Microfluidic platform for assessing pancreatic islet functionality through dielectric spectroscopy

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Human pancreatic islets are seldom assessed for dynamic responses to external stimuli. Thus, the elucidation of human islet functionality would provide insights into the progression of diabetes mellitus, evaluation of preparations for clinical transplantation, as well as for the development of novel therapeutics. The objective of this study was to develop a microfluidic platform for *in vitro* islet culture, allowing the multi-parametric investigation of islet response to chemical and biochemical stimuli. This was accomplished through the fabrication and implementation of a microfluidic platform that allowed the perfusion of islet culture while integrating real-time monitoring using impedance spectroscopy, through microfabricated, interdigitated electrodes located along the microchamber arrays. Real-time impedance measurements provide important dielectric parameters, such as cell membrane capacitance and cytoplasmic conductivity, representing proliferation, differentiation, viability, and functionality. The perfusion of varying glucose concentrations and monitoring of the resulting impedance of pancreatic islets were performed as proof-of-concept validation of the lab-on-chip platform. This novel technique to elucidate the underlying mechanisms that dictate islet functionality is presented, providing new information regarding islet function that could improve the evaluation of islet preparations for transplantation. In addition, it will lead to a better understanding of fundamental diabetes-related islet dysfunction and the development of therapeutics through evaluation of potential drug effects. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4929652>]

INTRODUCTION

Dielectric spectroscopy of cell suspensions has been successfully employed as a non-invasive and rapid method to elucidate information pertaining to biophysical properties. This includes cell membrane thickness as well as membrane and cytoplasmic dielectric parameters.¹ Recent efforts have also been made into dielectric measurements of more complex biological tissue such as cancer spheroids in order to monitor drug effects and interactions.² Other biological processes have also been dielectrically monitored such as cell cluster formation and epithelial cell differentiation.³ An important application of dielectric cell characterization and differentiation monitoring has been applied to human pancreatic islets to assess regeneration potential. The application of dielectric spectroscopy to an electrically actuated endocrine micro-organ such as pancreatic islets demonstrates the clinical applicability of this non-invasive monitoring tool. Recent efforts have also been explored to expand the utility of dielectric biological analysis and further exploit the high spatiotemporal resolution of measurement. This includes the integration of microfluidic systems and patterned electrodes for single cell measurements⁴ and subsequent

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correlation of dielectric parameters to biological functionality, enabling quantitative biology analyses. It is therefore pertinent to also extend this dielectric evaluation of cellular functionality to more complex tissues. The pancreatic islet presents a multi-cellular endocrine tissue that is biochemically stimulated and electrically excitable. Islets are in turn responsible for homeostatic regulation of blood glucose where adverse variation in functionality is implicated in serious metabolic diseases and onset of diabetes.⁵

Glucose-stimulated insulin release (GSIR) of islets is the most common and accepted assay for determining islet functionality. Typically, islets are subjected to low and high concentrations of glucose to evaluate their insulin stimulation index.⁶ Other secretagogues, such as 3-isobutyl-1-methylxanthine (IBMX), are introduced in order to raise and subsequently assess cyclic adenosine monophosphate (cAMP)-mediated insulin secretion.⁷ An insulin secretion profile representative of a given islet population can then be determined for functionality assessment. Employing dielectric spectroscopy may serve to better evaluate efficacy of therapeutic candidates, as new screening avenues are needed to monitor, stimulate, and assess pancreatic islets *in vitro*. A main challenge is that islets experience a decline in functionality and viability *in vitro* and cannot be stored long-term.⁸ It is therefore pertinent to also implement a physiologically relevant environment *in vitro* whilst integrating biosensing methods to rapidly and accurately assess islet response to stimuli. In addition, a tool that can facilitate smaller islet samples to evaluate dynamic islet functionality over a shorter time period is required to deal with the issues of low islet isolation yields and loss of islet functionality and viability *in vitro*. Furthermore, the required tool should have screening capability for therapeutic assessment and enable facile experimental setup. Microfluidic platforms can be used to overcome these challenges, as they consume less reagents and can facilitate smaller quantities of islets. These microfluidic platforms can comprise a physiologically relevant perfusion system with integrated biosensors for analysis and assessment of a given islet population.

