Microfluidic Devices for Stem Cell Isolation, Expansion and Differentiation

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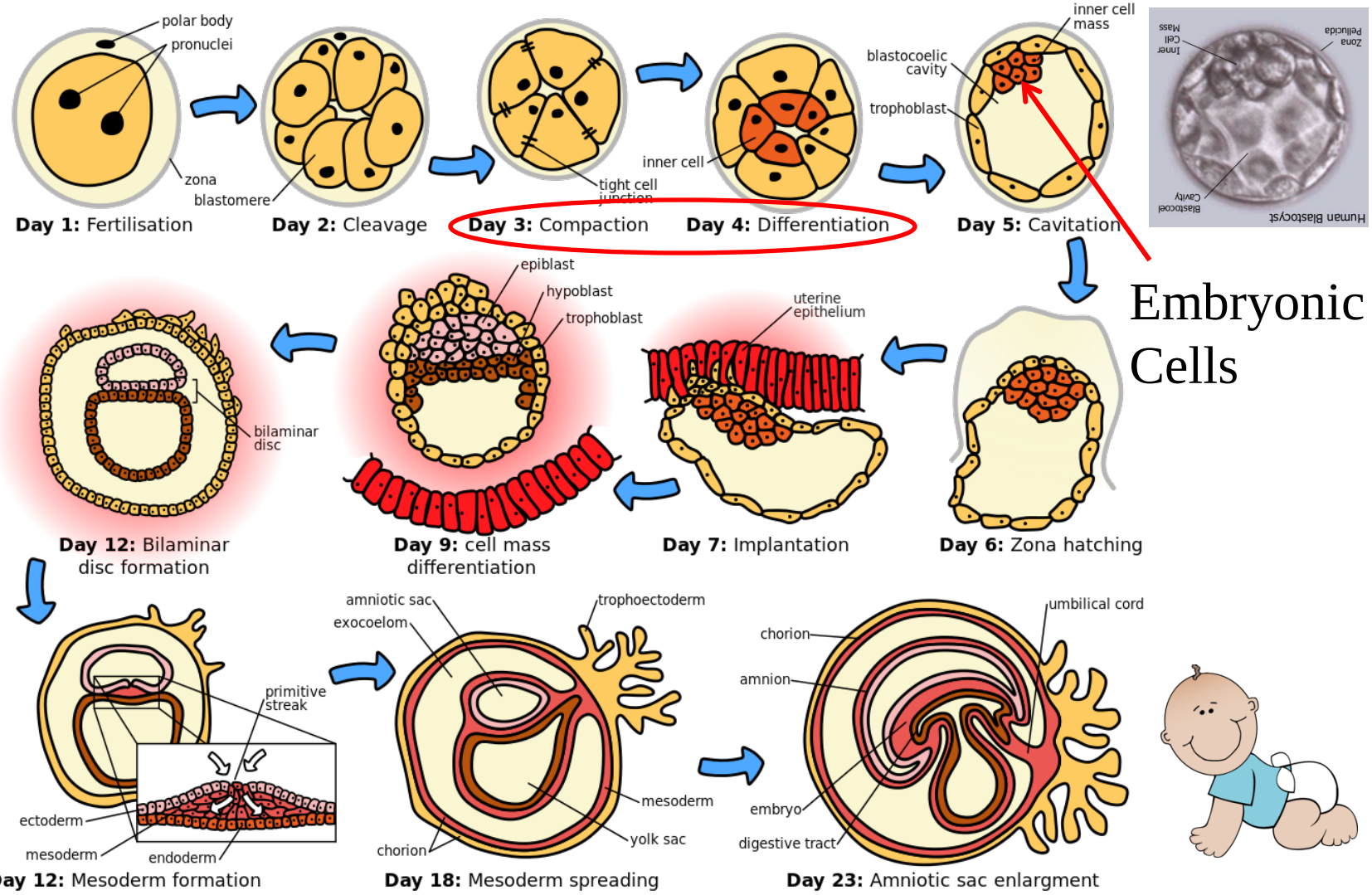
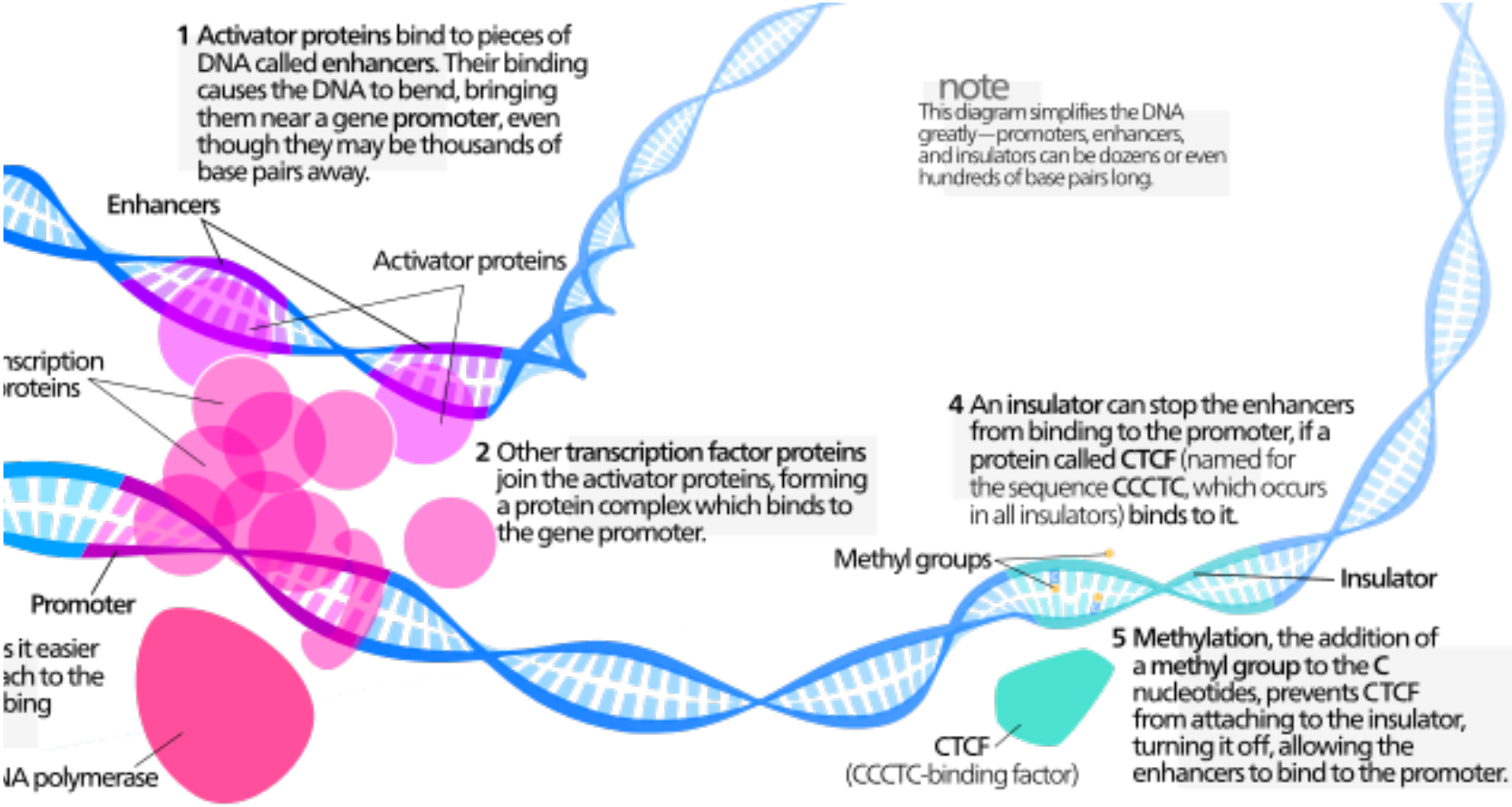
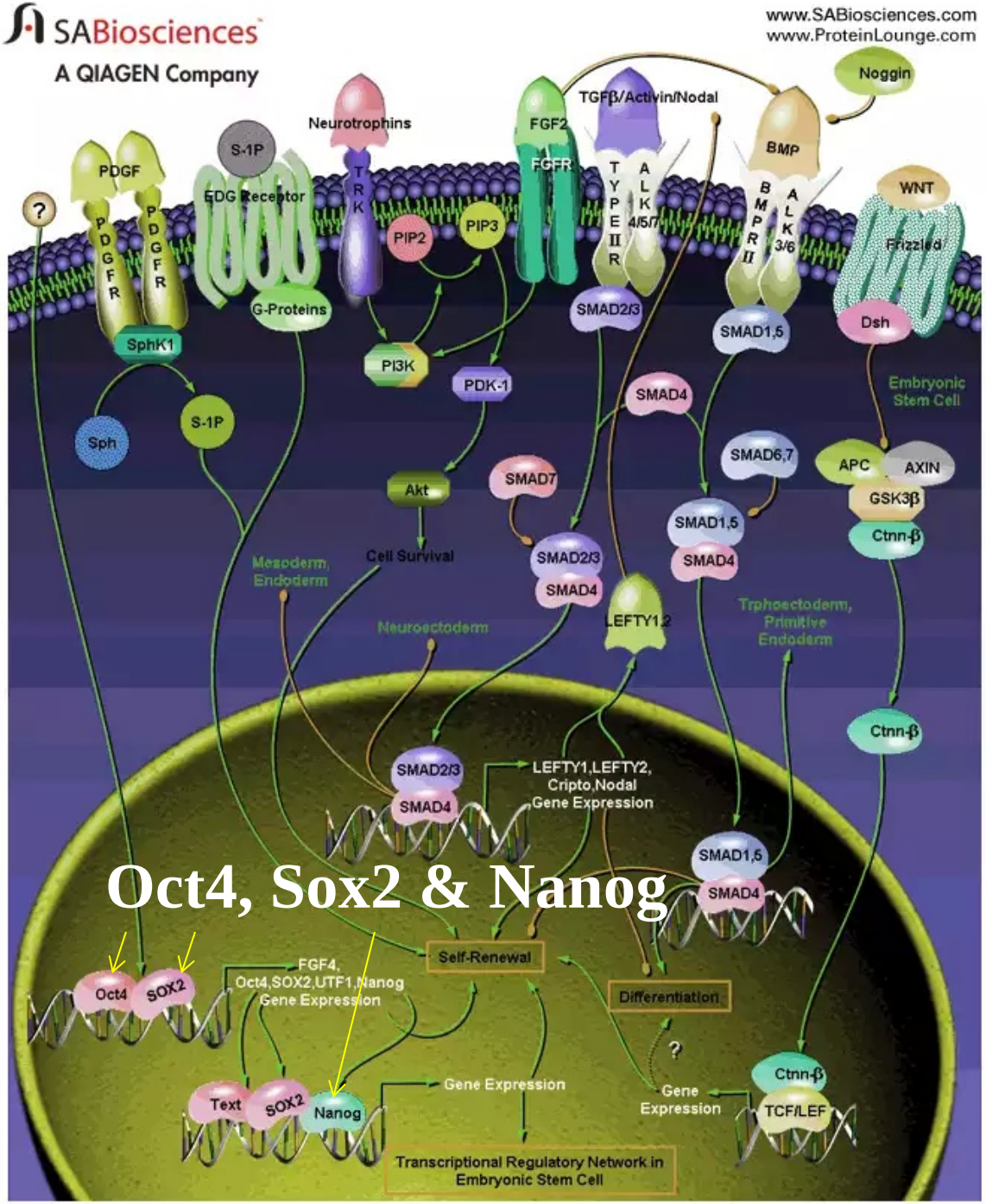
Early Development



