Supporting Information

In Situ Photocrosslinkable Hyaluronic Acid/Gelatin Hydrogel for Local Nitric Oxide Delivery

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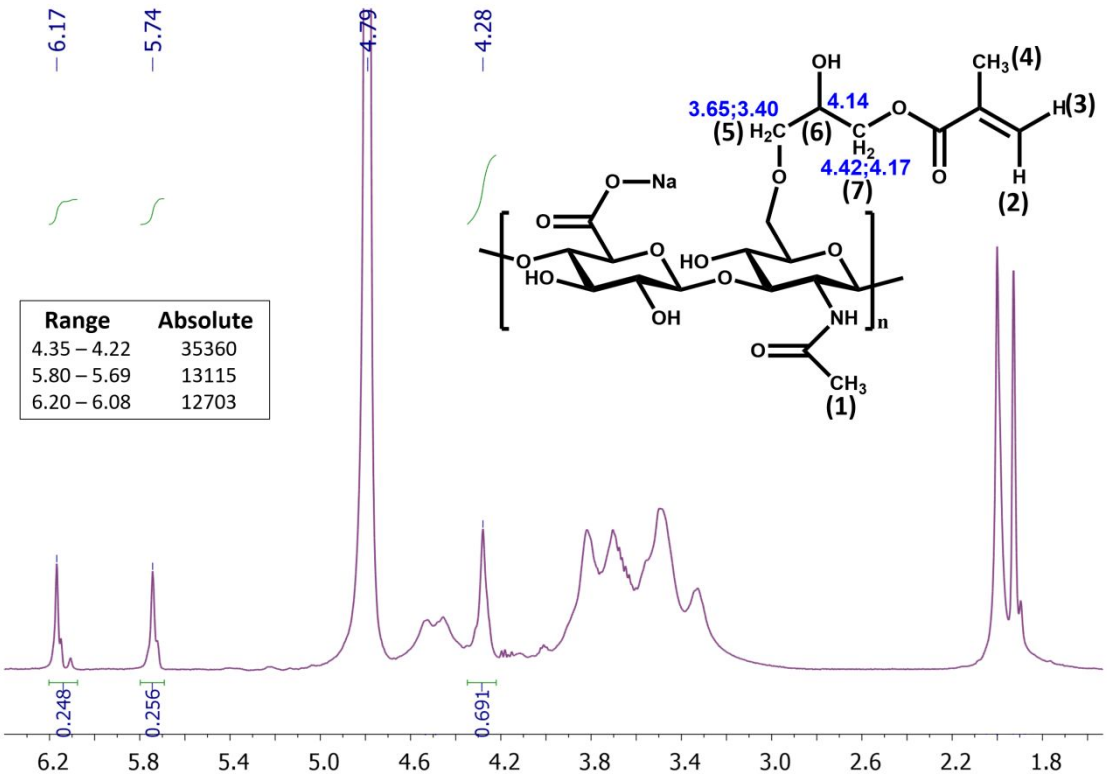

Figure S1. ^{1}H NMR spectrum of HAGMA showing the integration of the peaks at 6.17 (6.20 – 6.08) ppm and 5.74 (5.80 – 5.69) ppm, assigned to the terminal protons of the vinyl group (peaks 2 and 3), and the narrow peak at 4.28 (4.35 - 4.22) ppm, which is likely to result from the overlapping of the peaks of the methylene groups predicted to appear at 4.14 ppm (peak 6) and 4.42 and 4.17 ppm (peak 7). The peaks assigned to the protons of the methylene group of the glycidyl spacer (peak 5) are predicted to appear at 3.40 ppm

and 3.65 ppm. The positions of the peaks marked in blue in the chemical structure were calculate using the program (ChemDraw®).

