Supplementary Information

Spatially-controlled apoptosis induced by released nickel(II) within a magnetically responsive nanostructured biomaterial

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S1: Rheometry on calcium alginate gels containing MNPVs

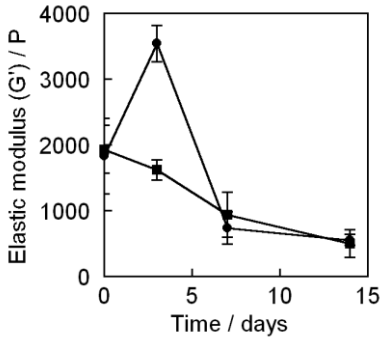

Figure S1. (a) Rheological studies of calcium alginate gels containing MNPVs. Changes in the elastic modulus over time for gels incubated in PBS at 37 °C (●) and for gels incubated in media supplemented with fetal bovine serum at 37 °C (■). Each value is an average of three measurements.

S2: Rate of 5/6-CF and Ni(II) diffusion out of 25 mm^3 calcium alginate blocks

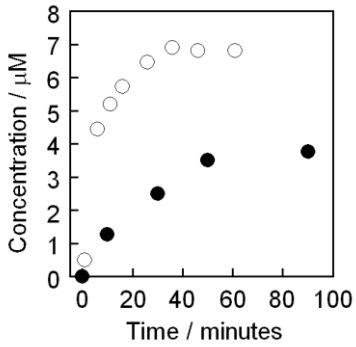

Figure S2. Rates of release from an alginate gel block at 37 °C for (○) 0.58 mM 5/6-CF and (●) 0.53 mM Ni(II).

S3: Comparison of cell responses after AMF-induced release of Ni(II) from MNPVs containing 7.7 mM or 15.4 mM Ni(II)

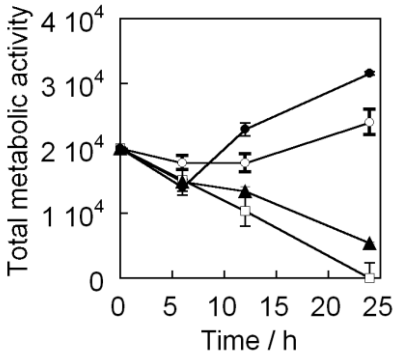

Figure S3. Post-AMF Alamar blue assay (normalised to cell seeding count) of 3T3 fibroblasts mixed with: (○) vesicles containing Ni(II) at 15.4 mM; (●) vesicles containing Ni(II) at 7.7 mM; (□) biotinylated MNP/Ni(II)-containing vesicle assemblies containing Ni(II) at 15.4 mM; (▲) biotinylated MNP/Ni(II)-containing vesicle assemblies containing Ni(II) at 7.7 mM.

S4: Release of 5/6-carboxyfluorescein (5/6-CF) from MNPVs composed of dioleoyl phosphatidylcholine (DOPC)

A modified procedure was used to measure 5/6-CF release from MNPVs in suspension. Vesicles with DSPE-PEG 2000-Biotin (2% mol/mol) in DPPC or DOPC (1 mL, 20 mM lipid concentration) were prepared by evaporation of a chloroform solution of the lipids to form a thin film. This film was rehydrated in 5/6-CF solution (1 mL, 0.05 M in phosphate buffer adjusted to pH 7.4). The resulting suspension was extruded (19×) through an 800 nm pore size polycarbonate membrane using an Avestin Liposofast extruder to give a suspension of unilamellar vesicles. In the case of DPPC vesicles, the extrusion temperature was above the T_m (approximately 41°C). Unencapsulated 5/6-CF was removed by GPC; the vesicle suspension (1 mL) was made up to 2.5 mL with the appropriate buffer solution and loaded onto an equilibrated column. Elution with buffer (3.5 mL) gave vesicles encapsulating 5/6-CF in 3.5 mL buffer. The volume was reduced by centrifugation of the suspension for 15 minutes (1500 g), yielding a compact plug of vesicles. The supernatant was carefully removed and vesicles resuspended in PBS to give 1 mL of suspension at 20 mM lipid.

The vesicle suspension (200 µL, 20 mM) was added to a sonicated solution of biotinylated nanoparticles (1.8 mL in PBS, final nanoparticle concentration 0.5 mg/mL). Avidin (50 µL of a 10 mg/ml solution) was added and the mixture briefly agitated. The samples were then left to aggregate at 37°C for approximately 40 minutes. After this time a magnetically responsive MNPV precipitate forms (~300 µL).

