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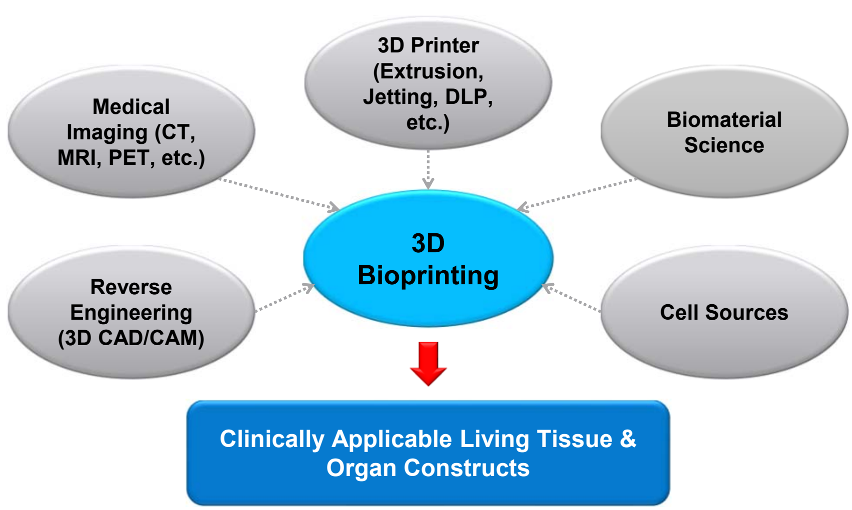
BIOINK DEVELOPMENT & PRINTABILITY FOR COMPLEX 3D PRINTING

Sang Jin Lee, Ph.D.

