Could new manufacturing technologies finally make the promise of stem cell therapies a reality?

Cell-based therapies are being widely recognised for their potential to treat a variety of medical conditions, but they face major obstacles toward commercialisation. Traditionally, they have been difficult and expensive to make in large quantities. Biopharmaceutical companies must develop manufacturing solutions that will enable them to efficiently and reproducibly create the huge amount of product needed to help patients around the world.

By Dr Ross Macdonald

Notwithstanding the challenges associated with commercial manufacture, cell-based therapies – and especially those involving stem cells – are having their day in the sun. There is a greater understanding of the biology of how cells in our body, particularly cells involved in repair and regeneration, interact with one another. Cell-cell interaction is hugely important because it helps to maintain structural integrity of tissues, enables co-ordinated functioning in multicellular organisms and synchronises responses in groups of cells[1].

The principle reason why cell-based therapies are gaining acceptance is data from research. Scientists have tested these therapies in animal models of disease and proven that stem cell therapies have great potential to treat a variety of therapeutic targets. Encouraging efficacy and safety data from studies in humans is now emerging, and several cell therapies have received marketing approval, facts which together are prompting the scientific and medical communities to view cell-based therapies as a viable potential future option for mainstream medical treatment.

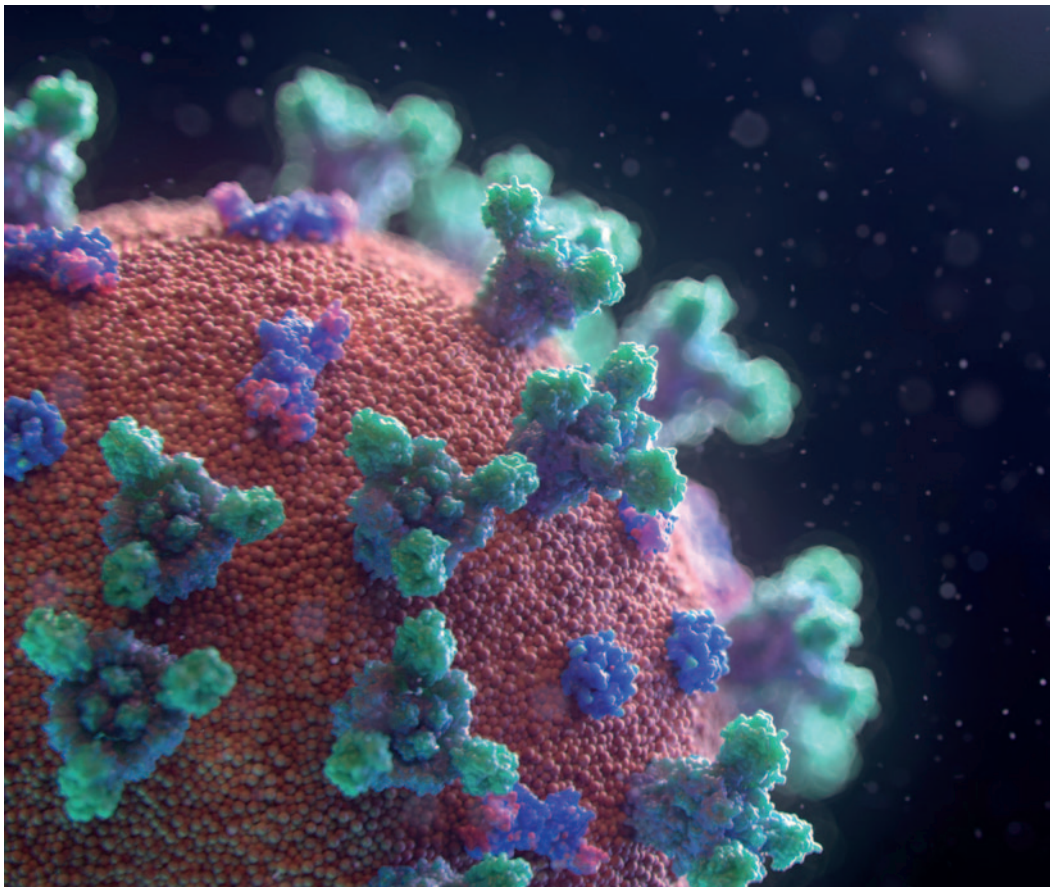
The body's raw materials

Stem cells are the body's raw materials, the cells from which all other types of cells are generated. Under the right conditions in the body or in a laboratory, stem cells divide to form more cells, called daughter cells[2].

