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MULTIFUNCTIONAL NANO–BIO MATERIALS WITHIN CELLULAR MACHINERY

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Abstract

Functional nanoscale materials that possess specific physical or chemical properties can leverage energy transduction *in vivo*. Once these materials integrate with biomolecules they combine physical properties of inorganic material and the biorecognition capabilities of bio-organic moieties. Such nano–bio hybrids can be interfaced with living cells, the elementary functional units of life. These nano–bio systems are capable of bio-manipulation or actuation via altering intracellular biochemical pathways. Thus, nano–bio conjugates are appealing for a wide range of applications from the life sciences and nanomedicine to catalysis and clean energy production. Here we highlight recent progress in our efforts to develop smart nano–bio hybrid materials, and to study their performance within cellular machinery under application of external stimuli, such as light or magnetic fields.

Keywords

Nano–bio interfaces; TiO₂ nanoparticles; ferromagnets; spin vortex state; magneto-mechanical actuation; apoptosis; cancer cells

1. Introduction

Functional nanoscale materials that possess intrinsic physical or chemical properties can leverage signal transduction *in vivo* under external stimuli, such as light, magnetic fields or mechanic force. Synthetic interfacial chemistry approaches enable the coupling of these materials with various bio-molecules capable of unique properties, such as biorecognition (Fig. 1). Hard materials integrated with biological molecules result in heterostructures that synergistically combine the properties of both inorganic and bioorganic moieties in synergistic, mutually enriching fashion. In addition, organic entities can affect physico-chemical properties of an inorganic moiety.

Nano–bio hybrids then can be interfaced with biological systems, such as a living cell, the smallest functional unit of life, for direct triggering of biochemical pathways via energy or information transduction. These systems are appealing for a wide range of applications in the life sciences, including gene sequencing and silencing, cell sorting and separation.

Furthermore, these smart nano–bio materials can be useful when introduced into more complex biological systems, such as the human body, and can serve as the basis of the new medical technology called *nanomedicine*, which is applicable for diagnostics, imaging, controlled drug delivery and therapy. Broadly defined, the nanobio interface is a novel area of science and technology that links physical and life sciences to create advanced materials in order to address the needs of the 21st century.

Here we discuss two examples of such nano–bio materials capable of signal transduction within cellular machinery under application of two different kinds of stimuli: light and alternating magnetic fields.

2. Biofunctionalized Titanium Dioxide Hybrids for Intracellular Red/Ox Signaling

Metal oxide TiO_2 is a semiconductor with a bulk band gap of 3.2 eV that can induce reactive oxygen species (ROS) in aqueous medium under UV light exposure. The surface of the metal oxide could be readily modified with various oxygen-containing functional groups, including carboxylic-, hydroxyl-, and phosphate.^{1–3} Our approach in the construction of TiO_2 nano–bio hybrids is based on application of naturally occurring dihydroxybenzenes. For instance, catecholamine neurotransmitter dopamine (**1**) or metabolically-related 3,4-dihydroxyphenylacetic acid (DOPAC) and L-3,4-dihydroxyphenylalanine (L-DOPA) (**2** and **3**) can serve as linkers between the semiconductor metal oxide particles and the biomolecules,^{4–8} see Fig. 2. Simple organisms are known to utilize proteins containing in their primary structures dihydroxybenzene motifs for adhesion to multiple materials; e.g. mussels use them to anchor to surfaces in turbulent aqueous environments.^{9,10} In our bio-inspired minimalist approach we use low molecular weight catecholate ligands for the coupling chemistry on the surface of metal oxide nanoparticles via “glueing” of carboxylic- or amino- groups suitable for further functionalization. In addition, chemisorption of electron donating dihydroxybenzenes ligands on the surface of TiO_2 nanoparticles reconstructs the nanocrystal surface, red shifting the absorption edge to the visible part of the spectrum below 750 nm.^{11,12} Modification of titanium dioxide particles with dihydroxybenzenes can affect the dynamics of localized charges drastically changing multiple light-induced radical chemistries initiated on the surface of the nanoparticles. As a result, the formation of reactive oxygen species (ROS) differs significantly between catecholate-modified and bare TiO_2 particles.⁵ The major ROS produced in this photoreaction is superoxide anion, Fig. 3, whereas hydroxyl radicals (OH) and singlet oxygen ($^1\text{O}_2$) are known to be the main products of photo-excitation of bare TiO_2 particles.^{13–17}

