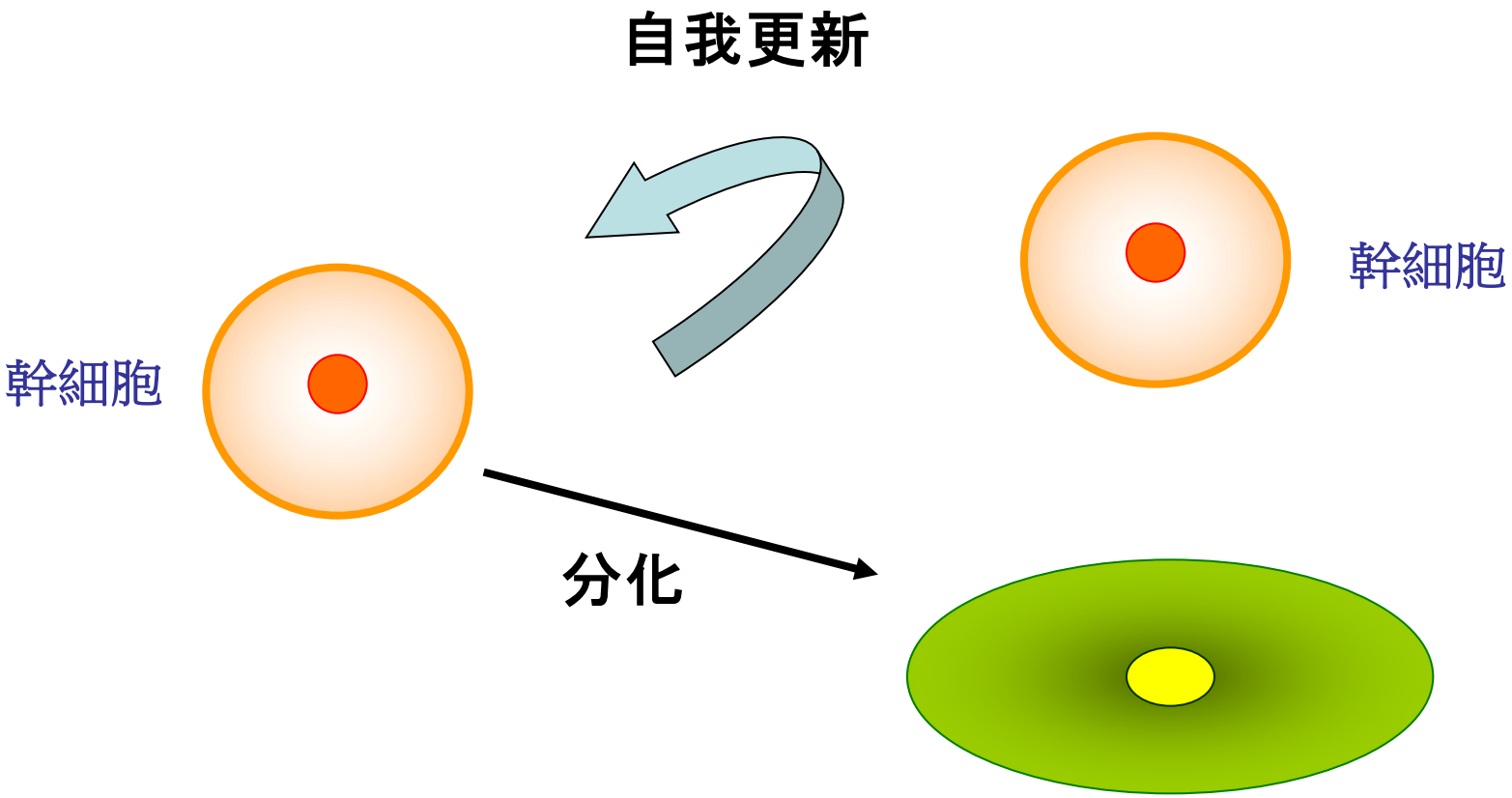
幹細胞-我們身體裏的孫悟空

中央研究院 基因體研究中心/細胞與個體生物研究所　幹細胞實驗室

郭紘志博士

- 1. 幹細胞的簡介
- 2. 胚胎幹細胞
- 3. 成體幹細胞
- 4. 細胞核轉移及治療性複製

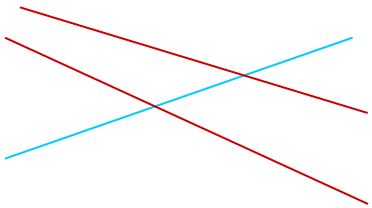
什麼是幹細胞?



幹細胞的種類:

依來源分類:

- 成體幹細胞
- 胚胎幹細胞



依分化潛力分類:

- 萬能幹細胞
- 多能幹細胞
- 單能幹細胞

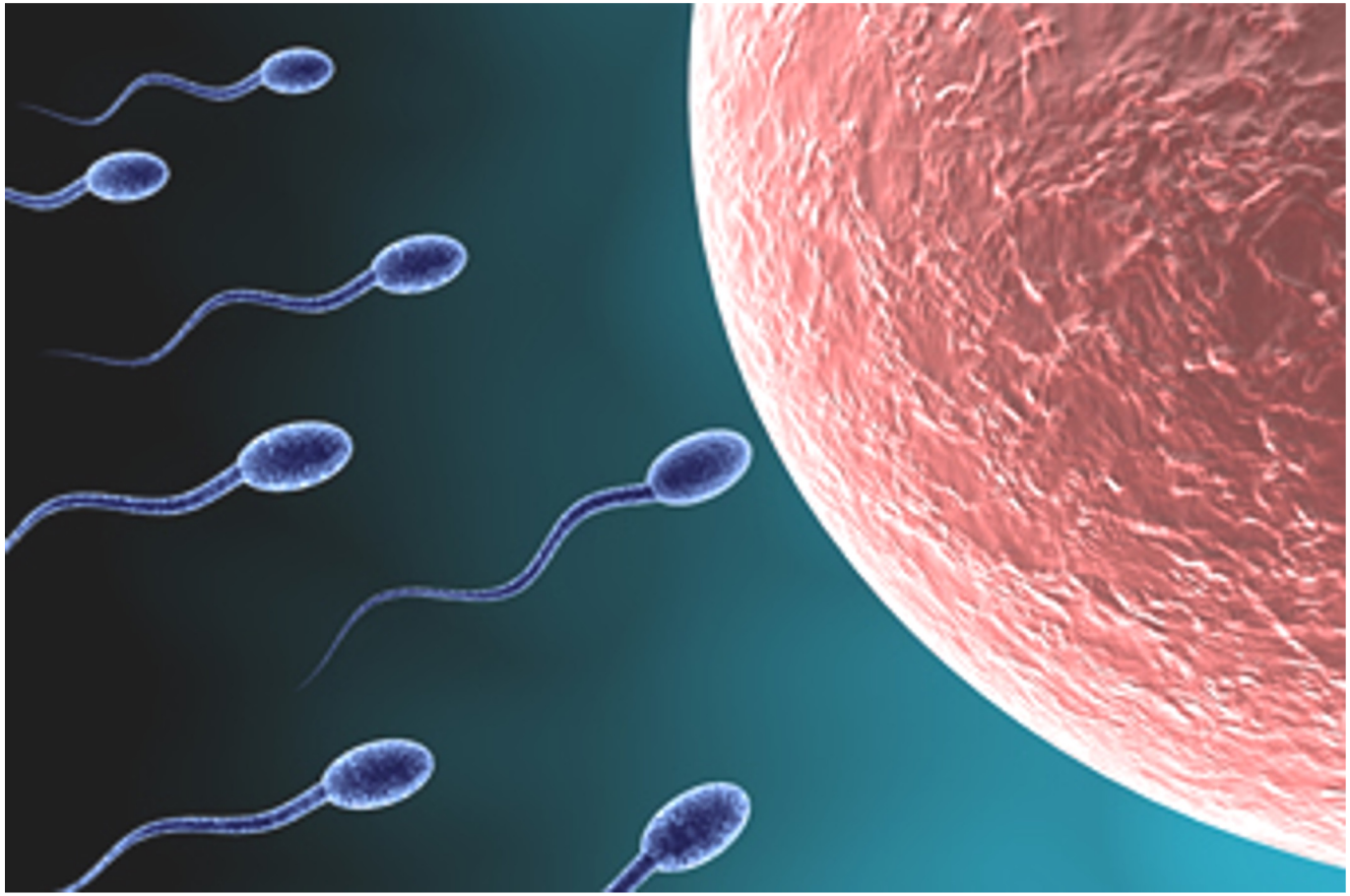
幹細胞的應用

- 醫學治療
  - 退化性疾病之治療
  - 損傷性疾病之治療
- 新藥物之開發
  - 毒物試驗
  - 藥物試驗
- 研究之應用

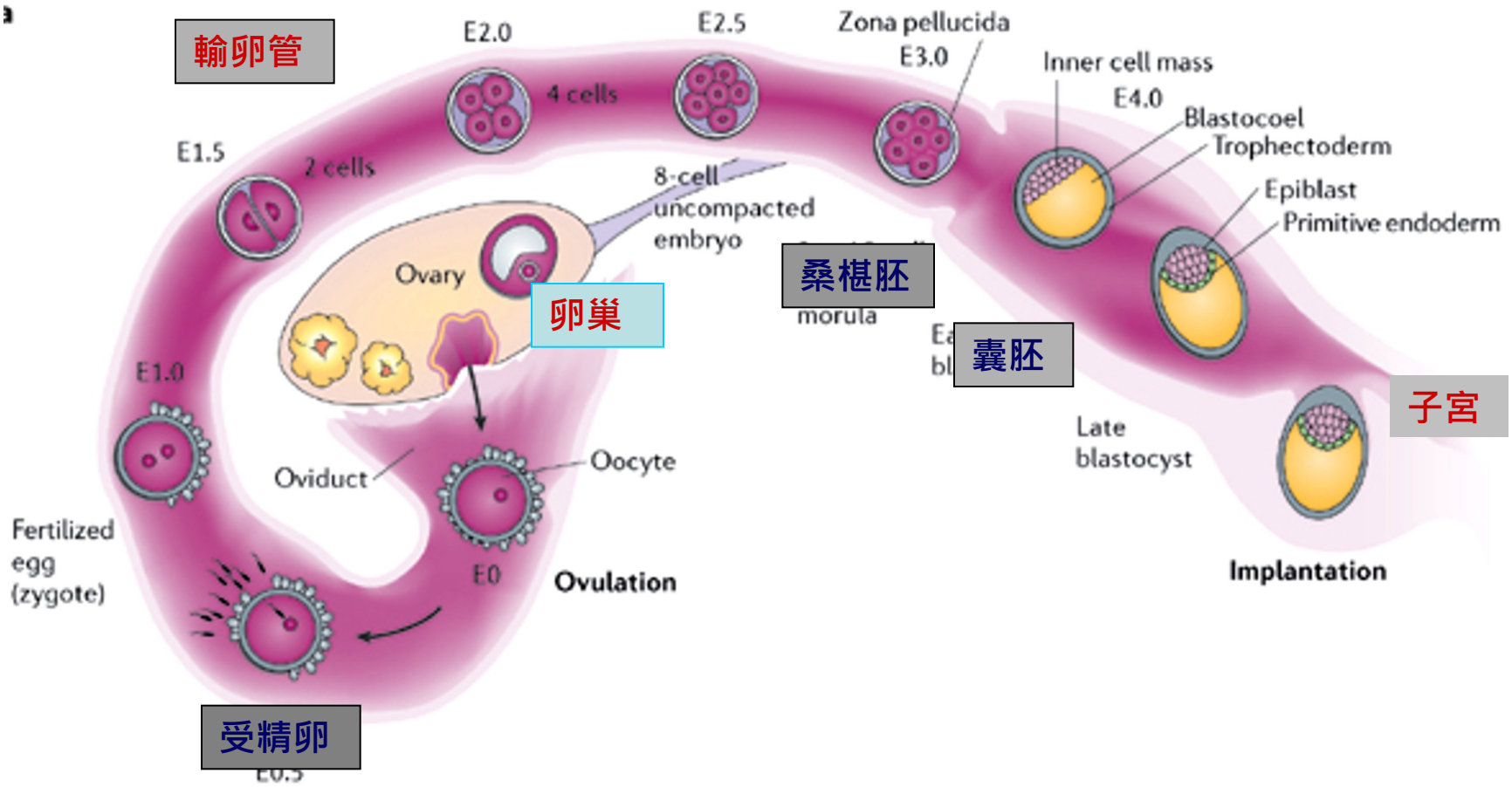
胚胎幹細胞

什麼是胚胎幹細胞?

- 從著床前胚胎所採集來的全能幹細胞
- 可在實驗室被大量，長期的培養並保有正常的遺伝組成
- 可分化成各種組織及細胞型態



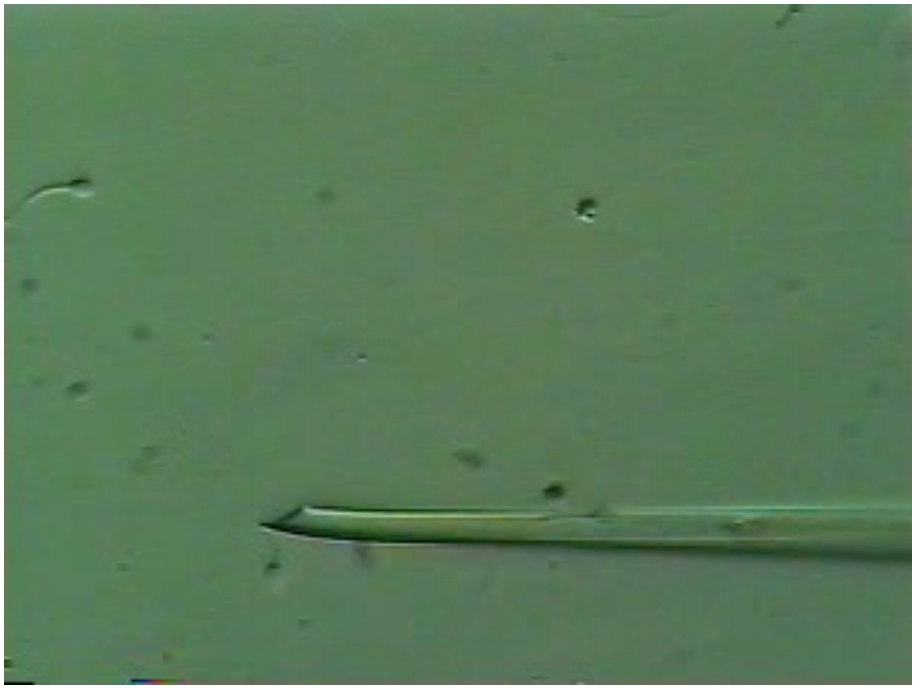
胚胎著床前發育　　我們都是這樣來的



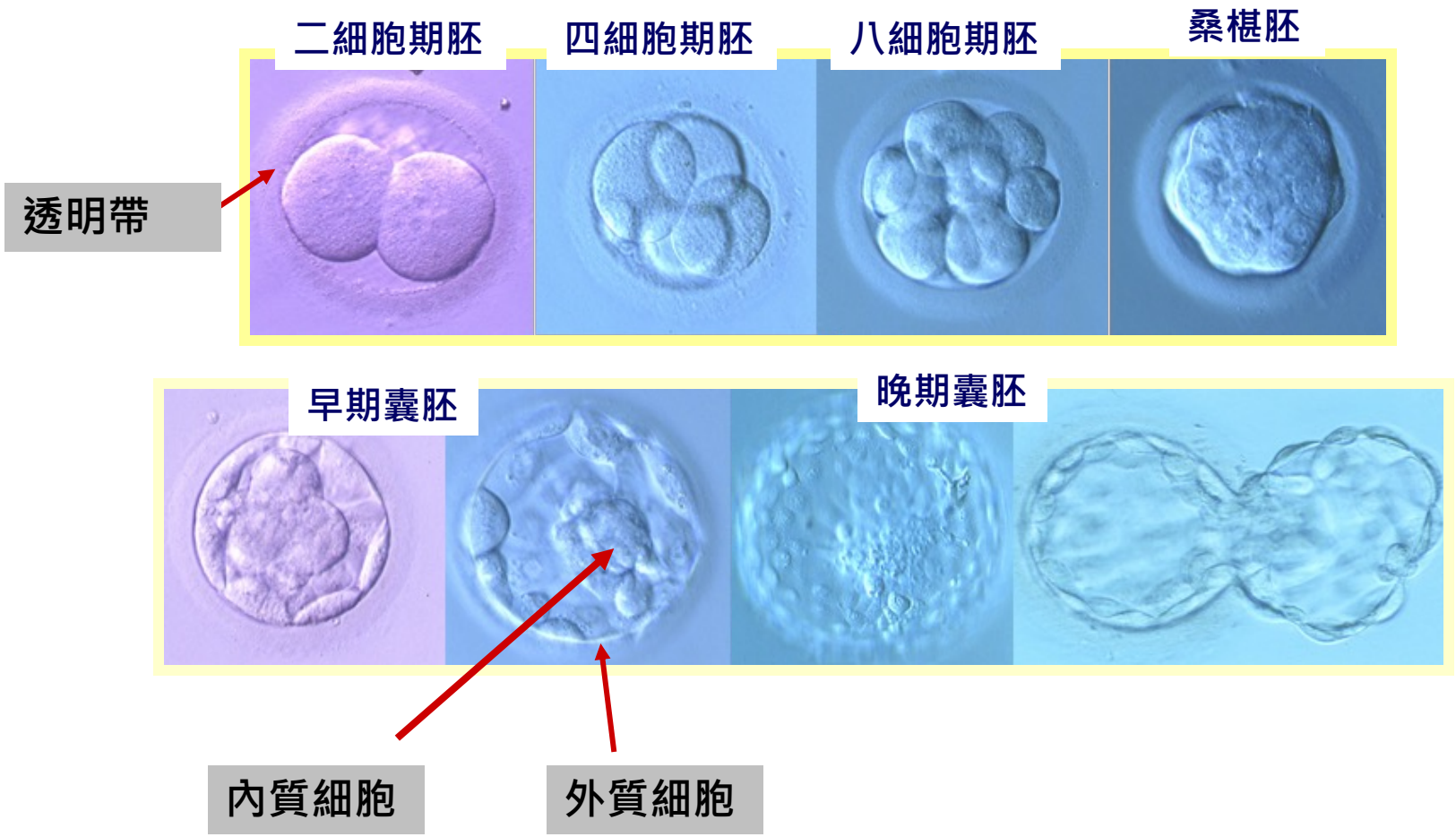
Nature Reviews Genetics 7, 185-199 (March 2006)

