Cell Printing

Post-Printing Considerations

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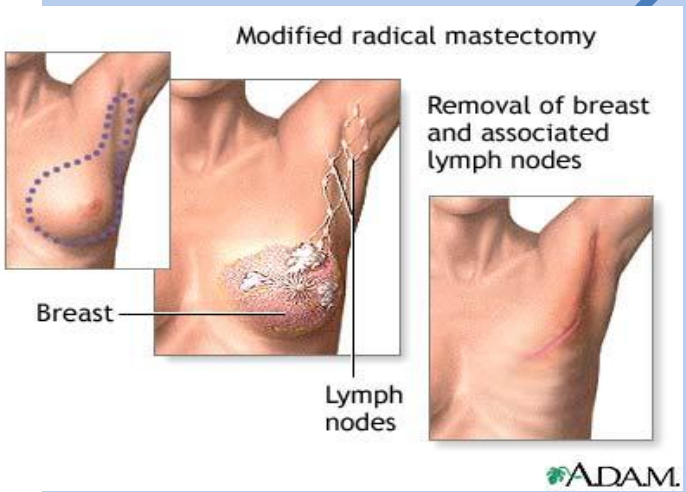
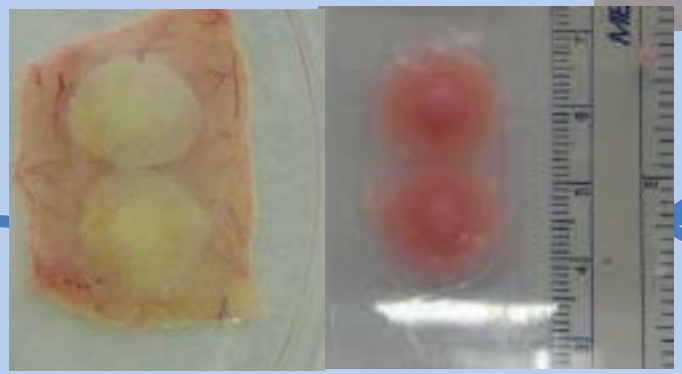
Co-founder & CSO, Tevido Biodevices, LLC

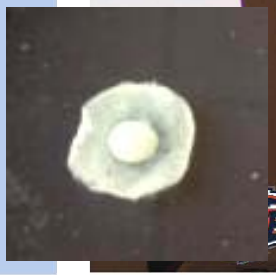
TeVido's *Nipple Areola Complex* Solution

TeVido BIODEVICES

Custom bio-printed grafts made from autologous cells



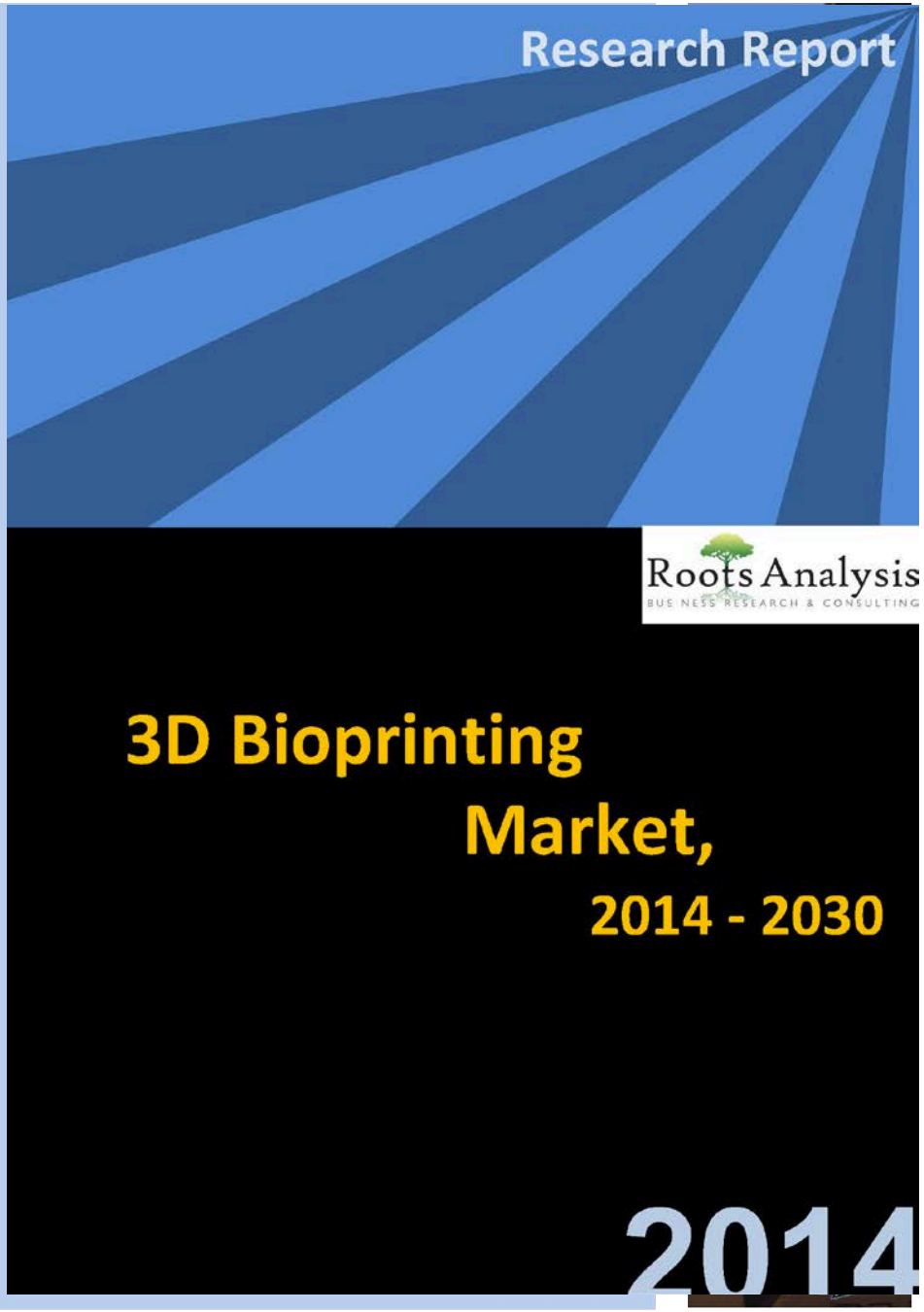




<u>3D bioprinting market</u>

$ 615 M within 10 years

$10 B by 2030

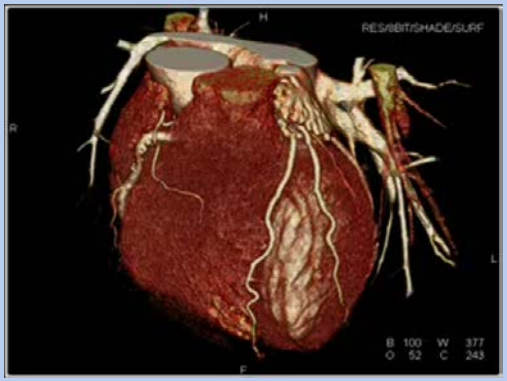


Organ Printing

Medical Imaging

(CT, MRI, 3D digitizer)

Virtual 3D image



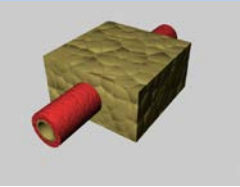
Regenerative Medicine

(Cell sources, growth factors, time dependence)

Organ Blueprint

Development

Virtual Organ



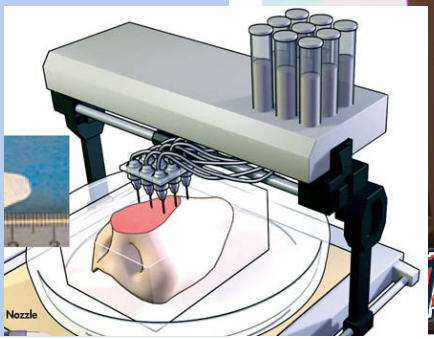
Sun, et al, BAB 2004 & CAD 2005

Biofabrication

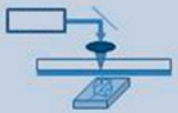
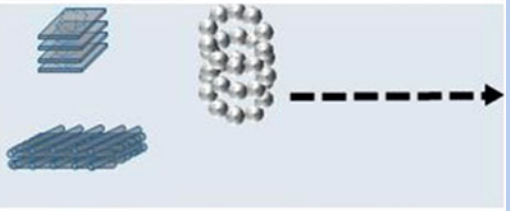
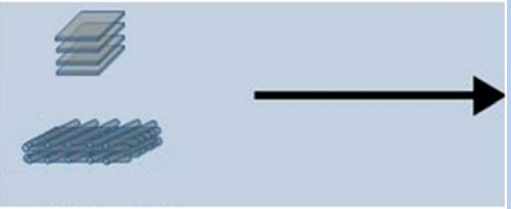
Biomaterials

(Hydrogels, ECM, Cells, growth factors)

Engineered Organ



Printing Tools

Printing tool	Capabilities
Laser Induced forward transfer 	
Inkjet	
Electrospray	
Extrusion pen	
Photopolymerization	

