Bio-3D Printing:
Challenges and Opportunities

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NSF Workshop on Additive Manufacturing for Health

Arlington VA
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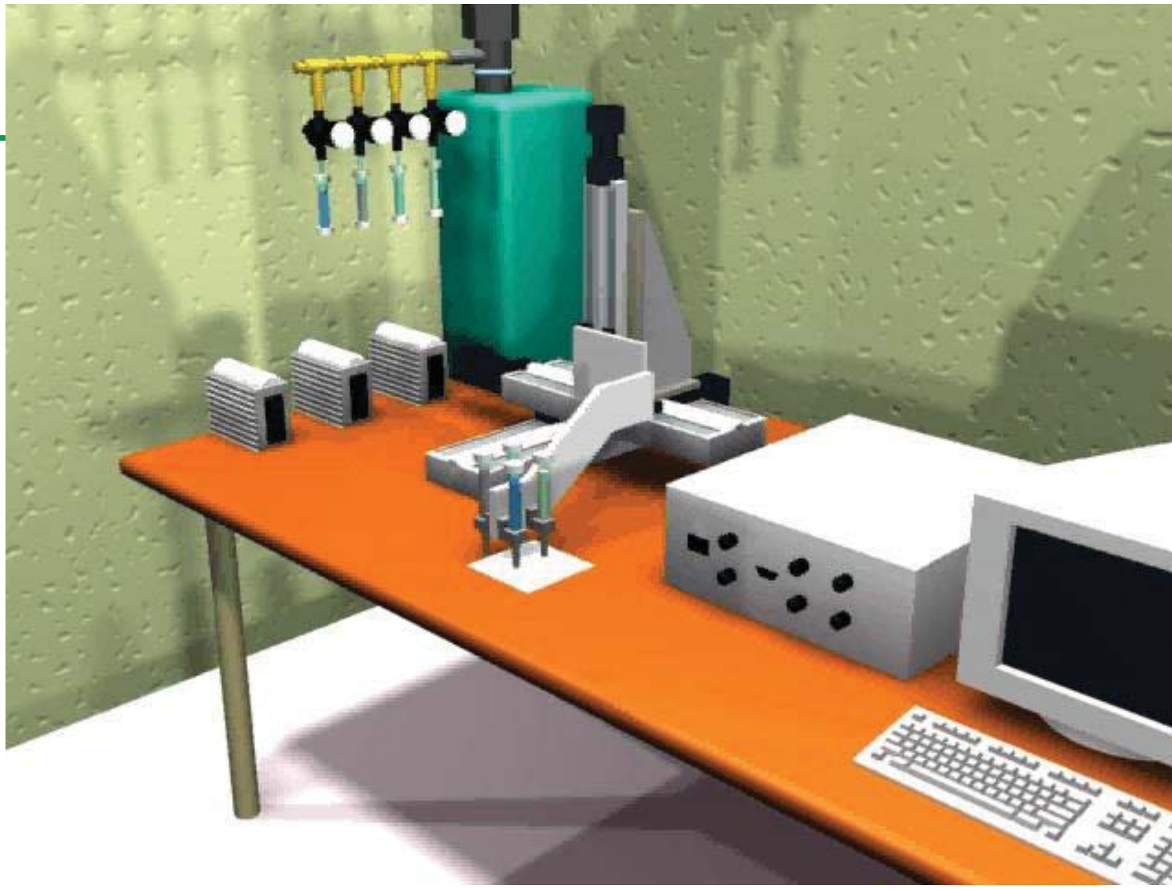
3D Printing

Innovation, Customization, Rapid Realization



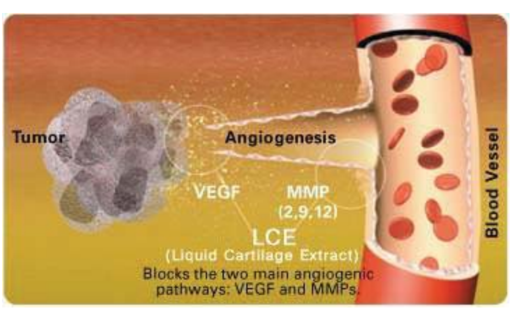
Directly Assembled 3D Biological Model by 3D Cell Printing

RGD Modified Surface

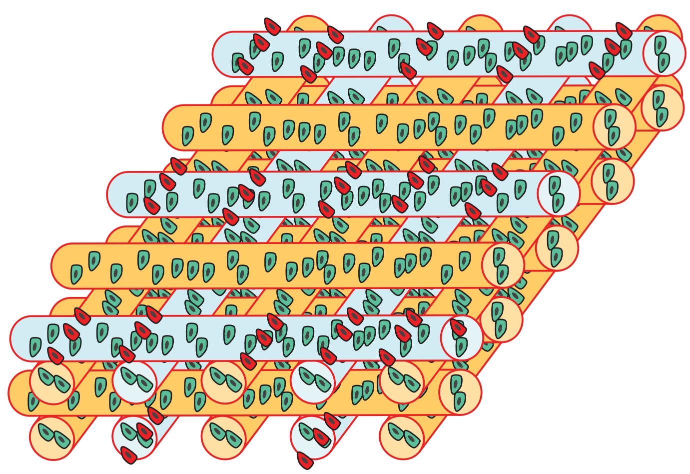
Unmodified Surface

Fibroblasts (Encasulated)

Endothelial (Applied to Surface)



http://www.angioworld.com/angiogenesis.htm



Direct Assembled Biological Model for Angiogenesis

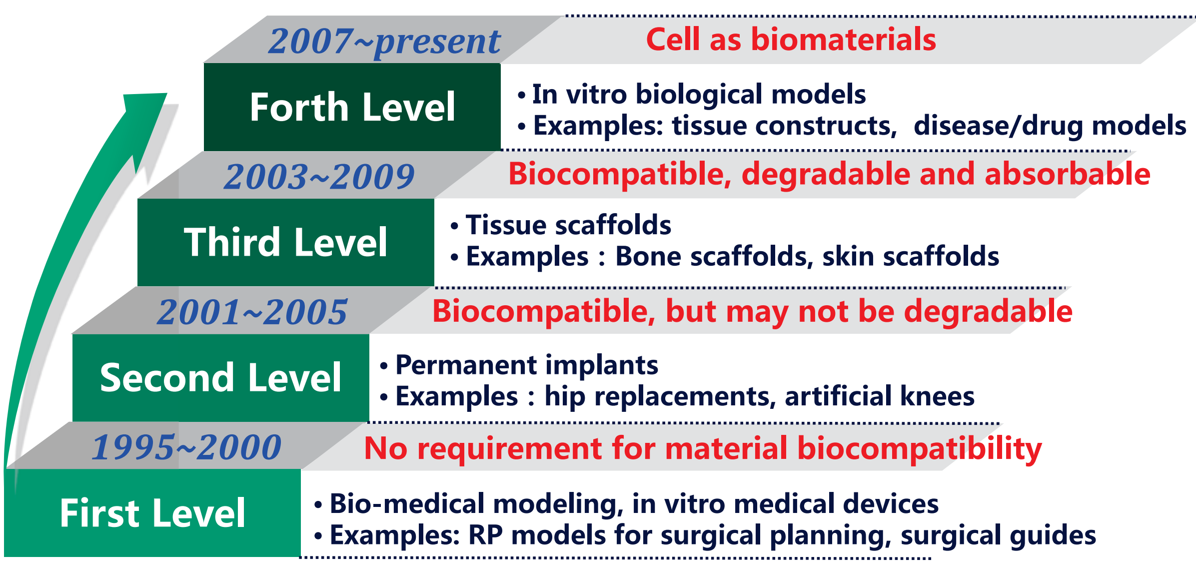
Bio-3D Printing

"Bio-3D Printing" uses biomaterials, cells, proteins or other biological compounds as building blocks to 3D Printing personalized (biomimetic) structures or *in vitro* functional biological models

--- according to a patient specific physiological structure, the designed cell microenvironment, or the required biological functions.

Bio-3D Printing: 4 Level Applications

- According to the technological development
- According to the requirements to biomaterials



Trend of Bio-3D Printing

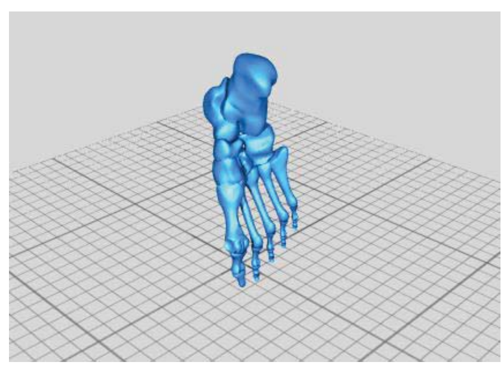
Application Level 1 to Level 3:

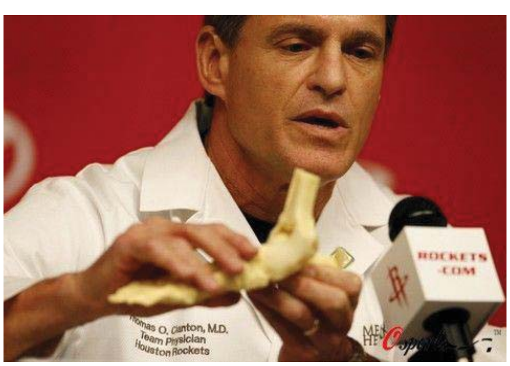
Enabling technologies

Translational Research

Commercialization

First Level: Bio-medical modeling, in vitro medical devices





Former NBA star Yao Ming's left foot suffered stress fractures after in a game in 2008, Rockets team physician Dr. Thomas Clanton explained to the reporters that the location, the severity of Yao's injury and the therapies would be used, using a foot skeleton model fabricated by 3D printing in the press conference.

First Level: Bio-medical modeling, in vitro medical devices

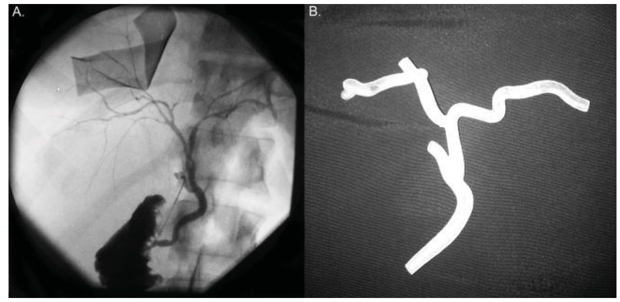


TABLE 2. Geometric Characteristics of 3D-Printed Liver Models and Corresponding Donor and Recipient Livers: Case 2

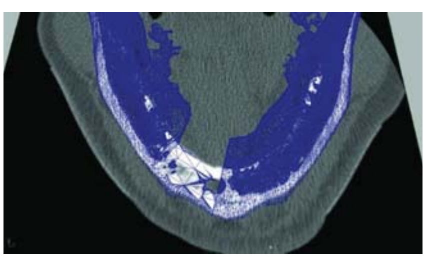
	Recipient's Liver		Donor's Right Liver Lobe	
	3D Print	Native Right Liver Lobe	3D Print	Native Right Liver Lobe
Volume (mL)	1235	1195	715	730
Length (cm)	17.5	17.5	15.4	15.3
Width (cm)	15.4	15.5	10	10.1
Height (cm)	7.5	7.5	9.5	9.5
Portal vein diameter (mm)	11	11.2	12	11.8
Right hepatic vein diameter (mm)	2.4	2.5	1.8	2
Left hepatic vein diameter (mm)	2.1	2	—	—

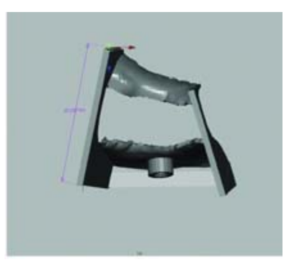
Liver model fabricated by 3D printing can be applied for preoperative planning in living donor liver transplantation.

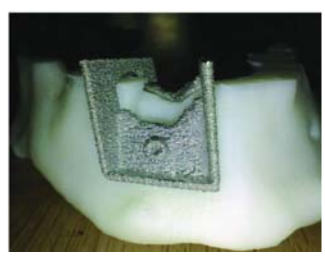
The preoperative identification of the vascular and biliary tract anatomy with 3-dimensional (3D) printing may allow better preoperative surgical planning, avert unnecessary surgery in patients with potentially unsuitable anatomy, and thereby decrease the complications of liver transplant surgery.

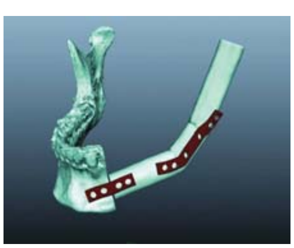
Nizar N. Zein, et al, Liver Transplantation, 2013

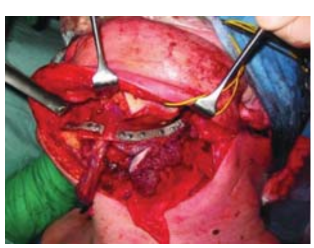
First Level: Bio-medical modeling, in vitro medical devices







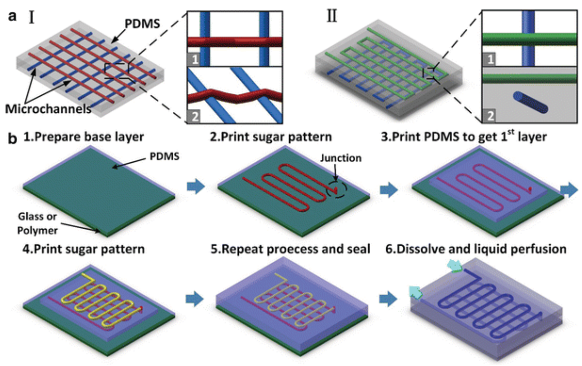


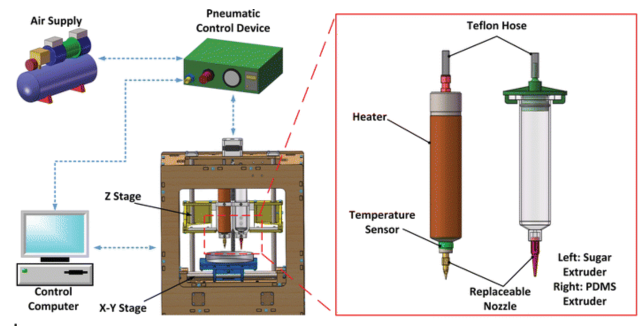


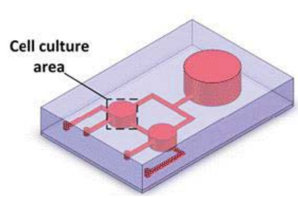
Patient-specific titanium reconstruction plates, cutting guide can be used in craniomaxillofacial surgeries.
Now, electron beam melting(EBM)-produced implants are fully usable under clinical conditions in reconstruction of acquired defects in the mandible.

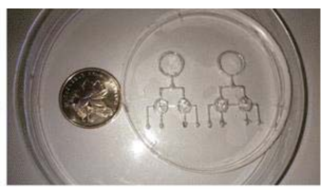
Per Dérand, et al. Craniomaxillofac Trauma Reconstr. 2012

First Level: Bio-medical modeling, in vitro medical devices









Researchers from Zhejiang University, China proposed a rapid manufacturing method to build microfluidic chips: they printed melted sugar to form a sacrificial layer including the channels inside the PDMS chip. After the curing of PDMS, the whole chip was put into water to dissolve the sacrificial layer and the channels were left hollow.

Yong He, et al., Microfluid Nanofluid, 2015

Second Level: Permanent implants

In 2013, POSTECH and Seoul St. Mary's Hospital, Korea successfully completed a facial surgery on a Mongolian child born without a nose or airway.
A new 'nose' including nostrils, nose bones and airways were made by 3DP and implanted into the patient's body. The surgery was a success.

Second Level: Permanent implants

It was demonstrated that stiffness-compatible implants can be fabricated for optimal stress shielding for bone regimes as well as bone cell ingrowth by 3D Printing(Electron beam melting, EBM) .

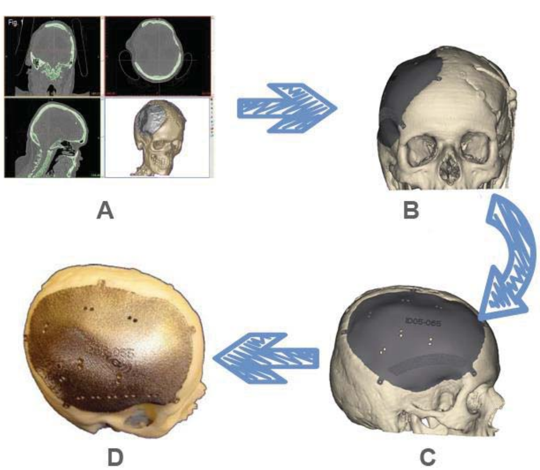
Lawrence E. Murr, et al., International Journal of Biomaterials, 2012

Second Level: Permanent implants

The implant surgery

Patient before surgery with indentation on the forehead

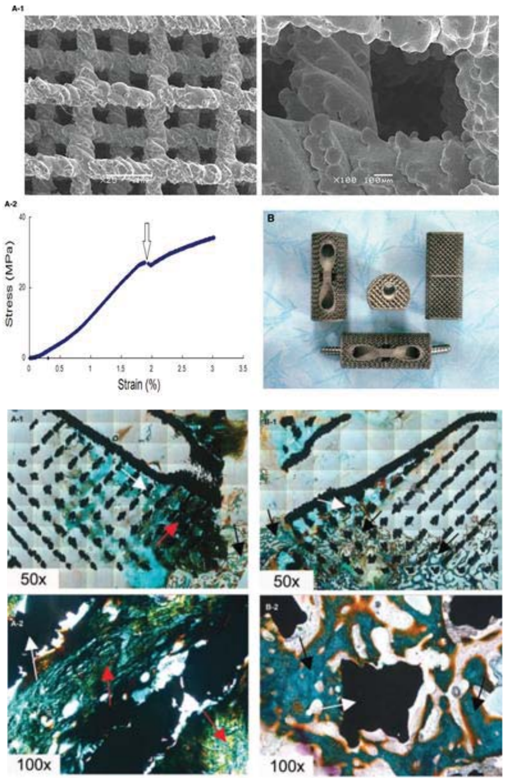
Patient after surgery with the forehead reconstructed cosmetically

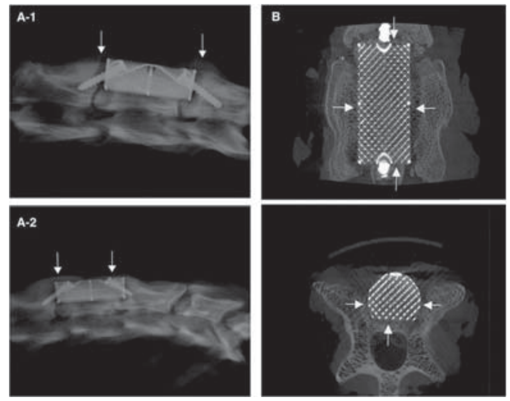


Custom Skull Implants sold by Materialise™

①Convert the CT scan data of the patient's head into an accurate 3D model which could be used for the design of a patient-specific implant.
②Design and select appropriate shapes of the implant.
③Fabricate the implant in titanium using a method of 3D printing called Electron Beam Melting(EBM).

