Supplementary Information for

Spatial Control of Chromosomal Location in a Live Cell with Functionalized Magnetic Particles

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S1. Bacterial magnetic particles

a)

b)

BMPs solution

Magnet bar

c)
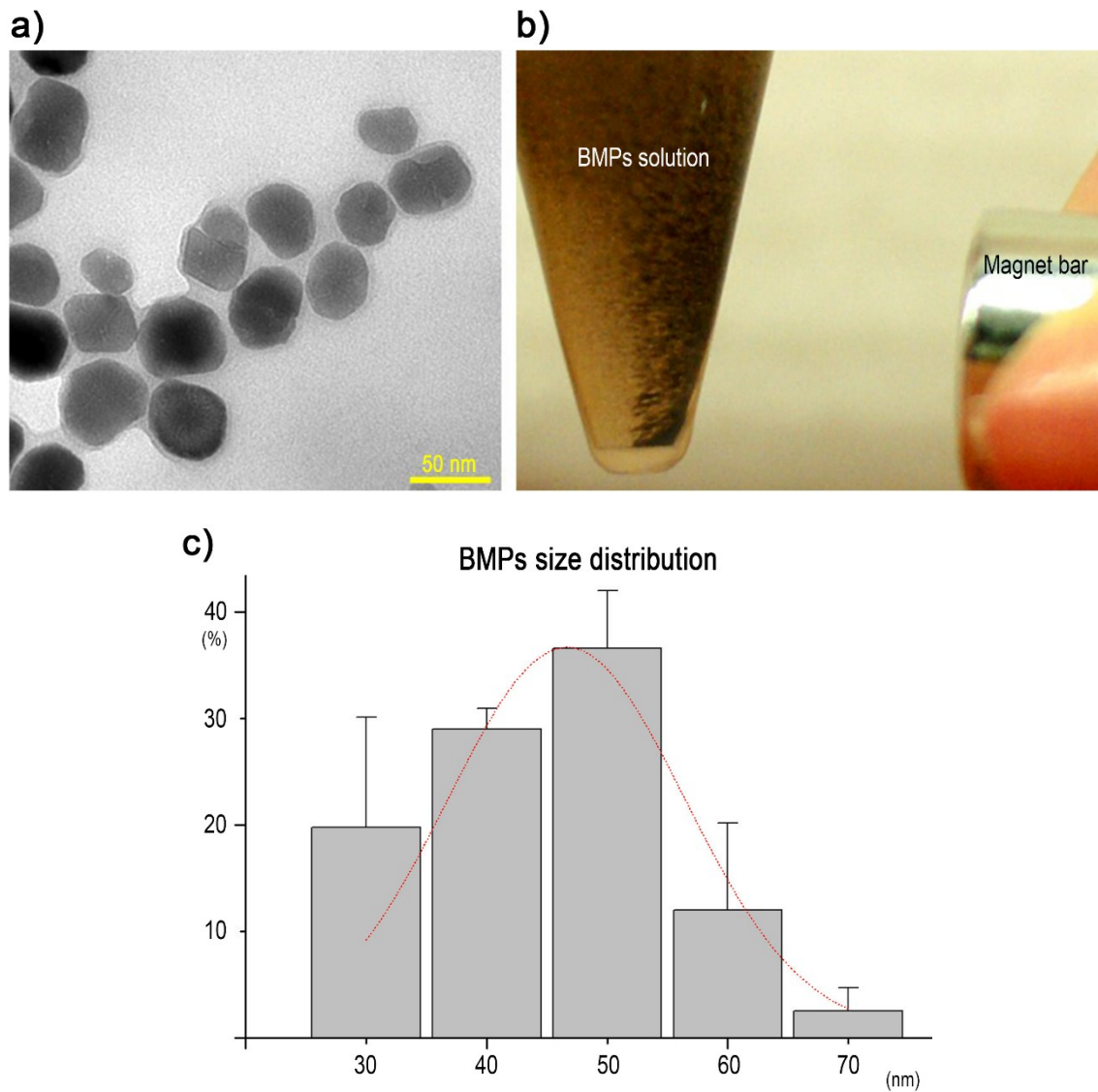
Fig. S1. Bacterial magnetic particles (BMPs). a) TEM image of collected BMPs. b) Photograph of concentrating process of BMPs using an external magnetic field. c) The distribution of BMPs size. The particle size of the BMPs was measured with Image J software using the obtained TEM images. The size distribution of BMPs ranges from 30 to 70 nm in diameter. The mean diameter of BMPs was about 49.6 ± 2.3 nm.