Continuous

Discrete

Low res

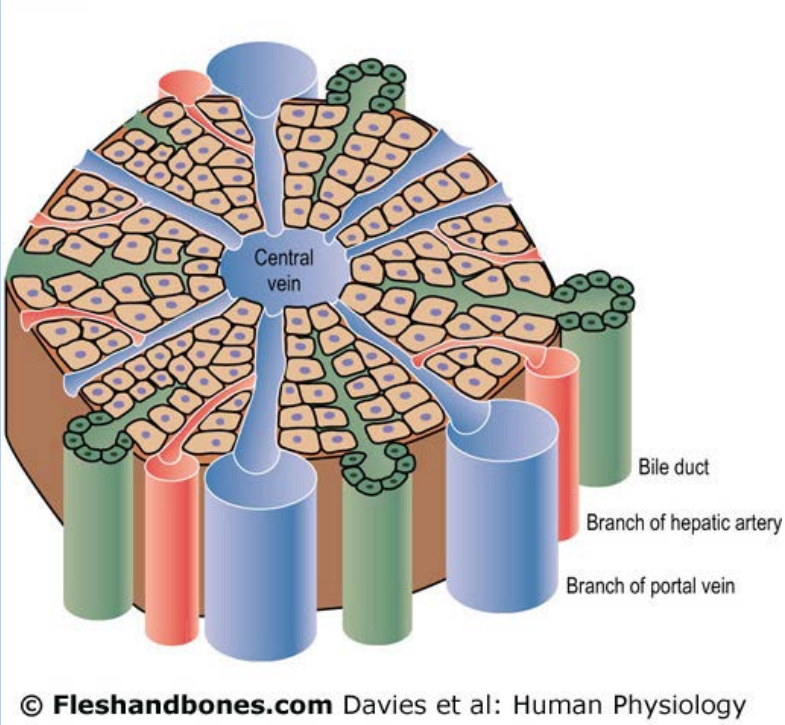
High res

From: Ringeisen et al. MRS Bulletin 2013

Why Inkjet Printing

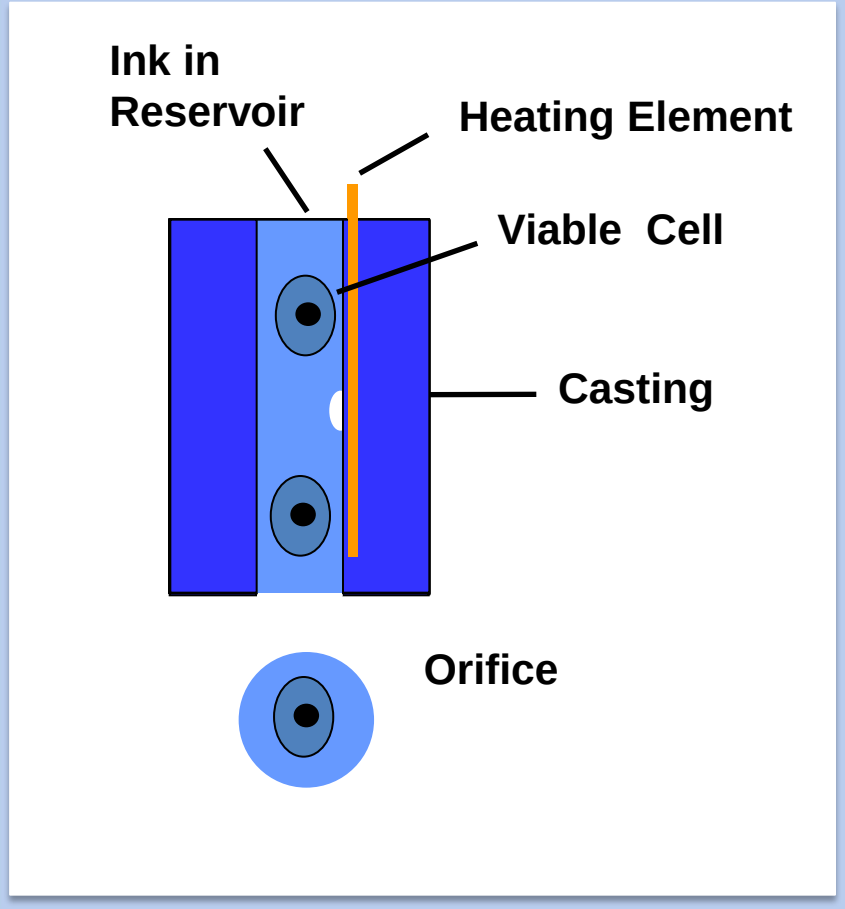
- Non contact technique
  - Drop-on-demand
- Rapid
  - >100,000,000 drops per second
- Quantitative
- Fully programmable
- Transportable
- Orifice/drop size 20µ to 60µ
- Many different inks

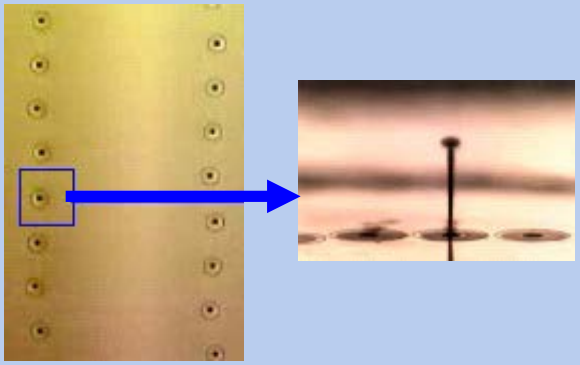
Structure of Liver

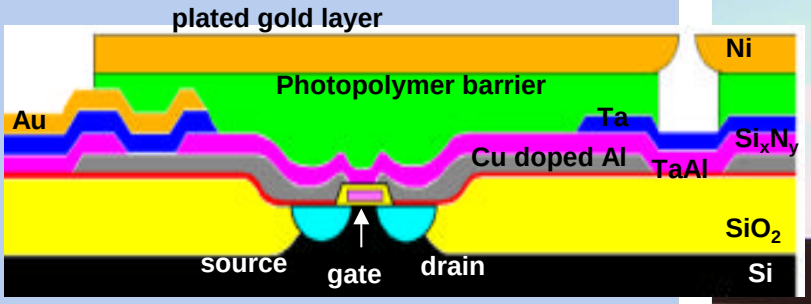


Thermal Inkjet

Print head



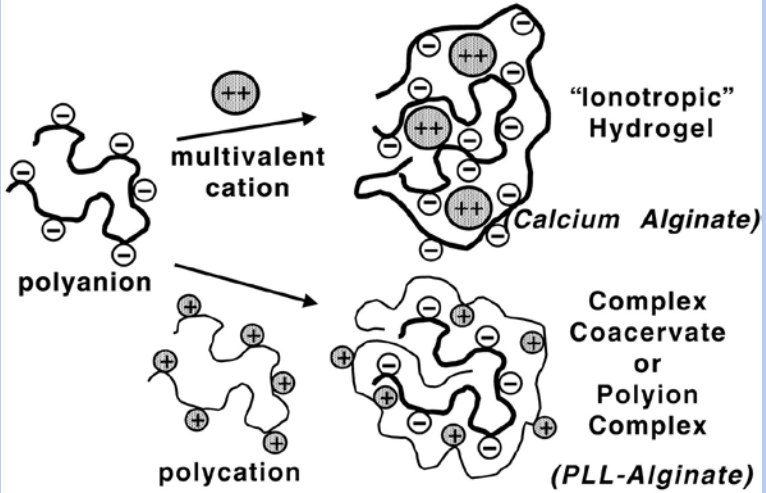


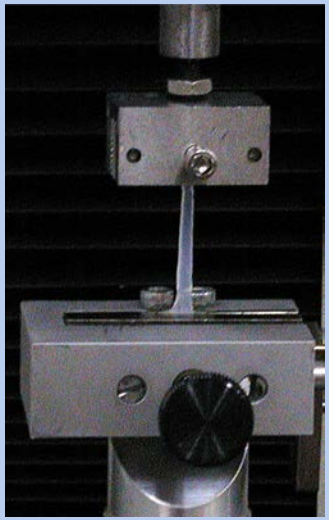


From: HP technotes

Physical Crosslinkers and Polymers

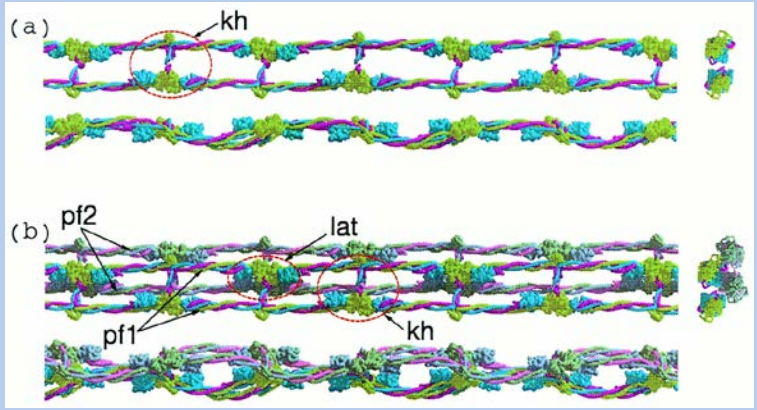
- Polyanions and (poly) cations
  - E.g. alginates

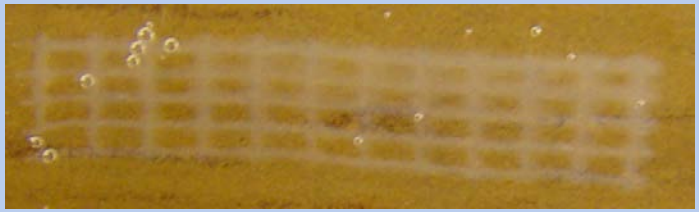


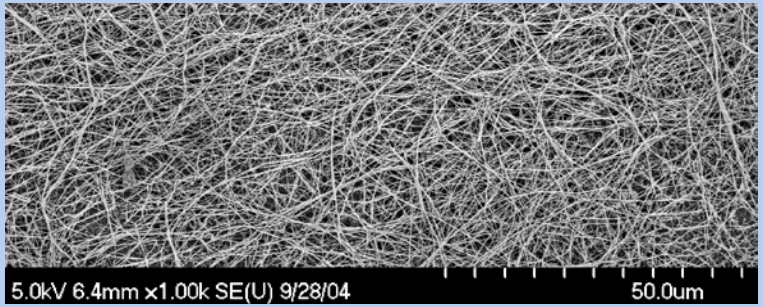


Self-Assembling Precursors

- Fibrinogen and thrombin

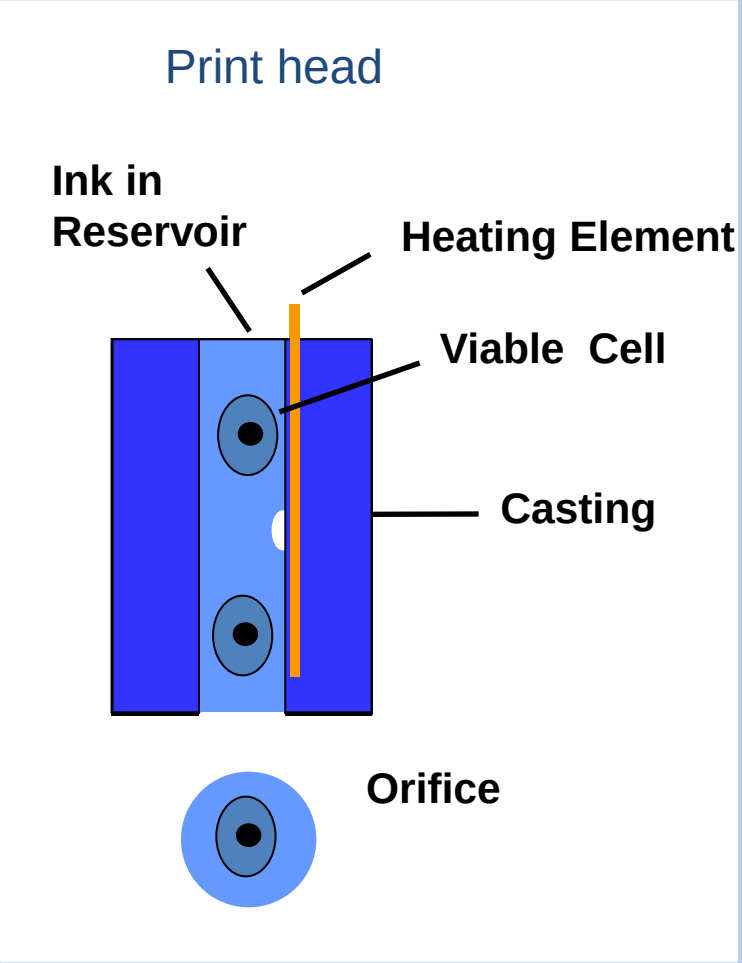






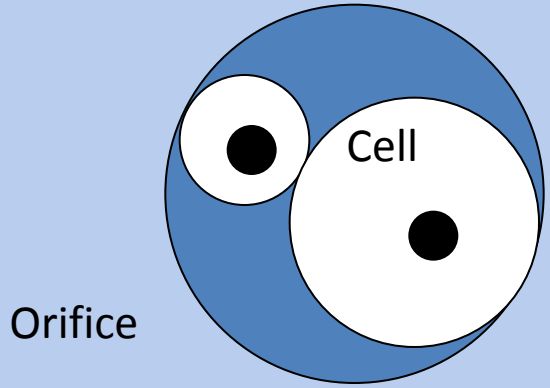
Inks Containing Cells

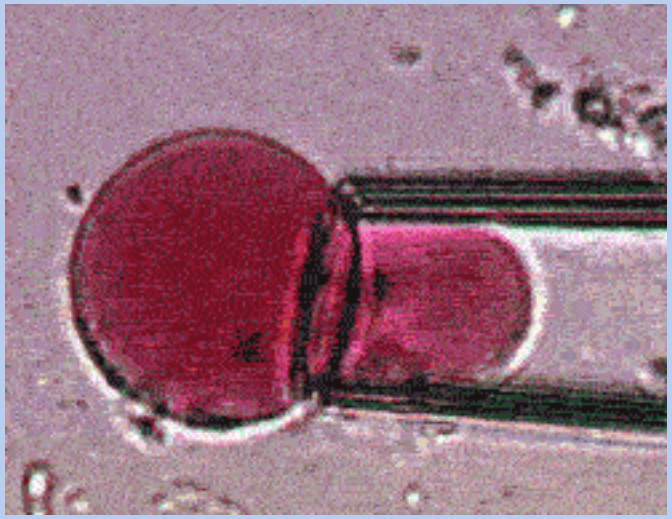
- Size concerns
- Enumeration
- Viability
- Support
- Fabrication



