Islet Viability and Potency Testing

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Vitacyte Webinars
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THE UNIVERSITY OF ARIZONA
College of Medicine

Department of Surgery

Commercial Interests & Nature of Relationships

I am a co-founder and stakeholder in Procyon Technologies LLC a start-up out of the University of Arizona that works on encapsulated cell therapies (with a focus on the treatment of T1D).

What Do We Want to Know?

Goals for Pre-Transplant Islet Quality Assessment

For a given islet preparation:

Is it safe to Transplant?

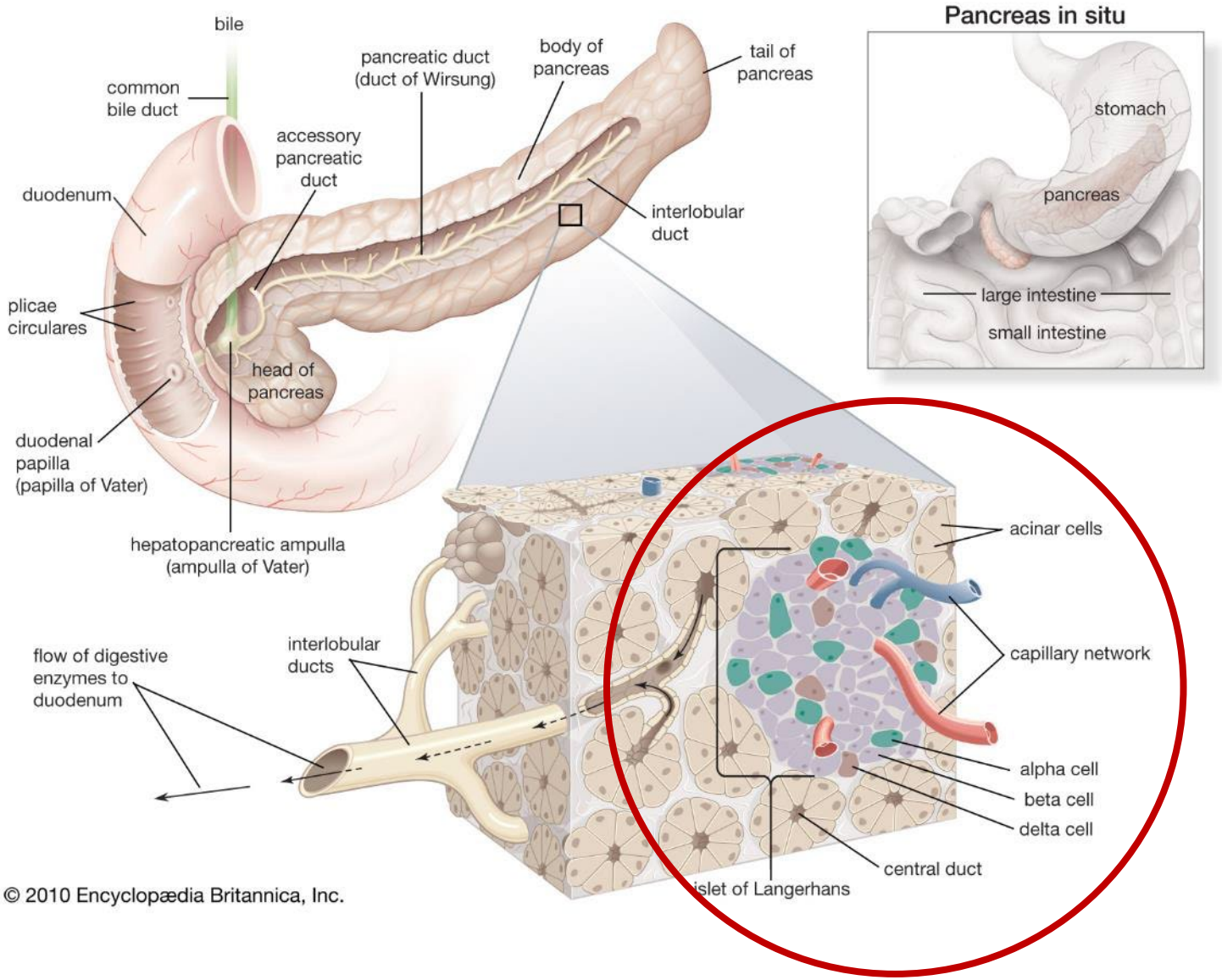
What is the Purity?

What is the “potency” or “dose”? E.g. Number of Viable, Functional β-Cells /Kg BW recipient.

Can we predict transplantation outcome using a set of real-time assays?

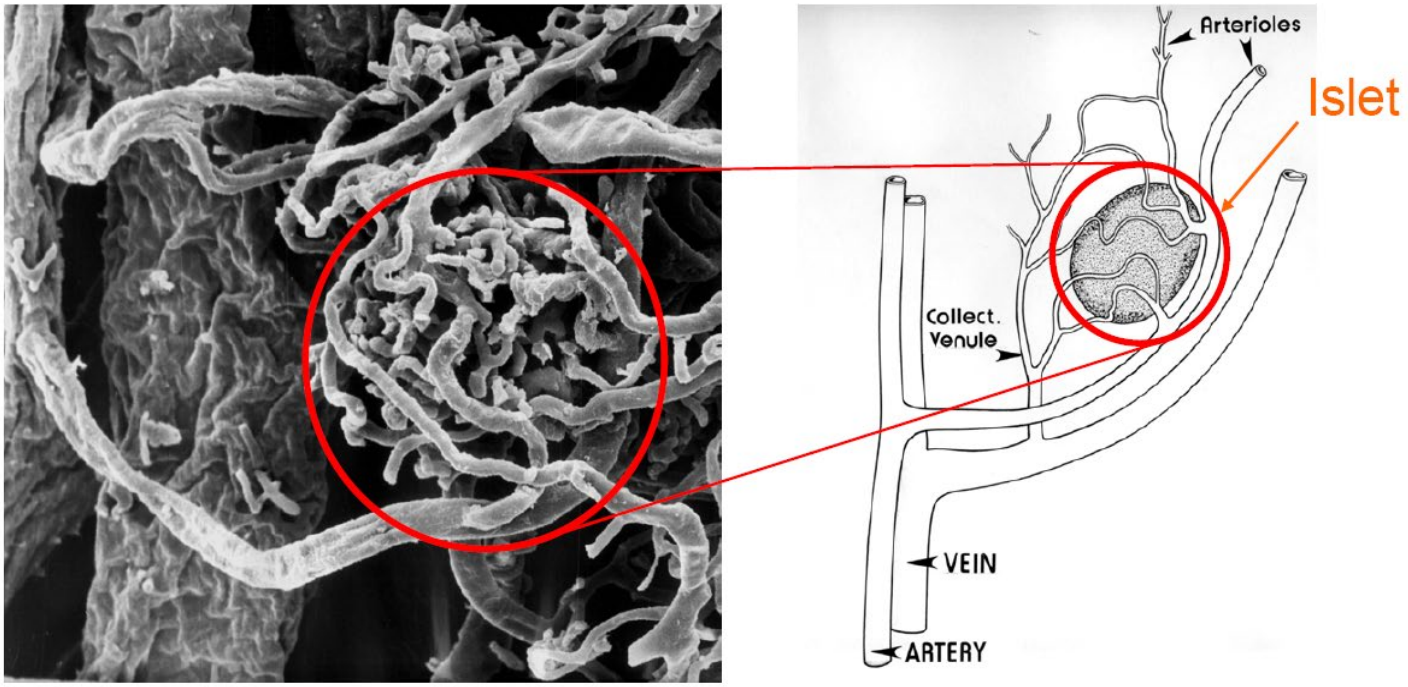
Islet Characteristics/ Mechanistic Information

Islet location and structure – islet contains multiple cell types



Islets are Complex "Organoids"

Microvasculature of Rodent Islets

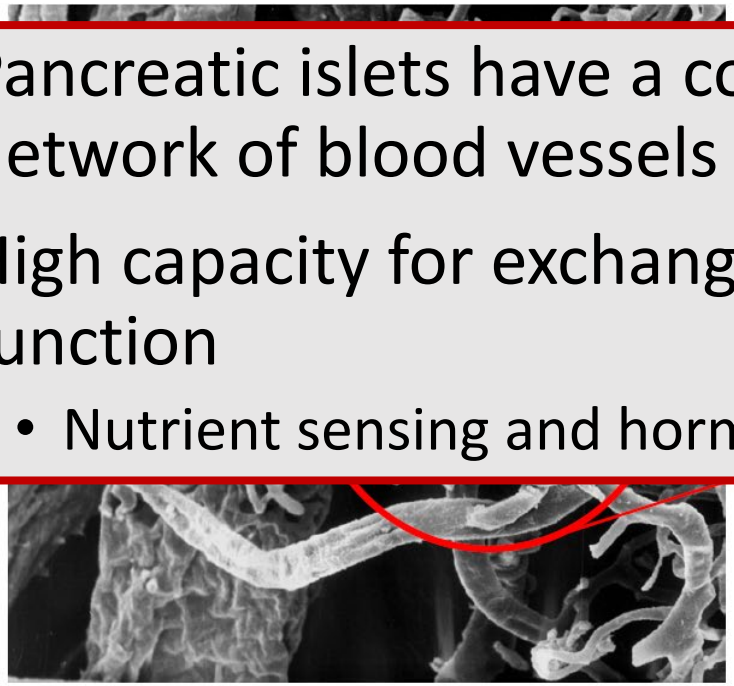


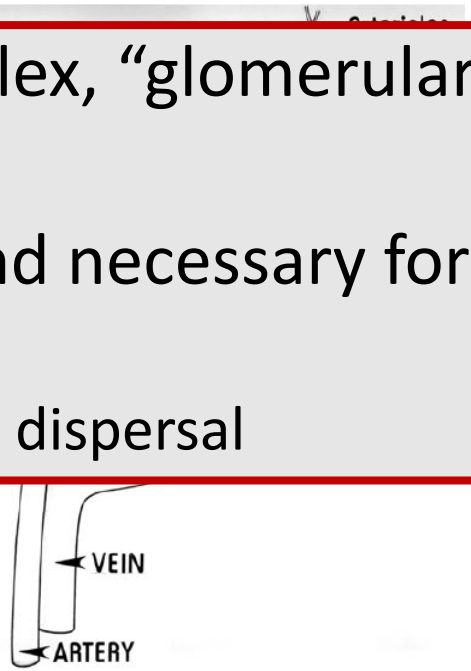
Bonner-Weir and Orci, Diabetes 1982

Islets are Complex "Organoids"

