

THE 20<sup>TH</sup> CONGRESS OF THE JAPANESE SOCIETY FOR REGENERATIVE MEDICINE

# LARGE-SCALE EXPANSION OF IPS CELLS IN TERUMO BCT QUANTUM<sup>®</sup> CLOSED AUTOMATED CELL CULTURE SYSTEM

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CiRA FOUNDATION  
FACILITY FOR IPS CELL THERAPY



## 第20回日本再生医療学会総会 筆頭発表者のCOI開示

筆頭演者は、過去1年間（1月～12月）において、  
本演題の発表に関して開示すべきCOIはありません。

# ABSTRACT

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MANUFACTURING OF CLINICAL-GRADE INDUCED PLURIPOTENT STEM CELLS (iPSC) REQUIRES EFFICIENT AND SCALABLE CELL EXPANSION PROTOCOLS THAT ALLOW FOR MAINTENANCE OF CELLS MORPHOLOGY, IMMUNOPHENOTYPE, GENETIC STABILITY AND PLURIPOTENCY UNDER CURRENT GOOD MANUFACTURING PRACTICE (cGMP) REQUIREMENTS.

TERUMO BCT QUANTUM® IS AN AUTOMATED, FUNCTIONALLY CLOSED PLATFORM DESIGNED TO SIMPLIFY TASKS ASSOCIATED WITH MANUAL CELL CULTURE. IT HAS BEEN USED FOR THE SCALABLE OF THE HUMAN MESENCHYMAL STROMAL CELLS (hMSCs).

HERE WE REPORT THE RESULTS OF OUR PROOF-OF-CONCEPT STUDY AIMED AT DEVELOPMENT OF IPSC EXPANSION PROTOCOL UTILIZING THE QUANTUM® SYSTEM. WE CULTURED IPSCS IN TWO DIFFERENT CULTURE SYSTEMS AND ATTEMPTED SEVERAL PROTOCOL OPTIMIZATIONS. AFTER EXPANSION OF THE CELLS IN QUANTUM® SYSTEM WE PERFORMED THE QUALITY ASSESSMENT IN ORDER TO COMPARE THE AUTOMATED PLATFORM WITH THE STANDARD MANUAL PROCEDURE.

THE QUANTUM® INSTRUMENT USED IN THIS STUDY WAS KINDLY PROVIDED BY TERUMO BCT JAPAN, INC.

# QUANTUM<sup>®</sup> CELL EXPANSION SYSTEM



QUANTUM DEVICE



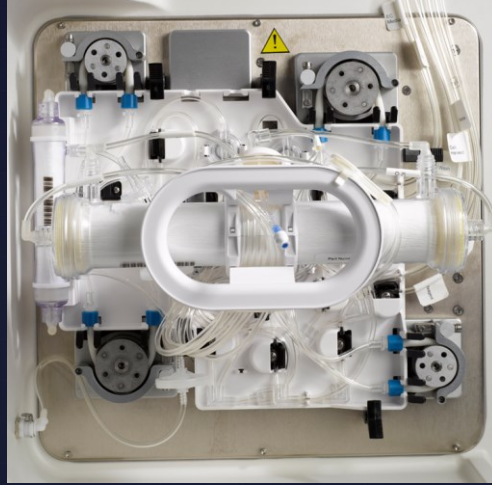
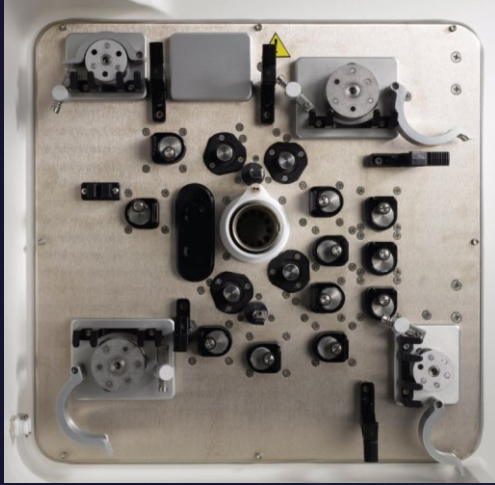
CELL EXPANSION SET



ACCESSORY SETS

TERUMOBCT

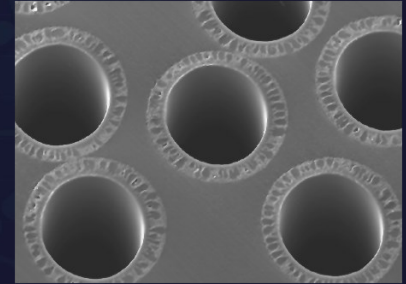
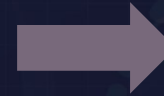
# INSIDE THE INCUBATOR



