

Three-Dimensional (3D) Cell Culture *In Situ* Histological Embedding

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The Trend & The Challenge

The paradigm shift from two-dimensional (2D) to 3D cell culture techniques¹ has grown rapidly with a 153% increase in 3D cell culture research publications from 2007 to 2008.² 3D culture conditions are associated with development into tissue-like structures, more similar to those formed in living organisms and better able to reproduce *in vivo*-like responses.³ Researchers are actively pursuing 3D cell culture techniques to gain the benefits of this more *in vivo* like environment, but have difficulties adapting 2D analysis techniques to 3D cell culture. Advances in 3D confocal imaging allow researchers to visualize larger scale 3D constructs and understand cell distribution and viability. Histological techniques are another useful way of qualitatively analyzing 3D cell constructs just as tissue biopsies have been analyzed over the years. Unfortunately, histology techniques commonly disrupt the *in vitro* cell culture microenvironment due to necessary sample manipulation. Current methods involve physically removing the sample from its *in vitro* culture vessel and placing the tissue or 3D cell construct within a histology cassette where it is embedded and polymerized in its manipulated state.

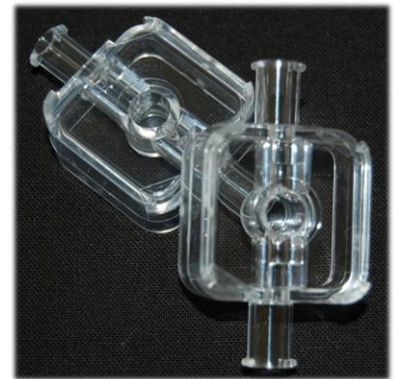
The Solution

3DKUBE™ 3D Cell Culture Plasticware can be used to embed a 3D cell-scaffold construct *in situ*, thereby, capturing the natural, non-manipulated *in vitro* 3D cellular environment. Disposable 3DKUBE plasticware provides a convenient and affordable platform to perform *in situ* embedding and processing of 3D cell culture samples for more accurate histological analysis and characterization.

The Demonstration

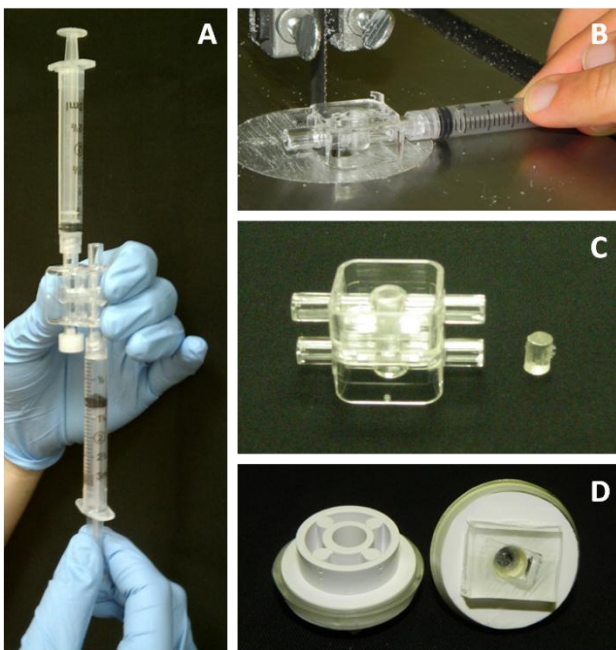
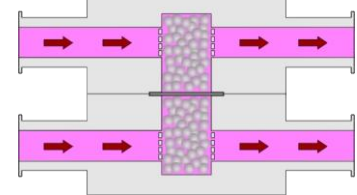
Alginate hydrogel beads (mean diameter = 1-2 mm) were crosslinked with suspended HepG2 human liver cells (ATCC) and cultured within the 3DKUBE plasticware 250 μ L chambers (2 chambers per 3DKUBE). Cells were maintained using a dynamic medium perfusion flow circuit at 1.0 mL/min. Novel histological techniques were used as endpoint analysis (at day 0, 2, and 6) as well as BD™ DiIC₁₂(3) Fluorescent Dye viability staining and *in situ* 3D laser confocal microscopy (Nikon Eclipse Ti).

