

# Stem Cells & Cell-Based Therapy

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# Current Life Science Topics

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1. Fundamentals in Basic Biochemistry & Cell Biology
2. Introduction to Tissue Engineering & Regenerative Medicine
3. Developmental Tissue Reconstruction
4. Wound Healing & Regeneration
5. Natural Tissue Composition & Cell-ECM Interaction
6. Stem Cells & Cell-Based Therapy
7. Biomaterials
8. *Mid-Term Exam*
9. Mechano-transduction & Bioreactors
10. Discussions on Tissue Reconstruction
11. Regulation & Ethics
12. AI in Current Life Science
13. Machine Learning & Github
14. Deep Neural Network
15. Convolutional Neural Network
16. *Final Exam*

# Study Materials

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**Principles of Regenerative Medicine by A. Atala A, Chapter 7**

**Vierbuchen T 2012 Molecular Cell**

**Vierbuchen T 2011 Nat Biotechnology**

**Takahashi K 2006 Cell**

**Research articles of CSDB group in NIH/NIDCR**

# classification of stem cells

# Definition of stem cells

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**Tissue-specific stem cell:**

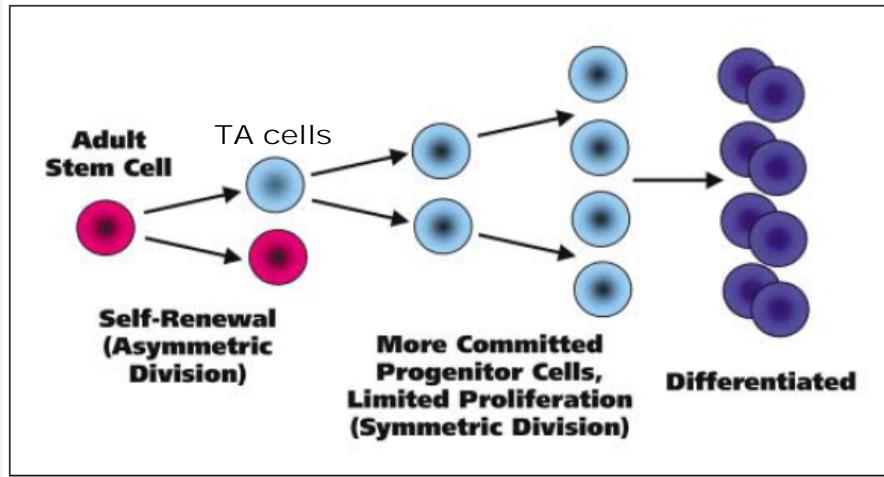
**A single stem cell has the ability to completely reconstitute an entire tissue in the body**

**Stem cell has the ability to self-renew and maintain itself for the lifetime of the organism**

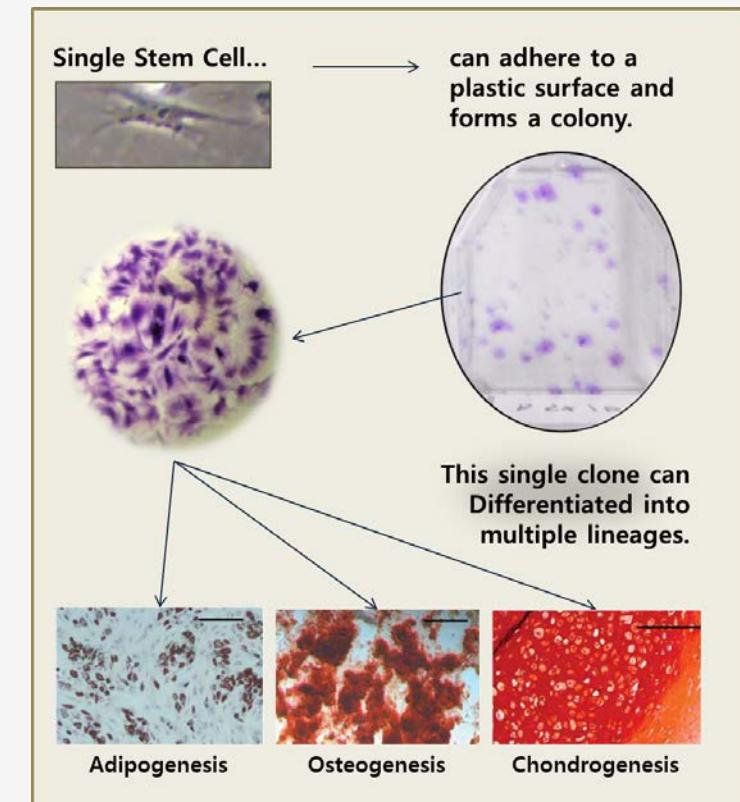
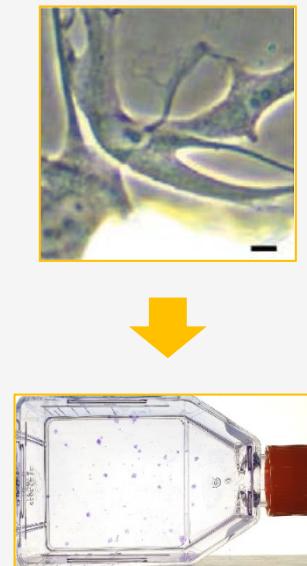
# What is a Stem Cell?

## A stem cell...

### 1. Self-renews



### 2. Forms multiple cell types.



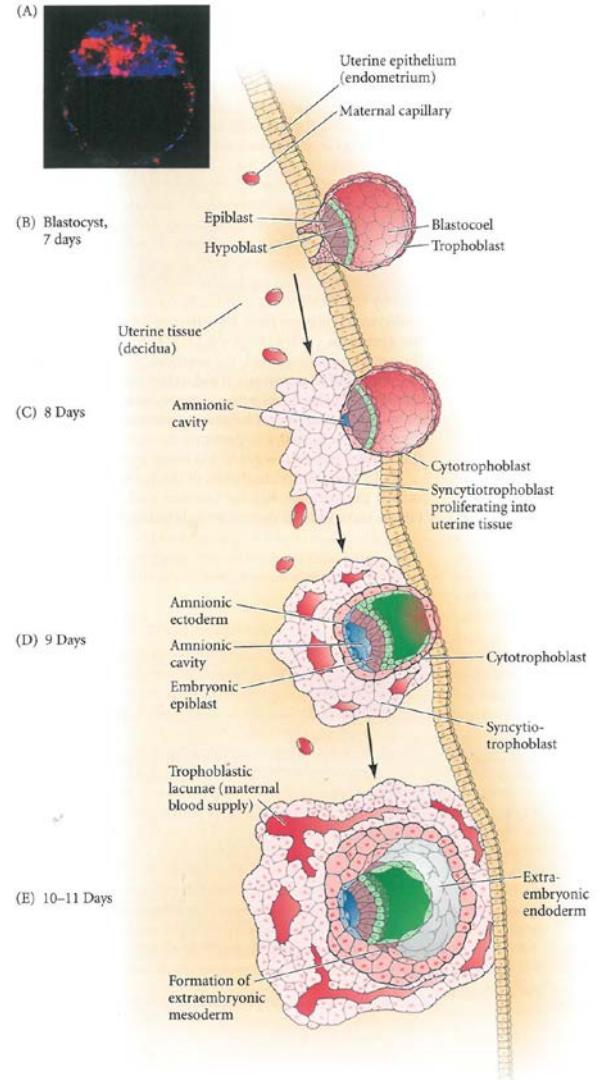
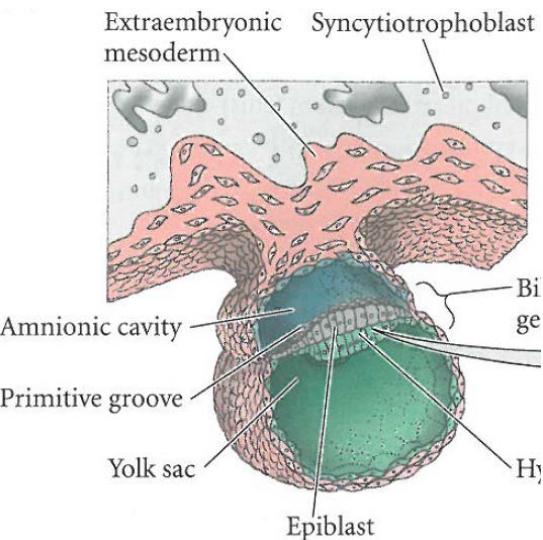
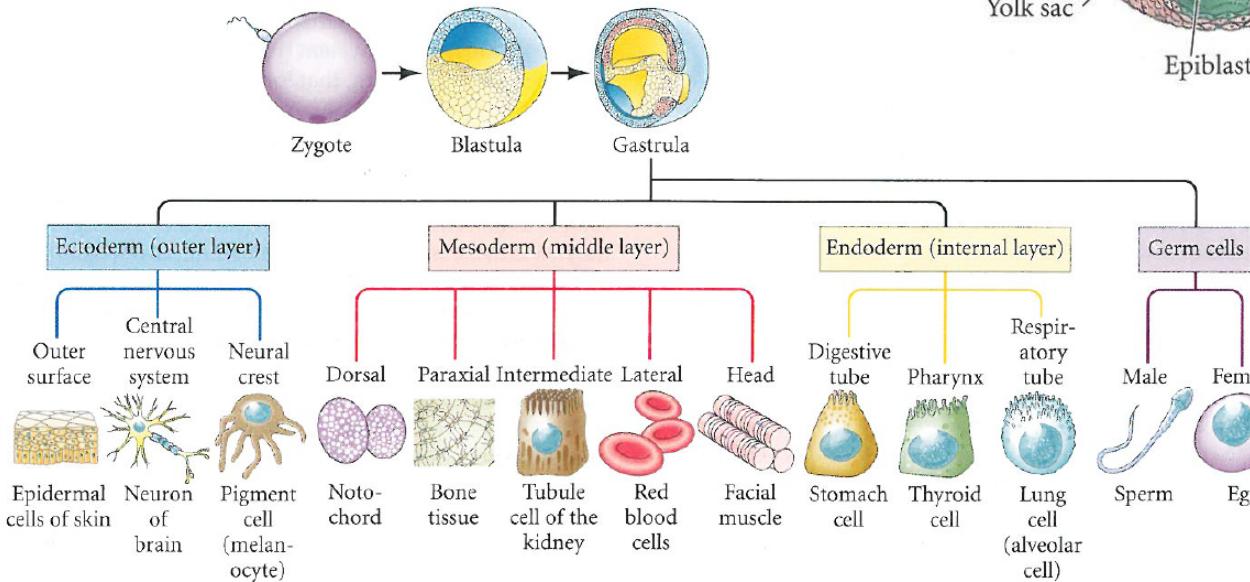
- Stem cell has the ability to self-renew and maintain itself for the lifetime of the organism
- Tissue-specific stem cell:  
A single stem cell has the ability to completely reconstitute an entire tissue in the body

A single stem cell can form a colony at low density culture.

Robey PG, JAMA

# Types of Stem Cells According to the Potential

- Totipotent stem cells**
- Pluripotent stem cells**
- Multipotent stem cells**  
Fetal or postnatal origin  
Mesenchymal stem cells  
Tissue-specific stem cells
- Unipotent Stem Cells/Progenitor Cells**



# Classification of Stem Cells according to its Origin

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**Autologous**

**Allogenic**

**Xenogenic**

**Established cell line**

# Postnatal stem cells

“A current read of the literature suggests that **virtually every tissue  
in the body** contains a cell that has some sort of **regenerative  
capacity.**”

(P. Robey 2005, Oral Biosci Med 2/3:83-90)

# **Source of post-natal stem cells and their functional aspect**

Craniofacial and Skeletal Diseases Branch,  
National Institute of Dental and Craniofacial Research,  
National Institutes of Health, DHHS

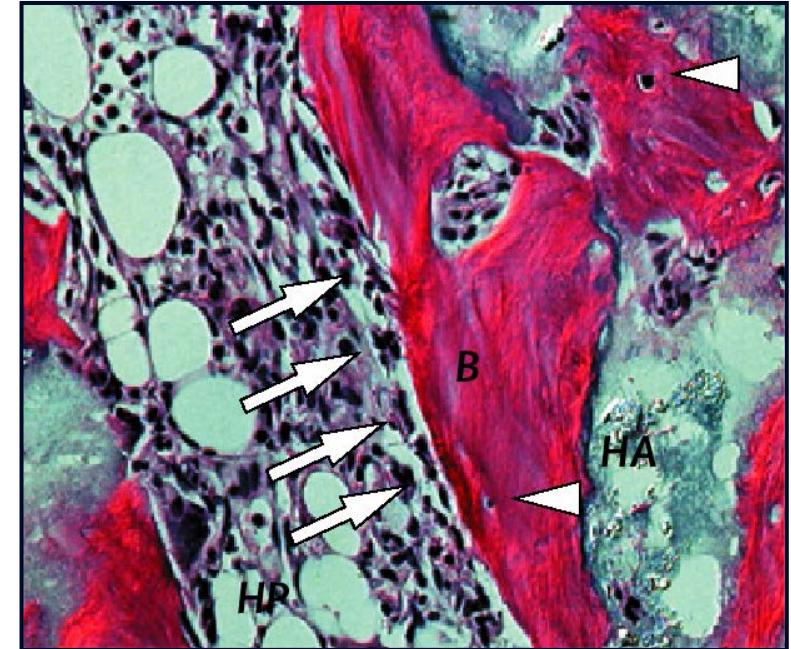


# Finding of Bone Marrow Stromal Cells (BMSCs)

Friedenstein 1966; Owen 1988

*"ex vivo expanded bone marrow stromal cells contain a subpopulation of post-natal stem cells"*

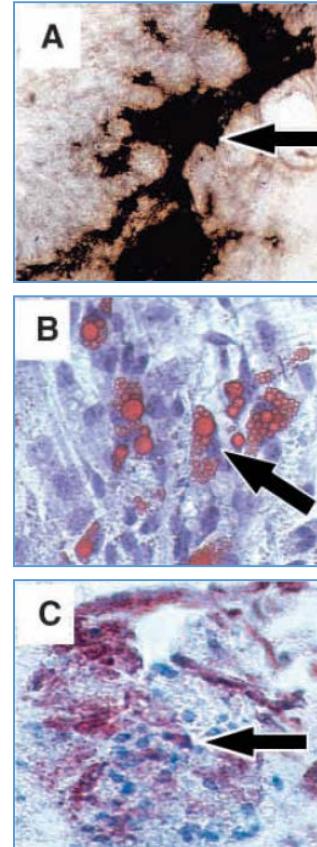
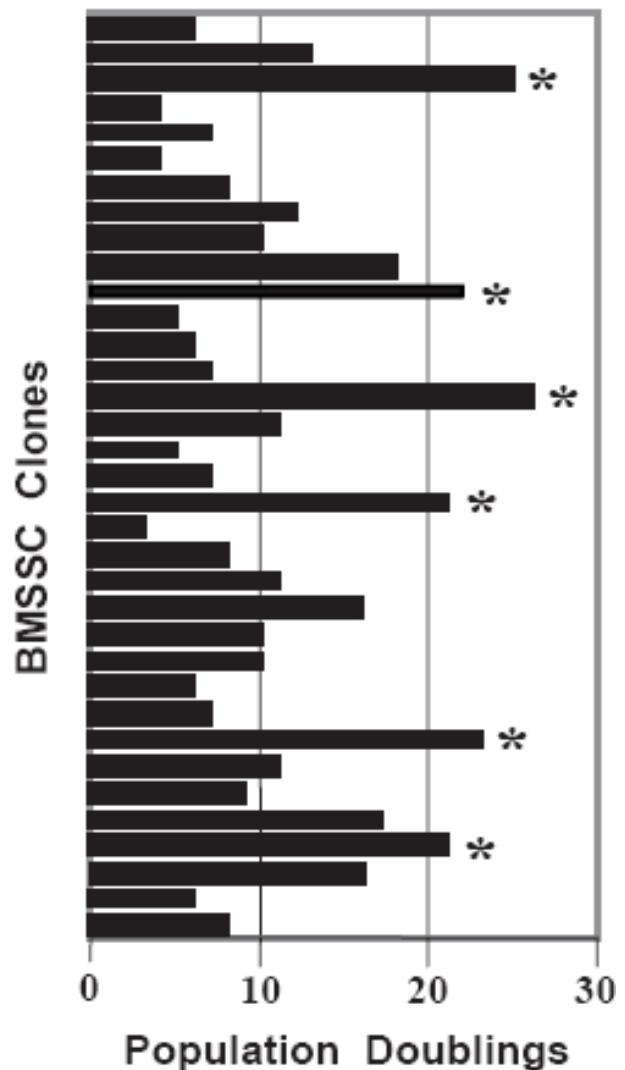
Clonal analysis indicates that between 10-20% of CFU-Fs are true SSCs, capable of completely regenerating a bone/marrow organ



- **Easily accessible, potentially central repository of post-natal stem cells of great utility**

# BMSCs (Bone Marrow Stromal Cells)

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S Gronthos, 2003 J Cell Sci. 116:1827-1835

# Bone Marrow as source of post-natal stem cell

**Bone marrow environment:  
home of the Hematopoietic Stem Cells (HSCs) and Multipotent  
Stromal Cells (MSCs)**

## HSC

- Non-adherent
- Circulating

## MSC

- Adherent and fibroblastic, non-circulating
- Can give rise to all major skeletal tissues
  - Cartilage, Bone, Myelosupportive stroma, Adipocyte, Fibrous connective tissue

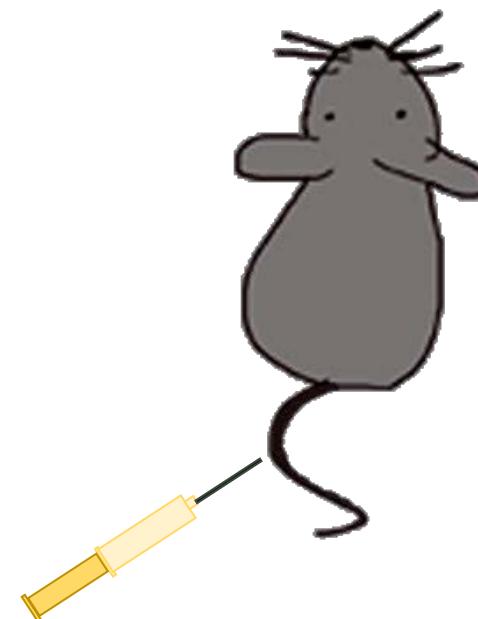


# Finding of Circulating Skeletal Stem Cells (CSSCs)

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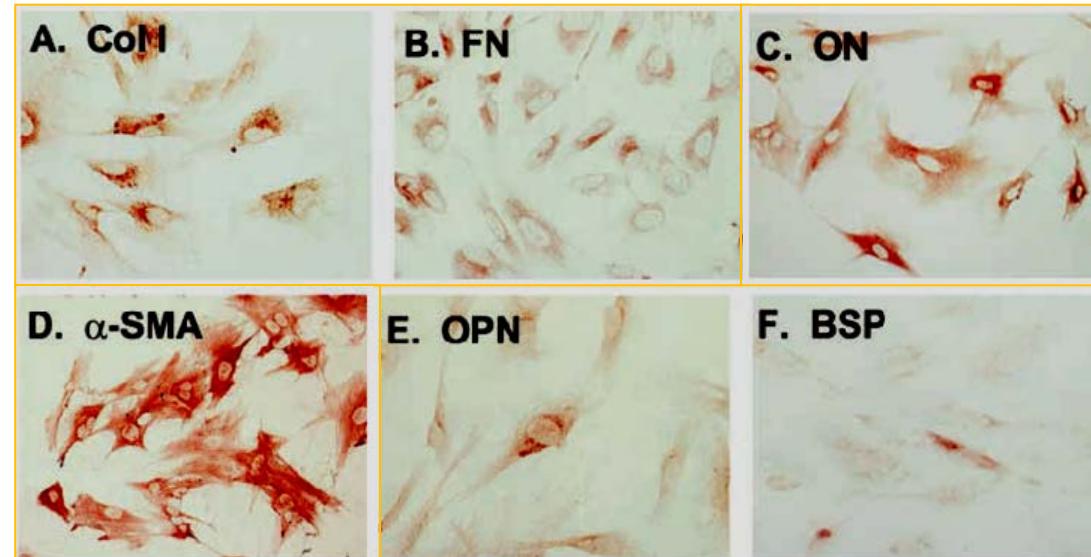
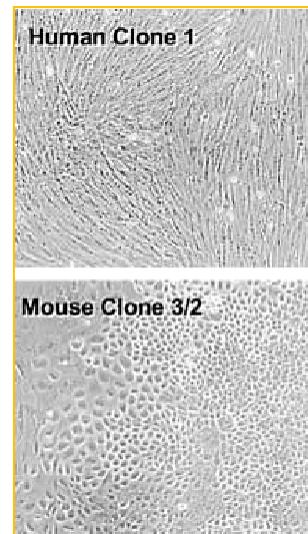
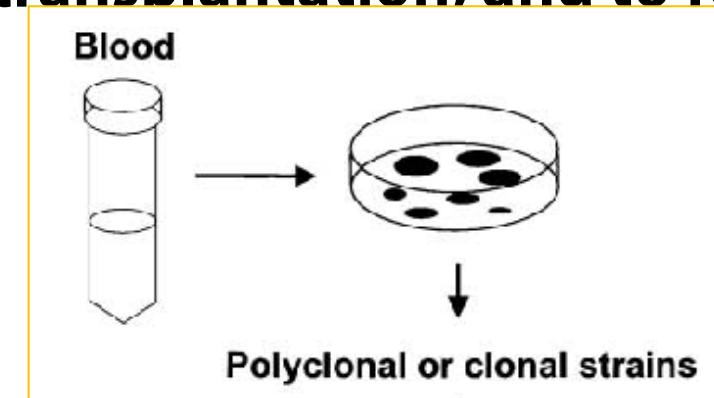
**Evidence of CSSCs reported by Ferrari et al. (1998) and Hou et al. (1999)**

- Both muscle and skeletal cells of donor origin differentiate within muscle and bone when marrow-derived cell populations are infused into the systemic circulation under some experimental conditions.
- These data provide evidence for at least some degree of systemic transplantability of progenitors of solid-phase mesodermal tissues.

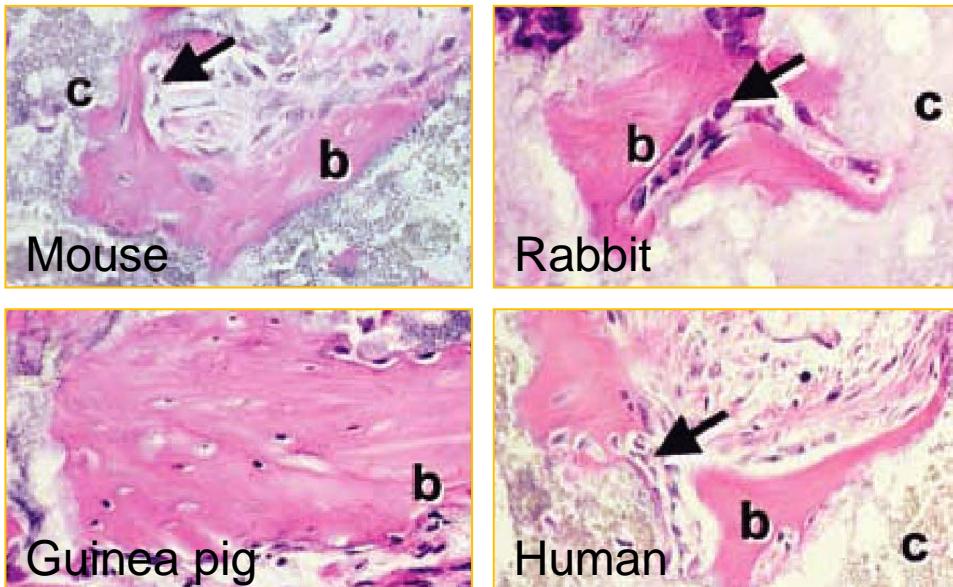
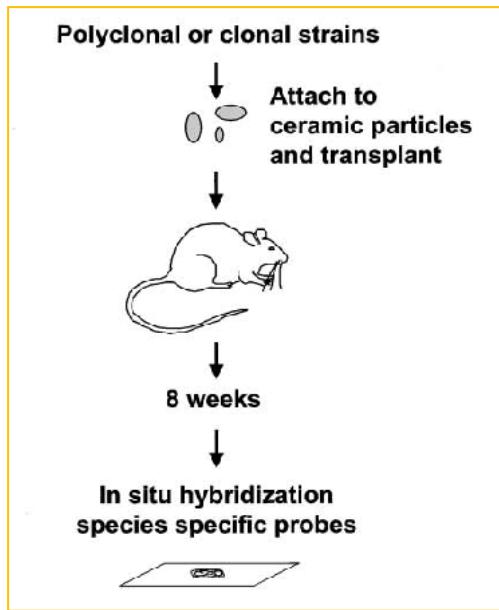


# Circulating Skeletal Stem cells (CSSCs) - S.A. Kuznetsov, 2001. JCB

Peripheral blood has been found to give rise to adherent, clonogenic cells that are able to form bone upon in vivo transplantation, and to form adipocytes and cartilage in vitro.



# Osteogenic differentiation of CSSCs



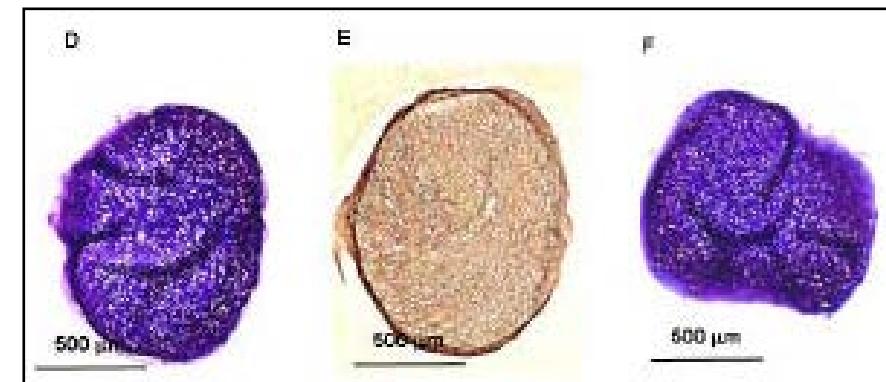
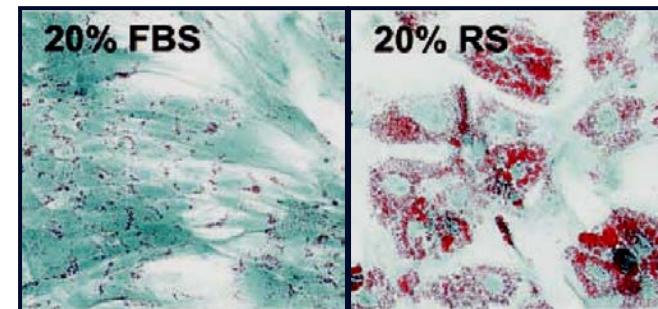
Animal species	# of donors	CFE/ $10^6$ cells	# of osteogenic clones/clones transplanted
Mouse	37	0.93	1/8
Rabbit	5	0.18	3/19
Guinea pig	5	2.7	2/4
Human	10	Rare	1/2

## Immunophenotyping

- (-) Hematopoietic (CD45, Cd14)
- (-) Endothelial (endogline, CD34)
- (+) Osteogenic markers (OPN, BSP)
- (+) Col I and Col III, FN, ON, a-SMA, CD44, VCAM-1, and  $\beta$ 1 integrin subunit
- Stro-1 was negative only in hCSSCs

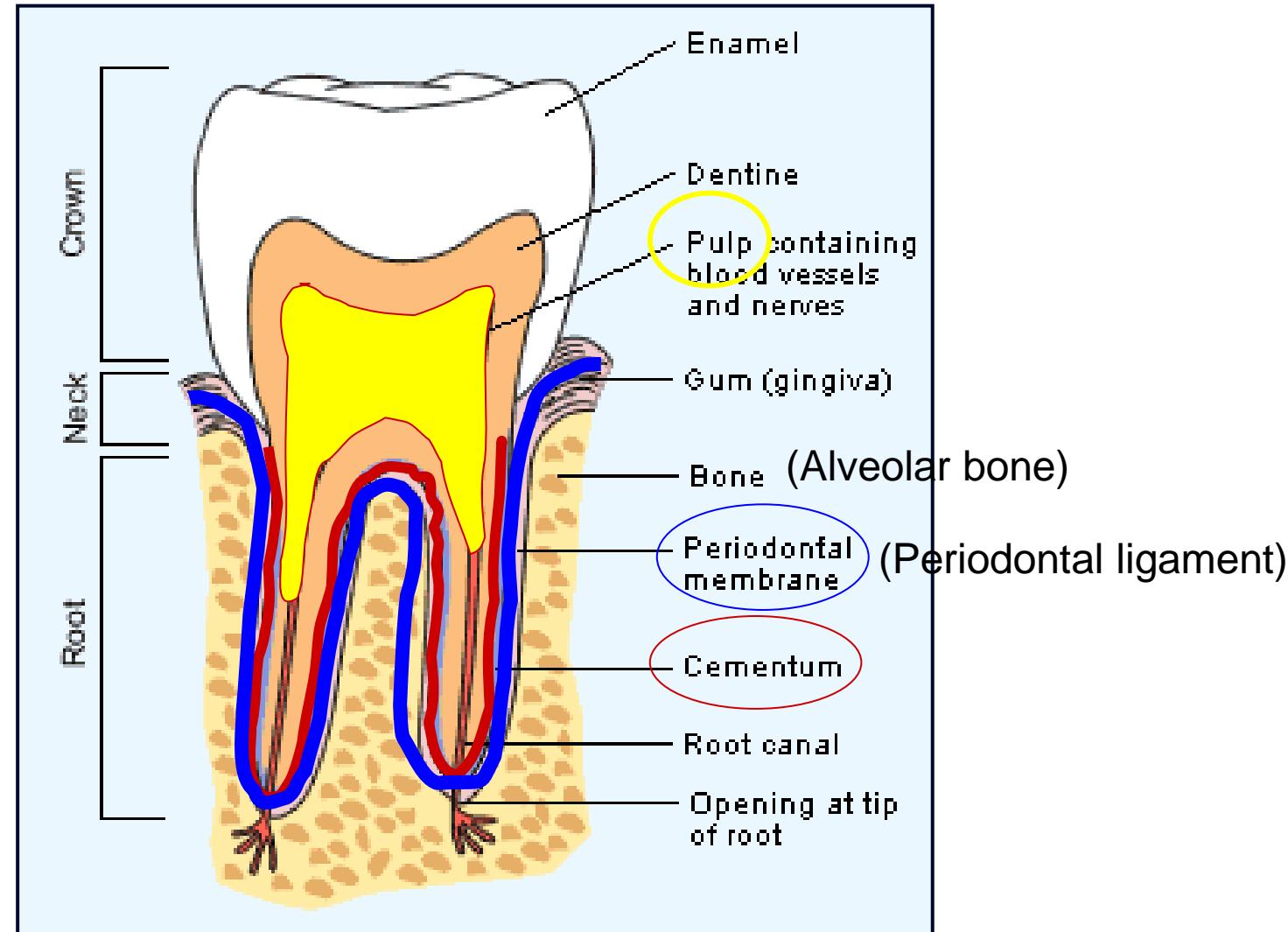
## Differentiation capacity of CSSCs

- Osteogenesis
- Adipogenesis
- Chondrogenesis



# Structure of normal tooth

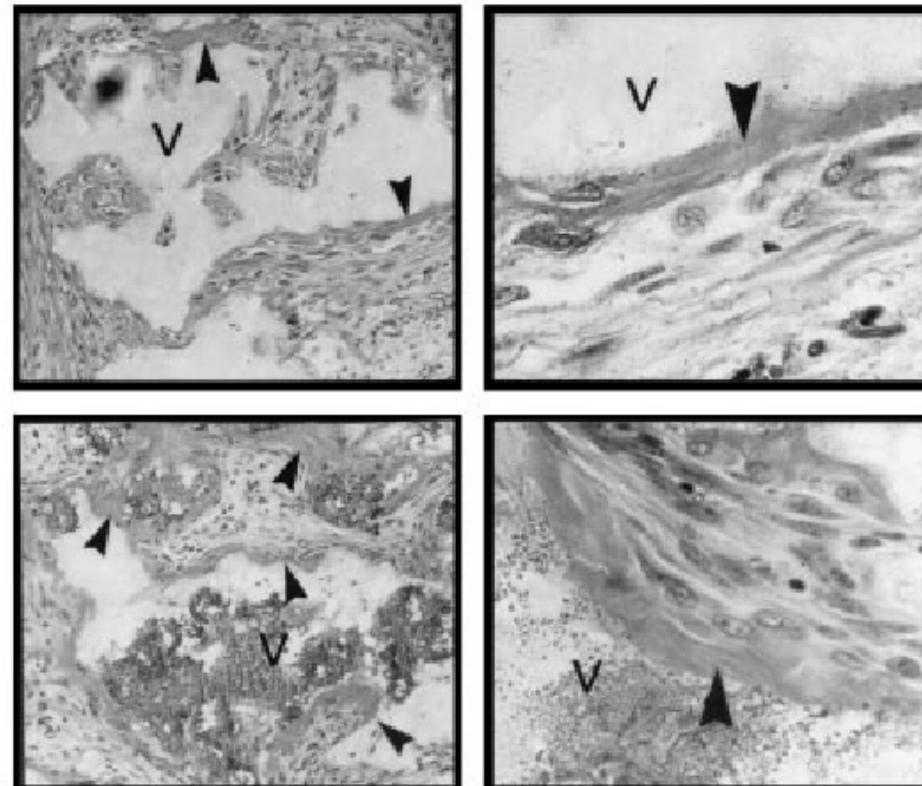
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# Normal human cementum-derived cells - W.J. Grzesik 1998. JBMR

Shavings of cementum were found to give rise to clonogenic cells capable of regenerating a cementum-like structure

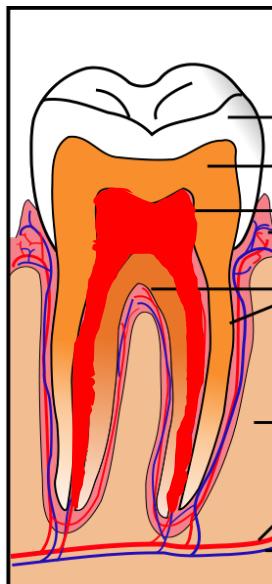
Cementum



# Dental Pulp Stem Cells (DPSCs) -M. Miura, 2003. PNAS



Songtao Shi DDS, Ph.D. with his daughter Julia



- Source of odontoblasts
- No or low adipogenic potential
- Stro-1 positive

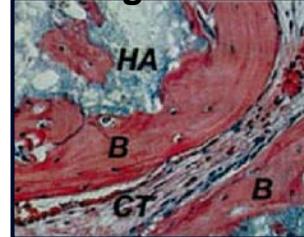
Differentiate into ...

