Electronic Supplementary Information (ESI)

A simple strategy for robust preparation and characterisation of hydrogels derived from chitosan and amino functional monomers for biomedical applications

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Hydrogels formation with different ratios of CS and six independent amino functional monomers, and their gelation time.

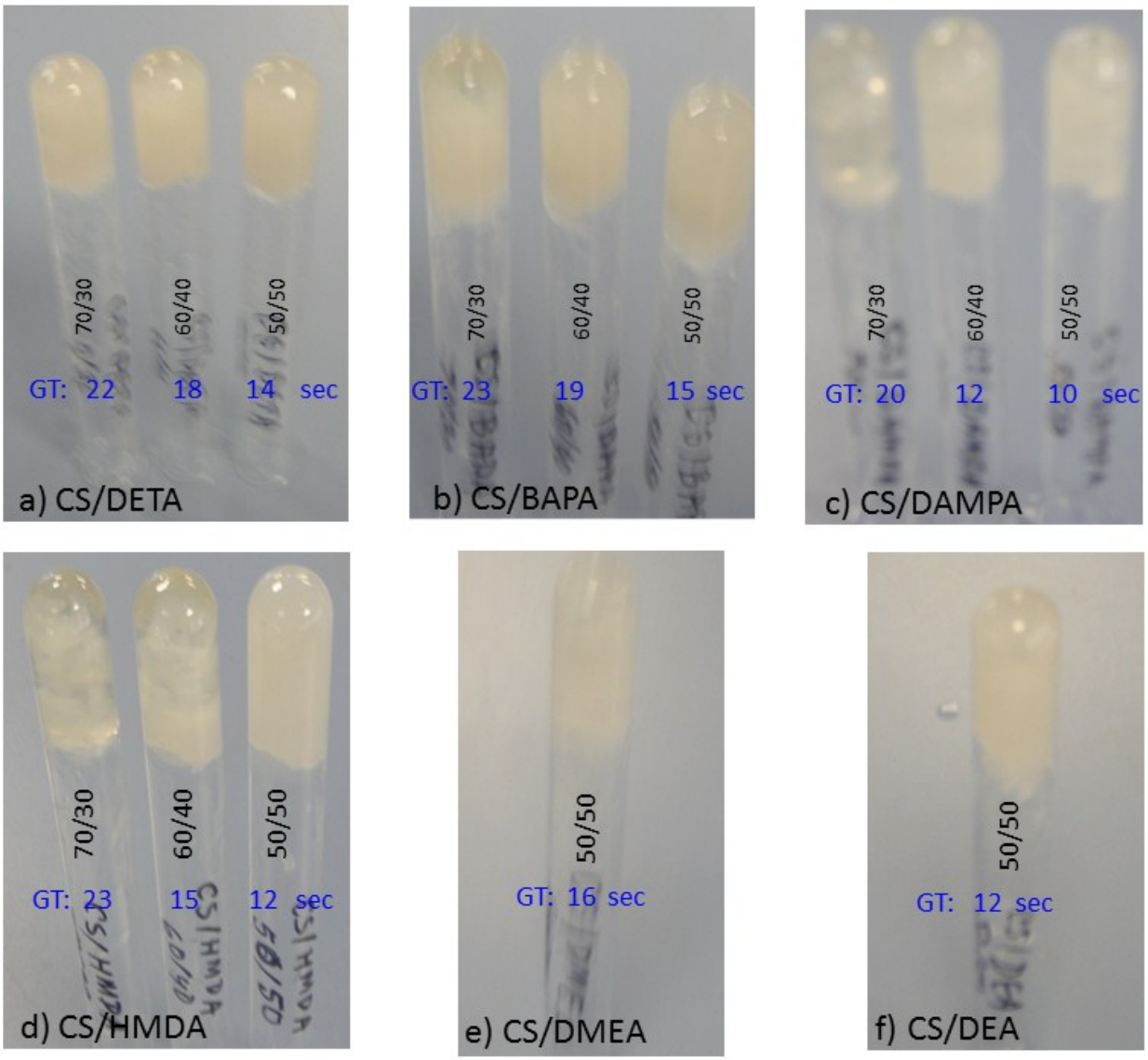


Fig. S1. Hydrogels formation *via* blending of CS and six independent AF monomers with various ratios. The average gelation time (GT) with three replicates for each ratio (e.g., CS/AF monomers: 70/30, 60/40 and/or 50/50) is presented: a) CS/DETA (70/30, 60/40 and 50/50), b) CS/BAPA (70/30, 60/40 and 50/50), c) CS/DAMPA (70/30, 60/40 and 50/50), d) CS/HMDA (70/30, 60/40 and 50/50), e) CS/DMEA (50/50) and CS/DEA (50/50).