Transcription Factors

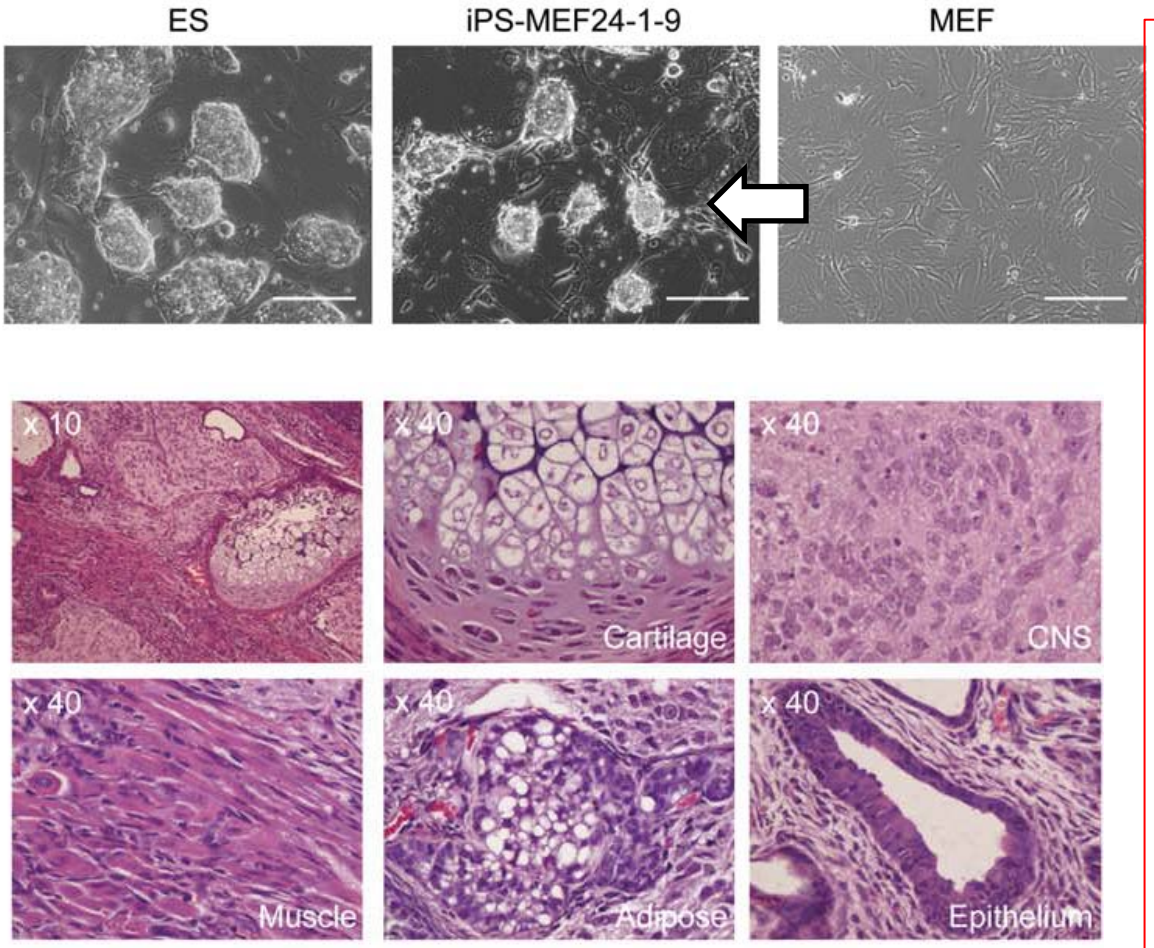




Transcription Regulatory Network in Embryonic Stem Cell

- Oct-3/4 plays a crucial role in maintaining pluripotency, which is exclusively expressed in pluripotent stem cells.
- Sox2 is associated with maintaining pluripotency similar to Oct-3/4.
- Nanog, along with Oct-3/4 and Sox2, is necessary in promoting pluripotency.

