Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. (July–Sept 2015) 85(3):711–717
DOI 10.1007/s40011-014-0430-8

REVIEW

Effects of Feeder Layers, Culture Media, Conditional Media, Growth Factors, and Passages Number on Stem Cell Optimization

Payam Ghasemi-Dehkordi · Mehdi Allahbakhshian-Farsani · Narges Abdian · Amin Mirzaeian · Morteza Hashemzadeh-Chaleshtori · Hamideh Jafari-Ghahfarokhi

Received: 20 April 2014 / Revised: 30 June 2014 / Accepted: 9 September 2014 / Published online: 18 October 2014

Abstract Stem cells are undifferentiated and self-renewal cells which could be obtained from the body or artificially derived from an adult somatic cell by forced expression of specific genes. In recent years stem cells are widely used in laboratory for tissue engineering and therapeutic applications. There are different factors and conditions that affect the stem cell culture such as feeder layers, atmosphere, kind of medium, growth factors, passages number, and conditional media with animal or human sources. Optimization of stem cell culture for medical approaches and regenerative medicine is important. Therefore, in the present study, the effect of these factors and agents on optimization of stem cell culture has been discussed. This review study showed that optimization of feeder layer, atmosphere, and using supplemented media with essential growth factors could help in maintaining the stem cells in undifferentiated state in vitro. The present study indicated that optimization of stem cell culture depends on the kind of each cell type and using stem cells in low passage number could decrease chromosomal abnormalities and DNA damages. For inhibiting the stem cell contamination by feeder cell lines, culture of these cells on feeder free systems like Matrigel matrix in conditioned media supplemented with essential growth factors is useful. Also, for eradicating immune system responses and reducing the risk of animal pathogen transfer, culture of stem cells on human feeder in optimized media is suitable for therapeutic approaches and regenerative medicine.

Keywords Stem cell · Feeder layer · Atmosphere · Culture media · Conditional media · Growth factor · Passages number

P. Ghasemi-Dehkordi · N. Abdian · M. Hashemzadeh-Chaleshtori (✉) · H. Jafari-Ghahfarokhi
Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Rahmatieh, 8813833435 Shahrekord, Iran
e-mail: biotechnologyskums@gmail.com

M. Allahbakhshian-Farsani
Department of Hematology and Blood Banking, Faculty of Allied Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

A. Mirzaeian
Clinical Biochemistry Research Center, Shahrekord University of Medical Sciences, Sharekord, Iran

Introduction

The optimization of stem cell culture is important in tissue engineering, therapeutic applications, regenerative medicine, and screening of drugs and toxins [1]. In laboratory, three types of stem cells are found. They are embryonic stem cells (ESCs), adult stem cells, and induced pluripotent stem cells (iPSCs). Adult stem cells like mesenchymal stem cells (MSC) and cancer stem cells are multipotent and have been isolated from numerous tissues such as bone marrow, adipose tissue, skin, placenta, thymus, and umbilical cord blood [2, 3]. Stem cells are widely used in various therapeutic approaches and can be obtained from somatic cells or artificially derived from adult somatic cells by forced expression of specific genes [4, 5]. ESCs are pluripotent cells derived from the inner cell mass of the blastocyst while iPSCs are the type of pluripotent stem cells derived from an adult somatic cell (typically human dermal fibroblasts (HDFs) isolated from newborn foreskin samples) by forced expression of specific genes such as OSKM (Sox2, Oct4, Klf4, and c-Myc) or SOLN (Sox2, Oct4, Lin28, and Nanog) combinations [6–8].

In optimization of stem cell culture different agents and factors such as kind of media, growth factors, passage

Table 1 The effects of different growth factors on stem cells culture

Growth factors	Roles
Activin-A, TGFβ1, and TGFβ3	Inhibit endodermal and ectodermal cells, increase proliferation of MSCs, but allow differentiation into mesodermal (muscle) cells.
RA, EGF, BMP-4, and bFGF	Activate ectodermal and mesodermal markers
βNGF and HGF	Differentiation into the three embryonic germ layers, including endoderm
LIF	Support mouse ESC self-renewal

