Development of a hybrid gelatin hydrogel platform for tissue engineering and protein delivery applications

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Supporting Information

Measurements

The physicochemical properties of the monomers, polymers and hydrogels were characterized by various standard methods. For Fourier transform infrared (FTIR) characterization, the dried samples were ground into powders and mixed with KBr at a sample/KBr ratio of 1:10 (w/w). FTIR spectra were then obtained with a PerkinElmer (Madison, WI) Nicolet Magana 560 FTIR spectrometer with Omnic software for data acquisition and analysis. ^{1}H NMR spectra were recorded with a Varian Unity Inova 400-MHz spectrometer (Palo Alto, CA). Deuterated water (D_2O-d_2) or deuterated dimethyl sulfoxide (DMSO-d_6) (Cambridge Isotope Laboratories, Andover, MA) with tetramethylsilane as an internal standard was used as the solvent. MestReNova software was used for the data analysis. Elemental analyses of the polymers/hydrogels were performed with a PE 2400 CHN elemental analyzer by Atlantic Microlab (Norcross, GA). The thermal property of the synthesized precursors (Arg-UPEAs and Pluronic) was characterized with a DSC 2920 (TA Instruments, New Castle, DE). The measurements were carried out from -10 to 200 °C at a heating rate of 10 °C/min and at a nitrogen gas flow rate of 25 mL/min. TA Universal Analysis software was used for thermal data analysis. For the MW measurement, Arg-UPEAs were prepared at a concentration of 1 mg/mL in a 0.1 % (w/v) LiCl in DMAc solution. The sample MWs were determined from a standard curve generated from polystyrene standards with MWs ranging from 841.7 kDa to 2.93 kDa that were chromatographed under the same conditions as the samples. The standard curve was generated from a 3rd order polynomial fit of the polystyrene standard MWs. Cell images were examined under a fluorescence microscope (Nikon TE2000-U DIC inverted microscope)

Table S1, Summary of developed Gel-MA/Arg-UPEA Hybrid Hydrogels

Hydrogels	Yield (%)/conversion percentage
Gel-MA/2-UArg-2-S(9:1, w/w)	91%
Gel-MA/2-UArg-2-S(4:1, w/w)	89%
Gel-MA/2-UArg-2-S(3:2, w/w)	93%
Gel-MA/2-UArg-4-S(9:1, w/w)	93%
Gel-MA/2-UArg-4-S(4:1, w/w)	95%
Gel-MA/2-UArg-4-S(3:2, w/w)	90%
Gel-MA/2-UArg-6-S(9:1, w/w)	92%
Gel-MA/2-UArg-6-S(4:1, w/w)	87%
Gel-MA/2-UArg-6-S(3:2, w/w)	88%

Synthesis and characterization of Gel-MA

Synthesis of Gel-MA was prepared according to previously published paper.[1] Briefly, 10.0 g of type A porcine skin gelatin was added into 100 mL of PBS buffer and dissolved by stirring at 60 °C using magnetic stirrer. 6.0 mL of methacrylic anhydride was then added to react with the gelatin solution under vigorous stirring for 3 h at 50 °C. Then, the reaction was stopped by a 5-fold dilution of the polymer solution with warm (40 °C) PBS. Salts and unreacted methacrylic anhydride were removed from the mixture by 1-week dialysis with 12-14 kDa cut-off in distilled water at 40°C. The reaction and purification temperatures were kept around or above 40 °C to avoid the possible gelation of gelatin or Gel-MA. White porous foam was then obtained by lyophilizing the solution for 1 week and was store at -80°C until further use. The degree of methacrylation was defined as the ratio of the number of methacrylamide groups tagged to gelatin to the number of amine groups in unreacted gelatin. Using ^{1}HNMR , such value was obtained by the integration of peaks at 7.4 ppm corresponding to the aromatic residues of gelatin, and peaks at 5.5 ppm and 5.7 ppm corresponding to methacrylamide groups.[2]