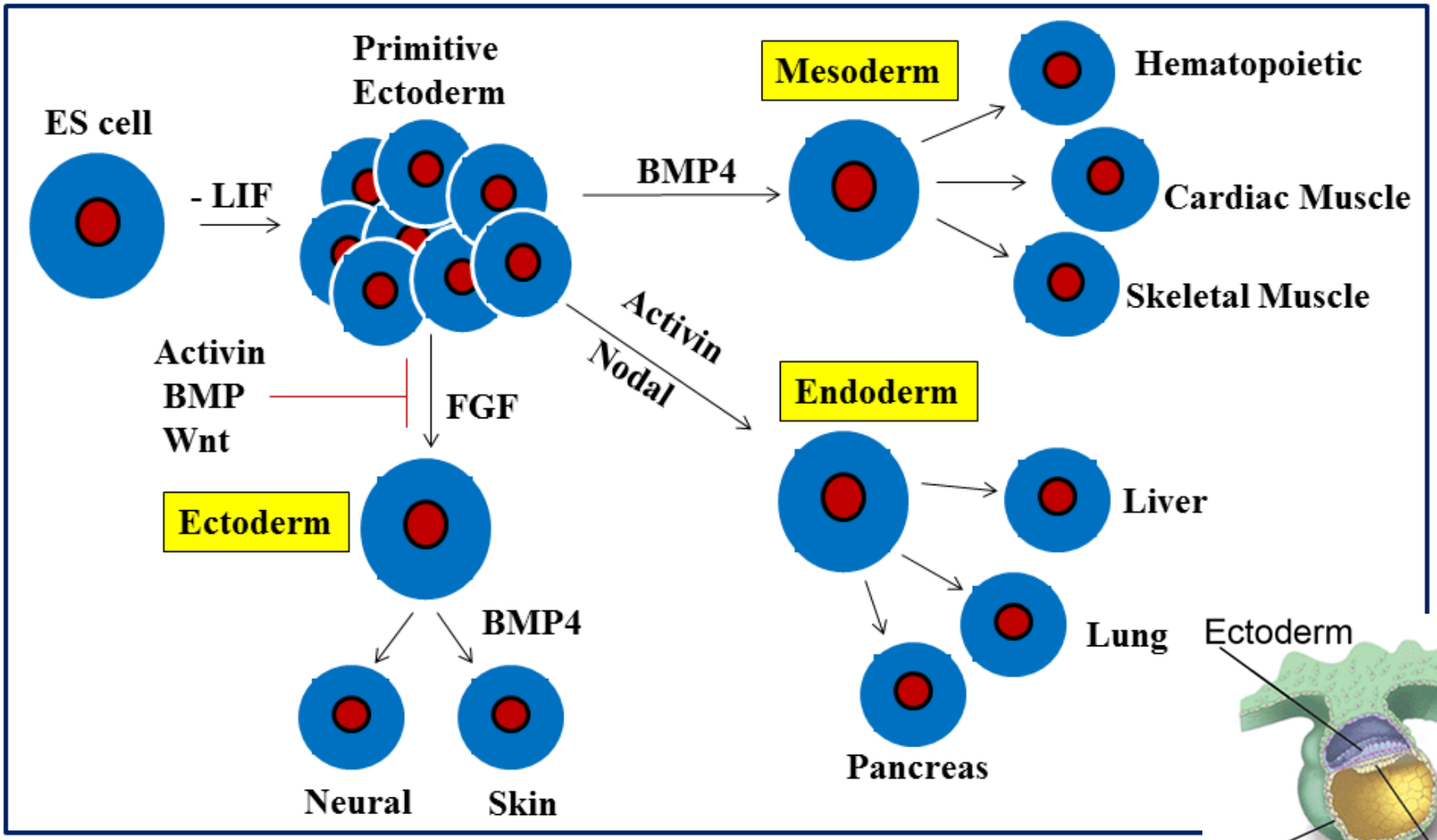
Induced pluripotent stem cell (iPSC)



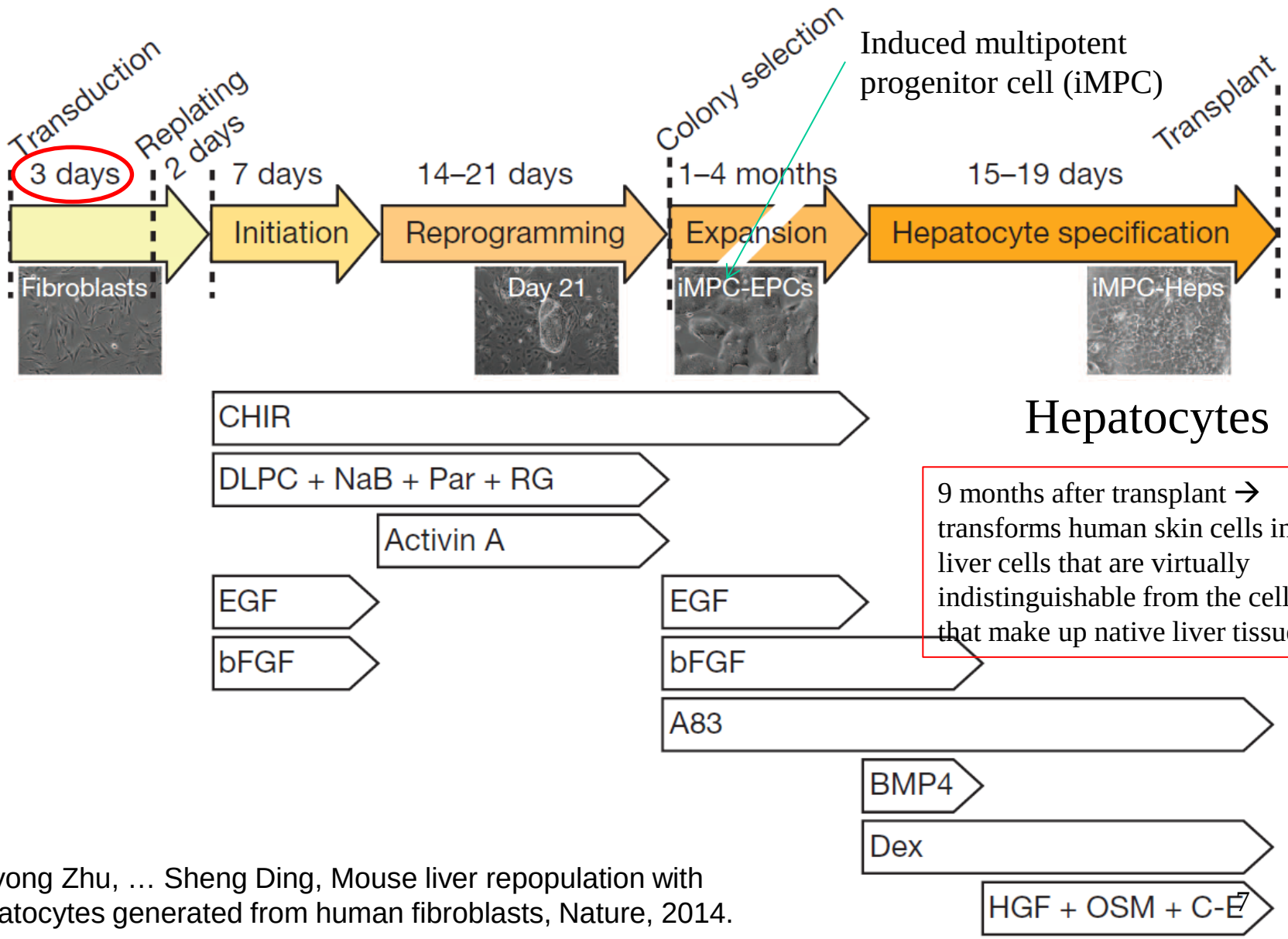
- **Shinya Yamanaka**
- **Mouse embryonic and adult fibroblast → iPSCs**
- **24 factors; retroviral transduction**
- **Oct3/4, Sox2, c-Myc, Klf4**
- **~ 16 days**
- **Extremely low yield**
- **Cancer cells!**
- **Nobel prize in 2012.**

