

Concise Review: The Evolution of Human Pluripotent Stem Cell Culture: From Feeder Cells to Synthetic Coatings

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ABSTRACT

Current practices to maintain human pluripotent stem cells (hPSCs), which include induced pluripotent stem cells and embryonic stem cells, in an undifferentiated state typically depend on the support of feeder cells such as mouse embryonic fibroblasts (MEFs) or an extracellular matrix such as Matrigel. Culture conditions that depend on these undefined support systems limit our ability to interpret mechanistic studies aimed at resolving how hPSCs interact with their extracellular environment to remain in a unique undifferentiated state and to make fate-changing lineage decisions. Likewise, the xenogeneic components of MEFs and Matrigel ultimately hinder our ability to use pluripo-

tent stem cells to treat debilitating human diseases. Many of these obstacles have been overcome by the development of synthetic coatings and bioreactors that support hPSC expansion and self-renewal within defined culture conditions that are free from xenogeneic contamination. The establishment of defined culture conditions and synthetic matrices will facilitate studies to more precisely probe the molecular basis of pluripotent stem cell self-renewal and differentiation. When combined with three-dimensional cultures in bioreactors, these systems will also enable large-scale expansion for future clinical applications. *STEM CELLS* 2013;31:1–7

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Like other stem cells, human pluripotent stem cells (hPSC) have the capacity for self-renewal and differentiation into specialized cell types. However, pluripotent cells are unique in their ability to self-renew indefinitely. They also feature the capacity to differentiate into all the approximately 200 specialized cell types of the body. These two fundamental characteristics make hPSCs a potential source of cells for regenerative medicine, drug discovery, disease modeling, and studies aimed to better understand human development. Human embryonic stem cells (hESCs) are isolated from the inner cell mass of the blastocyst [1], while human-induced pluripotent stem cells (hiPSCs) are derived by the overexpression of key transcription factors in somatic cells [2, 3]. Both of these PSCs share the expression of transcription factors (Oct4, Sox2, and Nanog), cell surface markers such as the stage-specific embryonic antigens (SSEA-3 and SSEA-4) the keratan sulfate-related antigens (TRA-1-60 and TRA-1-81), high telomerase, and alkaline phosphatase activity as well as the capacity to grow indefinitely in vitro when cultured under permissive conditions.

To maximize the potential of PSCs in regenerative medicine and for future transplantation studies, in vitro derivation and continuous culture conditions need to be performed using

good manufacturing practices. This objective was clear from the first derivation and prolonged culture of hESCs [1] and subsequently a rapid evolution in derivation and culture methods has been realized. The early culture conditions for hESCs were determined by effectively following the methods developed for mouse ESCs [4]. These early methods included coculture of hESCs with irradiated mouse embryonic fibroblasts (MEF) in an enriched culture medium containing fetal bovine serum. It soon became evident, however, that hESCs and mouse ESCs requirements for self-renewal are distinct. The principle difference between the two species is that the growth of undifferentiated hESCs cannot be maintained in feeder-free conditions in the presence of leukemia inhibitory factor, as it is possible for mouse ESCs [5].

Since the initial description of the successful derivation and culture of hESCs [1], several hundred lines of human ESCs and iPSCs have been derived and investigation of their biologic characteristics has contributed to the identification of key molecular pathways and transcription factors that are involved in the self-renewal and lineage differentiation of PSCs. This in turn has been translated into knowledge to optimize the culture conditions of PSCs. In this concise review, we summarize the evolution in hPSC culture and place an emphasis on the use of synthetic coatings as substrates to support the unlimited proliferation of hPSCs in vitro (Fig. 1; Table 1).

