

RESEARCH ARTICLE

Replication of an intervertebral disc using an in-house bioprinter:
A proof-of-concept study

Supplementary File

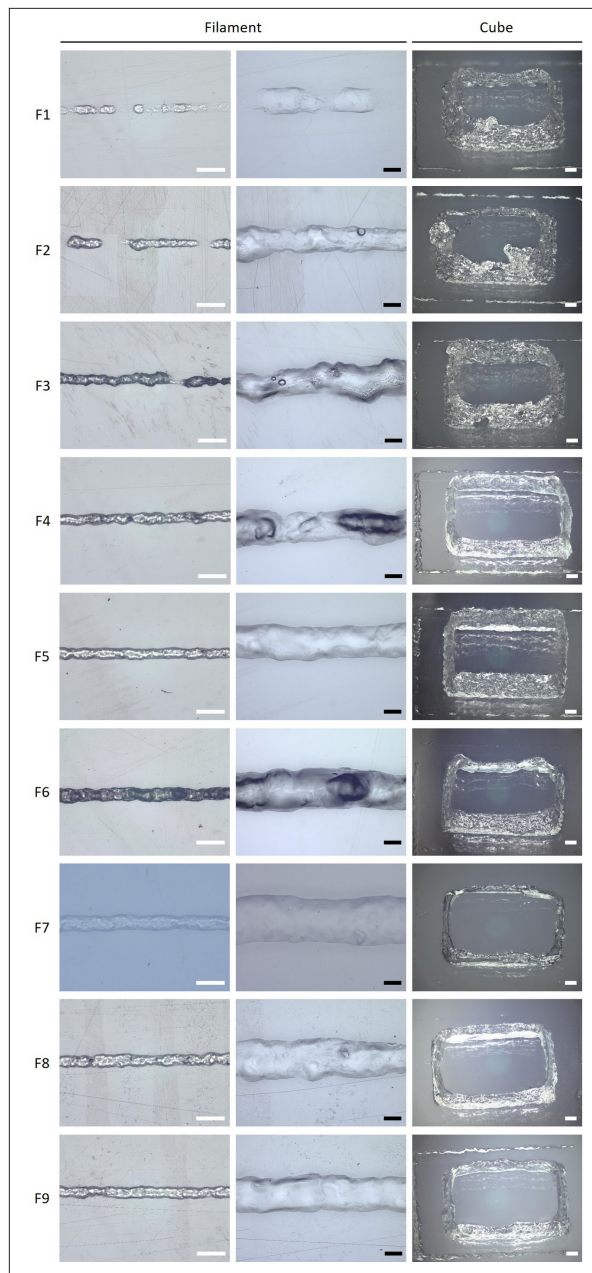


Figure S1. Representative images of filaments and cubes bioprinted with different ink formulations. Nine formulations of alginate, gelatin, and hyaluronic acid were extruded from the needle at 20 kPa and with a printing speed of 10 mm/s. The concentration of each component is presented in **Table 1**. Scale bars: 1 mm (white); 200 μ m (black).

