Cell Stem Cell
Forum

Generating iPSCs: Translating Cell Reprogramming Science into Scalable and Robust Biomanufacturing Strategies

Marli Silva,[1] Laurence Daheron,[2] Hannah Hurley,[3] Kim Bure,[4] Richard Barker,[3] Andrew J. Carr,[3,5] David Williams,[6] Hae-Won Kim,[7,8,9] Anna French,[1] Pete J. Coffey,[10,11] Justin J. Cooper-White,[12,13,14] Brock Reeve,[2] Mahendra Rao,[15] Evan Y. Snyder,[16,17,18] Kelvin S. Ng,[2,19,20] Benjamin E. Mead,[2,19,20] James A. Smith,[2,3] Jeffrey M. Karp,[2,19,20] David A. Brindley,[2,3,5,21] and Ivan Wall[1,7,8,*]
[1]Department of Biochemical Engineering, University College London, London, WC1H 0AH, UK
[2]Harvard Stem Cell Institute, Cambridge, MA 02138, USA
[3]The Oxford–UCL Centre for the Advancement of Sustainable Medical Innovation, University of Oxford, Oxford, OX3 9DU, UK
[4]TAP Biosystems, Royston, Hertfordshire, SG8 5WY, UK
[5]Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Nuffield Orthopaedic Centre, University of Oxford, Oxford, OX3 7LD, UK
[6]Centre for Biological Engineering, Wolfson School of Mechanical and Manufacturing Engineering, Loughborough University, Loughborough, LE11 3TU, UK
[7]Department of Nanobiomedical Science and BK21 Plus NBM Global Research Center of Regenerative Medicine, Dankook University, Cheonan 330-714, Republic of Korea
[8]Institute of Tissue Regeneration Engineering, Dankook University Graduate School, Cheonan 330-714, Republic of Korea
[9]Department of Dental Biomaterials, School of Dentistry, Dankook University, Shinbu-dong, Cheonan 330-714, Republic of Korea
[10]Ocular Biology and Therapeutics, Institute of Ophthalmology, University College London, London, EC1V 9EL, UK
[11]Neuroscience Research Institute, University of California, Santa Barbara, CA 93106-5060, USA
[12]Australian Institute for Bioengineering & Nanotechnology, The University of Queensland, St. Lucia, QLD 4072, Australia
[13]School of Chemical Engineering, The University of Queensland, St. Lucia, QLD 4072, Australia
[14]Materials Science and Engineering Division, CSIRO, Clayton, VIC 3169, Australia
[15]New York Stem Cell Foundation, New York, NY 10023, USA
[16]Burnham Medical Research Institute, La Jolla, CA 92037, USA
[17]Department of Pediatrics, University of California, San Diego, La Jolla, CA 92161, USA
[18]Sanford Consortium for Regenerative Medicine, La Jolla, CA 92037, USA
[19]Division of Biomedical Engineering, Department of Medicine, Center for Regenerative Therapeutics, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA
[20]Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA 02139, USA
[21]Centre for Behavioural Medicine, UCL School of Pharmacy, University College London, London WC1H 9JP, UK
*Correspondence: i.wall@ucl.ac.uk
http://dx.doi.org/10.1016/j.stem.2014.12.013

Induced pluripotent stem cells (iPSCs) have the potential to transform drug discovery and healthcare in the 21st century. However, successful commercialization will require standardized manufacturing platforms. Here we highlight the need to define standardized practices for iPSC generation and processing and discuss current challenges to the robust manufacture of iPSC products.

Introduction

Induced pluripotent stem cells (iPSCs) are powerful tools for research and drug discovery and may provide regenerative therapies for diseases that conventional medicine cannot cure presently. While methods for generating iPSCs are continually evolving in the laboratory, and there is substantial growth in patent activity in the global iPSC landscape (Roberts et al., 2014), significant hurdles remain for successful commercial translation, such as global harmonization of the regulatory landscape and attitudes to clinical adoption. A fundamental requirement for successful commercialization is the ability to translate iPSC science to the biomanufacturing community to create robust and consistent high-quality iPSC products at desired quantities that display acceptable levels of comparability across multiple lines.

