

Supplementary Information

Modulation of Cell-Cycle Progression by Hydrogen Peroxide-Mediated Cross-Linking and Degradation of Cell-Adhesive Hydrogels

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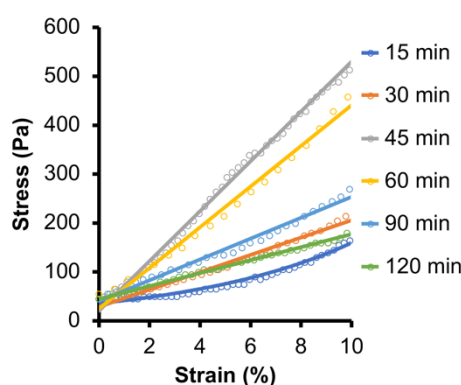


Figure S1. Stress–strain curves of Gelatin–Ph/HA–Ph hydrogel obtained from 3.0 *w/v*% Gelatin–Ph + 0.5 *w/v*% HA–Ph and 1 U/mL HRP exposed with various air containing H₂O₂ exposure time.

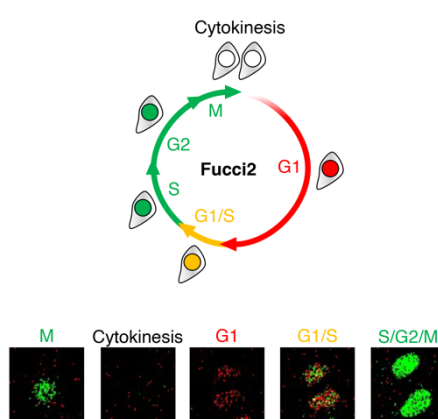


Figure S2. Diagram of the Fucci2 expression and representative confocal laser-scanning microscope observation of the Fucci2 expression. mCherry–hCdt1 is expressed in G1 phase shown as red fluorescence, mVenus–hGem expressed in late S/G2/M phase, and G1/S transition observed as yellow fluorescence (co-expression of red and green). Scale bar: 10 μ m.

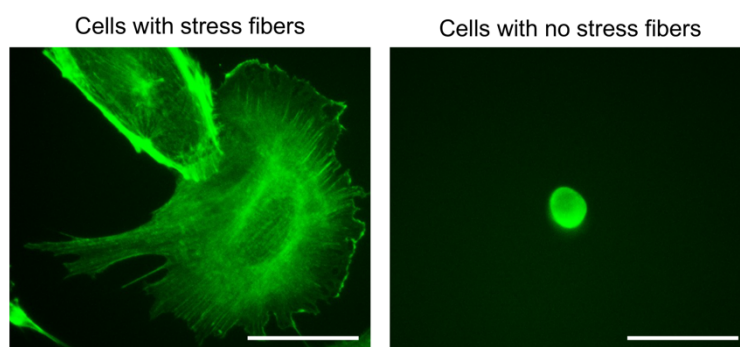


Figure S3. Representative image of cells with stress fibers and no stress fibers. Cells with stress fibers are characterized by the visible fibers in the cell's cytoplasm and nucleus. Cells: NMuMG/Fucci2 cells. Scale bars: 50 μm .

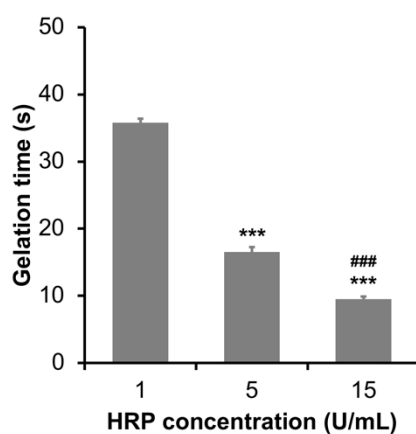


Figure S4. Gelation time of 3.0 $w/v\%$ Gelatin-Ph / 0.5 $w/v\%$ HA-Ph solutions containing 1, 5, and 15 U/mL HRP exposed to air containing H_2O_2 . Bar: S.E. ($n=5$). *** $p < 0.0005$, compared to 1 U/mL, ### $p < 0.0005$ compared to 5 U/mL, Tukey HSD.

