Chapter 1
Magnetic Field Parameters and Biological Sample Differences That Lead to Differential Bioeffects

Xin Zhang

Abstract We have to admit that the literature concerning bioeffects of electromagnetic fields is replete with reports that cannot be reproduced in other labs. Besides the intended or sub-conscious experimenter bias, which can be avoided or much reduced by the gold standard of blinded analysis, most inconsistences in the literature were actually caused by confounding effects, different magnetic field parameters, and biological sample differences. The goal of this chapter is to summarize the factors that contribute to the differential bioeffects of static magnetic fields (SMFs), including magnetic field exposure parameters, such as magnetic field types, magnetic flux density, homogeneousness, field direction and distribution, exposure time, as well as biological sample differences, including cell type, cell density, cell status, and other factors. It is clear that all these aspects are crucial for the diverse effects of SMFs on biological samples, which also lead to the seemingly lack of consistencies in literature. Therefore, we encourage people to not only perform double blinded analysis in independent studies, but also clearly describe the experimental details, including various magnetic field exposure parameters, biological samples, and experimental procedures. This will be crucial for people to perform further subjective analysis and mechanistic investigations.

Keywords Magnetic field (MF) · Static magnetic fields (SMFs) · Time-varying magnetic field (TVMF) · Dynamic magnetic field (DMF) · Magnetic field intensity · Gradient magnetic fields (GMF) · Differential effects of magnetic fields

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1.1 Introduction

Generally speaking, magnetobiology is the study of interaction between magnetic field (MF) and biological systems, which includes but not limited to the magnetic field-induced bioeffects and mechanisms, perception, and utilization of magnetic fields by organisms, as well as magnetic field related technologies. It is a multidisciplinary and interdisciplinary area that involves biology, physics, and chemistry (Fig. 1.1), which has made a tremendous progress in the past few decades.

Depending on whether the magnetic intensity changes over time, MFs can be divided into static magnetic field (SMF) or time-varying magnetic field (TVMF)/dynamic magnetic field (DMF), which can be further divided into different categories according to their frequency and other parameters. Depending on the magnetic field intensity, there are weak, moderate, strong (high), and ultra-strong (ultra-high) magnetic fields. Depending on the spatial distribution, there are homogeneous or inhomogeneous MFs. This book focuses on the biological effects of static magnetic field, which does not change the magnetic field intensity, direction, or distribution over a certain period of time. Here we will discuss the major variations in magnetic field parameters and their differential effects on biological objects.

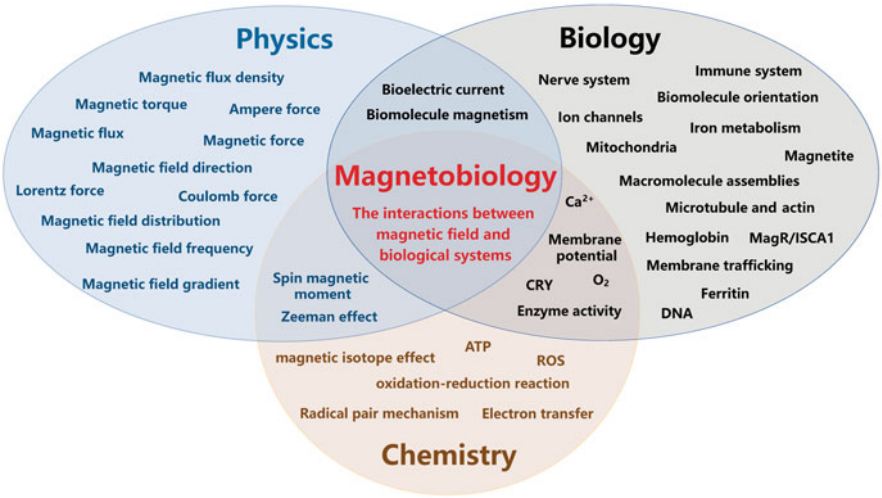

Fig. 1.1 Magnetobiology is an interdisciplinary research area

1.2 Magnetic Field Parameters That Influence Bioeffects

1.2.1 Static Magnetic Field vs. Time-Varying Magnetic Field

It is obvious that cells and living organisms respond very differently to SMFs vs. TVMFs. Multiple evidence showed that different types of magnetic fields of the same intensity could produce totally different effects on the same biological samples. For example, 0.4 mT 50 Hz and a 2 μT 1.8 GHz pulsed magnetic fields (PMFs) both increased epidermal growth factor receptor (EGFR) phosphorylation, which were reversed by incoherent ("noise") MF of the same intensities (Wang et al. 2010; Li et al. 2012). Our group has also reported that the cellular ATP levels in multiple cell lines were differentially affected by the 6 mT magnetic fields with 0 Hz, 50 Hz, and 120 Hz (Wang et al. 2018).

This book only focuses on SMFs because they have much fewer variable parameters and do not cause electric current or heat effects. Therefore, they have obvious advantages in basic research compared to time-varying magnetic fields. However, it should be pointed out that people are actually exposed to much more time-varying magnetic fields in everyday life, such as the 50 Hz or 60 Hz power frequency alternating current (AC) MFs from the power line, and radiofrequency MFs from cell phones. On the other side, people have also successfully developed a time-varying magnetic field-based FDA-approved medical device, transcranial magnetic stimulation (TMS), that can be used to treat depression and other medical conditions. The low frequency rotating MFs have also shown great medical potentials.

1.2.2 Different Magnetic Flux Density

According to the magnetic flux density, SMFs used in the biological effect studies could be classified as weak (<1 mT), moderate (1 mT–1 T), high (1–20 T), and ultra-high (20 T and above). It should be mentioned that the standard for defining the threshold of each category is different in various field. For example, for MRI (magnetic resonance imaging), people usually consider SMF higher than 5 T as ultra-high.

$$1\,\mathrm{T}\,(\mathrm{Tesla}) = 10{,}000\,\mathrm{G}\,(\mathrm{Gauss})$$

$$1\,\mathrm{G} = 100\,\mu\mathrm{T}.$$

Figure 1.2 shows some examples of different magnetic flux density generated by different sources. For example, electrical currents flowing through neurons in our brain will generate weak magnetic fields that can be recorded by sensitive magnetic detectors at the surface of the head; our planet earth generates weak but ubiquitous magnetic fields that can protect our planet from solar storms; permanent magnets

