

Culture medium, extracellular components and differentiation in culturing of human pluripotent stem cells

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Introduction

The cultivation of human pluripotent stem cells (hPSCs) has become an important factor in regenerative medicine and the development of drugs. Similar to other mammalian cells, human pluripotent stem cells are important in culture for the formation of microenvironment, including intercellular interactions, growth factors, and contact between cells and matrix [1]. The culture of stem cells is similar to that of standard mammalian cell culture, but differentiation or maintenance of undifferentiated state may be necessary for the purpose. Phenotypic characteristics in pluripotent stem cells (morphology, colony assessment, pluripotent potential) and in differentiated cells (morphology, differentiation markers, functionality) should be evaluated [1].

Common method used for mammalian cells such as selection of culture methods, culture medium exchange, subculture, cryopreservation, and thawing frozen stocks is needed for cultivation of hPSCs. However, there are many problems to be solved in relation to culturing hPSCs, despite its commonality with this general culture and its active use in regenerative medicine. (i) a lack of standardized protocols for cell culture, (ii) an absence of efficient assays of difficulties in growth, apoptosis, and spontaneous differentiation signals, (iii) impurities or heterogeneity arising during cell growth, and (iv) genomic instability related to chromosomal abnormalities and (v) potential tumorigenicity [2,3].

In this content, we will look at the characteristics, culture medium, extracellular components and differentiation in culturing of hPSCs culture

Basic characteristics of in vitro hPSCs culture

In order to maintain stem cell culture in vitro, it is necessary to provide an environment that imitates in vivo conditions to perform metabolic and biochemical processes while maintaining the phenotype and characteristics of the cells. Cell proliferation and differentiation have opposite actions, and cell proliferation tends to decrease in order to obtain differentiated cells [1].

Culture medium

Serum-using culture medium

The first-generation hPSCs medium generally used fetal bovine serum (FBS) and secretory components from mouse embryonic fibroblasts (MEFs) [3]. In particular, FBS is the most common growth-providing substance that provides a growth component in cell culture media. Advantages of serum use include important nutrients, growth factors, hormones, attachment factor trypsin inhibitors and protection agent, and serve as cryoprotectant in cryopreservation. Disadvantages

are that the components are not constant because the content of specific components is different for each unit of product, the standardization of product is difficult, animal-derived components is present, and it is difficult to predict the cell growth because it contains growth-inhibitory and growth-promoting activity [4]. The plating efficiency test used to compare cell growth promotion between serum batches is a relatively simple and widely used method. Human serum is used only for special purposes because it has the potential risk of human pathogenic viruses such as human immunodeficiency virus (HIV) or hepatitis C virus. Serum can be produced by specifying the maximum allowable levels of serum components such as immunoglobulins (cell growth inhibitory effects), endotoxins (indicators of bacterial contamination, strong mitogen), hemoglobin (a toxic contaminant indicative of hemolysis during the production of the serum) [4].

Serum-free culture medium

The disadvantages of the serum medium led to the development of serum-free medium, protein-free medium and chemically-defined medium.

Additives

In stem cell culture, proteins, hormones and growth factors and hydrolysates may be added to the basal medium. Proteins such as insulin, transferrin and serum albumin can be purified from animal sources or recombinantly produced in bacteria, yeast or plants and added for growth and differentiation of stem cells. Purified growth factors such as activin, platelet derived growth factor (PDGF), fibroblast growth factor (FGF), and epithelial growth factor (EGF) are often added to the stem cell culture medium [4].

Medium replenishment

Depletion of essential nutrients and the increase of acidic metabolites resulted in cell growth inhibition. Therefore, it is necessary to plan and practice the replacement (e.g. frequency and amount of medium) and timely passaging (e.g., split ratio).

Use of antibiotics

If appropriate facilities, equipment, sterile reagents and aseptic techniques are used, there is no need to use antibiotics during cell

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culture. Antibiotics are not routinely used to check for special cases such as culturing primary cells with high potential for contamination or mycoplasma contamination [4].

Extracellular components

Extracellular components contain a variety of organic matrices derived from animal cells, hydrogels, individual matrix proteins, synthetic surfaces, and xenogeneic-free materials. The main commercially available products include CELLstart™ (Invitrogen Inc.), which is produced only in human cells, StemAdhere™ (Primorigen Biosciences Inc.), which has a matrix with fully defined human proteins, Synthemax® with a unique synthetic peptide acrylate-R Surface (Corning Inc.). Matrigel-Matrigel is one of the most widely used extracellular components of feeder-free cultures of hPSCs. It is a basement membrane matrix rich in I, IV collagen, laminin, entactin, heparan sulfate proteoglycan, matrix metalloproteinases, undefined growth factors and chemical compounds [5]. It is not suitable for culturing hPSCs for clinical use. Many extracellular matrix proteins can be used to determine hPSCs self-renewal or lineage commitment. Vitronectin and specific laminin help maintain hPSCs self-renewal [3].