As "living" products, cells pose a range of biomanufacturing challenges. Variability in starting materials, process reagents, microenvironmental fluctuations, and stochastic events within a cell population, such as spontaneous cell cycle arrest, create inconsistencies that are challenging to control in manufacturing processes. The challenges for creating iPSC-based drug testing platforms or therapeutics are particularly significant because there are multiple methodologies for reprogramming cells to create iPSCs. From a biomanufacturing perspective this creates significant variability in input materials along with process variability that could translate to the iPSC product that is generated. Subsequent processing to derive differentiated target cell types that are comparable would consequently be affected.

The need for stratified therapies is driven by the heterogeneous nature of patients, not just in terms of disease status but also genetic background. With global efforts to generate large repositories of iPSCs, effort is required to ensure that the critical quality attributes of the resulting iPSCs meet consistent high quality. If different reprograming methodologies are used for producing iPSCs across different repositories (and within a single

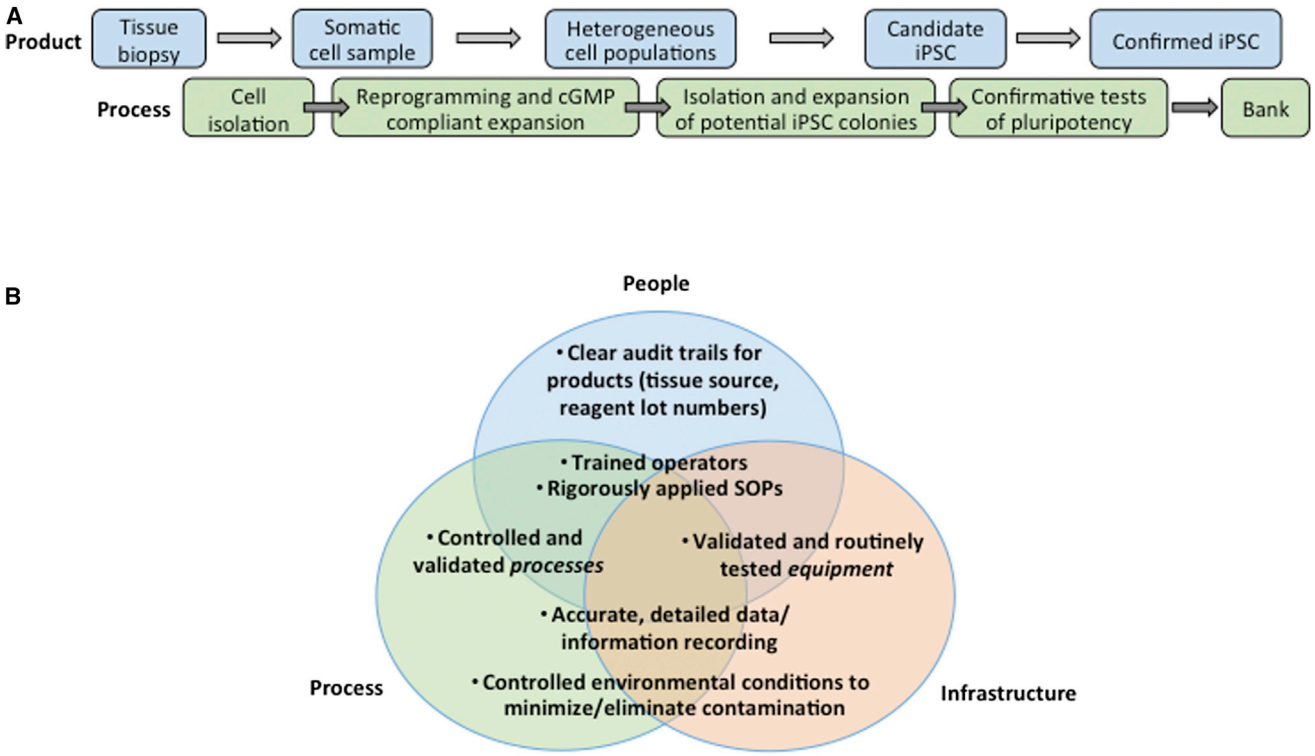

Figure 1. High-Level Process Map for iPSC Generation and Relationships between Operators and Processes
High level process map (A) outlining the transition of cell samples from raw input material to final product. In the case of the iPSC product, it is then itself an input material for differentiation processes. The process is then presented below to highlight the unit operations required to deliver the downstream product. The relationship between operators, process, and infrastructure (B) is critical in the development of robust, standardized manufacturing strategies that reproducibility yield material of consistent quality.

