胚芽干细胞研究：现在与未来

Embryonic Stem Cell Research: Present and Future

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Embryonic Stem Cell Research: Present and Future

Therapeutic Cloning: Where we are now?

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College of Medicine
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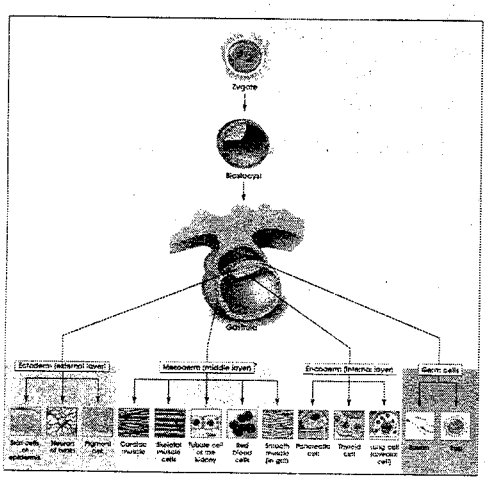
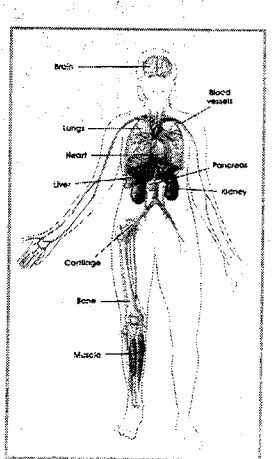
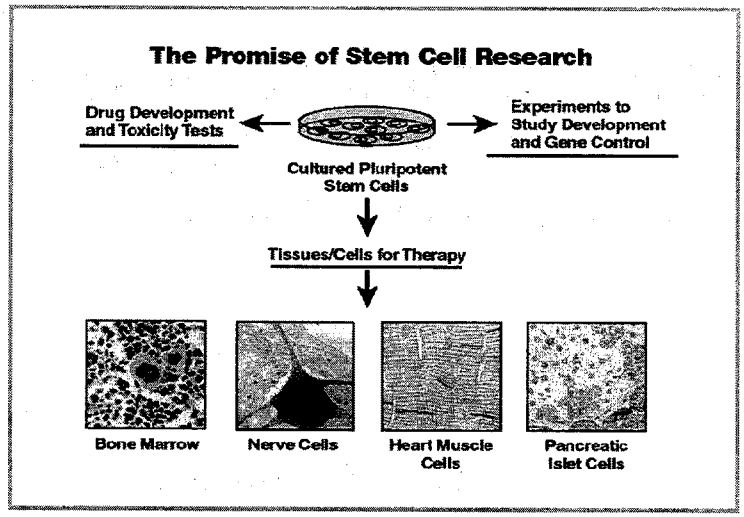
Purpose of Embryonic Stem Cell Research

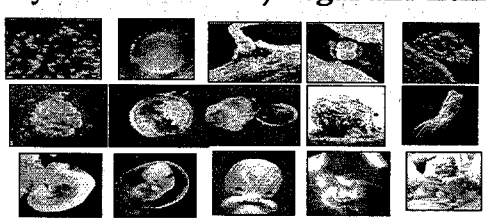






It has been hypothesized by scientists that stem cells may, at some point of future, become the basis for treating disease such as Parkinson's disease, diabetes and heart disease.

Embryonic Stem Cell ; Legal and Ethical Consideration



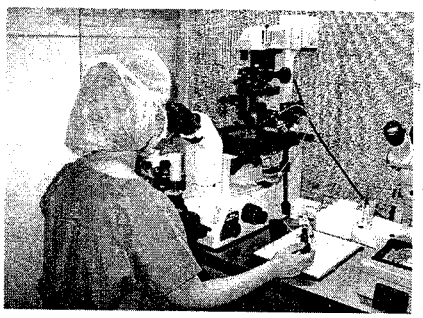
Normal Conception and Birth

Embryonic stem cells are found to be more clinically promising than adult stem cell but their research has been hindered by et hical consideration.

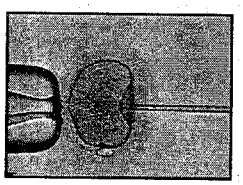
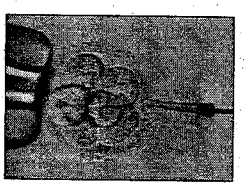
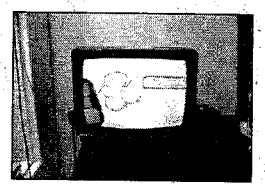
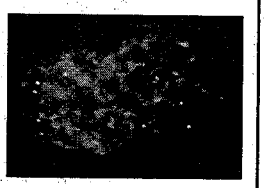
For those who believe the human embryo from the one cell stage onwards has absolute moral value, equal to that of a new born baby or an adult, any embryo research is ethically unacceptable.

If ES cells turn out to be the best route to cure a particular disease, then many would argue that it would be morally wrong not to use embryos that would otherwise be discarded.