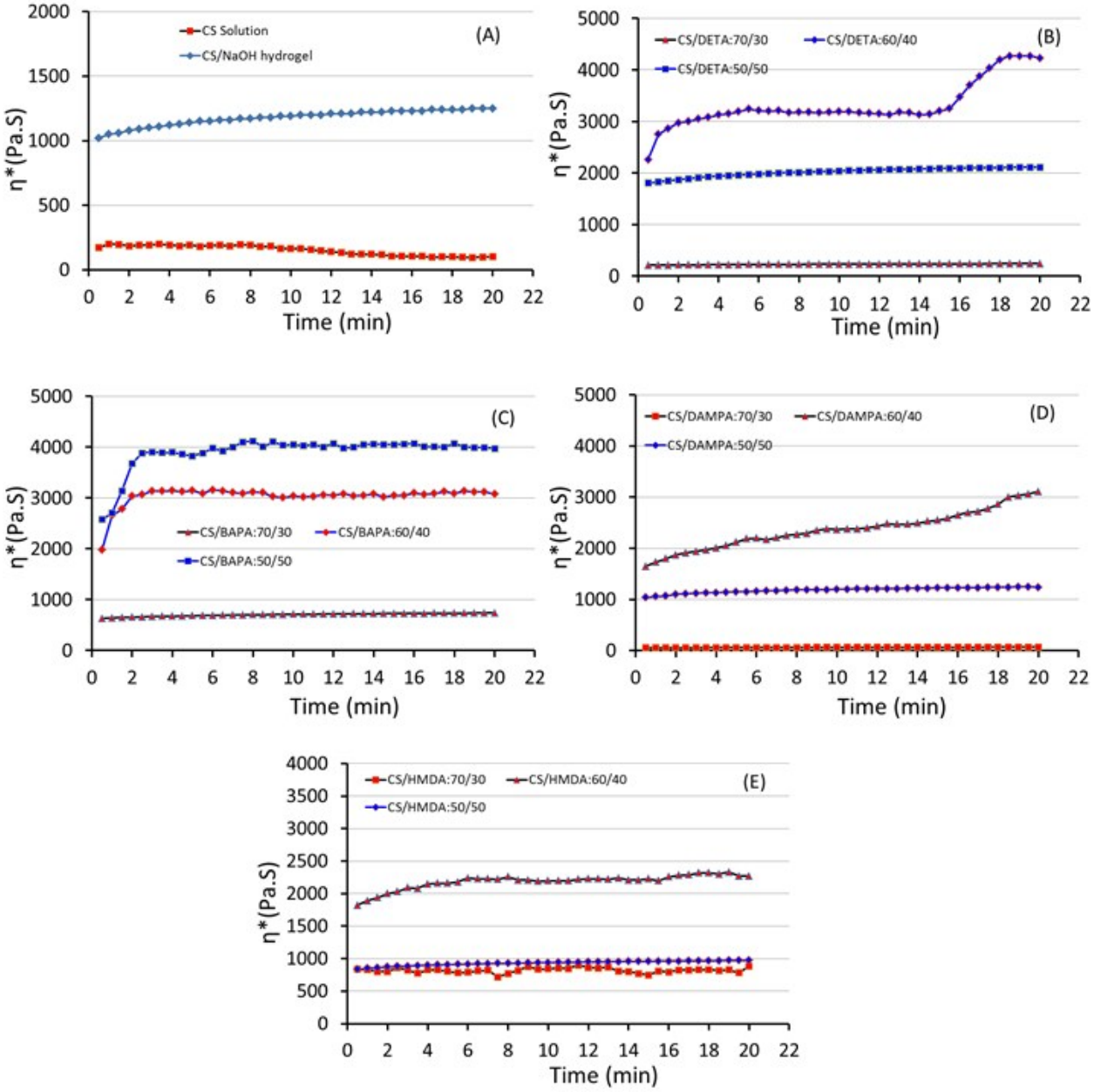
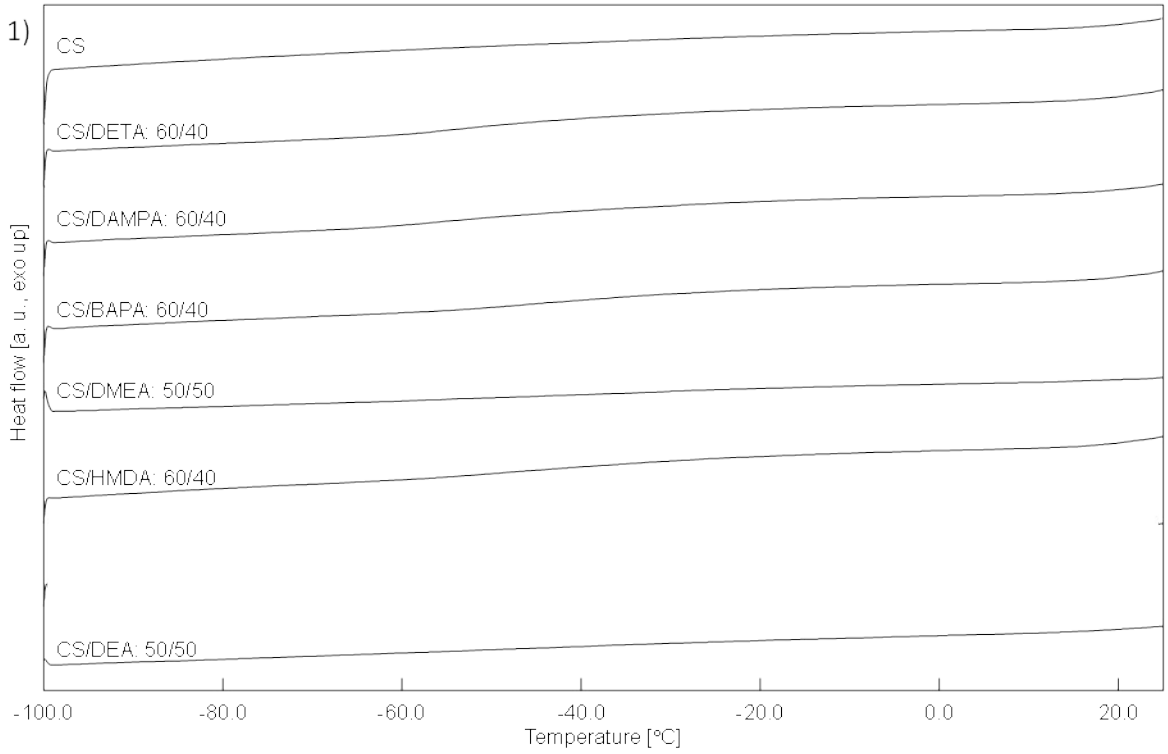