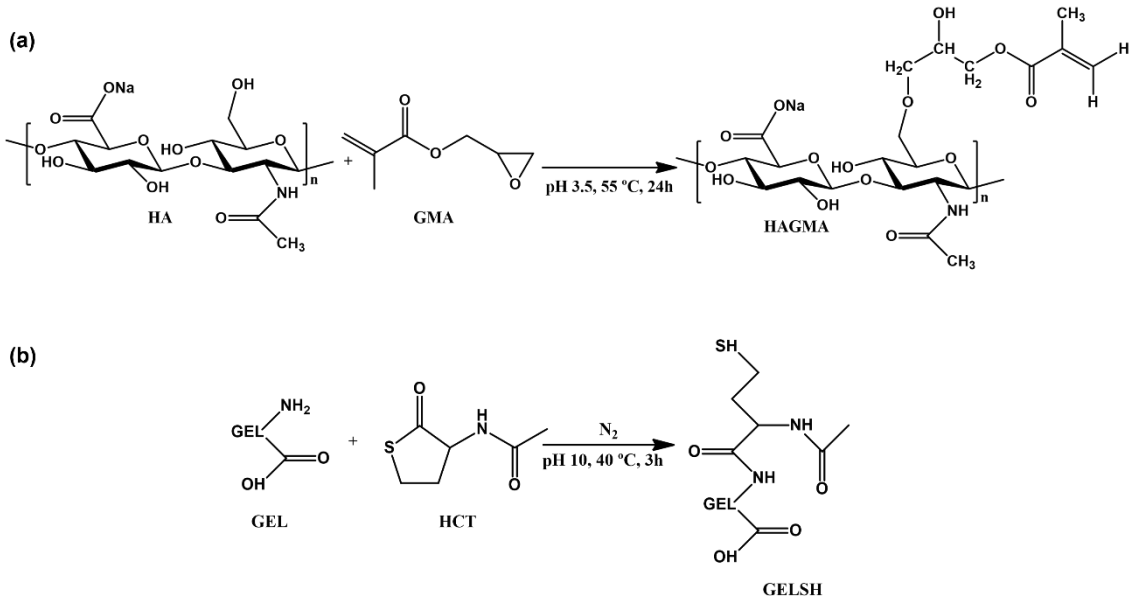

Figure S2. Scheme of the reaction between (a) hyaluronic acid (HA) and glycidyl methacrylate (GMA) leading to the formation of methacrylated hyaluronic acid (HAGMA), (b) gelatin (GEL) and N-acetyl homocysteine thiolactone (HCT) leading to the formation of thiolated gelatin (GELSH).

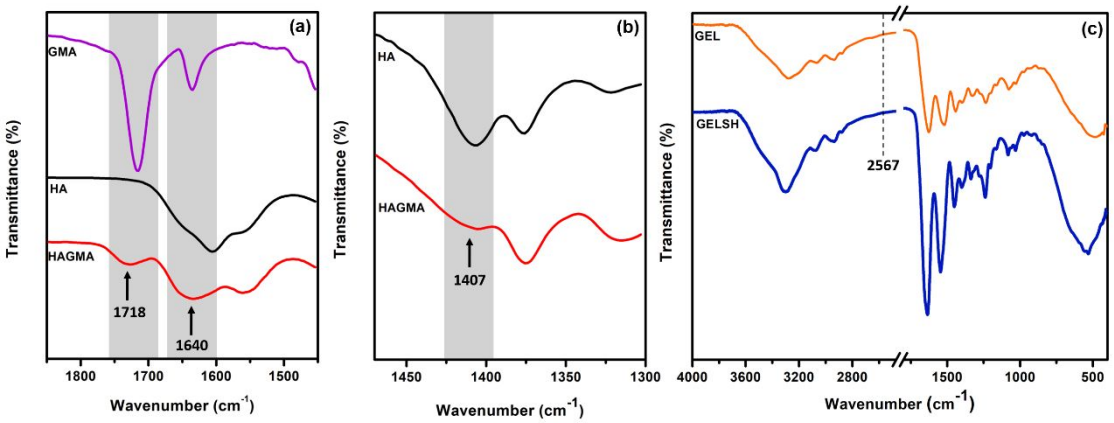

Figure S3. (a) FTIR-ATR spectra of HA, GMA, and HAGMA. The bands assigned to the C=O (1718 cm^{-1}) and C=C (1640 cm^{-1}) stretching vibrations of GMA, which are absent in the HA spectrum, become visible in the HAGMA spectrum. At the same time, the band at 1600 cm^{-1}, assigned to the hydroxyl of the N-acetyl-D-glucosamine portion is extinguished in the spectrum of HAGMA. These spectral changes reinforce the evidence that methacrylation occurs through ether bond formation between the epoxy ring of GMA and the hydroxyl group of the N-acetyl-D-glucosamine portion. (b) FTIR-ATR spectra of HAGMA and HA. The reduction in intensity of the HAGMA band at 1407 cm^{-1} assigned to the asymmetric stretching of the carboxyl group[1] is evidence that part of the methacrylation reactions occurs through the reaction of GMA with the protonated carboxyl group of the D-glucuronic acid portion of HA, as already reported.[2] (c) FTIR-ATR spectrum of GEL and GELSH.

