

Isolation of Islets of Langerhans for Transplantation in Diabetic Patients

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Symposium Interdivisional GMP Facility LUMC, a vital link between bench and bedside in Immuno, Cell, and Gene Therapy. Leiden (Netherlands) February 22, 2000

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Isolation of Islets of Langerhans for Transplantation in Type-1 Diabetic Patients

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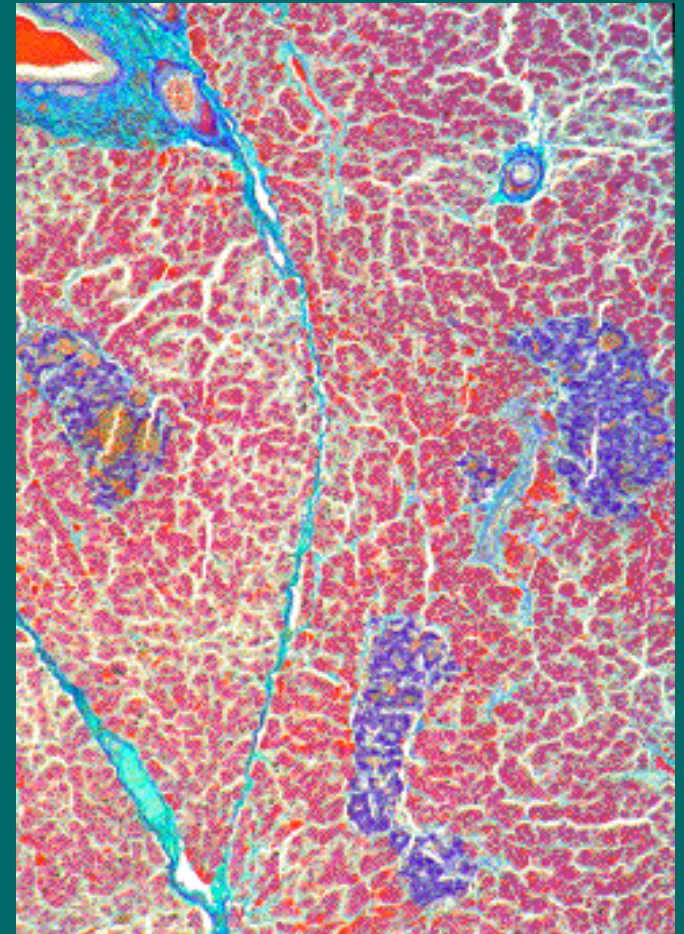
BURG@LUMC.NL[†]

Type-1 Diabetes Mellitus

- In industrialised countries 0.2-0.4% of the population is affected by type-1 diabetes ('insulin dependent diabetes')
- These patients have high blood sugar levels (high glucose) because the insulin-producing cells of the "islets of Langerhans" are destroyed
- ? What are "islets"...

Islets of Langerhans

- The human pancreas contains ~1 million of these “islets” (purple stained in picture):
 - Clusters of roughly 100 to a few 1000 cells that produce insulin
 - The size of an islet is up to 0.5 mm (Ø)
 - Total weight of all islets ~ 1 gramme:
 - » 1-2% of the whole pancreas
- The other 98-99% of the pancreas produces digestive enzymes for the breakdown of our food (‘exocrine tissue’)



Current therapies for type-1 diabetes

- The conventional treatment with daily insulin injections
 - sustains the patient's life, but
 - does not prevent high blood glucose levels ('hyperglycemia'),
 - which , after 20-30 years, may cause serious complications such as blindness, lower limb amputations, or kidney failure — requiring dialysis or a kidney transplant
 - the overall morbidity accounts for an 1/3 reduction of life expectancy
- For some patients: transplantation of the whole pancreas or only the islets is an option ...