Differentiation

Verifying of the pluripotent potential

Teratoma assay: The standard method for verifying the pluripotent potential of stem cells is the teratoma assay. However, this method is costly, time consuming, reproducibility problems and requires special expertise [4].

Embryoid body (EB) formation: Embryoid bodies are three-dimensional aggregates of pluripotent stem cells. If there is no anti-differentiation factor and the bottom of the culture flask is coated with an agar which is not easy to adhere cells, embryonic bodies form spontaneously in suspension culture [6]. Embryoid body formation is commonly used to confirm the pluripotency of human ESC and iPSCs and assesses the expression of specific genes and proteins in three embryonic layers [7,8]. The markers that indicate ectodermal differentiation are SRY (Sex Determining Region Y) -box 1 (Sox1), paired box 6 (Pax6), neural cell adhesion molecule (NCAM) and neuroectodermal stem cell marker (Nestin). Endodermal commitment markers include α -fetoprotein, cytokeratin's, somatostatin, bone morphogenetic protein 4 (BMP4), GATA binding protein 4 (GATA4), hepatocyte nuclear factor-4, and markers indicative of mesodermal differentiation include brachyury, α -cardiac actin, and the atrial natriuretic factor [4].

Factors affecting differentiation

Protocols: HPSCs can be differentiated into specific lines by differentiation protocols using a variety of differentiation culture media, matrices. Control of in vitro oxygen concentration, culture

structure (single layer /2D vs. suspension/3D), pathway modulator and a transcriptional memory of the cells may also affect differentiation [9-11].

Methods of passaging and culturing: Methods of passaging and culturing undifferentiated PSCs may impact their differentiation efficiency [8].

Transcriptional memory of the cells of origin: Transcriptional memory of the cells of origin may be retained in iPSCs at low-passages, which may affect their propensity to differentiate into specific lineages [12].

Importance of defined medium and matrix

To create a reproducible differentiation protocol, uncontrollable variables such as serum and co-culture must be removed, and preference should be given to use of the defined medium and matrix components.

References

1. Pames D, Bal-Price A, Simeonov A, Tagle D, Allen D, et al. (2017) Good cell culture practice for stem cells and stem-cell-derived models. *ALTEX* 34: 95-132. [[Crossref](#)]
2. Coecke S, Balls M, Bowe G, Davis J, Gstraunthaler G, et al. (2005) Guidance on good cell culture practice. a report of the second ECVAM task force on good cell culture practice. *Altern Lab Anim* 33: 261. [[Crossref](#)]
3. Chen KG, Mallon BS, McKay RD, Robey PG (2014) Human pluripotent stem cell culture: considerations for maintenance, expansion, and therapeutics. *Cell stem cell* 14: 13-26. [[Crossref](#)]
4. Pames D, Bal-Price A, Simeonov A, Tagle D, Allen D, et al. (2017) Good Cell Culture Practice for stem cells and stem-cell-derived models. *ALTEX* 34: 95-132. [[Crossref](#)]
5. Kleinman HK, McGarvey ML, Hassell JR, Martin GR (1983) Formation of a supramolecular complex is involved in the reconstitution of basement membrane components. *Biochemistry* 22: 4969-4974.
6. Kurosawa H (2007) Methods for inducing embryoid body formation: in vitro differentiation system of embryonic stem cells. *J Biosci Bioeng* 103: 389-398. [[Crossref](#)]
7. De Miguel MP, Fuentes-Julián S, Alcaina Y (2010) Pluripotent stem cells: origin, maintenance and induction. *Stem Cell Rev* 6: 633-649. [[Crossref](#)]
8. Pistollato F, Bremer-Hoffmann S, Healy L, Young L, Stacey G (2012) Standardization of pluripotent stem cell cultures for toxicity testing. *Expert Opin Drug Metab Toxicol* 8: 239-257. [[Crossref](#)]
9. Chen Y, Zeng D, Ding L, Li X-L, Liu X-T, et al. (2015) Three-dimensional poly-(ϵ -caprolactone) nanofibrous scaffolds directly promote the cardiomyocyte differentiation of murine-induced pluripotent stem cells through Wnt/ β -catenin signaling. *BMC Cell Biol* 16: 22. [[Crossref](#)]
10. Nagaoka M, Kobayashi M, Kawai C, Mallanna SK, Duncan SA (2015) Design of a vitronectin-based recombinant protein as a defined substrate for differentiation of human pluripotent stem cells into hepatocyte-like cells. *PLoS one* 10: e0136350.
11. Bose B, Sudheer PS (2016) *In vitro* differentiation of pluripotent stem cells into functional β islets under 2D and 3D culture conditions and *In vivo* preclinical validation of 3D islets. *Methods Mol Biol* 1341: 257-284. [[Crossref](#)]
12. Ohi Y, Qin H, Hong C, Blouin L, Polo JM, et al. (2011) Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPSC cells. *Nat Cell Biol* 13: 541-549. [[Crossref](#)]