Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

for

Programmable multilayer printing of mechanically-tunable 3D hydrogel co-culture system for high-throughput investigation of complex cellular behavior

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1 Experimental Section

1.1 Materials

Gelatin (from porcine skin, gel strength 300), 4-dimethylaminopyridine (DMAP), 4-methoxyphenol (4-MP), and glycidyl methacrylate (GMA) were purchased from Sigma Aldrich. Lithium bromide and dimethyl phenylphosphonite were purchased from Alfa Aesar. 2,4,6-trimethylbenzoyl chloride (TMBC) were purchased from Tokyo Chemical Industry, Japan.

1.2 Synthesis of methacrylic gelatin (MGel)

Gelatin (5 g), DMAP (0.5 g) and 4-MP (0.05 g) were first dissolved in DMSO at 50 ℃. Then, GMA (2 mL) was slowly added to the mixture and reacted for 48 hours at 50 ℃ under dry N_2. The product was purified by dialysis against deionized (DI) water and dried by lyophilization.

1.3 Synthesis of lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP)

TMBC was added dropwise to dimethyl phenylphosphonite under dry N_2. The reaction was stirred for 18 hours. Then, four-fold excess of lithium bromide in 2-butanone was added to the reaction mixture and then heated to 50 ℃, resulting in the formation of crystallized product. The mixture was cooled to room temperature for several hours, and then the product was obtained by filtration. The product was washed and filtered 3 times with 2-butanone and dried under vacuum.

2. Supplementary figures

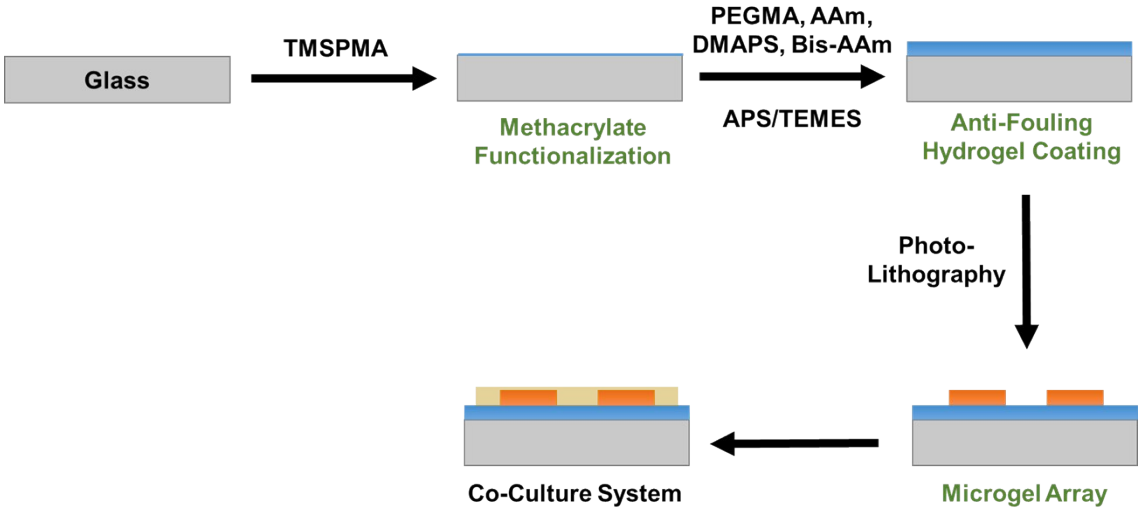

Figure S1. Schematic illustration of the process of developing 3D hydrogel co-culture system.

