Supplementary Information

High-throughput bioprinted 3D cultures for probing host-pathogen interactions in bioinspired microenvironments

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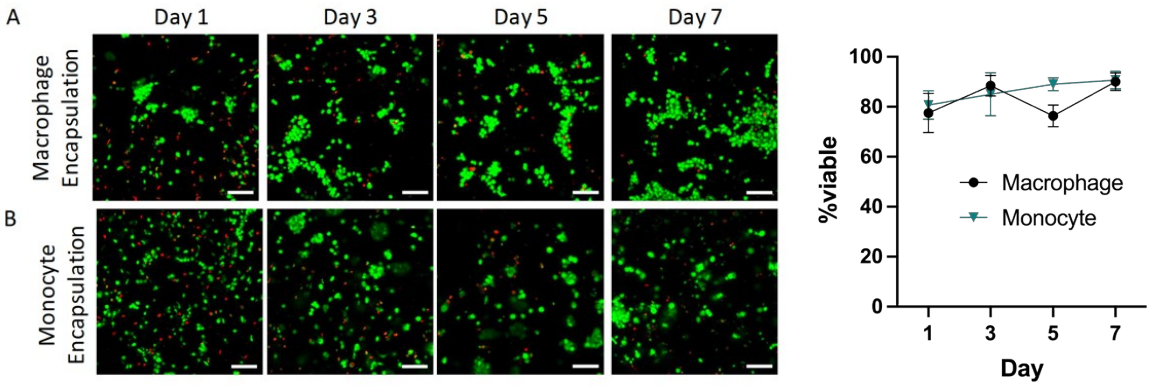

Figure S1: Live/Dead images comparing encapsulation strategy for dTHP-1 macrophages. Macrophages shown are encapsulated in 1.1 kPa PEG + RGD, GFOGER, YIGSR. (A) THP-1 cells were treated with 200 nM PMA 2 days prior to bioprinting and printed as differentiated THP-1 (dTHP-1) macrophages. (B) THP-1 monocytes were bioprinted and treated with PMA immediately following encapsulation (scale bar = 100 μm). Live cells are shown in green (calcein) and dead cells are shown in red (ethidium-homodimer-1).

