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Supplementary Materials for

Precise control of synthetic hydrogel network structure via linear, independent synthesis-swelling relationships

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Supplementary Materials

Table S1. Summary of PVA hydrogel formulations.

	Universal Synthesis Variables		**Standard, Polymer-Specific Synthesis Variables**		
Formulation ID	**Initial Polymer Volume Fraction**	**Expected Degree of Polymerization Between Crosslinks**	**Concentration (% w/w)**	**Molar ratio PVA/Glutaraldehyde (mol/mol)**	**PVA Number Average Molecular Weight ($\bar{M}_n$; g/mol)**
PVA-1	0.050	20	6.7%	40	33,884
PVA-2		30		60	
PVA-3		40		80	
PVA-4		50		100	
PVA-5		60		120	
PVA-6		70		140	
PVA-7	0.075	20	10.3%	40	
PVA-8		30		60	
PVA-9		40		80	
PVA-10		50		100	
PVA-11		60		120	
PVA-12		70		140	
PVA-13	0.100	20	14.1%	40	
PVA-14		30		60	
PVA-15		40		80	
PVA-16		50		100	
PVA-17		60		120	
PVA-18		70		140	

Table S2. Summary of PEGDA hydrogel formulations.

	Universal Synthesis Variables		**Standard, Polymer-Specific Synthesis Variables**			
Formulation ID	**Initial Polymer Volume Fraction**	**Expected Degree of Polymerization Between Crosslinks**	**Concentration (% w/w)**	**PEGDA Number Average Molecular Weight ($\bar{M}_n$; g/mol; $n = 6$)**	**Polydispersity Index**	**Extent of Functionalization (% of chain-ends)**
PEGDA-1	0.087	76	10%	3360 ± 10	1.08 ± 0.01	89%
PEGDA-2		139		6110 ± 70	1.07 ± 0.01	88%
PEGDA-3		202		8900 ± 800	1.11 ± 0.02	98%
PEGDA-4		432		19000 ± 400	1.14 ± 0.01	91%
PEGDA-5		589		25900 ± 500	1.21 ± 0.01	87%
PEGDA-6	0.130	76	15%	3360 ± 10	1.08 ± 0.01	89%
PEGDA-7		139		6110 ± 70	1.07 ± 0.01	91%
PEGDA-8		202		8900 ± 800	1.11 ± 0.02	97%
PEGDA-9		432		19000 ± 400	1.14 ± 0.01	91%
PEGDA-10		589		25900 ± 500	1.21 ± 0.01	90%
PEGDA-11	0.175	76	20%	3360 ± 10	1.08 ± 0.01	89%
PEGDA-12		139		6110 ± 70	1.07 ± 0.01	87%
PEGDA-13		202		8900 ± 800	1.11 ± 0.02	98%
PEGDA-14		432		19000 ± 400	1.14 ± 0.01	91%
PEGDA-15		589		25900 ± 500	1.21 ± 0.01	85%

