

Sulfobetaine as a zwitterionic mediator for 3D hydroxyapatite mineralization

Pingsheng Liu and Jie Song*

Department of Orthopedics & Physical Rehabilitation, Department of Cell and Developmental Biology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, USA

Classical Descriptions of Mineral Nucleation and Growth:

For a spherical nucleus, the activation energy of homogeneous nucleation ΔG_N^* , nucleation rate J_N , and mineral growth rate J_G can be described as Equations S1, S2 and S3[1]:

$$\Delta G_N^* = 16\pi\sigma^3 v^2 / 3(kT \ln S_R)^2 \quad \text{Eq-S1}$$

where σ is interfacial free energy per unit surface area, v is molecular volume, S_R is relative supersaturation, k is the Boltzmann constant, T is absolute temperature.

$$J_N = A \exp[-\Delta G_N^*/kT] \quad \text{Eq-S2}$$

where A is a pre-exponential factor.

$$J_G = k_2(S_A)^x \quad \text{Eq-S3}$$

where, k_2 is a rate constant, S_A is the absolute supersaturation, and $x \geq 1$.

References cited:

[1] Mann S. *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry*. Oxford: Oxford University Press; 2001.

Supplementary information

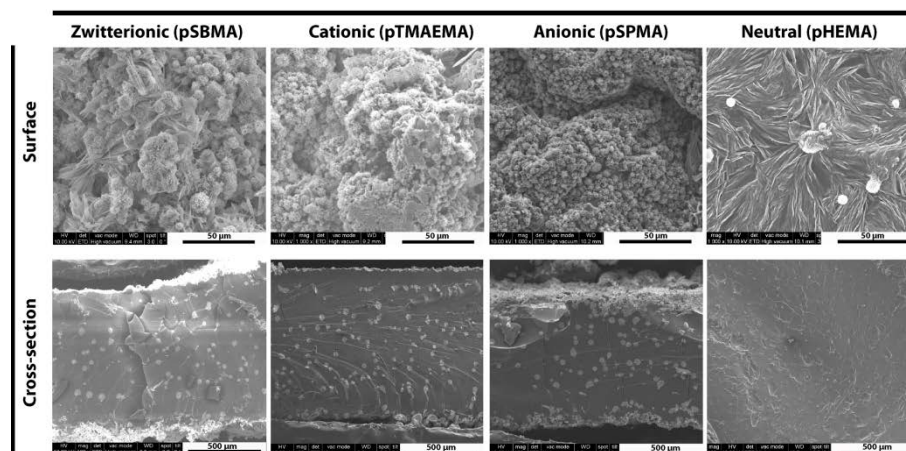


Figure S1. SEM micrographs revealing surface and cross-section mineral domain morphologies of the mineralized hydrogels containing identical crosslinker content (1.326 mol%) but varied side chain charges. All hydrogels were subjected to heterogeneous mineralization by controlled heating in an acidic solution of HA containing 2-M urea from 37 °C to 95 °C at a heating rate of 0.2 °C/min.

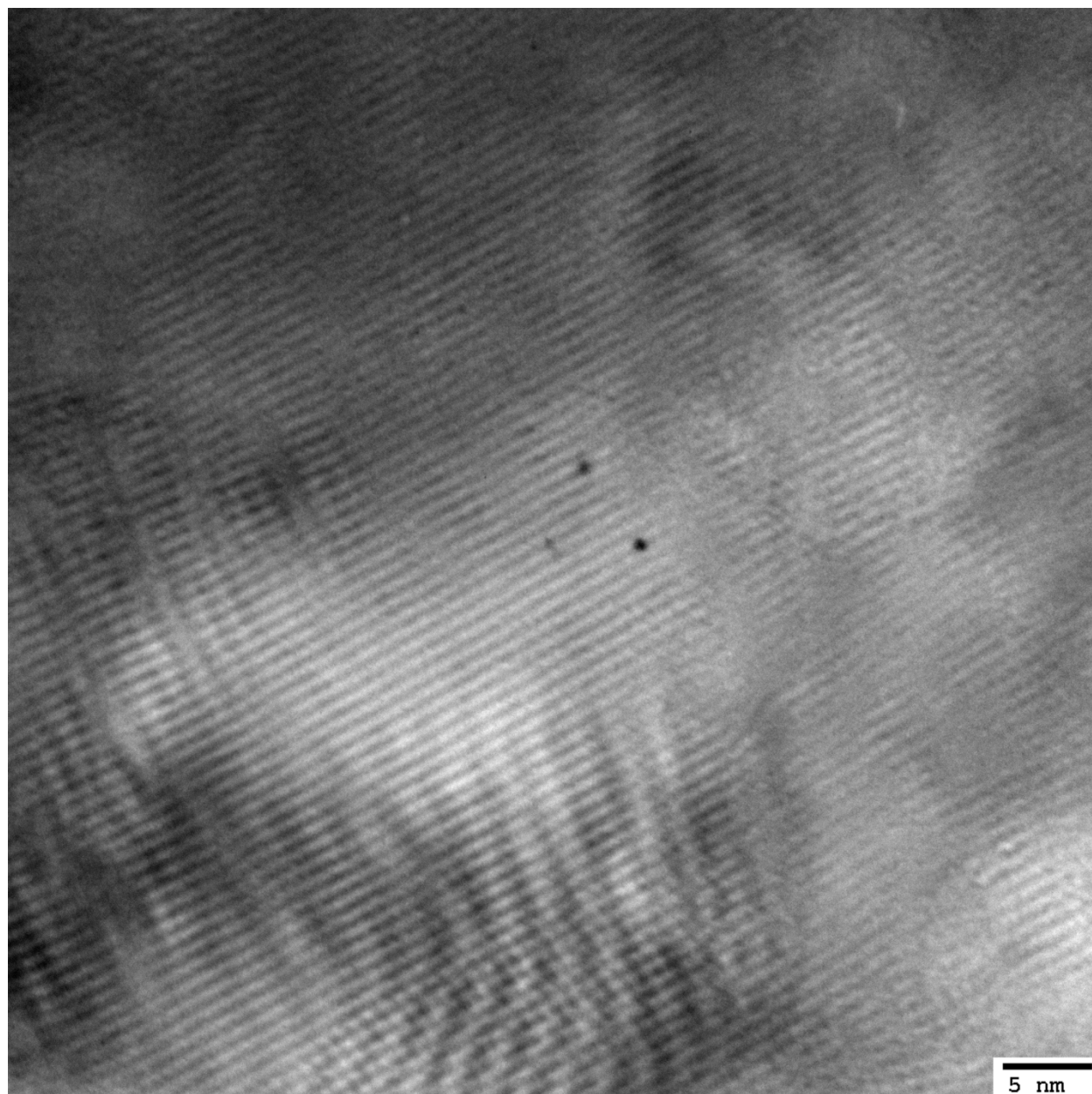


Figure S2. HR-TEM micrograph of the mineralized composite formed at the heating rate of 0.2 °C/min, suggesting predominantly crystalline phase of the mineral component.

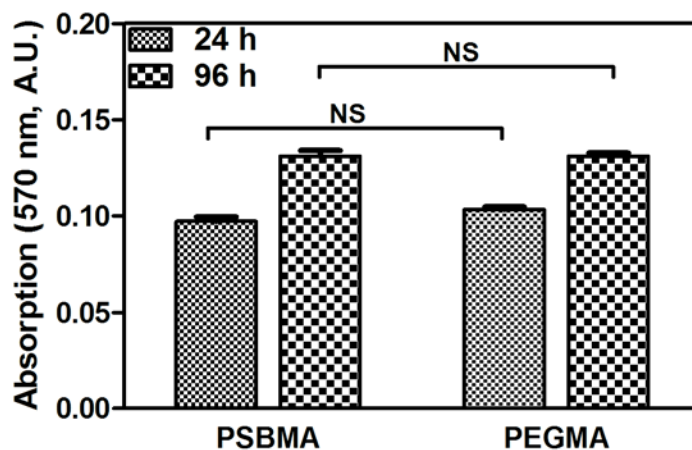


Figure S3. Cell viability of rMSC encapsulated by pSBMA and the PEGMA control (with identical crosslinker content of 1.33 mol%) as determined by MTT assay (n=3). No significant difference ($p > 0.05$) was observed between the cells encapsulated by the 2 hydrogel formulations at a given time, while significant increases in viable cells were detected over time in each hydrogel formulation. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparison.

