

Mapping Glycosaminoglycan-Hydroxyapatite Colloidal Gels as Potential Tissue Defect Fillers

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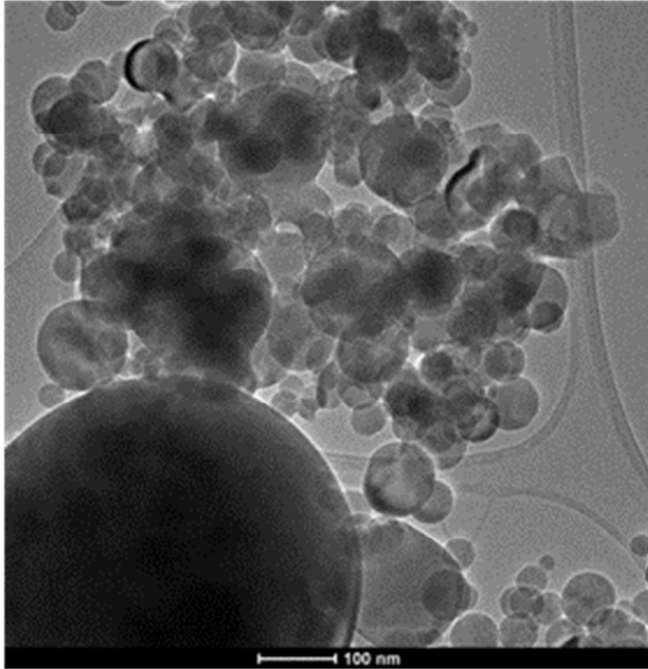
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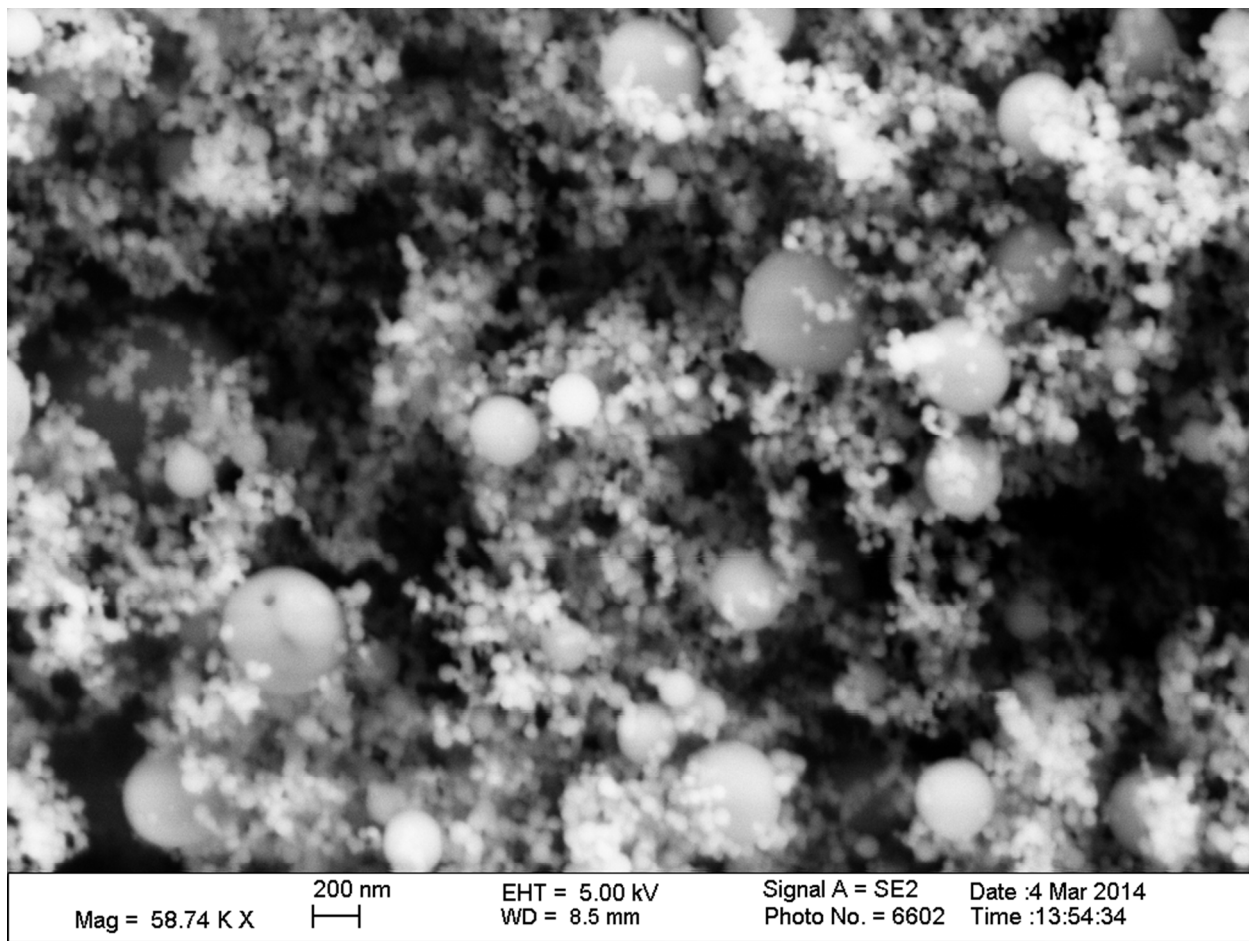
KEYWORDS: Hydroxyapatite; Glycosaminoglycan; Chondroitin Sulfate; Hyaluronic Acid; Colloidal Gel; Self-Assembling Fluid; Herschel-Bulkley Fluid; Tissue Defect Filler

