Cellular Replacement for Diabetes:

Bench to Bedside,

Progress and Challenges

Kenneth L. Brayman, M.D., Ph.D.
Department of Surgery
Division of Transplantation

University of Virginia Health System

Diabetes Epidemic

Worldwide increase - from 151 to >300 Million by 2025 (100% increase)

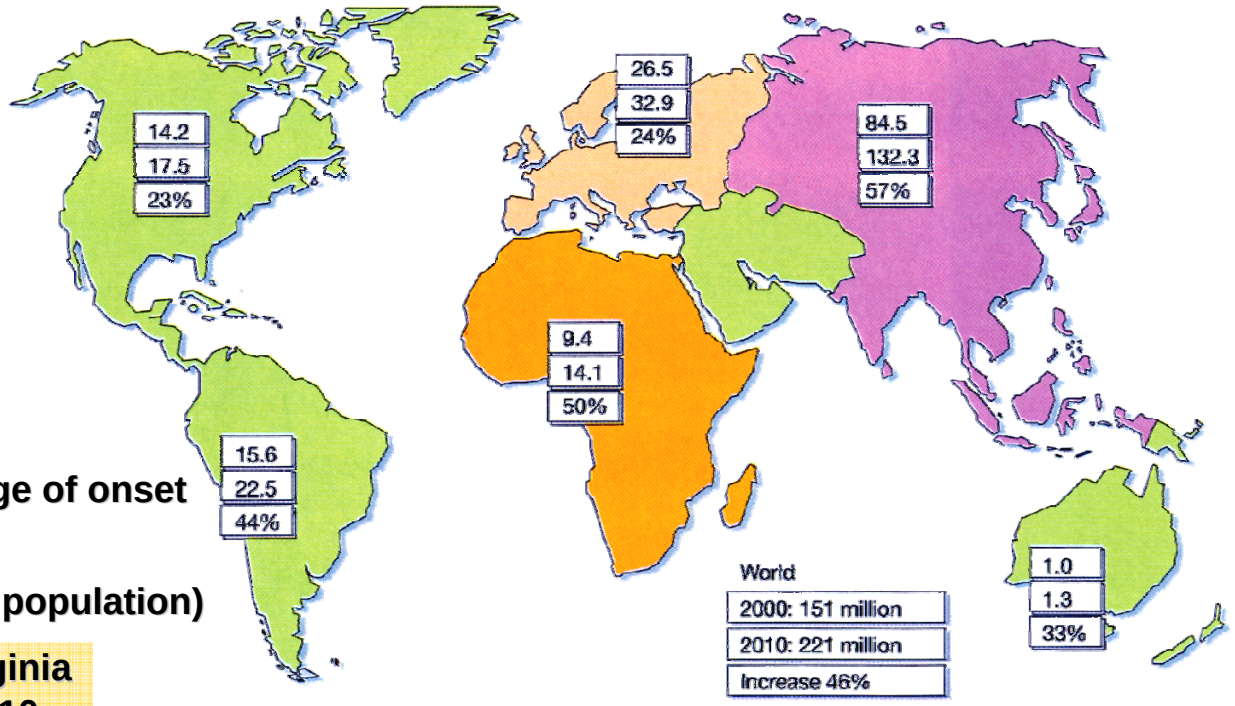
Commonwealth of Virginia
from 250,000 to >500,000 by 2025 (approximately 25,000 new cases annually)

Leading cause of renal failure, adult blindness, and non-traumatic amputation.

4 fold increase in rate of stroke and MI.

Life expectancy shortened by >15 years.

Annual health care costs >$100 Billion nationally.



TYPE 1 Diabetes

"juvenile" diabetes – early age of onset

≈20 to 25 Million by 2010
(≈ 10% of total diabetic population)

in the Commonwealth of Virginia
≈45,000 to 50,000 by 2010

costs difficult to judge
approx. 10% of household income (US)

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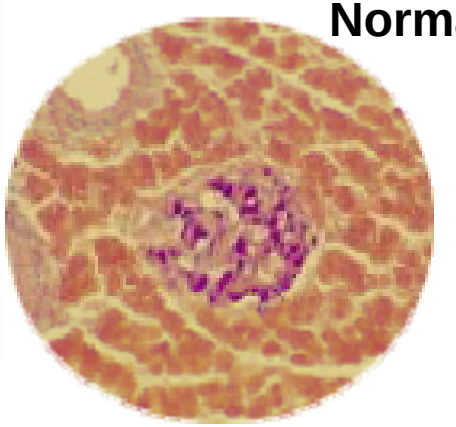
PURPOSE
of
CELLULAR REPLACEMENT THERAPY

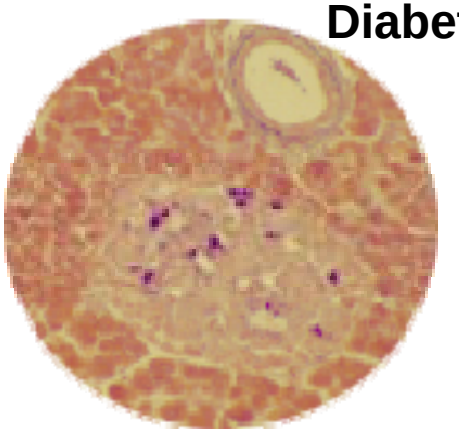
Restore or establish an insulin independent, normoglycemic state which hopefully will prevent development or progression of the secondary complications of diabetes

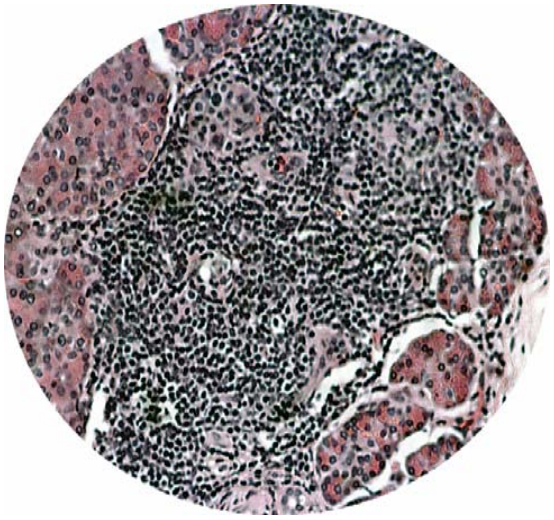
Diabetes Mellitus

Normal Pancreatic Islet



Diabetic Islet





immune system incorrectly targets β-cells
- **infiltrating T cells produce islet-toxic cytokines**
- **T cells (predominantly $CD4^+$) target autoantigens**
- **autoantibodies also produced**

β-cells destroyed leaving no insulin-production

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Cellular Replacement for Diabetes

3 basic approaches:

1. **Islet Cell Transplantation**
   replacement of insulin-producing cells with mature, functioning cells from cadaver organ donors

2. **Stem Cell Therapy to Regenerate Islet Function**
   replacement of insulin-producing cells with stem cell-derived insulin-producing cells
   a. stem cells isolated and differentiated *in vitro,* then transplanted
   b. stem cells isolated and transplanted, differentiate *in vitro*

3. **Stem Cell Therapy to Prevent Diabetes Onset**
   modification of host immune system by stem cell-derived immune modulatory cells

Islet Transplantation

The Edmonton Protocol:
A New Era of Human Islet Transplantation

The New England
Journal of Medicine

VOLUME 343 JULY 27, 2000 NUMBER 4

ISLET TRANSPLANTATION IN SEVEN PATIENTS WITH TYPE 1 DIABETES MELLITUS USING A GLUCOCORTICOID-FREE IMMUNOSUPPRESSIVE REGIMEN

A.M. JAMES SHAPIRO, M.B., B.S., JONATHAN R.T. LAKEY, PH.D., EDMOND A. RYAN, M.D., GREGORY S. KORBUTT, PH.D., ELLEN TOTH, M.D., GARTH L. WARNOCK, M.D., NORMAN M. KNETEMAN, M.D., AND RAY V. RAJOTTE, PH.D.

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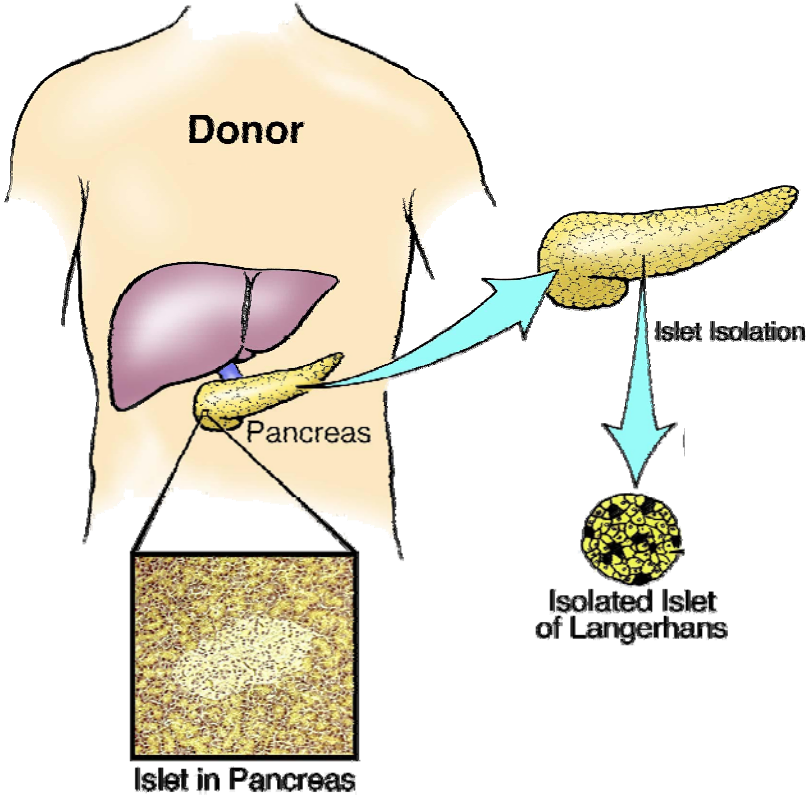
Pancreatic Islet Transplant Process



- Cadaveric pancreata procured.
- Islets isolated using automated method in approximately 6hrs.
- Islets cultured 36 to 48hrs to allow stabilization and recovery.

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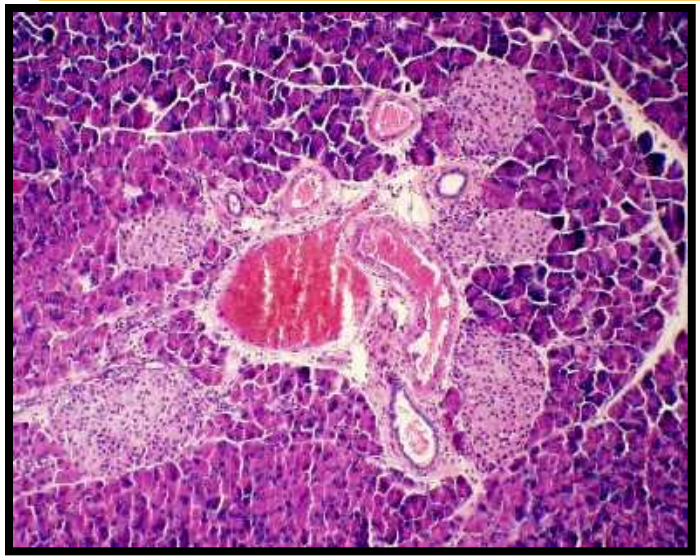
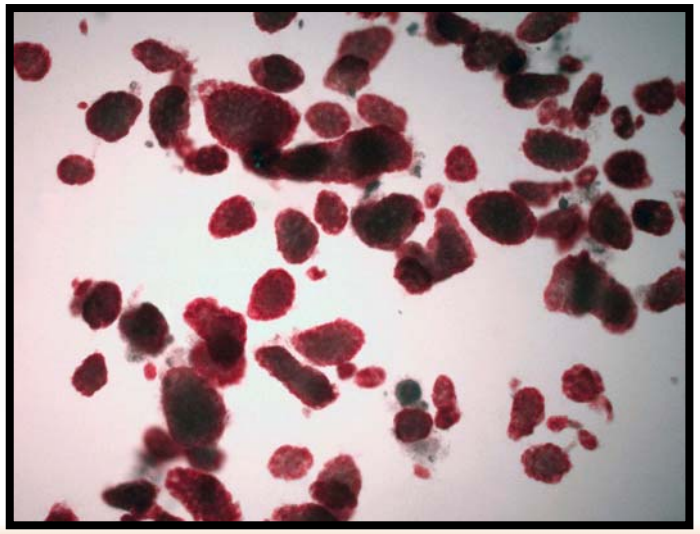
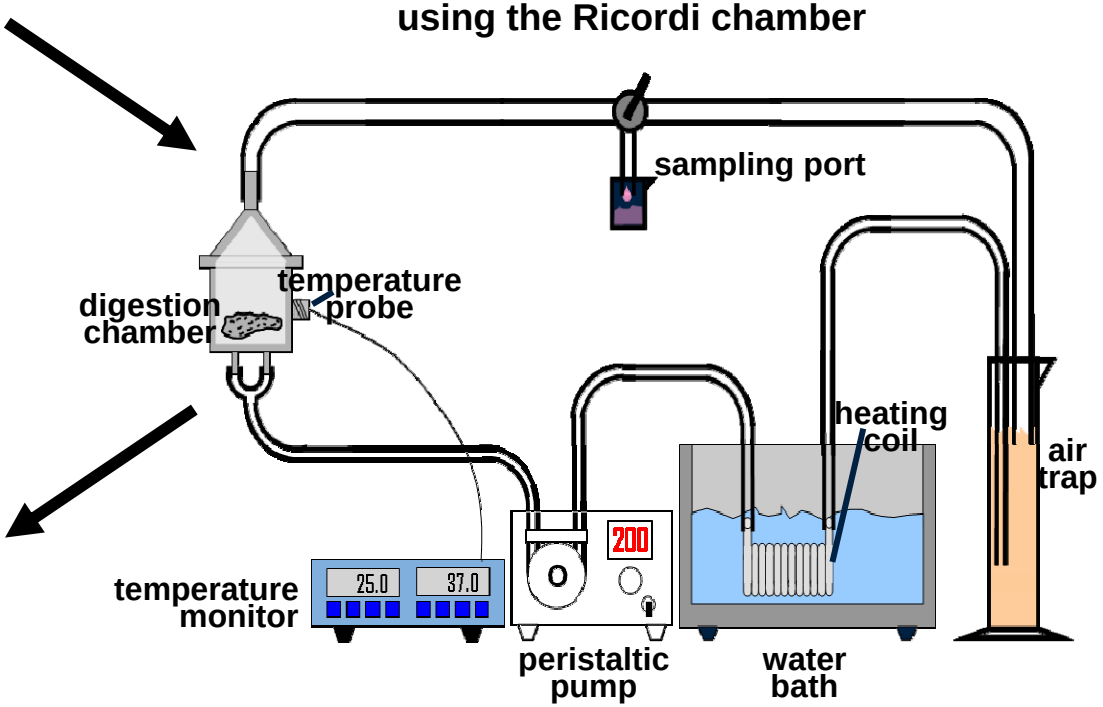
Islets of Langerhans







