An Emerging Revolution in Automated 3D Cell Culture

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Overview

- Uniquely engineered spherical microcarriers
- 150 – 300 µm in diameter
- Embedded paramagnetic particles manipulated by magnetic field
- Improved transfection
- Morphological control
- High survivability following cryopreservation
- Useful for high-throughput screening

Introduction

- Market pressure exists for new paradigms to meet the demands for more rapid and reproducible cell production to keep up with advances in cell-based assays used in drug discovery and development
- Novel automation platforms enable high productivity on three dimensional surfaces

Methods

- Global Eukaryotic Microcarrier (GEM)
- HeLa, HEK, and smooth muscle cells
- The Automated Partnership SelecT™
- New Brunswick Scientific Instruments NucleoCounter®
- Yellow Springs Instruments (YSI) 2700 SELECT™ Biochemistry Analyzer
- Nikon Eclipse T300 & TE 2000-E
- Kalypsys Systems high-throughput screening robot
- PerkinElmer® ViewLux™
- Amaxa Biosystems 96-Well Shuttle

Results

Transfection

- GFP expression was uniform and in similar concentrations in cells on GEMs as compared to conventional transfection (Figure 1)
- HeLa cells stayed on the microcarriers after the electroporation procedure

Morphological control

- 3D surface on GEMs resulted in even confluent growth (Figure 2) as compared to clustering effect on 2D plate
- Smooth muscle cells wrapped around GEMs (Figure 3A)
- HEK cells more columnar than those grown on a tissue culture flask (Figure 3B)

Cryopreservation

- HeLa cells were cryogenically preserved on microcarriers for three weeks
- Forskolin stimulated cAMP dose response showed similar functionality after thaw and recovery (Figure 4)

High-throughput screening

- GEMs covered with cells were readily dispensed from a Kalypsys Systems kinetic straight-tip bottle-fed dispensing robot into the wells (Figure 5)

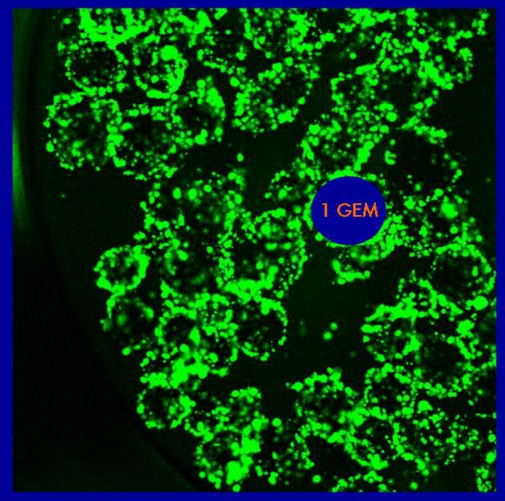


Figure 1 (above): Transfection of HeLa cells to express green fluorescent protein (GFP)
- Image taken at 24 hours using a confocal Nikon Edipse T300 with argon ion laser hookup at 40X and 7.40 gain

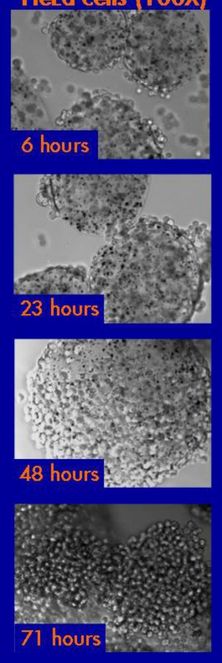


Figure 2 (right): Growth kinetics of HeLa cells over 3 day period. Images taken with Nikon Eclipse TE2000-E at 100X

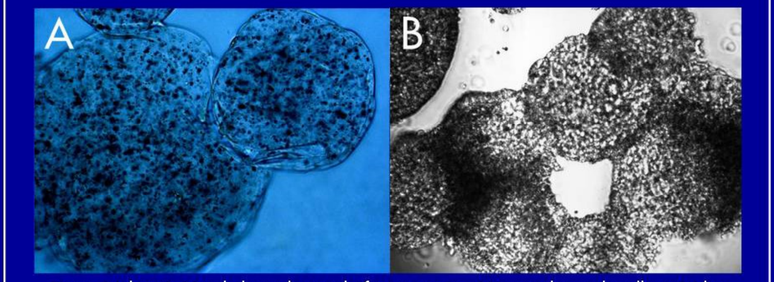


Figure 3 (above): Morphological control of microcarriers (A) smooth muscle cells at 24 hrs with blue filter (B) HEK cells at 5 days. Images taken with Nikon Eclipse TE2000-E at 40X

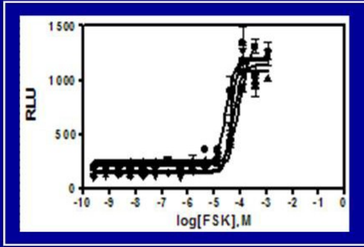
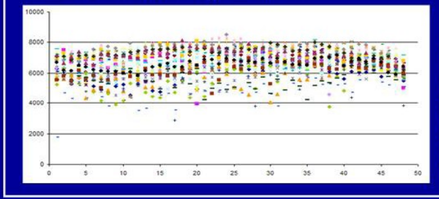
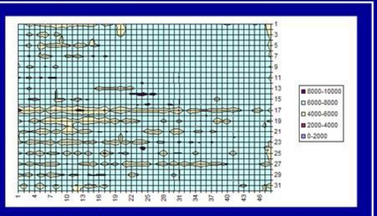


Figure 4:
Recovery following cryopreservation and pharmaceutical response
- ■ Cells frozen by traditional means
- ▲ Fresh cells
- ▼ Cells frozen on microcarriers at 50% confluence
- ● Cells frozen on microcarriers at 60% confluence
- ♦ Cells frozen on microcarriers at 90% confluence

No significant difference was observed between the five conditions

Figure 5:
High-throughput screening robotic dispense on to a 1536-well microplate
- The Cell Titer Glo® confirmation showed fluorescence that was measured by a PerkinElmer® ViewLux™
- Relative number of viable cells undergoing metabolism revealed by test for ATP





Vital statistics from heat map (above) and scatter plot (left):
- Mean: 6612.8 RLU/well
- Standard Deviation: 869.6 RLU/well
- Coefficient of Variation: 13.15 RLU/well
- Data suggests even cell distribution in plate

Conclusions

- Magnetic microcarrier culture offered improvements in yield, functionality, and convenience for all cells tested
- Cells grew to confluence in three days or fewer, averaging 750 cells/GEM
- Microcarrier-based cell manipulation was efficient for electroporation, robotic dispensing, and cryopreservation
- The convenience of microcarrier-based culture provided functional convenience of being able to pipette cells directly into a 1536-well microplate used in high-throughput screens without the need to passage