Microfluidic Devices for Stem Cell Isolation, Expansion and Differentiation

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Early Development

Day 1: Fertilisation
Day 2: Cleavage
Day 3: Compaction
Day 4: Differentiation
Day 5: Cavitation

Day 6: Zona hatching
Day 7: Implantation
Day 9: Cell mass differentiation
Day 12: Bilaminar disc formation
Day 18: Mesoderm spreading
Day 23: Amniotic sac enlargement
Transcription Factors

1. Activator proteins bind to pieces of DNA called enhancers. Their binding causes the DNA to bend, bringing them near a gene promoter, even though they may be thousands of base pairs away.

2. Other transcription factor proteins join the activator proteins, forming a protein complex which binds to the gene promoter.

3. An insulator can stop the enhancers from binding to the promoter, if a protein called CTCF (named for the sequence CCCTC, which occurs in all insulators) binds to it.

4. Methyl groups prevent CTCF from attaching to the insulator, turning it off, allowing the enhancers to bind to the promoter.

5. Methylation, the addition of a methyl group to the C nucleotides, prevents CTCF from attaching to the insulator.
Oct4, Sox2 & Nanog

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 \(\rightarrow\) iPSCs
- 24 factors; retroviral transduction
- Oct3/4, Sox2, c-Myc, Klf4
- \(~16\) days
- Extremely low yield
- Cancer cells!
- Nobel prize in 2012.

Pluripotent → Multipotent stem cells

ES cell

- LIF

Activin
BMP
Wnt

FGF

BMP4

Primative Ectoderm

Ectoderm

Mesoderm

Hematopoietic
Cardiac Muscle
Skeletal Muscle

Endoderm

Liver
Lung
Pancreas

Neural
Skin
Pluripotent → Multipotent stem cells

Induced multipotent progenitor cell (iMPC)

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

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
Multiple sites: Cancers

Crohn's disease
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.

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 may be 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.
A continuous-flow microbioreactor array was used 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

Cell Isolation
- Novel isolation strategies
- Control at single cell resolution

Cell Expansion & Differentiation
- Factor screening
- Process parameter optimization
- Control of autocrine/paracrine signalling effects

Cell Characterization
- Cell-based assays of identity and potency
- Real-time assessment of cell proliferation, differentiation state, and function
- Biochemical and biophysical property characterization

Cell Delivery
- Cell encapsulation
- Point of care patient cell processing
- Advanced material processing

Post-delivery Monitoring
- Point of care diagnostics
- Real-time sample-to-answer testing