ACCSCIENCE PUBLISHING

International Journal of Bioprinting

RESEARCH ARTICLE

Modeling inflammatory response using 3D bioprinting of polarized macrophages

Supplementary File

Supplementary information A: Optimization of collagenase treatment

The 3D-bioprinted constructs (10 × 10 × 0.8 mm^3) were washed with Dulbecco's phosphate-buffered saline (DPBS) and incubated with 200 µL of increasing concentrations (0, 0.25, 0.5, 1, 1.5, 2, and 2.5 mg/mL) of collagenase in Dulbecco's Modified Eagle Medium (DMEM) for 30 min at 37°C. The construct's appearance was observed at 2, 5, 10, 15, 20, and 30 min, with complete digestion of constructs (no residues of GelMA present) identified following 10-min treatment with 1 mg/mL collagenase. Figure S1 illustrates the alterations in the morphology of 3D-bioprinted THP-1 constructs following collagenase treatment.

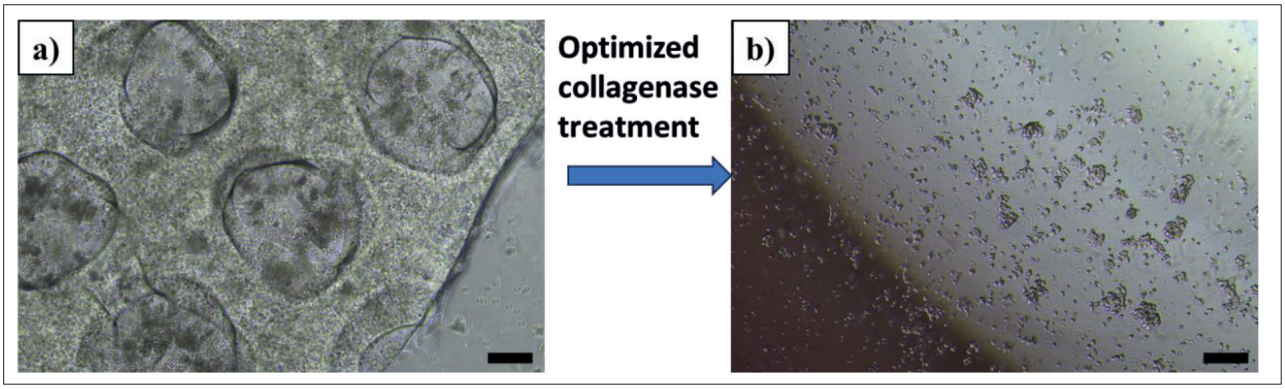

Figure S1. (**a**) Microscopic image depicting 3D-bioprinted THP-1 cells 3 days into the cell culture reveals resilient 3D-bioprinted filaments intertwined with cells. (**b**) Following collagenase treatment, the GelMA filaments underwent complete digestion, leaving only the cells suspended in the medium. Scale bar: 250 µm.

To test the effect of collagenase digestion on surface marker expression, M1-polarized macrophages were retrieved from the tissue culture plate, treated with 1 mg/mL collagenase for 10 min, and expression of surface markers CD11b and CD80 evaluated using flow cytometry. Surface marker expression was compared with that of untreated M1 cells, and no effect of collagenase treatment on CD11b and CD80 expression was observed (Figure S2).

