Derivation and Long-Term Culture of Transgene-Free Human Induced Pluripotent Stem Cells on Synthetic Substrates

LUIS GERARDO VILLA-DIAZ,a,b JIN KOO KIM,a,b JOERG LAHANN,b,c PAUL H. KREBSBACHa,b

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ABSTRACT

We describe a platform to derive, culture, and differentiate genomically stable, transgene-free human induced pluripotent stem cells (iPSCs) on a fully synthetic polymer substrate made of a grafted zwitterionic hydrogel: poly2-(methacryloyloxy)ethyl dimethyl-(3-sulfopropyl) ammonium hydroxide (PMEDSAH). Three independent transgene-free iPSC lines derived in these conditions demonstrated continuous self-renewal, genomic stability, and pluripotency in vitro and in vivo after up to 9 months of continuous in vitro culture on PMEDSAH-grafted plates. Together, these data demonstrate the strength this alternative platform offers to generate and maintain human iPSCs for regenerative medicine.

INTRODUCTION

The indefinite self-renewal and pluripotency capacity featured by human induced pluripotent stem cells (iPSCs) makes them a potential source of cells for regenerative medicine, drug discovery, disease modeling, and detailed study of self-renewal and differentiation. Since the first report demonstrating the derivation of human iPSCs [1], it was recognized that safety issues would need to be resolved for these cells to reach their full potential. The initial focus toward this goal was to avoid genomic integration of the overexpressed transgenes (OCT4 [also known as POU5F1], SOX2, KLF4, and c-MYC) in the resulting iPSC lines to limit the inherent risk of tumor formation. Since then, several methods [2–7] have been developed to generate transgene-free human iPSCs.

To achieve appropriate safety profiles for human applications, the microenvironment in which human iPSCs are derived and cultured must also be considered. Historically, the culture conditions for human iPSCs have followed methods developed for human embryonic stem cells (ESCs), which in turn were adapted from the culture of mouse ESCs [8]. Consequently, the majority of human iPSC lines have been derived and cultured in the presence of mouse embryonic fibroblasts, human fibroblasts, or autologously derived fibroblasts. However, these commonly used culture conditions encompass biosafety risks such as xenogeneic contamination by nonhuman sialic acids [9], mycoplasma, and viral and nonviral infectious agents and may be immunologically incompatible with human use [10].

Feeder-free systems have been implemented for culturing human PSCs using extracellular matrix proteins derived from either animal sources such as Matrigel [11] or synthetically produced proteins such as human recombinant (hr) laminin [12], E-cadherin [13], and vitronectin [14]. Recently, the derivation of human iPSCs on hr vitronectin [14] and hr laminins 511 fraction E8 [15] and S21 [16] was reported, demonstrating the feasibility of generating xenogeneic-free pluripotent stem cells in feeder-free conditions. Nevertheless, the use of recombinant proteins as a substrate for the derivation and long-term and large-scale culture of human iPSCs is not cost-effective. As an alternative, synthetic coatings have been developed to support growth of human ESCs and human iPSCs while maintaining pluripotency [8]. These synthetic substrates are superior to biological substrates with respect to cost effectiveness, stability, consistency, and ease of sterilization. Thus, the use of fully synthetic substrates for derivation of human iPSCs in xenogeneic-free and feeder-free conditions could represent an ideal culture system.

Here, we describe the derivation and long-term culture of human iPSC lines using a polymer-based substrate consisting of a grafted zwitterionic hydrogel: poly2-(methacryloyloxy)ethyl dimethyl-(3-sulfopropyl) ammonium hydroxide (PMEDSAH), which is extremely effective in sustaining the long-term feeder-free culture of human ESCs [17] and iPSCs [18]. Three independent human iPSC lines were derived and continuously cultured for 9 months on this chemically defined substrate.
Rigorous characterization of these cell lines demonstrated a fully reprogrammed character, in vitro and in vivo differentiation potential, as well as genomic stability.