3^DKUBE™
3D CELL CULTURE PLASTICWARE



Independent Chambers (n=2)

- Solid gasket insert
- Two independent samples

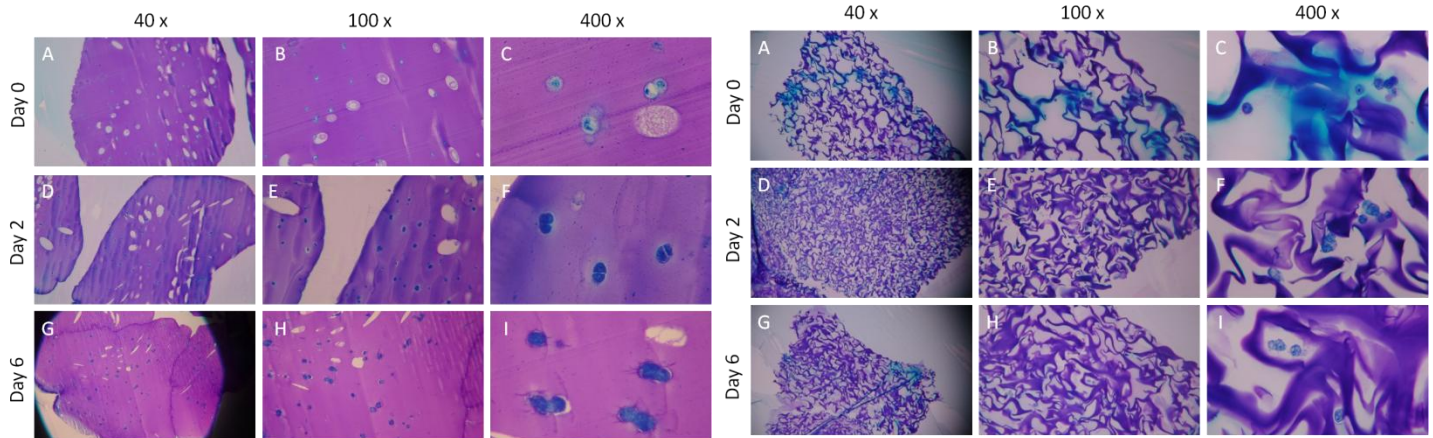


Novel histological techniques included *in situ* embedding and polymerization. HepG2 cells encapsulated in the alginate beads were embedded *in situ* by perfusing solutions of increasing concentration of glycol methacrylate (GMA) directly into the 3DKUBE chambers. Following manufacturer guidelines (Technovit® 7100), hardening agents were also perfused through the cell-scaffold construct *in situ* to create a fully polymerized cylinder that was removed from the plasticware using simple cutting methods. The polymerized cylinder containing the cell-scaffold constructs was attached to standard microtome mounting materials and sectioned according to manufacturer's guidelines. Samples were sectioned, mounted and stained with a Quik-Dip® Stain (Platinum Line).

In situ histology embedding in 3DKUBE™ 3D Cell Culture Plasticware. (A) Embedding agent is slowly perfused into the cell culture chamber; (B, C) after *in situ* polymerization, the sample can be removed from the surrounding plasticware; (D) the polymerized chamber contents can then be used with universal microtome mounting materials.

