TOPICAL REVIEW — Magnetism, magnetic materials, and interdisciplinary research

Using magnetic nanoparticles to manipulate biological objects*

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The use of magnetic nanoparticles (MNPs) for the manipulation of biological objects, including proteins, genes, cellular organelles, bacteria, cells, and organs, are reviewed. MNPs are popular candidates for controlling and probing biological objects with a magnetic force. In the past decade, progress in the synthesis and surface engineering of MNPs has further enhanced this popularity.

Keywords: magnetic nanoparticles, manipulation, biological objects

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1. Introduction

Cellular-scale manipulation of biological objects is critical for both fundamental biomedical studies and the large-scale separation/purification of biological products.[1–4] For example, the ability of optical tweezers to precisely position and manipulate micro/nano objects like particles, proteins, and DNAs allows researchers to study the strength of interactions between single molecules with picoNewton accuracy. Recombination technology produces recombinant proteins with a known affinity, which can be efficiently purified from crude extracts by affinity chromatography.

Among many technologies of manipulation at the scale of the cell and beyond, the magnetic manipulation is attractive due to its non-contact or action at a distance characteristic.[2,5] Typically, samples containing biological objects of interest are mixed with magnetic probes that could specifically recognize and label the interested objects. Then the samples are placed in a magnetic field, which isolates the labeled objects from the rest. As such, the magnetic manipulation is time-efficient, cost-effective, and easily scalable.

To achieve a successful manipulation, the key is to specifically magnetize biological objects of interest with magnetic labels which are usually magnetic micro/nano particles (MNPs). Those particles should be made of biocompatible materials in the dimension range from 1 nm to 10 μm, which allows them to fully interact with the biological objects. They can be coated with either inorganic (e.g., gold, silica, hydroxyapatite) or organic (e.g., dextran, polyvinyl alcohol (PVA), polyethylene glycol (PEG)) shells to prevent the aggregation caused by hydrophobic interaction and/or magnetic attraction. In the case of *in vivo* manipulation, a proper surface coating will be required to make the MNPs undetected by the immune system for a prolonged circulation time. Finally, a targeting agent like an antibody has to be incorporated for the recognition and labeling of the biological objects of interest.

Because of the importance of MNPs in the magnetic manipulation and other applications like the magnetic resonance imaging (MRI), which are not the focus here, the design and fabrication of MNPs have been frequently reviewed.[5–10] Instead of narrating similar stories, this review focuses on the latest progress in the magnetic manipulation of proteins, genes, bacteria, cellular organelles, cells, and organs. The goal is to provide an overview of the magnetic manipulation of biological objects (Fig. 1), aiming to help readers find the challenges and opportunities in this field.

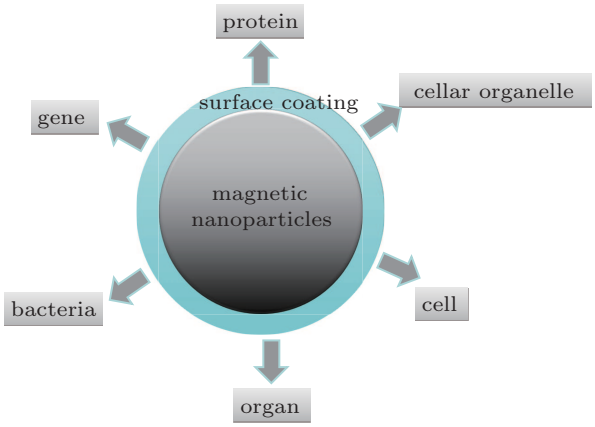


Fig. 1. The use of magnetic nanoparticles for the manipulation of biological objects.

2. Protein separation

The effective and efficient purification of proteins from crude extracts without changing their properties is important for both life science research and pharmaceutical

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production.[11–14] Among the many methods for large-scale protein separation, magnetic separation techniques have several advantages. First, they are cost-effective, with no need for expensive liquid chromatography systems, centrifuges, filters, or other equipment. The separation is also simple, which can be performed directly in crude samples. Second, the magnetic separation is gentle with a minimal potential to damage the proteins by shear forces. Finally, it concentrates the proteins without the need of ultrafiltration and/or ultracentrifugation.

Magnetic beads of 1 μm or larger (e.g., Dynabeads from Invitrogen) are already commercially available from a variety of companies and widely used in laboratories. Based on the success of microsized magnetic beads, MNPs are also adapted for protein separation because of their higher surface-to-volume ratio, better interaction with proteins, and longer retention time in the solution.[2,5,15]

An early example of manipulating proteins with MNPs was the recognition and separation of hexahistidine-tagged green fluorescent proteins (6His-GFP) from crude E. coli lysate. Specifically, sub-10 nm FePt MNPs were functionalized with nitrilotriacetic acid (NTA) with a thiol group acting as an anchor. Later, nickel ions were introduced to form the NTA–Ni^{2+} complex on the MNPs' surface, which specifically chelated the hexahistidine tag of 6His-GFP.[16,17] Then the FePt–NTA–Ni^{2+} complexes were mixed with crude E. coli lysate containing 6His-GFP. With ∼5 min incubation, 6His-GFP was extracted by a magnet and then detached from the MNPs using imidazole solution. Figure 2(a) is a schematic illustration of the binding process between 6His-GFP and the FePt–NTA–Ni^{2+} complex. Given the higher surface area, longer retention time in solution, and thus better interaction with proteins, those MNPs showed a higher purification yield for 6His-GFP (2–3 mg proteins/1 mg MNPs vs. 10–12 μg proteins/1 mg beads) – about 200 times higher than that of the commercial Dynabeads. The SDS polyacrylamide gel electrophoresis (SDS/PAGE) analysis of the separated proteins confirmed the presence and purity of extracted 6His-GFP (Fig. 2(b)).

