Title:

Glucose-stimulated insulin response of silicon nanopore-immunoprotected islets under convective transport

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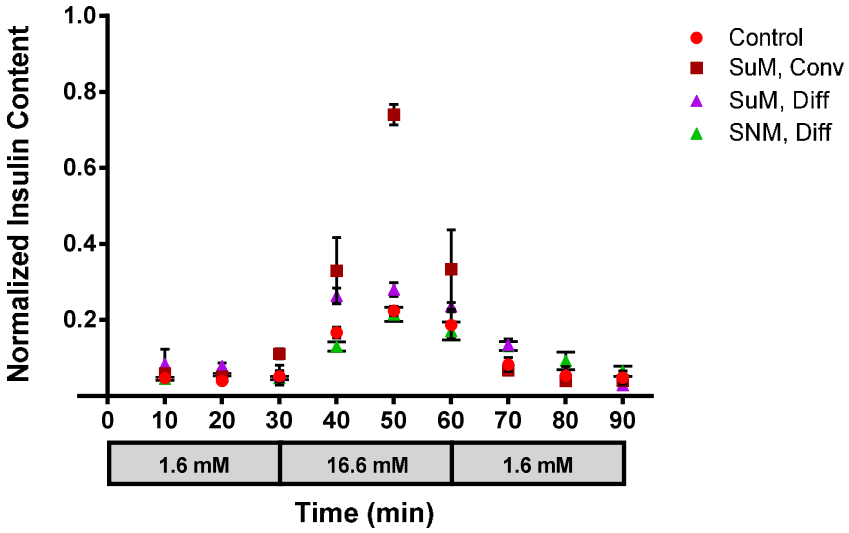
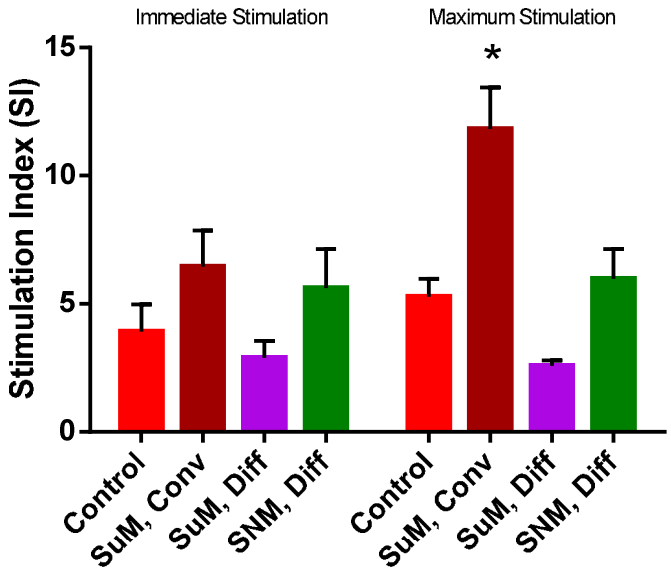
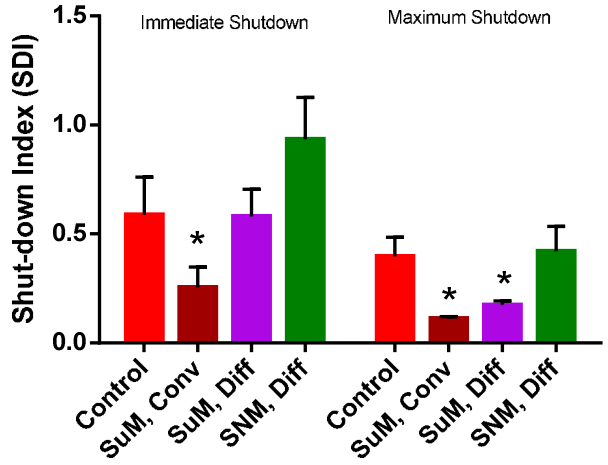
Figure S1. Glucose-insulin kinetics of membrane-encapsulated islets under convection and diffusion without cytokine exposure



a. Insulin release kinetics of membrane-encapsulated mouse islets during 90-minute low-high-low (1.6 mM, 16.6 mM, 1.6 mM) glucose stimulation under convective (2 psi) (Conv) and diffusive transport (Diff) without subjection to cytokines. The naked islets cultured under static conditions served as controls (Control). The SμM-encapsulated islets under convective transport (SμM, Conv) exhibited higher insulin secretion following stimulation at high glucose concentration and faster insulin release kinetics in response compared to SNM- and SμM-encapsulated islets under diffusive transport (SNM, Diff & SμM, Diff). (Mean±SEM, n≥3)