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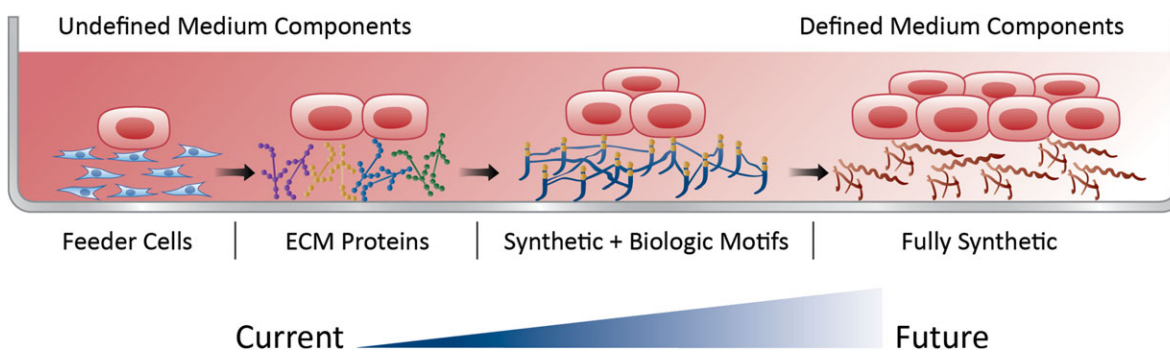


Figure 1. Evolution of human pluripotent stem cell (hPSC) culture. The *in vitro* culture of hPSCs has evolved to achieve xenogeneic-free and defined conditions. The illustration depicts this progression (left to right) from coculture with feeder cells and serum-containing medium, to feeder-independent cultures in chemical-defined medium. Feeder-free conditions have progressed from the use of complex combinations of ECM proteins like Matrigel as a substrate, to individual ECM molecules such as laminin-511, vitronectin, and fibronectin. The third generation substrates for hPSC culture are defined by the use of synthetic components in combination with biologic motifs such as RGD peptides. Advanced materials now provide a fully synthetic substrate that support clonal growth, derivation, and long-term culture of genomically stable hPSCs. The gradient transition from dark to light red color (left to right) indicates the complexity of the culture medium, from undefined to defined components. hPSCs are illustrated as the rectangular cells with a prominent red nuclei, while fibroblasts are shown as elongated cells with blue nuclei. Abbreviation: ECM, extracellular matrix.

RISKS ASSOCIATED WITH FEEDER CELLS AND XENOGENEIC COMPONENTS AND THEIR IMPEDIMENT TO MECHANISTIC STUDIES

Feeder cells such as MEFs support the self-renewal of hPSCs by the secretion of essential growth factors, cytokines, and extracellular matrices (ECM) such as transforming growth factor β , activin A, laminin-511, and vitronectin [25]. However, inconsistencies in expression and secretion of these factors by different feeder cells [25, 26] make it difficult to determine which components are indispensable for the support of hPSCs in an undifferentiated state. Moreover, the γ -irradiation of feeder cells not only impedes their proliferation but also induces apoptosis and subsequently alters the secretion of soluble factors and deposition of an ECM. All these factors may negatively affect the self-renewal and consistent culture of hPSCs [27]. Thus, the dynamic and undefined microenvironment that feeder cells create limits our ability to interpret mechanistic studies designed to understand the biology of hPSCs.

Feeder cells and their products can also be a source of pathogens for hPSCs. For example, in the coculture of hESCs and MEFs with animal-derived serum replacements, the detection of an immunogenic sialic acid (Neu5Gc) has been reported [28]. This is of particular concern because the presence of non-human sialic acid may induce an immune response upon transplantation of hPSC derivatives. Xenogeneic feeder cells and serum are also a common source of mycoplasma contamination. Because mycoplasmas compete with host cells for essential nutrients, mycoplasma contamination of cultured cells may compromise diverse aspects of cell physiology such as cell growth, phenotype, karyotype, and induction of cytokine expression. These infections often go undetected and consequently could alter the interpretation of key experimental observations (see [29] for literature review). In an effort to prevent xenogeneic contaminations, human feeder cells and serum have been proposed for the culture of hPSCs (see [30] for literature review). However, the risk of contamination by viral and nonviral infectious agents also exists when using human feeder cells (see [31] for literature review).

FEEDER-FREE CULTURE SYSTEMS AND THEIR BENEFITS IN UNDERSTANDING THE BIOLOGY OF hPSCs

One of the first examples of alternatives to feeder-dependent PSC cultures was the demonstration of long-term culture of hESCs on tissue culture plates coated with Matrigel in combination with MEF-conditioned medium [6]. Matrigel is composed mainly of laminin, collagen IV, heparin sulfate proteoglycans, entactin, and growth factors [32]. However, Matrigel is derived from Engelbreth-Holm-Swarm mouse sarcomas [32], exhibits lot-to-lot variability, and can introduce unwanted xenogeneic contaminants. Therefore, Matrigel is not an ideal substrate for feeder-free culture of hPSCs if the primary objective is to culture these cells for eventual human therapies. Nevertheless, Matrigel remains one of the most commonly used substrates and has served as an important starting point to define the requirements for hPSC growth and differentiation.

