Electronic Supplementary Information for

Precise Tuning of Facile One-Pot Gelatin Methacryloyl (GelMA) Synthesis

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Table S1. Comparison of three different GelMA synthesis methods

Parameters	Buffer and molarity	pH adjustment	MAA/ Gelatin (mL/g)	Gelatin conc. (w/v %)	Reaction time (h)	Reaction temperature (°C)	DS (%)
Conventional Methods	PBS	NA	(0.8-2)/1	10	1-3	50	>85
Sequential Method	CB 0.1 M	pH 9 (multiple)	total 0.1/1 (multiple)	10	3	50	97
Facile One-pot Method	CB 0.25 M	pH 9 (one-time)	0.1/1 (one-time)	10-20	1	35-50	96

Review of previous studies

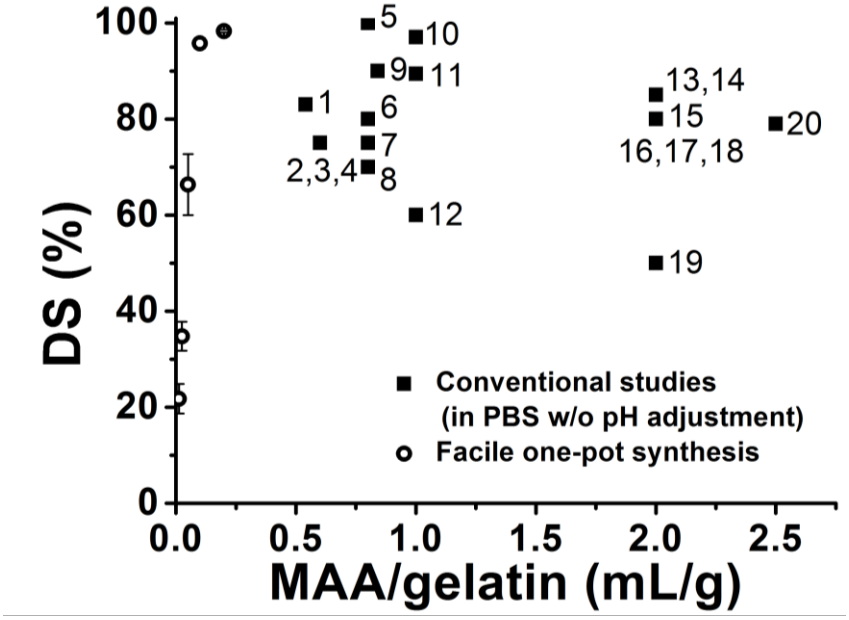

Figure S1. Comparison of DS *versus* MAA/gelatin feed ratio between conventional studies using PBS as a buffer without employment of pH adjustment (closed square) and facile one-pot synthesis (open circle). It indicates that the conventional method of GelMA synthesis has little controllability over DS. Each label corresponds to its related reference.[1-20] Some of the values were obtained from NMR measurement.[5,10,11,13,14,18-20] It is reported that NMR measurement tends to result in a higher DS value compared to colorimetric assays (e.g. TNBS).[21]

NMR results of time-dependent DS monitoring of GelMA synthesis through direct freeze-drying