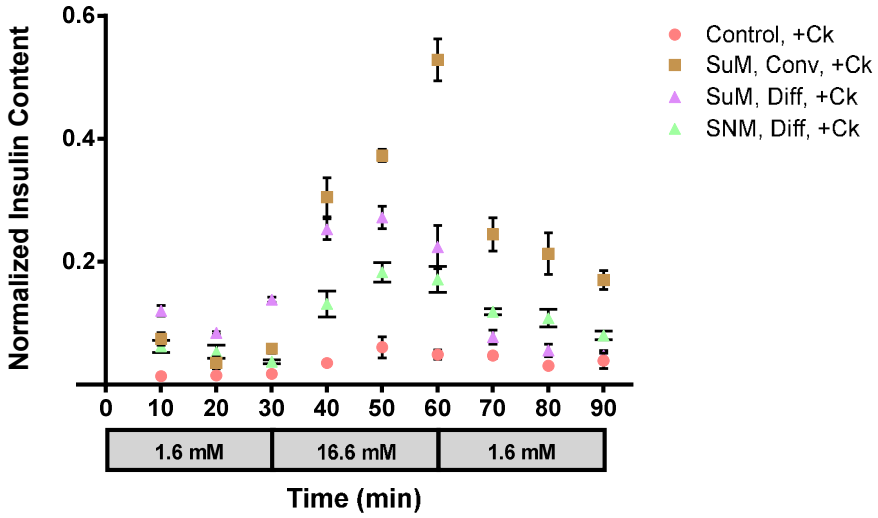


b. The stimulation index (SI) was calculated as the ratio of (1) the first insulin collection in the high glucose phase at 40 minutes to the last insulin collection point of the previous low glucose phase at 30 minutes (Immediate Stimulation), and (2) the highest insulin secretion in the high glucose phase to the last insulin collection point of the previous low glucose phase at 30 minutes (Maximum Stimulation). The SI indicates the magnitude of insulin released as stimulated by a higher concentration of glucose. Without cytokine exposure, SμM-encapsulated islets under convection (SμM, Conv) and diffusion (SμM, Diff), SNM-encapsulated islets under diffusion (SNM, Diff), and the naked islets cultured under static conditions (Control) all exhibited similar magnitude of glucose-induced insulin secretion (Immediate Stimulation). However, the SμM-encapsulated islets under convection (SμM, Conv) showed the highest magnitude of insulin secreted when the highest insulin secretion in the high glucose phase was used (Maximum Stimulation). (Mean±SEM, n≥3)

