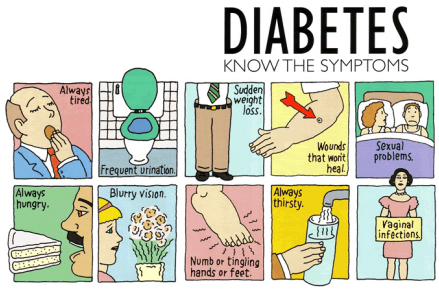
Diabetes

- The number of people with diabetes rose from 108 million in 1980 to 422 million in 2014.
- The global prevalence of diabetes among adults over 18 years of age rose from 4.7% in 1980 to 8.5% in 2014.
- Between 2000 and 2016, there was a 5% increase in premature mortality from diabetes.
- Diabetes prevalence has been rising more rapidly in low- and middle-income countries than in high-income countries.
- Almost half of all deaths attributable to high blood glucose occur before the age of 70 years. WHO estimates that diabetes was the seventh leading cause of death in 2016.
- A healthy diet, regular physical activity, maintaining a normal body weight and avoiding tobacco use are ways to prevent or delay the onset of type 2 diabetes.
- Diabetes can be treated and its consequences avoided or delayed with diet, physical activity, medication and regular screening and treatment for complications.

Diabetes

- Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation.

- Type 1: juvenile-onset diabetes, autoimmune destruction of beta-cells
- Type 2: adult-onset, familiar, insulin-resistance combined with reduced secretion



Current therapy for diabetes

No cure available
Support therapy: insulin (type 1), diet, exercise, oral medications (type 2)
Whole organ transplant requires strong immunosuppression (only in combination with kidney transplant).

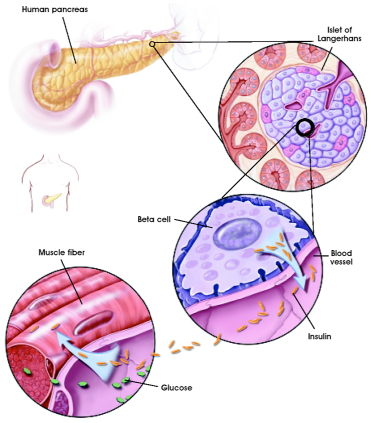
Insulin was discovered over 90 years by JJR Macleod at the University of Toronto.
The first patient, Leonard Thompson, at the time of treatment was on a starvation diet that was intended to extend his life for a few years. He was injected with a crude extract of bovine pancreas in January 1922 with an almost immediate effect on his glycosuria, blood glucose levels and general well-being. From that moment onward diabetes was no longer a fatal disease.

Medscape	www.medscape.com
1922	First clinical use of insulin
1920s	Short-acting bovine and porcine pancreas extracts
1930s	Improved purification Protamine-insulin complexes reported
1940s	NPH (neutral protamine Hagedorn) introduced
1950s	Lente and ultralente insulins
1970s	Highly purified (monocomponent) insulins
1980s	Premixed biphasic insulins Insulin pumps for CSII (continuous subcutaneous insulin infusion) Biosynthetic human insulin Pen injection devices
1990s	Rapid-acting insulin analogues
2000s	Long-acting insulin analogues

Source: Br J Diabetes Vasc Dis © 2004 Sherbourne Globe, Ltd

There have been many major breakthroughs since 1922, but none more important than the cloning and sequencing of the insulin gene in 1980, which brought about the introduction of unlimited supplies of **bacterially expressed human insulin** and the technology to modify the structure of the protein, such that there are now at least 6 rapid- acting or long-acting analogues.