Microvasculature of Rodent Islets

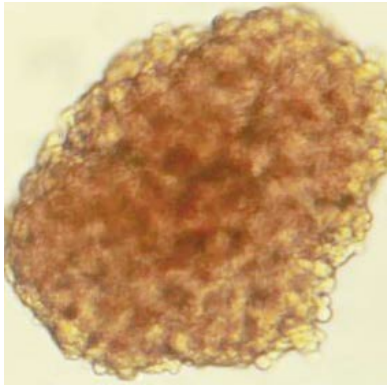
- Pancreatic islets have a complex, "glomerular-like" network of blood vessels
- High capacity for exchange and necessary for islet function
  - Nutrient sensing and hormone dispersal





Bonner-Weir and Orci, Diabetes 1982

Definition of an Islet Equivalent (IE)



Islet = Cell Cluster 50-400 µm in Diameter

150 µm Islet (Sphere) = 1 “Islet Equivalent” (IE)

1 IE has ~1500–2000 Cells

~50%-75% of these Cells are
Expected to be β-Cells that Produce Insulin

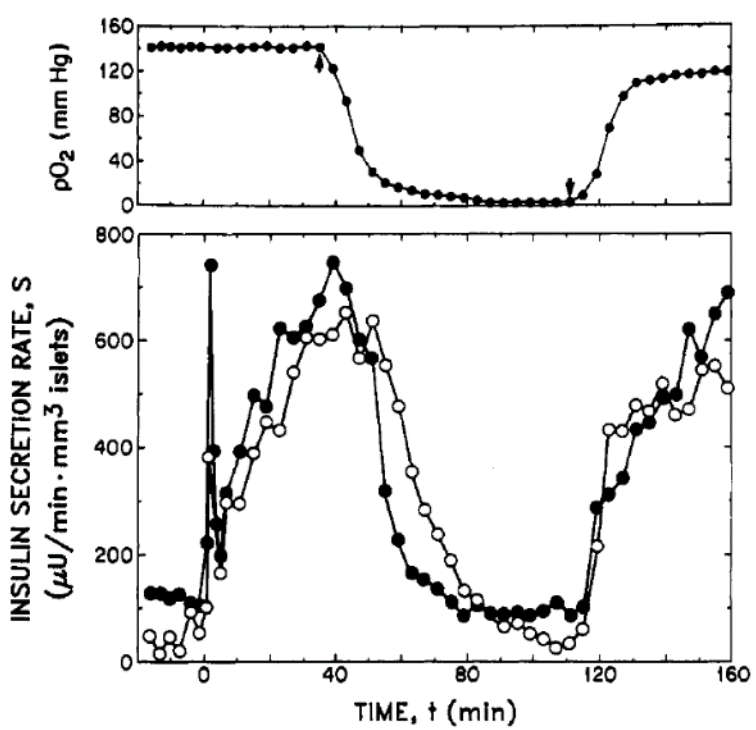
Islets also contain alpha-cells that produce glucagon as well as delta-cells that produce somatostatin

Islet (β-cell) function is highly sensitive to hypoxia

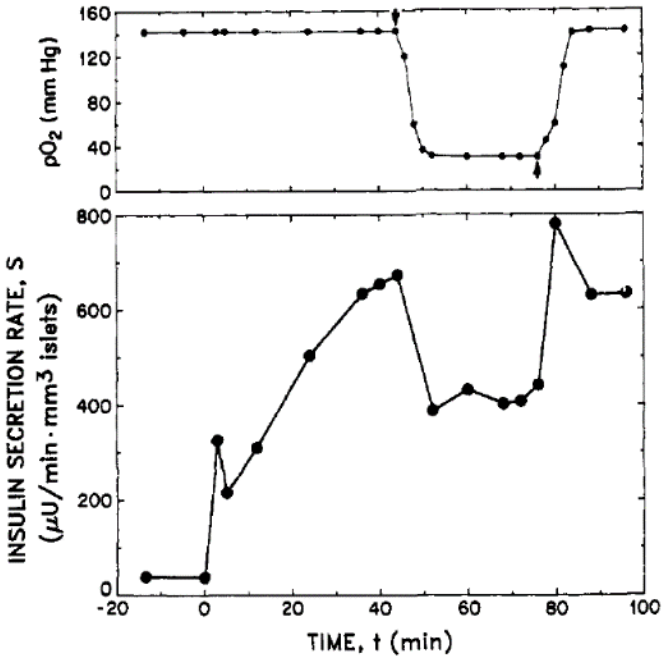
Biotechnol. Prog. **1991**, *7*, 359–368

A Microperifusion System with Environmental Control for Studying Insulin Secretion by Pancreatic Tissue

Keith E. Dionne,[†,‡] Clark K. Colton,[*,†] and Martin L. Yarmush[§]



Biotechnol. Prog., 1991, Vol. 7, No. 4



Islets are highly sensitive to hypoxia

β-cells are unable to effectively produce ATP anaerobically:
- low LDHα

Biochem. J. (2000) **352**, 373–380 (Printed in Great Britain)

Importance of lactate dehydrogenase for the regulation of glycolytic flux and insulin secretion in insulin-producing cells

Oscar ALCAZAR, Markus TIEDGE and Sigurd LENZEN[1]
Institute of Clinical Biochemistry, Hannover Medical School, D-30623 Hannover, Germany

Overexpression of LDHα impairs islet function

Overexpression of monocarboxylate transporter and lactate dehydrogenase alters insulin secretory responses to pyruvate and lactate in β cells

J. Clin. Invest. 104: 1621-1629, 1999

Hisamitsu Ishihara,[1] Haiyan Wang,[1] Lester R. Drewes,[2] and Claes B. Wollheim[1]

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A Preexistent Hypoxic Gene Signature Predicts Impaired Islet Graft Function and Glucose Homeostasis

James Cantley,*†[1] Stacey N. Walters,†‡[1] Min-Ho Jung,§ Anita Weinberg,†‡ Mark J. Cowley,†¶ P. Tess Whitworth,*† Warren Kaplan,†# Wayne J. Hawthorne,** Philip J. O'Connell,** Gordon Weir,§ and Shane T. Grey†‡

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- 6 hr exposure to hypoxia with HIF1α activation is sufficient to cause a persistent "hypoxic signature" e.g. LDH-α, resulting in long-term (months) impairment of insulin secretion

Original Basic Science—General

(*Transplantation* 2017;101: 2705–2712)

OPEN

Acute Ischemia Induced by High-Density Culture Increases Cytokine Expression and Diminishes the Function and Viability of Highly Purified Human Islets of Langerhans

Kate E. Smith, MS,[1,2] Amy C. Kelly, BS,[3] Catherine G. Min, MS,[1,2] Craig S. Weber, BS,[4] Fiona M. McCarthy, PhD,[3] Leah V. Steyn, PhD,[1] Vasudeo Badarinarayana, PhD,[5,6] J. Brett Stanton, BS,[1] Jennifer P. Kitzmann, MPH,[1] Peter Strop, PhD,[5,6] Angelika C. Gruessner, PhD,[7] Ronald M. Lynch, PhD,[4] Sean W. Limesand, PhD,[3] and Klearchos K. Papas, PhD[1]

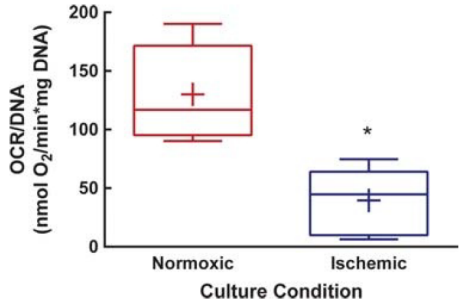

FIGURE 1. Human islet viability is reduced after acute ischemia. After 12 hours of control (normoxic) or ischemic exposure, islet viability was determined by OCR/DNA. Shown above are values for n = 8 paired experiments. *P = 0.01. Data mean are indicated by +, whiskers indicate minimum and maximum values. Box bounds indicate upper and lower quartiles, and the median value is indicated by the line within the box.

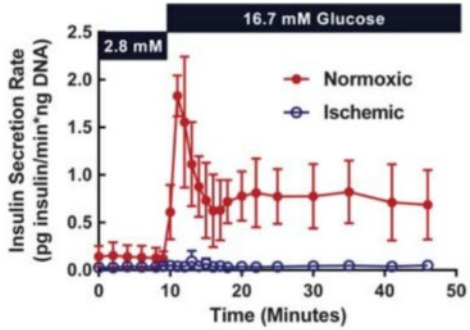

FIGURE 2. Human islet function is absent after acute ischemic exposure. To determine β cell function, GSIS was measured on a perifusion system. Shown above are the insulin secretion profiles for control and ischemic islets. The figure indicates mean ± SD values for n = 4 pairs of islets.

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TABLE 2.

