Islet Cell Biology Core

The Core is currently located on the fifth floor of Stemmler Hall in Diabetes Center space and is organized to provide routine services and access to diverse more sophisticated approaches and equipment that promise to advance the research capabilities and productivity of about 2 dozen investigators in the pancreatic islet cell field.

Background

Islet research at Penn has greatly expanded since the inception of the DERC in 1977 and has had a significant impact on the field during the last 25 years. The Diabetes Center has always been the nucleus of this program following the establishment of the laboratories of Drs. Clyde Barker and Franz Matschinsky in the late 1970s. Following the inception of the DERC in 1977 several investigators have been trained here and have since established islet cell research laboratories at other academic institutions and in industry (K. Braymen, B.Corkey, D. Dafoe, P. Drain, Y. Liang, M. Meglasson, J. Parker, M. Prentki, P. Ronner and W. Zawalich). Others have continued their career here or have been attracted to the faculty because of unique research opportunities (e.g. R. Ahima, M. Birnbaum, C. Barker, N. Doliba, C. Deng, K. Gooch, Y. Imai, K. Kaestner, L. Kubin, J. Markmann, F. Matschinsky, A. Naji, R. Simmons, D. Stoffers, C. Stanley, K. Teff, B Wolf ). A subgroup of these (Barker, Deng, Markmann, Matschinsky, Naji, Wolf) has established an NIDDK supported pancreatic islet transplant program under the leadership of Dr. Ali Naji.

At the present time, the Islet Cell Biology Core of the Penn Diabetes Center is designed to play a critical role in the scientific life of a multifaceted group of individuals studying islet cells in health and disease.

Scientific Purpose:

It is the purpose of this core to assist investigators who currently study or have plans to study independently or collaboratively various aspects of pancreatic islet cell biology. In order to accomplish this goal the Core provides four services:

- isolate, culture, and functionally assess pancreatic islets of rat and mouse including batch incubations, perifusions, respirometry, measurements of $Ca_{i}^{++}$, the P-potential (ATP, ADP, AMP and Pi) and other metabolites, hormone contents and release.

- Will perform extra corporal phenotyping of the endocrine pancreas of mouse and rat using the intact isolated perfused or minced perifused pancreas (both of these techniques to be fully established).

- maintains a broad and well characterized stock of transformed islet cells, grows large batches of such cells or generates pseudo islets by embedding such cells into agarose beads for dynamic studies of metabolism and hormone release.
provides in depth consultation and helps develop strategies how to use the services of the core optimally or will attempt to modify available technologies to solve particular problems.

**Personnel of the Islet Cell Biology Core**

Dr. Franz Matschinsky serves as core director and he is involved in all aspects of the operation of the core in accordance with the general principles of operating DERC core facilities. He sets priorities and makes job assignments. He closely interacts with users to achieve the highest benefits for them and the highest scientific standards of the work to be conducted.

Dr. Nicolai Doliba serves as the technical director. He is responsible for operating the Ca^{2+}, NAD(P)H imaging setup and maintain and operate the system for optical analysis of O_{2} consumption, participating also in the development of a more sensitive instrument. He participates in the planning of all islet studies that require the above approaches and supervises or performs many of the actual measurements. He also does cell work involving NMR technology. He is aided by Dr. C. Li (fully supported by Dr. Charles Stanley from Children’s Hospital). Drs. Doliba and Li are going to establish the isolated rat and mouse pancreas, methods previously operative in Dr. Matschinsky’s laboratory as documented by many publications. This team also does the $^{13}$C-and $^{31}$P-NMR studies using islet cell lines.

Carol Buettger: Ms. Buettger is a Senior Research Specialist with more than 40 years of experience who masters all technical aspects of tissue culture, in this particular case of culturing and maintaining stock of an extensive collection islet cell lines, of culturing islets, of performing hormone release assays with a multi well plate test with alpha- and beta-cell lines and GLUTag cells releasing GLP-1. She knows how to
generate pseudo islets by incorporating cells into agarose beads.

