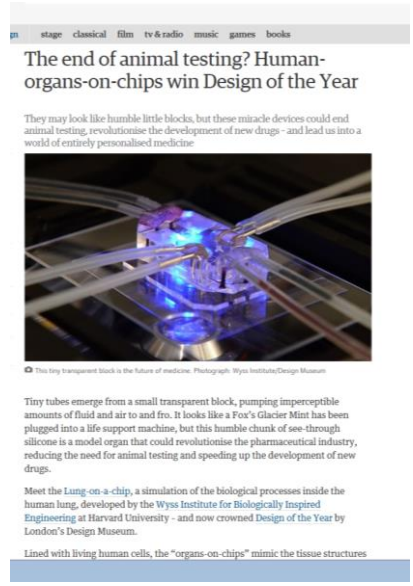
Organs on chips II



gn stage classical film tv & radio music games books

The end of animal testing? Human-organs-on-chips win Design of the Year

They may look like humble little blocks, but these miracle devices could end animal testing, revolutionise the development of new drugs - and lead us into a world of entirely personalised medicine

This tiny transparent block is the future of medicine. Photograph: Wyss Institute/Design Museum

Tiny tubes emerge from a small transparent block, pumping imperceptible amounts of fluid and air to and fro. It looks like a Fox's Glacier Mint has been plugged into a life support machine, but this humble chunk of see-through silicone is a model organ that could revolutionise the pharmaceutical industry, reducing the need for animal testing and speeding up the development of new drugs.

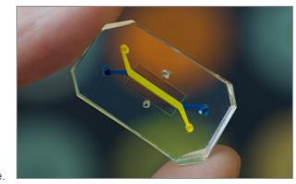
Meet the Lung-on-a-chip, a simulation of the biological processes inside the human lung, developed by the Wyss Institute for Biologically Inspired Engineering at Harvard University - and now crowned Design of the Year by London's Design Museum.

Lined with living human cells, the "organs-on-chips" mimic the tissue structures

Lung on chip part of permanent exhibition on Museum of Modern Art (2015)

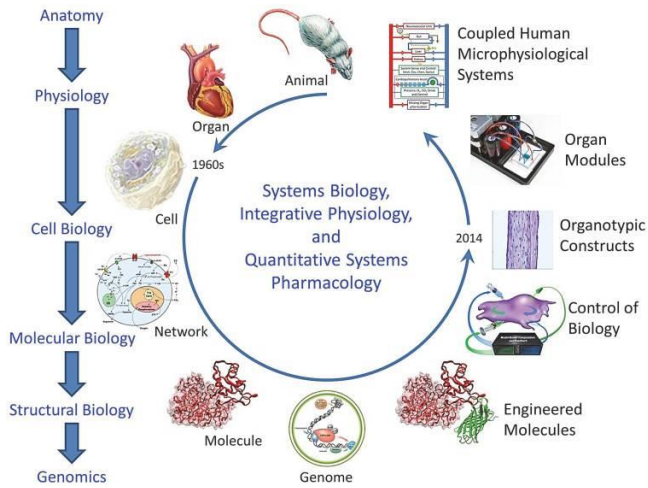
In the case of some of the new acquisitions included in this exhibition, the answer to that question—Is this for everyone?—is: *yes, hopefully, if not now, then in the future*. Before design objects, whether physical or digital, debut in the world, they undergo intensive prototyping. Even when they are conceptual, speculative, and not immediately viable, most design experiments are created to prompt dialogue and to anticipate concrete needs, problems, or conditions—in other words, to actively support a greater good to come.

Esoteric or specialized, perhaps, but universally remarkable in their balance of form, function, and vision, investigations like the Wyss Institute's Human Organs-on-Chips demonstrate new, radical intersections of synthetic biology and design. The Wyss Institute at

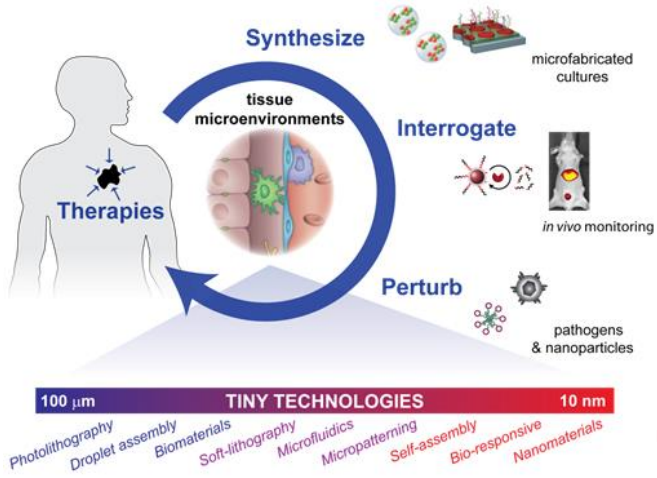


Donald Elliot Ingber, Dan Dongeun Huh, Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston Children's Hospital. Human Organs-on-Chips (Lung-on-Chip). 2008. Silicone rubber. The Museum of Modern Art, New York. Gift of the designers, 2015

Understanding physiology in a historic POV



Small approaches to biomedicine



Physiology

Molecular medicine

Organs that have been partially recreated in vitro using human cells

Liver
Lung
Intestines
Lymphoid system
Blood Brain Barrier
Heart
Tumors

Uterus
Kidney
Bone marrow
Skin
Glands
Brain

The Liver

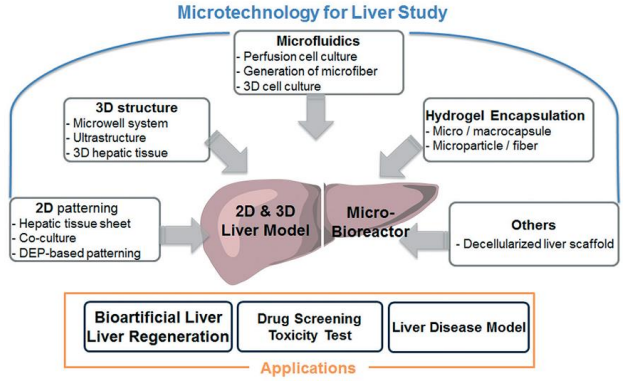
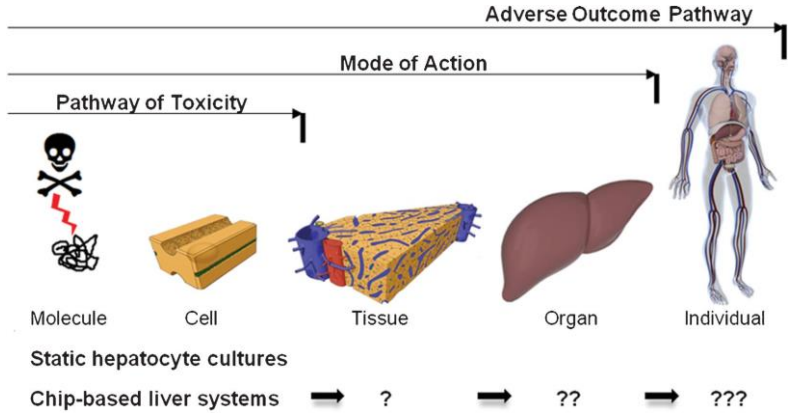


Fig. 1 Overview of the microtechnologies for liver study and their applications.