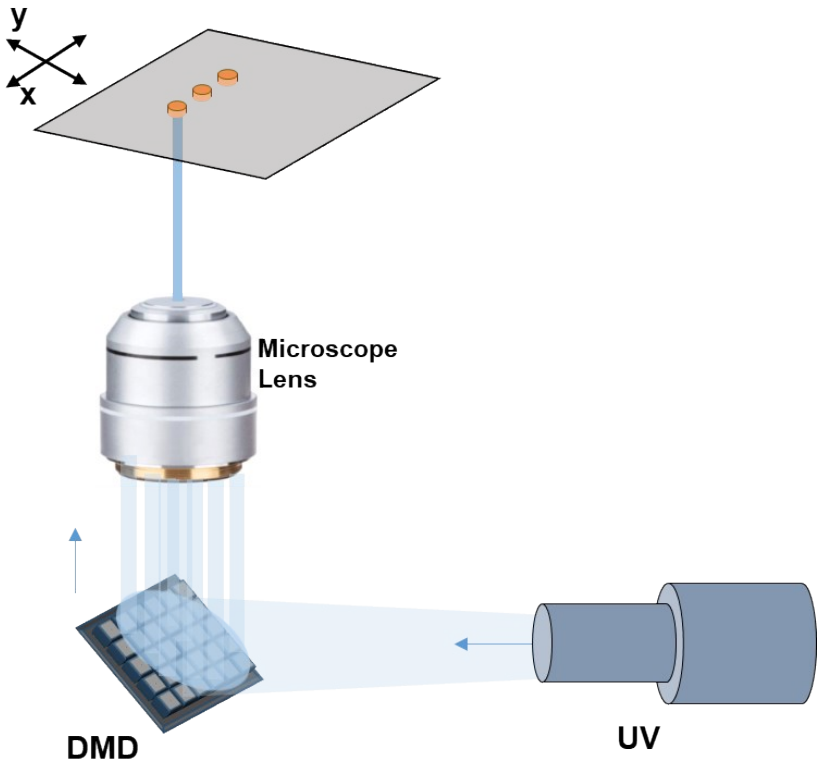

Figure S2. Schematic illustration of DMD-based printing system to generate microgel arrays.

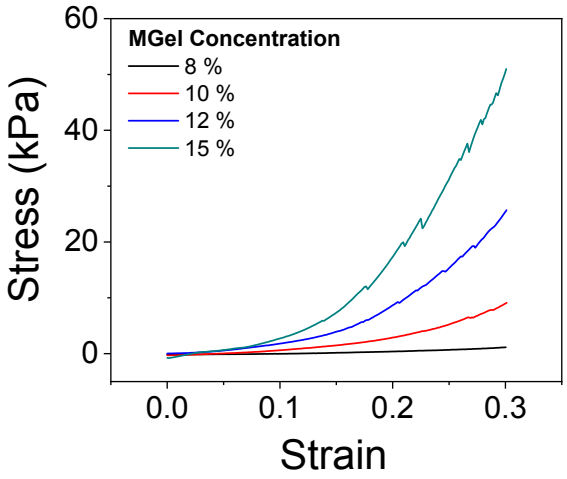

Figure S3. Stress-strain curves of MGel hydrogels with varying MGel concentrations obtained from uniaxial compression tests.