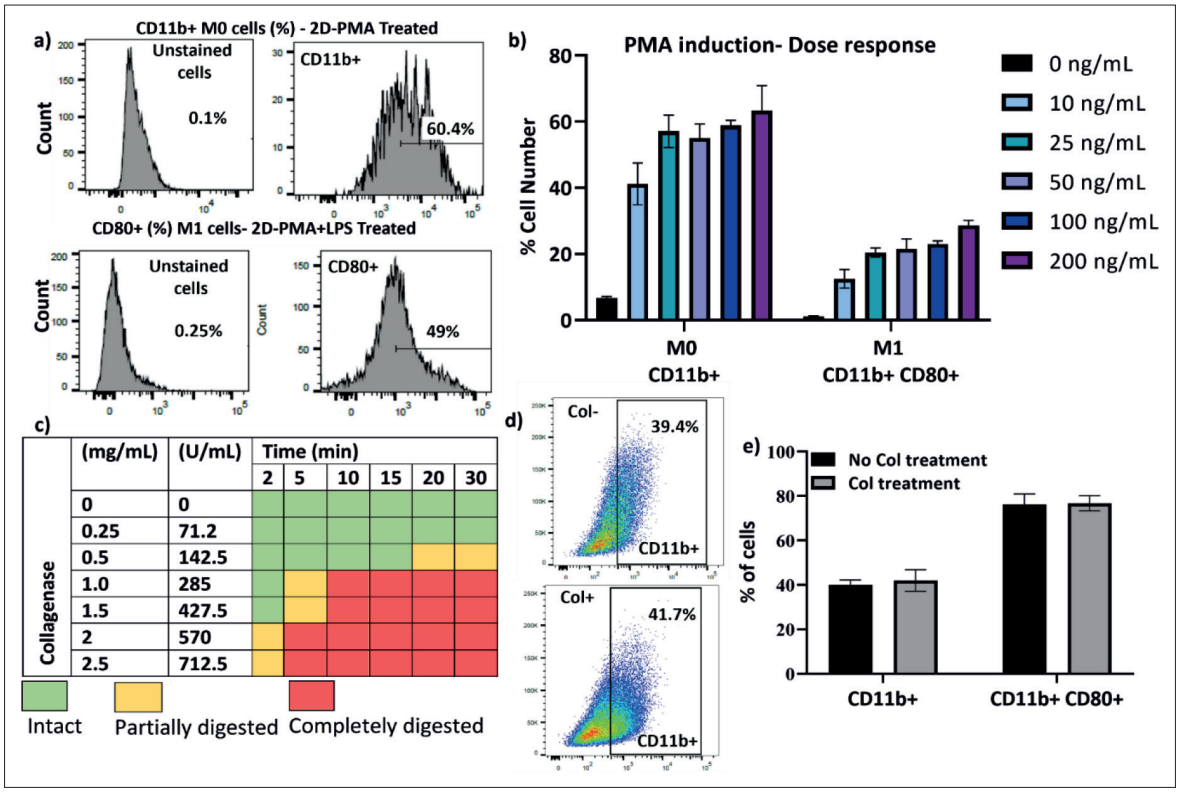

Figure S2. (**a**) Flow cytometry histogram represents the expression of CD11b and CD80 in macrophages cultured in 2D after phorbol 12-myristate 13-acetate (PMA) and lipopolysaccharide (LPS) treatment, respectively; (**b**) quantified CD11b and CD80 expression from the flow cytometry results; (**c**) table represents the extent of 3D-bioprinted GelMA digestion with different concentration of collagenase; (**d**) flow cytometry dot plot for CD11b and CD80 expression in the M1 macrophages before and after the collagenase treatment.

Supplementary information B: Cytotoxicity of ibuprofen concentration

The M1 cells, after 48 h of LPS induction, were treated with 0, 5, 10, and 15 μg/mL of ibuprofen (Ibu) for 1, 2, and 4 days. At the test time points, the 3D-bioprinted constructs were stained with 5 μg/mL fluorescein diacetate (FDA; Sigma-Aldrich, Australia) and 2 μg/mL propidium iodide (PI; Sigma-Aldrich, Australia) for 30 min, and imaged using Nikon Eclipse Ti confocal microscope. The results showed that none of the tested Ibu concentrations caused any cytotoxicity to M1 cells (Figure S3).

