The major correlate of aging is the gradual loss of regenerative capacity in most organs and tissues after birth

Nicolas-Sebastien Adam, Paris, Louvre

Rubens, Philadelphia

"The hound of Zeus, the tawny eagle, ... feasting on thy liver til he hath gnawn it black"
Aeschylys, Prometeus Bound

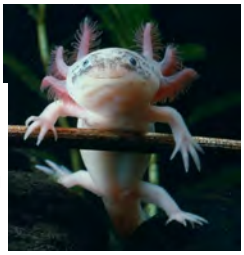
Regenerative responses in urodeles

Regeneration might be a primordial attribute of metazoan that has been lost subsequently for reasons that are not yet understood

An adult newt can regenerate:

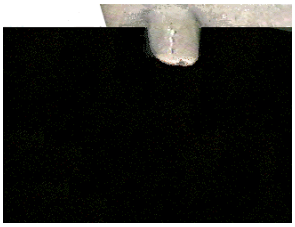
- **Jaws**
- **Lens**
- **Retina**
- **Large sections of the heart**
- **Limbs**
- **Tail**

Regenerative potential of *Ambystoma mexicanum* (Axolotl)



The axolotl does not heal by scarring and is capable of regenerating entire lost appendages in a period of months, and, in certain cases, more vital structures. Some have indeed been found restoring the less vital parts of their brains. They can also readily accept transplants from other individuals, including eyes and parts of the brain—restoring these alien organs to full functionality. In some cases, axolotls have been known to repair a damaged limb as well as regenerating an additional one, ending up with an extra appendage that makes them attractive to pet owners as a novelty.

Regenerative potential of *Ambystoma mexicanum* (Axolotl)



Patient requiring tissue regeneration

Myocardial infarction and heart failure
Liver cirrhosis
Need for epidermal and dermal substitution
Vascular grafts
Neurodegeneration and trauma
Bone and cartilage damage

Embryonic stem cells

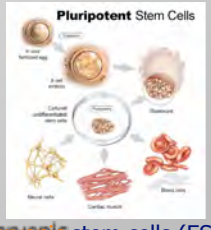
- From embryos
- Through cloning
- Through genetic reprogramming

Adult stem cells

What is a stem cell?

A cell that:

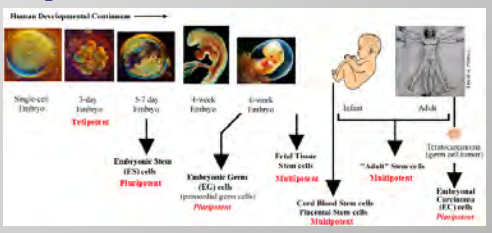
- is not differentiated
- is able to self-renewal
- can proliferate indefinitely ····· *stem cell ageing???*
- can generate many cell types
- supports development, tissue homeostasis and repair



Embryonic stem cells (ESC)

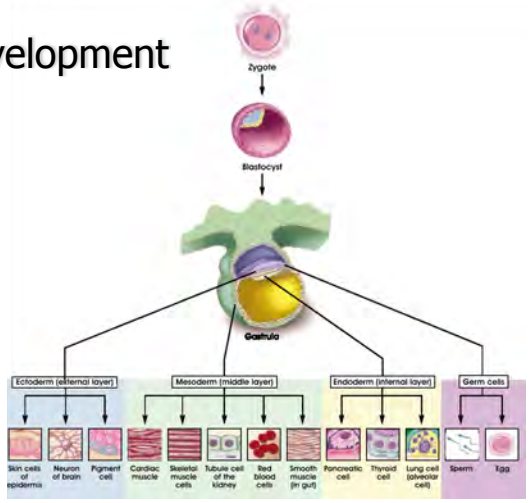
Adult stem cells (ASC)

Potency: the range of commitment options available to a cell



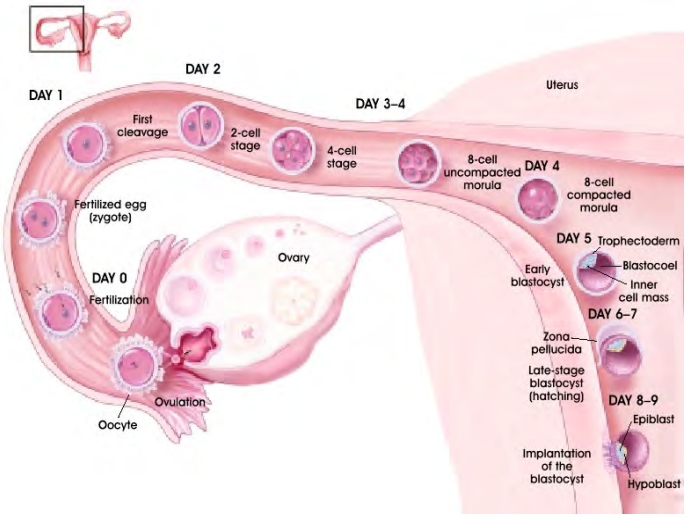
Totipotent: able to give rise to all the cells of the embryo and those that support its development in utero: Zygote
Pluripotent: able to give rise to cells derived from all three germ layers
Embryonic stem cells,
Embryonic Germ (EG) cells,
Embryonic Carcinoma (EC) Cells
Multipotent: able to give rise to a subset of cell lineages that constitute an entire tissue or tissues, e.g.Haematopoietic stem cells
Unipotent: able to differentiate into only one mature cell type.

Development

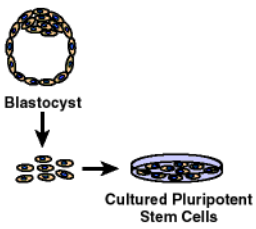


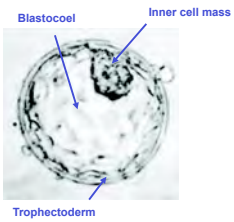
The Waddington epigenetic landscape

Fifty years ago, Conrad Waddington proposed an elegant model for the complex decision making process that takes place during differentiation. He compared the totipotent zygote to a marble poised to roll down a slope with branching ravines. It was thought that, just as a marble never rolls back up a slope of its own accord, the branching points during development define permanent decisions made by the cells that cannot be undone or reversed.



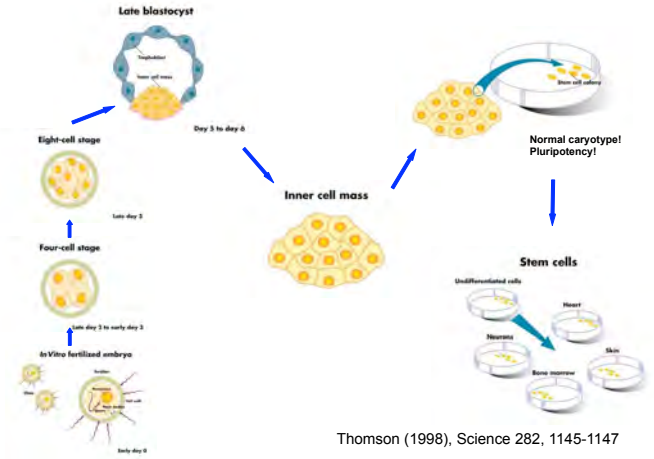
Establishment in culture of pluripotential cells from mouse embryos



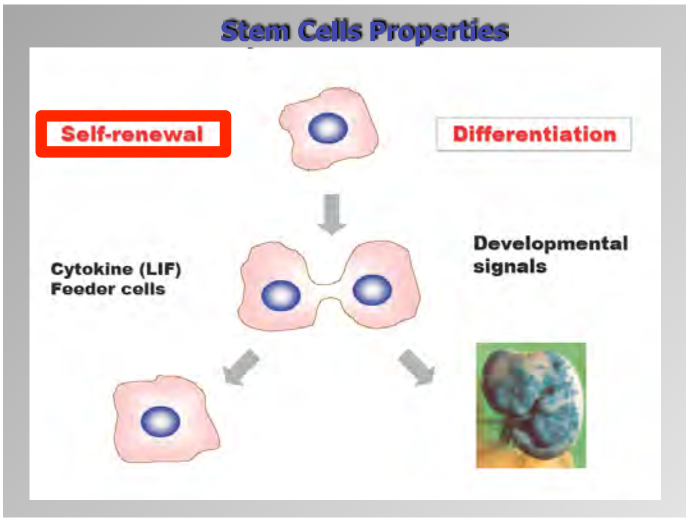


Evans MJ and Kaufman MH (1981), Nature 292, 154-156

Human Embryonic Stem cells



Thomson (1998), Science 282, 1145-1147



TRANSCRIPTIONAL PROFILE OF STEM CELLS

- Since all stem cells share fundamental biological properties, they may also share a set of molecular regulatory pathways
- Some components of these pathways may be preferentially expressed in stem cells
- It may be possible to define a general gene expression profile of the stem cell state, termed 'stemness'

