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Magnetic Nano-tweezers for Interrogating Biological Processes in Space and Time

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CONSPECTUS

The ability to sense and manipulate the state of biological systems has been extensively advanced during the last decade with the help of recent developments in physical tools. Unlike standard genetic and pharmacological perturbation techniques – knockdown, overexpression, small molecule inhibition – that provide a basic on/off switching capability, these physical tools provide the capacity to control the spatial, temporal, and mechanical properties of the biological targets.

Among the various physical cues, magnetism offers distinct advantages over light or electricity. Magnetic fields freely penetrate biological tissues and are already used for clinical applications. As one of the unique features, magnetic fields can be transformed into mechanical stimuli which can serve as a cue in regulating biological processes. However, their biological applications have been limited due to a lack of high-performance magnetism-to-mechanical force transducers with advanced spatiotemporal capabilities. In this account, we present recent developments in magnetic nano-tweezers (MNTs) as a high-performance tool for interrogating the spatiotemporal control of cells in living tissue.

MNTs are comprised of force-generating magnetic nanoparticles and field generators. Through proper design and the integration of individual components, MNTs deliver controlled mechanical stimulation to targeted biomolecules at any desired space and time. We first discuss about MNT configuration with different force-stimulation modes. By modulating geometry of the magnetic field generator, MNTs exert pulling, dipole-dipole attraction, and rotational forces to the target

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Notes

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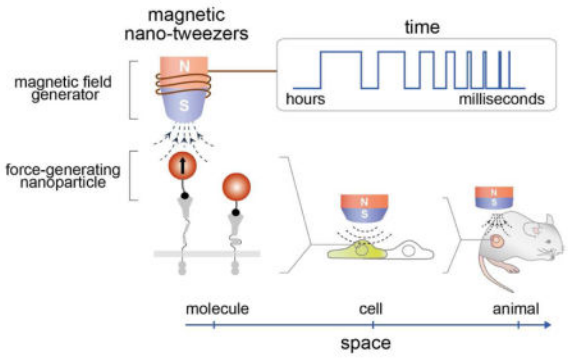
specifically and quantitatively. We discuss the key physical parameters determining force magnitude, which include magnetic field strength, magnetic field gradient, magnetic moment of the magnetic particle, as well as distance between the field generator and the particle. MNTs also can be used over a wide range of biological time scales — By simply adjusting the amplitude and phase of the applied current, MNTs based on electromagnets allow for dynamic control of the magnetic field from microseconds to hours. Chemical design and the nanoscale effects of magnetic particles are also essential for optimizing MNT performance. We discuss key strategies to develop magnetic nanoparticles with improved force-generation capabilities with a particular focus on the effects of size, shape, and composition of the nanoparticles. We then introduce various strategies and design considerations for target-specific biomechanical stimulations with MNTs. One-to-one particle-receptor engagement for delivering a defined force to the targeted receptor and the small size of the nanoparticles are important. Finally, we demonstrate the utility of MNTs for manipulating biological functions and activities with various spatial (single molecule/cell to organisms) and temporal resolution (microseconds to days).

MNTs have the potential to be utilized in many exciting applications across diverse biological systems spanning from fundamental biology investigations of spatial and mechanical signaling dynamics at the single-cell and systems levels to *in vivo* therapeutic applications.

Graphical Abstract



1. Introduction

Mechanical transducers, devices that convert one form of energy to another via mechanical elements, are commonly employed in many current areas of science and technology[1]. Cells utilize miniaturized single-molecule mechanical transducers that convert extracellular physical cues into intracellular biochemical or neuroelectrical signals, (i.e. mechanoreceptors).[2,3] Surprisingly, these tiny molecular transducers can detect spatial and temporal information related to the environment of the cell, such as the mechanical stiffness, and elicit corresponding cellular responses.[2–4] For example, the spatial distribution of integrin-binding ligands within the extracellular matrix differentially regulates downstream cell signaling processes, determining spreading, motility, polarization, and differentiation of the cell.[5–9] Auditory hair cells expressing mechanosensitive ion channels selectively detect oscillatory mechanical stimulation (*i.e.,* sound-induced mechanical motion of the inner ear basal membranes) of specific sound waves.[4,10,11]

Spatiotemporal tools for applying controlled stimulation to mechanoreceptors allow us to interrogate and understand the operating mechanisms underlying these complex mechanotransduction pathways. By measuring the cellular responses to mechanical perturbations in space and time, mechanoreceptors transducing cell signals can be systematically investigated. For several decades, various microscale tweezers, including atomic force microscopy, magnetic tweezers, and optical tweezers, have played prominent roles in the study of biological systems.[12] These tweezers have provided many exciting new discoveries owing to their strong force (~nanonewton, nN) generating capabilities. However, many of these techniques suffer from disadvantages associated with having rather large dimensions (~μm) and their mode of force stimulation is mostly limited to pulling mode (Figure 1a(i), Table 1).[13]