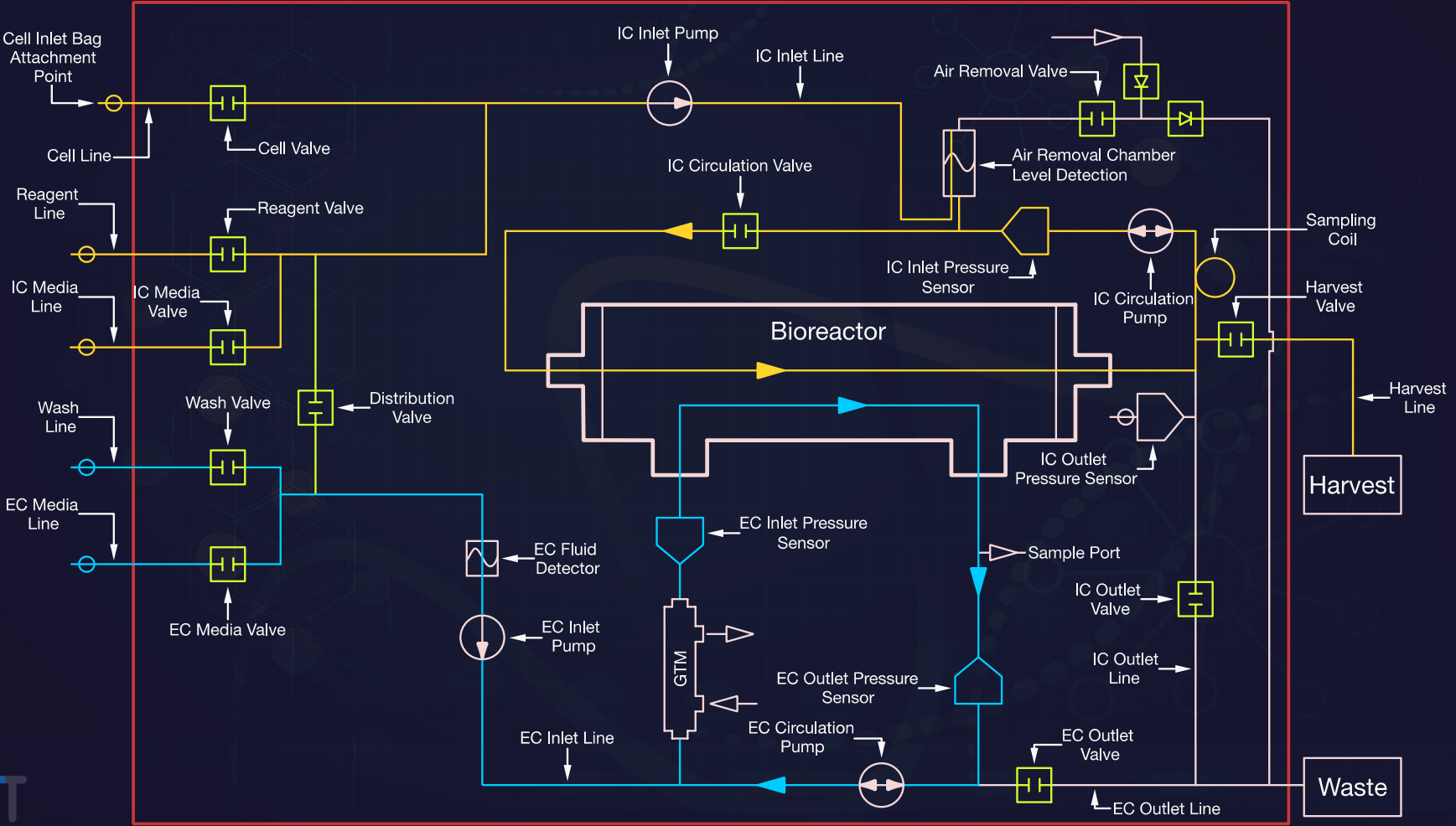
~ 10,000 SEMI-PERMEABLE  
HOLLOW FIBERS ( $\Phi \sim 200 \mu\text{m}$ )

~ 2.1 m<sup>2</sup> OF SURFACE AREA

INTRACAPILLARY (IC) &  
EXTRACAPILLARY (EC)  
COMPARTMENTS



# HYDRAULIC LAYOUT





# CONTROL SOFTWARE

Task Selection

Set Management

System Management

Washout

Load and Attach

Feed and Add

Release and Harvest

Custom

Close

Task

Configuration

Utilities

Pressure (mmHg)