胚胎的來源

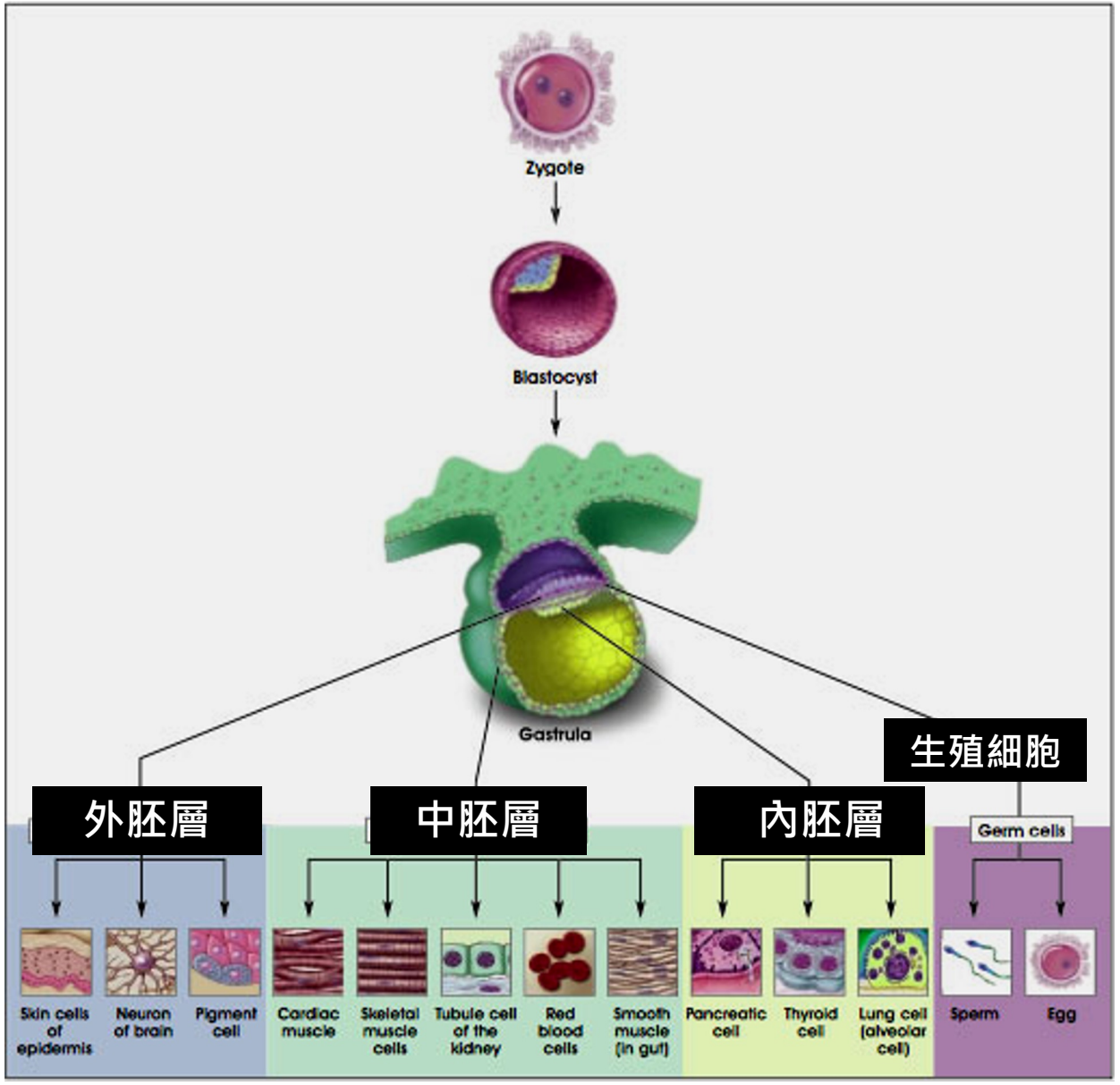
人工生殖後剩餘之胚胎



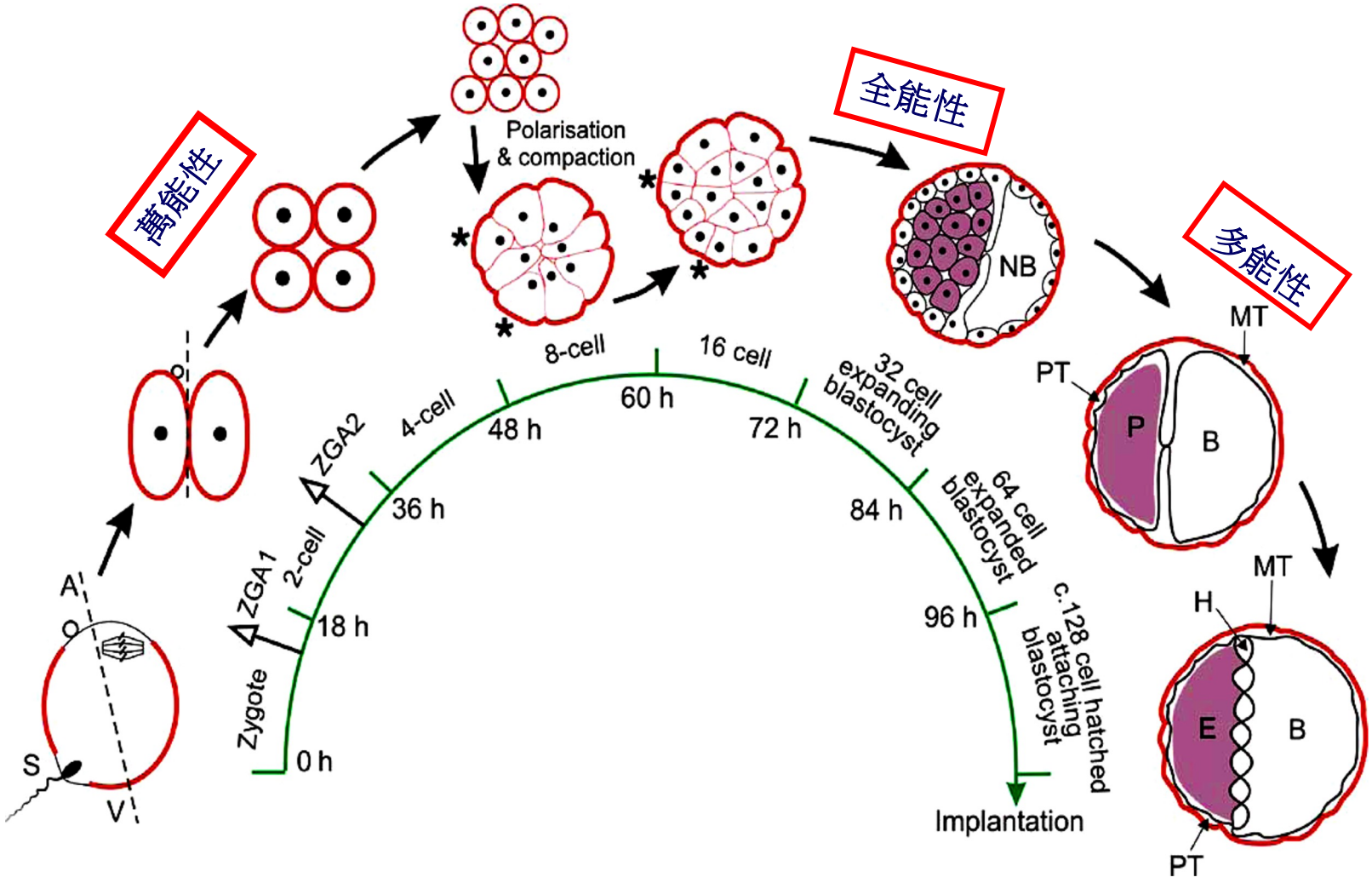
著床前胚胎發育



胚胎著床後發育


Zygote
Blastocyst
Gastrula
生殖細胞
外胚層
中胚層
内胚層
Germ cells
Skin cells of epidermis
Neuron of brain
Pigment cell
Cardiac muscle
Skeletal muscle cells
Tubule cell of the kidney
Red blood cells
Smooth muscle (in gut)
Pancreatic cell
Thyroid cell
Lung cell (alveolar cell)
Sperm
Egg

著床前胚胎發育



Johnson M.H. & Mcconnell J.M.L. (2004) Lineage allocation and cell polarity during mouse embryogenesis.Seminars in cell and developmental biology. 15, 583-597

胚胎幹細胞株的分離與建立

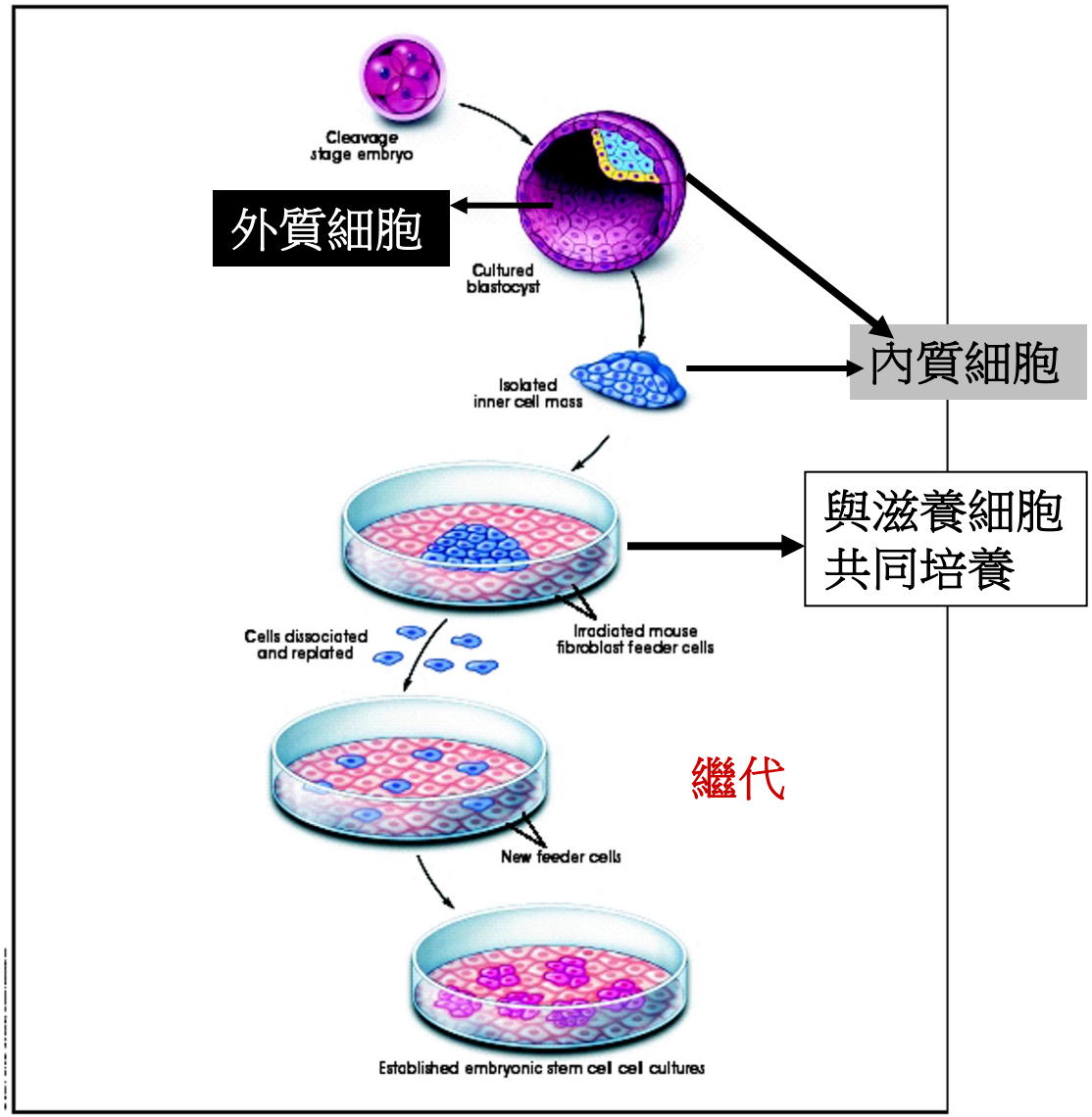
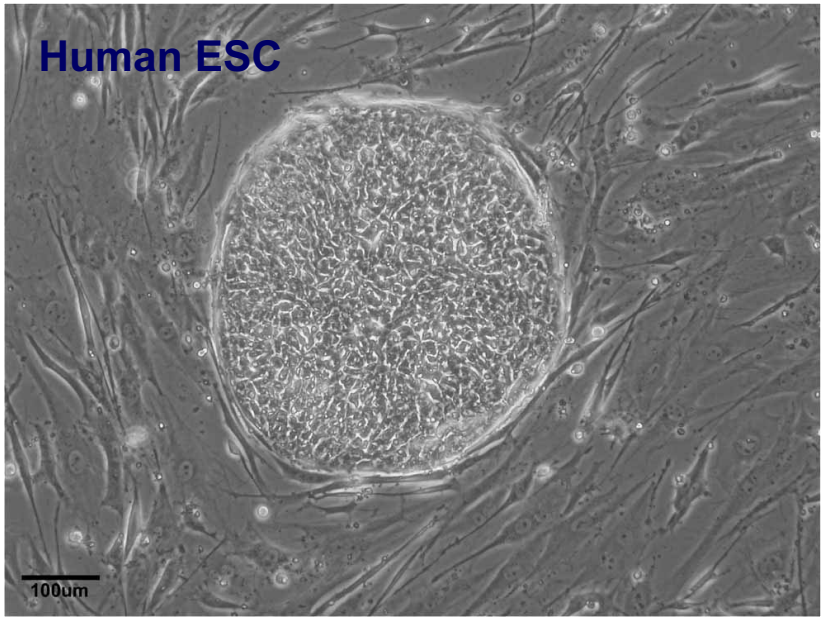
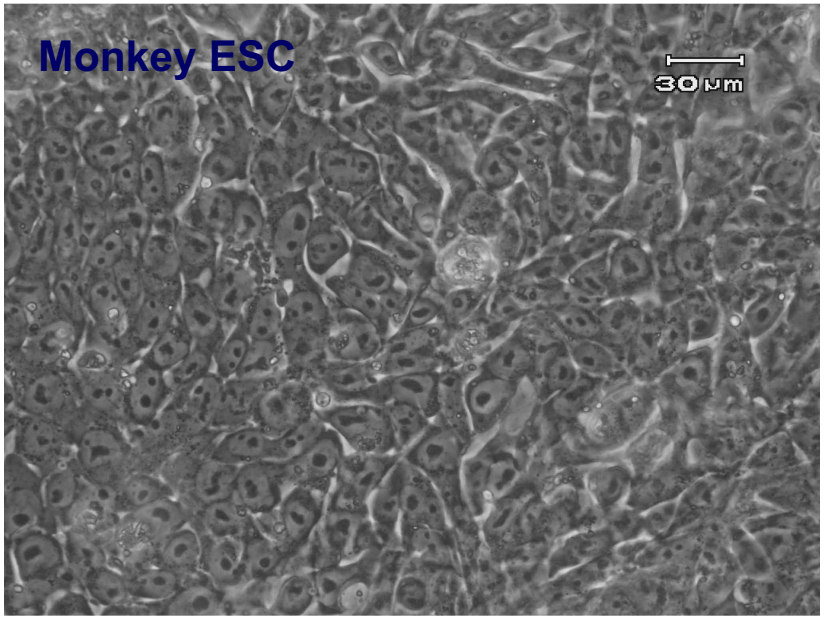


Figure C.1. Techniques for Generating Embryonic Stem Cell Cultures.

