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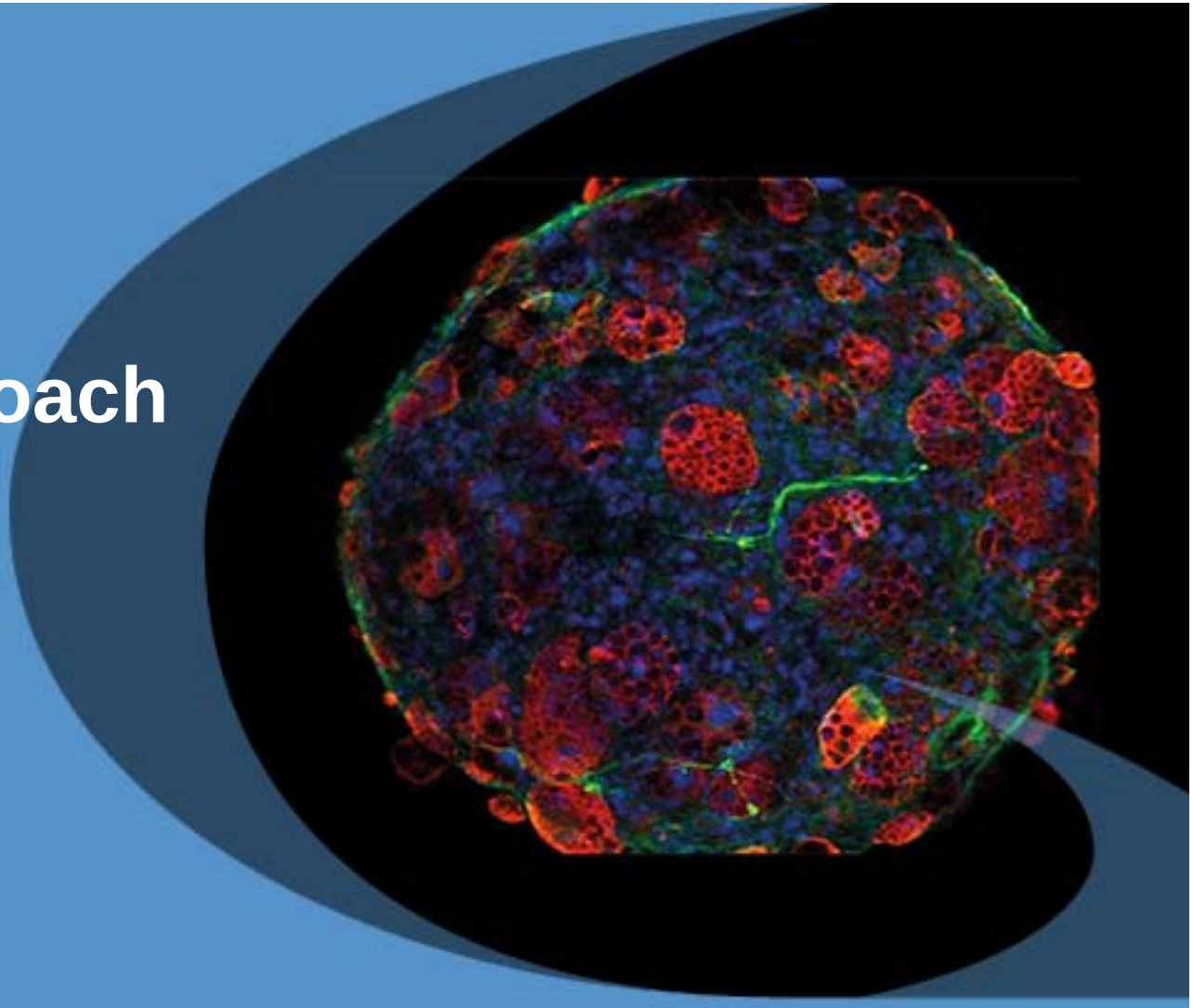
Magnetic cell culturing

The n3D approach



Greiner Bio-One GmbH
November 2015

Agenda

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- CELLSTAR® cell-repellent surface for 3D cell culture
- Magnetic cell culturing – The n3D approach
- Applications
- Products / Info material

Cell-repellent surface

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- Effectively prevents the process of cell-attachment
- Cells form aggregates or spheroids by self-assembly
- Technology: Stable chemical modification of PS surface

Cell-repellent surface for 3D applications

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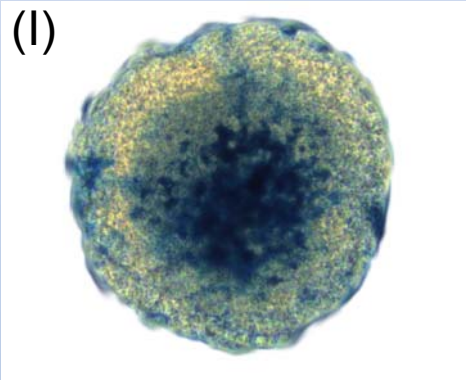
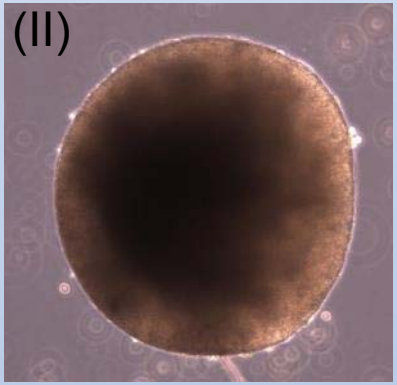
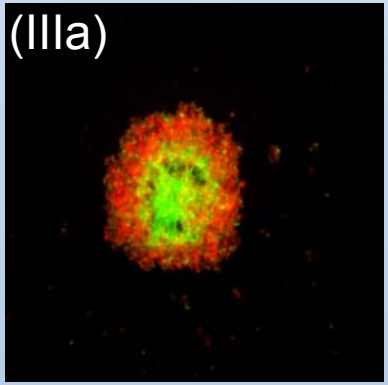
- Compound screening and toxicology studies with spheroids (e.g. tumor cells, liver cells), (I)
- Formation of stem cell aggregates, (II)
- Platform for magnetic cell culture (IIIa) and hydrogel scaffolds (IIIb)







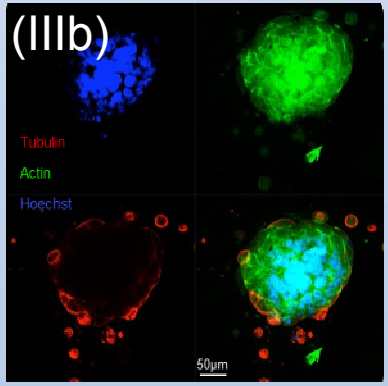


Image is courtesy of Celenys, Rouen (France)

Spheroid formation in 96 well U-bottom plates with cell-repellent surface

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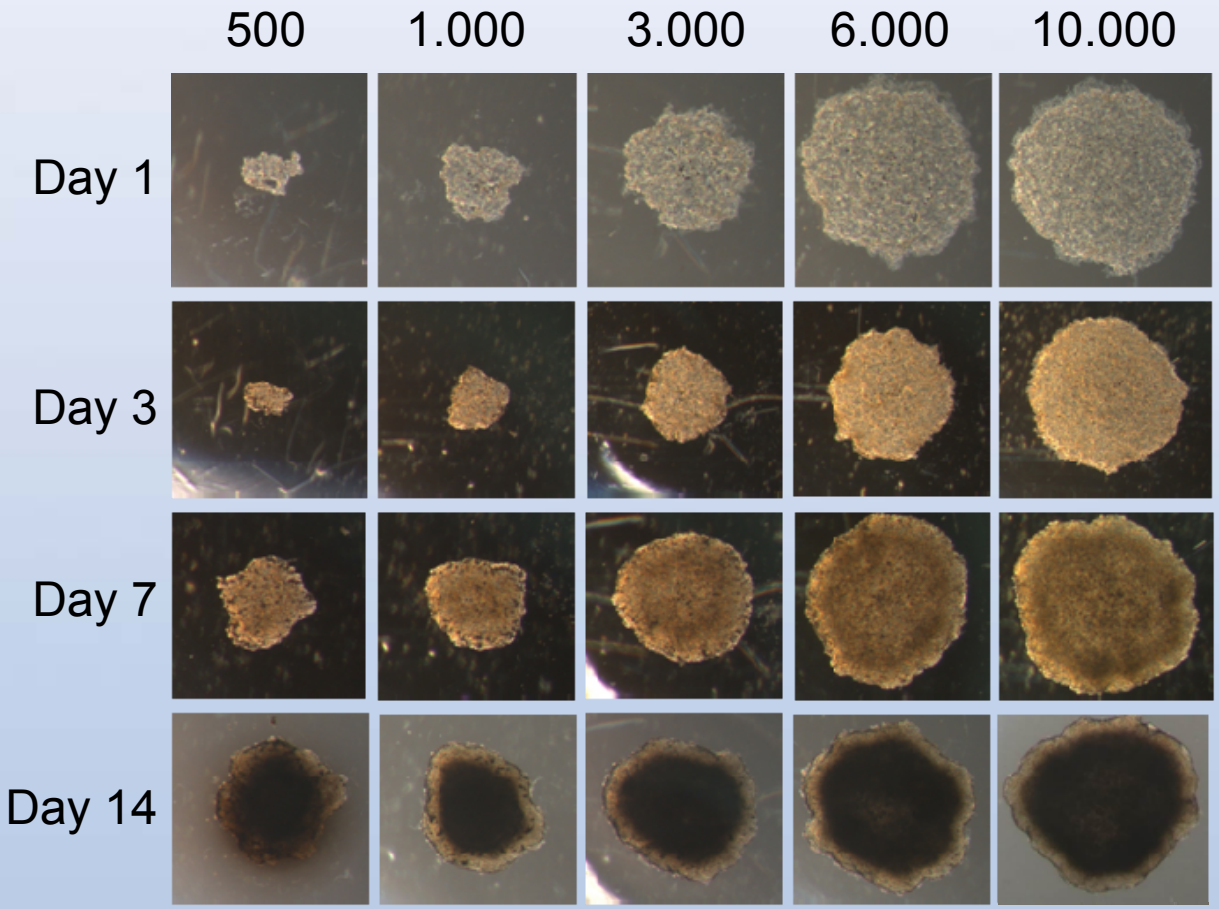

Tested cell lines:

- HeLa
- HepG2
- LNCaP
- HEK-239

GBO formats with cell-repellent surface

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- 655 970 96 well F-bottom, transparent (1 per bag / 6 per case)
- 655 976 96 well, black, µClear (8 per bag / 32 per case)
- 655 976-SIN 96 well, black, µClear (1 per bag / 32 per case)

- 650 970 96 well U-bottom (1 per bag / 6 per case)
- 650 979 96 well U-bottom (8 per bag / 32 per case)

- 651 970 96 well V-bottom (1 per bag / 6 per case)

- 781 970 384 well, F-Bottom, transparent (1 per bag / 60 per case)
- 781 976 384 well, black, µClear (8 per bag / 32 per case)
- 781 976-SIN 384 well, black, µClear (8 per bag / 32 per case)

- 657 970 6 Well Plate (1 per bag / 5 per case)
- 662 970 24 Well Plate (1 per bag / 5 per case)
- 677 970 48 Well Plate (1 per bag / 5 per case)

- 664 970 100 mm cell culture dish (1 per bag / 5 per case)
- 628 979 60 mm cell culture dish (10 per bag / 20 per case)
- 627 979 35 mm cell culture disc (10 per bag / 40 per case

- 660 985 T175 cell culture flask, filter screw cap (5 per bag / 5 per case)
- 658 985 T75 cell culture flask, filter screw cap (5 per bag / 15 per case)
- 690 985 T25 cell culture flask, filter screw cap (10 per bag / 20 per case)
- 660 980 T175 cell culture flask, standard screw cap (5 per bag / 5 per case)
- 658 980 T75 cell culture flask, standard screw cap (5 per bag / 15 per case)
- 690 980 T25 cell culture flask, standard screw cap (10 per bag / 20 per case)

Info material

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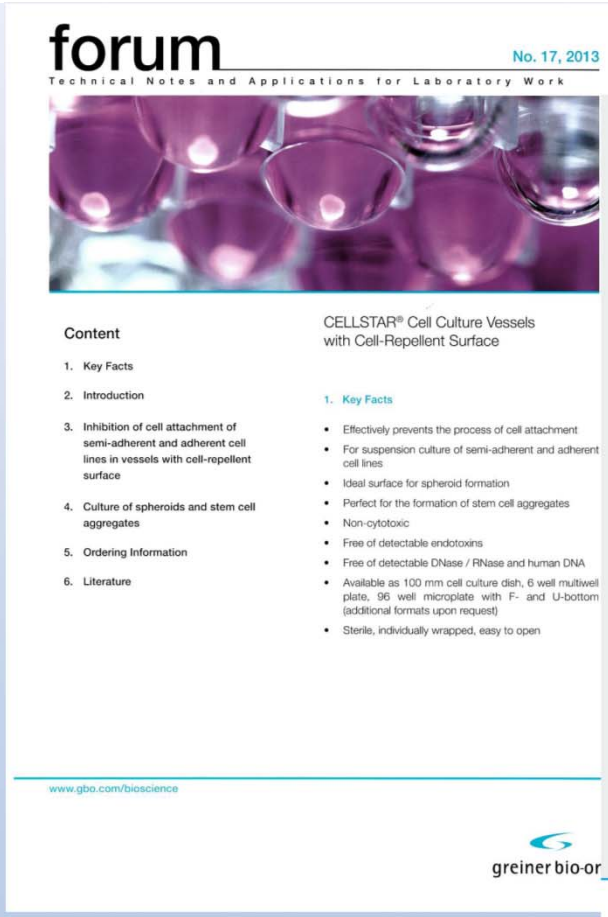
forum
Technical Notes and Applications for Laboratory Work
No. 17, 2013

Content

1. Key Facts
2. Introduction
3. Inhibition of cell attachment of semi-adherent and adherent cell lines in vessels with cell-repellent surface
4. Culture of spheroids and stem cell aggregates
5. Ordering Information
6. Literature

CELLSTAR® Cell Culture Vessels with Cell-Repellent Surface

1. Key Facts

- Effectively prevents the process of cell attachment
- For suspension culture of semi-adherent and adherent cell lines
- Ideal surface for spheroid formation
- Perfect for the formation of stem cell aggregates
- Non-cytotoxic
- Free of detectable endotoxins
- Free of detectable DNase / RNase and human DNA
- Available as 100 mm cell culture dish, 6 well multiwell plate, 96 well microplate with F- and U-bottom (additional formats upon request)
- Sterile, individually wrapped, easy to open

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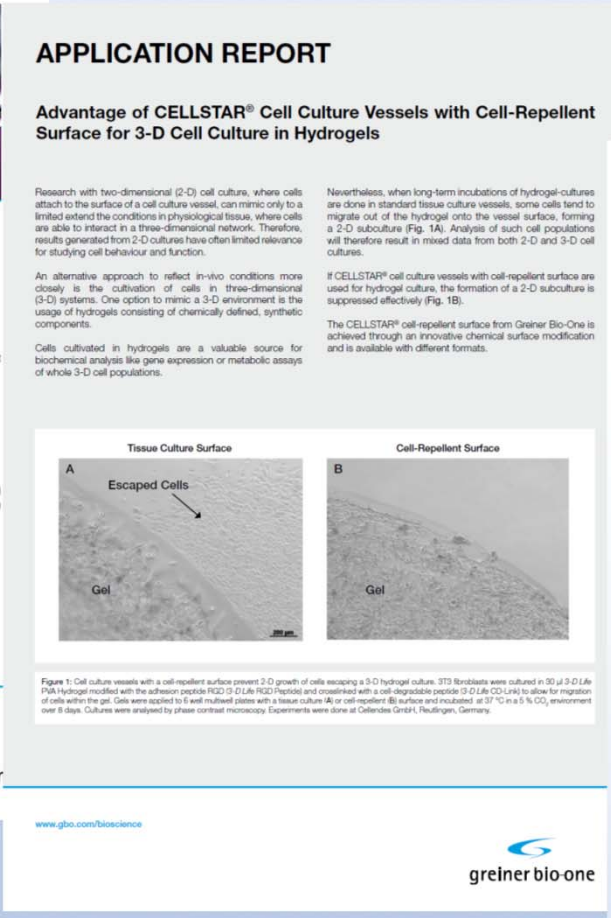
APPLICATION REPORT

Advantage of CELLSTAR® Cell Culture Vessels with Cell-Repellent Surface for 3-D Cell Culture in Hydrogels

Research with two-dimensional (2-D) cell culture, where cells attach to the surface of a cell culture vessel, can mimic only to a limited extend the conditions in physiological tissue, where cells are able to interact in a three-dimensional network. Therefore, results generated from 2-D cultures have often limited relevance for studying cell behaviour and function.

An alternative approach to reflect in-vivo conditions more closely is the cultivation of cells in three-dimensional (3-D) systems. One option to mimic a 3-D environment is the usage of hydrogels consisting of chemically defined, synthetic components.

Cells cultivated in hydrogels are a valuable source for biochemical analysis like gene expression or metabolic assays of whole 3-D cell populations.

