산부인과에서 줄기세포의 활용성

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What are ‘Stem Cells’

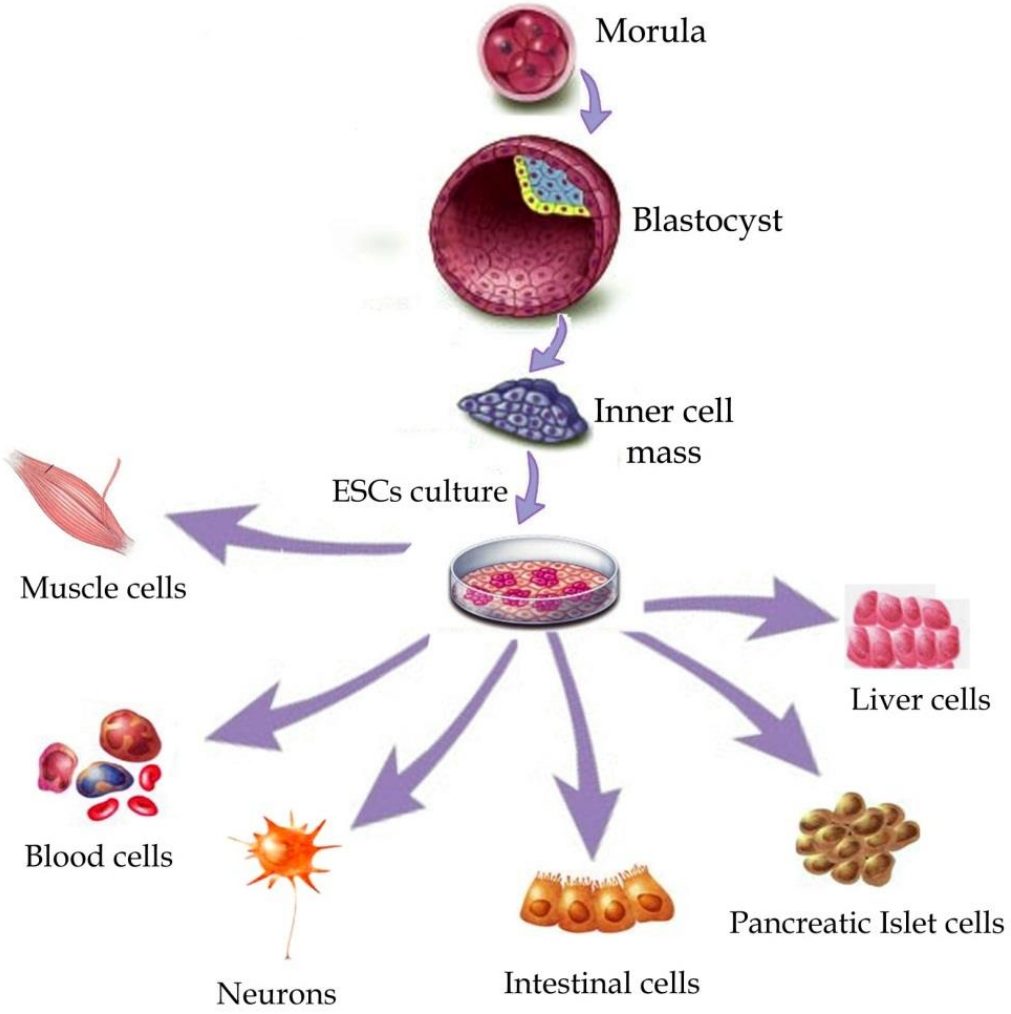
- Stem cells ***the definition***
  - Regenerative potential
  - Capacity and ability to differentiate into one or more cell types
  - Unlimited propagation of source
  - NOT uniformly characterized

Stem Cells

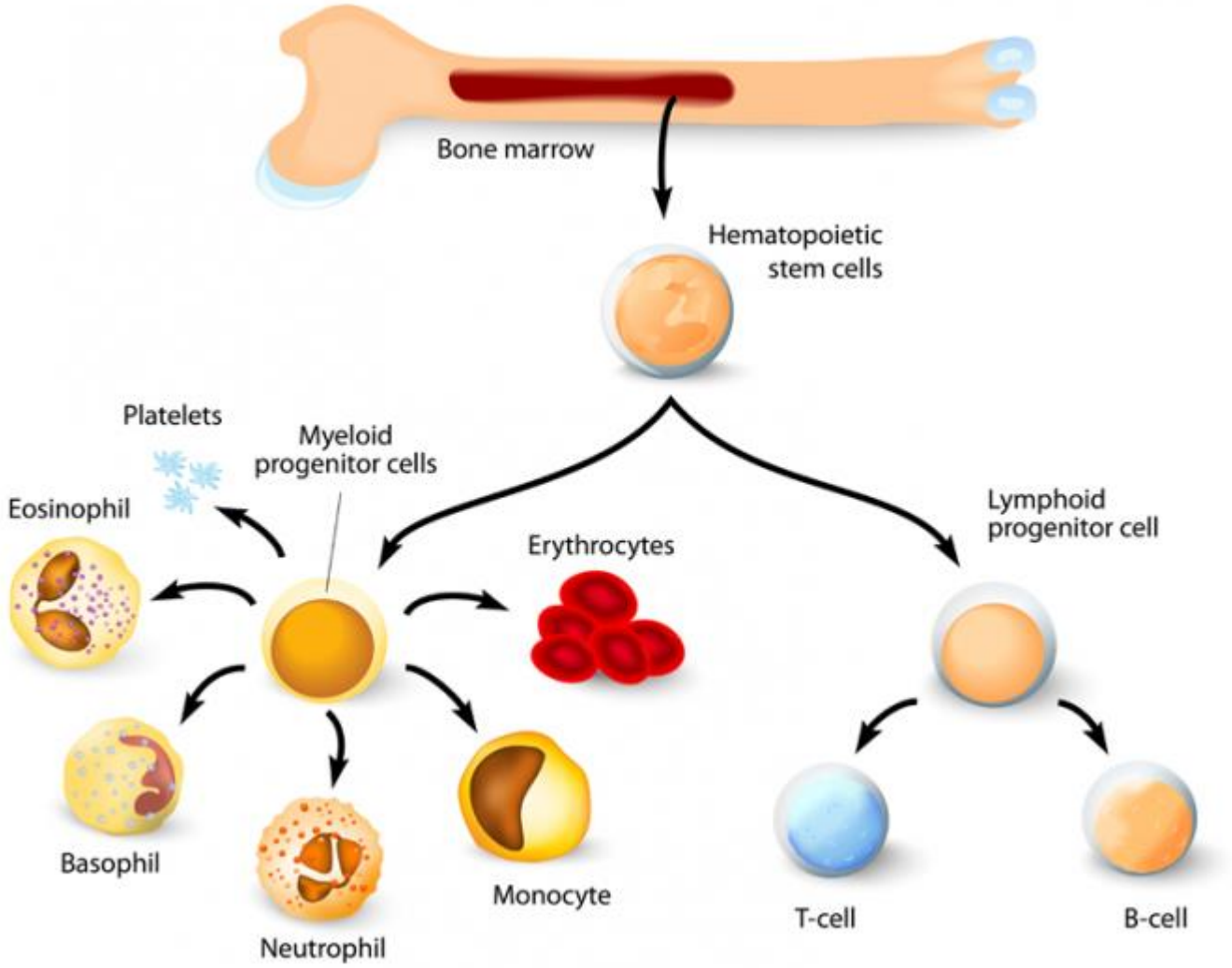
- Toti or pluripotent stem cells
  - typically embryonic or fetal in source
  - differentiate into any cell type
  - 안전성<<가능성
- Multipotent cells
  - mesenchymal stem cells (from blood, bone marrow, placenta, or adipose tissue),
  - capable of differentiating into multiple, but not all, cell types
- Unipotent
  - stem cells, of skin and muscle origin, most limited, capable of regeneration within only 1 cell type
  - 안정성>>가능성

Type of stem cell	What it can be	Examples
Totipotent cells	Each cell can develop into a new individual	Cells of embryo of 1-3 days
Pluripotent cells	Each cell can form any cell type (over 200)	Cells of blastocyst 5-14 days
Multipotent cells	Cells differentiate and can form a number of tissue types.	Fetal tissue, cord blood, adult cells

Classic ‘pluripotent’ stem cells
Embryonic stem cells



Multipotent cells



Source of stem cell

- Heterologous
  - 동종 타 개체 공여
  - Embryonic stem cell
- Autologous
  - 자가 공여
  - Adult stem cell, iPSc
- Allograft 동종 공여
- Xenograft 타종 공여
  - Porcine graft

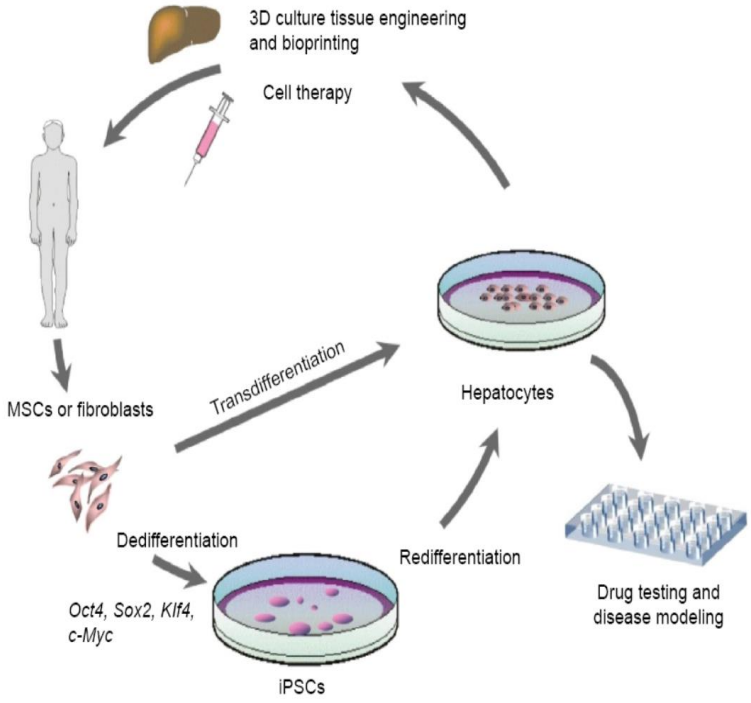
BACKGROUND

- 인구의 노령화로 2030년 기준, 여성의 경우 50세 이상이 인구의 약 50%전후가 될 것으로 추산 **Regenerative Medicine**

