The Rise of Direct Cell Reprogramming

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Stem cells as therapeutics: is the future finally here?

Is regenerative medicine poised to lead a revolution in the treatment of disease, as it unfolds its power to rebuild damaged or diseased tissue? There has been tremendous activity over the last decade in the development of stem cells for use as therapeutics. Today, there are over 5,000 “stem cell” trials registered on [ClinicalTrials.gov](https://clinicaltrials.gov/) and publications have increased dramatically in recent years, demonstrating the rapidly growing interest of the scientific community. However, for all its promise, stem cells are yet to deliver on this therapeutic potential, and this failure appears due to various technical challenges that still need to be overcome.

To explain this better, we need to go back to the beginning.

The cell is the basic building block of all living organisms and the human body is composed of trillions of cells. They provide structure for the body, take in nutrients from food, convert those nutrients into energy, and carry out specialized functions. These functionalities are determined by the genes that are expressed in that particular cell.

All cells in the body contain the same genes (~22,000), but only certain ones are “expressed” in each particular cell, making the proteins that determine the functions of that cell. A group of different cells working together to fulfil a function constitutes a tissue (e.g., neurons-astrocytes-oligodendrocytes working synergistically constitute neuronal tissue). A group of tissues working together to fulfill a bodily function constitutes an organ (e.g., the brain or the spinal cord). Finally, a concerted group of organs are re-grouped in a system (e.g. the brain and spinal cord constitute the Central Nervous System).

If cells make tissues, organs and systems, then what makes cells?

At the beginning of human life when the sperm fertilizes the egg, **totipotent cells** are born of the zygote. They possess the ability to become anything (placenta **and** embryo) and are present in such form up to the 16-cell stage (i.e., within the first couple of cell divisions after fertilization, up to 4 days post fertilisation).  Following this stage of totipotency, as the number of cells increases, **pluripotent cells**emerge**.**Pluripotent cells retain the ability to make any cell of the embryo (embryonic stem cells are pluripotent), but lose the ability to become placenta.

After 3 weeks post fertilisation, pluripotent cells begin to organize themselves into 3 germ layers (Ectoderm, Mesoderm, and Endoderm) and progressively differentiate towards a more defined state. Pluripotent stem cells have attracted significant attention since Yamanaka was able to reprogram “mature” fibroblasts (skin cells) to pluripotent stem cells (induced pluripotent stem cells or iPS cells).[[1]](http://www.bioinformant.com/direct-cell-reprogramming/%22%20%5Cl%20%22_ftn1) This pioneering work led Yamanaka to receive the Nobel Prize for Medicine in 2012.

However, iPS cells were abandoned by most scientific investigators as therapeutic candidates themselves, given their poor safety profile. (They go back to a pluripotent state, and in addition to the desired tissue, also form unwanted teratomas, which are rapidly growing tumors containing cells of all three germ layers). In fact, significant efforts have gone into attempting to differentiate these iPS cells into **multipotent stem cells** that are safe and potent therapeutic cell candidates.

**Multipotent stem cells** are specialized stem cells differentiated from one of the three germ layers, but restricted within a specific lineage. Each type of multipotent stem cell makes the specialized somatic cells of its lineage: for example, a neural stem cell is a multipotent stem cell that originates in the ectodermal germ layer and is the only multipotent stem cell that has the ability to make neurons, astrocytes and oligodendrocytes (and thus, neuronal tissue), and does not have the ability to form any other tissue.



iPS cells: great science but limited therapeutic potential due to safety, potency, and cost issues

iPS cells promised to be a boon for regenerative medicine. Researchers could take a person’s skin, blood, or other cells, reprogram them into iPS cells, and then use those to grow liver cells, neurons, or whatever was needed to treat a disease. This personalized therapy would get around the risk of immune rejection, and sidestep the ethical concerns of using cells derived from embryos and fetuses.

Unfortunately, the reality has been very different. Producing autologous iPS cell derived multipotent stem cells at a scale that can be used for clinical purposes has proved to be very difficult, time consuming, and costly. The only clinical trial using autologous iPS cell multipotent stem cells was halted in 2015 after only one person had received a treatment. As the lab prepared to treat the second trial participant, Yamanaka’s team identified two small genetic changes in both the patient’s iPS cells and the retinal pigment epithelium (RPE) cells derived from them.

There was no evidence that either mutation was associated with tumor formation, yet “to be on the safe side,” the trial was put on hold. In June 2016, the trial resumed, but was [switched to allogeneic](https://www.bioinformant.com/riken-resuming-first-ever-clinical-study-of-ipsc-derived-cells-in-humans/), rather than autologous cells, presumably for purposes of cost and time efficiency, as well as safety.  Thus, 10 years on from the discovery of iPS cells, iPS cells are no longer viewed as the key source of therapeutic cell candidates. They have become an important tool for modelling and investigating human diseases, as well as for screening drugs.

**iPS cells have become a lab workhorse — providing an unlimited supply of once-inaccessible human tissues with specific genetic mutations for research.**

Holy Grail: directly reprogrammed autologous multipotent stem cells

Today, autologous multipotent stem cell therapy is regarded by many as the “Holy Grail” for regenerating and replacing damaged, lost, or aged cells in organs. Bone marrow and fat (adipose) stem cells are effective therapeutic multipotent stem cells for replacing bone, cartilage and fat cells. However, they do not have the ability to replace cells in other organs, where cells are derived from other lineages.