number, feeder cell layers, atmosphere, and using conditional media (CM) with animal or human sources are effective [9, 10]. For proper maintenance in undifferentiated state and best growth the stem cells need feeder cell layers with animal or human origin such as mouse embryonic fibroblasts (MEFs) and HDFs. Feeder layer provides the important soluble factors for optimization of the stem cell growth. MEFs feeder secrete many factors that are essential for undifferentiated growth of iPSCs and ESCs in long-term period [6, 7, 11]. MEF feeder and using fetal bovine serum (FBS) in stem cell culture media could increase the immune response and the risk of exposure of these cells to zoonotic animal pathogens [11, 12]. Passage numbers could influence on the pluripotency of stem cells and long-term passages of stem cell culture could affect the chromosomal and cytogenetic stability of these cells in vitro [12, 13]. Furthermore, random insertion of lentiviral vectors contains gene delivery factors for reprogramming of host cell to iPSCs which could influence on cytogenetic stability of these cells [14]. Using the basic fibroblast growth factor (bFGF) and leukemia inhibitory factor (LIF) supplementation as well as kind of media and conditional media could affect on generation and pluripotent stability of stem cell lines [15, 16]. Preparing the optimal conditions and obtaining standardized stem cell culture are not easily achievable. So, in the present study, the effect of these factors and agents on optimization of stem cell culture was discussed.

Growth Factors

Growth factors such as bFGF, LIF, transforming growth factor beta 1 and 3 (TGF-β1 and TGF-β3), activin-A, bone morphogenic protein 4 (BMP-4), epidermal growth factor (EGF), hepatocyte growth factor (HGF), β nerve growth factor (βNGF), platelet derived growth factor BB (PDGF-BB), and retinoic acid (RA) have been used for supporting and maintaining the pluripotency of the stem cells [17, 18]. Each growth factor has a unique effect on undifferentiating state of stem cells and/or cell selection [19, 20] (Table 1). For example LIF affects on pluripotent stability and supports the expansion of animal stem cells like mouse embryonic stem cells (mESCs) but not human ESCs (hESCs) by activating the transcription factor STAT3 [15, 19]. The member of the bone morphogenetic protein (BMP) family, well known as an anti-neural factor increases MSC proliferation inducing osteogenesis. BMP with LIF could support and sustain mouse ESCs self-renewal by inducing Id1 through Smad activation in serum-free medium. Self-renewal response to BMP depends on continuous LIF signaling [15, 21]. The effect of Activin A and TGF-β1 is to maintain ESCs in undifferentiated state by inhibiting BMP signaling [9, 22–24].

The content of each growth factor used in stem cell culture is variable. The supplemented media with 4–10 ng/ml and 100 ng/ml of bFGF in feeder-dependent and feeder-free systems respectively are essential for maintaining the pluripotency of human stem cells and their long-term passages in the culture [25–27]. Furthermore, using 10 ng/ml LIF plus 10 ng/ml BMP can sustain ESCs in self-renewal state in serum-free media [15]. MEFs do not express bFGF but culture of hESCs on MEF feeder cells could affect on endogenous expression of bFGF at least 0.1 ng/ml and bFGF intracrine knockdown could induce differentiation of hESCs [28, 29]. Ludwig et al. [16] showed that high concentrations of bFGF could support feeder-independent growth of ESCs, but this condition included poorly defined serum and matrix components. In another study they described feeder-independent human ESC culture which includes protein components solely derived from recombinant sources or purified from human material. So, supplemented media with appropriate amount of growth factor could help to support and maintain the pluripotency of the stem cells.

Culture Media

In recent years, synthetic media supplemented with recombinant growth factors or cytokines are widely used for optimization of human and animal stem cell culture [9, 15]. The kind of culture media, pH condition, and their composition could affect the self-renewal or cell

differentiation and to maintain the pluripotency of mouse and human stem cells like hESCs, mESCs, miPSC, and hiPSCs in the culture. The stem cell requirements not only depend on the kind of stem cell culture but also on the species they originate from [17]. The culture media content including glucose, pyruvate, lactate, ammonia, and amino acids could affect each stem cell lines. Adding essential factors in the media is important for optimization of stem cell culture [30]. For example hiPSC need bFGF in their culture media while hESC need LIF. Adding Rho kinase inhibitor Y-27632 or the full length IL6RIL6 chimera to hiPSCs medium is effective for maintaining proliferation in an undifferentiated state [31, 32]. Use FBS in culture media could transmit the pathogens to human stem cells. Therefore, stem cell culture media supplemented with factors of animal origin are not suitable for stem cells that are used for therapeutic purposes [12, 13]. Nowadays, most stem cell culture media are serum-free like knockout DMEM and DMEM/F12 that contain growth factors and various additives including knockout serum (KSR), sodium pyruvate (NaPy), Glutamax, 2-Mercaptoethanol (2ME), members of the BMP family (which can either synergize with LIF to support mouse ESC self-renewal), and non-essential amino acids (NEAA) or growth factors such as bFGF and LIF [9, 15].

