

RESEARCH ARTICLE

Effect of tunable stiffness on immune responses in 3D-bioprinted alginate-gelatin scaffolds

Supplementary file



Figure S1. (A) *In vitro* Live/Dead assay of macrophage cultured on scaffolds at day 1; live cells were stained green, and dead cells were red. (B) *In vitro* Live/Dead assay of macrophage cultured on scaffolds at day 7; live cells were stained green, and dead cells were red; viewed with $50 \times$ magnification; scale bar = 500μ m.



Figure S2. (A) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 1; iNOS (red) and DAPI (blue). (B) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 1; TNF- α (red) and DAPI (blue). (C) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 1; Arg1 (red) and DAPI (blue). (D) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 1; Arg1 (red) and DAPI (blue). (D) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 1; Arg1 (red) and DAPI (blue). (D) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 1; Hz-10 (red) and DAPI (blue); viewed with 400× magnification; scale bar = 100 µm.



Figure S3. (A) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 3; iNOS (red) and DAPI (blue); (B) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 3; TNF- α (red) and DAPI (blue). (C) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 3; Arg1 (red) and DAPI (blue). (D) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 3; Hrg1 (red) and DAPI (blue). (D) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 3; Hrg1 (red) and DAPI (blue). (D) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 3; Hrg1 (red) and DAPI (blue). (D) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 3; Hrg1 (red) and DAPI (blue). (D) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 3; Hrg1 (red) and DAPI (blue). (D) *In vitro* immunofluorescence imaging of macrophages polarization cultured on scaffolds at day 3; Hrg1 (red) and DAPI (blue); viewed with 400× magnification; scale bar = 100 µm.



Figure S4. (A) Macroscopic imaging of residual 3D-bioprinted scaffolds at day 7 after subcutaneous implantation; scale bar = 5 mm. (B) Quantitative analysis of residual scaffolds area at day 7 after subcutaneous implantation. (C) Macroscopic imaging of residual 3D-bioprinted scaffolds at day 14 after subcutaneous implantation; scale bar = 5 mm. (D) Quantitative analysis of residual scaffolds area at day 14 after subcutaneous implantation.