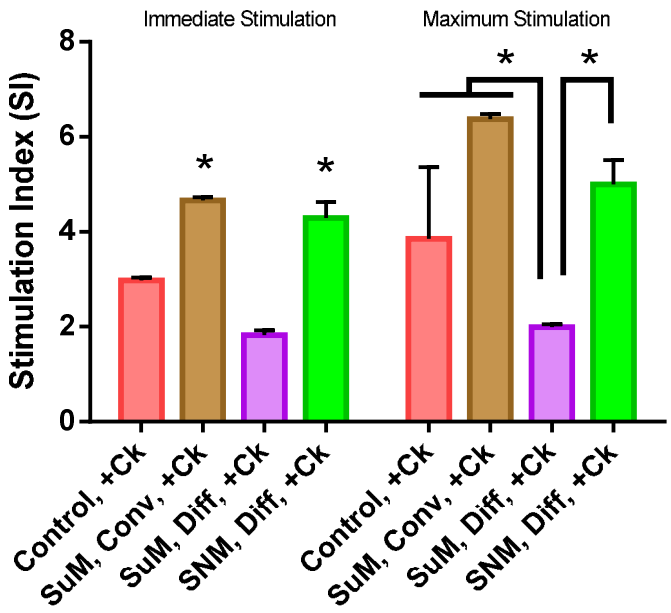


c. The shut-down index (SDI) was the ratio of (1) the first insulin collection point in the subsequent low glucose phase at 70 minutes to the last insulin collection point in the high glucose phase at 60 minutes (Immediate Shutdown), and (2) the lowest insulin secretion in the subsequent low glucose phase to the last insulin collection point in the high glucose phase at 60 minutes (Maximum Shutdown). The SDI reflects the magnitude of cessation in insulin production once glucose concentration returns to normal. Without cytokine exposure, SμM-encapsulated islets under convection (SμM, Conv) exhibited the highest magnitude of insulin reduction compared to the diffusive conditions (Control & SμM, Diff & SNM, Diff) as glucose dropped low (Immediate Shutdown). When the lowest insulin secretion in the low glucose phase was used, SμM-encapsulated islets under convection and diffusion (SμM, Conv & SμM, Diff) showed the largest magnitude of insulin reduction (Maximum Shutdown). (Mean±SEM, n≥3, *$p<0.05$)

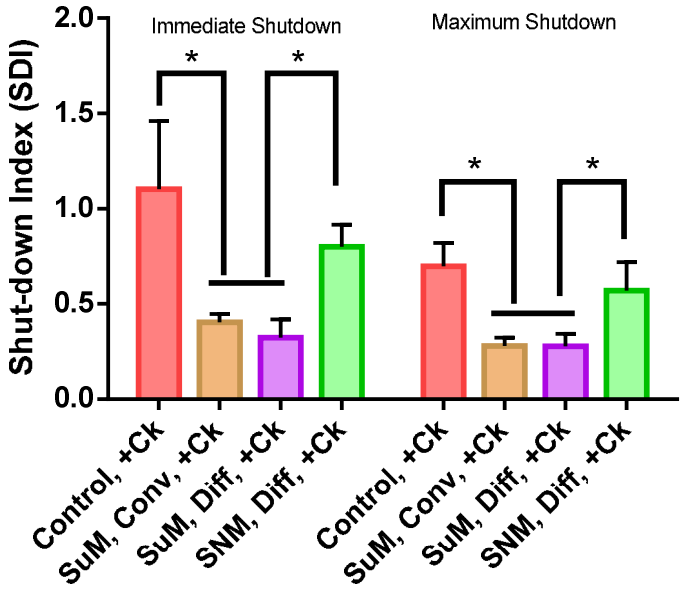
Figure S2. Glucose-insulin kinetics of membrane-encapsulated islets under convection and diffusion with cytokine exposure



a. Insulin release kinetics of membrane-encapsulated mouse islets during 90-minute low-high-low (1.6 mM, 16.6 mM, 1.6 mM) glucose stimulation under convective (2 psi) (Conv) and diffusive transport (Diff) with subjection to cytokines (+Ck). Experiments with cytokine exposure (+Ck) consisted of media containing TNF-α (2,000 U/mL), IFN-γ (1,000 U/mL), and IL-1β (10,000 U/mL). The naked islets cultured under static conditions served as controls (Control, +Ck). The SμM-encapsulated islets under convective transport (SμM, Conv, +Ck) exhibited higher insulin secretion and faster insulin release kinetics in response to stimulation at high glucose concentration compared to SNM- and SμM-encapsulation under diffusive transport (SNM, Diff, +Ck & SμM, Diff, +Ck) and naked islets cultured under static conditions (Control, +Ck). (Mean±SEM, n≥3)

