Cardiovascular Bioprinting

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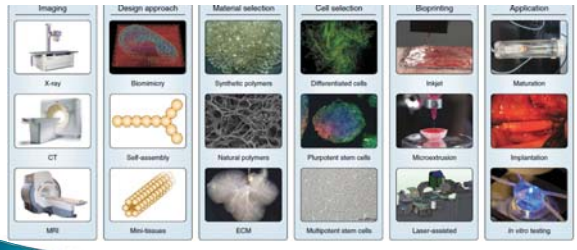
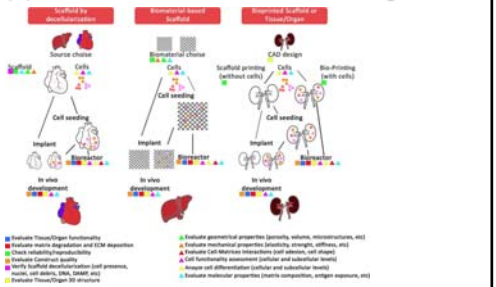
Pathway for Bioprinting 3D Tissue



Murphy, S. V., and A. Atala. "3d Bioprinting of Tissues and Organs." *Nature Biotechnology* 32, no. 8 (Aug 2014): 773–85.

Three Approaches to Tissue Building



Teodori, L., A. et al. "Three-Dimensional Imaging Technologies: A Priority for the Advancement of Tissue Engineering and a Challenge for the Imaging Community." *Journal of Biophotonics* 10, no. 1 (Jan 2017): 24–45.

Bioinks & Process Configurations

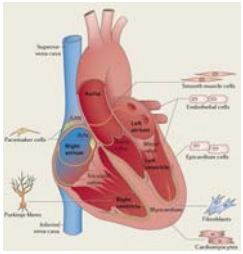
- Bioactive hydrogels such as gelatin, collagen, fibrin and peptide with capacity to support cell adhesion are usually implemented for cardiovascular bioprinting.
- Microcarriers
  - High specific surface area and bioactive environment for quick cell attachment and proliferation
  - Cells can be encapsulated.
- Scaffold-free cell spheroids generated by biofabrication approaches like hanging drop, micro-molded, microfluidics, and spinner flasks are also used in bioprinting
  - Deposited spheroids can fuse together and quickly generate into more mature constructs.
  - Enables co-culture of endothelial cells, smooth muscle cells, fibroblasts, cardiomyocytes and/or other related cardiovascular cells types.
  - Time consuming, mechanically weak structures, long time for remodelling/maturation.
- Extracellular matrix (ECM) from various native tissues.

Duan, B. "State-of-the-Art Review of 3d Bioprinting for Cardiovascular Tissue Engineering." *Annals of Biomedical Engineering* 45, no. 1 (Jan 2017): 195-209.

Myocardium

- Atherosclerosis and coronary artery occlusion leads to myocardial ischemia (angina) and possible myocardial infarction (heart attack).
- Fibroblasts/myofibroblasts migrate to the area of infarction and form non-contracting fibrotic scar tissue.
- Heart failure (inadequate pumping) can result, or even death.
- Coronary artery bypass can help ischemia, but severe loss of tissue may require ventricular assist, heart transplant or even an artificial heart.
- Cardiomyoplasty, the process of injecting cells into the myocardium, has low cell viability and poor integration.
- Myocardial tissue engineering requires a high density of CM and various supporting cells, vascularization and efficient oxygen exchange to generate synchronous contractions.

Heart Cell Populations



Xin M, Olson E N and Bassel-Duby R 2013 Nat. Rev. Mol. Cell Biol. 14 529-41

Pathway to Myocardium from MSC

Mesenchymal Stem Cells Differentiation Pathways & Lineage-Specific Markers

Heart muscle (myocardium) contain cardiomyocytes, cells that have an ability to contract in response to electrical stimulation

White Adipocytes
Brown Adipocytes
Myocytes
Chondrocytes
Osteocytes

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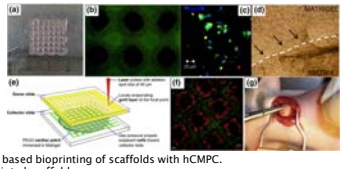
R&D Systems, https://www.rndsystems.com/research-area/mesenchymal-stem-cells

Bioprinting Myocardium



Extrusion based bioprinting of scaffolds with hCMPC.
a) Bioprinted scaffolds.
b) High cell viability of hCMPC.
c) Expression of human beta-integrin and Ki.
d) Migration of hCMPC after 3-week culture. Inkjet based bioprinting of cardiac patch.
e) Schematic bioprinting setup.
f) Patterned cells.
g) Patch implantation in vivo in a rat model.

