08/14/2019
SFF 2019
0815 hrs
Rm. 415AB

Material-structure-property Optimization and In-process Monitoring of 3D Printed Bone Tissue

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Acknowledgements

University of Nebraska-Funding
National Science Foundation (NSF).
CMMI 1739696
CMMI 1752069
CMMI 1719388

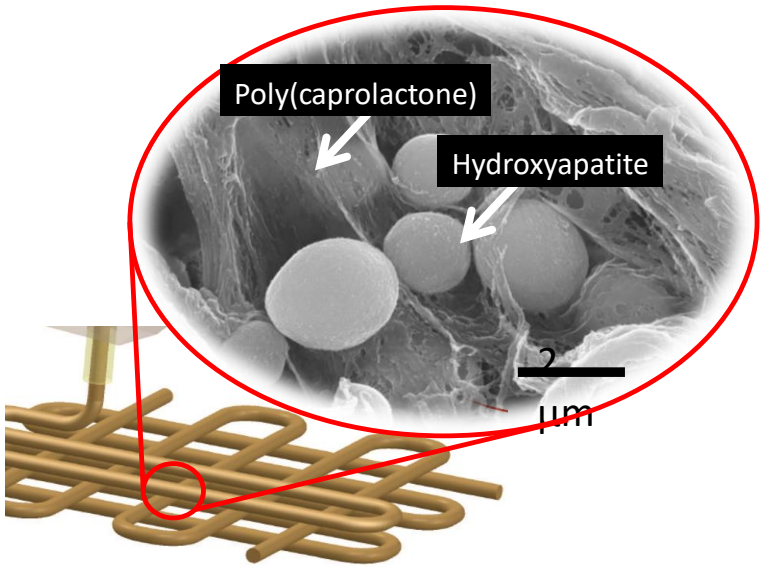
Defect-free 3D printing of biological constructs.

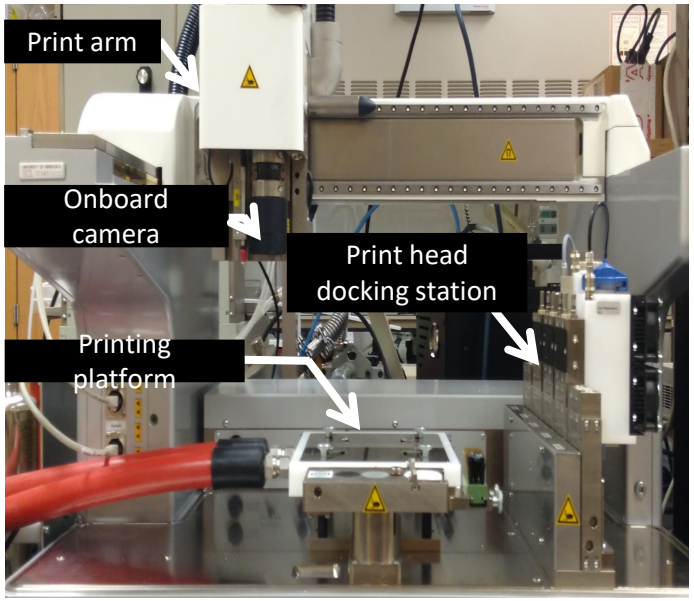
Objectives

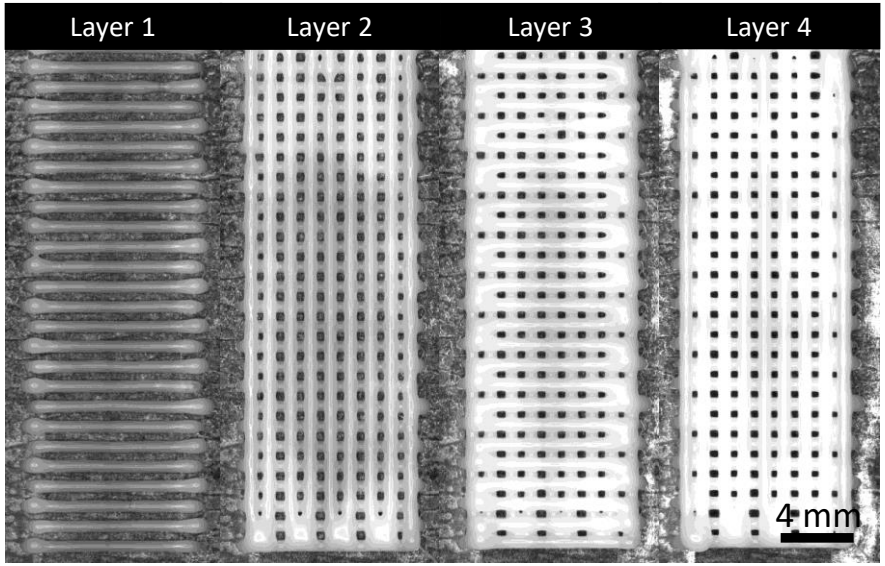
1. Optimization and testing of the bioprinted constructs in the context of extrusion-based printing of poly(caprolactone) (PCL)-hydroxyapatite (HAp) composite biological constructs.
2. Unveil the process- mechanical property relationships in printing of various PCL-HAp composites.
3. Demonstrate the use of using in-situ image-based monitoring for quantification of key quality-related aspects of biological constructs, such as geometric integrity.
4. Ascertain the *in vitro* osteoinductivity of the fabricated constructs.

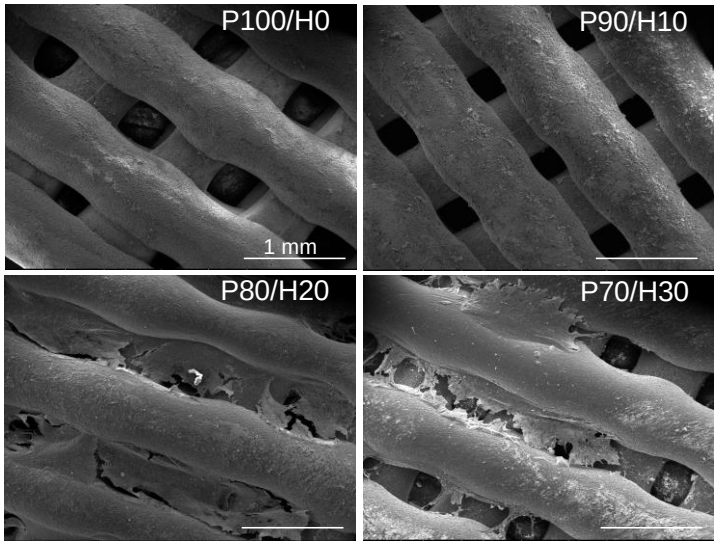
Process Parameters → In-Situ Sensor Data → Construct Quality
→Mechanical Properties → Cell Viability.

The Big Picture









Outline

- Background and Rationale
- Methods and Results
  - Optimizing Bioprinting Conditions
  - Mechanical Testing
  - Cell Viability
- Conclusions and Future Work

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Rationale

- Poly(caprolactone) (PCL) is the most popular biopolymer for bone tissue engineering
  - FDA-approved thermoplastic polymer
  - Slow degradation rate (ranging from months to a few years), which is comparable to the rate of bone regeneration.
- PCL is not *osteoinductive* (bone does not penetrate and grow into the polymer)
  - Hence, mixed with bioceramics and bioglasses
  - Scaffolds of PCL and Hydroxyapatite (Hap) have been successfully 3D printed and tested to induce osteogenesis in vitro and in vivo.

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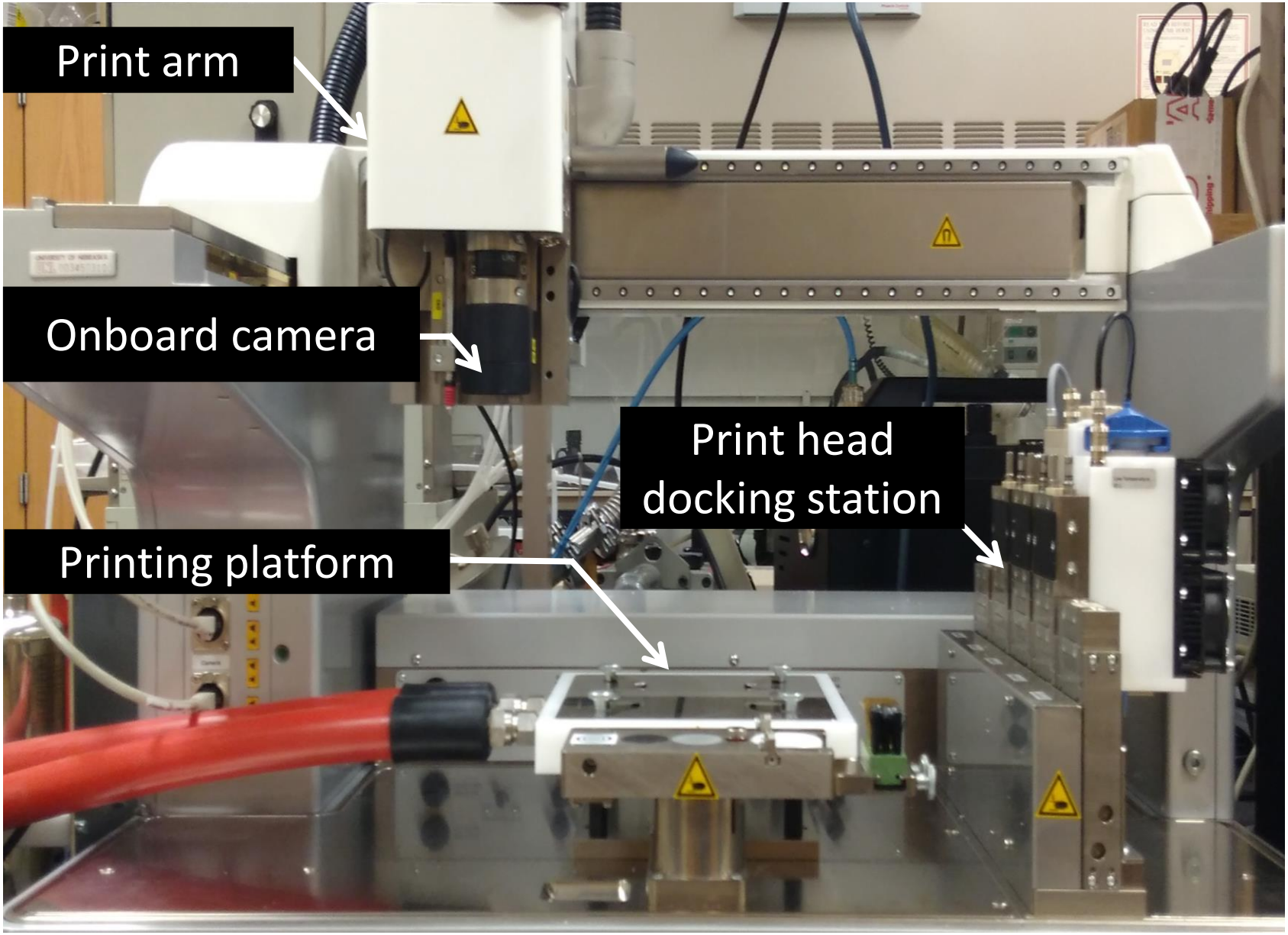
EnvisionTec Bioprinter with Onboard Camera

