Supplementary Materials for

Multifunctional biohybrid magnetite microrobots for imaging-guided therapy

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The PDF file includes:

- Section S1. Autofluorescence of biological materials
- Section S2. Dip-coating *S. platensis* with Fe$_3$O$_4$ NPs
- Section S3. Magnetic actuation of BMRs
- Section S4. Fluorescence properties and in vivo imaging
- Section S5. MR imaging in vitro and in vivo
- Section S6. Degradation of MSPs
- Section S7. Cytotoxicity to SiHa and 3T3 cell lines
- Fig. S1. Autofluorescence of biological materials with various structures.
- Fig. S2. Characterizing the autofluorescence of *S. platensis*, pine pollen, and *S. cerevisiae*.
- Fig. S3. Photostability tests in DI water for *S. platensis* (100 μg/ml), *S. cerevisiae* (1 mg/ml), and *C. salina* (100 μg/ml).
- Fig. S4. Characterization of *S. platensis* (i.e., MSP-0h) and Fe$_3$O$_4$ NPs.
- Fig. S5. ζ potential of *S. platensis* and Fe$_3$O$_4$ NP suspension at different pH.
- Fig. S6. Characterization of the magnetized *S. platensis*.
- Fig. S7. Magnetic hysteresis loops of MSP-6h/24h/72h (300 K, via VSM).
- Fig. S8. FESEM images of MSP-72h that has undergone a 5-min sonication treatment.
- Fig. S9. The periodically varying magnetic field for the actuation of MTS-24h.
- Fig. S10. Strength of the magnetic field generated by the permanent magnet B versus the distance d to its rotation axis.
- Fig. S11. Photostability test of MSP-72h.
- Fig. S12. Supplementary data for fluorescence-based in vivo imaging.
- Fig. S13. $T_2$-weighted MR imaging of MSP samples in vitro and in vivo.
Fig. S14. Degradation of MSP in 37°C DPBS solution.
Fig. S15. Supplementary data for cytotoxicity evaluation.
Fig. S16. CLSM imaging for 3T3 and SiHa cells cocultured with MSP-24h samples (0, 100, and 400 μg/ml) for 24 and 48 hours.
Table S1. Quantitative measurement of Fe contents in MSP-6h/24h/72h samples.
Table S2. Main distribution of body length (in pitches) for MSP samples under different conditions.
Table S3. Viscosity of various fluids for in vitro/in vivo swimming experiments.
Table S4. Quantitative data of the emission peaks for MSP-0h/6h/24h/72h samples in Fig. 3A.
Table S5. Quantitative data of the emission peaks for MSP-0h/24h/72h samples before and after degradation.
References (77–79)

Other Supplementary Material for this manuscript includes the following:
(available at robotics.sciencemag.org/cgi/content/full/2/12/eaaq1155/DC1)

Movie S1 (.avi format). Magnetic actuation of three-pitch MSP-72h in DI water using a rotating magnetic field (7.5 mT and 10 Hz).
Movie S2 (.avi format). Magnetic actuation of MCR-24h (θ = 90°) in DI water using a rotating magnetic field (7.5 mT and 5 Hz).
Movie S3 (.avi format). Magnetic actuation of MCR-24h (θ = 0°) in DI water using a rotating magnetic field (7.5 mT and 3 Hz).
Movie S4 (.avi format). Magnetic actuation of MTS-24h in water using a periodically varying magnetic field described in fig. S9.
Movie S5 (.avi format). Magnetic actuation of three-pitch MSP-24h in diluted blood using a rotating magnetic field (7 mT and 6 Hz).
Movie S6 (.avi format). Magnetic actuation of three-pitch MSP-24h in gastric juice using a rotating magnetic field (7 mT and 8 Hz).
Movie S7 (.avi format). Magnetic actuation of three-pitch MSP-24h in urine using a rotating magnetic field (7 mT and 4 Hz).
Movie S8 (.avi format). In vitro magnetic actuation of a MSP-72h swarm in PO (first pressed).
Section S1. Autofluorescence of biological materials

