Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Title

Advanced biphasic porous and injectable scaffold displays a fine balance between mechanical strength and remodeling capabilities essential for cartilage regeneration

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Figure S1.

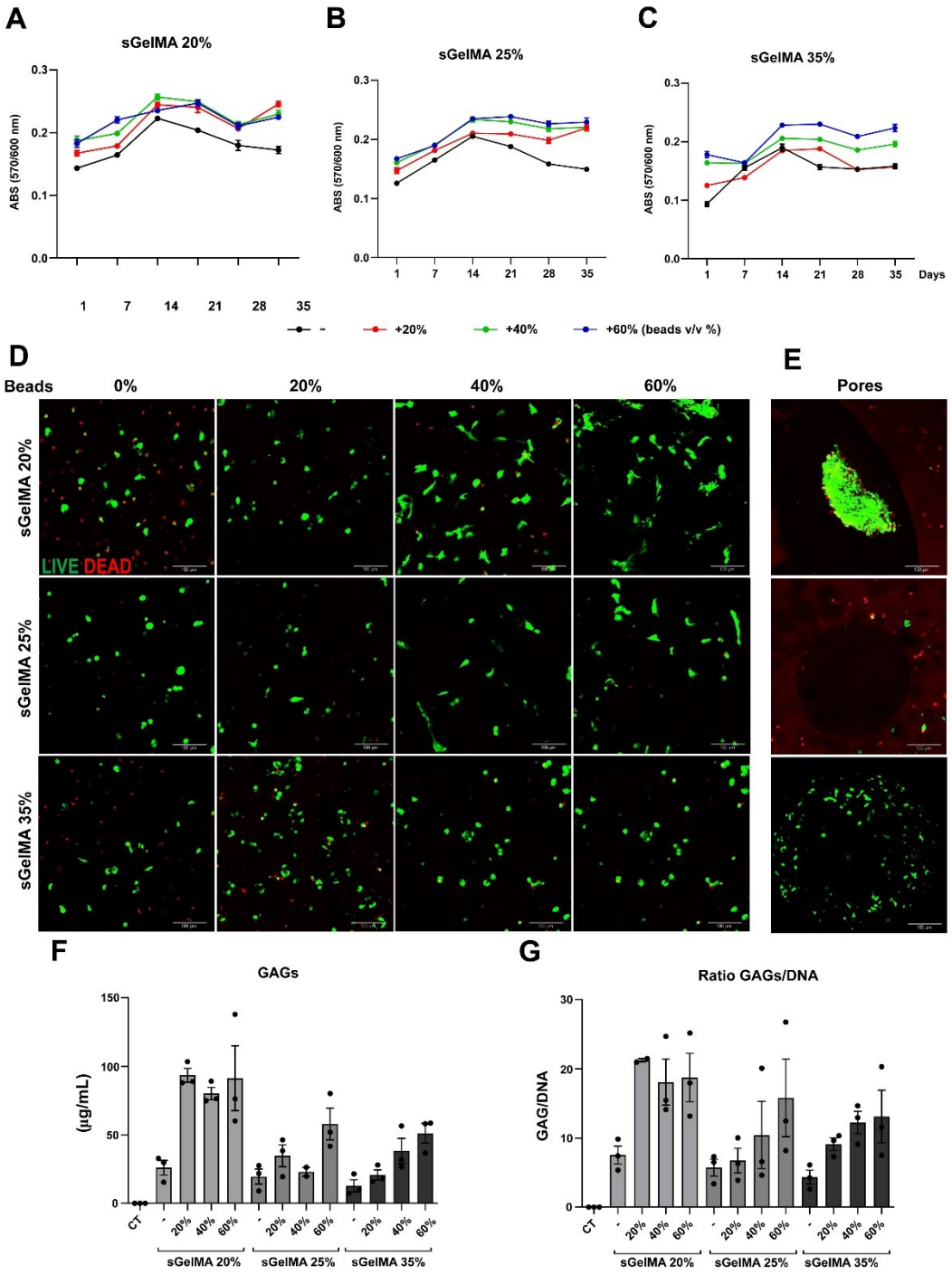

Figure S1. The metabolic activity of NHACk encapsulated in different sGelMA formulations was evaluated with PrestoBlue assay for 5 weeks. A) G20, B) G25, and C) G35 supplemented with 20, 40, and 60% of beads (v/v). D) Viability was evaluated in the same formulations with LIVE/DEAD staining, scale bar= 50 µm. E) Representative images of pores after the differentiation protocol from LIVE/DEAD images, scale bar= 50 µm. F) GAGs quantification after 5 weeks in all the formulations, (CT= empty hydrogels). G) GAGs normalized data concerning total DNA. n= 3 hydrogels for formulation.