Conditional Media (CM)

CM has been reported to maintain stem cells in undifferentiated state [33]. MEF and HDF conditioned media are widely used in stem cell culture to produce important factors such as LIF and bFGF to support the ESCs and iPSCs growth in vitro. Previous studies demonstrated that the growth of human stem cells on MEF feeders and on laminin- or Matrigel-coated matrix are supplemented with MEF-conditioned medium (MEF-CM) [33]. MEF-CM could increase the immune system interactions and transfer the animal pathogens to human stem cells.

In recent years for clinical applications human fetal and adult fibroblast feeders are used to support prolonged undifferentiation of ESC and iPSC in cell culture. For maintaining the human stem cells in undifferentiated state in long term culture many studies used MEF-CM as well as matrix components for the secretion of growth factors including bFGF, stem cell factor (SCF), and fetal liver tyrosine kinase-3 ligand (Flt3L) in stem cell media. Xu et al. [34] showed that using these and other growth factors in stem cell media could maintain undifferentiated hESCs in the absence of CM. The hESCs culture maintained in bFGF alone or in combination with other factors showed characteristics similar to MEF-CM control cultures, including morphology, surface marker, transcription factor expression, telomerase activity, differentiation, and karyotypic stability. These data demonstrated that hESCs can be maintained in non-conditioned medium using growth factors [34].

Feeder Layer

Feeder layer provides the suitable attachment substrate and important soluble factors for stem cells culture [35]. Different feeder layers such as MEF and HDF are commonly used to support the growth of stem cell culture. An important concern about using stem-cell therapy is the transmission of zoonotic infection of retrovirus to the recipients. MEF feeder could increase the immune response to check the exposure of the stem cells to exogenous antigens. The advantage of MEF feeder are secretion of factors that are essential for undifferentiated growth of stem cells in long-term period. Furthermore, separation of stem cells from MEFs in therapeutic aims is difficult. The MEF feeder could be replaced by HDF feeder cells or extracellular matrix like Matrigel. Human feeder cells provide suitable base to support the stem cells and reduce the risk of zoonosis and immune interactions [14, 36, 37].

Feeder-independent and xeno-free system of stem cells culture is essential for clinical approaches. The advantages of feeder-free culture conditions are easier to use and more amendable reproducibility to larger scale. Feeder free systems such as gelatin coated plastic dishes have been recently used for the culture of stem cells [38–40]. Feeder-free conditions could affect the morphology of stem cells and decreased expression of pluripotency markers in these cells. In feeder-free systems MEF-CM and Matrigel substrate are used which could increase the zoonotic pathogen transfer in therapeutic approaches. Matrigel contains laminin, collagen and growth factors of animal origin but its contents have not been defined clearly. It is very important substitute for optimal growth of stem cells and could maintain the stem cells in an undifferentiated state for a long time [9, 41, 42]. For optimization of Matrigel matrix coating in plates with non-gelled ones the Matrigel is used in cold conditions and then warmed to polymerize the Matrigel in the humidified incubator. Adult stem cells including MSC and cancer stem cells do not need feeder layer for attachment support [2, 3]. In Fig. 1 the cultures of human ESCs on different types of feeder layers as MEF, HDF and feeder free system (Matrigel) are shown.

Many studies are performed on using feeder layers for optimization of stem cells culture. Xu and their co-workers cultured hESC on Matrigel or laminin in MEF-CM and demonstrated that in a successful feeder-free hESC culture system the undifferentiated cells can be maintained for at least 130 population doublings for first time [14]. The T3HDF feeder and T3HDF-CM could be used to support