Inlet

IC

0

0

Differential

0

EC

0

0

37.0 °C

37.8 °C

8-06-2013 16:18

Idle

TERUMOBCT

Setup Confirmation: Load Cells With Circulation

Modify

Modify

Modify

Number of Steps: 3

Load IC Loop: Step 1

Chase ARC: Step 2

Load Bioreactor: Step 3

IC Inlet	Cell	IC Media	IC Media
IC Inlet Rate (mL/min)	50	50	50
IC Circulation Rate (mL/min)	139	139	41
EC Inlet	None	None	None
EC Inlet Rate (mL/min)	0.00	0.00	0.00
EC Circulation Rate (mL/min)	30	30	30
Outlet	EC Outlet	EC Outlet	EC Outlet
Rocker	In Motion: -90° to 180° Dwell Time: 1 sec	In Motion: -90° to 180° Dwell Time: 1 sec	In Motion: -90° to 180° Dwell Time: 1 sec
Stop Condition	Empty Bag	IC Volume 22 mL	IC Volume 63 mL
Estimated Fluid Needed (L)	Unknown	IC Media: 0.1	IC Media: 0.1
Omit or Include Step	Include	Include	Include

Reset

Start

Cancel

8-06-2013 16:45

Idle

User ID: 1111

TERUMOBCT

Idle

Task

Configuration

Utilities

Sign Out

IC Inlet

None

IC Inlet Rate 0.00 mL/min

IC Circulation Rate 0.00 mL/min

IC

EC Inlet

None

EC Inlet Rate 0.00 mL/min

EC Circulation Rate 0.00 mL/min

EC

EC Fluid Detector

Pressure (mmHg)

Inlet

IC

0

0

Differential

0

EC

0

0

37.0 °C

37.8 °C

8-14-2013 13:08

Idle

User ID: 1111

TERUMOBCT

Feed Cells

Task

Configuration

Utilities

Feed Cells: Step 1/1

IC Inlet

IC Media

IC Inlet Rate 0.1 mL/min

IC Circulation Rate 20 mL/min

IC

Outlet

IC Outlet

EC Inlet

None

EC Inlet Rate 0.00 mL/min

EC Circulation Rate 30 mL/min

EC

EC Fluid Detector

Pressure (mmHg)

Inlet

IC

0

0

Differential

0

EC

0

0

37.0 °C

37.8 °C

Task Finished In Unknown

Stop Condition Manual

Pause

Modify

Finish

8-06-2013 15:31

Feed Cells Step 1/1

TERUMOBCT

Feed Cells

Task

Configuration

Utilities

Sign Out

Feed Cells: Step 1/1

IC Inlet

IC Media

IC Inlet Rate 0.1 mL/min

IC Circulation Rate 20 mL/min

IC

Outlet

IC Outlet

EC Inlet

EC Media

EC Inlet Rate 5.0 mL/min

EC Circulation Rate 30 mL/min

EC

EC Fluid Detector

Pressure (mmHg)

Inlet

IC

0

0

Differential

0

EC

0

0

37.0 °C

37.8 °C

Task Finished In Unknown

Stop Condition Manual

Pause

Modify

Finish

8-07-2013 12:58

Feed Cells Step 1/1

User ID: 1111

TERUMOBCT

# EXPERIMENT 1 | iMATRIX-511 / AK03N | FEED: IC | OUT: IC

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- DAY - 01
  - CELL EXPANSION SET INSTALLATION AND PRIMING
  - COATING BIOREACTOR | iMATRIX-511 (5 mg)
- DAY 00
  - SEEDING CELLS | 201B7 iPSC P43  $1.88\text{E}+07$  CELLS
- DAY 01 ~ 06
  - LACTATE MEASUREMENT (INDICATOR OF CELL PROLIFERATION)
  - FEEDING CELLS | IC:  $0.1 \sim 1.6 \text{ mL/min} = 144 \sim 2,300 \text{ mL/24 h}$
  - CELL HARVEST | ACCUMAX
- RESULTS
  - TOTAL CELL COUNT (ESTIMATED):  $4.92 \sim 5.66 \text{ E}+08$

