Printing Considerations: Cells, Biologics

NIH Center for Engineering Complex Tissues (CECT)
June 8, 2018

Bhushan Mahadik, Ph.D.
Assistant Director, CECT
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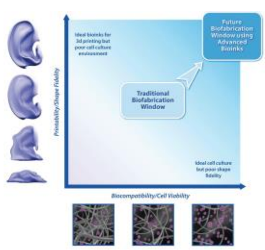
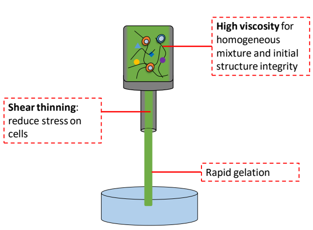
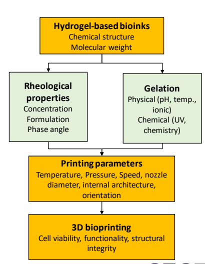
Biofabrication window



Kyle et al., Adv. Healthcare Mats. 2017, 6, 1700264

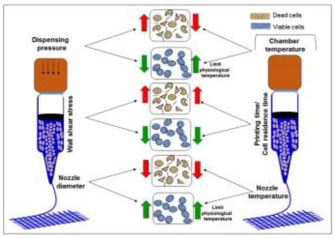
Important Bioink properties for extrusion





Adapted from WFIRM

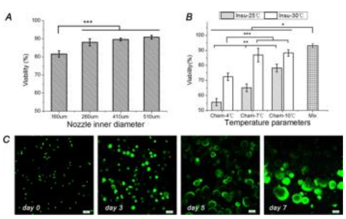
Impact of extrusion conditions



Adapted from Biofabrication Workshop, Rice University

Anwar and Tan. *Molecules.* 2016

Impact of extrusion conditions

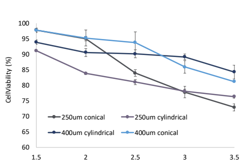


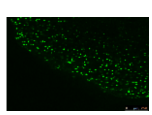
Adapted from Biofabrication Workshop, Rice University

Ouyang,et al. *Biofabrication.* 2015

Impact of extrusion conditions

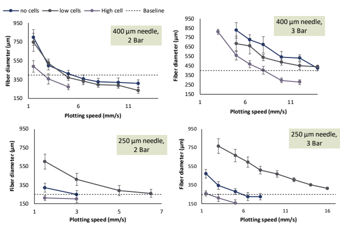
- Cell viability in printed fibrin

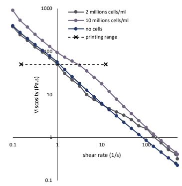




Slides courtesy Ms. Piard, TEBL

Impact of extrusion conditions





Slides courtesy Ms. Piard, TEBL

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Considerations: Bioink

Materials

ID	LA/GA Ratio	Molecular Weight	End Cap	Simplified Code
PLGA 1	50:50	9730Da	ester	0.5-10kD-ester
PLGA 2	50:50	30263Da	ester	0.5-30kD-ester
PLGA 3	60:40	42609Da	ester	0.6-42kD-ester
PLGA 4	60:40	33792Da	acid	0.6-34kD-acid
PLGA 5	85:15	61943Da	ester	0.85-62kD-ester

Printing conditions

	0.2 mm ID			0.4 mm ID		
Composition	Temperature (°C)	Pressure (bar)	Speed (mm/s)	Temperature (°C)	Pressure (bar)	Speed (mm/s)
0.5-10kD-ester	110	9	1.5	95	7.5	1.5
0.5-30kD-ester	135	9	0.7	125	8	1.5
0.6-42kD-ester	165	9	0.7	140	9	0.5
0.6-34kD-acid	150	9	1.5	130	8	1
0.85-62kD-ester	170	9	0.5	145	8	0.5

Increased LA ratio, molecular weight

Slides courtesy Dr. Guo, TEBL

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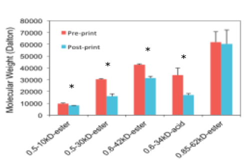
Considerations: Bioink