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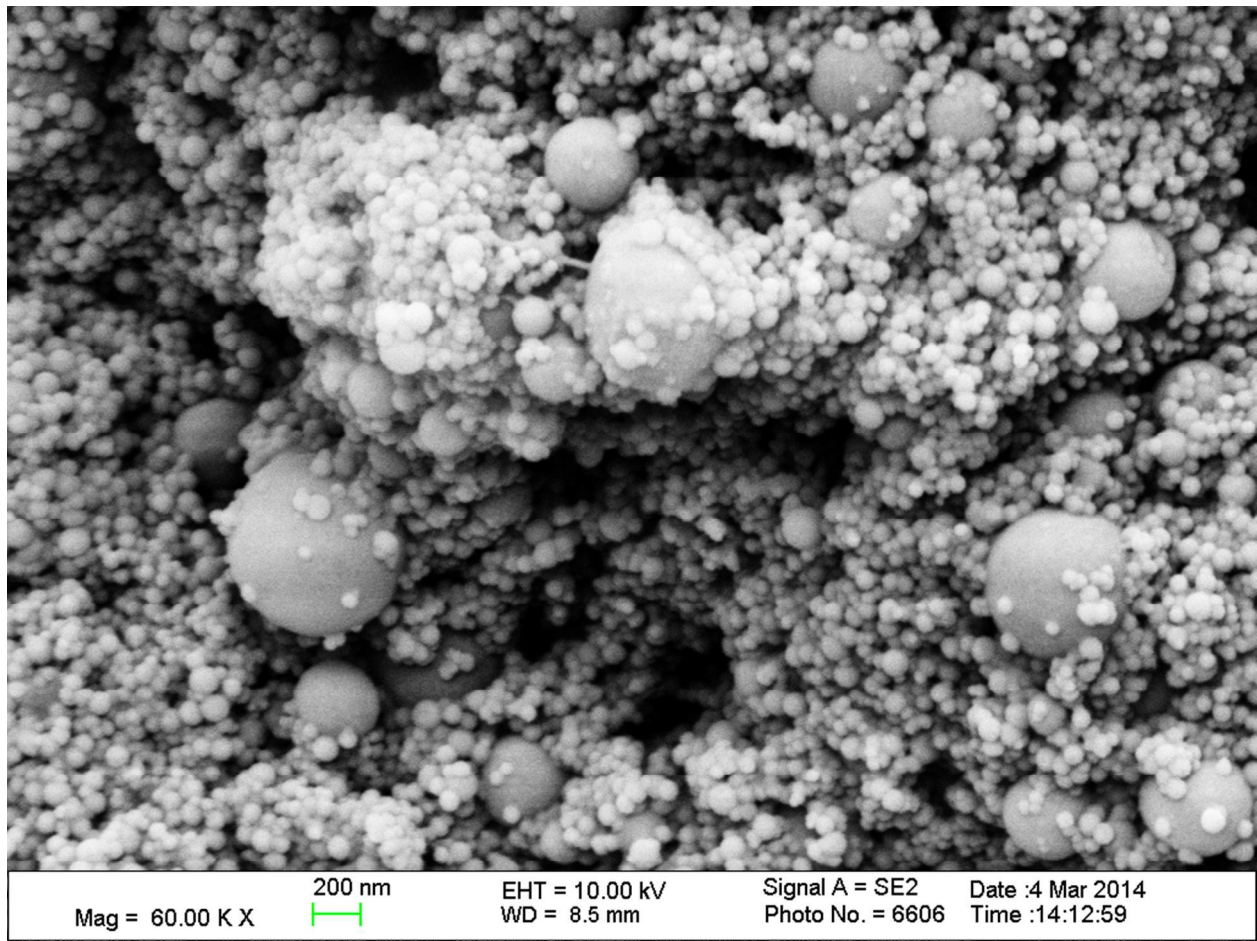
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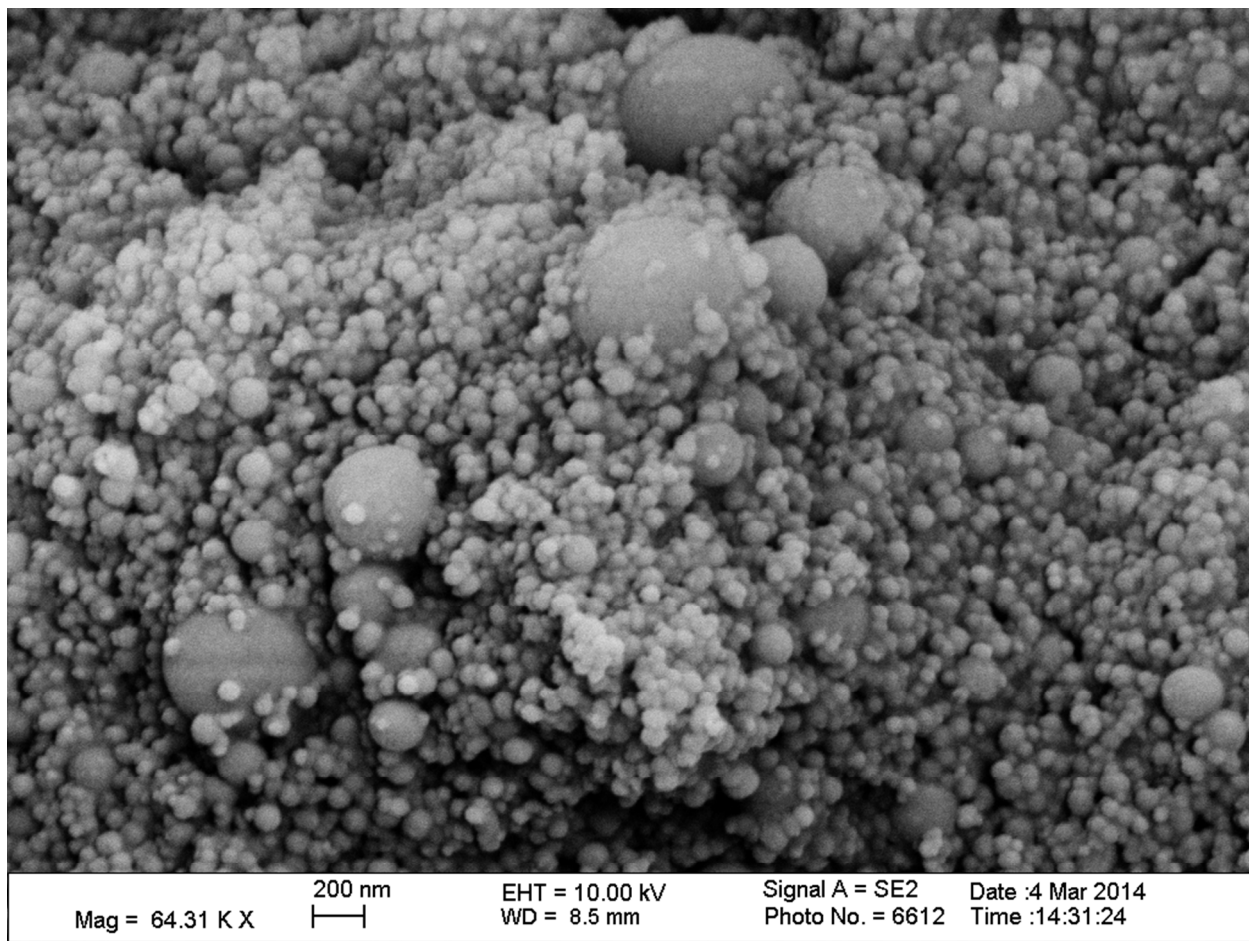
Supporting Figure 1: TEM image of HAP particles (scale bar = 100 nm).