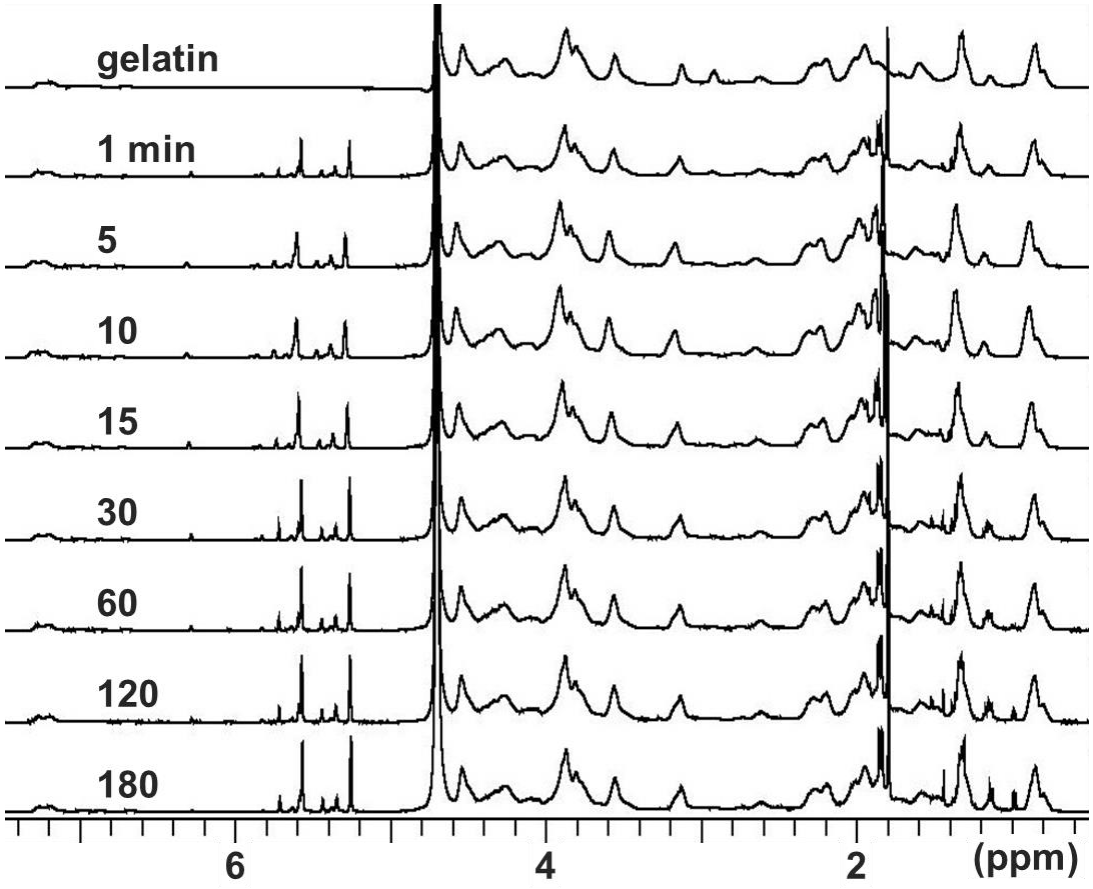

Figure S2. NMR spectra of samples, taken during GelMA synthesis conducted at standard conditions; CB (0.25 M), initial pH adjustment at pH 9, MAA/gelatin (0.1 mL/g), gelatin concentration at 10 w/v %, reaction temperature at 50 °C. Peaks at around 2.8 ppm, which correspond to methylene protons (2H) of unreacted lysine groups, disappeared almost completely after 60 min.