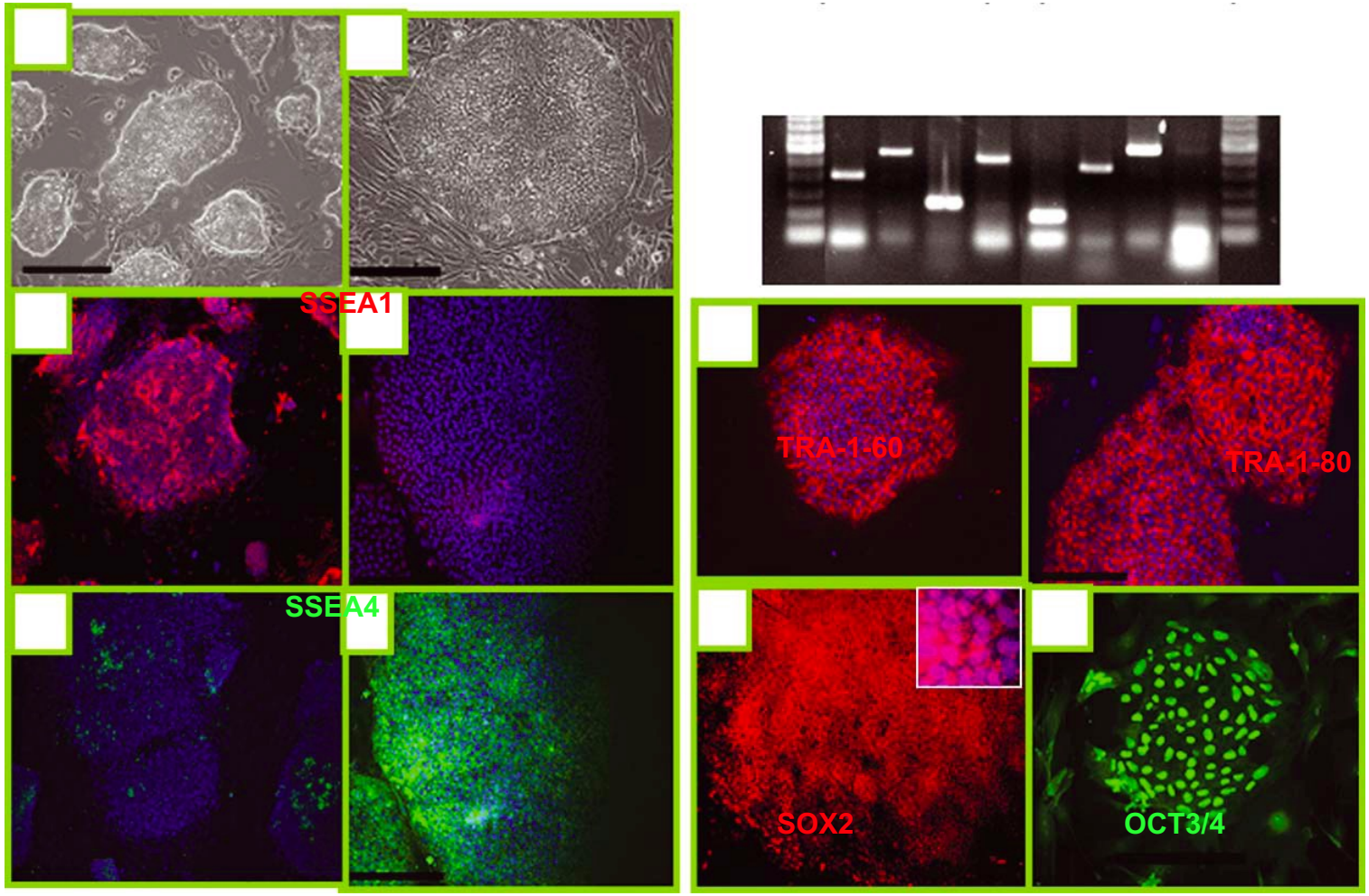
胚胎幹細胞就是長這樣子



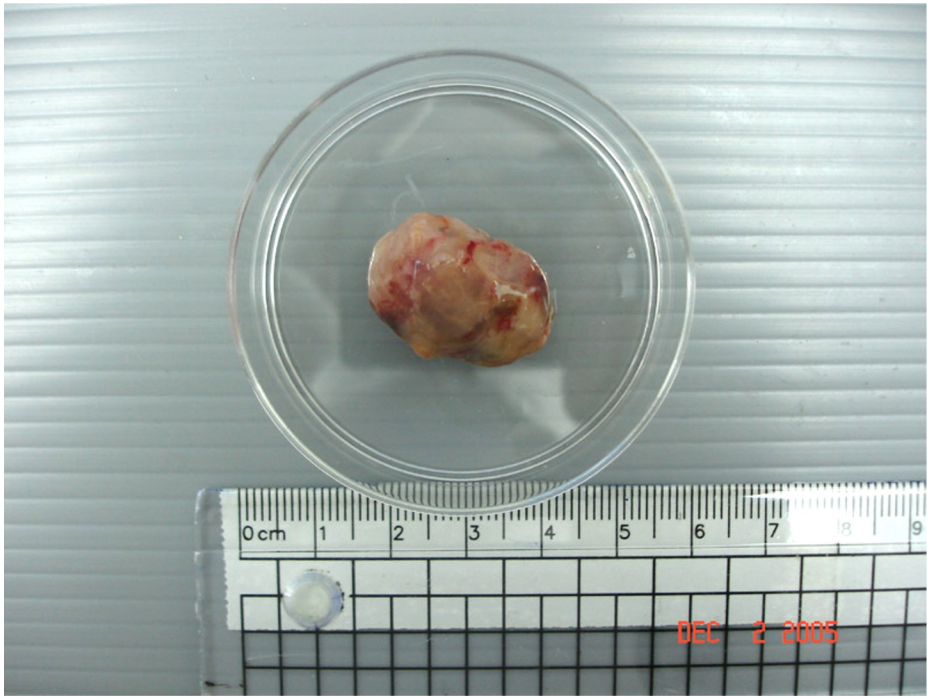


胚胎幹細胞的鑑定

胚胎幹細胞表達特定之基因與蛋白質

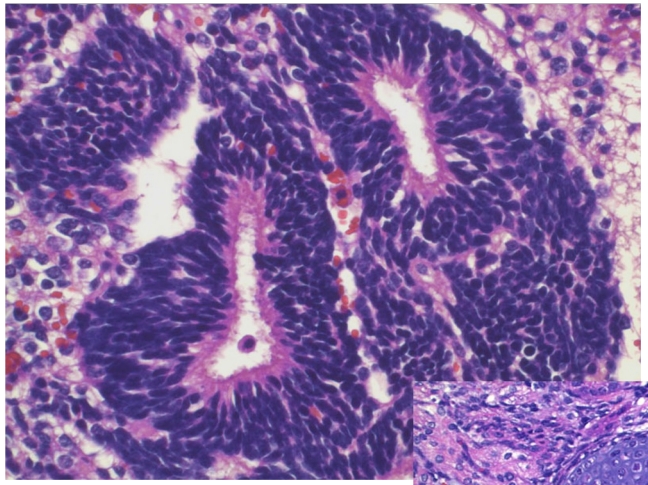


畸胎瘤的形成

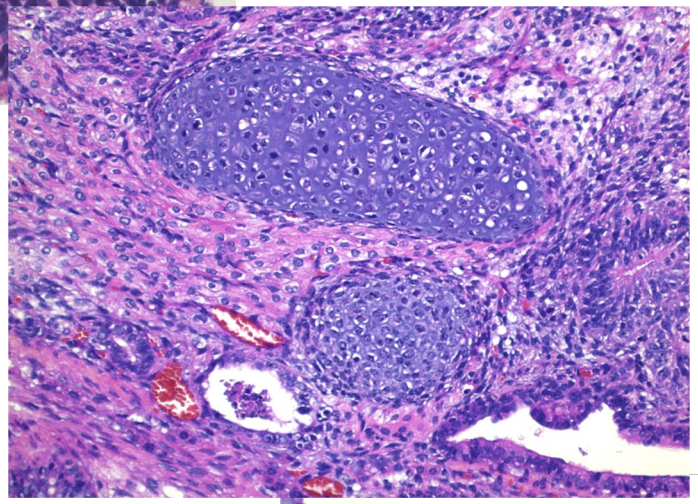


Ectoderm

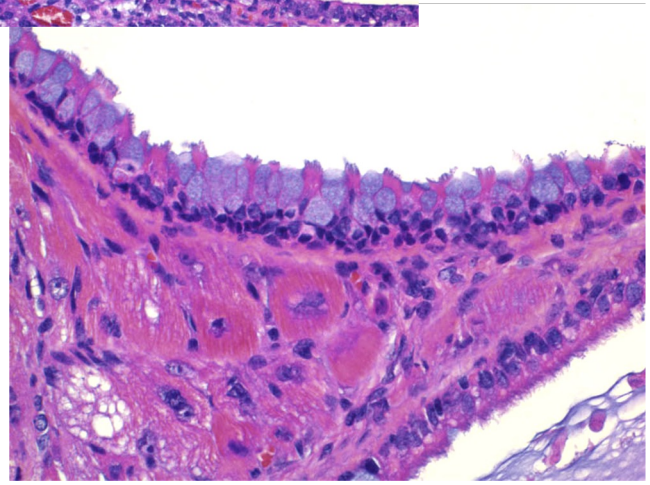
Neural Epithelium



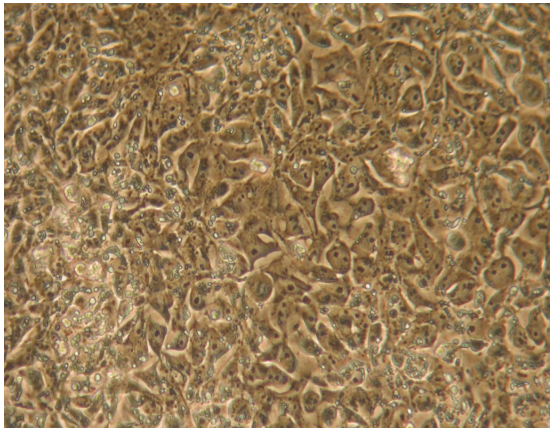
Mesoderm Cartilage

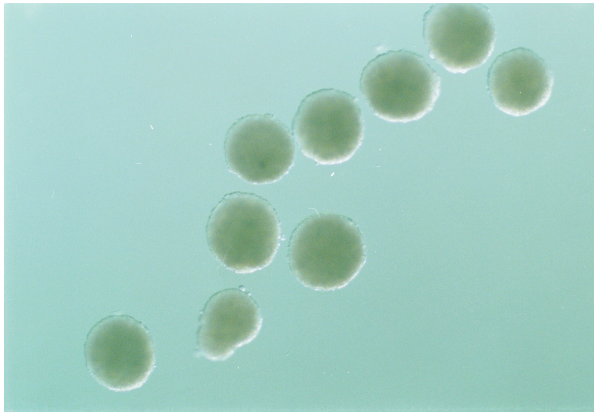


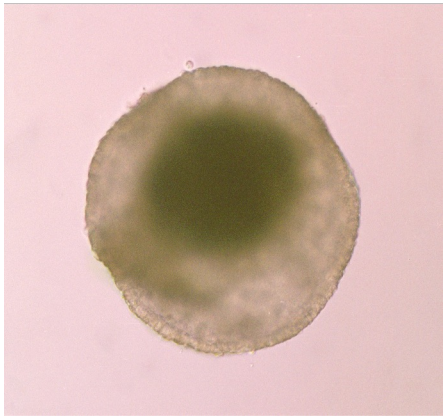
Endoderm Respiratory Epithelium



胚胎幹細胞的分化







Undifferentiated ES cells

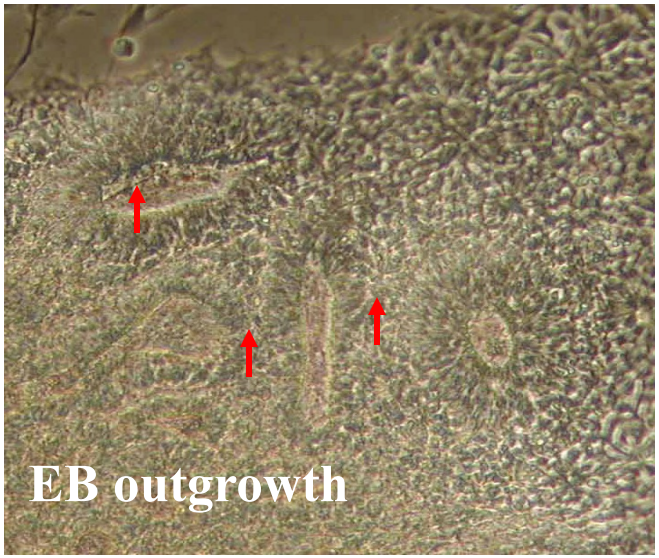
Day 2

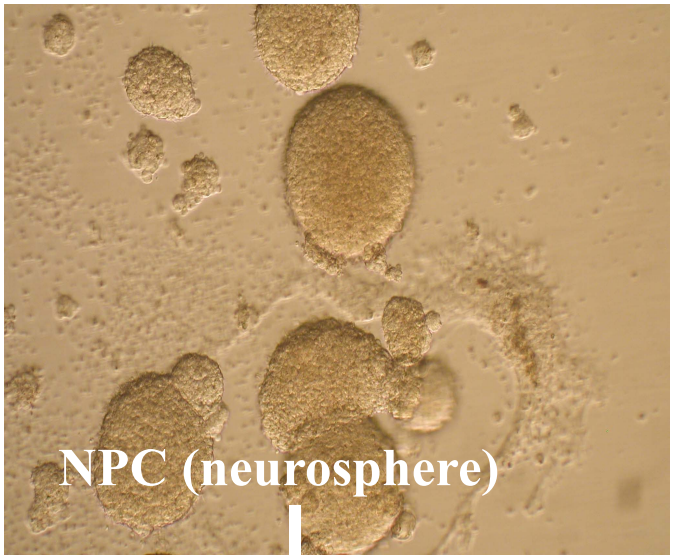
Simple EBs

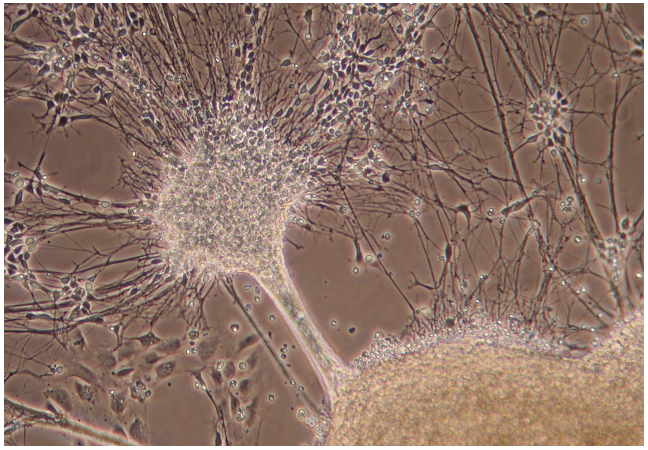
Day 5-15

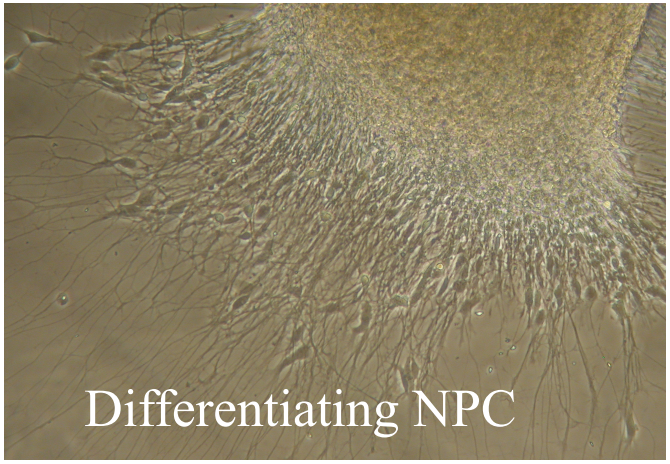
Complex (cystic) EBs

胚胎幹細胞可分化成神經細胞(外胚層)



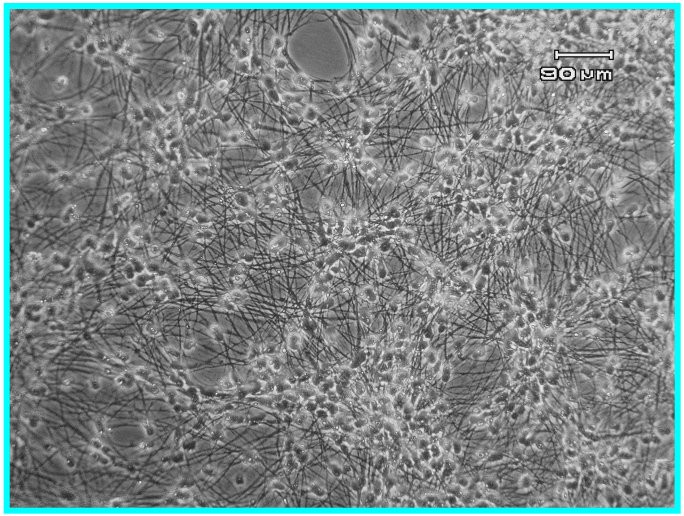




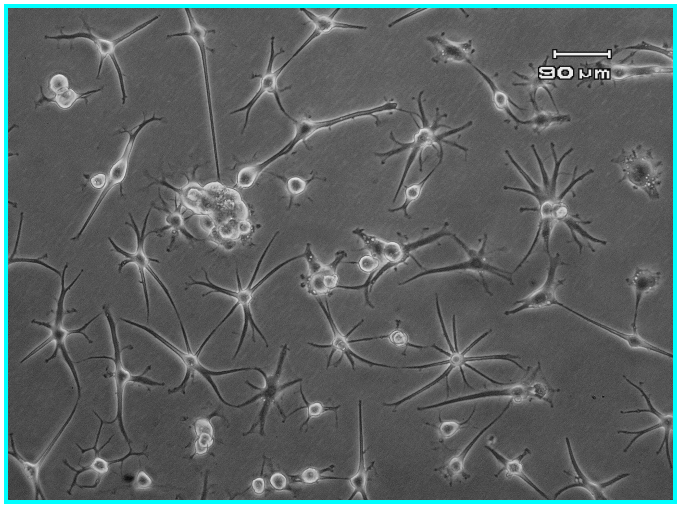


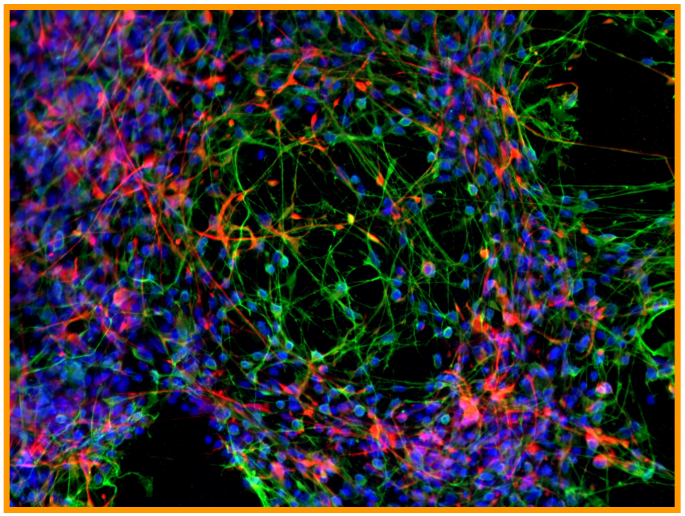
胚胎幹細胞可分化成神經細胞(外胚層)