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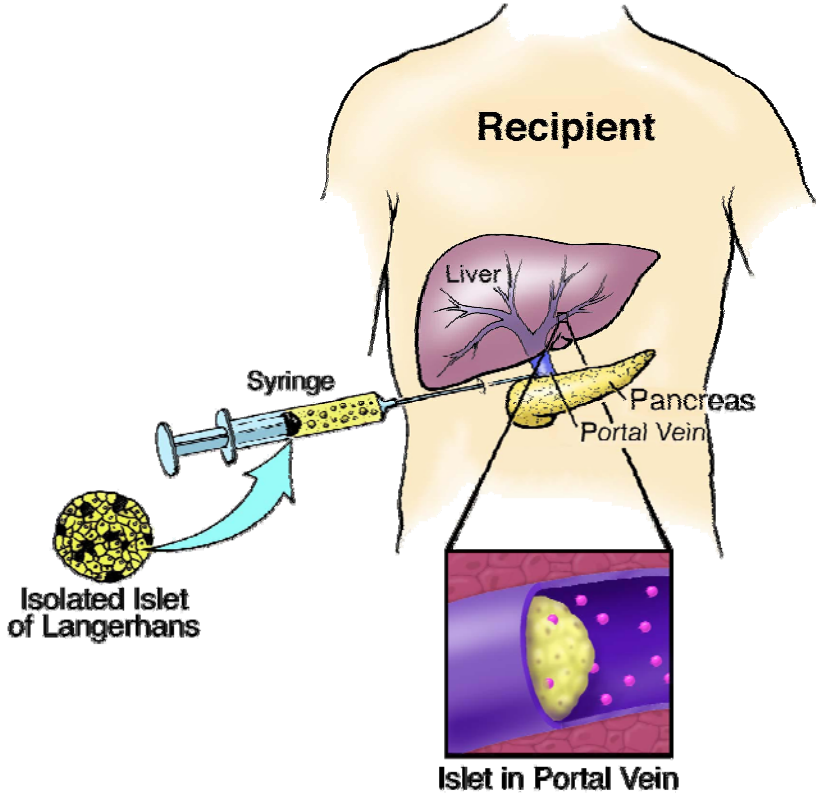

- Transplant recipient given light anesthetic.
- Interventional Radiologist determines location of portal vein of liver through fluoroscopic and ultrasound guidance.
- Catheter inserted through skin into recipient's liver and islets injected into portal vein.
  - infused over 20-6- minutes
  - heparin (35U/kg with islet infusion)
  - portal pressure monitored
- Almost immediately, pancreatic islets within host liver, begin producing insulin.
- hospital stay (2 days) to monitor patient

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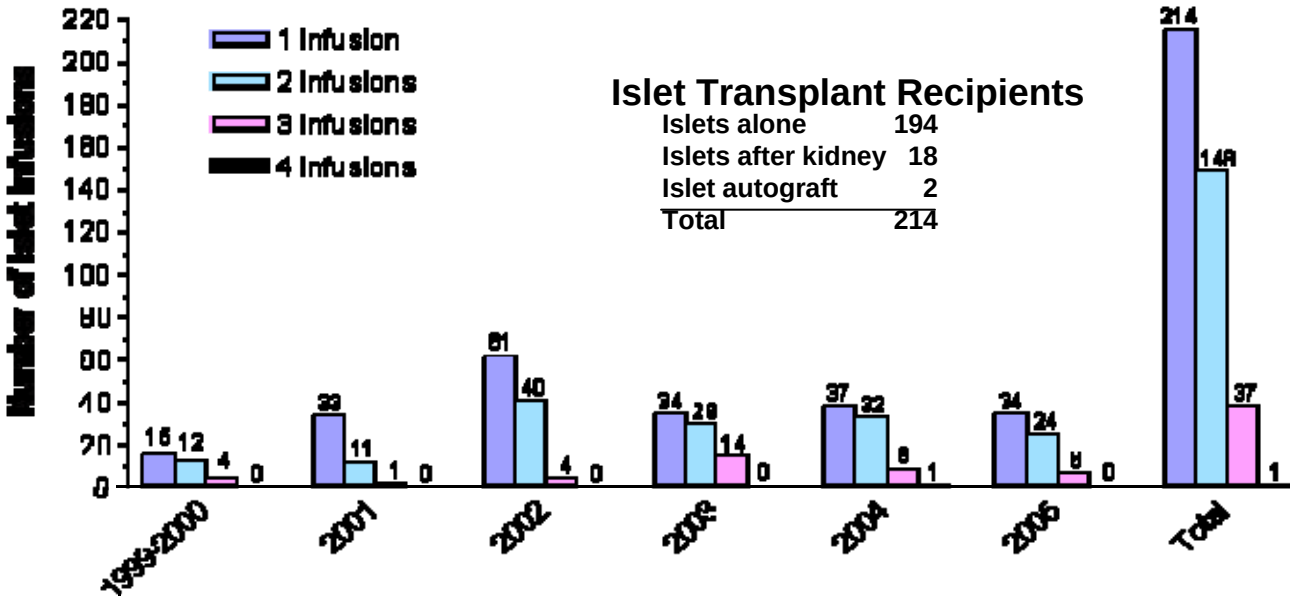
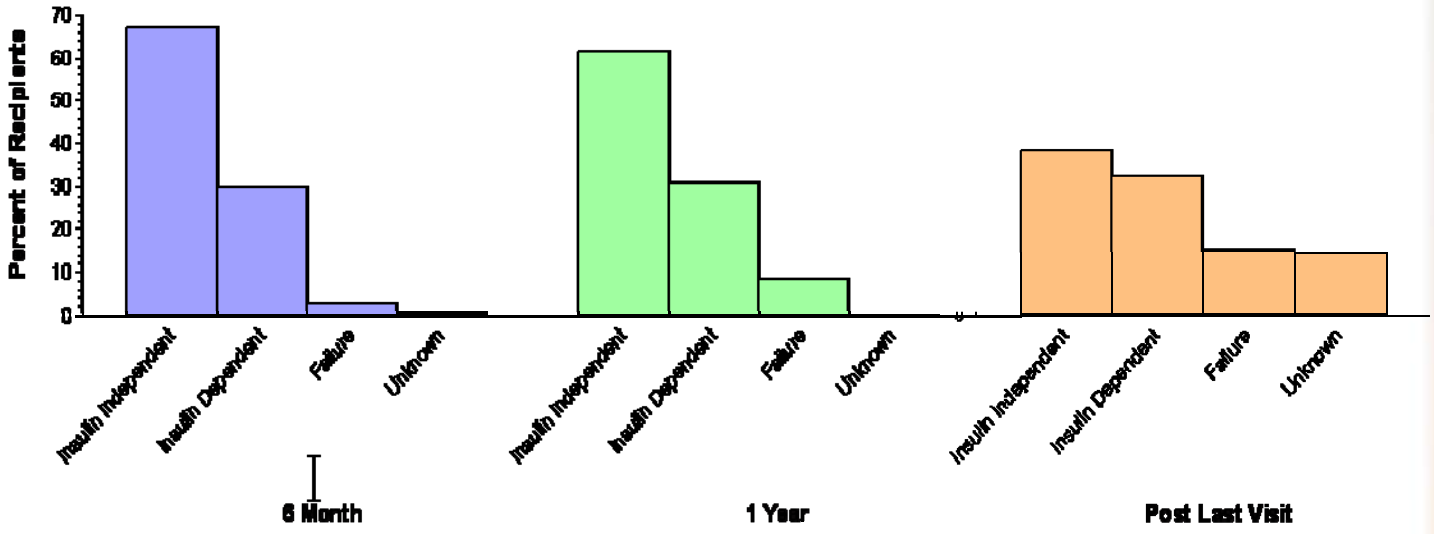




United States National Institute of Diabetes & Digestive & Kidney Diseases of the National Institutes of Health

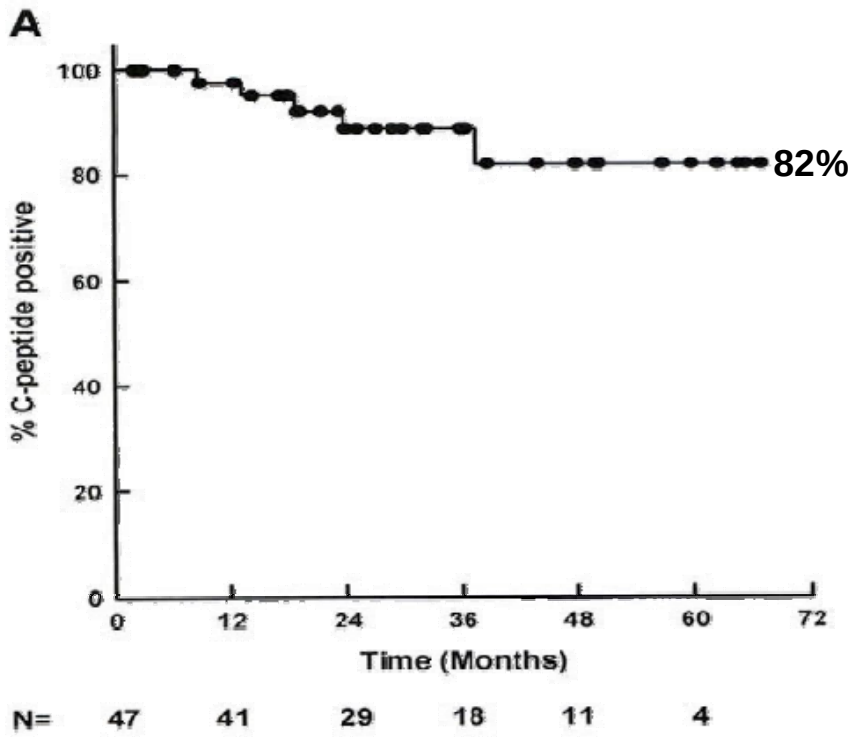
citr Collaborative Islet Transplant Registry

University of Virginia Health System

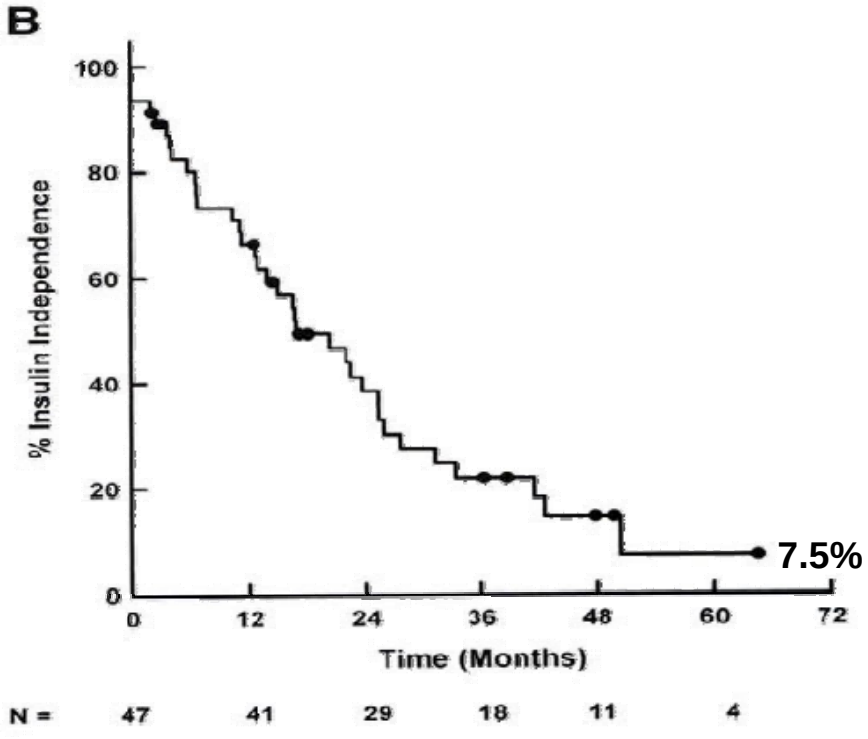
Islet Graft Function

Graft Survival vs. Insulin Independence

Graft Survival (C-peptide)



Insulin Independence



from: **Five Year Follow-Up After Clinical Islet transplantation**
Ryan, Paty, Senior, Bigam, Alfadhli, Kneteman, Lakey, and Shapiro
***Diabetes* 54:2060-2069; 2005**

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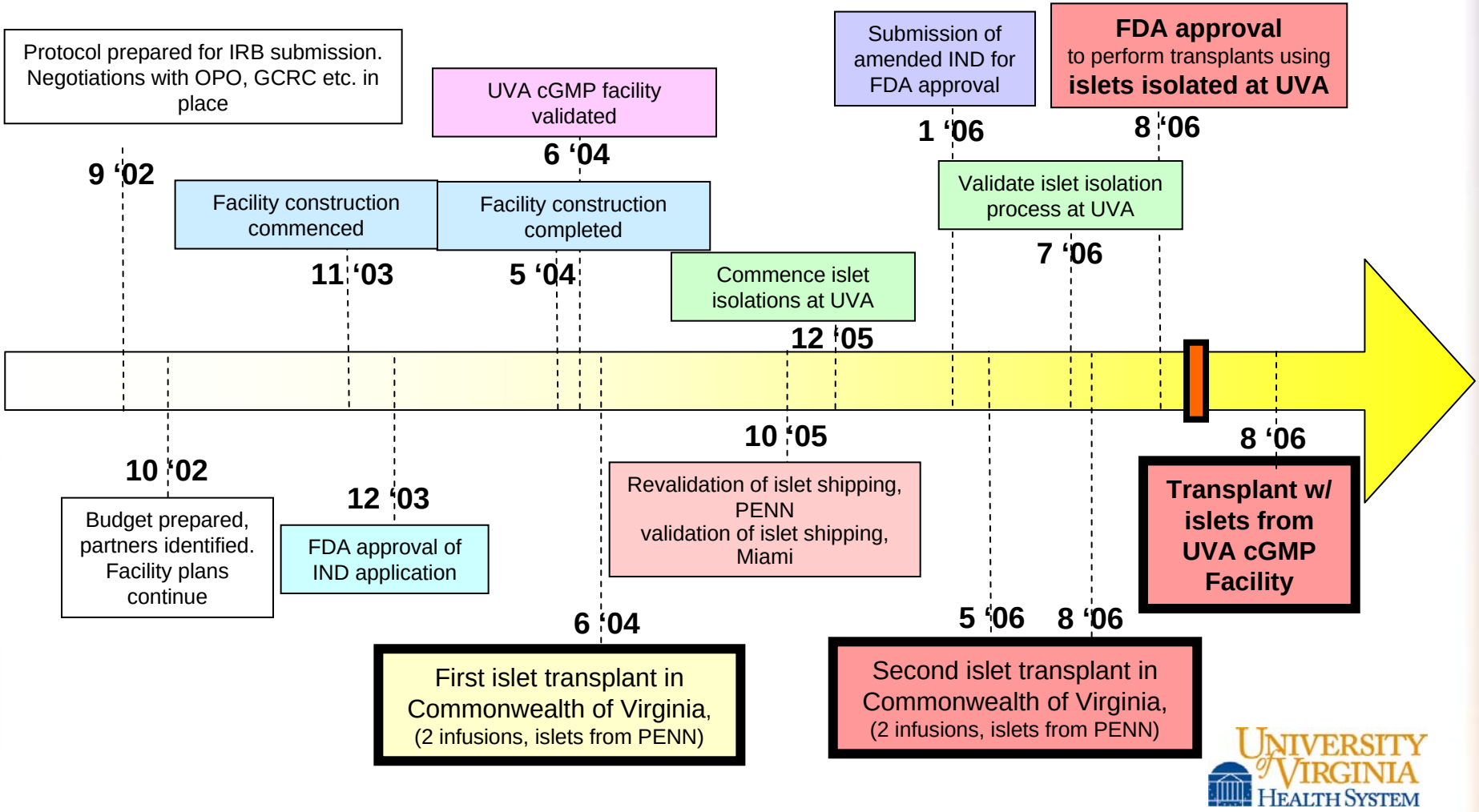
UVA Islet Transplantation Program