- **Induced pluripotent stem cells:** 체세포에 외부에서 배아줄기세포에서 주로 미분화상태를 유지하는 인자를 과발현 시켜서 배아줄기세포 유사상태로 만드는 것. "유도 만능줄기세포"

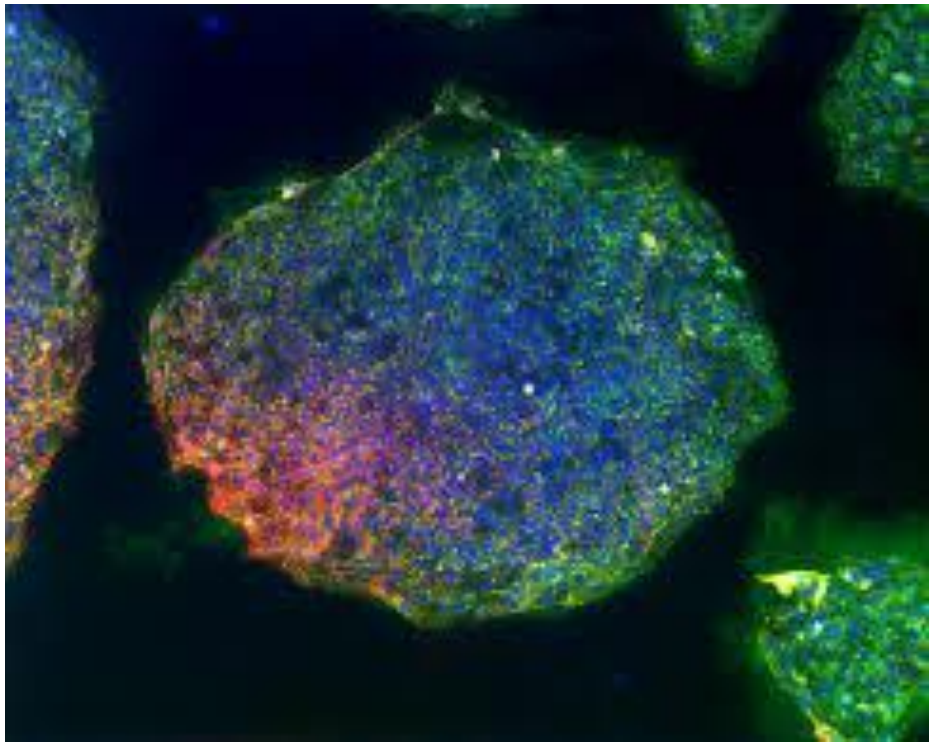
- 대표적으로 iPSc를 신경세포, 근골격계 세포, 췌장의 beta cell, 심근세포 등으로 분화하여 재생의학에 사용하려는 노력이 활발

- iPSc first proposed by Shinya Yamanaka (2006)-Noble Prize (2012)

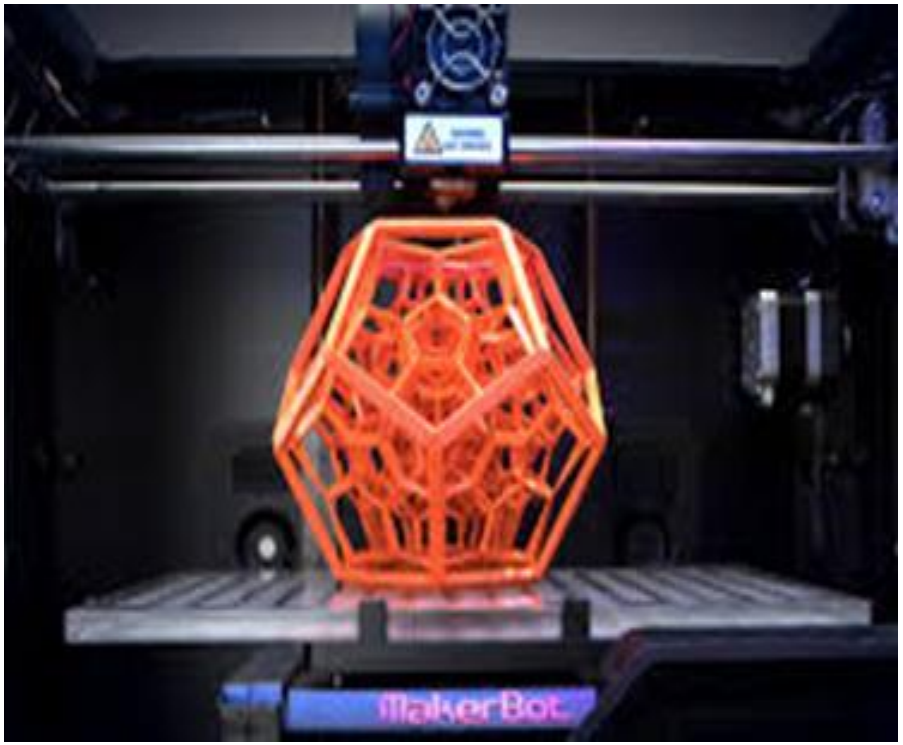


Regenerative medicine의 혁신

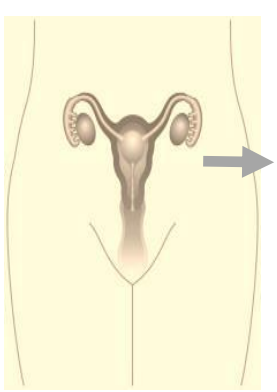
Stem cells-iPS cells



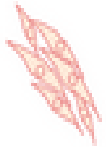
3D printing-scaffold



BACKGROUND



Female patient



Endometrial cells

iPS cells

Mesodermal Lineage differentiation

Hematopoietic stem cell

Autologous erythroblast differentiation

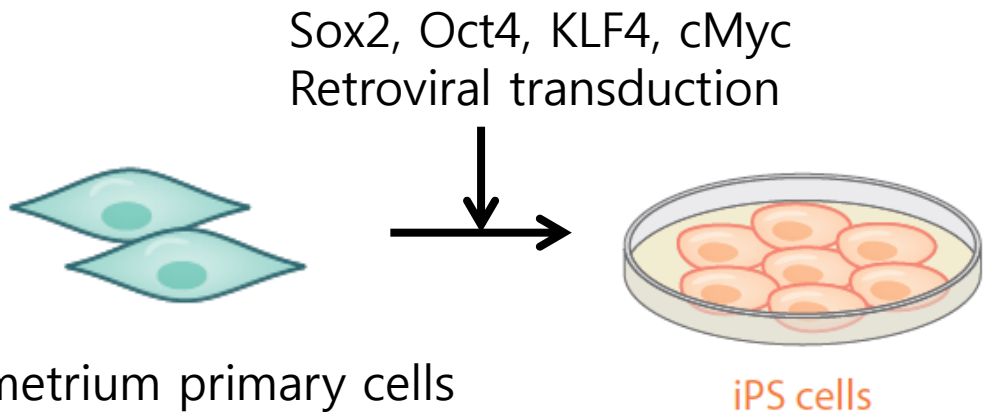
Myeloid progenitors

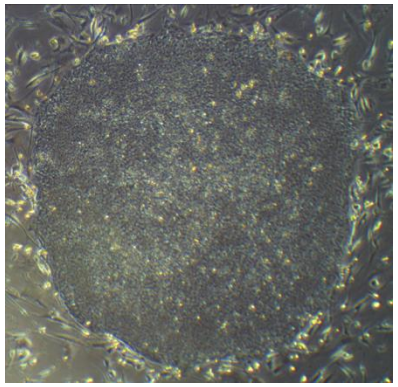
Osteoclastic differentiation

Osteoblastic differentiation

In vitro bone made for patient specific osteoporosis medication screening

iPS cell line from Endometrium





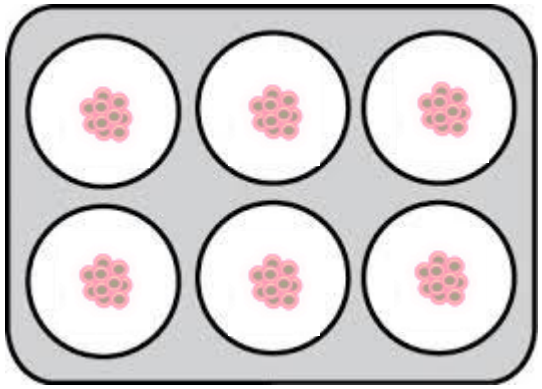
hENDO 1, hENDO 2, hENDO 7

Origin	Reprogramming	Donor	iPSC line
Endometrium	Retrovial vectors	hENDO1	hiPSC1
		hENDO2	hiPSC2
		hENDO7	hiPSC7

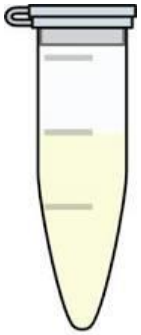
자궁내막→유핵적혈구

- Hysterectomy driven endometrial cells
- Retroviral reprogramming to iPSc (hiPSc1,2,7)
- OP9 hematopoietic differentiation system
- Erythroid differentiation of RBCs from hiPSC/OP9 co-culture cells
- Analysis of proliferation and morphology
- Differential counting of cultured erythroid cells

