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A Novel Flask Design for High Density Cell Culture

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Introduction

Abstract

The need for large quantities of cells for HTS cell-based assays continues to motivate organizations to look for methods for achieving larger cell numbers with minimal investment. In order to provide a solution it is imperative that the cells generated using such methods exhibit similar characteristics such as growth kinetics and response to stimuli. Therefore, we developed a novel cell culture flask, called the HYPERFlask™ cell culture vessel that has the same overall dimensions as a standard T175 flask but grows approximately 10 times as many cells. We demonstrate that a wide variety of cell types, both cell lines and primary, are able to grow to high densities in this novel cell culture vessel. Further, the cells grown in these flasks are identical to cells grown in conventional flasks for viability, cell cycle distribution, metabolic activity, growth rate and, most importantly, response to stimuli in cell based assays. These results show the HYPERFlask vessel is capable of expanding existing cell culture capacities ~10 fold.

Corning® HYPERFlask Cell Culture Vessel Advantages:

- Greater consistency of cells and assays
- Reduced processing and handling time
- 10 fold reduction in space utilization and disposal requirements

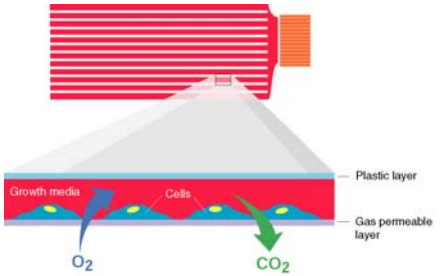

Figure 1: Corning® HYPERFlask Cell Culture Vessel Principal of Operation. Ten equivalent layers each containing the same surface area of a gas permeable material are joined together to form a multi-layered cell culture vessel. Cells attach and grow on the gas permeable material with gas exchange occurring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

Materials & Methods/Results

Materials

Cells: CHO-K1, Vero, HT1080 and M1WT2 cells were from ATCC. Normal human dermal fibroblasts were from Cambrex and HEK Cre-Luc were purchased from Panomics.

Media: Either IMDM or Ham's F12 from Invitrogen were used as basal medias. Fetal bovine serum (Invitrogen) at 10% was used in all cultures.

Vessels: T175 with vent caps were used as comparison to the HYPERFlask cell culture vessel and a competitor three layer flask.

Methods

Growth Studies: Cells were seeded between 2,500 -10,000 cells/cm^2, depending on culture duration and allowed to grow for 72-96 hours at which time the cultures were ~90% confluent. Cultures were harvested using HyQ®Tase (Hyclone), trypsin or PBS. Cells were counted either by hemocytometer, Coulter Counter Z2, or Guava Easycyte mini. For cell cycle analysis Guava cell cycle reagent was used and processed according to manufacturer's instructions. All experiments were conducted at least three times with three replicates per condition.

Signaling studies: Cells were plated at 2,500 cells/well into 384 well assay plates and cultured for 24 hr. Utilizing FLIPR® calcium 3 and steadylite HTS gene reporter assays, plates were processed following manufacturers' instructions. Plates were read on either LJL Analyst or FlexStation II 384 analyzers. Each assay was performed at least three times with at least six replicates per condition.

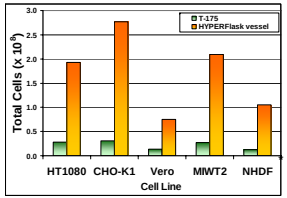

Figure 2: The HYPERFlask Cell Culture Vessel Can Be Used for a Variety of Cell Types. Each cell type was tested for cell growth in comparison to a standard T175. Each vessel was seeded with equivalent cells/cm^2 and cultured until the T175 reached ~90% confluence.
* For clarity values for Normal Human Dermal Fibroblasts (NHDF) are 10^7.

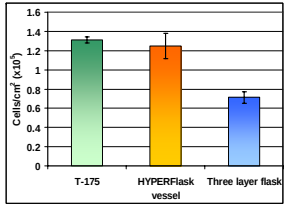

Figure 3: Cells Grow to Higher Density in the HYPERFlask Cell Culture Vessel than Other Multilayer Flasks. CHO-K1 cells were seeded at equal densities and grown for 96 hr in each vessel. Each layer of the vessels was individually harvested, cells enumerated and averaged together. There is a statistically significant greater density of cells in the Corning HYPERFlask vessel as compared to the three layer vessel ($p \leq 0.001$).

Results and Conclusion

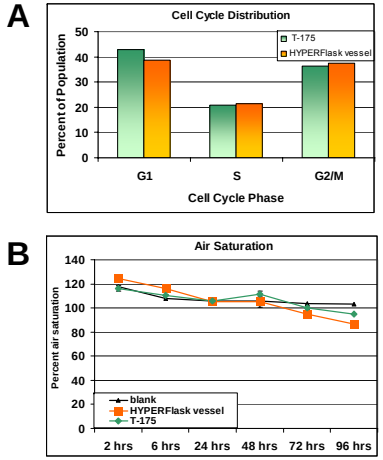

Figure 4: Cells Grown in the HYPERFlask Cell Culture Vessel Have the Same Growth Characteristics as Cells Grown in Traditional T-Flasks. CHO-K1 cells were grown in the indicated flasks and analyzed for cell cycle distribution (A). Additionally, cultures were monitored for metabolic activity using a Nova BioProfile™ 400 analyzer (B). An example for air saturation is shown. Similar results were seen for glucose, pH, lactate, and CO_2 amongst other metabolites (data not shown).

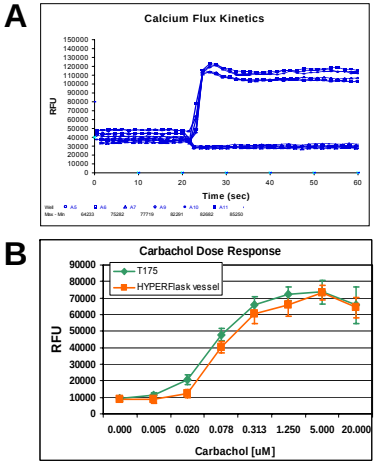

Figure 5: Cells Grown in the HYPERFlask Cell Culture Vessel in a Calcium Flux Cell Based Assay. Comparison of calcium flux kinetics (A) in M1WT2 cells grown in the HYPERFlask (A5-7) and in T175 flasks (A9-11), using the Calcium 3 kit, performed on the FlexStation (Molecular Devices, Inc.). Similar kinetics of Ca^{++} flux were observed from cells grown in either vessel. Comparison of the 4-parameter logistic curves (B) reveal similar dose response curves and EC50s.

Conclusions:

- **The new Corning HYPERFlask vessel is capable of growing a wide variety of cell types**
- **Cells grown in the HYPERFlask vessel have an identical cell cycle profile and growth characteristics as those grown in traditional T-flasks**
- **Cells grown in the HYPERFlask vessel have the same metabolic kinetics as cells grown in traditional T-flasks**
- **Assays performed with cells grown in the HYPERFlask vessel show similar dose response, sensitivities and kinetics as those from traditional T-flasks**