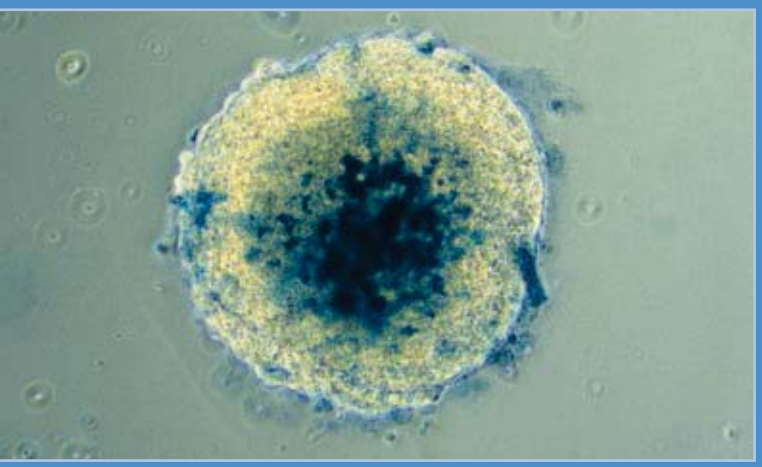
Your Power for Health

n3D Biosciences, Inc.

greiner bio-one



3D Cell Culture

With Products from Greiner Bio-One and Nano3D Biosciences

www.gbo.com/bioscience

Introduction

Why 3D Culture?

In preclinical drug discovery validation processes, monolayer cell cultures are still predominant. Nevertheless, 2D cultures can only mimic the conditions of physiological tissue to a limited extent, whereas cells *in vivo* are able to interact in a three-dimensional network. Therefore, results generated from 2D cultures may often be of limited relevance for clinical effectiveness and may contribute to high attrition rates in the drug development process.[1] The employment of spheroid cultures is regarded as a better rational to develop more predictive *in-vitro* screening assays for preclinical drug development, especially in cancer research.

In spheroid cultures, cells grow in a three-dimensional system with zones of cellular heterogeneity and nutrient and oxygen gradients, to more closely reflect the *in-vivo* tumor microenvironment (**Fig. 2**). Comparisons of spheroid cultures and 2D monolayer cultures showed functional differences in tumour cell lines, e.g. alterations in protein expression, phosphorylation patterns and responsiveness to inhibitor molecules.[2,3]

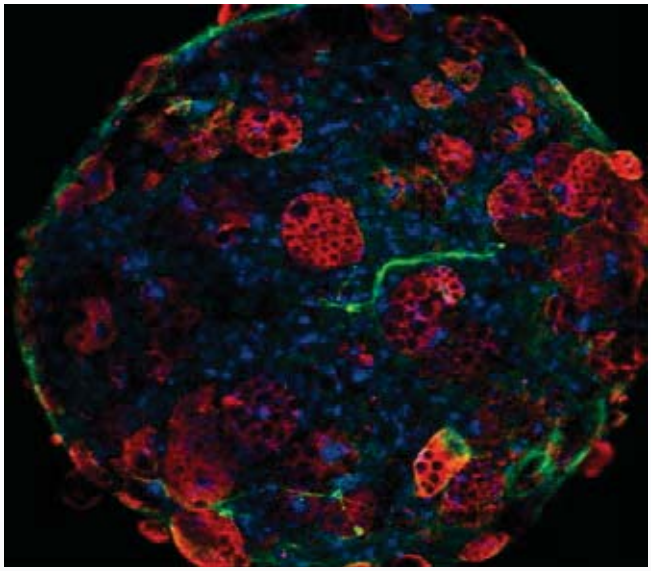


Figure 1: 3D Co-culture.
Daquinag, A. C., Souza, G. R. & Kolonin, M. G. Adipose tissue engineering in three-dimensional levitation tissue culture system based on magnetic nanoparticles. Tissue Eng. Part C. Methods 19, 336–44 (2013).