Nevertheless, when long-term incubations of hydrogel-cultures are done in standard tissue culture vessels, some cells tend to migrate out of the hydrogel onto the vessel surface, forming a 2-D subculture (Fig. 1A). Analysis of such cell populations will therefore result in mixed data from both 2-D and 3-D cell cultures.

If CELLSTAR® cell culture vessels with cell-repellent surface are used for hydrogel culture, the formation of a 2-D subculture is suppressed effectively (Fig. 1B).

The CELLSTAR® cell-repellent surface from Greiner Bio-One is achieved through an innovative chemical surface modification and is available with different formats.

Tissue Culture Surface
Cell-Repellent Surface
A
B
Escaped Cells
Gel
Gel

Figure 1: Cell culture vessels with a cell-repellent surface prevent 2-D growth of cells escaping a 3-D hydrogel culture. 3T3 fibroblasts were cultured in 30 µl *3-D Life* PVA Hydrogel modified with the adhesion peptide RGD (*3-D Life* RGD Peptide) and crosslinked with a cell-degradable peptide (*3-D Life* CD-Link) to allow for migration of cells within the gel. Gels were applied to 6 well multiwell plates with a tissue culture (A) or cell-repellent (B) surface and incubated at 37 °C in a 5 % CO_2 environment over 8 days. Cultures were analysed by phase contrast microscopy. Experiments were done at Cellendes GmbH, Reutlingen, Germany.

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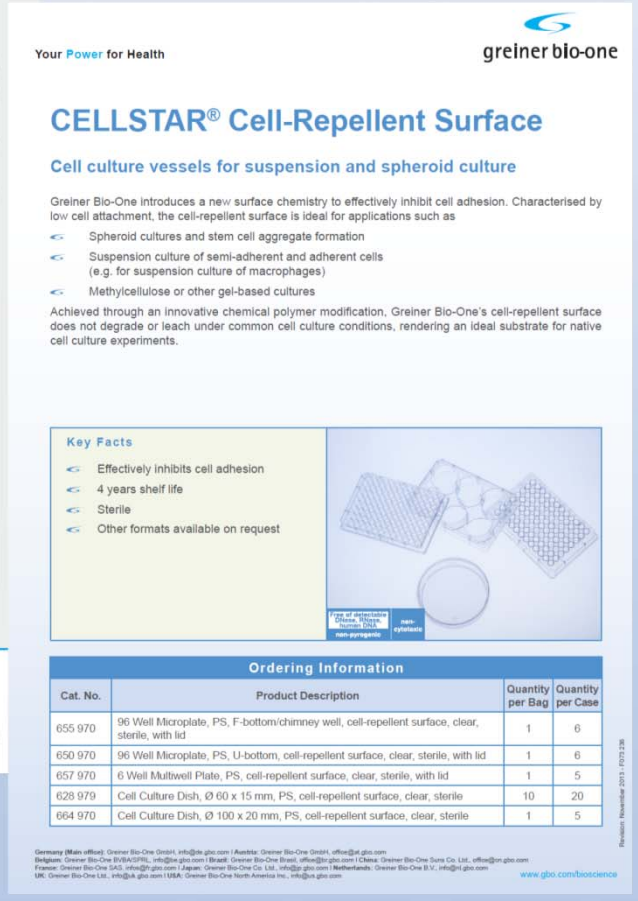
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CELLSTAR® Cell-Repellent Surface

Cell culture vessels for suspension and spheroid culture

Greiner Bio-One introduces a new surface chemistry to effectively inhibit cell adhesion. Characterised by low cell attachment, the cell-repellent surface is ideal for applications such as

- Spheroid cultures and stem cell aggregate formation
- Suspension culture of semi-adherent and adherent cells (e.g. for suspension culture of macrophages)
- Methylcellulose or other gel-based cultures

Achieved through an innovative chemical polymer modification, Greiner Bio-One's cell-repellent surface does not degrade or leach under common cell culture conditions, rendering an ideal substrate for native cell culture experiments.

Key Facts

- Effectively inhibits cell adhesion
- 4 years shelf life
- Sterile
- Other formats available on request

Ordering Information

Cat. No.	Product Description	Quantity per Bag	Quantity per Case
655 970	96 Well Microplate, PS, F-bottom/chimney well, cell-repellent surface, clear, sterile, with lid	1	6
650 970	96 Well Microplate, PS, U-bottom, cell-repellent surface, clear, sterile, with lid	1	6
657 970	6 Well Multiwell Plate, PS, cell-repellent surface, clear, sterile, with lid	1	5
628 979	Cell Culture Dish, Ø 60 x 15 mm, PS, cell-repellent surface, clear, sterile	10	20
664 970	Cell Culture Dish, Ø 100 x 20 mm, PS, cell-repellent surface, clear, sterile	1	5

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n3D Biosciences

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- Magnetic cell culturing technology invented by our partner

n3D Biosciences, Inc.

7000 Fannin Street
Suite 2140
Houston, TX 77030

- Technology requires F-bottom vessels with cell-repellent surface!

Basic principle: Magnetization of cells by adding NanoShuttle-PL

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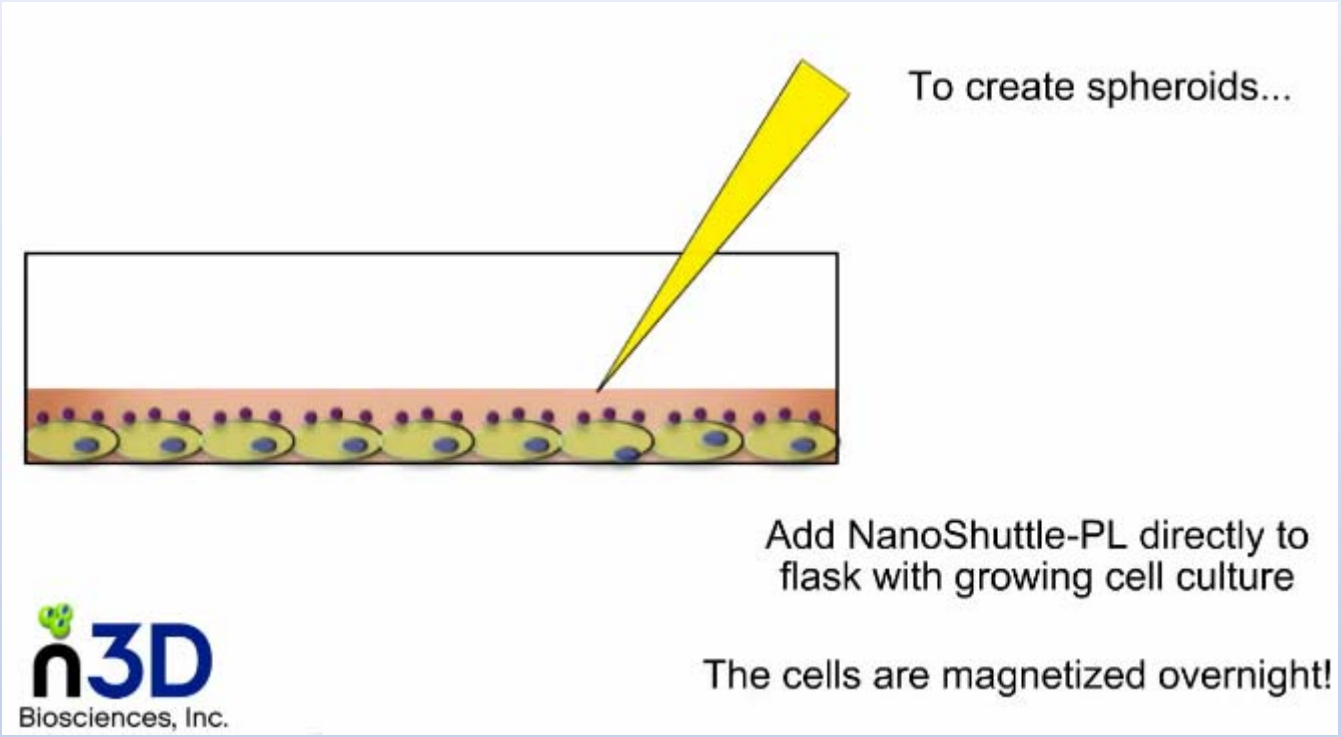

NanoShuttle™-PL

Biocompatible magnetic iron oxide - gold - nanoparticles (~50nm), coated with Poly-L-Lys, attaching electrostatically to the cell plasma membrane

Key features of the NanoShuttles™

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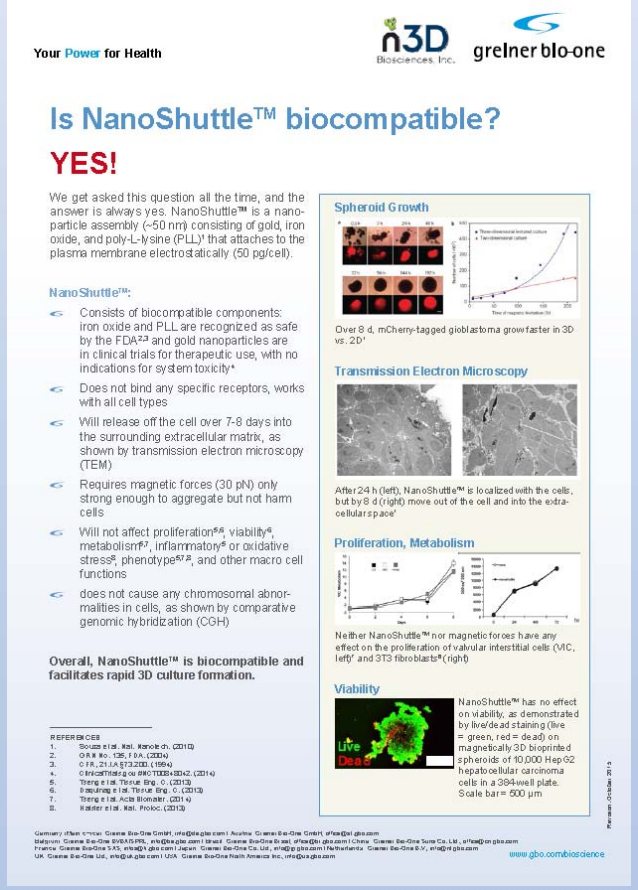
- No effect on viability, proliferation, inflammatory stress
- No interference on established analysis methods like e.g.
  - fluorescence assays
  - Western blot analysis
  - qRT-PCR
  - Viability assays



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n3D Biosciences, Inc. greiner bio-one

Is NanoShuttle™ biocompatible?

YES!

We get asked this question all the time, and the answer is always yes. NanoShuttle™ is a nanoparticle assembly (~50 nm) consisting of gold, iron oxide, and poly-L-lysine (PLL)[1] that attaches to the plasma membrane electrostatically (50 pg/cell).

NanoShuttle™:

- Consists of biocompatible components: iron oxide and PLL are recognized as safe by the FDA[2,3] and gold nanoparticles are in clinical trials for therapeutic use, with no indications for system toxicity[4]
- Does not bind any specific receptors, works with all cell types
- Will release off the cell over 7-8 days into the surrounding extracellular matrix, as shown by transmission electron microscopy (TEM)
- Requires magnetic forces (30 pN) only strong enough to aggregate but not harm cells
- Will not affect proliferation[5,6], viability[6], metabolism[6,7], inflammatory[8] or oxidative stress[8], phenotype[6,7,8], and other macro cell functions
- does not cause any chromosomal abnormalities in cells, as shown by comparative genomic hybridization (CGH)

Overall, NanoShuttle™ is biocompatible and facilitates rapid 3D culture formation.

REFERENCES
1. Souza et al., Nat. Nanotech. (2010)
2. GRAS no. 135, FDA, (2004)
3. CFR, 21 I.A §73.200, (1994)
4. ClinicalTrials.gov #NCT00933842, (2014)
5. Tseng et al. Tissue Eng. C. (2013)
6. Ruppinag et al. Tissue Eng. C. (2013)
7. Tseng et al. Acta Biomater. (2014)
8. Haisler et al. Nat. Protoc. (2013)

Spheroid Growth

Over 8 d, mCherry-tagged glioblastoma grow faster in 3D vs. 2D[1]

Transmission Electron Microscopy

After 24 h (left), NanoShuttle™ is localized with the cells, but by 8 d (right) move out of the cell and into the extracellular space[1]

Proliferation, Metabolism

Neither NanoShuttle™ nor magnetic forces have any effect on the proliferation of valvular interstitial cells (VIC, left)[7] and 3T3 fibroblasts[8] (right)

Viability

NanoShuttle™ has no effect on viability, as demonstrated by live/dead staining (live = green, red = dead) on magnetically 3D bioprinted spheroids of 10,000 Hep G2 hepatocellular carcinoma cells in a 384-well plate. Scale bar = 500 µm

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Basic principle

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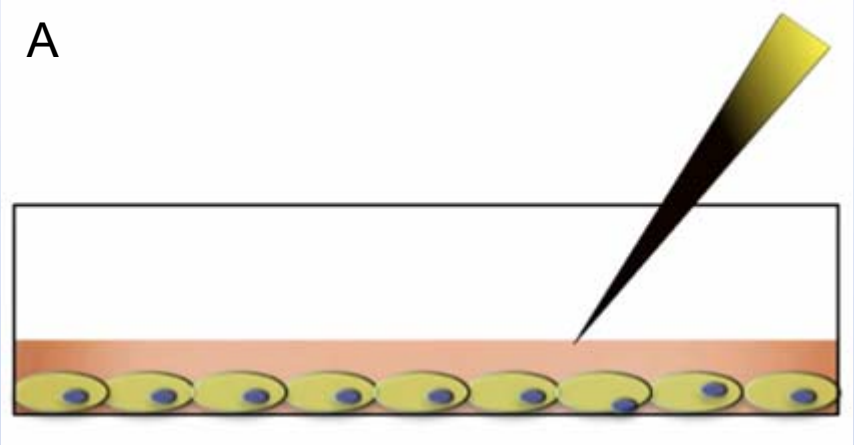

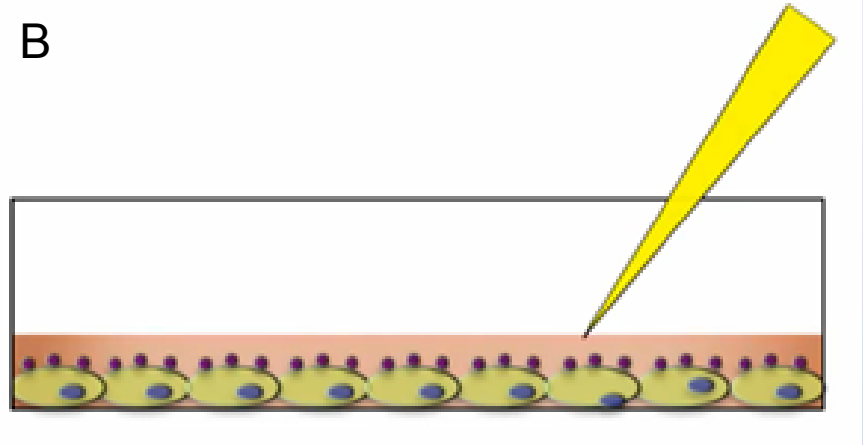

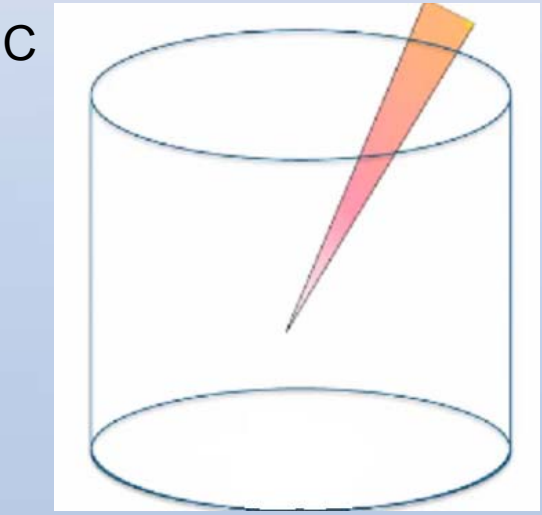

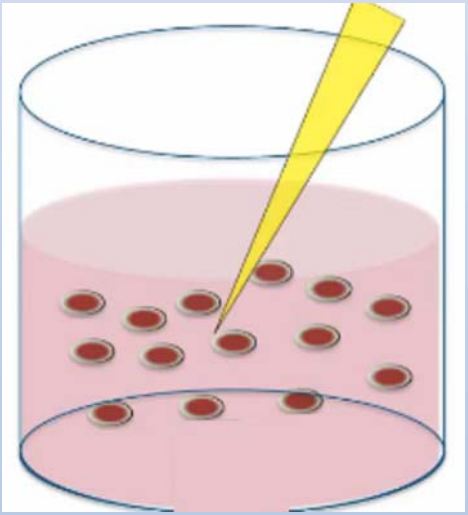

A cells in cell culture vessel
B add NanoShuttles to the cells and incubate overnight, harvest cells and
C/D transfer cells to wells of a cell repellent plate

