A Novel 10 Layer Flask For Use In The SelecT Automated Cell Culture System

Todd Upton PhD, Ana Maria P. Pardo, Hilary A. Sherman, Corning Incorporated, Kennebunk ME; Manuel Pomeranz, Stacey L. Szymanski, Kevin W. Huff, Alison Rush PhD, Merck Research Labs, No. Wales PA; Matt Golding, Alan Platt, Stephen Guy, Frank Tully, The Automation Partnership, Royston UK

Introduction

Abstract

The need for large quantities of cells to satisfy the demands of high throughput and ultra-high throughput screening continues to grow as more organizations continue to expand the use of cell based assays. As with all the reagents in a cell based screen, the cells used must be routinely available as well as consistent in their performance from screen to screen. Many users have found that the use of automated cell culture systems improves the availability of cells to include nights and weekends while improving the consistency of the cellular response in their assay system. To meet the growing demands of cells, we set out to design a novel cell culture vessel that would achieve a log scale increase in cell yield from a traditional T175 flask which could be used either manually or in automation. The flask, called the HYPERFlask™ cell culture vessel, utilizes a gas permeable layer to grow cells on 10 growth layers within the footprint of a T175. We demonstrate that the flask can be uniformly seeded, cultured and harvested utilizing protocols on the SelecT automated cell culture system from The Automation Partnership (TAP). Importantly, cell densities per unit growth area are similar to traditional single and multilayer vessels resulting in approximately ten times the cell yield as traditional cell culture vessels in the same footprint. Further, viabilities of cells harvested from the HYPERFlask vessel are identical to those from other vessels. Our data show that large quantities of cells can be grown effectively and reproducibly utilizing the HYPERFlask vessel and the SelecT automated cell culture system.

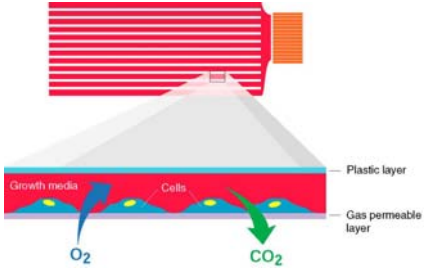

Figure 1: Corning® HYPERFlask Cell Culture Vessel Principal of Operation. Ten equivalent layers each containing the same surface area of a gas permeable material are joined together to form a multi-layered cell culture vessel. Cells attach and grow on the gas permeable material with gas exchange occurring through this layer. All fluid manipulations occur through the single neck which is contiguous with all layers.

Materials & Methods

Materials

Cells: CHO-K1 were from ATCC and used for these studies.

Media: IMDM was from Invitrogen. Fetal bovine serum (Invitrogen) was used at 10% in all cultures.

Vessels: The new Corning HYPERFlask cell culture vessel and a commercially available triple layer flask were used in these studies

Methods

Growth Studies: Cells were seeded between 20,000-30,000 cells/cm^2, depending on culture duration and allowed to grow for 72-96 hours at which time the cultures were harvested. Cultures were harvested using Accutase (Innovative Cell Technologies). Cells were counted on the SelecT. For each flask type processes for seeding and harvesting were first optimized individually whereupon the comparative experiments were then run. Additional moves specific to the HYPERFlask vessel were added to the standard operation modes for standard T175 and three layer flask protocols.

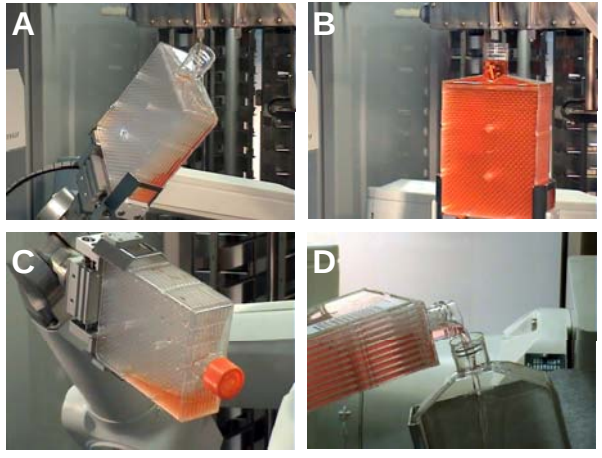

Figure 2: The Corning HYPERFlask vessel In Use In the TAP SelecT. Photo representation of the HYPERFlask vessel at various positions in the SelecT automated cell culture work station. A) Filling operation at the cocktail bar, B) HYPERFlask vessel after filling is complete, C) Inoculum equilibration prior to filling and, D) Pour off of cell supernatant into a collection vessel.

Results and Conclusion

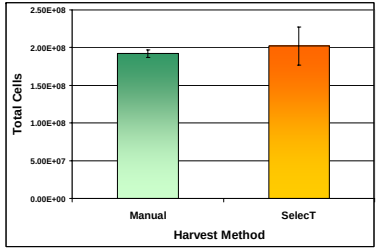

Figure 3: Yield of Cells is Equivalent in Manual and Automated Processes. CHO-K1 cells were seeded and harvested either manually or via the SelecT. Bars represent the average ± std error of 3 vessels per condition.

Vessel	Seed time	Harvest time
T175	4 min	31 min
Three Layer Flask	4.5 min	35 min
HYPERFlask vessel	7 min	25 min

Table 1: Greater Throughput Achieved With the Corning HYPERFlask Cell Culture Vessel. Seed and harvest times were determined from optimized processes developed during these studies.

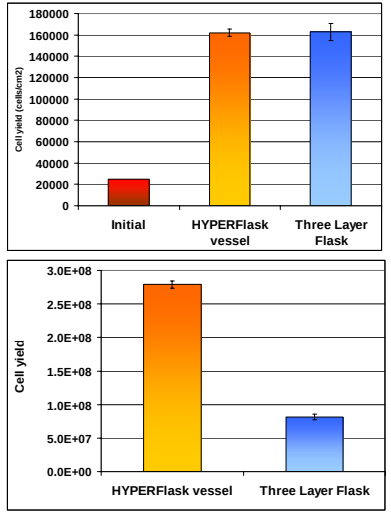

Figure 4: Increased Yield and Throughput of SelecT With the Corning HYPERFlask vessel. HYPERFlask vessels or three layer flasks were seeded and harvested using the SelecT and optimized processes for both vessels. A) Cell yield per cm^2 of each vessel B) Total cell yield from each vessel.

Conclusions:

- **The new Corning HYPERFlask vessel is designed and optimized to increase cell yield using the SelecT Automated Cell Culture System**
- **Harvesting time for the HYPERFlask vessel is 33% less than three layer flasks and 20% less than standard T175 flasks**
- **Cell yield from Corning HYPERFlask vessels are 3.4 times greater than three layer flasks and 10 fold greater than T175 flasks**
- **Cell yield from HYPERFlask vessels are equal for both manual and automated operations**

Acknowledgements: The authors recognize the contributions of Julie Kerby PhD, and Matt Lethridge