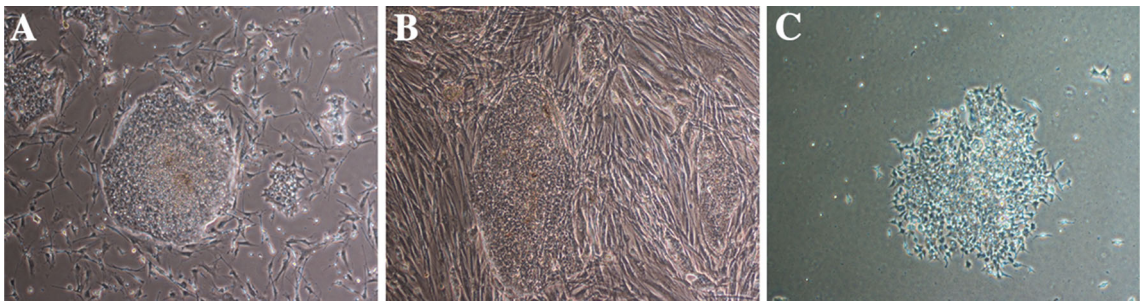

Fig. 1 The cultures of hESCs on different feeder layers **A** MEF feeder, **B** HDF feeder and **C** Matrigel matrix (feeder free system)

hESC for undifferentiated growth hence they would be useful for drug development and toxicity testing [43–45]. Stover and Schwartz [46] presented a protocol for adaptation of hiPSC to feeder-free conditions in chemically defined medium with enzymatic single-cell passaging and maintained the cells without karyotypic abnormalities. Quang et al. [25] suggested that each feeder cell type could prepare a microenvironment depending on the culture media composition which might support the culture of hESCs and hiPSCs line. So, feeder layers are important for optimal growth of stem cells and to maintain and support these cells in an undifferentiated state for a long term period in vitro [47, 48].

The Atmosphere

The atmosphere and temperature conditions are important for optimization of stem cell culture but the effect of temperature on stem cell cultures has not been thoroughly investigated. The O_2 range from 3 to 10 % and 5 to 10 % CO_2 are suitable for stem cell culture. Furthermore, increasing of atmospheric CO_2 leads to decrease the pH of the culture media. For the stabilization of pH of stem cell culture 3.7 g sodium bicarbonate ($NaHCO_3$) is added in 10 % CO_2 atmosphere. The culture media of stem cell contain 1.5–2.2 g/L $NaHCO_3$ in 5 % CO_2 atmosphere. If the concentration of $NaHCO_3$ is too high in standard CO_2 atmosphere in the incubator, the media become more alkaline and the pH increases which could affect the viability of stem cells [49, 50]. The optimum temperature for stem cell culture is commonly 37 °C but human spermatogonia need low temperature from 37 to 31 °C for increasing of DNA synthesis [17, 49]. Furthermore, the temperature effects on stem cell behaviour and on cytogenetic stability of these cells have not been clarified [17].

Passages Number

When the stem cell colonies are too dense and large or feeder cells become old the stem cells need to passage. Mechanical passaging or passaging combined with enzyme digestion (such as animal-derived collagenase, dispase, and trypsin) are used for dissociating the colonies into small clumps. Many researchers combine both enzymatic and mechanical passaging methods for long term maintenance of stem cells in vitro [51]. Recent studies showed that enzymatic passage and long term culture of ESCs could lead to chromosomal abnormalities which are important in therapeutic applications [12, 13, 52, 53]. However, another study indicated that chromosomal instability in long term culture is not frequent after prolonged passaging [12, 54]. Thomson et al. showed that human ESC passaged, using enzymatic methods, retain a normal karyotype but they observed cells passaged using EDTA, without trypsin, acquire an isochromosome p7 in three replicates [55]. Furthermore, the exposing time of enzyme for separation of stem cell colonies to single cells are important. They could affect cell morphology, cytogenetic stability and may lead to apoptosis [55, 56]. So, mechanical methods of passaging allow selective transfer of exclusively undifferentiated colonies and maintain better cytogenetic stability of stem cells [55]. Many studies indicated that the number of passages influence the pluripotency of stem cells like ESC and iPSC [56, 57]. The comparison of enzymatic and non-enzymatic patterns on dissociating adherent monolayers of MSC showed that exposure of trypsin-dissociated MSC to enzyme-free dissociation buffer for 1 h had no significant detrimental effect on cell viability [58]. Increasing of passage number could affect on cytogenetic stability of stem cells through mutations and DNA damages which could create genetic heterogeneity within the parental cell line [59, 60]. The study of Nagy et al. [61] showed that early passage of these cells are fully potent and very useful not only for ES cell-based genetic manipulations but also in defining optimal in vitro culture conditions for retaining the initial totipotency of ESC. Crisostomo et al. [62] showed that high passage number had an adverse effect on MSC activation and protection. Shiue et al. [63] investigated the influence of passage number and the duration of in vitro culture on the capacity of porcine inner cell mass (ICM)-derived cells including