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors, Kazutoshi Takahashi and Shinya Yamanaka, *Cell* 126, 663–676, August 25, 2006.

Pluripotent → Multipotent stem cells



Pluripotent → Multipotent stem cells



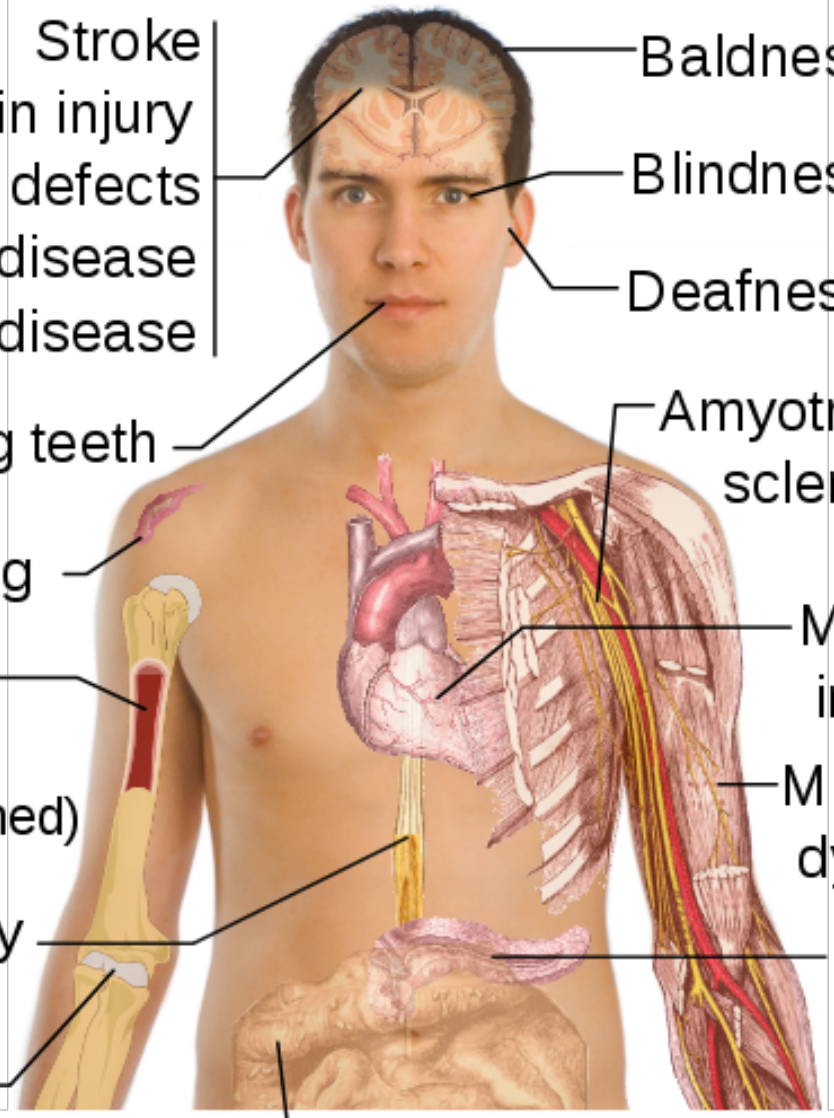
Hepatocytes

9 months after transplant → transforms human skin cells into liver cells that are virtually indistinguishable from the cells that make up native liver tissue.

Saiyong Zhu, … Sheng Ding, Mouse liver repopulation with hepatocytes generated from human fibroblasts, Nature, 2014.

