Novel Plasticware for Three-Dimensional (3D) Cell Co-culture

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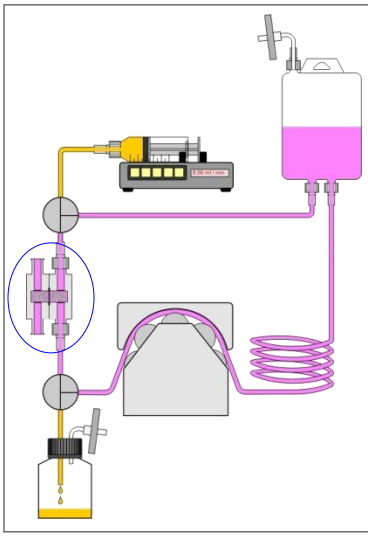
The Trend & The Challenge

A paradigm shift from two-dimensional (2D) to 3D cell culture techniques[1] has grown rapidly in recent years[2]. A shift from traditional monolayer culture to 3D affects cell function and behavior including morphology[3], gene expression[4] and similarity to the *in vivo* response[5]. To date, the majority of related research and commercial activity has focused on novel 3D scaffolds utilized in traditional 2D plasticware. Innovative 3D plasticware is needed to accommodate the spectrum of 3D scaffold configurations and materials, provide dynamic fluid medium exchange to sustain long-term cell viability and function, synergy with advanced imaging modalities, advanced co-culture of multiple cell types, and cost-effective.

The Solution

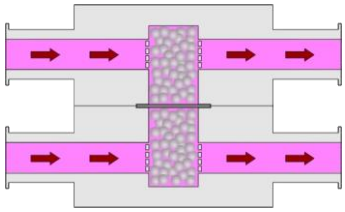
3DKUBE™ 3D Cell Culture Plasticware can be configured to permit independent culture (n=2) of single cell and mixed cell types or segregated co-culture (n=1) of multiple cell types within the dual chamber modular design. The assembly consists of two injection molded polystyrene modules and either a solid silicone gasket (independent chambers) or a silicone gasket ring plus polyethersulfone membrane insert with 0.45 µm porosity (segregated co-culture).



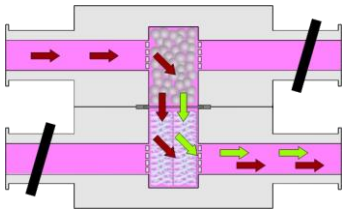
Perfusion Flow Circuits
Each culture chamber (circled in blue) can receive a cell-specific medium under variable and reversible flow conditions. Multiple pump drives can be used to perfuse the culture chamber (i.e., syringe, peristaltic, gravity). One of two flow circuits is shown here.

3D KUBE™
3D CELL CULTURE PLASTICWARE

Independent Chambers (n=2)
- Solid gasket insert
- Two independent samples



Segregated Co-culture (n=1)
- Gasket-membrane insert
- Physically segregates the different cell types
- Porous membrane allows soluble factor transfer (green)



The Demonstration

Study #1: Dynamic 3D Co-culture vs. Static 2D

Cell 1: HepG2 Human Liver Line [ATCC]
3D - Passage: 29, Seed: 7.5E+5 / chamber, 2D - Passage: 12, Seed: 1.0E+5 / well
Medium: Minimum essential eagle's medium, Sodium bicarbonate 1500mg/L, Sodium pyruvate 1mM, FBS 10%, Antibiotic 1%

Cell 2: MCF7 Human Breast Cancer Line [ATCC]
3D - Passage: 6, Seed: 7.5E+5 / chamber, 2D - Passage: 6, Seed: 9.0E+4 / well
Medium: Minimum essential eagle's medium, Sodium bicarbonate 1500mg/L, Sodium pyruvate 1mM, Bovine insulin 0.01mg/mL, FBS 10%, Antibiotic 1%

3D Scaffold: Alginate beads (cells encapsulated)
Scaffold Diameter: ~1mm
2D Well Plate: 12-well plates with no scaffold
Time Points: 2 and 6 days
Assays: Albumin production (HepG2), Cathepsin D activity (MCF7), Hoechst dye cell count, *in situ* 3D confocal laser microscopy (via 3DKUBE™ imaging window)

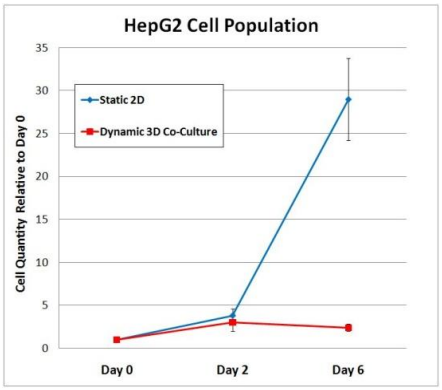
Study #2: Dynamic 3D Mono-culture - Cell Number & Metabolism Correlation

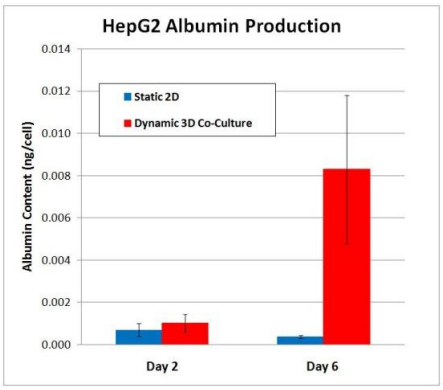
Cell: HepG2 Human Liver Line
Passage: 14, Seed: 6.3E+5 / chamber
Media: Minimum essential eagle's medium, Sodium bicarbonate 1500mg/L, Sodium pyruvate 1mM, FBS 10%, Antibiotic 1%