Cell Concentration vs Orifice Diameter

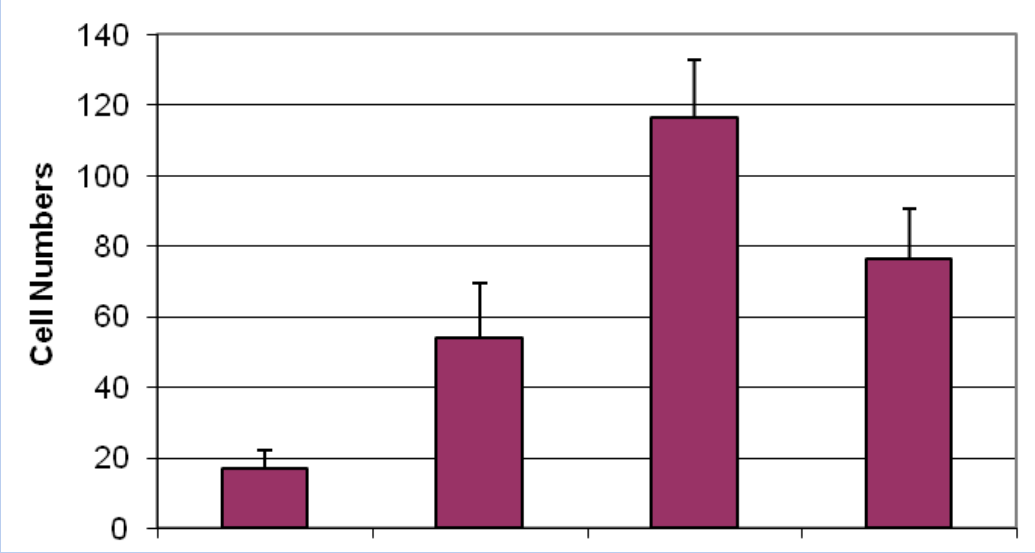
- Preferred concentration @ 1 cells/drop
- Cell size < Orifice diameter < 2x Cell size
- HP #26 Cartridge
  - Orifice size 50 microns
  - $8x10^6$ cells/ml = 1 cell/drop





Relation between ejected Cells and Cell Concentration

120 drops of ink were printed,
Cells were counted with a Coulter counter



Orifice size: 50 µm
Cell diameter: 30 µm
Drop volume 120 pl

Cell concentration, 10^6 cells/ml

Cui, X., Dean, D., Ruggeri, Z., & Boland, T., Biotechnology and bioengineering 106(6):963-9. 2010

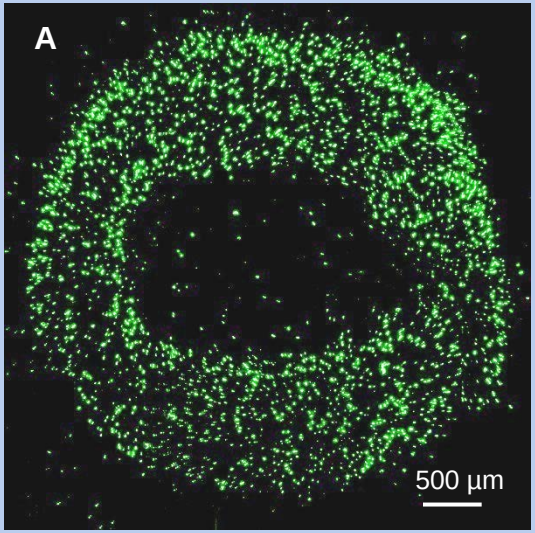
Printing of Primary Neurons

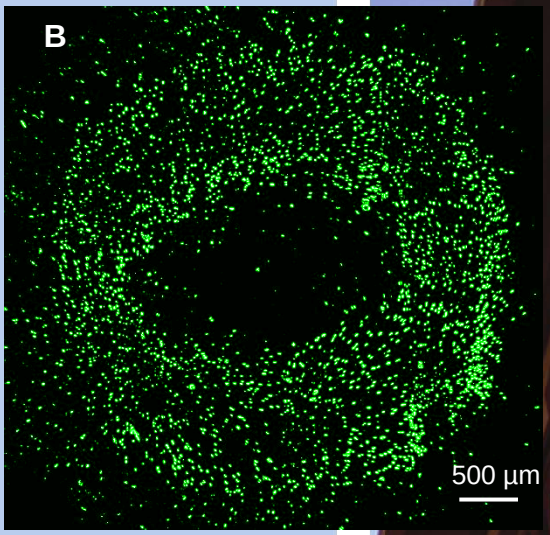
Anchorage dependent

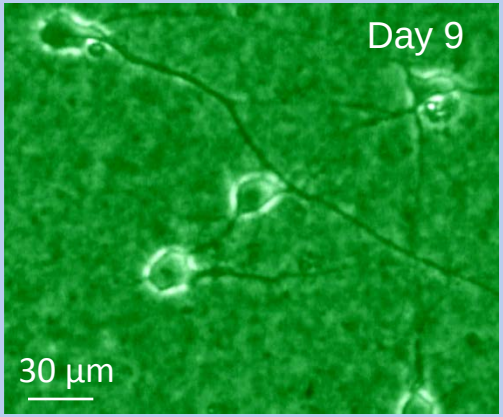
Cortical neurons

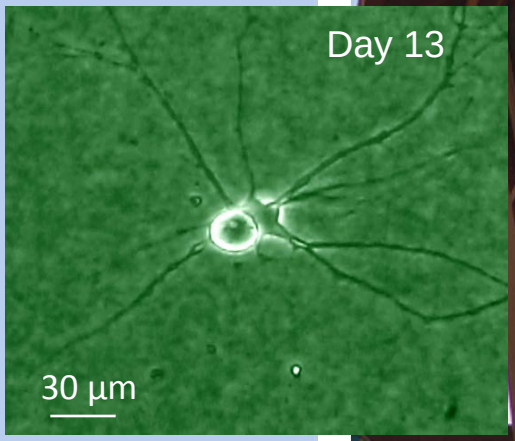
Hippocampal neurons

Phase contrast





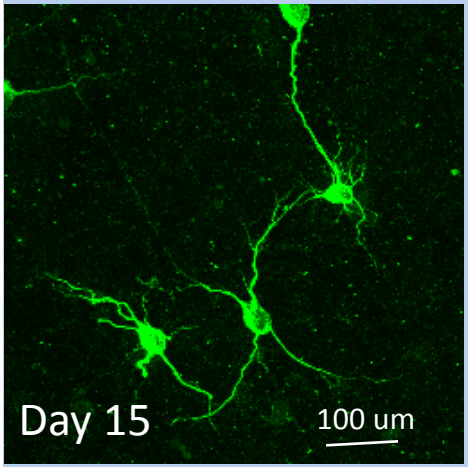


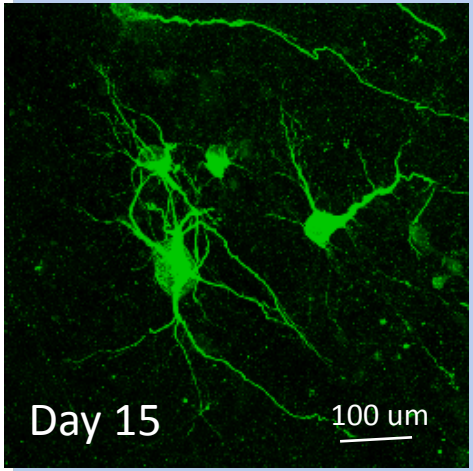


Xu T, Jin J, Gregory C, et al. Biomaterials 26 (2005)

Immunoassaying of Printed Neurons

Cortical Neurons

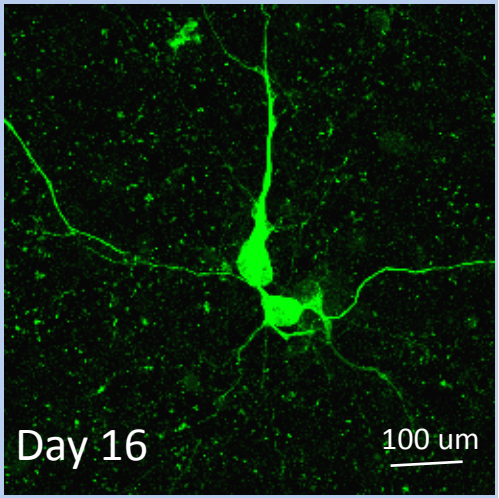


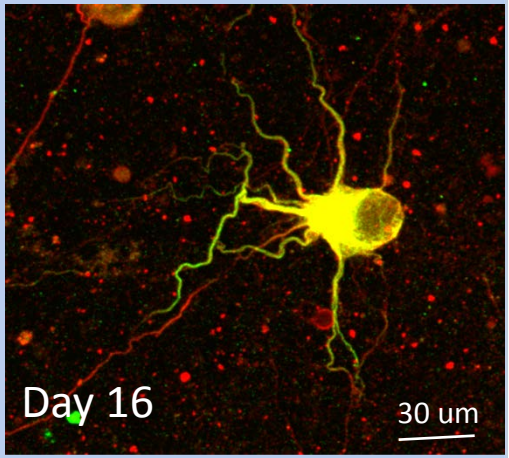


Neuron-specific markers:

- **Anti-MAP2 Ab:**
  Cell body and dendrites (green)
- **Anti-Neruofilament Ab:**
  Cell axons (red)

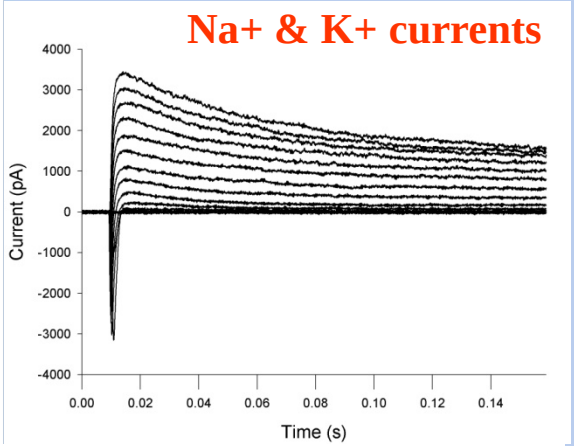
Hippocampal Neurons

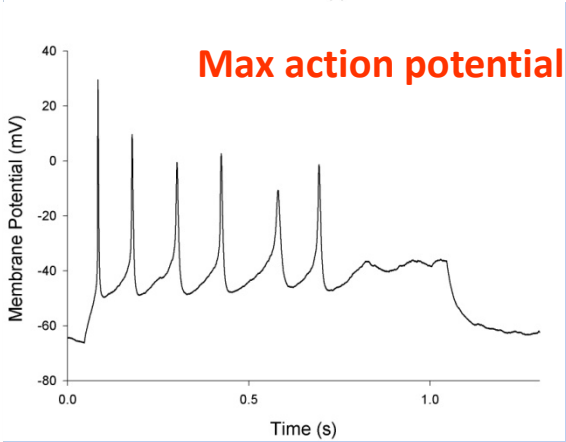




Xu T, et al. Biomaterials (2006)

