Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplemental Materials

Stereochemistry and Stoichiometry in Aliphatic Polyester Photopolymers for 3D Printing Tailored Biomaterial Scaffolds

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Key Words: Stereochemistry, Off-stoichiometry, Polyester, Digital Light Processing, Ring Opening Copolymerization

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Methods and Materials

Maleic anhydride (99%, Acros Organics), phenyl glycidol ether (>99.0%, Tokyo Chemical Industry), stannous octoate (Alfa Chemicals), diethylamine (99+%, Alfa Aesar), chloroform ($CHCl_3$, ACS grade, Sigma Aldrich), diethyl ether (ACS grade, Acros Organics), hydrochloric acid (HCl, 37% HCl 63% water, ACS grade, LabChem) were commercially available and used without purification unless otherwise stated. Solution state NMR spectra (500 MHz for ^1H and 125 MHz for ^{13}C) were analyzed on a Bruker 500 spectrometer; solid state NMR were analyzed using a 500MHz Varian NMR spectrometer; spectra were processed using Topspin v4.1.1 (Mestrelab Research, S.L., Santiago de Compostela, Spain). Chemical shifts were referenced to residual solvent peaks at δ = 7.26 ppm (^1H) and δ = 77.16 ppm (^{13}C) for $CDCl_3$. Size exclusion chromatography (SEC) was performed using an Agilent 1160 Infinity II Multi-Detector GPC/SEC System fitted with RI and ultraviolet (UV) detectors (λ = 309 nm) and PLGel 3 μm (50 × 7.5 mm) guard column and two PLGel 5 μm (300 × 7.5 mm) mixed-C columns with $CHCl_3$ (flow rate 2 mL/min, 40 °C). A 16-point calibration based on poly(styrene) standards (Easivial PM, Agilent) was applied for determination of molecular weights and dispersity ($Đ$).

Synthesis of Polyester: Poly(maleate-*co*-phenyl glycidol either) (PMPGE) was synthesized from performing ring opening co-polymerization on maleic anhydride (MA) (Argos Organics) and the epoxide, phenyl glycidol either (Tokyo Chemical Industry) using the tin catalyst stannous octuate. These components were combined in a molar ratio of 200:200:1 and synthesized at 120 °C for 48 hours in a closed round bottom flask. M_n: 9.15 kDa, $Đ_M$ = 1.55.

Purification of Polyester: Once the PMPGE was synthesized, the polyester was dissolved in CHCl3 using a 1:1 mass ratio of solvent to polymer. Once the polyester was dissolved, the solution was pipetted into a 3:1 volume to mass ratio of cold diethyl ether (-80 °C) to dissolved polyester.

Isomerization of Polyester: The purified PMPGE was dissolved in $CHCL_3$ using a 3:1 mass ratio of solvent to polyester until dissolved. Once dissolved, diethylamine (DEA) was added in a 1:0.8 molar ratio of polyester to DEA in a round bottom flask. The solution was stirred at 50 °C for twenty-four hours and was washed and pulled under vacuum to remove the remaining solvent. In a 2:1 volumetric ratio, the dissolved PFPGE is mixed with 1 M hydrochloric acid in a separation funnel, shaken, and the organic layer isolated (performed twice followed by once with brine). The solution was concentrated at 40 °C under vacuum.

Formulation of polyester resins: Stoichiometric amounts of PMPGE or PFPGE, along with 1,3,5-triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (trione, a reactive diluent) were added to a vial, and mixed with the 4-arm tetrathiol pentaerythritol tetrakis(3-mercaptopropionate) (PETMP) in stoichiometric amounts. Approximately 50 wt% of acetone was added to the resin to fully dissolve the reactive components and create a homogeneous mixture. To this was added Irgacure 819 (photoinitiator, 0.5 wt%) and was shaken for approximately thirty seconds until the photoinitator was fully dissolved in the resin. Off-stoichiometric ratios of 2:1, 5:1, 10:1, 20:1, and 30:1 of alkene from the polyesters to thiol were used along with formulations without the thiol crosslinker (W/O thiol) to create additional resins of the PMPGE and PFPGE with an excess of alkenes.

Photocrosslinking Kinetic ^1H NMR: Twelve samples of PMPGE and PFPGE resins were diluted in a 5 wt% $CDCl_3$ solution. Six of each resin formulation contained the polyester and 0.5 wt%

Irgacure 819 for the free radical crosslinking study while the other six or each resin formulation contained the polyester, stoichiometric amounts of PETMP, and 0.5 wt% Irgacure for the thiol-ene crosslinking study. Six time points were collected for each crosslinking mechanism and each polyester: 0 s, 30 s, 60 s, 90 s, 120 s, 150 s, and 240 s.

Solid state multi-CP quantitative ^{13}C NMR: All experiments were collected using 17kHz magic angle spinning (MAS) multi-cross polarization with a 500MHz Varian NMR spectrometer; testing was conducted at 300K. Data was collected using multi-CP with a total CP duration of 12.1ms with each spectrum collected over 18.5 hours with 5,000 scans per sample.

Rheological Characterization. Rheological analysis was performed using a TA Instruments DH3 rheometer (TA Instruments Inc, Delaware, USA) fitted with a Peltier parallel plate system (40 mm stainless steel plate with 0° surface, TA Instruments, New Castle, Delaware, USA). The two states of the polyester, PMPGE and PFPGE, were loaded onto the parallel plates (500 μm gap, 0° angle, 40 mm diameter stainless steel plate; standard TA Instruments' Peltier plate system with attached cooling unit) at 40 °C for 30 s to ensure a uniform polymer layer without overflow, and were then cooled to 25 °C. A flow sweep measured stress and viscosity by stepping the uni-rotational shear rate from 0.2 to 2000.0 s^{-1} at 25 °C for 20 step increments. The oscillatory temperature sweep was conducted at an angular frequency of 10.0 rad × s^{-1}, beginning at 5 °C and heating to 195 °C at a rate of 2 °C × min^{-1}.

Thermal Analysis: Thermal analysis samples were prepared by punching out a 4.0 mm diameter puck from cured photopolymer resins and taking 10.0 – 25.0 grams of the PMPGE and PFPGE.

The thermal analysis was performed using a TA instruments Q2000 DSC equipped with a compressed nitrogen gas tank. The samples were cooled to -90 °C, held isothermal for five minutes, then ramped at 10 °C × min^{-1} to 170 °C and again held isothermal for five minutes and that marked one cycle, three cycles were performed for each sample.

Dynamic Mechanical Analysis: Rectangular dynamic mechanical analysis (DMA) samples were prepared *via* 3D printing sample bars (2.0 cm × 0.5 cm × 0.2 cm) using a TA Instruments Q800 DMA equipped with a liquid nitrogen dewer. Samples were analyzed in tension mode 10 Hz, a preload force of 0.01 N, and were tested in multi-strain mode. Samples were cooled to -30 °C, held isothermal for 5 min, and then heated to failure at 2 °C × s^{-1}. Three samples were used in each analysis.

Mechanical Testing. Uniaxial tensile testing to failure was conducted using an Instron with a 100 kg load cell. The printed dogbone samples (modified ASTM Type IV) were pulled at 5 mm × min^{-1} until failure, with a minimum of 7 samples per composition tested.

Shape Memory Kinetics: A 10 mm film of the *cis* and *trans* states of the photopolymer resin were heated to 100 °C and bent around a glass vial until the two ends of the film were approximately 22 cm apart and cooled to 25 °C while holding the bent position. The film was then heated to 37, 50, 75, 100, and 125 °C and kept isothermal until it had regained its original curing position. The temperatures were adjusted accordingly using a ratio between the T_gs of the 1:1 and the 30:1 formulation of 1.7 for PMPGE and 1.2 for PFPGE. As the film was returning to its original position, a picture was taking every two seconds, including at the start of the isothermal state of

the film documenting the kinetics of the film as it returned to its original cure shape. The shape memory kinetics was quantified by measuring the distance from each end of the film as it returned to its original cure shape using ImageJ (NIH, Bethesda, Maryland). Using the start of the isothermal state as the starting point, the distance recorded at every two seconds was converted to percent strain as a function of time at each temperature.

Micro CT Imaging: The 3D printed scaffolds were scanned using the methods described by Merckle et al. with exception of thresholding at an intensity of 14000. [1]

Thermal Gravimetric Analysis: 4.0 mm diameter samples of cured photopolymer resin were prepared for thermal gravimetric analysis performed using a TA instruments Q600 equipped with a tank of compressed nitrogen gas. The samples were ramped at 10 °C/min to 120 °C and held isothermal for 30 minutes. The samples were ramped again at 10 °C/min to 500 °C then pushed to 1000 °C and held isothermal for another five minutes.