Figure S2

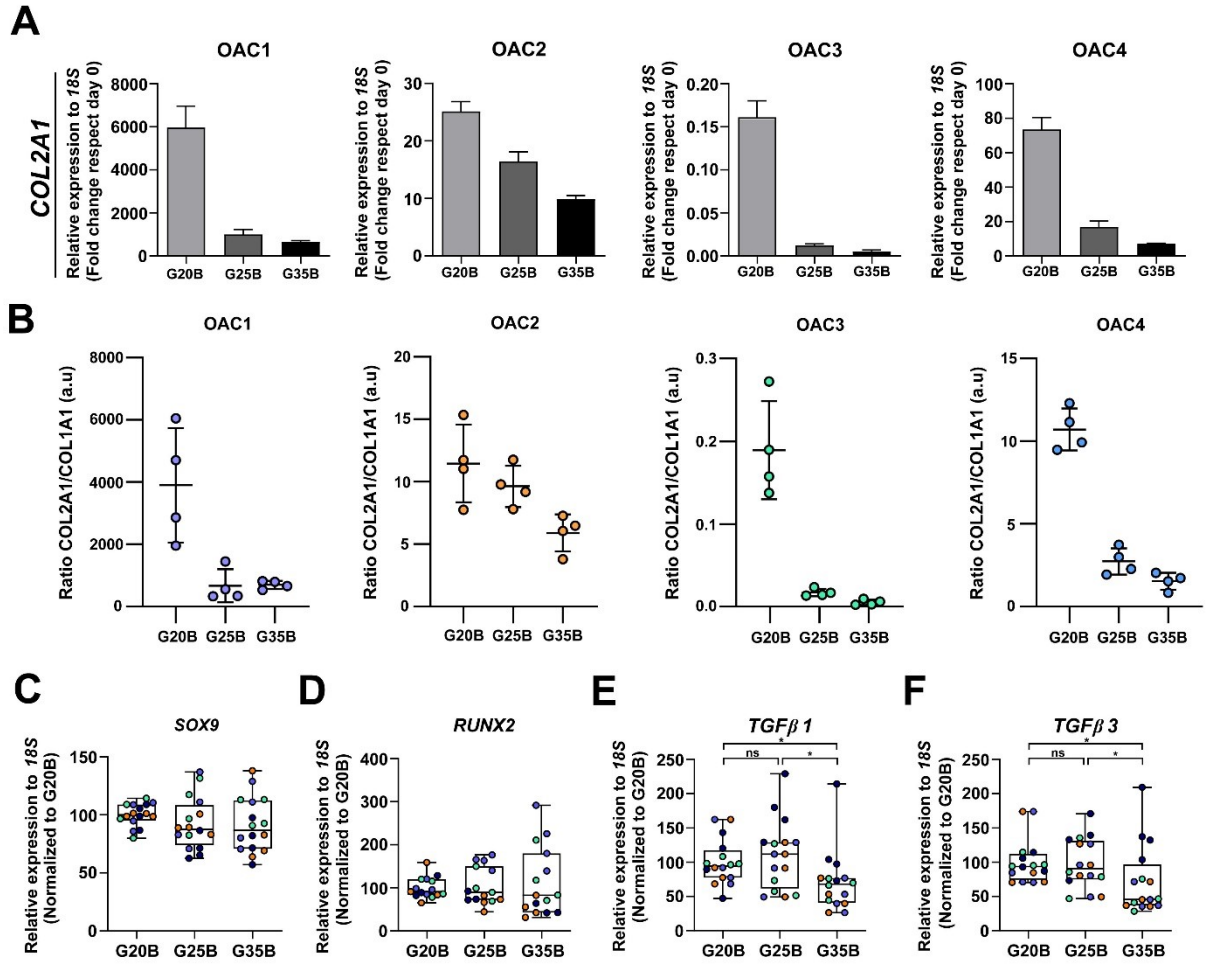

Figure S2. Expression of *COL2A1* in OACs isolated from different donors, fold-change is calculated concerning mRNA isolated from OACs at the moment of the encapsulation, *18S* was used as a housekeeping gene. Additionally, the ratio *COL2A1*/*COL1A1* is shown for each donor (B). Expression of genes of interest in G20B, G25B, and G35B, C) *SOX9*, D) *RUNX2*, E) *TGFβ1* and F) *TGFβ3*. n= 4 donors, n= 4 hydrogels per formulation. The data is shown as mean ± SEM and was analyzed with Kruskal-Wallis test and Dunn's multiple comparisons test, *p < 0.05. The data was normalized relative to G20B.