Habiba Najafi: Mrs. Najafi leads the islet cell isolation team and has worked in the FMM laboratory for 20 years, with an outstanding track record of isolating islets with the collagenase procedure, is an accomplished tissue culture technician and masters all physiological and biochemical procedures used to assess islet function. This includes measurements of glucose, glutamine, fatty acid, leucine and KIC metabolism using 14C or Tritium labeled substrate.

Qin Wei: Mrs. Wei masters all procedures associated with islet cell isolation, culture and functional testing. She works closely with the other members of the team such that the group is capable of isolating and processing larger batches of rat or mouse islets in the shortest possible time when needed for certain projects.

Description of Services and Facilities

Acute Functional Phenotyping of the Endocrine Pancreas: Responding to the specific needs of Center investigators the core uses the perfused, minced pancreas preparation (Ian Burr et al., J. Clin. Invest. 49:2097, 1970) to investigate fuel or drug stimulated insulin and glucagon release. The approach is uniquely suited to study the ontogeny of the endocrine pancreas, starting with neonatal pancreas minces and follow-ups through early adulthood. This procedure allows functional islet studies at a time when isolation of islets or perfusion of the intact pancreas may be difficult or not be possible.
Islet Isolation, Organ Culture and Delayed in vitro Functional Testing: There are the facilities and the personnel for isolating about 5,000 to 10,000 rat islets or 1000 to 1500 mouse islets per day using classical collagenase digestion combined with Ficoll gradient centrifugation with modification by the procedure of M.L. McDaniel et al (Meth. in Enzymol. 98:182-200, 1983).

The Core includes a dedicated tissue culture room with two hoods, four incubators, table top centrifuges and stereo as well as inverted microscopes. Rat and mouse islets may be cultured for as long as 14 days using a variety of culture conditions with virtually no endocrine cell losses but variable secretory function (depending on conditions).

Maintenance of Stock, Culturing and Characterization of Transformed Islet Cells: The use of islet cell lines derived from spontaneous insulinomas and from insulinomas of transgenic mice which carry transgenes constructed of the SV40 T-antigen and promoters of the major islet cell hormones insulin, glucagon, somatostatin continues to play an important role in islet cell research (S. Efrat, et al. Proc. Natl. Acad. Sci. USA .82,9037-9041,1980; S. Efrat, et al. Neuron 1,605-613, 1988; F. Radvanyi, et al. Molecular & Cell Biol. 13, 4223-4232, 1993). The islet cell resource assists investigators of the Center by maintaining stock of many useful cell lines and providing well characterized cultured cells for various experimental purposes. The Core has stocked early passages of the RIN-m5F, HIT-T15, INS-1, β-TC3, β-TC7, MIN-6 and has established a comprehensive collection of β-HC cells. It provides seed cultures and generates large batches of these cells for investigators (i.e. as many as 2 billion cells per harvest). Available technology of the core is the superfusion of cultured islet cells imbedded in agarose beads (modified from D.L. Foxall et al. Experim. Cell Res. 1.1:1:, 521-529, 1984). This paradigm allows dynamic studies with large amounts of 0.5 to 1.0 ml of packed islet cells for the application of NMR/or mass spectrometry and energy balance studies using A/V differences of O2, lactate/pyruvate and other parameters (e.g. determination of cytosolic pH) during fuel stimulation of hormone release. We have noted a declining demand for this resource, but predict that this approach will show an upsurge in the next grant period because metabolomic research will increase and make use of this resource.

Freshly isolated or cultured islets are functionally tested by several approaches available through the core:

- measuring insulin, glucagon and cAMP release in perifusion systems (in connection with the Radioimmunoassay Core)
- quantitating oxygen consumption in large batches of cells
- studying glucose metabolism using 15N, 14C or 3H labeled substrate (in collaboration with Dr. M. Yudkoff and Dr. I. Nissim; to be arranged case by case)
- investigating the behavior of free Ca2+ as well as the redox state of NAD(P)H using state of the art fluorescence imaging techniques
Under development: quantitating oxygen consumption of small groups of freshly isolated and cultured islet tissue using novel optical methods as developed by Dr. D. Wilson.