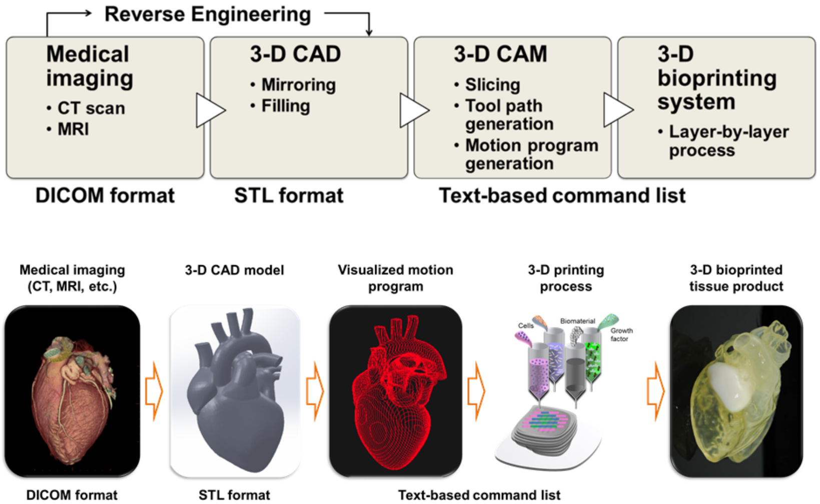
1

Definition of 3D Bioprinting



2

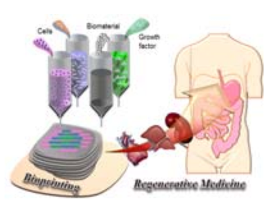
3D Bioprinting Workflow



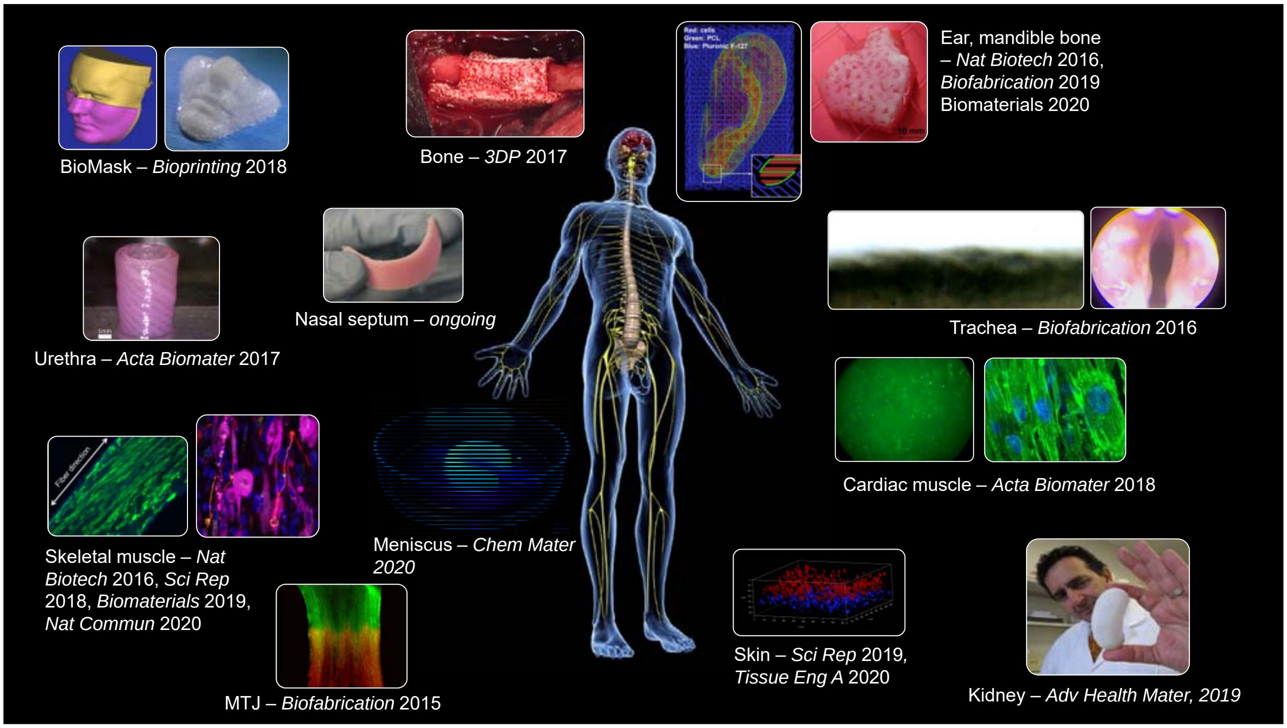
Integrated Tissue-Organ Printing (ITOP) System

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

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



Kang, Lee et al., Nat Biotechnol 2016



5

3D Bioprinting - Bioinks

- A major challenge for tissue and organ engineering is *the production of 3D biomimetic, cellular tissue constructs* of clinically relevant size and shape with structural integrity
- 3D bioprinting can print *cell-laden hydrogels to manufacture complex, multi-cellular living tissue constructs* that mimic the structure of native tissues
- *Bioinks* provide the biological microenvironment needed for the successful delivery of cells and biomaterials to discrete locations within 3D structures
- To improve and enhance the significance and innovation of this approach, it is critical to develop *standardized bioink systems*

6

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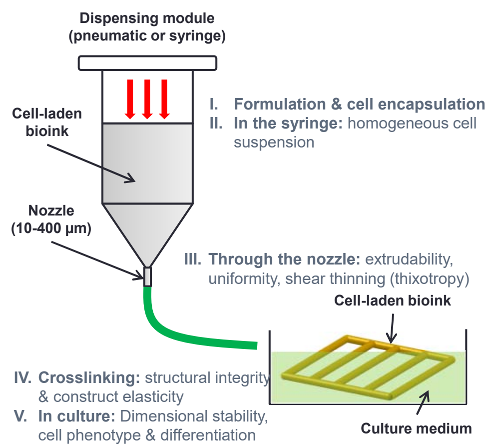
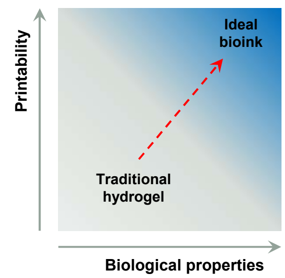
Hydrogel-based Extrusion Bioprinting

Required properties of bioinks



Working Definitions of Printability

1. **Extrudability**
   - How difficult is it to extrude the bioink?
   - Pressure required to extrude the bioink at a given flowrate
2. **Extrusion uniformity/accuracy**
   - Are the extrusion lines straight and uniform?
   - Length of an extrusion line's edge relative to a perfectly uniform filament
3. **Structural integrity**
   - Does the bioink hold its shape after extrusion?
   - Height of a printed structure



Biological properties
- Non-toxic
- Supporting cell growth
- Maintaining cell phenotype
- Accelerating tissue formation

