

Islet Cell Resource Center (ICR) Consortium 4th Annual Islet Workshop
Hyatt Regency Hotel, Newport Beach, CA Friday, October 3rd, 2008



University Hospital
Zurich

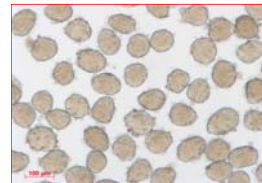
Pseudoislet biology and possible approach for transplantation

Roger Lehmann
Islet Transplantation Program
University Hospital Zürich

Overview

Introduction
Revascularisation, Hypoxia

Future
Goals
Take home
Message



Small
vs. Large
Islets

Pseudoislets
Advantages and Possible Problems

First contact with hypoxic islet cores

Hypoxia, 48h



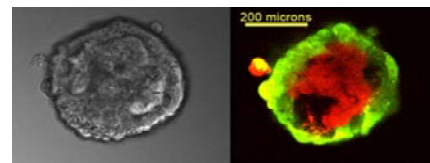
The FASEB Journal express article 10.1096/j.01-0403ge. Published online March 26, 2002.

Apoptosis in hypoxic human pancreatic islets correlates
with HIF-1 α expression

W. Moritz,* F. Meier,* D. Stroka,^{1,2} M. Giuliani,* P. Kugelmeier,* Ph. C. Nett,*
R. Lehmann,¹ D. Candinas,¹ M. Gassmann,^{1,2} and M. Weber*



Confocal Microscopy of large rat islet after 24 hours of culture



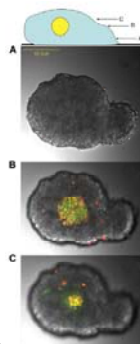
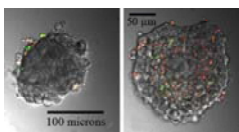
Fluorophors Sitox and Calcein: green = live, red = dead

Mac Gregor RR et al. AM J. Physiol Endocrinol Metab 2006; 290: E771-9



Apoptosis/Necrosis after 24 hrs of culture in large rat islet

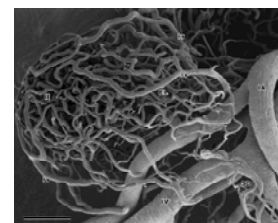
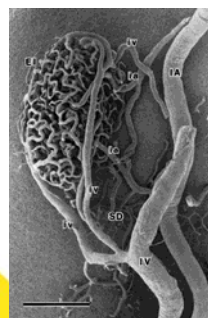
Cells lacking fluorescence = live
Green stain = apoptosis
Yellow/Red Stain = necrosis



Mac Gregor RR et al.
Am J. Physiol Endocrinol Metab 2006; 290: E771-9



Islet vascularization

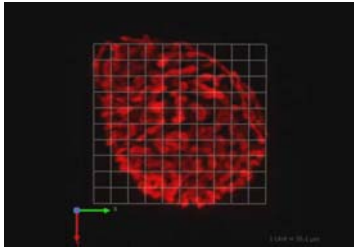


Islets of Langerhans: extremely well
perfused human tissue

Pictures: Murakami et al. Microsc. Res. and Technique 1997



Vascularization of Islets

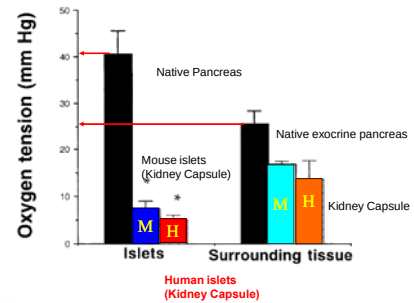


Islets of Langerhans = best vascularized tissue in human body
1% of pancreas mass, 20% of whole blood flow of pancreas



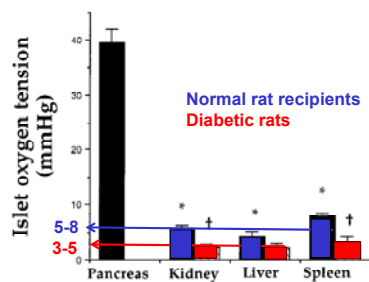
Speyer St et al. Nature Med 2008; 14: 574-8