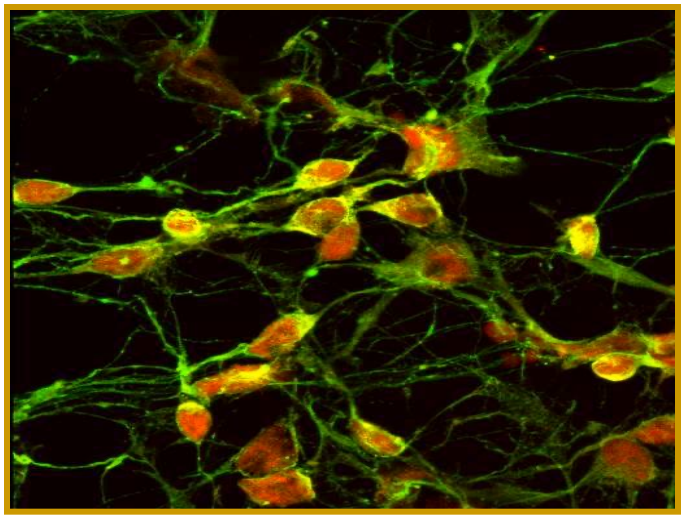
神經元細胞



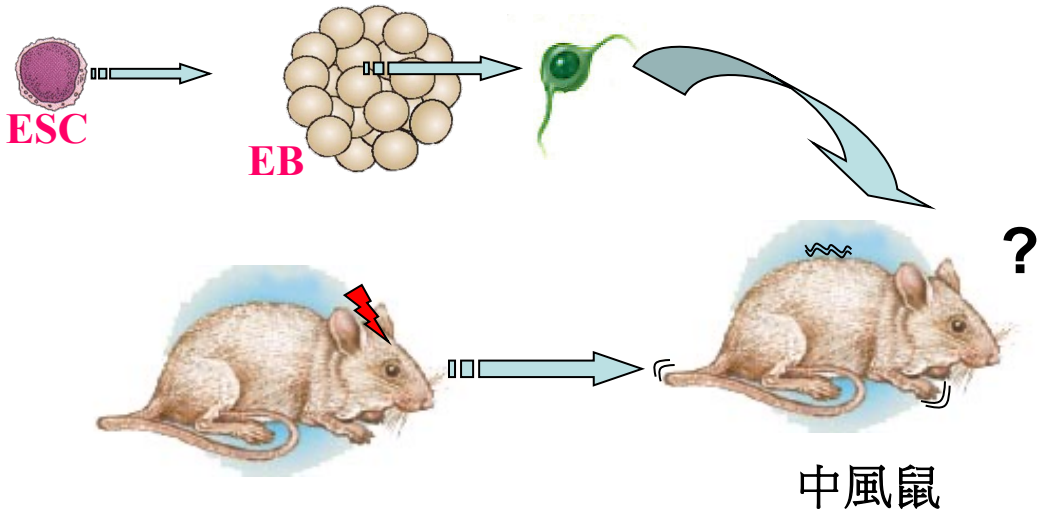
神經膠細胞

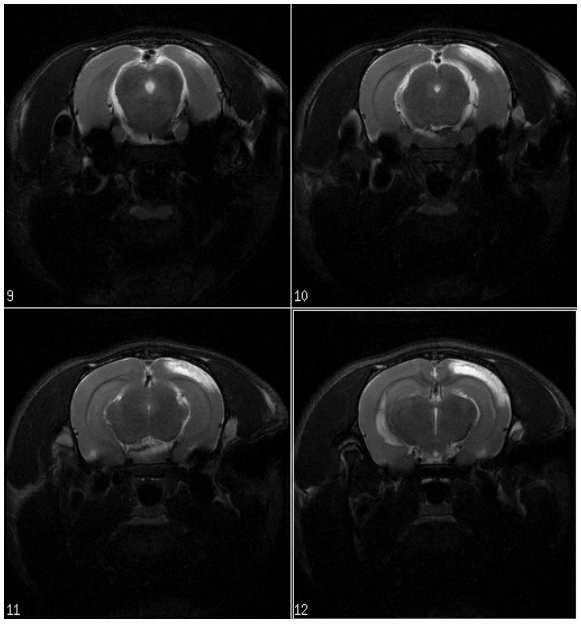




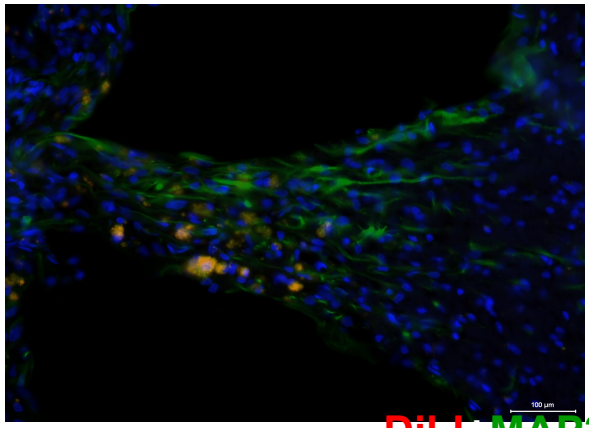


神經幹細胞之移植

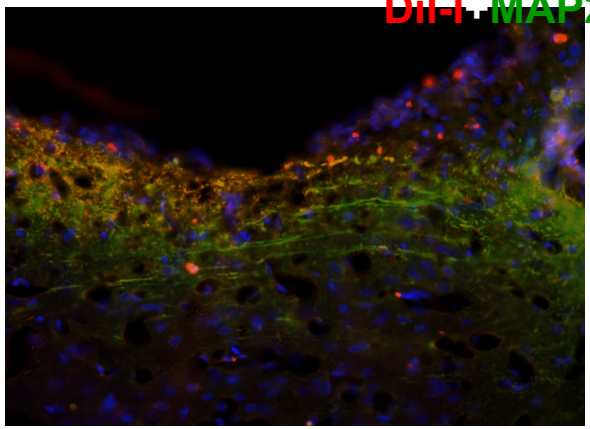




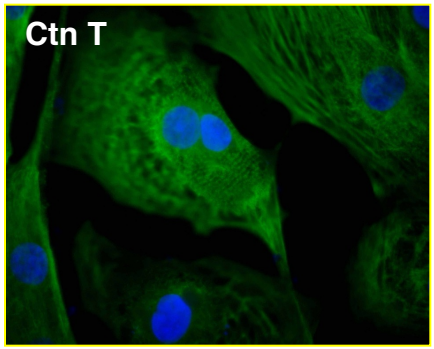
Nestin

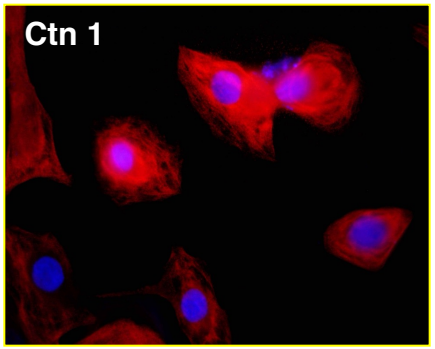


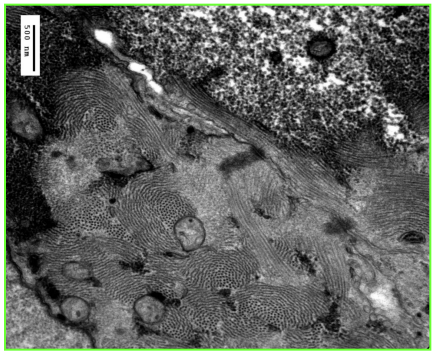
Dil-I+MAP2

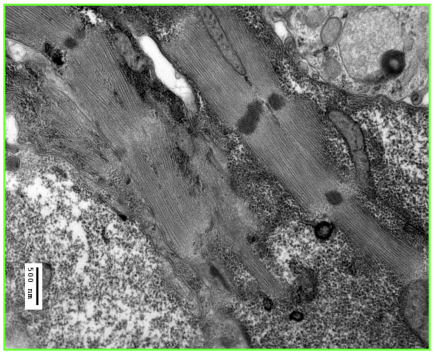


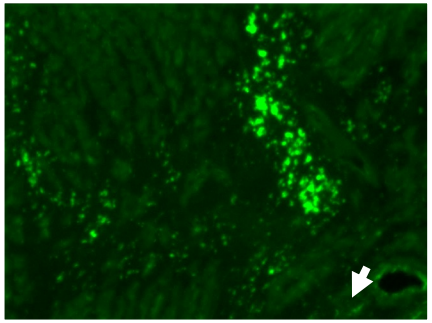
胚胎幹細胞可分化成心臟細胞(中胚層)



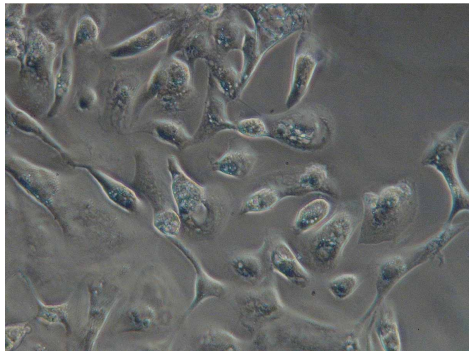


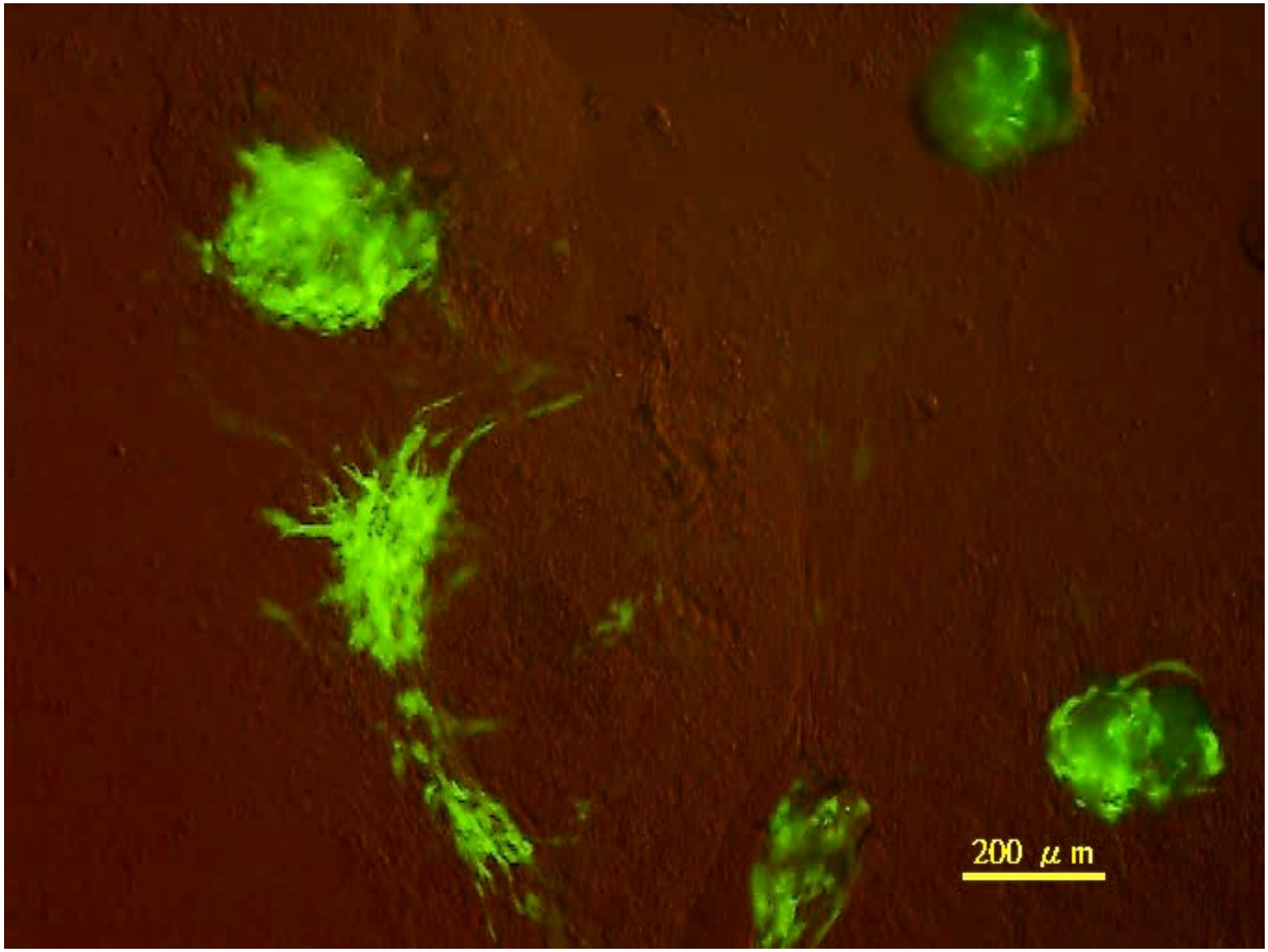


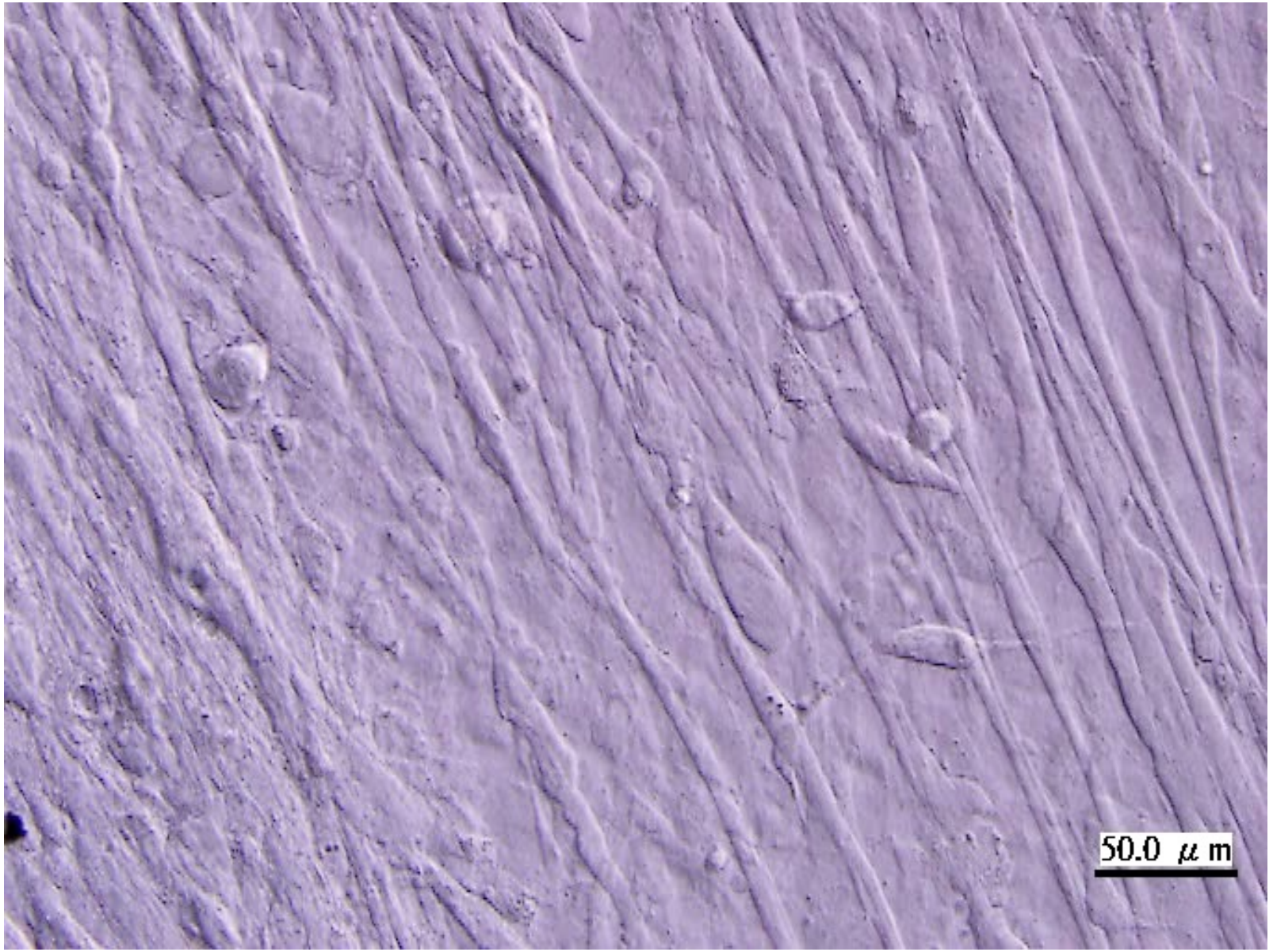




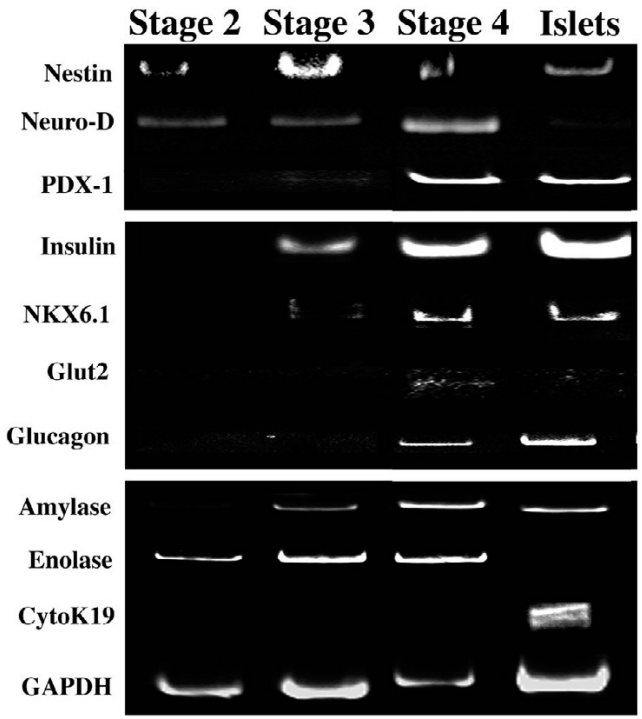
心肌梗塞鼠

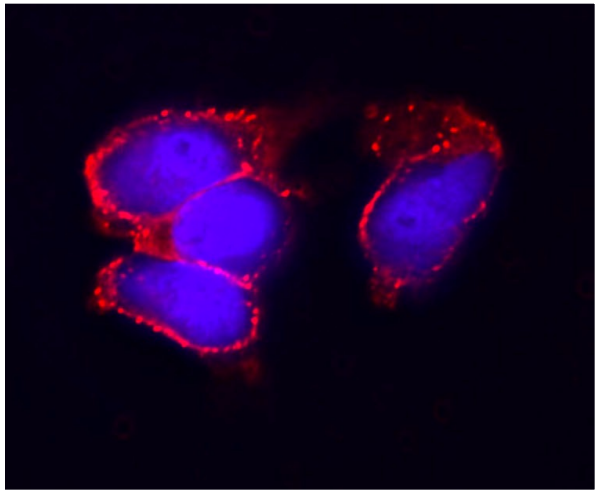


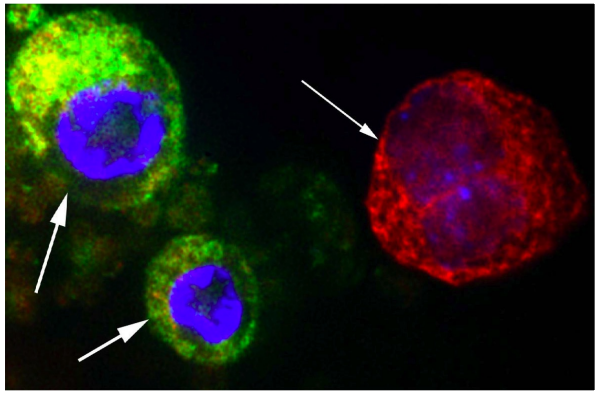

200 μm


50.0 μm

胚胎幹細胞可分化成胰臟細胞
(內胚層)







胚胎幹細胞還可分化成.....

皮膚細胞

肝臟細胞

生殖細胞 (卵子＆精子)

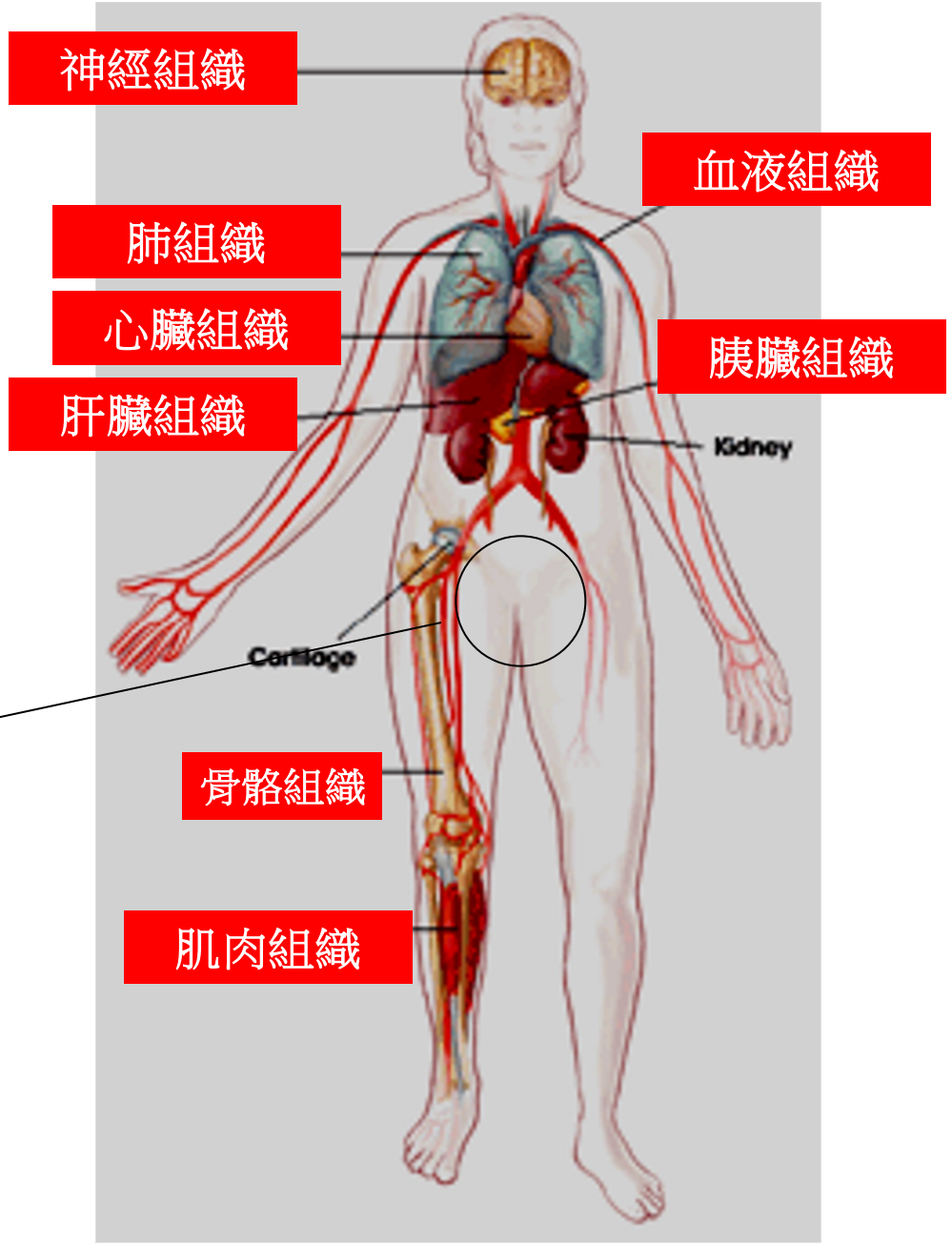
肌肉細胞

骨骼細胞

.....