**MATERIALS AND METHODS**

**Cell Culture**

Human gingival fibroblasts (hGF) were derived with approval of the University of Michigan Institutional Review Board. The derivation procedure followed a protocol detailed previously [19]. The two hGF cell lines labeled as hGF(1) and hGF(2) and human foreskin fibroblasts (hFFs; American Type Culture Collection, Manassas, VA, http://www.atcc.org) were cultured in dedicated incubators in high humidity at 37°C and 5% CO₂, with 10% fetal bovine serum (vol/vol) in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen, Carlsbad, CA, http://www.invitrogen.com) and 1× antibiotic/antimycotic solution (Invitrogen). This culture medium was changed during the reprogramming cycle and expansion of human iPSC colonies to human cell conditioned medium (hCCM; GlobalStem, Gaithersburg, MD, http://www.globalstem.com) supplemented with 4 ng/ml human recombinant basic fibroblast growth factor (GlobalStem), which is herein referred to as hCCM+. PMEDSAH-grafted plates (GPs) were used as a synthetic substrate during the reprogramming cycle and for the long-term culture of the derived human iPSC lines. The preparation of PMEDSAH-GPs has been detailed previously [20]. Briefly, the grafting was performed on tissue culture polystyrene (TCPs) plates (Becton, Dickinson and Company, Franklin Lakes, NJ, http://www.bd.com), and polymers were obtained from Sigma-Aldrich (St. Louis, MO, http://www.sigmaaldrich.com). Graft polymerization was performed using a 0.2 M solution of methacrylate monomers in a 4:1 mixture of water and ethanol. The TCPs plates were activated using a UV-ozone cleaner (Jelight, Irvine, CA, http://www.jelight.com) for 40 minutes. Surface-activated dishes were immersed into monomer solution at 80°C for 2.5 hours. The polymer-grafted dishes were subsequently cooled to 50°C and rinsed in 1% NaCl-water solution at the same temperature overnight. The grafted plates were cleaned by ultrasonication in deionized water and dried under a stream of nitrogen gas. The cell culture on PMEDSAH was performed as previously described [20]. Briefly, PMEDSAH plates were prepared as follows: after overnight UV exposure, plates were washed two times with phosphate-buffered saline followed by addition of hCCM+. Then plates were placed and maintained until and during use in incubators in high humidity at 37°C and 5% CO₂. If prepared PMEDSAH-GPs were not used within a week, hCCM+ was refreshed. Matrigel-coated plates (CPs) were prepared the day of intended use following manufacturer’s instructions (BD Matrigel hESC-qualified Matrix; BD Biosciences, San Diego, CA, http://www.bdbiosciences.com). Colonies of human iPSCs (hiPSCs) were passaged manually by cutting them into small clusters using either a pulled-glass pipette or the StemCell Tool (Invitrogen).

**Reprogramming Cycle**

The reprogramming cycle was performed with minor modifications to the protocol provided by the CytoTune iPS reprogramming kit (Invitrogen) using Sendai virus (SeV) vectors for human (h)OCT3/4, hSOX2, hKLF4, and hc-MYC. A schematic timeline of events is given in supplemental online Figure 1. A cell-doubling time calculation was performed for all three fibroblast cell lines to determine the initial cell plating density and to achieve a density of approximately 3.3 × 10⁵ cells per well of a six-well plate (Becton, Dickinson and Company) at the time of infection, 48 hours postplating. In total, approximately 1 × 10⁶ cells were infected with the SV vectors at a multiplicity of infection of 3. From day 1 to day 8, parental fibroblasts were cultured with fibroblast culture medium on six-well plates; at day 8, they were treated with 0.25% trypsin-EDTA (Gibco, Grand Island, NY, http://www.invitrogen.com), inactivated with fibroblast medium, and resuspended in hCCM+. The cell suspension of each parental fibroblast line was subdivided into six parts of equal volumes and distributed into three PMEDSAH-GPs and three Matrigel-CPs. All plates were 60 mm in diameter and were pre-equilibrated with hCCM+ supplemented with Rock inhibitor (10 μM; Enzo Life Sciences, Inc., Farmingdale, NY, http://www.enzolifesciences.com). This medium was replaced every 48 hours, until day 22 when clonal expansion was performed by selecting one human iPSC colony per cell line and manually passaged into a freshly prepared 35-mm PMEDSAH-GPs or Matrigel-CPs. The resulting subcloned colonies were continuously expanded for 9 months without Rock inhibitor and characterized to assure their full reprogramming into iPSCs. The expansion of colonies on Matrigel-CP was terminated at the time of reprogramming efficiency calculation.