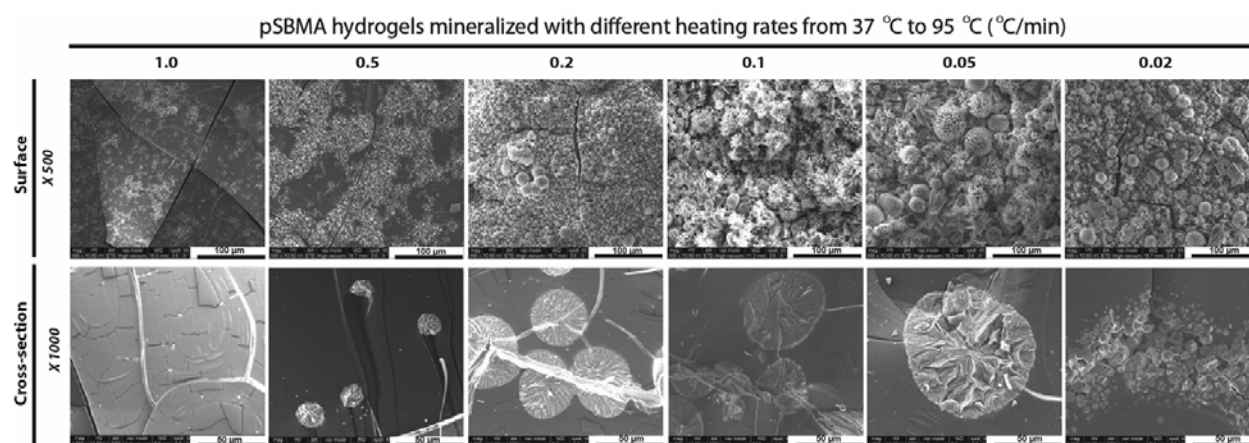


Figure S4. SEM micrographs of the surfaces and cross-sections of mineralized pSBMA hydrogels as a function of the heating rate. All mineralized hydrogel specimens contained the same crosslinker content of 0.265 mol%.

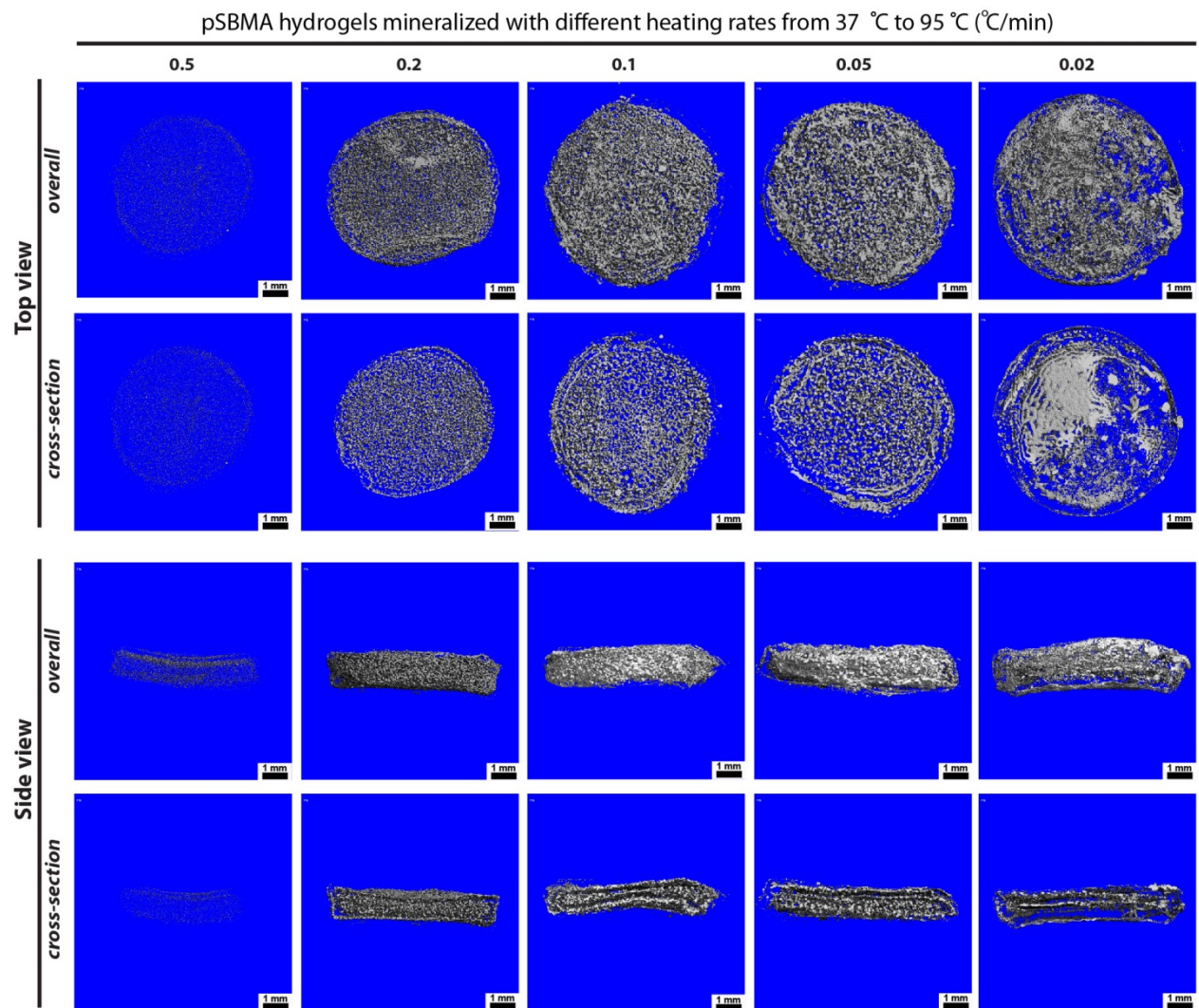


Figure S5. Reconstructed μ -CT images of mineralized pSBMA hydrogels obtained with various heating rates. All mineralized hydrogel specimens contained the same crosslinker content of 0.265 mol%.

