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A Review of Primary Pancreatic β-Cell Separation and Purification

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Abstract

The β-cells of the pancreatic islets of Langerhans are the only cells that produce and secrete insulin, a key metabolic hormone that plays a central role in the maintenance of glucose homeostasis in the body. Pancreatic β cell dysfunction plays an important role in the development of diabetes. Therefore, the study of the pathology and physiology of islet cells has become an integral part of the fight against diabetes. However, the separation and purification of them in living condition remains an obstacle for researchers. The current study of the separation and purification technology used for primary pancreatic β cells such as density gradient centrifugation, flow cytometry, magnetic-activated cell sorting, zinc-fluorescent probe sorting. They are effective but accompanied with inevitable shortcomings. Raman spectroscopy is a new technique for cell sorting. Based on Raman scattering, Raman spectroscopy has the ability to separate samples based on molecular organization signals, construction and relative content *in vitro*. Recently, our team has demonstrated that by using the method of Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) the four kinds of islet cells could be identified and discriminated by Raman spectroscopy. This may provide a label-free and non-invasive method to discriminate islet cell type in a randomly distributed mixed islet cell population via their physical property rather than by using antibodies or fluorescence labeling.

Keywords: β cell; Separation; Purification

Core Tips

Pancreatic β cell dysfunction plays an important role in the development of diabetes. Therefore, the study of the pathology and physiology of islet cells has become an integral part of the fight against diabetes. Based on a current study of the separation and purification technology used for primary pancreatic β cells, this paper aims to provide a summary to promote the development of basic research in the field of diabetes.

Introduction

Diabetes mellitus is a metabolic disorder characterized by an absolute or relative lack of insulin. According to epidemiological analyses to date [1-4], the morbidity from diabetes increases each year. As reported in a study from 1998 [5], diabetes occurrence in adults worldwide was 4.0% in 1995 and will rise to 5.4% in 2025. That is, the number of adults with diabetes will increase from 135 million in 1995 to 300 million in 2025. What is more, a study in 2010 [6], predicted that the number would reach 380 million in 2025. Based on the all-cause mortality determined by using the Global Burden of Disease (GBD) from 1990 to 2010, the number of people dying from diabetics was 30% of the increasing all-cause mortality. It is high time we take action to learn more about diabetes. Pancreatic cell dysfunction plays an important role in the development of diabetes. Thus, it is indispensable for us to research the physiology and pathology of pancreatic cells to defeat diabetes.

The pancreas in mammals has both exocrine and endocrine components. Acinar cells of the exocrine pancreas secrete pancreatic fluid. Pancreatic islets are collections of endocrine cells that are dispersed throughout the pancreas. Pancreatic islets are composed of four different types of endocrine cells: α-cells, which secrete glucagon, β-cells, which secrete insulin, δ-cells, which secrete somatostatin, and pp cells, which secrete pancreatic polypeptide. In addition, the islets, which are a highly vascularized micro-organ consisting of multiple cells, contain nerve cells, endothelial cells and lymphocytes. Previous studies of *in vitro* cultures of islet cells have used cloned cell lines from transplantable islet cell (α-cells or β-cells) tumors and primary cultured islet cells (isolated from living animal pancreas). In contrast to the tumor cells, the primary cultured islet cells more closely represent human biological characteristics, are more sensitive to changes in the external environment, and are more suitable for studying physiological mechanisms. Studies on cellular electrophysiology and cell dynamics, such as membrane ion channels, exocytosis, organelle transport, insulin and protein folding in the endoplasmic reticulum of cells, require purified and living islet cells. However, it is extremely difficult to isolate the endocrine cells, which limits the research using primary cultured islet cells at the single-cell level.

Density Gradient Centrifugation

In order to obtain purified pancreatic β cells, which are small insulin secretion organs, separation of pancreatic islets is an essential first step. Currently, the method for obtaining primary rat pancreatic islets relies mainly on collagenase isolation by density gradient centrifugation. Researchers have carried out many studies on gradient

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centrifugation media, collagenase perfusion and the efficiency of separation.