Direct cell reprogramming is uniquely designed to allow a patient’s cell to “jump” directly to the specialized multipotent stem cell of choice, thus tailor-making autologous multipotent stem cells needed to repair a damaged or diseased organ. Instead of making pluripotent stem cells from a patient’s cells and differentiating them to multipotent stem cells over an extended period of time, direct cell reprogramming produces directly the specialized multipotent stem cell of choice, solving the safety, high cost, and time constraints associated with iPS cells.

*In vitro* reprogramming better than *in vivo* reprogramming

The main approach for direct cell reprogramming is based on *in vitro* reprogramming of harvested somatic cells from an accessible source (blood, skin, or bone marrow) to the specialized multipotent stem cell type of choice, according to the indication or disease (i.e. a neural stem cell for CNS conditions, a cardiac stem cell for heart disease, etc.). This approach relies on using transcription factors and chromatin remodeling agents to permanently alter the expression patterns of the cells, locking them into their new multipotent stem cell state of choice.

**The key to ultimate clinical success with this approach involves selecting the most relevant cell subtype(s) for reprogramming, refraining from using genetic manipulation or viral vectors for changing the gene expression pattern, and achieving stable reprogramming.**

A second approach being investigated (chemically induced direct reprogramming) relies on using small molecules *in vivo* (drugs directly in patients) to induce cell-lineage reprogramming. Such a strategy could be cost-effective and might sound attractive. However, specific targeting of systemically administered small molecule drugs to specific cells/tissues (without affecting other cells or parts of the body) is still many years away.  Even after decades of research by pharmaceutical firms, targeted drug delivery remains a key problem for the industry. Hence, *in vivo*this approach would raise significant safety concerns, because the drugs could result in widespread effects (e.g. turning many or most of the cells within the body into neurons).

Directly reprogrammed multipotent stem cells are destined to be lineage-restricted stem cells that regenerate and grow defined tissues of their specific lineage. They provide “new seeds” that can actually re-grow the targeted damaged tissue or organ. (This unique feature is sometimes confused with the pluripotent stem cells that do not possess this feature, but instead can differentiate into some unwanted cell types too). This restricted ability also means that the right type of stem cell can and should be used in the particular tissue or organ to be treated. For example, hematopoietic or mesenchymal stem cells have limited regenerative and no cell replacement abilities if implanted into the CNS, whereas neural stem cells are the cells that have been evolved for this specific purpose.

General lack of awareness surrounding this feature has resulted in confusion, whereby for example, mesenchymal stem cells (MSCs) have been implanted into numerous unrelated tissues (e.g. the brain), and this has resulted in, as expected, limited beneficial effects. This approach can also be harmful, as one patient that received these MSCs injected into her eye actually started growing bone inside her eye.

Autologous, young reprogrammed cells better than allogeneic sourced cells

For any stem cell to correctly, efficiently, and permanently replace cells in a patient’s tissue, the stem cell has to be of the **correct lineage** and **autologous** (the patient’s own cell in the right organ or tissue), and adequately “young” to be able to do the job. So far, this has not happened within the stem cell field. The only exception to this is hematopoietic stem cell grafts (bone marrow transplants) that have been performed since the 1960s to support the cure of blood diseases, such as leukemia, a rare example of where allogeneic stem cells can be used. The procedure is more akin to a full organ transplant than to cell therapy, as the entire hematopoietic system is replaced after irradiation (killing off) of the patient’s old hematopoietic system. In the process, the white cells that would normally reject an allogeneic graft are killed off too.

Of course, this irradiation of the “native” tissue cannot be done for tissue such as the brain. It is important to note that even with closely matched hematopoietic stem cell (HSC) transplants, the success rate of these procedures is often less than 20%, even after more than 50 years of clinical refinement. As a comparison, *autologous* hematopoietic stem cell transplants (from cord blood) have had no graft failures. Still, this partial exception of hematopoietic stem cell transplants has unfortunately caused confusion about the abilities of other donor stem cells to avoid rejection.

For example, there is a perception that other types of donor stem cells will adequately graft into a patient if the patient is on immunosuppressants or if the particular type of somatic stem cells has a very low immune signature (as is, for example, the case with mesenchymal stem cells). The reality is that although these strategies allow donor stem cells to survive in the patient’s body for a period of time without being fully detected by the patient’s immune system, they are still ultimately killed off. In addition, any immunosuppressive regimen can cause serious side effects in patients.

As more is understood about the mechanisms of direct cell reprogramming, their specialized manufacturing, and the importance of autologous lineage-restricted stem cells, these unique multipotent stem cells have the potential to drive regenerative medicine into the clinic around the world and compliment small molecule drugs, proteins, oligonucleotides and medical devices, in tomorrow’s medical portfolio. This is good news for patients afflicted with some of the most challenging diseases and injuries, as well as for society at large, which is crumbling under the cost of chronic care management.

Footnotes

1. [Cell.](http://www.ncbi.nlm.nih.gov/pubmed/16904174) 2006 Aug 25;126(4):663-76. Epub 2006 Aug 10. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. [Takahashi K](http://www.ncbi.nlm.nih.gov/pubmed/?term=Takahashi%20K%5BAuthor%5D&cauthor=true&cauthor_uid=16904174), [Yamanaka S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Yamanaka%20S%5BAuthor%5D&cauthor=true&cauthor_uid=16904174).