After that early work, more MNPs-based platforms have been developed for the purification of histidine-tagged proteins, such as Fe_3O_4@SiO_2@polymer-NTA–Ni^{2+} MNPs and Fe_3O_4@Au–NTA–Ni^{2+} MNPs.[18,19] Unconventional chelating complexes were also examined. For example, MNPs with Ni exposure on the surface, namely Ni@NiO MNPs, were conjugated with imidazole for the purification of histidine-tagged proteins that could be detached by means of another imidazole solution later. Similarly, MNPs with a NiO-decorated SiO_2 shell were also suitable.[20] In addition, MNPs functionalized with the Cu^{2+}–terpyridine complex exhibited similar results.[21]

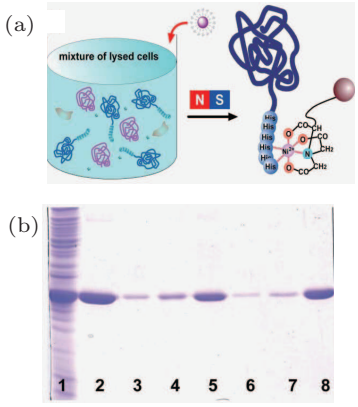


Fig. 2. Recognition and separation of hexahistidine-tagged green fluorescent proteins (6His-GFP) from crude E. coli lysate: (a) surface-modified FePt MNPs selectively bound 6His-GFP in a cell lysate; (b) SDS/PAGE analysis of the cell lysate (lane 1), the fraction (lane 2) washed off a commercial NTA-Ni^{2+} column; the fraction washed off the FePt MNPs separation agent using imidazole solution (10 mM, lane 3; 80 mM, lane 4; 500 mM, lane 5); fractions washed off the reused FePt MNPs separation agent using imidazole solution (10 mM, lane 6; 20 mM, lane 7; 500 mM, lane 8).[15]

Up to now, we have focused on the manipulation of polyhistidine-tagged proteins. However, we should be aware that there are other protein tags such as glutathione-S-transferase (GST) and maltose binding protein (MBP). By modifying MNPs with the corresponding glutathione (GSH), Xu *et al.* separated GST-GFP from a crude extract by forming a conjugate of MNP-GSH/GST-GFP. Then GFP was eluted from MNPs upon the enzymatic cleavage of the TEV (Tobacco Etch Virus) linker between the two proteins.[22] Xing *et al.* developed maltose-modified MNPs for the separation, purification, and immobilization of MBP-heparinase I fusion enzyme.[23] In addition to small molecular ligands, other targeting molecules like antibodies can be conjugated with MNPs for protein recognition and separation. For example, Matsunaga *et al.* developed a bacterial MNP with the protein-A coating. After being conjugated with anti-human insulin antibodies, the resulting antibody–protein-A–bacterial MNPs can bind and help identify human insulin in solutions.[24] Nichkova *et al.* used the antibodies to engineer luminescent MNPs with a Co:Nd:Fe_2O_3 magnetic core and a luminescent Eu:Gd_2O_3 shell for fluoroimmunoassay (Fig. 3). After the engineered MNPs captured the target proteins (e.g., human, rabbit, and mouse immunoglobulin Gs (IgGs)), the dye-labeled secondary reporter antibodies were added and formed a sandwich structure (i.e., MNP–protein–antibody) with both luminescent and fluorescent signals. The magnetic core of this system allowed the separation and purification of proteins, and the intrinsic luminescence from the shell of the MNPs served as an internal standard in the quantitative immunoassay.[25]

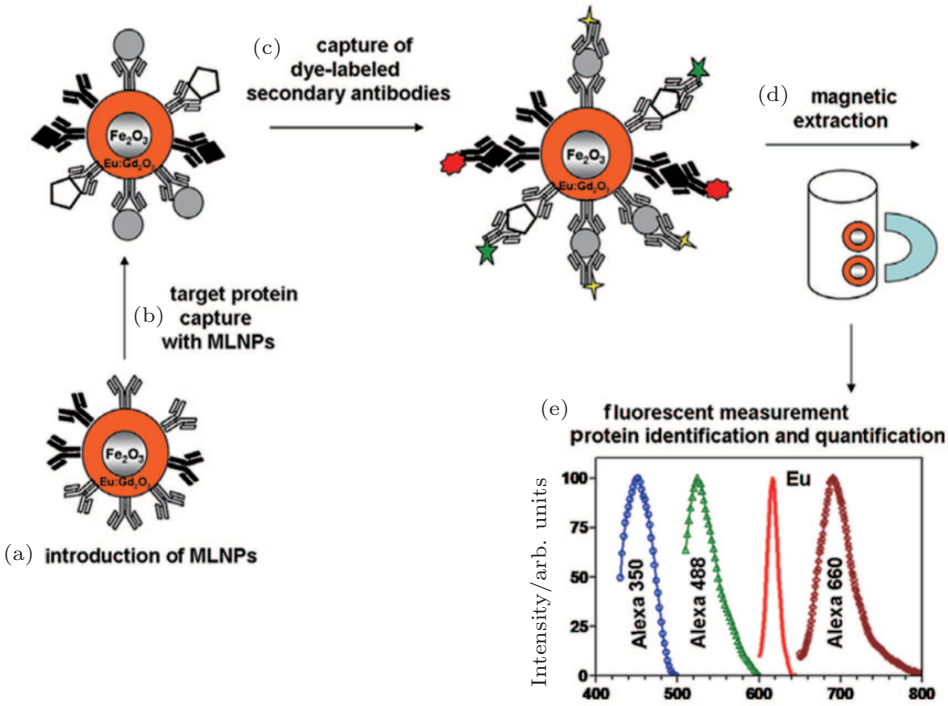


Fig. 3. Process of the multiplexed fluoroimmunoassay with magnetic luminescent nanoparticles (MLNPs): (a) introduction of antibody-modified MLNPs; (b) binding of the protein analytes on the MLNPs surface; (c) capture of the dye-labeled secondary (reporter) antibodies; (d) magnetic extraction; (e) fluorescence detection of reporters and MLNPs.[25]

3. Magnetofection

Another type of magnetic manipulation is magnetic transfection or magnetofection. It is well known that the development of an efficient system for delivering genes into targeted cells is an important strategy to understand gene/protein functions and to develop therapeutics. Considering the public concern about the safety of viral transfection, non-viral gene delivery is preferred but suffers from a low transfection efficiency.[26] Magnetofection has arisen as a promising approach to address this challenge. This method uses a magnetic field to concentrate MNPs complexed with plasmids into the cells. The concentrated genes can be released onsite via enzymatic cleavage of the cross-linking molecules, charge interactions, degradation of the polymer matrix, etc.[27,28]