S2. Concentration of H1-BMPs

BMPs conjugated with the histone H1 antibodies (H1-BMPs) were concentrated by a magnetic bar to remove the excess antibodies and washed several times with MES buffer (0.1x). After removal of the supernatants, the H1-BMPs were stored in buffer solution (0.3% Bovine serum albumin (BSA) in 1x PBS). We increased the purity of H1-BMPs using a magnetic bar (Figure S2). The concentration of H1-BMPs was measured by the inductively coupled plasma mass spectrometry (ICP, Shimadzu, Japan).
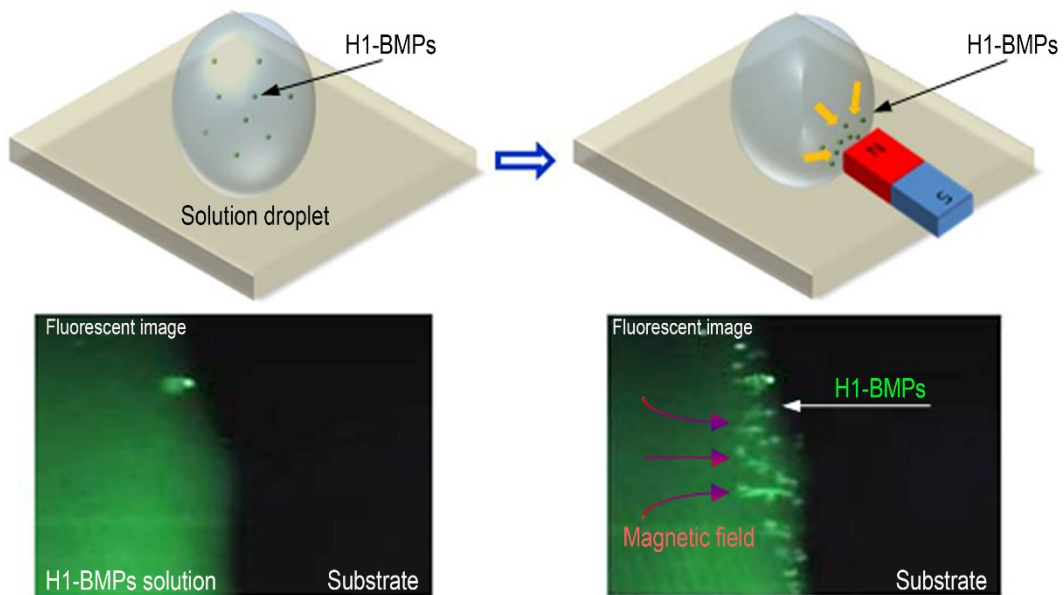
Fig. S2. Focusing of H1- BMPs by an external magnetic field to remove an excess of unbound histone H1 antibodies.

S3. Magnetic field vs distance

We used neodymium magnet to generate a strong magnetic field. Neodymium magnets of various sizes were placed at different distances from the sample, depending on experiments. The magnetic field vs. distance measured from the surface of a magnet at an interval of 1 mm using a Tesla meter (Kanetec, Japan).
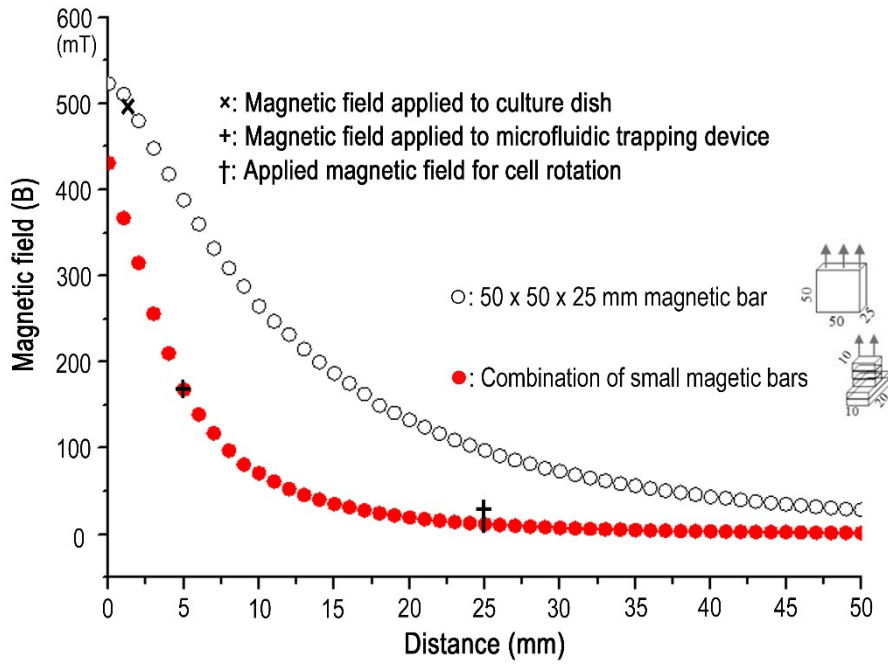
Fig. S3. Magnetic field vs distance