Degradation Characterization: Films of PMPGE and PFPGE at stoichiometric and off-stoichiometric ratios of 1.1:1, 1.5:1, 10:1, 30:1, and W/O thiol were printed and then cut into 5 mm pucks with 5 pucks at each ratio which were then measured for diameter, thickness, and mass and placed in small 2 mL vials. Each vial was filled with 0.5 mL of 0.1 mol NaOH solution (enough to fully submerge each puck). The vials were capped and placed into an incubator set to 37 °C with slow agitation. Every 3 days the vials were removed from the incubator and the pucks were

removed from solution, lightly dried, and measured again for thickness, diameter, and mass. The solution in each vial was wasted and replaced with fresh 0.1 mol NaOH Solution. The pucks were placed back into each vial fully submerged, the vials were capped and placed back into incubator under the same conditions. The same process was used with phosphate buffer solution; however, the solution did not need to be remade, just wasted, and refilled in each vial. This process was repeated every 3 days for 111 days then every 9 days until full degradation or experiment was called.

Spin Coating: The PMPGE 1:1, PMPGE 30:1. PFPGE 1:1, and PFPGE 30:1 mixtures were prepared by adding 5 wt% to chloroform and cured for 12 hours under UV light. Once cured the samples were spun onto 12mm glass slides for 10 secs at 50 RPM and another 10 secs at 70 RPM using a KW-4A spin coater. Following spin coated the glass were placed in a 120 °C oven for 24 hours for a final cure.

Cell Culture: RAW 264.7 cells were cultured in dulbecco's modified eagle's medium (DMEM) growth media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. THP-1 cells were cultured in RPMI-1640 medium with 0.05 mM 2-mercapoethanol and 10% FBS. Both cells were kept in a humidified 37 °C incubator with a 5% CO_2 atmosphere before being seeded onto the polymer spin coated slides. To prepare for the cytocompatibility study, the spin coated slides were immersed in isopropyl alcohol (IPA) for 1 hour. IPA was removed from the slides and replaced with 70% ethanol for 15 min, followed by exposure to UV light for 20 min. RAW 264.7 and THP-1 cells were seeded at a density of 10000 cells/mL in their respective growth

media. Cytocompatibility testing was conducted using CellTiter 96 AQueous one solution cell proliferation assay (MTS) after day 1, day 3, day 5, and day 7 followed by an absorbance measurement using a Synergy HTX multi-mode microplate reader from Biotek at 490 nm. Invitrogen CellTracker CMAC Blue Dye (7-amino-4-chloromethylcoumarin) was used to stain live cells which were then imaged using a Nikon eclipse Ti inverted fluorescence microscope.

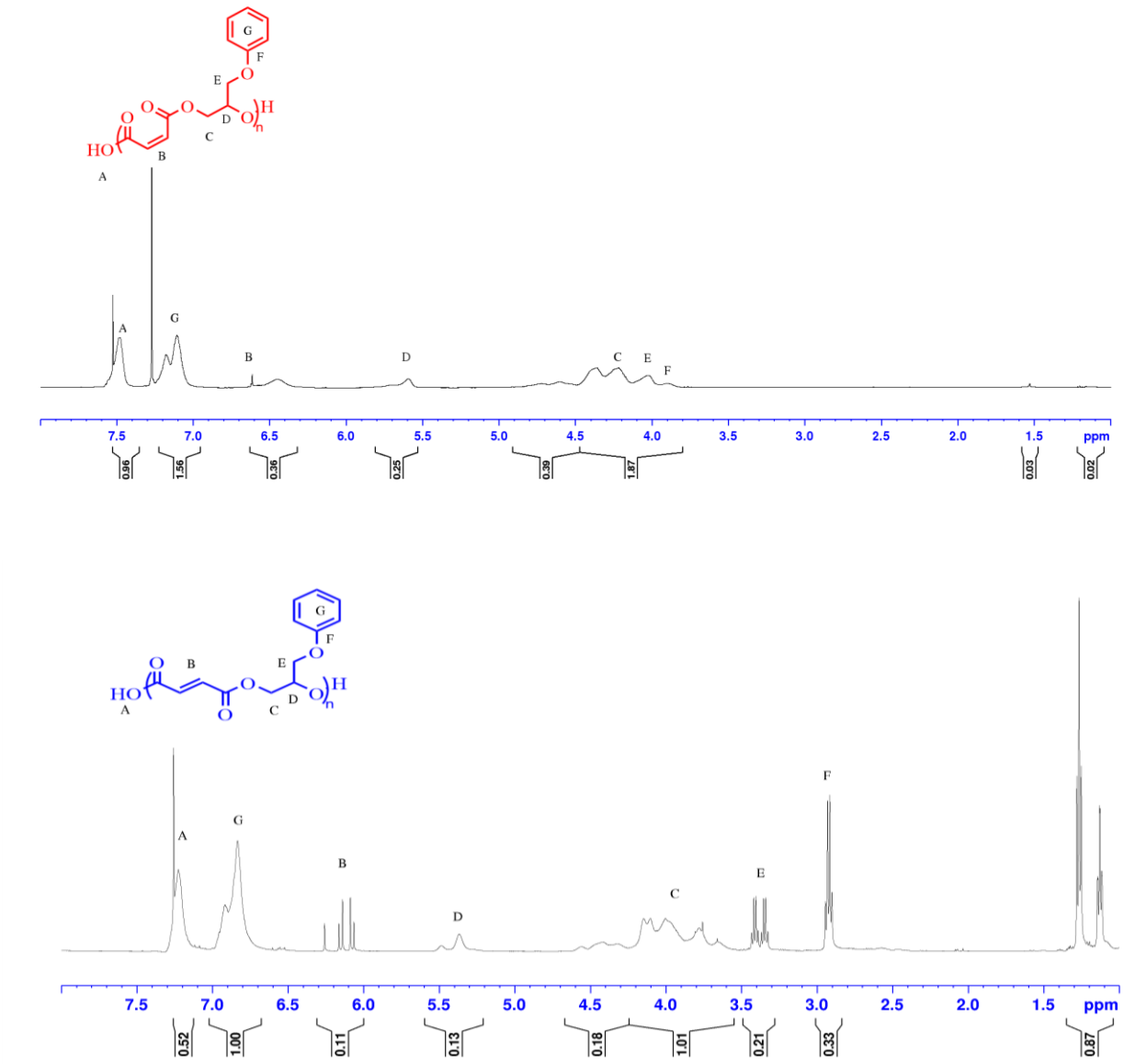

Figure S1: ^{1}H NMR spectra run at 300 MHz. The *cis* to *trans* isomerization of PMPGE (red) to PFPGE (blue) at the *B* alkene shift from 6.6 ppm to 6.3 ppm indicating a full isomerization. Axis was calibrated using $CDCl_3$ at 7.26 ppm.