From IVF to Cloned Human Embryonic Stem Cell



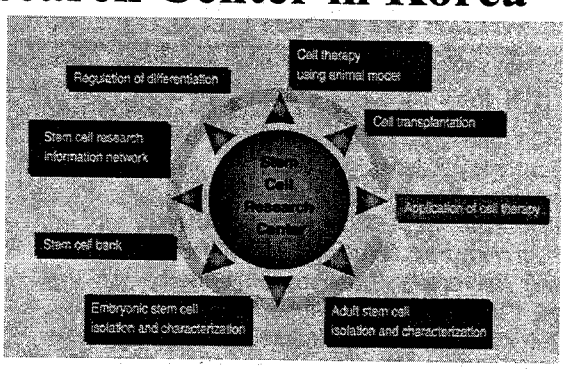
1. IVF-ET Program(1985)
2. Cryopreservation
3. Assisted Hatching
4. ICSI
5. Preimplantation Genetics
6. Embryo-coculturte systems
7. Human embryonic stem cell (2001.9~) : SNUhES 1,2,3 & 4
8. Cloned human embryonic stem cell (2004)

Stem Cell Research Center in Korea

Stem Cell Research Center 2002

www.stem.or.kr

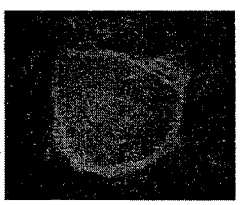
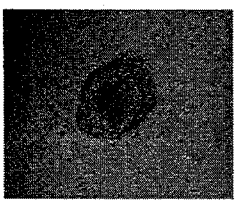
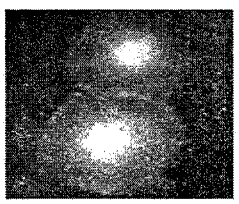


- Established: 2002.7
- Research Fund: 7.5 million US dollars / year
- Sponsor: Ministry of Science and Technology, Korea
- Location of Center: Seoul National University Hospital

Human Embryonic Stem Cell Bank (2002)

KSCRC REGISTRED STEM CELL LINES

- Fully characterized human ES Cell line : 36 lines
- Fully characterized human EG Cell line : 1 line
- US NIH registered human ES Cell line : 1 line (Miz-hES1)
- Cloned human ES Cell line : 1 line + 11 lines

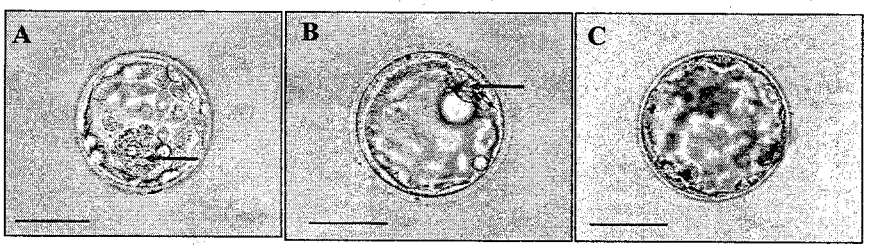
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health
Health | Grants | News | Science | Institutes | About NIH

Home Page
June 15, 2004

NIH Human Embryonic Stem Cell Registry

BresaGen, Inc., Athens, Georgia	4(2)
CyThera, Inc., San Diego, California	9
ES Cell International, Melbourne, Australia	6(5)
Geron Corporation, Menlo Park, California	7
Göteborg University, Göteborg, Sweden	19
Karolinska Institute, Stockholm, Sweden	6
Maria Infertility Hospital Medical, Seoul, Korea	3
MizMedi Hospital – Seoul National University, Seoul, Korea	1(1)
National Centre for Biological Sciences, Bangalore, India	3
Pochon CHA University, Seoul, Korea	2
Reliance Life Sciences, Mumbai, India	7
Technion University, Haifa, Israel	4(2)
University of California, San Francisco, California	2(1)
Wisconsin Alumni Research Foundation, Madison, Wisconsin	5(3)

Derivation of human embryonic stem cells depending on quality of blastocyst



The arrows indicate the ICM regions. Scale bar, 100 μm.

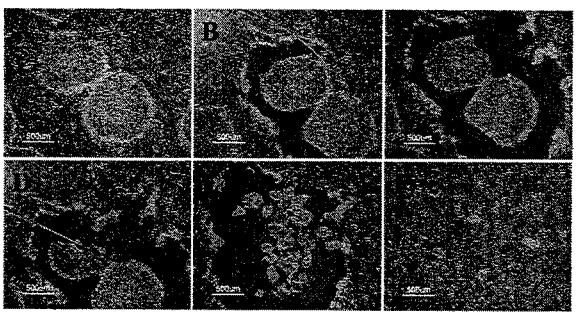
Blastocyst quality is one of the most important factors : Implantation and pregnancy

(A) Good blastocyst, which harbor large and distinct ICM ,
were processed via the immunosurgical method.

(B) Expanded blastocyst with small ICM
were processed via the partial embryo culture method.

(C) Blastocyst with poorly-defined ICM
were processed via the whole embryo culture method.

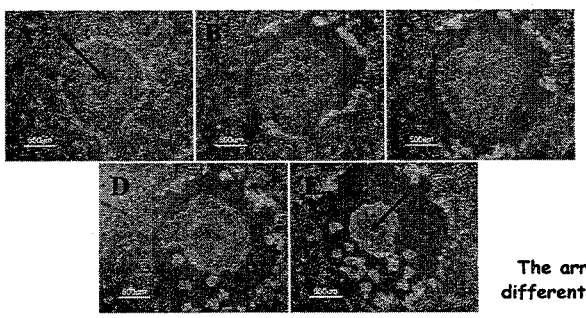
Mechanical transfer of hESC for maintenance (I)



Scale bar, 500 μm.