Oxygen tension in islets



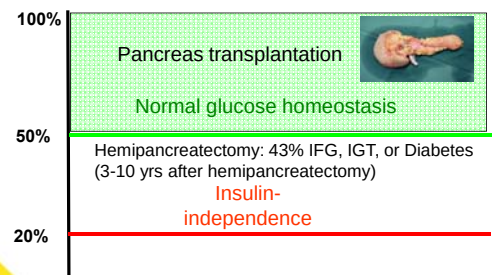
P.O. Carlsson et al. JCEM 2002; 87: 5418-23

Rat islet allotransplantation to different sites



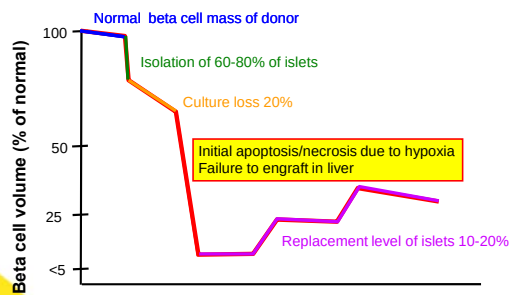
P.O. Carlsson et al. Diabetes 2001; 50: 489-95

Beta cell mass and glucose



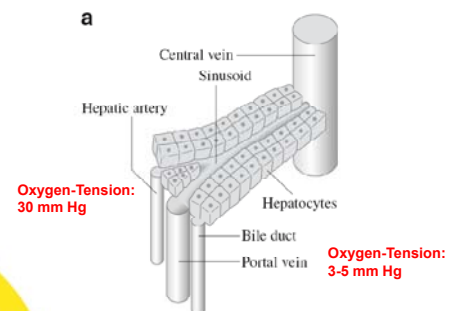
Korsgren O et al. Diabetologia 2000; 51: 227-32
Kumar AF et al. Diabetes Care 2008; 31: 1639-43

Beta-cell mass after islet transplantation



Korsgren O et al. Diabetologia 2000; 51: 227-32

Normal blood perfusion of the liver



Korsgren O et al. Diabetologia 2000; 51: 227-32

Large size of rodent islets as compared to portal veins ⇒ large necrotic areas

- Necrotic areas induce cascade of growth factors with hepatocyte proliferation ⇒ proliferation of transplanted islets and improved engraftment
- Islets are incorporated in newly formed liver parenchyma and induce revascularization by hepatic artery within the first week after transplantation

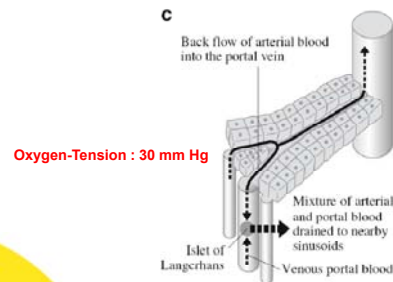
Normal Oxygen-Tension
in Islets: 40 mm Hg



Oxygen-Tension:
3-5 mm Hg

Korsgren O et al. Diabetologia 2008; 51: 227-32

Islet entrapped in portal vein (size restriction)

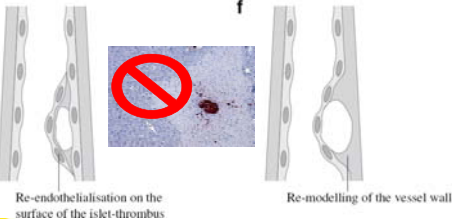


Low Pressure and low Oxygen-Tension:
< 3-5 mm Hg

Korsgren O et al. Diabetologia 2008; 51: 227-32

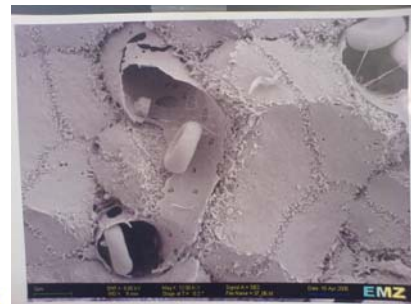
Revascularization of intraportally transplanted islets

- No vessel growth into lumen of another vessel (Barrier=basement membrane of endothelial cell)
- No significant gradients of growth factors (VEGF A, FGF)

