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# Moving toward Xeno-free Culture of Human Pluripotent Stem Cells

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## Abstract

Human pluripotent stem cells (hPSCs) were conventionally cultured on feeder cells that are isolated from mouse embryonic fibroblast (MEF). However, these culture components could contaminate the hPSCs and can limit the application of hPSCs for clinical use. On the other hand, we demonstrated that exogenous basic fibroblast growth factor (bFGF) could be omitted from the hPSC culture media if we used the suitable feeder cells. We also showed that although hPSCs can proliferate on the feeder-free culture system, however, genetic instability of hPSCs has been reported in such environment. Feeder cells enable hPSCs to maintain their pluripotency. The feeder cells are usually grown in a culture medium containing fetal bovine serum (FBS) prior to coculture with hPSCs. The use of FBS might limit the clinical application of hPSCs. We proposed the use of human cord blood-derived serum (hUCS) and showed a positive effect on culture of mesenchymal stem cells. The results showed that human foreskin fibroblasts (HFFs) cultured in hUCS-containing medium (HFF-hUCS) displayed fibroblastic features, short population doubling times, high proliferation rates, and normal karyotypes after prolonged culture. These studies of hPSC xeno-free culture have been growing in both basic research and clinical trial. The data regarding the current clinical trials of using hPSCs convince the researchers not only about the possibility of application of hPSCs for cell-based therapy, but also the quality of established hPSC lines. Most of the hPSC lines that were published in the literature and registered in the National Institute of Health (NIH), hPSCreg of the European Union are not Good Manufacturing Practice (GMP) grade cell lines. Since one of the goals of using hPSCs is therapeutic purpose, GMP for derivation, cultivation, and handling the hPSCs are required. This chapter also reviews the state-of-the-art xeno-free culture system of hPSCs in the respect of future clinical applications.

**Keywords:** xeno-free, pluripotent stem cells, cell culture, cell therapy, regenerative medicine

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## 1. Introduction

The ultimate goal of pluripotent stem cell research is to improve quality of life and patient treatment. The human pluripotent stem cells (hPSCs) can be classified as human embryonic stem cells (hESCs) and human-induced pluripotent stem cells (hiPSCs). hESCs can be derived from the inner cell mass (ICM) of the preimplantation embryos [1]. hiPSCs can be generated by reprogramming the somatic cells using the exogenous transcriptional factors and resulting in the pluripotent cells [2]. The successful derivation of hESCs and hiPSCs holds the great hope of treatment of incurable diseases, and the hPSC-related studies have been growing with regard to the potential of hPSCs.

hPSCs was firstly cultured in the medium containing the fetal bovine serum (FBS) and cocultured with mouse embryonic fibroblast (MEF) feeders [1, 2]. However, such conditions might introduce the contamination of animal pathogens to the hPSCs and make hPSCs unsuitable for clinical applications. Thus, elimination of all animal products during the derivation, long-term culture as well as differentiation of hPSCs is necessary prior to application of hPSCs in clinical cell therapy. Because the ultimate aim of pluripotent stem cell research is to improve quality of life and patient outcome, the more defined culture systems of hPSCs were progressively developed. The researchers firstly developed xeno-free culture system that consisted of a growth medium containing Knockout serum replacement (KO-SR) and basic fibroblast growth factor (bFGF) instead of FBS [3–5]. MEF feeder cells were replaced by the humanized feeder cells derived from human tissues. Later, the researchers have developed various xeno-free culture media, and xeno-free extracellular matrices which support the growth and pluripotency of hPSCs [6–10]. Recently, commercial xeno-free, defined culture media and extracellular matrix has been developed and available for the researchers.

## 2. Human pluripotent stem cells properties

hPSCs are widely accepted as the valuable source for cell-based therapy due to their ability of differentiation toward three embryonic germ layers and process cell division indefinitely [11]. Although the origin of hESCs and hiPSCs is different, they share similarities in the morphological appearances and the molecular levels. The unique morphological appearances of hPSCs can be identified by the tightly pack colonies with defined borders. The clear cytoplasm and distinctive nuclei of the individual cells can be observed. hPSCs expressed the pluripotent-related transcriptional factors including OCT-4, Nanog, Sox2, Nodal, hTERT, Rex1 as well as positive for stage-specific-embryonic antigen (SSEA)-3, SSEA -4, tumor-recognition antigen (TRA)-1-60 and -1-81, expressed high level of alkaline phosphatase and telomerase activity [1–

10]. Mellon et al. [12] and Guenther et al. [13] reported that there were no significant gene expression differences between hESCs and hiPSCs. However, some studies reported the differences in the methylation profile between hESCs and hiPSCs [14–16]. This can reflect the genetic and epigenetic abnormalities during the reprogramming process or even the differences of cell culture condition in different laboratories [17]. Although the similarities and differences between hESCs and hiPSCs are contradictory, the researchers around the world tried to standardize the culture conditions and characterization of hPSCs with the ultimate goal to achieve the development of the hPSC culture system for the future clinical application [18, 19].

### **3. Good Manufacturing Practice for human Pluripotent Stem Cells**

Since one of the goals of using hPSCs is therapeutic purpose, Good Manufacturing Practice (GMP) for derivation, cultivation, and handling the hPSCs are required. Most of the hPSC lines that were registered in the National Institute of Health (NIH), hPSCreg of the European Union as well as the data published in the literature are not GMP grade cell lines. Non-GMP grade cell lines are usually contacts to the animal products especially through the feeder cells, serum, and extracellular matrix [20, 21]. The researchers found that culture of hESCs on the feeder cells derived from the mouse embryonic tissues may contaminate nonhuman sialic acid (Neu5Gc) molecules, which can stimulate the immune rejection after transplantation [22]. Moreover, FBS that was supplemented in the culture medium of feeder cells might contain bovine pathogens such as bovine spongiform encephalitis (BSE) and limits the use of hPSCs for clinical applications [23]. Therefore, the materials for hPSC culture system that compliant for clinical application should be verified that the materials are GMP-grade and animal-free products. Importantly, the clinical-grade hPSCs should be generated under the ethical consideration guidelines that strictly followed the national law, from the traceable and healthy donors and the culture system should follow the standard operating procedures (SOPs) of GMP.

