Pig Islets as a Source of Islet Transplantation

Boris Draznin, MD, PhD
Professor of Medicine
Director, Adult Diabetes Program
Department of Medicine
University of Colorado Denver
• Destruction or severe malfunction of pancreatic islets is the core pathogenetic feature of diabetes.
• Cure of diabetes is impossible without restoration of pancreatic islet – β-cell – function.
• Pancreatic β – cells are the only cells capable of sensing ambient blood glucose and converting this information into regulated insulin secretion.
Strategies to restore β-cell function.

- Islet transplantation
- Stem cell derived insulin producing cells
- Non-islet cells genetically modified
- Islet cell regeneration
• T1DM - > 2,000,000 patients
• T2DM - > 20,000,000 patients in the US alone.
• Only around 1,000 cases of allogenic islet transplantation.
Islet Transplantation

• Intrahepatic allotransplantation with immune suppression is not the answer to treatment of Type 1 diabetes.
Xenotransplantation

- Porcine islet cells provide an essentially unlimited supply of cells for transplantation.
- Potential to do implants without immunosuppression.
Porcine Islets

• **Pro:** Porcine insulin
  • Large litters with robust islet number
  • Islets respond to the same physiological range of glucose as human islets
  • Ethical considerations

• **Con:** Humans express high titers of antibodies against Galactose (1,3) α-galactose residue present in most pig cells
  • Retroviruses
  • Immunosuppression.
Instant Blood-mediated Inflammatory Reaction (IBMIR)

- Direct exposure of pig islets to blood can result in their destruction, a phenomenon termed IBMIR.
- Reaction involves consumption of platelets, activation of neutrophils and monocytes, coagulation and complement systems.
Pig Viruses

- Pig Hep E
- Pig circovirus type 2
- Cytomegalovirus
- Pig lymphotrophic herpesvirus
- Others
Pig Endogenous Retrovirus (PERV)

- Part of the pig genome (with more than 100 of pre-viruses)
- PERV-A and PERV-B are infections to human cells in vitro
## Presence of infectious PERV in different pig breeds

<table>
<thead>
<tr>
<th>Pig breed</th>
<th>Cells</th>
<th>Infectious PERV</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Landrace</td>
<td>Endothelial cells</td>
<td>+</td>
<td>Martin, 2000</td>
</tr>
<tr>
<td>Landrace</td>
<td>Islets</td>
<td>+</td>
<td>Van der Laan, 2001</td>
</tr>
<tr>
<td>Yucatan micropig</td>
<td>PBMC</td>
<td>+</td>
<td>Martin, 1998</td>
</tr>
<tr>
<td>Gottinger minipig</td>
<td>Endothelial cells</td>
<td>+</td>
<td>Martin, 1998</td>
</tr>
<tr>
<td>Large White/Landrace</td>
<td>Islets</td>
<td>+</td>
<td>Deng, 2000</td>
</tr>
<tr>
<td>Miniature Swine Yorkshire</td>
<td>PBMC</td>
<td>+</td>
<td>Wilson, 1999</td>
</tr>
<tr>
<td>Miniature Swine D/D</td>
<td>PBMC, fetal neuronal cells</td>
<td>-</td>
<td>Dinsmore, 2000</td>
</tr>
<tr>
<td>Cambrough</td>
<td>PBMC, Sertoli, islets</td>
<td>-</td>
<td>Patience, 2002</td>
</tr>
<tr>
<td>Westran pig</td>
<td>PBMC, islets</td>
<td>-</td>
<td>Garkavenko, in prep.</td>
</tr>
<tr>
<td>BioCert pig</td>
<td>PBMC, islets, choroid plexus, liver cells</td>
<td>-</td>
<td>Simond, in prep.</td>
</tr>
</tbody>
</table>
• Specific Pathogen Free animals (SPF)
• Designated Pathogen Free (DPF) herds of pigs
Milestone experiments with Islet Xenotransplantation.
Cynomolgus macaques

Reversal of DM for more than 100 days with intraportal transplantation of cultured islets from the wild-type pigs with immunosuppression.

