Human Genetics & Embryology

Requena et al., Human Genet Embryol 2014, 4:1
DOI: 10.4172/2161-0436.1000120

Review Open Access

# The Future Challenges for the Clinical Application of Reprogrammed Cells

**Jordi Requena**[1], **Ana Belen Alvarez Palomo**[1], **Marti Farrera Sal**[1], **John Christodoulou**[2], **Josep M Canals**[3] **and Michael J Edel**[1,4*]

[1]*Control of Pluripotency Laboratory, Department of Physiological Sciences I, Faculty of Medicine, University of Barcelona, Hospital Clinic, Casanova 143, 08036, Barcelona, Spain*

[2]*Western Sydney Genetics Program, The Children's Hospital at Westmead, Disciplines of Paediatrics & Child Health and Genetic Medicine, Sydney Medical School, University of Sydney, Australia*

[3]*Laboratori de Cellules Mare i Medicina Regenerativa, Departament Biologia Cellular, Immunologia i Neurociències, Programa de Terapia Cellular, Facultat de Medicina, Universitat de Barcelona-IDIBAPS, Spain*

[4]*Senior Research Fellow at the University of Sydney Medical School, Faculty of Medicine, Westmead Children's Hospital, Division of Pediatrics and Child Health, Sydney, Australia*

***Corresponding author**: Michael J Edel, Senior Research Fellow at the University of Sydney Medical School, Faculty of Medicine, Westmead Children's Hospital, Division of Pediatrics and Child Health, Sydney, Australia, Tel: 0034 93 402 4518; E-mail: michaeledel@ub.edu

**Received date:** Jan 15, 2014, **Accepted date:** Feb 19, 2014, **Published date:** Mar 10, 2014



**Abstract**

Recent developments in the field of reprogramming somatic cells to induced pluripotent stem cells (iPSC) has brought the field closer to the exciting possibility of their future clinical application. A number of challenges remain to be addressed before clinical application of reprogrammed stem cells becomes a reality. The main issues are the threat of cancer, control of differentiation to tissue specific stem cells or body organs and the immune response of iPSC-derived cells. The future use of iPSC will require that they are cancer free, do not proliferate and can be contained to the specific injection site for tissue regeneration. This review discusses the recent advances that address these main challenges in the field of cellular reprogramming

**Keywords:** Cell reprogramming; Clinical grade iPSC; Immune response; Organ bioengineering

## Development of High Quality Clinical Grade Safe iPSC

The first method to make human iPSC used a retroviral vector delivery system, carrying the risk of transgene reactivation and insertional mutagenesis [1]. Since then many other groups have used the same methods to reprogram cells to pluripotency [2-4] among others. After that research efforts focused on searching different ways to induce pluripotency without suffering genetic changes in order to prevent transgenes from reactivating and avoiding the risk of genomic recombination or insertional mutagenesis. Some of these methods are; non-integration adenoviruses [5], expression plasmids [6], episomal vectors [7], piggy Bac transposition [8], Sendai virus [9], direct delivery of reprogramming proteins [10], synthetic modified mRNAs [11], chemical compounds [12] and synthetic self-replicative RNA replicons [13].

All though a wide range of methods have successfully reprogrammed somatic cells to a pluripotent state only three methods appear to be appropriate for reprogramming patient cells for cellular therapy: Sendai virus, mRNA and episomal vectors.

(1) Sendai virus is a powerful and transient gene expression vector, with the advantages of wide host specificity and low pathogenicity, but with the worrying disadvantage of strong immunogenicity response [9].

(2) Direct transfection of synthetic modified mRNA is a strategy that administrates mRNA modified to overcome innate antiviral immune responses. It presents the best option for future clinical applications because you can control the dose and has transient expression over 48 hours [11].

(3) Episomal vector reprogramming consists on introducing episomal genes that are expressed and replicate when the host cell divides and the episome is naturally lost when the iPSCs multiply [7].

Interestingly, the first human clinical study approved for iPSC transplantation therapies (RPE cells) in Japan, headed by the stem cell researcher Masayo Takahashi, uses the episomal vector strategy. All three methods eliminate the risk of genomic integration and insertional mutagenesis, are conceivable from the technical, scientific and ethical point of view.

