Technical Note:

Key Points to Consider in Spinner Flask Cell Culture

Introduction
Culturing cell growth in spinner flasks represents a crucial process that is part of a range of cell culture methodologies available to research and production scientists. Spinner flasks are used in growing suspension cells, anchorage dependent cells that have been adapted to suspension growth, and adherent cells on microcarriers on a microcarrier substrate. This guide explores optimal techniques for successful spinner flask culture implementation.

Mixing Speed
One of the critical attributes of successful spinner flask culture lies in identifying and maintaining the optimal rotation speed of the mixing element. Typically, this will range from 100 to 250 revolutions per minute (rpm). This precise range is crucial for cells like HEK293 suspension-adapted cells, as it ensures efficient nutrient distribution while simultaneously preventing excessive shear stresses that can damage cellular integrity. The specific speed within this range must be calibrated based on the individual cell line's characteristics and density, with more robust cell lines, such as HeLa S3 cells, being able to tolerate higher speeds within this range.

Cell Types
Spinner flask cultivation is well suited for both suspension cell lines, modified adherent cell lines, and adherent cells that are adapted to grow on microcarriers, as the system's design allows for optimal cell density, distribution and nutrient access throughout the culture medium.

Naturally suspended cell lines such as Chinese Hamster Ovary cells (CHO), Human Embryonic Kidney cells (HEK), insect cells such as Sf9 and Sf21, U937 cells and K562 cells, have all been extensively documented to grow extremely well in suspension cell culture, both spinner flasks and bioreactors. Many traditionally adherent cell lines have been successfully adapted for suspension growth in spinner flasks. Notable examples include HeLa S3 Cells and HEK293 suspension-adapted cells, which have been specifically modified to grow in suspension conditions. These adapted lines maintain their characteristic properties while benefiting from the scalability and efficiency of suspension culture, making them invaluable for large-scale protein production and biotechnology applications.

Numerous scientific papers have also been published demonstrating the efficient growth of shear sensitive human mesenchymal stem cells on microcarriers in spinner flasks (1), (2). Therefore, demonstrating the suitability of spinner flasks for even highly shear sensitive cell culture applications.

Design
The typical spinner flask design of a central stirrer shaft with attached impeller, and strategically positioned side arms, form the basis of a successful suspension culture platform. The stirrer shaft/impeller assembly can be driven either by a bottom mounted magnetic mixer, or for larger assemblies a top mounted direct overhead drive unit. Both mixing options provide gentle and continuous agitation while also ensuring uniform suspension and preventing cell aggregation.

The position and number of side arms on the spinner flask, and the range of port options available from the manufacturer, serve as critical access points allowing for efficient media replacement, cell addition, media addition, aeration, (including CO_2 enriched air when required) monitoring and sampling, while maintaining sterile conditions.

Spinner Flask Mixing Element Selection.

There are many different designs of mixing elements available, and selection of the appropriate mixing element is sometimes based on previous experience, or it can be based on previously published information, and sometimes on manufacturer's recommendations depending on the cell line being used and the product being expressed. Some mixing elements can only be used with a magnetic drive unit and some only with a top mounted drive system.

Scale Up

Scaling up a process from a small spinner flask to larger spinner flask volume is a relatively straight forward process. However, scaling up from a spinner flask to a stirred tank bioreactor can present a significant challenge because of the very different hydrodynamic properties of both types of systems.

Spinner flasks typically use large impellers that efficiently mix a high volume of culture media at low revolution rates per minute (rpm). In contrast stirred tank bioreactors have smaller impellers compared to the media volume in the system, and as such higher rpms are required to provide adequate mixing. Higher mixing speed equates to higher speeds generated at the edge of the impeller that is furthest from the central shaft, also called tip speed. Higher tip speed may create higher mixing forces within the culture media which result in higher shear forces on suspended cells, and the potential to dislodge adherent cells from microcarriers because of the more turbulent mixing conditions.

Conclusions

Spinner flask-based cell culture remains a cornerstone technique in modern cell culture applications, offering scalable solutions for growth of both suspension and adherent cells. When properly implemented with appropriate selection of the mixing element, optimal stirring speeds, and suitable material selection, this method provides exceptional results for both native suspension cells, adapted lines like HeLa S3 cells and microcarrier based cultures. Understanding and optimizing these key parameters ensures successful outcomes in your cell culture applications.

References

1. Zhang, B. et al. Enhancing mesenchymal stem cells cultivated on microcarrier supports via impeller design optimization for aggregated suspension. *Bioresour. Bioprocess* **(2023)** 10 (1): 89.
2. Jeske, J. et al. Agitation in a microcarrier based spinner flask bioreactor mediating homeostasis of human mesenchymal stem cells. *Biochem. Eng. J.* **(2021)**. 168: 107947.

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