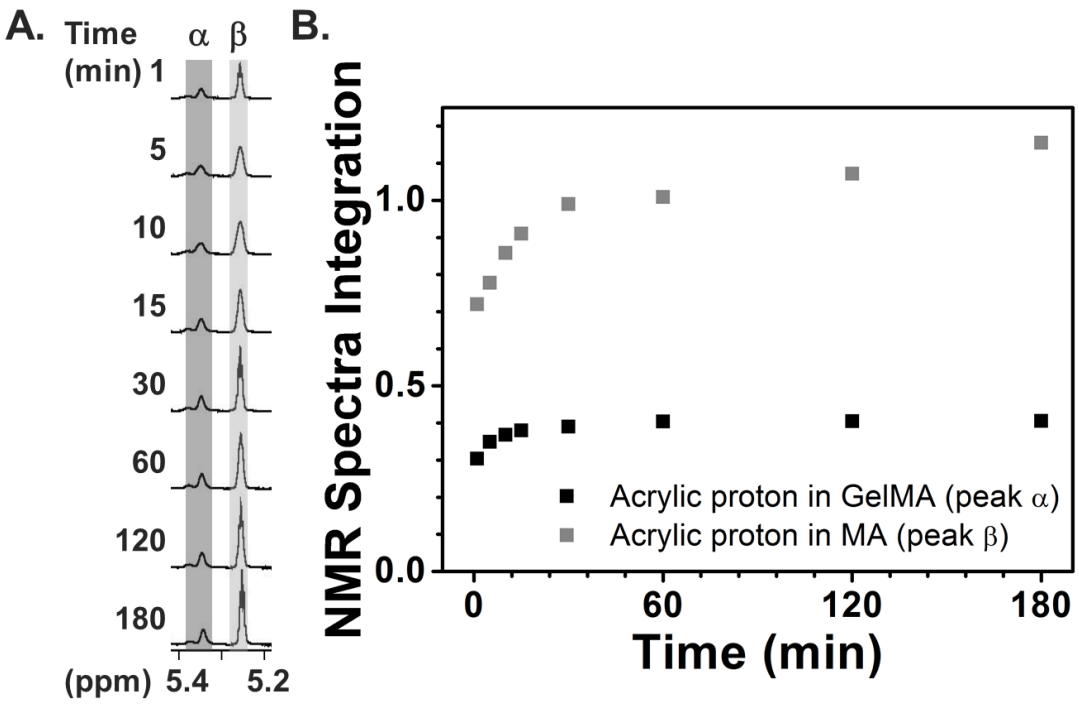

Figure S3. (A) Enlarged spectra of time-dependent NMR samples (Figure S2). Peak α at 5.35 ppm corresponds to an acrylic proton in methacrylated grafts of GelMA, and peak β at 5.25 ppm corresponds to an acrylic proton in the byproduct, methacrylic acid (MA). (B) Integrated NMR peaks of α and β. All the integrations are normalized to peaks of aromatic groups at around 7.2 ppm. Methacrylation reached a plateau after 60 min whereas production of methacrylic acid increased slightly. These results support that the GelMA reaction can be complete within 1 h. Also it suggests that there is a small amount of unreacted MAA after 1 h, being hydrolyzed further.

Mechanical properties of GelMA hydrogels

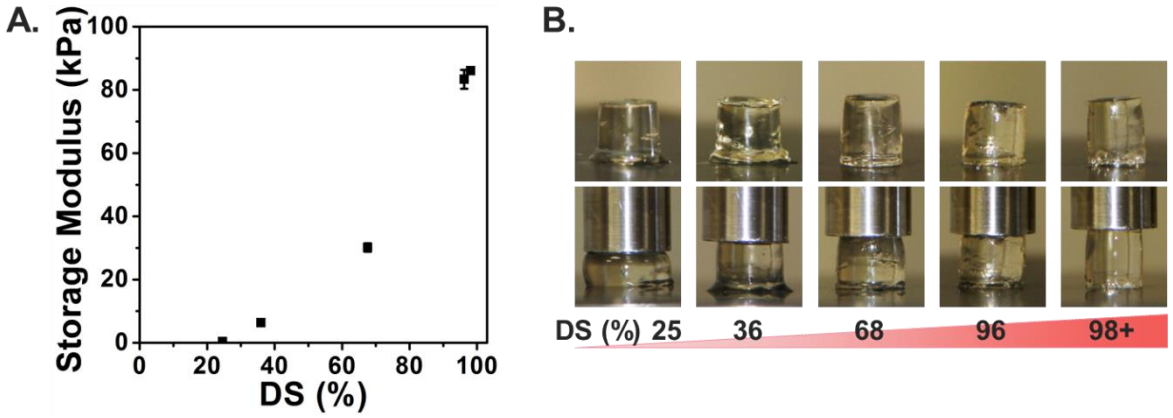

Figure S4. (A) Storage modulus of GelMA samples (30 w/v % in distilled watercontaining 1 w/v % I2959) with different DS. Strain was applied at 2% at frequency of 0.1-10 Hz during the measurement. (B) Demonstration of deformation of cylindrical-shaped GelMA hydrogels, (top) without load and (bottom) with a 1 N normal force applied. Plus (+) in DS denotes additional methacryloylation of the pendant hydroxyl group.

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