Electrophysiology of Printed Neurons



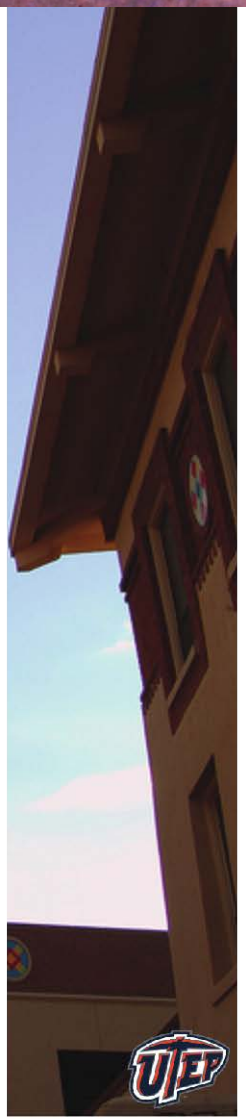


Hippocampal neurons (Day16)

Membrane properties of hippocampal cells at day 15				
	Printed	STD	Control	STD
VM (mV)	-35.7	3.6	-35.78	5.76
RN (MΩ)	382	185	220	92
CM (pF)	45.8	16.3	32.44	16.67
L τ0/τ1	1.80	0.31	1.86	0.45
τ0(ms)	22.74	8.73	11.11	5.26
TVCi1 (ms)			.01	0.4
Na current (p			455	897
K current (pA			439	709
Vthr(mv)			7.69	4.6
Ithr(pA)			108	22.8
Firing type	1.22	1.86	1.86	1.46
Max firing (Hz)	2.44	2.24	3.71	5.62
AP Ampl. (mV)	18.8	8.4	13.09	12.86
AP Dur(ms)	2.95	0.77	5.08	1.95
AHP Ampl. (mV)	-4.72	0.57	-4.08	0.97
AHP Dur.(ms)	74.5	31.7	59.39	19.51
n	9		9	

No statistical difference

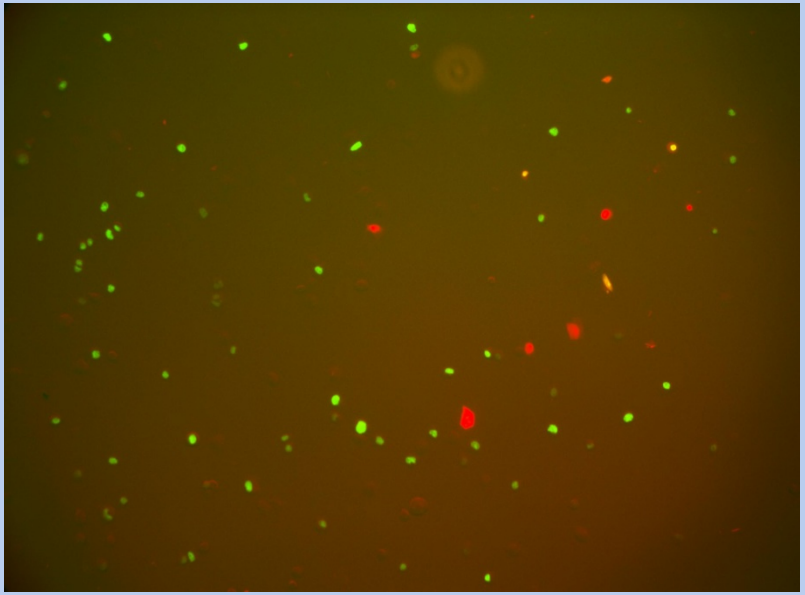
(P > 0.05)

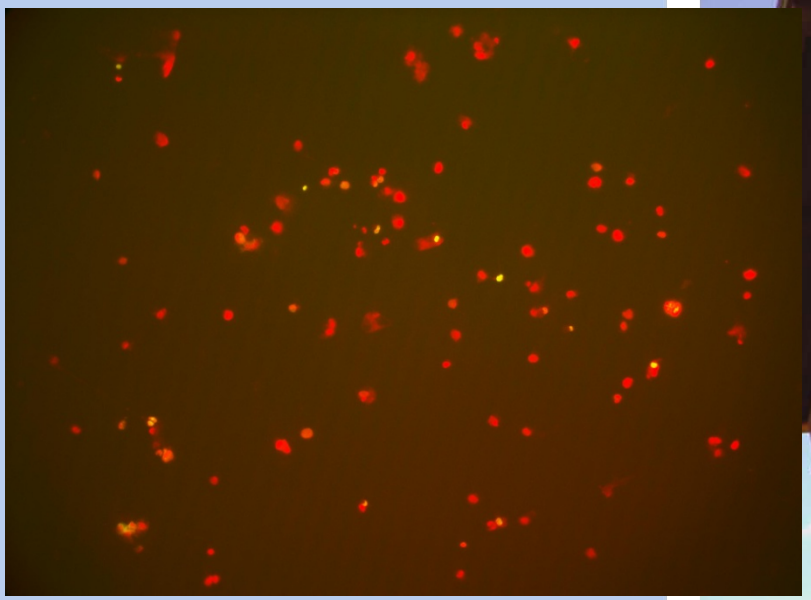


Xu T, et al. Biomaterials (2006)

Cell Membrane Porosity

Dye: Propidium iodide, MW ~700





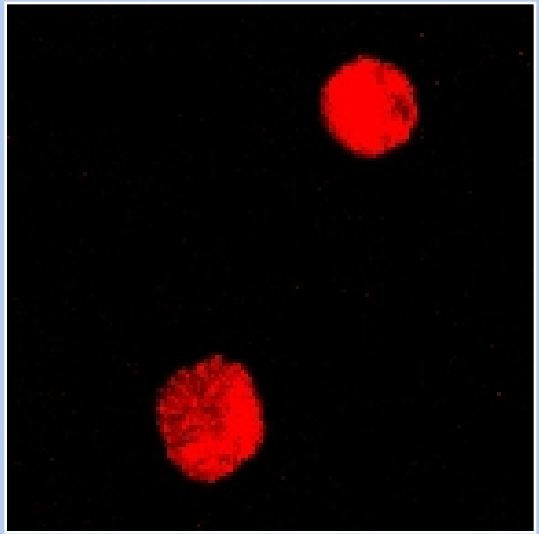
Control cells
5% pick up the dye

Printed cells
99% pick up the dye

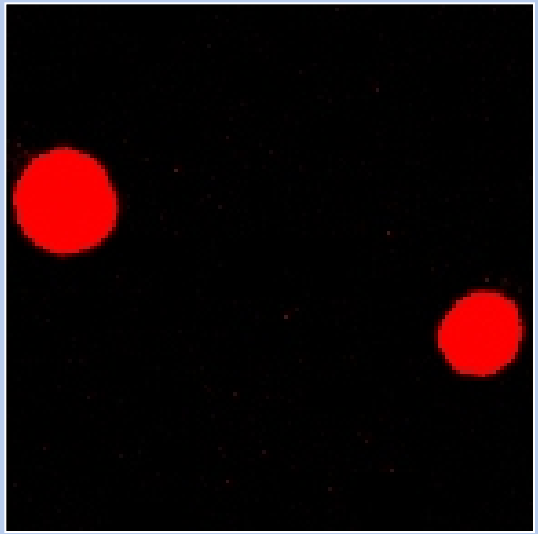
Cui, X., Dean, D., Ruggeri, Z., & Boland, T., Biotechnology and bioengineering 106(6):963-9. 2010

Cell Membrane Porosity

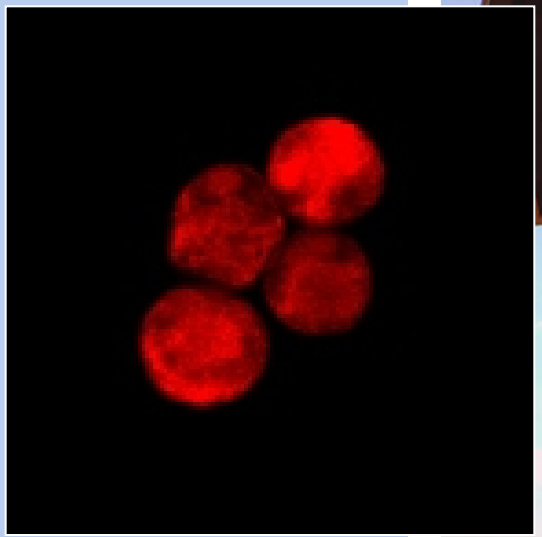
Dye: Dextran
Time: 15 min after printing



MW ~3,000



MW ~10,000



MW ~40,000

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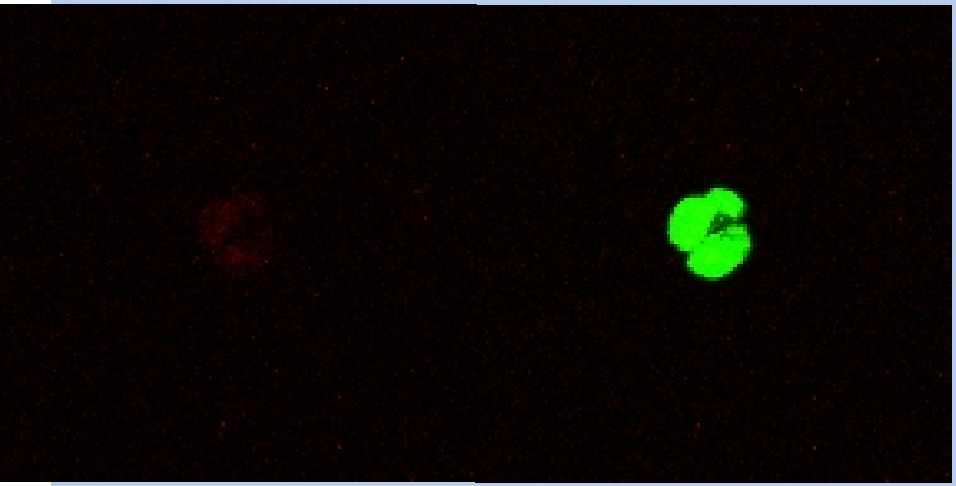
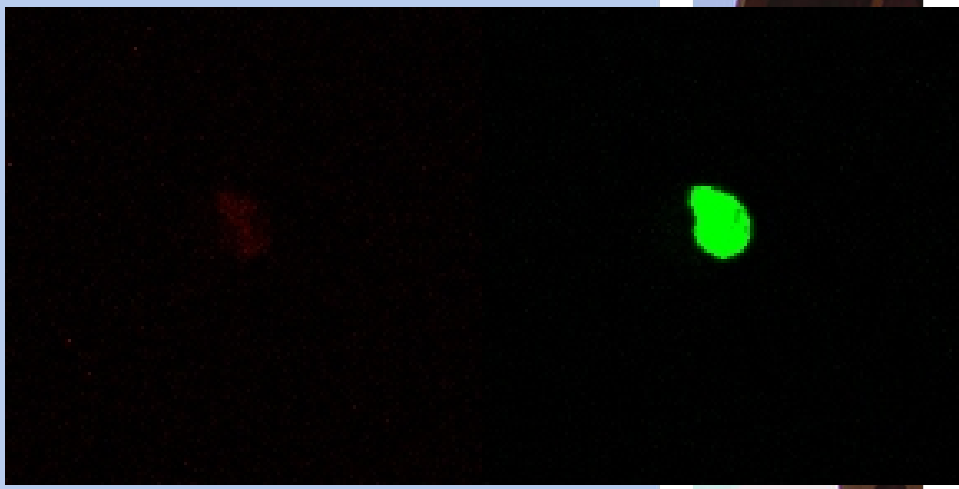
Cell Membrane Porosity