Progress to Date



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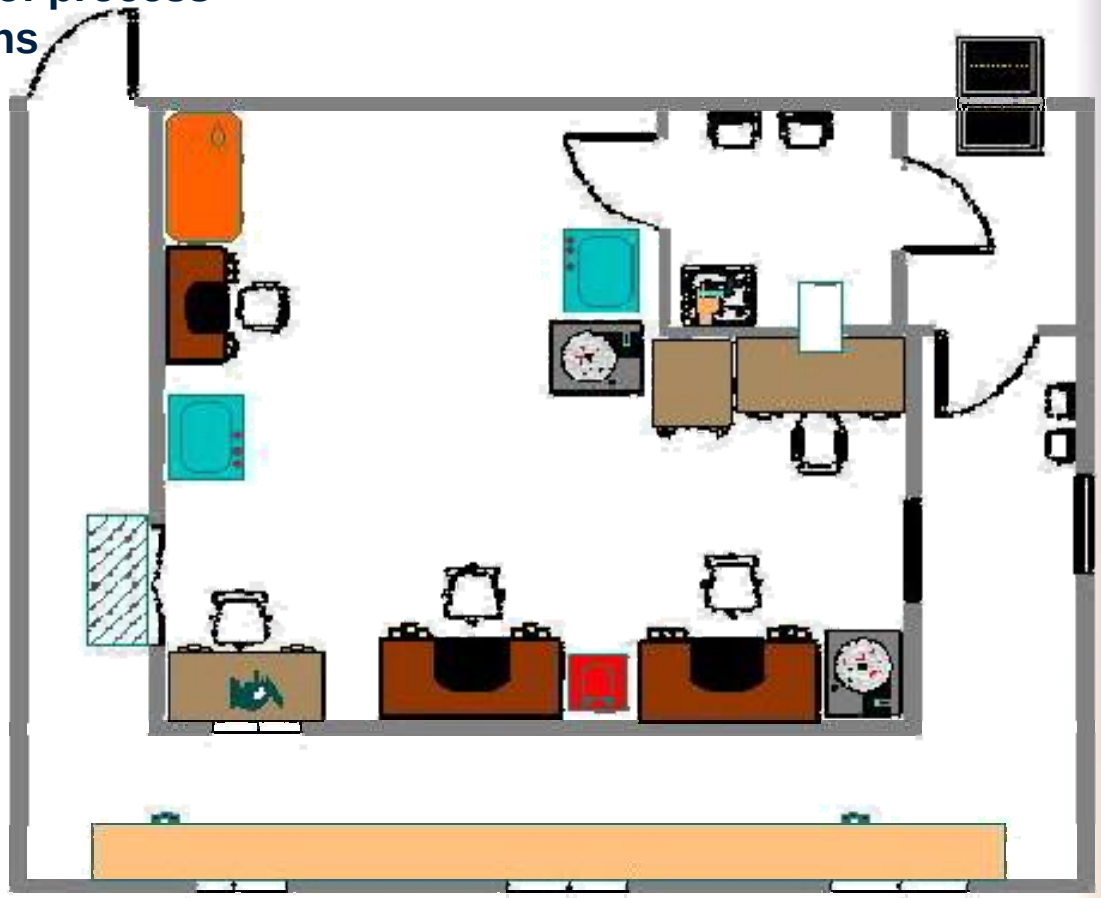
UVA cGMP Islet Isolation Facility

Layout follows process flow
Design follows movement in order of process
- **Distinct areas for distinct functions**
- **Support areas near,**
  - **but outside cleanroom**



Consists of 3 smaller rooms:
- **ante-chamber / autoclave room**
- **gowning room**
- **processing area**

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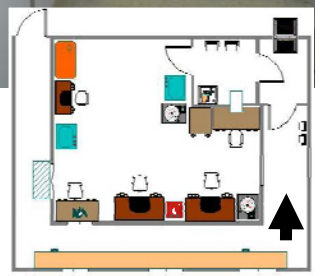
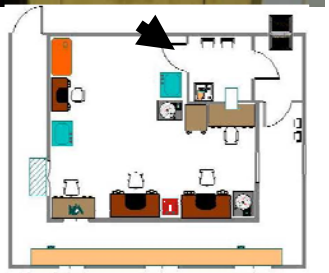
UVA cGMP Islet Isolation Facility





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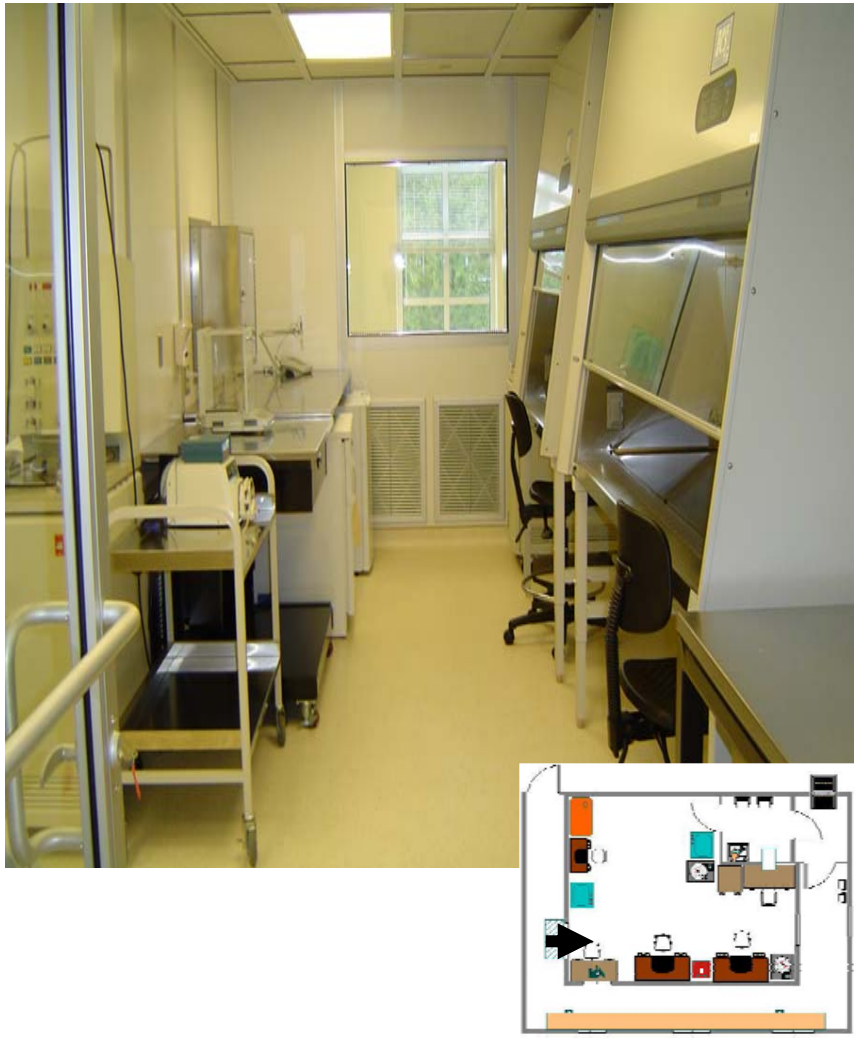
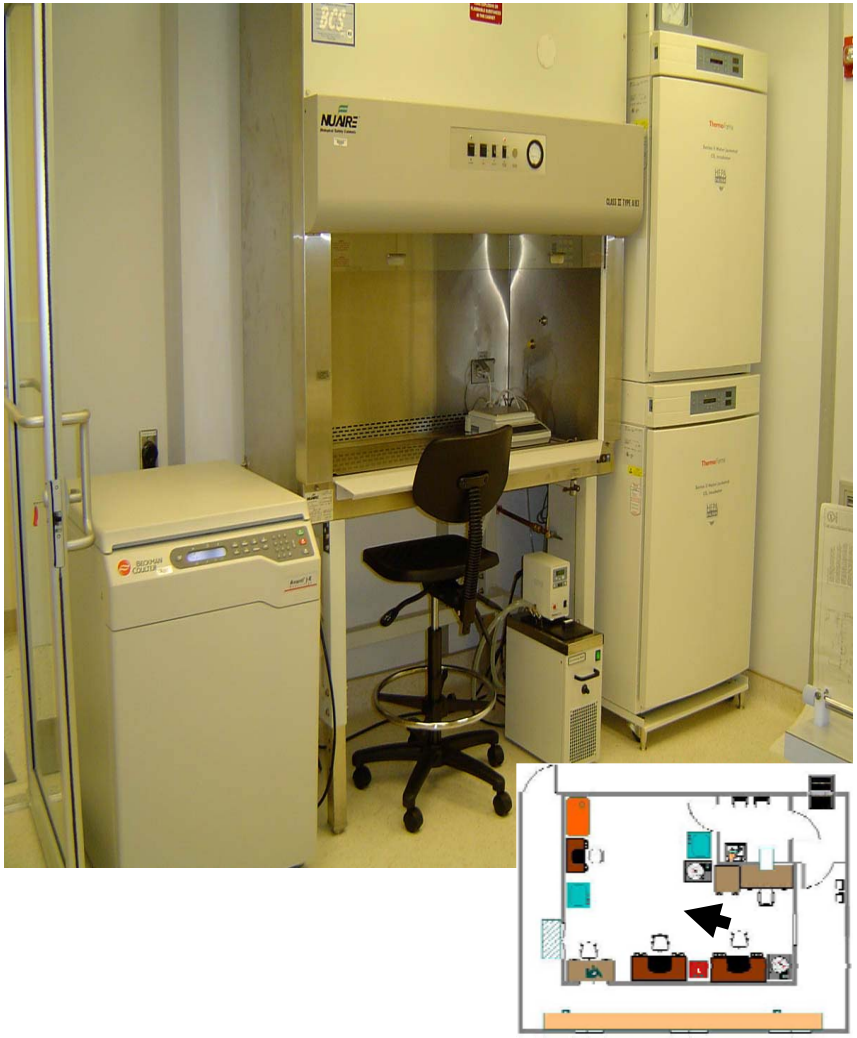
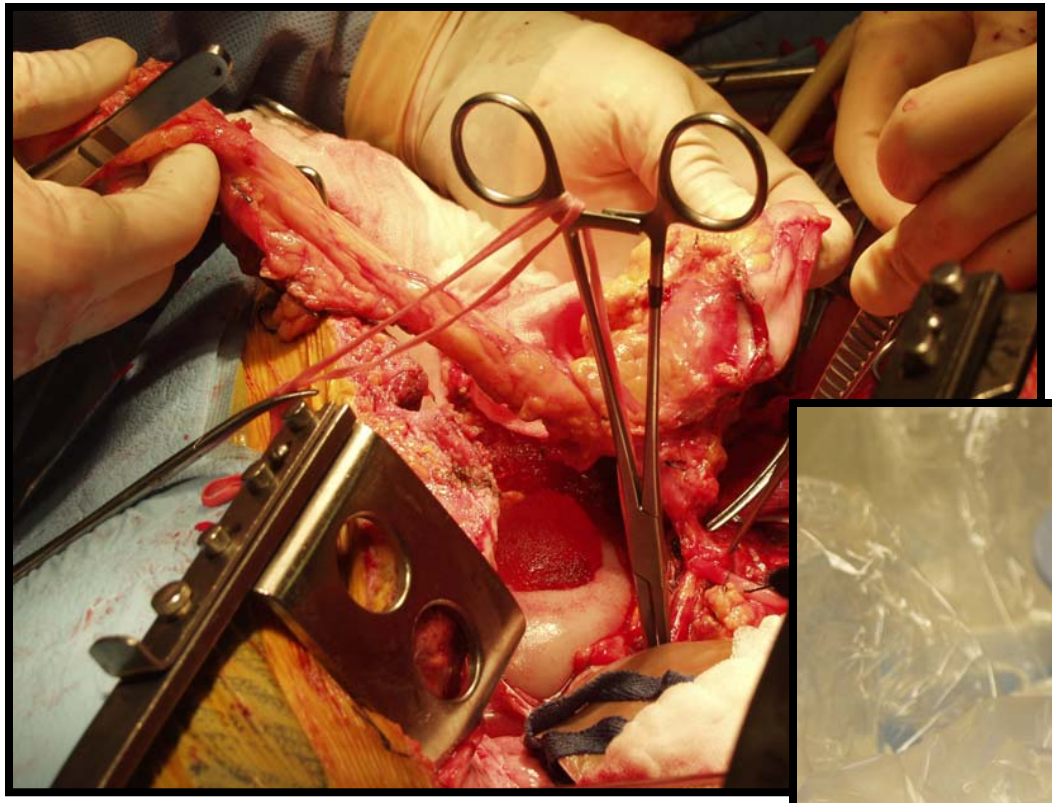
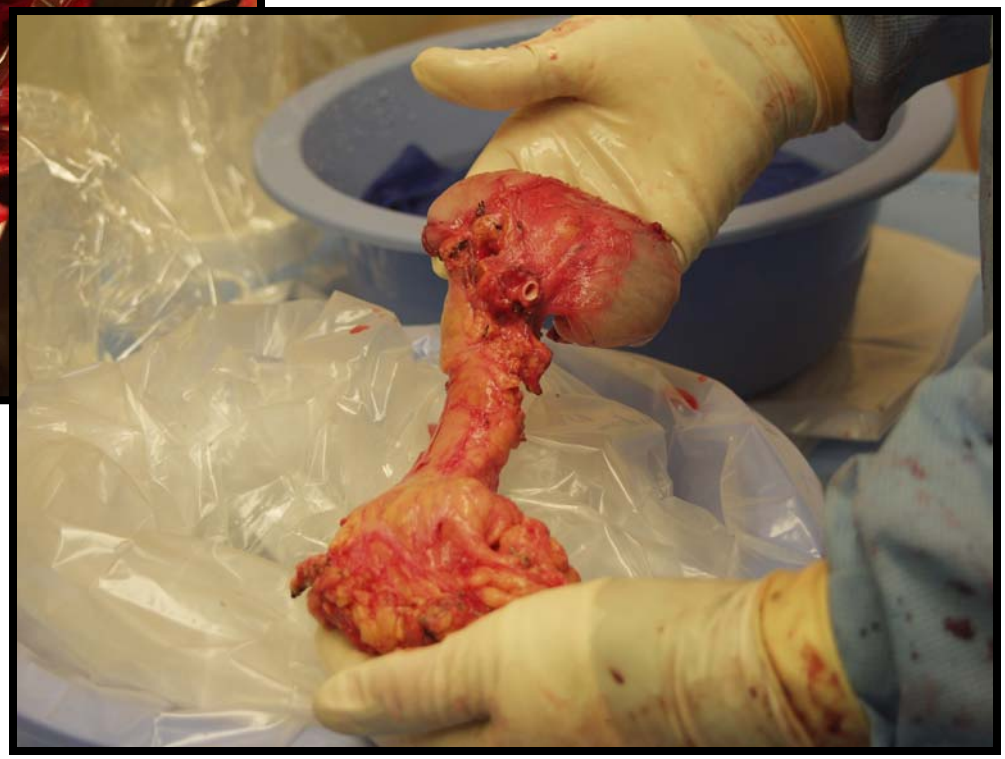
UVA cGMP Islet Isolation Facility



