Submitted to ADVANCED HEALTHCARE MATERIALS

Supporting Information

for *Adv. Healthcare Mater.*, DOI: 10.1002/adhm.201300260

A Magnetically Responsive Biomaterial System for Flexibly Regulating the Duration Between Pro- and Anti-Inflammatory Cytokine Deliveries

*By Anita E. Tolouei, Nihan Dülger, Rosa Ghatee, Stephen Kennedy**

Materials: Sodium alginate was donated from Pronova Biopolymers (Oslo, Norway) with an average molecular weight of ~250 kDa and with high guluronate content (Protoanal LF 20/40). Adipic acid dihydrazide (AAD), 1-ethyl-3-(dimethylaminopropyl) carbodiimide (EDC), MES, 1-hydroxybenzotriazole (HOBT), iron (II,III) oxide powder (< 5 micron), Phosphate Buffered Saline (PBS), Sigmacote®, bovine serum albumin (BSA), paraformaldehyde (PFA), Triton X-100, fluorescein isothiocyanate (FITC) labeled phalloidin, and 2-(4-Amidinophenryl)-6-indolecarbamidine dihydrochloride (DAPI) were all purchased from Sigma-Aldrich (St. Louis, MO). Mouse Monocyte Chemoattractant Protein-1 (MCP-1), Interferon Gamma (IFN-γ), Interleukin-4 (IL-4), Interleukin-10 (IL-10), and all Enzyme-linked Immunosorbent Assay (ELISA) kits and kit reagents were purchased from R&D Systems (Minneapolis, MN).

Macrophage culturing: RAW 264.7 mouse macrophages (ATCC, Manassas, VA) were used in these studies. Macrophages were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma) containing 10% fetal bovine serum (FBS, Sigma) and 1% penicillin-streptomycin (Sigma) in 75 cm^2 flasks at 37ºC and 5% CO_2 and split every 2-3 days at a 1:3 to 1:6 ratio, as recommended by the manufacturer.

Mechanical characterization of biphasic ferrogels before and after magnetic stimulation: Biphasic ferrogel (inner compartments) stiffness (Young's modulus) before and after magnetic stimulation was quantitatively measured in compression using an Instron Model 3345 (Norwood, MA). Prior to magnetic stimulation, biphasic ferrogels were placed

between the plates of the Instron and compressed (at 2 mm/min) until reaching 50% strain in order to produce a well-define elastic region on the stress-strain curve. Note that this compression resulted in deformation of only the iron-oxide-free region of the ferrogels and not the much stiffer/denser iron-oxide-laden region. Recorded stress-strain curves were analyzed using the Instron's Bluehill software package to extract moduli. Because this compression test could have damaged the gels, separate sets of gels were magnetically stimulated and mechanically tested after being exposed to magnetic stimulation profiles.

