

RESEARCH ARTICLE

Hydroxyapatite–alginate–gelatin bioinks for bioprinting: Printability, microarchitecture, and dental pulp stem cell viability and morphology

Supplementary File

Table S1. Full experimental design matrix, replicate structure for cell-free scaffold morphometric characterization and printing outcomes

Cell-free bioink composition (%)	Nozzle	Extrusion speed (mm/s)	# of layers	# open pores	Pore size (μm)	Printability index	Pore area (mm ²)	Pore perimeter (mm)
Alg–Gel–HAp(1)	20G	1	2	15	899	1.004	0.470	2.746
Alg–Gel–HAp(1)	20G	1	2	15	950	0.927	0.530	2.805
Alg–Gel–HAp(1)	20G	1	2	16	956	0.973	0.563	2.960
Alg–Gel–HAp(1)	20G	1	4	13	845	0.954	0.406	2.489
Alg–Gel–HAp(1)	20G	1	4	14	802	0.932	0.387	2.403
Alg–Gel–HAp(1)	20G	1	4	12	843	0.925	0.432	2.527
Alg–Gel–HAp(1)	20G	2	2	16	976	0.943	0.581	2.960
Alg–Gel–HAp(1)	20G	2	2	16	969	0.964	0.531	2.861
Alg–Gel–HAp(1)	20G	2	2	16	1,044	0.948	0.618	3.062
Alg–Gel–HAp(1)	20G	2	4	12	875	0.973	0.458	2.672
Alg–Gel–HAp(1)	20G	2	4	10	928	0.966	0.523	2.841
Alg–Gel–HAp(1)	20G	2	4	11	909	0.908	0.509	2.719
Alg–Gel–HAp(1)	20G	3	2	16	1,120	1.008	0.660	3.262
Alg–Gel–HAp(1)	20G	3	2	16	1,052	0.965	0.674	3.227
Alg–Gel–HAp(1)	20G	3	2	16	999	0.964	0.578	2.985
Alg–Gel–HAp(1)	20G	3	4	11	924	0.981	0.517	2.850
Alg–Gel–HAp(1)	20G	3	4	12	909	0.964	0.451	2.637
Alg–Gel–HAp(1)	20G	3	4	12	903	0.923	0.491	2.691
Alg–Gel–HAp(1)	22G	1	2	16	1,132	0.907	0.783	3.372
Alg–Gel–HAp(1)	22G	1	2	16	1,112	0.891	0.778	3.330
Alg–Gel–HAp(1)	22G	1	2	16	1,192	0.896	0.895	3.582
Alg–Gel–HAp(1)	22G	1	4	N.A.	N.A.	N.A.	N.A.	N.A.
Alg–Gel–HAp(1)	22G	1	4	N.A.	N.A.	N.A.	N.A.	N.A.
Alg–Gel–HAp(1)	22G	1	4	16	1,220	0.946	0.852	3.592
Alg–Gel–HAp(1)	22G	2	2	16	1,015	0.933	0.608	3.013
Alg–Gel–HAp(1)	22G	2	2	16	1,147	0.928	0.792	3.430
Alg–Gel–HAp(1)	22G	2	2	16	1,144	0.897	0.817	3.423
Alg–Gel–HAp(1)	22G	2	4	N.A.	N.A.	N.A.	N.A.	N.A.

(cont'd...)

Table S1. (Continued)

