

# Effects of Photoinitiators on Cell Viability, Physical Properties, and Microstructure in 3D Bioprinting

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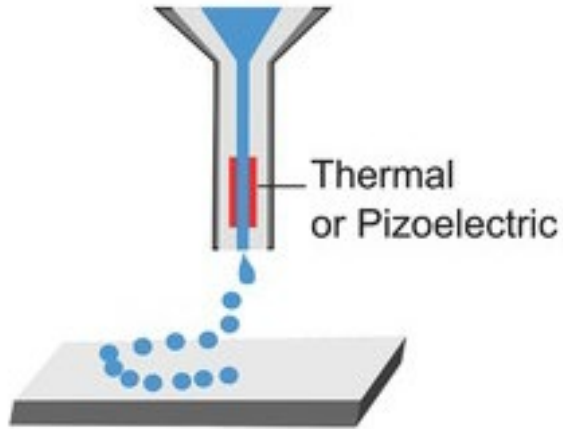
# Contents

- **Introduction and Background**
- **Materials and Methods**
- **Results**
- **Conclusions**

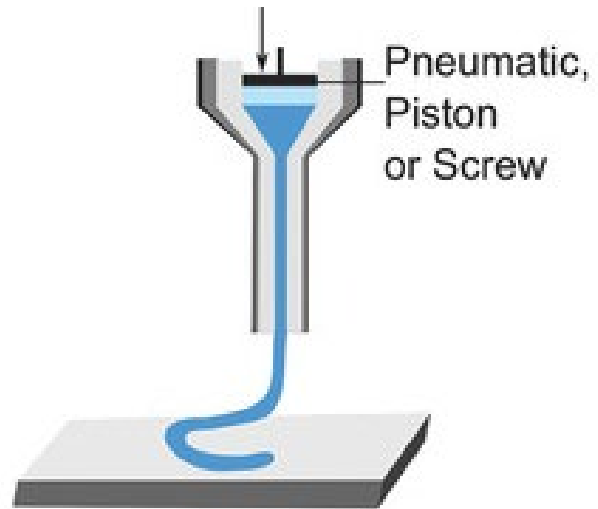


# Typical Bioprinting Techniques

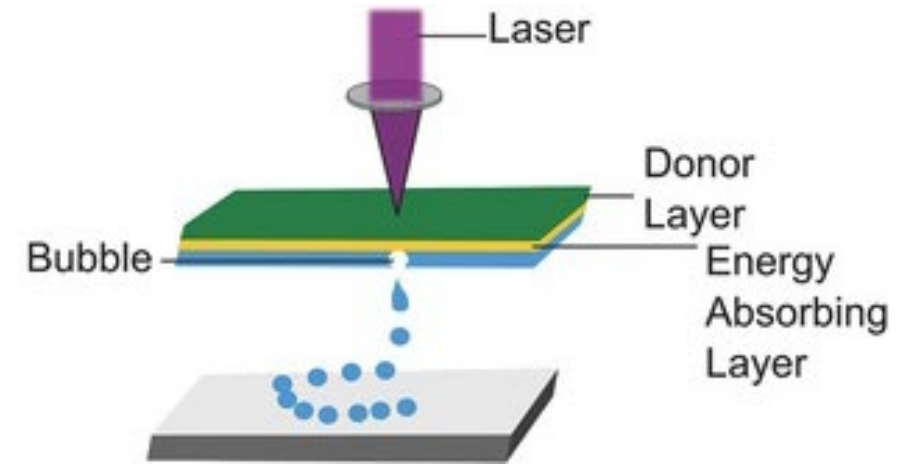
Inkjet-based Bioprinting



Microextrusion Bioprinting



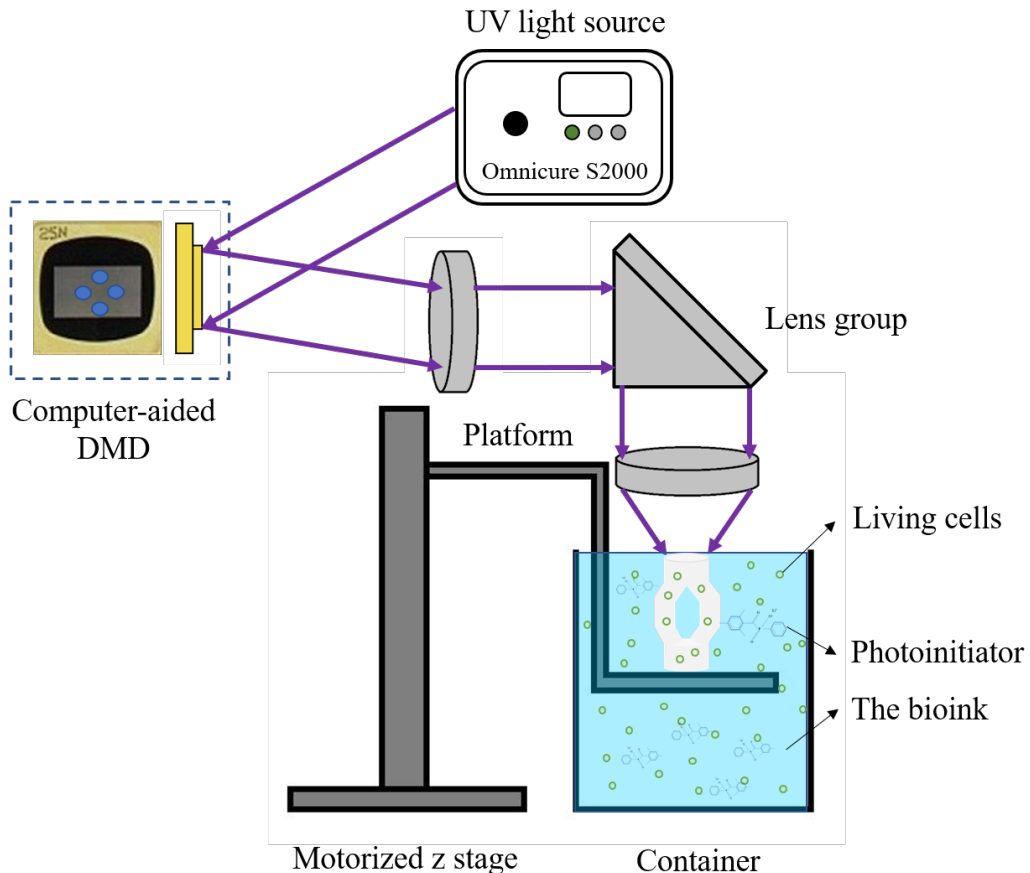
Laser-assisted Bioprinting



[Foyt2018]

# Typical Bioprinting Techniques

## Stereolithography-based Bioprinting



### Mechanism:

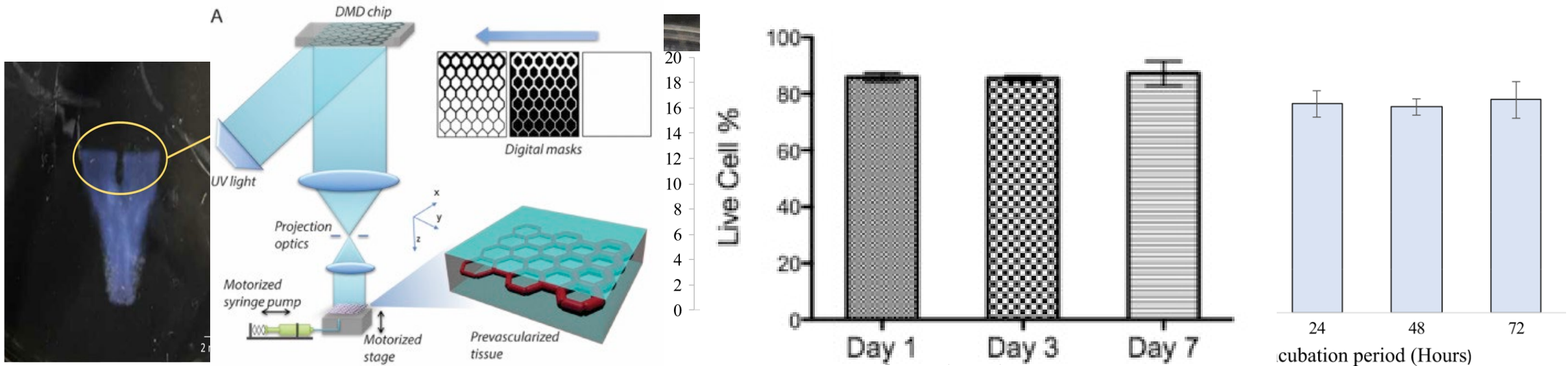
1. Divide 3D model into several 2D patterns
2. 2D pattern controlled by Digital micro-mirror device (DMD), projected onto the bioink (GelMA, photoinitiator, and living cells), and crosslinked upon UV irradiation
3. Z-stage moved down one layer thickness until completion of the full structure

### Advantages:

- High resolution
- High deposition accuracy
- High fabrication rate
- High cell viability

# Literature review

A prevascularized tissue fabricated with digital light processing (DLP) based 3D bioprinting printing (Krishnamoorthy2020) with a post-printing cell viability of 75% (Bioink 5% (w/v) GelMA 0.5% (w/v) Irgacure 2959 and NIH 3T3 fibroblasts) (Wadnap2019) and NIH 3T3 fibroblasts (the cell viability was measured and moderately after 7 days incubation time). The swelling ratio and the pore size of the hydrogel increases significantly within the first 24 hour incubation time.



[Zhu2018]  
 [Wadnap2019]  
 [Krishnamoorthy2020]



# Objectives

- To systematically study the effects of photoinitiator and printing time on cell viability during printing and the effect of photoinitiator and incubation time on cell viability after printing.
- To investigate the photoinitiator effect on the physical properties: swelling ratio and degradation rate.
- To characterize the photoinitiator effect on the microstructure (pore size) of the hydrogel.

