Pharmaceutical BIOPROCESSING

Limitless starting materials for large-scale manufacture of MSCs – what does the future hold?

"...induced pluripotent stem cell-derived mesenchymal stem cells have the potential to solve the biggest challenge associated with mesenchymal stem cell products – the quest for truly scalable, consistent manufacture."

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Mesenchymal stem (or stromal) cells (MSCs) are multipotent cells, found in a range of mammalian tissues. In recent years, there has been enormous interest in the therapeutic potential of MSCs, because of their ability to secrete various bioactive molecules, in addition to their immunoregulatory properties. MSCs have not been associated with induction of immune responses in unrelated recipients, so they can be used in an 'off the shelf' manner, without matching the donor to the recipient [1,2]. As of December 2012, the US FDA had received 66 distinct investigational new drug (IND) applications for MSC-based products [3], while there were over 450 clinical trials involving MSC-like products listed on the ClinicalTrials.gov website [4] as of March 2015. These trials involve an extremely wide range of indications, including hematological, cardiovascular, orthopedic, gastrointestinal and autoimmune disorders.

Although a number of different MSC isolation and expansion techniques have been used, all MSC products tested in clinical trials to date have been isolated from human tissue – most commonly bone marrow [2]. Impressive data have been generated, but these approaches have significant limitations in the context of large-scale manufacture. There are two fundamental problems: the number of MSCs that can be recovered from a tissue donation is relatively small; and there is a limit to the culture expansion capacity of MSCs.

For example, a typical bone marrow donation yields 10,000–20,000 MSCs [5,6], while typical clinical doses are between 35 and 350 million MSCs in an average adult [2], MSC populations can be significantly expanded in culture, but it has been reported that they enter senescence after 13–25 population doublings using conventional culture methods, and changes in their properties start to occur earlier during the expansion process [7]. Even if it was possible to consistently expand MSCs through 25 population doublings without affecting functionality, the yield from a typical bone marrow donation would be fewer than 7000 doses of 100 million cells.

In light of the therapeutic potential of MSCs for numerous common conditions, the commercial demand for a successful MSC product could run to millions of doses annually. Based on the example above, hundreds of new donations would be required each year to meet that level of demand. Even if such a scenario was feasible, it would be extremely costly. Aside from the expense associated with recruiting, screening and testing new donors, comparability testing on the final product would be required each time a new donation was used as starting material. Given the difficulty of correlating *in vitro* assays with *in vivo* effects of cellular products, and the well-established donor-to-donor variability with MSCs [8], it is likely that comparability testing would have to include *in vivo* studies [9].

In an effort to maximize the yield from each donation, novel cell culture techniques have been explored. Processes involving 3D microcarrier culture in bioreactors have shown promise, but challenges remain and

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most studies using these approaches have utilized small bioreactors that may not be scalable [10–12]. Additionally, even if successful scale-up to large bioreactors can be achieved, it cannot be assumed that MSCs expanded at large scale will retain the same functional properties, given the propensity of MSCs to change in culture. For example, it has been suggested that bone marrow MSCs expanded to produce 10,000 doses per donation may have limited clinical efficacy, in comparison to the type of modestly expanded MSCs that have been used in numerous successful academic clinical trials [8].

Rather than seeking to increase the number of MSCs that can be produced from a small starting population, another approach is to identify a much more plentiful, or ideally limitless starting material. This could facilitate large-scale manufacture without relying on an ongoing supply of new donations or excessive culture expansion of MSCs.

Pluripotent stem cells (PSCs) have an effectively infinite capacity to reproduce themselves without changing, in addition to the ability to differentiate into any other type of cell in the body. There are two types of PSCs – embryonic stem cells and induced pluripotent stem cells (iPSCs). Progress with embryonic stem cells has been hampered by ethical issues, but such concerns are not applicable to iPSCs, which are produced by reprogramming cells obtained from adult donors. It has been reported that even after 10^{72}-fold expansion in culture, iPSCs retain their ability to differentiate into all three germ-layers [13]. Consequently, a single iPSC bank has the potential to give rise to an effectively limitless number of cells.

The development of nonintegrating episomal reprogramming methods, which can generate iPSCs suitable for use in the manufacture of therapeutic products for humans, was a critically important step to facilitate the development of iPSC-derived MSCs (and indeed other types of iPSC-derived cells) [14–16]. Additionally, a reproducible and robust differentiation and expansion process is required, which gives rise to cells that display the phenotypic and functional properties that characterize MSCs isolated from adult tissues. Early methods of producing MSCs from PSCs involved the use of serum-containing media and/or animal-origin feeder layers [17,18], which are associated with batch-to-batch variability and risks of adventitious agent contamination and immunogenicity [19]. Subsequently, a group at the University of Wisconsin-Madison developed a process based on the differentiation of PSCs to an intermediate type of cell known as mesenchymoangioblasts and onward to MSCs, under serum-free conditions [20]. Their original process utilized mouse embryonic fibroblasts for maintenance of the PSCs, and co-culture with OP9 murine cells to produce the MSCs. However, more recent work has eliminated the use of xenogeneic cells, making the process well suited to the production of MSCs for human use. This technology is now being commercialized by Cynata Therapeutics Limited (Vic, Australia).

On a more general note, in September 2014, a patient in Japan became the first person worldwide to receive an iPSC-derived therapy – retinal pigment epithelium cells for age-related macular degeneration [21]. This was a major milestone in this emerging field, indicative of increasing comfort with the concept of using iPSC-derived cells in humans.

The next steps for iPSC-derived MSCs include additional preclinical proof of concept and safety studies, to supplement the studies completed thus far, followed by clinical trials in humans. With positive data from these trials, iPSC-derived MSCs have the potential to solve the biggest challenge associated with MSC products – the quest for truly scalable, consistent manufacture.

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