Signaling pathways enriched following acute ischemia in human islets

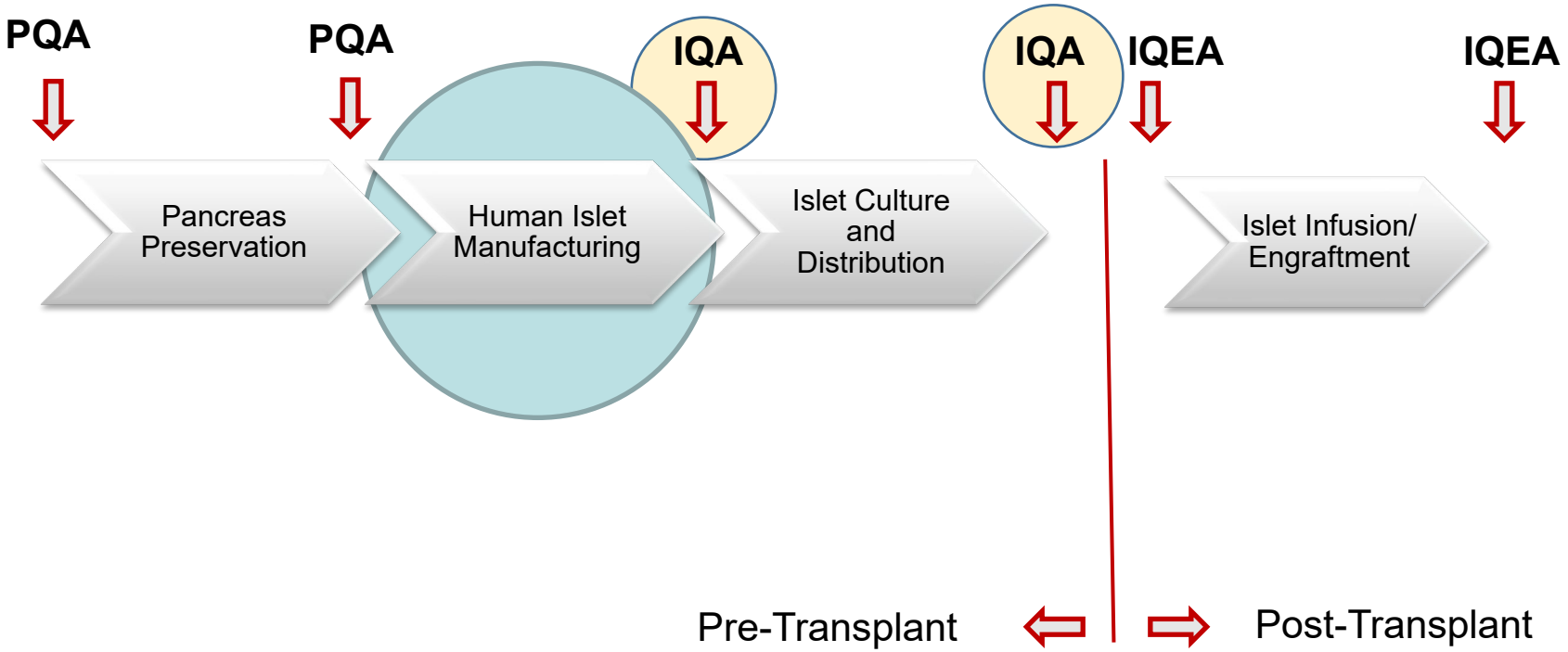
Canonical pathway	Database	DE genes	Corrected *P* value
TNF signaling pathway	KEGG PATHWAY	23	4.1×10^{-8}
Cytokine-cytokine receptor interaction	KEGG PATHWAY	25	4.1×10^{-4}
HIF-1-alpha transcription factor network	PID	17	4.3×10^{-4}
Cellular Senescence	Reactome	16	9.8×10^{-4}
Chemokine receptors bind chemokines	Reactome	9	9.8×10^{-4}
Extracellular matrix organization	Reactome	20	1.6×10^{-4}
NOD-like receptor signaling pathway	KEGG PATHWAY	10	2.7×10^{-3}
Cellular responses to stress	Reactome	18	3.3×10^{-3}
AP-1 transcription factor network	PID	15	4.8×10^{-3}
ATF-2 transcription factor network	PID	13	8.7×10^{-3}

Shown above is a summary of the most enriched pathways in ischemic human islets. Top pathways were determined for upregulated genes using KOBAS 2.0, drawing from KEGG, PID, and Reactome databases. Significance was defined as a $P < 0.05$ following Fisher exact test with Benjamini-Hochberg correction. The number of genes differentially expressed in islets and annotated in these pathways are presented in DE genes column and compared to the total number of genes annotated to that pathway in the databases to generate frequency. Note that although Malaria and Rheumatoid Arthritis appeared in the top 10 pathways shown above, they were excluded from the list due to appearance from nondisease specific inflammatory genes including CCL2, CCL20, CCL3L3, CSF2, CSF3, CXCL1, CXCL5, CXCL8, FLT1, FOS, HBA2, HBB, HGF, ICAM1, IL6, JUN, SELE, and VCAM1.

Do we need to worry about islet quality pre-Tx?

Islet Processing and Engraftment: Focus on the "islet"

Islets are exposed to a number of stresses in key steps from donor to ITx recipient



PQA: Pancreas Quality Assessment

IQA: Islet Quality Assessment

IQEA: Islet Quality and Engraftment Assessment

Transplant Proc 2001, 33(1-2): 1709

ELSEVIER

Challenges Toward Standardization of Islet Isolation Technology

C. Ricordi, J.R.T. Lakey, and B.J. Hering

SEVERAL efforts have been conducted toward improved islet isolation technology based on the principles established by the automated method for pancreatic islet isolation.[1] Several minor modifications of the procedure have been introduced over the last decade. However, it has been difficult to determine the real contribution of each change introduced in the islet isolation and purification process. In fact, modifications that may allow for extraction of an increased number of purified or semipurified islets from each donor pancreas may not necessarily reflect an increased viability and function of the final islet product. In addition, identification of parameters in islet processing that are predictive of insulin independence following human islet transplantation (ITx) has proven difficult. Nevertheless, controlled islet manufacturing processes and validated islet batch product release criteria will help in identifying variables that are predictive of ITx success and that could serve to further improve current methods in islet isolation, purification, and pretransplant in vitro culture.

Suitable assays for human islet product testing have yet to be identified, validated, and implemented, and the lack of insulin independence following single-donor islet transplantation has virtually eliminated the validation of quality control assays being predictive for insulin independence. In addition, an increasingly demanding regulatory framework, such as the one employed by the US FDA (Center for Biologics Evaluation and Research) for cellular and tissue-based products will impose standardization of islet product testing in the setting of clinical transplantation. Laws, regulations, and guidelines are already in place to address some of the quality control and product release criteria that may become applicable or required for any clinical islet transplant procedure (Table 1). The regulatory framework applies to the procurement, the manufacturing process, product safety testing, and product characterization of pancreatic islet tissue intended for transplantation. Product safety includes testing for sterility, mycoplasma, pyrogenicity/endotoxin, and freedom from adventitious agents. Product characterization includes identity, purity, cell number or amount of tissue, viability, potency, and stability of the final islet product. Product characterization can be performed on aliquots of the final islet preparation collected before transplantation. Presently, no selected test for islet quality assessment has proven predictive of successful islet transplantation, and more work will be necessary in the field to develop reliable and predictive tests that could be used to evaluate prospectively the suitability of each islet preparation for transplant applications.

Table 1. Laws, Regulations, and Guidelines for ITx

Laws
- Food, Drug, and Cosmetic Act and Public Health Service Act

Regulations
- 21 CFR 312, 610, 800, 1270: Safety, effectiveness; biological product standards; medical device standards; tissues intended for transplantation

Guidelines
- 1993: Statement for Somatic Cell and Gene Therapies
- 1997: Proposed Appendix to the Regulation of Cell and Tissue-Based Products
- 1998: Establishment Registration and Listing—proposed rule
- 1999: Donor Suitability Determination—proposed rule
- Good Tissue Practices (GTP)—under development
- 1998: Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy

REFERENCE

1. Ricordi C, Lacy P, Finke E, et al: Diabetes 37:413, 1988

From the University of Miami (C.R.), Miami, Florida; University of Alberta (J.R.T.L.), Edmonton, Alberta, Canada; and University of Minnesota (B.J.H.), Minneapolis, Minnesota.

Address reprint requests to Dr C. Ricordi, 1450 NW 10th Ave, Miami, FL 33136.

Transplantation Proceedings 2001, 33(1-2): 1709

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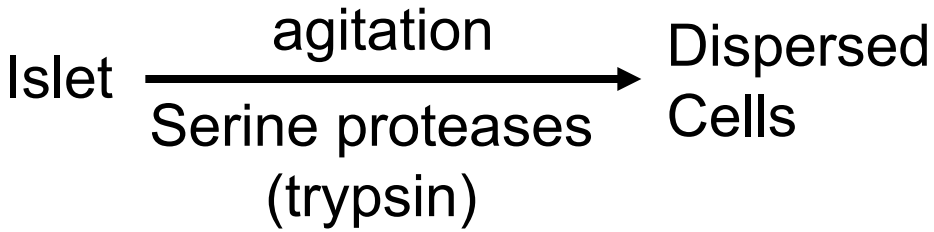
Institute for Cellular Transplantation

Why Are Islet Preparations Difficult To Characterize?

1. Islets are cellular aggregates.
   Variety of shapes and sizes
   Visual size estimation is
   - prone to error
   - operator dependent
   - large uncertainty
2. Human preparations have varying amounts of impurities.
   Distinguishing properties of islets/exocrine tissue difficult
3. The islet is a moving target.
   Damage occurs during
   - pancreas preservation
   - isolation
   - culture
   - shipment
   - post-Tx
4. Many techniques for cells are inapplicable to islets because the islets may not be usefully dissociated into cells.



