO_2 nanoparticles as a matrix for a glucose-responsive membrane. MnO_2 NPs show high reactivity towards H_2O_2 [10 - 12], while crosslinked BSA presents several advantages over other polymeric matrices previously applied in glucose-responsive nanocomposite materials (e.g., high biocompatibility; GOx/CAT immobilization can be processed in an aqueous medium quickly in one-step during membrane preparation). In this way, the combination of crosslinked BSA with MnO_2 NPs could greatly improve GOx stability and consequently lead to a better response to elevated glucose concentration for the controlled release of insulin.

EXPERIMENT

All chemicals were of analytical grade (Sigma-Aldrich unless indicated) and used without further purification. Membranes were prepared by crosslinking BSA with glutaraldehyde in the presence of 30 wt.% hydrogel NPs (380 ± 110 nm in pH 7.4 PBS and 157 ± 50 nm in pH 5.0 PBS; prepared as previously described [5-9]), 5 wt.% GOx, 1.6 wt.% CAT and or 10 wt.% MnO_2 NPs (NPs of 80 ± 30 nm were prepared as previously described [13]). Membranes containing GOx alone (1), GOx with CAT (2), GOx with MnO_2 (3), and GOx with CAT and MnO_2 (4) were prepared with a molar ratio of crosslinker to protein $X \approx 0.08$. The resultant membranes had a thickness of about 400 μm. Membrane samples (1 – 4, 10 × 10 × 1 mm) containing approximately 1mg of immobilized GOx were incubated in 200 mg/dL glucose in pH 7.4 PBS solution (20 mL) at 37°C and the H_2O_2 concentration was determined over time by using PeroXOquant™ assay kit (Pierce, USA). The relative stability of immobilized GOx in the membranes (1 – 4) was determined by measuring the GOx ability to lower the pH of a 200 mg/dL glucose solution in normal saline at 37°C over 10 consecutive days. SEM images of freeze-dried membranes were obtained with a Hitachi TM-1000 SEM microscope at 15kV. Bovine insulin (1 mg/mL in 10mM pH 7.4 PBS, 0.15M NaCl, 0.02M Pluronic F-68) was used in the permeation study. The release of insulin through the membranes at 37°C and at various glucose concentrations was measured by using a side-by-side diffusion cell system and a UV spectrophotometer (HP 8453 UV) [5, 6]. The glucose concentration in the solutions was alternated between hypoglycemic and hyperglycemic levels (100 – 400 mg/dL) in order to

simulate glucose fluctuations in diabetic patients. Permeated insulin through membranes was assayed by measuring insulin absorbance at 276 nm.

DISCUSSION

Preparation of glucose-responsive membranes

Glucose-responsive nanocomposite membranes were obtained by the crosslinking of BSA, GOx and CAT in the presence of MnO_2 and hydrogel NPs. Membranes were prepared in one quick step in aqueous medium, which can contribute greatly to preserving enzyme activities. Different membranes containing 30 wt% of hydrogel NPs and GOx (1), GOx/CAT (2), GOx/MnO_2 (3) or GOx/CAT/MnO_2 (4) were prepared. The immobilization of the enzymes GOX and CAT in the different membrane formulations were obtained by direct crosslinking with glutaraldehyde, while the hydrogel and MnO_2 NPs were integrated into the base membrane by entrapment during the crosslinking process. Due to the characteristic surface properties of metal oxide NPs (i.e. negatively charged surfaces and relatively large surface area), the conjugation of BSA, GOx and CAT with MnO_2 NPs prior to the crosslink process cannot be ruled out. In this context, the introduction of MnO_2 NPs into the membrane formulation could lead to higher efficiency in terms of enzyme immobilization. Additionally, the incorporation of MnO_2 NPs improved the mechanical strength of the membrane.