Table 2 The stem cells culture conditions based on each cell type

Stem cell types			Attachment support	Serum	Growth factors	Serum-free	Atmosphere	Passages number
Pluripotent	iPSCs	hiPSCs or miPSCs	Yes (feeder cells or extracellular matrix like Matrigel)	No	bFGF	Yes	5 to 10 % CO_2 and 3 to 10 % O_2	Early passages (1 to 7)
	ESCs	hESCs	Yes (feeder cells or extracellular matrix like matrigel)	No	bFGF	Yes	5 to 10 % CO_2 and 3 to 10 % O_2	Early passages (1 to 10)
		mESCs	Yes (feeder cells, or gelatin)	Yes	LIF	No	5 to 10 % CO_2 and 3 to 10 % O_2	Early passages (1 to 7)
Multipotent	Adult stem cells	Mesenchymal Stem cells (MSCs)	No	Yes	bFGF, EGF, RA, and TGF-β3	No	5 to 10 % CO_2 and 3 to 10 % O_2	Early passages (1 to 2)
		Cancer stem cells	No	No	Variable	Yes	5 to 10 % CO_2 and 3 to 10 % O_2	Early passages (1 to 4)

ESC in the generation of chimeric embryos. When passage number was less than 15, detrimental effects on integration ability are not showed [63]. Li et al. [56] used the technique called tetraploid embryo complementation. They demonstrated that increased ESC passage number negatively affected stem cell proliferative potential and ability of the cells to form an adult mouse. Kretlow et al. [43] showed that the increasing age and the number of passages had lineage dependent effects on bone marrow-derived stem cell (BMSC) differentiation potential [43]. The study of Allahbakhshian-Farsani et al. [60] conducted cytogenetic analysis of HDFs in early and late passages using karyotyping and comet assay showed that only early passages of HDFs culture are cytogenetically stable and could be used for genetic manipulation, nuclear transfer, and cell reprogramming to produce iPSCs. Their results indicated the occurrence of single strand breaks (SSBs) or DNA damage after many passages in the cells.

Conclusion

The previous researches and present investigation show that maintaining stem cells in undifferentiated state and using the therapeutic applications need optimized feeder layer and atmosphere, by adding supplemented media containing essential growth factors in their culture. Furthermore, the optimizations of stem cell culture conditions based on each cell type are unique and variable (Table 2).

The present study indicated that optimization of critical parameters such as pH, 3–10 % O_2 and 5–10 % CO_2 are suitable for stem cell culture. Furthermore, for clinical approaches using stem cells in low passage number could decrease cytogenetic instability and DNA damages. Although feeder cells are essential for supporting the stem cell culture but for avoiding the stem cells contamination with feeder cell line, culture of these cell on feeder free systems like Matrigel matrix in conditioned media or supplemented media with essential growth factors are useful. Therefore, for decreasing the immune system responses and controlling the risk of animal pathogen transfer, culture of stem cells on human feeder in optimized culture media is suitable for clinical approaches.

Acknowledgments This review study was supported through funds (Grant Nos: 91-11-4 and 91-11-6) provided by Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.

References

1. Hoffman LM, Carpenter MK (2005) Characterization and culture of human embryonic stem cells. Nat Biotechnol 23(6):699–708