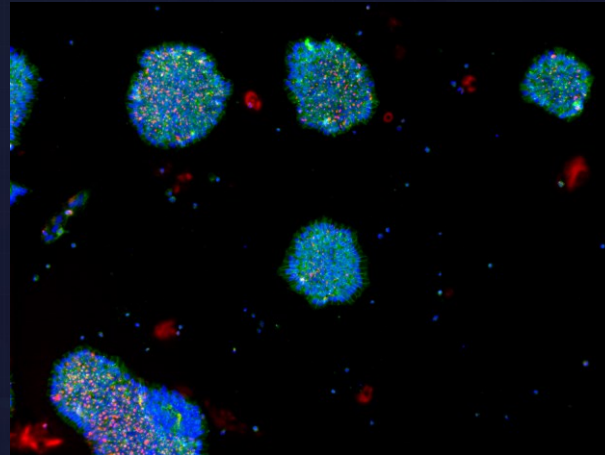
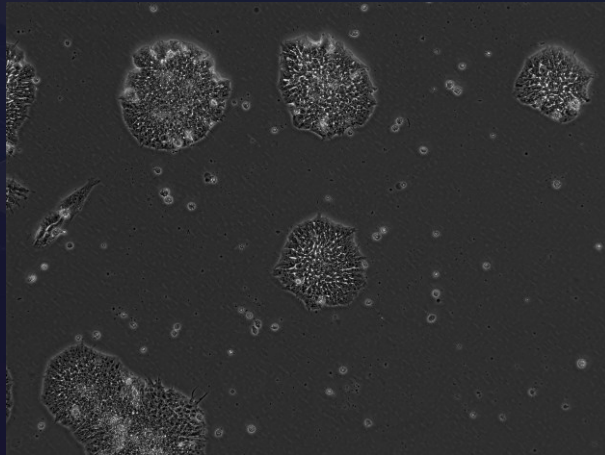
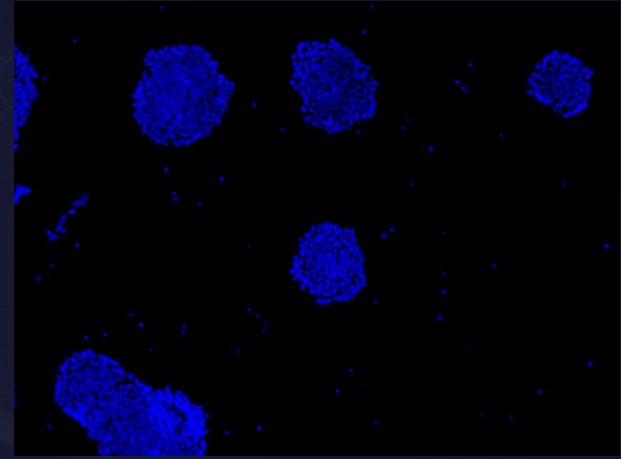
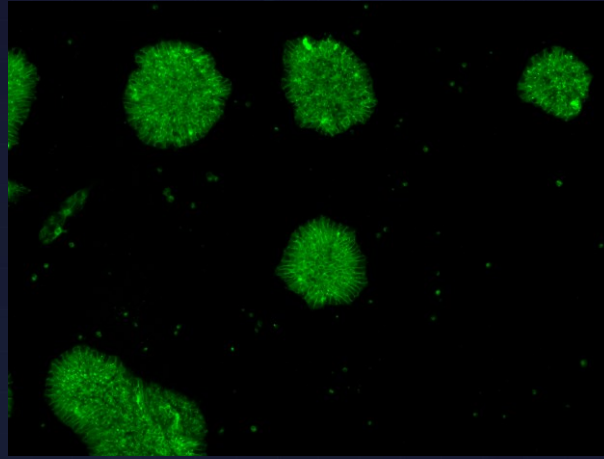
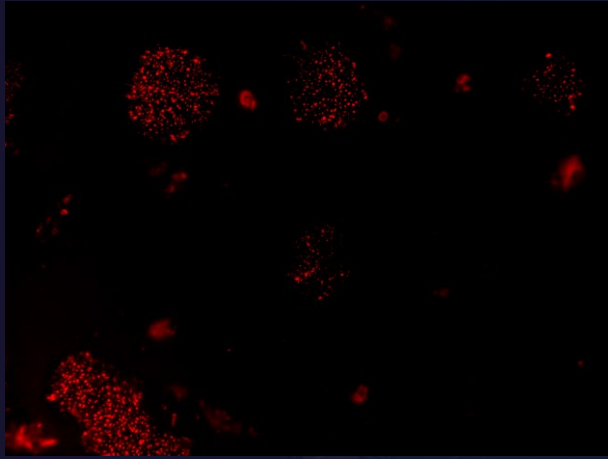
$1.88\text{E}+07$