A) At day 6, undifferentiated colonies shown on STO feeder layer.
B) The feeder layers pushed away from hESC colonies using the dissecting pipette.
C) Complete separation between feeder layer and hESC colonies.
D) Dissecting with pipette into small clumps.
E) Completely dissected clumps.
F) Transfer to new culture dish using the transfer pipette.

Mechanical transfer of hESC for maintenance(II)

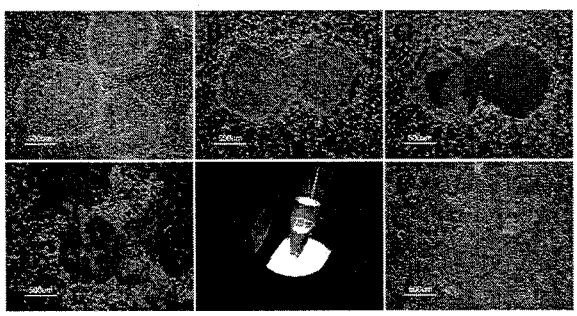


The arrow indicate differentiated hES cell

Mechanical separation and transfer of undifferentiated hESCs from differented cells

A) Differentiated cells at day 6, indicated by arrow within hESC colony.
B) The feeder layers pushed away from hESC colonies using the dissecting pipette.
C) Complete separation between feeder layer and hESC colony.
D) Separation of undifferentiated cells from differentiated cells using the dissecting pipette.
The undifferentiated cells are dissected into small clumps.
E) The differentiated cells remain, and all of the undifferentiated cells are dissected into small clumps.

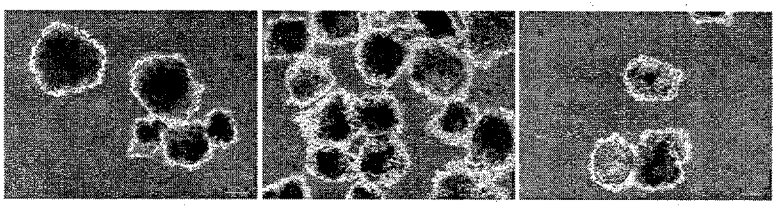
Enzymatic transfer for large quantities of cells



A) Undifferentiated hESC colonies were treated with collagenase.
B) After 30 minutes of enzyme treatment, the cells began to detach around the edges. At this time point, collagenase was removed and new medium was added.
C) The colonies lifted off the dish by gently pipetting with a 200-μl micropipette.
D) Multiple colonies completely detached from dish.
E) The detached hESC colonies were collected in a 15-ml conical tube, allowed to settle to bottom, and pipetted multiple times to make small clumps.
F) Small clumps transferred to new culture dish : various sizes.

Three kinds of human feeder support growth undifferentiated of hESC (II)