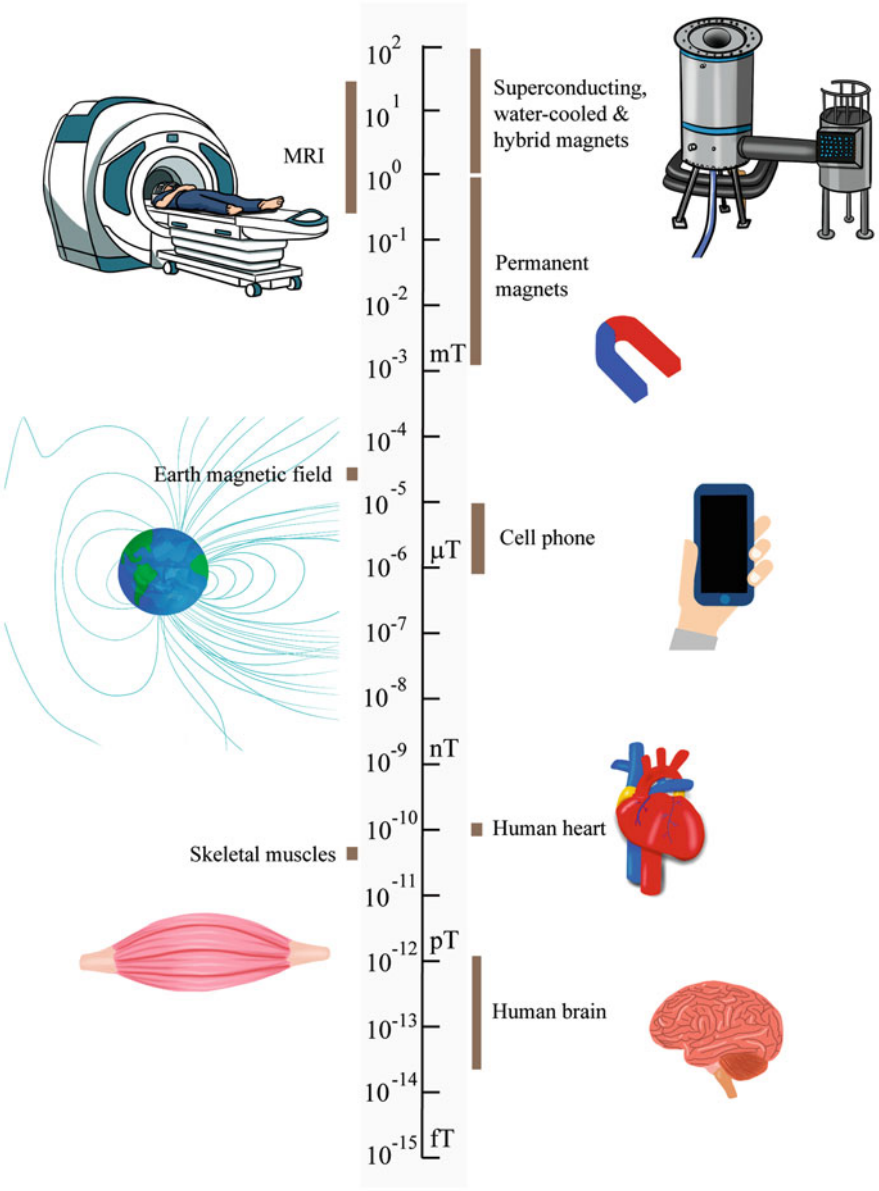

Fig. 1.2 Magnetic fields of different magnetic flux density. *T* tesla, *MRI* magnetic resonance imaging. (Illustration courtesy of Ding Joe Wang)

usually have moderate intensity, which are widely used in everyday life; most MRI machines in hospitals are within 0.5–3 T, while higher and lower intensity MRIs are also been developed for special circumstances; superconducting, water-cooled and hybrid magnets with ultra-high intensity are used for research and manufacturing.

1.2.2.1 Earth Magnetic Field (Geomagnetic Field)

For weak earth magnetic field, there are tremendous research in the past few decades, especially about magnetoreception. Overall, people are still debating on this topic and there are at least four different hypotheses (Fig. 1.3), including the radical pair mechanism (Fig. 1.3a), magnetite (Fig. 1.3b), electromagnetic induction (Fig. 1.3c), as well as the putative magnetoreceptor (MagR) (Fig. 1.3d). Since each hypothesis has its own limitations, more research is needed to unravel this mystery. Besides the contradictories between physical calculations and biological observations, it is possible that different organisms use different ways to sense the geomagnetic field, and there might be other undiscovered mechanisms between the complex biological

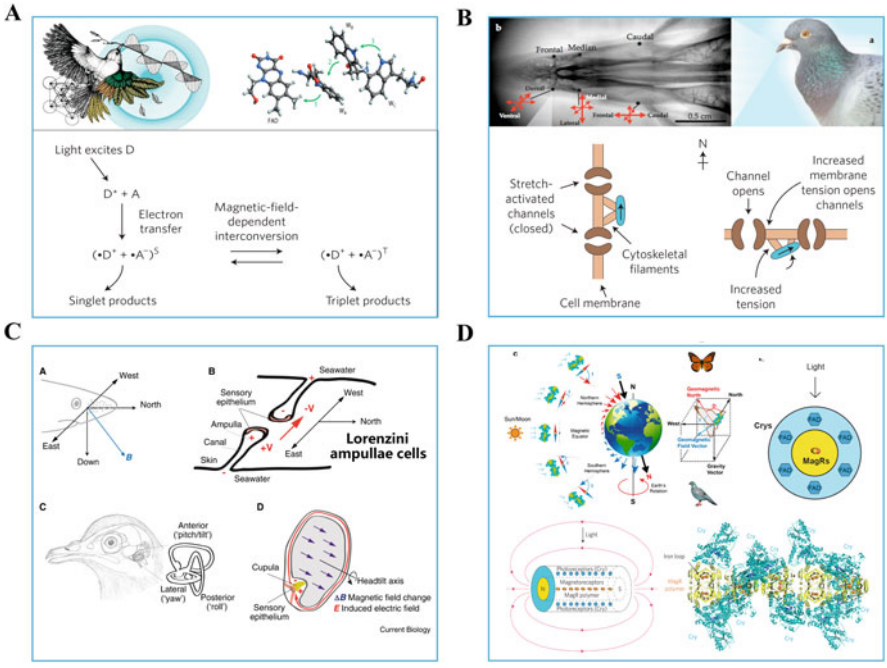

Fig. 1.3 Different hypotheses of magnetoreception. (**a**) The radical pair mechanism (RPM) hypothesis (Ritz et al. 2000; Ball 2011; Hore and Mouritsen 2016). (**b**) The magnetite hypothesis (Lohmann and Johnsen 2008; Lohmann 2016). (**c**) Electromagnetic induction hypothesis (Bellono et al. 2018; Nimpf et al. 2019; Winklhofer 2019). (**d**) The ISCA1 (iron–sulfur cluster assembly **a**)/ magnetoreceptor (MagR) hypothesis (Lohmann 2016; Qin et al. 2016). [Figures are adapted with permissions from (Ball 2011; Lohmann 2016; Winklhofer 2019)]