S4. H1-BMPs distribution in immature oocyte at GV and GVBD stage

Oocyte was stained with DAPI after cell fixation. The images were obtained from confocal laser scanning microscopy at intervals of 1 μm. Figure S4 shows that nuclear envelope disrupts the access of H1-BMPs to chromosomes. As a result, immature oocytes at GVBD stage were selected for subsequent experiments.
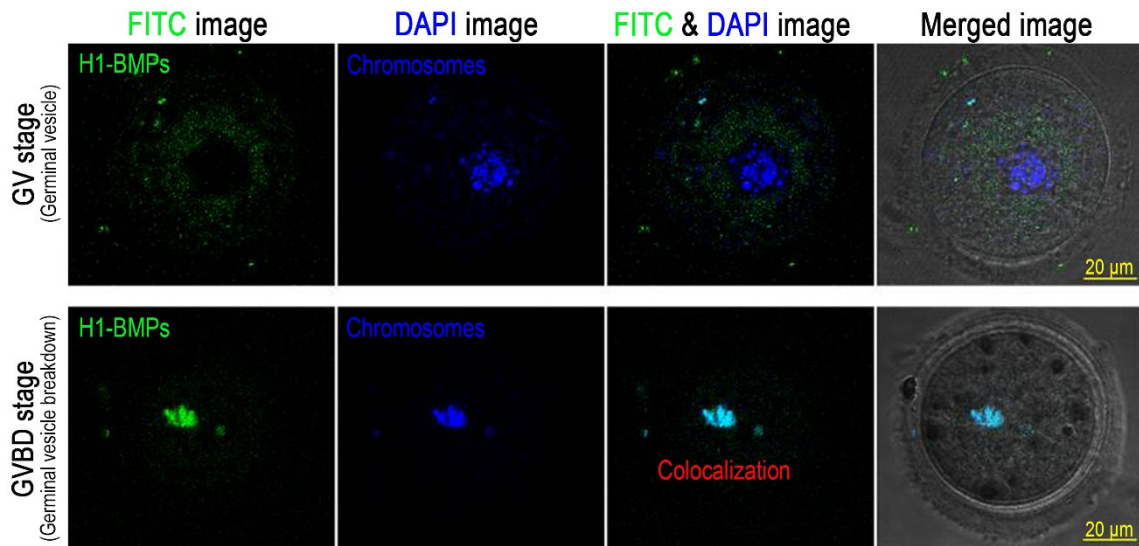
Fig. S4. H1-BMPs distribution in immature oocytes at germinal vesicle (GV) and germinal vesicle breakdown (GVBD) stage (green: H1-BMPs, blue: chromosomes).