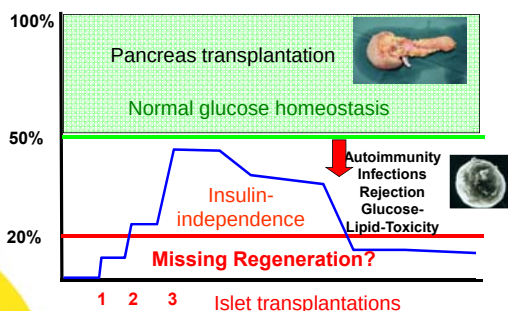


Korsgren O et al. Diabetologia 2008; 51: 227-32

Liver sinusoid with fenestration Vessel wall thickness at embolization site?



Beta cell mass and glucose



Korsgren O et al. Diabetologia 2008; 51: 227-32

Critical Obstacles for Long-Lasting Insulin Independence after Clinical Islet Transplantation

Limited mass of permanently engrafted islets

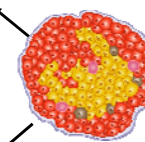
- Limited beta-cell reserve
- Cell loss by apoptosis induced by hypoxia and by other non-immunological, inflammatory mechanisms (IBMIR)

60-70% loss within 2 wks after Tx

No reliable marker for rejection

High metabolic demand

- Insulin resistance
- Diabetogenic
- Immunosuppression



Immunological Graft loss

- Autoimmunity
- Alloimmunity

Small vs. Large



Original Article

Superiority of Small Islets in Human Islet Transplantation

Roger Lehmann,¹ Richard A. Zuellig,¹ Patrick Kugelmeier,² Aurel Perren,³ Philipp B. Baenninger,¹ Wolfgang Moritz,⁴ Pierre-Alain Clavien,⁴ Markus Weber,⁵ Giangen A. Spinas¹



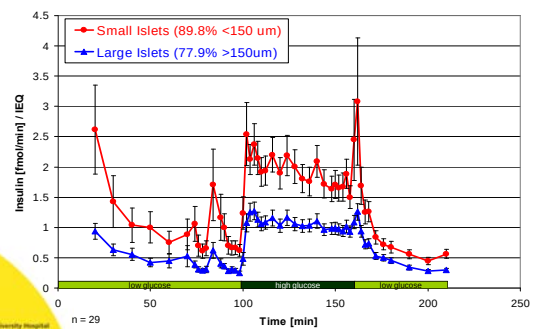
Lehmann R, Züllig R. et al., Diabetes 2007; 56: 594-603

Islet size distribution in human pancreas

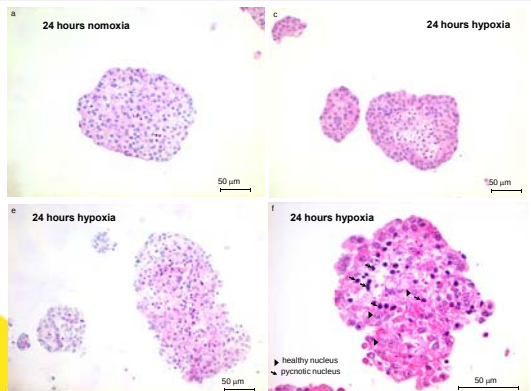
The human endocrine pancreas			
Islet diameter (µm)	Number of islets	% of total number of islets	% of total islet volume
<50	782,000	52%	5.4%
50-100	416,000	28%	17%
100-150	203,000	14%	30%
150-200	54,000	3.6%	26%
200-250	20,000	1.3%	15%
>250	3,000	0.2%	6.3%