DOI: 10.1039/C5LC00611B

The Liver

Why create liver?



Impossible to predict MoA and AOP from mono cell cultures.

The Liver

The liver microachitecture

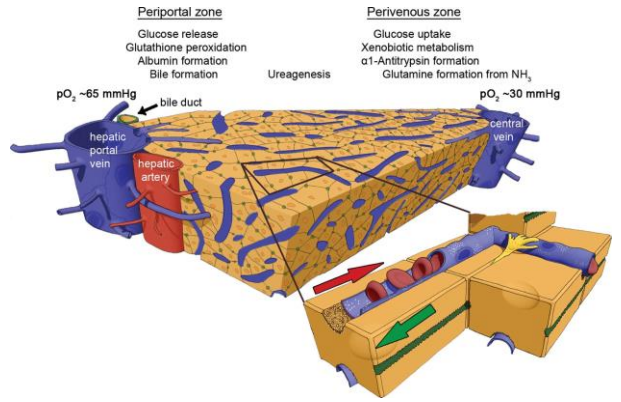
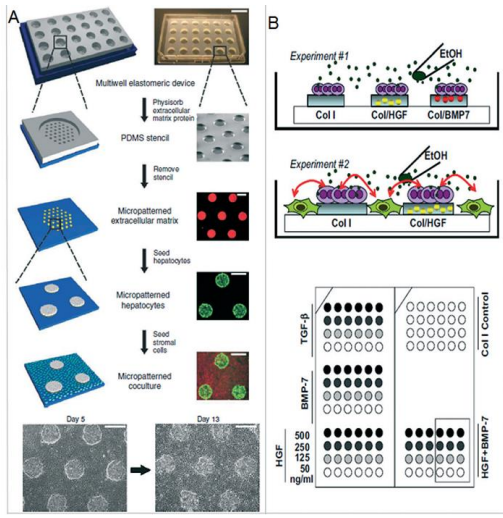


Fig. 1 Liver zonation at lobule level – architecture defines functionality. Periportal and perivenous zonal specialisation of hepatocyte activity is enabled by a sophisticated architecture of the liver lobule – the smallest functional unit of the liver. A stable oxygen gradient is ensured by a dynamic arrangement of blood flow from the outer surface to the centre of the lobule (red arrow), whilst a reverse bile flow takes place in segregated bile canaliculi (green arrow and channels). The space of Disse, generated by tight interactions between liver cells (brownish) and endothelial sinusoids (blue), accounts for efficient substance uptake. Ito cells (yellow) are responsible for matrix formation and remodelling in the space of Disse.

The Liver

Creating patterned cocultures



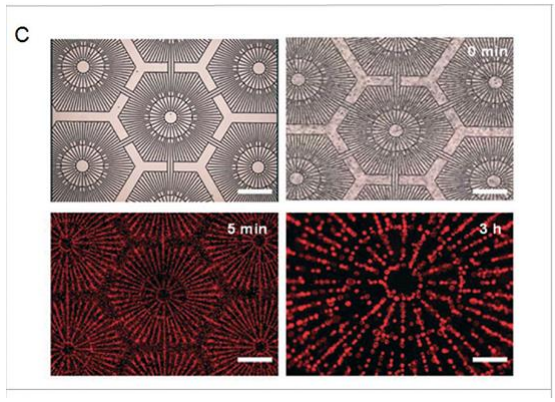
Colcultures keep hepatocytes happy

Model for
Hepatitis C virus (HCV)
Drug metabolism
Toxicity screening

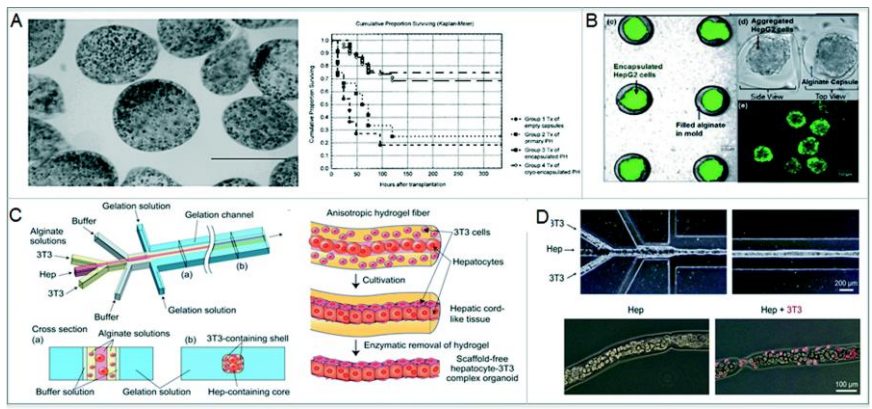
Bhatia N et al

Mimicking the sinusiodal architecture

Dielectrophoresis-based patterning of a heterogeneous, lobule-mimetic, radial pattern model of hepatocytes and endothelial cells.



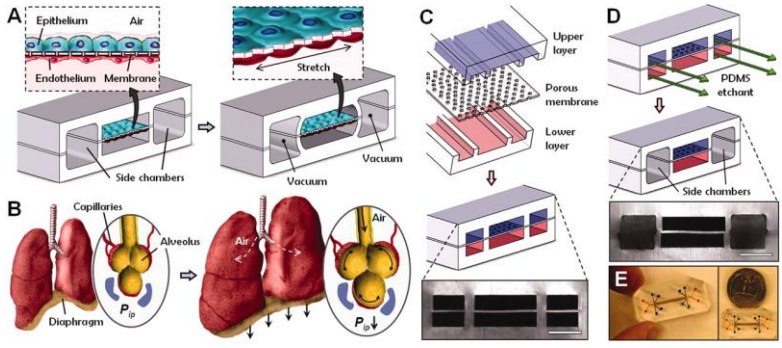
3D solutions



Examples

The lung

A breathing lung on a chip



Reconstituting Organ-Level Lung Functions on a Chip
Huh D et al. Science 25 June 2010: Vol. 328 no. 5986 pp. 1662-1668
DOI: 10.1126/science.1188302

