Supplementary Information (SI) for Biomaterials Science.
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Supporting information

Inflammation environment-adaptive matrix confinement for three-dimensional modulation of macrophages

Yilun Luo [1,2], Sentao Hu [1], Yan Li [1] and Lie Ma[*,1,3]

1 MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China

2 MOE Key Laboratory of Prevention and Treatment of Cardiovascular and Cerebrovascular Diseases, School of Medical Information Engineering, Gannan Medical University, Ganzhou 341000, China

3 Key Laboratory of Reproductive Dysfunction Management of Zhejiang Province, Assisted Reproduction Unit, Department of Obstetrics and Gynecology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, China

* Corresponding author: liema@zju.edu.cn

Morphological analysis of BMDMs

After 6-7 days in culture, BMDMs were digested by 0.25% trypsin (0.02% EDTA, Cienry, Zhejiang, China), collected and resuspended at a certain density of 5,000,000 cells/mL. The scaffolds were soaked in 75% ethanol solution for sterilization and washed with PBS ten times. And then, the cell solution (200 μL) was added to the scaffold and cultured in an incubator (37 ℃, 5% CO_2) to allow BMDMs attachment inside the scaffold. 800 μL basal medium was added to the cell culture plate 2 hours later.

Macrophages morphologies were investigated by staining the cytoskeleton. The cells were fixed in 4% paraformaldehyde for 30 min at 37 °C, permeabilized with 0.5% (v/v) Triton X-100 for 20 min at 4 °C, and then blocked with 3% (w/v) BSA in PBS for 80 min at 37 °C. Cells were incubated with rhodamine-labeled phalloidin (1:1000, Thermo Fisher Scientific, USA) in 1% (w/v) BSA solution at 4 °C overnight. Then the above solution was washed and then incubated with DAPI (1:500) in 1% (w/v) BSA solution at room temperature for 30 min. The cells were observed by confocal laser scanning microscope (CLSM, LSM-880, Zeiss, Germany).

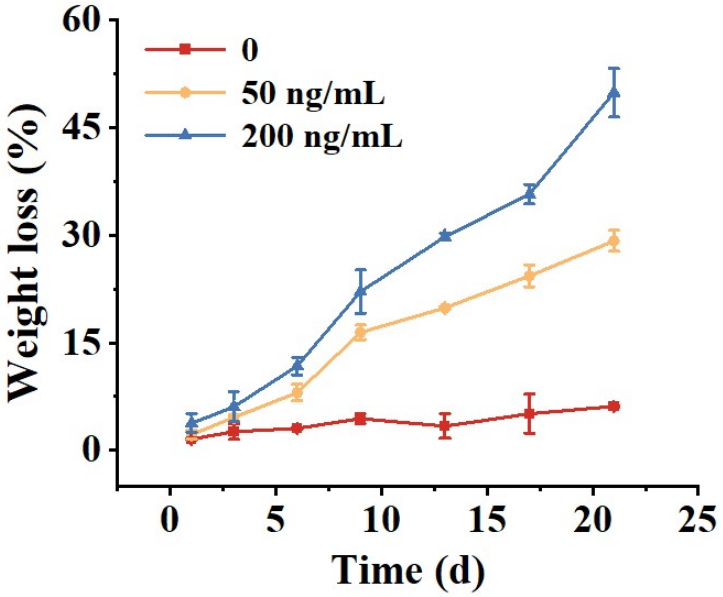

Figure S1. Weight loss of gelatin scaffolds at different time after treatment with 0, 50 and 200 ng/mL MMP-9.

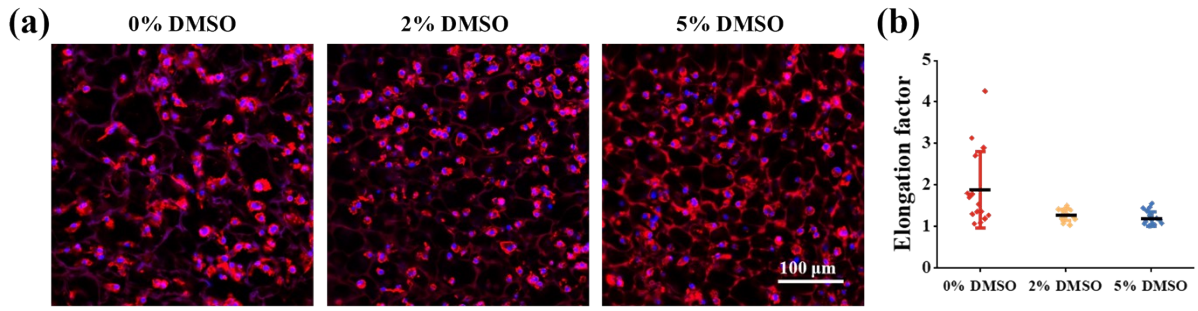

Figure S2. (a) CLSM images of macrophages stained for actin (red) and nuclei (blue) in auto-fluorescent gelatin scaffolds. Scale bar represents 100 µm. (b) Quantification of cell elongation.