7

Gillispie et al. Biofabrication. 2020

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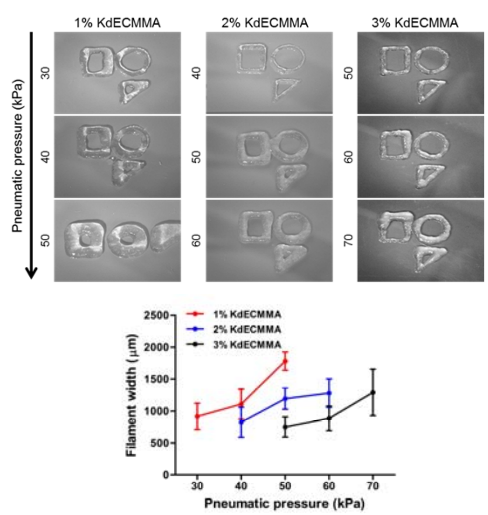
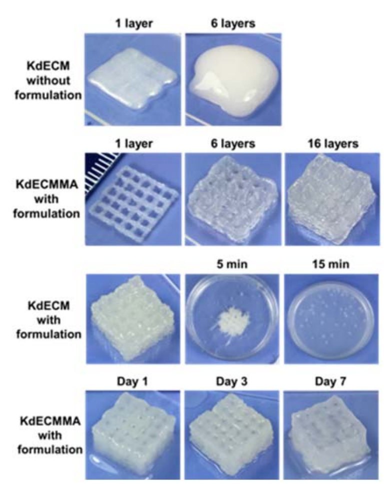
Determination of Printing Parameters





8

Ali et al., Adv Healthcare Mater 2019

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Hydrogels Available for Bioprinting

- **Hydrogels**
  - Collagen
  - Fibrinogen
  - Hyaluronic acid (HA)
  - Sodium alginate
  - Gelatin
  - Methyl cellulose
  - Gellan gum
  - Chitosan
  - Agarose
  - Xantan gum
  - Poly(ethylene glycol) (PEG)
  - Pluronic F127

Quantitative printability measurement is needed

- Most often, printability is described qualitatively
- Rheological measurement for the extrudability and shear-thinning property of materials
- Yet, predicting the final shape of a printed construct have been inconclusive
- The relationships between rheology and other aspects of printability are not fully understood - rheology cannot yet be used as a proxy for printability
- Direct measures of printability are currently needed in order to confirm the suitability of bioinks for specific bioprinting applications.

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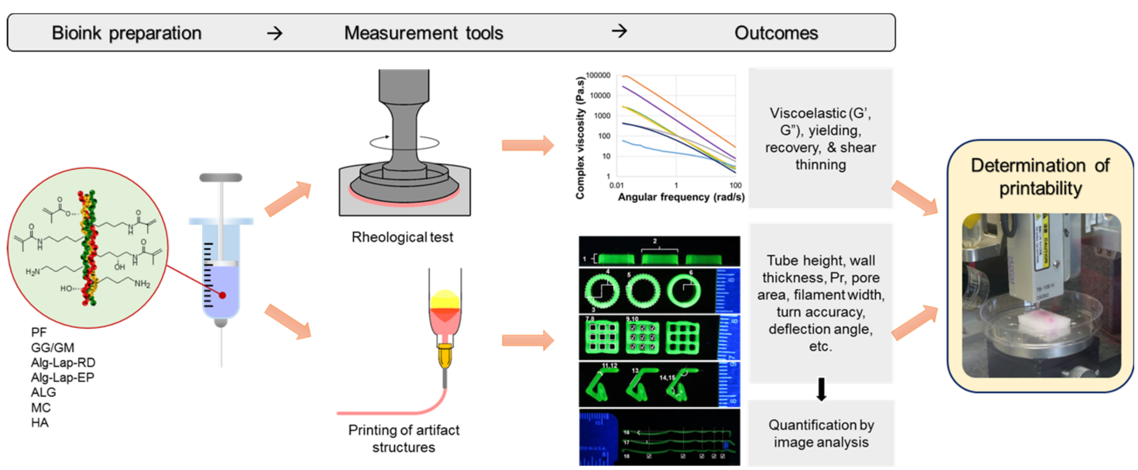
Strategy of Bioink Development



10

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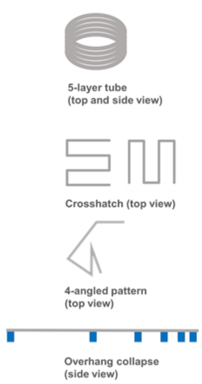
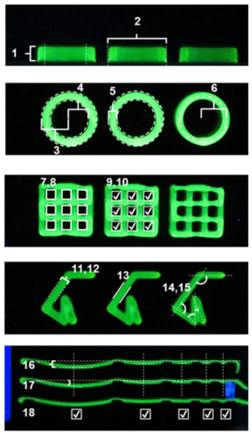
Bioink Artifact for Printability Measurement