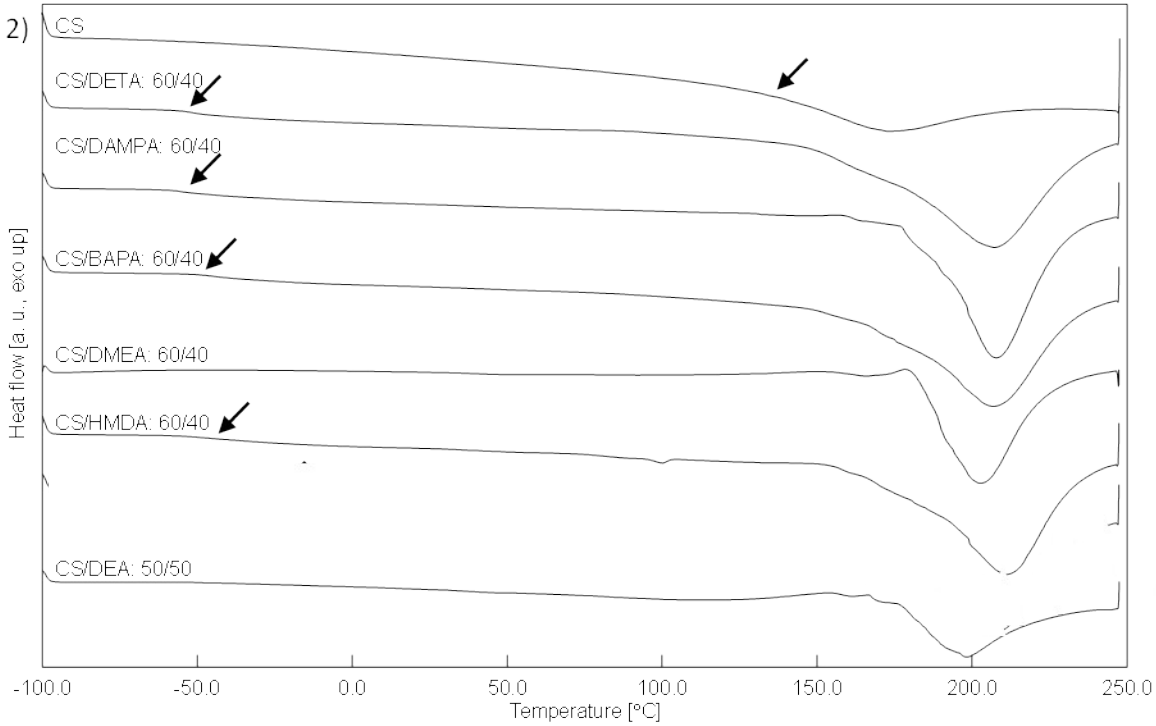