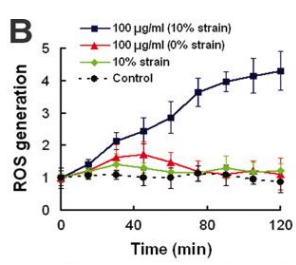
Examples

The lung

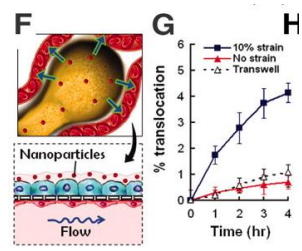
The model one step closer to reality

Stretching increased

inflammation/immune cell recruitment



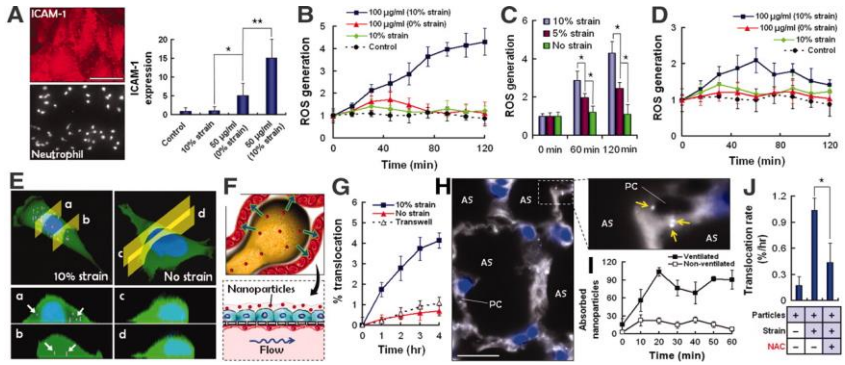
translocation of nanoparticles



DOI: 10.1126/science.1188302

Examples

Stretching increases nanoparticle translocation



https://www.youtube.com/watch?v=UDX7aGmYYRQ

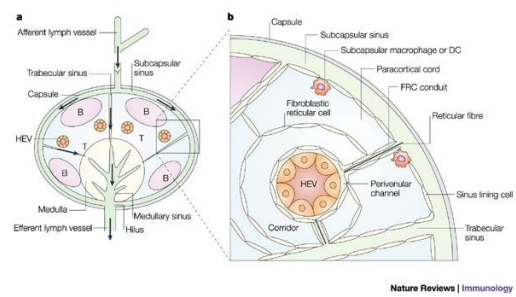
Examples

The lymph node

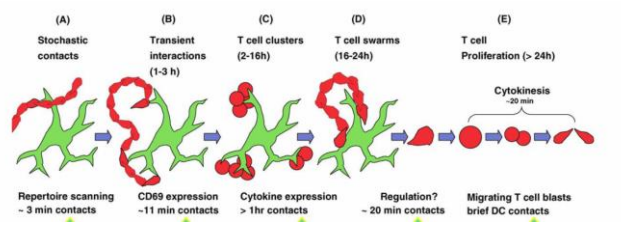
Own research: Lymph node-on-a-chip

Recreating lymph-node functionalities in the lab

- Shaping immunological memory
- Test vaccine efficiencies
- *In vitro* vaccination



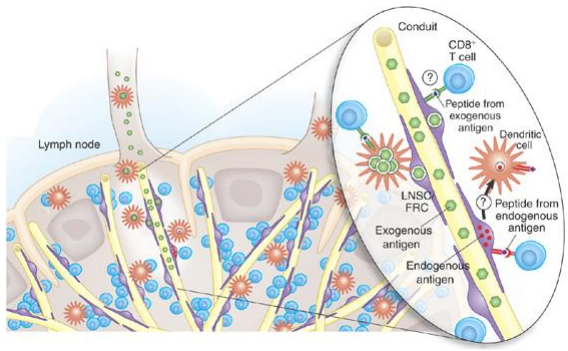
APC : T cell interaction process in lymph node

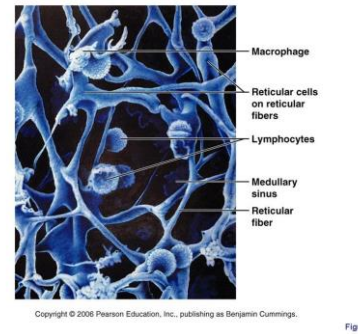


Lymphnodes

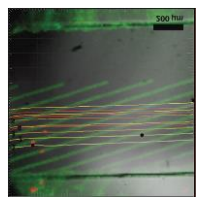
(immunotherapy of infectious diseases and cancer)

T cell priming in lymphnode: Naïve T cells serially interact with dendritic cells and stromal cells. Cell:cell contact is the key!



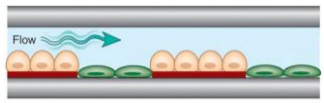


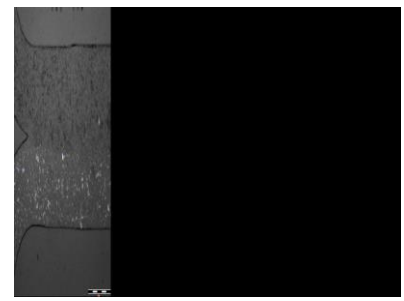
Lymphnode-on-a-chip

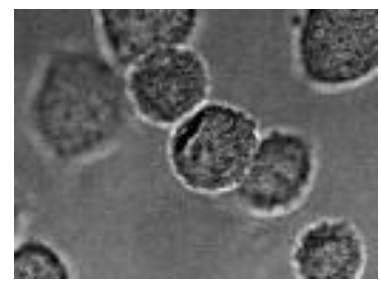


Patricia Rosa, IKM/NanoLab

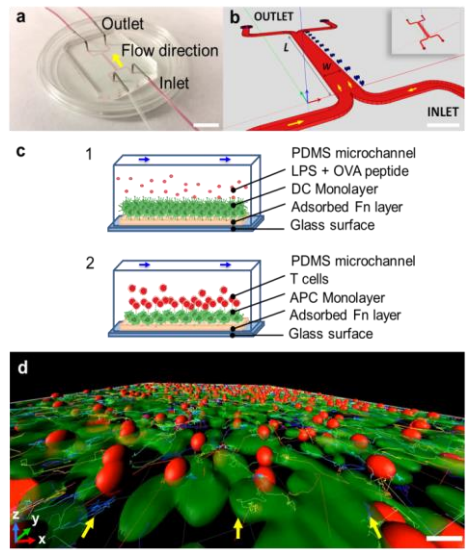
Øyvind Halaas, IKM



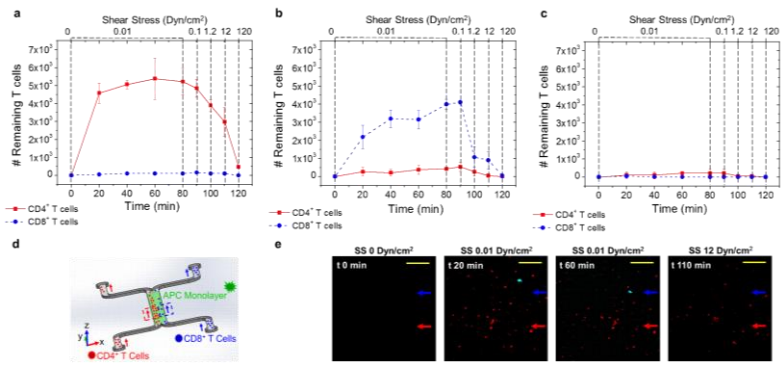




The principle



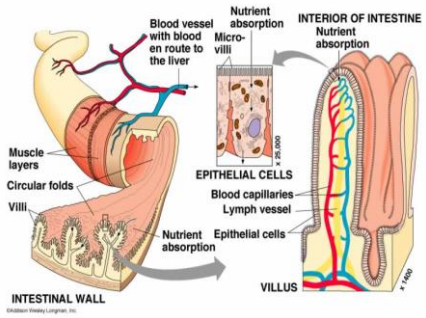
The data



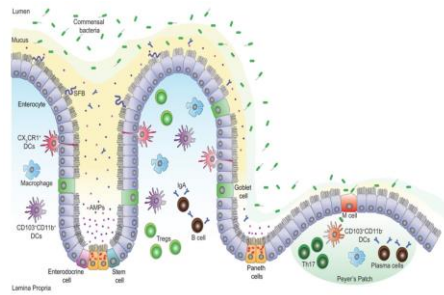
There is an antigen-specific shear-stress- and time-dependent adhesion of T cells to dendritic cells

