Elaboration of a Finite Element Model of Pancreatic Islet Dielectric Response to Gap Junction Expression and Insulin Release

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Graphical Abstract

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Abstract

Dielectric spectroscopy could potentially be a powerful tool to monitor isolated human pancreatic islets for applications in diabetes therapy and research. Isolated intact human islets provide the most relevant means to understand the cellular and molecular mechanisms associated with diabetes. The advantages of dielectric spectroscopy for continuous islet monitoring are that it is a non-invasive, inexpensive and real-time technique. We have previously assessed the dielectric response of human islet samples during stimulation and differentiation. Because of the complex geometry of islets, analytical solutions are not sufficiently representative to provide a

pertinent model of islet dielectric response. Here, we present a finite element dielectric model of a single intact islet that takes into account the tight packing of islet cells and intercellular junctions. The simulation yielded dielectric spectra characteristic of cell aggregates, similar to those produced with islets. In addition, the simulation showed that both exocytosis, such as what occurs during insulin secretion, and differential gap junction expression have significant effects on islet dielectric response. Since the progression of diabetes has some connections with dysfunctional islet gap junctions and insulin secretion, the ability to monitor these islet features with dielectric spectroscopy would benefit diabetes research.

Introduction

In vitro monitoring of isolated pancreatic islets has enabled better understanding of the cellular and molecular mechanisms related to the progression of diabetes mellitus. Currently, isolated intact human islets provide the best means of studying relevant cellular processes. Indeed, the choice of analytical methodology and technique is critical given the challenge of non-invasively monitoring islets – owing to their inherent complexity and functionality as micro-organs comprising various cell types. *In vitro* monitoring has many applications in developing islet specific treatments for diabetes and is already well established for type 2 diabetes drug discovery (1). Another application involves islet transplantation, which is a promising treatment for type 1 diabetes (2, 3). Prior to transplantation, it is vital to assess islet functionality and viability to optimize patient outcomes (3). For this task, *in vitro* analysis of isolated islet batches includes insulin secretion and oxygen consumption assays. Lastly, another emerging field encompasses developing techniques for islet cell regeneration and replacement (4-6). These techniques could yield tissues that display many of the features of islet cells, including glucose dose dependent insulin secretion. Hence, these tissues could substitute freshly isolated cadaveric islets for transplantation, overcoming challenges created by donor islet shortages and the lack of reliable methods to culture islets long term. *In vitro* monitoring is also used to identify the biochemical pathways and environmental cues involved in islet cell regeneration. Furthermore, to determine their suitability as islet substitutes, the functionality and phenotype of regenerated tissues must be compared against freshly isolated islets. This comparison is performed *in vitro* as a starting point, followed by more complex analyses involving transplantation into animal models.

Traditional assays for *in vitro* islet monitoring include those that assess hormone secretion, transcription factors, gene expression, cell metabolism and cell viability. These assays yield important information regarding islet response, but are often slow, expensive and require complex multistep preparations.

In vitro islet analyses would benefit from the development of real-time, facile and high throughput analytical tools that overcome the shortcomings of traditional assays. Due to the electrically excitable nature of β -cell insulin secretion, electric based analytical tools supply relevant information about islet activity *in vitro*. One electric based analytical tool is dielectric spectroscopy that could provide a rapid, non-invasive and label free method to assess intact islets. Indeed, using dielectric spectroscopy, we have measured the dielectric response of human islets *in vitro* and demonstrated that dielectric spectra obtained from islet samples are influenced by the islet stimulation state (7).

Insulin exocytosis could possibly be detected using dielectric spectroscopy due to secretion associated changes in β -cell membrane capacitance, as has been previously established with patch clamp measurements (8). This type of analysis is important since dysfunctional insulin secretion is a major factor in diabetes. Islet therapies can be developed by observing the insulin secretory response to secretagogues, drugs, biochemical agents and the extracellular environment. By taking advantage of the rapid and real-time capability of dielectric spectroscopy, large quantities of islet targeting drug candidates could be screened rapidly, speeding up drug development. Dielectric analysis of islet insulin secretion could also allow the evaluation of the functionality of isolated islets prior to transplantation into diabetic patients.