Table S1. Sequences of primers used in this study

Gene (human)	Forward primer (5'–3')	Reverse primer (5'–3')
Interleukin 1 beta (*IL-1β*)	CCACAGACCTTCCAGGAGAATG	GTGCAGTTCAGTGATCGTACAGG
Interleukin 6 (*IL-6*)	AGACAGCCACTCACCTCTTCAG	TTCTGCCAGTGCCTCTTTGCTG
Interleukin 12 subunit beta (*IL-12*β)	GACATTCTGCGTTCAGGTCCAG	CATTTTTGCGGCAGATGACCGTG
Nitric oxide synthase (*iNOS*)	GCTCTACACCTCCAATGTGACC	CTGCCGAGATTTGAGCCTCATG
Tumor necrosis factor-alpha (*TNF-α*)	CTCTTCTGCCTGCTGCACTTTG	ATGGGCTACAGGCTTGTCACTC
Cluster of differentiation 206 (*CD206*)	AGCCAACACCAGCTCCTCAAGA	CAAAACGCTCGCGCATTGTCCA
Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (*DC-SIGN*)	GCAGTCTTCCAGAAGTAACCGC	GCTCTCCTCTGTTCCAATACTGC
Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*)	TCAGCAATGCATCCTGCAC	TCTGGGTGGCAGTGATGGC
18S ribosomal RNA (*18S rRNA*)	TTCGGAACTGAGGCCATGAT	CGAACCTCCGACTTCGTTC

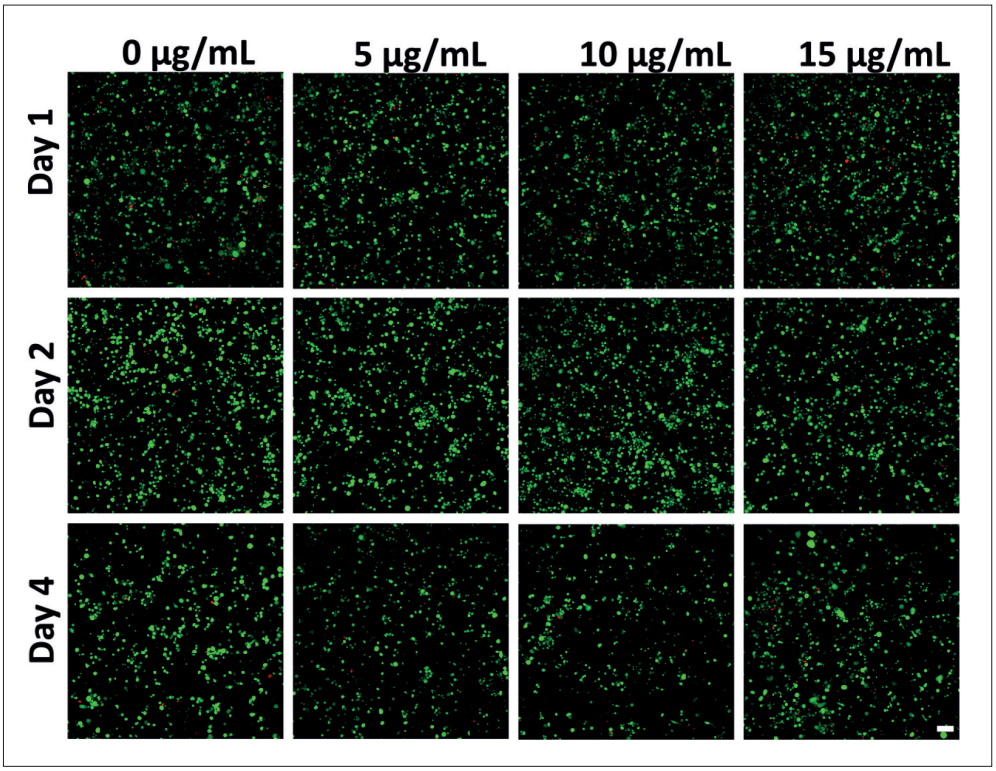

Figure S3. Confocal microscopic images after live/dead imaging of M1 cells treated with different concentrations of ibuprofen in 2D culture. Scale bar: 100 µm.