Three approaches

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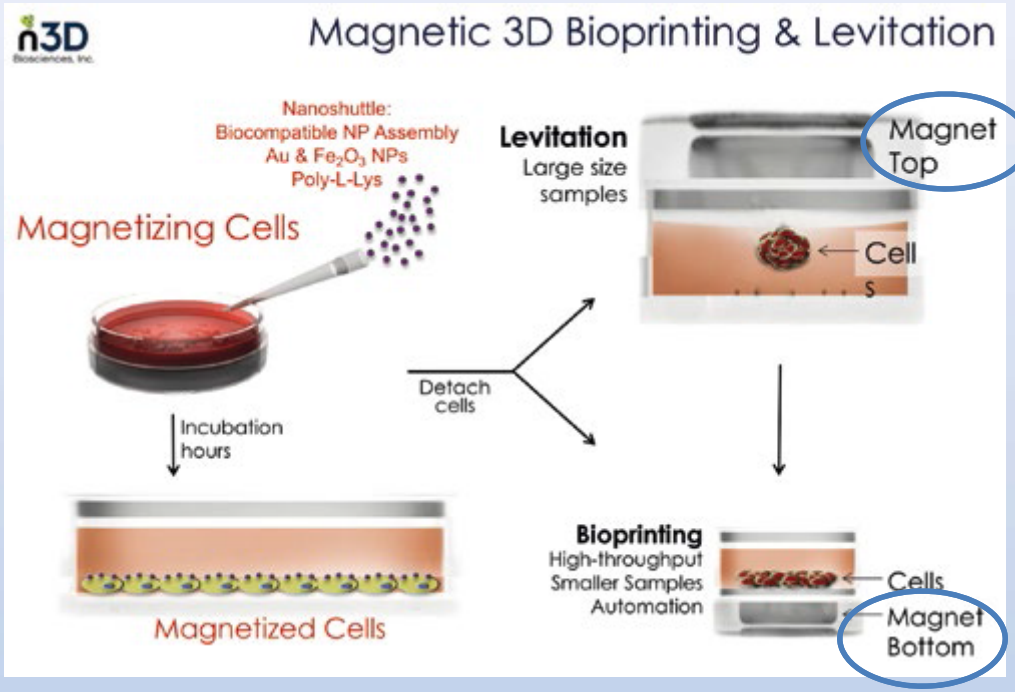
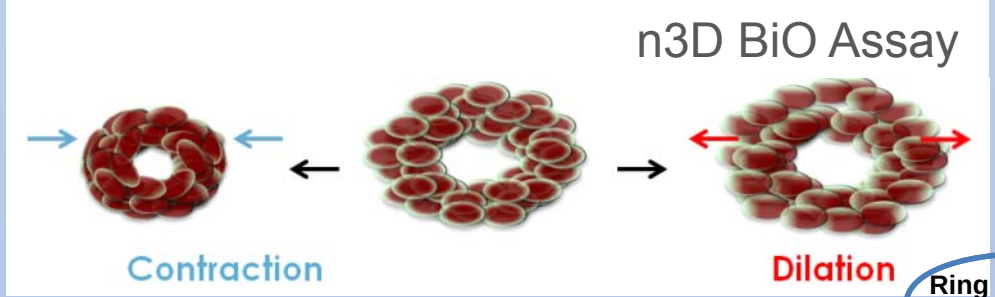


(I) Levitation
Floating 3D cell clusters
- Cell proliferation, ECM
- Organoids
- Genomics
- Protein analysis

(II) Bioprinting
3D spheroids at the well bottom
- Compound screening
- Toxicity screening
- Stem cell research



(III) n3D BiO Assay
3D ring formation
- Wound healing
- Toxicity screening
- Cardiovascular research

(I) Levitation – Magnet on top

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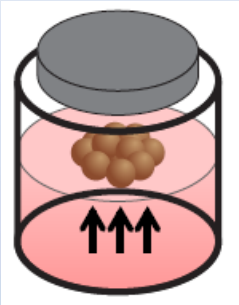
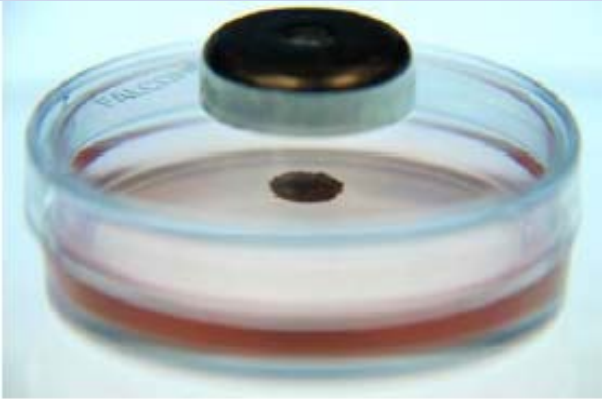
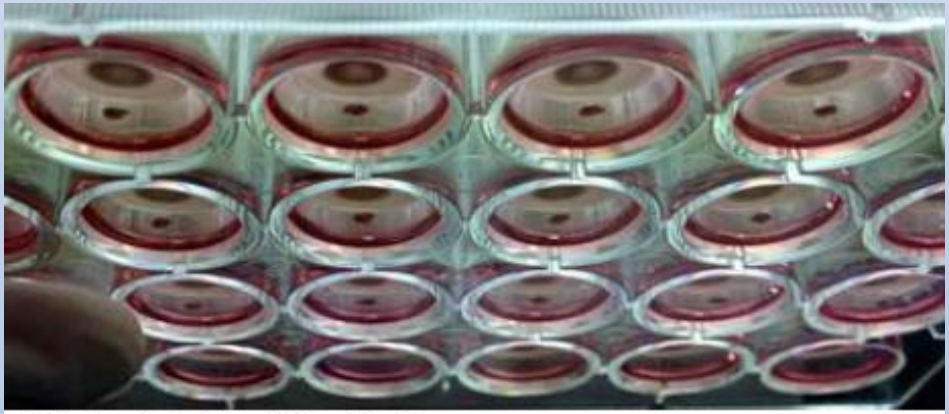
- Cell proliferation, ECM
- Organoids
- Genomics
- Protein analysis

Well Number	Magnetic Levitation 35 mm dish	6	24





Cells levitated in a 35 mm dish



Cells levitated in a 24-well plate

(I) Levitation – Magnet on top

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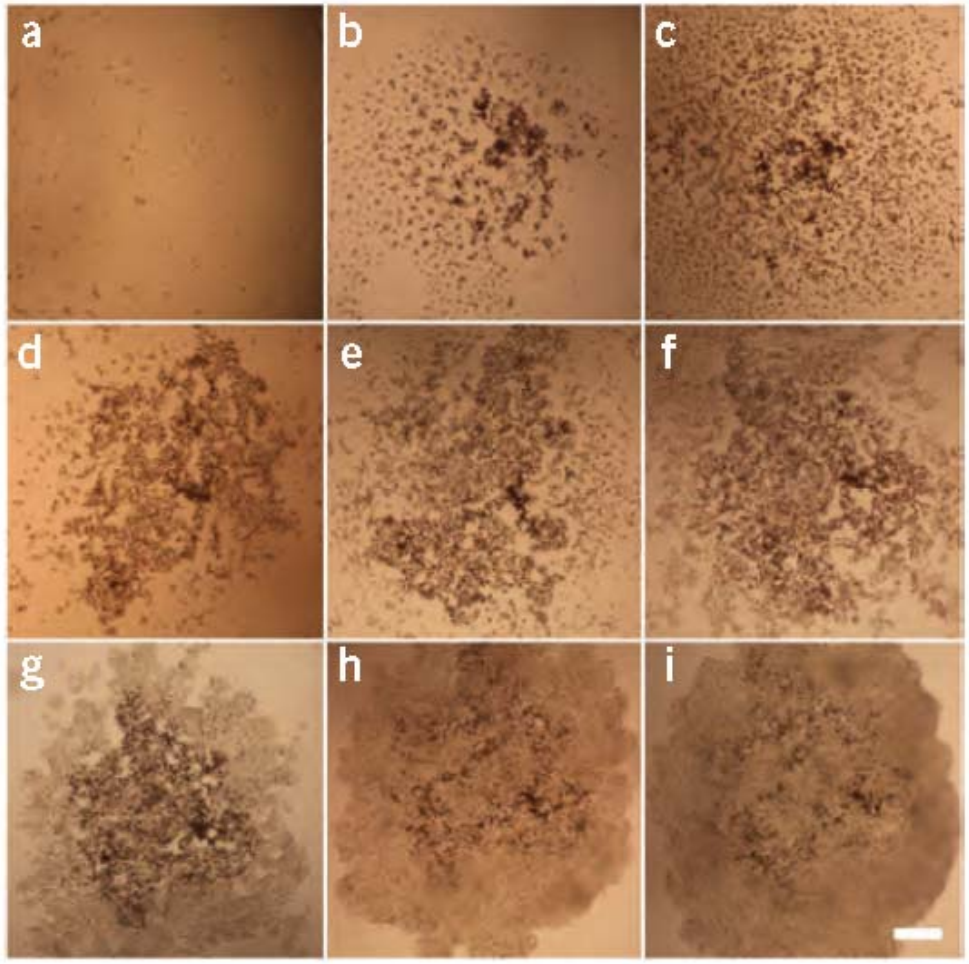

Figure 7 | Magnetically levitated 3D cultures of HepG2s. (**a**–**i**) After 0 min (**a**), 5 min (**b**), 15 min (**c**), 30 min (**d**), 45 min (**e**), 4 h (**f**), 24 h (**g**), 4 d (**h**) and 7 d (**i**). Scale bar, 250 μm.

Haisler et al., Nature Protocols, 2013

(I) Levitation – Magnet on top

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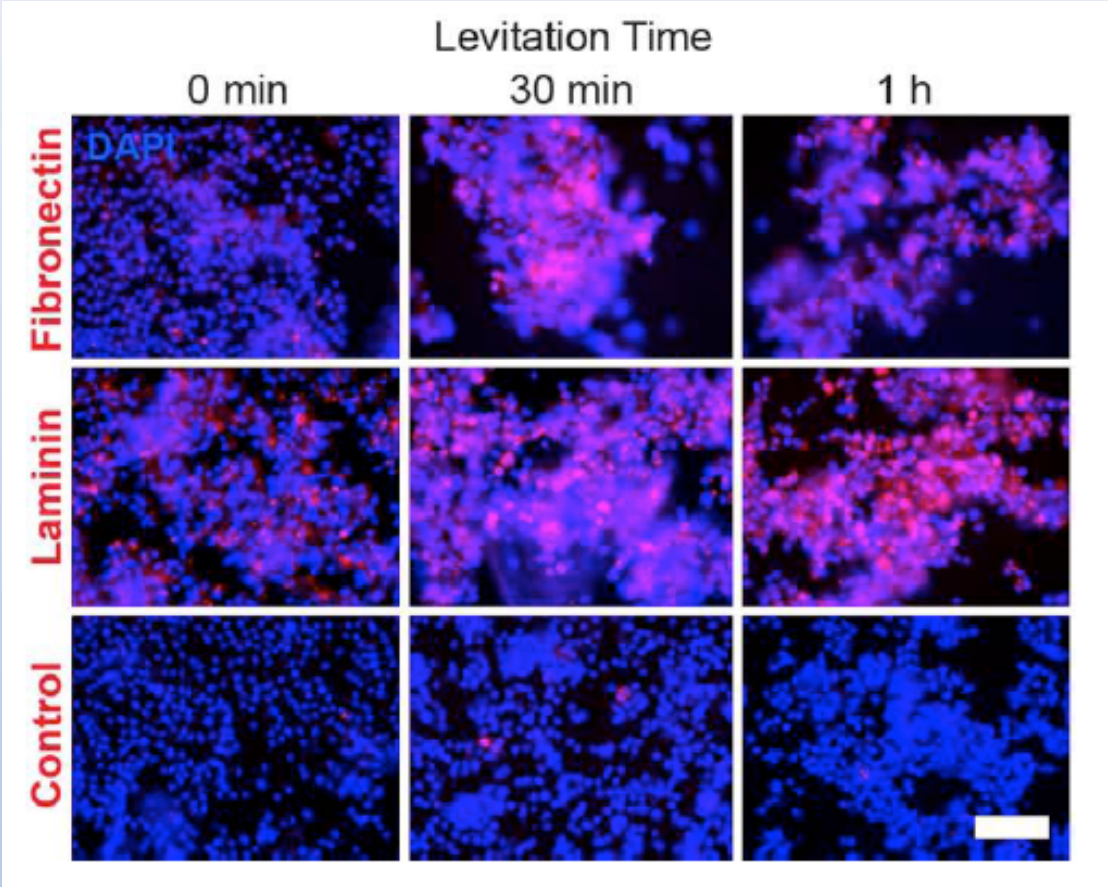
- Endogenous Extra-Cellular Matrix Formation



Immunohistochemical stains of levitated 3T3s for fibronectin (red) with varying levitation times. Nuclei are counterstained with DAPI (blue). Within an hour of levitation, 3T3s are extruding ECM in the form of fibronectin and laminin. Scale bar = 100 µm.

Haisler et al., Nature Protocols, 2013
Tseng et al. Tissue Engineering – C, 2013

(I) Levitation – Magnet on top

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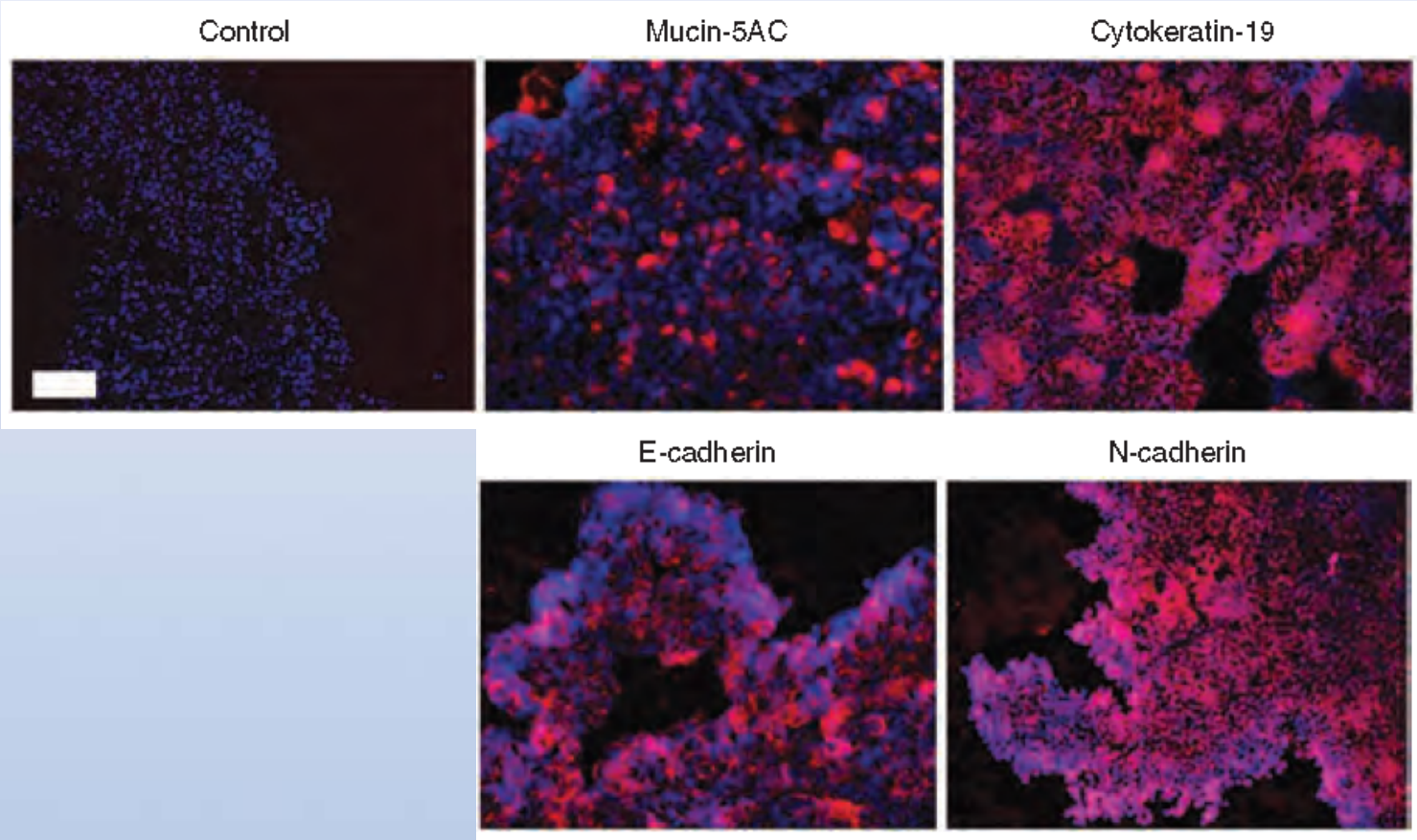

Immunohistochemical staining patterns of 3D cultures of A549s for mucin-5AC, cytokeratin-19, E-cadherin and N-cadherin. 175,000 cells per culture in 400 µl of medium. Positive staining patterns for mucin-5AC, cytokeratin-19 and E-cadherin verified epithelial phenotype and function, whereas N-cadherin demonstrated cell-cell interactions within the 3D culture. Scale bar, 100 µm.

