

RESEARCH ARTICLE

Three-dimensional bioprinting of gelatin methacryloyl hydrogels with a tri-layered vascularized architecture for full-thickness skin regeneration

Supplementary file

Supplementary method

For cell-laden printing, human umbilical vein endothelial cells (HUVECs) ($1 \times 10^6/\text{mL}$) were loaded into gelatin methacryloyl (GelMA) at concentrations of 3% (w/v), 5% (w/v), and 7% (w/v) and printed into 6-well plates. After printing, the samples were exposed to 405 nm blue light for 60 s for crosslinking, followed by the addition of culture medium and incubation. The culture medium was replaced every 3 days. Images of the samples were taken at Days 0, 3, 6, and 9 post-printing.

Supplementary result

As shown in **Figure S1**, on Day 3, HUVECs in the 3% (w/v) GelMA hydrogel group began to extend and elongate, while cells in the 5% (w/v) and 7% (w/v) GelMA hydrogel groups remained spherical. By Day 6, the 3% (w/v) GelMA hydrogel group exhibited significant microvascular formation, whereas the 5% (w/v) and 7% (w/v) GelMA hydrogel groups showed no noticeable cell extension. On Day 9, the 3% (w/v) GelMA hydrogel group demonstrated extensive microvascular formation, the 5% (w/v) GelMA hydrogel group showed slight cell elongation, and the 7% (w/v) GelMA hydrogel group still displayed no significant changes. In summary, the 3% (w/v) GelMA hydrogel is more suitable for culturing HUVECs.

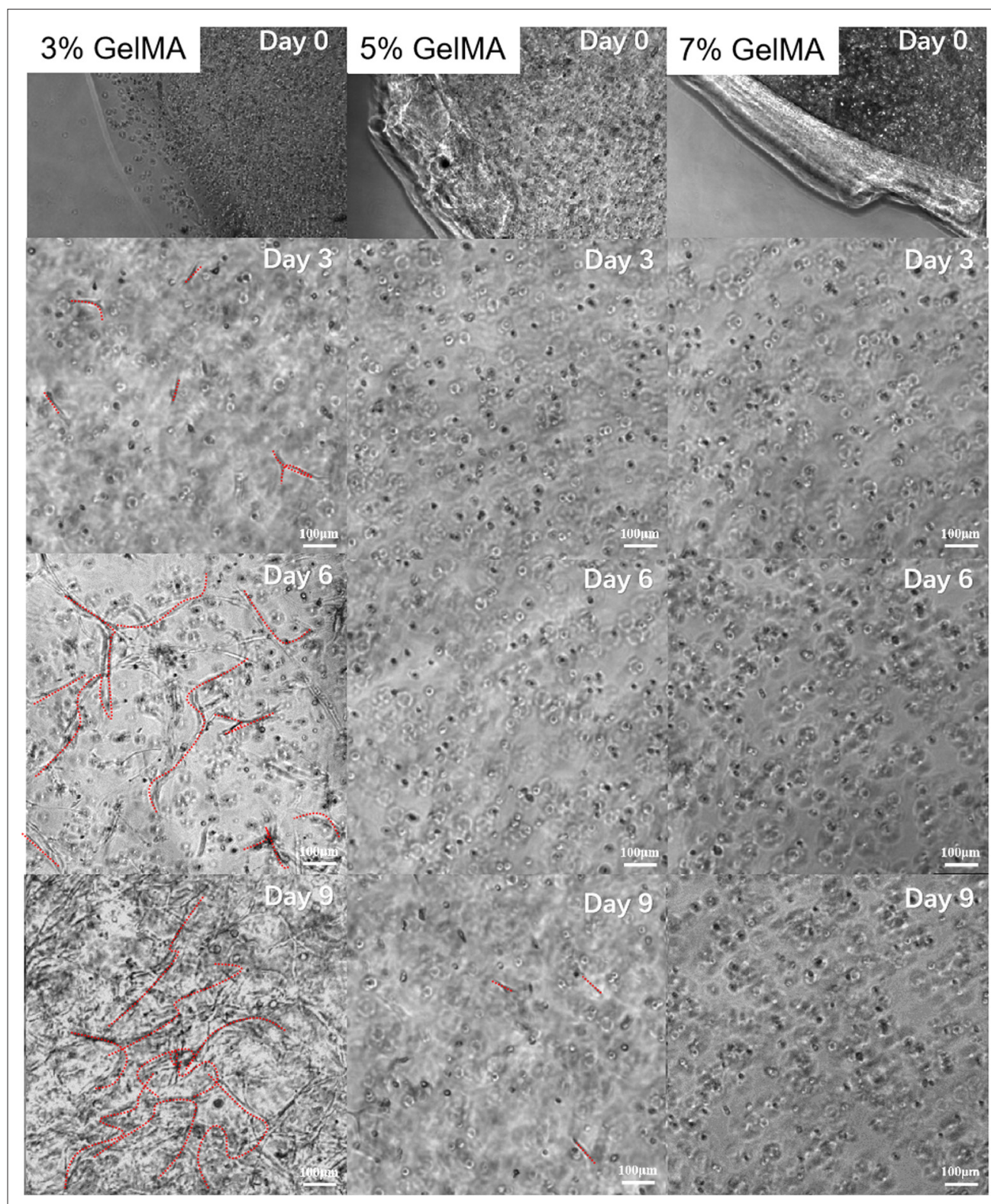


Figure S1. Bright-field images showing the morphology of the human umbilical vein endothelial cells cultured in 3% (w/v), 5% (w/v), and 7% (w/v) gelatin methacryloyl (GelMA) hydrogels at Days 0, 3, 6, and 9. The red dashed lines mark areas where partial cell extension and interconnected microvascular structures are observed. Scale bars: 100 μm; magnification: 40x.