Supporting Information

Release of Magnetic Nanoparticles from Cell-Encapsulating Biodegradable Nanobiomaterials

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MATERIALS AND METHODS

GelMA Synthesis. To synthesize GelMA, type A porcine skin gelatin 10% (w/v) was dissolved into Dulbecco's phosphate buffered saline (DPBS; GIBCO) at 50 °C for 1 hour. 94% methacrylic anhydride (MA) was added to the solution at the concentration of 0.8 mL MA/1g gelatin. The mixture was allowed to react for 2 hours under stirring conditions (500 RPM) at 50 °C, and then diluted by 3X using DPBS. The diluted solution was dialyzed in distilled water using a 12-14 kDa cutoff dialysis bag (Fisher Scientific) for 1 week at 40 °C, where distilled water was replaced daily. The solution was then lyophilized for another week to produce a final GelMA powder.

Preparation of Prepolymer Solution. The GelMA prepolymer solution was prepared by mixing (5% w/v) GelMA powder, 0.5% (w/v) photoinitiator (IgracureTM 2959; CIBA Chemicals), and varying concentrations (1%, 5%)(w/v) of dry iron (II, III) oxide nano-powder (<50 nm, Sigma Aldrich) in Dulbecco's phosphate buffered saline (DPBS). The prepolymer solution was then heated at 80 °C, and vortexed to generate a homogeneous mixture. The controls were MNP-free GelMA hydrogels.

Sample Preparation for Scanning Electron Microscopy (SEM). For SEM imaging, three sets (MNP-free controls, 1% MNP, 5% MNP (w/v)) hydrogels were fabricated using a polydimethylsiloxane (PDMS) mold. To fabricate the PDMS mold, VersaLASER cutting machine (Universal Laser Systems Inc., Scottsdale, AZ) was first used to achieve the hard PMMA mold, and then, soft PDMS mold was replicated by casting the mixture of PDMS and 10% (w/v) curing agent on the PMMA mold with 6 mm x 6 mm x 10 mm. Finally, PDMS mold was curved in oven at 80°C. After PDMS fabrication, the hydrogels were crosslinked using the UV settings of 6.9 mW/cm^2 for 40 seconds. The hydrogels were cast into cylinders with 6 mm diameter and 10 mm height and lyophilized (Labconco Corporation, Kansas City, MO) for 48 hours. Next, lyophilized hydrogel sponges were placed in tightly closed

containers for storage. For imaging the cross-sections, lyophilized MNP encapsulating hydrogels were placed in liquid nitrogen for 5 minutes. Next, frozen hydrogels were freeze-fractured with blades and also kept in liquid nitrogen for 1 minute. The freeze-fractured sectioned hydrogels were air dried in a laminar flow hood for 30 minutes, followed by mounting on 10 mm diameter aluminum SEM stubs (Ted Pella Inc., Redding, CA) with carbon tape. The mounted samples were sputter coated (Cressington Scientific Instruments Ltd., Watford, England) with Platinum/Palladium at 40 mA for 90 seconds in a chamber purged with Argon gas. After coating, samples were imaged with field emission SEM (Ultra 55, Carl Zeiss MicroImaging, LLC, Thornwood, NY) under high vacuum mode with secondary electron detector. The quantification of the porosity based on the SEM images was performed using NIH ImageJ software. 10-15 images for each group were used to quantify the porosity ratio with an automatic threshold that uses the mean profile of the surface as the base line. To evaluate the mean pore size distributions, SEM images were converted to binary images, which indicated separately the bulk (white) and the void (black) phases by NIH ImageJ software.

Statistical Analyses. To evaluate the effect of MNPs on hydrogel mechanical properties, porosity ratio, swelling behavior and degradation rate of hydrogels encapsulating cells and MNPs were analyzed by One-way Analysis of Variance (ANOVA) with Tukey post hoc test for multiple comparisons with statistical significance threshold set at 0.05 ($p<0.05$). The correlation between degradation rate and MNP release was analyzed by determining the Pearson product moment correlation coefficient with a significance threshold set at 0.05 ($p<0.05$). Data for mechanical tests, porosity ratio and swelling behavior were presented as mean ± standard error of the mean (SEM). Error bars in the plots for microgel degradation rates, release of MNPs from microgels and cell growth in microgels were presented as standard deviation. Statistical analyses were performed using GraphPad Prism (La Jolla, CA, USA).

