INTERVIEW

Advances in expansion technologies for the clinical-scale production of MSCs

Steve Oh is the Director of Stem Cell Bioprocessing and Institute Professor/Scientist of the Bioprocessing Technology Institute (BTI) and an expert in integrated stem cell bioprocessing for the manufacture of human pluripotent/embryonic and adult mesenchymal stem cells (MSCs) for cell therapies. Key recent achievements include high density production of pluripotent stem cells, cardiomyocytes, neuroprogenitors and red blood cells in suspension bioreactors. In the MSC area, methods of producing high quality MSCs, and primed towards cartilage and bone repair have been achieved. Recently a new assay for stem cell senescence useful for the quality control of stem cells for bioprocesses has been developed by his team. He was the International Society of Cellular Therapies (ISCT) Co-Vice President (Asia) from 2015 to 2017, and led the planning of the Sessions for Commercialisation of Cell Therapies for the Silver Jubilee (25 years) ISCT meeting at Suntec City, Singapore in 2016. He is also the current Vice President of the Singapore Stem Cell Society and holds an Adjunct Professorship at the Nanyang Technological University. Steve has founded two stem cell companies, Brilliant Research and Veristem, and is a veteran of 26 years in the biotech industry.

Q What do you see as the major bottlenecks for the manufacture of mesenchymal stromal cells?

The lack of a significant blockbuster result for mesenchymal stem/stromal cells (MSCs) in clinic is the major bottleneck for the manufacturing of these cells. Once their clinical efficacy is proven in one or two indications (e.g., stroke or lung cancer), the field will be quickly primed to develop new technologies for manufacturing.

It is also unclear if the current sources of MSCs are suited for clinical indications or will next-generation MSCs be required for better efficacy? And at the moment the lack of a big clinical success could be because the source of cells, MSCs, used in these clinical trials, are not quite the best cells for therapy. We need a next generation of mesenchymal stem cells. Just like when CAR-T started off, they had different T-cell receptors and finally, they came up with a very potent T-cell engineered receptor, which won in the clinic. Such a clinical win for MSCs will drive the field forward in terms of manufacturing.

Q With regard to expansion, planar cell culture systems are widely used for expansion of mesenchymal stromal cells. What are the factors that limit its use in large-scale manufacture of MSCs?

Planar cell culture systems are commonly used in laboratories and represent a cost-effective and easily operable way to achieve MSC expansion. These technologies are similar to the use of roller bottles in the beginning when recombinant proteins were produced. The production of erythropoietin, which is required in micrograms/ml, was adequate in these low-tech solutions but not when antibodies are required in milligrams/ml.

Likewise, planar culture systems are sufficient when making smaller doses of cells, say for up to 200 patients in a Phase 2 clinical trial. But it is not feasible beyond that for commercial scale manufacturing.

A considerably high number of flasks would be required to obtain the large cell numbers required in the clinics. This would be highly time consuming and labor intensive, but also limited by the low surface area per volume and lack of ability to monitor and control culture parameters in these systems, which would most likely result in variability in terms of cell numbers and quality. In addition, handling multiple flasks would increase the risk of bacterial contamination.

Everyone in the field realizes that planar culture systems are really only a stop gap solution until we master bioreactor-based cultures of MSCs that can yield 10s to 100s of liters. You get the high densities and good yields in controlled environments.

Q What are the critical parameters to consider when designing a cell expansion platform for MSCs?

Volumetric scalability is the key parameter to consider when designing an expansion platform for MSCs. It means that you should choose an operation where the cell performance is equivalent at 1, 10, 100 liters or larger scales. For instance, you did your process optimization for the best growth conditions at 1 liter. You should be able to replicate those conditions at 10 and 100 liters. This is easier said than done. Some of the parameters like mixing conditions, feeding regimen, controlling the low levels of waste products and maintaining cell yields all require creativity

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and genius of a bioprocess engineer to make the same yields as the process is scaled 10 times.

Volumetric scalability is the principle upon which proteins are made and I believe that's how the cell therapy industry will be shaped eventually – by having good controls at increasing volumes.

Q Studies have shown the effectiveness of using microcarrier-based bioreactors as alternative platforms for the scalable manufacture of MSCs. Can you elaborate on those?

Microcarrier-based bioreactors represent a robust alternative for the scalable expansion of MSCs. Microcarriers are essentially small particles, often spherical, that provide a large surface area per unit volume for anchorage-dependent cells like MSCs to adhere and expand. The increased surface area of microcarriers provides a very effective option for the attachment and cultivation of these cells in dynamic culture systems such as stirred bioreactors. Besides providing greater scalability compared to 2D planar systems, microcarrier-based manufacturing of MSCs is advantageous as it avoids consecutive passaging.