Owing to the unique function of molecular recognition, biological molecules linked to the nanoparticle surface can carry resulted bio-nano construct to cellular membrane antigens or sub-cellular compartments of interest limiting undesirable random distributions of nanoparticles within healthy tissues. Our nanoparticle–antibody (Ab) coupling strategy enabled the production of a nanobio conjugate that retained both photocatalytical and biorecognition capabilities.^{6–8} On the base of the standard ELISA assay the binding affinity of the TiO_2 -conjugated Ab was comparable to that of an unconjugated antibody with slight (up to 10%) loss of activity. Pronounced and human glioblastoma-specific phototoxicity of 5 nm TiO_2 particles tethered to an antibody an Ab via dihydroxybenzene bivalent linker was demonstrated.⁶ Moreover, the cytotoxicity effect depended on levels of the specific receptor over-expression on malignant cells; it reached 50% for U87 and over 80% for A172 cells. More importantly, no cytotoxicity toward normal human astrocytes was observed.⁶ In addition to a standard viability assay microscopy examination of light-induced dynamics of altering a cellular morphology can serve as a useful complementary approach to study

mechanisms of photo-induced cytotoxicity. Thus, on the basis of laser confocal analyses of A172 glioblastoma cell cultures, it is obvious that an apoptotic pathway was initiated. Cell morphology changes induced by the nano-bio hybrid application and exposure to white light are summarized in Fig. 4. All cells were pre-incubated with nano TiO_2 -Ab photocatalyst for 1 h under standard conditions. When no focused polychromatic white light was applied, the cells did not change and appeared intact, see Fig. 4, left.

Only 5 min of light illumination with intensity of 60 mw/cm^{-2} resulted in remarkable cell morphology changes. Thus, after 30 min following the light exposure some cells (marked with white arrows) undergo cell blebbing, a morphology change typical of apoptotic cells, see Fig. 4, center. Dramatic cell morphology changes were observed in 90 minutes following of post-light exposure. On Fig. 4, right, the majority of cancer cell appear to be dead, the cell bodies are rounded off, and cell membranes look shrunk.

We propose, that under visible light illumination that the TiO_2 -DOPAC-Ab system catalyses generation ROS, mainly superoxide anion.^{5,6} These electronically-activated species then serve as intra-cellular mediators altering cellular respiratory pathways for re-programming cancer cells death.

Reduced sensitivity of cancer cells toward apoptosis leads to inappropriate cell survival in comparison with normal cells, and consequent malignant progression. Therefore, selective induction of apoptosis in malignant cells is important for the anticancer therapeutic strategies, including those based on nano-bio platforms.¹⁸ The possibility of controlling the surface photochemistry using small organic electron-donating ligand opens future horizons for selective on-demand induction of redox signaling within the cellular machinery and the development of novel, intelligent approaches for cancer therapies.

3. Biofunctionalized Ferromagnetic Disks for Magnetomechanically-Induced Cellular Signalling

Beside their well-known application in the magnetic recording industry,¹⁹ nanomagnetic materials have been proposed for the life sciences, including cell separation technologies, magnetic resonance imaging (MRI) contrast enhancement, targeted drug delivery, magnetically induced hyperthermia and magnetofection.²⁰⁻²⁵

The advantage in applications of nanoscale magnetic materials in life sciences is in the high surface-area-to-volume ratio that makes possible a fusion of inorganic material with biological molecules to achieve a combination of properties in one designer material.²⁶

While most reports on applications have been of *superparamagnetic* materials, including their bio-conjugates, the interfacing of *ferromagnetic* nano-materials with biomolecules and biological systems is a principally novel approach.^{27,28}

In contrast to magnetic hyperthermia treatment modalities, we are exploring a different innovative pathway to achieve tumor regression (cytotoxicity effect) with nanomagnetic materials. Most experimental works to date have utilized super-paramagnetic particles, those having zero magnetic moment at remanence.^{20-26,29,30} The super-paramagnetic nature and typically small magnetization at saturation of these particles, requires a high frequency magnetic field to enable their detection and/or manipulation. Conversely, if larger particles are used to increase the magnetic moment, they tend magnetize even in absence of a magnetic field which can result in particle agglomeration (due to inter-particle dipolar interactions). Other challenges associated with the magnetic hyperthermia approach include the difficulty in controlling the growth process, broad size and shape distributions, and

variable structural and magnetic properties. Our approach is based on an application of lithographically-defined disks-shaped magnetic particles, see Fig. 5. Owing to the anisotropic shape of these planar structures with lateral dimensions less than their thickness, the spin arrangement often takes on a unique pattern known as a spin vortex state.^{31–34} The existence of the magnetic vortices in the microdisks was confirmed using Magnetic force microscopy (MFM) of the reference sample, as shown in Fig. 7.