On-chip perfusion can be manipulated to allow controlled delivery of stimulants, such as glucose and other secretagogues, to islets. In addition, microfluidic platforms can allow real-time measurements of islet secretion through continuous sampling of the islet effluent.⁹ In terms of quantitative measurement, enzyme-linked immunosorbent assay (ELISA) and western blots are common standard techniques for quantification of insulin secretion, providing preliminary assessments of functionality. Although mature and specific, these techniques lack dynamic assessment, are costly and time-consuming, and they involve multistep protocols that often result in error.¹⁰ To remedy these limitations, many microfluidic platforms have been developed to assess islet functionality by coupling with techniques including zinc sampling,¹¹ insulin ELISA,¹² Ca^{2+} ,^{13,14} and NADPH imaging.¹⁵ Many of the challenges with dynamic islet functionality analysis using insulin ELISA still remain when coupling with microfluidic systems. Although Ca^{2+} and NAD(P)H imaging can illuminate the complex intracellular mechanisms in association with insulin secretion, these imaging techniques require the use of confocal microscopy or two photon microscopy.¹⁶ The method of islet immobilization on different microfluidic platforms varies, including use of mechanical actuators¹⁵ and well arrays.^{9,17} Using actuators requires complicated islet loading procedures, prevents good perfusion around the islets, and subjects islets to mechanical stress. Well-type arrays enable immobilization of a small but sufficient quantity of islets for batch analysis of islet preparations. A critical parameter is the well diameter in order to facilitate single islet capture and prevent islet-islet binding. In addition, various means of microfluidic media delivery to islets exist, including continuous flow and fluid plugs.¹³ Creating optimal stimulation conditions for islet batches is simpler to achieve with continuous flow rather than with fluid plugs.

Given the challenges of evaluating the islet functionality of isolated batches, what is needed for measuring islet functionality is a rapid, simple, cost-effective, and noninvasive technique. Dielectric impedance spectroscopy could potentially fulfill this need. During dielectric impedance spectroscopy measurements, the sample is subjected to a frequency swept voltage signal while the resulting current is recorded, and the magnitude and phase of the current are converted into a dielectric impedance spectrum. Dielectric impedance spectroscopy is already a well-established technique for measuring a range of cell activities, including differentiation,

proliferation, morphology, migration, regeneration, and cell death.¹⁸ Given electrical characteristics of islet response, this technique provides a suitable non-invasive and label-free method for measurement of cell membrane dielectric parameters, which could be related to stimuli response and functionality assessment. Combining this technique with microfluidics allows the creation of an environment that facilitates islet capture with a continuous delivery of stimulants and real-time dielectric recording of islet response. Herein, using cleanroom microfabrication techniques, we present a microfluidic platform with these properties. An array of wells situated on the device captures individual islets in each well and enables stimulation of the islets under continuous media perfusion. Electrodes are situated directly beneath the wells for real-time measurement of dielectric impedance spectra. Islet insulin secretion has significant and measurable effects on the dielectric impedance spectra. With further studies to correlate dielectric signals with signaling effects, this technique could yield a potential tool to study islet functionality rapidly and noninvasively, providing a useful tool for drug screening applications and transplantation.

MATERIALS AND METHODS

Device preparation

In the Electrodes, Chamber wells, Mold preparation, PDMS channel preparation, and Device assembly sections, the construction of the microfluidic platform is presented in several steps: the fabrication of electrodes, wells, the polydimethylsiloxane (PDMS) mold, and the PDMS channels and the assembly of the device. The process is represented in Fig. 1(a) and Fig. S1 in the supplementary material.³⁴

Electrodes

The electrodes were prepared in a cleanroom environment. They had an interdigitated configuration with an electrode width of $300\text{ }\mu\text{m}$ and a separation of $200\text{ }\mu\text{m}$. Glass wafers (127 mm diameter, 1.95 mm thick), used as substrates for the electrode preparation, were cleaned with acetone and isopropyl alcohol (IPA) and air-dried. The substrate was then heated

FIG. 1. Microfluidic device fabrication and on-chip islet immobilization. (A) Left: Bare electrode on glass substrate. The electrode digit width is $300\text{ }\mu\text{m}$ with a separation of $200\text{ }\mu\text{m}$. Centre: Su-8 layer with wells ($300\text{ }\mu\text{m}$ diameter) on top electrodes. Right: PDMS channel layer bound on top wells and electrode. (B) Left: Close-up of well array on the electrodes. Right: Close-up of microfluidic channels on top well array. (C) Left: Electrode covered with a layer of empty wells prior to islet loading. Top right: Microfluidic device with islets immobilized within the chamber wells. Bottom right: Magnified image of an islet captured in a well.