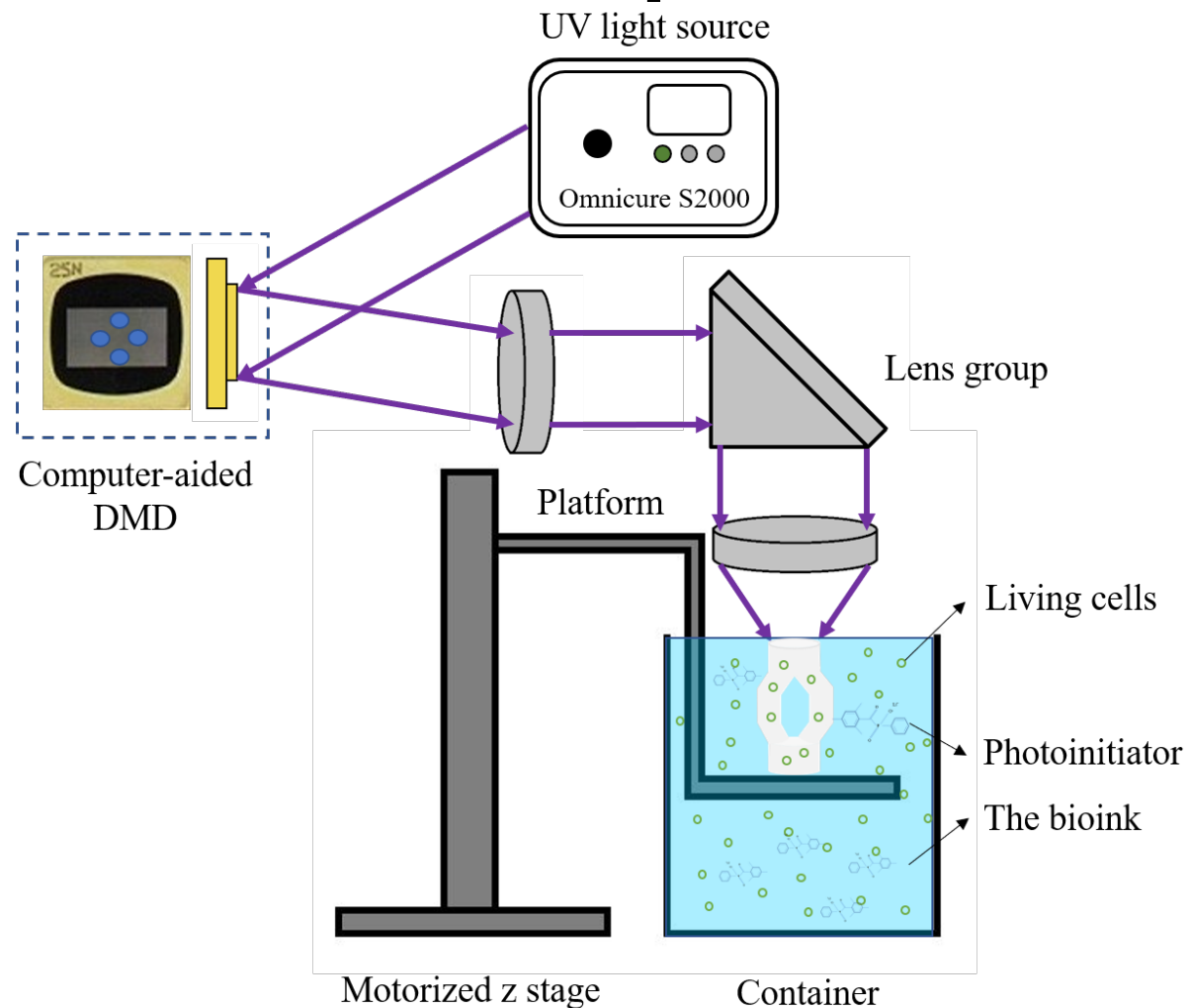


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# Experimental Setup



Key elements:

1. UV source
2. Digital micromirror device (DMD)
3. Lenses group
4. Bioink container
5. Motorized z-stage and platform



## Bioink:

- 5% (w/v) GelMA
- Photoinitiator (Irgacure 2959 or LAP) concentration:  
0.3 – 0.9% (w/v) with an interval of 0.2% (w/v)
- Cell concentration:  $1 \times 10^6$  cells/mL

Printing time (minute)	Photoinitiator concentration (%)	Incubation time (hour)
0	0.3	0
15	0.5	12
30	0.7	24
45	0.9	
60		

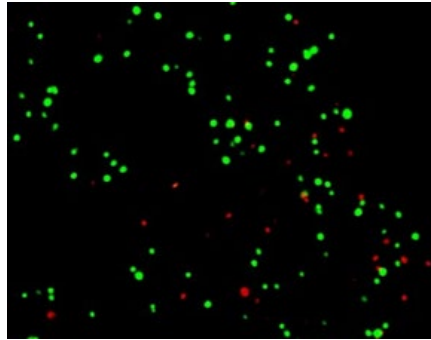


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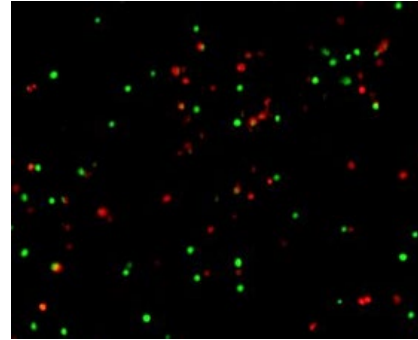
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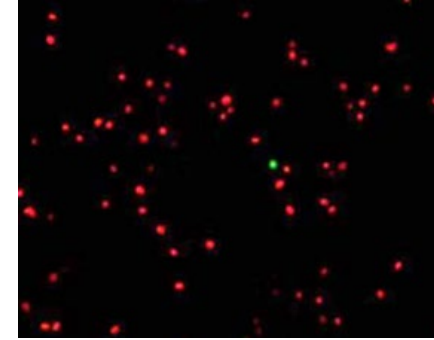
# Representative cell viability images during 3D bioprinting