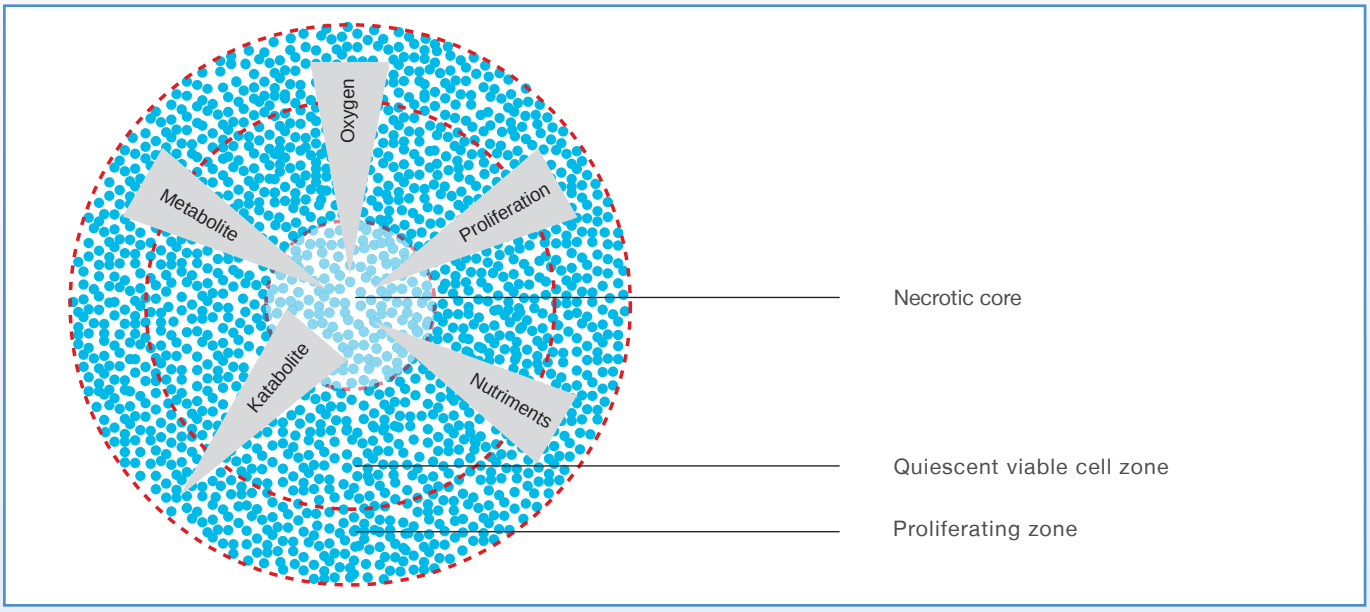


Figure 2: Schematic description of a tumour spheroid

1) Friedrich J. et al. (2007). Experimental anti-tumour therapy in 3-D: Spheroids - old hat or new challenge? Int J Rad Biol. 83(11-12):849-871.

2) Ekert J E et al. (2014) Three-dimensional lung tumor microenvironment modulates therapeutic compound responsiveness in vitro – implications for drug development. PLOS ONE 9(3): e92248.doi10.1371/journal.pone.0092248

3) Vinci, M et al. (2012) Advances in establishment and analysis of three-dimensional tumor spheroid-based functional assays for target validation and drug evaluation. BMC Biology 10:29.

The n3D Approach

Magnetic 3D Cell Culturing

The core technology of our partner Nano3D Biosciences (n3D) is the magnetization of cells with NanoShuttle™-PL. The cells can be aggregated with magnetic forces, either by levitation or printing, to form structurally and biologically representative 3D models *in vitro*.