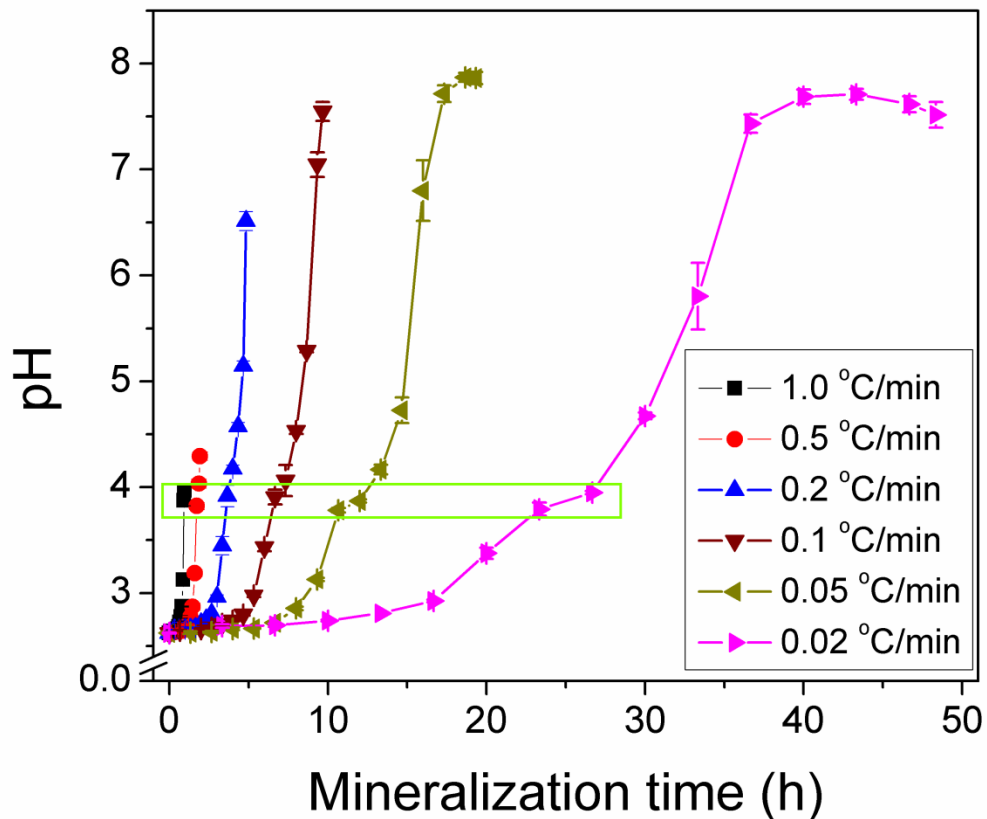


Figure S6. Temporal pH changes of the mineralization solution as a function of the heating rate and overall mineralization time (n=3). The first pH plateaus (from pH 3.8 to 4.0, indicated by the green box) observed in all six pH-mineralization time curves could be attributed to the deprotonation of H_2PO_4^- ions. All specimens contained the same hydrogel crosslinker content of 0.265 mol%.

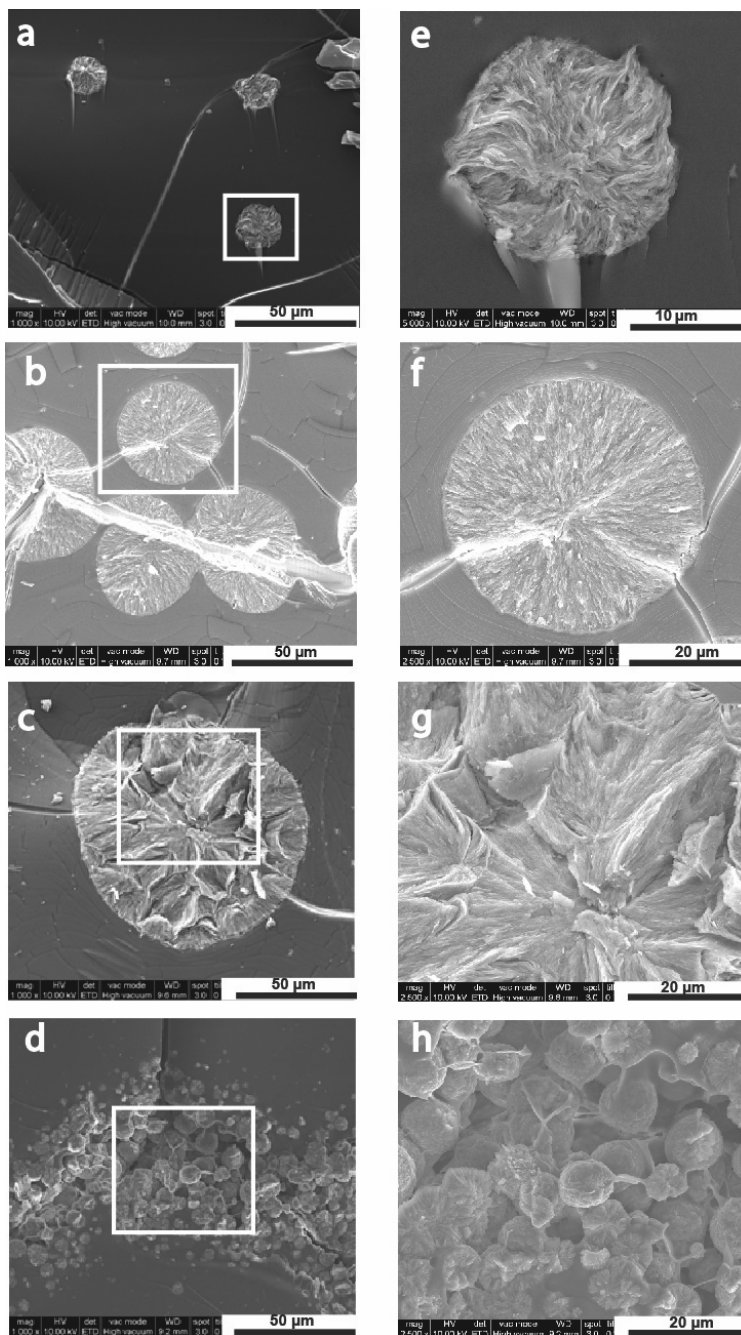


Figure S7. SEM micrographs revealing the different mineral nodule sizes and morphologies as a function of the heating rate. **a & e**, Specimen mineralized at 0.5 °C/min where small spherical mineral nodules were formed (type I). **b & f**, Specimen mineralized at 0.2 °C/min where large spherical mineral nodules were formed (type II). **c & g**, Specimen mineralized at 0.05 °C/min where larger spherical mineral domains containing multiple smaller nodules were formed, indicating a departure from a typical type II mineral morphology. **d & h**, Specimen mineralized at 0.02 °C/min where bicontinuous stripe-like mineral aggregates were formed (type III). All mineralized specimens contained the same crosslinker content of 0.265 mol%.

