# Self Healing

Over time the human body loses the ability to regenerate and heal itself. Autologous and allogeneic cells are shown to have the potential of reviving what our bodies are unable to do. However, many obstacles are restricting these therapies from coming to life

Eytan Abraham, Ayelet Chajut and Lena Pinzur at Pluristem Therapeutics

"If we examine the accomplishments of a man in his most advanced endeavours, in theory and in practice, we find that the cell has done all this long before him, with greater resourcefulness and much greater efficiency."

Albert Claude, Nobel Lecture, 1974.

Cells are extremely complex living units that have the capacity to form an organism, regenerate and heal. It is clear that as we grow older, the capacity of our bodies and our cells to regenerate and heal diminishes.

Can we harness the innate abilities of cells to restore tissues and treat diseases? The short answer is – yes, we can. We know how to isolate, grow and re-introduce cells into the human body. But how can we use them to actually heal specific diseases and conditions? Which cells should be used? The patient's own cells (autologous)

**Figure 1:** Packed bed bioreactor system containing 3D Fibracel carriers allows efficient and controlled cell culture

*Images: Pluristem Therapeutics*

or perhaps cells from other individuals (allogeneic)? Do the cells remain in one area and replace injured tissue or do they act transiently, facilitating a healing process and then disappearing?

## Cell Therapy Modalities

There are three central modalities of cell therapy: tissue engineering, cell differentiation and integration, and secretome mediated cell therapy.

**Tissue Engineering**
Growing a fully functional human kidney or heart in the laboratory would be an amazing breakthrough. Despite extensive work being done in this area, only limited success has been achieved in humans.

A central issue is mimicking the complexity of a whole organ – especially the need for each cell to be within several 100 microns from a blood vessel or capillary – and therefore there is a need to form organs that are not just a mass of cells of a certain type, but that also contain rich vasculature (1).

Another concern is imitating the structure and extracellular matrix (ECM) complexity of an organ. One of the promising concepts to tackle this issue is the use of de-celluralised organs, such as taking a human or animal organ, removing all the living cells – while maintaining the ECM structure and composition – and seeding new (human patient) cells that then grow into the existing structure.

To date, two partially successful tissue engineered cell-based products have been used in humans. The first is an artificial bladder, made from the patient's own urothelial and muscle cells, and seeded into a collagen matrix (2). The second is an artificial trachea (3,4). While these are both significant achievements, they are both relatively simple organs. There is a lot of work being done on growing more complex organs, for example the heart and liver, with the aim of transplantation into humans.

**Cell Differentiation and Integration**
To overcome the complexity and challenges of tissue engineering, cells in a scaffold or in suspension can be directly introduced to the damaged tissue via systemic (intravenous) or local administration. The injected cells are specialised in the function that the damaged tissue lacks and are integrated into the tissue following delivery. One of the major advantages of toti-potent or multi-potent stem cells is their differentiation potential. This can be implemented prior to or following cell delivery.

This approach can therefore be divided into two schemes: stem cells can be differentiated *in vitro* into the cells of interest by a variety of stimuli (soluble factors, bio-physical culture conditions), or can be guided towards differentiation *in vivo* by local cues provided by the surrounding niche within the target tissue.

For example, adipose tissue-derived mesenchymal stem cells (MSCs) were shown to be efficiently differentiated into insulin-producing-like cells when co-cultured *in vitro* with pancreatic islets, before being administered to treat an insulin-dependent diabetes mellitus animal model (5).

Another example is induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) that were observed to undergo full differentiation and functional integration into newly forming bone after being placed in critical-sized calvarial defect macroniche (6). Eventually, in both cases the therapeutic effect is achieved by specialised, functioning cells which are integrated within the target organ or tissue, thus replacing non-functional or absent tissue.

**Secretome Mediated Cell Therapy**
For many years, the main dogma in cell therapy postulated that delivery of cells to the region of interest – either as an engineered organ/tissue or as cells meant to replace damaged tissue – is an essential prerequisite for successful therapy. In the last decade, there has been a realisation that cells have the capacity to release soluble biomolecules, such as cytokines, chemokines and growth factors, which act in a paracrine or endocrine manner to facilitate self-healing of a damaged organ/tissue, and has led to the conclusion that cells can also act therapeutically from a distance.