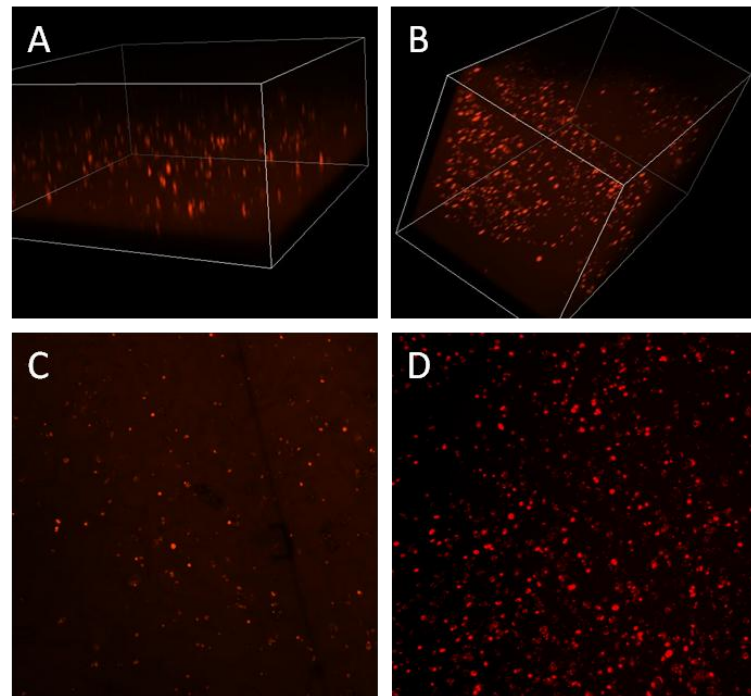
The Results
Histology Analysis via Phase Contrast Microscopy

DISCOVER. DEVELOP. DIAGNOSE.™
 KIYATEC 3D CELL CULTURE DELIVERS



HepG2 cells stained with a Quick-Dip® Stain making the scaffold material a purple/pink color while HepG2 cells appear blue. (Left): HepG2 cells encapsulated in alginate beads cultured under sustained perfusion for up to 6 days in 3DKUBE™ 3D Cell Culture Plasticware. The cell distribution at Day 0 (A-C) shows individual cells encapsulated by the matrix. As the cells proliferate (D-F) during the culture period, spheroids or cell clusters form by Day 6 (G-I) creating a more *in vivo*-like environment. **(Right):** HepG2 cells seeded via perfusion within Algimatrix™ and cultured under sustained perfusion for up to 6 days in 3DKUBE™ 3D Cell Culture Plasticware. An increase in spheroid formation occurs in Day 2 (F) and 6 (I) when compared to original cell seeding at Day 0 (C). (In partnership with the Clemson University Bioengineering Histology Laboratory)

3D Laser Confocal Microscopy



Histology sections of both HepG2 encapsulated alginate beads and Algimatrix™ show well distributed cell populations throughout the 3D scaffold constructs. HepG2 cells created spheroids or cell clusters at the later time points suggesting a more *in vivo* like environment. 3D laser confocal images of fluorescent stained HepG2 cells confirm cell viability within the scaffold construct, demonstrating adequate mass transfer at the center of the scaffold within the 3DKUBE.

The Conclusion

3DKUBE™ 3D Cell Culture Plasticware was demonstrated to be a potentially universal standardized platform for conducting 3D cell culture experiments and facilitating easy and affordable endpoint evaluation via *in situ* histology embedding and processing.

Special attention was paid to viability at the interior of the 3D cell-scaffold constructs, a significant concern for 3D cell culture. Both 3D laser confocal microscopy and histological analysis display excellent cell viability for HepG2 cells throughout the alginate beads and Algimatrix™ at day 6.

In situ 3D laser confocal microscopy images of viable HepG2 cells (stained with BD™ DiI_{C12}(3) fluorescent dye) cultured in Algimatrix™ (A and C) and encapsulated within alginate beads (B and D) at 100x total magnification. Images A and B are 3D stacks and images C and D are compressed stacks. (In partnership with the Clemson University Jordan Hall Imaging Facility)

References

1. Prestwich GD. Simplifying the extracellular matrix for 3-D cell culture and tissue engineering: a pragmatic approach. *J Cell Biochem* **2007**, 101:1370-1383.
2. www.3dcellculture.com, **2010**.
3. Mazzoleni G. (2009) *Genes Nutr*, **4**, 13.