- Cells are damaged: anoikis
- Cells are lost
- Recovered cells are likely not representative of original islet

Islet Potency – Nude Mouse Bioassay

Islet assessment for transplantation

Klearchos K. Papas[a,b], Thomas M. Suszynski[a] and Clark K. Colton[b]

Table 3 Strengths and limitations of the diabetic nude mouse bioassay

Assay	Strengths	Limitations
Nude mouse bioassay	Most reliable in-vivo assessment of islet potency Results correlate with clinical outcome	Assay can only be used retrospectively (days to weeks for outcomes) Impure preparations may yield false-negative transplant outcomes The severity and duration of the diabetic state of the mouse affects the predictive outcome of the assay Islets are transplanted into the kidney capsule, not into the hepatic portal system (thereby not fully representing the current clinical protocol) Mice are susceptible to developing other conditions (e.g., infection) that can also affect outcome Does not account for immunologic rejection or the effect of immunosuppressive agents on islets The assay carries several practical challenges (e.g., induction of diabetes needs to be timed with islet isolation)

Current Opinion in Organ Transplantation 2009, 14:674–682

Papas KK, *Sensitivity and Specificity of the Nude Mouse Bioassay to the Clinical Islet Allotransplantation Outcome.* ICR-ABCC, City of Hope. PDF: https://icr.coh.org/docs/PDF%20Powerpoint%20Conversions/Papas%20Animal%20Study%20Slides.pdf

Transplantation 2008; 86: 360-363

Quantitative In Vivo Islet Potency Assay in Normoglycemic Nude Mice Correlates With Primary Graft Function After Clinical Transplantation

Robert Caiazzo,[1,2] Valery Gmyr,[1,3] Bertrand Kremer,[1] Thomas Hubert,[1] Benoit Soudan,[4] Bruno Lukowiak,[1,3] Brigitte Vandewalle,[1] Marie-Christine Vantyghem,[5] Francois Pattou,[1,2,3,6] and Julie Kerr-Conte[1,3]

TABLE 1. Correlation between graft characteristics and C-peptide increase in man

Criteria	Mean±SE	r^2	*P*
Islet mass (IEQ/kg)	5366±1127	0.17	0.31
Islet number (n/kg)	5661±282	0.17	0.30
Beta cell number (10^6 cells/kg)	2.9±0.5	0.09	0.50
Islet viability (%)	93±1	0.40	0.12
Islet purity (%)	51±6	0.13	0.38
ATP content (pmoles/40IE)	129.9±26.5	0.33	0.18
insulin secretion stimulation index	2.3±0.6	0.07	0.56
hCP in mice (ng/mL)	1.3±0.5	0.85	0.003[a]

[a] Statistically significant.
hCP, human C-peptide; IEQ, islet equivalent.

The only assay significantly correlated with clinical outcome was human C-peptide in mice

Islet assessment for transplantation

Klearchos K. Papas[a,b], Thomas M. Suszynski[a] and Clark K. Colton[b]

[a]Department of Surgery, Schulze Diabetes Institute, University of Minnesota, Minneapolis, Minnesota and [b]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

Current Opinion in Organ Transplantation 2009, 14:674–682

Table 1 Product release criteria for clinical islet preparation

Type of test	Product test	Specification	Type of sample
Safety	Endotoxin	<5 EU/kg	Supernatant of islet suspension in transplant medium
	Gram stain	No organisms detected within limits of assay	
Identity	Islet count (IE/kg)	5000–20 000 (first transplant) 3000–20 000 (re-transplants)	Islets in transplant medium
	Purity	≥30%	
Viability	Dye exclusion (FDA/PI)	≥70%	Islets after overnight culture and in transplant medium
Potency	Glucose-stimulated insulin release (ELISA)	Stimulation Index >1	Islets after overnight culture

DTZ, dithizone; EU, endotoxin unit; FDA/PI, fluorescein diacetate/propidium iodide; IE, islet equivalent. Islet equivalent defined as a volume of islet tissue equal to that of a sphere having a 150-μm diameter (as given in [15]).

Islet assessment for transplantation

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Theoretically when combined, information on identity, viability and potency as described in this TABLE 1 should provide information on the number of viable/functional β-cells transplanted/Kg BW recipient (and should be predictive of transplant outcome in the absence of immune rejection)

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However, this may not be true unless:

1) Measurement of IE number, viability, and purity, is accurate and precise;
2) β-cell fraction/islet is relatively constant (or is measured and accounted for);
3) β-cell function (insulin secretion) is not impaired or it is within acceptable limits.

Islet assessment for transplantation

Klearchos K. Papas[a,b], Thomas M. Suszynski[a] and Clark K. Colton[b]

Table 2 Strengths and limitations of assays currently used prior to islet product release for clinical transplantation

Assay	Strengths	Limitations
Islet count (IE)	Relatively easy to perform counts Experienced islet isolation centers have standardized procedures	Visual assessment of 3D islet in 2D planes contributes to error Sample may not be representative of whole preparation Presence of contaminant tissue (e.g., exocrine cells, ganglia, etc.) may complicate counts
Purity (DTZ)	Stain differentiates between exocrine and islet tissue Relative ease of use Rapid assessment	Visual assessment of 3D islet in 2D planes contributes to error Provides no information regarding viability of preparation
Cell membrane integrity (FDA/PI)	Relative ease of use Can be performed prospectively Fractional viability can be estimated by dye exclusion	Visual assessment of 3D islet in 2D planes contributes to error Impossible to identify irreversibly damaged cells whose plasma membranes have not yet been permeabilized FDA may be additionally cleaved by lipases or esterases from nonendocrine tissue, overestimating the true islet viability Visual counting is operator dependent Background fluorescence (with certain combinations or high concentrations of dyes) can obscure approximations Counterstain may not provide enough contrast Dyes rely on diffusion to penetrate into islet core Lack of correlation with mitochondrial function assays, NMB, and clinical outcomes Does not discriminate endocrine (islet) from exocrine (contaminant) tissue
Glucose-stimulated insulin secretion	May provide information regarding potency of islet preparation	Unable to predict true islet potency or transplant outcome Islets may not be as responsive to glucose stimulus *in vitro* but may still reverse diabetes *in vivo* Difficult to account for degranulation of β-cells following glucose stimulus or 'leaky' cells with damaged plasma membranes

2D, two-dimensional; 3D, three-dimensional; DTZ, dithizone; FDA/PI, fluorescein diacetate/propidium iodide; IE, islet equivalent; NMB, nude mouse bioassay. Islet equivalent is defined as a volume of islet tissue equal to that of a sphere having a 150-μm diameter (as given in [15]).

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Islet count (IE)	Relatively easy to perform counts Experienced islet isolation centers have standardized procedures	Visual assessment of 3D islet in 2D planes contributes to error Sample may not be representative of whole preparation Presence of contaminant tissue (e.g., exocrine cells, ganglia, etc.)
		Difficult to account for degranulation of β-cells following glucose stimulus or 'leaky' cells with damaged plasma membranes

2D, two-dimensional; 3D, three-dimensional; DTZ, dithizone; FDA/PI, fluorescein diacetate/propidium iodide; IE, islet equivalent; NMB, nude mouse bioassay. Islet equivalent is defined as a volume of islet tissue equal to that of a sphere having a 150-μm diameter (as given in [15]).

Current Opinion in Organ Transplantation 2009, 14:674–682

Notes:

1) Stimulation Index (ratio of glucose stimulated over basal insulin secretion in a static incubation setting) by itself cannot be predictive of Transplant outcome; However, it may be proven more useful when combined with information such as specific basal and glucose stimulated insulin secretion rate, especially if obtained with Dynamic (Perifusion) assays.

2) It may be important to consider α-cell function and glucagon release.

What Are the Characteristics of Interest? and What Tools Are Available?

- **Quantity of tissue**
  - Volume
  - Number of Cells
  - Composition
- **Viability**
  - Membrane Integrity
  - Mitochondrial Function
  - Apoptosis
- **Potency**
  - Glucose Stimulated Insulin Release (Static/Dynamic – Perifusion)
  - Immunodeficient Mouse Transplant

Institute for Cellular Transplantation

Quantity of Tissue – Current Release Test Methods

Characterization of islet preparations. In: Cellular Transplantation. Elsevier; 2007:85-133.

Type of Quantity	Tissue Assayed	Parameter Measured	Method
Volume	Islet Preparation	Tissue volume	• Packed cell volume of tissue pellet
		Islet volume	• Insulin content • Dithizone (DTZ) staining Visual counting / Image analysis } Enumeration of islet equivalents (IE)
Number of Cells	Islet Preparation	Total DNA Total intact cell nuclei	• DNA content • Nuclei counting
Cell Composition	Islet Preparation	Volume fraction islets	• DTZ staining • Morphology (light microscopy)
		Individual cell types	• Ultrastructural analysis (electron microscopy), immunohistochemistry
	Dispersed Cells	Individual cell types	• Differential staining (laser scanning cytometry)

Institute for Cellular Transplantation

Quantity of Tissue – Improved Test Methods

Characterization of islet preparations. In: Cellular Transplantation. Elsevier; 2007:85-133.

Type of Quantity	Tissue Assayed	Parameter Measured	Method
Volume	Islet Preparation	Tissue volume	• Packed cell volume of tissue pellet
		Islet volume	• Insulin content • Dithizone (DTZ) staining Visual counting / Image analysis } Enumeration of islet equivalents (IE)
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STEM CELLS TRANSLATIONALMEDICINE 2016;5:1338–1344

STEM CELLS TRANSLATIONAL MEDICINE

ENABLING TECHNOLOGIES FOR CELL-BASED CLINICAL TRANSLATION

Concise Review: Markers for Assessing Human Stem Cell-Derived Implants as β-Cell Replacement in Type 1 Diabetes

DANIEL PIPELEERS,[a,b] THOMAS ROBERT,[a] INES DE MESMAEKER,[a] ZHIDONG LING[a,b]