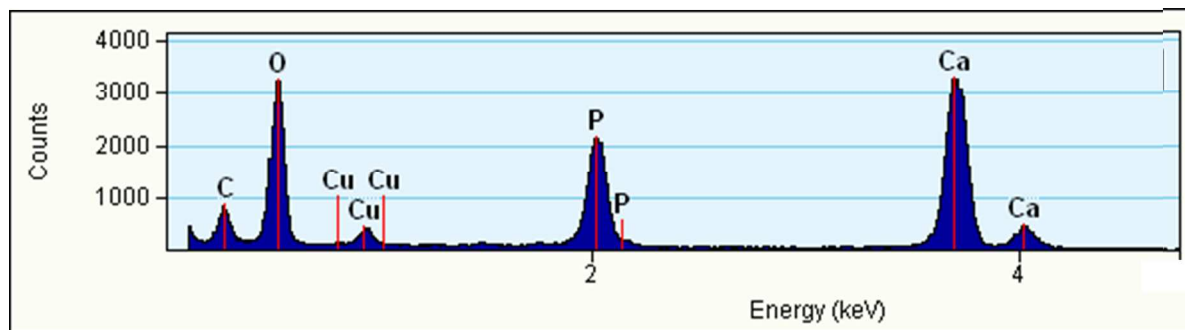
Supporting Figure 2: SEM image of HAP particles (60% w/v) (scale bar = 200 nm)



