

3D Automated Cell Culture Using Existing Hamilton Platform Technologies

Overview

- Cultured cells are critical for drug discovery and biotechnology applications. Automated cell culture reduces cost and assay failure rates.
- The 3D CellHOST™ is a scalable, automated 3D culture system suitable for many diverse cell types.
- The 3D CellHOST cultured CHO cells to significant numbers with little operator oversight, no contamination, and 96% viability.

Introduction

As the use of high content screening increases, so does the need for more physiologically relevant cells. Automated 3D cell culture systems will produce these cells. The 3D CellHOST uses the GEM™, a magnetic alginate microcarrier, to perform 3D cell culture with existing Hamilton liquid handling technologies. This system:

1. Promotes *in-vivo* cell phenotypes
2. Offers small volume high yield culture
3. Is compatible with current automated technologies

Materials & Methods

- Global Eukaryotic Microcarrier (GEM) – Global Cell Solutions, Inc (GCS), Charlottesville, VA
- GCS BioLevigator™; an impeller free bioreactor
- Hamilton STARlet™ liquid handling platform – Hamilton Bonaduz AG, Bonaduz, Switzerland
- Cell Culture Media
- Chinese Hamster Ovary Cells (CHO)
- New Brunswick Scientific NucleoCounter™

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1: Global Cell Solutions, Inc, Charlottesville, VA; 2: Hamilton Bonaduz AG, Bonaduz, Switzerland