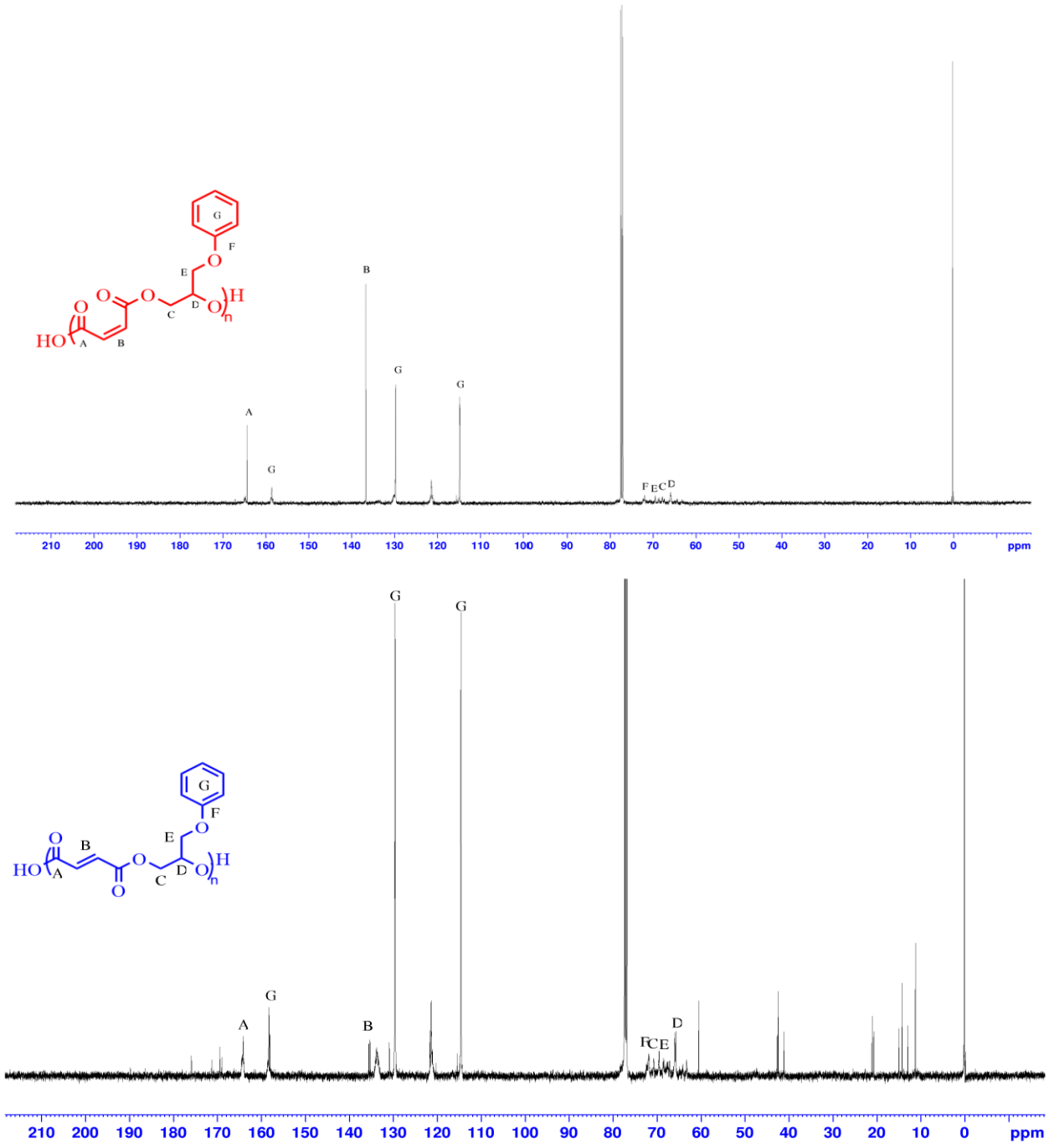

Figure S2: ^{13}C NMR spectra run at 125 MHz of PMPGE (red) and PFPGE (blue). Axis was calibrated using $CDCl_3$ 77.16 ppm.

Table S1: GPC was run using an Agilent series 1100 HPLC calibrated using polystyrene standards and $CHCl_3$.

Formulation	M_w **(kDa)**	$Đ_m$	T_g **(°C) DSC**	T_F **(°C) Rheology**
PMPGE	9.16	1.55	-2.80	66.9
PFPGE	12.8	1.56	17.4	92.2

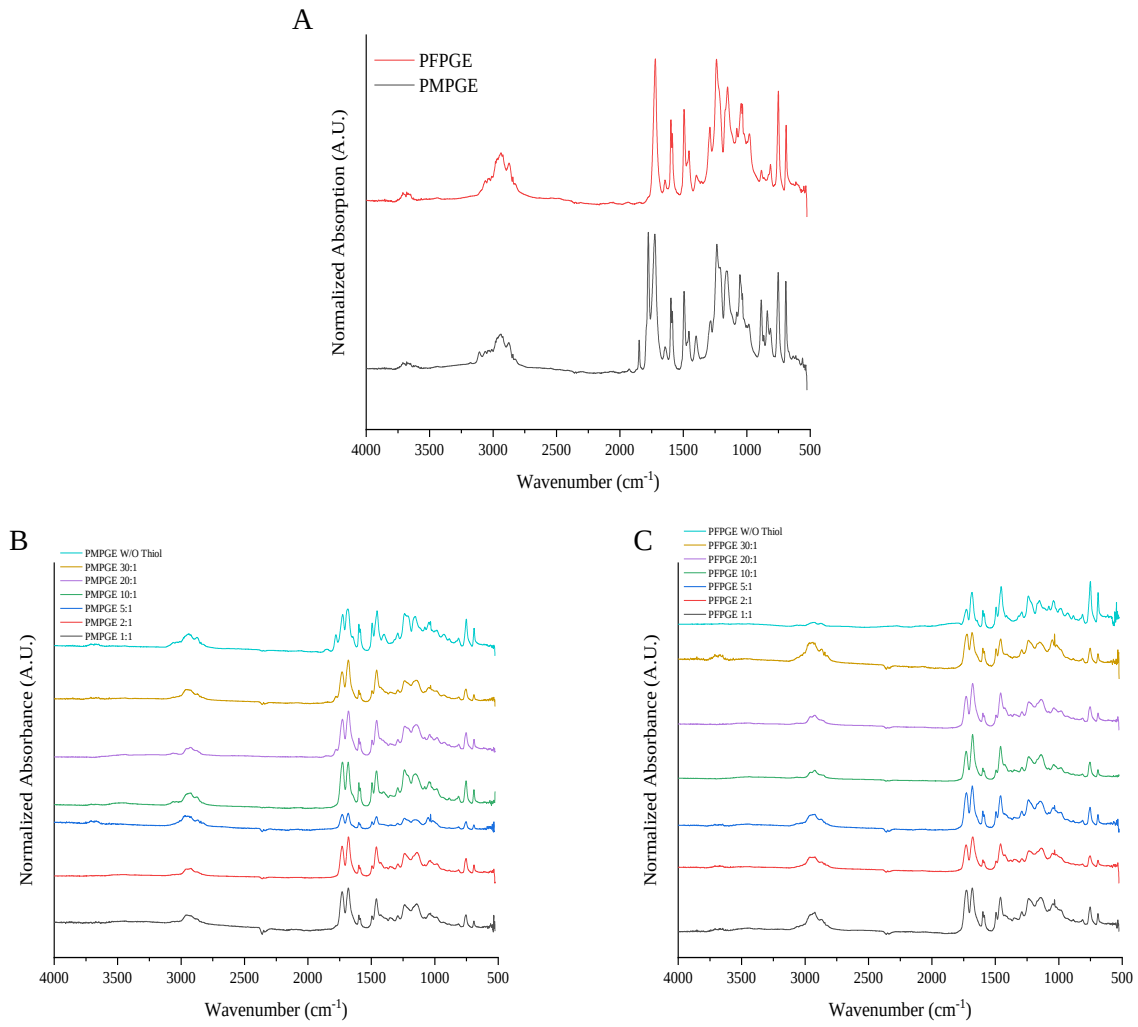

Figure S3: FT-IR spectroscopy of PFPGE and PMPGE polyester thermoplastics (A) and thermosetting films (B, C, respectively).

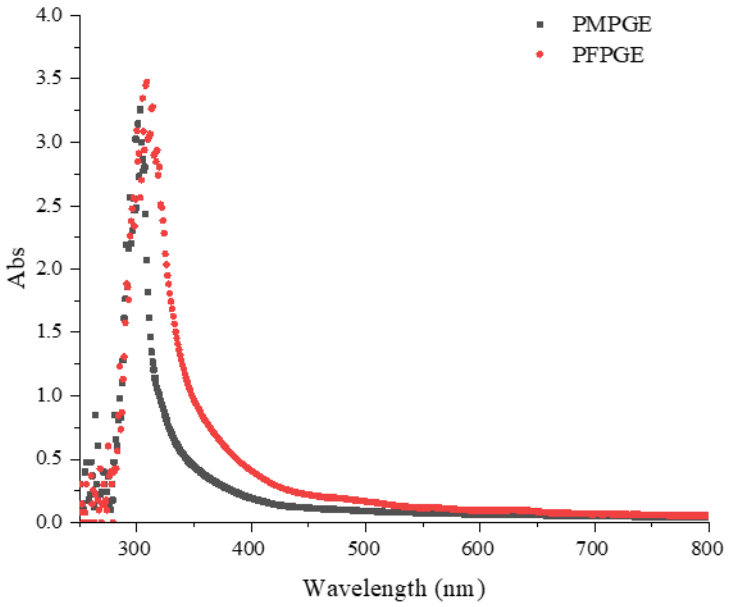

Figure S4: Ultraviolet visible spectroscopy of the PMPGE and PFPGE polyesters dissolved in $CHCl_3$ on a wavelength scan ranging from 200 to 800 nm.