Odontoblast-like cells  
Form dentin/pulp-like complex

Adipogenesis



Osteogenesis



S. Gronthos, 2000 PNAS

# Periodontal Ligament Stem Cells (PDLSCs) -B.-M. Seo, 2004. Lancet

Cells isolated from the periodontal ligament formed both PDL and cementum like structures upon transplantation

PDL contains heterogeneous cell populations

- Cementoblasts
- Osteoblasts

Multipotent - osteogenic, adipogenic

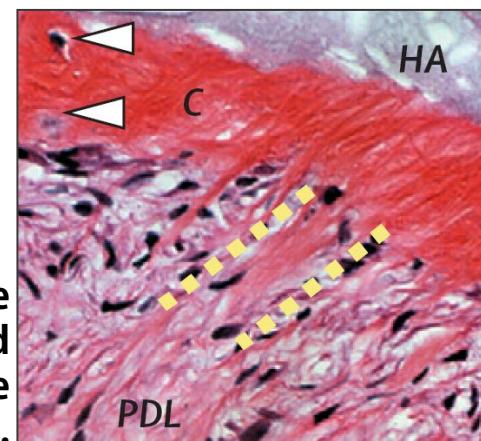
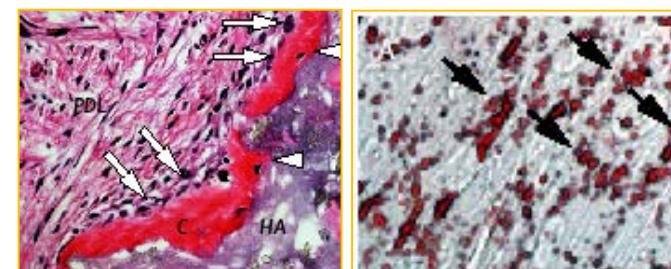
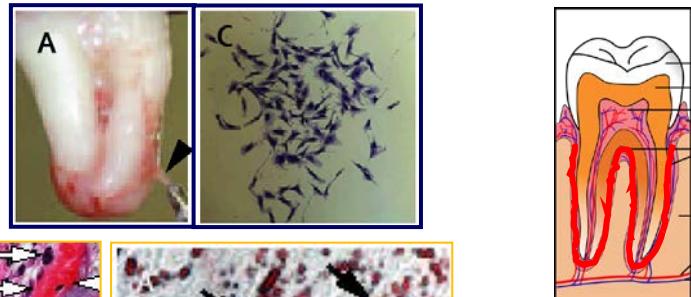
Source of odontoblasts

Stro-1 positive

Differentiate into ...

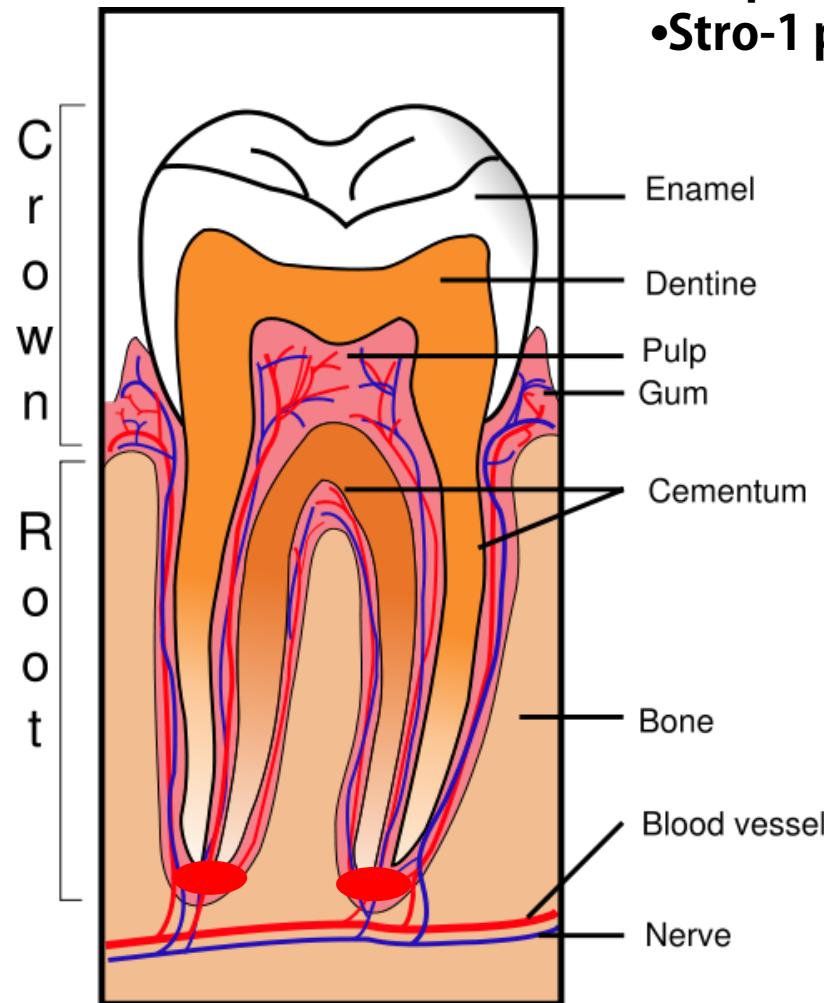
- PDL-like tissue (Col I-positive)
- Form cementum/PDL-like structure

Transplanted PDLSCs formed cementum-like structures (C) that connected to newly formed collagen fibres (yellow dashed lines), similar to the structure of Sharpey's fibre.

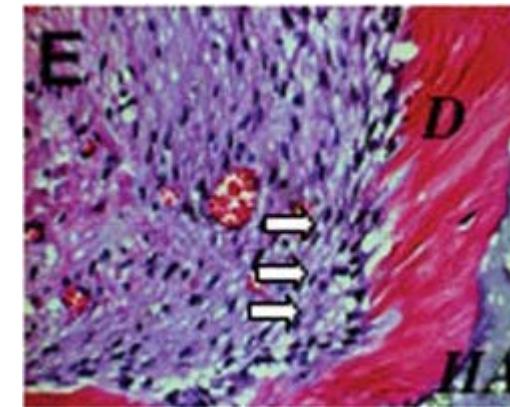


# SCAPs (Stem Cells from Apical Papilla)

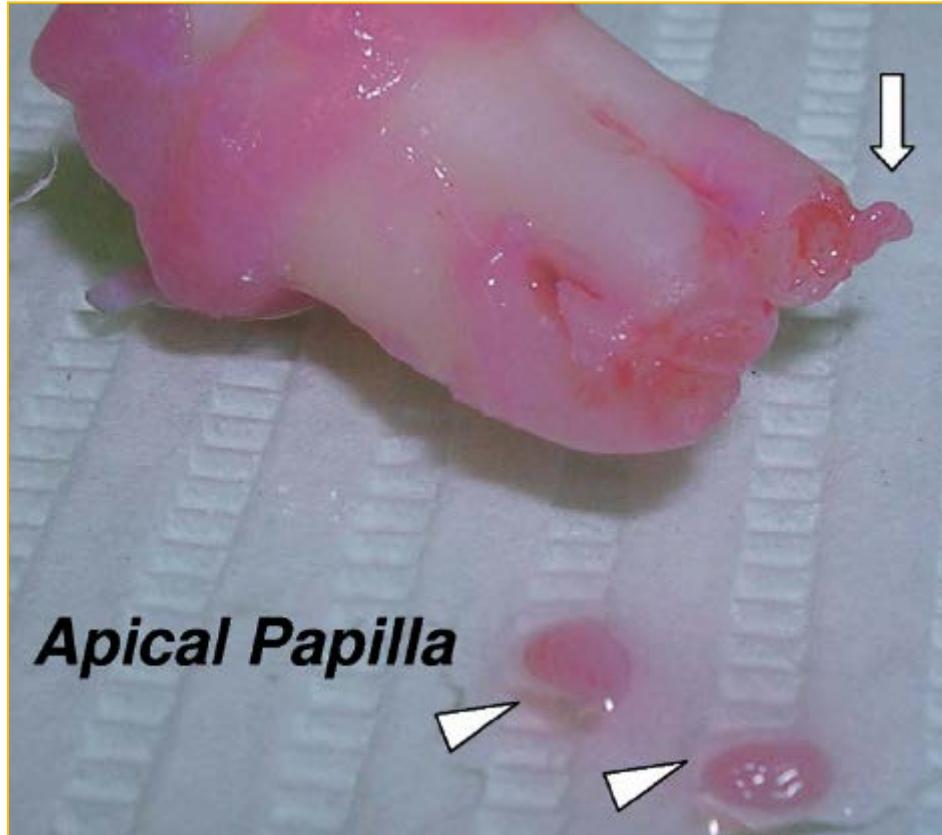
- Appears to be the primary source of odontoblasts
- Responsible for the formation of root dentin
- Stro-1 positive



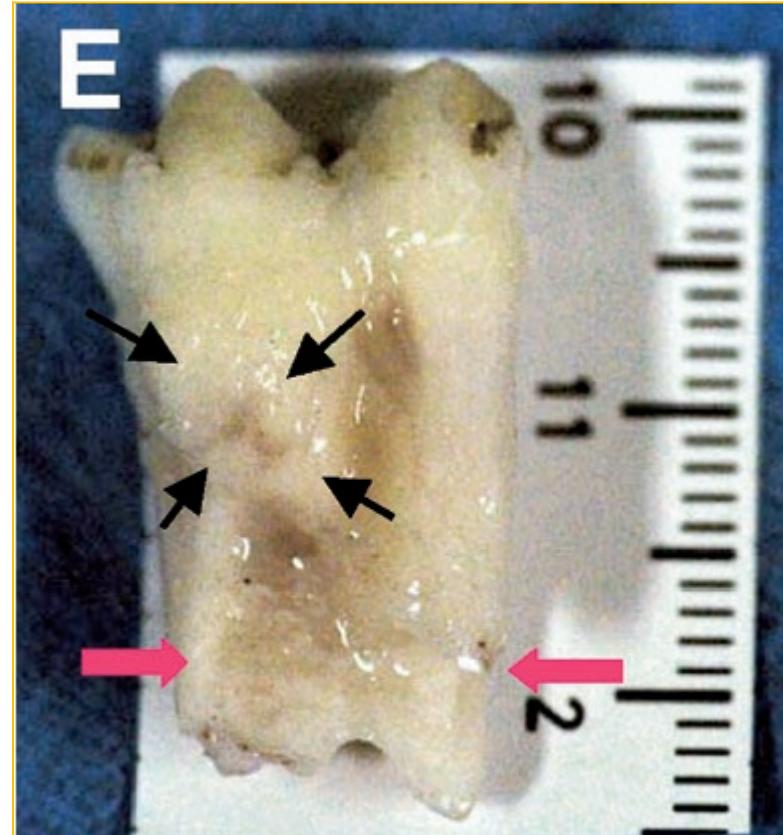
Showed significantly greater ...  
BrdU uptake rate  
Number of population doublings  
Tissue regeneration capacity  
Number of Stro-1 positive cells  
(compared with DPSCs)



W. Sonoyama, 2006 PLoS ONE



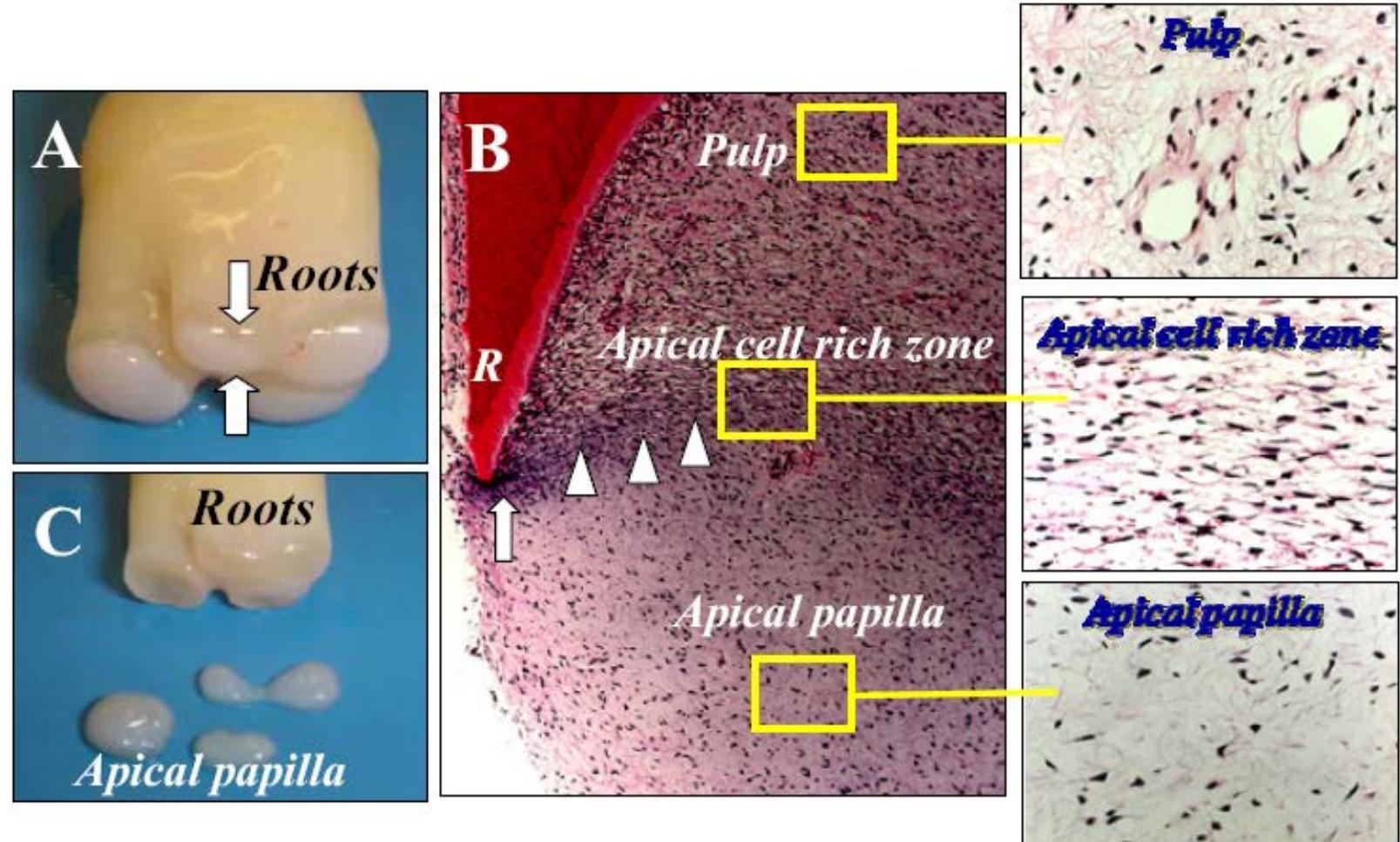
An extracted human third molar depicting three immature roots with two pieces of apical papilla being removed from their apices (arrow heads) and one piece of apical papilla being peeled away from the root end but not completely detached (arrow).



## Minipig

When distal buccal root apical papilla of the lower first molar was surgically removed from a 9-month-old minipig, the distal buccal root stopped developing at the 3-month follow-up (black arrows), but other roots show a normal growth and development (red arrows).

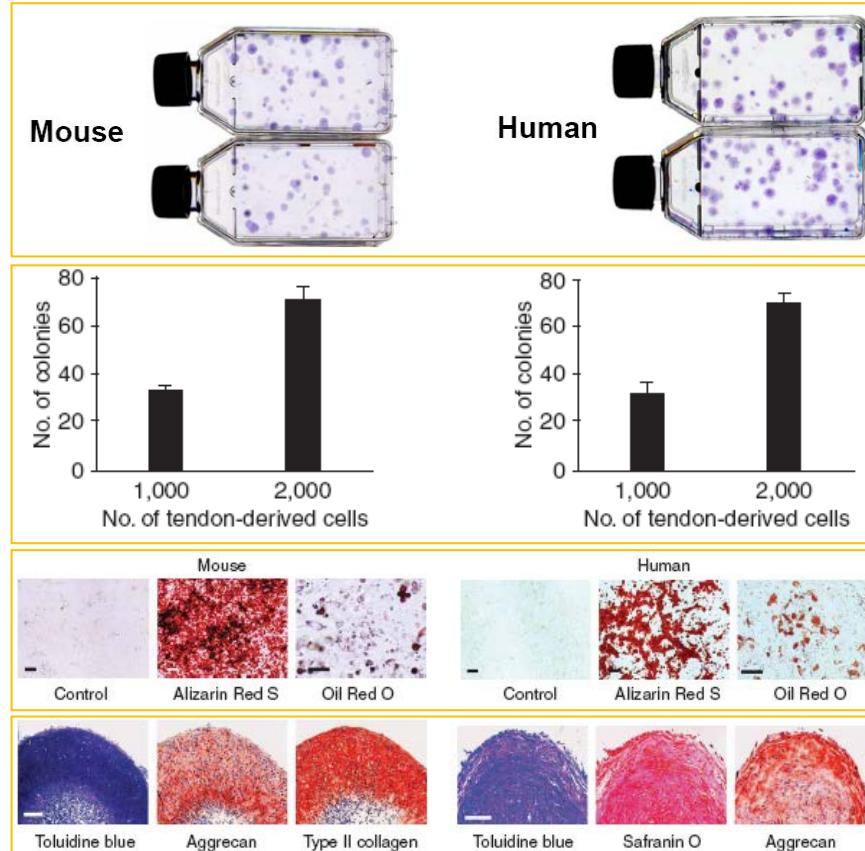
GTJ Huang 2008 J Endodo 34:645



W Sonoyama, 2008 J Endodo 34:166

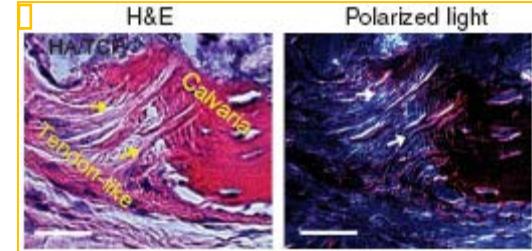
**The anatomy of the apical papilla. (A) An extracted human third molar depicting root attached to the root apical papilla (open arrows) at developmental stage. (B) Hematoxylin and eosin staining of human developing root (R) depicting epithelial diaphragm (open arrows) and apical cell rich zone (open arrowheads). (C) Harvested root apical papilla for stem cell isolation.**

# Tendon Stem Cells (TSCs)



*Y. Bi, 2007 Nature Med*

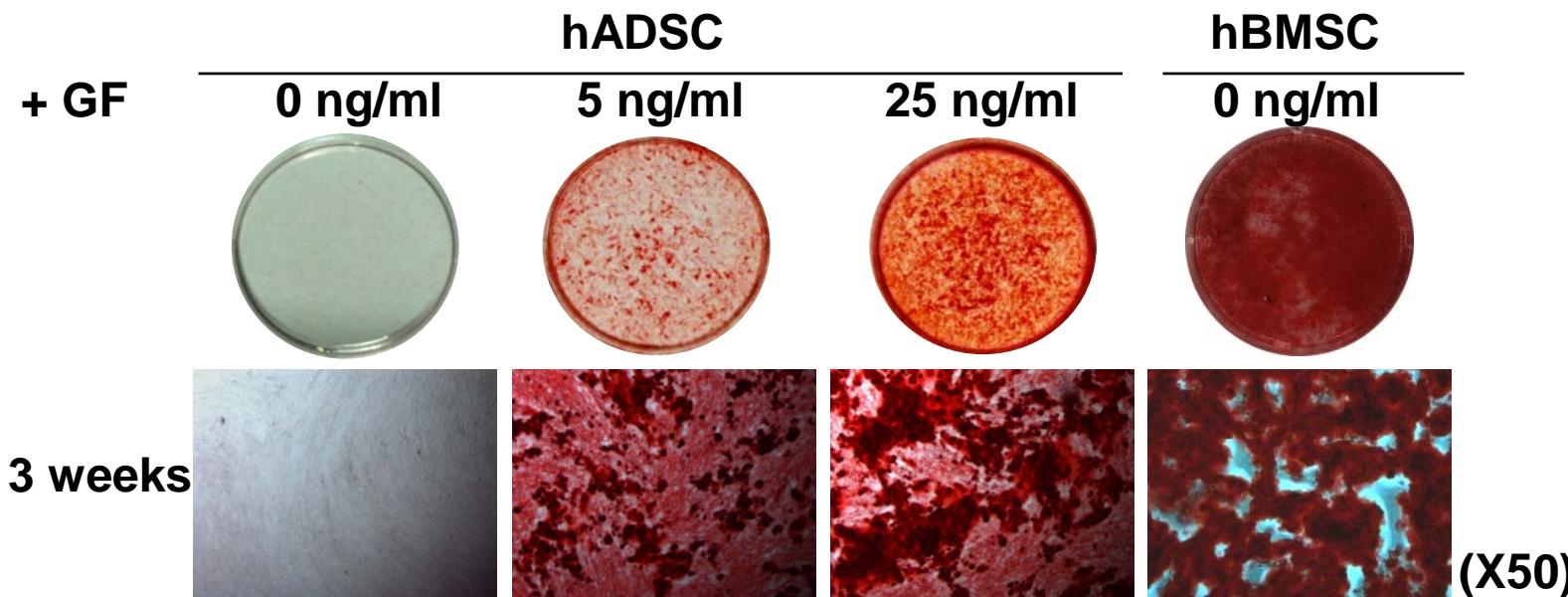
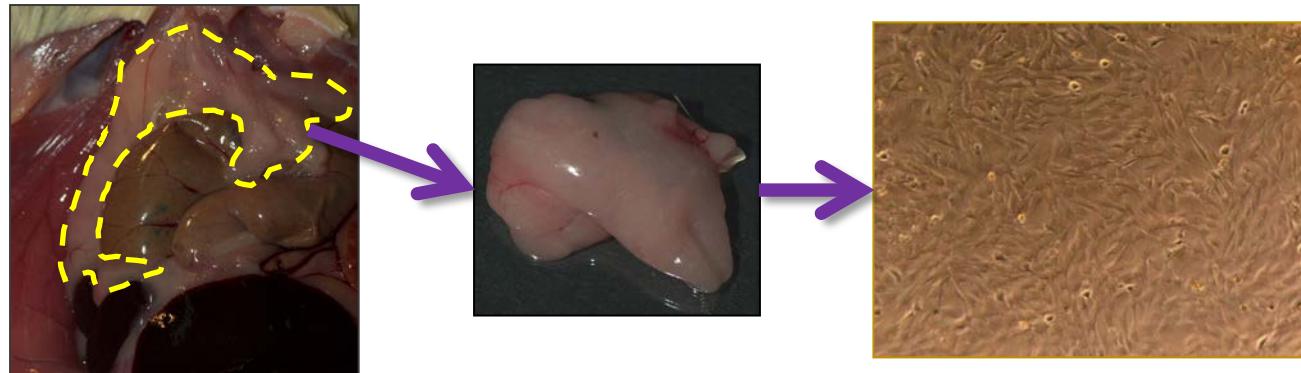
## Sharpey's fiber



- **Stem cells found in tendon**
- **Differentiation capacity**
  - **Osteogenesis**
  - **Adipogenesis**
  - **Chondrogenesis**
- **Immunophenotyping**
  - (+) for BMSC markers
  - (-) for HSC and endothelial cell markers

# ADSCs (Adipose Derived Stem Cells)

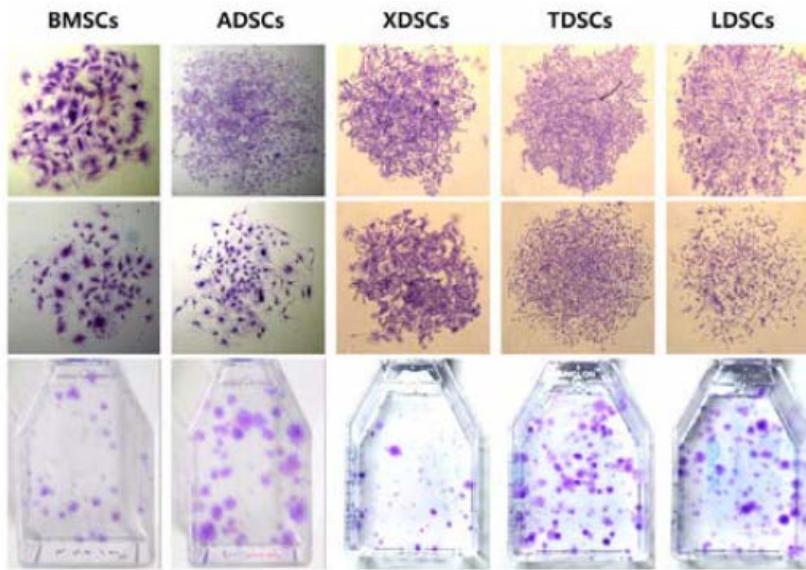
"Most promising among the non-skeletal stem cells"



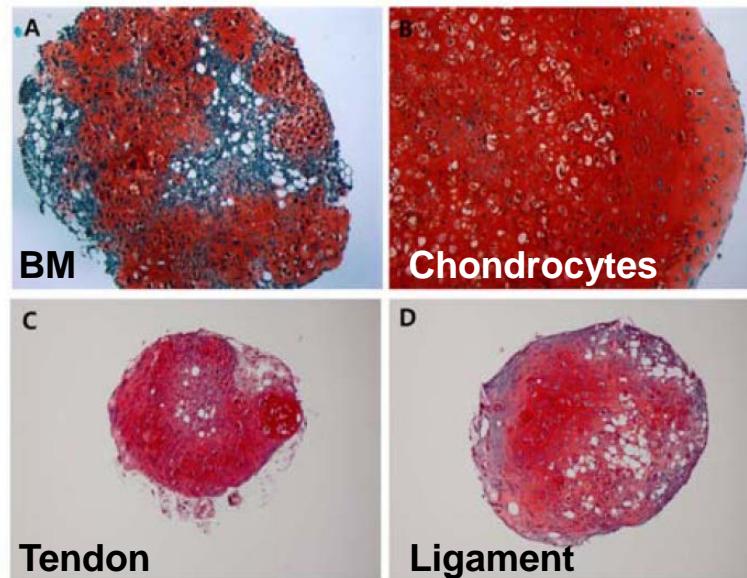
**표 1. 발견된 다양한 성체줄기세포**

성체줄기세포	유래조직	Immuno-phenotype	분화능
골수줄기세포 (MSCs, BMSCs)	골수	CD29 <sup>+</sup> , CD73 <sup>+</sup> , CD90 <sup>+</sup> , CD105 <sup>+</sup> , CD146 <sup>+</sup> , CD45 <sup>-</sup> , CD34 <sup>-</sup> , CD14 <sup>-</sup> , CD11b <sup>-</sup> , CD19 <sup>-</sup> , HLA-DR <sup>-</sup>	뼈, 지방, 연골, 근육, 신경계 등
지방줄기세포 (ASCs, ADSCs)	지방조직	CD29 <sup>+</sup> , CD44 <sup>+</sup> , CD71 <sup>+</sup> , CD90 <sup>+</sup> , CD105 <sup>+</sup> , Stro-1 <sup>+</sup> CD31 <sup>-</sup> , CD34 <sup>-</sup> , CD45 <sup>-</sup>	지방, 뼈, 연골 등
근육줄기세포(Muscle Stem Cells)	근육	CD49d <sup>+</sup> , CD73 <sup>+</sup> , CD90 <sup>+</sup> , CD105 <sup>+</sup>	지방, 뼈, 연골
건 줄기세포 (TSCs)	건(Tendon)	BMSC markers <sup>+</sup> CD45 <sup>-</sup> , CD34 <sup>-</sup> , CD14 <sup>-</sup> , CD11b <sup>-</sup>	뼈, 지방, 연골
치수줄기세포 (DPSCs)	치아	Stro-1 <sup>+</sup> , $\alpha$ -SMA <sup>+</sup> Cd44 <sup>+</sup> , CD90 <sup>+</sup> , Cd105 <sup>+</sup> , CD146 <sup>+</sup> , Cd166 <sup>+</sup>	뼈, 지방
치아인대줄기세포 (PDLSCs)	치아 주변조직	Stro-1 <sup>+</sup> , $\alpha$ -SMA <sup>+</sup> CD44 <sup>+</sup> , CD90 <sup>+</sup> , CD105 <sup>+</sup> , CD166 <sup>+</sup> , CD146 <sup>+</sup>	뼈, 지방
Apical Papilla 유래 줄기세포(SCAP)	치아 주변조직	Stro-1 <sup>+</sup> , $\alpha$ -SMA <sup>+</sup> , ALP <sup>+</sup> , CD105 <sup>+</sup> , CD146 <sup>+</sup> , NeuN <sup>+</sup> , Nestin <sup>+</sup> , $\beta$ III Tubulin <sup>+</sup> ,	뼈, 지방
Circulating stem cells	말초혈액	OPN <sup>+</sup> , BSP <sup>+</sup> , ON <sup>+</sup> , $\alpha$ -SMA <sup>+</sup> , CD44 <sup>+</sup> , VCAM-1 <sup>+</sup> , CD29 <sup>+</sup> CD45 <sup>-</sup> , CD14 <sup>-</sup> , CD34 <sup>-</sup> , CD105 <sup>-</sup>	뼈, 지방, 연골
Substance P 가동화 줄기세포	말초혈액	CD29 <sup>+</sup> , CD105 <sup>+</sup> , CD106 <sup>+</sup> , CD90 <sup>+</sup> , $\alpha$ -SMA <sup>+</sup> , CD11b <sup>-</sup> ,	뼈, 지방, 연골

## Colony-forming

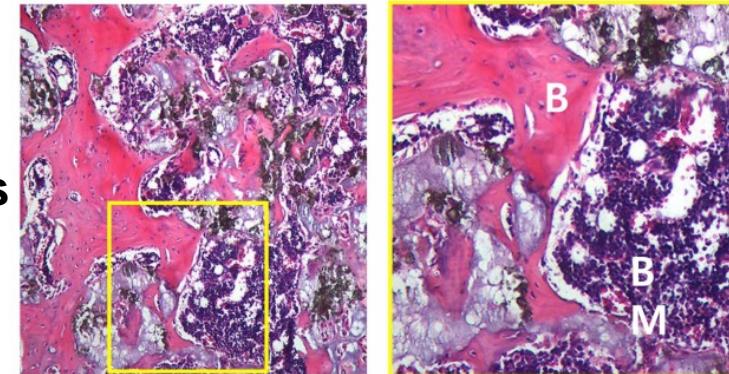


## Chondrogenesis

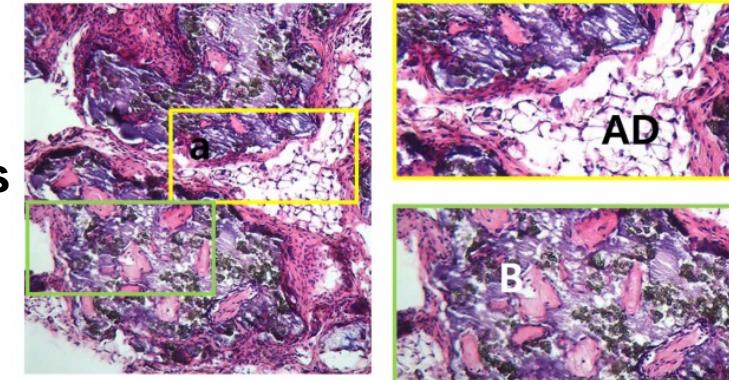


## *In vivo* osteogenesis

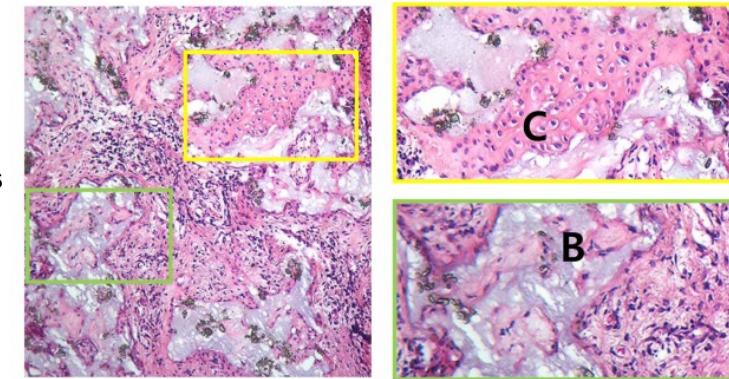
BMSCs



ADSCs



Chondrocytes



## *In vitro adipogenesis*

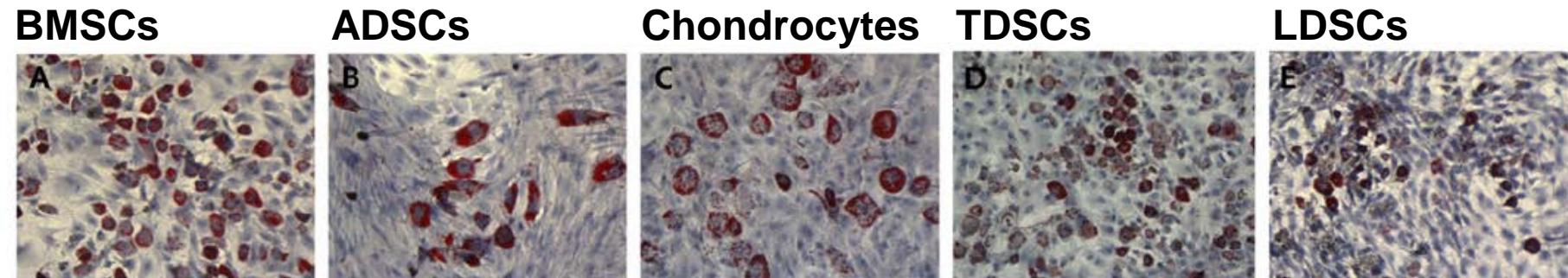


표 2. 성체줄기세포의 특성 비교분석<sup>17</sup>

성체줄기세포	<i>In vitro</i> 분화능			<i>In vivo</i> 분화능 <sup>**</sup>	실용화 가능성
	Osteogenesis	Adipogenesis	Chondrogenesis		
BMSCs	+++++	++	++	B + BM	++++
ADSCs	++	+	NC*	B + AD	+++
XDSCs***	+	+	++++	B + C	+++
TLSCs	+	++	+++	B	+
LDSCs	+	++	+++	B	+

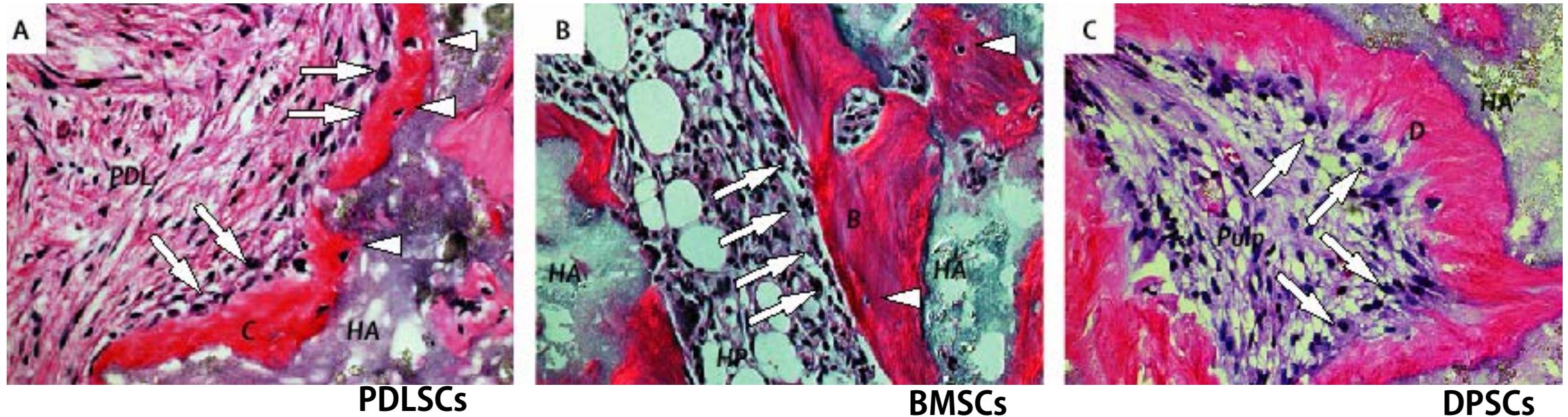
\* Not Confirmed

\*\* B: Bone matrix, BM: Bone marrow, AD: Adipose tissue, C: Cartilage

\*\*\* XDSCs: xiphoid process cartilage-derived stem cells

# Relatedness and differentiation capacity of post-natal skeletal stem cells

Protein compositions of bone, dentin, cementum and associated tissues are very similar, yet the organization of the extracellular matrix produced upon *in vivo* transplantation by bone marrow stromal cells, dental pulp cells and PDL cells are quite distinctive.