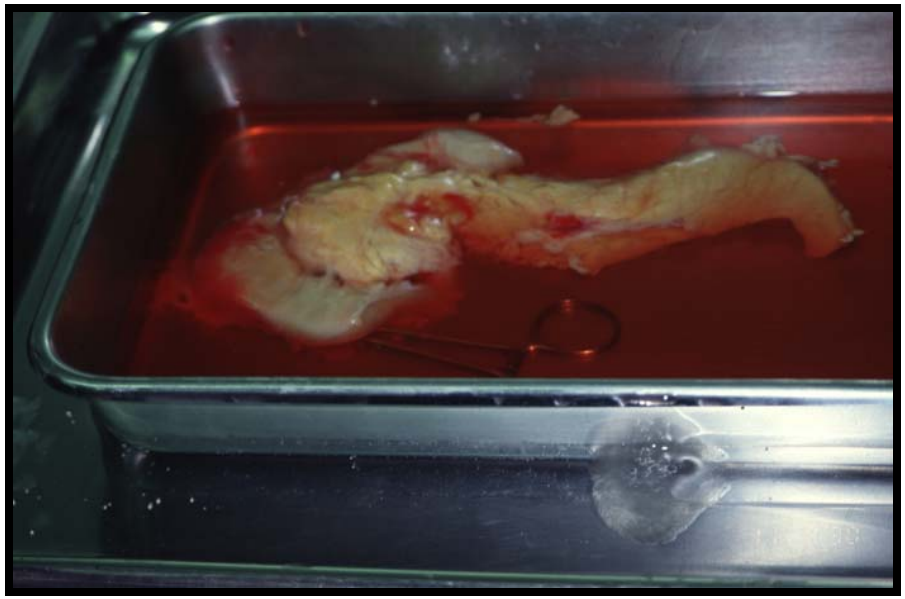


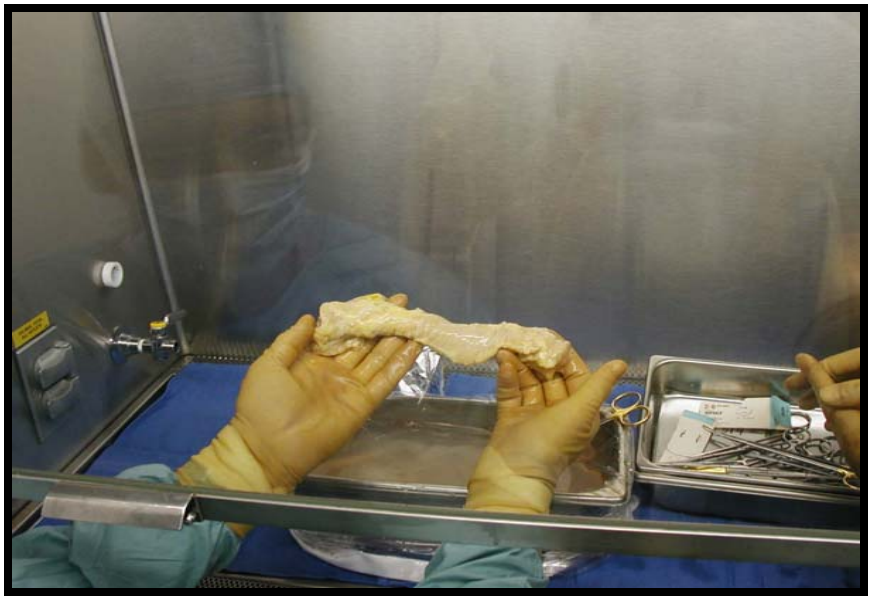
Pancreas Procurement

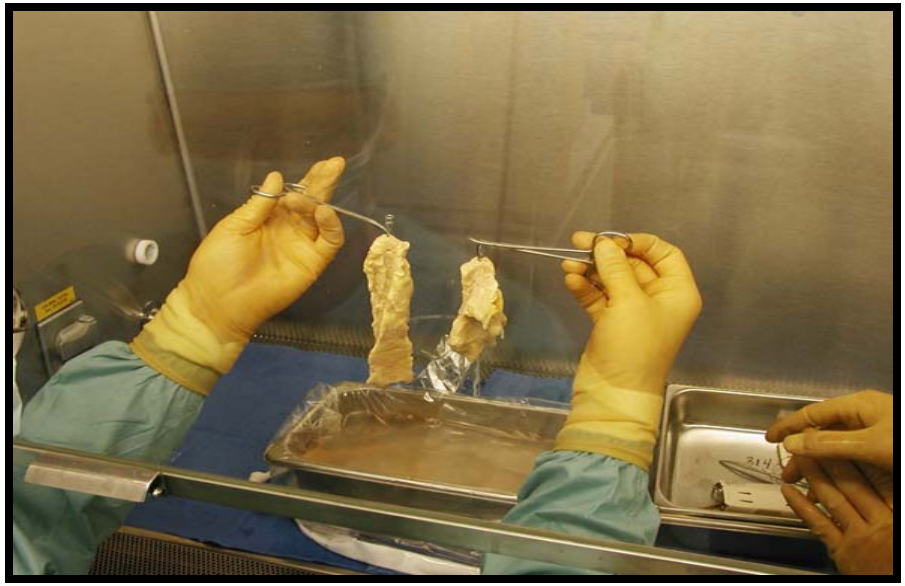


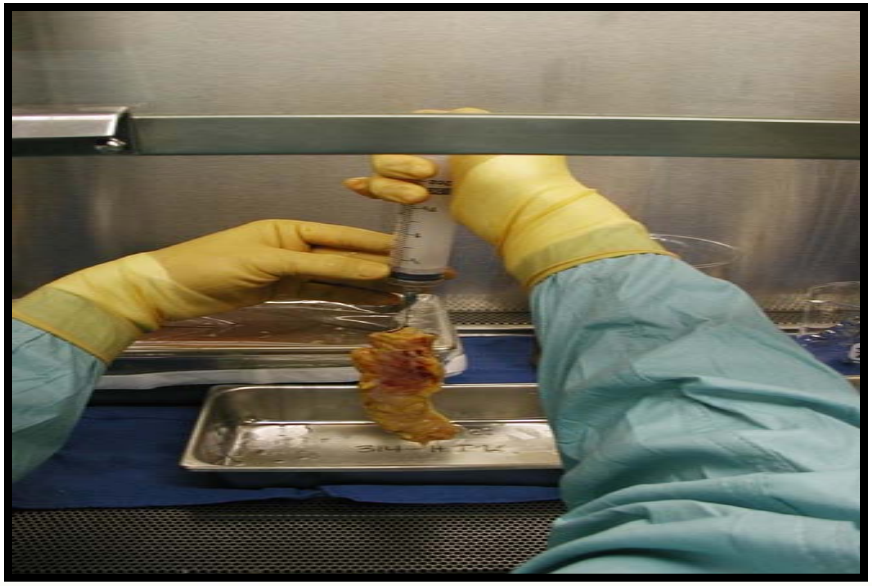


Organ Preparation

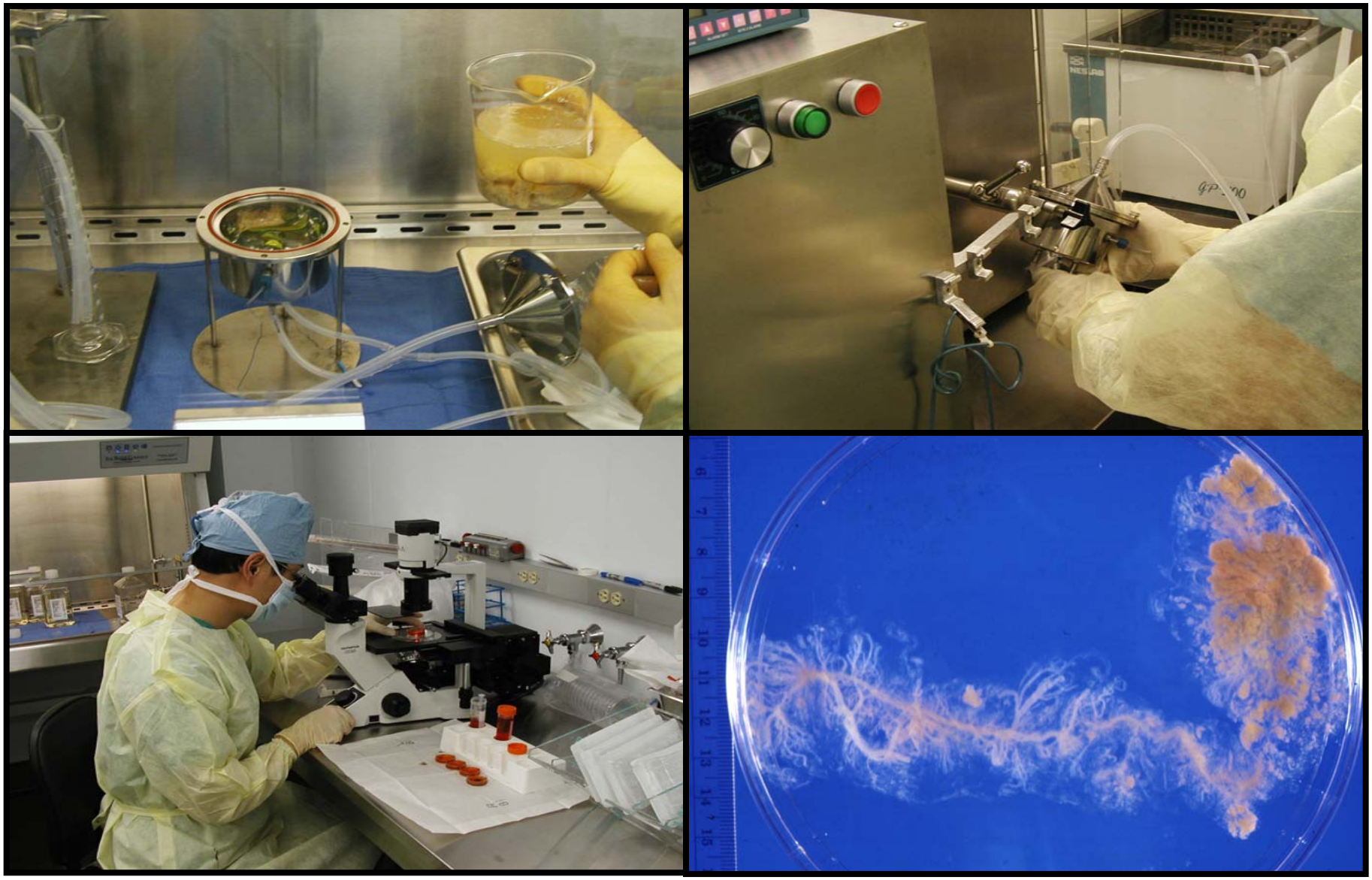




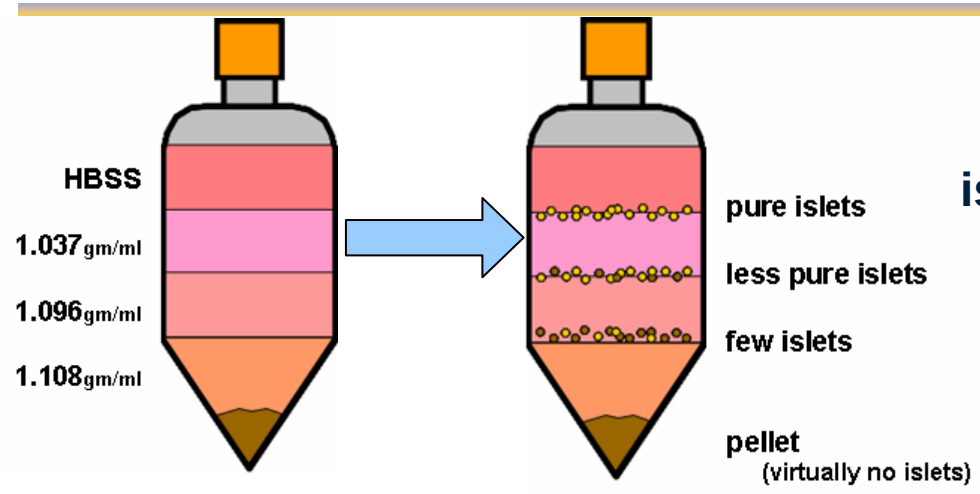




Digestion

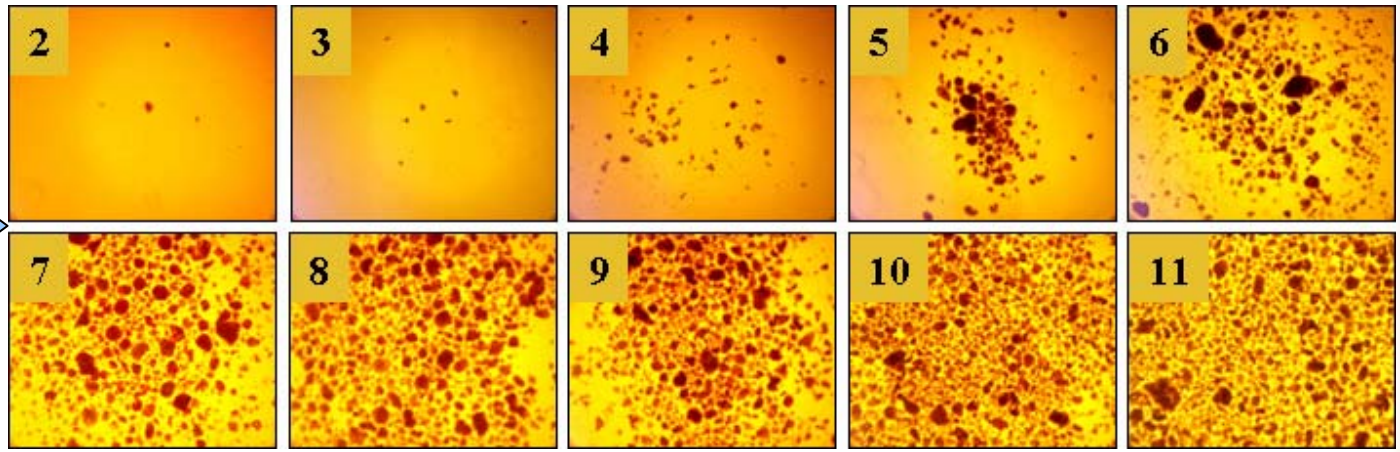


Islet Purification on Density Gradients



isolated islets are less dense than other pancreatic cells, & separate to level of their density, above the non-islet tissue.