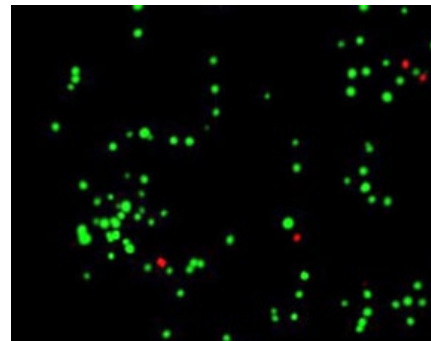
0.5% Irgacure 2959  
at 30-minute



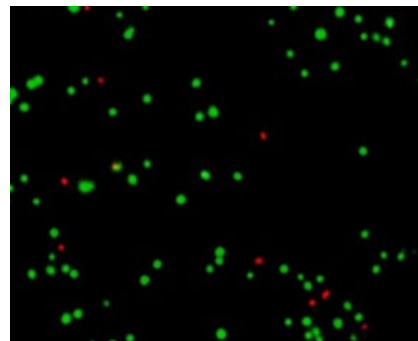
0.7% Irgacure 2959  
at 30-minute



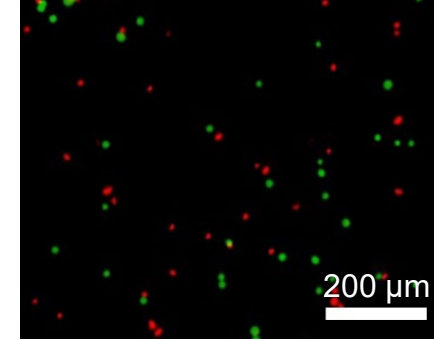
0.9% Irgacure 2959  
at 30-minute



0.5% LAP  
at 0-minute

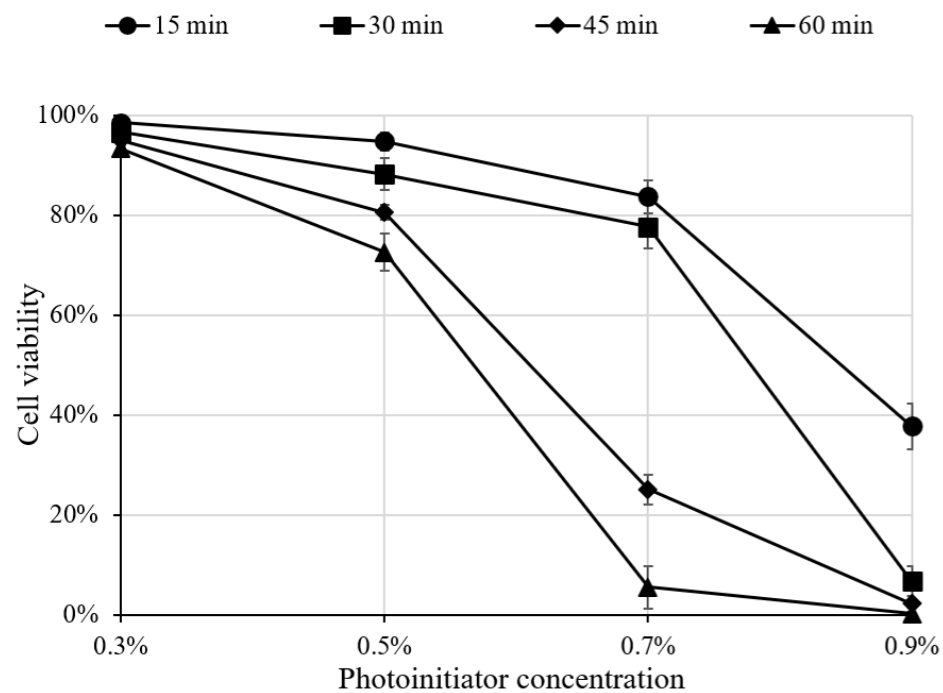


0.5% LAP  
at 30-minute