to 100 °C to evaporate any solvents and then cooled in preparation for spin coating. LOR5B™ (Microchem, Newton, MA) was spin-coated (1000 rpm, 30 s) onto the substrate and baked at 180 °C for 5 min. The substrate was cooled in preparation to spin-coat the next layer. Microposit™ S1813™ (Shipley, Marlborough, MA) was spin-coated (3950 rpm, 30 s) and baked at 115 °C for 1 min. The substrate was then exposed to UV light (124.3 mJ/cm²) under a chrome photomask with the electrode patterns. Finally, the substrate was developed in Microposit MF®-319 (Shipley) for 50 s. The substrate was immediately washed with deionized water and air-dried. A 10 nm titanium adhesion layer and a 50 nm gold layer were electron beam deposited onto the substrate. The substrate was then immersed in Microposit Remover 1165 (Shipley) at 70 °C to lift off the metal layer and yield the patterned electrodes. The sample was removed from the cleanroom and the substrate was cut, using a diamond saw, into individual devices (43.5 cm × 31.5 cm) containing two electrodes each—one for performing raw islet measurements, while the other is used to determine blank media values in order to arrive at differential impedance data.

Chamber wells

The wells were designed with a diameter of 300 μm and a depth of 200 μm. First, the devices were treated with piranha solution (3:1 mixture of sulfuric acid and hydrogen peroxide) to clean the surfaces. Inside the cleanroom, the devices were cleaned with acetone and IPA and air-dried. The devices were then heated to 100 °C to evaporate any solvents and cooled in preparation for spin-coating. A first Su-8 2050 (Microchem) layer was spin coated at 2000 rpm and baked at 65 °C for 4 min and 95 °C for 12 min. The device was cooled in preparation to spin-coat the next layer. This process was repeated to create a second Su-8 2050 layer. A third layer was spin-coated at 3000 rpm and baked at 65 °C for 5 min and 95 °C for 15 min. The device was then exposed to UV light (525 mJ/cm²) under a 350 nm long-pass filter and a patterned chrome photomask. Microscopic alignment was performed to position the wells directly above the electrodes. After exposure, the sample was baked at 65 °C for 10 min and 95 °C for 20 min. The device was then maintained at 65 °C for 5 min and cooled for 5 min using a chill plate to reduce thermal stress. The device was developed in Su-8 developer (Microchem) for ~25 min and then washed with more Su-8 developer and IPA. When washing with IPA, a milky substance seeping from the devices is an indication that the Su-8 2050 is not sufficiently developed and that the device must be put back into the developer. If a milky substance does not appear, the device is air-dried and then removed from the cleanroom. Note that the quality of the Su-8 layer is highly dependent on the substrate dimensions, bake times and temperatures, and UV exposure time. Deviation from a successful fabrication protocol could result in cracks in and delamination of the Su-8 layer. All conditions were optimized to prevent these problems from arising.

Mold preparation

The channels were designed as a serpentine path with a width of 400 μm and height of 225 μm. The mold for the PDMS channels was prepared in a cleanroom environment. A silicon wafer was used as a substrate. The substrate was cleaned with acetone and IPA, heated to 100 °C to evaporate any solvents, and then cooled in preparation for spin-coating. A first Su-8 2050 layer was spin-coated at 1000 rpm and baked at 65 °C for 7 min and 95 °C for 30 min. The substrate was then cooled in preparation to spin-coat the next layer. A second layer was spin-coated at 3000 rpm and baked at 65 °C for 2 min and 95 °C for 8 min. The substrate was then exposed to UV light (350 mJ/cm²) under a 350 nm long-pass filter and a patterned chrome photomask. The substrate was developed in Su-8 developer for 17 min and then washed with more Su-8 developer and IPA. The substrate was air-dried and then removed from the cleanroom. Outside the cleanroom, the substrate was baked at 65 °C for 30 min. Then, trichloro (1H 1H 2H 2H perfluorooctyl) silane was vacuum-deposited onto the substrate to prevent PDMS adhesion.

PDMS channel preparation

PDMS was prepared by mixing the base and curing agent at a 10:1 weight ratio. The PDMS was then poured over the mold, degassed to remove any bubbles, and oven cured overnight at 60°C. The PDMS was then peeled from the mold and cut into individual channels. Holes were punched at the inlets and outlets of the PDMS channels with a 1.2 mm coring tool. PDMS and curing agent were obtained from Dow Corning (Midland, MI).