In 1990, Suylichem et al [7] used seven different density gradient-forming materials to compare their efficacy in rat islet purification. The results show that albumin, dextran-40 and metrizamide were the three gradient separation medias with the highest isolation efficiency for islets. Metrizamide resulted in significantly higher yields than Ficoll, in terms of both the numbers of islets and there *in vitro* function; the best results were obtained with metrizamide and dextran-40. In 1996, James Shapiro et al [8], found that intraductal distention of the pancreas with collagenase followed by stationary warm incubation improved the recovery of the islets of Langerhans in the rat and reduced the cost. In 2008, Y Yuan et al [9] found that high-purity islet cells with good viability can be obtained through improved collagenase perfusion and a gradient centrifugation method; the viability and purity of the islet cells obtained were greater than 90%. In 2011, Mccall et al [10] published a study of the separation and purification of islets using a Ficoll, Histopaque, dextran or iodixanol gradient. The results show that use of a Ficoll or Histopaque gradient led to the purest and most viable islets in terms of functionality, compared to dextran and iodixanol; although islets isolated by Ficoll gradient had the highest glucose-stimulated insulin release *in vitro*, the Ficoll gradient performed similarly to Histopaque and dextran gradients *in vivo*. However, isolating islets by Ficoll gradient was more expensive.

To purify pancreatic β cells, in 1981, Pipeleers et al [11] isolated and dissociated islets by using Krebs-Ringer solution buffered with Hepes and centrifugal elutriation; then, density gradient centrifugation was performed with diluted iso-osmotic Percoll to obtain individual α, β, and δ cells. However, pancreatic β cell diameter and the rate of sedimentation are not constant parameters of cells from the islets of Langerhans; for example, the diameter and sedimentation rate change during cell division, growth and maturation. Therefore, the results of this method are not very reliable.

Flow Cytometry

Flow cytometry has been employed as a method to separate fluorescent single cells or particulates under high velocity flow according to fluorescence intensity and their low forward angle light scattering properties. Different cells have different physical and chemical properties, such as size and specific proteins and antigens [12-15]. By using automated flow cytometry, ten thousand cells can be analyzed and sorted simultaneously, and multiple parameters can be present in one cell.

Pancreatic islets are composed of four different types of endocrine cells: α, β, δ and pp. The volume of α and β cells is 200~600 μm^3, while the β cell volume is 600~1500 μm^3, which is significantly greater than that of non-β cells [16]. According to this difference, in 1982, DA Nielsen et al [17] used light-scattering flow cytometry to isolate and purify islet cells. The results show that α cells scattered less intensely and were concentrated on the left side of the islet cell peak, while δ cells were localized to the far-right side, indicating a higher intrinsic light scattering property of the δ cells. The more abundant β cells were located at the center of the islet cell peak. Since that time, light-scattering flow cytometry has become a new way to isolate and purify islet cells. In the same year, Rabinovitch A et al [18], validated and improved this method. However, it is hard to obtain highly purified β cells by only the size of cells. In 1985, Pipeleers et al [19], used flow cytometry to isolate and purify β cells by the fluorescence intensity, and the separation rate of β cells was improved. After that, many researchers purified β cells by using this method [20-22].

Magnetic-Activated Cell Sorting

Immunomagnetic Beads (IMBs) are composed of carrier microspheres and immune ligands. The immune ligands can bind to active proteins. The antibodies on the beads can be specifically combined with the corresponding antigens to form antigen-antibody-magnetic bead immune complexes. The complexes move mechanically in a strong magnetic field, separating the complexes from other substances to separate the specific antigens.

