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Two major hurdles in regenerative medicine: Tissue shortage & rejection
Therapeutic cloning
(somatic cell nuclear transfer)

Cell reprogramming
(induced pluripotent stem cells)
1. Donor cells
2. IVF embryos
3. Human-human
4. Human-bovine
5. Human-rabbit
Sequential detection of the *Neo* gene in peripheral blood from cow #31

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Cow DNA (negative control)</td>
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<tr>
<td>2</td>
<td>Post-transplant (0 week)</td>
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<tr>
<td>3</td>
<td>Post-transplant (2 wks)</td>
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<tr>
<td>4</td>
<td>Post-transplant (4 wks)</td>
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<tr>
<td>5</td>
<td>Post-transplant (7 wks)</td>
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<td>6</td>
<td>Post-transplant (12 wks)</td>
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<tr>
<td>7-10</td>
<td>Single colony detection on 12 wk blood sample</td>
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<td>11</td>
<td>Positive control</td>
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<td>12</td>
<td>Water control</td>
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<td>M</td>
<td>Molecular Marker</td>
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The *Neo* gene was present in peripheral white blood cells following fetal liver stem cells from a cloned fetal cow into the original adult. We also detected the marker gene in primitive blood progenitor cells.

WBC colony from cloned stem cells
Cardiovascular disease costs the US $329 billion annually.
• Parkinson’s ($6 billion/yr)
• Stroke ($45 billion/yr)
• Spinal cord injury ($10 billion/yr)
• Epilepsy ($3 billion/yr)
• Alzheimer’s ($100 billion/yr)
• Multiple sclerosis ($10 billion/yr)

• Visual disorders/AMD ($50 billion/yr)
Retinal Degenerative Diseases

- Age-related macular degeneration (ARMD) alone affects over 30 million people worldwide
  - Leading cause of blindness in patients over 60 yrs old in the US
  - Degeneration of retinal pigment epithelium (RPE) plays a critical role in the pathogenesis of AMRD as well as retinitis pigmentosa and other retinal degenerative diseases
- Transplanted RPE cells have been shown to rescue host photoreceptors and attenuate loss of visual function in animals
- Stem cell-derived RPE could be a potentially important source of tissue for subretinal transplantation in patients
Anatomy & Function of RPE

FUNCTIONS OF RPE

• Immune barrier
• Absorption of stray light
• Vit A metabolism & transport
• Phagocytosis of shed photoreceptor segments
Advantages of ECS-derived Tissues for Regenerative Medicine

- Unlimited supply
- Can be derived under GMP conditions pathogen-free
- Can be produced with minimal batch to batch variation
- Can be thoroughly characterized to ensure optimal performance
RPE can be generated from hES cells

Over 2 dozen hES cell lines studied – all reproducibly generated RPE lines that could be passaged, characterized, and expanded

• ACT-derived hES cell lines (14 different lines studied)
  MA01  MA40  MA126
  MA03  MA99  MA127
  MA04  MAJ1  MA128
  MA09  MA99  MA129
  MA14  MA128  MA133

• HHMI/Harvard (8 different hESC lines studied)
  HUES1  HUES5  HUES8
  HUES2  HUES6  HUES10
  HUES3  HUES7

• WiCell (3 different hESC lines studied)
  H1/WA01  H7/WA07  H9/WA09
Stages of RPE isolation from spontaneously differentiating hES cells

- 35 mm plate
- One of the clusters
- Cell suspension at plating
- 4 days
- 7 days
- Passage 1 -- 25 days

Magnification: x0.75, x200, x100, x200, x200, x200
hES-RPE express RPE markers (bestrophin & CRALBP)

**Immunostaining**
- CRALBP x400
- CRALBP x200

**Western blot**
- Mw
- -- 78
- -- 46
- -- 32

- bestrophin
- CRALBP
hES-RPE express RPE65 and PEDF

RT-PCR

1 – fetal RPE
2 – hES-RPE
Phagocytosis of latex beads (electron microscopy)
RPE transplantation into subretinal space of RCS rats

Collaboration with Ray Lund

RCS rats become blind in several weeks due to RPE degeneration and photoreceptor death

Tests:
Head tracking (behavior)
Electroretinogram (ERG)
Histology

_in vitro assessment:_
Molecular markers of RPE morphology and behavior
Hemangioblasts derived from hESC
Hemangioblasts Generated from Human ES Cells

40X

10X
BCs form vessels with both endothelial & functional smooth muscle cells

Vessels have the ability to contract when exposed to carbachol
Flow ratio (Ischemia/ctl limb)

BC cells
Control

Restoration of blood flow to ischemic limbs

(Days)
Survival rate after myocardial infarction
End-stage renal disease will cost US $1 trillion during the coming decade
Is it possible to generate hES cells without destroying embryos?

• The most basic objection to ES cell research is that it deprives embryos of any further potential to develop into complete human beings.

• For a decade, PGD has been used successfully to remove a single cell (blastomere) for genetic testing without interfering with the developmental potential of the biopsied embryo. Several thousand healthy babies have been born using this procedure.

• Question: Can such a biopsied cell be used to generate ES cells?
Biopsy Procedure

Live Young

Biopsied 23/47 (49%)
Non-Biopsied 38/75 (51%)
Human embryonic stem cell lines derived from single blastomeres

Irina Klimanskaya, Young Chung, Sandy Becker, Shi-Jiang Lu & Robert Lanza

The derivation of human embryonic stem (ES) cells in mice indicates that it might be feasible to derive them using a single-cell approach without the need for blastomere splitting. This method involves the use of single-cell isolation techniques and the development of genetic markers to identify and select embryonic stem cells. In this study, the authors describe the isolation of human embryonic stem cell lines derived from single blastomeres, demonstrating the potential for their use in regenerative medicine and therapy.

Human Embryonic Stem Cell Lines Generated without Embryo Destruction

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First hESC lines created without destroying embryos

hESC lines derived from single cells without embryo destruction. The biopsied embryos were allowed to develop to the blastocyst stage (they remain viable & frozen).
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