



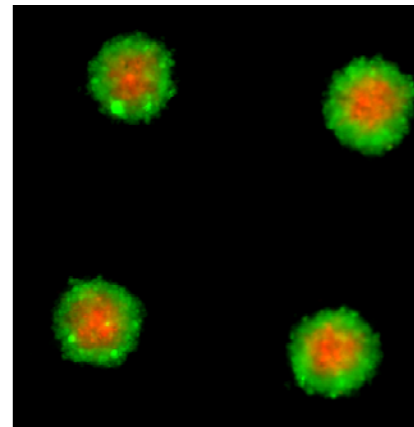
microtissues[®]
natural 3D™

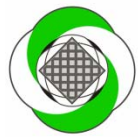
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The 3D Petri Dish[®]

A New Cutting-Edge Culture Format

The 3D Petri Dish[®] grows cells in three dimensions and forms multi-cellular microtissues that more closely mimic tissues *in vivo*





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The 3D Petri Dish[®] is now available from Sigma-Aldrich[®]

The **3D Petri Dish**[®] is an easy-to-use 3D cell culture system that creates a natural 3D environment by maximizing cell-cell interactions. **Its scaffold-free.**

Autoclavable precision micro-molds for the creation of spheroids, neurospheres, hepatospheres, cardiospheres, embryoid bodies, and much more.

A growing list of over 40 different cell types (primary cells & cell lines) form 3D microtissues in the **3D Petri Dish**[®]

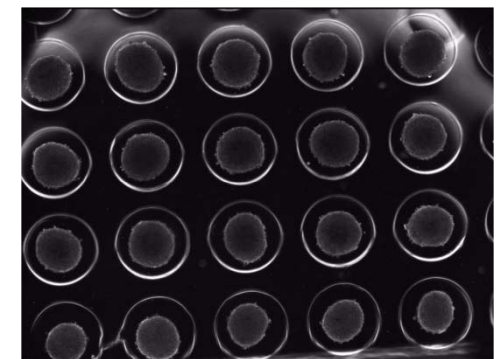


precision micro-molds

12 well format		
Z764000-6EA	12-256	256 small spheroids
Z764019-6EA	12-81	81 large spheroids
Z764094-6EA	12-1	spheroids, mixed pack
Z764035-6EA	12-36TO	36 toroids
Z764027-6EA	12-60TR	60 rods

24 well format		
Z764043-6EA	24-96	96 small spheroids
Z764051-6EA	24-35	35 large spheroids
Z764108-6EA	24-1	spheroids, mixed pack
Z764078-6EA	24-24TR	24 rods
Z764086-6EA	24-H	1 honeycomb

Tech pack		
Z764116-8EA	8-1	1 of each micro-mold, pk of 8



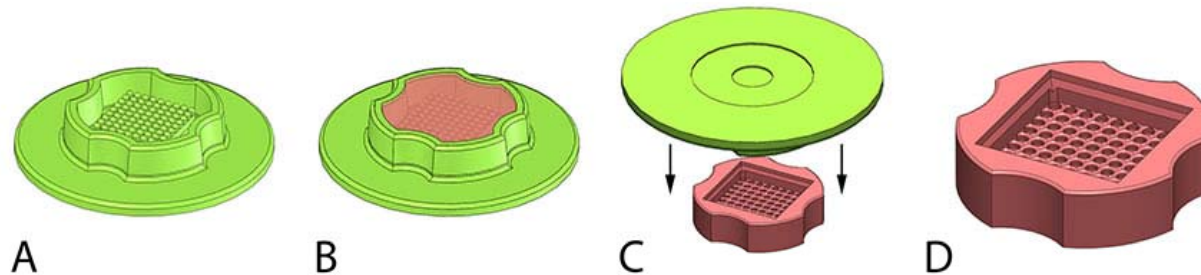
spheroids of human mesenchymal stem cells



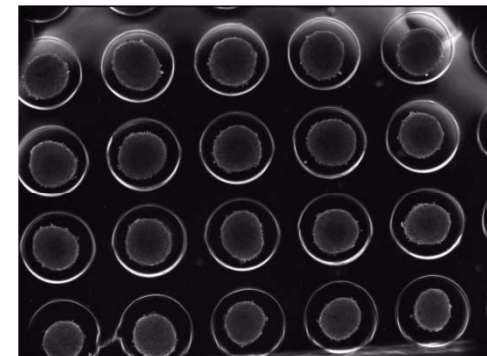
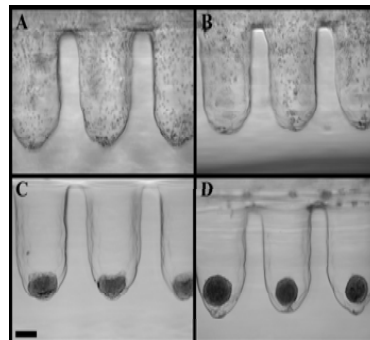
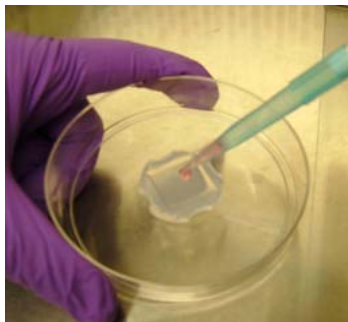
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Micro-mold casting system



