Human Pluripotent Stem Cell Research for Regenerative Medicine and Drug Discovery

Our Multidisciplinary Academia-Industry Collaboration Project in Japan

Norio Nakatsuji
Professor and Founding Director
Institute for Integrated Cell-Material Sciences
Kyoto University
Cell-Material Integration for Stem Cell Research

- Embryo
  - Cell_growth
  - Reprogramming
  - iPS cells
  - ES/iPS cells
  - ES/iPS Cells
  - Cell Growth
  - Differentiation
  - Cell-based Therapy
  - Cell Biology Tools
  - Disease Model Study
How to deliver safe and effective stem cell therapy to many patients at affordable cost

Key Targets

• **Large-scale** production of **high-quality** stem cells (e.g. human pluripotent stem cells)

• **Robust** and **reliable** production of **high-quality** differentiated cells for cell transplantation therapy

• All steps and procedures at **lower cost** with reliable **quality control**
Our Academia-industry collaboration in Japan (2011-2014)

Development of evaluation machines and reagents

Defined/robust medium with low molecular compounds

Development of cell culture substrate and materials

Development of cryo-preservation method

Development of cryo-preservation method

Development of large scale culture method

(1) Development of defined/robust mass culture and cryo-preservation technology

(2) Development of quality evaluation system of human stem cells

(3) Development of quality control and stable supply technology of human stem cells

Automated large-scale stem cell culture system

Quality evaluation system

Automated large-scale stem cell culture system

Human ES cells

Human iPS cells

Accurate cell shipment system

Imaging

Tokyo Univ. Prof. Nakauchi

Kyoto Univ. Prof. Nakatsuji

NIBIO Dr. Mizuguchi

Keio Univ. Prof. Okano

Chiba Univ. Prof. Iwama

Tokyo Univ. Prof. Nakauchi
Multidisciplinary Research of Human Pluripotent Stem Cells

1. Novel 3D culture system for large-scale production of human pluripotent stem cells

2. Cytokine-free and xeno-free chemical induction of cardiomyocyte differentiation
Development of large-scale culture and quality control system for human pluripotent stem cell lines

1000ml
500ml
50ml
70ml
30ml
10ml
T175 Flask
T75 Flask
100mm Dish

1~10 L

1~10 L
>>10 L
Expression of pluripotency markers in more than 98% cells after > 50 passages.

Frozen section

Otsuji et al. *Stem Cell Reports* (April 2014)
From conventional adherent 2D culture to 3D sphere culture for large-scale production of human pluripotent stem cells.
Detailed morphological study of the hPSC spheres with electron microscopy by Heuser Lab shows homogenous undifferentiated cell population.

Otsuji et al. *Stem Cell Reports* (April 2014)

TEM by Dr. Yoshimura *(Heuser Lab)*
Expansion rate of hPSCs in the sphere culture with passaging every 5 days (unpublished data)

*Otsuji et al. Stem Cell Reports* (April 2014)

**hESCs (KhES-1 line)**

**hiPSCs (253G1 line)**
Maintenance of pluripotency & normal karyotype in sphere culture of hPSCs
Current 3D culture system needs stirring/agitation devices that may cause cell damages by stronger than adequate shear stress for keeping suspension.

Process engineering of human pluripotent stem cells for clinical application.

Figure I. Schematic diagrams of bioreactor systems for stem cell culture: (a) micro-bioreactor, (b) slowly turning lateral vessels and (c) stirred-tank bioreactors.
Low-Acyll Gellan Gum Polymer (GG)

(A) Repeat unit of GG.

(B) Stereo view of GG (Chandrasekaran & Thailambal, 1990). Two double-helices are crosslinked by calcium ions.

(C) Apparent viscosities and settling rates of GG and methylcellulose (MC). Asterisks, no settling.

(D) Polystyrene beads at various concentrations of GG.
Inhibition of sphere sedimentation by polymer: Gellan Gum enables very simple 3D culture system at low concentration

<table>
<thead>
<tr>
<th>Gellan Gum</th>
<th>0.00%</th>
<th>0.01%</th>
<th>0.015%</th>
<th>0.02%</th>
</tr>
</thead>
</table>

After 20 hrs

hES cells (KhES-1 line)

Otsuji et al. *Stem Cell Reports* (April 2014)
Gellan Gum Polymer inhibits sedimentation of cell spheres without gel formation or viscosity increase

Otsuji et al. *Stem Cell Reports* (April 2014)
Bag culture of hESCs (KhES-1 line) using 200ml gas-permeable bag

Capacity: $1.5 \sim 2.0 \times 10^8$ cells / 200 ml

Otsuji et al. *Stem Cell Reports* (April 2014)
Multidisciplinary Research of Human Pluripotent Stem Cells

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Human ES/iPS Cell Lines

Directed Differentiation to Cardiomyocytes

- Clinical grade
- Mass production
- High efficiency
- Maturation
- Cost-effective

Cardiac cells

- Drug screening
- Heart disease model
- Cell Therapy

Minami et al. Cell Reports 2012

Itsunari MINAMI
Kazuhiro AIBA
We discovered a novel small molecules KY02111 that promotes cardiac differentiation efficiently.
KY02111 is a novel type WNT inhibitor acting downstream of GSK3β and APC.

![Diagram showing Wnt pathway and KY02111's effect on gene expression and cardiac differentiation.]

Graphs showing cell luminescence under different treatments: Control, KY02111, XAV939, and IWP2. The graph on the right compares Topflash and SW480 with APC mutation.

Bar charts illustrate normalized luminescence values for each condition:
- Control: 100%
- KY02111: 7943%
- XAV939: 1621%
- IWP2: 5910%

Additional images show fluorescence microscopy images for DMSO, KY, XAV, and IWP conditions with BIO, KY+BIO, XAV+BIO, and IWP+BIO treatments.
Efficient and Robust Cardiac Differentiation under cytokine- and xeno-free condition

Efficiency of cardiac differentiation

Beating colonies on Day 21

FACS analysis
Characterization of KY02111-induced cardiac cells

Cardiac gene expression

The expression of cardiac markers

MLC2v: ventricular cardiomyocyte
MLC2a: atrial cardiomyocyte

FACS analysis
Cellular structures of hPSC-derived cardiomyocytes

- Organized sarcomere structure
- Desmosomes and intercalated disk
- Sarcoplasmonic reticulum
HERG channel QT prolongation test

Multi-electrode recording

Control
400ms

E4031(HERG blocker)
630ms

Patch-clamp recording

pretreatment
E4031
E4031 + Chromanol293B

Action potential prolongation

QT prolongation

KY02111 promotes electrophysiological maturation
Serum-, cytotokine- and xeno-free cardiac differentiation method of hES/iPS cells using chemical compounds including KY02111

Minami et al. Cell Reports 2012

Itsunari MINAMI

Kazuhiro AIBA

Robust
Simple
Cost effective

Human ES/iPSCs

Serum-free
Cytokine-free
Xeno-free
(Clinical grade)

BIO
CHIR99021

Known chemicals

XAV939

Discovered Chemical

KY02111

Small molecules

90-98%
Cardiac cells
(High efficiency)

Relatively maturated cells

Cardiac troponin T

Cardiac cells

Mesoderm cells
Collaborators

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