Gelatin-Methacrylated Hyaluronic Acid Microgels for Neural Tissue Generation *In Vitro*

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Introduction

- Hyaluronic acid (**HA**) is a linear glycosaminoglycan composed of repeating polymeric disaccharides found in the extracellular matrix (**ECM**) of many tissues, including the central nervous system.
- When HA undergoes esterification with methacrylic anhydride (**AMA**), methacrylated hyaluronic acid (**HAMA**) is produced. Hydrogels provide a 3D scaffolding that supports cell survival.
- Injectable hydrogels are a promising platform for tissue engineering due to their ability to conform to a wound and their minimally invasive administration. Many injectable hydrogels are nonporous, leading to cellular isolation and entrapment.
- Injectable microporous hydrogels are particularly attractive for neural tissue engineering, offering a minimally invasive delivery and supportive framework for neural cell infiltration.

Objective

Building on previous work with gelatin-based microgels, this study explores the addition of HAMA–a key component of the native neural ECM–to gelatin microgels to improve biocompatibility and support neural tissue generation *in vitro.*

Methodology

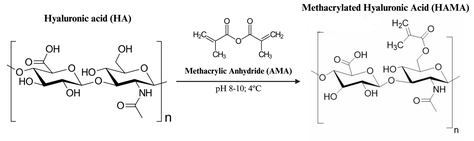


Figure 1: Synthesis of HAMA

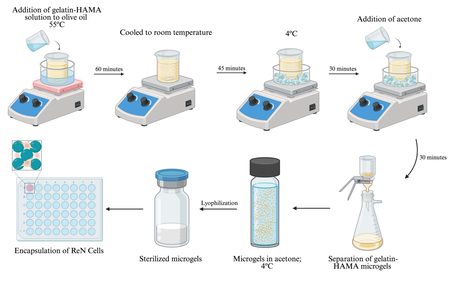


Figure 2: Synthesis of HAMA microgels using water-in-oil emulsion technique

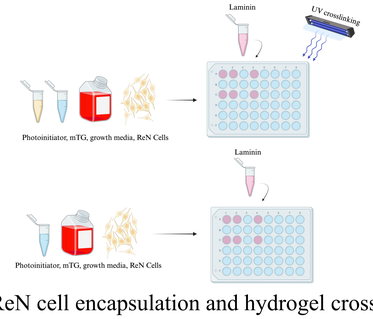
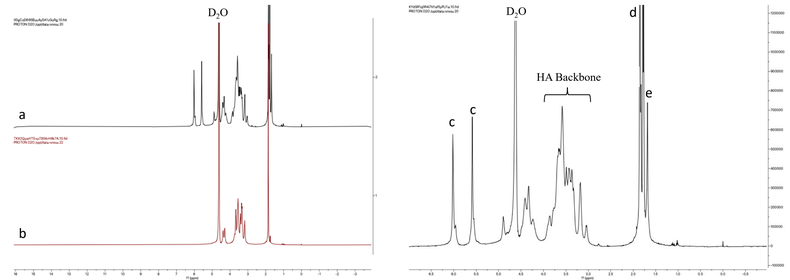


Figure 3: ReN cell encapsulation and hydrogel cross-linking

Results

Chemical Analysis



$$DM_{vinyl} = \frac{(2.06/2)}{(11.70/10)} = \frac{1.03}{1.17} = 0.88$$

NMR Peaks
c) Peaks at 5.6 and 6.1 ppm confirm methacrylation of HA
d) Methyl protons on HA backbone at 1.9 ppm
e) Methacrylate protons at 1.8 ppm

Figure 4: ^{1}H NMR spectra for HAMA (a) and HA (b). Peaks at 5.6 and 6.1 ppm (c) represent the 2 vinyl protons per methacrylate group. Vinyl protons were used to determine degree of methacrylation, which was 88%.