Recently, as a new enabling nanoscale tool, magnetic nano-tweezers (MNTs) have been developed for the controlled stimulation of targeted receptors.[13–22] MNTs use magnetic nanoparticles with sizes comparable to those of conventional proteins as a force-generating component (Figure 1a(ii)). Therefore, MNTs can target specific cells, deliver a defined mechanical force at a desired location and time by tuning the configuration of the external magnets, and hence regulate cellular responses. They can create multiple modes of force stimulation, including pulling, dipolar attraction, and rotation (Figure 1a(iii)). This capacity of the tool has opened exciting opportunities for interrogating cell signaling that are not available with conventional microscale tweezers. Regulation of cell signaling on various biological spatial scales from macroscopic organisms/tissues, to single cells, and even to single molecules is possible by manipulating the nanoparticles with an external magnetic field (Figure 1b(i)).[13–20] This ability has enabled the remote control of animal development and behaviors,[14,15] single-cell and multi-cell signaling control,[13,15–22] subcellular localized aggregation of receptors,[13,20] and single receptor activation.[13,20] MNTs also allow for receptor manipulation over wide ranges of temporal scales that cover the dynamics of most biological events. Extremely fast (*e.g.,* sound perception; μs-ms) or slow (*e.g.,* tissue pressure changes during development; hours to days) mechanotransduction processes can be precisely recapitulated using MNTs (Figure 1b(ii)).[21,22]

To summarize the features of MNTs presented here, we provide a comparison with the features of conventional tweezers in Table 1.[12,23,24]

2. Modes of force stimulation in MNTs

MNTs are comprised of two components: force-generating magnetic nanoparticles and an external magnetic field generator. By using a gradient or uniform magnetic field, several different modes of force stimulation can be achieved to steer the magnetic nanoparticles. Here, we discuss basic principles of force-generation using different field application modes. Construction of external magnetic field generators required for these modes is described in previous publications by our lab and other researchers.[20,22,25]

2.1. Pulling mode under gradient magnetic field

The forces involved in cellular events span a broad range from a few femtonewtons (fN) to sub-nanonewtons.[12,26] Single-membrane protein diffusion based on Brownian motion is

typically on the order of tens of femtonewtons.[14,27] In contrast, the rupture force of a single protein domain-domain interaction is roughly estimated to be 1–100 piconewtons (pN),[28] and forces exerted by single cells (*e.g.,* hair cell deflection) can reach sub-nanonewtons.[29,30]

Magnetic nanoparticles in a gradient magnetic field (Figure 2a(i)) experience a pulling force (Figure 2a(ii)) proportional to the number of magnetic nanoparticles (n), the magnetic moment of the nanoparticles (m), and the magnetic field gradient (∇B), where $F_{pulling} = n \cdot m \cdot \nabla B$, $B \gg k_B T/m$ (k_B, Boltzmann constant; T, temperature).[31–33] Typically, a single magnetic nanoparticle can have a pulling force in the femtonewton range, which is sufficient for the translocation of membrane receptors requiring *ca.* 0.1 femtonewton (Figure 2a(ii), orange and Figure 2b).[13,14,20]

The pulling force generated by magnetic nanoparticles can be easily increased to the piconewton range by i) shortening the magnet-to-particle distance or ii) producing clusters of magnetic nanoparticles. When magnetic nanoparticles are positioned within hundreds of nanometers of the magnet, individual nanoparticles can exert piconewton forces due to the steep magnetic field gradient.[13,20] A conformational change can be triggered in proteins with such a force magnitude.[13,20] Labeling a cell with multiple nanoparticles (*i.e.,* clusters of $10^3 – 10^5$ magnetic nanoparticles) through either the binding of surface receptors or intracellular transfection can exert stronger forces (Figure 2a(ii), green and blue).[21,22] The advantage is that MNTs can apply piconewton-range forces to targets located a few centimeters (~ 3 cm) away from the magnet. For example, these clusters of magnetic nanoparticles allow for the remote *in vivo* manipulation of cell displacement (Figure 2c, top) and migration in animals (Figure 2c, bottom).[21,22]

2.2. Dipolar attraction and rotation modes under uniform magnetic field

A magnetic field generator design that exerts uniform magnetic fields provides another way to steer magnetic nanoparticles. In this case, the magnetic field induces magnetization of the nanoparticles; however, due to the field uniformity (∇B < 50 T/m, Figure 2d), the nanoparticles do not experience a pulling force arising from the field gradient. Instead, when the nanoparticles are in close proximity (< 100 nm) to one another, they experience magnetic dipole-dipole attraction force, which can induce aggregation (Figure 2e).[34] When two magnetic particles are placed under the uniform magnetic field, force ($F_{m_1 m_2}$)is defined as follows[34]:

$$F_{m_1 m_2} \propto \frac{\vec{m}_1 \cdot \vec{m}_2}{r^3}(1 - 3cos^2\theta)$$

where, r is interparticle distance, $\vec{m}_1$, $\vec{m}_2$ are magnetic dipole moments of nanoparticles, and θ is angle between the direction of external magnetic field and the centerline of two magnetic nanoparticles. This magnetic dipolar attraction force can be in the range of tens of femtonewtons at a relatively weak external magnetic field strength with a long working distance (1–10 cm).[14,15]