2. Musina RA, Bekchanova ES, Sukhikh GT (2005) Comparison of mesenchymal stem cells obtained from different human tissues. Bull Exp Biol Med 139(4):504–509
3. Singh S, Deka D, Mulinti R, Sood NK, Agrawal RK (2014) Isolation, culture, in vitro differentiation and characterization of canine adult mesenchymal stem cells. Proc Natl Acad Sci, India, Sect B Biol Sci. doi:10.1007/s40011-014-0309-8
4. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318(5858):1917–1920
5. Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, Blake J, Schwager C, Eckstein V, Ansorge W (2005) Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol 33(11):1402–1416
6. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5):861–872
7. Liao J, Wu Z, Wang Y, Cheng L, Cui C, Gao Y, Chen T, Rao L, Chen S, Jia N (2008) Enhanced efficiency of generating induced pluripotent stem (iPS) cells from human somatic cells by a combination of six transcription factors. Cell Res 18(5):600–603
8. Nasri M, Karimi A, Farsani MA (2014) Production, purification and titration of a lentivirus-based vector for gene delivery purposes. Cytotechnology. doi:10.1007/s10616-013-9652-5
9. Van Der Sanden B, Dhobb M, Berger F, Wion D (2010) Optimizing stem cell culture. J Cell Biochem 111(4):801–807
10. Pera MF, Reubinoff B, Trounson A (2000) Human embryonic stem cells. J Cell Sci 113(1):5–10
11. Yao S, Chen S, Clark J, Hao E, Beattie GM, Hayek A, Ding S (2006) Long-term self-renewal and directed differentiation of human embryonic stem cells in chemically defined conditions. Proc Natl Acad Sci 103:6907–6912
12. Buzzard JJ, Gough NM, Crook JM, Colman A (2004) Karyotype of human ES cells during extended culture. Nat Biotechnol 22:381–382
13. Mitalipova MM, Rao RR, Hoyer DM, Johnson JA, Meisner LF, Jones KL, Dalton S, Stice SL (2005) Preserving the genetic integrity of human embryonic stem cells. Nat Biotechnol 23(1):19–20
14. Xu C, Inokuma MS, Denham J, Golds K, Kundu P, Gold JD, Carpenter MK (2001) Feeder-free growth of undifferentiated human embryonic stem cells. Nat Biotechnol 19:971–974
15. Ying QL, Nichols J, Chambers I, Smith A (2003) BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell 115:281–292
16. Ludwig TE, Levenstein ME, Jones JM, Berggren WT, Mitchen ER, Frane JL, Crandall LJ, Daigh CA, Conard KR, Piekarczyk MS (2006) Derivation of human embryonic stem cells in defined conditions. Nat Biotechnol 24(2):185–187
17. Axelrod BW, Wion D (2012) Stem cell culture: optimizing amidst the complexity. Stem Cells Cancer Stem Cells 8:3–12
18. Davidson JM, Zoia O, Liu JM (1993) Modulation of transforming growth factor-beta 1 stimulated elastin and collagen production and proliferation in porcine vascular smooth muscle cells and skin fibroblasts by basic fibroblast growth factor, transforming growth factor-α, and insulin-like growth factor-I. J Cell Physiol 155(1):149–156
19. Schuldiner M, Yanuka O, Itskovitz-Eldor J, Melton DA, Benvenisty N (2000) Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. Proc Natl Acad Sci 97(21):11307–11312
20. Xiao L, Yuan X, Sharkis SJ (2006) Activin a maintains self-renewal and regulates fibroblast growth factor, Wnt, and bone morphogenic protein pathways in human embryonic stem cells. Stem Cells 24:1476–1486