Key Words. Diabetes • Insulin • Transplantation • Cell therapy • Encapsulation

Dow

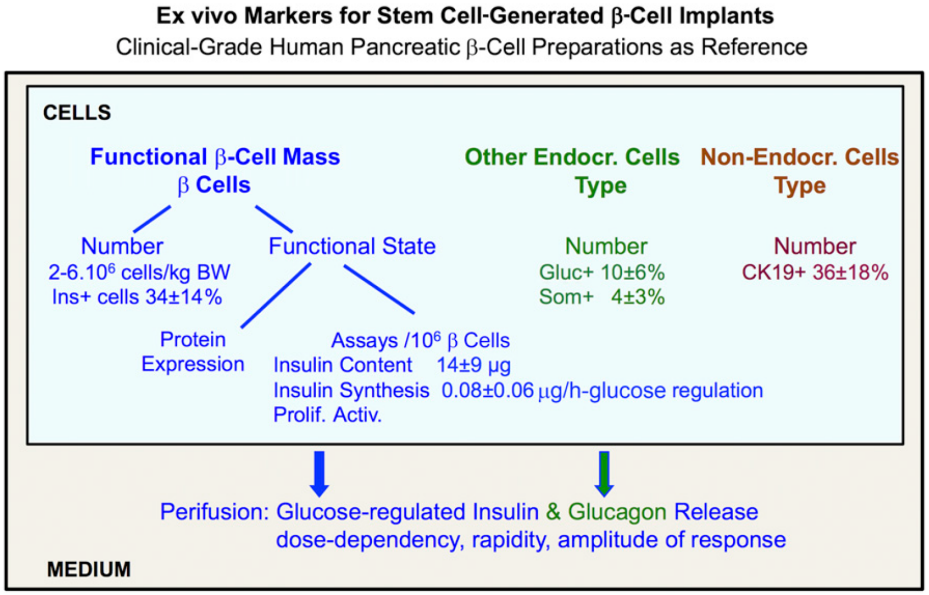


Figure 2. Ex vivo markers for stem cell-generated β-cell implants. Ex vivo markers analyze retrieved implants for their cellular composition and functions (see text for references). Data are compared with those collected for clinical grafts prepared from human pancreatic β-cell isolates. Figure shows data for this reference preparation, which requires minimally 2.10^6 β cells per kilogram of BW to achieve a metabolic effect in recipients with type 1 diabetes. Abbreviations: BW, body weight; Endocr., endocrine; Ins., insulin; Prolif. Activ., proliferation activities.

β-cell fraction is highly variable in human islet preparations

RAPID COMMUNICATION

Assessment of Human Pancreatic Islet Architecture and Composition by Laser Scanning Confocal Microscopy

Volume 53(9): 1087–1097, 2005
Journal of Histochemistry & Cytochemistry
http://www.jhc.org

Marcela Brissova, Michael J. Fowler, Wendell E. Nicholson, Anita Chu, Boaz Hirshberg, David M. Harlan, and Alvin C. Powers

Department of Medicine, Division of Diabetes, Endocrinology, and Metabolism, Vanderbilt University School of Medicine, Nashville, Tennessee (MB,MJF,WEN,AC,ACP); Islet and Autoimmunity Branch of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland (BH,DMH); and VA Tennessee Valley Healthcare System, Nashville, Tennessee (ACP)

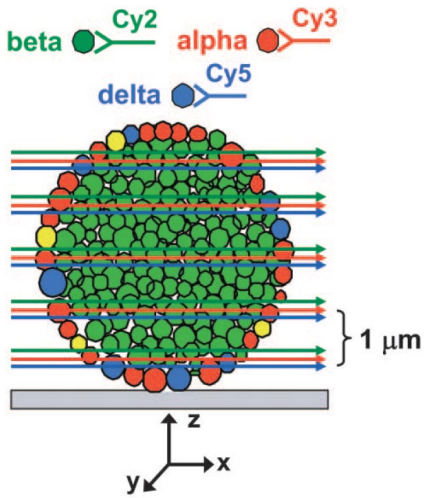
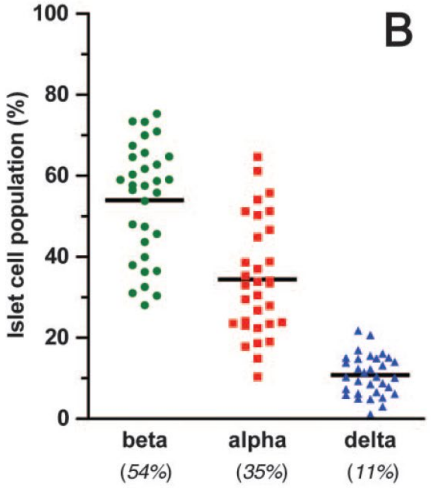
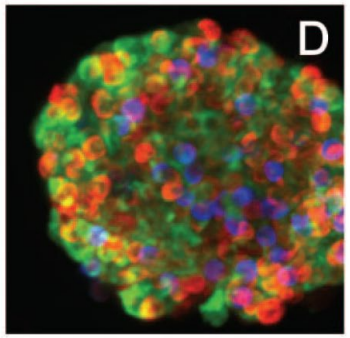


Figure 1 Schematic representation of optical sectioning of isolated islets by confocal laser scanning microscopy. Islet cell types are illustrated in four different colors: β cells, green; α cells, red; δ cells, blue; PP cells, yellow. Antibodies applied to islet hormones for islet cell labeling are shown schematically at the top. Red, green, and blue arrows represent image overlay of α, β, and δ cells in a single focal plane (optical slice). x,y,z refer to axis. Optical slices through islet were acquired by moving focal plane (x,y) along z-axis from the bottom to the top of the islet at 1-µm increments. Using image analysis software, individual optical sections were assembled into a three-dimensional (3-D) stack and projected as a 0° view with respect to the y-axis (head-on projection).





β-cell fraction is highly variable in human islet preparations

AJT 2005, 5: 1635-1645

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doi: 10.1111/j.1600-6

A Novel Method for the Assessment of Cellular Composition and Beta-Cell Viability in Human Islet Preparations

Hirohito Ichii[a,c], Luca Inverardi[a], Antonello Pileggi[a], R. Damaris Molano[a], Over Cabrera[a], Alejandro Caicedo[a], Shari Messinger[b], Yoshikazu Kuroda[c], Per-Olof Berggren[a,d] and Camillo Ricordi[a,*]

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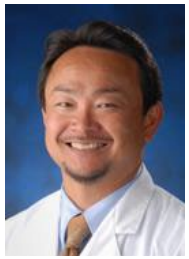
[c] *Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kobe University, Kobe, Japan and* [d] *Department of Molecular Medicine, Rolf Luft Center for Diabetes Research, Karolinska Institutet, SE-171 76 Stockholm, Sweden*
* *Corresponding author: Camillo Ricordi, ricordi@miami.edu*



Ichii et al.

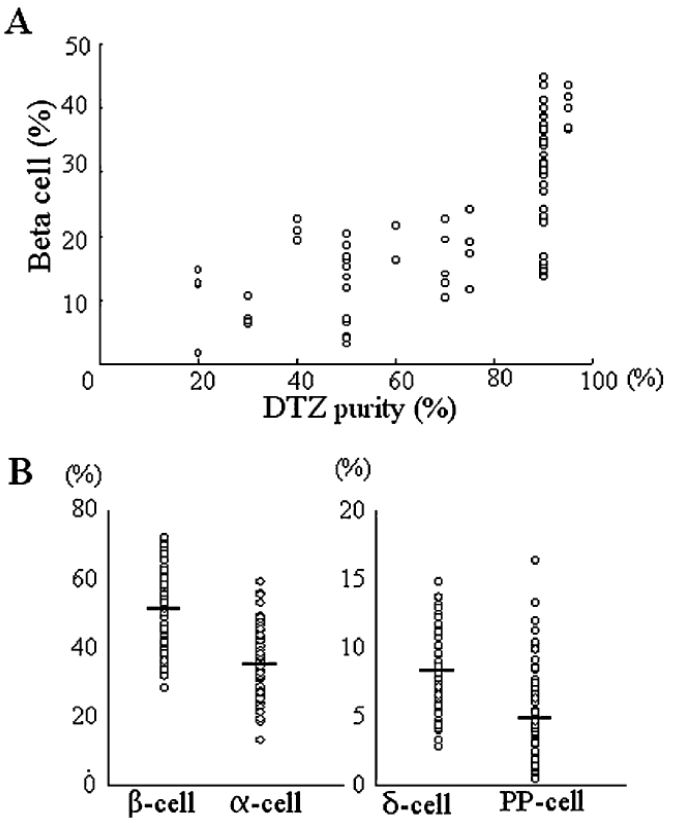


Figure 2: Beta cell content variability in individual human islet preparation. A. Relation between ß-cell percentage in whole islet preparations and purity, the latter assessed by DTZ staining. Beta-cell percentage was calculated as fraction of insulin positive cells over all cells (not only the endocrine subsets). Results were obtained by analyzing more than 60 preparations. B. Percentages of cells belonging to the indicated endocrine subsets were calculated and expressed as fraction over endocrine cells only, excluding nonendocrine cells from computation. Results were obtained by analysis of over 60 preparations.

β-cell fraction is highly variable in human islet preparations

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[a] Diabetes Research Institute, [b] Department of Epidemiology and Public Health, University of Miami, Leonard M. Miller School of Medicine, Miami, FL
[c] Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kobe University, Kobe, Japan and [d] Department of Molecular Medicine, Rolf Luft Center

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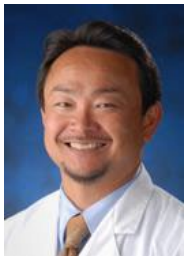
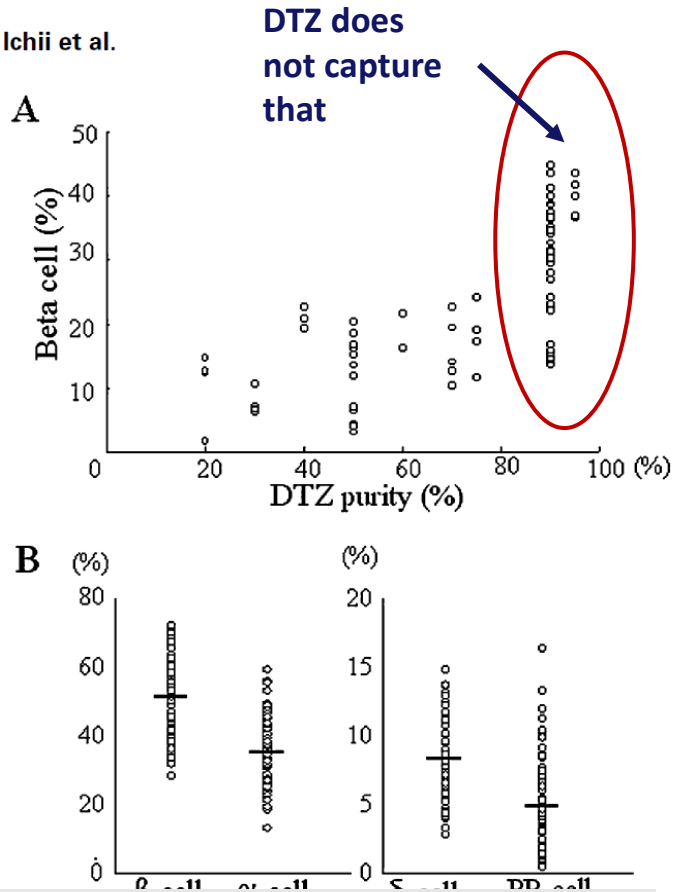


Ichii et al.



