Impact of Islet Size on Graft Function

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Core Cell Death in Low-Insulin Secreting Islets

MacGregor et al, 2006
Human Islet Survival

Day 1

Day 12
Are Decreased Functional Outcomes for Large LIS Islets Due to Diffusion Barrier?
Dynamic partial differential equations for a spherical islet were solved numerically using finite-difference method in spherical coordinates.

Confirmed with 2 NBDG diffusion experiments.
Will reducing the diffusion barrier in large islets improve their function?

1) Cut islet into segments
2) Engineer islets
3) Make islets porous
• IMRA FCPA μJ laser
• Yb-fiber oscillator / amplifier system 1 μm wavelength output.
• Pulse widths to < 500 fs
Islets isolated from donor pancreas

- Disperse - maintain high cell viability
- Centrifuge onto biomaterial patch
How can we prove that islets are porous?
Optical Density (arbitrary units)

Intact Islets Porous Islets

*
Increasing Surface Area Increased Viability
A bar graph shows the comparison of Insulin Content (ng/IE) between low glucose and high glucose conditions. The graph includes two categories: Large and Small. The y-axis represents the Insulin Content (ng/IE) ranging from 0 to 90. The x-axis represents the glucose conditions: low glucose and high glucose.
Is there something inherently different between small and large islets?

Are these different sub-populations of islets?
Morphological analysis, first reported differences in size distribution, number and volume in several species (Haist & Pugh, *Am. J. Physiol.* 1947)

Morphological differences in human islets (Saito et al., *J. Exp. Med.* 1978)

3-D reconstruction demonstrates 2 types of islets (Baetens et al, *Science*, 1979)


Type of cell | Large Islets | Small Islets
--- | --- | ---
Beta (insulin) | 52% | 57%
Alpha (glucagon) | 38% | 30%
Delta (somatostatin) | 11% | 6%
Total DNA content was 3 times higher in small islets than large when normalized for EI (rats).

Is there a difference in genetic regulation in large and small islets?
<table>
<thead>
<tr>
<th>Gene (protein product)</th>
<th>Fold difference in expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reg 1 Regenerating Gene</td>
<td>7.11 greater in small islets</td>
</tr>
<tr>
<td>Atk1 Protein kinase B</td>
<td>6.10 greater in small islets</td>
</tr>
<tr>
<td>Pdx-1 Pancreatic Duodenal Homeobox-1</td>
<td>2.41 greater in large islets</td>
</tr>
<tr>
<td>VEGF Vascular endothelial growth factor</td>
<td>12.04 greater in large islets</td>
</tr>
<tr>
<td>IAPP Islet Amyloid Polypeptide</td>
<td>2.55 greater in small islets</td>
</tr>
<tr>
<td>LDLR Low Density Lipoprotein Receptor</td>
<td>4.26 greater in large islets</td>
</tr>
<tr>
<td>Ins2 Pre-insulin</td>
<td>2.97 greater in large islets</td>
</tr>
<tr>
<td>Irs2 Insulin receptor substrate</td>
<td>15.27 greater in large islets</td>
</tr>
<tr>
<td>Ddit3 (part of CHOP 10) (differentiation and apoptosis)</td>
<td>4.56 greater in large islets</td>
</tr>
<tr>
<td>HNF4a Hepatocyte Nuclear Factor (beta cell development)</td>
<td>2.38 greater in small islets</td>
</tr>
<tr>
<td>GLUT 2/Slc2a2 Glucose transporter</td>
<td>8.88 greater in large islets</td>
</tr>
<tr>
<td>NOS2 Nitric Oxide Synthase</td>
<td>3.71 greater in small islets</td>
</tr>
<tr>
<td>Total Protein Content in an islet</td>
<td>µg/islet</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Small</td>
<td>0.04</td>
</tr>
<tr>
<td>Large</td>
<td>0.35</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Protein Content per volume</th>
<th>µg/IE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>0.58</td>
</tr>
<tr>
<td>Large</td>
<td>0.10</td>
</tr>
</tbody>
</table>
2D PAGE

Small > Large  Red
Large > Small  Blue
Islet Subpopulations

- **Small**: High insulin secreting islets
  - Under 125 μm diameter

- **Large**: Low insulin secreting islets
  - Over 150 μm diameter

- **CL**: Clear islets
  - Range from 5 – 200 μm
Summary

• Islet sub-populations are associated with different functions.
• Small islets are more viable in vitro
• Small islets secrete more insulin in vitro
• Transplantation with small islets reverse diabetes
• Reducing the diffusion barrier in large islets increases viability, but does not improve in vitro insulin secretion or islet transplantation outcome
• Small islets have greater insulin content
• Ultrastructural differences exist between large and small islets
• Small islets have a higher % of beta cells
• Small islets have more total protein content
• Selective genes are expressed in detectible levels in large islets that are not detected in small islets
• Selective proteins are found in either small or large islets
Summary

Why do we care?
Acknowledgements

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Sympathetic nerve fibers

Large diameter, myelinated

Small diameter thinly myelinated and medium diameter unmyelinated peptidergic

Sympathetic nerve fibers

Lindsay et al., 2006
Takahashi et al., Science 2002
Islet Diameter (μm)

<table>
<thead>
<tr>
<th>Islet Diameter (μm)</th>
<th>Number of Islets</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
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<tr>
<td>200</td>
<td>20</td>
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<td>300</td>
<td>30</td>
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<td>400</td>
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<tr>
<td>500</td>
<td>50</td>
</tr>
<tr>
<td>600</td>
<td>60</td>
</tr>
</tbody>
</table>

- **Small Islets**
- **Large Islets**
Distribution of Cells the Same in Both

Figure 7

A.

B.

MacGregor et al, 2006