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

Institute for Integrated Cell-Material Sciences

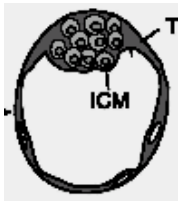
Kyoto University Founded October 2007

iCeMS
KYOTO UNIVERSITY
WPI Research Center

wpi World Premier International Research Center Initiative

KYOTO UNIVERSITY
KYOTO JAPAN
FOUNDED 1897

Cell-Material Integration for Stem Cell Research



Embryo

ES cells

Cell Growth

Reprogramming

iPS cells

ES/iPS Cells

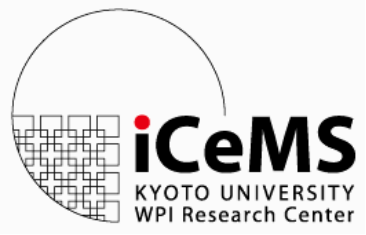
Cell-based Therapy

Differentiation

Cell Biology Tools
Disease Model Study

How to deliver safe and effective stem cell therapy to many patients at affordable cost

Norio Nakatsuji @



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)

日産化学工業株式会社
NISSAN CHEMICAL INDUSTRIES, LTD.

ReproCELL

NIPRO

Imaging

HAMAMATSU

ReproCELL

Human ES cells

Human iPS cells

TaKaRa

Defined/robust medium with low molecular compounds

Development of cell culture substrate and materials

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

Development of cryo-preservation method

Development of large scale culture method

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

Development of evaluation machines and reagents

GENETEIN

SHIMADZU

SUMITOMO BAKELITE CO., LTD.

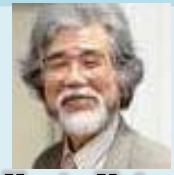
Automated large-scale stem cell culture system

Quality evaluation system

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

Accurate cell shipment system

Academia



Kyoto Univ.
Prof. Nakatsuji

NIBIO
Dr. Mizuguchi

Keio Univ.
Prof. Okano

Chiba Univ.
Prof. Iwama

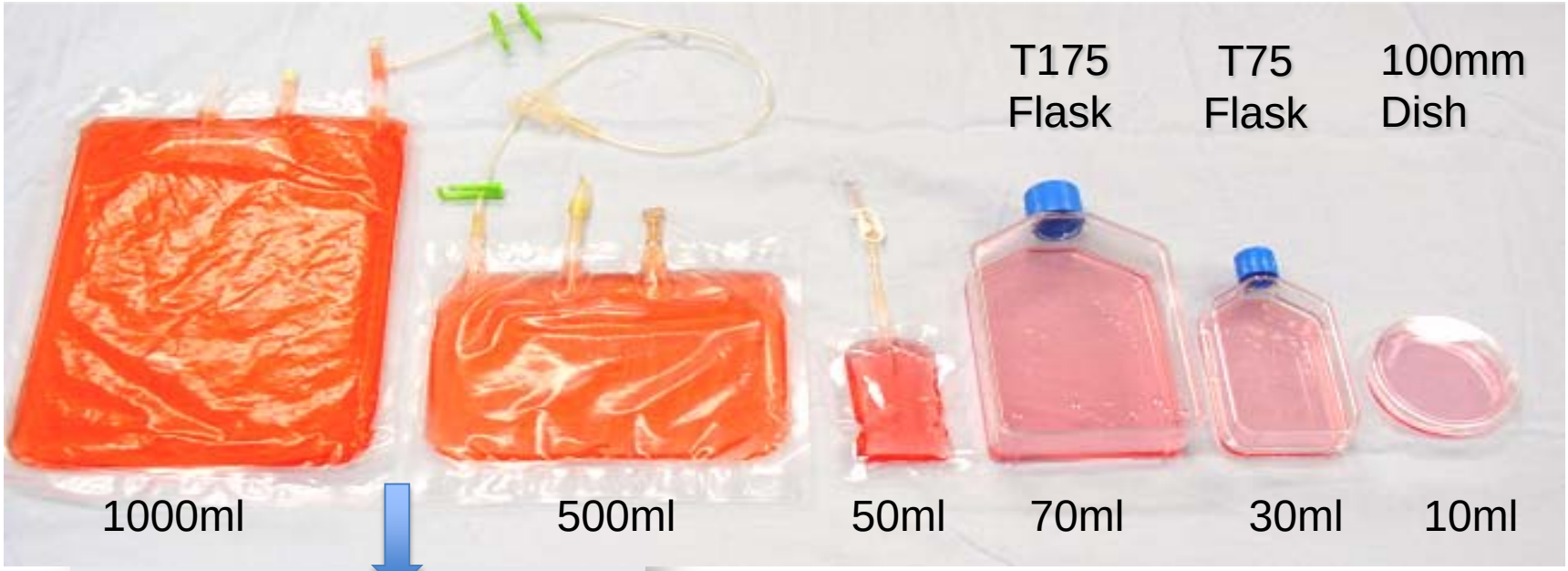
Tokyo Univ.
Prof. Nakauchi

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



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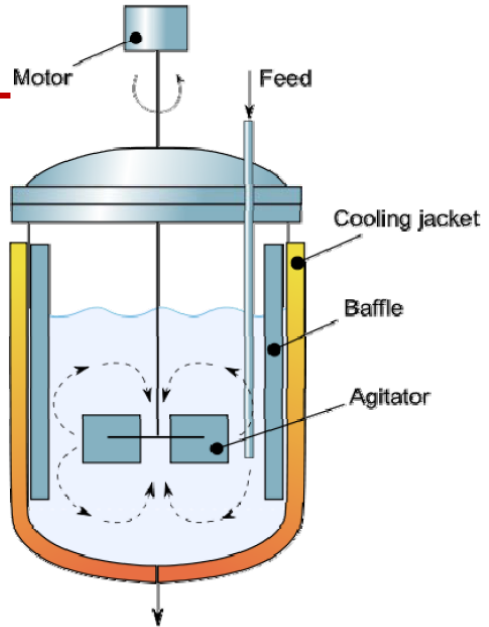
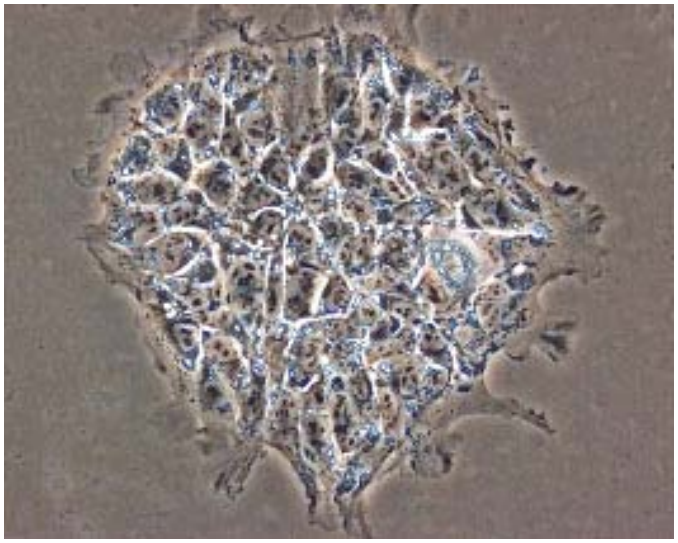
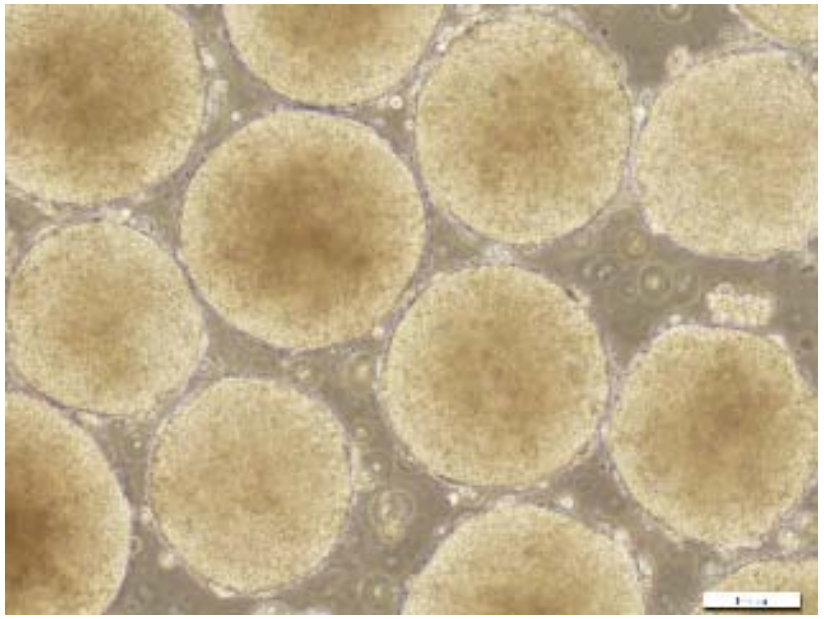
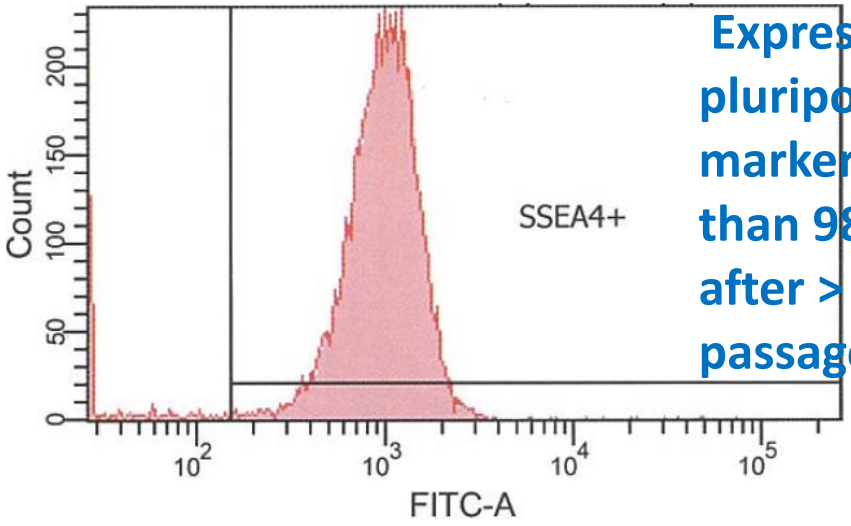


Otsuji et al. *Stem Cell Reports* (April 2014)