Quenching of H_2O_2 by CAT and/or MnO_2 NPs and stability of immobilized GOx

MnO_2 NPs and CAT, known as hydrogen peroxide scavengers, were introduced to the membrane formulations in order to quench the H_2O_2 produced during GOx turnover cycles. The rate of H_2O_2 generated by membranes 1 – 4 is compared in Fig. 1a. It can be seen from the plots that the accumulation of H_2O_2 by membranes containing the H_2O_2 quencher (CAT, MnO_2 or their combination) was lower than that for the control membrane with GOx alone. The lowest H_2O_2 accumulation was observed for the membrane containing both CAT and MnO_2 NPs. In this membrane ca. 80% of the hydrogen peroxide produced by GOx was quenched by the combination of both scavengers.

The stability of the immobilized GOx in different membranes was also studied. The results in Fig. 1b show that the introduction of an H_2O_2 scavenger (MnO_2 and/or CAT) in the membrane formulation helped to maintain GOx stability over the period of time studied. For the membrane containing the combination of MnO_2 NPs and CAT, the activity of the immobilized GOx was preserved up to 8 days. The higher GOx stability in this formulation can be attributed to lower accumulation of H_2O_2 for this membrane and structural stabilization of GOx by MnO_2 NPs.

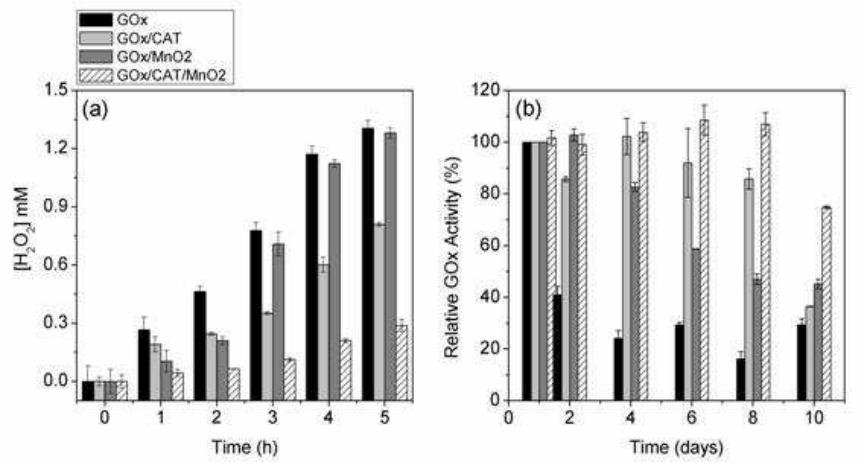

Figure 1. (a) H_2O_2 generated by immobilized GOx in membranes 1 – 4. H_2O_2 is the product of the oxidation of glucose to gluconic acid by GOx. **(b)** Stability of immobilized GOx in the different membranes formulations. Data points are represented as mean ± SD (n = 3).

Morphology of membranes

The morphologies of membranes made with or without MnO_2 NPs (membranes 2 and 4, respectively) are compared in Fig. 2. The images clearly show that the incorporation of MnO_2 NPs into the formulation led to different membrane morphology. Unlike the membrane 2 (Fig. 2a), membrane 4 (Fig. 2b) possesses a homogeneous spongy structure. The bright dots observed in the Fig. 2b can be attributed to MnO_2 NPs aggregates distributed in the polymeric matrix. This can explain the better mechanical properties observed for membranes made with MnO_2 NPs. The incorporation of inorganic NPs into soft materials normally leads to reinforced hybrid materials with improved mechanical properties [14].