Distinct from other force microscopy tools, magnetic particles can rotate under a rotating external magnetic field.[35] A magnetic particle having magnetic moment (m) can rotate to align with the direction of external magnetic field (B), and hence generate torque (τ), where $\tau = m \times B$ (Figure 2f).[36] This rotating mode has been extensively used for investigating the dynamics of single molecules such as supercoiled DNA with magnetic particles.[37,38] Also, magnetic twisting cytometry on cells has been demonstrated for studying force transmission across the cell membrane and assessing cell stiffness.[39,40]

3. Temporal resolution of MNTs

MNTs based on permanent magnets can provide only a static magnetic field. In this case, the temporal resolution (*i.e.,* magnetic field on/off) for the fast mode can be on the order of a few seconds. On the other hand, MNTs based on electromagnets allow for dynamic control of the magnetic field and hence the magnetic nanoparticles. By simply adjusting the amplitude and phase of the applied current, the magnetic field strength and orientation can be manipulated. This unique property of electromagnets provides programmed and rapid manipulation of magnetic particles and hence the targeted biomolecules with high temporal precision. Recently, a micro-electromagnet comprising a permalloy needle (tip diameter: 10 μm) housed inside a water cooling jacket was developed.[21,22] It can selectively generate a magnetic field within a single cell with a 50 μs temporal resolution without noticeable heat generation. This ability of the micro-electromagnets is particularly useful for investigating rapid mechanotransduction pathways such as hearing at the single-cell level.[21,22]

4. Design of force-generating magnetic nanoparticles

Magnetic nanoparticles are conjugated to targeting moieties for the selective stimulation of desired targets. Several critical considerations should be taken into account when designing magnetic nanoparticles and conjugating them with targeting moieties to produce a force-generating component.

4.1. Magnetic nanoparticles

Among the various types of magnetic nanoparticles, ferrite-based nanoparticles are most commonly utilized for MNTs due to their excellent biocompatibility. Typical ferrite (that is, iron oxide) nanoparticles can usually exert a few tens of femtonewtons force under magnetic field.[16] The force exertion capability of these magnetic nanoparticles can be improved by engineering ferrite nanoparticles (Figure 3a).[41–44] By tuning the size (4–100 nm)[41,44], composition (Ni, Mn, Co and Zn doping)[41,42] and shape (spheres and cubes),[43] single ferrite nanoparticles can exert forces up to sub-piconewtons.[13–15,17,20–22]

The size of magnetic nanoparticles should be carefully considered in terms of the desired cell labeling density.[13] Cell membranes are densely covered by a carbohydrate matrix forming the glycocalyx.[45] This sugar layer serves as a permeability barrier to foreign materials, limiting the access of large particles to the cell surface receptors. Hence, the number of nanoparticles that can bind to a cell surface is inversely correlated with particle size. For example, under identical labeling conditions, magnetic nanoparticles (50 nm) have a 10^4–10^5 times higher target labeling efficiency than magnetic microbeads (2.8 μm).[13]

Typically, particles that range from 10–50 nm in diameter are ideal, where relatively large particles are suitable for force microscopy applications that require strong force generation and small nanoparticles are beneficial for maximizing receptor labeling and minimizing nonspecific perturbation.[13,20]

In contrast to conventional microscale tweezers, to observe nanoscale particles, additional optical moieties are required since nanoparticles are too small to be detected by optical microscopy due to the diffraction limit of light. Fluorescent dyes, quantum dots, and gold nanoparticles can be used for this purpose. For example, magnetoplasmonic nanoparticles (MPNs) that have gold shells as an imaging component allow for the real-time monitoring of single nanoparticles under an optical microscope (Figure 3b(i)).[13,20] The scattering colors can also be precisely tuned from 550 to 620 nm depending on the gold shell thickness (Figure 3b(ii, iii)).

4.2. Targeting moieties

Several targeting strategies have been employed for magnetic nanoparticle-receptor labeling. Among them, a common strategy is to use antibody-antigen interactions, where antibodies specific to the target receptors are covalently conjugated to the nanoparticles via chemical crosslinkers. Magnetic nanoparticles with anti-Tie2 and anti-death receptor 4 (DR 4) antibodies were synthesized via carbodiimide crosslinker chemistry and used for targeting and controlling Tie2 and DR 4 receptors, respectively.[14,17] The other method is to decorate magnetic nanoparticles with native ligands that selectively bind to target receptors via ligand-receptor interactions (*e.g.,* IgE-FcεRI[16] or delta-like ligand (Dll)-Notch[13]). The genetically encoded targeting of receptors with magnetic nanoparticles is also possible via recombinant DNA technology. In this case, various polypeptide protein tags such as Myc-, His- and SNAP-tags can be inserted into a target receptor sequence and labelled with the corresponding antibody-conjugated or small molecule labeled magnetic nanoparticles.[13,20]

While various targeting strategies are available, several important design considerations for the targeting domain should be taken into account. To deliver a defined force to the targeted receptor, a one-to-one particle-receptor engagement with minimized nonspecific receptor perturbation is important. The presence of multiple ligands per nanoparticle is not desirable because the force generated by a single particle is distributed across multiple receptors.[13,20] Also, the presence of multiple ligands can induce receptor crosslinking and change the receptor dynamics, including diffusion, translocation, and signal transduction.[13,14,20] The simplest way to achieve monovalent conjugation is through the stoichiometric control of the targeting moieties and magnetic nanoparticles.[13,14,20] For example, nanoparticles bearing a single oligonucleotide can be obtained via either steric exclusion or charge-based valency discrimination. Targeting biomolecules can be further conjugated via complementary DNA or RNA hybridization or end-functionalization of the oligonucleotide strand.