Potential uses of

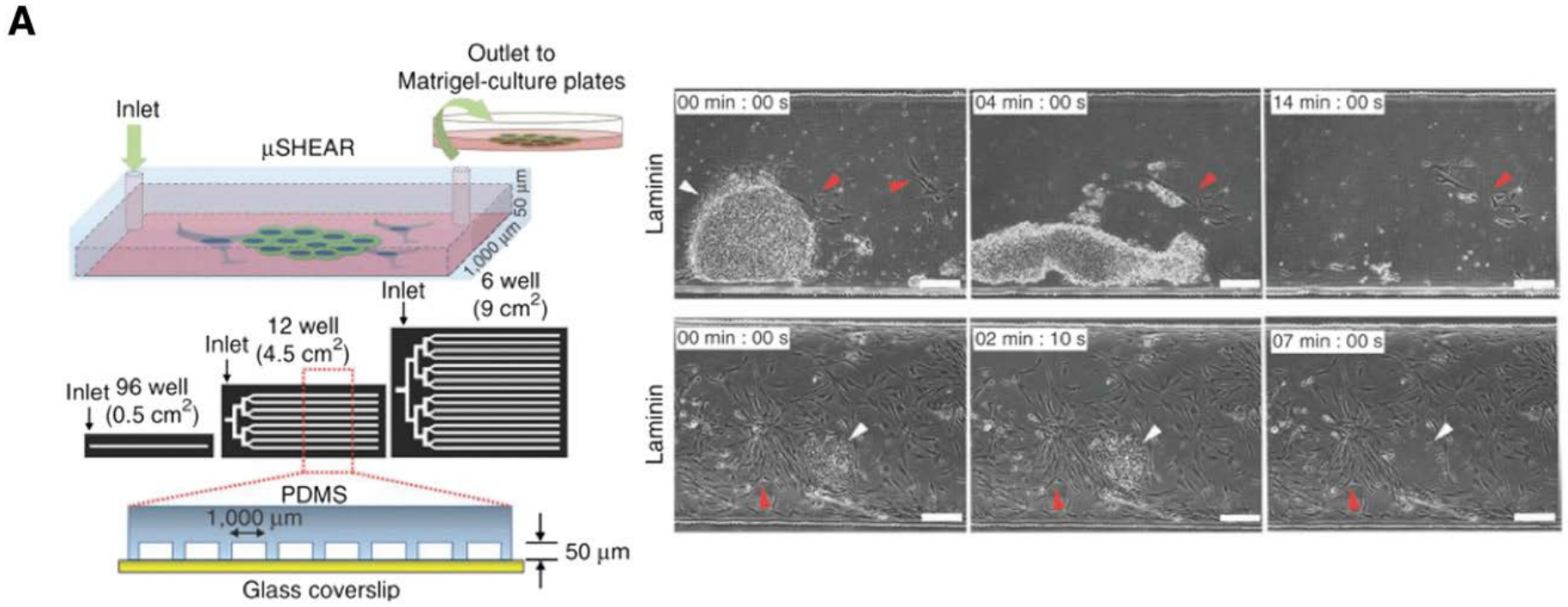
Stem cells



Stroke
Traumatic brain injury
Learning defects
Alzheimer's disease
Parkinson's disease
Missing teeth
Wound healing
Bone marrow transplantation (currently established)
Spinal cord injury
Osteoarthritis
Rheumatoid arthritis
Baldness
Blindness
Deafness
Amyotrophic lateral-sclerosis
Myocardial infarction
Muscular dystrophy
Diabetes
Crohn's disease
Multiple sites: Cancers

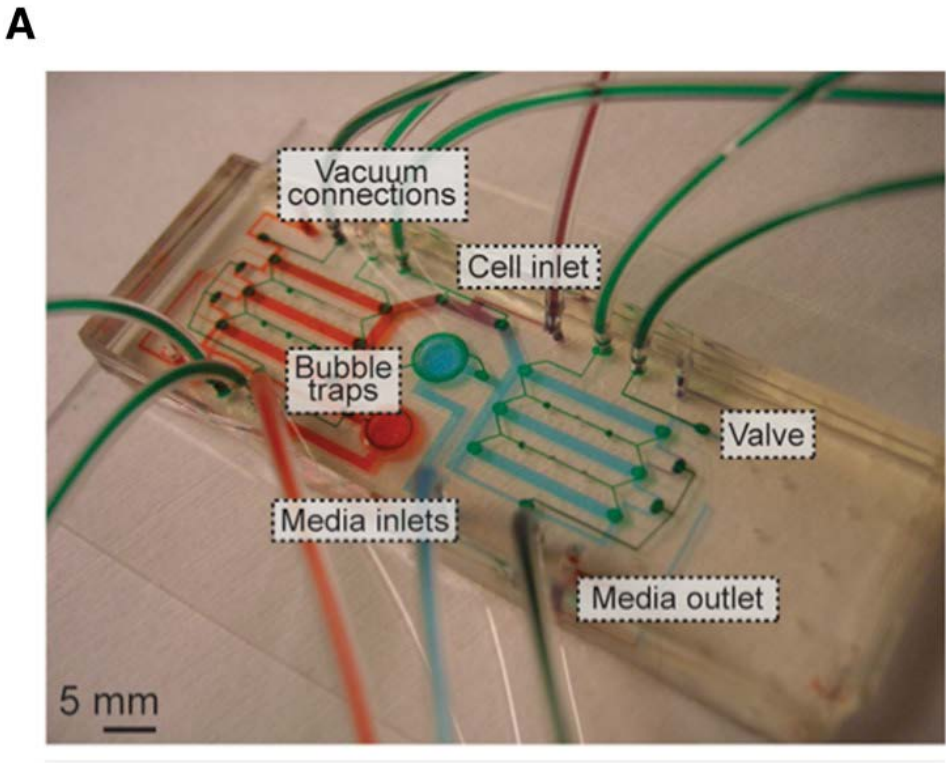
A system for shear force-based purification of human pluripotent stem cells from IMR90 fibroblasts.