Device assembly

The Su-8 well layer on the device was bound to the PDMS channel layer. First, the devices were cleaned with IPA and air-dried, after which (3-Aminopropyl) triethoxysilane (APTES) (Sigma Aldrich, St. Louis, MO) was vacuum-deposited onto the devices for 2 h. The PDMS channel layer was exposed to O₂ plasma to activate the surface. Using a microscope, the PDMS channels were manually aligned with the wells on the device and then bound together. The assembled device was kept at 70°C under a force of 30–60 N for 15 min.¹⁹ The devices were removed from the hotplate, and tygon tubing (ID: 0.02 in., OD: 0.06 in.) was inserted into the inlets and outlets of the device.

Human islet isolation procedure

Human pancreases were retrieved from brain-dead cadaveric multi-organ donors under the coordination of Transplant Quebec after the expressed research consent was obtained. Testing was conducted on all of the organ donors, and only pancreases from donors suitable for clinical solid organ transplant were retrieved. The cold ischemic time between aortic cross-clamp and the initiation of the warm closed-loop pancreas digestion was kept below 8 h. All steps were carried out aseptically in the cleanroom facility at McGill University Health Centre Human Islet Transplant Laboratory (MHITL). Pancreatic islets of Langerhans were isolated, purified, and cultured as described previously.²⁰ Briefly, after inspection and surface decontamination of the pancreas was performed, the spleen, duodenum, and extraneous tissues were removed. The pancreas was loaded intraductally with cold Clzyme (Collagenase HA; VitaCyte LLP, Indianapolis, IN) and neutral protease (Serva Electrophoresis GMBH, Heidelberg, Germany) enzymes. Once properly distended, the pancreas was cut into pieces and transferred to a digestion chamber for warm digestion at 37°C in a closed-loop circuit. Upon identification of approximately 50% free, intact islets, digestion was stopped using ice-cold dilution solution containing 10% normal human AB serum. The digest was collected in 250 ml centrifuge tubes, pelleted, and washed with cold media containing 10% normal human AB serum to combine the pellets. No more than 20 g of pellet per purification run was centrifuged, using a COBE 2991 cell processor on a continuous density (iodixanol) gradient. After a 5 min centrifugation at 2400 rpm, purified fractions were collected and the gradient was washed off. Fractions of varying purity were quantified (zinc-chelating dye dithizone) and cultured in separate non-treated 175 cm² cell culture flasks with vented caps. Islet fractions with over 85% purity were routinely obtained. Release testing was performed at the MHITL to assess preparation for viability by fluorescence-based membrane exclusion dye (ethidium bromide, dead cells; SYTO-13, live cells) and GSIR assays. Samples of pre-culture preparations were sent off to microbiology for confirmation of sterility.

Islet culture and microfluidic GSIR assay

Upon receiving islets from the MHITL, the medium was replaced with recovery media supplemented with human serum albumin (2.5%). The islets were stored in non-adherent flasks in an incubator set at 37°C and 5% CO₂. All experiments were performed 48 h post-isolation to ensure sufficient cellular recovery. Islet stimulation medium for the experimental run was prepared using Krebs solution as a base. Krebs solution was prepared using NaCl [137 mM], KCl [4.7 mM], KH₂PO₄ [1.2 mM], MgSO₄·7H₂O [1.2 mM], CaCl₂·2H₂O [2.5 mM], and NaHCO₃

[25 mM]. The pH of the solution was adjusted to 7.4 by adding NaOH and HCl as required. The Krebs solution was used to make solutions containing low glucose [2.8 mM], high glucose [16.7 mM], and high glucose with IBMX [50 μ M]; 0.1% BSA was added to each solution. The solutions were sterile filtered in a tissue culture hood and kept in a refrigerator until use. All chemicals were obtained from Sigma Aldrich.

In preparation for loading onto the microfluidic platform, the islets were aspirated with a pipette and placed into a syringe without a plunger. The needle of the syringe was attached to the inlet tubing of the device, and a peristaltic pump was placed at the outlet of the device to pull islets from the syringe into the inlet tubing. The islets then entered the device and settled into subsequent wells, as shown in Fig. 1(c). Once all of the islets settled into wells, the device was attached to the impedance analyzer through an electrical interface. This interface is composed of spring loaded pins that via shielded cables connects one electrode to the impedance analyzer's high voltage/high current terminal, while the other electrode is connected to the low voltage/low current terminal. The device and the electrical interface were then kept in an incubator at 37°C and 5% CO₂ for the duration of the experiment, with the perfusion rate set to 10 μ l/min. The islets were stimulated with low glucose for 1 h to adapt them to the new environment. Continuous dielectric impedance measurements were recorded with a spectrum every minute, using an Agilent 4294A impedance analyzer (Agilent, Palo Alto, CA) for the GSIR assay. The spectra were recorded using 201 points/spectra at a frequency range of 40 Hz–10 MHz and at an amplitude of 100 mV. During the glucose challenge test, the islets were stimulated for 36 min with each of the following glucose concentrations: low glucose, high glucose, high glucose with IBMX, and low glucose. Islet effluent was collected in tubes at 6-min intervals. The tubes were then used for ELISA quantification of secreted insulin.