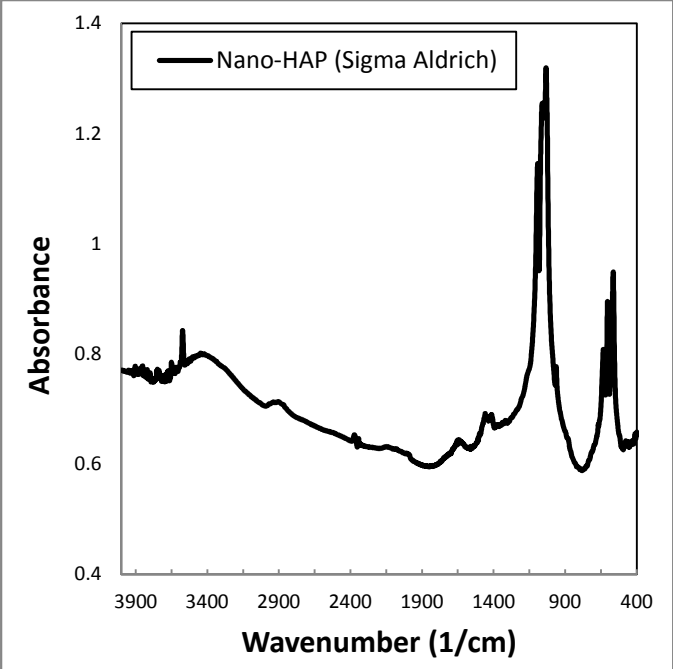
Supporting Figure 3: SEM image of CS-HAP (15%-60% w/v) colloidal gel (scale bar = 200 nm)



