

3D Bioprinting Applications for Microfluidic Chip Design and Porous Scaffolds



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Background

There has long been scarcity in donor tissue for use in both transplants and experimental settings. 3D bioprinting enables production of structures that mimic organic tissue.

- Bioprinting is a method of extrusion printing which utilizes biomaterials to 3D print living cells and create biological structures
- Hydrogels are a 3D network of polymers which swell to absorb water, enabling 3D cell culture by mimicking ECM properties
- Bioink consists of a composite of a biocompatible hydrogel, cell growth media, and cells
- 3D printed structures facilitate multi-dimensional cell culture for applications in regenerative medicine

Creating a bioink which prints with ease in a range of conditions and has tunable physical and chemical properties has presented a considerable challenge thus far. We propose a method of generating monodisperse microgels to use as a bioink for optimal cellular responses.



Fig 1. BioassemblyBot 400 Advanced Solutions Life Sciences

Research Objectives

- Synthesize smaller microgels for use in bioprinting for cell encapsulation
- Utilize TSIM software to design a 3D-printed microfluidic chip to produce monodisperse microgels
- Print with microgels to form a porous scaffold

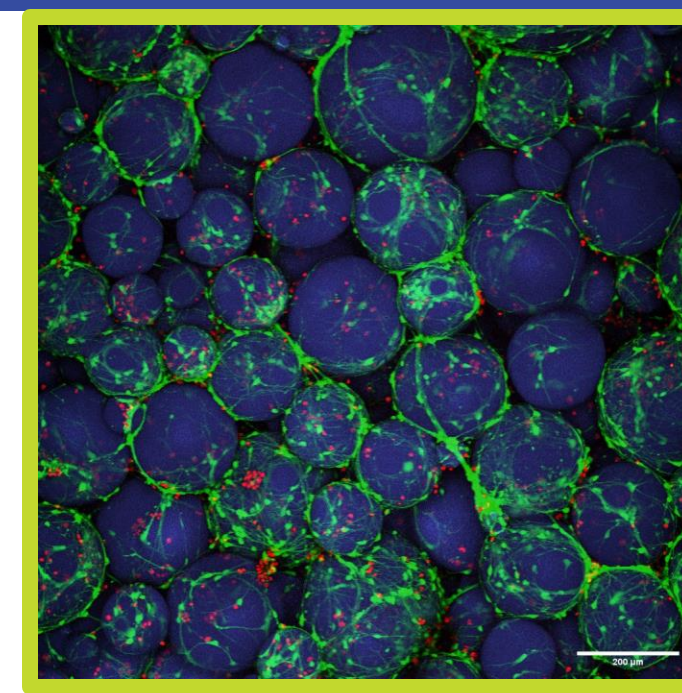


Fig 2. Cell growth in microgel scaffold; microgels stained blue; scale bar 200 μm

Bulk Emulsion Microgel Synthesis

Microgels are spherical hydrogels which form a porous scaffold providing cells with space to grow and proliferate for applications in 3D cell culture. Bulk emulsion is one of the most efficient methods for microgel production, although microgel size varies.

The initial formulation for microgels utilized a 10% gelatin solution, which was improved to produce smaller microgels by manipulating polymer concentration, emulsifiers, and stirring speed.

Bulk Emulsion

- 5% w/v Gelatin, 2% w/v Tween 20, dissolved in DI water
- Solution is added dropwise to oil bath heated to 50°C, stirred at 400 RPM
- Heat turned off after 45 min, once near room temperature, ice added to water bath
- Microgels dehydrated with acetone; vacuum filtered dry, rehydrated for imaging

