ACCSCIENCE PUBLISHING

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RESEARCH ARTICLE

Dexamethasone-loaded PLGA porous microspheres used as 3D-bioprinted bioink promote tissue-engineered cartilage regeneration

Supplementary File

To assess the printability of each bioink, the experiment was conducted under controlled room temperature (20–22°C), with specific nozzle and platform temperatures set at 19 and 23°C, respectively, using a 400 μm needle for extrusion. The extrusion pressure was meticulously adjusted until achieving smooth filamentous extrusion. The evaluation of the constructs was conducted in the aspects of interlayer adhesion, shape stability, surface smoothness, and construct morphology (**Figure S1**). It was observed that all five groups of constructs retained their intended shapes effectively. Following the printing of six layers, there were no instances of collapse or local deformations noted among the constructs. The interlayer connections demonstrated tightness, with no occurrence of sliding or detachment throughout the printing process. Additionally, it was noted that the PLGA-dex15 MPs@GelMA group exhibited rough extrusion lines characterized by irregular edges and void structures within the constructs. From the 3D printing of the second layer, both the GelMA control group and the PLGA-dex60 MPs@GelMA group were capable of extruding filamentous structures and completing the printing process. However, the uniformity of extrusion lines and the clarity of construct edges were poor, and they also failed to accurately form a 10 mm × 10 mm cross-section. In contrast, the PLGA-dex0 MPs@GelMA and PLGA-dex30 MPs@GelMA groups demonstrated the ability to produce sharp square cross-sections on the second, fourth, and sixth layers, featuring more uniform and smooth extrusion lines. The surface of the lines appeared smoother, with voids in the mesh printing structure distributed more evenly and regularly, resulting in more consistent rectangular shapes. As a result, it was concluded that constructs composed of PLGA-dex0 MPs and PLGA-dex30 MPs bioinks exhibited superior printability through extrusion-based 3D bioprinting.

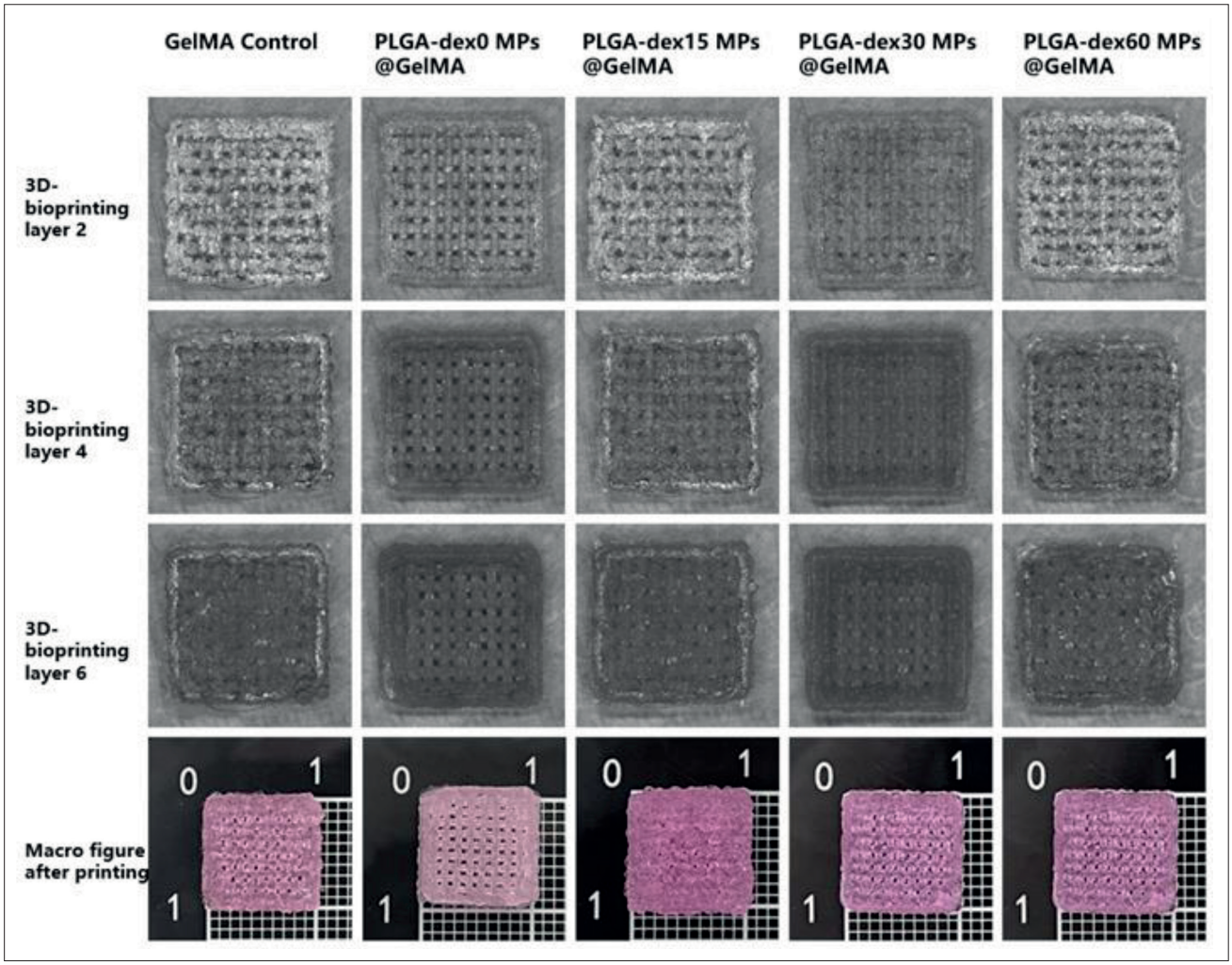

Figure S1. Images of constructs captured immediately after 3D bioprinting.