Fig. S2. Complex viscosity (η*) of hydrogel biomaterials is plotted as a function of time. (A) CS solution and CS/NaOH hydrogel: (B) CS/DETA (ratios: 70/30, 60/40 and 50/50), (C) CS/BAPA (ratios: 70/30, 60/40 and 50/50); (D) CS/DAMPA (ratios: 70/30, 60/40 and 50/50); (E) CS/HMDA (ratios: 70/30, 60/40 and 50/50).

Table S1. Band assignments of major absorptions in IR spectra of CS, CS/DETA, CS/DAMPA, CS/BAPA, CS/HMDA, CS/DMEA and CS/DEA dried samples 3800-600 cm^{-1}.

CS		CS/DETA: 60/40		CS/DAMPA: 60/40		CS/BAPA: 60/40		CS/HMDA: 60/40		CS/DMEA: 50/50		CS/DEA: 50/50	
Wave number (cm^{-1})	Intensity	Wave number (cm^{-1})	Intensity	Wave number (cm^{-1})	Intensity	Wave number (cm^{-1})	Intensity	Wave number (cm^{-1})	Intensity	Wave number (cm^{-1})	Intensity	Wave number (cm^{-1})	Intensity
3441	61	3389	75	3395	40	3380	48	3396	80	3421	79	3367	63
		3281	74	3263	42							3277	64
				3054	40	3050	48					3091	67
2932	65	2906	73	2925	37	2927	47	2939	77	2925	81	2928	67
2870	65	2868	73	2864	37			2856	78	2876	80	2876	67
								2662	80				
								2567	82			2498	78
2144	90	2163	89	2178	71	2189	70	2189	90			2126	90
1643	74			1635	49	1635	52	1647	80	1654	83	1654	73
1557	72	1558	70	1559	34	1558	46	1558	77	1559	80	1558	68
				1465	49			1541	76				
1407	73	1402	73	1401	36	1399	47	1405	77	1407	82	1407	71
1327	76	1318	80	1334	51	1335	54	1336	83	1336	85	1319	78
1263	80							1258	89	1262	89	1263	86
				1228	70								
						1200	79	1200	93				
1155	75	1149	83	1150	49	1150	57	1152	85	1153	83	1154	72
		1066	78	1073	37	1073	49	1073	82	1084	81	1081	65
		1030	79	1030	39	1012	53	1018	81	1027	82	1031	67
944	81							958	90			944	81
896	84	921	90	921	64	921	73	919	90	896	91	897	86
		815	93	832	79	815	85	815	95				
				760	81	762	81	735	94				
653	82	652	86	652	59	653	62	650	85	653	90	653	87
618	84	618	90	617	69	618	72	617	91	619	91	614	87


1)
CS
CS/DETA: 60/40
CS/DAMPA: 60/40
CS/BAPA: 60/40
CS/DMEA: 50/50
CS/HMDA: 60/40
CS/DEA: 50/50
Heat flow [a. u., exo up]
-100.0
-80.0
-60.0
-40.0
-20.0
0.0
20.0
Temperature [°C]


2)
CS
CS/DETA: 60/40
CS/DAMPA: 60/40
CS/BAPA: 60/40
CS/DMEA: 60/40
CS/HMDA: 60/40
CS/DEA: 50/50
Heat flow [a. u., exo up]
-100.0
-50.0
0.0
50.0
100.0
150.0
200.0
250.0
Temperature [°C]