In magnetofection, MNPs are usually coated with positively charged molecules that allow the negatively charged plasmids to be associated with them.[28,29] For example, MNPs for magnetofection can be made by coating an iron oxide core with a cationic copolymer, which is comprised of short chain polyethylenimine (PEI) and PEG grafted to chitosan. This system incorporates the biocompatibility of chitosan, the steric stabilization of PEG, and the positive charge of PEI so that it can bind, protect, and deliver plasmids into tumor cells both *in vitro* and *in vivo*.[30] In another example, Chari *et al.* compared magnetofection with other transfection methods for oligodendrocyte precursor cells (OPCs), finding that the transfection efficacies obtained using the magnetofection methods are highly competitive with or better than current widely used nonviral transfection methods (e.g., electroporation and lipofection) with the additional critical advantage of high cell viability.[31]

Although MNPs are categorized as non-viral vectors, they could be incorporated with other viral or non-viral platforms to maximize the delivery efficiency. Byrne *et al.* conjugated virus vectors (i.e., recombinant adeno-associated virus 2, rAAV) onto MNPs (Fig. 4). With the MNP–virus complex, the amount of vector required was significantly reduced to 1% of the previous quantity for the same transfection efficiency.[32] Similar results have been obtained with smaller MNPs. For example, Tanaka *et al.* mixed 29 nm maghemite MNPs with the hemagglutinating virus of Japan envelope vector (HVJ-E) to enhance the transfection efficiency. They found that the MNPs with protamine sulfate that gives a cationic surface charge significantly enhance the transfection efficiency *in vitro* due to the enhanced association of HVJ-E with the cell membrane in the presence of a magnetic force. MNPs coated with heparin enhance the transfection efficiency *in vivo*. Thus, the surface chemistry of the MNPs needs to be tailored to meet specific demands.[33] Wenzel *et al.* reported the transduction of murine embryonic stem cells by an MNP-assisted lentiviral gene transfer. By the combined use of MNPs and lentivirus, they achieved a four-fold increase of the number of transduced cells in comparison to the lentivirus only.[34]

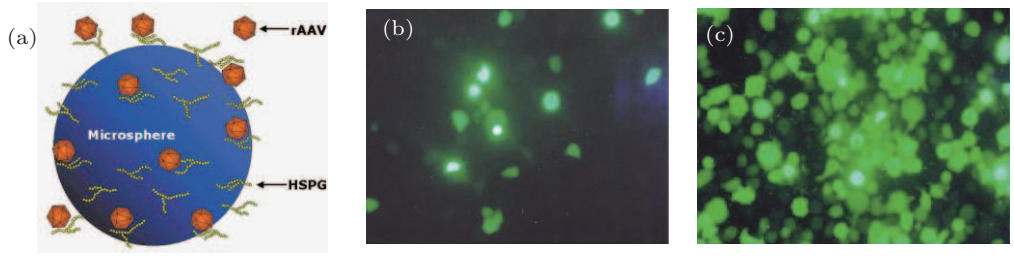


Fig. 4. Magnetic microparticle–rAAV complex (MNP–rAAV) for cell transfection: (a) illustration of MNP–rAAV; (b) C12S cells were infected with free rAAV encoding the gene for green fluorescent protein (GFP); (c) C12S cells were infected with MNP–rAAV encoding the gene for GFP under a magnet field.[32]

4. Manipulation of cellular organelles

An organelle is a specialized subunit within a cell that has specific functions and is usually separately enclosed within its own lipid bilayer. The ability to manipulate and remotely control specific cellular organelles *in vitro* and *in vivo* would provide a powerful tool for investigating the cell function and signaling pathways. Recent representative examples that utilize MNPs for organelle manipulation are summarized in Table 1.

Table 1. Magnetic nanoparticles for the manipulation of cellular organelles.

Nanoparticle	Size/nm	Ligand/antibody	Organelle	Reference
$Zn_{0.4}Fe_{2.6}O_4$ MNPs	15	antibody for death receptor 4	membrane	[35]
Superparamagnetic NPs, Ademtech	480	guanine nucleotide exchange factor	membrane	[36]
Iron oxide NPs	120	RanGTP protein/guanine exchange factor RCC1	cytoskeleton	[37]
Iron oxide	14	proteins recognize EGFR	lysosome	[38]
Anti-mouse IgG1 microbeads	50	anti-TrkB agonist antibody	endosome	[39]
Bacteria MNPs (Fe_3O_4)	50	cytochrome c-specific binding aptamers	mitochondria	[40]

A cell's membrane separates the interior of the cell from the extracellular components. It consists of a lipid bilayer with embedded proteins. Some proteins face the cell exterior and act as receptors for particular messengers. These are the terminations of signaling pathways that regulate the cell activities. By noticing this phenomenon, researchers realize that the cell signaling pathways could be manually controlled with a magnetic field if MNPs are bound to the interesting receptors. For example, Cheon *et al.* turned on the apoptosis cell signaling with zinc-doped iron oxide ($Zn_{0.4}Fe_{2.6}O_4$) MNPs that were coated with death receptor 4 antibodies (DR4A) of DLD-1 colon cancer cells. Briefly, the researchers labeled DLD-1 colon cancer cells with MNPs–DR4A first. Then a magnetic field was applied, which aggregated the MNP-bound DR4s to trigger the apoptosis signaling pathways. In addition to cells, the apoptotic morphological changes of zebrafish were also achieved with this technology.[35] Similarly, the same group triggered tubulogenesis with MNPs that were labeled with TiMo214 antibodies for Tie2 receptors.[41] Recently, Dahan *et al.* reported the local remodeling of the actin cytoskeleton and morphological changes of living cells with magnetic manipulation. They functionalized MNPs with the catalytic domain of TIAM1 ($TIAM^{DHPH}$, AS 1033–1406), a specific guanine nucleotide exchange factor (GEF) that activates Rac1, a Rho-GTPase known to induce morphological changes upon activation. As the TIAM–MNPs were localized to the cell membrane by a magnetic tip, they activated Rho-GTPase and created a protrusion and subsequently an actin comet (Fig. 5).[36]

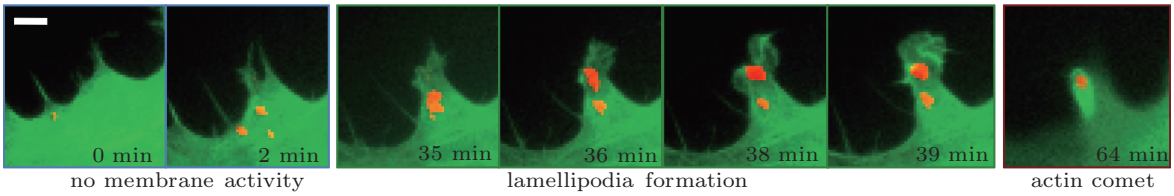


Fig. 5. TIAM–MNPs were magnetically localized into an inactive area, which created a protrusion and subsequently an actin comet. Scale bar is 1 μm.[36]