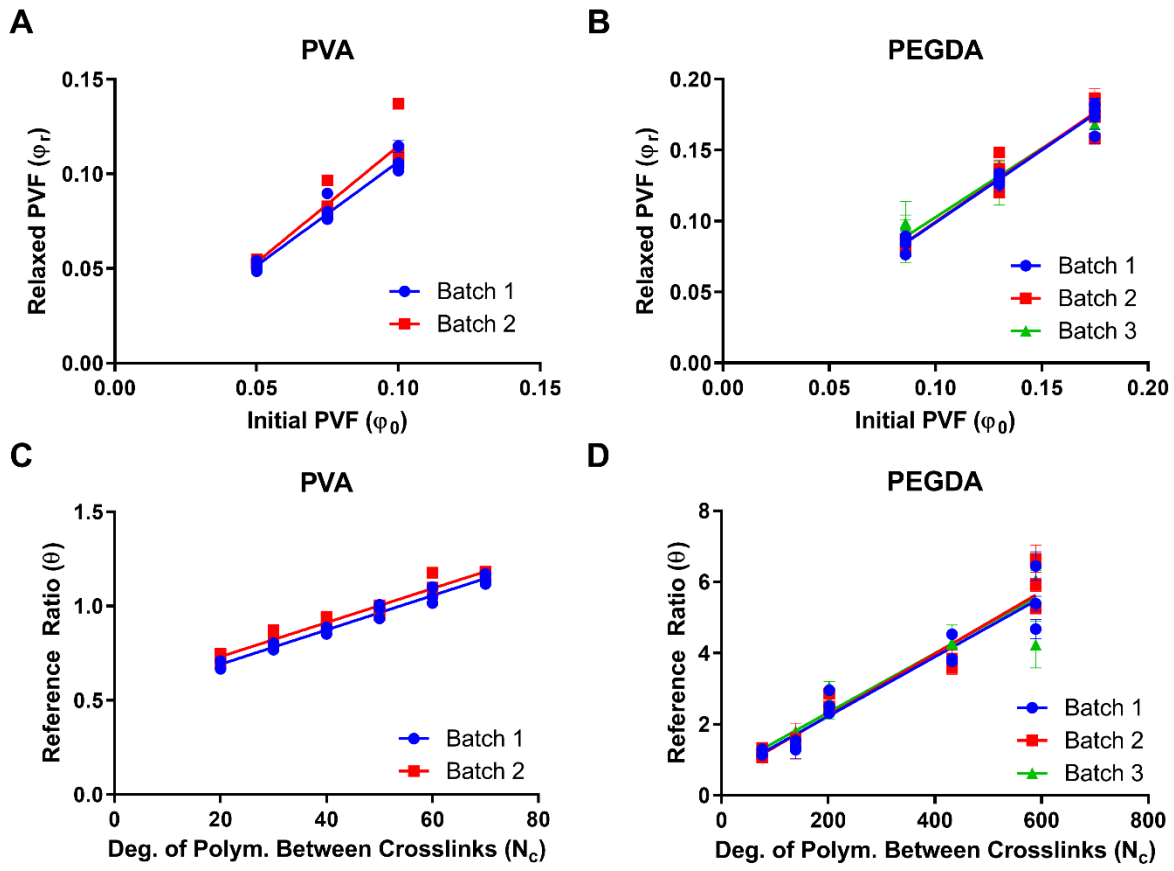

Figure S1. Batch comparison for the relationships between synthesis and swelling in PVA and PEGDA hydrogels. Initial polymer volume fraction (φ_0) correlates to the relaxed polymer volume fraction (φ_r) in (**A**) two batches of PVA hydrogels and (**B**) three batches of PEGDA hydrogels. Expected degree of polymerization between crosslinks (N_c) correlates to reference ratio (θ) in (**C**) two batches of PVA hydrogels and (**D**) three batches of PEGDA hydrogels. Solid lines represent the best linear fit for each batch. Error bars represent standard deviations ($n = 3$ for PVA; $n = 4$ for PEGDA).

Table S3. Fit values for each linear relationship.