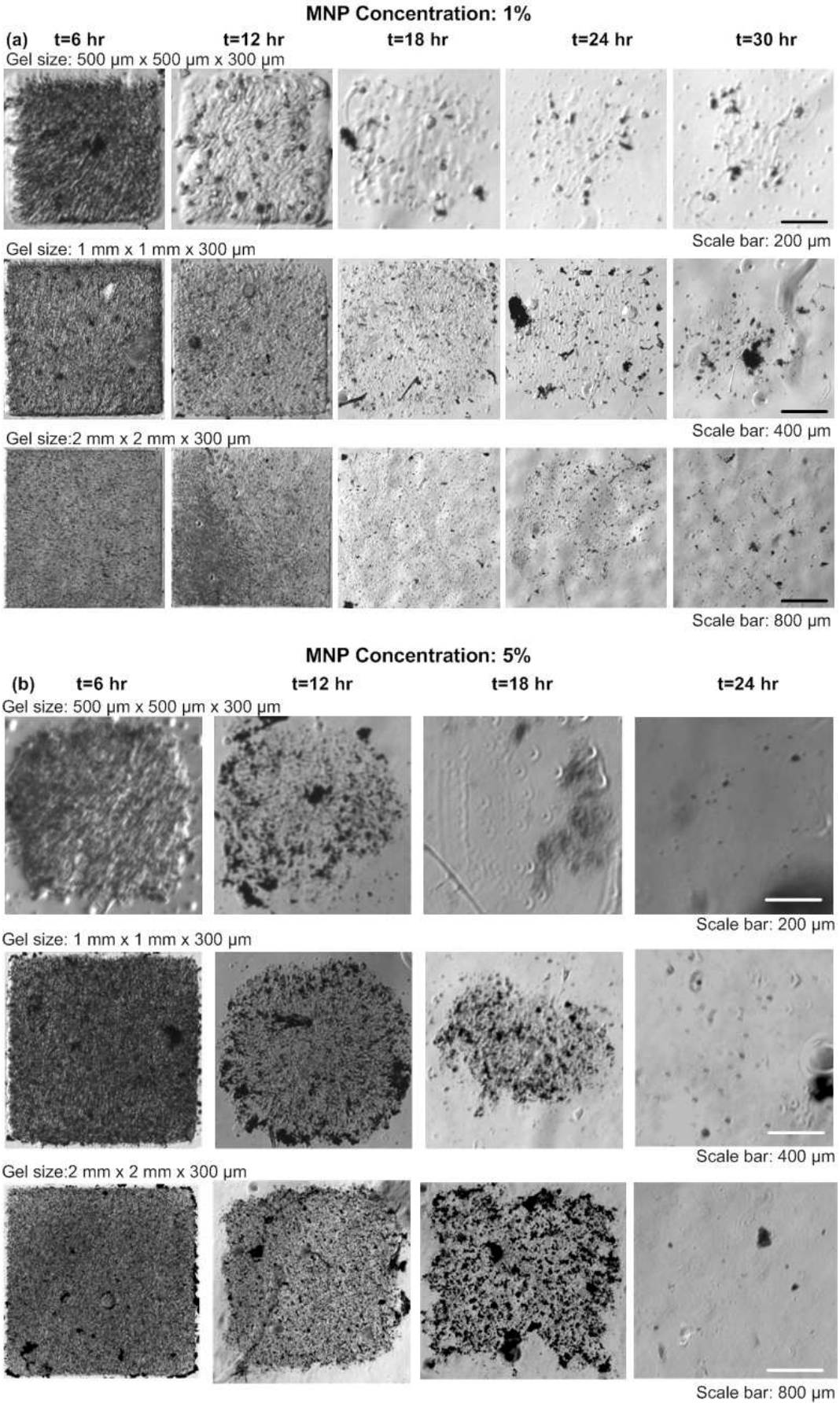
MNP Concentration: 1%
(a) t=6 hr
t=12 hr
t=18 hr
t=24 hr
t=30 hr
Gel size: 500 μm x 500 μm x 300 μm
Scale bar: 200 μm
Gel size: 1 mm x 1 mm x 300 μm
Scale bar: 400 μm
Gel size:2 mm x 2 mm x 300 μm
Scale bar: 800 μm
MNP Concentration: 5%
(b) t=6 hr
t=12 hr
t=18 hr
t=24 hr
Gel size: 500 μm x 500 μm x 300 μm
Scale bar: 200 μm
Gel size: 1 mm x 1 mm x 300 μm
Scale bar: 400 μm
Gel size:2 mm x 2 mm x 300 μm
Scale bar: 800 μm

FIGURE S1. Degradation profile of hydrogels cultured in 2 U/mL collagenase type I. (a) Image profile of typical hydrogels at different time points for each sample (500 μm, 1 mm, 2 mm). These samples were fabricated using 1% MNP (w/v) prepolymer solution. (b) Image profile of individual hydrogels for each sample for 5% MNP hydrogels.