3) Embryoid body formation

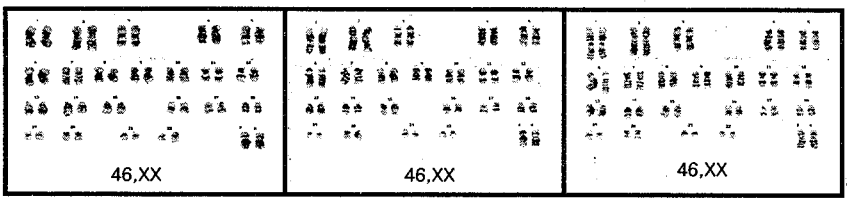


hES9 P15 -day5 EB(X100) *AF Cells*

hES9 P15 -day5 EB(X100) Foreskin

hES9 P15 -day5 EB(X100) *CVS*

4) Karyotype

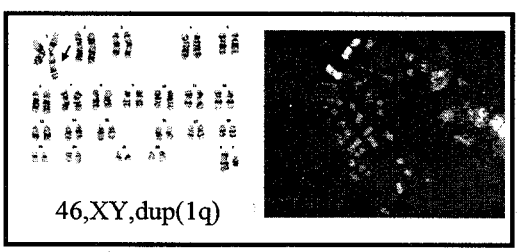


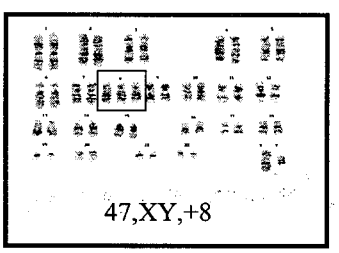
hES9 P11 *AF Cells*

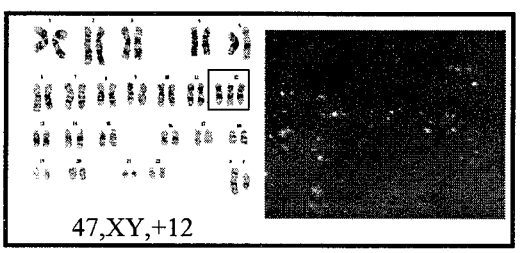
hES9 P11 Foreskin

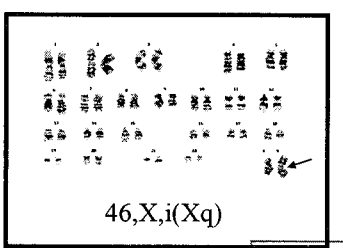
hES9 P11 *CVS*

The occurrence of chromosomal abnormalities in SNUhES cell lines

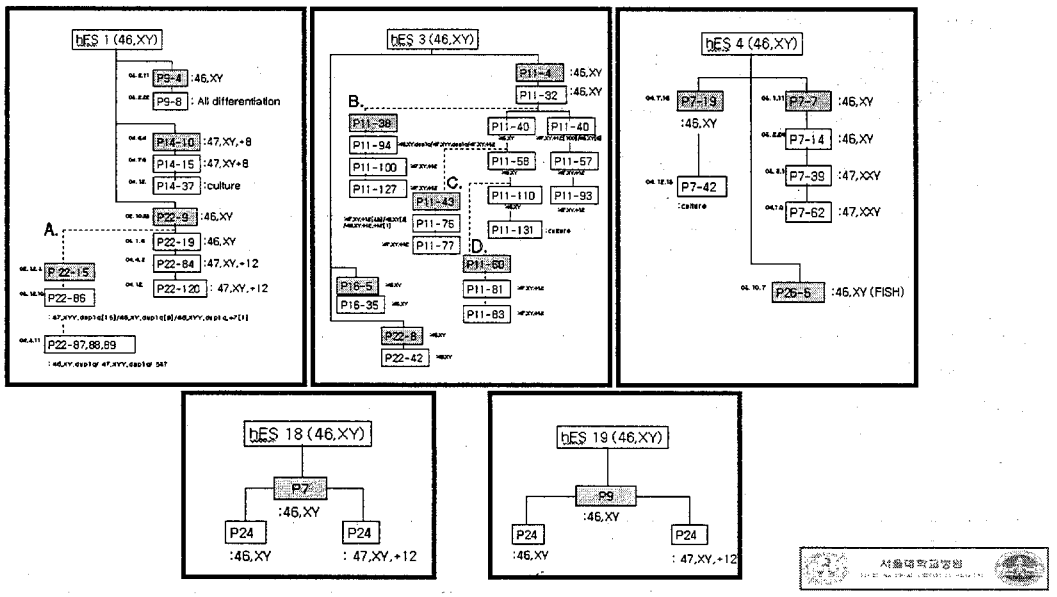




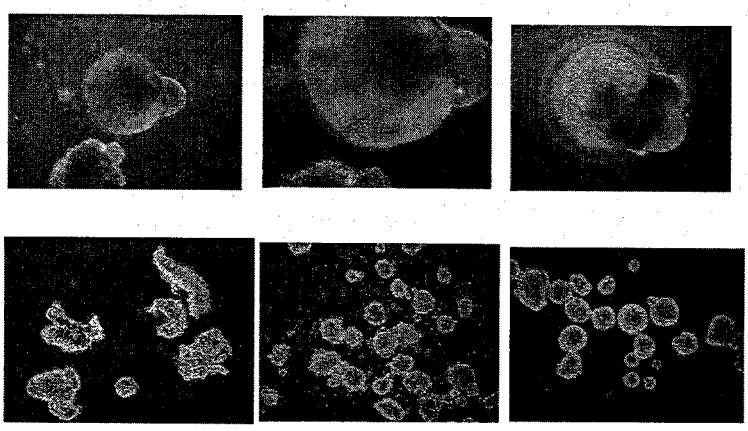




Karyotype pedigrees of SNUhES cell lines

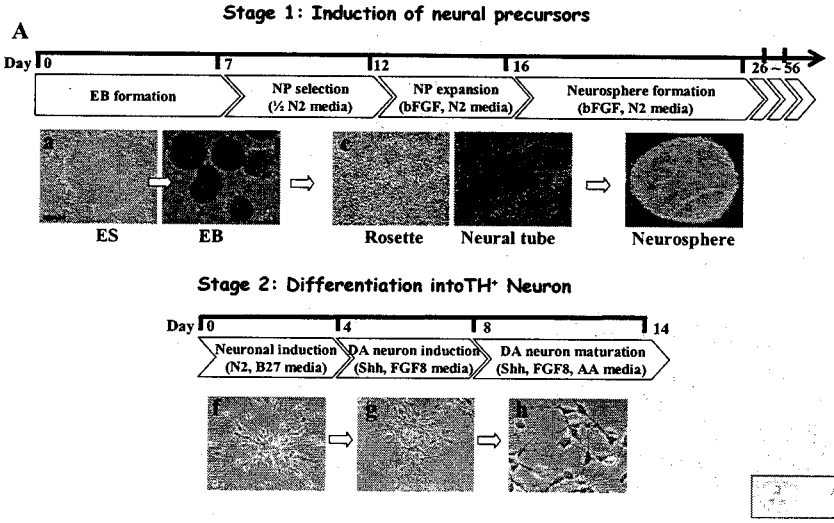


Differentiation of hESCs



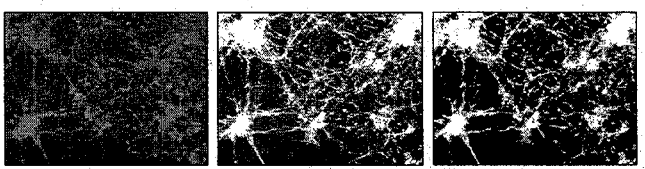
Differentiation of hESCs: Neurons (Ectoderm)

♦ Differentiation protocols



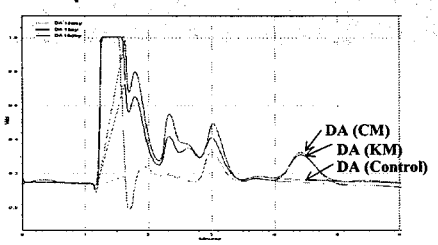
Differentiation of hESCs: Neurons (Ectoderm)