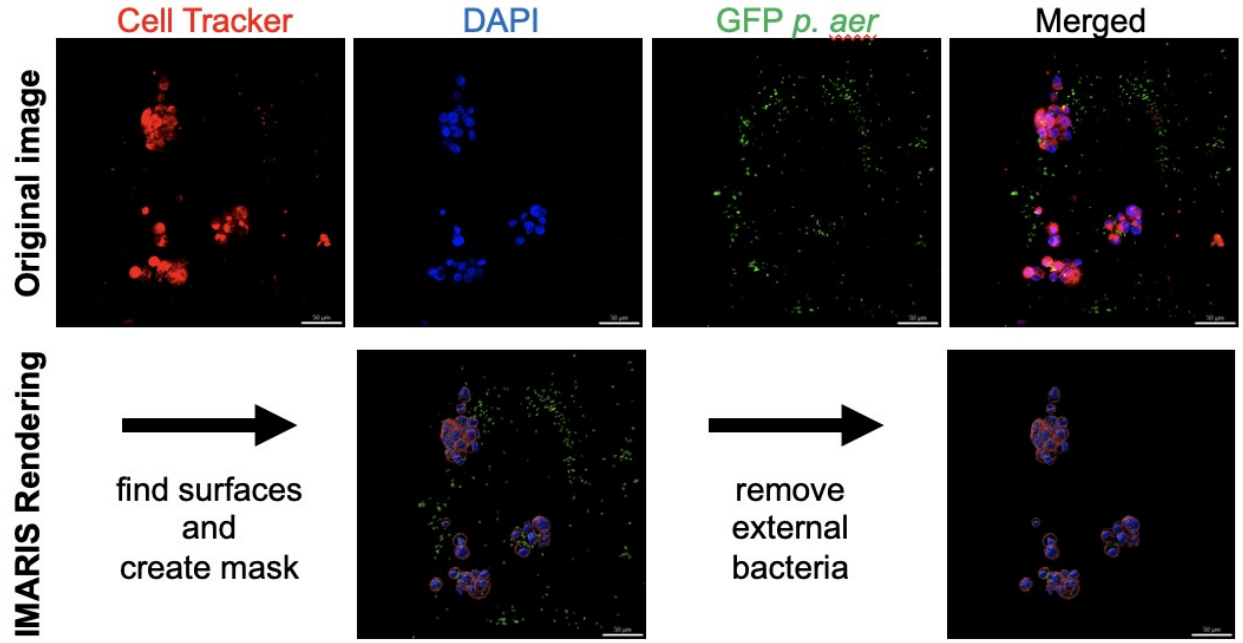

Figure S2: Representative images of bacteria invasion. GFP-expressing *P. aeruginosa* were added to macrophages labeled with cell tracker deep red cultured in 1.1 kPa hydrogels, where samples were processed, including fixation and nuclear staining with DAPI, and imaged. Images were processed through IMARIS software to visualize and quantify engulfed bacteria.

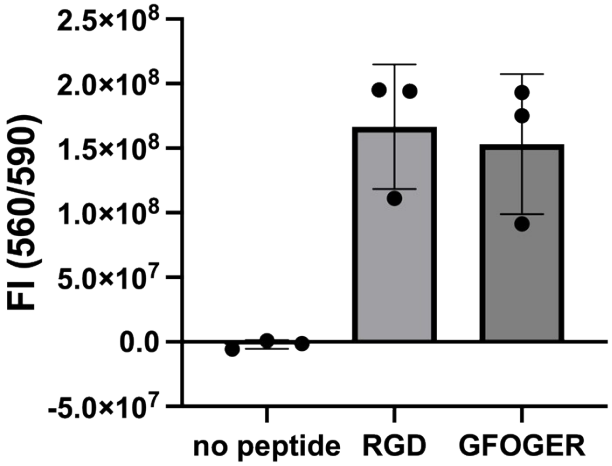

Figure S3: Alamar blue metabolic activity data for dTHP-1 macrophages encapsulated in 1.1 kPa hydrogels for 3 days. Cell encapsulated in bioprinted PEG-peptide hydrogel-based synthetic ECMs without adhesive peptides were not metabolically active. Metabolic activity of differentiated THP-1 macrophage (200 nm PMA) measured via fluorescence intensity of alamarBlue™ excitation [ex.] 590 nm and emission [em.] 560 nm. alamarBlue™ was added in a 1:10 dilution and incubated for 4 hours prior to analysis.

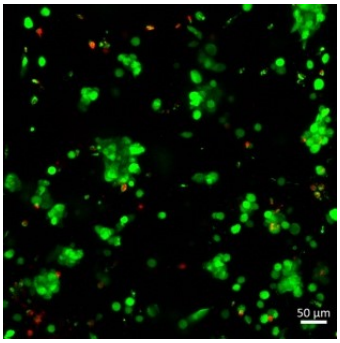

Figure S4: Live/Dead™ imaging of THP-1 macrophages in 1.1 kPa RGD, GFOGER, YIGSR, Hyaluronic acid hydrogel on day 3 after encapsulation. Live cells are shown in green (calcein) and dead cells are shown in red (ethidium-homodimer-1).