Dye: Dextran
Time: 15 min after printing





MW ~70,000

MW ~3,000

Control Cells

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Pore Transients

Time after Printing	PI (16Å)	Dextran 3000 (28Å)	Dextran 10000 (46Å)	Dextran 40000 (90Å)	Dextran 70000 (120Å)
0.25 hour	□	□	□	□	X
1 hour	□	□	X	X	X
1.5 hours	□	X	X	X	X
2 hours	X	X	X	X	X

□: molecules entered cells; X: molecules didn't enter cells

Apoptosis

- Measured by APO-BrdU TUNEL Assay kit (Invitrogen)

- Apoptotic ratio
  - Printed cells: 3.5 ± 1.3%
  - Pipetted cells: 3.2 ± 1.6%
  - Not significantly different

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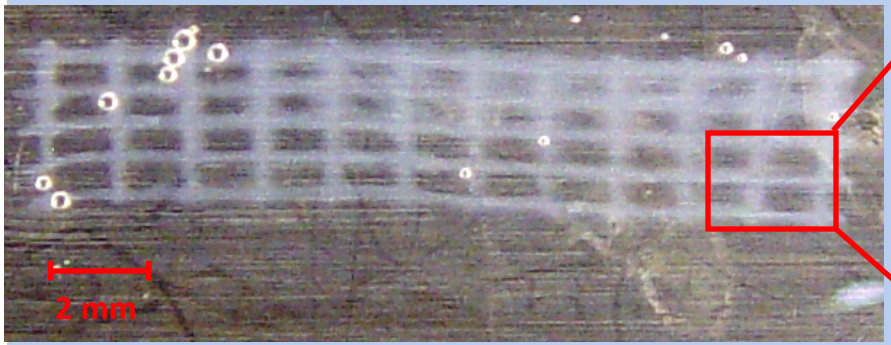
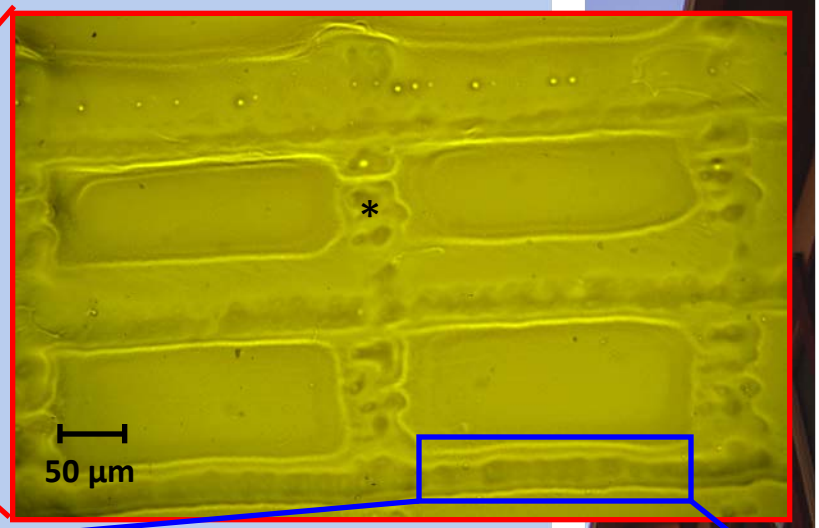
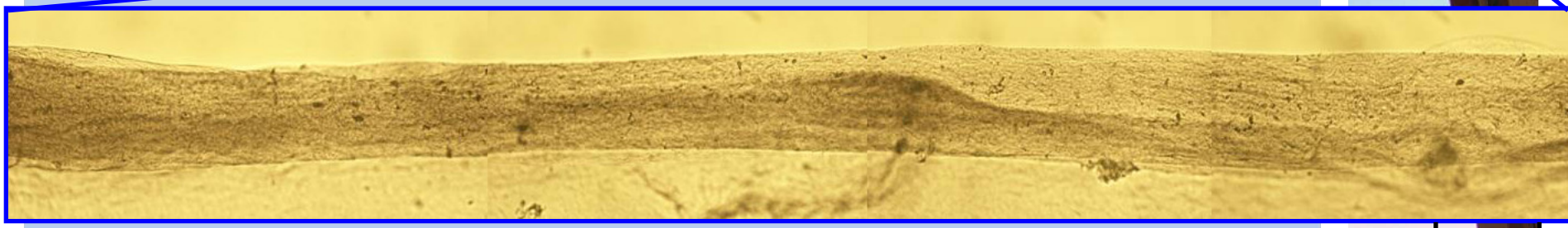
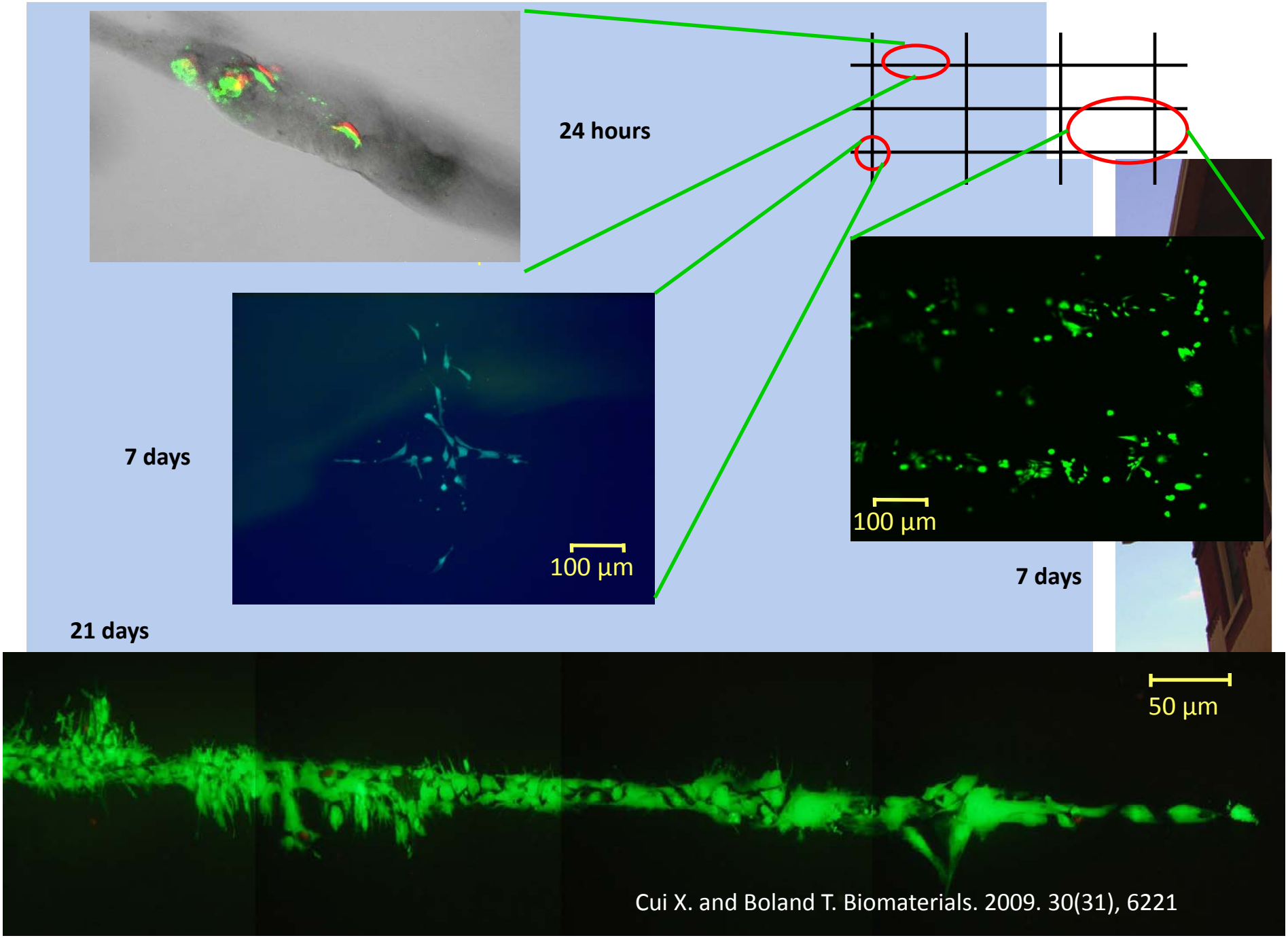
Light Microscope Characterizations





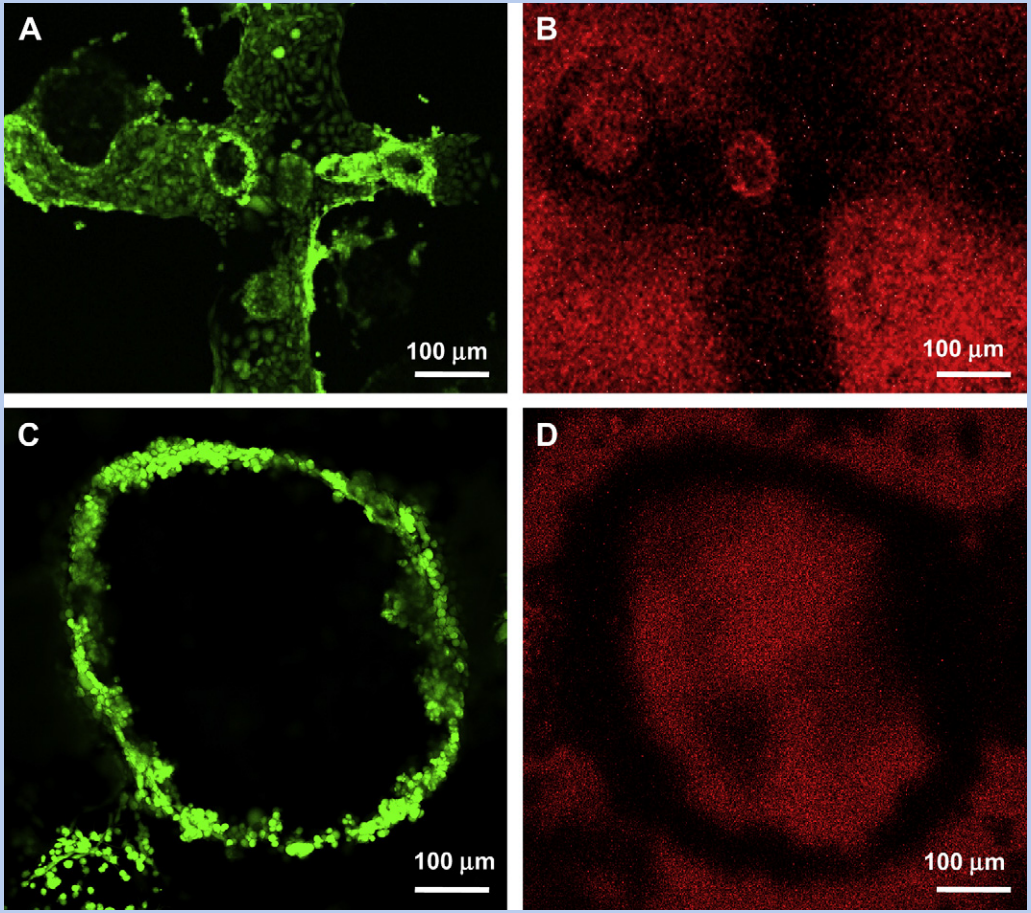


Cui X. and Boland T. Biomaterials. 2009. 30(31), 6221



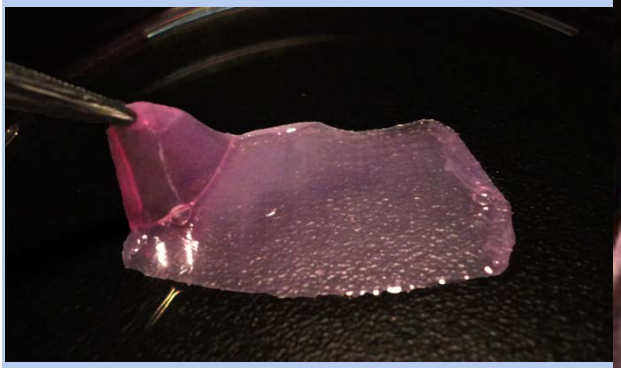
Cui X. and Boland T. Biomaterials. 2009. 30(31), 6221

