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Supporting Information

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Fabrication and Characterization of Magnetic Microrobots for Three-Dimensional Cell Culture and Targeted Transportation

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Fabrication and Characterization of Magnetic Microrobots for Three-dimensional Cell Culture and Targeted Transportation

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Supporting Information

Exposure tests to optimize the parameters for microrobot fabrication by 3D laser lithography

Table S1. Test parameters for 3D laser lithography using a Nanoscribe (Nanoscribe, Germany) with an oil-immersion 100× Zeiss objective lens.

Slice distance[a] [μm]	Scan speed[b] [μm s^{-1}]	Laser power[c] [mW]
1.2	50	2.0
0.9	100	3.2
0.6	200	4.4
0.3		5.6

[a] Slice distance is the distance between adjacent horizontal lines. [b] Scan speed and [c] laser power determine the total laser power delivered to the exposed spot. The combination of these parameters determines the quality of the structure, as shown in **Figure S1**.

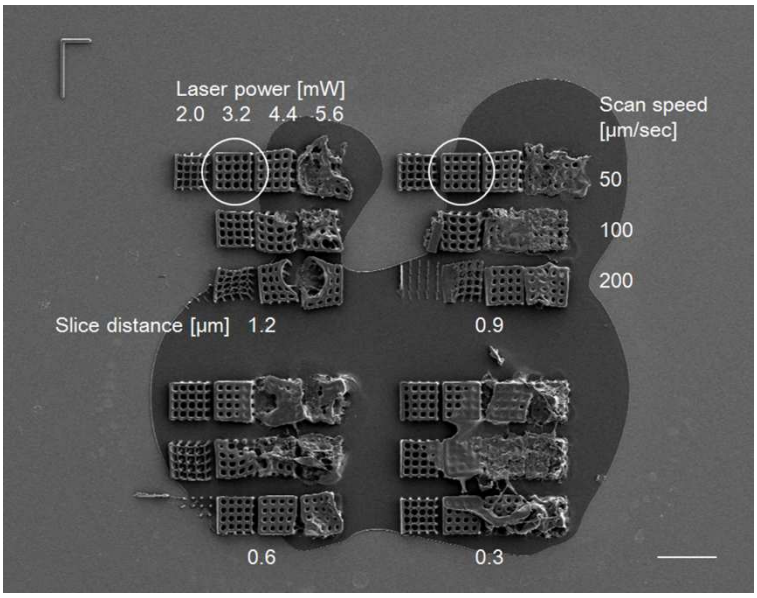

Figure S1. Scanning electron microscopy (SEM) image of parametric test results using SU-8 with test structures. The slice distance, scan speed, and laser power were varied as listed in Table S1. Two well-defined structures were fabricated with optimum parameters; 1.2 and 0.9 μm for the slice distance, 50 μm s^{-1} for the scan speed, and 3.2 mW s^{-1} for the laser power. The scale bar is 100 μm.

Various designs of microrobots

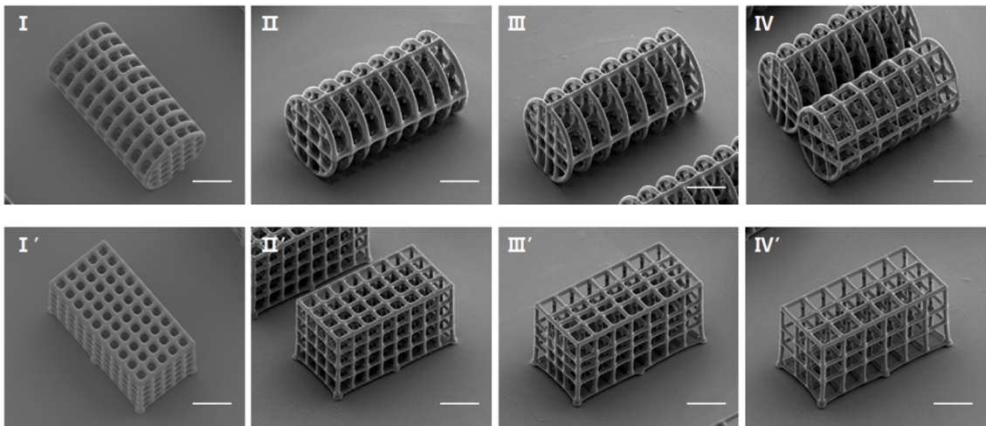

Figure S2. SEM images of various designs of the fabricated microrobots. The design values are listed in Table 1. The pore sizes of the microrobots are 10, 13, 17, and 21 μm for I (I′), II (II′), III (III′), and IV (IV′), respectively, which covers most of the biological cell sizes. The

scale bar is 40 μm.