Therefore, it is important to measure β-cell fraction

preparation. A. Relation between ß-cell percentage in whole islet preparations and purity, the latter assessed by DTZ staining. Beta-cell percentage was calculated as fraction of insulin positive cells over all cells (not only the endocrine subsets). Results were obtained by analyzing more than 60 preparations. B. Percentages of cells belonging to the indicated endocrine subsets were calculated and expressed as fraction over endocrine cells only, excluding nonendocrine cells from computation. Results were obtained by analysis of over 60 preparations.

Islet Preparation Purity Is Overestimated, and Less Pure Fractions Have Lower Post-Culture Viability Before Clinical Allotransplantation

J.P. Kitzmann[a,b], T. Karatzas[a,c], K.R. Mueller[a,b], E.S. Avgoustiniatos[a], A.C. Gruessner[b], A.N. Balamurugan[a], M.D. Bellin[a], B.J. Hering[a], and K.K. Papas[a,b,*]

[a]Department of Surgery, University of Arizona, Tucson, Arizona; [b]Schulze Diabetes Institute, University of Minnesota, Minneapolis, Minnesota; [c]Second Department of Propedeutic Surgery, School of Medicine, University of Athens, Athens, Greece

Transplant Proc. 2014 ; 46(6): 1953–1955. doi:10.1016/j.transproceed.2014.06.011.

Islet Fraction Purity (by DTZ):
Pure: >70% (avg: 84.2%)
Less Pure: 30-69% (avg: 39.2%)

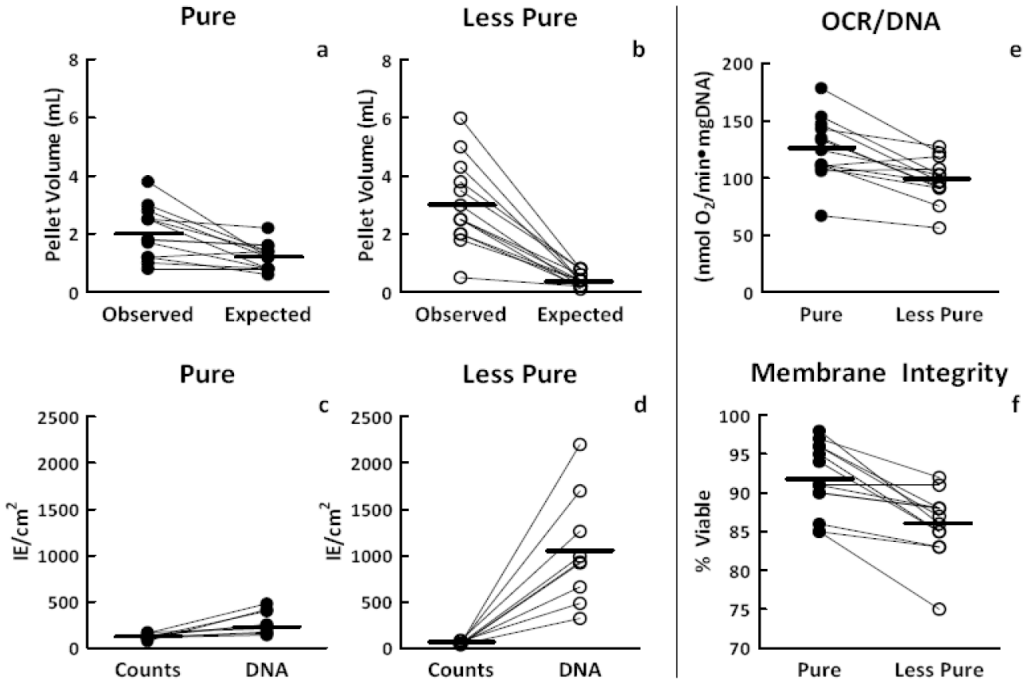


Fig 1.

Observed values plotted against theoretical expected valuesfor (a, b) pellet volume, (c,d) islet density (islet equivalents [IE]/cm^2) in culture flasks as measured by counts and DNA, and (e,f) islet preparation viability for separate purity fractions as measured by oxygen consumption rate normalized to DNA content (OCR/DNA) and membrane integrity staining for 13 clinical islet preparations.

Institute for Cellular Transplantation

Viability of Tissue – Current Release Test Methods

Type of Assay	Tissue Assayed	Method
Cell Membrane Integrity	Islet Preparation	**Live/Dead (Membrane Permeable)** Fluorescein Diacetate (FDA)/Propidium Iodide (PI) SYTO 13/Ethidium Bromide (EB) **All/Dead** LDS 751/Sytox Orange **Dead** Trypan Blue Quantitative assay via Nuclei Counting- 7- AAD
Mitochondrial Function	Islet Preparation	Redox state of the cell-Tetrazolium salts MTT, MTS Oxidative phosphorylation-Oxygen consumption rate (OCR) Energetic State-[ATP], [ATP]/[ADP], ATP production rate
	Dispersed Cells	Mitochondrial membrane potential (MMP)-Fluorescent dyes JC-1, TMRE (Flow Cytometry)
Apoptotic Events	Dispersed Cells	Early: Signaling pathway – Caspase activation Late: Nucleosome DNA fragmentation
	Fixed Tissue or Cells	Phosphatidyl serine translocation – Annexin V DNA fragmentation – TUNEL

Characterization of islet preparations. In: Cellular Transplantation. Elsevier; 2007:85-133.

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Apoptotic Events	Dispersed Cells	Early: Signaling pathway – Caspase activation Late: Nucleosome DNA fragmentation
	Fixed Tissue or Cells	Phosphatidyl serine translocation – Annexin V DNA fragmentation – TUNEL

Note: if islets are dissociated, there may be inaccurate data. Therefore, it is important to explore assays that maintain aggregate structure.

Examples of (Prospective) Assays with Attempts to Relate to/Predict Nude Mouse Bioassay Tx Outcome (Retrospective)

Oxygen Consumption Rate (OCR) Parameters

If OCR per viable cell ~ constant

Parameter	Proportional To	Measure of
OCR	$\frac{\text{Number of viable cells}}{\text{Volume of viable tissue}}$	Amount of good tissue
DNA	$\frac{\text{Number of cells}}{\text{Total tissue volume}}$	Total amount of tissue
$\frac{\text{OCR}}{\text{DNA}}$	$\frac{\text{Viable tissue volume}}{\text{Total tissue volume}}$	Quality of the tissue
$\frac{\text{OCR/DNA}}{(\text{OCR/DNA})_v}$	= Fractional Viability	

Assuming function is not impaired, viable β-cell (OCR) dose per Kg recipient BW would be expected to predict Tx outcome in the absence of immune rejection.

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Blackwell Munksgaard

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doi: 10.1111/j.1600-6143.2006.01655.x

Brief Communication

Human Islet Oxygen Consumption Rate and DNA Measurements Predict Diabetes Reversal in Nude Mice

K. K. Papas[a,*], C. K. Colton[b], R. A. Nelson[c], P. R. Rozak[a], E. S. Avgoustiniatos[a], W. E. Scott III[a], G. M. Wildey[a], A. Pisania[b], G. C. Weir[d] and B. J. Hering[a]

[a]Diabetes Institute for Immunology and Transplantation, Department of Surgery, University of Minnesota, Minneapolis, MN [b]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA [c]Administrative and Bioinformatics Coordinating Center (ABCC) for the Islet Cell Resource (ICR) Center Consortium, City of Hope National Medical Center and Beckman Research Institute, Duarte, CA [d]Joslin Diabetes Center, Harvard Medical School, Boston, MA

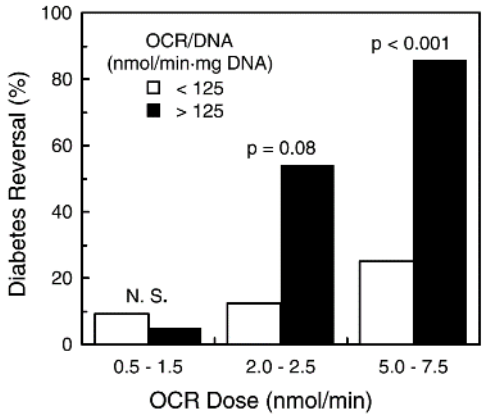

Figure 2: Rates of diabetes reversal (DR) in athymic nude mice for 3 OCR dose groups (low, 0.5–1.5 nmol/min; medium, 2–2.5 nmol/min; and high, 5–7.5 nmol/min) when transplanted islets were of low (OCR/DNA <125 nmol/min—mg DNA) or higher viability (OCR/DNA >125 nmol/min—mg DNA). N.S. = nonsignificant.