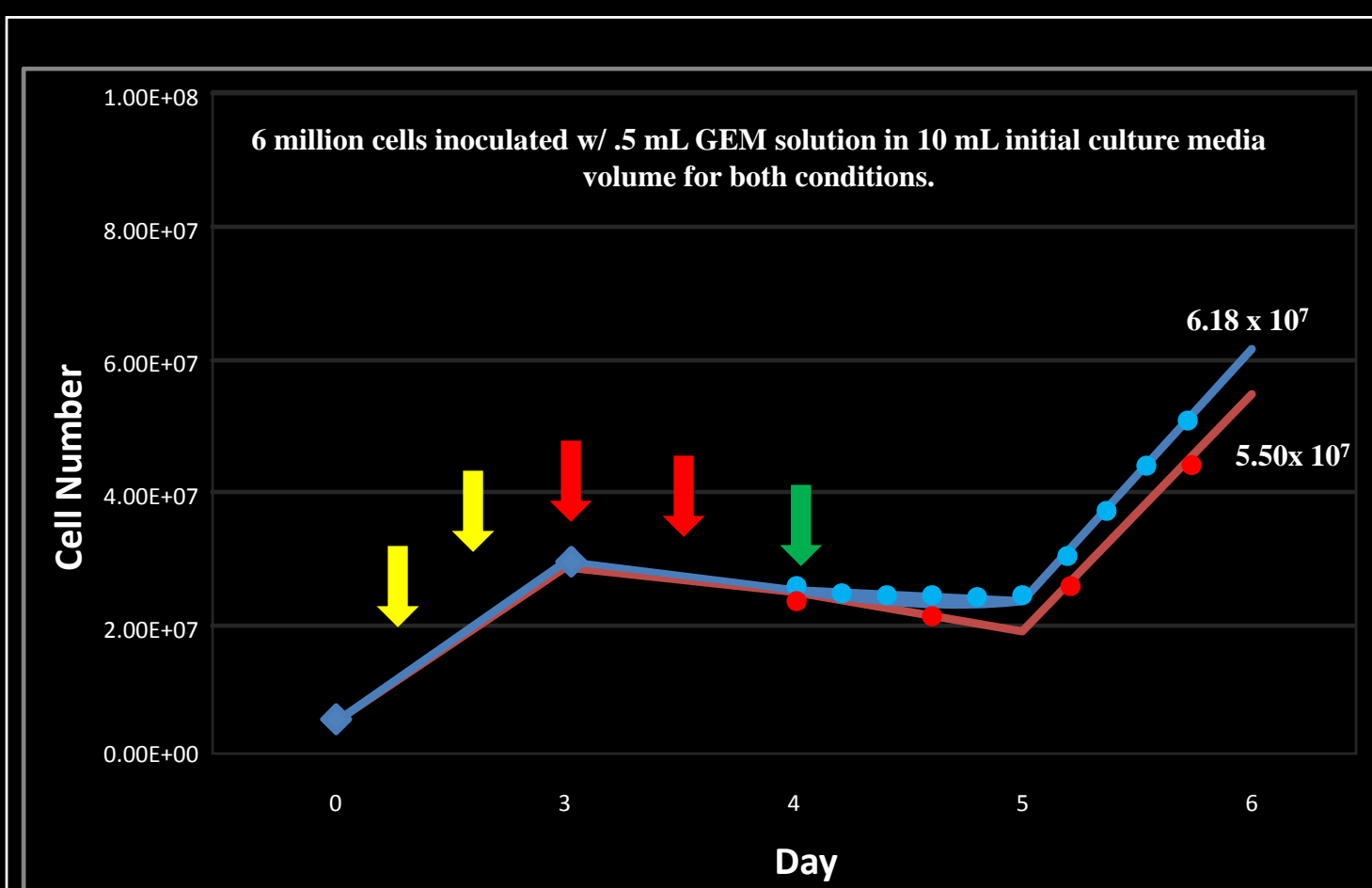


Fig. 1: CHO Culture

CHO cultures were expanded in the 3D CellHOST. Yellow arrows signify addition of 10 mL media to each culture, red the exchange of 15 mL media from both cultures, and green the start of different frequencies of 15 mL media exchange to every 4h (blue) or 12h (red).