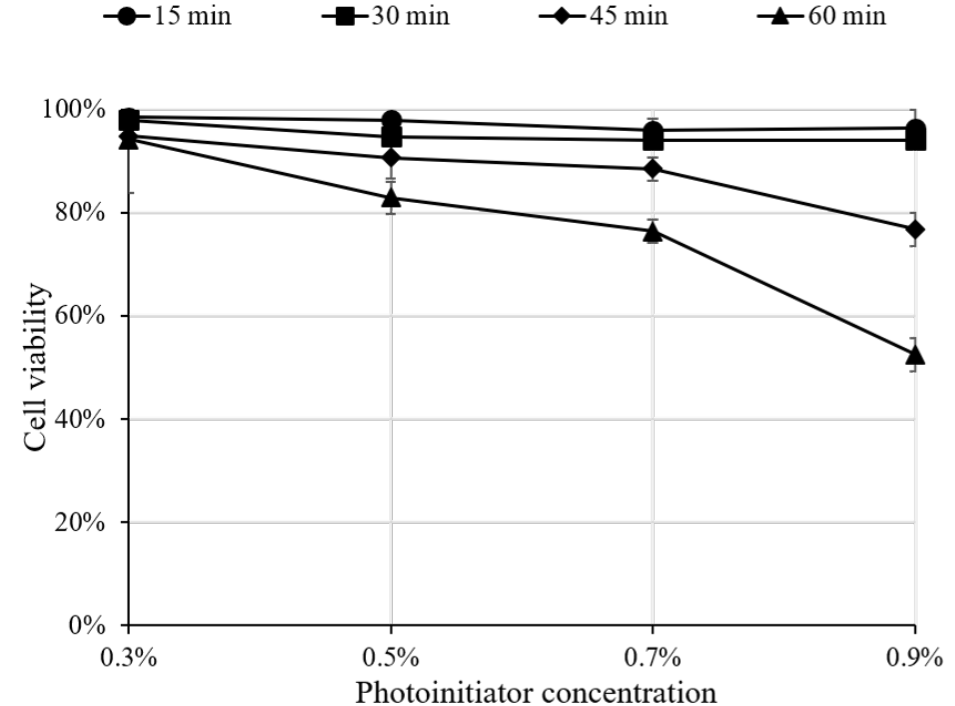


0.5% LAP  
at 60-minute

# Effect of photoinitiator on cell viability during printing



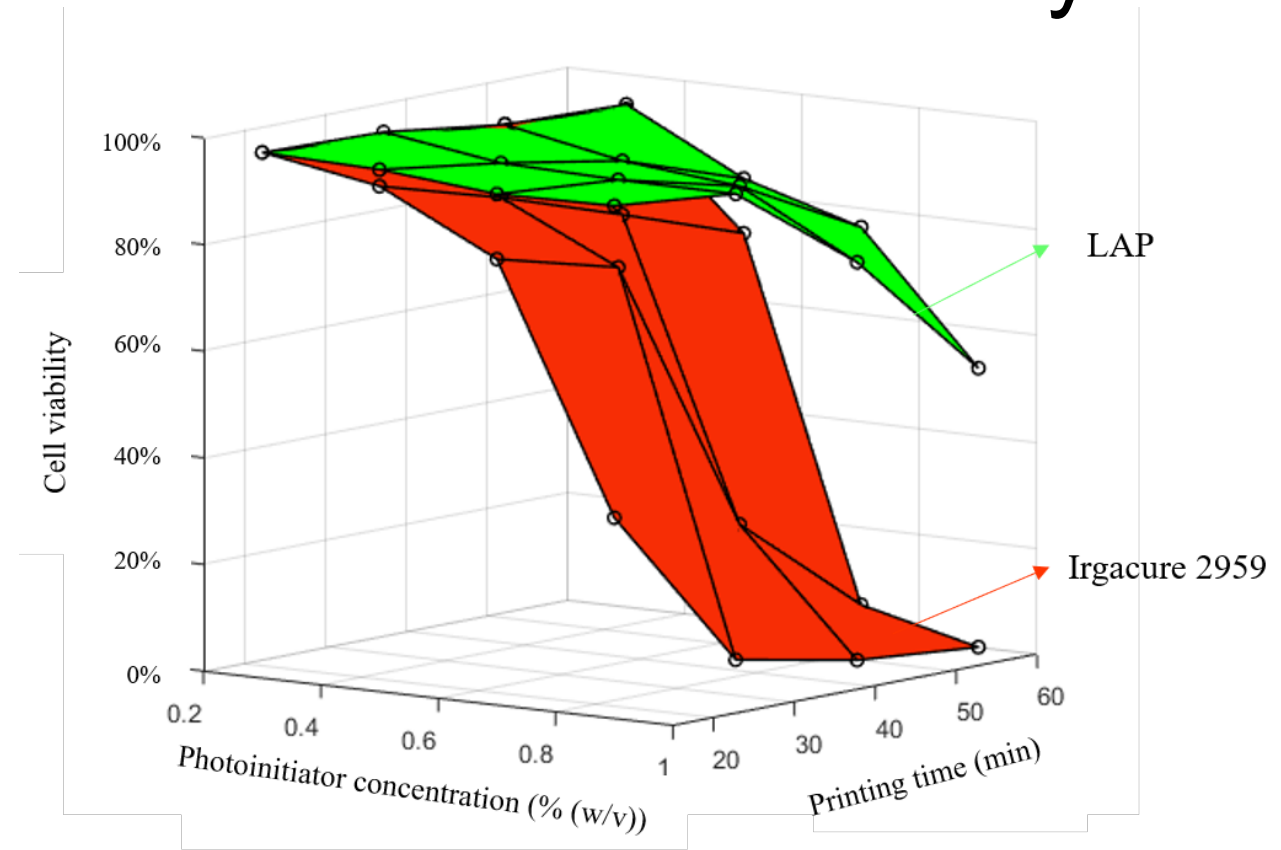
Irgacure 2959



LAP

- For lower concentrations, both photoinitiators are suitable for 3D bioprinting
- For higher concentrations, only LAP are suitable for short-time 3D bioprinting