Synthesis of Arg-UPEA

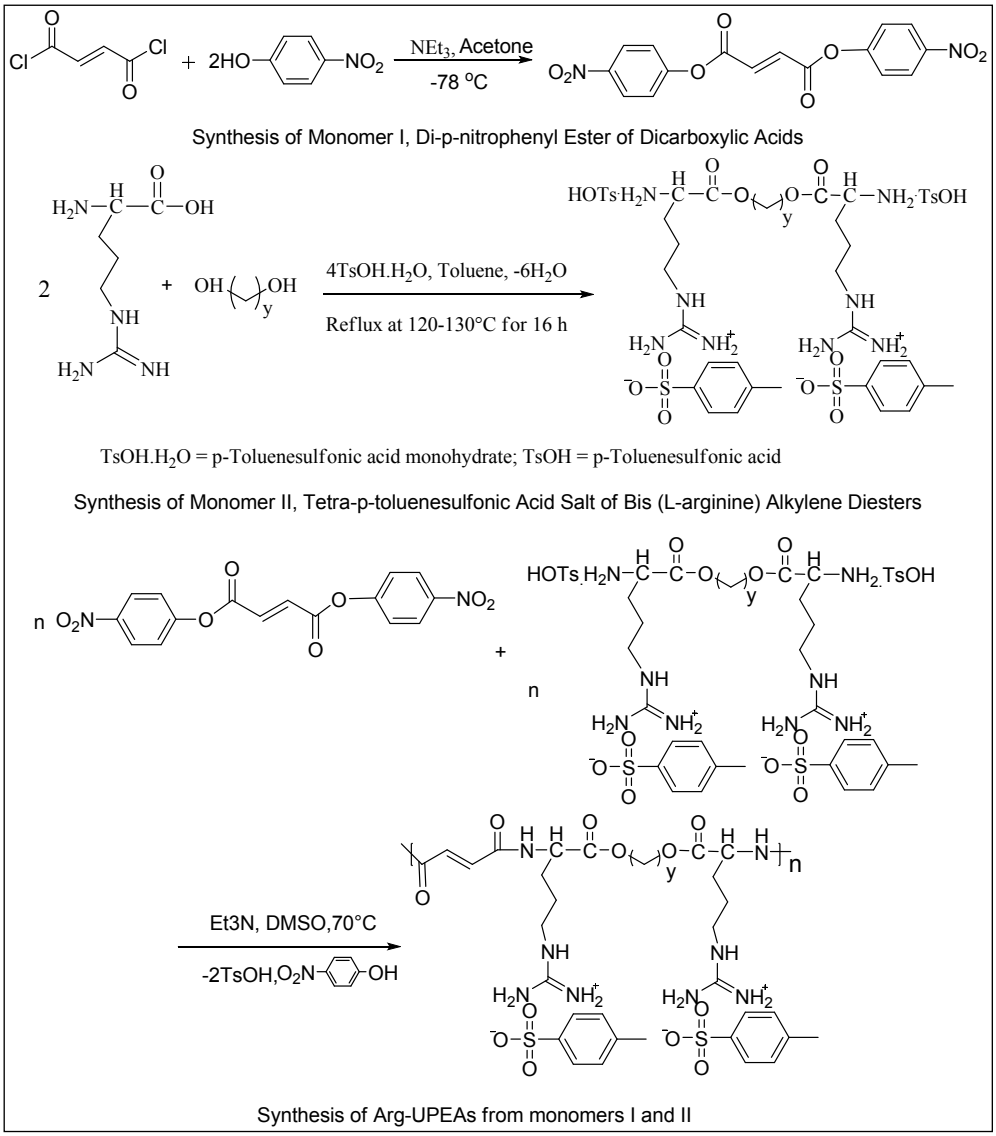

Figure S1, Synthesis of Arg-UPEAs

[1]HNMR spectrum of precursors

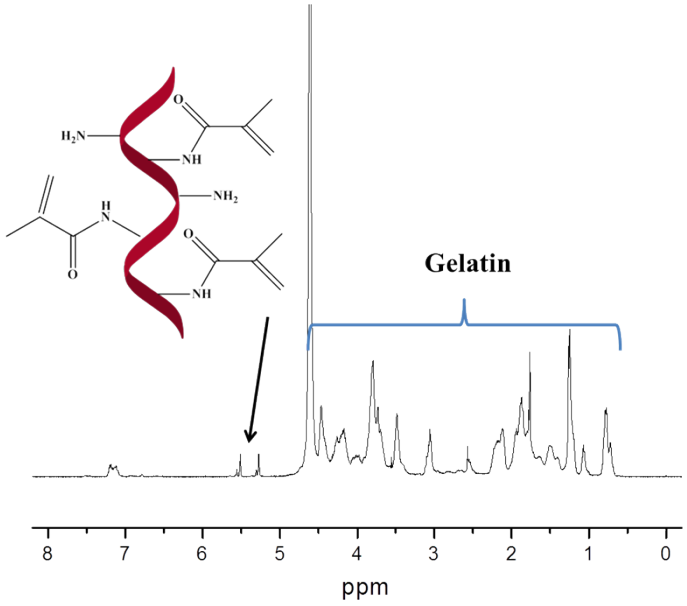

Figure S2, [1]HNMR spectrum of Gel-MA (D_2O as solvent)