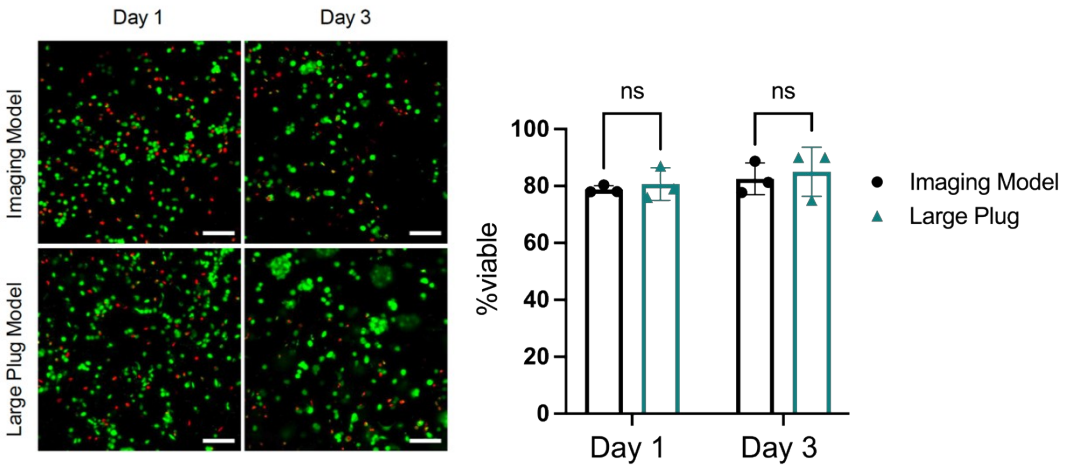

Figure S5: Live/Dead images comparing large plug and imaging models in 1.1 kPa PEG + RGD, GFOGER, YIGSR. Viabilities between imaging and large plug models are not significantly (ns) different (scale bar = 100 µm). Live cells are shown in green (calcein) and dead cells are shown in red (ethidium-homodimer-1).

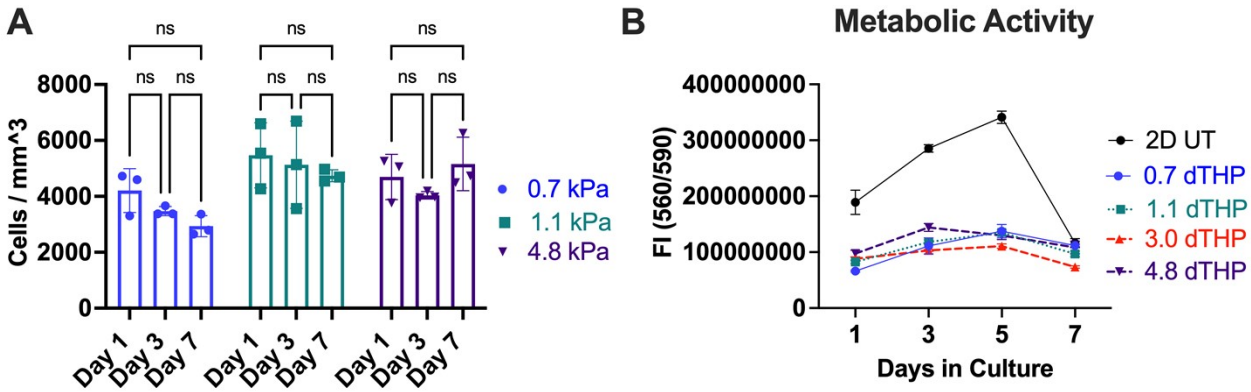

Figure S6: Cell counts and metabolic activity (alamarBlue™) data of cells cultured in hydrogels for 7 days. (A) Cell counts were performed using Volocity Software to analyze number of nuclei. Nuclei were stained after fixation (4% PFA) using Hoechst 33342 solution (ThermoFisher Scientific) (1:200) to stain nuclei, visualized by confocal microscopy (LSM 800, Zeiss; 10x objective and frame size of 1024 × 1024, 180 µm z-stack, 8 µm/stack). (B) Metabolic activity of differentiated THP-1 macrophage (200 nm PMA) measured via fluorescence intensity of alamarBlue™ excitation [ex.] 590 nm and emission [em.] 560 nm. alamarBlue™ was added in a 1:10 dilution and incubated for 4 hours prior to analysis.