**Reprogramming Efficiency Calculation**

The reprogramming efficiency was calculated for each cell line of human iPSCs derived on PMEDSAH-GPs and Matrigel-CPs as follows: the total number of undifferentiated colonies present in the original PMEDSAH-GPs and Matrigel-CPs plus one at day 30 post-infection was divided by the corresponding number of parental fibroblasts found in each substrate at the day of infection. A colony was considered undifferentiated if it had well defined borders and a high nucleus:cytoplasm ratio and stained positively for alkaline phosphatase.

**Evaluation of Pluripotency**

Pluripotency was tested in vivo by a teratoma formation assay 6 months after derivation and continuous in vitro culture. Briefly, undifferentiated cells (~5 × 10⁶ cells per cell line) from each derived human iPSC line on PMEDSAH-GPs were injected subcutaneously into NOD.CB17-Prkdcscid/NcrCrl mice (Charles River Laboratories, Wilmington, MA, http://www.crv.com) to induce teratomas. When tumors become palpable after 8–12 weeks, mice were euthanized, and tumors were isolated and processed for histologic analysis. A certified pathologist at the Pathology Core for Animal Research at University of Michigan Medical School analyzed and identified tissue representative from the three germ layers in hematoxylin/eosin-stained slides.

For in vitro analysis of pluripotency, embryoid body (EB) formation and directed cell-lineage differentiation from undifferentiated human iPSCs derived and cultured on PMEDSAH-GPs were performed 3, 6, and 9 months after derivation and continuous in vitro culture, using established protocols [21]. Undifferentiated human iPSCs were induced to differentiate in basal medium consisted of DMEM/F12 (Invitrogen) supplemented with 1× N2 (Invitrogen), 1× B27 (Invitrogen), 2 mM L-glutamine, 0.11 mM 2-mercaptoethanol, 1 mM nonessential amino acids, and 0.5 mg/ml bovine serum albumin (fraction V; Sigma-Aldrich). To
induce neuronal differentiation, 100 ng/ml human recombinant Noggin (Stemgent, Cambridge, MA, https://www.stemgent.com) was added to the basal medium, and cells were cultured in this condition for 8 days. For definitive endoderm differentiation, 100 ng/ml human recombinant activin A (Stemgent) was added to basal medium, and cells were cultured for 9 days. The induction to cardiomyocyte differentiation consisted of two sequential steps; first, cells were cultured in basal medium plus 50 ng/ml human recombinant BMP4 (Stemgent) and 50 ng/ml human recombinant activin A for 4 days and then further cultured in basal medium for an additional 10 days.

**Quantitative Real-Time PCR**

Total RNA was extracted using TRIzol (Invitrogen) and purified using RNeasy Mini Kit (Qiagen) and DNase I treatment. One milligram of total RNA was reverse transcribed into cDNA using Superscript III Reverse Transcriptase (Invitrogen). Two microliters of diluted reverse transcribed CDNA (reverse transcription [RT] reaction, 1:5 in RNase-free water) were amplified in a polymerase chain reaction (PCR) assay (30 µl) using TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA, http://www.appliedbiosystems.com), target primers, and probes (unlabeled PCR primers and a carboxyfluorescein dye-labeled TaqMan minor groove binderprobe) (Applied Biosystems). Gene expression was determined by quantitative real-time PCR on an ABI Prism 7700 Sequence Detection System (Applied Biosystems). The relative expression levels of target genes were analyzed by the comparative ΔΔCt method using β-actin as an internal control. Subsequently, expression levels of investigated genes were normalized to expression levels of control samples and reported as fold changes.

**RT-PCR Analysis**

Total RNA was reverse transcribed using SuperScript One-Step RT-PCR with platinum Taq (Invitrogen). In a single reaction (25 µl), 0.5 µg of total RNA, and 20 pmol of forward and reverse primers were used (supplemental online Table 3). The CDNA synthesis and predenaturation were carried out at 95°C for 2 minutes. The PCR amplification was performed for 35 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. The final extension cycle was run at 72°C for 10 minutes. Finally, 10 µl of PCR product was loaded onto a 1.0% agarose gel and size-fractionated.