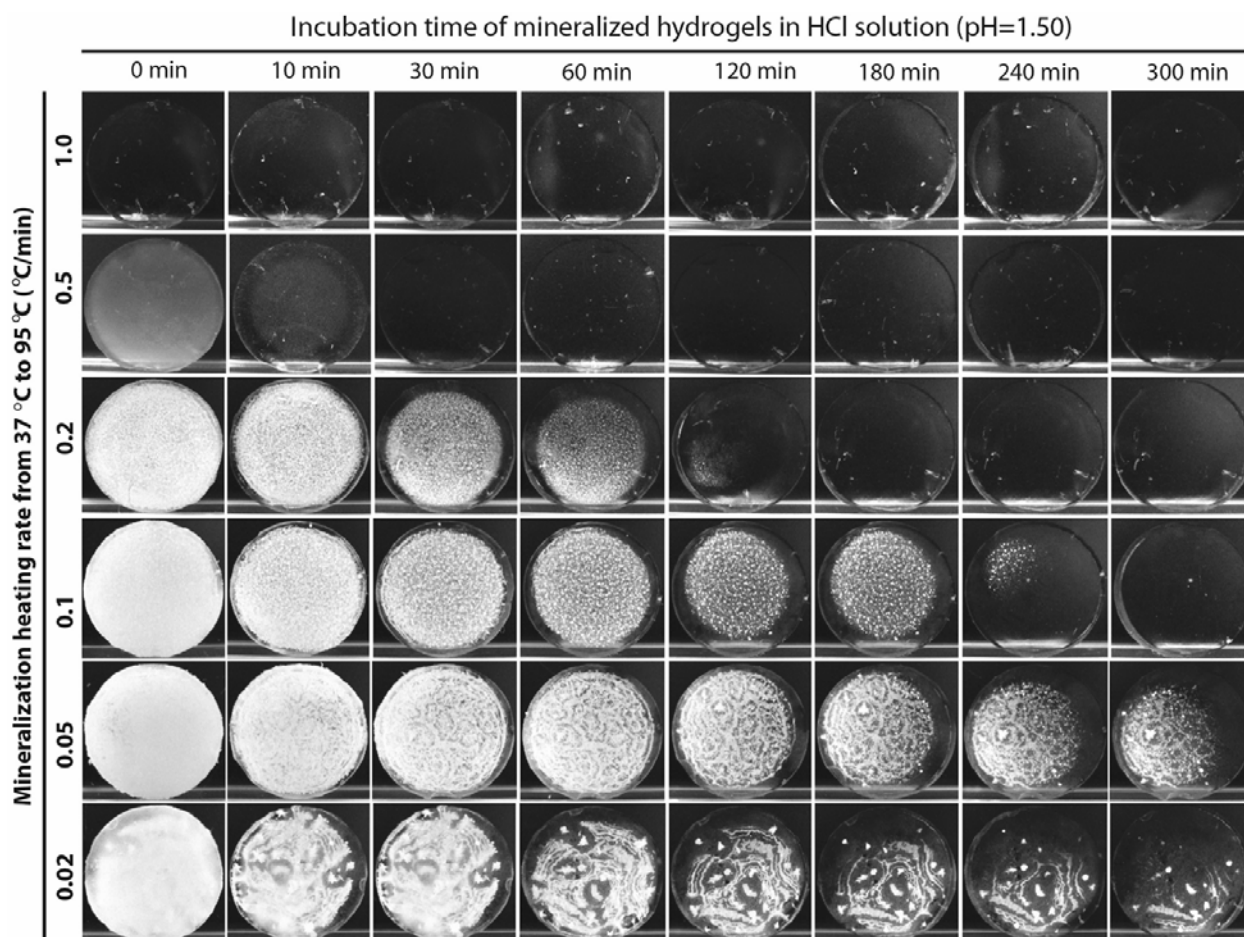


Figure S8. Digital photograph images of the mineralized pSBMA hydrogels at various time points of the acid treatment. All mineralized hydrogel specimens contained the same crosslinker content of 0.265 mol%.

Supplementary information

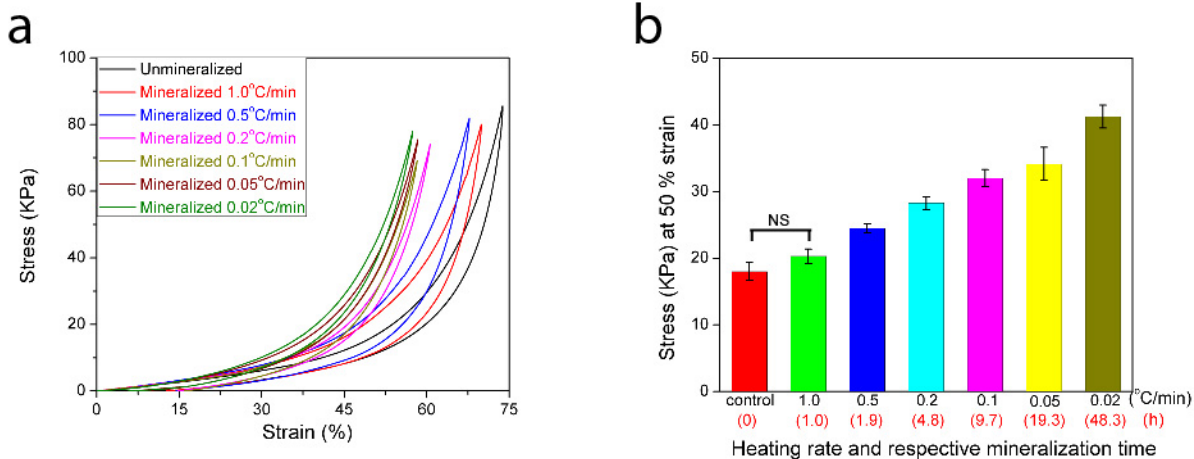


Figure S9. Compressive mechanical properties of the mineralized pSBMA hydrogels as a function of the heating rate. **a**, Compressive stress-strain curves of hydrated specimens as a function of the heating rate of their mineralizations. The data were recorded on a Q800 DMA equipped with a submersion compression fixture at 25 °C. **b**, Stress at 50 % compressive strain of the mineralized specimens as a function of the heating rate and the respective mineralization time. Statistical significance was determined by one-way ANOVA with Tukey’s multiple comparison tests. Pairwise comparisons with unmineralized pSBMA hydrogel are statistically significant ($P < 0.05$) unless denoted as NS (not significant). Complete pairwise comparisons are in shown in Supplementary Tables S8. All mineralized hydrogel specimens contained the same crosslinker content of 0.265 mol %.

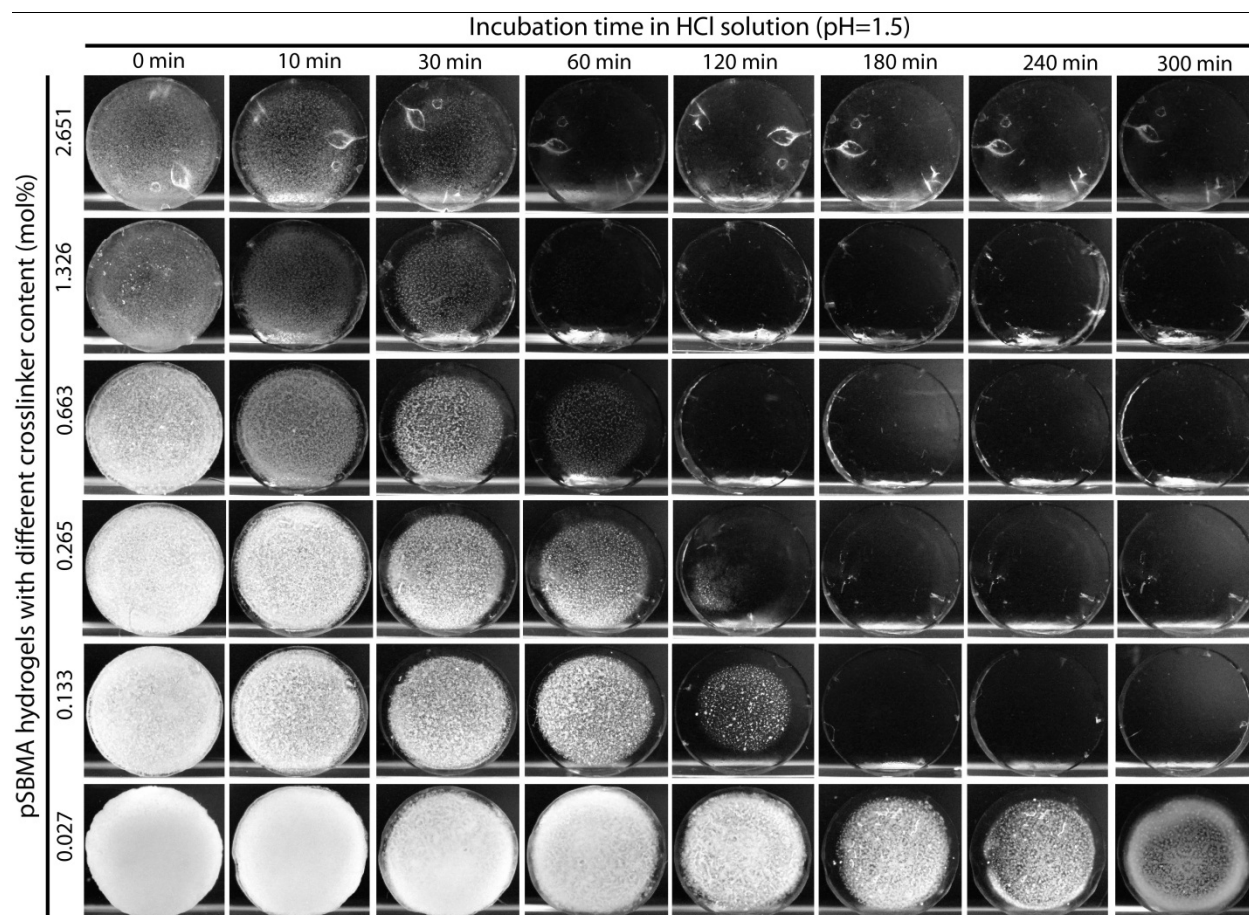


Figure S10. Digital photograph images of the mineralized hydrogels with different crosslinker contents at various time points of the acid treatment. All the hydrogel specimens were mineralized with a heating rate of 0.2 °C/min.