At present transplantation is the only way to reach normal glucose control:

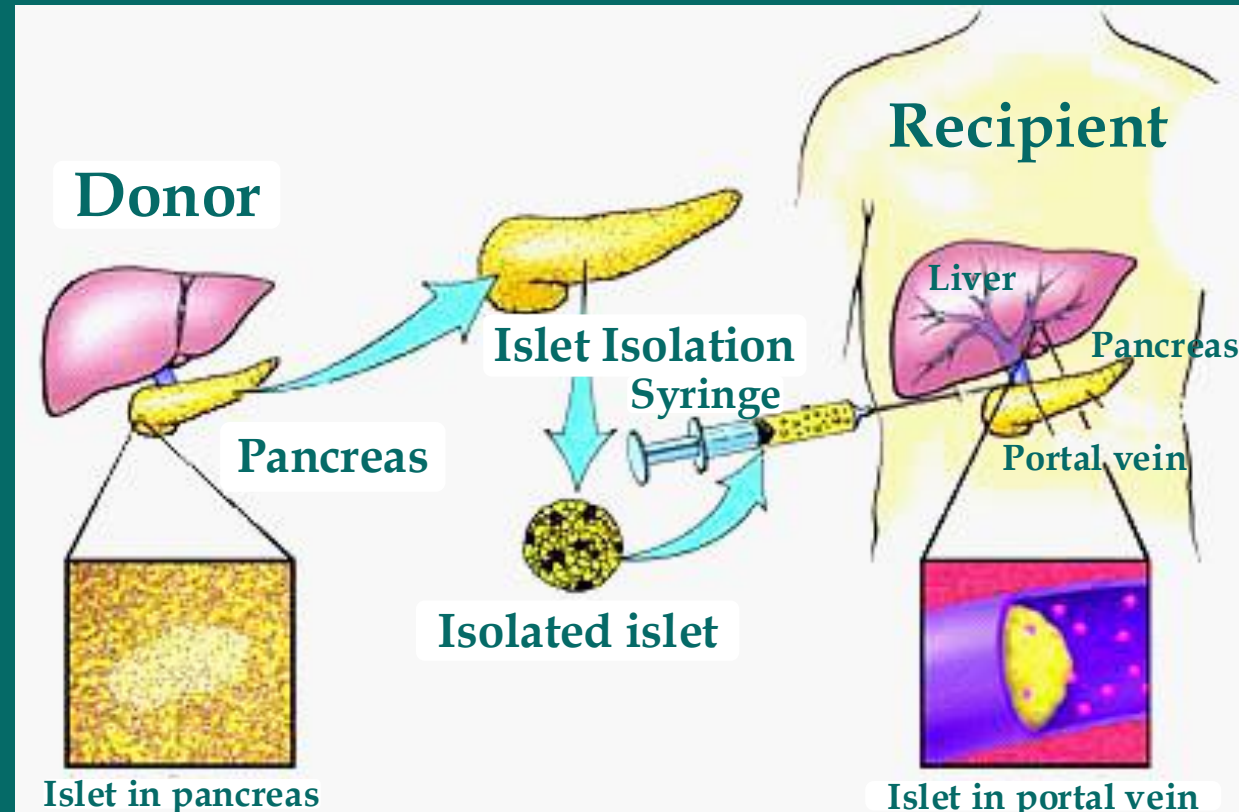
- Transplantation of the whole pancreas
 - Now > 10,000 pancreas grafts have been performed
 - The pancreas is generally only transplanted together with a kidney, in diabetic patients that need a kidney for renal failure
 - Currently, an > 90% patient- and ~ 80% graft survival are reported
 - » normal basal glucose levels,
 - » and no insulin injections required
- Transplantation of only the islets
 - ? Why transplant the whole pancreas

Transplantation of only the islets of Langerhans: after isolation from the pancreas

- ? Why transplant the whole pancreas
 - Only the islets need to be replaced in people with type 1 diabetes
 - Note: the other (exocrine) tissue of the pancreas is the major source of surgical complications in pancreas transplants
- An islet transplant is a less invasive (safer) procedure —a ‘simple’ injection of 1-2 ml islet tissue — and less costly
 - The islet transplant is performed in a radiology suite where the portal vein can be visualized by CT scan
 - A local anesthetic is administered and then a needle is placed in the portal vein and the islets are injected over 15 minutes