**Chromosomal Stability Studies Using High-Resolution aCGH and G-Banding Karyotype**

The array-based comparative genomic hybridization (aCGH) 135K Stem Array (Ambry Genetics, Aliso Viejo, CA, http://www.ambrygen.com) was performed by Cell Line Genetics (Madison, WI, http://www.clgenetics.com) on cells continuously passaged for 9 months as follows: genomic DNA quality and concentration were determined with a NanoView spectrophotometer. The aberration detection settings were: minimum probe, 5; significance threshold, 1.0×10E-7; high gain, 1.0; gain, 0.3; loss, −0.3; big loss, −1.1. For standard G-banding karyotyping, chromosomes from at least 20 cells at the metaphase stage were prepared using standard protocols and analyzed by Cell Line Genetics.

**Western Blot Analysis**

The following antibodies were used: antibody to Sendai virus protein (1:1,000; MBL International Corp., Woburn, MA, http://www.mblintl.com), Oct4-specific antibody (1:2,000; Santa Cruz Biotechnology Inc., Santa Cruz, CA, http://www.scbt.com), Nanog-specific antibody (1:1,000; Cell Signaling Technology, Beverly, MA, http://www.cellsignal.com), α-tubulin-specific antibody (1:2,000; Santa Cruz Biotechnology Inc.), and β-actin-specific antibody (1:1,000; Cell Signaling Technology). Whole cell lysates were prepared from cells, separated on 7.5% SDS-polyacrylamide gel, and transferred to polyvinylidene difluoride membranes. The membranes were incubated with 5% milk in TBST (wt/vol) for 1 hour and then incubated with primary antibodies overnight at 4°C. Blots were incubated with peroxidase-coupled secondary antibodies (Promega, Madison, WI, http://www.promega.com) for 1 hour, and protein expression was detected with SuperSignal West Pico Chemiluminescent Substrate (ThermoScientific, Waltham, MA, http://www.thermoscientific.com).

**Immunocytochemistry**

Cells were fixed in 2% paraformaldehyde (wt/vol) for 30 minutes at room temperature and, if required, permeabilized with 0.1% Triton X-100 (vol/vol) for 10 minutes. Primary antibodies were diluted in 1% normal serum (vol/vol) and incubated overnight at 4°C and detected by respective secondary antibodies. Samples were imaged and captured using a Leica DM IRB inverted microscope (Leica, Heerbrugg, Switzerland, http://www.leica.com) with an Olympus (Center Valley, PA, http://www.olympusamerica.com) DP-30 CCD camera (Microscopy and Image Analysis Laboratory, University of Michigan, Ann Arbor, MI). The following antibodies were used: GATA4-specific antibody (R&D Systems Inc., Minneapolis, MN, http://www.rndsystems.com), HNF-3b/FoxA2-specific antibody (R&D Systems, Inc.), SOX1-specific antibody (R&D Systems, Inc.), PAX6-specific antibody (Abcam, Cambridge, U.K., http://www.abcam.com), SSEA-3-specific antibody (Millipore, Billerica, MA, http://www.millipore.com), TRA-1-81-specific antibody (Santa Cruz Biotechnology Inc.), TRA-1-81-specific antibody (Santa Cruz Biotechnology Inc.), SSEA4-specific antibody (Santa Cruz Biotechnology Inc.), Oct3/4-specific antibody (Santa Cruz Biotechnology Inc.), Nanog-specific antibody (Cell Signaling Technology), and Sox2-specific antibody (Millipore).
**Bisulfite Sequencing**

Genomic DNA (1 mg) was treated with components of the CpGene DNA modification kit (Chemicon, Temecula, CA, http://www.chemicon.com), according to the manufacturer’s recommendations. Treated DNA was purified with QIAquick column (Qiagen, Hilden, Germany, http://www.qiagen.com). The promoter regions of human OCT4 and NANOG genes were amplified by PCR. The PCR products were subcloned into pCR2.1-TOPO. Ten clones of each sample were verified by sequencing with the M13 universal primer. Primer sequences [22, 23] used for PCR amplification are shown in supplemental online Table 3.

**Statistical Analysis**

Experiments were performed in triplicate, and the data are expressed as mean values ± SD and analyzed by an unpaired t test. Levels of statistical significance were set at \( p < .05 \).