A magnetic vortex is characterized by an in-plane flux-closure spin distribution with net zero magnetization in the absence of a magnetic field, see Fig. 6, inset. The latter is important since it eliminates long-range magnetostatic forces that are otherwise responsible for particle agglomeration. The disks are composed of a 50 nm-thick, one micron-diameter 20:80% iron–nickel magnetic core, encapsulated into a 5 nm-thick layer of gold shell. They were prepared by magnetron sputtering and optical lithography.²⁷ Group 3d transition metals comprise the magnetic core and provide high values of the saturation magnetization and a corresponding ability to induce a high magnetomotive force, while the gold shell provides protective and biocompatibility functions, as well as a substrate for subsequent surface coupling chemistry steps. Because of their anisotropic (flat) shape, the magnetic particles respond by mechanical torque to an external magnetic field stimulus, similar to how a compass needle aligns with an external magnetic field. Typically, a ~ 10 millisecond duration field pulse of ~10 Gauss amplitude is sufficient to initiate field-driven disk alignment via mechanical torque. A consequence is that the magnetic vortices can be used as mediators to deliver the energy of an external source (the magnetic field) to the cell membrane, to which it attaches itself, via magneto-mechanical coupling.

The disks functionalized with specific Ab were targeted to the surface of human N10 glioblastoma cells, see Fig. 5 (right bottom corner). As can be seen from a light microscopy and AFM studies (Figs. 8 and 9) an application of *ac* fields of 90 Oe and 10–20 Hz for only 10 min resulted in drastic cell morphology changes. Figures 8 and 9 compare representative optical and AFM images of the control N10 cells pre-incubated with MD–mAb (left), and the cells after 90 Oe/20 Hz *ac* magnetic field exposure (right) followed by a 1 hr recovery culture. As a result of the *ac* field application the cells lose their membrane integrity, their shape rounds and flattens, and nuclear material gets re-distributed.

Moreover, significant nuclear DNA damage as result of the magneto-mechanical stimulus application was revealed using TUNEL assay.²⁸ These experimental data lead us to propose that the applied magneto-mechanical cell stimulation results in the induction of apoptosis.

The disks are attached to a cell surface via ligand-antigen interactions. In a general case, the dynamic equation of motion can be written in its general form as follows:

$$g d^2 \beta / dt^2 + F V \eta d \beta / dt + k \beta = m(t) H(t),$$

where $\beta(t)$ is the disk rotation angle, g — the disk moment of inertia, F — the disk shape factor, V — the disk volume, η — the viscosity, k — the cell stiffness, and $m(t)$ and $H(t)$ are a time varying magnetic moment of the disk and the applied magnetic field, respectively. Thermal fluctuations can be ignored. This equation can be solved numerically to extract $\beta(t)$ as a function of the system parameters. We can make an order of magnitude estimate of the force that can be applied to the cell using magnetic disks. The disk has a diameter of $2r = 1 \mu\text{m}$ and thickness $h = 60 \text{ nm}$. The disk volume is $V = \pi r^2 h = 4.712 \times 10^{-20} \text{ m}^3$. The saturation magnetization, M_s , for the Permalloy is $8 \times 10^5 \text{ A/m}$. An upper limit of the applied forces can be estimated by considering the problem in a quasi-static regime. This simplification can be justified for time-varying fields with the pulse duration longer than

$(FV_\eta)/k = \sim 10^{-4} - 10^{-5}$ s. when no substantial energy dissipation due to viscous forces is expected. The angle α between the disk plane and applied field H is largely unknown and lies in a range of $0-90^\circ$; therefore we compare three model cases of $\alpha = 10^\circ, 45^\circ$ and 80° (Fig. 10). From the experimental and micromagnetic hysteresis loops we find that a field of 0.01 T (100 Oe) induces an in-plane component of the magnetization of $\sim 0.2, \sim 0.1$ and ~ 0.05 of M_s for $10^\circ, 45^\circ$, and 80° , respectively.