Figure S3

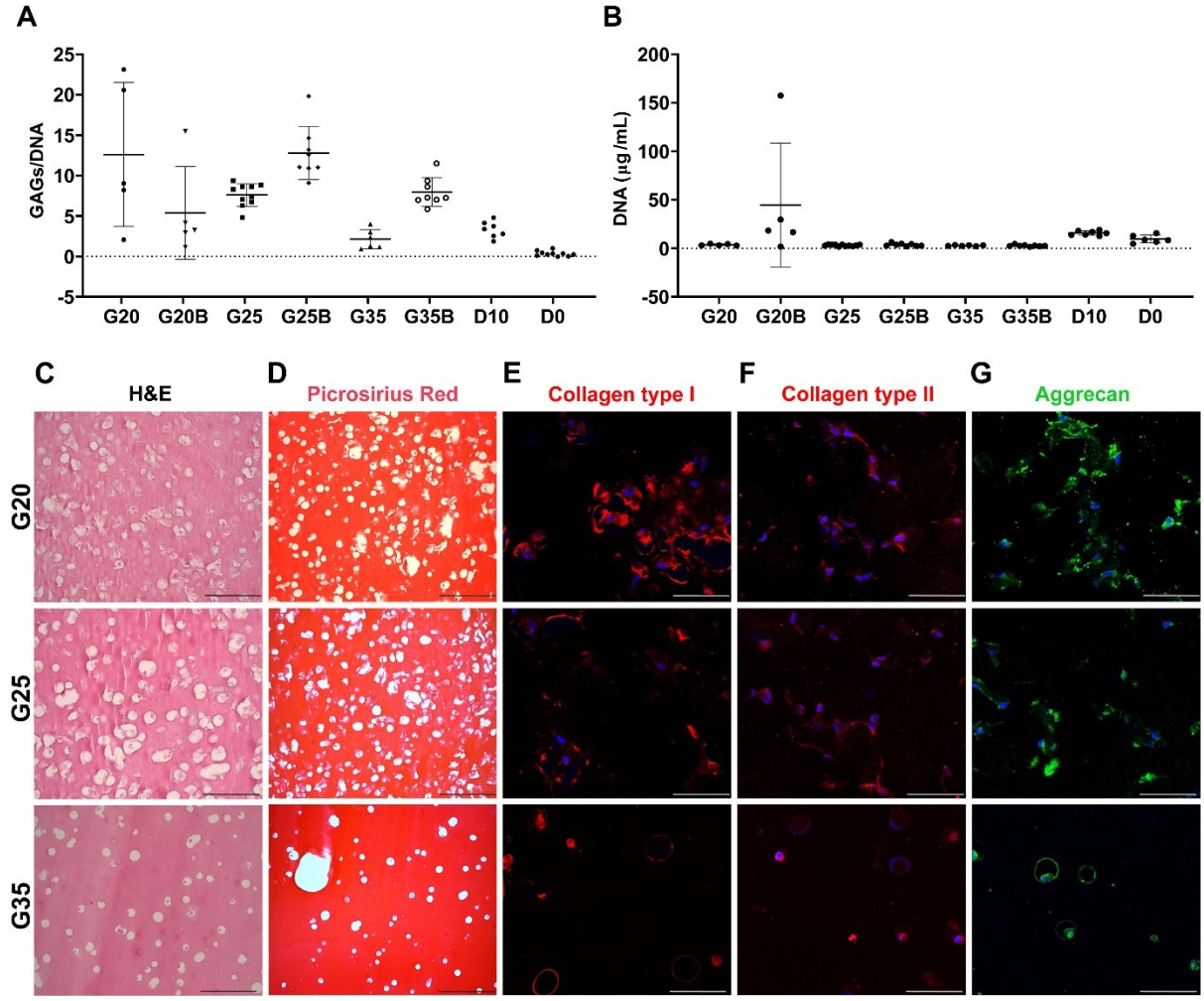

Figure S3. A) GAGs data normalized by total DNA in hydrogels recovered after the in vivo assay. B) Total DNA from hydrogels recovered after the assay. Histological analysis of the non-porous samples after the in vitro and in vivo differentiation: C) H&E, D) Picrosirius Red, E) Collagen type 1, F) Collagen type 2, G) Aggrecan, scale bar= 50 µm.