Experiment on magnetic manipulation

After fabrication of the microrobots on a glass substrate, each microrobot was detached from the substrate by a probe manipulator. The microrobots were immersed in a plastic container filled with DI water. The container was placed in the center of a magnetic manipulator, and exposed to external magnetic fields. For translational motion, an external magnetic field gradient was applied to the microrobot, generated by current through the coils inside of the magnetic manipulator. The rotational frequency and magnetic field intensity were examined in a previous study of helical microrobots.[1]

An external magnetic field gradient of 800 mT m^{-1} was applied along the *x*-axis with *z*-aligned microrobots to demonstrate translational motion, as shown in Figures 2a,c and Video S1. Figures 2b,d and Video S2 show rotational motion with a rotation frequency of 3 Hz. For the cylindrical microrobots, the translational velocity was ~50 μm s^{-1} (~1/3 body lengths per second) using a field gradient of 800 mT m^{-1}; the locomotion efficiency was lower than that reported for helical microrobots.[2] We demonstrated rolling motion for high-speed locomotion, as shown in Figures 2e and Video S3. For a rotation frequency of 4 Hz, the velocity of the rolling motion was 277 μm s^{-1} (1.85 body lengths per second); this was five times greater than the translational velocity. In Figure 2f and Video S4, a 2-Hz rotation field was applied to reduce the resistive force for targeted control.

Materials and method for cell culturing

Cell culture and SEM inspection: Prior to cell seeding, the microrobot fabricated on a glass wafer was coated with 10 μg mL^{-1} of poly-L-lysine (PLL) (Sigma Chemical, USA), followed by sterilization using an autoclave and ultraviolet irradiation. The human embryonic kidney

(HEK) 293 cells were grown on dishes in Dulbecco's Modification of Eagle's medium (DMEM) (Thermo, USA), supplemented with 10% fetal bovine serum (FBS) (Thermo, USA) and 1% antibiotics at 37°C under 5% CO_2. HEK293 cells (1×10^6 cells per mL) were seeded in the culture dish containing the microrobot. The dish was stored in an incubator at 37°C under 5% CO_2. The cells were cultured for four days, and the growth media was changed every other day. After four days, we immersed the microrobot in 4% paraformaldehyde (PFA) solution, and stored the microrobot at 4°C overnight. Lastly, the microrobot was coated with platinum for examination using SEM.

Immunocytochemistry assay: First, we affixed the HEK 293 cells cultured in the PLL-coated microrobots using a 4% PFA solution. The cells were immersed in 0.5% Triton X-100, which contained Dulbecco's phosphate-buffered solution (DPBS) (Thermo, USA), at room temperature. After 10 min, the cells were incubated with DPBS containing 4% normal donkey serum (NDS) (Jackson ImmunoResearch, USA) for 1 h. To label β-actin, we incubated the cells with antibodies against β-actin (Chemicon, Germany) diluted at 1:500 at 4°C for 2 h. Subsequently, the cells were washed twice with DPBS and incubated with fluorescein isothiocyanate (FITC) conjugated antibodies (Jackson Immuno Research, USA), diluted at 1:1000 for cytoskeletal staining at room temperature for 2 h. We visualized the nucleus of cells cultured in the microrobot using DAPI (Vector, USA) and β-actin under a confocal microscope (LMS 700, Zeiss, Germany). Confocal microscopy images were taken of the microrobots on a glass substrate, as shown in Figure S3. The microrobot contained cells inside of its structure, and was detached for wireless manipulation.

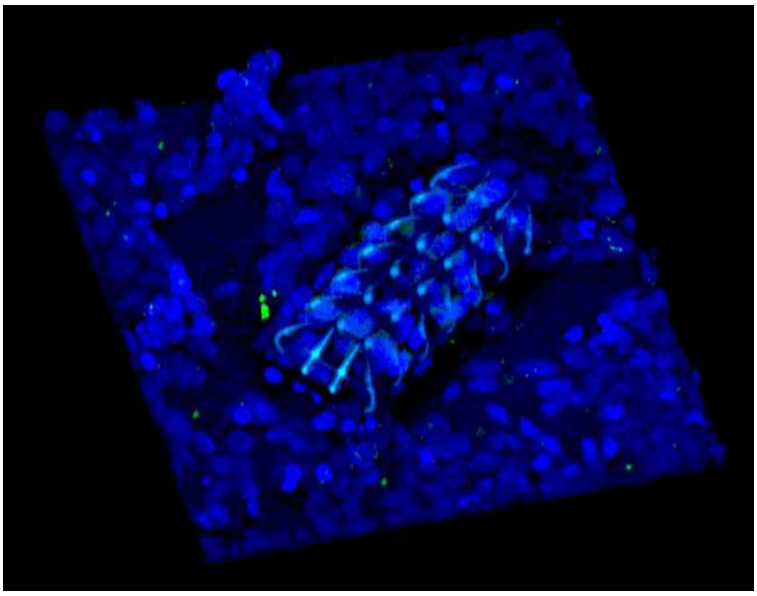

Figure S3. Confocal microscopy image of a cylindrical microrobot on a glass substrate after staining the cells.

Video clips

Video S1. 1-D translational manipulation of microrobots along the *x*-axis with 800 mT m^{-1} of magnetic field gradient on *z*-aligned microrobots.

Video S2. 1-D rotational manipulation of microrobots using a 3-Hz rotating field.

Video S3. Synchronized swimming for microrobots with rolling motion with a rotation frequency of 4 Hz.

Video S4. Targeted control of microrobots using a rotation frequency of 2 Hz.

References

[1] S. Tottori, L. Zhang, F. Qiu, K.K. Krawczyk, A. Franco-Obregón, B.J. Nelson, *Adv. Mat.* **2012**, 24, 811.

[2] S. Schuerle, S. Erni, M. Flink, B.E. Kratochvil, and B.J. Nelson, *IEEE Tran. Magn.* **2013**, 49, 321.