Pancreas structure

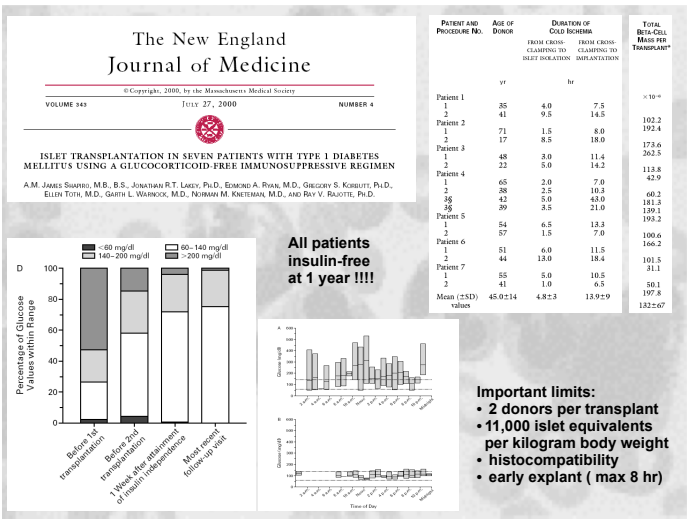


Langherans islets:

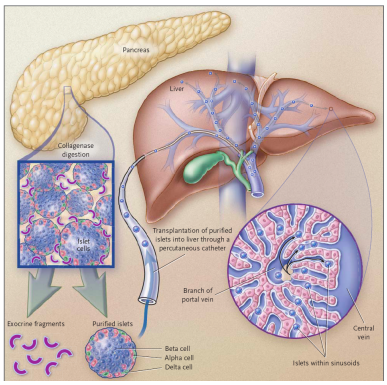
- Different cell types:
  - Alpha cells producing glucagon (15–20% of total islet cells)
  - Beta cells producing insulin and amylin (65–80%)
  - Delta cells producing somatostatin (3–10%)
  - PP cells (gamma cells) producing pancreatic polypeptide (3–5%)
  - Epsilon cells producing ghrelin (<1%)
- Complex interplay in glucose metabolism regulation
- Digestive enzymes secreted by exocrine pancreatic tissue
- Islet transplantation better than whole organ and beta-cell transplantation

Table 1. Synopsis of Reports of Successful Islet Transplantation in Patients with Type 1 Diabetes.*

Study	Year of Report	No. of Recipients and Size of Transplant	Outcome
Largiader et al.[19]	1980	1 Recipient of pancreas microfragments containing 200,000 islets	Insulin-independent with normal glucose level at 9½ mo
Scharp et al.[22]	1990	1 Recipient of 800,000 islets	Insulin-independent at 22 days
Tzakis et al.[23]	1990	9 Patients with cancer and abdominal exenteration without diabetes received 205,000–746,000 islets	Normal glycosylated hemoglobin values in 5 patients, with some receiving insulin supplementation
Warnock et al.[24]	1991	1 Recipient of 611,000 islets	Insulin-independent with normal glucose levels at 3 mo
Scharp et al.[25]	1991	First 9 patients receiving 6161±911 to 13,916±356 islets/kg of body weight	3 Transplantations failed; 4 had measurable C-peptide levels for up to 10 mo but not insulin-independent; 2 with normal glucose levels and insulin-independent for 1–5 mo
Warnock et al.[26]	1992	4 Recipients of 261,000–896,000 fresh and cryopreserved islets	3 Had measurable C-peptide levels for 1–8 mo, but not insulin-independent; 1 insulin-independent for 1 yr
Gores et al.[27]	1993	2 Recipients of 502,000–528,000 islets	1 Had measurable C-peptide levels but not insulin-independent at 9 mo; 1 with normal glucose levels and insulin-independent at 8 mo
Soon-Shiong et al.[28]	1994	1 Recipient of 678,000 encapsulated islets	Insulin-independent with normal glucose levels at 9 mo
Carroll et al.[29]	1995	1 Patient with cancer and abdominal exenteration without diabetes	Insulin-independent with normal glycosylated hemoglobin values at 3 yr
Luzi et al.[30]	1996	15 Recipients of 98,587–1,294,125 islets	8 Had C-peptide levels >1.4 ng/liter; 4 insulin-independent with glycosylated hemoglobin values of 5.6–7.2 percent at 1–8 mo
Alejandro et al.[31]	1997	8 Recipients of 478,000–1,271,000 islet equivalents	2 Insulin-independent at 1 mo and 2 insulin-independent at 6 yr with normal to near-normal glycosylated hemoglobin values
Secchi et al.[32]	1997	20 Recipients of 3461–14,488 islet equivalents/kg	9 Had measurable C-peptide levels with decreased need for insulin; 6 insulin-independent at 3–11 mo; 1 insulin-independent at 48 mo; all with normal or near-normal glycosylated hemoglobin values
Keymeulen et al.[33]	1998	7 Recipients of 2100–5300 islet equivalents/kg	3 Had measurable C-peptide levels for >1 yr; 2 insulin-independent with normal to near-normal glycosylated hemoglobin values for 1 yr
Oberholzer et al.[34]	2000	13 Recipients of 199,000–863,000 islets	All had measurable C-peptide levels for >3 mo; 5 of 8 had normal C-peptide levels >1 yr; 2 patients insulin-independent at 4 and 36 mo
Shapiro et al.[35]	2000	7 Recipients of 11,546±1604 islets	All insulin-independent at 4–15 mo with 6-month glycosylated hemoglobin values of 5.7±0.2 percent