The **advantages** of magnetic cell culture include:

- Mimicking native tissue environment
- Rapid 3D model formation within hours
- No specialized equipment, media, or artificial substrate
- Easy to handle / no sample loss
- Allows co-culture

With magnetized spheroids, solution addition and removal is made easy by using magnetic force to hold them in a stationary position during aspiration, thereby limiting spheroid loss. Spheroids can also be picked up and transferred between vessels using magnetic tools such as the MagPen™.[4]
NanoShuttle™-PL consists of gold, iron oxide, and poly-L-lysine.[5] NanoShuttle™-PL magnetizes cells by electrostatically attaching to cell membranes during an overnight static incubation. Magnetized cells will appear peppered with dark nanoparticles after incubation. NanoShuttle™-PL will stay attached to the cell membrane for up to 8 days, at which point it's released into the 3D culture.[5] NanoShuttle™-PL is biocompatible, having no effect on metabolism, proliferation, and inflammatory stress[6-7], and even encouraging proliferation in 3D.[5,8] Additionally, it does not interfere with experimental techniques, such as fluorescence[9], or Western blotting.[10]

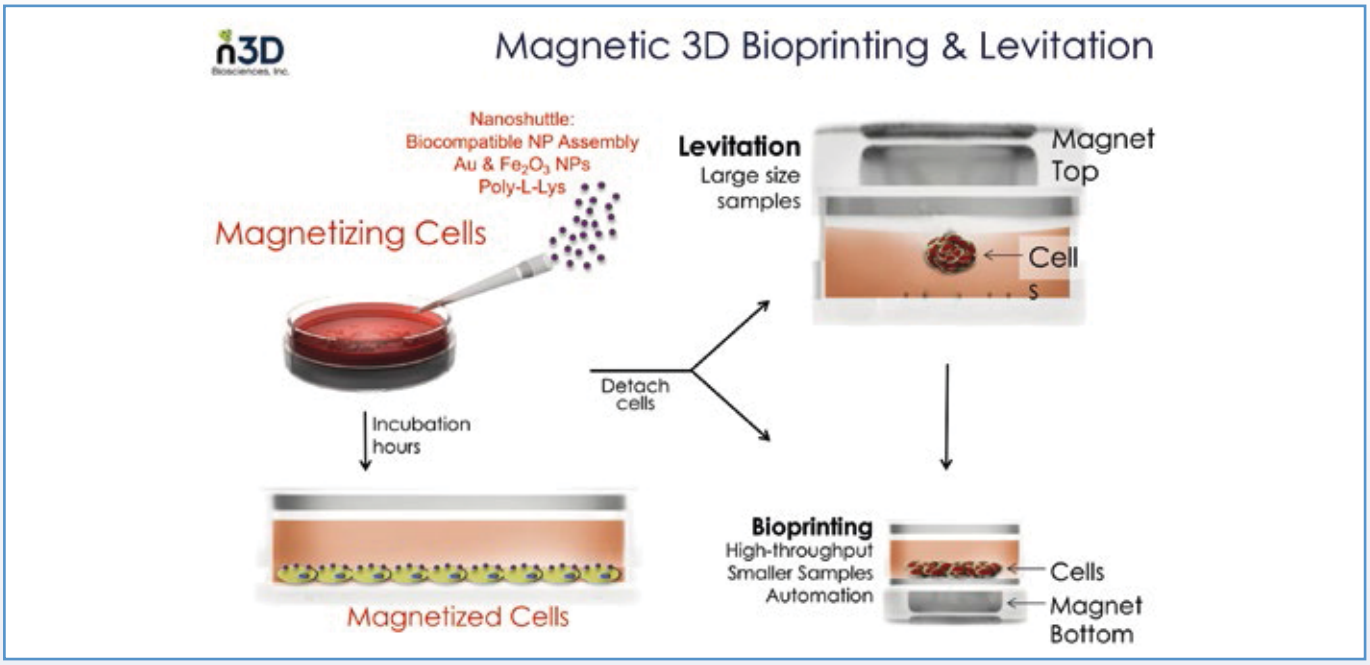


Figure 3: Magnetic 3D bioprinting and levitation

4) Tseng, H. et al. Assembly of a three-dimensional multitype bronchiole coculture model using magnetic levitation. Tissue Eng. Part C. Methods 19, 665–75 (2013).
5) Souza, G. R. et al. Three-dimensional tissue culture based on magnetic cell levitation. Nat. Nanotechnol. 5, 291–6 (2010).
6) Tseng, H. et al. Assembly of a three-dimensional multitype bronchiole coculture model using magnetic levitation. Tissue Eng. Part C. Methods 19, 665–75 (2013).
7) Tseng, H. et al. A three-dimensional co-culture model of the aortic valve using magnetic levitation. Acta Biomater. 10, 173–82 (2014).
8) Castro-Chavez, F., Vickers, K. C., Lee, J. S., Tung, C.-H. & Morrisett, J. D. Effect of lyso-phosphatidylcholine and Schnurri-3 on osteogenic transdifferentiation of vascular smooth muscle cells to calcifying vascular cells in 3D culture. Biochim. Biophys. Acta 1830, 3828–34 (2013).