Printing patterns

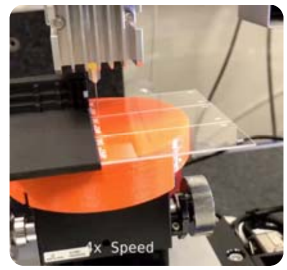


Printing outcomes



Printability measurement

Structure	View	Printability Aspect	Measures	Calculation
5-Layer Tube	Side	• Stack multiple layers	[1]Height	Direct measurement
			[2]Width	Direct measurement
5-Layer Tube	Top	• Stack multiple layers	[3]External radius	$r_e = \sqrt{A_e/_\pi}$
		• Arc accuracy	[4]Internal radius	$r_i = \sqrt{A_i/_\pi}$
			[5]Wall thickness	$T = r_e - r_i$
			[6]Radial accuracy	$r_a = \frac{(r_e + r_i)/_2}{4} \times 100\%$
Crosshatch	Top	• Form horizontal pores	[7]*Pr*	$Pr = L_p^2/_{16A_p}$
			[8]Area of pores	Direct measurement
			[9]# of broken pores	Direct measurement
			[10]# of filled pores	Direct measurement
4-angled pattern	Top	• Sharp turns	[11]Filament width	Direct measurement
		• Single filament dimensions	[12]Standard deviation of filament width	$\sigma_w = \sqrt{\frac{\sum(w_i - \bar{w})^2}{N_w}}$
			[13]Uniformity ratio	$U = \frac{(P_1 + P_2)/_2}{L_f}$
			[14]Turn angle	Direct measurement
			[15]Turn angle error	$\theta_e = \theta_m - \theta_t$
Overhang Collapse	Side	• Span gaps unsupported	[16]Deflection at midpoint	Direct measurement
			[17]Angle of deflection	$\theta_d = \sin^{-1}(^D/_{0.5G})$
			[18]Spanning success rate	Direct measurement



The artifact possesses excellent ease of use, printing in **less than 10 min**, using less than **0.4 mL of bioink**, and an **automated image analysis** process.

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11

11

Selected Testing Bioink Formulations

Abbr.	Formulation	Selection criteria	Printing conditions				
			Pressure (kPa)	Flowrate (mm³/min)	Feedrate (mm/min)	Layer height (μm)	Nozzle size (μm)
PF	40% Pluronic F127	Standard bioink	258	84	150	420	330
GG/GM	1.2% Gellan Gum + 4% GelMA	Testing formulation	164				
Alg-Lap-RD	1% Alginate + 6% Laponite RD	2nd high printability comparator	140				
Alg-Lap-EP	1% Alginate + 6% Laponite EP	Testing formulation	75				
ALG	7% Alginate	Viscose hydrogel	742				
MC	8% Methylcellulose	Poor shape fidelity	602				
HA	3% Hyaluronic Acid	Poor shape fidelity	174				

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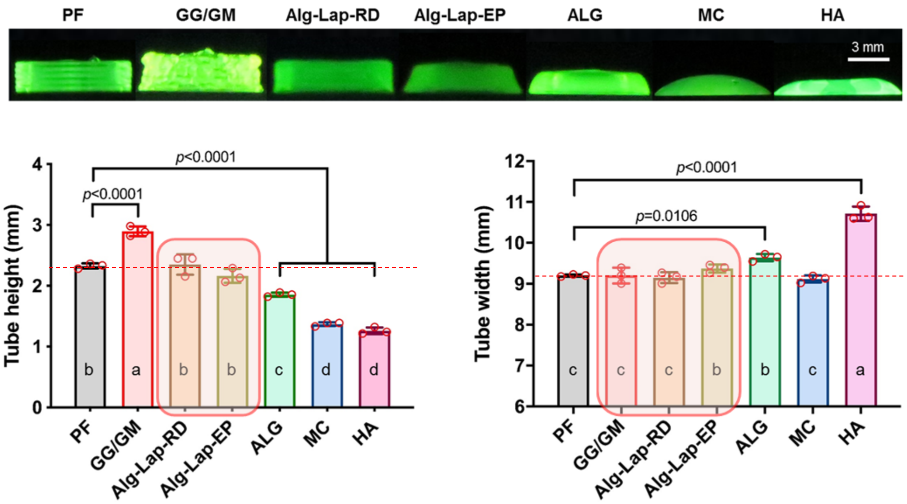
12

12

5-layer Tube (Side View)