system and their interaction with the geomagnetic field. Moreover, it is also possible that these models are not mutually exclusive (Xie 2022). There are many reviews that people can get information on this topic and we will also discuss about them in Chap. 4.

1.2.2.2 Moderate and High SMFs (1 mT–20 T)

The most commonly seen SMFs in current research and in daily life are permanent magnets, such as the magnets on refrigerators, toys, and accessories, which are usually not very strong (below 1 T), unless they are fabricated after special design. In addition, the core component of the MRI machines in most hospitals provides SMFs with field intensities usually range between 0.5 and 3 T. Because of the public sensitivity, the question of the possible effects of SMFs of 0.5–9.4 T, the range of the MRI machines in current hospitals and clinical research, on human health is of paramount interest. The MRI process involves a combination of homogenous SMF, gradient SMF, and pulsed radiofrequency magnetic fields. Currently, the MRI scanners are considered to be safe if used properly. Studies show that 7 T ultra-high field MRI is well tolerated by humans without excessive discomfort (Miyakoshi 2006; Simko 2007; Heilmaier et al. 2011), DNA damage (Fatahi et al. 2016), or other cellular abnormalities (Sakurai et al. 1999). At the same time, since stronger magnets could give better resolution and more detection possibilities, the researchers and engineers are continuously investigating on MRI machines with stronger SMFs. In fact, 21.1 T MRI has already been developed and applied on rodent brain.

1.2.2.3 Ultra-High Static Magnetic Fields (>20 T)

Due to technical limitations, the biological effects of strong field of ≥20 T have not been systematically investigated until recent few years. Although the ultra-high field NMR (nuclear magnetic resonance) machines currently available can generate around 20 T SMFs, they have very narrow bore size that is impractical to accommodate cell culture plates. In addition, the animal and human cells need to be cultured with accurate temperature, humidity, and gas control, which make the NMR machines unsuitable to do these experiments. For the limited number of large-bore SMF equipment that can generate ≥20 T ultra-high SMFs, they are mostly used for material science and physical science studies. People need to construct special sample holders to make these magnets appropriate to study biological samples such as animal and human cells, as well as other small animal models.

In the past few years, we have constructed a series of incubation system to match the large-bore ultra-high magnets. They can provide accurate temperature and gas control for cell cultures and small animals, which enabled us to perform cellular (Zhang et al. 2017b; Tao et al. 2020) and animal studies (Tian et al. 2018, 2019, 2021; Lv et al. 2022; Khan et al. 2022) above 20 T. For example, we have examined

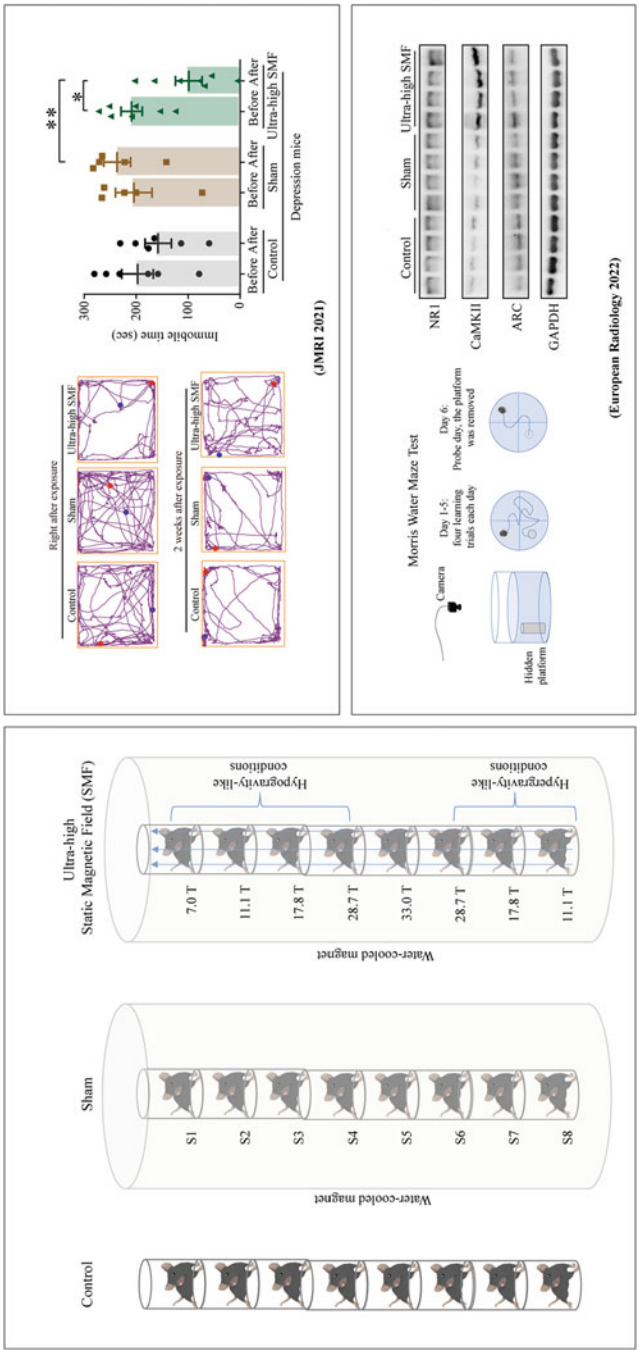

Fig. 1.4 Short-term ultra-high SMF exposure on healthy mice. [Figures are adapted with permission from (Lv et al. 2022; Khan et al. 2022). $*p < 0.05$, $**p < 0.01$]

the effects of 1-h SMF exposure up to 33 T and 2-h SMF exposure up to 23 T on healthy mice (Fig. 1.4), which did not cause obvious detrimental effects. On the contrary, it is interesting that these short-term treatment of ultra-high SMFs showed anti-depressive and improved memory effects on mice.