9) Daquinag, A. C., Souza, G. R. & Kolonin, M. G. Adipose tissue engineering in three-dimensional levitation tissue culture system based on magnetic nanoparticles. Tissue Eng. Part C. Methods 19, 336–44 (2013).
10) Molina, J. R., Hayashi, Y., Stephens, C. & Georgescu, M.-M. Invasive glioblastoma cells acquire stemness and increased Akt activation. Neoplasia 12, 453–63 (2010).

CELLSTAR® Cell Culture Vessels with Cell-Repellent Surface

The Perfect Match for Magnetic Cell Culture

For the successful application of magnetic 3D cell culturing, attachment of the cells to the surface of the culture vessel used must be avoided. Therefore standard tissue culture products, which are optimized to enhance conditions for cell attachment, cannot be used. The Greiner Bio-One CELLSTAR® cell culture vessels with cell-repellent surface (**Fig. 4**) effectively prevent cell attachment and therefore provide the perfect match for the n3D magnetic cell culturing approach.

The cell-repellent properties are achieved through an innovative chemical modification of the vessel surface. All cell culture vessels with cell-repellent surface are sterilized by irradiation (SAL of 10^{-3}) and controlled for absence of detectable endotoxins, DNase/RNase and human DNA. Vessels with a cell-repellent surface additionally demonstrate no cytotoxic effects. Evaluation of cytotoxicity is done in accordance to EN ISO 10993-5 with mammalian cell lines.

As with all Greiner Bio-One microplates, cell-repellent surface microplates are manufactured with a footprint that conforms to the recommendations of the American National Standards Institute (ANSI 1-2004) to guarantee compatibility with all widely used lab equipment.

Cell culture vessels equipped with the Greiner Bio-One cell-repellent surface present an ideal platform for cultivating suspension cultures of semi-adherent and adherent cell lines as well as the formation of stem cell aggregates and spheroids either with or without magnetic approach.