Characterization of dielectric spectra

Dielectric spectra were characterized using the Cole–Cole equation with two terms to characterize the double dispersion. Obtaining the Cole–Cole parameters from the spectra allows a quantitative approach for tracking changes in both dispersions throughout the experiment

$$\varepsilon^* = \varepsilon_h + \frac{\Delta\varepsilon_1}{\left(1 + i\frac{f}{f_{c1}}\right)^{\beta_1}} + \frac{\Delta\varepsilon_2}{\left(1 + i\frac{f}{f_{c2}}\right)^{\beta_2}} + \frac{\kappa_l}{i\varepsilon_0 2\pi f}. \quad (1)$$

In Eq. (1), ε^* is the complex permittivity, $\Delta\varepsilon$ is the intensity of the dispersion, ε_0 is permittivity of a vacuum, f_c is the characteristic frequency, β is the Cole–Cole parameter ($0 < \beta < 1$), $i^2 = -1$, ω is angular frequency, where $\omega = 2\pi f$, κ_l is the low-frequency limit of conductivity, and f is the frequency of the applied field. The subscripts 1 and 2 denote the first and second dispersions, respectively. The dielectric spectra were fitted to the Cole–Cole equation using the Levenberg–Marquardt least squares algorithm in MATLAB.

Statistical analysis

The number of biological replicates is $n = 3$ for all experiments performed. Statistical significance was determined by one-way analysis of variance (ANOVA) with *post hoc* Bonferroni's test, or paired Student's *t* test, when applicable. Differences were considered significant when $P < 0.05$ or less. Data fitting to the Cole–Cole relation possessed a R^2 value of > 0.99 .

RESULTS AND DISCUSSION

Device design and simulations of fluid flow and shear stress

Design of device

The first consideration of the platform design was to enable testing of the functionality of a population of islets, giving more representative results for an isolated batch. Hence, a 15×15

array of wells was chosen to randomly capture numerous islets and subject them to fluid flow from a single channel. The size of the wells was selected at $300\ \mu\text{m}$ to standardize islet functionality through size normalization. Larger islets $>300\ \mu\text{m}$ do not settle in wells and are removed with the effluent. This is beneficial considering that larger islets $>300\ \mu\text{m}$ (classes 7 and 8) are less responsive to glucose stimulation and in turn secrete less,²¹ leading to a less representative analysis of islet functionality. The selective capture in the wells removes variation of islet response due to size, producing a comparative analysis by establishing a baseline of islet functionality by the most physiological abundant and prevalent size distribution. The device design was optimized to reduce shear stress, ensure sufficient mass transfer to the islets, and maximize dielectric signal penetration into the islets (Figs. S2, S3, and S4 in the supplementary material).³⁴

Insulin release

The line plot data shown in Fig. 2(a) show the insulin release profile from the islets throughout the glucose challenge test. These measurements were obtained from insulin ELISA using effluent from the microfluidic device. Of particular importance are the large spikes in insulin secretion in the middle of the test, when the islets were subjected to high glucose stimulation, followed by the largest spike in the presence of IBMX. The insulin release was also represented in the traditional manner, by averaging the insulin secretion over 36 min intervals. As expected, the averaged secretion plot showed the highest insulin release during high glucose and IBMX stimulation, with lower averaged secretion during the low glucose stimulation. The expected insulin release profile confirms that the device does not hinder normal islet functionality. In addition, since this batch of islets is functional, they are ideal for exploring the behavior of dielectric spectra during a glucose challenge test.^{22,23} The insulin release is highly temporal and variable, as seen in the 6 min interval line plot. When insulin release was represented in the traditional manner, by averaging insulin secretion over a 36-min interval, the variation is hidden, as demonstrated by the bar plots shown in Fig. 2(a). As such, the sensitivity and resolution of ELISA are lost when performing traditional cluster analysis.²³ In clinical studies, C-peptide measurements taken from human volunteers over several hours have demonstrated that insulin secretion is pulsatile both after meals and in non-stimulatory conditions.²⁴ Thus, these findings illustrate that insulin secretion from the native pancreatic islets is pulsatile