1.2.2.4 Magnetic Flux Density-Induced Differences

Numerous studies have shown that the magnetic flux density is a key factor that causes the bioeffect differences. Moreover, the different magnetic flux densities and their effects on biological samples need to be examined case by case.

In many cases, SMFs with higher flux density could generate stronger phenotypes, or phenotypes that are not inducible by SMFs of lower flux density. For example, erythrocytes (red blood cells, RBCs) could be aligned by SMFs with their disk planes parallel to the SMF direction and the orientation degree was dependent on SMF intensity (Higashi et al. 1993). Specifically, 1 T SMF had only detectable alignment effect on erythrocytes while 4 T high SMF induced almost 100% alignment (Higashi et al. 1993). Moreover, Prina-Mello et al. reported that the p-JNK level was increased in rat cortical neuron cells after exposure to 2 T and 5 T SMFs but not the weaker SMFs of 0.1–1 T (Prina-Mello et al. 2006). In addition, our lab recently showed that the human nasopharyngeal cancer CNE-2Z cell and human colon cancer HCT116 cell proliferation could be inhibited by SMFs in a magnetic field intensity dependent manner (Zhang et al. 2016). Specifically, 1 T SMF exposure for 3 days reduced CNE-2Z and HCT116 cell number by ~15% and 9 T SMF for 3 days reduced their cell number by over 30%. In contrast, 0.05 T SMF did not have significant effects on these two cells (Zhang et al. 2016). Okano et al. found that moderate intensity gradient SMF of 0.7 T (Bmax) significantly reduced the nerve conduction velocity of frog nerve C fibers but gradient SMF of 0.21 T (Bmax) did not (Okano et al. 2012). Our recent findings showed that 1–9 T moderate and strong magnetic fields can affect EGFR orientation to inhibit it activity and cancer cell growth while weaker SMFs cannot (Zhang et al. 2016). In addition, we found that 27 T ultra-strong SMF can affect spindle orientations in cells while moderate intensity SMFs cannot (Zhang et al. 2017b).

Although multiple studies show that some biological effects are directly correlated with the SMF intensity in a linear relationship and the higher magnetic field intensities are frequently associated with stronger phenotypes (Bras et al. 1998; Takashima et al. 2004; Glade and Tabony 2005; Guevorkian and Valles Jr. 2006), there are also studies showing that SMFs of different density may have different or even opposite biological effects compared to lower SMFs. For example, Ghibelli et al. showed that 6 mT SMF had an anti-apoptotic activity, but 1 T SMF potentiated the apoptotic effects of small molecules (Ghibelli et al. 2006). Morris et al. showed that application of a 10 or 70 mT, but not a 400 mT, SMF for 15 or 30 min immediately following histamine-induced edema resulted in a significant reduction in edema formation (Morris and Skalak 2008). In 2014, the Shang group compared the effect of hypomagnetic field of 500 nT, moderate SMF of 0.2 T, and high SMF of

16 T for their effects on mineral elements in osteoblast MC3T3-E1 cells (Zhang et al. 2014b). They found that both hypo and moderate magnetic fields reduced osteoblast differentiation but the 16 T high magnetic field increased osteoblast differentiation. In addition, hypomagnetic field did not affect mineral elements levels but moderate MF increased iron content and high magnetic field increased all mineral elements except copper (Zhang et al. 2014b). In addition, study in Shang's group demonstrated that 500 nT and 0.2 T SMFs promoted osteoclast differentiation, formation, and resorption, while 16 T had an inhibitory effect (Zhang et al. 2017a).

Therefore, different magnetic flux density could induce completely different effects at various biological systems. As Ghibelli et al. have mentioned in their paper, the lack of a direct intensity-response curve may explain the existence of so many contradictory reports in the literature (Ghibelli et al. 2006).

1.2.3 Homogeneous vs. Inhomogeneous Magnetic Field

Depending on the spatial distribution of magnetic fields, SMFs can be classified as homogeneous (uniform) SMF and inhomogeneous (gradient) SMF, in which the field strength can be spatially constant or different. In most cases, both homogeneous and inhomogeneous MFs are present in the same system. For the electromagnets designed for SMFs, the center of the magnet usually can provide a homogeneous magnetic field, as long as the samples are placed within a certain range. However, if the samples are placed far away from the center, the magnetic field usually becomes inhomogeneous. For example, although the center of the MRI machine has a homogeneous magnetic field, MRI workers who stand step away from the MRI machines receive an inhomogeneous (gradient) SMF. SMFs generated by most permanent magnets are inhomogeneous.

Here we show the magnetic flux density distributions on the surfaces of 4 different permanent magnets in our lab to show the diversity (Fig. 1.5). Even for a rectangular-shaped magnet that produces an evenly distributed flux density at the XY direction parallel to the magnet surface (Fig. 1.5a, b), there is still a gradient along the *Z*/ vertical direction.

The magnetic forces used in magnetic levitation belong to the inhomogeneous SMFs. The magnetic flux density decreases along the upward direction away from the center so that the forces can point to the upward direction to balance gravity. The magnetic force acting on diamagnetic object is repulsive and if it is stronger than gravity, the object will be levitated. The famous "flying frog" used a 16 T superconducting magnet that provided a SMF with a gradient that is large enough to balance the gravity of the frog when it was placed at the upper part of the magnet, away from the center (Fig. 1.6). Apparently, magnetic levitation can only be achieved in inhomogeneous SMFs, but not in pulsed magnetic fields or homogeneous SMFs.

Besides the flying frog, there is another excellent example of using magnetic levitation to "fly" much smaller living objects, single cells. In 2015, Durmus et al.