The flow of culture medium over cells results in shear forces being applied to the cells. This isolation exploited approximately twofold differences in substrate adhesion strength between hPSCs and non-pluripotent cells.



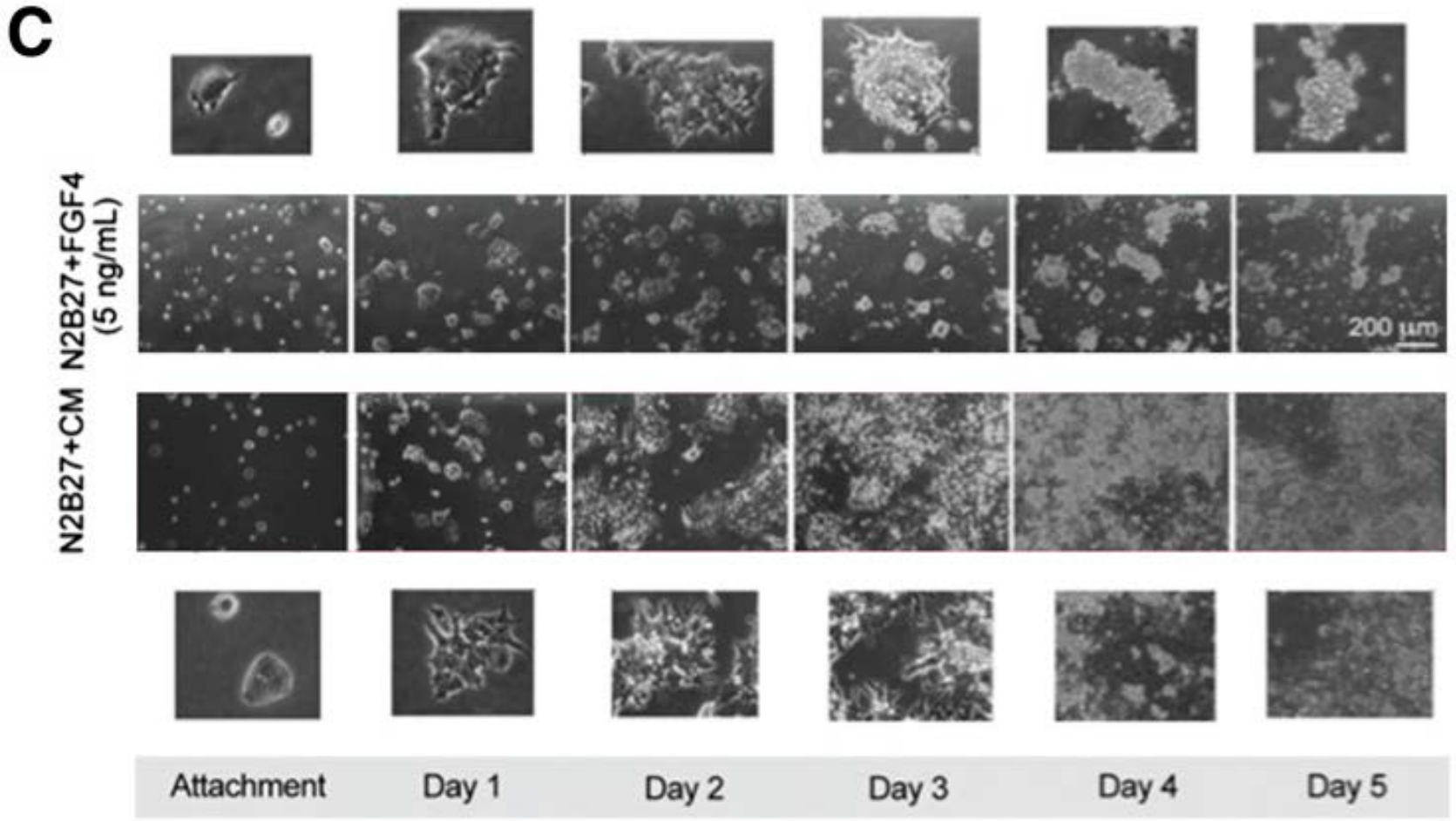
Concise Review: Microfluidic Technology Platforms: Poised to Accelerate Development and Translation of Stem Cell-Derived Therapies; Titmarsh et al., *Stem Cells Trans Med* 2014, 3:81-90