Schematical representation of the islet transplantation procedure:

- A pancreas is procured from a cadaveric organ donor
- The pancreas is taken to the islet lab where the islets are isolated
- The islets are kept alive in culture for some days for quality control of the prep
- Then, the islets are injected in the portal vein and carried by the blood into the liver where they lodge



Focus on the Islet Isolation Procedure

- Currently islet transplantation is less successful than pancreas transplantation.
- One of the problems is the isolation of a sufficient *number of purified, and viable*, islets from a donor pancreas
- The next slides illustrate the islet isolation process: work that we will carry on in the GMP-facility of the LUMC within weeks

The islet isolation begins with ...

- the preparation of a donor pancreas
 - first, the pancreas is cleaned from fat and other tissues
- Next, the organ is injected with a solution containing collagenase
 - at a later stage — when the pancreas is warmed to 37°C — this collagenase will breakdown (digest) the connective tissue in the pancreas, that will fall apart: thus liberating the islets



For the digestion of the pancreas ...

- The pancreas is placed in a chamber containing glass beads ('knikkers'), and the chamber is placed in a 'shaker' -->
- The chamber is shaken, and perfused using a roller-pump with a collagenase solution that is heated in a waterbath to 37°C



Schematic representation of the digestion of the pancreas (recirculation and dilution)

- The pancreas is in a steel chamber with glass beads ('knikkers')
- A screen in the chamber allows only particles < 0.5 mm to pass
- The collagenase solution is pumped through a heating coil — warmed to 37°C — and recirculated through the chamber
- The pancreas is progressively broken down into small pieces and after ~ 30 min when most islets are free the recirculation and heating is stopped
- Next, over another ~ 30 min a cold solution (no collagenase) is pumped into the chamber, and the suspension leaving the chamber is collected