The individual components of Matrigel exhibit varying degrees of support for PSCs. Laminin-coated surfaces support the growth of hESCs [6]. In contrast, when cultured on fibronectin and collagen IV, the self-renewal of hESCs is compromised [6]. It has also been reported that the specific laminin isoforms -111, -332, and -511 support the adhesion and proliferation of undifferentiated hESCs, while isoforms -211 and 411 do not [13]. In addition, it has been shown that supportive feeder cells [25] and hESCs [6, 13] produce laminin isoforms -511/-521 and express the integrin $\alpha 6 \beta 1$ receptor, the primary receptor for these laminin isoforms [33]. The laminin isoform -511 is abundant in the embryonic basement membrane [34] and is also supportive of mouse ESC cultures [35]. Therefore, these findings suggest that mechanisms responsible for self-renewal of hPSCs *in vitro* may be conserved between species.

Because integrins are the principal cell surface receptors that mediate cell-ECM interactions, it is likely that the identification of integrins in hPSCs signal the use of other supporting ECM proteins for self-renewal of these pluripotent stem cells. Following this hypothesis, vitronectin has been shown to

Table 1. Summary of substrates and cell culture media used for feeder-free culture of human pluripotent stem cells

Substrate coating	Cell culture medium (chemically defined)*	Cell lines tested (passages) ^a	Cell characterization ^b	Substrate				References	
				Sterilization capability	Relative Cost	Source of derivation	Stability at RT ^c		Preparation
Matrigel, laminin	MEF-CM+FGF2 ^d	H1, H7, H9, H14 (25)	Full	No	Expensive	Xenogeneic	No	Absorption	Xu et al. [6]
Laminin	X-VIVO10+GF ^{2e}	H1 (40)	Full	No	Expensive	Human protein	No	Absorption	Li et al. [7]
Matrigel, fibronectin	HESCO ^f	H9, BG01 (23)	Full	No	Expensive	Xenogeneic	No	Absorption	Lu et al. [8]
Matrigel,	DMEM/F12, N2, B12... ^g	H1, HSF6 (22)	Full	No	Expensive	Xenogeneic	No	Absorption	Yao et al. [9]
Matrigel, 4ECM ^h	mTeSR ⁱ	H1, H7, H9, H14 (25)	Full	No	Expensive	Xenogeneic	No	Absorption	Ludwig et al. [10]
Matrigel	StemPro ^j	H1, BG01, BG02, BG03, CyT49 (25)	Full	No	Expensive	Xenogeneic	No	Absorption	Wang et al. [11]
Laminin-derived peptides	MEF-CM+FGF2	H1, H9 (Short-term: 7-10 days)	Partial	No	Expensive	Protein	No	Peptide synthesis	Derda et al. [12]
rh laminin-511	MEF-CM+FGF2	KhES-1, KhES-2, KhES-3 (10)	Partial	No	Expensive	Recombinant human protein	No	Absorption	Miyazaki et al. [13]
rh vitronectin	mTeSR ^k	HUES1, HES2, NL3 (5)	Partial	No	Expensive	Recombinant human protein	No	Absorption	Braam et al. [14]
	E-8 ^k	H1, H9, hiPSCs (20)	Full	No	Expensive	Recombinant human protein	No	Absorption	Chen et al. [15]
rh E-cadherin	MEF-CM+FGF2, mTeSR ^k	H1, H9, hiPSCs (37)	Full	No	Expensive	Recombinant human protein	No	Absorption	Nagaoka et al. [16]
PMVE-alt-MA	StemPro ^k	HUES1, HUES9, hiPSCs (5)	Partial	Yes	Inexpensive	Synthetic coating	Yes	Free radical polymerization	Brafman et al. [17]
Heparin-binding GKKQ	mTeSR ^k	H1, H7, H9, H13, H14, IMR-90-1 iPSCs, DF19-97T iPSCs (17)	Partial	No	Expensive	Synthetic coating	No	Chemisorption	Klim et al. [18]
Hit 9	mTeSR ^k	BG01, WIBR3 (10)	Full	Yes	Inexpensive	Synthetic coating	Yes	Photo-polymerization	Mei et al. [19]
Synthemax	X-VIVO10+GF ² , mTeSR ^k	H1 and H7 (10)	Full	Yes	Expensive	Synthetic coating	Yes	Photo-polymerization & chemical conjugation of peptide	Melkounian et al. [20]
APMAAM	mTeSR ^k	H1, H9 (20)	Partial	Yes	Inexpensive	Synthetic coating	Yes	Photo-polymerization	Irwin et al. [21]
PMEDSAH	MEF-CM+FGF2, hCCM+FGF2, StemPro ^k	H1, H7, H9, CHB-8, CHB-10, hiPSCs (20)	Full	Yes	Inexpensive	Synthetic coating	Yes	Surface initiated polymerization	Villa-Diaz et al. [22-24]

