ACCSCIENCE PUBLISHING

International Journal of Bioprinting

RESEARCH ARTICLE

Optimization of 3D bioprinting of mouse preosteoblasts using nanofibrillated cellulose hydrogels

Supplementary file

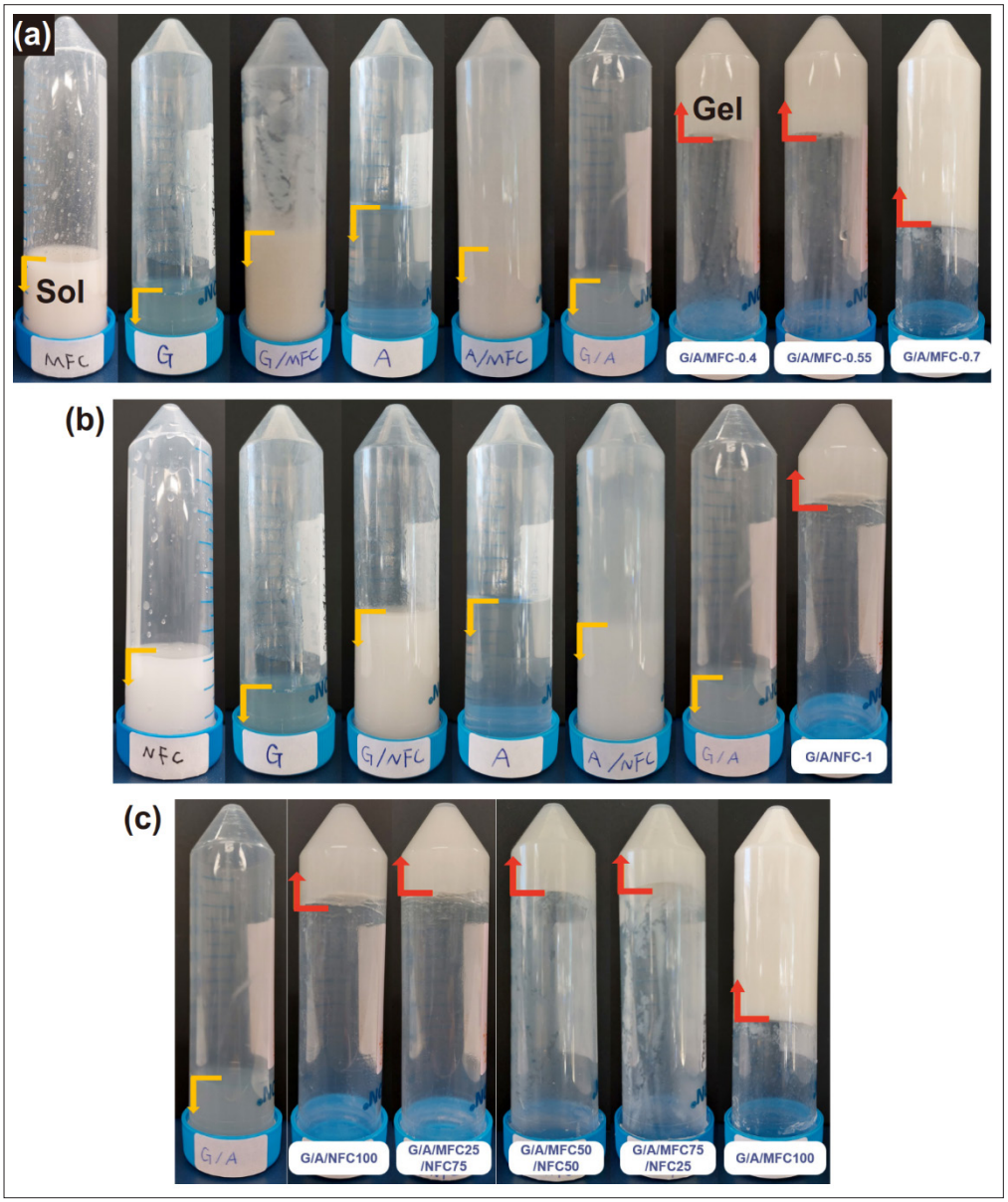

Figure S1. Images of the benchmarks for cellulose-composited G/A formulations inverted for 1 h. All benchmarks flow to the opposite end of the sample tube, demonstrating a sol-like state. Abbreviation: G/A: Gelatin methacryloyl/alginate; NFC: Nanofibrillated cellulose.

Figure S2. Line width (mm) of G/A/MFC100 filaments extruded from a 27G nozzle at different printing speeds and dispensing pressures. Abbreviations: G/A: Gelatin methacryloyl/alginate; MFC: Microfibrillated cellulose.