Table S2: Peak locations and full width half maxes of the representative UV-vis traces of PMPGE and PFPGE

Sample	Peak Location (nm)	Full Width Half Max (nm)
PMPGE	303	290-310
PFPGE	309	290-331

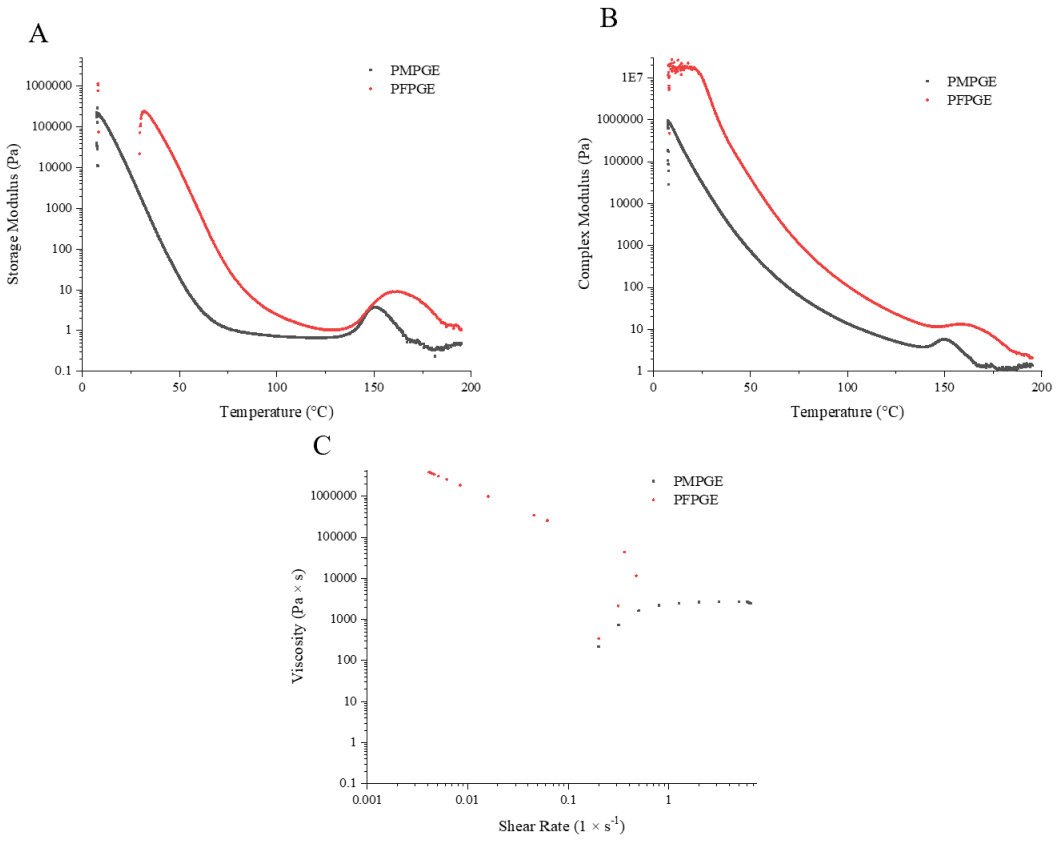

Figure S5: PMPGE and PFPGE polyester thermoplastic rheological properties were analyzed on a temperature sweep (A) and (B) (temperature sweep procedure) and a flow sweep (C) (flow sweep procedure).

Table S3: Averaged T_g and G', G'' moduli values at the T_gs of the PMPGE and PFPGE polyester thermoplastics recorded from the rheological oscillatory thermal sweep.

Polyester	T_F **(°C)**	G' **(Pa)**	G'' **(Pa)**	**Complex Modulus (Pa)**
PMPGE	66.9±3.37	1.35±0.400	124±11.8	120±5.56
PFPGE	92.2±2.41	4.39±0.698	201±11.0	205±6.23

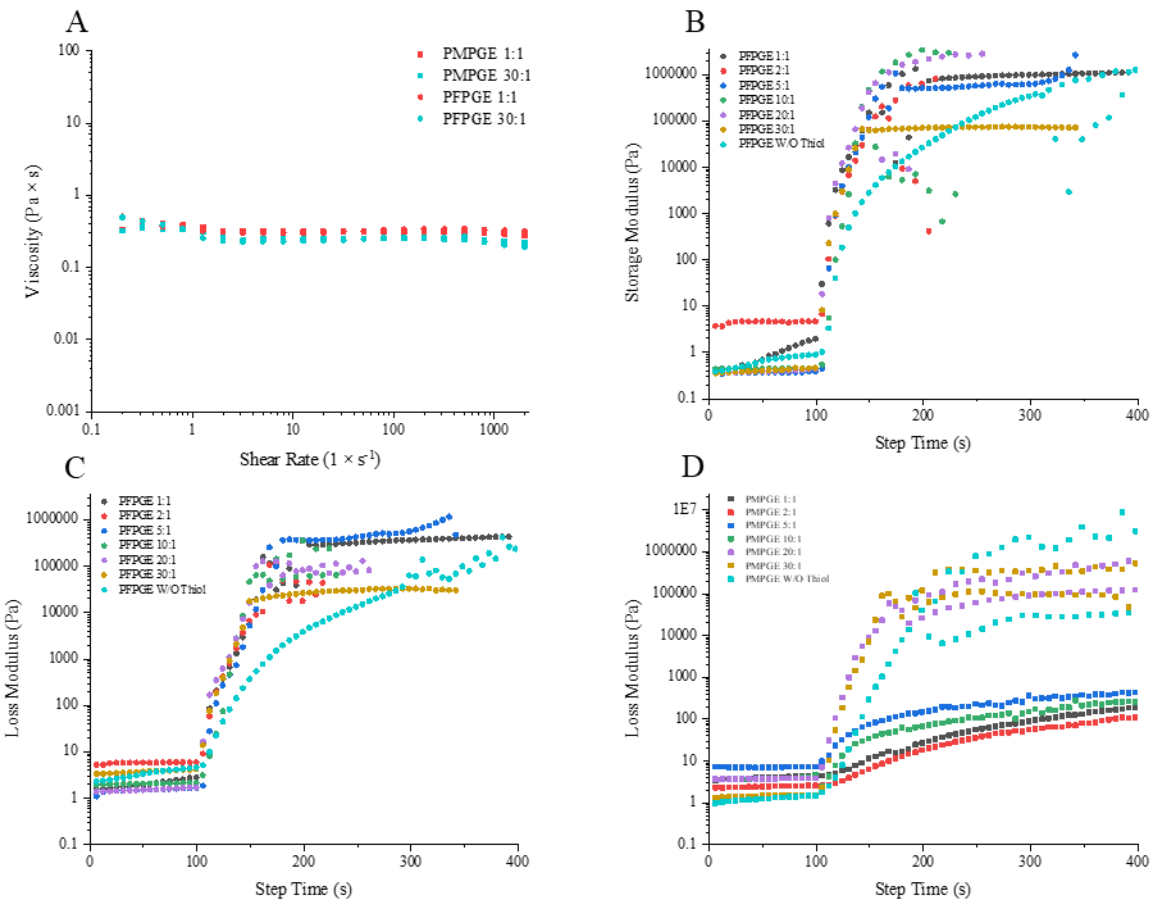

Figure S6: Shear rate sweep of the photopolymer resin (A, tested at 25 °C, 0.1 to 100 radians × sec^{-1}), photocrosslinking behaviors of PMPGE and PFPGE displaying respective *G'* (B) and *G''* (C) and (D) behavior during a rheological oscillation at an angular frequency of 10.0 rad × s^{-1} conducted at 25 °C being exposed to 405 nm wavelength ultraviolet light at the 100 s mark.

Table S4: Averages of *G'* and *G''* crossover times of PFPGE and PMPGE resins during photocrosslinking of differing stoichiometric ratio resins (alkene:thiol).