The gastrointestinal tract

Macroarchitecture



Microarchitecture



9m long, 30m^2 area, persitaltic movement
Barrier for microbes, sites of nutrition
Diseases (inflammatory bowel diseases, cancer, obesity, drug absorption)

Examples

Gut in a dish (today's state of the art)

Adsorption through intestinal epithelium in Transwell® assay

ADME (Adsorption, Distribution, Metabolization, Excretion for drug development)

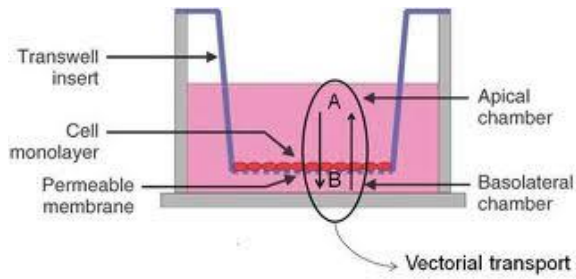
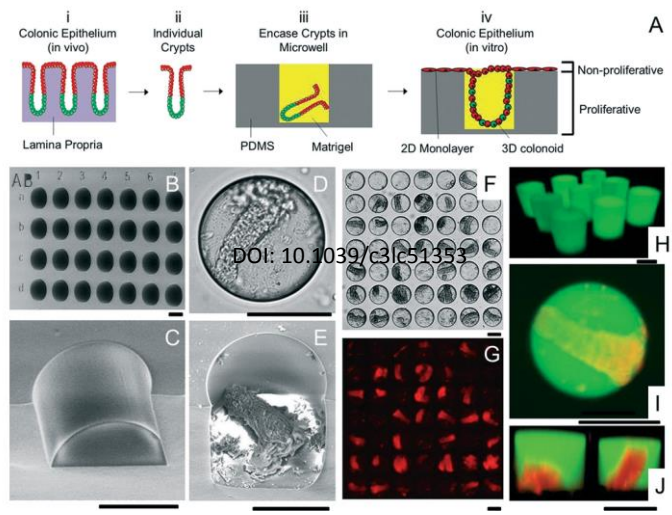


Table 2. *In vitro* culture models of the small intestine: cellular composition and relevant applications[a]

Cells	Application	Unique characteristics	Significant results	Refs
Caco-2 and HT29-MTX	Predicting iron bioavailability	Incorporated goblet cells that secrete mucus	Co-cultures of Caco-2 and HT29-MTX cells exhibited lower ferritin than Caco-2 monolayers	[62]
Caco-2 and HT29-MTX	Mercury transport	Incorporated goblet cells that secrete mucus	Introduction of HT29-MTX cells increased the permeability of the transport marker Lucifer Yellow	[63]
Caco-2	Drug permeability	Used collagen hydrogel with authentic size and shape of intestinal villi	3D models utilizing collagen hydrogels had similar TEER values to rat ileum (~50 Ω/cm^2)	[64]
Caco-2, HT-29, and T84	Inflamed intestinal mucosa model	Addition of proinflammatory stimuli and incorporation of macrophages and dendritic cells	Caco-2 cells exhibited increased expression and release of IL-8, whereas HT-29 and T84 showed no response to the stimuli	[16]
Caco-2	Body-on-a-chip device	Microfabrication of villi using photoresist on silicon substrates	Complete Caco-2 cell coverage on SU-8 membranes and tight junction proteins expression throughout the cell layer	[67]
Caco-2	Drug permeability	Microfluidic device with suspended Caco-2 cells	Ten drugs with a wide range of permeabilities were tested. Close correlation to *in vivo* data was observed	[74]
Caco-2	Gut-on-a-chip	Microfluidic device with peristalsis-like motions	Physiological fluid flow and shear stress across apical surface accelerated cell differentiation compared to static cultures. TEER values of the organ on a chip were threefold to fourfold higher than static cultures	[17]
Caco-2 and hMVECs	Nutrition and drug absorption	Co-culture environment in a dynamic bioreactor system	Cells in 3D perfusion system differentiated and proliferated faster than static cultures	[13]
Caco-2, MEFs and HT29-MTX	Drug absorption	3D co-culture system with MEF-embedded collagen extracellular matrix	Immunostaining of ZO-1 and P-glycoprotein tight junction proteins showed lower expression in 3D co-cultures than 2D Caco-2 monocultures	[82]

http://dx.doi.org/10.1016/j.tibtech.2014.04.006

Capture and 3D culturing of individual villi



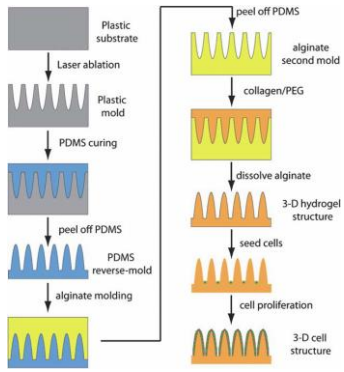
DOI: 10.1039/c3lc51353

Examples

The GI tract

Human intenstinal villi tissue model

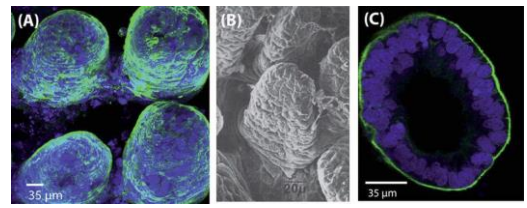
Fabrication strategy



Caco-2 cells on microstructrues
Green=actin
Blue=nuclei

SEM of clinical sample of human jejuna villi

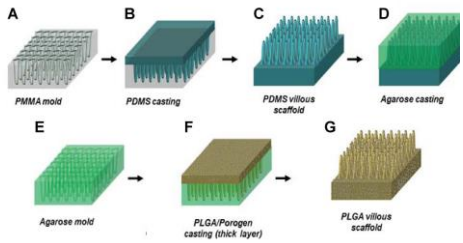
Confocal of model:
Green=actin
Blue=nuclei