### **4. Derivation of human pluripotent stem cells**

The recent advances in GMP derivation of hPSC lines are using xeno-free reagents/materials and replacing the mouse feeders with GMP-qualified human feeders or recombinant human proteins [24–28]. Regarding the xeno-free hPSC derivation, there are several steps that should be considered in order to meet the GMP or criteria for clinical grade hPSCs.

For hESC derivation, the zona pellucida of the blastocyst embryos has to be removed prior to isolation of the ICM. The zona pellucida could be digested by the acid Tyrode's solution or pronase [3–5, 29]. To avoid the destructive effect of the acid or enzyme, the laser-assisted zona pellucida removal and the mechanical cut using surgical blade or needle were successfully applied [30–32]. Enzymatic removal, laser assisted, and mechanical dissection of zona pellucida are the xeno-free procedures due to these methods has no contact to the animal

materials. However, further step for derivation of hESCs is separation of the ICM from the trophectoderm (TE) which the embryo or ICM might contact to the animal products. Traditionally, the TE is separated from the ICM by the procedure called immunosurgery. The immunosurgery technique involves the selective lyses of the TE but not the ICM, resulting to the TE-free ICM. The use of anti-human serum combined with complement mediated lysis using guinea pig serum made the immunosurgery technique unsuitable for derivation of xeno-free hESCs. Therefore, other means such as microdissection of blastocysts using fine needle, laser-assisted biopsy, and whole blastocyst culture are the promising techniques for xeno-free ICM isolation [30–32]. The isolated ICM is subsequently plated and cultured for generation of hESC lines. The following procedures including culture conditions, cell propagation, and cryopreservation have to manage under the xeno-free conditions.

For hPSC derivation, the early step for derivation of xeno-free hPSCs is isolation and culture of the somatic cells prior to reprogramming. The somatic cells should be isolated under the aseptic condition with the minimal invasive technique. It has been reported that hPSCs can be generated by using several somatic cell types such as skin fibroblasts, peripheral blood mononuclear cells and even the cells isolated from urine [2, 33, 34]. Replacement of the animal products by using human grade or recombinant synthetic products during the isolation and culture of somatic cells is necessary. For example, human dermal fibroblasts can be isolated and propagated in the culture medium supplemented with human serum. These fibroblast cells can multiply under the xeno-free condition and suitable for using as the starting cells for reprogramming. We proposed the use of human cord blood-derived serum (hUCS) and showed a positive effect on culture of mesenchymal stem cells. The results showed that human foreskin fibroblasts (HFFs) cultured in hUCS-containing medium (HFF-hUCS) displayed fibroblastic features, high proliferation rates, short population doubling times, and normal karyotypes after prolonged culture. Inactivated HFF-hUCS expressed important genes, including Activin A, FGF2, and transforming growth factor beta-1 (TGF $\beta$ 1), which have been implicated in the maintenance of hPSC pluripotency. Moreover, hPSC lines maintained pluripotency, differentiation capacities, and karyotypic stability after being cocultured for extended period with inactivated HFF-hUCS. Therefore, the results demonstrated the benefit of hUCS for hPSC culture system.

However, one of the bottle-neck of derivation of xeno-free hPSCs is the method for reprogramming of the somatic cells. Originally, the exogenous genes were delivered to the somatic cells by means of virus such as lentivirus or retrovirus [2, 35]. Because the integration of lentiviral- or retroviral genome to the host (somatic cells) genome might occur during the reprogramming process, thus the hPSCs generated by lentivirus- or retrovirus are not the ideal hPSCs for the clinical use. The clinical applications will require hiPSCs that are free of exogenous DNA and that can be manufactured through GMP. Recently, reprogramming by protein, microRNA, episomal vectors, or Sendai virus, a nonintegrating virus is considered suitable for delivery of the exogenous genes into the somatic cells [36]. We demonstrated recently that the transgene-free hPSCs can be generated by the temperature-sensitive strain of Sendai virus and the viral particles were clearly eliminated from the established hPSCs by the

heat treatment [37]. Thus, derivation of transgene-free hPSCs is a very important step for generation of xeno-free hPSCs that match the future clinical applications.

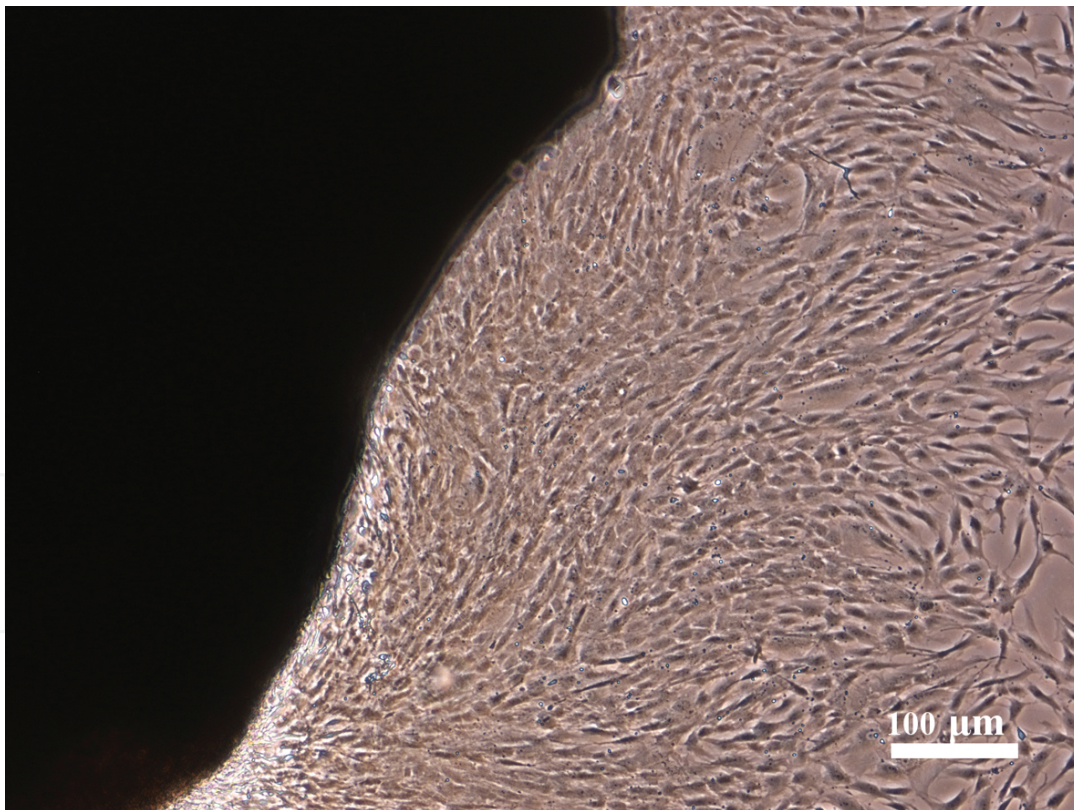
## 5. Culture of human pluripotent stem cells