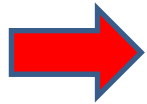
- A. Autoclave sterilize micro-mold
- B. Fill micro-mold with sterile molten agarose (2%)
- C. Separate gelled agarose from flexible micro-mold
- D. Place gel in multi-well plate and equilibrate with cell culture medium
- E. Seed with mono-dispersed cells
- F. Cells settle into micro-wells of agarose, aggregate and self-assemble spheroids





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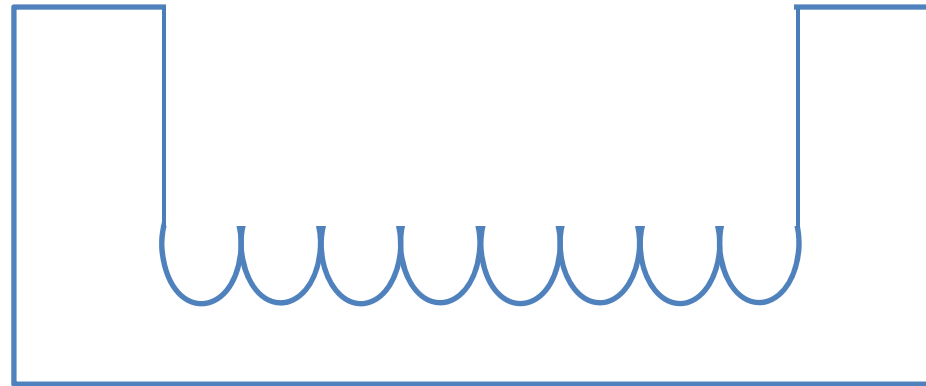


Remove gelled agarose from micro-mold

Fill large chamber with mono-dispersed cells (190μl)

Cells settle by gravity to bottoms of many micro-wells

Cells aggregate and self-assemble many spheroids

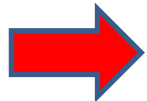




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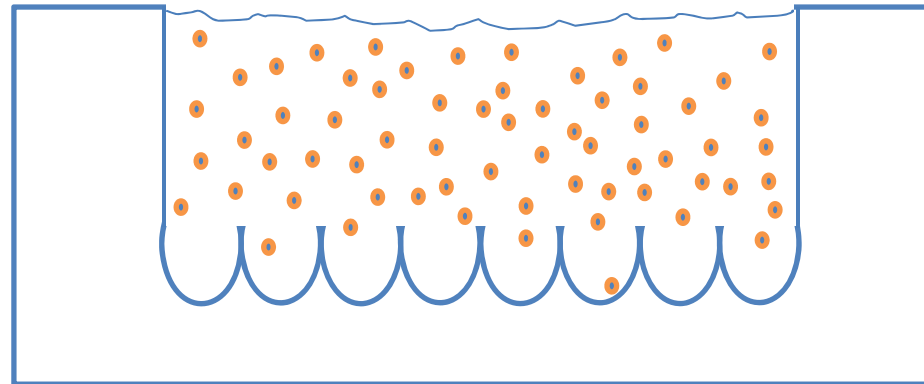
Remove agarose from micro-mold



Fill large chamber with mono-dispersed cells (190μl)

Cells settle by gravity to bottoms of many micro-wells

Cells aggregate and self-assemble many spheroids



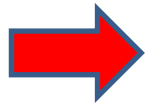


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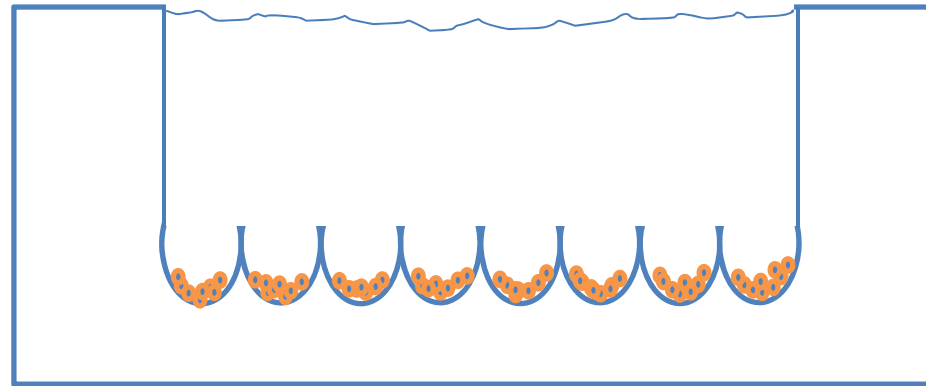
Remove agarose from micro-mold

Fill large chamber with mono-dispersed cells (190μl)