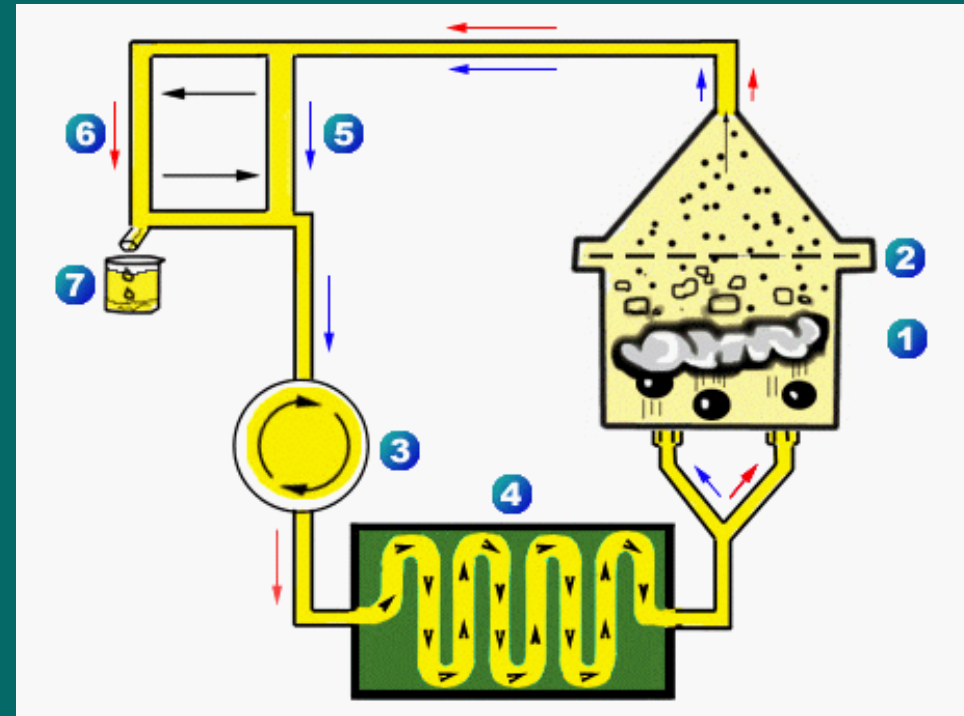


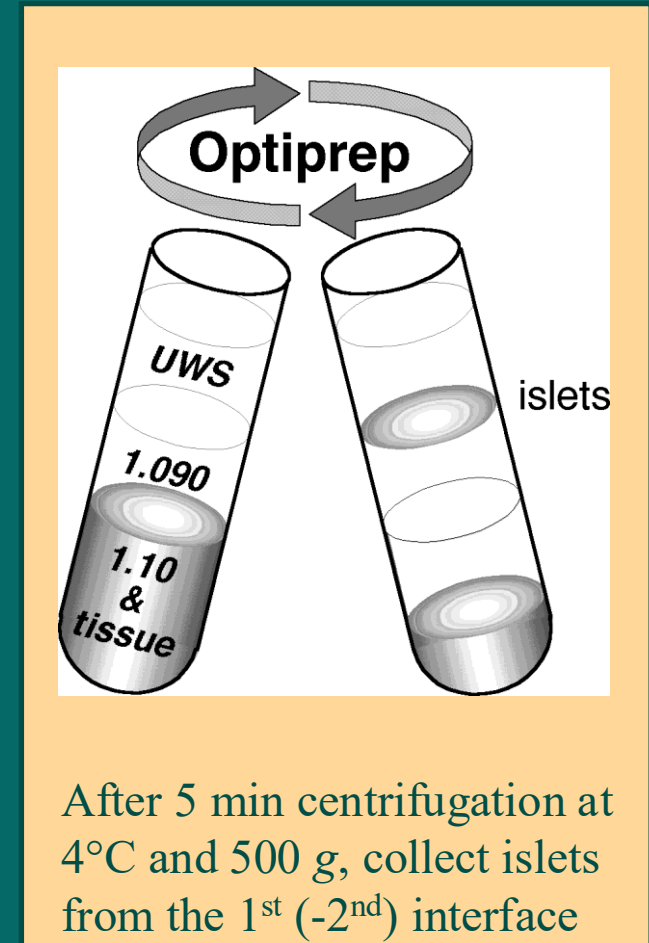
Illustration of the dilution phase ...

- Fresh cold solution (left) is pumped into the chamber
 - the heating coil is out of the waterbath
- The tissue suspension with liberated(‘isolated’) islets is collected in another flask
- Next, the solution is centrifuged, to prepare the tissue for purification ..



The islets are purified in a density gradient: as explained here with the 'tube-method'

- Islets usually have a low density as compared to the exocrine pancreatic fragments ('acini')
- Thus, to separate ('purify') the islets from the rest of the pancreatic tissue:
 - the tissue is mixed in tubes with a purification solution ('OptiPrep') with a high density of e.g. 1.10 g/ml
 - on top of this bottom solution, a solution is layered with a lower density (e.g. 1.090)
- Next during centrifugation the islets float to the top, and the rest of the pancreatic tissue sinks to the bottom of the tubes