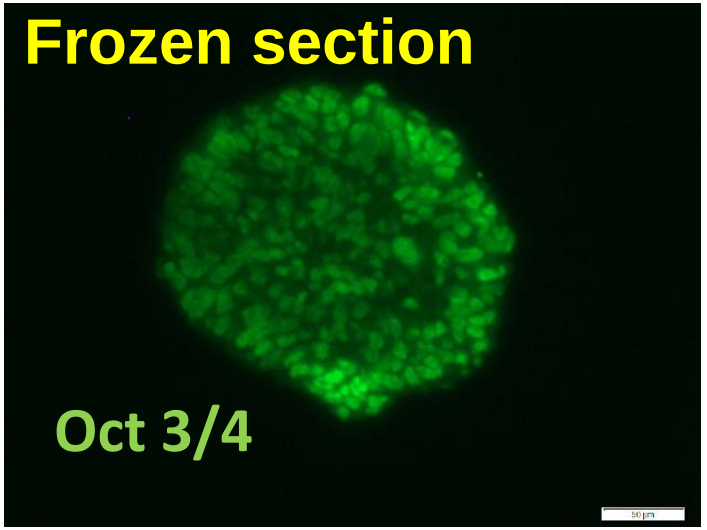
From conventional adherent 2D culture to 3D sphere culture for large-scale production of human pluripotent stem cells





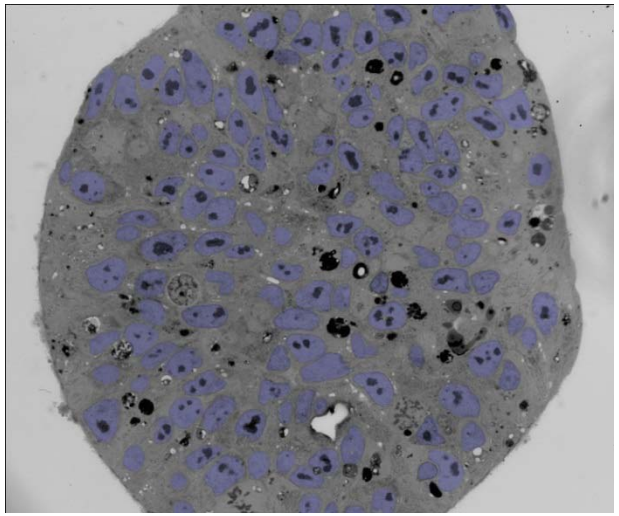


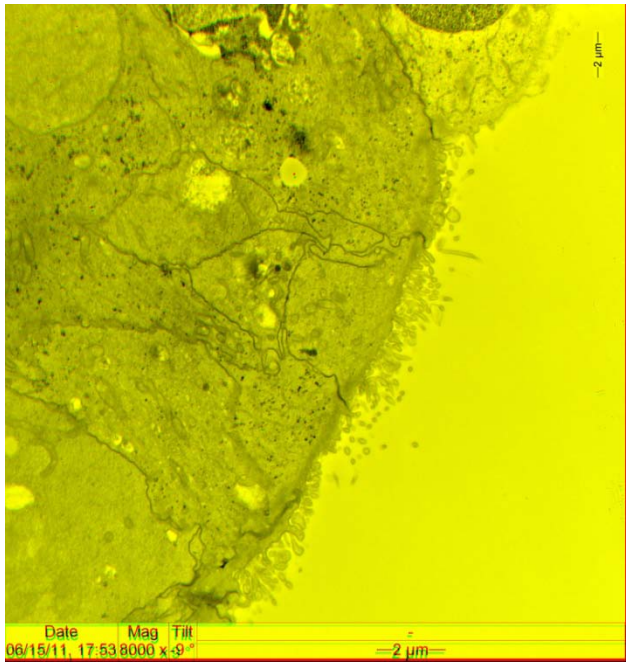
Expression of pluripotency markers in more than 98 % cells after > 50 passages



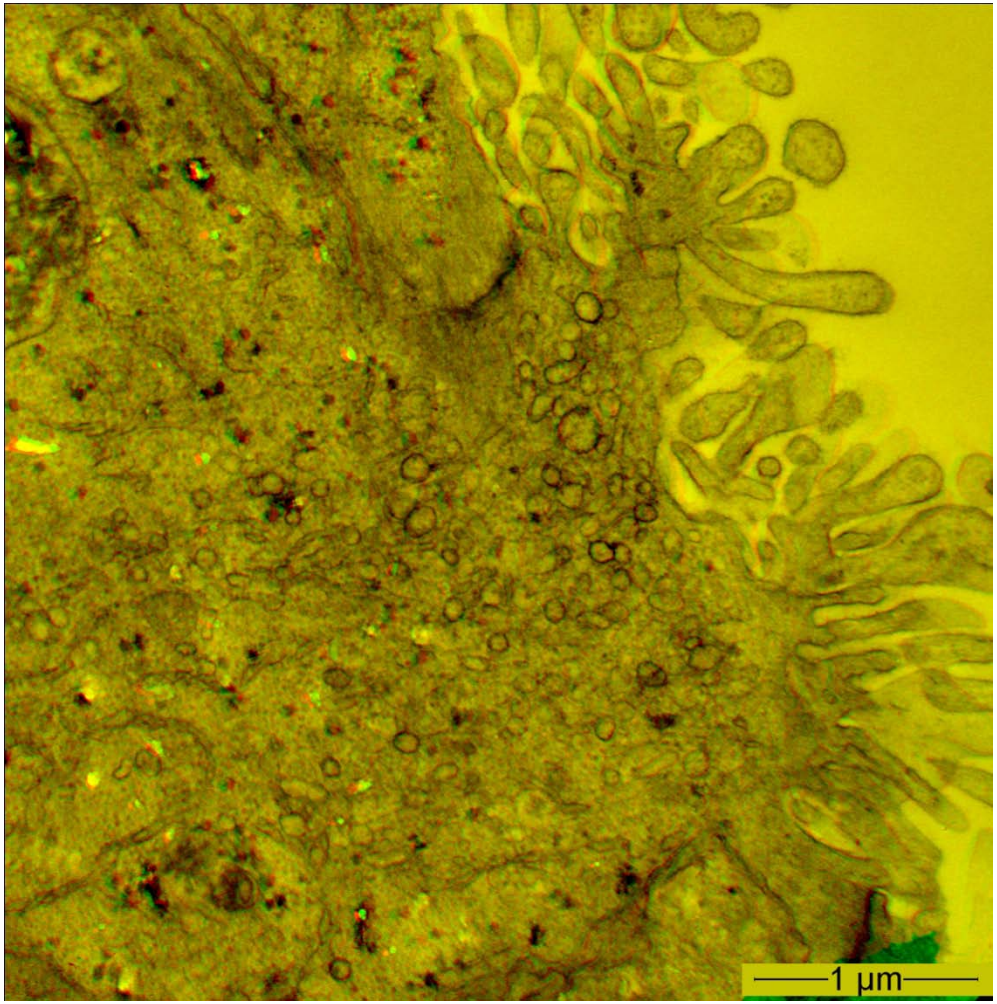
Detailed morphological study of the hPSC spheres with electron microscopy by Heuser Lab shows homogenious undifferentiated cell population

Otsuji et al.
Stem Cell Reports
(April 2014)