The live/dead staining of cells cultured post-bioprinting using the five bioinks is shown in Figure S2A. The average optical density (AOD) values of green fluorescence indicating live cells were subjected to statistical analysis (Figure S2B), which revealed slight variations among the five groups. However, no statistically significant differences in fluorescence intensity were observed ($p > 0.05$).

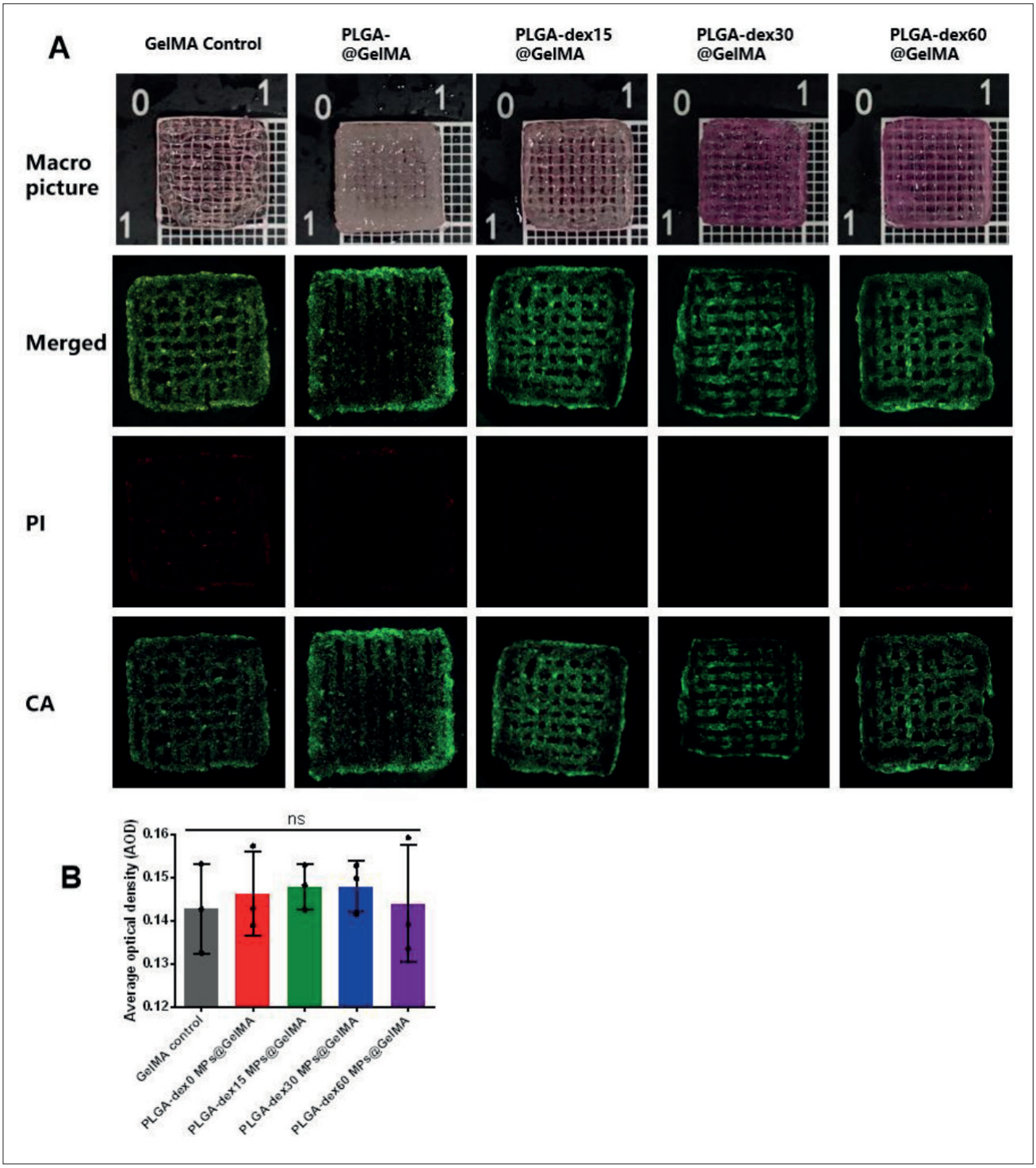

Figure S2. Live/dead staining reveals consistent cell viability across five bioinks. (A) Calcein AM/PI staining of 3D-printed scaffolds cultured *in vitro* for 24 h (green fluorescence represents live cells; red fluorescence represents dead cells). (B) Statistics of average optical density (AOD) values of live cells (indicated by green fluorescence). Abbreviations: CA, calcein acetoxymethyl ester; PI, propidium Iodide.