In 1979, Professor John Ugelstad and his colleagues developed a technique to produce uniform sized polymeric microspheres [23]. Using this technique, they produced symmetrical polymeric microspheres (1.5-100 μm diameters). Prior to that, NASA experts thought that this would be possible only in the absence of gravity. Now, the balls made of composite paramagnetic material are called Dynabeads. Dynabeads are a type of homogeneous, super paramagnetic and single scattering polymeric microspheres synthesized by gamma Fe_2O_3 and Fe_3O_4 magnetic materials. Each microsphere is coated with a layer of polymeric material as a carrier for the adsorption and binding of various molecules. The homogeneity of the shape and size of Dynabeads ensures the consistency of the microsphere surface physical and chemical properties, and the acquisition of high quality results and the reproducibility of the results are established based on these characteristics. The immunomagnetic purification method using the Dynabead system can separate insulin-containing β cells from a mixed rat islet cell population. Dynabeads are uniform, paramagnetic particles coated with specific antibodies. Single rat islet cells were initially incubated with a beta-cell surface specific antibody (K14D10 mouse IgG) for 20-60 min. A suspension of Dynabeads coated with a secondary antibody (anti-mouse IgG) was added for another 15min, after which the Dynabead-coated cells were instantaneously pelleted upon contact between the tube and a magnet (Dynal MPC). Immunocytochemistry was used to confirm that the Dynabead-coated cells contained insulin and to quantify the efficiency of the method [24]. After the cell specific magnetism is tagged, these cells are selected by a separation column in a strong, stable magnetic field. The matrix in the separation column creates a high gradient magnetic field. The cells that are magnetically labeled remain in the column while the unlabeled cells flow out. When the separation column is removed from the magnetic field, the magnetic marker cells in the column will be eluted, so that both labeled and unlabeled cell components can be obtained. The reaction between magnetic antibodies and magnetic markers can be completed in a few minutes. Because of the small size of the magnetic beads, the beads do not cause mechanical stress on the cells, and the reaction between the magnetic antibody and the magnetic label can be completed in a few minutes so that the incubation time is short, and the operation is fast. Dynabeads form a stable colloidal solution that neither precipitates nor condenses in the magnetic field. The size and components (iron oxide and polysaccharide) of the micro-beads make them

Table 1: Comparison of different separation methods for β cells.

Method	Separation mechanism	Advantage	Disadvantage
Density gradient centrifugation	Utilizes the fact that different islet cells have different sizes and sedimentation rates	No confounding factors	Low yields
		Easy and simple to perform	Purity rate of approximately 80-90%
			Reagent is toxic
Flow cytometry	β cells are purified according to cell auto fluorescence and side scattering	No confounding factors	Low function in isolated cells
		High-throughput sorting	Does not apply to human β cells
		High purity	Expensive equipment Purity depends on cytoactivity
Magnetic-activated cell sorting	The antigens on the β cell surface and a specific antibody (K14D10 mouse immunoglobulin G) and immunomagnetic beads are combined into antigen-antibody-magnetic bead immune complexes for separation	High purity	Confounding factors
		Easy and simple to perform	Immunomagnetic beads are expensive
			Occurs outside of a physiological environment
Zinc-fluorescent probe sorting	Utilizes the large number of zinc ions, which play an important role in the synthesis and release of insulin, in β cells	Applies to human β cells	Confounding factors
			Zinc-fluorescent probe is expensive
Transfection with adenovirus vector	Recombinant adenovirus is used to infect β cells to cause expression of a new globulin- green fluorescent protein	Applies to human β cells	Confounding factors
			Difficult to do follow-up research

biodegradable. They will not activate cells or affect the function and viability of the cells, and the physiological function of the cells remains unchanged. The magnetic beads do not need to be removed, so the positively sorted cells (i.e., the magnetically labeled cells) can be used immediately for analysis and subsequent experiments. The Magnetic Activated Cell Sorting system can isolate pure cell populations with high purity and viability and yield better survival rates. According to the difference in cell frequency and marker expression levels, the purity of Magnetic Activated Cell Sorting-isolated cells reaches 95%-99.9%, and the recovery rate is >90%.