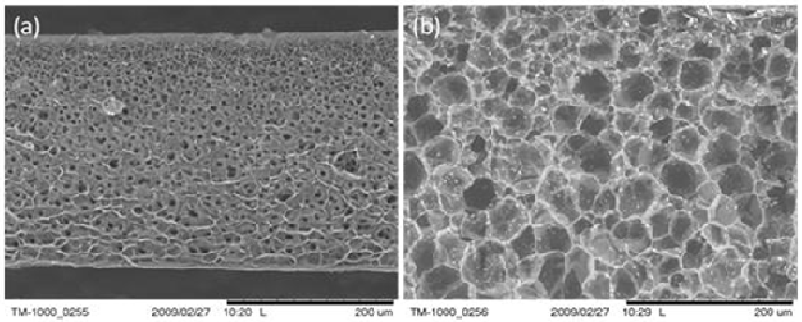

Figure 2. SEM images of the surfaces of **(a)** membrane 2 (GOx/ CAT) and **(b)** membrane 3 (GOx/ CAT/ MnO_2 NPs). The bright dots revels MnO_2 NPs aggregates distributed in the polymeric matrix.

Insulin permeation in response to glucose concentration

The permeation of insulin across membrane 4 was determined as a function of time and glucose concentration relevant to diabetic patients (normal glucose, 100 mg/dL and hypoglycemic glucose, 200 and 400 mg/dL). The glucose-responsive insulin permeation across the membrane containing both MnO_2 NPs and CAT is showed in Fig. 3a. As depicted in the rate

of insulin permeation increases with increasing glucose concentration in the medium. At normal glucose levels a small amount of insulin was permeated across the membrane (3.9 x 10^{-4} mg insulin/ min); while a four-fold increase in insulin release was observed when glucose concentration was increased to hyperglycemic levels (16.8 x 10^{-4} mg insulin/ min). The regulated profile of the system was demonstrated by determining the cumulative permeation of insulin in several alternated cycles (Fig. 3b). The permeation of insulin increased at hypoglycemic glucose levels (from 5.8 x 10^{-4} to 15.7 x 10^{-4} mg insulin/ min) and decreased (to 6.9 x 10^{-4} mg insulin/ min) when the glucose level was dropped to a normal level. This behavior demonstrated the glucose-regulated profile of the membrane.

The glucose-controlled permeability of insulin can be ascribed to the pH-dependent swelling and shrinking behavior of the impregnated hydrogel NPs. At hyperglycemic glucose levels, a high rate of glucose diffusion into the membrane leads to faster conversion of glucose to gluconic acid by GOx, thereby decreasing the local pH and causing the shrinkage of the hydrogel nanoparticles. As a result, the porosity of the membrane increases leading to higher insulin permeation. Once the glucose concentration is dropped to normal levels the process is reversed, and the insulin permeation is decreased. This proposed mechanism is illustrated in Fig. 4.

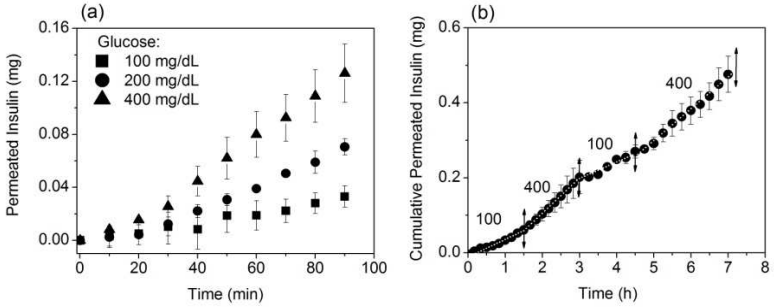

Figure 3. (a) Profile of insulin permeated across membrane 4 (containing GOx and both H_2O_2 quenchers, MnO_2 NPs and CAT) in different glucose concentrations; (b) cumulative permeated insulin in response to abrupt changes in glucose concentration (between 100 and 400 mg/dL) in several consecutive cycles. The numbers in the graph indicate the glucose concentration in the medium (mg/dL). Data points are represented as mean ± SD (n = 3).