Korsgren et al., Transplantation 79: 1289-1293, 2005

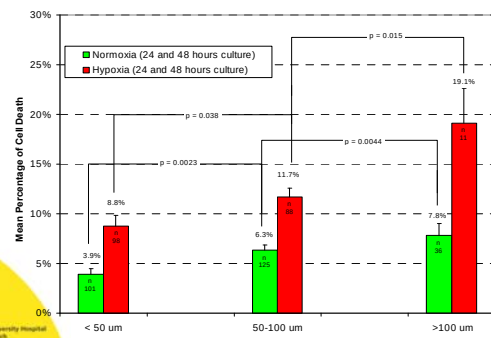
Insulin secretion during perfusion

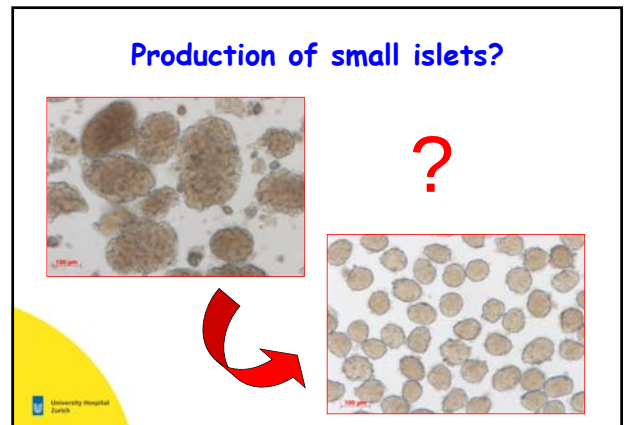
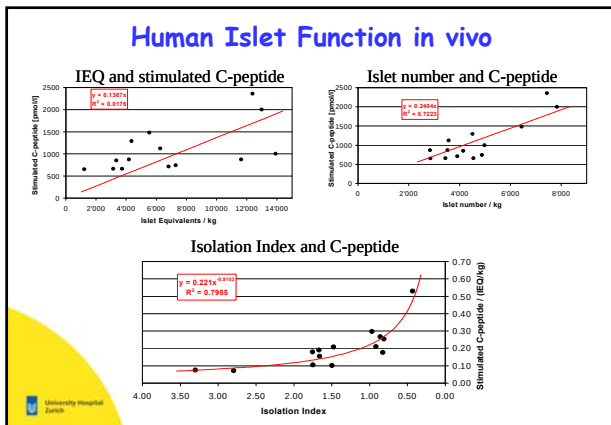
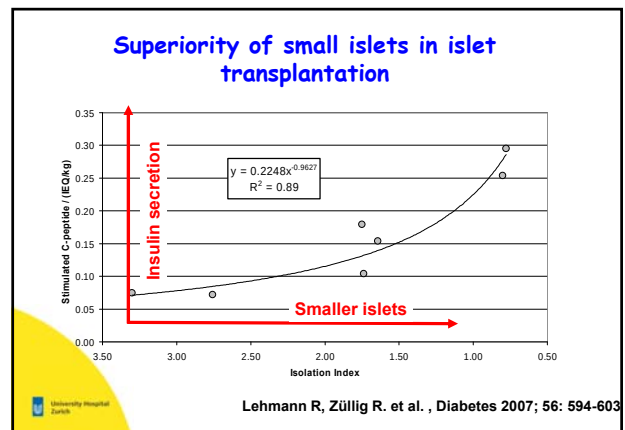
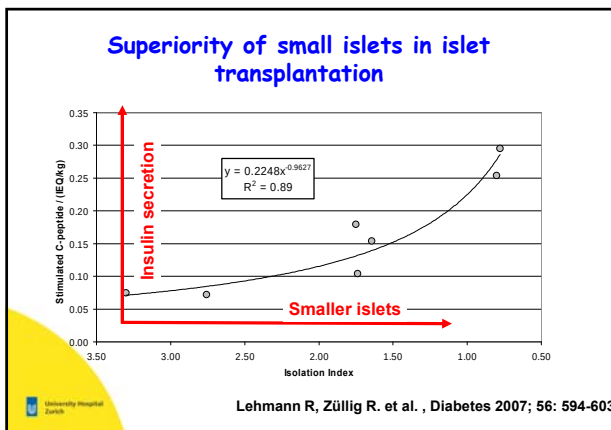
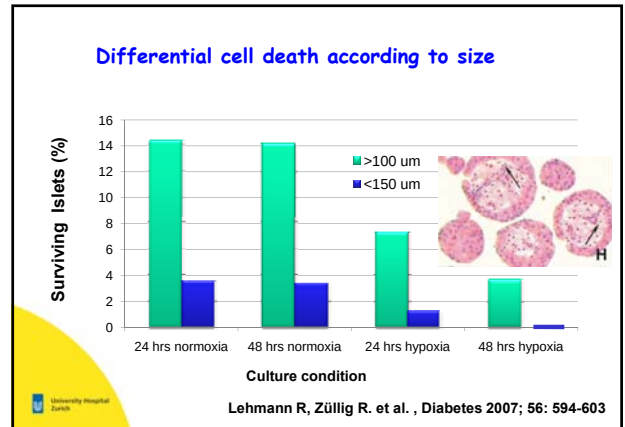
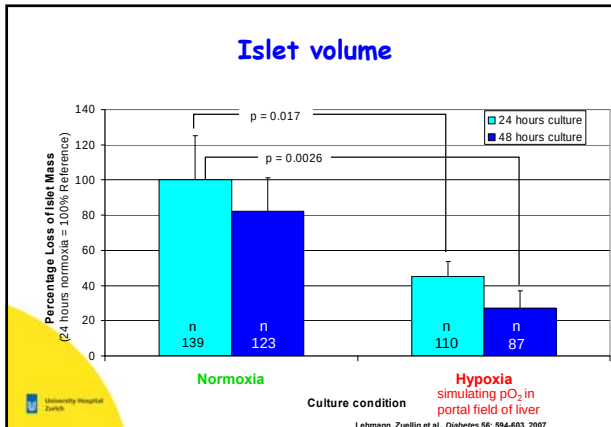