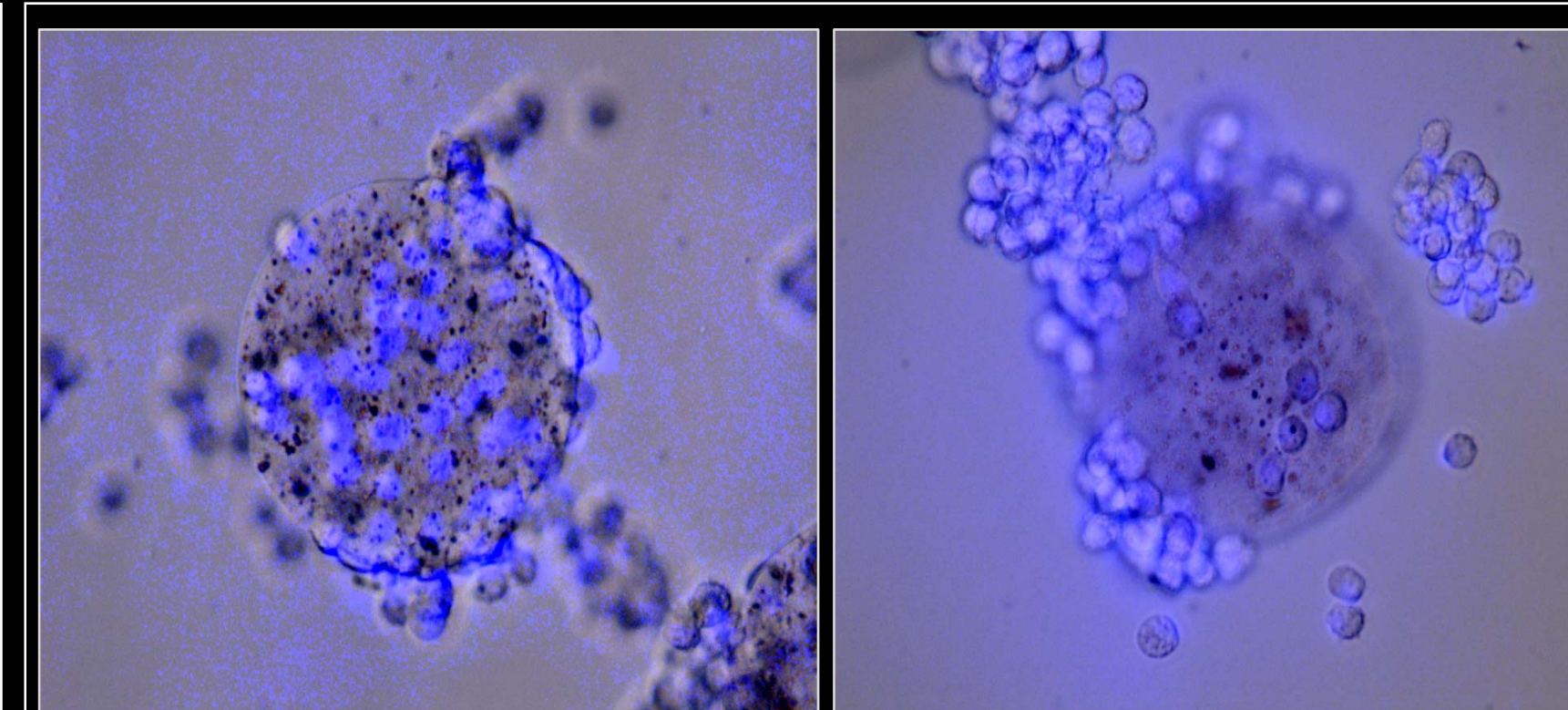


Fig. 2: Hoechst stain of CHO cells growing on GEMs

Results

- Cell growth is dependent on media exchange frequency.
- The 3D CellHOST can automate 3D cell culture, trypsinization and release of cells from GEMs, and dispensing of cells for assays.
- The 3D CellHOST can run autonomously for an extended period, reduce overhead costs, and save time for lab personnel.