This results in a total induced magnetic moment m per disk of $\sim 7.54 \times 10^{-15}, \sim 3.77 \times 10^{-15}$ and $\sim 1.88 \times 10^{-15}$ Am² for each model case. The magnetic torque $m H \sin(\alpha)$ can be estimated as follows:

$$\begin{aligned} 7.54 \times 10^{-15} \text{ Am}^2 \times 0.01 \text{ T} \times \sin(10^\circ) &= 1.31 \times 10^{-17} \text{ Nm}, \\ 3.77 \times 10^{-15} \text{ Am}^2 \times 0.01 \text{ T} \times \sin(45^\circ) &= 2.67 \times 10^{-17} \text{ Nm}, \\ 1.88 \times 10^{-15} \text{ Am}^2 \times 0.01 \text{ T} \times \sin(80^\circ) &= 1.85 \times 10^{-17} \text{ Nm}. \end{aligned}$$

Therefore, the force applied to the cell at the disk edge can then be estimated as: ~ 26 , and 53 and 37 pN for $\alpha = 10^\circ, 45^\circ$ and 80° , respectively. Important to note, is that this is an upper limit of the force that can be achieved at the disk edge in the quasi-static regime using material and field parameters of our experiment. Thus, we find that the magnetic-vortex induced forces are in the ~ 10 s pN range, whereas >100 s pN forces are needed to physically rupture the cell membrane. However, forces as small as a few pN were reported to be sufficient to activate a single ion channel. Furthermore, a theoretically calculated energy barrier to create a pore in the cell membrane comparable in diameter to its thickness (~ 7 nm) using rotational forces produced by a magnetic particle is of order $\sim 30 k_B T$, where $k_B = 1.38 \times 10^{-23}$ J/K is the Boltzmann constant, and T is temperature. For comparison, the Zeeman energy of the magnetic vortex subjected to 100 Oe field is of $\sim 10^{-15}$ J, e.g., of order $\sim 1000 k_B T$. Finally, let us estimate the rotation angle of the microdisks attached to the cell surface. The magnetic torque applied to the MD-mAb-cell complex is counterbalanced by a restoring forces that originates from the cell elasticity: $\tau \beta(t) = m(t) H(t)$. Therefore, $\beta(t) = m(t) H(t) / \tau$, where τ is the elastic torque, $\beta(t)$, $m(t)$ and $H(t)$ are the time varying deflection angle, magnetic moment of the disk, and the applied field, respectively. The maximum elastic torque τ can be written as kS , where k is the cell rotational stiffness, and $S = \pi r^2$ is the disk attachment area. The cell stiffness k can be written as $k = SE/L$, where E is the elastic (Young's) modulus, S is the area, and L is the length of the deformed element. Young's modulus for the tumor cell is ~ 1 kPa.³⁵ By choosing L equal to the disk diameter, we find $k = \pi E r / 2 = 0.78 \times 10^{-3}$ N/m. Therefore, with $S = \pi (500 \text{ nm})^2 = 7.854 \times 10^{-13} \text{ m}^2$, we calculate that the elastic torque $\tau = k \times S$ is $\sim 0.62 \times 10^{-15}$ Nm. This results in the maximum disk rotation angle $\beta_{\max} = m \times H / \tau = \{1.2, 2.5 \text{ and } 1.7\}$ degrees, or a lateral displacement of the disk edges $r \sin(\beta_{\max}) = \{10.5, 21.8 \text{ and } 14.8\}$ nm for the angles $\alpha = 10^\circ, 45^\circ$ and 80° , respectively. The above mentioned displacement is comparable with the average thickness of the membrane lipid bilayer (of ~ 7 nm), and therefore most likely will result in pore formation in the membrane. Thus, the magnetic vortex forces appear to be strong enough to mechanically actuate the existing ion channels, as well as to alter the barrier function of the cell membrane through formation of ~ 10 nm pores. The later could lead to altered molecular transport into and out of the cells, therefore altering the intra cellular machinery function. To clarify this possibility, we have performed experiments on imaging of intercellular calcium re-localization during application of *ac* magnetic field (10 Hz/90 Oe) in N10 glioblastoma cells. The role of the calcium as a universal secondary messenger in execution of the cell-death process is well-established and is reported in connection with the studies of drug-induced cytotoxicity.