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**RESULTS**

**Derivation of Human Induced Pluripotent Stem Cells on PMEDSAH-GP**

The parental cell lines, two derived from adult gingival tissue (hGF) and a commercial cell line from newborn foreskin (hFF), were initially cultured and infected with the reprogramming factors introduced by SeV on tissue culture plastic plates, and 5 days postinfection were subcultured, equally divided, and reseeded on PMEDSAH-GPs and Matrigel-CPs (used as control) in hCCM+ (supplemental online Fig. 1). During this period and until the first passage of colonies, the medium was supplemented with Rock inhibitor. Nine days postinfection, colonies were observed on both PMEDSAH-GPs and Matrigel-CPs. At day 16 postinfection, two colonies growing on PMEDSAH-GPs were subcloned to evaluate the expression of Nanog and alkaline phosphatase activity (supplemental online Fig. 1). At day 22 postinfection, one colony from each of the three parental lines growing on both PMEDSAH-GPs and Matrigel-CPs was selected, manually dissected, and reseeded on new substrates. Subsequently, six human iPSC lines were established, two from each parental cell line, from which one was derived and propagated on PMEDSAH-GPs and the other on Matrigel-CPs. At day 30 postinfection, the reprogramming efficiency was calculated and ranged from 0.0004% to 0.0006%. There was no statistical difference \( (p > .05) \) between parental fibroblasts and substrates. The number of colonies generated in each condition \( (178 ± 75 \text{ [average ± SD]} \) exceeded the required number to establish and characterize independent human iPSC lines. This suggests that PMEDSAH-GP is an effective platform to generate human iPSCs.

**Characterization of Human iPSCs Derived on PMEDSAH-GPs**

The three human iPSC lines derived on PMEDSAH-GPs were subcultured on grafted plates of the same synthetic substrate for approximately 170 cell doublings over a period of 9 months and continuously characterized to demonstrate their fully reprogrammed character. Initially, Western blot analysis demonstrated that parental fibroblasts did not express Oct4. In contrast, Oct4 was highly expressed in cells undergoing reprogramming and in fully established human iPSCs, 3 months after derivation and culture (Fig. 1A). SeV protein levels were not detected in parental fibroblasts but were high 3 days postinfection and subsequently decreased to undetectable levels in human iPSCs (Fig. 1A). RNA expression of SeV was also tested by RT-PCR, showing no detectable levels in established human iPSC lines (supplemental online Fig. 2). The expression of Nanog in all iPSC lines was detected by Western blot analysis (Fig. 1B), and immunocytochemistry revealed the continuous expression of pluripotent markers: Oct4, SSEA3, SSEA4, TRA-1-60, TRA-1-81, and Sox2 (Fig. 1C). Bisulfite sequencing analysis demonstrated that promoters of OCT4 and NANOG were highly methylated in parental cell lines and converted to a largely unmethylated state in established human iPSCs, 3 months after derivation and continuous culture (Fig. 1D).

The pluripotency of the human iPSC lines derived and cultured on PMEDSAH-GPs was tested up to 9 months after derivation and continuous in vitro culture, by EB formation, in vitro cell lineage differentiation, and teratoma formation. In vitro, clusters of iPSC lines formed EBs when cultured in suspension (Fig. 2A) and expressed genes representative of the three germ layers: ectoderm, endoderm, and mesoderm (data not shown). Furthermore, specific cell lineage differentiation on PMEDSAH-GPs was directed via chemically defined conditions using well-established protocols [21]. For neural differentiation, treatment with Noggin resulted in upregulation of Pax6 RNA levels (Fig. 2B) and dual expression of Pax6 and Sox1 in treated cells (Fig. 2C). Activin A treatment induced the differentiation of human iPSCs into definitive endoderm/pancreatic cells, as indicated by high RNA levels of SOX17 and FOXA2 (Fig. 2B), and detection of FoxA2+ cells (Fig. 2D). Cardiac muscle lineage differentiation in response to Activin A and BMP4 treatment represented in vitro mesodermal lineage development, which was confirmed by the upregulation of T, TNNI3, NXX2.5, and HESX1 (Fig. 2B) and by expression of Gata4 (Fig. 2E). Teratoma analysis confirmed the pluripotency of these cell lines in vivo, as observed by identification of mature cell such as neurons, cartilage, gut glandular epithelium, and other differentiated tissues (Fig. 2F–2H).

**Genomic Stability of Human iPSCs Derived and Cultured on PMEDSAH-GPs**

The genetic stability of human iPSCs derived on PMEDSAH-GPs was analyzed 9 months after derivation and continuous in vitro culture. Standard G-band analysis demonstrated normal male chromosomal karyotypes for all three human iPSC lines (Fig. 3A), whereas aCGH analysis detected minimal gains and losses in specific chromosomal regions in all cell lines (Fig. 3B; supplemental online Table 1). It was observed that some of the aberrations were recurrent in two of the three human iPSC lines and only in one occasion (chromosome 9q34.3) in all cell lines (supplemental online Table 2).