Another potential application for dielectric spectroscopy is for analyzing gap junctions, which are situated in the cell membrane and allow ions to move between adjacent islet cells. Gap junction activity has been shown to electrically couple islet cells, to synchronize β -cell calcium ion oscillations and to affect insulin secretion (9). For instance, in cell lines that exhibit glucose induced insulin secretion, loss of the gap junction protein connexin36 (Cx36) was implicated in altered insulin release and de-synchronization of Ca^{2+} oscillations (10). In another insulin secreting cell line, both increased or decreased levels of Cx36 protein resulted in reduced insulin content and glucose induced insulin release (11). Likewise, islet Cx36 knockout mouse models exhibited suppressed glucose induced insulin secretion (12). These studies suggest an important role of gap junctions in regulating islet functionality. Moreover, islet cells have been shown to

differentially express Cx36 in response to stimulation and environmental factors. In one study, Cx36 mRNA levels in human pancreatic islets correlated with insulin expression (13). Additionally, environmental factors such as chronic hyperglycemia and conditions of lipotoxicity have a suggested effect on Cx36 expression (14). Hence, in order to provide an analysis of islet functionality and stimulus response, dielectric modelling of islets needs to take into account gap junction activity.

Dielectric spectroscopy could further clarify the relationship between gap junction expression and islet functionality. Gap junction dysfunction, which desynchronizes β -cells and affects secretion, could be assessed dielectrically to develop islet pathological models. This type of investigation is particularly relevant given the putative relationship between islet gap junctions and diabetes (14, 15). As such, gap junctions are an interesting target for pharmacological modulators (14). The effects of environmental factors, particularly those that arise during diabetes, and glucose stimulation on islet gap junction expression could also be further elaborated.

Since dielectric spectroscopy lacks the direct measurements offered by traditional assays, dielectric models are required to interpret the dielectric response of islets in terms of their physiology. Dielectric models for complex cell aggregates are well established (16) and are useful since they approximate islet morphology. These analytical models are derived from the Maxwell Wagner Effect and yield electrical parameters that can be translated into physiological mechanisms, for example, those occurring during islet insulin secretion. Secretion related mechanisms include insulin vesicle-cell membrane binding activity, which increases membrane capacitance, an electrical parameter. An applicable analytic model for islets is the vesicle inclusion model (17) comprising a membrane bound suspension of vesicles with the vesicles representing single cells. However, the analytical models are not sufficiently representative of islets as they do not account for electrically significant structures that connect islet cells, such as tight and gap junctions (18).

The effect of gap junctions on dielectric spectra of cells and tissues can be modelled numerically (13, 16). Numerical models also give better approximations of the cell-cell electrical interactions that occur between cells packed together by adherens and tight junctions (16). Finite element analysis (FEA) allows elaboration of models that are more representative of islet morphology and more realistically simulate islet dielectric response. In this work, we elaborate a

novel FEA model based on human islet architecture where discrete compartments represent individual islet cells, which are aggregated into a sphere to simulate the islet structure. The electrical properties were set accordingly for the intracellular and extracellular spaces, along with the cell membranes. The junctions between islet cells (gap, tight and adherens) which are well distributed across the entire islet, were determined from confocal immunofluorescent imaging of isolated human islets. Accordingly, the elaborated FEA model contains gap junctions between cells and adjoining membranes representing adherens and tight junctions. The shape of the model dielectric spectra was comparable with the experimental islet dielectric spectra obtained in our previous work (7, 18). Gap junction density between the model islet cells was varied to evaluate the effect of this parameter on the dielectric spectra. Further, to simulate insulin secretion, changes in model dielectric spectra as a function of membrane capacitance variations were investigated.

Materials and Methods

Islet Isolation and Fixation

Islets were fixed in 10% formalin for 48 hours at 4°C. The islets were then washed in phosphate buffered saline (PBS), morning and evening, for 2 days, followed by a final wash on the morning of the third day. Between these washes, islets were stored at 4°C. On the evening of the third day, the islets were placed in 70% ethanol and stored at 4°C.