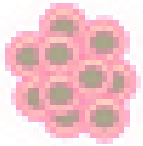
Phase I: OP9 cell and iPS coculture



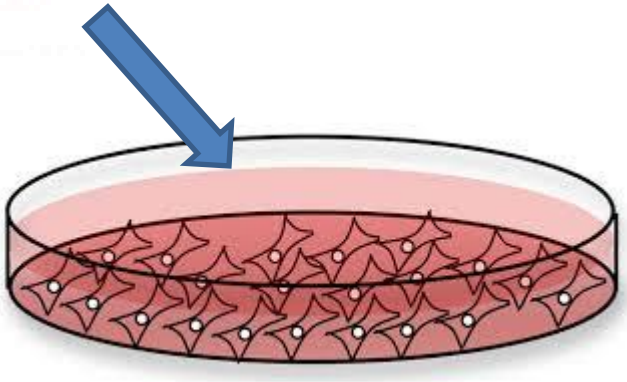
iPS cell culture in 6 well



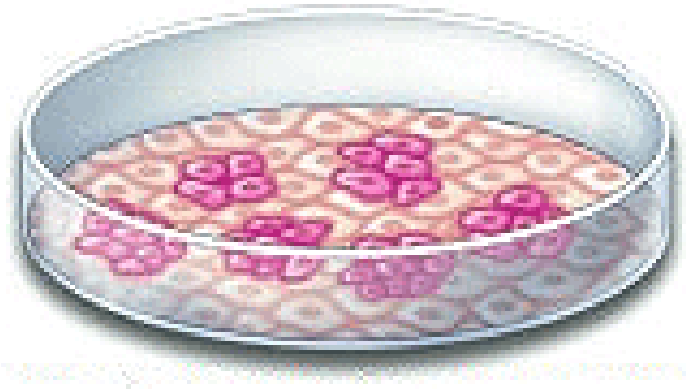
Collagen IV (1mgml^{-1}), 1mL, 5 min



iPS cells (7day, 1X10^{6})

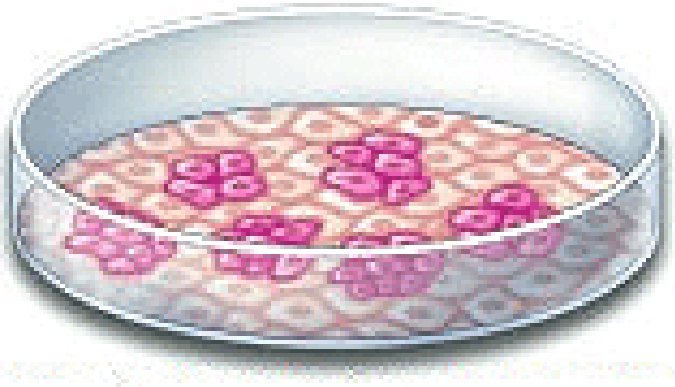


8 day OP9 cell culture

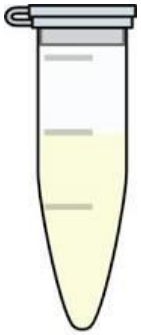


OP9 cell and iPS **coculture**

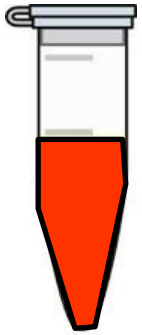
Phase I: OP9 cell and iPS coculture



OP9 cell and iPS coculture (9day)



Collagen IV ($1mgml^{-1}$) 5mL, 30 min



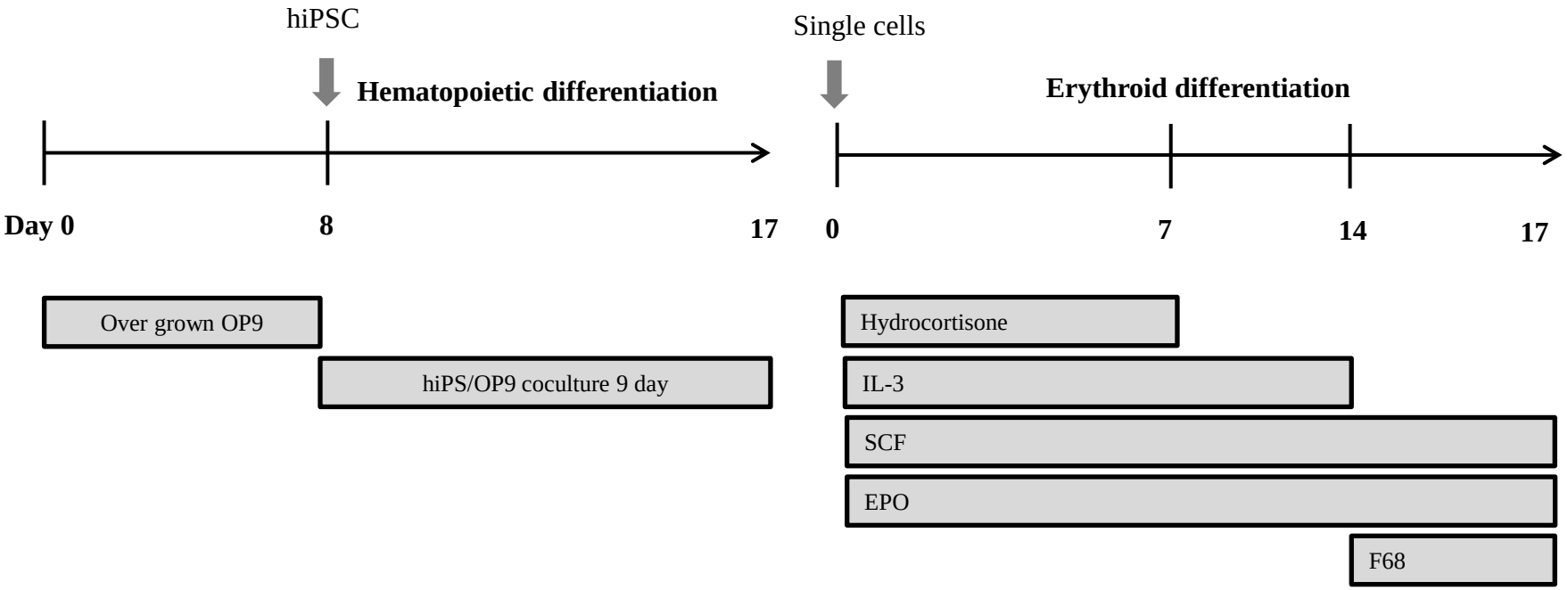
Trypsin EDTA (0.05%) 5mL, 15 min

FACS

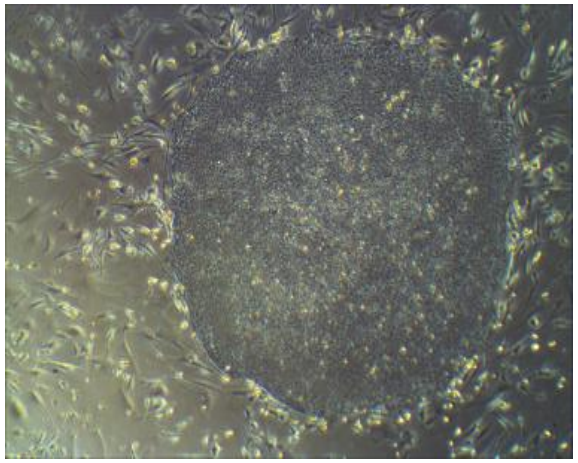
(sample당 $5X10^5$ 씩 사용)

(VOL.6 NO.3, 2011, NATURE PROTPCPLS, published online 17 February 2011; doi:10.1038/nprot.2010.184)

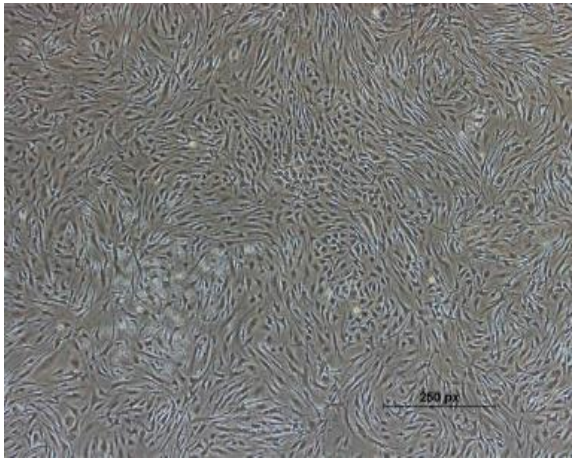
Erythroid differentiation Protocol



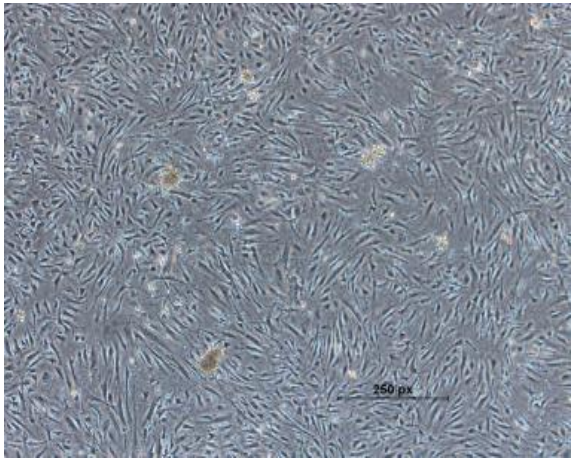
Overview of heiPSc differentiation to erythroblast differentiation



