Supporting Information

Photo-responsive hydrogels with photoswitchable mechanical properties allow time-resolved analysis of cellular responses to matrix stiffening

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Tables of prepolymer formulations.

Table S1. Formulations with ratios of AM/BIS/AZO monomers in DMF and EtOH.

Formula	Monomer/Crosslinker			Solvent System						
	AM (M)	BIS (mM)	AZO (mM)	EtOH (μL)	DMF (μL)	DMSO (μL)	H_2O (μL)	PBS[a] (μL)	Heating[b]	Observation[c]
1	1.65	26.26	10.12	--	500	--	500	225	--	x
2	1.72	27.37	--	--	225	--	725	225	--	x
3	1.65	26.26	--	--	500	--	500	225	--	x
4	1.84	29.22	--	--	150	--	725	225	--	hydrogel
5	1.84	29.22	--	225	150	--	500	225	--	hydrogel
6	1.84	29.22	11.26	--	150	--	725	225	--	precipitation
7	1.84	29.22	5.63	225	150	--	500	225	--	precipitation
8	1.92	30.60	2.95	225	100	--	500	225	--	precipitation
9	1.92	30.60	2.95	225	100	--	500	225	70°C	hydrogel
10	0.41	6.59	--	100	--	--	625	250	--	hydrogel
11	0.46	7.33	--	45	--	--	580	250	--	hydrogel
12	0.41	6.59	--	45	100	--	580	250	--	hydrogel
13	0.46	7.33	--	45	200	--	380	250	--	x
14	0.46	7.33	--	--	625	--	--	250	--	x
15	0.41	6.59	--	413	100	--	212	250	--	x

[a] PBS: phosphate buffered saline

[b] "--" in this column indicates reaction was performed at room temperature.

[c] "x" in this column indicates no gel formation observed, mixture remained liquid

Table S2. Formulations with minimal AM/BIS monomers in different solvent systems.

Formula	Monomer/Crosslinker			Solvent System						
	AM (M)	BIS (mM)	AZO (mM)	EtOH (µL)	DMF (µL)	DMSO (µL)	H_2O (µL)	PBS[a] (µL)	Heating[b]	Observation[c]
16	0.46	7.33	--	625	--	--		250	--	x
17	0.46	7.33	--	--	--	--	625	250	--	x
18	2.81	0.14	--	--	--	--	765	225	--	hydrogel
19	2.81	0.11	--	--	--	--	765	225	--	hydrogel
20	2.81	0.07	--	--	--	--	765	225	--	hydrogel
21	2.79	0.14	--	225	--	--	550	225	--	hydrogel
22	2.79	0.03	--	225	--	--	550	225	--	x

(a) PBS: phosphate buffered saline

(b) "--" in this column indicates reaction was performed at room temperature.

(c) "x" in this column indicates no gel formation observed, mixture remained liquid

Table S3. Formulation of AM/BIS/AZO polymer heated during polymerisation.

Formula	Monomer/Crosslinker			Solvent System						
	AM (M)	BIS (mM)	AZO (mM)	EtOH (µL)	DMF (µL)	DMSO (µL)	H_2O (µL)	PBS[(a)] (µL)	Heating[(b)]	Observation[(c)]
23	2.87	45.68	4.40	100	--	--	600	--	70°C	hydrogel
24	0.80	12.72	6.13	200	--	--	300	--	70°C	hydrogel
25	1.32	20.92	10.08	100	--	--	200	--	70°C	hydrogel
26	0.45	7.13	3.43	400	--	--	500	--	70°C	x
27	0.45	7.13	3.43	--	--	--	900	--	70°C	precipitate
28	0.45	7.13	3.43	--	--	200	700	--	70°C	precipitate
29	0.45	7.13	3.43	--	--	200	700	--	70°C	precipitate
30	0.45	7.13	3.43	--	--	200	700	--	70°C	precipitate
31	0.45	7.13	3.43	--	--	600	300	--	70°C	precipitate
32	0.45	7.13	3.43	--	--	600	300	--	70°C	precipitate
33	0.40	6.36	3.06	100	--	--	100	800	70°C	precipitate

(a) PBS: phosphate buffered saline

(b) "--" in this column indicates reaction was performed at room temperature.

(c) "x" in this column indicates no gel formation observed, mixture remained liquid

Table S4. Formulations with high concentrations of monomers.