Immunofluorescent (IF) Solution Preparation

10xPBS:glycine was prepared by adding NaCl (7.6g), Na₂HPO₄ (1.88g), NaH₂PO₄ (0.414g) and glycine (7.5g) to water (100ml). 10xIF-wash was prepared by adding NaCl (7.6g), Na₂PO₄ (1.88g), NaH₂PO₄ (0.414g), NaN₃ (0.5g), bovine serum albumin (BSA) (1g), Triton X-100 (2ml) and Tween-20 (0.5ml) to water (100ml). The pH of the 10xPBS:glycine wash and the 10xIF wash was adjusted to 7.4 followed by filter sterilization. Islets were stained separately for hormones (insulin, glucagon and somatostatin) and cell junction proteins (Zonula Occludens-1 or ZO-1, Cx36 and E-cadherin). For islet hormone staining, primary antibody solution was prepared by adding rabbit polyclonal anti insulin (1:100), mouse monoclonal anti glucagon (1:100) and rat monoclonal anti somatostatin (1:200) to goat serum. For islet hormone staining, the respective

secondary antibody solution contained goat anti rabbit Cy3 (1:400), goat anti mouse Alexa Fluor 647 (1:1000) and goat anti rat FITC (1:400) in goat serum. For cell junction staining, primary antibody solution was prepared by adding rabbit polyclonal anti ZO-1 (1:200), mouse monoclonal anti Cx36 (1:200), and rat monoclonal anti E-cadherin (1:200) to goat serum. For cell junction staining, respective secondary antibody solution contained goat anti rabbit Cy3 (1:1000), goat anti mouse Alexa Fluor 647 (1:1000) and goat anti rat FITC (1:400) in goat serum. The secondary antibodies were protected from light.

Islet Immunofluorescent Staining

Ethanol was removed from islets and replaced with PBS using serial dilutions (90%, 75%, 50%, 25%, 10% and finally 0% ethanol), 15 minutes each. To keep the fixed islets immobilized during staining and imaging, they were suspended in cold matrigel (BD biosciences) which solidifies at room temperature. The islets were then rinsed 3 times, 10 minutes each, in 1xPBS:glycine. The islets were then permeabilized for 10 minutes with 0.5% Triton X-100 in PBS. Next, the islets were blocked in goat serum for 1.5 hours and subsequently incubated with primary antibody solution for 2 hours on a rocker. Afterwards, islets were rinsed 3 times, 20 minutes each, with 1xIF wash. In all further steps, the islet samples were protected from light to preserve the fluorescent labels. Islets were incubated in secondary antibody solution for 1 hour and then rinsed 3 times, 20 minutes each, with 1xIF wash. Subsequently, islets were stained with Hoechst 33342 (1:1000) in PBS for 10 minutes. Finally, the islet sample was mounted on slides using a mounting solution, capped with a coverslip and stored at 4°C until imaging with the Zeiss LSM 510 confocal system.

Numerical Calculation of Complex Permittivity by FEA

Numerical calculations were performed by FEA using COMSOL Multiphysics with an AC/DC Module (COMSOL, Sweden). Quasi-electrostatic and time harmonics conditions were adopted over a frequency range from 1 kHz to 100 MHz. The cytoplasm and the external medium, represented by subdomains i and a, have complex conductivities of κ_i^* and κ_a^* respectively. Complex conductivity κ^* and complex permittivity ε^* are related as $\kappa^* = j\omega\varepsilon_0\varepsilon^*$, where $j^2 = -1$, ω is the angular frequency and ε_0 is the vacuum permittivity. The interface between the subdomains has an impedance of d/κ_m^* , where d and κ_m^* represent the thickness and the complex

conductivity of the membrane, respectively. Thin layer approximation (TLA) was adopted (19) by assuming a negligible membrane thickness – while employing a membrane impedance of d/κ_m^* – in order to reduce computational tasks. The following boundary conditions were applied when solving for the potential distribution. The subdomains are situated inside a cube with potentials of 1V and 0V at the top and bottom, respectively. The current density is zero across the four faces of the cube, i.e., electric insulation conditions. The interface between subdomains i and a has a current density J_m that is given by

$$-n_i \cdot \kappa_i^* \nabla V_i = n_a \cdot \kappa_a^* \nabla V_a = J_m \quad (1)$$

$$J_m = \kappa_m^* / d (V_i - V_a) \quad (2)$$

where n_i and n_a are outward normal to the boundaries of subdomains i and a, and V_i and V_a are the electric potentials of subdomains i and a at the interface.