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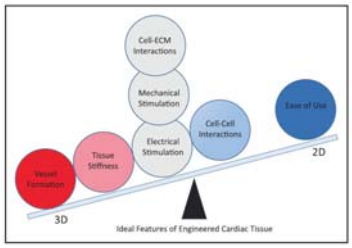
3D vs 2D



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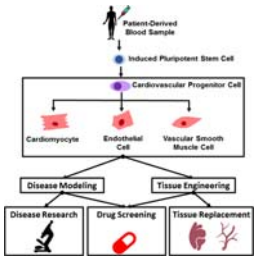
Borovjagin, V. Anton, M. Brenda Ogle, L. Joel Berry, and L. Jianyi Zhang. "From Microscale Devices to 3d Printing: Advances in Fabrication of 3d Cardiovascular Tissues." *Circulation Research* 120, no. 1 (2017): 150-65.

Application of iPSCs



Anderson, C. W., et al. "Stem Cells in Cardiovascular Medicine: The Road to Regenerative Therapies." *Current Cardiology Reports* 19, no. 4 (Apr 2017).

- iPSCs have the combined attributes of being autologous, easily accessible, and not ethically dubious.
- These qualities make them a prime candidate for both experimental studies in different genetic backgrounds and engineering therapies.
- Reprogramming and differentiation to a specific cell lineage requires time, so interventions using this cell source would be limited to nonurgent.

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Bioprinting Techniques

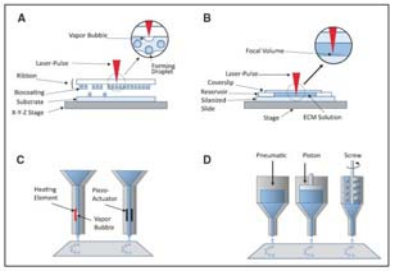
Bioprinting is usually accomplished using a combination of gel and cells.

A. Laser induced forward transfer.

B. Multiphoton excitation-based printing.

C. Inkjet.

D. Extrusion



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Features

Table 2. Features of the Current 3D Bioprinting Approaches

Feature	3D Bioprinting Approaches			
	Laser-Assisted	Multiphoton Excitation	Inkjet	Microextrusion
Resolution	High	Very high	Medium	Low
Droplet size	>20 μm	300 nm to 3 μm	50 to 300 μm	100 μm to 1 mm
Printer speed	Medium (200–1600 μm/s)	Slow (1 mm³/h)	Fast (1–10 000 droplets/s)	Medium (10–1000 μm/s)
Cell viability	High	Low	Medium	High
Cost	High	High	Low	Medium
Primary advantage(s)	Single cell manipulation, no clogging associated with nozzles, wide viscosity range	Can print ECM exclusively, not dependent on high viscosity of bioink	Gradients can be generated by altering droplet size, low cost	High cell density can be used
Primary disadvantage(s)	High cost, time-consuming, technically challenging	Cells cannot be deposited with printing, end product mm small scale	Nozzle clogging, low droplet directionality	Limited number of biomaterials used to date
References	69, 70	71	72–75	76

ECM indicates extracellular matrix.

Borovjagin, V. Anton, M. Brenda Ogle, L. Joel Berry, and L. Jianyi Zhang. "From Microscale Devices to 3d Printing: Advances in Fabrication of 3d Cardiovascular Tissues." *Circulation Research* 120, no. 1 (2017): 150-65.

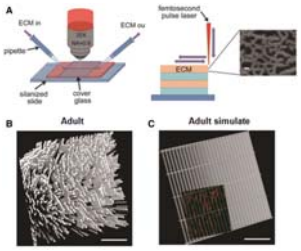
Human-induced Pluripotent Stem Cell-Derived Cardiac Muscle Patch (hCMP)...

hCMP fabrication via 3-dimensional multiphoton excited (3D-MPE) printing.

A. The extracellular matrix (ECM) and associated crosslinking solution are passed through the optical interrogation path.

B. Heart of an adult mouse were immunofluorescently stained for the presence of fibronectin and scanned via multiphoton excited.

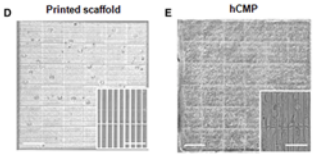
C. The distribution of fibronectin in the native tissue was simulated in a template.