5. Spatiotemporal manipulation of cell signaling with MNTs

5.1. Manipulation of biological activities on different spatial scales

Early attempts to use magnetic nano-tweezers focused on stimulating a group of cells located in the proximity of a magnetic needle in a cell culture. Aggregation and activation of FcεRI receptors in cells was reported using IgE-coated magnetic nanoparticles.[16] Similarly, microtubule polymerization could be regulated by magnetic nanoparticles through induced localized aggregation of Rho-GTPase and Ran GTP.[18,19]

The applicability of MNTs can be further extended to *in vivo* systems.[14,15] Magnetic nanoparticles were delivered into the yolk of a zebrafish embryo to target the ovarian TNF receptors (OTRs). Then, a uniform magnetic field was applied to induce aggregation of the OTRs by using the dipolar attraction mode to trigger apoptotic signaling (Figure 4a(i)).[14] A characteristic phenotypic defect in the tail region of zebra fish was observed for the magnetically stimulated group compared with the negative control groups (Figure 4a(ii)). This approach could be further applied to a larger animal model by implementing the magnetic tandem apoptotic trigger (mTAT) concept in a system for treating multidrug resistant (MDR) cancer.[15] The mTAT system could stimulate both extrinsic (*i.e.,* DR 4 signaling) and intrinsic (*i.e.,* DNA damage) apoptotic signals through magnetically controlled receptor aggregation and intracellular anti-cancer drug release (Figure 4b(i)). This ability led to the complete removal of MDR cancer from a mouse xenograft model, which was not possible with chemical drug treatment alone (Figure 4b(ii)).

In contrast to the dipolar attraction mode, the usage of the pulling mode in *in vivo* applications is often limited due to exponential decay of the magnetic field strength. However, when loaded with clusters of magnetic nanoparticles (~10^5), cells can experience a piconewton-level pulling force even when located a few centimeters away from the magnet. Such magnetic nanoparticles loaded cells, termed magnetically-labeled cells (Mag-Cells), can be pulled by external magnet and thus can be delivered to a target location within *in vivo* tissue environments such as a rat brain (Figure 4c(i–iii).[46]

By implementing suitable magnetic nanoparticles and improved magnet designs, MNTs can be used for single-cell and subcellular signaling control. Specifically, under a gradient magnetic field with a magnet-to-particle distance of sub-micrometers, magnetic nanoparticles can exert force on targeted Notch receptors, leading to mechanical activation of Notch receptor signaling (Figure 5a(i)). In a model Gal4/upstream activation sequence (UAS) reporter system, the transcriptional profiles of targeted cells could be systematically tuned at any desired location with single-cell resolution (Figure 5a(ii)). Single-cell apoptosis has also been demonstrated with a m-TAT system (Figure 5a(iii, iv)).[15] A pair of micro-magnets applied a uniform magnetic field only to the targeted cell region, induced DR 4 aggregation and initiated apoptosis signaling. MNTs further allowed for interrogation of the subcellular receptor signaling dynamics under a gradient magnetic field (Figure 5b).[13,20] Magnetic nanoparticles could induce the localized aggregation of cadherin and hence promote stable adherens junction formation at a desired subcellular location.

MNTs can also be used for the single-molecule activation of mechanoreceptors in live cells (Figure 5c).[13,20] The key advantages of MNT-based single-molecule studies in live cells compared with conventional single-molecule force microscopy are that monovalent nanoparticles ensure one-to-one engagement of the force-generating particle with the targeted receptor and do not perturb the dynamic properties and mechanical environment of the receptors.

5.2. Toward the interrogation of rapid mechanotransduction dynamics

The time scale of biological events varies from microseconds to days.[47] Among them, sound perception is one of the most rapid mechanotransduction process.[4,10,11] Human hair cells sense the mechanical vibration of sound in the frequency range of 20–20,000 Hz.[11] While MNTs that cover biological time scales of minute to hours (Notch receptor signaling-based DNA transcription/translation[13,20]) and days (apoptosis[14] and angiogenesis[17]) have been successfully demonstrated, those with high-speed capabilities have not been possible until recently.

To interrogate the fast dynamic mechanotransduction process, ultra-fast MNTs consisting of 40 nm Zn-doped ferrite nanocubes and a micro-electromagnet were developed.[21,22] Individual hair bundles labeled with magnetic nanoparticles can be deflected with an applied magnetic field (Figure 6a). Approximately 1,400 magnetic nanoparticles are bound to each hair bundle, and they provide a pulling force of up to 280 pN. Hair bundle deflection induces mechanical tension in the tip links and opens the ion channels, allowing for an influx of Ca^{2+}. The gating of the mechanosensitive ion channels coincides with the applied magnetic pulses from calcium imaging (Figure 6b). Indeed, ultra-fast MNTs can provide bundle oscillations of up to 50 kHz. This temporal resolution is 1,000 times faster than that of conventional MNTs (Figure 6c). When the hair bundle is stimulated with the audible frequencies of humans, its oscillations remain phase-locked over the tested frequencies (Figure 6d).