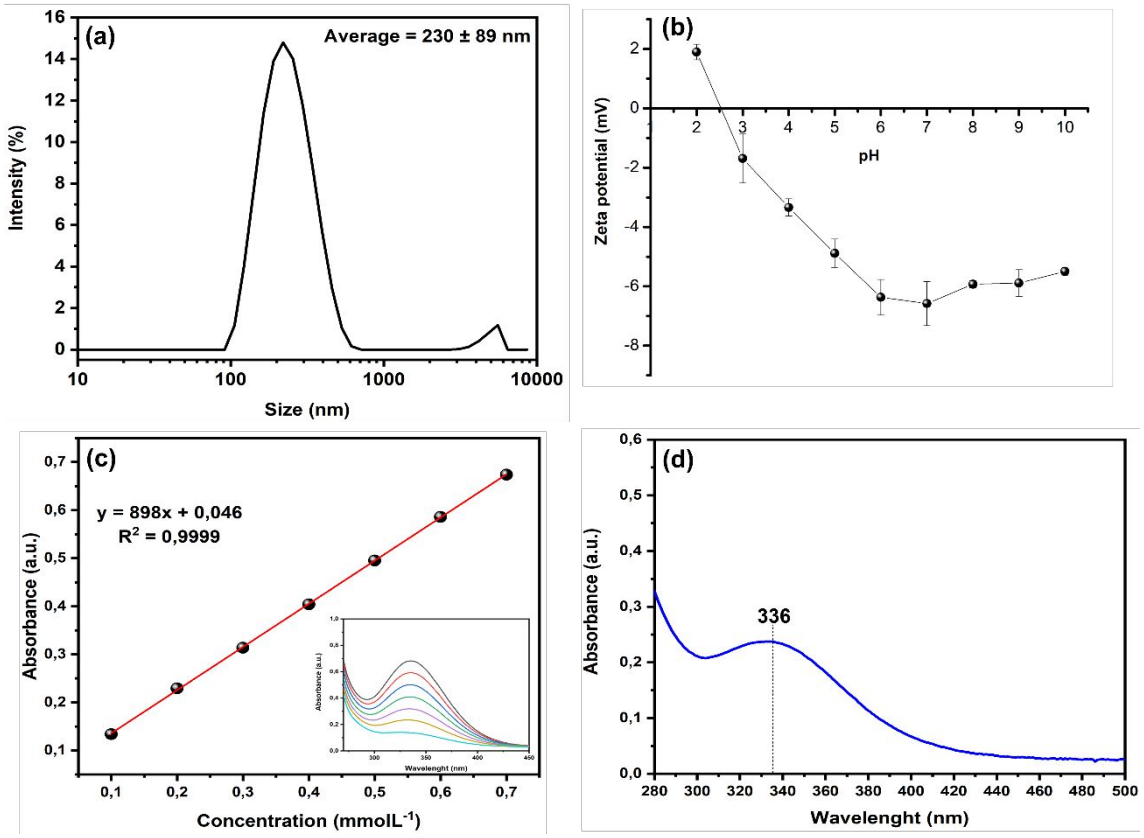

Figure S4. (a) Mean hydrodynamic diameters of NPPLGA-GSNO obtained by dynamic light scattering. (b) Surface charge measured by zeta potential of NPPLGA-GSNO as a function of the pH. (c) Calibration curve of GSNO in DMSO at concentrations range of 0.1 to 0.7 mmol L^{-1} measured at 336 nm. (d) UV-Vis absorption spectrum of NPPLGA-GSNO dispersed in DMSO.