Modern islet isolation technology



- Availability of a healthy pancreas from a brain-dead donor
- Same technique used to procure a pancreas for whole-organ transplantation
- Pancreas duct cannulation and collagenase infusion
- Islet purification by density-gradient centrifugation
- Infusion into the portal vein

The Journal of Clinical Investigation http://www.jci.org Volume 114 Number 7 October 2004

Science in medicine

Challenges facing islet transplantation for the treatment of type 1 diabetes mellitus

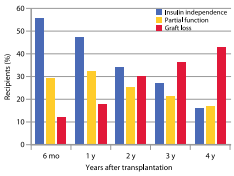
Kristina I. Rother and David M. Harlan

Islet and Autoimmunity Branch, National Institutes of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland, USA.

Compared to pancreas transplantation, islet transplantation is easier, has lower morbidity and permits storage of the islet graft (cryopreservation for banking)

Yet, islet transplantation does not offer permanent cure of hyperglycemia for all diabetic patients in need

- Only 10% maintain insulin independence after 15 months



- High number of islets is required: 850,000 with Edmonton protocol, 300,000 with autotransplantation after pancreatectomy
- Imbalance between supply and demand. Eligible patients have had T1D for >5 years, are aged 18-65, have poor diabetes control
- Significant side effects due to immunosuppression

Source: Collaborative Islet Transplant Registry (CITR)

Immunosuppressive regimen that avoids the use of diabetogenic glucocorticoids

Table 1
Systemic side effects commonly associated with the immunosuppressive agents typically administered following islet transplant

Immunosuppressant (brand name)	Drug classification	Common and important side effects (Phase of drug administration)
Rapamycin, also known as Sirolimus (Rapamune)	Macrocyclic lactone	Hyperlipidemia, antiproliferation (e.g., anemia, diarrhea) (Maintenance)
FK506, also known as Tacrolimus (Prograf)	Calcineurin inhibitor	Hypertension, nephrotoxicity, CNS effects (e.g., tremor), diabetogenicity (Maintenance)
Daclizumab (Zenapax)	mAb-binding IL-2 receptor α subunit	May increase risk of infections; hypersensitivity (Induction)

- An endpoint more rigorous than insulin independence at 1 year after transplant needs to be met
- What do current studies suggest regarding the impact of islet transplantation on patient survival and quality of life?