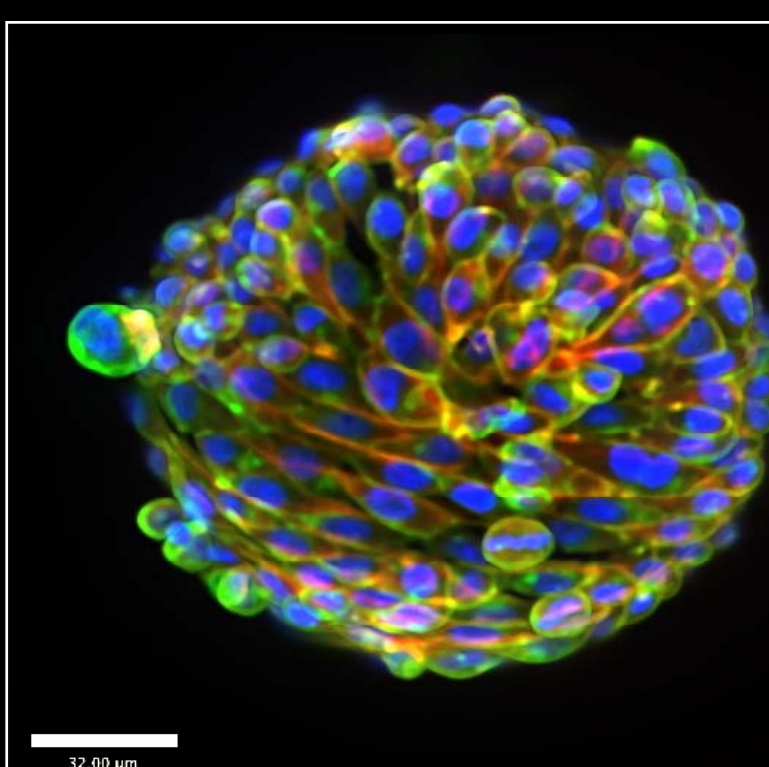


Fig. 3: CHO cells growing on the GEM

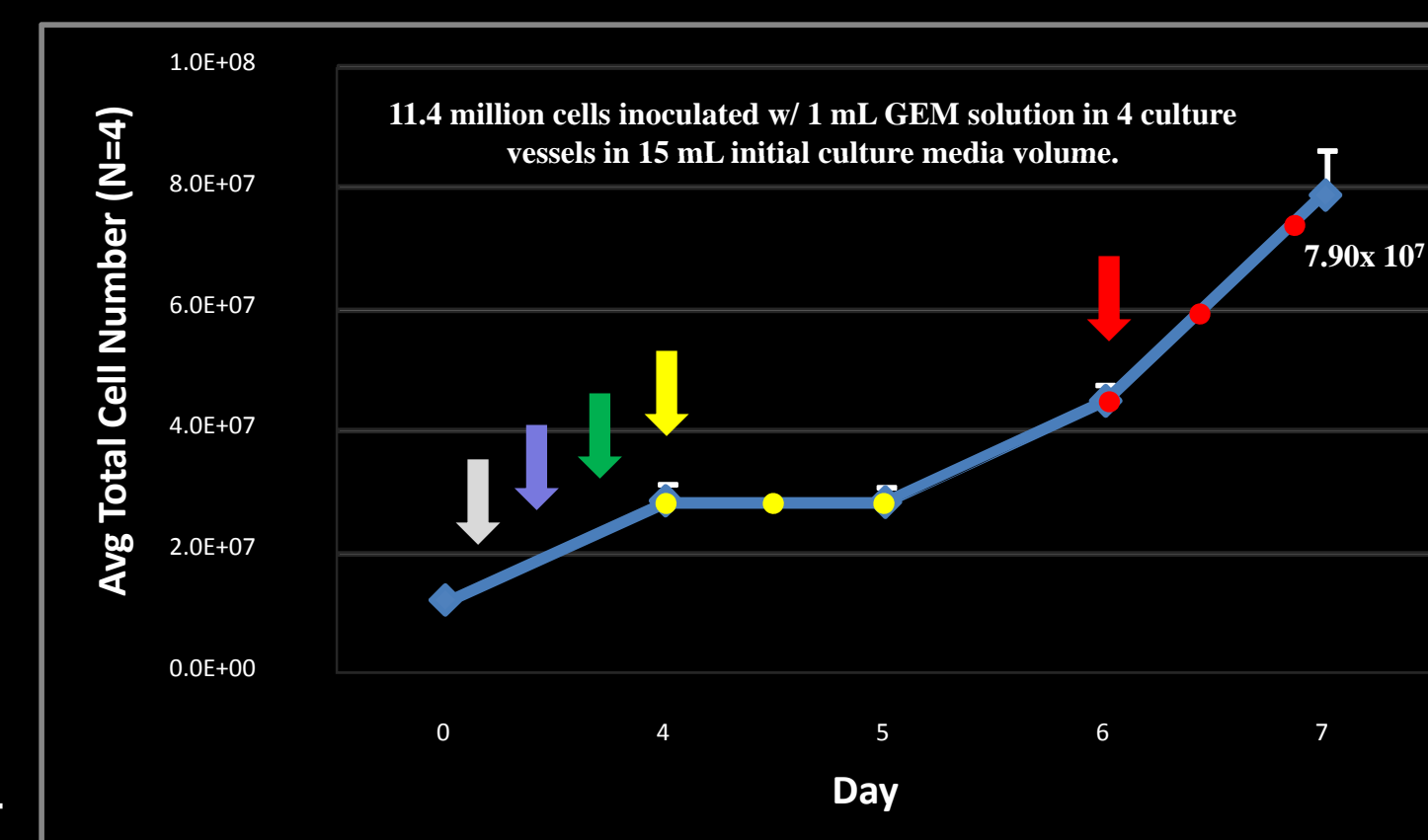
Fluorescent stain of DAPI (blue), wheat germ agglutinin (green), and phalloidin (red). The GEM is non-autofluorescent.

Fig. 4: STARlet manipulations for culture media exchange



Fig. 6: Automated Expansion

CHO cells from Fig. 1 were resuspended and expanded to yield 317 million cells with 96% viability. Grey arrow: 5 mL media addition; Blue: 10 mL media addition; Green: 11 mL media removal, 19 mL addition; Yellow: start of three 19 mL (half volume) media exchanges, spaced 8 hours apart; Red: start of three 10 mL media exchanges, 8 hours apart.



Conclusions

- The magnetic GEM permits precise control of cell position, and is thus uniquely suited for automating 3D cell culture.
- Protocols designed for a bench top system were easily automated.
- Media replenishment can be optimized to positively affect cell growth rate.
- Automated culture reduces contamination and frees valuable operator time.