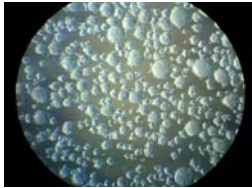
Lehmann, Zuellig et al., Diabetes 56: 594-603, 2007



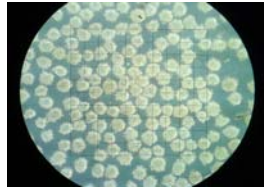
Cell death during Normoxia and Hypoxia (HE-Staining)





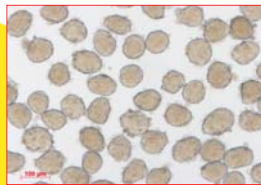
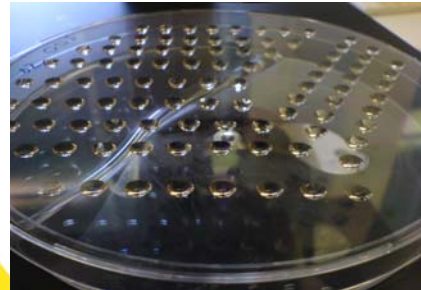


Stem cells kept as floating spheres: no adherence-mediated differentiation, but cluster size still varies: morphogen gradients, hypoxia, etc.



Stem cells in hanging drops: defined environment, defined cell number and cluster size: morphogen gradients defined, hypoxia situation defined, gravity enforced cluster formation, etc. „embryoid bodies“ experimentation possible

Hanging drop technology



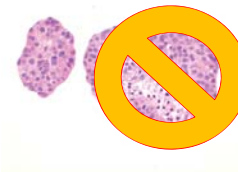
Technique of Pseudoislets



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Islet Transplantation Program
University Hospital Zürich

Rational: Transforming large islets into small islets?



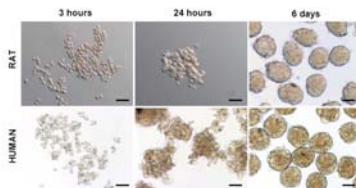
Large islets disappear

50 μ m

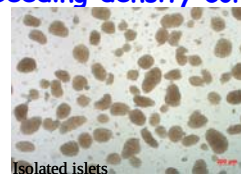
Simulation Hypoxia in Liver (1% O₂)
after 24 hours

Generating small islets with a defined size

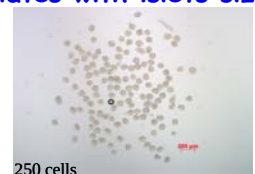
Isolated rat islets $\xrightarrow{\text{Trypsin dissociation}}$ Single cells $\xrightarrow{\text{Counting seeding}}$ „hanging drop“ culture