FIG. 2. Islet insulin release profile during microfluidic GSIR assay and dielectric spectra obtained on-chip. (A) The insulin stimulation index for the GSIR assay is presented in the traditional manner (bar graphs representing averaged insulin release in 36 min intervals for each stimulation). Also, superimposed is a plot of the temporal 6-min interval in measuring the insulin release for the same GSIR assay. The insulin release data were obtained from insulin ELISA. (B) An example of capacitance spectra for a blank (islet-free) microfluidic device, a device filled with islets (raw) and resulting differential spectrum showing distinct dispersions. The differential spectrum was taken by subtracting the blank measurement (stimulation media) from the raw islet measurement at the 45-min mark (during high glucose stimulation). Note that the differential spectrum is free of the effects of the double layer capacitance and yields a defined double dispersion.

in vivo. Pulsatile secretion has also been observed under *in vitro* conditions using isolated human islets in perfusion chambers.²⁵ It is thus imperative to take into account pulsatile/variable secretion patterns and the physiological triggers that cause them, in order to arrive at a more accurate description of functionality.

Due to the illustrated variable and pulsatile nature of insulin secretion, quantifying insulin secretion to determine islet quality can be inefficient and often not reproducible. The variable secretion shows a more complex relationship between stimulation and secretion, making it more challenging to correlate both. For instance, when the islets were subjected to 16.7 mM high glucose stimulation, insulin secretion was sometimes low, being in the trough between peaks. The traditional ELISA tests for insulin secretion, which average insulin secretion over a greater time interval during static conditions, do not resolve these oscillations. Taking dynamic insulin measurements continuously over a shorter time period requires a more tedious preparation of the ELISA setup and presents difficulties in detecting smaller volumes of insulin samples. Dielectric spectroscopy, on the other hand, provides measurements with high temporal resolution (1 min for this particular analyzer). With greater understanding of the spectra, there is potential for dielectric spectroscopy to provide a simpler and more rapid technique to assess islet secretion, and in turn, functionality, during a glucose challenge test.

Dielectric spectra

Dielectric spectra of islets during GSIR

First, the dielectric effect of the Su-8 2050 well array layer was determined by comparing the spectra with that of bare electrodes. The Su-8 2050 raises the capacitance over the entire spectrum in the order of picofarads, which is negligible compared to the capacitance of the media. For islet experiments, the recorded dielectric spectra were plotted, using MATLAB, to observe changes in the dispersion patterns during glucose stimulations throughout the course of the experiment. Blank spectra, without islets, were recorded on the microfluidic platform using the same experimental conditions as with islets. Hence, the device was kept in an incubator at 37 °C and 5% CO₂ while perfusing for 36 min of each: 2.8 mM low glucose, 16.7 mM high glucose, 16.7 mM high glucose/50 μ M IBMX, and 2.8 mM low glucose. The dielectric spectra shifted little during this experiment, illustrating that the glucose and IBMX concentrations do not have a major role in changing the shape of the dielectric spectra. When subtracting the islet spectra from the blank spectra (yielding the differential spectra), the effect of the double layer capacitance at low frequency (LF) was eliminated, yielding clearly distinguishable dispersions arising from stimulated islets, as shown in Fig. 2(b), using example spectra extracted from islet stimulation with high glucose during the experimental glucose challenge. These two dispersions represent the islet contribution to the dielectric spectra with the effects of the media removed.

Following the subtraction of the blank from islet measurements, the differential spectra were separated into four plots based on the islet stimulation: initial low glucose, high glucose, high glucose/IBMX, and final low glucose (Fig. 3). The plots enable the observation of shifts within the spectra throughout the experiment. At the beginning, during the low glucose measurements, little change was observed in the spectra. When the islets were stimulated with high glucose, large shifts in the spectra were observed, making the dispersions more pronounced. These shifts were also observed during the high glucose/IBMX and the final low glucose stimulation, in which the dispersions become less pronounced and then more pronounced again. During the final low glucose stimulation, the dielectric spectra shifted towards higher frequency.