^aPassages: indicated the number of passages that cells were cultured at time of karyotype analysis.

^bCell characterization: partial includes pluripotent stem cell marker expression (Oct4 also known as Pou5f1, Sox2, TRA-1-60, TRA-1-81, SSEA3, and SSEA4), in vitro differentiation into derivatives of three germ layers, and karyotype analysis. Full characterization complements the partial characterization with in vivo differentiation through teratoma assay.

^cRT: room temperature.

^dMEF-CM+FGF2: conditioned medium from MEF supplemented with rh FGF 2.

^eX-VIVO 10+GF: X-VIVO10 medium, nonessential amino acids (NEAA), L-glutamine (L-glu), β-mercaptoethanol (β-Mer), rh stem cell factor, rh Flt3 ligand, rh leukemia inhibitory factor.

^fHESCO: DMEM/F12 medium, knockout serum replacer, NEAA, L-glu, β-Mer, bovine serum albumin (BSA), Insulin (Ins), transferrin (Transf), FGF2, B-cell activating factor, Wnt3a, cholesterol.

^gDMEM/F12, N2, B12: DMEM/F12 medium, N2 medium, B12 medium, NEAA, L-glu, β-Mer, BSA, rh FGF2.

^h4-ECM: combination of collagen IV, fibronectin, laminin, and vitronectin.

ⁱmTeSR: DMEM/F12 medium, NEAA, L-glu, β-Mer, BSA, Ins, Transf, rh FGF2, transforming growth factor (TGF) β1, pipecolic acid, lithium chloride, γ-aminobutyric acid.

^jStemPro: DMEM/F12 medium, BSA, Trans, rh FGF2, IGF-1, activin A, heregulin 1β.

^kE-8: DMEM/F12 medium, NaHCO₃, selenium, ascorbic acid, Ins, Transf, rh FGF2, TGFβ1.

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; FGF, fibroblast growth factor; MEF, mouse embryonic fibroblasts; rh, recombinant human.

support hESC self-renewal via integrin $\alpha V\beta 5$ [14]. Similarly, E-cadherin, which mediates cell-cell interactions and has been involved in hESC colony formation [36] and self-renewal [37], has been used as a substrate for long-term culture of hPSCs [16]. The use of recombinant human (rh) laminin -511 [13], rh vitronectin [14], and rh E-cadherin [16] represents significant milestones in the culture of hPSCs because they were the first examples of defined and xenogeneic-free substrates.

FROM NONDEFINED TO DEFINED CONDITIONS: CULTURE MEDIUM AND SUPPORTIVE SUBSTRATES

While this review places an emphasis on the cell culture substrates, one must acknowledge that the culture medium plays an essential role in achieving a defined culture system for hPSCs. As mentioned above, the secretion of soluble factors by feeder cells influences the fate of PSCs in self-renewal and differentiation. Consequently, conditioned medium from feeder cells has been commonly used to culture hPSCs in feeder-free conditions. However, due to its undefined characteristics, variability, and the risks associated with contaminants, more rigorous practices include the use of chemically defined culture medium. While several culture media formulations [7–11, 15] have been developed to support the culture of hPSCs in feeder-free conditions, it should be noted that all these media formulations were developed using Matrigel as a substrate. Additionally, some media formulations have also been shown to be effective when used with individual ECM proteins like fibronectin [8], laminin [7], or vitronectin [15] and with synthetic substrates [17–22] that support undifferentiated hPSC proliferation.