S5. Collection and prepartion of oocytes

Animal care and handling were conducted in accordance with the policy and regulation for the care and use of laboratory animals at Seoul National University (SNU-101214-1 and SNU-130808-1-2). Six-week-old C57BL/6 female mice were superovulated with the injection of 5 IU/ml Pregnant Mare Serum Gonadotropin (PMSG, Folligon, Intervet International, Boxmeer, Netherlands) and 5 IU/ml human Chorionic Gonadotropin (hCG, Pregnyl, Organon, Oss, the Netherlands) was administered 48 hrs post-PMSG injection. Subsequently, cumulus-oocyte-complex (COC) was retrieved from oviduct 17 hrs post-hCG injection. Cumulus cells of COC retrieved from a mouse ovary were enzymatically removed with 200 µl hyaluronidase solution (80 IU/mL, Irvine Scientific, USA) for 1 min. The oocytes were pipetted in and out through a fine-bore glass pipette to loosen the weakened cumulus mass, and then transferred to fresh M16 medium (Sigma, USA). For cell cycle arrest which can prevent the spontaneous chromosome movement during experiment, 0.3 µM nocodazole treatment (Sigma Aldrich, USA) in dimethylsulfoxide solution (DMSO, Sigma, USA) was carried out. The nocodazole was diluted in M16 medium to yield a final concentration of 2.1 nM. H1-BMPs with final concentration 1 µg/ml were added in culture medium and a weak magnetic field was applied at the bottom of the culture dish to increase cellular uptake[27]. The H1-BMPs remaining outside the cells were removed by pippeting after 16~18 hrs. Then, immature oocytes at the germinal vesicle breakdown (GVBD) stage were selected for subsequent experiments. Confocal laser scanning microscopy (CLSM, Zeiss, USA) was used to obtain images.