# Expression features of PDLSCs, BMSCs, and DPSCs

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## Immunophenotyping

- (-) hematopoietic markers
- (+) connective tissue markers
- (+) endothelial markers
- (+) smooth muscle markers

**Markers consistently expressed by these different populations:**

- Stro-1
- SMA

# Chronicles of stem cell research

# Key Research Events in Stem Cell Field

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- 1908: The term "stem cell" was proposed for scientific use by the [Russian histologist Alexander Maksimov](#) (1874 - 1928) at congress of hematologic society in [Berlin](#). It postulated existence of haematopoietic stem cells.
- 1952 Briggs & King: Nuclear Transfer of amphibian Rana ppiens.
- 1960s: [Joseph Altman](#) and Gopal Das present scientific evidence of adult [neurogenesis](#), ongoing stem cell activity in the brain; their reports contradict [Cajal](#)'s "no new neurons" dogma and are largely ignored.
- 1962: Gurdon made the discovery that the specialization of cells is reversible. (Replacing the immature cell nucleus in an egg cell of a frog with the nucleus from a mature cell that was derived from the intestine of a tadpole. This modified egg cell developed into a normal tadpole.)
- 1963: [McCulloch](#) and [Till](#) illustrate the presence of self-renewing cells in mouse bone marrow.
- 1966 Friedenstein, 1988 Owen: Finding of Bone Marrow Stromal Cells (BMSCs)
- 1968: [Bone marrow transplant](#) between two siblings successfully treats [SCID](#).
- 1978: [Haematopoietic stem cells](#) are discovered in human [cord blood](#).
- 1981: Illmensee & Hoppe - the first report of cloning an adult mammal. (Birth of three cloned mice after NT from innercell mass cell into enucleated zygotes)
- 1981: Mouse [embryonic stem cells](#) are derived from the [inner cell mass](#) by scientists [Martin Evans](#), [Matthew Kaufman](#), and [Gail R. Martin](#). Gail Martin is attributed for coining the term "Embryonic Stem Cell".
- 1984: McGrath & Solter: "cloning of mammals by simple NT was biologically impossible, mainly due to the rapid loss of totipotency of the embryonic cells. This conclusion affected research in this field profoundly. -> This contradiction to other research did not withstand the test of time.
- 1986: Willadsen - Mammalian blastomeres NT into enucleated oocyte. (Sheep)
- 1988: Stice & Robl - Mammalian blastomeres NT into enucleated oocyte. (Rabbits)
- 1989: Prather - Mammalian blastomeres NT into enucleated oocyte. (Pigs)
- 1993: Cheong - Mammalian blastomeres NT into enucleated oocyte. (Mice)
- 1994: Sims & First - Mammalian blastomeres NT into enucleated oocyte. (Cows)
- 1997: Meng - Mammalian blastomeres NT into enucleated oocyte. (Monkeys)
- 1996: Campbell - The full potential of somatic cloning in mammals became evident for the first time. (Cultured cell line fused to enucleated oocyte -> Transferred to foster-mother -> Result in two healthy cloned sheep. ( "Morag" & "Megan" )
- 1997: Wilmut - "Dolly" The first mammal cloned from an adult mammary epithelial cell. (from same lab. as Morag & Megan)

- 1992: [Neural stem cells](#) are cultured *in vitro* as neurospheres.
- 1998: [James Thomson](#) and coworkers derive the first human embryonic [stem cell line](#) at the [University of Wisconsin - Madison](#).
- 1998: John Gearhart (Johns Hopkins University) extracted germ cells from fetal gonadal tissue (primordial germ cells) before developing pluripotent stem cell lines from the original extract.
- 2000s: Several reports of [adult stem cell](#) plasticity are published.
- 2001: Scientists at [Advanced Cell Technology](#) clone first early (four- to six-cell stage) human embryos for the purpose of generating embryonic stem cells.
- 2003: Dr. Songtao Shi of NIH discovers new source of adult stem cells in children's primary teeth (DPSCs).
- 2004 - 2005: Korean researcher [Hwang Woo-Suk](#) claims to have created several human [embryonic stem cell](#) lines from unfertilised human [oocytes](#). The lines were later shown to be fabricated.
- 2005: Researchers at [Kingston University](#) in [England](#) claim to have discovered a third category of stem cell, dubbed cord-blood-derived embryonic-like stem cells (CBEs), derived from umbilical [cord blood](#). The group claims these cells are able to differentiate into more types of tissue than adult stem cells.
- 2005: Researchers at [UC Irvine](#)'s Reeve-Irvine Research Center are able to partially restore the ability of rats with paralyzed spines to walk through the injection of human [neural stem cells](#).
- April 2006 Scientists at the University of Illinois at Chicago identified [novel stem cells](#) from the [umbilical cord blood](#) with [embryonic](#) and [hematopoietic](#) characteristics.
- August 2006: Mouse [Induced pluripotent stem cells](#): the journal [Cell](#) publishes Kazutoshi Takahashi and [Shinya Yamanaka](#).
- November 2006: Yong Zhao et al. revealed the [immune regulation of T lymphocytes](#) by [Cord Blood-Derived Multipotent Stem Cells \(CB-SCs\)](#).
- October 2006: Scientists at [Newcastle University](#) in England create the first ever artificial liver cells using umbilical cord blood stem cells.
- January 2007: Scientists at [Wake Forest University](#) led by Dr. [Anthony Atala](#) and [Harvard University](#) report discovery of a new type of stem cell in [amniotic fluid](#). This may potentially provide an alternative to embryonic stem cells for use in research and therapy.
- June 2007: Research reported by three different groups shows that normal skin cells can be reprogrammed to an embryonic state in mice. In the same month, scientist [Shoukhrat Mitalipov](#) reports the first successful creation of a primate stem cell line through [somatic cell nuclear transfer](#).

- October 2007: [Mario Capecchi](#), [Martin Evans](#), and [Oliver Smithies](#) win the 2007 [Nobel Prize for Physiology or Medicine](#) for their work on embryonic stem cells from mice using gene targeting strategies producing genetically engineered mice (known as [knockout mice](#)) for gene research.
- November 2007: Human induced pluripotent stem cells: Two similar papers released by their respective journals prior to formal publication: in [Cell](#) by [Kazutoshi Takahashi](#) and [Shinya Yamanaka](#), "Induction of pluripotent stem cells from adult human fibroblasts by defined factors", and in [Science](#) by [Junying Yu](#), et al., from the research group of [James Thomson](#), "Induced pluripotent stem cell lines derived from human somatic cells": pluripotent stem cells generated from mature human fibroblasts. It is possible now to produce a stem cell from almost any other human cell instead of using embryos as needed previously, albeit the risk of [tumorigenesis](#) due to [c-myc](#) and [retroviral gene transfer](#) remains to be determined.
- January 2008: Robert Lanza and colleagues at Advanced Cell Technology and UCSF create the first human embryonic stem cells without destruction of the embryo
- January 2008: Development of human cloned blastocysts following [somatic cell nuclear transfer](#) with adult fibroblasts
- February 2008: Generation of pluripotent stem cells from adult mouse liver and stomach: these iPS cells seem to be more similar to embryonic stem cells than the previously developed iPS cells and not tumorigenic, moreover genes that are required for iPS cells do not need to be inserted into specific sites, which encourages the development of non-viral reprogramming techniques.
- March 2008-The first published study of successful cartilage regeneration in the human knee using autologous adult mesenchymal stem cells is published by clinicians from Regenerative Sciences<sup>[1]</sup>
- October 2008: Sabine Conrad and colleagues at Tübingen, Germany generate [pluripotent stem cells](#) from spermatogonial cells of adult human testis by culturing the cells in vitro under [leukemia inhibitory factor](#) (LIF) supplementation.
- 30 October 2008: Embryonic-like stem cells from a single human hair.
- January 2009: Yong Zhao and colleagues confirmed the reversal of autoimmune-caused type 1 diabetes by [Cord Blood-Derived Multipotent Stem Cells \(CB-SCs\)](#) in an animal experiment.

- January 2009: Yong Zhao and colleagues confirmed the reversal of autoimmune-caused type 1 diabetes by [Cord Blood-Derived Multipotent Stem Cells \(CB-SCs\)](#) in an animal experiment.
- 1 March 2009: Andras Nagy, Keisuke Kaji, *et al.* discover a way to produce embryonic-like stem cells from normal adult cells by using a novel "wrapping" procedure to deliver specific genes to adult cells to reprogram them into stem cells without the risks of using a virus to make the change. The use of [electroporation](#) is said to allow for the temporary insertion of genes into the cell.
- 28 May 2009 Kim *et al.* announced that they had devised a way to manipulate skin cells to create patient specific "induced pluripotent stem cells" (iPS), claiming it to be the 'ultimate stem cell solution'.
- 11 October 2010 First trial of embryonic stem cells in humans.<sup>[1]</sup>
- 25 October 2010: Ishikawa *et al.* write in the Journal of Experimental Medicine that research shows that transplanted cells that contain their new host's nuclear DNA could still be rejected by the individual's immune system due to foreign [mitochondrial DNA](#). Tissues made from a person's stem cells could therefore be rejected, because mitochondrial genomes tend to accumulate mutations.
- 2011: [Israeli](#) scientist Inbar Friedrich Ben-Nun led a team which produced the first stem cells from endangered species, a breakthrough that could save animals in danger of extinction.
- January 2012: The human clinical trial of treating [type 1 diabetes](#) with [lymphocyte modification](#) using [Cord Blood-Derived Multipotent Stem Cells \(CB-SCs\)](#) achieved an improvement of C-peptide levels, reduced the median glycated hemoglobin A1C (HbA1c) values, and decreased the median daily dose of insulin in both human patient groups with and without residual beta cell function. Yong Zhao's [Stem Cell Educator Therapy](#) appears "so simple and so safe"
- 2012: Katsuhiko Hayashi et al. reported in the Journal [Science](#) that they used mouse skin cells to create stem cells and then used these stem cells to create mouse eggs. These eggs were then fertilized and produced healthy baby offspring. These latter mice were able to have their own babies.
- 2012: Shinya Yamanaka and John Gurdon were awarded the Nobel Prize for Physiology or Medicine for the discovery that mature cells can be converted to stem cells. (John Gurdon's research in 1962 and Shinya Yamanaka's research in 2006)

# Types of stem cells according to potential

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**Totipotent stem cells**

**Pluripotent stem cells**

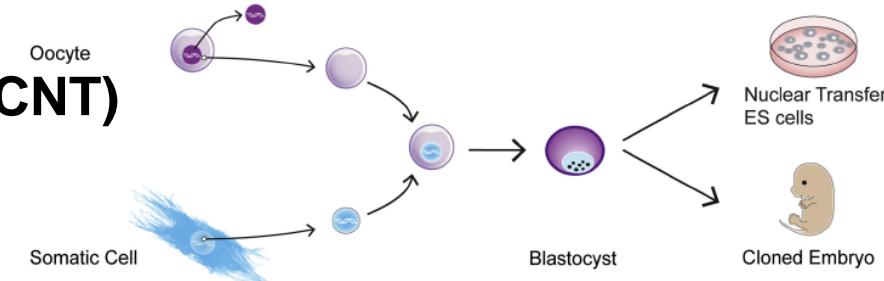
**Multipotent fetal stem cells**

**Multipotent adult stem cells (=postnatal stem cells)**

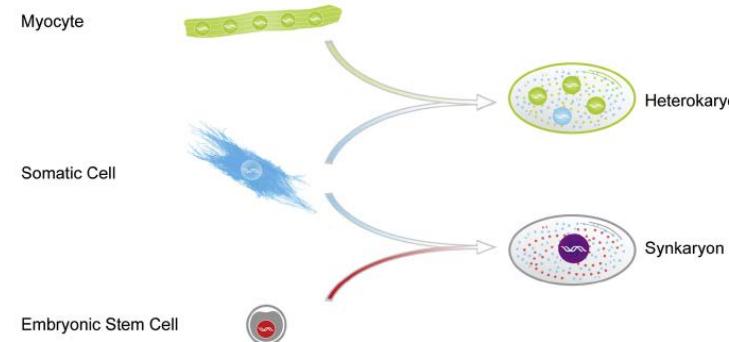
**Unipotent Stem Cells**

# Experimental Systems for Pluripotent State

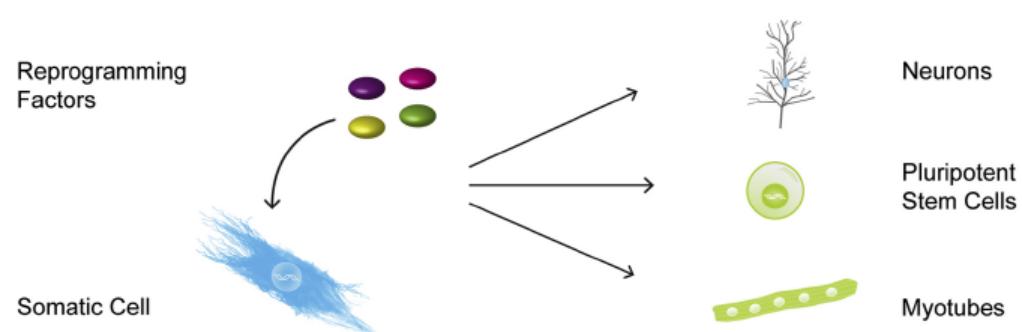
- Somatic Cell Nuclear Transfer (SCNT)

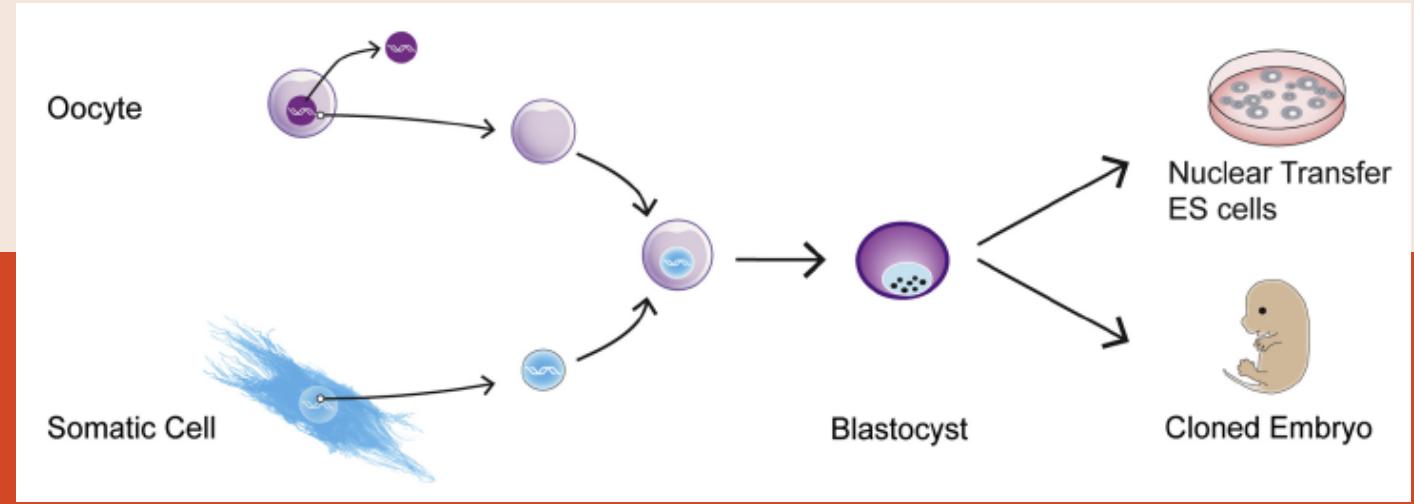


- Cell Fusion



- Transcription Factor Transduction





# Somatic cell nuclear transfer (SCnt)

# Somatic Cell Nuclear Transfer (SCNT)

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SCNT has been successful in a total of 16 species, including…

- Sheep (Wilmut et al., 1997)
- Cow (Kato et al., 1998)
- Mouse (Wakayama et al., 1998)
- Goat (Baguisi et al., 1999)
- Pig (Polejaeva et al., 2000; Onishi et al., 2000)
- Cat (Shin et al., 2002)
- Rabbit (Chesne et al., 2002)
- Mule (Woods et al., 2003)
- Horse (Galli et al., 2003)
- Rat (Zhou et al., 2003)
- Dog (Lee et al., 2005) - questioned in the context of the scandal of Hwang, and eventually confirmed as a genuine clone by microsatellite analysis and mitochondrial genotyping (Lee & Park 2006; Parker et al., 2006)
- Ferret (Li et al., 2006)
- Red deer (Berg et al., 2007)
- Buffalo (Shi et al., 2007)
- Gray wolf (Oh et al., 2008)
- Camel (Wani et al., 2010)

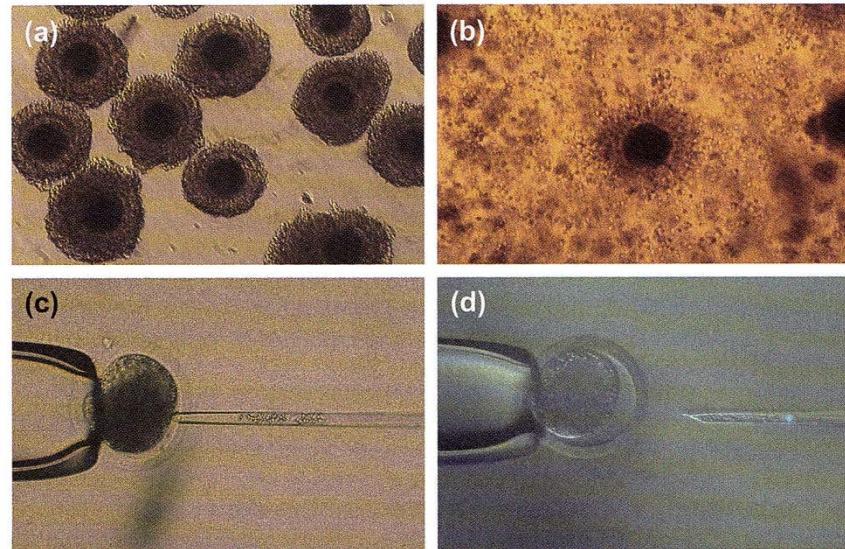
# Technical Aspects of Somatic Cell Nuclear Transfer (SCNT)

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## Steps of somatic cloning protocols

- Collection & enucleation of the recipient oocyte (cytoplasm)
- Preparation and subzonal transfer of the donor cell
- Fusion of the two components
- Activation of the reconstructed complex
- Temporary culture of the reconstructed embryo
- Transfer to a foster mother or storage in liquid nitrogen

# In vitro Maturation and enucleation of porcine oocytes



## Areas benefit from SCNT:

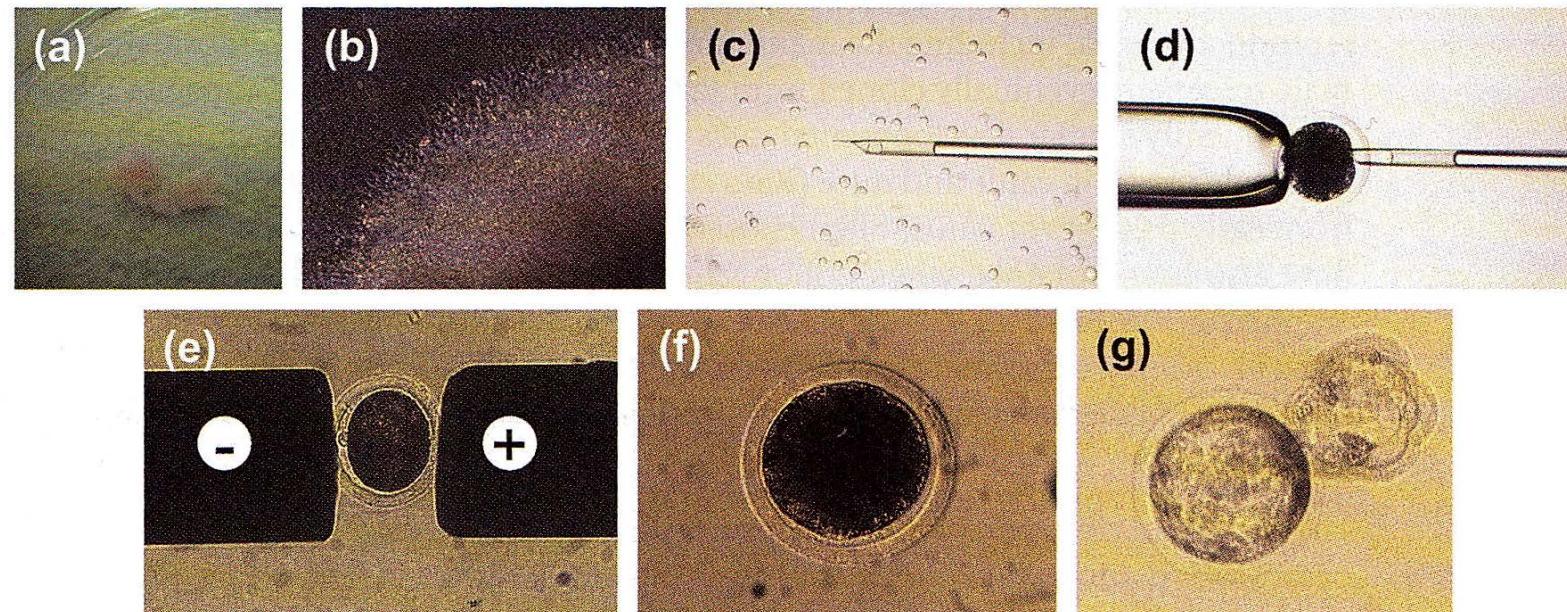
- Basic biological research
- Agricultural applications
- Biomedical applications

## Shortcoming: low efficiency due to biological stress

<ex: Dolly>

- 277 Oocytes used
- 29 Embryogenesis
- 3 Births
- Dolly: the only one that lived

# From donor cell production to cloned blastocysts



# Investigation on the Underlying Mechanisms for Successful Somatic Nuclear Transfer

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## Initial hypothesis for the limited success of SCNT:

- Clones only arose from a subpopulation of adult stem cells. (Hochedlinger & Jaenisch, 2002)

**The most dramatic epigenetic reprogramming occurs in SCNT when the expression profile of a differentiated cell is abolished and a new embryo-specific expression profile is established that drives embryonic and fetal development (Niemann et al., 2008)**

## Evidences of cell fate plasticity exhibited by somatic cells

- Treatment with 5-azacytidine (DNA methylation inhibitor) caused fibroblasts to spontaneously differentiate into muscle and fat cells. (Taylor & Jones, 1979)
- Multinucleated myotubes could dominantly reprogram nuclei from other cell types to express muscle-specific gene products in heterokaryons. (Artificially fused cells that maintain distinct nuclei)
- Fusion of embryonic stem cells and fibroblasts could activate pluripotency markers in somatic nuclei
- Yamanaka and colleagues hypothesized that reprogramming factors could be identified by their specific expression in pluripotent cell types

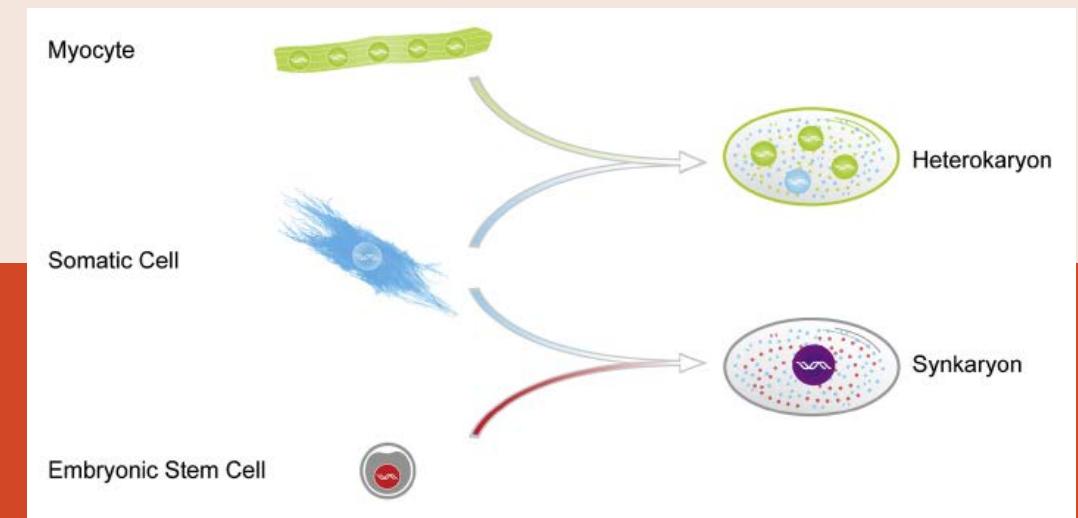
Reprogramming from one somatic cell state to another would theoretically require a highly specific erasure of the epigenetic marks of one lineage, followed by the establishment of a new set of epigenetic features characteristic of the new cell state.

For these reasons it was assumed that lineage reprogramming was possible between closely related cell types, such as...

- Fibroblast-myocytes
- Lymphocytes-macrophages
- Astrocytes-neurons
- These are likely to share some epigenetic features as a result of their recent descent from a common progenitor cell and would thus provide a chromatin landscape that was permissive for reprogramming factor binding and activity

~~Many reports suggested that reprogramming between distantly related somatic cells might not be possible. Limit exists in cell reprogramming by nuclear transfer!~~

# Reprogramming by cell fusion



# Fusion-Mediated Reprogramming

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**Cell fusion experiments indicated that cellular identity affected the transcriptional response to ectopic trans-acting factors.**

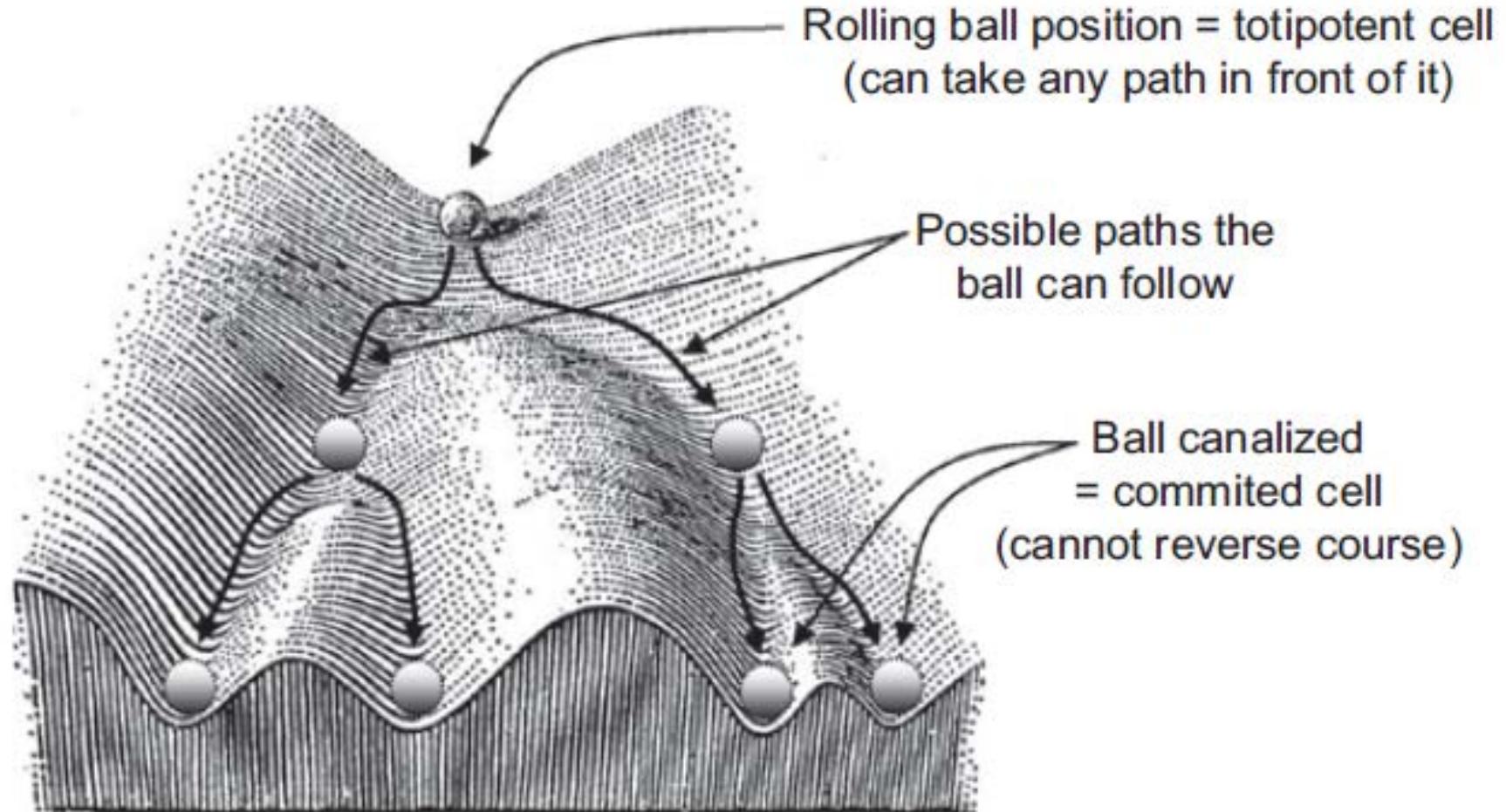
**Myotube heterokaryons could activate muscle-specific genes in nuclei of cells derived from all three developmental germ layers.**

**However, nuclei from endodermal and ectodermal lineages exhibited slower kinetics of transcriptional activation.**

**: This suggest that the lineage-specific patterns of epigenetic information determined the response to the reprogramming factors found in myotubes.**

**ES cells are needed to induce fusion-mediated pluripotency (Ethical issue)**

# Waddington' s Epigenetic Landscape



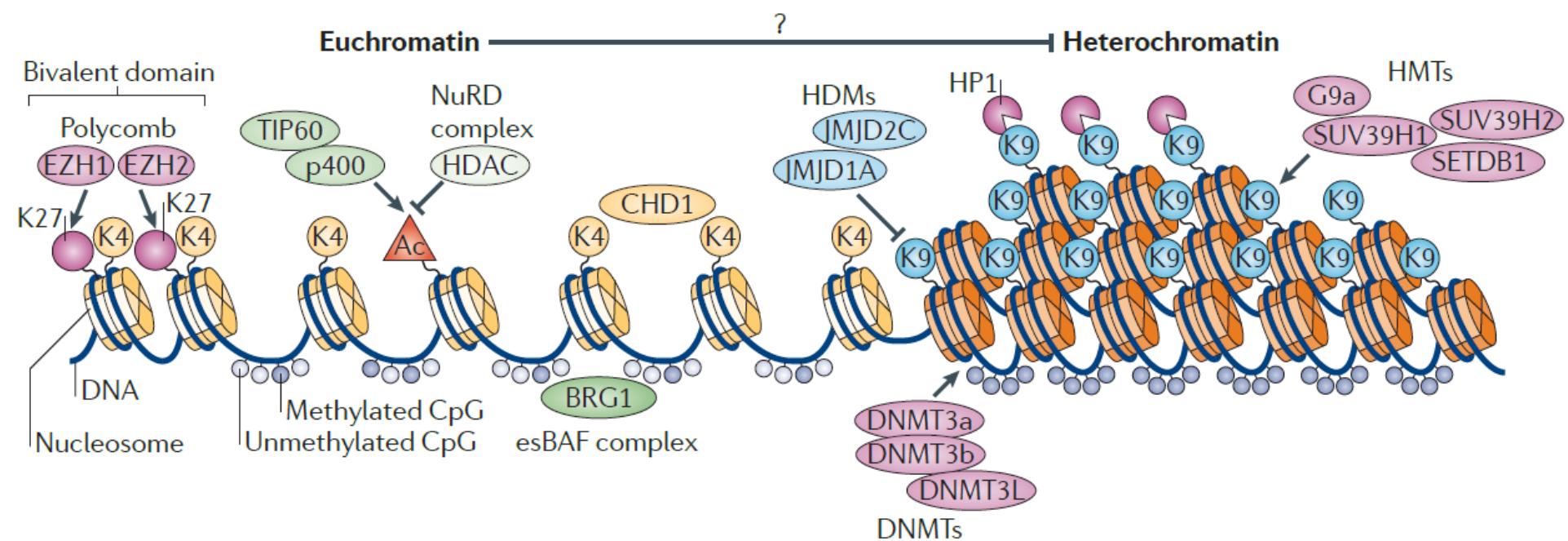
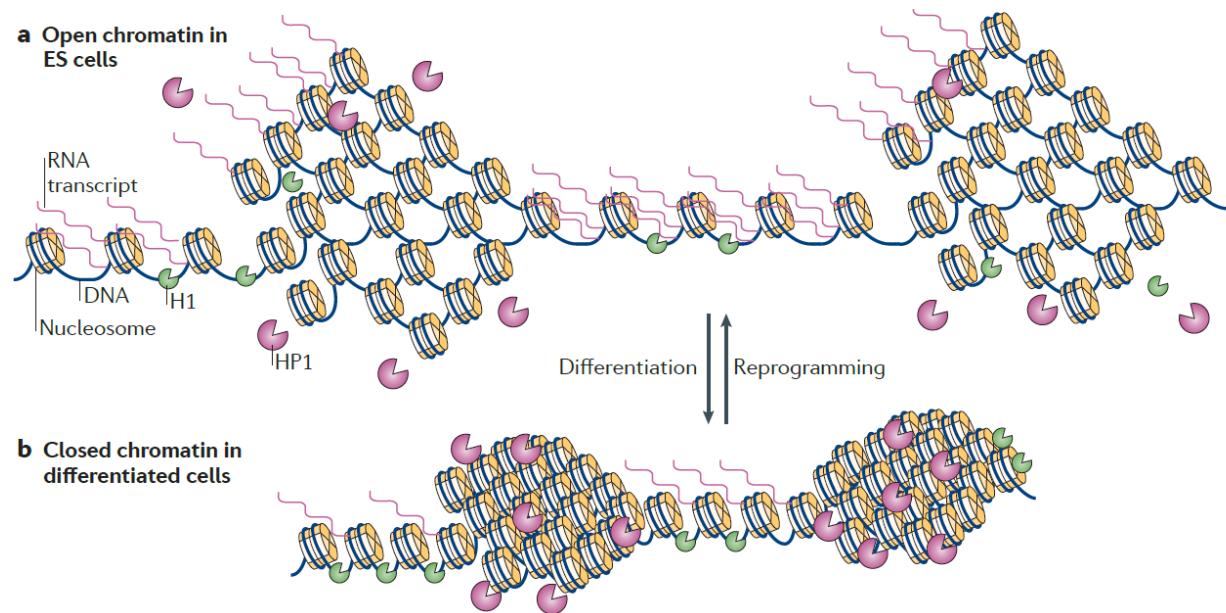
Choudhuri S 2011 Toxicol Mechanisms and Methods

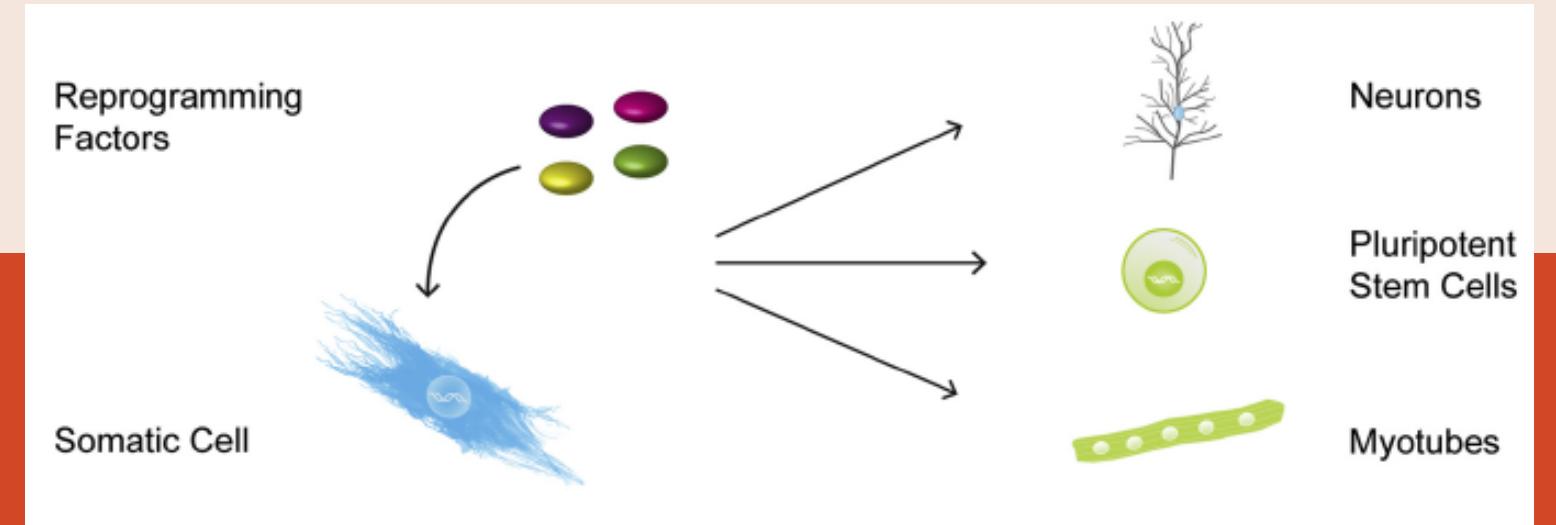
# Epigenetics

- Study of changes in gene expression or cellular phenotype, caused by mechanisms other than changes in the underlying DNA sequence (such as DNA methylation and histone modification).