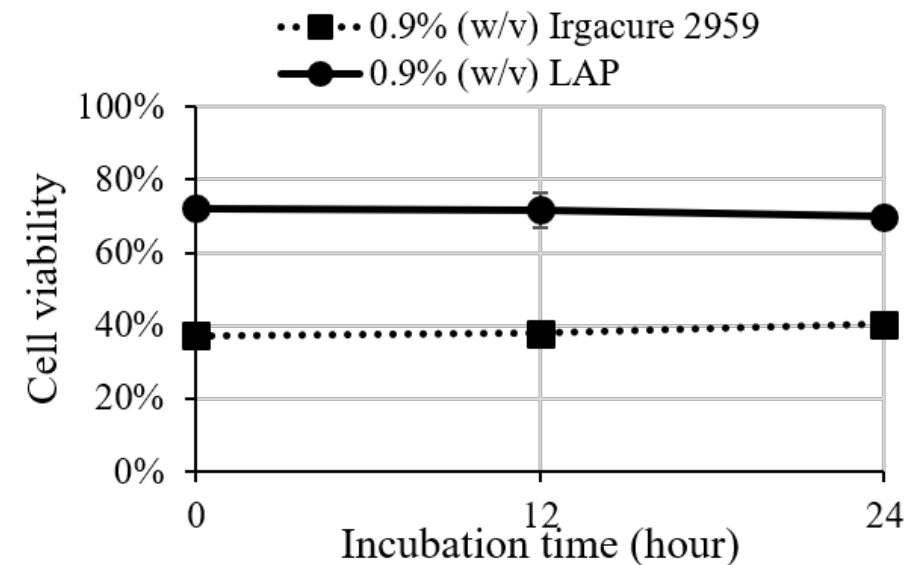
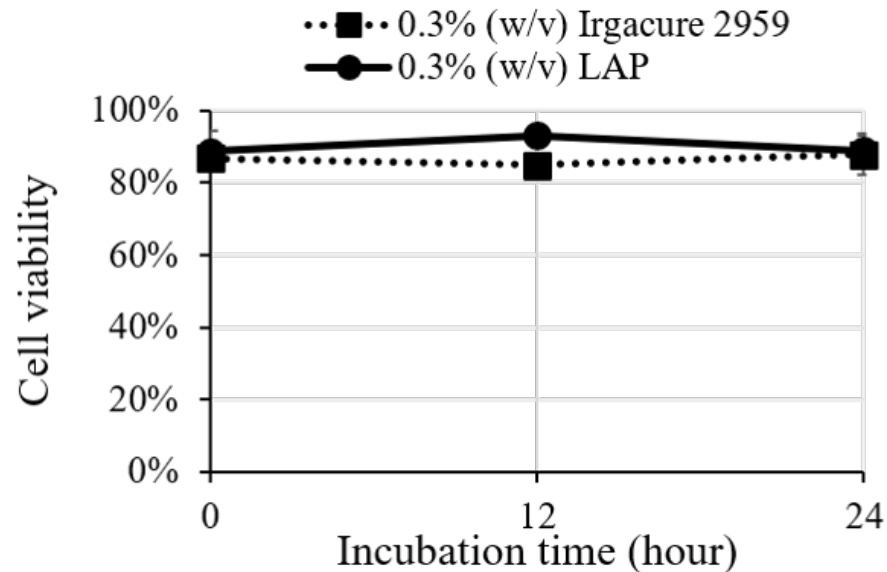
# Photoinitiator effect on cell viability during printing



- The cell viability decreases with printing time and photoinitiator concentration
- At the same concentration, the cell viability using LAP is higher