1. Ovary was punctured with needle

2. Oocytes were released from ovary

3. Cumulus was removed by hyaluronidase treatment

4. Oocytes were treated with nocodazole

5. H1-BMPs were added into cells

6. H1-BMPs were introduced into cells

Magnetic field

7. A weak magnetic field was applied to cells for 3~4 hrs

8. Extracellular H1-BMPs were removed and immature cells were selected

Magnetic field

9. An external magnetic field was applied to cells

10. After experiment, cells were fixed with formaldehyde

11. Chormosomes were stained with DAPI or Hoechst

12. Immages were obtained by confocal microscopy

Fig. S5. Experimental procedure

S6. Vacuum-assisted microfluidic cell trapping device

We fabricated a vacuum-assisted microfluidic cell trapping device using a silicon-on-glass (SOG) wafer and a transparent polydimethylsiloxane (PDMS, Dow corning, USA). First, the trapping device was fabricated with two etching process such as 400 µm thick Si and 30 µm thick glass using deep-reactive ion etching (DRIE). The aluminum was used as the mask of the deep reactive ion etching (DRIE) process. After etching the glass with DRIE, the aluminum layer was removed. Next, the microchannel was fabricated via PDMS replication process. A mold was created with SU-8, spin-coated and patterned. The PDMS mixture (curing agent: PDMS = 1: 10) was placed in a vacuum chamber for 1 hr to remove the trapped gases, and then poured onto the mold. After PDMS curing, the separate parts were assembled into the final microfluidic trapping device using a biocompatible UV epoxy. The oocytes in the medium chamber were trapped at the holes, which were connected to the vacuum chamber. Considering the size of the oocytes, the holes in various sizes between 20-50 µm were designed to find the optimal capturing condition. Chamber pressure was controlled by a micromanipulator (IM-9B and 9C, Narishige, Japan), which was attached to inverted microscope (TE 2000-E, Nikon, Japan). To monitor the dynamic chromosome movement, a portable incubator (IC-L-10, Chamlide, Korea) which can maintain constant temperature, humidity, and CO_2 was used.
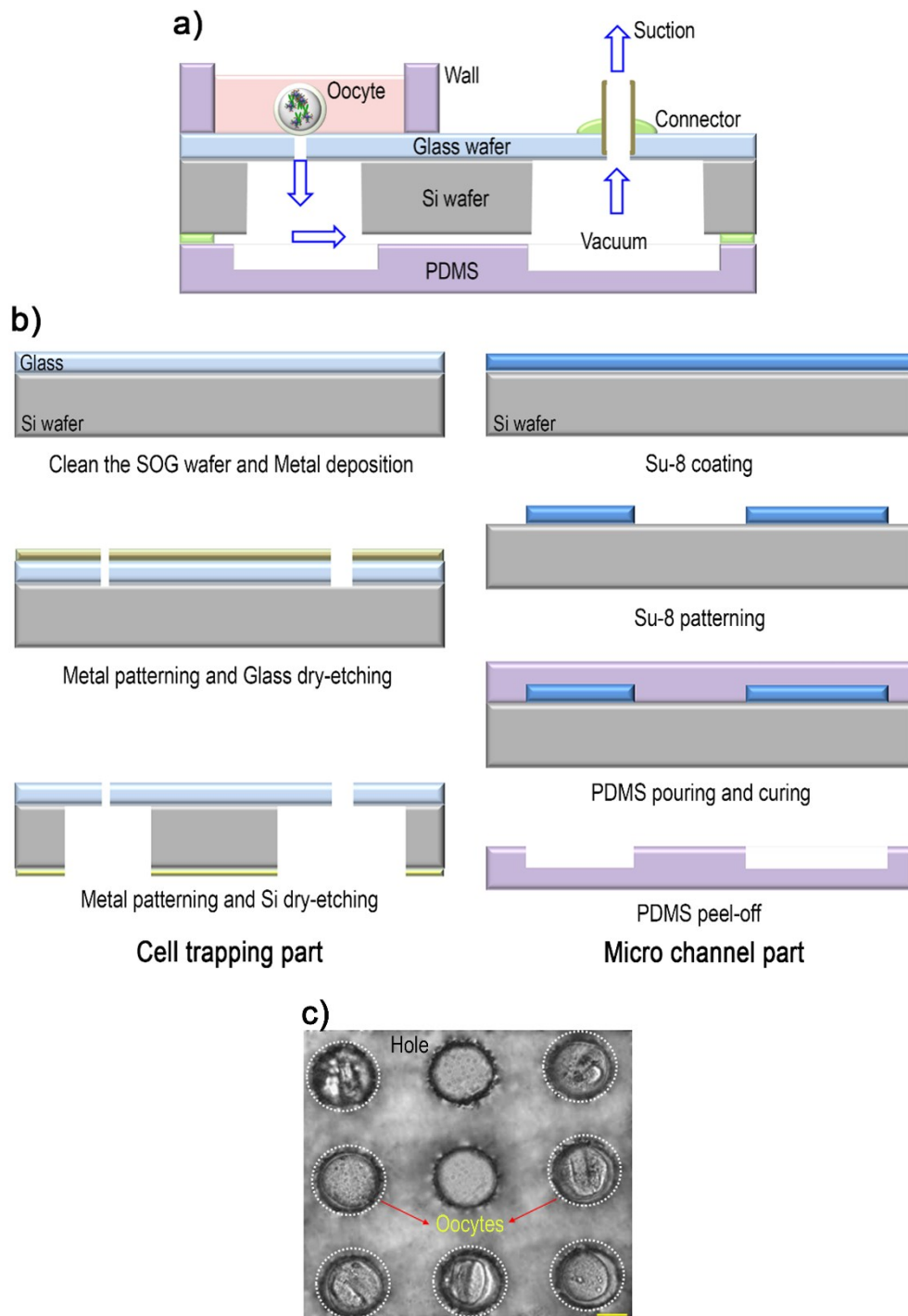
Fig. S6. Vacuum-assisted microfluidic cell trapping device. a) Schematic diagram of a vacuum-assisted microfluidic cell device. b) Process flow. c) The trapped cells image in the holes. Scale bar is 20 μm.

Section 1. Approximatecaculation of the exerted magnetic force on chromosome

Cellular uptake of particles can be affected by many factors, such as particle size, types of cell lines and cell densities, compositions of the particles and surface properties[1-3]. Currently it is hard to identify the cellular uptake efficiency because only small number of oocytes was used in our experiment. However, the magnetic force can be roughly estimated. In a separate our experiment with national institute of health (NIH) 3T3 cells, ~40% cellular uptake was measured[4]. With this uptake efficiency and 50% binding efficiency assumed, 20% of the H1-BMPs on the projected area should be delivered and bound to the chromosomes. When 1 μl H1-BMP solution (Density, ~5000 kg/m^3) was added in a culture dish (ø: 35 mm), there should be 6.4×10^{12} (H1-BMPs/m^2). Thus, the number of the H1-BMPs on the projected area of a single oocyte (~60 μm) were ~2×10^4. If 3.6 × 10^3 H1-BMPs conjugated with the chromosomes, it can produce a total force of ~2.9 nN in the culture dish and ~1.5 nN in the microfluidic trapping device experiments.

References

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