COBE 2991 Centrifuge



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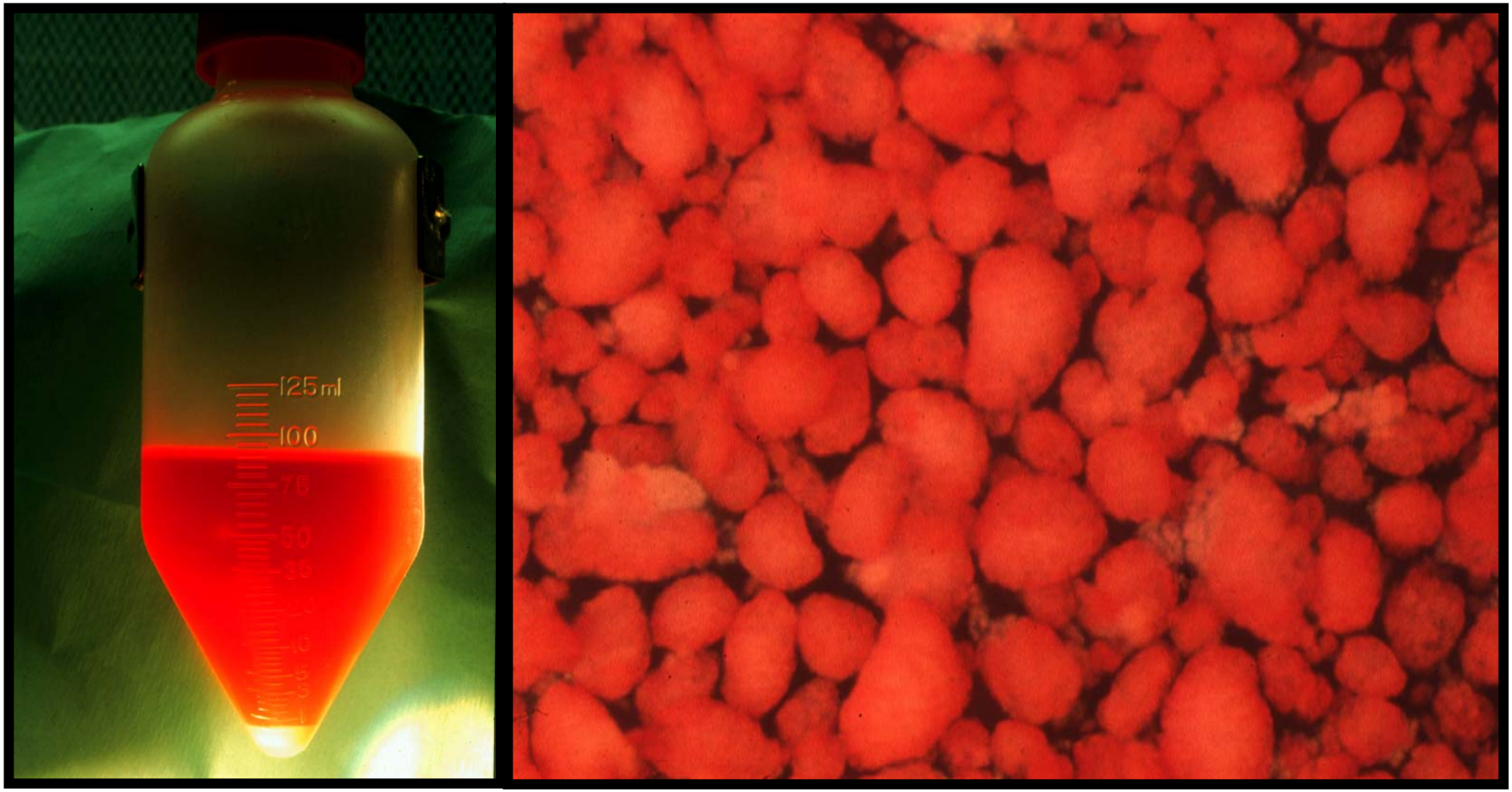
Islet Product for Transplantation



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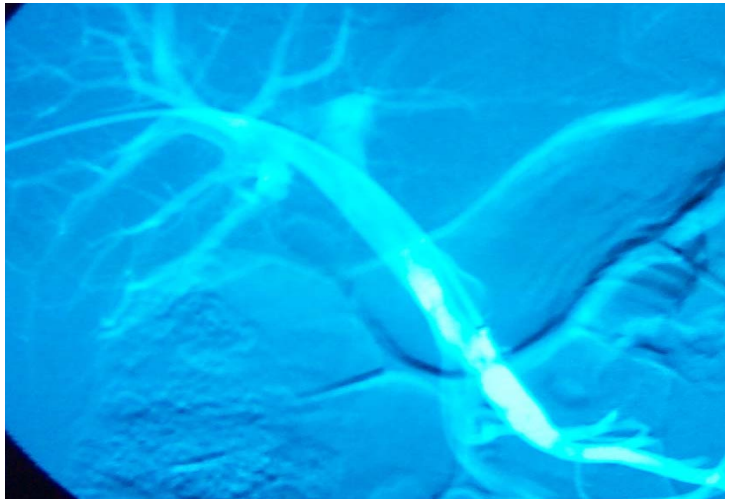

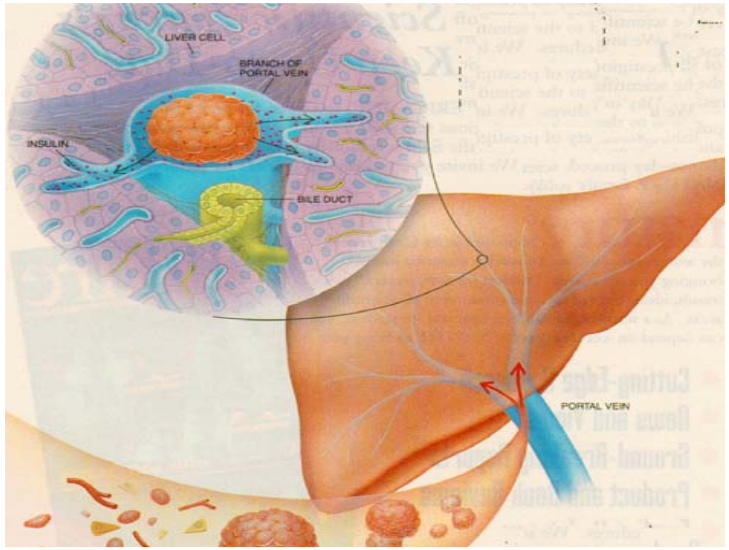

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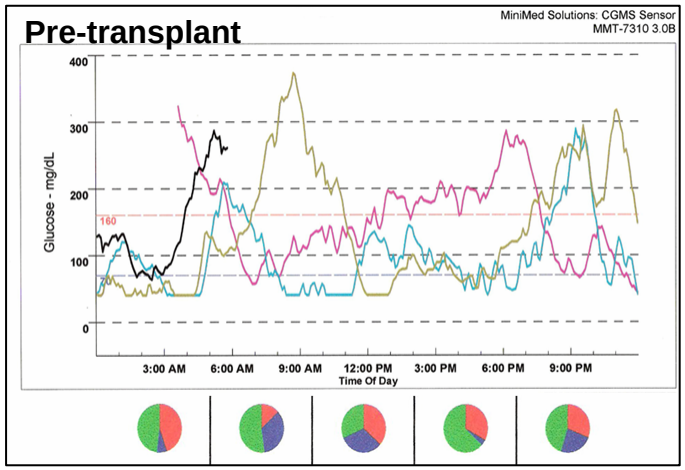
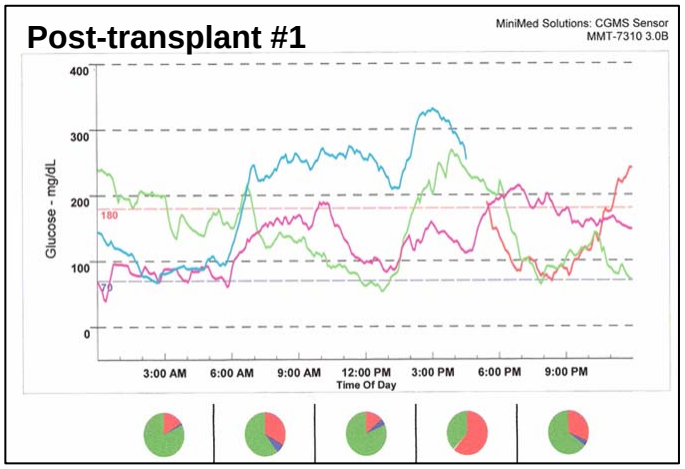
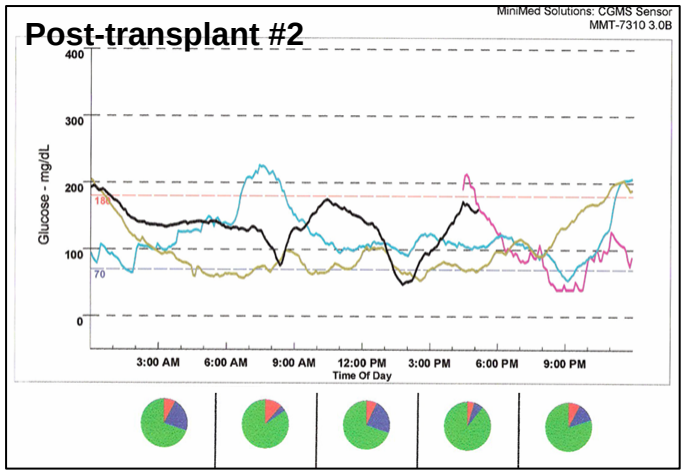
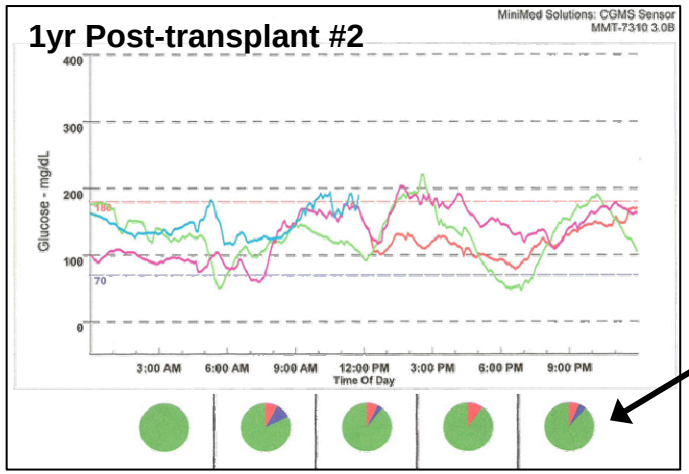
Continuous Glucose Monitoring System:

Assessment of Blood Glucose Excursions









UVA patient 1

pre-transplant

post-transplant #1 (1mo post #1)

post-transplant #2 (1mo post #2)

post-transplant (1yr post #2)

green areas represent percent time within normal BG range

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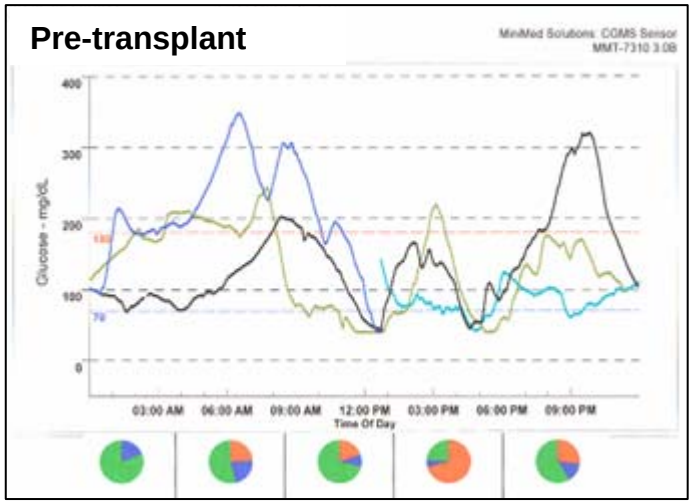
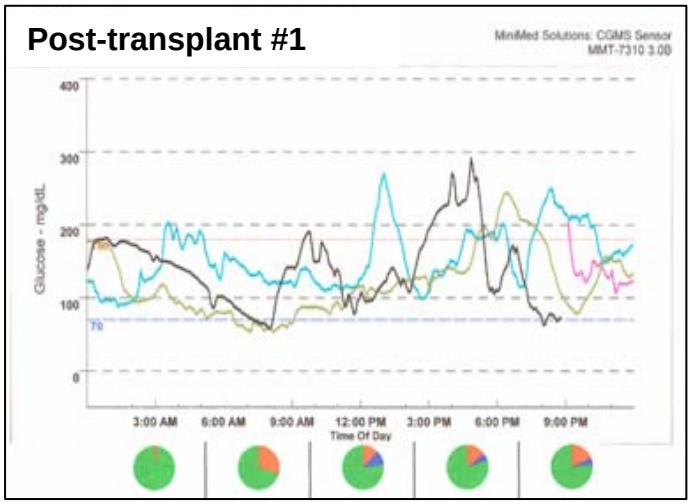


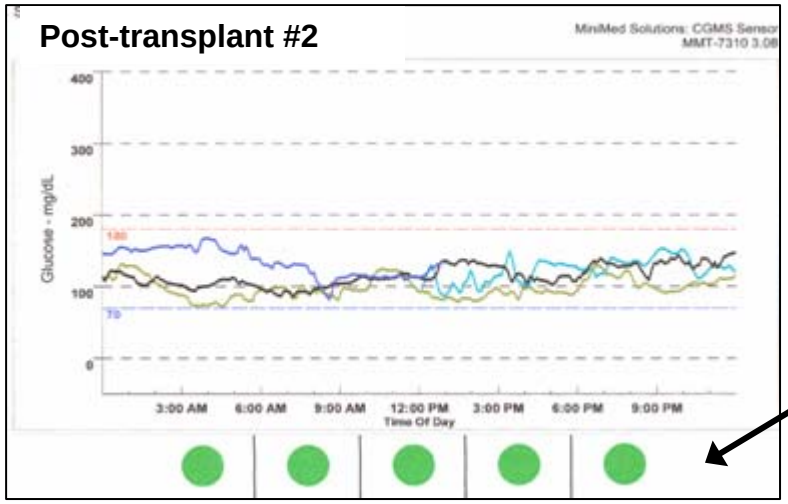


UVA patient 2

pre-transplant

post-transplant #1 (24 da post #1)

post-transplant #2 (24 da post #2)



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   modification of host immune system by stem cell-derived immune modulatory cells

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Basic definitions

stem cell
immature (not fully differentiated) cell, still capable of proliferation and differentiation into a variety of cells

pleuripotent stem cell
capable of differentiating into virtually any cell

multipotent stem cell
capable of differentiating into a variety of cells, but limited to a specific class of cell

mesenchymal stem cell (MSC)
usually refers to a stem cell derived from a bone marrow source

progenitor cell
immature cell, capable of proliferation and differentiation into a specific type or class of cells; usually refers to the immediate precursor of the final differentiated cell type

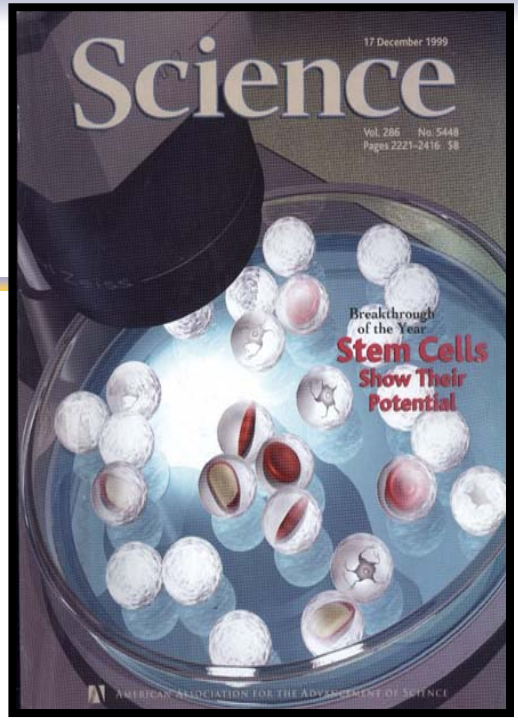
Stem Cells

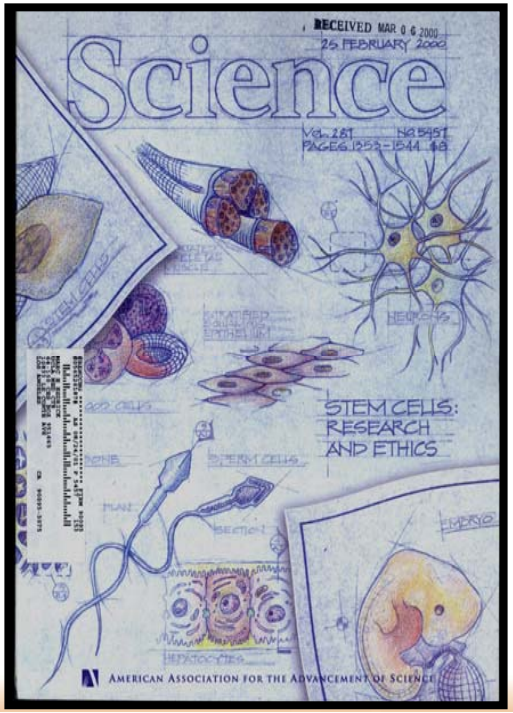
"…given the enormous promise of stem cells to the development of new therapies for the most devastating diseases,

when a <u>readily available source of stem cells</u> is identified,

it is not too unrealistic to say that this research will revolutionize the practice of medicine and improve the quality and length of life."