Haisler et al., Nature Protocols, 2013
Tseng et al. Tissue Engineering – C, 2013

Magnetic Pen for the assembly of co-cultures

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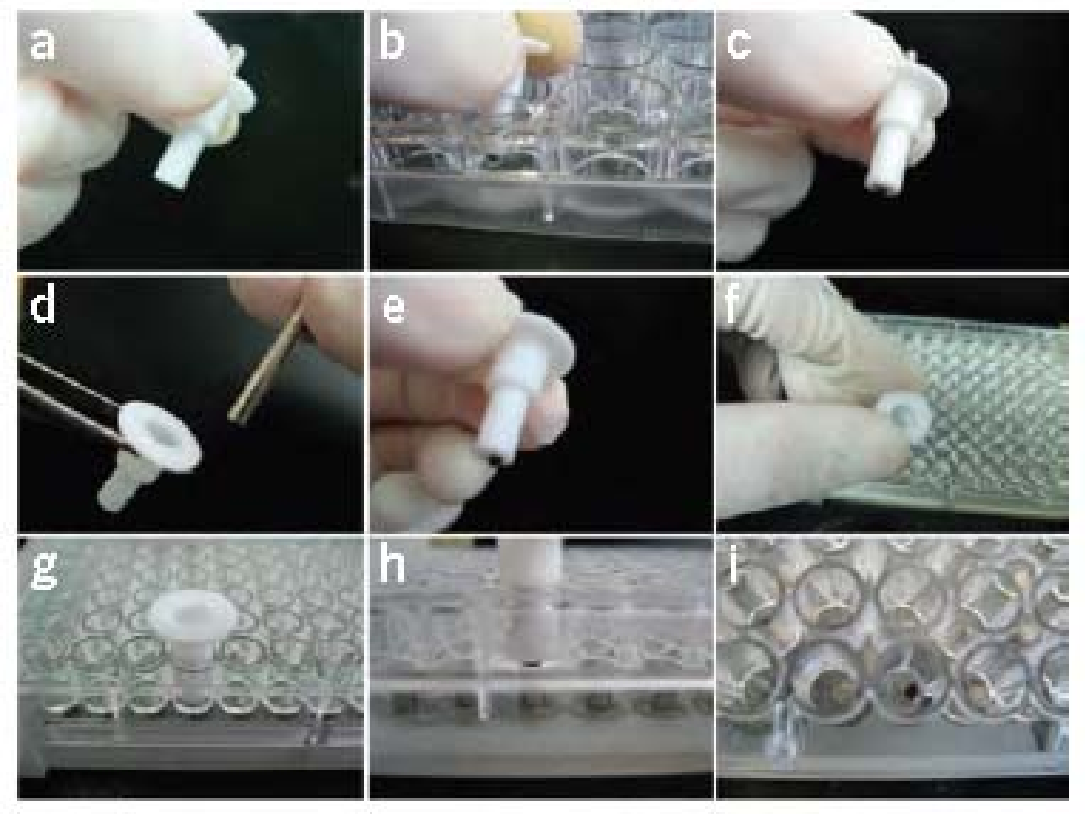
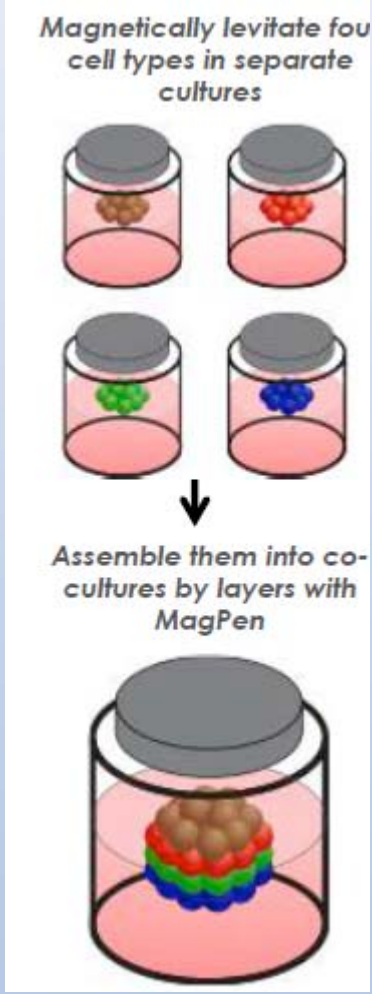
- Transfer of magnetized 3D cultures





Tseng et al. Tissue Engineering - C, January 2013

Bronchiole Co-Culture

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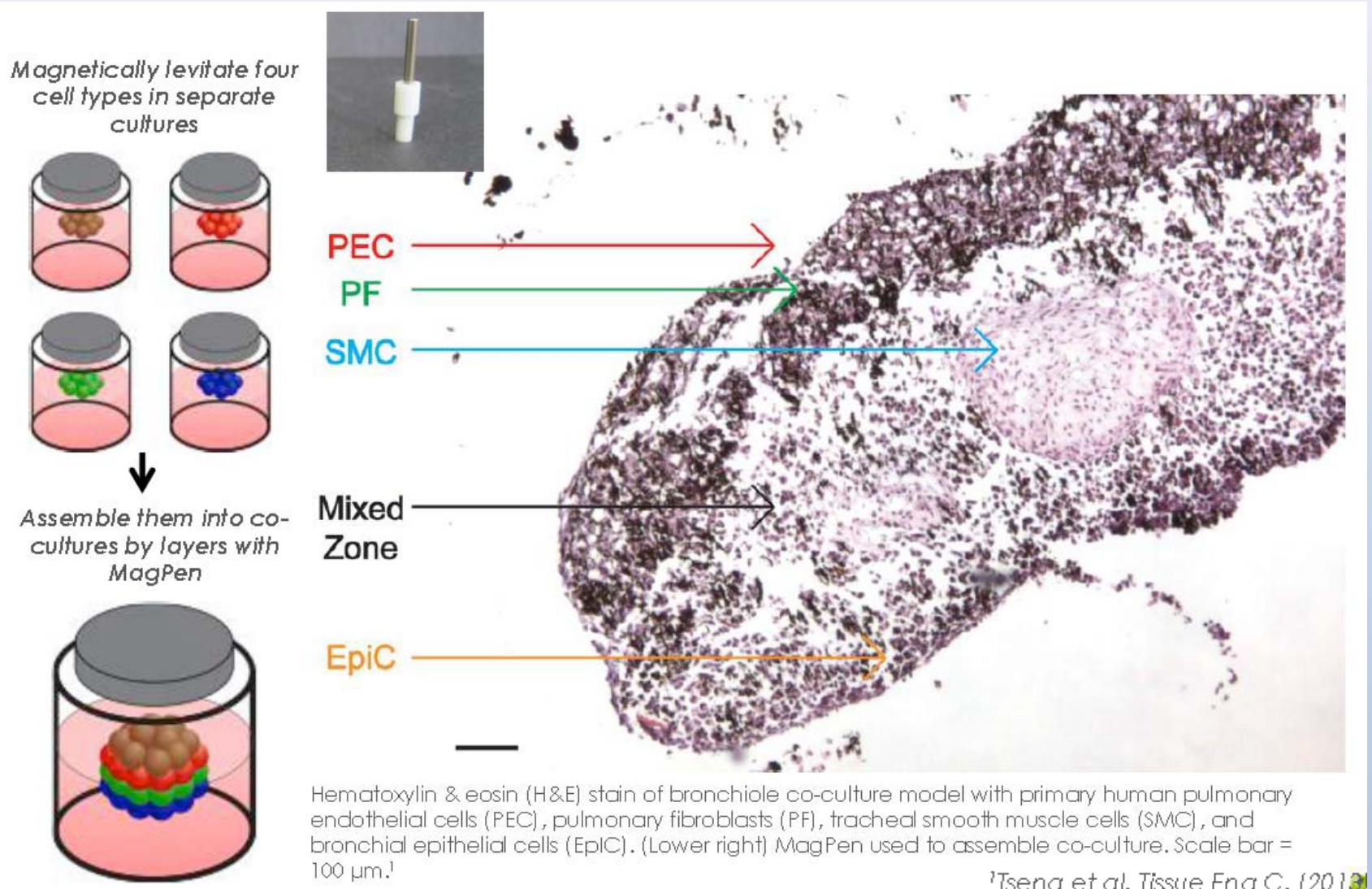

Hematoxylin & eosin (H&E) stain of bronchiole co-culture model with primary human pulmonary endothelial cells (PEC), pulmonary fibroblasts (PF), tracheal smooth muscle cells (SMC), and bronchial epithelial cells (EpIC). (Lower right) MagPen used to assemble co-culture. Scale bar = 100 µm.[1]

[1]Tseng et al. Tissue Eng C. (2013)

Co-cultures

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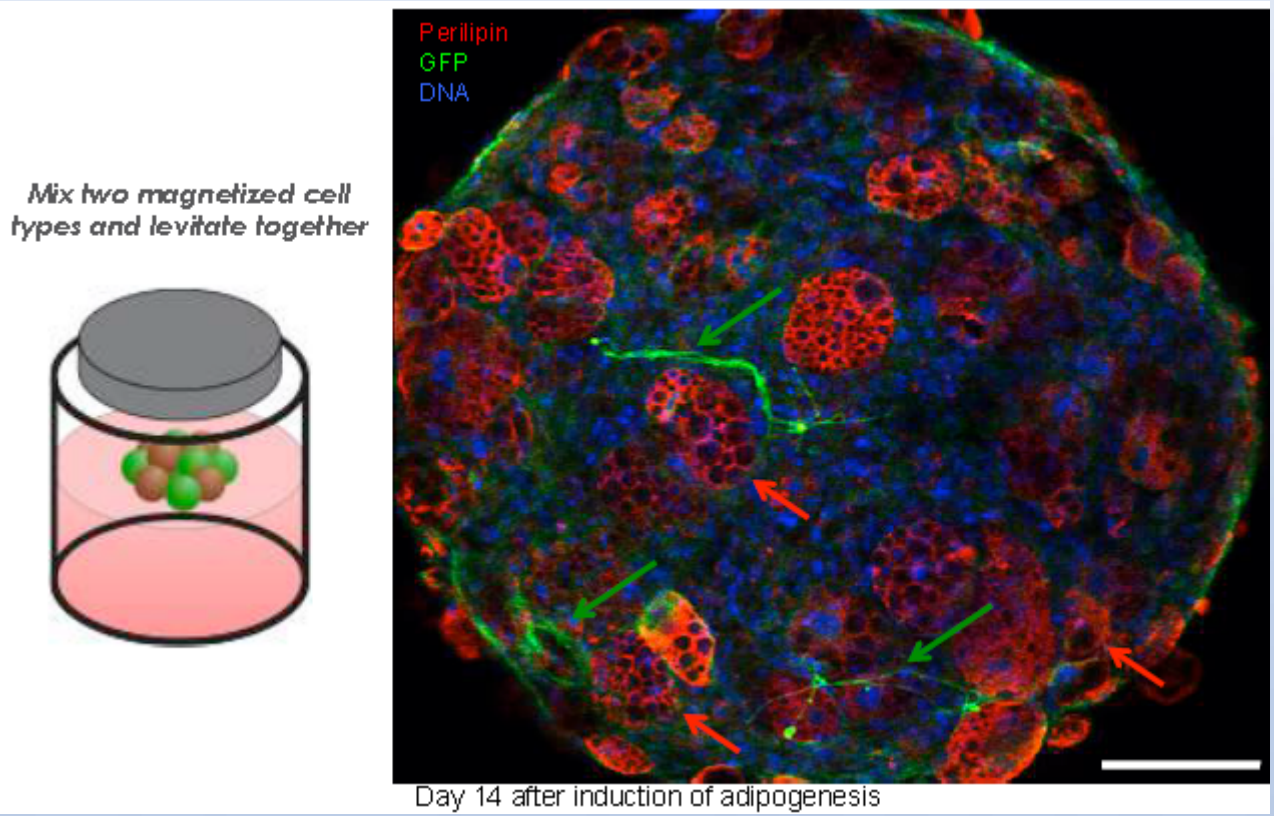
- Adiposphères with endothelial network formation and adipocyte differentiation



Whole-mount immunofluorescence showing bEND.3-GFP endothelial cells formed microvessels within the adiposphere.

Daquinag et al. Tissue Engineering - C, October 2012

Invasion Assay & Co-Culture

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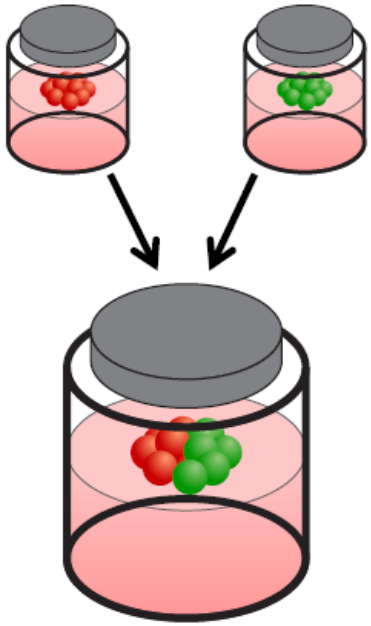
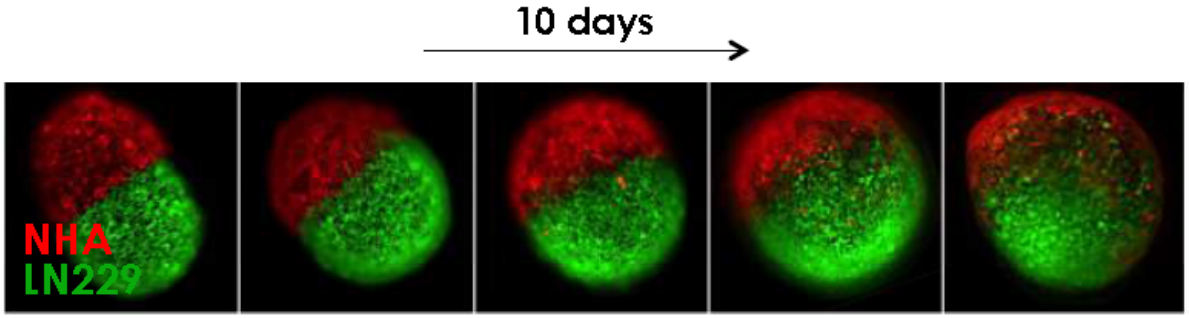
Levitate astrocytes (red) and glioblastoma (green) separately



Add to same well and allow to glioblastoma to compete and invade



Invasion assay of normal human astrocytes (NHA, red) and glioblastoma (LN229, green) over 10 days[1,2]

[1]Souza et al. Nat Nanotech. (2010); [2]Molina et al. Neoplasia. (2010)

(II) Magnetic 3D Bioprinting

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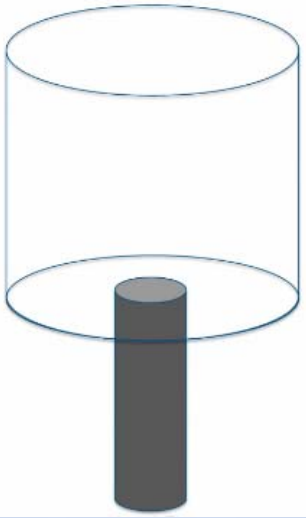
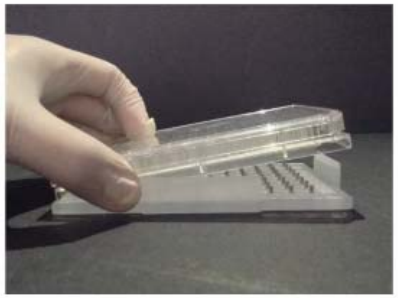
- Compound screening
- Toxicity screening
- Stem cell applications

Well Number	Spheroid Bioprinting	
	96	384

(1)

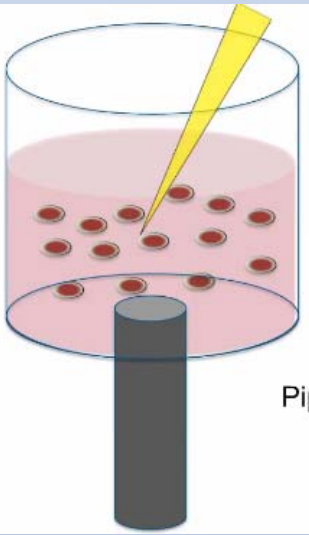


Take a cell-repellent 96 or 384 well plate and place on the spheroid drive



n3D Biosciences, Inc.

(2)



Pipet magnetized cells into each well

n3D Biosciences, Inc.

(II) 3D Bioprinting – Magnet at bottom

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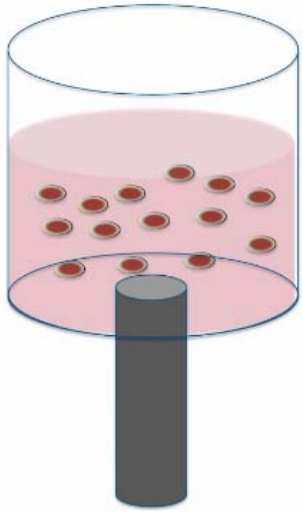
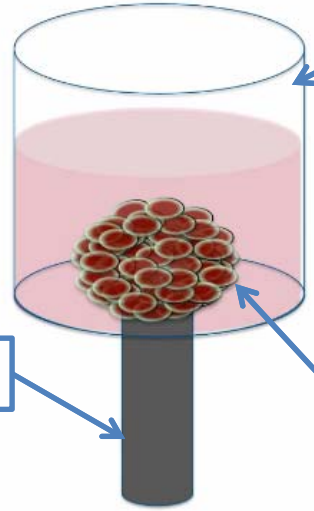
(3)



The magnet will attract cells to the bottom of the well

n3D Biosciences, Inc.