Seeding density correlates with islets size



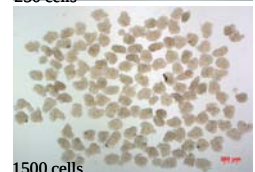
Isolated islets



250 cells



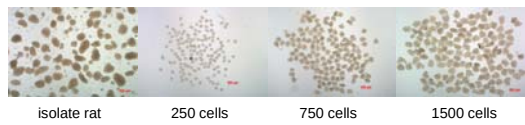
750 cells



1500 cells

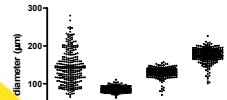
Submitted 2008

Seeding density correlates with islets size

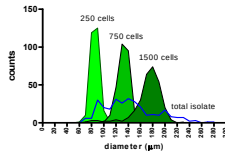


isolate rat 250 cells 750 cells 1500 cells

rat islet size distribution

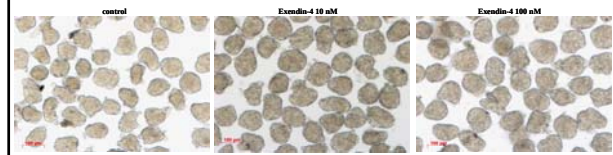


rat islet size histogram



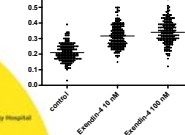
Submitted 2008

GLP-1 improves islet cell survival after dissociation

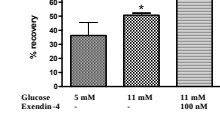


250 cells pseudoislets

IEQ distribution

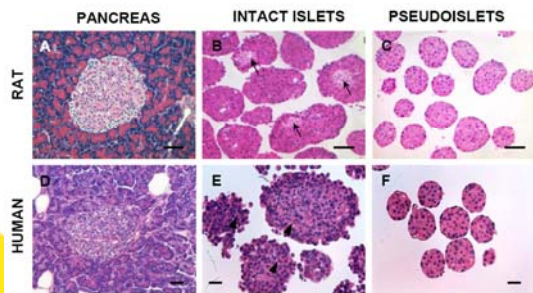


% recovery



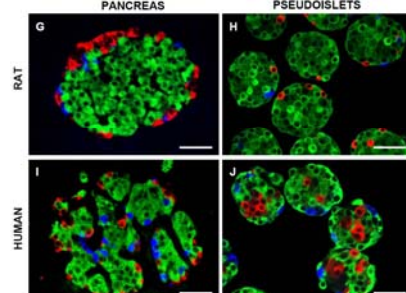
Submitted 2008

Cellular composition and islet architecture is preserved in reaggregated pseudoislets



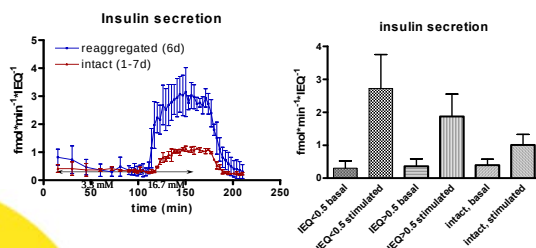
Submitted 2008

Cellular composition and islet architecture is preserved in reaggregated pseudoislets