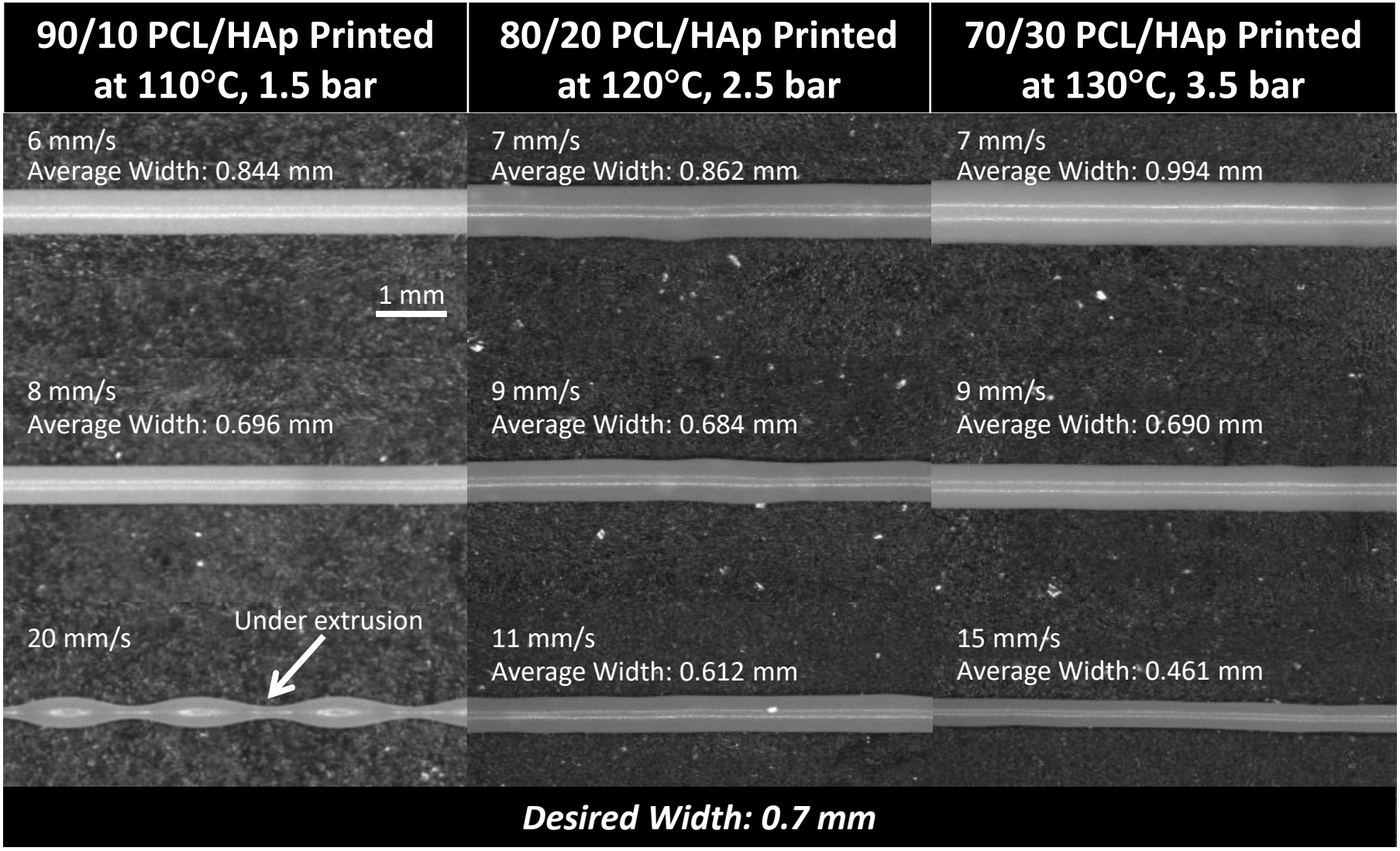


Evolutionary Optimization Experimental Methodology

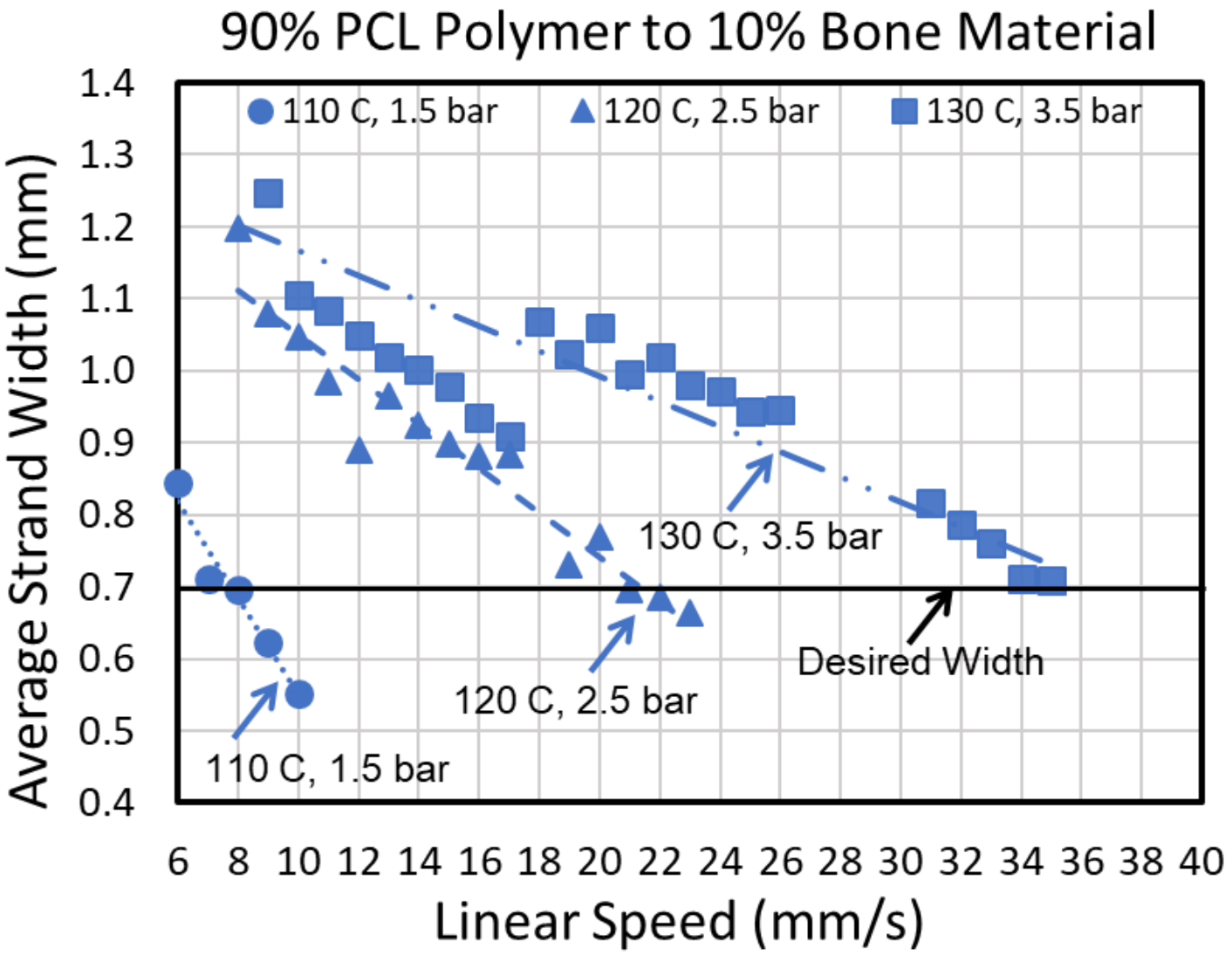
There a large number of printing variables.

Not possible to do a conventional factorial design of experiment.

Linear Speed Optimization
Using image analysis of single strands

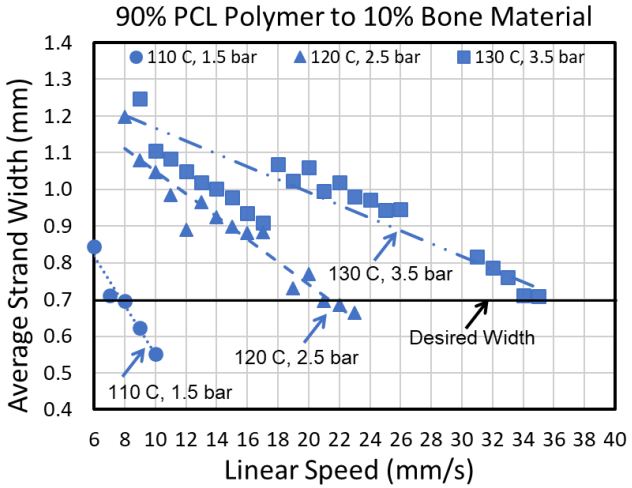


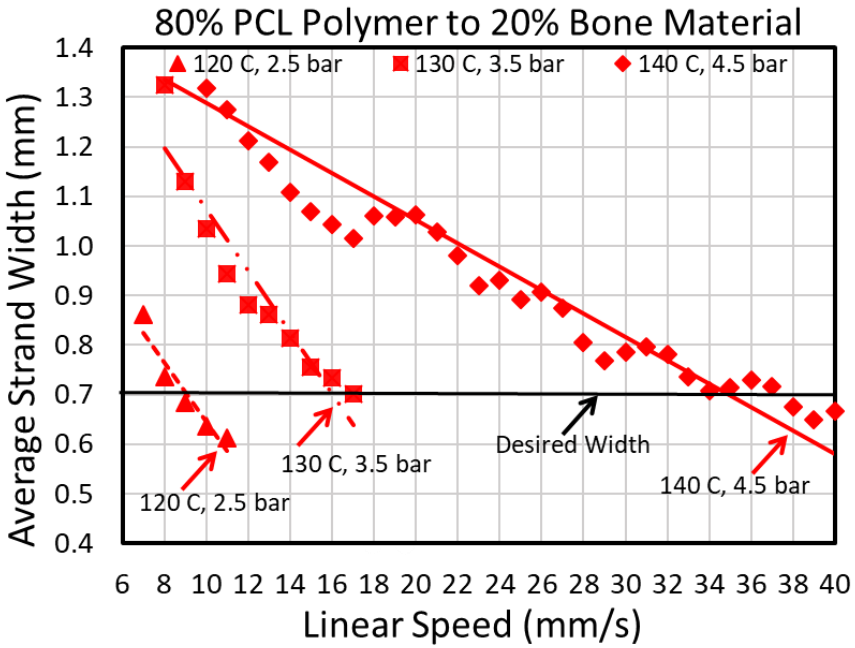
Infeasible to obtain the desired strand width at some conditions