Noncontact-mode force stimulation is another unique capacity of ultra-fast MNTs that has led to new findings in hair cell dynamics in response to mechanical perturbation. Specifically, the noncontact-mode is beneficial for the readout cellular components involved in the oscillatory dynamics of free-standing hair bundles.[22] From exponential fitting of the recovery motions of hair bundles after magnetic deflection (Figure 6e(i)), two relaxation components with different time scales (τ_{fast} and τ_{slow}) were observed (Figure 6e(ii)). τ_{fast} and τ_{slow} were closely related to the passive physical properties of hair bundles and the active actomyosin interactions, respectively. Additionally, the active recovery motions of hair bundles showed unique frequency-dependent characteristics, where basal, middle, and apical hair bundles could effectively respond to their respective ranges of frequency (Figure 6e(iii)). These experiments provided new insights into the frequency selection mechanisms of hair cells along the tonotopic axis.

6. Conclusion and outlook

In this account, we present a review of novel and potentially generalizable strategies for studying biological activities with MNTs. Nano-tweezers provide key advantages over

conventional force microscopy tools, particularly for interrogating and manipulating the spatiotemporal dynamics of cellular signaling and living organisms with high precision. Limitations of the systems also exist and include a relatively high magnetic field gradient for strong pulling force exertion and poor accessibility to intracellular targets. Further improvements may be achieved by developing nanoparticles that have higher magnetization values, implementing spatiotemporally optimized magnets with more precise field control (*e.g.,* superconducting magnetic coils), and developing genetically encoded magnetic nanoparticles beyond the current ferritin system (*e.g.,* genetic expression of magnetosomes[48–50] in mammalian systems). With their unique and versatile capabilities, nano-tweezers are being developed to investigate many important biological questions during developmental, physiological, and pathological processes and to provide new therapeutic interventions.

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Biographies

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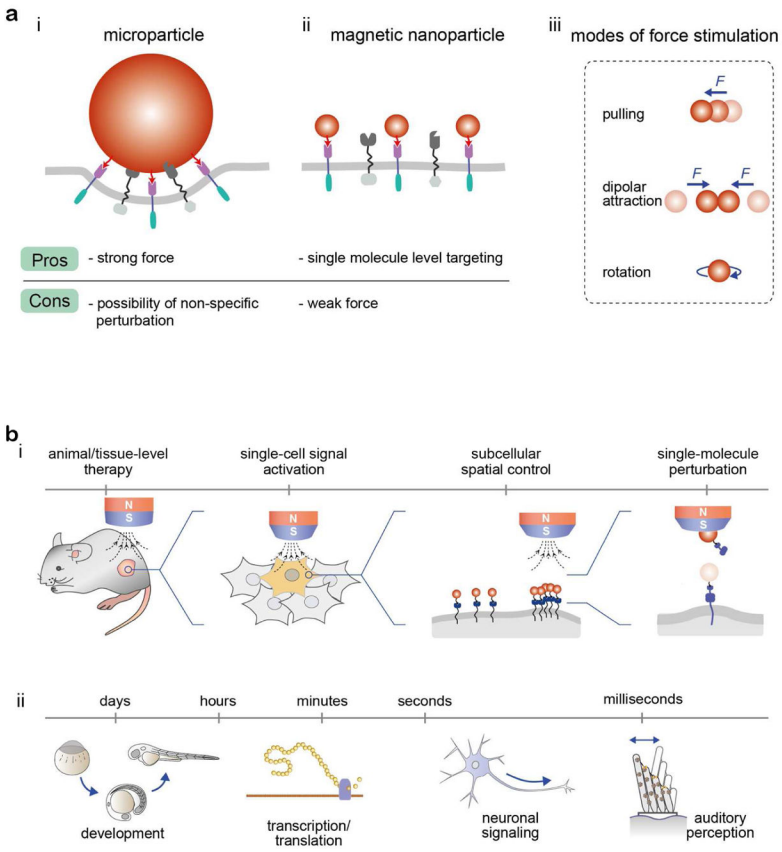

Figure 1.

(a) Comparison between micro- and nanoparticles as force generation components with microscale (i) and nanoscale (ii) tweezers, respectively. (iii) Modes of force stimulation. (b) Applications of magnetic nano-tweezers (MNTs) in the regulation of biological process on various spatial and temporal scales. (i) Regulation of cell signaling on various biological spatial scales from organisms/tissues, to single cells and to single molecules is possible by manipulating the nanoparticles with an external magnetic field. (ii) Extremely fast or slow mechanotransduction processes can be precisely recapitulated using MNTs.

