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INTRODUCTION

Homogeneous large-scale cultivation of anchoragedependent animal cells for the production of therapeutic protein is made possible by cultivating the cells on small solid spherical particles called microcarriers, which are suspended in growth medium. Cells attach and spread on the microcarrier beads and form a monolayer (van Wezel, 1985). In this study, Pharmacia Cytodex 3$_w$ microcarriers having a hydrated density of 1040 kg m^{-3} have been employed in phosphate buffer saline (PBS) solution. The low solid–liquid density difference is sufficient to prevent flotation (van Wezel, 1985), but adequate stirring is also necessary to maintain the particles in suspension and prevent settling. Complete suspension of the particles would maximize the surface area of contact between the cells and the liquid medium for mass transfer processes. Hirtenstein et al. (1982) observed that traditional magnetic stirrers as used in spinner flasks provide 'laminar' flow with poor vertical movement, and do not create an even suspension of microcarriers at stirring speeds compatible with the growth of attached cells. Sedimentation of microcarriers was observed under the impeller shaft and at the periphery of the base.

A primary concern in tissue cell culture is the susceptibility of the cells to damage due to fluid dynamic generated stresses, which may arise from agitation and aeration. Such a phenomenon is commonly called 'shear damage' and it is often a rather loose qualitative concept rather than quantitative. According to Cherry and Papoutsakis (1986), there are three potential damage mechanisms to the cells: collision among cell-covered microcarriers; collision with parts of the reactor (especially the impeller); and interaction with turbulent eddies. Clark and Hirtenstein (1981) reported that for 1 litre volume cultures of Vero cells on Cytodex 1, if the stirring speeds were too high, cells would detach from microcarriers, particularly during mitosis; and if speeds were too low, the microcarriers did not circulate in the medium and cell growth was poor. The optimal stirring speed occasionally varied between cultures of different cell types and also between stages of the culture cycle, with best results obtained by changing from a static phase to low and then high speed as the culture progressed. A final speed of 60 rev min^{-1} during the exponential growth phase was found optimal. However, it was noted that the optimum speed was dependent on the impeller and vessel design.

Sinskey et al. (1981) compared the growth of chicken embryonic fibroblasts (CEF) at agitation rates ranging

from 60 to 200 rev min21 in 0.1 and 1.0 l vessels after 24 h of gentle agitation to allow the cells to attach to the microcarriers. Only at 200 rev min21 in the 0.1 l vessel did cell death occur. Cell deterioration was indicated when the value of an 'integrated shear factor' (ISF), which was related to the tip speed and the smallest distance between impeller tip and the wall, was greater than 90 s21. Estimating an ISF of 15 s21 in a 1000 l vessel operating at 60 rev min21, a better performance was expected on a larger scale. However, the ISF has no fundamental basis in fluid dynamics.

van Wezel (1985) had suggested that large-scale cultivation could be carried out in standard commercially available bioreactors that can provide slow agitation to maintain the microcarriers in suspension without pulverizing them. A rounded bottom tank was recommended to prevent the formation of dead zones. The combination of a propellertype stirrer with large blades and a rounded base vessel was found suitable. Propellers are axially pumping stirrers, which at the optimal impeller-to-tank diameter ratio, are among the best for solid suspension (Ibrahim and Nienow, 1996). There was no mention of the impeller and tank relative dimensions in van Wezel's (1985) work. Hirtenstein et al. (1982) had reported for Cytodex 1 microcarrier culture of 3 mg ml21, 50–70% greater yields using a bulb-shaped rod moving in a culture vessel with a rounded, indented base compared to traditional spinner vessels, as the improved vessel geometry eliminated accumulation on the vessel base. The use of a large paddle, although found to improve yields, created erratic stirring motion with many eddies forming in the wake of the paddle and thus they considered that it might not be compatible with the growth of cells. Clark and Hirtenstein (1981) experimented with a large screw impeller, which fitted closely to the rounded contour of the culture vessel to provide good lift at very slow speeds. Flexibility in equipment design and function was recommended in order to accommodate various culture procedures necessary for cell yields.