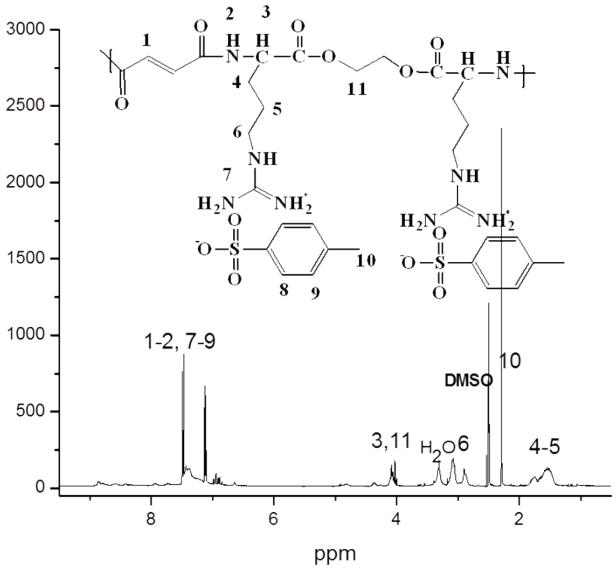

Figure S3, [1]HNMR spectrum of 2-UArg-2-S (DMSO as solvent)

Examples of stress-strain curves

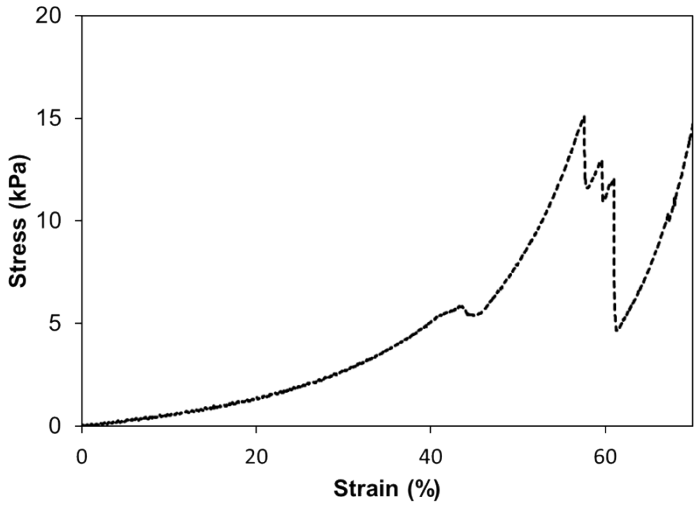

Figure S4, Stress-strain curve of Gel-MA/2-UArg-2-S (3/2, w/w)