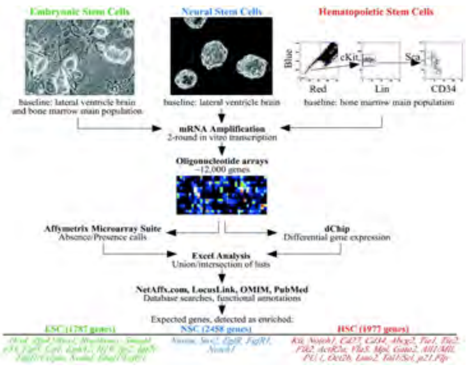
Burns & Zon (2002) Fig. 1

"Stemness": Transcriptional Profiling of Embryonic and Adult Stem Cells

Miguel Ramalho-Santos,[1] Soonsang Yoon,[2] Yumi Matsuzaki,[2] Richard C. Mulligan,[2] Douglas A. Melton[1]*

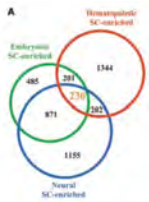
SCIENCE VOL 298 18 OCTOBER 2002

The **transcriptional profiles** of mouse embryonic, neural and hematopoietic stem cells were compared to define a genetic program for stem cells



The 216 genes enriched in all three stem cells.

Category	Genes
Signaling (35)	F2r (Thrombin R), Growth Hormone R, Integrin a6/b1, Adam9, Bystin, Ryk, Pkd2, shaker K+ channel b3, Grb1, Gab1, Kras2, Cttn, Cops 4/7a, Smad1/2, Tbrg1, Stam, Statip1, Cish2, Rock2, Yes, Yap, Ptpn2, Ppp1r2, Ywhab (14-3-3b), Ywhhb (14-3-3a), Auxotrophin, Trip6, Gfer (ALR), Upp, ESTs highly similar to Gap, ESTs highly similar to PPP2R1B, ESTs moderately similar to Jak3
Transcriptional regulation (14)	MyoD family inhibitor, Tead2, Yap, Four and a half LIM, Zfx, Zfp54, Rnf4, Chromodomain Helicase 1, Etl1, 4 ESTs highly similar to Zfp
DNA repair (4)	Ercc5, Xrcc5 (Ku80), Msh2 (MutS2), Rad23b
Cell cycle regulation (13)	Cyclin D1, P21, Cdkap1, Cell cycle progression 2, Gas2, CenpC, Wild-type p53 induced 1, Tmk, Uhrp, Gfr3, ESTs highly similar to exportin 1, ESTs highly similar to CAD, ESTs similar to Map4kk3
Cell death (3)	Gas2, Pdcd2, Wild-type p53 induced 1
RNA processing (9)	Sfrs3, Snrp1c, Phax, NOL5, RNA cyclase, ESTs highly similar to Sfrsb, ESTs highly similar to Prp6, ESTs highly similar to Nop56, ESTs highly similar to Ddx1
Translation (6)	Eif4ebp1, Eif4g2, Mrps31, Mrpl17, Mrpl34, ESTs highly similar to Eif3s1
Protein folding, chaperones (9)	Hspa1l (Hsc70t), Hspa4 (Hsp110), Dnajb6 (Mr Dnaj), Hspp12, Tcp1-rs1, peptidylprolyl isomerase C, FKBP 9, ESTs moderately similar to Fkbp13
Ubiquitin pathway (12)	Ube2d2, Ariadne 1, F-box only 8, Ubiquitin Protease XX, Urhp, Auxotrophin, Tpp2, Cops 4/7a, Nyren18 (Nub1), ESTs moderately similar to Ubc13 (bendless), ESTs highly similar to proteasome 26S subunit, non-ATPase, 12 (p55)
Vesicle traffic (5)	Rab18, Rabggtb, Stxbp3, Sec23a, ESTs moderately similar to Coatomer delta
Toxic stress response (6)	Abcb1 (Mdr1), Gsta4, Gclm, Thioredoxin reductase, Thioredoxin-like 32kD, Laptm4a
Other (8)	Reticulocalbin, Supt15h, Pla2g6, Acadm, Sacs, Pex7, Tjp1, Gcat
Unknown (180)	EST clusters with little or no homologies



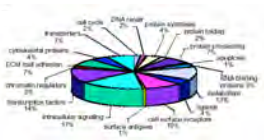
Venn diagram of the number of genes enriched in each stem cell population and their overlaps.

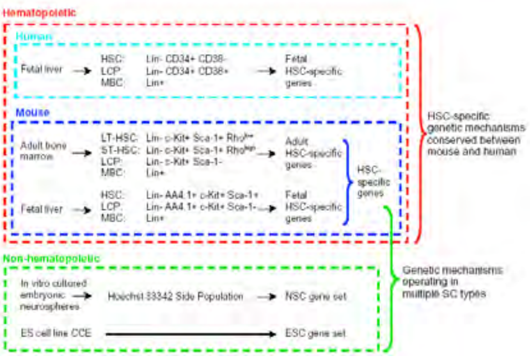
Note the high overlap between ESC- and NSC-enriched genes.

A Stem Cell Molecular Signature

Natalia B. Ivanova, John T. Dimos, Christoph Schaniel, Jason A. Hackney, Kateri A. Moore, Ihor R. Lemischka*

18 OCTOBER 2002 VOL 298 SCIENCE





Because all SCs share fundamental biological properties, they may share a core set of molecular regulatory pathways. Thus, it was tempting to define a general gene expression profile of the SC "state" (by using Affymetrix oligonucleotide arrays).

Intrinsically expressed transcription factors and extrinsically supplied cytokines are required to maintain ES cell pluripotency

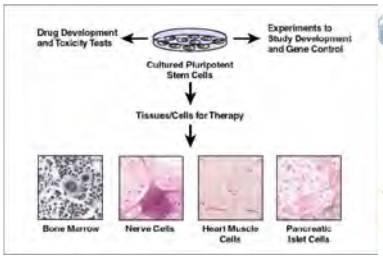
ES cells express characteristic transcription factors:

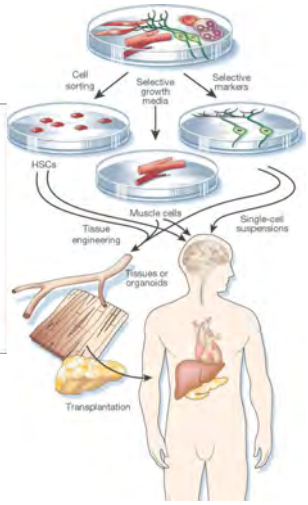
- **Oct-4**, encoded by *POU5f1*, a class V POU (Pit-Oct-Unc) domain protein
- **Nanog**, a novel homeodomain protein
- **Sox2**, an SRY-related HMG box protein
- Cell-surface markers are also expressed:
  - mES cells express SSEA-1 (stage specific embryonic antigen)
  - hES cells express SSEA-3 and SSEA-4
  - mES and hES cells express TRA1-60, TRA-81 and alkaline phosphatase

Mouse ES cells must also receive the cytokine LIF

Human ES cells are LIF-independent (Wnt signalling is the basis of pluripotency in human ES cells)

Transplantation of ES-derived cells

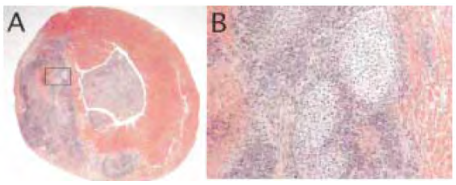




Major drawbacks
- medical concerns
- moral concerns
- political concerns
- immune rejection

ES cells transplanted into the heart develop into a teratoma:

