Supplementary Material

Alginate/GelMA Microparticles via Oil-free Interface Shearing for Untethered Magnetic Microbots

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Section S1: Synthesis of GelMA

Firstly, 10 wt % gelatin (Type A, Sigma, USA) was added to phosphate buffer solution (1×PBS, Labshark, China) and rapidly stirred to dissolve in a water bath to 50 °C. Then, after complete dissolution of the gelatin, 0.5 % (v/v) methacrylic anhydride (MA, Aladdin, China) was added dropwise to the gelatin solution, with continuous stirring. And the reaction was carried out in the dark at 50 °C for 2 hours. Afterward, the heating was stopped, and a reaction solution with double the volume of PBS was added to dilute the reaction mixture and terminate the reaction between gelatin and MA. The solution was filtered using medium-speed filter paper to remove flocculent by-products. The filtered solution was poured into a dialysis bag (molecular weight cutoff 14 kDa) and dialyzed with deionized water at 40°C, with water changed three times daily, for a total of 7 days. The dialyzed GelMA solution was placed in a freeze dryer (Scientz-12N/A, Scientz, China) and freeze-dried for 2 days until a white porous foam was obtained. The final product was stored in the dark at -20 °C.

Section S2: Experimental equipment for microparticle preparation and characterization

The experimental setup for microparticle preparation primarily consists of a signal generator (DG2052, Rigol, China), amplifier (SA-PA010, WuxiShiao, China), injection pump (LSP01-1A TW, Longer, China), vibrator (Vibration generator, Dayangkejiao, China) and precision tip needle (21G,outer diameter: 0.81 mm, inner diameter: 0.51 mm). The temperature control module (XH-W3002, Huilide, China) is integrated into the experimental setup to maintain a constant temperature of the prepolymer liquid within the tubing. A neodymium magnet with 60×10×2 mm^3 cylinder exhibiting a static magnetic field at the surface of 120 mT. This alters the magnetic environment within the container, and a magnetometer (WT10A, WEITE, China) is used to measure the central intensity of the magnetic field. A 365 nm UV lamp (UV-030A，DAZZLE，ShenZhen) was used to photocure GelMA in the microparticles.

The magnetic control device is composed of a signal generator (FY8300S, FeelElec Ltd., China) and a two-channel power amplifier (DPA-1698, JUNCTEK Ltd., China). The magnetic control device is placed under the camera equipped with 4× microscopic lens (Olympus Ltd., Japan) and the trajectory video of the microrobots is captured by the camera (Fujifilm, Japan).

The rotational rheometer (MCR 302e, AntonPaar, Austria) was to measure the viscosity of the prepolymer. The production process of microparticles was monitored using a high-speed camera equipped with 60 mm F 2.8 macro lens (X-H2S, Fujifilm, Japan). Optical images of the microparticles were obtained by an inverted microscope (TMV8000, China). The alginate/ GelMA microparticles morphology features were observed by scanning electron microscopy (SEM) (SEM3100, CIQTEK Quantum Science & Technology, China). The magnetic property measurement system (SQUID-VSM, Quantum Design, USA) was used to measure the pure Fe_3O_4 NPs and alginate/GelMA microparticle in the room temperature. The dimensions of patella removed from the rats are measured using a 3D ultra depth of field digital microscope (Easyzoom, Motic, China)

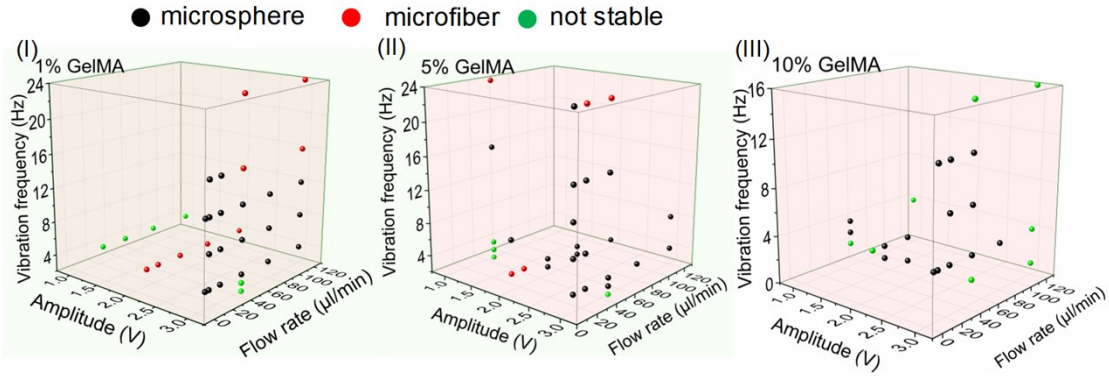

Fig.S1. Adjustable parameter domain of Q, f_s and vibration amplitude for generating microparticles. (Black: microparticle, Red: microfiber, Green: not stable).