Microscale 3-D hydrogel scaffold for biomimetic gastrointestinal (GI) tract model
Jong Hwan Sung et al Lab Chip, 2011,11, 389-392 DOI: 10.1039/C0LC00273A

The GI tract

Differential adhesion of probiotic/pathogenic bacteria to crypt/villi



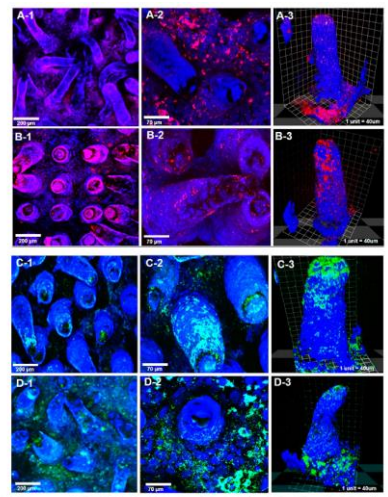


Figure 1. Confocal microscopy of PLGA scaffolds cultured for 21 days with Caco-2 (blue), and 2 h with bacteria (red and green). A = Nissle, B = LAB, C = ST, and D = PAO1. 20× magnification shows full coverage of scaffolds with Caco-2 and bacteria (A-D1) with a zoomed in 40× magnification enabling visualisation of individual bacteria (A-D2), and 3-D rendering shows bacteria adhering selectively to different locations along the crypt–villus axis on an individual villus measuring 500 µm (A-D3).

The GI tract

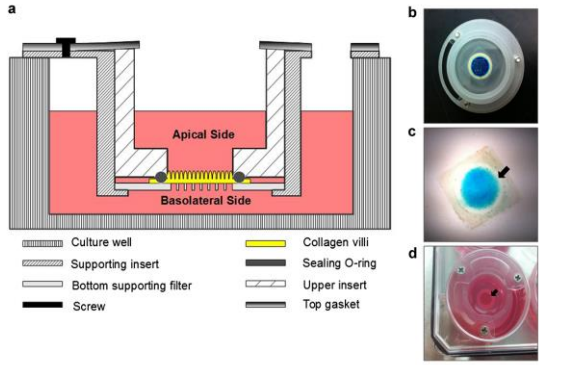
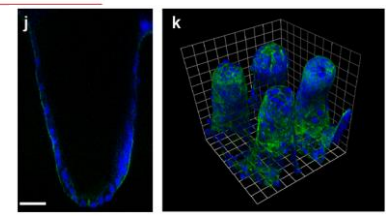


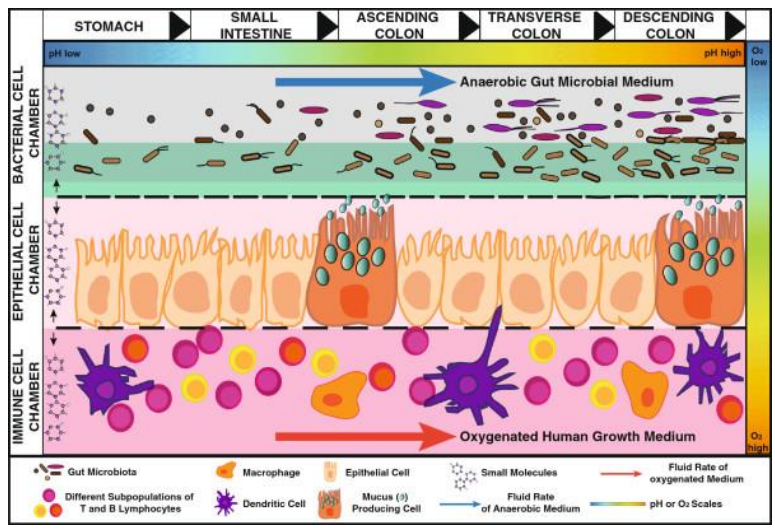
Figure 1. Overview of the insert kit (**a**) schematic of the insert design (**b**) dye test result of the insert, an O-ring was used to completely seal the villous insert within the culture well and prevent short-circuiting around the test area. (**c**) When the scaffolding was removed from the culture well, the scaffolding layer did not rupture and the dye remained within the villous test area (arrow points to the O-ring sealing area). (**d**) An image of a modified well inserted in a six-well plate (arrow points to the area actually covered by synthetic villi). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/bit]