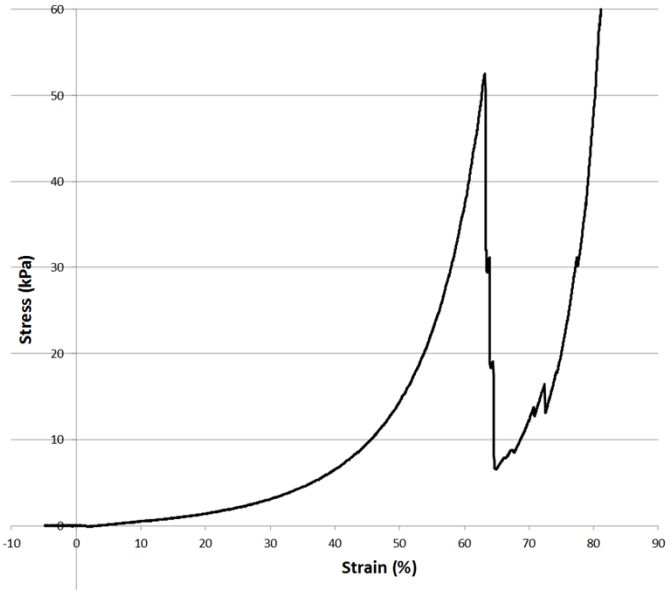

Figure S5, Gel-MA/2-UArg-6-S (3/2, w/w)

FIIR of precursors and hydrogels

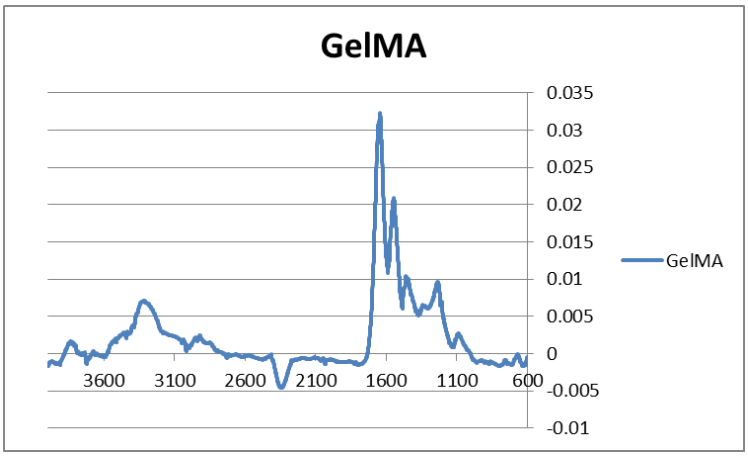

Figure S6, Gel-MA

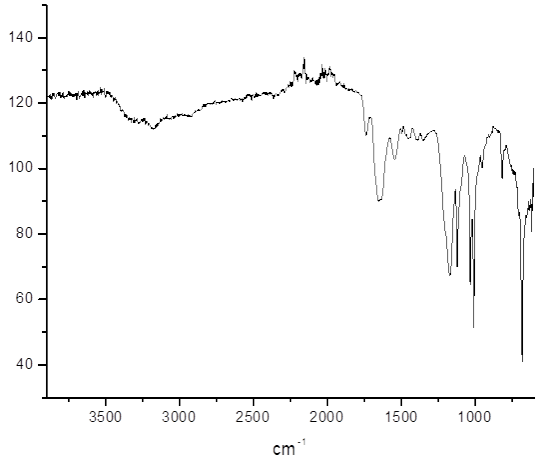

Figure S7, 2-UArg-2-S

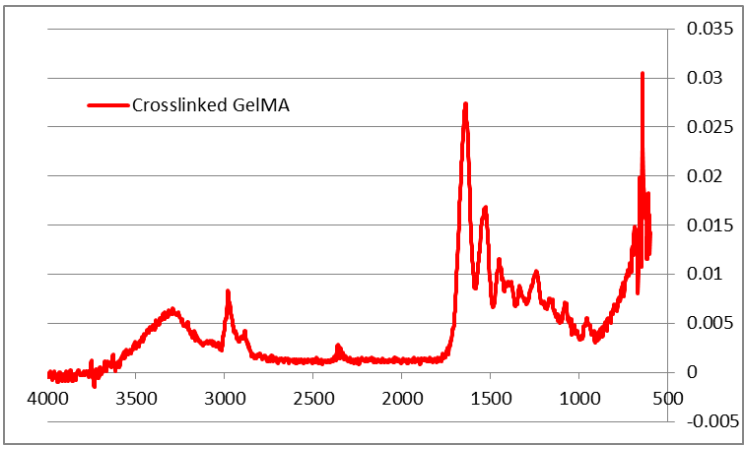

Figure S8, Crosslinked Gel-MA (Gel-MA hydrogel)