Rhesus macaques

- Total pancreatectomy
- 50,000 IEQ/kg of neonatal porcine islets
- 2 animals – no immunosuppression: rejection in 4-5 days; no insulin independence; no porcine C-peptide; post-mortem – T-cells, macrophages – cell-mediated rejection
- Immunosuppressed animals – sustained insulin independence, graft survival > 140 days, no PERV transmission.

Cardona K et al: Nature Medicine 12:3-4-306, 2006
Prerequisites for 
Xenotransplantation

• Cells must be free from any xenotic agent
• Islets must be uncontaminated, undamaged, free of exocrine tissue
• Anti-rejection strategy should not be based on immunosuppression.
Cells free from any xenotic agent

- Source herd must be free of infections capable of being transmitted to man,
- Must be housed in the DPF facilities,
- Must be checked for infection status frequently.
Discovered in 1806; Area 220 sq miles; Temp 35-65º F; humid, cloudy and very windy.
## Viruses in New Zealand and Auckland Island herd

<table>
<thead>
<tr>
<th>Virus</th>
<th>Prevalence in general pig Population (PCR)</th>
<th>Prevalence in BioCert herd (PCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt; 20 weeks</td>
<td>7 days</td>
</tr>
<tr>
<td>PCMV</td>
<td>70%</td>
<td>Not detected</td>
</tr>
<tr>
<td>PLHV</td>
<td>95%</td>
<td>Not detected</td>
</tr>
<tr>
<td>PCV1</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>PCV2</td>
<td>96%</td>
<td>10%</td>
</tr>
<tr>
<td>HepEV</td>
<td>90% (14 weeks)</td>
<td>Not detected</td>
</tr>
<tr>
<td>EMCV</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td><strong>Conventional pathogens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AuJD</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>BVD</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>PPV</td>
<td>Present</td>
<td>Not detected</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>Present</td>
<td>Not detected</td>
</tr>
<tr>
<td>Leptospiroses</td>
<td>Present</td>
<td>Not detected</td>
</tr>
<tr>
<td><em>Mycoplasma hyopneumoniae</em></td>
<td>Present</td>
<td>Not detected</td>
</tr>
</tbody>
</table>
Uncontaminated
Islet Isolation

- Stress to the tissue
- Harsh enzymatic digestion
- Encapsulation
- Storage
- Transport
- Viability at the time of transplantation
Undamaged

Free floating islet day 15 culture. Insulin producing cells red Zinc staining DTZ.
Viable

Porcine Free Islets AOPI staining

Viability > 95%
Exocrine free (amylase)
Microencapsulatation

- Surrounding of islet cells with a highly biocompatible biopolymer called alginate which reduces the host’s immune response to the implanted islets.
- Alginate coat allows insulin, glucose, oxygen and other nutrients to diffuse freely, while blocking antibodies and T-cells.
Alginate

• Polyanionic carbohydrate that gels in the presence of cations, such as calcium chloride.
Capsules

- No defects
- Exclude immunoglobulins, T-cells, macrophages and complement
- Allow free passage of insulin, nutrients and oxygen
- Biocompatible
- Rugged and long lasting
Capsules

- Manufactured alginate must have requisite guluronic/mannuronic acid ratio, be free of endotoxine, protein and polyphenols.
- Optimal concentration, viscosity, molecular size, and range.
DiaBecell-e Encapsulated Neonatal Porcine Islets
Islet Maximum Insulin Release

uU/100 IEQ/h

Day

DB 4 5-7 12 15 16-7 18 21-2 25 26 29 32 40

** ** **
STZ Diabetic Primate Study

Time Course Monkey Achieving Insulin Independence
Blood Glucose and Insulin Dose

-2 -1 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37

Weeks Post Transplant

BG (mmol/L) Insulin dose (unit/day)