The net effect of improved glycemia control produced by the transplant, when balanced against the immunosuppressive-associated hypertension, hyperlipidemia, and decreased renal function, may actually decrease quality of life and increase mortality

Alternative sources of cells oh physiologically regulated insulin secretion

1. **Expanding islet cellular mass in vitro**

   Inexorable decline in insulin production
   Islets are mini-organs

2. **Islets from species other than humans**

   Humans express high titers of antibodies against a galactose residue present on most pig cells (*historically pigs were the first source of insulin for diabetes treatment*)

3. **Promotion of β-cell differentiation from stem cells**

XENOTRANSPLANTATION

Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates

Bernhard J Hering[1], Martin Wijkstrom[1], Melanie L Graham[1], Maria Hårdstedt[2], Tor C Aasheim[1], Tun Jie[2], Jeffrey D Ansite[1], Masahiko Nakano[1], Jane Cheng[2], Wei Li[2], Kathleen Moran[2], Uwe Christians[2], Colleen Finnegan[2], Charles D Mills[1], David E Sutherland[1], Pratima Bansal-Pakala[1], Michael P Murtaugh[3], Nicole Kirchhof[4] & Henk-Jan Schuurman[5]

NATURE MEDICINE VOLUME 12 | NUMBER 3 | MARCH 2006

Problem of immune rejection

- use of transgenic pigs that do not express xenogenic surface antigens
- islet embedding in alginate microcapsules
- influence the recipient's immune system

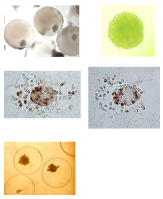
About 100 swine at the University of Minnesota's Schulze Diabetes Institute in Minneapolis constitute the first herd in the country specially bred to supply insulin-secreting pancreatic islets for people with diabetes.

Research Paper

Clinical Benefit of Islet Xenotransplantation for the Treatment of Type 1 Diabetes

Shinichi Matsumoto MD,a,*, Adrian Abalovich MD,b, Carlos Wechsler MD,b, ShaunWynyard PhD,, Robert B. Elliott MD,c,d

EBioMedicine 12 (2016) 255–262

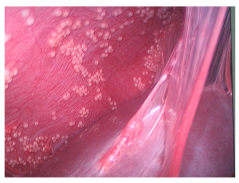


Encapsulated porcine neonatal islets transplanted into type 1 diabetic patients (8 patients).

Patients with high dose group could maintain HbA1c < 7% > 600 days with reduced hypoglycemic events.

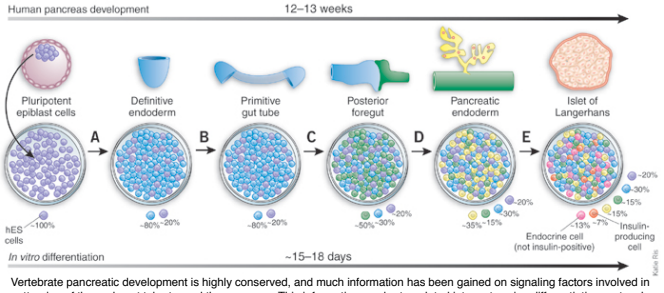
No PERV infection in all patients

B



Insulin dependent diabetes mellitus can be successfully treated by human islet cell transplantation. However the shortage of donated human pancreas is the major issue. Islet transplantation using clinical grade porcine pancreas is a promising treatment to alleviate the shortage of donated human pancreas.
In this study, we transplanted encapsulated neonatal porcine islets into 8 insulin dependent diabetic patients. There was no porcine endogenous retrovirus infection. All patients reduced HbA1c levels which indicated glycemic controls were improved.
Encapsulated neonatal porcine islet transplantation appears safe and efficacious to improve glycemic control for insulin dependent diabetic patients.