The culture conditions that allow hPSCs to maintain their pluripotency is the complex environment and still under investigation. The researchers proved that the pluripotency of hPSCs is controlled by the TGF pathway which involves the cooperation of Activin and Nodal signaling pathway [38]. The pathways that related to the maintenance of the pluripotency of hPSCs might be provided by the secretion of cytokines, growth factors, extracellular matrix from the feeder cells, synthetic substrates, or culture medium. The improvement of culture system is not only necessary for maintenance of hPSC pluripotency but also for large-scale propagation of hPSCs prior to cell transplantation. The aims of our study were to determine (i) the effect of exogenous bFGF supplementation in the hPSC culture media on the morphology and gene expression of inactivated human cesarean scar fibroblasts (HSFs) and (ii) the feasibility of using the inactivated HSFs as the feeder cells for culturing the hPSCs. Our results showed that the cells shrunk and an increase in gap between the cells were observed in the inactivated HSFs that were cultured in 4 and 8 ng/ml but not in 0 ng/ml bFGF. Expression of Activin A, bFGF, TGF- $\beta$ , and BMP4 was similar between inactivated HSFs cultured in 0, 4, and 8 ng/ml bFGF. After two hPSC lines including hESC line (Chula2.hES) and hiPSC line (PFX12) were cocultured with three conditions of inactivated HSFs for more than ten passages, the hPSCs lines were subjected to characterization. The results showed that the hESCs and hiPSCs cultured in 0, 4, and 8 ng/ml bFGF could maintain their undifferentiation state, differentiate in vitro into three embryonic germ layers, and maintain their normal karyotype. In conclusion, exogenous bFGF supplementation in the culture medium can be omitted when using HSFs as the feeder cells for culturing the hPSCs.

### 5.1. Feeder-dependent culture system

Feeder-dependent culture system referred to the culture system that the hPSCs grown by the support of feeder cells. The feeder-dependent culture system is the traditional system that was developed for generation and propagation of hPSCs [1–6]. Feeder cells secreted cytokines, extracellular matrices and provided the niches that support the growth and maintenance of pluripotency of hPSCs. Generally, MEFs are used as the feeder for culture of hPSCs. Due to, MEFs might stimulate the immune response of the patients after transplantation of hPSCs [22]. Therefore, the feeder cells that isolated from human tissues are the better choice for culture of the clinical-grade hPSCs. Several studies demonstrated that the supportive feeder cells of hPSCs could be derived from the human tissues. Hovatta et al. [3] firstly demonstrated that HFFs supported the derivation and culture of hESCs. HFFs supported the pluripotency of hPSCs by expression of the key genes such as TFG, FGF2, and Activin A which have been implicated in the maintenance of hPSC pluripotency [39]. Ma et al. [40], the HFFs produced interleukin-6 and can be used for coculture with mouse embryonic stem cells. However, not all the HFF lines exhibited the characteristics of supportive feeder cells. The data confirmed that the foreskin fibroblasts from the different donor secreted different amount of growth

factors, cytokines, and specific genes related to pluripotency [39, 41]. Therefore, it is necessary to explore the new supportive feeder cell types in order to develop the xeno-free feeder dependent culture system of hPSCs. Up to date, feeder cells derived from other sources of human tissues, for example, human adult fallopian tubal fibroblasts, human fetal skin, human adult skin, human fetal muscle, human adult muscle, human adult marrow cells, human umbilical cord mesenchymal stem cells, human amniotic epithelial cells, and human iPS cell derived fibroblast-like cells had been reported as the supportive feeder cells for culture of the hPSCs [42–45]. In addition, genetic modification of human feeder cells such as immortal HFFs or bFGF-secreting HFFs has been developed and proved to be useful for culture of hPSCs [46, 47]. Although replacement of MEFs by human feeder cells can reduce the risk of contamination of mouse pathogens to the hPSCs, the use of FBS in the feeder cell culture medium does not meet the xeno-free condition of hPSCs. We recently demonstrated that HFFs cultured in the medium containing human umbilical cord blood serum (hUCBS) retain their supportive feeder characteristics for maintenance of hPSC lines [48]. In addition, we used hUCBS for isolation and culture of cesarean scar-derived fibroblasts (**Figure 1**). The xeno-free fibroblasts will be used for the feeder cells and the starting cells for generation of transgene-free hPSCs.



**Figure 1. Isolation of fibroblast cells from human cesarean scar tissue.** Fibroblast cells grew out from the tissue after culture for 7 days in the culture medium containing human umbilical cord blood serum (hUCBS). Scale bar = 100  $\mu\text{m}$ .

To generate the clinical-grade human feeder cells, the cells should be derived under the culture system containing humanized or recombinant GMP materials and reagents. Interestingly,

Prathalingam et al. [27] demonstrated that the clinical-grade feeder cells can be derived and cultured in the medium containing the FBS that was derived under the GMP condition. Prior to use of the feeder cells for derivation of the new hPSC lines or routinely coculture with the existing hPSC lines, the clinical-grade human feeder cells should be tested for the potential of the cells to support the proliferation of hPSCs. Although the standard protocol for characterization of the feeder cells is lacking, several methods have been adopted. Inactivation of feeder cells by irradiation or Mitomycin C treatment is the method for inactivation of feeder cell proliferation prior to use for coculture with hPSCs. After being inactivated, the feeder cells should be able to secrete the enough amounts of cytokines and growth factors into the culture medium. Moreover, the extracellular matrices provided by the feeder play important roles for maintenance of the pluripotency of hPSCs [7]. The cells that easy to apoptosis after the inactivation process might probably not be a good supportive feeder cells.