(4)



Well of a microplate

A spheroid forms!

You can leave the spheroids on the magnet between 15 min to overnight to make a competent spheroid

Magnet

Bioprinted cells

n3D Biosciences, Inc.

(II) 3D Bioprinting – Magnet at bottom

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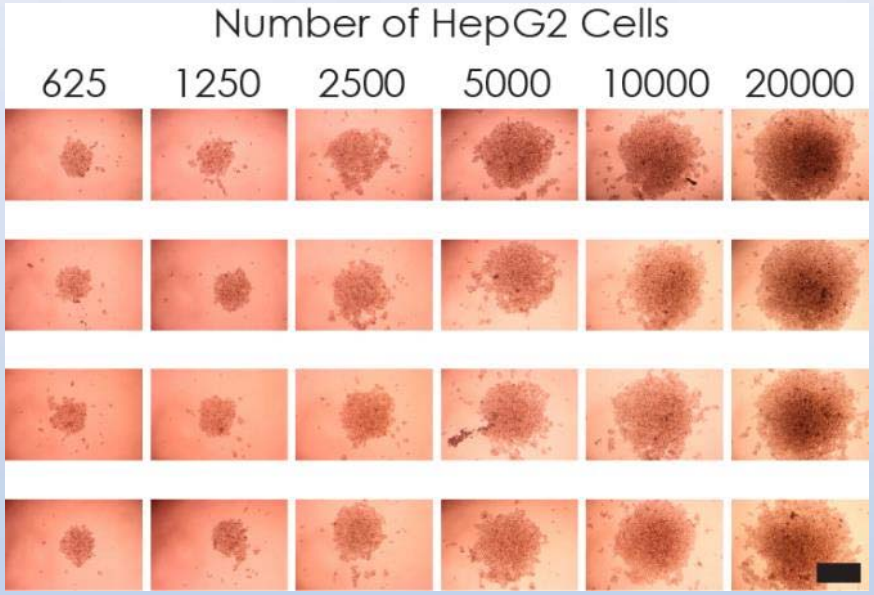

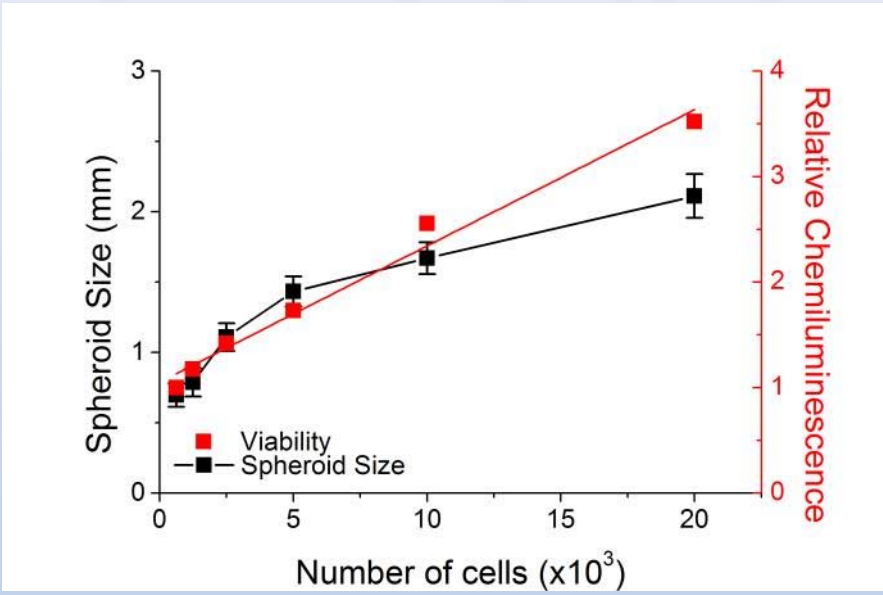

Left: Magnetically 3D bioprinted spheroids of HepG2 hepatocytes in a 384-well plate of various cell numbers after 15 min of printing. Right: Spheroid size and viability (CellTiter-Glo, Promega) as a function of cell number. Scale bar = 500 µm.

(II) Compound / Toxicity screening with bioprinted spheroids

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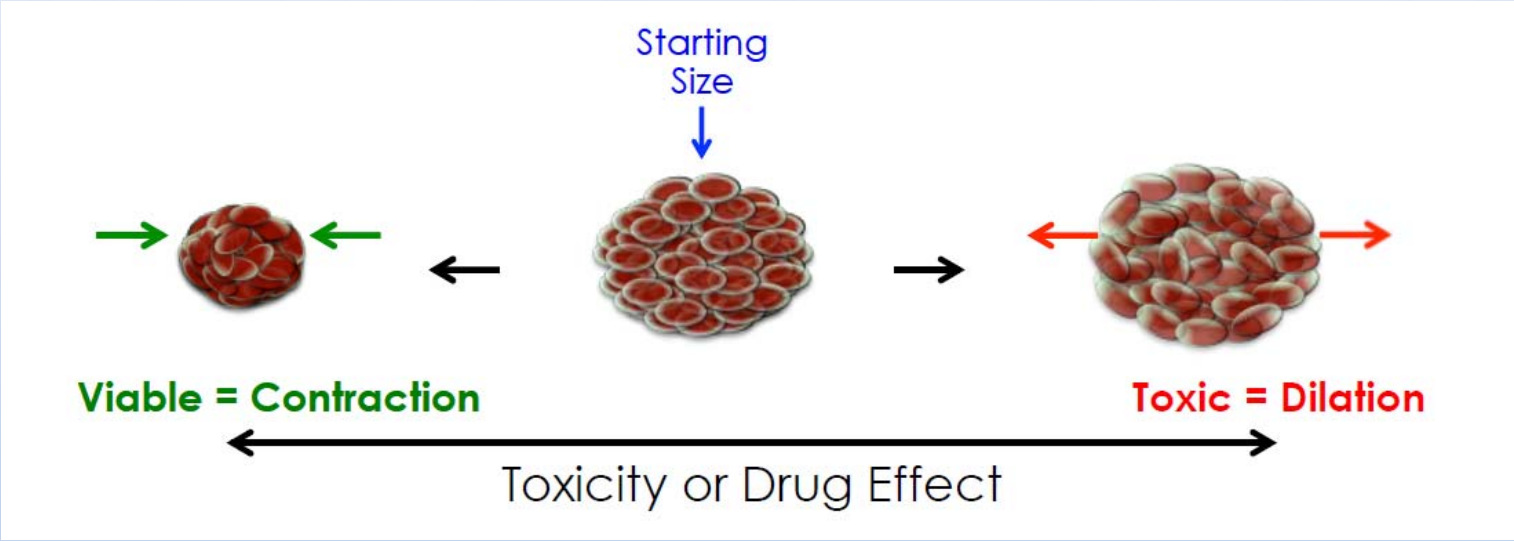

Assay is monitored over 2 days

Spheroid formation in 96 well U-bottom plates with cell-repellent surface

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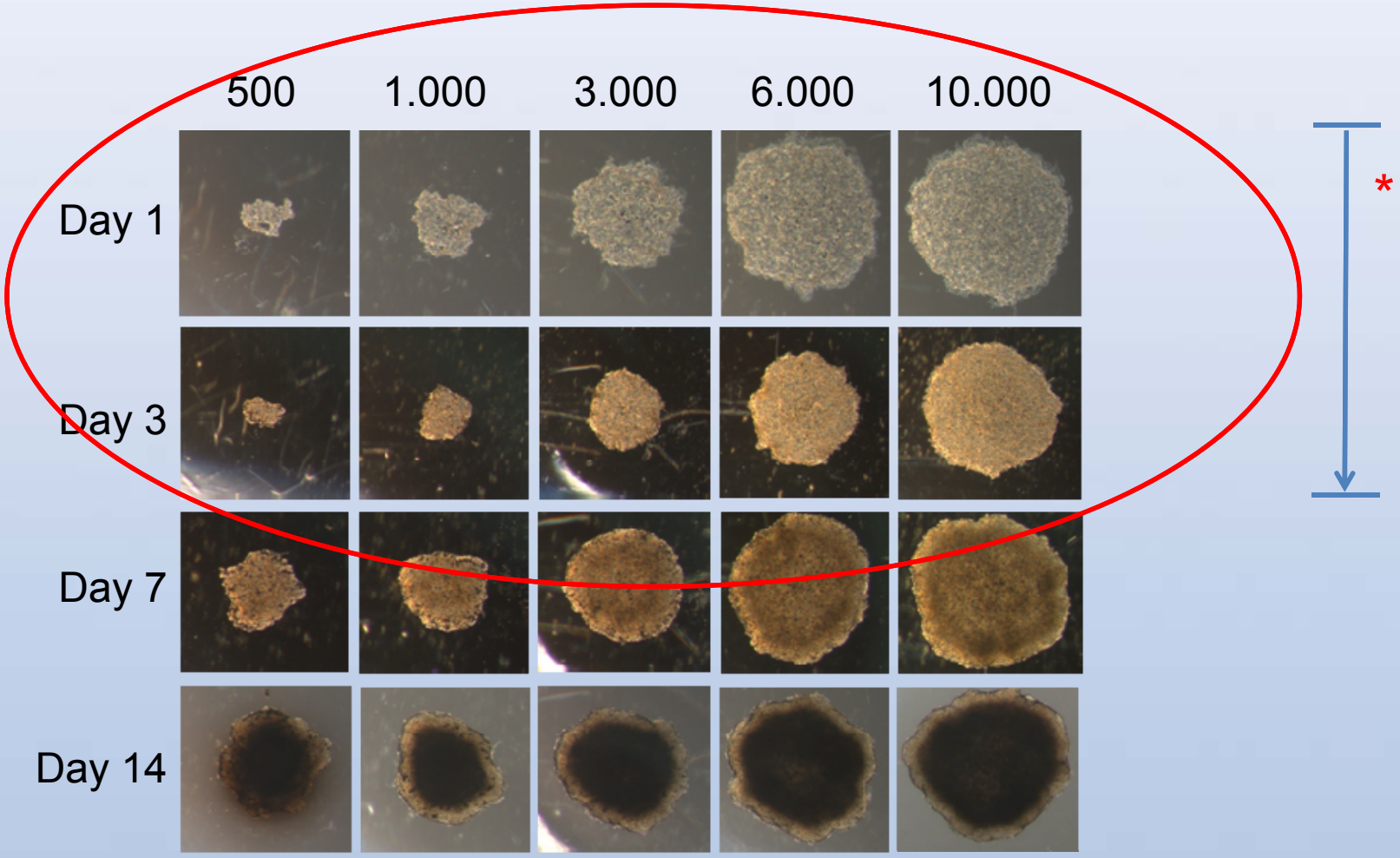

*Contraction of cells from day 1 to day 3!

(II) Toxicity assay

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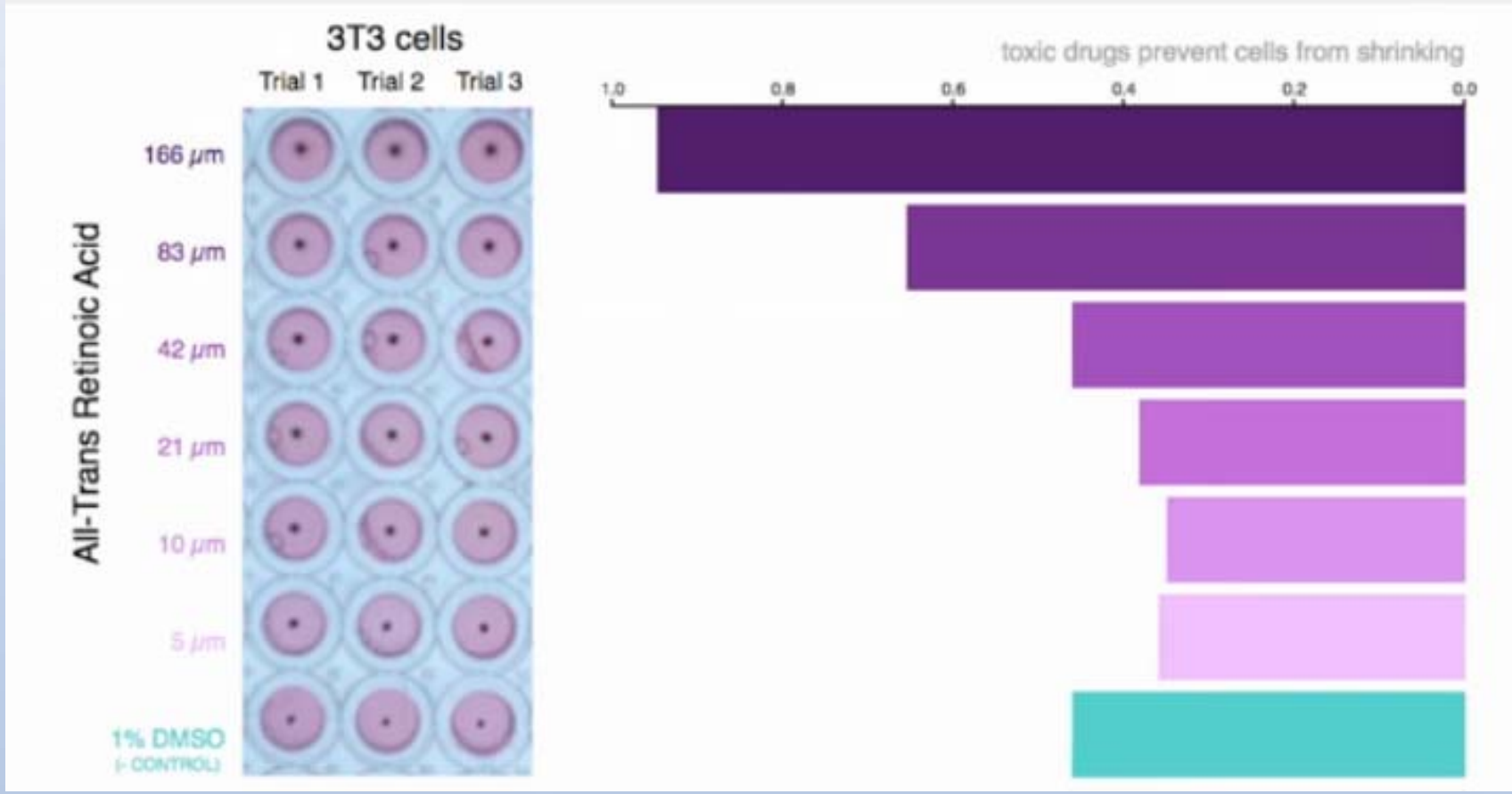
Reduction of spheroid diameter = cells are viable (see low drug concentrations)



http://youtu.be/WqXLCEv1eKI

(II) Toxicity assay

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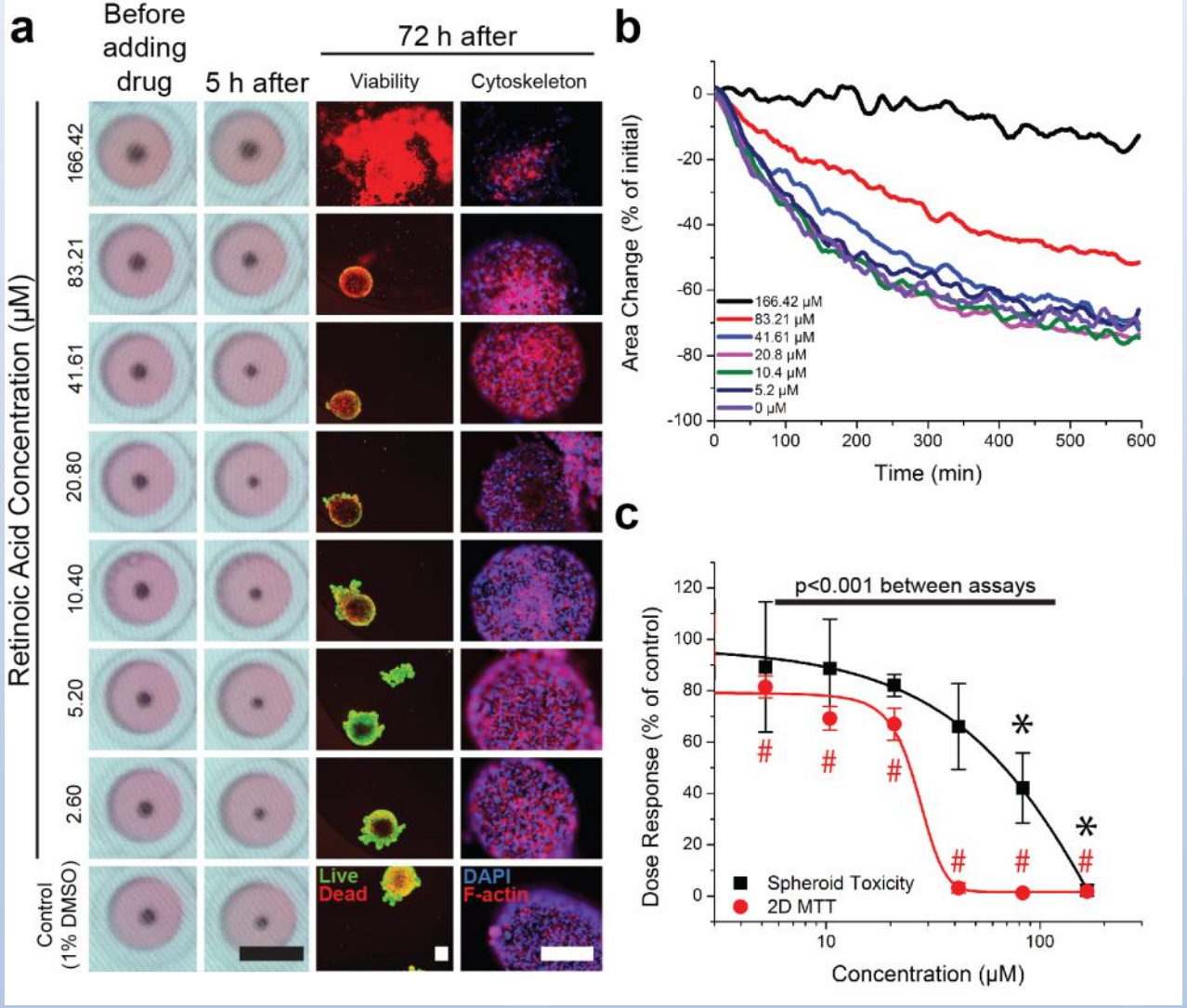

