Microgel Production for Precision 3D Bioprinting Applications

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Introduction

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 and facilitate multi-dimensional cell culture.

- Bioprinting uses bioink, a composite of a biocompatible hydrogel, cell growth media, and live cells, to 3D print biological structures
- Hydrogels are a 3D network of polymers which swell to absorb water, enabling 3D cell culture by mimicking ECM properties
- Microgels are spherical hydrogels which form a porous scaffold providing cells with space to grow, spread, and form cell-to-cell connections
- Compared to bulk hydrogel scaffolds which trap cells, microgel scaffolds produced from monodisperse microgels have uniform pores which aid cell proliferation

Fig 1. BioAssemblyBot 400
Advanced Solutions Life Sciences

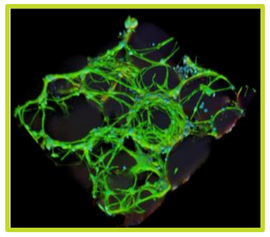

Fig 2. Confocal image of neural stem cells differentiated into neurons

Research Objectives:

Develop a method to synthesize small, monodisperse, gelatin microgels for use in bioprinting and cell encapsulation.

- Utilize TSIM software to design and produce a 3D-printed microfluidic chip
- Regulate temperature during chip operation
- Produce monodisperse gelatin microgels
- Print with microgels to form a porous scaffold

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

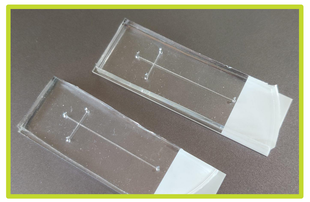

Fig 5. Microfluidic chips

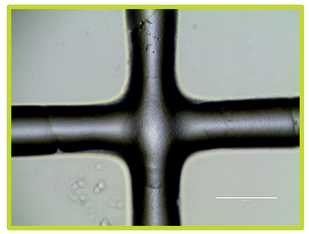

Fig 6. 4-way intersection;
scale bar 1000 µm

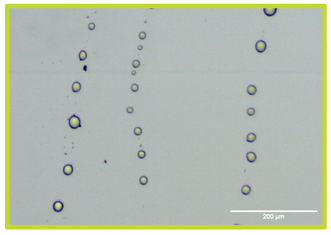

Fig 8. Microgels from microfluidic chip;
scale bar 200 µm

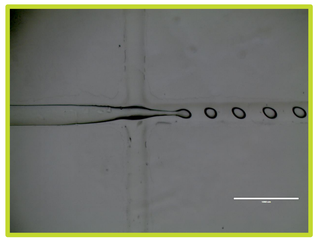

Fig 7. Droplet formation in channel;
scale bar 1000 µm

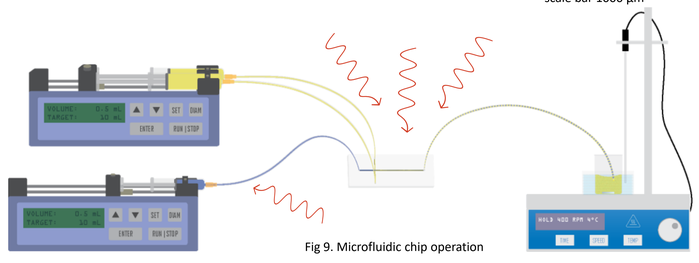

Fig 9. Microfluidic chip operation

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

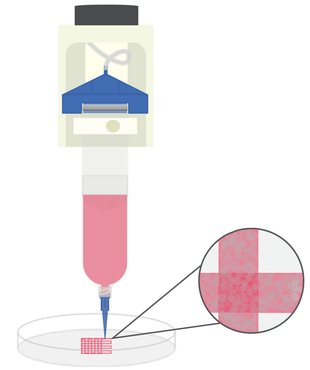

Fig 11. Microgel bioprinting

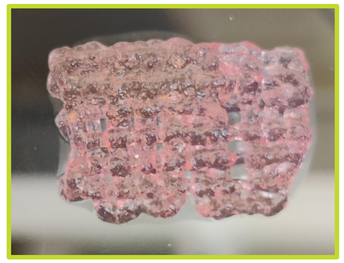

Fig 12. Microgel bioprinted grid

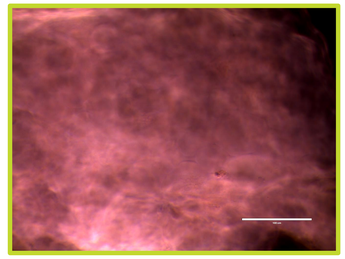

Fig 13. Light microscope image of printed microgels;
scale bar 100 µm

Bulk Emulsion Microgel Synthesis

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

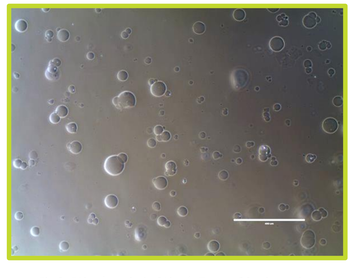

Fig 3. 5% w/v gelatin 2% w/v Tween 20 microgels;
scale bar 400 µm

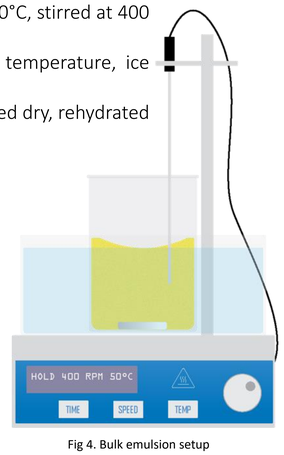

Fig 4. Bulk emulsion setup

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

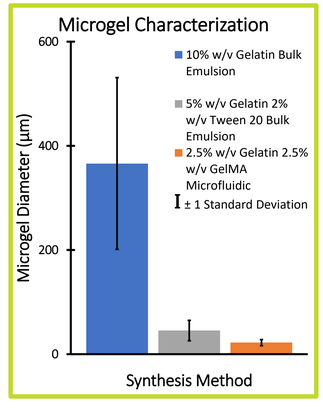

Fig 10. Microgel diameters and synthesis methods

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 flow rates. 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 (Ca^{2+})
- 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

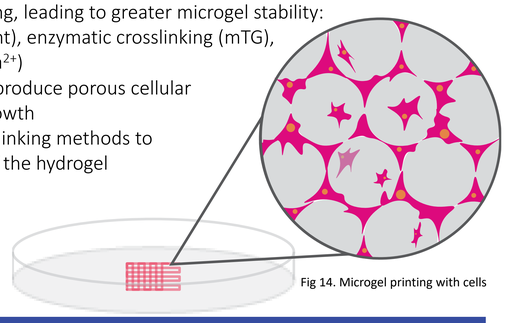

Fig 14. Microgel printing with cells

Acknowledgements

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