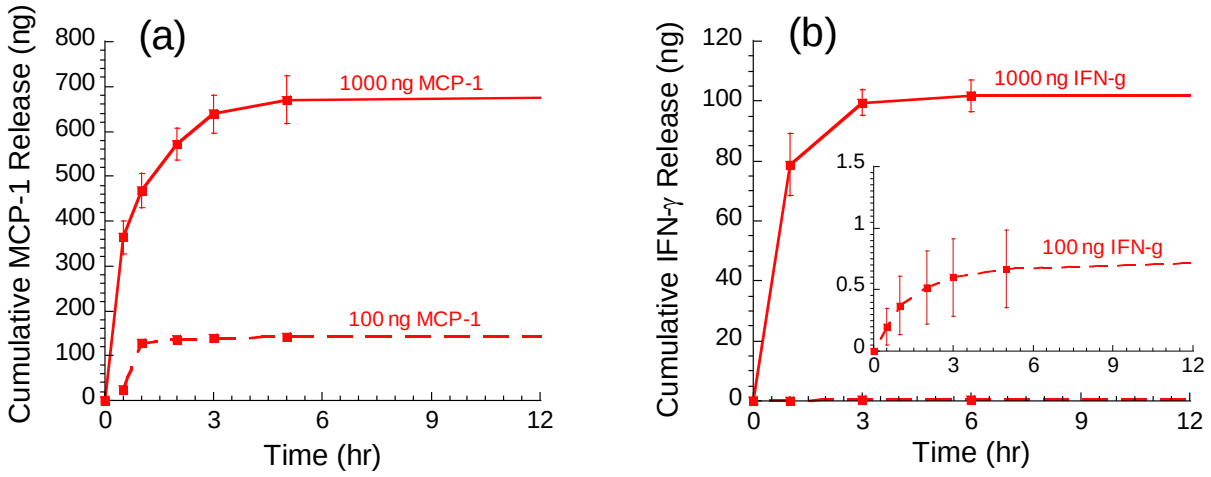

Figure S1. Outer porous gelatin scaffolds rapidly released MCP-1 and IFN-γ in the first 12 hours. (a) Cumulative MCP-1 release during the first 12-hours of experimentation for gelatin scaffolds loaded with 1000 ng (solid red) and 100 ng (dashed red) MCP-1. (b) Cumulative IFN-γ release during the first 12-hours of experimentation for gelatin scaffolds loaded with 1000 ng (solid red) and 100 ng (dashed red) IFN-γ. Inset: zoom-in of cumulative release for 100 ng-loaded scaffolds. For parts (a) and (b), N = 4.

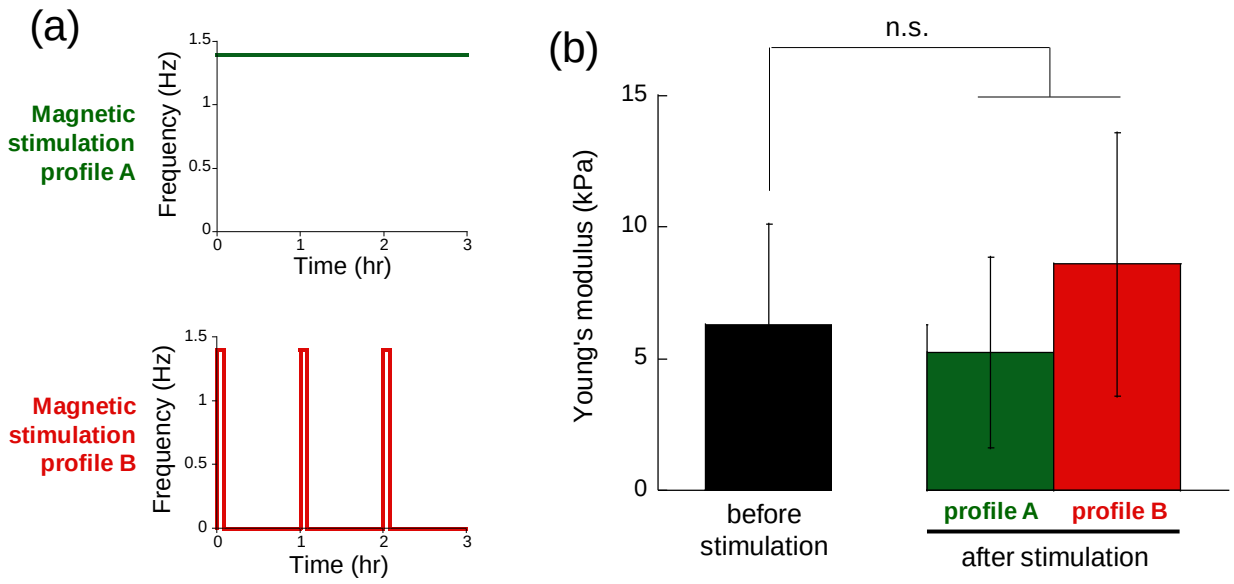

Figure S2. Ferrogel mechanics did not significantly change due to magnetic stimulation. (a) Schematics of the two stimulation profiles used: continuous stimulation at 1.4 Hz for 3 hours (top) and periodic stimulation at 1.4 Hz for 5 min every hour on the hour for 3 hours. (b) Young's modulus of ferrogels before stimulation (black), after stimulation profile A (green), and after stimulation profile B (red). N = 4.

<< MovieS1.avi >>

Movie S1. 3D z-stack reconstruction of macrophages recruited to the bottom 170 μm of a porous gelatin scaffold. Scaffold and macrophages were stained to identify f-actin (green) and cell nuclei (blue).

<< MovieS2.mov >>

Movie S2. Biphasic ferrogel being cyclically compressed at 1.4 Hz using a magnet on our custom rocker setup.