# Abstract

Mammalian cells offer superior cellular machinery for the production of complex biological products. These cells provide proper post-translational processing machinery for recombinant protein expression to acquire the desired folding for optimal activity. With this advantage, mammalian cells have become the preferred choice for the production of biologicals, e.g. biotherapeutic proteins, monoclonal antibodies, and vaccines. These cells may grow either attached to a solid surface for their growth (adherent cells) or, where adapted, as suspension cultures. In order to grow these cells efficiently, a bioreactor is therefore required.

Bioreactors play a key role in the production of biologicals. Due to the continuous advancement of medicine and the healthcare industry, the demand for biological drugs has increased in the last three decades. This has placed a significant pressure on the biopharmaceutical industry to meet this increasing demand and has become a key driving force behind the need to develop better, safer and more economical bioreactor designs and culture processes.

Continuous stirred tank bioreactor is the norm for production of many bioproducts. However, in the context of growing mammalian cells, these bioreactors exert high shear forces to cells due to the impeller speed, bubble disruption, and foam formation. In addition, at a large scale, improper mass transfer impairs the performance of cell lines and achieving high cell densities and prolonged viability with correct glycosylation of a secreted proteins is still a challenge during scale-up.

Many cell lines for example Vero cells, are difficult cells to adapt into suspension cultures. Fixed bed bioreactors have been used for the propagation of such adherent cells for the production of biologicals. Fixed-bed bioreactors and the use of microcarriers provide an alternative platform for their growth. However, to achieve the high cell density requirements, a high surface area for the adherent cells are required, leading to an elevated cost of production (mainly from microcarriers) and ensuing a costly and technically challenging scaling-up of these systems. Other designs such as single-use bioreactors and novel bioreactors based on different mixing and mass transfer principles have been explored, but their utilization is limited from laboratory to pilot scale. Hence, a comprehensive design which would be suitable for a large variety of cell lines to produce high-yielding products with the lowest cost and risk in the shortest span of time is still sought.

In the current research, two approaches were investigated to address these challenges. Firstly, a horizontal tubular bioreactor (HTB) with a spiral impeller was designed and fabricated for the propagation of suspended mammalian cells with a focus to achieve cell density (≥ 5 x 106 cells mL-1) by improving mass transfer whilst reducing hydrodynamic shear and energy requirements through surface aeration.

The second approach is to test the adaptation of adherent Vero cells into single-cell suspension culture in serum-free media by treating them with an anti-cancer drug, Puromycin amino nucleoside (PAN). The absence of a supporting surface for cell growth (e.g. microcarriers) and serum-free conditions are expected to reduce the cost of manufacturing and to achieve higher productivity of biological production per unit volume of bioreactor.

In the first approach, the horizontal tubular vessel was designed to achieve the final volume of approximately 5.0 L. A key component of the bioreactor design was on the design of the impeller as this feature dictates mixing patterns and mass transfer. Different aspect ratios (geometric configurations) were used to design the spiral impeller by considering various parameters such as impeller diameter, the pitch of the blade, pitch angle, height of the blade, the thickness of the blade, clearance efficiency and the position of the heating element. Another important aspect of the prototype design was incorporating an external magnetically coupled motor drive which assisted in not only in aseptic handling but also reduction in mechanical stress and generation of fewer particles, which is important for cleanroom operations. The side plate was designed with the appropriate number of addition ports to allow execution of batches with minimum cross-contamination and for the ease of operation.

Thereafter, the engineering characterization of the HTB was carried out. The performance of the HTB was evaluated for (i) oxygen mass transfer **()** through the dynamic gassing-in method, (ii) mixing time and fluid flow by tracer and phenolphthalein method, (iii) minimum stirring speed **()** through alginate beads mimicking cell loading and modelling through modifying Zwietering equation, (iv) power consumption through heat calorimetry (temperature method) and (v) shear stress by determining specific death constant at different impeller speeds.

The general characterization profile of HTB has shown that at high agitation speed, homogeneity and mass transfer efficiency improved while power consumption increases with an increase in agitation speed. The bioreactor operated well at 2 L and 3 L capacity when the impeller is 40 - 90 % immersed in the liquid. The maximum mass transfer coefficient **()** of 16 h-1 was measured with a 3 L volume with an impeller speed of 500 rpm. These results are comparable with the other culture systems of the same scale.

The HTB was also tested for suitability to grow mammalian cells. Three batches were carried out, of which one was with the Chinese hamster ovary (CHO) cells expressing the somatic angiotensin-converting enzyme (sACE) and the two with plain CHO cells without expressing any recombinant protein. The maximum cell density achieved was of 5.48 x 106 cells mL-1 with plain CHO cells and 4.14 x 106 cells mL-1 with CHO cells expressing sACE with a maximum productivity of 465 mU mL-1. The specific death rate constant of 0.025 (h-1) was obtained when impeller speed was increase from 150 rpm (normal) to 300 rpm (induced shear) for 72 h.

In this study, CHO cells have been successfully adapted to suspension in serum-free conditions using the slow weaning of serum method and propagated in the HTB whereas Vero cells have been adapted successfully to serum-free media in adherent conditions. Attempt to suspend Vero cells based on literature using the weaning method remains timeous. Therefore, an alternative approach was explored using an anti-cancer drug (PAN) which is known to suppress the expression of integrin (cell adhesion receptors). The expectations from this approach was that the suppression of integrin would allow cells to detach and grow as a suspended culture (Krishnamurti et al., 2001). The results indicated that the anti-cancerous drug may have modulated the structure and function of the integrin which resulted in dislodging of the cells from the surface and form clumps which were viable for a week in suspension culture without increase in cell density. The viability of the cell clumps and few suspended cells were tested by re-seeding of these cells back to tissue culture (TC) flasks in serum-containing media without the presence of PAN. The culture in the TC flask regained confluency in the 2-3 day which confirms the viability of the cells and the likeliness of integrin re-modulating itself in the absence of PAN. As the suspended Vero cells did not grow, they were not tested for growth in the HTB.

To investigate the biological activity of these Vero cells, Isothermal microcalorimetry was used to evaluate the heat generation profile of the Vero cells quantitatively before and after drug treatment. The heat flow data (metabolic heat) from the treated and normal cells showed a distinct decrease in the heat generation profile which indicated that the treated cells were viable but not as active as the normal (non-treated) cells. It was evident from the heat flow data obtained for the PAN-treated Vero cells (-0.13 µW) from that of non-treated cells (13.12 µW) and thereafter when PAN-treated Vero cells regrown in serum-containing media, they regain their metabolic activities which were indicated by their heat flow values as positive control (9.30 µW), 100 µg mL-1 (10.12 µW), 200 µg mL-1 (10.18 µW), and 250 µg mL1 (9.15 µW).

It is recommended that more batches at different agitation speeds in combination with different airflow rate would further unravel the suitability of HTB for the growth of mammalian cells and stringently decode the optimum process conditions (operational window) to achieve high cell densities with extended longevity. Additionally, changes in the pitch of the impeller blades could result in the improved fluid flow profile, mixing and mass transfer while drawing low power input. Subsequently, different modes of operation, e.g. fed-batch or continuous operation are suggested to investigate the suitability of the HTB for integrity, sterility, and possible higher productivities and yields of products.

In suspending Vero cells, it is recommended that a thorough investigation of the drug-treated cell integrin profile is examined through fluorescence-activated cell sorting (FACS) which would give details of the inhibition of the different integrin subunits. This information could form the basis of adapting cell-lines into suspension in a single step, which is otherwise difficult to adapt.