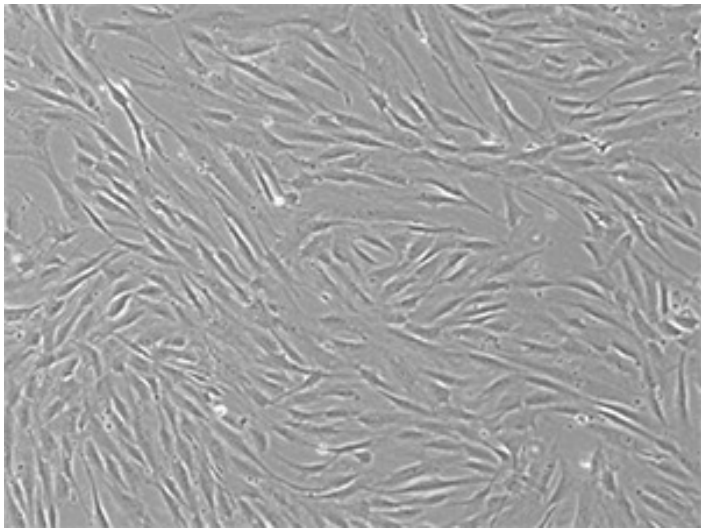
2. 成體幹細胞

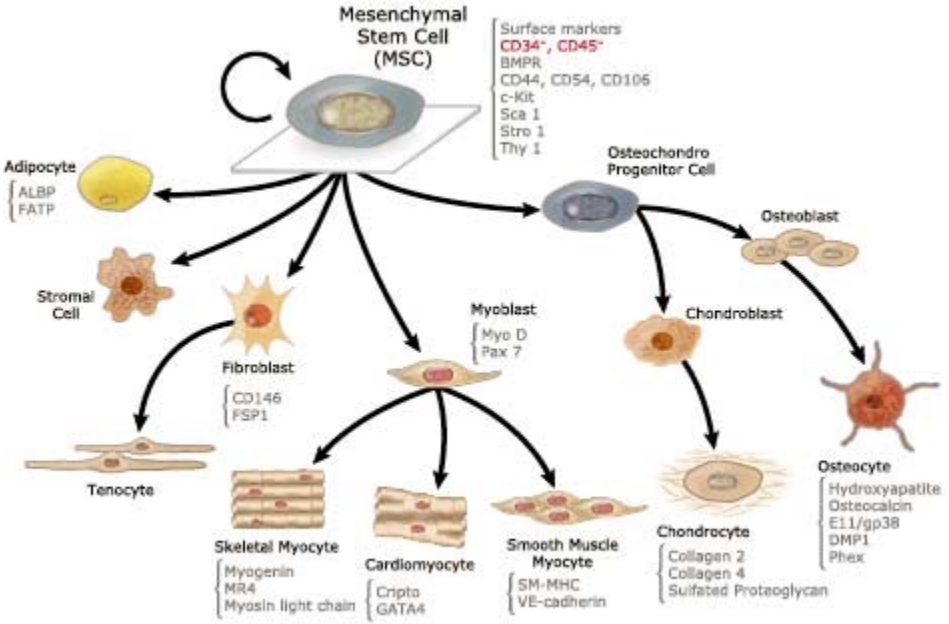
成體幹細胞存在於我們體內的許多器官



間葉幹細胞

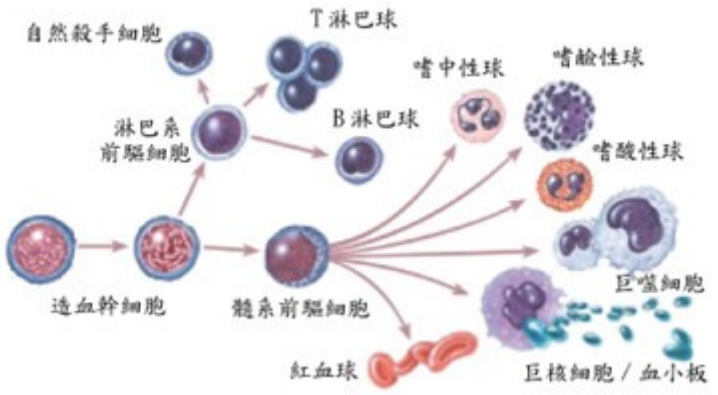
- 多重來源:骨髓　脂肪組織　羊水 臍帶血.....等
- 是一種多能幹細胞, 主要可分化為中胚層細胞, 如骨細胞, 肌肉細胞及脂肪細胞
- 可使用於多種疾病之治療, 但療效還有爭議

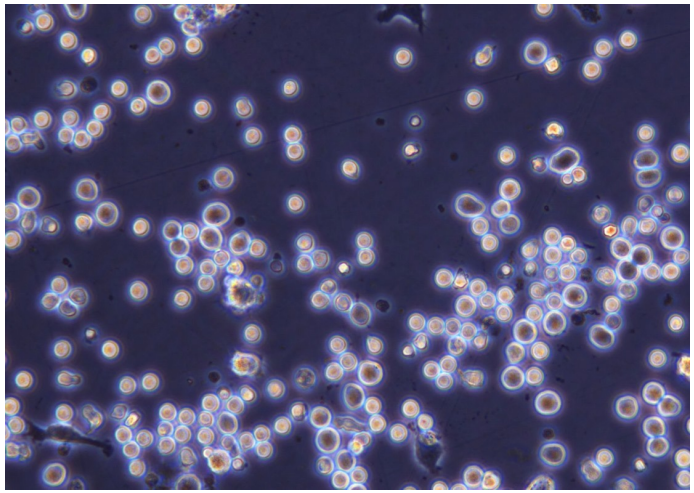




造血幹細胞

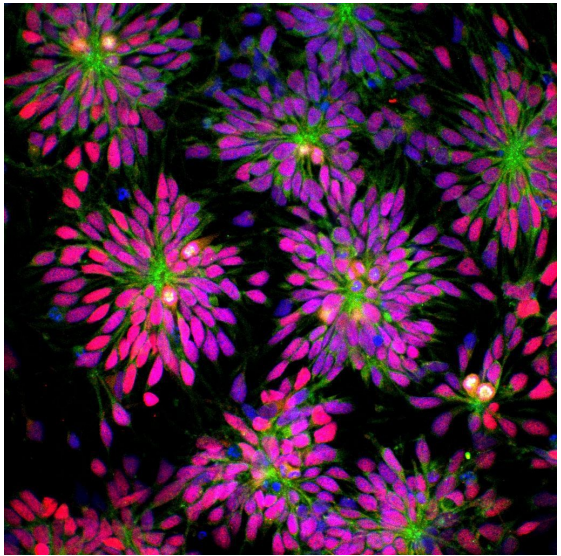
- 多數存在於骨髓，少數可於血液中發現。臍帶血中也被發現有大量的造血幹細胞
- 功能主要是用於補充我們體內所失去的血球細胞
- 可用於治療血液系統疾病
- 骨髓庫及臍帶血庫