- extensive replacement of the ventricular wall and cavity with tumor, with central necrosis
- Multiple nodules of cartilage (mesodermal) at the interface with host myocardium
- Several poorly differentiated epithelial cells
- Gut epithelium and ciliated respiratory epithelium (endodermal)
- Stratified squamous epithelium (ectodermal)



C. Murry (University of Washington, Seattle)

Clinical use of ES cells

ACT Advanced Cell Technology

Advanced Cell Technology Receives FDA Clearance For the First Clinical Trial Using Embryonic Stem Cells to Treat Macular Degeneration

FDA Lifts Clinical Hold, Company to Commence a Phase I/II Clinical Trial at Multiple Centers

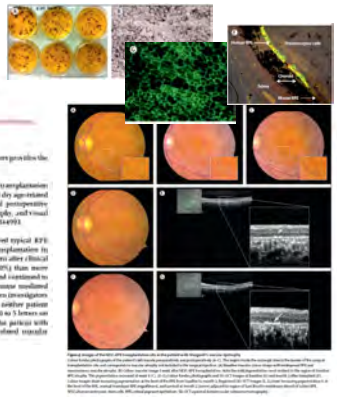
MARLBOROUGH, MA – November 22, 2010 – Advanced Cell Technology, Inc. ("ACT" OTCBB: ACTC) announced today that the US Food and Drug Administration (FDA) has cleared the Company's Investigational New Drug (IND) application to immediately initiate a Phase I/II multicenter clinical trial using retinal cells derived from human embryonic stem cells (hESCs) in ten patients with Stargardt's Macular Dystrophy (SMD), one of the most common forms of juvenile macular degeneration in the world. The decision removes the clinical hold that the FDA had placed on the trial.

Safety and Tolerability of Sub-retinal Transplantation of hESC Derived RPE (MA09-hRPE) Cells in Patients With Advanced Dry Age Related Macular Degeneration (Dry AMD)

Sponsor: Advanced Cell Technology

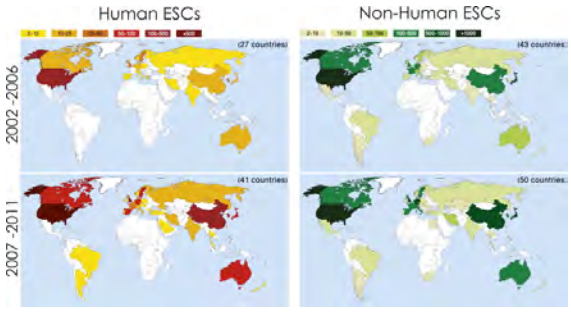
ClinicalTrials.gov Identifier: NCT01344993

Embryonic stem cell trials for macular degeneration: a preliminary report



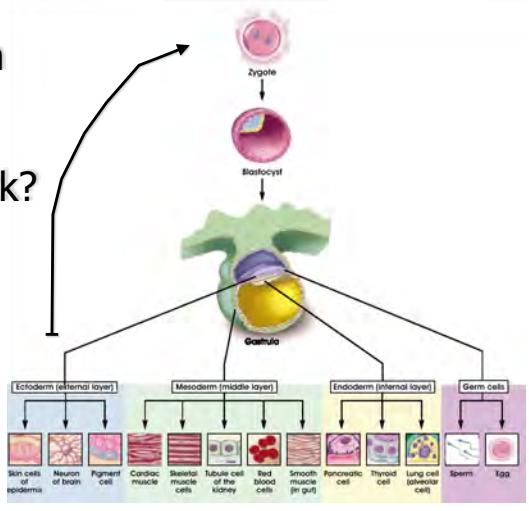
www.thelancet.com Vol 379 February 25, 2012

Expanding the boundaries of ES cells



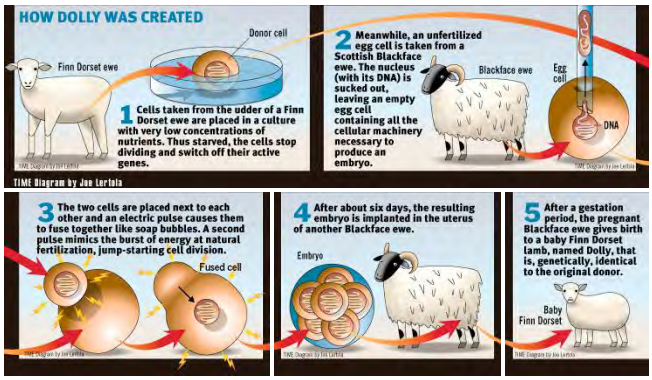
ESC Research Distribution throughout the WorldWorld maps comparing the distribution of stem cell research throughout the world between two 5 year periods: 2002–2006 and 2007–2011. The numbers of publications involving human and nonhuman ESCs were assessed separately and are thus presented in separate maps. Nonhuman ESCs are mostly, but not exclusively, mouse ESCs. The maps are color-coded by the absolute number of articles published by laboratories from each country. The total number of contributing countries during the examined years appears in the upper right side of each map. Articles dealing with iPSCs were removed from the analysis. Quantification of articles was carried out using "ISI Web of Science"

Can we go back?



CLONING and NUCLEAR REPROGRAMMING: running backward along cell differentiation

Cloning



Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei

T. Wakayama*†, A. C. F. Perry*‡, M. Zuccotti*§, K. R. Johnson‖ & R. Yanagimachi*

* Department of Anatomy and Reproductive Biology, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii 96822, USA
† Department of Veterinary Anatomy, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
‡ Department of Signalling, Babraham Institute, Cambridge CB2 4AT, UK
§ Dipartimento Biologia Animale, Laboratorio Biologia dello Sviluppo, University of Pavia, Piazza Botta 10, 27100, Pavia, Italy
‖ Jackson Laboratory, 600 Main Street, Bar Harbor, Maine 04609, USA

NATURE | VOL 394 | 23 JULY 1998

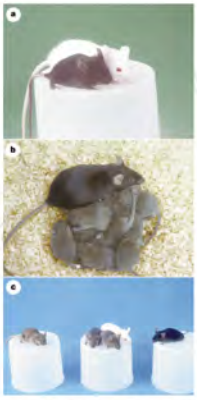


Figure 2 Cloned mice. a, The first surviving cloned mouse, Cumulina (born 3 October 1997) at four weeks (foreground) with her foster mother. b, Cumulina at 2.5 months with the pups she produced following mating with a CD-1 (albino) male. c, Two BIC2F1-derived, cloned, agouti young (centre) in front of their albino foster mother (CD-1), and a BIC2F1 oocyte donor (black, right). The two agouti offspring in the centre are clones (identical 'twin' sisters) of the agouti BIC2F1 cumulus donor shown on the left, and are two of the offspring described in series C (see text) and Table 2.

Production of goats by somatic cell nuclear transfer

Alexander Baguisi[2,7†], Esmail Behboodi[1†], David T. Melican[1], Julie S. Pollock[1], Margaret M. Destrempes[1], Christine Cammuso[1], Jennifer L. Williams[1], Scott D. Nims[1], Catherine A. Porter[1], Patricia Midura[1], Monica J. Palacios[2,3], Sandra L. Ayres[3], Richard S. Denniston[3], Michael L. Hayes[3], Carol A. Ziomek[1], Harry M. Meade[1], Robert A. Godke[2], William G. Gavin[1], Eric W. Overström[2,3*], and Yann Echelard[1*]

NATURE BIOTECHNOLOGY VOL 17 MAY 1999

Cloned pigs produced by nuclear transfer from adult somatic cells

Irina A. Polejaeva*, Shu-Hung Chen*, Todd D. Vaught*, Raymond L. Page*, June Mullins*, Sayapa Ball*, Yifan Dai*, Jeremy Boone*, Shawn Walker*, David L. Ayares*, Alan Colman* & Keith H. S. Campbell*†

NATURE | VOL 407 | 7 SEPTEMBER 2000

Since the first report of live mammals produced by nuclear transfer from a cultured differentiated cell population in 1995 (ref. 1), successful development has been obtained in sheep[2,3], cattle[4], mice[5] and goats[6] using a variety of somatic cell types as nuclear donors. The methodology used for embryo reconstruction in each of these species is essentially similar: diploid donor nuclei have been transplanted into enucleated MII oocytes that are activated on, or after transfer. In sheep[2] and goat[6] pre-activated oocytes have also proved successful as cytoplast recipients. The reconstructed embryos are then cultured and selected embryos transferred to surrogate recipients for development to term. In pigs, nuclear transfer has been significantly less successful; a single piglet was reported after transfer of a blastomere nucleus from a four-cell embryo to an enucleated oocyte[7]; however, no live offspring were obtained in studies using somatic cells such as diploid or mitotic fetal fibroblasts as nuclear donors[8,9]. The development of embryos reconstructed by nuclear transfer is dependent upon a range of factors. Here we investigate some of these factors and report the successful production of cloned piglets from a cultured adult somatic cell population using a new nuclear transfer procedure.