Directed differentiation of hES or iPS cells to insulin-producing cells by mimicking embryonic development



Vertebrate pancreatic development is highly conserved, and much information has been gained on signaling factors involved in patterning of the early gut tube toward the pancreas. This information can be translated into a stepwise differentiation protocol that includes sequential exposure to:

(i) FGF10 and the hedgehog-signaling inhibitor cyclopamine, and removal of activin A (inducing the primitive gut-tube markers HNF1B and HNF4A)

(ii) retinoic acid inducing the posterior gut-tube markers HNF6, PDX1 and HLXB9

(iii) exendin-4 with DAPT-mediated inhibition of Notch signaling inducing pancreatic epithelial markers including endocrine progenitor markers such as NKX6-1, NKX2-2, NGN3 and PAX4

(iv) exendin-4, IGF1 and HGF (inducing PAX6, NEUROD1, ISL1 and hormone-gene expression).

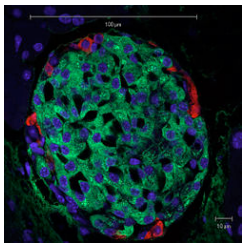
Protocol for the differentiation of pluripotent cells in functional islets

- D0-2: induce formation of Definite Endoderm by high concentrations (100 ng/ml) of activin A, which mimics the effects of nodal signaling in the early embryo

- D2-4: specification of the pancreas, by adding retinoic acid and inhibiting endogenous sonic hedgehog signaling with cyclopamine

-D4-6:formation of the pancreatic cell types by adding FGF and inhibiting the actions of activin A, which at this stage would push the cells towards liver lineages

- D7-9: inhibit Delta/Notch signaling, by use of a γ-secretase inhibitor, to enrich for a population of endocrine progenitors



To date it has not been possible to differentiate these progenitors further into fully functional β-cells; however when placed under the kidney capsule or epididymal fat pad of immunocompromised mice, the progenitors, after 12 weeks or so, secrete human C-peptide in a manner that responds to a glucose tolerance test and can rescue hyperglycemia if the mice are subsequently treated with streptozotocin, which kills mouse but not human β-cells

Markers of functional β-cells

MAFA: a basic leucine zipper transcription factor expressed in mature β cells and absent in pancreatic progenitors and other cell types

NEUROD1: downstream factor of NGN3 expressed in most pancreatic endocrine cells, including β cells)

PDX1/NKX 6.1: restricted coexpression in β cells

Functional features of β-cells

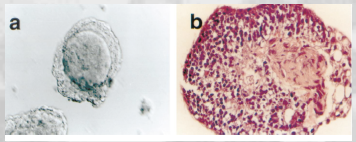
Glucose-stimulated insulin secretion (GSIS)

C-peptide secretion

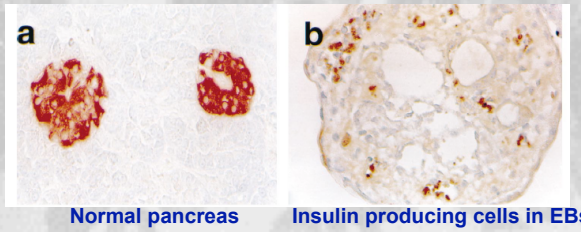
Rapid Publication

Insulin Production by Human Embryonic Stem Cells

Suheir Assady,[1] Gila Maor,[1] Michal Amit,[1] Joseph Itskovitz-Eldor,[1,2] Karl L. Skorecki,[1,2] and Maty Tzukerman[2]