One major concern of feeder-dependent culture system is coating the culture vessel with the xeno-free matrix in order to enhance the attachment of feeder cells to the surface of the culture vessel. Typically, porcine-skin-derived gelatin was used for coating the culture vessel prior of seeding the inactivated feeder cells. To meet the criteria of xeno-free condition, porcine-skin-derived gelatin should be replaced by recombinant gelatin. Tannenbaum et al. [26] developed the platform of derivation of xeno-free and GMP-grade hESCs by using recombinant gelatin and they found no adverse effect of recombinant gelatin on the pluripotency of hESCs. In addition, Ding et al. [49] showed that human cord blood serum can be used for coating the culture vessels instead of the porcine-skin-derived gelatin for derivation and maintenance of hPSCs.

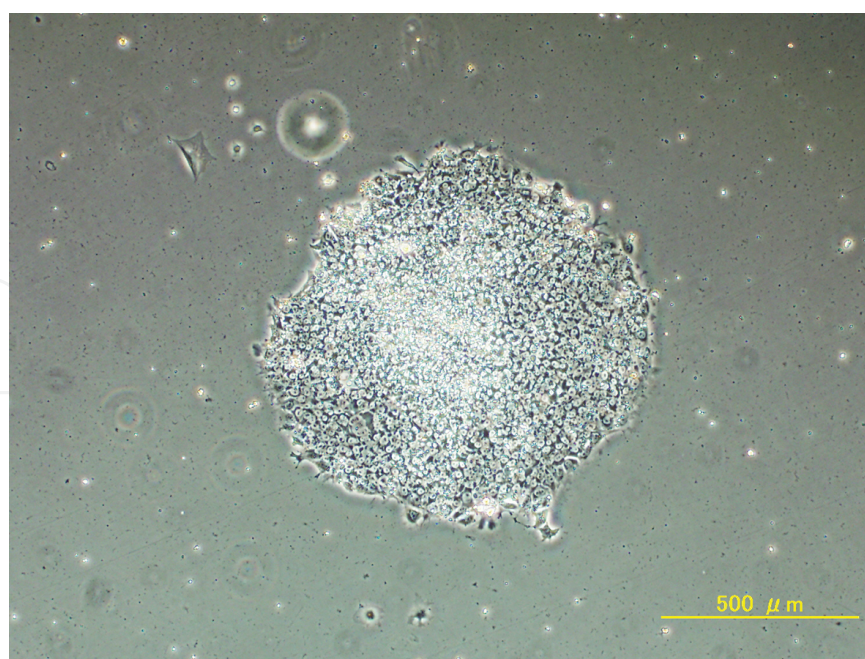
## 5.2. Feeder-free culture system

It has been proposed that coculture of hPSCs with the feeder layer might contaminate the harmful substance and cause the risk of graft rejection, viral or bacterial infection, or zoonoses [50]. To culture hPSCs under the feeder-free condition, several media formulations have been developed that eliminate the use of fetal bovine serum and reduce or eliminate all animal-derived components. Therefore, development of feeder-free and xeno-free culture system for hPSCs by the use of xeno-free reagents and matrices in every cell-handling process is important for obtaining clinical-grade hPSCs.

Feeder-free culture system is applied for culturing hPSCs without the feeder layers but the matrices such as Matrigel or recombinant human proteins, together with the commercially defined hPSC culture medium [51, 52]. Although the genetic and epigenetic instability could be detected in hPSCs grown under feeder-free condition [53–55], the feeder-free condition together with defined culture medium is still important because it allows the hPSCs to proliferate robustly.

In order to propagate and maintain the pluripotency of hPSCs under the feeder-free condition, it is necessary to provide the cell adhesion coating that support the proliferation of hPSCs. Matrigel, the solubilized basement matrix extracted from mouse Engelbreth-Holm-Swarm sarcoma is the widely used matrix. The major components of Matrigel include laminin, collagen IV, heparan sulfate proteoglycan, and entactin. In addition, Matrigel contains growth

factors such as TGF- $\beta$ , fibroblast growth factor, TPA, and insulin-like growth factor [56, 57]. Matrigel provides the complex extracellular matrices that support the cell communication and the suitable microenvironment for the hPSCs to grow as well as maintain their pluripotency. Although hPSCs proliferated robustly on Matrigel, the origin of Matrigel makes this matrix unsuitable for maintaining the clinical-grade hPSCs. Recently, Ding et al. [49] developed the humanized ECM by using umbilical cord blood serum (UCBS) as the matrix for derivation and culture of hPSCs. The best result was obtained when UCBS was used in combination with the basal medium supplemented with bFGF, fibronectin, and Y-27632. Other humanized matrices, for example, human placental-derived ECM and H9-hESC embryoid body-derived ECM have been proved to support the pluripotency of hPSCs [58, 59]. The hPSC lines cultured with the ECM derived from both types of human tissues or cells in the defined serum and xeno-free culture media such as TeSR2, sustained their pluripotency after prolonged culture. However, lot-to-lot variations in the individual serum donor or tissue-derived ECM can affect the effectiveness of the serum or ECM in maintaining the pluripotent state of hPSCs. In order to develop the defined-feeder-free cell adhesion coating, individual extracellular matrix (ECM) proteins have been used instead of Matrigel. Several ECM including laminin, fibronectin, vitronectin, and collagen support the proliferation of hPSCs. Those ECM can be used as single or in various combinations. Interestingly, the studies have demonstrated that recombinant laminin-511 and recombinant vitronectin enable to support the growth and maintain the pluripotency of hPSCs [60, 61]. To improve feeder-free culture system, cell adhesion proteins can be added to ECM component. E-cadherin, the cell adhesion protein improved the attachment and expansion of hPSCs when the cells were cultured in defined media such as mTeSR1 and xeno-free media, TeSR2 [62]. Moreover, recombinant E-cadherin fusion protein supported the proliferation of the other pluripotent cells [63]. Additional developments in defined culture surfaces include synthetic peptide coatings such as Synthemax, Peptide acrylate surfaces (PAS), or synthetic polymers have been proved to support the growth and pluripotency of hPSCs after prolonged culture [64]. The combination of human ECM proteins or cell adhesion molecules and synthetic biomaterials with well-designed surfaces and/or structures in the presence of a chemically defined medium containing recombinant growth factors would offer a xeno-free alternative to feeder cells for culturing hPSCs and maintaining their pluripotency. Because the xeno-free and feeder-free culture system is suitable for generation of hPSCs for clinical application, we reprogrammed CD34<sup>+</sup> cells by the temperature-sensitive Sendai viral vectors carries OCT-4, SOX2, KLF4 and c-MYC, on Pronectin F plus (Sanyo Chemical Industries, Kyoto, Japan) coating matrix and cultured the cells in ReproFF (ReproCELL, Yokohama, Japan) culture medium (**Figure 2**). We will use this newly established hPSCs for further development of xeno-free culture system. In our study [48], the hPSC lines were derived and cultured on HFF feeder layer. The hPSC lines were continuously maintained on either mitomycin-C inactivated HFF-FBS or mitomycin-C inactivated HFF-hUCS in serum-free hPSC culture medium. The serum-free hPSC culture medium comprising 80% Knockout Dulbecco's modified Eagles' medium (KO-DMEM), 20% KO-SR, 1% nonessential amino acid, 1% Glutamax, 1% penicillin-streptomycin, 0.1 mM  $\beta$ -mercaptoethanol (all from Invitrogen), and 8 ng/ml bFGF (R&D Systems, Minneapolis, MN, USA).



