THE 20TH CONGRESS OF THE JAPANESE SOCIETY FOR REGENERATIVE MEDICINE

LARGE-SCALE EXPANSION OF iPS CELLS IN TERUMO BCT QUANTUM® CLOSED AUTOMATED CELL CULTURE SYSTEM

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CiRA FOUNDATION

FACILITY FOR iPS CELL THERAPY

CiRA Foundation

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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

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.

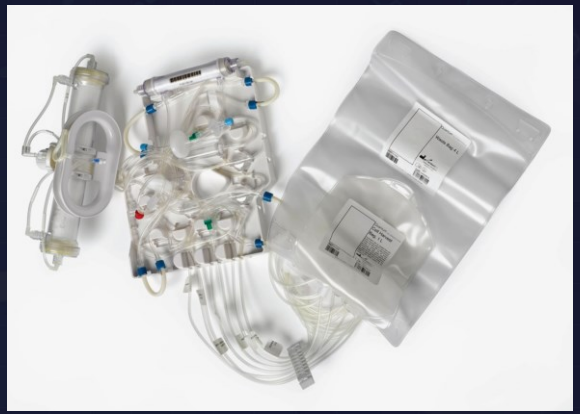
TERUMO BCT QUANTUM® IS AN AUTOMATED, FUNCTIONALLY CLOSED PLATFORM DESIGNED TO SIMPLIFY TASKS ASSOCIATED WITH MANUAL CELL CULTURE. IT HAS BEEN USED FOR THE SCALABLE OF THE HUMAN MESENCHYMAL STROMAL CELLS (hMSCs).

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.

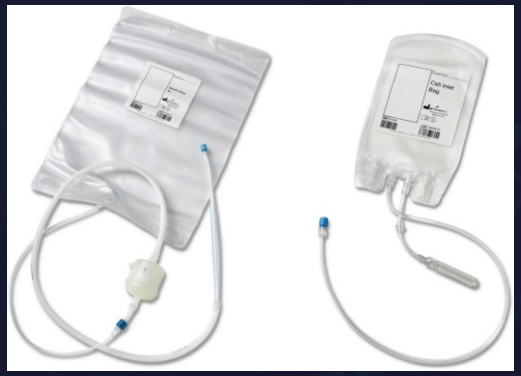
THE QUANTUM® INSTRUMENT USED IN THIS STUDY WAS KINDLY PROVIDED BY TERUMO BCT JAPAN, INC.

