ROLE OF EQUIPMENT IN THE MANUFACTURING OF PANCREATIC ISLETS

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Why Islets?

Micro-organs

Glucose sensors

Produce glucagon

Produce insulin

Release insulin when needed

Maintain normoglycemia

Insulin
Glucagon
Somatostatin
FDA does not regulate transplantation of whole vascularized organs.

Tx of islet cells meet criteria for regulation as a biologic.
Pancreatic Islet Cells

- Biologic Product according to Section 351 of PHSA

- Somatic cell therapy according to FDA
  - Not a “practice of medicine”
  - Considered experimental in the U.S.
  - Clinical studies must be performed under IND (21 CFR Part 312) or BLA (21 CFR Part 600, 601 & 610)

- Drug according to Federal Food, Drug and Cosmetic Act

Prior to marketing the product as therapy for Type 1 diabetic patients the following need to be demonstrated by the manufacturer:

- Safety (sterility)
- Purity (Endotoxin)
- Potency (viability, dose, stability, composition & biological activity)
- Effectiveness (reproducibility, consistency)

Complex nature of pancreatic islets (final product)

Limited ability to characterize final product prior to administration into a patient

Control of the Source Material

Control of the Manufacturing Process
Regulatory Requirements

1. Current Good Manufacturing Practices
   (cGMP’s, 21 CFR Part 210 & 211)
   - minimum requirements of any manufacturing process
   - Equipment (qualification, validation, & calibration)
   - Control of components (qualification)
     - Receipt and storage
     - Testing, approval and use
   - Process and product control (standardization, testing)

2. Current Good Tissue Practices
   (cGTPs, 21 CFR Part 1271)
   - Govern donor eligibility rules and other tissue practices
   - Prevention of introduction, transmission and spread of communicable diseases
     - HIV 1 & 2, HBV, HCV, TSE, Treponema pallidum, HTLV I/II
     - Donor qualification (social, medical history & physical examination)
3. Biologic Products
(21 CFR Part 600, 601 & 610)
- govern standards for biologics
- Product testing for
  - Sterility, identity, purity & potency
- Product licensure

4. Standards For Cellular Therapy Product Services, 2nd Ed.
- Quality Systems
- Safety of
  - Procurement
  - Processing
  - Storage
  - Administration

5. FACT-JACIE
   International Standards for Cellular Therapy Product Collection, Processing and Administration, 3rd Ed.
Evolution of islet isolation process…

… is a story of imagination & innovation
Initial attempts to isolate islet cells from a donor pancreas involved crude & disruptive mechanical methods.
Can Glucose Stimulate Insulin Release “In Vitro”?

Isolation and Culture of the Islets of Langerhans of the Guinea Pig

STANISLAW MOSKALEWSKI
Department of Histology and Embryology, School of Medicine, Warsaw, Poland

Received November 3, 1964

Islets of Langerhans of guinea pigs were isolated by means of collagenase and identified with a dissecting microscope. Organ culture of the islets was started in a natural medium with various glucose concentrations. The islets were examined histologically and their general morphology was noted, as well as the state of the specific granules in the alpha and beta cells. Both types of cells underwent partial degranulation. At high glucose concentration the beta cells were more degranulated and their nuclei were noticeably hypertrophied. No distinct differences in the morphology of alpha cells at low and high glucose concentrations could be detected. It is concluded that the influence of glucose upon the islands in vivo and in vitro is in principle similar.
Islet Isolation and Transplantation

1967  Lacy  →  islet isolation using intraductal distention

1969  Lindall  →  Islet purification by density gradients

1981  Horaguchi  →  collagenase via duct perfusion

1984  Gray  →  large scale isolation of human islets
Automated Method for Isolation of Human Pancreatic Islets

CAMILLO RICORDI, PAUL E. LACY, EDWARD H. FINKE, BARBARA J. OLACK, AND DAVID W. SCHARP
Scientific Concerns

**Concern:** control of source material
- Donor selection & testing
- Organ preservation

**Concern:** process control

**Concern:** control of final product
Required Equipment (general)

- BSC’s
- CO₂ incubators \((37^\circ C & 22^\circ C)\)
- Refrigerators
- Freezers
- Refrigerated centrifuges
- Inverted microscope (Light/fluorescent & camera)
- T° Monitoring System
- Peristaltic Pumps
Required Equipment (general)

- Densitometer
- Waterbaths (37° & 45°)
- Automatic Pipette Aids
- Balances
- pH meter
- Computer & printer
- Heat sealer
- Microplate reader (fluorescent & spectrophotometric)
- Manual cell counter
- Glass washer
- Autoclave
Islet Isolation – Specific Equipment

- Organ transport set (oxygenated PFC/UW)
- Continuous pancreas perfusion apparatus (cold/warm) & perfusion tray
- Chamber, screen & hollow stainless steel beads
- Ricordi isolator with heating, cooling, pressure control, and flow & pH monitoring capabilities
- COBE 2991 Cell Processor
- Islet shipping container
- Ricordi infusion bag