Linear Relationship	***Slope***	***Intercept***	***R^2***
Fig. 2A: *PVA φ_0 vs. φ_r (Global)*	1.17 ± 0.03	-0.0062 ± 0.0024	0.933
Fig. 2B: *PEGDA φ_0 vs. φ_r (Global)*	1.01 ± 0.02	-0.0001 ± 0.0024	0.947
Fig. 2C: *PVA N_c vs. θ (Global)*	0.0090 ± 0.0002	0.53 ± 0.01	0.945
Fig. 2D: *PEGDA N_c vs. θ (Global)*	0.0085 ± 0.0002	0.57 ± 0.07	0.913
Fig. 3A: *PVA N_c vs. θ (Global)*	0.0075 ± 0.0002	0.53 ± 0.02	0.982
Fig. 3B: *PAAm N_c vs. θ*	0.0109 ± 0.0007	0.69 ± 0.03	0.988
Fig. 3C: *PEGDMA N_c vs. θ*	0.0052 ± 0.0001	0.61 ± 0.02	0.998
Fig. 4C: *PVA N_c vs. θ ($\varphi_0 = 0.050$)*	0.0058 ± 0.0002	0.30 ± 0.01	0.995
Fig. 4C: *PVA N_c vs. θ ($\varphi_0 = 0.075$)*	0.0080 ± 0.0003	0.43 ± 0.01	0.995
Fig. 4C: *PVA N_c vs. θ ($\varphi_0 = 0.100$)*	0.0100 ± 0.0004	0.54 ± 0.02	0.995
Fig. 4D: *PEGDA N_c vs. θ ($\varphi_0 = 0.086$)*	0.0052 ± 0.0002	0.99 ± 0.06	0.996
Fig. 4D: *PEGDA N_c vs. θ ($\varphi_0 = 0.130$)*	0.0065 ± 0.0003	1.31 ± 0.09	0.995
Fig. 4D: *PEGDA N_c vs. θ ($\varphi_0 = 0.175$)*	0.0080 ± 0.0003	1.57 ± 0.09	0.997
Fig. 6B: *GelMA φ_0 vs. φ_r (Global)*	0.64 ± 0.35	0.0206 ± 0.0168	0.094
Fig. 6C: *GelMA N_c vs. θ (Global)*	-0.0056 ± 0.0013	2.09 ± 0.10	0.382
Supp. Fig. 1A, Batch 1: *PVA φ_0 vs. φ_r*	1.10 ± 0.05	-0.0034 ± 0.0038	0.970
Supp. Fig. 1A, Batch 2: *PVA φ_0 vs. φ_r*	1.23 ± 0.08	-0.0083 ± 0.0064	0.933
Supp. Fig. 1B, Batch 1: *PEGDA φ_0 vs. φ_r*	1.02 ± 0.04	-0.0031 ± 0.0059	0.977
Supp. Fig. 1B, Batch 2: *PEGDA φ_0 vs. φ_r*	1.03 ± 0.06	-0.0032 ± 0.0086	0.953
Supp. Fig. 1B, Batch 3: *PEGDA φ_0 vs. φ_r*	0.97 ± 0.05	0.0059 ± 0.0066	0.968
Supp. Fig. 1B, Global: *PEGDA φ_0 vs. φ_r*	1.01	-0.0001	
Supp. Fig. 1C, Batch 1: *PVA N_c vs. θ*	0.0091 ± 0.0004	0.51 ± 0.02	0.973
Supp. Fig. 1C, Batch 2: *PVA N_c vs. θ*	0.0090 ± 0.0005	0.55 ± 0.02	0.959
Supp. Fig. 1D, Batch 1: *PEGDA N_c vs. θ*	0.0084 ± 0.0006	0.55 ± 0.22	0.930
Supp. Fig. 1D, Batch 2: *PEGDA N_c vs. θ*	0.0087 ± 0.0006	0.49 ± 0.21	0.940
Supp. Fig. 1D, Batch 3: *PEGDA N_c vs. θ*	0.0083 ± 0.0007	0.67 ± 0.26	0.905
Supp. Fig. 1D, Global: *PEGDA N_c vs. θ*	0.0085	0.57	

Table S4. Network parameters for PVA, PEGDA, and GelMA hydrogels.

System	*Symbol*	*Value*	*Unit*	*Ref.*
All	V_1	18	mL/mol	(*16, 17, 22*)
PVA	χ	0.494	N/A	(*17, 41*)
	C_∞	8.3	N/A	(*42, 43*)
	$\bar{M}_r$	44	g/mol	(*22, 41*)
	ρ_p	1.27	g/mL	(*17, 22, 41*)
	f	4	N/A	(*44*)
	λ	2	N/A	a
	$\bar{l}$	0.154	nm	(*16, 17, 22, 42*)
	$\bar{M}_n$	33,884	g/mol	b
PEGDA	χ	0.426	N/A	(*13, 15, 45*)
	C_∞	4	N/A	(*15, 46*)
	$\bar{M}_r$	44	g/mol	c
	ρ_p	1.18	g/mL	(*13, 47, 48*)
	f	>100	N/A	(*13*)
	λ	3	N/A	a
	$\bar{l}$	0.15	nm	(*15, 46, 49*)
	γ	0.02-0.15	N/A	d
GelMA	χ	0.49	N/A	(*50*)
	C_∞	5.3	N/A	(*39*)
	$\bar{M}_r$	91.2	g/mol	(*14*)
	ρ_p	1.35	g/mL	(*14*)
	f	~4	N/A	e
	λ	3	N/A	a
	$\bar{l}$	0.144	nm	(*51*)
	$\bar{M}_n$	62,600	g/mol	(*14*)

a) Determined based on the repeating unit's chemical structure.

b) Measured by a third-party service. (EAG Laboratories, Maryland Heights, MO)

c) Calculated based on the repeating unit's chemical structure.

d) Calculated based on extent of functionalization data.

e) Assumed based on crosslinking scheme.