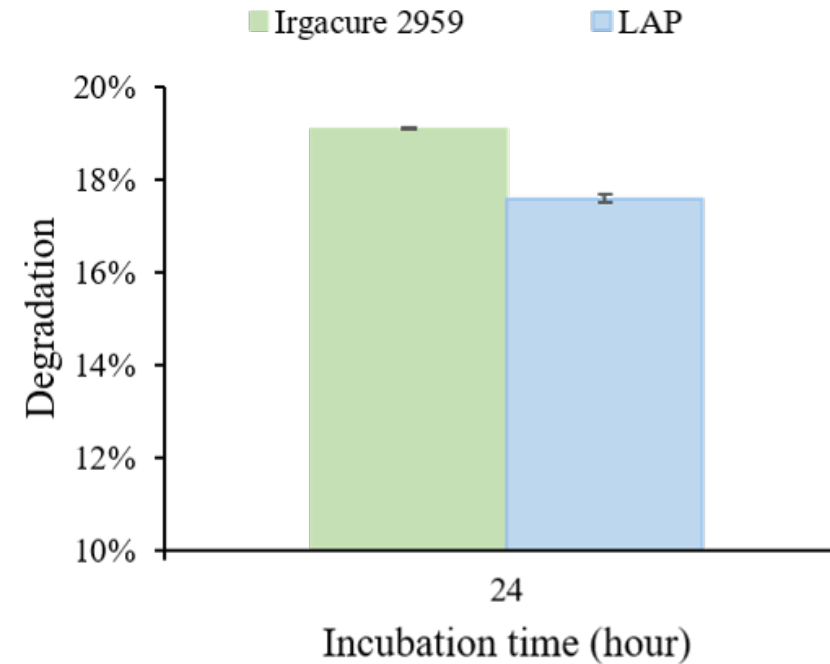
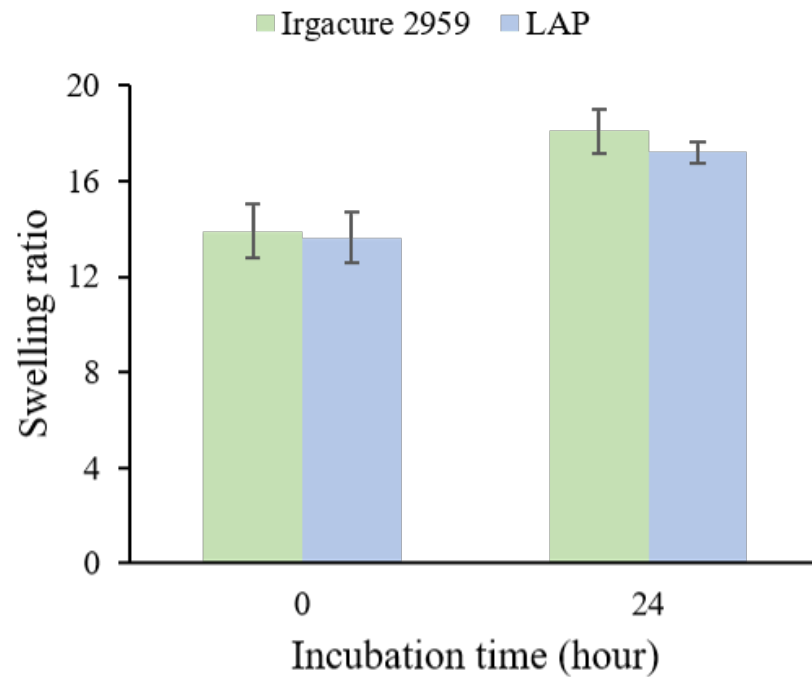


# Effect of photoinitiator on cell viability after printing



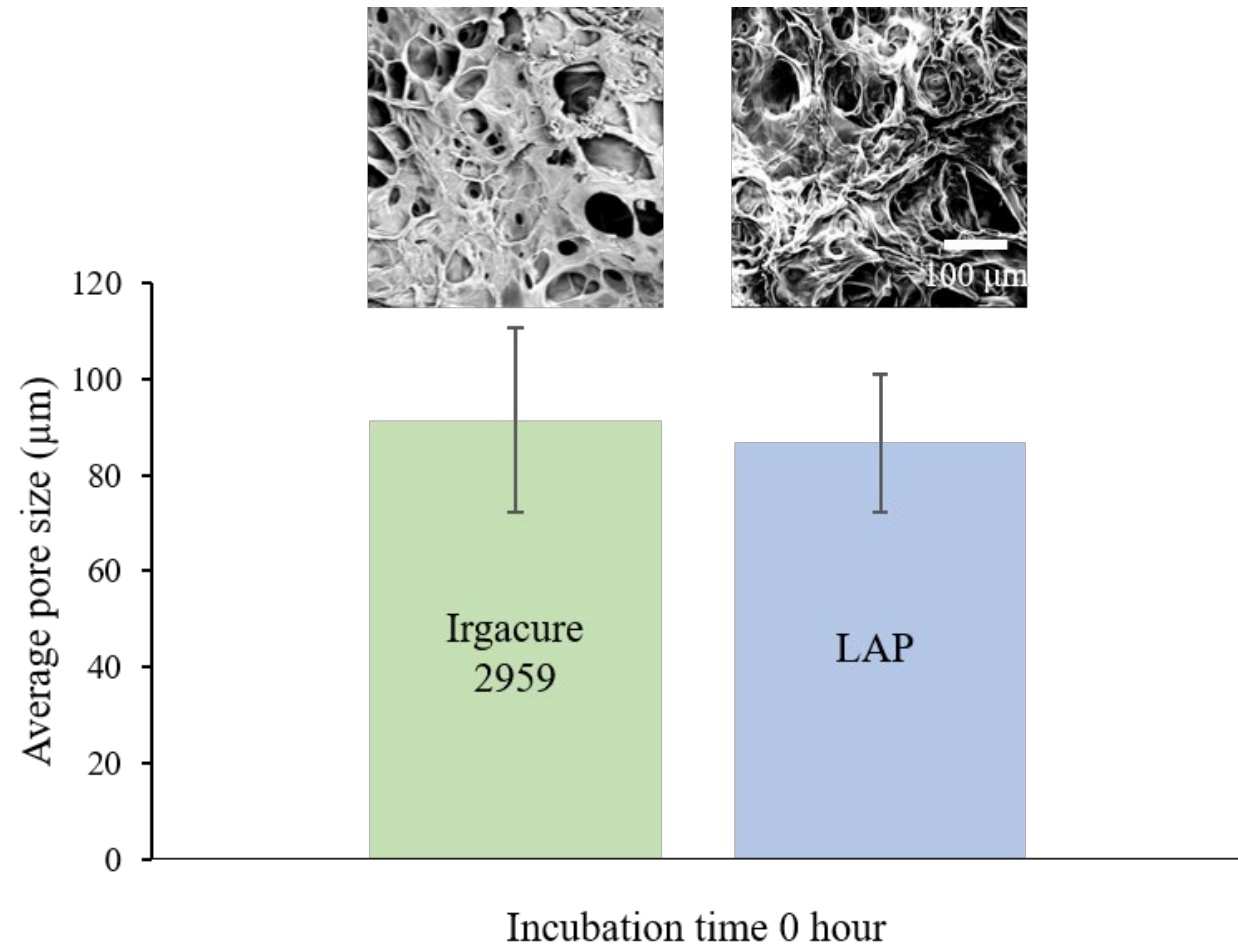
- Both types of photoinitiators have negligible effects on post-printing cell viability