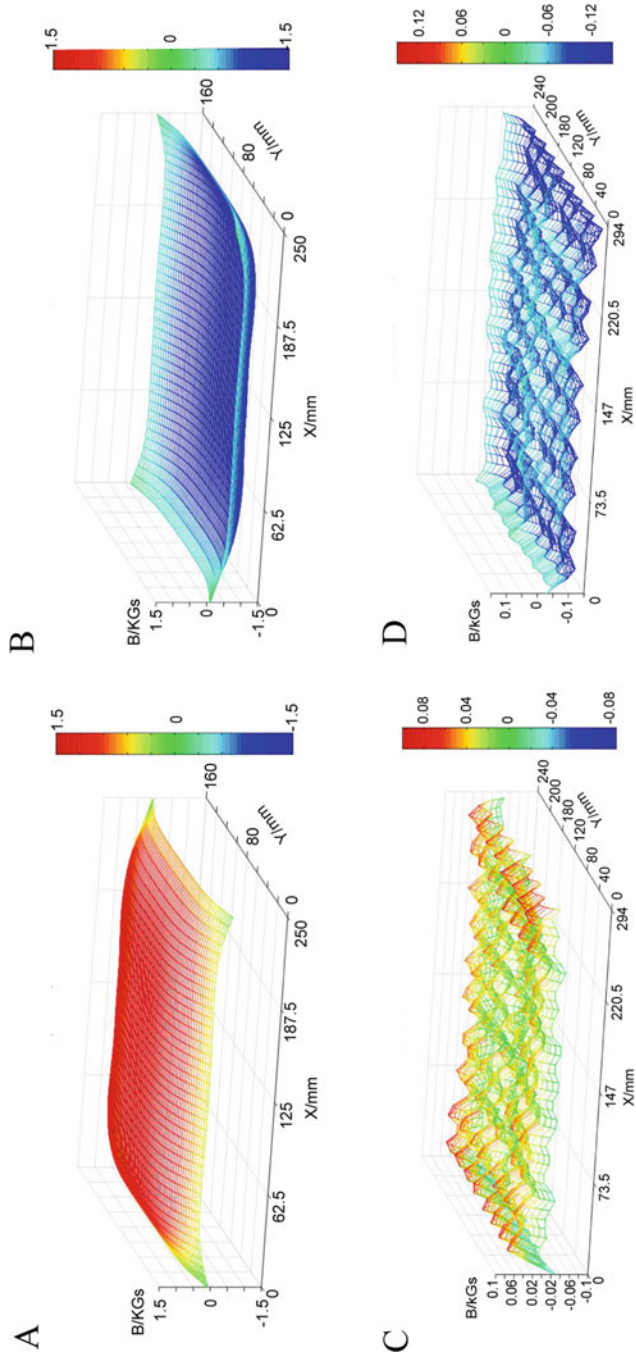

Fig. 1.5 Magnetic field distributions on the surface of 4 different permanent magnets. (**a**) Vertically upward direction with horizontal homogeneousness. (**b**) Vertically downward direction with horizontal homogeneousness. (**c**) Vertically upward direction with horizontal inhomogeneousness. (**d**) Vertically downward direction with horizontal inhomogeneousness

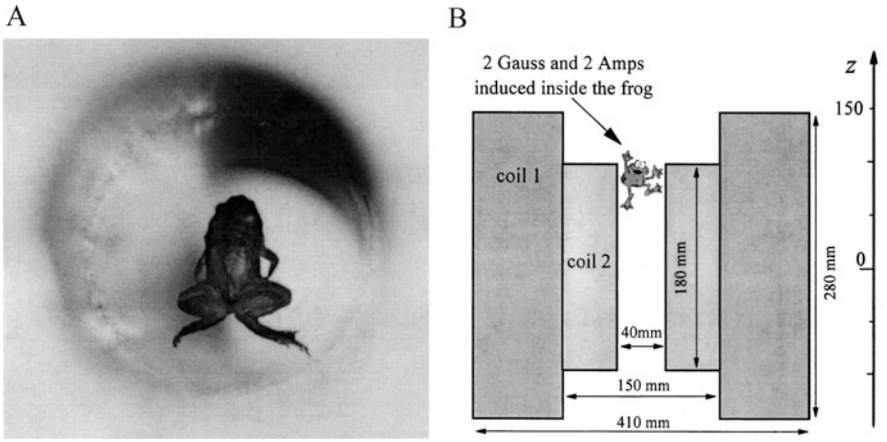

Fig. 1.6 The flying frog. (**a**) A small frog levitated in the stable zone within a 16 T magnet. (**b**) Illustration of the position of the frog within the magnet. [Reprinted with permission from (Geim and Simon 2000). Copyright © AIP Publishing LLC]

made a small magnetic levitation platform (Fig. 1.7a). This is based on the principle that each cell has a unique cellular magnetic signature, predominantly owing to the formation of intracellular paramagnetic reactive oxygen species (ROS). For example, cancer cells, white blood cells (WBC), and red blood cells (RBCs) are all different from each other (Fig. 1.7b). Apparently, this platform is much smaller than the one that is needed to fly a frog (Fig. 1.7c) and the magnetic field strength is also much weaker (Fig. 1.7d) because cells are much smaller and lighter than frogs. They actually used permanent magnets of moderate intensity (hundreds of millitesla) in this platform (Fig. 1.7d). This relatively simple set up actually can give ultrasensitive density measurements because each cell has a unique levitation profile (Fig. 1.7e) (Durmus et al. 2015). They proposed that this technique could be used in label-free identification and monitor of heterogeneous biological changes in various physiological conditions, including drug screening in personalized medicine.

In fact, multiple groups have utilized magnetic levitation technique to mimic the "weightless" condition and study its effects on cells. For example, the Shang group did a series of studies to investigate the effect of SMF with a vertical gradient using a large gradient ultra-strong magnet (Qian et al. 2009, 2013; Di et al. 2012). They compared the samples when they were placed at 0 gradient (1 g, indicate that the gravity is normal), or at above or down the magnet center, where the magnetic force is upward (0 g) or downward (2 g), respectively. The "0 g" position mimics the weightless condition and the "2 g" position has the double gravity forces in the downward direction. Since "0 g" and "2 g" have identical magnetic field intensity of around 12.5 T and the magnetic field direction (B) is upward at both positions, their only difference is the direction of magnetic force. At "0 g" position, the magnetic force that is equivalent to the gravity in the opposite direction so that "0 g" can be used to investigate the effect of weightless condition. At "2 g" the magnetic force is