A microtubule is a major component of the cytoskeleton and is important in a number of cellular processes including maintaining the cell structure. The polymerization of microtubules is controlled by cell-signaling pathways. Gueroui *et al.* showed that MNPs conjugated with key regulatory proteins can artificially control, in time and space, the Ran/RCC1 signaling pathway that regulates the cell cytoskeleton. In the presence of a magnetic field, MNPs coated with RanGTP proteins induce microtubule fibers to assemble into asymmetric arrays of polarized fibers in Xenopus laevis egg extracts. The orientation of the fibers is dictated by the direction of the magnetic force. When the researchers locally concentrated MNPs that were conjugated with the upstream guanine nucleotide exchange factor RCC1, the assembly of microtubule fibers could be induced over a greater range of distances than RanGTP–MNPs.[37]

Another organelle that could be manipulated is lysosome. In cancer treatment, increasing the permeability of lysosomes can be an effective strategy to promote cell death. Rinaldi *et al.* utilized MNPs to target and induce the permeabilization of lysosomes under the action of an alternating magnetic field (AMF) (Fig. 6). They coated 14 nm iron oxide MNPs with carboxymethyl dextran and proteins that recognized the epidermal growth factor receptor (EGFR) overexpressed in cancer cell membranes. Functionalized MNPs rapidly concentrated inside the lysosomes upon the cell internalization. Under AMF, heat was generated around the MNPs, damaging the lysosome membrane. This observation suggests the possibility of remotely triggering lysosomal death pathways in cancer cells with MNPs.[38]

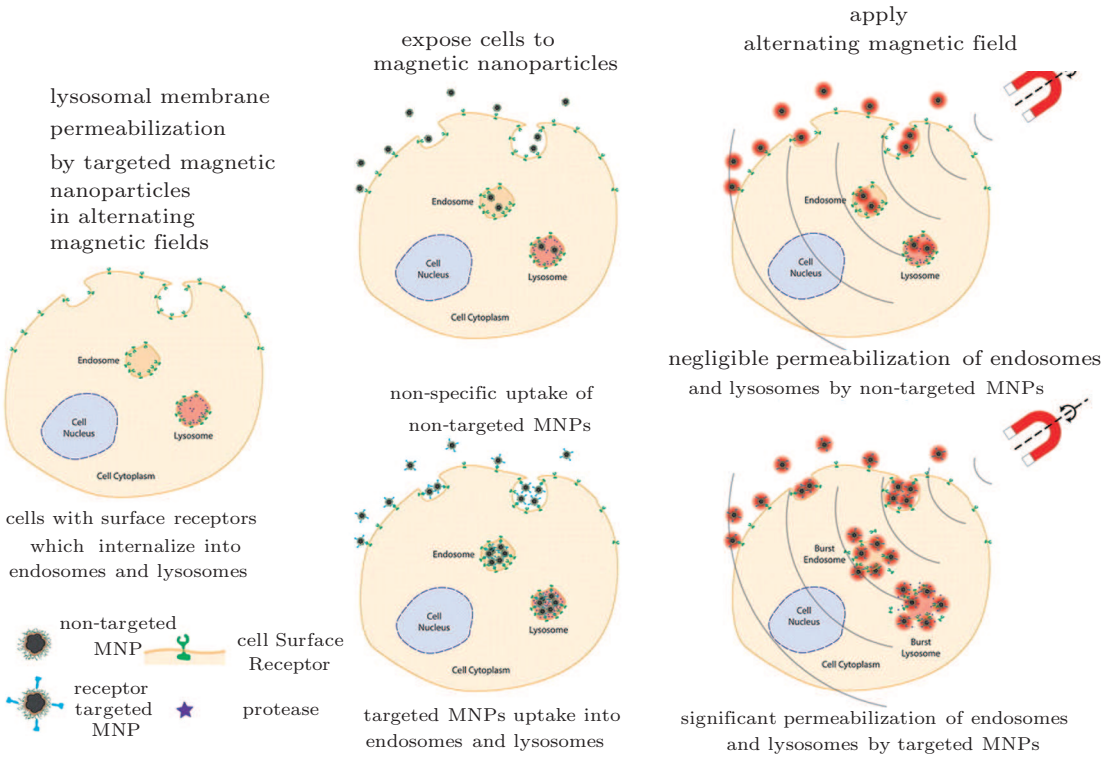


Fig. 6. Schematic diagram of lysosomal membrane permeabilization by MNPs with an AMF: nontargeted MNPs were taken up by a nonspecific mechanism while targeted MNPs were taken up into endosomes and lysosomes due to receptor mediated endocytosis. When an AMF was applied, both types of MNPs dissipated heat. Targeted MNPs delivered heat specifically to endosomes/lysosomes, resulting in membrane permeabilization.[38]

Signaling endosomes are critical long-range communication links used by neurons in the central and peripheral nervous systems during development and regeneration.[42] Therefore, it is vital to study their localization and related functions in regulating neurite growth. To demonstrate this idea, Goldberg *et al.* activated tropomyosin-related kinase B (TrkB) signaling endosomes with MNP-based magnetic manipulation.[39] Specifically, they coated 50 nm MNPs with the anti-TrkB agonist antibody (fMNPs). The antibody facilitated the rapid MNP endocytosis into TrkB signaling endosomes. Purified retinal ganglion cells (RGCs), lacking neurites, were incubated overnight with either fMNPs or cMNPs (control MNPs, conjugated with IgG antibodies) and then plated on laminin-coated coverslips where they could extend new neurites (Figs. 7(a) and 7(b)). fMNPs and cMNPs were detected in 95% and 7% of RGC somas, and 80% and 0% of RGC neurites and growth cones, respectively (Figs. 7(c) and 7(d)). To alter the transportation of fMNP signaling endo-

somes, defined magnetic forces were applied to fMNP-loaded RGCs via an electromagnetic needle. A 15-pN magnetic force was applied to cMNP- or fMNP-loaded RGCs. Figures 7(e) and 7(f) show that a continuous magnetic force failed to alter the growth cone motility or neurite growth in cMNP-loaded RGCs, while it was sufficient to halt the neurite growth without retraction in fMNP-loaded RGCs. So manipulating MNP-signaling endosomes by a focal magnetic field can alter the growth cone motility and halt the neurite growth in neurons of both the peripheral and central nervous systems.