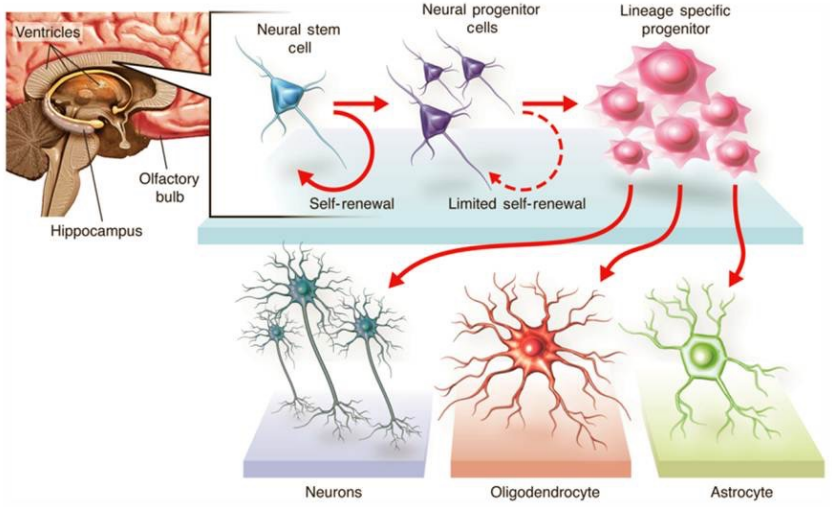




神經幹細胞

- 主要純在於大腦中的海馬迴及側腦室區域
- 可分化為神經元，神經膠細胞及寡樹突細胞
- 可用於治療中樞神經疾病

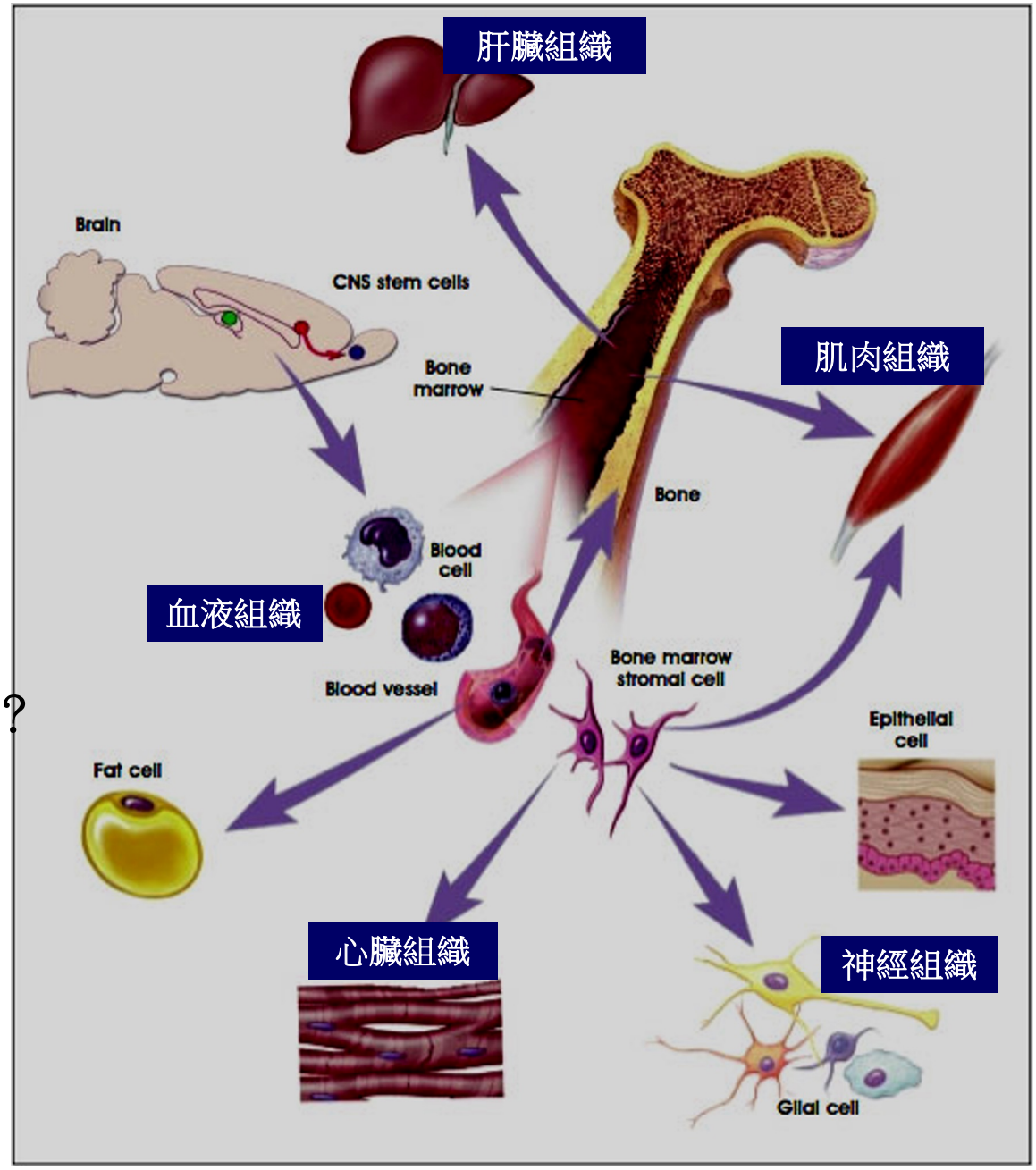




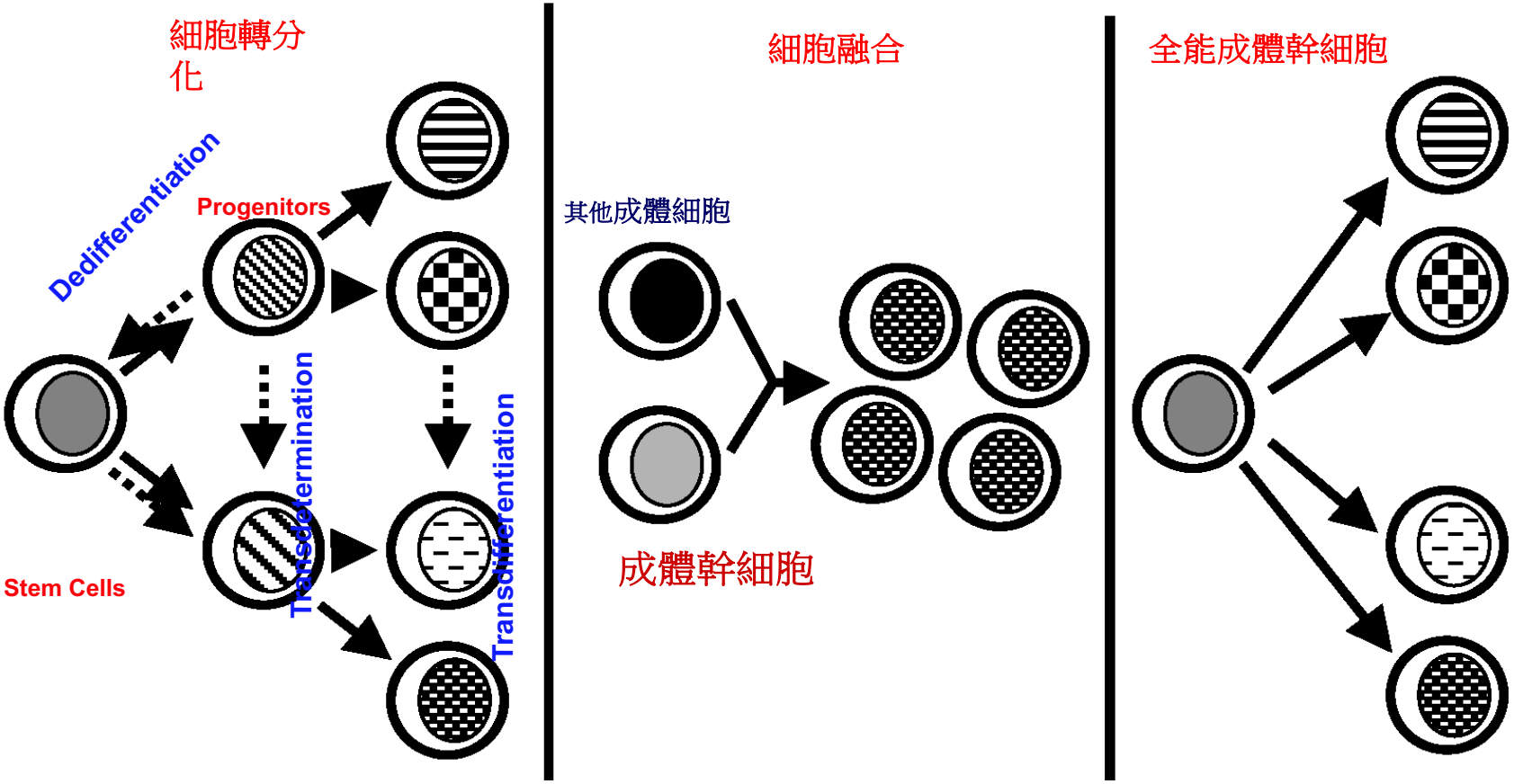
有趣的
成體幹細胞
可塑性

骨骼幹細胞 & 神經幹細胞

具有異想不到的分化潛能嗎？？



成體幹細胞可塑性之可能機轉



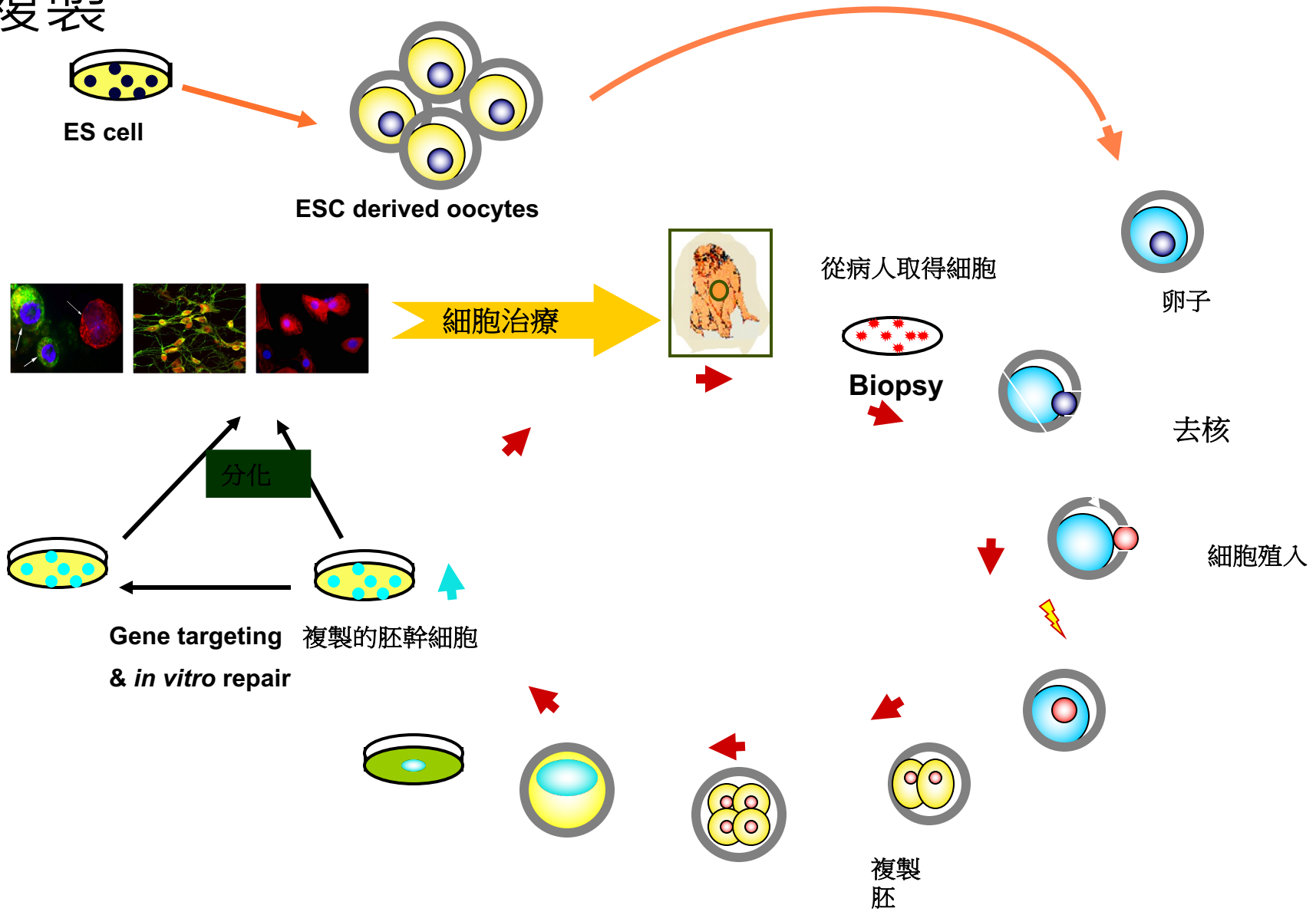
成體幹細胞與胚胎幹細胞之比較

- 較無道德爭議
- 數量稀少不易增殖
- 分化潛力較小
- 可自體移植不會引起免疫排斥反應

- 道德爭議
- 數量多可大量增殖
- 分化潛力較小
- 會引起免疫排斥反應

體細胞核轉植技術

克服細胞移殖排斥之方法--細胞核轉移及治療性複製



體細胞核轉植(複製)技術

愛丁堡皇家博物館


TIME
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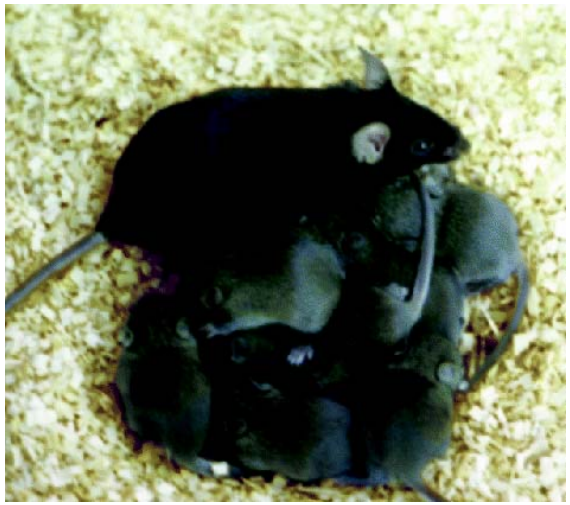
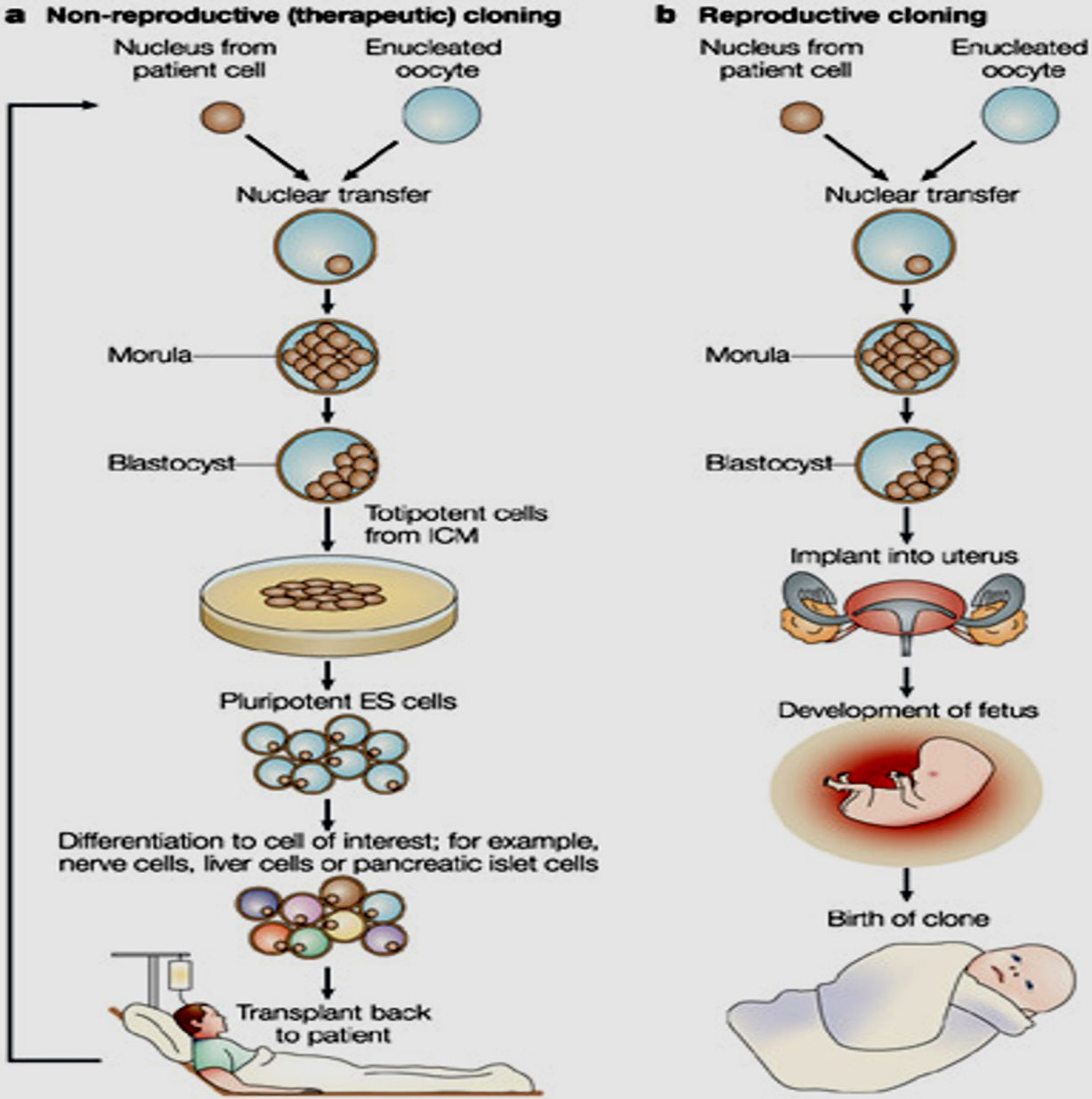
TECH AND COMPUTER NEWS
Will There Ever Be Another You?
A SPECIAL REPORT ON CLONING

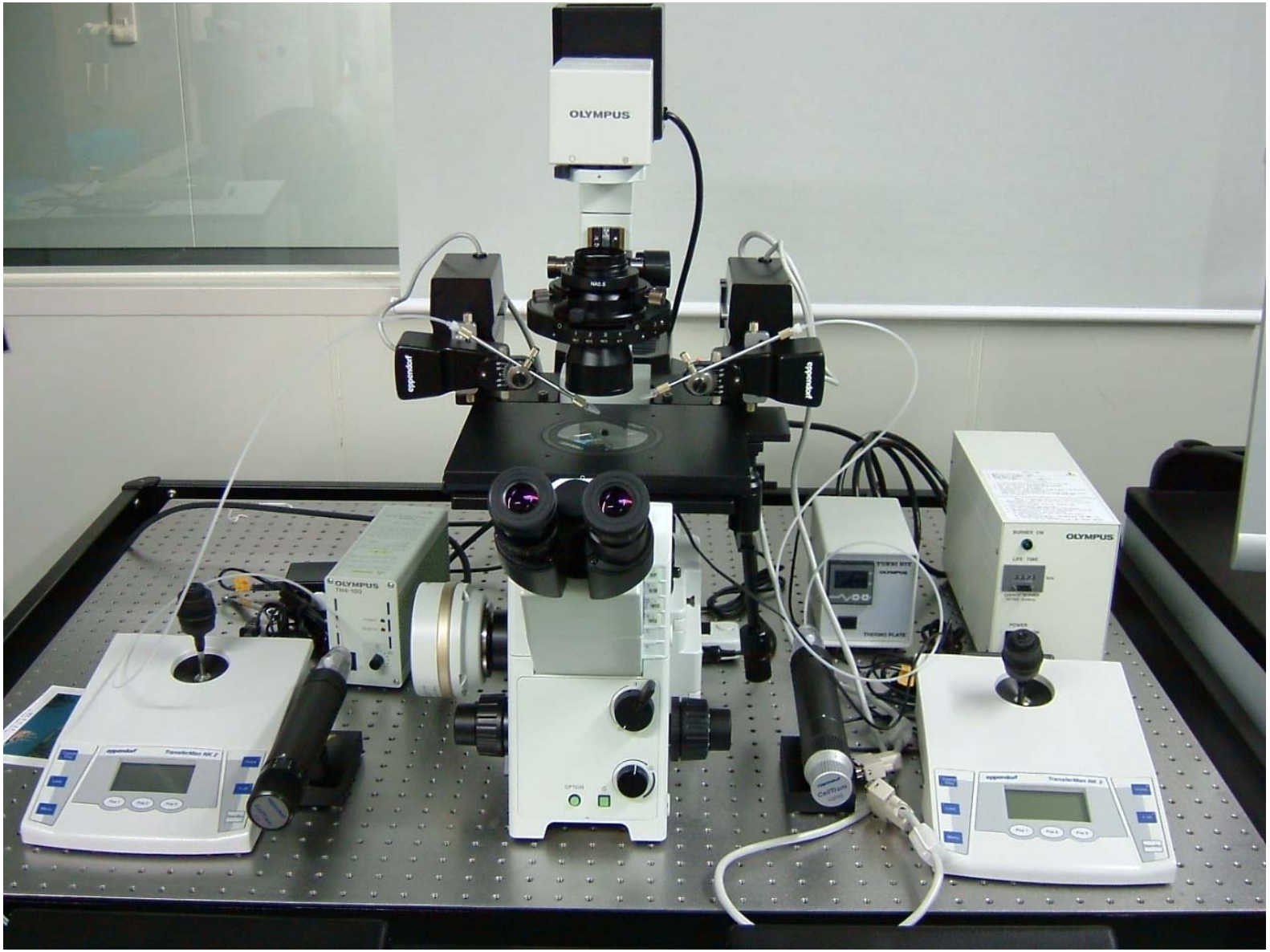



a Non-reproductive (therapeutic) cloning
Nucleus from patient cell
Enucleated oocyte
Nuclear transfer
Morula
Blastocyst
Totipotent cells from ICM
Pluripotent ES cells
Differentiation to cell of interest; for example, nerve cells, liver cells or pancreatic islet cells
Transplant back to patient
b Reproductive cloning
Nucleus from patient cell
Enucleated oocyte
Nuclear transfer
Morula
Blastocyst
Implant into uterus
Development of fetus
Birth of clone

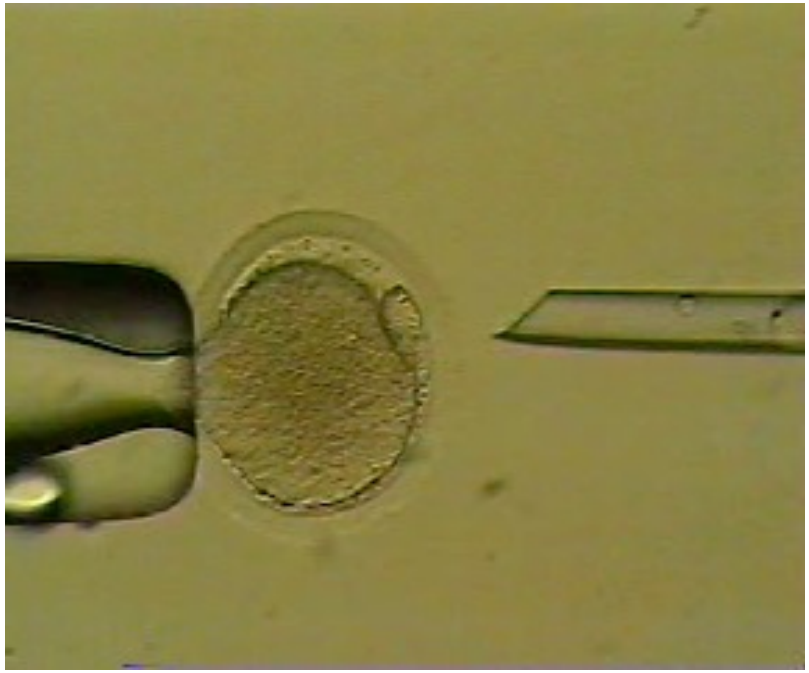
治療性複製

生殖性複製

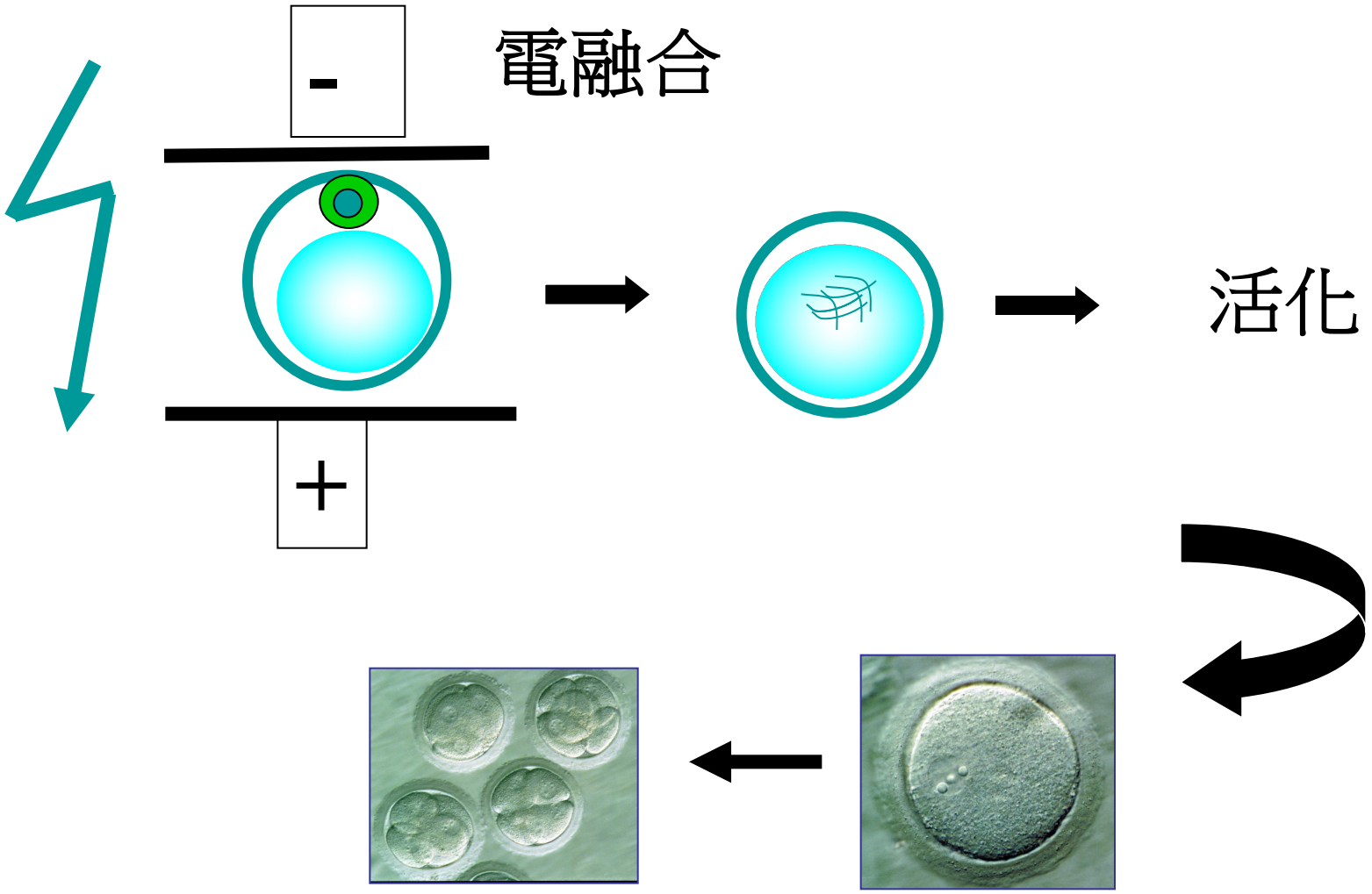
顯微操作器



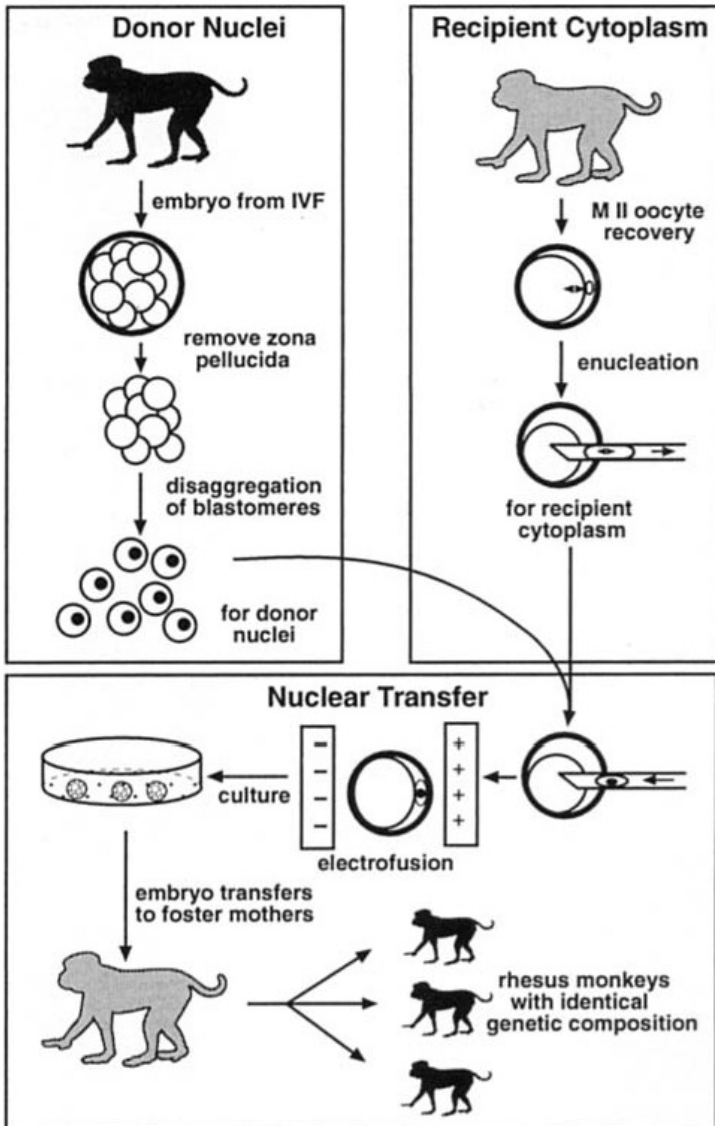
細胞核轉移技術



細胞核轉移技術

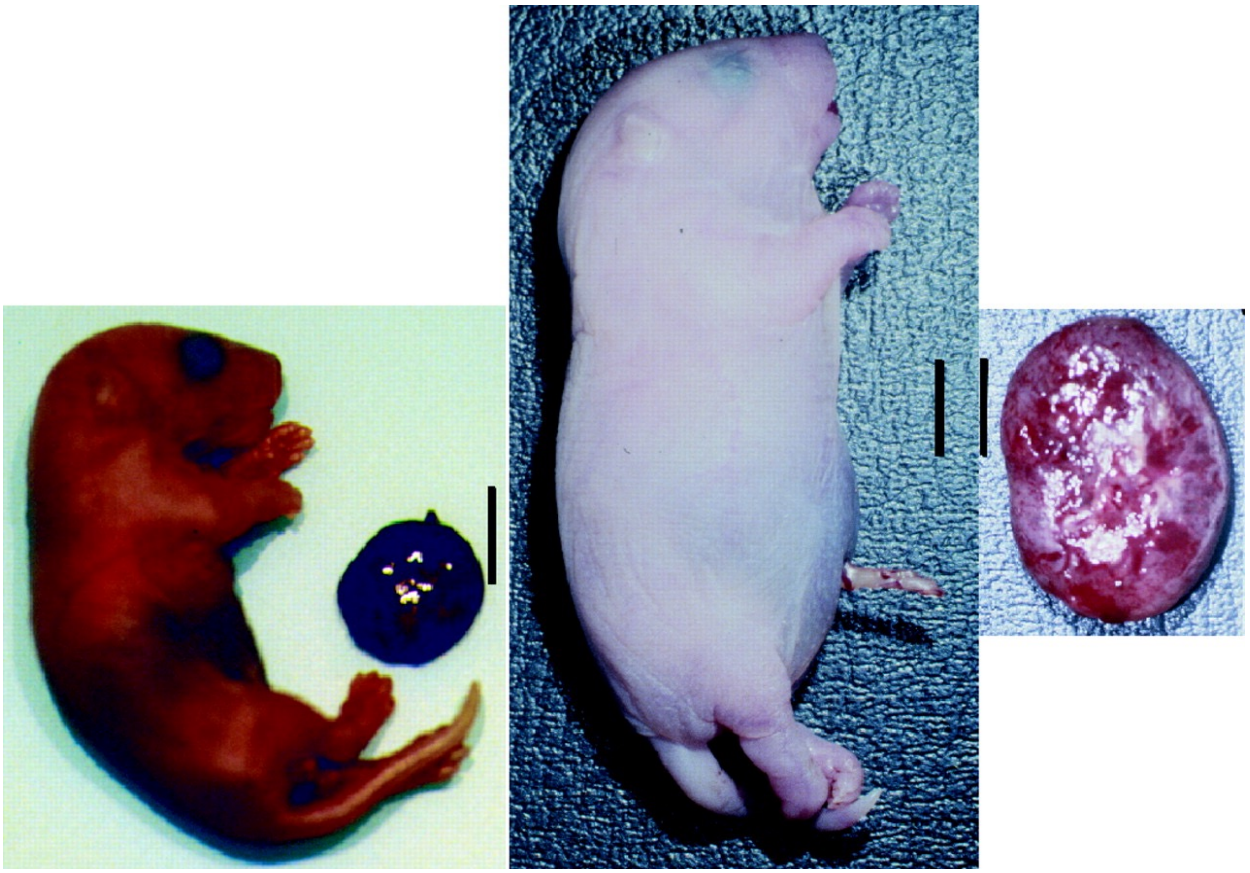


複製猴



Meng et al. (1997)Rhesus monkeys produced by nuclear transfer. Bio Repro. 57 (2)