Toxicity Screening with 3T3 spheroids

(II) Stem cell research

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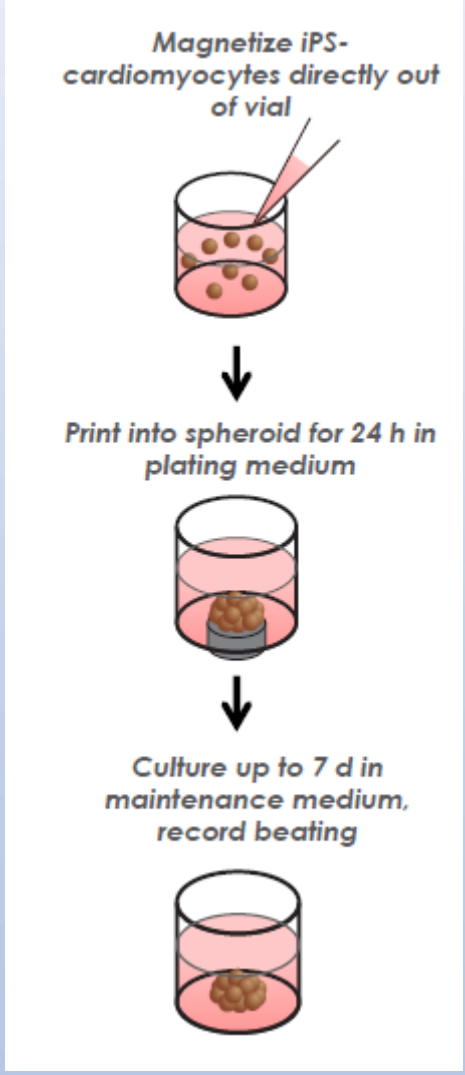

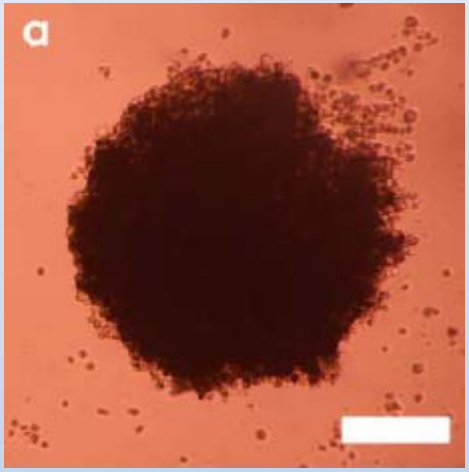

- iPSC Cardiomyocytes

https://www.youtube.com/watch?v=3PtyZicjQ3c

(III)n3D BiO Assay / Ring Drive

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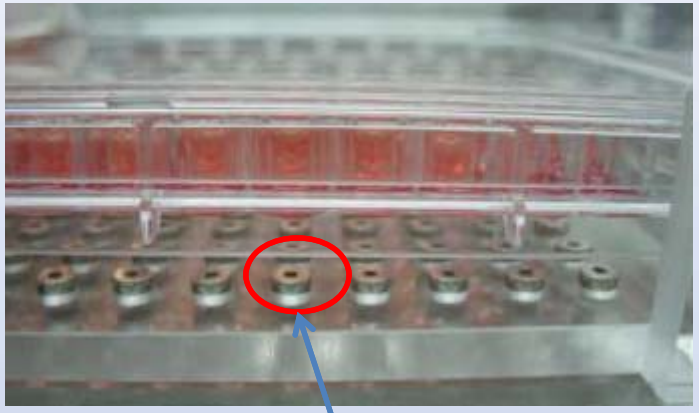
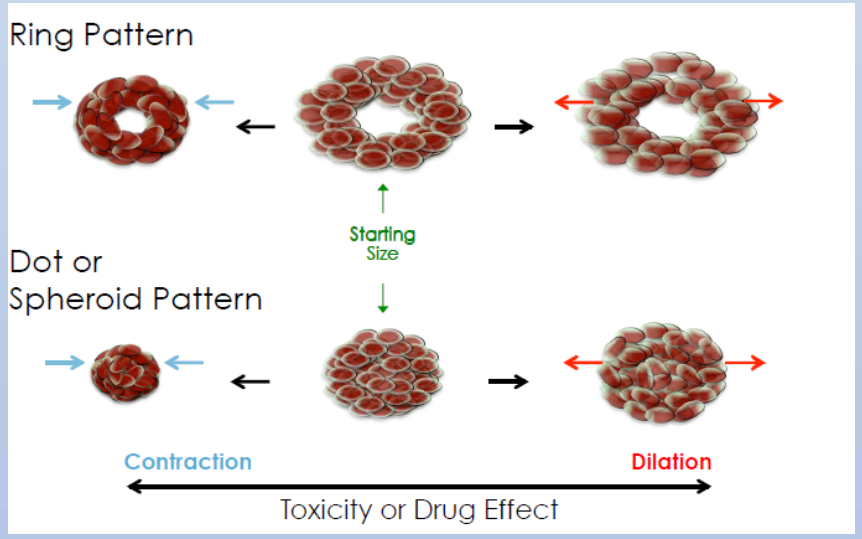
- Animal-free assays / cosmetics industry
- Toxicity screening
- Cardiovascular research



Ring Drive – ring-shaped magnet

https://www.youtube.com/watch?v=OwDhfBQvWis

Formation 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



(III)n3D BiO Assay / Ring Drive

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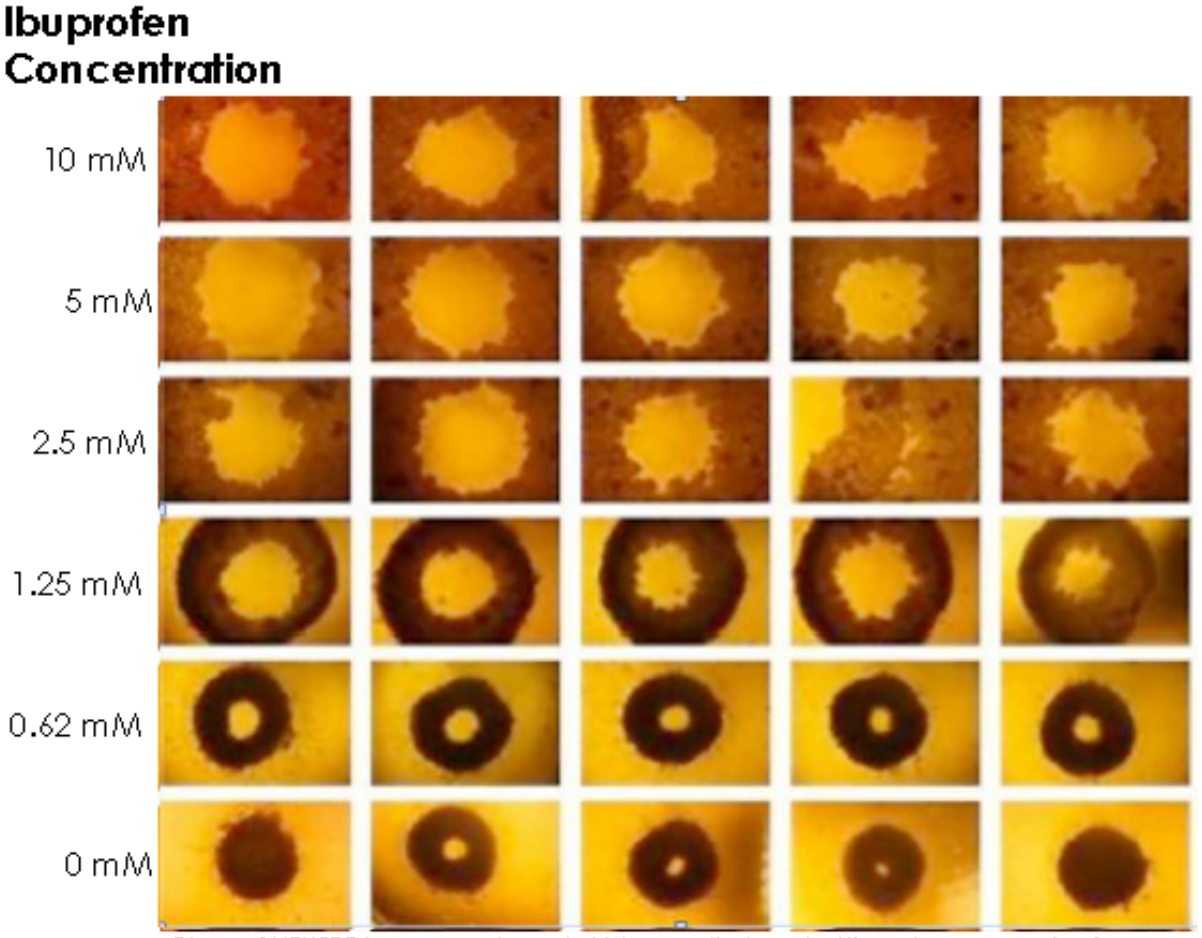
Closing the void = cells are viable (see low drug concentrations)



Rings of HEK293 human embryonic kidney cells dosed with various amounts of ibuprofen.[1]

[1]Timm et al. Sci Rep. (2013)

Imaging - iPOD™ vs. Microscope

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Contraction/shrinkage of spheroids can be 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.

Imaging - iPOD™ vs. Microscope

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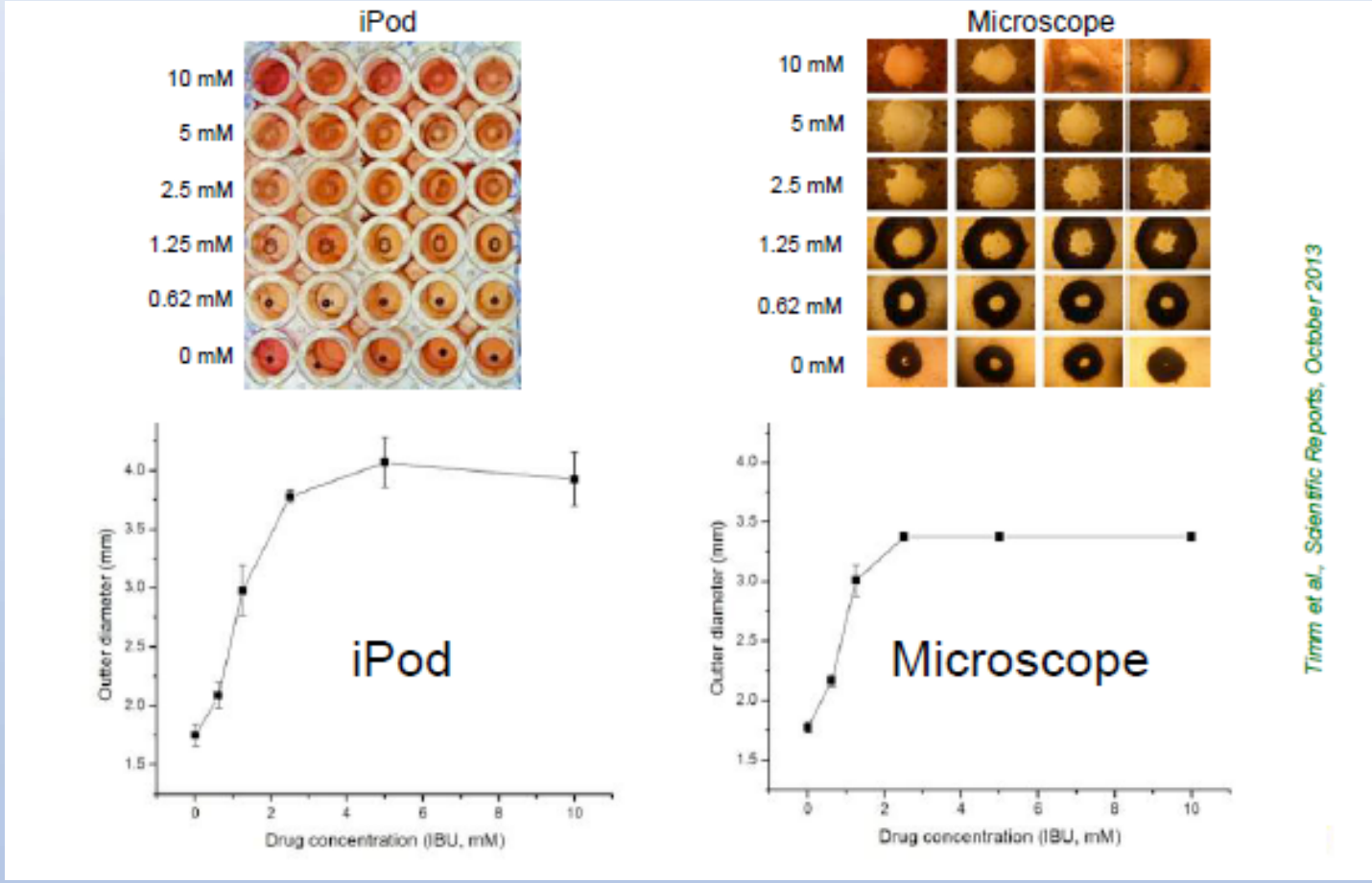
Easy imaging with the iPOD™