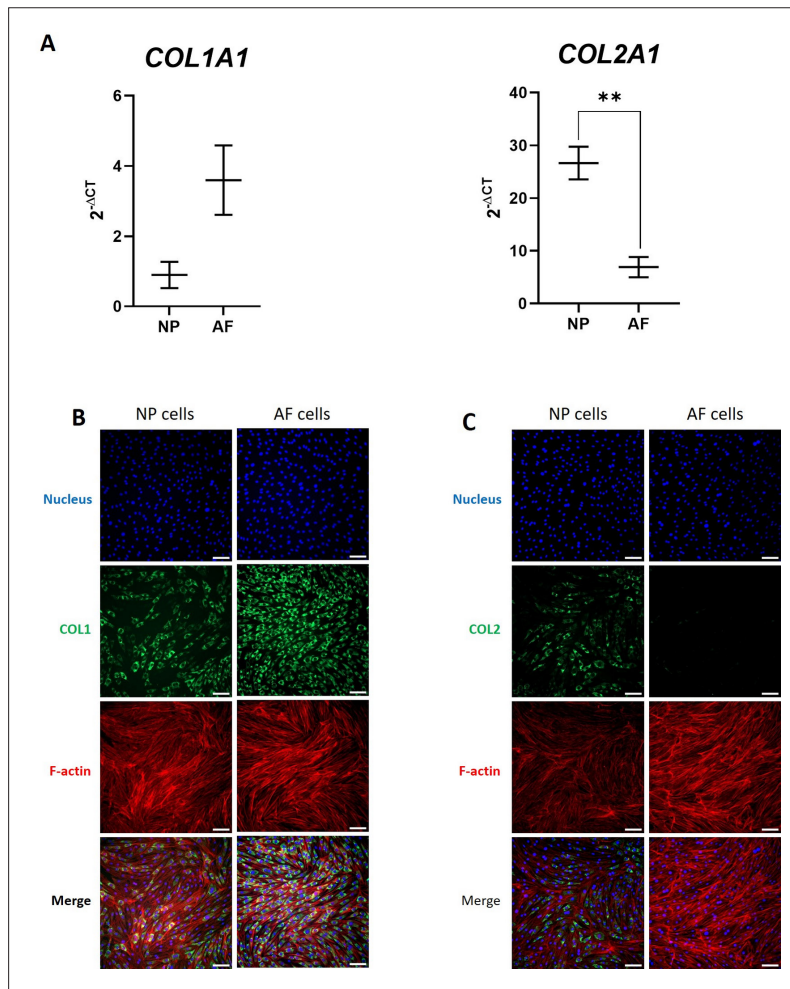


Figure S2. Expression of collagen type 1 and type 2 in ovine *nucleus pulposus* (NP) and *annulus fibrosus* (AF). (A) Gene expression in NP and AF cells. (B and C) Representative images of collagen type 1 (B) and collagen type 2 (C) expression by NP and AF cells. Scale bars: 100 μ m. Results are expressed as the mean \pm standard error of the mean ($n = 3$ independent experiments). $**p < 0.01$ indicates a significant difference.

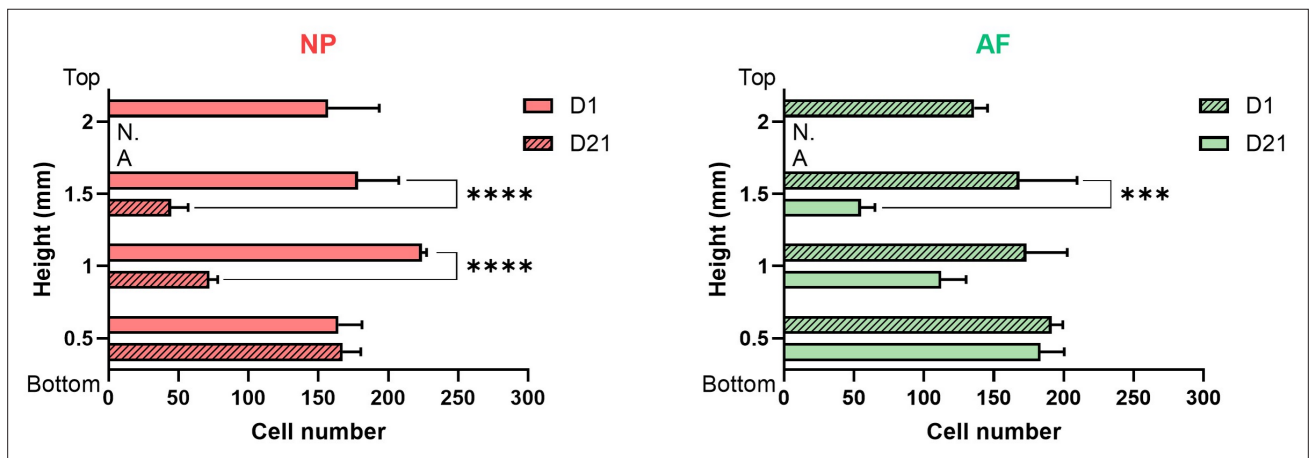


Figure S3. Distribution of *nucleus pulposus* (NP; left) and *annulus fibrosus* (AF; right) cells in the bioprinted intervertebral disc constructs on days 1 and 21. Results are expressed as the mean \pm standard deviation ($n = 3$ experimental replicates). $***p < 0.001$; $****p < 0.0001$. Abbreviation: N.A., not applicable.