Embryoid bodies

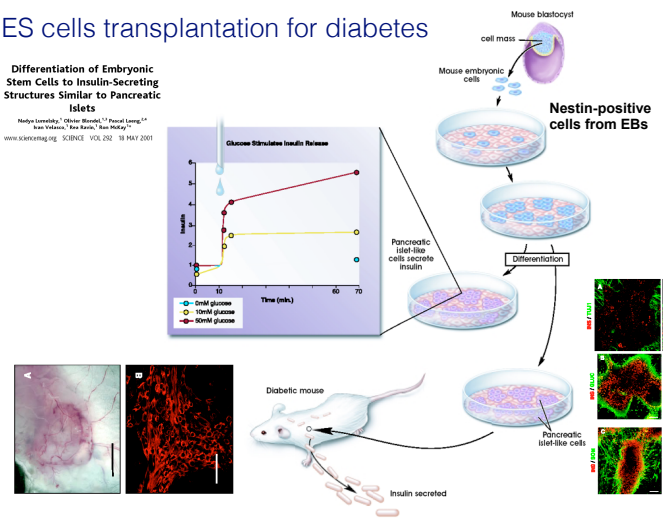


Pdx-1 expression

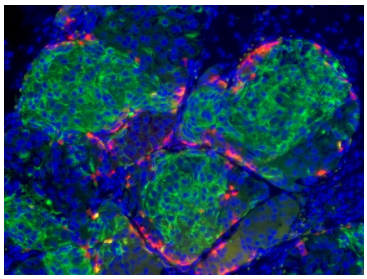
Normal pancreas

Insulin producing cells in EBs

ES cells transplantation for diabetes



From stem cells to billions of human insulin-producing cells



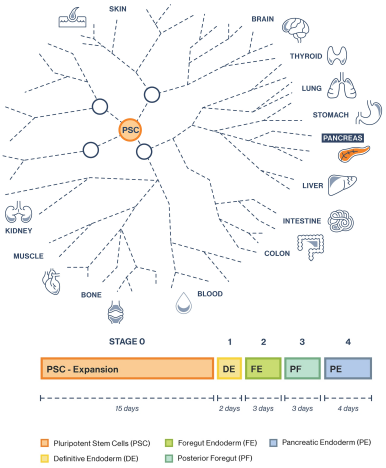
The generation of insulin-producing pancreatic β cells from stem cells in vitro would provide an unprecedented cell source for drug discovery and cell transplantation therapy in diabetes. However, insulin-producing cells previously generated from human pluripotent stem cells (hPSC) lack many functional characteristics of bona fide β cells. Here, we report a scalable differentiation protocol that can generate hundreds of millions of glucose-responsive β cells from hPSC in vitro. These stem-cell-derived β cells (SC-β) express markers found in mature β cells, flux Ca^{2+} in response to glucose, package insulin into secretory granules, and secrete quantities of insulin comparable to adult β cells in response to multiple sequential glucose challenges in vitro. Furthermore, these cells secrete human insulin into the serum of mice shortly after transplantation in a glucose-regulated manner, and transplantation of these cells ameliorates hyperglycemia in diabetic mice.

Generation of Functional Human Pancreatic β Cells In Vitro

Felicia W. Pagliuca,[1,3] Jeffrey R. Millman,[1,3] Mads Gürtler,[1,3] Michael Segel,[1] Alana Van Dervort,[1] Jennifer Hyoje Ryu,[1] Quinn P. Peterson,[1] Dale Greiner,[2] and Douglas A. Melton[1,*]
[1]Department of Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138, USA
[2]Diabetes Center of Excellence, University of Massachusetts Medical School, 368 Plantation Street, AS7-2051, Worcester, MA 01605, USA
[3]Co-first author
*Correspondence: dmelton@harvard.edu
http://dx.doi.org/10.1016/j.cell.2014.09.040

Cell *159*, 428–439, October 9, 2014 ©2014 Elsevier Inc.

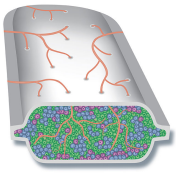
VIACYTE
Regenerating Health™



The process mimics the natural development of the human pancreas. During each step, prescribed types and amounts of growth factors, growth media, and supplements direct pluripotent stem cells to progress along the differentiation pathway until they become PEC-01 cells. Once implanted under the skin of a patient, PEC-01 cells, which are contained within an implantation device, have been designed to mature into functional beta cells and other cells of the islet that control blood glucose levels.

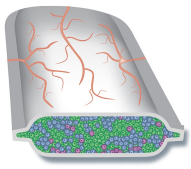
VIACYTE
Regenerating Health™

PEC-Direct (VC-02)



The pouch is designed to allow blood vessels to enter the device and directly interact with the implanted PEC-01 cells. Vascularization is intended to allow for robust and consistent engraftment but will necessitate the use of immune suppression therapy because the implanted cells are not hidden from the immune system.