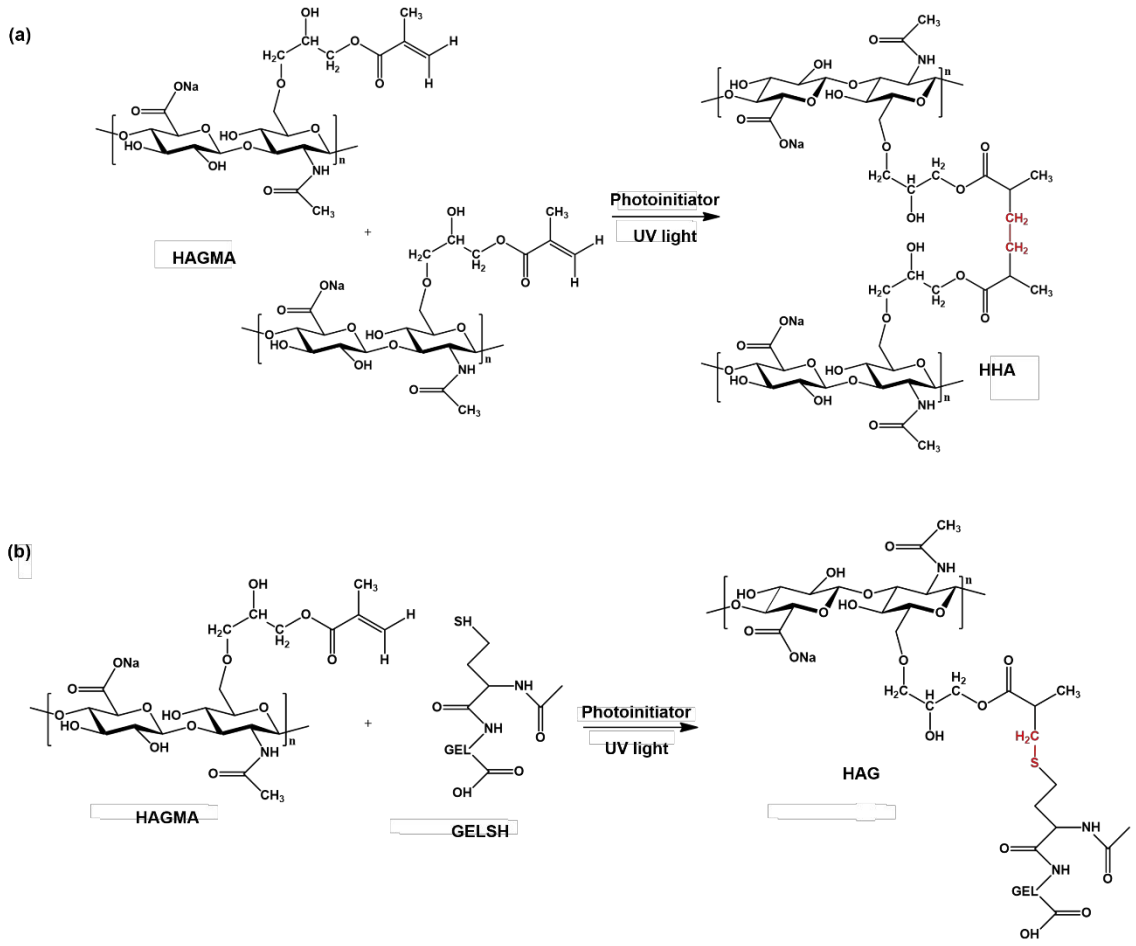

Figure S5. (a) Scheme of the photocrosslinking reaction between vinyl groups of HAGMA leading to the formation of HHA. (b) Scheme of the reaction between the vinyl group of HGGMA and the thiol group of GELSH, leading to the formation of thiol-ene bond in HAG.