Fig. S1. Autofluorescence of biological materials with various structures. The bright-field and fluorescence images of *S. cerevisiae* and *R. rubrum* were taken by a Leica SP8 confocal laser scanning microscopy (CLSM) with excitation at 405 nm; of human hair, pine pollen, kapok fiber and lotus vessel, at 488 nm; of *C. reinhardtii*, *C. salina*, *T. subcordiformis* and *S. platensis*, at 552 nm. In particular, fluorescence images of human hair and kapok fiber were obtained through projection of 3D stacks using ImageJ®. The fluorescence spectra were recorded using a Hatachi F-7000 Fluorescence Spectrophotometer at a scanning rate of 1200 nm/min. The concentration for *S. cerevisiae* and pine pollen tests was respectively 4 mg/mL and 2 mg/mL, and for *R. rubrum*, *C. reinhardtii*, *C. salina*, *T. subcordiformis* and *S. platensis* it was all 100 µg/mL (note that the control of concentration does not apply to human hair, kapok fiber and lotus vessel). All experiments were conducted in DI water.
Fig. S2. Characterizing the autofluorescence of *S. platensis*, pine pollen, and *S. cerevisiae*.

(A) Fluorescence emission spectra of 100 µg/mL *S. platensis*, 2 mg/mL pine pollen and 1 mg/mL *S. cerevisiae* in various solutions. Quantitative analysis for the effect of sample concentration on the (B) full width at half maximum (FWHM), (C) intensity of emission peak and (D) wavelength of emission peak.

Autofluorescence, also known as natural fluorescence, has been found in a variety of biological materials (33, 34). It is attributed to naturally occurring fluorescent compounds such as flavins, nicotinamide-adenine dinucleotide/dinucleotide phosphate, lipofuscin, advanced glycation end-products, protoporphyrin IX, lignin, holocellulose, chlorophyll and drosophila, and so on. In the literature, this phenomenon has frequently been considered as an adverse effect, which interferes with the normal function of artificial fluorescent markers (34). Herein, we demonstrate in this report that biological autofluorescence, if properly harnessed, can actually work as cost-effective fluorescent tags for *in vitro* and *in vivo* imaging of micro- and nano-robots. Better yet, compared with artificial markers such as fluorescent dyes and quantum dots, biological
fluorescent tags are usually more stable, meanwhile, less vulnerable to external conditions, owing to the fact that the fluorescent compounds are intrinsically embedded in intact biological structures (52, 53).

Biological autofluorescence in nature is found to exhibit broad emission spectra, from ultraviolet (wavelength: 100-400 nm) to near-infrared (wavelength: 750-2500 nm). This provides abundant fluorophore options for the preparation of inherently fluorescent robots. Shown in fig. S1 are the autofluorescence characteristics of ten distinct biological materials, including rod-like \textit{Saccharomyces cerevisiae} (\textit{S. cerevisiae}) and \textit{Rhodospirillum rubrum} (\textit{R. rubrum}), filamentous human hair and kapok fiber, ellipsoidal pine pollen and \textit{Tetraselmis subcordiformis} (\textit{T. subcordiformis}), helical lotus vessel and \textit{Spirulina platensis} (\textit{S. platensis}), and spherical \textit{Chlamydomonas reinhardtii} (\textit{C. reinhardtii}) and \textit{Chlorella salina} (\textit{C. salina}). Specifically, factors that would affect their autofluorescence are investigated taking \textit{S. platensis}, pine pollen and \textit{S. cerevisiae} as examples. As illustrated by fig. S2, the fluorescence performance varies, depending not only on the type and concentration of solvents, but also pH values of the solution. Interestingly, all samples tested manifest significant fluorescent signals in physiological media such as 5\% dextrose, Dulbecco’s Phosphate Buffered Saline (DPBS) and cell growth media (CGM). Photostability of the autofluorescence is also examined, which turns out to be rather good, with steady fluorescent signal detected even after continuous illumination of 1h (fig. S3).

**Fig. S3. Photostability tests in DI water for \textit{S. platensis} (100 μg/ml), \textit{S. cerevisiae} (1 mg/ml), and \textit{C. salina} (100 μg/ml).** For \textit{S. platensis}, the fluorescent intensity was recorded at 651 nm with 552 nm excitation; for \textit{S. cerevisiae}, at 527 nm with 445 nm excitation; for \textit{C. salina}, at 690 nm with 552 nm excitation.