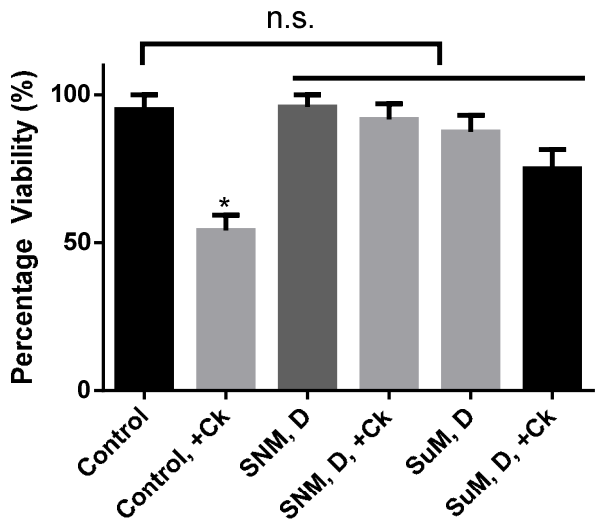


b. The stimulation index (SI) was calculated as the ratio of (1) the first insulin collection in the high glucose phase at 40 minutes to the last insulin collection point of the previous low glucose phase at 30 minutes (Immediate Stimulation), and (2) the highest insulin secretion in the high glucose phase to the last insulin collection point of the previous low glucose phase at 30 minutes (Maximum Stimulation). The SI indicates the magnitude of insulin released as stimulated by a higher concentration of glucose. With cytokine exposure (+Ck), SμM-encapsulated islets under convection (SμM, Conv) and SNM-encapsulated islets under diffusion (SNM, Diff) exhibited high level of magnitude in glucose-induced insulin secretion (Immediate Stimulation). The SμM-encapsulated islets under convection (SμM, Conv) showed an increase in the magnitude of insulin secretion when the highest insulin secretion in the high glucose phase was used (Maximum Stimulation). (Mean±SEM, n≥3, *p<0.05)

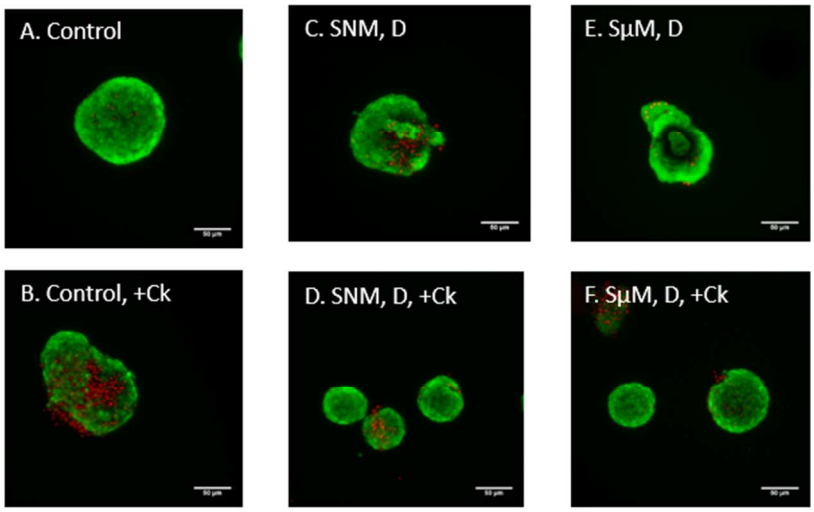


c. The shut-down index (SDI) was the ratio of (1) the first insulin collection point in the subsequent low glucose phase at 70 minutes to the last insulin collection point in the high glucose phase at 60 minutes (Immediate Shutdown), and (2) the lowest insulin secretion in the subsequent low glucose phase to the last insulin collection point in the high glucose phase at 60 minutes (Maximum Shutdown). The SDI reflects the magnitude of cessation in insulin production once glucose concentration returns to normal. With cytokine exposure (+Ck), the SμM-encapsulated islets under convection (SμM, Conv) and under diffusion (SμM, Diff) both exhibited the highest magnitude of insulin reduction compared to the naked islet culture (Control) and SNM-encapsulation under diffusion (SNM, Diff) as glucose dropped low (Immediate Shutdown & Maximum Shutdown). (Mean±SEM, n≥3, *p<0.05)