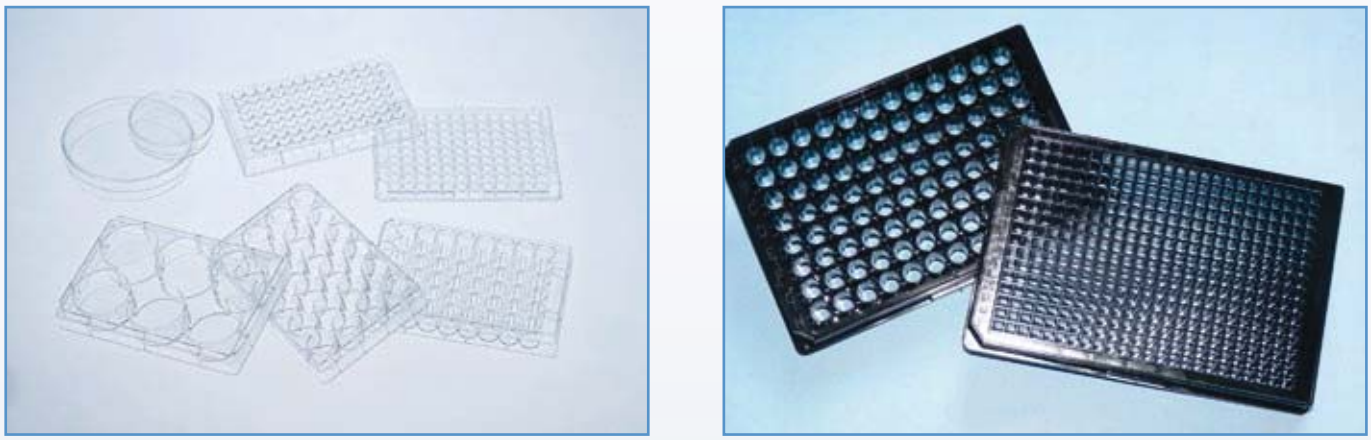


Figure 4: CELLSTAR® cell culture vessels with cell-repellent surface in clear version (left) and black version with µClear® film bottom (right).

Figure 5: Single spheroids in a CELLSTAR® 96 well cell culture microplate (U-bottom) with cell-repellent surface

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Further information on CELLSTAR® cell-repellent surface can be found in the Download Panel on our website www.gbo.com:

→ **Forum No. 17: CELLSTAR® Cell Culture Vessels with Cell-Repellent Surface** (F073 777)

Magnetic 3D Cell Culturing

Spheroid Bioprinting

Magnetic 3D bioprinting is a rapid and effective tool to print spheroids that are representative of native cellular environments in an easy to handle manner. While other spheroid systems can mimic native cellular environments, they take a long time to form and are difficult to handle/retrieve. Magnetic 3D bioprinting addresses these issues by utilizing n3D's core technology, magnetizing cells with NanoShuttle™-PL to print spheroids. In magnetic 3D bioprinting, cells incubated with NanoShuttle™-PL overnight are printed into spheroids by placing magnetized cells atop a drive of magnets, fashioned below each well of a standard microplate.[11] The magnets below each well aggregate the cells using mild magnetic forces to form a spheroid at the well bottom. In only 15 minutes to a few hours, the plate of spheroids can be removed from the magnet drive for short to long-term culture.

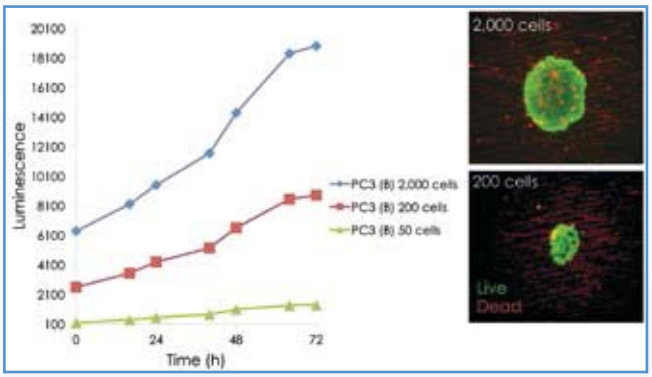

Figure 6: Viability of PC3 spheroids of various sizes as measured by (left) the real-time CellTiter-Glo assay (Promega, Madison, WI) and (right) live/dead staining.