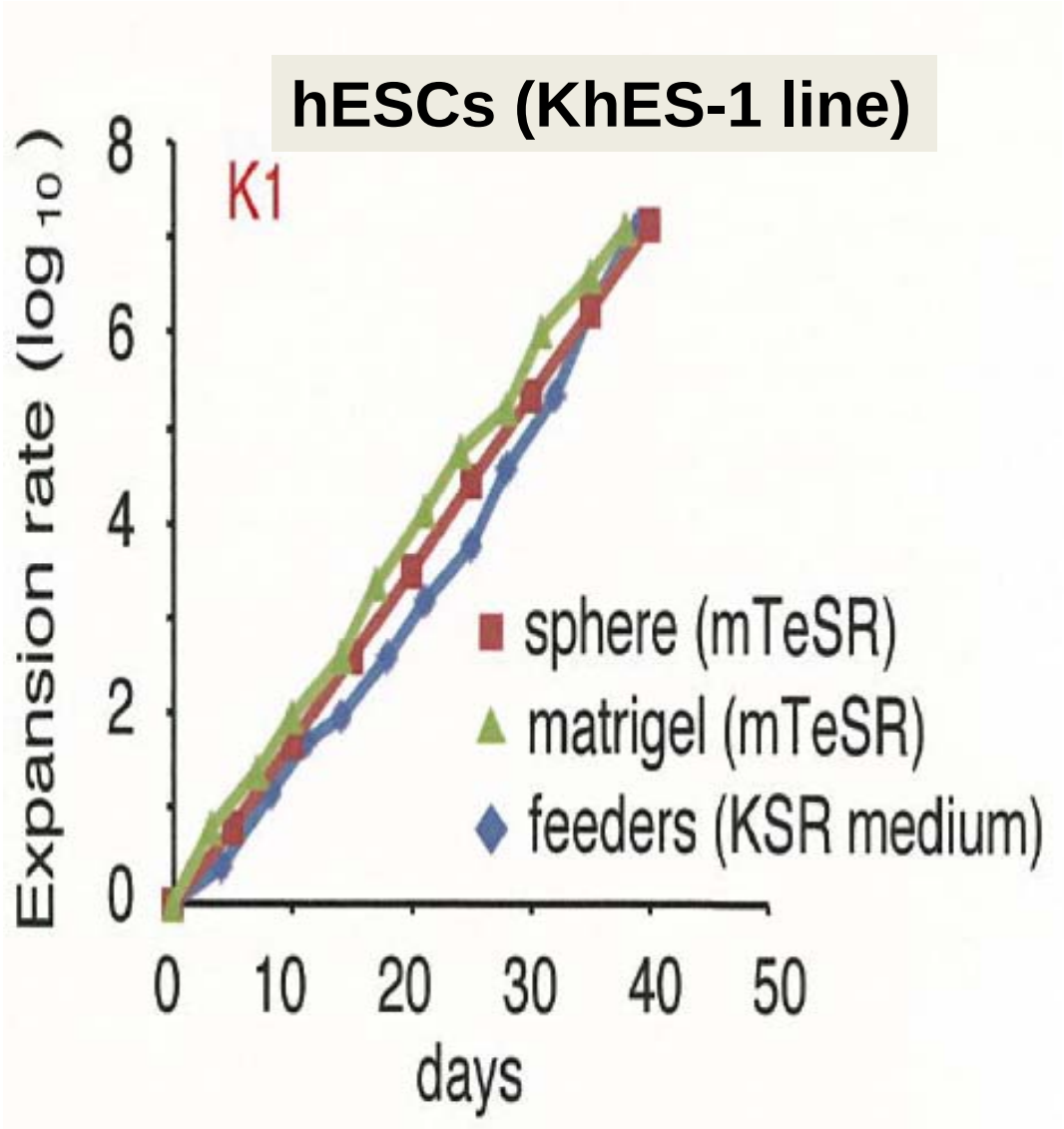


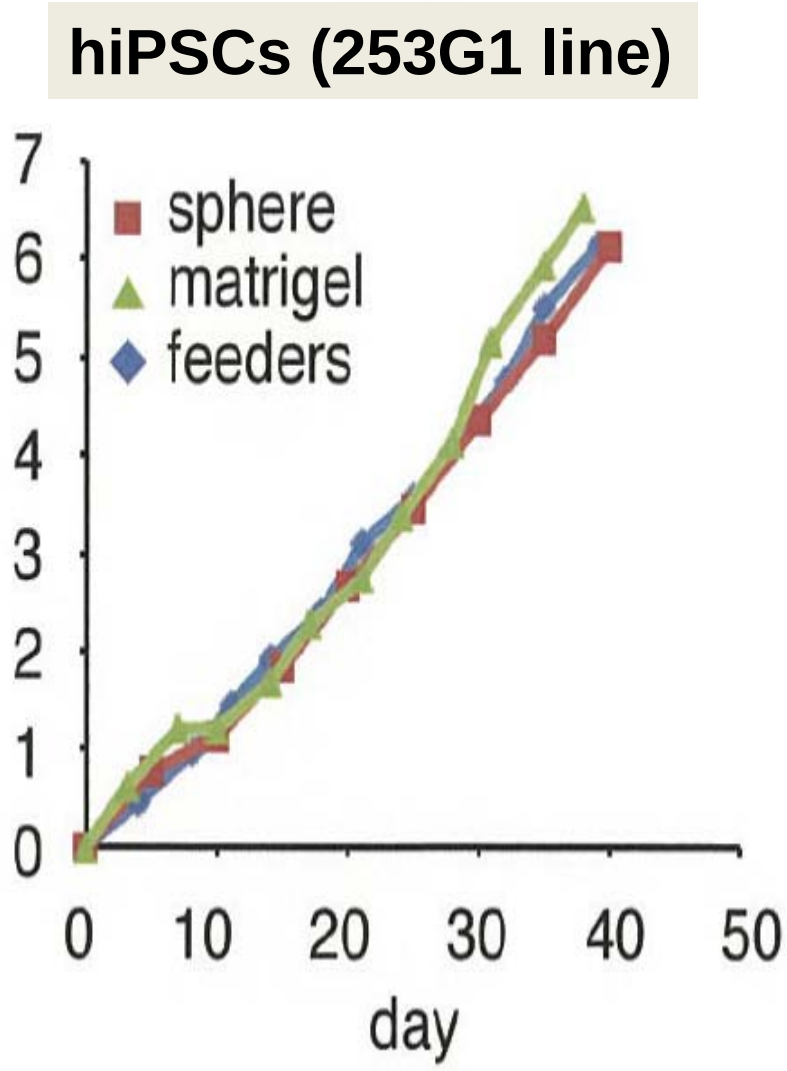
TEM by Dr. Yoshimura (Heuser Lab)



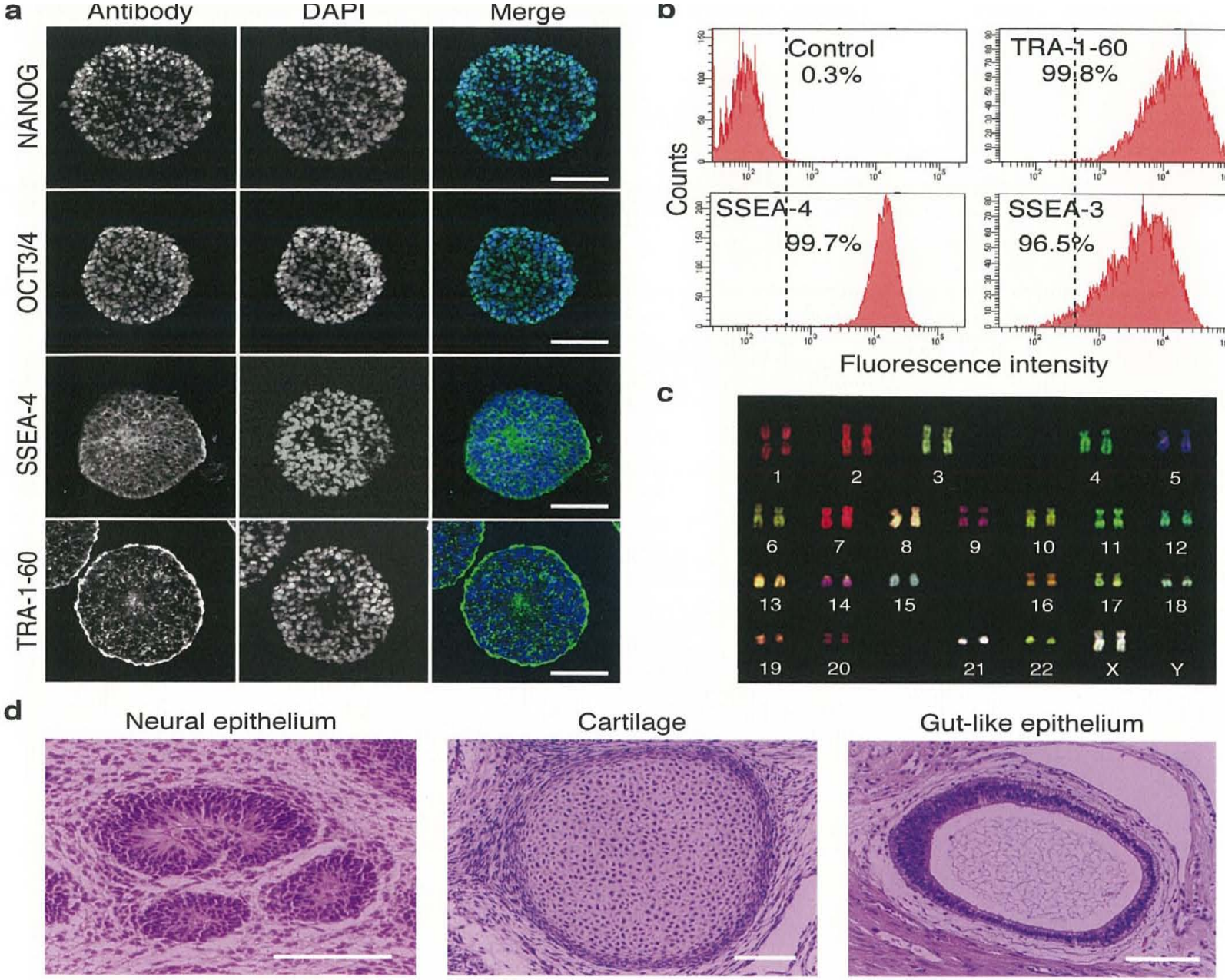
Otsuji et al. *Stem Cell Reports* (April 2014)

Expansion rate of hPSCs in the sphere culture with passaging every 5 days (unpublished data)



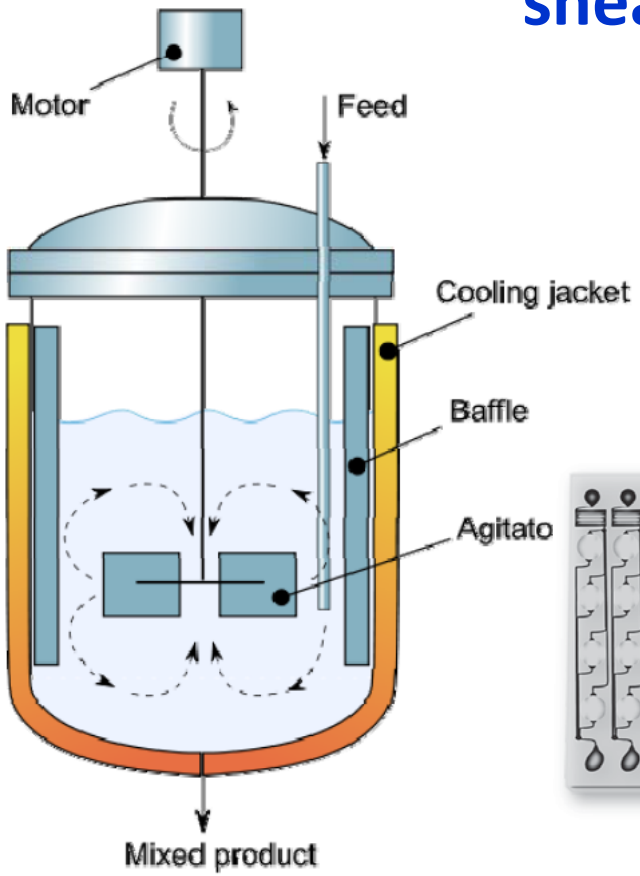


Maintenance of pluripotency & normal karyotype in sphere culture of hPSCs

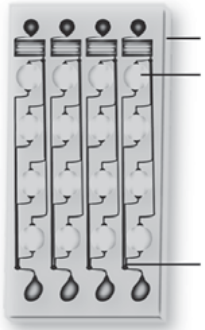


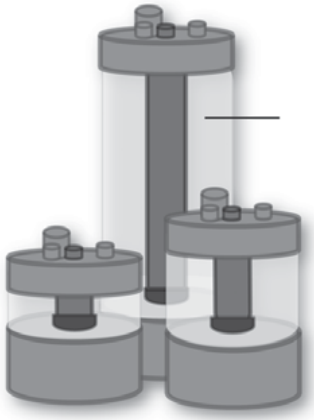
Current 3D culture system needs stirring /agitation devices that may cause cell damages by stronger than adequate shear stress for keeping suspension

File:Agitated vessel.svg
From Wikipedia, the free encyclopedia



Process engineering of human pluripotent stem cells for clinical application.
Margarida Serra, Catarina Brito, and Paula M. Alves.
Trends in Biotechnology 2012





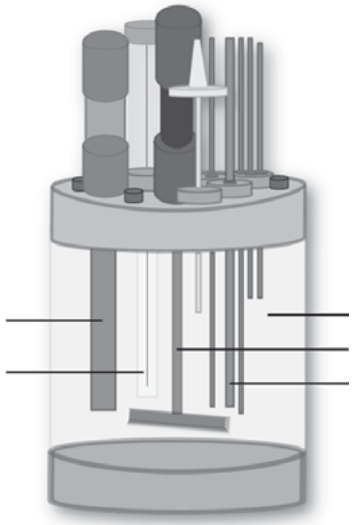


Figure I. Schematic diagrams of bioreactor systems for stem cell culture: (a) micro-bioreactor, (b) slowly turning lateral vessels and (c) stirred-tank bioreactors.

