Synthesis and Characterization of Tunable Gelatin Methacrylate Hydrogels

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Introduction

Synthesis of gelatin methacrylate (GelMA) can be achieved through the reaction of methacrylic anhydride with gelatin. Production of GelMA hydrogels is useful for 3D printing due to their known biocompatibility with cells [2]. The generation of biomaterials for advanced therapies involves the seeding of human cells into a scaffold or encapsulation into a biomaterial before 3D printing. Biomaterials need to be catered to the types of cells that will be incorporated. Gelatin methacrylate-based 3D printing materials face challenges with batch-to-batch consistency and drawbacks with print resolution due to gelatin's melting point but are still very useful for applications which involve emulating soft tissues in areas like the brain [4]. ReNcell VM is a multipotent progenitor cell which can differentiate into three cell types within the human central nervous system. The fragility of gelatin hydrogels can help to emulate the natural environment in which ReNcell VM is derived. In this project, different batches of GelMA were synthesized at UNH Manchester and their degrees of methacrylate functionalization were assessed using ^{1}H NMR [3, 5, 6]. ReNcell VM were encapsulated into GelMA as single cells or neurospheres, then 3D printed using the Corning Matribot to assess both the printability and biocompatibility of our different GelMA batches.

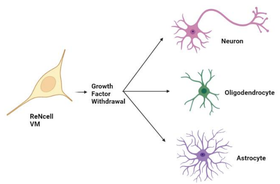

Figure 1a: ReNcell VM differentiation into three lineages.

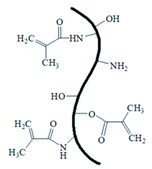

Figure 1b: General structure of Gelatin Methacrylate [2].

Methods

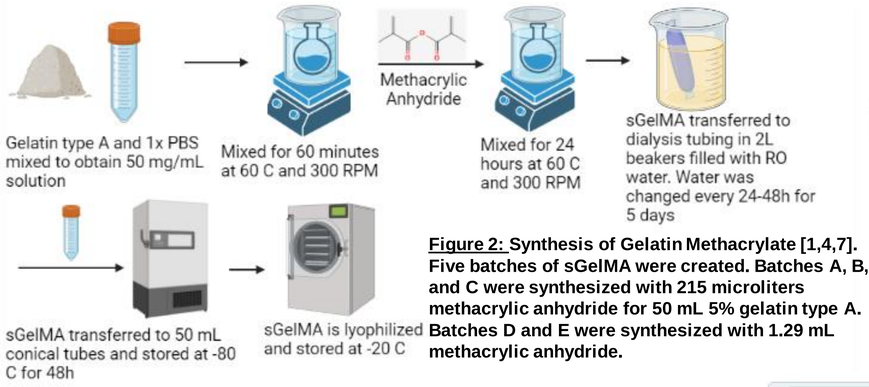

Figure 2: Synthesis of Gelatin Methacrylate [1,4,7]. Five batches of sGelMA were created. Batches A, B, and C were synthesized with 215 microliters methacrylic anhydride for 50 mL 5% gelatin type A. Batches D and E were synthesized with 1.29 mL methacrylic anhydride.

Figure 3: Reconstitution procedure for sGelMA with RwoPS media or 1x PBS.

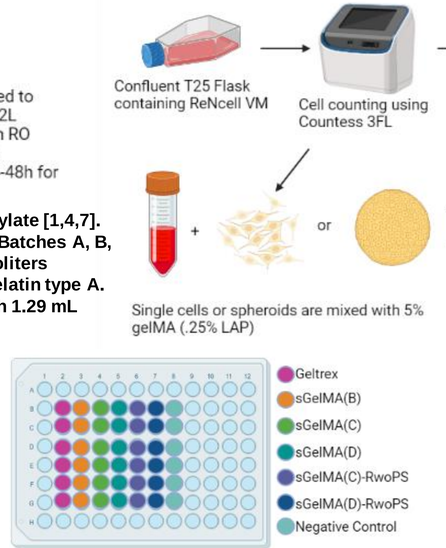

Figure 4: Preparation of ReNcell VM for 3D Printing using the Corning Matribot. Neurospheres were grown for 72 hours before printing. Both single cells and neurospheres were printed onto 48-well plates and grown for 10 days. Immunocytochemistry was performed on single cells after 48 hours and after 4 days and 11 days for neurospheres [7, 8].