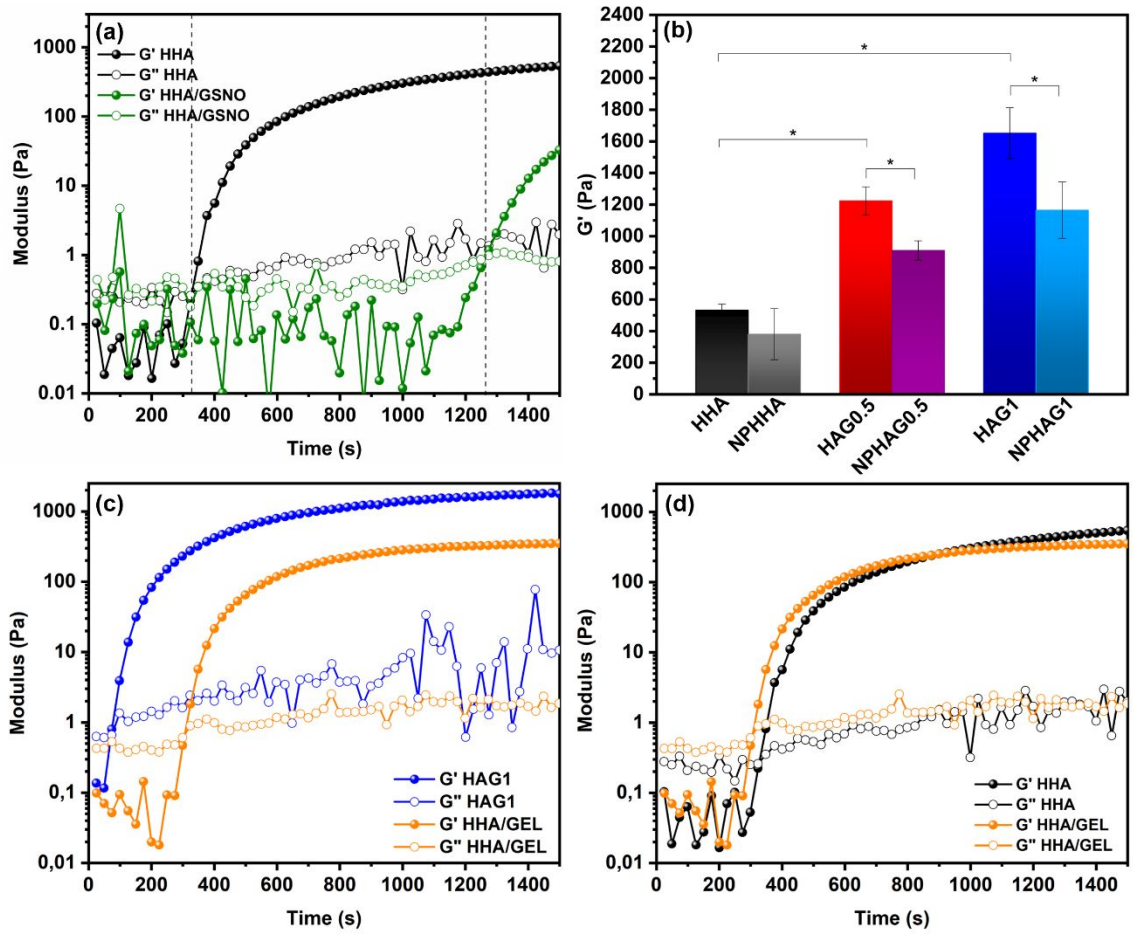

Figure S6. (a) Variation of the G' and G" moduli as a function of the irradiation time for HHA in the absence and in the presence of free GSNO at the concentration of 0.15 mmol L^{-1}. The dashed lines indicate the crossing points of the of G' and G" curves, which characterize the gelation processes (b) Bar graph of the elastic modulus of HHA, NPHHA, HAG0.5, NPHAG0.5, HAG1 and NPHAG1. (c) Variation of the G' and G" moduli as a function of the irradiation time for HAG1 and for a physical mixture of HAGMA and non-sulfhydrylated gelatin (HHA/GEL), at the same HAGMA:GELSH mass ratio as used for the preparation of HAG1. (d) Variation of the G' and G" moduli as a function of the

irradiation time for HHA and for a physical mixture of HAGMA and non-sulfhydrylated gelatin (HHA/GEL), at the same HAGMA:GELSH mass ratio as used for the preparation of HAG1.