-NIH, May 2000





<u>Postnatal (Adult) Stem Cells</u>

- **Limited amounts/donor morbidity**
- **Limited plasticity?**
- **Multiple potential sources**
  - **umbilical cord blood**
  - **bone marrow**
  - **adipose tissue (fat)**

MSCs and ASCs:

Uncannily Similar

- **Marrow vs. Subcutaneous adipose depot**
- **Stromal cell fraction**
- **Isolation via adherence to plastic**
- **morphology**
- **Transcriptome**
- **Cell surface/CD markers (integrin alpha 4; L-selectin)**
- **Anti-inflammatory properties**
- **Angiogenic properties**
- **Homing**
- **In vitro developmental plasticity**
- **In vivo tissue repair**

but there is one major difference…

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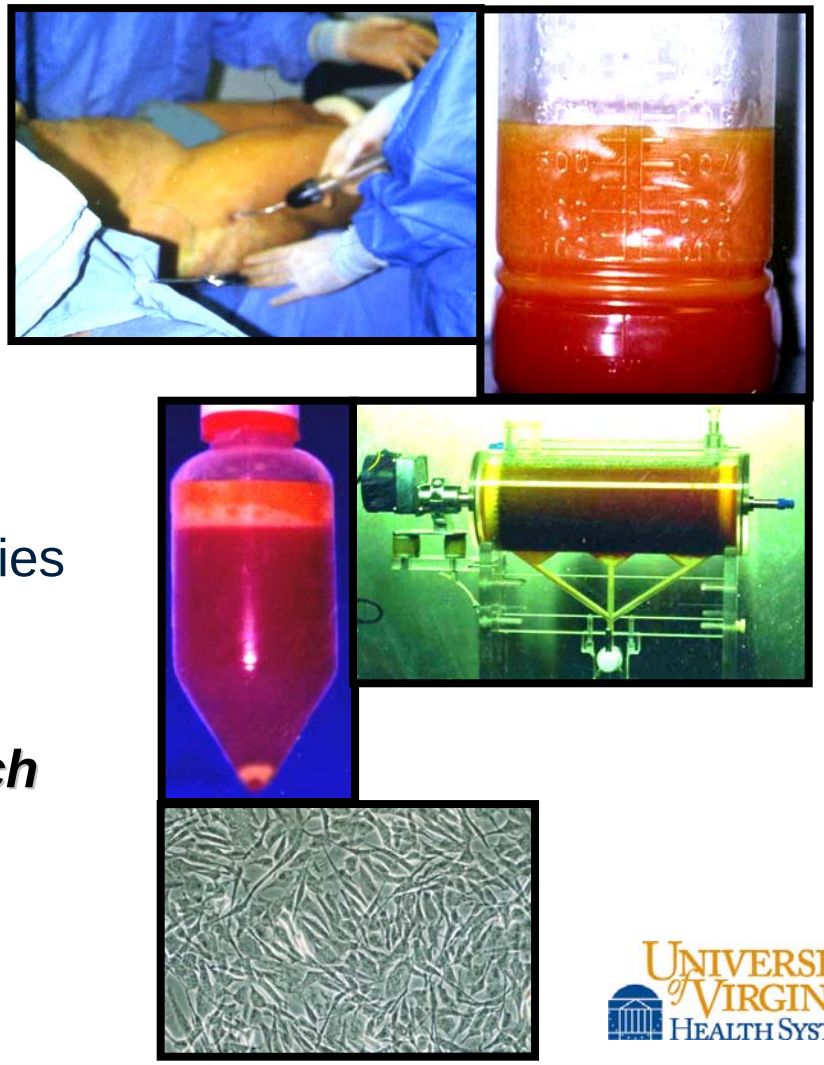
Human Fat as a Potential Stem Cell Reservoir

Human Fat is:

- abundant
- expendable
- renewable
- easy to harvest
- appealing to donor
- may permit autologous strategies

potential to translate research into clinical practice…



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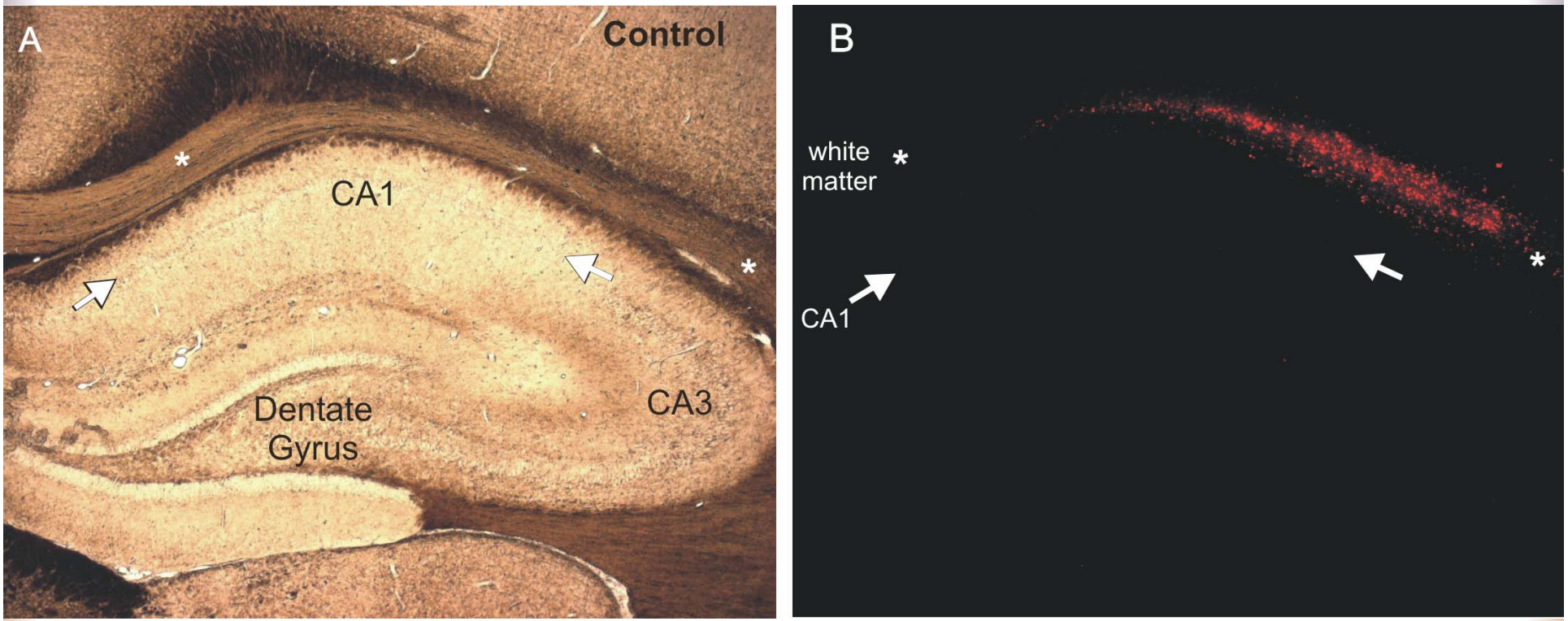
hASCs in the Rat CNS

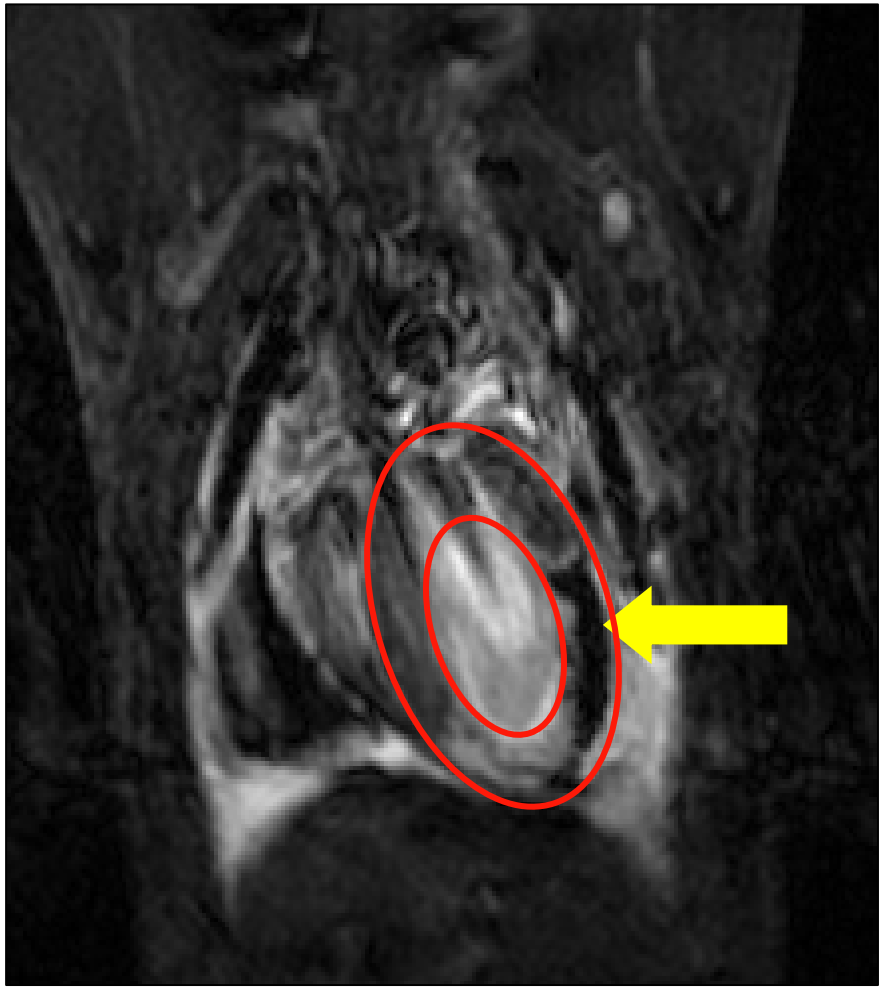


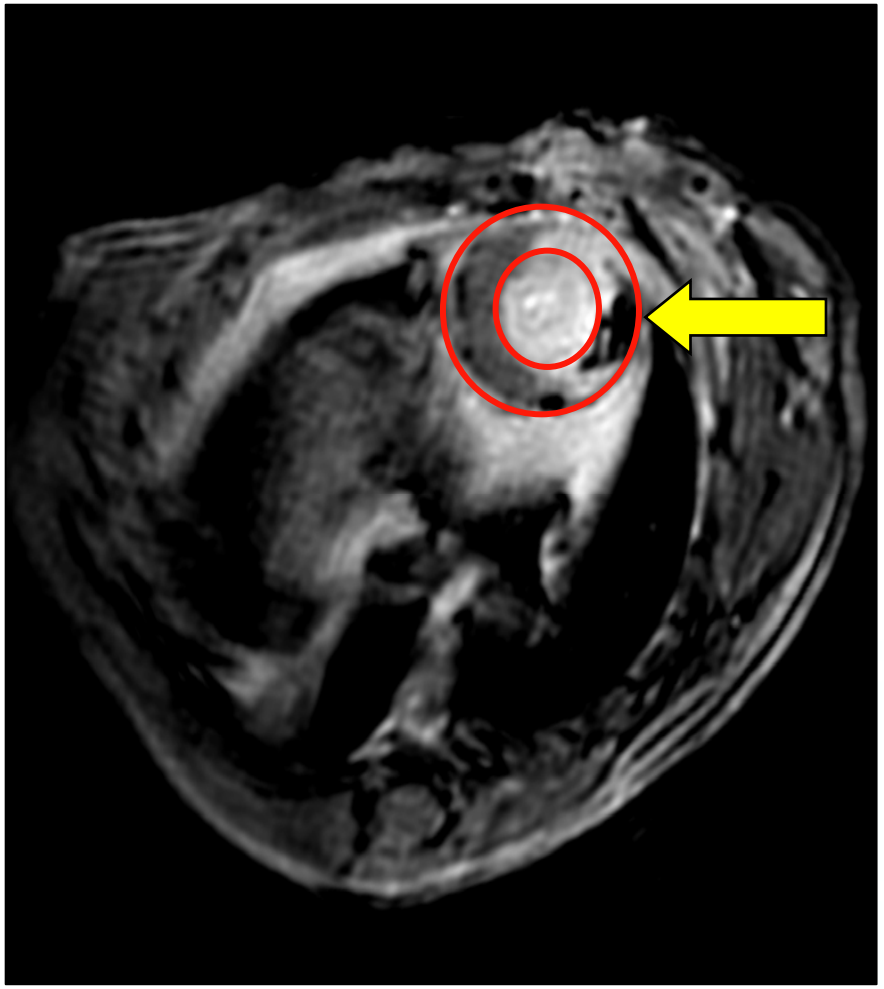
Kevin Lee, et al

University of Virginia Health System

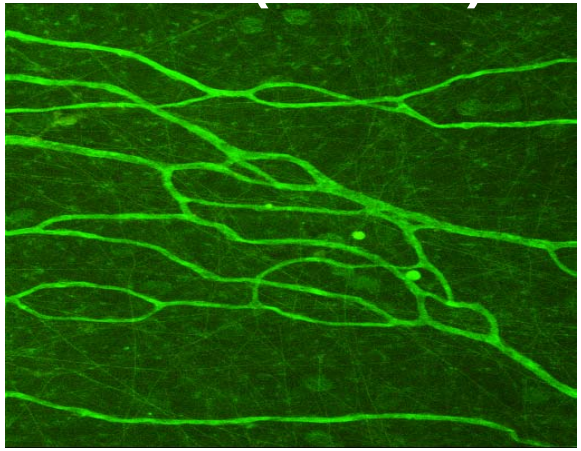
Human ASCs in the Mouse Heart

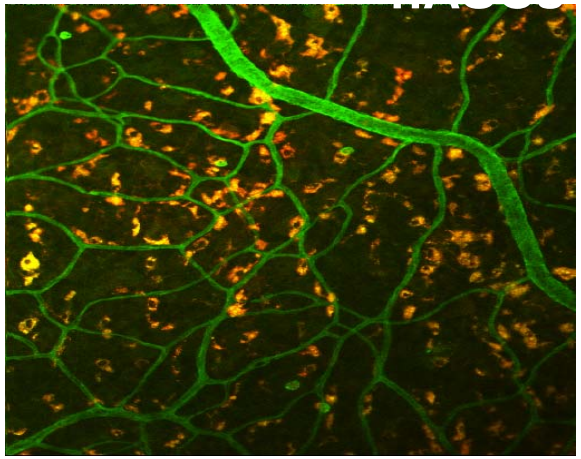
Results: Cell Distribution in Gd-Enhanced Images on Day 1 post-MI