- Material properties might alter due to a variety of reasons



Slides courtesy Dr. Guo, TEBL

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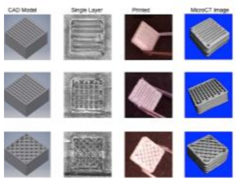
Considerations: Bioink

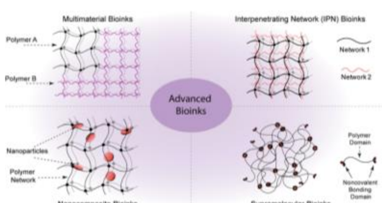


Parameters Affecting Printing Precision

Needle Diameter 0.2 mm ID	Interaction coefficient	p-value	Needle Diameter 0.4 mm ID	Interaction coefficient	p-value
LA/GA ratio	Significant	0.0017	LA/GA ratio	Not significant	0.91
Molecular weight	Significant	0.00019	Molecular weight	Not significant	0.22
Endcap	Significant	0.028	Endcap	Significant	0.010
Pressure	N/A	N/A	Pressure	Significant	0.048
Temperature	Significant	0.000068	Temperature	Significant	0.025
Speed	Significant	0.022	Speed	Significant	0.0025
Pattern	Not significant	0.78	Pattern	Not significant	0.88

T Guo, TR Holzberg, CG Lim, F Gao, A Gargava, JE Trachtenberg, AG Mikos, and JP Fisher. 3D Printing PLGA: A Quantitative Examination of the Effects of Polymer Composition and Printing Parameters on Print Resolution. Biofabrication. 9: 024101 (2017).

Considerations: Bioink

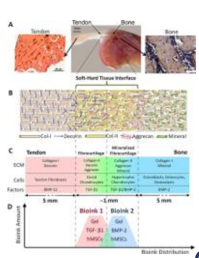


Adapted from Biofabrication Workshop, Rice University

Chimene, et al. *Annals of Biomedical Engineering*. 2

Printing of bioactive moieties

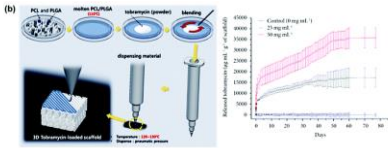
- Growth factors and biomolecules can help guide region-specific tissue regeneration



Adapted from Biofabrication Workshop, Rice University

Gurkan, et al. *Mol Pharm.* 2014.

Homogenous immobolization



Geiger, et al. *Adv. Drug Delivery Rev.* 2003.

Adapted from Biofabrication Workshop, Rice University

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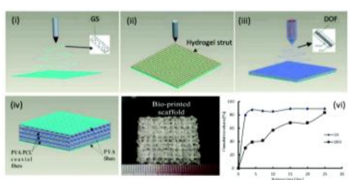
Heterogenous immobolization

- For temporal release of gentamycin sulfate and deferoxamine
- Blend electrospinning of polyvinyl alcohol 124-gentamycin sulfate (PVA–GS) fibers
- 3D printing for gelatin–sodium alginate struts
- coaxial electrospinning of PVA–DFO/PCL fibers



Adapted from Biofabrication Workshop, Rice University

Liu, et al. *Polym. Eng. Sci.* 2016.

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Takeaways

- Several factors to be considered when
  - choosing the appropriate bioink
  - Printing procedure and impact on cells
  - Incorporating biological cues
- Post-processing impacts functionality just as much as pre- and during-
- Alternatives available, but have to be tailored to specific applications

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Printing strategies and examples

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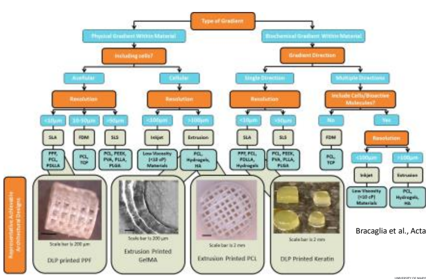
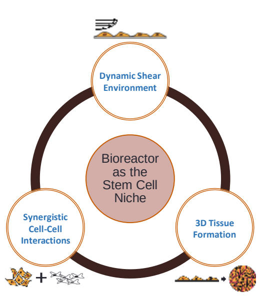
Choosing a 3D Printing Technique



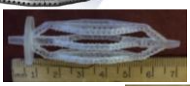
Bracaglia et al., Acta Biomaterialia, 2017

Bioreactors

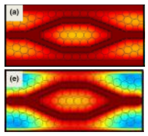


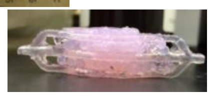
3D Printed Vascular Network

3D Printed Template of a perfusable vascular network



Pore size and spacing affect nutrient exchange





Ball et al., Annals of Biomedical Engineering, Vol. 44, No. 12, December 2016

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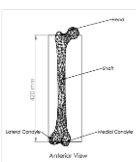
Bioreactor Scale-up



BNB Nguyen, et al., Tissue Engineering Part A. 22: 263-271 (2016).

