Technical Note:

Flow Characterization of a Spinner Flask for Mouse Induced Pluripotent Stem Cell (iPSC) Culture Application.

Introduction

Since the development of pluripotent stem cells, such as embryonic stem (ES) cells and induced pluripotent stem cells (iPSC's), the focus of regenerative medicine has moved from organ transplantation to cell therapy. However, one of the major shortcomings of stem cell therapy is that a significant number of cells are required to provide an effective therapeutic treatment. Generating the number of cells required using static culture processes is not practical, so many researchers have focused their research on techniques to transfer static production to microcarrier based suspended cell culture processes in either spinner flasks or stirred tank bioreactors.

Spinner flask bioreactors are widely used in cell culture applications due to their widespread availability, ease of use, they can be easily adjusted to use with multiple cell types, and they provide homogeneous conditions for cell growth throughout the process and can produce a relatively high cell count. Most published studies have correlated the number of cells directly to the stirring rate of the spinner flask, this does not take into account the effects of flow dynamics and hydrodynamic forces on the ability of the spinner flask to grow cells.

Procedure

Fluorescent particles of a diameter of approx. 31um were seeded into 50mL of distilled water in a Bellco 100mL spinner flask. The impeller was driven by a top mounted stepped drive motor that was fully controllable through a motion controller. The fluorescent particles were illuminated by a Nd:YAG laser and the effects on the dispersion of the particles in the liquid column by the rotation of the impeller were recorded by a high-speed camera.

Three areas of flow were studied

- Flow profile in the meridional plane
- Flow profile in the azimuthal plane
- Fluid interaction at the bottom surface of the spinner flask

Rotational speed of the impeller was varied between 20rpm and 45rpm, in 5rpm increments, with 6 measurements being taken at each rotational speed

- 1 measurement in the meridional plane
- 3 measurements at 3 different heights in the azimuthal plane
- 2 measurements near the bottom wall in the azimuthal plane.

A parallel study was conducted to measure the effect of hydrodynamic force on live mouse OG2 iPS cells attached to Cytodex 3 microcarriers grown at 37°C, with 5% CO_2 for 7 days with 50% of the culture medium replaced daily. 3 replicates were undertaken at each of the agitation speeds.

Results

<u>Flow profile in the meridional plane</u>

- As the impeller spins fluid accelerates radially outwards to wards the side wall of the flask.
- The fluid then travels downwards to the bottom wall of the vessel before traveling inwards and then upwards in the center of the flask producing a global clockwise flow

- At each spin rate the same flow pattern is observed with the flow achieved being maximum near the bottom wall and the surrounding impeller area.
- At low spin rates the fluid circulates smoothly from the bottom of the flask upwards.
- At high spin rates the fluid is being pushed downwards, producing a much more abrupt change in direction at the side wall/bottom wall junction, with a corresponding higher vertical velocity in the center of the rotation.
- The vertical velocity provides lift for the microcarriers to keep them in suspension throughout the culture procedure
- The level of vortex development, vorticity, also increases as rotational speed is increased. The level of vorticity is highest in the region immediately behind the flat impeller during rotation.
- The level of shear is highest in the region close to the bottom of the flask, with noticeable shear around the vortex

<u>Flow profile in the azimuthal plane</u>

- Azimuthal flow is the primary velocity component in the spinner flask with floe rotating like a solid body rotation.
- There is a thin boundary layer around the sidewall of the flask where flow slows due to friction with the side wall.
- The rotating impeller provides the driving force for flow and therefore the regio around the impeller has the highest velocity.
- Flow is more homogeneous the further away for the impeller measurements are taken.
- Shear stress generated depends on the velocity of the liquid column, with side wall shear being lowest at the lowest speed tested and increasing as the rotational speed increased.
- The sharp edges of the impeller provide both a strong force to agitate the fluid but also produce a significant vortex gradient, which translates to high shear.

<u>Mouse iPS cell culture</u>

- Cells cultured at a rotational speed at or higher than 28rpm exhibited significantly lower normalized cell densities than cells grown in static culture.
- Mouse iPS cells are very susceptible to the negative impacts of shear
- Cytodex 3 microcarriers have a very similar density to water, are neutrally buoyant and follow the fluid flow in the flask.
- Declining cell count with increased speed shows that mouse iPS cells are not able to maintain secure attachment to the microcarriers at the higher level of shear stress associated with higher rotational speeds.
- The highest cell count was achieved at 25rpm. This cell count represented a 20-25% increase over the cell count obtained in the static culture control.
- At 25rpm the cells were also able to maintain their pluripotency properties which is critical for larger scale iPS culture development.
- Reducing the rotational speed too far could have the effect of reducing the mixing effect which would have counter-intuitive effects on the ability of microcarrier based cells to remain in suspension.

Conclusions

- Optimal mouse OG2 iPS cell growth was observed at a rotational impeller speed of 25rpm
- Higher shear stress at higher rotational speeds had a significant and negative impact on cell growth and cell viability.
- Speeds below 25rpm were not able to maintain a homogeneous suspension of Cytodex 3 microcarriers.

- Optimal conditions for other cell lines or using different microcarriers would need to be defined through experimental procedures.2025
- Understanding the flow mechanics of the culture vessel being used would allow the required culture conditions to be more easily transferred to different types of culture vessels.
- The narrow range of spin rates within which there was optimal cell growth demonstrates that great care must be taken in ensuring the impeller spins at an accurate rate in the cell culture process.
- This relates directly to the accuracy of the drive system used, either a top mounted direct drive motor, or a bottom mounted magnetic stir plate. Whichever ius used it should be routinely calibrated for accuracy.

<u>References.</u>

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