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

Claudia R. Gordijo, Adam J. Shuhendler and Xiao Yu Wu*

Leslie L. Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada.

sxy.wu@utoronto.ca

UNIVERSITY OF TORONTO

Introduction

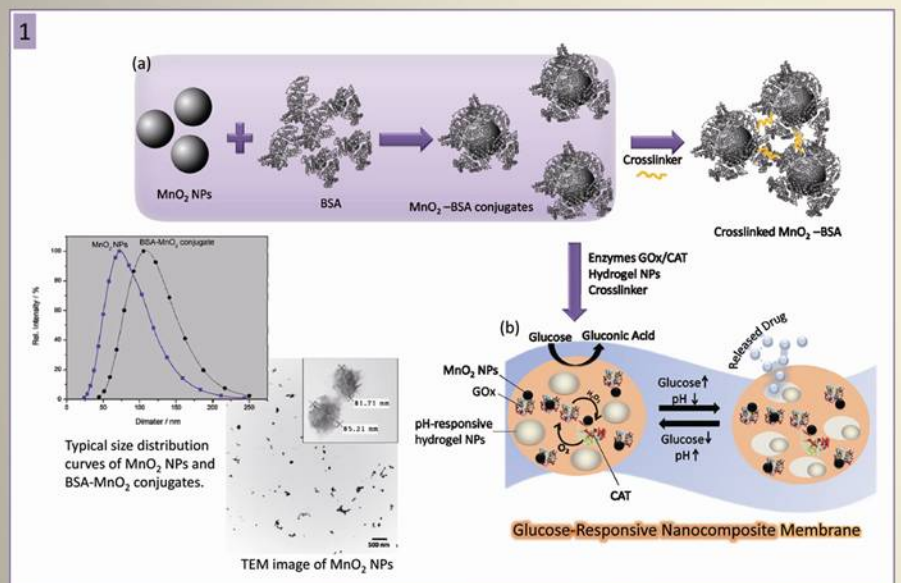
Diabetes mellitus is a major public health problem that affects more 246 million people worldwide. It is a disorder in glucose regulation, characterized by the accumulation of glucose in the blood due to the inability of the pancreas to secrete insulin or the body to respond to insulin. The conventional way of controlling glycemia in insulin-dependent diabetic patients is the frequent self-administration of insulin injections, which often results in hypoglycemia along with poor patient compliance. A more effective approach to delivering insulin in direct response to blood glucose levels (mimicking a healthy human pancreas) is thus highly desirable. For this propose, smart nanomaterials have been investigated for glucose-modulated insulin delivery. Our group has been developing glucose-responsive nanocomposite membranes able to control the permeation of insulin in response to glucose concentration. Our approach involves the incorporation of pH-sensitive hydrogel NPs and the enzyme glucose oxidase (GOx) into a polymeric matrix. 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, leading to the formation of an interconnected porous framework that increases insulin permeation across the membrane (Fig. 1b). In this system, catalase (CAT) is normally co-immobilized with GOx in order to quench the H_2O_2 produced during GOx turn over cycles and replenish ½ of the O_2 consumed by GOx reaction. 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 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 bovine serum albumin (BSA) and MnO_2 nanoparticles as a matrix for a glucose-responsive membrane. MnO_2 NPs shows high reactivity towards H_2O_2 while crosslinked BSA presents several advantages over other polymeric matrices previously applied; 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 glucose concentration for the controlled release of insulin.

1 – Glucose-Responsive Nacomposite Membrane

Membrane preparation: multifunctional MnO_2 NPs were conjugated with bovine serum albumin (MnO_2 – BSA) and enzymes GOx and CAT. A membrane was prepared by crosslinking these biomolecule-MnO_2 conjugates with glutaraldehyde in the presence of pH-responsive poly(N-isopropylacrylamide-co-methacrylic acid) (PNIPAM/MAA) hydrogel nanoparticles.

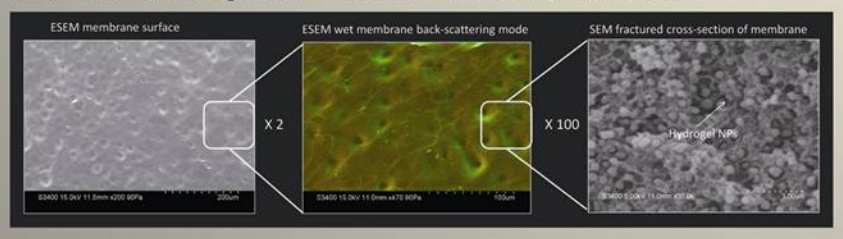
1



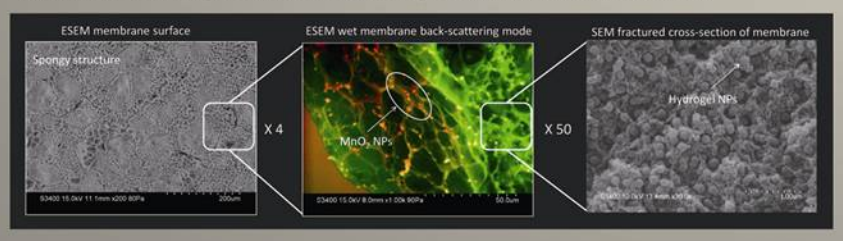
2 – Effect of MnO_2 NPs on Morphology of the Membrane