## Epigenetic Memory

- Remnants of transcriptional properties or chromatin features typical of the starting cell type after reprogramming

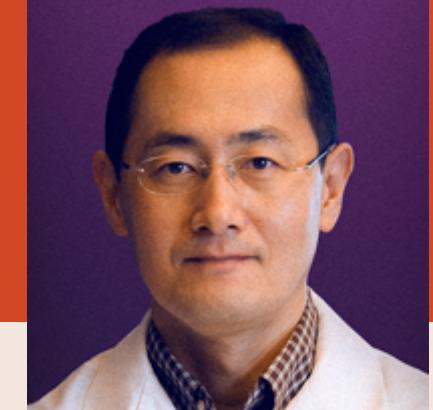




# Ips & direct conversion

[Takahashi K 2006 Cell]

SHINYA YAMANAKA  
2012 NOBEL PRIZE  
IN MEDICINE



KHU

# Comparison of Reprogrammed cells and ES cells

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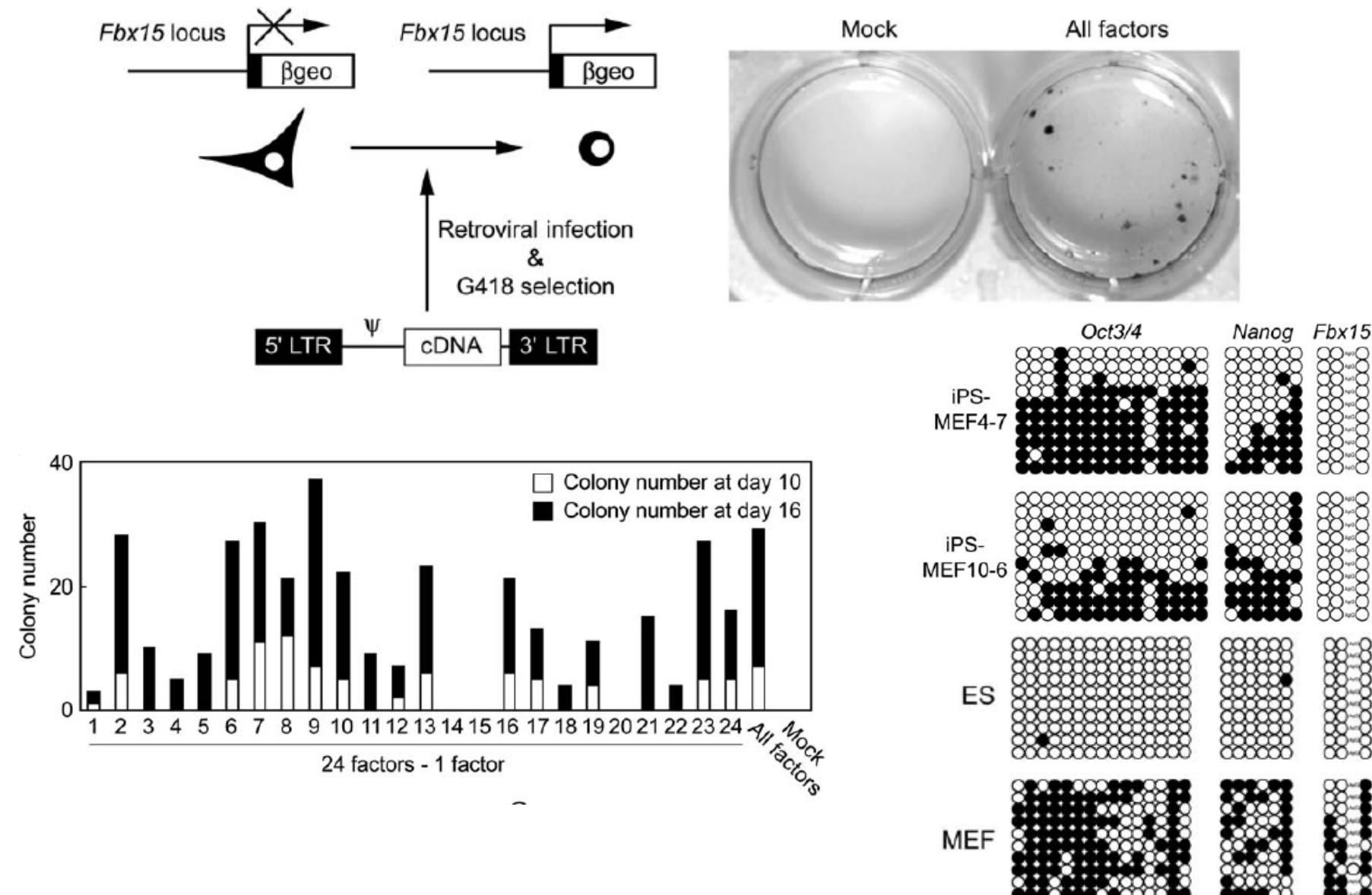
## Similarity

- Cell morphology & growth rate
- Gene expression & chromatin modification
- Pluripotency
- Teratoma formation

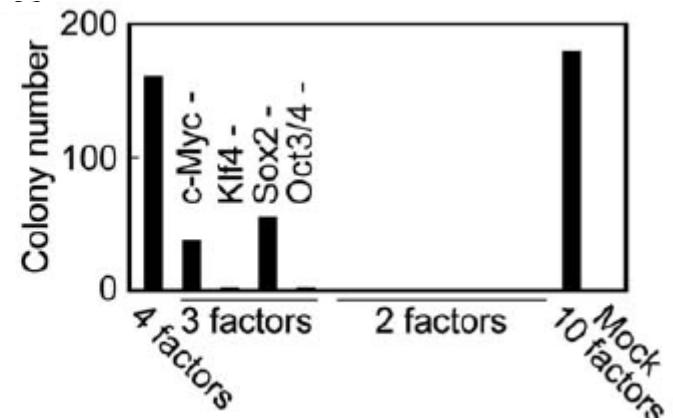
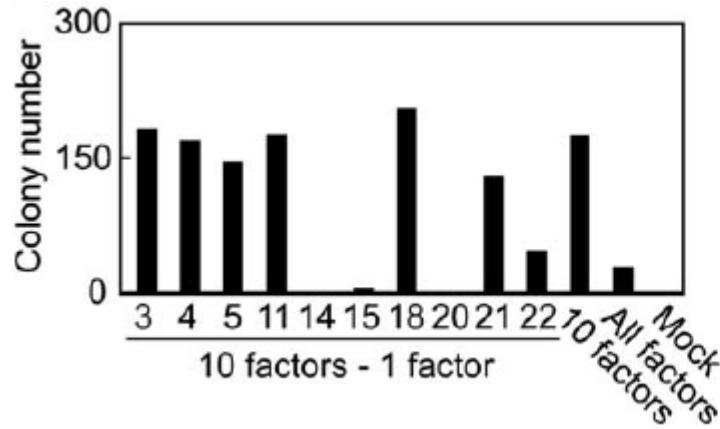
## Advantageous trait of reprogrammed cells

- Customized cell therapeutic agent
- Beyond the religious & ethical controversy
- Can be utilized for customized drug screening

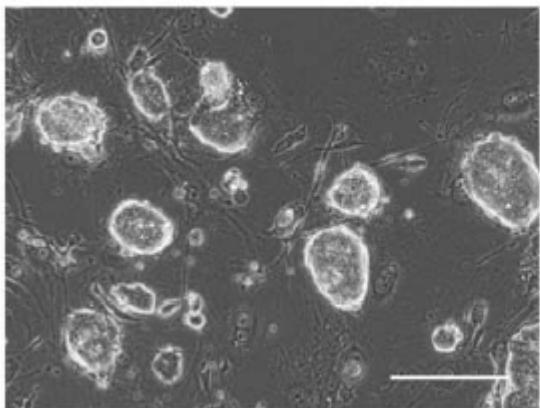
# Assay system for induced pluri-potency



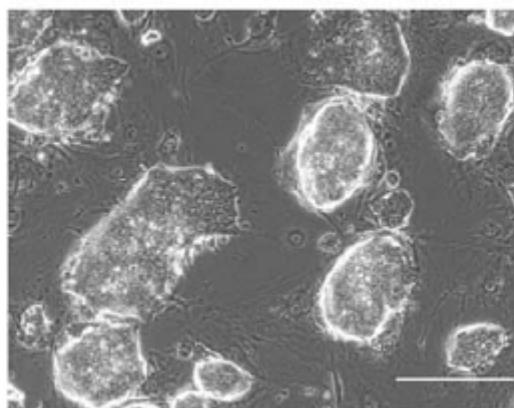
# Narrowing down the Candidate Factors



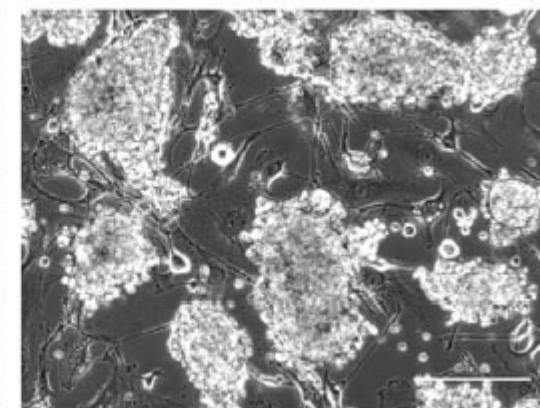
iPS-MEF4-7



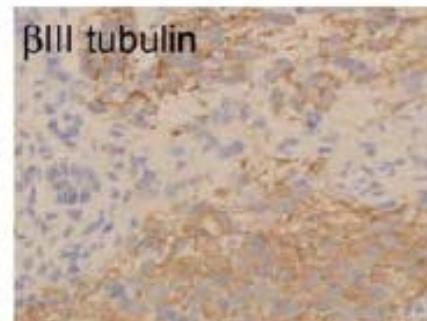
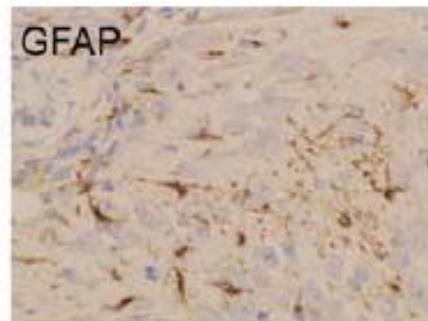
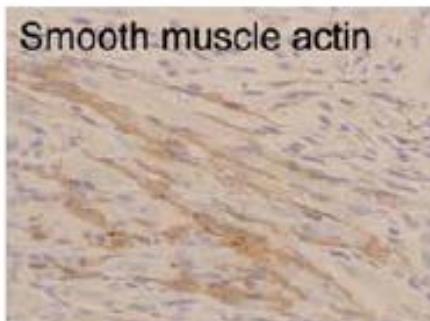
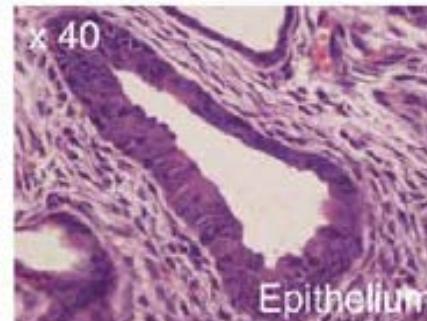
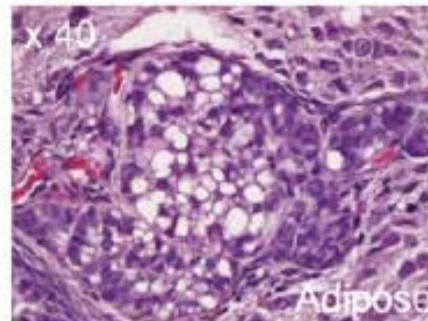
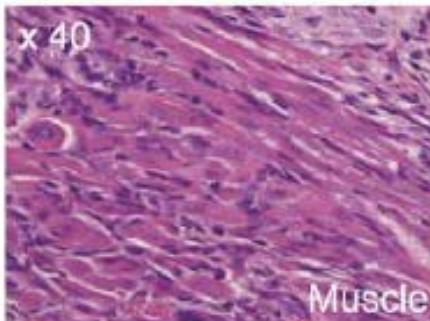
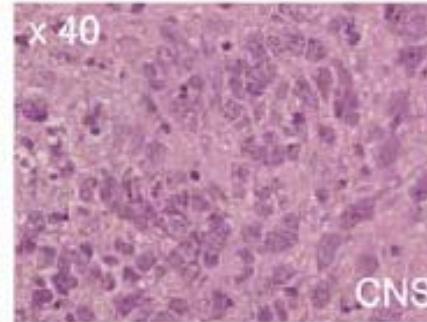
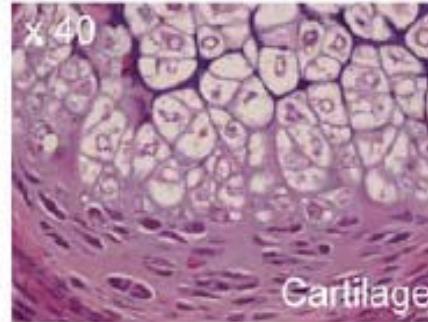
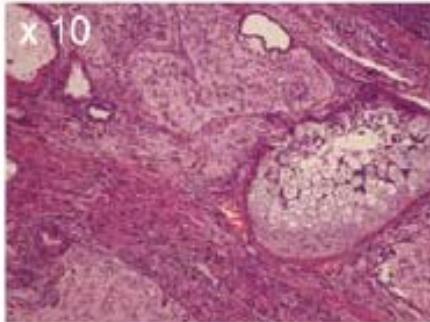
iPS-MEF10-6



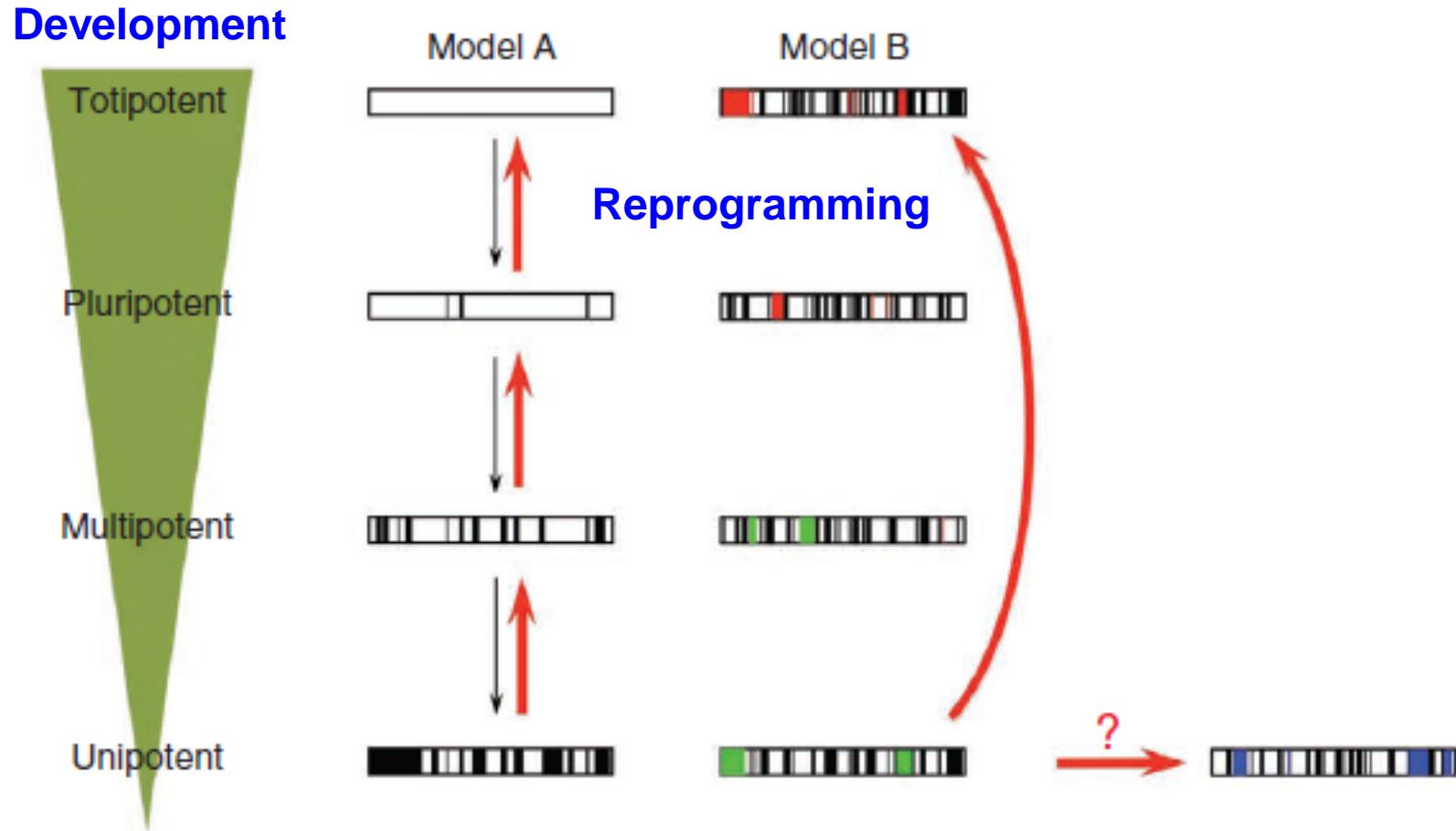
iPS-MEF3-3



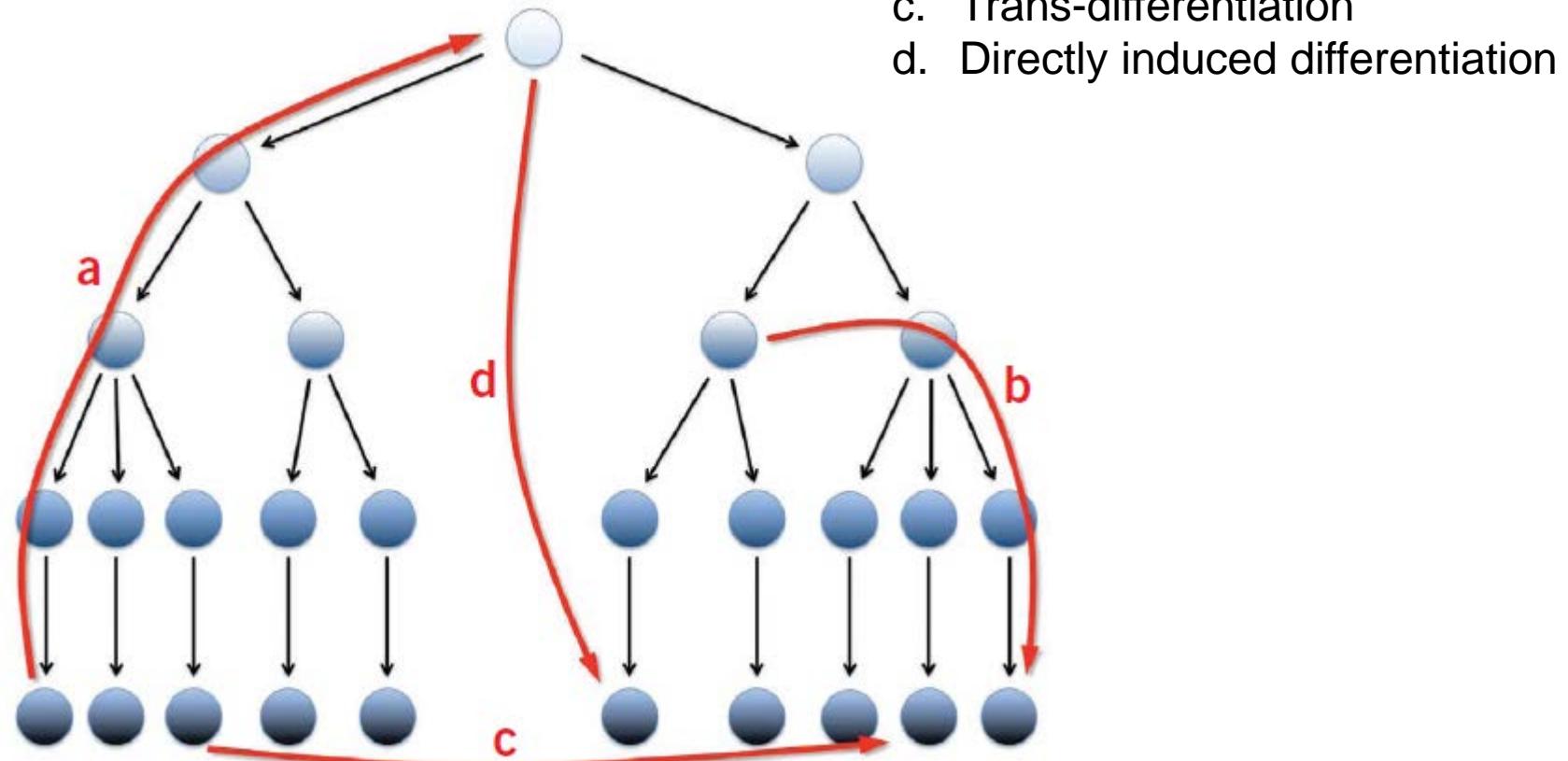
# Pluripotency of iPS Cells Derived from MEFs



# Epigenetic Models of Development and Reprogramming

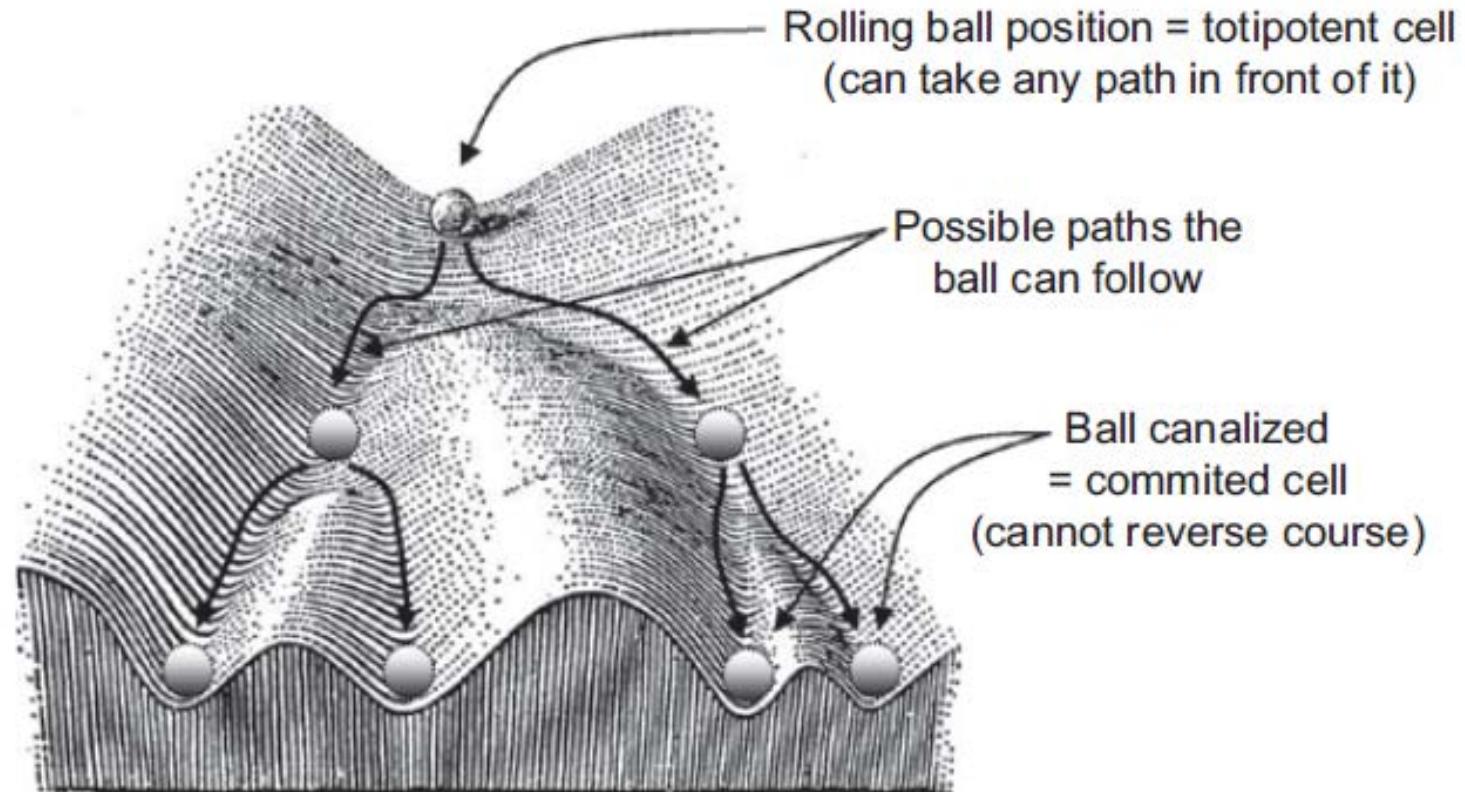


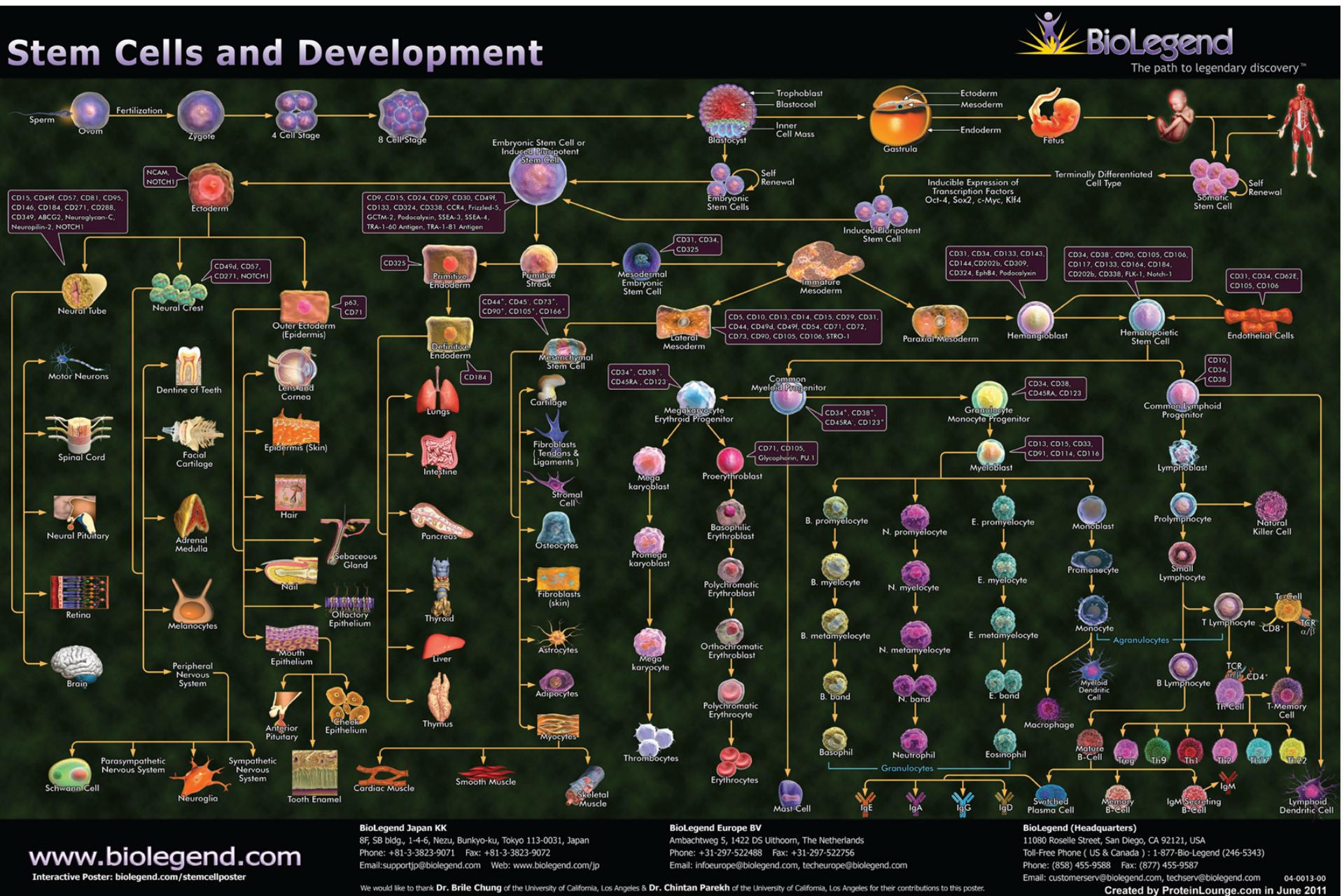
# Various Modes of Induced Cell Fate Changes



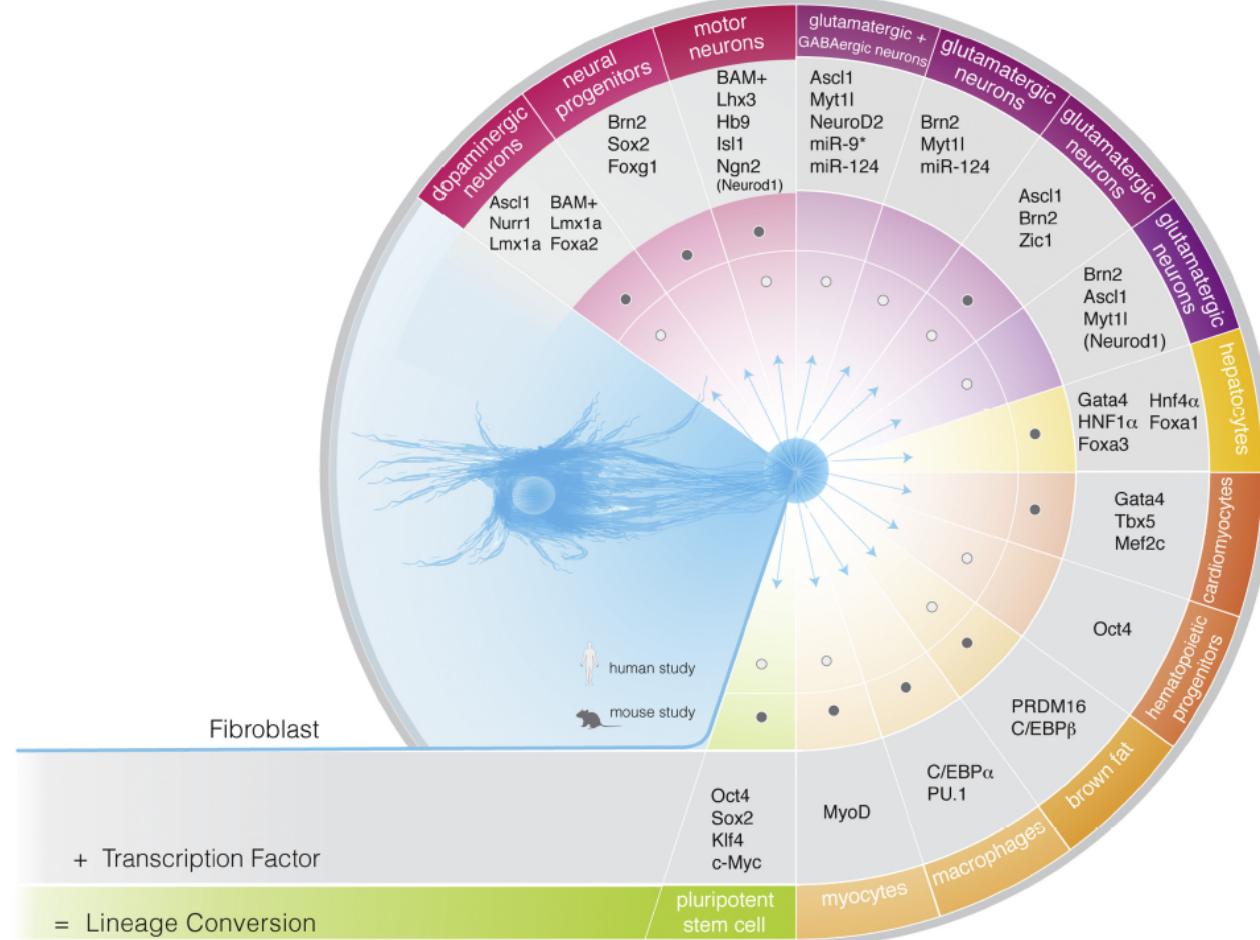
# Waddington' s Epigenetic Landscape

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# Direct Conversion



**Figure 2. Transcription Factor-Mediated Conversion of Fibroblasts into Diverse Cellular Lineages**

Summary of the diverse cell types generated directly from mouse and human fibroblasts by lineage reprogramming. Factors listed in parentheses are required for reprogramming human cells but not for mouse cells. References (starting from the bottom left of the figure and going counterclockwise): Ambasudhan et al., 2011; Caiazzo et al., 2011; Davis et al., 1987; Feng et al., 2008; Huang et al., 2011; Ieda et al., 2010; Kajimura et al., 2009; Lujan et al., 2012; Pang et al., 2011; Pfisterer et al., 2011; Qiang et al., 2011; Sekiya and Suzuki, 2011; Son et al., 2011; Szabo et al., 2010; Takahashi and Yamanaka, 2006; Yoo et al., 2011.