Cells settle by gravity to bottoms of many micro-wells

Cells aggregate and self-assemble many spheroids





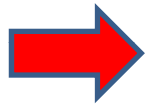
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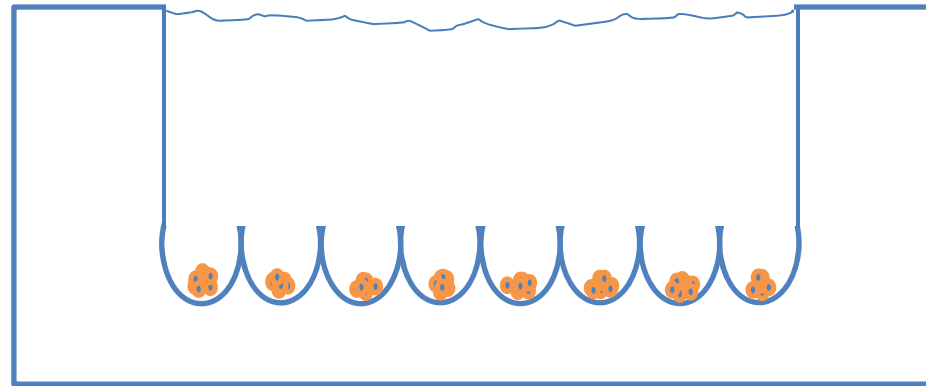
Remove agarose from micro-mold

Fill large chamber with mono-dispersed cells (190μl)

Cells settle by gravity to bottoms of many micro-wells



Cells aggregate and self-assemble many spheroids





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The 3D Petri Dish[®] is scaffold-free

Multi-cellular spheroids form at the bottom of nonadhesive agarose micro-wells

Cells aggregate and self-assemble 3D spheroids

No scaffold, no added ECM, no attachment factors

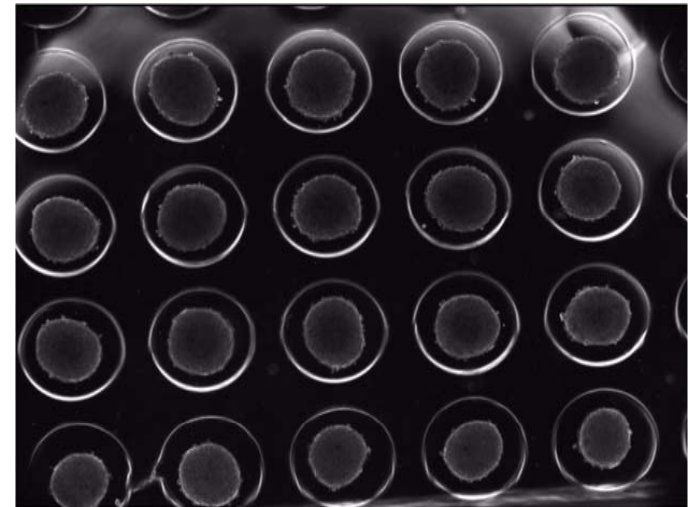
Cell-cell interactions and cell-cell communication are maximized