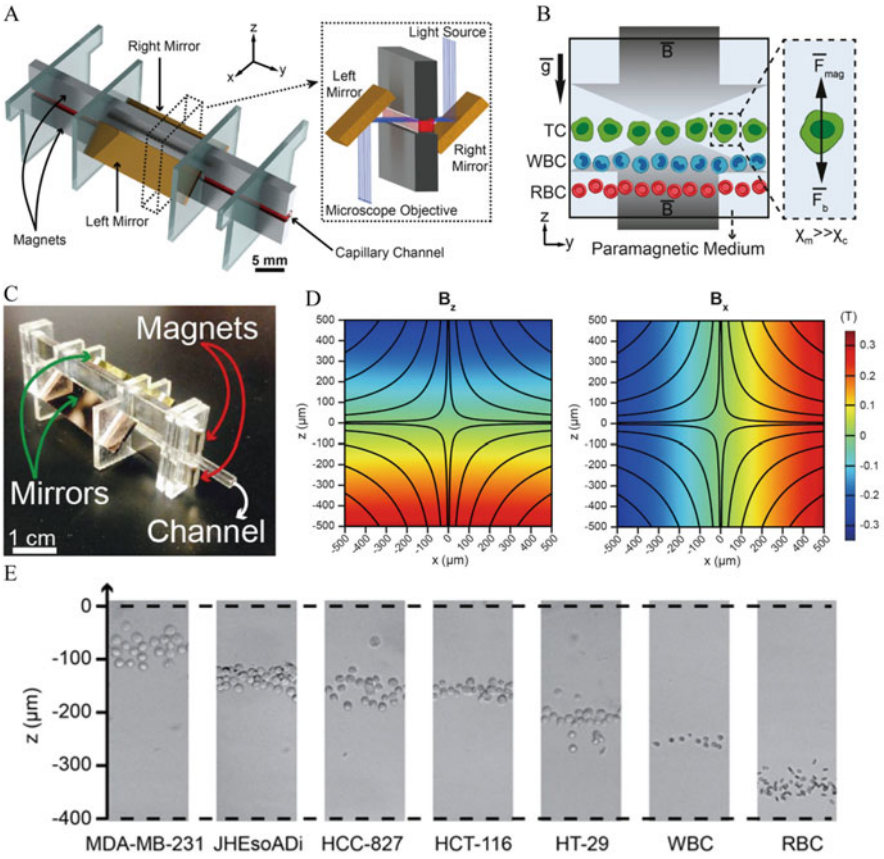

Fig. 1.7 Magnetic levitation of single cells using a densitometry platform, the MagDense cell density meter. (**a**) Illustration of the platform. (**b**) Final equilibrium height of cells in MagDense. Owing to the magnetic induction (B) and gravity (g), cells are levitated in the channel and are focused in an equilibrium plane where magnetic forces (Fmag) and buoyancy forces (Fb) equilibrate each other. Magnetic susceptibility of the medium (χm) is chosen to be bigger than the cells' magnetic susceptibility (χc). Different cell types with different densities, such as cancer cells (TC), WBC, and RBC, are separated from each other. (**c**) Photograph of densitometry platform. Capillary channel is introduced between two permanent neodymium magnets whose same poles are facing each other. (**d**) FEM simulation results showing *z* and *x* component of magnetic induction (Bz, Bx) inside the channel. Total magnetic induction (Bz + Bx) is also presented as streamlines on the images. (**e**) Distribution of cancer and blood cells in the MagDense along the channel. [The figures were adapted from (Durmus et al. 2015) (open access)]

the same as the gravity so that it mimics the double weight condition. In the meantime, the "1 g" position provides homogeneous SMF with no gradient so that it can be used to investigate the effect of magnetic field itself. Their results show that the magnetic field and the reduced gravity worked together to affect integrin protein expression in osteoblast-like cells. Moreover, MTT assays also revealed that the 12–16 T SMFs could increase the cell number/viability of osteosarcoma MG-63 and

osteoblast MC3T3-E1 cells since all three positions increased the MTT assay reading. However, they observed the difference between "1 g" of 16 T to "0 g" and "2 g" of 12 T, which is more likely due to the 4 T difference in magnetic field intensity.

SMF homogeneousness often can directly impact the biological effects. This is not surprising because the magnetic force acting on any particular object is proportional to the magnetic flux density, field gradient, and the magnetic susceptibility of the object. Magnetic fields with low or no field gradients can be used to induce a magnetic torque, rather than a magnetic force, which act on magnetic objects to move them along magnetic gradients. For example, Kiss et al. compared the homogeneous and inhomogeneous SMFs generated by permanent magnets and found that although both homogeneous and inhomogeneous SMFs of moderate intensity can significantly reduce pain in mice, the spatial SMF gradient might be responsible for the pain relief rather than the exposure to the SMF itself (Kiss et al. 2013). In addition, the SMFs with high gradient have been applied in red blood cell separation as well as malaria-infected red blood cell separation and diagnosis (Owen 1978; Paul et al. 1981; Nam et al. 2013).

1.2.4 Exposure Time

People are exposed to increasing amount of electromagnetic radiation these days from multiple sources, such as mobile phones and power lines, whose effects on human health are still debated. One of the constricting factors is that long-term exposure effects are lacking. In contrast, the human exposure to most SMFs, except for earth magnetic field, is only for a limited time. For example, the duration of the MRI examinations in hospitals is less than an hour. Even for people who work with MRI, the exposure time is relative limited. So far there are no known detrimental effects of repetitive MRI exposure on human bodies, as long as they follow the MRI instructions. Long-term SMF effects are discussed in Chap. 14 of this book, which summarizes experimental data on animals and humans that were subjected to SMFs for more than 2 weeks, either continuously or intermittently.