**Section S2. Dip-coating \textit{S. platensis} with Fe\textsubscript{3}O\textsubscript{4} NPs**

**Section S2.1. Characterization of \textit{S. platensis} and Fe\textsubscript{3}O\textsubscript{4} NP suspension**

\textit{S. platensis}, widely distributed in nature, is known for possessing a wealth of biocompounds such as biopeptides, biopolymers, carbohydrates, essential fatty acids, and so on (62, 63). As shown in fig. S4 (A and B), wet and freeze-dried samples of \textit{S. platensis} appear in green and dark-green colors, respectively (the freeze-drying process was performed in an iShinBioBase freeze dryer, TFD8503). Optical image in fig. S4C reveals that \textit{S. platensis} in DI water naturally presents helical morphology with a pitch of about 55 μm and a cross-sectional diameter of about 10 μm. The helical structure can overall be preserved after the freeze-drying treatment, though accompanied by severe shrinking (about 5-6 μm) as suggested by fig. S4 (D and E). High-
magnification FESEM imaging shows the clean surface of free-dried *S. platensis* (fig. S4E). And EDX test detects the existence of C, N, O, P and S elements (fig. S4F).

![Fig. S4](image)

**Fig. S4. Characterization of *S. platensis* (i.e., MSP-0h) and Fe₃O₄ NPs.** Digital images of (A) the *S. platensis* in DI water and (B) the freeze-dried *S. platensis*. (C) Optical image of the *S. platensis* in DI water, in which the sample was processed with sonication (20 kHz, 0.35 w/cm²) for 15 min. (D) Low- and (E) high-magnification FESEM images of the freeze-dried *S. platensis*. (F) EDX spectrum of the freeze-dried *S. platensis*, in which the Si element is due to the Si wafer that has been used as substrate for SEM samples. (G, H and I) Digital image, XRD pattern and size distribution of the Fe₃O₄ NP suspension. The inset of (I) shows its bright-field TEM image with a scale bar of 100 µm. The size distribution was determined by DLS measurement using Zetasizer Nano ZS90 (Malvern Instruments Ltd, UK).

The Fe₃O₄ NP suspensions (ca. pH = 2.1) were stocked in DI water at 4°C. Normally, Fe₃O₄ NPs can be well dispersed in DI water for two weeks without extra disturbance processing (fig. S4G). Even if they precipitate from the suspension solution, ultrasound can be employed to resuspend them. XRD was used to confirm the magnetite phase of the suspension (fig. S4H). And dynamic light scattering (DLS) measurement together with TEM imaging was employed to examine the shape and size of the Fe₃O₄ NPs, which were observed to present pseudo-spherical morphology with an average diameter of about 50 nm (fig. S4I).

**Section S2.2. Mechanism of BMR dip-coating**

In a typical dip-coating process for the fabrication of BMRs (Fig. 1A, main text), the mixture solution of *S. platensis* and Fe₃O₄ NP suspension has a pH value of ca. 3.0 (measured by a CHEETAH PHS-3C pH meter). As the zeta potential measurements (fig. S5), the surface charges
on the *S. platensis* and the Fe$_3$O$_4$ NP at such a pH are opposite. This leads to an electrostatic force that bonds the Fe$_3$O$_4$ NPs to the *S. platensis*, resulting in random formation of discrete Fe$_3$O$_4$ aggregates on the *S. platensis*. Meanwhile, organic compounds from the membrane and cytoplasm of *S. platensis*, e.g., proteins, polysaccharide chains and amino acid residues, might arrive at these aggregates via diffusion and exert a weakening effect on the electropositivity of the Fe$_3$O$_4$ NPs there, rendering them only marginally positive, or even more remarkable, reversing their apparent charge to electronegativity (31, 77-79). This effect counters the electrostatic repulsion between the Fe$_3$O$_4$ aggregates formed on the *S. platensis* and Fe$_3$O$_4$ NPs suspended in the bulk solution, enabling the former as nuclei for the growth of a layer of Fe$_3$O$_4$ coating. Based on the same principle, subsequent layers of Fe$_3$O$_4$ coating will grow. Since both the diffusion of compounds from the cell and the diffusion of Fe$_3$O$_4$ NPs from the bulk solution are time-dependent, the thickness of the Fe$_3$O$_4$ coating is correlated to the duration of the dip-coating process. With the dipping time extended from 6h to 72h, the distribution of Fe$_3$O$_4$ NPs on the *S. platensis* surface evolves from sparse to dense till the formation of a shell-like coating (Fig. 1A). This time-dependent feature enables us to tailor the magnetic property as well as the inherited functionalities of the *MSP*.

![Fig. S5. ζ potential of *S. platensis* and Fe$_3$O$_4$ NP suspension at different pH. These measurements were conducted using a JS94J micro-electrophoresis apparatus of POWEREACH® (Shanghai zhongchen digital technic apparatus co., ltd, China).](image_url)

**Fig. S5. ζ potential of *S. platensis* and Fe$_3$O$_4$ NP suspension at different pH.** These measurements were conducted using a JS94J micro-electrophoresis apparatus of POWEREACH® (Shanghai zhongchen digital technic apparatus co., ltd, China).