Facilities, Equipment and Collaborations:

The Islet Cell Biology Core is located on the Fifth floor of Stemmler Hall in the Diabetes Center space. There is about 750 SF of dedicated laboratory space, equipped for islet isolation, perifusion and metabolic testing. It is also used for preparing pseudoislets from transformed cells and for testing cells functionally. The Core uses a 300 SF tissue culture room for culturing islets and transformed cells. The Core has also established a computerized image analysis system (the Zeiss Atto-Fluor System) for studying Ca^{2+} transients and native NAD(P)H fluorescence. Apparatuses for isolated pancreas perfusions and optical microrespirometry are being reestablished or are available, respectively. The equipment includes in addition centrifuges, microscopes, perifusion pumps, oxygen electrodes, water baths, 2 computerized gradient mixers to generate any desired stimulus profile for islet and pseudoislet perfusion studies. We have access to state of the art NMR machines located at CHOP through Dr. Susan Wehrli, a long time collaborator. We have also established a close working relationship with Drs. M. Yudkoff and I. Nissim from Children’s Hospital of Philadelphia to perform heavy isotope studies of isolated islets using ^{15}N or ^{13}C labeled substrates.

Highlights of Core Activities:

1) In collaboration with Dr. David Wilson we were able to establish a prototype apparatus for measuring oxygen consumption of perifused pancreatic islets during insulin secretion stimulated by fuels, neuroendocrine modifiers and drugs. This optical method is based on the phosphorescence quenching by oxygen and is significantly more sensitive and maintains far more stable baselines than the common oxygen electrode based assays (see Figure 1). We have applied the method to two projects. Pancreatic islets from SUR1^/- mice were investigated and it was observed that insulin release and energy metabolism do not change in parallel consistent with current views of exocytosis which has a low requirement for ATP. In another project it was observed that epigallocatechingallate (one of the active ingredients of green tea) inhibits glutamate dehydrogenase, glutamine induced insulin release and the associated enhanced respiration. These studies have been published in the JBC and AJP (references 52 and 54). These investigations justify our proposal to develop a next generation of respirometers allowing to study 50-100 islets rather than the currently needed 500-1000. The project illustrates how the Core interacts productively with scientists from other fields.

2) We were able to assemble a unique team of investigators under the umbrella of the ICBC (Drs. Kelley, Li, Matschinsky, Nissim, Stanley and Yudkoff) which resulted in the successful application of heavy isotope technology for the study of glutamine metabolism in a mouse model of GDH linked hypoglycemia. We discovered that GDH in pancreatic islet tissue operates in the oxidative direction only, providing an explanation for many observations in man and with animal or cell models. This work exemplifies what we consider as an ideal activity of this core.

3) Under Dr. Matschinsky's direction it was possible to show for the first time that a newly discovered glucokinase activator drug enhanced glucose induced insulin release in normal rodent and human islets and enhanced insulin secretion from defective human islets. It is anticipated that glucokinase activators will be developed to a useful drug therapy for type 2 diabetes. This high light shows how interaction between
industry (in this case Hoffman La Roche, the inventor of these cpds) and academia contributes significantly to translational research benefiting diabetics.

Figure 1: A new system for studying respiration and secretory function of isolated perifused pancreatic islets. Panel A: Scheme of perfusion system for measurement of oxygen consumption. 1) glass perfusion chamber; 2) peristaltic pump; 3) water bath (37°C) and perfusion buffer reservoirs; 4) tank with gas mixture (20% O₂ and 5%CO₂ balanced with N₂); 5) gas exchanger (artificial lung); 6) fraction collector (Waters Division of Millipore); 7) excitation (524 nm) and emission (690 nm) light guides (at the angle of 90 to each other) for measuring phosphorescence lifetimes in outflow; 8) computer.