Supplementary information

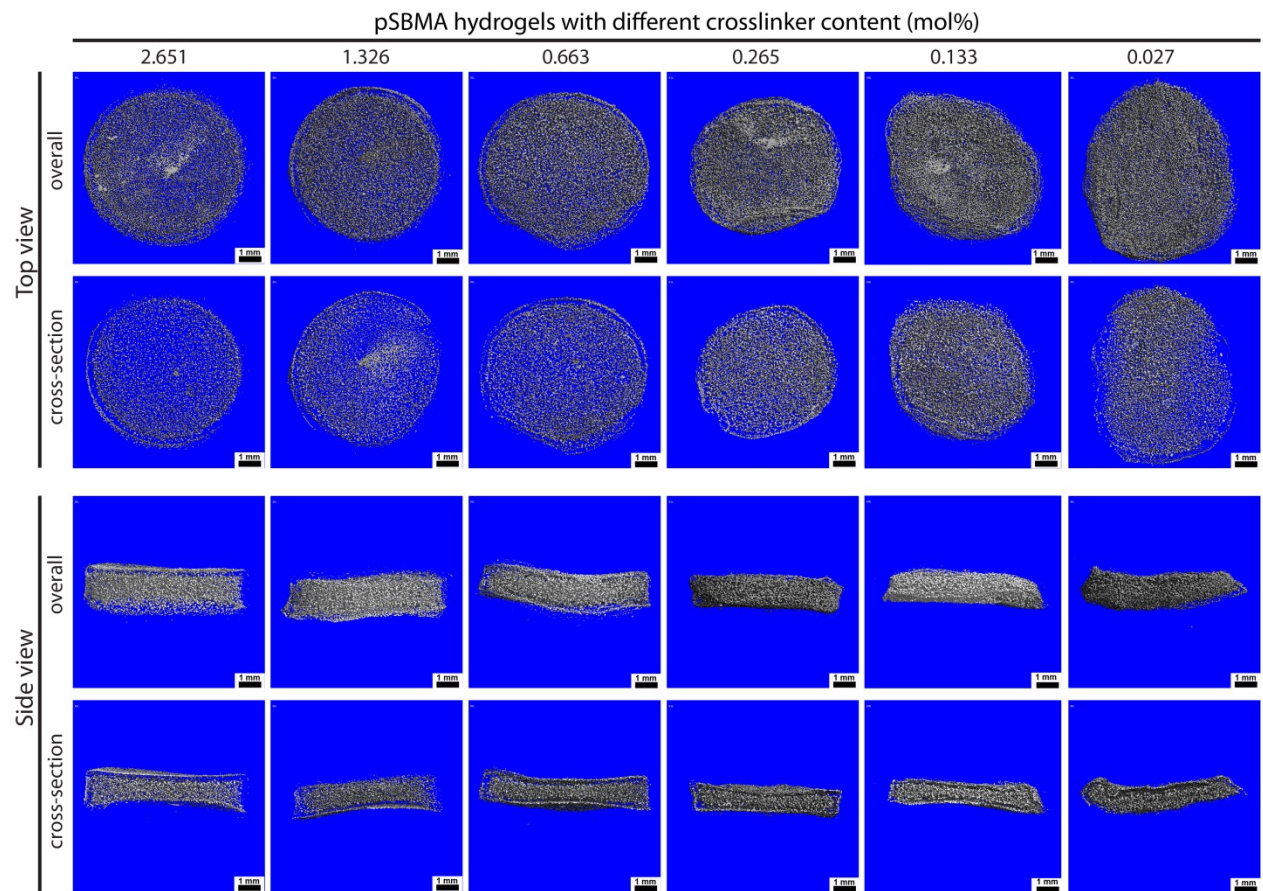


Figure S11. Reconstructed μ -CT images of mineralized pSBMA hydrogels containing different crosslinker content. All specimens were mineralized with a heating rate of $0.2\text{ }^{\circ}\text{C}/\text{min}$.

Supplementary information

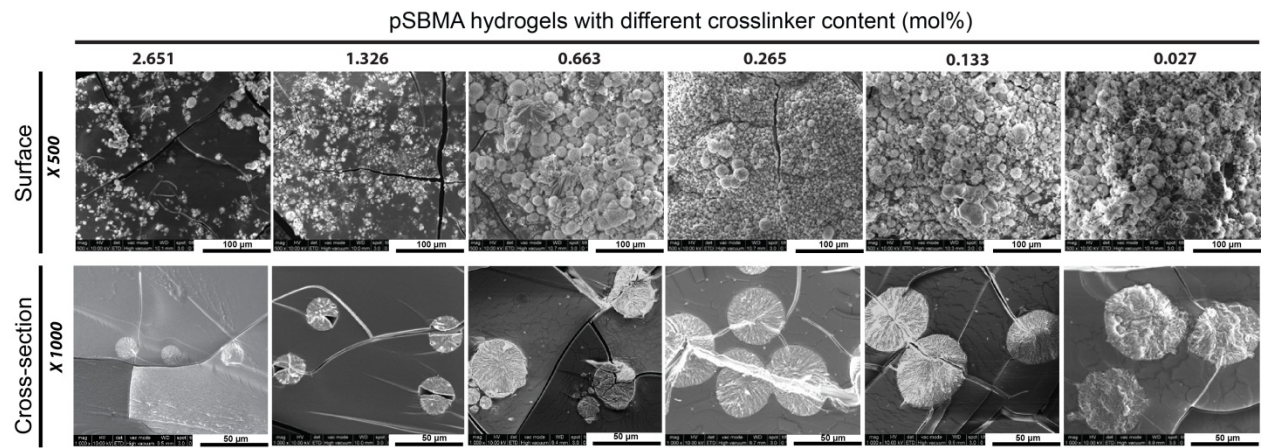


Figure S12. SEM micrographs of the surfaces and cross-sections of the mineralized pSBMA hydrogels containing different crosslinker contents. All hydrogel specimens were mineralized with a heating rate of 0.2 °C/min.

