Bio-3DP: Challenges and Opportunities

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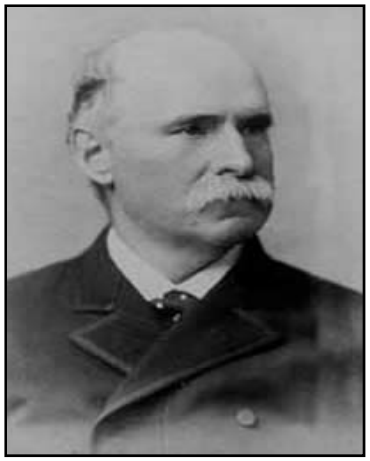
3D Cell Printing for In Vitro Biological Model
Society for Technology in Anesthesia Annual Meeting
2016.1.9

Drexel University

Philadelphia, PA, USA



Drexel a hundred twenty years ago

A private university founded by Anthony Drexel in 1891

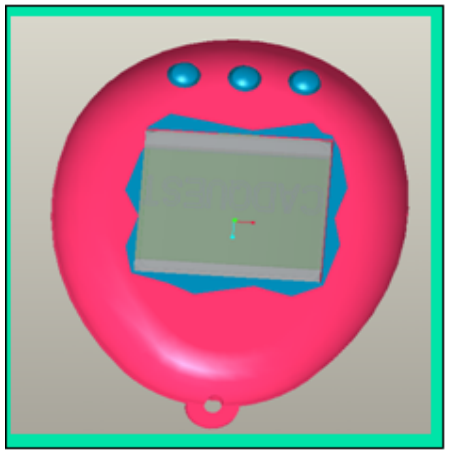
- **~27,000 students**
- **College of**
  - **Engineering**
  - **Business**
  - **Medicine**
  - **Law**
  - **Science, IST, Education, etc**
- **Largest Engineering and Medical Colleges as private universities in US**

Biomanufacturing Engineering Research Center

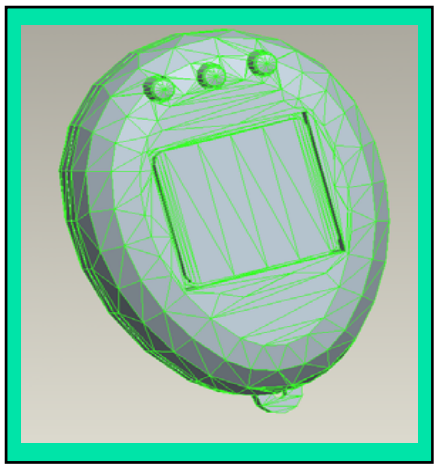
Bio-Manufacturing
Engineering Research Institute
清华大学生物制造工程研究所

- **Biomanufacturing and Rapid Forming Key Laboratory - Beijing**
- **International Collaborative Center for Biomanufacturing - Beijing**
- **Biomanufacturing Engineering Laboratory – Shenzhen**
- **8 Faculty: 3 Full P, 3 Associate P, 1 Assist. 1 Senior Engineer**
- **4 Post Doctors; > 40 graduate students**

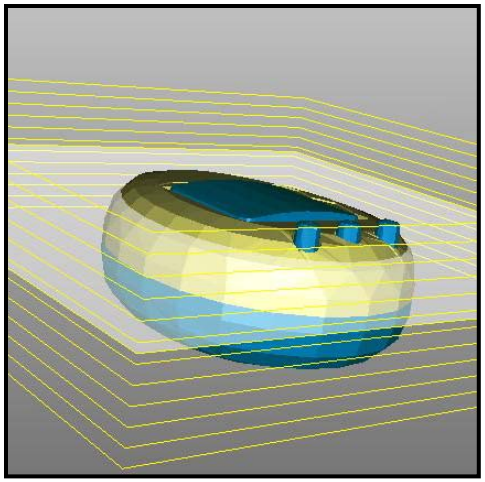
3D Printing Process



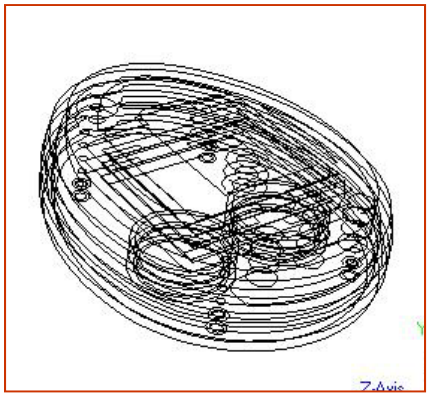
CAD solid model



STL format



slicing



Process tool-path

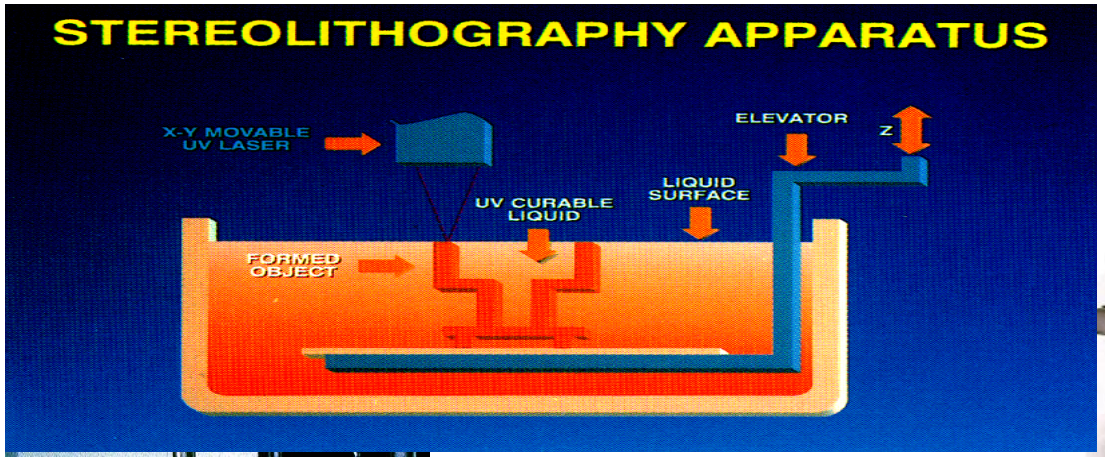
Rapid Prototyping
SLA
SLS
FDM
3DP
…

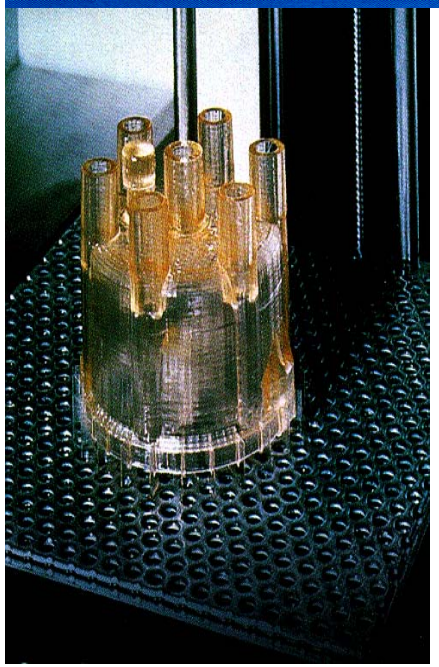
Printing

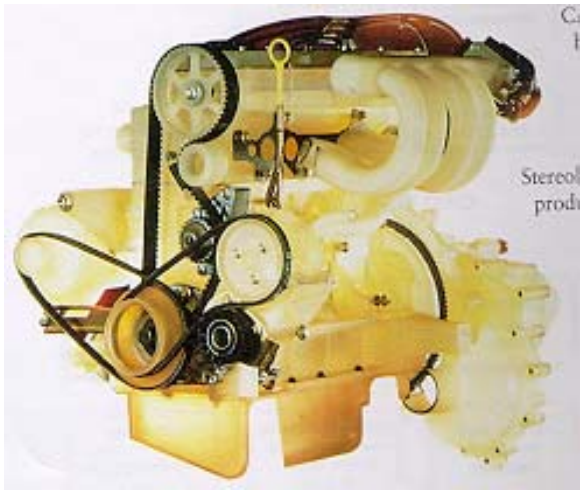
RP Part

3D Printing

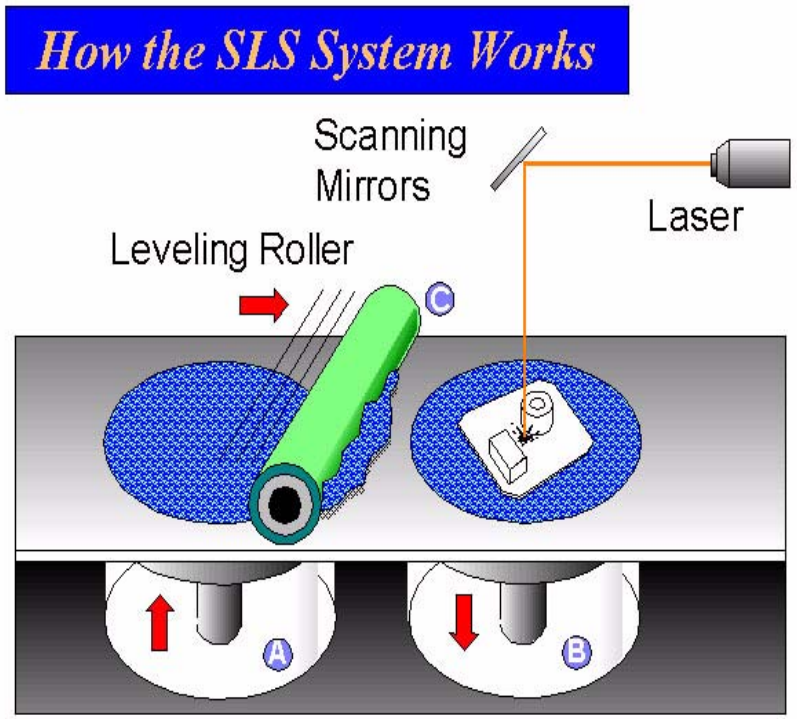
StereoLithography Apparatus (SLA)







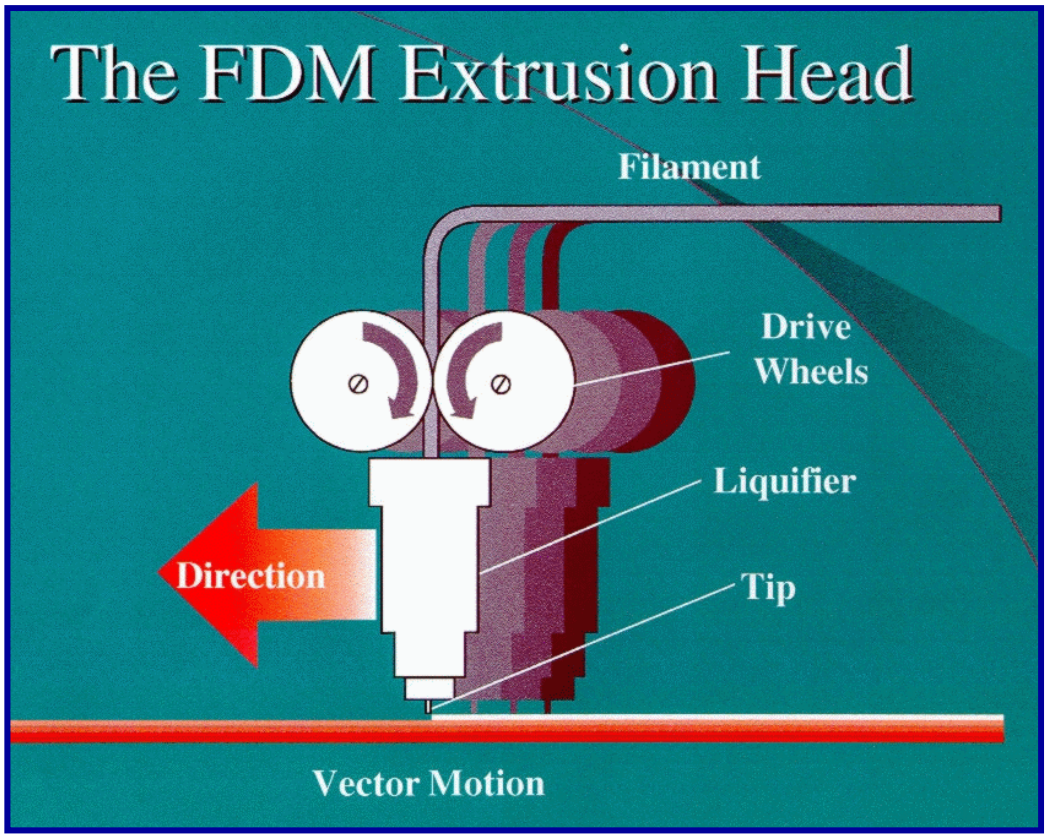
Selective Laser Sintering (SLS)



DTM / 3D System

- Fusing Powdered thermo-elastic material with heat from laser beam.
- A thin layer of powered thermoplastic material is evenly spread, by a roller, over the build region. Then, the pattern of the corresponding part cross section is “drawn” by the laser on the powder surface.
- With amorphous materials, the laser heat causes powder particles to soften and bind to one another at their points of contact, forming a solid mass. This process is called fusing or sintering.

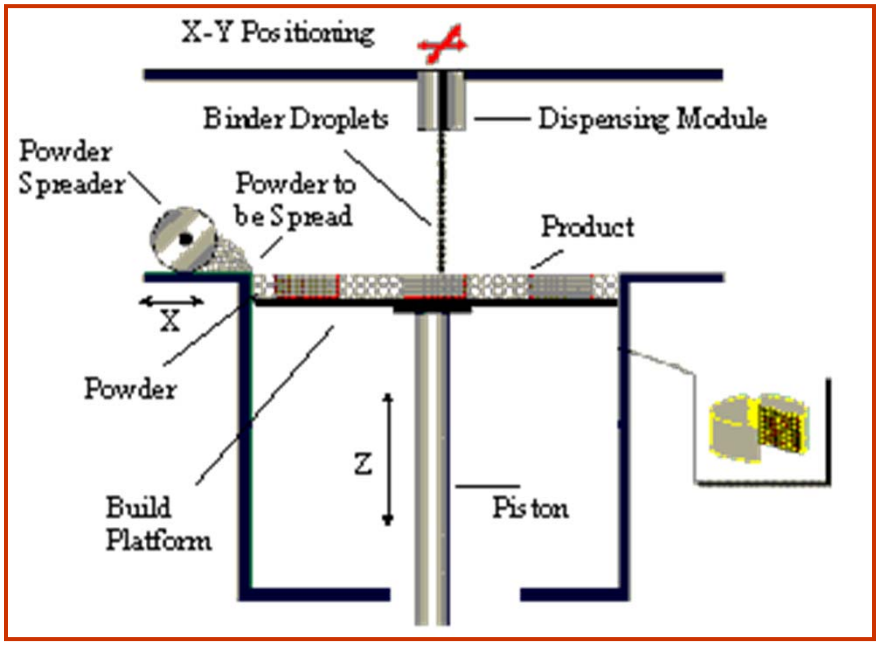
Fused Deposition Modeling (FDM)

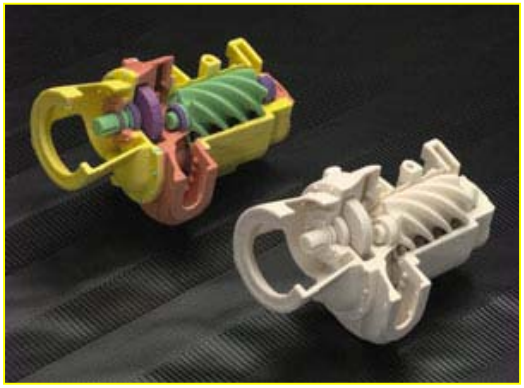


Stratasys, Eden Prairie, MN

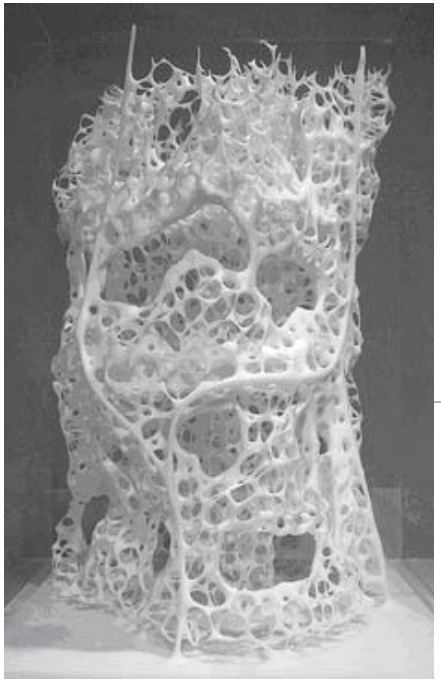
- FDM uses thermoplastic wire-like filaments which are melted in the delivery head.
- The material is extruded from the head and deposited on a layer-by-layer basis.
- Rapid solidification (approximately 1/10 second) of the molten laminate material from the modeling filament.
- Favored material – ABS plastic.

3D Printing

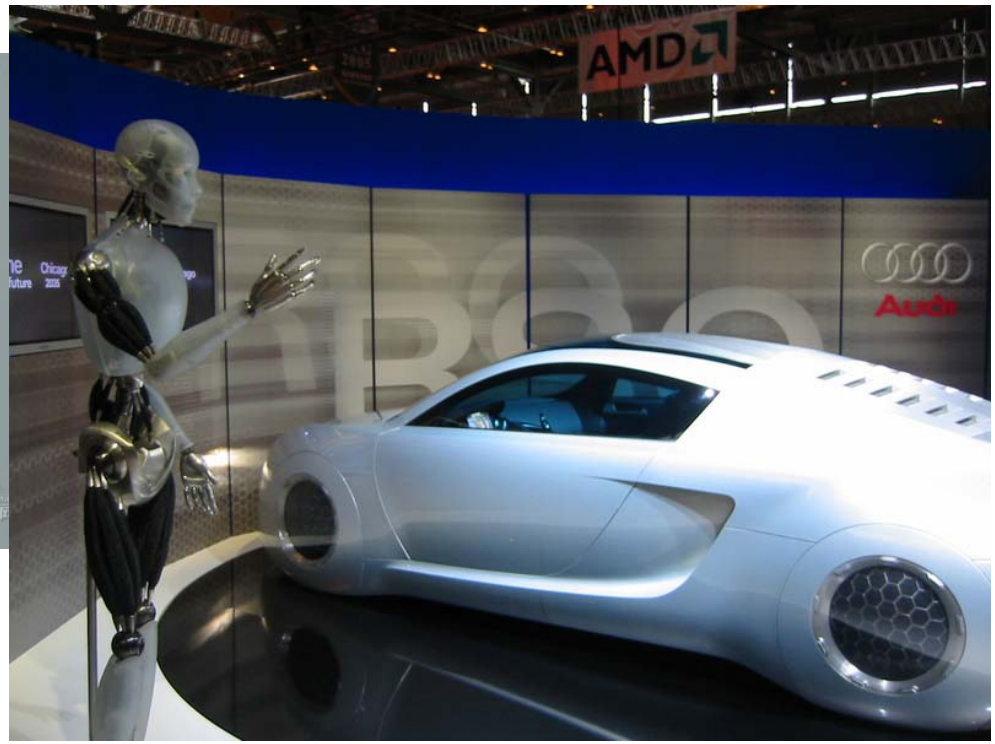

X-Y Positioning
Binder Droplets
Dispensing Module
Powder Spreader
Powder to be Spread
Product
X
Powder
Z
Build Platform
Piston



3DP Applications



3DP Applications



Product Innovation

http://www.cnbeta.com/articles/243136.htm

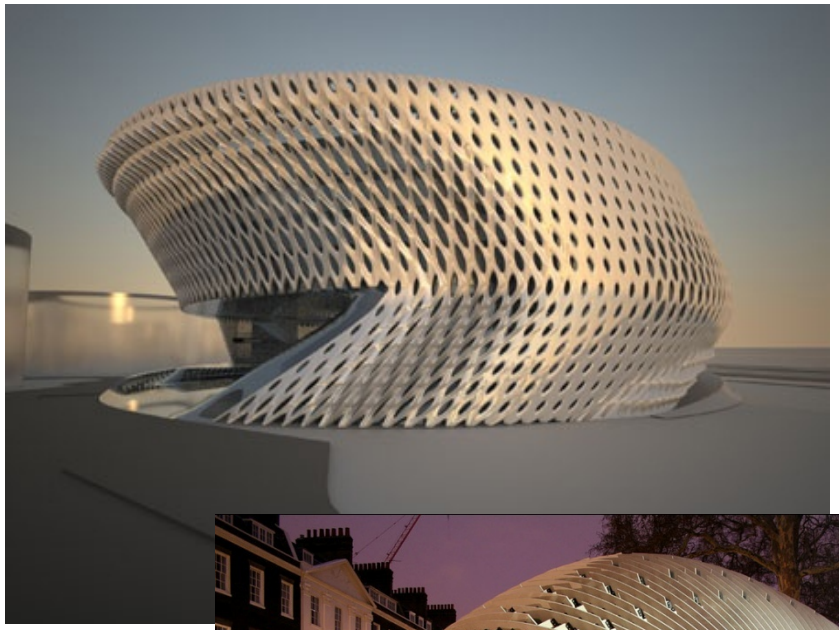
3DP Applications

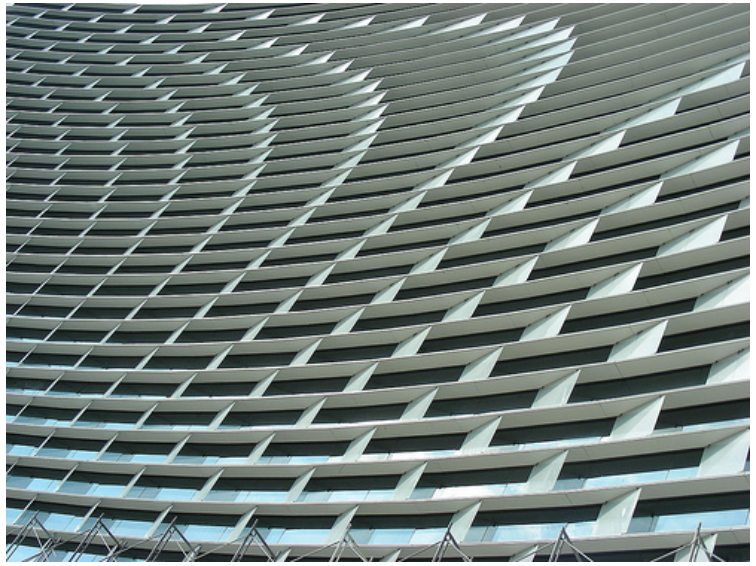
英发明世界首辆通过电脑打印出来的自行车(图)