Daughter cells become new stem cells or specialised cells with specific functions, such as blood cells, brain cells, heart muscle cells or bone cells. No other cell in the body has the natural ability to generate new types of cells[3].

Embryonic stem cells come from embryos. While use of this type of stem cell to generate cell therapies shows promise for treating or even curing many diseases, there is significant resistance to their mainstream use. Those who oppose such use of human embryonic stem cells argue it is unethical because the process of obtaining these cells destroys the embryo, an outcome which is considered by many to amount to murder.

Adult stem cells are found in many adult tissues, such as bone marrow or fat. These are undifferentiated cells found throughout the body that divide to replenish dying cells and regenerate damaged



Recent research has shown mesenchymal stem cells (MSCs) to be a useful adjunct for treating patients with serious, ongoing complications due to COVID-19 infection. *Image: Fusion Medical Animation*

tissues. Adult stem cells can self-renew and differentiate to generate the cell types of the organ from which they originate – potentially regenerating the entire organ from a few cells. They can be harvested from the patient and have been used successfully for many years as an adjunct in the treatment of leukaemia and related bone/blood cancers through bone marrow transplants[4].

Perinatal stem cells are found in amniotic fluid, placental tissue and umbilical cord blood[5]. These cells are formed around the time of birth, and because they are believed to be more primitive than stem cells in bone marrow and fat tissue, they have great therapeutic potential. Some new parents bank perinatal tissue and cord blood in a private blood bank with hopes that it will help to treat their child if they have diseases in the future.

Induced pluripotent stem cells (iPSCs) are adult cells that are altered in the laboratory to have properties of embryonic stem cells. This type of cell can become a multipurpose research and clinical tool for understanding and modelling diseases, developing and screening candidate drugs and deriving cell therapies for regenerative medicine[6]. Because they are not derived from embryos (and yet have many of the properties thereof) they are not associated with the ethical controversy mentioned above. This renders iPSCs as a particularly valuable and useful starting material for the production of cell therapy products.

Another type of stem cell, *mesenchymal stem cells* (MSCs), are multipotent adult stem cells present in small amounts in several tissues, including umbilical cord, bone marrow and fat tissue. Mesenchymal stem cells can self-renew by dividing and can differentiate into multiple tissues including bone, cartilage, muscle and fat cells, and connective tissue. Because of their unique and powerful immunomodulatory properties, MSCs are the subject of intense research around the world. Many biopharmaceutical companies are utilising MSCs to create new drug therapies and several MSC-based products are now being commercialised in major markets.

A main advantage of cell-based therapies is a high level of specificity. Most pharmaceutical compounds not only impact specific targeted bodily processes, but commonly have 'off-target' actions. That lack of specificity and precision is the reason why drug therapies commonly have side effects,

Because cell-based therapies have enormous business potential, biopharma companies are forging scientific and business partnerships that will assist in moving these new technologies forward.
Image: ThisisEngineering RAEng

ranging from minor to dangerously life-threatening. Cell-based therapies have the unique ability to target and enhance the body's own processes – which is a huge therapeutic advantage over other medicines – and, at least in the case of MSCs, have demonstrated that they cause no major adverse effects[7].

Obstacles to overcome

The task of obtaining stem cells, however, presents significant obstacles. Donating bone marrow for stem cell harvesting is a surgical procedure that is painful and requires a hospital stay. Donating fat tissue is highly invasive as well. Both require many donors to obtain a substantial number of stem cells. Moreover, the cells that are obtained still require massive expansion (through causing the cells to divide) in culture. This process has been shown to cause the properties of the cells to change, in particular losing their therapeutic potency in a well-described process of senescence.

Another problem with patients donating their own material – called an autologous process – is that treatment options are limited. An alternative approach, called allogeneic, is referred to as an 'off-the-shelf' approach that provides a product that can be used in many patients.