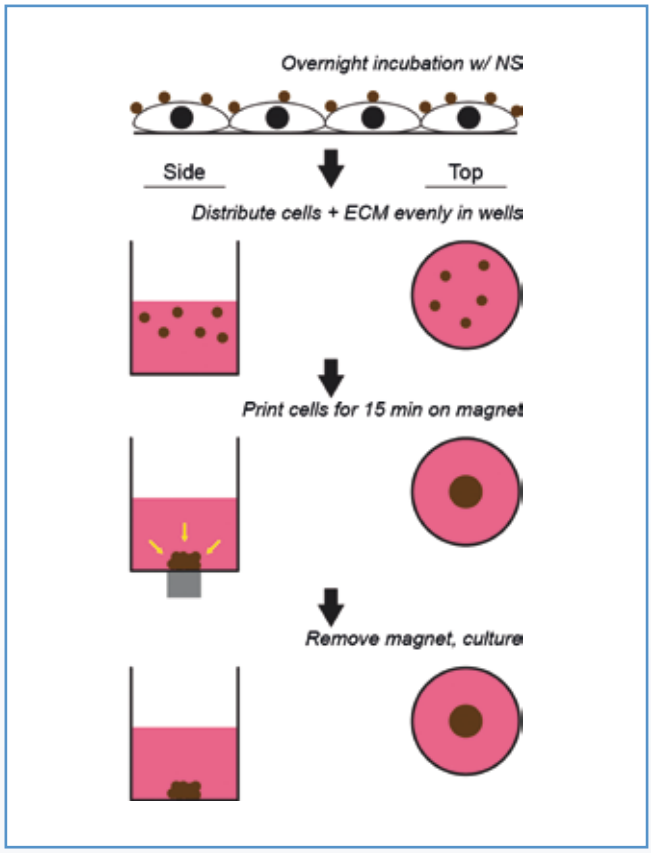

Figure 7: Magnetic 3D bioprinting

Watch our video
"Magnetic 3D Bioprinting Spheroids"

http://youtu.be/0g8PICDdqvA

Magnetic forces can also be used to create co-cultures with fine spatial organization.[12]

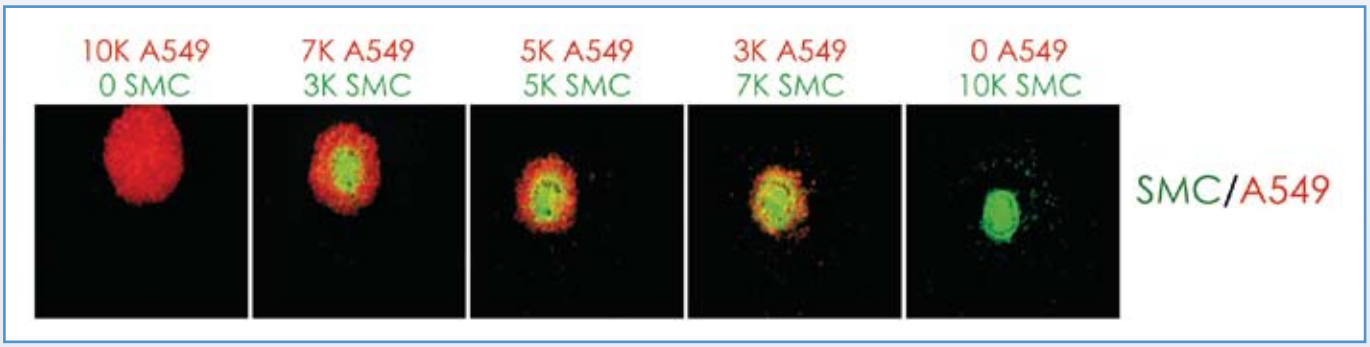

Figure 8: Co-culture of primary human tracheal smooth muscle cells (green) and A549 human alveolar epithelial lung adenocarcinoma cells (red) at varying ratios.

11) Timm, D. M. et al. A high-throughput three-dimensional cell migration assay for toxicity screening with mobile device-based macroscopic image analysis. Sci. Rep. 3, 3000 (2013)

12) Tseng, H. et al. Assembly of a three-dimensional multitype bronchiole coculture model using magnetic levitation. Tissue Eng. Part C. Methods 19, 665–75 (2013).

Magnetic 3D Cell Culturing

Magnetic Levitation

Magnetic levitation is an easy tool to recreate native tissue environments *in vitro*. Cells are magnetized with NanoShuttle™-PL through overnight incubation and dispensed into a cell-repellent dish or multiwell plate, where they are levitated off the bottom by a magnet above the plate.[13] In levitating cells off the substrate bottom, the magnetic forces work as an invisible scaffold that rapidly aggregates cells to induce cell-cell interactions and ECM synthesis. The resultant 3D culture is formed without any artificial substrate or specialized media or equipment and can be cultured long-term.[14]