来源： 北京科普之窗 【字体：大 中 小】

“空气自行车”比普通钢铝结构自行车轻65%，但却一样坚固。

3DP Applications





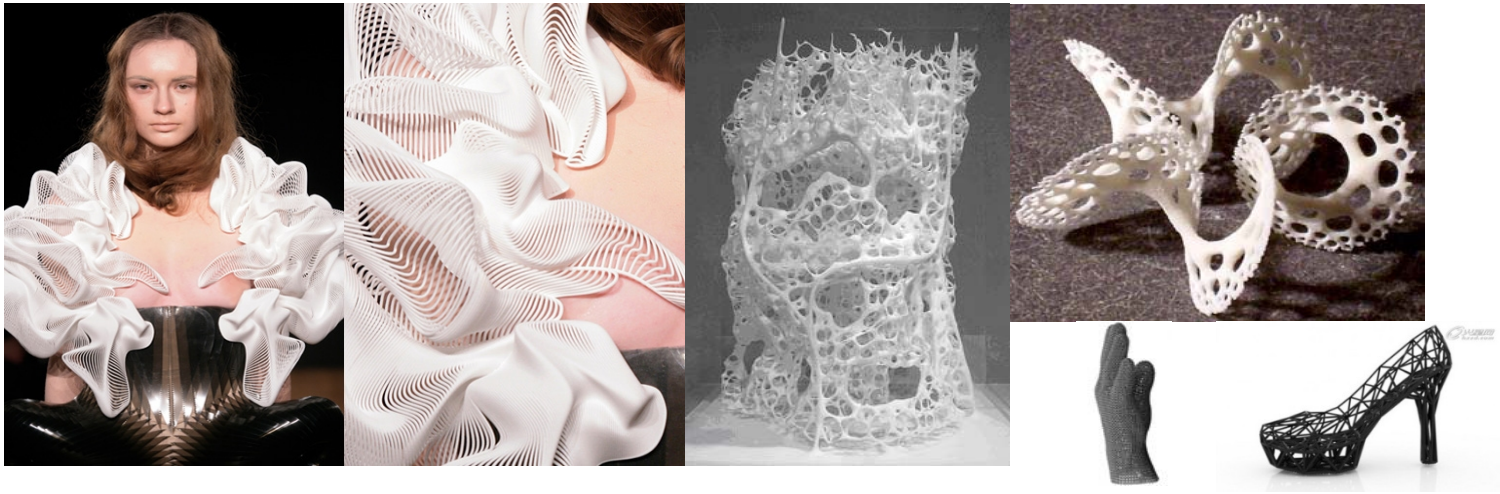
3DP Applications

3DP vase

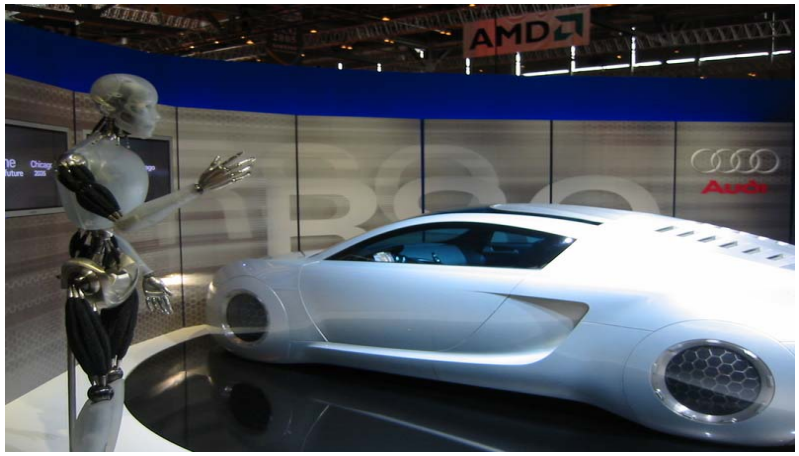
http://www.3d-print.cn/

3DP cake and chocolate
http://www.guokr.com/article/4170/

3D Printing



Innovation, Customization, Rapid Realization



3D Cell Printing

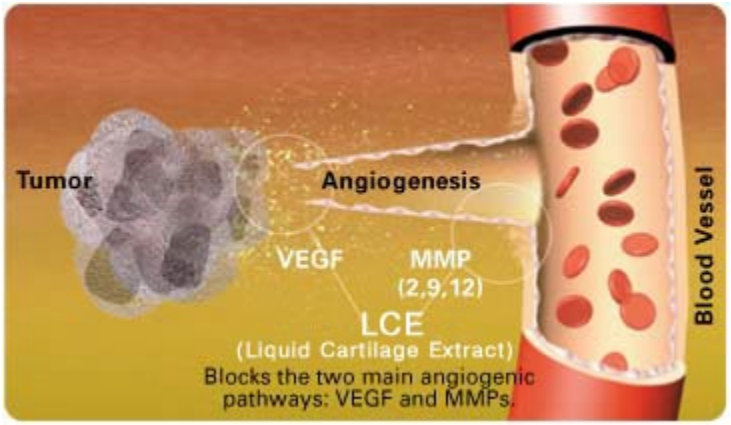
Directly Assembled 3D Biological Model by 3D Cell Printing

RGD Modified Surface

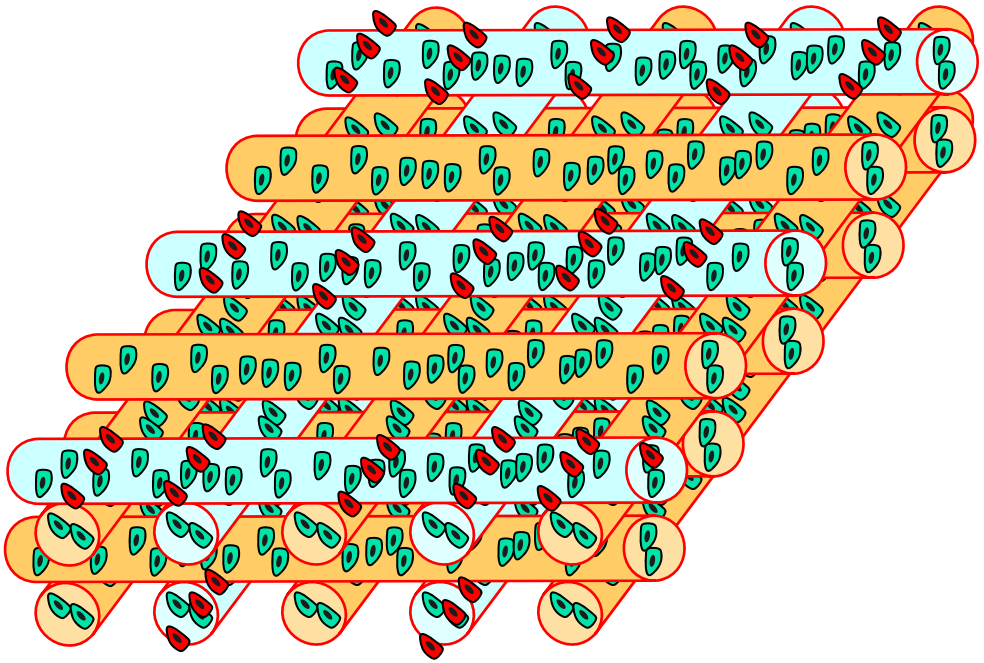
Unmodified Surface

Fibroblasts (Encasulated)

Endothelial (Applied to Surface)



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



Direct Assembled Biological Model for Angiogenesis

Morss-Clyne & Sun: 2008 Biomaterial Congress;
Tissue Engineering, 2010, Biofabrication, 2010

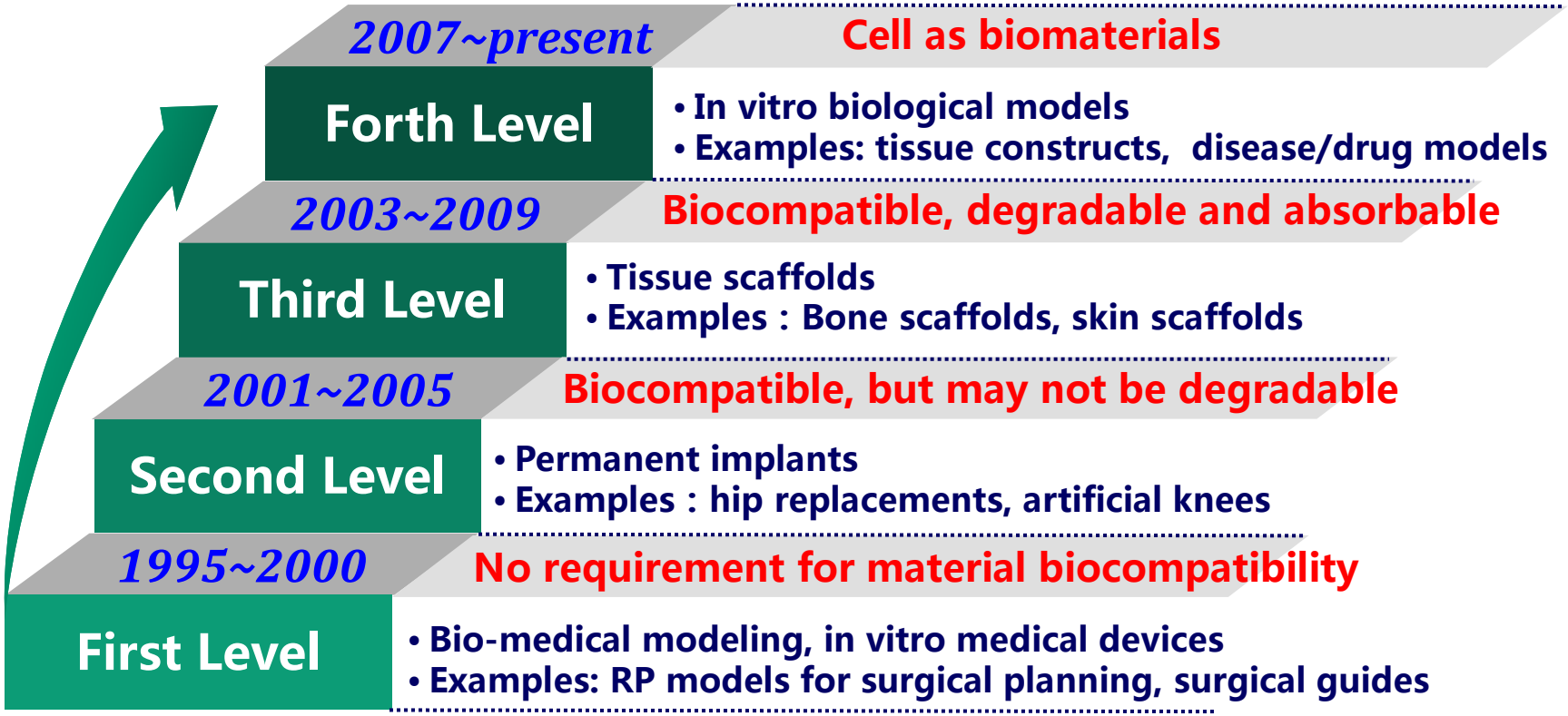
Bio-3D Printing

"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



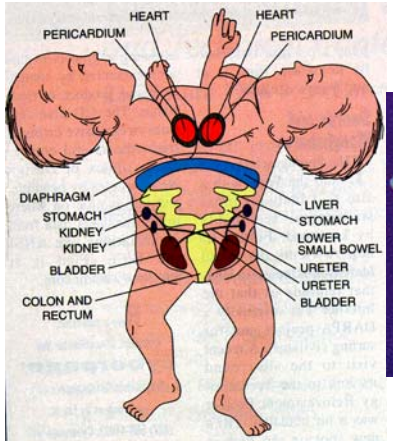
Application Level 1 to Level 3:

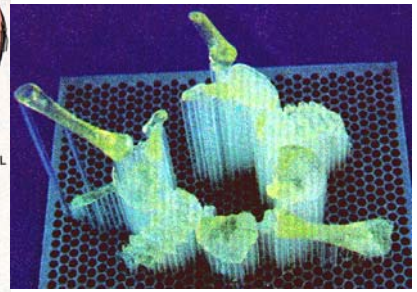
Enabling technologies

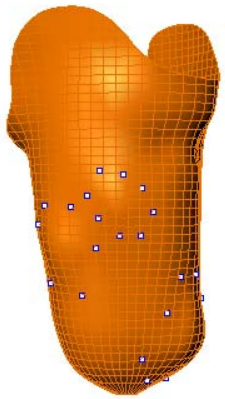
Translational Research

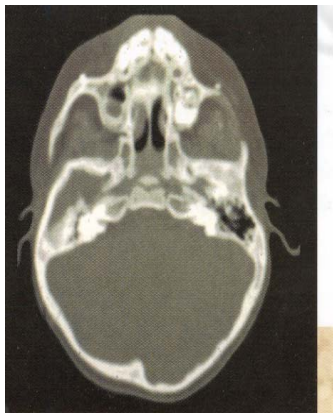
Commercialization

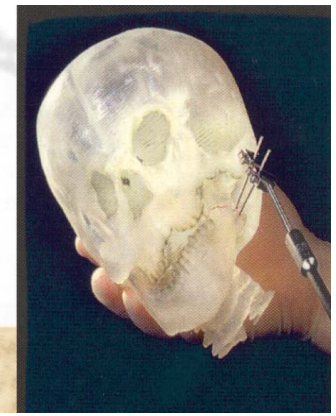
Bio-3DP: Personalized Medical Modeling and Implants







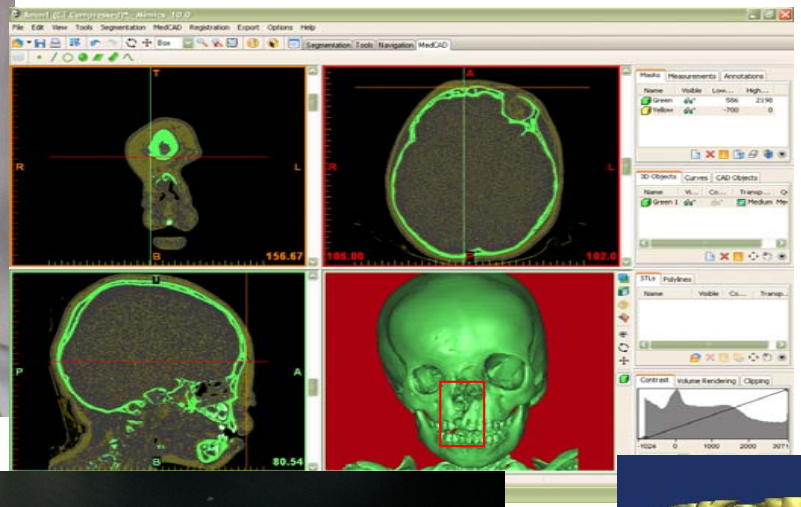


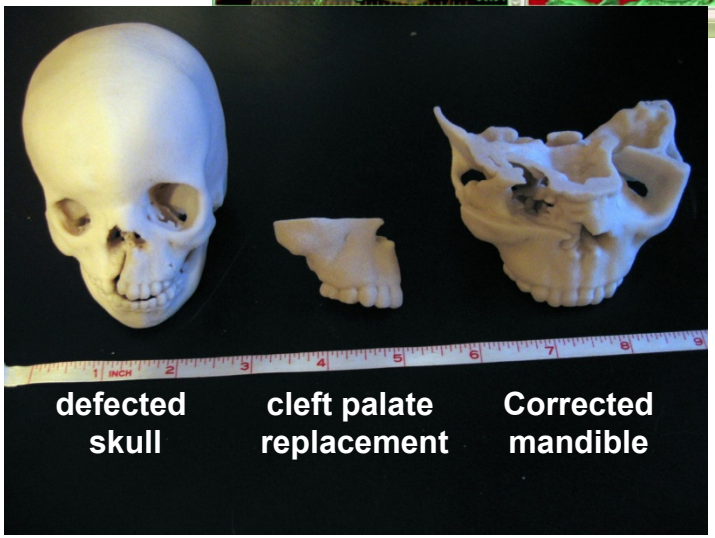


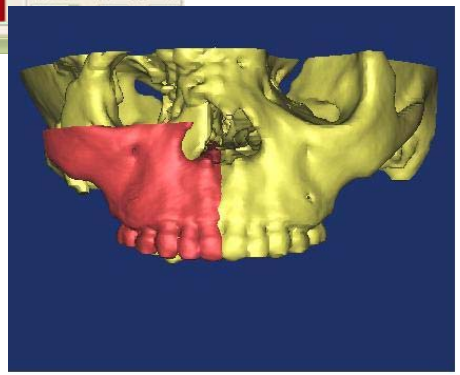
Application：plastic surgery, surgical planning, prosthesis

Biomodeling of Cleft Palate Deformities

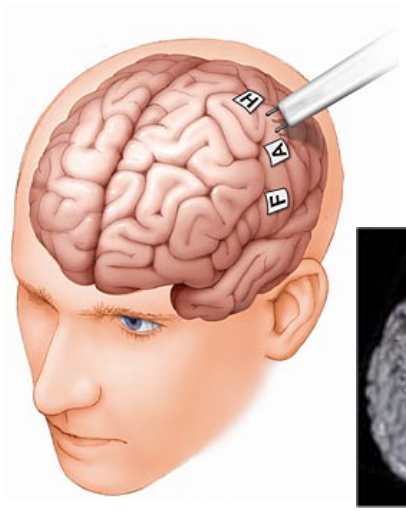
- Dr. HD Nah (CHOP)

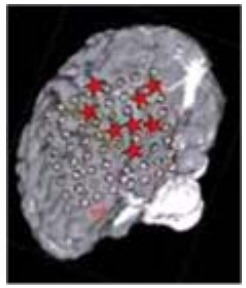


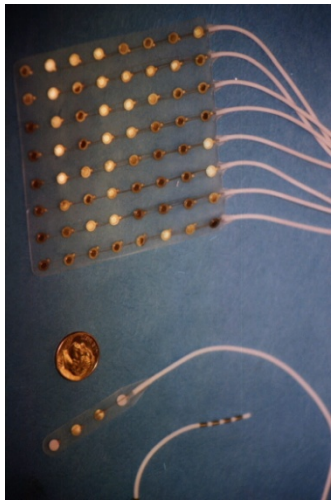


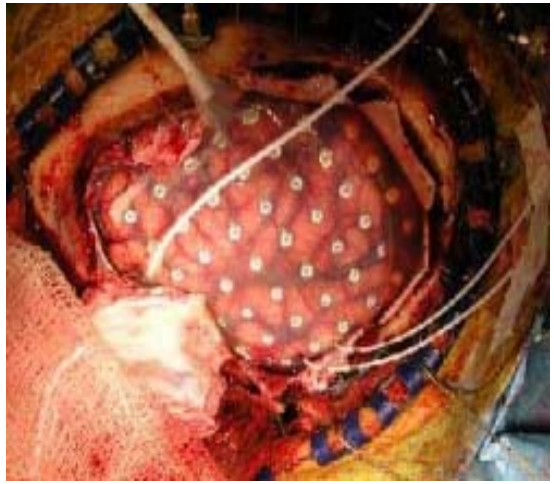


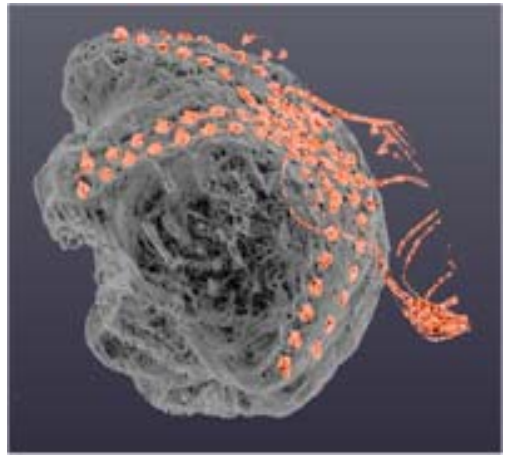
Biomodeling of Epileptic Regions of Brain with Electrodes - Dr. Sperling (TJU)

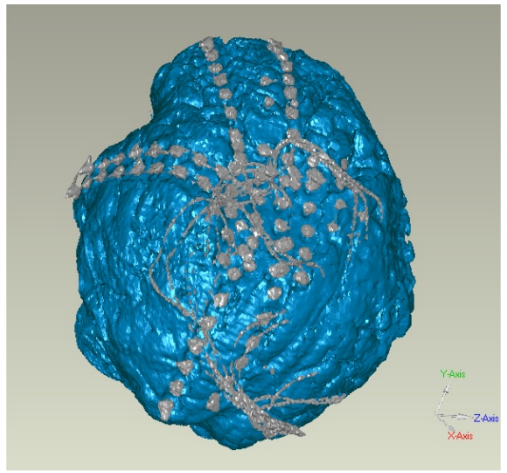


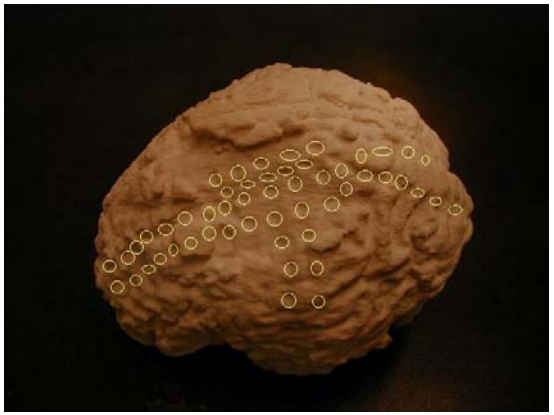




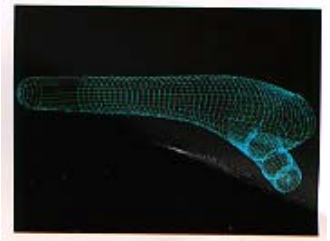






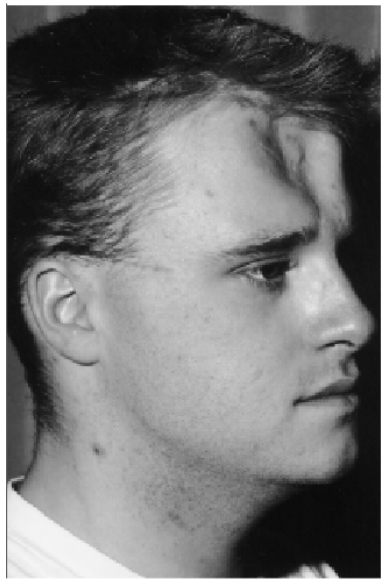
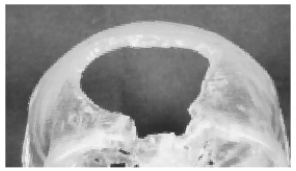
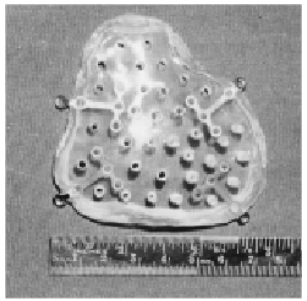


Bio – 3D Printing
Customized Implants

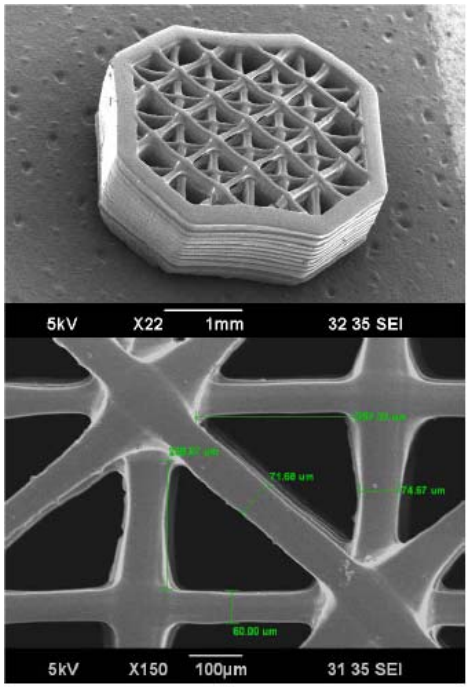






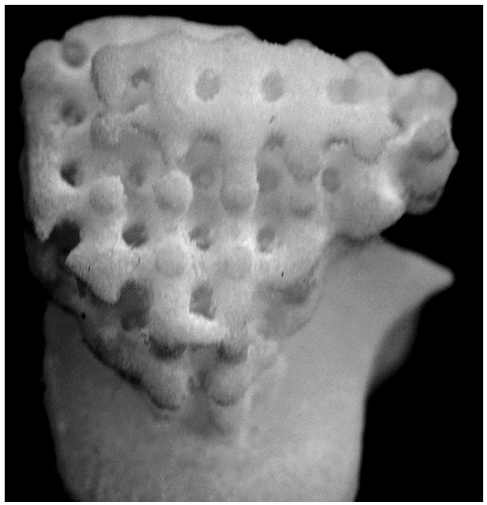

Scaffolds by Conventional 3DP Processes



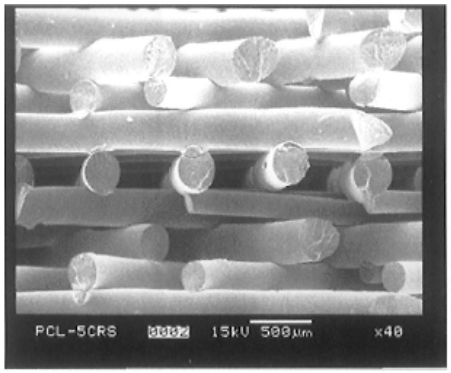
SLA builds concept-verification models of its tensegrity structures

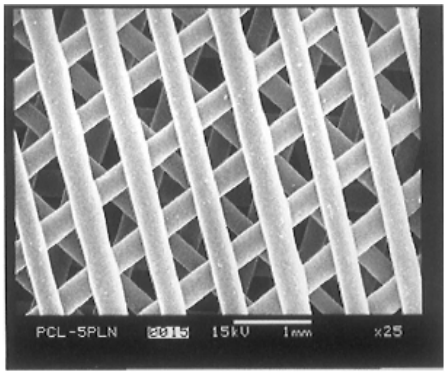
70 μm in diameter.