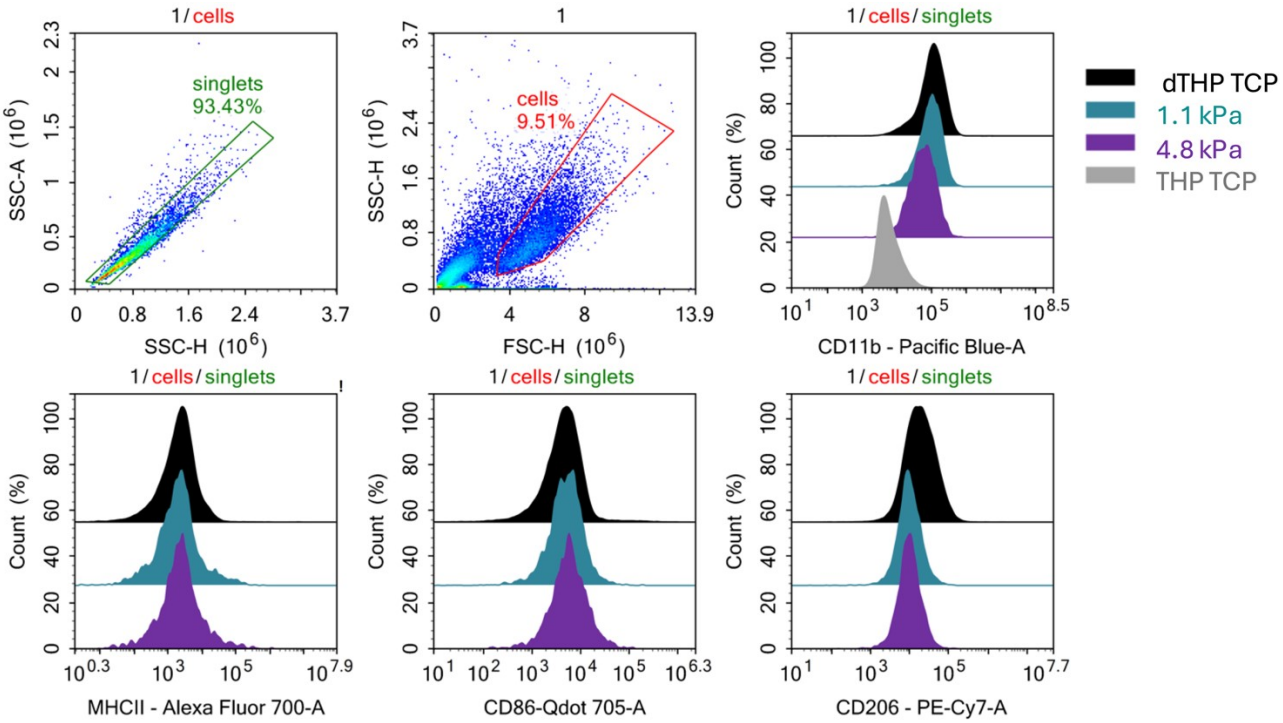

Figure S7: Representative flow cytometry gating scheme for macrophage surface markers. First cells were identified and gated on singlets, then singlets were analyzed for respective markers.