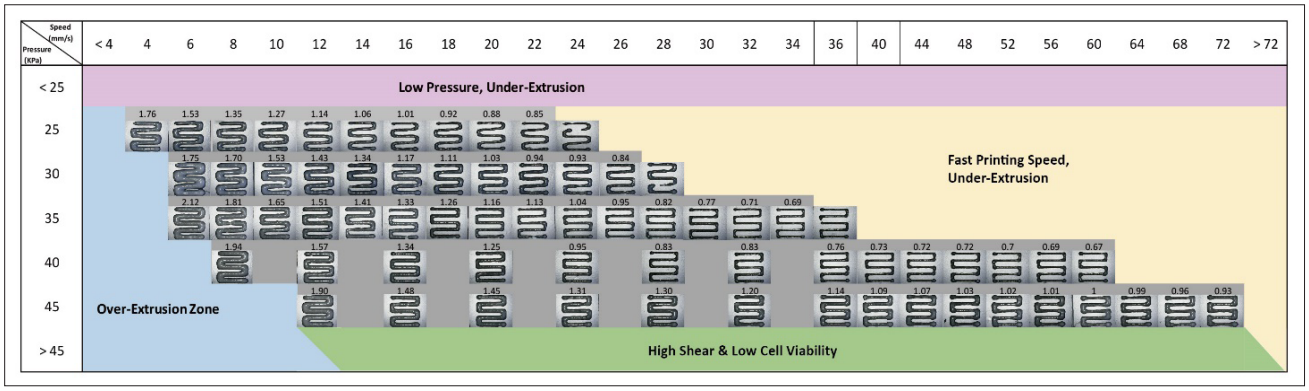

Figure S3. Line width (mm) of G/A/MFC100 filaments extruded from a nozzle of 22G at different printing speeds and dispensing pressures. Abbreviations: G/A: Gelatin methacryloyl/alginate; MFC: Microfibrillated cellulose.

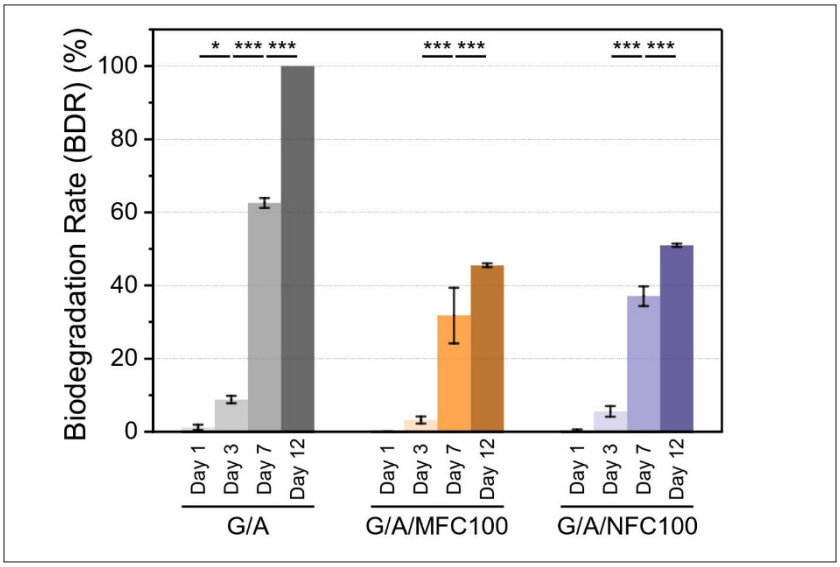

Figure S4. Biodegradation rates (BDR) of G/A, G/A/MFC100, and G/A/NFC100 after 1, 3, 7, and 12 days of immersion in 1× dPBS with 5 U/mL collagenase type II. Either MFC or NFC significantly prolongs the biodegradation of ICE hydrogels. BDR of G/A/NFC100 is slightly faster than that of G/A/MFC100. Abbreviations: dPBS: Dulbecco's phosphate-buffered saline; G/A: Gelatin methacryloyl/alginate; ICE: Ion–covalent entanglement; MFC: Microfibrillated cellulose; NFC: Nanofibrillated cellulose.