$26 \sim 30\text{X}$

$4.92 \sim 5.66\text{E}+08$



# TRA-1-60 / SSEA4 LIVE STAINING | D05 AFTER HARVEST



10X

# EXPERIMENT 2 | LN 521 / mTeSR PLUS | FEED: IC | OUT: IC

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- DAY - 01
  - CELL EXPANSION SET INSTALLATION AND PRIMING
  - COATING BIOREACTOR | LAMININ 521 (5 mg)
- DAY 00
  - SEEDING CELLS: 201B7 iPSC | P26 |  $2E+07$  CELLS
- DAY 01 ~ 03
  - FEEDING CELLS | IC:  $0.1 \sim 0.3$  mL/min = 144 ~ 430 mL/24 h
  - AFTER INITIAL RISE, LACTATE LEVELS START TO DECLINE INDICATING CELL LOSS
- DAY 04 ~ 07
  - FEEDING CELLS | IC:  $0.3$  mL/min = 430 mL/24 h
  - CONTINUOUS DECLINE OF LACTATE LEVELS
  - EXPERIMENT TERMINATED

## WHY CULTURE FAILED?

HYPOTHESIS: BECAUSE OF THE WEAKER CELL ATTACHMENT IN LN521/mTeSR+ SYSTEM HIGH CIRCULATION RATE IN IC LOOP MIGHT HAVE CAUSED CELL DETACHMENT

# EXPERIMENT 3 | LN 521 / mTeSR PLUS | FEED: IC | OUT: IC

- DAY - 01
  - CELL EXPANSION SET INSTALLATION AND PRIMING
  - COATING BIOREACTOR | LAMININ 521 (5 mg)

## • DAY 00

- SEEDING CELLS: 201B7 iPSC | P27 |  $2\text{E}+07$  CELLS

## • DAY 01 ~ 03

- FEEDING CELLS | IC:  $0.1 \sim 0.3$  mL/min =  $144 \sim 430$  mL/24 h
- AFTER INITIAL RISE, LACTATE LEVELS START TO DECLINE INDICATING CELL LOSS

CELLS FEEDING STRATEGY CHANGE – FEED: EC | OUT: EC

## • DAY 04 ~ 10

- FEEDING CELLS | EC :  $0.3 \sim 2.0$  mL/min =  $144 \sim 2,900$  mL/24 h
- CELL HARVEST | EDTA

## • RESULTS

- TOTAL CELL COUNT:  $5.2\text{E}+08$

FEED: EC | OUT: EC

$\sim 1\text{E}+07$  (ESTIMATED)

$\sim 50\text{X}$

$5.2\text{E}+08$

# PROTOCOL OPTIMIZATIONS AND NEXT PLANS

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- FINDING OPTIMAL FEEDING STRATEGY
  - INLET: IC / EC
  - OUTLET: IC / EC
  - FULL MEDIUM IN IC LOOP / BASAL MEDIUM IN EC LOOP
  - SLOW-RELEASE FGF2
- ESTIMATION OF MINIMAL REQUIRED CELL NUMBER
  - SEEDING CELLS |  $\sim 1E+06$
- SAMPLING MATERIAL FROM IC LOOP
  - BETTER ASSESSMENT OF CULTURE STATUS
- EXTENSIVE QUALITY CHECK OF CELLS EXPANDED IN TERUMO QUANTUM SYSTEM
  - GENETIC STABILITY
  - PLURIPOTENCY

# CONCLUSIONS

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- ALTHOUGH SYSTEM EVALUATION IS STILL IN PROGRESS, WE ARE CONVINCED THAT QUANTUM® IS PLATFORM SUITABLE FOR EXPANSION OF iPS CELLS
- HOLLOW FIBER BIOREACTOR WITH TWO INDEPENDENT CIRCULATION LOOPS OFFERS INTERESTING OPTIONS FOR FEEDING PROTOCOL OPTIMIZATION
- WE ARE PLANNING TO USE TERUMO BCT QUANTUM® CELL EXPANSION SYSTEM IN OUR CELL PROCESSING CENTER
- WE HOPE THAT COLLABORATION WITH TERUMO BCT WILL RESULT IN THE SIGNIFICANT IMPROVEMENT OF THE PLATFORM

# ACKNOWLEDGEMENTS

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THE AUTHORS WOULD LIKE TO THANK TERUMO BCT JAPAN FOR PROVIDING QUANTUM® CELL EXPANSION SYSTEM AND TECHNICAL ASSISTANCE FOR THIS STUDY.

THIS RESEARCH WAS SUPPORTED BY JAPAN AGENCY FOR MEDICAL RESEARCH AND DEVELOPMENT (AMED) UNDER GRANT NUMBER JP20bm0104001.



The background is a dark blue gradient with a subtle grid pattern. It features several abstract elements: a vertical column of hexagons on the left, a large wavy line curving across the middle, and several molecular or network-like structures composed of circles and lines. The text "THANK YOU" is centered in a white, sans-serif font.

THANK YOU