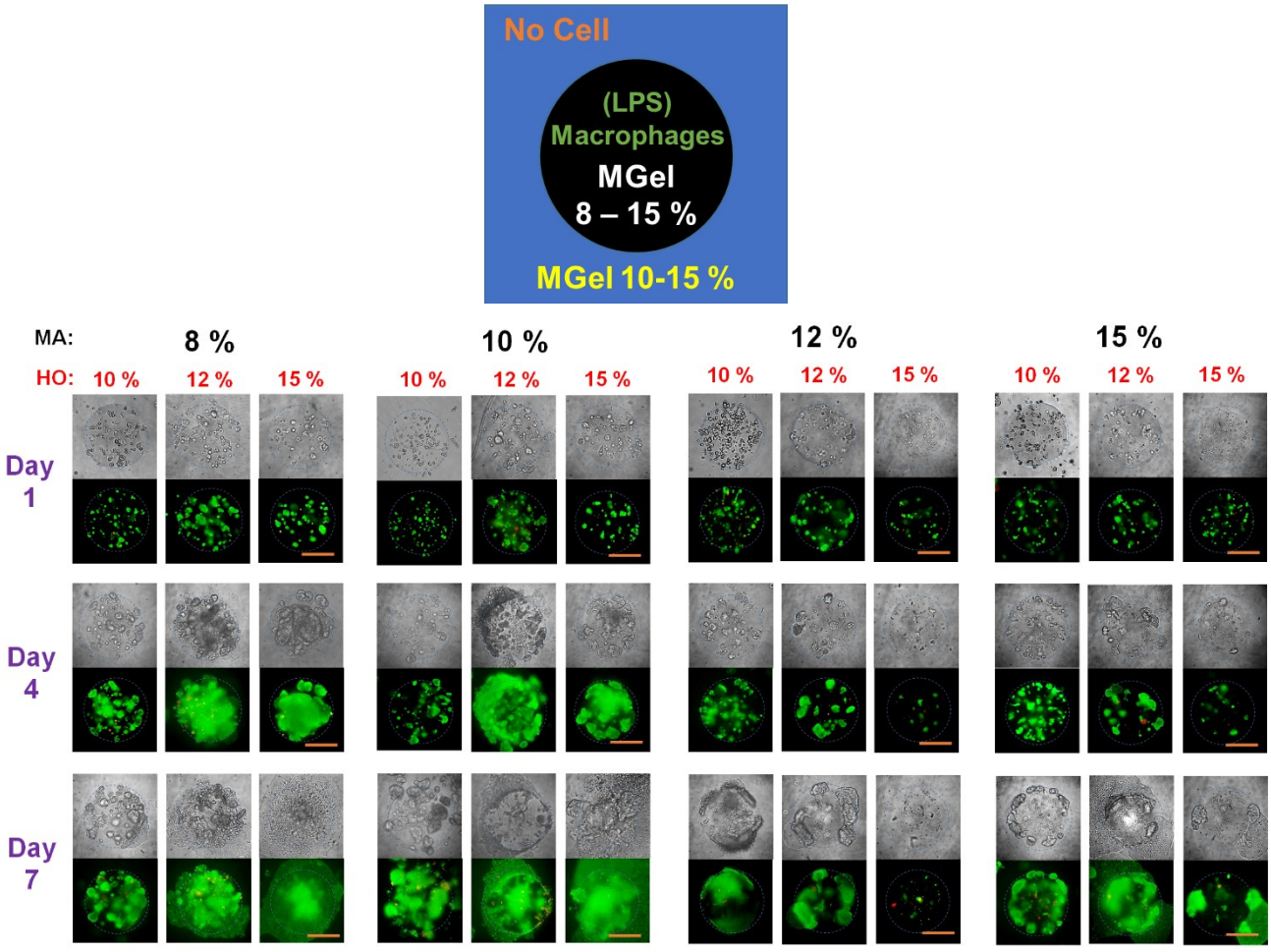

Figure S4. Optical (top) and fluorescent (bottom) microscopic images of macrophages encapsulated in microgel array (MA), covered with hydrogel overlay (HO) without cells (scale bar: 200 μm). The cells were treated with LPS to induce Mϕ polarization, and cultured up to 7 days. The cells were fluorescently labeled to visualize live (green) and dead (red) cells. The MGel concentrations of MA and HO were varied 8-15 % and 10-15 %, respectively.

