Form 3

Abstract of Thesis

Name （ Nath Suman Chandra）	
Title	Operational Design for High-density Culture of Human Induced Pluripotent Stem Cells in Suspension (ヒト人工多能性幹細胞の懸濁高密度培養のための操作設計)

Abstract of Thesis

Large numbers of human induced pluripotent stem cells (hiPSCs) are required for cell therapy applications. Suspension culture of hiPSCs is superior to obtain large numbers of cells in comparison to adherent culture since high cell number (10^8-10^9 cells), as well as better control of process parameters can be obtained in stirred suspension bioreactor. In the conventional suspension culture, hiPSCs show initial cell loss during seeding due to single cell dissociation, low growth rate due to aggregate heterogeneity, and size limitation during late-stage of culture which results in low cell density. Moreover, toxic metabolites (lactic acid, ammonium, etc.) accumulate in the culture medium which requires frequent medium change which along with remove autocrine factors from medium in conventional suspension culture. Since autocrine factors potentiate the growth of hiPSCs, removal of these components from culture medium also results in low cell density in suspension culture. By considering these limitations, this study aimed at establishing a simple and scalable method by improving the operational design of hiPSC culture for obtaining high cell density with efficient recycling of autocrine factors in suspension.

In chapter 1, size- and time-dependent growth properties of hiPSC aggregate was investigated for setting up a boundary condition in which hiPSCs can maintain high growth rate in suspension culture. hiPSCs showed size-dependent growth heterogeneity during early-stage, and time-dependent collagen type I accumulation on the aggregate periphery during late-stage of culture. Thus, considerations for initial cell number and culture time were important to maintain the balance between the secretion of autocrine factors, and the accumulation of extracellular matrix on the aggregate periphery to achieve preferable growth of hiPSCs in single aggregate culture.

In chapter 2, a simple method for *in situ* break-up of hiPSC aggregate was established by revealing the temporary effect of botulinum hemagglutinin (HA) for the disruption of E-cadherin mediated cell-cell connections in suspension culture. This method allowed high-density culture of hiPSCs through the break-up of aggregates into small sizes without initial cell loss during seeding in suspension culture. It also provided the simplification of culture operations without enzymatic treatment or washing in suspension culture.

In chapter 3, a culture medium refinement method was established by using a dialysis filter by which toxic metabolites (lactic acid, ammonium, etc.) was removed from the medium, and bFGF, TGF-β1, and other autocrine factors were recycled efficiently for the expansion of hiPSCs in suspension culture. This method allowed expansion of hiPSCs by changing the medium only once and recycling growth factors, as well as other autocrine factors that promoted a cost-effective platform in suspension culture.

In chapter 4, medium refinement system was combined with aggregate break-up by HA during late-stage of culture for obtaining high-density of hiPSCs in suspension. By using this system, higher growth rate as well as higher cell density was obtained by a minimum supplementation of growth factors which provided 21-times higher yield on culture medium in comparison to the conventional suspension culture.

Overall, a simple and scalable method for high-density culture of hiPSCs was established by setting up a boundary condition for maintaining high growth rate, and breaking up the aggregates into small sizes with HA in suspension culture. This method also provided the simplification of passage operations without enzymatic treatment or washing in suspension culture. Moreover, the integration of medium refinement system with aggregate break-up by HA provided high-density of hiPSCs with high yield on culture medium by efficient recycling of autocrine factors in suspension culture. Thus, the improved operational design of hiPSC culture provided a cost-effective platform for obtaining high cell density with the simplification of passage operations which will promote simple closed-system for large-scale expansion of hiPSCs in suspension culture.

論文審査の結果の要旨及び担当者

氏　名	（　Nath Suman Chandra　）	
	（職）	氏　名
論文審査担当者	主 査　教授	紀ノ岡　正博
	副 査　教授	福崎　英一郎
	副 査　教授	渡邉　肇
	副 査　教授	村中　俊哉
	副 査　教授	大政　健史
	副 査　教授	藤山　和仁
	副 査　教授	仁平　卓也
	副 査　教授	永井　健治

論文審査の結果の要旨

以上のように、本論文はヒト iPS 幹細胞の懸濁培養時において，細胞集塊形成および集塊径制御の観点から，高密度懸濁培養の実現を報告している．

第 1 章では，初期の細胞数が異なる細胞集塊を個別に培養することで，集塊細胞数と増殖能力の関係を示し，増殖能力が，集塊細胞数と培養時間それぞれに依存し，集塊細胞数が最適な範囲が存在することを見出した．よって，懸濁培養においては，集塊細胞数の制御が必要であることを結論付けた．

第 2 章では，増殖の低下を引き起こす大きな集塊を，ボツリヌス菌由来のヘマグルチニン（HA）の添加により，集塊を小さくすることを見出した．特に，HA 添加後 9 h において，集塊崩壊することが分かり，その後，HA による崩壊効果は消滅することが分かった．この一時的な細胞集塊崩壊効果は，集塊崩壊後の培養液からの HA 除去操作を不要とし，細胞集塊を崩壊・形成を繰り返すことによる高密度培養を簡便い実施できることを示唆した．

第 3 章では，高密度培養を行う際の老廃物処理ならびに高価値成分の最少添加を実現するために，透析を伴う培養システムを構築した．本システムにより，乳酸，アンモニアの毒性成分の除去およびサイトカイン（bFGF や TGF-β の）の高価値成分の最少添加を実現し，高密度培養でも，必須成分の枯渇を防ぐことができた．

第 4 章では，これまでの知見を統合し，高密度培養システムを構築した．特に，培養中，HA 添加にて，細胞集塊崩壊を引き起こすことで集塊細胞数を制御し，かつ，透析による培地成分の保持により，増殖能を高く維持し，高密度培養を実現した．

以上のように、本論文は細胞集塊の増殖特性の理解ならびに集塊制御に基づく培養法によって，ヒト iPS 細胞の高密度培養を実現する統合システムを提案している．よって本論文は博士論文として価値あるものと認める。