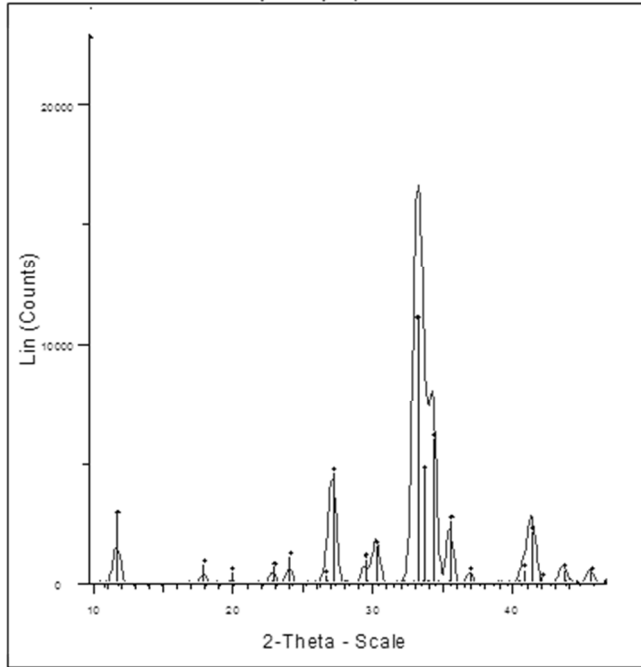
Supporting Figure 4: SEM image of HA-HAP (15%-60% w/v) colloidal gel (scale bar = 200 nm)



Supporting Figure 5: EDS spectra of HAP particles



Supporting Figure 6: FTIR spectra of HAP particles. ^{15, 16}



Supporting Figure 7: XRD of HAP particles (curve) compared to ICDD (vertical lines) HAP standard. ^{15, 16}

Supporting Table 1: CS-HAP rheological properties estimated by H-B fluid model

[CS] (% w/v)	[HAP]		τ_y (Yield Stress) (Pa)			K (Consistency Index) (Pa·s ⁿ)			n (Flow Behavior Index)			RMSE
	(% w/v)	ϕ (% v/v)	Average	±	95% CI	Average	±	95% CI	Average	±	95% CI	
CS 10%	0%	0.0%	0.01	±	0.07	0.04	±	0.01	0.93	±	0.02	0.06
	5%	3.1%	0.05	±	0.10	0.14	±	0.01	0.86	±	0.01	0.10
	10%	6.1%	0.52	±	0.55	0.11	±	0.03	0.93	±	0.04	0.57
	20%	12.3%	0.96	±	0.61	0.24	±	0.05	0.85	±	0.03	0.61
	40%	24.6%	10.46	±	2.39	0.87	±	0.28	0.77	±	0.05	2.20
	80%	49.2%	1403.00	±	1193.00	38.34	±	52.66	0.28	±	2.84	286.60
CS 15%	0%	0.0%	-0.23	±	0.24	0.34	±	0.03	0.81	±	0.02	0.20
	7.5%	4.6%	0.24	±	0.54	0.30	±	0.04	0.86	±	0.02	0.53
	15%	9.2%	0.31	±	0.61	0.51	±	0.06	0.81	±	0.02	0.59
	30%	18.4%	2.33	±	0.72	1.52	±	0.10	0.73	±	0.01	0.63
	60%	36.9%	218.50	±	21.10	2.69	±	1.23	0.80	±	0.06	3.51
	—	—	—	±	—	—	±	—	—	±	—	—
CS 20%	0%	0.0%	0.13	±	0.16	0.43	±	0.02	0.85	±	0.01	0.14
	10%	6.1%	-0.65	±	0.65	1.08	±	0.07	0.78	±	0.01	0.60
	20%	12.3%	-0.72	±	0.84	2.23	±	0.13	0.71	±	0.01	0.73
	40%	24.6%	11.66	±	3.67	4.06	±	0.55	0.72	±	0.02	3.22
	80%	49.2%	1350.00	±	68.00	3.14	±	2.49	0.93	±	0.11	31.10
	—	—	—	±	—	—	±	—	—	±	—	—
CS 30%	0%	0.0%	0.15	±	1.48	1.34	±	0.13	0.83	±	0.01	1.44
	15%	9.2%	-2.23	±	1.27	4.02	±	0.19	0.73	±	0.01	1.11
	30%	18.4%	-2.06	±	3.67	7.97	±	0.56	0.72	±	0.01	3.18
	60%	36.9%	134.70	±	25.70	20.20	±	3.77	0.73	±	0.03	22.56
	—	—	—	±	—	—	±	—	—	±	—	—
	—	—	—	±	—	—	±	—	—	±	—	—
CS 40%	0%	0.0%	-1.86	±	1.26	3.19	±	0.13	0.80	±	0.01	1.19
	20%	12.3%	-11.56	±	8.84	10.39	±	1.37	0.71	±	0.02	7.65
	40%	24.6%	3.09	±	13.97	16.55	±	1.69	0.77	±	0.02	12.82
	80%	49.2%	958.80	±	368.20	382.40	±	205.90	0.52	±	0.10	125.80
	—	—	—	±	—	—	±	—	—	±	—	—
	—	—	—	±	—	—	±	—	—	±	—	—
CS 60%	0%	0.0%	-7.96	±	3.28	10.47	±	0.36	0.78	±	0.01	3.06
	30%	18.4%	-65.26	±	83.57	44.58	±	12.56	0.72	±	0.04	72.90
	60%	36.9%	112.60	±	47.50	199.30	±	17.20	0.68	±	0.02	24.16
	—	—	—	±	—	—	±	—	—	±	—	—
	—	—	—	±	—	—	±	—	—	±	—	—
	—	—	—	±	—	—	±	—	—	±	—	—
CS 80%	0%	0.0%	-81.25	±	101.09	48.17	±	18.46	0.74	±	0.06	79.21
	40%	24.6%	11.33	±	55.17	111.00	±	14.80	0.78	±	0.03	33.02
	—	—	—	±	—	—	±	—	—	±	—	—
	—	—	—	±	—	—	±	—	—	±	—	—
	—	—	—	±	—	—	±	—	—	±	—	—
	—	—	—	±	—	—	±	—	—	±	—	—