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



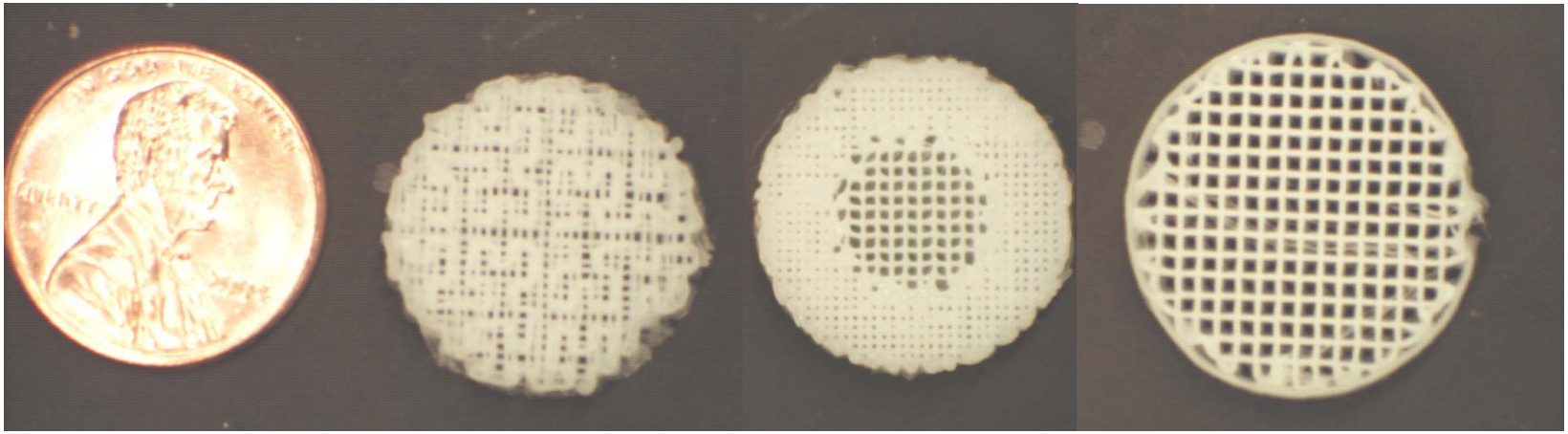
(Das & Hollister Group, UM\, SLS)





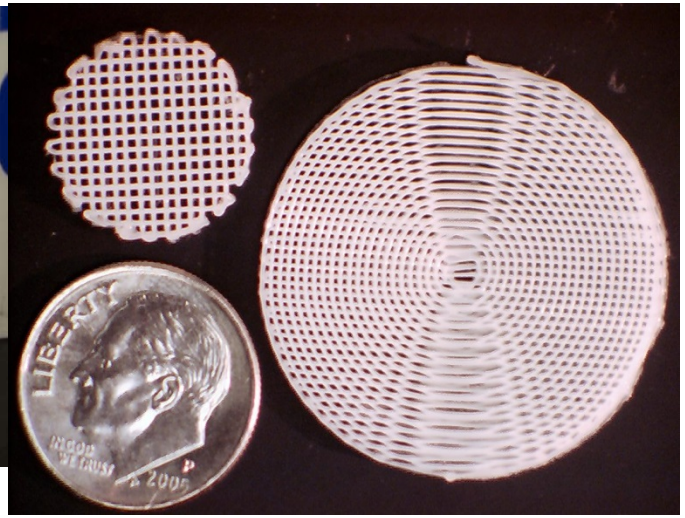
D. Hutchmacher group, FDM)

Scaffolds Fabricated by Precision Extrusion Deposition Technique



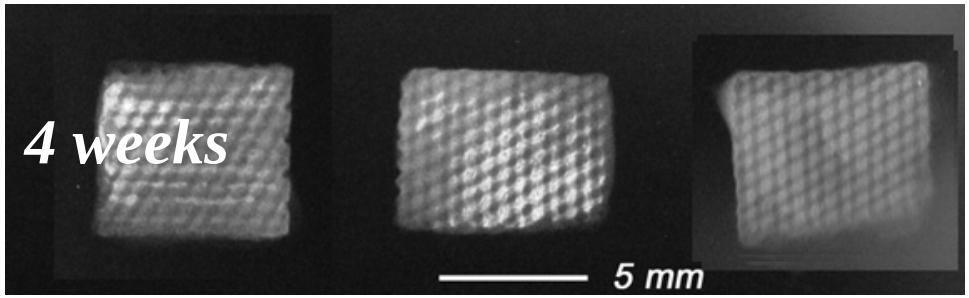
Material:
Poly-ε-Caprolactone (PCL)

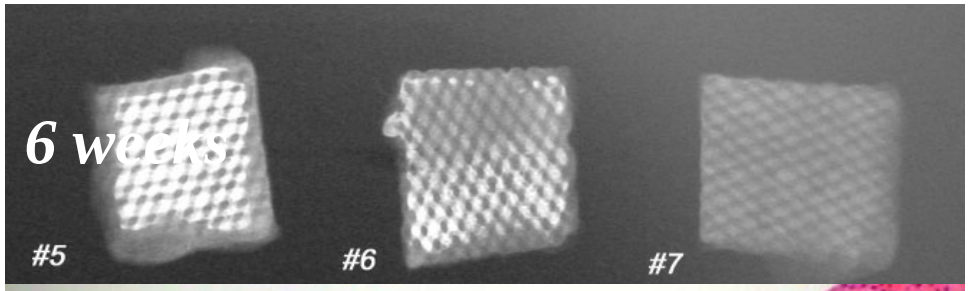
Average pore size:
~ 200 μm
Smallest strut: 100 μm

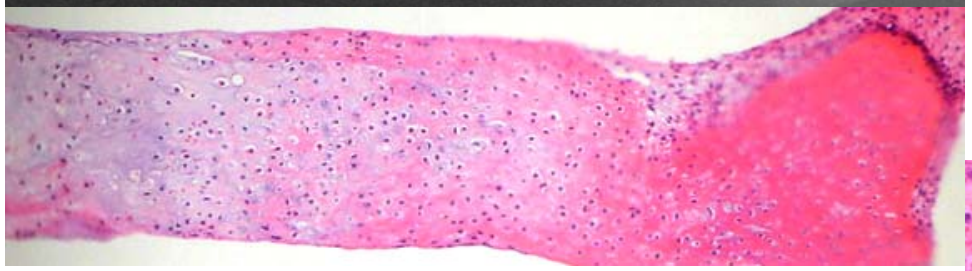


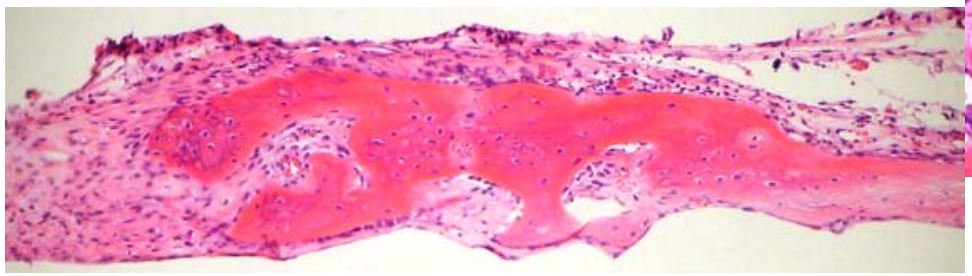
Darling et al, JBMB, 2005
Wang, et al, RPJ, 2005
Starly et al, CAD 2006
Shor et al, Biomaterials, 2007

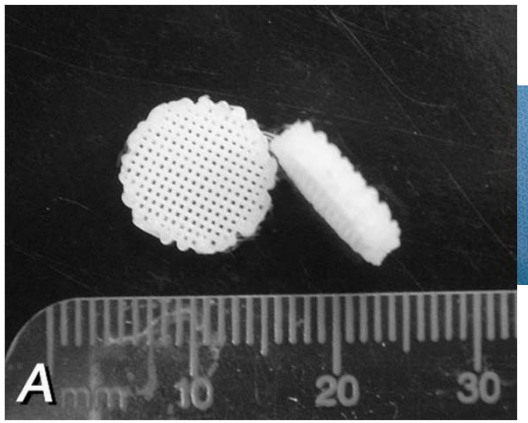
Nude Mouse SC Osteogenesis (collaborate with Dr. H. An, MUSC)

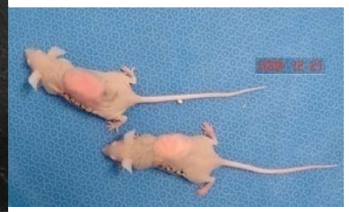






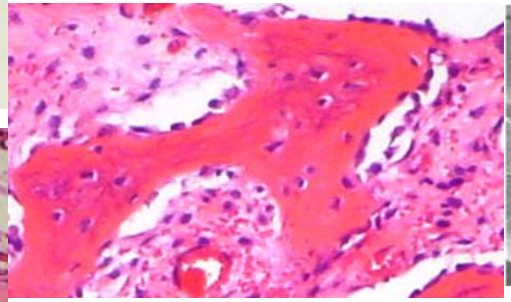


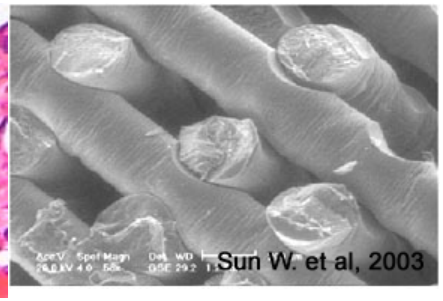




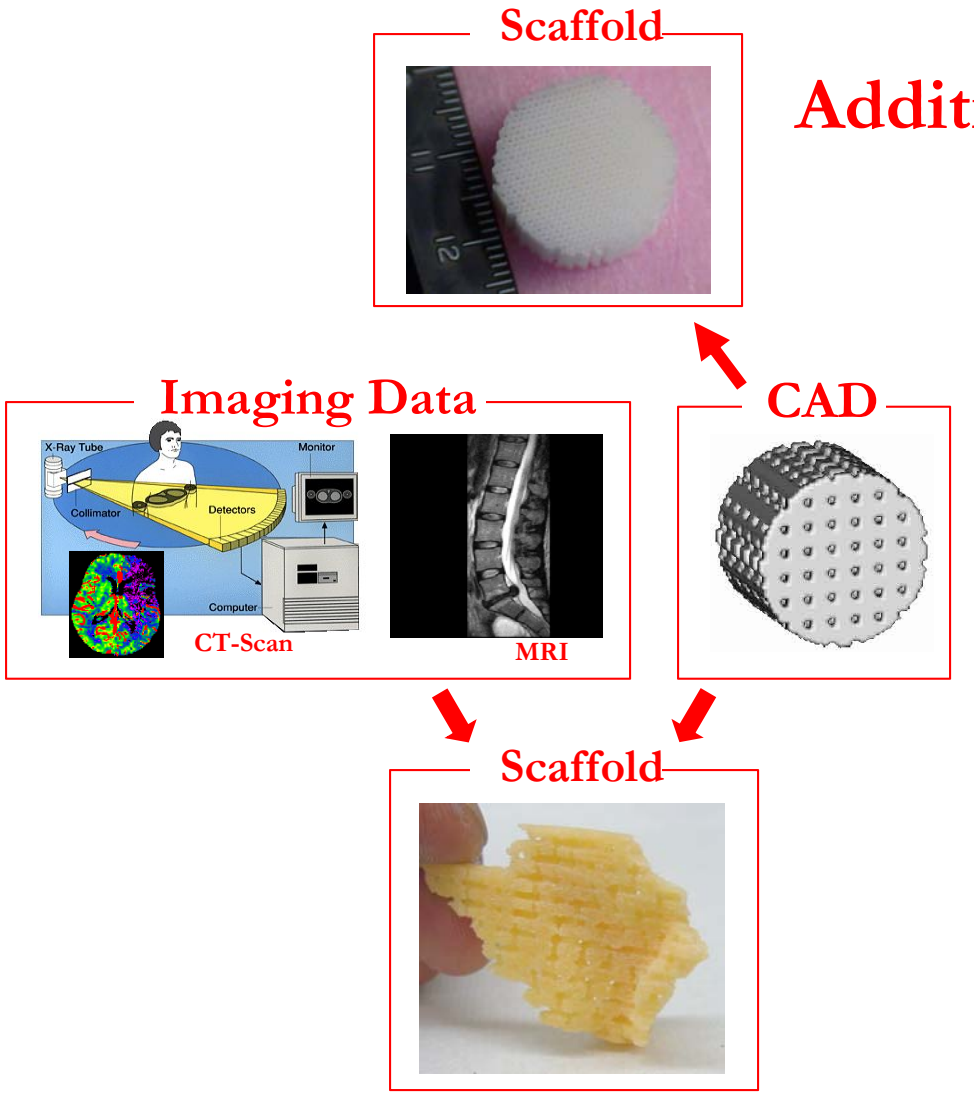
Nude Mouse
Subcutaneous Osteogenesis
Printed poly ε-Caprolactone scaffold

PCL scaffold + bovine osteoblasts





Tissue Scaffold Fabrication - Direct Methods by 3DP



Additive Manufacturing (3DP)

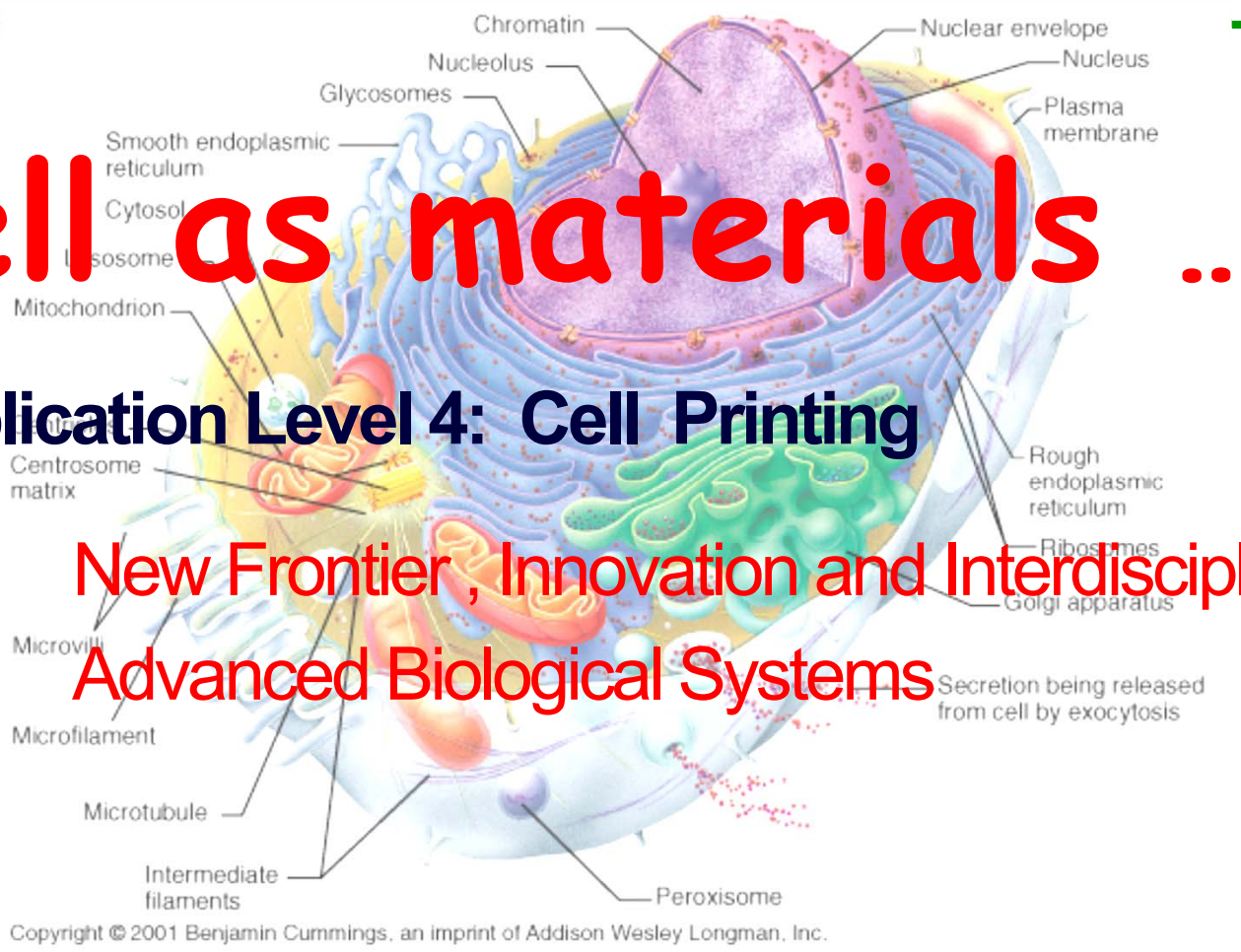
- CT / MRI – CAD – SFF:
  - FDM
  - SLS
  - 3DP – Theriform Process
- Advantages:
  - Biomimetic
  - No restriction on shape
  - High control capability
  - Consistent – reproducible
- Disadvantages:
  - Limited resolution
  - Not a cell-friendly environment
    - Harsh Heat
    - Toxic Solvents
   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

cell as materials …

Application Level 4: Cell Printing

New Frontier , Innovation and Interdisciplinary

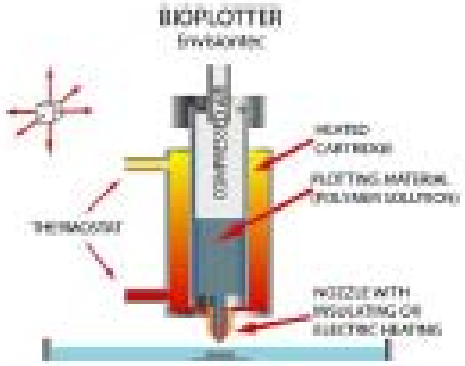
Advanced Biological Systems



Enabling Engineering Processes for Cell Printing / Assembly

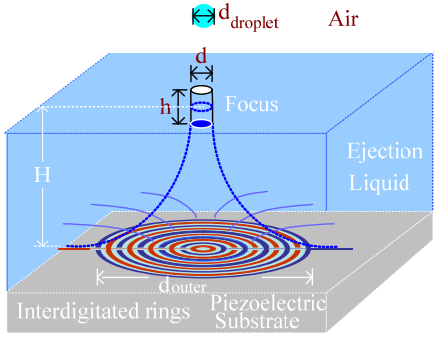
Inkjet

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



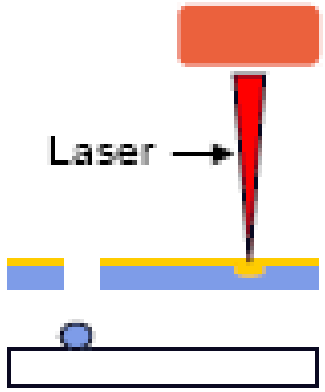
Bio-plotting

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



Acoustic Droplet Ejection

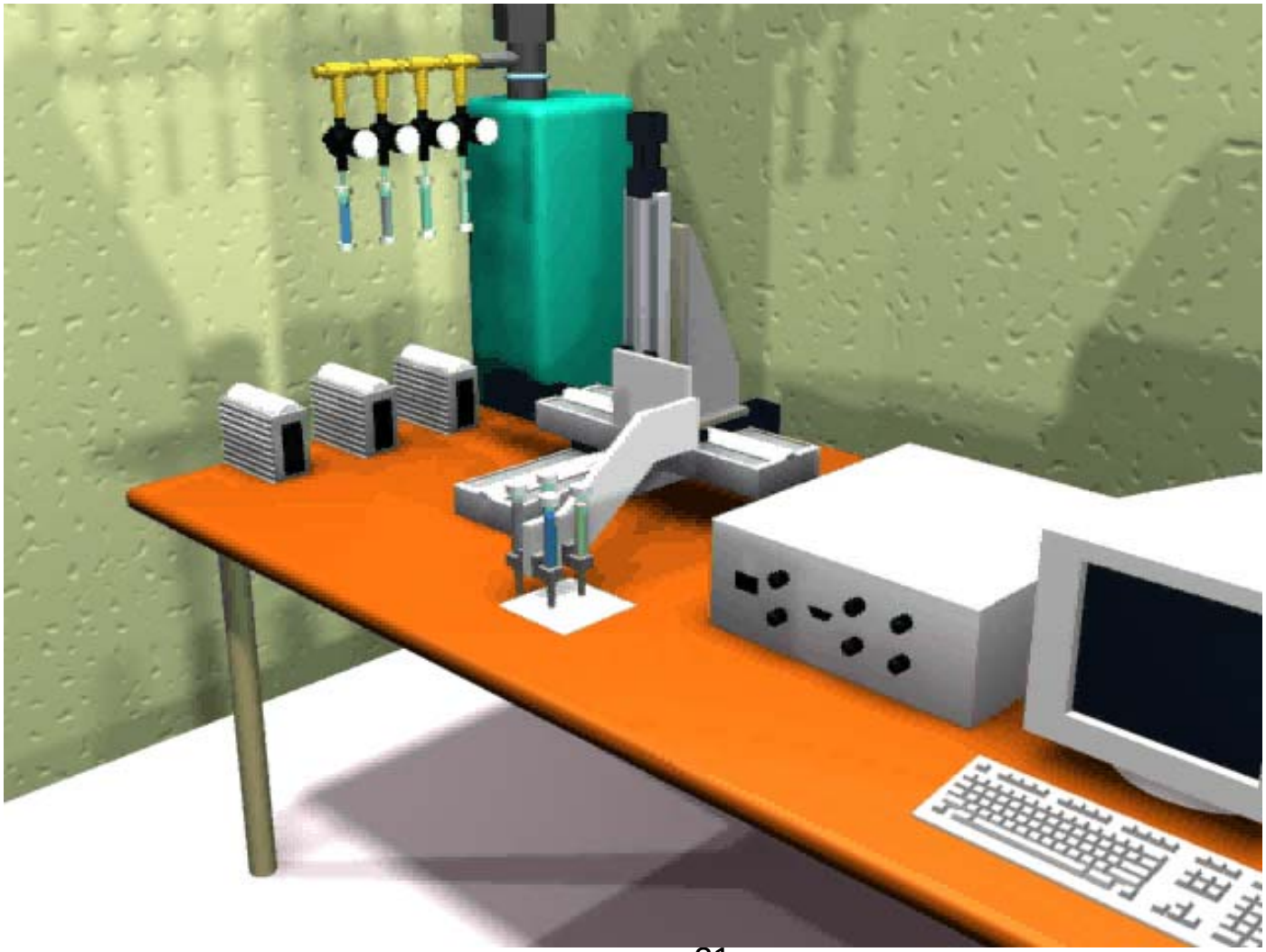
Stanford
(Demirci)