Oxygen partial pressure was recorded by phosphorescence lifetimes of an oxygen-sensitive porphyrin (palladium-mesotetra (4-carboxyphenyl) porphyrin dendrimer). The inflow oxygen tension was measured in the absence of islets in the chamber before and after each experiment. This technique has outstanding sensitivity and unmatched stability. Panel B: Oxygen consumption rate and insulin release in response to step changes in glucose concentration. Islets were subjected to step increases in glucose concentration from 0 → 3 → 6 → 12 → 24 mM and then switched back to zero glucose. Insulin release and glucose concentrations were measured in outflow samples and were correlated with measurements of oxygen tension also in the effluent. Increases in glucose concentrations led to a step increases in oxygen consumption reaching a plateau each time within about 20 min (see reference 54).

4) Using the islet core it was possible to perform perhaps the first comprehensive functional study with isolated pancreatic islets from normal and diabetic human pancreas donors. This was a collaboration between this core and the PENN islet transplant program directed by Dr. A. Naji. The study clearly showed that the function of β-cells from diabetic donors was impaired as evidenced by reduced insulin secretion resulting from stimulation with glucose or a physiological amino acid mixture. There clearly is a functional defect not just a reduction of β-cell mass in islet tissue from type 2 diabetics! This is an example of close and critical cooperation between the DERC and an independent program project. (see reference 31)
Figure 2: Changes in intracellular Ca$^{2+}$ concentration in islets and INS-1 cells.
Panel A: Effect of IBMX and glucose on intracellular Ca$^{2+}$ concentration in control and SUR1$^{-/-}$ islets. Panel B: Effect of glyburide on intracellular Ca$^{2+}$ concentration in INS-1 cells treated with scrambled or SCHAD siRNA. The changes in cytosolic Ca$^{2+}$ concentration were recorded using Fura-2AM. The Ca$^{2+}$ tracings are typical examples (n=3).

List of Publications of Work Supported by the ICBC (2002-2006):


18. Selak MA, Storey BT, Peterside IE, Simmons RA:


19. Peterside IE, Selak MA, and Simmons RA:


Governance and Fees

We ask users, who require repeated services, to pay for animal purchase cost and per diems and contribute to cost of collagenase, Ficoll, tissue culture reagents and other materials.

- It costs the Core $400 to culture 2 billion cells when production is geared up (excluding personnel).

- An NMR study using $^{13}$C or $^{13}$P containing metabolites to study the energetics of $\beta$-cells and involving a reasonable (n) of 20 experiments would cost the investigator or a group of investigators $8000-9000$, which is considered much less than required if an independent laboratory would generate the material not including training and startup cost.

- The actual cost to the Core of preparing isolated islets (fresh or cultured) is the result of prices for animals, collagenase, Ficoll, plastic ware, media and fetal calf serum and amounts to $250/750-1000$ cultured and about 2/3 that for freshly isolated islets. This does not include personnel costs.

- Responding to a recommendation of the External Advising Committee the core offer the option of services for fixed fees for two services of the core rather than ask for provision of reagents: islet isolation and cell expansion. We will charge $75$ for isolation of islets from 1 adult rat or from 3-5 adult mice treated as one batch. Culturing the islets will add $30/2$ days of culturing each islets harvest.

- We will continue to provide services of "side order" size without charge (i.e $\leq 100$ islets). We will also continue to offer initial services free of charge e.g. $\leq 500$ islets 2-3 times, or batches of transformed cells $\leq 1$ billion 2-3 times for the purpose of pilot studies to facilitate islet research.

- Occasional microscopic imaging and metabolic rate measurements (O$_2$-consumption) are performed free of charge as are pilot perifusion studies designed to test the viability of islets that have been prepared by users. Extensive projects involving the latter procedures will require special arrangements because of limited capacity and associated cost.