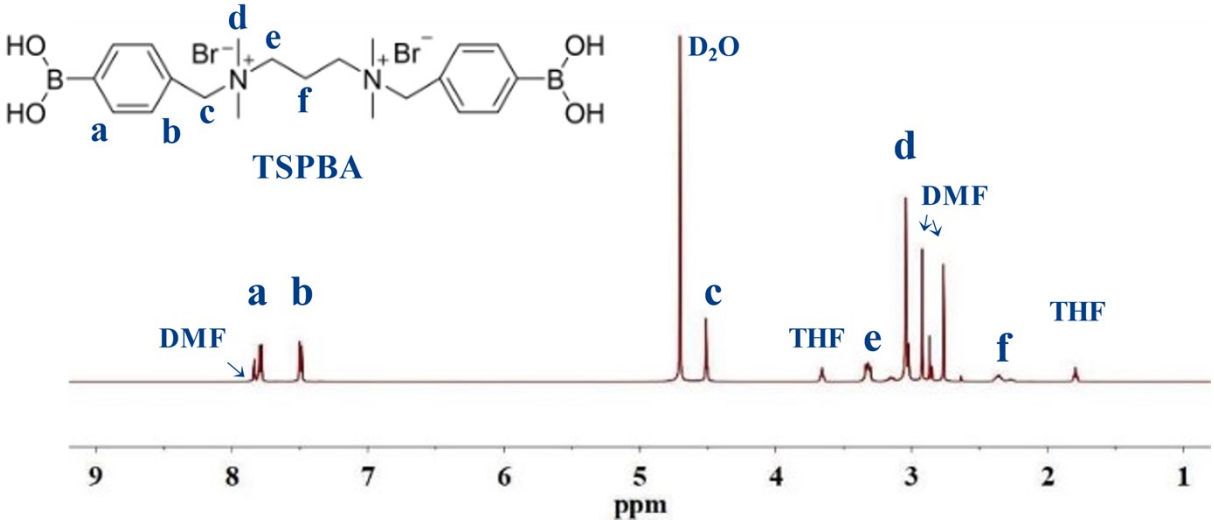

Figure S3. ^{1}H NMR characterizations of TSPBA.

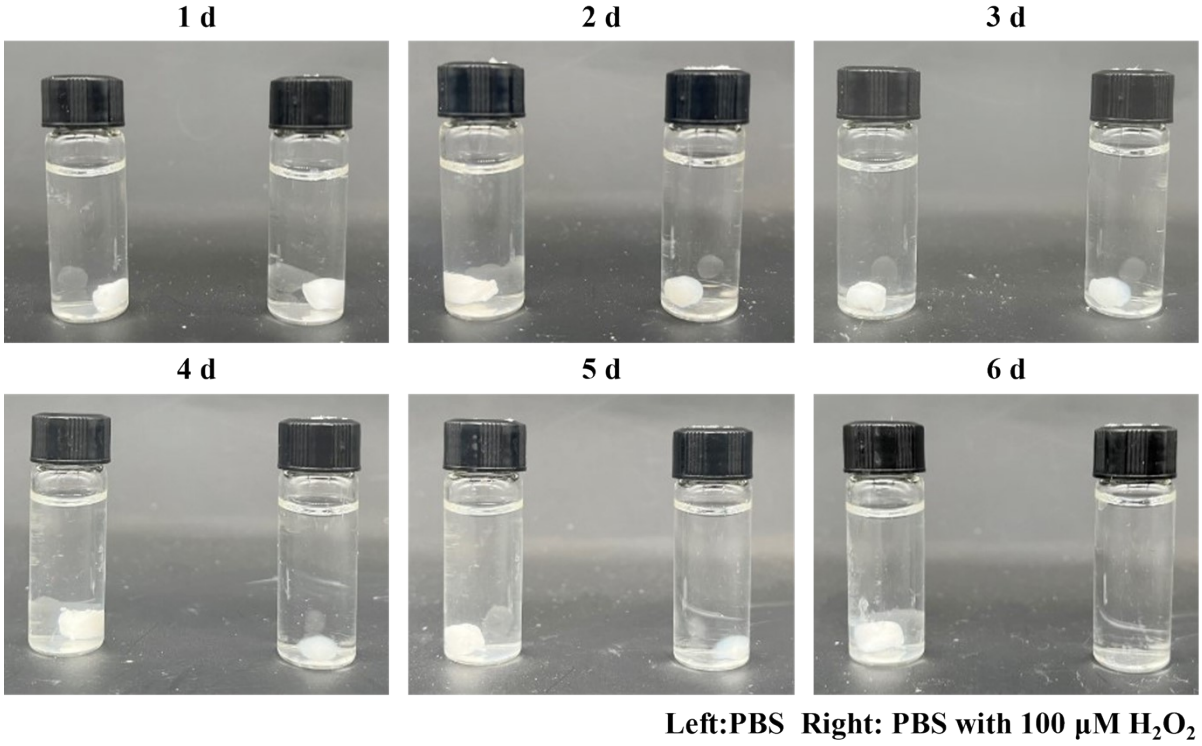

Figure S4. H_2O_2-triggered hydrogel disassembly. TSPBA-PVA-gelatin hydrogels were incubated with PBS and 100 μM H_2O_2.

