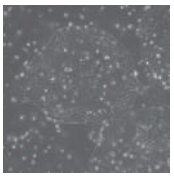
Freezing and Thawing experiment for large volume of human iPS cell-derived cardiomyocytes

Upon the development of cell-processed products and regenerative medical products, scale-up of cell culture process is considered to be essential. However, there is concern that to achieve scale-up could affect the efficiency of cell culture or other processes. Besides, in case of using autologous cells, the cells can't be used for treatment immediately because it takes long time to cultivate after collection. For this reason, allogeneic cells (ES cells, iPS cells), cells donated by donors have been attracting attention recently. By collecting and cultivating a lot of cells from donors beforehand, they can be used for treatment sooner. This time we evaluate cryopreservation of human iPS cell-derived cardiomyocytes for treatment volume based on the success of cryopreservation of undifferentiated iPS cells at preliminary experiment. Also, we used CryoMACS Freezing Bag and ThawSTAR CB the same as preliminary experiment.

Preliminary experiment

Conditions

- 【Cells】 undifferentiated human iPS cells
- 【Volume】 70 mL cell suspension
- 【Concentration】 5×10^6 cells/mL
- 【Freezing medium】 STEM-CELLBANKER®
- 【Freezing period】 approx. 1.5 months
- 【Bag】 CryoMACS Freezing Bag 250 mL

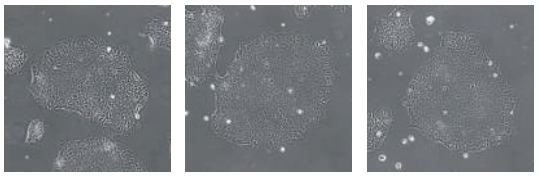


Before freezing

CryoMACS Freezing Bag after thawing with ThawSTAR CB

Results

- 【Thawing time】 30 min. (47 x 2 mL tubes : 90 min.)
- 【Viability after thawing】 95.5%
- 【Proliferation rate after thawing】
  Seeded cells : 0.1×10^6 cells, Culture period : 4 days
  ・250 mL bag : 2.55 times
  ・Control 1 tube : 2.49 times
  ・47 × 2 mL tubes : 1.85 times



47 tubes　250 mL bag　Control(1 tube)

Conclusion In large scale of freezing and thawing, using bag allows us to increase work efficiency and reduce damage to cells.

Experiment

In this experiment, cardiomyocytes derived from human iPS cells were used, and we froze and thawed the cells twice.

ThawSTAR CB

- 【Cells】 Cardiomyocytes derived from human iPS cells
  Cells were collected in 15-17 days after differentiation.
- 【Volume】 20 mL cell suspension
- 【Concentration】 5×10^6 cells/mL
- 【Freezing period】 1-2 weeks at -150 C
- 【Bag】 CryoMACS Freezing Bag 50 mL
  50 mL bag is equivalent to 14 × 2 mL tubes (total liquid volume : 21 mL)

1	2	3	4	5
Create and paste a label	Freezing	Cryopreservation	Thawing	Cell culture

- Working time and post-thawing viability

∗ 14 tubes (total liquid volume : 21 mL)