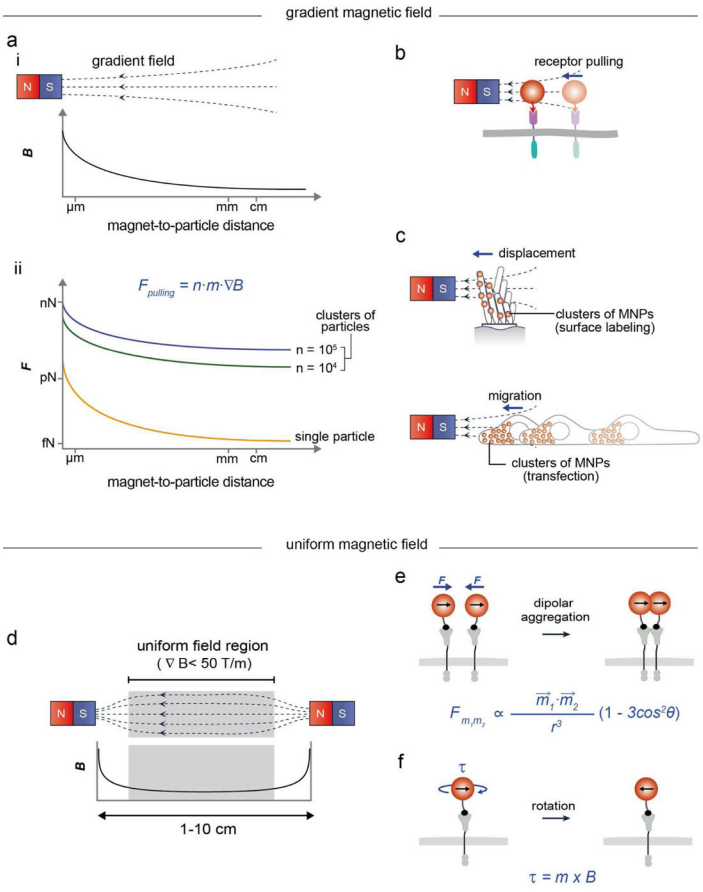

Figure 2.

Modes of force stimulation in MNTs. (a–c) Pulling force modes. (a) Gradient magnetic field generated by a single magnet (i) and the corresponding force magnitude exerted by magnetic nanoparticles (MNPs) as a function of the magnet-to-particle distance and the number of MNPs (ii). (b) The pulling force exerted by a single MNP allows for the translocation of membrane receptors. (c) The displacement (top) and migration (bottom) of cells can induced with clusters of MNPs. (d–f) Dipolar attraction and rotation modes. (c) Uniform magnetic field generated between two magnets. (e, f) Dipolar attraction between magnetic nanoparticles induces the receptor clustering (e) and the rotation of particles for torque generation (f). n, number of nanoparticles; $\vec{m}$, magnetic moment; B, magnetic field strength; ∇B, magnetic field gradient, r, distance between nanoparticles θ, angle between the direction of external magnetic field and the centerline of two magnetic nanoparticles; τ, torque.

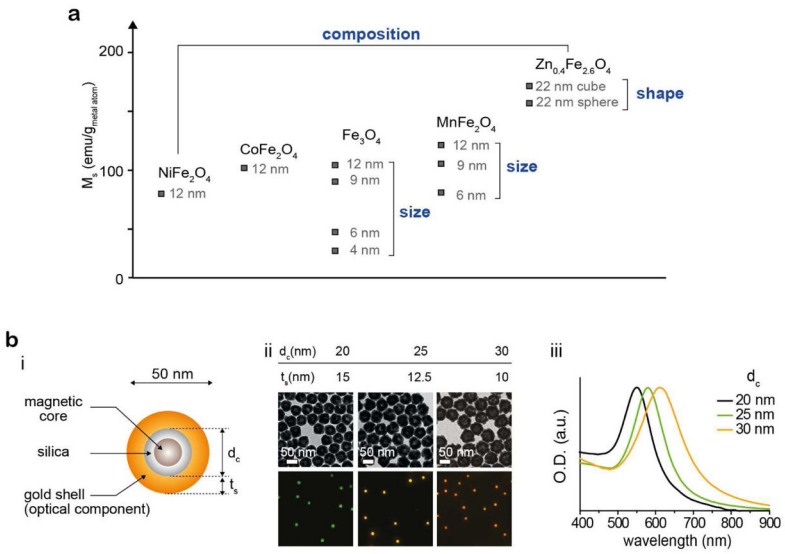

Figure 3.

(a) Magnetism engineering of magnetic nanoparticles for effective force generation. By tuning the size, composition, and shape of magnetic nanoparticles, a higher saturation magnetization (M_S), which is a critical factor for force generation, can be achieved. (b) Magnetoplasmonic nanoparticles (MPNs). (i) A MPN is comprised of a magnetic core and a plasmonic shell that serve as the force-generating and imaging components, respectively. (ii) Silica core size (d_C)-dependent plasmonic absorption shift. t_S indicates thickness of gold shells. TEM images (top) of 50 nm MPNs with 20 nm (left), 25 nm (middle) and 30 nm (right) silica cores and their corresponding scattering images (bottom). (iii) Absorption spectra of 50 nm MPNs with 20 nm, 25 nm and 30 nm silica cores. Reproduced with permission from ref 20, copyright 2017 Nature Publishing Group.