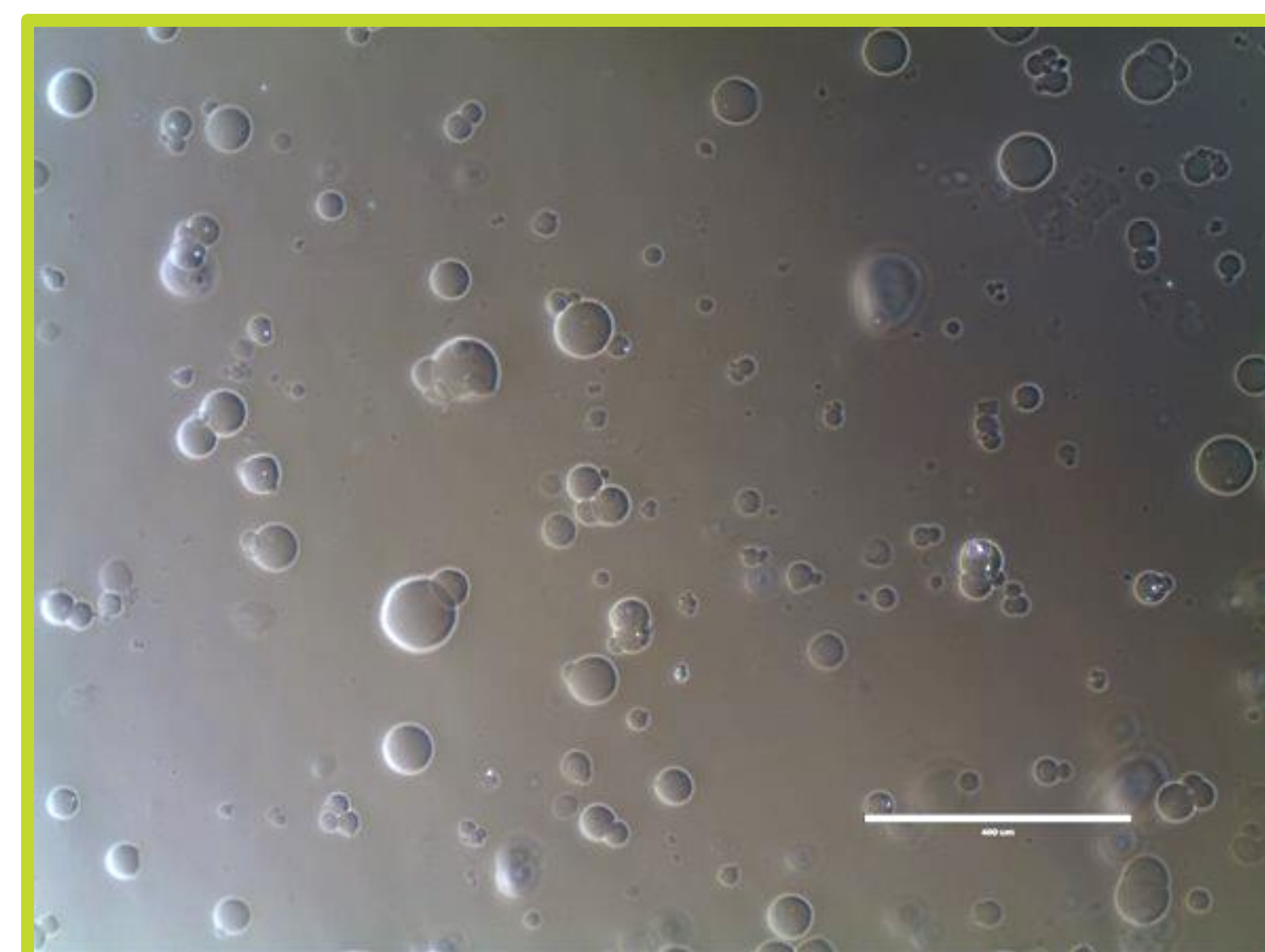


Fig 3. 5% w/v Gelatin 2% w/v Tween 20 Microgels; scale bar 400 μm

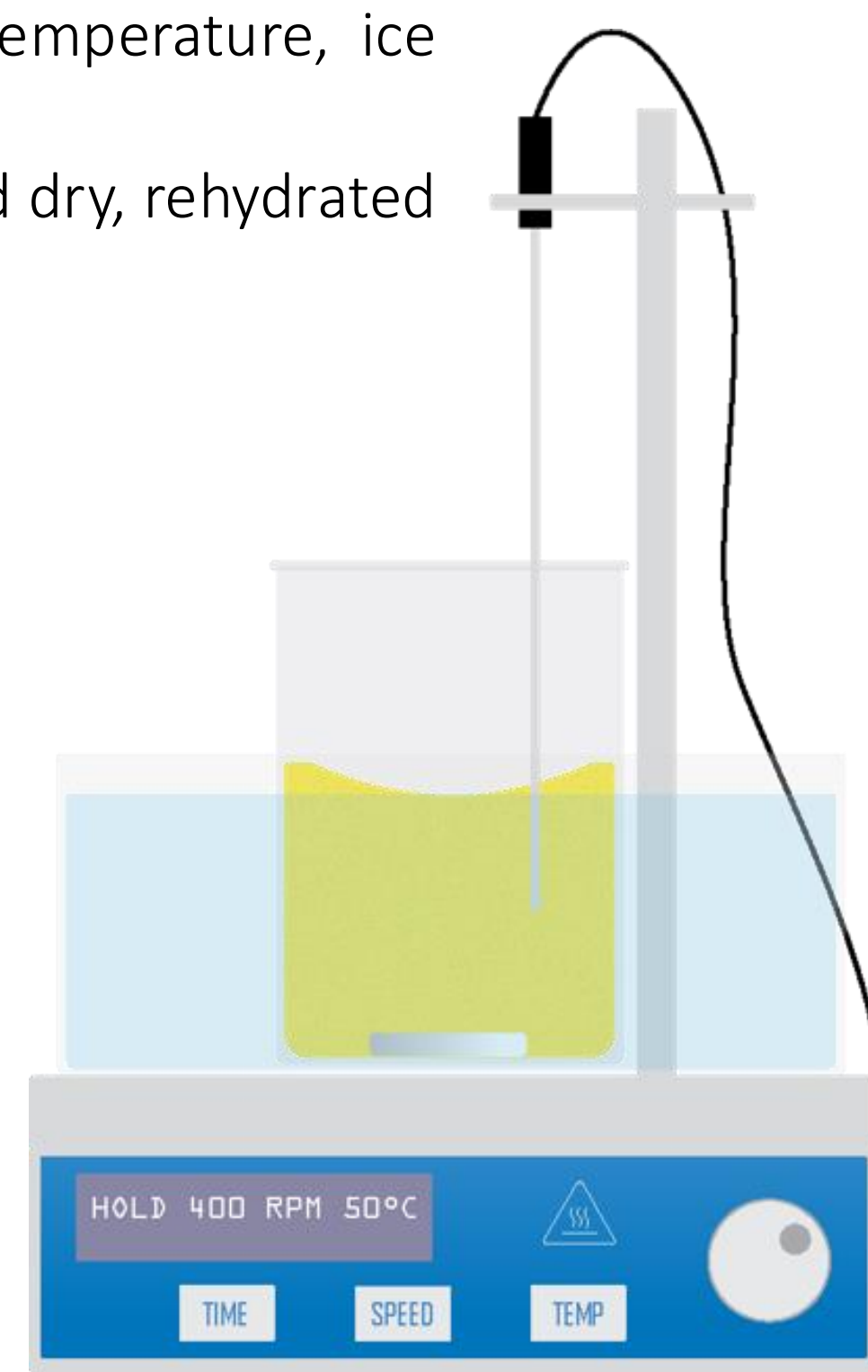


Fig 4. Bulk emulsion setup

3D Printed Microfluidic Chip

Microfluidic chips have microscale channels through which fluids can flow at controlled rates, making it possible to form droplets of uniform size and shape.