Dispersions are typical for dielectric spectra obtained for biological cells. Two important dispersions in the frequency range used here are the α - and β -dispersions. In previous studies using *E. coli* cells, the α -dispersion was found to be caused by the ion cloud outside and/or inside the cell wall, while the higher-frequency β -dispersion was caused by charging at the membranes.²⁶ Each of these dispersions can also have sub-dispersions. To understand the dispersions obtained in this experiment, the data were compared with that of previous studies on multicellular clusters. For instance, Asami and Irimajiri²⁷ obtained dielectric measurements of the embryogenesis of frog eggs. During the morula phase, consisting of cells (blastomeres) aggregated in a spheroid, a

FIG. 3. Variation of the differential islet dielectric spectra throughout the on-chip GSIR assay. The first recorded spectrum is blue, the last spectrum is red, and each subsequent spectrum is an additional color hue. The plots are divided based on the perfused stimulation media, as indicated in each. Note the (1) initial low glucose stability in terms of electrical properties, (2) electrical property shift during high glucose, (3) shift in high/IBMX towards less pronounced then more pronounced dispersion again, indicating cell signaling in response to stimulation, and (4) the reversion back to the same electrical state as first low glucose.

double dispersion was observed in dielectric spectra.²⁷ Moreover, using finite element analysis (FEA) modeling, computer generated dielectric spectra were obtained from the vesicle inclusion model (illustrated in Fig. S5A in the supplementary material),³⁴ which consists of a single shell surrounding membrane-bounded vesicles.²⁸ Typically, the model is represented with an outer shell and vesicle membranes possessing low conductivity, while the surrounding medium and internal regions are highly conductive. Irimajiri *et al.*²⁸ showed that when the vesicle size was reduced in the vesicle inclusion model, thus increasing the number of vesicles, the dielectric spectra separated into two β -sub dispersions. This model was translated for islets and used in this study, with the numerous packed vesicles representing individual islet cells. The outer shell accounts for the basement membrane composed of extracellular matrix (ECM), surrounding the inner cell cluster—the dielectric parameters of which may be estimated to be equivalent to the high conductivity of extracellular media and cell cytoplasm. Past studies have shown the negligible contribution of basement membrane on dielectric relaxation of enclosed cell clusters.³ Given these findings, in our own vesicle inclusion model, we effectively eliminated the dielectric contribution of the outer membrane by setting its dielectric properties equivalent to the surrounding media. As a result, our computer generated dielectric spectra contain only a single dispersion. Previous dielectric modelling work included gap junctions that are not accounted for in the vesicle inclusion model. The high LF sub-dispersion was due to the gap junctions interconnecting cells within the cluster—resulting in high charge density accumulation,²⁹ while high frequency (HF) sub-dispersion was caused by the cell-cell interactions, resulting in dipole-dipole relaxation.²⁹ We therefore note that the two dispersions observed in this study represent the two aforementioned HF and LF β -sub dispersions. These sub-dispersions dominate that the spectra and other dispersions, such as the α -dispersion, are too insignificant to be detected. The distinct β -dispersions representing islet cell clusters may be utilized to further delineate functionality and response to stimuli based on cell membrane and cytoplasmic dielectric parameters.

Interpreting shifts in islet dielectric spectra during GSIR

Because insulin granules fuse with the cell plasma membrane, they alter the membrane capacitance, which we believe contributes to the large shifts in the dielectric spectra. This could

explain why the dielectric spectra are relatively stable during the initial low glucose stimulation and then shift towards lower frequencies during high glucose stimulation. Previous studies support these claims, in which step increases in membrane capacitance were observed almost instantaneously when inducing depolarization in human β -cells.³⁰ Membrane capacitance measurements have also been able to resolve exocytotic fusion of single insulin vesicles and endocytotic events in mouse β -cells, observed as membrane capacitance on-steps and off-steps, respectively.³¹ Because our experiment was composed of several islets, the data reflect the combined membrane capacitance changes due to exocytotic and endocytotic events of multiple β -cells. This could explain the oscillatory behavior of the cell dielectric parameters, which will be discussed in subsequent sections. The exocytotic events should be particularly pronounced during high glucose and high glucose/IBMX stimulation, as shown in the insulin release profile in Fig. 2(a). However, the dielectric spectra shift in a different manner during high glucose/IBMX stimulation as compared to high glucose stimulation. This is not surprising given the different biochemical reactions and mechanisms that occur between glucose-stimulated insulin release and IBMX/cAMP-mediated insulin release.

Furthermore, membrane recovery and endocytosis should have opposite effects on membrane capacitance and permittivity, which could shift the dielectric spectra back to its baseline during reduced secretion. Indeed, previous research has reported a “recovery” of β -cell membrane capacitance sometime after stimulation for mouse³¹ and rat³² islets. The shift in the dielectric spectra towards higher frequencies during final low glucose stimulation (Fig. 3) could represent membrane recovery after prolonged stimulation. Other mechanisms besides changes in membrane capacitance can contribute to shifts in islet dielectric spectra. We demonstrated how islet substructures affect the dielectric spectra using analytical models in supplementary material Fig. S5.³⁴ Our own analytical modelling, along with previous computer simulations of the double shell model, showed that the conductivities of the cell membrane and cytoplasm all had large effects on the dielectric spectrum.³³ As such, we also believe that ion channel activities prior to secretion and during IBMX stimulation can affect membrane and cytoplasm conductivity and alter the dielectric spectra. This effect can explain some of the large shifts in the dielectric spectra during and after IBMX stimulation. However, we do not rule out the possibility that more complex mechanisms within the islet contribute to shifts in the dielectric spectra, such as recruitment and trafficking of the low-conductivity insulin vesicles.