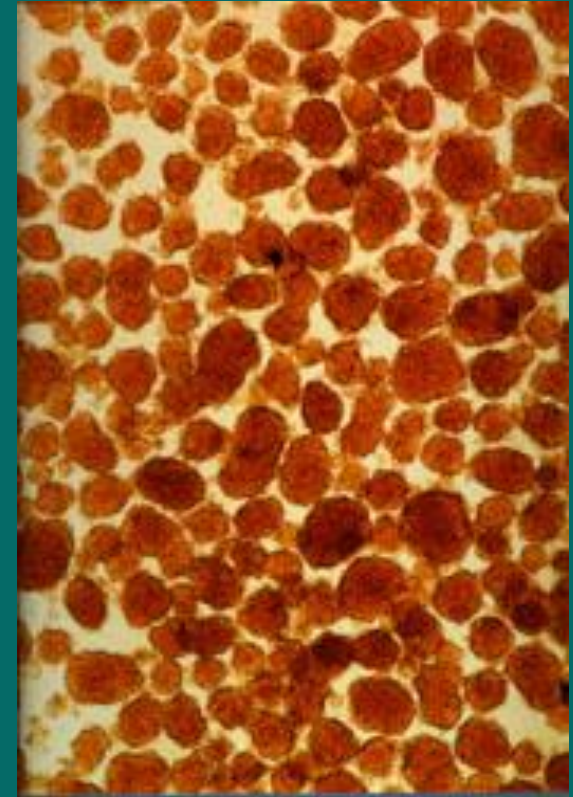
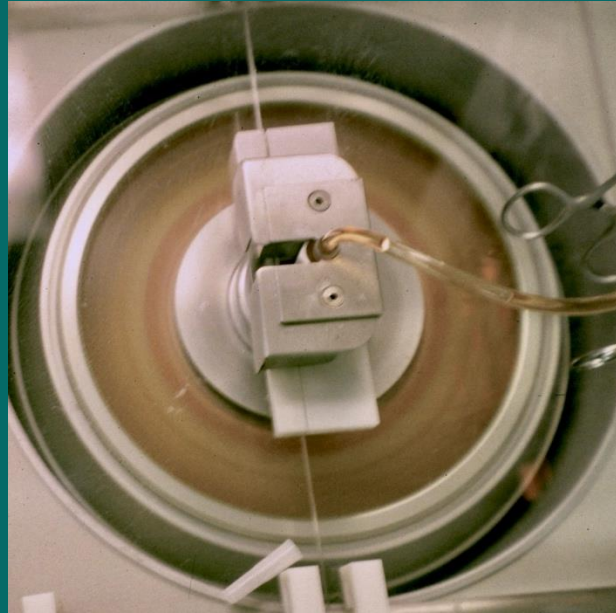
Rather than handling many tubes, a special centrifuge is generally used for the purification: the 'Cobe'

- First the tissue is mixed with the bottom density solution, and loaded in a blood-bag
- By gravity drainage the suspension is transferred to a large centrifuge bag in the Cobe
- Next — while the centrifuge is spinning — a low density solution is pumped on top of the bottom solution



Purification in the Cobe centrifuge

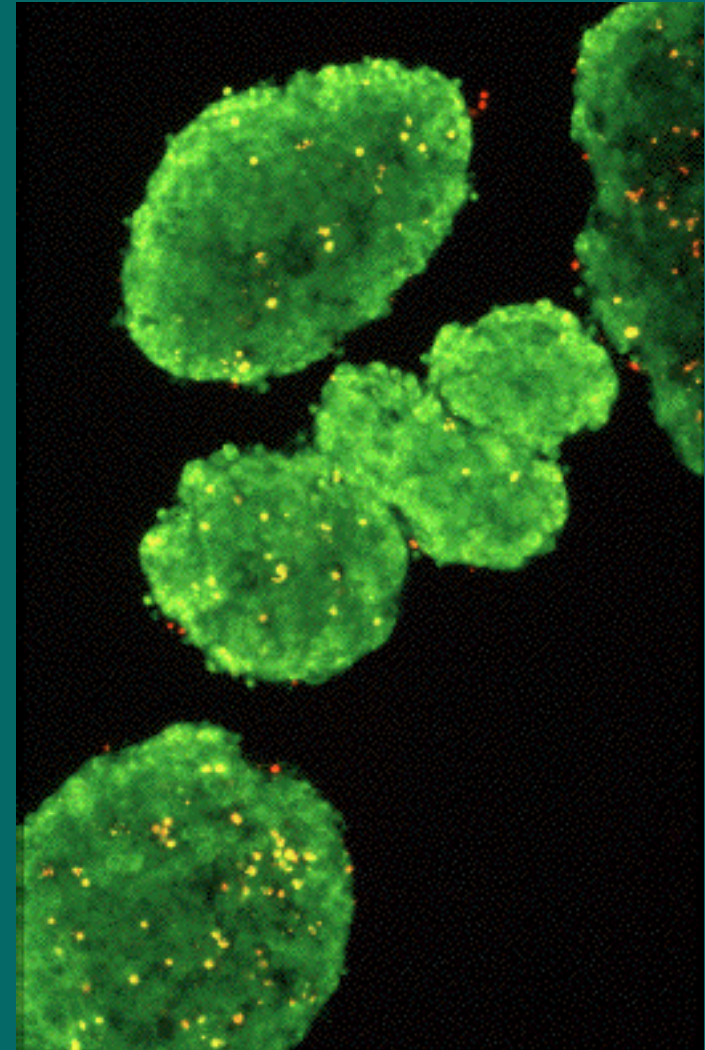
- Finally, after 5 min the gradient is slowly pushed out of the centrifuge bag and the purified islets are collected
- Before purification:



- After:
purified islets

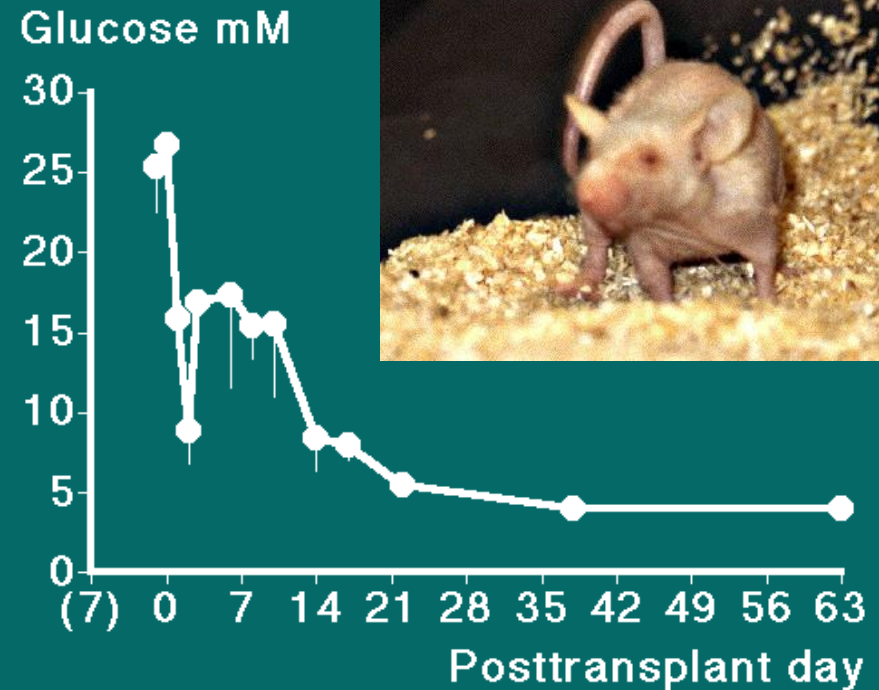
Culture and Quality Control before Transplantation

- The islets are kept in 'culture' for ~ 1-2 weeks and the quality is tested before the islets are considered for a transplant
- Pre-transplant QC includes:
 - Sterility testing
 - Assessing the islet number and purity
 - Viability testing: using specific stains that color viable cells green, and dying or dead cells red or yellow