QUANTUM® CELL EXPANSION SYSTEM

QUANTUM DEVICE



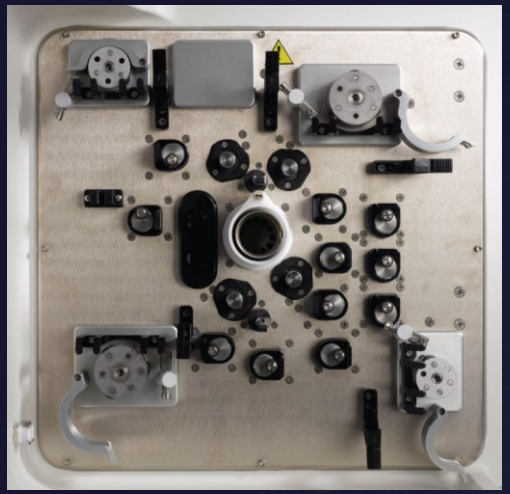
CELL EXPANSION SET

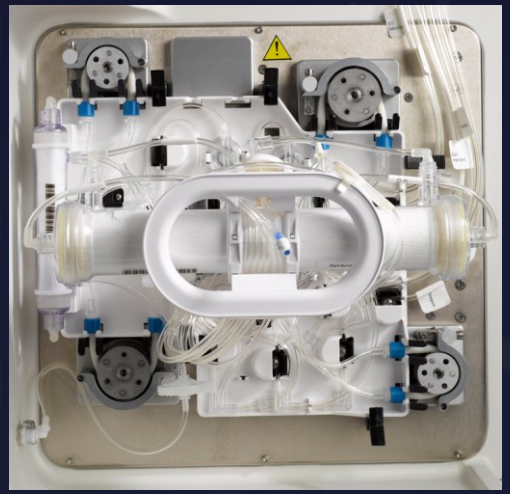


ACCESSORY SETS

TERUMOBCT

INSIDE THE INCUBATOR

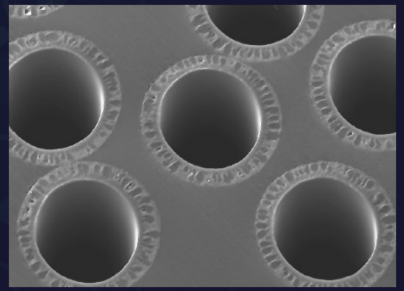




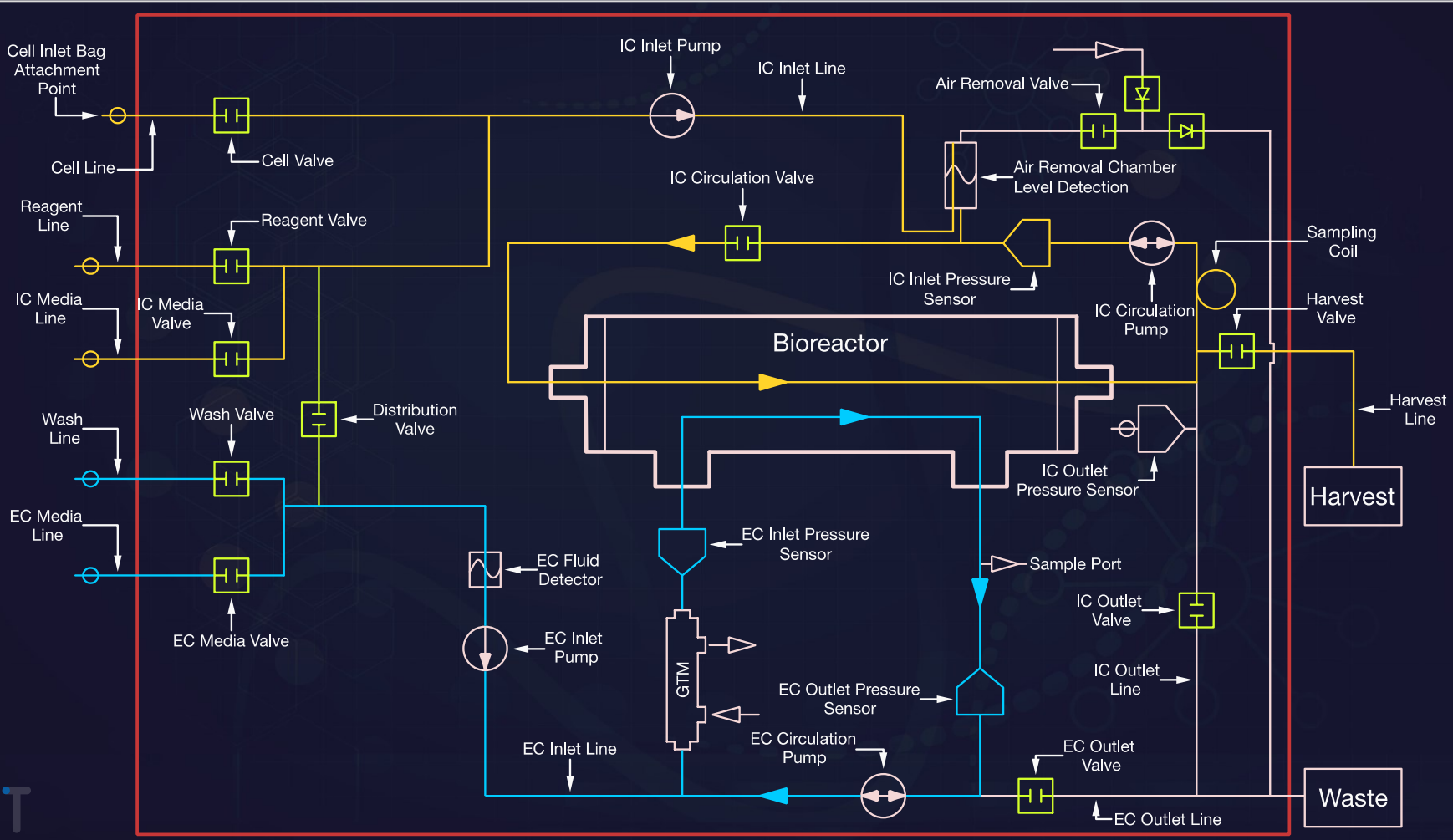
~ 10,000 SEMI-PERMEABLE HOLLOW FIBERS (Φ ~200 µm)