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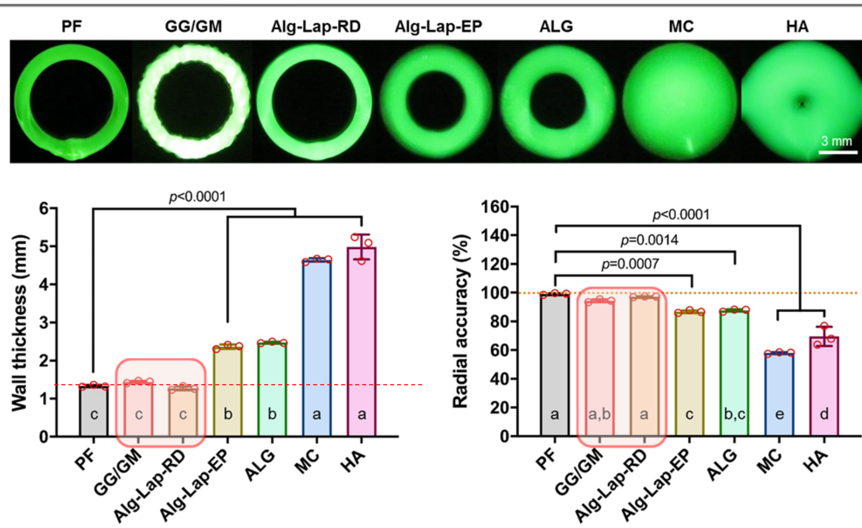
5-layer Tube (Top View)



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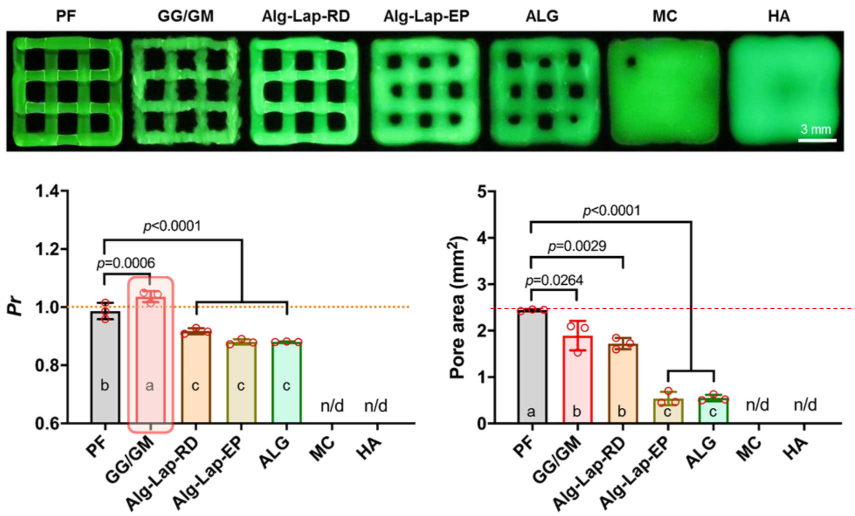
Crosshatch



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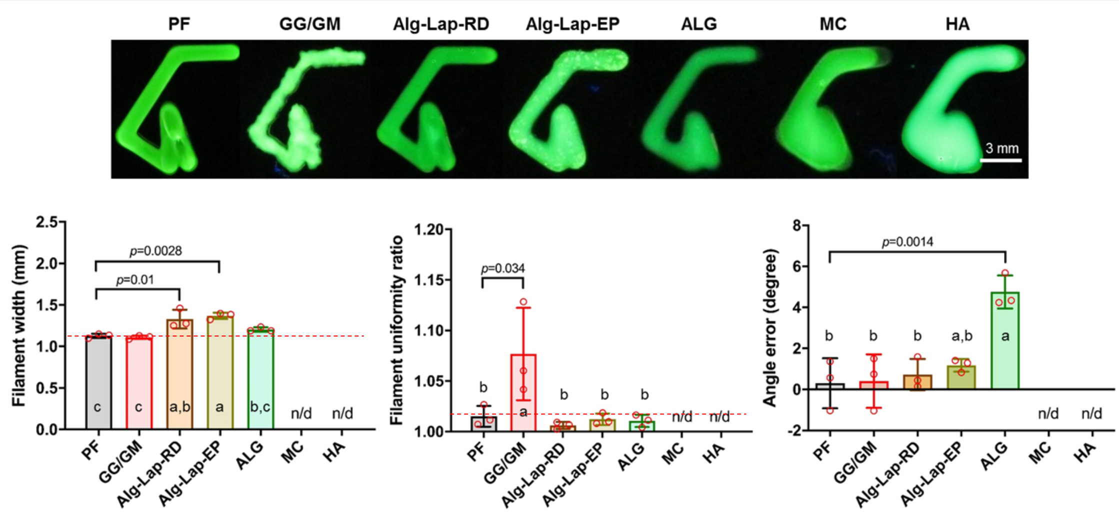
15