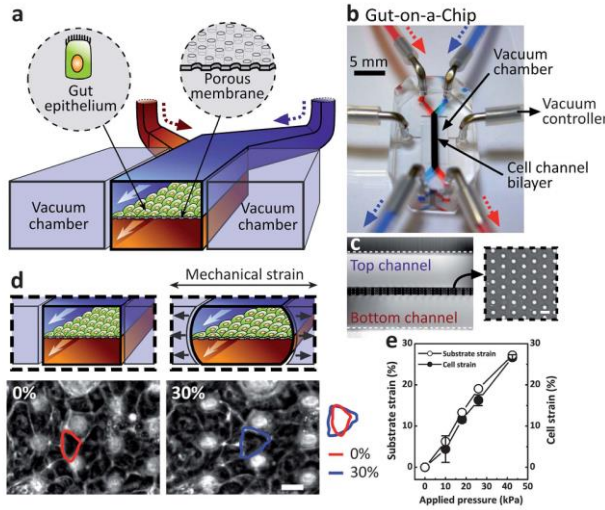
DOI 10.1002/bit.24518

The GI tract

The microenvironment in the gut

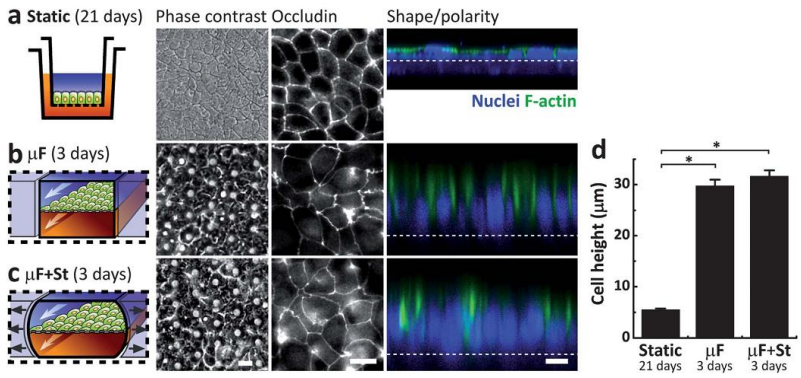


Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow



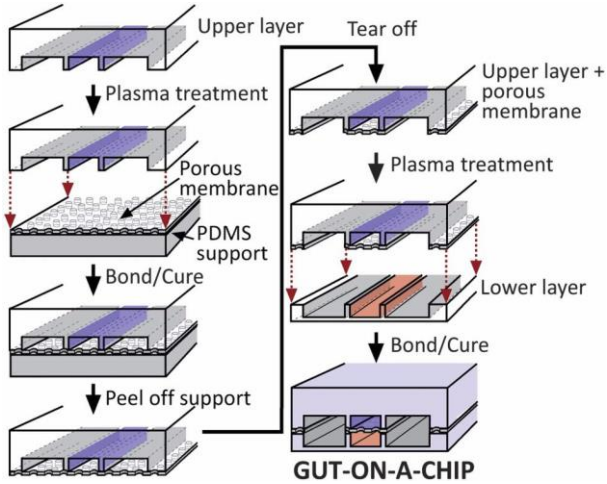
DOI: 10.1039/C2LC40074J

Adding mechanical stress improves model

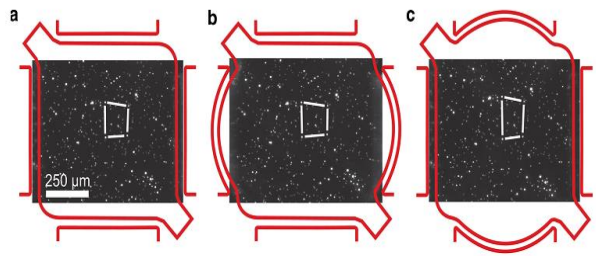


Fabrication strategy, gut-on-a-chip

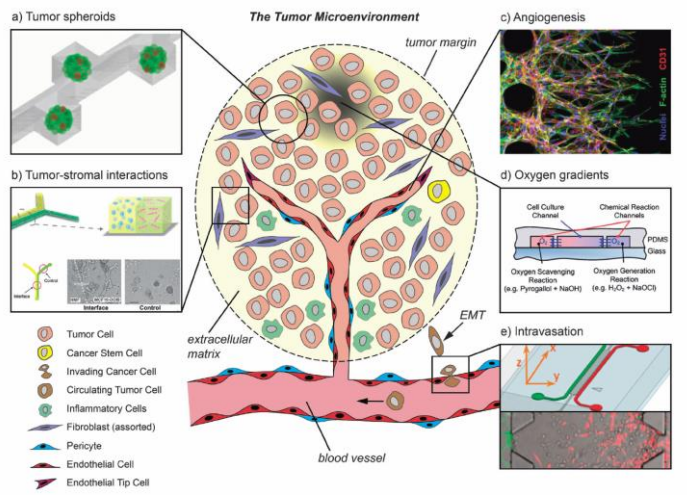
(see example from lung-on-a-chip)



Next challenges: add 2D stretching behaviour
(how about true peristalsis?)



Tumor-on-a-chip

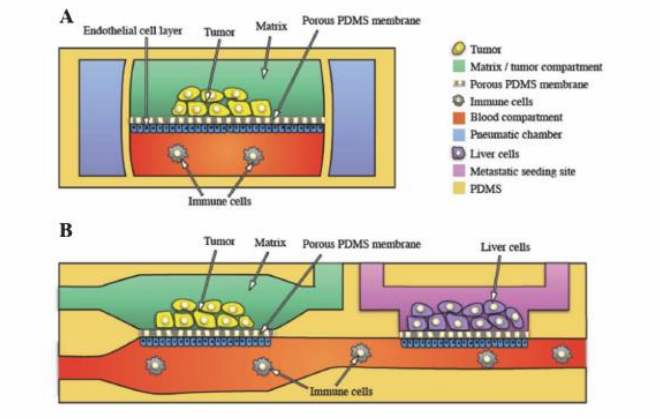


Cells, tissues, and organs on chips: challenges and opportunities for the cancer tumor microenvironment Edmond W. K. Young*a
Integr. Biol., 2013,5, 1096-1109 DOI: 10.1039/C3IB40076J

Table 1. Examples of 3D Tumor Models

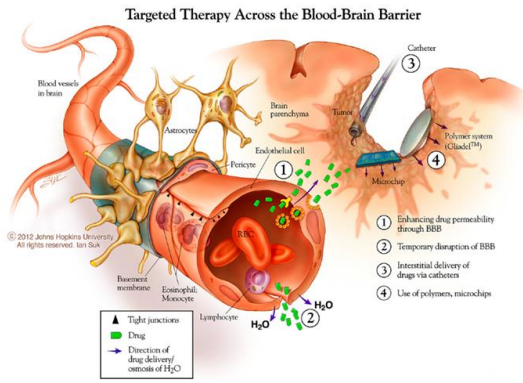
Tumor Model	Description	Advantages	Disadvantages
Multicellular spheroid[91,159,163–171,188,189]	Somewhat spherical cell aggregate are formed spontaneously in dishes or by culturing on treated substrates	Works for a variety of normal and tumor cell lines Mimics tumor heterogeneity Can be supplemented with sandwich cultures for additional analyses	Difficult to control growth Does not account for blood-vessel barrier to nutrients
Hollow fiber[158,193]	Cells are seeded into hollow fibers (often made of polyvinylidine fluoride (PVDF)) to form solid masses	Works for a variety of cell lines Mimics tumor heterogeneity Cells cultured in biocompatible fibers can be implanted into mice for *in vivo* studies as well	Fiber wall constrains culture growth Fiber wall presents artificial barrier that excludes application to gene therapy studies
Multicellular layer (MCL)[194–200]	Cells are seeded onto semi-permeable support membrane often coated with collagen and multiple layers (often as much as 20) accumulate in culture	Planar geometry enables direct flux measurements Mimics tumor heterogeneity Growth can be reasonably controlled Can be used for some cells incapable of growing spheroids	Does not account for blood-vessel barrier to nutrients

Tumor on a chip – interaction with immune system and liver



The Blood Brain Barrier

BBB



Understanding:

- Drug delivery
  The BBB stops 98-100% of drugs
- Brain tumors
- Degenerative diseases

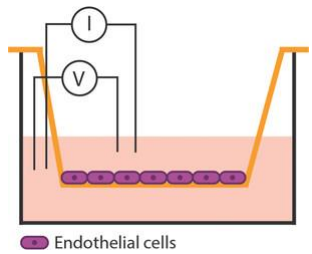
https://www.youtube.com/watch?v=v_mBLzYf91U#t=109

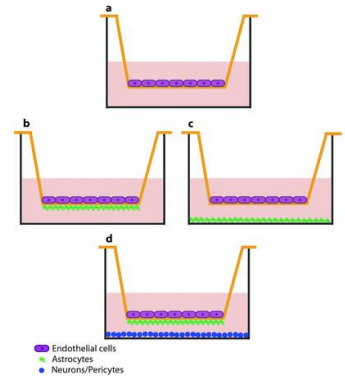
BBB

The original setup Transwell assays

Cells seeded in monolayer on a permaebale membrane

Transport of drug molecules and electrophysiology through the monolayer





Multicellular setups

BBB

BBB-on-a-chip

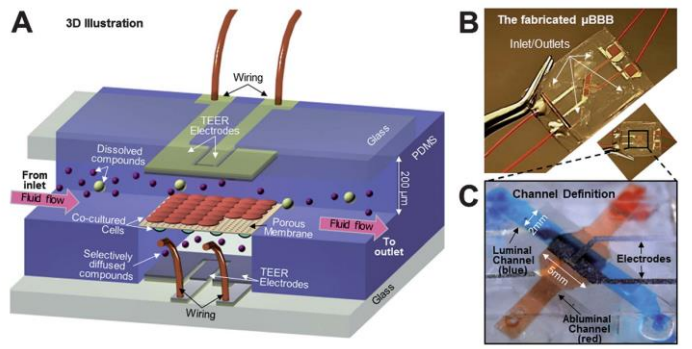


Fig. 2 Structure and design of the developed µBBB. (A) The µBBB system comprises two perpendicular flow channels. (B) The fully fabricated µBBB chip. (C) Close-up view. Channels model the lumenal (blue) and ablumenal (red) sides of the neurovascular unit. Endothelial cells and astrocytes are respectively cultured on the lumenal and ablumenal sides of the enclosed porous membrane. Channel heights are 200 µm, and channel widths are 2mm (lumen) and 5mm (albumen).