• Reduce variability
• Simplify process
Manufacturing Controls

Control of Source Material

- Source material – pancreata, from deceased heart-beating donors
- Can not be controlled in a traditional sense
- Establishment of acceptance criteria (both inclusion & exclusion)
  - Age: 15 – 65 years old
  - Acceptable cause of death
  - CIT: ≤ 12 hrs
  - Cold storage: UW, UW/PFC, HTK, HTK/PFC
  - Acceptable physical examination, social & medical history
  - Negative for HIV 1 & 2, HBV, HCV, TSE, *Treponema pallidum*, HTLV I/II, WNV
Islet Isolation – Specific Equipment

Oxygenated PFC/UW Transport

Outlet

Inlet: O₂ 95%
CO₂ 5%

EC or UW

PFC

Ice

Pancreas

Improved human islet isolation outcome from marginal donors following addition of oxygenated perfluorocarbon to the cold-storage solution.


<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>AGE</th>
<th>CIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFC+UW*</td>
<td>15</td>
<td>56.7±5.4</td>
<td>7.6±5.5</td>
</tr>
<tr>
<td>UW</td>
<td>18</td>
<td>55.9±4.2</td>
<td>8.4±3.2</td>
</tr>
</tbody>
</table>

* 8 preparations transplanted
1 patient off insulin after receiving a single islet preparation
Manufacturing Controls

**Process Controls**
*(goal: to optimize & standardize the manufacturing process)*

- Control of raw materials through reagent qualification
- Control of process, according to cGMP
  - Critical process controls
  - Standardization of standard operating procedures (SOP)
- Tracking of final product to donor organ / tissue
- Qualification and validation of equipment
- Lot-to-lot reproducibility
  - In-process specifications
  - Final product specifications (lot release criteria)
Islet Isolation – Specific Equipment

Continuous Pancreas Perfusion System

- Touch-screen controls
- Automatically pumps fluid at a constant pressure
- Automatic adjustment of the flow rate to maintain constant pressure
- Automatic $T^\circ$ control
- Capacity to download data to PC with Perfusion Data Acquisition software
Islet Isolation – Specific Equipment

The Ricordi Chamber
Reusable Ricordi Chamber

- Ultem Polyetherimide - tough, rigid biocompatible plastic of superior thermostatic strength
- Resistant to high $T^\circ$, can be repeatedly sterilized
- Comes with stainless steel screen & O-ring
Disposable Ricordi Chamber

• Durastar – clear, tough, chemically resistant plastic
• Clamp replaces stainless steel screws
• Sold individually packed & autoclaved
• Comes with stainless steel screen & O-ring
• New generation of shakers
• Touch-screen user interface
• Controlled heating & cooling
• Capacity to monitor $T^\circ$, flow rate, pH & shaking parameters (stroke length, frequency of vertical and rotational strokes)
• Data saved on a memory card
Multiparametric Monitoring of the Islet Isolation Procedure: A Potential Tool for In-process Corrections of Critical Physiological Factors.
Fraker C, Montelongo J, Szust J, Khan A and Ricordi C.
Improved Human Islet Isolation Using a New Enzyme Blend, Liberase™.


- Reduced Endotoxin
- Improved islet yields
Assessment of a Novel Two-Component Enzyme Preparation for Human Islet Isolation and Transplantation.


- Low Endotoxin
- Improved islet quality
- GMP grade

• Comparable islet yield
Profound Degradation of Serva’s Collagenase NB1 vs. Roche’s Liberase HI Revealed by Ion-Exchange HPLC on ProteinPak DEAE 5PW Column in Imidazole.HCl Gradient Buffer System at pH 6.3

**Liberase HI**

- Collagenase II
  - 38.5%
- Collagenase Ia
  - 37.1%
- Collagenase Ib
  - 8.8%

**Collagenase NB1**

- Collagenase IIb
  - 24.4%
- Collagenase IIa
  - 19.9%
- Collagenase Ia
  - 10.0%
- Collagenase Ia
  - 35.5%
Roche’s Thermolysin vs. Serva’s Neutral Protease: Homogeneity and Trio-teeth as a Result of Separation by Ion-Exchange HPLC on ProteinPak DEAE 5PW Column in L-Histidine.HCl Gradient Buffering System at pH 5.5

**Thermolysin**
- Single Peak
- 90.2%

**Neutral Protease NP**
- 1st Peak 22.2%
- 2nd Peak 29.3%
- 3rd Peak 20.2%

Sample: Thermolysin (Lot#93467920 ExMar08/75µg/250µl)
Eluant A: 5 mM L-Histidine.HCl pH 5.5, containing 1 mM CaCl
Eluant B: 0.2 M NaCl in Buffer A
Gradient Profile: 100% B→10% B in 15 min→10% B→10% B
Flow Rate: 1 ml/min
Column: WATERS PROTEIN PAK DEAE 5PW 7.5 mm x 7.5 cm
Detector A: Model 1716 var UV/VIS, 280 nm STD, 0.0025 AUFS