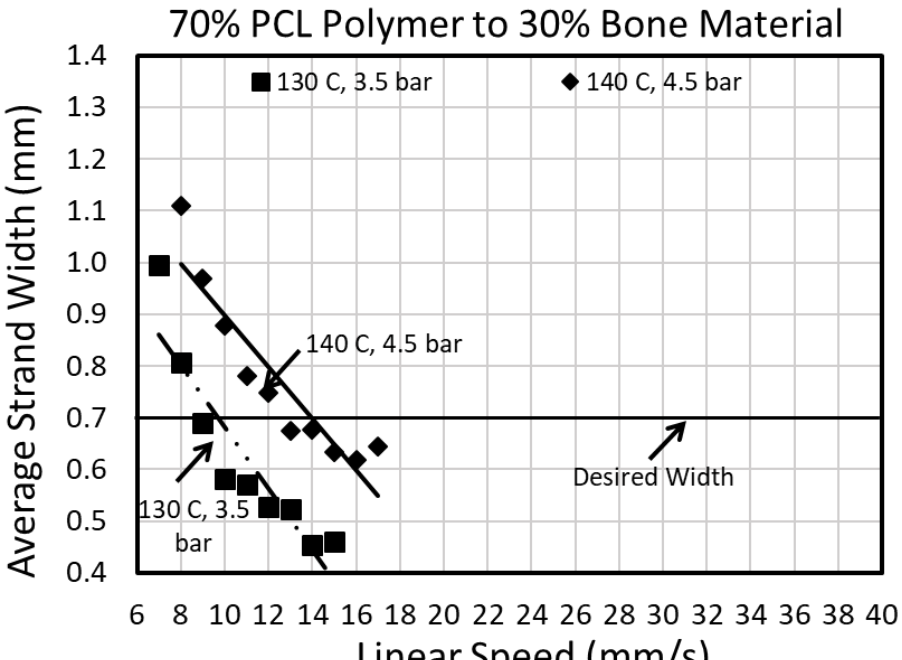


Every material composition has its own “sweet spot”

Need to understand and explain the rheology of the material.







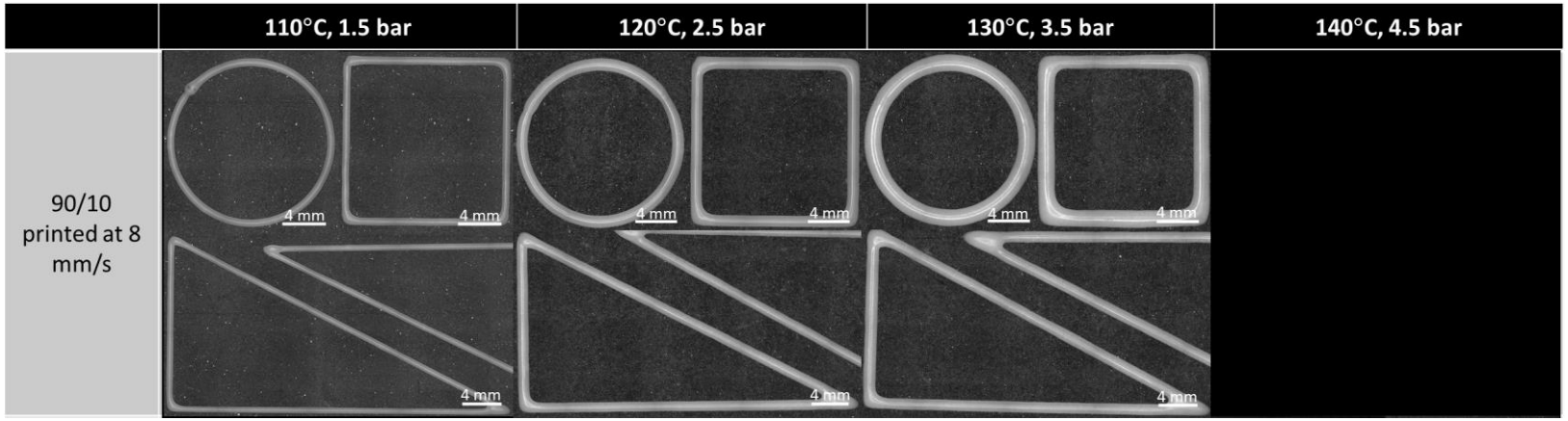
Finding the sweet spot requires trial-and-error in the absence of a model-based approach.

		Condition 1 110°C, 1.5 bar	Condition 2 120°C, 2.5 bar	Condition 3 130°C, 3.5 bar	Condition 4 140°C, 4.5 bar
Composition: %PCL/HAp	90/10	8 mm/s	21 mm/s	35 mm/s	>40 mm/s
	80/20	<5 mm/s	9 mm/s	17 mm/s	37 mm/s
	70/30	<5 mm/s	<5 mm/s	9 mm/s	13 mm/s

Printing single strands is trivially simple.

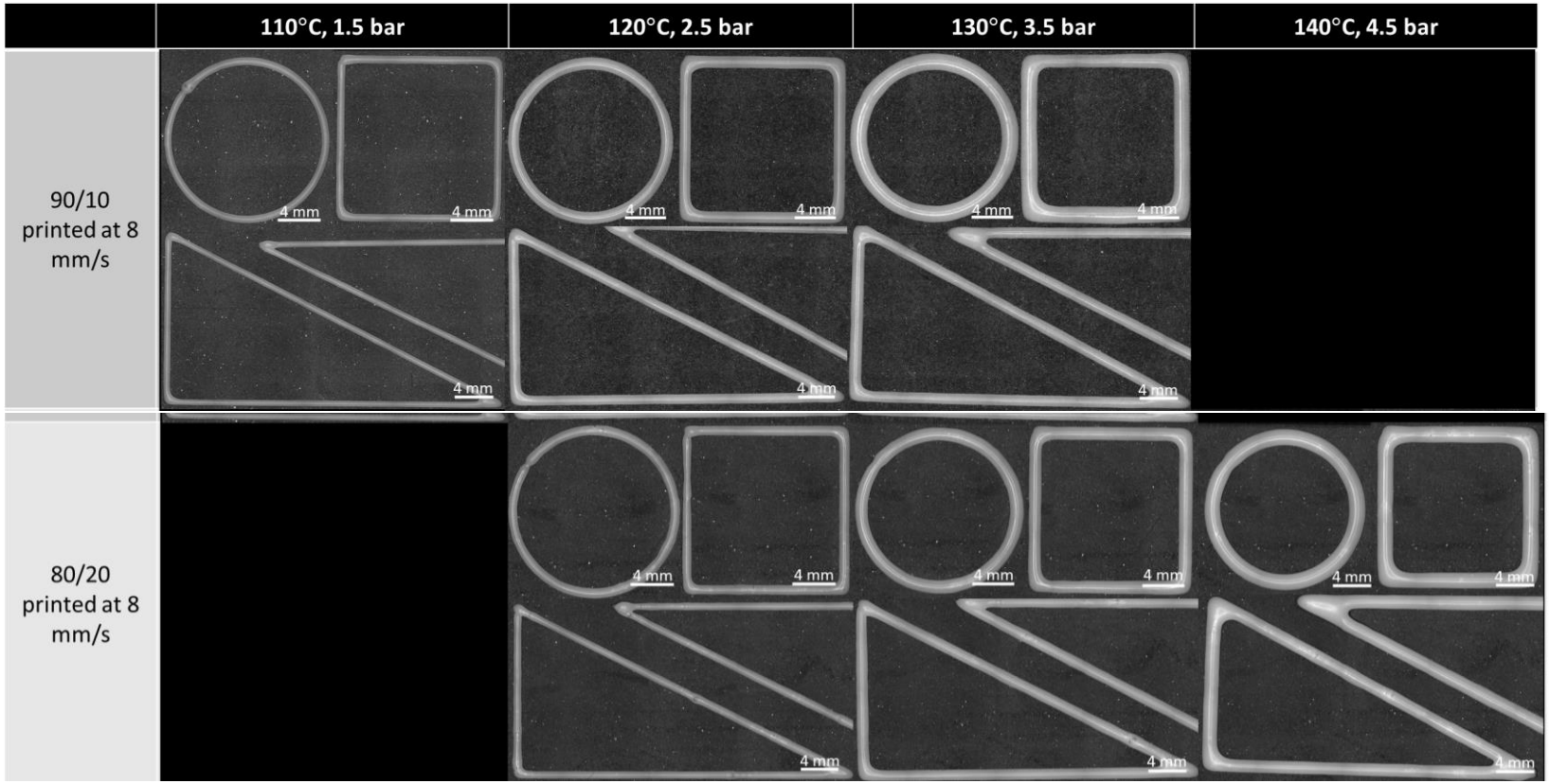
Geometric integrity is important.