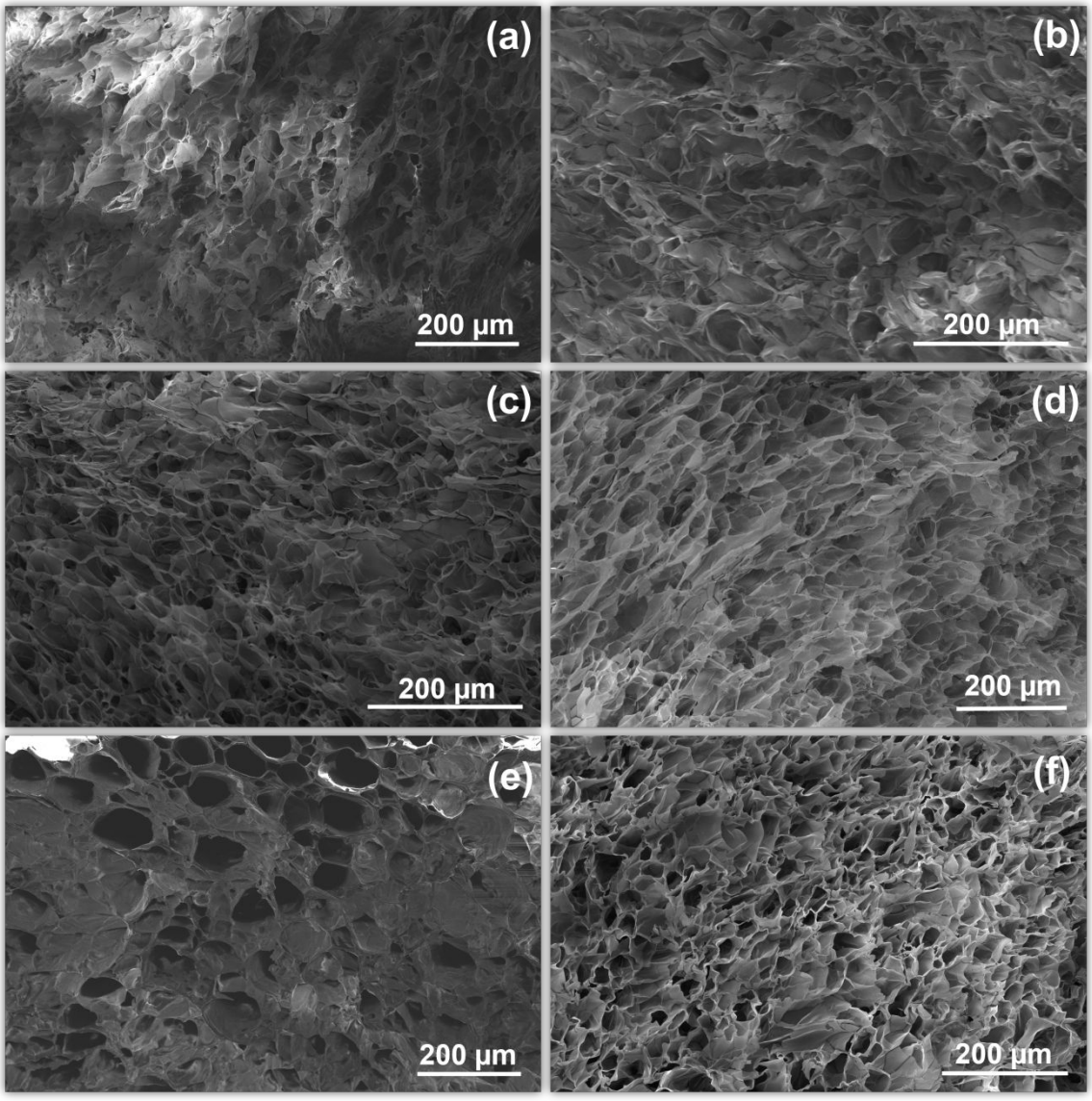

Figure S7. SEM images of (a) HHA, (b) NPHHA, (c) HAG0.5, (d) NPHAG0.5, (e) HAG1, and (f) NPHAG1.