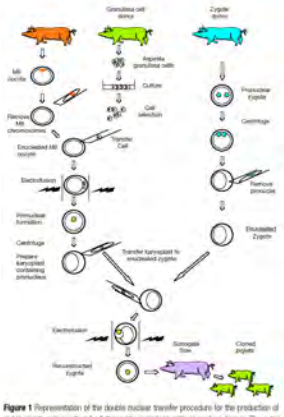


Figure 1 Representation of the double nuclear transfer procedure for the production of viable piglets using cultured adult somatic granulosa cells as nuclear donors. The outer circle in all the oocytes and embryos denotes the zona pellucida; the inner circle denotes the cell membrane.

Cloned rabbits produced by nuclear transfer from adult somatic cells

Patrick Chesné*, Pierre G. Adenot*, Céline Viglietta*, Michel Baratte*, Laurent Boulanger*, and Jean-Paul Renard*†

We have developed a method to produce live somatic clones in the rabbit, one of the mammalian species considered up to now as difficult to clone. To do so, we have modified current cloning protocols proven successful in other species by taking into account both the rapid kinetics of the cell cycle of rabbit embryos and the narrow window of time for their implantation after transfer into foster recipients. Although our method still has a low level of efficiency, it has produced several clones now proven to be fertile. Our work indicates that cloning can probably be carried out successfully in any mammalian species by taking into account physiological features of their oocytes and embryos. Our results will contribute to extending the use of rabbit models for biomedical research.

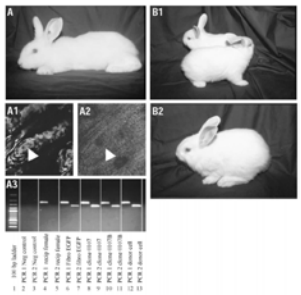


Figure 3. Rabbits born from somatic nuclear transfer. (A) Cloned rabbit 0107 with corresponding controls. (A1) expression of the EGFP protein fluorescence (arrowhead) detected by confocal microscopy from hair follicles obtained from an ear biopsy at 1 month of age; (A2) the same under transmission light; (A3) amplifications of the *EGFP* transgene (PCR 2) and of the exon 10 of the *CFTR* gene used as DNA quality control (PCR 1) with expected fragment sizes of 240 bp for the *CFTR* gene and 350 bp for the *EGFP* transgene. This confirms that rabbit 0107 and its littermate 107b (who died 1 day after birth) were derived from the donor cumulus cell. (B1, B2) Three other rabbits from two different litters; rabbits in B1 have now proved to be fertile.


brief communications

A cat cloned by nuclear transplantation

Biol Reprod 1997 Aug;57(2):454-9

Rhesus monkeys produced by nuclear transfer.

Meng L., Ely JJ, Stouffer RL., Wolf DP.

Division of Reproductive Sciences, Oregon Regional Primate Research Center, Beaverton 97006-3499, USA.

Genetically identical nonhuman primates can provide a powerful animal model for gene therapy and research activities where the physiological parameters directly or indirectly under study are heritable. Here we demonstrate that nuclear transfer is a viable technology for the production of identical rhesus macaques. Oocytes recovered from gonadotropin-treated females were enucleated by aspiration of the first polar body and underlying ooplasm, then activated by cycloheximide exposure. Individual diploid blastomeres, recovered from in vitro-fertilization-produced embryos (either fresh or frozen-thawed) and used as nuclear donors, were injected under the zona pellucida of enucleated (chromosome-free) oocytes and fused by electric pulses. The reconstituted embryos were cocultured on buffalo rat liver cells before cryostorage and transfer to synchronized host mothers. Of the 9 females receiving a total of 29 reconstituted embryos, 3 became pregnant, with two live births resulting, one male and one female. The parentage of both infants was established unequivocally by genotype analysis at 7 highly variable short tandem repeat loci.


Neti and Ditto

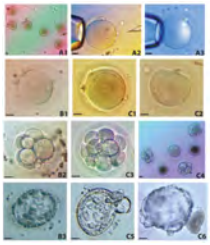
Although Dolly was born 15 years ago, cloning human embryos became possible only recently

STEM CELLS
TECHNOLOGY DEVELOPMENT

Development of Human Cloned Blastocysts Following Somatic Cell Nuclear Transfer with Adult Fibroblasts

STEM CELLS 2008;26:485–493 www.StemCells.com

Human Embryos Derived by Somatic Cell Nuclear Transfer Using an Alternative Enucleation Approach



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,[3] 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,[3] Curie Ahn,[5] Jung Hye Hwang,[6] Ky Young Park,[7] Jose B. Cibelli,[8] Shin Yong Moon[5]*

Somatic cell nuclear transfer (SCNT) technology has recently been used to generate animals with a common genetic composition. In this study, we report the derivation of a pluripotent embryonic stem (ES) cell line (SCNT-hES-1) from a cloned human blastocyst. The SCNT-hES-1 cells displayed typical ES cell morphology and cell surface markers and were capable of differentiating into embryoid bodies in vitro and of forming teratomas in vivo containing cell derivatives from all three embryonic germ layers in severe combined immunodeficient mice. After continuous proliferation for more than 70 passages, SCNT-hES-1 cells maintained normal karyotypes and were genetically identical to the somatic nuclear donor cells. Although we cannot completely exclude the possibility that the cells had a parthenogenetic origin, imprinting analyses support a SCNT origin of the derived human ES cells.

[1]College of Veterinary Medicine, [2]School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea. [3]Medical Research Center, MizMedi Hospital, Seoul, 135-280, Korea. [4]Gachon Medical School, Incheon, 417-840, Korea. [5]College of Medicine, Seoul National University, Seoul, 110-744, Korea. [6]School of Medicine, Hanyang University, Seoul, 471-701, Korea. [7]College of Natural Science, Sunchon National University, Sunchon, 540-742, Korea. [8]Department of Animal Science-Physiology, Michigan State University, East Lansing, MI 48824, USA.

SCIENCE VOL 303 12 MARCH 2004

Woo-Suk Hwang & the problem of research misconduct

"He was a national hero in South Korea, his research lab was probably one of the best funded in the world, and he flew first class anywhere he wanted, any time he wanted, for free, courtesy of Korean Air. He was treated like a rock star. His spectacular fall from one of the most envied positions in science plays out like a Greek tragedy."

Stephen Minger: *The Fall of a Scientific "Rock Star"*. BBC online: Tuesday, 10 January 2006, 17:53 GMT. http://news.bbc.co.uk/1/hi/sci/tech/4599974.stm

Originally published in Science Express on 12 February 2004
Science 12 March 2004:
Vol. 303. no. 5664, pp. 1669 - 1674
DOI: 10.1126/science.1094515

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REPORTS

<u>This article has been retracted</u>

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,[3] 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,[3] Curie Ahn,[5] Jung Hye Hwang,[6] Ky Young Park,[7] Jose B. Cibelli,[8] Shin Yong Moon[5]*

Somatic cell nuclear transfer (SCNT) technology has recently been used to generate animals with a common genetic composition. In this study, we report the derivation of a pluripotent embryonic stem (ES) cell line (SCNT-hES-1) from a cloned human blastocyst. The SCNT-hES-1 cells displayed typical ES cell morphology and cell surface markers and were capable of differentiating into embryoid bodies in vitro and of forming teratomas in vivo containing cell derivatives from all three embryonic germ layers in severe combined immunodeficient mice. After continuous proliferation for more than 70 passages, SCNT-hES-1 cells maintained normal karyotypes and were genetically identical to the somatic nuclear donor cells. Although we cannot completely exclude the possibility that the cells had a parthenogenetic origin, imprinting analyses support a SCNT origin of the derived human ES cells.