The Circle-Square-Diamond Test



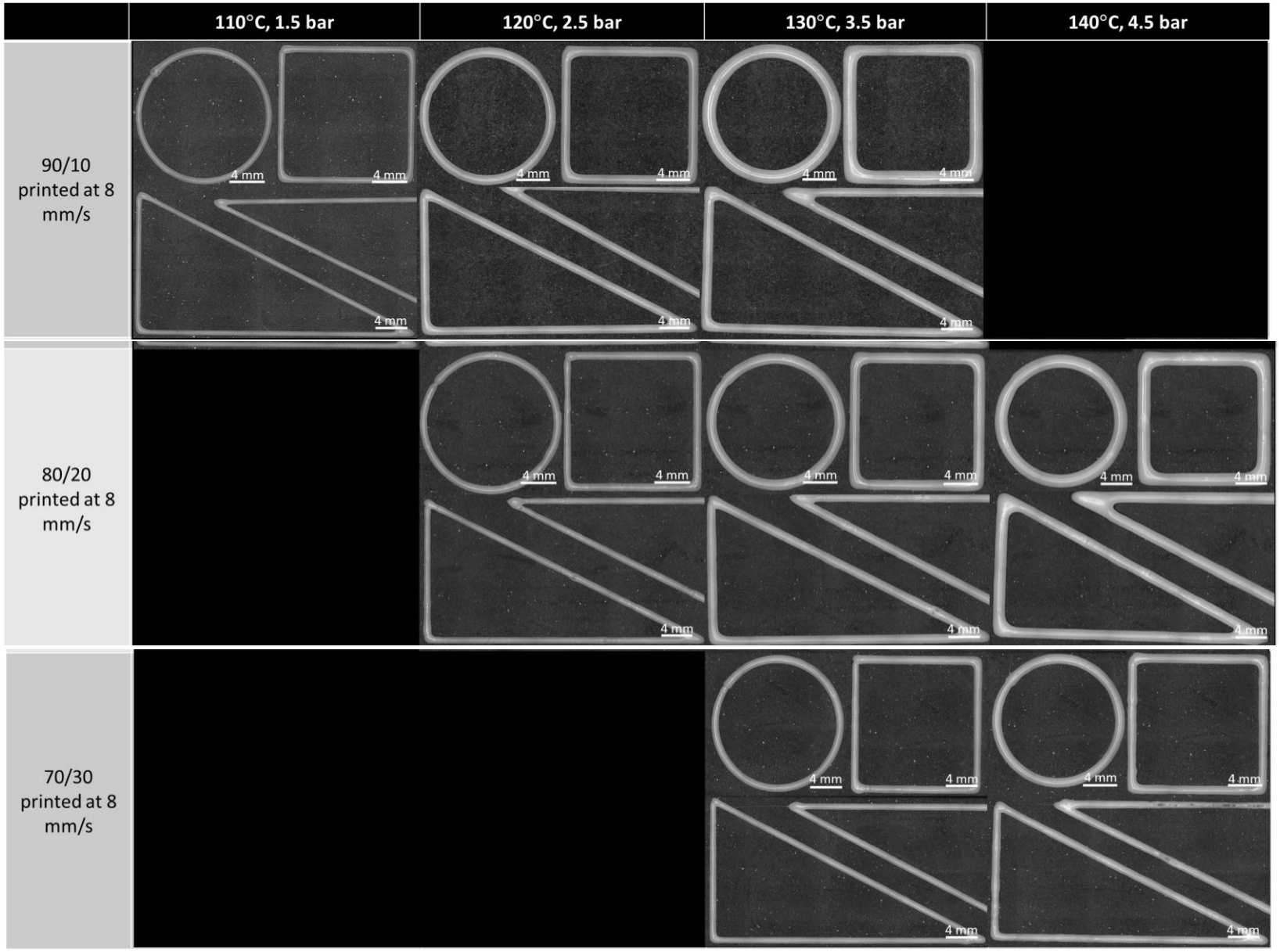
Increasing temperature and pressure aids deposition (viscosity decreases) upto a certain threshold

The Circle-Square-Diamond Test



Not all conditions are conducive to print (there is a sweet spot)

The Circle-Square-Diamond Test



Circularity is used to quantify geometric integrity

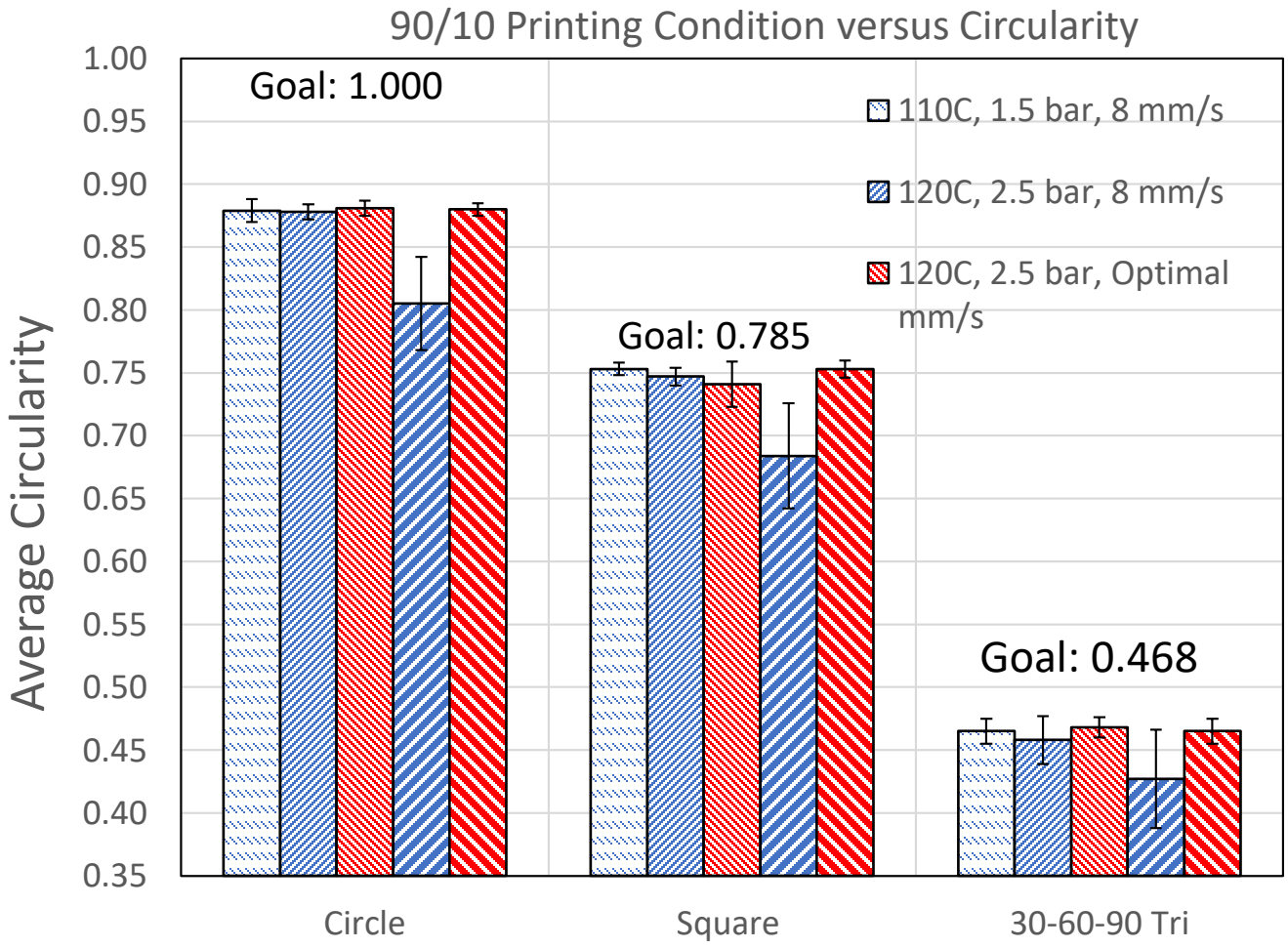
$$C = (4\pi A)/ P^2$$

C is circularity (dimensionless)

A is the area (mm^2), and P is perimeter (mm).

Quantifying Geometric Integrity

Ten circularity measurements per shape and condition



Avoid printing circular scaffolds.

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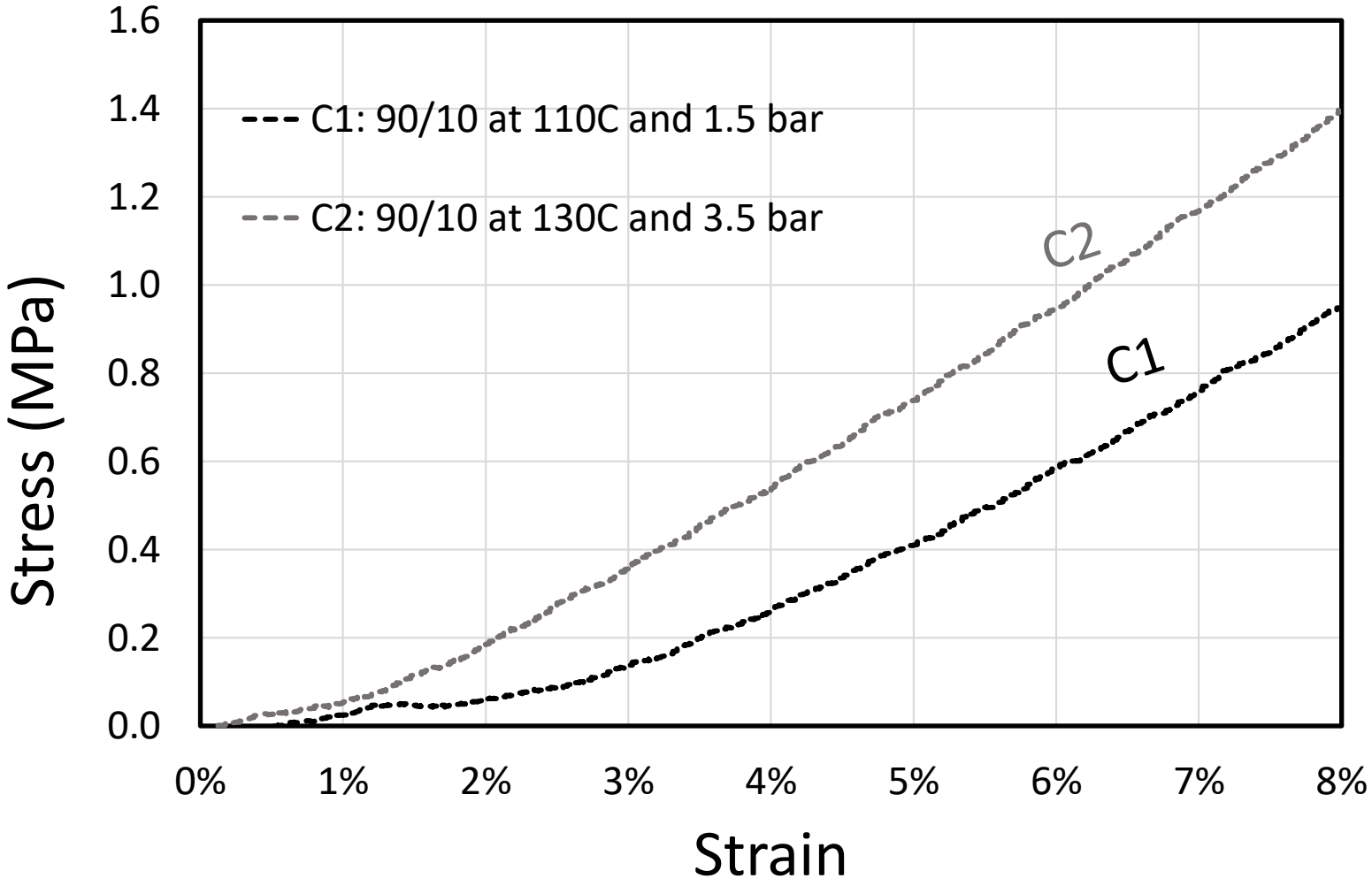
Mechanical Compressive Testing

Compressive testing of solid 10 mm x 10 mm x2.5 mm samples was conducted.