Fig.S1 shows the adjustable range of the Q, f_s and vibration amplitude for the referenced state. The black dots represent the available microparticles, and the red dots represent the microfiber. The green dots represent the unstable state. For 10% GelMA concentration hydrogel prepolymer, only microparticles and unstable states are observed. Stable microparticles can be formed when the f_s is 20 Hz.

Section S3: Cytotoxicity tests

Human osteosarcoma cells (Saos-2) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum. The cells were seeded into petri dishes and incubated at 37C in a 5% CO_2 cell culture incubator (WJ-80A, CIMO Medical Instrument). Prior to co-culture, the prepared GelMA /alginate hydrogel microparticles with 5% wt Fe_3O_4 NPs were immersed in 75% ethanol for 20 minutes. Then the samples were immersed in PBS solution for 60 minutes, with the PBS solution being replaced every 30 minutes. Finally, the sample was sterilized by exposure to UV light for 1 hour, then samples were immersed in 1.5 mL of DMEM medium

supplemented with 10% fetal bovine serum for 20 minutes. Saos-2 cells were digested down using trypsin, and 10 μL of cell suspension was added to the cell counting plate (Countstar BioTech), and the cells were counted using an manual method with the density of 1.5 × 10^5 cells/ml. The Saos-2 cells at equivalent density were seeded in petri dishes as the control group, while the cells were co-cultured with the hydrogel microparticles for a duration of 3 days as experimental group. Calcein AM/PI staining was performed on control and experimental groups at the first day, second day, and third day to distinguish live cells (calcein-AM staining) from dead cells (calcein-PI staining). and The cells were observed using fluorescence microscopy (CSIM-130). The fluorescene images of the experimental group in three different regions on different days were compared to those of the control group. The number of live (green fluorescence) and dead (red fluorescence) cells in each region was calculated using the cell counting tool (ImageJ NIH). The cell viability of co-cultured cells was calculated as the percentage of live cells relative to the total number of cells (live cells + dead cells).

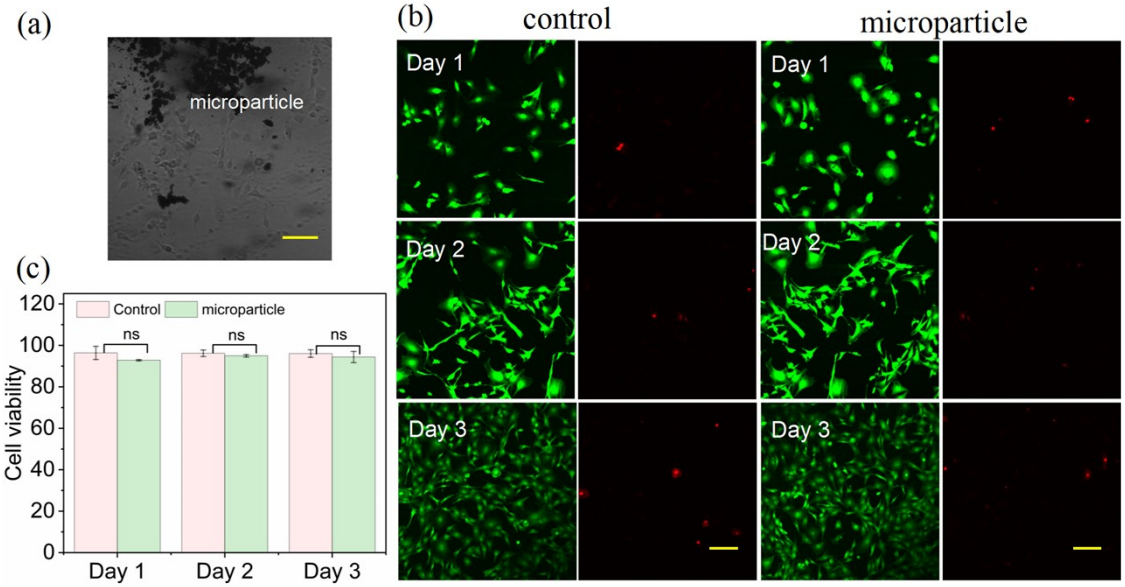

Fig. S2 Biocompatibility properties of hydrogels microparticles. (a) photographs of co-cultures of cells and hydrogel microparticle ((n=3, ns: P>0.05)). (b) Cytotoxic tests of microparticles by the Live/Dead Staining Kit (scale bar, 100 μm).(c) Cell viability of Saos-2 cells only and co-cultured with hydrogel microparticles for 3 days.