**Table 2 iPS cell reprogramming versus direct lineage conversion**

	iPS cell reprogramming	Direct lineage conversion
Cell division	Required	Not required
Reprogramming dynamics	Slow	Fast
Reprogramming efficiency	Low	High
Potential tumor risk	High	Low
Target cell generation	Two steps <sup>a</sup>	One step
Cell scaling	Feasible	Limited
Screening numerous individuals	Laborious	Feasible

<sup>a</sup>Reprogramming followed by targeted differentiation.

**표 3. Pluripotency를 얻기 위한 실험적 방법 비교**

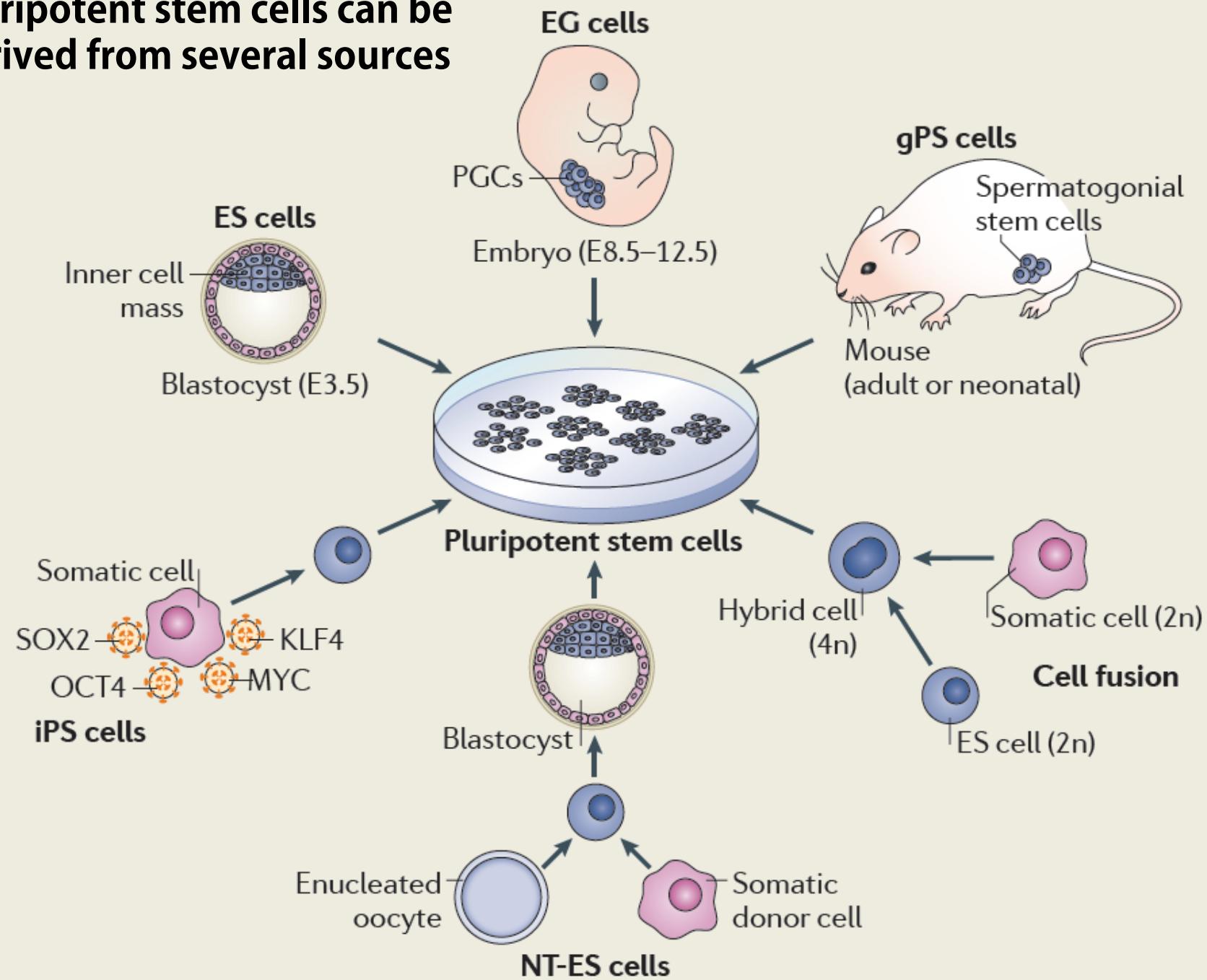
	SCNT	Cell Fusion	iPS Cells
생식세포 이용	이용	이용	이용하지 않음
배아형성 여부	포함	포함하지 않음	포함하지 않음
Plasticity	Pluripotent	제한적	Pluripotent
염색체의 배수성(Ploidy)	2n	4n	2n
기술적 난점	Donor와 recipient 모두 stress가 커서 성공율이 극히 낮음.	Fusion rate 낮음	Teratoma 형성 제어 필요

## \* How to overcome shortcomings of iPSCs?

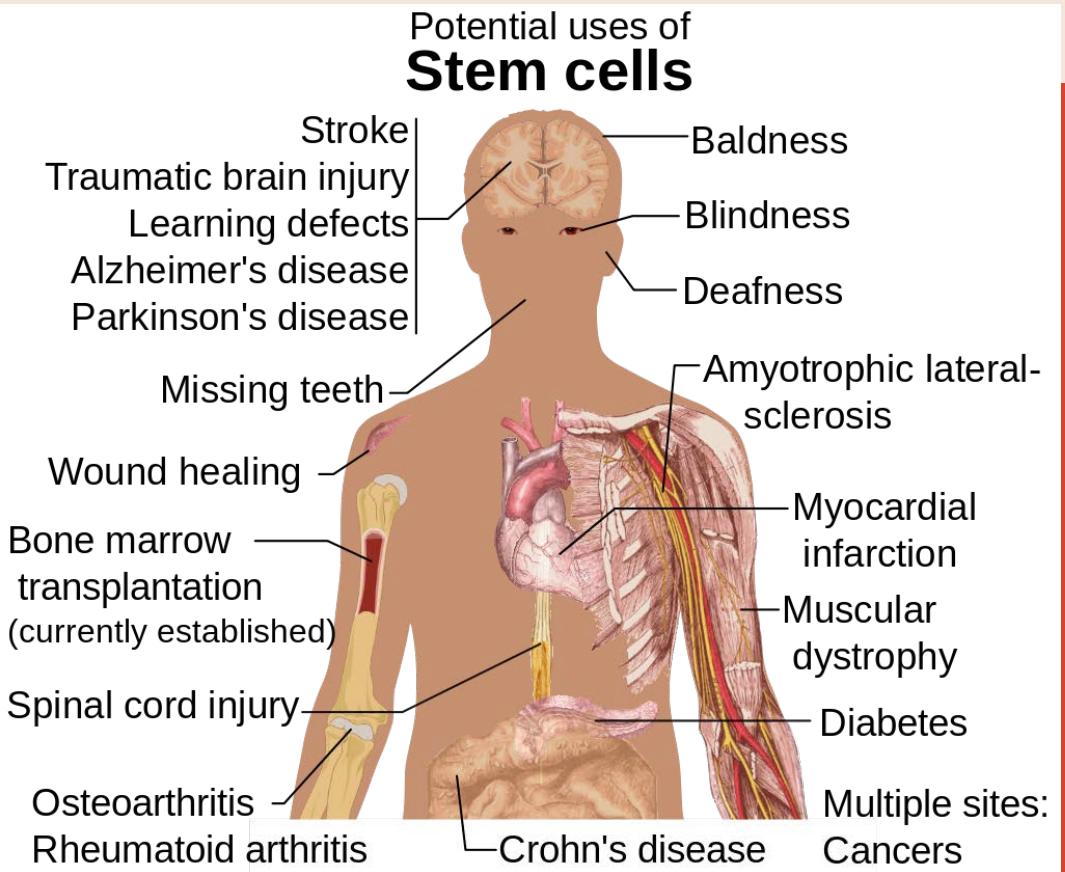
- Teratoma formation
- Random mutation induced by “knock in” method

1. Screening of reprogramming-inducing small molecules
2. Protein delivery rather than gene delivery
3. Non-integrating episomal vector
4. Mechanical or environmental reprogramming factor

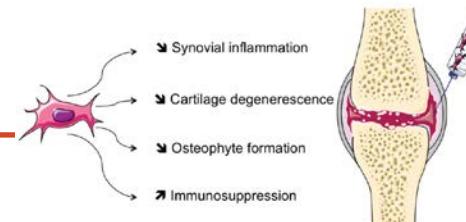
# Pluripotent stem cells can be derived from several sources



# cell-based therapy



# Cell-based therapy



## Regenerative function of SCs

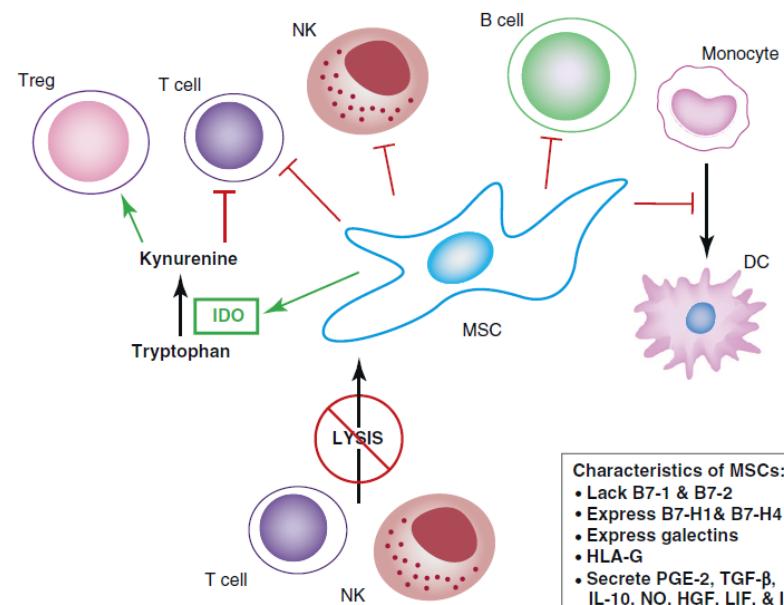
Trophic effect

Immune modulatory function

GVHD (Graft-versus-host disease)

Autoimmune disease

- Systemic injection
- Local injection



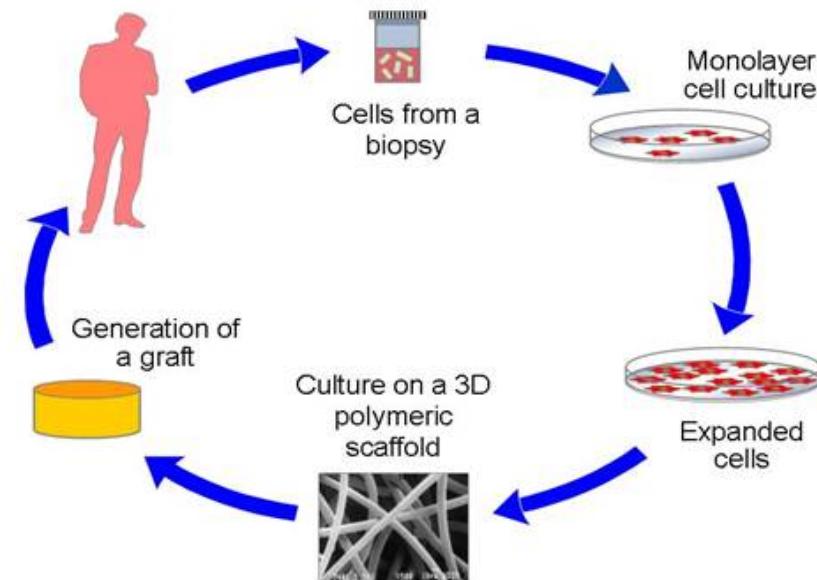
## Tissue Engineering

3D reconstruction of tissue

- Shape & Function

- Combinatorial approach
  - Biomaterials
  - Cells
  - Bioactive factors (GFs)

### Basic principles of Tissue engineering



# 국내외 줄기세포치료제 제품 현황

*Classification of stem cells according to its origin - Autologous/ Allogenic /Xenogenic stem cells*

**As of 2019, cord blood therapies are the only FDA approved, stem-cell based therapies in the United States. These cord blood therapies are currently limited to treating patients with blood disorders. Furthermore, Europe, Japan and South Korea have only seen a small handful of approved stem-cell based products.**

# MSCs Therapy - Disease types

Rheumatoid Arthritis

Osteoarthritis

Stroke

Arterial Disease

Paralysis

Inflammatory diseases

Acute GVHD (Graft versus host disease)

Chronic GVHD

Autoimmune disease

Sepsis

Rheumatoid arthritis

Mastocytosis

Chronic Granulomatous Diseases

Cancer

Lymphoma

Neuroblastoma

Myeloma

Leukemia

Hematologic Neoplasm

Chronic Myeloproliferative Disorders

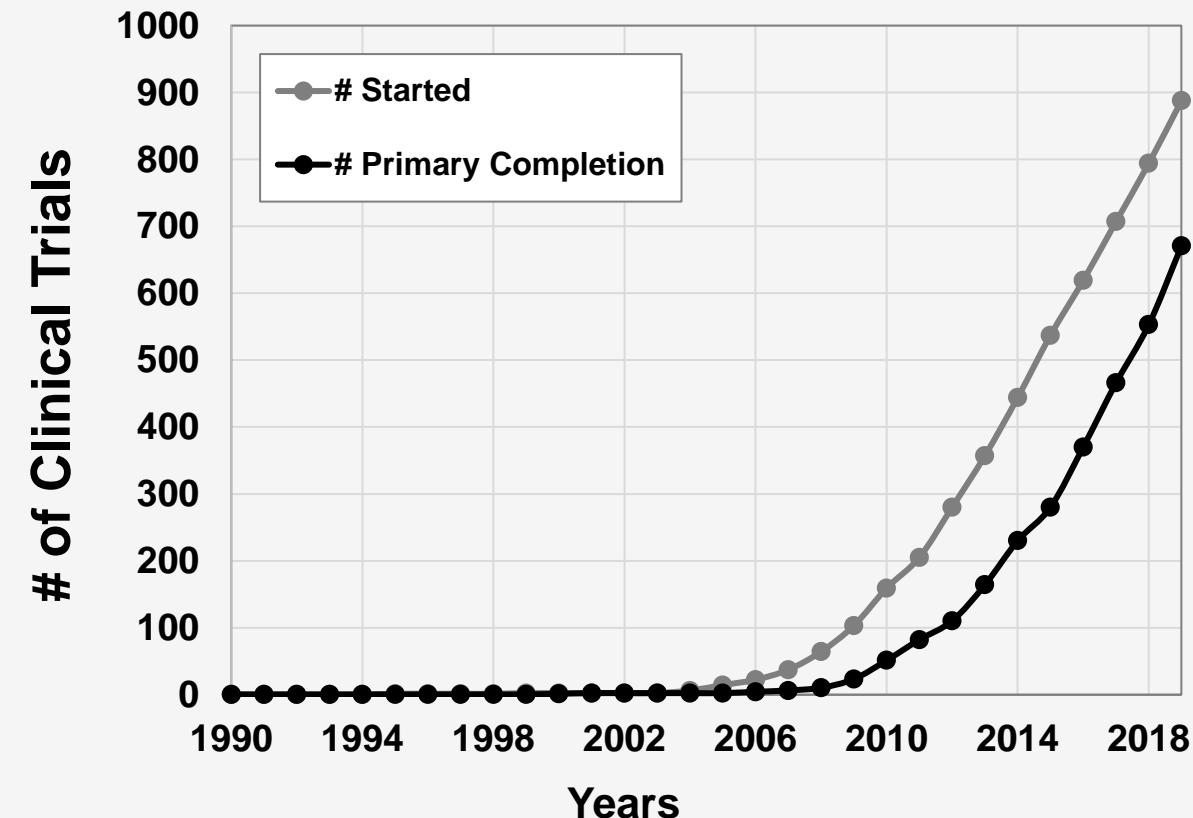
Fungus Disease

Amyloidosis

Death

Fibrosis

*Data from Clinicaltrials.gov*

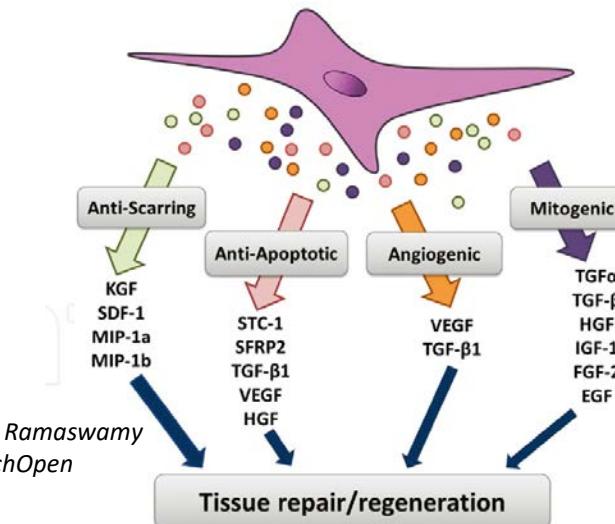
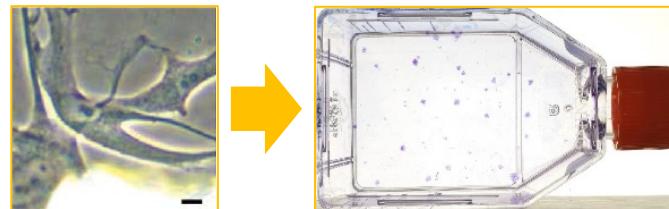


\* Intervention = Mesenchymal stem cells

# Stem Cells in Regenerative Therapy

## - Mechanisms of the Approved Therapeutic Agents

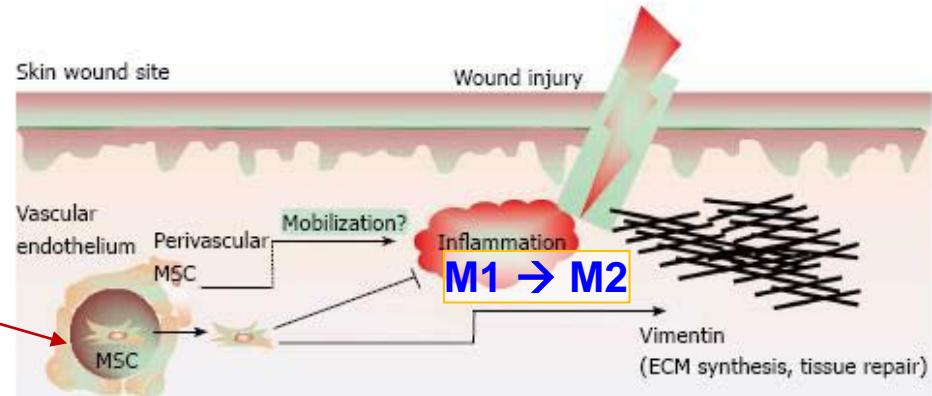
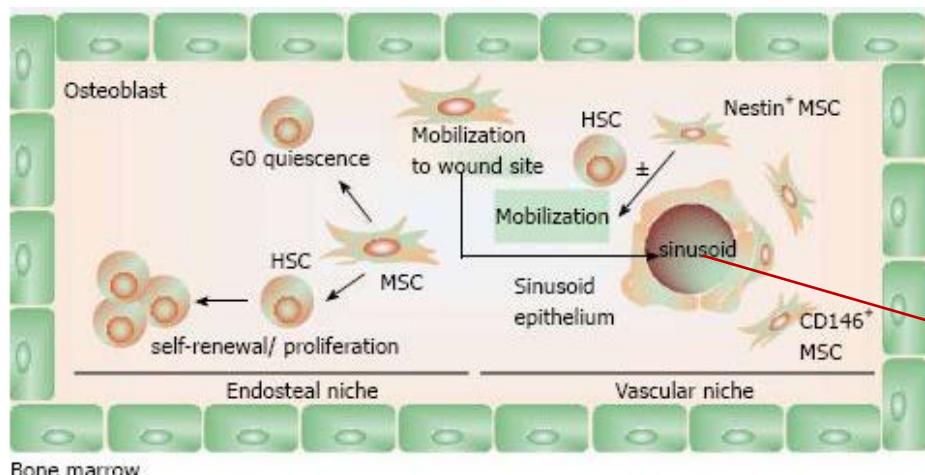
### 1. Trophic effect



### 2. Direct tissue regeneration



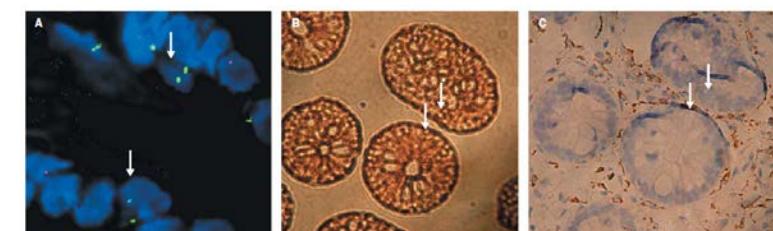
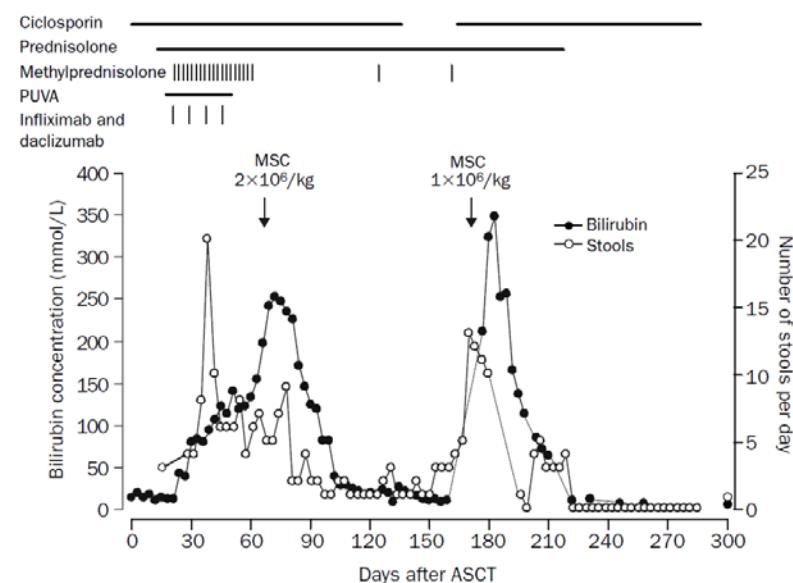
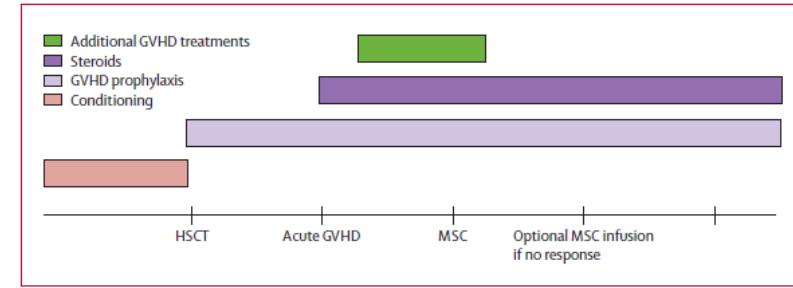
### 3. Immune- modulation



Glenn JD 2014 World J Stem Cells

# MSCs Therapy - Targeting GVHD (*Le Blanc K 2004 & 2008 Lancet*)

- **9-year-old boy** with acute lymphoblastic leukemia
- Transplantation of blood stem cells
  - Preconditioning with cyclophosphamide (120mg/kg)
  - Fractional total body irradiation (3Gy for 4 days)
  - HLA-A, HLA-B, HLA-DRb1 identical, unrelated female donor
  - Immunosuppression: Thymoglobulin (6 mg/kg), ciclosporin, methotrexate
- Day11: developed a **maculopapular rash of the thorax and back**
- Day22: developed **diarrhea and abdominal pain** requiring morphine
- Day24: **stop eating, Bilirubin & ALT rose**
  - PUPA treatment, Infliximab, Daclizumab
- Day70: **developed grade IV GVHD**
  - Diarrhea up to 20 times a day
- **Day73: MSCs given intravenously (donor: mother)**
  - 4 days later: diarrhea fell to twice a day,
  - 5 days later: bilirubin declined
  - 2 weeks later: resumed oral food intake
- Day143: DNA analysis showed the presence of minimal residual disease
- Day150: had **diarrhea again** without abdominal pain
- **Day170: second MSCs transplantation** form the same batch
  - Diarrhea back to normal after 1 week and start to eat again
- **Day220: went home**



Le Blanc K 2004, 2008 Lancet

	Measure	Number of patients
Recipients		
Recipient age (years)		
Male, female		
Child, adult		
Diagnosis		
AML		
ALL		
CML	7	
CLL	2	
JMML	4	
Multiple myeloma	2	
Myeloproliferative disorder	1	
Myelodysplastic syndrome	6	
Lymphoma	1	
Non-malignant disorders	10	
Solid tumour	2	
Disease stage: early, late†	21, 24	
Donors and cells		
Female donor to male recipient	10	
Male donor to female recipient	9	
HLA-identical sibling	19	
Unrelated A, B, DR $\beta$ 1 identical	25	
Mismatched donor	6	
Unrelated CB (matched, mismatched)	3,2	
Stem cell source (BM, PBSC, BM+PBSC, CB)	19, 30, 1, 5	
GVHD prophylaxis		
Ciclosporin	4	
Ciclosporin + methotrexate	38	
Ciclosporin + MMF	5	
Ciclosporin + prednisolone	6	
Other	2	
ATG, ALG, alemtuzumab	30, 1, 5	
Dose		
Nucleated cell dose $\times 10^8$ /kg	5.9 (0.17-20.6)	
CD34+ cell dose $\times 10^6$ /kg	8 (0.15-28)	
Cytomegalovirus serology		
Negative in donor and recipient	14	
Positive in donor and recipient	20	
Positive in donor or recipient	21	
Data are numbers unless otherwise indicated. ALG=anti-lymphocyte globulin. ALL=acute lymphoblastic leukaemia. AML=acute myeloid leukaemia. ATG=antithymocyte globulin. BM=bone marrow. CB=cord blood. CLL=chronic lymphocytic leukaemia. CML=chronic myeloid leukaemia. JMML=juvenile myelomonocytic leukaemia. MMF=mycophenolate mofetil. PBSC=peripheral-blood stem cell. *Median (min-max range). †Early=non-malignant disease, first complete remission, first chronic phase; late=beyond these stages at time of transplant.		
Table 1: Characteristics of patients and treatment		

Our patient had progressive severe GVHD that was unresponsive to all therapy. Mesenchymal stem-cell treatment had a striking immunosuppressive effect. In our

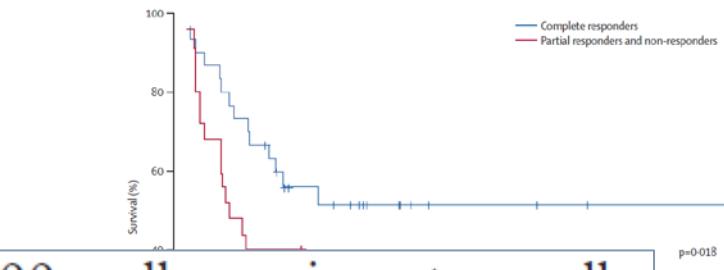
Two:

gut+skin/gut+liver/liver+skin

15,7,4

Three

19



In our experience of 1000 allogeneic stem-cell transplantations, 25 patients developed grade IV acute GVHD. This is the only patient with such severe disease who is still alive. The other 24 patients died a median of 2 months after transplantation (range 0.5–5.5). This case encourages prospective, controlled studies with mesenchymal stem cells for prophylaxis and treatment of GVHD. The effects of these stem cells could also be explored in other patients who are in need of immune modulation and tissue repair, such as organ transplant recipients and patients with severe autoimmune diseases.

Fifth line

2

ATG=antithymocyte globulin. MMF=mycophenolate mofetil. MSC=mesenchymal stem cell. PUVA=psoralen and ultraviolet-A irradiation. \*Numbers in brackets had immunosuppressive therapy at time of MSC infusion. †One Thymoglobulin, Genzyme, USA; one ATGAM, Upjohn, USA.

Table 2: GVHD grade and organ involvement

	Survival	12	6	24
Limited chronic GVHD	2	0	2	
Extensive chronic GVHD	4	2	6	

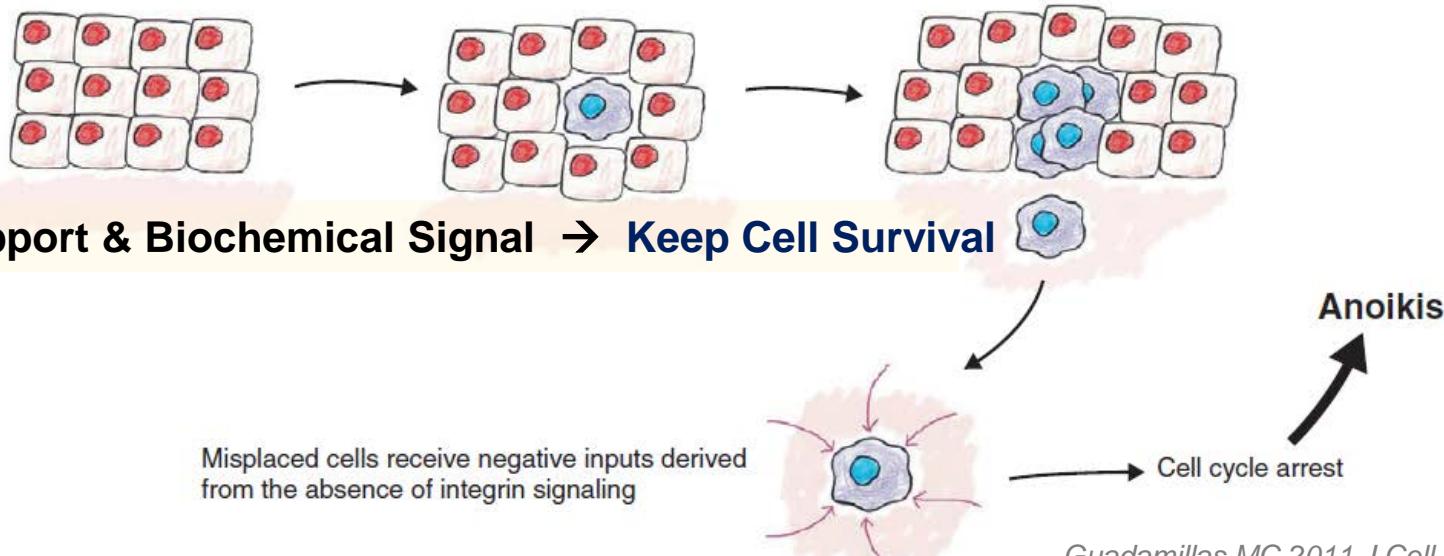
\*At last data collection, March, 2007.