1	2	4	
7.5 min.	6 min.	60 min.	Total : 1 hour or more　Viability: 94%

∗ 50 mL bag(liquid volume : 20 mL)　1st experiment

1	2	4	
3.6 min.	3 min.	10 min.	Total : 20 minutes or less　Viability: 91%

∗ 50 mL bag(liquid volume : 20 mL)　2nd experiment

1	2	4	
3.6 min.	3 min.	10 min.	Total : 20 minutes or less　Viability: 91.5%

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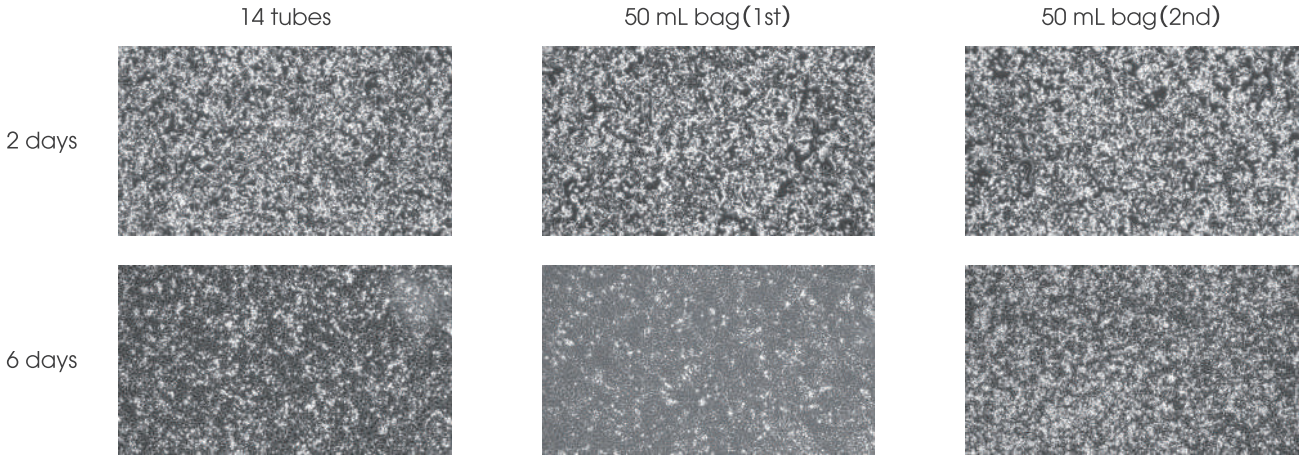
5 Evaluation after cell culture

Post-thawing adhesive culture

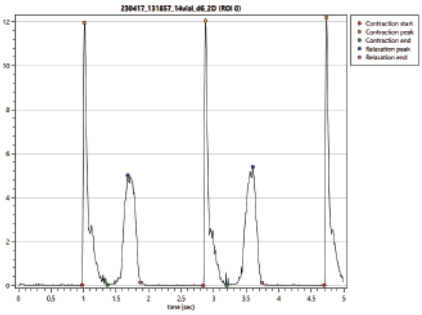


Adhesiveness of 50 mL bag cells was equivalent to that of 14 tubes. There was no difference.

Motion analysis(SONY SI8000) : After 6 days of post-thawing adhesive culture

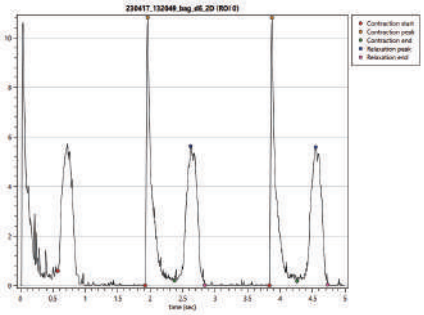
14 tubes

Beat rate: 32.3/min



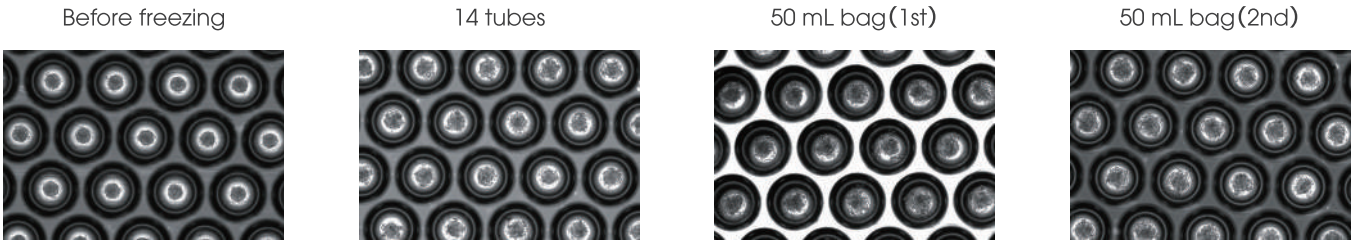
50 mL Bag

Beat rate: 31.4/min



Beat rate of 50 mL bag cells was equivalent to that of 14 tubes. There was no difference.

Ability to generate cardiac spheroids



Ability to generate cardiac spheroids using 50 mL bag cells was equivalent to that using 14 tubes. There was no difference.

DATA : provided by the research group of Dr. Shugo Tohyama, Department of Cardiology, Keio University School of Medicine

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