Second Level: Permanent implants

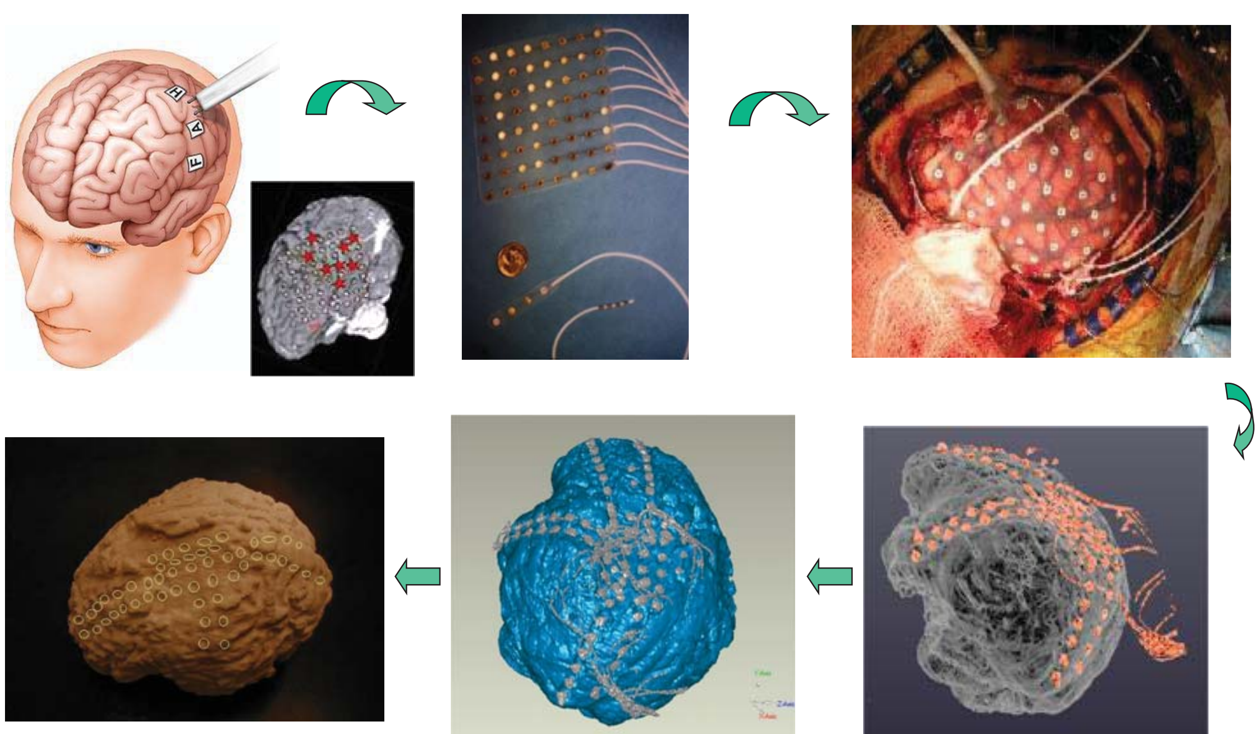




In 2014, Dr. Zhongjun Liu from Peking University Third Hospital, China successfully implanted the first 3D-printed vertebra in a 12-year-old boy with cancer in his spinal cord. The titanium bone substitute fabricated by Electron Beam Melting was full of small holes that let natural bone grow inside.

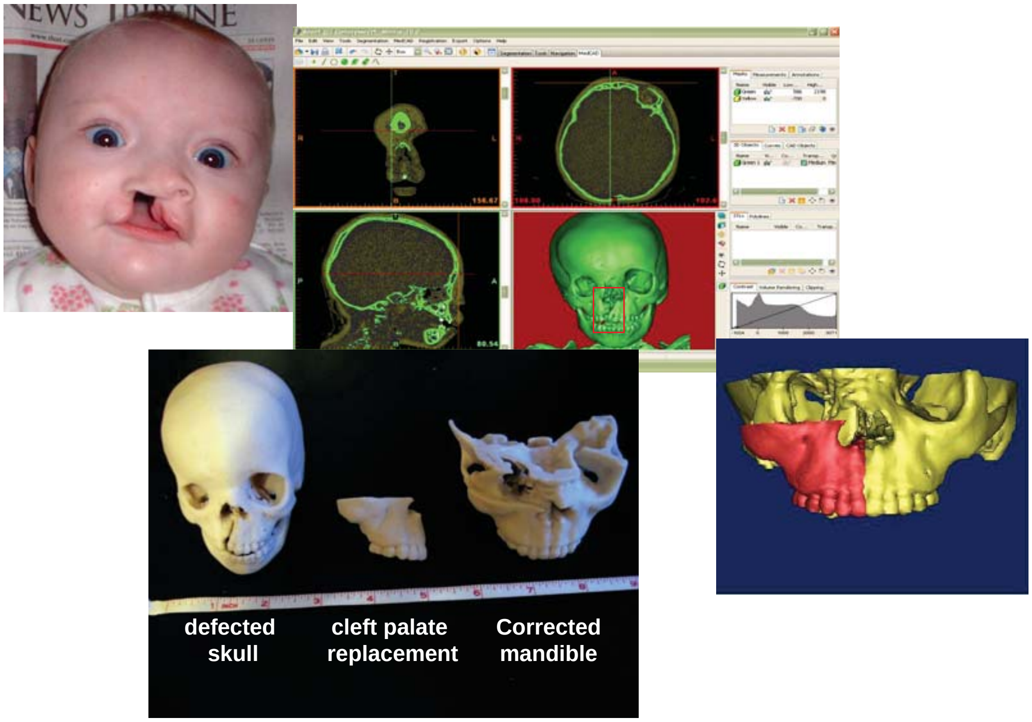
Yang J, et al., Spine, 2014

Biomodeling of Epileptic Regions of Brain with Electrodes - Dr. Sperling (TJU)

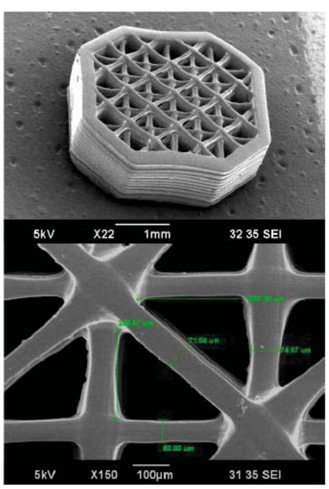


Biomodeling of Cleft Palate Deformities

- Dr. HD Nah (CHOP)



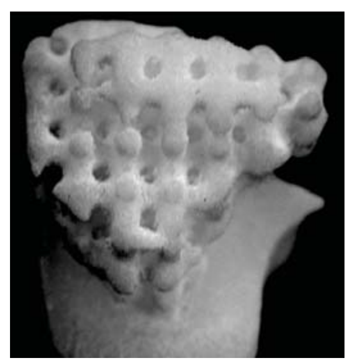
Scaffolds by Conventional 3DP Processes



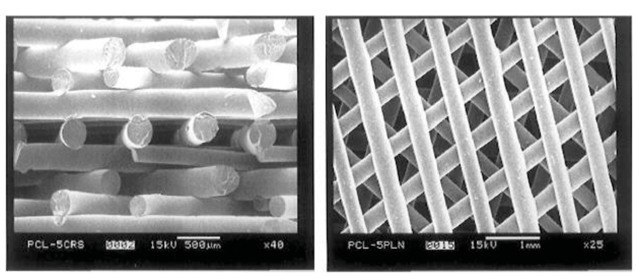
SLA builds concept-verification models of its tensegrity structures

70 μm in diameter.

SJ Lee & DW Cho (POSTECH, Korea, Micro-SLA))

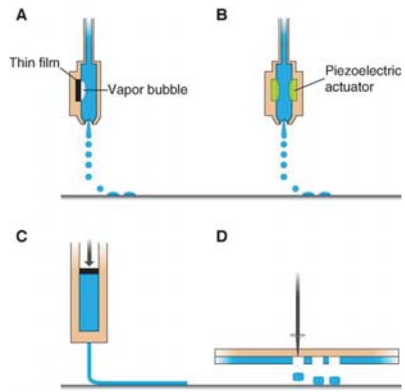


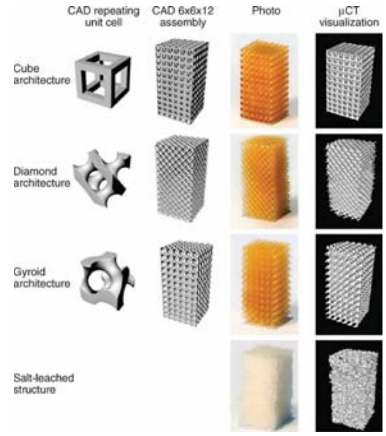
(Das & Hollister Group, UM\, SLS)

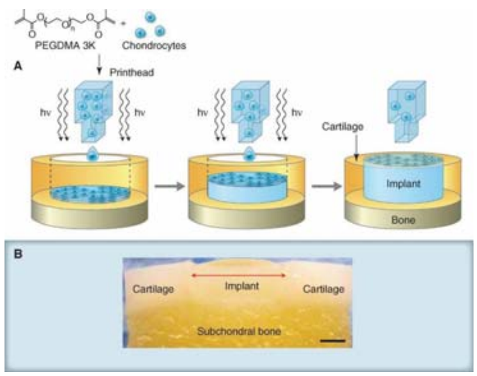


D. Hutchmacher group, FDM)

Third Level: Tissue scaffolds





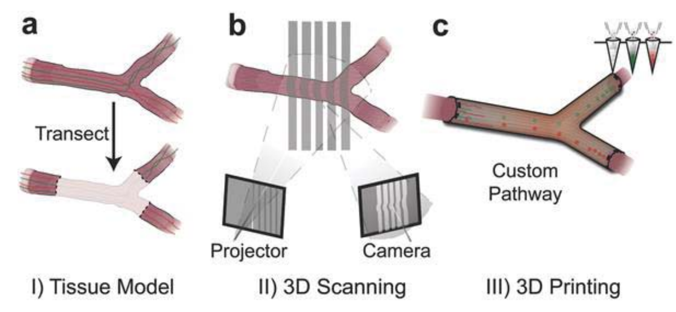


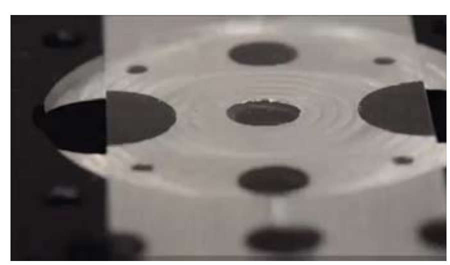
Various techniques 3D printing have been invented to fabricate scaffolds. Tomographic reconstruction, and numerical modeling methods have allowed researchers to design a range of complex internal scaffold architectures with a range of length scales.

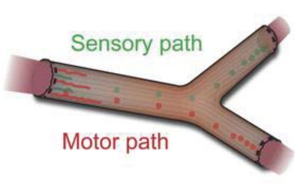
New materials have been developed, for example, a poly(ethylene glycol) dimethacrylate (PEGDMA) solution can be printed to repair cartilage with a simultaneous photopolymerization process.

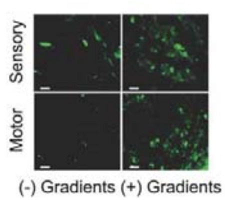
Brian Derby, Science, 2012

Third Level: Tissue scaffolds







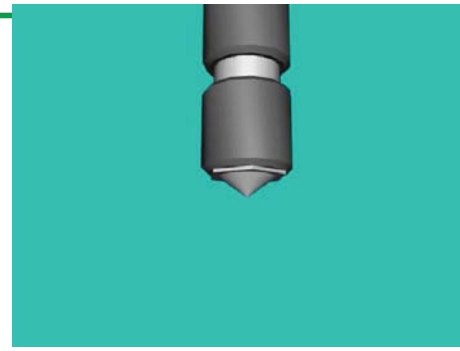


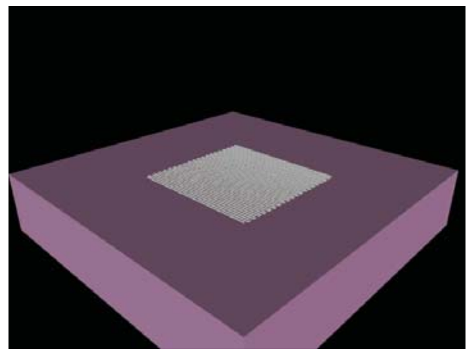
Prof. McAlpine from University of Minnesota developed a first-of-its-kind, 3D-printed guide that helps regrow both the sensory and motor functions of complex nerves after injury.

Incorporated into the guide were 3D-printed chemical cues to promote both motor and sensory nerve regeneration. The guide was then implanted into a rat by surgically grafting it to the cut ends of the nerve.
Within about 10 to 12 weeks, the rat's ability to walk again was improved.

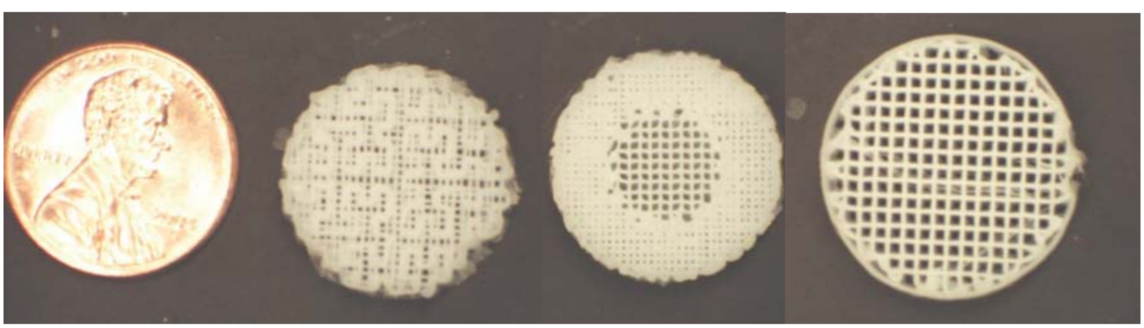
Johnson B N, et al., Advanced Functional Materials, 2015

Precision Extrusion Deposition





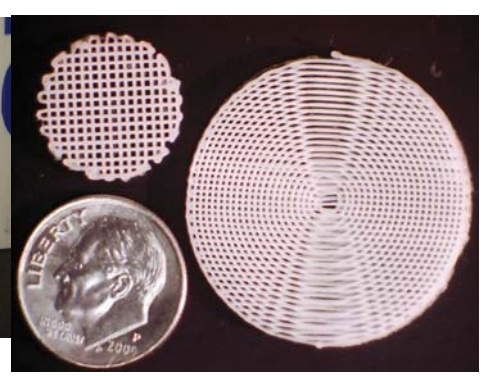
Scaffolds Fabricated by Precision Extrusion Deposition Technique



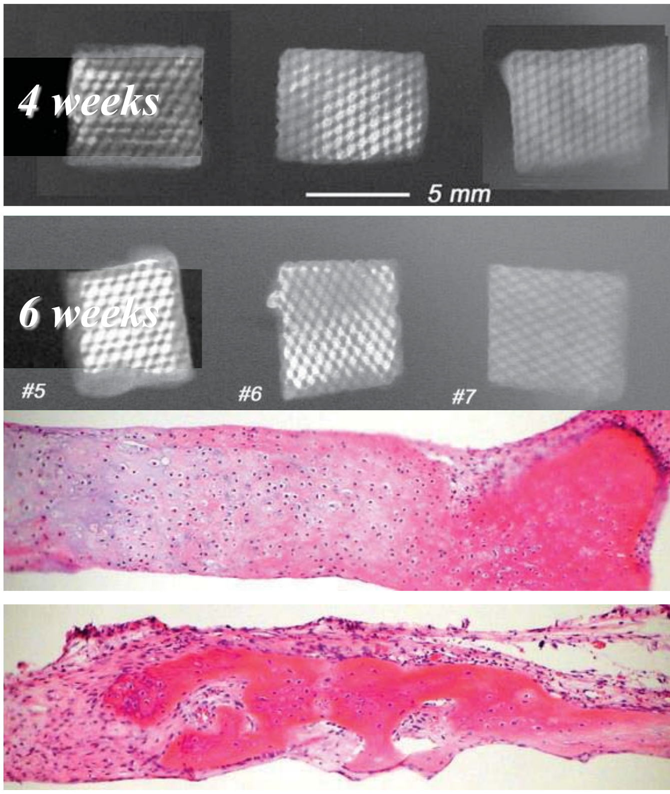
Material:
Poly-ε-Caprolactone (PCL)