Microgel Characterization

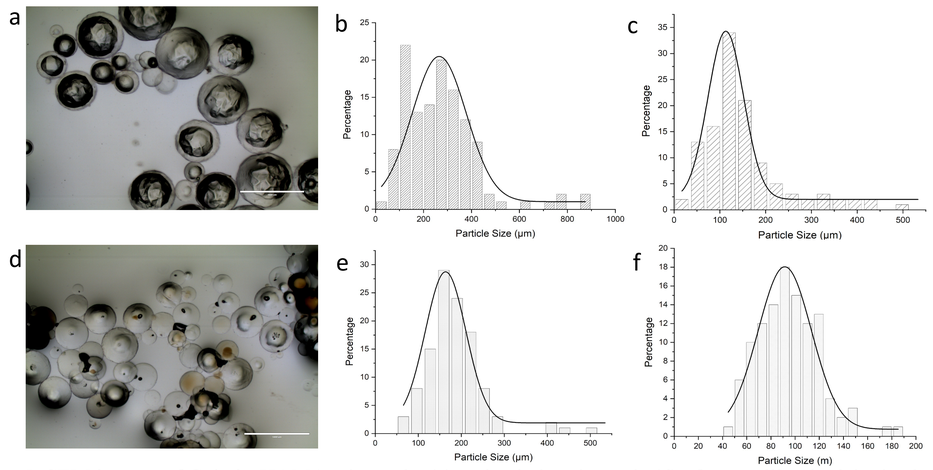


Figure 5: SEM images of Gelatin-HAMA microgels (a) and gelatin microgels (d) after swelling in deionized water. (b) Size distribution of hydrated gelatin-HAMA microgels (average diameter = 265 μm ±157 μm) and (c) dry gelatin-HAMA microgels (average diameter = 123 μm ±91 μm). (e) Size distribution of hydrated gelatin microgels (average diameter = 163 μm ±70 μm) and (f) dry gelatin microgels (average diameter = 91 μm ±26 μm)

Cellular Viability in Gelatin-HAMA Hydrogels versus Gelatin Hydrogels

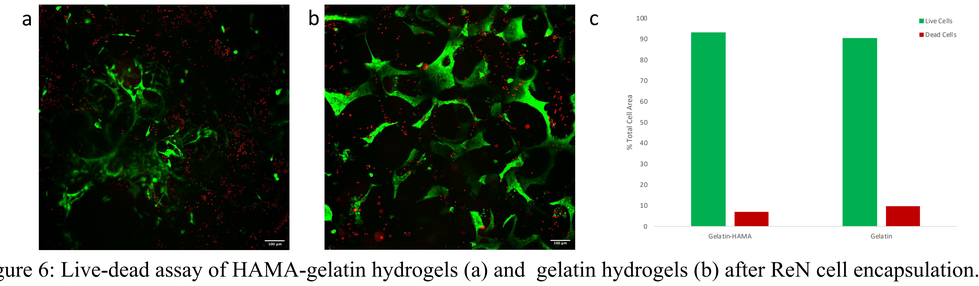


Figure 6: Live-dead assay of HAMA-gelatin hydrogels (a) and gelatin hydrogels (b) after ReN cell encapsulation. Green indicates live cells while red indicates dead cells. (c) Total green versus red area was measured to compare cell viability.

Gelatin-HAMA Hydrogel Characterization

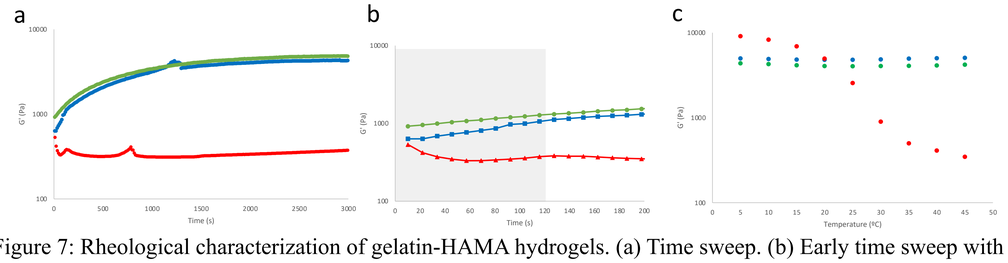


Figure 7: Rheological characterization of gelatin-HAMA hydrogels. (a) Time sweep. (b) Early time sweep with shaded region indicating UV exposure period (0-120 s). (c) Temperature sweep. Red: HAMA-gelatin + UV only. Green: HAMA-gelatin + mTG. Blue: HAMA-gelatin + MTG + UV.

Conclusions

- Methacrylation of HA was successful, shown by a high degree of methacrylation (88%). This enabled effective crosslinking and hydrogel formation.
- Gelatin-HAMA microgels exhibited a larger average diameter compared to gelatin-only microgels.
- Cell viability (%) was comparable between gelatin-only and gelatin-HAMA hydrogels, but gelatin-only supported higher overall cell counts.
- Despite lower cell density, gelatin-HAMA hydrogels may be a promising option for scaffolding that more closely mimics the native ECM.
- These findings suggest that while HAMA incorporation is feasible and structurally supportive, further optimization is needed to improve the microenvironment for neural cell viability and function.
- Continued research will focus on optimizing hydrogel composition and properties to more effectively mimic the native neural ECM and enhance neural tissue formation.
- Future studies may look at differentiation and multicellular interactions within the gelatin-HAMA hydrogels.

Acknowledgements

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