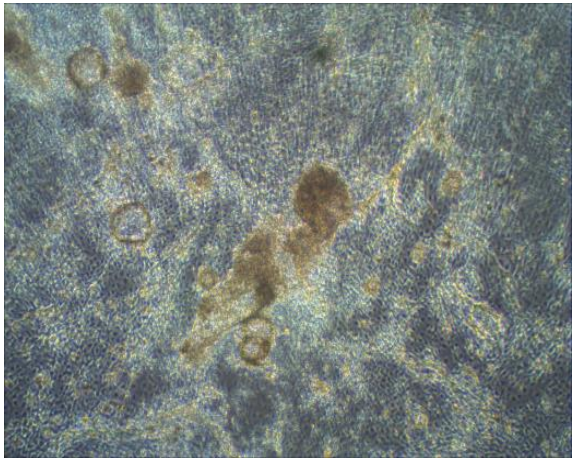
hiPSC (Day 7, 1X10^6)



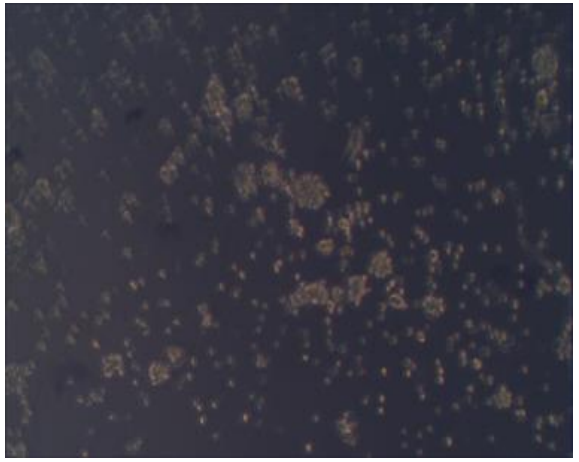
Overgrown OP9 (Day 8)



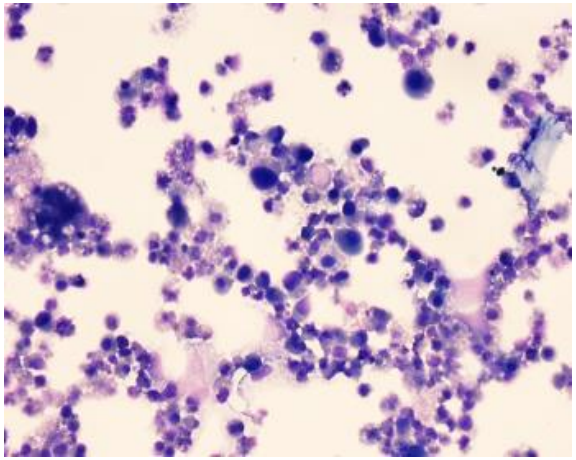
hiPSC/OP9 coculture Day 0



hiPSC/OP9 coculture Day 9

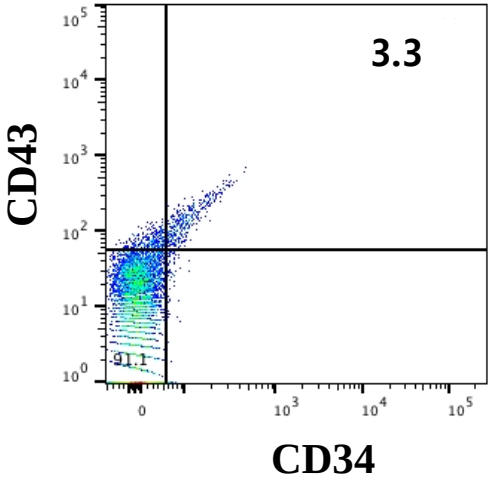


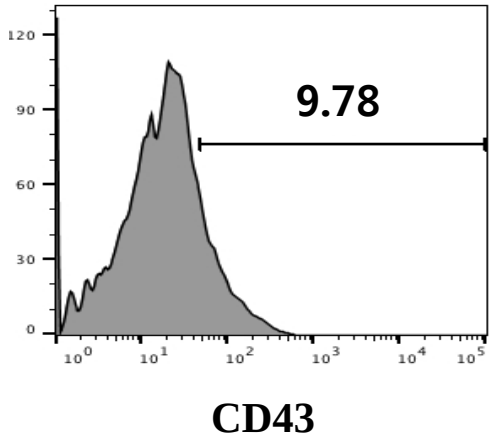
Erythroblast differentiation

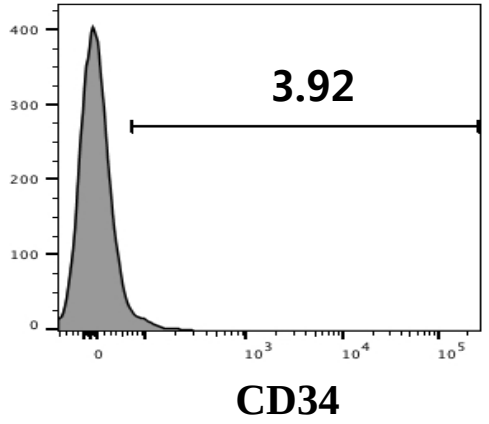


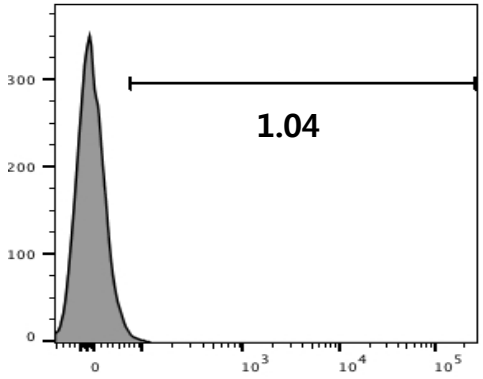
Erythroblast differentiation (Wright-Giemsa staining)

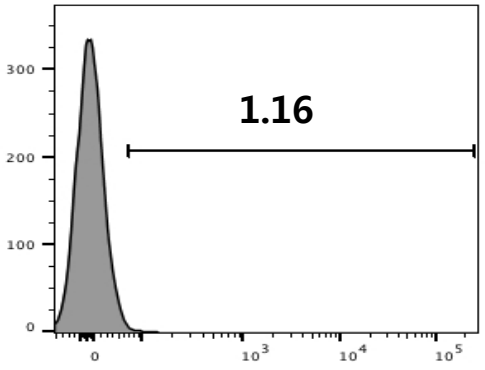
Flow cytometric analysis of hematopoietic differentiation on OP9