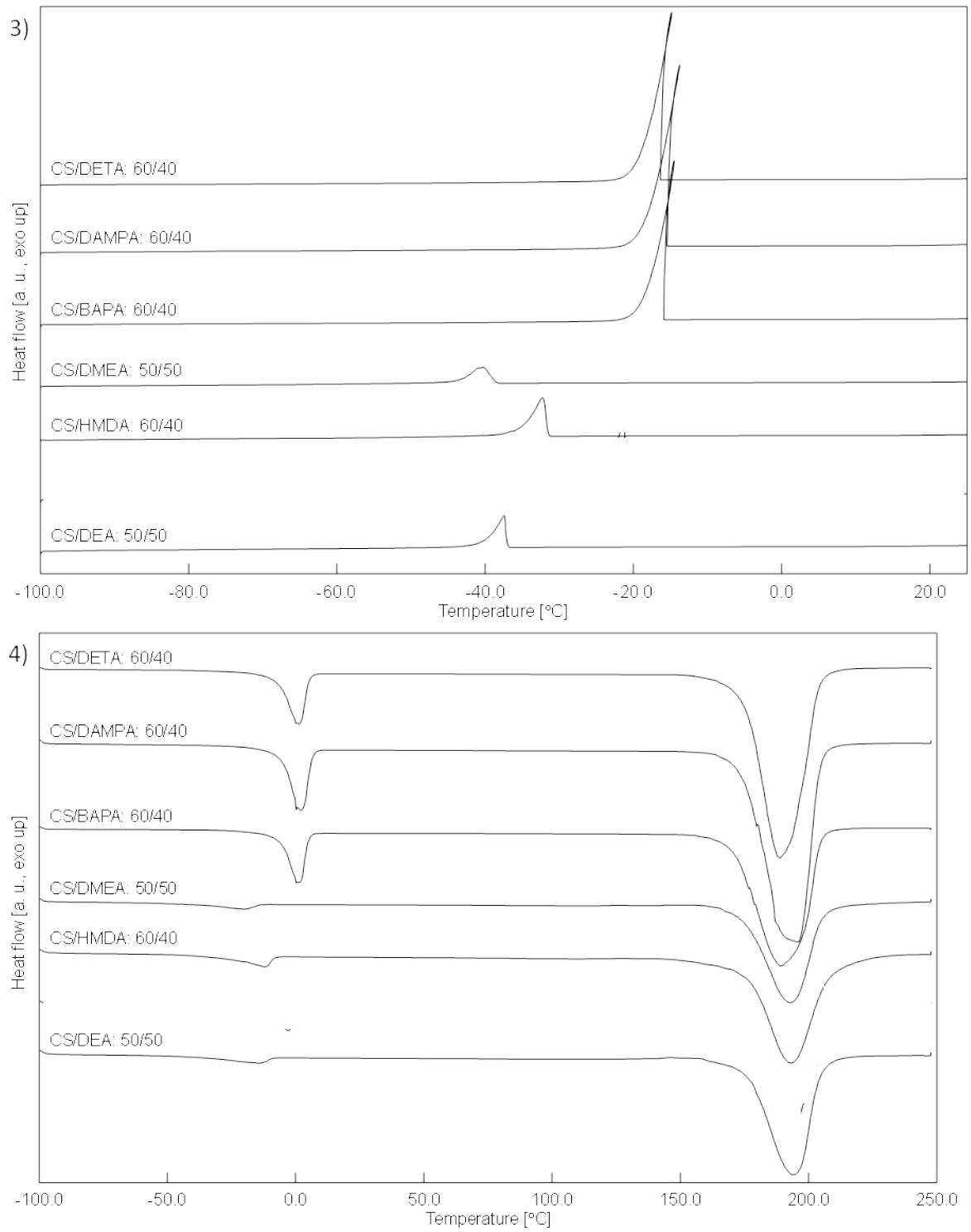


Fig. S3. DSC profiles of CS and its blend samples both heating and cooling scans for in both dried and in hydrated states: 1) Cooling scan of dried samples, 2) Heating scan of dried samples, 3) Cooling scan of hydrated samples, and 4) Heating scan of hydrated samples. Glass transition temperature (T_g) in dried state was arrowed in 2).