Formulation	**Time to *G'* and *G''* Crossover (s)**	**Formulation**	**Time to *G'* and *G''* Crossover (s)**
PMPGE 1:1	132.4±3.6	PFPGE 1:1	15.0±8.8
PMPGE 2:1	126.3±3.6	PFPGE 2:1	8.8±0.0
PMPGE 5:1	106.0±0.8	PFPGE 5:1	8.7±0.0
PMPGE 10:1	109.7±3.6	PFPGE 10:1	8.7±8.8
PMPGE 20:1	8.7±0.5	PFPGE 20:1	5.6±4.5
PMPGE 30:1	11.8±4.4	PFPGE 30:1	8.7±0.0
PMPGE W/O Thiol	18.0±0.1	PFPGE W/O Thiol	14.9±0.1

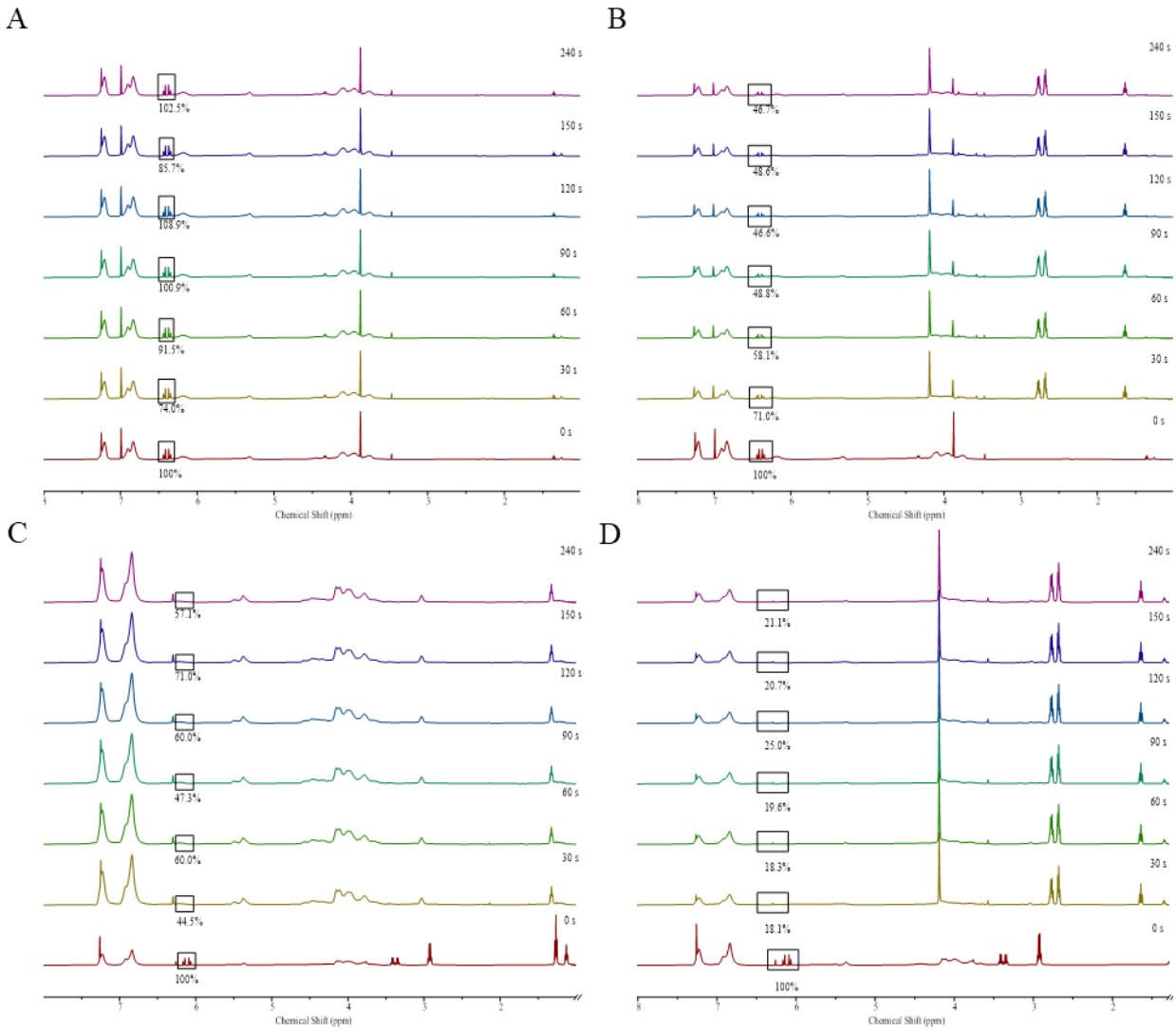

Figure S7: ^{1}H NMR spectra of free radical and thiol-ene crosslinking kinetics showing the consumption of polyester alkenes through selected time points of irradiation of PMPGE free radical crosslinking (A) PFPGE free radical crosslinking (B) PMPGE thiol-ene crosslinking (C) and PFPGE thiol-ene crosslinking (D).

Table S5: Percentage of PMPGE and PFPGE polyester alkene consumption with corresponding time points throughout a 4 minute period of irradiation *via* free radical and thiol-ene crosslinking

Time (s)	PMPGE TH	PMPGE FR	PFPGE TH	PFPGE FR
0	100	100	100	100
30	71.0	74.0	18.1	44.5
60	58.1	91.5	18.3	60.0
90	48.8	100.9	19.6	47.3
120	46.6	108.9	25.0	60.0
150	48.6	85.7	20.7	71.0
240	46.7	102.5	21.1	57.1

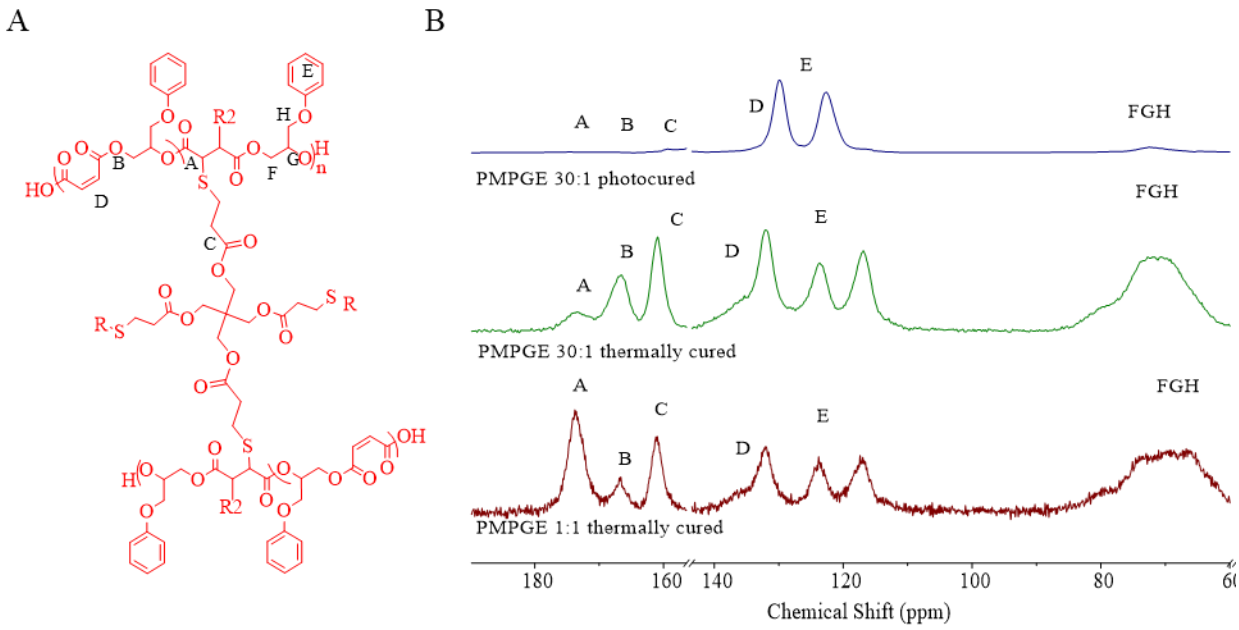

Figure S8: Representative PMPGE network structure after thiol-ene "click" crosslinking (A) MAS multi-CP solid state ^{13}C NMR spectra of photoset networks.