Low-Acyl Gellan Gum Polymer (GG)

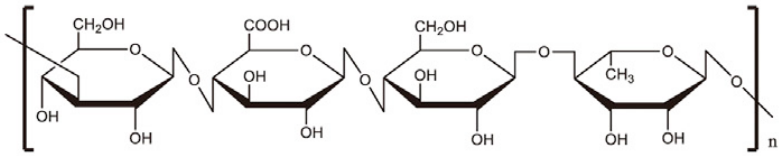
(A) Repeat unit of GG.

(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.

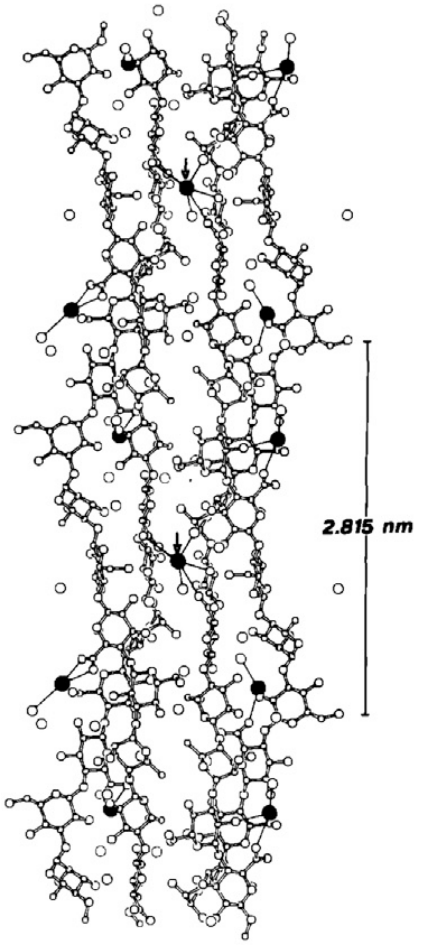
(D) Polystyrene beads at various concentrations of GG.

A

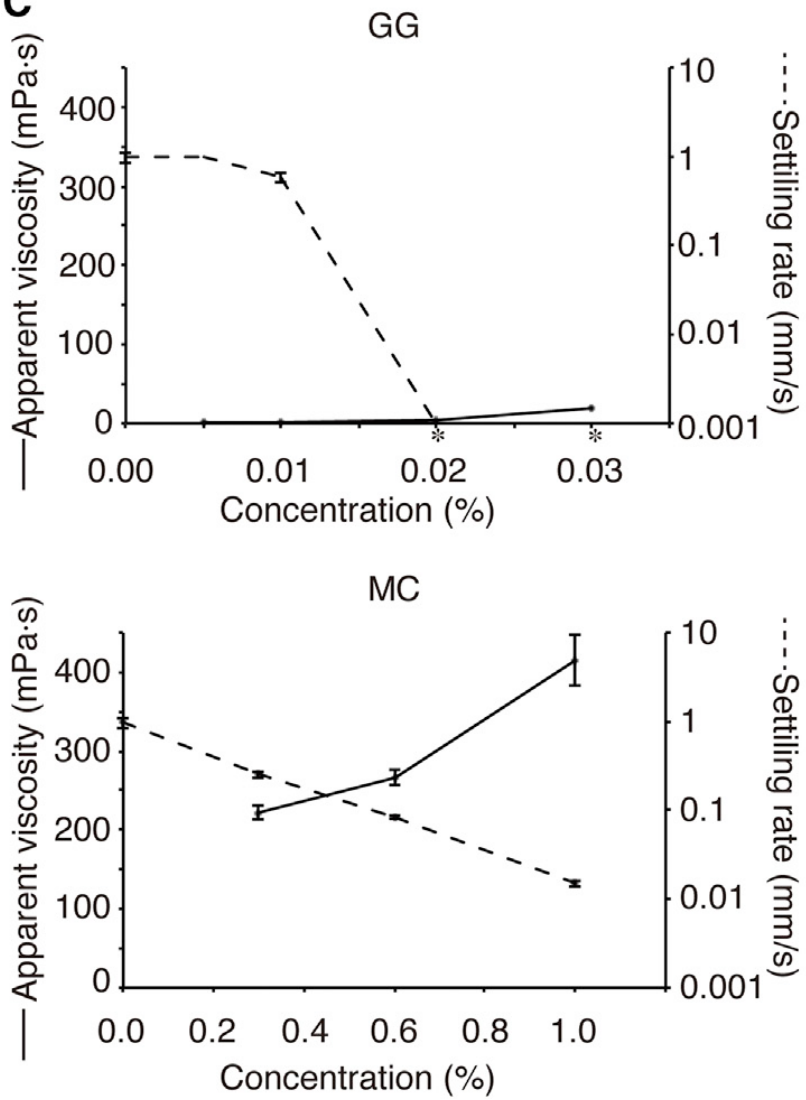


[D-Glc(β1→4)D-GlcA(β1→4)D-Glc(β1→4)L-Rha(α1→3)]n

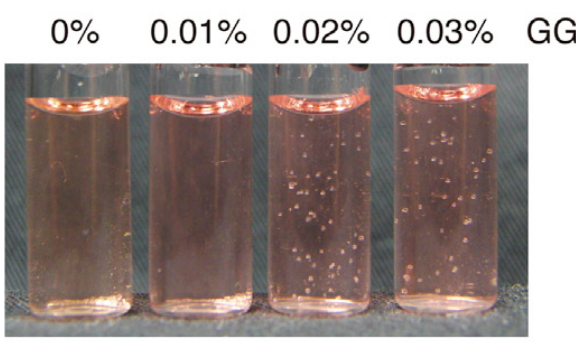
B



C

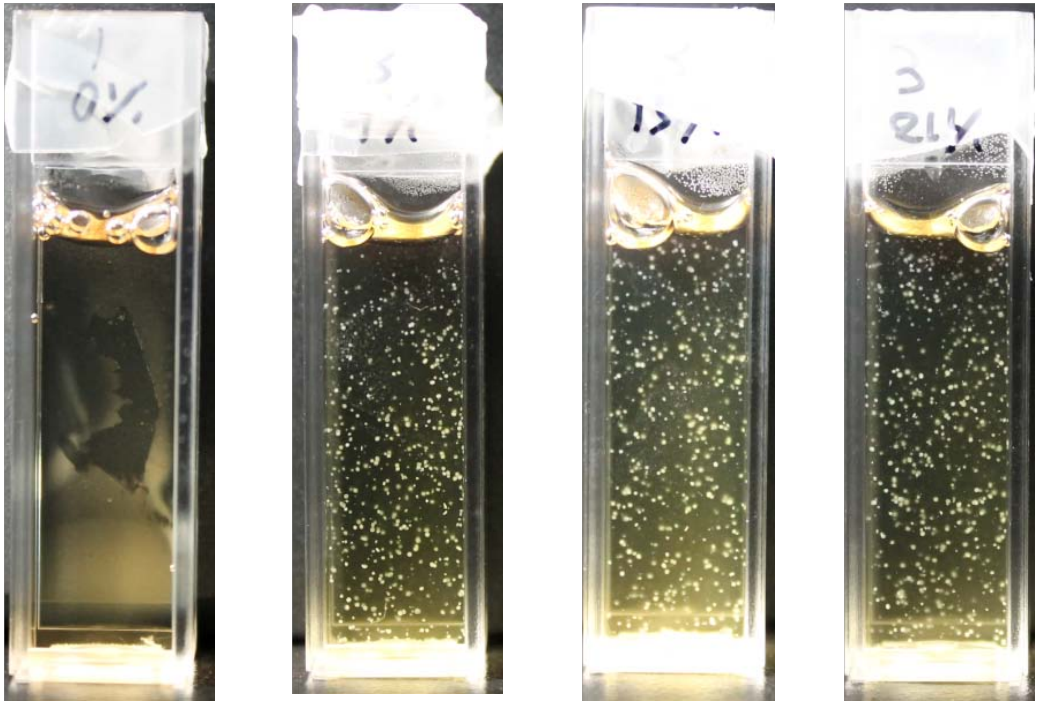


D



Inhibition of sphere sedimentation by polymer:
Gellan Gum enables very simple 3D culture system at low concentration