PEC-Encap (VC-01)



The pouch is designed to fully contain the implanted cells but still allow vital nutrients, such as oxygen, glucose, insulin, to travel between the cells inside the device and the blood vessels, which grow along the outside of the device. This device is also designed to prevent immune cells from directly contacting the implanted cells. The Encaptra® system prevents immune rejection and immune sensitization.

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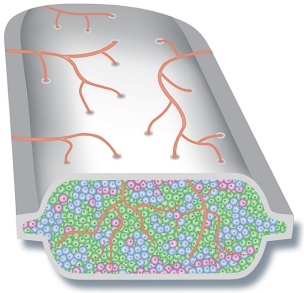
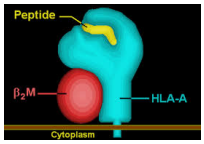
PEC-QT (VCTX210)



Using gene editing on the pluripotent stem cell offers the potential to protect implanted cells from the patient's immune system by ex vivo editing immune-modulatory genes.
ViaCyte and CRISPR Therapeutics, a leading company in the gene-editing space, are collaborating to discover, develop, and commercialize an immune-evasive islet replacement treatment for diabetes, which we refer to as the PEC-QT program.

IMMUNE-MODULATION STRATEGIES

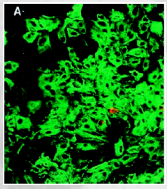
Human leukocyte antigen (HLA) mismatching is the major molecular mechanism of immune rejection in allo- or xenografts.



- Elimination of HLA-A genes
- Knocking out the β2-microglobulin (B2M) gene, which abolishes all HLA class I molecules
- Targeted overexpression of PDL1-CTLA4Ig in β cells

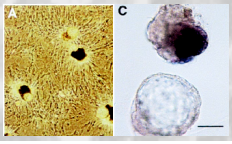
In vitro cultivation of human islets from expanded ductal tissue

Susan Bonner-Weir*, Monica Taneja, Gordon C. Weir, Krystyna Tatarkiewicz, Ki-Ho Song, Arun Sharma, and John J. O'Neil

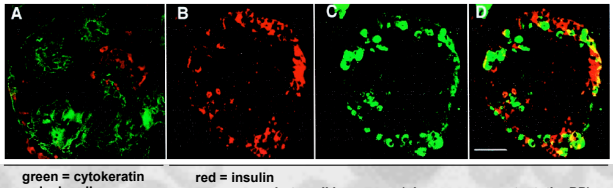


Primary ductal cells
green = cytokeratin
red = insulin

Insulin secretion according to G concentration
In vitro expansion, autologous transplantation



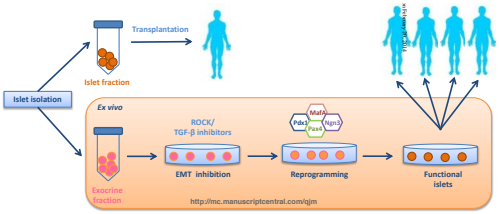
CHIBs rising from a monolayer of ductal cells
(dithizone staining)



green = cytokeratin
red = insulin

red = insulin
green = non beta-cell hormones (glucagons, somatostatin, PP)

Reprogramming Adult Cell Types towards β-Cells



Suppression of Epithelial-to-Mesenchymal Transitioning Enhances Ex Vivo Reprogramming of Human Exocrine Pancreatic Tissue Toward Functional Insulin-Producing β-Like Cells

Lima MJ, Muir KR, Docherty HM, Drummond R, McGowan NW, Forbes S, Heremans Y, Houbracken I, Ross JA, Forbes SJ, Ravassard P, Heimberg H, Casey J, Docherty K.

Diabetes. 2013 Aug;62(8):2821-33.