repository), then comparing cell products from different sources may be challenging. Such discrepancies are problematic because different cell product characteristics arising from multiple genetic backgrounds need to be understood from a stratified medicine perspective independent of any product variation arising from technical input variability. Therefore, a robust and standardized methodology will reduce the impact of technical variability on the iPSC product. A solid understanding of the manufacturing processes and the relationship between key factors and their impact on the cellular product is thus needed (Figure 1).

Compared to protocols established in individual research groups, stricter standards are needed to develop and validate manufacturing processes and the associated iPSC banks (Turner et al., 2013). In particular, there remains uncertainty around the manufacturing and regulatory challenges concerning human leukocyte antigen-matched (HLA) master cell banks, in addition to regulatory and social uncertainty around this new class of drug discovery platform and therapeutic. With many different methods available to generate iPSCs, it is increasingly important to define optimal manufacturing platforms to meet the regulatory and quality assurance demands of the biomanufacturing industry while also minimizing cost of goods. To this end, we highlight here various aspects of iPSC generation and processing that must be considered for developing standardized iPSC products.

Current Technologies for iPSC Generation

Significant global effort has been made to develop methods to generate iPSCs more rapidly, safely, and efficiently. Increasingly, such scientific advances are yielding commercial products and services; notably, Life Technologies (now acquired by Thermo Fisher) has licensed assay technologies for efficient generation (CytoTune-iPS 2.0) and characterization/validation (TaqMan hPSC Scorecard) of iPSCs.

Today, multiple methods can be used to generate iPSCs (Figures 2 and S1) and key publications of factors delivered are reviewed elsewhere (Theunissen and Jaenisch, 2014). Creating iPSCs that are fit for purpose, either drug discovery or, more challengingly, therapeutics, will require a wide understanding of the biological characteristics and process conditions. Critical considerations to meet cGMP guidelines include cell line derivation, potential contaminants and adventitious agents, desired features and how these features can be assayed, and reliability/reproducibility in the generation of a safe and efficacious end-product. The first major consideration for generating iPSCs is, however, the choice of primary cell source for reprogramming.

Cell Material

Because primary cell "input" material is typically heterogeneous, each single reprogrammed "output" cell might differ.