The combination of chemically defined medium and xenogeneic-free biological substrates represents significant progress in the generation of clinically compliant culture systems for hPSCs. Nevertheless, for large-scale expansion of hPSCs in chemically defined and clinically compliant conditions, biological substrates have drawbacks that must be overcome. These barriers include factors such as batch-to-batch variability, limited scalability, difficulty in isolation, expense to manufacture, and the need to ensure pathogen-free conditions. As important alternatives, synthetic substrates that support the proliferation of undifferentiated hPSCs have been developed [17–20, 22]. These synthetic environments will likely prove to be superior because they exhibit little batch-to-batch variation, are defined, are reproducible, are stable, are amenable to standard sterilization techniques, and can be readily tuned to meet the culture requirements for different hPSC lines.

Due to the anchorage-dependent nature of hPSCs, synthetic substrates must allow cell adhesion, spreading, self-renewal, and subsequent colony formation of undifferentiated hPSCs. Furthermore, hPSCs cultured on synthetic substrates must retain the unique nature of pluripotency and must not develop chromosomal and genomic abnormalities. In addition, synthetic coatings should demonstrate efficacy in the long-term culture of multiple stem cell lines/types, be compatible with common biomedical sterilization methods, be cost-effective and have the potential to be scaled-up for commercial purposes.

To create defined synthetic substrates that allow the long-term propagation of hPSCs, several materials and material combinations have been developed and tested. For example, peptide or protein-based systems, polymers and polymers in conjunction with biomolecules have been developed. Many of these systems exploit biologic moieties to achieve appropriate

culture conditions. The success of this approach was realized when the heparin-binding peptide GKKQFRHRNRKG was conjugated to an alkanethiol self-assembled monolayer for the long-term culture of hESCs [18]. Similarly, arrays of laminin-derived peptides have also been shown to support hESCs [12].

Among the polymer-based substrates is the zwitterionic hydrogel, poly2-(methacryloyloxy)ethyl dimethyl-(3-sulfopropyl)ammonium hydroxide (PMEDSAH), that has been developed using a surface initiated graft polymerization technique. The surface coatings composed of PMEDSAH are fully synthetic and are extremely effective in sustaining the long-term expansion of hESCs and hiPSCs [22–24]. Other polymers coatings for hPSCs include polymer Hit9 [19] and the amino-propylmethacrylamide (APMAAm) [21], a methacrylamide containing polymer. Both of these polymers are fabricated by photopolymerization. Poly(methyl vinyl ether-alt-maleic anhydride) (PMVE-alt-MA), an anhydride containing polymer coating generated by radical polymerization [17], is another synthetic material that supports hPSCs. Other research groups have taken a hybrid approach, using polymers as the base substrate and then modifying the surface with biomolecules. For example, Synthemax (Corning), an acrylate polymer modified with amino-containing peptides, has been shown to be effective in the prolonged culture of multiple hESC lines [20].

Of all the synthetic surfaces detailed thus far, only PMEDSAH [22, 23] and APMAAm [21] have been reported to maintain hPSCs for greater than 20 passages in defined and xenogeneic-free culture medium. Because of the great number of potential applications in regenerative medicine and tissue engineering using hPSCs, it is desirable for culture substrates to maintain numerous cell lines and cell types in an undifferentiated state. To date, all the substrates detailed in this review meet the criteria of having been used in the culture of multiple hESC and hiPSC lines.

Biomedical products such as culture plates to expand PSCs must be batch sterilized before use and thus it is important for synthetic substrates to be compatible with common sterilization techniques such as electron beam- and γ -radiation. However, substrates with biologic components such as proteins and peptides do not readily lend themselves to common sterilization methods because the sterilization treatment may denature or degrade the biologic moieties. At this time, only Synthemax and PMEDSAH have shown compatibility with common sterilization methods [20, 22, 38]. Furthermore, the inclusion of peptides and proteins in stem cell culture substrates may not allow the surfaces to be reused because biomolecules are known to undergo degradation from metalloproteinases secreted by cultured cells [39]. This observation should also be considered as a factor for future clinical adoption, as the inclusion of biomolecules leads to increased costs. Due to the large number of stem cells that will likely be needed for cell-based regenerative therapies [40], it will be important to use processes to generate substrates that can be scaled-up from the bench top to high-throughput manufacturing processes. Modifying surfaces with peptides and proteins can also hinder scale-up as it increases process complexity.