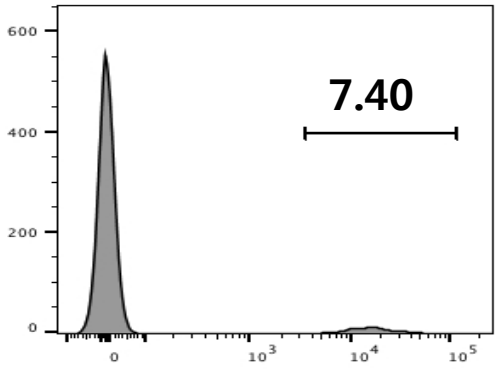




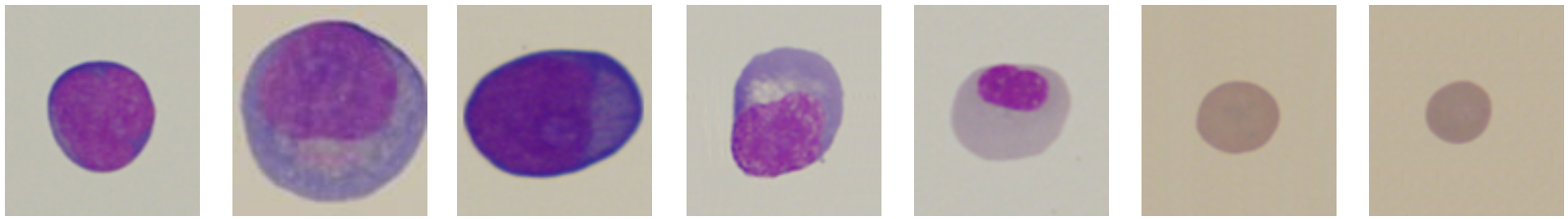








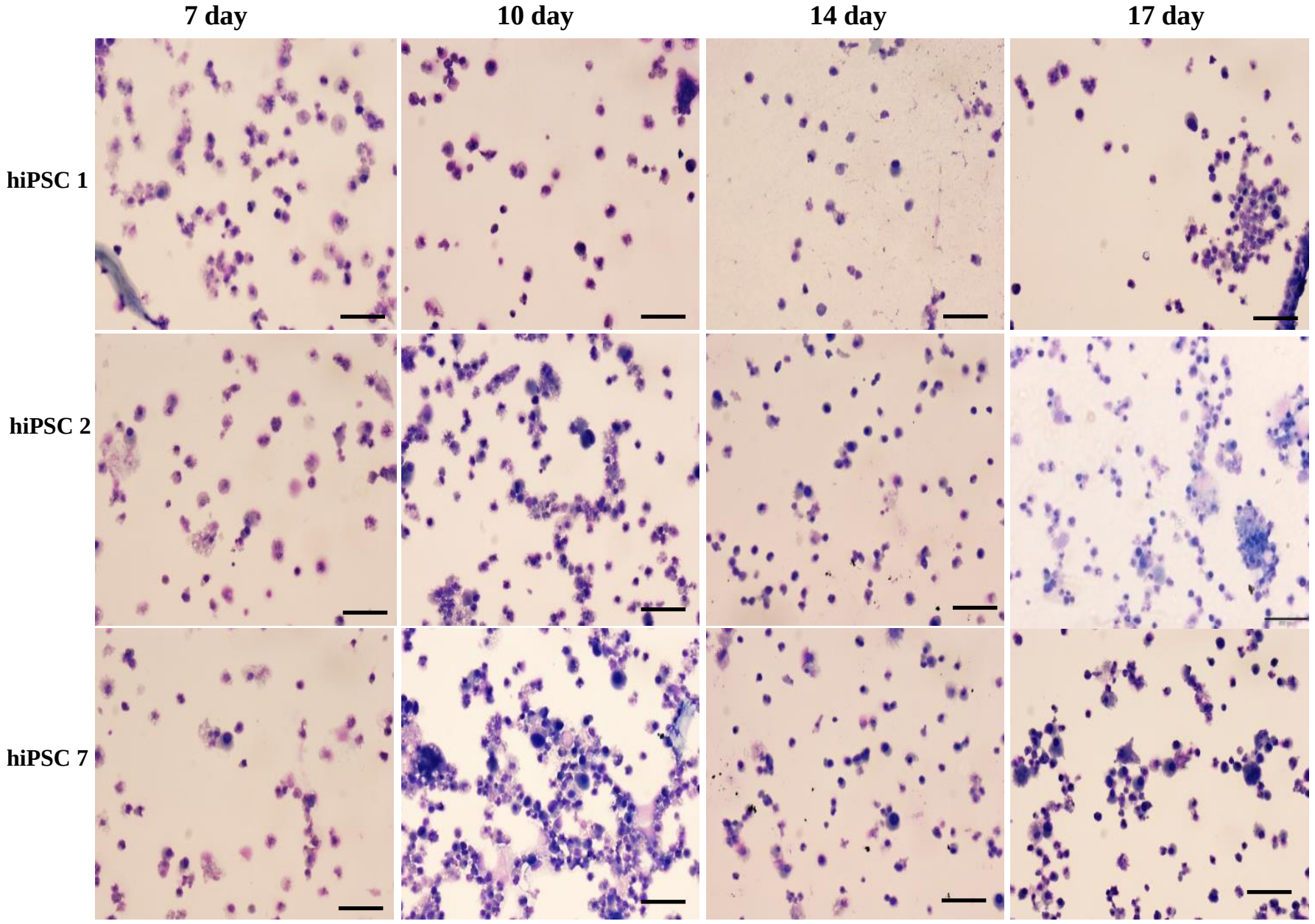
Normal RBC differentiation



Mononuclear | Proerythroblast | Basophilic normoblast | Polychomatic n. | Orthochromatic n. | Reticulocyte | Mature RBC

0 4 7 14 21

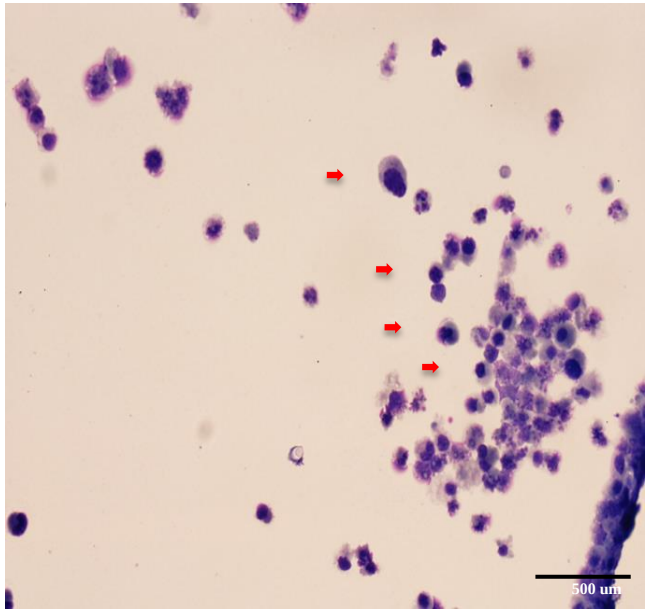
Days differentiation

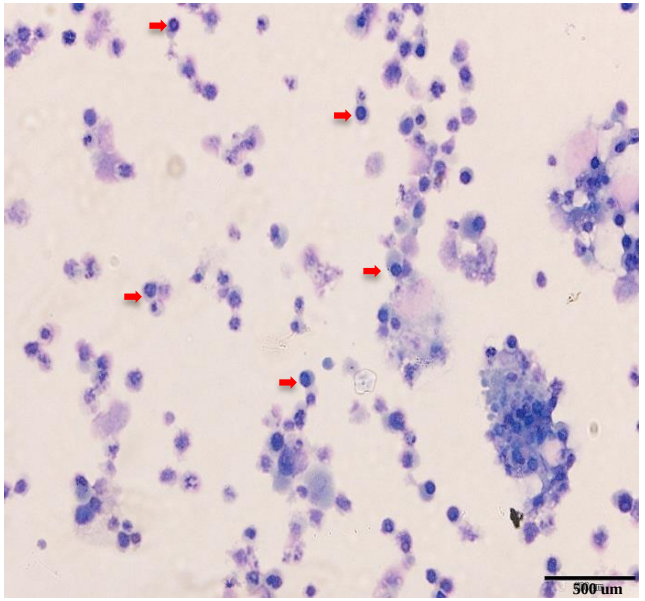

7 day
10 day
14 day
17 day
hiPSC 1
hiPSC 2
hiPSC 7
500 um

IPS MNC RBC differentiation

hiPSC 1

17 day



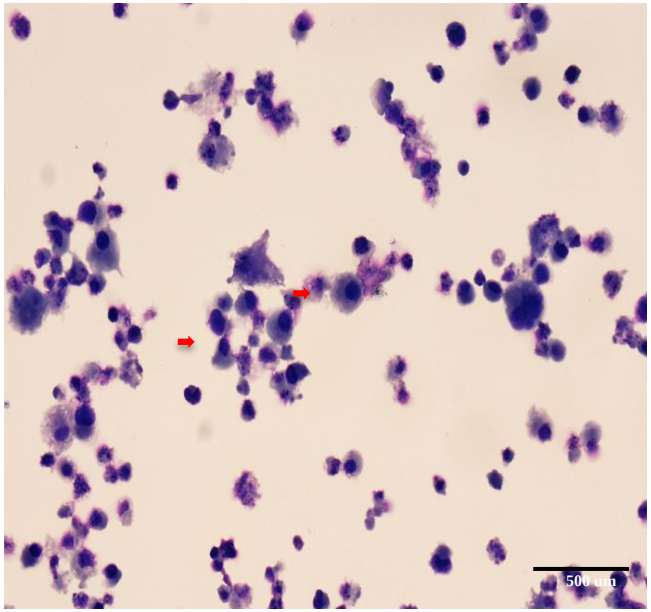


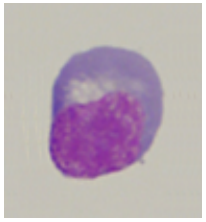
hiPSC 2

17 day

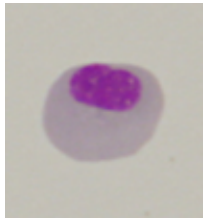
hiPSC 7

17 day



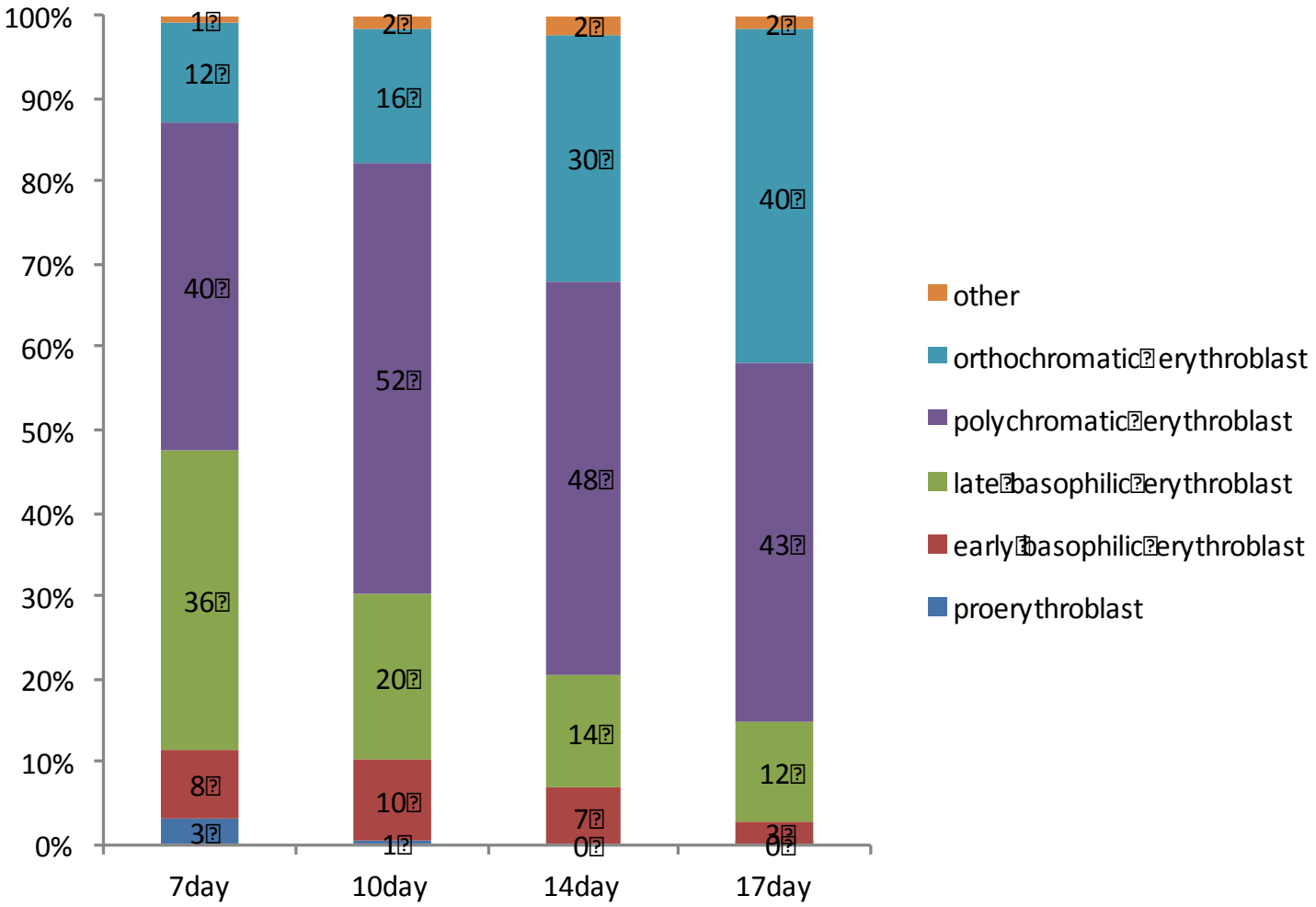


Polychomatic n.