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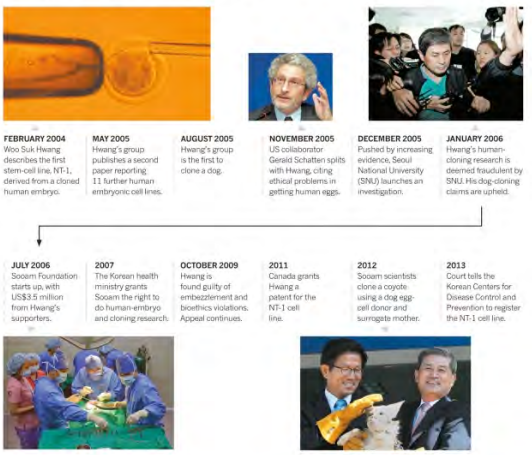
[5] College of Medicine, Seoul National University, Seoul, 110-744, Korea

THE RISE AND FALL AND RISE OF WOO SUK HWANG



FEBRUARY 2004 Woo Suk Hwang describes the first stem-cell line, NT-1, derived from a cloned human embryo.

MAY 2005 Hwang's group publishes a second paper reporting 11 further human embryonic cell lines.

AUGUST 2005 Hwang's group is the first to clone a dog.

NOVEMBER 2005 US collaborator Gerald Schatten splits with Hwang, citing ethical problems in getting human eggs.

DECEMBER 2005 Pushed by increasing evidence, Seoul National University (SNU) launches an investigation.

JANUARY 2006 Hwang's human-cloning research is deemed fraudulent by SNU. His dog-cloning claims are upheld.

JULY 2006 Sooam Foundation starts up, with US$3.5 million from Hwang's supporters.

2007 The Korean health ministry grants Sooam the right to do human-embryo and cloning research.

OCTOBER 2009 Hwang is found guilty of embezzlement and bioethics violations. Appeal continues.

2011 Canada grants Hwang a patent for the NT-1 cell line.

2012 Sooam scientists clone a coyote using a dog egg-cell donor and surrogate mother.

2013 Court tells the Korean Centers for Disease Control and Prevention to register the NT-1 cell line.

Woo-Suk Hwang, cloning of Snuppy

Hwang WS, *et al.* (2005). "Dogs cloned from adult somatic cells". *Nature* **436** (7051): 641. PMID 16079832 DOI:10.1038/436641a.

theguardian

SHORTCUTS
BLOG
A SIDEWAYS LOOK AT THE NEWS

If your dog is about to die, why not clone it?

A researcher in South Korea claims he can clone your pet. All he needs is some tissue from the animal and £66,000

Spot the difference ... The spots on a dalmatian clone will be different from the original. Photograph: Alamy

Insung Hwang's business, according to his website, is "healing broken hearts". Specifically those of people who have lost a beloved dog. Now he is to offer his therapeutic services in the UK.

PER SAPERNE DI PIÙ
www.continental.com
www.state.gov
19

Stati Uniti

Cinque cloni di Trakr, il cane-eroe trovò l'ultimo superstite dell'11/9

Trakr, il cane eroe dell'11 settembre

WASHINGTON — Trakr, il cane-eroe dell'11 settembre trovò l'ultimo sopravvissuto a Ground Zero, è stato clonato. A eseguire l'operazione, in Corea del Sud, è stato il controverso professore Hwang Woo-Suk, che nel 2005 clonò il primo cane. È stato lo stesso padrone di Trakr a volere che il suo cane, morto due mesi fa, venisse clonato. E ora cinque piccole "copie" dell'eroe canino potranno essere comprate per 100 mila dollari.

Applications of cloning

- Treatment of human infertility NO!
- Transgenic animals for drug production
- Genetic rescue of endangered mammals
- Animal organs for human xenotransplantation
- Therapeutic cloning for human stem cell therapy
- Human tissue and organ engineering
- Rescue of genetic defect by ex vivo gene therapy

Reproductive cloning for human infertility, besides ethically questionable, is highly inefficient

Low efficiency of nuclear transfer
High rate of abnormal embryonic development
"Large offspring syndrome"
- placental abnormalities
- fetal overgrowth
- respiratory failure
- high incidence of neonatal abnormalities

Are there any normal cloned mammals?

The finding that cloned mice, produced by transfer of nuclei from cumulus cells, develop obesity but do not transmit the phenotype to their offspring provides further evidence that cloned embryos are vulnerable to epigenetic change. (pages 262–267)

IAN WILMUT

NATURE MEDICINE • VOLUME 8 • NUMBER 3 • MARCH 2002

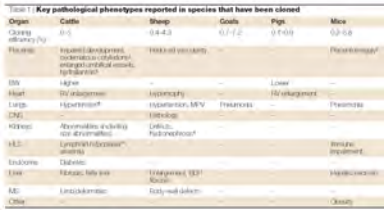
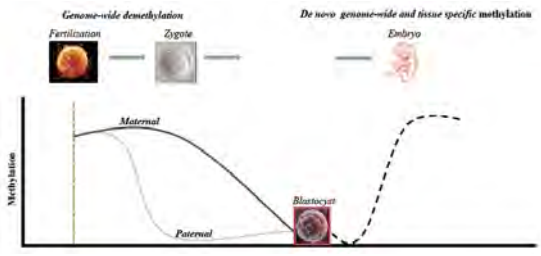


Table 1 | **Key pathological phenotypes reported in species that have been cloned**

Organ	Cattle	Sheep	Goats	Pigs	Mice
Placenta	Impaired development, oedematous cotyledons/ enlarged umbilical vessels, hydrallantois*	Thickened vessels			Placentomegaly*
Liver	Higher			Lower	
Heart	RV enlargement	Hypertrophy		RV enlargement	
Lungs	Hypertension*	Hypertension, RVH	Pneumonia		Pneumonia
CNS		Lissencephaly			
Kidneys	Abnormalities including size abnormalities	Urinary tract abnormalities, Pyelonephritis*			
Immune system	Lymphohistiocytosis*, anaemia				Immune impairment
Endocrine	Diabetes				
Liver	Fibrosis, fatty liver	Enlarged, fatty, fibrotic			Hepatocytomegaly
Muscle	Limb deformities	Stiffness, body-wall defects			
Other					Obesity

Normal embryo development requires extensive genome demethylation



Between fertilization and implantation, the embryo demethylates most of its genes, with the exception of imprinted and some repeat genes. The maintenance of imprinted genes through the preimplantation period is essential for normal embryonic development. However, demethylation of other genes is important to make the genome broadly available to the undifferentiated and developing embryo.

Demethylation in the embryo may help remove the epigenetic modifications acquired during parental gametogenesis

Human somatic cell nuclear transfer and cloning

The Ethics Committee of the American Society for Reproductive Medicine

American Society for Reproductive Medicine, Birmingham, Alabama

This document presents arguments that conclude that it is unethical to use somatic cell nuclear transfer (SCNT) for infertility treatment due to concerns about safety; the unknown impact of SCNT on children, families, and society; and the availability of other ethically acceptable means of assisted reproduction. This document replaces the ASRM Ethics Committee report titled, "Human somatic cell nuclear transfer (cloning)," last published in Fertil Steril 2000;74:873-6. (Fertil Steril® 2012;98:804–7. ©2012 by American Society for Reproductive Medicine.)

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VOL. 98 NO. 4 / OCTOBER 2012

Applications of cloning

- Treatment of human infertility NO!
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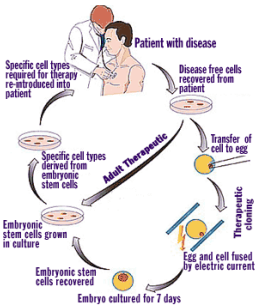
- Genetic rescue of endangered mammals
- Animal organs for human xenotransplantation
- Therapeutic cloning for human stem cell production for tissue and organ regeneration
- Rescue of genetic defect by ex vivo gene therapy

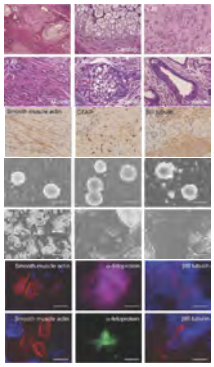
Therapeutic cloning



Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Cell 126, 663-676, August 25, 2006 ©2006 Elsevier Inc.

Induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, **Oct3/4, Sox2, c-Myc**, and **Klf4** in the FBX15 locus, under ES cell culture conditions.