Cell-free bioink composition (%)	Nozzle	Extrusion speed (mm/s)	# of layers	# open pores	Pore size (µm)	Printability index	Pore area (mm ²)	Pore perimeter (mm)
Alg-Gel-HAp(1)	22G	2	4	N.A.	N.A.	N.A.	N.A.	N.A.
Alg-Gel-HAp(1)	22G	2	4	15	1,102	0.923	0.654	3.107
Alg-Gel-HAp(1)	22G	3	2	16	1,185	0.930	0.644	3.095
Alg-Gel-HAp(1)	22G	3	2	16	1,078	0.932	0.707	3.246
Alg-Gel-HAp(1)	22G	3	2	16	1,095	0.944	0.729	3.318
Alg-Gel-HAp(1)	22G	3	4	N.A.	N.A.	N.A.	N.A.	N.A.
Alg-Gel-HAp(1)	22G	3	4	N.A.	N.A.	N.A.	N.A.	N.A.
Alg-Gel-HAp(1)	22G	3	4	16	1,267	0.941	0.929	3.740
Alg-Gel-HAp(2)	20G	1	2	14	988	0.874	0.608	2.914
Alg-Gel-HAp(2)	20G	1	2	14	1,001	0.881	0.613	2.939
Alg-Gel-HAp(2)	20G	1	2	14	956	0.887	0.540	2.767
Alg-Gel-HAp(2)	20G	1	4	9	917	0.946	0.454	2.622
Alg-Gel-HAp(2)	20G	1	4	8	1,030	0.980	0.558	2.959
Alg-Gel-HAp(2)	20G	1	4	8	907	0.931	0.443	2.569
Alg-Gel-HAp(2)	20G	2	2	16	1,056	1.011	0.600	3.116
Alg-Gel-HAp(2)	20G	2	2	15	908	0.853	0.511	2.640
Alg-Gel-HAp(2)	20G	2	2	13	965	0.875	0.558	2.794
Alg-Gel-HAp(2)	20G	2	4	8	875	0.928	0.426	2.516
Alg-Gel-HAp(2)	20G	2	4	8	829	0.911	0.386	2.371
Alg-Gel-HAp(2)	20G	2	4	8	905	0.899	0.468	2.594
Alg-Gel-HAp(2)	20G	3	2	16	1,005	0.886	0.586	2.882
Alg-Gel-HAp(2)	20G	3	2	13	907	0.868	0.486	2.597
Alg-Gel-HAp(2)	20G	3	2	15	943	0.876	0.518	2.695
Alg-Gel-HAp(2)	20G	3	4	8	879	0.902	0.437	2.511
Alg-Gel-HAp(2)	20G	3	4	8	927	0.891	0.492	2.649
Alg-Gel-HAp(2)	20G	3	4	8	951	0.930	0.485	2.686
Alg-Gel-HAp(2)	22G	1	2	16	1,226	0.915	0.876	3.582
Alg-Gel-HAp(2)	22G	1	2	16	1,142	0.910	0.767	3.341
Alg-Gel-HAp(2)	22G	1	2	16	1,005	0.874	0.595	2.885
Alg-Gel-HAp(2)	22G	1	4	16	1,017	0.885	0.616	2.955
Alg-Gel-HAp(2)	22G	1	4	15	978	0.882	0.551	2.788
Alg-Gel-HAp(2)	22G	1	4	16	1,107	0.893	0.718	3.203
Alg-Gel-HAp(2)	22G	2	2	16	1,202	0.906	0.862	3.534
Alg-Gel-HAp(2)	22G	2	2	16	1,248	0.928	0.908	3.671
Alg-Gel-HAp(2)	22G	2	2	15	1,102	0.923	0.654	3.107
Alg-Gel-HAp(2)	22G	2	4	14	999	0.975	0.510	2.821
Alg-Gel-HAp(2)	22G	2	4	13	1,075	0.899	0.665	3.095
Alg-Gel-HAp(2)	22G	2	4	15	1,158	0.885	0.794	3.353

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Table S1. (Continued)

Cell-free bioink composition (%)	Nozzle	Extrusion speed (mm/s)	# of layers	# open pores	Pore size (μm)	Printability index	Pore area (mm^2)	Pore perimeter (mm)
Alg-Gel-HAp(2)	22G	3	2	16	1,165	0.939	0.790	3.445
Alg-Gel-HAp(2)	22G	3	2	16	1,316	0.931	0.978	3.817
Alg-Gel-HAp(2)	22G	3	2	13	1,080	0.904	0.663	3.097
Alg-Gel-HAp(2)	22G	3	4	16	1,163	0.883	0.823	3.409
Alg-Gel-HAp(2)	22G	3	4	16	1,025	0.885	0.613	2.946
Alg-Gel-HAp(2)	22G	3	4	16	1,226	0.915	0.876	3.582

Notes: N.A.: Not Applicable. Structural integrity and pore geometry could not be reliably quantified.

