For many years, there has been great interest in approaches to the replacement of insulin-producing beta cells in individuals with diabetes.

Cellular replacement therapies carry the hope to achieve physiologic glucose control, thereby reducing or eliminating the need for daily medication, as well as preventing long-term diabetic complications.
Cellular treatments seem specifically suited for type 1 diabetes because primary destruction of beta cells is the central pathological process in this disease.

In addition, patients with advanced type 2 diabetes and only relative beta cell insufficiency could benefit from replacement therapy.
Transplantation of Whole Donor Pancreas

- It has been successfully performed in a large number of patients with type 1 diabetes.
- The need for intense immunosuppression, as well as the difficulties associated with drainage of exocrine pancreatic fluid, result in undesirable morbidity.
The Transplantation Of Isolated Pancreatic Islets

- The transplantation of isolated pancreatic islets into the livers of type 1 diabetic patients had been largely unsuccessful for many years.

- Recently, the newly devised “Edmonton Protocol” for islet transplantation provided unprecedented positive results (Shapiro et al, 2000)
Scarcity of Islet Cells For Transplantation

- The amount of donor islet tissue is severely limited.
- The Edmonton Protocol requires the utilization of islets from 2 to 4 donor pancreata for the successful transplantation of a single patient.
- Using current protocols, less than 0.5% of needy recipients can be treated.
Possible Approaches To Overcome The Shortage Of Donor Pancreata

- **Direct expansion of beta cells** *in vitro* for use in transplantation (limited proliferative potential of fully differentiated beta cells)
- **Genetic manipulation** of an unrelated cell type to secrete insulin in a glucose responsive manner (difficult, to accurately mimic the complex regulatory circuits of a beta cell)
- **Expansion and subsequent differentiation** of stem or progenitor cells
Expansion And Subsequent Differentiation Of Stem Or Progenitor Cells

This approach seems promising

The proliferative capacity of beta cells *in vivo* is limited and that new beta cells are mainly generated via their differentiation from undifferentiated progenitor cells

The formation of new islet tissue via the differentiation of stem/progenitor cells in adult pancreata is referred to as *islet neogenesis*
In the beginning...

Egg + Sperm
Cell division...

zygote → morula
The Blastocyst

Outer cell layer $\Rightarrow$ placenta

Inner mass $\Rightarrow$ baby
And then...
The Blastocyst

Inner mass $\Rightarrow$ embryonic stem cells
Cell Development

Plastic → Specialized
Totipotent stem cells - cells produced by the first few divisions of the cell. So can form any cell of the embryo as well as the placenta.
Pluripotent – these cells differentiate into cells derived from the three germ cell layers.

Eg: embryonic stem cell, embryonic germ cell and embryonic carcinoma cells.
Multipotent – these cells can produce cells of a closely related family of cells.

Eg: haematopoietic stem cells, neural and mesenchymal stem cells
Unipotent – these cells only produce one cell type, but have the property of self renewal which distinguishes them from the non stem cells.
## Kinds of Stem Cells

<table>
<thead>
<tr>
<th>Stem cell type</th>
<th>Description</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Totipotent</strong></td>
<td>Each cell can develop into a new individual</td>
<td>Cells from early (1-3 days) embryos</td>
</tr>
<tr>
<td><strong>Pluripotent</strong></td>
<td>Cells can form any (over 200) cell types</td>
<td>Some cells of blastocyst (5 to 14 days)</td>
</tr>
<tr>
<td><strong>Multipotent</strong></td>
<td>Cells differentiated, but can form a number of other tissues</td>
<td>Fetal tissue, cord blood, and adult stem cells</td>
</tr>
</tbody>
</table>
Stages of Embryogenesis:
- **Day 1**: Fertilized egg
- **Day 2**: 2-cell embryo
- **Day 3-4**: Multi-cell embryo
- **Day 5-6**: Blastocyst
- **Day 11-14**: Tissue Differentiation
Derivation and Use of Embryonic Stem Cell Lines

Isolate inner cell mass (destroys embryo)

Day 5-6 Blastocyst

Outer cells (forms placenta)

Inner cells (forms fetus)

Culture cells

“Special sauce” (largely unknown)

Heart repaired

Kidney

Liver

Heart muscle
Cell Potency Types

- **Totipotent** - fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent.