Direct proof that factor secretion plays a major role in some cells' mechanisms of action came from studies showing that using only mediums in which the cells have grown – conditioned medium (CM) – can elicit beneficial therapeutic effects in multiple animal disease models. Examples include CM from bone marrow-derived MSCs in lung injury or in cisplatin-induced renal injury models (7,8).

However, using a CM approach does not give the cells the opportunity to alter their secreted factor repertoire following 'sensing' the disease conditions *in vivo*. When using live cells as the drug – administered either via local (with a scaffold) or systemic administration routes – the cells remain viable for a relatively short period of time, such as days or weeks. During this time, the cells 'sense' the environment and secrete relevant therapeutic factors which activate different underlying disease healing pathways.

For example, hypoxic stress present in ischemic organs (such as following acute myocardial infarction or stroke) was found to increase the production and secretion of angiogenic factors from MSCs, such as vascular endothelial growth factor and basic fibroblast growth factor, in injured hearts, or insulin-like growth factor 1 that was detected in the brain core and ischemic border zone three days after induction of focal cerebral ischemia in rat models (9,10). The use of cells as 'protein factories', which sense the environment and actively secrete therapeutic factors, is gaining ground.

## Possible Tissue Sources

There are two central models for cell therapy: autologous – cells taken from the patient to treat the same patient; and allogeneic – cells taken from a donor to treat others. In both cases, when discussing possible tissue sources, each modality has a particular cell type

**Figure 2:** A device for rapid and precise thawing of cell vials at the point of care

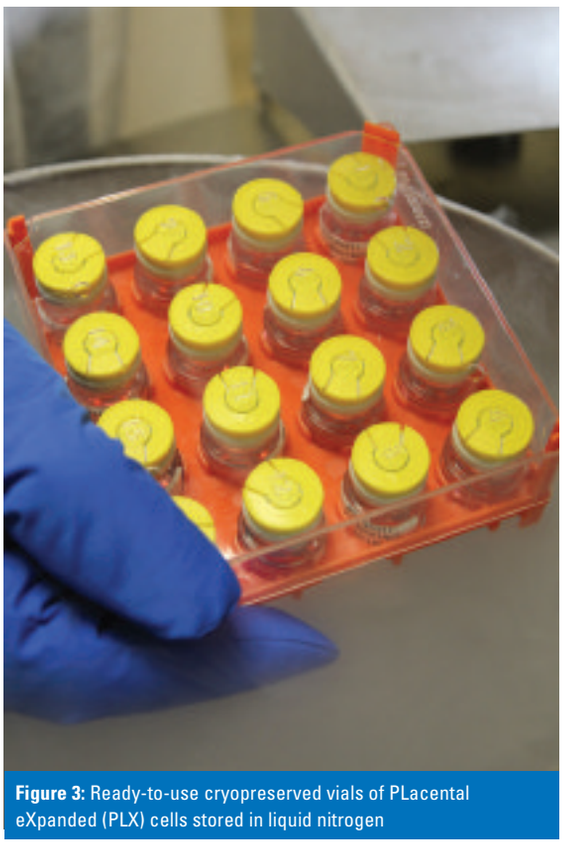

**Figure 3:** Ready-to-use cryopreserved vials of PLacental eXpanded (PLX) cells stored in liquid nitrogen

which is more commonly used and, consequentially, its own cell source.

When dealing with secretome mediated cell therapies, the cells of interest will often be MSCs or stromal cells with a low differentiation capacity, but with the ability to elicit *in vivo* effects via secretion of various proteins. These cells do not integrate into the tissue but instead die after several days or weeks.

The tissue sources for such cells vary widely. MSCs have been successfully isolated from bone marrow, adipose tissue, placenta, amniotic membranes, cord vein, cord blood, peripheral blood and alveolar epithelium; they were shown to be immuno-modulatory and of low immunogenicity, thus allowing allogeneic implantation, with no need for human leukocyte antigen matching (11,12). Other cell types, such as mature endothelial cells, have also been shown to have therapeutic potential which is based on their secretome (13).