Various tissues present in teratomas derived from iPS

Neural tissues and muscles in teratomas

In vitro embryoid body formation and differentiation

In vitro differentiation into all three germ layers.

These cells, which were designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes.

1- Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers.

2- Following injection into blastocysts, iPS cells contributed to mouse embryonic development, but embryos failed to develop beyond mid-gestation stage.

iPS derivation from human skin cells

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]

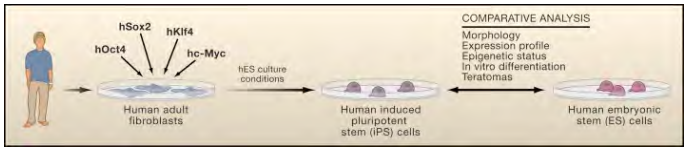
DOI 10.1016/j.cell.2007.11.019

Reprogramming of human somatic cells to pluripotency with defined factors

Vol 451|10 January 2008|doi:10.1038/nature06534

Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

www.sciencemag.org SCIENCE VOL 318 21 DECEMBER 2007



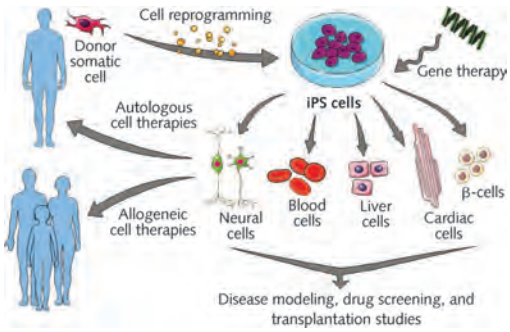
Induced pluripotent stem cells: the new patient?

Milena Bellin[1], Maria C. Marchetto[2], Fred H. Gage[2] and Christine L. Mummery[1]

Method of reprogramming	Delivery method	Percentage of publications*	Refs (disease modelling)
Integrating			
Viral*	Retrovirus	76%	37,49,50,59,62-67, 71-73,76,77,95-134
	Lentivirus	20%	38,51,59,61,68-70,72,74, 99,104,131,135-138
Non-viral	Transposon (excisable)	–	–
Non-Integrating			
Viral*	Adenovirus	–	–
	Sendai virus	–	–
Non-viral	mRNA	–	–
	miRNA	–	–
	Small molecules	–	–
	Episomal vectors	4%	124,139,140
	Protein	–	–

*Approximate calculation was performed by Pubmed advanced searching on disease modelling studies that used iPS cells over the past 5 years. *National regulations surrounding the use of retroviruses, lentiviruses and Sendai viruses vary among countries (for example, in the USA retrovirally-derived cell lines are considered virus-free after two passages, whereas in parts of Europe these cell lines can never leave bio-safety level II laboratories).

Medical use of iPS cells: iPS as an ethical alternative to ES cells?



Derivation and cardiomyocyte differentiation of induced pluripotent stem cells from heart failure patients

Limor Zwi-Dantsis[1,2], Irit Huber[1], Marhal Habib[1], Aaron Winterstern[1], Amira Gepstein[1], Gil Arbel[1] and Lior Gepstein[1,2,*]
+ Author Affiliations
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Received April 4, 2011.
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Accepted March 22, 2012.

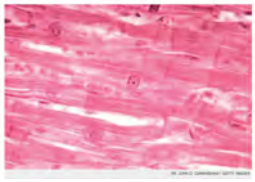
Abstract

Aims Myocardial cell replacement therapies are hampered by a paucity of sources for human cardiomyocytes and by the expected immune rejection of allogeneic cell grafts. The ability to derive patient-specific human-induced pluripotent stem cells (hiPSCs) may provide a solution to these challenges. We aimed to derive hiPSCs from heart failure (HF) patients, to induce their cardiomyocyte differentiation, to characterize the generated hiPSC-derived cardiomyocytes (hiPSC-CMs), and to evaluate their ability to integrate with pre-existing cardiac tissue.

Methods and results Dermal fibroblasts from two HF patients were reprogrammed by retroviral delivery of *Oct4, Sox2*, and *Klf4* or by using an excisable polycistronic lentiviral vector. The resulting HF-hiPSCs displayed adequate reprogramming properties and could be induced to differentiate into cardiomyocytes with the same efficiency as control hiPSCs (derived from human foreskin fibroblasts). Gene expression and immunostaining studies confirmed the cardiomyocyte phenotype of the differentiating HF-hiPSC-CMs. Multi-electrode array recordings revealed the development of a functional cardiac syncytium and adequate chronotropic responses to adrenergic and cholinergic stimulation. Next, functional integration and synchronized electrical activities were demonstrated between hiPSC-CMs and neonatal rat cardiomyocytes in co-culture studies. Finally, *in vivo* transplantation studies in the rat heart revealed the ability of the HF-hiPSC-CMs to engraft, survive, and structurally integrate with host cardiomyocytes.

HEART DISEASE

Scientists Turn Human Skin Cells Into Healthy Heart Cells



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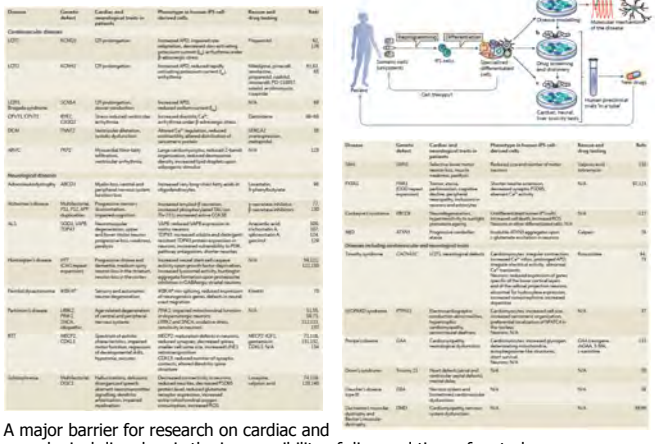
News › Science › Stem cells

Skin from heart attack patients transformed into beating heart cells

The heart cells created from patients' skin were at the same stage of development as those of a newborn baby

Ian Sample, science correspondent
guardian.co.uk, Wednesday 23 May 2012 00.06 BST
Comments (38)

Disease modelling using human iPS



A major barrier for research on cardiac and neurological disorders is the inaccessibility of diseased tissue for study

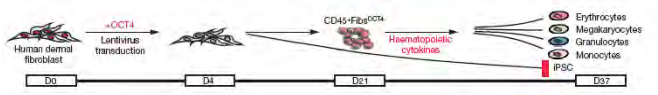
iPS vs ES cells

- Although they have similar phenotypes they are not identical
- Significant differences in their gene expression profile
- iPS cells retain an epigenetic memory of their tissue of origin
- Several methods for reprogramming now exist
- iPS cells can cause teratoma - 2 of the 4 transgenes are known to be oncogenic; retroviruses and lentiviruses used as vectors can cause insertional mutagenesis
- The source and age of donor cells can affect reprogramming (the more differentiated the donor cell is the more difficult it is to wind back its developmental clock)

Transdifferentiation: the direct reprogramming of one somatic cell type into another without a stem cell intermediate

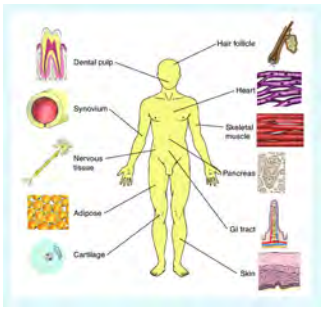
ARTICLE

Direct conversion of human fibroblasts to multilineage blood progenitors

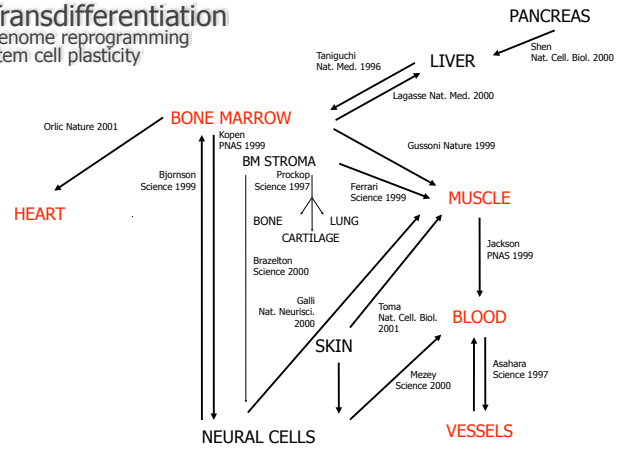


Sources of adult stem cells

- Bone marrow: *HSC and MSC*
- Peripheral blood: *HSC, hemangioblast?*
- Brain and spinal cord: *neural stem cells*
- Skin: *epidermal stem cells*
- Liver: *oval cells*
- Pancreas: *ductal stem cells*
- Eye: *corneal and retinal stem cells*
- Skeletal muscle: *satellite cells and SP*
- Heart: *cardiac stem cells*