Chip Production

- Design and print chip design with BioassemblyBot using 25% w/v Pluronic F127 in DI water
- Pour PDMS over printed design and cure
- Bond PDMS mold to glass microscope slide with plasma cleaner

Chip Operation

- Set continuous phase pump to 12 μl/min with two 3ml syringes
 - 2% w/v Tween 20 in olive oil
- Set dispersed phase pump to 1.5 μl/min with one 3ml syringe
 - 2.5% w/v gelatin, 2.5% w/v GelMA, 0.5% w/v Irgacure 2959 in DI water
- Cover dispersed phase pump with heating pad
- Deposit microgels into oil cooled by an ice bath with a stir bar

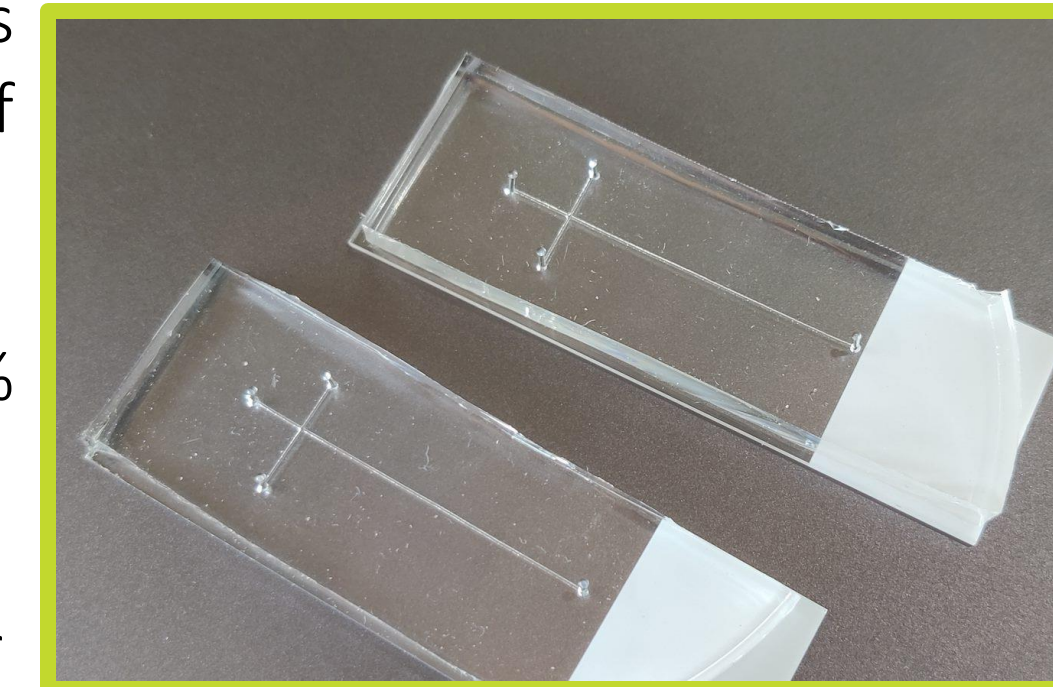


Fig 5. Microfluidic chips

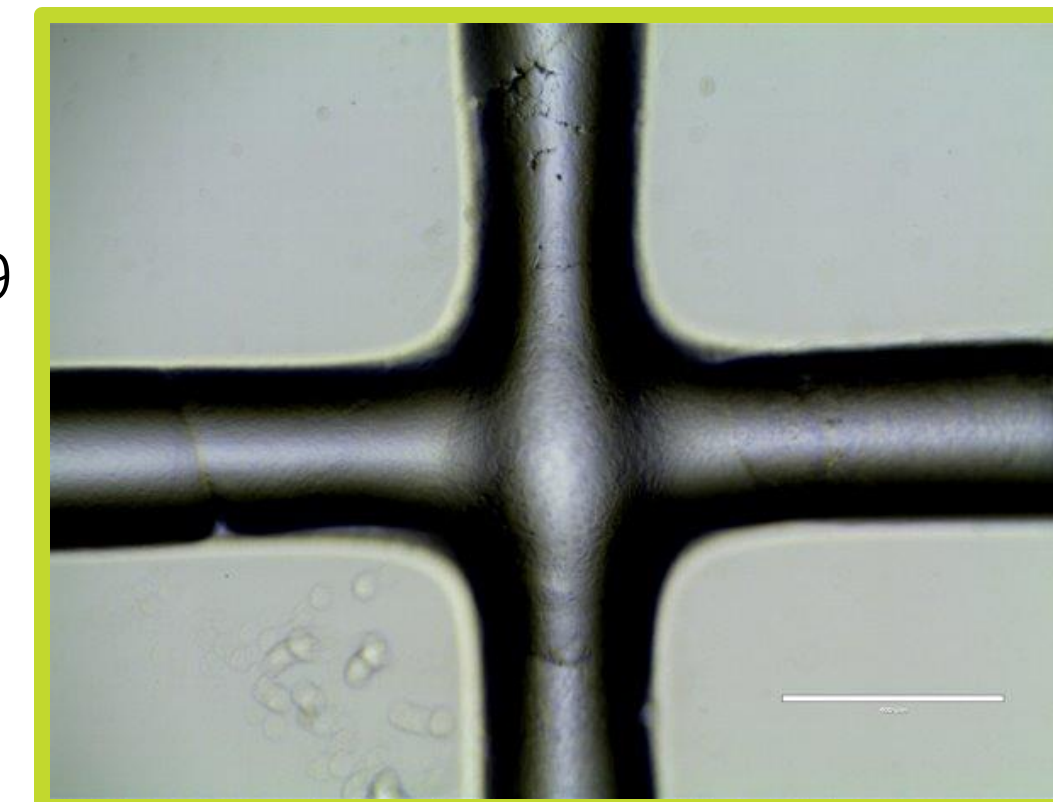


Fig 6. 4-way intersection; scale bar 1000 μm

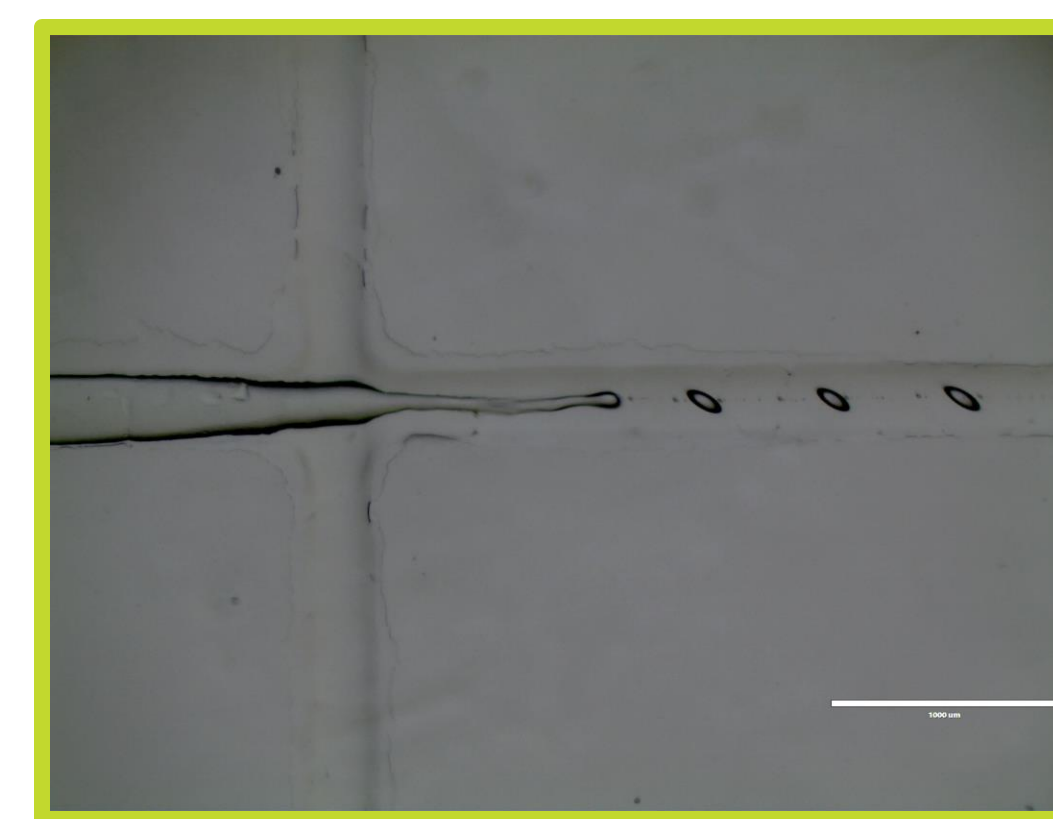