Laser-Assisting

NRL
INSERM-France
(Guillemot)
LDW-Clemson

Cell Deposition for Freeform Fabrication of 3D Tissue Constructs



Dr. W. Sun, NSF RAM Workshop Keynote Presentation

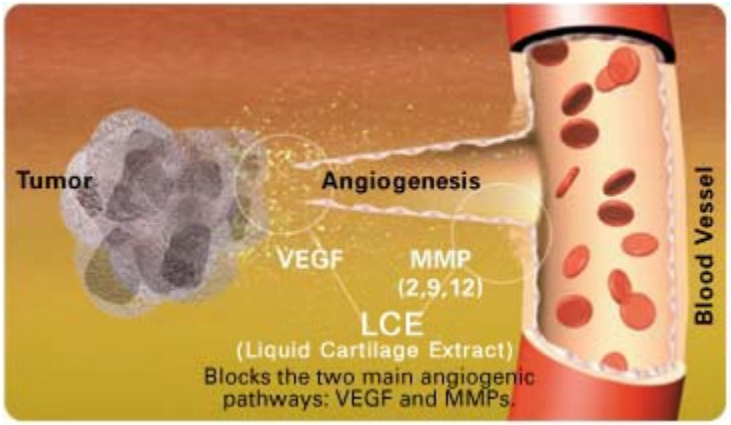
基于生物3D打印的仿生生物构建模型

含生物因子表面

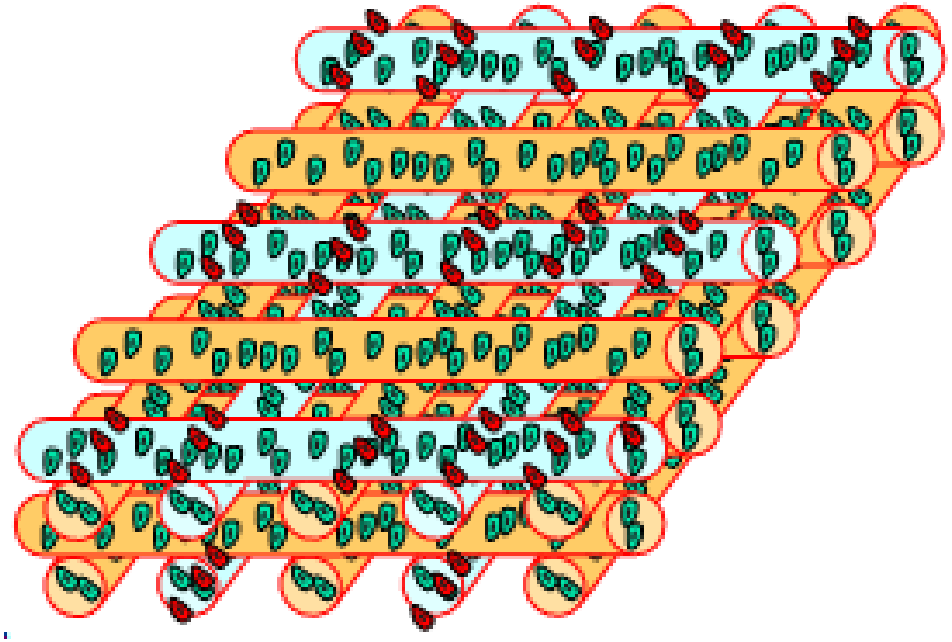
无生物因子表面

成纤维细胞

内皮细胞 、干细胞 （作用表面）



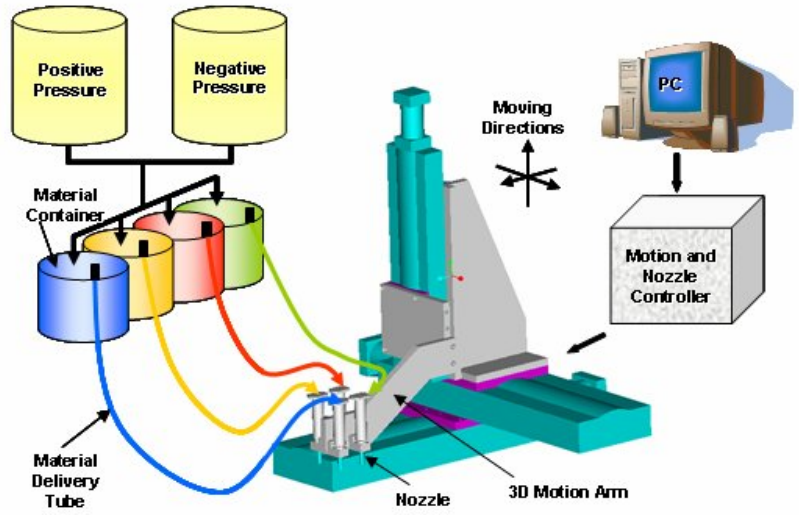
http://www.angioworld.com/angiogenesis.htm



基于细胞打印技术组装的血管再生体外生物学模型

将细胞，干细胞， 生长因子和细胞基质材料用细胞打印方式在三维空间进行可控组装，制造体外生物结构体模型，

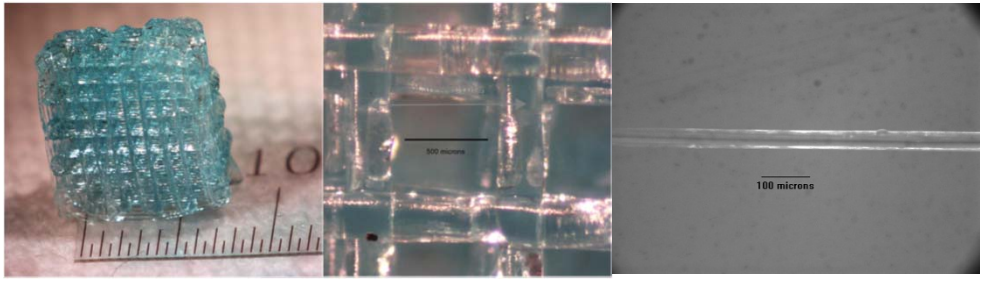
Multi-nuzzle 3D Cell Deposition System



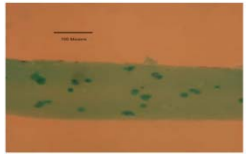
Multi-nozzle systems:
- Precision extruding
- Solenoid-actuated
- Piezoelectric
- Pneumatic syringe
- Pneumatic spray

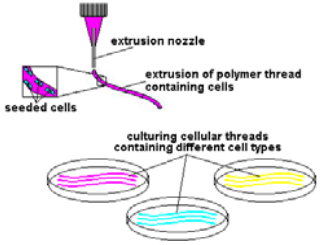
Biopolymer:
- Hydrogel-Alginate/Chitosan
- Fibrin, Collagen
- Matrigel

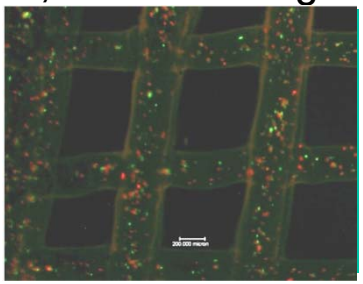
Cells:
- Endothelial
- Cardiomyoblasts
- Fibroblast
- Chondrocytes
- Osteoblasts
- Sm. muscle cells
- Hepatocyte
- MCF-7
- Huh7.5.1



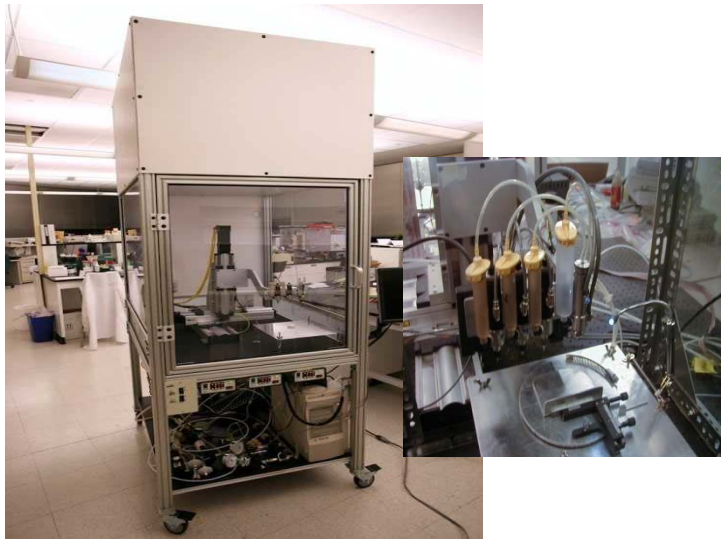
40 layers, 275 micro strand pattern, 38 micro single strand







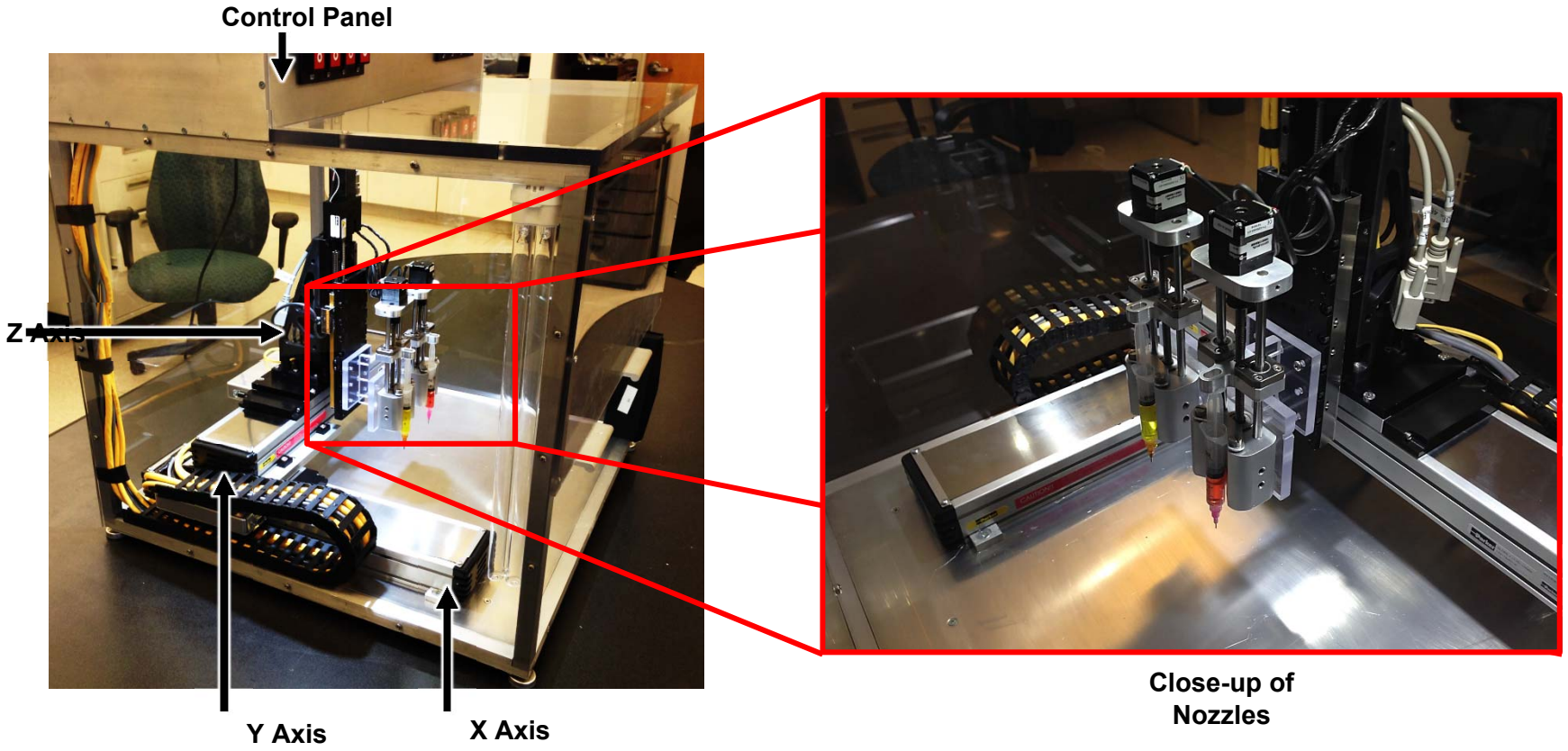
Cell deposition, cellular thread, cell viability



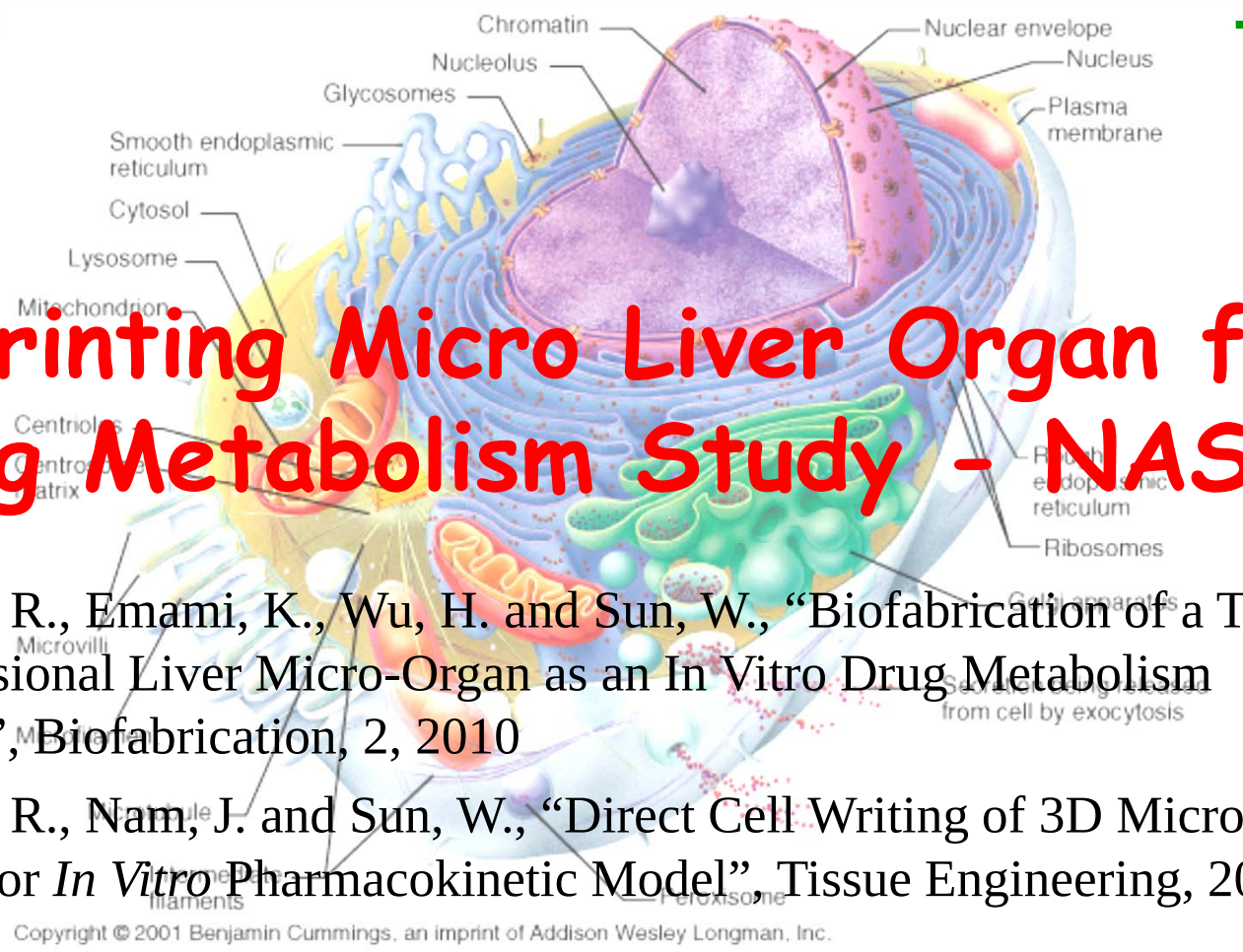
US Patent #: 8639484 B2

Heterogeneous Bio-Printing

Multi-nozzle Direct Cell Printing



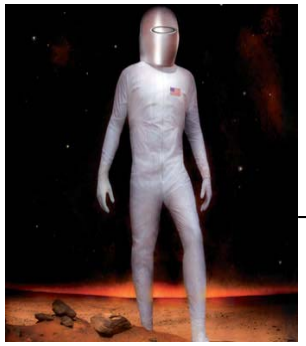
Bioprinting Micro Liver Organ for Drug Metabolism Study - NASA



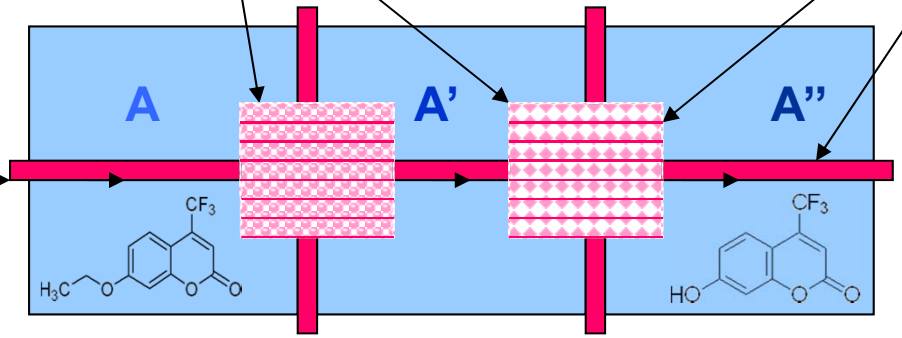
- Chang, R., Emami, K., Wu, H. and Sun, W., "Biofabrication of a Three-Dimensional Liver Micro-Organ as an In Vitro Drug Metabolism Model", Biofabrication, 2, 2010
- Chang, R., Nam, J. and Sun, W., "Direct Cell Writing of 3D Micro-organ for *In Vitro* Pharmacokinetic Model", Tissue Engineering, 2008

Bioprinting Micro Liver Organ for Anti-radiation Drug Metabolism Study
(NSAS-USRA-09940-008)

NASA's Interest - Safe plenary exploration & Mars Landing



Drug A



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

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

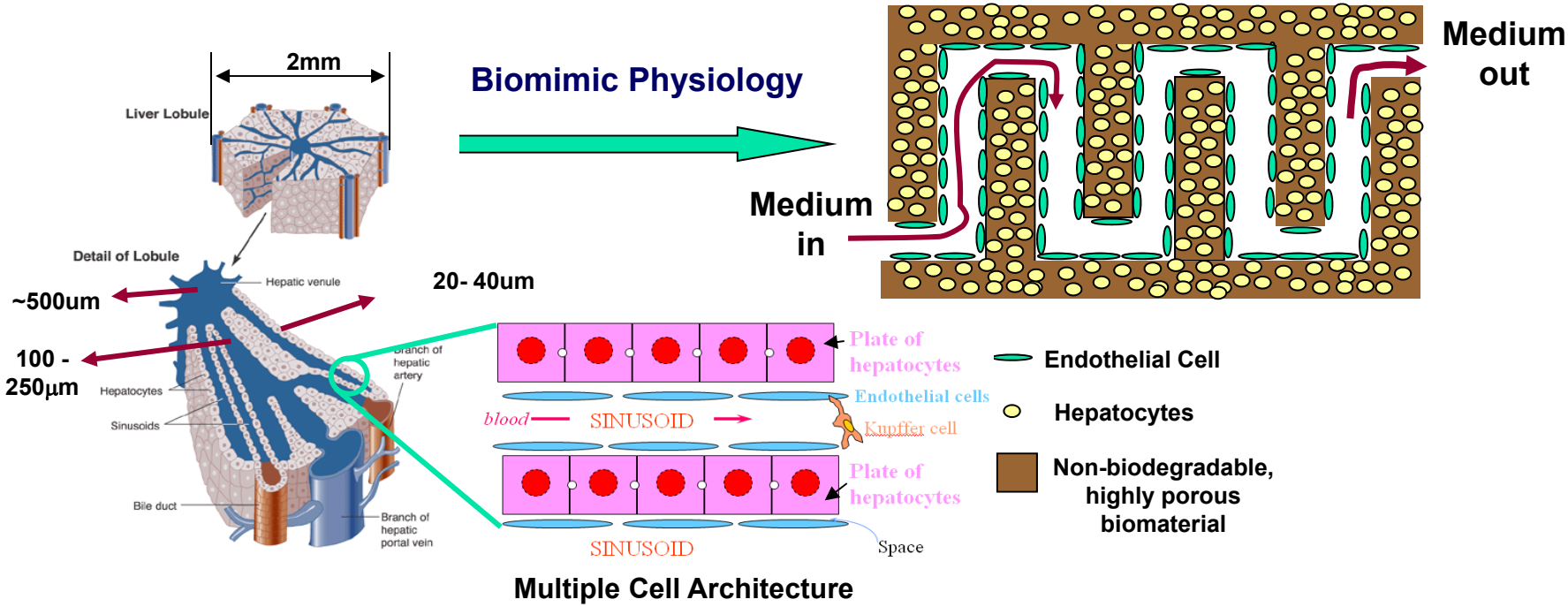
Micro-Organ Device

Liver

Target Organ
Heart
Brain
Mussel
Bone
…

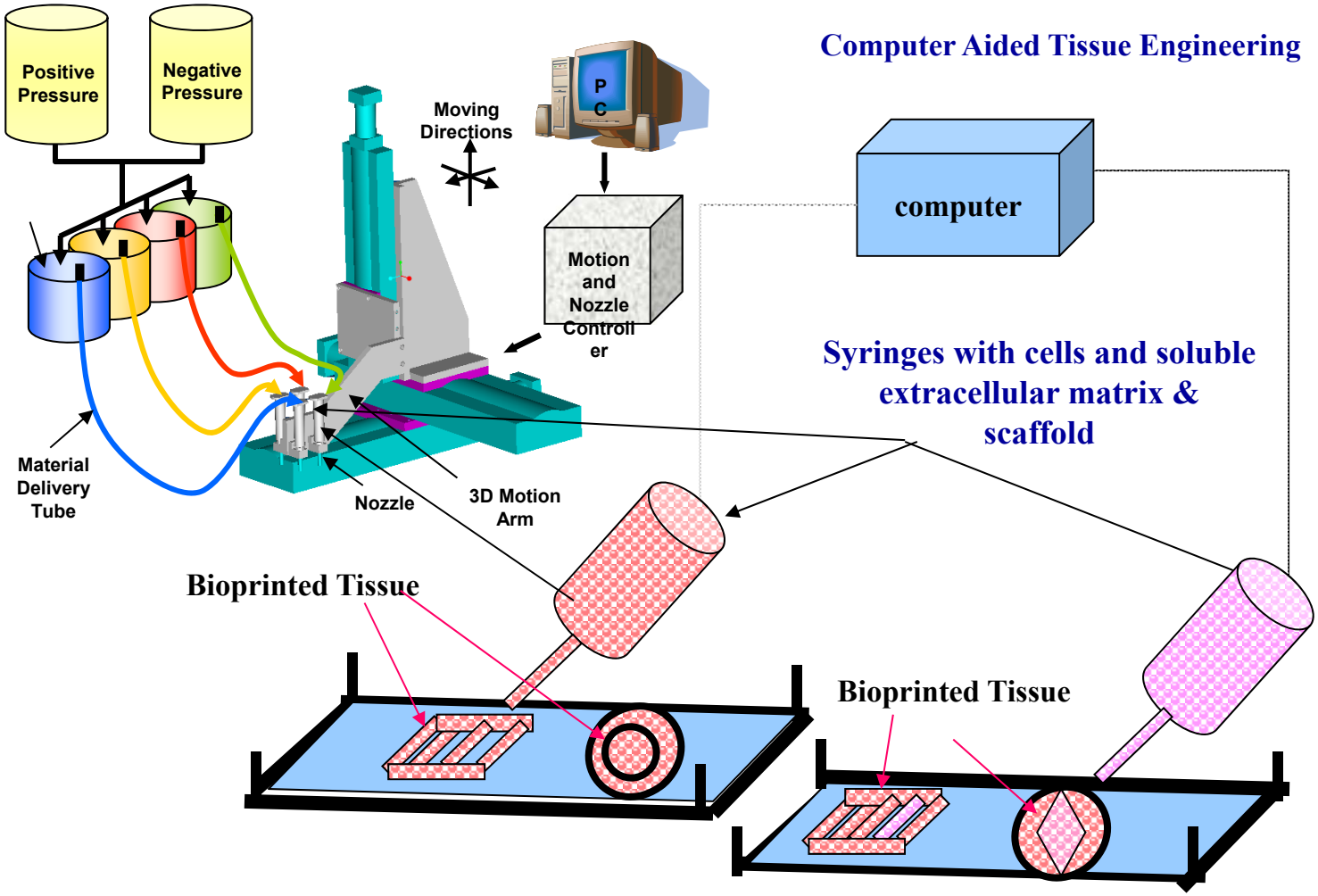
Micro-Organ Device for drug conversion study (A, A', A" with multiple micro-organs)
Schematic drug metabolic conversion from EFC → HFC