Figure S3. *In vitro* viability of mouse islets

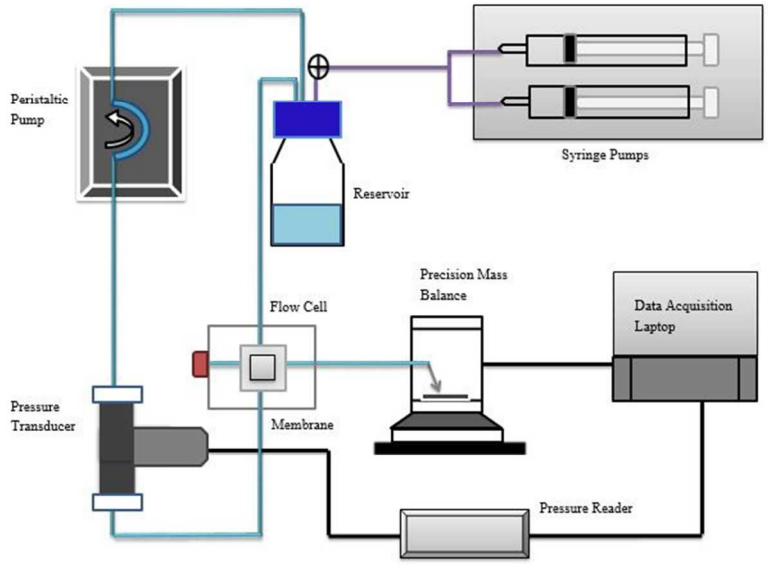


a. Viability of mouse islets was measured following the 90-minute low-high-low (1.6 mM, 16.6 mM, 1.6 mM) glucose stimulation in which islets were subjected to the mock-loop circuit with (+Ck) or without cytokine exposure for SNM- and SμM-encapsulation under diffusion (SNM, D & SμM, D). The naked islet culture under static culture with cytokine exposure (Control, +Ck) showed significantly less viability compared to all other conditions. (Mean±SEM, n≥3, *p<0.05)



b. Viable (green) and dead (red) cells were stained for control static culture without cytokines (A: Control), control static culture with cytokines (B: Control, +Ck), SNM-encapsulated mouse islets under diffusion without cytokines (C: SNM, D), SNM-encapsulated mouse islets under diffusion with cytokines (D: SNM, D, +Ck), SμM-encapsulated mouse islets under diffusion without cytokines (E: SμM, D), and SμM-encapsulated mouse islets under diffusion with cytokines (F: SμM, D, +Ck). Experiments with cytokine exposure (indicated by +Ck) consisted of media containing TNF-α, IFN-γ, and IL-1β. The control static culture with cytokines (B: Control, +Ck) showed significant level of islet damage compared to all other conditions.

Figure S4. Hydraulic permeability for SNM pore size characterization



Schematic diagram of the hydraulic permeability testing system. Air was applied through a pressure regulator into the liquid reservoir. A peristaltic pump circulated this liquid through the flow cell with enclosed membrane. The flow cell connected to a differential pressure transducer that was automatically controlled by a data acquisition laptop to adjust the transmembrane pressure. The permeated ultrafiltrate was collected into a liquid container on top of a precision

mass balance. Data from the differential pressure transducer and the mass balance were automatically collected and stored in a data acquisition laptop.

Table S1. The rate of change in insulin secretion. The rate of change in insulin production was calculated based on the slopes of curves that were fitted on glucose-insulin kinetics graphs to describe the quickness of insulin being secreted as glucose concentration changes. The SμM-encapsulated mouse islets under diffusion without cytokine exposure (SμM, Diff) showed similar rate of insulin secretion in glucose-induced stimulation and a slightly faster insulin cessation compared with the SμM-encapsulated mouse islets under diffusion with cytokine exposure (SμM, Diff, +Ck).

Experimental Condition	Low-High Glucose Stimulation (10^{-2})	High-Low Glucose Shut-Down (10^{-2})
SμM, Diff	1.13	-0.73
SμM, Diff, +Ck	1.15	-1.46