Gao, E., B. M. Ogle, J. Zhang *et al.* "Myocardial Tissue Engineering with Cells Derived from Human-Induced Pluripotent Stem Cells and a Native-Like, High-Resolution, 3-Dimensionally Printed Scaffold." *Circulation Research* 120, no. 8 (2017): 1318-25.

D. Native-like ECM scaffold. scaffold was seeded with human-induced pluripotent stem cells (hiPSC)-derived cardiomyocytes (CMs), endothelial cells (ECs), and smooth muscle cells (SMCs) to generate the hCMPs

E. The complete hCMP .



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hCMP Engraft & Survive after Transplantation..

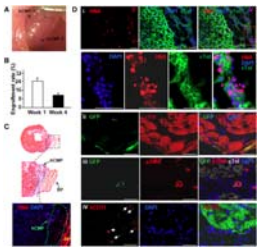
hCMP engraft and survive after transplantation into the hearts of mice with myocardial infarction (MI). MI was surgically induced in mice.

A. Transplanted hCMPs on the mouse heart.

B. Engraftment rate MI + hCMP at 1 and 4 wk post infarct.

C. hCMP on the epicardial surface a wk 4.

D. Region of patch in MI + hCMP animals at wk 4 were immunofluorescently stained for the presence of HNA, cardiac troponin I (cTnI), green fluorescent protein (GFP), α-smooth muscle actin (α-SMA), and the human isoform of the endothelial marker CD31 (hCD31).



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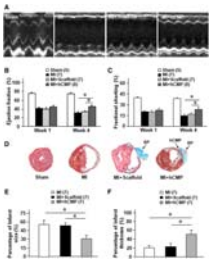
Reduced Infarct Size…

hCMP transplantation improves cardiac function and reduces infarct size after myocardial infarction (MI).

A. Echocardiographic assessments of (B) left ventricular ejection fraction and (C) fractional shortening.

D-F. Sections of hearts from animals in different groups were (D) Masson trichrome stained for histological assessments of (E) infarct size and (F) infarct wall thickness.



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Vascularization Strategy

- Vascularization is necessary for oxygen transfer, deliver nutrients, remove metabolic waste and promote the circulation of immune cells.
- In vitro 3D printing:
  - Generation of vascular constructs by self-assembly of cells.
  - Generation of microvasculatures by inkjet based bioprinting.
  - Generation of bioprinted constructs with growth factor delivery.
  - Coaxial nozzle assisted 3D bioprinting of vasculature.
  - Generation of channel based vascularized constructs.

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Vasculature

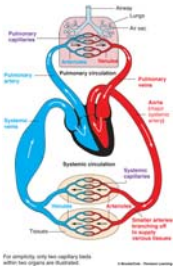

Image Courtesy of Brook/Cole - Thomson Learning

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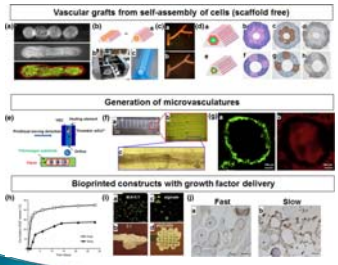
Bioprinting Vascular Structures



a) Cell fusion of spheroids in collagen type1 hydrogel.
b) Tubular strictures with cellular cylinders.
c) Fusion of multicellular spheroids into braches.
d) Double layer vascular wall.
e) HMEC and fibrin channel scaffold - thermal inkjet printer.
f) Fibrin scaffold.
g) Ring shaped microvasculature.
h) Cumulative release of VEGF.
i) Hydrogel mixture and tubulogenesis assay vessel formation in EPC seeded scaffolds.

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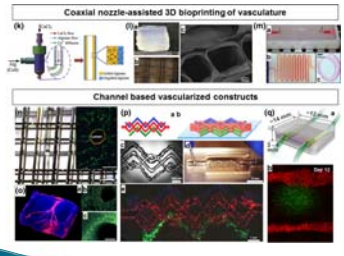
Vascular Structures...



k) 3D alginate structure with built-in microchannels.
l) Hollow alginate filaments.
m) Alginate based vasculature.
n) Carbohydrate-glass filament-architecture and vascular lumen with endothelial monolayer after removing sacrificial filament and perfusion.
o) Gelatin based constructs with branched bioprinted agarose templates.
p) Heterogeneous engineered tissue construct
q) Dual channel by bioprinting sacrificial gelatin within fibrin.