Croughan et al. (1987) reported optimal growth of human diploid fibroblasts (FS-4 cells) grown on Cytodex 1 microcarriers at a concentration of 3 g/l in 100 ml cultures. Various stirring speeds were tested using a cylindrical bar of D/T ¼ 0.69 positioned at one-third of the liquid height. The stirrer was observed to create a 'radial flow pattern' and a speed of 60 rev min21 was required to achieve complete suspension of the microcarriers. At 140 rev min21 and higher speeds, the growth declined and it was assumed to be entirely due to excessive agitation. They proposed a model, which predicts that the hydrodynamic cell death is proportional to (_1T)0:75 where _1T is the mean specific energy dissipation rate, which is equivalent to the specific power input (W kg21), that is, _1T ¼ P=rlV: They also stated that the damage would be insignificant as long as

the Kolgomoroff eddy scale, l_K, is larger than the particle size of interest based on the equation $l_K = (n^3/\bar{\varepsilon}_T)^{1/4}$ where n is kinematic viscosity. Aunins et al. (1986) estimated that for microcarriers of 180 mm, the specific power input should be less than 1×10^{23} W kg^{-1} for negligible cell death. Cherry and Papoutsakis (1989) showed that smaller microcarriers beads reduced cell death and increased cell growth rate.

In a study on scale-up effects on membrane oxygenation in 0.5 l and 10 l stirred vessels with four flat-blade and four pitched-blade impellers used together on a single shaft, Aunins et al. (1986) reported that the major effect of scale-up at constant power input per unit volume on mass transfer rate achievable in the reactor is the loss of interfacial area per unit volume. From the mass transfer coefficient correlations, it was evident that increasing the power input to the culture would only affect the volumetric mass transfer by $P^{0.23}$, while the cell death rate, based on the work of Croughan et al. (1987) will increase as $P^{0.75}$. For suspension cultures with power inputs lower than that causing cell death, increasing the power input could be an option for enhancing mass transfer. The $\bar{\varepsilon}_T$ for paddle impellers of D/T = 0.547 and 0.813 in a 500 ml vessel for a range of speeds up to 250 rev min^{-1} (Aunins et al., 1989) was significantly lower than the values estimated from literature correlations. This showed that scale-up ought to be carried out with more consideration given to the geometrical parameters of the system concerned.

In the above work, there is a trend that suggests cell damage is associated with high power inputs or high mean specific energy dissipation rates, $\bar{\varepsilon}_T$. Extensive work has been undertaken on particle suspension in standard stirred reactors to determine agitator types and tank geometries that enable this condition to be achieved with low values of mean specific energy dissipation rates, $(\bar{\varepsilon}_T)_{js}$ (Nienow, 1997a). However, such work has not been utilized for optimizing the geometry of stirred bioreactors for microcarrier culture. Therefore, it was decided to study the suspension behaviour of microcarriers in a PBS solution using impeller/tank geometries known to require low $(\bar{\varepsilon}_T)_{js}$ values. Thus, the agitator speed required to just fully suspend the particles, N_{js}, was observed and the power imparted into the stirred vessel, P_{js}, at this speed was also estimated as set out below. The impellers used were axially downward pumping Chemineer hydrofoil HE-3s of different diameter, one or a pair of Ekato Inter MIG impellers and a pitched six-blade mixed-flow turbine in a tank of flat and modified bases. At their optimal D/T ratios, these impellers have been shown to be efficient for the suspension of denser particles (Ibrahim and Nienow, 1996). Their performance in the microcarrier system would indicate the feasibility of using these geometries in shear-sensitive microcarrier cultures.

In addition, from the generic mixing perspective, most previous studies on solid suspension in stirred vessels have used particles significantly denser than the liquid in which they are suspended (typically Dr of at least 500 kg m23) (Zwietering, 1958; Ibrahim and Nienow, 1996). The most commonly accepted way of correlating the solid suspension characteristics of an impeller (Nienow, 1997a) is via the equation developed by Zwietering (1958):