A microfluidic chip for perfusion culture of cells

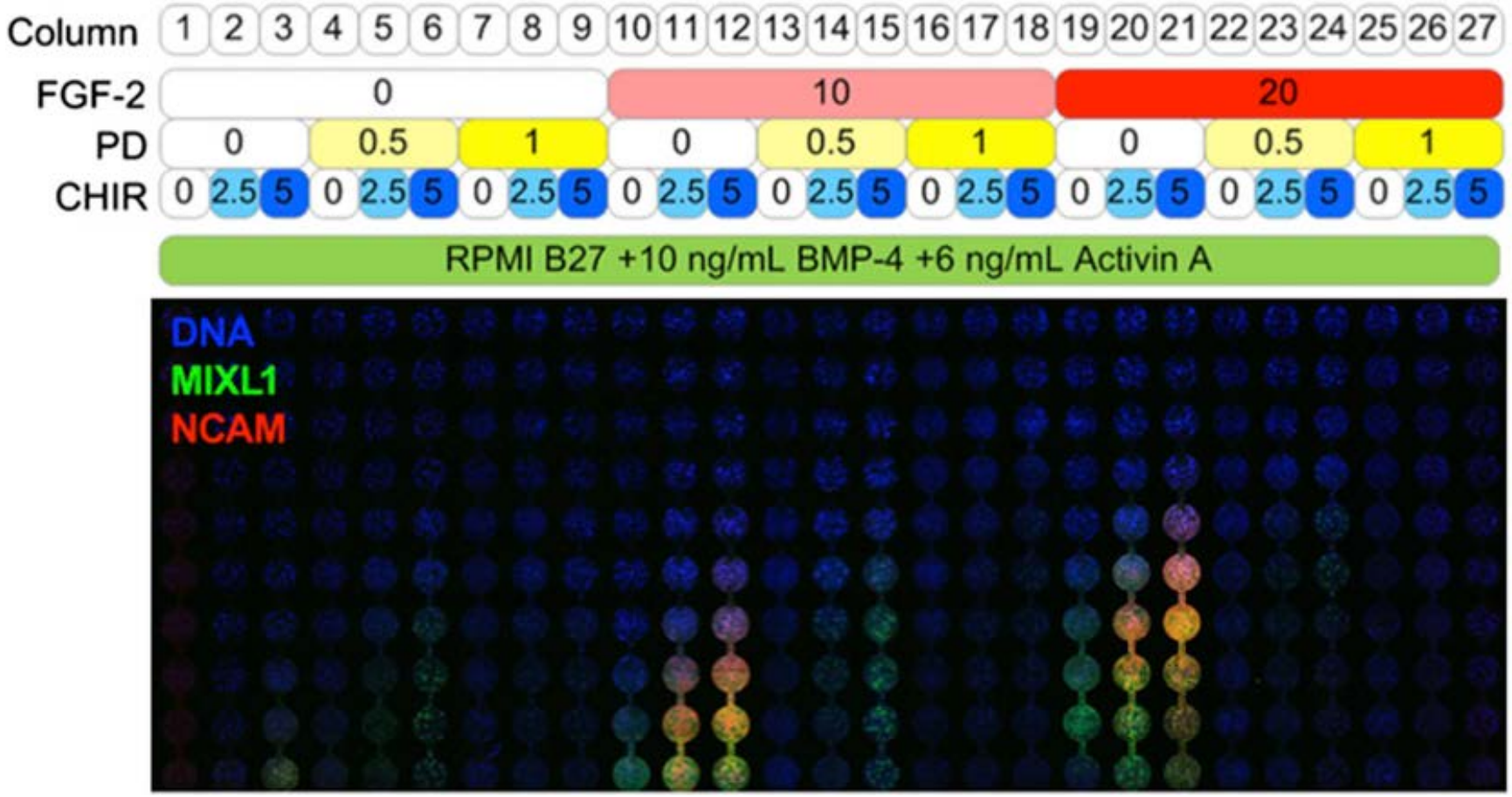


One intrinsic advantage of microfluidic technology compared with traditional cell culture and analysis platforms is the precision with which fluid flow maybe manipulated. This permits unrivalled regulation, spatially and temporally, of both the biophysical parameters (e.g., shear stress due to the convective flow of medium) and biochemical parameters (e.g., nutrient and growth factor level variations due to medium turnover rates) of the cellular microenvironment for implementing cell-based assays and optimizing stem cell culture and differentiation.

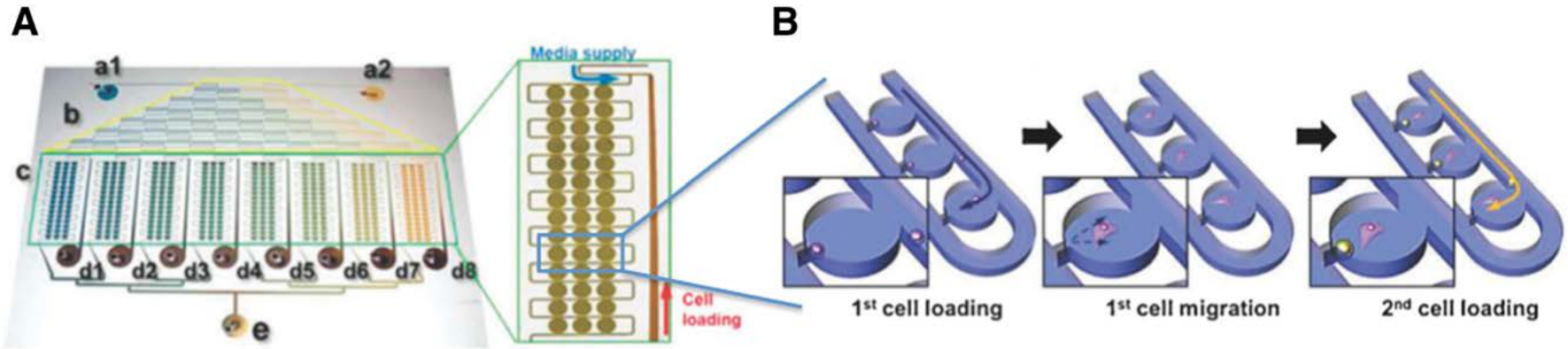
Continuous microfluidic perfusion of culture medium is used to examine differentiation of mouse embryonic stem cells into Sox1-positive neuroectoderm.



Acontinuous-flowmicrobioreactor arraywasused to generate various combinations of FGF-2, the MEK inhibitor PD0325901, and the Wnt activator CHIR99021



An example of a microfluidic coculture system to study cell-cell interactions at the single-cell level.



Microfluidic technologies add insight to cell therapy processes