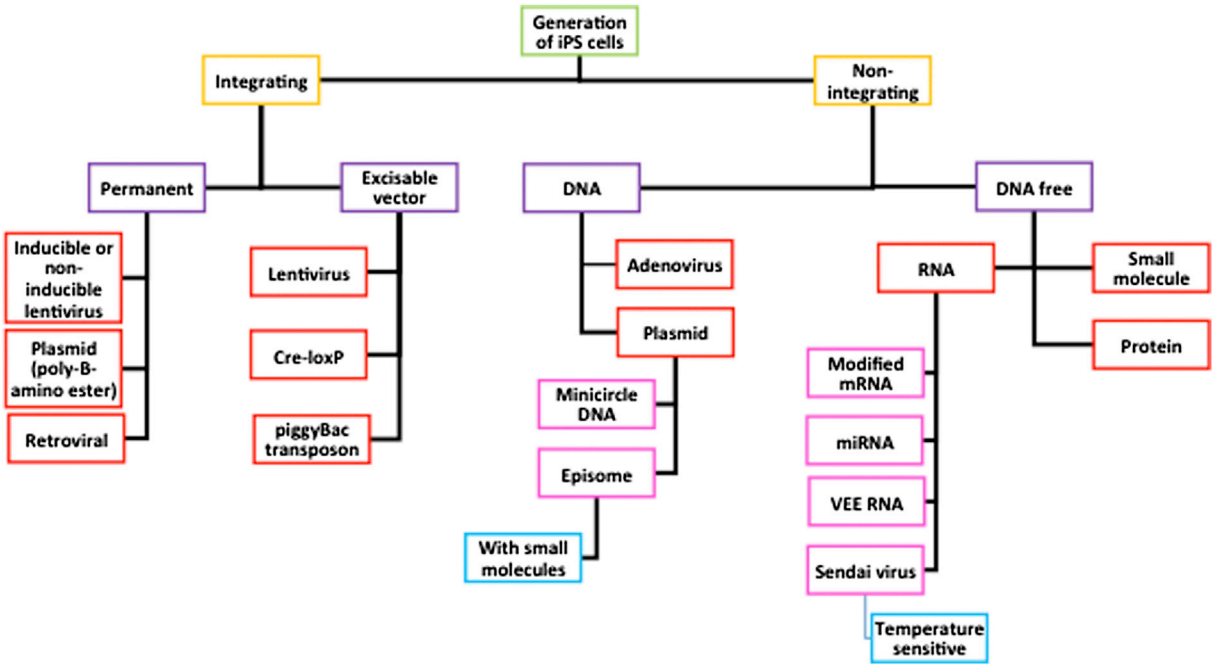

Figure 2. Delivery Methods for Molecular Reprogramming of Cells in the Generation of iPSCs
Numerous candidate technologies have potential applications in the manufacturing pathway to create iPSCs.

Reducing or eliminating heterogeneity in the input material by pre-selecting a particular cell subset (e.g., selecting mesenchymal cells on the basis of a panel of surface epitopes) could create a more robust product. Although the field has not yet settled on the optimum starting cell for iPSC products, convincing arguments have been made for using multipotent stem cells, particularly those from cord blood, which are highly unlikely to have accumulated epigenetic changes, point mutations, or chromosomal damage typical of older cells (Jacoby et al., 2012). For the generation of disease-specific cell lines for research purposes, however, blood may be a better candidate due to broader patient accessibility.

Aside from biological considerations, the choice of source material will be influenced to a large degree by the ease with which it can be obtained from the donor with informed consent for tissue/cell removal, reprogramming, expansion, and storage. The legal requirements to cover ethical and regulatory standards must be met before source tissue/cells can be obtained—including informed consent based on the understanding of the full scope of iPSC generation including commercial opportunities.

Cell Reprograming

Advances in reprograming methods now provide multiple vectors for gene delivery, techniques that avoid the use of oncogenic transgenes, and even DNA-free methods (Figures 2 and S1).

Fundamental for manufacturing cell therapies is product stability, which in part underpins clinical safety. While phenotypic stability will be challenging to gauge when initially generating iPSC banks, any measurable characteristics of iPSCs that indicate genomic stability and phenotypic fluctuations would greatly facilitate future development of iPSC-derived products. This is where a clear distinction is drawn between integrating vectors and non-integrating vectors and DNA-free methods. Unlike integrating vectors, non-integrating vectors achieve reprogramming without inserting material into the host genome and thus avoid the risk of insertional mutagenesis.

Non-integrating episomes can achieve reprogramming within 30 days, and methodologic improvements that bypass the need for serial transfection (Yu et al., 2011) have created one of the simplest processes with minimal manual processing that could be translated to automated platforms with ease. Minicircle vectors are episomes that lack plasmid backbones, making them potentially advantageous in terms of safety. However, overall reprogramming efficiency of episomal methods is low compared with other non-integrating methods such as Sendai virus or mRNA transfection.

The Sendai virus is also popular as a non-integrating reprograming vector because the method is simple and, when temperature-sensitive Sendai viruses such as that already used for c-*myc* (Ban et al., 2011) are adopted, removal of Sendai RNA from iPSCs by a temperature shift will be possible. Also based on the Sendai virus, the CytoTune 2.0 kit (Life Technologies) is available off-the-shelf with validated protocols for generating stable transgene-free iPSCs with little cytotoxicity and much more rapid virus elimination than that enabled by previous kits. Such methods are not only very amenable to process development but would, if adopted as part of cGMP protocols, also satisfy regulatory requirements for therapeutics, where full removal of viral material will be desirable or necessary. The cost, however, remains high.