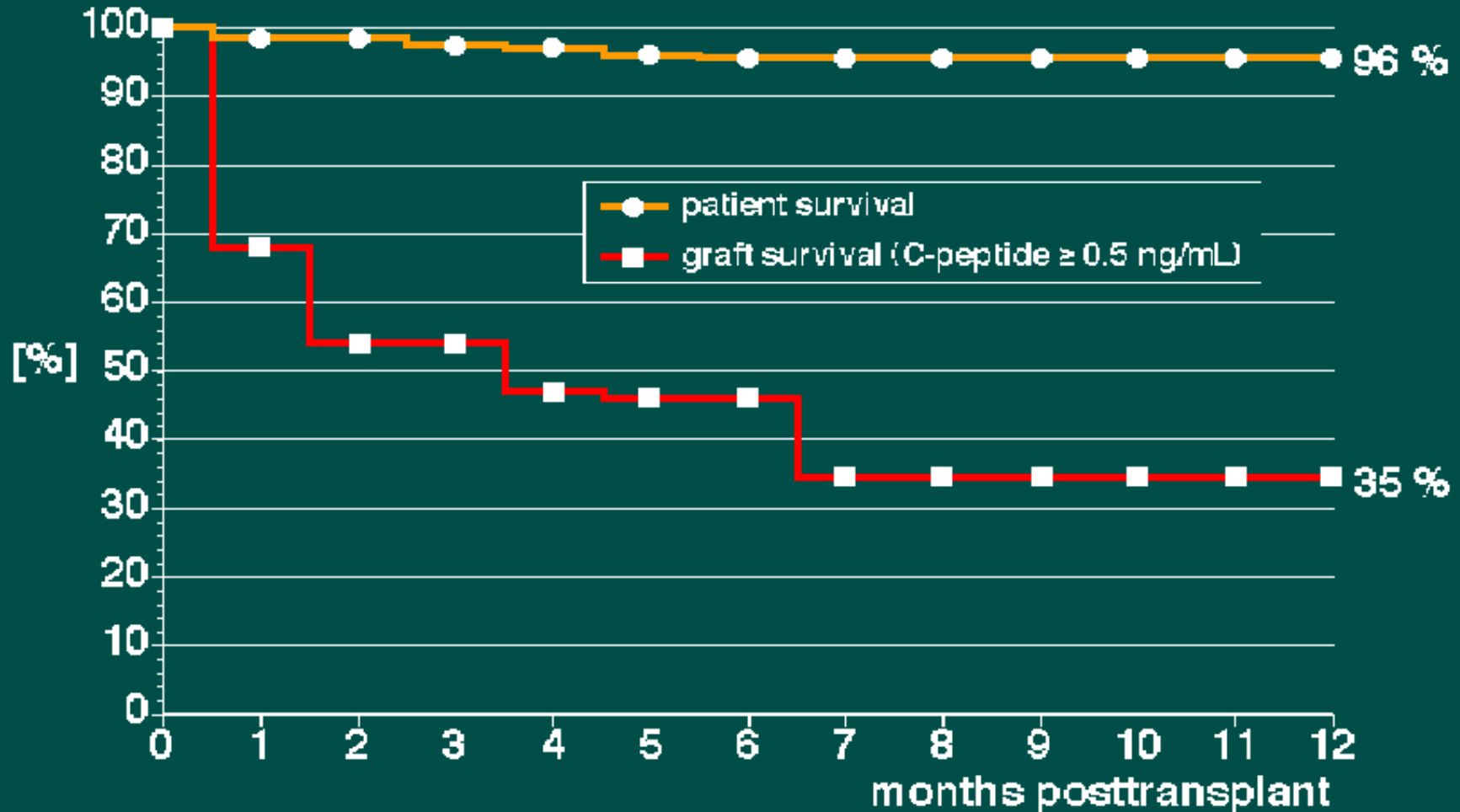


Peri-transplant the islet function is tested by transplanting a sample of the islets in nude mice

- Nude mice are unable to reject foreign tissue
- First, the mice are made diabetic by an injection of streptozotocin (destroys insulin-producing cells)
- Next the human islets are infused under the kidney capsule in the diabetic animals
- Results are usually obtained in 2-3 weeks



Patient and Graft Survival of 200 Islet Allografts in Type-1 Diabetic Recipients (1990-1997)



What are the benefits of an islet transplant?

- Worldwide, about 10% of the recipients were able to completely stop insulin injections and about one-third of the recipients benefited from a significantly improved glucose control after transplantation
- Patients plagued by life-threatening reactions to insulin injections (hypoglycemia), could benefit immediately from an islet transplant
- Even islet recipients who still required some insulin after transplantation have maintained normal glucose control (HbA1c levels) and were relieved of the worries related to hypoglycemia because they regain their ability to sense low blood sugar levels

