Supplementary Information

Horseradish Peroxidase-Catalyzed Hydrogelation of Fish Gelatin with Tunable Mechanical Properties and Biocompatibility

Cytocompatibility test

FGH polymer was dissolved in DMEM with different concentrations (5; 7.5 and 10 wt%) and filtered using a syringe filter (pore size of 0.2 μm). The cytotoxicity of polymer solutions was evaluated by the cell proliferation assay kit (WST-1 assay) as previously reported.[1-3] Briefly, hDFBs were seeded in a 24 well-plate with the density of 3 x 10^4 cells/well and incubated with 1 mL of culture media (DMEM supplemented with 10% FBS and 1% PS) for 24 h. Afterward, the media were removed and cells were incubated with 1 mL of polymer soultions for further 24 h. Then, the media were replaced with 1 mL of DMEM containing 10% WST-1 solution and incubated for 1 h. Subsequently, 100 μL of the solution was transferred to a 96 well-plate and the the optical density was measured at 450 nm using a microplate reader (VersaMax Tunable Microplate reader, Molecular Devices, USA). The cell viability was expressed as a ratio of control cells (TCPS, cells treated with only DMEM and defined as 100% viable). The cell viability was also determined by a live/dead assay kit (Invitrogen, USA). Briefly, cells in each well were incubated with 500 μL of mixture consisting of 2 mM of acetomethoxy derivate of calcein (calcein AM) and 4 mM of ethidium homodimer-1(EthD-1) at 37 °C for 30 min. Then, cell morphologies were observed by a fluorescence microscope (TE2000, Nikon, Japan), in which live cells were stained green and dead cells were stained red.

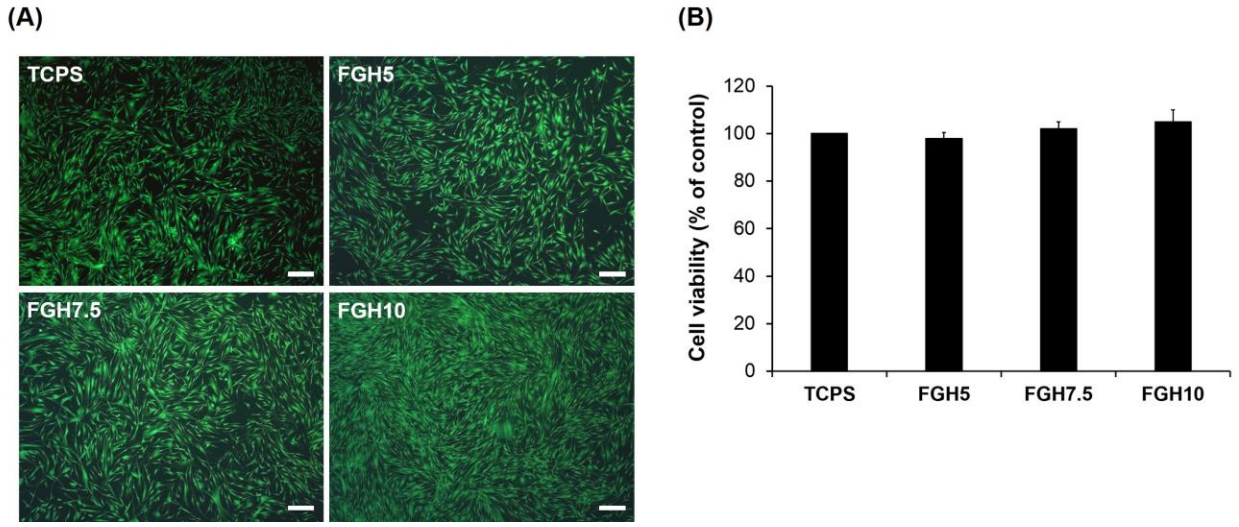

Figure S1. The cytocompatibility of FGH polymer solutions: (A) Live/Dead staining of hDFBs cultured with different FGH polymer concentrations for 24 h (scale bars represent 100 μm) and (B) Quantitative analysis of hDFBs viability by the WST-1 assay. There was no significant difference in cell viability between the FGH groups and the control group (TCPS) ($P > 0.05$), indicating the excellent biocompatibility of FGH polymer.

H_2O_2 measurement

The amount of residual H_2O_2 released from FGH hydrogels was determined by Pierce quantitative peroxide assay kits (Thermo Scientific Pierce, Rockford, IL, USA).[1, 4] In brief, 100 μL hydrogels formed by different FGH concentrations were prepared in a 48-well plate and incubated with 1 mL of PBS solution at 37 ^oC. After 30 min of incubation, 10 μL of solution was transferred to a 96 well-plate and mixed with 100 μL of premixed working reagent solution, according to the supplier's protocol. The mixture was incubated at room temperature for 15 min, then the light absorbance was measured at 595 nm using a microplate reader (VersaMax Tunable Microplate reader, Molecular Devices, USA). The released H_2O_2 concentration was calculated from a calibration curve of known H_2O_2 concentrations (0-100 μM).

Table S1. H_2O_2 release from the FGH hydrogel matrices after incubating with PBS for 30 min

Hydrogel	**H_2O_2 concentration (μM)**
FGH5	5.6 ± 2.4
FGH7.5	2.9 ± 1.5
FGH10	No detect

Table S2. Sequences of PCR primers for RT-PCR analysis

Gene	**Primer sequence**
Col I	Forward: CCAGAAGAACTGGTACATCA Reverse: CCGCCATACTCGAACTGGAA
Col III	Forward: AGGGGAGCTGGCTACTTCTC Reverse: CGGATCCTGAGTCACAGACA
FN	Forward: ATGATGAGGTGCACGTGTGT Reverse: CTCTTCATGACGCTTGTGGA
GAPDH	Forward: ATGACTCCACTCACGGCAAA Reverse: ATGATGACCCTTTTGGCTCC

References

[1] Kim BY, Lee Y, Son JY, Park KM and Park KD. Dual Enzyme-Triggered In Situ Crosslinkable Gelatin Hydrogels for Artificial Cellular Microenvironments. *Macromolecular bioscience.* 2016; 16: 1570-1576.

[2] Lee Y, Bae JW, Oh DH, Park KM, Chun YW, Sung HJ and Park KD. In Situ Forming Gelatin-Based Tissue Adhesives and Their Phenolic Content-Driven Properties. *Journal of Materials Chemistry B.* 2013; 1: 2407.

[3] Hoang Thi TT, Lee JS, Lee Y, Park KM and Park KD. Enhanced Cellular Activity in Gelatin-Poly(Ethylene Glycol) Hydrogels without Compromising Gel Stiffness. *Macromolecular bioscience*. 2016; 16: 334-340.

[4] Lee Y, Choi KH, Park KM, Lee JM, Park BJ and Park KD. In Situ Forming and H_2O_2-Releasing Hydrogels for Treatment of Drug-Resistant Bacterial Infections. *ACS applied materials & interfaces*. 2017; 9: 16890-16899.