### section S2.3. Characterization of the magnetized *S. platensis* (*MSP*)

Compared to raw *S. platensis*, MSP appears darker in color, either as suspension in DI water or in freeze-dried form (fig. S6, A and B). Optical image of the *MSP* (fig. S6C) demonstrates its natural morphology as helix. FESEM images (fig. S6, D and E) show enlarged views of the *MSP*-24h and *MSP*-72h samples as in Fig. 1A of the main text, from which the distribution of Fe$_3$O$_4$ NPs on the surface can be clearly observed. For *MSP*-24h, there are numerous yet still not adequate nanoparticles to fully encase the surface, whereas for *MSP*-72h the distribution of nanoparticles are much denser and form a tight shell-like coating on the surface. Through a cross-sectional FESEM image, the thickness of the coating can be roughly measured as 650 nm to 950 nm (fig. S6F). The cross-sectional sample was prepared as follows: first, embed the freezing-dried *MSP*-72h samples into epoxy resin adhesive; second, cut a slice from the above mixture with diamond blades and put it on a silicon substrate; third, remove the epoxy resin adhesive using acetone.

The *MSP* samples were also examined by EDX, XRD and FTIR analyses. The results for *MSP*-6h are shown in fig. S6 (G to I), from which the composition of the coating can be confirmed as Fe$_3$O$_4$. Quantitative data of Fe contents contained by three *MSP* samples (i.e., *MSP*--
were extracted from the EDX analysis and listed in table S1. Apparently, the absolute amount of Fe in MSP is positively correlated to the length of dip-coating time. The magnetic properties of these MSP samples were characterized using a Vibrating Sample Magnetometer (VSM, PPMS Model 6000 Quantum Design). As shown in fig. S7, they are all superparamagnetic, and the saturation magnetization $M_s$ is 62.73 emu/g, 33.72 emu/g, and 9.16 emu/g for MSP-72h, MSP-24h, and MSP-6h, respectively.

**Fig. S6.** Characterization of the magnetized *S. platensis*. Digital images of (A) MSP-24h in DI water and (B) freeze-dried MSP-24h. (C) Optical image of MSP-24h in DI water, in which the S. platensis has gone through sonication (20 kHz, 0.35 w/cm$^2$) for 10 min. (D) and (E) FESEM images of MSP-24h and MSP-72h in enlarged views. (F) FESEM image of a cross-section from MSP-72h. (G) EDX spectrum of gold(Au)-coated MSP-6h (placed on a Si substrate). (H) XRD and (I) FTIR patterns of MSP-6h in comparison with those of MSP-0h.

**Table S1. Quantitative measurement of Fe contents in MSP-6h/24h/72h samples.**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Average values (wt%)</th>
<th>Error values (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSP-6h</td>
<td>3.18</td>
<td>± 2.00</td>
</tr>
<tr>
<td>MSP-24h</td>
<td>15.08</td>
<td>± 1.60</td>
</tr>
<tr>
<td>MSP-72h</td>
<td>23.50</td>
<td>± 1.92</td>
</tr>
</tbody>
</table>
Fig. S7. Magnetic hysteresis loops of MSP-6h/24h/72h (300 K, via VSM).

To attest the stability of the Fe₃O₄ coating, sonication (20 kHz, 0.35 w/cm²) was applied to treat MSP-72h for 5 min. The samples after sonication were then examined with FESEM, which still present a compact Fe₃O₄ shell on the surface (fig. S8) without discernable difference in comparison with the untreated samples as in Fig. 1A.

Fig. S8. FESEM images of MSP-72h that has undergone a 5-min sonication treatment. The unlabeled scale bar in the enlarged view (inset) represents 1 µm.
Section S3. Magnetic actuation of BMRs

All MSP samples used for magnetic actuation and imaging were fabricated from *S. platensis* treated with 10 min sonication prior to the dip-coating process. According to table S2, the body lengths were mainly 2-7 pitches for MSP-6h, and 2-5 pitches for MSP-24h/72h. We also employed low-magnification optical microscopy to count the number of MSPs in 20 µL 25 µg/mL MSP-6h/24h/72h samples, respectively. Each sample was counted twice by two different people. There were $63 \pm 4$ MSP-6h, $66 \pm 4$ MSP-24h and $62 \pm 5$ MSP-72h. Based on the conversion relation between the above 20 µL 25 µg/mL solution (containing 0.5 µg MSP sample) and MSP numbers, the number of MSPs in other solutions can be estimated.