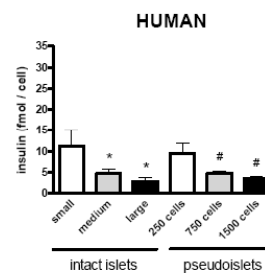
Submitted 2008

Islet Function of Pseudoislets remains intact



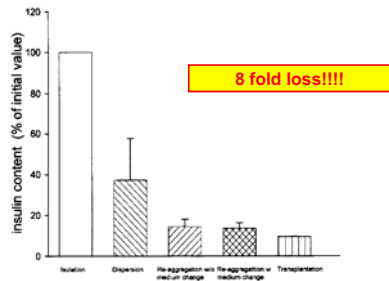
Submitted 2008

Insulin Secretion of human „pseudoislets“



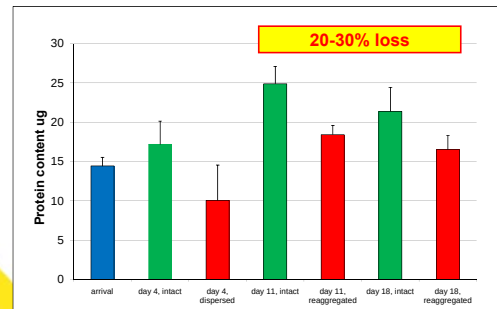
Submitted 2008

Potential Drawbacks: Cell Loss

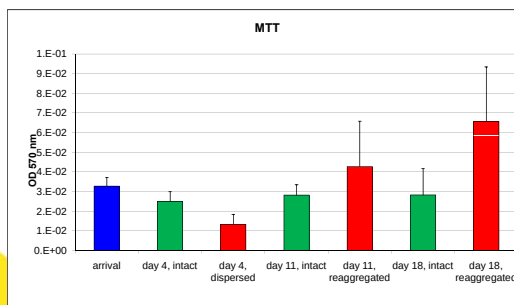


Callewaert H. et al. Cell Transplantation 2007; 16: 527-37

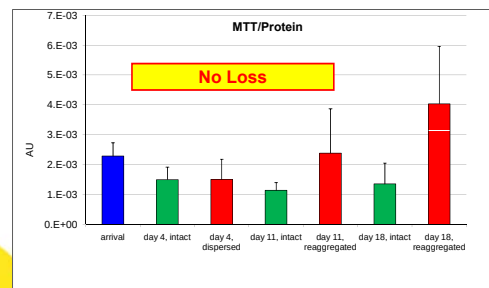
Potential Drawbacks: Cell Loss



Potential Drawbacks: Cell Loss

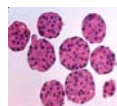
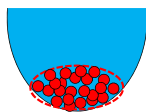
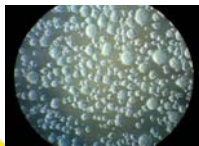


Potential Drawbacks: Cell Loss



Reasons for different cell loss?

- Mechanical vs. enzymatical dissociation; very gentle
- Rotary shaker vs. Hanging drops (gravity)
- Loss of nonaggregated islets by medium change
- Smaller islets and better diffusion



Advantages of Pseudoislets for Clinical Islet Transplantation

Limited mass of permanently engrafted islets ✓

- Limited beta-cell reserve
- Cell loss by apoptosis induced by hypoxia and by other non-immunological, inflammatory mechanisms (IBMIR)

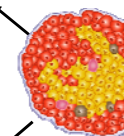
High metabolic Demand ✓

- Insulin resistance
- Diabetogenic
- Immunosuppression

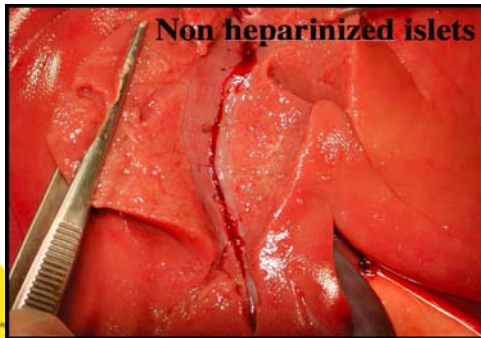
No reliable marker for rejection

Immunological Graft loss ✓

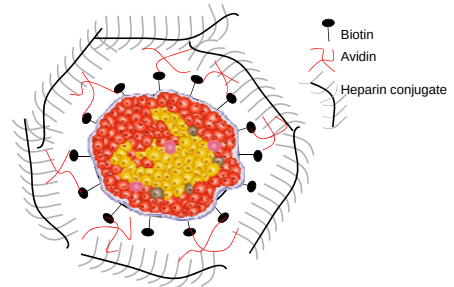
- Autoimmunity
- Alloimmunity



IBMIR: Immediate Blood-Mediated Immune Reaction

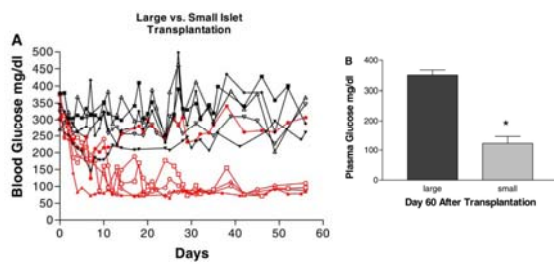


Heparinized Islets



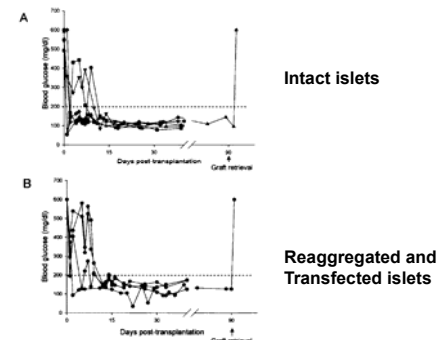
Cabric S. et al, Diabetes 2007; 51: 2008-15