Fig 7. Droplet formation in channel; scale bar 1000 μm

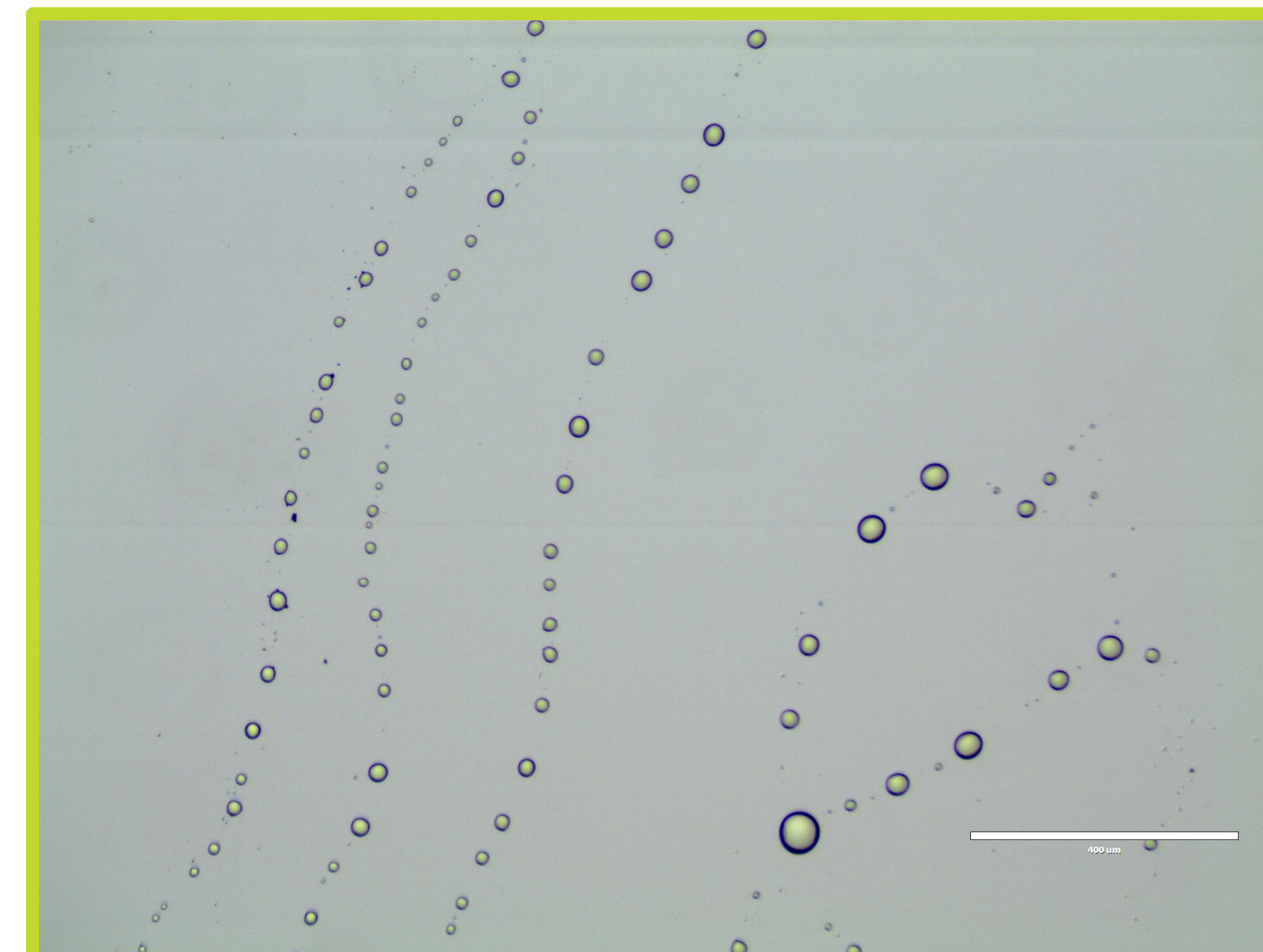


Fig 8. Microgels from microfluidic chip; scale bar 400 μm

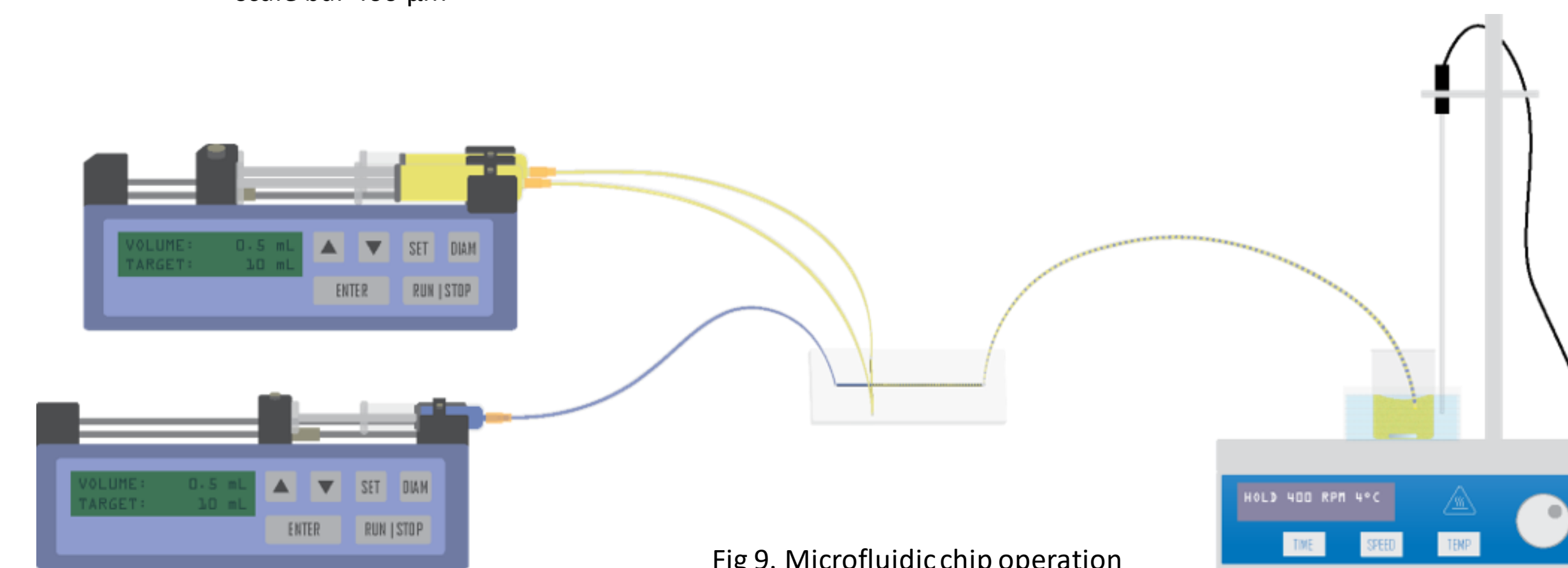


Fig 9. Microfluidic chip operation

Microgel Results

The microfluidic chip produced microgels of much smaller size that would be ideal for cell encapsulation. Smaller microgels also result in a hydrogel of greater stiffness due to a larger surface area for crosslinking to occur across.

Table 1. Microgel diameters (μm) by synthesis method

	Bulk Emulsion		Microfluidic Chip
	10% w/v Gelatin	5% w/v Gelatin 2% w/v Tween 20	2.5% w/v Gelatin 2.5% w/v GelMA
Mean	417.0	45.6	28.3
Standard Deviation	164.9	19.6	5.9
Polydispersity Index	0.156	0.185	0.044