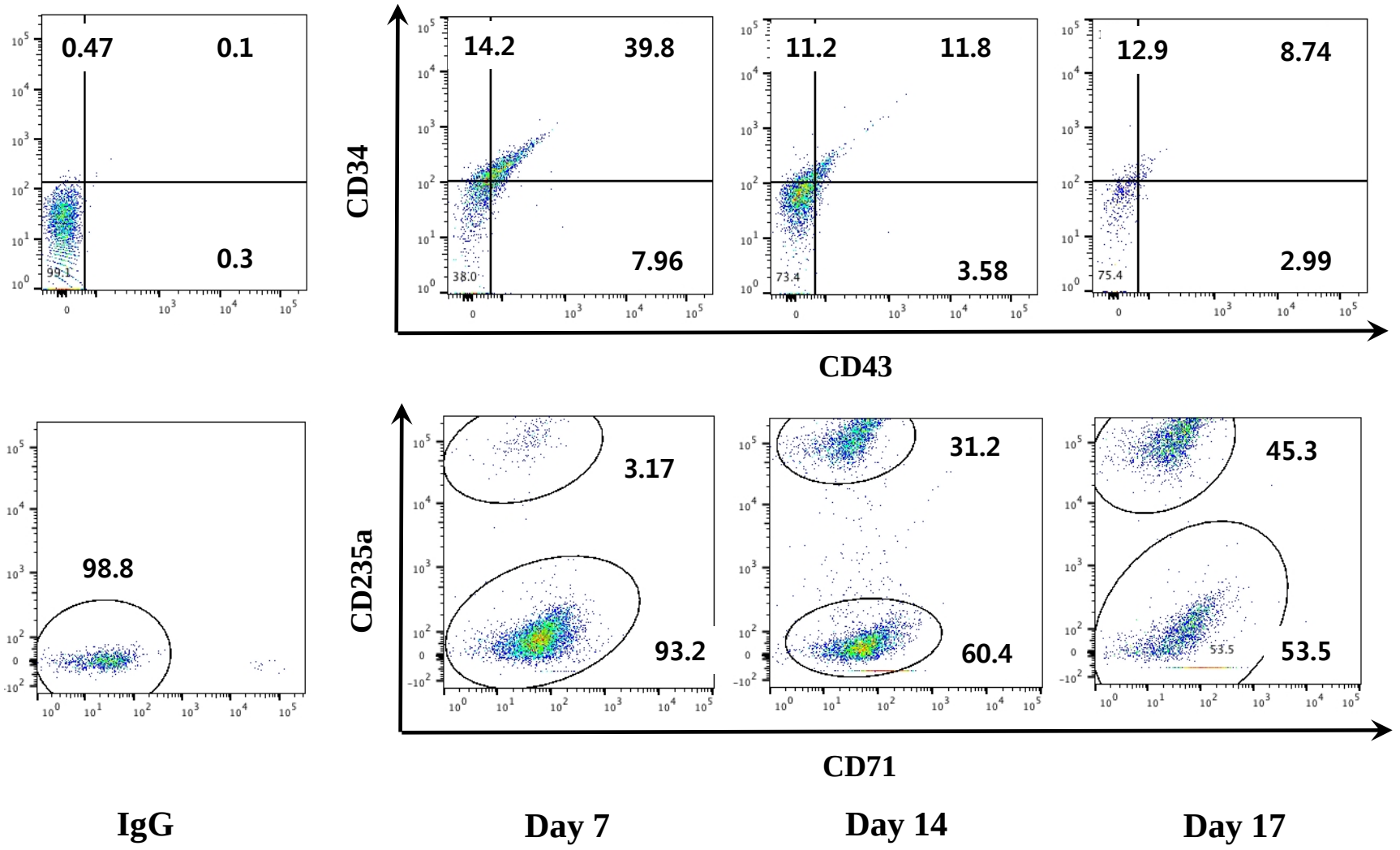


Orthochromatic n.

Comparison of cell morphological changes



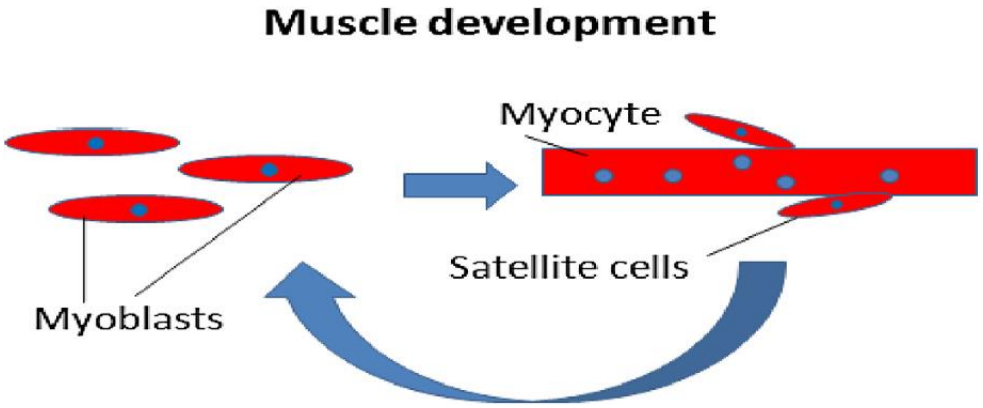
Flow cytometric analysis of erythroblast differentiation culture



임상적으로는 앞으로 어떠한 추가적인 유용성이 있겠는가?

Uses of Stem Cells in gynecology

- SUI and pelvic floor dysfunction
  - Bone marrow(BMSC) and adipose-derived mesenchymal stem cells (ADSC): animal studies
  - muscle-derived stem cells (MDSC) (cultured from patient thigh or deltoid biopsies): human studies



- Admninstration Routes
  - Intraurethral
  - Periurethral
  - Intravenous (IV)

Localized injections make inherent sense!

TABLE 1
Summary stem cell research in stress urinary incontinence

Author	Cell source	Methods	Results/conclusions	Complications
Kim et al[12]	Rat BMSC	Rat population Injury: bilateral pudendal nerve transection Injection: periurethral 4 wk outcome measures	Restoration LPP/CP in transplant group Muscle masses noted in urethra Conclude efficacy could be due to improved contraction ± bulking effect	None Presence "muscle masses" noted
Cruz et al[13]	Rat BMSC labeled with GFP	Rat population Injury: vaginal distension Injection: IV 4 and 10 d outcome measure	4 d: GFP in urethra, vagina, rectum, and levator 10 d: Increased GFP in urethra IV BMSC able to home to sites of injury	None
Xu et al[14]	Rat MDSC labeled w GFP ± Fibrin Glue	Rat population Injury: bilateral pudendal transection Injection: periurethral 1, 4 wk functional evaluation 4 wk histology	Transplant: LPP increased with and without fibrin glue vs injury without transplant No significant difference in function with fibrin glue, but + increased cell survival and microvessel density	None Transplant group: + increased thickness of muscle with variable fiber orientation
Corcos et al[15]	Rat BMSC	Rat population Injury: bilateral pudendal transection Injection: intrasphincteric 4 wk postevaluation	Resolution of LPP to baseline in Transplant group	None
Wu et al[16]	Rat ADSC	Rat population Injury: pudendal nerve (crush) Injection: periurethral, 3 sites Sacrifice at 3, 7, 14 d posttransplantation	Transplantation group: increased LPP, CP, FUL, vs no transplant Urethra: structural resolution to baseline with transplantation	None
Lim et al[18]	HUCB with fluorescent label	Rat population Injury: periurethral electrocautery Injection: 2 sites, lateral wall midurethra Evaluation 2, 4 wk	Transplants: LPP and histologic improvement vs control BUT no labeled cells found at 4 wk Impact attributed to paracrine effect of cytokines and growth factors	None
Lin et al[19]	Rat ADSC, labeled	Rat population Injury: vaginal distension and bilateral oopherectomy Injection: intraurethral or IV Evaluation at 4 wk	Significant improvement in LPP with either transplantation method + Detection cells at 4 wk + Homing demonstrated in IV transplantation	None
Zhao et al[20]	Rat ADSC rat ± nerve growth factor (NGF) ± encapsulated polylactic-coglycolic acid	Rat population Injury: bilateral pudendal transection Injection: periurethral Evaluation at 8 wk	Significant improvement LPP and muscle proportion and neuronal density when ADSC + NGF + Capsule Other ADSC groups improved but did not return to baseline	None
Kinebuchi et al[24]	Rat BMSC Also injected 1 group with CFM	Rat population Injury: urethrolysis and injection toxin Injection: periurethral 13 wk evaluation	LPP increased in transplant group, but not significant vs CFM injection +GFP showed fusion Less striated muscle in CFM vs MSC or control group Some contribution growth factors/cytokines from medium alone seen	None

Lane. Stem cells. Am J Obstet Gynecol 2012. (continued)