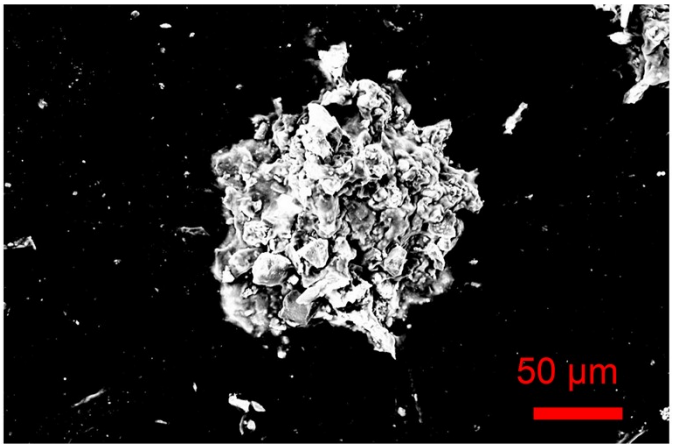

Fig.S3. (a) SEM image of hydrogel microparticle.

The image of hydrogel microparticle using SEM reveals that the microparticles exhibited a spherical structure shown in Fig.S3.

Section S4: Preparation and tensile tests of the hydrogel sample

Tensile testing of the hydrogels was performed using a universal tensile testing machine (REGER RGM10). A sensor with a range of 100 N was used and the loading rate was set to 10 mm/min. The tensile stress (δ) was calculated as $\delta = F/S$, where F is the tensile load (N) and S is the original cross-sectional area (m^2). The tensile strain (ε) is defined as $\varepsilon = (L - L_0)/L_0 \times 100\ \%$, where L_0 is the initial length (mm) of the hydrogel, and L is the breaking length (mm) of the hydrogel

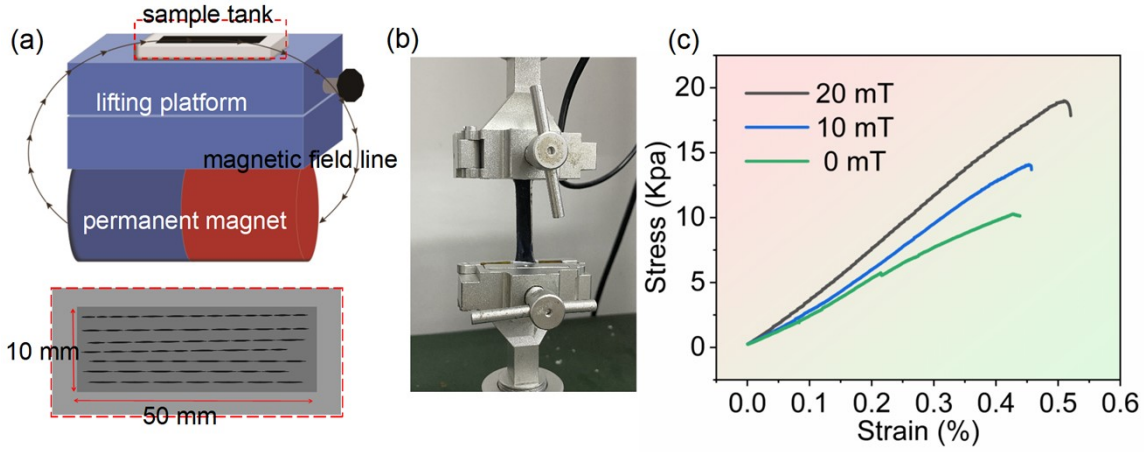

Fig.S4. Magnetic field on the mechanical properties of the microparticles (a) Schematic diagram of a device guiding the distribution of Fe_3O_4 NPs in hydrogel sample. (b) Photograph of tensile experiment. (c) Tensile curves of GelMA/alginate hydrogels supported with Fe_3O_4 NPs with the different magnetic field intensity.