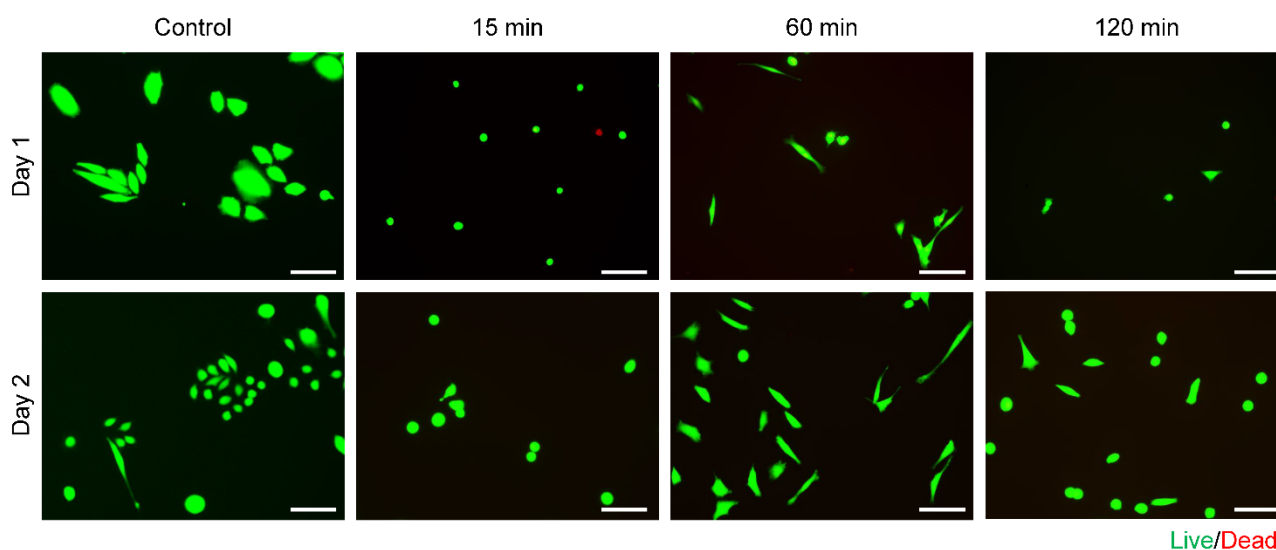


Figure S5. HeLa/Fucci2 cells stained with Calcein-AM (green) and propidium iodide (PI, red) indicating live and dead cells, respectively. Scale bar: 100 μm .

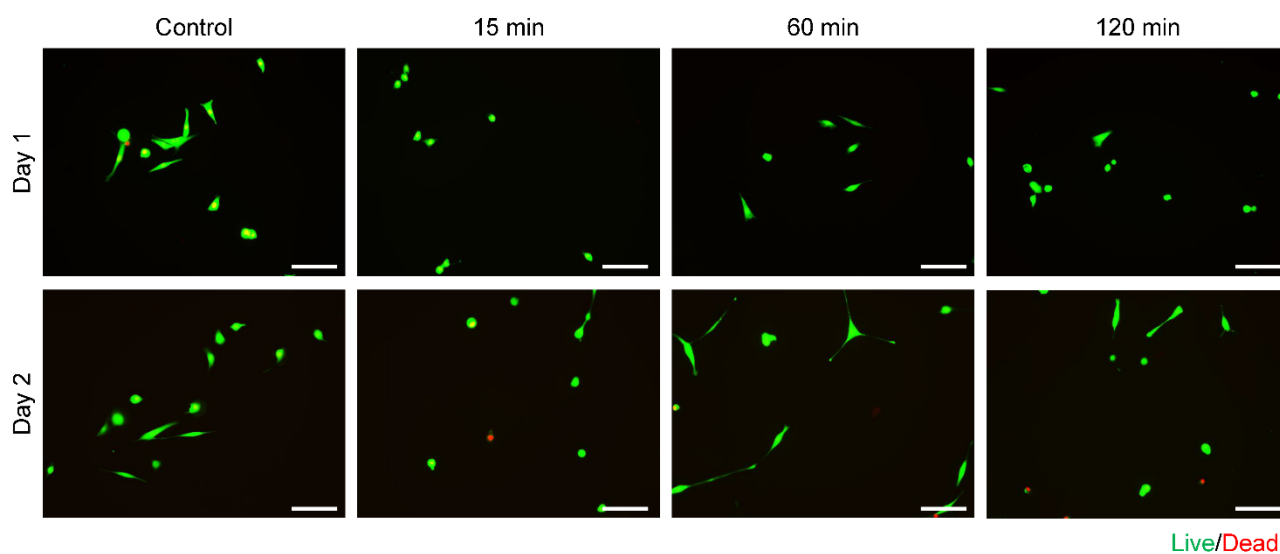


Figure S6. Fluorescence micrograph of live and dead NMuMG/Fucci2 cells stained with Calcein-AM and PI. Scale bar: 100 μm.

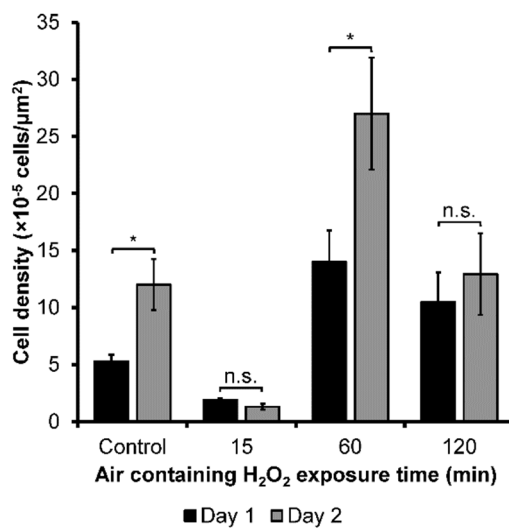


Figure S7. Density of HeLa/Fucci2 cells on Gelatin-Ph/H A-Ph composite hydrogel obtained from different H₂O₂ exposure time. Bar: S.E. (n=5). **p* < 0.05, n.s.: no significant difference (*p* > 0.05), Tukey HSD.