Cell density is akin to normal tissue

No lot-to-lot variation

No-synthetic scaffold

No artificially stiff substrate



spheroids of human mesenchymal stem cells

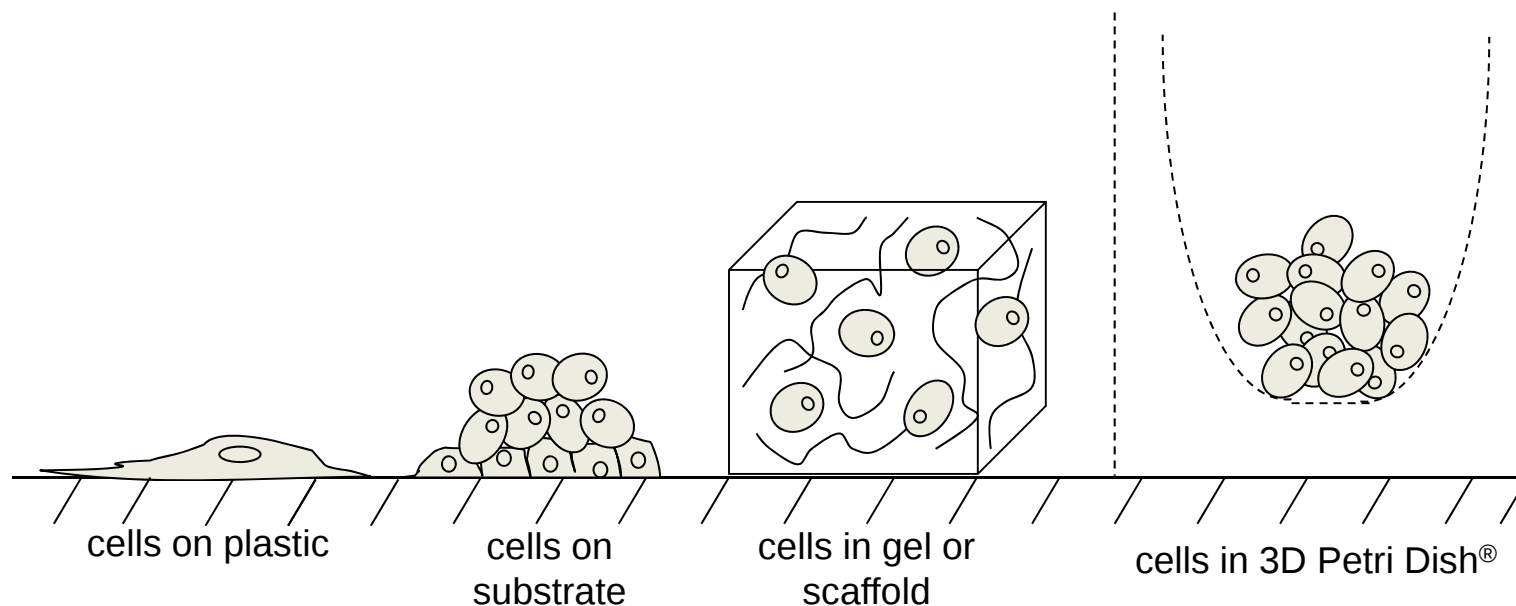


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Culture spheroids in a stable uniform environment

- won't dry out
- won't spill out
- won't fall from a drop
- surrounded by culture medium on all sides
- culture long term
- replenish media, test drugs, add growth factors, sequential differentiation

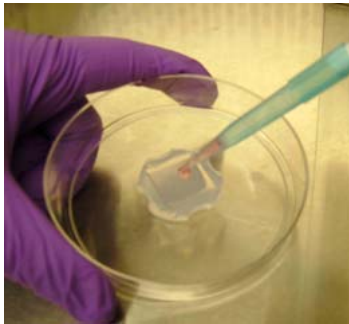




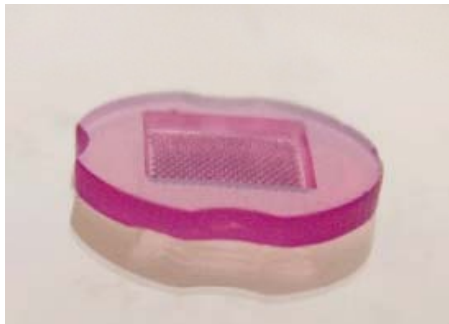
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Make 100s of spheroids in a single pipeting step



12 well format		
Z764000-6EA	12-256	256 small spheroids
Z764019-6EA	12-81	81 large spheroids



24 well format		
Z764043-6EA	24-96	96 small spheroids
Z764051-6EA	24-35	35 large spheroids





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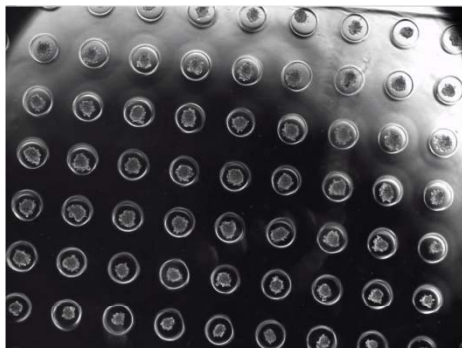
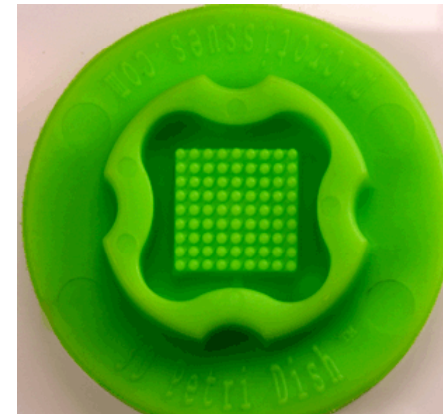
Control spheroid size by micro-mold choice



large spheroids of human
mesenchymal stem cells

Z764019-6EA
12-81

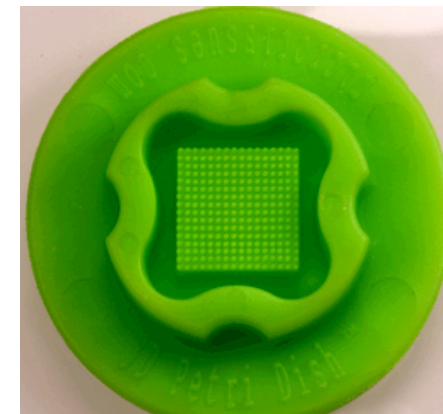
81 large spheroids
micro-well diameter
~800μm
micro-well depth
~800μm