♦ Immunocytochemistry of TH positive neurons



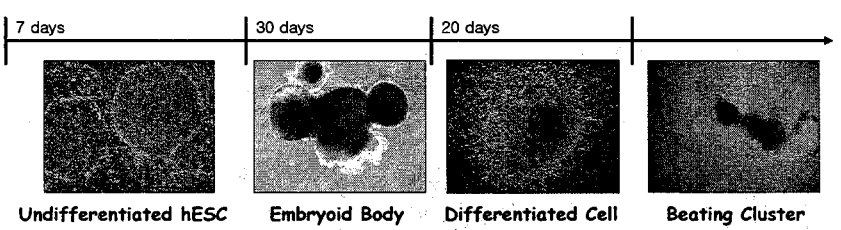
TH positive cells | βIII tubulin positive neurons | TH positive neurons

♦ HPLC analysis for dopamine secretion into the culture media

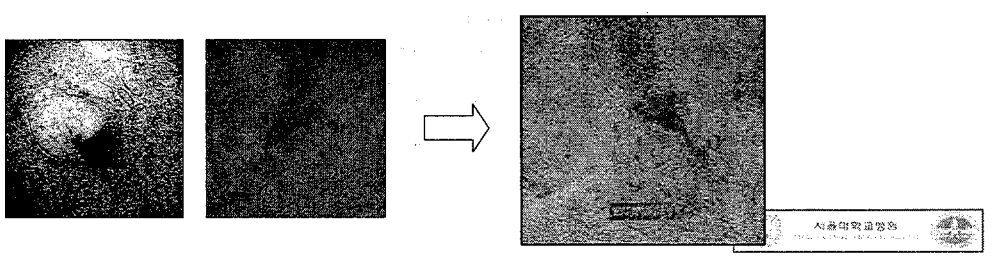


Differentiation of hESCs: Cardiomyocytes (Mesoderm)

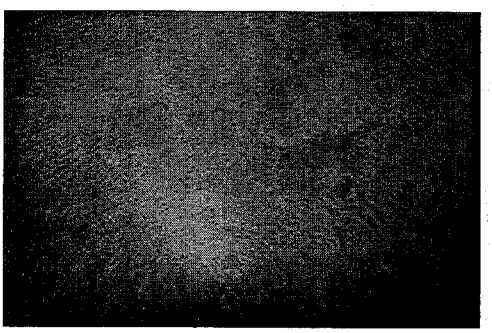
♦ Differentiation protocols



♦ Morphology of beating cluster from hES cells



BMP2-Induced Differentiation



▶ **Culture Condition**

suspension 30 days
attachment 20 days
with 0.6 ng/ml BMP2

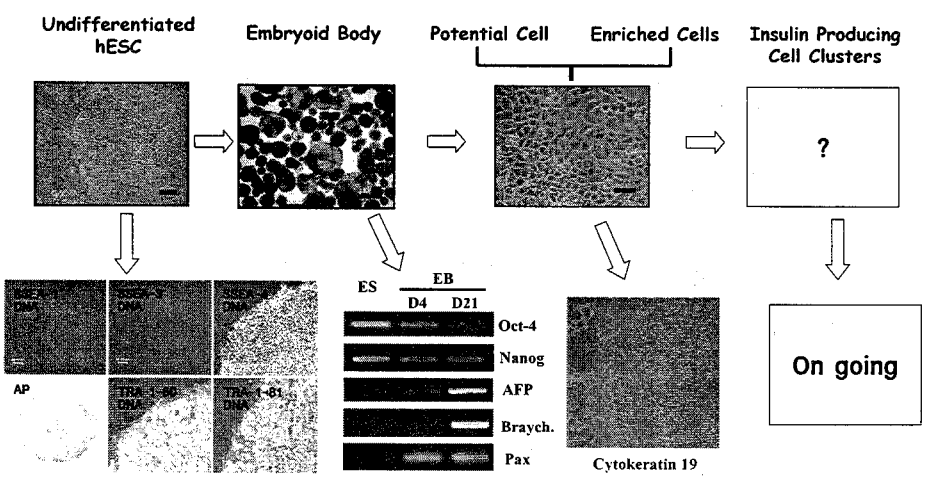
▶ **Beating**

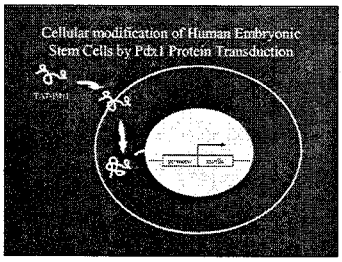
- **sensitive to temperature change**
- **keep beating until now**
- **25 / min**

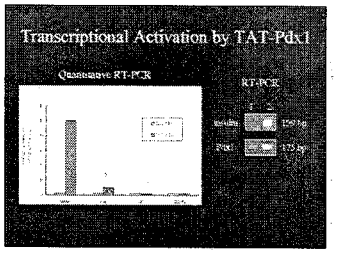
Cardiomyocytes derived from hES cell express cardiac specific markers and generate beating cluster.• FGF2 and BMP2 appear to enhance cardiomyocyte differentiation from hES cells.