To investigate whether calcium ion signaling is involved in the observed magnetic-vortex-driven cell death, the N10 cells were preloaded with Fluo-4AM, a calcium indicator dye (Invitrogen, Molecular Probes) and studied using dual-wavelength laser confocal microscopy. A magnetic coil was mounted on the microscope sample stage. Figure 11 shows semi-quantitative data on changes in intracellular concentration of calcium during *ac* field application in a nominally calcium-free Hank's buffer solution. Fluorescence intensity was detected in areas (a), (b) and (c) corresponding to three cells with different initial fluorescence intensities due to different steps of a life cycle. Total fluorescence (including cells and background) was measured as an area (d). Once the *ac* field is applied, noticeable changes in fluorescence were detected. Thus, for cells (b) and (c), a steadily increasing of the fluorescence intensity (up to twofold) was observed. In contrast, for cell (a), with originally higher calcium concentration, an initial sharp slope was followed by a smooth increase of the calcium level. The global increase in the cytoplasmic calcium triggers the simultaneous release of cyto-chrome *c* from mitochondria, and the formation of the apoptosome and activation of specific protease caspases. Caspases together with nucleases then finalize the cell death process by cleaving protein substrates and nuclear DNA. Importantly, the number of TUNEL-stained cells was observed to fall remarkably (~50%) after the addition of 20 μM Z-VAD-FMK, an inhibitor of caspase proteases, the key role-players in apoptosis. Therefore, we can anticipate that the magnetic vortex mediated mechanical stimulus may eventually be converted into a downstream chemical signal cascade able to initiate apoptosis and cell destruction. Altogether these confocal microscopy data confirmed our proposal on magneto-mechanically-induced ionic channels actuation or/and cell membrane pore formation altering calcium homeostasis and resulting in re-programming of important intracellular biochemical pathways. Future studies, including application of specific inhibitors of stretch-activated membrane protein complexes and experiments with controlled concentrations of calcium in cell culture media may help to further clarify intriguing details of the proposed mechanism.

Benefits of the proposed approach include application of unprecedentedly low frequency *ac* fields for triggering cancer cell apoptosis via direct energy transfer to a single cell, and transformation of magnetic field-induced mechanical forces to ionic currents signals.

The above described results were obtained using ~1 micron diameter disks. However, the spin vortex concept is scalable down to much smaller dimensions.^{33,36–40} Figure 12 shows the magnetic phase diagram, calculated using the Landau–Lifshitz–Gilbert micromagnetic approach. Magnetic vortices in deep submicron disks were demonstrated experimentally by means of Lorenz and photoelectron emission microscopies. It is clear from the energy minimization viewpoint that the magnetic vortex state remains stable (“ground”) spin state for magnetically soft disks down to the deep sub-100-nm disk diameter. Mass production of such nanodisks can be achieved via nanoimprint lithography. Figure 13 shows typical structures that can be obtained. Small diameter disk patterns can be defined in polymer resists. The disk detachment from the substrate (after gold/magnetic/gold films deposition) can be achieved using bi-layer polymer resist structures and ultrasonic-assisted lift-off processes. Importantly, nanoimprint lithography is based on a “stamp and repeat” approach, e.g. using a master mask that can be employed many times. It is a low cost, mass production method with a narrow size distribution of the final structures. A decrease in the disk volume will decrease the total magnetic moment m per disk (because of the reduced particle volume). The magnetic torque, $m \times H$, will also decrease accordingly. However, the torque that drives the rotational motion of the nanodisks can readily be recovered by increasing the magnetic field amplitude.

For instance, a ten times smaller volume disk (e.g. using ~ 300 nm diameter, ~ 17 nm thin disks instead of currently used ones), will experience the same magnetic torque if subjected

to ~ 2.7 kOe field, instead of the ~ 90 Oe used in Fig. 8. Stronger magnetic fields are easy to achieve since the required magnetic field frequency only a few tens of Hz. To summarize this section, we believe that there are no major conceptual or feasibility problems to use nanosize magnetic disks to further advance the concept of magneto-mechanical cancer cell destruction and its use for *in vivo* applications.

4. Conclusion

Taken together, we presented two examples of applications of nano–bio hybrids, based on semiconductor or ferromagnetic materials chemically grafted with biomolecules for controlled biological manipulation and actuation via triggering of important biochemical pathways leading to apoptosis. Apoptosis is one of the main types of programmed cell death used by nature for elimination of damaged or worn-out cells without local inflammation. The fundamental importance of correct execution of apoptosis is evident from the many human diseases with atypical apoptosis, including cancer. During cancer development, various imbalances can arise in the apoptotic machinery. Consequently, sensitivity towards apoptosis is progressively reduced, which ultimately leads to inappropriate cell survival, multiple resistance to cytotoxic drugs, and malignant progression.⁴ Induction of apoptosis plays a central role in the investigation of therapeutic activation of apoptosis in malignant cells as a potential anticancer strategy.¹⁸

Living cells interact with their environment through a complex network of chemical signals. These signals vary in healthy and diseased cells. Intelligent nanoscale materials capable of spatially-controlled application of external cues, such as redox species or mechanical force, at the cell membrane can alter intracellular signaling and, therefore, cell behavior. Development of such materials opens new horizons in life sciences and medicine.