BIOLOGY OF SYNTHETIC SUBSTRATE-PSC INTERACTIONS: MAINTENANCE OF SELF- RENEWAL AND CONTROL OF DIFFERENTIATION

Several strategies have been used to develop synthetic substrates for long-term hPSC culture. While the molecular

mechanisms responsible for this support are not yet fully characterized, the influence of material properties on cells is currently an area of intense investigation. For substrates that present cell adhesion elements such as heparin-binding peptides [18] and laminin-derived peptides [12], the mechanism of support for hPSCs may be due to these biological-mimicking components. It is possible that substrates like PMVE-altMA [17] and PMEDSAH [22], which contain carboxyl and sulfonyl groups, mimic the functional characteristics of heparin, and in this way, support hPSCs. However, other material properties, including hydrophilicity/hydrophobicity, surface roughness, and stiffness of the culture substrate, can influence hPSC behavior. For example, the wettability properties of polymer coatings affect the adhesion of hPSCs. In this case, hydrophobic materials are less permissive [22]. The roughness of the substrate also impacts the fate of hPSCs. In terms of adhesion, proliferation, and self-renewal, surfaces categorized as smooth by a root-mean-square roughness (Rq) of 1 nm provide superior support to hESCs over nanorough surfaces with an Rq = 75–150 nm [41]. Similarly, it has been shown that rigid substrates maintain self-renewal of hESCs, while on soft substrates stem cells are more prone to differentiate. These data demonstrate that hESCs are mechanosensitive and increase their cytoskeleton contractility with substrate rigidity [42].

The chemical properties of the substrate also affect the structural conformation of proteins that interact with the synthetic coating. The source of the protein may be from factors that are used to coat the surface, factors that are added to the culture medium, and those factors that are secreted as stem cells adhere to their physical environment in vitro [19]. For example, the network structure of Matrigel and its ability to support attachment and self-renewal of hESCs change depending on the surface on which it is adsorbed [43]. In the case of supplementary proteins, it has been shown that APMAAm surfaces adsorb bovine serum albumin from the culture medium in an unfolded state, allowing the proliferation of undifferentiated hESCs [21]. Taken together, these examples illustrate the importance and complexity of the role the microenvironment plays in self-renewal and differentiation of hPSCs and suggests that synthetic substrates that support hPSCs exhibit both chemical and mechanical properties that support hPSC proliferation.

CULTURE OF PLURIPOTENT STEM CELLS IN THREE-DIMENSIONAL BIOREACTORS

While the establishment of synthetic substrates and defined culture conditions for hPSCs allows us to more precisely probe the molecular basis of pluripotent stem cell self-renewal and differentiation, the large-scale expansion of these cells for future clinical use could be accelerated by the development of three-dimensional cell-suspension cultures [40]. It is accepted that hPSCs grow as adherent colonies and upon detachment from supportive substrates, stem cells randomly differentiate and the formation of embryoid bodies (EBs) is enabled. However, with the proper culture medium, undifferentiated hPSCs can proliferate in suspension as spheroid clumps. Homogenous populations of small cells with large nuclei can survive and retain the expression of pluripotent stem cell markers and the capability to differentiate in vivo and in vitro [44–47]. Interestingly, when hPSC spheroid clumps are cultured in suspension with serum-containing medium, cystic EBs form and evidence of differentiation is observed [44].

The long-term and large-scale propagation of both hESCs and hiPSCs in bioreactors with serum-free medium and without microcarriers in both static and dynamic cultures has been reported [44–47]. Under these culture conditions, hPSCs have been expanded for over 20 passages [44] and the proliferation rates, although varying from study to study, have been reported to be as high as 20-fold in 6 days, which is higher than that reported for feeder cell cultures (approximately fivefold) or feeder-free cultures (approximately ninefold) for the same period of time [46]. It has been calculated that an initial seeding density of 5×10^7 hPSCs per spinner flask would result in a yield of at least 1.6×10^9 cells over five passages [47].