Gellan Gum 0.00% 0.01% 0.015% 0.02%

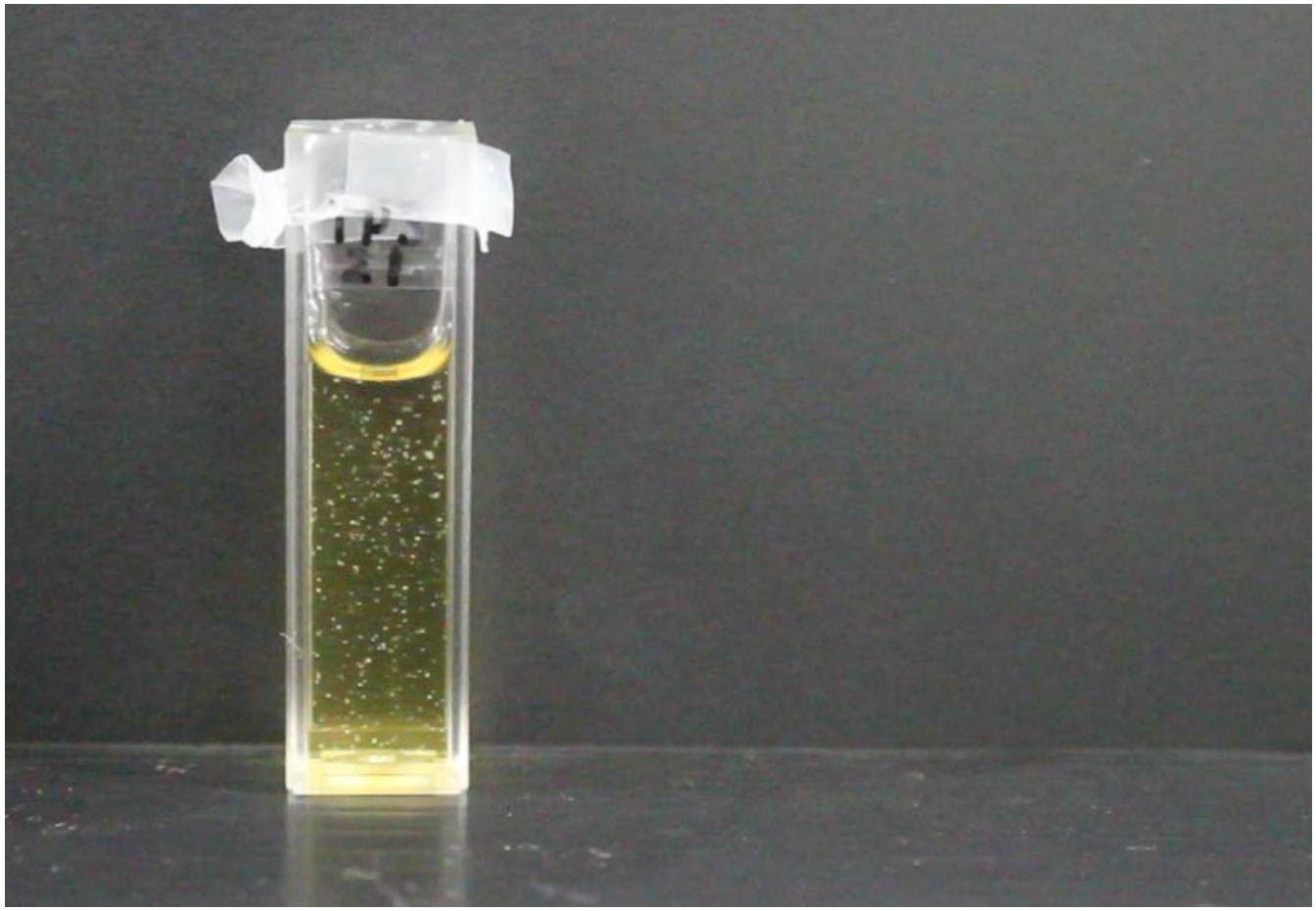


After 20 hrs

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

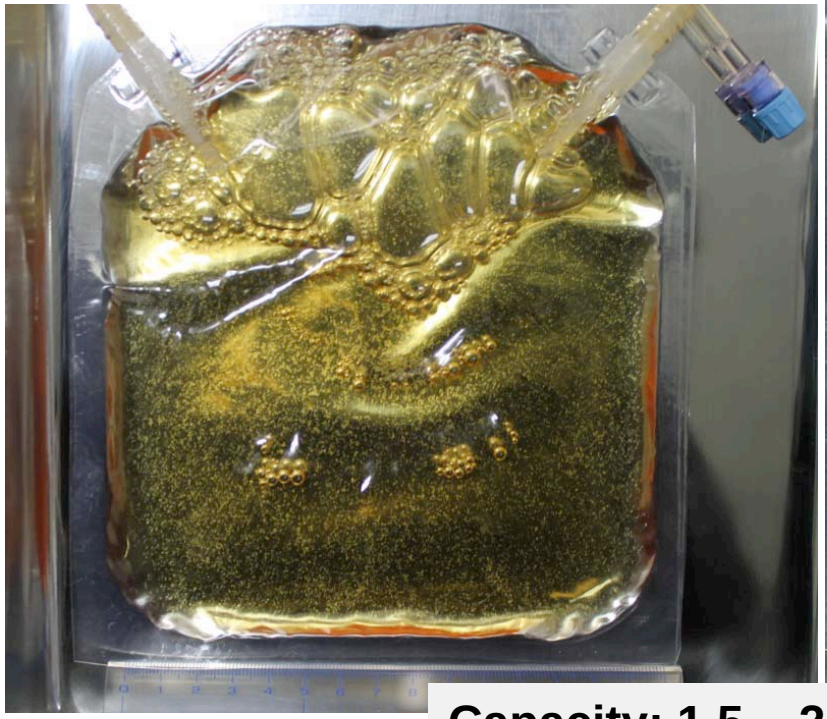


Otsuji et al. *Stem Cell Reports* (April 2014)

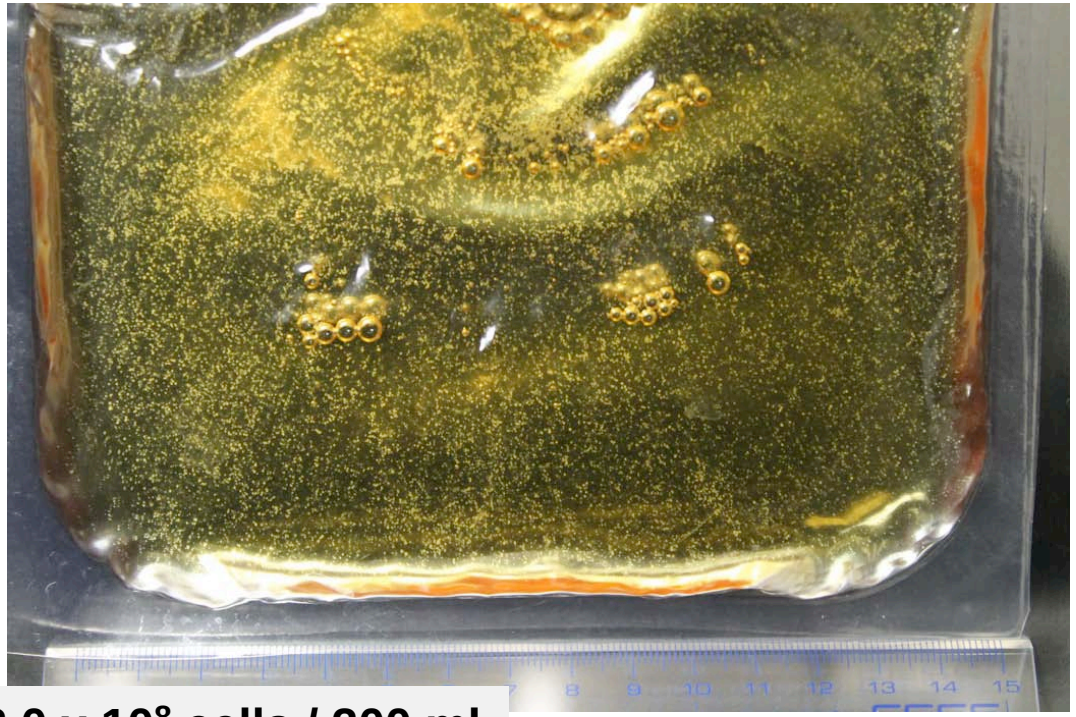
Bag culture of hESCs (KhES-1 line)
using 200ml gas-permeable bag