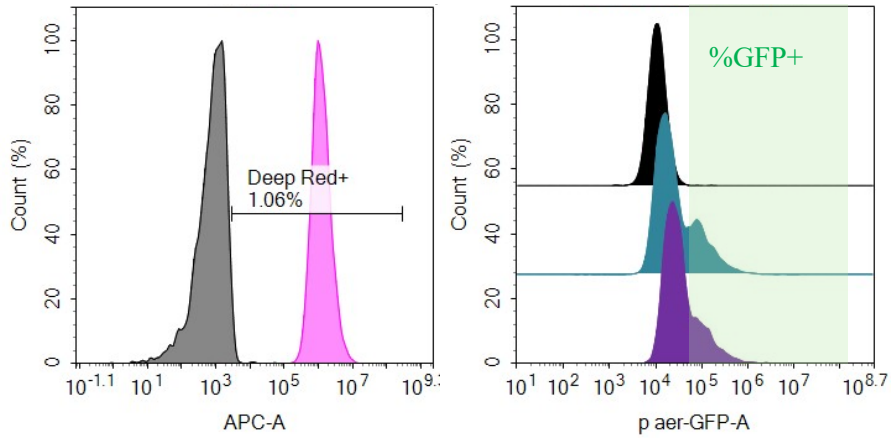

Figure S8: Representative flow cytometry gating scheme for phagocytosis of *P. aeruginosa*. Macrophages were labeled with Cell Tracker Deep Red prior to invasion. Macrophages were gated on cells then singlets (S3). Deep Red+ cells were then analyzed for GFP fluorescence to identify the percentage of cells that phagocytosed bacteria (% phagocytosis).

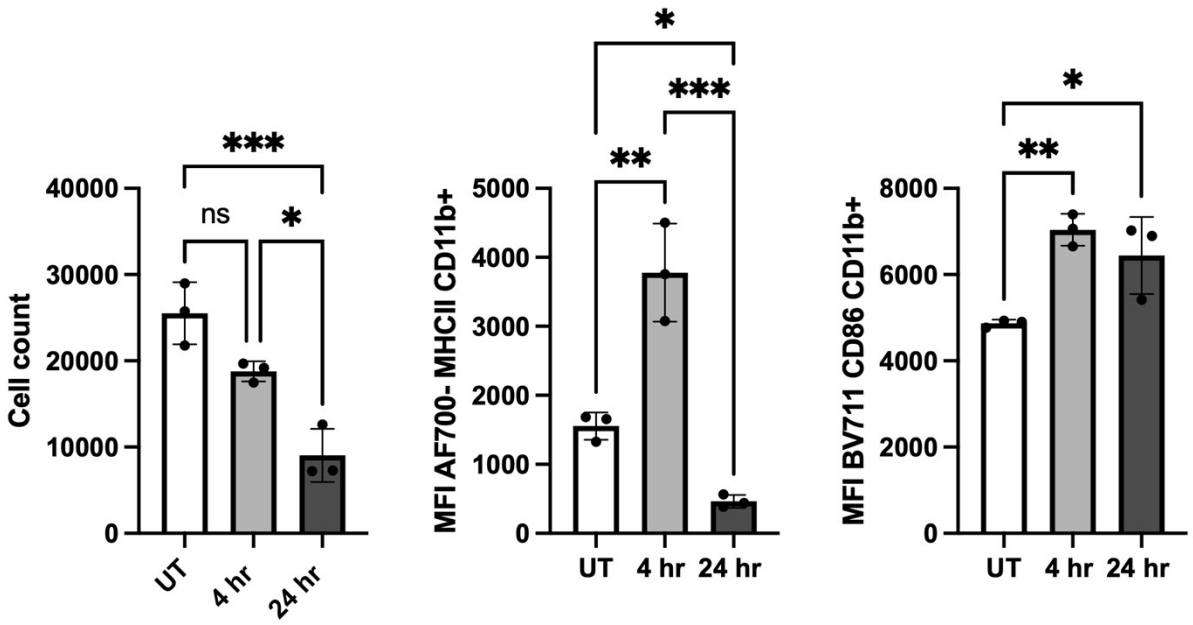

Figure S9: Flow cytometry results quantifying macrophage response to *P. aeruginosa* after 4-hour and 24-hour invasion. 4 μL (OD = 2) *P. aeruginosa* culture was added to dTHP-1 macrophages encapsulated in 1.1 kPa hydrogels at day 4 and incubated for either 4 or 24 hours. After 4 or 24 hours, cultures were treated with gentamicin, then macrophages were retrieved from hydrogels and analyzed via flow cytometry. Statistical analyses were performed using Tukey's post-hoc test with one-way ANOVA (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).