DERIVATION AND LONG-TERM CULTURE OF hPSCS ON SYNTHETIC SUBSTRATES AND IN CELL-SUSPENSION CULTURES: PHENOTYPIC, GENETIC, AND EPIGENETIC STABILITY

As the field of regenerative medicine advances toward the development of in vitro disease models from hPSC lines, both directed genetic modification and derivation of new stem cell lines need to be performed. This implies the successful expansion of genetically modified single cells into an entire population of hPSCs. Therefore, synthetic substrates should support clonal growth of PSCs. To date, the proliferation of undifferentiated single hPSCs has been reported on hit-9 surfaces [19], while the derivation of new hiPSC lines on defined substrates has been achieved on rh vitronectin [15] and in suspension conditions with the addition of fibronectin, laminin, and gelatin in the culture medium [45]. These newly derived populations of hPSCs were shown to be phenotypically similar to cells derived on feeder cells. They expressed the characteristic markers of PSCs, showed comparable cell-doubling rates, and demonstrated the ability to differentiate into derivatives of the three germ layers in vitro and in vivo. However, equally important is the finding that derivation and long-term culture of hPSCs on synthetic substrates and in bioreactors may be accomplished without the introduction of genomic abnormalities that may generate a selective advantage such as a greater propensity for self-renewal [48]. Chromosomal abnormalities have been reported in hPSCs after prolonged culture as well as in early passages. These aberrations commonly involve nonrandom gains of chromosomes 12, 17, 20, and X, or fragments of these chromosomes [49] as detected by standard G-banding metaphase karyotype analysis. High-resolution genome-wide analysis using array-based comparative genomic hybridization techniques have shown recurrent alterations in the same regions as well as others not detected by standard karyotyping methods. One alteration frequently reported is an amplification in the 20q11.21 region that includes genes such as *DNMT3B*, *ID1*, *HM13*, and *BCL2L1* [49, 50], which have been shown to be involved in cell proliferation, inhibition of differentiation and apoptosis [51–53], and may provide a strong selective advantage in culture compared to normal cells. To date, all studies investigating the expansion of hPSCs on synthetic substrates have reported normal karyotypes using the low-resolution G-banding technique. However, for more rigorous interpretation of biological studies and a greater safety profile for eventual cell therapy, high-resolution genome-wide studies should be performed.

The epigenetic stability of hPSCs is also important to consider when using or developing new culture systems. The epigenetic status of genes can change dynamically with culture

time and has been shown to be highly variable among differentiated hPSC lines and between sibling lines [49]. There have been no systematic reports of potential epigenetic changes when hPSC are cultured on synthetic substrates or in suspension. However, this will be an important consideration in light of recent studies that related derivation and culture conditions of female hiPSCs with erosion of DNA methylation and gene expression on the inactive X chromosome. Such changes have the potential to affect disease modeling, differentiation potential and clinical applications (see [54] for review).

FUTURE DIRECTIONS

The current knowledge of synthetic substrates and their characteristics responsible for supporting the proliferation of undifferentiated hPSCs will likely continue to evolve. A new and deeper understanding of how chemical moieties support or direct biologic behavior will lead to improvement in, and development of, new synthetic substrates and will improve our understanding of the biology of pluripotent stem cells. Using the tuning capacity in chemical synthesis of polymer substrates, it will be possible to investigate the response of hPSCs to custom tailored chemical and mechanical signals to maintain self-renewal or to perhaps induce cell-lineage-specific differentiation. Initial examples of this potential have been demonstrated with mesenchymal stem cells, where ma-

trix elasticity of the culture substrate contributed to lineage progression toward neurons, myoblasts, and osteoblasts [55]. One can envision that in combination with high-throughput screenings, the use of small molecules, gene-transfection libraries, and directed chemical manipulations, that synthetic substrates will facilitate the development of defined culture conditions for multiple cell-lineage commitment of hPSCs. In this regard, the impact that synthetic substrates and chemically defined, xenogeneic-free medium for the culture of hPSCs is already high and research in this area will continue to play a prominent role in the development of strategies to use hPSCs to treat debilitating human diseases.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors declare not conflict of interest.

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