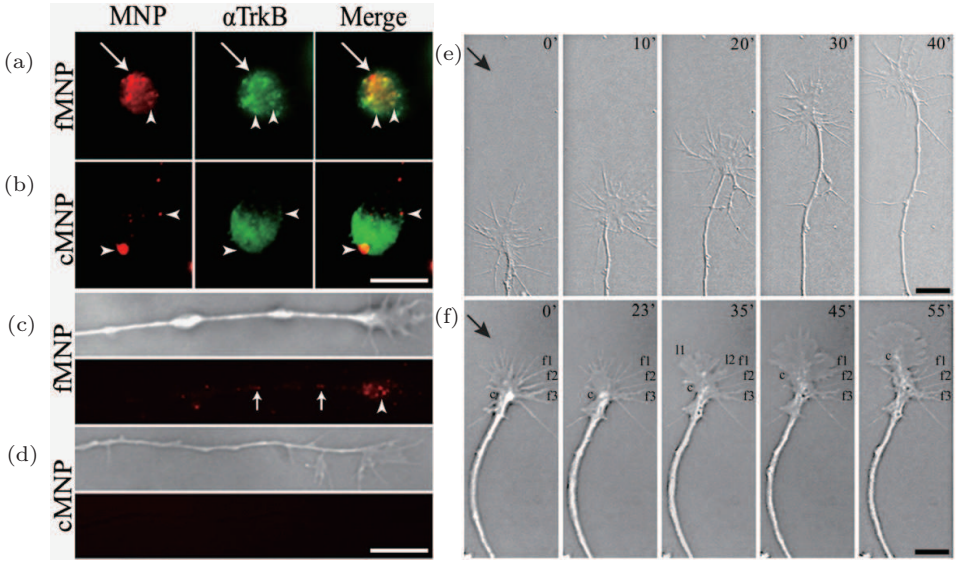


Fig. 7. FMNP signaling endosomes are transported into nascent RGC neurites, and a focal magnetic force alters growth cone mobility and halts neurite growth. (a) FMNP puncta were robust in RGC somas (arrowheads) and co-localized with TrkB in most fMNP puncta (arrows); (b) cMNPs were also detected as puncta (arrowheads) in some RGC somas, but these puncta were less numerous and usually failed to co-localize with TrkB. Differential interference contrast (DIC) and fluorescent images of (c) an fMNP-loaded RGC demonstrated anterograde transport into nascent neurites (arrows) and growth cones (arrowhead); (d) a cMNP-loaded RGC did not migrate into either the neurite or growth cone. (Scale bars: 10 μm.) (e) In control RGCs, a constant 15-pN force failed to alter either growth cone motility or neurite growth rate. This growth cone extended new lamella and filopodia, and the neurite continued to grow at ∼50 μm/h throughout the recorded time period. (f) In fMNP-loaded RGCs, a 15-pN force applied for 3 min was sufficient to immobilize both lamellar and filopodial protrusions in the peripheral domain and halt neurite growth. Time in minutes (t) is indicated. The electromagnet tip is indicated by black arrows. (Scale bars: 5 μm.)[39]

The last, but not the least, type of cellular organelles to be manipulated is mitochondria, which have both vital and lethal functions in several physiological and pathological processes.[43] Besides generating most of the cell's energy, mitochondria are also involved in signaling, cellular differentiation, cell death and growth. Cha *et al.* labeled mitochondria with MNPs and applied an external magnetic field, which led to reduced cell viability. The MNPs were 50 nm single domain Fe_3O_4 and labeled with cytochrome c-specific binding aptamers.[40]

5. Separation and detection of bacteria

Pathogen detection (e.g., bacteria, viruses) is another exciting application of magnetic manipulation. Currently, the identification of microbial pathogens relies upon the conventional clinical microbiology monitoring approaches. The standard culture and susceptibility tests permit pathogen identification but are laborious, time-consuming, and expensive, and require labile natural products.[44] The conventional techniques also do not lend themselves well to managing large numbers of environmental or clinical samples. To quickly determine the presence of a pathogen for the benefit of society, researchers and clinicians need tools that are cheap and quick, but still reliable and accurate. One solution is to utilize MNPs for the labeling, isolation, and analysis of pathogens. This strategy is easy and cheap, and allows the enrichment of the pathogen from samples for sensitive detection at ultralow concentrations without tedious procedures.[45]

One representative example is the isolation and detection of Gram-positive bacteria. It is well known that an antibiotic, vancomycin (Van), specifically binds the terminal peptide (D-Ala-D-Ala) on the cell wall of Gram-positive bacteria.[46,47] Therefore, the combination of Van with MNPs allows the recognition and isolation of Gram-positive bacteria with magnetic separation. To achieve this, FePt MNPs were functionalized with Van molecules through the surface ligand exchange with bis(vancomycin) cystamide. Each as-prepared FePt–Van MNP contained 8 to 9 Van molecules.[45] Then the FePt–Van MNPs were mixed with a sample containing bacteria for about 10 min before being isolated with a magnet. As illustrated in Fig. 8(a), bacteria and FePt–Van MNPs formed aggregates after being separated from the solution with a mag-

net. The significant size difference allowed the researchers to distinguish the bacteria from the aggregates with a scanning electron microscope (SEM). As shown in Fig. 8(b), there was a uniform binding of FePt–Van MNPs onto the surface membranes of the Gram-positive bacteria such as E. faecium (VanA), but few particles could be found on the surfaces of Gram-negative bacteria such as E. coli. With this technology, the researchers could detect low counts of bacteria in human blood in cases where the PCR based detection could be inaccurate. Given that SEM is impractical in clinical settings, fluorescent markers could be grafted onto MNPs for the sensitive detection of pathogens with optical modalities.[48] Besides SEM and florescence, other analytical techniques can be coupled with biofunctional MNPs for bacteria detection. For example, Chen *et al.* reported combining biofunctional MNPs with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), a useful tool for the characterization of microorganisms.[49–51]