The current density over the top or bottom boundary of the cube is integrated to calculate the current through the cube. The current and the applied voltage provide the admittance Y of the cubic system of dimensions $l \times l \times l$. The effective complex conductivity κ^* and complex permittivity ε^* are obtained from the admittance by

$$\kappa^* = j\omega\varepsilon_0\varepsilon^* = Y/l = \kappa + j\omega\varepsilon_0\varepsilon \quad (3)$$

Results and Discussion

Confocal Immunofluorescent Imaging of Islets

The insulin staining showed a normal distribution of insulin containing cells throughout the islet, confirming the islet fixing and staining procedure along with the quality of the islet samples (Figure 1). As expected the islets displayed lesser quantities of glucagon and somatostatin containing cells. Immunofluorescence staining showed that the cell junctions, ZO-1, Cx36 and E-cadherin, are well distributed throughout the islet (Figure 2). Similar observations for Cx36 were previously made for human islets, in which Cx36 was observed mostly in discrete spots in β cells (13). The distribution of Cx36 in these images demonstrates the large degree of electrical coupling between islet cells, agreeing with the requirement of gap junctions to synchronize β -cell electrical activity. In addition, the distribution of ZO-1 tight junctions and E-cadherin in these confocal images is in line with previous observations for islets. Tight junctions have been observed in detail on the membranes of human islet cells (13, 20). E-cadherin is a notable β -cell junction (21) and is suggested to regulate proper mouse islet aggregation *in vivo* (22). The

distribution of tight and adherens junctions indicate the tightly packed arrangement of cells within the islet. As a result, the volume fraction of the islet cells within the cluster is significantly larger compared to the inter-cell space. The FEA model was built to account for observations made by confocal imaging of islets. The resulting FEA model geometry is composed of a tightly packed cubic cell aggregate with adjoining cell membranes connected by at least one gap junction.

Figure 1 Confocal microscopy images of islet hormones. Hormone staining of islet cells showing insulin (top left), glucagon (bottom left) and somatostatin (top right). The merged images show nuclei stained blue with Hoechst 33342 (bottom right).

Figure 2 Confocal microscopy images of islet cell junctions. Cell junction staining of islet cells showing Cx36 gap junctions (left), E-cadherin adherens junctions (center), and ZO-1 tight junctions (right). Nuclei are stained blue using Hoechst 33342.

FEA Model of a Pancreatic Islet: Geometry, Dielectric Parameters and Response

Islet morphology – and associated gap junction adjoining cells – is represented through the computer aided design (CAD) model in Figure 3. This model employed in the FEA simulation is composed of two overlapping layers of cubes, possessing six faces each $\sim 10\ \mu\text{m}$ in length, representing cells. The first layer is the central cell; the second engulfing layer is composed of 12 cells adjoined to each face and corner of the first layer cube cell, thus engulfing the central cell; this gives a total of 27 cells representing an islet cellular cluster. Fenestrations within the islet are represented by a spacing of $\sim 10\ \text{nm}$ between cells. FEA calculations were carried out with the following parameter values: $P=0.0585$ for islet volume fraction and $P_i=0.95$ for volume fraction of cells within the islet. Permittivity and conductivity of suspension media (ϵ_s, κ_s) and cytoplasm (ϵ_a, κ_a) are evaluated as $\epsilon_s=\epsilon_a=80$ and $\kappa_s=\kappa_a=1\ \text{S m}^{-1}$. While permittivity and conductivity of cell membranes (ϵ_m, κ_m) are evaluated as $\epsilon_m=5$ and $\kappa_m=0\ \text{S m}^{-1}$ with a membrane thickness $d=5\ \text{nm}$. Because the islet is composed mostly of β cells, this cell type dominates the islet dielectric signal. Therefore, the model is composed solely of β cells.