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

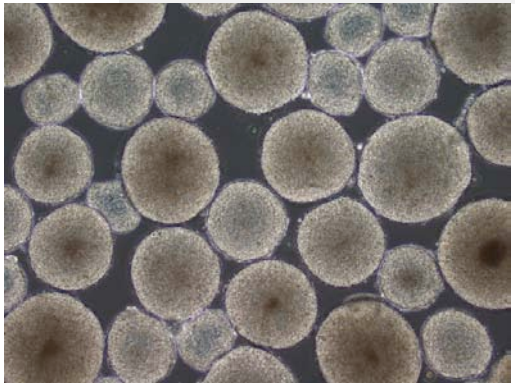
5 cm

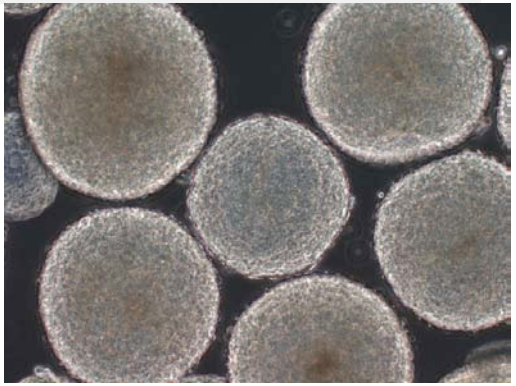


5 cm



Capacity: 1.5 ~ 2.0 x 10^8 cells / 200 ml



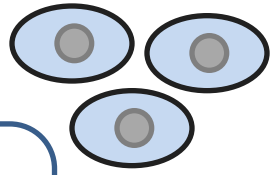


Multidisciplinary Research of Human Pluripotent Stem Cells

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

Human ES/iPS Cell Lines



Directed Differentiation to Cardiomyocytes

Cost-effective

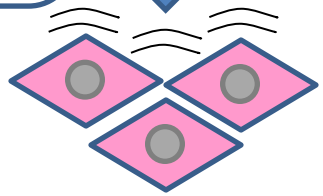
Clinical grade

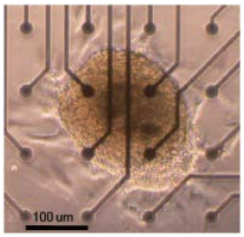
High efficiency

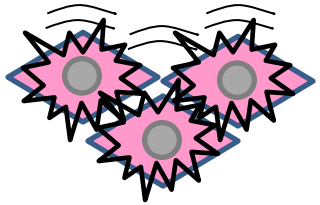
Mass production

Cardiac cells

Maturation







Cell Therapy

Drug screening

Heart disease model

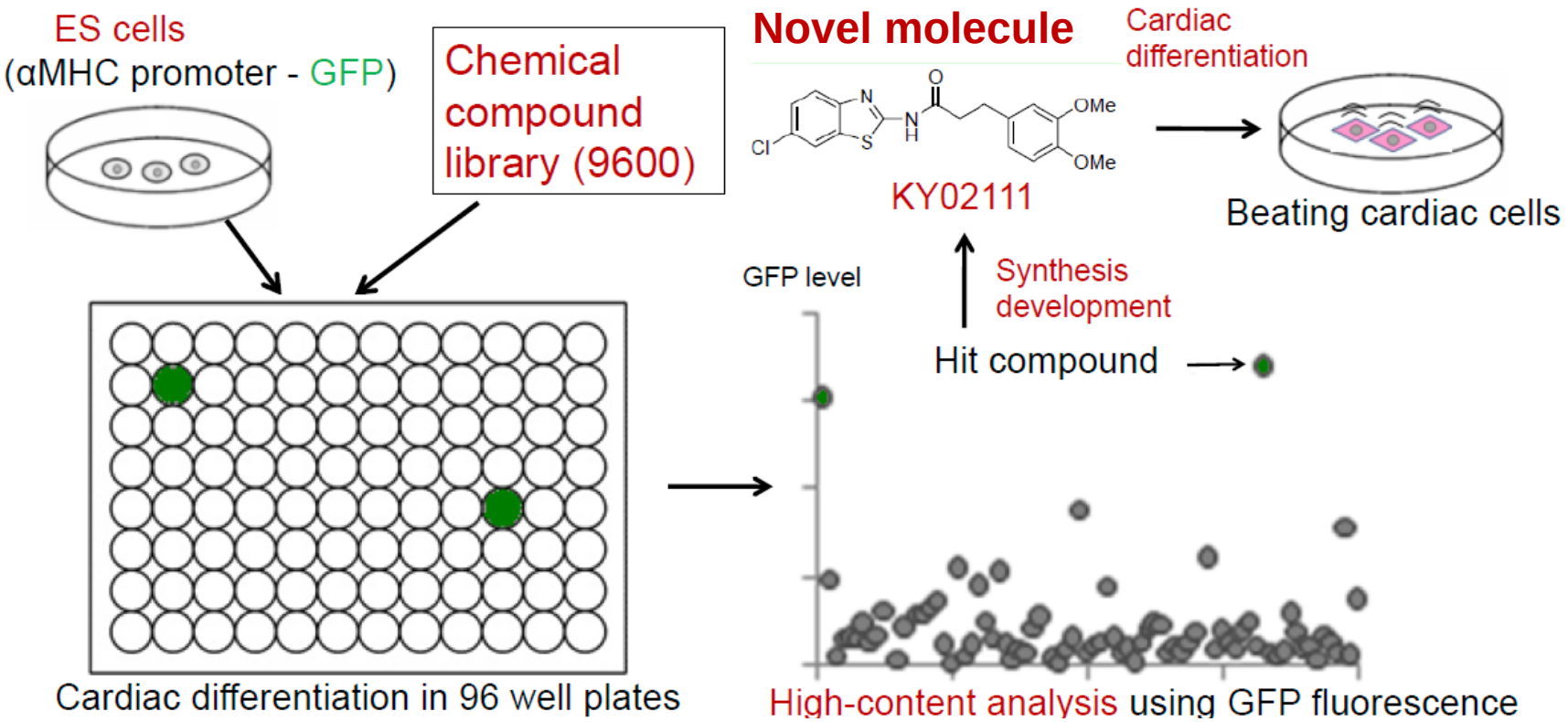
Itsunari MINAMI

Kazuhiro AIBA

Minami et al.
Cell Reports
2012

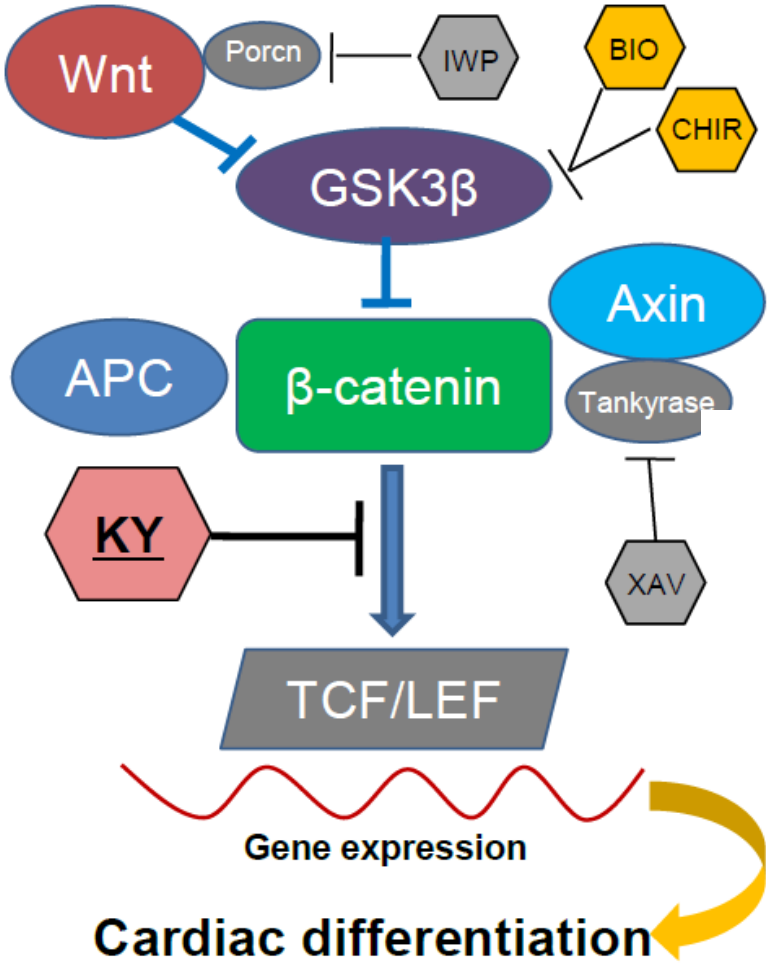
Cell-based chemical library screening using ES cells

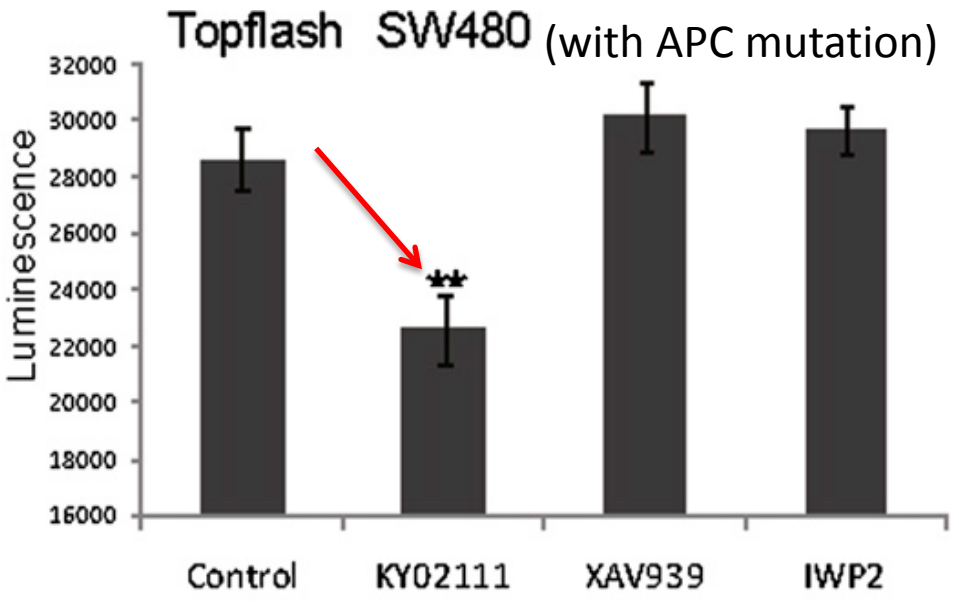
Nakatsuji Lab and Uesugi Lab

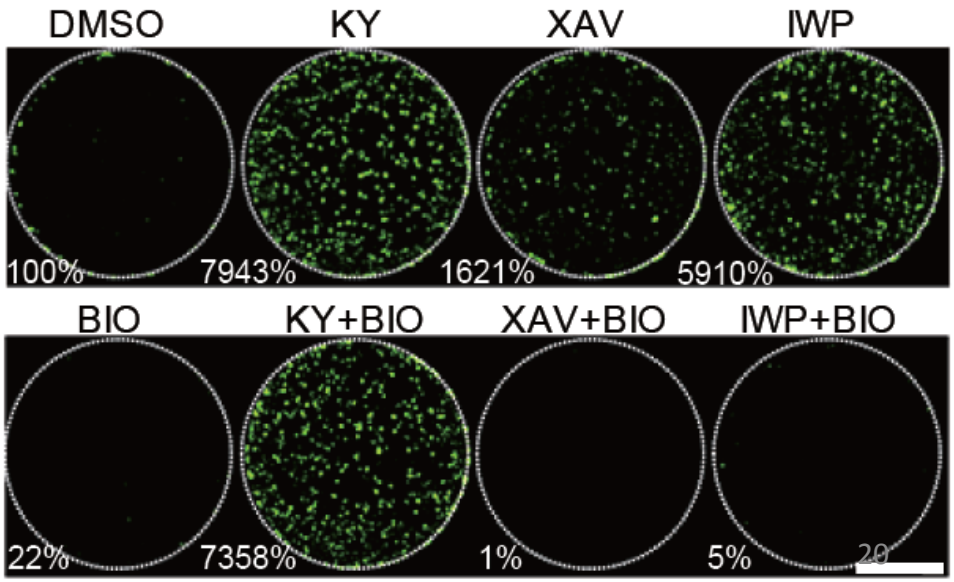


We discovered a novel small molecules KY02111 that promotes cardiac differentiation efficiently

KY02111 is a novel type WNT inhibitor acting downstream of GSK3β and APC

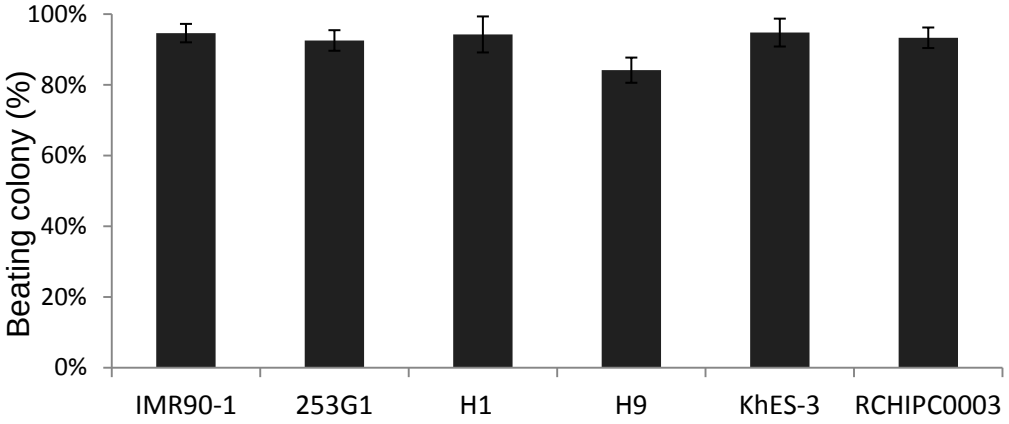


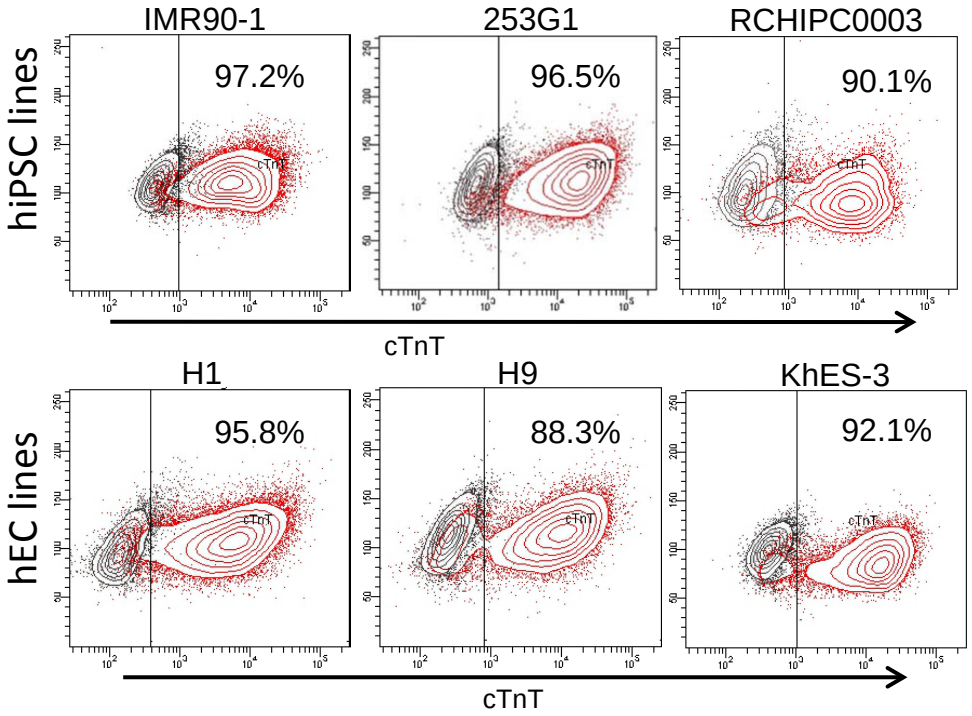




Efficient and Robust Cardiac Differentiation under cytokine- and xeno-free condition