Direct delivery of mRNA or microRNA (miRNA), which eliminates the use of DNA and viruses, has been developed

as a safer reprogramming strategy. Exogenous mRNA can be modified to dampen cytotoxicity and antiviral defense mechanisms in the cell (Warren et al., 2010). This method can achieve one of the highest reprogramming efficiencies among non-integrative techniques. Current methodologies require daily transfection but may be easily automated, making it an exciting candidate for routine biomanufacture. Yet, work remains in confirming the reproducibility and mitigating commercial risks before this methodology can serve as a viable tool for manufacturing iPSCs.

Total removal of nucleic acids for reprograming by using peptide-based delivery of transcription factors or small molecules instead could utilize established biomanufacturing pathways to generate the reprograming material. From a bioprocessing perspective, small molecules are easier to define and control for platform standardization and regulatory approval.

Process Optimization for Reprograming iPSCs

In any stem cell manufacturing endeavor, cost of reagents and media typically represents the most substantial fraction of total production costs. Furthermore, bioprocessing operations traditionally rely on iteratively optimizing each stage, which adds significant cost and off-process analysis requirements. Process optimization using small-scale, high-throughput testing devices that require microliter quantities of culture media yet allow standardized comparative assessment of cell product quality would greatly contribute toward a commercially viable process. Although still in the early stages of development, such devices can provide real-time or rapid readouts of cell product attributes, using minimal cell numbers and media in microbioreactor arrays. These microdevices can be multiplexed, permitting multiple bioprocessing parameters to be assessed in factorial or combinatorial manners, and are being used to address gaps in our knowledge of each process step, such as the impact of media exchange and shear stress, media composition and timing of factor provision, and paracrine/autocrine signaling on cellular behaviors and quality (Titmarsh et al., 2013). Due to their flexibility and portability, such microdevices have utility for pre-screening multiple cell inputs and reprogramming reagents for the optimal reprogramming regime that delivers the most consistent quality product in terms of transfection efficiency and yield, which can subsequently be mathematically modeled as part of Quality by Design engineering approaches to optimize larger scale operations for subsequent clinical-scale iPSC expansion. Finally, they would facilitate standardized product testing of iPSCs from diverse genetic backgrounds and could provide a platform to perform comparability studies with genetically diverse cells versus gene-edited single pluripotent cell lines that are not confounded by diverse genetic backgrounds.

iPSC Selection and Validation

The cell reprogramming process is not 100% efficient and consequently a heterogeneous mixture of iPSCs, partially reprogrammed cells, and partially differentiated cells is produced. Therefore, iPSCs must be isolated from the mixed population for further use. Many cell-sorting methods, particularly those depending on a single parameter, such as morphology-based colony picking, may not discern between partially reprogrammed cells and iPSCs. Ideally, an industrial-scale selection process would have the capability to be replicated robustly and the capacity to screen thousands of cell lines with very high accuracy.

Selecting colonies based on morphology is an option favored at lab-scale due to the ability of well-trained operators to identify appropriate colonies. It may even be the best method for generating different iPSC lines in parallel, given that large numbers of cell lines need to be generated from multiple primary sources to cover the immunological variation among patients. However, operator bias is difficult to control and manual picking is labor-intensive and, thus, prohibitive at large scales (Meissner et al., 2007). Algorithms for automated colony selection based on morphology could remove operator bias but would require significant validation against the gold standard: the eyes and expertise of a skilled operator.

Incorporation of a reporter or selection tool (e.g., antibiotic selection) to positively select iPSCs is typically considered a more accurate alternative to colony picking. However, adding a reporter/selection tool may introduce additional risk, particularly in cells intended for therapeutic use. Downstream elimination of reporters/selectors, using principles similar to the temperature-sensitive Sendai virus, may lower such risks, albeit with higher process complexity.