It is possible to obtain enough stem cells for research purposes through donation and expansion in culture, but when it comes to global commercialisation, the fact that the cells are difficult and expensive to make in large quantities is a major barrier. At the end of the day, if efficacy and safety are demonstrated but the product cannot be manufactured in sufficient quantities to treat patients in need, it is not a product at all: it is just an interesting piece of biology.

Cell-based medicines will be a major force in future medical treatments, but the challenge is making enough of them, economically and consistently. Besides the need to be able to meet demand, a cardinal requirement of product manufacturing, is making the product exactly the same every time to ensure a consistent response from patients.

Groundbreaking clinical trials for MSCs

In recent years, clinical trials in humans have evaluated MSCs as a possible therapy for a very wide range of diseases including graft-versus-host disease (GvHD), lupus, cardiac disease, osteoarthritis, pulmonary disease, stroke and lumbar degenerative disc disease. While some of these studies have shown promise, a consistent challenge remains in manufacturing sufficient quantities of product without reliance upon multiple tissue donors.

In 2016, Australian biotech company Cynata Therapeutics started blazing the trail for the study of iPSC-derived MSC therapies when the company received approval to launch the world's first clinical trial of an allogeneic iPSC-derived MSC (CYP-001) for steroid-resistant acute graft-versus-host disease (GvHD). iPSCs are derived from mature cells such as skin or blood cells and reprogrammed back into an embryonic-like, pluripotent state. A pluripotent stem cell is the most versatile one – it can replicate itself indefinitely and differentiate into any other type of cell in the body. This provides a unique solution to the challenges associated with conventional manufacturing approaches which depend on a constant supply of new donors and the inherent product inconsistency that comes with sourcing starting materials from different donors.

Since an iPSC-derived MSC cell therapy product had never been tested in a human clinical trial before, the global medical and scientific community eagerly awaited the outcome. The results of the Phase I trial showed compelling efficacy data for treating GvHD and also no treatment-related adverse effects. The positive outcome of this trial provides a sound foundation to proceed to further clinical studies investigating the safety and efficacy of iPSC-derived MSCs in GvHD and also other potential diseases.

Fujifilm now has an exclusive licence to develop CYP-001 for GvHD and expects to launch a Phase II trial of the therapy before the end of 2020.

In 2020, Cynata expects to launch one of the largest MSC therapy clinical trials to date. Researchers will enroll 448 patients in a Phase II

trial that will evaluate the company's Cymerus™ iPSC-derived MSC platform for treating osteoarthritis. The trial will assess the effect of Cymerus on clinical outcomes and knee joint structure for osteoarthritis patients over a two-year period[8].

If MSCs can cause a joint damaged by osteoarthritis to repair itself or slow down the process of joint degeneration, it can potentially delay further intervention or prevent the use of analgesics or surgery. If the joint is treated early in the course of the disease, it is possible to stop the cycle of pain, exacerbation and destruction.

Proven effectiveness of MSCs

A recent review of MSC therapies published in *Cell Death & Disease* cites the potential for MSCs to treat cardiovascular disease: "In the cardiovascular system, MSCs can protect the myocardium by reducing the level of inflammation, promoting the differentiation of myocardial cells around infarct areas and angiogenesis, increasing apoptosis resistance and inhibiting fibrosis, which are ideal qualities for cardiovascular repair"[10].

In *Therapeutic Delivery*, a review of recent clinical studies listed diseases for which MSCs are being evaluated: "These clinical trials employ MSCs for a multitude of different purposes in different disease states, including tissue replacement in musculoskeletal, cardiac and liver diseases, and as immunomodulatory cells to mitigate graft-versus-host disease, organ transplant rejection and autoimmune disorders"[11].

These and many other published studies underscore the growing need to establish a robust, consistent and commercially-viable manufacturing solution beyond the traditional reliance upon multiple-donor-derived material.

MSCs in the battle against COVID-19

Recent research has shown MSCs to be a useful adjunct for treating patients with serious, ongoing complications due to COVID-19 infection. MSCs are not inherently antiviral and they do not act as vaccines, but they appear to support patient recovery, particularly from complications involving the lungs.