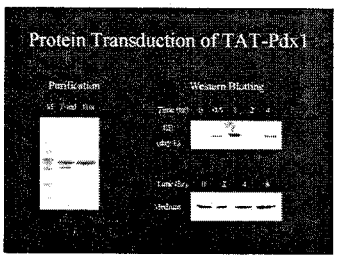
Differentiation of hESCs: Insulin producing cells (Endoderm)

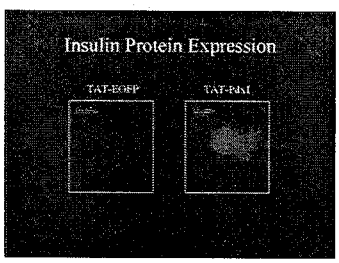
♦ Differentiation protocols



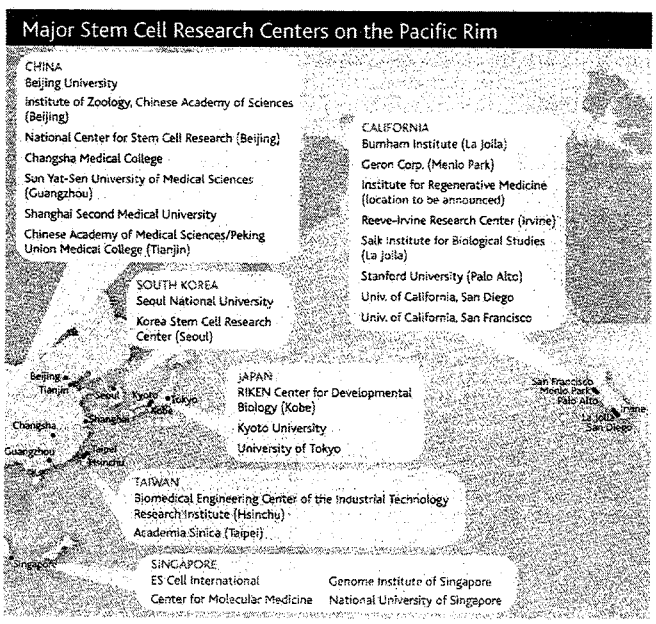


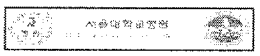




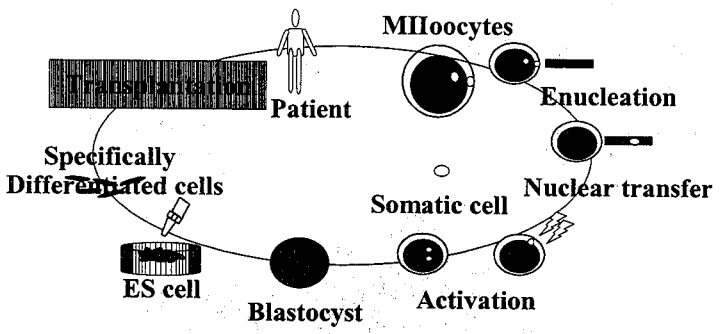


Kwon YD, Moon SY: Cellular Manipulation of Human Embryonic Stem Cells By TAT-PDX 1 Protein Transduction. Molecular therapy 12;28-32. July 2005



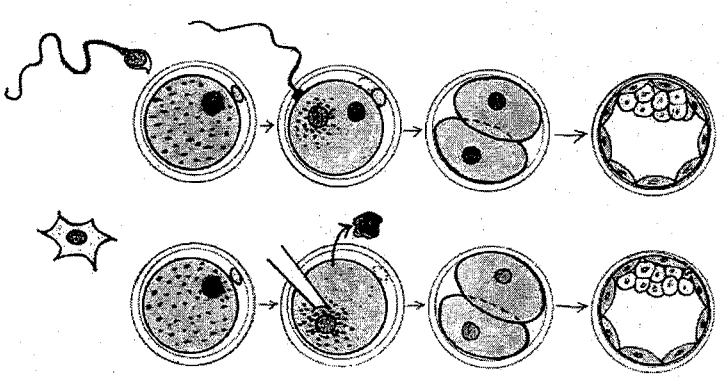


Therapeutic Cloning (Somatic Cell Nuclear Transfer)



For ethical and practical reasons, SCNT is not permitted in manycountries. The principal objection is that it might facilitate reproductive cloning in humans.This technology begins with thepatient's own cells, the transplanted tissue wouldnot be rejected by patient's immune system.

IVF / Cloned Blastocyst



Genomic replacement → creating customized hESCs

"Potential and Possibility"

Cloned Cow (1999) & Pigs (2002)
Hwang et al.

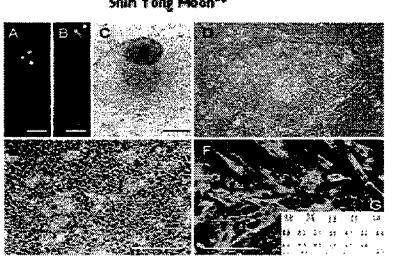
Miz-hES1 cells (2001)
Yoon et al.