On the other hand, when the therapy modality is based on implantation, differentiation, integration and function of the cells within the treated tissue or scaffold, the cells of interest should be of a high differentiation potential. One of the best tissue sources for such cells is the embryo; however, ethical considerations make the use of this cell source problematic. Moreover, ESCs are not immunoquiescent, so the issue of rejection is an obstacle for their clinical use.

For these reasons, iPSCs have become an attractive alternative to ESCs. In 2006, Kazutoshi Takahashi and Shinya Yamanaka were the first to demonstrate that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only a few defined factors (14). Human iPSCs are a potential source of patient-specific pluripotent stem cells that would bypass immune rejection due to their autologous source (15).

Today, several groups have shown that iPSCs can be generated from different cell types, such as neuronal progenitor cells, keratinocytes, hepatocytes, B cells, fibroblasts, kidneys, muscles, and adrenal glands (16). However, many obstacles still remain, including assuring that these cells do not pose a tumourigenic threat, plus ensuring that they do indeed integrate into the target organ and function as intended for long periods of time. The diversity of cell sources and technologies for cell therapy allows for flexibility and more than one approach to treating various conditions.

## Cell Culture Process

Contrary to chemical compounds, which are produced by a chemical process and are then relatively stable, or even compared to biological products, such as antibodies, live cells are a different type of therapeutic entirely. Cells are the basic structural, functional and biological unit of all living organisms; they are sensitive to environmental changes, sensing and responding even to subtle changes by transiently or permanently changing their biology. This is true from the beginning of the expansion process, through to the point at which they are injected into the patient, and in many cases they continue to respond even after injection.

These characteristics allow for manipulation of cells *in vitro*. For example, subtle changes in culture conditions, such as pH or oxygen levels, may result in a shift of gene expression and a subsequent change in protein production or differentiation capacity of the cells. Such changes may fundamentally change the cell potency. For this reason, control over environmental parameters at all stages – from isolation, through to culture and shipping and injection/implantation – is extremely important.

Conversely, the capacity to induce changes in the cell translates to the potential of developing novel products based on the alteration of

“Production of cells for therapy presents significant challenges, and requires complex and costly manufacturing processes. One of the emerging solutions to both process control and cell numbers is the use of 3D scaffolds or cell aggregates in bioreactors for cell production”

culture conditions. This can result in the development of several novel therapeutic products from the same cell source.

Another aspect of the cell culture process – especially in allogeneic cell therapy – in which many doses might be produced from one source (as opposed to autologous cell therapy) is the quantity of cells needed. We might consider how many cells it takes to make a heart. There are about two billion muscle cells in an adult heart, and many cell therapy treatments require hundreds of millions of cells per dose. So treating 10,000 patients with a dose of 300 million cells each would require 3 trillion cells. It is extremely difficult and inefficient to produce such vast amounts of cells using traditional plastic flasks.

Production of cells for therapy presents significant challenges, and requires complex and costly manufacturing processes. One of the emerging solutions to both process control and cell numbers is the use of 3D scaffolds or cell aggregates in bioreactors for cell production. The bioreactor environment allows for full real-time control over culture parameters, while the culture in 3D environments – such as fibres, hydrogels and cell aggregates – allows for multi-fold increased efficiency in the number of cells that can be produced per given volume, and a reduction in the cost of goods.

Several companies now offer a combination of bioreactor systems with 3D scaffold-based cell culture. For example, Fibracel-based proprietary 3D bioreactor-based culture systems are fully compliant with Good Manufacturing Practice, automated, and can achieve a 70-fold improvement in cell/volume efficiency as compared with standard tissue culture plates or 2D cell factory platforms. Such efficiencies – resulting from 3D cell culture – represent a better approach for reaching the cell numbers needed to turn cell therapy into a global industry (17).

## Conclusion

Cell therapies signify the potential of a new era in medicine. They harness the innate capacity of our cells to heal our bodies. In many respects, the use of cell therapy is akin to turning back the clock. Over time, our cells and organs lose their ability to self-heal; making use of cells – whether they are mature cells from a donor, iPSCs, ECSs or autologous cells from the patient which are concentrated or manipulated – have the potential to restore lost function and heal disease.