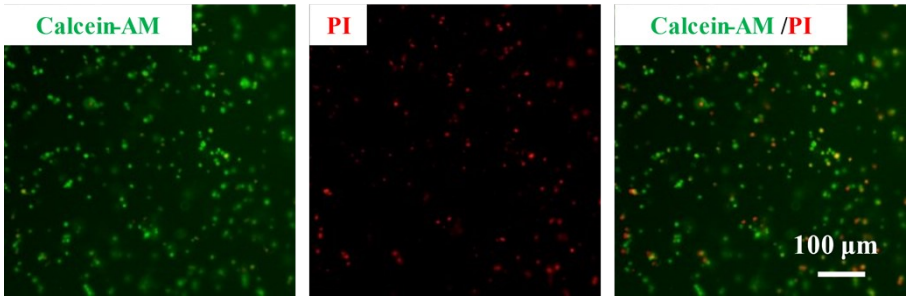

Figure S5. Fluorescent images of BMDMs in TSPBA-PVA hydrogels by CLSM after being cultured for 3 d. The live and dead BMSCs were stained with Calcein-AM (green) and PI (red), respectively. Scale bar represents 100 μm.

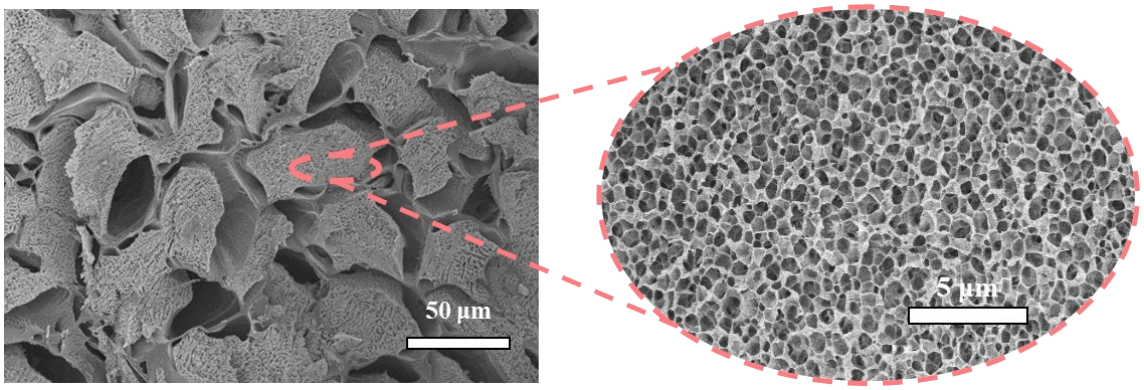

Figure S6. SEM images of hydrogel-filled scaffold with different magnifications.

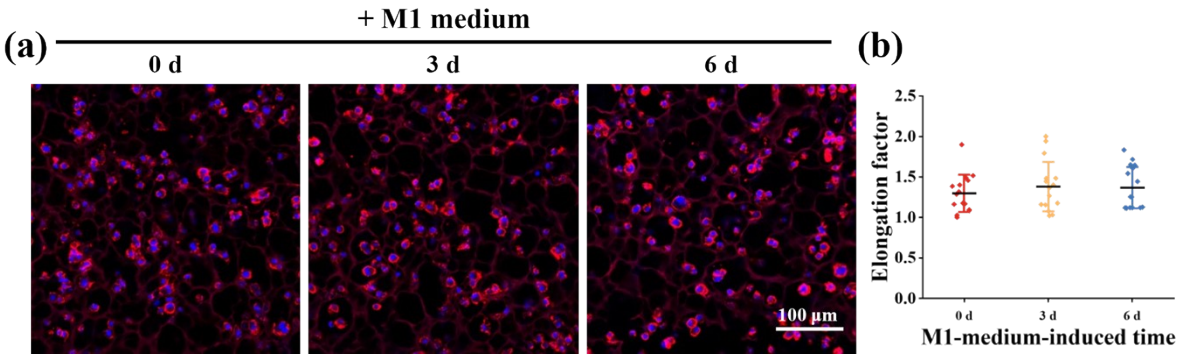

Figure S7. (a) CLSM images of macrophages stained for actin (red) and nuclei (blue) in auto-fluorescent BMDMs-laden hydrogel-filled scaffold after treatment with M1-polarized medium for 0 d, 3 d and 6 d. Scale bar represents 100 μm. (b) Quantification of cell elongation.