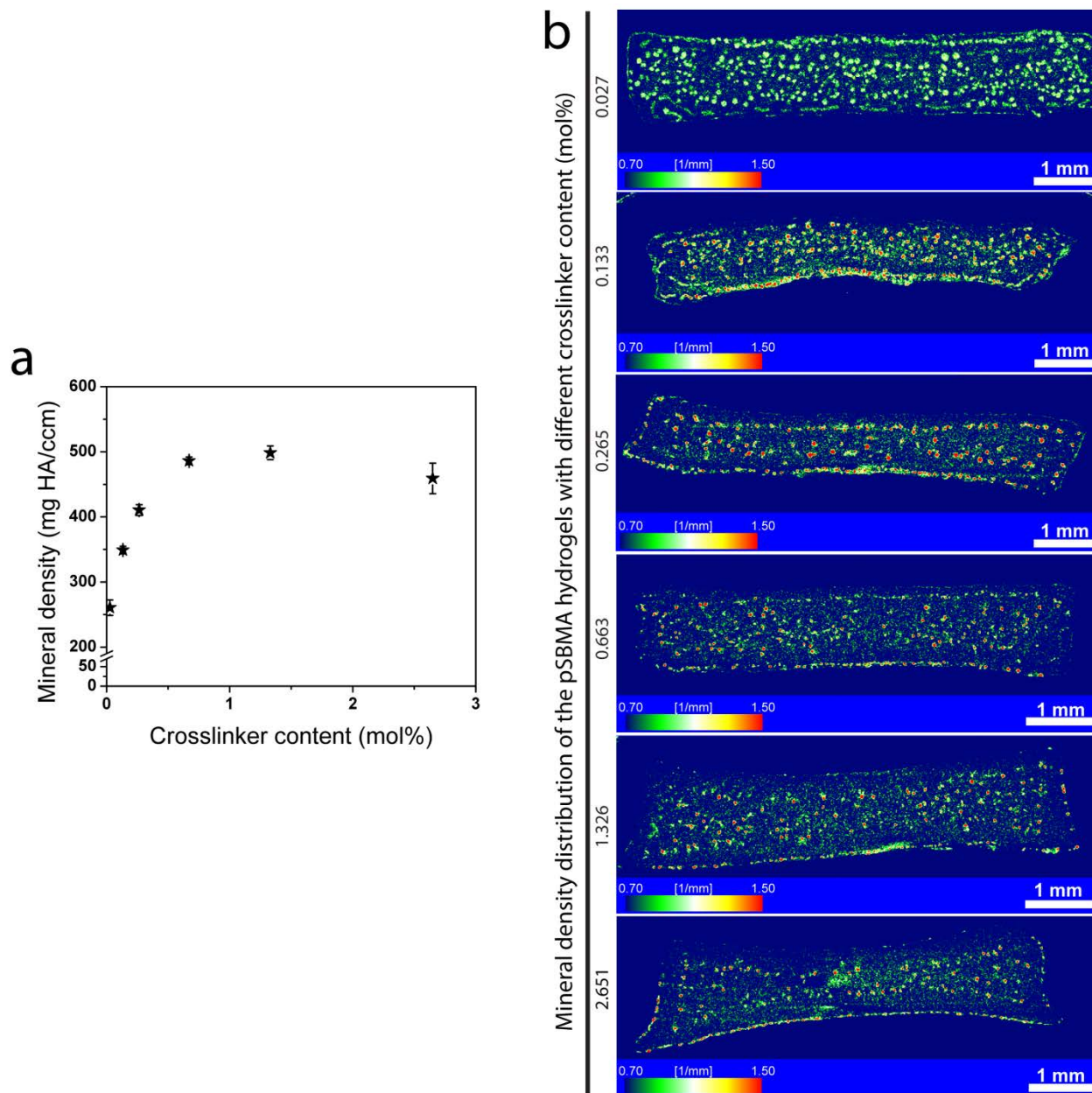


Figure S13. Mineral density (a) distribution colored mapping (b) of pSBMA hydrogels as a function of crosslinking content (determined by μ -CT). The mineral density increased gradually with the increase of crosslinker content (obviously from 0.027 to 0.265 mol%, but not so clear from 0.265 to 2.651 mol% due to the mineral size). All specimens were mineralized with a heating rate of 0.2 °C/min. Complete pairwise comparisons of mineral density are shown in Supplementary Table S11.

Table S1. Preparation of hydrogels with different side chain charges for mineralization (No.1-4) and cyto-compatibility (No. 5-7) studies.

No.	Monomer	Monomer amount	Crosslinker amount	VA-086 stock solution ^c	Milli-Q water	Cell culture medium ^d
		(mmol)	(μ L)	(μ L)	(μ L)	(μ L)
1	HEMA	2	50 ^a	100	1850	0
2	SBMA	2	50 ^a	100	1850	0
3	TMEAMA	2	50 ^a	100	1850	0
4	SPMA	2	50 ^a	100	1850	0
5	SBMA	2	17.9 ^b	100	0	1882.1
6	TMEAMA	2	35.8 ^b	100	0	1864.2
7	SPMA	2	53.8 ^b	100	0	1846.2

^a 10 % (v/v) ethanol solution of EGDMA.

^b PEGDMA ($M_n=750$; radical inhibitors were removed by passing through an aluminum oxide column).

The amount of PEGDMA used for each hydrogel was adjusted to ensure comparable equilibrated volumes of pSBMA, pTMEAMA, and pSPMA gels in culture media upon cell encapsulation for the cytotoxicity study.

^c 2 % (w/v) VA-086/PBS solution.

^d α -MEM supplemented with 20% FBS, 1% penicillin, 1% streptomycin, and 2% glutamine.

Table S2. Preparation and mineralization conditions of zwitterionic pSBMA hydrogels

No.	SBMA Monomer (g)	EGDMA stock solution ^a (μ L)	Crosslinker content (mol%)	I-2959 stock solution ^b (μ L)	Heating rate ($^{\circ}$ C/min)	Mineralization time ^c (min)	Milli-Q water (μ L)
1	3.36	600	2.651	1200	0.2	290	660
2	3.36	300	1.326	1200	0.2	290	960
3	3.36	150	0.663	1200	0.2	290	1110
4	3.36	60	0.265	1200	0.2	290	1200
5	3.36	30	0.133	1200	0.2	290	1230
6	3.36	6	0.027	1200	0.2	290	1254
7	3.36	4.5	0.020	1200	0.2	290	1255.5
8	3.36	3	0.013	1200	0.2	290	1257
9	3.36	60	0.265	1200	1	58	1200
10	3.36	60	0.265	1200	0.5	116	1200
11	3.36	60	0.265	1200	0.2	290	1200
12	3.36	60	0.265	1200	0.1	580	1200
13	3.36	60	0.265	1200	0.05	1160	1200
14	3.36	60	0.265	1200	0.02	2900	1200
15	3.36	6	0.027	1200	0.05	1160	1254

^a 10 % (v/v) ethanol solution of EGDMA.

^b 0.25% (w/v) water-acetone (19:1) solution of I-2959.

^c Total mineralization time from 37-95 $^{\circ}$ C.

Supplementary information

Table S3. Sampling intervals for the monitoring of pH changes of the mineralization solution with different heating rates.

Heating rate (°C/min)	1.0	0.5	0.2	0.1	0.05	0.02
Overall mineralization time (min)	58	116	290	580	1160	2900
Interval time (min)	4	8	20	40	80	200
Overall time points	15	15	15	15	15	15

Supplementary information

Table S4 Complete statistical significance data (p values) of the mineral volume pairwise comparisons as a function of heating rate (corresponding to Fig. 5c) as determined by one-way ANOVA with Tukey’s multiple comparison tests.

Comparison of Groups ^a	Mean Diff	SEM	q Value	Prob	Alpha	Sig ^b	LCL	UCL
0.2 vs 0.5	4.034	0.54536	10.46077	1.75E-04	0.05	S	2.23916	5.82884
0.1 vs 0.5	4.4537	0.54536	11.54912	7.47E-05	0.05	S	2.65886	6.24854
0.1 vs 0.2	0.4197	0.54536	1.08835	0.93377	0.05	NS	-1.37514	2.21454
0.05 vs 0.5	5.41697	0.54536	14.04701	1.30E-05	0.05	S	3.62213	7.21181
0.05 vs 0.2	1.38297	0.54536	3.58624	0.15792	0.05	NS	-0.41187	3.17781
0.05 vs 0.1	0.96327	0.54536	2.4979	0.44078	0.05	NS	-0.83157	2.75811
0.02 vs 0.5	4.10743	0.54536	10.65119	1.50E-04	0.05	S	2.31259	5.90227
0.02 vs 0.2	0.07343	0.54536	0.19042	0.99991	0.05	NS	-1.72141	1.86827
0.02 vs 0.1	-0.34627	0.54536	0.89792	0.96568	0.05	NS	-2.14111	1.44857
0.02 vs 0.05	-1.30953	0.54536	3.39582	0.19198	0.05	NS	-3.10437	0.48531

^a The different experimental groups are referred to as “X vs Y”, where X and Y stand for the respective heating rate (°C/min).

^b S indicates $p < 0.05$ (significant); NS indicates $p > 0.05$ (not significant).

Table S5. Complete statistical significance data (p values) of the calcium content pairwise comparisons as a function of heating rate (corresponding to Fig. 5d) as determined by one-way ANOVA with Tukey's multiple comparison tests.