- Glucose
- Insulin

Tx 1
Tx 2
IVGTT
STZ Diabetic Primate Study

Insulin in post HPLC Eluates IVGTT 5 months post 2nd TX

- Human
- Porcine Insulin

Elution Time (min) vs. Insulin (relative)

- Standards
- IVGTT 30 min
- IVGTT 60 min
- IVGTT 120 min
Clean encapsulated islets released from small clusters retrieved 12 weeks after transplantation. 100% viable islets (AO/PI). No signs of cellular debris attached to the surface of the capsules.
Clinical data
## Results of Human Trial

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>No. of Implants</th>
<th>Follow-up (Weeks)</th>
<th>Insulin Dose (Units/day)</th>
<th>% Dose Reduction</th>
<th>HbA1c (%)</th>
<th>Current Mean Glucose (mMol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre-Implant</td>
<td>Current</td>
<td>Pre-Implant</td>
<td>Current</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>96</td>
<td>113</td>
<td>76</td>
<td>33</td>
<td>7.1</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>84</td>
<td>22</td>
<td>0</td>
<td>100</td>
<td>8.2</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>72</td>
<td>60</td>
<td>53</td>
<td>12</td>
<td>10.0</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>60</td>
<td>30</td>
<td>27</td>
<td>10</td>
<td>7.6</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>30</td>
<td>68</td>
<td>48</td>
<td>29</td>
<td>9.8</td>
</tr>
<tr>
<td>6*</td>
<td>1</td>
<td>20*</td>
<td>41</td>
<td>57*</td>
<td>-</td>
<td>8.5</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>18</td>
<td>37</td>
<td>0</td>
<td>100</td>
<td>8.3</td>
</tr>
</tbody>
</table>
Insulin Detection in Post HPLC Eluates
Patient#1 before and after glucagon stimulation

Elution Time

Insulin (Relative)

Porcine Insulin

Human Insulin
Showing Reduction in Mean Blood Glucose and Range of Excursions Despite Minimal Post Implant Insulin Dose Reduction
Patient Implant – Recovered Cells
Future of Islet Xenotransplantation

- Supply and demand issues
- Safety and efficacy
- Length of benefits
- Long term safety
Candidates for Xinotransplantation

- Brittle diabetes with end stage kidney disease
- Severe hypoglycemia with attempts to optimize care
Summary

- Careful manufacture of encapsulated islets from neonatal pigs yields a product that shows promise as a treatment of T1DM;
- Regulatory concerns are around PERV – which increasingly appears to be a non-issue. Other xenoses can be avoided.
Thanks for your kind attention...
## Provisional results

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Number of Implants</th>
<th>Follow Up (wks)</th>
<th>Insulin Pre-Tx</th>
<th>% of pre-Tx</th>
<th>HbA1c Pre-Tx</th>
<th>HbA1c Current</th>
<th>Glucose (mean last 3 months follow up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>68</td>
<td>113</td>
<td>76</td>
<td>33</td>
<td>7.1</td>
<td>6.1</td>
</tr>
<tr>
<td>2</td>
<td>2*</td>
<td>60</td>
<td>22</td>
<td>13</td>
<td>43</td>
<td>8.2</td>
<td>7.1</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>48</td>
<td>60</td>
<td>55</td>
<td>8</td>
<td>10</td>
<td>7.4</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>36</td>
<td>30</td>
<td>27</td>
<td>10</td>
<td>7.6</td>
<td>6.5</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>30</td>
<td>68</td>
<td>48</td>
<td>29</td>
<td>9.8</td>
<td>7.2</td>
</tr>
<tr>
<td>6**</td>
<td>1</td>
<td>20</td>
<td>41</td>
<td>57</td>
<td>0</td>
<td>8.5</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>4</td>
<td>37</td>
<td>20</td>
<td>46</td>
<td>8.3</td>
<td>4.8</td>
</tr>
</tbody>
</table>

*3rd transplant has just occurred

**Lost to follow up so far

**Mean = 24.1%**

8.5 6.5