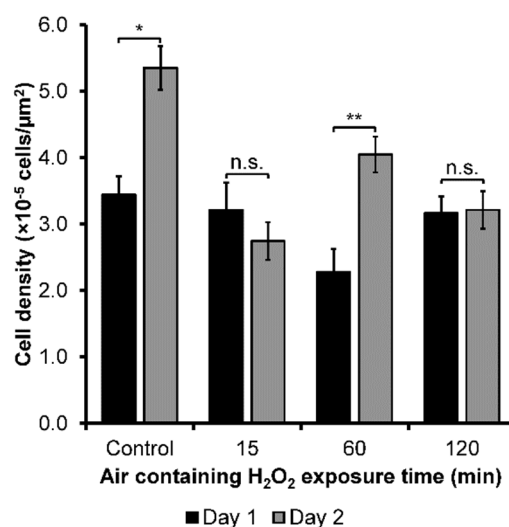


Figure S8. NMuMG/Fucci2 cell density cultured on Gelatin-Ph/HA-Ph hydrogel obtained from varying H₂O₂ exposure time. Bar: S.E. ($n=5$). * $p<0.05$, ** $p<0.005$, n.s.: no significant difference, Tukey HSD.

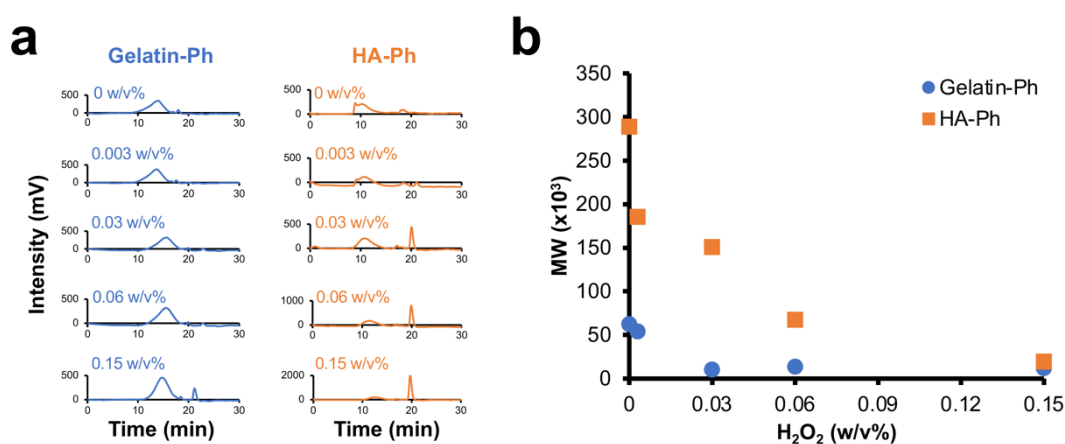


Figure S9. Degradation of Gelatin-Ph and HA-Ph polymer by H₂O₂. (a) Intensity-time curve of 3.0 w/v% Gelatin-Ph and 0.5 w/v% HA-Ph treated by 0%, 0.003%, 0.03%, 0.06% and 0.15% (w/v%) H₂O₂ solution. (b) Peak top molecular weight, relative to the polyethylene glycol (standard), of 3.0 w/v% Gelatin-Ph and 0.5 w/v% HA-Ph after overnight incubation with H₂O₂. LC conditions: separation was performed on a SHIMADZU HPLC system equipped with Shodex OHpak SB-804 HQ, 8.0 mmID x 300 mmL column; mobile phase PBS at pH 7.4; flow rate, 0.7 mL/min; temperature, 25°C.

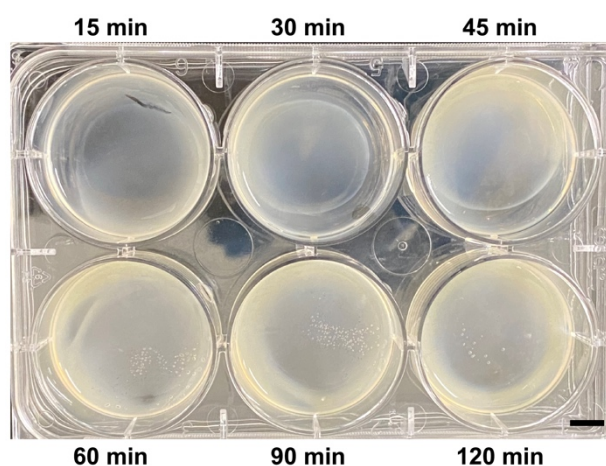


Figure S10. Phase separation in Gelatin-Ph/HA-Ph composite hydrogel fabricated after exposure to H₂O₂ for 15 to 120 min. Notice the cloudy appearance of the hydrogel in extended exposure time, indicating phase-separation occurrence. Scale bar: 5 mm.