- Urinary incontinence 관련 연구가 가장 활발

★효과가 직접 반영될 가능성
★Multipotent 혹은 unipotent 한 세포들로 연구가 이루어져 안정성 높음

TABLE 1
Summary stem cell research in stress urinary incontinence *(continued)*

Author	Cell source	Methods	Results/conclusions	Complications
Imamura et al[10]	Rabbit BMSC	Rabbit population Injury: cryoinjury Injection: intraurethral Evaluation at 1, 2 wk	LPP higher in transplant vs control at 2 wk	None
Fu et al[25]	Rat ADSC	Rat population Injury: vaginal distention Injection: posterior urethra at bladder neck Follow-up 1, 3 mo	Max bladder capacity, LPP, and muscle thickness increased in transplant group	None
Carr et al[21]	Autologous MDSC	Human: 8 women SUI Injection: up to 4 circumferential urethral injections 12 mo data	5 women + improvement 1 woman = total continence 2 of the improved still proceeded with midurethral sling 3 did not continue follow-up	None
Lee et al[22]	Human cord blood	Human: 39 women w/SUI Injection: transurethral 1, 3, 12 mo follow-up	12 months: 26 women (72.2%) had >50% improvement In selection of 10 women, MUCP increased >30 cm H_2O	None
Sebe et al[23]	Autologous muscle progenitor cells	Human: 12 women persistent, severe SUI w fixed urethra s/p prior failed surgery Injection: intrasphincteric Follow-up to 12 mo	3 patients dry: subjective diary and pad test 7 decreased with pad test, but no change after incontinence episodes 2 some worsening	None

ADSC, adipose derived mesenchymal stem cells; *BMSC*, bone marrow-derived mesenchymal stem cells; *CFM*, cell free medium; *CP*, closure pressure (urethral); *FUL*, functional urethral length; *GFP*, green fluorescent protein; *HUCB*, human umbilical cord blood; *LPP*, leak point pressure; *MDSC*, muscle derived stem cells; *NGF*, nerve growth factor; *SUI*, stress urinary incontinence.

Lane. Stem cells. Am J Obstet Gynecol 2012.

Evidence for Stem cell therapy for Asherman's syndrome

- eMSCs can be isolated as CD146 PDGF-R β cells (platelet derived growth factor receptor-β)
  - which identified their perivascular location in both the functional and basal layers of human endometrium
  - regenerating endometrium
  - Gargett, Taylor Group

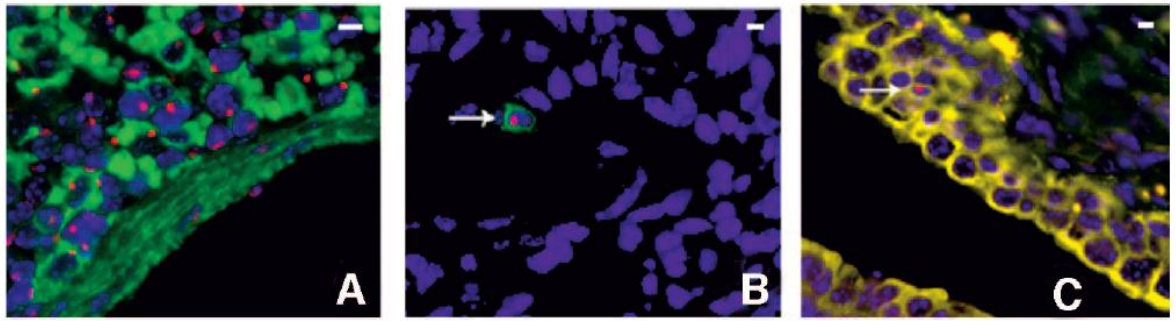


Figure 2. Y chromosome, cytokeratin, and CD45 immunofluorescence. Immunofluorescence staining of Y chromosome (red), cytokeratin (yellow), and CD45 (green) demonstrates differentiated bone marrow-derived endometrial epithelium cells in the transplanted female mice uteri. **(A):** Y chromosome signal in lymphoid cells (spleen) demonstrating expression of CD45 (pan leukocyte marker). **(B):** Y chromosome-positive and CD45-positive cell demonstrating a transient leukocyte in endometrium. **(C):** Y chromosome-positive, cytokeratin-positive, and CD45-negative cell in transplanted endometrium. This cell is a differentiated epithelial cell (cytokeratin+), not a leukocyte (CD45−), and is of donor origin (Y+). Three-micrometer paraffin sections; nuclei are stained blue with 4,6-diamidino-2-phenylindole. Original magnification ×400. Bar = 10 microns.

Du et al, 2007

Repeated implantation failure/Asherman's syndrome

Effect of stem cell application on Asherman syndrome, an experimental rat model

Sevtap Kilic · Beril Yuksel · F. Pinarli · A. Albayrak · B. Boztok · T. Delibasi

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Abstract
Purpose We evaluate the effect of stem cells to induce endometrial proliferation and angiogenesis on Asherman Syndrome (AS).
Methods The experimental study was performed in stemcell research laboratory. Forty Wistar-Albino rats were divided according to groups. In group1 ($n=10$) to establish the model; trichloroacetic acid was injected to right uterine horn. Two weeks later, intrauterine synechia was confirmed. In group2 ($n=10$), 2 weeks later, 2×106 mesenchymal stem cells (MSC) were injected into right uterine horn followed by three intraperitoneal injections of MSCs. In group3 ($n=10$), daily oral estrogen was initiated on the second week. In group4 ($n=10$), MSC injections and oral estrogen was given together. The amount of fibrosis, vascularisation, inflammation and immunohistochemical staining with vascular endothelial growth factor (VEGF), proliferating cell nuclear antigen (PCNA) and Ki-67 were evaluated in the uterine tissues.
Results In all treatment groups; fibrosis decreased but vascularisation and immunhistohemical stainings increased in the experimental side. The amount of fibrosis, vascularisation, Ki-67 and PCNA scores were similar between group2 and 3. In group4, comparing to group2, less fibrosis but more Ki-67, PCNA and VEGF staining was observed.
Conclusion Stem cells, when added to estrogen, are a highly effective alternative to induce regeneration of endometrium in Asherman Syndrome therapy.

Keywords Intrauterine adhesion · Intrauterine synechia · VEGF · PCNA · Ki-67

Introduction

Asherman Syndrome (AS) is defined as intrauterine adhesions

Intrauterine transplantation of autologous bone marrow derived mesenchymal stem cells followed by conception in a patient of severe intrauterine adhesions*

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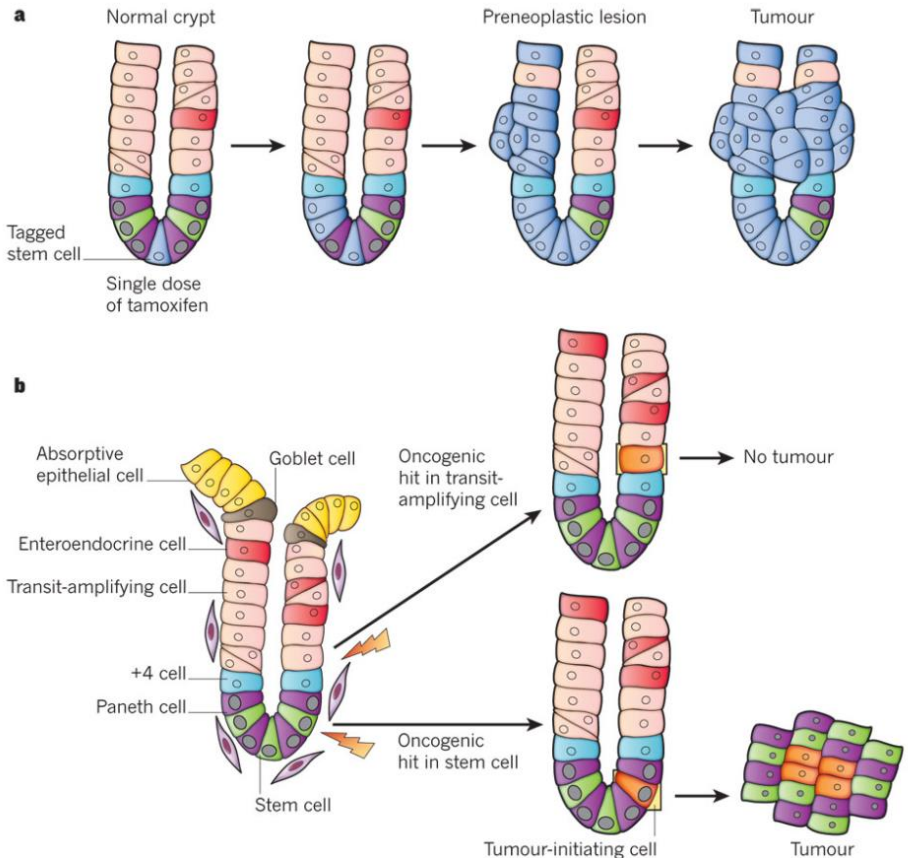
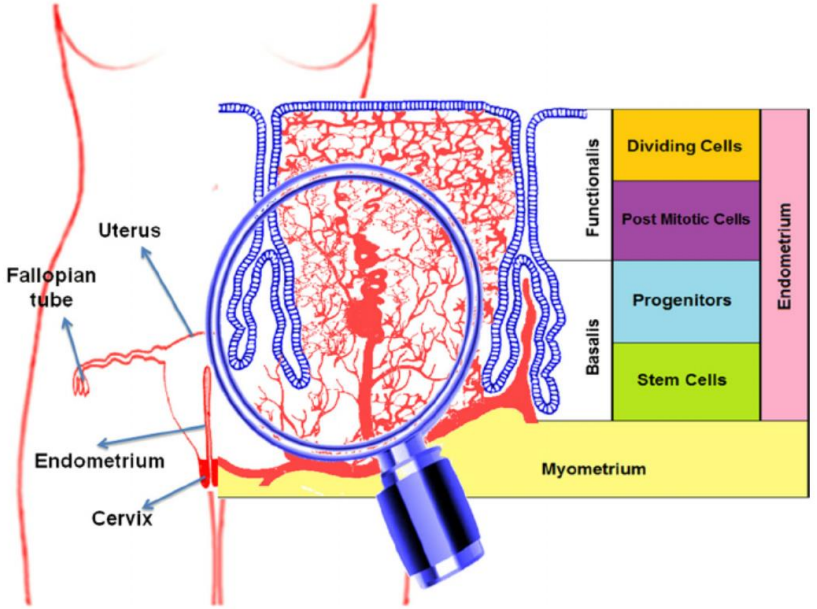
Potential uses in the future for endometrium induced diseases