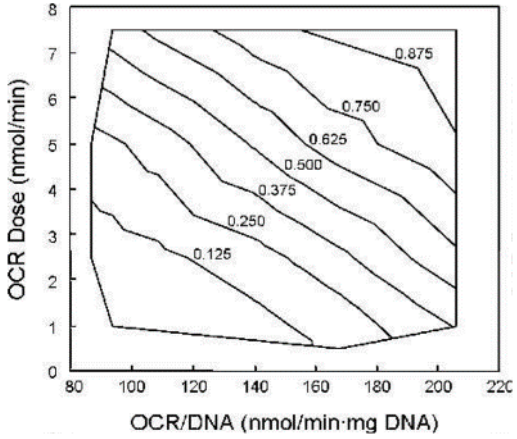

Figure 3: Probability of diabetes reversal (DR) in the athymic nude mouse bioassay (NMB) as a function of transplanted OCR (a measure of the transplanted viable tissue volume) and OCR/DNA (a measure of islet fractional viability). The model was based on outcomes from 86 mouse transplants. The model equation and optimization parameters are provided in the text.

J Invest Surg 2010, 23(1): 28-34

Prediction of Marginal Mass Required for Successful Islet Transplantation

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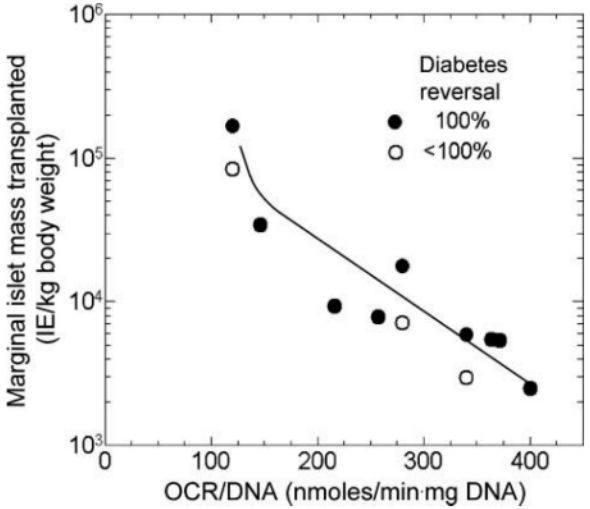

Figure 2. Dependence of normalized marginal islet mass on OCR/DNA. Filled circles correspond to the lowest OCR dose that produced DR in 100% of samples. Open circles correspond to the next lowest OCR dose (at which less than 100% of the sample cured). The straight line portion of the curve was determined by least squares regression of all data for which OCR/DNA ≥ 146-nmol/min mg DNA.

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Additional studies attempting to relate in vitro islet quality assays to diabetic mouse transplant outcome

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- Ichii H, et al. A novel method for the assessment of cellular composition and beta-cell viability in human islet preparations. American Journal of Transplantation. 2005;5(7):1635-1645.

All employed parameters related to oxygen consumption rate/mitochondrial function

Assays with attempts to relate to/predict clinical Tx outcome

RESEARCH ARTICLE

Islet Oxygen Consumption Rate (OCR) Dose Predicts Insulin Independence in Clinical Islet Autotransplantation

Klearchos K. Papas[1,2,3]*, Melena D. Bellin[2,3], David E. R. Sutherland[2,3], Thomas M. Suszynski[2,3], Jennifer P. Kitzmann[1,2,3], Efstathios S. Avgoustiniatos[2,3], Angelika C. Gruessner[1], Kathryn R. Mueller[1,2,3], Gregory J. Beilman[2], Appakalai N. Balamurugan[2,3], Gopalakrishnan Loganathan[2,3], Clark K. Colton[4], Maria Koulmanda[5], Gordon C. Weir[6], Josh J. Wilhelm[2,3], Dajun Qian[7], Joyce C. Niland[7], Bernhard J. Hering[2,3]

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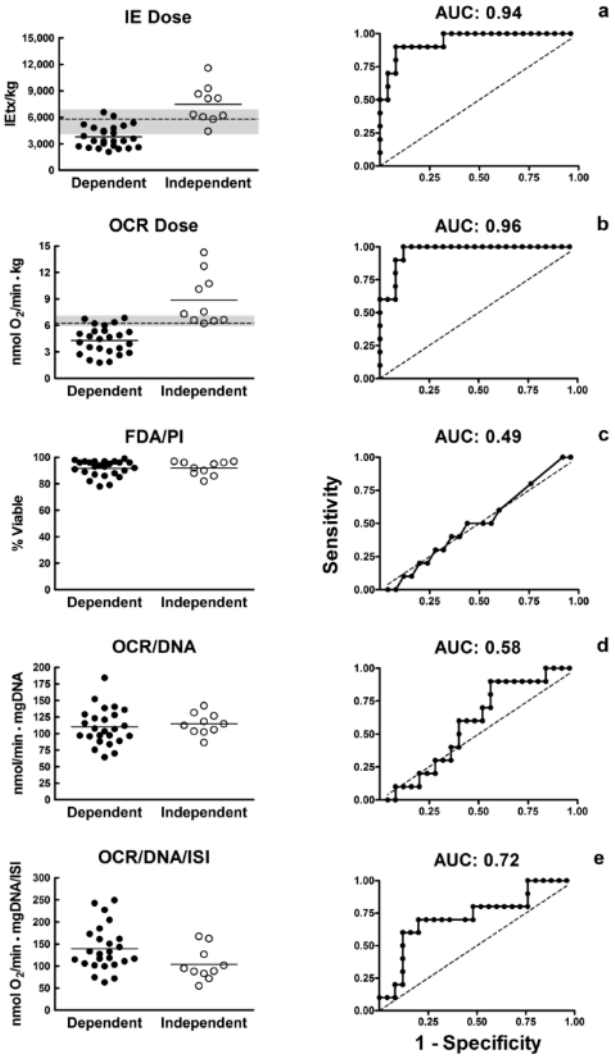
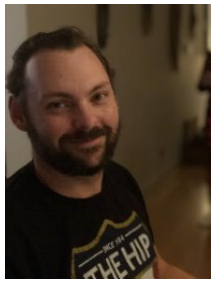


Fig 2. Overlap and correlation of islet characterization methods with clinical transplant outcome.

Transplant Proc 2014, 46(6):1985-1988



ELSEVIER CrossMark

Islet Oxygen Consumption Rate Dose Predicts Insulin Independence for First Clinical Islet Allotransplants

J.P. Kitzmann[a], D. O'Gorman[b], T. Kin[b], A.C. Gruessner[a], P. Senior[b], S. Imes[b], R.W. Gruessner[a], A.M.J. Shapiro[b], and K.K. Papas[a,*]

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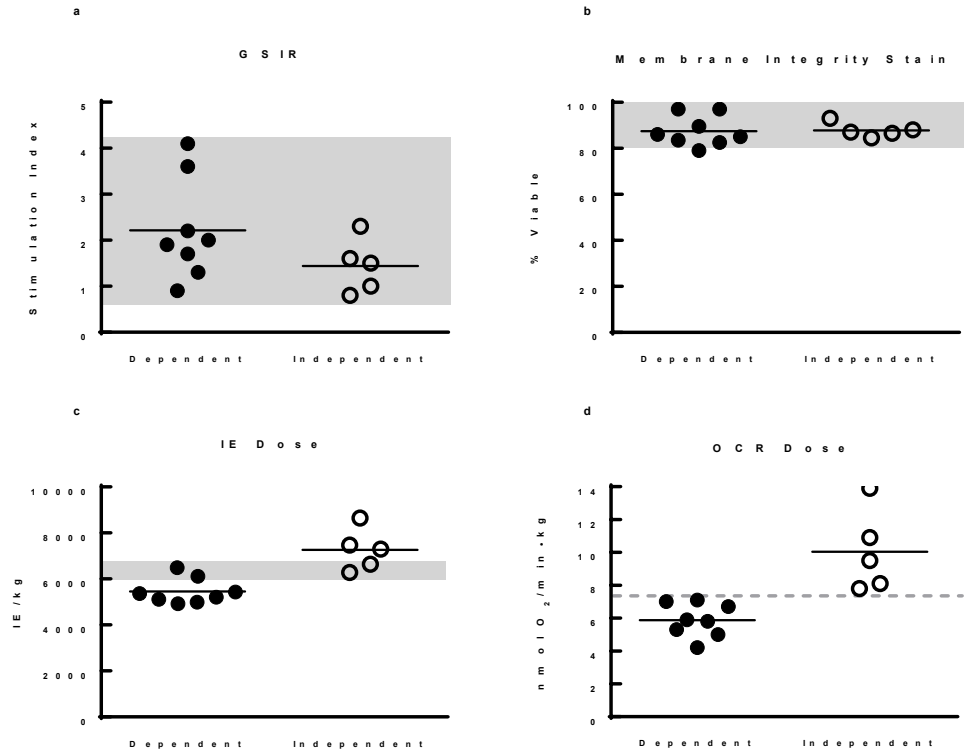

Funding that Enabled OCR Islet Potency Tests (Since 1999)

NIH Grants:

R44-DK069865
R01-DK063108-01A1
NCRR ICR U4Z 16606
U01DK070431-09
U42RR016598
148/U42RR017673
1R43DK075211-01A2
NIHNCRRRFARR001-002
R43DK069865
30.6693.912611

JDRF Grants:

JDRF Center for Islet Transplantation at Harvard Medical School
7-2005-1167

NIH National Institutes of Health
Turning Discovery Into Health

NIH Small Business Innovation Research (SBIR)
Small Business Technology Transfer (STTR)

NIH National Institute of Diabetes and Digestive and Kidney Diseases

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

City of Hope

Summary

While great progress has been made in the field:

- There is a need to further develop and refine real-time predictive potency tests for clinical islet allotransplantation.
- Islet nuclei counts and DNA measurements may further improve islet dosing especially when combined with measurements or β-cell (and α-cell) fraction.
- Measurements of islet preparation purity should be further refined and the relationship between islet purity and transplant outcome should be further explored.
- Viability and potency assays based on mitochondrial function (i.e. Oxygen Consumption Rate) appear to be useful and should be further explored.
- Attempts to correlate to clinical outcome should take into account viable (and functional) β-cell dose.