21. Waldrip WR, Bikoff EK, Hoodless PA, Wrana JL, Robertson EJ (1998) Smad2 signalling in extraembryonic tissues determines anterior–posterior polarity of the early mouse embryo. Cell 92:797–808
22. Rao BM, Zandstra PW (2005) Culture development for human embryonic stem cell propagation: molecular aspects and challenges. Curr Opin Biotechnol 16:568–576
23. Adelman CA, Chattopadhyay S, Bieker JJ (2002) The BMP/BMPR/Smad pathway directs expression of the erythroid-specific EKLF and GATA1 transcription factors during embryoid body differentiation in serum-free media. Development 129:539–549
24. Pera MF, Andrade J, Houssami S, Reubinoff B, Trounson A, Stanley EG, Ward-van Oostwaard D, Mummery C (2004) Regulation of human embryonic stem cell differentiation by BMP-2 and its antagonist noggin. J Cell Sci 117(7):1269–1280
25. Quang T, Marquez M, Blanco G, Zhao Y (2014) Dosage and cell line dependent inhibitory effect of bFGF supplement in human pluripotent stem cell culture on inactivated human Mesenchymal stem cells. PLoS ONE 9(1):e86031
26. Pucéat M (2007) TGFbeta in the differentiation of embryonic stem cells. Cardiovasc Res 74(2):256–261
27. Brimble SN, Zeng X, Weiler DA, Luo Y, Liu Y, Lyons IG, Freed WJ, Robins AJ, Rao MS (2001) Schulz TC (2004) Karyotypic stability, genotyping, differentiation, feeder-free maintenance, and gene expression sampling in three human embryonic stem cell lines derived prior to August 9. Stem Cell Dev 13(6):585–597
28. Eiselleova L, Matulka K, Kriz V, Kunova M, Schmidtova Z, Neradil J, Tichy B, Dvorakova D, Pospisilova S, Hampl A (2009) A complex role for FGF-2 in self-renewal, survival, and adhesion of human embryonic stem cells. Stem Cells 27:1847–1857
29. Park Y, Choi IY, Lee SJ, Lee SR, Sung HJ, Kim JH, Yoo YD, Geum DH, Kim SH, Kim BS (2010) Undifferentiated propagation of the human embryonic stem cell lines, H1 and HSF6, on human placenta-derived feeder cells without basic fibroblast growth factor supplementation. Stem Cells Dev 19(11):1713–1722
30. Becker T, Hitzmann B, Muffler K, Portner R, Reardon KF, Stahl F, Ulber R (2007) Future aspects of bioprocess monitoring. Adv Biochem Eng Biotechnol 105:249–293
31. Watanabe K, Ueno M, Kamiya D, Nishiyama A, Matsumura M, Wataya T, Takahashi JB, Nishikawa S, Nishikawa SI, Muguruma K, Sasai YA (2007) Rock inhibitor permits survival of dissociated human embryonic stem cells. Nat Biotechnol 25(6):681–686
32. Amit M, Laevsky I, Miropolsky Y, Shariki K, Peri M, Itskovitz-Eldor J (2011) Dynamic suspension culture for scalable expansion of undifferentiated human pluripotent stem cells. Nat Protoc 6(5):572–579
33. Richards M, Fong C-Y, Chan W-K, Wong P-C, Bongso A (2002) Human feeders support prolonged undifferentiated growth of human inner cell masses and embryonic stem cells. Nat Biotechnol 20:933–936
34. Xu C, Rosler E, Jiang J, Lebkowski JS, Gold JD, O'Sullivan C, Delavan-Boorsma K, Mok M, Bronstein A, Carpenter MK (2005) Basic fibroblast growth factor supports undifferentiated human embryonic stem cell growth without conditioned medium. Stem Cells 23(3):315–323
35. Skottman H, Hovatta O (2006) Culture conditions for human embryonic stem cells. Reproduction 132(5):691–698
36. Amit M, Margulets V, Segev H, Shariki K, Laevsky I, Coleman R, Itskovitz-Eldor J (2003) Human feeder layers for human embryonic stem cells. Biol Reprod 68(6):2150–2156