Transdifferentiation
Genome reprogramming
Stem cell plasticity

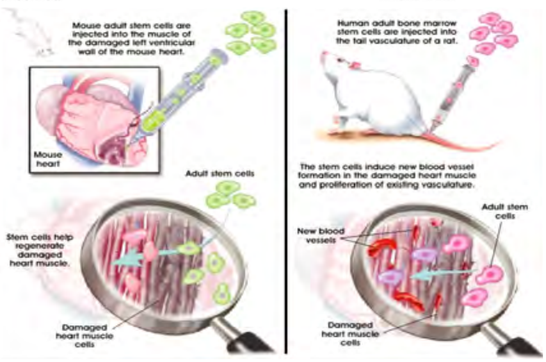


Bone marrow stem cells can repair damaged heart muscle?

Bone marrow cells regenerate infarcted myocardium

Donald Orlic†, Jan Kajstura*, Stefano Chimenti*, Igor Jakoniuk*, Stacie M. Anderson†, Baosheng Li*, James Pickel‡, Ronald McKay‡, Bernardo Nadal-Ginard*, David M. Bodine†, Annarosa Leri* & Piero Anversa*

Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function



Bone marrow cells regenerate infarcted myocardium

Donald Orlic†, Jan Kajstura*, Stefano Chimenti*, Igor Jakoniuk*, Stacie M. Anderson†, Baosheng Li*, James Pickel‡, Ronald McKay‡, Bernardo Nadal-Ginard*, David M. Bodine†, Annarosa Leri* & Piero Anversa*

* *Department of Medicine, New York Medical College, Valhalla, New York 10595, USA*
† *Hematopoiesis Section, Genetics and Molecular Biology Branch, NHGRI, and*
‡ *Laboratory of Molecular Biology, NINDS, NIH, Bethesda, Maryland 20892, USA*

Myocardial infarction leads to loss of tissue and impairment of cardiac performance. The remaining myocytes are unable to reconstitute the necrotic tissue, and the post-infarcted heart deteriorates with time[1]. Injury to a target organ is sensed by distant stem cells, which migrate to the site of damage and undergo alternate stem cell differentiation[2–5]; these events promote structural and functional repair[6–8]. This high degree of stem cell plasticity prompted us to test whether dead myocardium could be restored by transplanting bone marrow cells in infarcted mice. We sorted lineage-negative (Lin^-) bone marrow cells from transgenic mice expressing enhanced green fluorescent protein[9] by fluorescence-activated cell sorting on the basis of *c-kit* expression[10]. Shortly after coronary ligation, Lin^- *c-kit*^{POS} cells were injected in the contracting wall bordering the infarct. Here we report that newly formed myocardium occupied 68% of the infarcted portion of the ventricle 9 days after transplanting the bone marrow cells. The developing tissue comprised proliferating myocytes and vascular structures. Our studies indicate that locally delivered bone marrow cells can generate *de novo* myocardium, ameliorating the outcome of coronary artery disease.

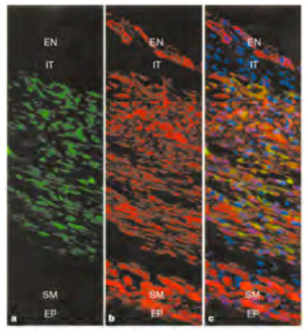


Figure 2 Myocardial infarct injected with Lin^- c-kit^{POS} cells; myocardium is regenerating from endocardium (EN) to epicardium (EP). **a**, EGFP (green), **b**, cardiac myosin (red), **c**, combination of EGFP and myosin (red–green), and propidium iodide stained nuclei (blue). Infarcted tissue (IT) can be seen in the subendocardium; spared myocytes (SM) can be seen in the subepicardium. Original magnification, ×250 (**a–c**).

The Bone Marrow—Cardiac Axis of Myocardial Regeneration

Table 2. Summary of Major Cell-Based/Cytokine Clinical Trials

Study	Method of Delivery	Patients Treated/ Control	Placebo/ Control	Cell Type Cell Number or Dose	Time of Cell Delivery (Days Post-MI)	Results	Reference
Strauer	Intracoronary	10/10 30 (CPC)	Control	BM-MNC 9×10^6 to 2.8×10^7	7	Improved contractility and reduced infarct size	Strauer et al, 2002[103]
TOPCARE-AMI	Intracoronary	29 (BM-MNC)	N/A	CPC 1.3×10^7 BM-MNC 2.4×10^8	3-7	Improved EF and reduced infarct size	Assmus et al, 2002[104] Britten et al, 2003[105] Schachinger et al, 2004[106]
BOOST	Intracoronary	30/30	Control	BM-MNC 24×10^9	6	Improved EF at 6 mo No difference at 18 mo	Wollert et al, 2004[107] Meyer et al, 2006[108]
Janssens	Intracoronary	33/34	Placebo	BM-MNC 3.0×10^8 cells	1	No effect	Janssens et al, 2006[109]
Chen	Intracoronary	34/35	Placebo	MSC 48×10^{10} to 60×10^{10}	18	Improved and perfusion at 3 mo	Chen et al, 2004[110]
REPAIR-AMI	Intracoronary	102/102	Placebo	BM-MNC 2.4×10^8	4	Improved EF and reduced infarct size at 4 mo	Schachinger et al, 2006[111]
ASTAMI	Intracoronary	100	Control	BM-MNC 8.7×10^7	5-8	No difference at 6 mo	Lunde et al, 2006[112]
FIRSTLINE-AMI	Mobilization	25/25	Control	G-CSF 10 μg/kg BW	0-6 1QD	Improved EF and remodeling at 4 mo	Ince et al, 2005[113]
STEMMI	Mobilization	39/39	Placebo	G-CSF 10 μg/kg BW	0-6 1QD	No difference at 6 mo	Ripa et al, 2006[114]
REVIVAL II	Mobilization	56/58	Placebo	G-CSF 10 μg/kg BW	0-5 1QD	No difference at 6 mo	Zohlnhoefer et al, 2006[115]

Few studies with placebo control group: no reduction in the infarcted area!

G-CSF: discontinuation because of restenosis

Progress in Cardiovascular Diseases, Vol. 50 No. 1 (July/August), 2007: pp 18-30

Adult Bone Marrow–Derived Cells for Cardiac Repair

A Systematic Review and Meta-analysis

ARCH INTERN MED/VOL 167, MAY 28, 2007

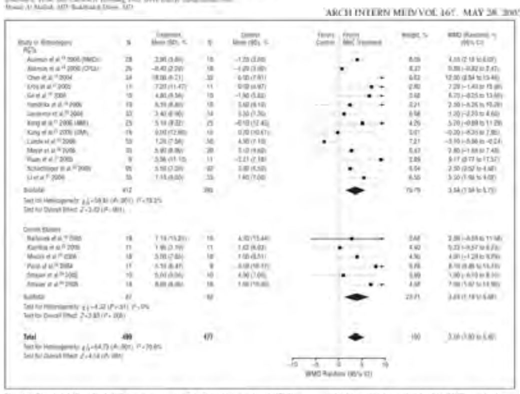


Figure 2. Forest plot of unadjusted difference in mean (with 95% confidence intervals (CIs)) improvement in left ventricular ejection fraction (LVEF) in patients treated with bone marrow–derived cells (BMCs) compared with controls. The figure shows the summary of cohort studies and randomized controlled trials (RCTs). Transplantation with BMCs resulted in a 3.66% (95% CI, 1.93% to 5.40%) increase in mean LVEF. The overall effect was statistically significant in favor of BMC therapy. AMI indicates acute myocardial infarction; CPCs, circulating progenitor cells; OMI, old myocardial infarction; and WMD, weighted mean difference.