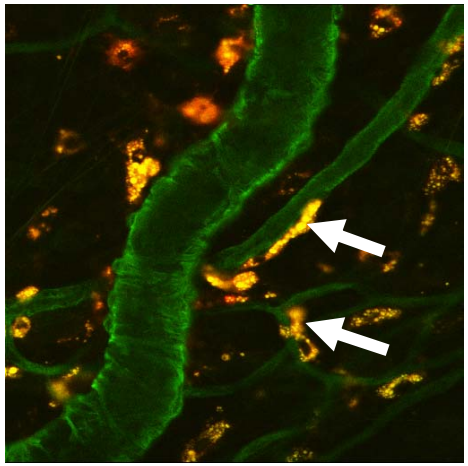


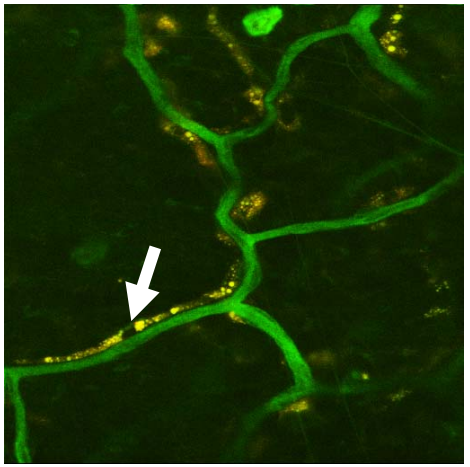
Microvascular Remodeling in the Nude rat mesentery: 60 days after hASC injection

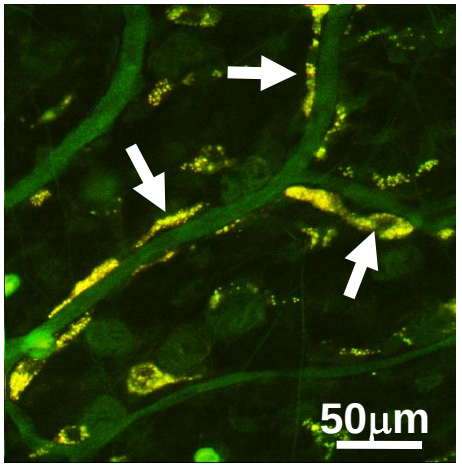




64% of hASCs associate with microvessels in a morphology similar to pericytes (elongated along vessels).







green - FITC-SMA & FITC-BSI Lectin
red - DiI-labeled hASCs

Shayn Peirce, et al.

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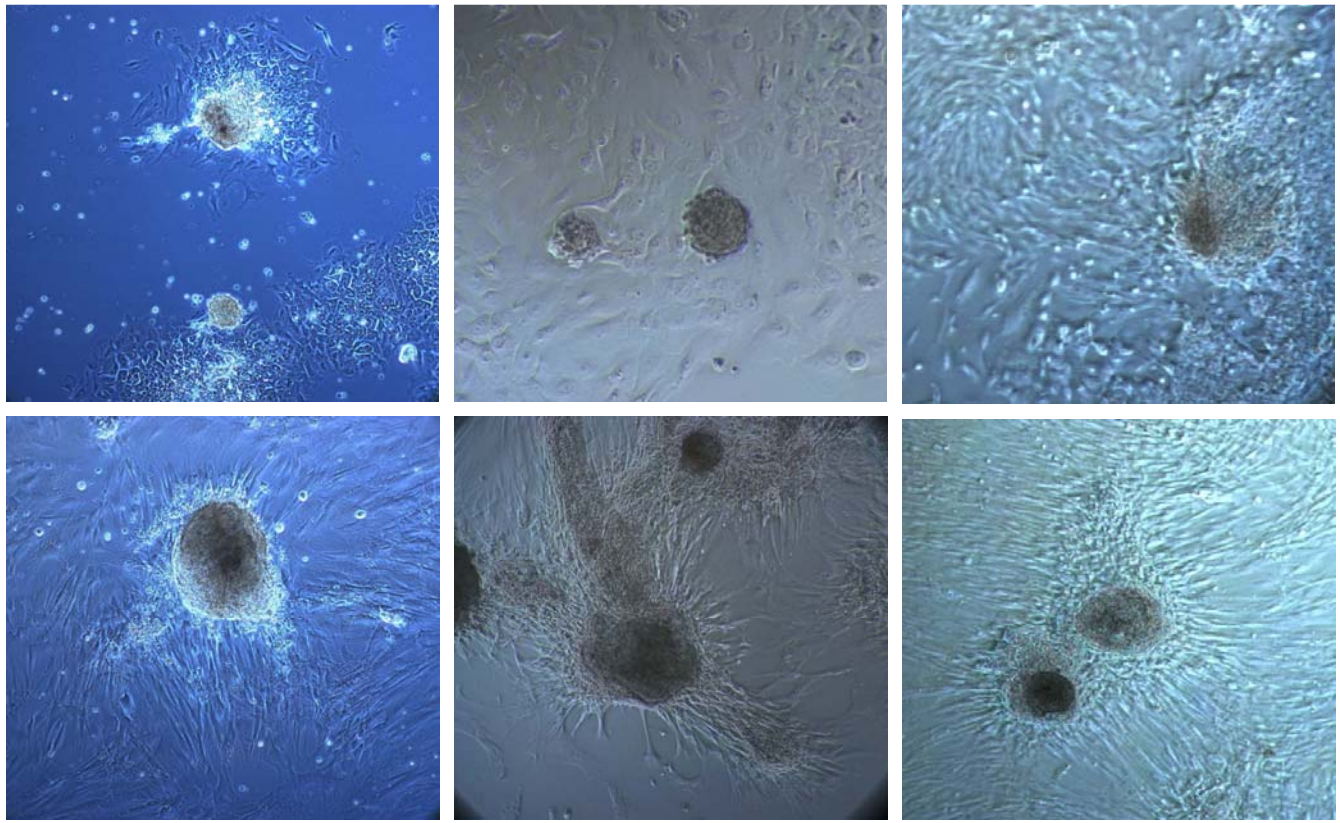
Human Islet-ASC Co-culture



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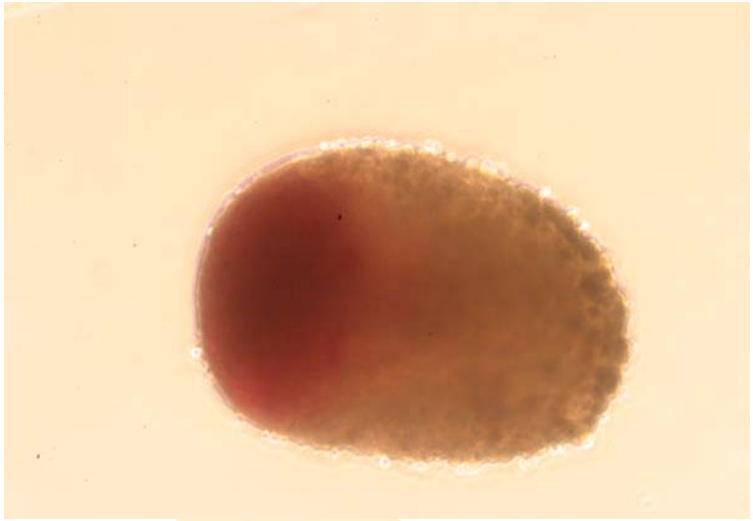
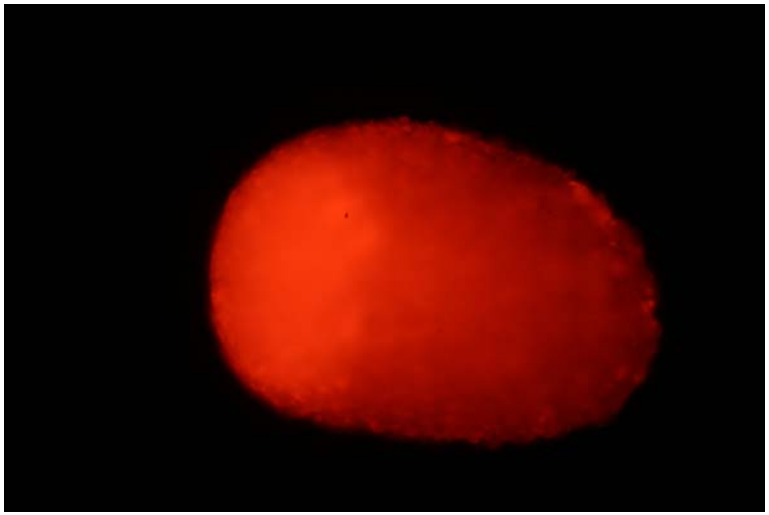
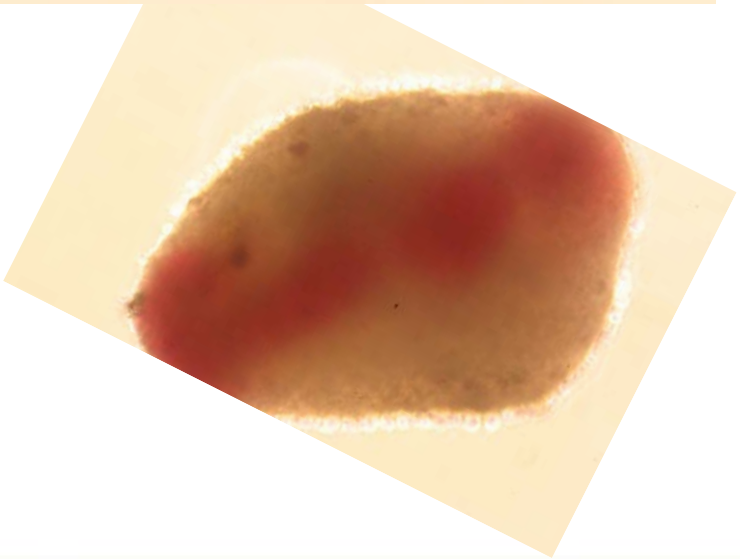
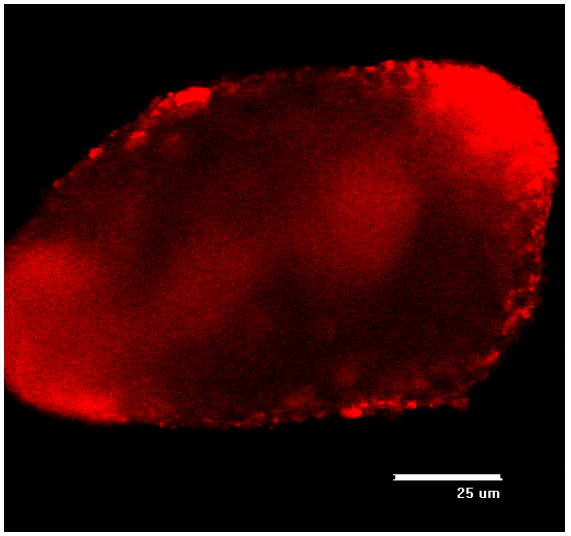
Precedence and Potential

self-assembled islet/ASC spheroids









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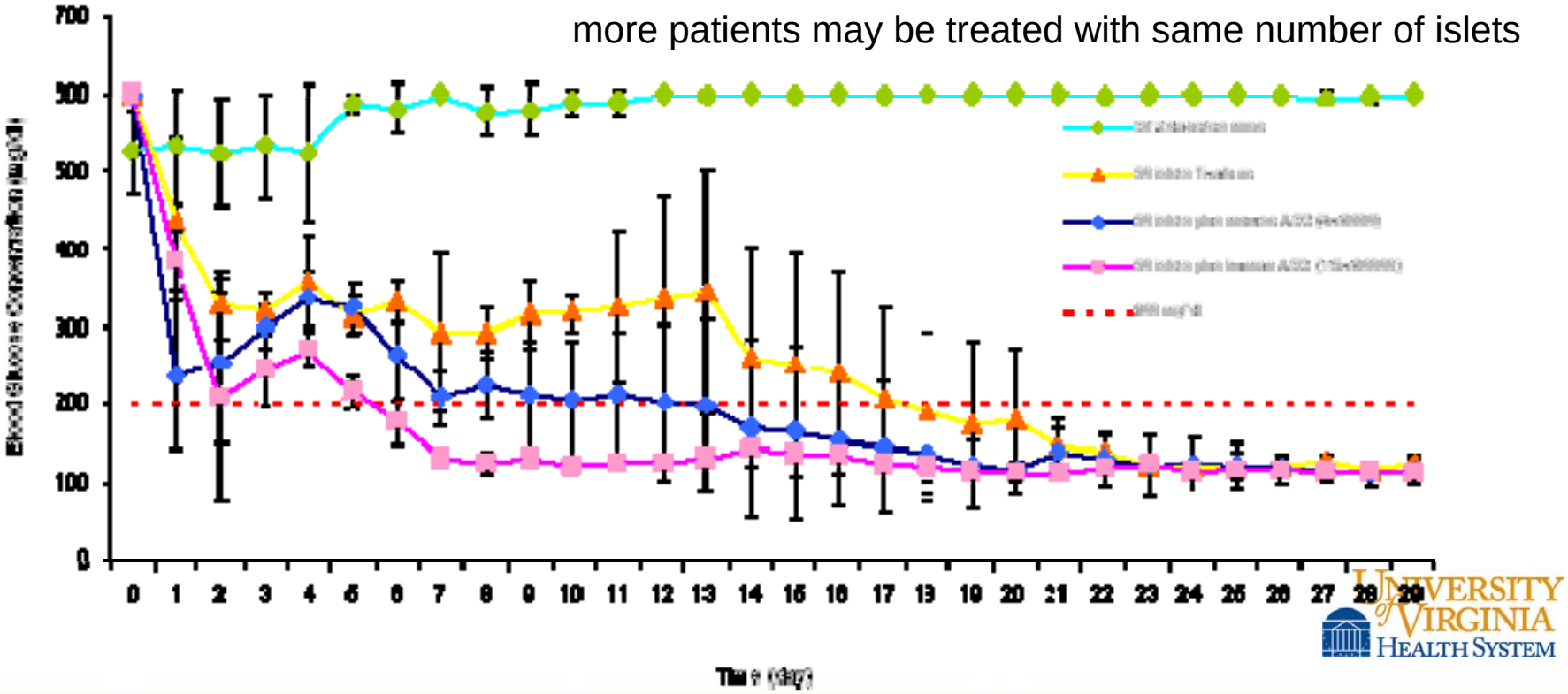
ASC / Islet Co-Transplant

Islet/ASC Co-Transplants may ameliorate transplant success

- reduce number of islets needed to achieve “cure”
- shorten time post-transplant to cure

potential impact: reduced number of islets needed for transplant

more patients may be treated with same number of islets



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ASCs and Diabetes

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Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells

Katharina Timper [a,1], Dalma Seboek [a,1], Michael Eberhardt [a], Philippe Linscheid [a], Mirjam Christ-Crain [b], Ulrich Keller [a,b], Beat Müller [a,b], Henryk Zulewski [a,b,*]

[a] *Department of Research, University Hospital, Basel, Switzerland*
[b] *Division of Endocrinology, Diabetes and Clinical Nutrition, University Hospital, Basel, Switzerland*

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Abstract

Mesenchymal stem cells (MSC) from mouse bone marrow were shown to adopt a pancreatic endocrine phenotype in vitro and to reverse diabetes in an animal model. MSC from human bone marrow and adipose tissue represent very similar cell populations with comparable phenotypes. Adipose tissue is abundant and easily accessible and could thus also harbor cells with the potential to differentiate in insulin producing cells. We isolated human adipose tissue-derived MSC from four healthy donors. During the proliferation period, the cells expressed the stem cell markers nestin, ABCG2, SCF, Thy-1 as well as the pancreatic endocrine transcription factor Isl-1. The cells were induced to differentiate into a pancreatic endocrine phenotype by defined culture conditions within 3 days. Using quantitative PCR a down-regulation of ABCG2 and up-regulation of pancreatic developmental transcription factors Isl-1, Ipf-1, and Ngn3 were observed together with induction of the islet hormones insulin, glucagon, and somatostatin.