Sinusoid Flow Pattern Design to Biomimic Liver Physiology

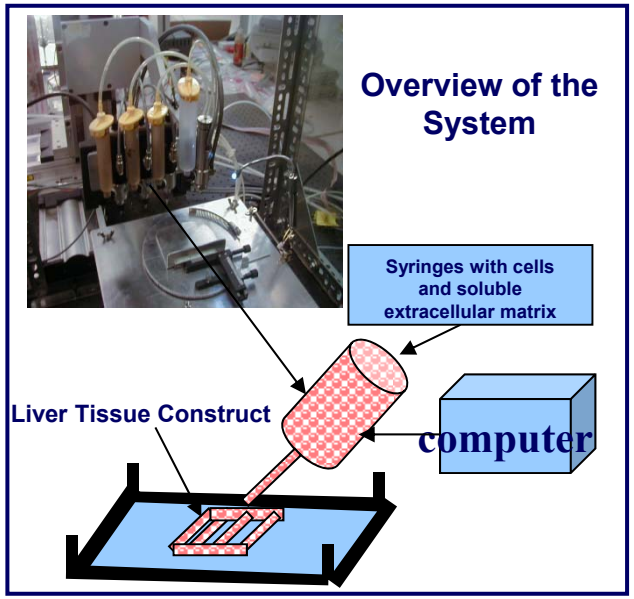


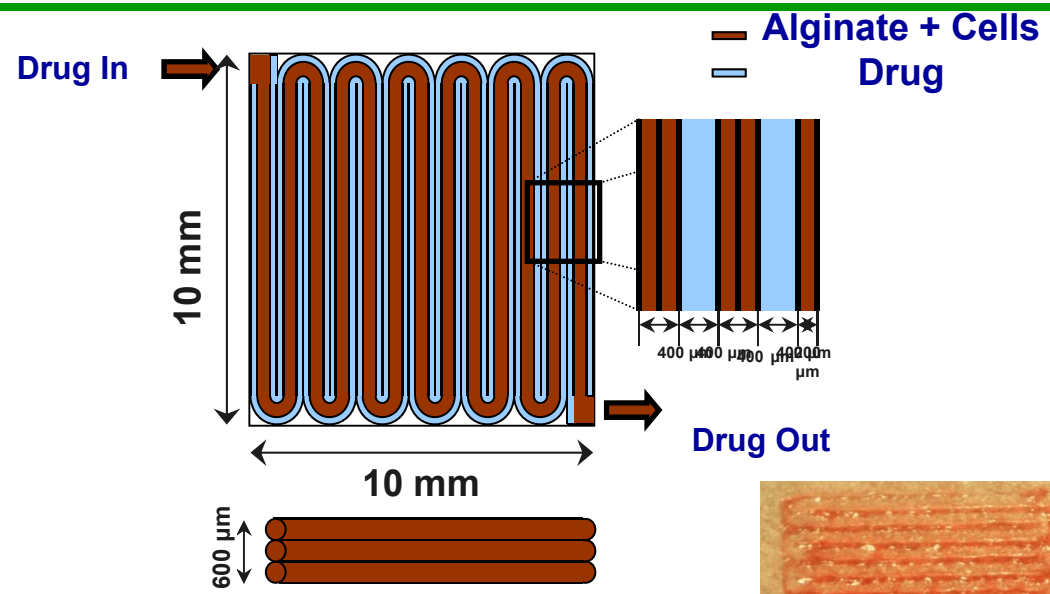
- hepatic vascular system (capillaries) is configured in sinusoidal pattern → design the sinusoidal micro-fluidic channel patterns to biomimic *in vivo* liver microstructure.
- Channel dimensions and strut widths vary from 50μm to 250μm, flow varies from 1ml/min to 5ml/min.

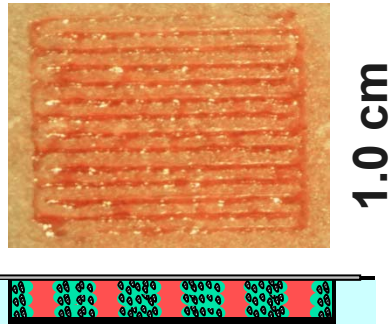
Bioprinting Micro Liver Organ Models



Liver Tissue Construct
Physiological Structure Formation

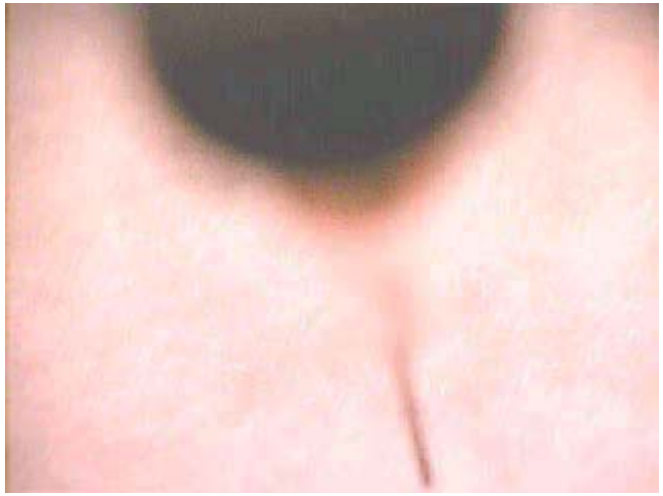




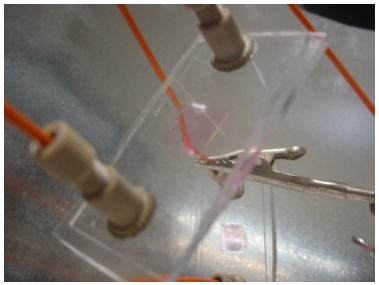


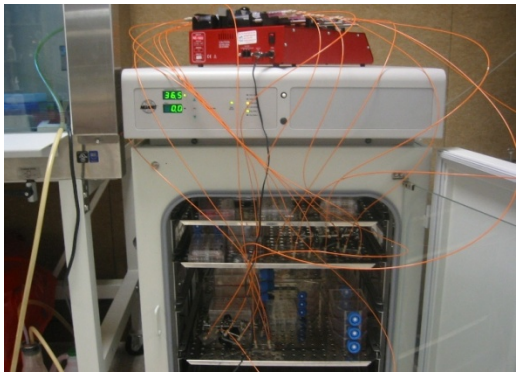
Process Parameters

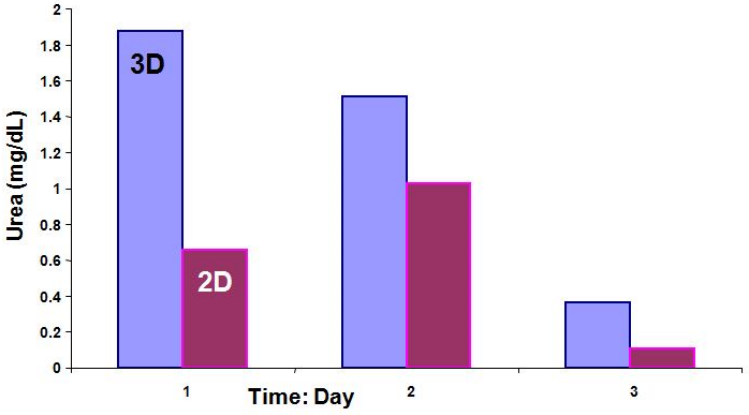
- Valve Type: Pneumatic Microvalve
- Pressure: 2.0 psi
- Motion Velocity: 10 mm/s
- Alginate Conc.: 3.0% w/v
- $CaCl_2$ Crosslinking Conc.: 5.0% w/v
- Nozzle Tip Size: 200 um

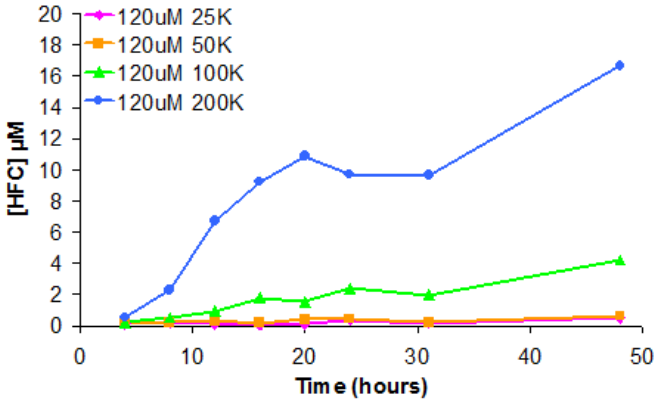


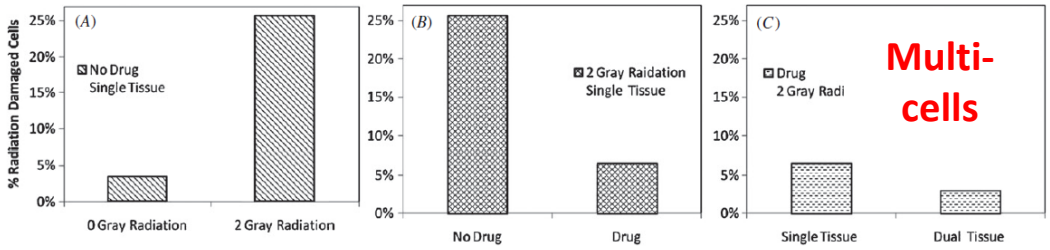
Integration of Bioprinted Liver Chamber with Microfluidic Device









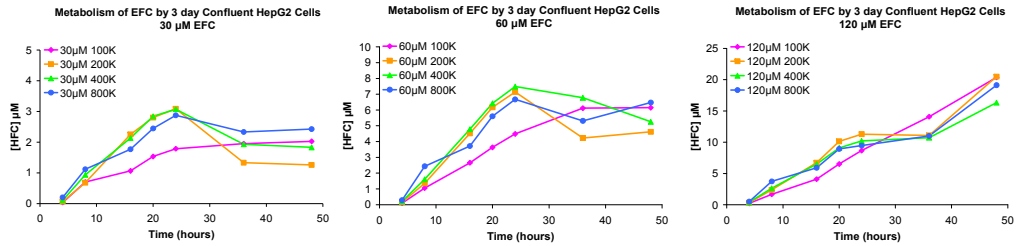


Multi-cells

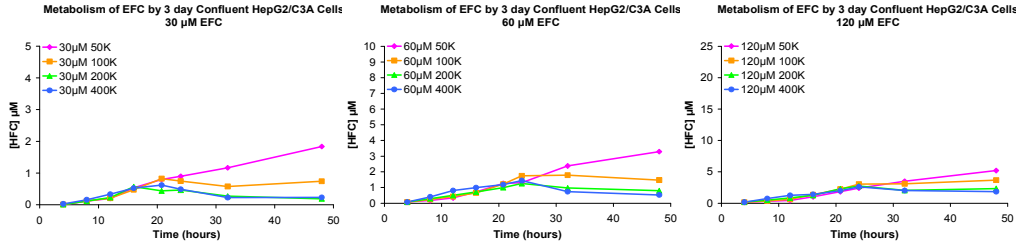
Results for Drug Metabolism Study

- 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

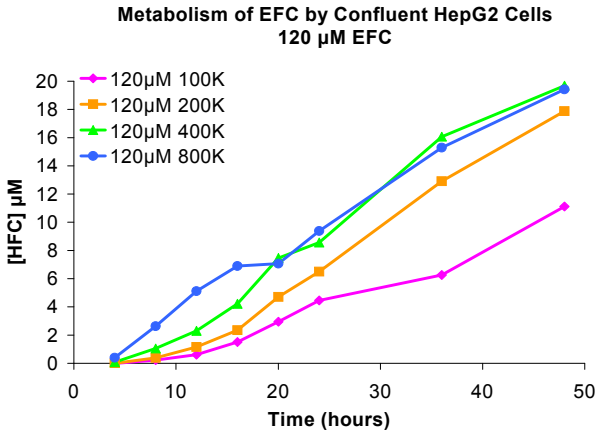
HepG2 cells metabolism

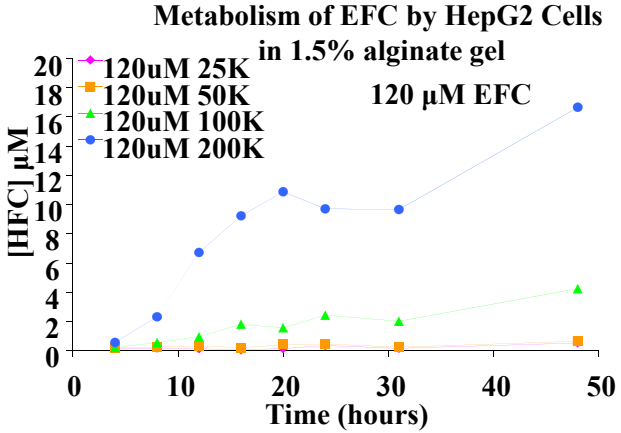


HepG2/C3A cells metabolism



* 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.

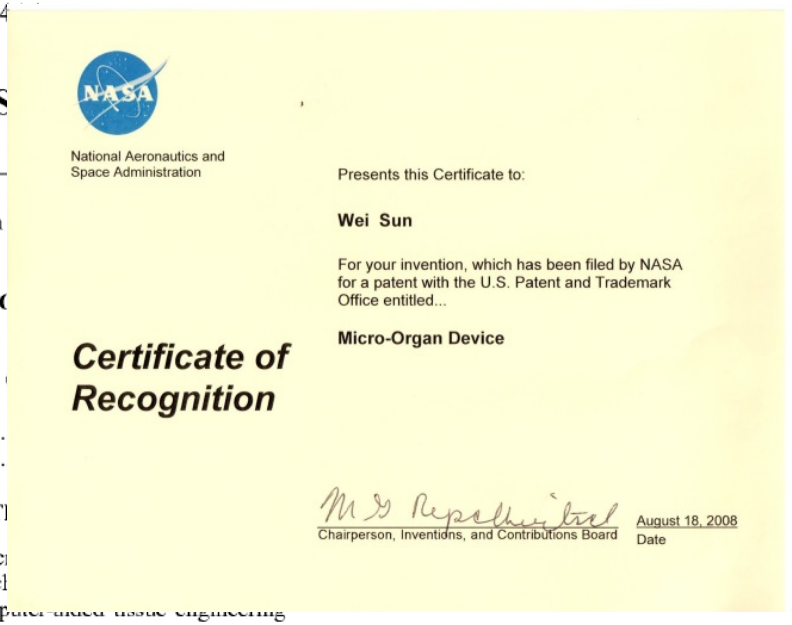
Publication

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

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

(57) **ABST**

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



National Aeronautics and Space Administration

Presents this Certificate to:

Wei Sun

For your invention, which has been filed by NASA for a patent with the U.S. Patent and Trademark Office entitled...

Micro-Organ Device

Certificate of Recognition

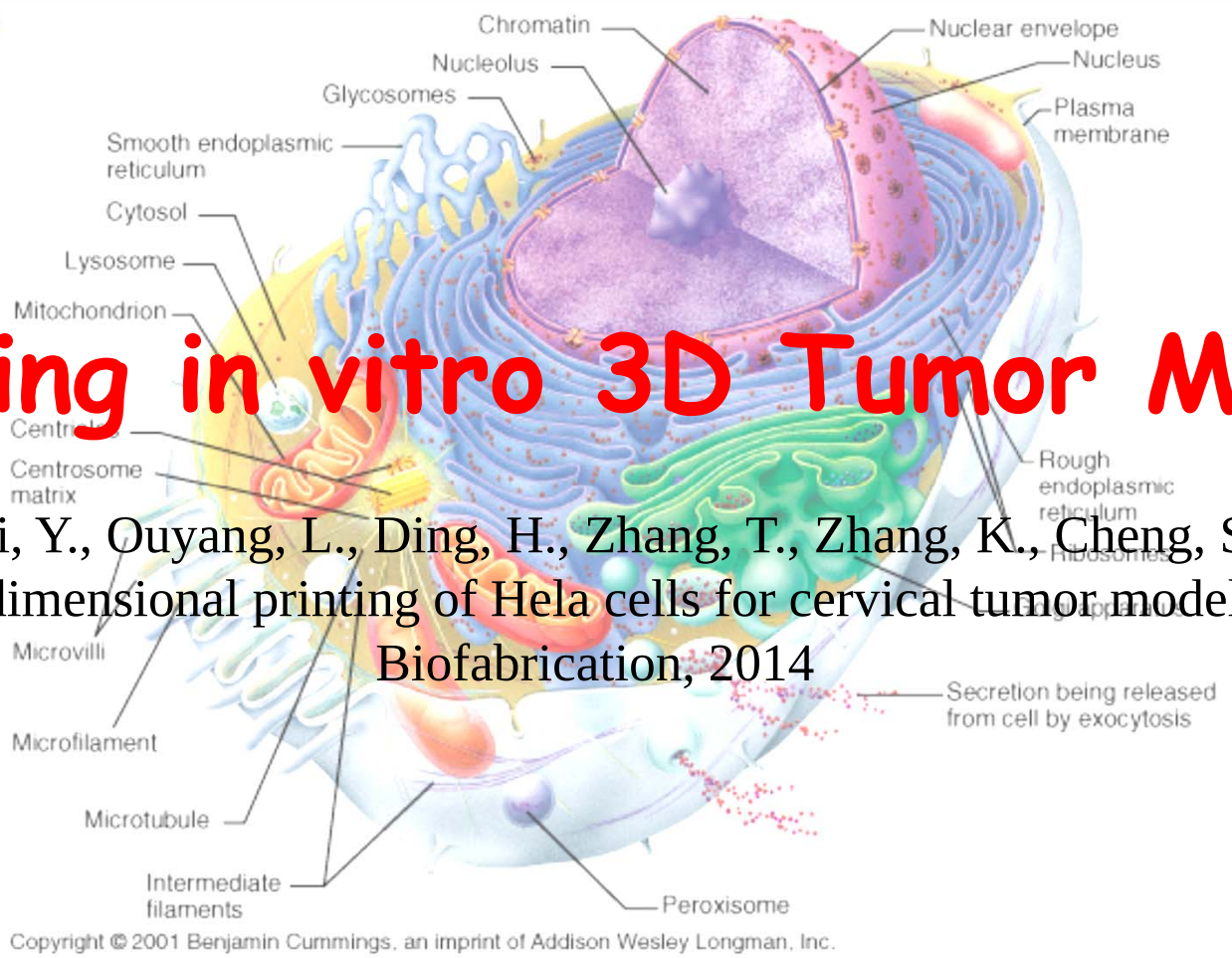
Chairperson, Inventions, and Contributions Board

August 18, 2008
Date

NASA-Micro-Organ Patents: US8343740; US8580546

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

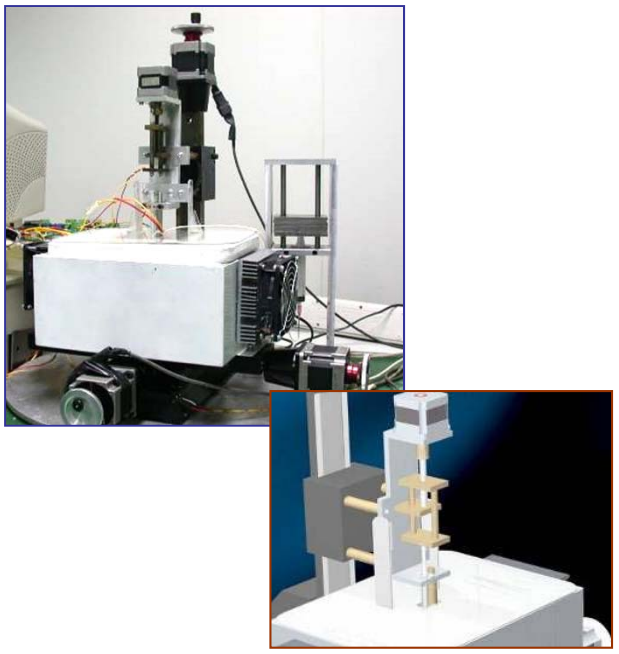
Printing in vitro 3D Tumor Model



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)

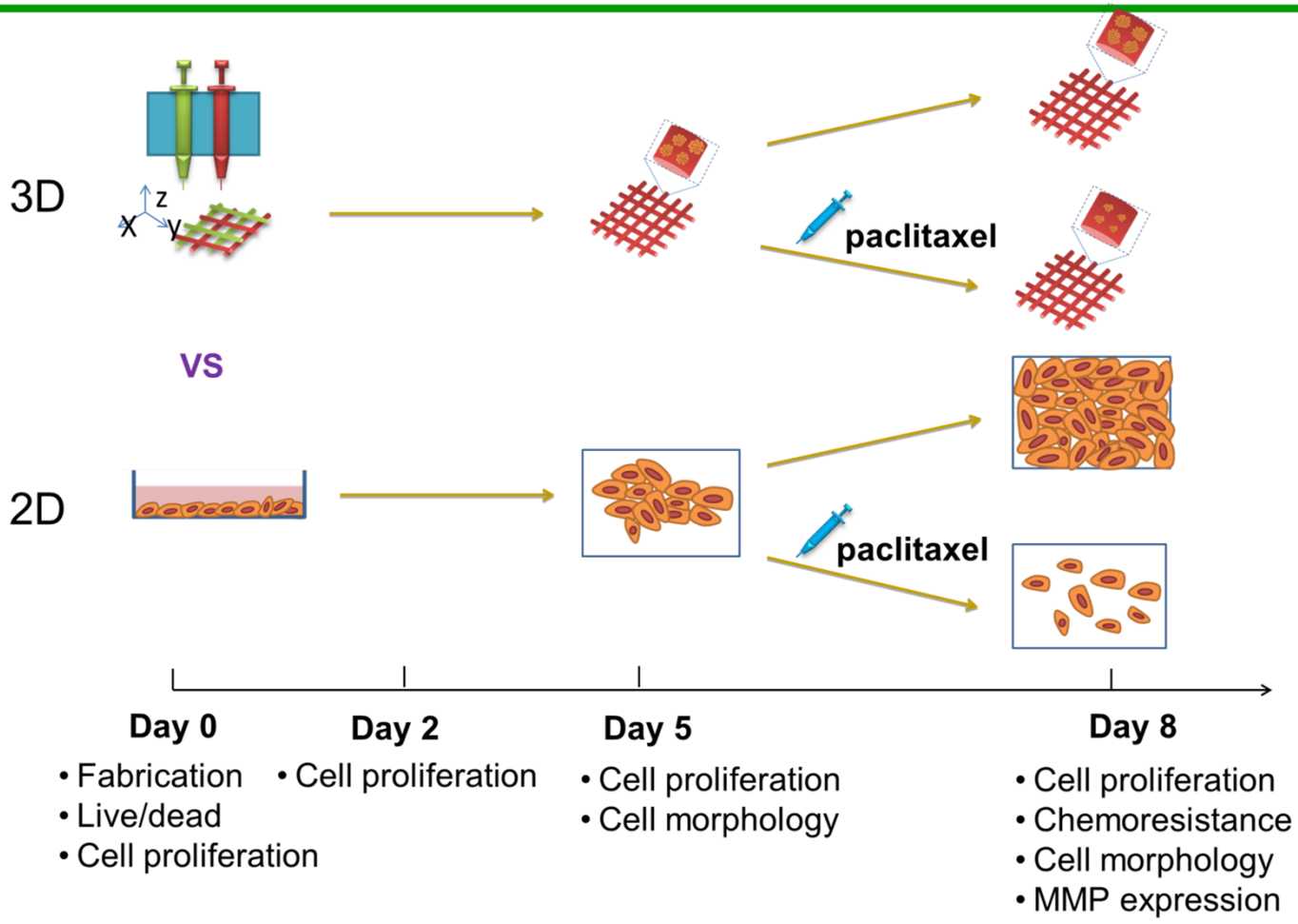


Single Nozzle System

Dual Nozzle System

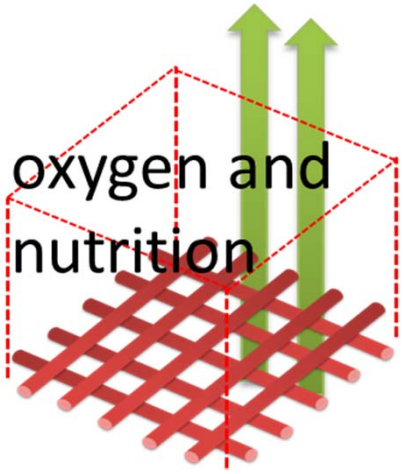
Assembly Cancer Cells for In Vitro Tumor Model