複製動物通常有不同之異常



K. Eggan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 6209 (2001).

可以複製人嗎???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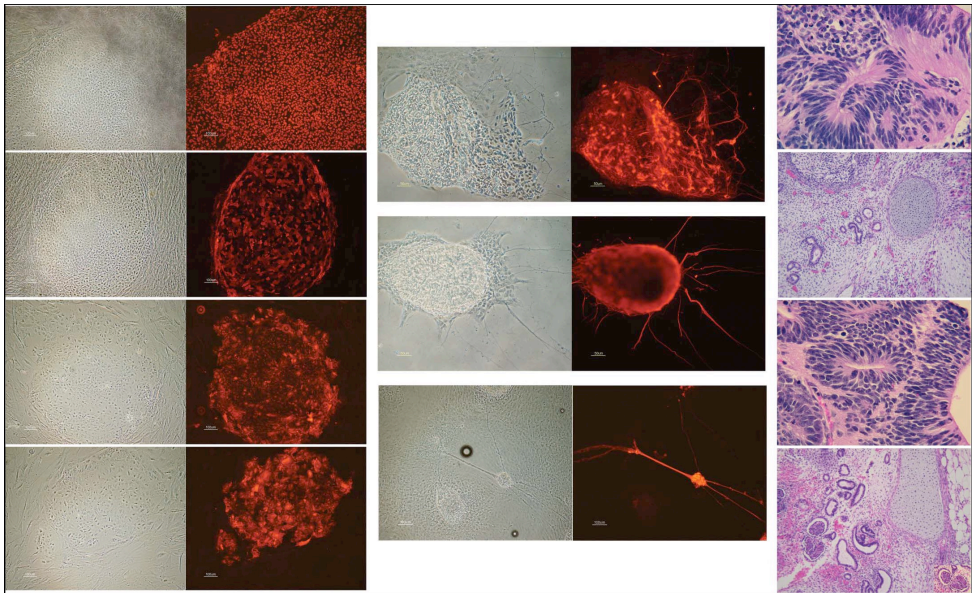
A SPECIAL REPORT ON CLONING


Newsweek
March 10, 1997 : $2.95
THE LINCOLN BEDROOM
Was the White House for Sale?
Can We Clone Humans?
By Sharon Begley

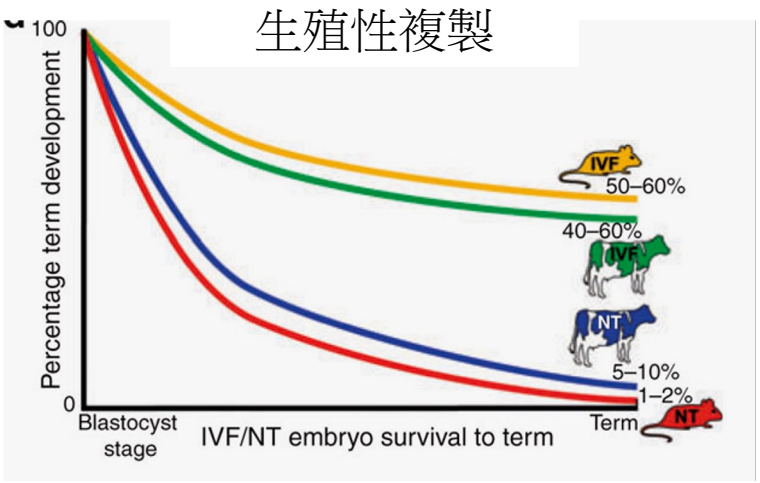
人類治療性複製之路

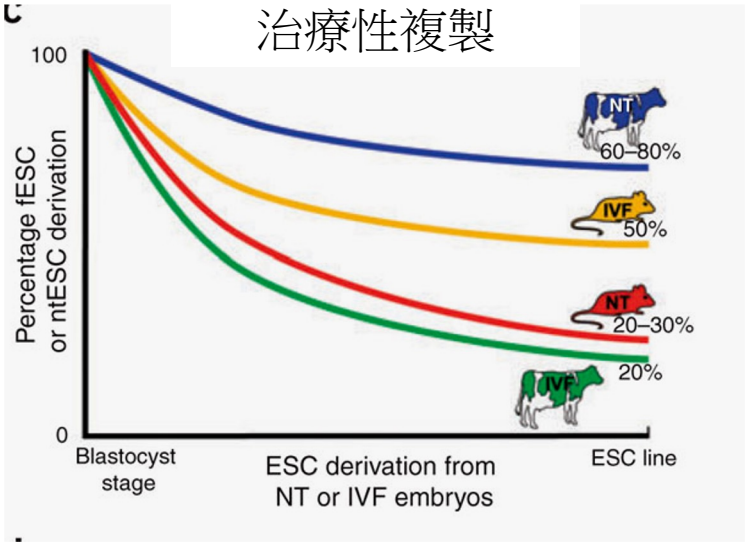
令人振奮的進展



2007年奧勒岡靈長類研究中心建立第一株恆河猴複製胚胎幹細胞

治療性複製的可行性





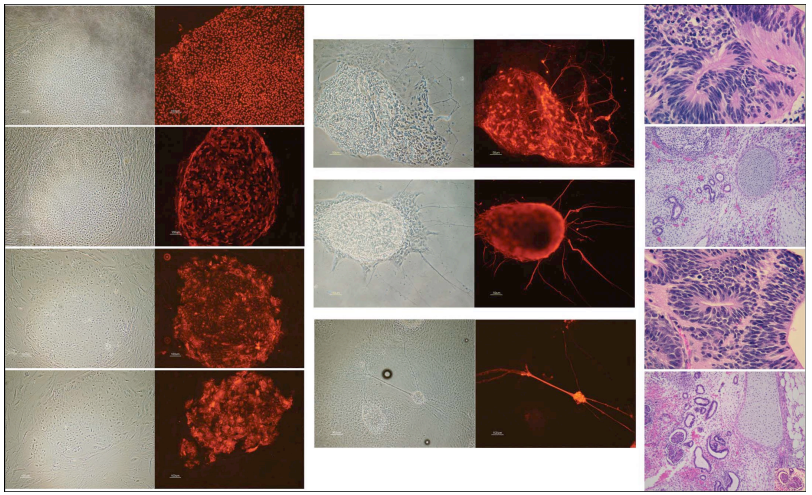
- 恆河猴治療性複製之效綠率低- 僅大約200分之1
- 執行須昂貴之儀器設備及專門技術人員
- 人類卵子取得困難
- 倫理爭議

***Nature Genetics* 39**, 295 - 302 (2007)

人類治療性複製之路

令人心碎的開始

令人振奮的進展



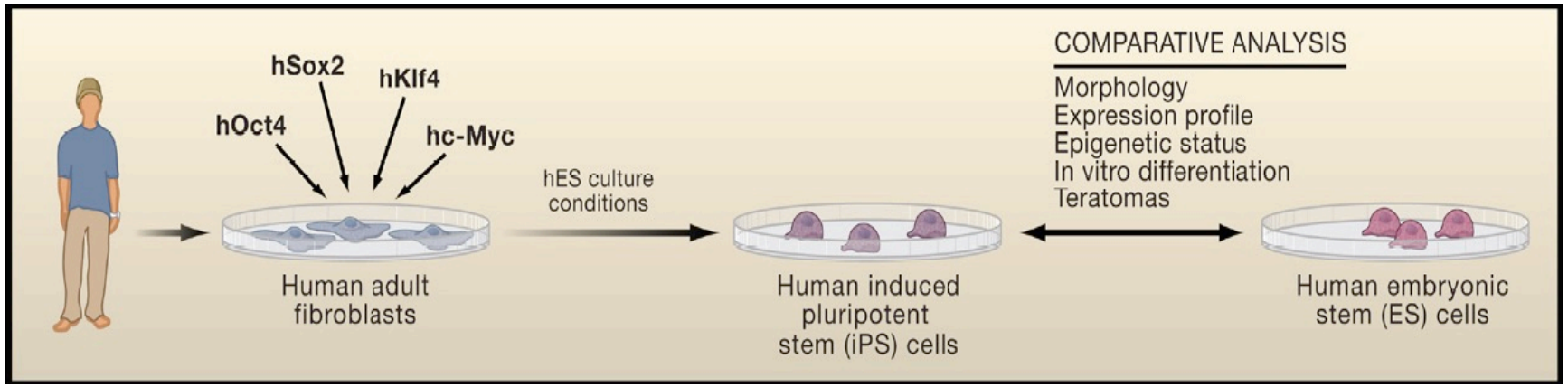
2007年奧勒岡靈長類研究中心建立第一株恆河猴複製胚胎幹細胞

2013年奧勒岡靈長類研究中心建立第一株人類複製胚胎幹細胞

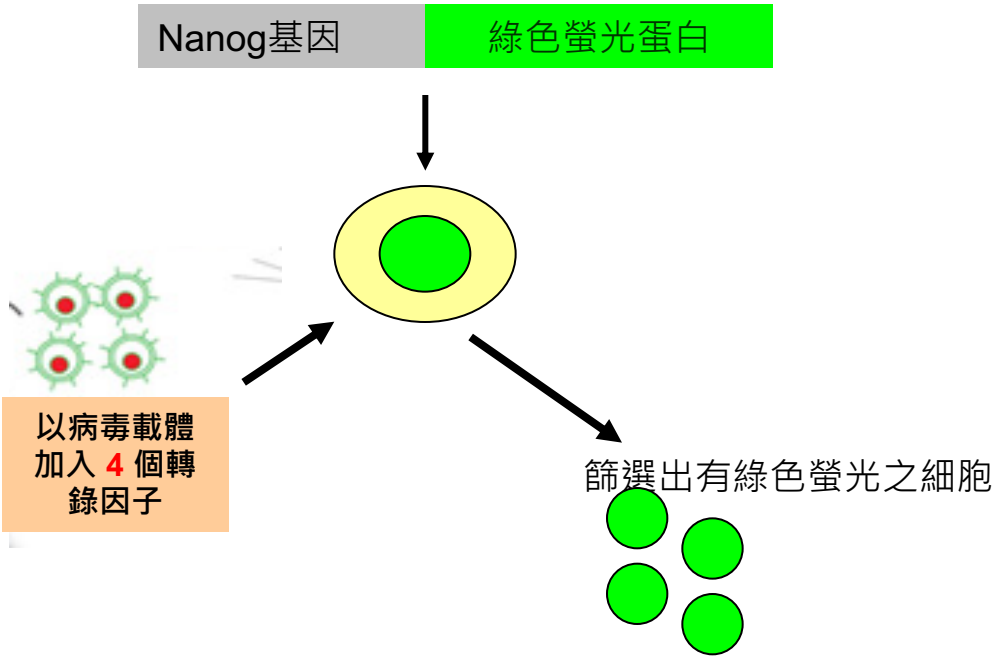
- 恆河猴治療性複製之效綠率低- 僅大約200分之1
- 執行須昂貴之儀器設備及專門技術人員
- 人類卵子取得困難
- 倫理爭議

誘導式全能性幹細胞

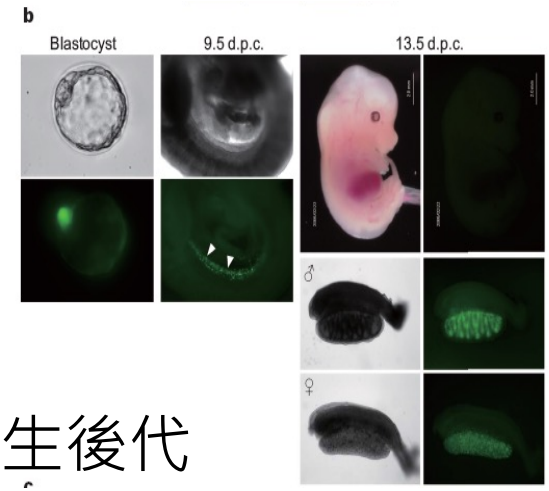
誘導式全能性幹細胞


hSox2
hKlf4
hOct4
hc-Myc
hES culture conditions
COMPARATIVE ANALYSIS
Morphology
Expression profile
Epigenetic status
In vitro differentiation
Teratomas
Human adult fibroblasts
Human induced pluripotent stem (iPS) cells
Human embryonic stem (ES) cells