Table S2. ANOVA analysis for printability results

Response variable	Factor	F	p-value	ω^2	Sig.
Open pores	HAp Level	55.17	<0.001	0.003	***
Open pores	Nozzle	228.92	<0.001	0.011	***
Open pores	Extrusion Speed	1.76	0.185	<0.001	ns
Open pores	Num. Layers	204.63	<0.001	0.010	***
Pore size (μm)	HAp Level	1.25	0.269	<0.001	ns
Pore size (μm)	Nozzle	209.21	<0.001	0.009	***
Pore size (μm)	Extrusion Speed	3.27	0.048	<0.001	*
Pore size (μm)	Num. Layers	19.83	<0.001	0.001	***
Printability index	HAp Level	21.40	<0.001	<0.001	***
Printability index	Nozzle	3.78	0.058	<0.001	ns
Printability index	Extrusion Speed	0.10	0.902	<0.001	ns
Printability index	Num. Layers	0.49	0.489	<0.001	ns
Pore area (mm^2)	HAp Level	2.50	0.122	<0.001	ns
Pore area (mm^2)	Nozzle	202.50	<0.001	0.034	***
Pore area (mm^2)	Extrusion Speed	3.01	0.060	0.001	ns
Pore area (mm^2)	Num. Layers	27.80	<0.001	0.005	***
Pore perimeter (mm)	HAp Level	7.84	0.008	<0.001	**
Pore perimeter (mm)	Nozzle	176.05	<0.001	0.008	***
Pore perimeter (mm)	Extrusion Speed	2.62	0.084	<0.001	ns
Pore perimeter (mm)	Num. Layers	26.65	<0.001	0.001	***

Notes: Effect sizes reported as omega-squared (ω^2); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant; $n = 66$ observations.

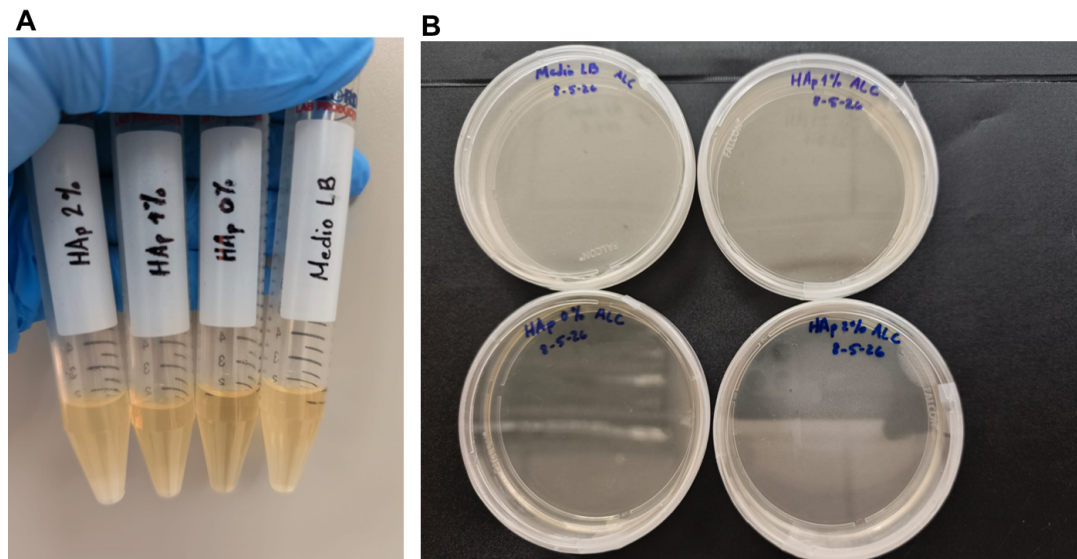


Figure S1. Microbiological sterility assessment of composite biomaterial formulations after UV irradiation. (A) Visual appearance of liquid Lysogeny Broth (LB) medium inoculated with 0% (control), 1%, and 2% HAp formulations after 72h of incubation at 37°C, demonstrating medium translucency and no macroscopic turbidity. (B) Representative macrographs of solid LB-agar plates showing no visible colony-forming units after 72 h, supporting the efficacy of the sterilization protocol and aseptic handling prior to cell encapsulation.