Zinc-Fluorescent Probe Sorting

An important feature distinguishing beta cells from other islet cells is that they contain large numbers of zinc ions, which play an important role in the synthesis and release of insulin. Based on this characteristic, a non-toxic zinc-sensitive fluorescent probe can be used to selectively label zinc in viable beta cells and then exhibit excitation and emission wavelengths in the visible spectrum to separate and purify beta cells. In 2001, Lukowiak et al [25] isolated beta cells using Newport Green, a probe excitable at 485nm with a dissociation constant in the micro molar range corresponding to a low affinity for zinc. The loading of the lipophilic esterified form of Newport Green is easy, rapid, specific, and non-toxic to cells. The probe, which is an ester, is cell permeable and can spread through the cell membrane. In the cell, when catalyzed by intracellular esterase, the ester will produce a cellular fluorescent indicator for zinc. Confocal microscopy can detect the intense fluorescence associated with secretory granules. Regression analyses show a good relationship between zinc fluorescence and islet number (r=0.98) and between zinc fluorescence and insulin content (r=0.81). This type of zinc probe can specifically label living cells in the visible spectrum; therefore, this method appears to be very promising for isolating beta cells for both clinical and research applications.

Transfection with Adenovirus Vectors

A recombinant adenovirus can infect the islets of the pancreas, integrating adenovirus genes into beta cells' insulin expression gene. Under the control of the insulin promoter, the β cells then express a new globulin-GFP. Under light at 50mw and 488nm, the β cells can be isolated and purified by flow cytometry based on the self-fluorescence of GFP.

In 1998, K Meyer et al selectively labeled human beta-cells with Green Fluorescent Protein (GFP), allowing for their sorting by flow cytometry. Human islet cells were infected with a replication-defective (attenuated) recombinant adenovirus expressing GFP driven by the rat insulin I promoter (Ad-RIP-GFP) for targeted expression in beta-cells or beta-galactosidase driven by the promiscuous cytomegalovirus (CMV) promoter (Ad-CMV-beta-gal) as a control [26]. While the majority of islet cells can be infected by adenovirus, as shown by infection with the Ad-CMV-beta-gal control, increased fluorescence after infection with Ad-RIP-GFP was limited to insulin-containing beta-cells. Infection of islet cells with Ad-RIP-GFP resulted in the reproducible appearance of a population of intensely fluorescent cells when analyzed by flow cytometry. These cells were sorted using a Fluorescence-Activated Cell Sorter (FACS) and were shown by immunofluorescence to consist of >95% beta-cells. The targeted expression of GFP thus allows for the preparation of human beta-cells purified close to homogeneity. This method should be readily applicable in any laboratory with FACS capability.

Raman Spectroscopy

Raman scattering was discovered by Sir Chandrasekhara Venkata Raman in 1928; however, Raman spectroscopy was not successfully applied to the analysis of living cells and tissues until the early 1990s. In 1990, PUPPELS et al [27] Applied Raman spectroscopy to successfully separate a single cell. Due to its unique advantages, Raman spectroscopy has become a powerful tool in cytology research. At present, analysis of cellular dynamics and distinguishing cells [28-30], which are two important aspects of single cell study via Raman spectroscopy, have been used to differentiate tumor cells from normal cells [31], leukemia cells [32], variations in red blood cell [33] and rat islet β cells [34].

In our recent work, the Raman spectra detection was performed on rat islet cells. By using Raman spectroscopy, subtle intrinsic differences between different types of islet cells can be detected. The cross-validation test shows that the classification accuracy for α, β, δ and pp cells is 95.7%, 92.3%, 77% and 100% respectively (the results have not yet been published).

Conclusion

As (Table 1) shows, the traditional method for pancreatic endocrine cell purification cannot "Both Fish and Bear's Paw Obtained" in the field of purity rate, cell activity and do not introduce of exotic confounding factors. These antibody labels, fluorescent probes, fluorescent protein even transfection virus not only take into new confounding factors, but also influence cell viability and cell physiology experiment. Presumably, Raman spectroscopy can be used to sort unmodified pancreatic cells based on mass. For instance, Raman spectroscopy separates cells without injury and without adding stains, antibodies, or hybrid materials and retains cytoactivity, which allows researchers to continue experiments, providing reliable models of original pancreatic α, β, δ or pp cells for diabetes research and greatly advancing the development of basic diabetes research.

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