DOI: 10.1039/c2lc40094d

BBB

A comparison reveal that dynamic co-cultures are better

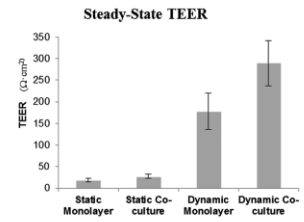


Fig. 7 Steady-state TEER levels of each base condition. Dynamic cultures reached significantly higher TEER levels than static cultures. For both systems, co-cultures developed higher TEER levels than endothelial monolayers alone.

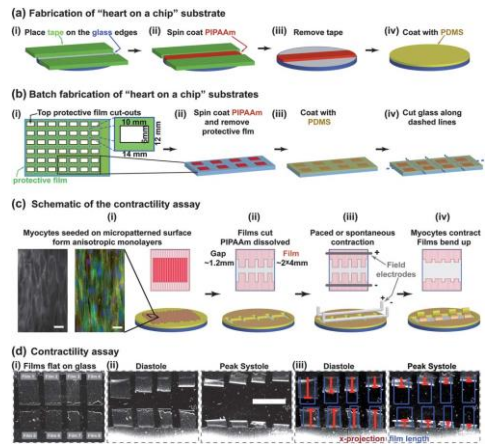
Examples

The heart

Heart on a chip

Standardization of myocytes on chip

- Contractility
- Action potential propagation
- Cytoskeletal architecture
- Real time monitoring



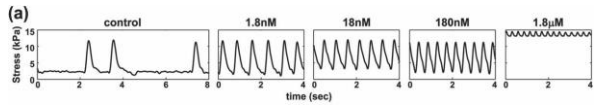
Ensembles of engineered cardiac tissues for physiological and pharmacological study: heart on a chip. Grosberg A et al, Lab Chip. 2011 Dec 21;11(24):4165-73. Epub 2011 Nov 10.

Examples

The heart

Application of the heart on chip

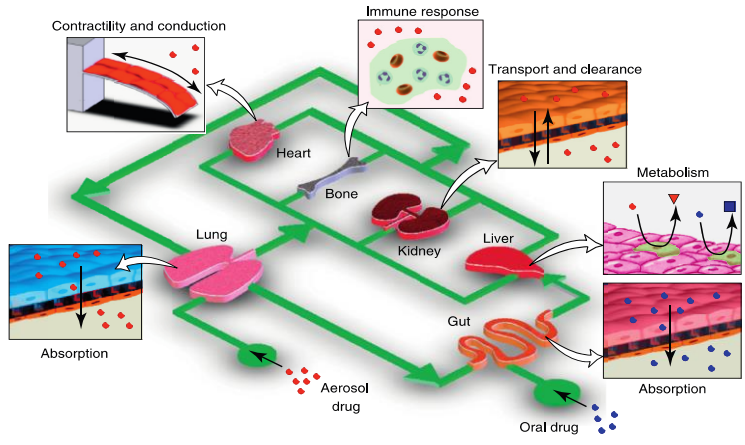
Epinephrine response is in accordance with expectations



Ensembles of engineered cardiac tissues for physiological and pharmacological study: heart on a chip. Grosberg A et al, Lab Chip. 2011 Dec 21;11(24):4165-73. Epub 2011 Nov 10.

Beyond state of the art

Human-on-a-chip



Huh et al. 2011. From 3D cell culture to organs-on-chips. *Trends in Cell Biology*, 21, 745-754.

Integration

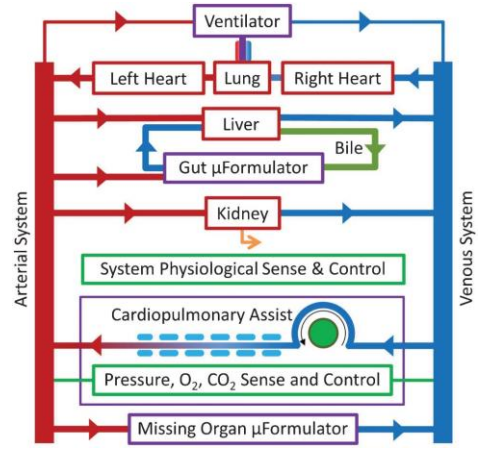


Fig. 2 The mHu Advanced Tissue-engineered Human Ectypal Network Analyzer (ATHENA), a milliHuman (*Homo chippus*) being developed by Los Alamos National Lab, Vanderbilt University, Charité Universitätsmedizin Berlin, University of California–San Francisco, Harvard University, and CFD Research Corporation with the support of the Defense Threat Reduction Agency (DTRA).[2,3] Figure from Wikswo *et al.*, 2013, with permission.[1]

Scaling and systems biology for integrating multiple organs-on-a-chip
John P. Wikswo, Erica L. Curtis, Zachary E. Eagleton, Brian C. Evans, Ayeeshik Kole, Lucas H. Hofmeister and William J. Matloff

Lab Chip, 2013, 13, 3496-3511
DOI: 10.1039/C3LC50243K
From themed collection Lab-on-a-Chip: 2013

Internal scaling of body components

Table 1 Allometric scaling coefficients and organ masses for a Hu, mHu, and µHu based upon primate data. Coefficients from Stahl, 1965[12]

			Human		milliHuman (mHu)		microHuman (µHu)		Organ mass ratios	
		Body mass:	60 kg		60 g		60 mg			
Organ	A	B	M, g	Organ/Body	M, g	Organ/Body	M, mg	Organ/Body	M_{mHu}/M_{Hu}	$M_{\mu Hu}/M_{Hu}$
Liver	33.2	0.93	1496	2.5%	2.4	4.0%	3.9	6.6%	1.62E-03	2.63E-06
Brain	85	0.66[a]	1268	2.1%	13	22%	139	232%	1.05E-02	1.10E-04
Lungs	9.7	0.94	455	0.76%	0.69	1.2%	1.0	1.7%	1.51E-03	2.29E-06
Heart	5.2	0.97	276	0.46%	0.34	0.57%	0.42	0.70%	1.23E-03	1.51E-06
Kidneys	6.3	0.87	222	0.37%	0.54	0.91%	1.3	2.2%	2.45E-03	6.03E-06
Pancreas	2.0	0.91	83	0.14%	0.15	0.26%	0.29	0.48%	1.86E-03	3.47E-06
Spleen	1.5	0.85	49	0.081%	0.14	0.23%	0.39	0.64%	2.82E-03	7.94E-06
Thyroid	0.15	1.12	15	0.025%	0.0064	0.01%	0.0028	0.0047%	4.37E-04	1.91E-07
Adrenals	0.53	0.7	9.3	0.016%	0.07	0.12%	0.59	0.98%	7.94E-03	6.31E-05
Pituitary	0.03		0.49	0.00081%	0.0044	0.0074%	0.040	0.067%	9.12E-03	8.32E-05

[a] Coefficients for human brain scaling: 80–90. The corresponding number for monkeys is 20–30, and great apes 30–40.