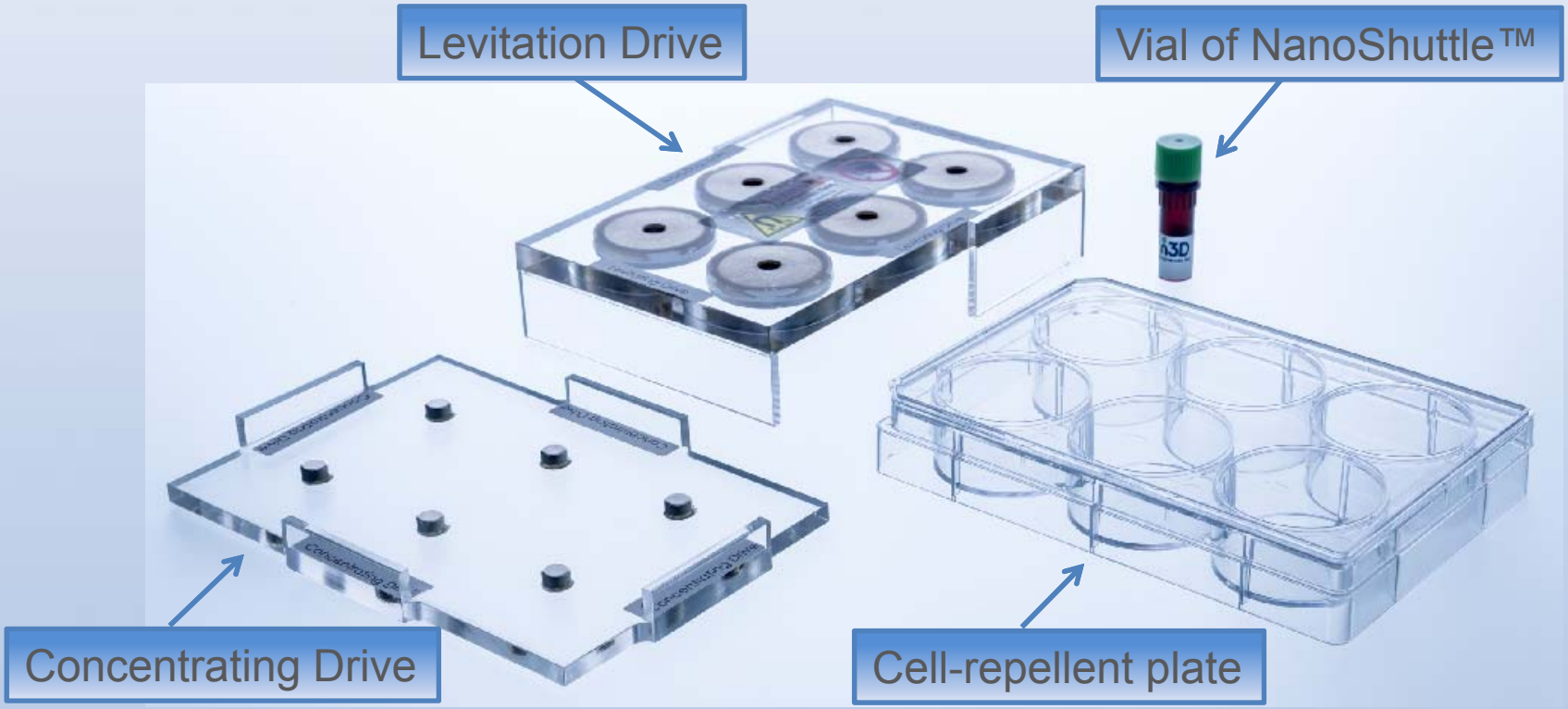
Impact of Ibuprofen on HEK 239



Products

greiner bio-one
Your Power for Health

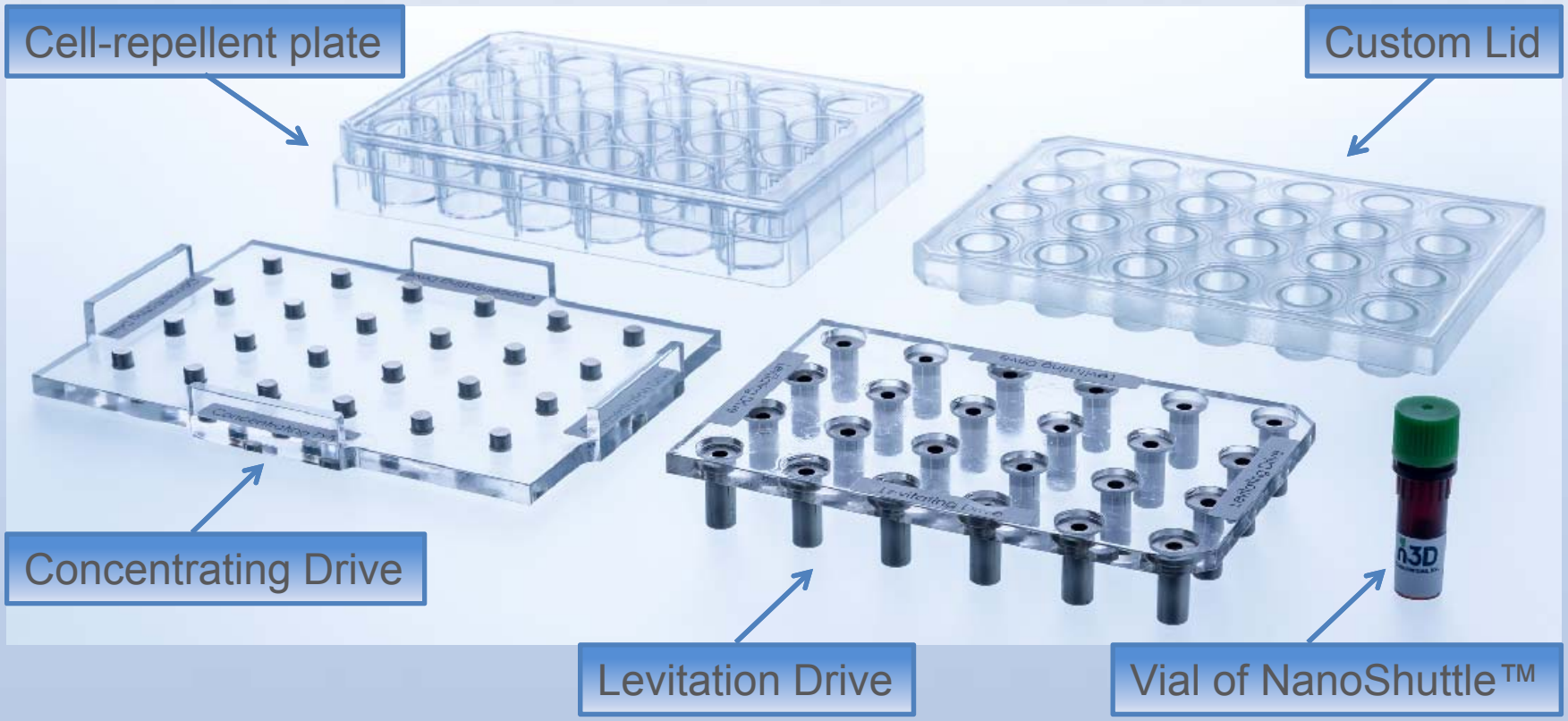
- Levitation
  - 657 840 6-Well Bio-Assembler Kit (available on stock)



Products

greiner bio-one
Your Power for Health

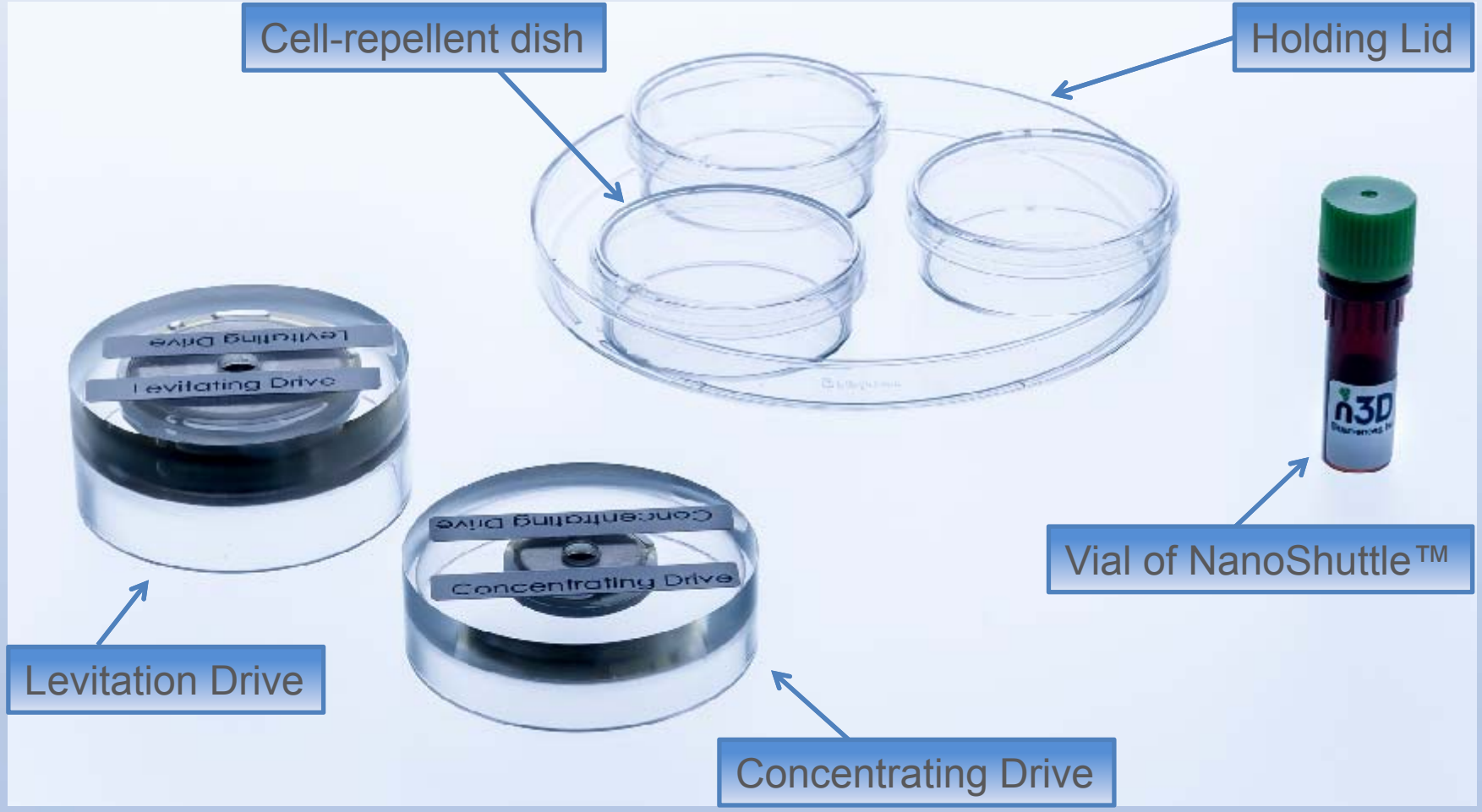
- Levitation
  - 662 840 24-Well Bio-Assembler Kit (available on stock)



Products

greiner bio-one
Your Power for Health

- Levitation
  - 627 840 Single-Well Bio-Assembler Kit (available on request)



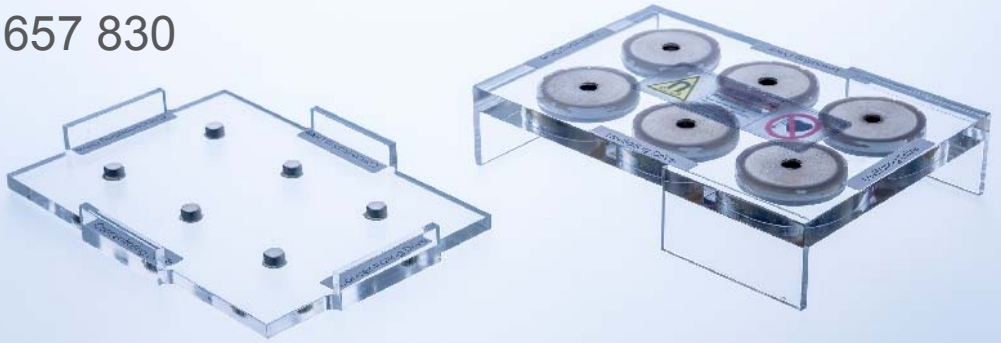
Products

greiner bio-one
Your Power for Health

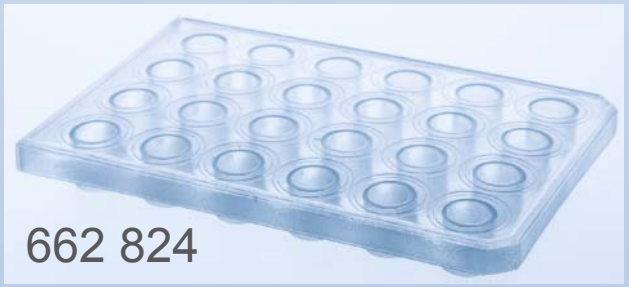
- Levitation
  - Magnets (single-, 6-, 24-well) and 24-Well lid (on request)

627 830

657 830



662 830

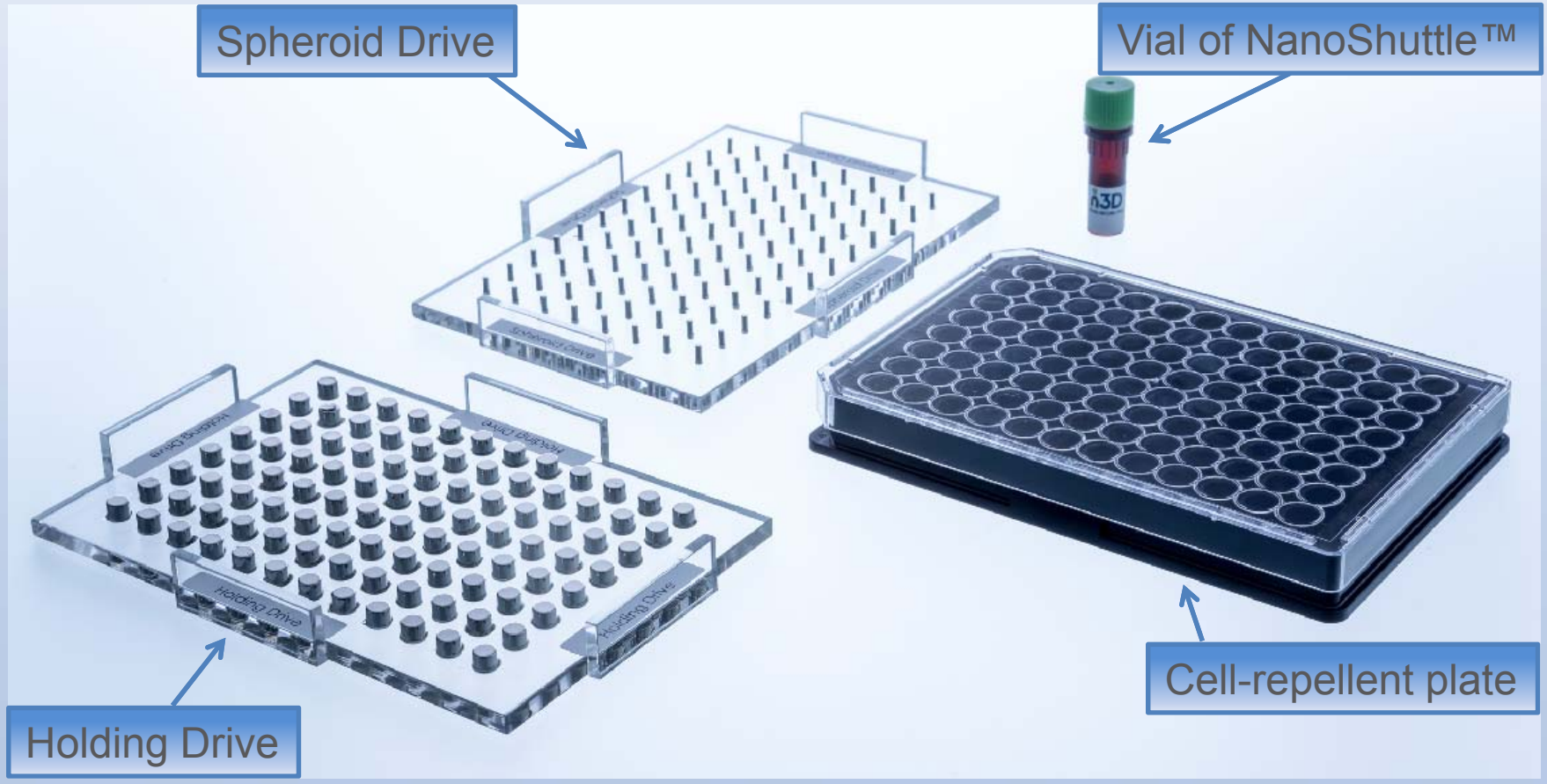


662 824

Products

greiner bio-one
Your Power for Health

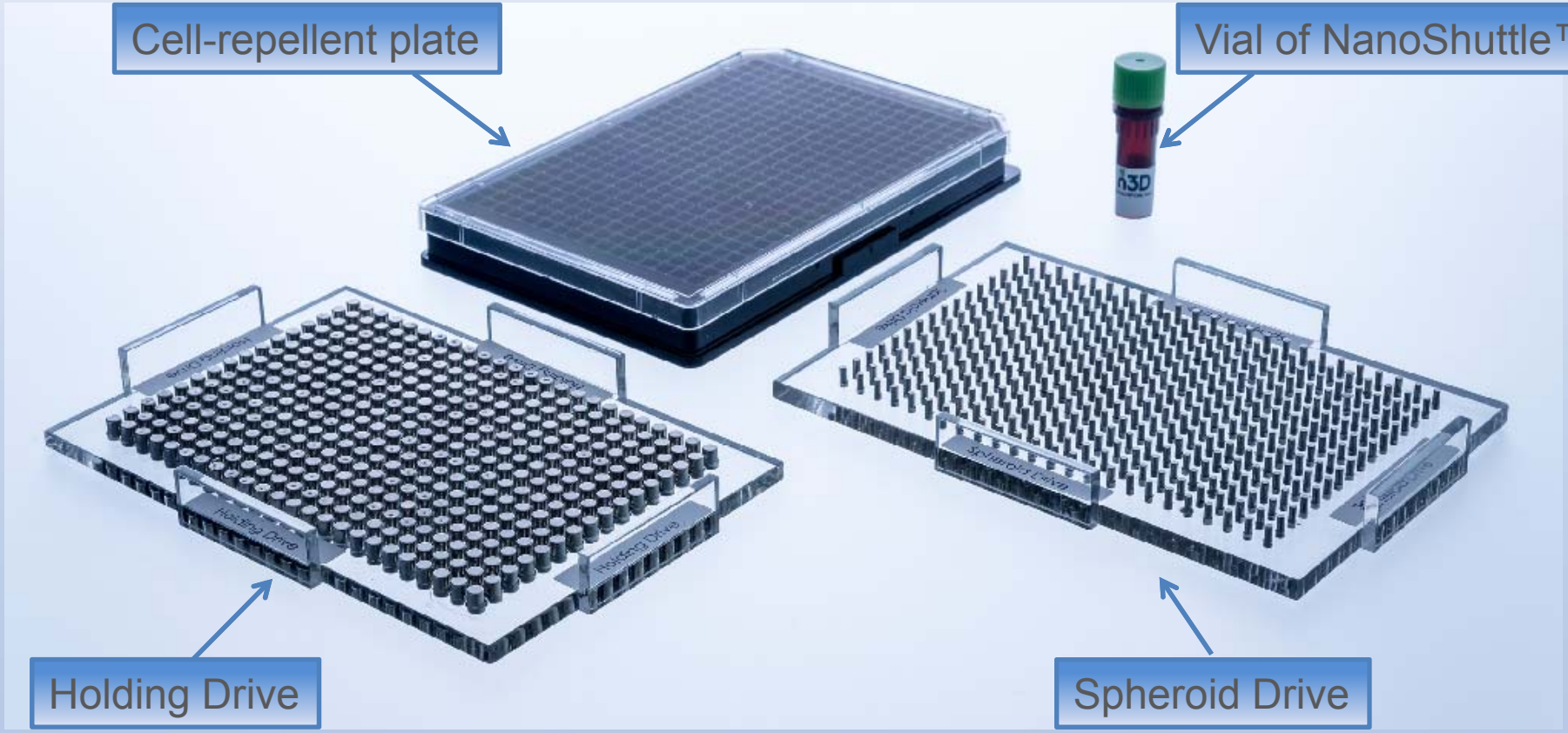
- Bioprinting
  - 655 841 96-Well Bioprinting Kit – Black Plates (on stock)



