

論文の内容の要旨

論文題目 Development of High Density 3D Dynamic Suspension Culture for Human Induced Pluripotent Stem Cells Expansion and Hepatic Differentiation
(ヒト人工多能性幹細胞の増殖と肝分化のための高密度三次元的懸濁培養法の開発)

氏 名 F u a d G a n d h i T o r i z a l

ファド ガンジ トリザル

Human induced pluripotent stem cells (hiPSCs) hold a great potential in wide range area of various application regarding their capability in differentiating into any kind of human cells. In order to achieve this application, a higher amount of the cells were required for industrial or clinical use. Dynamic suspension culture is one of the methods to generate the higher amount of hiPSCs which preserved better pluripotent capability by stimulating the cell-cell interaction which plays an important role both in cellular growth and differentiation.

The first chapter introduced the potential of the hiPSCs to generate the hepatocyte like cells (iHeps) as a source of hepatocyte for various application, such as regenerative medicine or cell based assays. Despite of its advantages, several problem still persist regarding the expansion and differentiation efficiency. The inefficient usage of growth factor cause the requirement of an expensive cost. Although the cost reduction can be minimized by using the suspension culture, their dynamic culture environment rise a problem related to mechanical stress. In this study, we address these problem by developing the high density suspension culture to fully utilize the cellular self maintenance by their native secreted substance (Cytokine or growth factors) to reduce the external growth factor usage while continuously remove the toxic cellular product and continuously deliver the micromolecule nutritions. In order to achieve

the low mechanical stress environment, gellan gum biopolymer was added to the culture medium in combination with multicompartment dialysis system.

Chapter 2 describe the utilization of dialysis culture and gellan gum solution in culture medium for high density hiPSCs expansion in rotational culture. Our initial study show that the miniaturized dialysis culture system showing good performance to selectively retain the macromolecule at the culture compartment while continuously exchange the micromolecule between two compartment. In addition, the gellan gum supplementation in culture medium represent its capability to reduce the cellular injury caused by excess mechanical stress without affecting the permeability of the dialysis membrane. By using this combined culture system, the high density (HD) hiPSCs expansion was resulted 8 times higher cell yield compared to the conventional culture system. This decent proliferation achieved by decent maintenance of culture environment by continuous toxic metabolite micromolecule removal and supply nutrition through medium refinement of dialysis culture. By retain the circulating growth factor both from natively secreted by the cells and external supplementation, the larger number of the hiPSCs can be produced by administrating the equivalent dose of growth factor as conventional suspension culture. In consequence, the cost reduction can be reduced up to approximately 8 times from conventional suspension culture. Moreover, the hiPSCs expanded in high density culture exhibit improved pluripotency preservation and differentiation capability.

Chapter 3 describe the optimization of hepatic differentiation based on single spheroid size generated from different cell number by using ultra low attachment 96 well plates. The morphological alteration resulted from smaller inoculated spheroid (less than 250 μm in diameter) which turned into cyst like structure followed by decrease in cell yield and downregulation of mature hepatic marker. Based on previous study, the failure of Wnt/ β -catenin inhibition at hepatoblast differentiation stage. Our result indicated that the Wnt/ β -catenin

signaling was upregulated in iHeps generated from smaller spheroid followed by suppression of hepatic nuclear factor HNF4 α which responsible for directing the differentiation into hepatic lineage. Based on this finding, we can obtain the best result when initiated the differentiation with more than 250 μ m hiPSCs spheroid. It is also revealed that the hepatic differentiation performed by using the three dimensional structure can significantly reduce the tendency to shifting the hepatic differentiation into cholangiocyte lineage which improving the hepatic differentiation.

In chapter 4, we describe the utilization of high density hiPSCs culture from chapter 2 for hepatic differentiation by using the optimized spheroid size to initiate the differentiation as described in chapter 3 by adapting the three dimensional hepatic differentiation with specific stage Wnt/ β -catenin signaling inhibition. The investigation of the effect of gellan gum supplementation was confirmed before we perform the high density hepatic differentiation. Decent growth and differentiation performance was resulted from the iHeps group with gellan gum supplementation. Afterwards, with the similar principles with the HD expansion, the HD hepatic endoderm differentiation was performed and resulted at approximately 7 times higher than conventional suspension culture with the increasing differentiation capability at higher density. This indicate that the utilization of the circulated growth factor can be effectively reduce the necessity of growth factor supplementation which also impacted the significant cost reduction up to 7 times.

Chapter 5 summarize the overall conclusion and future perspectives of this study. The environmentally controlled high density culture by dialysis and gellan gum was showing their capability to preserve the pluripotency and enhanced the differentiation by minimum growth factor usage. In general, this study provide efficient methodologies and culture platform design for the cost effective, high density, scalable, and decent cellular quality. The high-density

culture by dialysis and gellan gum biopolymer can be extended in larger scalable hiPSCs expansion or differentiation might be important to obtain a large number of expected yields for the actual purpose. In order to address the problem in oxygenation, nutrient transfer, toxic metabolite removal, full utilization of cellular self-maintenance, and spheroid uniformity the larger scalable culture system such as our proposed O-shaped dialysis culture system design in combination with low acyl gellan gum can be applied to achieve low shear stress culture environment for the hiPSCs expansion and differentiation to realize the clinical and industrial application such as liver regeneration.