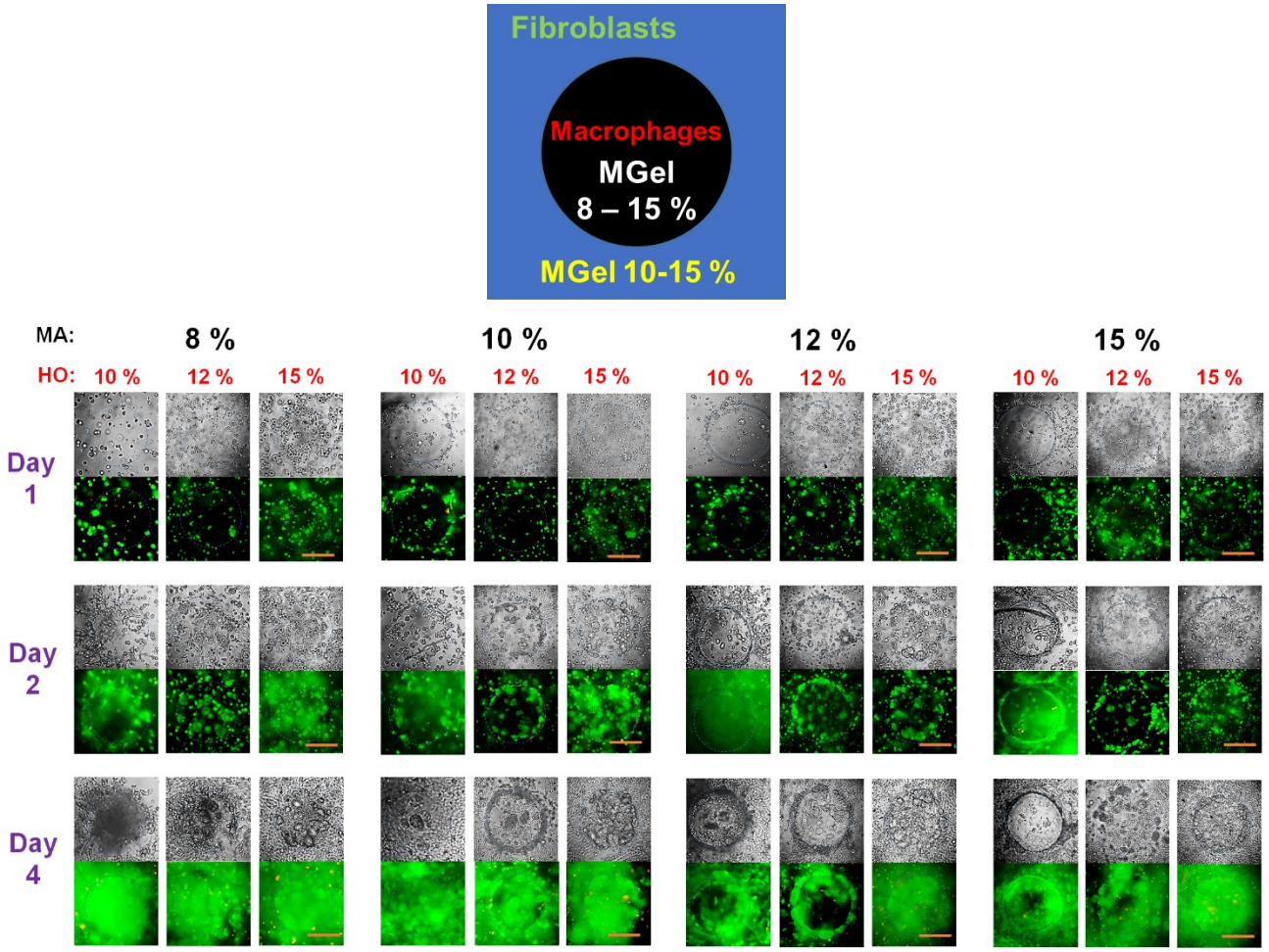

Figure S5. Optical (top) and fluorescent (bottom) microscopic images of macrophages encapsulated in microgel array (MA), covered with hydrogel overlay (HO) encapsulated with fibroblasts (scale bar: 200 μm), and cultured up to 4 days. The cells were fluorescently labeled to visualize live (green) and dead (red) cells. The MGel concentrations of MA and HO were varied 8-15 % and 10-15 %, respectively.