**Figure 2. Human-induced pluripotent stem cell culture on feeder-free condition.** The transgene-free human-induced pluripotent stem cells generated from CD34<sup>+</sup> cells and culture in feeder-free culture system. Scale bar = 500 μm.

## 6. Contamination concern

Prior to clinical application, xeno-free hPSC lines should be tested and evaluated for their biological safety. The evaluation should follow the guidelines that developed for clinical trials or therapies. The hPSC lines should be free from serious pathogenic microorganisms such as human immunodeficiency virus (HIV) and human papilloma virus (HPV). On the other hand, hPSC lines should also be free from mycoplasma contamination. Although Romorini [65] demonstrated the successful elimination of the mycoplasma contamination in their newly established hPSC lines but the mycoplasma-free newly established cell line is the ideal cell line for therapeutic application. Moreover, the level of endotoxin in the culture medium of hPSCs should meet the requirement of the national or international standard. Other biological test, for example, injection of the hiPSCs into the chorioallantoic and yolk sac of the chicken for testing unknown pathogenic microorganisms can be performed [25].

## 7. Clinical trial and therapeutic application

The clinical trial and therapeutic application requires hPSCs that were derived, cultured, and differentiated under the xeno-free and GMP conditions. Despite controversies and difficulties, the clinical trials using hESCs started in 2010 and continue ongoing. Geron Corporation, the biotechnology company based in California, USA began a clinical trial in patients with spinal

cord injuries using hESCs in 2010. The patient was transplanted with oligodendrocyte precursor cells derived from hESCs. Unfortunately, the project was discontinued a year later because the company changed their business strategy. There is no official data that has been published from the study of Geron. However, the project has been started and continued again by Advance Cell Technology, the biotechnology company based in Massachusetts. The company just recently published their preclinical safety data [66]. In 2012, the clinical trials using hESC-derivative cells were initiated and conducted in the USA. Advance Cell Technology, performed the clinical trials in the patients with Stargardt's macular degeneration and age-related dry macular degeneration, which caused the loss of photoreceptor and results in blindness. Retinal-pigmented epithelial (RPE) cells derived from hESCs were transplanted to replace the degenerated cells. Interestingly, the visual improvement of the patients had been preliminarily reported [67, 68]. ViaCyte, the company based in California, USA reported the successful surgical transplantation of pancreatic precursor cells derived from hESCs for treatment of Type I diabetes [69]. Recently, the Japanese researchers from RIKEN institute performed the first clinical trial using hiPSC-derived cells for treatment of age-related macular degeneration. Autologous hPSCs were differentiated to retinal-pigmented epithelial (RPE) sheet and later transplanted to the patient. However, the second trial was cancelled due to the occurring of genetic instability of the cells prior to transplantation [70]. Besides the clinical trials mentioned above, the hPSC-based products for the treatment of Parkinson's disease and others diseases are currently in the pipeline [71].

## 8. Perspective of collaboration

Although clinically compliant hPSC has been derived, limitations in scale of production and high costs for culture of hPSCs remain significant challenges. Besides efficiency and safety, the development of hPSC-based cell therapies are expected to be expensive, time consuming and might face the clinical failure. Therefore, the collaboration between academy, SME and large pharmaceutical company can take the advantage of opportunities to tap into various sources of support. Funding for basic research carried out in the academic setting is available from national research grant agencies and institutes. In the private sector, SMEs have the possibility to attract venture capital to fund efforts in regenerative medicine. In addition, the pharmaceutical companies have the possibility to directly support the project using their internal resources. Besides individually applying for grants or securing other type of funding, the partners can also join together and obtain consortium grants or support based on collaborative networks from international initiatives such as the Framework Programs funded by the European Union. We had developed the similar model by carried out the project with the two European partners, The University of Copenhagen, Denmark and BioTalentum, Ltd, Hungary. The project was funded by the European Union under the Framework 7 through the Marie Curies Actions. The results of the project were not only the publications [72] but also transferring the researchers and knowledge between the academy and industry. We believed that having research partners cover both non-profit and for-profit organizations is of great advantage when developing hPSC-based cell therapy.

## 9. Conclusion

To enable a xeno-free culture system capable of clinical use, it is important to examine the sources of components used in hPSC derivation, culture, including the reagents used for handling cells during passaging and the reagents used for cryopreservation. The sufficient protocols and documentations, development of hPSC derivation and culture conditions under the strict cleanroom and the use of GMP-grade reagents and materials should be prepared. In addition to the technical issues involved, establishment of the donor consent documentation appropriately and utilize forms that specifically states that the cells will be used for cell therapy purposes have to be prepared. The clinical grade hPSC lines that derived completely under the GMP conditions will be a valuable source for the future clinical therapies.