Besides the usual spheroid MNPs, core-shell MNPs are also suitable. For example, in the case of Ag@Fe_2O_3 yolk-shell MNPs, the magnetic Fe_2O_3 shell could be functionalized with glucose molecules as anchors for bacteria attachment. Simultaneously, the porous Fe_2O_3 shell facilitates the release of Ag ions and/or Ag NPs, which act as broad-spectrum antibacterial agents. In a bacteria-elimination experiment with drinking water, Ag@Fe_2O_3-Glucose MNPs not only captured bacteria as precipitates, but also inhibited bacterial growth in both supernatant and precipitates.[52] Another example was magnetite@silica core/shell MNPs, which were coated with antiseptic polyhexamethylene biguanide that bound lipid A of G^+ bacteria.[53,54]

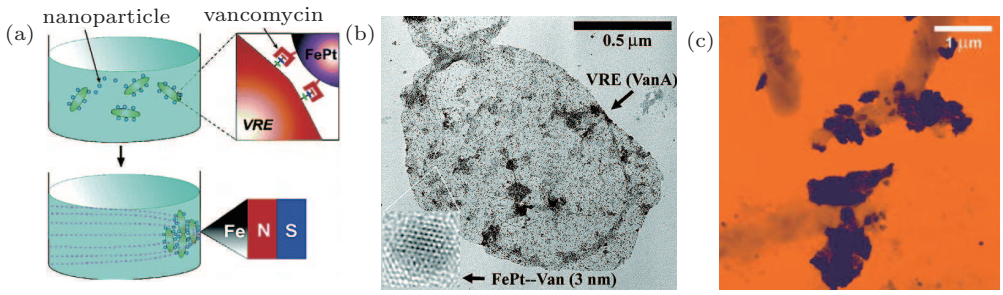


Fig. 8. FePt–Van MNPs for bacteria capturing: (a) illustration of the capture of bacteria by FePt–Van MNPs; TEM images of bacteria captured (b) E. faecium (VanA), (c) E. coli.[15,45]

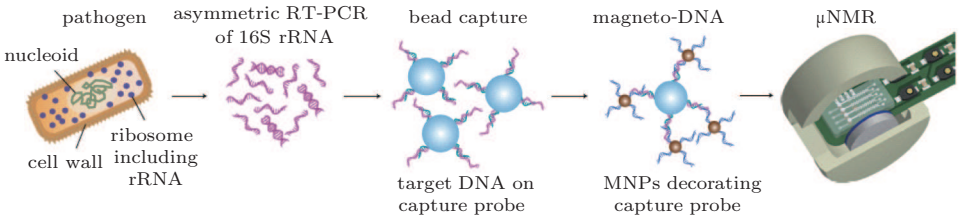


Fig. 9. The magneto-DNA assay for the detection of bacterial 16S rRNA: total RNA is extracted from the specimen, and the 16S rRNA is amplified by asymmetric RT-PCR. Single-strand DNA of the amplified product is then captured by beads conjugated to capture probes, before hybridizing with MNPs to form a magnetic sandwich complex. Samples are subsequently analyzed using a μNMR system.[55]

All the technologies discussed so far include similar procedures, including anchoring MNPs to the bacterial surface, magnetic separation with a magnet, and analyzing samples with imaging technologies. Although basically simpler than the traditional technologies for bacterial separation and detection, further analysis with additional imaging technologies is still needed to identify and quantify the captured bacteria. Weissleder and Lee minimized this requirement by developing a magneto-DNA probe capable of rapid and specific profiling of pathogens directly in clinical samples. As illustrated in Fig. 9, the magneto-DNA assay is based on a sandwich hybridization technique wherein two oligonucleotide probes are bound to each end of the target nucleic acid. First of all, the total RNA is extracted from a specimen, and then target regions within the 16S rRNA are amplified by asymmetric reverse transcription-PCR (RT-PCR) to produce a large quantity of single-strand DNA with only sense (or antisense) sequences. The resultant DNA is then captured by polymeric microspheres conjugated with probe oligonucleotides (the bead capture probe). Subsequently, the overhanging edges of the target DNA are hybridized with MNP-detection probe conjugates (the MNP–detection probe). These magnetically la-

beled beads shorten the transverse relaxation rate (R_2) of a sample, which is detected by a miniaturized μNMR device. This method was proved to be robust and rapid in simultaneously diagnosing a panel of 13 bacterial species in clinical specimens within 2 h.[55] Lowery *et al.* also developed a nucleic acid-based bacterial detection platform. They first mechanically lysed Candida cells, and selectively amplified the released DNA by the PCR technique. MNPs with complementary "capture probes" could then bind to the amplified DNA. By then, the free MNPs had clustered together and allowed the detection of bacteria with the T2 magnetic resonance platform (T2MR). With this technology, the researchers successfully identified 8 candidemic patients from 24 patients by whole-blood sample analysis.[56]

6. Manipulation of cells

Magnetic labeling of living cells creates opportunities for numerous biomedical applications, from individual cell manipulation to MRI tracking. While non-specific labeling of various types of cells with MNPs has been reported, it is more important to achieve specific binding.[57] MNPs are versatile to be functionalized with biomolecules to selectively target specific cells such as tumor cells, progenitor cells, or stem cells. For example, the specific binding of MNPs to stem cells enables the visualization and manipulation of their biodistribution.[58]

In 2000, Weissleder *et al.* reported *in vivo* tracking and recovery of progenitor cells using TAT peptide-derivative MNPs.[59] This technology was based on 5 nm MNPs stabilized by a 20 nm thick crosslinked aminated dextran coating. As-prepared 45 nm MNPs were derivatized with an average of four HIV-1 TAT peptides covalently attached to the dextran coating. In addition, the dextran coating was labeled with ^{111}In for concomitant nuclear imaging. The final MNPs were fluorescent, magnetic, and radioactive. The multi-functional MNPs were efficiently internalized into hematopoietic and neural progenitor cells in quantities up to 10–30 pg of iron per cell. The iron incorporation did not affect the viability, differentiation, or proliferation of the cells. After intravenous injection with immune-deficient mice, 4% of MNP-labeled cells were found by MRI to home to bone marrows. Finally, those magnetically labeled cells in the bone marrow could be recovered by magnetic separation columns. The localization and retrieval of cell populations revealed in this technology enables a detailed analysis of specific stem cell and organ interactions that is critical for advancing the therapeutic use of stem cells.