Table S5. All literature values considered for network parameters.

System	*Parameter*	*Symbol*	*Parameter Value*	*Unit*	*Ref.*
All	Molar volume of the solvent (water)	V_1	18	mL/mol	(*16, 17, 22*)
All	Molar volume of the solvent (water)	V_1	18.1	mL/mol	(*13, 42*)
PVA	Flory's Polymer-Solvent Interaction Parameter	χ	0.490-0.510	N/A	(*22*)
PVA	Flory's Polymer-Solvent Interaction Parameter	χ	0.494	N/A	(*17, 41*)
PVA	Flory's Polymer-Solvent Interaction Parameter	χ	0.495	N/A	(*42*)
PVA	FPS Interaction Parameter, 0th Order	χ_0	0.474-0.481	N/A	(*18*)
PVA	FPS Interaction Parameter, 1st Order	χ_1	0.387-0.416	N/A	(*18*)
PVA	Flory Characteristic Ratio	C_∞	8.9	N/A	(*22, 46*)
PVA	Flory Characteristic Ratio	C_∞	8.4	N/A	(*17*)
PVA	Flory Characteristic Ratio	C_∞	8.3	N/A	(*42, 43*)
PVA	Molecular weight of the repeating unit	$\bar{M}_r$	44	g/mol	(*22, 41*)
PVA	Density of the dry polymer	ρ_d	1.269	g/mL	(*17, 22, 41*)
PVA	Junction functionality	f	4	N/A	(*44*)
PVA	Number of atoms in the repeating unit backbone	λ	2	N/A	a
PVA	Weighted average bond length	$\bar{l}$	0.154	nm	(*16, 17, 22, 42*)
PVA	Number average molecular weight of the dry polymer	$\bar{M}_n$	33,884	g/mol	b
PEGDA	Flory's Polymer-Solvent Interaction Parameter	χ	0.426	N/A	(*13, 15, 45*)
PEGDA	Flory's Polymer-Solvent Interaction Parameter	χ	0.5	N/A	(*52*)
PEGDA	Flory's Polymer-Solvent Interaction Parameter	χ	0.5-0.52	N/A	(*53*)
PEGDA	Flory Characteristic Ratio (PEG) (At Theta Point)	C_∞	4	N/A	(*15, 46*)
PEGDA	Flory Characteristic Ratio (PEG) (At 140 °C)	C_∞	5.6	N/A	(*54*)
PEGDA	Molecular weight of the repeating unit	$\bar{M}_r$	62.07	g/mol	(*49*)
PEGDA	Molecular weight of the repeating unit	$\bar{M}_r$	44	g/mol	c
PEGDA	Density of the dry polymer	ρ_d	1.12	g/mL	(*15*)
PEGDA	Density of the dry polymer	ρ_d	1.07	g/mL	(*53*)

PEGDA	Density of the dry polymer	ρ_d	1.18	g/mL	(*13, 47, 48*)
PEGDA	Junction functionality	f	>100	N/A	(*13*)
PEGDA	Junction functionality	f	?	N/A	e
PEGDA	Number of atoms in the repeating unit backbone	λ	3	N/A	a
PEGDA	C-O bond length	l_{C-O}	0.143	nm	(*46, 49*)
PEGDA	C-C bond length	l_{C-C}	0.153	nm	(*46*)
PEGDA	C-C bond length	l_{C-C}	0.154	nm	(*49*)
PEGDA	Weighted average bond length	$\bar{l}$	0.15	nm	(*15, 46, 49*)
PEGDA	Frequency of chain-end defects	γ	0.04-0.12	N/A	d
GelMA	Flory's Polymer-Solvent Interaction Parameter	χ	0.49	N/A	(*50*)
GelMA	Flory's Polymer-Solvent Interaction Parameter	χ	0.48	N/A	(*55*)
GelMA	Flory's Polymer-Solvent Interaction Parameter	χ	0.562-0.639	N/A	(*39*)
GelMA	Flory's Polymer-Solvent Interaction Parameter	χ	0.497	N/A	(*56*)
GelMA	Flory Characteristic Ratio	C_∞	5.3	N/A	(*39*)
GelMA	Flory Characteristic Ratio	C_∞	8.26	N/A	(*56*)
GelMA	Flory Characteristic Ratio	C_∞	8.87	N/A	(*14*)
GelMA	Average molecular weight of the repeating unit	$\bar{M}_r$	91.3	g/mol	(*39*)
GelMA	Average molecular weight of the repeating unit	$\bar{M}_r$	91.2	g/mol	(*14*)
GelMA	Average molecular weight of the repeating unit	$\bar{M}_r$	94.7	g/mol	(*56*)
GelMA	Density of the dry polymer	ρ_d	1.345	g/mL	(*57*)
GelMA	Density of the dry polymer	ρ_d	1.33-1.36	g/mL	(*58*)
GelMA	Density of the dry polymer	ρ_d	1.35	g/mL	(*14*)
GelMA	Junction functionality	f	~4	N/A	e
GelMA	Number of atoms in the repeating unit backbone	λ	3	N/A	a
GelMA	Ca-C bond length (Ca Carbon has R-group)	$l_{C\alpha-C}$	0.153	nm	(*51*)
GelMA	C-N bond length	l_{C-N}	0.133	nm	(*51*)
GelMA	N-Ca bond length	$l_{N-C\alpha}$	0.146	nm	(*51*)
GelMA	Weighted average bond length	$\bar{l}$	0.144	nm	(*51*)
GelMA	Total peptide bond length	$\bar{l}_{pep}$	0.428	nm	(*14*)
GelMA	Number average molecular weight of the dry polymer	$\bar{M}_n$	62600	g/mol	(*14*)
GelMA	Frequency of Lysine Residues	FoL	0.0286	mol/mol	(*14*)