The other types of cells that can grow on microcarriers are pluripotent stem cells. Unlike MSCs, which grow as monolayers, pluripotent stem cells like to clump together as aggregates. Microcarriers also provide the environment to allow them to clump and eventually these grow as colonies of about 400–500 microns in diameter.

The cells that can grow in suspension in bioreactors without the need for microcarriers are T cells and hematopoietic stem cells. They grow as single cells floating around in their liquid and are amenable to be put inside bioreactors where you control the environment that you can feed the oxygen levels, essential nutrients and growth factors.

Q Apart from the microcarrier-based culture cell systems, are there other alternative platforms being used for MSCs?

Hollow fiber bioreactors and multi-plate technologies are the other culture systems commonly used for the clinical scale expansion of MSCs. These are all scale-out systems that need multiple units.

Hollow fiber bioreactors are a 3D culture system that consist of fibers fixed into a module with cells typically seeded on the outside of the porous fibers and media delivered through the fiber lumen. This creates a versatile culture system with superior mass transport in which high cell densities can be reached. Studies conducted for early stage clinical studies, for instance Phase 1 and Phase 2, use hollow fiber bioreactors and each hollow fiber bioreactor has the capability to make enough for 10–20 doses of cells for patients.

To scale up, you would need to increase the number of hollow fiber units and so instead of having volumetric scalability, you are essentially

having to buy multiple units for more patients. Therefore, the approach is less cost effective.

Multi-stack cell factories offer another alternative for MSC expansion and by simply increasing the size and number of stacks, these systems offer large surface areas. Those stack plates can make about 20–40 patient doses at a time. However, when you start getting into hundreds and thousands of patients, these technologies will not be able to meet those needs.

Q There have been reports showing efficacy of expansion using a serum-free medium compared to serum containing medium formulation. What are your thoughts?

Serum-containing medium derived from animals, for instance, fetal bovine serum has been a gold-standard cell culture supplement used in laboratories for the expansion of MSCs. However, its use in clinic has been discouraged by regulatory authorities due to the lack of standardization in its preparation and to avoid xenogeneic immune reactions in the host.

People are now increasingly using low serum-based media and supplementing it with platelet lysates as source of growth factors. However, the problem is that currently when you try to grow some of these MSCs in suspension cultures, they do not perform as well in the serum-free media as they do in serum-containing media. Therefore, additional components need to be added to preserve their viability, or supplements like lipids or carrier molecules, to allow them to grow in a low serum environment to replace the serum function. These are problems that can be solved and serum-free defined media will become the standard of choice eventually to maintain consistency of production and this will be driven by the need for therapy and large doses.

Q Are there differences in expansion protocol for allogeneic versus autologous MSCs, and what are the challenges for each?

The autologous platform is patient-specific and if you develop something for autologous MSCs, it's unlikely that it will be suitable for allogeneic platform. These are the T-flasks and tray-based culture methods. Whereas, systems such as spinner flasks or bioreactors that are optimized and developed for allogeneic platform will be able to work for autologous production.

For autologous platform, you wouldn't have to use serum-free media because it's individual patients, and you won't use it for large patient populations.

Q What are the challenges that remain for the microcarrier-based systems to be fully implemented in the cell therapy manufacturing sector?

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A few of the bioprocess parameters that need further optimization for upstream cell expansion in microcarrier-based systems are aeration and medium feeding regime. Additional optimizations are also needed to address the downstream processing of cells after expansion, involving the separation of cells from the culture platform, cell washing and subsequent volume reduction, and storage/preservation till use.

Harvesting the cells off the microcarriers in a fast-enough time to avoid cells from re-clumping and losing viability is a real challenge. Better mixing conditions or alternatively better designs of bioreactors would be important as you get to higher cell densities in these bioreactors. We also need to assure the efficient recovery of MSC-based products free of any bead particulates. Currently this is one of the factors that limit the progression of MSCs into the clinics. These are challenges that can be solved, but we need more bioprocess engineers in the field to solve them. And not just scientists.

Q Where do you see the next opportunities for advancing the use of MSCs for cell therapy applications?

Besides the cells themselves, recent evidences suggest the importance of using secretions derived from MSCs, including exosomes, in rendering functions similar to those of MSCs. The production and purification protocols for those will be unique because you're not making cells now, but trying to concentrate the proteins or extracellular vesicles secreted by the cells and retaining the cells in the bioreactor.

As mentioned for the blockbuster cell therapy, maybe we need to have engineered MSC lines that have a more effective therapeutic response or better survivability in the host. If you engineer those cells, again, their growth characteristics inside the bioreactor might be different or they might grow better in the bioreactor than in donor-derived MSCs. This might open up a lot of interesting challenges ahead of us.

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