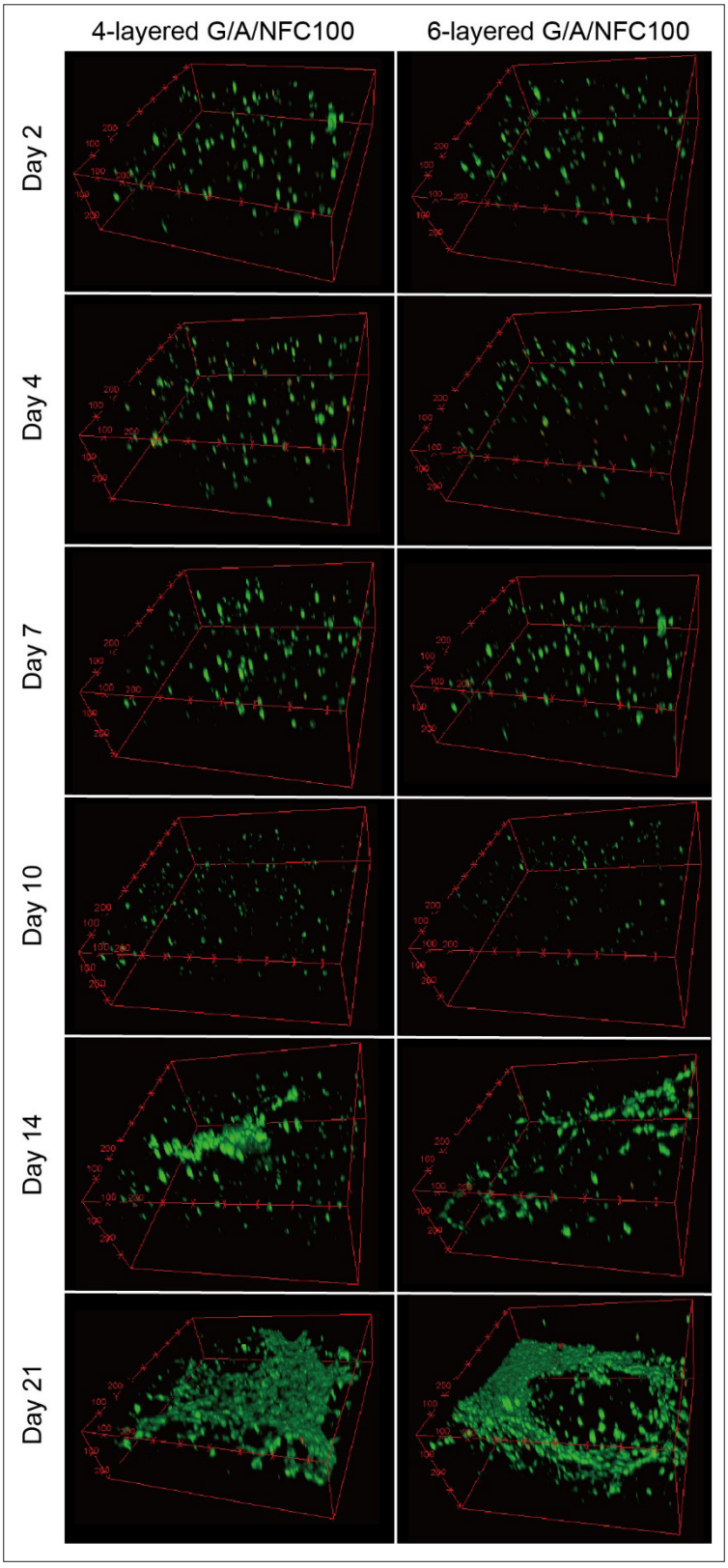

Figure S5. Three-dimensional view images of cellular morphology in 4- and 6-layered G/A/NFC100 scaffolds over 21 days. The minimum scale on the coordinate axis is 100 **μm**. Abbreviations: G/A: Gelatin methacryloyl/alginate; NFC: Nanofibrillated cellulose.