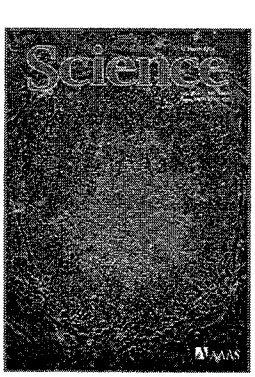
Establishment of
SCNT-hES-1
(2003)

Establishment of cloned hESC Line by Somatic Cell Nuclear Transfer

Evidence of a Pluripotent Human Embryonic Stem Cell Line Derived from a Cloned Blastocyst

Woo Suk Hwang,[1,2*] Young June Ryu,[1] Jong Hyuk Park,[2] Eul Soon Park,[1] Eu Gene Lee,[1] Ja Min Koo,[4] Hyun Yong Jeon,[1] Byeong Chun Lee,[1] Sung Keun Kang,[1] Sun Jong Kim,[2] Curie Ahn,[5] Jung Hye Hwang,[6] Ky Young Park,[7] Jose B. Cibelli,[8] Shin Yong Moon[2*]





2004 2005

Sciencexpress Report

Patient-Specific Embryonic Stem Cells Derived from Human SCNT Blastocysts

Woo Suk Hwang,[1,2*] Sung Il Roh,[3] Byeong Chun Lee,[1] Sung Keun Kang,[1] Dae Kee Kwon,[1] Sue Kim,[1] Sun Jong Kim,[3] Sun Woo Park,[1] Hee Sun Kwon,[3] Chang Kyu Lee,[2] Jung Bok Lee,[1] Jin Mee Kim,[3] Curie Ahn,[4] Sun Ha Paek,[4] Sang Sik Chang,[5] Jung Jin Koo,[5] Hyun Soo Yoon,[6] Jung Hye Hwang,[6] Youn Young Hwang,[6] Ye Soo Park,[6] Sun Kyung Oh,[4] Hee Sun Kim,[4] Jong Hyuk Park,[7] Shin Yong Moon,[4] Gerald Schatten[7*]

Collaborative Research

According to SCRC Guideline

Before beginning any experiments, we obtained approval from IRB on Human Subjects Research and Ethics Committee.

Oocyte Retrieval
Hanyang University

IRB Approval

Control to Prevent Human Reproductive Cloning
Stem Cell Research Center (SCRC), Korea

SCNT → Cloned hES Cells
Seoul National University

Derivation of Patient-specific NT- hES Cell Liness

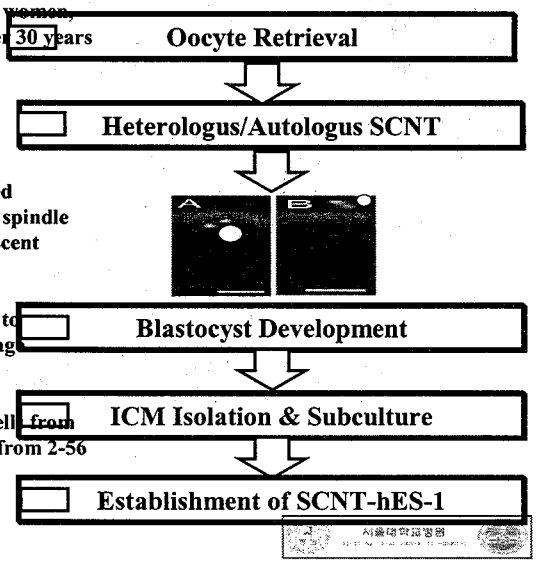
185 oocytes were donated for these studies by eighteen women, of which 125 oocytes were donated by ten women under 30 years old.

The donor's fibroblast cell was transferred back into enucleated oocyte.

To directly confirm that the oocyte's DNA was removed during enucleation, we imaged the extruded DNA MII spindle complex from every oocyte with Hoechst 33342 fluorescent DNA dye.

By allowing 2 hours for reprogramming, we were able to develop about 25% of the embryos to the blastocyst stage.

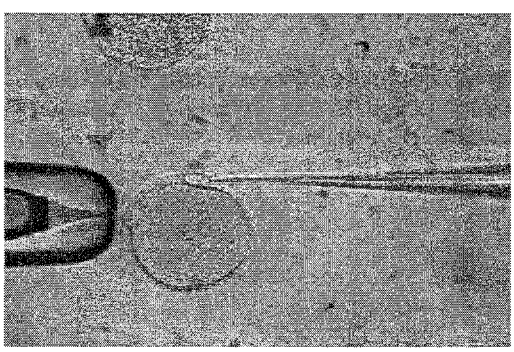
Eleven SCNT-ES cell line was derived using somatic cells from SCI, JD and CHG patients of both sexes and ranging from 2-56 years old.



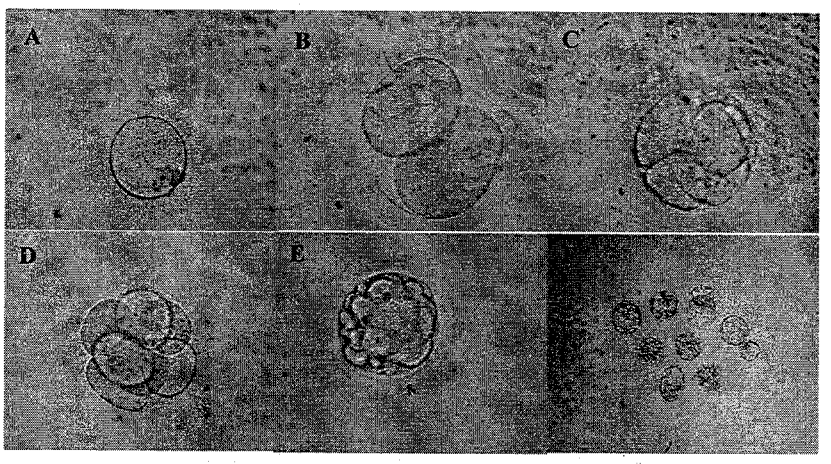
Derivation of Cloned hES Cells

We squeeze the MII oocyte so that the DNA spindle complex is extruded through a small hole in the zona pellucida and for lessening damages on the oocyte.