Formula	Monomer/Crosslinker			Solvent System						
	AM (M)	BIS (mM)	AZO (mM)	EtOH (μL)	DMF (μL)	DMSO (μL)	H_2O (μL)	PBS[(a)] (μL)	Heating[(b)]	Observation[(c)]
34	0.50	--	0.35	--	--	--	880	--	--	precipitate
35	0.80	0.28	4.46	--	--	--	650	--	--	precipitate
36	0.66	0.23	3.68	--	--	350	450	--	--	x
37	0.66	0.23	3.68	--	--	250	550	--	--	x
38	0.66	0.23	3.68	250	--	--	550	--	--	x
39	2.56	0.08	3.55	225	--	--	420	225	--	precipitate
40	2.30	0.07	3.19	325	--	--	420	225	--	precipitate
41	1.72	0.07	3.19	425	--	--	320	225	--	precipitate
42	1.15	0.07	3.19	525	--	--	220	225	--	precipitate
43	1.76	--	3.26	425	--	--	300	225	--	precipitate
44	1.76	--	3.26	--	--	225	500	225	--	precipitate
45	1.72	0.07	3.19	--	--	225	520	225	--	x
46	0.59	0.07	--	--	--	225	500	225	--	x
47	1.15	0.07	3.19	--	--	225	520	225	--	x
48	0.57	0.07	3.19	--	--	325	420	225	--	x

[(a)] PBS: phosphate buffered saline

[(b)] "--" in this column indicates reaction was performed at room temperature.

[(c)] "x" in this column indicates no gel formation observed, mixture remained liquid

Table S5. Formulations of AM/BIS/AZO monomers with various solvents.

Formula	Monomer/Crosslinker			Solvent System						
	AM (M)	BIS (mM)	AZO (mM)	EtOH (µL)	DMF (µL)	DMSO (µL)	H_2O (µL)	PBS[a] (µL)	Heating[b]	Observation[c]
49	2.30	0.07	1.59	325	--	--	420	225	--	hydrogel
50	1.72	0.07	1.59	425	--	--	320	225	--	hydrogel
51	1.15	0.07	1.59	525	--	--	220	225	--	hydrogel
52	1.76	--	3.26	--	--	225	500	225	--	hydrogel
53	1.76	0.04	1.63	--	--	225	500	225	--	hydrogel
54	1.17	0.04	1.63	--	--	125	600	225	--	precipitate
55	1.17	0.04	1.63	--	--	100	625	225	--	hydrogel
56	1.76	--	1.63	--	--	225	300	425	--	hydrogel
57	1.06	--	1.47	--	--	100	200	750	--	precipitate
58	1.06	--	1.47	--	--	100	725	225	--	precipitate
59	1.17	--	1.63	--	--	225	200	525	--	precipitate
60	1.06	--	1.47	--	--	100	725	225	--	hydrogel
61	0.59	--	1.63	--	--	100	625	225	--	x
62	1.17	0.04	1.63	--	--	225	500	225	--	hydrogel
63	0.59	--	1.63	--	--	225	100	625	--	x
64	1.76	--	0.81	--	--	225	300	425	--	hydrogel
65	1.17	--	0.41	--	--	113	200	637	--	hydrogel
66	1.13	--	0.16	--	--	45	200	737	--	hydrogel
67	1.17	--	0.81	--	--	225	200	525	--	hydrogel
68	1.17	0.04	0.41	--	--	113	200	637	--	hydrogel
69	1.17	0.04	0.16	--	--	45	200	705	--	hydrogel

(a) PBS: phosphate buffered saline

(b) "--" in this column indicates reaction was performed at room temperature.

(c) "x" in this column indicates no gel formation observed, mixture remained liquid

Table S5. Continued.

Formula	Monomer/Crosslinker			Solvent System						
	AM (M)	BIS (mM)	AZO (mM)	EtOH (μL)	DMF (μL)	DMSO (μL)	H_2O (μL)	PBS[(a)] (μL)	Heating[(b)]	Observation[(c)]
70	1.76	--	8.14	200	--	225	300	225	--	hydrogel
71	1.59	--	7.37	225	--	100	300	425	--	precipitate
72	1.56	--	7.23	120	--	220	300	430	--	precipitate
73	1.56	--	7.23	220	--	220	300	330	--	precipitate
74	2.45	--	7.27	225	--	100	375	150	--	precipitate
75	2.20	--	3.40	225	--	100	360	225	--	hydrogel

(a) PBS: phosphate buffered saline

(b) "--" in this column indicates reaction was performed at room temperature.