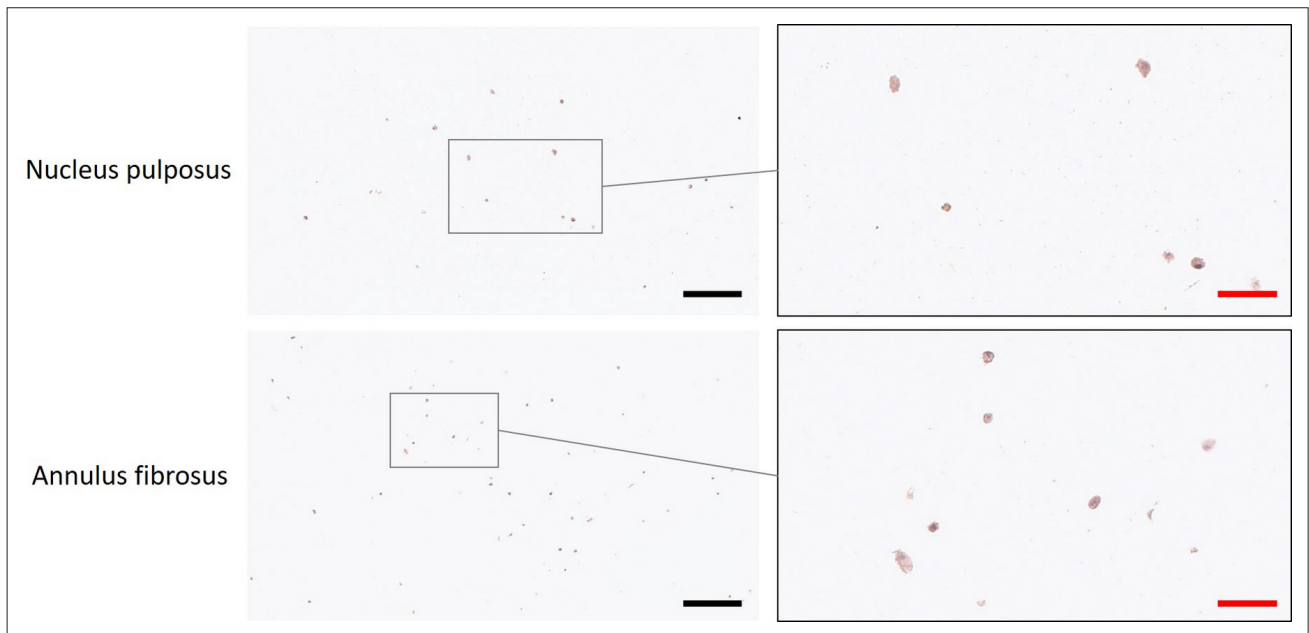


Figure S4. Negative control of collagen type 1 immunohistochemistry of intervertebral disc (IVD) constructs cultured for 21 days. Scale bars: 200 μm (black); 50 μm (red).

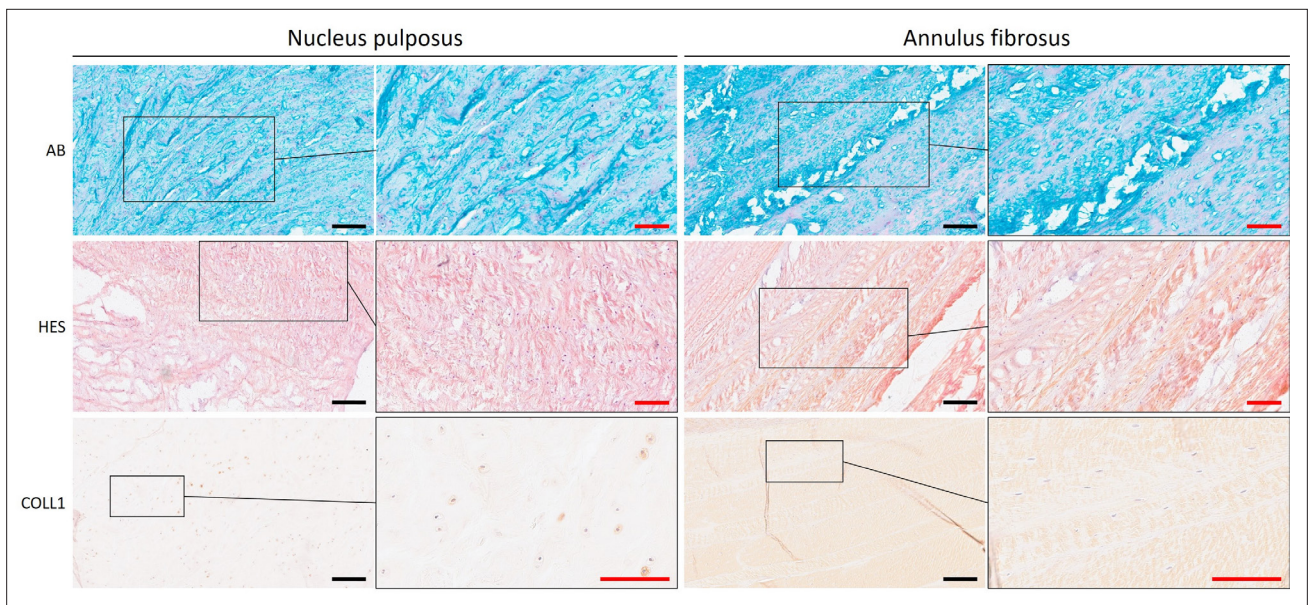


Figure S5. Histological analysis of a sheep intervertebral disc (IVD). Representative images of Alcian blue (AB; top row), hematoxylin-eosin-saffron (HES; middle row), and collagen type 1 (COL1; bottom row) staining of *nucleus pulposus* and *annulus fibrosus*. Scale bars: 200 μm (black); 100 μm (red).