All the FEA simulations of islet dielectric response in this work yielded capacitance spectra that contained a double dispersion, which is characteristic of cell aggregates (16). This double dispersion has also been observed experimentally during *in vitro* dielectric analysis of isolated human islets (7, 18). The low frequency dispersion is attributed to the presence of gap junctions (16, 23). The high frequency dispersion is associated with interfacial polarization of concentrated cell suspensions(16, 23), such as what occurs due to the tight packing of islet cells.

Figure 3 Finite element model of islets. Top-side (A) and side view (B) of the islet model showing cells arranged in a spherical cluster. (C) Close up of a single cell showing junctions between adjacent cells.

FEA Modelling of Single and Multiple Gap Junctions between Adjacent Islet Cells

In order to simulate the effect of gap junctions, 2 different models are employed whereby neighboring cells are joined by one (Figure 4a) or five (Figure 4b) cylindrical junctions of diameter ~ 10 nm, emulating single and multiple gap junctions, respectively. These 2 models still maintain the ubiquitous distribution of gap junctions within the islet as observed with confocal immunofluorescent imaging. However, the 2 models represent the effects of low and high gap junction density between adjacent cells, which arise due to differential gap junction expression. The FEA simulations are performed using the aforementioned parameters applied to the geometries in Figure 3. The capacitance and conductance spectra obtained from the simulation are shown in Figure 5. Increasing the number of gap junctions between adjacent model islet cells moves the dielectric spectra towards higher frequencies between 1 kHz and slightly below 1MHz. This range represents the largest changes in the dielectric spectra and corresponds to the low frequency dispersion, agreeing with the relationship between this dispersion and gap junctions. At around 1MHz to 10MHz, the dielectric spectra shifts to lower frequencies when increasing gap junctions, creating a smaller effect on the high frequency dispersion. The overall effect of increasing the amount of gap junctions is to move both dispersions closer together. In

addition, increased gap junctions raises the conductivity spectra in the region between 10 kHz and 1 MHz.

Figure 4 Close-up showing a single cell connected to neighboring cells through 10nm cylindrical junctions. Neighboring cells are connected by either (A) 1 or (B) 5 junctions. The cubes, inter-cube space and the cylindrical junctions represent cells, the extracellular space and gap junctions respectively.

Figure 5 Simulated Dielectric response of single vs. multiple gap junctions between adjacent islet cells. Capacitance (left) and conductance (right) spectra for single vs. multiple gap junctions between adjacent cells.

FEA Modelling of Islet Cell Membrane Capacitance Variations

To investigate the effects of insulin secretion as a function of membrane capacitance, four FEA simulations were performed by applying membrane permittivity values of $\epsilon_m=5, 6, 7.5,$ and 10 .

This corresponds to membrane capacitance of $C_m=2.78, 3.34, 4.17$, and 5.57 pF, respectively. These values were calculated using the following equation for capacitance of concentric spheres.

$$C_m = \frac{4\pi\epsilon_0\epsilon_m}{\frac{1}{R_1} - \frac{1}{R_2}} \quad (4)$$

$R_1=5\mu\text{m}$ and $R_2=5.005\mu\text{m}$ represent the inner and outer radii of a single cell within the islet, where the difference assumes the membrane thickness $d_m=R_2-R_1=5\text{nm}$. These capacitance values were selected based on patch clamp investigations with human islets, in which depolarization evoked insulin exocytosis triggered increases in β cell membrane capacitance by up to hundreds of femtofarads (24, 25). The geometric model of a single gap junction was employed as shown in Figure 4a. Increasing the permittivity of the model cell membranes simulates the increase in membrane capacitance associated with the fusion of vesicles during insulin exocytosis. Because of the synchronization of β cell insulin secretion, the model assumes a homogeneous secretory behavior for all cells. Therefore increases in cell membrane permittivity are applied uniformly to all model cells. The resultant dielectric effects are shown as changes in the capacitance and conductance spectra in Figure 6. The most pronounced effect of increasing the cell membrane permittivity is to raise the capacitance spectrum in the regions immediately before and after the low frequency dispersion. Specifically, the capacitance increases below 10 kHz and increases again between 10 kHz and 1 MHz. Above 1MHz , the capacitance decreases slightly relative to the other changes observed in the dielectric spectra. When increasing cell membrane capacitance, the conductivity spectra shift to lower frequencies, especially in the region between 100 kHz and 10 MHz.