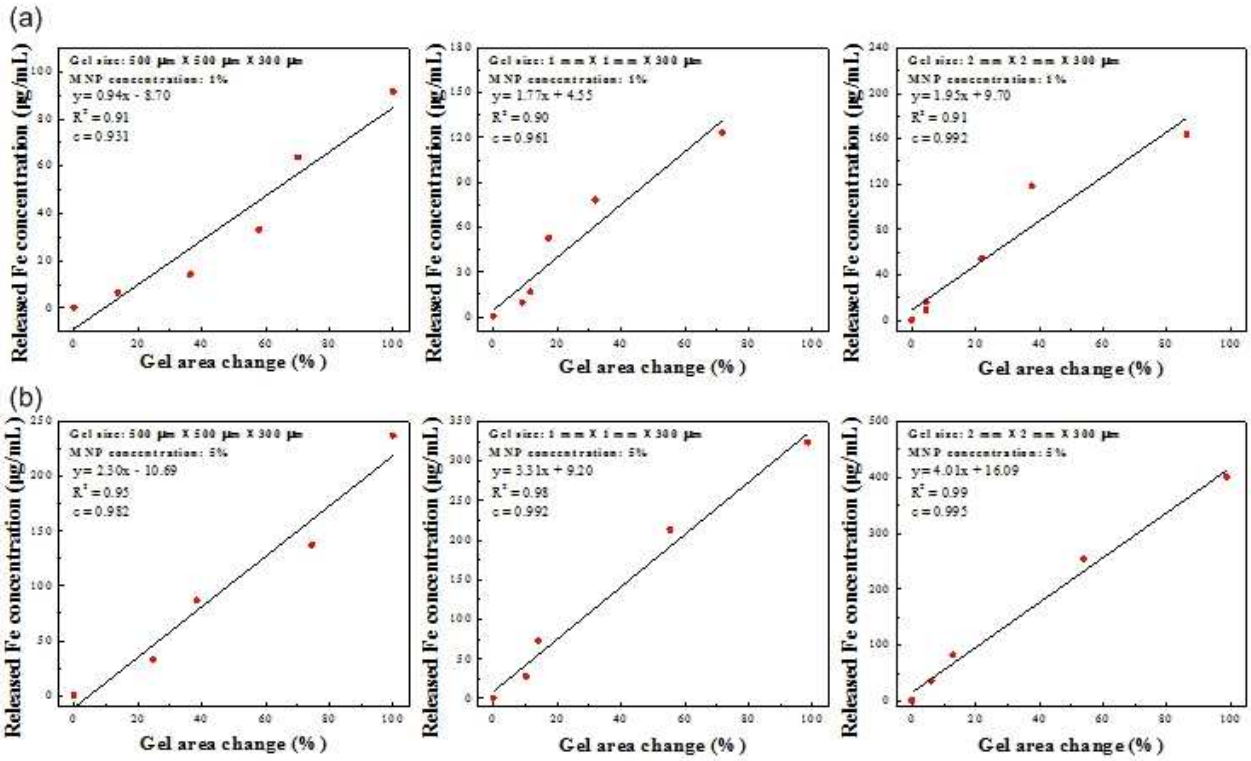

FIGURE S2. Release profile of MNPs during hydrogel degradation as measured by ICP-AES. The correlation between the degradation of hydrogels of different sizes and Fe concentration released from gels encapsulating (a) 1% MNP and (b) 5% MNP. The results indicated that the release of encapsulated MNPs is directly proportional to the degradation of hydrogels. "c" represents the Pearson product moment correlation coefficient.