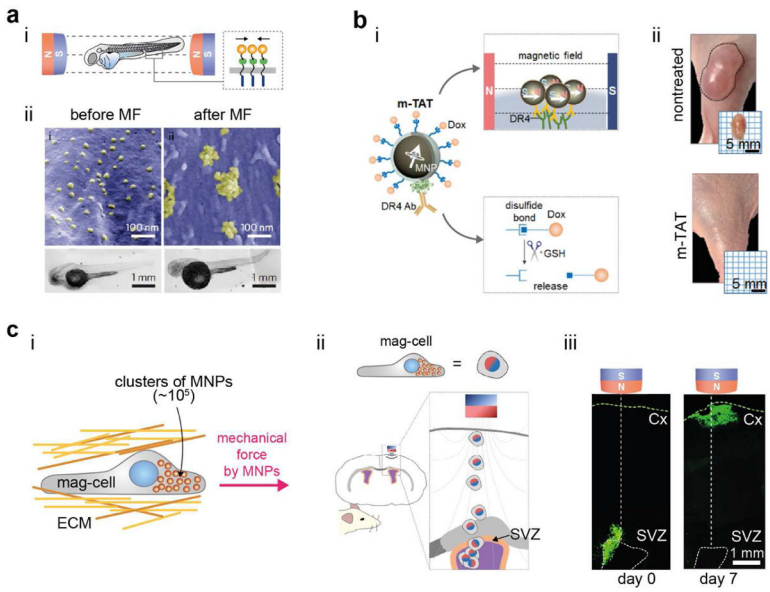

Figure 4.

In vivo manipulation of biological activities with MNTs at the tissue level. (a) Magnetic activation of apoptotic signaling in zebra fish. (i) The aggregation of ovarian TNF receptors (OTRs) triggered by the dipolar attraction force induce apoptosis. (ii) SEM images of aggregated magnetic nanoparticles on the cell surface and the corresponding tail region bending. (b) Schematic illustration of magnetic tandem apoptosis trigger (m-TAT) and its dual modes of stimulation (i), and photographs of xenograft tumor-bearing mice obtained after treatment (day 14) (ii). (c) Guiding of cell migration by using a magnetic pulling force. Magnetically labelled cells (mag-cell) can remotely control cell migration (i) and thus deliver cells from the subventricular zone (SVZ) to the cortex (Cx) within a rat brain (ii). (iii) Immunohistochemistry images showing magnetism-guided cell migration within a rat brain. The green color indicates magnetic nanoparticle-loaded cells. Reproduced with permission from ref 14, copyright 2012 Nature Publishing Group; Reproduced with permission from refs 15, 46, Copyright 2016, 2018 American Chemical Society.

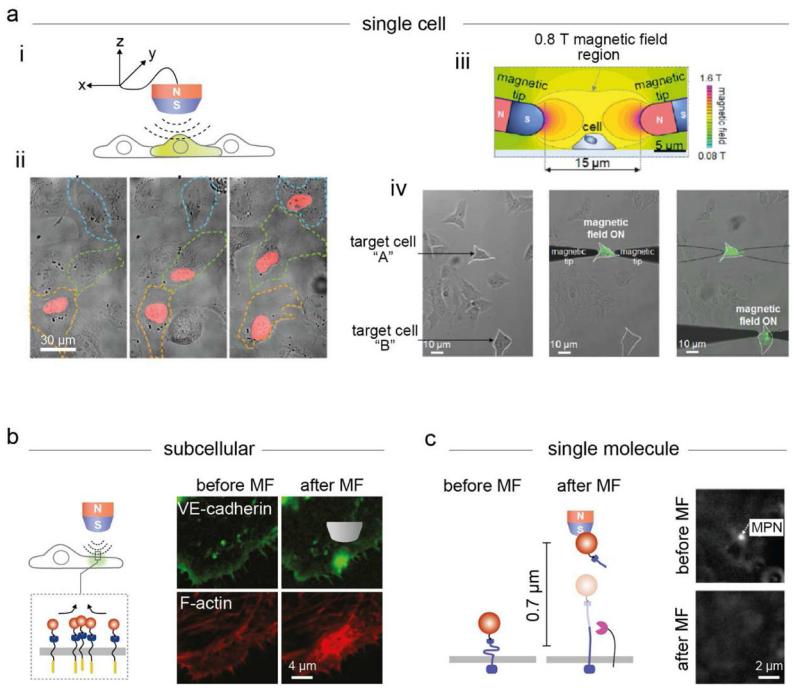

Figure 5.