37. Lee JB, Song JM, Lee JE, Park JH, Kim SJ, Roh SI, Yoon HS (2004) Available human feeder cells for the maintenance of human embryonic stem cells. Reproduction 128(6):727–735
38. Lei T, Jacob S, Ajil-Zaraa I, Dubuisson JB, Irion O, Jaconi M, Feki A (2007) Xeno-free derivation and culture of human embryonic stem cells: current status, problems and challenges. Cell Res 17(8):682–688
39. Su Z, Frye C, Bae KM, Kelley V, Vieweg J (2008) Differentiation of human embryonic stem cells into immunostimulatory dendritic cells under feeder-free culture conditions. Clin Cancer Res 14(19):6207–6217
40. Amit M, Shariki C, Margulets V, Itskovitz-Eldor J (2004) Feeder layer- and serum-free culture of human embryonic stem cells. Biol Reprod 70:837–845
41. Rodin S, Domogatskaya A, Strom S, Hansson EM, Chien KR, Inzunza J, Hovatta O, Tryggvason K (2010) Long-termself-renewal of human pluripotent stemcells on human recombinant laminin-511. Nat Biotechnol 28:611–615
42. Wang G, Zhang H, Zhao Y, Li J, Cai J, Wang P, Meng S, Feng J, Miao C, Ding M (2005) Noggin and bFGF cooperate to maintain the pluripotency of human embryonic stem cells in the absence of feeder layers. Biochem Biophys Res Commun 330(3):934–942
43. Kretlow JD, Jin YQ, Liu W, Zhang WJ, Hong TH, Zhou G, Baggett LS, Mikos AG, Cao Y (2008) Donor age and cell passage affects differentiation potential of murine bone marrow-derived stem cells. BMC Cell Biol 9(1):60
44. Klimanskaya I, Chung Y, Meisner L, Johnson J, West MD, Lanza R (2005) Human embryonic stem cells derived without feeder cells. Lancet 365(9471):1636–1641
45. Tsai Z-Y, Singh S, Yu S-L, Chou C-H, Li S-L (2010) A feeder-free culture using autogeneic conditioned medium for undifferentiated growth of human embryonic stem cells: comparative expression profiles of mRNAs, microRNAs and proteins among different feeders and conditioned media. BMC Cell Biol 11:76
46. Stover AE, Schwartz PH (2011) Adaptation of human pluripotent stem cells to feeder-free conditions in chemically defined medium with enzymatic single-cell passaging. Methods Mol Biol 767:137–146
47. Inzunza J, Gertow K, Strömberg MA, Matilainen E, Blennow E, Skottman H, Wolbank S, Ährlund-Richter L, Hovatta O (2005) Derivation of human embryonic stem cell lines in serum replacement medium using postnatal human fibroblasts as feeder cells. Stem Cells 23(4):544–549
48. Hovatta O, Mikkola M, Gertow K, Strömberg AM, Inzunza J, Hreinsson J, Rozell B, Blennow E, Andäng M, Ährlund-Richter L (2003) A culture system using human foreskin fibroblasts as feeder cells allows production of human embryonic stem cells. Hum Reprod 18(7):1404–1409
49. Nakamura M, Namiki M, Okuyama A, Matsui T, Doy Y, Takeyama M, Fujioka H, Nishimune Y, Matsumoto K, Sonoda T (1987) Temperature sensitivity of human spermatogonia and spermatocytes in vitro. Arch Androl 19(2):127–132
50. Waldman SD, Couto DC, Omelon SJ, Kandel RA (2004) Effect of sodium bicarbonate on extracellular pH, matrix accumulation, and morphology of cultured articular chondrocytes. Tissue Eng 10(11–12):1633–1640
51. Oh SK, Kim HS, Park YB, Seol HW, Kim YY, Cho MS, Ku SY, Choi YM, Kim D-W, Moon SY (2005) Methods for expansion of human embryonic stem cells. Stem Cells 23:605–609
52. Draper JS, Smith K, Gokhale P, Moore HD, Maltby E, Johnson J, Meisner L, Zwaka TP, Thomson JA, Andrews PW (2004) Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. Nat Biotechnol 22:53–54
53. Rosler ES, Fisk GJ, Ares X, Irving J, Miura T, Rao MS, Carpenter MK (2004) Long-term culture of human embryonic stem cells in feeder-free conditions. Dev Dyn 229:259–274
54. Ulloa-Montoya F, Verfaillieb CM, Hu W-S (2005) Culture systems for pluripotent stem cells. J Biosci Bioeng 100(1):12–27
55. Thomson A, Wojtacha D, Hewitt Z, Priddle H, Sottile V, Di Domenico A, Fletcher J, Waterfall M, Corrales NL, Ansell R, McWhir J (2008) Human embryonic stem cells passaged using enzymatic methods retain a normal karyotype and express CD30. Clon Stem Cells 10(1):89–106
56. Li XY, Jia Q, Di KQ, Gao SM, Wen XH, Zhou RY, Wei W, Wang LZ (2007) Passage number affects the pluripotency of mouse embryonic stem cells as judged by tetraploid embryo aggregation. Cell Tissue Res 327(3):607–614
57. Wang ZQ, Kiefer F, Urbanek P, Wagner EF (1997) Generation of completely embryonic stem cell-derived mutant mice using tetraploid blastocyst injection. Mech Dev 62:137–145
58. Heng BC, Cowan CM, Basu S (2009) Comparison of enzymatic and non-enzymatic means of dissociating adherent monolayers of mesenchymal stem cells. Biol Proced Online 11:161–169
59. Hughes P, Marshall D, Reid Y, Parkes H, Gelber C (2007) The costs of using unauthenticated, over-passaged cell lines: how much more data do we need? Biotechniques 43(5):575–586
60. Allahbakhshian-Farsani M, Abdian N, Ghasemi-Dehkordi P, Sadeghiani M, Saffari-Chaleshtori J, Hashemzadeh-Chaleshtori M, Khosravi-Farsani S (2014) Cytogenetic analysis of human dermal fibroblasts (HDFs) in early and late passages using both karyotyping and comet assay techniques. Cytotechnol 66:815–822
61. Nagy A, Rossant J, Nagy R, Abramow-Newerly W, Roder JC (1993) Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. Proc Natl Acad Sci 90(18):8424–8428
62. Crisostomo PR, Wang M, Wairiuko GM, Morrell ED, Terrell AM, Seshadri P, Nam UH, Meldrum DR (2006) High passage number of stem cells adversely affects stem cell activation and myocardial protection. Shock 26(6):575–580
63. Shiue YL, Liou JF, Shiau JW, Yang JR, Chen YH, Tailiu JJ, Chen LR (2006) In vitro culture period but not the passage number influences the capacity of chimera production of inner cell mass and its deriving cells from porcine embryos. Anim Reprod Sci 93:134–143