經由Nanog報導基因
可篩選出具有生殖潛力之誘導式全能幹細胞

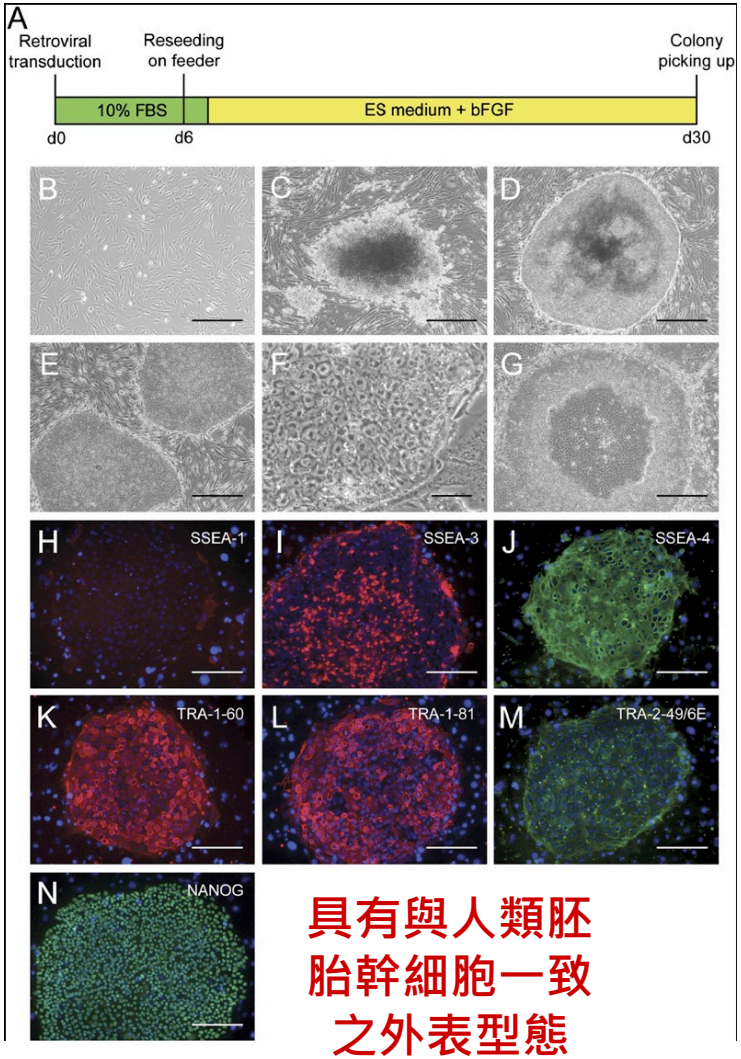


會貢獻至生殖組織



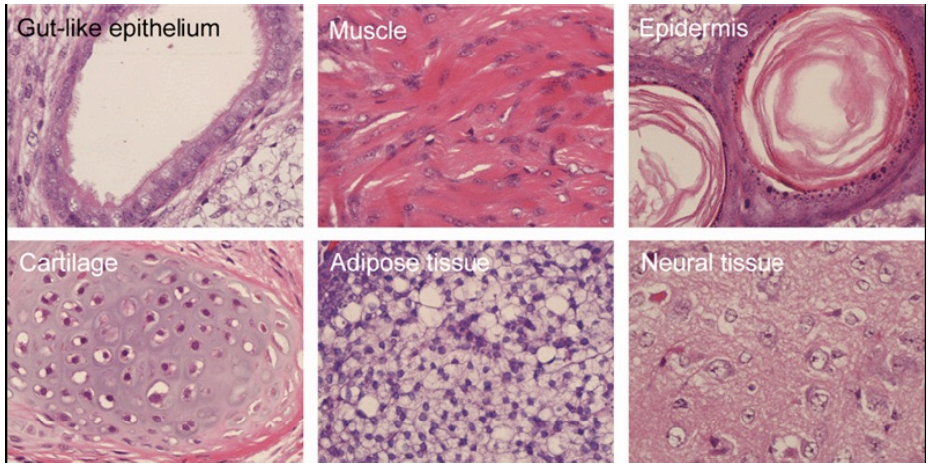
可產生後代

人類誘導式全能幹細胞之誕生

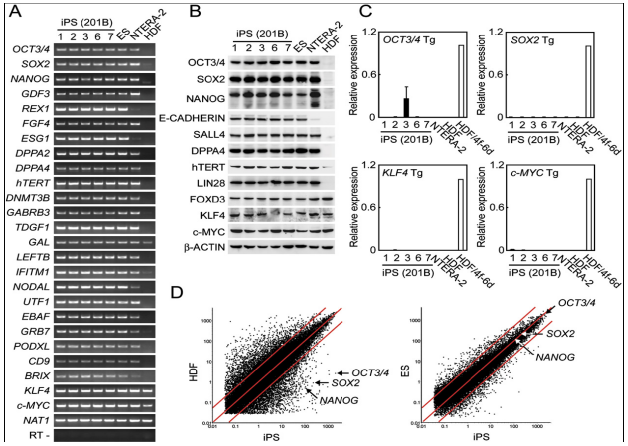


具有與人類胚胎幹細胞一致之外表型態

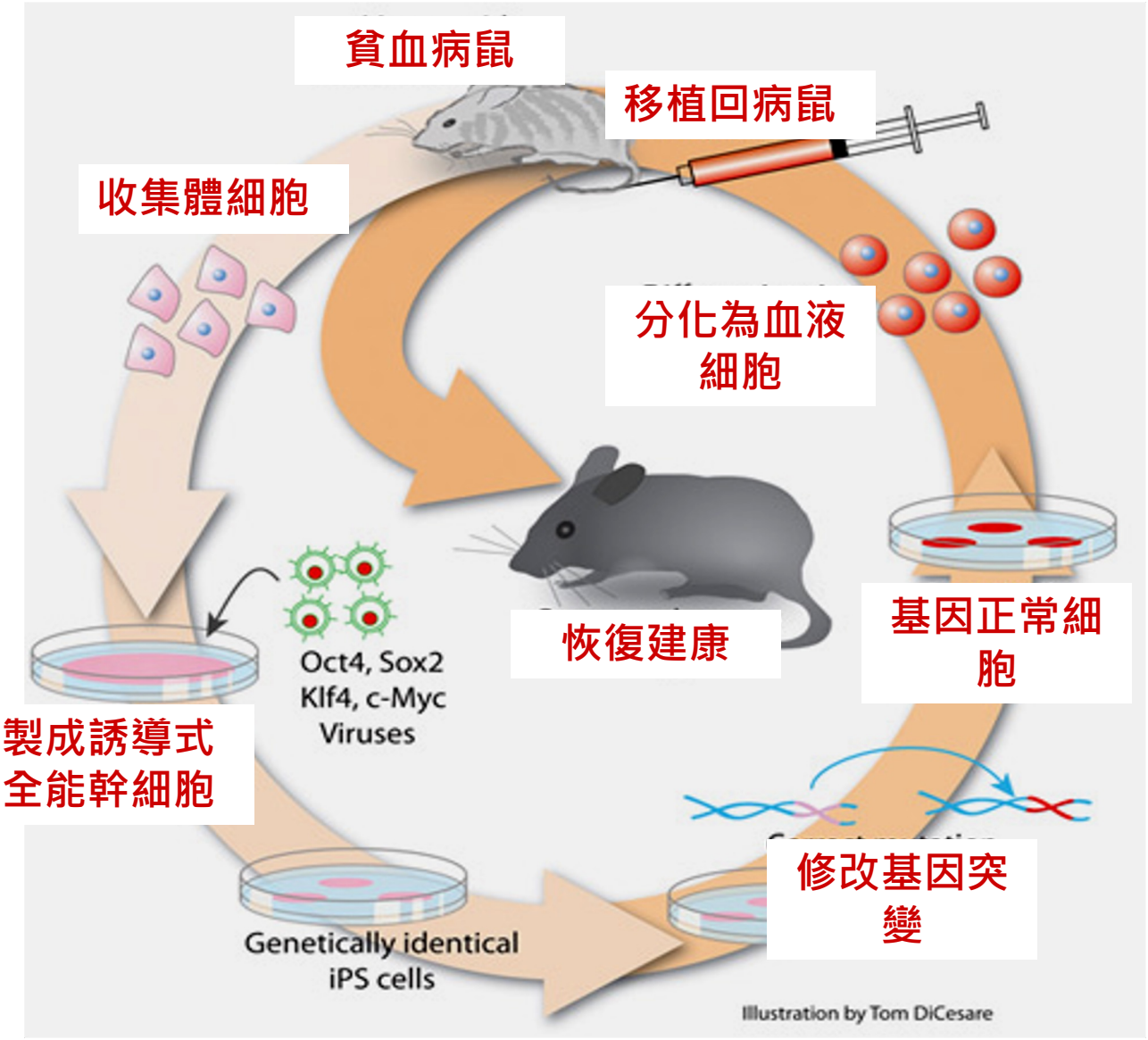
會形成畸胎瘤



具有與人類胚胎幹細胞一致之基因表現



誘導式全能幹細胞用於疾病治療之可能性



Science

誘導式全能幹細胞之問題 1

因**c-myc**基因之再活化而導致腫瘤之產生

導致誘導式全能幹細胞問題之可能原因

- 使用c-myc基因導致腫瘤之發生

  解決方法-----不使用c-myc基因

- 病毒載體隨機插入細胞基因體中，導致重要基因功能受到影響

  解決方法-----不使用病毒載體，而以其他方法將基因導入

ARTICLE

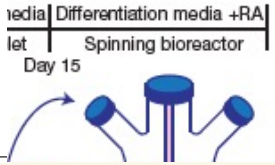
doi:10.1038/nature12517

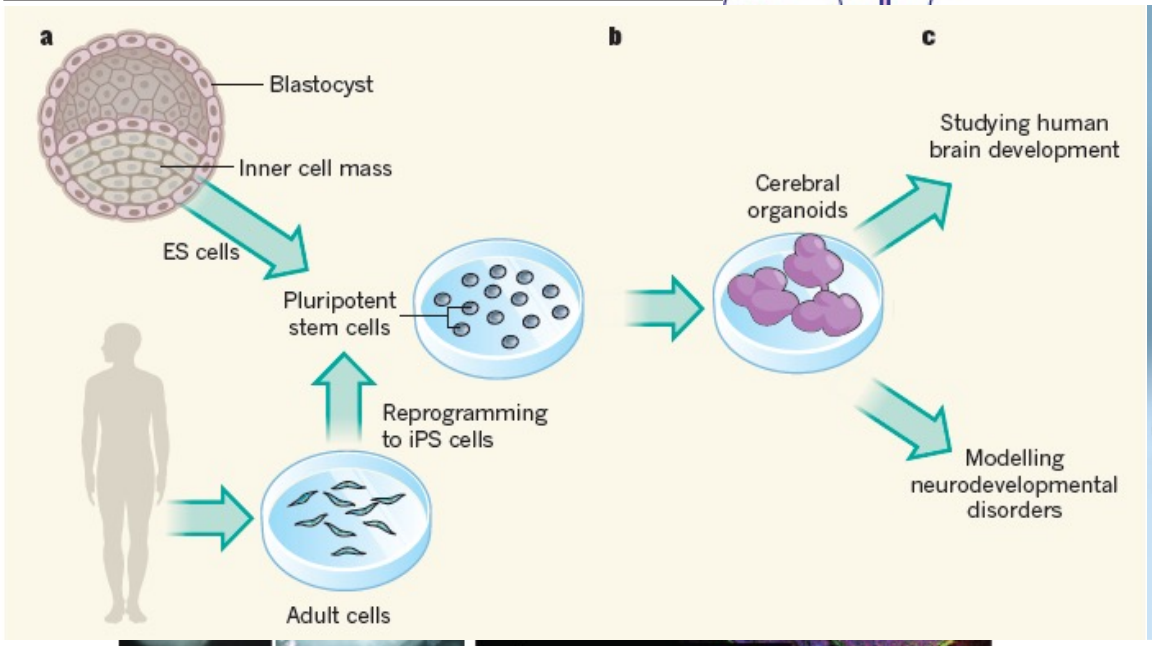
Cerebral organoids model human brain development and microcephaly

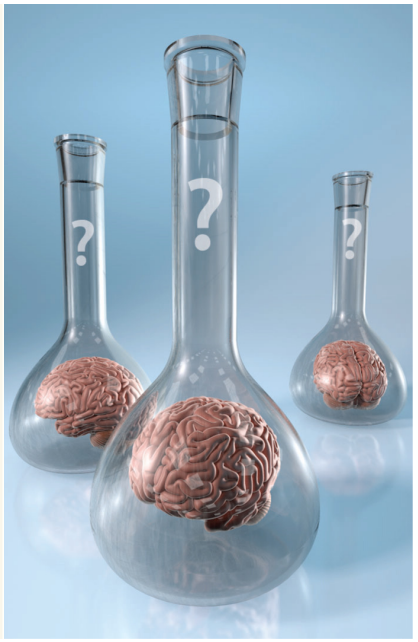
Madeline A. Lancaster[1], Magdalena Renner[1], Carol-Anne Martin[2], Daniel Wenzel[1], Louise S. Bicknell[2], Matthew E. Hurles[3], Tessa Homfray[4], Josef M. Penninger[1], Andrew P. Jackson[2] & Juergen A. Knoblich[1]

DEVELOPMENTAL NEUROSCIENCE

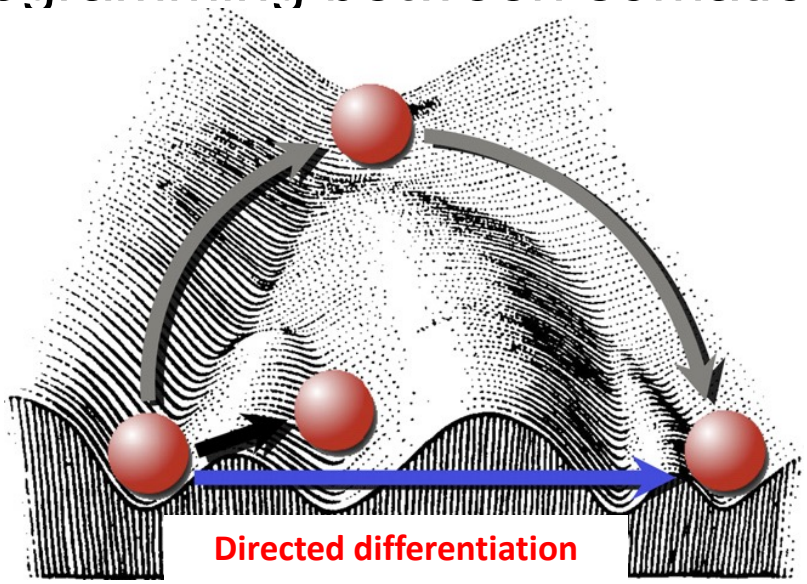
Miniature human brains







Direct reprogramming between somatic cell types



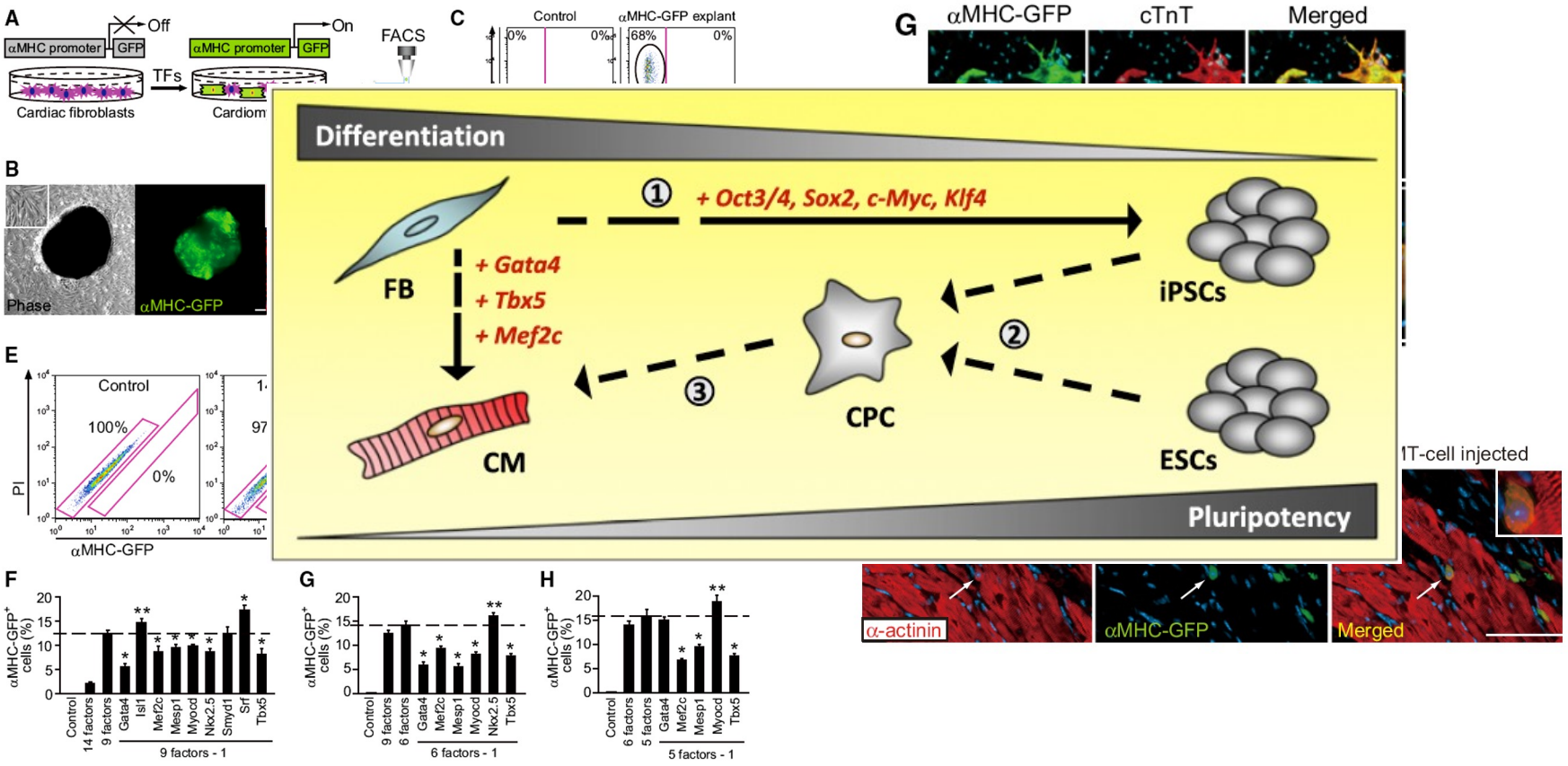
Targeted cells	Inducing factors	Reprogrammed cells	Efficiency	Reference
embryonic, adult fibroblasts	Oct3/4, Sox2, c-Myc, Klf4	pluripotent stem cells	<0.1%	(Takahashi and Yamanaka, 2006)
pancreatic exocrine cells	Ngn3, Pdx1, Mafa	pancreatic β cells	1.8-7.7% up to 19.5%	(Zhou et al., 2008)
embryonic, adult fibroblasts	Ascl1, Brn2, Myt1l	neurons	>20%	(Vierbuchen et al., 2010)
embryonic, adult fibroblasts	Gata4, Mef2c, Tbx5	cardiomyocytes	20%	(Ieda et al., 2010)

Cell stem cell, 7

Cell, 142

Direct Reprogramming of Fibroblasts into Functional Cardiomyocytesby Defined Factors

Masaki Ieda,1,2,3,6,* Ji-Dong Fu,1,2,3 Paul Delgado-Olguin,1,2,4 Vasanth Vedantham,1,5 Yohei Hayashi,1, Benoit G. Bruneau,1,2,4 and Deepak Srivastava1,2,3,*

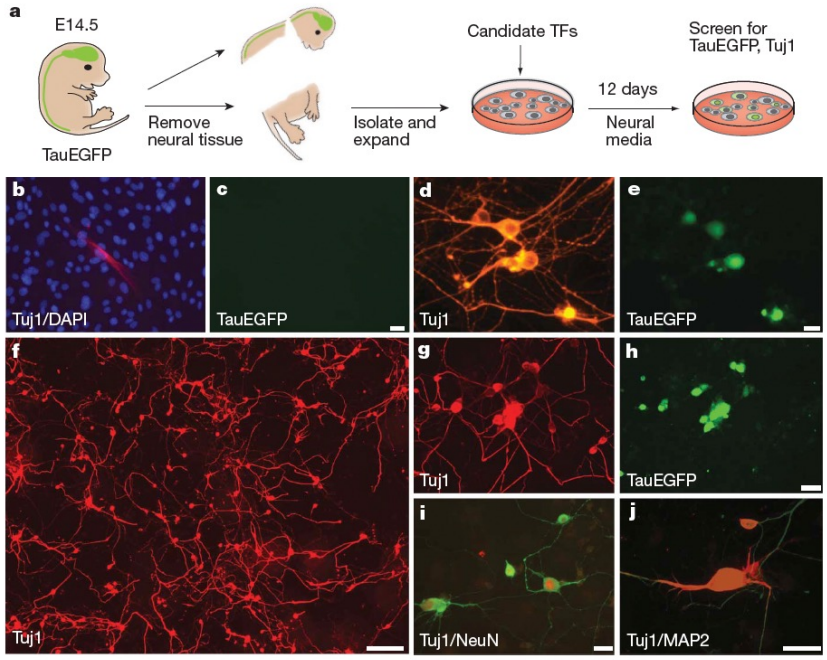


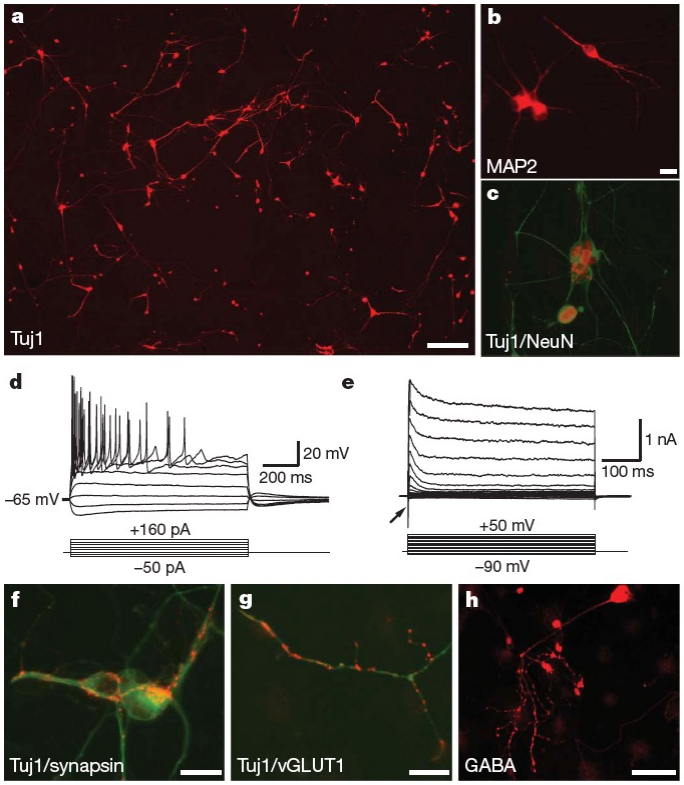
20 factors➔Gata4, Mef2c, Tbx5

Nature 463,

Direct conversion of fibroblasts to functional neurons by defined factorsThomas

Vierbuchen1,2, Austin Ostermeier1,2, Zhiping P. Pang3, Yuko Kokubu1, Thomas C. Südhof3,4 & Marius Wernig1,2





19 factors ➔Ascl1, Brn2, Myt1l

結論

- 成體幹細胞與胚胎幹細胞雖有不同之特性但一樣重要
- 幹細胞對未來之醫療將有極大之貢獻但目前仍有問題亟待克服
- 醫學科技之應用與倫理道德之為難