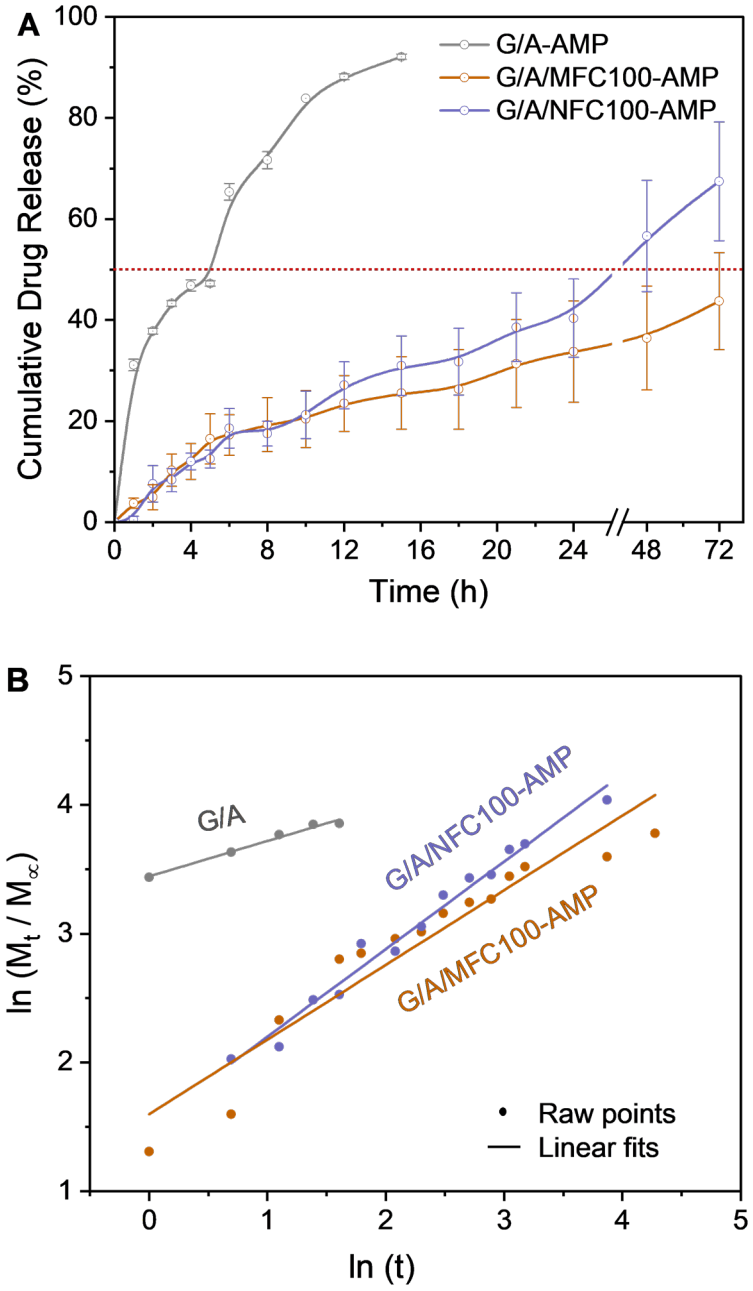

Figure S6. (A) Representative curves of cumulative drug released from G/A-AMP, G/A/MFC100-AMP, and G/A/NFC100-AMP hydrogel discs over time. (B) Linear fits of the first 60% cumulative drug release according to **Equation IX**. Abbreviations: AMP: Ampicillin sodium salt; G/A: Gelatin methacryloyl/alginate; MFC: Microfibrillated cellulose; NFC: Nanofibrillated cellulose.

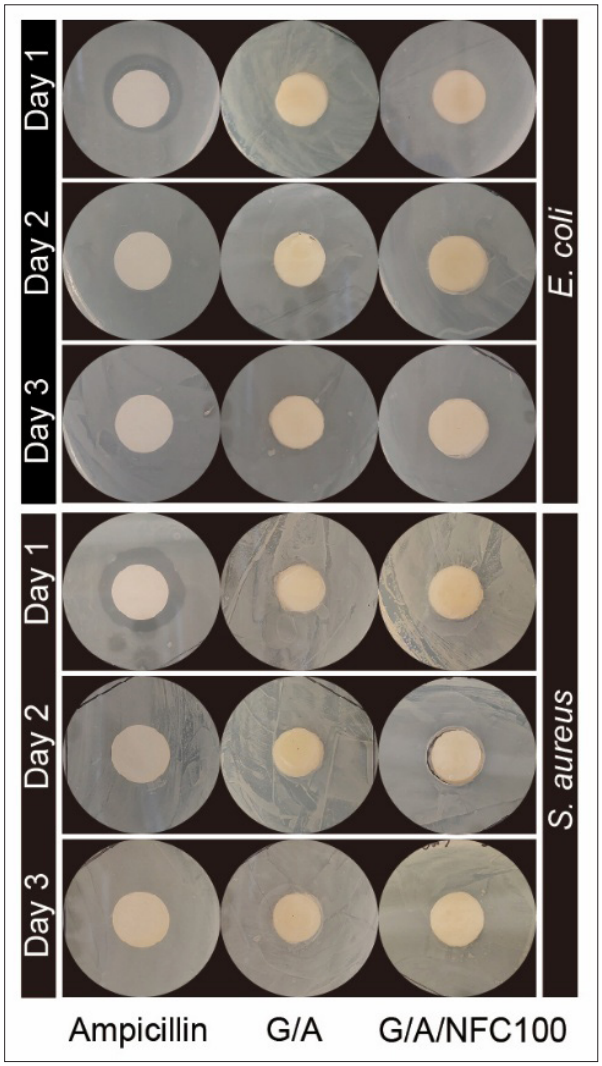

Figure S7. Images of inhibitory zones for positive (ampicillin-loaded filter paper discs) and negative (G/A and G/A/NFC100) controls against *E. coli* and *S. aureus* over incubation of 3 days in agar disc diffusion assay. Abbreviations: G/A: Gelatin methacryloyl/alginate; NFC: Nanofibrillated cellulose.

Supplementary Videos

Video S1. Mechanical stability of the square structure in three consecutive press-relax cycles.
Video S2. Mechanical stability of the five-pointed star structure in three consecutive press-relax cycles.
Video S3. Mechanical stability of the tubular structure in three consecutive press-relax cycles.