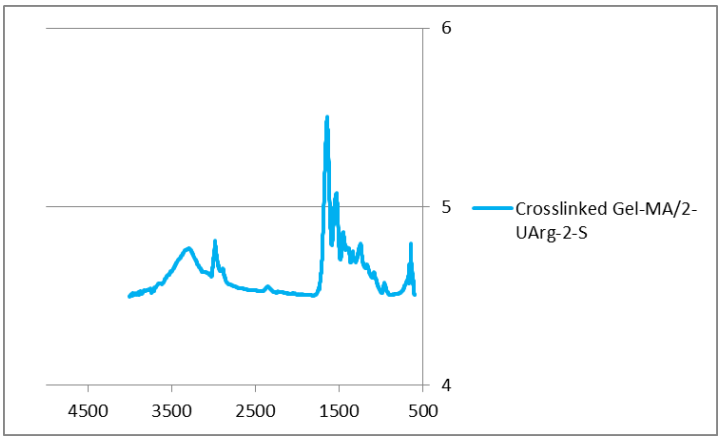

Figure S9, Gel-MA/2-UArg-2-S 4/1, w/w)

Cell culture

Hela cells, NIH 3T3 fibroblasts and J774 macrophages were purchased from ATCC. Cells and macrophages were cultured at 37 °C in 5 % CO_2 in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10 % FBS (Invitrogen), and 1 % each of penicillin–streptomycin. Cells or macrophages were used from passages 10–20. Media was changed every 2 days. Cells or macrophages were grown to 70 % confluence before splitting or harvesting.

Bioactivity of released BMP-2

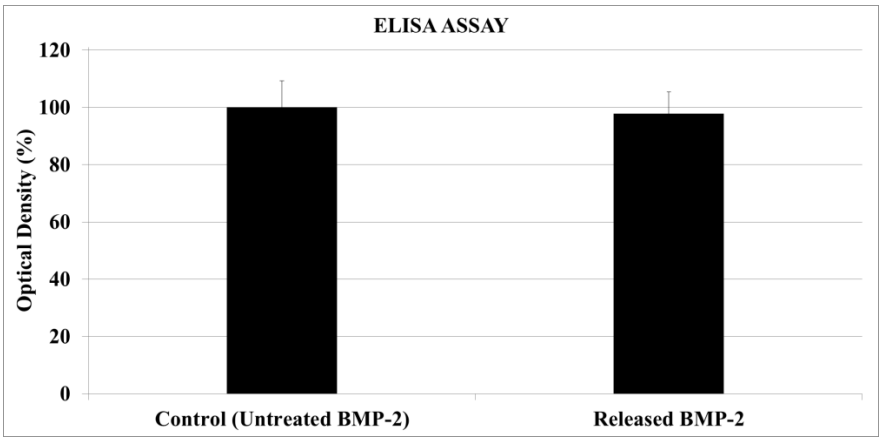

Figure S10, bioactivity comparison of untreated BMP-2 and released BMP-2

Statistics

Where appropriate, the data are presented as mean ± standard error of the mean calculated over at least three data points. Significant differences compared to control groups were evaluated by unpaired Student's t-test or Dunnet test at p 0.05, and between more than two groups by Tukey's test with or without one-way ANOVA analysis of variance. JMP software (version 8.0, from SAS Company) was used for data analysis.

References

1. A. I. Van Den Bulcke, B. Bogdanov, N. De Rooze, E. H. Schacht, M. Cornelissen and H. Berghmans, *Biomacromolecules*, 2000, **1**, 31-38.
2. H. Shin, B. D. Olsen and A. Khademhosseini, *Biomaterials*, 2012, **33**, 3143-3152.