Sample: Neutral Protease NP, 125µg in 250µl
Eluant A: 5 mM L-Histidine.HCl pH 5.5, containing 1 mM CaCl
Eluant B: 0.2 M NaCl in Buffer A
Gradient Profile: 100% B→10% B in 15 min→10% B→10% B→10% B
Flow Rate: 1 ml/min
Column: WATERS PROTEIN PAK DEAE 5PW 7.5 mm x 7.5 cm
Detector A: Model 1716 var UV/VIS, 280 nm STD, 0.0025 AUFS
Islet Isolation – Specific Equipment

COBE 2991 Cell Processor

• FDA approved Class II device (# BK840009, cleared Date: 08-JUN-1984
• Used as a large capacity centrifuge, @ 4°C
• Operated in a semi-automated fashion with continuous Ficoll gradients
Rescue purification maximizes the use of human islet preparations for transplantation.
Manufacturing Controls

Specifications for In-Process Testing

- Identity (visual inspection using DTZ)
- Potency
  - Insulin release assay: ≥ 1
  - Islet quantity: ≥ 5,000 IEQ/kg,
  - Viability: ≥ 70%
    - Cellular composition and β-cell fractional viability
- Purity: ≥ 30%
- Safety (sterility testing for aerobic, anaerobic and fungal organisms: no growth)


Relationship between β-cell viability index and in vivo transplantation success rate (reversal rate).

<table>
<thead>
<tr>
<th>Index</th>
<th>x ≤ 0.2</th>
<th>0.2 &lt; x ≤ 0.3</th>
<th>0.3 &lt; x ≤ 0.4</th>
<th>0.4 &lt; x</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>13</td>
<td>30</td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td>Reversal rate</td>
<td>0%</td>
<td>30%</td>
<td>69%</td>
<td>100%</td>
</tr>
</tbody>
</table>

x = β-cell content in islet x β-cell viability

prospectively assess and correlate β-cell mass and fractional viability of an islet product to its functional performance
Islet Isolation – Specific Equipment

Islet Shipping Device

- Insulated plastic coolers
- Several room T° Sebra gel packs on the bottom / sides
- One bag / metal shelf (x3) in the device
- Cells are in gas permeable bags
- T° monitoring devices enclosed
- Shipping T° is room T°
- Cooler sealed with tape & transported upright
- Containers validated
Shipment of human islets for transplantation.
Manufacturing Controls

Control of the Final Product

- Each preparation is considered to be a product lot
- Each product lot must be tested before release for transplant
- Testing determined by
  - Regulatory requirements
  - Manufacturers
- Each test must contribute meaningful scientific information about the final product
Manufacturing Controls

Specifications for Lot Release Testing

- Identity (visual inspection of product by DTZ & in its final labeled container)
- Product Volume (< 200 ml/bag)
- Potency (insulin release assay: ≥ 1; islet quantity: ≥ 5,000 IEQ/kg; viability: ≥ 70%)
- Purity (≥ 30%)
- Safety (Gram Stain: negative; Endotoxin: < 5 EU/kg)
The Bag Method for Islet Cell Infusion.
Baidal D. A., Tatiana Froud T, Ferreira J. V., Khan A., Alejandro R and Ricordi C.
Camillo Ricordi, Chairperson  
James Shapiro and Bernhard Hering, Co-Chairpersons  

Phase II Pilot Clinical Trials & Phase III Licensure

|------------|----------|--------------|-----------------|

• Subjects are screened for inclusion/exclusion criteria common to all trials  
• All sites have identical manufacturing procedures except for modifications specific to phase 2 trials

<table>
<thead>
<tr>
<th>Randomization</th>
<th>Randomization</th>
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<th>Randomization</th>
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<tbody>
<tr>
<td>LEA29Y Phase 2 Study (N=12)</td>
<td>Exenatide and Lysophylline Phase 2 Studies (N=12)</td>
<td>DSG Phase 2 Study (N=20)</td>
<td>Rituximab Phase 2 Study (N=12)</td>
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</table>

Multi-center Single Arm Phase 3 Trial of Islet Transplantation for T1D
Islet Isolation: The Future

Oxygenated PFC/UW Transport

Oxygenated Tissue Culture

Transplant Devices
Enhanced oxygenation promotes beta-cell differentiation in vitro.

Fraker C.A., Alvarez S., Papadopoulos P., Giraldo J., Gu W., Ricordi C., Inverardi L., Domínguez-Bendala J.
Reversal of diabetes by pancreatic islet transplantation into a subcutaneous, neovascularized device.

Pileggi A., Molano R. D., Ricordi C., Zahr E., Collins J., Valdes R. and Inverardi L.