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## References

- [1] Thomson JA, Itskovitz-Eldor S, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. (1998). Embryonic stem cells lines derived from human blastocysts. *Science* 282: 1145–1147.
- [2] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131: 861–872.
- [3] Hovatta O, Mikkola M, Gertow K, Stromberg AM, Inzunza J, Hreinsson J, et al. (2003). A culture system using human foreskin fibroblasts as feeder cells allows production of human embryonic stem cells. *Hum. Reprod.* 18(7): 1404–1409.

- [4] Inzunza J, Gertow J, Stromberg MA, Matilainen E, Blennow E, Skottman H, et al. (2005). Derivation of human embryonic stem cell lines in serum replacement medium using postnatal human fibroblasts as feeder cells. *Stem Cell*. 23(4): 544–549.
- [5] Pruksananonda K, Rungsiwiwut R, Numchaisrika P, Ahnonkitpanit V, Virutamasen P. (2009). Development of human embryonic stem cell derivation. *J. Med. Assoc. Thai*. 92: 443–450.
- [6] Ellerstorm C, Strehl R, Moya K, Andersson K, Bergh C, Lundin K, et al. (2006). Derivation of a xeno-free human embryonic stem cell line. *Stem Cells* 24(10): 2170–2176.
- [7] Meng G, Liu S, Li X, Krawetz R, Rancourt DE. (2010). Extracellular matrix isolated from foreskin fibroblasts supports long-term xeno-free human embryonic stem cell culture. *Stem Cells Dev*. 19(4): 547–556.
- [8] Vuoristo S, Toivonen S, Weltner J, Mikkola M, Ustinov J, Trokovic R, et al. (2013). A novel feeder-free culture system for human pluripotent stem cell culture and induced pluripotent stem cell derivation. *PLoS One* 8(10): e76205.
- [9] Desai N, Judgin J, Goldberg J, Falcone T. (2013). Development of a xeno-free non-contact co-culture system for derivation and maintenance of embryonic stem cells using a novel human endometrial cell line. *J. Assist. Reprod. Genet.* 30(5): 609–615.
- [10] Montes R, Ligerio G, Sanchez L, Catalina P, de la Cueva T, Nieto A, Melen GJ, Rubio R, Garcia-Castro J, Bueno C, Menedez P. (2009). Feeder-free maintenance of hESCs in mesenchymal stem cell-conditioned media: distinct requirements of TGF-beta and IGF-II. *Cell Res*. 19(6): 698–709.
- [11] Philonenko ES, Shutova MV, Chestkov IV, Lagarkova MA, Kiselev SL. (2011). Current progress and potential practical application for human pluripotent stem cells. *Int. Rev. Cell Mol. Biol.* 292: 153–169.
- [12] Mallon BS, Hamilton RS, Kozhich OA, Johnson KR, Fann YC, Rao MS, et al. (2014). Comparison of the molecular profiles of human embryonic and induced pluripotent stem cells of isogenic origin. *Stem Cell Res*. 12(2): 376–385.
- [13] Guenther MG, Frampton GM, Soldner F, Hockemeyer D, Mitalipova M, Jaenisch R, et al. (2010). Chromatin structure and gene expression programs of human embryonic and induced pluripotent stem cells. *Cell Stem Cell* 7(2): 249–257.
- [14] Bock C, Kiskinis E, Verstappen G, Gu H, Boulting G, Smith ZD, et al. (2011). Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. *Cell* 144(3): 439–452.
- [15] Deng J, Shoemaker R, Xie B, Gore A, LeProust EM, Antosiewicz-Bourget J, et al. (2009). Targets bisulfite sequencing reveals changes in DNA methylation associated with nuclear reprogramming. *Nat. Biotechnol.* 27(4): 353–360.

- [16] Nashun B, Hill PW, Hajkova P. (2015). Reprogramming of cell fate: epigenetic memory and the erasure of memories past. *EMBO J.* 34(10): 1296–1308.
- [17] Wutz A. (2012). Epigenetic alterations in human pluripotent stem cells: a tale of two cultures. *Cell Stem Cell* 11(1): 9–15.
- [18] International Stem Cell Initiative, Adewumi O, Aflatoonian B, Ahrlund-Richter L, Amit M, Andrews PW, et al. (2007). Characterization of human embryonic stem cells by the International Stem Cell Initiative. *Nat. Biotechnol.* 25(7): 803–816.
- [19] Andrews PW, Baker D, Benvenisty N, Miranda B, Bruce K, Brüstle O, et al. (2015). Points to consider in the development of seed stocks of pluripotent stem cells for clinical applications: International Stem Cell Banking Initiative (ISCBI). *Regen. Med.* 10(2 Suppl): 1–44.
- [20] Shetty P, Bharucha K, Tanavde V. (2007). Human umbilical cord blood serum can replace fetal bovine serum in the culture of mesenchymal stem cells. *Cell Biol. Int.* 31: 293–298.
- [21] Kocaoemer A, Kern S, Kluter H, Bieback K. (2005). Human AB serum and thrombin-activated platelet-rich plasma are suitable alternatives to fetal calf serum for expansion of mesenchymal stem cells from adipose tissue. *Stem Cells* 25(5): 1270–1278.
- [22] Martin MJ, Muotri A, Gage F, Varkj A. (2005). Human embryonic stem cells express an immunogenic nonhuman sialic acid. *Nat. Med.* 11(2): 228–232.
- [23] Chou ML, Bailey A, Avory T, Tanimoto J, Burnouf T. (2015). Removal of transmissible spongiform encephalopathy prion from large volume of cell culture media supplemented with fetal bovine serum by using hollow fiber anion-exchange membrane chromatography. *PLoS One* 10(4): e0122300.
- [24] Durruthy-Durruthy J, Briggs SF, Awe J, Ramathal CY, Karumbayaram S, Lee PC, Heidmann JD, Clark A, Karakikes I, Loh KM, Wu JC, Hoffman AR, Byrne J, Reijo Pera RA, Sebastiano V. (2014). Rapid and efficient conversion of integration-free human induced pluripotent stem cells to GMP-grade culture conditions. *PLoS One* 9(4): e94231.
- [25] Wang J, Hao J, Bai D, Gu Q, Han W, Wang L, et al. (2015). Generation of clinical-grade human induced pluripotent stem cells in xeno-free conditions. *Stem Cell Res. Ther.* 6(1): 223.
- [26] Tannenbaum SE, Turetsky TT, Singer O, Aizenman E, Kirshberg S, Ilouz N, et al. (2012). Derivation of xeno-free and GMP-grade human embryonic stem cells—platforms for the future clinical applications. *PLoS One* 7(6): e35325.
- [27] Prathalingam N, Ferguson L, Young L, Lietz G, Oldershaw R, Healy L, et al. (2012). Production and validation of a good manufacturing practice grade human fibroblast like for supporting human embryonic stem cells derivation and culture. *Stem Cell Res Ther.* 3(2): 12.