small spheroids of human
mesenchymal stem cells

Z764000-6EA
12-256

256 small spheroids
micro-well diameter
~400μm
micro-well depth
~800μm



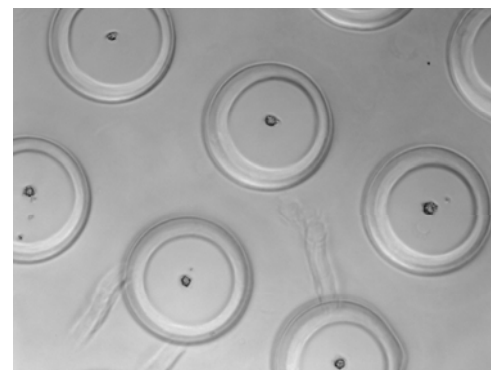
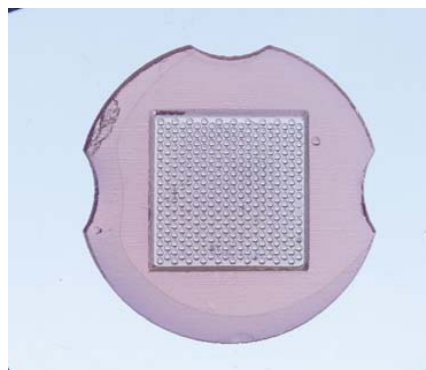
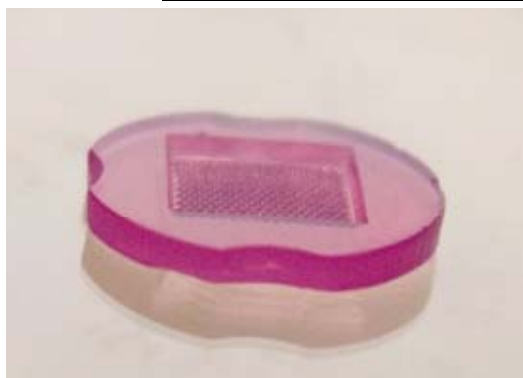


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Control spheroid size by number of cells seeded

Cell seeding numbers for different sized spheroids.		# 12-256	# 24-96
		12 well plate Small spheroids: 256	24 well plate Small spheroids: 96
Spheroid diameter (μm)	~Cells/spheroid	Total cells seeded (cells/190 μl)	Total cells seeded (cells/75 μl)
50	15	3,840/190 μl	1,440/75 μl
100	125	32,000/190 μl	12,000/75 μl
150	421	107,000/190 μl	40,000/75 μl
200	1,000	256,000/190 μl	96,000/75 μl
250	1,953	500,000/190 μl	187,000/75 μl

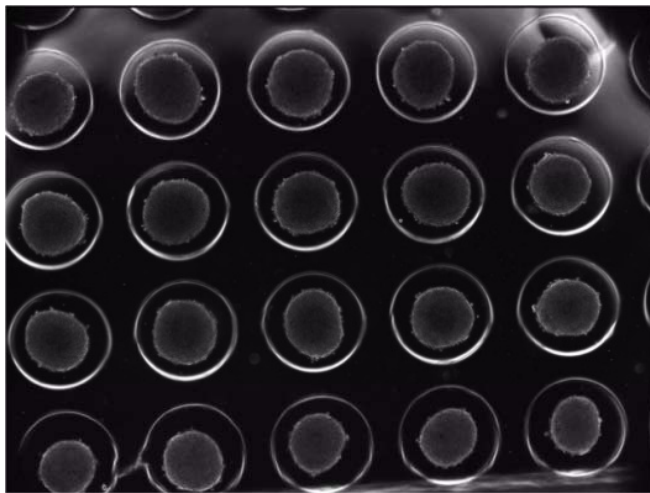




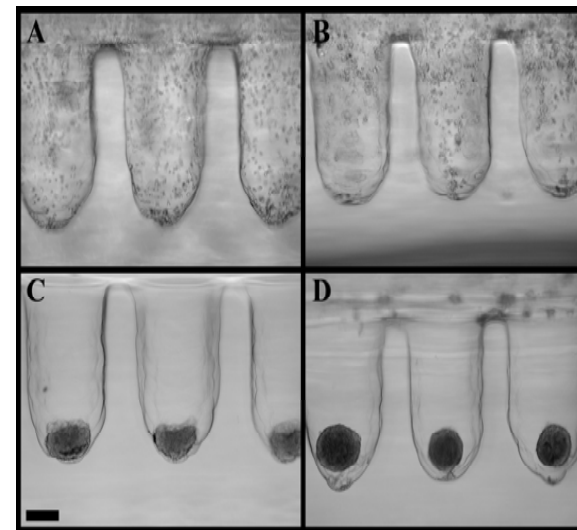
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Form spheroids in an array on the same optical plane