(c) "x" in this column indicates no gel formation observed, mixture remained liquid

Table S6. Formulations of AM/AZO polymer using improved conditions based on previous formulations.

Formula	Monomer/Crosslinker			Solvent System						
	AM (M)	BIS (mM)	AZO (mM)	EtOH (μL)	DMF (μL)	DMSO (μL)	H_2O (μL)	PBS[(a)] (μL)	Heating[(b)]	Observation[(c)]
76	2.20	--	6.79	225	--	100	360	225	--	hydrogel with precipitated particulates
77	2.20	--	5.10	225	--	100	360	225	--	hydrogel with precipitated particulates
78	2.20	--	3.40	180	--	60	360	310	--	hydrogel
79	2.20	--	6.79	180	--	60	360	310	--	hydrogel with precipitated particulates
80	2.20	--	3.40	100	--	60	360	390	--	hydrogel with precipitated particulates
81	2.20	--	3.40	100	--	80	360	370	--	hydrogel
82	2.03	--	3.13	100	--	80	360	450	--	hydrogel with precipitated particulates
83	4.05	--	3.13	100	--	80	720	90	--	hydrogel
84	1.01	--	3.13	100	--	80	180	630	--	precipitation
85	4.05	--	3.13	100	--	160	720	10	--	x
86	4.40	--	6.79	100	--	80	720	10	--	precipitation

(a) PBS: phosphate buffered saline

(b) "--" in this column indicates reaction was performed at room temperature.

(c) "x" in this column indicates no gel formation observed, mixture remained liquid

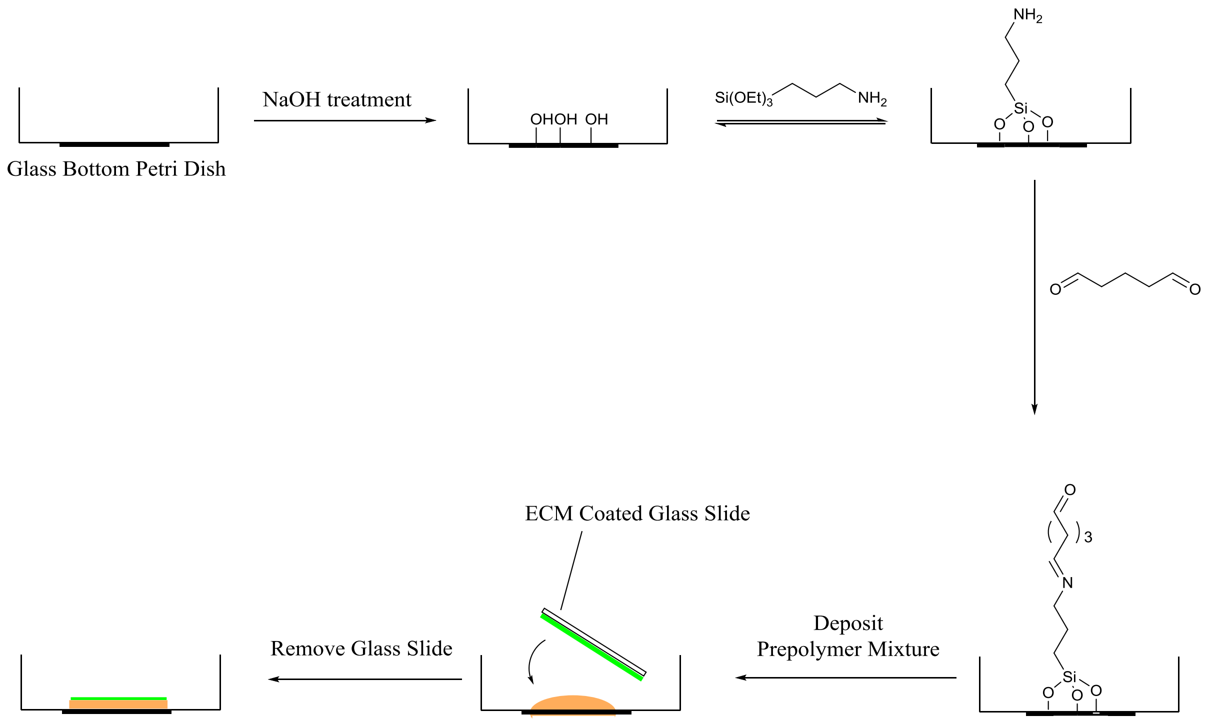

Figure S1. Schematic diagram of the substrate preparation for cell culture.