Comparison of Groups ^a	Mean Diff	SEM	q Value	Prob	Alpha	Sig ^b	LCL	UCL
0.5 vs 1	0.1052	0.06855	2.17039	0.65095	0.05	NS	-0.12505	0.33545
0.2 vs 1	0.52673	0.06855	10.8671	6.48E-05	0.05	S	0.29649	0.75698
0.2 vs 0.5	0.42153	0.06855	8.69671	5.45E-04	0.05	S	0.19129	0.65178
0.1 vs 1	0.7996	0.06855	16.49664	7.08E-07	0.05	S	0.56935	1.02985
0.1 vs 0.5	0.6944	0.06855	14.32625	3.60E-06	0.05	S	0.46415	0.92465
0.1 vs 0.2	0.27287	0.06855	5.62955	0.01752	0.05	S	0.04262	0.50311
0.05 vs 1	0.8674	0.06855	17.89543	3.24E-07	0.05	S	0.63715	1.09765
0.05 vs 0.5	0.7622	0.06855	15.72504	1.26E-06	0.05	S	0.53195	0.99245
0.05 vs 0.2	0.34067	0.06855	7.02834	0.0034	0.05	S	0.11042	0.57091
0.05 vs 0.1	0.0678	0.06855	1.39879	0.91288	0.05	NS	-0.16245	0.29805
0.02 vs 1	0.7942	0.06855	16.38524	7.70E-07	0.05	S	0.56395	1.02445
0.02 vs 0.5	0.689	0.06855	14.21484	3.92E-06	0.05	S	0.45875	0.91925
0.02 vs 0.2	0.26747	0.06855	5.51814	0.02001	0.05	S	0.03722	0.49771
0.02 vs 0.1	-0.0054	0.06855	0.11141	1	0.05	NS	-0.23565	0.22485
0.02 vs 0.05	-0.0732	0.06855	1.5102	0.88491	0.05	NS	-0.30345	0.15705

^a The different experimental groups are referred to as "X vs Y", where X and Y stand for the respective heating rate (°C/min).

^b S indicates $p < 0.05$ (significant); NS indicates $p > 0.05$ (not significant).

Supplementary information

Table S6. Complete statistical significance data (p values) of the mineral density pairwise comparisons as a function of heating rate (corresponding to Supplementary Fig. 5e) as determined by one-way ANOVA with Tukey's multiple comparison tests.

Comparison of Groups ^a	Mean Diff	SEM	q Value	Prob	Alpha	Sig ^b	LCL	UCL
0.2 vs 0.5	34.85333	21.91505	2.24914	0.53417	0.05	NS	-37.2709	106.9775
0.1 vs 0.5	85.19043	21.91505	5.49748	0.01986	0.05	S	13.06623	157.3146
0.1 vs 0.2	50.3371	21.91505	3.24833	0.22254	0.05	NS	-21.7871	122.4613
0.05 vs 0.5	88.58147	21.91505	5.71631	0.01567	0.05	S	16.45726	160.7057
0.05 vs 0.2	53.72813	21.91505	3.46716	0.17853	0.05	NS	-18.3961	125.8523
0.05 vs 0.1	3.39103	21.91505	0.21883	0.99985	0.05	NS	-68.7332	75.51524
0.02 vs 0.5	140.2873	21.91505	9.05296	5.78E-04	0.05	S	68.16306	212.4115
0.02 vs 0.2	105.4339	21.91505	6.80382	0.00498	0.05	S	33.30973	177.5581
0.02 vs 0.1	55.09683	21.91505	3.55549	0.16303	0.05	NS	-17.0274	127.221
0.02 vs 0.05	51.7058	21.91505	3.33666	0.20378	0.05	NS	-20.4184	123.83

^a The different experimental groups are referred to as "X vs Y", where X and Y stand for the respective heating rate (°C/min).

^b S indicates $p < 0.05$ (significant); NS indicates $p > 0.05$ (not significant).

Supplementary information

Table S7. Complete statistical significance data (p values) of the mineral anisotropy pairwise comparisons as a function of heating rate (corresponding to Fig. 5f) as determined by one-way ANOVA with Tukey's multiple comparison tests.

Comparison of Groups ^a	Mean Diff	SEM	q Value	Prob	Alpha	Sig ^b	LCL	UCL
0.2 vs 0.5	0.01047	0.03599	0.41125	0.99816	0.05	NS	-0.10799	0.12892
0.1 vs 0.5	0.09867	0.03599	3.87676	0.11633	0.05	NS	-0.01979	0.21712
0.1 vs 0.2	0.0882	0.03599	3.46551	0.17883	0.05	NS	-0.03026	0.20666
0.05 vs 0.5	0.1633	0.03599	6.4163	0.00744	0.05	S	0.04484	0.28176
0.05 vs 0.2	0.15283	0.03599	6.00505	0.0115	0.05	S	0.03438	0.27129
0.05 vs 0.1	0.06463	0.03599	2.53954	0.42599	0.05	NS	-0.05382	0.18309
0.02 vs 0.5	0.37287	0.03599	14.65048	8.86E-06	0.05	S	0.25441	0.49132
0.02 vs 0.2	0.3624	0.03599	14.23923	1.15E-05	0.05	S	0.24394	0.48086
0.02 vs 0.1	0.2742	0.03599	10.77372	1.36E-04	0.05	S	0.15574	0.39266
0.02 vs 0.05	0.20957	0.03599	8.23418	0.00122	0.05	S	0.09111	0.32802

^a The different experimental groups are referred to as "X vs Y", where X and Y stand for the respective heating rate (°C/min).

^b S indicates $p < 0.05$ (significant); NS indicates $p > 0.05$ (not significant).

Table S8. Complete statistical significance data (p values) of the mechanical property pairwise comparisons as a function of heating rate (corresponding to Supplementary Figure S8b) as determined by one-way ANOVA with Tukey's multiple comparison tests.

Comparison of Groups ^a	Mean Diff	SEM	q Value	Prob	Alpha	Sig ^b	LCL	UCL
1 vs control	2.3	1.4682	2.21543	0.70375	0.05	NS	-2.7133	7.3133
0.5 vs control	6.48	1.4682	6.24172	0.00817	0.05	S	1.4667	11.4933
0.5 vs 1	4.18	1.4682	4.0263	0.13304	0.05	NS	-0.8333	9.1933
0.2 vs control	10.27667	1.4682	9.89878	1.00E-04	0.05	S	5.26337	15.28997
0.2 vs 1	7.97667	1.4682	7.68335	0.00133	0.05	S	2.96337	12.98997
0.2 vs 0.5	3.79667	1.4682	3.65706	0.20213	0.05	NS	-1.21663	8.80997
0.1 vs control	14.03333	1.4682	13.51731	2.66E-06	0.05	S	9.02003	19.04663
0.1 vs 1	11.73333	1.4682	11.30188	2.27E-05	0.05	S	6.72003	16.74663
0.1 vs 0.5	7.55333	1.4682	7.27559	0.0022	0.05	S	2.54003	12.56663
0.1 vs 0.2	3.75667	1.4682	3.61853	0.2108	0.05	NS	-1.25663	8.76997
0.05 vs control	16.14667	1.4682	15.55293	4.24E-07	0.05	S	11.13337	21.15997
0.05 vs 1	13.84667	1.4682	13.3375	3.15E-06	0.05	S	8.83337	18.85997
0.05 vs 0.5	9.66667	1.4682	9.31121	1.93E-04	0.05	S	4.65337	14.67997
0.05 vs 0.2	5.87	1.4682	5.65415	0.01742	0.05	S	0.8567	10.8833
0.05 vs 0.1	2.11333	1.4682	2.03562	0.77316	0.05	NS	-2.89997	7.12663
0.02 vs control	23.25333	1.4682	22.39827	3.43E-07	0.05	S	18.24003	28.26663
0.02 vs 1	20.95333	1.4682	20.18285	1.18E-07	0.05	S	15.94003	25.96663
0.02 vs 0.5	16.77333	1.4682	16.15655	2.66E-07	0.05	S	11.76003	21.78663
0.02 vs 0.2	12.97667	1.4682	12.49949	6.94E-06	0.05	S	7.96337	17.98997
0.02 vs 0.1	9.22	1.4682	8.88097	3.17E-04	0.05	S	4.2067	14.2333
0.02 vs 0.05	7.10667	1.4682	6.84534	0.00378	0.05	S	2.09337	12.11997

^a The different experimental groups are referred to as "X vs Y", where X and Y stand for the respective heating rate (°C/min), while "control" is the unmineralized pSBMA hydrogel.