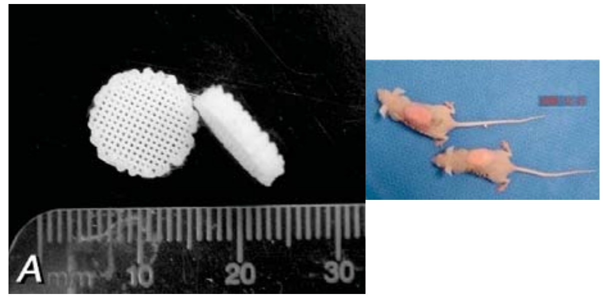
Average pore size:
~ 200 μm
Smallest strut: 100 μm

Darling et al, JBMB, 2005
Wang, et al, RPJ, 2005
Starly et al, CAD 2006
Shor et al, Biomaterials, 2007



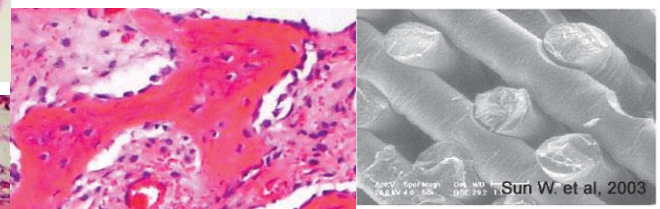
Nude Mouse SC Osteogenesis (collaborate with Dr. H. An, MUSC)





Nude Mouse
Subcutaneous Osteogenesis
Printed poly ε-Caprolactone scaffold

PCL scaffold + bovine osteoblasts



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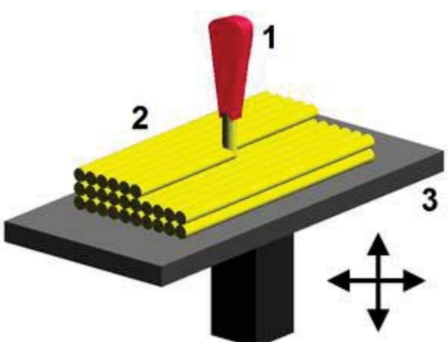
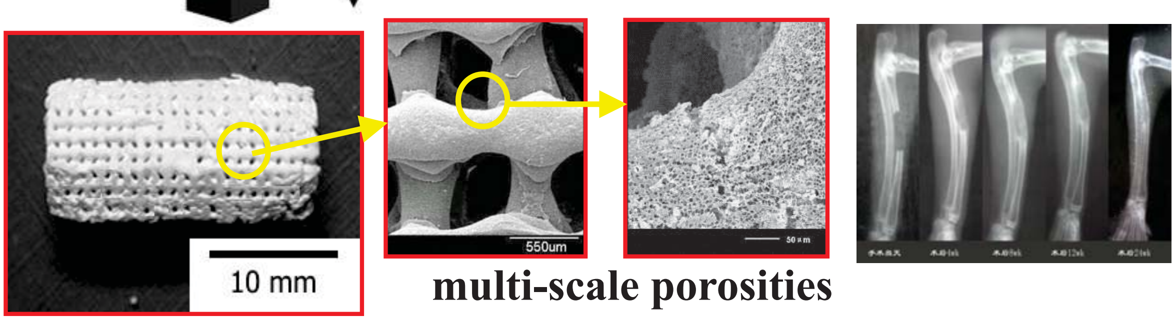
Low-temperature deposition for fabrication of multi-scale pores tissue scaffolds



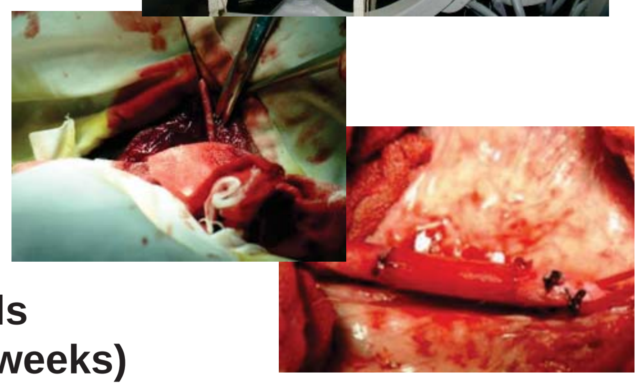
PLA-TCP



multi-scale porosities

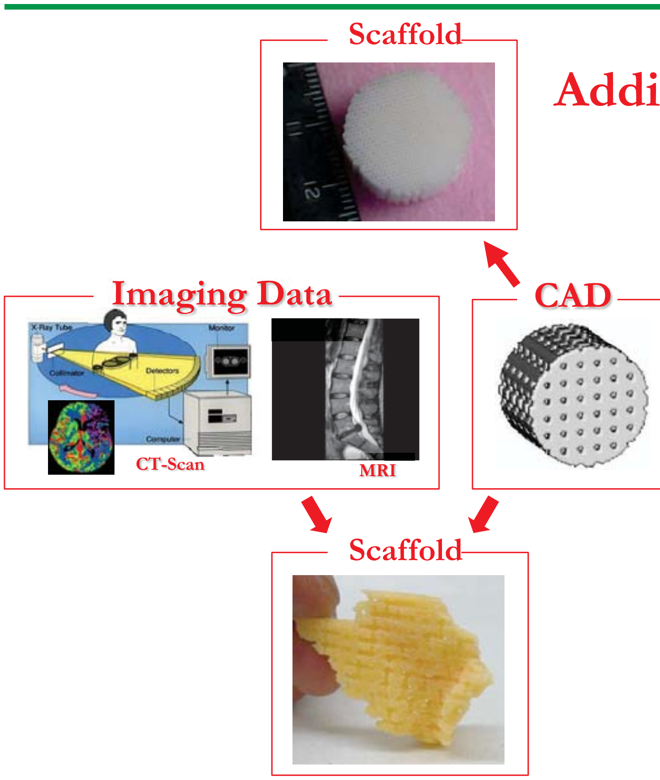
Low-temperature Deposition for 3DP of Tissue Scaffolds (Tsinghua)

3DP for Engineering Blood Vessels



Animal Models
(Mice and Dog-24 weeks)

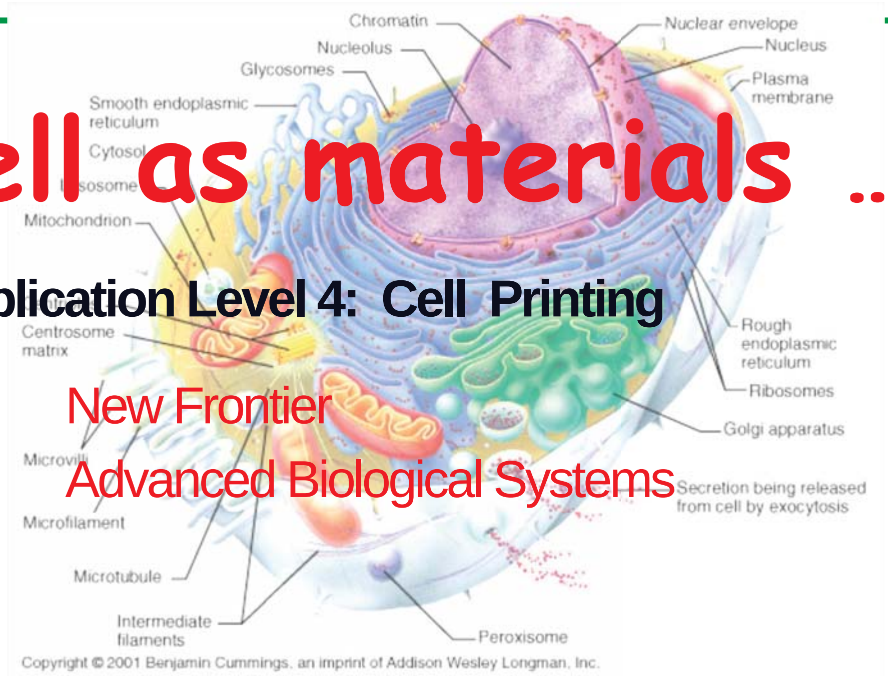
Tissue Scaffold Fabrication - Direct Methods by 3DP



Additive Manufacturing (3DP)

- CT/MRI – CAD – SFF:
  - FDM
  - SLS
  - 3DP – Theriform Process
- Advantages:
  - Biomimetic
  - No restriction on shape
  - High control capability
  - Consistent – reproducible
- Disadvantages:
  - Limited resolution
  - Not a cell-friendly environment
    - Harsh Heat
    - Toxic Solvents
    - Non-Sterile

cell as materials …

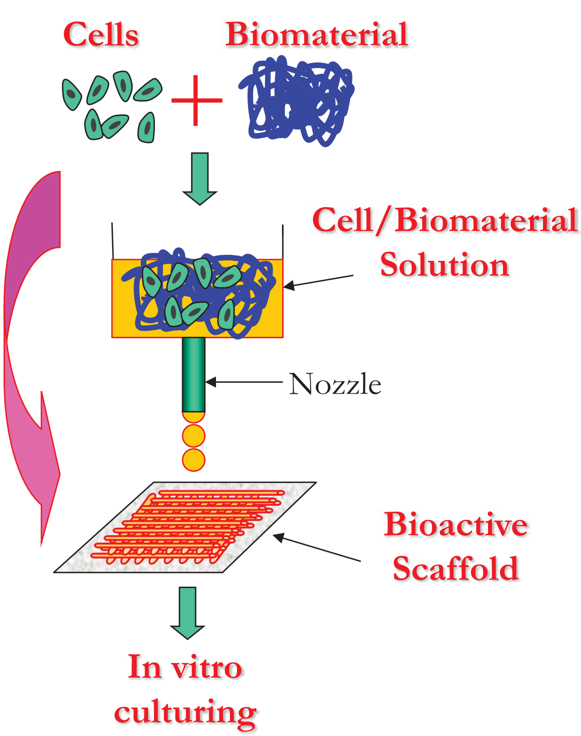


Application Level 4: Cell Printing

New Frontier

Advanced Biological Systems

Printing Cell with Gel

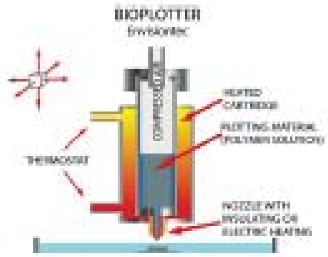


- **Cell/Biomaterial solution is delivered to form a designed 3D architecture**
- **Seeding or printing cells simultaneously with the construction of scaffolds**
- **Advantages:**
  - Controlled cell density and spatial distribution
  - Assisted cell seeding and migration to allow the construction of large thick tissue scaffold
  - Incorporation of hybrid scaffolding materials, growth factors and nutrients to form heterogeneous scaffolds
  - Consistent, automated and reproducible

Enabling Engineering Processes for Cell Printing/Assembly

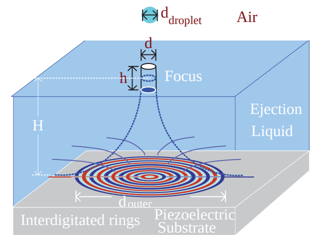
Inkjet

(thermal, piezo)
Dimatix, Clemson
U. Manchester
Epson



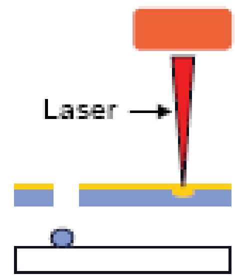
Bio-plotting

(extrusion through syringe)
Envisiontech, Organovo
Tsinghua, Drexel, POSTECH
Harvard (J. Lewis)
Invivo BAT- Sciperio



Acoustic Droplet Ejection

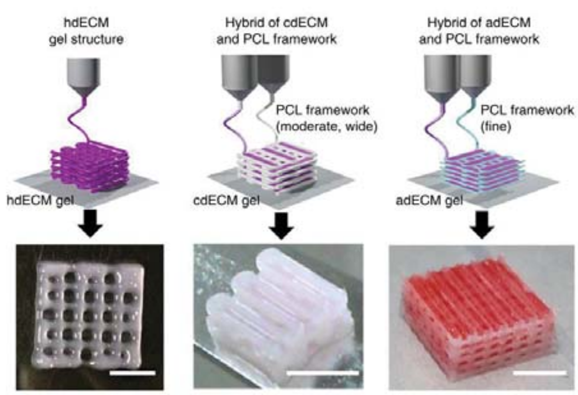
Stanford
(Demirci)

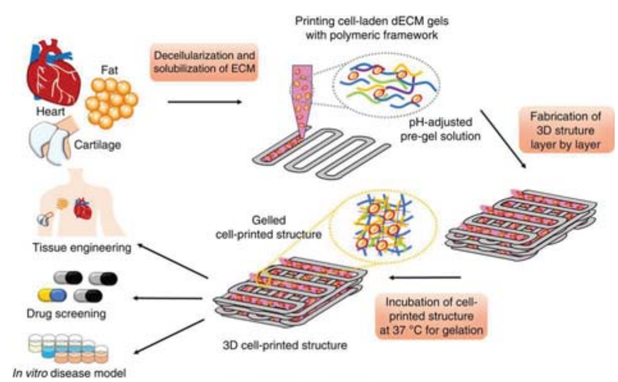


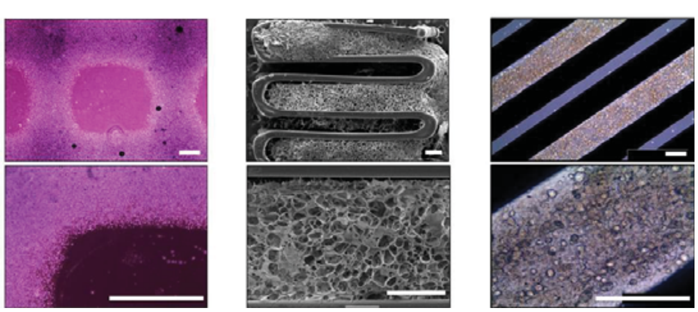
Laser-Assisting

NRL
INSERM-France
(Guillemot)
LDW-Clemson

Forth Level: In vitro biological models



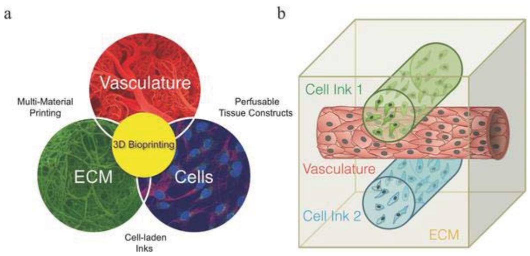


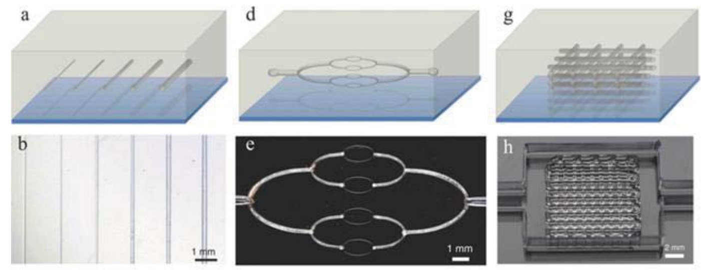


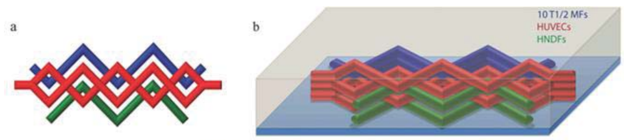
Prof. Dong-Woo Cho's group from POSTECH, Korea printed cell-laden decellularized extracellular matrix(dECM) from cartilage tissue, heart tissue, adipose tissue to form functional tissue *in vitro*. The results showed that dECM was capable of providing crucial cues for cells engraftment, survival and long-term function.

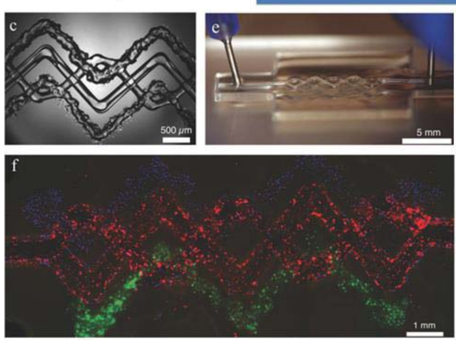
Pati, F., et al., Nat Commun, 2014

Forth Level: In vitro biological models





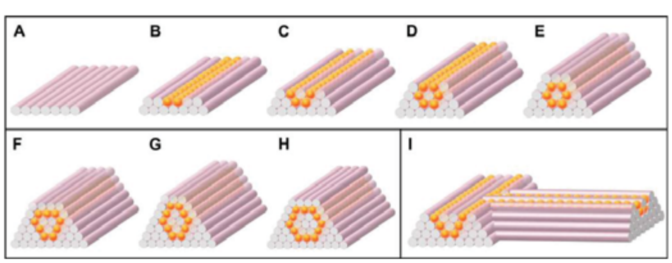


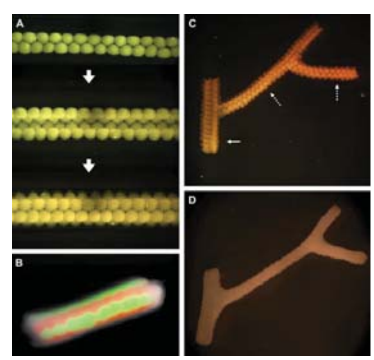


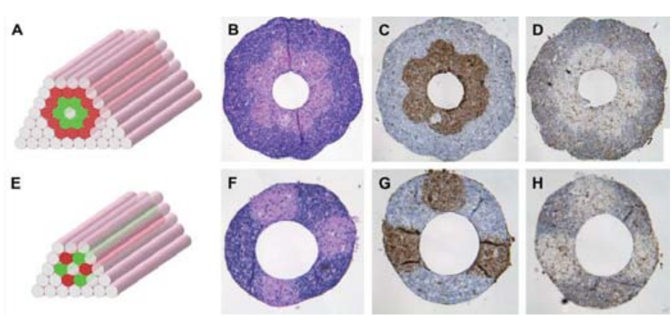
Prof. J.A. Lewis's group from Harvard University printed vascularized, heterogeneous cell-laden tissue constructs successfully using a modified bioprinter. In this study, two kinds of cells on both sides of the artificial blood vessels had normal development.
The construction of the microvascular structure and the realization of their basic functions may help to solve the big problem that cells in a big scaffold always suffer from lacking of oxygen and nutrients.

