Cell Viability in 3D Culture using *in situ* Bioluminescence

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The Trend & The Challenge

The paradigm shift from two-dimensional (2D) to 3D cell culture techniques[1] has grown rapidly with a 153% increase in 3D cell culture research publications from 2007 to 2008.[2] As compared to 2D, 3D culture conditions help organize cells into tissue-like structures that are more similar in form and function.[3] Oncology researchers are actively pursuing 3D cell culture techniques to gain the benefits of this more biologically-relevant environment in drug discovery and development, but barriers of lack of a universal and cost-effective 3D culture system have prevented widespread adoption of 3D techniques.

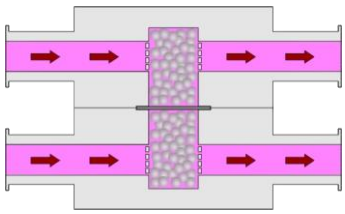
The Solution

3DKUBE™ 3D Cell Culture Plasticware can be used in conjunction with 3D Biotek polycaprolactone (PCL) scaffolds to provide a perfused 3D cell culture environment suitable for cell attachment and growth *in vitro*. The following preliminary study demonstrates the compatibility of 3D Biotek's PCL scaffold and 3DKUBE plasticware by creating a standard curve based on a variety of cell seeding densities and using luminescence as a method of *in situ* analysis within the 3DKUBE plasticware. The SHEP-luc cell line, neuroblastoma cells with stable expression of luciferase, was chosen for this study to demonstrate cell attachment and viability through *in situ* analysis. The luciferase expression can be quantified through luminescence and then correlated with cell number to create a standard curve showing cell attachment, viability, proliferation, or response to a drug.

3D KUBE™
3D CELL CULTURE PLASTICWARE

Independent Chambers (n=2)

- Solid gasket insert
- Two independent samples



The Demonstration

A 3D Biotek PCL scaffold disc was placed in the bottom of 3DKUBE™ 3D cell culture plasticware chambers. A male luer plug was then placed on each perfusion port of the 3DKUBE plasticware (Figure 1). The PCL scaffold disc was seeded with SHEP-luc cells through a direct seeding approach by placing 100 µl cell suspension (a range of 1.0E+5 - 7.5E+5 cells/ml in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin) directly on the scaffold. The 3DKUBE plasticware was placed in an incubator (37°C and 5% CO_2) for two hours under static conditions to allow for cell attachment to the PCL scaffold.

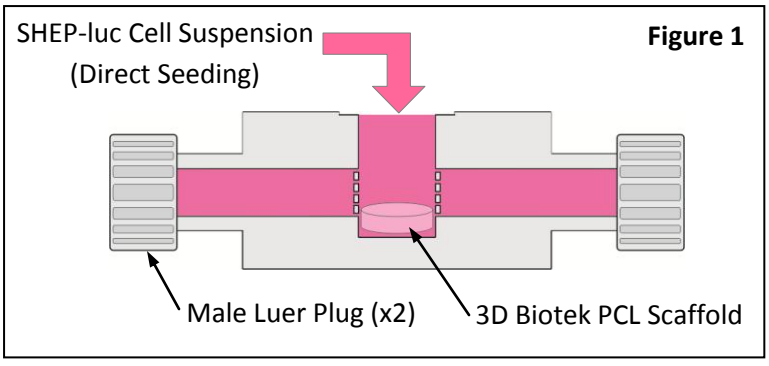


Figure 1

Cell culture medium was removed and replaced with 100 µl fresh medium, followed by 100 µl of a 0.104 µg/µl solution of VivoGlo™ (Promega Corporation) reagent. The 3DKUBE plasticware was then incubated for 10 minutes followed by luminescence measurement using KIYATEC's spectrometer plate reader adapter (Figure 3) and a plate reader (Wallac Victor 2).