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Fig. 1. Synthetic interfacial chemistry approaches enable the coupling the nanomaterials with various biomolecules offering bio-recognition properties.

Fig. 2. Naturally-occurring dihydroxybenzenes (1) dopamine, (2) 3,4-dihydroxyphenylacetic acid (DOPAC) and (3) L-3,4-dihydroxyphenylalanine (L-DOPA) can be used as linkers between semiconductor metal oxide particles and biomolecules.

Fig. 3. Nanobio hybrid consisted of 5 nm TiO_2 and an anti-body tethered via DOPAC linker (right two images-atomic force microscopy) recognizes and binds specifically to a surface receptor. Visible light photo-excitation of the nano-bio hybrid in an aqueous solution results in formation of the various ROS. ROS, mainly superoxide cause cell membrane damage, permeability changes and cell death.

Fig. 4.
Laser confocal microscope images of the A172 glioblastoma cell cultures.

Fig. 5.

Left: The concept of targeted magneto-mechanical cancer cell destruction using disk-shaped magnetic particles possessing a spin vortex ground state. The microdisks gold shell is biofunctionalized with an antibody, specifically targeting human glioblastoma cells. When an *ac* magnetic field is applied, the magnetic disks oscillate compromising the cell membrane integrity and initiating spin vortex-mediated programmed cell death. Right top: A representative optical microscope image of the dried suspension of 50 nm thick, $\sim 1 \mu\text{m}$ diameter iron-nickel 20:80 (permalloy) disks coated with a 5 nm thick layer of gold on each side. Right bottom: High resolution optical images (200X objective lens magnification) of the magnetic disks interfaced with glioblastoma cells.

Fig. 6.

A room temperature magnetic hysteresis loop for a dried sample (red symbols) measured with a VSM for MDs in an acetone solution cooled in the presence of a 50 Oe magnetic field below the freezing point of -95°C (black symbols). The data agree with the concept of magnetization reversal due to nucleation and annihilation of the magnetic vortices. In zero field (remanent state) the vortex core is in the center of each disk, whereas under application of a magnetic field the vortex shifts (reversibly) to develop a magnetization component parallel to the field (see the two representative spin structures shown in the insert). The dashed blue area depicts the magnetic field range used in the cell experiments.

Fig. 7. MFM images of dot array grown simultaneously with our MDs sample. The ~10–20 nm “dark” or “light” spot in the center of each disk is the signature of the magnetic vortex state.

Fig. 8.

Representative X50 optical microscope images of cancer cells for two distinctive cases. Left image: Binding experiments: Human glioma N10 cells incubated with IL13-functionalized disks; no field was applied. The morphology of the cell is essentially the same as for the control sample (e.g., cells without magnetic disks; not shown). Right image: an optical micrograph of the cells incubated with Ab-functionalized disks after a 20 Hz/90 Oe *ac* magnetic field was applied for 10 min.

Fig. 9. 2D and 3D AFM images of control (conjugated with MDs, but untreated) cell (upper images) and treated N10 glioma cell subjected to 90 Oe/20 Hz magnetic field (lower images).

Fig. 10.
Schematic representation of microdisk attached to the cancer cell surface.

Fig. 11.

Calcium fluorescence image of the N10 cells of the same area prior (left) and after (right) application of magnetic field. The plot (center) shows time-evolution of the calcium signal for three difference cells (a, b and c) as well as for whole area of microscope view (d).

Fig. 12.

Calculated magnetic phase diagram of $\text{Fe}_{20}\text{Ni}_{80}$ nanodisk. The spin vortex state remains the ground state down to sub-100 nm diameter size. See also Ref. 36 with analytical calculations.

Fig. 13. Scanning electron microscope image of ~30 nm lines and ~50 nm disk structures fabricated with Imprio 50 tool (courtesy of Molecular Imprints Inc.) Nanoimprint lithography enables replicating large area experimental structures in hours using step and flash imprint lithography rather than in weeks with the e-beam tool.