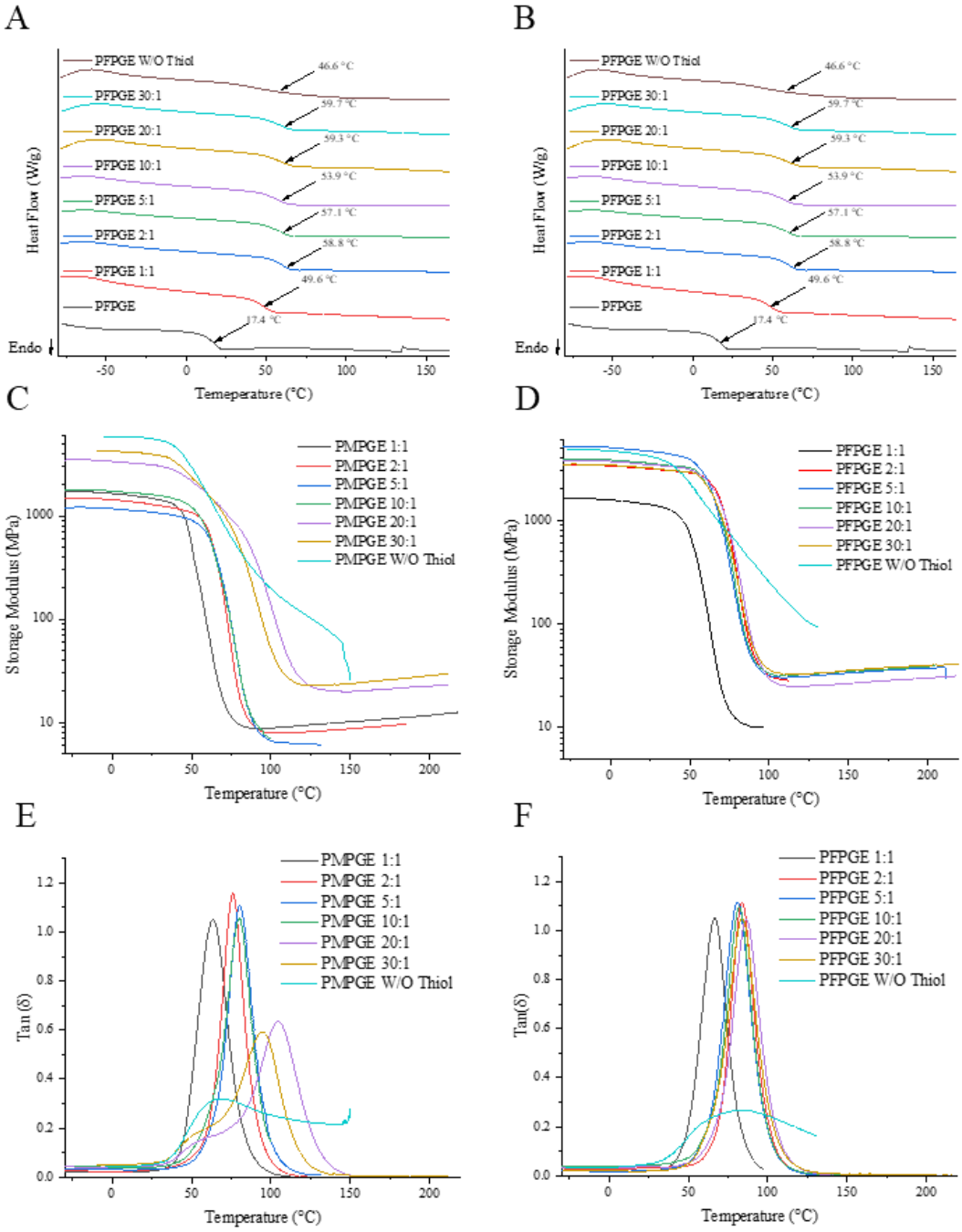

Figure S9: Representative DSC thermograms (A-B), and storage moduli (C-D), and tan (δ) as functions of temperature (E-F) of PMPGE and PFPGE thermoset materials. DMA samples temperature was swept from -30 °C to 220 °C at 2 °C × min^{-1}. DSC sample heating was over 3 cycles at 10 °C × min^{-1} (n = 3).

Table S6: T_g, storage (E'), loss (E'') moduli comparisons of PMPGE and PFPGE films.

Formulation	T_g (°C) DSC	T_g (°C) DMA	E' 20 °C - T_g (MPa)	E' at T_g (MPa)	E' 20 °C + T_g (MPa)	E'' 20 °C - T_g (MPa)	E'' at T_g (MPa)	E'' 20 °C + T_g (MPa)
PMPGE W/O Thiol	33.5±0.80	65.5±2.33	3493±274	1034±113	271±36.7	605±96.1	331±36.8	90.8±8.12
PMPGE 1:1	47.7±2.65	65.6±2.2	1014±97.3	41.9±3.9	9.27±0.7	167±33.3	43.6±2.5	1.45±0.2
PMPGE 2:1	54.8±0.07	74.5±1.49	1112±183	44.8±6.16	10.9±2.58	123±8.07	51.9±6.77	3.72±2.89
PMPGE 5:1	60.8±0.45	80.7±1.27	827±250	40.9±15.9	9.80±4.15	146±46.4	43.7±15.4	1.43±0.65
PMPGE 10:1	58.8±0.29	79.6±0.85	678±24.5	32.1±1.01	5.20±4.52	160±14.7	33.8±0.82	1.33±1.44
PMPGE 20:1	74.44±2.48	104±0.26	600±39.6	79.0±4.62	23.8±1.84	175±19.8	51.1±4.28	4.81±0.49
PMPGE 30:1	81.0±1.43	100±6.73	692±72.8	93.7±8.32	29.9±7.43	199±26.2	55.4±8.11	8.65±6.63
PFPGE W/O Thiol	46.6±0.46	85.8±2.35	1394±309	548±102	238±39.9	347±73.9	156±31.1	57.9±12.5
PFPGE 1:1	49.6±0.96	70.8±3.67	994±257	44.9±8.36	10.0±0.80	118±11.5	49.1±9.19	1.16±0.19
PFPGE 2:1	58.8±0.51	81.8±1.78	2676±493	135±15.8	32.3±2.10	313±72.3	152±18.3	4.49±1.15
PFPGE 5:1	57.1±0.31	80.4±0.63	2359±170	121±4.68	29.9±1.41	393±31.8	134±5.60	4.51±0.64
PFPGE 10:1	53.9±0.09	78.8±6.09	2293±396	118±6.42	28.6±3.87	358±53.3	128±8.27	5.77±2.10
PFPGE 20:1	59.3±0.71	85.1±1.07	2039±257	119±8.67	28.5±2.55	385±28.6	125±10.5	4.42±0.37
PFPGE 30:1	59.7±0.59	83.3±0.64	2090±371	136±2.69	33.9±0.23	405±25.8	143±2.16	6.26±0.46