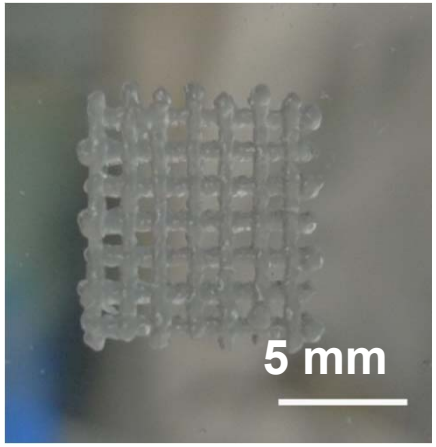
(NSFC 2012 E05 Key Research Project; Tsinghua)



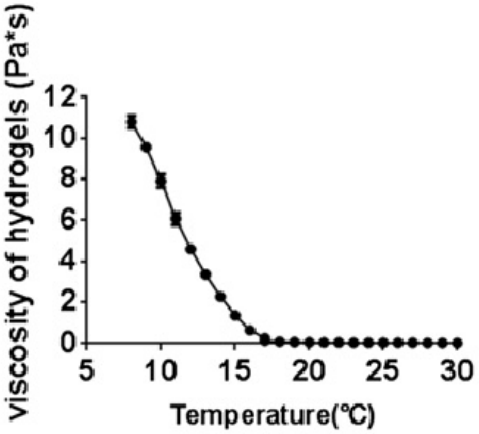
Biomaterials: Gelatin-Alginate-Fibrin Cells: Hela

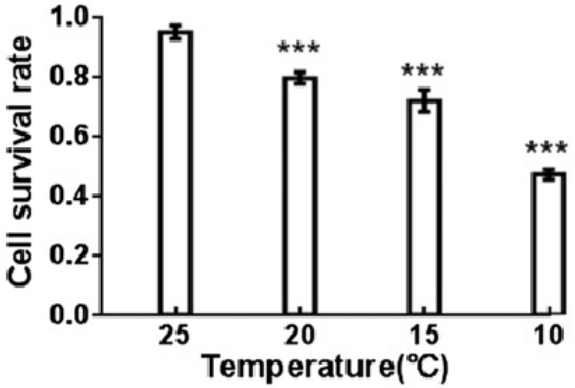
Printing of 3D cervical tumor models





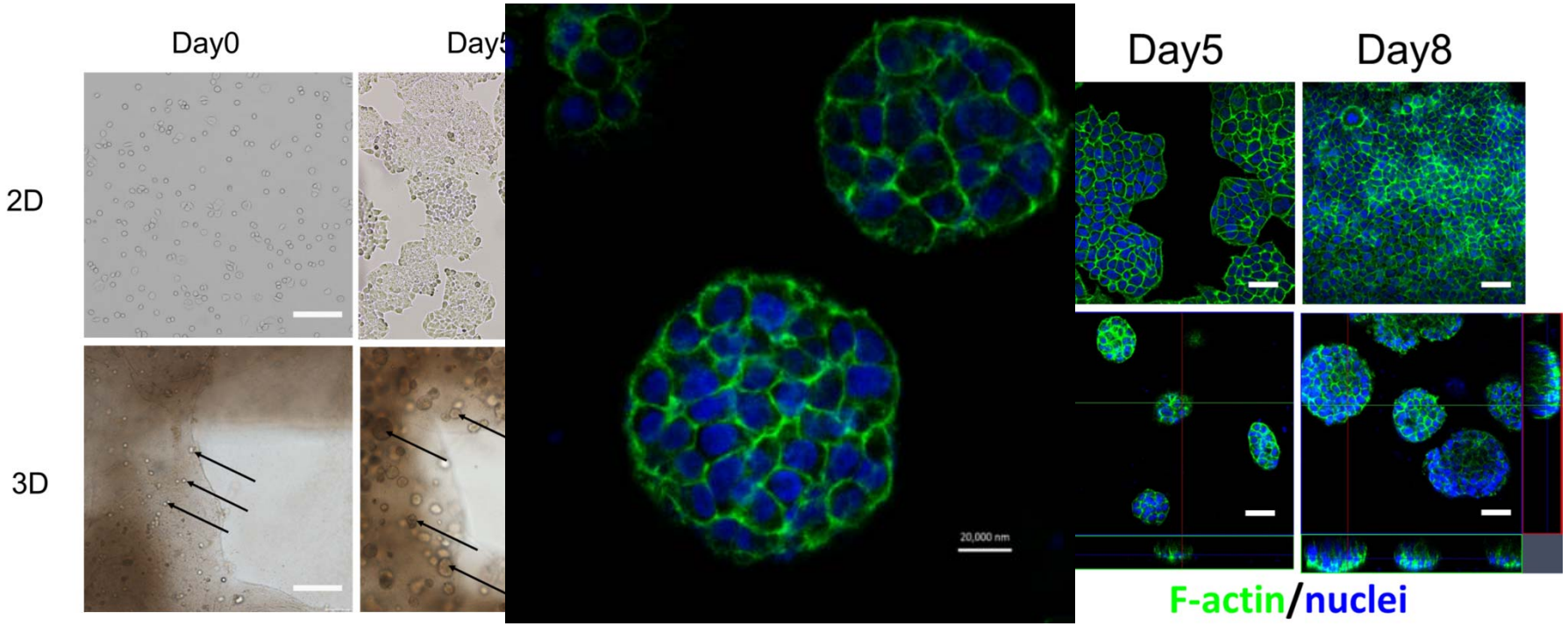
- A grid structure with high supply of oxygen and nutrition was designed and printed.





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

Cellular morphology in 2D vs 3D cultures



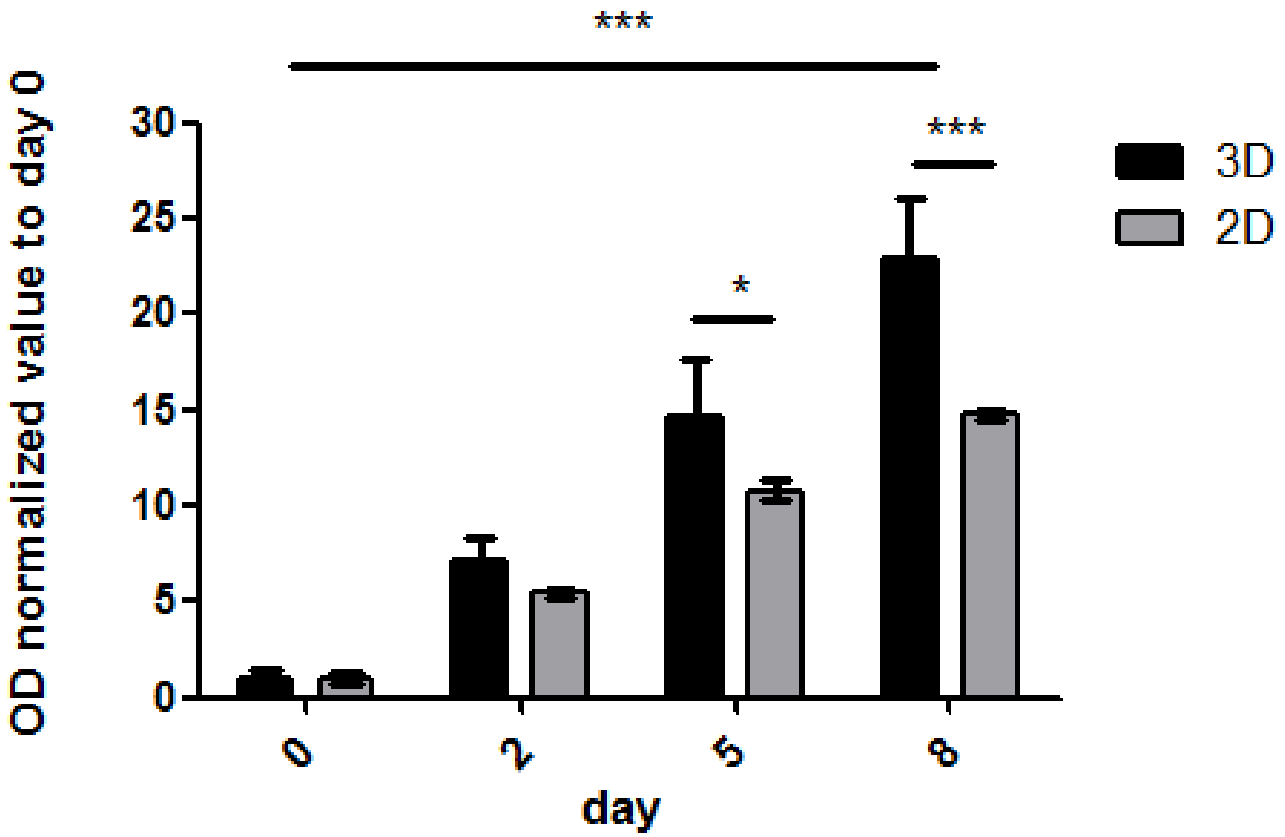
Scale bar, 200 μm

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.

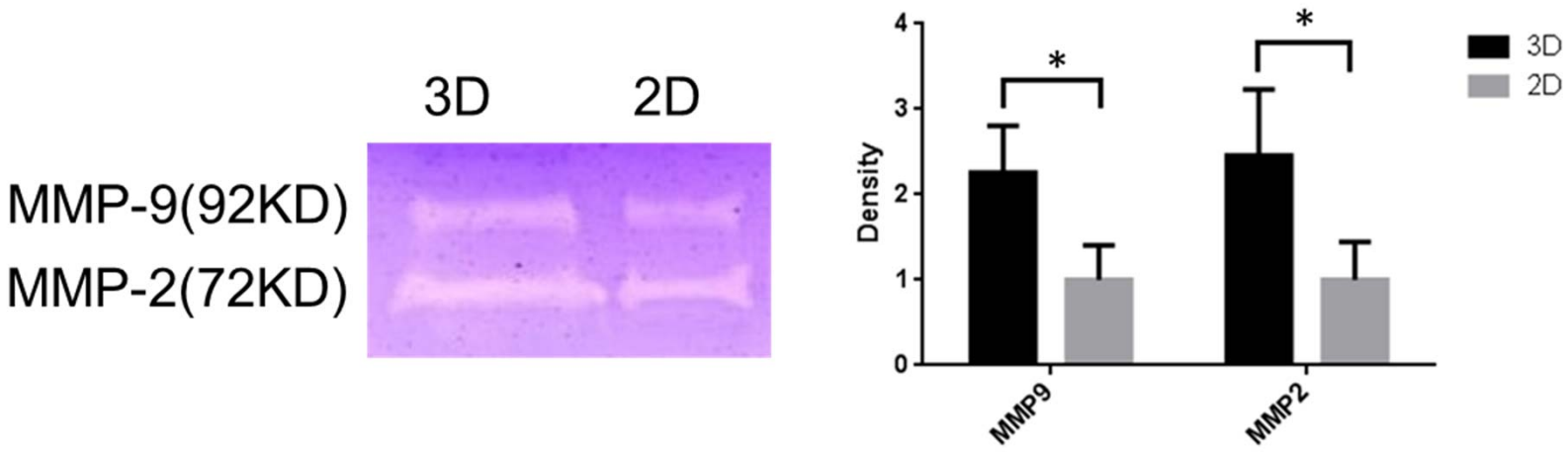
Zhao Y, Yao R, Ouyang L, et al. Three-dimensional printing of Hela cells for cervical tumor model in vitro , Biofabrication, 2014, 6(3): 035001.

Cell proliferation



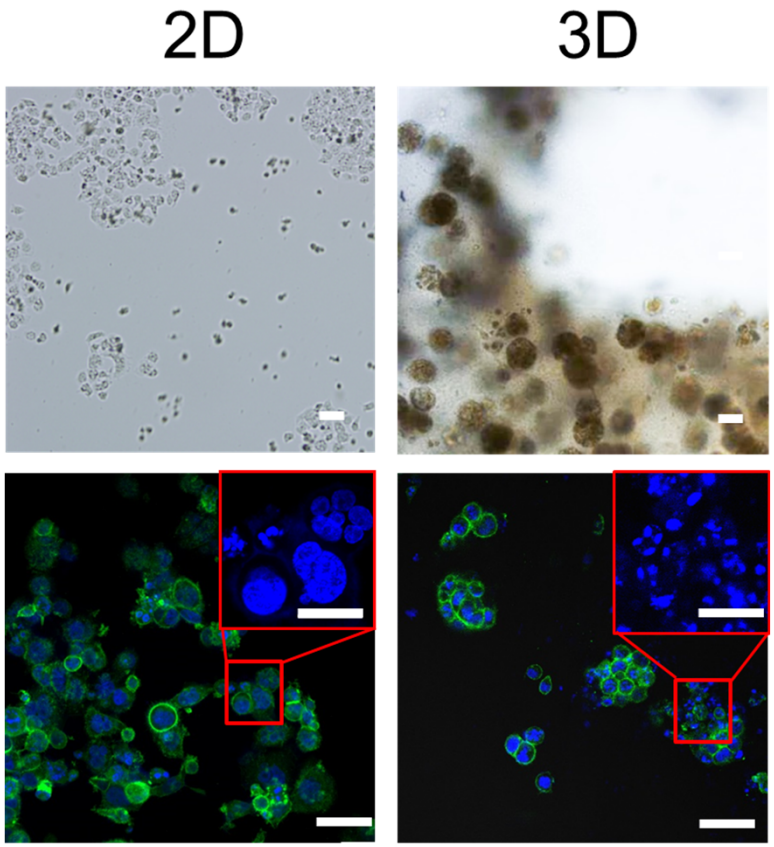
Results: Hela cells in 2D culture plates proliferated more slowly than in printed 3D constructs.

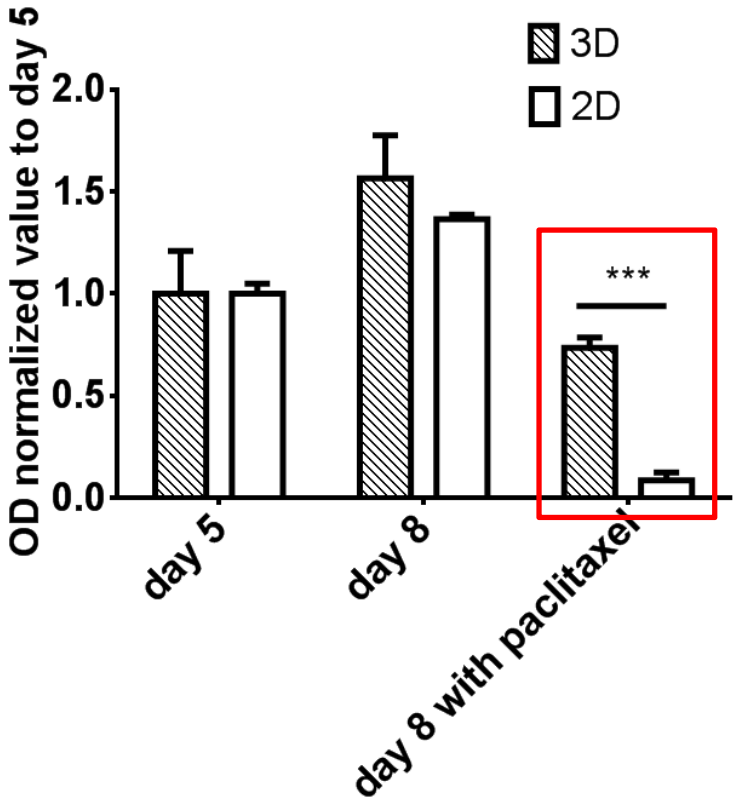
MMP expression



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**

	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**

	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

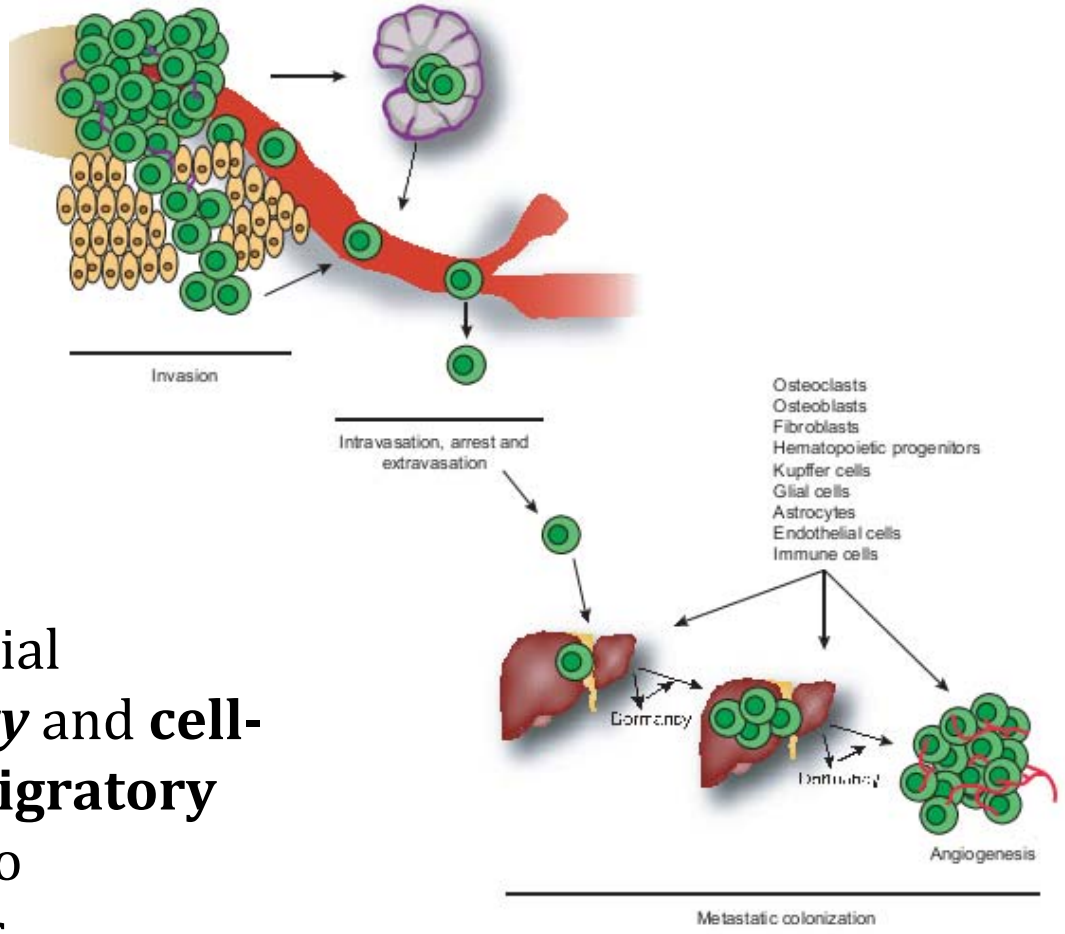
(unpublished data)

- 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



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

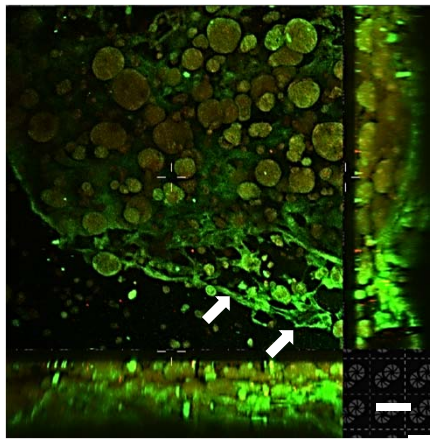
Steeg, P. S. *Nature medicine* (2006) *12*(8), 895-904.