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Bioreactor Scale-up

2.5 L osteogenic media flowing at **240 ml/min**
250 mL of 2% alginate for cell encapsulation within **7200 alginate beads**
1000mL of 2% alginate to fill empty space in culture chamber with **30,000 alginate beads**
Approximately **800 x 10^6 hMSCs**
Sterilizing air filter on media flask to increase gas exchange

BNB Nguyen, et al., Tissue Engineering Part A. 22: 263-271 (2016).

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Bioreactor Scale-up

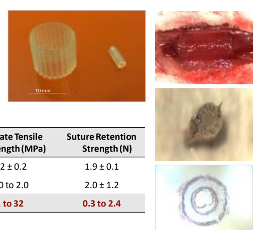
Volume of construct is **200 cm^3**

- 2.5 L osteogenic media flowing at 240 ml/min
- 250 mL of 2% alginate for cell encapsulation within 7200 alginate beads
- 1000mL of 2% alginate to fill empty space in culture chamber with 30,000 alginate beads
- Approximately 800 x 10^6 hMSCs
- Sterilizing air filter on media flask to increase gas exchange

BNB Nguyen, et al., Tissue Engineering Part A. 22: 263-271 (2016).

3D Printed Vascular Grafts

- Grafts printed from poly(propylene fumarate) using a direct-light processing and crosslinked with UV light
- Mechanical properties similar to vessels used in autologous grafts



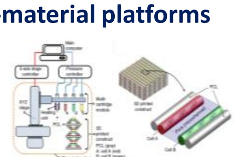
	Modulus (MPa)	Ultimate Tensile Strength (MPa)	Suture Retention Strength (N)
Human Saphenous Vein	6.7 ± 1.3	2.2 ± 0.2	1.9 ± 0.1
Human Femoral Artery	9.0 to 12.0	1.0 to 2.0	2.0 ± 1.2
3D Printed Graft	11 to 176	1 to 32	0.3 to 2.4

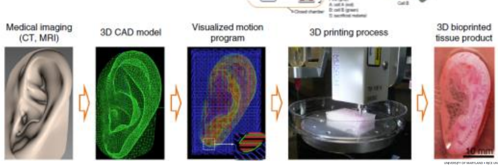
AJ Melchiorri, et al.,. Advanced Healthcare Materials. 5: 319-325 (2015).

Custom-made, Multi-material platforms

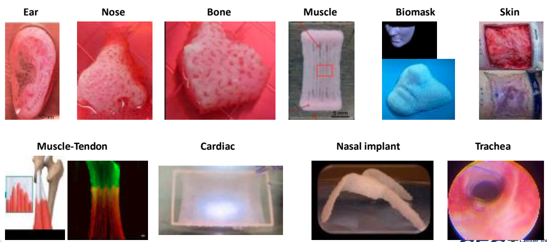
Integrated Tissue-Organ Printer (ITOP)

Kang H-W, Lee SJ et al. *Nat Biotechnol*. 2016





Custom-made, Multi-material platforms



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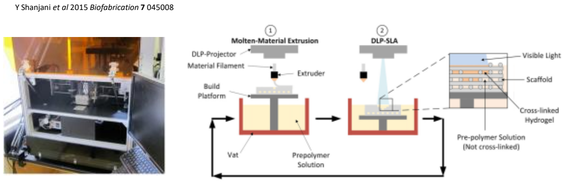
Kang H-W, Lee SJ et al. *Nat Biotechnol.* 2016