The samples were exposed to a 392 kHz AMF pulse for 300 s. Release was quantified after 45 minutes by taking aliquots (200 µL) of the sample supernatant. These were diluted with PBS (1.8 mL) and the fluorescence emission intensity at 520 nm recorded (ex. 490 nm). The 5/6-CF concentration in the supernatant was determined by calibration of fluorescence intensity values onto a standard curve. Percentage release was determined by obtaining maximum values through heating the samples to > 50°C for at least 1 h (DPPC MNPVs) or addition of Triton X-100 (DOPC MNPVs) and measuring the fluorescence intensity.

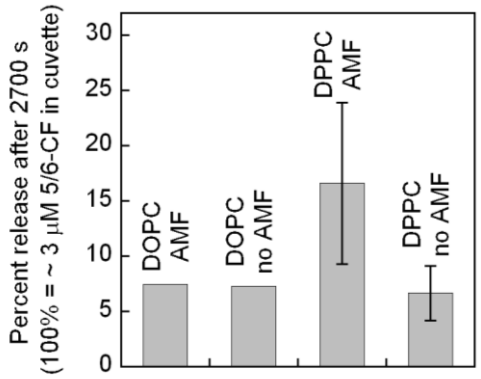

Figure S4. Comparison of percentage release after 2700 s from MNPVs composed of DOPC or DPPC after exposure to a 392 kHz AMF for 300 s. After 14 h, release from AMF-exposed DPPC MNPVs was (50 ± 10) %.

S5: Microscopy images of MNPVs before and after exposure to a 392 kHz AMF.

Rhodamine-DHPE labeled MNPVs (20 mM DPPC lipid in PBS, 0.1 % mol/mol Rh-DHPE) were exposed to a 300 s AMF. Fluorescence microscopy images taken before and after exposure to the AMF (samples diluted to 2 mM lipid) did not reveal significant differences in aggregate morphology, indicating that cross-linking interactions remain present and vesicle-like structures are preserved. The integrity of the vesicle membranes cannot be visualized using LUVs, but Baglioni and co-workers have shown release of a fluorescent dye from GUVs after exposure to a low frequency AMF (200 Hz) is due to perturbation of the GUV membrane but not membrane collapse.[1]

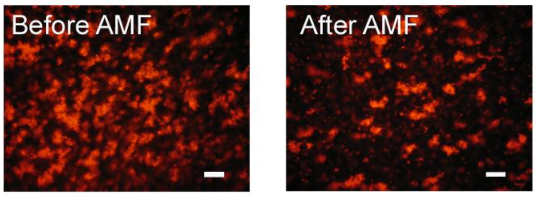

Figure S5. Fluorescence microscopy images of rhodamine-DHPE labeled MNPVs both before and after exposure to an AMF (300 s). Samples diluted to 2 mM lipid, scale bar 10 μm.

S6: Rheometry on calcium alginate gels containing MNPVs

Induction heating was carried out with a water-cooled EASYHEAT 0224 induction heater (2.0 kW, 150-400 kHz) with an EASYHEAT 300P workhead, purchased from Cheltenham Induction Heating, Gloucestershire GL52 6RU, U.K. A generalized procedure is as follows. Samples were placed inside an insulating jacket within the coils of the induction heater and the sample exposed to an AMF pulse (240 s or 300 s, 392 kHz frequency AMF, field strength calculated as 42 kA/m (2 turn solenoid, n = 140 turns/m and I = 300 A).

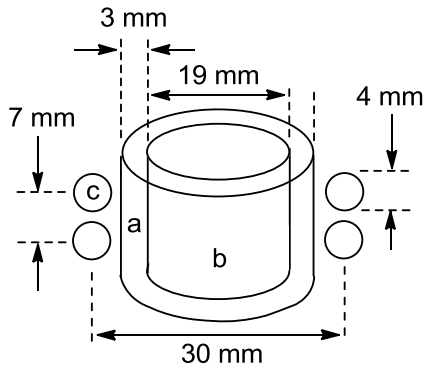

Figure S6. Schematic representation of the inductive coils used to generate the AMF

[1] S. Nappini, T. Al Kayal, D. Berti, B. Nordèn and P. Baglioni, *J. Phys. Chem. Lett*. 2011, **2**, 713-718