~ 2.1 m^2 OF SURFACE AREA

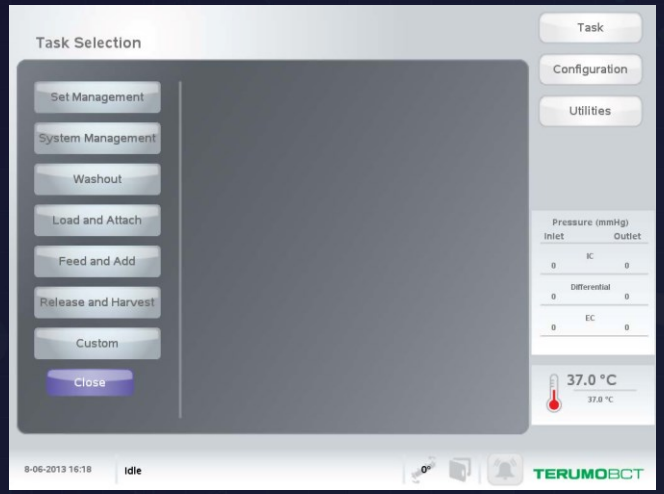
INTRACAPILLARY (IC) & EXTRACAPILLARY (EC) COMPARTMENTS

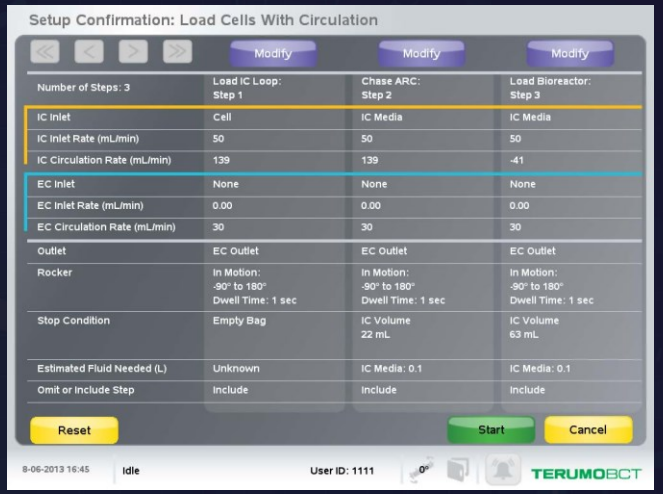


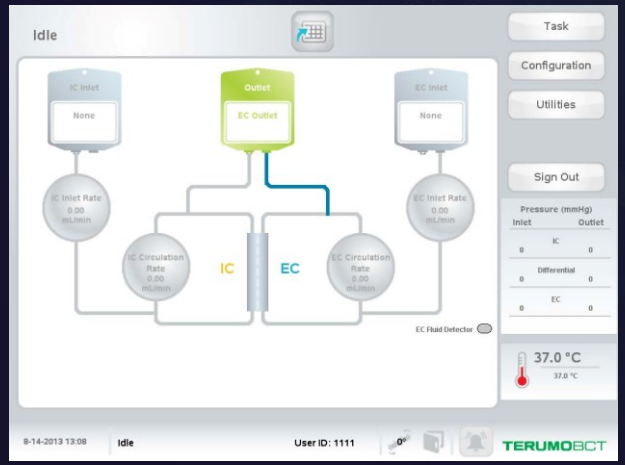
HYDRAULIC LAYOUT

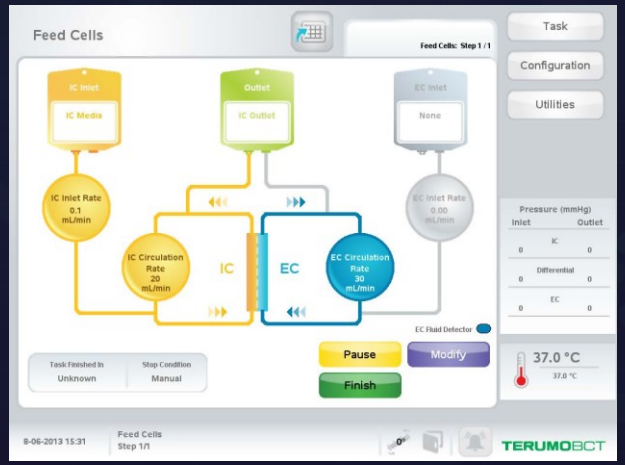


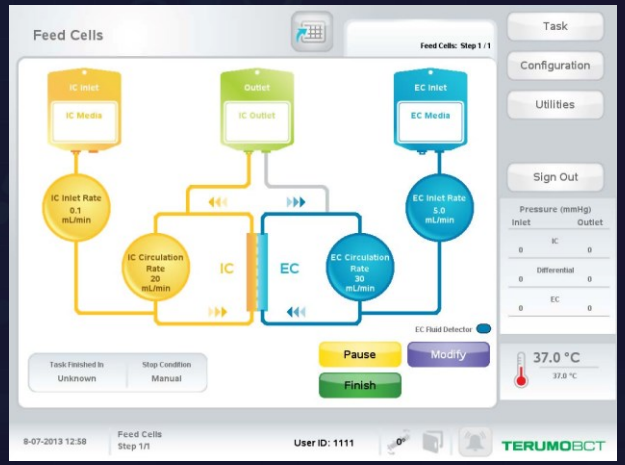
CONTROL SOFTWARE





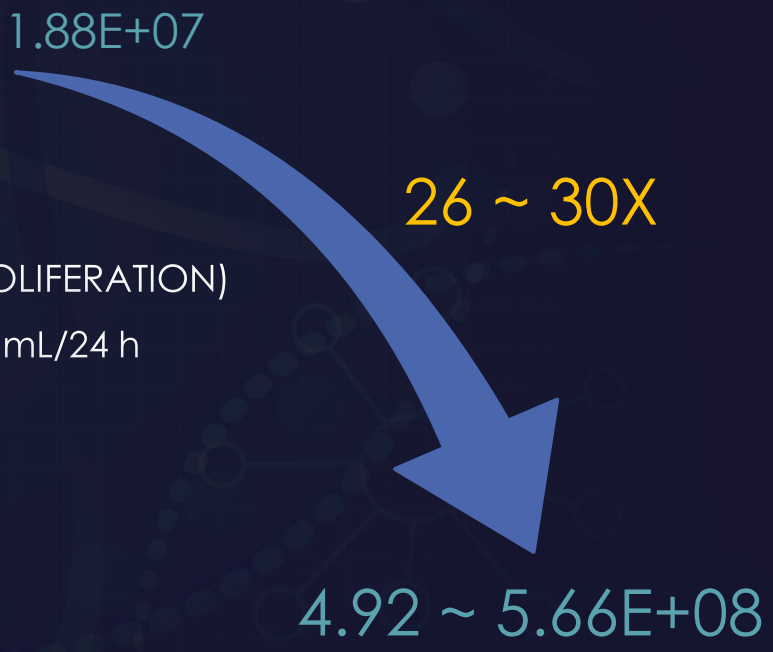




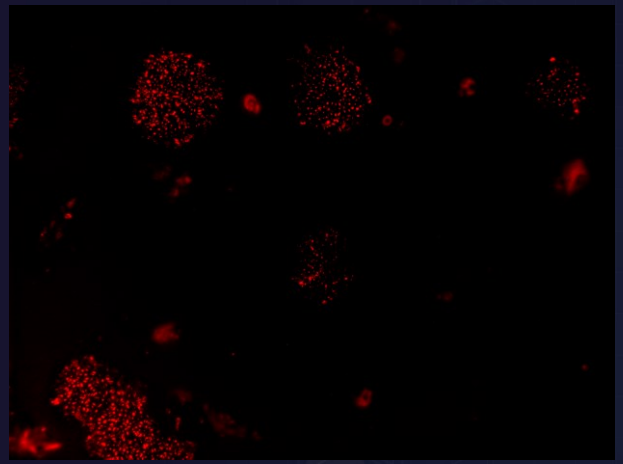
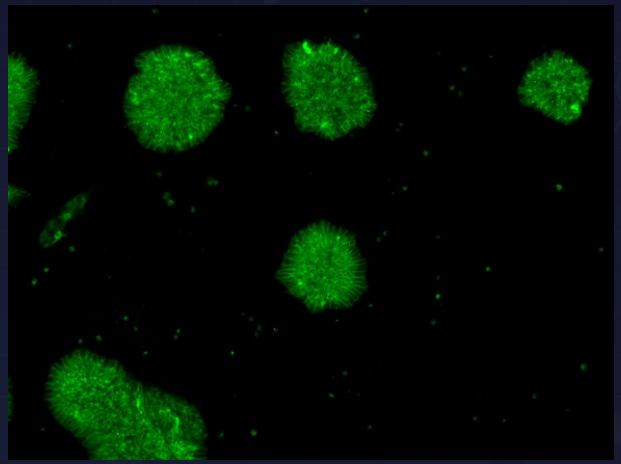
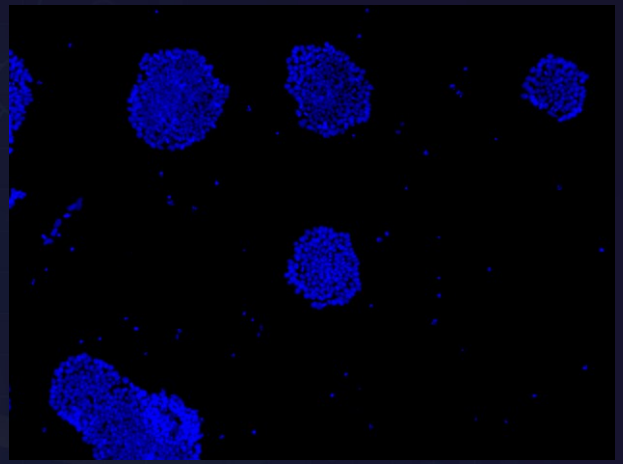
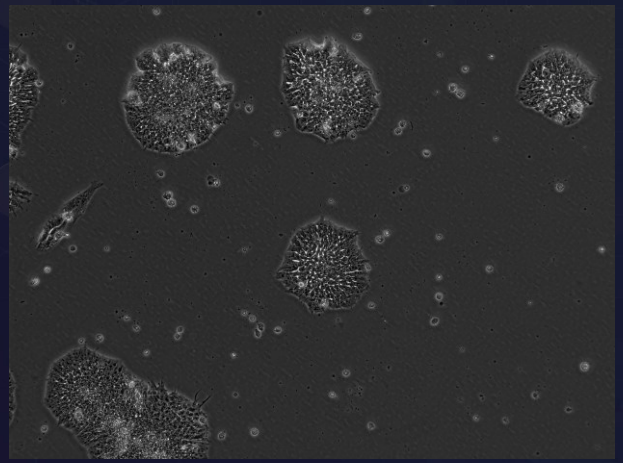