Printed Microvasculature Channel Integrity (14 Days)

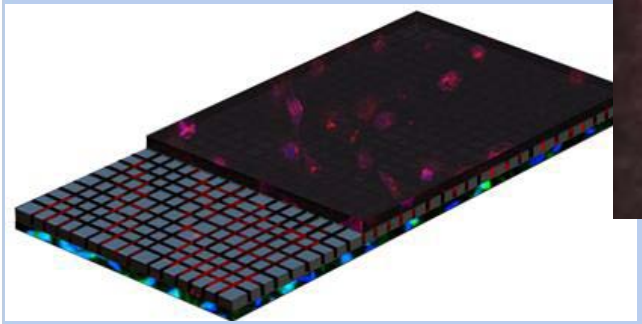


Cui X. and Boland T. Biomaterials. 2009. 30(31), 6221

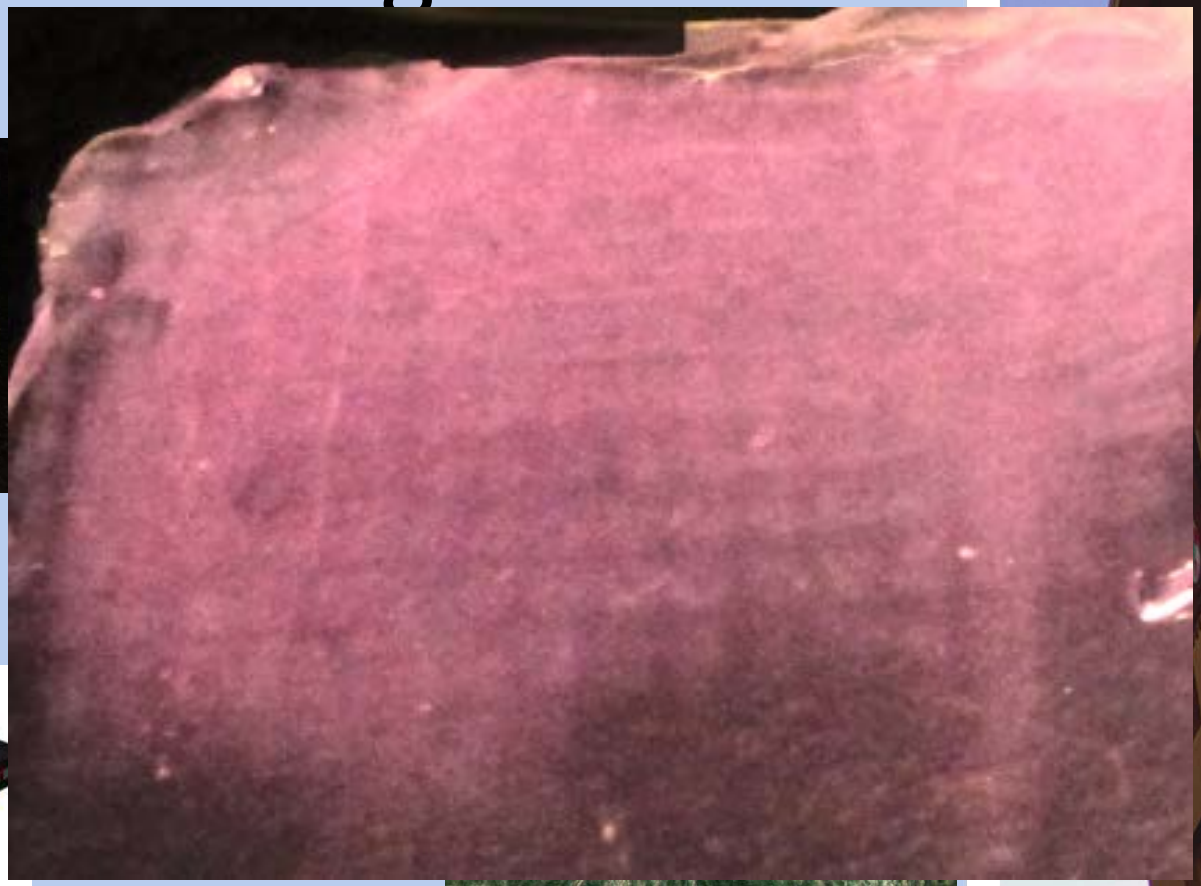
Applications: Printing Skin

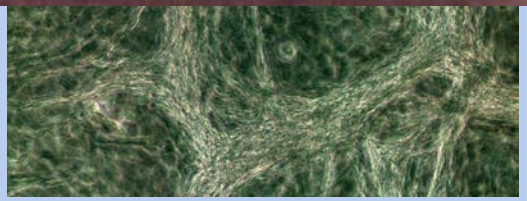


Printed immediately after fabrication

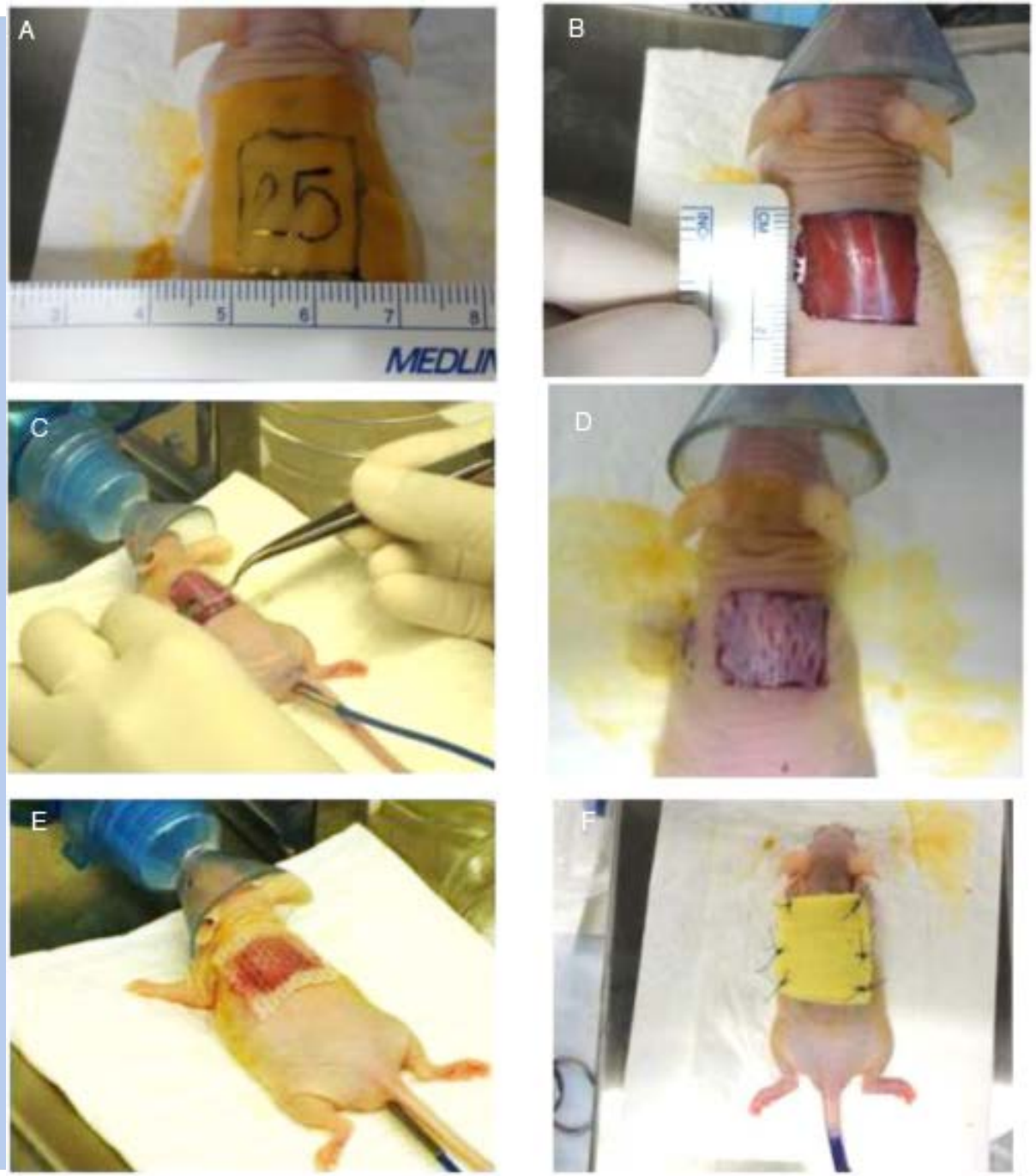


Printed Patterns



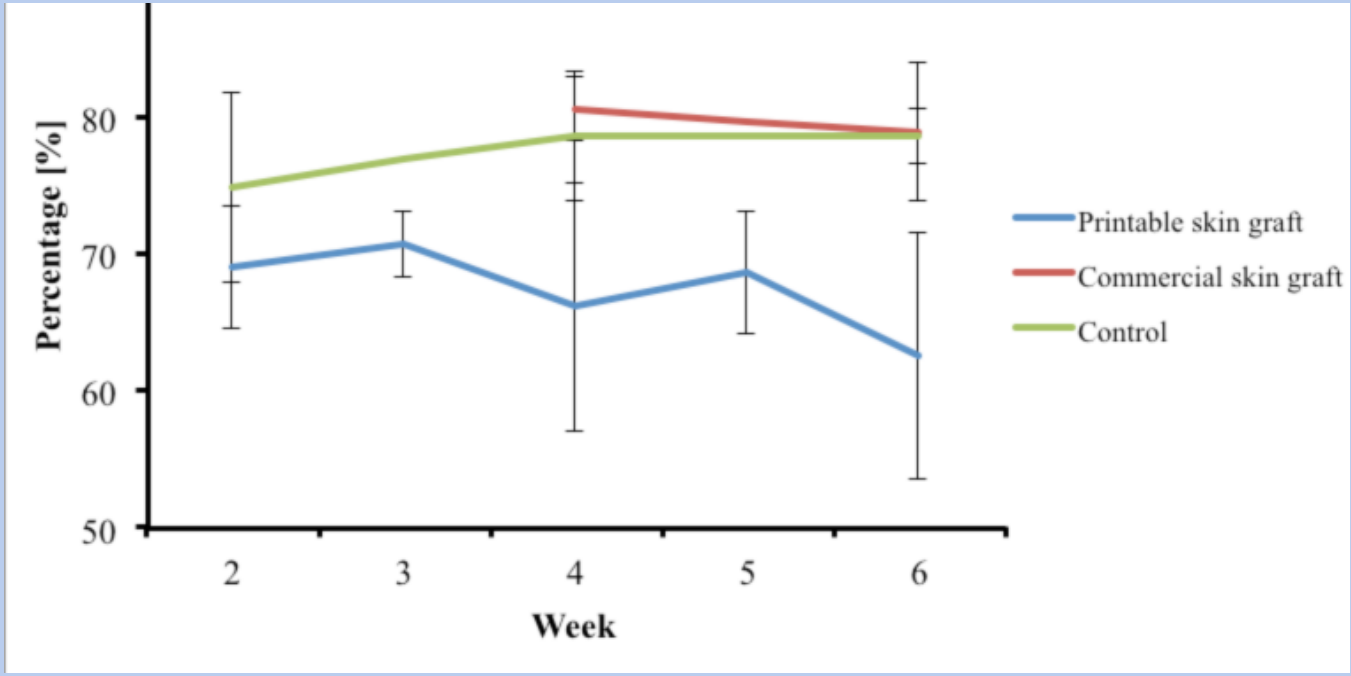


Skin graft 7 days after printing (10X phase contrast image)

