Cell Culture Scale Up Utilizing Disposable Spinner Flasks

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Introduction

Background

A majority of approved and developing biotherapeutics are ultimately produced in large stainless steel stirred tank bioreactors. It has been known for some time that recapitulation of the growth conditions of these very large scale bioreactors early in the process speeds process development. Historically this has been done in glass spinner flasks. More recently users have switched to disposable shaker flasks for their convenience and reproducibility. However the mechanism of agitation is unlike stirred tanks and they are not easily monitored and controlled. Therefore we compared several new disposable spinner flasks to traditional glass spinner flasks. We demonstrate that a polystyrene based disposable spinner flask performed as well as, and sometimes better than, traditional glass spinner vessels. Importantly, the design of the polystyrene flask allowed for the direct inoculation of cryopreserved cells into the flask. This eliminated several scale up steps as well as reduced the time to high density growth. Finally, this design was amenable to on-line monitoring and controlling, greatly increasing it's utility over other disposable vessels.

Corning® Disposable Spinner Flasks:

Advantages:

- ❖ Gamma sterilized
- ❖ Double bagged for use in GMP conditions
- ❖ No need for siliconization

Parameter	125mL	500mL
Nominal Volume	125 mL	500 mL
Vessel Height (in/mm)	5.7 / 145	8.0 / 203.2
Diameter (in/mm)	2.5 / 63.5	3.4 / 87.3
Footprint (in/mm)	4.4 /114.8	5.5 /139.7
Center Cap Diameter	70 mm	100 mm
Sidearm Opening (in/mm)	0.74 / 18.8	1.5 /38.1
Sidearm Cap	25 mm GL 25	45 mm GL 45
Paddle Height (in/mm)	1.97 / 50.0	2.4 / 61.0
Width (in/mm)	1.57 / 39.9	1.98 / 50.3
Minimum Drive Coupling	2 rpm	2 rpm
ALINCO Magnet	12,800 Gauss induction	12,800 Gauss induction

Materials & Methods

Materials

Cells: Serum free adapted CHO-S cells (Gibco, part# 11619-012) were thawed according to manufacturer's instructions.

Vessels: Corning® disposable spinner flasks (Cat # 3152 and 3153) and Bellco glass spinners (Cat # 1965-00250 and 1965-02500) were used in these studies. Corning® SS4L slow speed stirrer, Cat. # 440814, was used for rotation in all studies.

Methods

Thaw from cryopreservation: Frozen vials of serum free adapted CHO-S cells were thawed and placed into pre-warmed CD CHO media (Gibco, part # 10743-029) supplemented with 1% HT solution (Gibco, part#11867-030) and 8mM L-glutamine (complete medium). Viable cell concentration was determined via trypan blue exclusion and dilutions made to seed a T75 flask and a 125 ml disposable spinner flask at a concentration of 2.0 x10^5 viable cells/ml. Cultures were incubated at 37°C and 5% CO_2. Spinner flask cultures were placed on a magnetic stir plate set to 60 rpm. Daily counts of both vessels were taken until a cell concentration of 1.0 x10^6 cell/ml was achieved.

Spinner Flask Cultures: Cells from plastic spinners started from thaw (above) were seeded into flasks at 2x10^5 of pre-warmed complete CD CHO media. Cultures were incubated at 37°C and 5% CO_2, spinner flasks were placed on magnetic stir plates set to 60 or 120 rpm, where applicable. Daily counts from each flask were taken until cultures reached stationary phase.

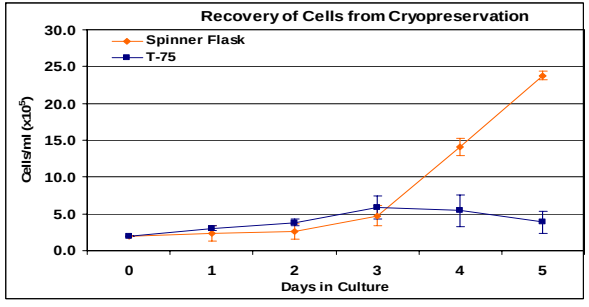

Figure 1: Thaw of CHO-S cells directly into disposable spinner flasks. Frozen vials (1.5 x 10^7 cells) of CHO-S cells were rapidly thawed. Equal amounts of cells per ml were placed in each vessel. Counts are the average ± S.D. from 3 independent experiments.

Results

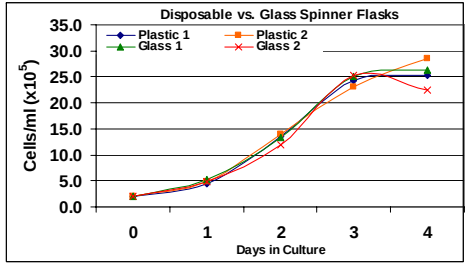

Figure 2: Cell Growth Comparison between Plastic Disposable and Glass Spinner Flasks. Equal numbers of cells were seeded into each vessel and stirred at 60 rpm until the completion of the growth period. Cell counts were taken each day. Representative experiment shown.

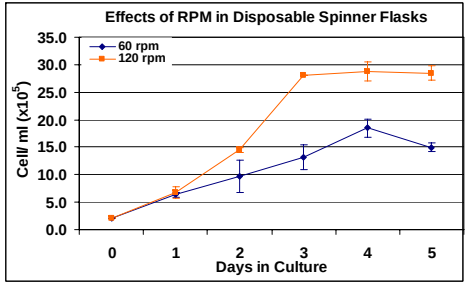

Figure 3: Cell Growth Comparison in Disposable Plastic Spinner Flasks. Equal numbers of cells were seeded into each vessel and stirred at 60 and 120 rpm until the completion of the growth period. Cell counts were taken each day. Representative experiment shown.

Conclusions:

- **Disposable plastic spinner flasks can be used to inoculate directly from frozen vials.**
- **Disposable plastic spinner flasks show equal growth performance to traditional glass spinner flasks.**
- **Disposable plastic spinner flasks can sustain growth at 120 rpm for extended culture periods.**
- **Growth in disposable spinner flasks can be easily scaled from small scale to large scale.**