David B, Kolesky, et al, Advanced Materials, 2014

Forth Level: In vitro biological models



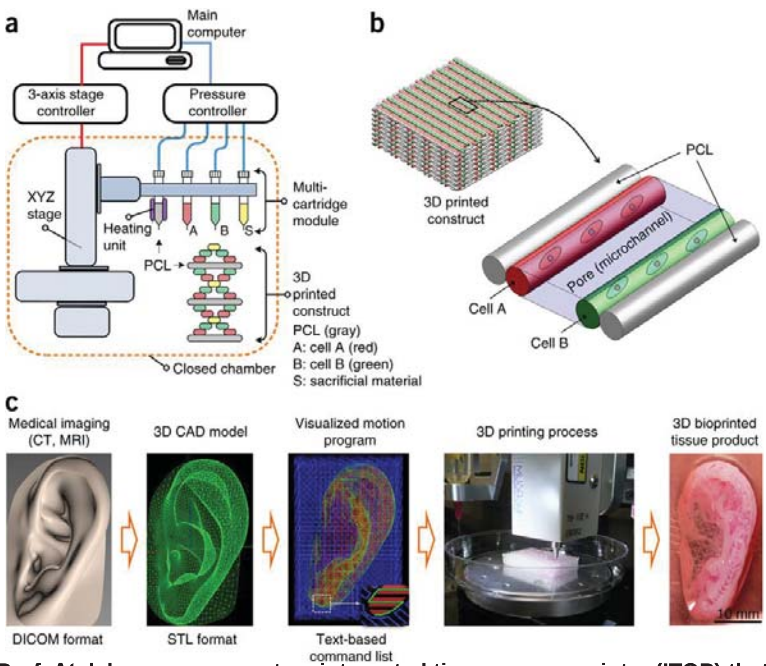




Prof. Forgacs and his colleagues used multicellular spheroids concomitantly with agarose rods as bio-inks to print vessels *in vitro*, the diameter of each spheroid is 300-500μm and the vessel is 900-2500μm wide. It took 5-7 days for the spheroids to fuse together to form final tubular construct.

C. Norotte, et al., Biomaterials, 2009

Forth Level: In vitro biological models

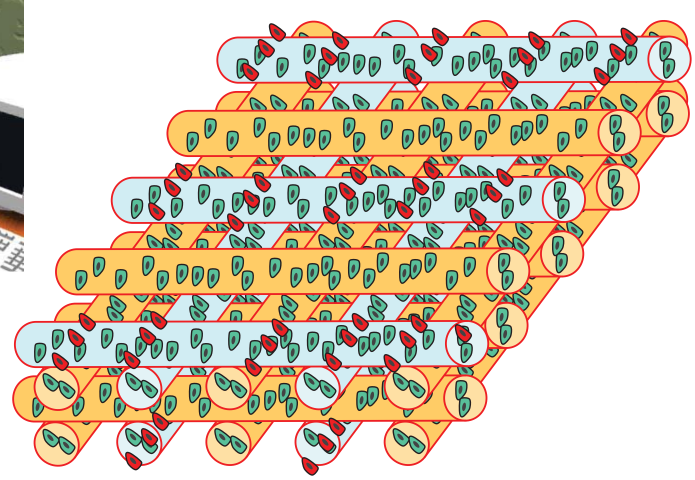


Kang, H. W., et al, Nat Biotechnol., 2016

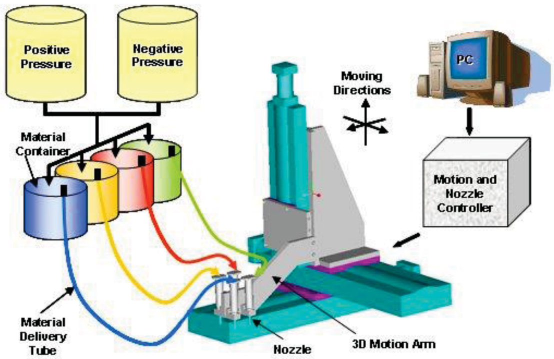
Prof. Atala's group present an integrated tissue–organ printer (ITOP) that can fabricate stable, human-scale tissue *in vitro.* PCL, as the support material, brought the ideal strength of the whole structure. Pluronic F-127 hydrogel was used as a sacrificial outer layer to support the 3D architecture of the dispensed cell-laden structures before crosslinking, which could leave micro-channels in the structures after dissolution. Two kinds of call-laden hydrogels and the micro-channels made the tissue more complicated.

33

Multi-nozzle Direct Cell Printing



Multi-nuzzle 3D Cell Deposition System



Multi-nozzle systems:

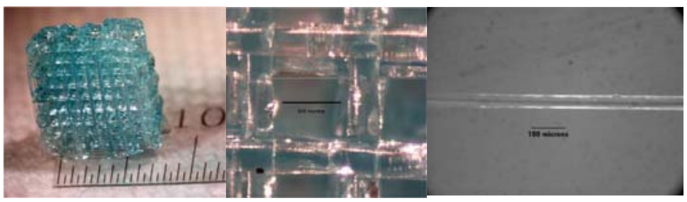
- Precision extruding
- Solenoid-actuated
- Piezoelectric
- Pneumatic syringe
- Pneumatic spray

Biopolymer:

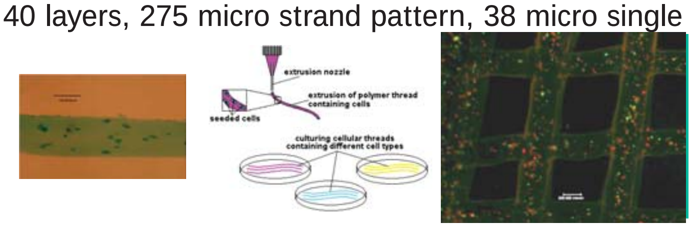
- Hydrogel-Alginate/Chitosan
- Fibrin, Collagen
- Matrigel

Cells:

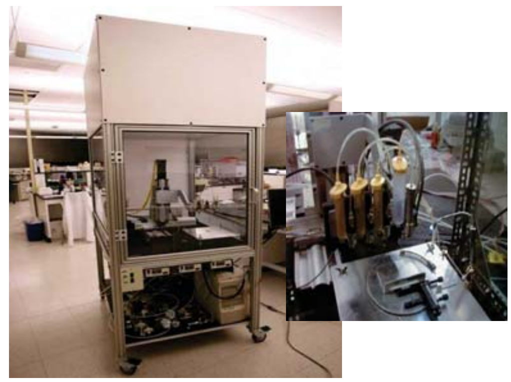
- Endothelial
- Cardiomyoblasts
- Fibroblast
- Chondrocytes
- Osteoblasts
- Sm. muscle cells
- Hepatocyte
- MCF-7
- Huh7.5.1



40 layers, 275 micro strand pattern, 38 micro single strand



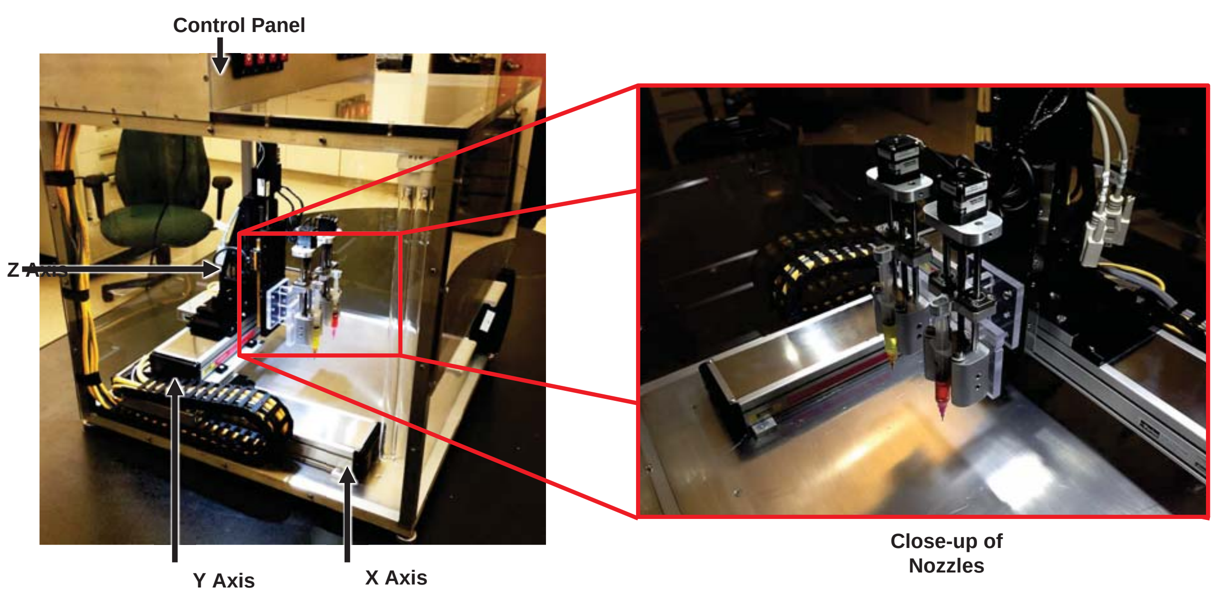
Cell deposition, cellular thread, cell viability

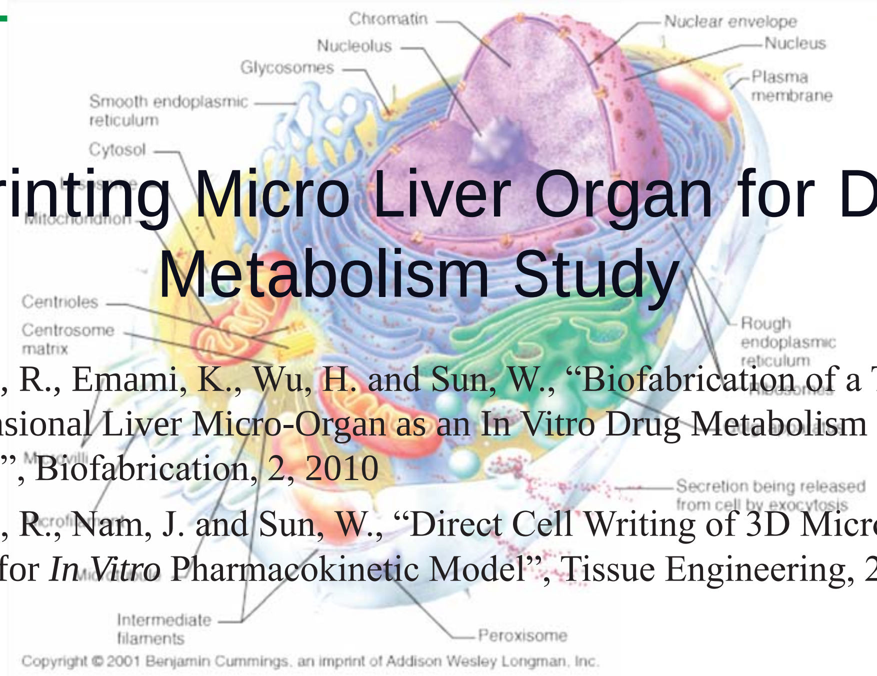


US Patent #: 8639484 (2003)

Heterogeneous Bio-Printing

Multi-nozzle Direct Cell Printing





Bioprinting Micro Liver Organ for Drug Metabolism Study

- Chang, R., Emami, K., Wu, H. and Sun, W., "Biofabrication of a Three-Dimensional Liver Micro-Organ as an In Vitro Drug Metabolism Model", Biofabrication, 2, 2010
- Chang, R., Nam, J. and Sun, W., "Direct Cell Writing of 3D Micro-organ for *In Vitro* Pharmacokinetic Model", Tissue Engineering, 2008

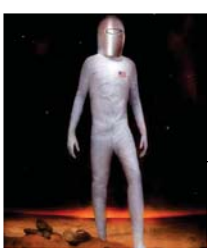
Bioprinting Micro Liver Organ for Anti-radiation Drug Metabolism Study

(NSAS-USRA-09940-008)

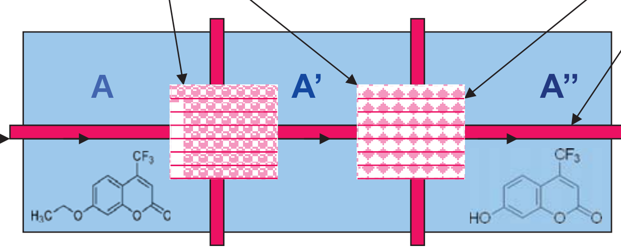
NASA's Interest - Safe plenary exploration & Mars Landing

Bioprinted Micro-organs

Microfluidic channels



Drug A



7-Ethoxy-4-(trifluoromethyl) coumarin (EFC)

7-Hydroxy-4-(trifluoromethyl) coumarin (HFC)

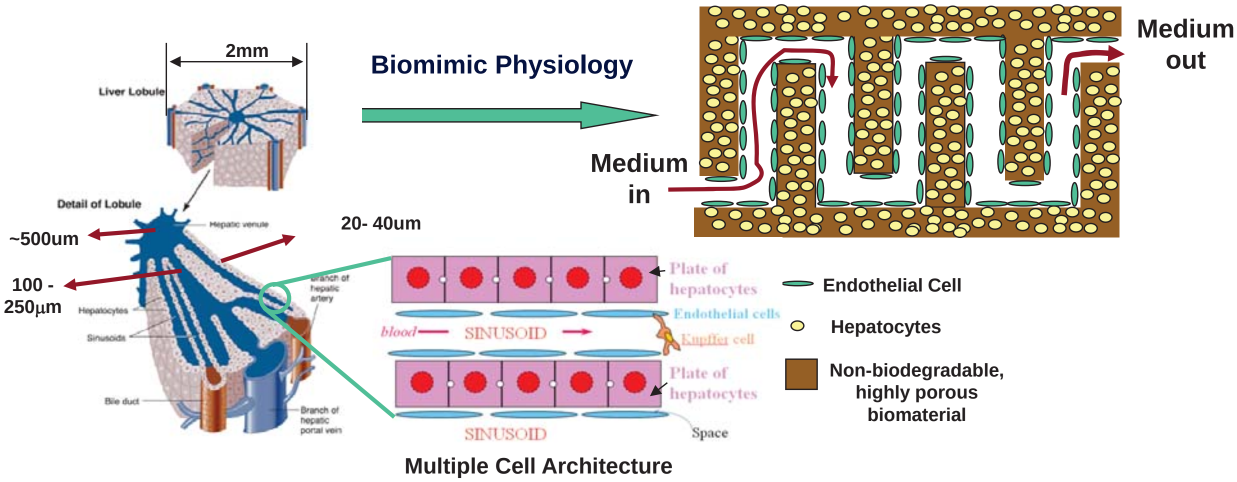
Micro-Organ Device

Liver

Target Organ
Heart
Brain
Mussel
Bone
…

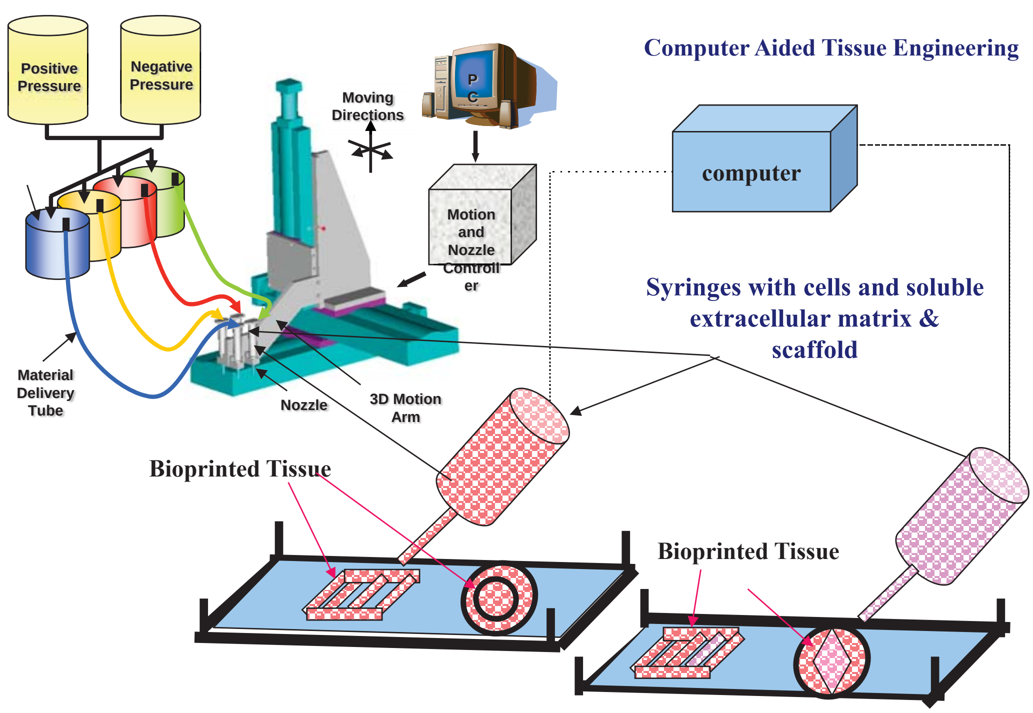
Micro-Organ Device for drug conversion study (A, A', A" with multiple micro-organs)
Schematic drug metabolic conversion from EFC → HFC

Sinusoid Flow Pattern Design to Biomimic Liver Physiology

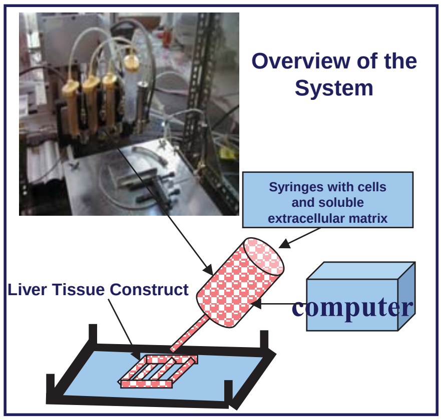


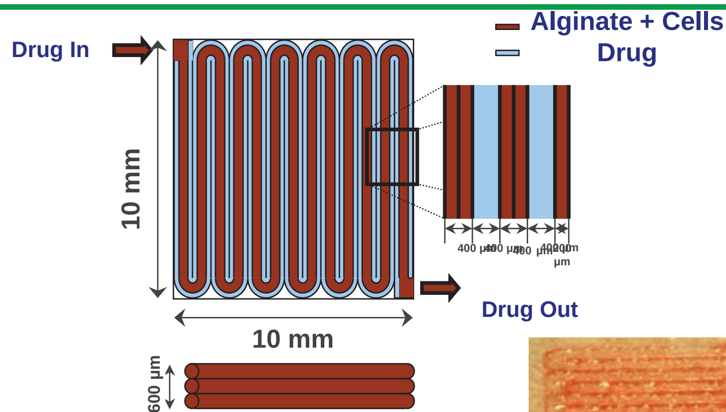
- hepatic vascular system (capillaries) is configured in sinusoidal pattern → design the sinusoidal micro-fluidic channel patterns to biomimic *in vivo* liver microstructure.
- Channel dimensions and strut widths vary from 50μm to 250μm, flow varies from 1ml/min to 5ml/min.