- Four samples were prepared with theoretically solid box architectures for both the lowest and highest print condition and optimal linear print speeds for a composition.
- Compress to a strain of 8% over the course of a minute.
- Compressive modulus was determined as the slope of the linear region of the stress-strain curve.

Mechanical Strength → *f*(process conditions)

Higher printing temperature and pressure relates to higher compressive strength.



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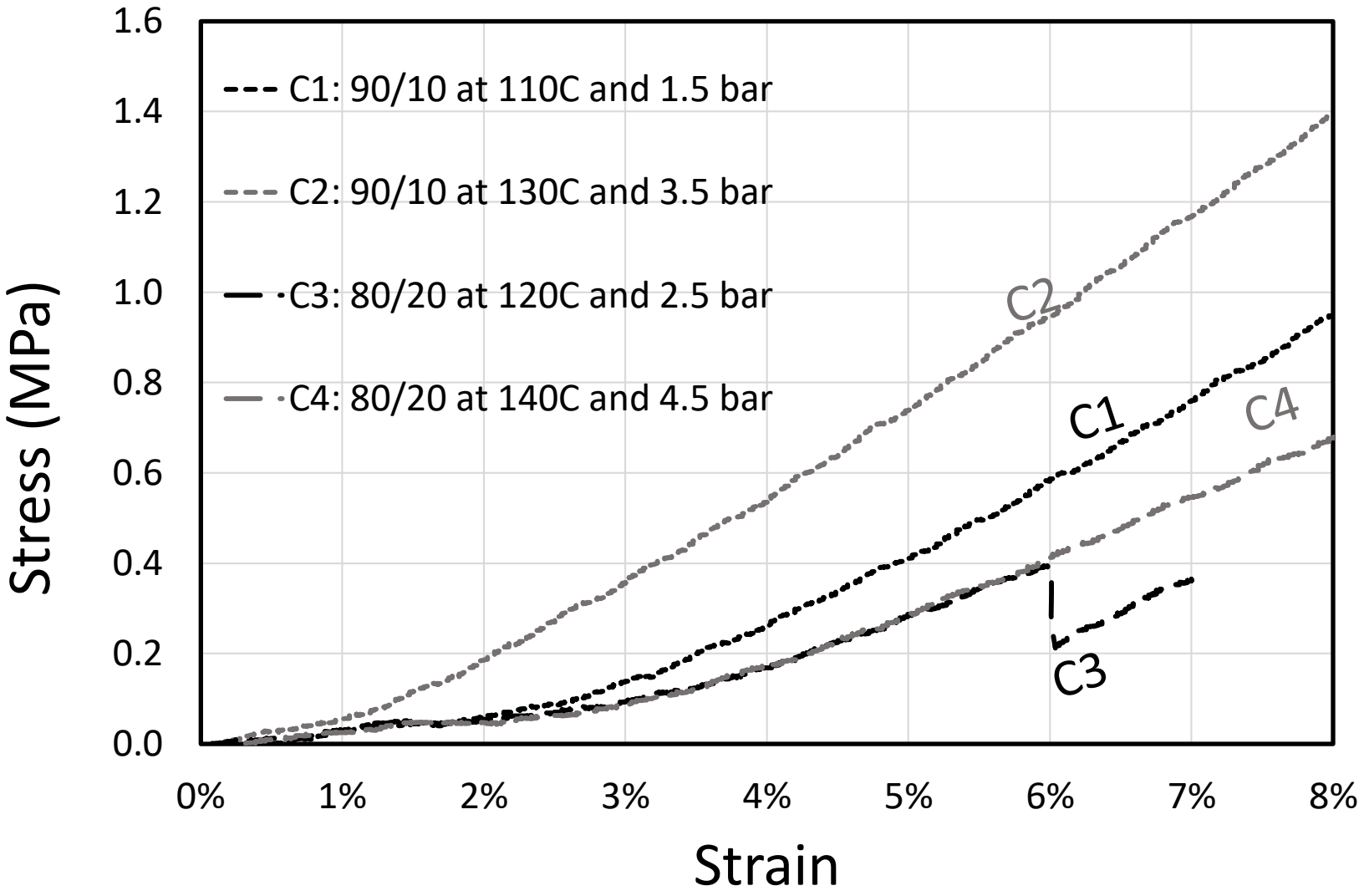

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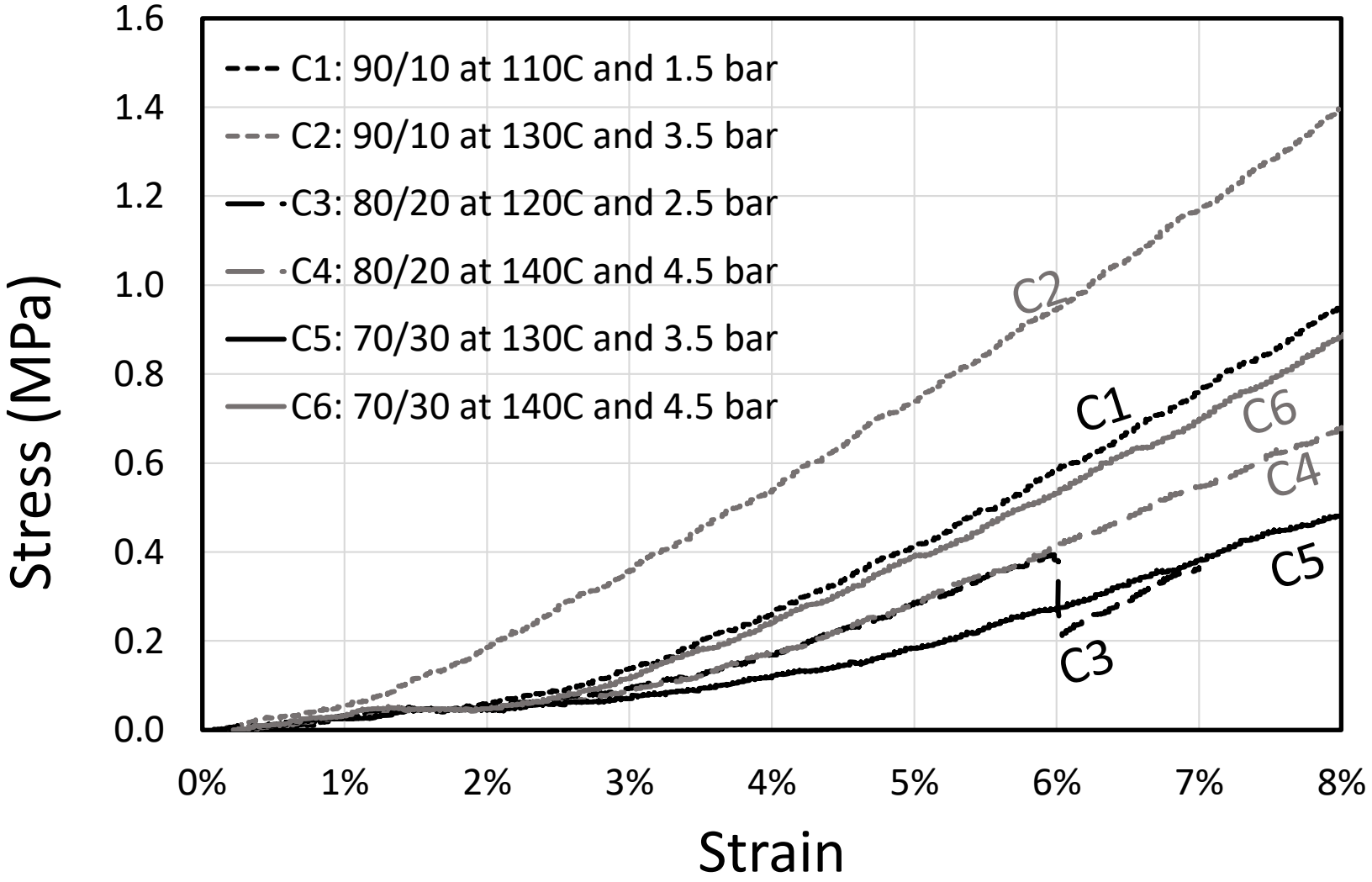
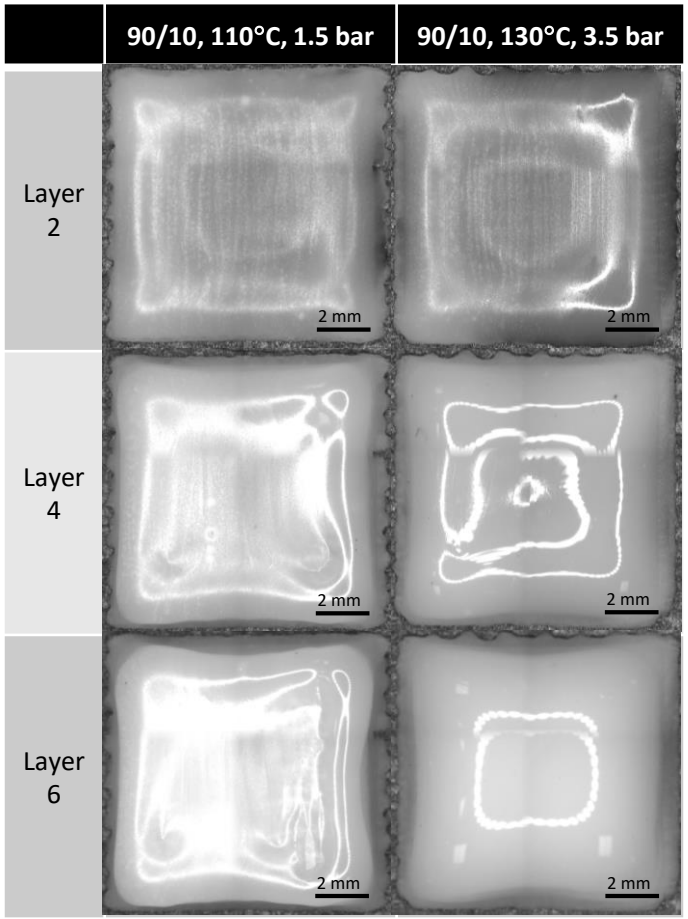
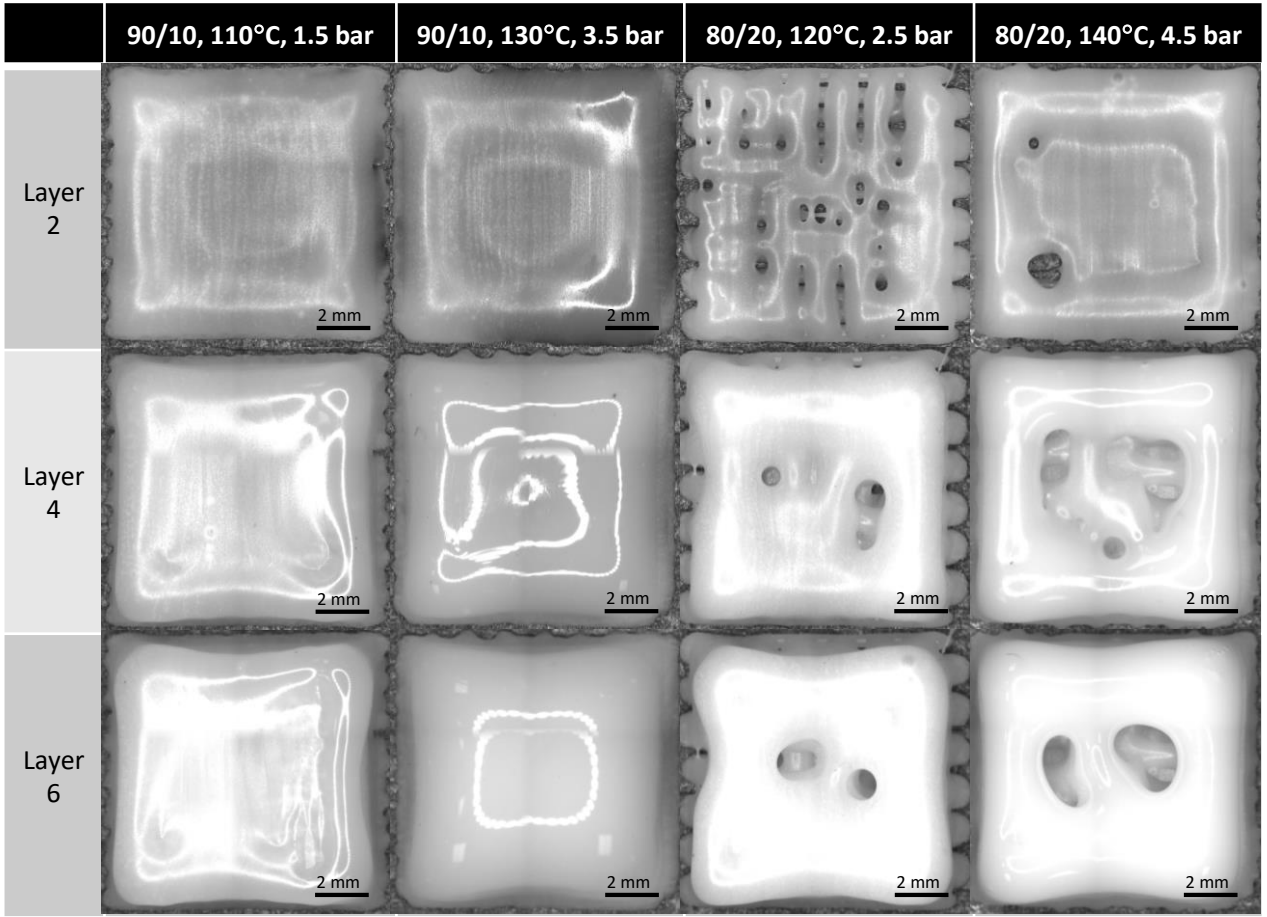


