Non-invasive Imaging to Assess Transplanted Islets

Alvin C. Powers
Vanderbilt University
Today

- Rationale and challenges for imaging pancreatic islets
- Overview of imaging modalities being used
- Bioluminescence to assess transplanted pancreatic islets
Pancreatic Islets Are an Imaging Challenge

Islets size ($\leq 250 \ \mu m$) is less than resolution of imaging modalities (CT, MRI)
Non-Immune Barriers to Improving Islet Transplantation

- Transplanted, intra-hepatic islets are relatively inaccessible
- Techniques to non-invasively assess or image islet mass are not available.
- Difficult to study islet survival following transplantation (must rely on islet function).
- Cannot assess interventions to sustain or improve islet survival after transplantation.
Features of Ideal Islet Imaging Modality

- Non-invasive and allow for serial, in vivo measurements in the same animal or person
- Non-toxic to islet cells
- Useful for study of islets in native pancreas and after transplantation
- Applicable to murine models
- Adaptable for human imaging
So what do you want to know from islet imaging?

- Should it measure islet cell mass or number? Should it reflect beta cell number of islet cell number?
- Should it reflect function or health of beta cells? The metabolic state and insulin secretory capacity of the beta cell will fluctuate in different physiologic and pathophysiologic conditions.
- Is spatial resolution of islets important?

Islet mass ≠ Islet function
Approaches to Image Pancreatic Islets

- Utilize an islet-specific protein or process
- Introduce a reporter into islet cells
Features/Approaches That Could be Useful for Imaging

- Unique glucose metabolism (GLUT2, glucokinase)
- Islet or beta cell-specific (or enriched) cell surface markers
- High concentration of zinc
Features/Approaches That Could be Useful for Imaging

- Unique glucose metabolism (GLUT2, glucokinase)
- Islet or beta cell-specific (or enriched) cell surface markers
- High concentration of zinc
- In pancreas, islets are highly vascularized with a fenestrated endothelium
- MRI
- PET
- Bioluminescence Imaging
Non-invasive Assessment of Islets Using Bioluminescence
Bioluminescence Reaction

Luciferase + Luciferin + ATP + O₂ → Luciferase-Luciferin + AMP + PP_i

Luciferase-Luciferin + AMP + O₂ → Oxyluciferin* + CO₂ + AMP

Oxyluciferin* → Oxyluciferin + hν

Nucleus

Luciferase

Oxygen

ATP

D-Luciferin

Photons
Islet Transplantation Model

Adapted from JDRF figure
NOD-SCID Mouse Model

- Lack B- and T-Lymphocytes
- NOD background further reduces immunity because of NK cell deficiency
- Accept xenografts
- Do not develop insulitis or diabetes
- Allow long-term expression of adenoviral DNA
- Species-specific insulin assay to distinguish human insulin from endogenous mouse insulin
Bioluminescence of Transplanted Islets

Adenovirus encoding luciferase → Culture → Transplant

Image with CCD Camera
Quantify Photon Emission
In vitro Bioluminescence in Human islets

A. Islets (IEQ)/well

B. 

$\text{Luminescence } \times 10^{6}$

(Integrated Gray Units)

$r^2 = 0.9808$

$n = 3$ wells

# of Islets/well

$r^2 = 0.9808$

$n = 3$ wells

<table>
<thead>
<tr>
<th># of Islets/well</th>
<th>Luminescence $\times 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>500</td>
<td>4</td>
</tr>
<tr>
<td>1000</td>
<td>16</td>
</tr>
</tbody>
</table>
Imaging Transplanted Islets

Kidney Capsule (human islets)

Liver (mouse islets)

Luminescent Rod

Photon counts

500 IEQ  1000 IEQ  2000 IEQ
Imaging Transplanted Islets

Kidney Capsule (human islets)

Liver (mouse islets)

Kidney Capsule (luminescent rod)
Standardization of Imaging Using Luminescent Bead Camera

A

B

C

D

Intensity Normalized to 0 Degrees

Angle of Rotation [Degrees]

Intensity Normalized to 0 Degrees

Angle of Rotation [Degrees]
Absorption of Photons by Surrounding Tissues

**Diagram:**
- Camera Aperture
- Air: 50 cm
- Skin: 0.025 cm
- Renal Bead
- Liver: 0.1 cm
- Hepatic Bead

**Graphs:**
- **D:** Photon Counts: 2000
- **E:** Photon Counts: 500
- **F:** Ratio of In Vitro Intensity to In Vivo Intensity vs. Weeks Post Implant
  - Renal
  - Hepatic