The range of conditions that can potentially be treated by cell therapy is vast: from rebuilding cartilage in joints to replacing skin or bone tissue; from muscle regeneration to treating neurodegenerative diseases; and from ischemic to auto-immune conditions. To date, there are several commercially available cell therapies, and the hundreds of ongoing clinical trials indicate that the number of therapies will rise to include many other diseases – including ailments which are increasing due to the ageing population.

The complexity and novelty of cell therapy mean that topics such as mechanism of action, regulation, production, potency and product shipping will need to be addressed by biotechnology and pharmaceutical companies. However, despite these challenges, cell therapy holds huge promise for the future of healthcare.

**Acknowledgement**

Eytan Abraham would like to thank Dr Ayelet Chajut and Lena Pinzur for their significant contribution to this article.

References

1. Auger FA, Gibot L and Lacroix D, The pivotal role of vascularization in tissue engineering, *Annu Rev Biomed Eng* 15: pp177-200, 2013
2. Atala A *et al*, Tissue-engineered autologous bladders for patients needing cystoplasty, *Lancet* 367(9518): pp1,241-1,246, 2006
3. Vogel G, Trachea transplants test the limits, *Science* 340(6130): pp266-268, 2013
4. Elliott MJ *et al*, Stem-cell-based, tissue engineered tracheal replacement in a child: a 2-year follow-up study, *Lancet* 380(9846): pp994-1,000, 2012
5. Karaoz E *et al*, Adipose tissue-derived mesenchymal stromal cells efficiently differentiate into insulin-producing cells in pancreatic islet microenvironment both *in vitro* and *in vivo*, *Cytotherapy* 15(5): pp557-570, 2013
6. Levi B *et al*, *In vivo* directed differentiation of pluripotent stem cells for skeletal regeneration, *Proc Natl Acad Sci USA* 109(50): pp20,379-20,384, 2012
7. Waszak P *et al*, Preconditioning enhances the paracrine effect of mesenchymal stem cells in preventing oxygen-induced neonatal lung injury in rats, *Stem Cells Dev* 21(15): pp2,789-2,797, 2012

8. Zarjou A *et al*, Paracrine effects of mesenchymal stem cells in cisplatin-induced renal injury require heme oxygenase-1, *Am J Physiol Renal Physiol* 300(1): pp254-262, 2011
9. Gnecchi M *et al*, Paracrine mechanisms in adult stem cell signaling and therapy, *Circ Res* 103(11): pp1,204-1,219, 2008
10. Wakabayashi K *et al*, Transplantation of human mesenchymal stem cells promotes functional improvement and increased expression of neurotrophic factors in a rat focal cerebral ischemia model, *J Neurosci Res* 88(5): pp1,017-1,025, 2008
11. Hass R *et al*, Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC, *Cell Commun Signal* 9: p12, 2011
12. Morandi F *et al*, Immunogenicity of human mesenchymal stem cells in HLA-class I-restricted T-cell responses against viral or tumor-associated antigens, *Stem Cells* 26(5): pp1,275-1,287, 2008
13. Nathan A, Nugent MA and Edelman ER, Tissue engineered perivascular endothelial cell implants regulate vascular injury, *Proc Natl Acad Sci USA* 92(18): pp8,130-8,134, 1995
14. Takahashi K and Yamanaka S, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, *Cell* 126(4): pp663-676, 2006
15. Park IH *et al*, Generation of human-induced pluripotent stem cells, *Nat Protoc* 3(7): pp1,180-1,186, 2008
16. Patel M and Yang S, Advances in reprogramming somatic cells to induced pluripotent stem cells, *Stem Cell Rev* 6(3): pp367-380, 2010
17. Rowley J *et al*, Meeting lot-size challenges of manufacturing adherent cells for therapy, *Bioprocess International* 10(S3): pp16-22, 2012


## About the author


Dr Eytan Abraham joined Pluristem Therapeutics in 2010. He leads Pluristem's Product Innovation group which develops the company's pipeline of cell therapies. He has extensive experience in molecular and developmental biology, *in vitro* assays and animal models, as well as 2D/3D cell culture, bioreactors, bioprocessing and commercialisation. Eytan conducted his post-doctoral training in cell therapy and tissue engineering at the Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology and at Harvard Medical School. He holds a PhD in developmental biology from the University of Maryland Biotechnology Institute. **Email: eytana@pluristem.com**