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Heart Valves

- Leaflets and root walls mainly contain valve interstitial cells (VIC) and smooth muscle cells (SMC), respectively, with valvular endothelial cells (VEC) covered on the surface.
- Causes of heart valve disease include congenital heart disease, rheumatic fever, cardiomyopathy, heart attack, prior endocarditis infection, and age.
- Tissue engineering has great potential to address current limitations of non-living prosthetics by providing living constructs that can grow, remodel and integrate in the patients.

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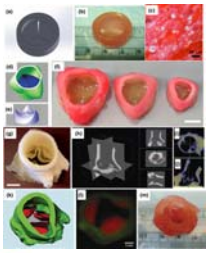
Bioprinting a Heart Valve



a-c) Flat valve.
d-f) Axisymmetric valve.
g-m) Anatomical valve.
a, d, e, k) Valve model.
b, f, m) Bioprinted valve.
c) Safranin-O staining showed GAG deposition.
h) μCT scan slices and their reconstruction.
i, j) Valve scan segmentation into separate STLs for the leaflet and the root.
l) Fluorescent image of first printed two layers of aortic valve conduit.

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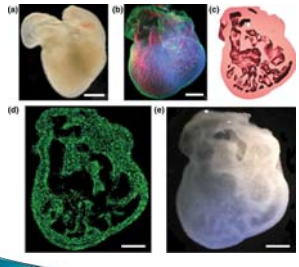
Bioprinting a Whole Heart



Scaffolds Based on 3D Imaging Data

a) Image of explanted embryonic chick heart.
b) 3D image of the embryonic chick heart stained for fibronectin (green), nuclei (blue), and F-actin (red).
c) Cross section of the 3D CAD model of the embryonic heart.
d) Cross section of the 3D printed heart (fluorescent alginate-green)/
e) 3D printed heart with internal structure visible through the translucent heart wall.

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Summary

- iPSCs have the combined attributes of being autologous, easily accessible, and not ethically dubious.
- hCMP transplantation has been shown to improve cardiac function and reduces infarct size after myocardial infarction (MI) in a mouse.
- 3D bioprinting may be useful in developing heart valves and vascular structures.
- Bioprinting a whole heart is a technological challenge.

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Appendix

Table 1 Cardiovascular stem cell therapies in the USA

Disease	Type	Status	Time frame	Cell source	Phase	Delivery mechanism	Clinical trial ID
Ischemic cardiomyopathy	Interventional	Completed	September 12, 2005–June 05, 2015	Autologous BMSC	1	Intramyocardial injection	NCT00535203
Chronic ischemic left ventricular dysfunction	Interventional	Ongoing	December 02, 2013–	Allogenic hMSC	2	Transendocardial injection	NCT02013674
General aging	Interventional	Ongoing	July 18, 2016–	Allogenic hMSC	2	Intravenous injection	NCT02065245
Coronary artery disease	Interventional	Ongoing	April 10, 2006–	Autologous BMSC-derived aldehyde dehydrogenase bright cells	1	Intramyocardial injection	NCT00114366
Ischemic cardiomyopathy	Interventional	Completed	May 15, 2007–October 15, 2014	Autologous c-kit+ cardiac stem cells	1	Intracoronary injection	NCT00474461
Peripheral arterial disease	Interventional	Recruiting	April 25, 2016–	Autologous ADSC	1	Intravenous injection or intramuscular injection	NCT02756884
Congenital heart disease	Interventional	Ongoing	January 29, 2008–	BMSC	2	Cardiomyoplasty	NCT01034007
Myocardial infarction	Interventional	Completed	July 2014–April 2016	Allogenic mesenchymal bone marrow cells	3	Intravenous injection	NCT02672267
Acute myocardial infarction	Interventional	Ongoing	June 2014–	Allogenic cardiac stem cells	2	Intracoronary infusion	NCT02439398
Cardiomyopathy	Observational	Recruiting	June 2014–	hiPSC	–	–	NCT02417311
Chronic myocardial ischemia	Interventional	Recruiting	January 2016–	Autologous MSC	2	Intramyocardial injection	NCT02462330
Cell type			No. of trials	% Observational		% Interventional	
hiPSC			5	100		0	
ADSC/BMSC			41	4.9		95.1	

Examples of recently completed or ongoing stem cell-based therapy trials. Total trials listed on clinicaltrials.gov for cardiovascular diseases are given for each cell type. Currently, there are no clinical trials using embryonic stem cells in the USA, so this category was omitted from this table

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