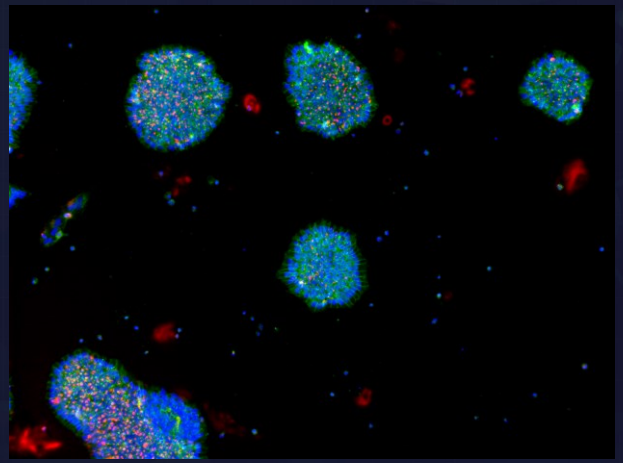


EXPERIMENT 1 | iMATRIX-511 / AK03N | FEED: IC | OUT: IC

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



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

10X

201B7_iPSC_P44_TER_A2-07_200406_D05_CH1-2-3-4_02

EXPERIMENT 2 | 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 | P26 | 2E+07 CELLS
- DAY 01 ~ 03
  - FEEDING CELLS | IC: 0.1 ~ 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 | 2E+07 CELLS
- DAY 01 ~ 03
  - FEEDING CELLS | IC: 0.1 ~ 0.3 mL/min = 144 ~ 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 ~ 2.0 mL/min = 144 ~ 2,900 mL/24 h
  - CELL HARVEST | EDTA
- RESULTS
  - TOTAL CELL COUNT: 5.2 E+08

FEED: EC | OUT: EC

~ 1E+07 (ESTIMATED)

~ 50X

5.2E+08

PROTOCOL OPTIMIZATIONS AND NEXT PLANS

- 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 | ~ 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

- 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

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

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THANK YOU