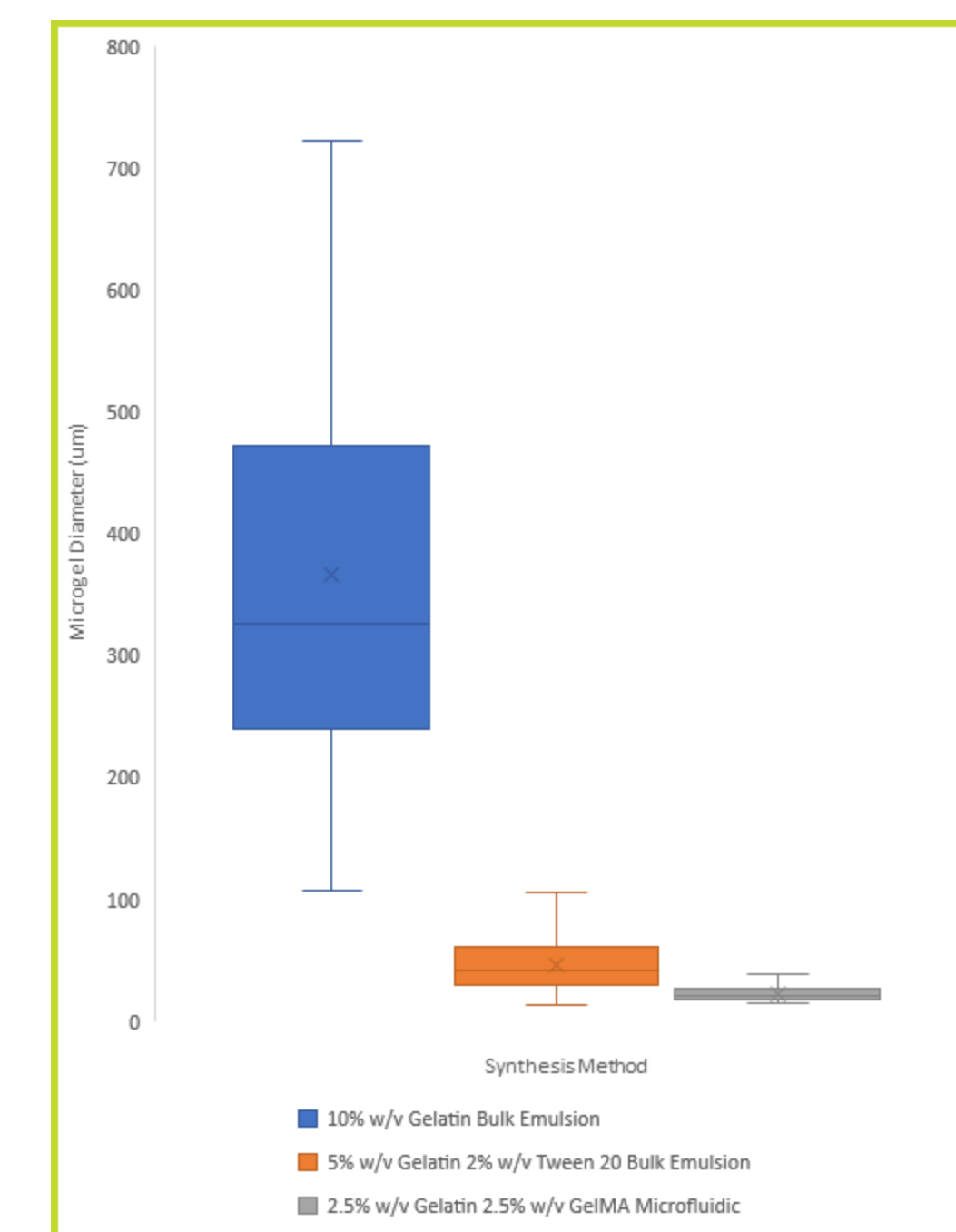


Fig 10. Microgel diameters and synthesis methods

Bioprinting with Microgels

Microgels are suitable for bioprinting because they do not require temperature manipulation and can be printed at low pressures through a tapered tip, reducing stress on cells.

- 8% w/v microgels consisting of 2.5% w/v gelatin and GelMA each are rehydrated in cell media with 0.5% w/v Irgacure 2959
- Printing at 2 PSI through an 18-gauge tapered needle produced the grid in Fig 12
- These samples were then crosslinked with UV light and an mTG bath