Individual points are reported (average ± 95% CI) from triplicate studies and overall model fit reported as root-mean-squared error (RMSE).

Supporting Table 2: HA-HAP rheological properties estimated by H-B fluid model

[HA] (% w/v)	[HAP]		τ_y (Yield Stress) (Pa)			K (Consistency Index) (Pa·s ⁿ)			n (Flow Behavior Index)			RMSE
	(% w/v)	ϕ (% v/v)	Average	±	95% CI	Average	±	95% CI	Average	±	95% CI	
HA 10%	0%	0.0%	0.26	±	0.21	0.11	±	0.01	0.98	±	0.02	0.21
	5%	3.1%	0.39	±	0.35	0.10	±	0.01	0.99	±	0.03	0.38
	10%	6.1%	0.52	±	0.50	0.06	±	0.01	1.05	±	0.04	0.56
	20%	12.3%	0.37	±	1.68	0.55	±	0.15	0.83	±	0.04	1.64
	40%	24.6%	7.24	±	2.94	0.85	±	0.22	0.86	±	0.04	2.94
	80%	49.2%	912.40	±	266.60	105.90	±	133.20	0.37	±	0.16	53.06
HA 15%	0%	0.0%	1.04	±	1.41	0.17	±	0.04	1.01	±	0.09	1.59
	7.5%	4.6%	1.41	±	1.31	0.34	±	0.05	1.01	±	0.02	1.43
	15%	9.2%	2.97	±	1.93	0.63	±	0.09	0.97	±	0.02	2.07
	30%	18.4%	7.41	±	0.09	2.32	±	0.09	0.87	±	0.01	1.18
	60%	36.9%	132.60	±	17.80	46.66	±	4.10	0.63	±	0.01	13.50
	—	—	—	±	—	—	±	—	—	±	—	—
HA 20%	0%	0.0%	1.10	±	1.44	0.49	±	0.05	1.03	±	0.01	1.59
	10%	6.1%	0.46	±	0.36	1.15	±	0.02	0.96	±	0.00	0.38
	20%	12.3%	-0.24	±	2.32	3.07	±	0.15	0.89	±	0.01	2.37
	40%	24.6%	-4.49	±	23.44	14.81	±	2.76	0.77	±	0.03	21.64
	80%	49.2%	263.00	±	521.90	759.90	±	373.10	0.39	±	0.08	113.10
	—	—	—	±	—	—	±	—	—	±	—	—
HA 30%	0%	0.0%	-5.02	±	6.54	3.40	±	0.32	0.95	±	0.01	6.93
	15%	9.2%	-32.50	±	44.75	12.94	±	4.32	0.81	±	0.05	43.00
	30%	18.4%	-17.48	±	28.59	22.31	±	3.69	0.82	±	0.03	24.54
	60%	36.9%	-22.98	±	111.90	234.10	±	44.40	0.65	±	0.04	53.53
	—	—	—	±	—	—	±	—	—	±	—	—
	—	—	—	±	—	—	±	—	—	±	—	—
HA 40%	0%	0.0%	-37.25	±	49.85	15.06	±	5.34	0.86	±	0.06	44.50
	20%	12.3%	-33.06	±	43.35	30.27	±	6.78	0.85	±	0.04	33.44
	40%	24.6%	-221.30	±	376.60	223.10	±	151.80	0.65	±	0.13	178.20
	—	—	—	±	—	—	±	—	—	±	—	—
	—	—	—	±	—	—	±	—	—	±	—	—
	—	—	—	±	—	—	±	—	—	±	—	—