It has been demonstrated by multiple studies that exposure time is a key factor that contributes to the differential effects of magnetic fields on biological samples. For example, in 2003, Chionna et al. found that human lymphoma U937 cells exposed to 6 mT SMF showed cell surface microvilli shape change after 24 h exposure, but they have distorted cell shape after longer exposure (Chionna et al. 2003). In 2005, Chionna et al. found that cytoskeleton was also modified in a time-dependent manner in human liver cancer HepG2 cells exposed to 6 mT SMF (Chionna et al. 2005). In 2008, Strieth et al. found that prolongation of the exposure time from 1 min to up to 3 h increased the 587 mT SMF-induced reduction effects on red blood cell velocity and functional vessel density (Strieth et al. 2008). In 2009, Rosen and Chastney exposed GH3 cells to 0.5 T SMF for different time points and found that the effects on cell growth are time-dependent. After 1-week 0.5 T SMF

exposure, the cell growth of GH3 cells was reduced by 22% but returned to control level in a week after magnetic field retrieval. After 4-week 0.5 T SMF exposure, the cell growth of rat pituitary tumor GH3 cells was reduced to 51% and returned back to control level after 4 weeks after magnetic field retrieval (Rosen and Chastney 2009). In 2011, Sullivan et al. found that ROS in fetal human lung fibroblast WI-38 cells was significantly increased by 18 h of moderate intensity SMF exposure but not 5 days of exposure (Sullivan et al. 2011) although the underlying mechanism is still unknown. Also in 2011, Tatarov et al. tested the effect of 100 mT SMF on mice bearing metastatic breast tumor EpH4-MEK-Bcl2 cells. They found that exposure of the mice to magnetic fields for 3 h or 6 h, but not 1 h, daily for as long as 4 weeks suppressed tumor growth (Tatarov et al. 2011). In 2014, Gellrich et al. found that although both SMF single exposure and repeated exposure increased the blood vessel leakiness and reduced functional tumor microvessels, the repeated SMF exposure had stronger effects (Gellrich et al. 2014). In 2021, Zhao et al. show that a gradient SMF can increase the ROS levels in osteosarcoma stem cell 1 day after exposure, but not 3 or 5 days (Zhao et al. 2021). All these studies show that the SMF exposure time is a key factor for their effects on biological systems and people should keep the exposure time in mind when they design their own experiments or analyze the result from literature.

1.2.5 Magnetic Poles and Magnetic Field Directions

As we mentioned above, magnetic flux density, gradient, exposure time are all important factors that contribute to the magnetic field-induced bioeffect variations. However, most people would not pay much attention to the magnetic poles or magnetic field direction during their research, which actually caused a lot of variations in the literature. In fact, I think it is one of the most underestimated confounders that caused inconsistencies in the field of electromagnetic research, which deserves a separate review dedicated to this topic. Whether different magnetic poles can really cause different bioeffects as claimed in some magnetic therapy websites? Is it because of the magnetic pole or magnetic field direction? Does magnetic field direction affect all types of bioeffects? What are the underlying mechanisms? More details can be found in Chap. 2 of this book, in which we summarize and analyze all reported studies we can find to get answers to the above-mentioned questions.

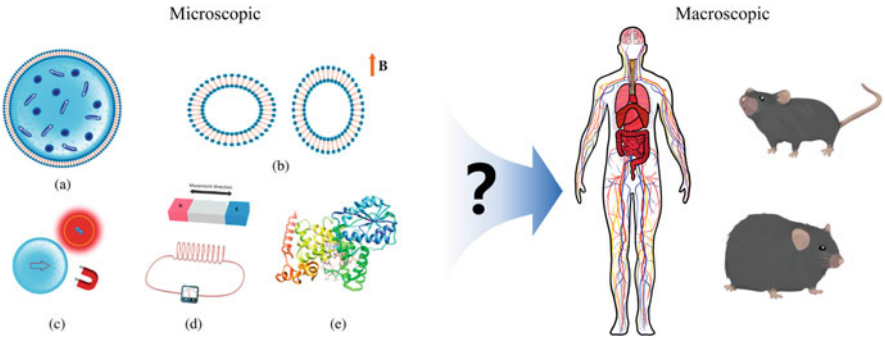

Fig. 1.8 The gap between microscopic mechanisms and macroscopic bioeffects. The left part was reprinted with permission from (Torbati et al. 2022). (The right part illustration courtesy of Shu-tong Maggie Wang and Ding Joe Wang)

1.3 Biological Sample Variations That Influence Magnetic Field-Induced Bioeffects

It is already known that magnetic field can affect biomolecules, electric current, free radicals, membrane potentials, etc., and magnetic properties of the biological samples can also determine their responses to externally applied magnetic fields, which will be discussed in detail in Chaps. 3, 4, and 5 of this book. In a recently published review by Torbati et al. a unified mathematical framework that couples nonlinear deformation and electromagnetic behaviors as germane for soft biological entities is summarized by the authors, which provide enormously valuable foundations for future research. However, since living organisms are complex systems that involve a large number of different types of tissues, cells molecules, and dynamic processes, how to translate the known physics, chemistry principles into the macroscopic phenomenon is still a difficult task (Fig. 1.8).

As the most fundamental unit of living organism, the cell is the place where the microscopic mechanism meets macroscopic phenomenon. Here we discuss some commonly seen biological sample variations at cellular level that have shown to be able to influence magnetic field-induced bioeffects, including cell type, cell density, and cell status. Beyond the cellular level, the phycological and pathological status of living organisms could also produce significantly different magnetic field bioeffects (our unpublished data), which should also cause some attention.

1.3.1 Cell Type-Dependent Cellular Effects of Static Magnetic Fields

Besides the various parameters of the MFs, different cell types in individual studies often have distinct genetic background, which makes them respond to the magnetic

fields differentially. For example, as early as in 1992, Short et al. showed that a 4.7 T SMF could alter the ability of human malignant melanoma cells attachment onto the tissue culture plate, but had no effect on normal human fibroblasts (Short et al. 1992). In 1999 and 2003, Pacini et al. found that a 0.2 T SMF-induced obvious morphology change in human neuronal FNC-B4 cell and human skin fibroblast cells but did not affect mouse leukemia or human breast carcinoma cells (Pacini et al. 1999, 2003). In 2004, Ogiue-Ikeda and Ueno compared three different cell lines for their orientation changes under an 8 T SMF for 60 h exposure. They found that while the smooth muscle A7r5 cells and human glioma GI-1 cells could be aligned along the field direction of the 8 T SMF, the human kidney HEK293 cells were not aligned (Ogiue-Ikeda and Ueno 2004). In 2010, the ultra-high magnetic field of 16 T did not cause obvious changes in unicellular yeast (Anton-Leberre et al. 2010) but could induce frog egg division alteration (Denegre et al. 1998). In 2011, Sullivan et al. showed that moderate intensity (35–120 mT) SMF could affect attachment and growth of human fibroblast cells as well as growth of human melanoma cells, but not attachment or growth of adult adipose stem cells (Sullivan et al. 2011). In 2013, Vergallo et al. showed that inhomogeneous SMF (476 mT) exposure caused toxic effects on lymphocytes but not on macrophages (Vergallo et al. 2013). These studies all show that different cell types respond to SMFs differently.