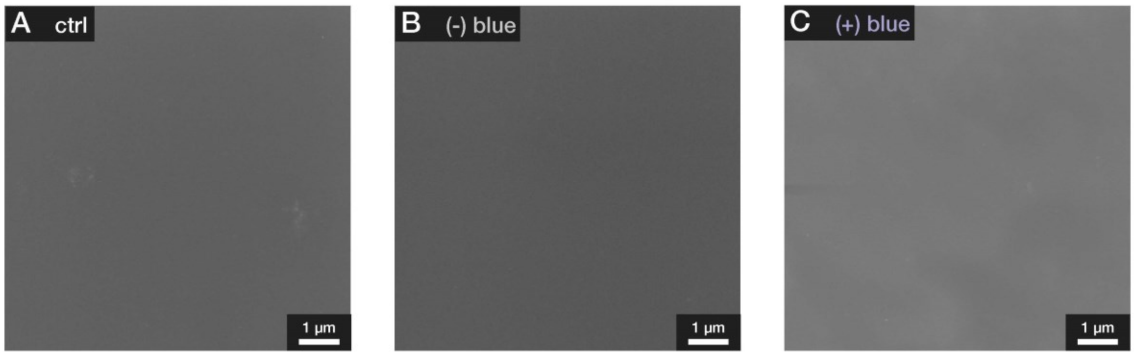

Figure S2. Low magnification ESEM micrographs of photo-responsive hydrogels before and after irradiation. The images were recorded following the treatments indicated in Figure 4A at the point of "stiffness measurement". (A) Control ("ctrl") sample: AZO hydrogels not subjected to irradiation. (B) "(-) blue" sample: hydrogel treated with UV (365 nm) irradiation. (C) "(+) blue" sample: hydrogel treated with UV (365 nm) and subsequent blue light (490 nm) irradiation.