Explanation of Poor Compression Results (In-situ Images of the worst performing sample from printing)



Defects are the root cause of poor mechanical performance.

Explanation of Poor Compression Results
(Pictures of the worst performing sample from printing)



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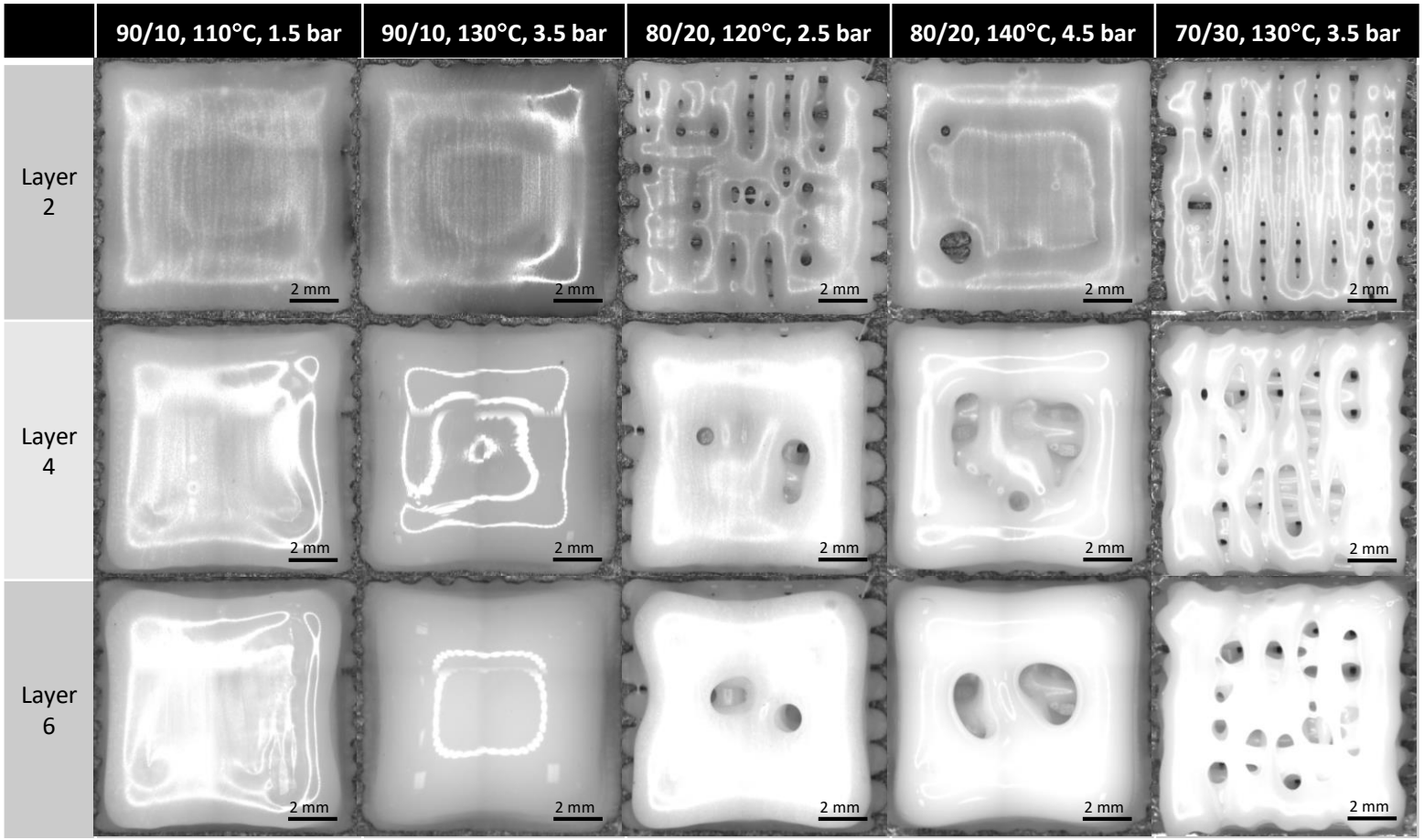
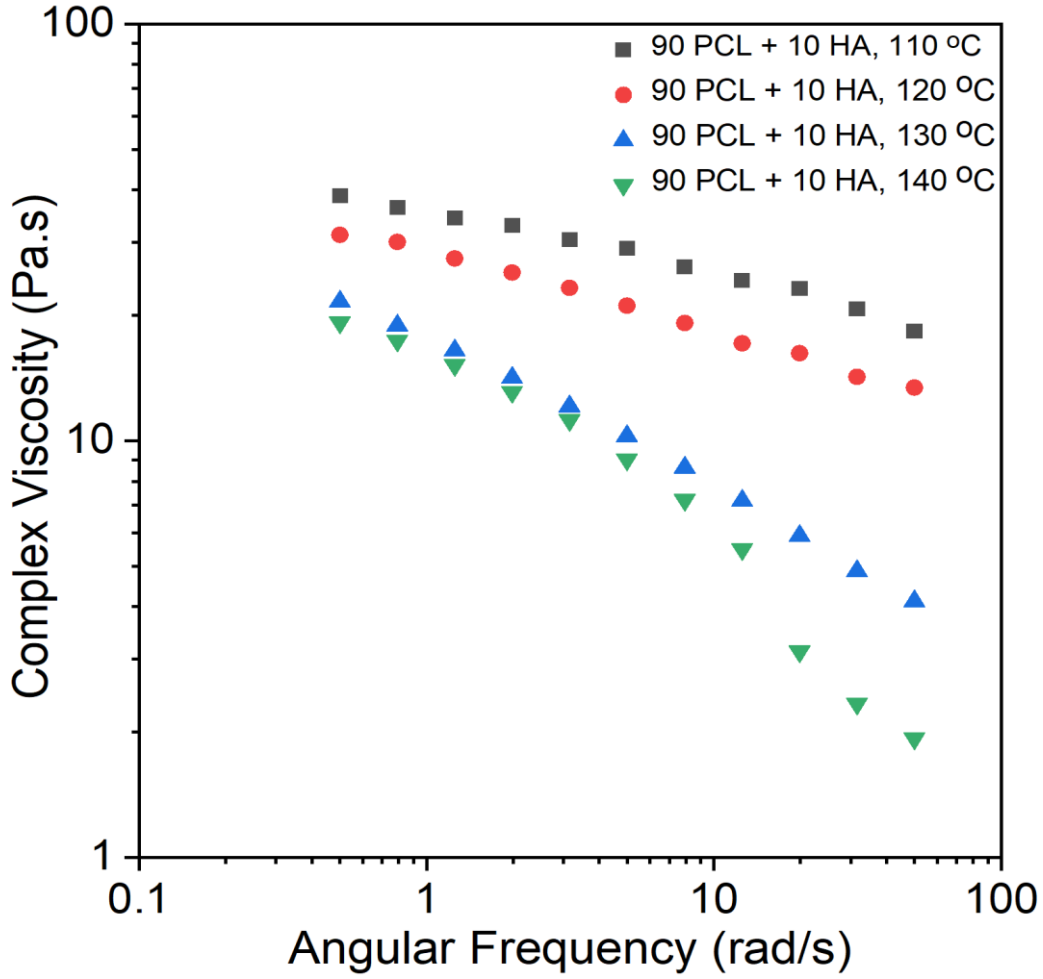


Defects are the root cause of poor mechanical performance.