# Effect of photoinitiator on the physical properties



- Samples with Irgacure 2959: greater swelling ratio and faster degradation rate

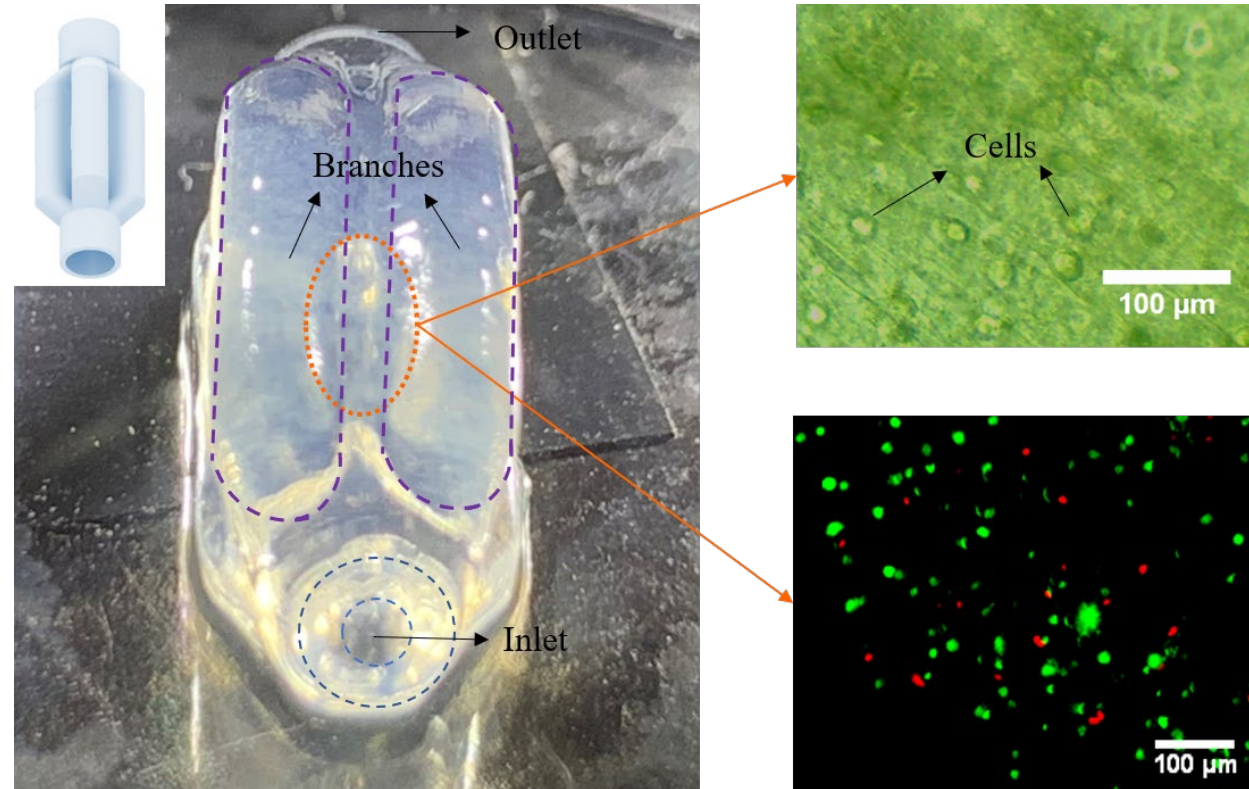
# Effect of photoinitiator on microstructure of hydrogel



- Samples cured with Irgacure 2959: slightly larger average pore size



# Fabrication of a 3D vascular-like construct



- Most cells survive with a post-printing cell viability of 80%
- The actual measured dimensions show good shape fidelity

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# Conclusions

- The cell viability during 3D bioprinting generally decreases with the increase of the photoinitiator concentration and printing time for both Irgacure 2959 and LAP;
- the photoinitiators Irgacure 2959 and LAP have negligible effects on the cell viability after 3D bioprinting; and
- GelMA samples cured with Irgacure 2959 have slightly larger pore size, faster degradation rate, and greater swelling ratio after 3D bioprinting compared to those cured with LAP.



# Acknowledgment

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# Bibliography

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[Zhu2018] Zhu, W., Qu, X., Zhu, J., Ma, X., Patel, S., Liu, J., Wang, P., Lai, C. S. E., Gou, M., and Xu, Y., 2017, "Direct 3D bioprinting of prevascularized tissue constructs with complex microarchitecture," *Biomaterials*, 124, pp. 106-115.



# Thank You!

