Colloids and Surfaces B: Biointerfaces 148 (2016) 474–480

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Elaboration of a finite element model of pancreatic islet dielectric response to gap junction expression and insulin release

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ARTICLE INFO

Article history:
Received 30 May 2016
Received in revised form 7 September 2016
Accepted 8 September 2016
Available online 16 September 2016

Keywords:
Islets of Langerhans
Dielectric spectroscopy
Dielectric cell models
Cell aggregates
Membrane capacitance
Gap junctions

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.

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1. 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

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http://dx.doi.org/10.1016/j.colsurfb.2016.09.012
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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.

2. Materials and methods

2.1. Islet isolation and fixation

Islets were fixed in 10% formalin for 48 h 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.

2.2. Immunofluorescent (IF) solution preparation

10xPBS:glycine was prepared by adding NaCl (7.6 g), Na_2HPO_4 (1.88 g), NaH_2PO_4 (0.414 g) and glycine (7.5 g) to water (100 ml). 10xIF-wash was prepared by adding NaCl (7.6 g), Na_2PO_4 (1.88 g), NaH_2PO_4 (0.414 g), NaN_3 (0.5 g), bovine serum albumin (BSA) (1 g), Triton X-100 (2 ml) and Tween-20 (0.5 ml) to water (100 ml). 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.

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