- **Pluripotent stem cells** are the descendants of totipotent cells and can differentiate into cells derived from any of the three germ layers. (embryonic stem cells)
Embryonic Stem Cells

- Obtained when cultures of cells are taken from the epiblast tissue of the inner cell mass of a blastocyst or earlier stage embryos.
- A blastocyst is an early stage embryo approximately 4 to 5 days old in humans and consisting of 50–150 cells.
Embryonic Stem Cells

Cont...

- Can be coaxed into developing all 220 types of cells found in the human body (e.g. blood cells, heart cells, brain cells, nerve cells, etc).

- Derived from human embryos in a process that causes the death of the embryos.
Mice Embryonic Stem Cells

- Mouse embryonic stem cells with fluorescent marker.
Stem Cell Cultivation

1. In Vitro Fertilized Egg
2. Blastocyst Stage (5-7 days old)
3. Inner Stem Cell Mass
4. Cultured Undifferentiated Stem Cells
5. Specialized Cells:
   a. blood cells
   b. neural cells
   c. muscle cells
Blastocyst Diagram

fertilized egg → Totipotent cells → Blastocyst

inner cell mass (pluripotent cells)
cells isolated from the ICM
cultured pluripotent stem cells
Five Day Pre-Embryo

http://www.nationalgeographic.com/ngm/
Adult Stem Cells

- Not as versatile for research purposes specific to certain cell types, such as blood, intestines, skin, and muscle.

- Extracted from an umbilical cord, or a child's or adult's body.

- The term "adult stem cell" may be misleading because both children and adults have them.
Adult Stem Cells Continued

- Difficult to extract, yet plentiful.
- Taken from the patient's own body, ensuring an exact DNA match so the body's immune system never rejects them.
Benefits of Stem Cell Research:

*Possible Treatments for:

- Alzheimer’s
- Parkinson’s
- Spinal Cord injuries
- Heart Damage
- Muscle Damage
- Brain Damage
- Stroke Damage
- Sickle Cell Anemia
- Surface Wound Healing
- Jawbone Replacement
- Skull Replacement
More Benefits:

- Stem Cell Research may provide treatment to over 26 different types of Cancers including:
  - Lymphoma
  - Leukemia
  - Brain Cancer
  - Breast Cancer
  - Ovarian Cancer
  - Testicular Cancer

- Can provide treatment to illnesses ranging from Cardiovascular diseases to Bladder diseases.

<table>
<thead>
<tr>
<th>Medical Condition</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular disease</td>
<td>58 million</td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>30 million</td>
</tr>
<tr>
<td>Diabetes</td>
<td>16 million</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>10 million</td>
</tr>
<tr>
<td>Cancer</td>
<td>8.2 million</td>
</tr>
<tr>
<td>Alzheimer's disease</td>
<td>5.5 million</td>
</tr>
<tr>
<td>Parkinson's disease</td>
<td>5.5 million</td>
</tr>
<tr>
<td>Burns (severe)</td>
<td>0.3 million</td>
</tr>
<tr>
<td>Spinal-cord injuries</td>
<td>0.25 million</td>
</tr>
<tr>
<td>Birth defects</td>
<td>0.15 million/year</td>
</tr>
</tbody>
</table>

Source: Derived from the [National Academy of Sciences](https://www.nationalacademies.org) web site.
<table>
<thead>
<tr>
<th>Characteristics Of Stem, Progenitor And Precursor Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Differentiation potential</strong></td>
</tr>
<tr>
<td>Stem cell</td>
</tr>
<tr>
<td>Progenitor cell</td>
</tr>
<tr>
<td>Precursor cell</td>
</tr>
</tbody>
</table>
The first specific progenitor cells for the pancreas are characterized by the expression of the transcription factor Pdx-1.