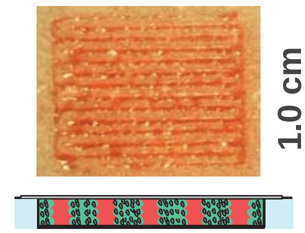
Bioprinting Micro Liver Organ Models



Liver Tissue Construct
Physiological Structure Formation

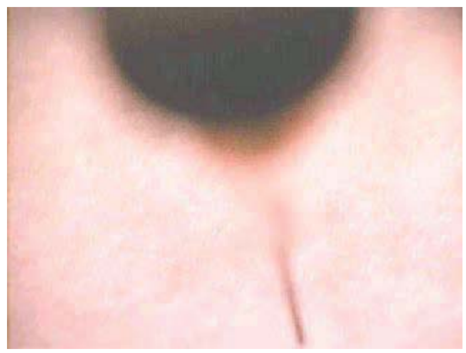




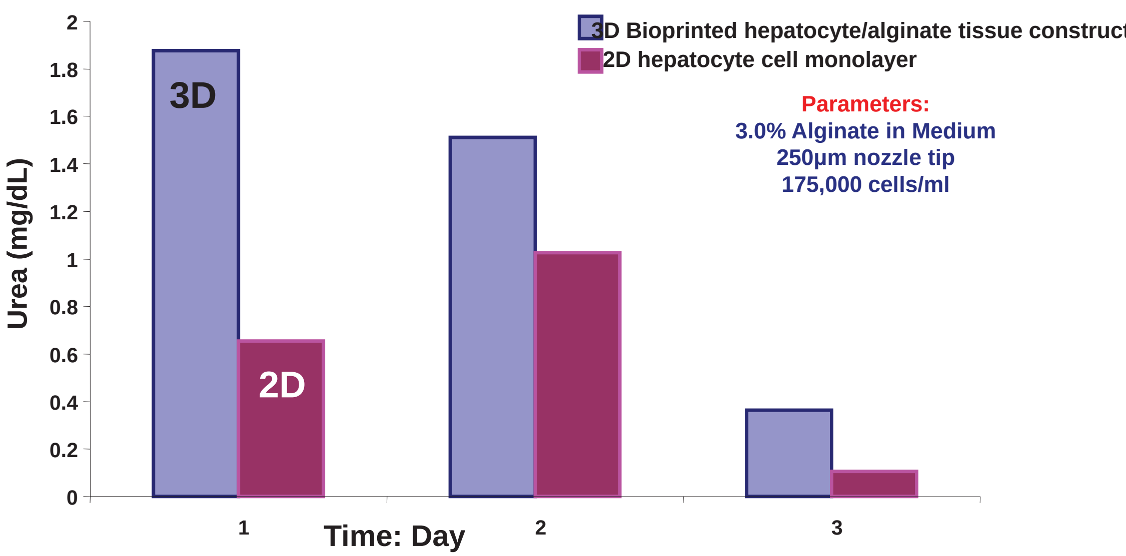


Process Parameters

- Valve Type: Pneumatic Microvalve
- Pressure: 2.0 psi
- Motion Velocity: 10 mm/s
- Alginate Conc.: 3.0% w/v
- $CaCl_2$ Crosslinking Conc.: 5.0% w/v
- Nozzle Tip Size: 200 um



A Better 3D Biological Model to Simulate Liver Physiology



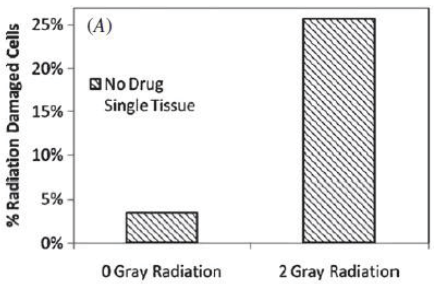
Results shows a better Urea Synthesis of hepatocytes in 3D configuration

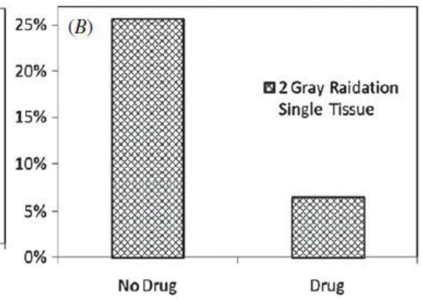
Dual Cell Damage under Radiation

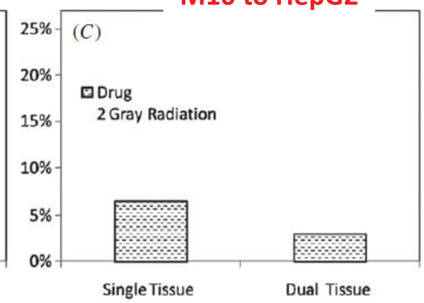
HepG2 surrounded by Endothelial Barrier

Radiation Treatment 2 Gray dose of ^{137}Cs gamma source

Dual Chip M10 to HepG2





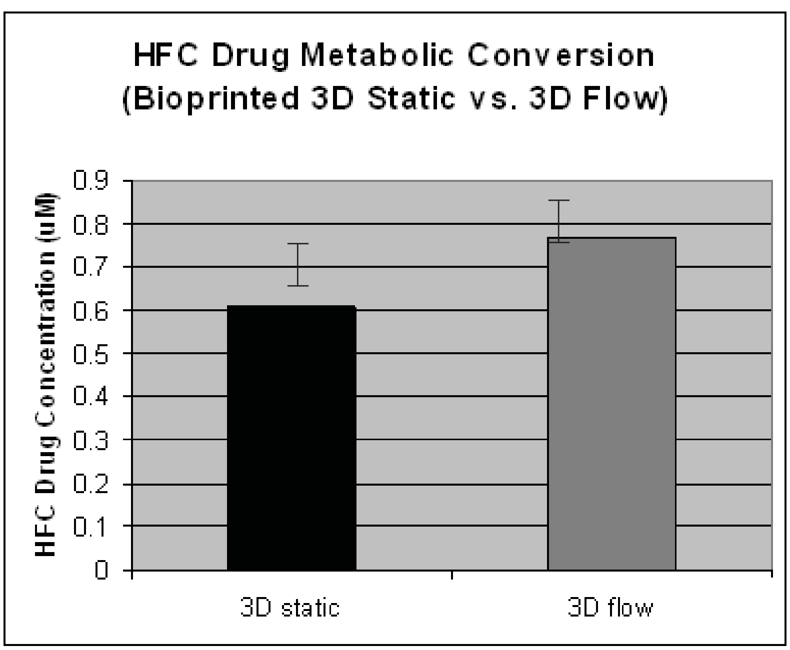


Micronuclei occur in small amounts during the experimental process (4% damaged cells). Radiation causes elevated levels of micronuclei (26% damaged cells).

Drug (Amifostine) shields the cells from some radiation damage (8% damaged cells).

Dual tissue model improves the effectiveness of the drug by a two fold decrease in radiation damage to hepatocytes after 2 Gy of radiation (3% damaged cells).

Comparison of Drug Metabolic Conversion Under Static and Dynamic Flow Conditions

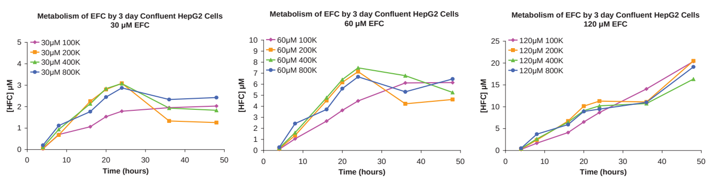


Metabolic drug conversion of bioprinted 3D tissue constructs under static vs dynamic flow conditions (0.25 µL/hr) with 4hr residence time in tissue chamber

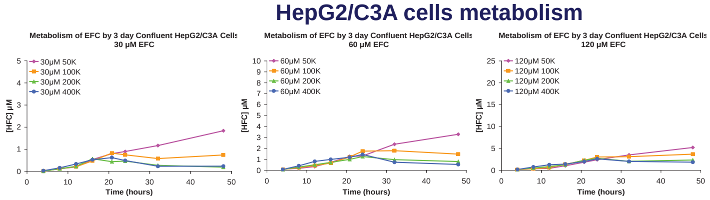
Results for Drug Metabolism Study

- Effect of cell type
- Effect of varying material & media volume
- Effect of media concentration
- Effect of cell confluency
- Effect of drug flow perfusion
- Effect of an alternative biopolymer

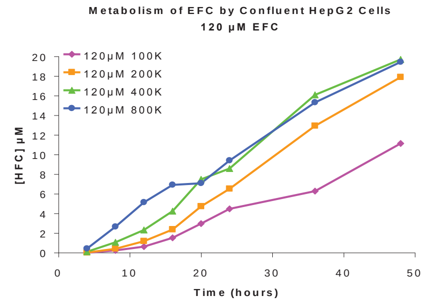
HepG2 cells metabolism

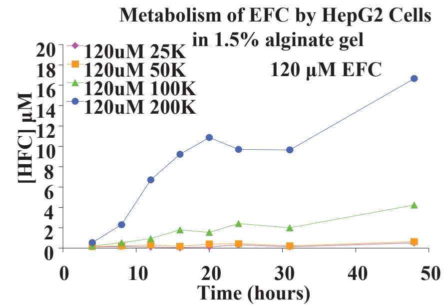


HepG2/C3A cells metabolism

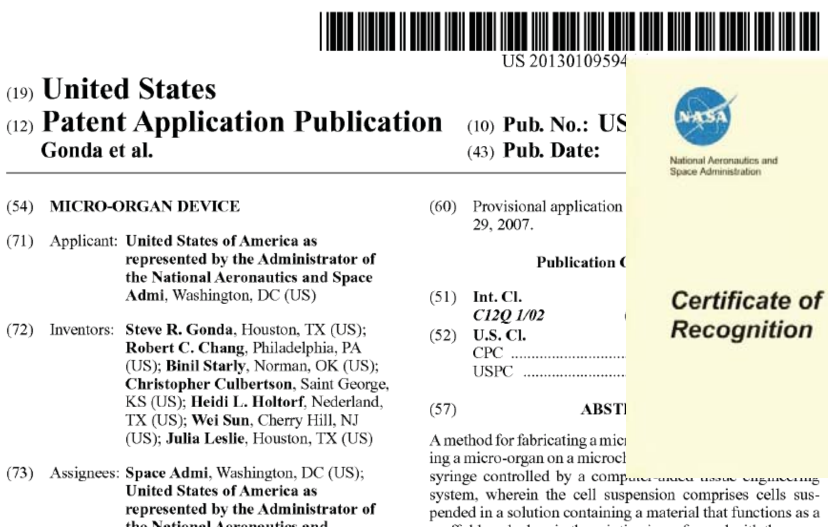


*** HepG2 cells metabolize EFC better than C3A subclone**





Related Patents and Publications



US 20130109594

(19) **United States**

(12) **Patent Application Publication**

Gonda et al.

(10) **Pub. No.: US**

(43) **Pub. Date:**

(54) **MICRO-ORGAN DEVICE**

(71) Applicant: **United States of America as represented by the Administrator of the National Aeronautics and Space Admi**, Washington, DC (US)

(72) Inventors: **Steve R. Gonda**, Houston, TX (US); **Robert C. Chang**, Philadelphia, PA (US); **Binil Starly**, Norman, OK (US); **Christopher Culbertson**, Saint George, KS (US); **Heidi L. Holtorf**, Nederland, TX (US); **Wei Sun**, Cherry Hill, NJ (US); **Julia Leslie**, Houston, TX (US)

(73) Assignees: **Space Admi**, Washington, DC (US); **United States of America as represented by the Administrator of**

(60) Provisional application ... 29, 2007.

Publication C

(51) **Int. Cl.**
C12Q 1/02

(52) **U.S. Cl.**
CPC ...
USPC ...

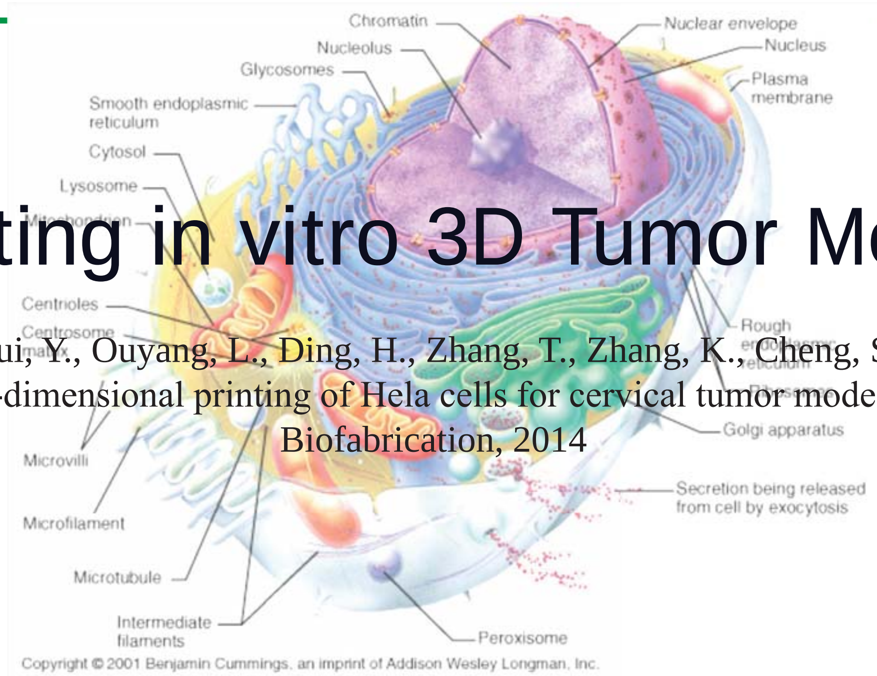
(57) **ABSTI**

A method for fabricating a micr... ing a micro-organ on a microcl... syringe controlled by a comp... system, wherein the cell suspension comprises cells suspended in a solution containing a material that functions as a

NASA-Micro-Organ Patents: US8343740; US8580546

Chang et al, Tissue Engineering, 2007 & 2008,
Snyder et al, Biofabrication (2011, 2014),
Hamid et al, Biofabrication (2014)

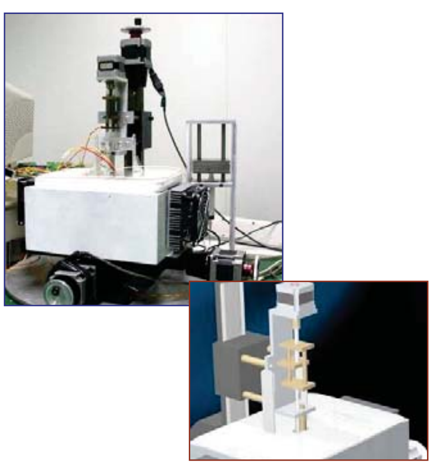
Printing in vitro 3D Tumor Model



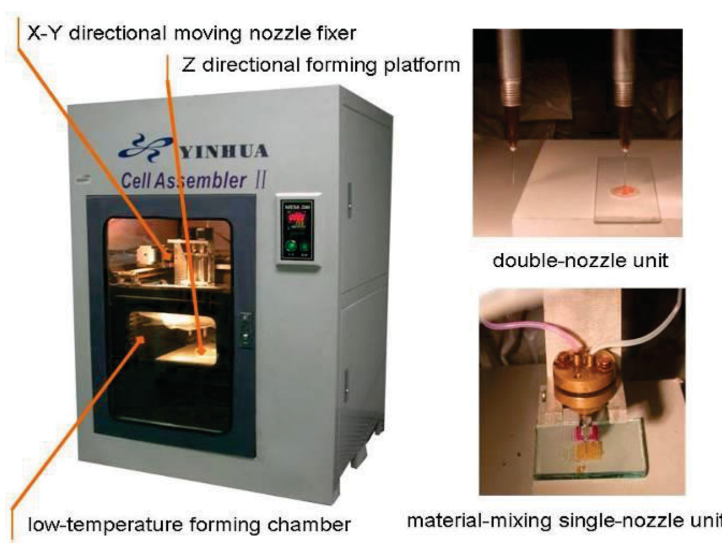
Zhao, Y., Rui, Y., Ouyang, L., Ding, H., Zhang, T., Zhang, K., Cheng, S. and Sun, W. "Three-dimensional printing of Hela cells for cervical tumor model in vitro", Biofabrication, 2014

3D Cell Direct Assembly

(Tsinghua University, China)