										OAC4		
									G35B	G20B	G25B	G35B
									13	0.78 ± 0.24	7.87 ± 2.10	6.44 ± 2.21	5.28 ± 1.68
									01	0.01 ± 0.01	73.5 ± 19.4	16.9 ± 9.73	6.95 ± 1.48
									04	0.08 ± 0.06	2.46 ± 0.86	0.73 ± 0.61	0.23 ± 0.03
									18	0.81 ± 0.10	3.45 ± 0.51	2.46 ± 0.38	2.66 ± 0.58
									64	12.3 ± 8.68	1.99 ± 0.22	2.75 ± 0.47	5.64 ± 3.10
COMP	38.7 ± 11.3	21.2 ± 3.85	13.4 ± 3.21	86.9 ± 27.7	34.6 ± 10.3	22.6 ± 8.20	5.33 ± 2.05	2.58 ± 0.56	1.84 ± 0.67	16.3 ± 5.10	8.17 ± 4.66	2.61 ± 0.88	
COL6A1	0.40 ± 0.14	0.38 ± 0.16	0.98 ± 0.28	0.18 ± 0.04	0.16 ± 0.07	0.13 ± 0.03	0.36 ± 0.07	0.34 ± 0.10	0.54 ± 0.20	1.27 ± 0.30	1.64 ± 0.48	2.78 ± 1.16	
COL3A1	2.79 ± 0.49	2.80 ± 0.27	2.14 ± 0.26	9.69 ± 4.68	8.58 ± 2.29	4.67 ± 2.25	3.09 ± 0.45	2.95 ± 0.88	1.90 ± 0.54	5.07 ± 0.63	4.09 ± 1.10	2.81 ± 1.20	
COL10A1	92.7 ± 31.4	106 ± 72.1	176 ± 44.6	202 ± 127	157 ± 63.4	282 ± 131	21.7 ± 5.61	47.0 ± 19.9	88.2 ± 43.7	188 ± 57.1	255 ± 78.1	605 ± 373	
TGFβ1	1.21 ± 0.19	1.19 ± 0.27	1.19 ± 0.12	4.33 ± 1.73	4.12 ± 1.40	2.12 ± 0.87	4.33 ± 1.73	4.12 ± 1.40	2.12 ± 0.87	2.09 ± 0.29	1.61 ± 0.67	1.34 ± 0.28	
TGFβ3	1.03 ± 0.34	1.02 ± 0.29	1.97 ± 0.84	5.98 ± 2.80	5.26 ± 1.84	2.83 ± 1.06	5.98 ± 2.80	5.26 ± 1.84	2.83 ± 1.06	1.94 ± 0.38	1.68 ± 0.69	0.93 ± 0.39	
ADAMTS4	6.47 ± 1.73	9.13 ± 1.36	11.2 ± 1.04	30.0 ± 6.42	22.16 ± 4.22	21.0 ± 3.37	8.44 ± 0.98	12.0 ± 2.37	9.90 ± 3.17	55.7 ± 18.4	64.2 ± 16.8	75.9 ± 21.9	
TIMP1	0.24 ± 0.07	0.15 ± 0.02	0.30 ± 0.08	3.84 ± 1.60	4.75 ± 1.26	3.99 ± 1.25	2.24 ± 0.14	2.36 ± 0.34	1.77 ± 0.61	1.12 ± 0.29	0.92 ± 0.19	0.85 ± 0.12	
TIMP2	0.11 ± 0.04	0.09 ± 0.03	0.21 ± 0.04	1.75 ± 0.84	1.88 ± 0.72	0.98 ± 0.29	0.93 ± 0.11	1.07 ± 0.22	1.01 ± 0.39	0.90 ± 0.19	1.11 ± 0.45	1.04 ± 0.30	
TIMP3	2.93 ± 0.43	2.91 ± 0.88	3.53 ± 0.95	30.2 ± 15.5	34.5 ± 14.5	20.9 ± 4.19	6.15 ± 1.48	6.23 ± 1.57	5.69 ± 2.64	6.80 ± 2.35	6.47 ± 1.83	4.64 ± 1.39	
MMP1	5.55 ± 4.90	15.4 ± 6.85	33.5 ± 8.69	26.6 ± 6.95	70.7 ± 17.6	193 ± 98.06	31.5 ± 8.55	42.8 ± 8.82	47.5 ± 5.16	30.2 ± 6.68	227 ± 63.0	455 ± 117	
MMP3	37.1 ± 7.39	107 ± 41.5	488 ± 196	23.6 ± 7.51	415 ± 98.2	4604 ± 4734	89.8 ± 33.8	229 ± 54.8	150 ± 56.4	117 ± 106	2732 ± 1235	3827± 2078	
MMP13	37.9 ± 14.1	65.2 ± 18.2	79.1 ± 18.16	359 ± 171	316 ± 67.6	369 ± 318	60.8 ± 25.3	63.9 ± 16.7	84.9 ± 46.9	400 ± 190	787 ± 337	886 ± 402	
RUNX2	0.95 ± 0.16	1.57 ± 0.24	2.04 ± 0.89	9.04 ± 3.55	6.01 ± 1.76	4.80 ± 2.00	1.21 ± 0.36	1.23 ± 0.47	1.46 ± 0.72	19.7 ± 5.24	14.6 ± 3.10	8.84 ± 2.46	

mRNA samples (day 35 relative to day 0), for all four OAC
alized to their respective *18S*.
), n= 4 hydrogels for condition.