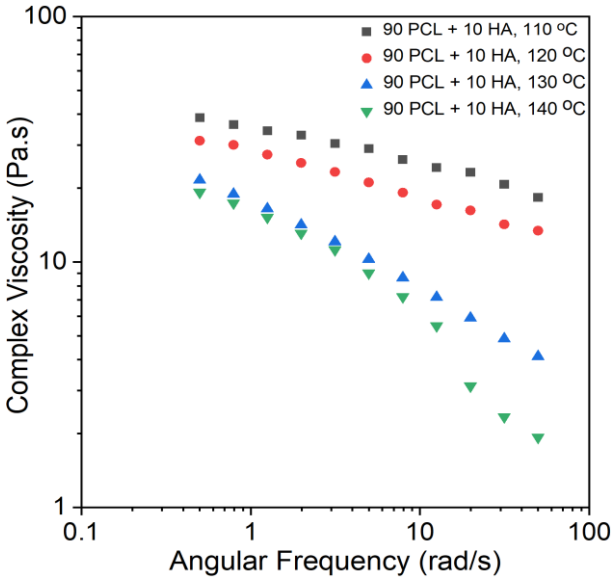
Rheology Testing

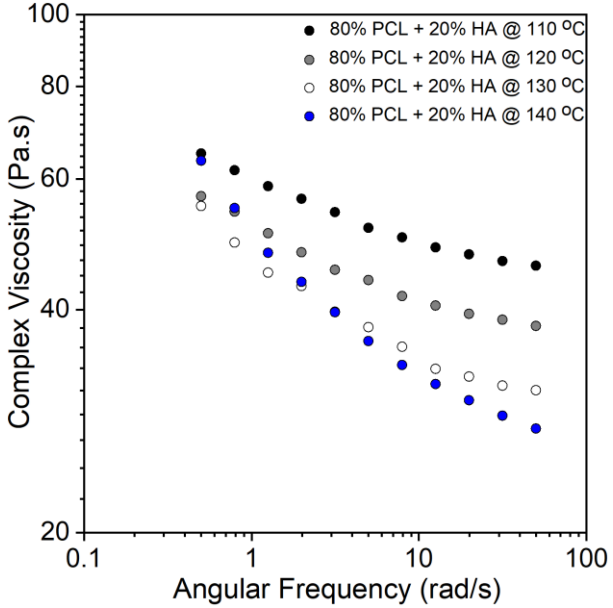
To assess the rheological properties of the developed composites during the extrusion process, a rotational rheometer was used to perform routine oscillatory experiments.

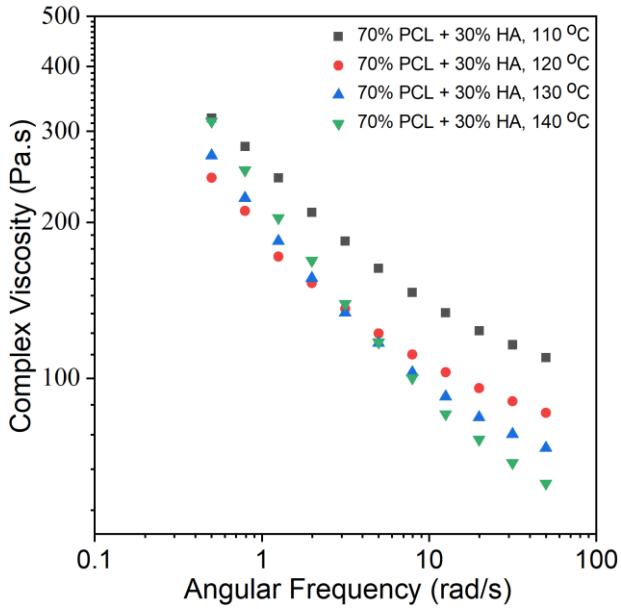
Shear thinning behavior is observed.



Adding HAp increases the viscosity.





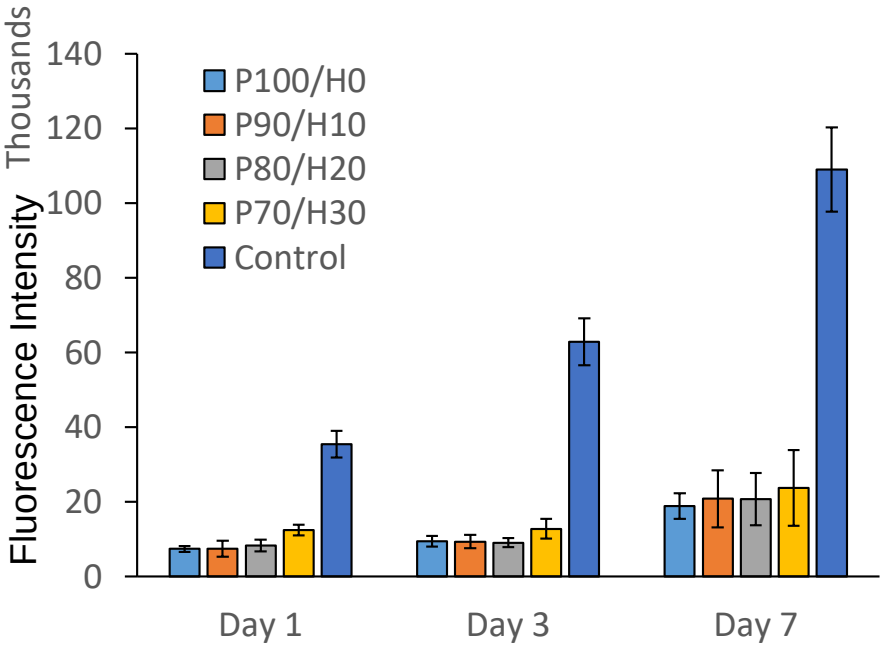


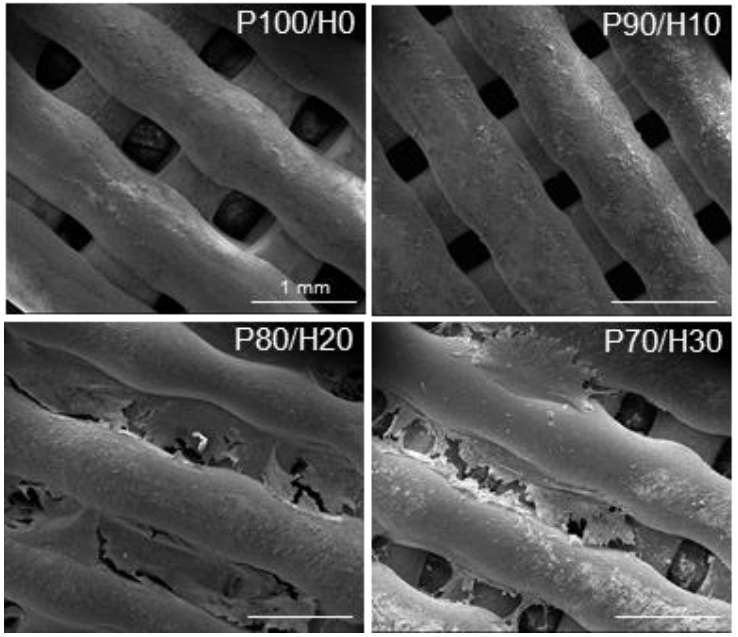
Cell Viability

Effectiveness of the engineered scaffolds in bone tissue engineering is demonstrated by in vitro culture of human mesenchymal stem cells (hMSCs).

Higher HAp concentration correlates to enhanced cell attachment and proliferation.

The viability and proliferation rate of hMSCs were quantified by measuring their metabolic activities using PrestoBlue Cell Viability Reagent on days 1, 3, and 7