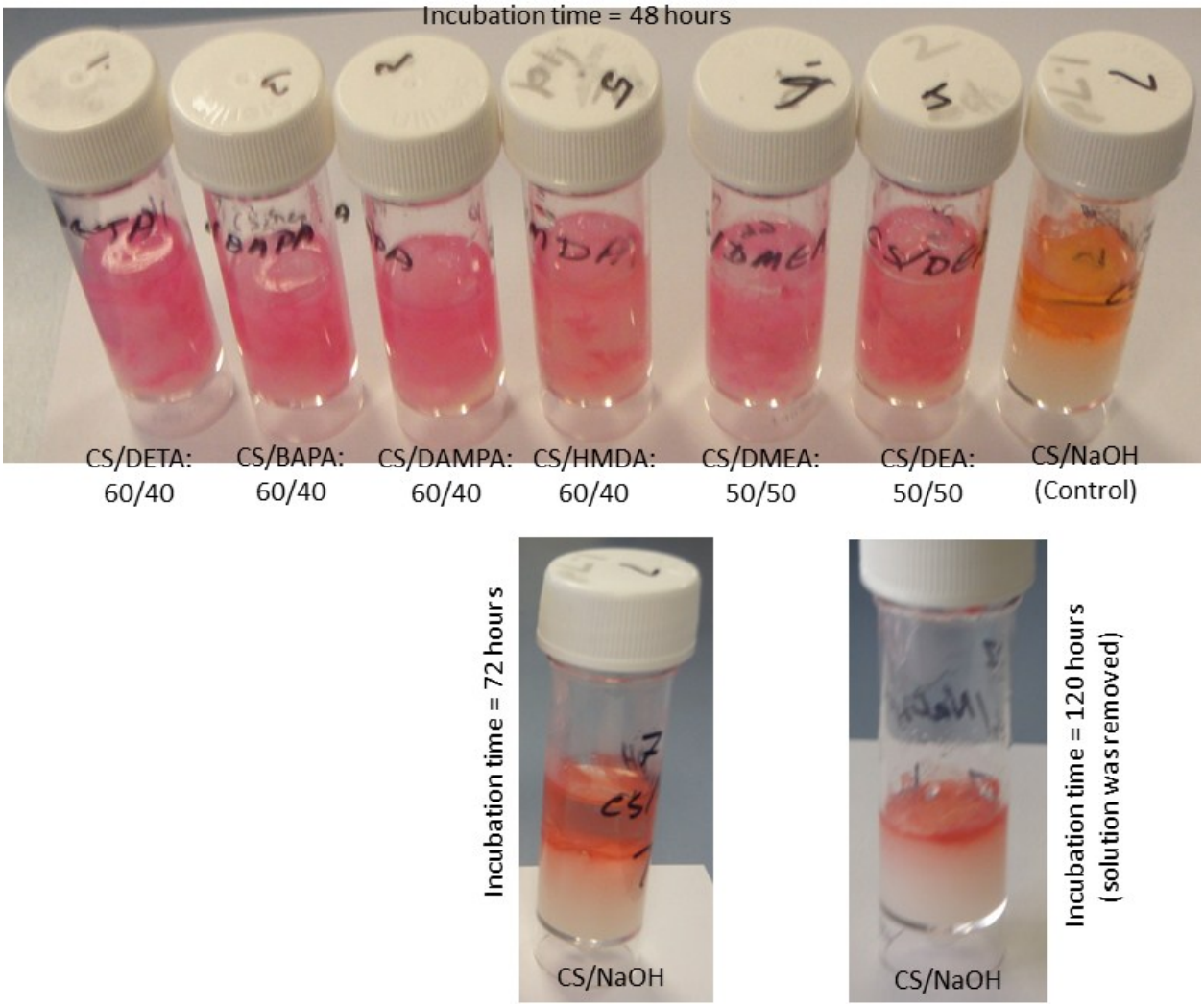


Fig. S4. The loading of RR120 molecules is showing uniform red color distribution into CS/DETA (60/40), CS/BAPA (60/40), CS/DAMPA (60/40), CS/HMDA (60/40), CS/DMEA (50/50) and CS/DEA (50/50) hydrogels. In contrary CS/NaOH hydrogel (control) did not interact with RR120 molecules and therefore no or very little adsorption was detected even after 120 hours of incubation time (bottom right).