array of spheroids of
mesenchymal stem cells



same optical plane

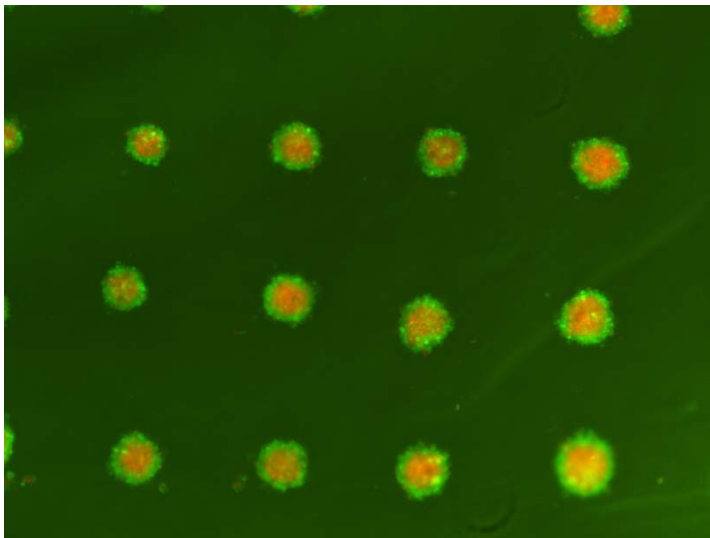
- ordered, addressable array
- image replicates
- automation
- time lapse, phase contrast, fluorescent microscopy



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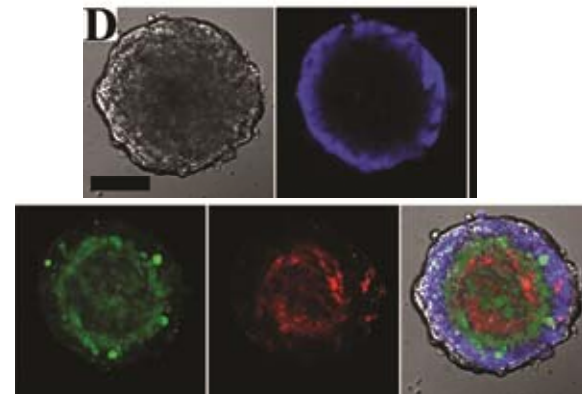
Form mixed spheroids with different cell types



1:1 mix, hepatocytes (green) fibroblasts (red)

- control ratio of cells
- maximize heterotypic cell interactions
- tumor cell–stromal cell interactions

3 cell types



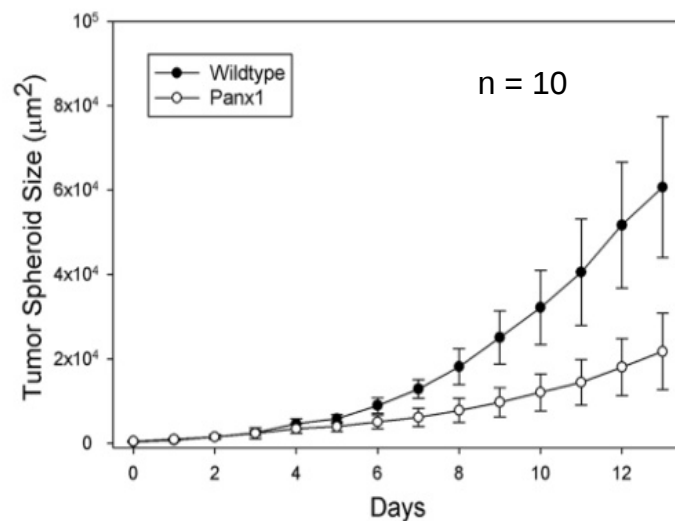
DIC microscopy; hepatocytes (blue)
fibroblasts (green); HUVEC (red); and
their composite image



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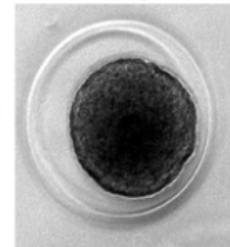
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Culture spheroids long term in a stable environment Grow clonal spheroids from single cells

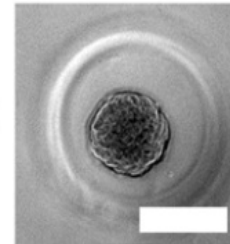


10 Days

Wildtype



Panx1



Seed ~1 cell/micro-well,
quantify spheroid
growth over 2 weeks

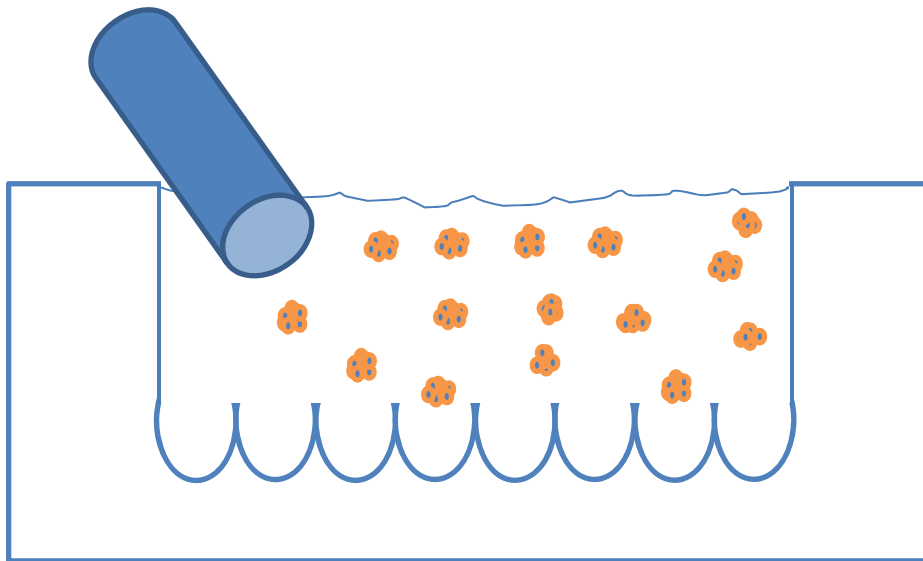
- won't dry out, won't spill out, won't fall from a drop
- surrounded by culture medium on all sides
- replenish media, test drugs, add growth factors
- sequential differentiation
- cancer stem cells