Notes

Notes added in this online reprint August 24, 2026

- Isolation of Islets of Langerhans for Transplantation in Diabetic Patients • Symposium GMP Facility LUMC 2000 • Michel van der Burg • Miracles.Media • @1MEMO 20260824_1 • Talk, Tuesday February 22, 2000 at 11:20, Symposium Interdivisional GMP Facility LUMC, a vital link between bench and bedside in Immuno, Cell, and Gene Therapy. Leiden University Medical Center, Leiden (Netherlands) • Digital remastered 2026 edition* by author, publisher, Michel van der Burg • Miracles.Media
- * Digitally remastered presentation, using the images from the original slides handout, and the handout of the text spoken during the original presentation , now read using my personal synthesized voice. This 2026 edition is supplemented with a Notes section, including the event program , front and back cover slides with a June 2003 photo of the LUMC building, and my ~ 2003 portrait from the LUMC Intranet 'facebook'.
- † Obsolete LUMC email address. Contact address : michelvanderburg.com/contact/
- The symposium concluded with the festive opening of the Interdivisional GMP Facility LUMC.
- Citation info : Isolation of Islets of Langerhans for Transplantation in Diabetic Patients • Symposium GMP Facility LUMC 2000 • Michel van der Burg • Miracles.Media • @1MEMO 20260824_1

Notes

Program leaflet



LEIDEN UNIVERSITY MEDICAL CENTER

SYMPOSIUM

**The Interdivisional GMP
Facility LUMC, a vital link
between bench and bedside
in Immuno, Cell, and
Gene Therapy**

**LUMC, gebouw 1,
collegezaal 1,
22 februari 2000**

Program

- 09.00-09.30: ontvangst/koffie
- 09.30-09.40: P. Vermeij
Opening
- 09.40-10.00: A.R. Wafelman
Cleanroom facility of the LUMC.
Development and production of new
immunotherapeutics
- 10.00-10.20: J.H.F. Falkenburg
Generation under GMP conditions of
T cell lines recognizing
hematological malignancies for
adoptive immunotherapy
- 10.20-10.40: A.R.P.M. Valentijn
The laboratory for GMP peptide
synthesis
- 10.40-11.00: T. Mutis
Generation of peptide-specific T cell
responses for adoptive
immunotherapy
- 11.00-11.30: Coffee break

- 11.20-11.45: M.P.M. van der Burg
Isolation of Islets of Langerhans for
transplantation
- 11.45-12.00: C.J.M. Melief
Vaccination against cervix carcinoma
associated HPV with peptide-loaded
dendritic cells
- 12.00-12.15: S. Osanto
Gene therapy and immunotherapy of
solid tumors
- 12.15-12.30: M.H.M. Heemskerk
Immunotherapy using gene-modified
T cells
- 12.30-12.45: M.W. Schilham
Generation of EBV-specific T cells
for clinical use
- 12.45-13.00: R.J. O'Reilly (New York)
Immunotherapy and gene therapy:
closing remarks
- 13.00-14.00: Lunch
- 14.00-14.30: Official opening of IGFL by
Dr.Ir.P.C.J.Leijh, Member of the
Board of Directors LUMC; first
exhibition of the film on the GMP
facility.

Speakers:

- dr.M.P.M.van der Burg, department of Surgery,
LUMC
- prof.dr.J.H.F.Falkenburg, department of Hematology,
LUMC
- mevr.dr.M.H.M.Heemskerk, department of
Hematology, LUMC
- prof.dr.C.J.M.Melief, department of
Immunohematology and Bloodbank, LUMC
- dr.T.Mutis, department of Immunohematology and
Bloodbank, LUMC
- mevr.dr.S.Osanto, department of Oncology, LUMC
- prof.dr.R.J. O'Reilly MD, Bone Marrow
Transplantation Services, Memorial Sloan-Kettering
Cancer Center, New York, USA
- dr.M.W.Schilham, department of Pediatrics, LUMC
- dr.A.R.P.M.Valentijn, department of
Immunohematology and Bloodbank, LUMC
- prof.dr.P.Vermeij, department of Clinical Pharmacy
and Toxicology, LUMC
- dr.A.R.Wafelman, department of Clinical Pharmacy
and Toxicology, LUMC

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