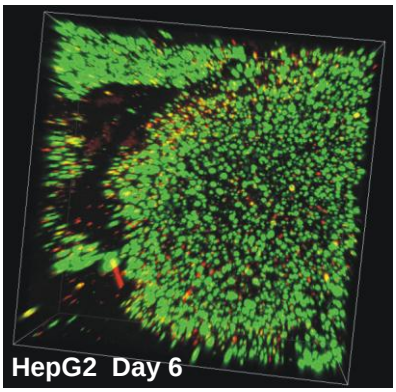
3D Scaffold: Alginate beads (cells encapsulated)
Scaffold Diameter: ~1mm
Time Points: 0, 2 and 6 days
Assays: Hoechst dye cell count, AlamarBlue cell metabolism, *in situ* 3D confocal laser microscopy

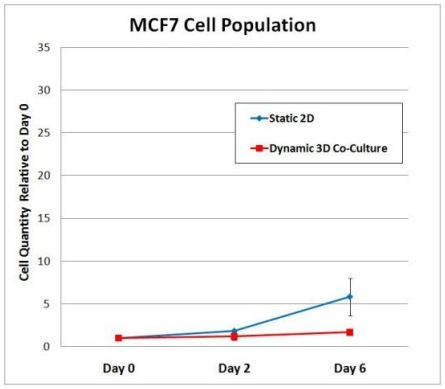
The Results

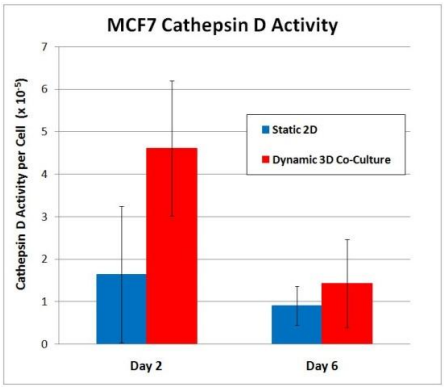
Study #1: Dynamic 3D Co-culture vs. Static 2D

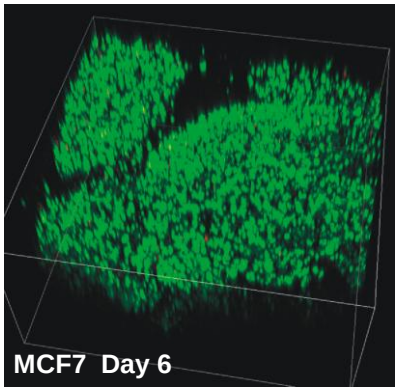






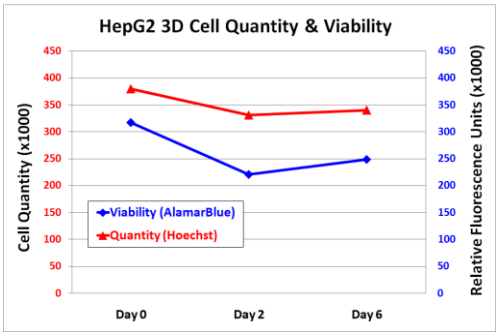


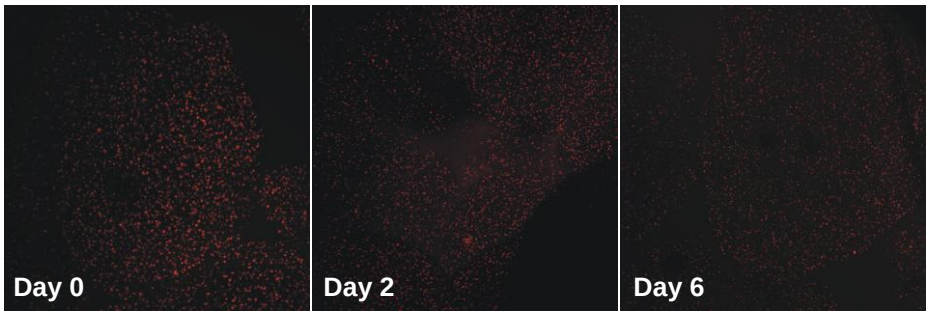




3D cell populations demonstrated stable growth curves while 2D culture grew in an exponential fashion. Numerical trends in cell function assays demonstrated good physiological response by cells in 3D culture while 2D cells were limited or trended down in their response. 3D confocal microscopy provided good images of cells stained with calcein and ethidium homodimer-1. 3D images (HepG2 top, MCF7 bottom) were taken at 40x total magnification. Image depth was limited by the alginate bead scaffold material. Other scaffold materials have allowed imaging depth up to 1 mm.

Study #2: Dynamic 3D Mono-culture - Cell Number & Metabolism Correlation





Hoechst cell count and alamarBlue cell metabolism assays demonstrated excellent correlation of the averages (0.995) in assessing the 3D cell populations at various timepoints. 3D confocal microscopy and DiIC12(3) fluorescent dye proved useful for visual affirmation of cell quantity trending and distribution over 6 days as imaged at 40x total magnification.

The Conclusion

3DKUBE™ 3D Cell Culture Plasticware has demonstrated to be a potentially universal standardized platform, adaptable to multiple cell types, scaffolds, imaging and assays as well as segregated co-culture experiments.

References

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3. Lan SF, Safiejko-Mroczka B, Starly B. Long-term cultivation of HepG2 liver cells encapsulated in alginate hydrogels: a study of cell viability, morphology, and drug metabolism. *Toxicol In Vitro* **2010**, 24(4):1314-23.
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