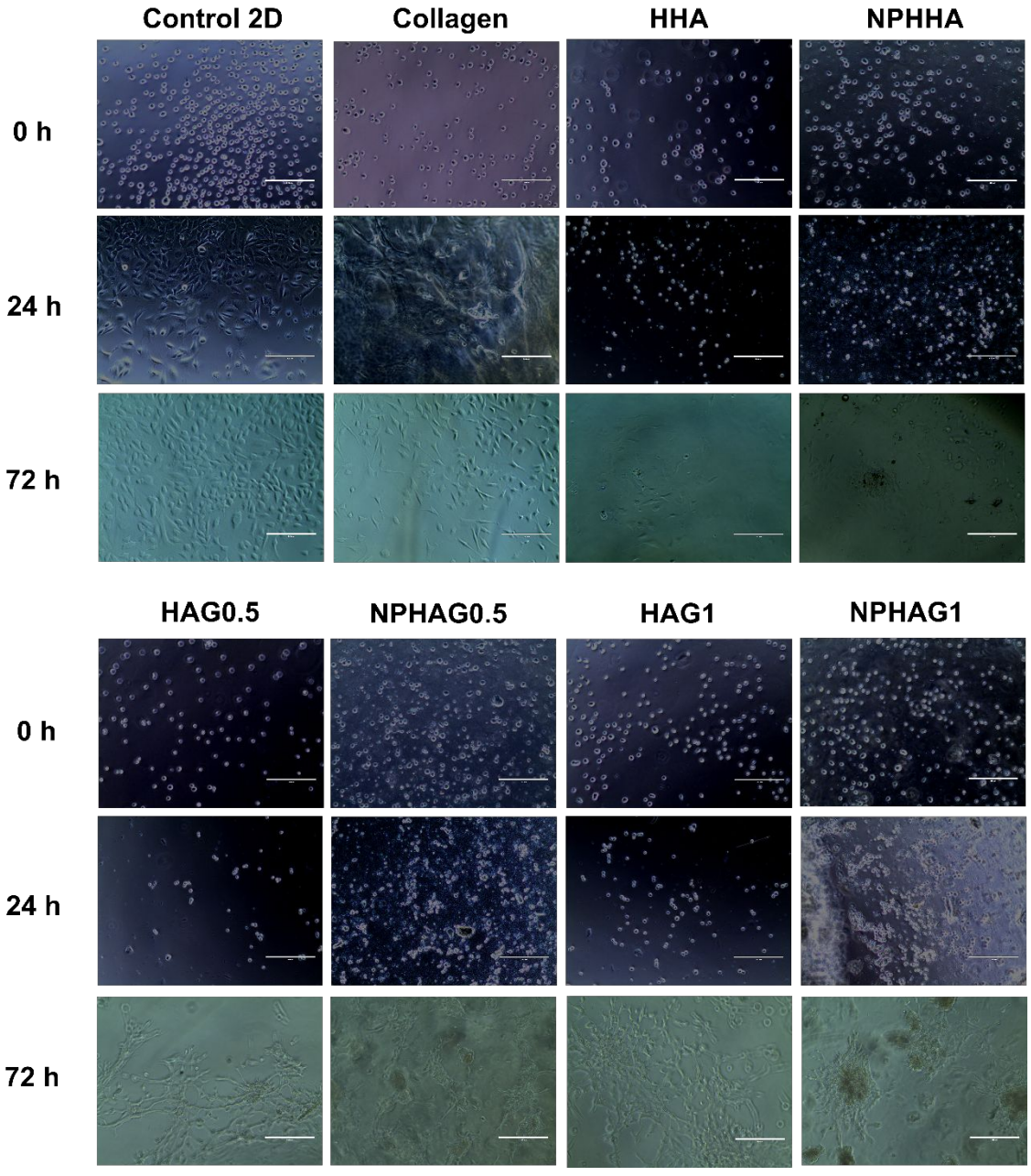

Figure S8. Optical microscopy images of fibroblasts adhesion on the controls (2D control and collagen), and HHA, NPHHA, HAG0.5, NPHAG0.5, HAG1, NPHAG1 hydrogels after 0 h, 24 h and 72 h of incubation.

REFERENCES

(1) Silverstein, R. M.; Webster, F. X.; Kiemle, D. J.; Bryce, D. L. *Spectrometric Identification of Organic Compounds*, 7th ed.; The State University of New York, College of Environmental Science and Forestry, 2005.

(2) Reis, A. V.; Fajardo, A. R.; Schuquel, I. T.; Guilherme, M. R.; Vidotti, G. J.; Rubira, A. F.; Muniz, E. C. Reaction of Glycidyl Methacrylate at the Hydroxyl and Carboxylic Groups of Poly(Vinyl Alcohol) and Poly(Acrylic Acid): Is This Reaction Mechanism Still Unclear? *J. Org. Chem*. **2009**, 74 (10), 3750-3757.