In recent years there has been more of a focus to make the reprogramming procedure more efficient rather than improving the quality of iPSC. Recent work by Rais et al has solved the challenge of inefficiency of reprogramming with the discovery that the knockdown of the epigenetic modifier, methyl-binding protein 3 (Mbd3) in combination with provision of the four factors Oct4, Sox2, Klf4 and c-Myc results in almost 100% of somatic cells reprogramming into iPSC [14]. However, as pointed out by the authors, whether Mbd3 knockdown increases the quality of iPSC remains to be tested.

The quality of differentiated cell types to be used for cell replacement therapy is dependent on the quality of the starting material. To develop high quality iPSC, the best method will most likely be to use modified RNA transfection methods using Oct4 and Sox2 with new pluripotency factors in a cell type that removes the use of oncogenic Klf4 and c-Myc, and using defined media that are xeno free, with GMP grade cell culture conditions [15-17]. The development of a protocol to make safe GMP-grade iPSC does not exist at the time of writing this review.

The risk of cancer from iPSC and their derived cells has been studied extensively in mouse models [18-21]. Previous studies that have compared embryonic stem cells (ESC) and iPSC have revealed

that the starting cell and differentiation protocol are critical in determining the threat of cancer of iPSC [20]. Other studies comparing ESC with iPSC have revealed a distinctive gene expression signature between them, implicating the cell cycle, which is an important regulator of cancer [21]. IPSC have been shown to have a higher tumorigenic potential than ES cells [20,21]. It has been suggested that four-factor iPSC have a higher tumorigenic potential than three-factor iPSC. Therefore the search for new pluripotency factors that can replace Klf4 and c-Myc, and cell lines that can be reprogrammed with just Oct4 and Sox2, have been addressed [15,23]. The application of these new pluripotency factors to make cancer free iPSC and their differentiated progeny is yet to be tested but it is expected to be lower than the use of the conventional four factors that include c-Myc and Klf4 oncogenes. The testing of iPSC lines for their capacity to induce benign tumours will have to be tested on a case-by-case basis before the cell type could be used in clinical trials.

## Efficient Cell Differentiation Protocols

A common misunderstanding in the application of iPSC technology is that iPSC will be injected for cell therapy. The injected cell type is actually more likely to be the differentiated progenitor cells from iPSC, and we have demonstrated that there is 10-fold reduction in the tumorigenicity of differentiated iPSC, equivalent to embryonic stem cells [4,18]. The idea that some iPSC cells may be remaining following differentiation can be addressed by a purification step using flow cytometry, and then "spiking" experiments with as few as 10 to 100 iPSC are added back to the purified differentiated cells and tested their capacity to be tumorigenic *in vivo*. Recently this concern has been alternatively addressed by the selective elimination of these remaining Nanog-positives iPSCs by the inhibition of stearoyl-coA desaturase [24]. This method induced apoptosis in the undifferentiated residual iPSCs but not in the iPSC-derived cardiomyocytes they were differentiating to. Then, using an animal model, those cardiomyocytes displayed the capability of engrafting and surviving in an infarcted myocardium. But, although the tumorigenic-iPSCs associated threat can be bypassed to enhance the safety of therapeutic iPSC-derived cellular transplantation, the treatment still will present the requirement of repeated periodical transplantation.

Another important issue still to be addressed for the application of iPSC-differentiated cells is to identify the cell type responsible for regeneration of a tissue. For example for cardiac muscle repair it is postulated that a fully differentiated cardiomyocyte de-differentiates into a "progenitor" cardiac muscle cell type that then proliferates and heals the injury [25]. Identification and characterisation of the de-differentiated cardiomyocyte is then essential to know what cell type to differentiate iPSC towards. This would apply to tissues and organs of the human body and calls for a return to basic research methods, such as lineage tracing to identify cell types responsible for bona fide tissue regeneration. For instance, the dopaminergic neurons lost in Parkinson's disease diverge from the many neuronal subtypes lost in Alzheimer disease that might be specifically needed for regeneration and recovery in these neurodegenerative disorders.

## Future Applications: Bioengineering Body Organs with iPSC

Bioengineering of body organs using iPSC is perhaps one of the most powerful applications for the future clinical use given the shortage of suitable body organs and waiting lists for organ transplantation worldwide. The bioengineering of adult organs from iPSC is still at the embryological level with fully functional and transplantational organs still to be created. However, three recent publications have made huge leaps in that direction, with the generation of brain, liver and recently, kidney-like buds from the group of Juan Carlos Izpisua Belmonte [26].