The incorporation of MnO_2 NPs into the membrane formulation led to different membrane morphology. Unlike the membrane made with crosslinked BSA (Fig. 2a), SEM images of the nanohybrid membranes (made with corsslinked MnO_2 – BSA) revealed a complex sponge-like structure with MnO_2 and hydrogel nanoparticles homogeneously distributed in the thin cavity wall of the base membrane (Fig. 2b).

a) Membrane without MnO_2 NPs: crosslinked BSA + enzymes + hydrogel nanoparticles



b) Membrane with MnO_2 NPs: crosslinked BSA-MnO_2 + enzymes + hydrogel nanoparticles



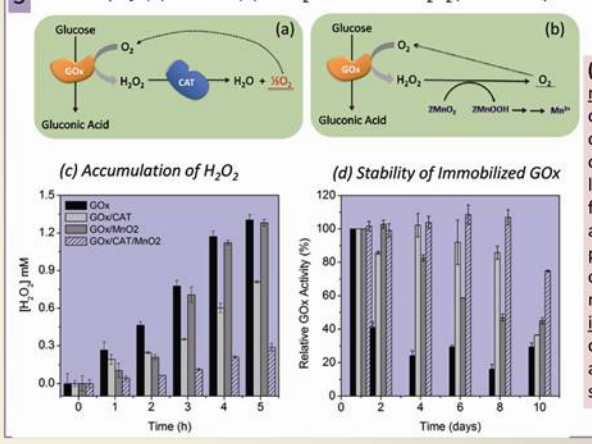
The authors gratefully acknowledge the Natural Sciences and Engineering Research Council of Canada & BioDiscovery Toronto for the financial support, Dr Hui Yu Huang for the initial studies with crosslinked BSA membranes.

BioDiscovery Toronto · NSERC CRSNG · Faculty of Medicine, University of Toronto Banting and Best Diabetes Centre

3 – Quenching of H_2O_2 by CAT/MnO_2 NPs and Stability of Immobilized GOx

3

Activity of (a) CAT and (b) MnO_2 NPs towards H_2O_2 produced by GOx reaction



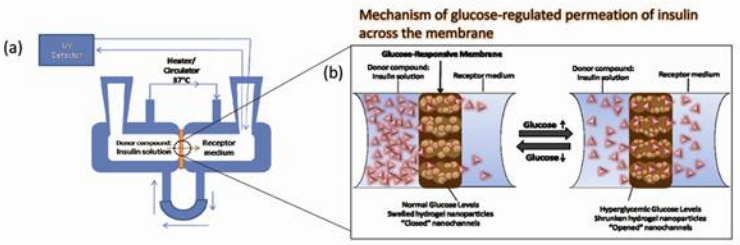
(c) Accumulation of H_2O_2

(d) Stability of Immobilized GOx

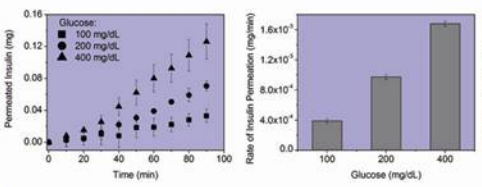
(c) The accumulation of H_2O_2 by membranes containing the H_2O_2 quencher (CAT, MnO_2 or their combination) was lower than that for the control membrane with GOx alone. The lowest H_2O_2 accumulation was observed for the membrane containing both CAT and MnO_2 NPs: ca. 80% of the H_2O_2 produced by GOx was quenched by the combination CAT/MnO_2. For this membrane, **(d)** the activity of the immobilized GOx was preserved up to 8 days, probably due to the lower accumulation of H_2O_2 and structural stabilization of GOx by MnO_2 NPs.

4 –Insulin Permeation in Response to Glucose Concentration

4 The *in vitro* release of insulin was measured by using a *side-by-side diffusion cell system coupled to an UV spectrophotometer* **(a)**. The glucose concentration in the release medium was alternated between hypoglycemic and hyperglycemic levels (100 – 400 mg/dL) in order to simulate glucose fluctuations in diabetic patients.

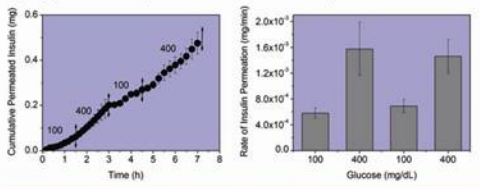


c) Profile of insulin permeated across the nanocomposite membrane containing GOx and both H_2O_2 quenchers (MnO_2 NPs and CAT) in different glucose concentrations.



The rate of insulin permeation increases with increasing glucose concentration in the medium. At normal glucose levels a small amount of insulin is permeated across the membrane; a four-fold increase in insulin release was observed when glucose concentration was increased to hyperglycemic levels.

d) Cumulative permeated insulin in response to abrupt changes in glucose concentration (between normal and hyperglycemic levels) in several consecutive cycles.



The glucose-regulated profile of the system is demonstrated by determining the cumulative permeation of insulin in several alternated cycles. The permeation of insulin increases at hypoglycemic glucose levels and decreases when the glucose level is dropped to a normal level.

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 and insulin release attenuator. 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 directly reflected on the improvement of the long term GOx stability in this membrane. The glucose-responsiveness of the system allows for regulated insulin permeation across the membrane in response to normal or hyperglycemic glucose concentrations.

References

K. Zhang, X. Y. Wu, *Journal of Controlled Release*, 2002, 80, 169. / K. Zhang, X. Y. Wu, *Biomaterials*, 2004, 25, 5281.