Individual points are reported (Average ± 95% CI) from triplicate studies and overall model fit reported as root-mean-squared error (RMSE).

Supporting Table 3: Pure HAP rheological properties estimated by H-B fluid model

[HAP]		τ_y (Yield Stress) (Pa)			K (Consistency Index) (Pa-s ⁿ)			n (Flow Behavior Index)			RMSE
(% w/v)	ϕ (% v/v)	Average	±	95% CI	Average	±	95% CI	Average	±	95% CI	—
0%	0.0%	0.00	±	0.00	0.001	±	0.00	0.95	±	0.18	0.01
5%	3.1%	-0.04	±	0.13	0.002	±	0.05	1.05	±	0.24	0.07
7.5%	4.6%	0.00	±	0.10	0.001	±	0.05	1.07	±	0.20	0.02
10%	6.1%	0.00	±	0.20	0.002	±	0.05	1.05	±	0.25	0.21
15%	9.2%	-0.06	±	0.18	0.01	±	0.02	0.90	±	0.19	0.16
20%	12.3%	-0.15	±	0.28	0.03	±	0.05	0.81	±	0.17	0.24
30%	18.4%	-9.57	±	18.69	0.97	±	18.05	0.91	±	0.15	0.92
40%	24.6%	-11.07	±	23.76	2.78	±	21.94	0.90	±	0.18	1.89
60%	36.9%	12.68	±	122.42	35.29	±	86.47	0.85	±	0.43	29.42
80%	49.2%	1124.87	±	1245.02	21.98	±	53.86	0.56	±	0.83	384.31

Individual points are reported (Average \pm 95% CI) from triplicate studies and overall model fit reported as root-mean-squared error (RMSE).

Supporting Table 4: Viscoelastic recovery of GAG (15%) – HAP (60%) mixtures compared to pure components

	[GAG]	[HAP]	Phase I: Initial			Phase II: Disruption			Phase III: Recovery			G' Recovery (5 min) (%)
			G' (kPa)	G'' (kPa)	δ (°)	G' (kPa)	G'' (kPa)	δ (°)	G' (kPa)	G'' (kPa)	δ (°)	
Pure CS	15%	—	8.00E-05	1.88E-03	88	2.40E-04	1.57E-03	81	8.00E-05	1.90E-03	88	100%
Mixture	15%	60%	7.8	1.9	14	9.75E-04	2.34E-02	88	4.95	1.42	16	64%
Pure HAP	—	60%	878	86.7	6	1.10E-03	1.12E-02	84	810	82.8	6	92%
Pure HA	15%	—	1.40E-04	1.70E-03	85	2.30E-04	1.79E-03	83	1.40E-04	1.73E-03	85	100%
Mixture	15%	60%	175	20.2	7	1.58E-03	1.39E-01	89	149	16.8	6	85%
Pure HAP	—	60%	878	86.7	6	1.10E-03	1.12E-02	84	810	82.8	6	92%