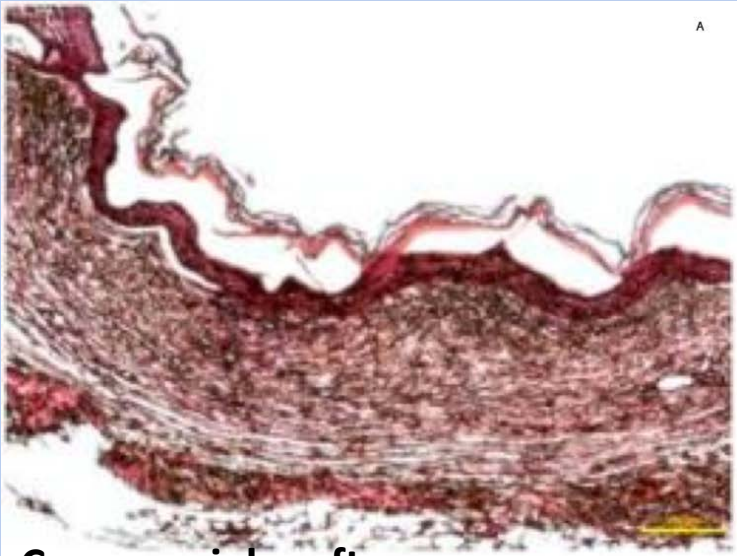

A
25
MEDLIN
B
C
D
E
F

UTEP

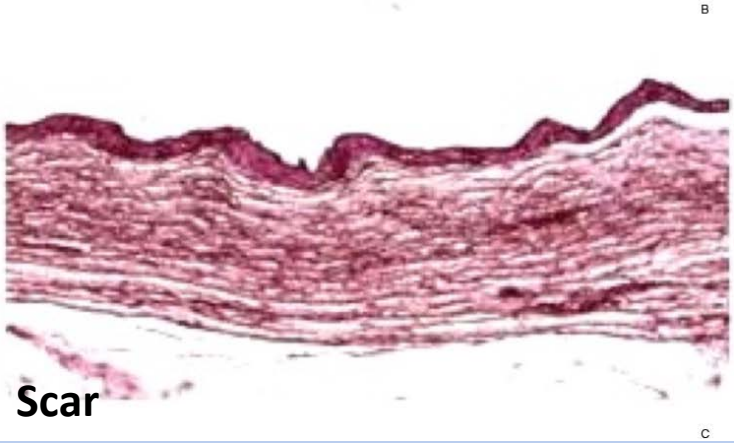
Wound Contraction



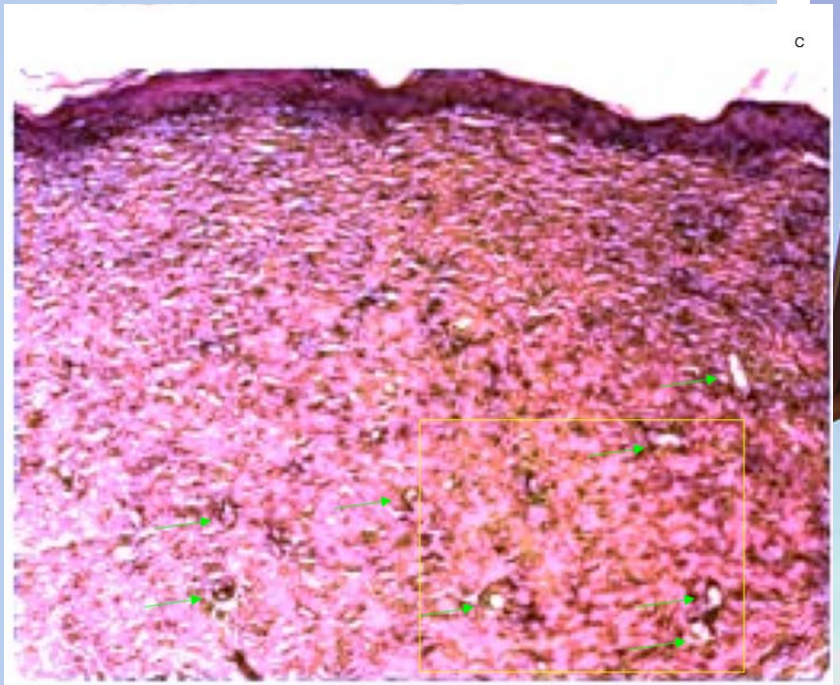
Histological Evaluation



Commercial graft

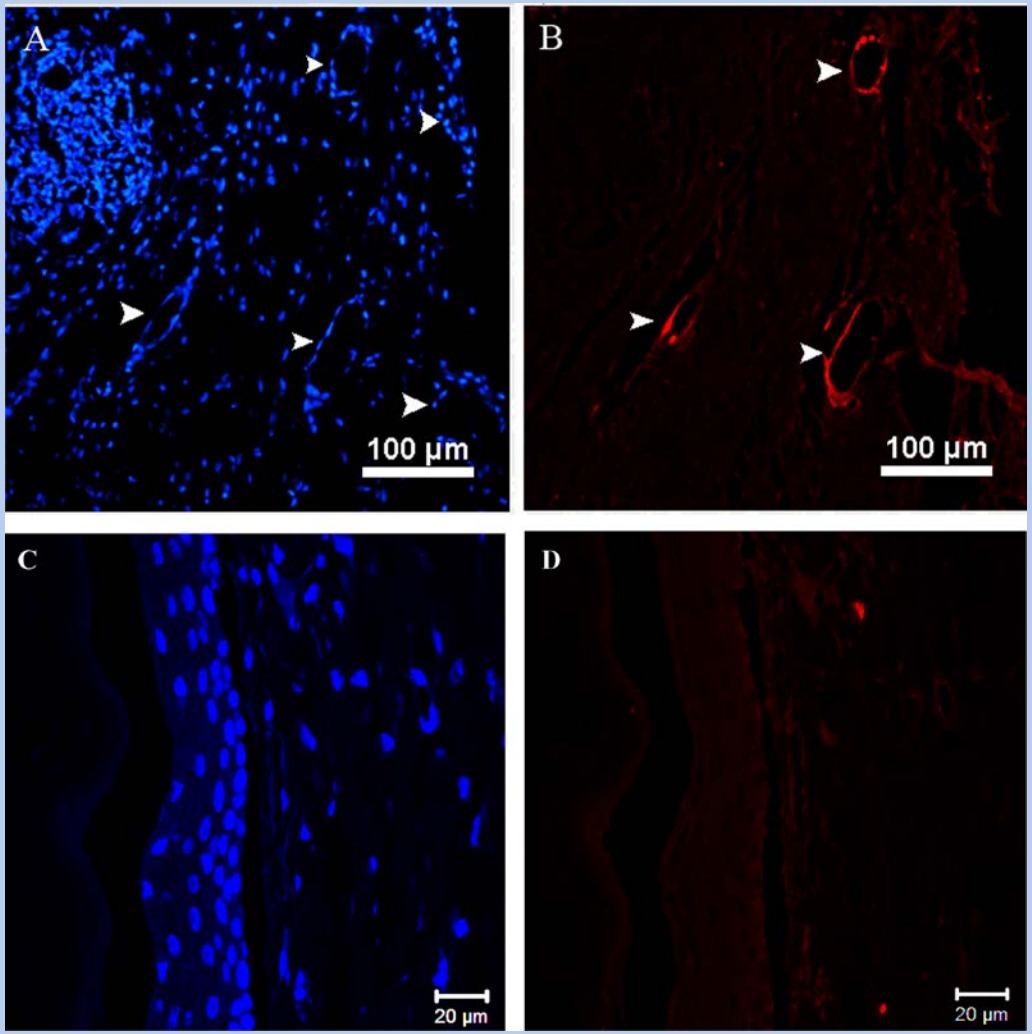


Scar



Printed graft

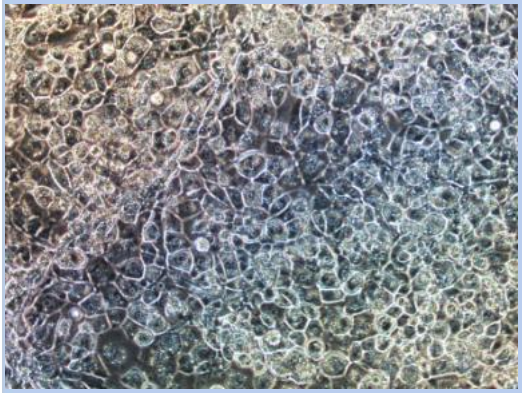
Human Cells integrate into Skin



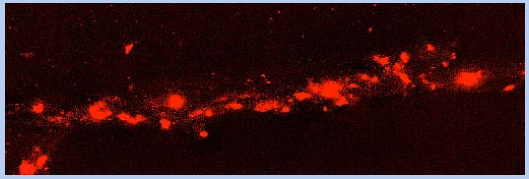
Inkjet Printed Skin

Commercial Product

Printing Adipose Tissue: Postprinting considerations



Pre-adipocytes differentiation day 5



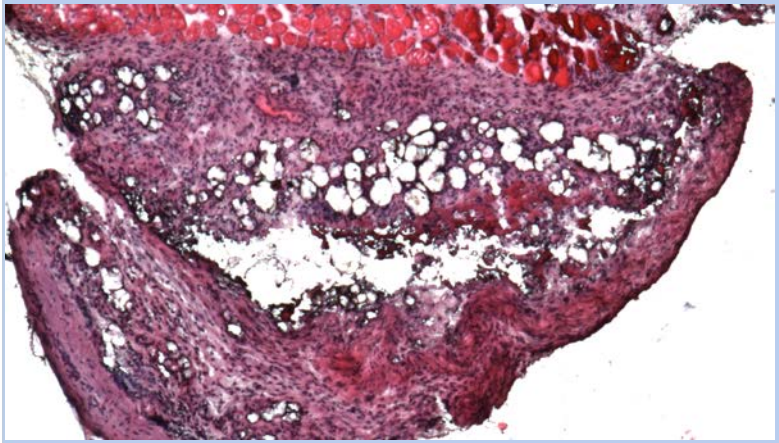
Pre-vascularized tubes

Printed adipocytes

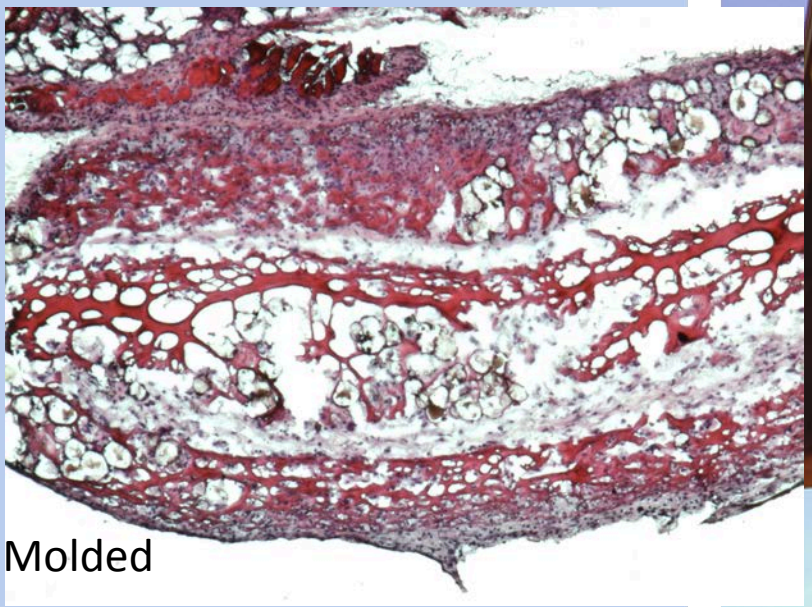
Implanted tissue

Explanted tissue

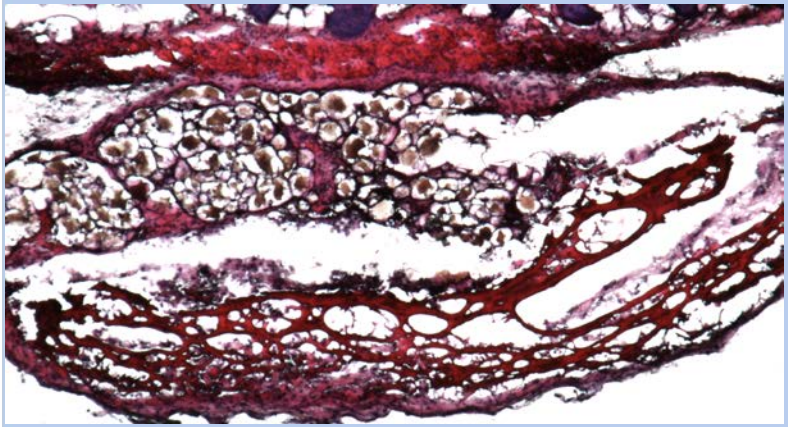
Printed Adipose Tissue: Biocompatibility