**Table S2. Main distribution of body length (in pitches) for MSP samples under different conditions.** The MSP samples below were fabricated from *S. platensis* subject to different duration (0/3/10/15min) of sonication treatment (20 kHz, 0.35 w/cm²) of the *S. platensis* before the dip-coating process. The statistical analysis was based on low-magnification optical microscopy results in 20 µL 25 µg/mL MSP solutions.

<table>
<thead>
<tr>
<th>Samples</th>
<th>0 min MDBL</th>
<th>3 min MDBL</th>
<th>10 min MDBL</th>
<th>15 min MDBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSP-0h</td>
<td>&gt; 20</td>
<td>6 - 15</td>
<td>3 - 7</td>
<td>0 - 4</td>
</tr>
<tr>
<td>MSP-6h</td>
<td>&gt; 20</td>
<td>6 - 12</td>
<td>2 - 7</td>
<td>0 - 3</td>
</tr>
<tr>
<td>MSP-24h</td>
<td>&gt; 20</td>
<td>5 - 10</td>
<td>2 - 5</td>
<td>0 - 3</td>
</tr>
<tr>
<td>MSP-72h</td>
<td>&gt; 20</td>
<td>5 - 8</td>
<td>2 - 5</td>
<td>0 - 3</td>
</tr>
</tbody>
</table>

**Table S3. Viscosity of various fluids for in vitro/in vivo swimming experiments.** The viscosity values were measured under a shear rate of 100 s⁻¹ with a Physica MCR 301 rheometer at 25°C. The pH values were measured with a CHEETAH PHS-3C pH meter also at 25°C. All biofluids including blood, gastric juice and urine were extracted from SD rats. Diluted blood was obtained by adding DPBS into the whole blood at a volume ratio of 4:1; Gastric juice by centrifugal separation of food residue from the rat’s stomach. Peanut oil (PO, first pressed) was purchased from market as a solvent for *in vivo* MR imaging.

<table>
<thead>
<tr>
<th>Fluidic media</th>
<th>Viscosity (mPa.s)</th>
<th>pH values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (DI)</td>
<td>$1.014769 \pm 0.026277$</td>
<td>$6.8 \pm 0.3$</td>
</tr>
<tr>
<td>Diluted blood</td>
<td>$3.567220 \pm 0.487326$</td>
<td>$6.9 \pm 0.5$</td>
</tr>
<tr>
<td>Gastric juice</td>
<td>$5.802531 \pm 0.075662$</td>
<td>$2.0 \pm 0.3$</td>
</tr>
<tr>
<td>Urine</td>
<td>$1.462367 \pm 0.028471$</td>
<td>$5.3 \pm 0.2$</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>$52.054850 \pm 0.076623$</td>
<td>-</td>
</tr>
</tbody>
</table>
Fig. S9. The periodically varying magnetic field for the actuation of MTS-24h. Only 0s to 5s is shown here.

Fig. S10. Strength of the magnetic field generated by the permanent magnet B versus the distance $d$ to its rotation axis. The $B$ values were measured with a LakeShore 410 Gaussmeter. The inset shows how the permanent magnet is mounted on an 80BL brushless DC motor (Time Brilliant, Beijing, China).
Section S4. Fluorescence properties and in vivo imaging

Table S4. Quantitative data of the emission peaks for MSP-0h/6h/24h/72h samples in Fig. 3A.