Institute for Cellular Transplantation (ICT)

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* Alexandra Hoeger
* Dr. Nathanial Hart
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* Katie Cocchi
* Hannah Douglas
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* Diana Molano

UA Collaborators including:

BIO 5

Dr. Jennifer Barton

UA BME

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Lynch Lab

Ron Lynch

Limesand Lab

Sean Limesand
Melissa Davis

**former lab member, no longer part of the ICT*

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Extra Slides

A Simplified Approach to Human Islet Quality Assessment

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TABLE 2. Evaluation of the predictive success of each islet quality test

Receiver operator characteristic analysis

Test (n=42)	AUC[a] (>0.8)	*P*	Test cutoff value	Sensitivity (% Detect +)	Specificity (% Detect −)	Likelihood ratio (>3)
Yield (IEQ/g)	0.695	0.036	3671	46.2	81.3	2.5
GSIS (S.I.)	0.692	0.038	3.8	65.4	68.8	2.1
MMP+ % (flow cytometry)	0.887	<0.0001	80.1	76.9	81.3	4.1
ATP/ADP (HPLC)	0.749	0.011	7.2	52.0	92.9	7.3

Multivariate discriminant analysis

Test (n=42)	Match between observed and predicted classifications of in vivo islet function[b] Sensitivity (%)	Specificity (%)	Overall rate of successful classification
MMP+ATP/ADP	75.0	84.6	81.0
MMP+GSIS	81.3	84.6	83.3
ATP/ADP+GSIS	43.8	80.8	66.7
MMP+ATP/ADP+GSIS	87.5	84.6	85.7

[c]Islet Quality Score=% of MMP$^+$ islet cells×(0.087)+GSIS SI×(0.15)+ ATP/ADP×(0.25)−9.20

	Good (n=16)	Poor (n=26)	P^d
Islet Quality Scores (mean±SD)	1.22±0.73	−0.75±1.13	<0.0001

[a] Receiver operator characteristic analysis (Graphpad Prism Software).
[b] Data shown are the rates of successful matching between observed and predicted isolation classifications based on multivariate discriminant analysis (Statistica software) of each islet quality test either alone or in combination (n=42).
[c] Formula determined by multivariate discriminate analysis of all 42 human islet preparations. Data shown are the mean±SD.
[d] *P* value calculated by two-tailed Student's *t* test.
AUC, area under the curve; MMP, mitochondrial membrane potential; HPLC, high-performance liquid chromatography; GSIS, glucose-stimulated insulin secretion.

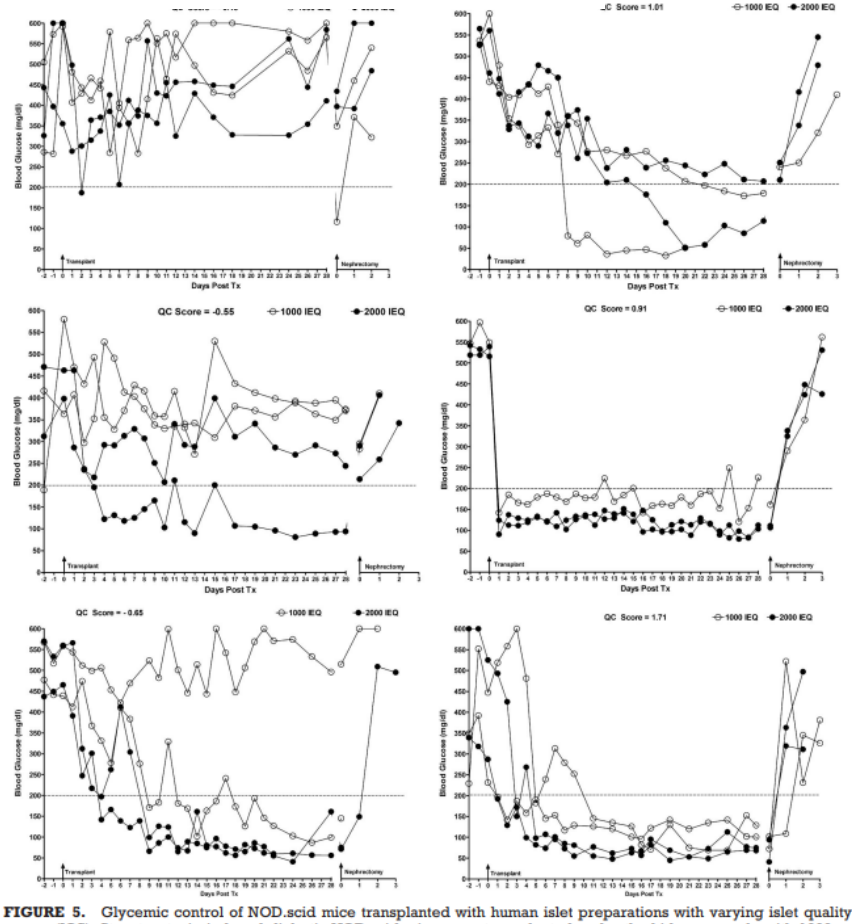

FIGURE 5. Glycemic control of NOD.scid mice transplanted with human islet preparations with varying islet quality scores (IQS). Streptozotocin-induced diabetic NOD.scid mice were transplanted under the kidney capsule with 1000 or 2000 IEQ doses of human islet preparations with IQS less than 0 (left panels) or more than 0 (right panels). Mice were considered cured if blood glucose levels fell below 200 mg/dL within 14 days of transplant and were maintained at or below that level until return to the hyperglycemic state after nephrectomy of the graft bearing kidney. IEQ, islet equivalent.

The Use of the BD Oxygen Biosensor System to Assess Isolated Human Islets of Langerhans: Oxygen Consumption as a Potential Measure of Islet Potency

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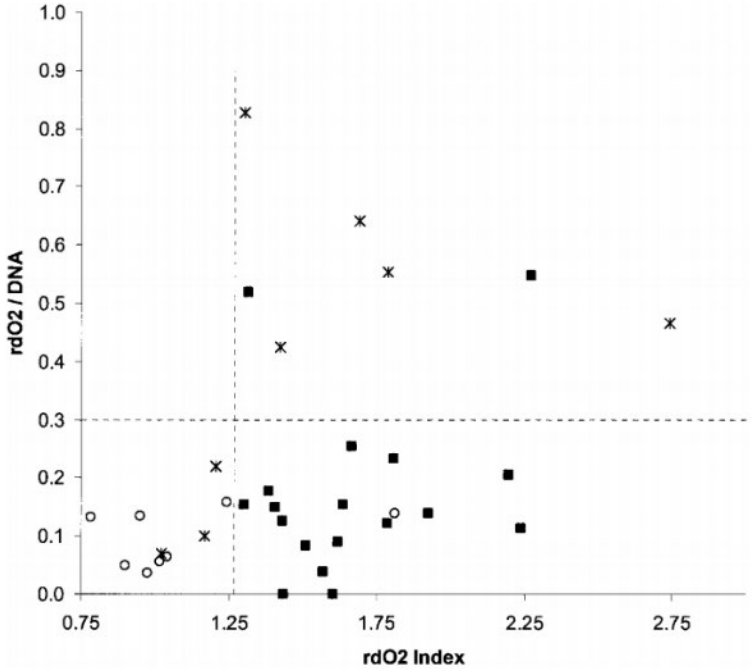

Figure 6. Transplant effectiveness as a function of both the OCR index and the DNA-normalized rdO_2 value. Indicated are preparations for which at least one of the replicate transplantations failed to reverse (circles), preparations where all replicates reversed within 3 days (X), and preparations where all animals reversed, but not all within 3 days (squares).

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The Islet Size to Oxygen Consumption Ratio Reliably Predicts Reversal of Diabetes Posttransplant

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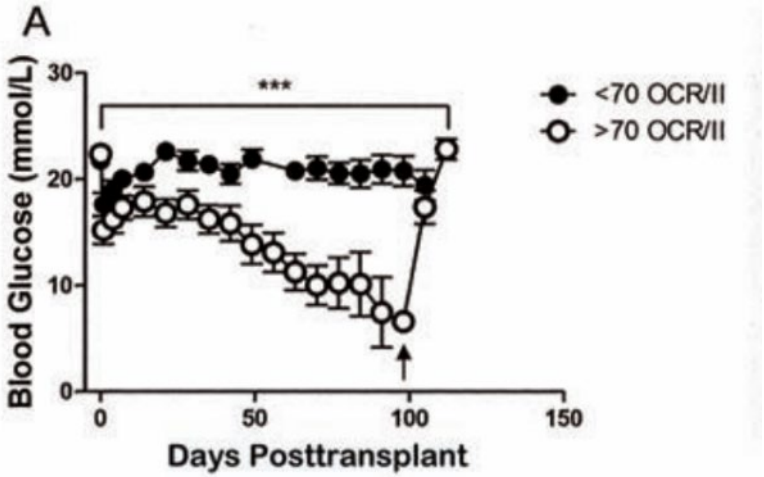
Table 1. Statistical Analysis Correlating In Vitro Islet Functional Assays to Reversal of Diabetes

	ROC Curve Analysis				Logistic Regression	
Assay	AUC	95% Confidence Limits	*p* Value	*n*	Intercept	Slope (SE)
Viability stain (AO/EtBr)	0.59 ± 0.09	0.42–0.76	0.30	45	–1.92	0.019 (0.019)
Glucose stimulated insulin index	0.54 ± 0.09	0.37–0.71	0.23	53	–1.35	0.54 (0.45)
Islet index (IEQ/A.I.)	0.63 ± 0.07	0.49–0.76	0.12	73	0.65	–0.73 (0.46)
OCR/DNA	0.68 ± 0.07	0.54–0.81	0.004	77	–1.65	0.009 (0.003)
OCR/II	0.79 ± 0.06	0.67–0.90	0.0002	75	–2.12	0.026 (0.007)

A.I., actual islet number; AO, acridine orange; AUC, area under the curve ± SEM; EtBr, ethidium bromide; IEQ, islet equivalent (volume of islet tissue equal to that of a sphere having a 150-µm diameter); II, islet index (IEQ/A.I); OCR, oxygen consumption rates; ROC, receiver operating characteristic; SE, standard error.