^b S indicates $p < 0.05$ (significant); NS indicates $p > 0.05$ (not significant).

Table S9. Complete statistical significance data (p values) of the mineral volume pairwise comparisons as a function of crosslinker content (corresponding to Fig. 6d) as determined by one-way ANOVA with Tukey's multiple comparison tests.

Comparison of Groups ^a	Mean Diff	SEM	q Value	Prob	Alpha	Sig ^b	LCL	UCL
1.326 vs 2.651	0.54723	0.39724	1.94822	0.73894	0.05	NS	-0.78705	1.88152
0.663 vs 2.651	0.84683	0.39724	3.01484	0.33398	0.05	NS	-0.48745	2.18112
0.663 vs 1.326	0.2996	0.39724	1.06661	0.97033	0.05	NS	-1.03469	1.63389
0.265 vs 2.651	2.90927	0.39724	10.35736	1.04E-04	0.05	S	1.57498	4.24355
0.265 vs 1.326	2.36203	0.39724	8.40914	7.39E-04	0.05	S	1.02775	3.69632
0.265 vs 0.663	2.06243	0.39724	7.34253	0.00238	0.05	S	0.72815	3.39672
0.133 vs 2.651	3.9868	0.39724	14.19352	3.99E-06	0.05	S	2.65251	5.32109
0.133 vs 1.326	3.43957	0.39724	12.2453	1.92E-05	0.05	S	2.10528	4.77385
0.133 vs 0.663	3.13997	0.39724	11.17868	4.88E-05	0.05	S	1.80568	4.47425
0.133 vs 0.265	1.07753	0.39724	3.83616	0.14337	0.05	NS	-0.25675	2.41182
0.027 vs 2.651	3.59913	0.39724	12.81338	1.20E-05	0.05	S	2.26485	4.93342
0.027 vs 1.326	3.0519	0.39724	10.86516	6.49E-05	0.05	S	1.71761	4.38619
0.027 vs 0.663	2.7523	0.39724	9.79854	1.79E-04	0.05	S	1.41801	4.08659
0.027 vs 0.265	0.68987	0.39724	2.45601	0.5354	0.05	NS	-0.64442	2.02415
0.027 vs 0.133	-0.38767	0.39724	1.38014	0.91713	0.05	NS	-1.72195	0.94662

^a The different experimental groups are referred to as "X vs Y", where X and Y stand for the respective crosslinker content (mol%).

^b S indicates $p < 0.05$ (significant); NS indicates $p > 0.05$ (not significant).

Supplementary information

Table S10. Complete statistical significance data (p values) of the calcium content pairwise comparisons as a function of crosslinker content (corresponding to Fig. 6e) as determined by one-way ANOVA with Tukey's multiple comparison tests.

Comparison of Groups ^a	Mean Diff	SEM	q Value	Prob	Alpha	Sig ^b	LCL	UCL
1.326 vs 2.651	0.15893	0.05492	4.09227	0.10756	0.05	NS	-0.02555	0.34342
0.663 vs 2.651	0.14113	0.05492	3.63395	0.17872	0.05	NS	-0.04335	0.32562
0.663 vs 1.326	-0.0178	0.05492	0.45832	0.99939	0.05	NS	-0.20229	0.16669
0.265 vs 2.651	0.35167	0.05492	9.05484	3.76E-04	0.05	S	0.16718	0.53615
0.265 vs 1.326	0.19273	0.05492	4.96257	0.03885	0.05	S	0.00825	0.37722
0.265 vs 0.663	0.21053	0.05492	5.42089	0.02248	0.05	S	0.02605	0.39502
0.133 vs 2.651	0.56207	0.05492	14.47229	3.22E-06	0.05	S	0.37758	0.74655
0.133 vs 1.326	0.40313	0.05492	10.38002	1.02E-04	0.05	S	0.21865	0.58762
0.133 vs 0.663	0.42093	0.05492	10.83834	6.65E-05	0.05	S	0.23645	0.60542
0.133 vs 0.265	0.2104	0.05492	5.41745	0.02257	0.05	S	0.02591	0.39489
0.027 vs 2.651	0.92847	0.05492	23.90649	2.39E-07	0.05	S	0.74398	1.11295
0.027 vs 1.326	0.76953	0.05492	19.81421	1.16E-07	0.05	S	0.58505	0.95402
0.027 vs 0.663	0.78733	0.05492	20.27253	2.54E-07	0.05	S	0.60285	0.97182
0.027 vs 0.265	0.5768	0.05492	14.85165	2.42E-06	0.05	S	0.39231	0.76129
0.027 vs 0.133	0.3664	0.05492	9.4342	2.56E-04	0.05	S	0.18191	0.55089

^a The different experimental groups are referred to as "X vs Y", where X and Y stand for the respective crosslinker content (mol%).

^b S indicates $p < 0.05$ (significant); NS indicates $p > 0.05$ (not significant).

Supplementary information

Table S11. Complete statistical significance data (p values) of the mineral density pairwise comparisons as a function of crosslinker content (corresponding to Supplementary Fig. S13a) as determined by one-way ANOVA with Tukey's multiple comparison tests.

Comparison of Groups ^a	Mean Diff	SEM	q Value	Prob	Alpha	Sig ^b	LCL	UCL
1.326 vs 2.651	39.262	12.30355	4.51291	0.06614	0.05	NS	-2.06465	80.58865
0.663 vs 2.651	27.004	12.30355	3.10393	0.30696	0.05	NS	-14.3227	68.33065
0.663 vs 1.326	-12.258	12.30355	1.40898	0.9105	0.05	NS	-53.5847	29.06865
0.265 vs 2.651	-48.6787	12.30355	5.5953	0.01825	0.05	S	-90.0053	-7.35202
0.265 vs 1.326	-87.9407	12.30355	10.10821	1.32E-04	0.05	S	-129.267	-46.614
0.265 vs 0.663	-75.6827	12.30355	8.69923	5.44E-04	0.05	S	-117.009	-34.356
0.133 vs 2.651	-110.069	12.30355	12.65176	1.37E-05	0.05	S	-151.396	-68.7427
0.133 vs 1.326	-149.331	12.30355	17.16467	4.24E-07	0.05	S	-190.658	-108.005
0.133 vs 0.663	-137.073	12.30355	15.75569	1.23E-06	0.05	S	-178.4	-95.7467
0.133 vs 0.265	-61.3907	12.30355	7.05646	0.00329	0.05	S	-102.717	-20.064
0.027 vs 2.651	-195.468	12.30355	22.46778	9.89E-07	0.05	S	-236.795	-154.141
0.027 vs 1.326	-234.73	12.30355	26.98069	0	0.05	S	-276.057	-193.403
0.027 vs 0.663	-222.472	12.30355	25.57171	9.85E-08	0.05	S	-263.799	-181.145
0.027 vs 0.265	-146.789	12.30355	16.87248	5.32E-07	0.05	S	-188.116	-105.463
0.027 vs 0.133	-85.3987	12.30355	9.81602	1.76E-04	0.05	S	-126.725	-44.072

^a The different experimental groups are referred to as "X vs Y", where X and Y stand for the respective crosslinker content (mol%).

^b S indicates $p < 0.05$ (significant); NS indicates $p > 0.05$ (not significant).