The most accurate method for iPSC identification is immunoselection based on cell surface antigens using fluorescence- or magnetic-activated cell sorting. These methods enable multiparametric selection to a very high purity. Removal of the selection antibodies will be important for ensuring that iPSCs are biologically functional for subsequent processing and directed differentiation. Even if clinical-grade antibodies are used, removal of sorting antibodies from the final product may be required, especially in clinical applications, and the additional processing will almost certainly impact cell quality. The application of existing clinical-grade cell sorting (e.g., Miltenyi CliniMACs) offers a very sound platform for selection, purification, and validation of iPSCs and provides automated, closed-system, minimal handling capacity.

True confirmation of pluripotency typically uses an in vivo teratoma assay, regarded as the best validation method for human iPSCs at present. However, this gold standard is an expensive and laborious assay, taking 2–3 months to perform, and it's hardly practical for scale-up or high-throughput adoption. In vitro assays that can conclusively confirm pluripotency would substantially streamline manufacturing of iPSCs. Recent Q-PCR-based methods offer rapid throughput and further analysis of differentiation and are now possible using the standardized *TaqMan* hPSC Scorecard (Life Technologies) by comparing transcripts to a reference standard. In vitro embryoid-body-based assays that assess differentiation toward specific lineages under optimized conditions are also better suited to high-throughput, with measurement of protein and tissue induction in addition to that of gene transcripts. However, in vitro gene transcription and directed differentiation assays may not be sufficient in the present regulatory environment. Recent developments such as a novel in vitro teratoma assay may offer unique solutions (Whitworth et al., 2014).

Establishing robust standardized assays to adequately characterize iPSCs currently presents one of the biggest obstacles to commercialization due to the quantity and complexity of tests required. The overarching goal of biomanufacturing is to streamline validation processes by reducing the number of assays, improving assay efficiency without compromising safety, or developing standardized assays to improve reproducibility between different manufacturers. As our understanding of iPSCs increases, the possibility to rethink validation procedures will advance, making the creation of production platforms for cell banking more feasible. Critically, we need to define a robust set of criteria to ensure that a consensus standard of quality is achieved for cell therapy and drug discovery applications.

Bottlenecks and Next Steps

Ultimately, the goal of generating iPSCs is the identification and development of novel therapies. Economically sound platforms are necessary to give a high degree of reproducibility for the production of multiple iPSC lines. This would enable the establishment of cell banks with quality-assured, off-the-shelf iPSC products for multiple applications, while covering the immunological diversity of the patient pool. Current progress in this direction is notable, though a substantial change is still required in standards, cell line manufacturing, and validation to support an effective transition from research to generation of clinical-grade iPSCs. For production of universal iPSC banks, process maps and product critical quality attributes must be clearly defined. This remains the main bottleneck in advancing iPSC banking because reprogramming and validation methods are currently varied and consensus is lacking. Outlining appropriate regulator-informed process maps that incorporate defined and controllable process parameters at every possible step will help to shape a biomanufacturing platform that delivers a robust iPSC product of consistent quality. In creating large iPSC banks, fundamental science would benefit substantially from the standardization of reprogramming methods early on so that consistency and reproducibility in the generation of iPSC products can be achieved, both of which are critical for successful commercial translation.

SUPPLEMENTAL INFORMATION

Supplemental Information for this article includes one figure and can be found with this article online at http://dx.doi.org/10.1016/j.stem.2014.12.013.

ACKNOWLEDGMENTS

We wish to express our sincere thanks to the following organizations that have contributed to the CASMI Translational Stem Cell Consortium: GE Healthcare, CCRM, TAP Biosystems (now Sartorius Stedim), Lonza, CIRM, SENS Research Foundation, UK Cell Therapy Catapult, NIH Centre for Regenerative Medicine, NYSCF, Thermo Fisher Scientific, Eisai, Medipost (US), Medipost (Korea), Celgene, Oxford Biomedica, and Roche. H.W.K. and I.W. are supported by the Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (grant No.2009-0093829). J.M.K. was supported by National Institutes of Health grant HL095722. D.A.B. gratefully acknowledges support from the SENS Research Foundation. R.B. is a non-executive director of Celgene Corp. B.R. is on the board of directors of Pathfinder Cell Therapy. K.E.B. is an employee of Sartorius-Stedim Biotech. D.A.B. is a stockholder in Translation Ventures Ltd.

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