Pdx-1 is expressed early in development (embryonic day 8.5 in the mouse) and its expression is required for the initial pancreatic anlage to bud from the endodermal epithelium.
The expression of neurogenin 3 (Ngn3), a basic helix-loop-helix transcription factor, at embryonic development day 9.5 defines the first definitive endocrine cell precursors.

From then on, a cascade of several transcription factors leads to the formation of all endocrine lineages of the islets of Langerhans.
Embryonic Stem Cells

- ES cells are derived from the inner cell mass (ICM) of the embryonic blastocyst.
- Mouse ES cells were first isolated 20 years ago, while human and other primate ES cells were isolated more recently, in 1998.
The Blastocyst
Because the establishment of ES cell lines \textit{in vitro} involves the destruction of a potentially viable embryo, the use of human ES cells for such purposes is ethically controversial.
Selective ESC Differentiation

- The basic concept of stem cell biology is that an undifferentiated cell can be isolated at some stage of development, expanded along a differentiation pathway until the desired type of cell or tissue is achieved.

- Embryonic stem cells (ESC) can be differentiated into insulin-producing cells by manipulating culture conditions.
Selection for nestin-expressing ESC in culture, can be stimulated to differentiate towards a β-cell-like phenotype.

The use of pax4 or pdx-1 (transcription factors associated with β-cell lineage) can yield promising results.
Challenges For Utilizing ES Cells As A Source Of Beta Cells For Transplantation

- Unregulated differentiation into other cell types
- Teratoma formation from remaining undifferentiated cells have to be prevented after transplantation
- Maturity of the cells generated
- Safety (lack of tumorigenicity) of ES cell derived transplants
**Fetal Stem Cells**

- Fetal islet-like clusters (ILCs), obtained from both human and porcine pancreata, have been evaluated for their potential as a source of beta cells.

- These ILCs contain a large proportion of undifferentiated progenitor cells that only differentiate into fully mature β-cells after transplantation.
The *fetal human pancreas* could become a valuable source of expandable beta cell progenitors in the future.

Similarly, attempts are under way to isolate a multipotential liver/pancreas stem cell from the fetal liver.
Attention is currently focused on 2 sets of cells within the pancreas that could be candidates for a therapeutic application as islet progenitors:

1. Cells of the pancreatic ducts
2. Nestin-positive islet-derived progenitor cells (NIPS)
1. Cells Of The Pancreatic Ducts

- The expansion of cells from a crude preparation of mouse pancreatic ducts derived from nonobese diabetic (NOD) mice generated insulin producing cells (ILCs).
- Upon transplantation into diabetic NOD mice, these ILCs significantly lowered the plasma glucose levels of the animals.
- However, the specific cells in the pancreatic ducts that are the progenitors and give rise to the insulin-producing cells remain to be identified and characterized.
2. Nestin-positive Islet-derived Progenitor Cells (NIPS)

- Cells expressing the intermediate filament protein nestin, a marker of neural stem cells, can be isolated from human and rodent islets and expanded \textit{in vitro}

- Insulin, glucagon and Pdx-1 expression, as well as low-level insulin secretion, can be detected in these cultures after the addition of differentiating cytokines and growth factors

- These cells also form ILCs \textit{in vitro}, a process that is markedly enhanced by the addition of the insulinotropic, neogenic hormone glucagon-like peptide-1 (GLP-1)
Hepatic Oval Cells

- The close anatomical proximity of pancreas and liver development in the primitive foregut during embryogenesis has prompted attempts to isolate pancreatic progenitor cells from a subpopulation of cells in adult liver.

- Recently, Yang et al (2002) reported the \textit{in vitro} generation of ILCs from rat liver cell preparations enriched for hepatic oval cells.
Adult Stem Cell Plasticity

“Transdifferentiation”

- It was commonly accepted until recently that tissue development is a unidirectional pathway in which cells become increasingly restricted in their differentiation potential.