Manipulation of biological activities with MNTs from the single-cell to the single-molecule level. (a) Single-cell signaling control. (i) Mechanical activation of Notch receptor signaling. (ii) The mCherry fluorescence increases under sequential magnetic field application to 3 target cells. (iii) Magnetic field simulation for single-cell-level apoptosis induction by m-TAT. (iv) Sequential activation of apoptosis signaling. The green fluorescence signal (FITC-annexin V) indicates the induction of apoptosis. (b) Interrogation of subcellular VE-cadherin-mediated adherens junction formation: (top) MPN-induced VE-cadherin clustering; (bottom) F-actin assembly recruitment. (c) Single-molecule activation of mechanoreceptors in live cells. Under the application of a strong pulling force (9 pN, top), the immediate detachment of the MPN is detected under dark-field microscopy (bottom). Reproduced from ref 13, Copyright 2016, with permission from Elsevier; Reproduced with permission from ref 15, Copyright 2016 American Chemical Society; Reproduced with permission from ref 20, copyright 2017 Nature Publishing Group.

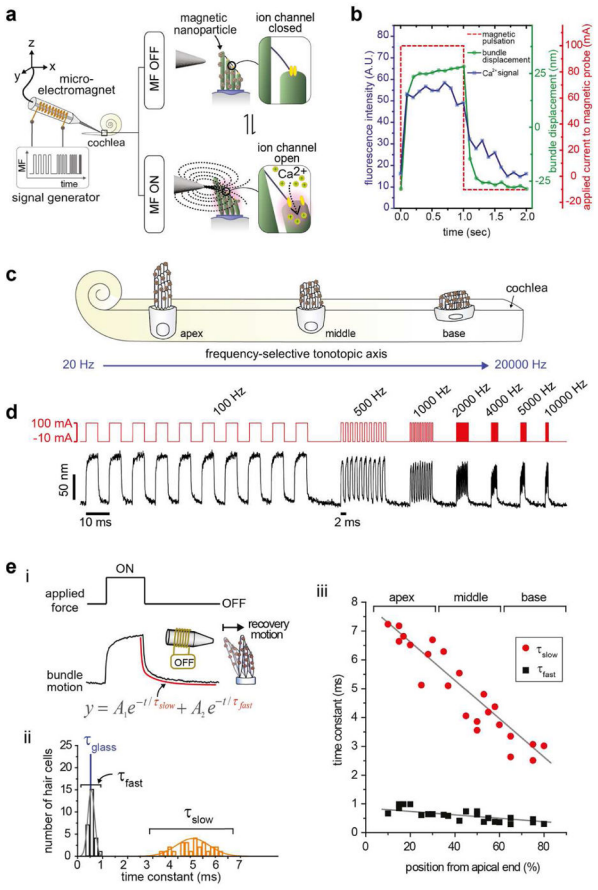

Figure 6.

MNTs for studying fast mechanotransduction dynamics with the non-contact mode. (a) Schematic illustration of the high-frequency magnetic control of hair bundle oscillation. Hair bundles labeled with 40 nm Zn-doped ferrite nanocubes are pulled with a micro-electromagnet. Mechanical tension on the tip links, induced by hair bundle deflection, opens the ion channels, creating an influx of Ca^{2+}. (b) Gating of the mechanosensitive ion channels in a hair bundle using MNTs. The Ca^{2+} influx in the bundle coincides with the applied magnetic pulses. (c) Hearing range of human hair cells. Hair cells are located along the tonotopic axis of the cochlea and detect specific resonance frequencies. (d) Magnetic entrainment of hair bundle oscillations; pulse frequencies of 100, 500, 1000, 2000, 4000, 5000, and 10000 Hz are applied (top), and the hair bundle displacements are observed (bottom). (e) MNTs for interrogating the biomechanics of the hair bundle recovery process. (i) The recovery motion of the deflected hair bundle is tracked and fitted with second-order exponential functions (equation, red line) to extract two biological components (τ_{slow} and τ_{fast}). (ii) Distribution of τ_{slow} and τ_{fast} of the middle-region hair bundle. The time constant of a glass fiber (τ_{glass}, blue) is shown as a passive object for comparison. (iii) Tonotopic dependency of the time constants measured along the entire tonotopic axis from the apical to the basal region of the cochlea. Reproduced with permission from refs 21, 22, Copyright 2014, 2016 American Chemical Society.

Table 1

Comparison of magnetic nano-tweezers with conventional tweezers*.

	Nanoscale tweezers		Conventional tweezers		
	Magnetic nano-tweezers (MNTs)		Magnetic tweezers	Optical tweezers	AFM
Probe type	Single magnetic nanoparticle	Clusters of magnetic nanoparticles	Magnetic microparticles	Polystyrene or silica microparticles	AFM Cantilever
Primary mode of force stimulation	- Pulling - Dipolar-attraction - Rotation	- Pulling	- Pulling - Rotation	- Pulling	- Pulling - Push
Force range	Weak (~ hundreds of fN)	Strong (~ sub-nN)	Strong (10^{-2} – 10^{2} pN)	Strong (10^{-1} – 10^{2} pN)	Very strong (10^{1} – 10^{4} pN)
Target dimension	Single molecule to cell and tissue	Single cell to tissue	Single molecule to cell and tissue	Single molecule to single cell	Single molecule to single cell
Target labeling density (probes/cell)	High (10^{1}–10^{5} probes/cell#)		Low (one or a few probes/cell)	Low (one or a few probes/cell)	Low (one probe/cell)

*general observation, but can be varied depending on the experimental condition, instrument, and setup

#depends on the target receptor density