Table 4: GVHD response and outcome

# Maximizing Stem Cell Activity by Cell Surface Engineering

# Anoikis

- apoptosis promoted by attachment-deprived state  
(Stupack & Cheresh 2002; Redding & Juliano 2005)



- Role of ECM on cell survival:
  - ECM is reservoir of GFs
  - Laminin-5, tenascin-C, & Decorin has EGF-like repeats  
-> binds & activate EGFR -> Transduce survival signal

- Attachment-deprived state
  - FAK inactivation
  - Loss of PI-3K & Src activation
  - BAX activation & translocation to mitochondria
  - Apoptosis

# Cell Surface Engineering for Advanced Cell Therapy

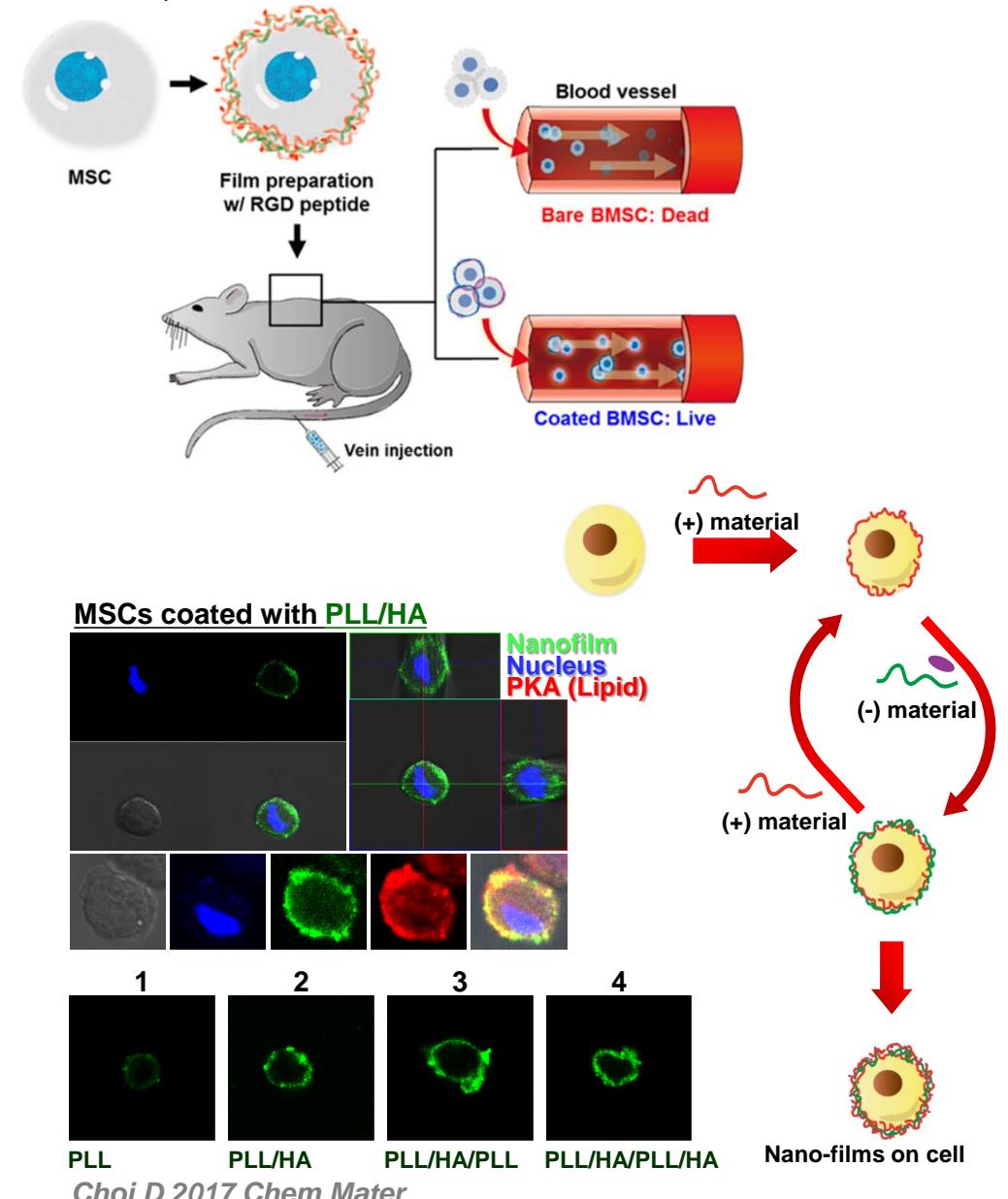
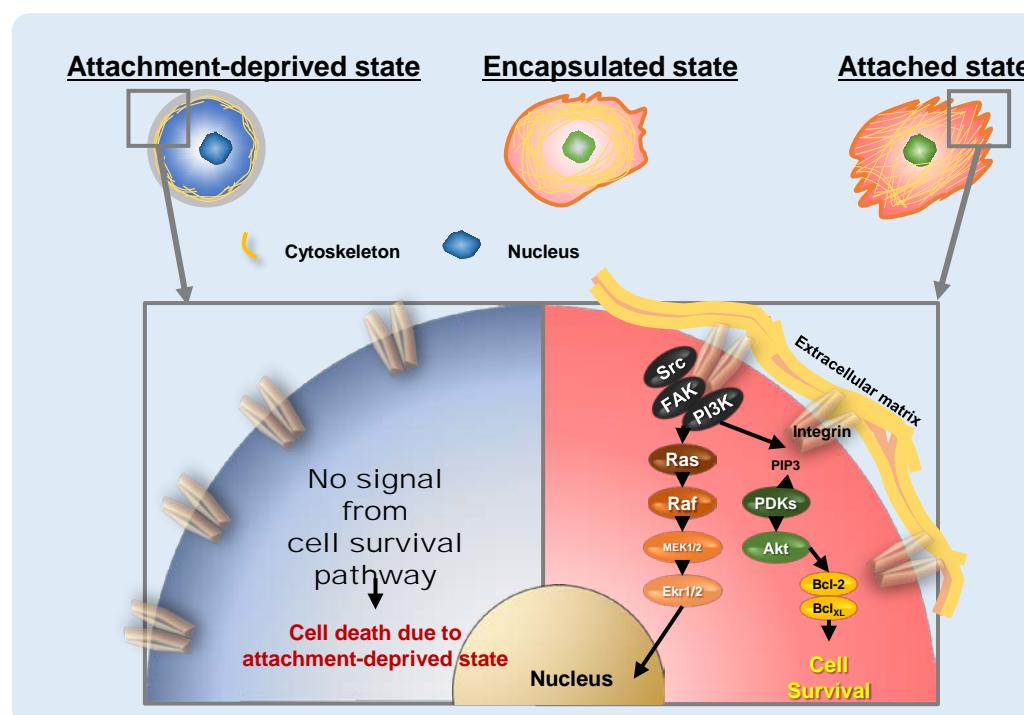
## Systemic Transplantation of MSCs

### Fate of MSCs after systemic transplantation

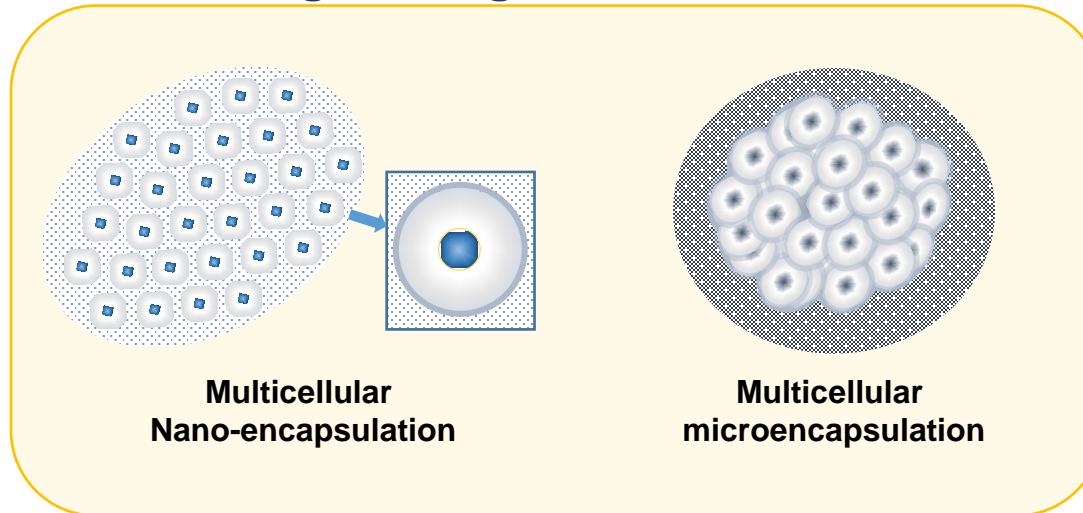
- Extremely low cell survival due to anoikis
  - MSCs eventually undergo apoptosis
- Poor retention at the intended target site

### Two Possible Approach

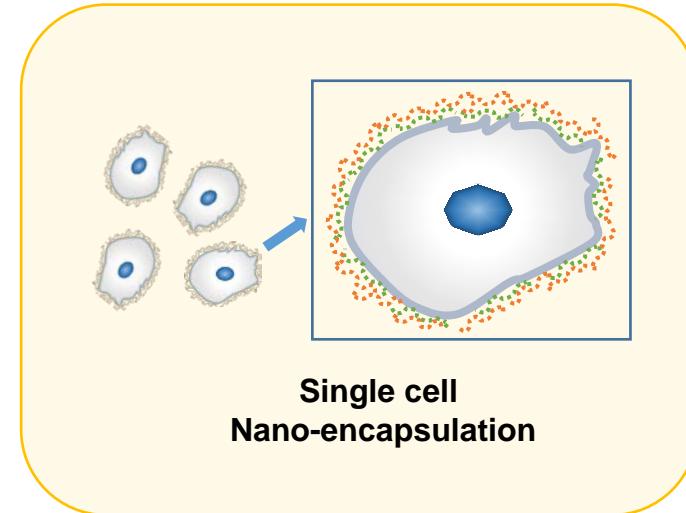
- Scale-up manufacturing for high-dose administration  
(Limitation by maximum tolerate dose)
- Boosting cell survival (maximizing cell activity to minimize the dose)



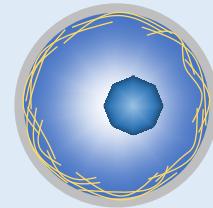
## Tissue Engineering



## Cell Therapy

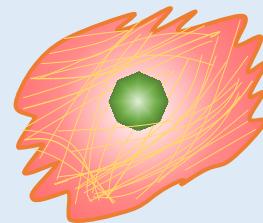


### Attachment-deprived state



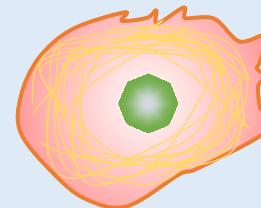
Cytoskeleton

### Attached state



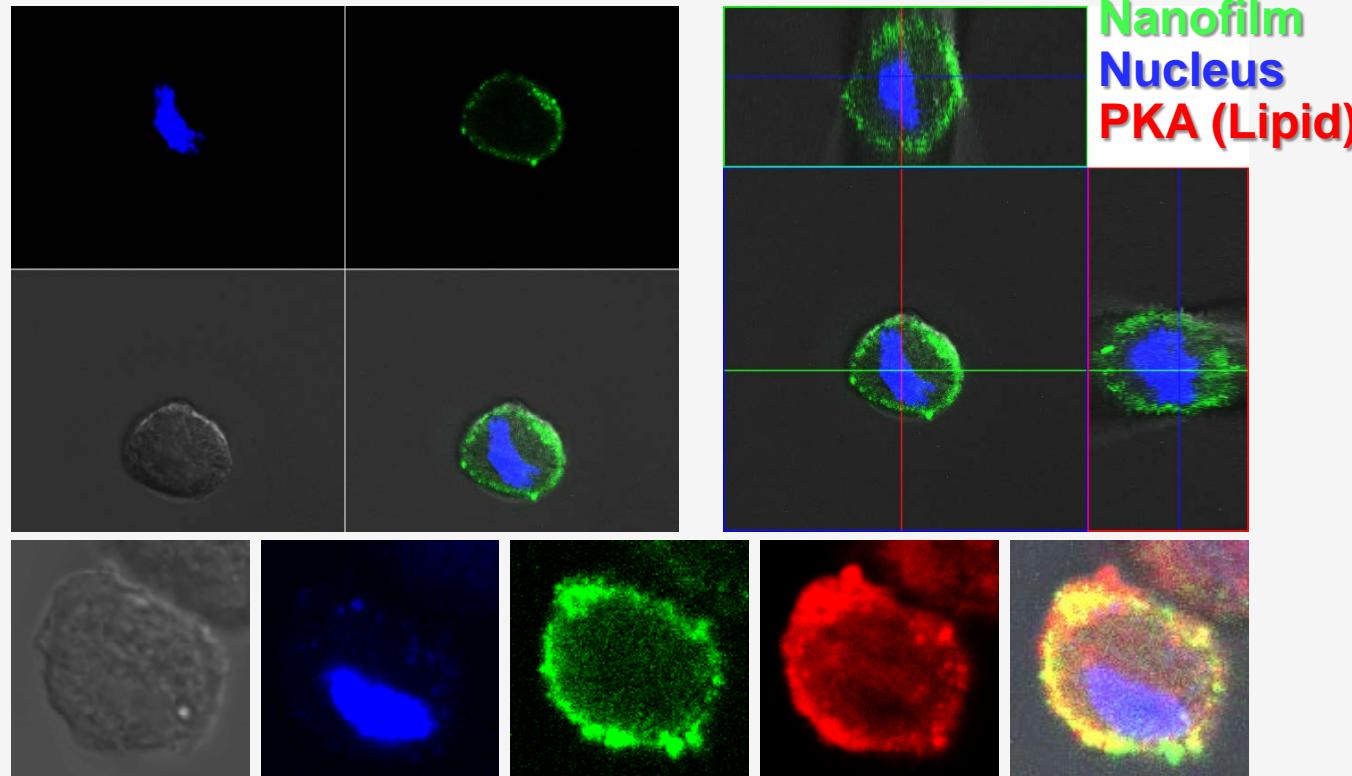
Nucleus

### Surface-modified state



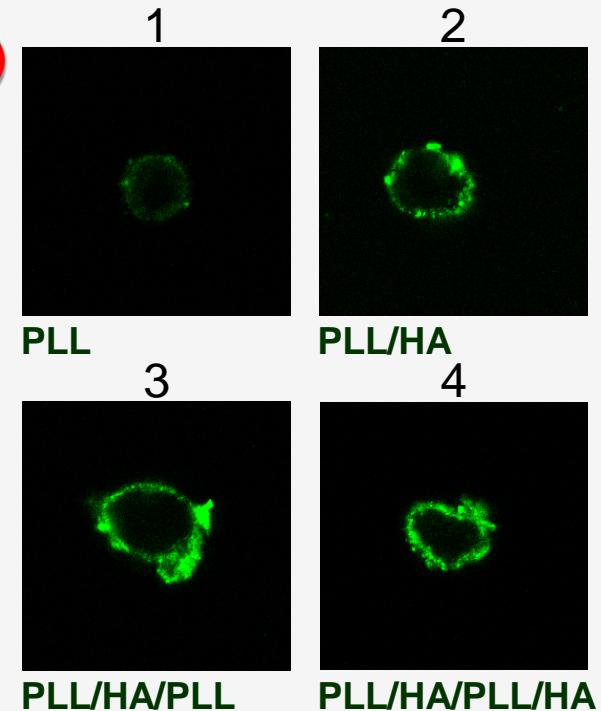
# Nanofilm on MSCs

**MSCs coated with PLL/HA**



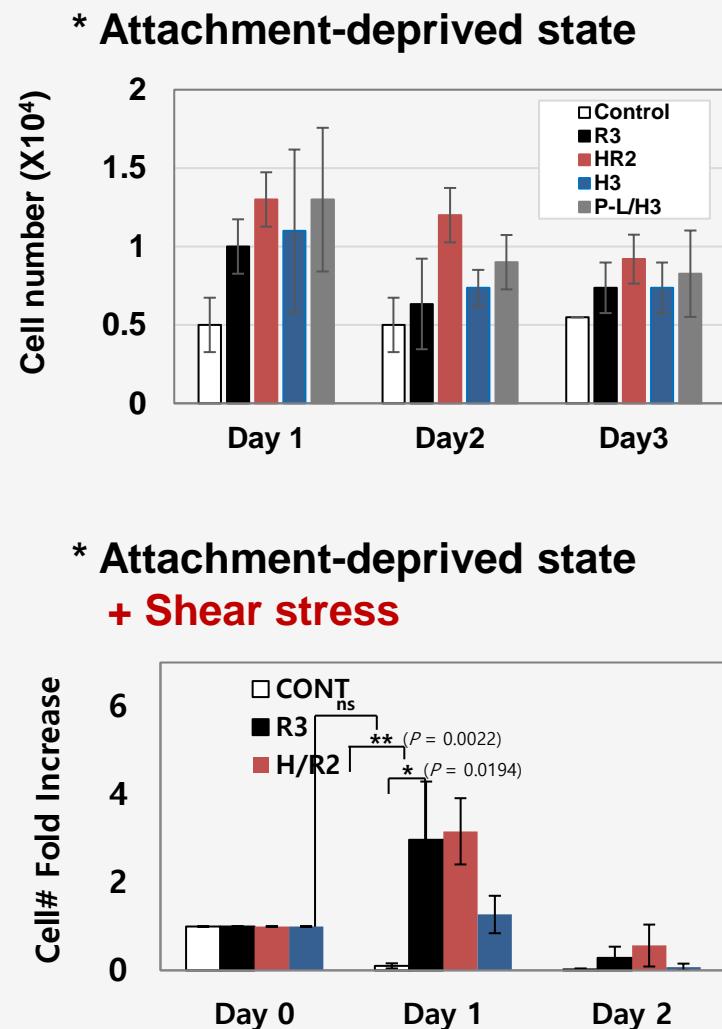
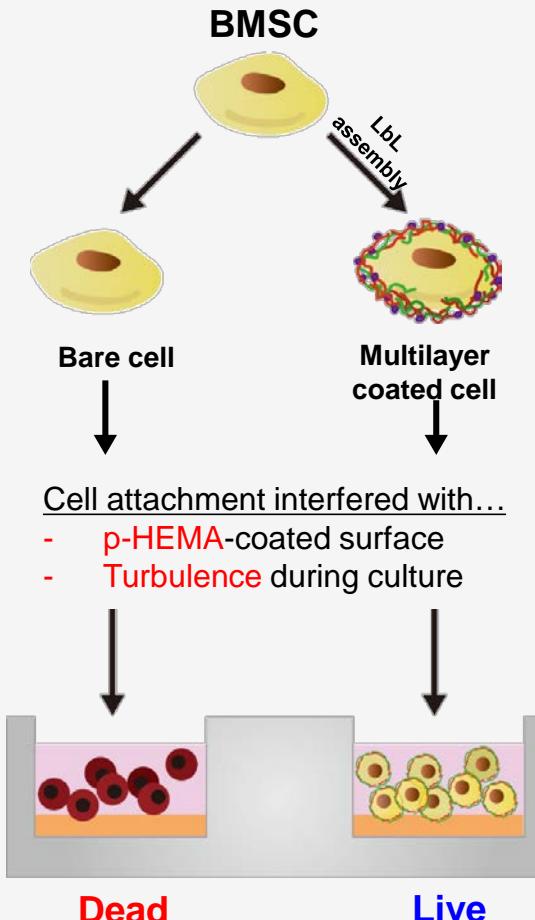
Choi D 2017 Chem Mater

**Number of Layers (PLL/HA)**

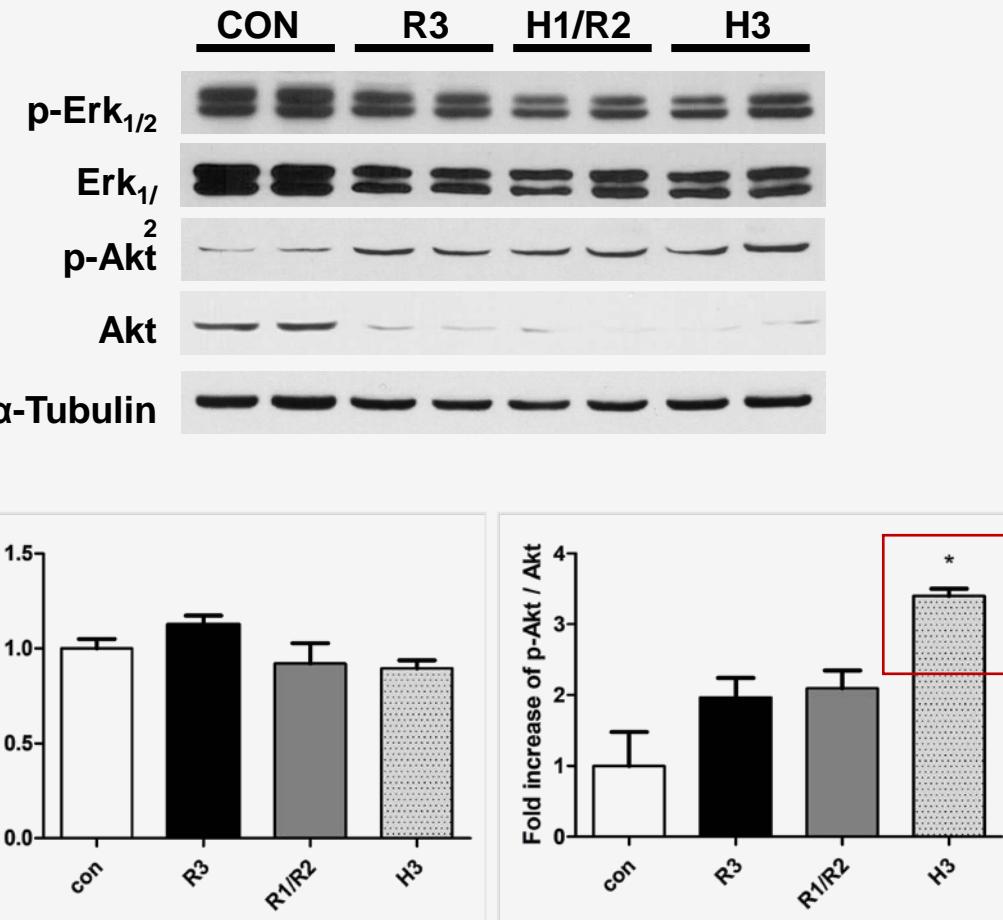


# Effect of Nanofilm on *In Vitro* Survival

R3	(PLL/RGD) <sub>3</sub>	H3	(PLL/HA) <sub>3</sub>
H/R2	(PLL/HA)/(PLL/RGD) <sub>2</sub>	P-R/H	(PLL-RGD/HA) <sub>3</sub>



\* Survival Signaling Status



# Cell Encapsulation

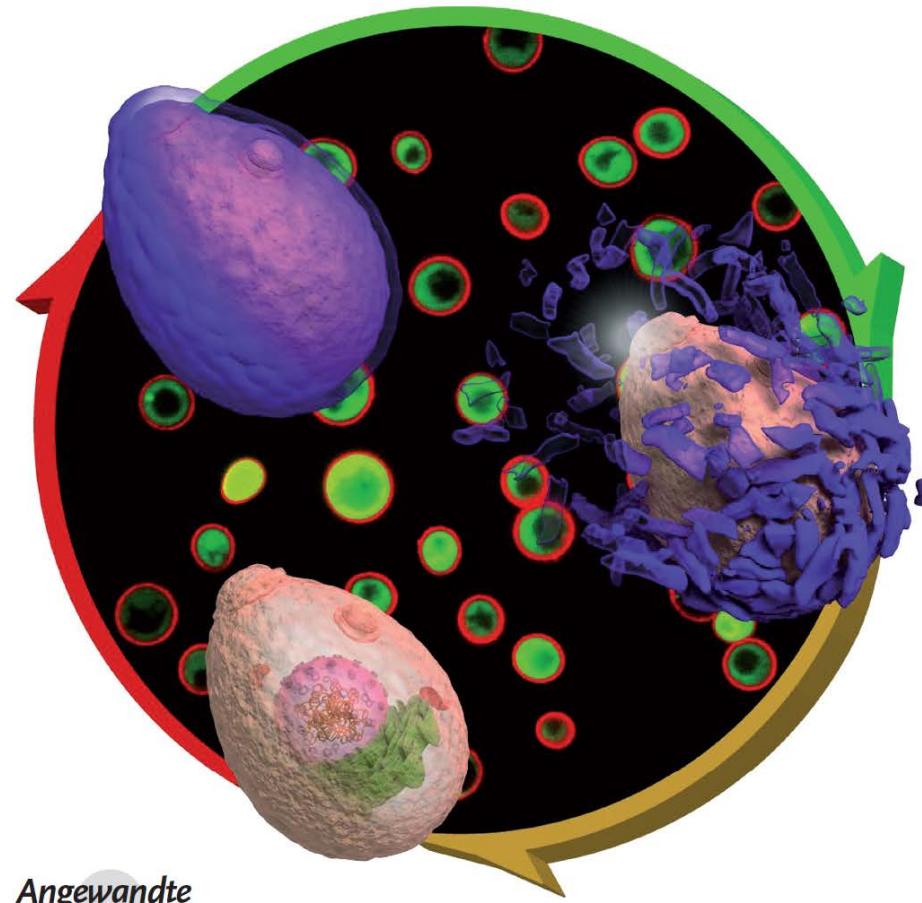
Lee JK et al., 2018 Chemistry-A European Journal

Artificial Spores

DOI: 10.1002/anie.201405905

## A Cytoprotective and Degradable Metal–Polyphenol Nanoshell for Single-Cell Encapsulation\*\*

Ji Hun Park, Kyunghwan Kim, Juno Lee, Ji Yu Choi, Daewha Hong,  
Sung Ho Yang, Frank Caruso,\* Younghoon Lee,\* and Insung S. Choi\*

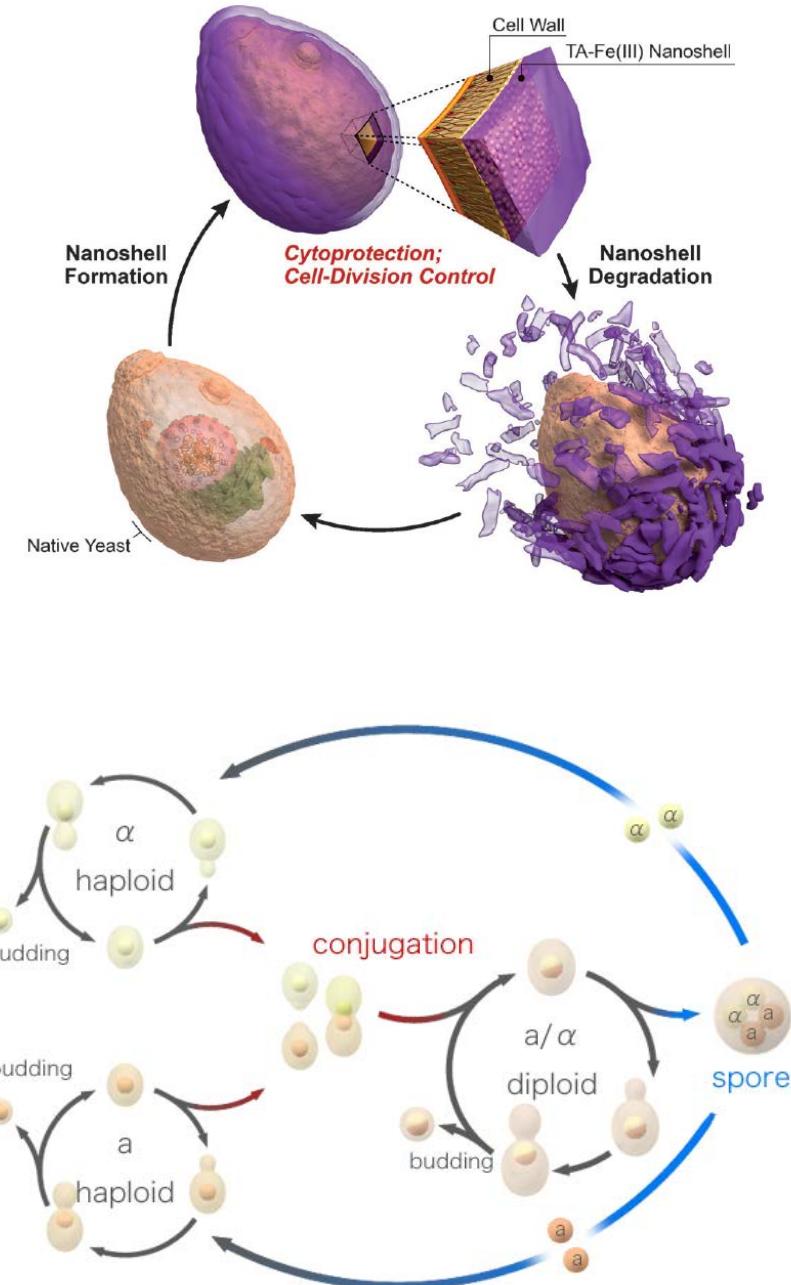


Angewandte  
Chemie  
International Edition

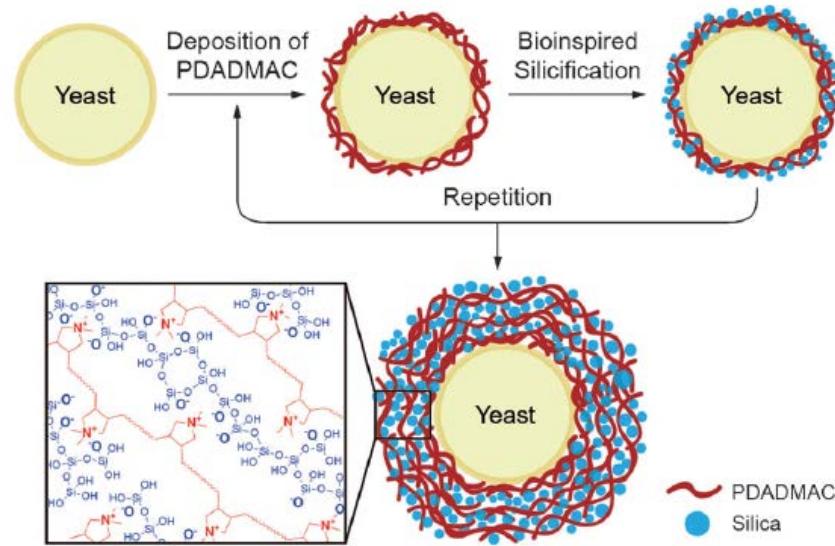
12420 Wiley Online Library

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Angew. Chem. Int. Ed. 2014, 53, 12420–12425

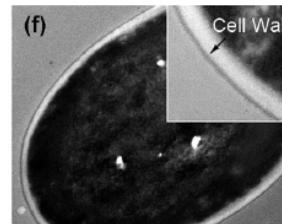
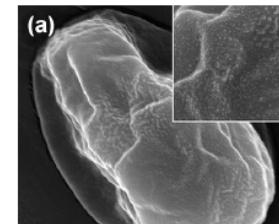


# Yeast@LbL assembly

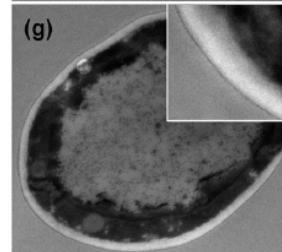
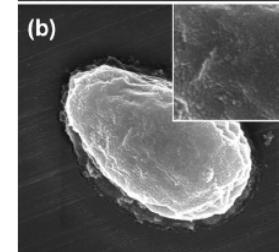


PDADMAC. Poly(diallyldimethylammonium chloride)

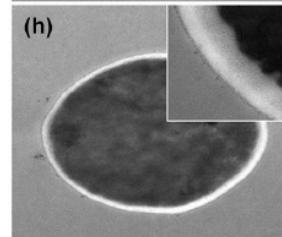
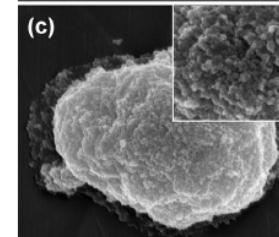
Control



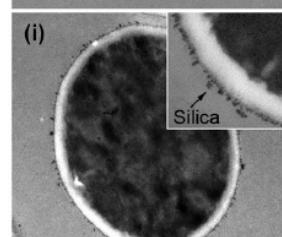
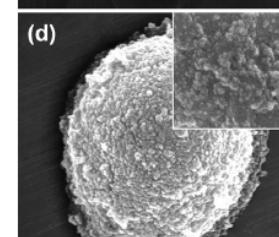
1 Layer



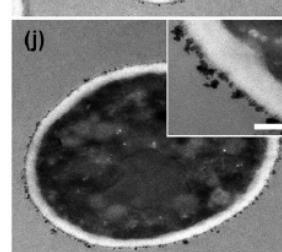
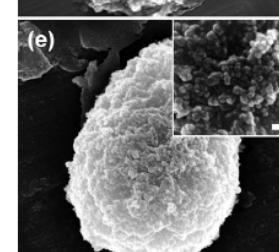
3 Layers



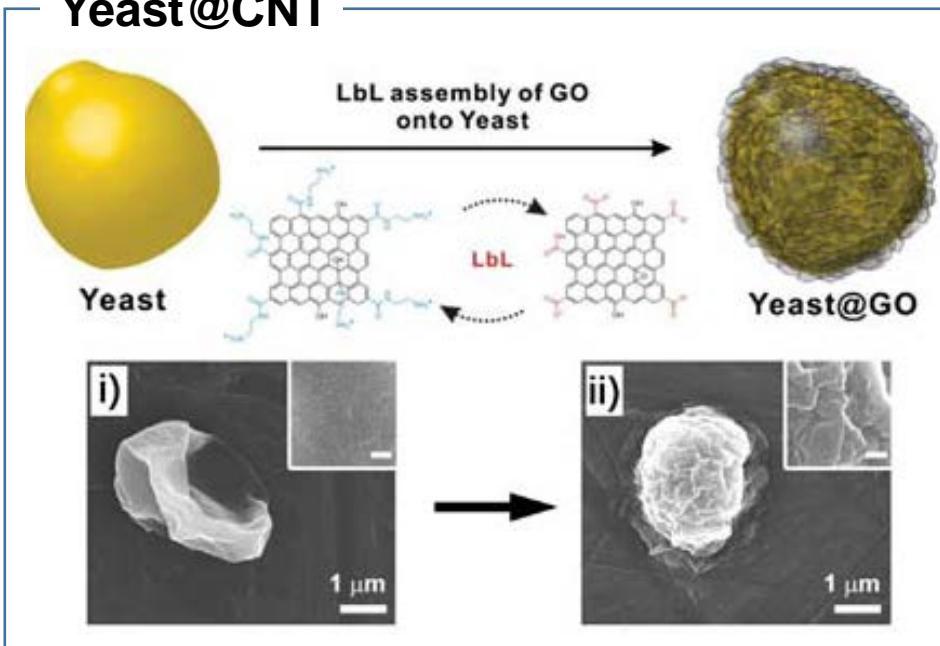
5 Layers

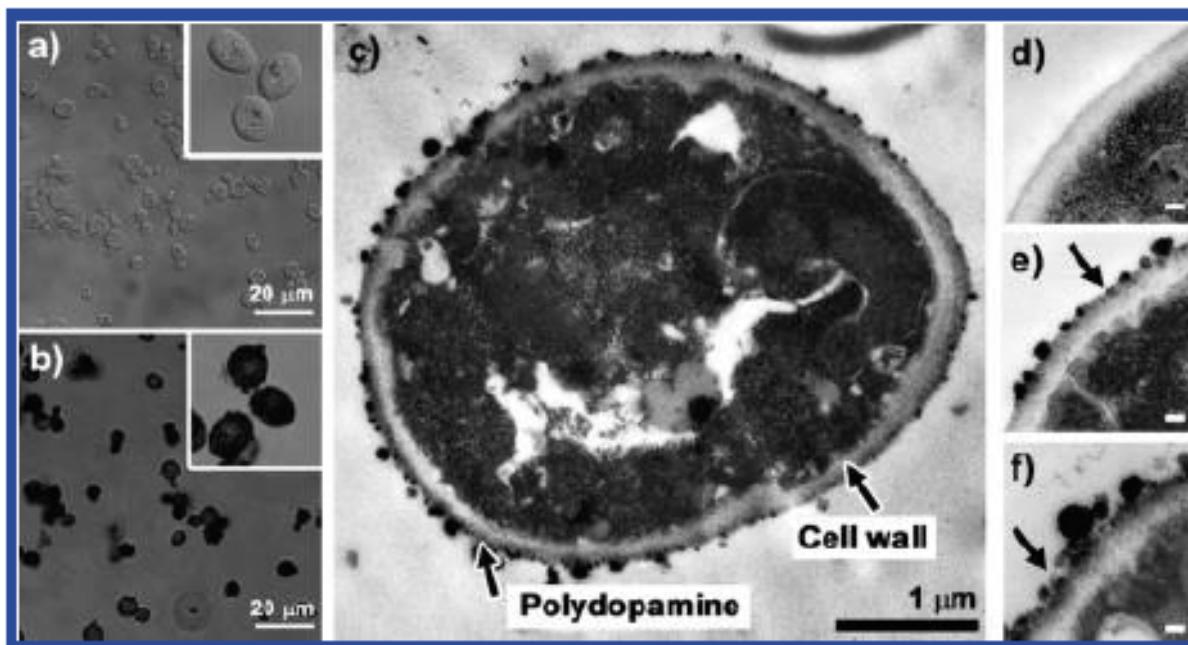
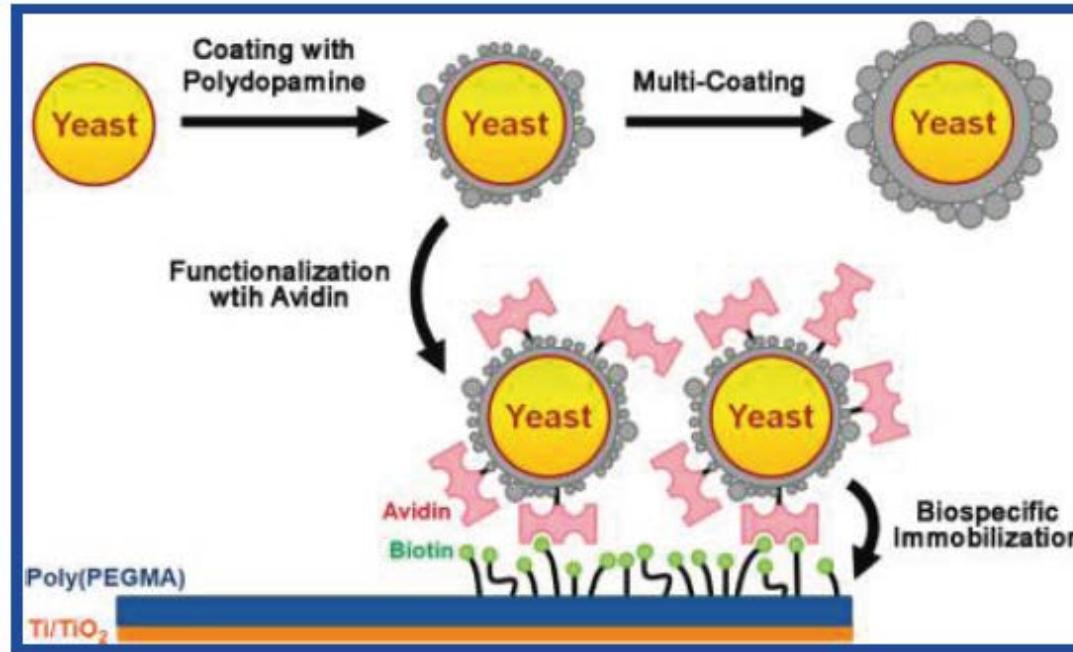