Stem cells

Lost in translation

Kenneth R. Chien

The potential use of stem cells as agents of repair in human disease makes them the subject of high-profile studies. But we should be wary of prematurely pushing laboratory research into clinical practice.

Figure 1 Two strategies used to show that bone-marrow stem cells do not take on the role of damaged heart cells. a, Murry *et al.*[2] isolated and purified genetically modified bone-marrow stem cells from mice. The modification 'tagged' the cells (with *LacZ*), enabling them to be detected in the recipient mouse heart, into which the cells were directly injected. Closer inspection of the recipient heart showed that the label could not be detected in heart muscle cells. b, Similar results were shown by Balsam *et al.*[3], although the approach was slightly different. Donor bone-marrow stem cells were transfused directly into the circulation of recipients. Again, the tag (GFP; green fluorescent protein) could not be detected in heart muscle cells of the donor; indeed, the bone-marrow cells continued to differentiate into blood cells while in the heart.

Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium

Leora B. Balsam[1], Amy J. Wagers[2,3], Julie L. Christensen[2,3], Theo Kofidis[1], Irving L. Weissman[2,3] & Robert C. Robbins[1]

[1]*Departments of Cardiothoracic Surgery,* [2]*Pathology, and* [3]*Developmental Biology, Stanford University School of Medicine, Stanford, California 94305, USA*

Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts

Charles E. Murry[1], Mark H. Soonpaa[2], Hans Reinecke[1], Hidehiro Nakajima[2], Hisako O. Nakajima[2], Michael Rubart[2], Kishore B. S. Pasumarthi[2*], Jitka Ismail Virag[1], Stephen H. Bartelmez[3], Veronica Poppa[1], Gillian Bradford[2], Joshua D. Dowell[2], David A. Williams[2*] & Loren J. Field[2]

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[2]*Wells Center for Pediatric Research, Indiana University, 1044 West Walnut Street, R4 Bldg, Room W376, Indianapolis 46202-5225, USA*
[3]*Department of Pathobiology, University of Washington, Seattle, Washington 98195, USA*

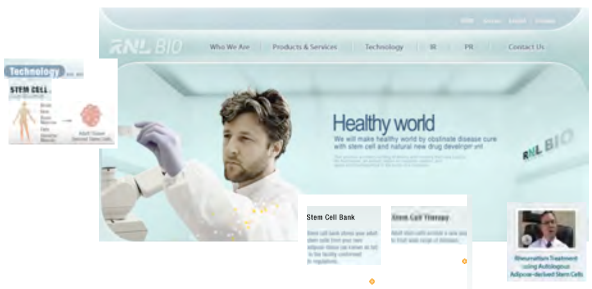
NATURE | doi:10.1038/nature02460 | www.nature.com/nature

Stem Cell Tourism

NewScientist

First case of alleged stem-cell fraud enters US courts

Six residents of Los Angeles, California, are suing South Korean company RNL Bio and associates in a Californian court for alleged fraud. They claim the company convinced them to travel to clinics in South Korea, China or Mexico to donate fat tissue and have stem cells from it re-administered to cure diseases and even reverse ageing.



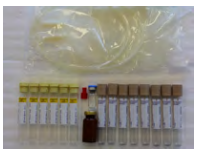
New Scientist, 13 July 2012

AdiStem™

AdiLight-1™ (LED)

Activates Adipose-Derived Stem Cells through photomodulation. Also used in autologous platelet rich plasma preparations as it modulates cytokine release from monocytes.

Made to order. 4 weeks delivery.

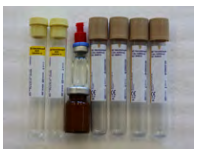


AdiLight-1™ Kits (Intravenous)

For Extraction of Adipose-Derived Stem Cells from 100ml to 400 ml of Lipoaspirated Fat.

For laboratory or research use only.

Used in clinical trials for lung diseases, brain injury and autism.



AdiLight-1™ Mini-Kits (Osteoarthritis)

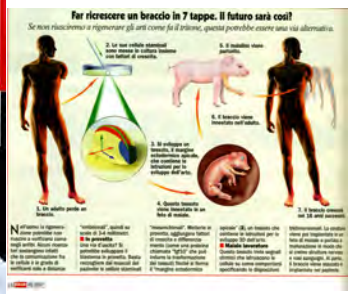
For Extraction of Adipose-Derived Stem Cells from 50ml of Lipoaspirated Fat.

Used in topical procedures such as intra-articular injection for osteoarthritis of the knee and hip, cosmetic surgery and acne scarring, dermal injection, stem cell enriched fat transfer, wounds and chronic ulcers.

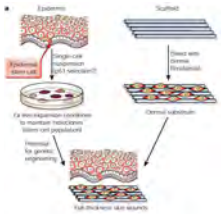
AdiStem®
Technology

Stem Cell Therapy is Happening Now

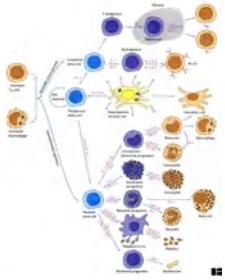
Cell - Enhanced Anti Aging



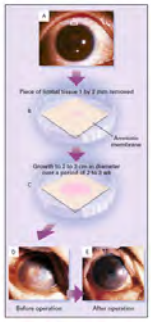
Skin autografts produced by stem cell derived keratinocytes



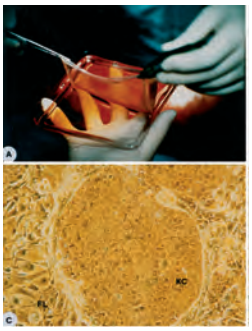
Hematopoietic stem cells



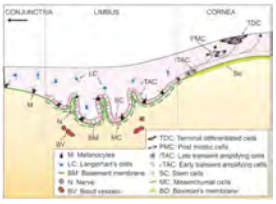
Limbal epithelial stem cells for corneal regeneration

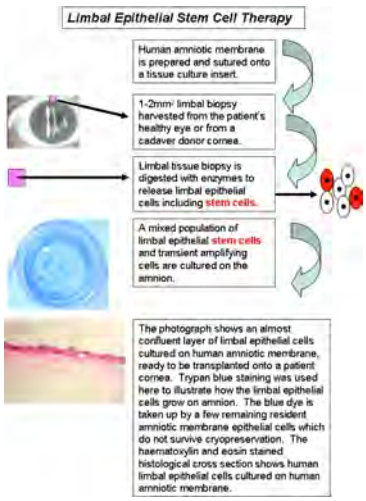


THE CONTROL OF EPIDERMAL STEM CELLS (HOLOCLONES) IN THE TREATMENT OF MASSIVE FULL-THICKNESS BURNS WITH AUTOLOGOUS KERATINOCYTES CULTURED ON FIBRIN[1]



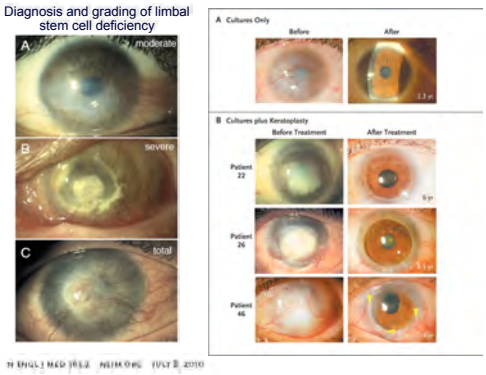
Limbal Stem Cells





Limbal Stem-Cell Therapy and Long-Term Corneal Regeneration

Paolo Rama, M.D., Stanislav Matuska, M.D., Giorgio Paganoni, M.D., Alessandra Spinelli, M.D., Michele De Luca, M.D., and Graziella Pellegrini, Ph.D.



All eyes had total LSCD, complete corneal opacification, and stromal scarring. Vision was reduced to counting fingers or perceiving hand movements. Autologous LSC cultures successfully regenerated functional corneal epithelium. To improve their visual acuity after grafting, the patients underwent penetrating keratoplasty. In all eyes, the engrafted LSC resurfaced the donor stroma. At the last follow-up visits, all eyes were covered by stable corneal epithelium. In Patient 46, the follow-up image shows that the conjunctival vessels stop at the conjunctival–corneal boundary (arrowheads); they do not invade the restored corneal surface.

N ENGL J MED 363;2 NEJM.ORG JULY 8, 2010