- Several studies done both \textit{in vitro} and \textit{in vivo} suggest that transformation of one adult cell type into a completely unrelated tissue is possible.
Multipotent Adult Progenitor Cells (MAPCs)

- The most striking results with regard to adult stem cell plasticity were recently presented in a cell population from human and rodent bone marrow (multipotent adult progenitor cells, MAPCs).
- MAPCs demonstrated an unlimited lifespan and differentiation potential reminiscent of embryonic stem cells.
- Functional hepatocytes could be generated from MAPCs \textit{in vitro}, and so, the derivation of pancreatic endocrine cells seems possible.
Advantages of Adult Stem Cells

- The use of adult tissue-derived stem cells would be preferable over ES cells, not only for ethical reasons, but also because the tumorigenicity of adult cells appears to be far lower than that of ES cells.

- It might also be possible to harvest adult stem cells from patients for expansion \textit{ex vivo} and then transplantation into the patients as isografts, thus avoiding the need for donor recruitment and immunosuppression.
Stem Cells Derived From Haemopoietic Organs

- Bone marrow harbors cells that can become parenchymal cells after entering the liver, intestine, skin, lung, skeletal muscle, heart muscle, and central nervous system, in rodent models and in human recipients of marrow transplantation.

- 1-2 months after bone-marrow transplantation, donor-derived cells are found in pancreatic islets of recipient mice

- These cells express insulin and genetic markers of β cells
In overtly diabetic mice whose β cells have been destroyed by streptozotocin, bone-marrow transplantation normalized blood glucose and insulin concentrations.
Bone-marrow Transplantation As A Therapeutic Approach For β-cell Replacement

- Transplanted hemopoietic stem cells can transdifferentiate into pancreatic islet cells
- In islets, marrow-derived cells can differentiate into endothelial cells which stimulate the proliferation of local pancreatic progenitors to insulin-producing cells
Bone-marrow transplantation induces microchimerism

In non-obese diabetic (NOD) mice, an autoimmune model of type 1 diabetes, transplanted with marrow before development of autoimmune diabetes, chimerism prevents diabetes mellitus

Donor immunoregulatory cells may have prevented the host cells from becoming autoreactive against β cells
Splenic Mesenchymal Cells

- Transplantation of mesenchymal cells from the spleen combined with complete Freund's adjuvant led to reversal of diabetes accompanied by regeneration of insulin-producing islets.
- The transplanted splenic mesenchymal cells differentiate into β cells.
- Thus splenic mesenchymal cells transplanted under certain conditions seem not only to keep immune destruction of islets in check, but also can transdifferentiate into pancreatic β cells.
Potential Stem/Progenitor Cells For The Treatment Of Diabetes
Approaches For The Use Of Stem Cells In The Treatment Of Diabetes

- Stem cells isolated from embryonic or fetal tissues and adult organs are expanded *in vitro*.
- They may then either be differentiated *in vitro* into glucose-responsive insulin-producing “islets” for transplantation into the liver of diabetic individuals or
- Administered into the circulation of diabetic patients where they “home in” on injured islets and differentiate into insulin-producing cells.
- Another approach is to administer “stem cell stimulators” such as drugs, growth factors, or hormones (GLP-1) to stimulate endogenous stem cells to differentiate into insulin-producing cells.
Different Approaches For The Use Of Stem Cells In The Treatment Of Diabetes

- Isolate stem cells
- Expand in vitro
  - Differentiate into “islets” (beta cells) in vitro
  - Administer stem cells systemically
    - Stem cells “home in” on injured islets and differentiate
    - Stimulate differentiation of endogenous stem cells (GLP-1)
- Transplant into liver
Conclusions

- Cellular replacement therapy may offer the best approach to achieve physiologic glucose control in diabetic patients.
- The expansion and subsequent differentiation of stem cells, be they of embryonic, fetal, or adult origin, appear to have considerable potential to overcome the shortage of donor organs.
Thank You