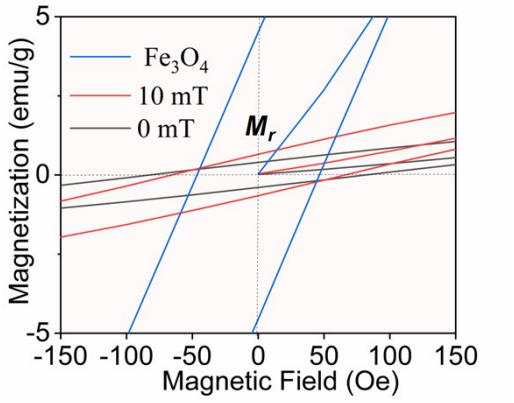

Fig.S5 .Enlarged hysteresis loops.

Section S5: Swelling test

In briefly, the prepared three cylindrical hydrogel samples were immersed in PBS for overnight to get a swelling equilibrium at room temperatue (24 ℃). Then, after wiping off the surface water of the swollen hydrogels, the weight (W_s) was recorded. The dry weight (W_d) of samples was obtained after further frozen and lyophilized using freeze drier. The swelling ratio (Q_o) was calculated by Equation. :

$$Q_o = (W_s - W_d)/W_d \qquad (1)$$

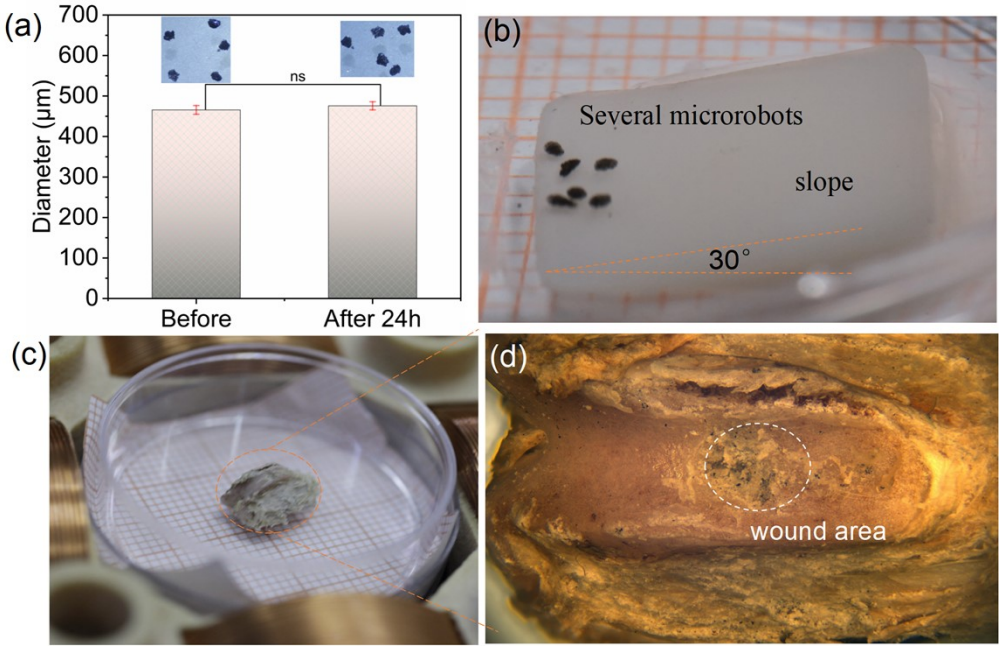

Fig.S6 (a) Diameter change of the microparticles upon submergence in PBS (n=5, ns: P>0.05) (b) A group of microparticle robots. (c) Photograph of patella under the RMF. (d) Three-dimensional reconstruction of the patella.

Section S6: Wound experiment

The wound experiment of patellar surface follows below steps:

(1) Anesthesia:

Firstly, the rats were anesthetized using 3% concentration of isoflurane mixture gas. Then, the concentration of anesthesia gas was maintained at 2%, and surgical modeling was performed.

(2) Modeling of articular cartilage defect:

The hair at the knee joint of the rat was shaved with the skin expose. After iodophor disinfection, a scalpel was used to longitudinally cut the cortical and muscular layers in the medial patellar ligament of the right knee of rats. And the patella was withdrawn to the opposite side to expose the internal joint.

(3) A cylindrical defect with a diameter of 1.0mm and a depth of 0.5mm was constructed by hand-held bone drill at the lower edge of the patella.