Custom-made, Multi-material platforms

Hybprinter

Y Shanjani *et al* 2015 *Biofabrication* **7** 045008



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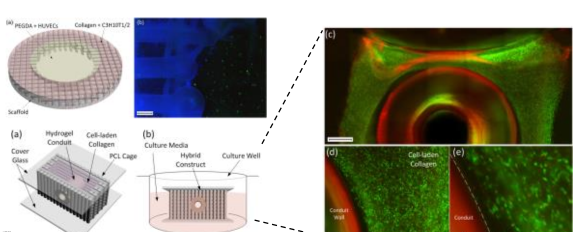
Custom-made, Multi-material platforms



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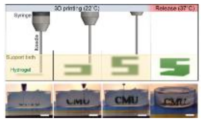
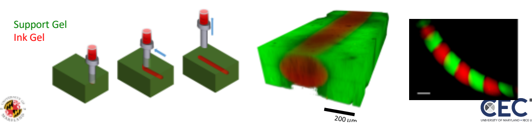
Novel biomaterials and techniques

- Gel-in-gel printing methods
- In situ polymerization methods



Hinton et al., Sci. Adv. 2015 Oct; 1(9)



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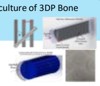
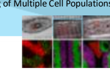
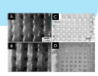
TR&D1: Bioreactor Culture
- 3DP Bioreactor Chambers
- Dynamic Coculture in Bioreactor Chambers
- Dynamic Coculture of 3DP Bone Mimetic



TR&D2: Cell Printing
- Cell Positioning in 3DP Tissues
- Cell-Substrate Printing to Control Microenvironment
- Bioprinting of Multiple Cell Populations



TR&D3: Complex Scaffolds
- Composite 3DP Scaffolds
- 3DP Scaffolds with Controlled Release
- Heterogeneously Distributed Growth Factors for Tissue Repair

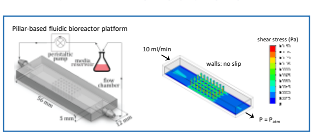


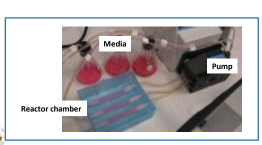
TR&D 1: 3D Printed Bioreactors for Cell Culture
Dr. John P. Fisher, University of Maryland

TR&D 2: Bioprinting Patterning for Cell-Laden Constructs
Dr. Anthony Atala and Dr. James J. Yoo, Wake Forest University

TR&D 3: Bioprinting for Complex Scaffold Fabrication
Dr. Antonios G. Mikos, Rice University

TR&D1: Bioreactors





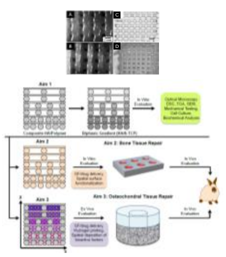
TR&D2: Live Cell Patterning

The ITOP can concurrently print synthetic biodegradable polymers and cell-laden hydrogels in a singe tissue construct with clinically applicable size, shape, and structural integrity for clinical applications

- Generation of 3D freeform shaped constructs with precision
  - Multiple cell types, biomaterials, drugs
- High strength constructs
  - Gel and polymeric materials (~12)
- Printing resolution
  - Cell printing: ≥ 50 µm
  - Structural material printing: ≥ 2 µm

TR&D3: Complex Heterogeneous Scaffolds

- **Develop a multi-material 3D printing system** for the fabrication of complex bone and osteochondral scaffolds
  - Tunable material compositions
  - Patterned loading of growth factors
- Multi-material 3D printing system **translatable to lower-cost 3DP systems**
- **Spatial deposition of transitional gradients** (pore, ceramics, GFs) can mimic zonal organization
- **Spatial manipulation of signaling properties** to recapitulate tissue growth and regeneration in terms of composition and strength



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A NIBIB / NIH Biomedical Technology Resource Center
Aiming to Grow the 3D Printing & Bioprinting Community

John Fisher (University of Maryland): 3D Printed Bioreactors for Dynamic Cell Culture
Antonios Mikos (Rice University): Bioprinting for Complex Scaffold Fabrication
Anthony Atala & James Yoo (Wake Forest University): Bioprinting for Cell-Laden Constructs

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Dr. Antonios Mikos, Rice
Dr. James Yoo, Wake Forest

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