<table>
<thead>
<tr>
<th>Samples</th>
<th>FWHM (nm)</th>
<th>Peak position (nm)</th>
<th>Peak height (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSP-0h</td>
<td>52.1</td>
<td>651.0</td>
<td>6.5</td>
</tr>
<tr>
<td>MSP-6h</td>
<td>56.7</td>
<td>651.2</td>
<td>5.3</td>
</tr>
<tr>
<td>MSP-24h</td>
<td>57.2</td>
<td>657.8</td>
<td>4.0</td>
</tr>
<tr>
<td>MSP-72h</td>
<td>57.7</td>
<td>658.4</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Fig. S11. Photostability test of MSP-72h. (A) Sedimentation phenomenon of MSP-72h during the photostability test. From 0~30 min, the proportion of the sample sinking to the bottom area marked with red rectangle increases as time is prolonged. Further to that, no notable increase can be found as suggested by the comparison between 60 min and 30 min. The trend of this sedimentation is in line with the variation of the fluorescence intensity in Fig. 3B of the main text. (B) Fluorescence images of individual MSP-72h subject to 0 min, 30 min and 60 min illumination (light intensity, 20W/cm²).
Fig. S12. Supplementary data for fluorescence-based in vivo imaging. (A) Fluorescence intensity of MSP-24h (300 µL, 800 µg/mL) in the intraperitoneal cavity of nude mic Balb/c athymic mice. The magnetic needle was removed at t = 22 min. Quantitative analysis of the fluorescence intensity (indicated by “Avg radiance” in vivo) in the subcutaneous tissue at different (B) sample concentration and (C) residence time. The injection volume was 100 µL for MSP-6h/24h/72h. (D) Quantitative analysis of the fluorescence intensity in the intraperitoneal cavity at different residence time. The injection volume is all 300 µL for MSP-6h/24h/72h. For both (C) and (D), the concentration of the injected sample is 800 µg/mL.
**Section S5. MR imaging in vitro and in vivo**

**Fig. S13.** $T_2$-weighted MR imaging of MSP samples in vitro and in vivo. (A) MR images of MSP-0h/6h/24h/72h in porcine skin gelatin (Type A) at different sample concentrations. Note that the MR images at high concentrations (e.g., 400 µg/mL and 800 µg/mL) were associated with susceptibility artifacts, resulting in deflected contrast shapes. (B and C) Cross-sectional MR imaging of SD rats’ stomachs (blue dotted lines) filled with merely PO (1 mL) and MSP-PO mixture (1 mL, 8 mg/mL), respectively. In (C), prior to MR imaging, the rat was placed for 12 min under a static magnetic field generated by the permanent magnet (without rotation).
Section S6. Degradation of MSPs

Fig. S14. Degradation of MSP in 37°C DPBS solution. (A) Fluorescence intensity of MSP-0h/6h/24h/72h (100 µg/mL) against degradation time. (B) MSP-0h, (C) MSP-24h and (D) MSP-72h before degradation and after degradation of 6h, 72h and 168h, respectively.

Table S5. Quantitative data of the emission peaks for MSP-0h/24h/72h samples before and after degradation. The time lengths in parentheses match those in fig. S14 (B to D).

<table>
<thead>
<tr>
<th>Samples</th>
<th>FWHM (nm)</th>
<th>Peak position (nm)</th>
<th>Peak height (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSP-0h (0h)</td>
<td>51.5</td>
<td>654.4</td>
<td>8.6</td>
</tr>
<tr>
<td>MSP-0h (6h)</td>
<td>49.7</td>
<td>645.8</td>
<td>19.5</td>
</tr>
<tr>
<td>MSP-24h (0h)</td>
<td>57.4</td>
<td>651.6</td>
<td>4.0</td>
</tr>
<tr>
<td>MSP-24h (72h)</td>
<td>54.1</td>
<td>645.6</td>
<td>6.4</td>
</tr>
<tr>
<td>MSP-72h (0h)</td>
<td>58.6</td>
<td>655.6</td>
<td>3.3</td>
</tr>
<tr>
<td>MSP-72h (168h)</td>
<td>55.3</td>
<td>644.8</td>
<td>4.2</td>
</tr>
</tbody>
</table>
Section S7. Cytotoxicity to SiHa and 3T3 cell lines

Fig. S15. Supplementary data for cytotoxicity evaluation. MTT assay for (A) SiHa cancer cell line and (B) 3T3 normal cell line with pure Fe₃O₄ NPs. MTT assay for SiHa cancer cell line with (C) MSP-24h and (D) MSP-72h samples that have gone through 24h pre-degradation in cell growth media. The 0-concentration group with 100% cell viability is taken as the control group. Other six groups of concentration, i.e., 25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/mL and 800 µg/mL, are examined and compared with the control group. * means $P < 0.05$, which is considered as statistical significance. Further, ** means $P < 0.01$, and *** means $P < 0.001$. 
Fig. S16. CLSM imaging for 3T3 and SiHa cells cocultured with MSP-24h samples (0, 100, and 400 μg/ml) for 24 and 48 hours. (A) 3T3 normal cell line. (B) SiHa cancer cell line. In the images, blue color excited at 405 nm represents cell nuclei; green color excited at 488 nm the cytoskeleton; and red color excited at 552 nm the MSP-24h. The fluorescent images were captured using a Zeiss LSM 700 CLSM. All images share the same scale bar of 50 μm.