Perez *et al.* investigated the role of MNP valency in the nondestructive magnetic-relaxation-mediated detection and magnetic isolation of cells in complex media, such as tumor cells in blood and bacteria in milk.[60] They conjugated a small molecule, folic acid, at two different densities (low-folate and high-folate) on polyacrylic-acid-coated iron oxide MNPs for labeling cancer cells expressing the folate receptors. They found that the multivalent high-folate MNPs performed better than the low-folate MNPs with higher sensitivity, faster detection kinetics, and more efficient magnetic isolation of cancer cells. The multivalent high-folate MNPs allowed the detection of a single cancer cell in a blood sample within 15 min.

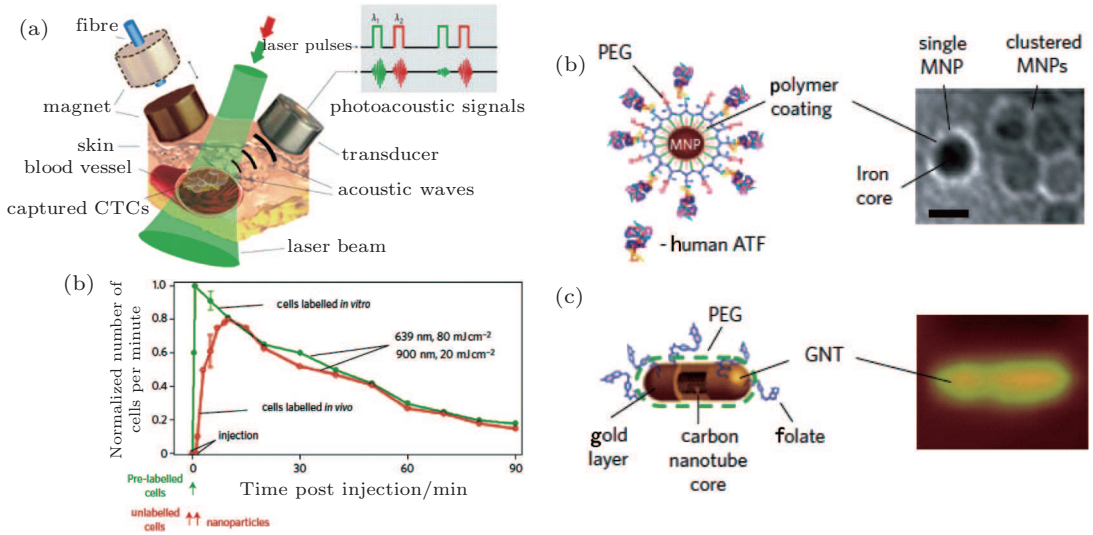


Fig. 10. Capturing CTCs with MNPs and photoacoustic detection: (a) schematic illustration of the magnetic enrichment of CTCs and the subsequent two-color photoacoustic detection; (b) structure (left) and TEM image (right) of MNPs; scale bar is 10 nm; (c) structure (left) and topographic atomic force microscopy image (right) of a gold carbon nanotube; (d) photoacoustic monitoring of CTCs in the abdominal vessel using fiber-based photoacoustic flow cytometry. The graph shows the clearance of cells labeled with the MNP cocktail *in vitro* before injection (green curve with filled circles) and those labeled with the MNP cocktail *in vivo* after sequential injections of unlabelled cells alone and then MNPs alone (red curve with open circles).[61]

Besides the pre-labeling of cells with MNPs *in vitro*, as discussed above, cells can also be labeled *in vivo*. As shown in Fig. 10, Zharov *et al.* developed a method to capture circulating tumor cells (CTCs) in the bloodstream of mice by means of MNPs.[61] Specifically, MNPs (Fig. 10(b)) were functionalized with the amino-terminal fragment of the urokinase plasminogen activator (uPA) that could specifically bind to receptors highly expressed on many types of cancer cells. Once systematically administered, MNPs recognized and bound CTCs, which could be concentrated in a blood vessel near the skin with a magnet. To allow the detection of the concentrated CTCs, the researchers introduced a second component, gold carbon nanotubes, as contrast agents for photoacoustic imaging (Fig. 10(c)). The occurrence of natural CTCs and the MNP targeting *in vivo* were demonstrated by intravenously injecting unlabelled CTCs followed by the injection of a cocktail containing the gold carbon nanotubes and MNPs. Flash photoacoustic signals gradually increased within 8–10 min after the injection of the MNPs (red curve in Fig. 10(d)), exhibiting a good match with the photoacoustic signals observed from the cells labeled *in vitro* (green curve in Fig. 10(d)).

Beyond the simple isolation and enrichment of interesting cells, the magnetic manipulation of cells could also help tissue engineering. An exciting example is three-dimensional (3D) cell culture technology that uses magnetic forces to levitate cells while they divide and grow.[62] Compared with cell cultures grown on flat surfaces, 3D cell cultures tend to form tissues that more closely resemble those inside the body. As shown in Fig. 11, hydrogel encapsulating MNPs and filamentous bacteriophage is levitated in a medium by placing a coin-sized magnet atop the dish's lid. More interestingly, through spatially controlling the magnetic field, the geometry of the cell mass can be manipulated and multicellular clustering of different cell types in co-culture can be achieved (bottom images of Fig. 11).[62]

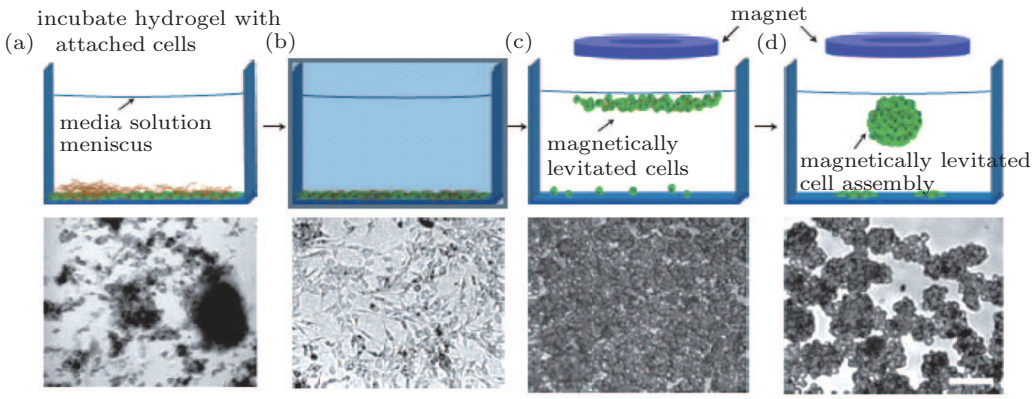


Fig. 11. 3D cell culture with magnetic levitation: (top row) stages of the general cell levitation strategy; (bottom row) the corresponding optical micrograph of neural stem cells at each stage. Scale bar is 30 mm.[62]