**DISCUSSION**

Three independent human iPSC lines derived in xenogeneic and integration-free conditions demonstrated continuous self-renewal, genomic stability, and pluripotency in vitro and in vivo after up to 9 months of continuous in vitro culture on PMEDSAH-GPs. These results demonstrate that PMEDSAH-GPs support the derivation and long-term self-renewal of genomically stable human iPSGs, as well as differentiation of into derivatives of the three germ lineages in chemically defined conditions. The fully reprogrammed character of the cell lines was demonstrated by continuous expression of
protein markers related to human pluripotent stem cells, the demethylated status of both Nanog and Oct4 promoters in these cells, and the capability to differentiate both in vivo and in vitro into representative cells and tissues of all three germ layers.

The human iPSC lines derived in these conditions were transgene-free because nonintegrating Sendai virus constructs encoding OCT4, SOX2, KLF4, and c-MYC were used to induce the reprogramming. The Sendai viruses are nonsegmented...
negative-strand RNA viruses that do not have a DNA phase and thus do not integrate into the genome of infected cells [24]. In addition, Sendai virus constructs were also selected to induce reprogramming because of their highly effective infection rates and simplicity of use compared with other methods that require multiple transfections to achieve strong gene expression, continuous protein translation, or further manipulations to excise integrated constructs. Indeed, our data demonstrated the effectiveness of these vectors to induce reprogramming on PMEDSAH-GPs. Our results demonstrate that in fully reprogrammed cells, RNA expression and protein levels of Sendai virus become undetectable after the establishment of fully reprogrammed cells (Fig. 1; supplemental online Fig. 2). We have also derived human iPSCs using Cre/LoxP constructs on PMEDSAH-GPs (data not shown), which suggests that other nonintegrating methods could be used in combination with this synthetic substrate to derive transgene-free human iPSC lines.

It has been established that during long-term culture of pluripotent stem cells, chromosomal changes may occur, and these aberrations may confer growth advantages and disrupt differentiation capacity [25]. In addition, it has been reported that SOX2, c-MYC, and KLF4 may be duplicated in retrovirus-generated iPSCs because of genomic integration [26]. Therefore, we evaluated the genomic stability of the three human iPSC lines derived and propagated continuously on PMEDSAH-GPs after 9 months (Fig. 3). The standard G-banding assay demonstrated a normal karyotype.
for all three cell lines, confirming previous results with hESC culture on PMEDSAH-GPs [17]. The aCGH analysis demonstrated minimum modifications in the three cell lines. However, these mutations do not match chromosomal loci where key stem cell and cancer-related genes are contained [26] or where genes related to culture adaption, such as BCL2L1, are localized [27]. Because the aCGH analysis was performed only in parental cells and after 9 months of continuous culture of the derived human iPSC lines, we could not determine the time at which these mutations occurred, or whether other mutations were corrected [27]. Thus, these results indicate the genomic stability of human iPSCs derived and cultured long term on PMEDSAH-GPs.

During the preparation of this work, it was reported that a new chemically defined and xeno-free medium supports the derivation of human iPSCs on vitronectin [14]. When this medium was used in combination with PMEDSAH-GPs, only pre-iPSC colonies were formed, and cells were not induced to be fully reprogrammed (data not shown). This observation indicates that further optimization of chemically defined media is required to achieve a complete chemically defined and xeno-free platform for the derivation and culture of human iPSCs on PMEDSAH-GPs, and work is in progress to achieve this goal.

CONCLUSION

This study demonstrated a feeder-free platform to derive, expand, and differentiate genomically stable and transgene-free human iPSCs. This platform has the potential to be in compliance with good tissue practice and good manufacturing practice. Compared with currently used methods, the conditions presented here represent an alternative to the goal of generation and long-term propagation of human iPSCs with potential for clinical application.

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AUTHOR CONTRIBUTIONS

L.G.V.-D.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; J.K.K.: collection and/or assembly of data, data analysis and interpretation; J.L.: provision of study material, data analysis and interpretation, final approval of manuscript; P.H.K.: conception and design, financial support, data analysis and interpretation, manuscript writing, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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