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From themed collection Lab-on-a-Chip: 2013

Scaling of organ function is non-linear

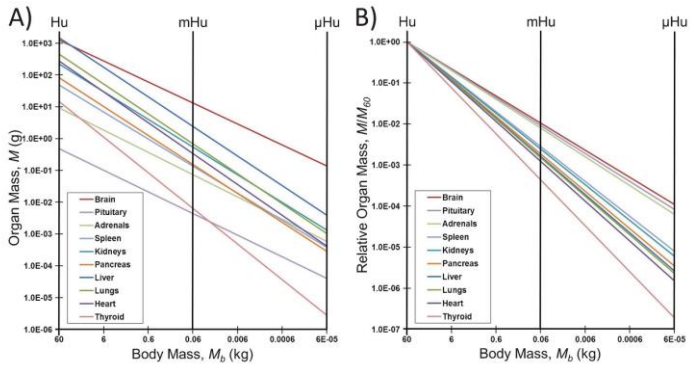
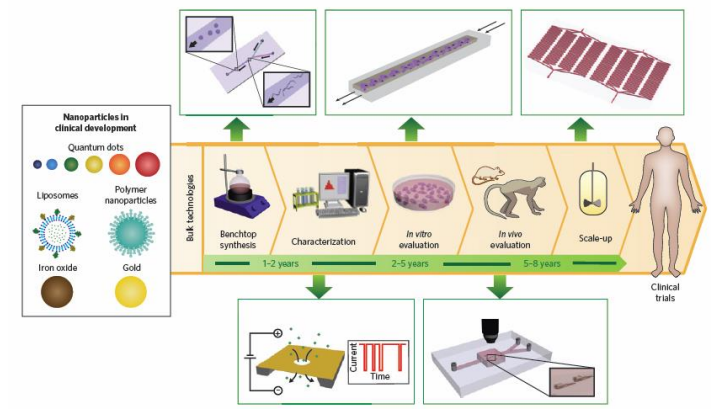


Fig. 1 How allometric scaling might (mis)inform mHu and µHu scaling when known power laws[12] are used to extrapolate from humans. A) Organ mass in grams. B) The mass of each organ relative to that for a 1.0 Hu. Note the range in allometric slopes for different organs, and that a 10^6 reduction in body mass leads to only a 10^4 reduction in the mass of the brain, pituitary, and adrenals, leading to a µBrain with twice the mass of the µHuman.

When integrating organs on a milli/micro-scale, different organs are given different weights

Beyond state of the art

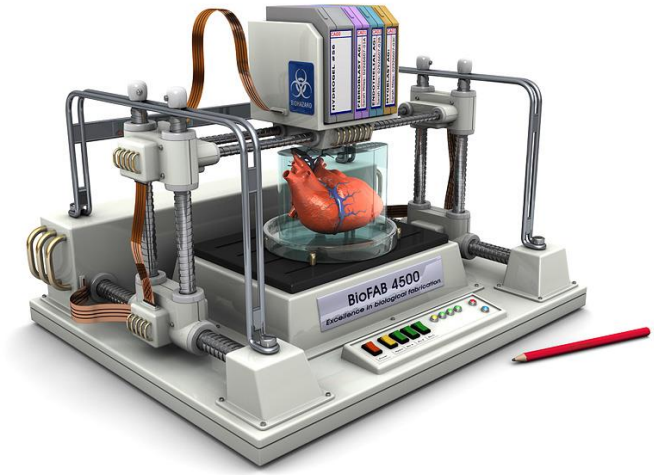
Integrated microtechnologies for development of next generation nanopharmaceuticals



Microfluidic technologies for accelerating the clinical translation of nanoparticles.
Pedro M. Valencia et al Nature Nanotechnology 7, 623–629 (2012)
doi:10.1038/nnano.2012.168

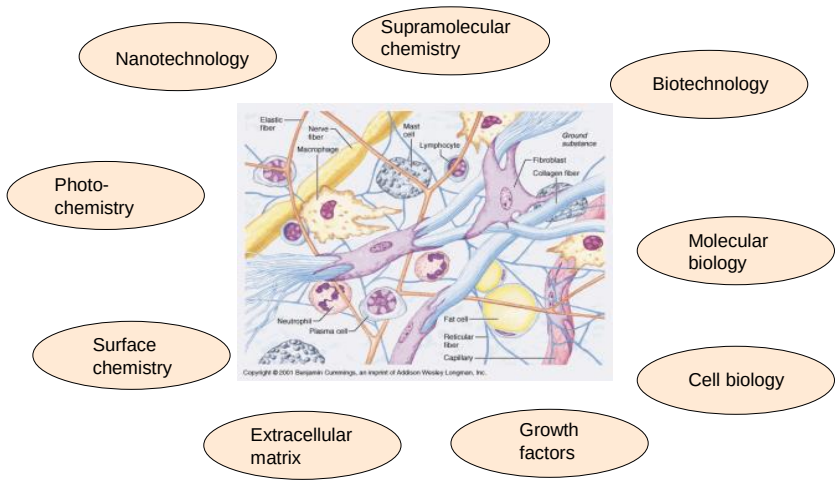
Beyond state of the art

A 3D bioprinter /plotter



Beyond state of the art

3D cell scaffolds – on a chip



Summary and conclusions

A wide range of nanotechnologies available at NTNU NanoLab
+

A wide range of biotechnology know-how at NTNU
+

A wide range of biomedical questions and tools available at NTNU/StOlavs
=

Opportunities for integrated and excellent science for better health

Pensum papers

Organs-on-chips at the frontiers of drug discovery
Eric W. Esch, Anthony Bahinski, Dongeun Huh
Nat Rev Drug Discov. . 2015 Apr; 14(4): 248–260. .doi: 10.1038/nrd4539

Microfluidic 3D cell culture: from tools to tissue models.
van Duinen V, **Trietsch** SJ, Joore J, Vulto P, Hankemeier T.
Curr Opin Biotechnol. 2015 Dec;35:118-26. doi: 10.1016/j.copbio.2015.05.002.