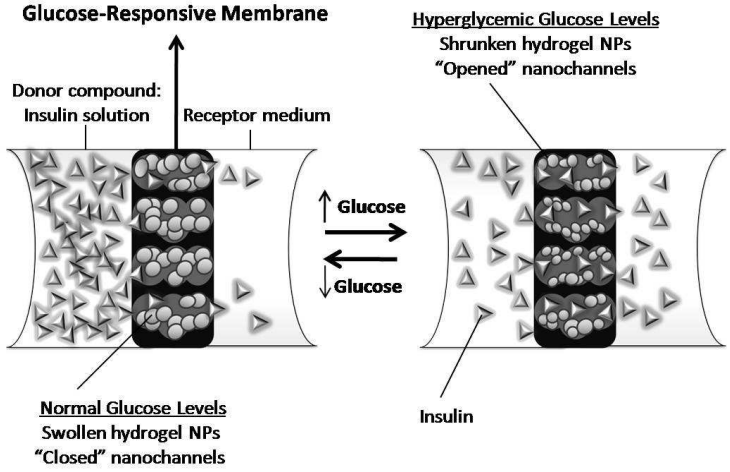

Figure 4. Schematic representation of the glucose-regulated permeation of insulin across the membrane.

CONCLUSIONS

For the first time a bio-inorganic nanocomposite membrane was prepared for the glucose-modulated release of insulin. The membrane acts as a glucose sensor due to the oxidation of glucose by the immobilized GOx, and as an insulin release attenuator stemmed from the volume phase transition of imbedded pH-sensitive nanohydrogels. The combination of MnO_2 nanoparticles with the enzyme CAT successfully quenched up to 80% of the undesirable hydrogen peroxide produced during GOx cycles, which improved the long term stability of GOx in the membrane. The glucose-responsiveness of the system allows for regulated insulin permeation across the membrane in response to variation in glucose concentration.

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REFERENCES

1. J. C. Pickup, Z. L. Zhi, F. Khan, T. Saxl, D. J. S. Birch, *Diabetes Metab. Res. Rev.*, **2008**, *24*, 604.
2. R. S. Parker, in *Encyclopedia of Biomaterials and Biomedical Engineering*, Vol. 1 (Eds: G. E. Wnek, G. L, Bowlin), Informa Healthcare, USA, **2008**, Ch. Insulin Deliver.
3. V. Ravaine, C. Ancla, B. Catargi, *J. Controlled Release* **2008**, *132*, 2.
4. N. A. Pepas, C. D. Bures, in *Encyclopedia of Biomaterials and Biomedical Engineering*, Vol. 1 (Eds: G. E. Wnek, G. L, Bowlin), Informa Healthcare, USA, **2008**, Ch. Glucose-Responsive Hydrogels.
5. K. Zhang, X. Y. Wu, *J. Controlled Release* **2002**, *80*, 169;
6. K. Zhang, X. Y. Wu, *Biomaterials* **2004**, *25*, 5281.
7. H.Y. Huang, J. Shaw, C. Yip, X.Y. Wu, *Pharm. Res.* **2008**, 25, 1150.
8. F. Yam, X.Y. Wu, Q. Zhang, in *Controlled Drug Delivery: Designing Technology for the Future,* (Ed: K. Park), ACS, Washington, DC, **2000**, p. 263.
9. X.Y. Wu, P.I. Lee, *Pharm. Res*. **1993**, 10, 1544.
10. X. Luo, A. Morrin, A. J. Killard, M. R. Smyth, *Electroanalysis* **2006**, *4*, 319.
11. X.-L. Luo, J.-J. Xu, W. Zhao, H.-Y. Chen, *Biosens. Biolectron.* **2004**, *19*, 1295.
12. J.-J. Xu, H.-Y. Chen, *Electrochem. Commun.* **2007**, *9*, 2611.
13. T. Fujimoto, Y. Mizukoshi, Y. Nagata, Y. Maeda, R. Oshima, *Scr. Matter.* **2001**, *44*, 2183.
14. M. K. Shin, G. M. Spinks, S. R. Shin, S. I. Kim, S. J. Kim, *Adv. Mater.* **2009**, *21*, 1.