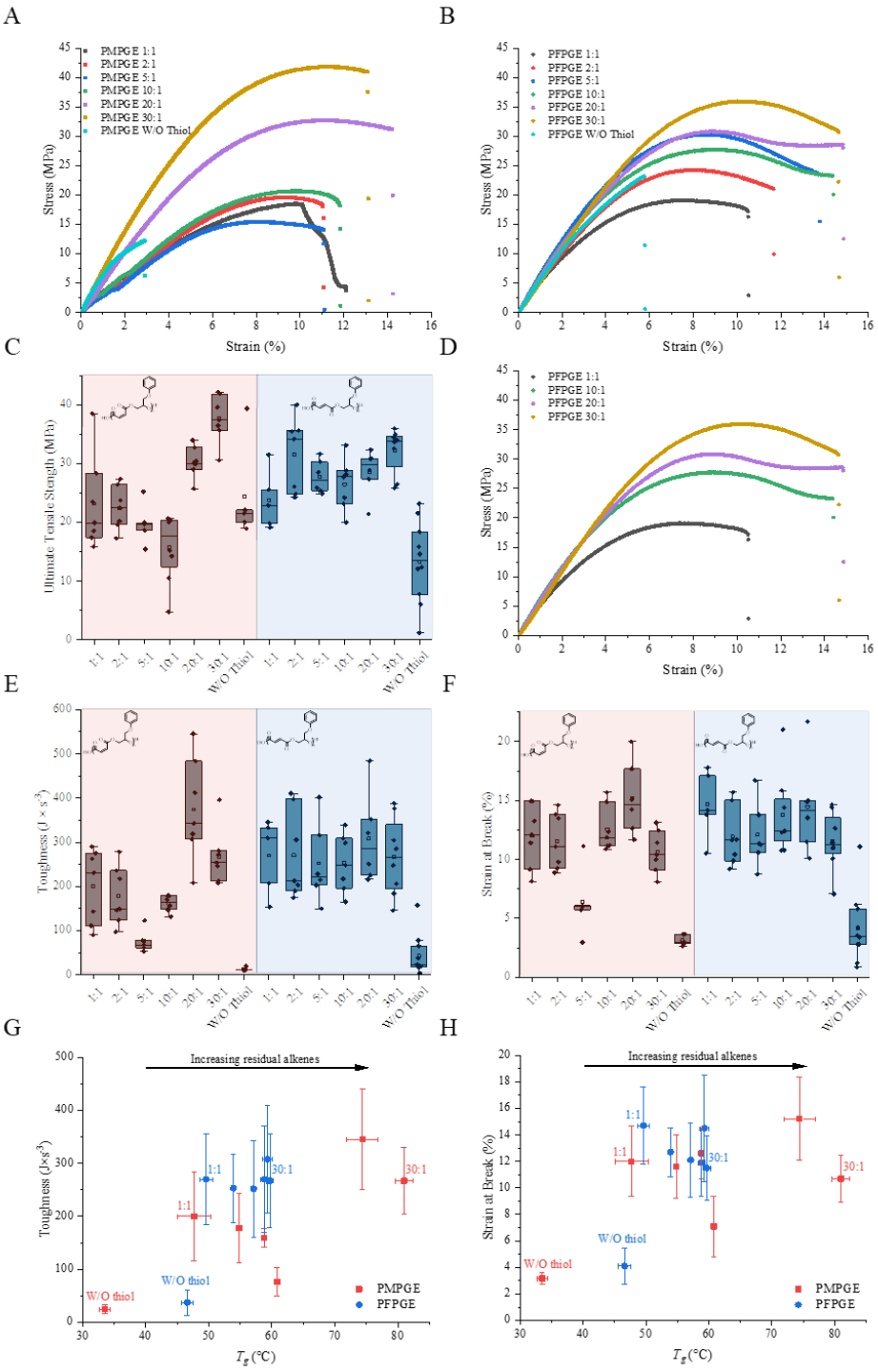

Figure S10: Representative monotonic uniaxial tensile plots of crosslinked photoset materials (A-B), box and whisker plots of ultimate tensile strength, toughness, and strain at break of PMPGE and PFPGE crosslinked photoset materials (C, E-F), representative PFPGE monotonic uniaxial tensile curves of crosslinked photoset materials (D), and toughness and strain at break as a function of T_g (G-H). The crosslinked photoset materials were tested as modified ASTM Type IV dogbones, extension rate of 5 mm × min^{-1} at ambient conditions.

Table S7: Comparison of monotonic uniaxial tensile mechanical properties of PMPGE and PFPGE photosets.

Formulation	Elastic Modulus (MPa)	Ultimate Strength (MPa)	Strain at Break (%)	Toughness ($J \times m^{-3}$)
PMPGE 1:1	401±103	23.1±7.98	12.0±2.66	200±83.6
PMPGE 2:1	324±38.2	22.4±3.68	11.6±2.39	178±66.2
PMPGE 5:1	355±92.9	19.7±3.54	7.09±2.27	76.4±27.5
PMPGE 10:1	293±40.5	18.2±2.82	12.6±1.94	159±17.9
PMPGE 20:1	490±43.2	30.3±2.90	15.2±3.13	345±94.7
PMPGE 30:1	636±81.9	37.7±4.02	10.7±1.80	267±63.1
PMPGE W/O thiol	478±111	13.2±3.89	3.16±0.46	24.4±8.46
PFPGE 1:1	463±91.4	23.8±5.05	14.7±2.91	270±84.8
PFPGE 2:1	548±69.6	31.5±6.32	11.9±2.51	270±100
PFPGE 5:1	545±45.0	27.8±2.83	12.1±2.78	252±91.0
PFPGE 10:1	534±58.5	26.5±4.33	12.7±1.85	253±64.5
PFPGE 20:1	536±92.3	28.6±3.93	14.5±4.04	308±102
PFPGE 30:1	576±68.5	32.2±3.88	11.5±2.45	267±87.9
PFPGE W/O thiol	438±89.7	14.9±4.55	4.10±1.38	37.3±24.5

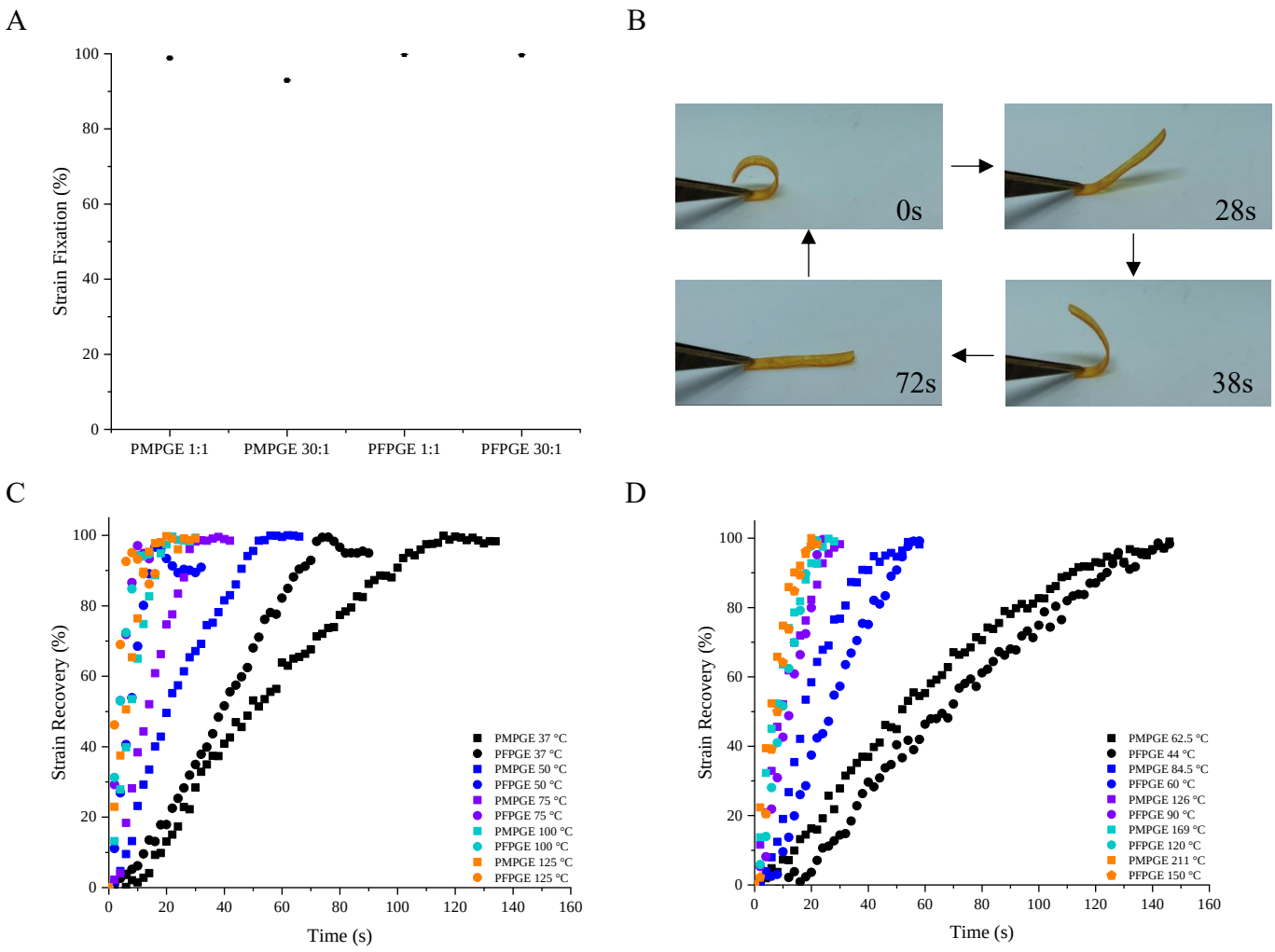

Figure S11: Strain fixation of PMPGE and PFPGE 1:1 and 30:1 photosets at twelve hours, fixed at 25 °C (A), representative recovery behavior of PMPGE 1:1 film at 75 °C over 75 s (B), representative strain recovery (% strain) of PMPGE 1:1 and PFPGE 1:1 (C), and PMPGE 30:1, and PFPGE 30:1 (D).