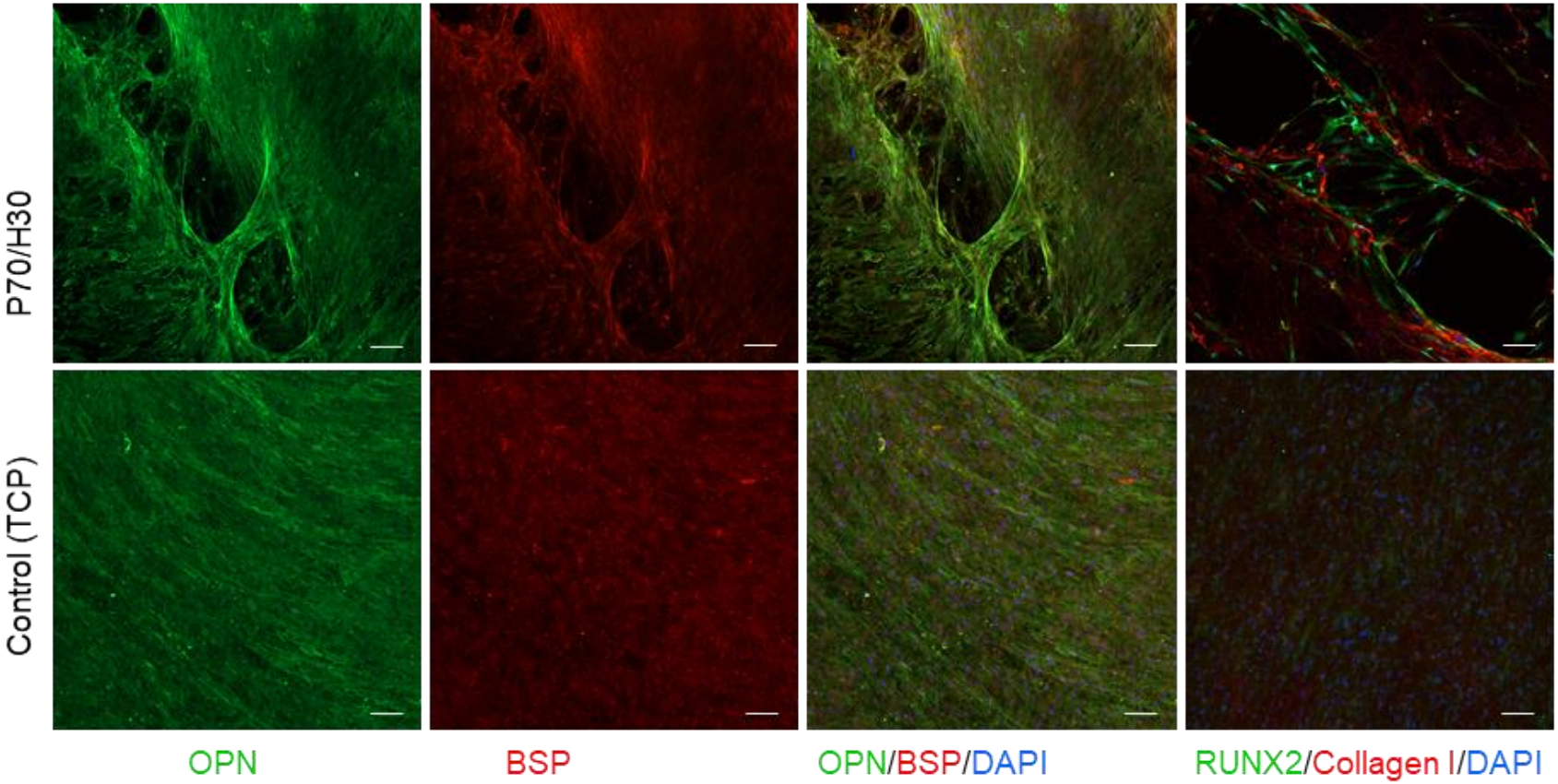


70/30 suggests the positive role of HAp in supporting cell attachment and proliferation.

Immunostaining

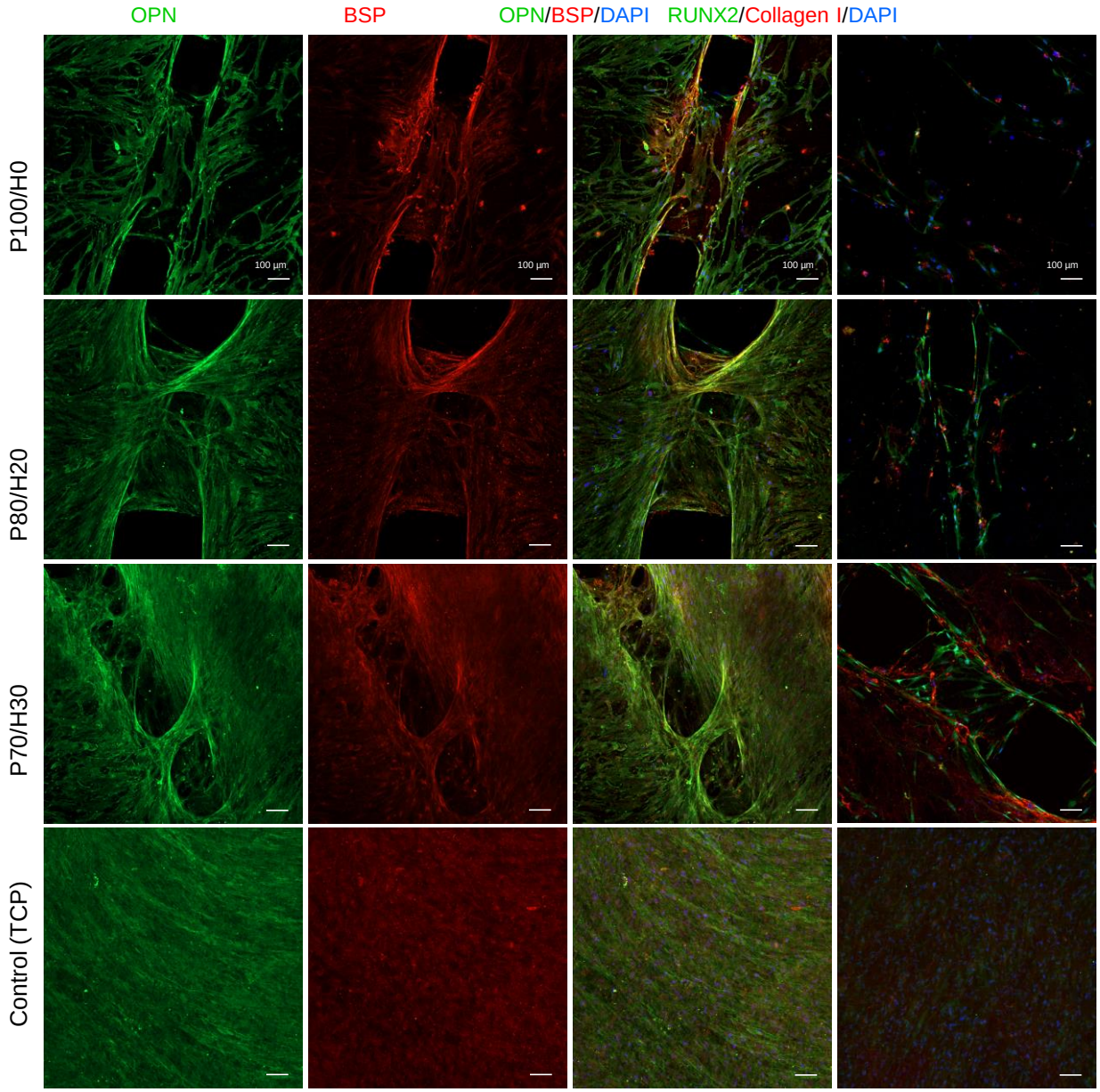
Cellular expression of osteopontin (OPN) and bone sialoprotein (BSP) after 28 days of seeding confirmed the osteogenic maturation of hMSCs

Immunostaining results demonstrates the viability of 3D printed bone tissues



Both OPN and BSP were present in printed samples.

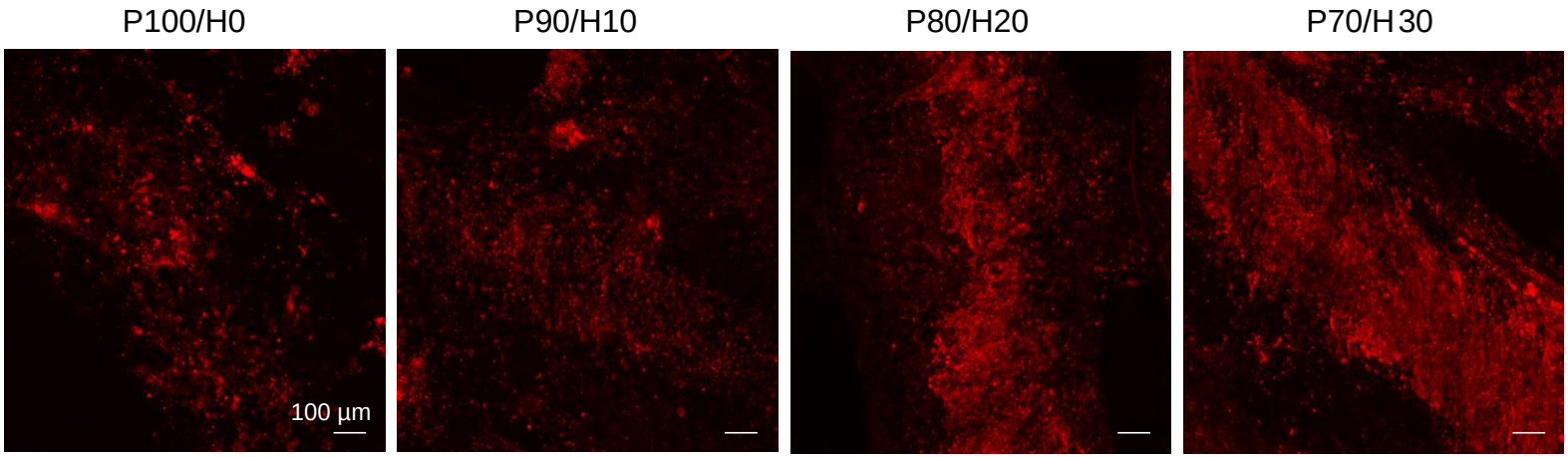
Differentiated cells on samples significantly increased from PCL100/H0 to PCL70/H30 samples.

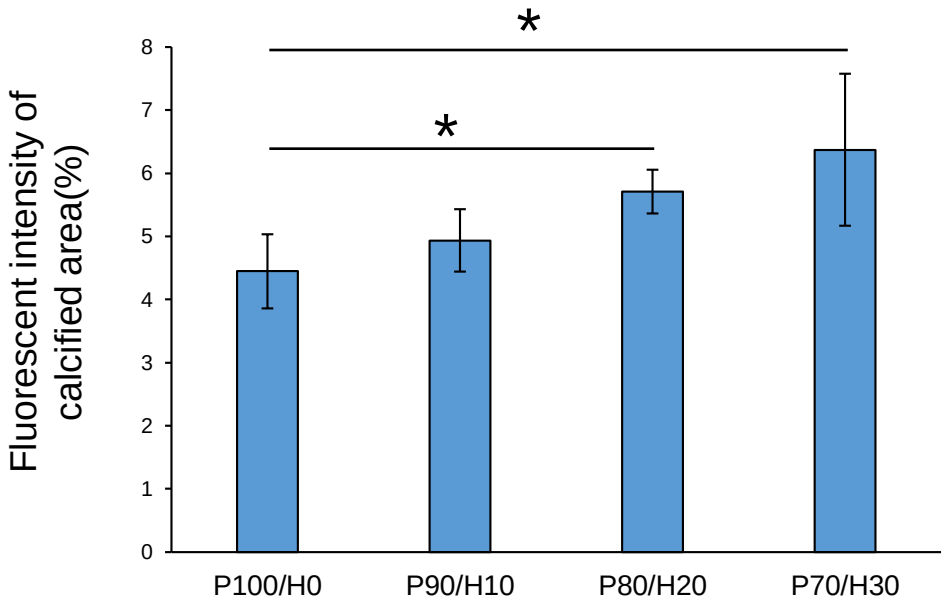


Xylenol Orange Staining

- Osteodifferentiated cells in the late stage of their differentiation have a high calcium deposition.
- To investigate the functional mineralization of differentiated cells, xylenol orange staining was performed on seeded constructs on day 35 of post differentiation.

Calcification area was significantly increased by increasing the HA percentage





Statistically significant increase in calcification with increase in HAp

Conclusions

- Higher concentrations of HAp increases osteoinductivity.
- Defects influence properties (hence in-situ monitoring is key)
- Need to model and explain the process-structure relationships.