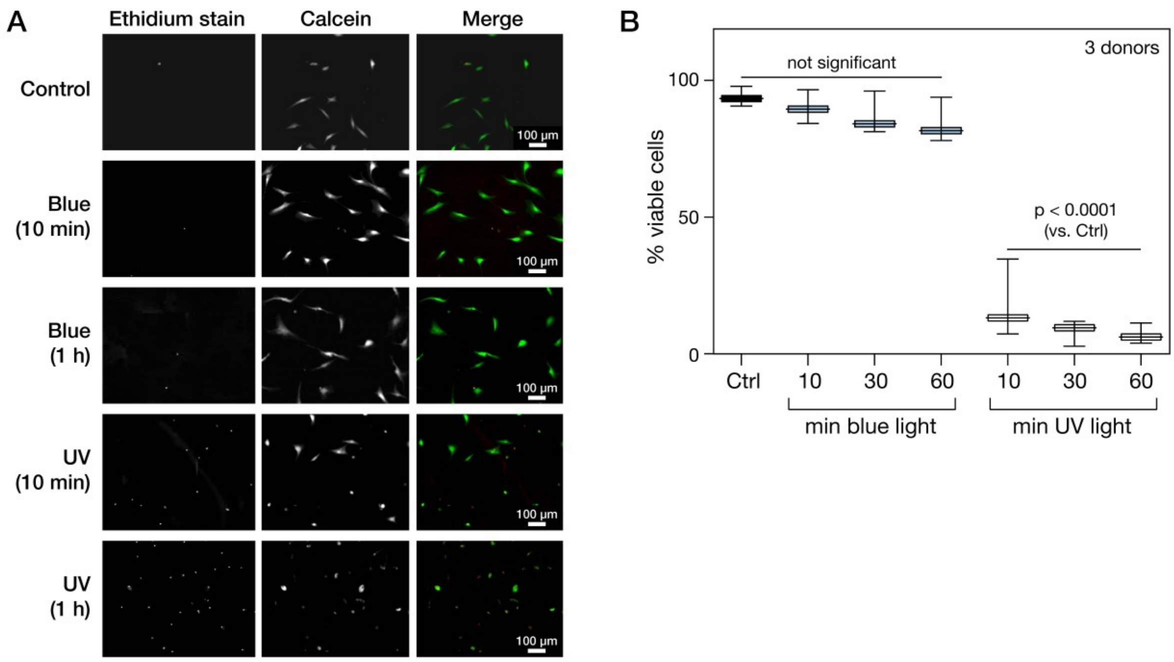

Figure S3. Cell viability following UV or blue light irradiation. Live cells are indicated by green fluorescence due to the generation of free calcein by intracellular esterases, while dead cells exhibit red fluorescence from ethidium, following loss of cell membrane integrity. (A) Primary human MSCs were subjected to different durations of UV (365 nm) or blue light (490 nm) irradiation. Cell viability was assayed by ethidium and calcein staining (scale bar = 100 µm). (B) Quantitative analysis of cell viability images. Exposure to blue light for up to 1 hour did not significantly reduce cell viability. Exposure to 10 minutes of UV irradiation significantly reduced cell viability to 18 ± 8 % (± S.E.M.; $n > 177$ measurements, cells from three donors; p-values indicated from ANOVA testing).

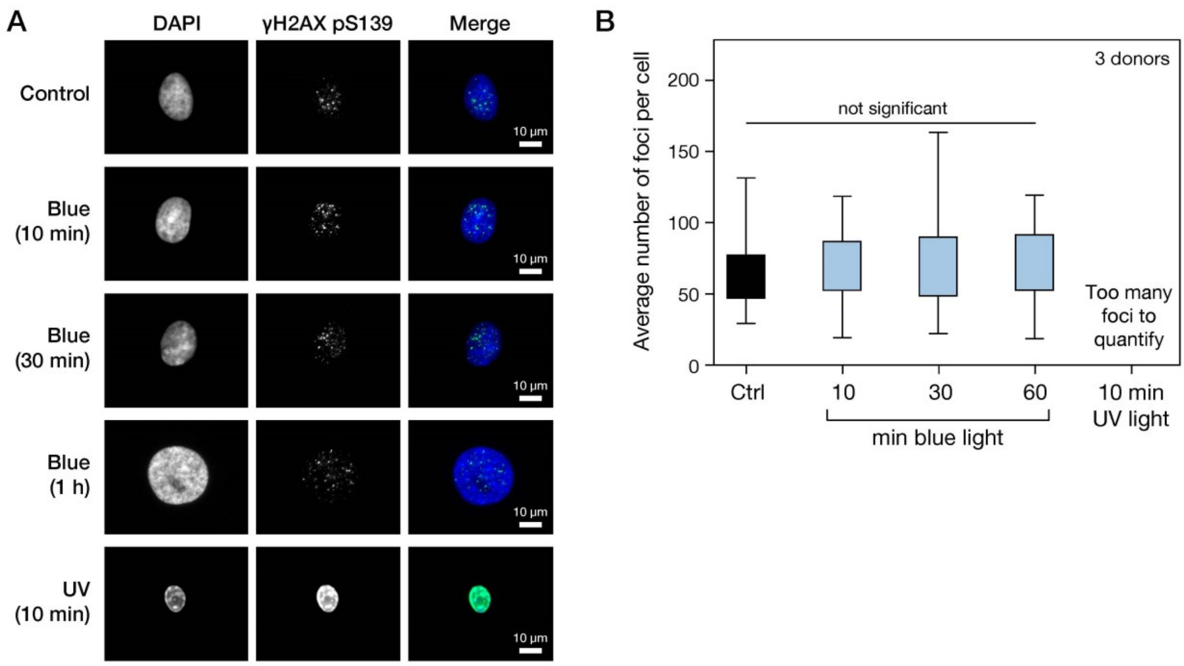

Figure S4. DNA damage following UV or blue light irradiation. (A) Primary human MSCs subjected to UV or blue light irradiation were imaged with the nuclei DAPI stained and immuno-stained against γH_{2A}X pS139 (scale bar = 10 μm). (B) Quantification of the number of γH_{2A}X pS139 foci per nucleus. Blue light exposure for up to 1 hour did not significantly increase the number of foci, but UV exposure of just 10 minutes created more foci than could be resolved with the microscope ($n > 57$ measurements, cells from three donors; significance from ANOVA testing).