In COVID-19, inflammatory cells can release a large quantity of cytokines – a 'cytokine storm' – an overreaction of the immune system. When this occurs, the immune system is unable to modulate the response following the initial defensive reaction against the virus. Inflammation caused by the immune system continues unchecked and the lungs flood with fluid, making it difficult to breathe. Eventually, a prolonged cytokine storm will shut down breathing completely.

MSCs used in patients affected by a cytokine storm may balance the immune response to stop the overreaction without switching it off completely, so the immune system can continue to fight the COVID-19 infection. In recent months, Beijing 302 Military Hospital of China and Zhongnan Hospital of Wuhan University conducted trials to assess safety and efficacy of MSC therapy in patients infected with COVID-19 who acquired pneumonia, a life-threatening consequence of the infection.

Acute respiratory distress syndrome (ARDS), an inflammatory process that causes a build-up of fluid in the lungs and respiratory failure, has been observed in patients who have COVID-19. Several published studies show that MSCs could be effective in treating ARDS caused by infection, trauma or sepsis and this has prompted the initiation of a number of clinical trials of MSCs in COVID-19 in the US and elsewhere. Cynata recently published positive preclinical results about the utility of Cymerus MSCs as a treatment for ARDS and plans to commence a trial in COVID-19 patients.

Cynata has shown that its Cymerus MSCs are highly potent in addressing many pathologies associated with lung diseases, pneumonia-induced sepsis, as well as cytokine release syndrome (CRS), a systemic inflammatory response that can be triggered by a variety of factors, including infections and certain drugs.

A manufacturing challenge

There are different approaches to manufacturing MSC-based therapies but all first-generation companies rely on donor-derived stem cells, which requires many donors. However, it is challenging for the requirement for consistency to be met if the stem cell donor is different each time.

One approach, employed by Cynata, is to create MSCs that are derived from induced pluripotent stem cells (iPSCs). The unique manufacturing solution does not require multiple donors. An original blood donation from a single donor was all that was required to initiate the process, obviating the requirement to continuously find and screen new donors.

Through the cost-effective Cymerus manufacturing approach, MSCs can be produced in uniform batches in essentially limitless quantities with sustained potency. Consequently, the technology addresses a critical shortcoming in the production of MSCs for therapeutic use, which is the ability to achieve economic manufacture at commercial scale

References

1 ScienceDirect. Cell-Cell Interaction. (2001). https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/cell-cell-interaction.

2 Mayo Clinic. Stem Cells: What They Are and What They Do. 2019. https://www.mayoclinic.org/tests-procedures/bone-marrow-transplant/in-depth/stem-cells/art-20048117.

3 Mayo Clinic. Stem Cells: What They Are and What They Do. 2019. https://www.mayoclinic.org/tests-procedures/bone-marrow-transplant/in-depth/stem-cells/art-20048117.

4 Science Daily. Adult Stem Cell. 2020. https://www.sciencedaily.com/terms/adult_stem_cell.htm

5 Perinatal Stem Cell Society. 2020. https://www.perinatalstemcells.com.

6 National Institutes of Health. The Promise of Induced Pluripotent Stem Cells (iPSCs). 2020. https://stemcells.nih.gov/info/Regenerative_Medicine/2006Chapter10.htm.

7 Technology Networks. Exploring the Utility of Stem Cell Therapy for COVID-19. 2020. https://www.technologynetworks.com/biopharma/articles/exploring-the-utility-of-stem-cell-therapy-for-covid-19-334068.

8 Clinical Leader. Stem Cell Therapy Trial Offers Hope to Osteoarthritis Patients. (2020). https://www.clinicalleader.com/doc/stem-cell-therapy-trial-offers-hope-to-osteoarthritis-patients-0001.

9 Shu-Bin, Fang et al. Small extracellular vesicles derived from human MSCs prevent allergic airway inflammation via immunomodulation on pulmonary macrophages. Cell Death & Disease. (2020).