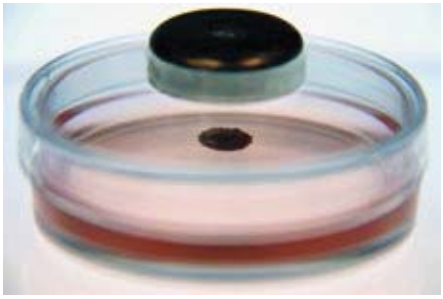

Figure 9: Magnetic levitation in a cell culture dish

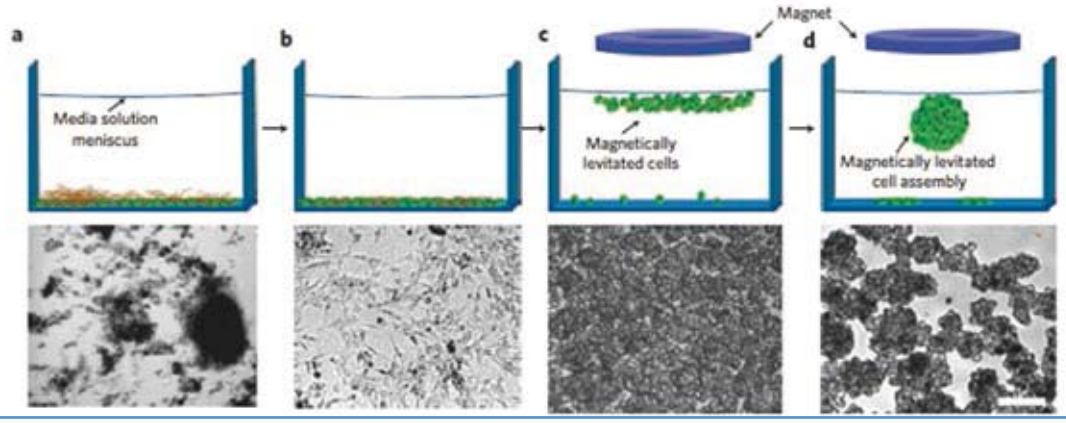

Figure 10: Three-dimensional cell culture with magnetic-based levitation

Watch our video
"3D Cell Culturing with Bio-Assembler"
https://www.youtube.com/watch?v=RKOn348qQ4c#t=11

13) Souza, G. R. et al. Three-dimensional tissue culture based on magnetic cell levitation. Nat. Nanotechnol. 5, 291–6 (2010).

14) Daquinag, A. C., Souza, G. R. & Kolonin, M. G. Adipose tissue engineering in three-dimensional levitation tissue culture system based on magnetic nanoparticles. Tissue Eng. Part C. Methods 19, 336–44 (2013).

What 3D platform is best for you?

	Magnetic Levitation			Spheroid Bioprinting	
Well Number	35 mm dish	6	24	96	384
Application					
Cancer				■	
Cardiotoxicity					■
Hepatotoxicity					■
Wound Healing				■	
Viability Assays				■	
Organoids	■	■	■	■	■
Genomics	■	■	■		
Western Blotting	■	■	■		

High-Throughput Compound Screening in 3D

Toxicity Testing with the n3D BiO Assay

The BiO Assay combines a 3D cell culture environment with high-throughput and high-content testing to effectively predict *in-vivo* response *in vitro*. The current standards for compound screening are animal models; while representing human tissues of interest, these models are expensive, scarce, and present ethical challenges. On the other end, *in-vitro* assays poorly mimic native cellular environments and thus human *in-vivo* response, but offer high-throughput testing with ease. Thus, there is a demand for *in-vitro* assays that are both predictive of human *in-vivo* response and high-throughput, for which the BiO Assay can fulfill.

Magnetic Printing of Rings

Based on magnetic 3D bioprinting, cells magnetized with NanoShuttle™-PL are printed into spheroids and rings. Immediately after printing, these structures will shrink/close, as a function of cell migration, viability and proliferation.[15] Shrinkage is captured using a compact imaging kit (n3Dock) with an iPod™ programmed by a freely available app (Experiment Assistant) to image whole plates at specific intervals, forgoing the need to image well-by-well under a microscope. Shrinkage is complete within a day, and images are batch processed to rapidly yield toxicity data. Moreover, as shrinkage is label-free, the remaining rings or spheroids are available for further experimentation (IHC, Western blot, genomics, etc.).