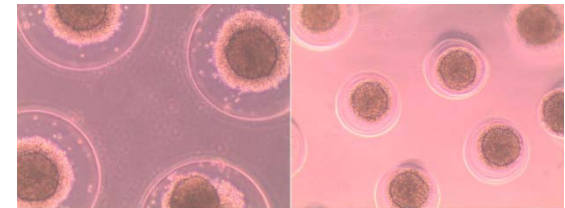
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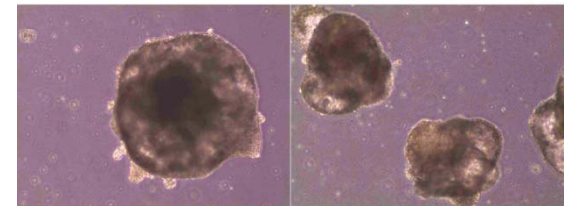
Harvest by flushing spheroids out of micro-wells with a pipette



embryoid bodies



81well-5K EB d1 256well-2K EB d1



81well-5K EB d5 256well-2K EB d5

Image courtesy of J Bruder
Max Planck

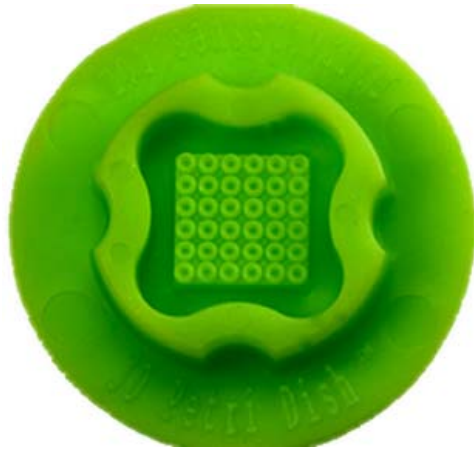
- no gel to dissolve, no enzymatic treatment
- spheroids remain intact and unaltered
- harvest spheroids for RT-PCR, Western blots, histology, immuno-staining
- harvest spheroids for transplantation, for tumor implantation, for cell therapy



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Form complex shapes for novel applications



12 well format		
Z764035-6EA	12-36TO	36 toroids
Z764027-6EA	12-60TR	60 rods



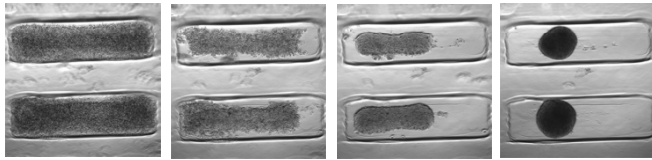
24 well format		
Z764078-6EA	24-24TR	24 rods
Z764086-6EA	24-H	1 honeycomb



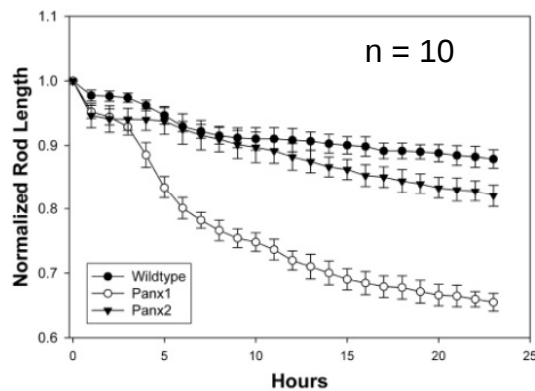
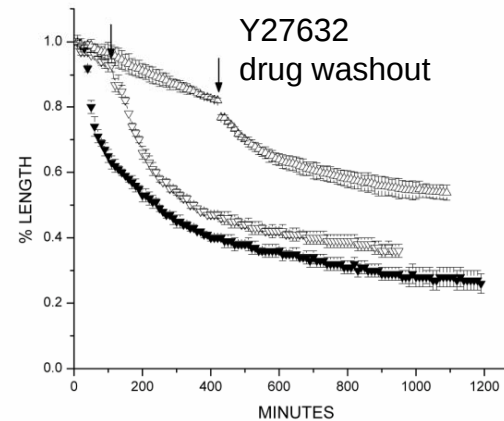