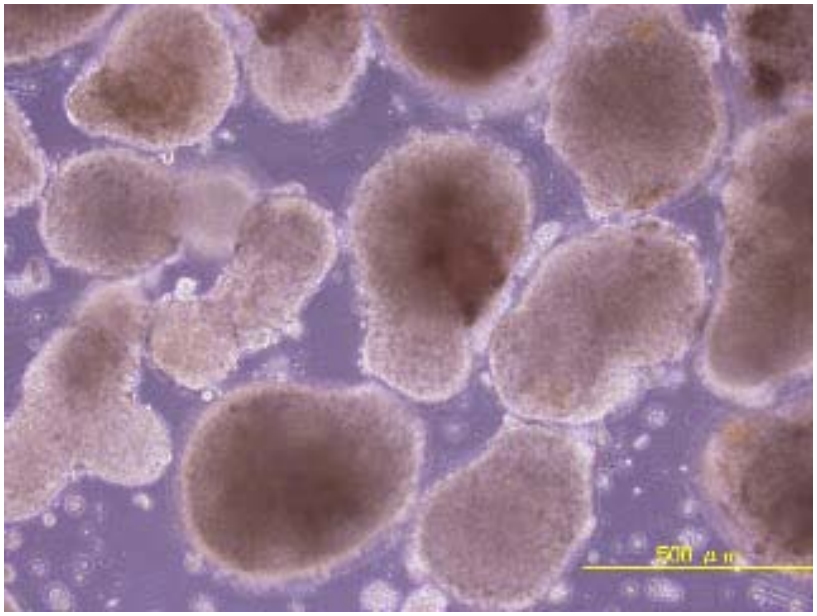
Efficiency of cardiac differentiation





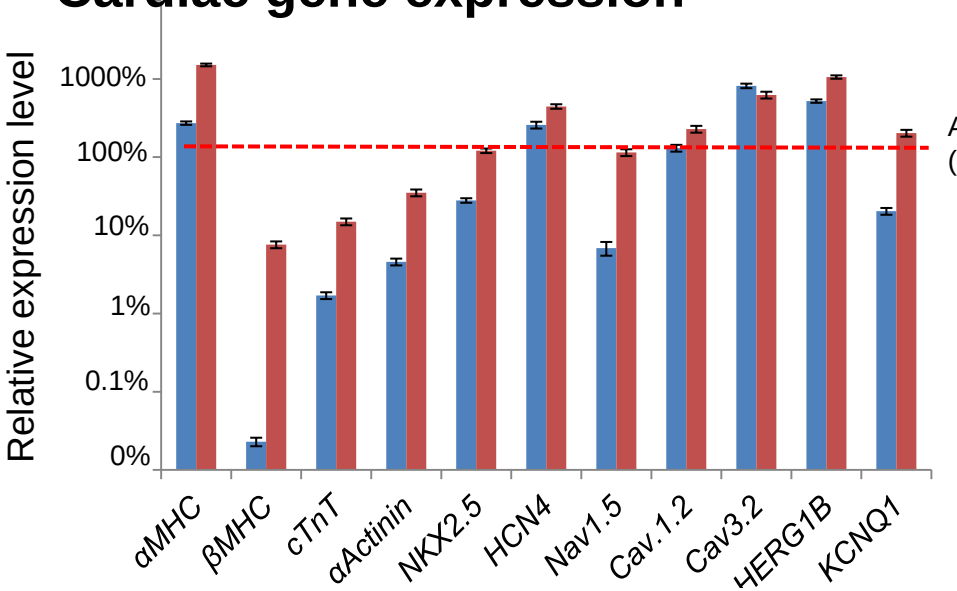
FACS analysis

Beating colonies on Day 21

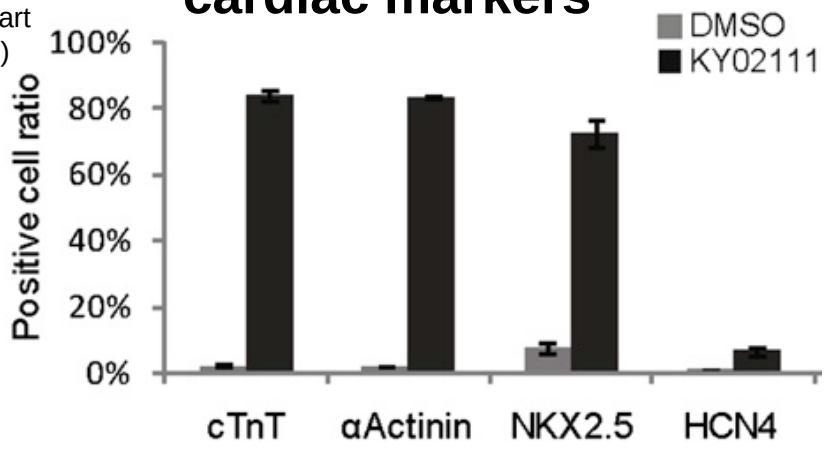


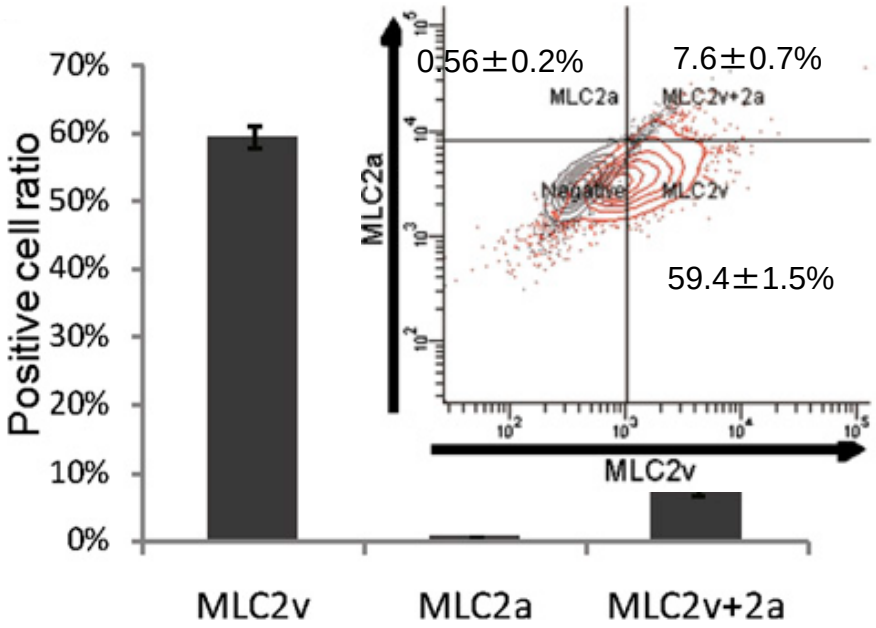
Characterization of KY02111-induced cardiac cells