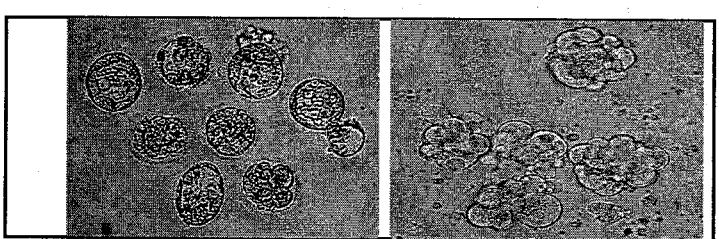
Enucleation, confirmation of the oocyte's DNA removal, NT, fusion, and activation were performed.



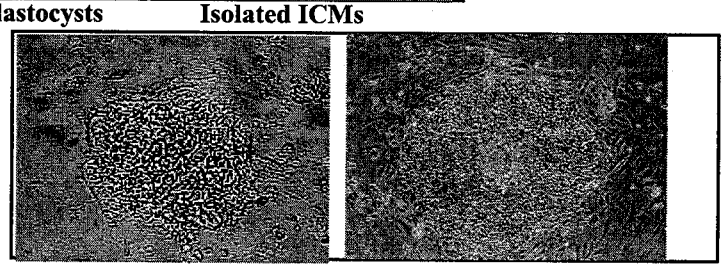
Development of human cloned emrbyos



Establishment of Cloned Human Embryonic Stem Cell



Cloned human blastocysts Isolated ICMs

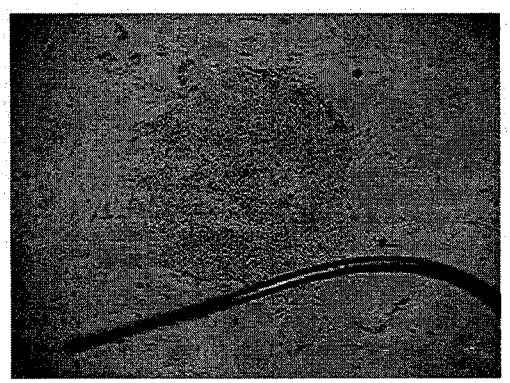


ICM outgrowth SCNT-hES-1 (>140th passage)

Protocols for subculture of hES cells

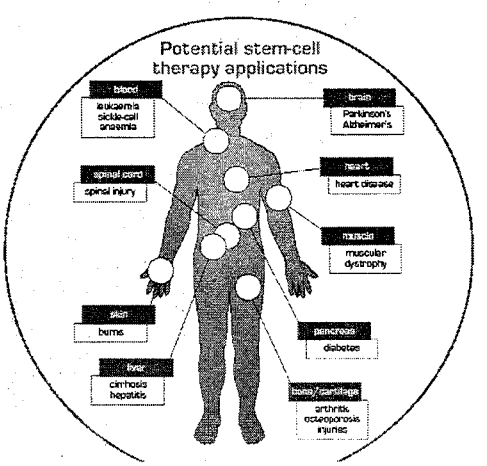
Subculture in every 7-9 days

- **Trypisn (0.05%) or collagenase IV (200 units/ml or 1 mg/ml) : may induce abnormal karyotype (trisomy 12, 17 or 18)**
- **Mechanical dissociation: a glass pipet (our lab) or hooked needle**



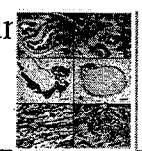
Embryonic Stem Cells for Cell-therapy

- **Establishment of embryonic stem cells**
- **Differentiation of pluripotent stem cells**
- **Isolation and Separation of Differentiated cells**
- **Transplantation of Differentiated Cells**
- **Functional evaluation of transplanted cells**



Safety Consideration in Cell-Based Therapy

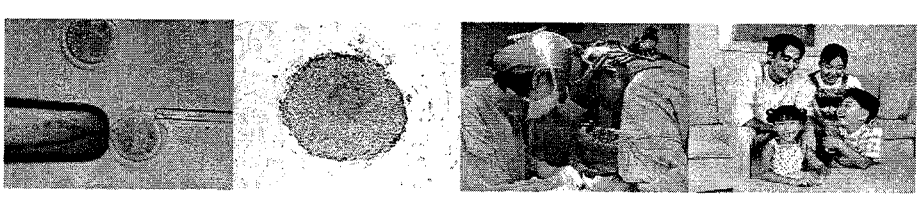
1. Whether cells can be derived that are histocompatible with every individual?
2. Whether transplanted pluripotent stem cells will form tumors or otherwise differentiate improperly or inappropriately after transplantation
3. Infectious agents that could be present in embryo-derived pluripotent stem cells or acquired by stem cells in feeder-dependent culture containing bovine serur



Conclusion

•Patient-specific stem cells derived in this study are now expected to provide cells in a disease state that can be used to understand disease progression and assist in drug development.

•In addition, prior to use in the clinic, biological properties of the patient-specific NT-hESCs must be defined, reliable differentiation procedures must be established, and the cells must be free of contaminating undifferentiated cells and potential pathogens.



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