Cell culture medium was removed from samples after VivoGlo™ assay completion and rinsed with PBS before replacing with 200 µl Steady-Glo® (Promega Corporation) solution (1:1 ratio of cell culture medium and Steady-Glo® reagent). Cell-scaffold constructs were incubated within the 3DKUBE plasticware for 2 minutes before reading the luminescence again using KIYATEC's spectrometer plate reader adapter and a plate reader.

The Results

Cell Seeding Standard Curve Measured through Luminescence

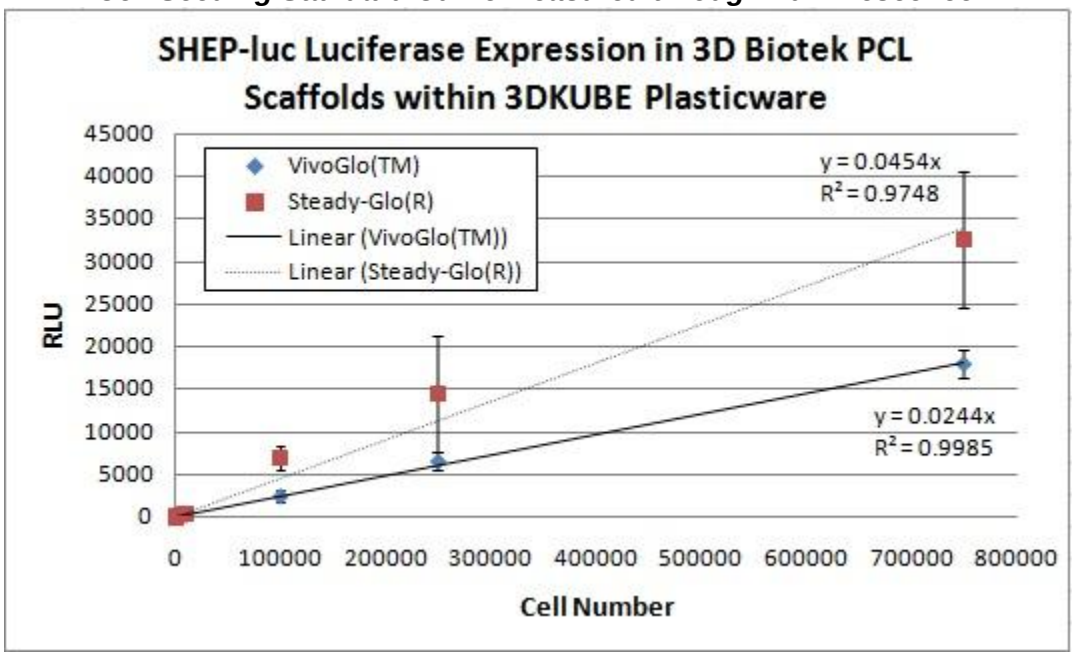


Figure 2: Luciferase expression of SHEP-luc cells seeded on 3D Biotek's PCL scaffold cultured within KIYATEC's 3DKUBE™ 3D Cell Culture Plasticware. Both viable and endpoint luciferase-based assays (VivoGlo™ and Steady-Glo®, respectively) demonstrate a linear correlation between luciferase expression and cell seeding number. RLU denotes Relative Luminescence Units.

The Conclusion

3DKUBE™ 3D Cell Culture Plasticware loaded with 3D Biotek PCL scaffold can be used to develop a 3D cell culture attachment and viability assay using a luciferase reporter cell system. A greater number of cells can be grown and analysed directly in the 3DKUBE than what is possible in the corresponding surface area (96 well plate) of a traditional 2D culture system (data not shown). *In situ* analysis is aided by the "docking" of the 3DKUBE into the KIYATEC spec adapter, which can be used with any spectrophotometer system.

Figure 3

This system represents a novel technique to measure important cellular functions, such as adhesion, viability, proliferation, and cell death, in response to any chemical or stimulus. The 3DKUBE loaded with 3D Biotek PCL scaffolds will be an important tool in life science research and discovery.

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.