Ring versus Spheroid

The BiO Assay can be used to track the shrinkage of both rings and spheroids. While both shapes will shrink similarly and are assayed identically, the different shapes can represent different situations. For rings, closure of the ring can represent wound-healing, wherein cells are working to close the void in the middle of the ring. Additionally, rings can represent similarly shaped tissues, like blood vessels, where dilation and contraction can be assayed.

For spheroids, shrinkage is related to spheroid assembly, with the assay macroscopically measuring how well the cells are interacting and migrating to build a competent structure.

Watch our video
"BiO Assay Experiments - Living Data"
https://www.youtube.com/watch?v=qMh8wxMGQN4

Watch our video
"3T3 vs. ATRA Dot new"
https://www.youtube.com/watch?v=JD0BB5Wpm20

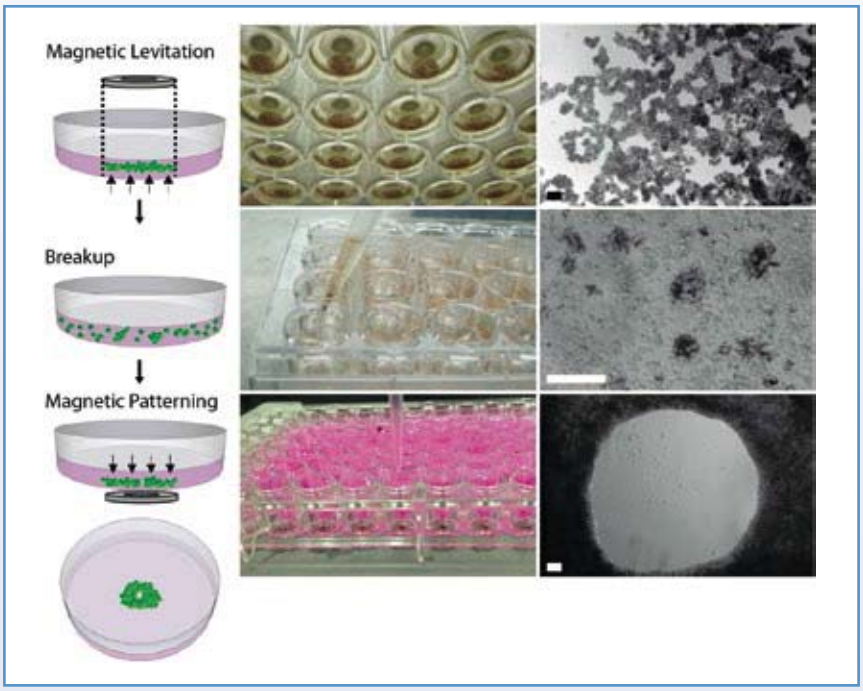

Figure 11: Magnetic printing of rings

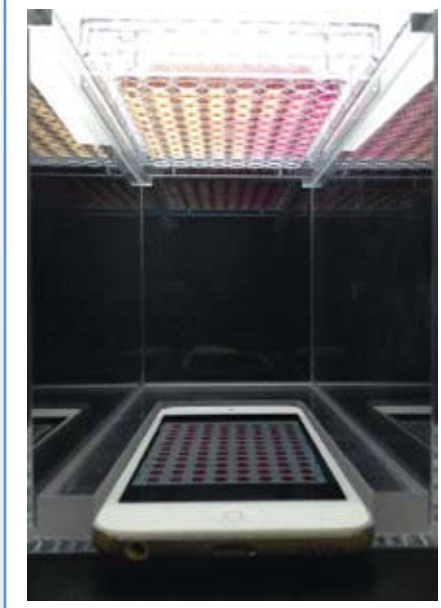

Figure 12: n3Dock - iPod -based imaging

15) Souza, G. R. et al. Three-dimensional tissue culture based on magnetic cell levitation. Nat. Nanotechnol. 5, 291–6 (2010).