a) Determined based on the repeating unit's chemical structure.
b) Measured by a third-party service. (EAG Laboratories, Maryland Heights, MO)

c) Calculated based on the repeating unit's chemical structure.
d) Calculated based on extent of functionalization data.
e) Assumed based on crosslinking scheme.

Table S6. Summary of GelMA hydrogel formulations.

	Universal Synthesis Variables		**Standard, Polymer-Specific Synthesis Variables**		
Formulation ID	**Initial Polymer Volume Fraction**	**Expected Degree of Polymerization Between Crosslinks**	**Concentration (% w/w)**	**Extent of Functionalization (% of amino acids)**	**GelMA Number Average Molecular Weight ($\bar{M}_n$; g/mol)**
GelMA-1	0.030	37	4%	0.027	62638
GelMA-2		78		0.013	
GelMA-3		100		0.010	
GelMA-4	0.045	37	6%	0.027	
GelMA-5		78		0.013	
GelMA-6		100		0.010	
GelMA-7	0.061	37	8%	0.027	
GelMA-8		78		0.013	
GelMA-9		100		0.010	

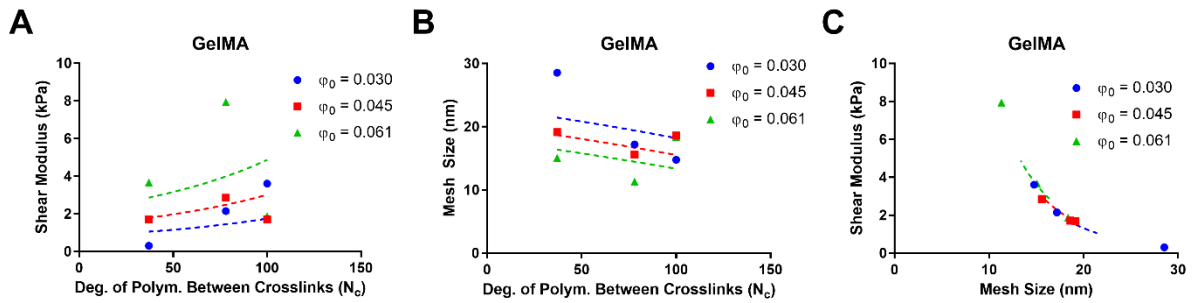

Figure S2. Secondary swollen polymer network model analysis of GelMA Hydrogels. Data were grouped by expected degree of polymerization between crosslinks (N_c) and initial polymer volume fraction (φ_0). Symbols represent predictions based on swelling data, and dotted lines represent predictions based on linear fits of the synthesis-swelling relationships. (**A**) Shear modulus increased with decreasing degree of polymerization between crosslinks. (**B**) Mesh size decreased with degree of polymerization between crosslinks (**C**) Formulations were predicted to fit along a master inverse curve relating shear modulus and mesh size.

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