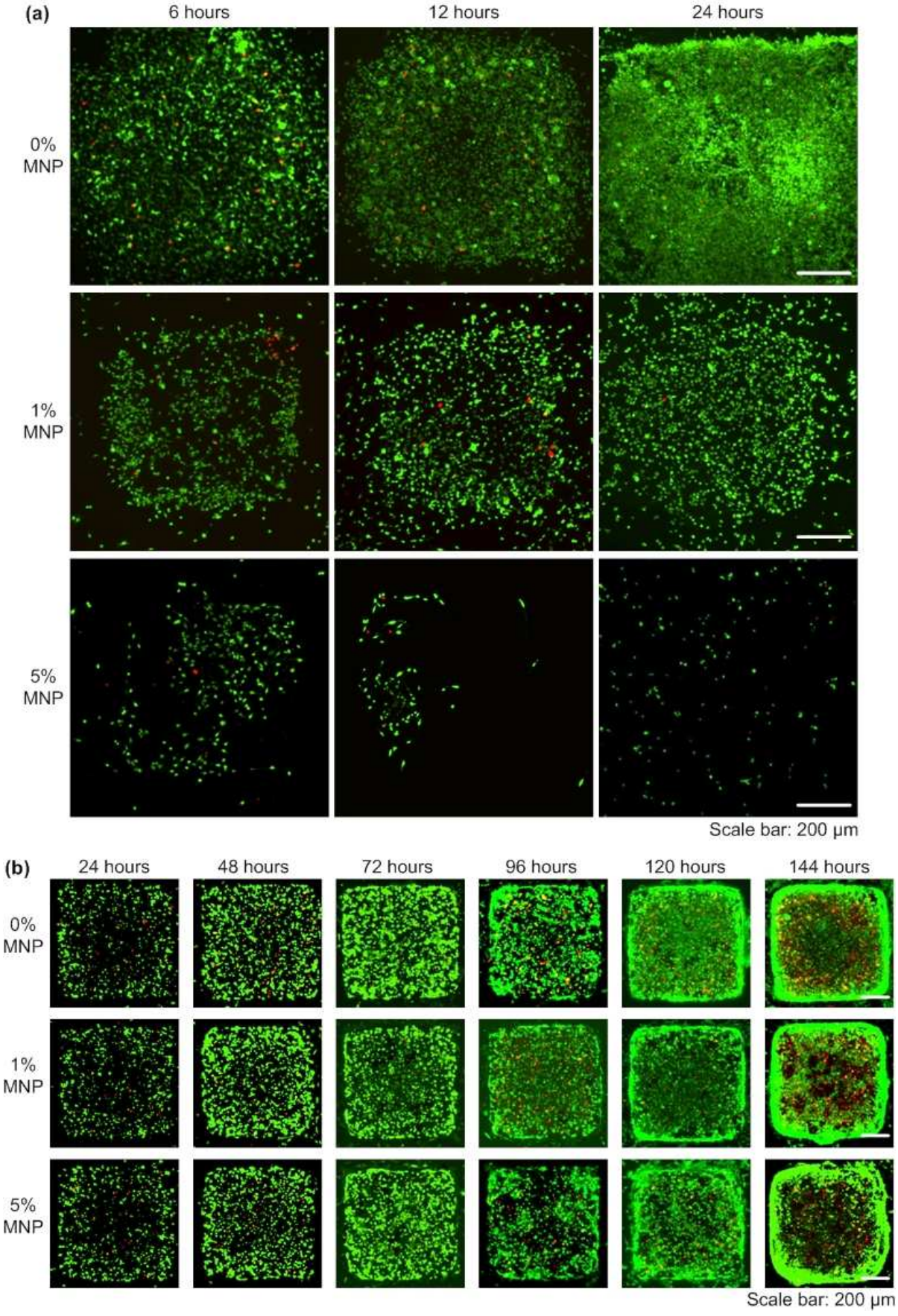
(a)
6 hours
12 hours
24 hours
0% MNP
1% MNP
5% MNP
Scale bar: 200 μm
(b)
24 hours
48 hours
72 hours
96 hours
120 hours
144 hours
0% MNP
1% MNP
5% MNP
Scale bar: 200 μm

FIGURE S3. Cell growth in MNP encapsulating hydrogels. (a) 1 mm x 1 mm x 300 μm hydrogels encapsulating 3T3 cells and MNPs were cultured in 3T3 medium with collagenase type I. Fluorescence live/dead images of were taken at t=6, 12, 24 hours. (b) 1 mm x 1 mm x 300 μm hydrogels encapsulating 3T3 cells and MNPs were cultured in 3T3 medium without collagenase. Fluorescence live/dead images of were taken at t= 24, 48, 72, 96, 120, 144 hours. Red and green dots indicate dead and live cells, respectively.