7 Layers

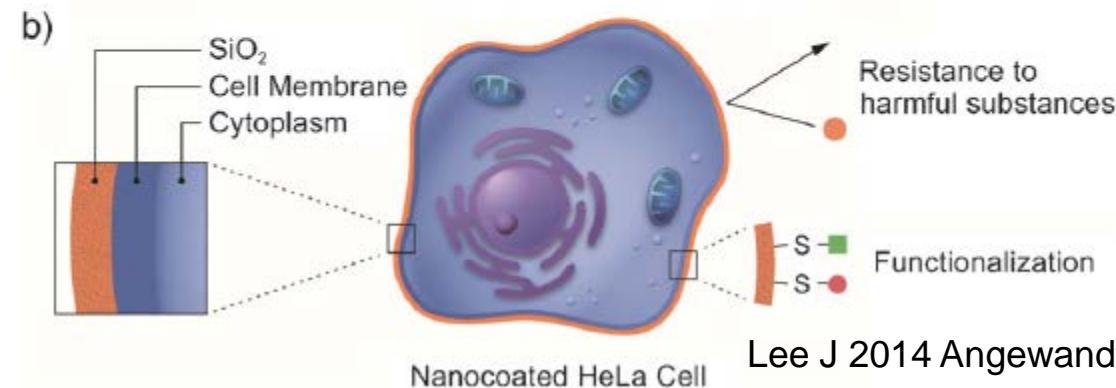
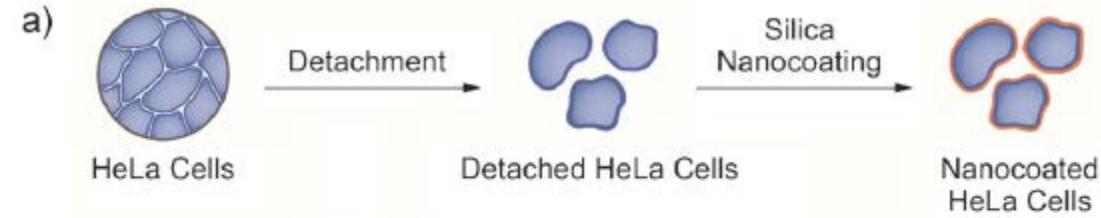


# Yeast@CNT

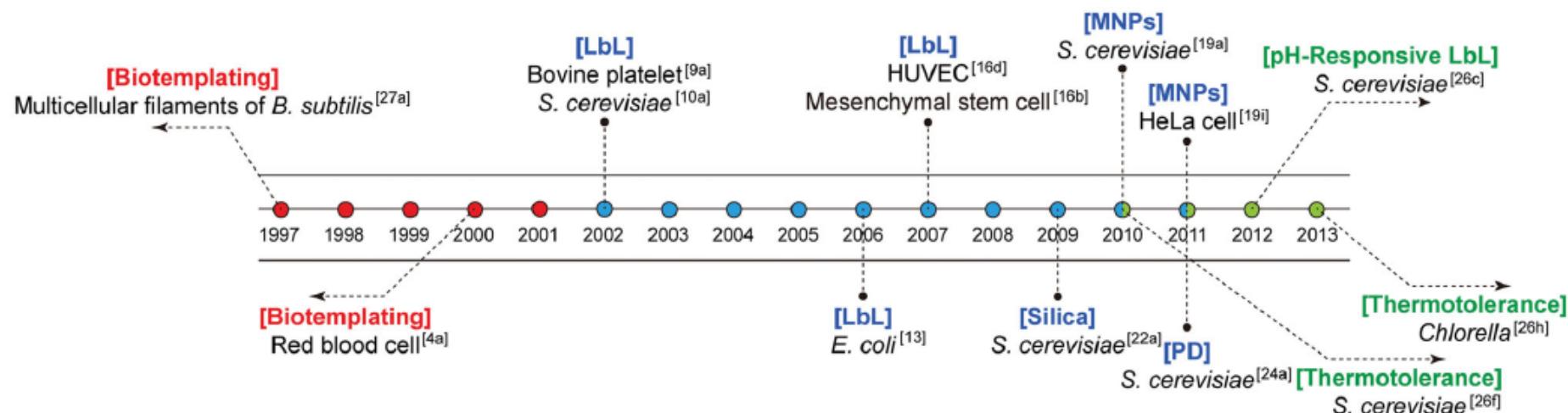




# Silica coating on mammalian cells



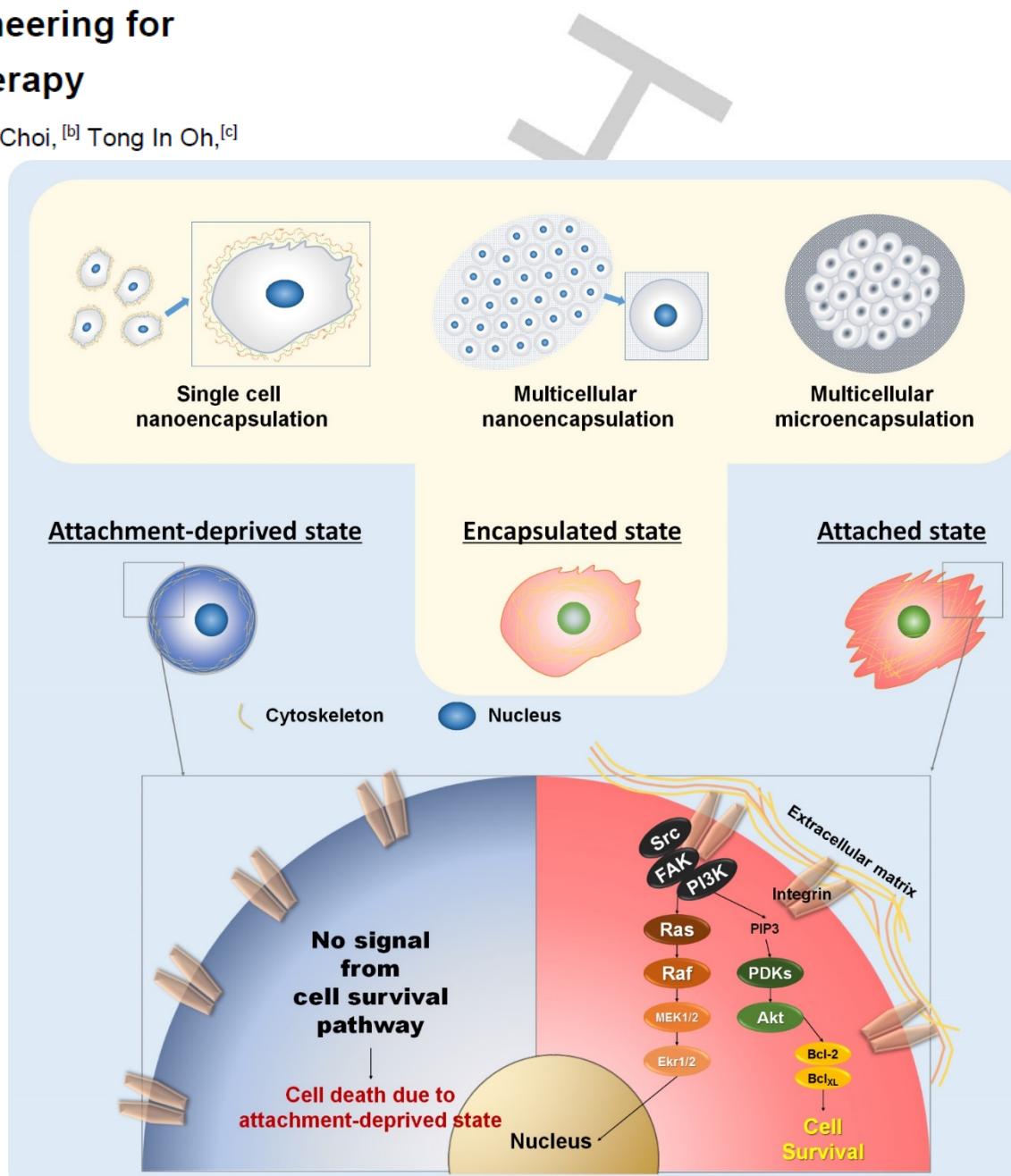
Lee J 2014 Angewandte Communication



Park JH 2014 Adv Mater

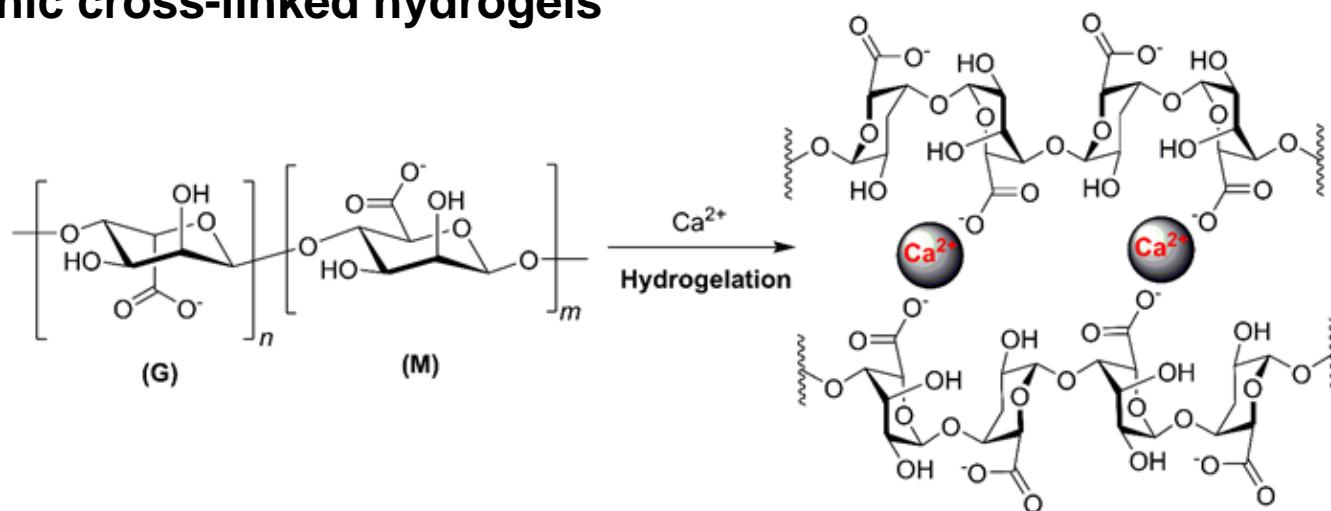
# Cell-Surface Engineering for Advanced Cell Therapy

Jungkyu K. Lee,<sup>[a]</sup> Insung S. Choi,<sup>[b]</sup> Tong In Oh,<sup>[c]</sup>  
and EunAh Lee<sup>\*[d]</sup>

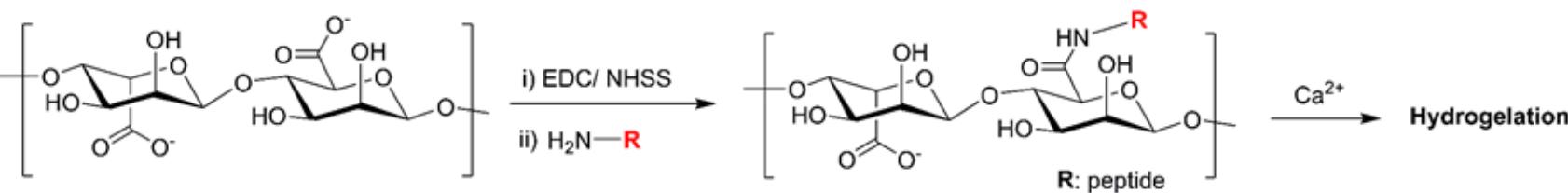


# Ionic cross-linked hydrogels

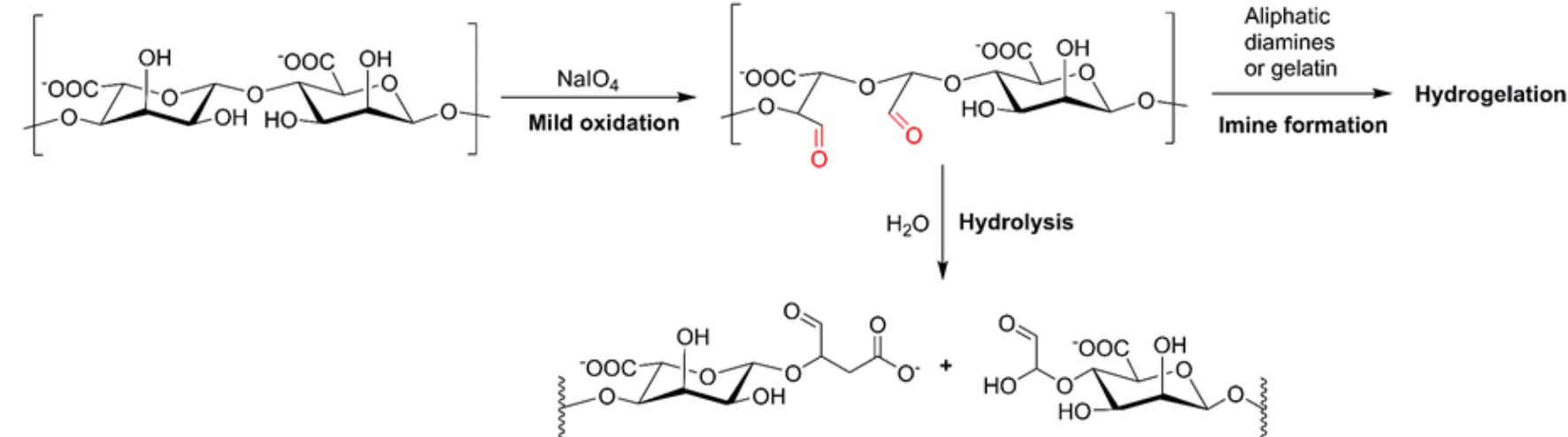
(a)



(b)

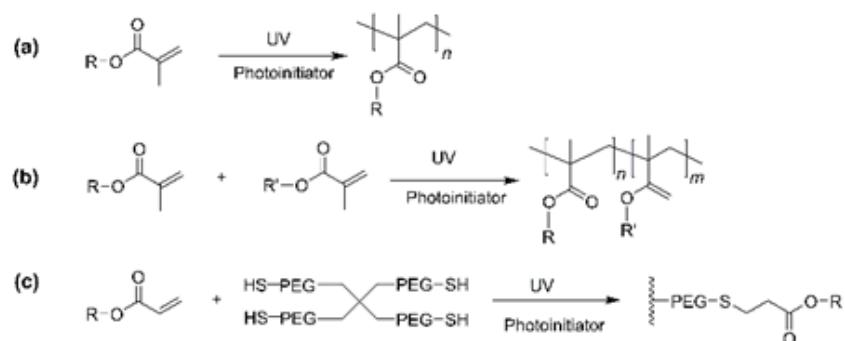


(c)

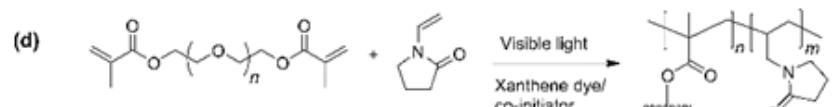


# Covalently cross-linked hydrogels

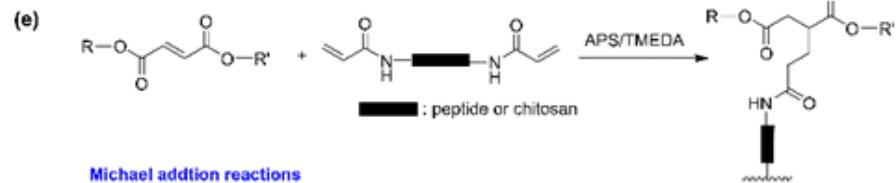
## UV-induced free radical polymerization



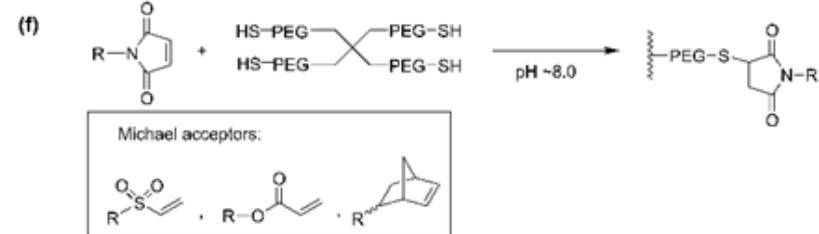
## Visible light-induced free radical polymerization



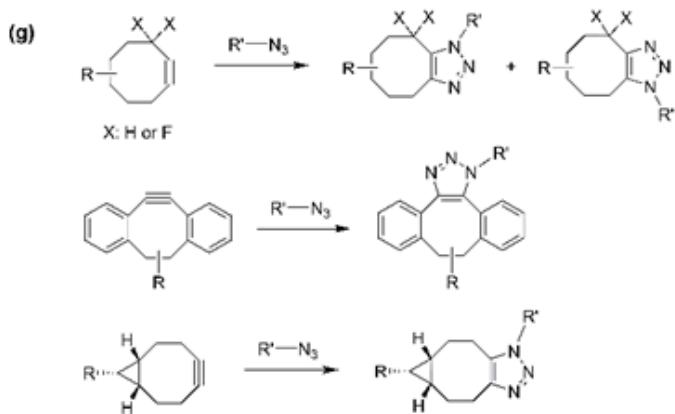
## Redox-initiated free radical polymerization



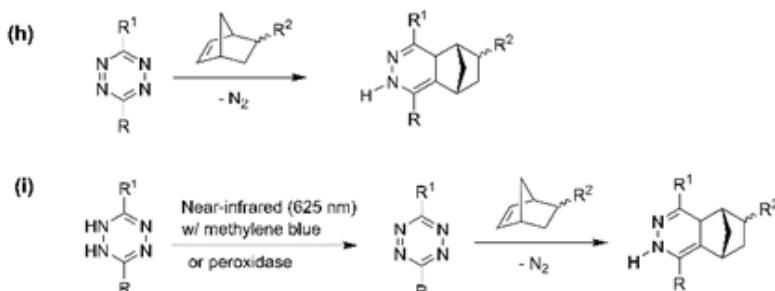
## Michael addition reactions



## Click Chemistry

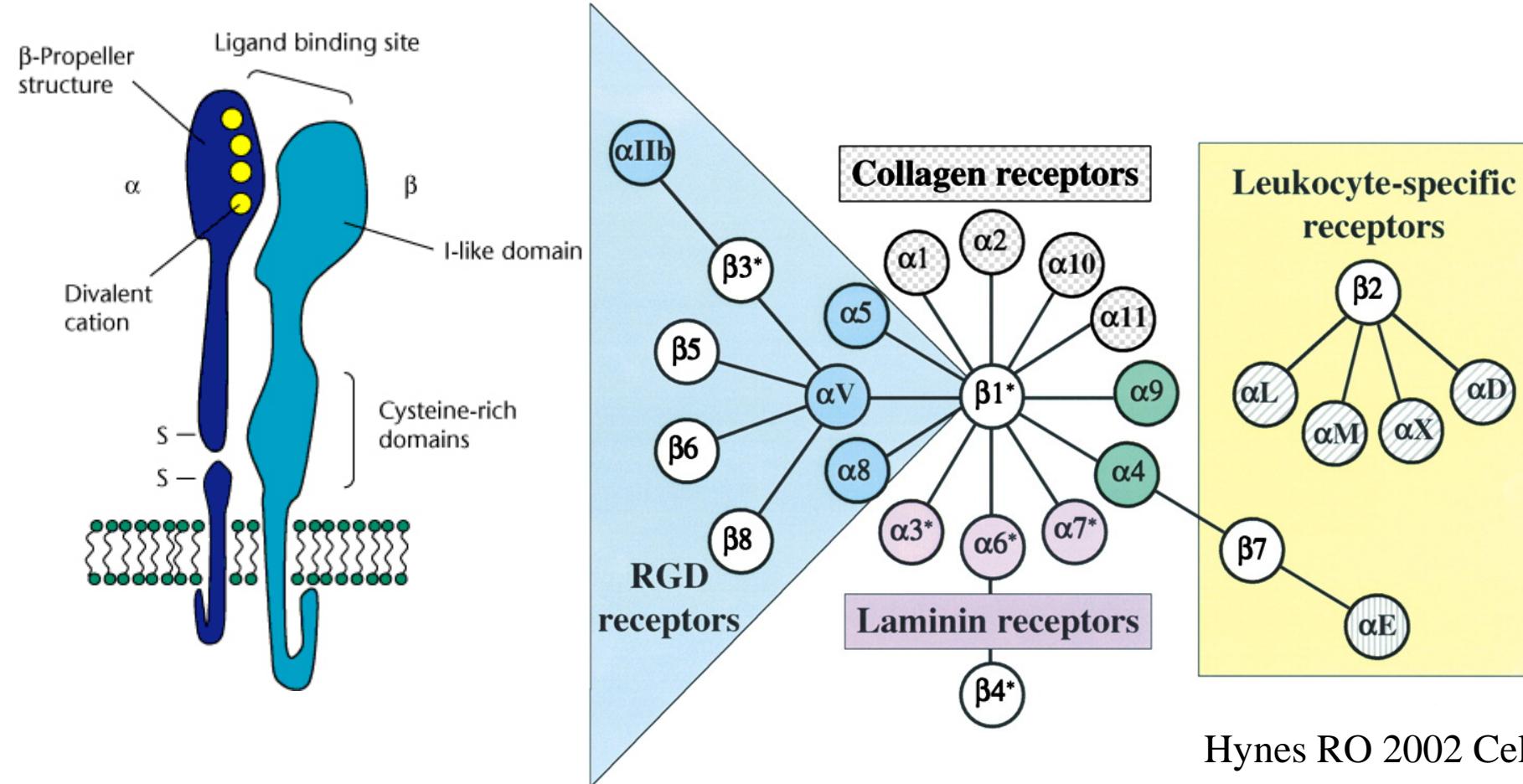


## Tetrazine Chemistry

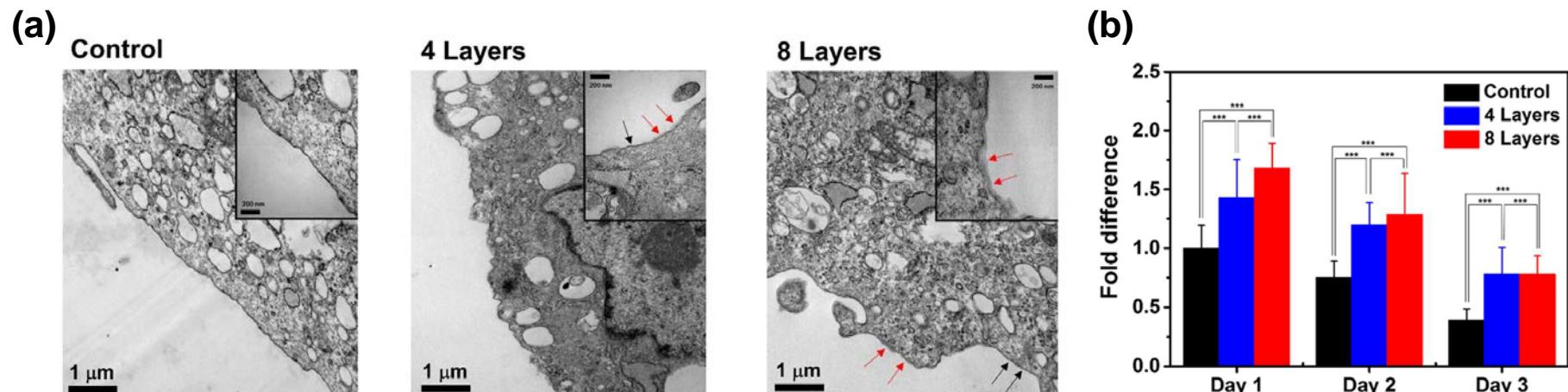
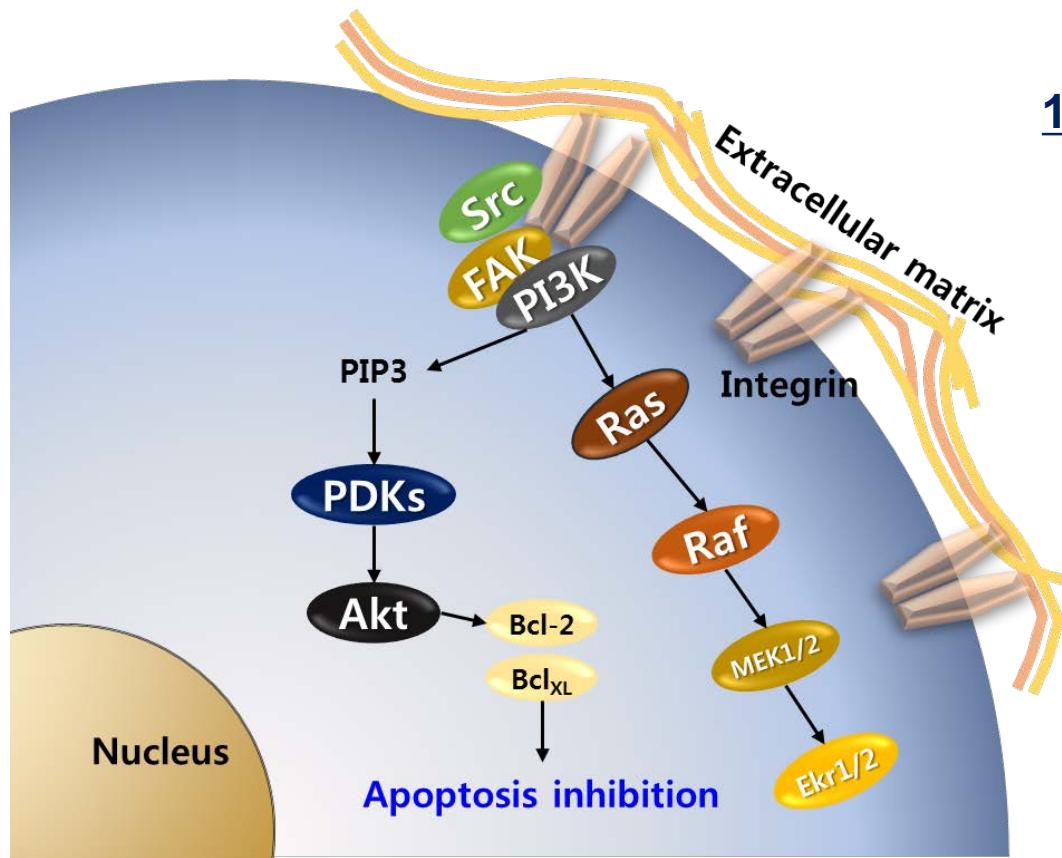


# Functional Aspects of Cell Encapsulation

## 1. Cytoprotective effects of cell encapsulation on the attachment-deprived state

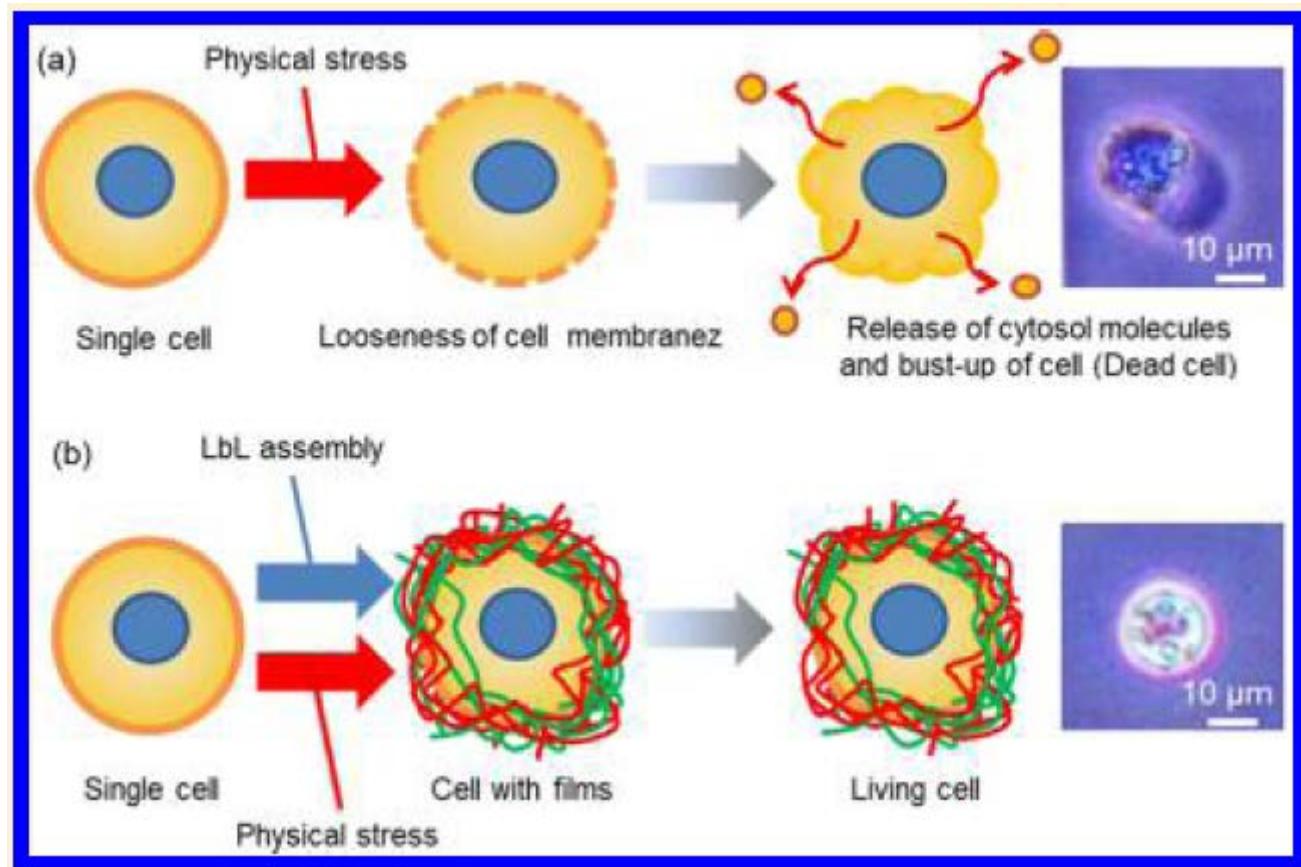


## 1.1 Inhibition of Apoptosis



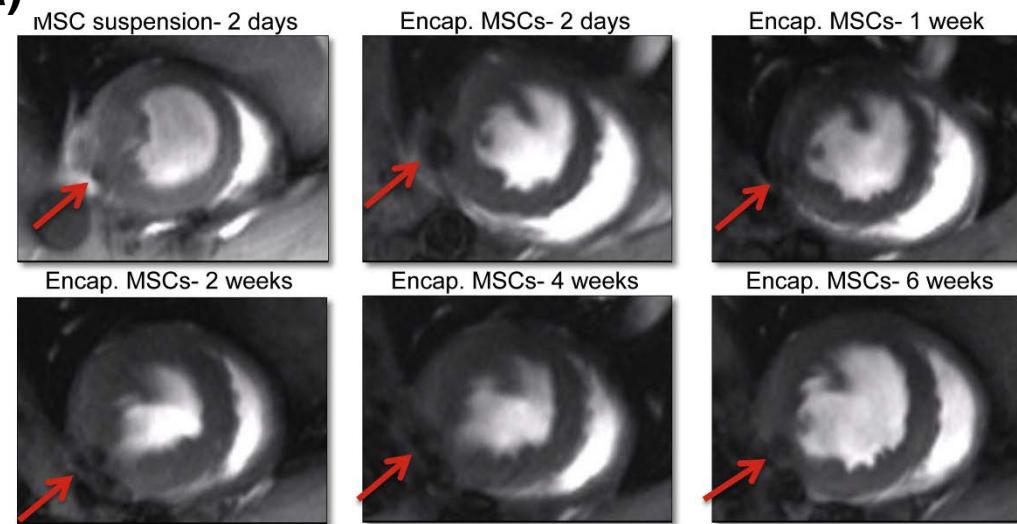
## 1.2 Resistance to physical and chemical stresses

- LbL assembly rescued hepatocyte carcinoma (HepG2) cells from apoptosis induced by mechanical stress

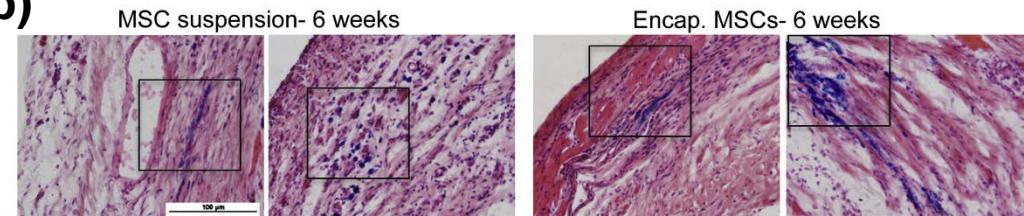


### 1.3 Better retention at the transplantation site

(a)