Keywords: Mesenchymal stem cells; Isl-1; Human; Adipose tissue; Nestin; ABCG2; Differentiation; Insulin; Glucagon

Mesenchymal stem cells have been initially described as clonal, plastic adherent cells from bone marrow [1] capable of differentiating into adipocytes, chondrocytes, and osteo-

secreting cells in vitro and to reverse hyperglycemia in an animal model of diabetes [16]. Similarly, mesenchymal CD45-negative precursor cells from mouse spleen were able

adipose-derived adult stem cells can be driven to differentiate into insulin-producing cells *in vitro*

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ASCs and Diabetes

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DOI 10.1007/s00534-005-0983-2

Journal of HBP Surgery

Regenerative medicine of the pancreatic β cells

Satoko Yamada and Itaru Kojima

Institute for Molecular and Cellular Regulation, Gunma University, Maebashi 371-8512, Japan

Abstract
Diabetes mellitus is a metabolic disorder that affects millions of people. The number of patients suffering from diabetes continues to increase all over the world. Both type 1 and type 2 diabetes result from an inadequate mass of functioning β cells. To achieve the ultimate goal of curing diabetes in the future, the mechanism of the regenerative process of the adult human pancreas must be elucidated. In this review, we first summarize the regenerative processes of the pancreas observed in animal models in vivo, and approaches to promote the regeneration of the pancreas in vivo. Next we consider other new approaches, such as stem cell research and cell-based therapy, for the cure of diabetes in the future. Based on the innovative success of the Edmonton protocol, islet transplantation has been considered to be a new therapeutic option for the treatment of diabetes. However, a serious shortage of donor pancreata is a critical problem. We suggest that the following issues should be solved in order to realize cell-based therapy. The first is to establish a source of stem/progenitor cells that will multiply easily in vitro and maintain their property as progenitor cells. The probable use of adult stem cells will circumvent potential ethical problems, and autotransplantation will become possible. The most difficult and as yet unsolved issue is how to differentiate these cells and acquire fully functional islets. Further investigations to understand the regenerative process of the adult pancreas and the appropriate induction of stem cell differentiation will help to establish cell-based therapy in diabetes.

levels can be controlled to some extent by multiple injections of insulin or by oral hypoglycemic agents, but the ideal glycemic control has not yet been perfectly achieved by these conventional treatments. Most of all, type 1 diabetes is a chronic metabolic disorder in which pancreatic islet β-cells are irreversibly destroyed by autoimmunity. In these patients, an almost complete loss of functional islet β cells leads to a long-lasting, absolute deficiency of insulin secretion. They are suffering from unstable glycemic control, and incomplete compensation for glucose homeostasis leads to irreversible diabetic complications. Frequent, recurrent hypoglycemia unawareness is extremely dangerous and can be fatal. The real cure for type 1 diabetes is the replacement of pancreatic β cells. In this regard, the surgical treatment of diabetes, i.e., successful pancreas transplantation, has the possibility to cure diabetes. In a recent report, the worldwide, 3-year organ survival rate for simultaneous kidney and pancreas transplantation had improved to approximately 70%–80%.[1] These results were obtained in a highly selected group of type 1 diabetic patients who had severe difficulties in achieving glycemic control. Nowadays, the American Diabetes Association recommends pancreas transplantation for patients with unacceptably poor metabolic control and quality of life despite optimum medical treatment. An innovative success for pancreatic islet transplantation (the Edmonton

Table 1. Potential adult stem cells that differentiate to insulin-producing cells

Cell source	Animals	Reference
Pancreatic stem cells		
Ductal stem cells	m, h, p	49, 50, 51, 52, 56
Intraislet stem cells	h, r, canine	54, 55, 56
Acinar cells	r	59
Liver stem cells		
Oval cells	r	65
Liver-epithelial cells	r	66
Small hepatocytes	r	68
Intestinal epithelial cells	m	70
Bone marrow-derived cells	m, h, r	76, 77, 81, 82
Duct cells of the salivary gland	r, m	83, 84
Amniotic epithelial cells	h	85

m, mouse; h, human; r, rat

University of Virginia Health System

ASCs and Diabetes

STEM CELLS®
Original Article

Adult Pancreas Generates Multipotent Stem Cells and Pancreatic and Nonpancreatic Progeny

YONG CHOI, MALANCHA TA, FOUAD ATOUF, NADYA LUMELSKY

National Institute of Diabetes and Digestive and Kidney Diseases,
National Institutes of Health, Bethesda, Maryland, USA

Key Words. Stem cells • Pancreatic islet • Differentiation • Multipotent stem cells • Diabetes

ABSTRACT

Strategies designed to produce functional cells from stem cells or from mature cells hold great promise for treatment of different cell-degenerative diseases. Type 1 and type 2 diabetes are examples of such diseases. Although different in origin, both involve inadequate cell mass of insulin-producing β cells, the most abundant cell type of pancreatic islets of Langerhans. Practical realization of such strategies is highly dependent on the elucidation of physiological mechanisms responsible for generation of new β cells in the pancreas, which at this time are poorly defined. The in vitro differentiation systems allowing generation of new β cells provide a valuable experimental tool for studying these mechanisms. Few such systems are currently available. In this work, we present an in vitro differentiation system, derived from adult mouse pancreas, capable of generating insulin-producing β-like cells, which self-organize into islet-like cell clusters (ILCCs) during the course of the culture. Surprisingly, we found that along with the ILCCs, multiple cell types with phenotypic characteristics of embryonic central nervous system and neural crest are also generated. Moreover, several embryonic stem cell–specific genes are induced during the course of these cultures. These results suggest that the adult pancreas may contain cells competent to give rise to new endocrine and neural cells. *Stem Cells 2004;22:1070–1084*

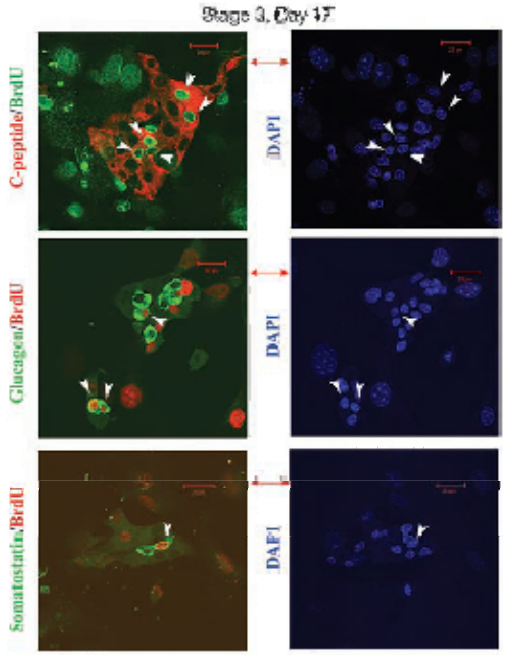

Figure 2. New hormone cells are generated in NEF cultures. Each row shows split images of the same microscopic field. Left, immunostaining for the markers, as indicated at right; nuclear DAPI staining. (A): Immunocytochemical analysis of C-peptide/BrdU 24-hour pulse experiment. Several C-peptide+/BrdU+ cells and their corresponding nuclei on the left are marked with arrowheads. Scale bar = 20 μm (Zeiss Axiovert 2 Plus). (B): BrdU pulse chase analysis of endocrine hormone-producing cells. Top, general scheme of BrdU pulse chase analysis. Immunocytochemical analysis of hormone+/BrdU+ cells. Several hormone+/BrdU+ cells and their corresponding nuclei on the right are marked with arrowheads (Zeiss LSM510). Scale bar = 20 μm. Abbreviations: BrdU, bromodeoxyuridine; NEF, islet-enriched fraction; IHC, immunohistochemistry.

INTRODUCTION

Insulin injections alleviate hyperglycemia in most patients with diabetes. However, they do not provide dynamic control of glucose homeostasis. Consequently, patients with long- is severely hampered by the shortage of islets available for transplantation. If functional β-cells and islets could be generated ex vivo, present severe islet shortage could be overcome. Another possible approach for restoration of islet cell

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The International Journal of Biochemistry & Cell Biology 38 (2006) 498–503

Medicine in focus

Islet neogenesis: A potential therapeutic tool in type 1 diabetes

Mark Lipsett, Reid Aikin[1], Mauro Castellarin[1], Stephen Hanley[1], Al-Maleek Jamal[1], Simon Laganiere[1], Lawrence Rosenberg*

Centre for Pancreatic Diseases at the McGill University Health Centre, Department of Surgery, McGill University, C9-128 Montreal General Hospital, 1650 Cedar Avenue, Montreal, Que., Canada H3G 1A4

Available online 27 September 2005

Abstract

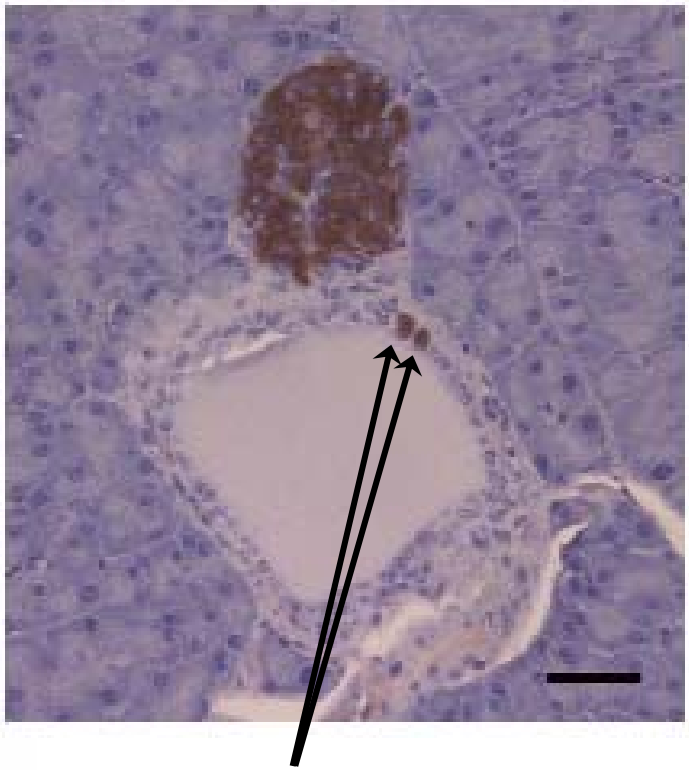
Current therapies for type 1 diabetes, including fastidious blood glucose monitoring and multiple daily insulin injections, are not sufficient to prevent complications of the disease. Though pancreas and possibly islet transplantation can prevent the progression of complications, the scarcity of donor organs limits widespread application of these approaches. Understanding the mechanisms of β-cell mass expansion as well as the means to exploit these pathways has enabled researchers to develop new strategies to expand and maintain islet cell mass. Potential new therapeutic avenues include ex vivo islet expansion and improved viability of islets prior to implantation, as well as the endogenous expansion of β-cell mass within the diabetic patient. Islet neogenesis, through stem cell activation and/or transdifferentiation of mature fully differentiated cells, has been proposed as a means of β-cell mass expansion. Finally, any successful new therapy for type 1 diabetes via β-cell mass expansion will require prevention of β-cell death and maintenance of long-term endocrine function.

Keywords: Epithelial growth factor (EGF); Gastrin; Glucagon-like peptide-1 (GLP-1); Islet neogenesis associated protein (INGAP); Pancreatic plasticity; Stem cell therapy

1. Introduction

Type 1 diabetes mellitus afflicts millions of individuals worldwide and its prevalence and incidence continue to rise annually. This disease results from autoimmune-mediated destruction of the insulin producing β-cells of

Despite the widespread use of meticulous blood glucose monitoring and new insulin formulations, most individuals with diabetes will still develop the devastating secondary complications of the disease. Clinical studies suggest that strict blood glucose control by intensified insulin treatment may attenuate or delay, but not prevent



possible *in vivo* induction of proliferation in islet progenitor cells

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Cellular Replacement for Diabetes

3 basic approaches:

1. **Islet Cell Transplantation**
   replacement of insulin-producing cells with mature, functioning cells from cadaver organ donors

2. **Stem Cell Therapy to Regenerate Islet Function**
   replacement of insulin-producing cells with stem cell-derived insulin-producing cells
   a. stem cells isolated and differentiated *in vitro,* then transplanted
   b. stem cells isolated and transplanted, differentiate *in vitro*

3. **Stem Cell Therapy to Prevent Diabetes Onset**
   modification of host immune system by stem cell-derived immune modulatory cells

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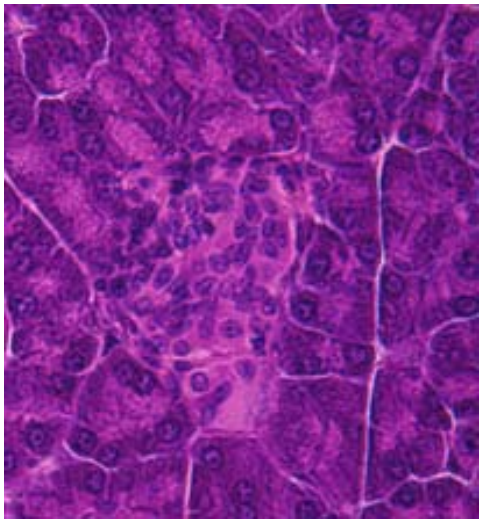
Cellular Replacement for Diabetes

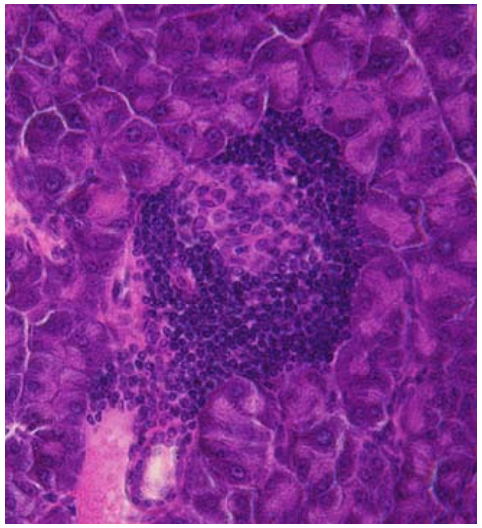
ASC Therapy in the Spontaneously Diabetic NOD mouse

ASC Therapy may delay diabetes onset

Treatment group	n	number diabetic	Age at diabetes onset (days)	mean (days)
ASC + immunosuppression	7	2	162, 214	188±26
ASC + vehicle	8	4	115, 128, 149, 152	136±9
vehicle alone	8	3	170, 173, 185	176±5



normal, healthy NOD islet



NOD islet under immune attack

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Cellular Replacement for Diabetes

Multiple approaches of cellular replacement therapy in diabetes:

Islet Cell Transplantation

- **promising, but insufficient tissue to treat the extremely large number of potential patients**

Stem Cell Therapy

- **ethical issues in use and source of stem cells**
  - **use of adult-derived stem cells may obviate concerns**
- **novel alternate sources of adult stem cells may provide a plentiful source of supply of these cells**

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