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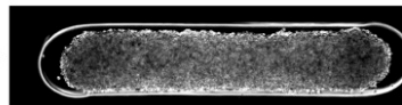
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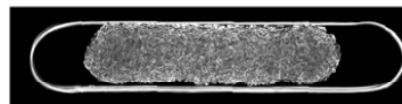
human fibroblasts seeded in troughs
form multi-cellular rods that self
assemble and contract over 12 hours



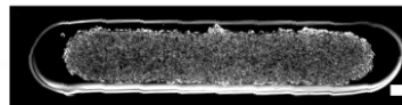
Wildtype



Panx1



Panx2



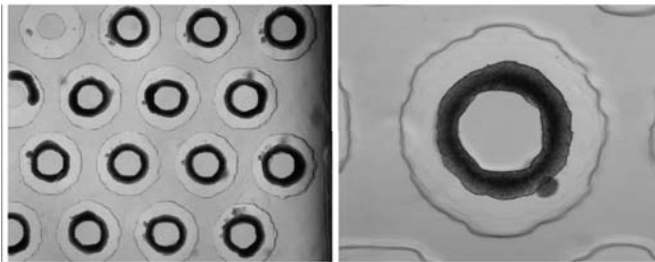
Troughs:
2,200 μm long
400 μm wide
800 μm deep
round bottom trough
~ 25,000 cells/trough

- quantify cell-cell adhesion
- drug testing, genetic manipulations
- cell-cell adhesion of cell mixtures

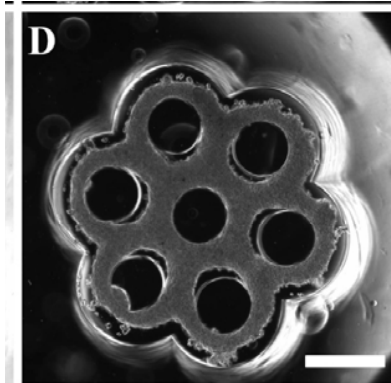
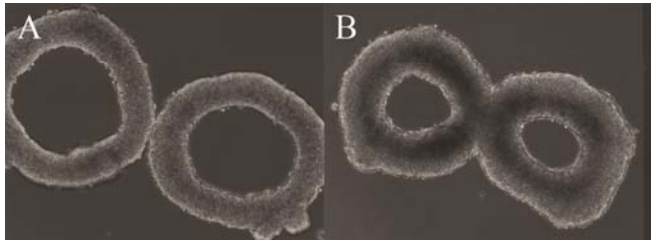


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toroid outer diameter 1.4 mm
diameter of inner peg 600 μm
circular trough width 400 μm
circular trough depth 800 μm



honeycomb size 3.4 mm
diameter of pegs 600 μm
trough width 400 μm
trough depth 800 μm



- developmental biology
- tissue engineering
- cell migration



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A growing list of cell types have been used in the 3D Petri Dish[®]

Primary cells

Human dermal fibroblasts
Rat cardiac myocytes
Rat cardiac fibroblasts
Human umbilical vein endothelial cells (HUVEC)
Calf pulmonary artery endothelial cells (CPAE)
Human theca cells
Murine neurons from the hippocampus
Human mesenchymal stem cells
Zebrafish ectoderm progenitor cells
Zebrafish mesoderm progenitor cells
Human mesenchymal stem cells
Embryoid bodies
Murine neural stem cells
Human chondrocytes
Bovine chondrocytes
Rat aortic smooth muscle cells

Cell lines

Murine fibroblasts (3T3)
Murine endothelial cells (bEnd3)
Rat endothelial cells (RBE4)
Human breast cancer cells (MCF-7)
Human breast cancer cells (T47D)
Human breast cancer cells (MDA-MB-231)
Human breast cancer cells (Hs-578T)
Human cervical cancer cells (HeLa)
Human epithelial carcinoma cells (A431)
Human hepatocytes (HepG2)
Rat hepatocytes (H35)
Human mesothelioma (M28)
Human mesothelioma (REN)
Human granulosa cells (KGN)
Human trophoblast cells (TCL)
Rat neuroblastoma cells (RG2)
Rat glioma cells (9L)
Rat glioma cells (C6)
Rat astrocytes (A7)
Murine neuroblastoma cells (B104)
Retinal pigment epithelial cells (ARPE-19)
Human embryonic kidney cells (HEK)
Rat endothelial cells (RBE4)
Murine endothelial cells (bEnd3)
Human non-small lung cancer cells (A549)
Human non-small lung cancer cells (H1299)

➤ **If your cells are adherent adhesive cells,
they will self-assemble in the 3D Petri Dish[®]**



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The 3D Petri Dish[®] is now available from Sigma-Aldrich[®]

PRODUCT SUPPORT

Visit our website (www.microtissues.com)

Detailed protocols
FAQs
Published papers
List of cells
Tech support
Email your questions