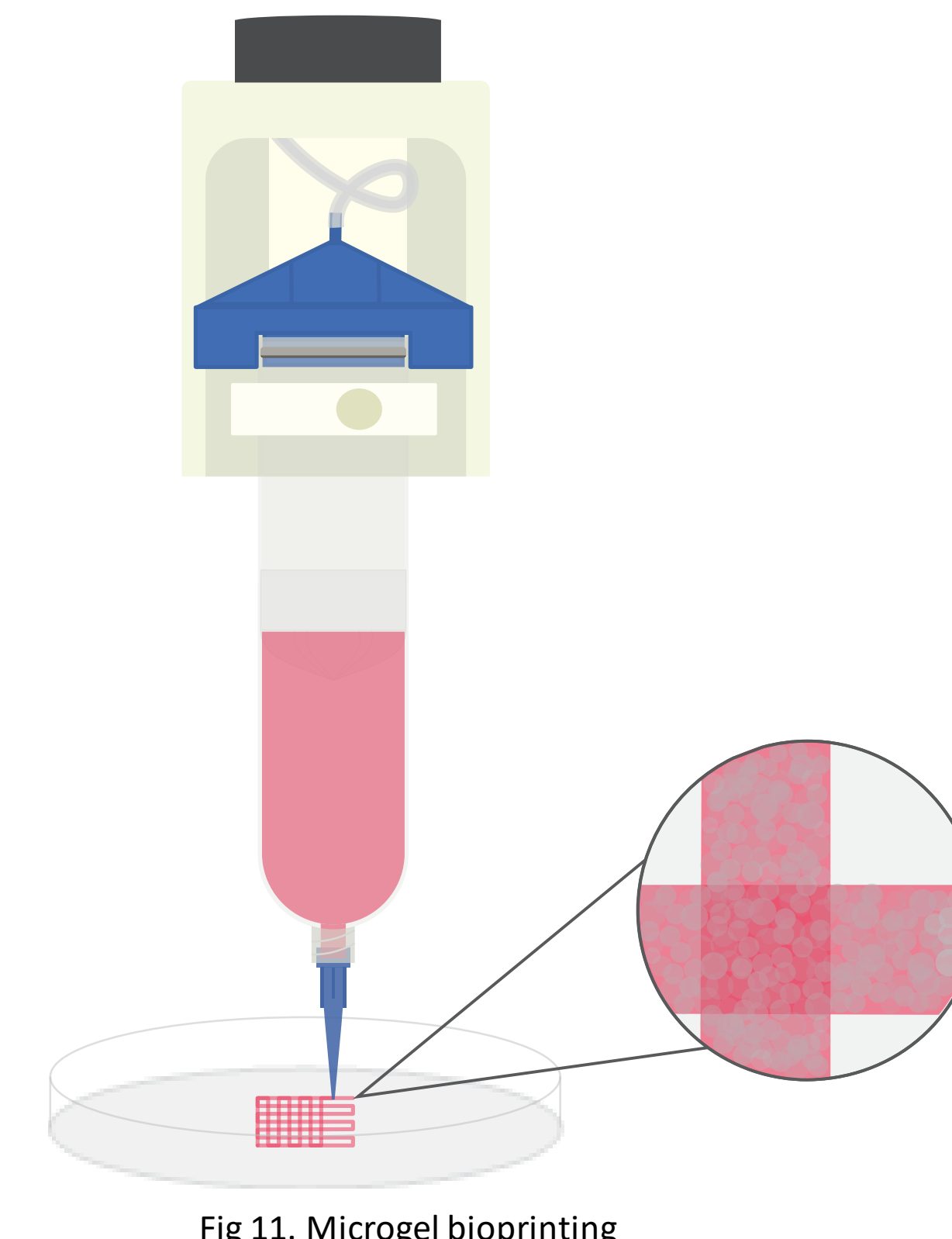


Fig 11. Microgel bioprinting

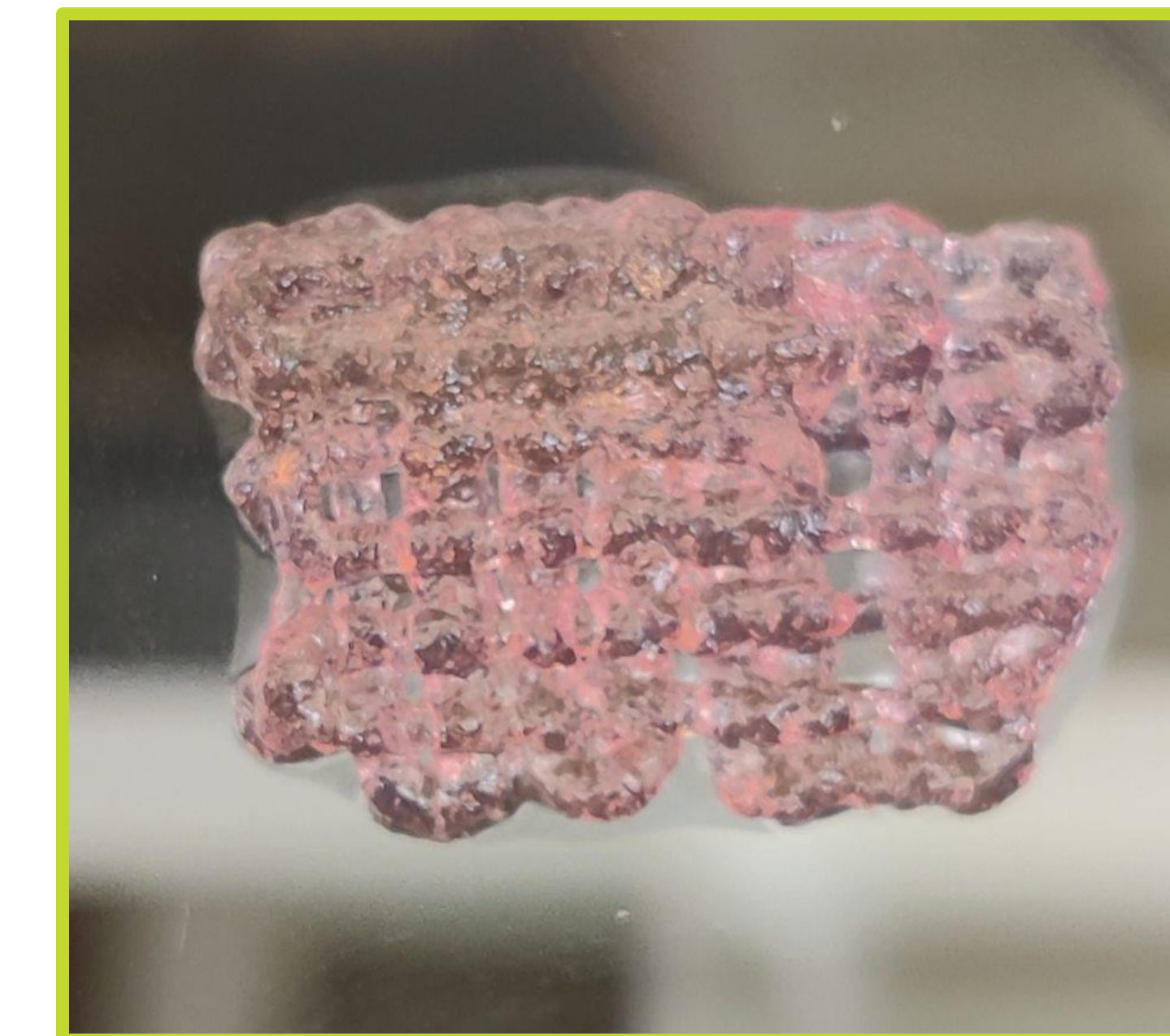


Fig 12. Microgel bioprinted grid

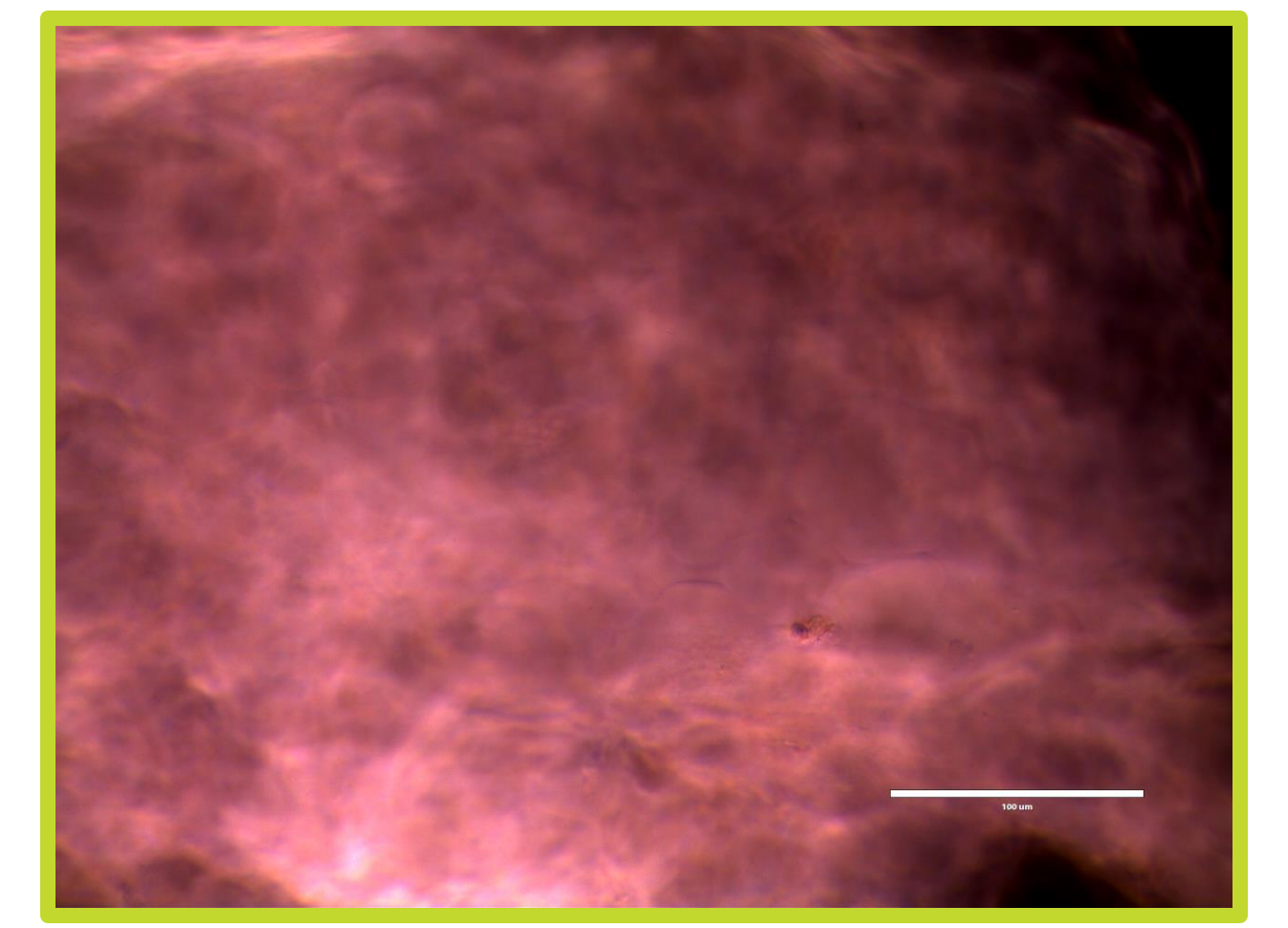


Fig 13. Light microscope image of printed microgels; scale bar 100 μm

Conclusions and Future Work

3D-printed microgels have a wide range of applications, from controlled drug delivery to cell encapsulation. Microgel size is an important factor for varying applications and can be manipulated by adjusting polymer