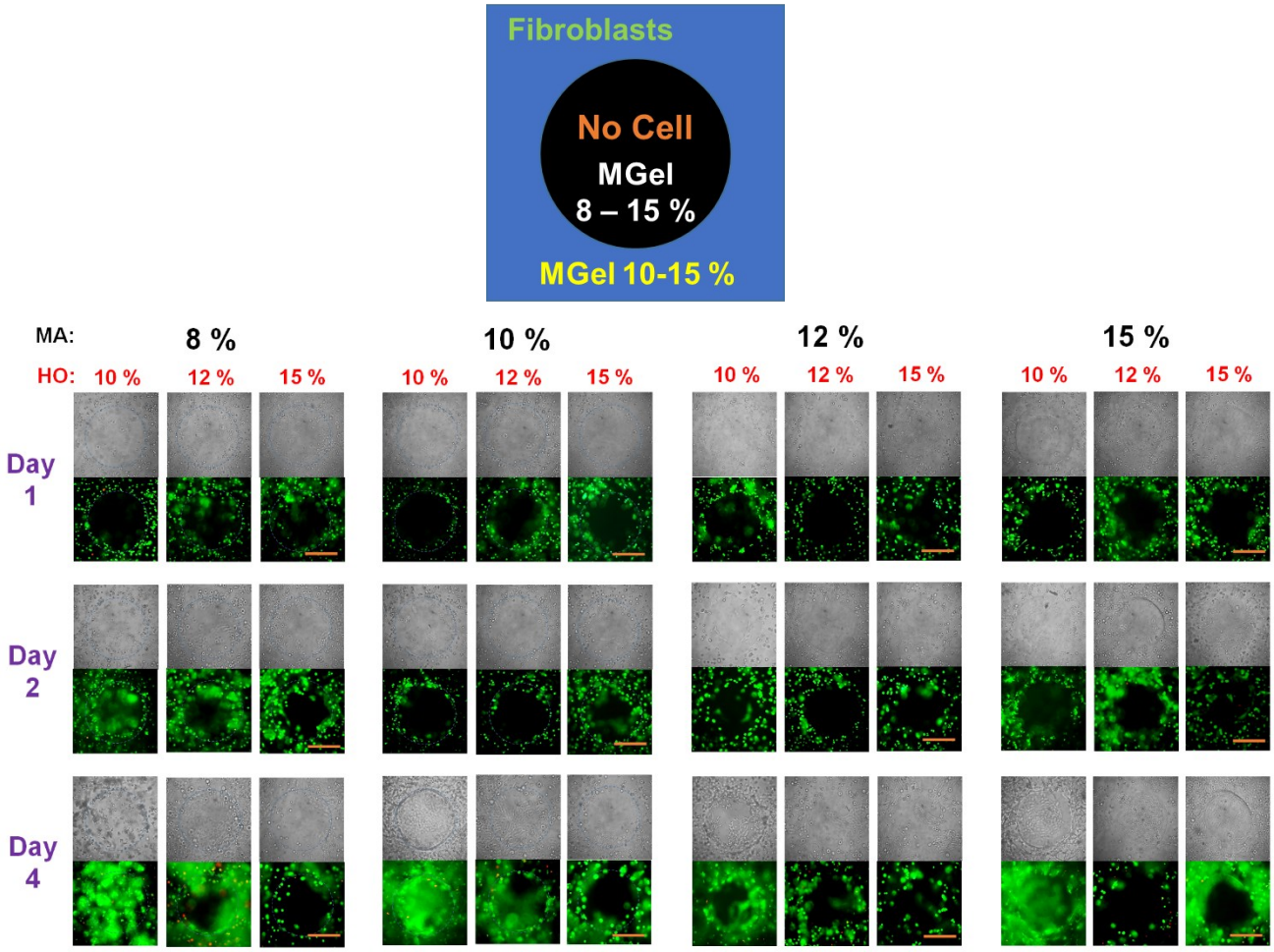

Figure S6. Optical (top) and fluorescent (bottom) microscopic images of fibroblasts encapsulated in hydrogel overlay (HO), without any cells encapsulated in microgel array (MA), and cultured up to 4 days (scale bar: 200 μm). The cells were fluorescently labeled to visualize live (green) and dead (red) cells. The MGel concentrations of MA and HO were varied 8-15 % and 10-15 %, respectively.

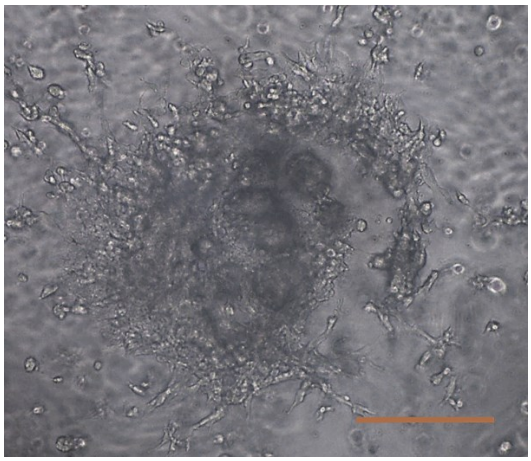

Figure S7. A representative magnified optical microscopic image of fibroblasts in HO aligning towards macrophages in MA, as demonstrated in Figure S4 (scale bar: 200 μm).