Figure 5: 96-well plate layout for the cytotoxicity analysis of single ReNCell VM encapsulated in 5% sGelMA using resazurin. 1-, 2-, and 3-hour fluorescence measurements were obtained using the Spectramax i3. Resazurin was added 24 hours after plating.

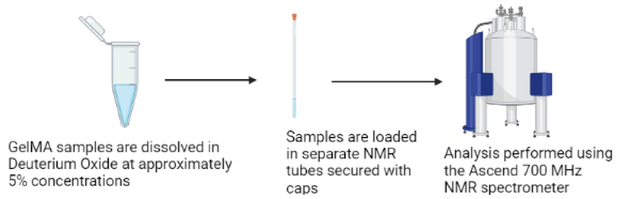

Figure 6: Preparation of GelMA samples for analysis using the Ascend 700 MHz NMR Spectrometer at UNH Durham.

Data & Results

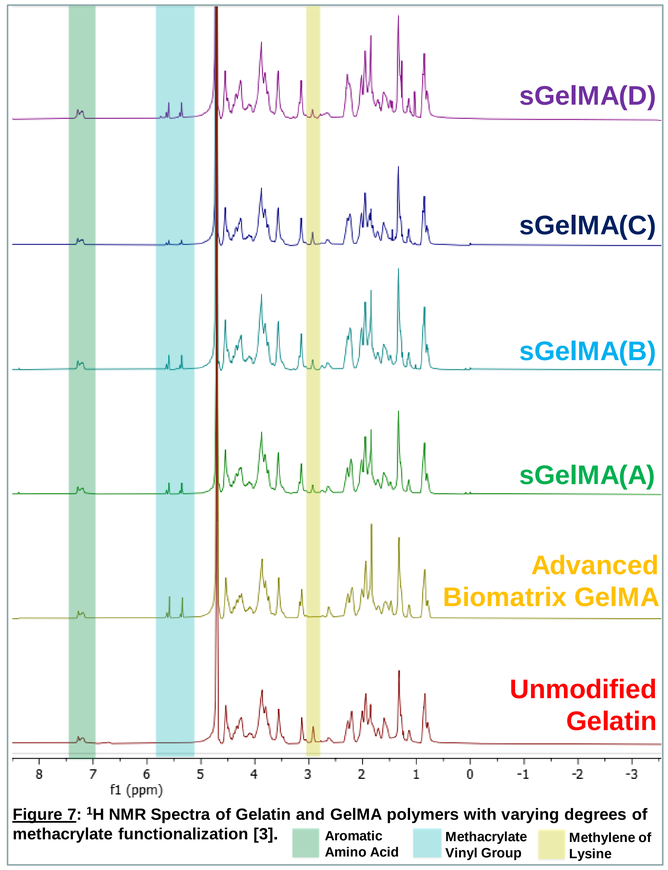

Figure 7: ^{1}H NMR Spectra of Gelatin and GelMA polymers with varying degrees of methacrylate functionalization [3].

	Gelatin (g) / MAA (mL)	Methylene of Lysine Relative Protons	Methacrylate Vinyl Group Relative Protons	Ratio
Unmodified Gelatin	5 g / 0.43 mL	0.94	0.02	1:0
Advanced Biomatrix GelMA	Unknown	0.56	1.34	1:3
sGelMA(A)	5 g / 0.43 mL	0.61	0.64	1:1
sGelMA(B)	5 g / 0.43 mL	0.45	0.76	1:2
sGelMA(C)	5 g / 0.43 mL	0.74	0.34	2:1
sGelMA(D)	5 g / 1.29 mL	0.52	0.93	1:2

Figure 8: Relative proton signals describing the functionalization of the primary amine in lysine (2.9 ppm) with methacrylate (5.5 ppm). Data was normalized to an aromatic amino acid signal from 7.1-7.5 ppm and the relative integration values were compared between unmodified gelatin and GelMA.