Summary of EMT Study

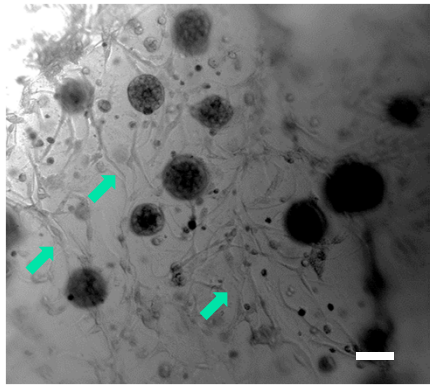
- **EMT phenomenon was observed in *in vitro* cervical tumor model established by three-dimensional printing of Hela cells from the following:**
  - **Morphology**
  - **Protein**
  - **Gene**
- **The down-regulation of epithelial marker E-cadherin was significant higher in 3D than 2D sample;**
- **The up-regulation of mesenchymal markers N-cadherin and snail were significant higher in 3D than 2D sample; however the up-regulation of vimentin in 3D and 2D samples were similar.**
- **These 3D printed cervical tumor model could be used for further cancer progression study: tumor metastasis in co-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**

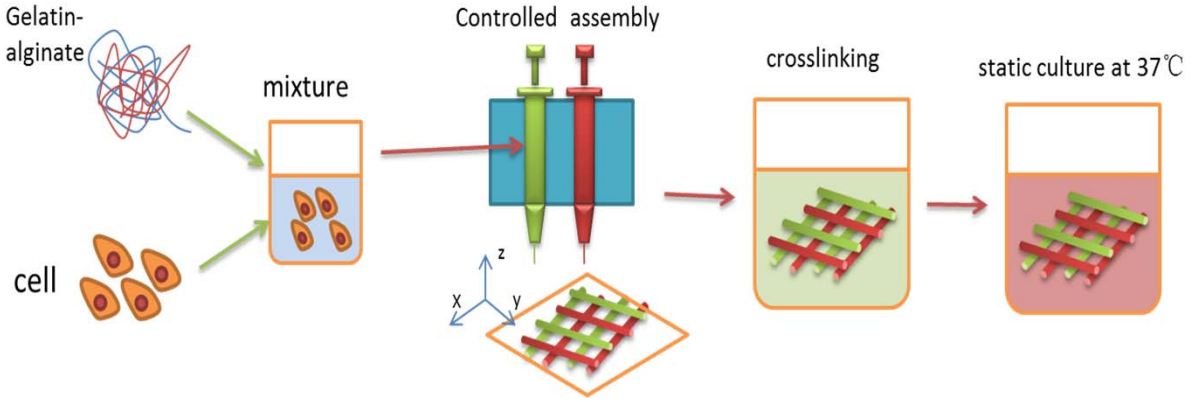


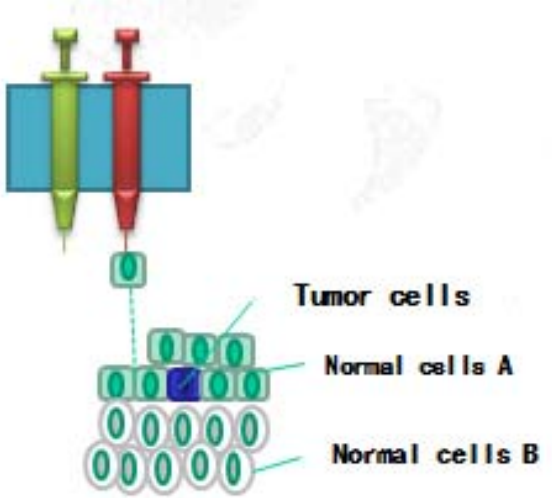
- **Clinical:**
  - **- for personalized cancer treatment**
  - **- cancer drug testing**

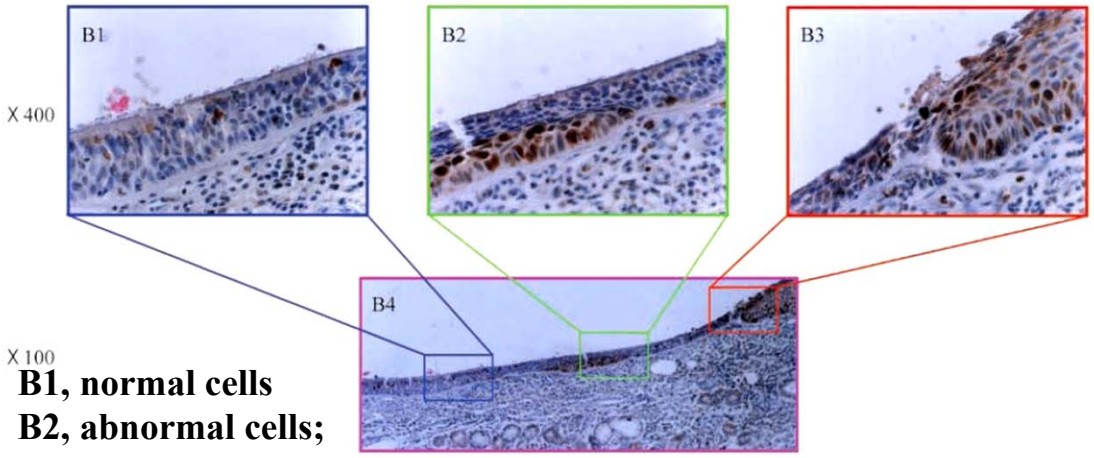


Assembly Cancer Cells for In Vitro Tumor Model

(NSFC 2012 E05 Key Research Project; Tsinghua)

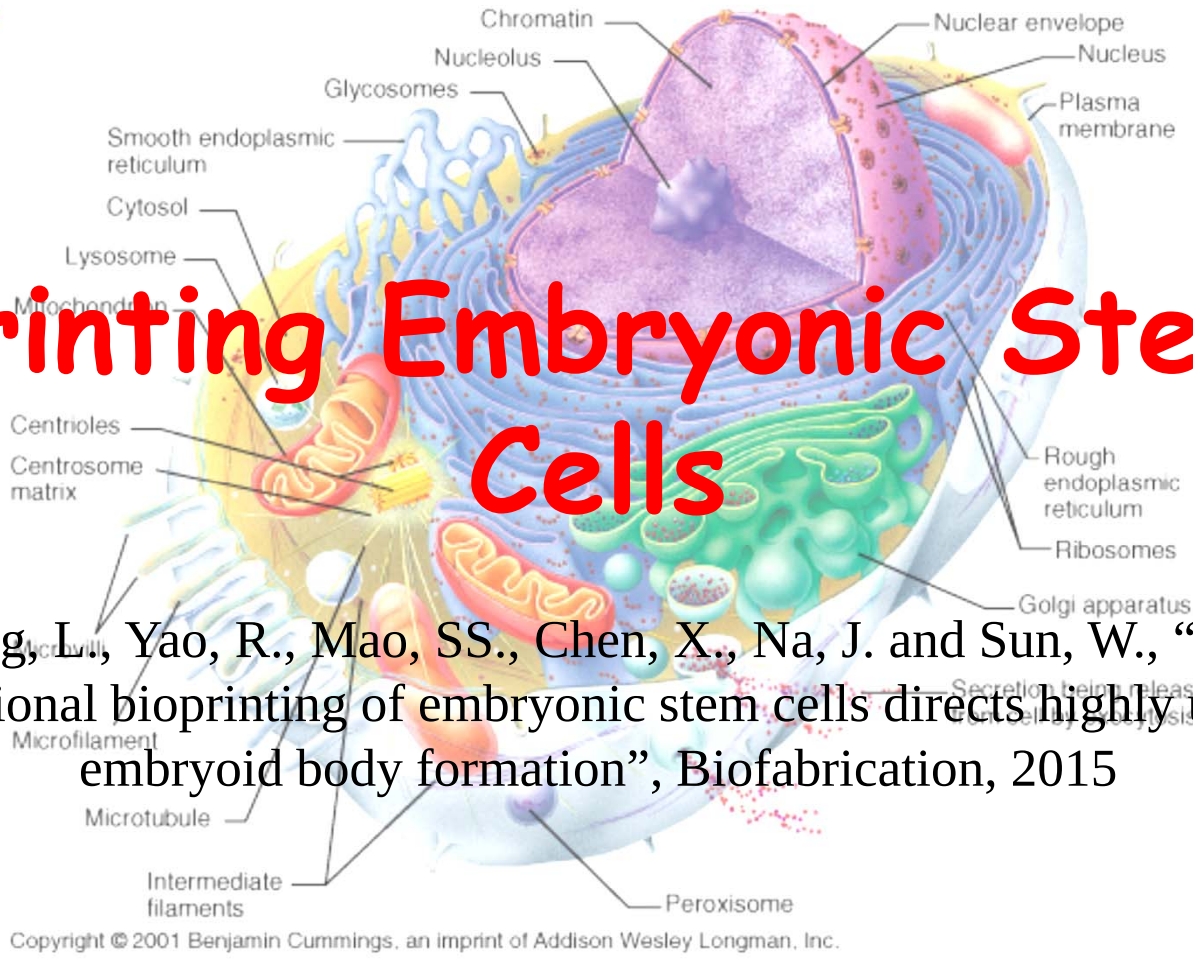






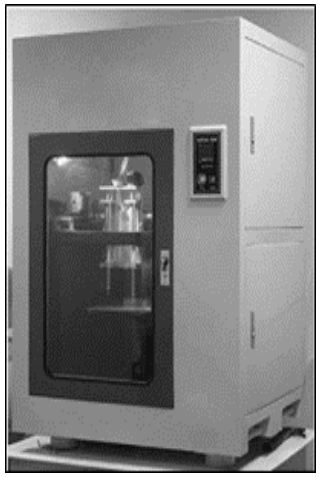
B1, normal cells
B2, abnormal cells;
B3, cancer cells;
Clinic Cancer Res 2006; 12(4): 1121.

Printing Embryonic Stem Cells

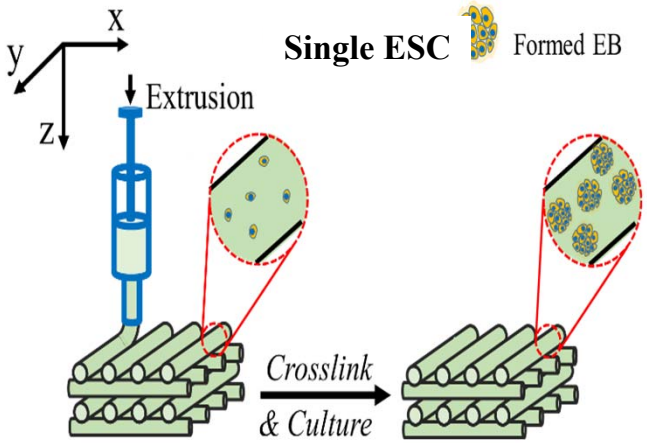


Ouyang, L., Yao, R., Mao, SS., Chen, X., Na, J. and Sun, W., "Three-dimensional bioprinting of embryonic stem cells directs highly uniform embryoid body formation", Biofabrication, 2015

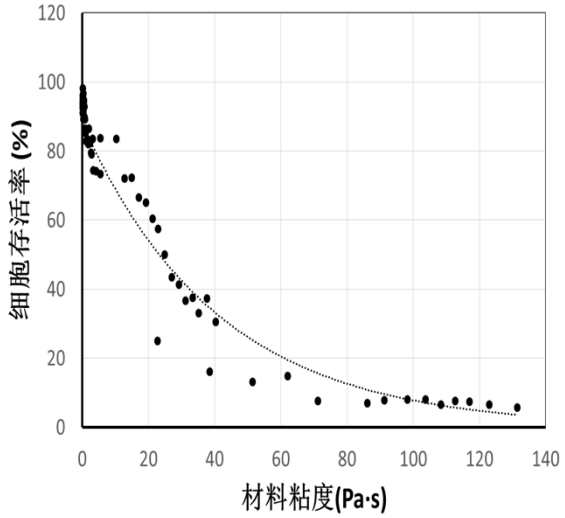
Printing Embryonic Stem Cells



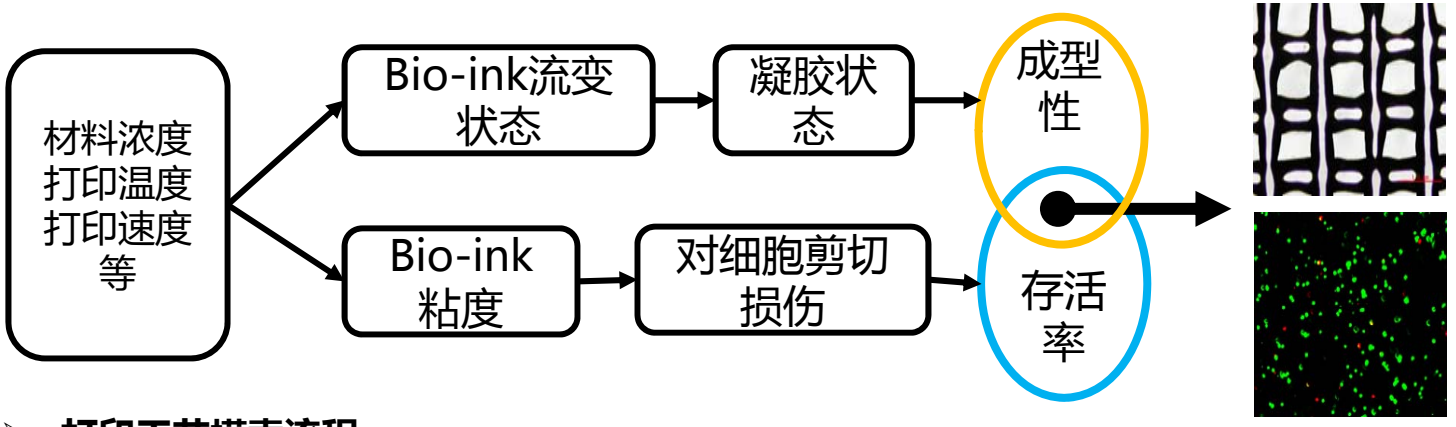
➢ 细胞三维打印机（清华大学）



➢ 打印示意图



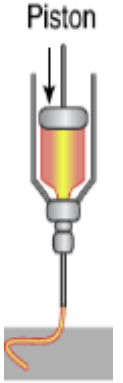
➢ mESC存活率随材料粘度变化



➢ 打印工艺摸索流程

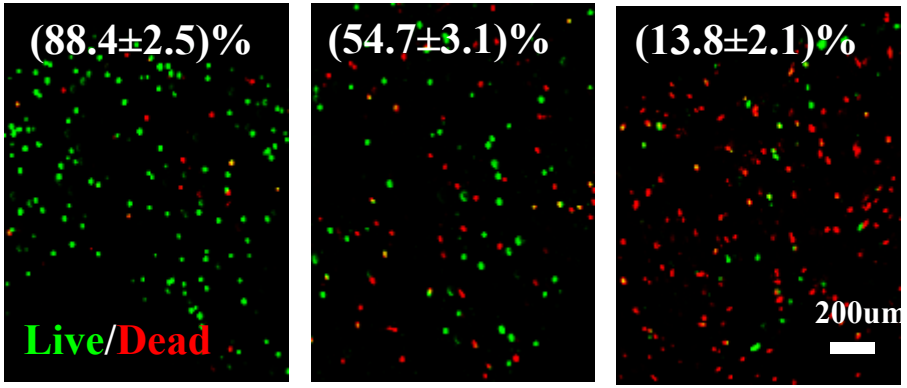
Printing Embryonic Stem Cells

- **Cell sensitivity with physicochemical stimulate**



The same printing parameters

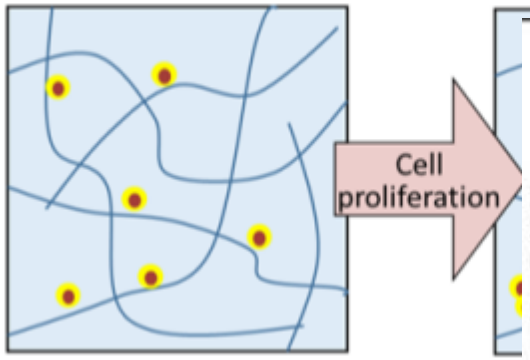
7.5%Gel+1%alg+1million cells/ml; chamber temp 22.5C;printing flux 3.4ul/s; printing speed 5mm/s

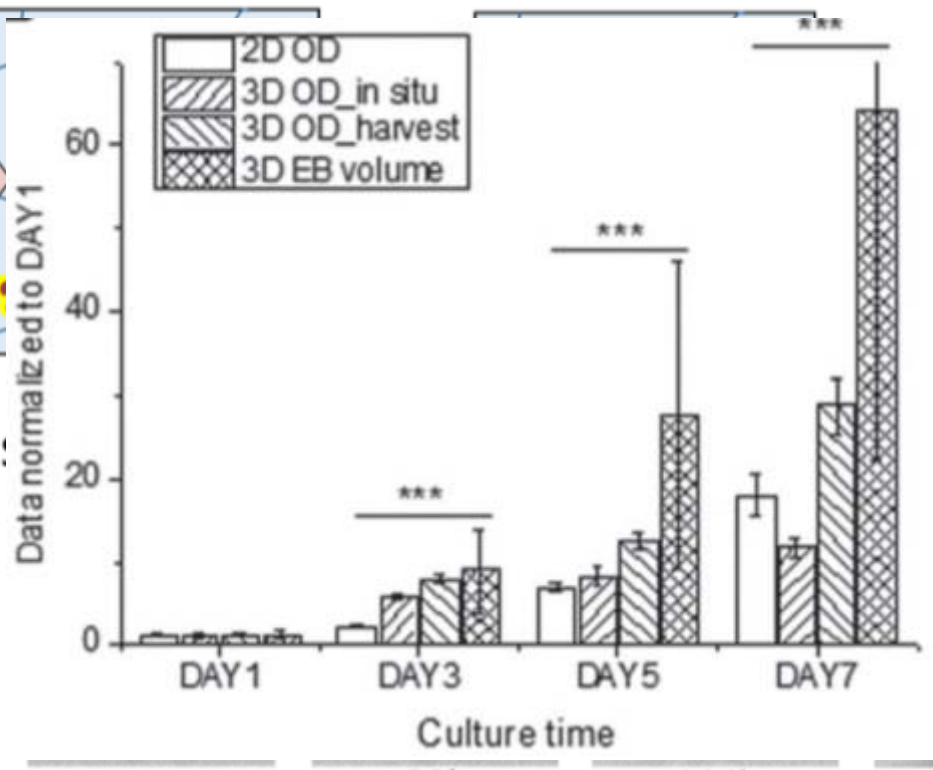


Hela Cell **C2C12 Cell** **ES Cells**

ESC proliferation and EB formation

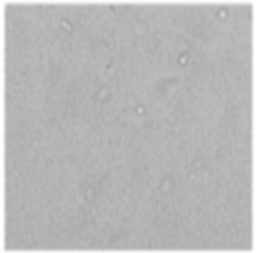
- EB formation within gels

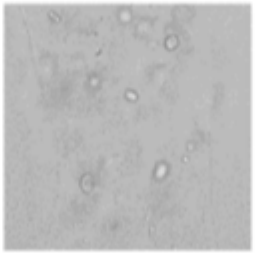




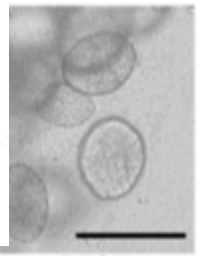
EB formation is mainly based on cell proliferation

- The actual proces





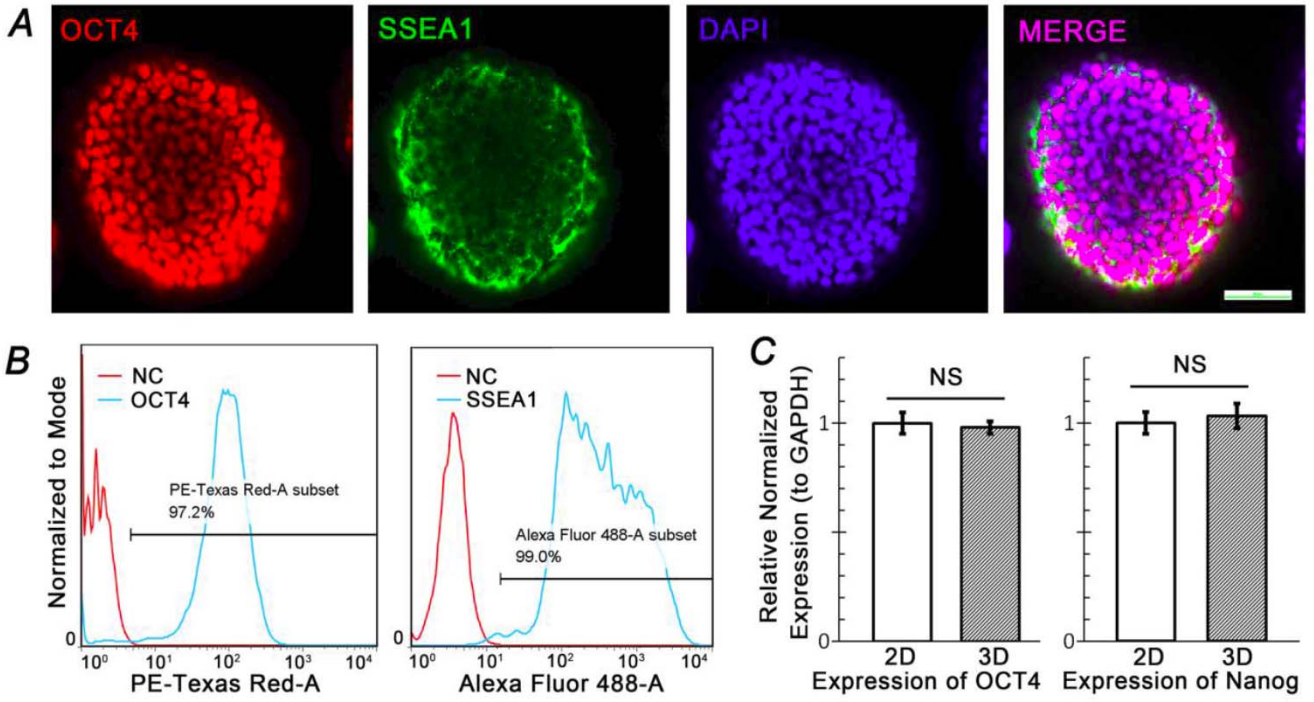
66h 90h 114h



Bar:200um

Maintenance of pluripotency

- **Nearly 100% cells maintain pluripotency**



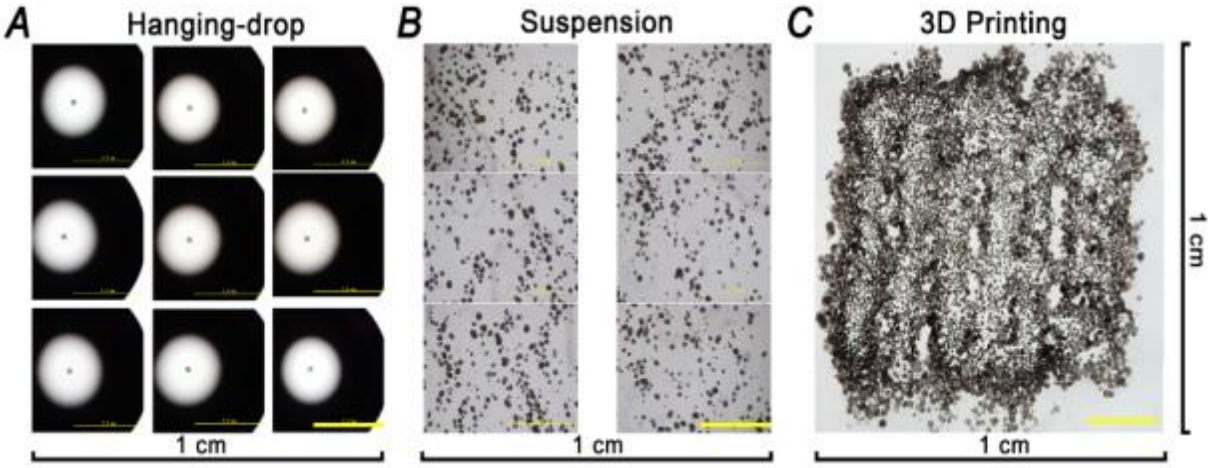
- **The maintenance of pluripotency after one week**