- [28] Chen VC, Couture SM, Ye J, Lin Z, Hua G, Huang HI, et al. (2012). Scalable GMP compliant suspension culture system of human ES cells. *Stem Cell Res.* 8(3): 388–402.
- [29] Pruksananonda K, Rungsiwiwut R, Numchaisrika P, Ahnonkitpanit V, Isarasena N, Virutamasen P. (2012). Eighteen-year cryopreservation does not negatively affect the pluripotency of human embryos: evidence from embryonic stem cell derivation. *BioRes Open Access.* 1: 166–173.
- [30] Kim HS, Oh SK, Park YB, Ahn HJ, Sung KC, Kang MJ, Lee LA, Suh CS, Kim SH, Kim DW, Moon SY. (2005). Methods for derivation of human embryonic stem cells. *Stem Cells* 23: 1228–1233.
- [31] Strom S, Rodriguez-Wallberg K, Holm F, Bergstrom R, Eklund L, et al. (2010). No relationship between embryo morphology and successful derivation of human embryonic stem cell lines. *PLoS One* 5: e15329.
- [32] Turetsky T, Aizenman E, Gil Y, Weinberg N, Shufaro Y, Revel A, et al. (2008). Laser-assisted derivation of human embryonic stem cell lines from IVF embryos after preimplantation genetic diagnosis. *Hum. Reprod.* 23(1): 46–53.
- [33] Trokovic R, Weltner J, Nishimura K, Ohtaka M, Nakanishi M, Salomaa V, et al. (2014). Advanced feeder-free generation of induced pluripotent stem cells directly from blood cells. *Stem Cells Transl. Med.* 3(12): 1402–1409.
- [34] Zhang SZ, Li HF, Ma LX, Qian WJ, Wang ZF. (2015). Urine-derived induced pluripotent stem cells as a modeling tool for paroxysmal kinesigenic dyskinesia. *Biol. Open.* 4(12): 1744–1752.
- [35] Li W, Zhou H, Abujarour R, Zhu S, Young Joo J, Lin T, et al. (2009). Generation of human-induced pluripotent stem cells in the absence of exogenous Sox2. *Cell* 27(12): 2992–3000.
- [36] Zhou YY, Zeng F. (2013) Integration-free methods for generating induced pluripotent stem cells. *Genomics, Proteomics Bioinf.* 11(5): 284–287.
- [37] Rungsiwiwut R, Pavarajarn W, Numchaisrika P, Virutamasen P, Pruksananonda K. (2016). Transgene-free human induced pluripotent stem cell line (HS5-SV.hiPS) generated from caesarean scar-derived fibroblasts. *Stem Cell Res.* 16(1): 10–13.
- [38] James D, Levine AJ, Besser D, Hemmati-Brivanlou A. (2005). TGFbeta/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells. *Development* 132(6): 1273–1282.
- [39] Eiselleova L, Peterkova I, Neradil J, Slaninova I, Hampl A, Dvorak P. (2008). Comparative study of mouse and human feeder cells for human embryonic stem cells. *Int. J. Dev. Biol.* 52(4): 353–363.

- [40] Ma Y, Gu J, Li C, Wei X, Tang F, Shi G, et al. (2012). Human foreskin fibroblast produces interleukin-6 to support derivation and self-renewal of mouse embryonic stem cells. *Stem Cell Res. Ther.* 3(4): 29.
- [41] Mamidi MK, Pal R, Mori NA, Arumugam G, Thrichelvam ST, Noor PJ, et al. (2011). Co-culture of mesenchymal-like stromal cells derived from human foreskin permits long term propagation and differentiation of human embryonic stem cells. *J. Cell. Biochem.* 112(5): 1353–1363.
- [42] Cheng L, Hammond H, Ye Z, Zhan X, Dravid G. (2003). Human adult marrow cells support prolonged expansion of human embryonic stem cells in culture. *Stem Cells* 21(2): 131–142.
- [43] Ding DC, Shyu WC, Lin SZ, Liu HW, Chiou SH, Chu TY. (2012). Human umbilical cord mesenchymal stem cells support nontumorigenic expansion of human embryonic stem cells. *Cell Transplant.* 21(7): 1515–1527.
- [44] Ávila-Gonzalez D, Vega-Hernandez E, Regalado-Hernandez JC, De la Jara-Diaz JF, Garcia-Castro IL, Molina-Hernandez A, et al. (2015). Human amniotic epithelial cells as feeder layer to derived and maintain human embryonic stem cells from poor-quality embryos. *Stem Cell Res.* 15(2): 322–324.
- [45] Du SH, Tay JC, Chen C, Tay FC, Tan WK, Li ZD, et al. (2015). Human iPS cell-derived fibroblast-like cells as feeder layers for iPS cell derivation and expansion. *J. Biosci. Bioeng.* 120(2): 210–217.
- [46] Saxena S, Hanwate M, Deb K, Sharma V, Totey S. (2008). FGF2 secreting human fibroblast feeder cells: a novel culture system for human embryonic stem cells. *Mol. Reprod. Dev.* 75(10): 1523–1532.
- [47] Unger C, Gao S, Cohen M, Jaconi M, Bergstrom R, Holm F, et al. (2009). Immortalized human skin fibroblast feeder cells support growth and maintenance of both human embryonic and induced pluripotent stem cells. *Hum. Reprod.* 24(10): 2567–2581.
- [48] Rungsiwiwut R, Ingrungruenglert P, Numchaisrika P, Virutamasen P, Phermthai T, Pruksananonda K. (2016). Human umbilical cord blood-derived serum for culturing the supportive feeder cells of human pluripotent stem cell lines. *Stem Cells Int.* 2016: 4626048.
- [49] Ding Y, Yang H, Yu L, Xu CL, Zeng Y, Qiu Y, et al. (2015). Feeder-free and xeno-free culture of human pluripotent stem cells using UCBS matrix. *Cell Biol. Int.* 39: 1111–1119.
- [50] Skottman H, Hovatta O. (2006). Culture conditions for human embryonic stem cells. *Reproduction* 132(5): 691–698.
- [51] Amit M, Shariki C, Margulets V, Itskovitz-Eldor J. (2004). Feeder layer- and serum-free culture of human embryonic stem cells. *Biol. Reprod.* 70(3): 837–845.