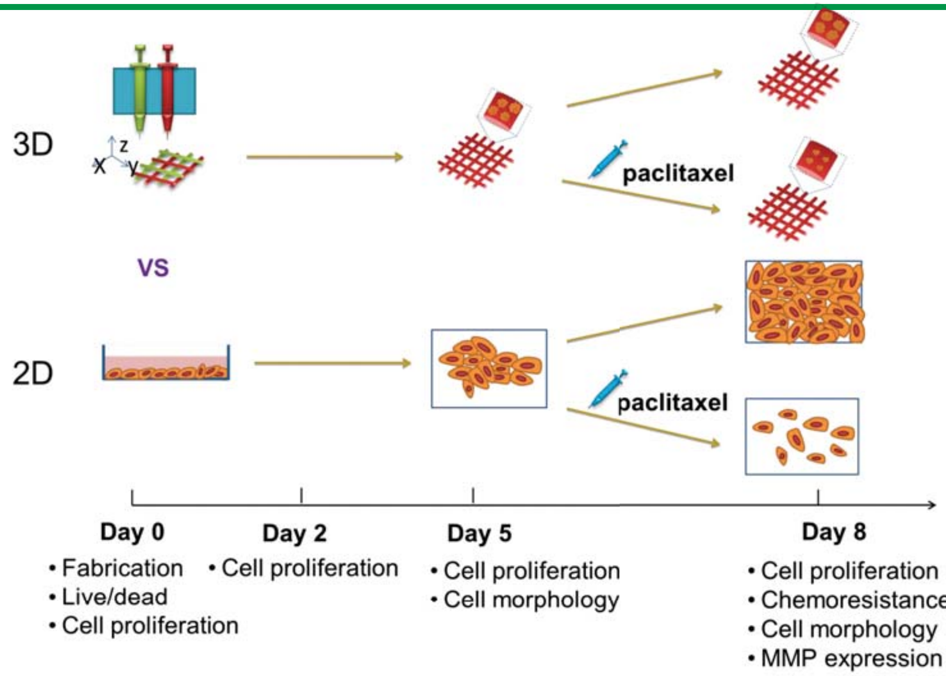
Single Nozzle System



Dual Nozzle System

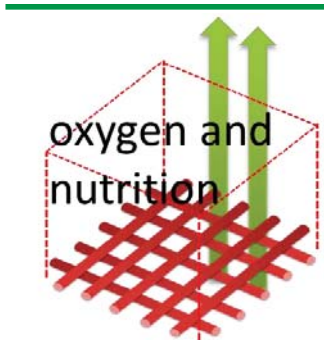
Assembly Cancer Cells for In Vitro Tumor Model

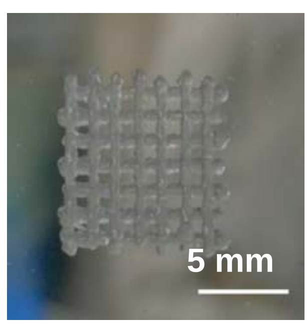
(NSFC 2012 E05 Key Research Project; Tsinghua)



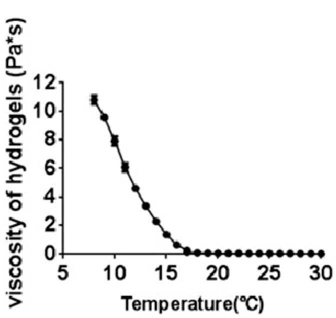
Biomaterials: Gelatin-Alginate-Fibrin   Cells: Hela

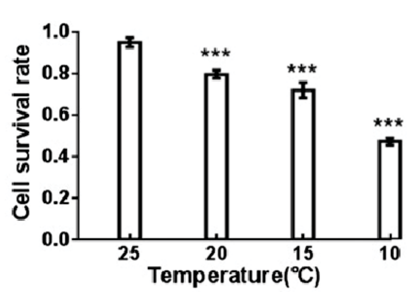
Printing of 3D cervical tumor models





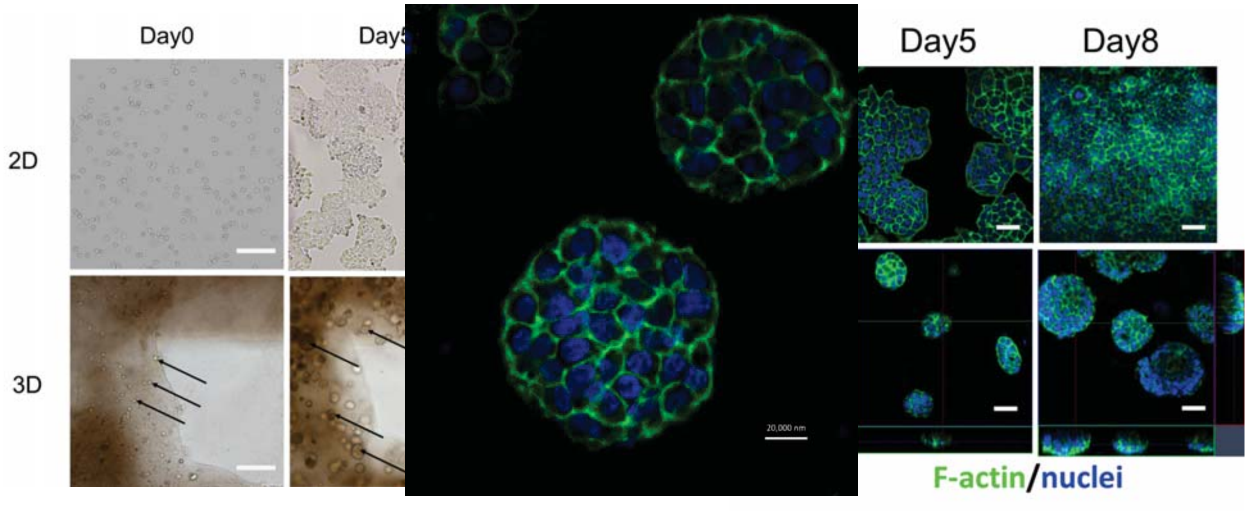
- **A grid structure with high supply of oxygen and nutrition was designed and printed.**





- **The printing process was optimized by adjusting the temperature, as well as the viscosity.**

Cellular morphology in 2D vs 3D cultures



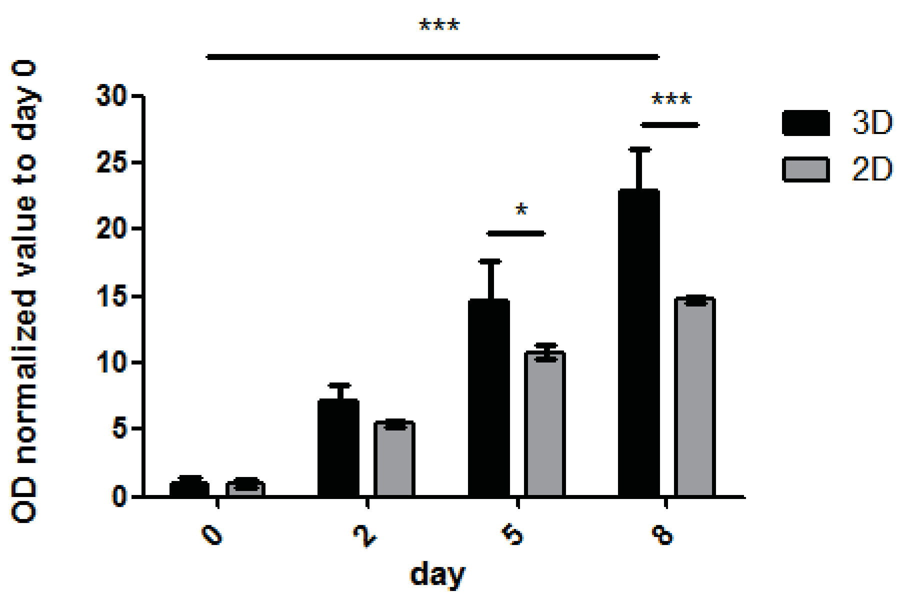
Scale bar, 200 μm

Scale bar, 50 μm

Results: Compared with 2D planar culture, Hela cells in 3D Hela/hydrogel constructs showed spheroid morphology on day 5 and day 8.

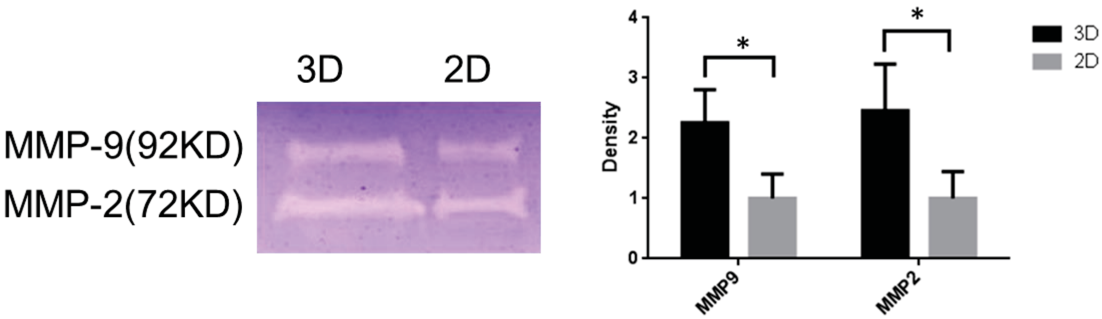
Zhao Y, Yao R, Ouyang L, et al. Three-dimensional printing of Hela cells for cervical tumor model in vitro , Biofabrication, 2014, 6(3): 035001.

Cell proliferation



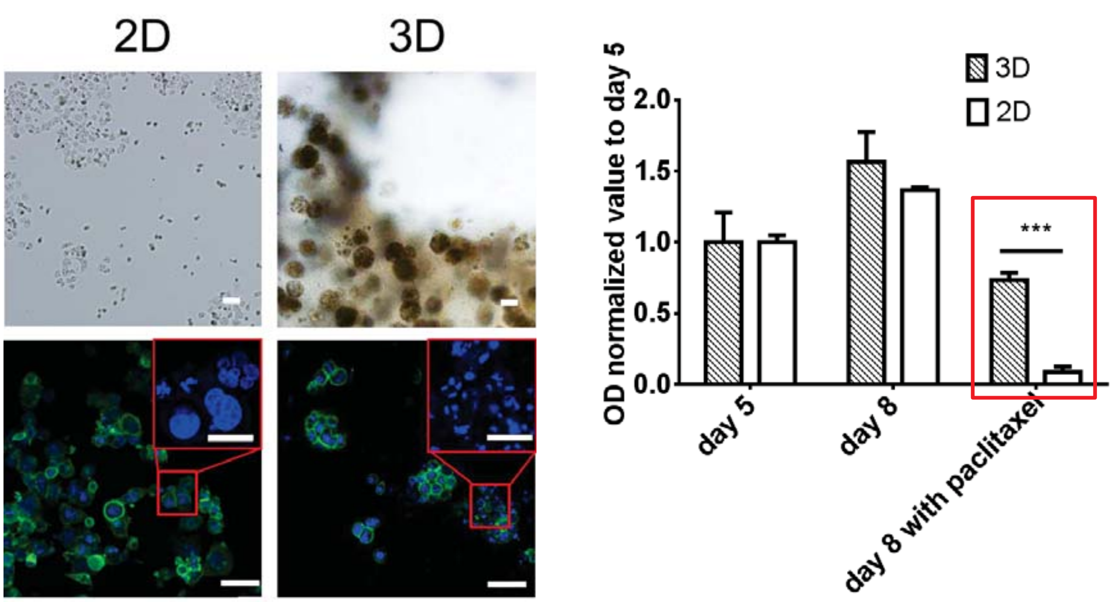
Results: Hela cells in 2D culture plates proliferated more slowly than in printed 3D constructs.

MMP expression



Results: Hela cells in 3D cultures showed enhanced expression of MMP-2 and MMP-9 compared with 2D planar culture.

Chemoresistances



Results: Enhanced chemoresistances were observed in 3D printed Hela/hydrogel constructs compared with 2D planar cell culture

Results of A549 Gene Chip

(unpublished data)

- **3D vs 2D: up-regulation 350**

1	ProbeName	FC ([3D] vs [2D])	Log FC ([3D] vs [2D])	Regulation ([3D] vs [2D])	2D.txt:gProcessedSignal(normalized)	3D.txt:gProcessedSignal(normalized)	GeneSymbol
2	A_23_P93641	5184.368	12.339952	up	-6.169976	6.169976	AKR1B10
3	A_33_P3380992	2573.9856	11.329788	up	-5.664894	5.664894	AKR1B15
4	A_24_P129341	2122.8735	11.051803	up	-5.5259013	5.5259013	AKR1B10
5	A_33_P3238433	927.3118	9.856911	up	-4.9284554	4.9284554	ALDH3A1
6	A_23_P207850	613.54785	9.261032	up	-4.630516	4.630516	TNS4
7	A_23_P3038	525.1893	9.036694	up	-4.518347	4.518347	GPX2
8	A_23_P20484	506.50415	8.98443	up	-4.492215	4.492215	FGL1
9	A_33_P3249046	378.7719	8.565186	up	-4.282593	4.282593	CLDN2
10	A_24_P42136	305.03247	8.252819	up	-4.1264095	4.1264095	KRT18
11	A_23_P373708	242.86235	7.923995	up	-3.9619975	3.9619975	KRT18P55
12	A_23_P208788	228.82758	7.838117	up	-3.9190586	3.9190586	C19orf33
13	A_23_P24129	180.36168	7.494749	up	-3.7473745	3.7473745	DKK1
14	A_33_P3209229	179.32521	7.4864345	up	-3.7432172	3.7432172	RAB26
15	A_23_P140450	156.06555	7.2860084	up	-3.6430042	3.6430042	SLC27A2
16	A_33_P3369844	151.19601	7.2402763	up	-3.6201382	3.6201382	CD24
17	A_24_P277367	146.88818	7.1985745	up	-3.5992873	3.5992873	CXCL5
18	A_32_P78681	133.54102	7.061139	up	-3.5305696	3.5305696	GLP2R
19	A_23_P58266	131.67162	7.0408006	up	-3.5204003	3.5204003	S100P
20	A_32_P8546	129.79428	7.020083	up	-3.5100415	3.5100415	LINC00473
21	A_23_P14083	123.01455	6.942685	up	-3.4713426	3.4713426	AMIGO2
22	A_32_P151544	122.290245	6.9341655	up	-3.4670825	3.467083	KRT18
23	A_23_P66798	121.98167	6.9305205	up	-3.4652603	3.4652603	KRT19
24	A_23_P359214	117.42778	6.87563	up	-3.437815	3.437815	LINC00842
25	A_21_P0000121	110.744	6.791085	up	-3.3955424	3.3955424	C19orf81
26	A_33_P3307495	106.93456	6.7405844	up	-3.3702922	3.3702922	STRA6
27	A_24_P190472	103.700806	6.6962833	up	-3.3481417	3.3481417	SLPI
28	A_33_P3368750	98.96262	6.628812	up	-3.314406	3.314406	PAQR5
29	A_33_P3398331	97.916435	6.613479	up	-3.3067396	3.3067396	MMP24
30	A_23_P207507	85.050835	6.4102535	up	-3.2051268	3.2051268	ABCC3
31	A_33_P3387621	78.90782	6.3020964	up	-3.1510482	3.1510482	RHPN2
32	A_33_P3262191	76.12815	6.250358	up	-3.125179	3.125179	CPNE7

Results of A549 Gene Chip

(unpublished data)

- **3D vs 2D: down-regulation 669**

1	ProbeName	FC ([3D] vs [2D])	Log FC ([3D] vs [2D])	Regulation ([3D] vs [2D])	2D.txt:gProcessedSignal(normalized)	3D.txt:gProcessedSignal(normalized)	GeneSymbol
2	A_23_P64873	-13207.168	-13.6890335	down	6.8445168	-6.8445168	DCN
3	A_33_P3304668	-5152.3115	-12.331004	down	6.165502	-6.165502	COL1A1
4	A_23_P69497	-2104.4207	-11.039207	down	5.5196037	-5.5196037	CLEC3B
5	A_23_P110791	-1875.3447	-10.87294	down	5.43647	-5.43647	CSF1R
6	A_33_P3215640	-1821.5233	-10.83093	down	5.415465	-5.415465	PI16
7	A_24_P935491	-1591.2158	-10.635914	down	5.317957	-5.317957	COL3A1
8	A_19_P00323082	-1396.927	-10.448041	down	5.2240205	-5.2240205	H19
9	A_23_P105562	-1376.2347	-10.426511	down	5.2132554	-5.2132554	VWF
10	A_23_P100660	-1123.8364	-10.134216	down	5.067108	-5.067108	SERPINF1
11	A_24_P270460	-997.88214	-9.962726	down	4.981363	-4.981363	IFI27
12	A_33_P3708413	-957.8089	-9.903594	down	4.951797	-4.951797	MFAP5
13	A_33_P3400763	-901.4472	-9.816099	down	4.9080496	-4.9080496	PLIN4
14	A_23_P111583	-820.0962	-9.679649	down	4.8398247	-4.8398247	CD36
15	A_33_P3220837	-808.1203	-9.658426	down	4.829213	-4.829213	MAFB
16	A_23_P161439	-711.2433	-9.474199	down	4.7370996	-4.7370996	ADIRF
17	A_23_P372834	-641.63336	-9.325605	down	4.6628027	-4.6628027	AQP1
18	A_32_P140139	-625.6108	-9.289122	down	4.644561	-4.644561	F13A1
19	A_32_P74409	-596.08203	-9.219367	down	4.6096835	-4.6096835	C11orf96
20	A_23_P152305	-575.42334	-9.16848	down	4.58424	-4.58424	CDH11
21	A_23_P214080	-551.56665	-9.107391	down	4.5536957	-4.5536957	EGR1
22	A_23_P48596	-512.8726	-9.002457	down	4.5012283	-4.5012283	RNASE1
23	A_23_P47709	-473.4242	-8.88699	down	4.443495	-4.443495	FOLR2
24	A_23_P203173	-462.28973	-8.8526535	down	4.4263268	-4.4263268	IL10RA
25	A_23_P200741	-446.05472	-8.801077	down	4.4005384	-4.4005384	DPT
26	A_33_P3258362	-441.1705	-8.7851925	down	4.3925962	-4.3925962	HBA2
27	A_23_P3312	-392.2162	-8.615505	down	4.3077526	-4.3077526	ISLR
28	A_33_P3295203	-382.8008	-8.58045	down	4.290225	-4.290225	HAS1
29	A_33_P3262635	-382.31528	-8.578619	down	4.2893095	-4.2893095	CECR1
30	A_33_P3409062	-381.56693	-8.575792	down	4.287896	-4.287896	TYROBP
31	A_23_P131676	-380.94693	-8.573446	down	4.286723	-4.286723	ACKR3
32	A_33_P3246833	-379.9234	-8.569565	down	4.2847824	-4.2847824	IL1RN

Results of A549 Gene Chip

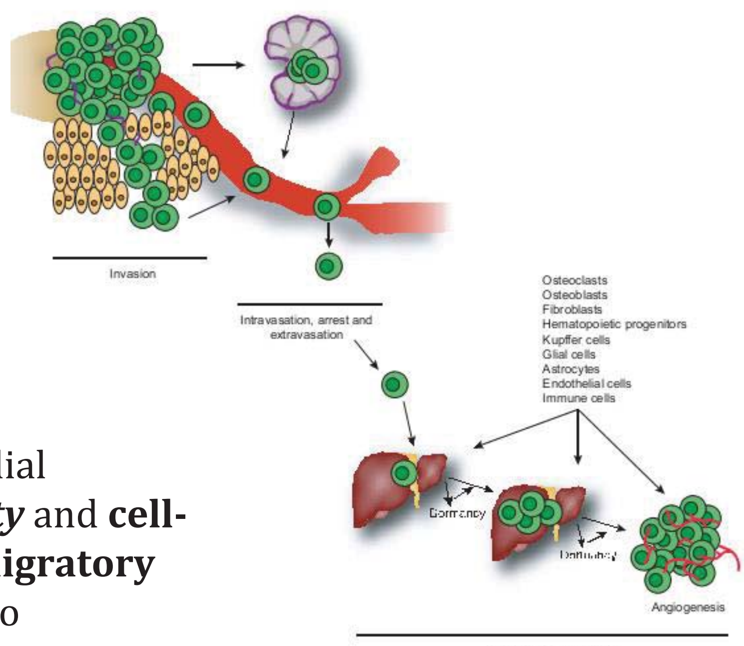
(unpublished data)

- Overall conclusion:
  - In 3D printing models compared with 2D culture, genes related to tumor cell proliferation, drug resistance, invasion and migration were mostly upregulated
  - In 3D printing models compared with 2D culture, genes related to tumor cell apoptosis, cytoskeleton synthesis were mostly upregulated
  - Genes related to cell morphology and cell-matrix interactions were drastically changed in 3D model compared with 2D culture.