The recent bioengineering of a liver bud, when transplanted into mice, was able to rescue drug-induced liver failure, providing perhaps the best example to date of iPSC bioengineered organs [27]. The method involves combining iPSC differentiated to hepatic endoderm mixed with human mesenchymal stem cells and human umbilical endothelial cells. This mix of cells is then placed in a bi-dimensional matrigel layer, with it self-organizing into a three-dimensional system termed iPSC-derived liver buds. The liver buds were able to produce albumin (liver-specific protein) and were able to metabolize drugs ketoprofen and debrisoquine. The presence of human umbilical endothelial cells in the starting cell mixture provided the iPSC-derived liver buds with blood vessels that connected with the host vessels within 48h, after implantation in mice. The authors highlighted that the newly formed vascular system, together with the 3D structure seemed to be the key for successful engraftment and maturation of the liver buds [27].

Cerebral organoids similar to structures seen in the first 8 weeks of development of the brain have been made by culturing neuroectoderm derived from human iPSC in a three dimensional culture system [28]. The method involved iPSC that were differentiated into embryoid bodies, which were then differentiated into neuroectoderm. The neuroectodermal tissue was then cultured in a three dimensional system using matrigel droplets as a scaffold, and subsequently transferred to a bioreactor. Remarkably the three-dimensional tissue produced heterogeneous regions similar to human brain. Although the authors did not intend to use the organ like structure for regenerative purposes, the cerebral organoids did recapitulate features of human cortical development [28]. Given that mice and human brains have highly complex and integrated structures, the authors suggested that these organoids could be good candidates for the study of human brain development as well as the modelling of brain pathologies.

The group led by Juan Carlos Izpisua Belmonte have recently made kidney progenitor like ureteric buds that can make 3D organ-like structure *in vitro* [26]. Differentiation of pluripotent cells into renal cell lines has had limited success but they reported the successful differentiation of human pluripotent cells into ureteric-bud-committed renal progenitor-like cells. The differentiated cells displayed specific expression of kidney progenitor markers when cultured under specific media conditions. They went on to demonstrate that maturation into ureteric bud structures was successful with the establishment of a three-dimensional culture system that enabled differentiated human cells to assemble and integrate alongside murine cells for the formation of chimeric ureteric buds. Taken together, the data provide a new method for the study of kidney disease lineages commitment and the future possibility of creating a 3D kidney [26].

Concerning the hypothetical transition of 3D organs or pseudo-organs to the clinic, some other questions still must be addressed. Once iPSCs have been derived into fully differentiated cells or into specific progenitors for a certain therapy and when the resulting cell population is completely free of pluripotent cells it has to be determined the amount of cells which the formed tissue may have in order to be able to suitably engraft into the host transplanted area. The

tissue tridimensional structure has to be developed *in vitro*, for instance by using collagen scaffolds, as has been shown for spinal cord injury in rats [29].

Another matter is that the hypothetical *in vitro* grown graft need to establish new blood capillary vessels in the minimum possible time, as the grafted cells feeding capability depends on this feat. It would be interesting to discover the chemical molecules whose administration in the grafted area may help this angiogenic function. For example, recently it has been published that the providing of synthetic modified mRNA coding for VEGF-A was successfully used for inducing myocardial regeneration after infarcted injury in mouse heart [30]. It may be considered then, the use of this mVEGF-A, or a functionally similar molecule, is a source for enhancing the angiogenic process between the host body and the grafted tissue.

## Assessment of Immune Response with Autologous Cell Therapy

One of the main possible advantages of iPSC technology for clinical application is the potential to use autologous cell or body organ transplantation therapy, removing the need for immune suppression drugs and their associated side effects. It follows that iPSC derived from patient cells that are differentiated into a cell type, and then transplanted back into the same patient, are unlikely to provoke an immune response, because the cells are derived from the same individual. However, the reprogramming process itself has a major impact on many cellular functions such as cell skeleton, cell cycle and the epigenetic landscape in its bid to win back the cell clock. It is possible then that the process of reprogramming itself could re-set the immune system machinery different to the starting cell. This could be especially true for retrovirally generated iPSC or for iPSC clones that have not been fully reprogrammed to ground state pluripotency. Indeed, work by Zhao et al. recently demonstrated anti-graft T cell responses were potent enough to prevent the formation of teratomas in mice [31]. This was not observed with embryonic stem cells (ESC), suggesting the method to reprogram cells to the pluripotent state itself influences the immune responses within the host.