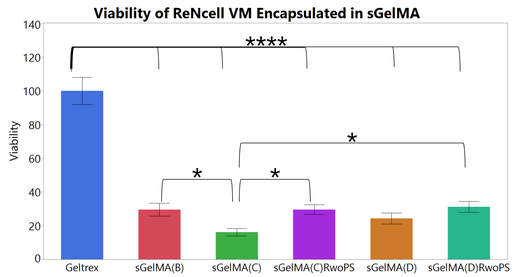

Figure 9: Viability of ReNcell VM plated on Geltrex or encapsulated in synthesized GelMA (.25% LAP) after 1 hour. There was a statistically significant difference between cells grown on Geltrex vs all other conditions ($p < .0001$). Cells encapsulated in sGelMA(D)RwoPS had higher viability compared to sGelMA(C) ($p = .0163$). Cells encapsulated in sGelMA(B) had higher viability compared to sGelMA(C) ($p = .0322$). Cells encapsulated in sGelMA(C)RwoPS had higher viability compared to sGelMA(C) ($p = .0324$).

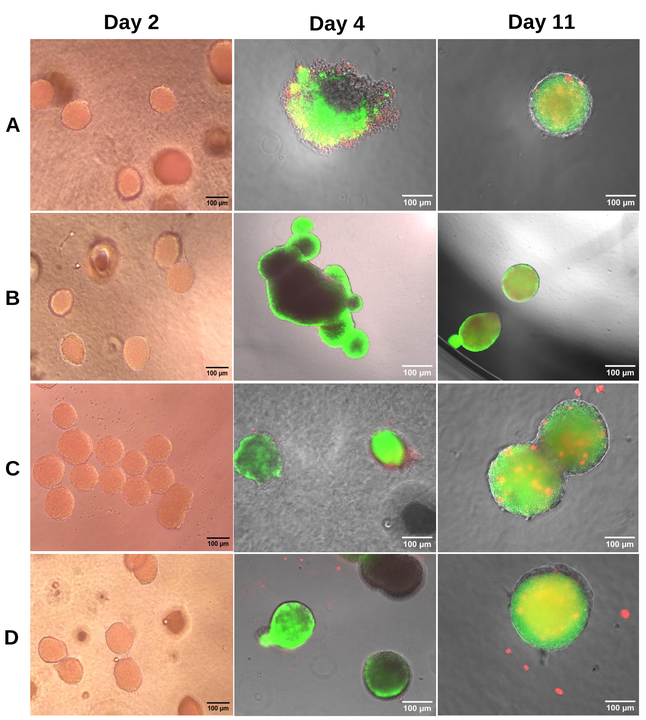

Figure 10: Brightfield and fluorescence microscopy of ReNcell VM neurospheres 3D printed in sGelMA. Live/dead cell staining was performed using Calcein AM and Propidium Iodide. By day five, both sGelMA(C) and sGelMA(C)/RwoPS hydrogels appeared to dissolve, but the cells remained alive and adhered to the bottom of the 48-well plates. At day 11, ReNcell VM remained mostly encapsulated in SGelMA(D) and sGelMA(D)/RwoPS hydrogels.
A: sGelMA(C) B: sGelMA(C)/RwoPS C: sGelMA(D) D: sGelMA(D)/RwoPS

Future Directions

Advanced Biomatrix GelMA had the highest ratio of methacrylate functionalization compared to all batches of sGelMA tested. When different types of sGelMA were 3D printed with ReNcell VM, batches A, B, and C dissolved by day 5. Some cells remained alive and appeared to attach to the bottom of the well plates. SGelMA(D) 3D prints did not completely dissolve. This is likely related to the degrees of methacrylate functionalization. The rate at which GelMA dissolves in aqueous solution can be tuned to cater its properties for a specific cell type. The use of ReNcell VM neurospheres may have a protective effect during the 3D printing process and provide better results compared to printing with single cells. The survival of neurospheres after day 11 suggests that an extruder temperature of 10 C is a viable choice when 3D printing. GelMA alone is likely insufficient for high 3D printing resolution. Mixing other materials, such as silica nanoparticles, hydrocarbon polymers, or peptides which are known to promote cell adhesion may improve fidelity and cell viability. More research is required to further improve GelMA synthesis and better understand its uses in regenerative medicine.

Acknowledgments

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