Epithelial-Mesenchymal Transitions 上皮-简充质转变 （EMT) study

- **Tumor metastasis is the main cause (90%) lending to end stage death:**

To study tumor metastasis by our *in vitro* model

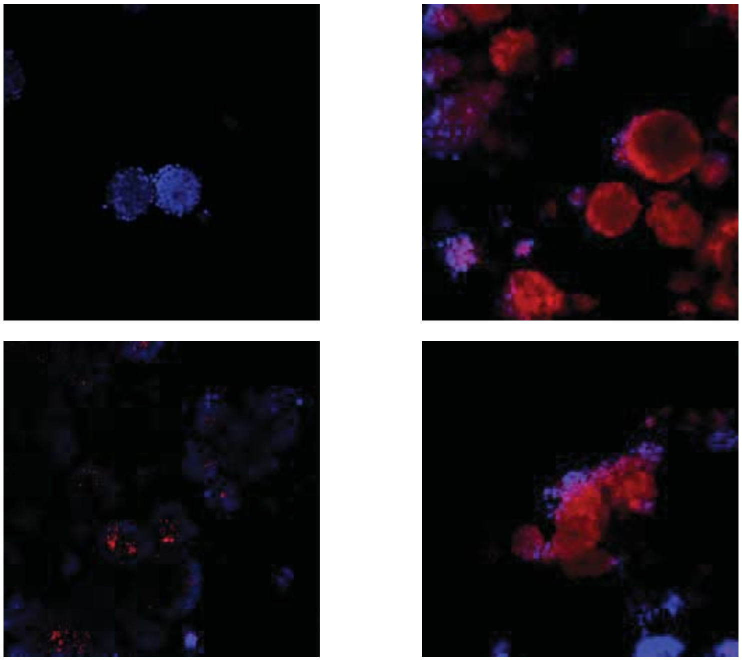


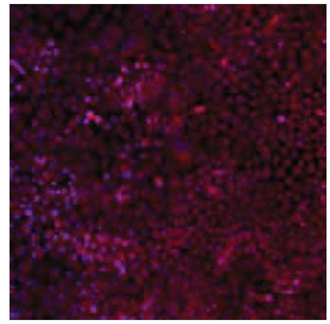
Steeg, P. S. *Nature medicine* (2006) *12*(8), 895-904.

- A process by which epithelial cells ***lose their cell polarity*** and **cell-cell adhesion**, and **gain migratory** and **invasive properties** to become mesenchymal cells

Histology-N-cadherin/dapi

3D-Control **3D-Sample** **2D-Sample**

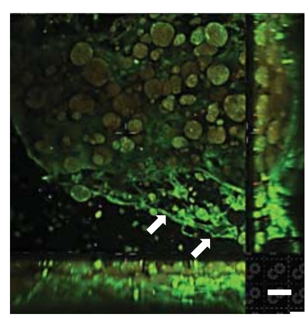


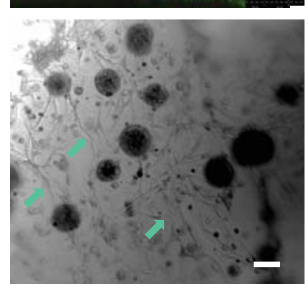


- N-cadherin expression was up-regulated in 3D sample compared to 3D control;
- N-cadherin expression was also observed in the sample of 2D culture model.

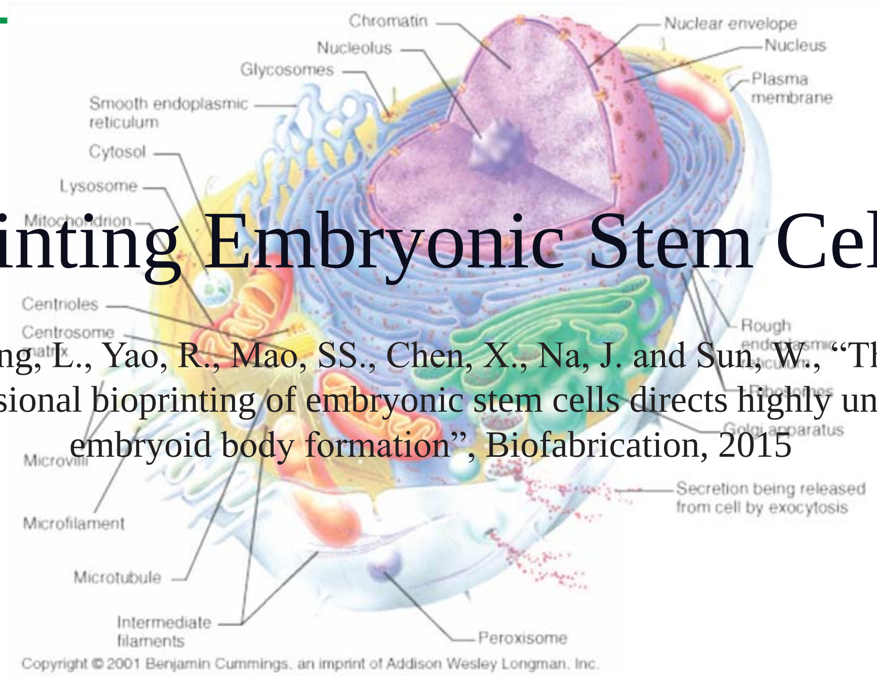
On-going study

- **It is still a simple tumor-like model, not a real tumor model**
  - introducing blood vessels
  - printing heterogeneous cells
  - looking at cell communicates





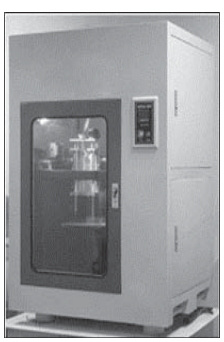
- **Clinical:**
  - for personalized cancer treatment
  - cancer drug testing



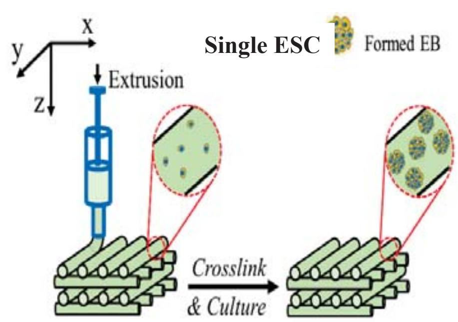
Printing Embryonic Stem Cells

Ouyang, L., Yao, R., Mao, SS., Chen, X., Na, J. and Sun, W., “Three-dimensional bioprinting of embryonic stem cells directs highly uniform embryoid body formation”, Biofabrication, 2015

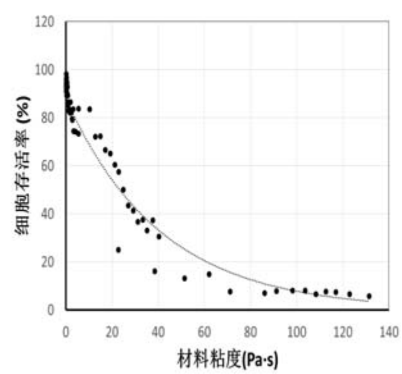
Printing Embryonic Stem Cells



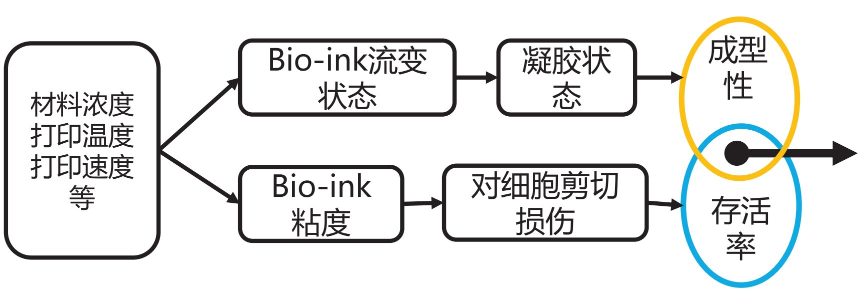
➢ 细胞三维打印机（清华大学）

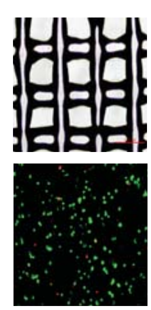


➢ 打印示意图



➢ mESC存活率随材料粘度变化

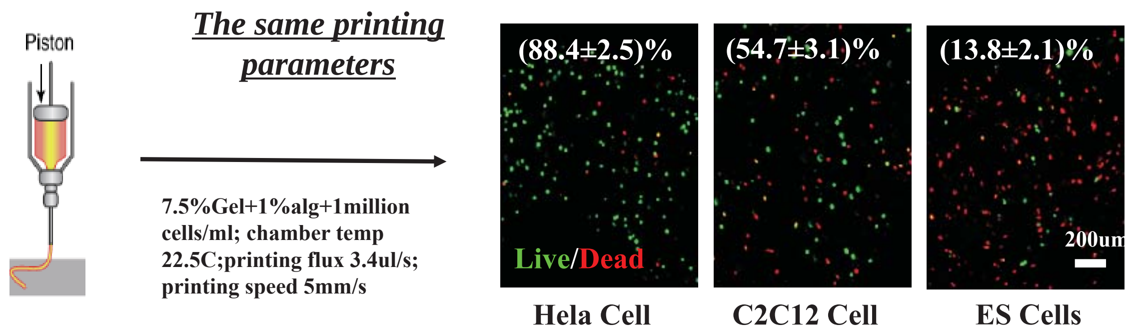




➢ 打印工艺摸索流程

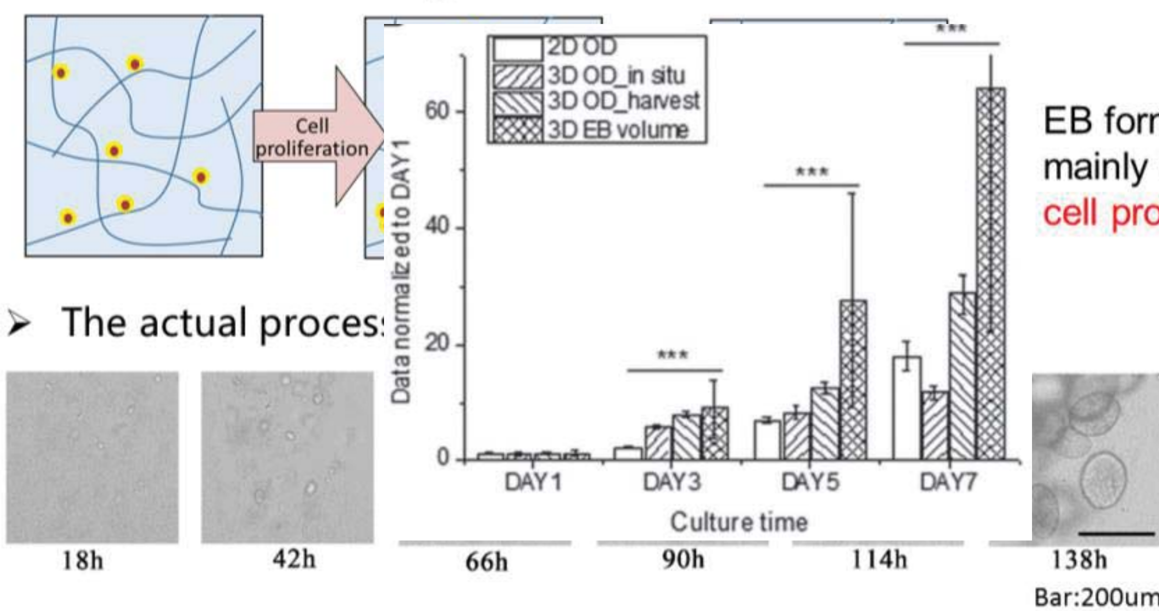
Printing Embryonic Stem Cells

- **Cell sensitivity** with physicochemical stimulate



ESC proliferation and EB formation

- EB formation within gels

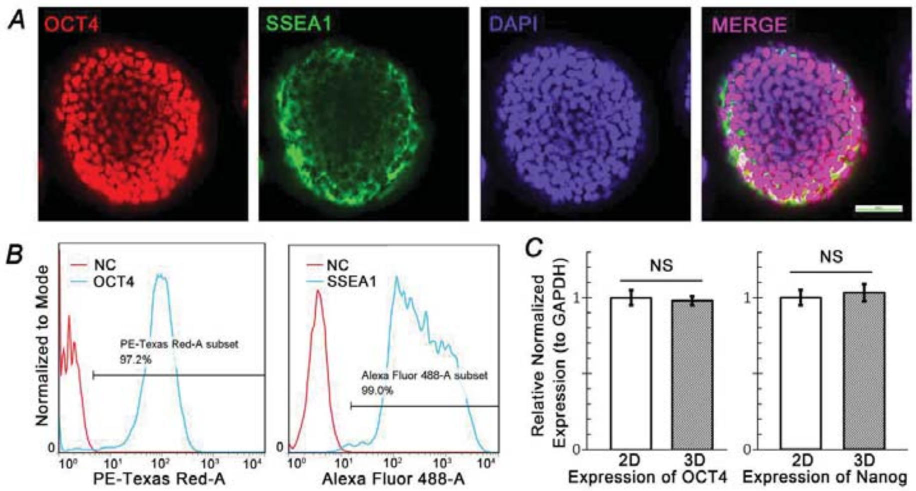


EB formation is mainly based on cell proliferation

- The actual proces

Maintenance of pluripotency

- **Nearly 100% cells maintain pluripotency**



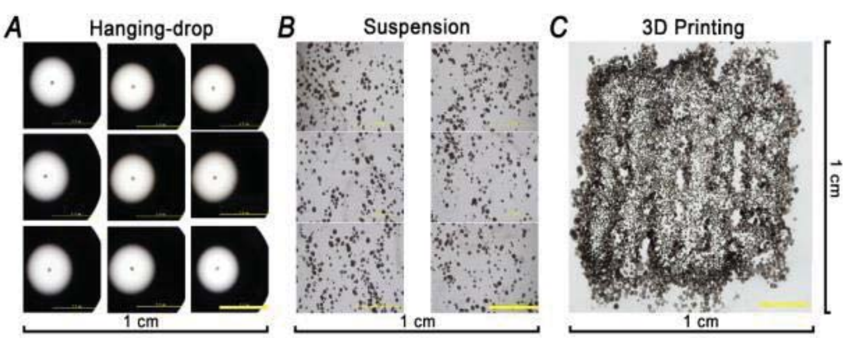
➢ **The maintenance of pluripotency after one week**

Bar:50um

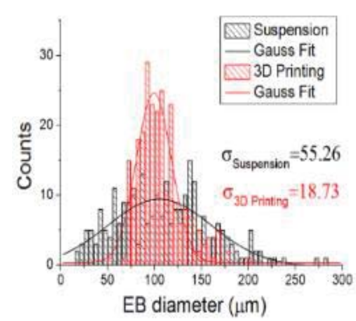
Comparison with other methods

- EB size can be controlled by culture time and bioink composition
- EB yield: higher than hanging-drop (10~1000X) and suspension (10~100X)
- EB uniformity: better than suspension (3X)

