

## 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 <sup>2</sup> ) | 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.                |

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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 <sup>2</sup> ) | 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)

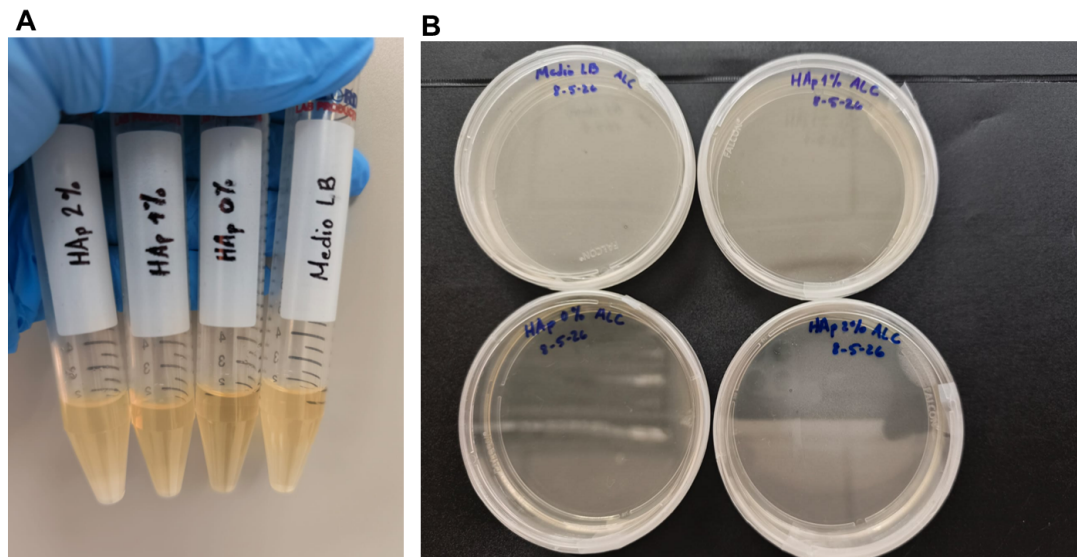
| Cell-free bioink composition (%) | Nozzle | Extrusion speed (mm/s) | # of layers | # open pores | Pore size (μm) | Printability index | Pore area (mm <sup>2</sup> ) | Pore perimeter (mm) |
|----------------------------------|--------|------------------------|-------------|--------------|----------------|--------------------|------------------------------|---------------------|
| 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 <sup>2</sup> | 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 <sup>2</sup> ) | HAp Level       | 2.50   | 0.122   | <0.001         | ns   |
| Pore area (mm <sup>2</sup> ) | Nozzle          | 202.50 | <0.001  | 0.034          | ***  |
| Pore area (mm <sup>2</sup> ) | Extrusion Speed | 3.01   | 0.060   | 0.001          | ns   |
| Pore area (mm <sup>2</sup> ) | 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<sup>2</sup>); \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , 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.