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Microcarrier culture in stem cell bioprocessing

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Steve Oh is currently Principal Scientist and Director of the Bioprocess Internship Programme at the Bioprocessing Technology Institute, one of the biomedical research institutes under the Agency for Science Technology and Research. The focus of the Bioprocessing Technology Institute's research is on developing simple yet rigorous and reproducible methods for producing high quality adherent stem cells (human pluripotent, multipotent stem cells and their differentiated derivatives, e.g., cardiomyocytes, neural cells and osteoblasts), for applications such as identifying drug candidates and tissue repair. Microcarriers have the versatility of different shapes, sizes, charges and the ability to present diverse extracellular matrices or peptides on their surfaces for cell expansion and differentiation. Microcarriers also enable adherent dependent stem cells to be cultured in suspension bioreactors instead of plasticware, which can readily be transferred to commercial manufacturing. They are also developing monitoring tools for stem cell bioprocessing. Their work is collaborative both within the Biomedical Research Council, cross disciplinary with the Science and Engineering Research Council, and also engages clinicians. Some of these partners include GIS, IMB, SBIC, SIMTech, IMRE, I2R in the Agency for Science Technology and Research family and the National University Hospital. Steve Oh recently spoke to *Pharmaceutical Bioprocessing* about the field of stem cell bioprocessing.

Interview conducted by Alice O'Hare, Commissioning Editor.

» What led you to your work on microcarrier culture for stem cell production?

Jerry Chan, a clinician at the National University of Singapore was looking for a more efficient method of generating human mesenchymal stem cells (MSCs) as he needed to deliver between 300 and 500 million cells to seed each scaffold to regenerate bone defects. He had previously tried to culture MSCs as monolayers and found it very laborious [Chan J, Pers. Comm.]. We felt that large amounts of MSCs could be easily achieved by cultivating them on spherical microcarriers in stirred bioreactors.

Furthermore, many late-stage clinical trials are underway that require large doses of MSCs [1] and we believe a microcarrier-based platform for stem cell expansion in bioreactors is the way forward to generate allogeneic (and autologous) large numbers of MSCs for cellular therapy.

» What would you say has been the biggest development in this field since its inception in 1967?

The explosion of clinical trials carried out with MSCs during the last few years by companies and research institutions. Some clinical indications include: graft versus host disease, Crohn's disease, bone and cartilage regeneration and myocardial infarction [1].

In 2012, two MSC products were approved for Osiris and Medipost and there are many clinical trials in Phase III. Commercialization of these cell therapy products will drive the development of large scale MSC production in microcarrier cultures.

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» What would you say are the main advantages of a 3D microcarrier culture, when compared with traditional monolayer cultures?

There are two major advantages: microcarrier cultures provide a much higher cell density per unit volume than monolayer cultures, which depends on the amount of surface area available, and microcarrier cultures enable production of MSCs in a single vessel, controlled bioreactor versus manual handling of multi-tray cultures.

» In your opinion, what is the biggest challenge faced by this field at the moment and how is this challenge being overcome?

Developing an inexpensive xeno-free GMP-compatible serum-free media for microcarrier-based culture. Currently, the commercially available serum-free media work well for monolayer MSC cultures but not on microcarriers. To address this, extensive multiparameter studies should be carried out in order to identify the critical parameters that enable MSC growth in stirred microcarrier cultures.

» What matters need to be considered in terms of choice of bioreactor for microcarrier culturing?

Various matters must be considered. Configuration of the reactor and the agitator type are important for achieving homogeneous distribution of the microcarrier particles in the bulk of the medium. In addition, round bottom vessels are important to ensure even distribution of microcarriers; since flat bottom vessels will lead to settling at the edges. Low shear is required for suspension of microcarrier-based cultures. Lastly, larger pitch-type impellers are preferred as small diameter impellers require high speeds, which we have found to cause spontaneous differentiation of stem cells and reduced cell growth.

» What would you say is the biggest challenge when manufacturing stem cells for bone tissue engineering?

This includes processes from efficient cell harvesting, storing the cells for long periods without losing activity and efficient seeding of the cells on the scaffolds at the point of usage. Due to the large doses of cells needed for bone tissue engineering, currently multiple T-flasks have to be harvested with trypsin manually, which takes hours to process. This can reduce the viability of the MSC and degrade the extracellular matrix thereby affecting their bone formation ability when seeded into scaffolds.

» Looking at novel research in harvesting cells from microcarrier culture; what do you believe holds most promise?

Harvesting from microcarriers is relatively straight forward if they are exposed to vigorous agitation with a shaker at high speeds to remove them from the surfaces, followed by filtration through a 40 µm filter; all this can be completed within 5 min. This protocol is described at the laboratory-scale in our recent article [2].

» Would you say that the field of stem cell bioprocessing is a multidisciplinary one? What can other disciplines offer this field?

Yes, I would say that there are several disciplines that can contribute to the field, including:

- Cell biology – understanding the pathways of potency and differentiation and developing new molecules to do this more efficiently;
- Analytics and assays – development of methods to analyze and understand cell potency, development of diagnostic methods for on-line monitoring of cells and the cytokines they secrete as a measure of their potency;
- Process engineering – engineering novel reactors as well as devices for cell harvesting;
- Storage and freezing – new methods for long-term storage of the harvested cells.

» Conversely, what can this field offer to other disciplines?

The microcarrier bioprocessing systems offer an improved method for generating high quality and yields of stem cells and differentiated cells for tissue engineering purposes, and for preclinical and clinical trials where cell requirements are high.

» What future work are you planning to expand on your research?

We are investigating the reasons for the improved 3D and *in vivo* bone differentiation of MSCs harvested from microcarrier cultures compared with ones harvested from 2D monolayers. This will be done by exploring the kinetics of expression of bone differentiation markers in the two different systems, that is, microcarriers versus monolayer MSC cultures.

» What other research is your team working on within the field of stem cell bioprocessing?

We are working on three main projects at the moment. First, the development of biodegradable microcarriers that can support growth of MSCs on microcarriers in bioreactors. That is, cell-covered microcarriers that can be transplanted directly *in vivo*. Second, the development of serum-free medium for propagation of MSCs in microcarrier cultures. Third, we are investigating the use of MSCs in bone and cartilage therapy.

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