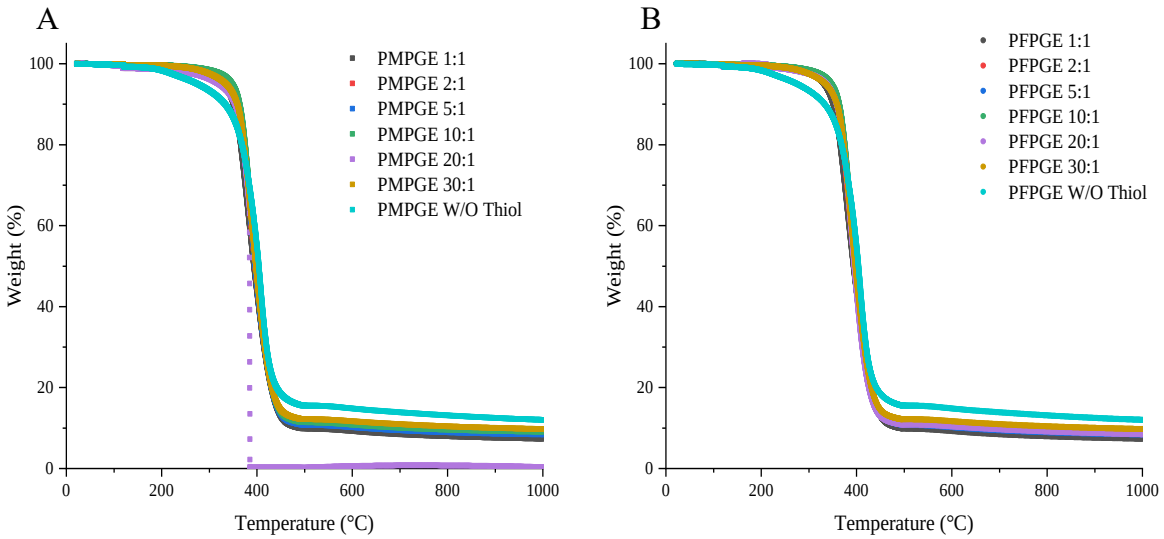

Figure S12: Thermogravimetric analysis (TGA) of PMPGE (A) and PFPGE (B) thermosets heated at 10 °C × min^{-1}.

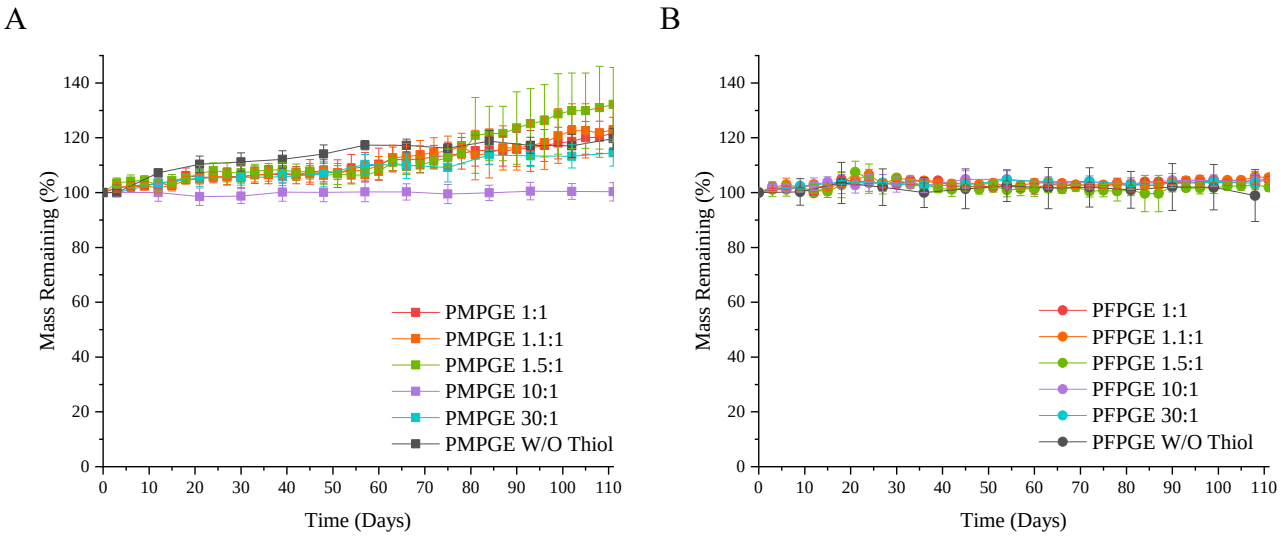

Figure S13: PMPGE (A) and PFPGE (B) thermoset film *in vitro* gravimetric changes after immersion in phosphate buffer saline solution (PBS) over 110 days when incubated at 37 °C, ambient atmosphere.

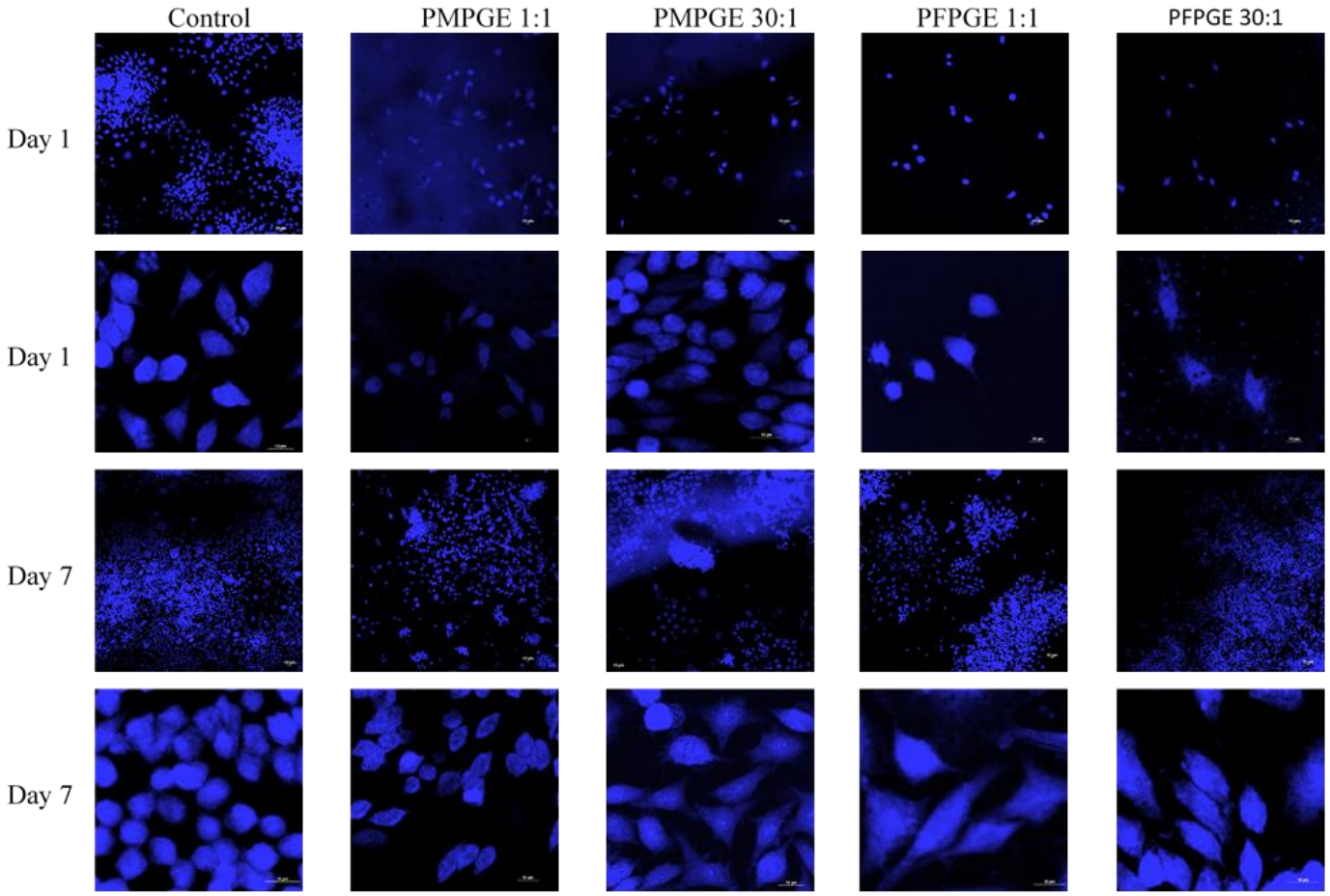

Figure S14: Cytocompatibility study conducted on a control, PMPGE and PFPGE 1:1 and 30:1 formulations. Fluorescent images were taken of the entire substrate as well as up close (10 µm scale bars) on the first- and seventh-day post cell seeding showing the cell proliferation as well as the increasing aspect ratio of the cells.

References

[1] D Merckle, E Constant, A.C. Weems, "Linalool derivatives for natural product-based 4D printing resins " *ACS Sustainable Chemistry & Engineering,* 2021.