This simulation did not assess the effects of changes in the islet membrane potential which occur prior to insulin secretion. In human islet cells, the membrane potential oscillates with millisecond resolution (24) and hence it is impractical to measure these changes dielectrically using a frequency sweep. Previous simulation work has shown that the membrane potential determines a low frequency α dispersion (26). This dispersion is much weaker compared to the double dispersions obtained in this simulation and observed experimentally for islets. Therefore, the influence of the membrane potential on the islet dielectric response is small and hence not included in this simulation.

Figure 6 **Simulated dielectric response of varying islet cell membrane capacitance.** Capacitance (left) and conductance spectra (right) for various cell membrane permittivity (ϵ_m) values.

FEA Modelling of Islet Cell Cytoplasm Conductivity Variations

Changes in islet cell cytoplasm conductivity were simulated to distinguish the dielectric effects of this parameter from gap junction activity and membrane capacitance variations. The cytoplasm conductivity of the FEA model cells were varied between 0.25 and 0.5 S/m for the geometries that included single and multiple gap junctions. The resultant changes in the dispersions can be described in terms of the Cole-Cole model of dielectric relaxation

$$\epsilon^* = \epsilon_h + \sum_n \frac{\Delta\epsilon_n}{(1+j\omega\tau_n)^\beta} \quad (5)$$

where ϵ_h is the high-frequency limit of relative permittivity, $\Delta\epsilon$ the relaxation intensity, τ the relaxation time, ω is the characteristic frequency, κ_l is the low-frequency limit of conductivity, β is the Cole-Cole parameter ($0 < \beta \leq 1$) describing the different spectral shapes resulting from fluctuating relaxation curves/times, and n represents the number of relaxation terms. For both the single and multiple gap junction geometries, increasing the cytoplasm conductivity shifts the entire capacitance spectra to higher frequencies (Figure 7). In other words, regardless of the gap junction geometry, higher cytoplasmic conductivities cause a rightward lateral shift, affecting the characteristic frequency, ω of the dielectric spectra/relaxation. In contrast, changes in gap junction activity and membrane capacitance have greater effects at certain frequency ranges and affect the two dispersions differently. These effects cause a significant change in the relaxation time of both dispersions, hence altering the spectral shape and β , the Cole-Cole parameter.

Therefore, the dielectric response to changes in cytoplasm conductivity, gap junction activity and membrane capacitance can be distinguished.

Figure 7 Simulated dielectric response to changing cytoplasmic conductivity. The simulation geometries include single and multiple gap junctions between adjacent cells. The cytoplasm conductivity (k_{cyt}) was varied between 0.25 and 0.5 S/m.

Conclusion

The presented FEA model of islet dielectric response extends beyond dielectric analytical models by accounting for key features of islets, namely their tightly packed cell aggregate morphology and inter-cell junctions. The simulations yielded dielectric spectra containing double dispersions, which are characteristic for cell aggregates and that were previously observed experimentally for islets. This model has applications in interpreting results from *in vitro* dielectric analysis of islets, providing a pertinent assessment of islet functionality and stimulus response. In particular, the simulated dielectric spectra were sensitive to islet gap junction density and membrane capacitance, which highlight the potential of dielectric spectroscopy to assess differential gap junction expression and insulin secretion respectively. This assessment is important given the role of gap junctions and insulin secretion in islet functionality and pathologies. Moreover, dielectric analysis of islets could be used as a real-time *in vitro* monitoring technique to better

delineate islet physiology and to assist in developing treatments for diabetes. Drug screening, investigation of islet regeneration/replacement and assessing donor islet quality for transplantation into diabetic patients are other applications of the proposed methodology. This model can also be extended to improve the understanding of the dielectric response of cell aggregates *in vitro*. These aggregates include spheroids and embryonic bodies that are used to study organ/tumor behavior and tissue morphogenesis (27-29). Given the interest in the relationship between gap junctions and cancer (30), dielectric modelling of gap junctions is particularly relevant.

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