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# Modulation of monocytic leukemia cell function and survival by high gradient magnetic fields and mathematical modeling studies

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ABSTRACT

The influence of spatially modulated high gradient magnetic fields on cellular functions of human THP-1 leukemia cells is studied. We demonstrate that arrays of high-gradient micrometer-sized magnets induce i) cell swelling, ii) prolonged increased ROS production, and iii) inhibit cell proliferation, and iv) elicit apoptosis of THP-1 monocytic leukemia cells in the absence of chemical or biological agents. Mathematical modeling indicates that mechanical stress exerted on the cells by high magnetic gradient forces is responsible for triggering cell swelling and formation of reactive oxygen species followed by apoptosis. We discuss physical aspects of controlling cell functions by focused magnetic gradient forces, *i.e.* by a noninvasive and nondestructive physical approach.



## 1. Introduction

It is well known that disruption of apoptotic signaling leads to continuous proliferation, which is associated with tumor initiation, progression and metastasis. Various intrinsic and extrinsic mechanisms leading to cellular apoptosis have been well documented. For cancer therapy, specifically designed anticancer agents promote apoptosis by targeting intrinsic and extrinsic pathways. However, several studies suggest that tumor-like growth can also be suppressed by truly mechanical effects [1–4]. Yet, application of externally applied mechanical forces to control cell proliferation, tumor growth, and apoptosis has been poorly investigated and the mechanisms involved are not fully understood.

Cells do not have a fixed standard size, and their volume changes depend on their functional state [5]. For example, migrating cells constantly adapt their volume according to the intracellular hydrostatic pressure and neutrophils swell rapidly upon activation with the standard chemoattractant fMLP [6]. Besides, externally applied mechanical forces may affect cell volume, and on the long run, also cell fate, and differentiation [7]. Cell volume alterations, which rely on the spatially and temporally coordinated function of ion channels and transporters, regulate proliferation, programmed cell death, cell metabolism, and secretion [5]. Moreover, extreme perturbations of the cell volume are characteristic features of various disease states such as diabetes mellitus, dehydration states, cardiac and brain ischemia, and in the context of therapeutic intervention during brain edema [8].

Most studies in the field of cellular volume control focused on short-term processes of volume regulation. Indeed, a cell can rapidly adjust its volume when facing changes in extra- or intracellular osmolarity [5]. Thus, in a hypotonic environment, initial cell swelling leads to activation of ion transporters (e.g. increased $K^+$ and $Cl^-$ efflux) within the first minute after exposure, which decreases about 10 min later resulting in cell shrinkage. Such changes in ion homeostasis were proposed to be relevant for the induction and progression of apoptosis rather than swelling alone [9]. In fact, cells possess extremely large membrane reserves and, in the case of non-spherical cells, can increase their volume by several-fold mainly by shape transition, or by recruiting extra membrane from pre-existing surfaces and intracellular membrane reserves thereby avoiding membrane rupture [9].

Exploitation of mechanical regulation of cell functionality, raises the question, how physically meaningful and controllable mechanical stress can be applied to a cell. There may be several ways, as for example, by i) application of hydrostatic [4] or osmotic pressure [3], ii) by application of shear stress, e.g. by fluid flow

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[10,11], or iii) by transmission of mechanical forces directly to a cell membrane by a micro-disk oscillating in an a.c.-magnetic field [12] and iv) by mechanical tension induced by nanoparticles [13]. In this study, we propose a new route of applying mechanical stress to individual cells though a spatially modulated high gradient magnetic field generated by high-performance micro-magnet arrays.

Environmental exposure to electromagnetic stray fields prompted already numerous studies suggesting biological effects in response to electromagnetic or static magnetic field exposure [14,15]. However, many investigations, especially those dealing with the biological impact of weak magnetic fields, are hampered by shortcomings in experimental design and often lack reproducibility [15]. Appropriate magnetic fields, however, might indeed either directly or indirectly affect cell functions. In contrast to electric fields, magnetic fields are much less attenuated by tissues, which is why they can penetrate into deeper tissue layers, where they could modulate biochemical processes, which are linked to changes in signaling pathways and cell biological events [14].

However, typical magnetic gradient forces are too small to overcome intracellular electric forces when alteration of cell functionality is the goal. In this respect, studies on high-gradient magnetic fields are of particular interest. Modern powerful superconducting magnets can provide magnetic gradient values of about $10^3$ $T^2/m$. By contrast, the magnetic field of the earth has a strength of about $5 \times 10^{-5}$ T, whereas modern magnetic resonance imagers develop up to 1.5–8 T [16]. Indeed, the effects of magnetic fields with gradients of about $10^3$ $T^2/m$ were shown to alter the subcellular morphology of osteoblast-like cells [17]. In particular, it has been shown that high-gradient magnetic fields cause expansion of the endoplasmic reticulum and swelling of mitochondria and microvilli, combined with a slight increase in viability, but they remained without effect on basic osteoblast functions [9]. It was hypothesized that diamagnetic levitation may play a major role in the observed effects. On the other hand, magnetic field gradients of ~2 T/m were demonstrated to inhibit proliferation of human endothelial cells and impaired angiogenesis *in vivo* [18].