10 Guo, Yajun, Yu, Yunsheng, Hu, Shijun, Chen, Yueqiu and Shen, Zhenya. The therapeutic potential of mesenchymal stem cells for cardiovascular diseases. Cell Death & Disease. (2020).

Continued on page 22

Continued from page 21

11 Helmy, Karim Y, Patel, Shyam A, Silverio, Kimberly, Pliner, Lillian and Rameshwar, Pranela. Stem cells and regenerative medicine: accomplishments to date and future promise. Ther Deliv. (2010).

without requiring multiple donors or excessive culture expansion of the derived MSCs. This opens up a wide range of therapeutic and manufacturing possibilities.

Other developers of MSC products have recognised the challenges inherent in manufacturing a living drug for eventual commercialisation and have sought processes that might improve the scalability of cell culture processes. Most of these centre around developments in three-dimensional (3D) bioreactor technology. For example, both Mesoblast and Pluristem utilise bioreactor systems to manufacture their allogeneic MSCs derived from bone marrow and from placental tissue donations, respectively. These manufacturing processes seek to enable commercial scale production. Manufacturing scale-up is a major focus in order to meet projected commercial demands and reduce costs.

Promising partnerships

Because cell-based therapies have enormous business potential, biopharma companies are forging scientific and business partnerships that will assist in moving these new technologies forward.

Fujifilm has grown its capabilities in cell therapies and has made regenerative medicine a main part of its healthcare mission. Cynata licensed CYP-001 to Fujifilm following the successful completion of the Phase I clinical trial in GvHD, where all safety and efficacy endpoints were achieved.

Regeneus Ltd teamed with AGC Asahi Glass, a manufacturer of glass, chemicals, high-tech materials and biopharmaceuticals, for the manufacture and commercialisation of Regeneus' Progenza, a patented, off-the-shelf stem cell technology platform for osteoarthritis and other therapeutic applications for the Japanese market.

Mesoblast Limited partnered with Tasly Pharmaceutical Group to develop Mesoblast's allogeneic mesenchymal precursor cell (MPC) product candidates MPC-150-IM for treating and preventing chronic heart failure and MPC-25-IC for treating heart attacks.

Astellas Pharma, Inc joined forces with Ocata Therapeutics, Inc to develop cell-based therapies that address the unmet medical needs of patients suffering from severe ophthalmic diseases.

Takeda and TiGenix entered into an agreement for development of Cx601, the leading investigational therapy in TiGenix's pipeline. Cx601 is a suspension of allogeneic expanded adipose-derived stem cells (eASC) for treating complex perianal fistulas in patients with non-active/mildly active luminal Crohn's disease. Takeda subsequently acquired TiGenix.

A daunting challenge: getting MSCs to market

Research demonstrating the safety and efficacy of MSCs has raised great interest in their eventual therapeutic potential. The unique and potent properties of MSCs offer great promise as targeted therapies that enhance the body's own processes to fight a variety of diseases, from osteoarthritis to autoimmunity to cardiovascular diseases to the recent ravages of COVID-19.

But manufacturing roadblocks have historically been a major challenge for companies developing cell-based therapies. When an MSC is taken from a single donor, it is exceedingly difficult to upscale production and derive a commercially-viable, robust and consistent manufacturing process.

The current reliance on multiple donors and massive expansion in culture of the derived cells leads to quality inconsistency and variability in the overall yield of the final cell product. Moreover, conventional donor-based processes result in long process times and high costs that restrict scalability and broad applicability across indications.

New technology, however, that allows efficient, cost-effective production of MSCs is emerging. This groundbreaking approach is enabling the creation of cell-based therapies at scale, cost-effectively, and with consistent quality and enduring potency. As innovative technologies overcome the inherent challenges of first-generation manufacturing methods, MSCs are on track to potentially become widely available for therapeutic use to help patients around the world. DDW

Dr Ross Macdonald *is Managing Director and Chief Executive Officer at Cynata Therapeutics Ltd, an Australian clinical-stage stem cell and regenerative medicine company focused on the development of therapies based on Cymerus, its proprietary therapeutic stem cell platform technology. Dr Macdonald has more than 20 years' experience and a track record of success in pharmaceutical and biotechnology businesses.*