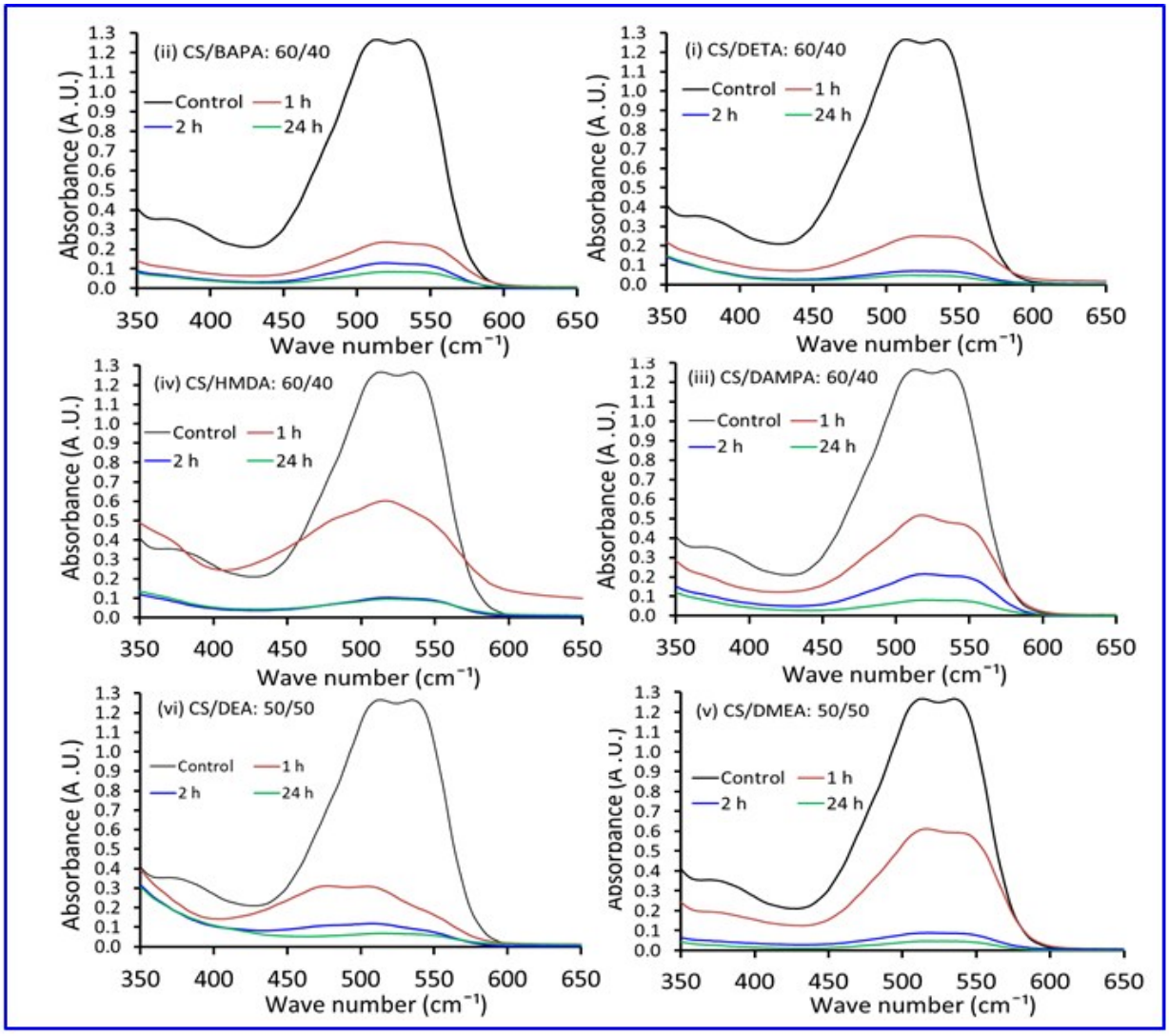


Fig. S5. UV-Vis spectroscopic absorption spectra of RR120 solution after different time of incubation with hydrogels [(i) CS/DETA (60/40), (ii) CS/BAPA (60/40), (iii) CS/DAMPA (60/40), (iv) CS/HMDA (60/40), (v) CS/DMEA (50/50) and (vi) CS/DEA (50/50)] compared with unloaded RR120 solution. RR120 solution was incubated at 37 ºC for a particular time up to 120 hours. After 24 hours incubation the spectral differences were insignificant.

