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Alvin Nienow
Loughborough University

Agitation Strategies for the Culture and Detachment of Human Mesenchymal Stem Cells (hMSCs) from Microcarriers in Multiple Bioreactor Platforms

Alvin W Nienow,
Centre for Biological Engineering, Loughborough University, Leicestershire,LE11 3TU, UK

Unlike cell culture for biopharmaceuticals, where the product of interest is usually a recombinant protein, for regenerative medicine, the cells form the basis of the therapeutic. For the expansion of cells for allogeneic purposes, work, mainly in spinner flasks, has led to culture on microcarriers. For processes involving mass transfer and reaction with particles in stirred reactors (e.g., crystallization, catalytic reaction), it is essential that particles are at least just fully suspended, agitator speed N_{JS}, for effective mass transfer to and from the particles. Though gentle agitation has generally been recommended, it has not previously been defined in such a precise way as the minimum effective agitation intensity for cell culture on microcarriers where transfer of nutrients to and metabolites from them is essential. This criterion has been applied here for four sizes of stirred bioreactor (15 mL ambr™ (Sartorius Stedim), 125 mL spinner flask, 250 mL DASGIP (Eppendorf) and 5 L Sartorius Stedim). If the agitation intensity at N_{JS} for the particular bioreactor adversely affects the quality and quantity of the cells, then that configuration is inappropriate for cell culture.

In addition, it is critical that the stem cells are successfully detached and separated from the microcarriers in a manner that again does not adversely affect cell quality or the quantity. Indeed, effective cell recovery will reduce overall cost of goods by increasing process efficiency and enabling process intensification. However, surprisingly, few published studies have harvested greater than millilitre samples of the microcarrier culture, typically by enzymatic digestion aided by extensional flow using a pipette. At larger scales, such an approach becomes impractical and in addition, the enzymes can also damage the cells if exposure is prolonged. Thus, a new method is required. Given the sensitivity of particles of the size of microcarriers to abrasion (or if crystals, to secondary nucleation), it was decided to try a short period of intense agitation at agitator speeds significantly greater than N_{JS} to enhances the removal of the cells by the action of the enzyme.

This presentation outlines our work using N_{JS} for cell culture in the four different bioreactors and the new technique for detaching cells in-situ in the three smallest. In total, cells from four donors were used with two microcarrier with and without surface coatings (two types), four enzymes and three growth media (with and without serum), a total of 22 different combinations.

Stresses on cells on microcarriers may come from turbulence and from microcarrier impacts with themselves and with impellers. For turbulence, it has generally been considered that if the Kolmogorov scale of turbulence, λ_K is greater than ~ 60% of the size of the microcarrier (d_{micro} = ~200 μm), damage to attached cells should not occur. For the stresses from impacts, they increase very dramatically with increases in agitator speed, N ($\propto N^{\sim 4}$). The latter concept led to the use of an enhanced agitator speed (~ 2 to 5N_{JS}) being used during enzymic detachment for 7 minutes. Once detached, the cells were smaller than λ_K and thus cells should not be damaged.

To suspend cells in the rectangular ambr™ required a high N_{JS} which led to $\lambda_K = \sim 0.25 d_{micro}$, much smaller than has generally been accepted can be used without impacting process performance. Yet the cells grew well and maintained the desired quality attributes. With the spinner flask, $\lambda_K = \sim 0.6 d_{micro}$ but the growth was similar and again the quality attributes were maintained. The results were essentially the same in both the DASGIP and Sartorius bioreactors though $\lambda_K = \sim 0.3 d_{micro}$.

After detachment, cells were separated from the microcarriers by filtration and in each case, > 95% cells were recovered regardless of the bioreactor, the detachment enzyme, the microcarrier or the donor. In addition, the cells always met the desired quality attributes and were able to proliferate.

These criteria for culture and detachment, well grounded in agitation theory seem a promising approach to scale up; and for comparing the effectiveness of different bioreactors. The relatively high agitation intensities at N_{JS} leading to λ_K values much smaller than generally accepted as appropriate for cell culture is rather notable. That finding along with the new detachment technique may also interest manufacturers using microcarrier culture with other animal cells such as CHO for vaccines.

Reference
A.W. Nienow et al., Biochem. Eng. J., accepted for publication, Aug., 2015