4-Angled Pattern



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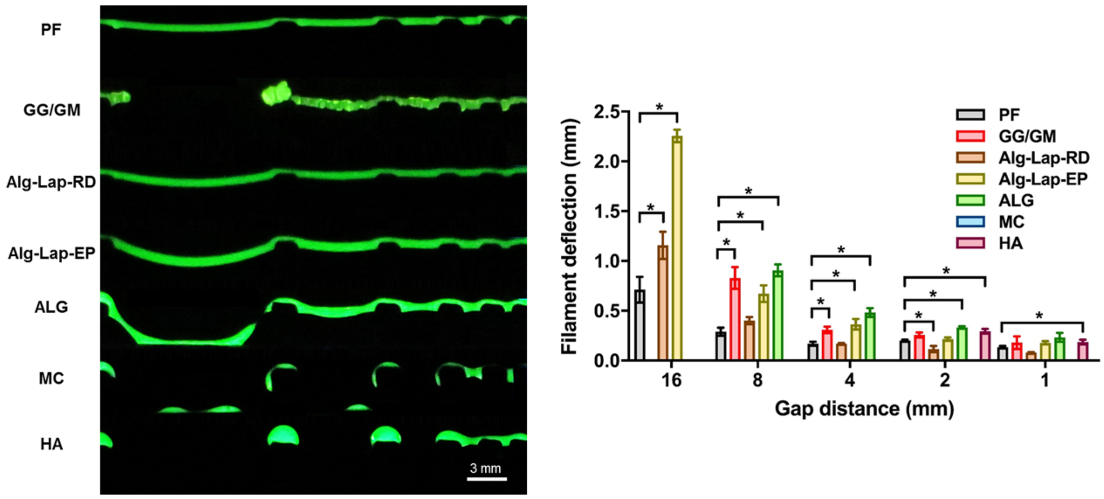
16

16

Overhang Collapse



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17

Printability Outcomes

Abbr.	Printing conditions									
	Tube height	Tube width	Wall thickness	Radial accuracy	*Pr*	Pore area	Filament width	Uniformity	Angle error	Filament deflection
PF (standard)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
GG/GM	++	+++	+++	+++	+++	+++	+++	+	+++	+
Alg-Lap-RD	+++	+++	+++	+++	++	++	++	+++	+++	+++
Alg-Lap-EP	+++	+++	++	++	+	+	++	+++	+++	++
ALG	+	++	++	++	+	+	+++	+++	+	+
MC	+	+	+	+	n/d	n/d	n/d	n/d	n/d	+
HA	+	+	+	+	n/d	n/d	n/d	n/d	n/d	+

+++ Good; ++ Intermediate; + Poor; n/d: not detectable

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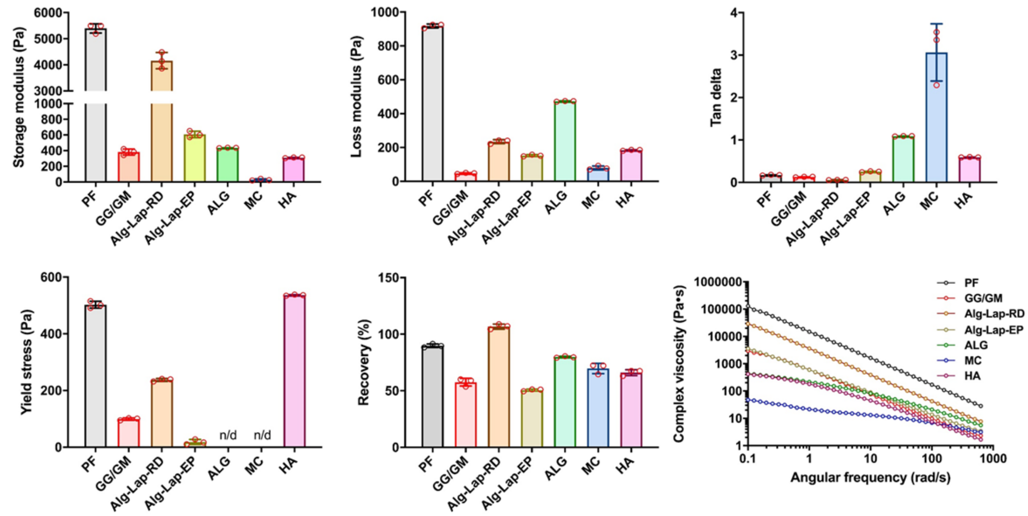
18