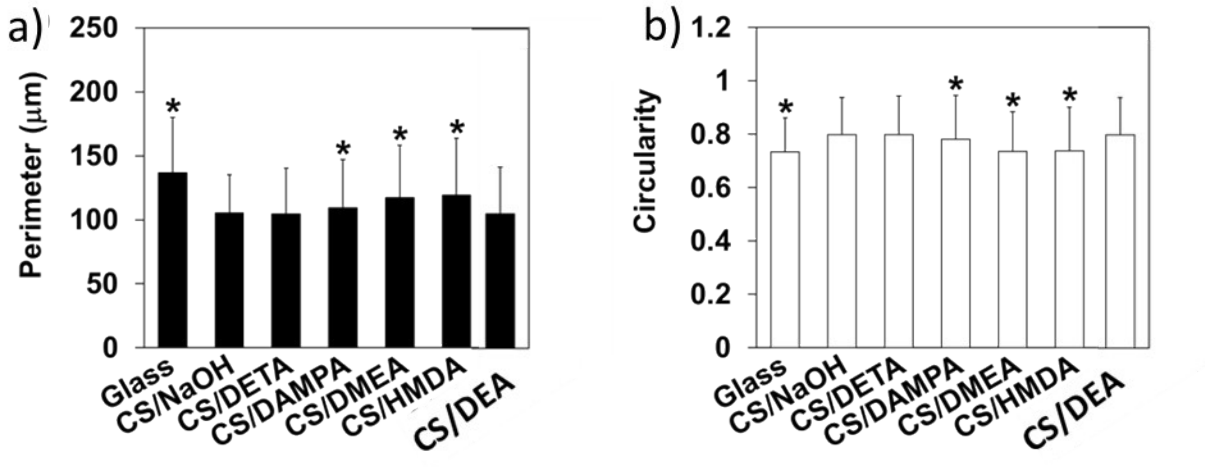


Fig. S6. Quantitative analysis of HT-1080 cell morphology is presented on CS-based hydrogels. (a -b) HT-1080 cells cultured into each gel and a slide glass for 24 h were stained for nuclei and F-actin. Cell area and perimeter of adhered cells were determined using 4-10 images (100-300 cells/image) randomly acquired from each sample. The circularity of cells was calculated as $4\pi \times$ (cell area) / (perimeter)^2. Values are expressed as the mean ± S.D. (*, $P < 0.01$ vs. CS/NaOH, n = 1,000).

Cell growth inside the hydrogel

Method

At first, HeLa cells were mixed into the CS solution, followed by then a required amount of DETA (10%) solution was added into the (CS-HeLa) mixture with a ratio of CS/DETA (60/40), and stirred using a tip. After HG formation, medium was added onto the HG and wash gently several times. Cells were cultured in 5% CO_2 atmosphere at 37 °C for 24 h. After the incubation, viable cells and cell nuclei were stained using Calcein AM (Dojindo Laboratories, Kumamoto, Japan) and Hochest 33342 [2-(4-Ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5-bi-1H -benzimidazole trihydrochloride] according to the manufacture's protocols, respectively. Images were acquired using a microscope (BZ-X710; KEYENCE Corp., Osaka, Japan) and presented in Fig. S7.

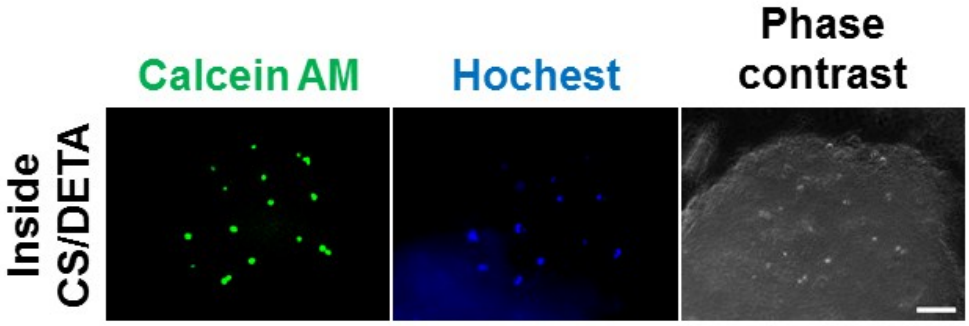


Fig. S7. Live cells staining inside CS/DETA (60/40) hydrogel after 24 h culture. Green: Calcein-AM-positive cells indicate viable cells (left image); Blue: Hochest 33342 (cell nuclei) (middle image) and phase contrast image (right). Scale bar =100 μm.