**Text:**
- Absorption of Photons by Surrounding Tissues
- Camera Aperture
- Air: 50 cm
- Skin: 0.025 cm
- Renal Bead
- Liver: 0.1 cm
- Hepatic Bead

**Graph F:**
- X-axis: Weeks Post Implant
- Y-axis: Ratio of In Vitro Intensity to In Vivo Intensity
Bioluminescence of Islets in Kidney or Liver

- 200 mouse islets
- Imaged two weeks post-transplant
Bioluminescence is Influenced by Site of Transplantation

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bead</strong></td>
<td>0.2384 ± 0.0261</td>
<td>0.0645 ± 0.0140</td>
</tr>
<tr>
<td><strong>100 islets</strong></td>
<td>0.0476</td>
<td>0.0116</td>
</tr>
<tr>
<td><strong>200 islets</strong></td>
<td>0.0284</td>
<td>0.0112</td>
</tr>
</tbody>
</table>
Bioluminescence of Transplanted Murine Islets

In vivo bioluminescence (photon counts)

- Liver
- Kidney

# of Murine Islets Transplanted

- 50
- 100
- 200
Transplanted Human Islet Number and Luminescence

$r^2 = 0.9946$
$n = 3 - 4$ mice

$r^2 = 0.9755$
$n = 3 - 4$ mice
Bioluminescence of Transplanted Islets

- Dependent of level of luciferase expression within mouse or human islets; (requires ATP and oxygen and viable islets)
- Optical scattering properties of tissue in which luciferase-expressing islet reside and tissues through which emitted light must exit the animal influence bioluminescence.
- If these are considered in calculations, bioluminescence reflects transplanted islet # and function.
Bioluminescence of Transplanted Murine Islets Beneath Renal Capsule

In vivo bioluminescence (x10^6) (photon counts)

Weeks post-transplantation

Tx (50 islets)
Bioluminescence of Transplanted Murine Islets

Intrahepatic

In vivo bioluminescence (x10^6) (photon counts)

Weeks post-transplantation

Tx (125 islets)
Decline in Bioluminescence of Transplanted Islets

- Large decline (> 60%) suggesting islet loss in first week after transplantation
- Beginning 2 weeks post-transplant, greater loss from intra-hepatic islets of all types
  - Liver is unfriendly site?
  - Destroyed by immune attack against luciferase or adenoviral/primate proteins?
- Islets no longer express luciferase
  - Cell division and progeny cells no longer express luciferase
Alternative Approaches

- Lentivirus (D. Kaufman, UCLA)
- AAV virus
- Transgenic expression of luciferase
Bioluminescence to Assess Transplanted Islets

- Non-invasive
- Sensitive (detect 25-50 transplanted mouse islets)
- Photon generation likely reflects islet cell number (and maybe islet function)
- Poor spatial resolution
- Allows for serial measurements of intra-hepatic islets
- Allows testing of interventions to increase or sustain transplanted islet mass
## Bioluminescence as Islet Imaging Modality

<table>
<thead>
<tr>
<th>Feature</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-invasive and allow for serial, in vivo measurements in the same animal</td>
<td>Yes</td>
</tr>
<tr>
<td>Non-toxic to islet cells</td>
<td>Yes</td>
</tr>
<tr>
<td>Useful for study of islets in native pancreas and after transplantation (in kidney and liver)</td>
<td>Probably</td>
</tr>
<tr>
<td>Applicable to murine models</td>
<td>Yes</td>
</tr>
<tr>
<td>Adaptable for human imaging</td>
<td>No</td>
</tr>
</tbody>
</table>
Co-Registration of Multiple Imaging Modalities and Biologic Information

- All imaging modalities will have limitations.
- No single modality will answer all questions about islets in pancreas or transplanted islets.
- Understanding islet survival and function will require integration of complementary imaging modalities and physiology in animal models and in humans.
Acknowledgements

- Marcela Brissova
- Michael Fowler
- Wendell Nicholson
- A. Radhika
- Alena Shostak
- Greg Poffenberger
- Zhongyi Chen
- Craig Hauck
- Jeanelle Kantz
- Chunhua Dai
- P. Brahmachary
- Qing Cai
- E. Duco Jansen
- John Virostko
- Masa Shiota
- Mark Magnuson
- Maureen Gannon
- David Piston
- John Gore
- David Harlan
- Boaz Hirschberg
- Graeme Bell
- Soo Young Park
- Daniel Kaufman
- Mark Atkinson

JDRF
NIH
VA