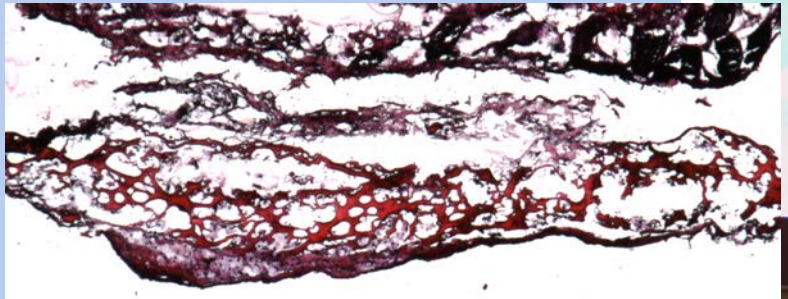
Injected



Molded

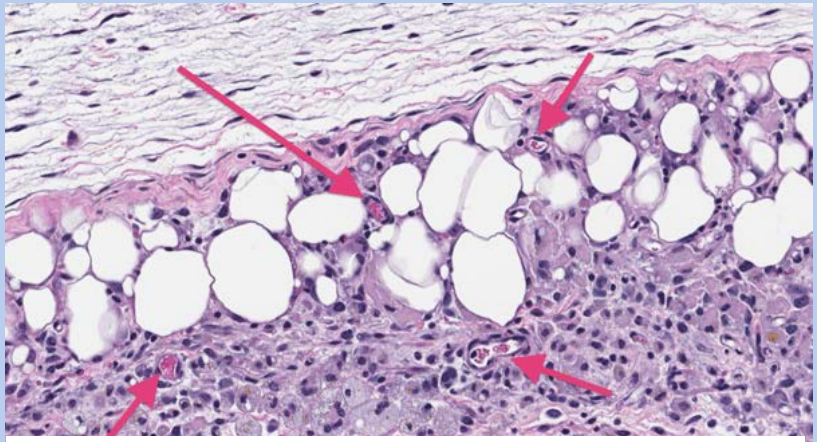


Printed vasculature

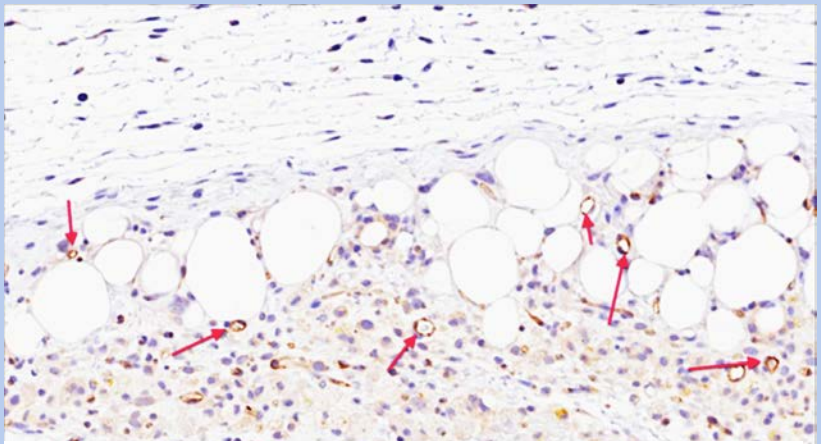


Printed vasculature + stem cells

Printed Adipose Tissue: Vascular Infusion

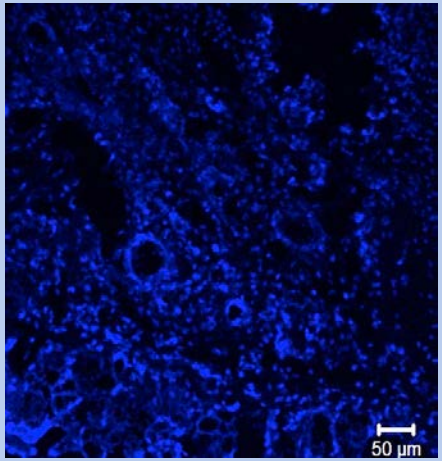


H&E

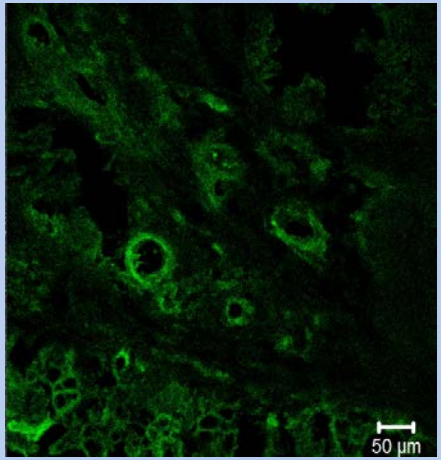


CD31

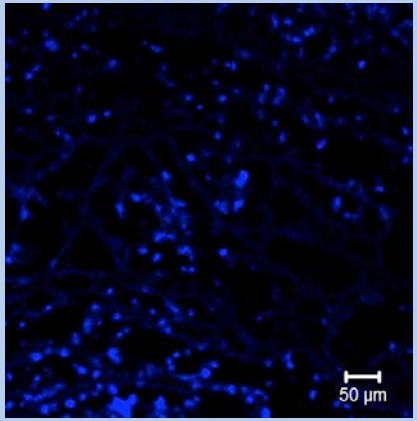
Printed Adipose Tissue: Vascularization



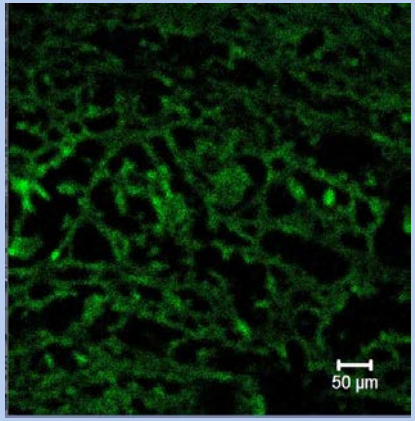
Printed (cell nuclei)



Printed (CD34)

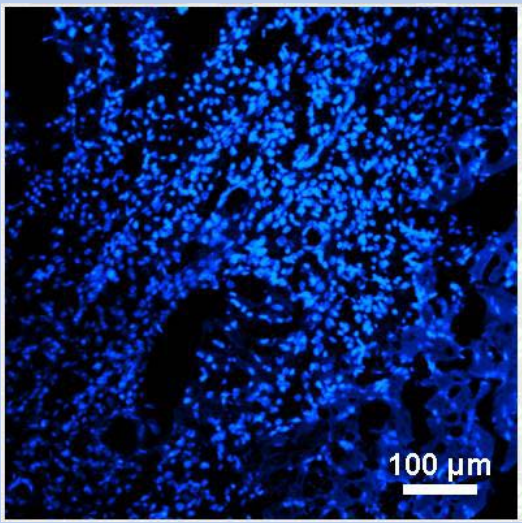


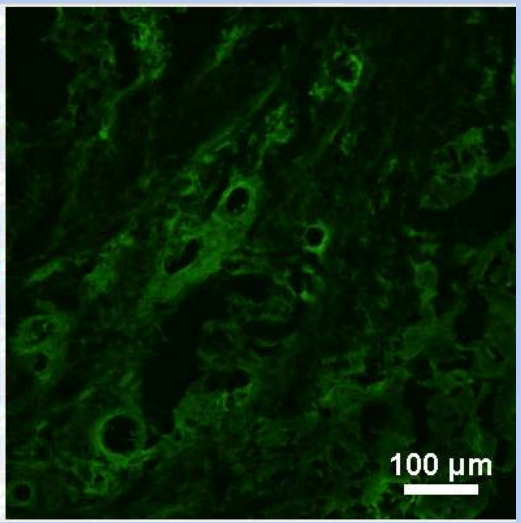
Molded (cell nuclei)

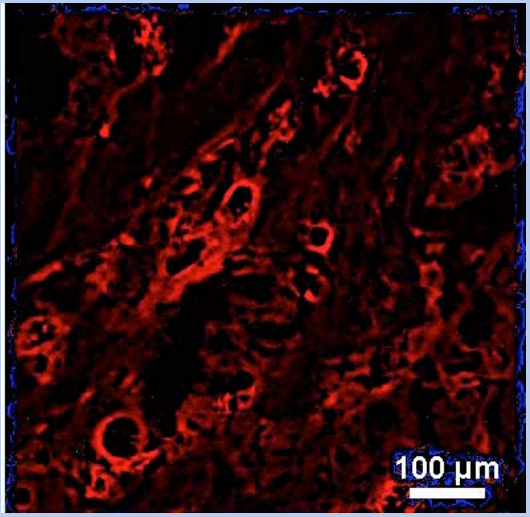


Molded (CD34)

Printed Adipose Tissue: Cell Source

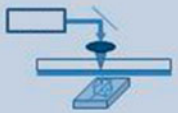
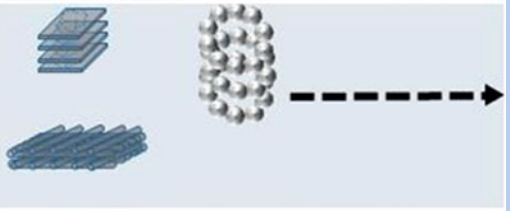
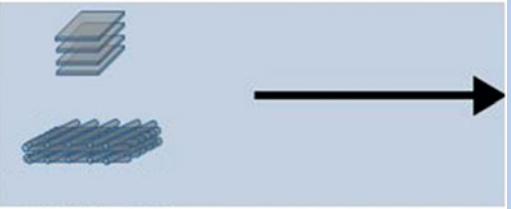






Printed (anti Human nuclear stain)

Other Printing Methods

Printing tool	Capabilities
Laser Induced forward transfer 	
Inkjet	
Electrospray	
Extrusion pen	
Photopolymerization	

Continuous

Discrete

Low res

High res

Ringeisen et al. MRS Bulletin 2013

Acknowledgements

MUSC
MEDICAL UNIVERSITY of SOUTH CAROLINA

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NSF

NASA