- [52] Xu C, Inokuma MS, Denham J, Golds K, Kundu P, Gold JD, et al. (2001). Feeder-free growth of undifferentiated human embryonic stem cells. *Nat. Biotechnol.* 19(10): 971–974.
- [53] Catalina P, Montes R, Ligerio G, Sanchez L, de la Cueva T, Bueno C, et al. (2008). Human ESCs predisposition to karyotypic instability: Is a matter of culture adaptation or differential vulnerability among hESC lines due to inherent properties? *Mol. Cancer* 7: 76.
- [54] Draper JS, Smith K, Gokhale P, Moore HD, Maltby E, Johnson J, et al. (2004). Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nat. Biotechnol.* 22(1): 53–54.
- [55] Garitaonandia I, Amir H, Boscolo FS, Wambua GK, Schultheisz HL, Sabatini K, et al. (2015). Increased risk of genetic and epigenetic instability in human embryonic stem cells associated with specific culture conditions. *PLoS One* 10(2): e0118307.
- [56] Kleinman HK, Martin GR. (2005). Matrigel: basement membrane matrix with biological activity. *Semin. Cancer Biol.* 15(5): 378–385.
- [57] Ludwig TE, Bergendahl V, Levenstein ME, Yu J, Probasco MD, Thomson JA. (2006). Feeder-independent culture of human embryonic stem cells. *Nat. Methods* 3(8): 637–646.
- [58] Fu X, Toh WS, Liu H, Lu K, Li M, Hande MP, et al. (2010). Autologous feeder cells from embryoid body outgrowth support the long-term growth of human embryonic stem cells more effectively than those from direct differentiation. *Tissue Eng. Part C Methods* 16(4): 719–733.
- [59] Fukusumi H, Shofuda T, Kanematsu D, Yamamoto A, Suemizu H, Nakamura M, et al. (2013). Feeder-free generation and long-term culture of human induced pluripotent stem cells using pericellular matrix of decidual derived mesenchymal cells. *PLoS One* 8(1): e55226.
- [60] Chen. (2011). Chemically defined conditions for human iPSC derivation and culture. *Nat. Methods* 8(5): 424–429.
- [61] Rodin. (2010). Long-term self-renewal of human pluripotent stem cells on human recombinant laminin-511. *Nat. Biotechnol.* 28(6): 611–615.
- [62] Braam SR. (2008). Recombinant vitronectin is a functionally defined substrate that supports human embryonic stem cell self-renewal via  $\alpha$ 5 $\beta$ 1 integrin. *Stem Cells* 26(9): 2257–2265.
- [63] Nagaoka M, Si-Taved K, Akaike T, Duncan SA. (2010). Culture of human pluripotent stem cells using completely defined conditions on a recombinant E-cadherin substratum. *BMC Dev. Biol.* 2(10): 60.
- [64] Enam S, Jin S. (2015). Substrate for clinical application of stem cells. *World J. Stem Cells* 7(2): 243–252.

- [65] Romorini L, Riva DA, Bluguermann C, Videla Richardson GA, Scassa ME, Sevillever GE, et al. (2013). Effect of antibiotics against *Mycoplasma* sp. On human embryonic stem cells undifferentiated status, pluripotency, cell viability and growth. *PLoS One*. 8(7): e70267.
- [66] Priest CA, Manley NC, Denham J, Wirth ED 3rd, Lebkowski JS. (2015). Preclinical safety of human embryonic stem cell-derived oligodendrocyte progenitors supporting clinical trials in spinal cord injury. *Regen. Med.* 10(8): 939–958.
- [67] Schwart SD, Hubschman JP, Heilwell G, Franco-Cardenas V, Pan CK, et al. (2012). Embryonic stem cell trials for macular degeneration: a preliminary report. *Lancet* 379(9817): 713–720.
- [68] Schwart SD, Regillo CD, Lam BL, Elliott D, Rosenfeld PJ, Gregori NZ, et al. (2015). Human embryonic stem cell-derived retinal pigmented epithelium in patients with age-related macular degeneration and Stargardt’s macular dystrophy: follow-up of two open-label phase 1/2 studies. *Lancet* 385(9967): 509–516.
- [69] Schulz TC. (2015). Concise review: manufacturing of pancreatic endoderm cells for clinical trials in type 1 diabetes. *Stem Cell Transl. Med.* 4(8): 927–931.
- [70] Garber K. (2015). RIKEN suspends first clinical trial involving induced pluripotent stem cells. *Nat. Biotechnol.* 33: 890–891.
- [71] Hunsberger JG, Rao M, Kurtzberg J, Bulte JW, Atala A, LaFerla FM, et al. (2016). Accelerating stem cell trials for Alzheimer’s disease. *Lancet Neurol.* 15: 219–230.
- [72] Rungsiwiwut R, Manolertthewan C, Numchaisrika P, Ahnonkitpanit V, Virutamasen P, Techakumphu M, Pruksananonda K. (2013). The ROCK inhibitor Y-26732 enhances the survival and proliferation of human embryonic stem cell-derived neural progenitor cells upon dissociation. *Cells Tissues Organs* 198(2): 127–138.