This disconcerting discovery has since been cast into doubt with two publications on the topic. The group of Guha et al. found that differentiated cells derived from syngeneic iPSC were not rejected after transplantation [32]. Moreover, Araki et al. compared the immunogenicity of differentiated skin and bone marrow tissue derived from integration-free mouse iPSC (generated by episomal vectors) and ESC-derived tissue, and did not observe any differences between the two groups [33].

To take this further, in research using non-human primates, Morizane et al. found that autologous transplantation of the iPSC-derived cells generated a minimal immune response compared with allografts both in the nonhuman primate brains in the absence of immunosupression [34]. They went on to suggest that immunosupression is not necessary for autologous transplantation of iPSC-derived neural cells into brain [34]. Therefore, the current level of knowledge would suggest that autologous transplantation of the iPSC-derived cells do not cause an immune response, or at worst a minimal response that would not need immunosupression. This would set the scene for future clinical transplantation of iPSC in various human diseases.

## Conclusion

A number of challenges remain to be clarified for iPSC before the technology becomes a reality for use in the clinic, including the immune response, the threat of cancer and the development of robust differentiation protocols, and the processes for the establishment of organ bioengineering. The threat of cancer of differentiated cells may be minimal, as fully reprogrammed iPSC have only been shown to form benign teratomas that pose little threat to a patient, but nevertheless a cause of concern for the long term in case of those benign cells might mutate and become malignant. If a patient were to benefit from five years extended life from iPSC derived cell transplantation instead of death or long term suffering, then iPSC transplantation may outweigh the threat of benign tumours or the remote long-term (10 years) possibility of malignancy. Further research is warranted to determine the true long term threat of cancer of iPSC derived cells for transplantation, and this needs to be tested on a case by case basis before clinical trial for any cell line might be planned for use. The immune response of iPSC derived cells may appear minimal, although further investigation in more detail would put to rest lingering doubt about the true immune response of iPSC derived cells. Finally, the differentiation of iPSC to the right cell type and organ bioengineering still require much work for many human diseases before clinical application might becom a reality. Work in this direction suggests that some of the first human diseases to be treated with iPSC derived cells will be eye diseases such as macular degradation (MD) and possibly spinal cord injury. Clinical trials for iPSC derived RPE to treat MD is currently approved in Japan, and the stem cell world waits in anticipation for their results.

## Author Statement

1. We confirm that we support open access publishing to allow unlimited access and high publicity of our published paper.

2. This review paper has not been published elsewhere (or submitted to another ournal).

3. The authors declare no competing financial interests.

## Acknowledgments

M.J.E. (RYC-2010-06512) is supported by the Program Ramon y Cajal and by project grant BFU2011–26596.

## References

1. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, et al. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131: 861-872.
2. Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, et al. (2008) Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotechnol 26: 1276-1284.
3. Edel MJ, Menchon C, Menendez S, Consiglio A, Raya A, et al. (2010) Rem2 GTPase maintains survival of human embryonic stem cells as well as enhancing reprogramming by regulating p53 and cyclin D1. Genes Dev 24: 561-573.
4. McLenachan S, Menchón C, Raya A, Consiglio A, Edel MJ (2012) Cyclin A1 is essential for setting the pluripotent state and reducing tumorigenicity of induced pluripotent stem cells. Stem Cells Dev 21: 2891-2899.
5. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K (2008) Induced pluripotent stem cells generated without viral integration. Science 322: 945-949.

6. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S (2008) Generation of mouse induced pluripotent stem cells without viral vectors. Science 322: 949-953.
7. Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, et al. (2009) Human induced pluripotent stem cells free of vector and transgene sequences. Science 324: 797-801.
8. Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, et al. (2009) piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 458: 766-770.
9. Fusaki N, Ban H, Nishiyama A, Saeki K, Hasegawa M (2009) Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B Phys Biol Sci 85: 348-362.
10. Kim D, Kim CH, Moon JI, Chung YG, Chang MY, et al. (2009) Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell 4: 472-476.
11. Warren L., Philip D. Manos, Tim Ahfeldt, Yuin-Han Loh, Hu Li, et al. (2010) Highly Efficient Reprogramming to Pluripotency and Directed Differentiation of Human Cells with Synthetic Modified mRNA. Cell Stem Cell 7: 618-630.
12. Masuda S, Wu J, Hishida T, Pandian GN, Sugiyama H, et al. (2013) Chemically induced pluripotent stem cells (CiPSCs): a transgene-free approach. J Mol Cell Biol 5: 354-355.
13. Yoshioka N, Gros E, Li HR, Kumar S, Deacon DC, et al. (2013) Efficient generation of human iPSCs by a synthetic self-replicative RNA. Cell Stem Cell 13: 246-254.
14. Rais Y, A Zviran, S Geula, O Gafni, E Chomsky, et al. (2013) Deterministic direct reprogramming of somatic cells to pluripotency. Nature 502:65-70.
15. Giorgetti A, Montserrat N, Rodriguez-Piza I, Azqueta C, Veiga A, et al. (2010) Generation of induced pluripotent stem cells from human cord blood cells with only two factors: Oct4 and Sox2. Nat Protoc 5: 811-820.
16. Rodríguez-Pizà I, Richaud-Patin Y, Vassena R, González F, Barrero MJ, et al. (2010) Reprogramming of human fibroblasts to induced pluripotent stem cells under xeno-free conditions. Stem Cells 28: 36-44.
17. Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, et al. (2010) Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 7: 618-630.
18. Alvarez Palomo AB, S McLenachan, J Requena, C Menchon, C Barrot, et al. (2013) Plant hormones increase efficiency of reprogramming mouse somatic cells to induced pluripotent stem cells and reduces tumorigenicity. Stem Cells Dev 23: 586-593.
19. Kiuru M, Boyer JL, O'Connor TP, Crystal RG (2009) Genetic control of wayward pluripotent stem cells and their progeny after transplantation. Cell Stem Cell 4: 289-300.
20. Miura K, Okada Y, Aoi T, Okada A, Takahashi K, et al. (2009) Variation in the safety of induced pluripotent stem cell lines. Nat Biotechnol 27: 743-745.
21. Okita K, Ichisaka T, Yamanaka S (2007) Generation of germline-competent induced pluripotent stem cells. Nature 448: 313-317.
22. Chin MH1, Mason MJ, Xie W, Volinia S, Singer M, et al. (2009) Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Cell Stem Cell 5: 111-123.
23. Montserrat N, Nivet E, Sancho-Martinez I, Hishida T, Kumar S, et al. (2013) Reprogramming of human fibroblasts to pluripotency with lineage specifiers. Cell Stem Cell 13: 341-350.
24. Zhang L, Pan Y, Qin G, Chen L, Chatterjee TK, et al. (2014) Inhibition of stearoyl-coA desaturase selectively eliminates tumorigenic Nanog-positive cells: Improving the safety of iPS cell transplantation to myocardium. Cell Cycle 13.
25. Jopling C, Sleep E, Raya M, Martí M, Raya A, et al. (2010) Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. Nature 464: 606-609.
26. Xia Y, Nivet E, Sancho-Martinez I, Gallegos T, Suzuki K, et al. (2013) Directed differentiation of human pluripotent cells to ureteric bud kidney progenitor-like cells. Nat Cell Biol 15: 1507-1515.
27. Takebe T, K Sekine, M Enomura, H Koike, M Kimura, et al. (2013) Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 499: 481-484.
28. Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, et al. (2013) Cerebral organoids model human brain development and microcephaly. Nature 501: 373-379.
29. Shi Q, Gao W, Han X, Zhu X, Sun J, et al. (2014) Collagen scaffolds modified with collagen-binding bFGF promotes the neural regeneration in a rat hemisected spinal cord injury model. Sci China Life Sci 57: 232-240.
30. Zangi L, Lui KO, von Gise A, Ma Q, Ebina W, et al. (2013) Modified mRNA directs the fate of heart progenitor cells and induces vascular regeneration after myocardial infarction. Nat Biotechnol 31: 898-907.
31. Zhao T, Zhang ZN, Rong Z, Xu Y (2011) Immunogenicity of induced pluripotent stem cells. Nature 474: 212-215.
32. Guha P, Morgan JW, Mostoslavsky G, Rodrigues NP, Boyd AS (2013) Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells. Cell Stem Cell 12: 407-412.
33. Araki R, Uda M, Hoki Y, Sunayama M, Nakamura M, et al. (2013) Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. Nature 494: 100-104.
34. Morizane A, Doi D, Takahashi J (2013) Neural induction with a dopaminergic phenotype from human pluripotent stem cells through a feeder-free floating aggregation culture Methods Mol Biol 1018: 11-19.