Dielectric parameters of islet dispersion from Cole-Cole fitting

Each dielectric parameter of the Cole–Cole equation changes with a unique pattern during the glucose challenge test, reflecting changes in the islet electrical properties related to stimulation and secretion. These parameters provide a more precise and temporal characterization of islet response to stimuli. These parameters—the first and second dispersion intensities ($\Delta\epsilon_{1,2}$), characteristic frequencies ($f_{c1,2}$), and Cole-Cole parameters ($\beta_{1,2}$)—are plotted in Fig. 4. The intensity of the first dispersion is about an order of magnitude larger than that of the second dispersion. This result parallels a previous study that measured the embryogenesis of frog eggs in the morula stage, in that the intensity of the first dispersion becomes progressively larger than that of the second dispersion.²⁷ All of the dielectric parameters were relatively stable below 36 min during low glucose stimulation, reflecting the small change in dielectric spectra for this time period, shown in Fig. 3, and reduced insulin secretion, shown in Fig. 2(a). After introducing high glucose to the microfluidic device, islet response is triggered and there is a large increase in insulin secretion. The dielectric spectra start shifting, which is reflected in the large, even step-like changes in all dielectric parameters at around 36 min. At this point, the dielectric parameters could reflect increases in membrane capacitance due to insulin vesicle fusion events and changes in ion channel activity. After islets were stimulated with IBMX, insulin secretion increased further and the dielectric spectra started moving back towards higher frequencies. The rapid changes in the first characteristic frequency and the first Cole-Cole parameter after 80 min could reflect IBMX associated islet response. When the islets are stimulated once again with low glucose, the dielectric spectra shift towards higher frequencies. At this point, around

FIG. 4. Variation of dielectric parameters from the Cole-Cole equation during the on-chip islet GSIR assay. These parameters are the first and second dispersion intensities ($\Delta\epsilon_{1,2}$), characteristic frequencies ($f_{c1,2}$), and Cole-Cole parameters ($\beta_{1,2}$).

108 min, large changes in the dielectric parameters are exhibited, particularly in the characteristic frequency of the first and second dispersions and the first Cole-Cole parameter. As previously discussed regarding past studies on single β -cells, the cell membrane does not recover immediately after increased secretion, which could explain why the dielectric parameters continue changing during renewed low glucose stimulation.

The Cole-Cole parameter patterns of both dispersions reflect the widening and sharpening of dispersions at the various stages of glucose stimulation—analogue of spectral changes as shown in Fig. 3. These variations are indicative of changes in cellular properties arising from secretory patterns such as the increased insulin release of 36 min following low-to-high glucose stimulation—which may be utilized as an indication of functional islet stimulation index. Therefore, the correlation of islet cellular dielectric parameter variation in response to glucose stimulation may provide a rapid and facile methodology of evaluating islet functionality, as illustrated by quantifiable and significant peak shifts indicating dynamic secretory responses.

CONCLUSIONS

In this work, we presented a microfluidic device with on-chip dielectric spectroscopy sensors as a viable platform with the potential to assess islet functionality and response to glucose stimuli. Within the microfluidic device, islets are immobilized under constant perfusion in order to simulate physiological conditions. Dielectric spectroscopy was chosen as an exploratory method to assess islet functionality, as it is a rapid, real-time, and noninvasive technique.

Electrical simulations showed that the configuration of the device's electrodes and wells enable sufficient electric field penetration into the islet so as to permit detection of changes in islet membrane permittivity. The islets create a characteristic double dispersion in the dielectric spectra, and these dispersions are sensitive to the islet response during a glucose challenge test. The vesicle inclusion model demonstrated that changes in islet membrane permittivity resulting from the fusion of insulin vesicles during secretion affect the dielectric spectra. Hence, we demonstrated that dielectric spectroscopy has potential to track islet stimuli response. With further understanding of the relationship between dielectric spectroscopy and islet functionality, this platform has numerous applications for islet research. These applications include screening islet quality prior to transplantation and in other situations where islet secretion assessment is needed, such as during drug screening and islet regeneration studies.

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