Rheological Properties



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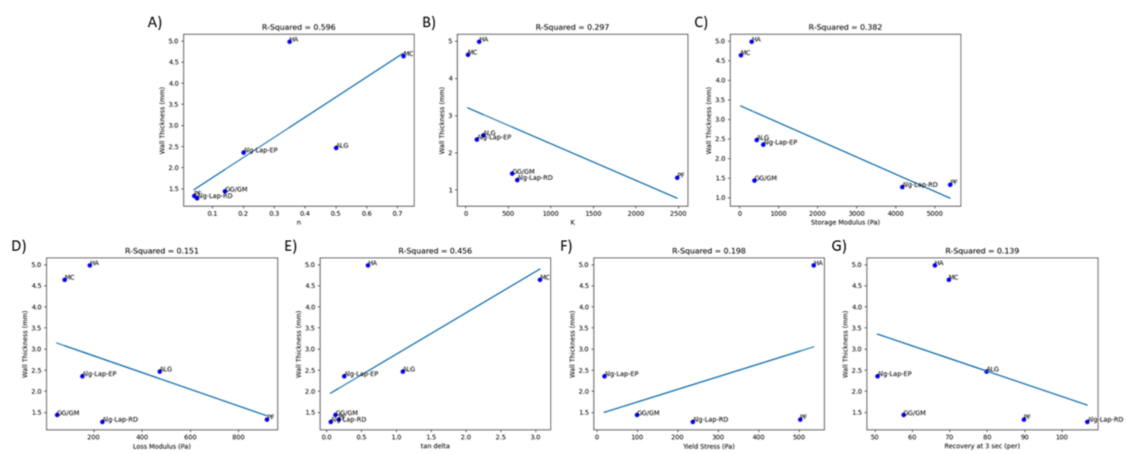
19

Rheology vs. Printability (Linear Regression Analysis)



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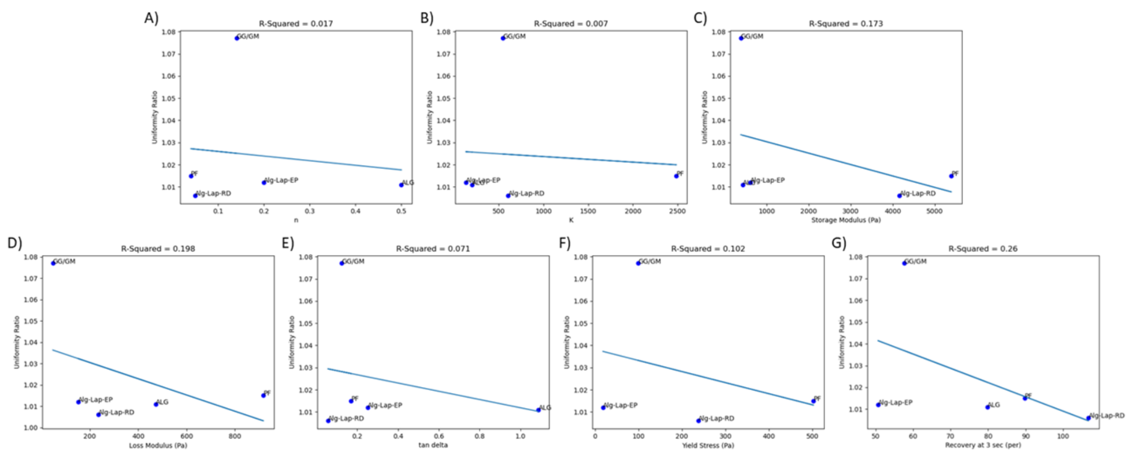
20

Rheology vs. Printability (Linear Regression Analysis)



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21

21

Rheological Properties vs. Printability

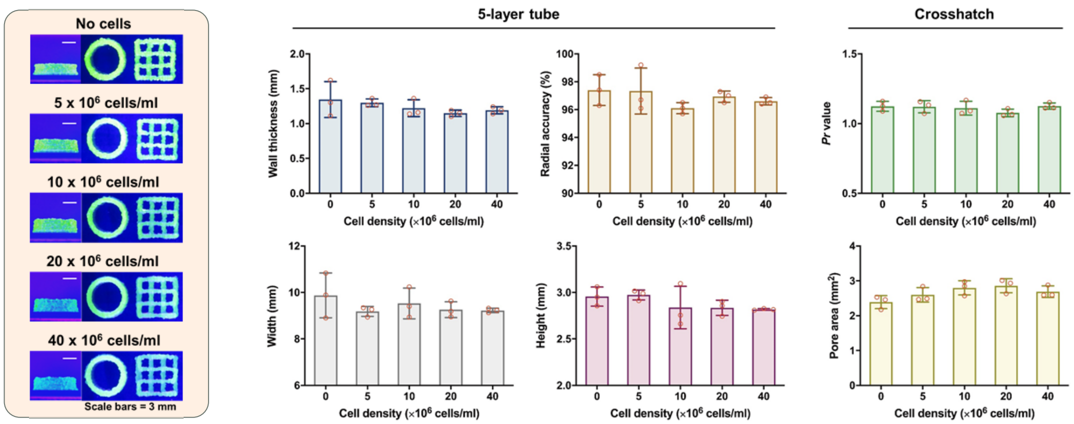
- Rheological measurement is valuable insight into the bioink's shear-thinning, viscoelastic, yielding, and recovery properties
- Loss modulus (G") is not predictive of printing outcomes
- Rheological measures are not predictive of uniformity, except, low G" may be an indicator of poor uniformity
- No rheological parameter alone was able to predict relative printability
- Printing outcomes must be measured directly rather than inferred from rheology