➢ EB yields of three methods



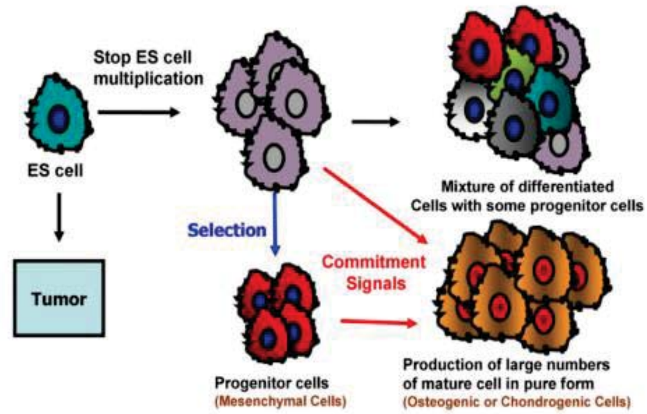
➢ EB uniformity

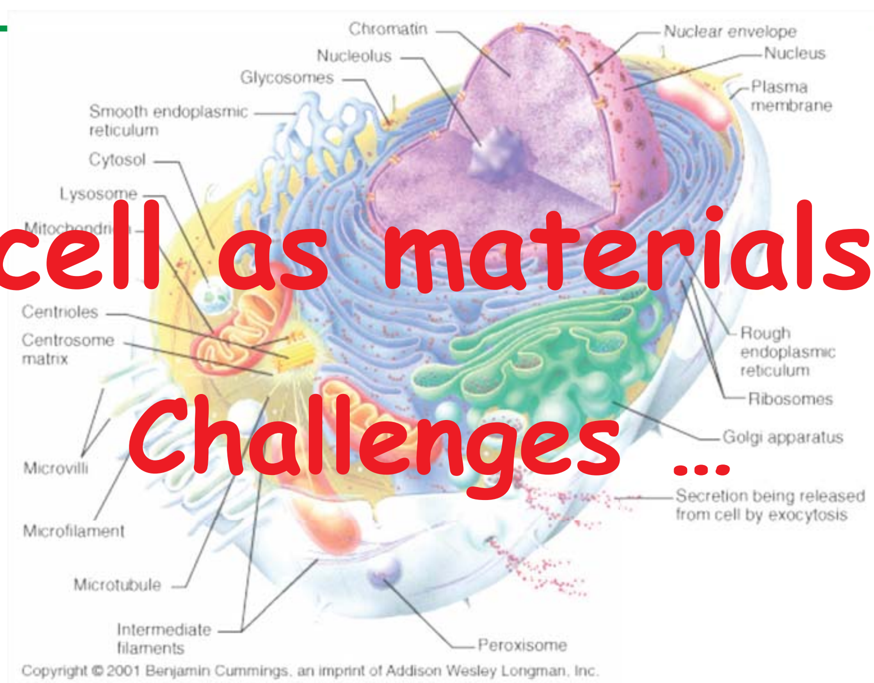


Ongoing work

- ESCs early differentiation in the construct
  - Study the factors that influence the differentiation fate of three germ layers
  - Like EB size, printing parameters

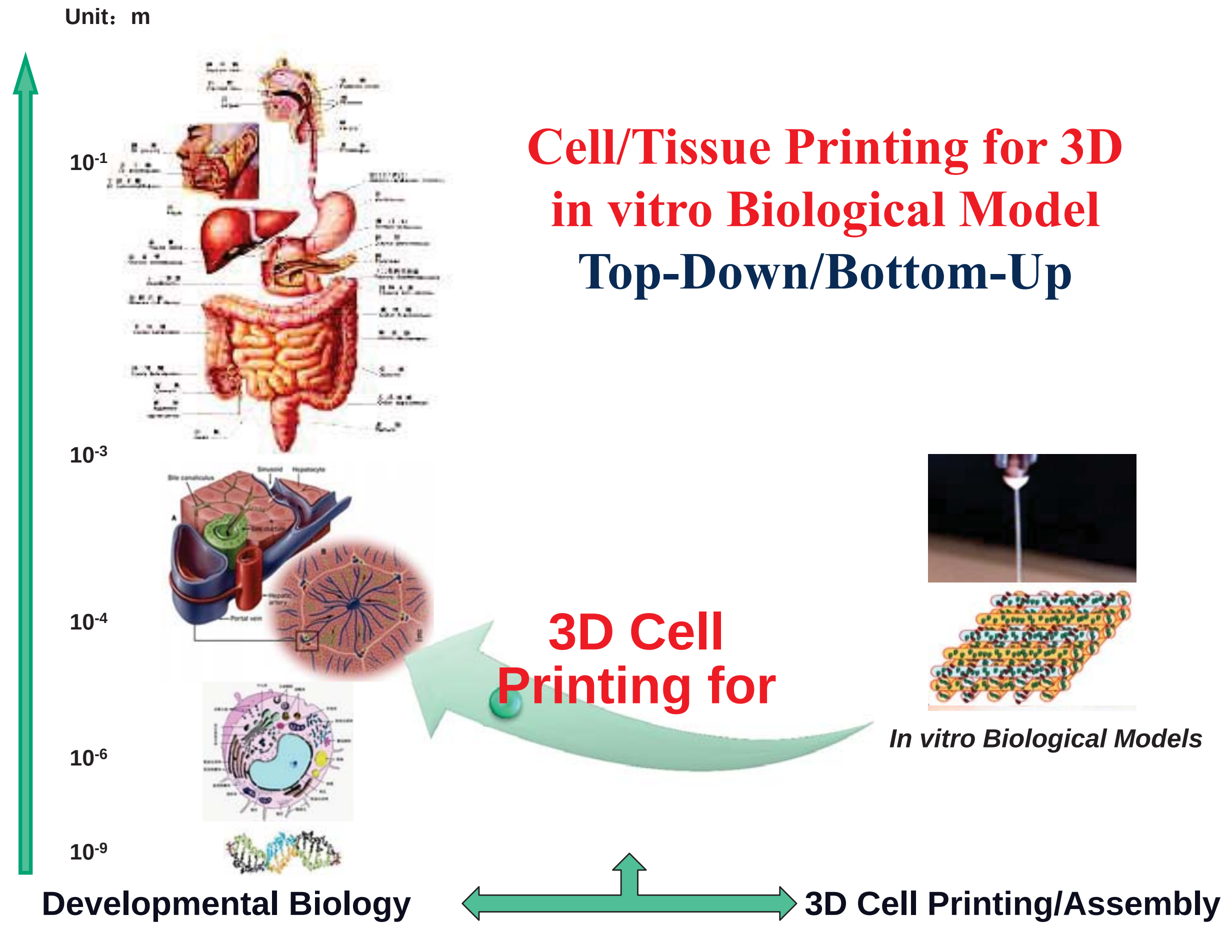
- **ESCs induced differentiation in the construct for microtissue fabrication and regulation studies**





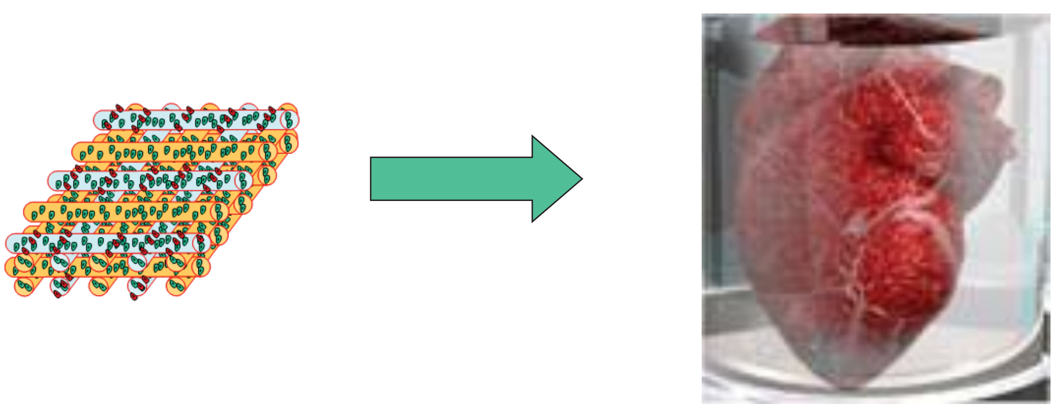
cell as materials

Challenges …



We may need a different biology ...

- **A knowledge gap for understanding of 3D printed biological Model**



Beyond the developmental biology and petri dishes ...

Challenge in Materials …

- **Lack of Bio-INK and/or cell delivery medium**
  - go with cells (as the cell delivery medium)
  - grow with cells (as supporting ECM and regulators

- **Limited material available:** Hydrogel, Alginate, Collagens etc

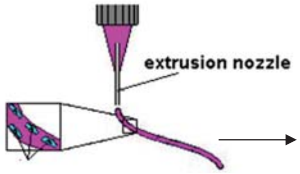
Structure and Bio-Function

Challenge in Printing …

- **Printing multi-type cells simultaneously**
- **Printing/patterning single cell**
- **Effect of printing process to cells**
  - temperature controlled environment
  - cell injury

- **Post printing**
  - structure integrity and stability
  - 3D co-culture to simulate human physiology

Printing will injure cells

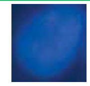


Bio-deposition of cell-hydrogel thread

Mechanical Disturbances by Cell Deposition Process

Deposition Pressure
Deposition Speed
Nozzle Diameter
Nozzle Length
Hydrogel Properties

Healthy cell with desired phenotype

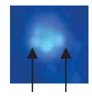


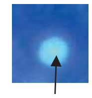
Normal nucleus

Injured

- Recovery
- Altered phenotype
- Apoptosis

Dead

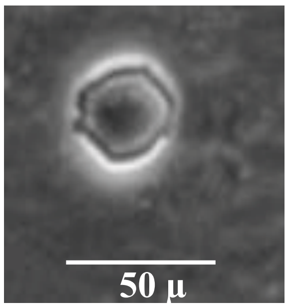




Karyolysis
Fragmentation an dissolution of nucleus

Pyknosis
Contraction of nuclear chromatin followed by nuclear condensation

Printing at 150 µ nozzle and p=40psi



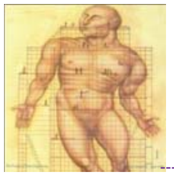
Optical Microscopy

Fluorescent Microscopy showing disintegrated nucleus suggesting irreversible injury and cell death

Opportunities ...

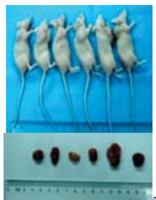
3DP In Vitro Model for Drug Testing

人体模型



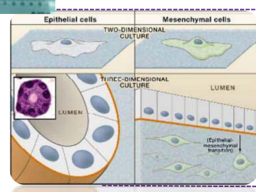
- **Limited human clinical trials**
- Not feasible for testing
- Ethic issues

动物模型



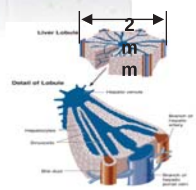
- Cell micro-environment different from human
- Different immune system
- **Different from human clinical trials**

二维模型



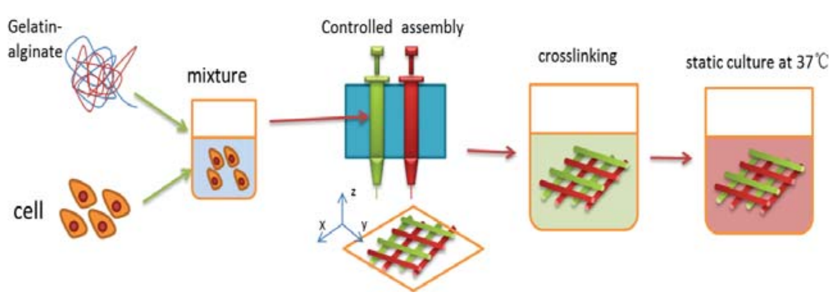
- Not a true physiological environment
- **Difficult to simulate 3D tissue**
- Not reliable to cancer drug testing

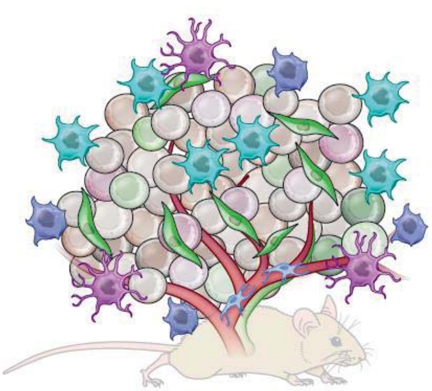
三维模型

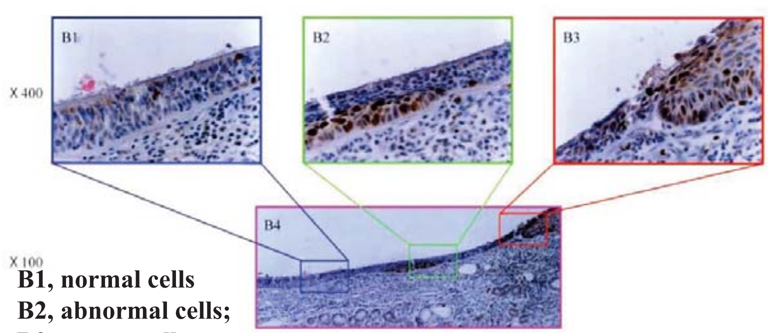


- **Simulated physiological model**
- **More close to 3D human tissue**
- **Reduce using animals**

Assembly Cancer Cells for In Vitro Tumor Model







B1, normal cells
B2, abnormal cells;
B3, cancer cells;
Clinic Cancer Res 2006; 12(4): 1121.

An Envision …

In 10 years, every major cell culture lab in the U.S. will be equipped with an easy-to-use Cell-Additive-Manufacturing system (i.e., Cell Dispensing System) as a standard biological labware. Biologists will use this system on daily basis to fabricate 3D cell aggregates or cell assembly models and use them for conducting 3D cell culture studies, disease and drug studies, and/or for regenerating tissue substitutes.

This is similar to the scenario we have envisioned for placing a desktop RP system in every engineering office.

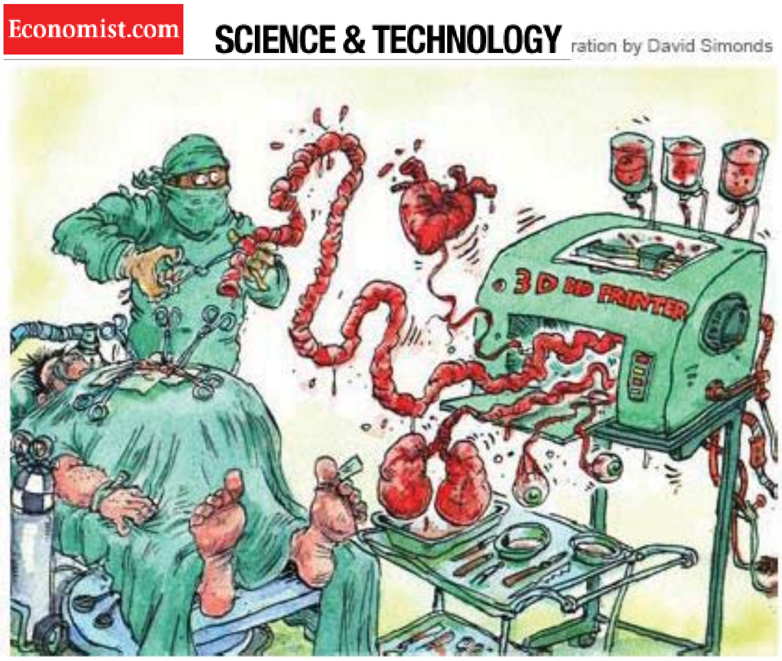
We envision the above killer applications will lead to a multi-billion dollar AM market.

Presented at "Roadmap for Additive Manufacturing", National Science Foundation, March 11, 2009

Roadmap for Biomanufacturing
NSF (2009.03.11)

2-3 Y	5 Y	10 Y	15 Y
Biomodeling of Implants/Prostheses And Tissue Scaffolds, Cell Printing	BAM with Disease model BAM with Drug model	BAM of functional tissue	Organ Printing
Implants, Prosthesis, Cell Printing	Disease/Drug Models	Functional Tissue Structures	Organ Printing

Printing Body Parts

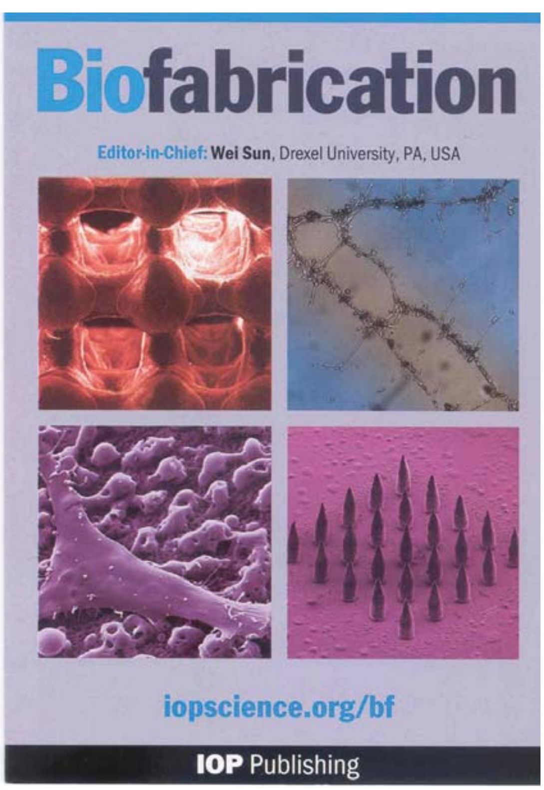


A machine that prints organs is coming to market
Feb 18th 2010, The Economist print edition

IOP Publishing

Biofabrication

Impact Factor: 4.3



Biofabrication: Use cells, biomaterials and bioactive compounds as building blocks through the means of physical, chemical, biological, and engineering processes to fabricate biological systems and/or therapeutical products.

Scope: biofabrication processes, process science, modeling and design, and applications to:

- Bioprinting of cells, tissues and organs
- Cell/Protein printing, patterning and assemblies
- Cell assemblies for disease, drug, and tissue substitute models
- Biochips, biosensors and cell-integrated micro-fluidic devices
- Tissue scaffolds, medical devices and Computer-aided tissue engineering
- Integrated bio/micro- and nano-fabrication
- Synthesis biology
- Others…..

Thank you!