Cardiac gene expression



The expression of cardiac markers

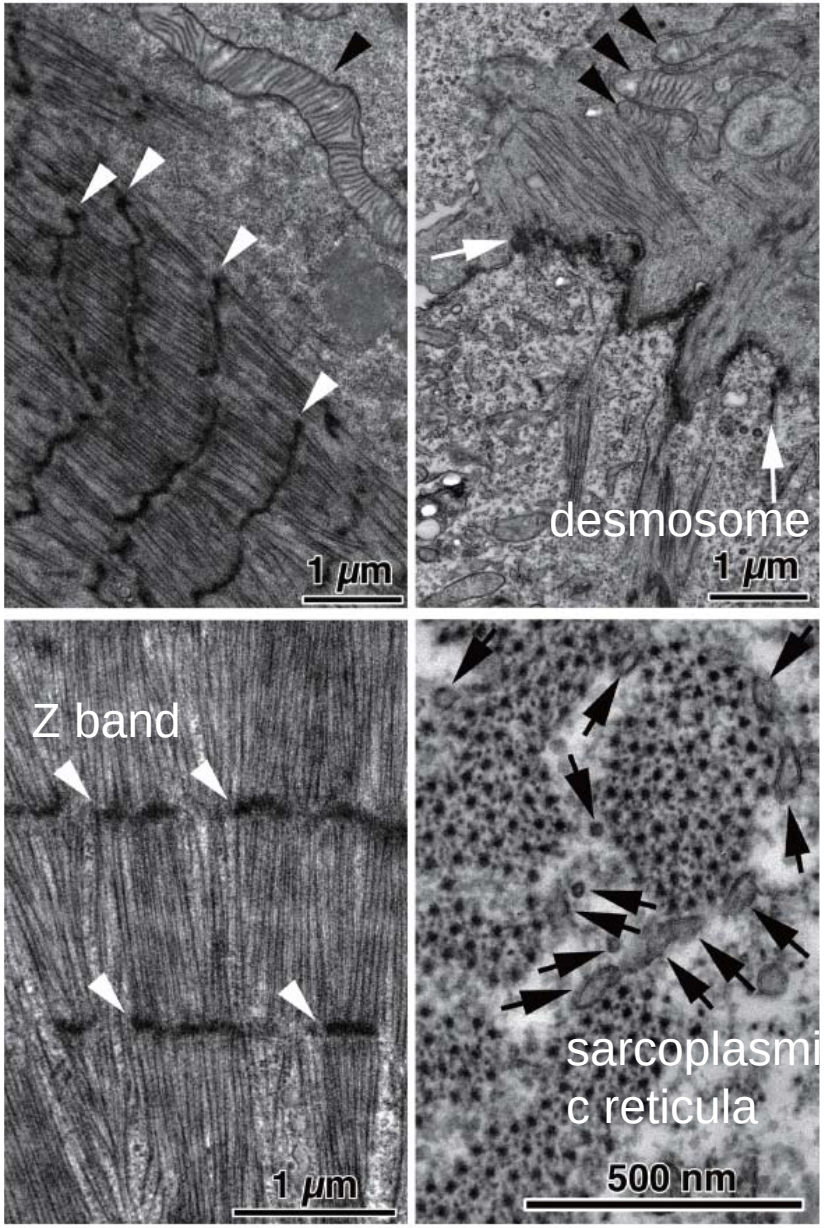


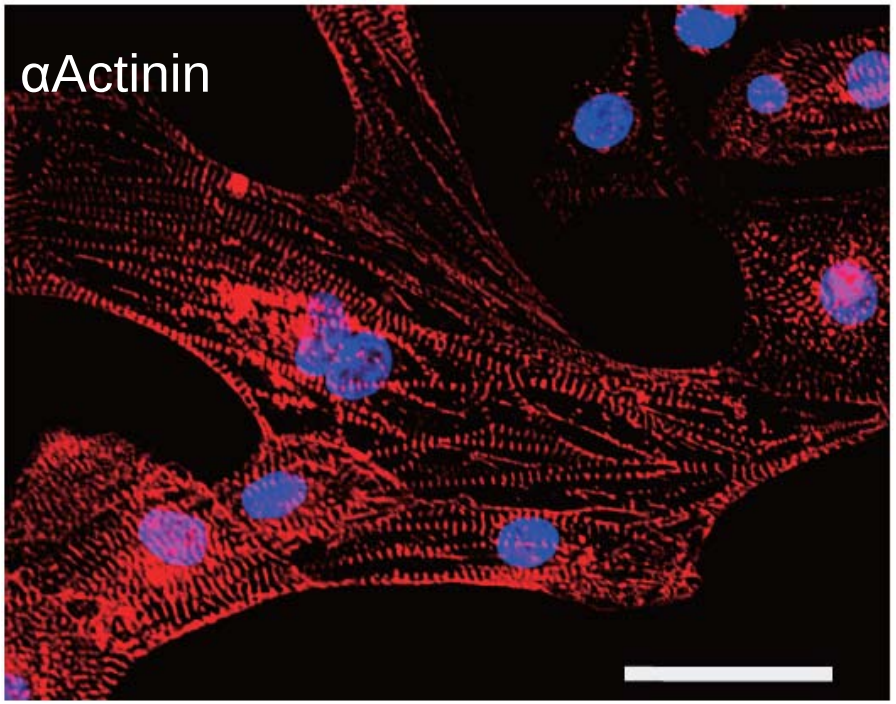


MLC2v: ventricular cardiomyocyte
MLC2a: atrial cardiomyocyte

FACS analysis

Cellular structures of hPSC-derived cardiomyocytes

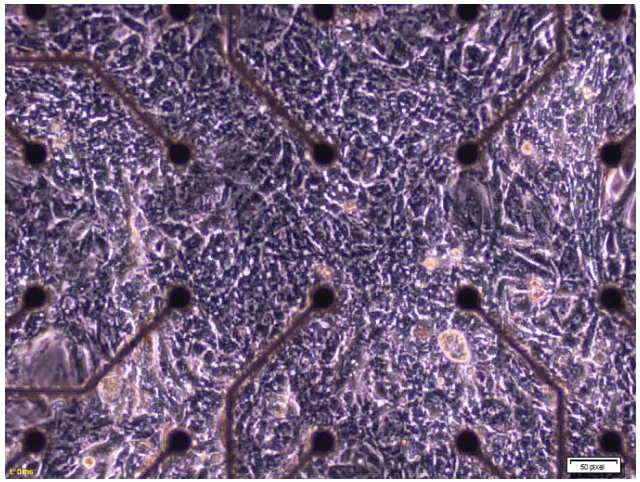




- ✓ Organized sarcomere structure
- ✓ Desmosomes and intercalated disk
- ✓ Sarcoplasmic reticulum

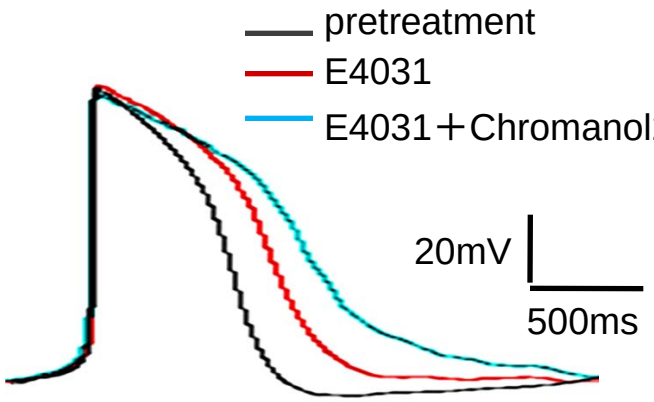
HERG channel QT prolongation test

Multi-electrode recording



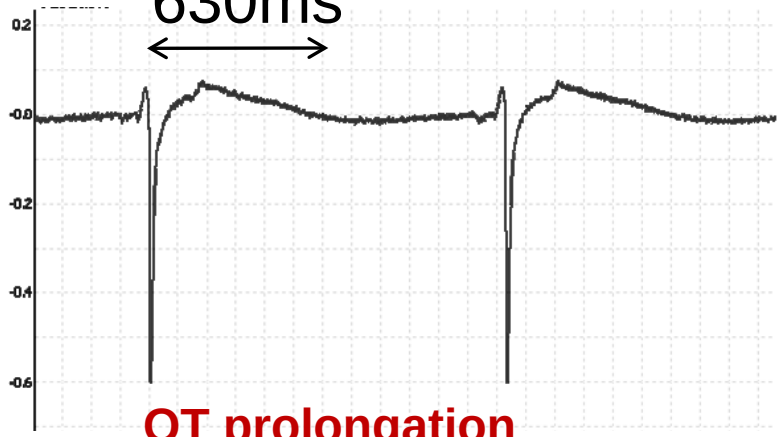
Control
400ms

Patch-clamp recording



Action potential prolongation

E4031(HERG blocker)
630ms

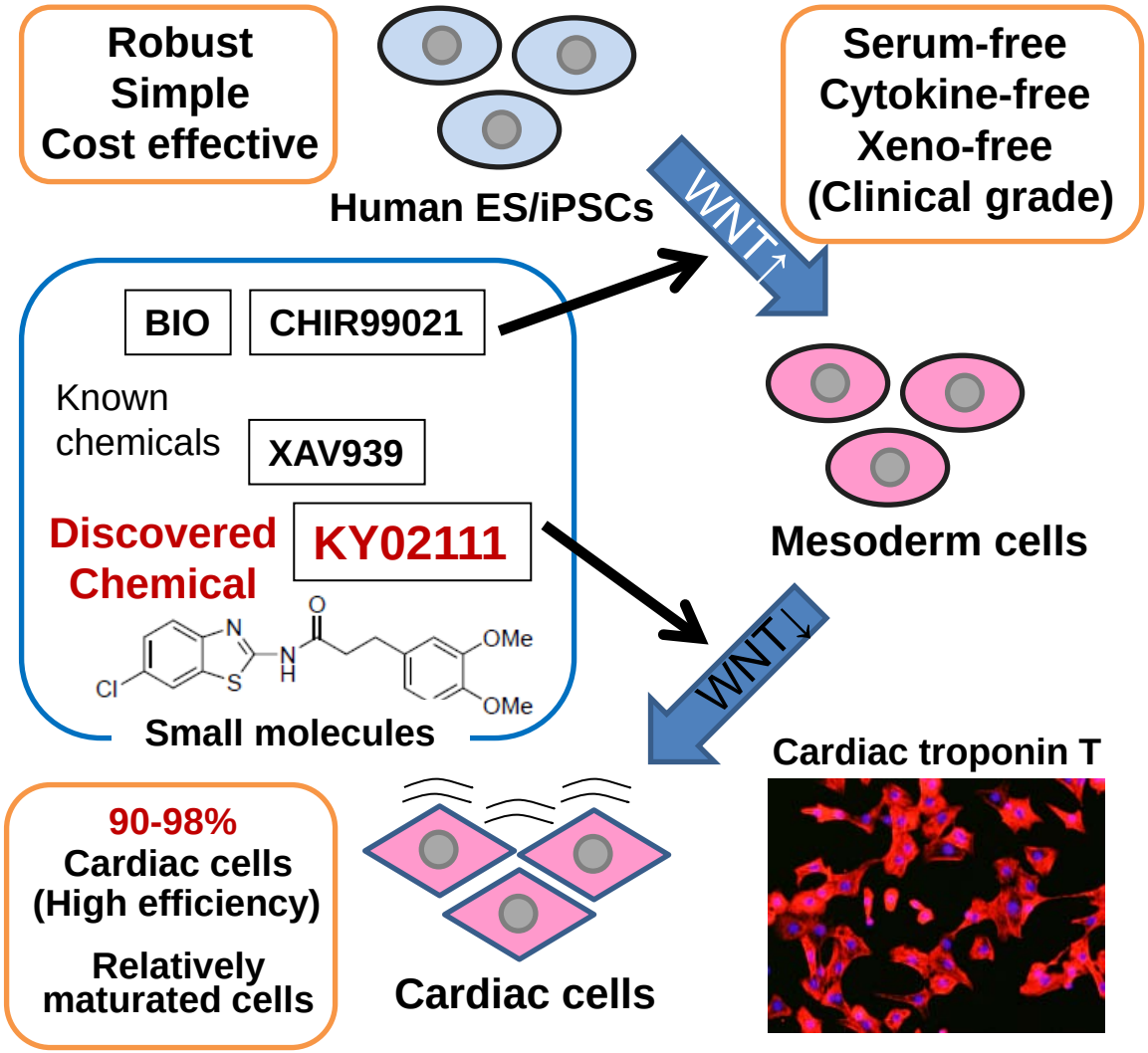


QT prolongation

KY02111 promotes electrophysiological maturation

Serum-, cyotokine- 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

Collaborators

iCeMS, Kyoto University

- **Norio NAKATSUJI**
- **Kazuhiro AIBA**
- **Itsunari MINAMI**
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