- *Thus, standardization of printability measurement is essential for bioink development*

22

22

Effect of Cell Density on Printability



Gillispie et al., Tisuse Eng 2020

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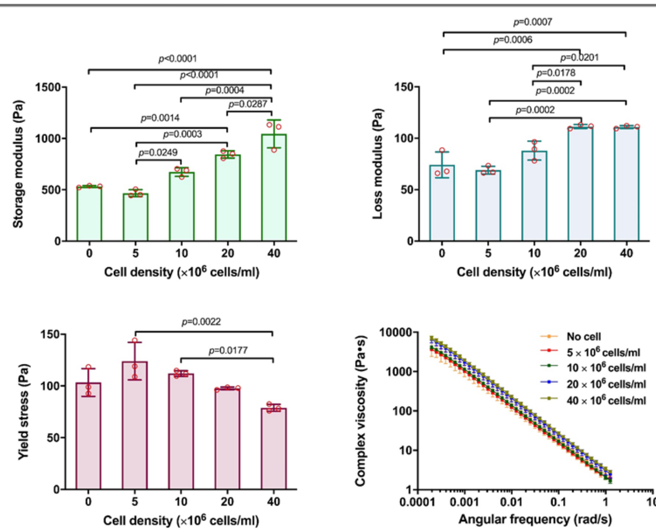
Effect of Cell Density on Rheological Properties



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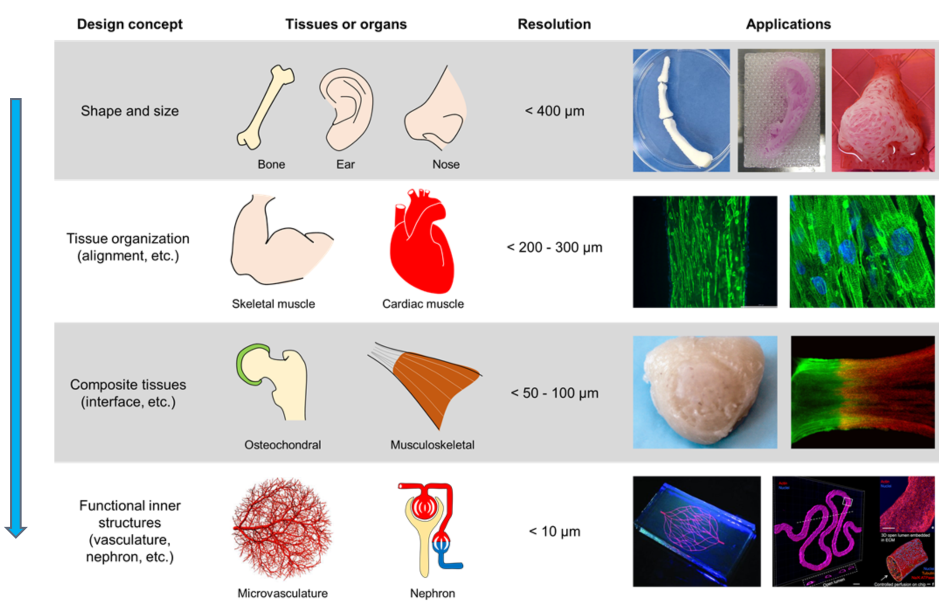
Effect of Cell Density on Printability

- The effect of cell density on printing outcomes have been investigated in the GelMA/GG composite bioink
- No effect on printability was seen for cell densities up to 40 × 10^6 cells/mL
- Rheological measures showed some variation between the bioinks with different cell densities.
- Both storage modulus (G') and loss modulus (G") increased moderately as cell density increased
- Yield stress showed slight changes, initially increasing as cells were introduced at 5 × 10^6/mL and then decreasing from there as cell density increased
- All bioinks showed similar shear-thinning abilities with analogous *K* and *n* constants



Moroni et al. Nat Rev Mater 2018

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48

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49