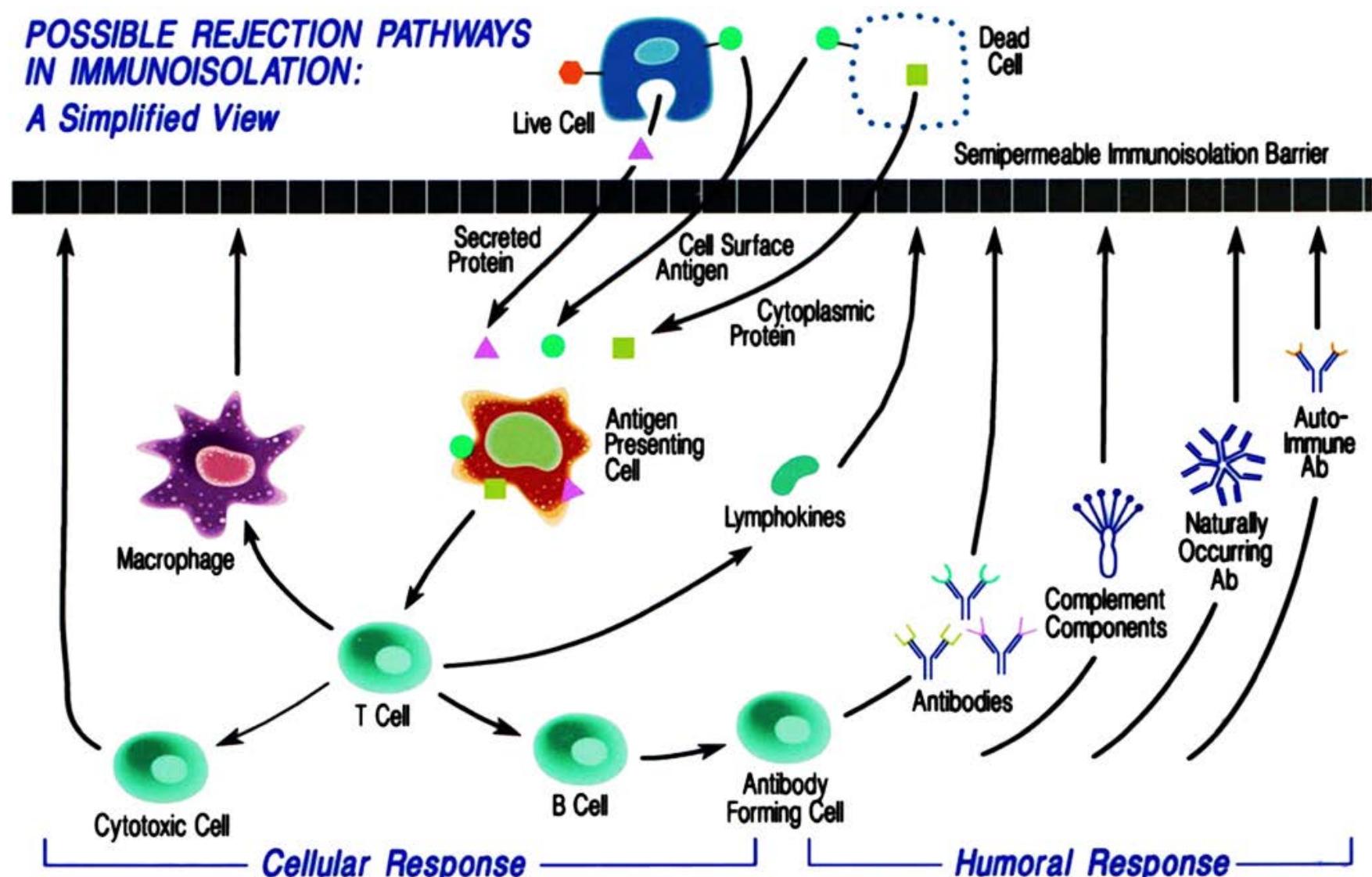


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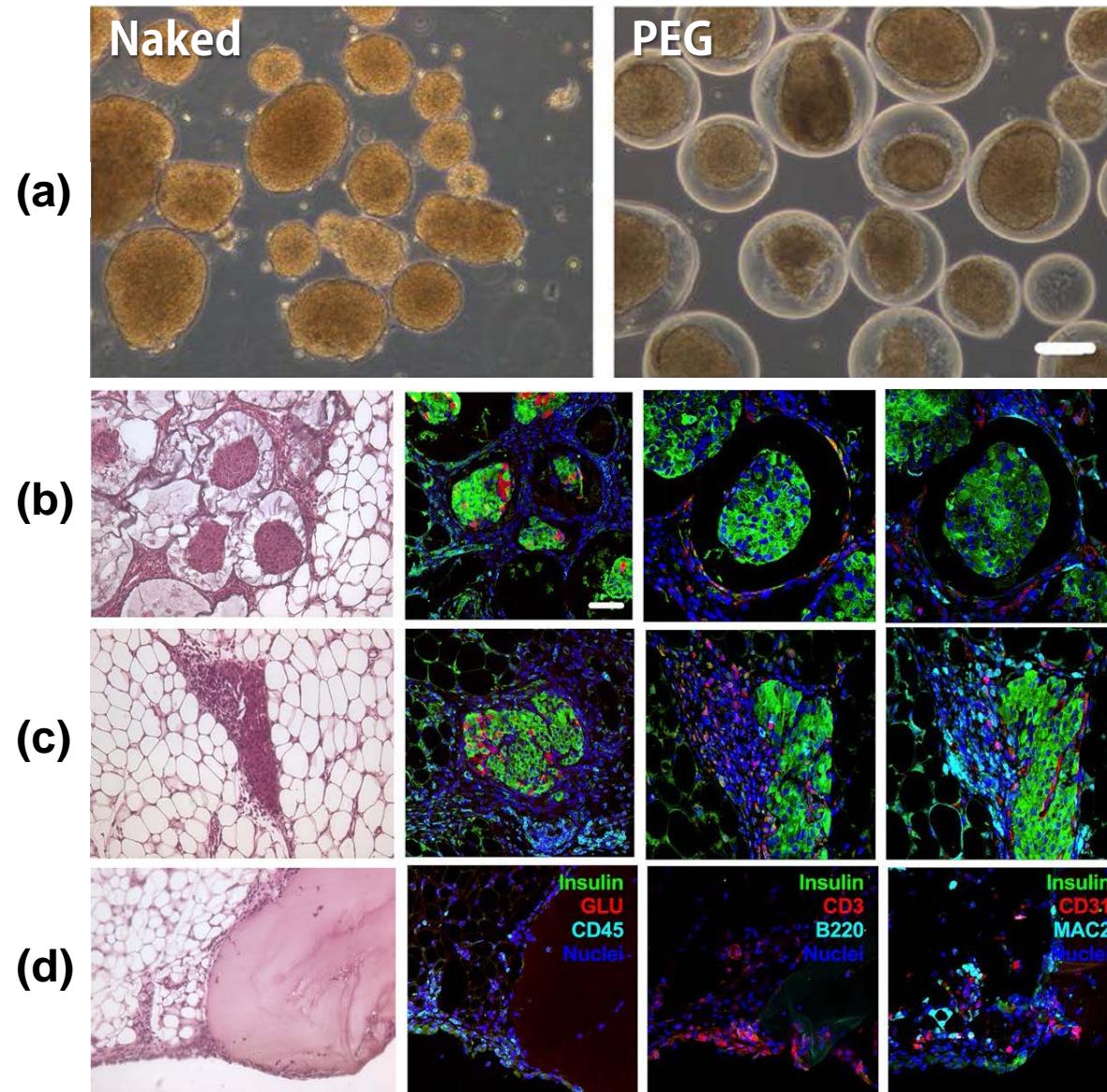


Blocki A 2017 Biomaterials

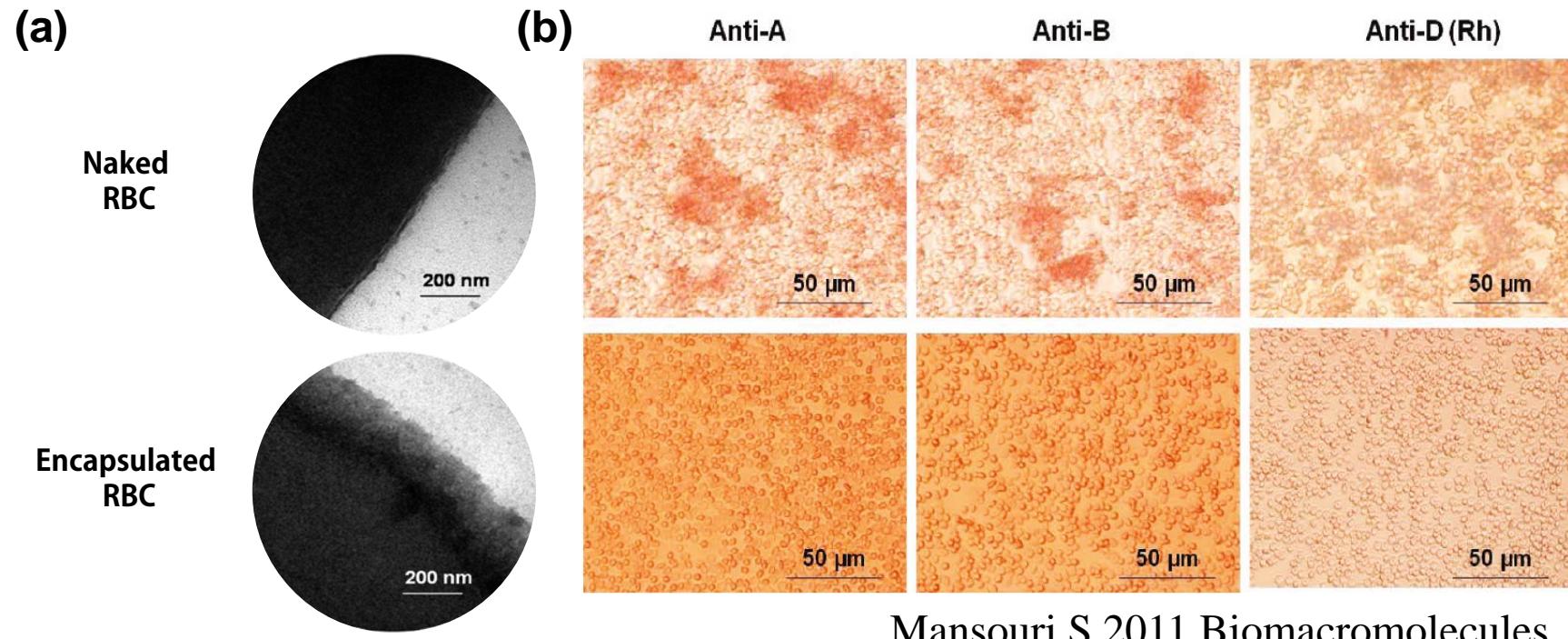
## 2. Cytoprotective effects against immune reactions



## 2.1 Pancreatic islet cells

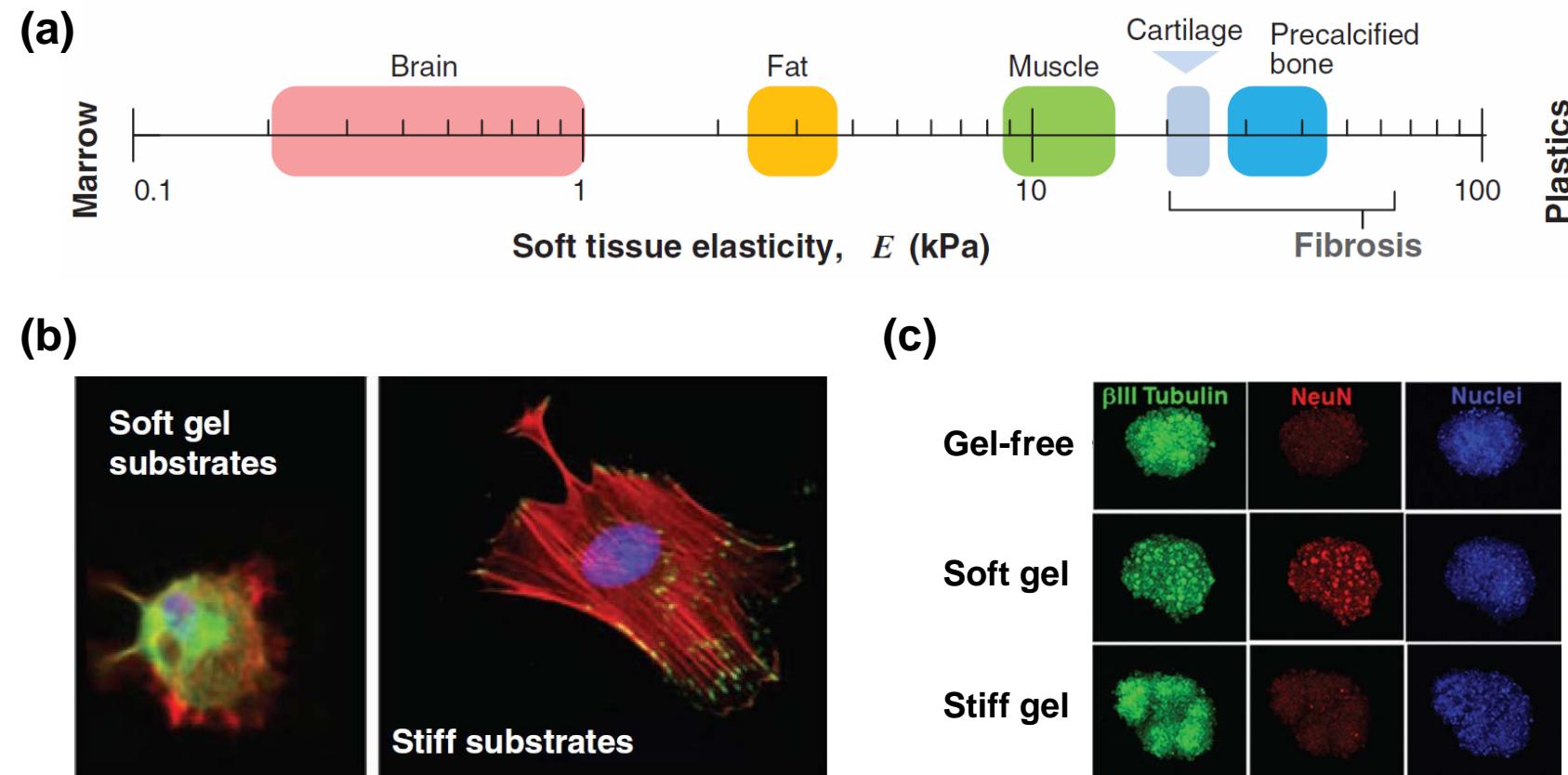


## 2.2 Red Blood Cells



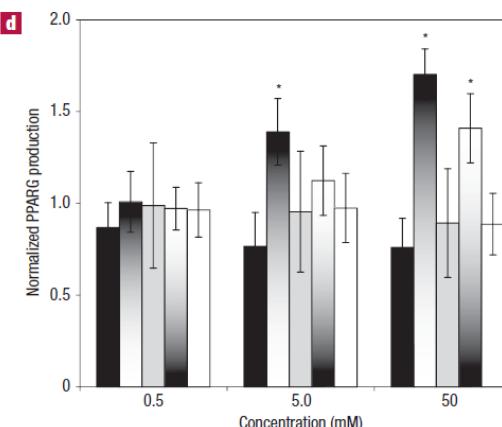
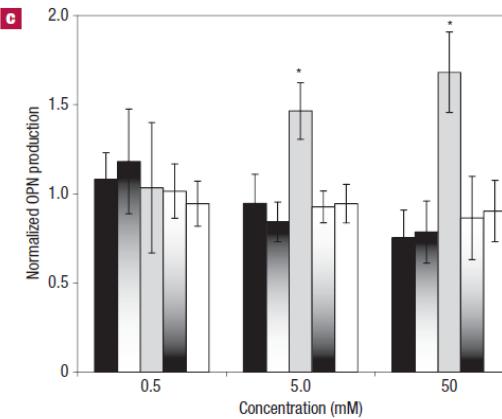
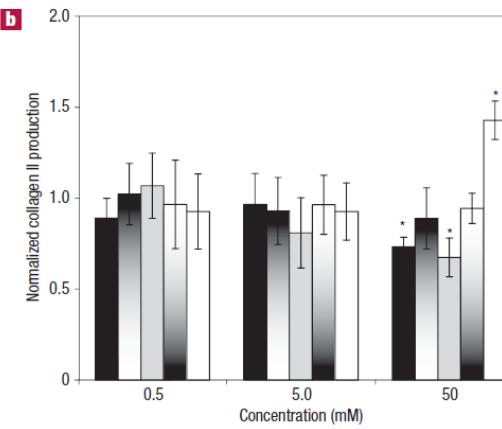
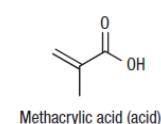
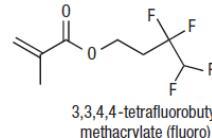
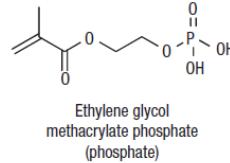
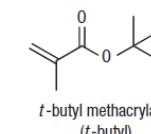
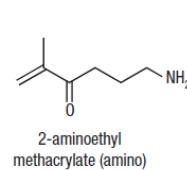
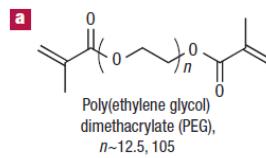
### 3. Modulatory effect of hydrogel encapsulation on differentiation tendency

#### 3.1 Modulation of differentiation based on stiffness of biomaterials

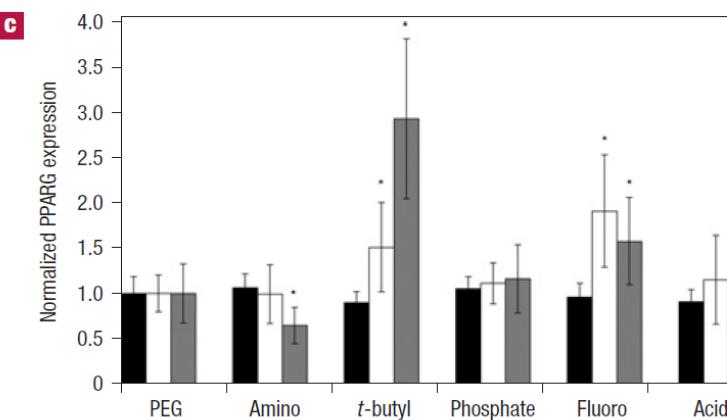
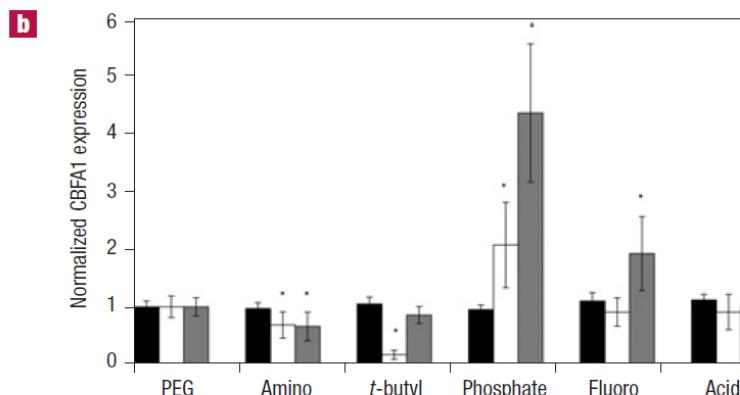
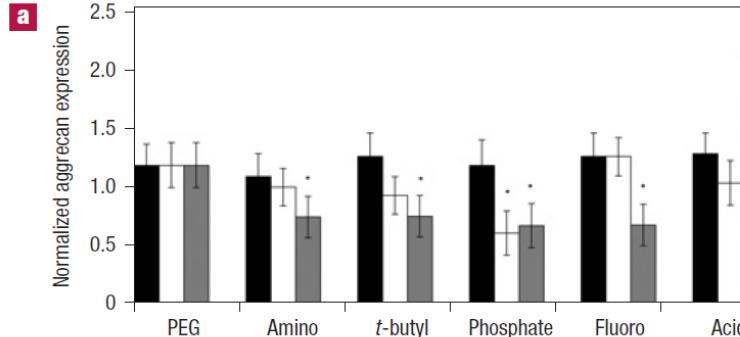


Wu S 2017 J Materials Chemistry B

### 3.2 Modulation of differentiation based on chemical groups in hydrogels



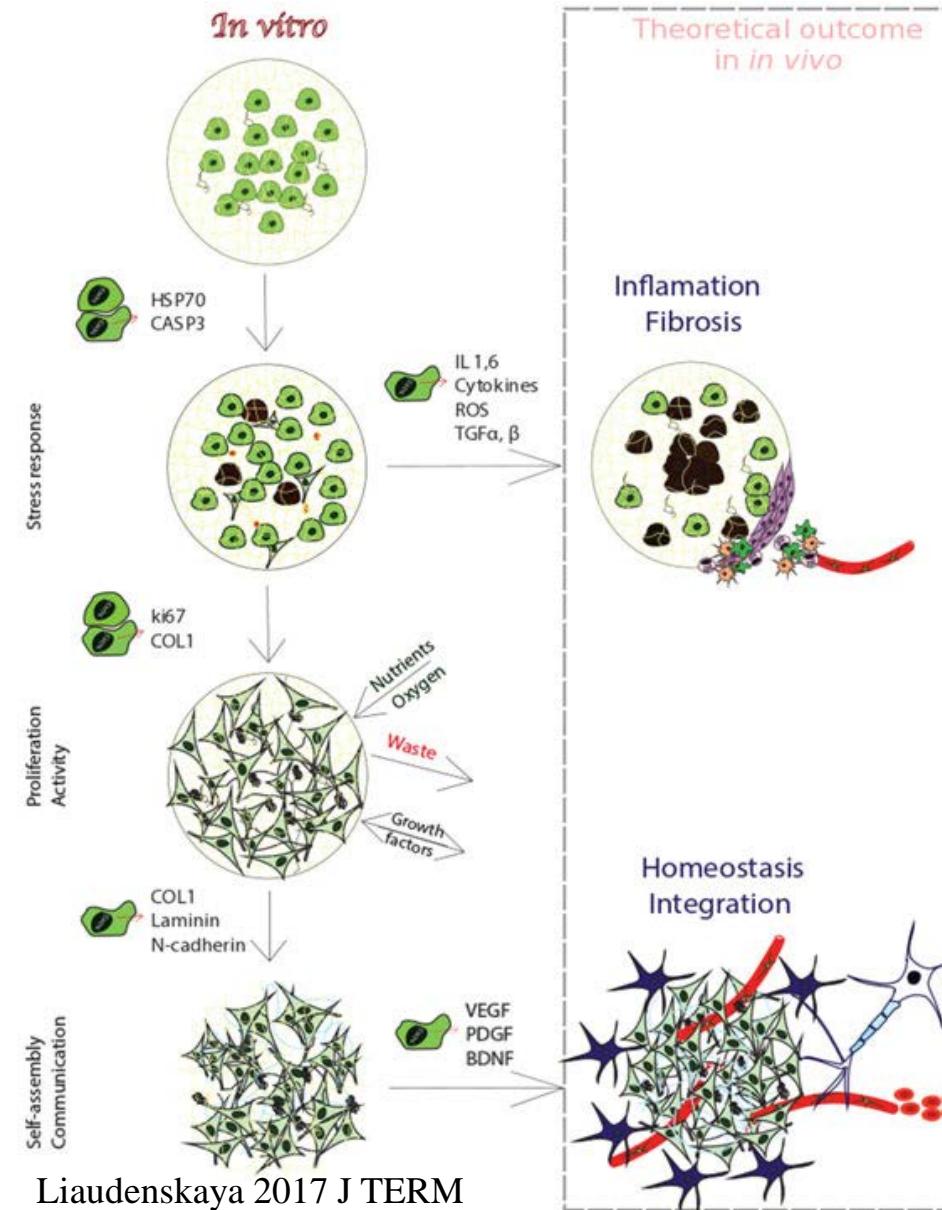
No evidence of better cell survival.  
Focused on differentiation.



## 4. Effects of cell encapsulation on cell proliferation, “hatching”, maturation, and tissue integration of transplants

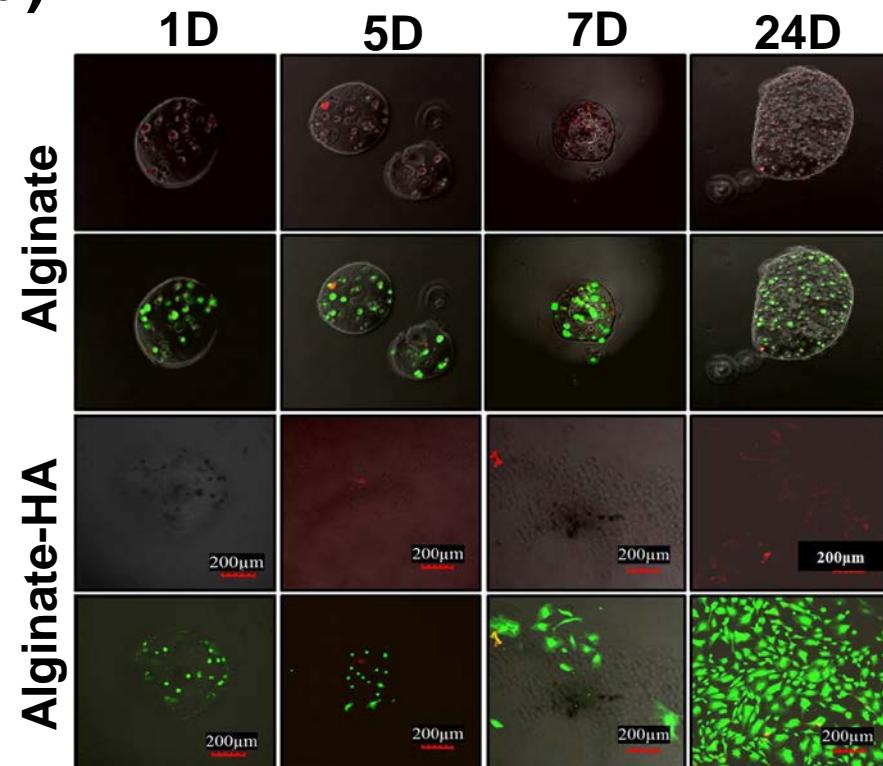
### 4.1 Effect of encapsulation on cell-cell interaction status

### 4.2 Effects of cell encapsulation on cell proliferation, maturation, and tissue regeneration after transplantation of encapsulated cells

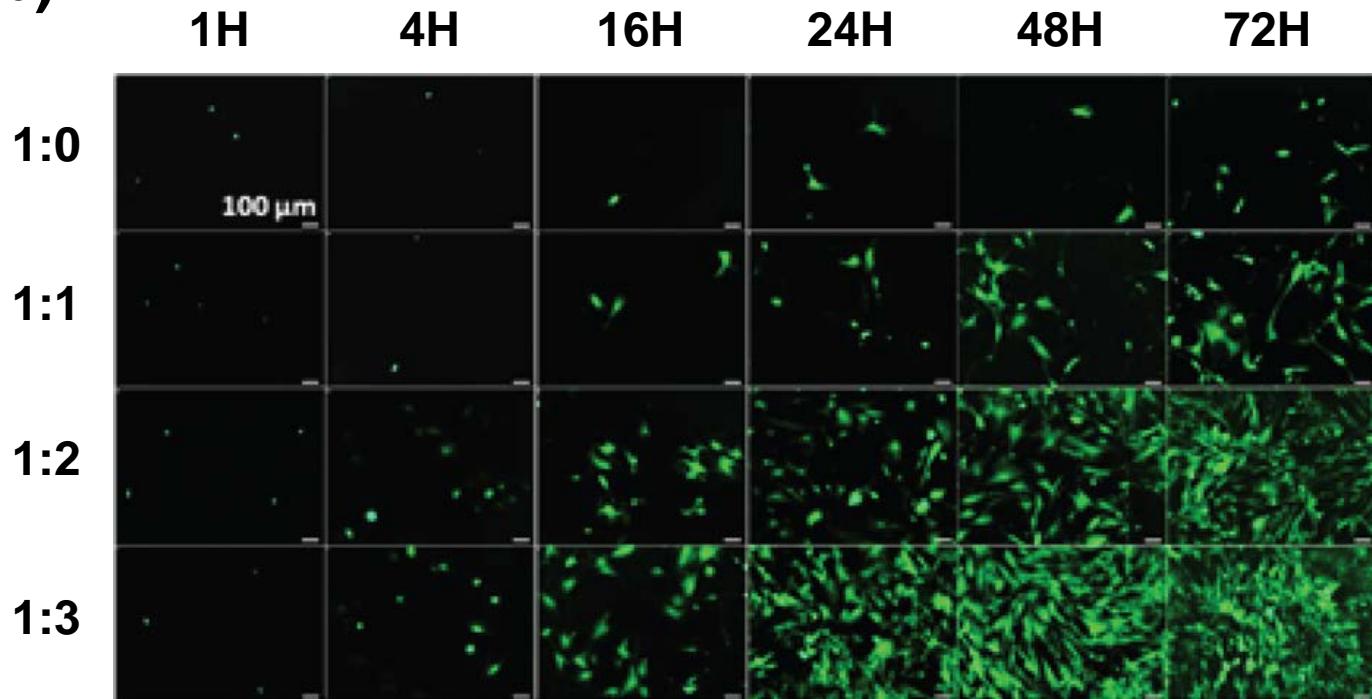


### 4.3 “Hatching” of encapsulated cells

(a)



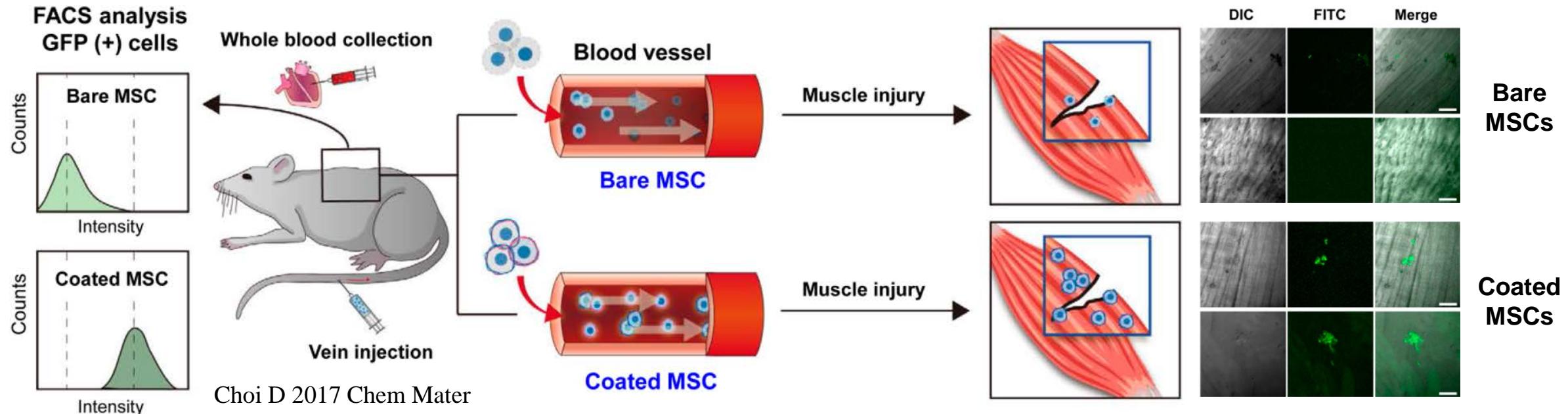
(b)



(Agarose : Gelatin)

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## 4.4 Possible applications of encapsulated cells



# Animal models

# Defect & Injury Model

**Contusion model for SCI (Spinal Cord Injury)**

**Cornea wound model**

**In vivo osteogenesis**

- Craniotomy model
- Ectopic BM model
- Fracture model

**Autologous Chondrocyte Implantation (ACI) model**

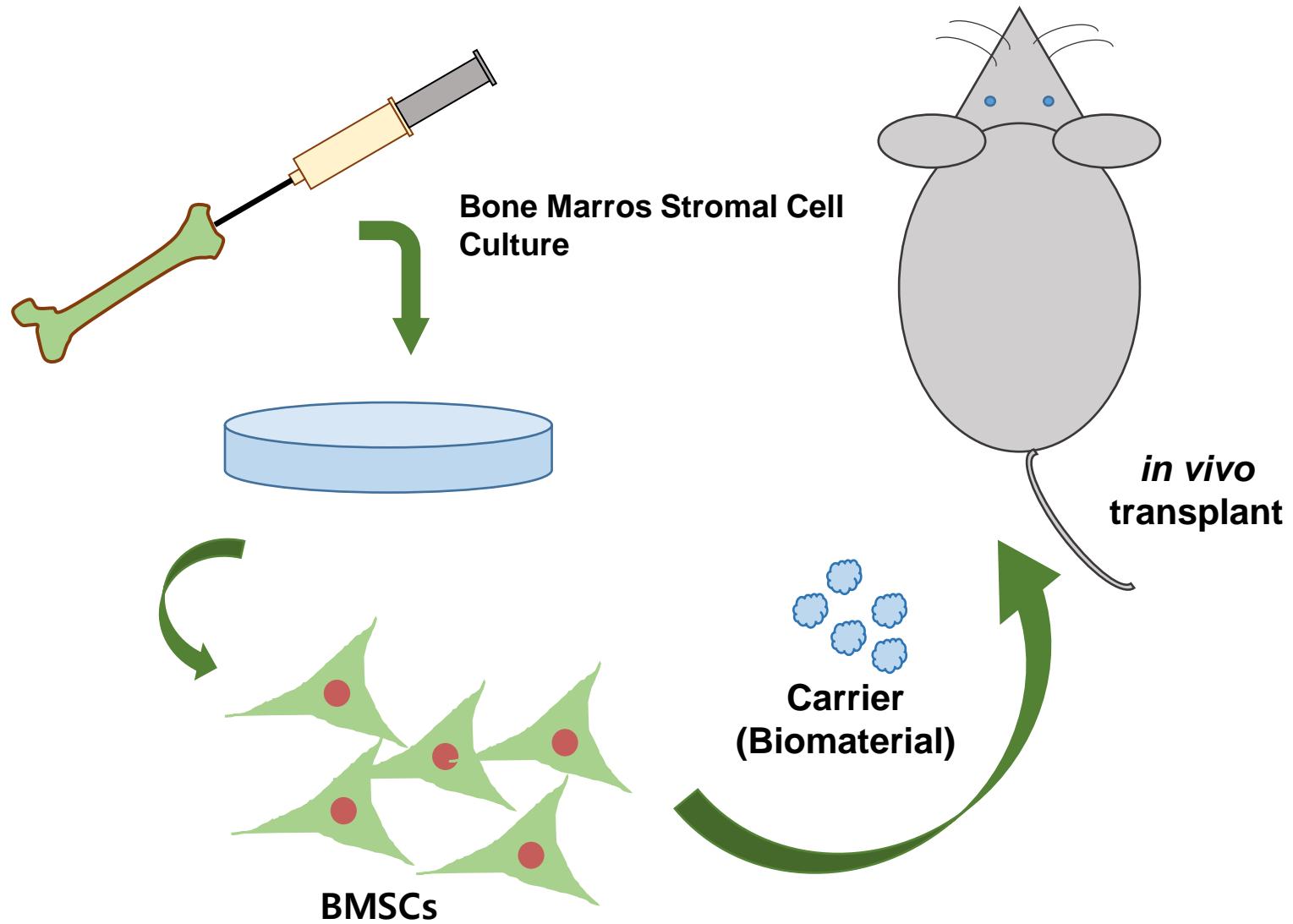


Cornea wound model



Craniotomy model

# Ectopic Bone Model



# Disease Model

## Diabetes mellitus model (Streptozotocin-induced model)

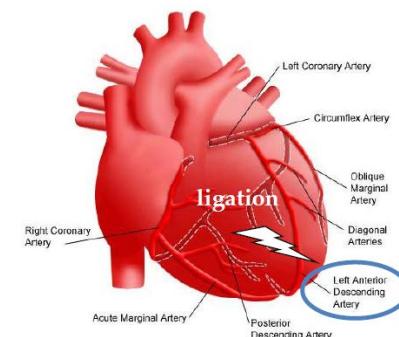
### Ovariectomy



## Collagen-induced rheumatoid arthritis (RA) model

## Collagenase-induced osteo-arthritis (OA) model

## MI (Myocardial infarction)



**EOD**