Arrays of high-performance micro-magnets generating fields with spatial dimensions comparable to cell sizes have recently been developed [19–23]. These micro-magnet arrays produce extremely high magnetic field gradients up to $10^6$ $T^2/m$ at their surface [24], and have been used to levitate cells in a paramagnetic solution [21] and to initiate interconnected networks in stem cells [22]. The goal of this work was to study the effects of high gradient magnetic fields on the function of monocytic leukemia cells. Our study may help to elucidate the interplay between mechanics and biochemistry that regulates functional cell behavior and which may ultimately facilitate the use of magnetic fields for the control of biochemical processes.

## 2. Materials and methods

### 2.1. High performance micro-magnet arrays and calculations of magnetic field distributions

The micro-magnet arrays were fabricated by high rate sputter deposition [ND07] onto pre-patterned silicon substrates [20,22]. Arrays of square Si features of lateral size $50 \times 50$ $\mu m^2$ or $100 \times 100$ $\mu m^2$, and a depth of 100 μm, were prepared by Deep Reactive Ion Etching. Tantalum (100 nm)/neodymium/iron/boron alloy (NdFeB, 35 μm)/tantalum (100 nm) trilayers were deposited at a temperature of 450 °C, and annealed at 750 °C for 10 min. Such films display an out of plane crystallographic texture with remanent magnetization values of up to 1.2 T. The resulting micro-magnet arrays were named “M50” and “M100”, respectively. A high-performance NdFeB cubic bulk magnet of 1 $cm^3$ generating a low-gradient magnetic field was used for comparison. The arrays and the bulk magnet were placed below thin-bottomed (180 μm) cell culture devices to analyze their effects on cultured cells. Distributions of the magnetic field and its gradient were calculated with the help of explicit expressions for the magnetic stray fields of a magnetized slab [25,26].

### 2.2. Experimental setup

THP-1 cells (30 000) seeded onto thin-bottomed ibidi μ-slides (Munich, Germany) in 150 μl growth medium (RPMI 1640 supplemented with 10% FCS, 50 U penicillin and 500 μg/ml streptomycin) were exposed either to arrays of M50 and M100 micro-magnets or to a NdFeB bulk magnet (1 $cm^3$) positioned at the distance of 180 μm to growing cells. In some experiments, the M100 micro-magnet array was positioned at a distance of 5 mm to the growing cells. For imaging and counting, a Diaphot microscope (Nikon Inc.) and ImageJ software (NIH) were used. Alternatively, cell membranes were stained with CellMask™ Deep Red (Invitrogen) [27,28] and analyzed microscopically.

### 2.3. ROS analysis

THP-1 cells were exposed either to static high-gradient magnetic fields (M50, M100) or low gradient magnetic field (NdFeB bulk magnet) for 24 h. ROS production was assessed with carboxy-$H_2$DCFDA as ROS-sensitive dye [29]; cell membranes were stained with CellMask™ Deep Red, cell nuclei were labeled with DAPI. Fluorescent images were recorded with a Diaphot microscope. ImageJ was used for data processing.

### 2.4. Apoptosis assay

Phosphatidylserine expression was determined by binding of fluorescein isothiocyanate-labeled annexin V (Roche Diagnostics) [29]. The cells were exposed to high-gradient magnetic fields (M50, M100) or a low-gradient magnetic field (NdFeB bulk magnet) for 24 h and were subsequently labeled with annexin V (green) and propidium iodide nuclear stain (red). Fluorescent digital photomicrographs were quantified using ImageJ.

### 2.5. Statistical analysis

Quantitative results are expressed as mean ± SEM. Results were analyzed by the Newman–Keuls test using Statistica software (StatSoft, Tulsa, OK). Differences were considered statistically significant at $^*p < 0.05$.

## 3. Results

### 3.1. Effects of high-gradient magnetic fields on THP-1 cell functionality

Arrays with micro-magnets of $50 \times 50$ μm and $100 \times 100$ μm, named M50 and M100, respectively, were positioned directly beneath the cell culture substratum, which allows focusing the magnetic gradient on the proliferating leukemia cells. The geometry and properties of the micro-magnets array are described in greater detail in the *Materials and Methods* Section. To prevent the potential toxicity of the material, the micro-magnet arrays were located outside the ibidi μ-slides separated by a 180 μm-thick membrane. Prolonged exposure to static high-gradient magnetic fields induced swelling of monocytic THP-1 cells. It should be noted, that THP-1 cells are growing in suspension and were therefore able to migrate slowly across the surface of the slides during the experiment. Exposure of the monocytic cells for 24 h to high-gradient magnetic fields generated by M100 micro-magnet arrays induced cell swelling with a gain in diameter of about 24% and a corresponding volume increase of 89% (Fig. 1). The M50 micro-magnet array, which generates a lower magnetic field gradient induced a smaller swelling effect with increases in diameter and volume by 15% and 51%, respectively.

The observed effects were rather dependent on the strength of the magnetic field gradient but not on the strength of the magnetic field as evidenced by the use of a conventional high-performance bulk magnet that yielded a magnetic field strength at least one order of magnitude larger than that of the micro-magnet arrays. Despite its superior magnetic field strength, the bulk magnet failed to trigger any cell swelling (Fig. 1).

Besides the cell swelling, prolonged exposure to static high-gradient magnetic fields generated by the M50 and M100 micro-magnet arrays positioned at a distance of 180 μm triggered formation of reactive oxygen species (ROS) (Fig. 2). ROS production has also been observed in cells exposed to hypotonic stress, which induces transient cell swelling [8]. Again, cells exposed to the bulk