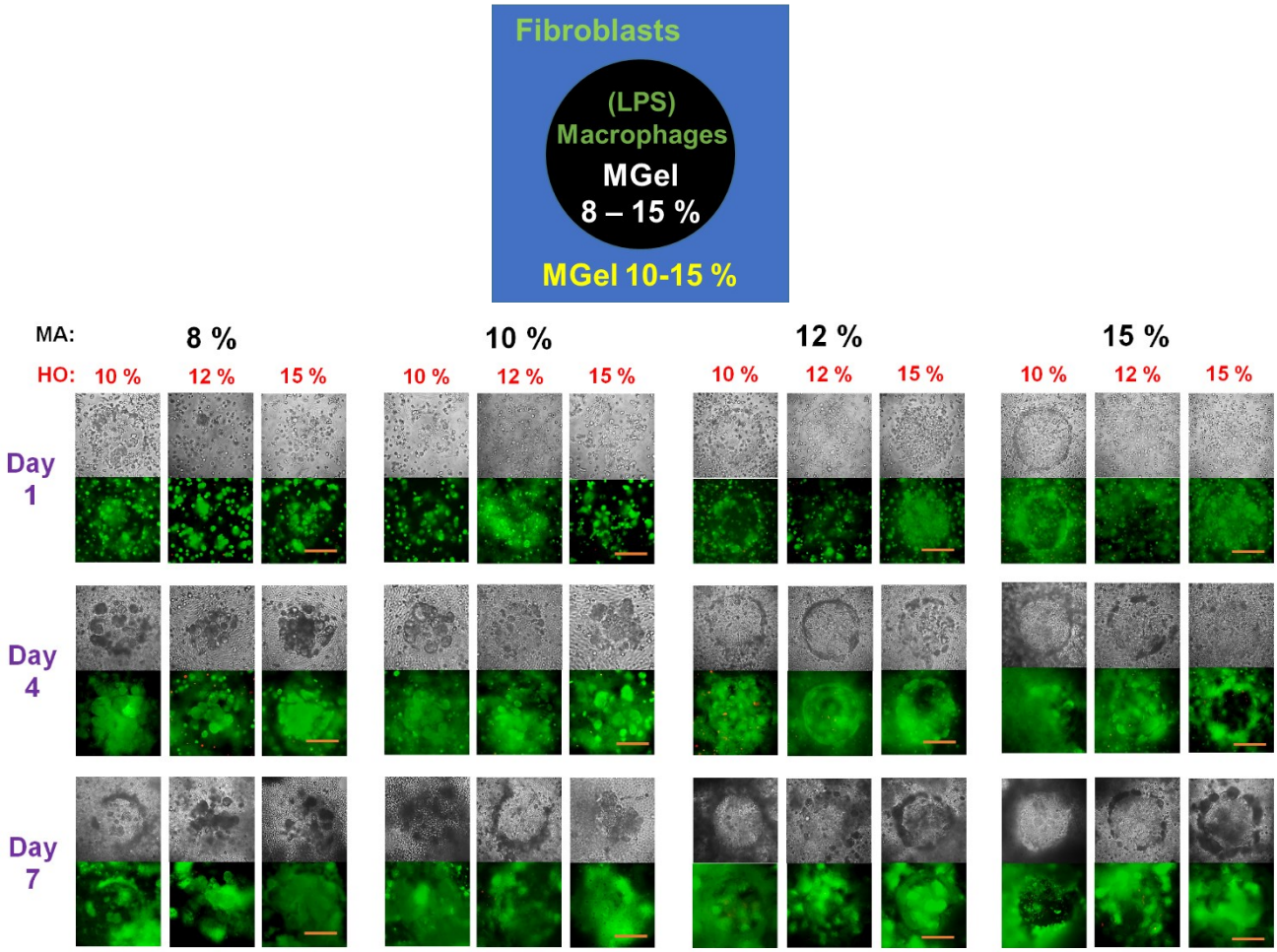

Figure S8. Optical (top) and fluorescent (bottom) microscopic images of macrophages encapsulated in microgel array (MA), covered with hydrogel overlay (HO) encapsulated with fibroblasts (scale bar: 200 μm). The cells were treated with LPS to induce Mϕ polarization, and cultured up to 7 days. The cells were fluorescently labeled to visualize live (green) and dead (red) cells. The MGel concentrations of MA and HO were varied 8-15 % and 10-15 %, respectively.