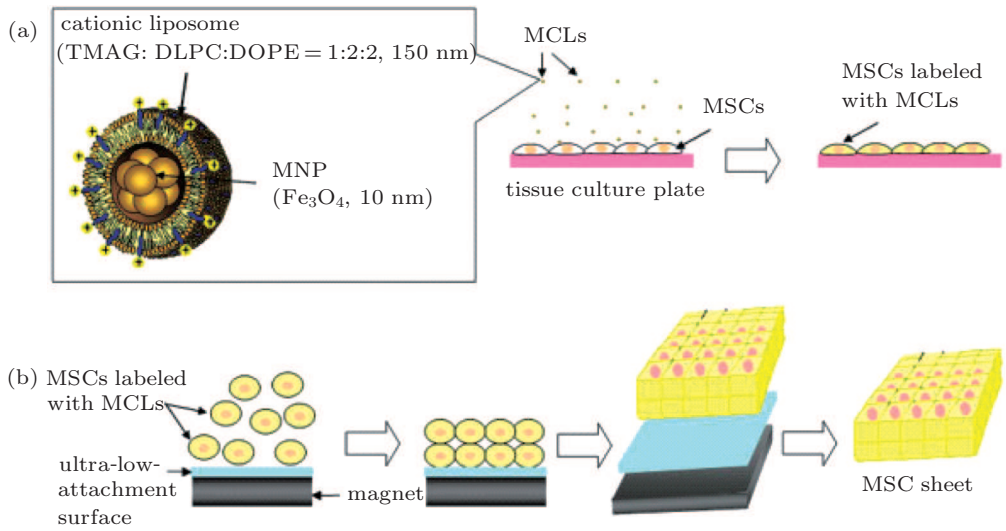


Fig. 12. Schematic illustration of the procedure for MSC sheet construction: (a) MCLs structure and the labeling process of MSCs with MCLs; (b) construction of MSC sheet with magnetic manipulation.[63]

Honda *et al.* took one step further and developed a novel tissue engineering strategy wherein a magnetic force was used to construct 3D tissue without scaffolds (Fig. 12). Briefly, human mesenchymal stem cells (MSCs) were labeled with magnetite cationic liposomes (MCLs) before being seeded onto an ultra-low attachment culture surface. By placing a magnet (4000 G) on the reverse side, MSCs formed multilayered sheet-like structures after a 24-h culture period. More interestingly, the sheets preserved the ability to differentiate into osteoblasts, adipocytes, or chondrocytes after a 21-day culture period using an induction medium. They proved the potential applicability of this technology by harvesting and transplanting the MSC sheets into bone defects in nude rats with successful bone regeneration.[63]

7. Manipulation of organs

Finally, through manipulating the cellular organelles and cells, we can even control the functions of organs. Pralle *et al.* reported a novel approach based on radio-frequency (RF) magnetic-field heating of MNPs to remotely activate temperature-sensitive cation channels in cells, including neurons.[64] And they adapted the approach to remotely trigger the behavioral responses of Caenorhabditis elegans worms. Fluorescence was used throughout their experiments as a nanometer-scale thermometer, showing that the heat was generated locally to the membrane without cytoplasmic heating. Specifically, 6 nm manganese ferrite ($MnFe_2O_4$) MNPs conjugated with streptavidin were targeted to human embryonic kidney cells (HEK 293) expressing TRPV1 and TN-XL via the biotinylated AP-CEP-TM membrane protein. Within 15 s of applying the RF magnetic field (40 MHz, 8.4 G), they detected an increase of the cytosolic calcium concentration from about 100 nM to 1.6 μM. The increase was attributed to a calcium influx through TRPV1 channels which could be thermally activated at around 42 °C.[65] In control experiments, cells with MNPs but without TRPV1 channels and cells with TRPV1 channels but without MNPs showed no calcium influx upon the application of the same RF magnetic field. Furthermore, using the MNP-labeled hippocampal neurons that express TRPV1, researchers demonstrated remotely stimulating neurons without cellular damage. For remotely triggering a behavioral response in the Caenorhabditis elegans worms, all sensory neurons in the amphid region were targeted by the PEG-phospholipid coated MNPs. As in Fig. 13, while a RF magnetic field was applied, within 5 s, fluorescence indicated a local temperature of 34 °C; the worms halted their forward locomotion, and soon afterwards reversed; just like encountering a heated probe.

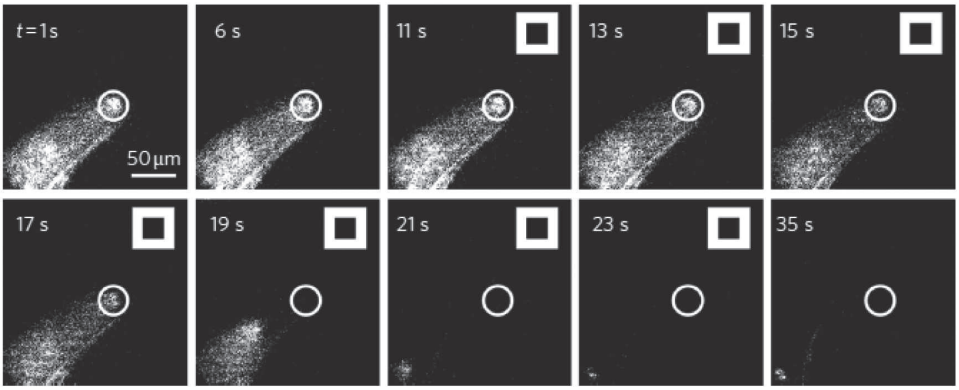


Fig. 13. Remote stimulation of thermal avoidance response in C. elegans. Fluorescence image sequence of the head region of a C. elegans worm labeled with fluorescein-PEG-coated MNPs and anaesthetized with sodium azide.[64]

8. Conclusion

In summary, the synergy between size, structure, and unique magnetic properties of MNPs makes them key players in the non-contact manipulation of biological objects. The successes and the clear promises described above indicate that these platforms will advance our understanding in the life sciences and enhance our ability to utilize them for helping suffering patients.

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