In vivo function of small islets



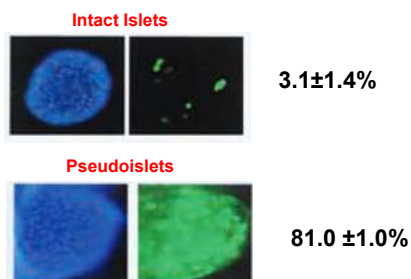
Mac Gregor RR et al. AM J. Physiol Endocrinol Metab 2006; 290: E771-9

In vivo function of reaggregated islets in mice



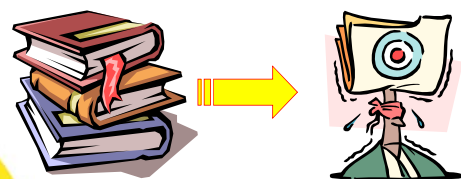
Callewaert H. et al. Cell Transplantation 2007; 16: 527-37

Transfection-Rates with Lentivirus in Pseudoislets (90 hours MOI 5)



Callewaert H. et al. Cell Transplantation 2007; 16: 527-37

Take home messages



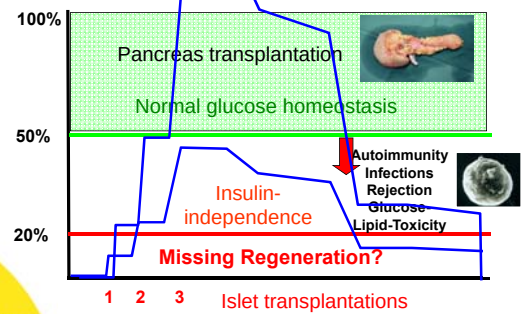
Conclusions

- Production of islets with a defined size and intact function and architecture = possible
- Loss of islet mass by hypoxia reduced by small islets (rats) and possible production of small pseudoislets
- Cell loss during production compensated by better function
- Use of growth factors and manipulation during culture = possible



University Hospital Zurich

Beta cell mass and glucose



Korsgren O et al. Diabetologia 200; 51: 227-32

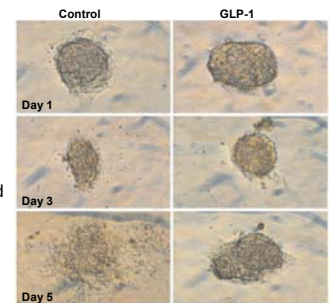
Major Problems in Islet Transplantation Future research?



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GLP-1 maintains islet morphology in culture

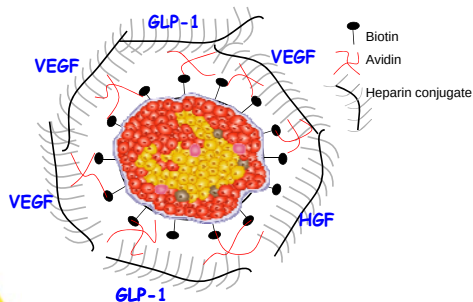
- Cultivated islets lose organisation after 5 days of culture
- After 5 days: 45% Islets Loss of 3-D Structure
- Only 15% loss of 3-D structure in GLP-1 treated islets ($p < 0.01$ vs Kontrolle)



University Hospital Zurich

Farilla L, et al. Endocrinology. 2003;144:5149-5158

Heparinized Islets



University Hospital Zurich

Pseudoislets: Potential Advantages

- Time for ideal immunosuppressive conditions
- Time for induction of immune tolerance
- Better function of islets
- Manipulation of islets
 - Higher transfection rates as compared to whole islets reaggregation
 - Coating of islets
 - Supplementation of growth factors
 - Transfection with local immunosuppressant factors



University Hospital Zurich