and emulsifier concentrations in addition to stirring speed. Bioprinted microfluidic chips are a feasible technology to produce uniform microgels.

- Revise formulation of dispersed phase in microfluidic chip to contain 2.5% w/v gelatin, 2.5% w/v GelMA, and 1% w/v alginate
 - Enables additional crosslinking, leading to greater microgel stability: photopolymerization (UV light), enzymatic crosslinking (mTG), and chemical crosslinking (Ca²⁺)
- Bioprint cells with microgels to produce porous cellular scaffolding for enhanced cell growth
- Utilize minimally cytotoxic crosslinking methods to optimize the biocompatibility of the hydrogel

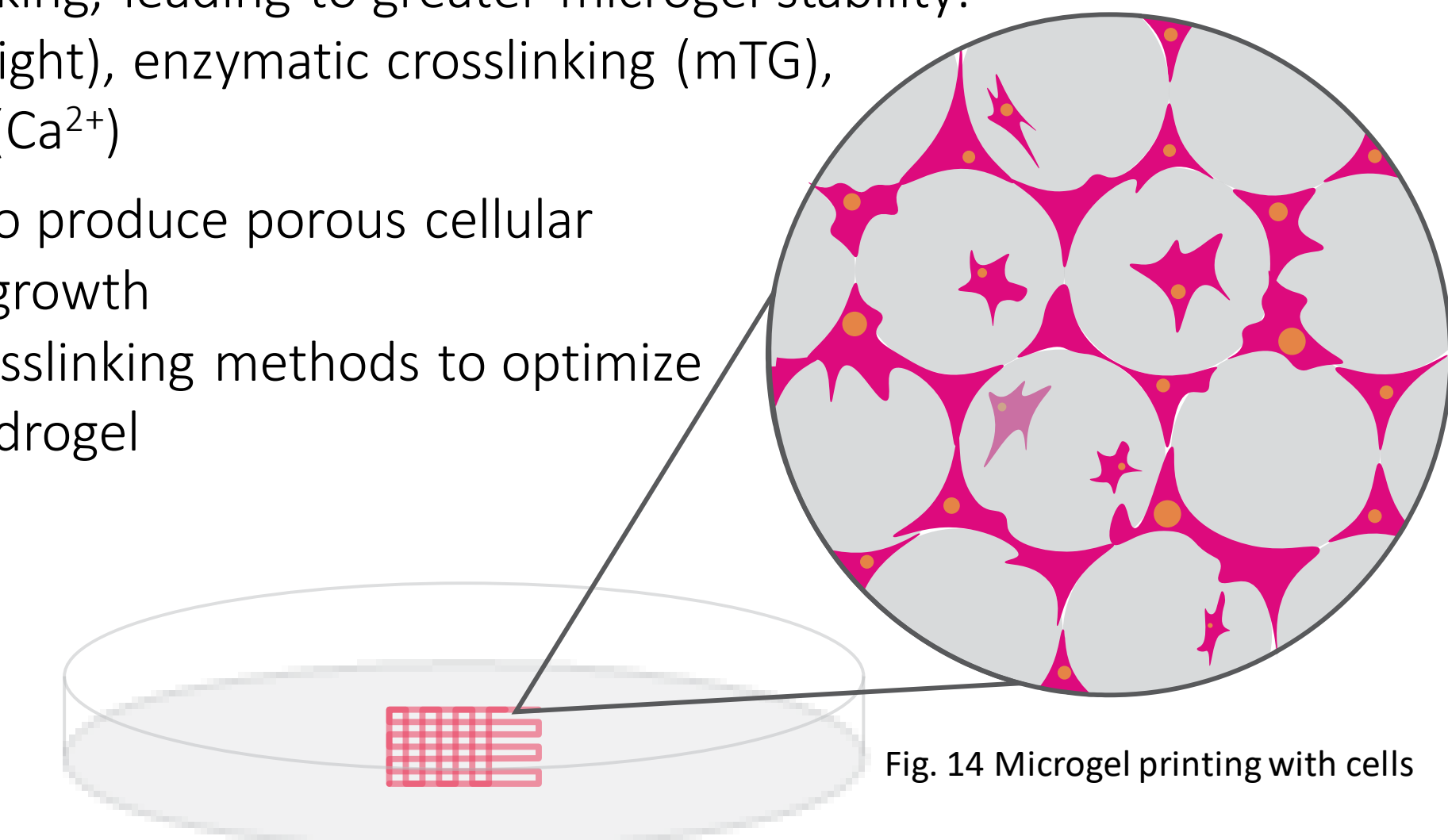


Fig 14. Microgel printing with cells

Acknowledgements

This research was sponsored by NH BioMade and funded by NSF Award #IIA 1757371 administered by NH EPSCoR at UNH. Thank you to Mrinal Ganash for assisting with operation of the 3D bioprinter and to Jack Reynolds for collaboration on formulating smaller bulk emulsion microgels.