Bar:50um

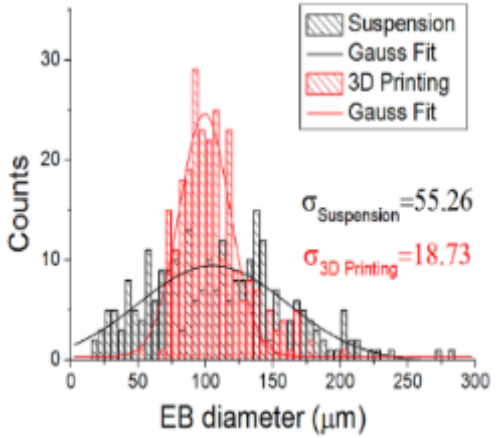
Comparison with other methods

- 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**



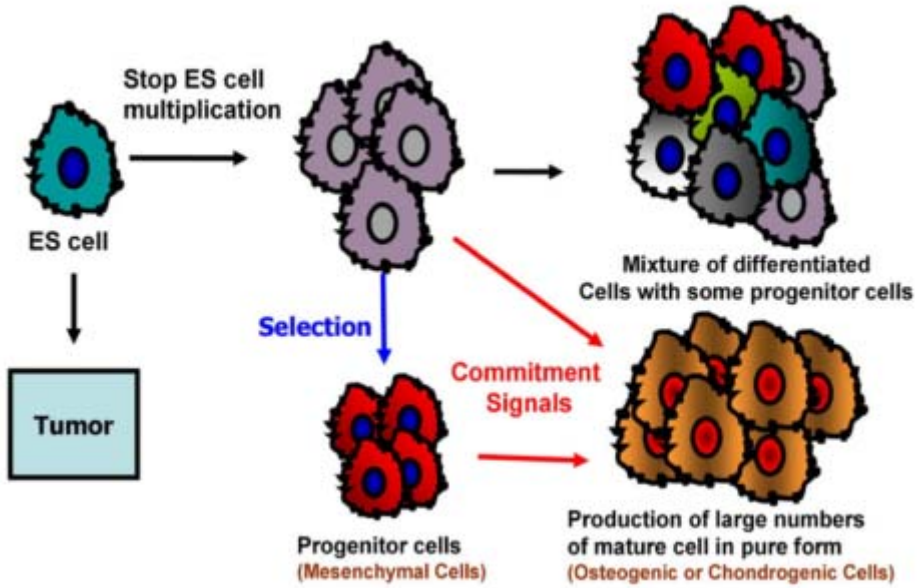
➢ **EB uniformity**



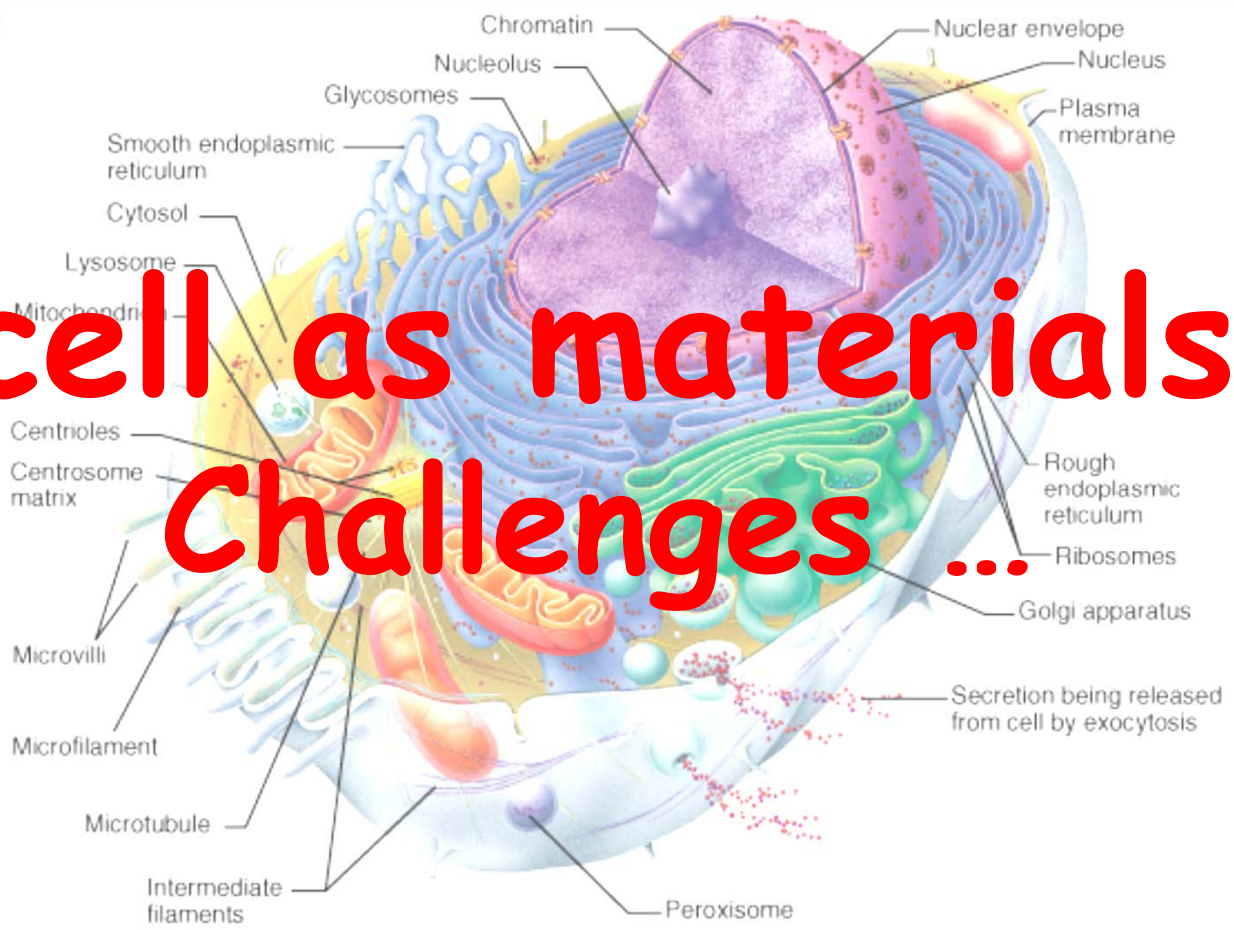
Ongoing work

- 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**

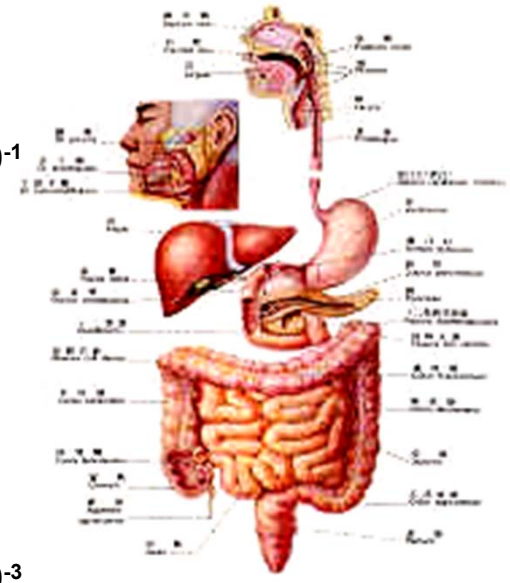


cell as materials Challenges …

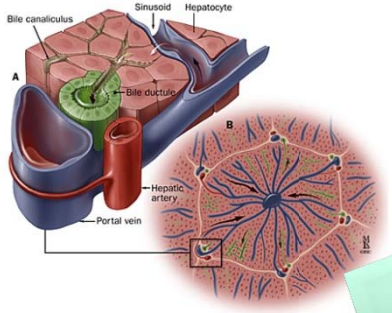


Unit：m

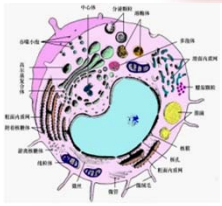
10^{-1}



10^{-3}



10^{-4}



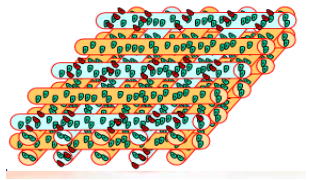
10^{-6}

10^{-9}

Cell/Tissue Printing for 3D in vitro Biological Model

Top-Down/Bottom-Up

3D Cell Printing for



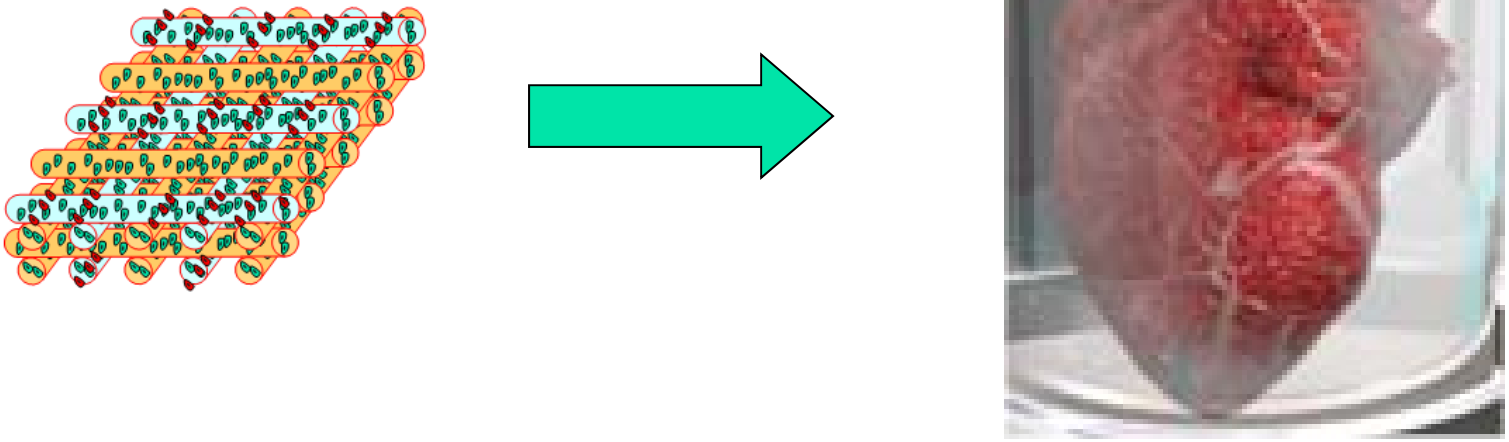
In vitro Biological Models

Developmental Biology

3D Cell Printing/Assembly

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

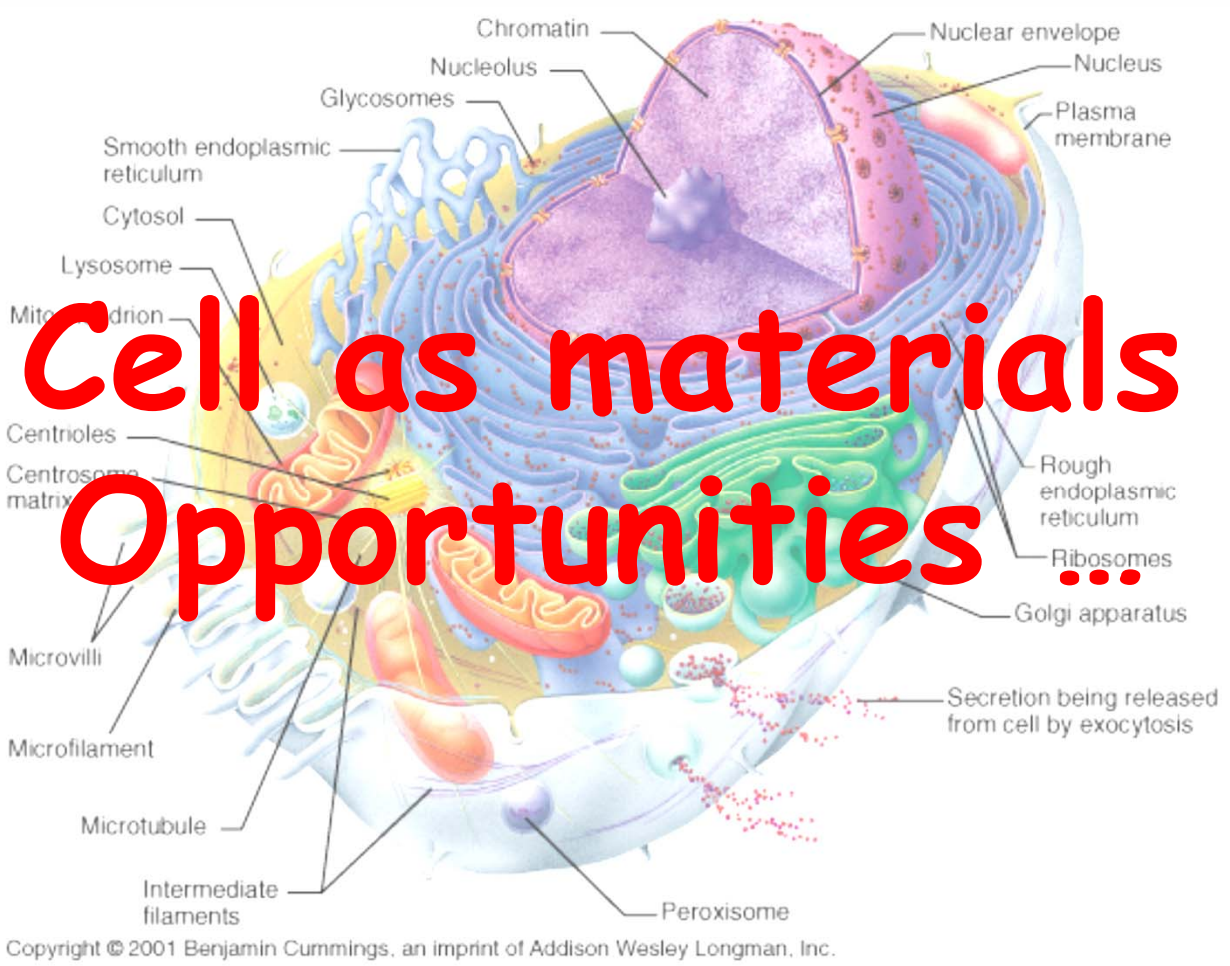
Structure and Function

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

Cell as materials Opportunities …



Evolving of Tissue Engineering

Tissue Science & Engineering*(2007)

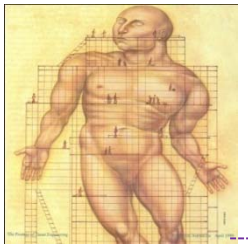
"The use of physical, chemical, biological, and engineering processes to control and direct the aggregate behavior of cells"

- Regenerative Medicine
  - more on cells, particularly on Stem Cells
- 3D Physiological or Disease Models
  - For better study disease pathogenesis and for developing molecular therapeutics
- Pharmacokinetic Models to replace animal testing
  - For drug screening and testing
- Cell/Tissue on Chip
  - For detection of bio/chemical threat agents

** Advancing Tissue Science & Engineering: A multi-angency strategic plan, June 2007*

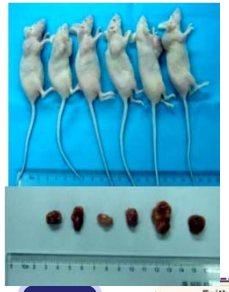
3DP In Vitro Model for Drug Testing

人体模型



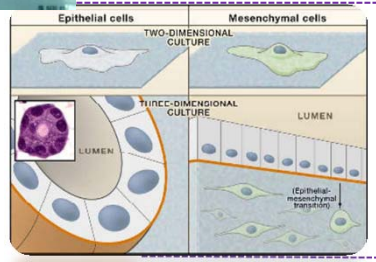
- **Limited human clinical trials**
- Not feasible for testing
- Ethic issues

动物模型



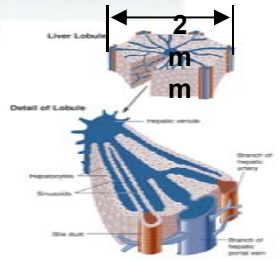
- Cell micro-environment different from human
- Different immune system
- **Different from human clinical trials**

二维模型



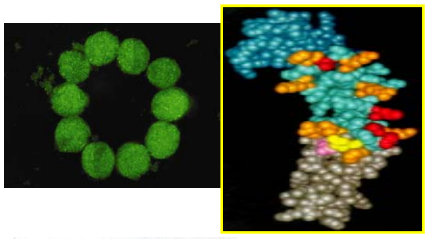
- Not a true physiological environment
- **Difficult to simulate 3D tissue**
- Not reliable to cancer drug testing

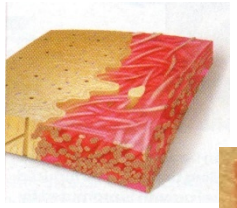
三维模型

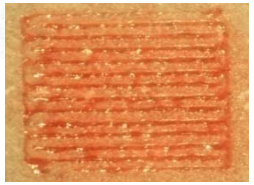


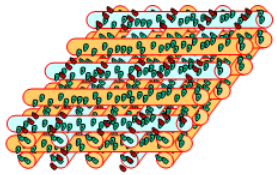
- **Simulated physiological model**
- **More close to 3D human tissue**
- **Reduce using animals**

The Needs of Bio-3DP:
Tissue/Organ Printing for Regenerative Medicine

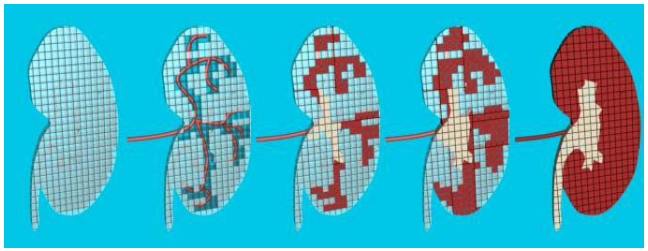


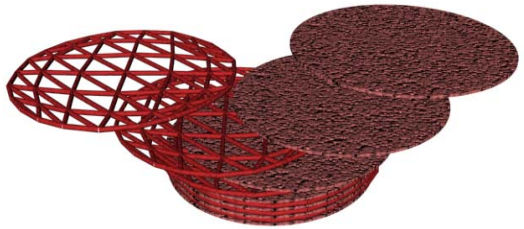




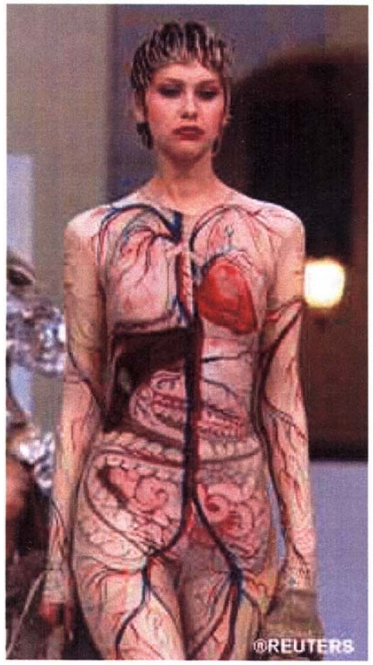


Cell/tissue printing & patterning





Biofabrication of tissue precursors and assembly

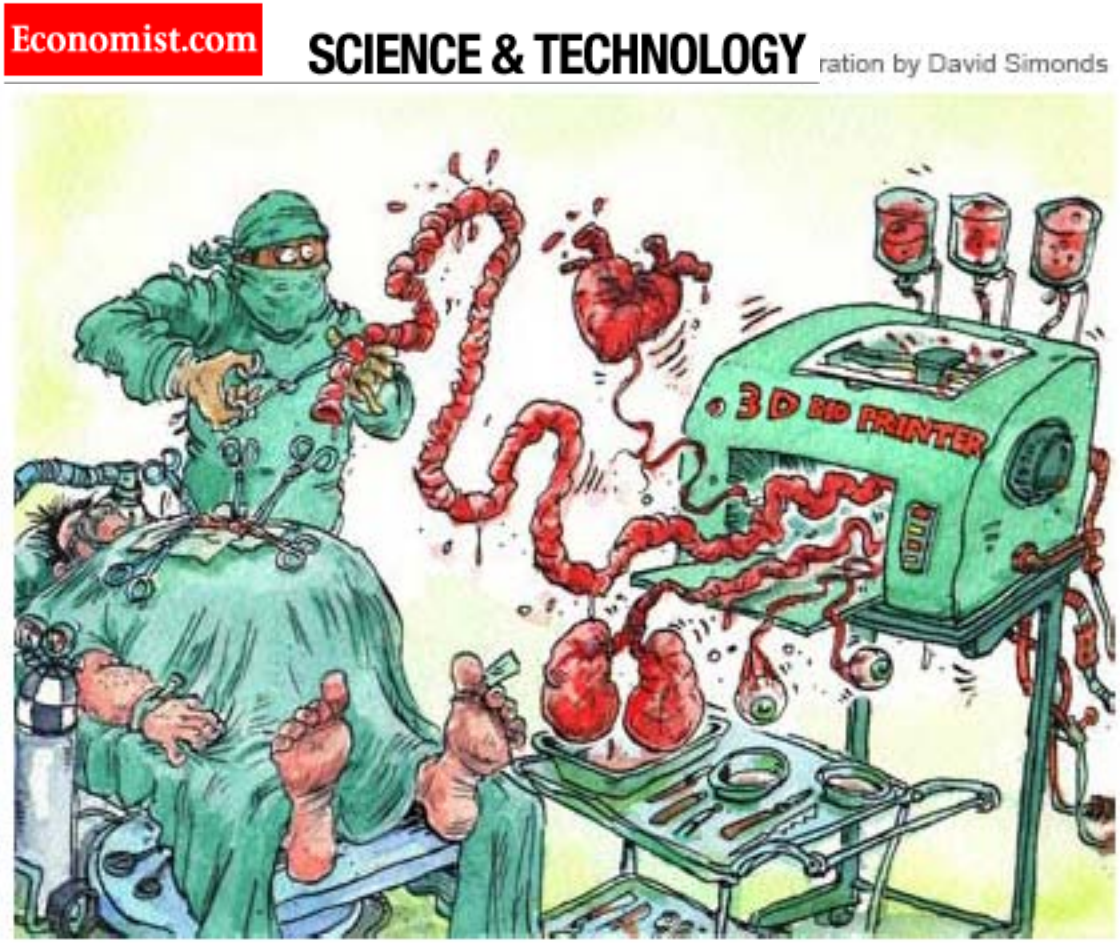


Organ printing

Image courtesy by Dr. V. Mironov

Sun, et al, BAB 2004, CAD 2005, Biofabrication, 2009

Printing Body Parts



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

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Collaborators

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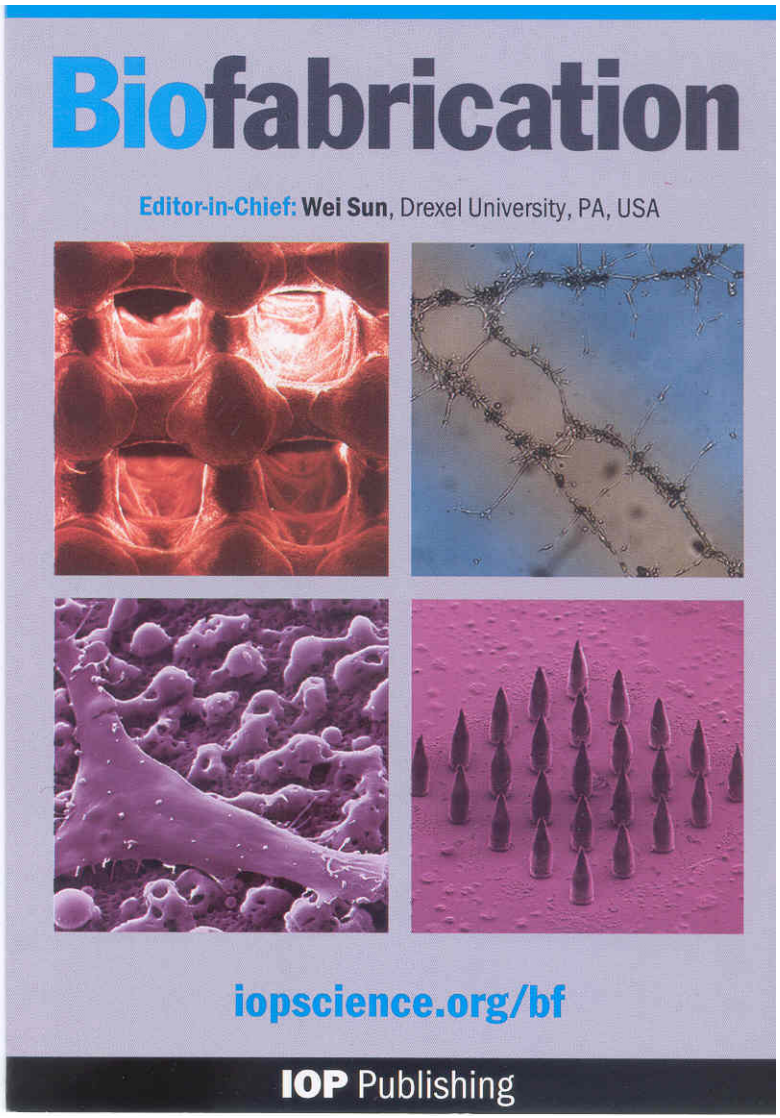
Thank you!

IOP Publishing

Biofabrication

Impact Factor: 4.302



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…..