Artificial Gametes from stem cells

- Somatic cell nuclear transfer of patient nucleus into donor embryonic stem cells *(Tachibana et al. 2013)*

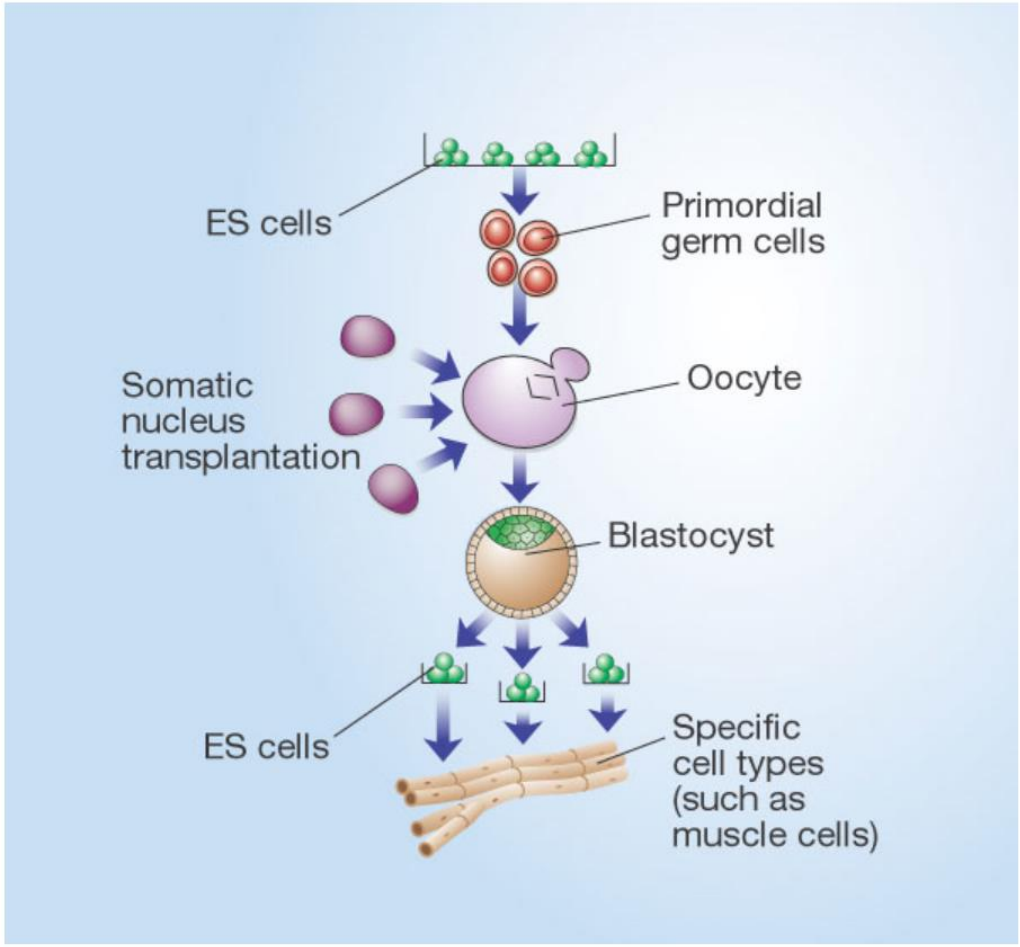
- iPSc driven oocytes *(Hayashi et al. 2012~)*

Table I Studies demonstrating possible routes to create artificial gametes.

Route creating artificial gamete	Most advanced outcomes reached[a]					
	Animal model			Human		
	Gamete	Fertilization	Offspring	Gamete	Fertilization	Offspring
Artificial sperm from male						
(1) *In vitro* differentiation of germline stem cells (GSCs)	1	—	2	—	—	—
(2) *In vitro* proliferation of GSCs followed by autotransplantation	3, 4, 5	6	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23	—	—	—
(3) *In vitro* differentiation of embryonic stem cells (ESCs)	—	24, 25, 26	27	28, 29, 30, 31, 32	—	—
(4) *In vitro* differentiation of ESCs followed by autotransplantation	33	—	34, 35, 36, 37			—
(5) *In vitro* differentiation of induced pluripotent stem cell (iPSCs)	38	—	—	29, 39, 31	—	—
(6) *In vitro* differentiation of iPSCs followed by autotransplantation	40	—	35	—	—	—
(7) *In vitro* somatic cell transformation into sperm without documented transitional cell types	—	—	—	—	—	—
(8) *In vivo* somatic cell transformation into sperm without documented transitional cell types	—	—	41	—	—	—
Artificial oocyte from female						
(1) *In vitro* differentiation of GSCs	42	—	—	42	—	—
(2) *In vitro* proliferation of GSCs followed by autotransplantation	—	42	43	42	—	—
(3) *In vitro* differentiation of ESCs	26, 44, 45, 46	—	47, 48	28	—	—
(4) *In vitro* differentiation of ESCs followed by autotransplantation	—	—	—	—	—	—
(5) *In vitro* differentiation of iPSCs	—	—	47, 48	—	—	—
(6) *In vitro* differentiation of iPSCs followed by autotransplantation	—	—	—	—	—	—
(7) *In vitro* somatic cell transformation into oocytes without documented transitional cell types	49, 50	—	—	51, 52	—	—
(8) *In vivo* somatic cell transformation into oocytes without documented transitional cell types	53, 54	—	—	—	—	—
(9) Haploidization by transplantation of a somatic cell nucleus into an enucleated donor oocyte	55, 56, 57, 58, 59, 60, 61	62	—	58, 63	59, 64, 65	—
Artificial oocytes from a male	44	24	—	—	—	—
Artificial sperm from a female	—	66, 67	68, 69, 70	39	—	—

ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; GSC, germline stem cell; —, refers to no publication reporting on the respective outcome as a furthest end-point.
[a]Numbers (Supplementary data) indicate the appropriate reference with the respective outcome as furthest end-point.

Oocyte production form stem cells (ES/iPSc)



NATIONAL / SCIENCE & HEALTH

Japanese team produces massive number of eggs iPS cells

KYODO

FUKUOKA – A team of researchers has achieved a world's first by using induced pluripotent stem cells from a mouse tail to produce a large number of eggs in vitro, according to a study published in the British science journal Nature.

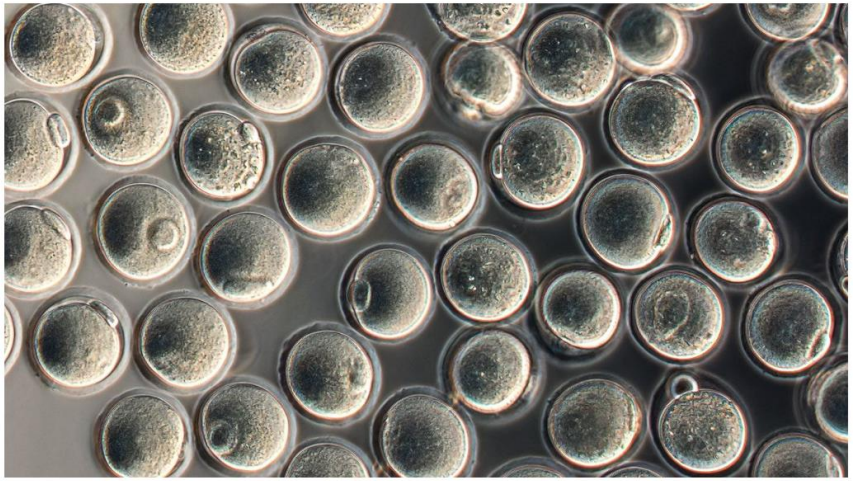
Until now, mouse iPS cells had to be transplanted to a different mouse ovary for eggs to become capable of fertilization, but the team, consisting of researchers from Kyushu University, Kyoto University and other institutions, achieved the process using only cultures.

Further improving the technology could within several years open up the possibility of producing human eggs from iPS cells, said Kyushu University professor Katsuhiko Hayashi, one of the researchers.

The technology may also help "shed light on the cause of infertility" if the team can replicate the egg creation process using iPS cells derived from infertile women, and "be useful for the conservation of endangered species" if a large number of eggs can be created in vitro, Hayashi said.

Somatic cell driven germline cells and offspring

– Hayashi et al. 2016



Egg cells derived in the lab from embryonic stem cells.

O. Hikabe *et. al.*, *Nature* 538, 7625 (20 October 2016) © MacMillian Publisher Ltd.

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Mouse egg cells made entirely in the lab give rise to healthy offspring

By **Gretchen Vogel** | Oct. 17, 2016 , 11:00 AM

summer, the scientists showed that they could **keep developing mouse ovaries growing in the lab** and make them produce mature, fertile eggs.

Mice derived from labmade eggs were normal, fertile adults.

O. Hikabe *et. al.*, *Nature* 538, 7625 (20 October 2016) © MacMillian Publisher Ltd.

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