Products

greiner bio-one
Your Power for Health

- Bioprinting
  - 781 841 384-Well Bioprinting Kit – Black Plates (on stock)



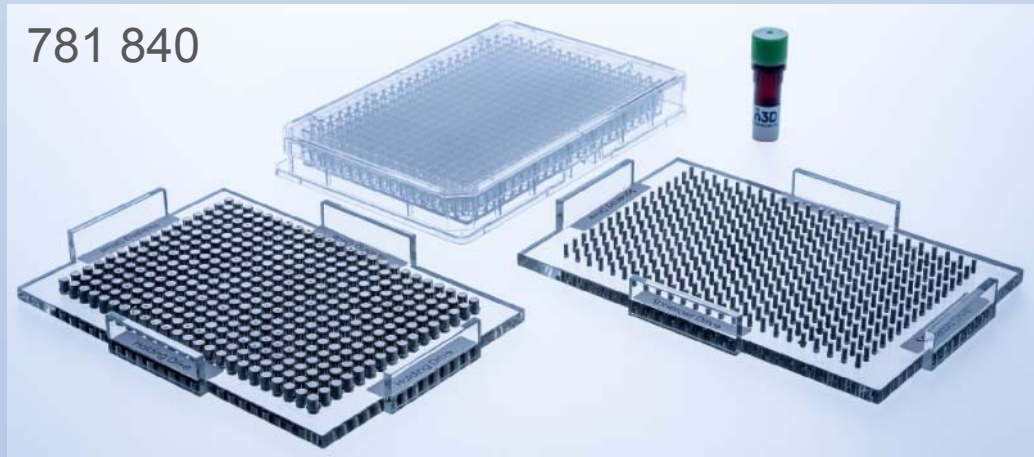
Products

greiner bio-one
Your Power for Health

- Bioprinting
  - 96-Well and 384-Well Bioprinting Kit with clear plates (on request)

655 840

781 840

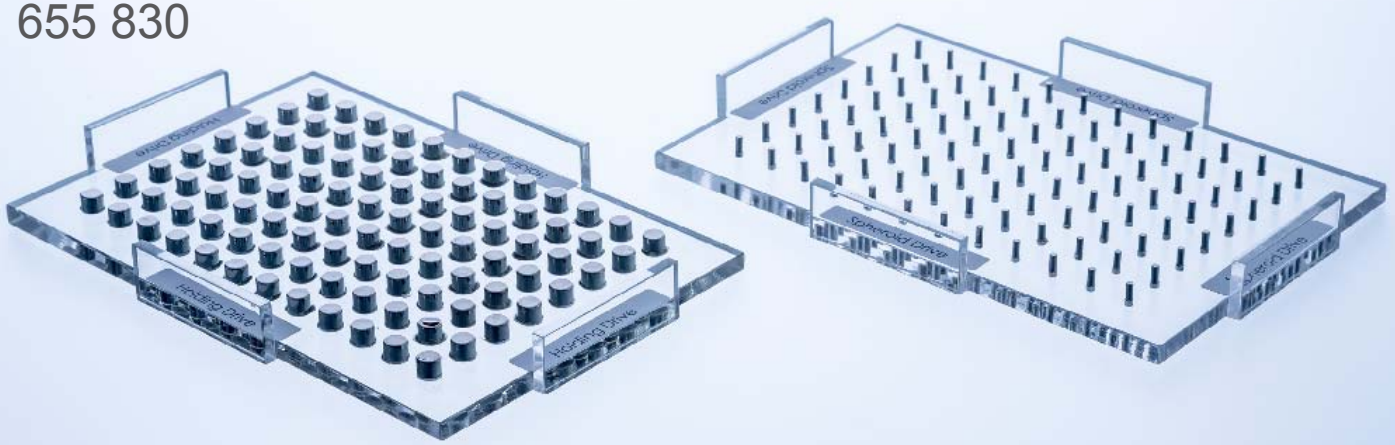


Products

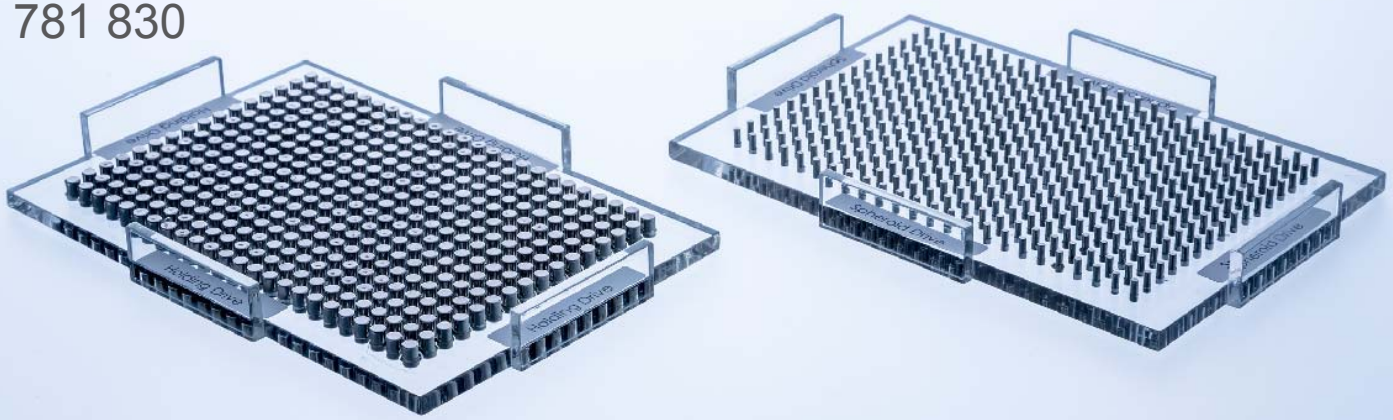
greiner bio-one
Your Power for Health

- Bioprinting
  - 96-Well Drives and 384-Well Drives (available on stock)

655 830



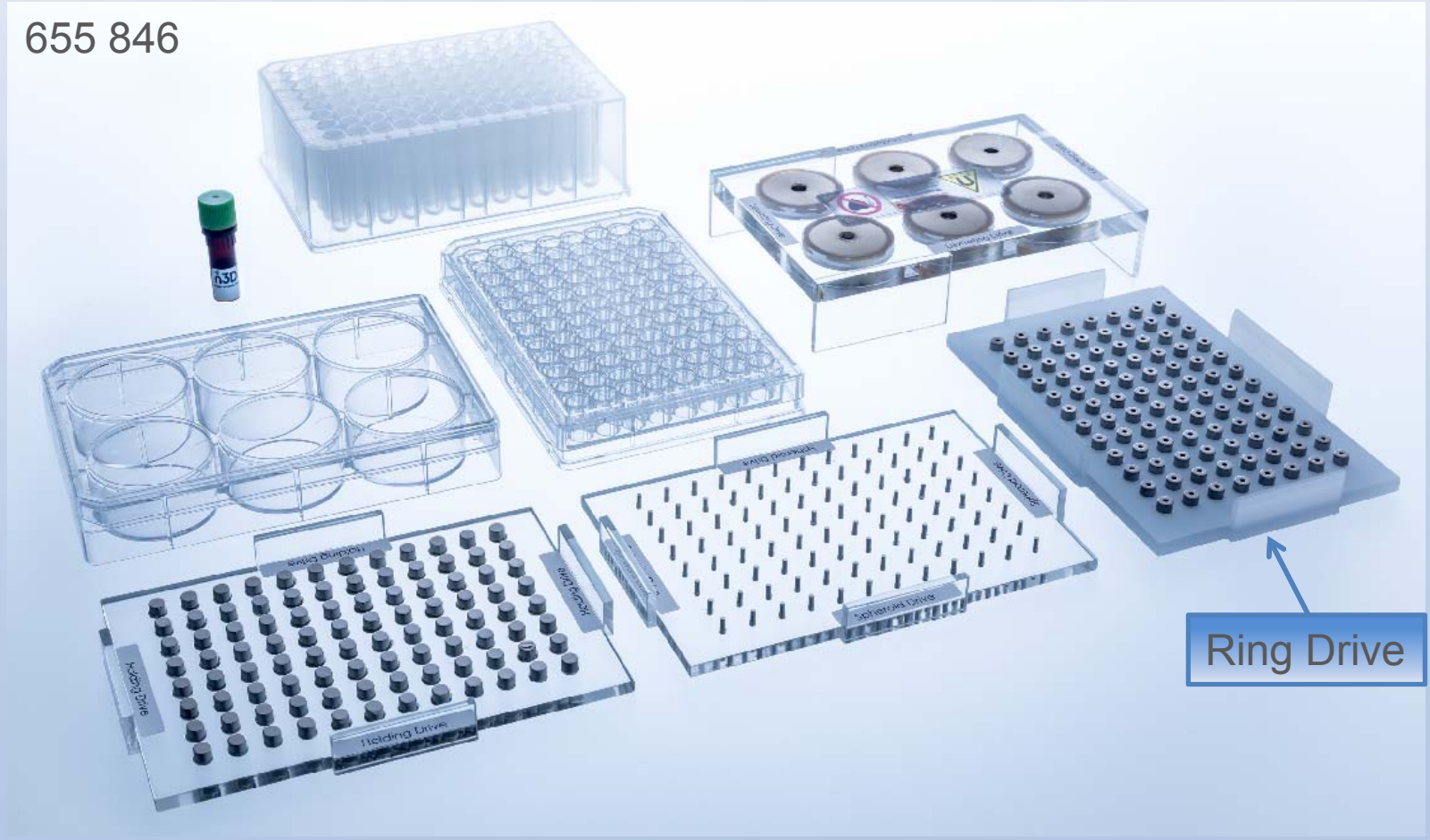
781 830



Products

greiner bio-one
Your Power for Health

- BiO Assay Kits (Toxicity, wound healing)
  - 96 Well and 384 Well Kits 781 846 (available on request)



Products

greiner bio-one
Your Power for Health

- BiO Assay Kits & n3Dock Imaging System
  - 96-Well and 384-Well System 781 849 (available on request)

Products

greiner bio-one
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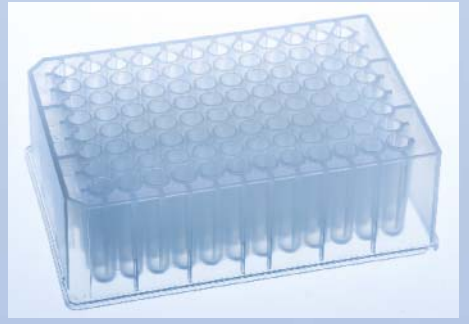
- Consumables
  - 657 841, 657 843, 657 846 NanoShuttle™-PL Refill

  - 657 850 MagPen™

  - 780 261 96-Well Mixing Plate



Products

greiner bio-one
Your Power for Health

- Consumables
  - 657 847 NanoShuttle™-PL Refill 6 with FREE iPOD™ (on request), FREE iPod™ with purchase of either 655 849 or 781 849

  - 657 860 n3Dock Imaging Kit (on request)

Products

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- Consumables
  - 657 810 Battery Power for n3Dock (on request)

  - Technology requires F- bottom vessels with cell-repellent surface!

Attention!

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- Warning note

n3D Biosciences, Inc.

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PRECAUTIONS FOR HANDLING BIO-ASSEMBLER MAGNETS

Thank you for purchasing products from nano3D Biosciences, Inc. For your safety and for proper care of your equipment, please note that the Bio-Assembler™ contains strong neodymium magnets that must be handled with extreme care.

When storing magnets in proximity to other magnets or materials that are attracted to magnets, take precautions so that objects do not slam together. Neodymium magnets are brittle and can shatter or crack, sometimes producing dangerous fragments moving at high speeds. Fingers can also be severely pinched between magnets or between magnets and certain metals. Large magnets can be difficult to separate from other magnets or certain metals if they are allowed to come into contact.

Persons with pacemakers or similar medical devices should not come near Bio-Assembler magnets.

Bio-Assembler magnets can damage magnetic media such as credit cards, magnetic ID cards, televisions, computer memory, and computer monitors. Keep magnets at least 30 cm (12 in.) from these devices.

Neodymium magnets should not be burned or machined. They will lose their magnetic properties if heated above 80 °C (175 °F).

Bio-Assembler magnets are not toys. They should only be used for their intended purpose of levitated cell culture. Children should not be allowed to play with them.

If you have need of further information, please contact us:

Greiner Bio-One GmbH
Maybachstrasse 2
72636 Frickenhausen
Germany
E-Mail: info@de.gbo.com
www.gbo.com/bioscience

Why magnetic cell culturing?

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Your Power for Health

- Allows spheroid culture of cell lines, which do not form spheroids by self-assembly
- Rapid spheroid formation
- Formation of one spheroid per well in F-bottom plates (solid and µClear®) with perfect optical properties
- Compatible with automated HTS approaches (384 well)
- No loss of spheroids during media exchange or washing steps
- Animal-free test method for cosmetics

Comparison cell-repellent vs n3D

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Your Power for Health

	Cell-repellent	n3D
Costs	☺ ☺	☺
Formats to cultivate 1 spheroid / well)	96 well U- bottom , clear 384 well to come	96, 384 well F-bottom stanard + µclear
Optics	☺ (U-bottom)	☺ ☺ ☺
Manual media exchange	☺	☺ ☺ ☺
Automated media exchange (HTS)		☺ ☺ ☺
Not self-assembling cell lines	☹	☺ ☺ ☺

Info material

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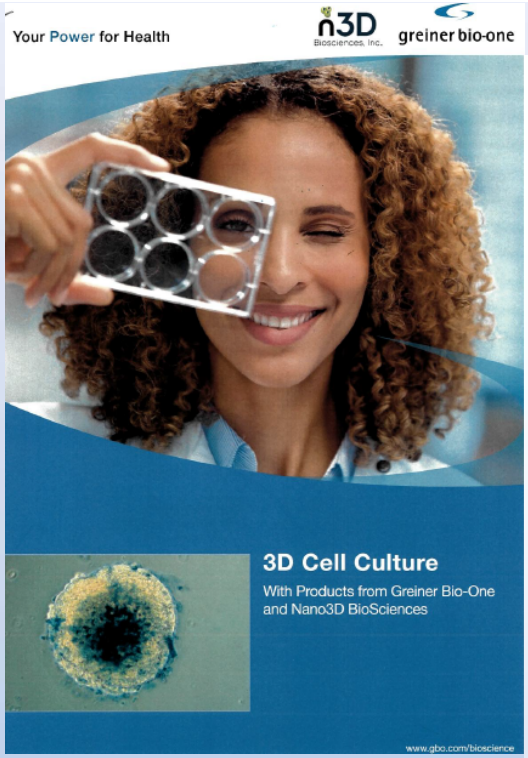

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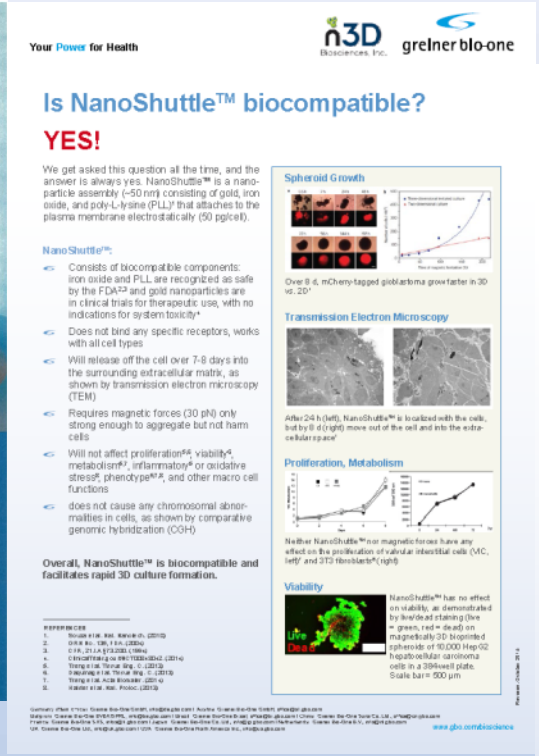

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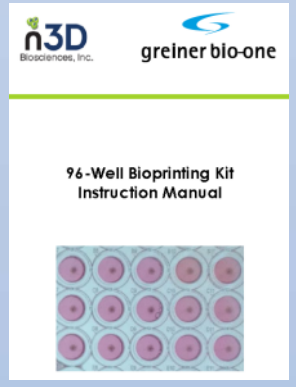

Instruction Manual for every kit available

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