The different cellular effects of SMFs on various cell types may because these cells were originated from different tissues. Since different tissues have totally distinct biological functions and genetic background, it is not surprising that they have different responses to SMF exposure. However, evidences show that even for cells from the same tissue, their response to the same SMF can be very different. For example, the Shang group has made series of progresses about the impact of SMFs on different types of bone cells. For example, they not only found that the differentiation and mineral elements can be differentially affected by low, moderate, and ultra-high SMFs (Zhang et al. 2014b) but also found that different types of bone cells have obviously different cellular responses. The Shang group compared the effects of 500 nT, 0.2 T, and 16 T SMFs on osteoblast MC3T3-E1 cells (Zhang et al. 2014b) and osteoclast differentiation from pre-osteoclast Raw264.7 cells (Zhang et al. 2017a). They found that both hypo and moderate SMFs reduced osteoblast differentiation but promoted osteoclast differentiation, formation, and resorption. In contrast, 16 T high SMF increased osteoblast differentiation and inhibited osteoclast differentiation. Therefore, the osteoblast and osteoclast cells responded totally opposite to these SMFs. Their studies revealed some parameters that could be used as a physical therapy for various bone disorders. They also summarized the effects of SMFs on bone in a very informative review (Zhang et al. 2014a).

It is interesting that many studies indicate that SMFs could have inhibitory effects on cancer cells but not non-cancer cells. For example, Aldinucci et al. found that 4.75 T SMF significantly inhibited Jurkat leukemia cell proliferation but did not affect normal lymphomonocytes (Aldinucci et al. 2003). Rayman et al. showed that growth of a few cancer cell lines can be inhibited by 7 T SMF (Raylman et al. 1996), but a few other studies showed that even 10–13 T strong SMFs did not induce obvious changes in non-cancer cells such as CHO (Chinese hamster ovary) cells or

human fibroblast cells (Nakahara et al. 2002; Zhao et al. 2010). These results indicate that cell type is a very important factor that contributes to the differential responses of cells to SMFs. Recently, we found that EGFR and its downstream pathway play key roles in the SMF-induced cell proliferation inhibition. Our results showed that although CHO cells did not respond to moderate (1 T) or strong (9 T) SMFs, the transfected EGFR, but not the kinase-dead mutant of EGFR, could convert the SMF-insensitive CHO cells into SMF-sensitive cells and their cell growth could be inhibited by moderate and strong SMFs. Detailed mechanisms will be discussed in Chap. 9 of this book, which focuses on the potential application of SMFs in cancer treatment.

Most individual studies so far have only investigated one or very few types of cells, which is not sufficient enough for people to comprehensively understand the effects of the magnetic fields on cells. Therefore, comparing different cell types side-by-side for their responses to the magnetic fields is strongly needed. In our recent work, we compared 15 different kinds of cells, including human cells and some rodent cells for their responses to 1 T SMF. Our results confirmed that SMFs could induce completely opposite effects in different cell types. However, since the biological systems are very complicated, the knowledge we have is still very limited. More studies are definitely needed for people to get a more complete understanding for the effects of SMFs on different types of cells.

1.3.2 Cell Plating Density-Dependent Cellular Effects of Static Magnetic Fields

We found that the cell density also played a very important role in SMF-induced cellular effects (Zhang et al. 2017c). We originally found this by accident, when we were investigating the effects of 1 T SMF on human CNE-2Z nasopharyngeal cancer cell proliferation. We got diverse results when we plated the cells at different cell densities. To verify this observation, we seeded CNE-2Z cells at 4 different cell densities and examined them side-by-side. We found that at lower cell density, 1 T SMF treatment for 2 days did not inhibit CNE-2Z cell proliferation and there was even a tendency of increased cell number after SMF treatment. However, when the cells were seeded at higher densities, it was interesting that 1 T SMF could consistently inhibit CNE-2Z cell proliferation. These results demonstrate that cell density can directly influence the effect of 1 T SMF on CNE-2Z cells.

We suspected that the cell density-induced variations must at least partly contribute to the lack of consistencies in the literature. Most researchers, including us, did not really pay enough attention to the cell density before, or at least did not realize that the cell density could cause such dramatic variations in the experimental outcomes. However, it has been shown that cell density could directly cause variations in cell growth rate, protein expression, alterations in some signaling pathways (Macieira 1967; Holley et al. 1977; McClain and Edelman 1980;

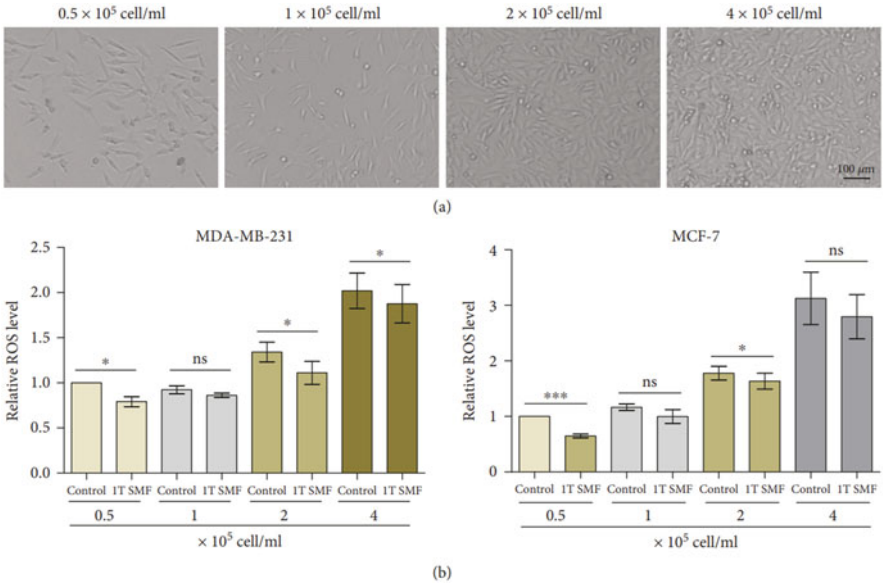

Fig. 1.9 Cells plated at different density have different ROS levels. Two types of breast cancer cell lines were plated at four different densities and subjected to an inhomogeneous SMF at 1 T (max). *ns* not significant; $*p < 0.05$; $***p < 0.005$. [Reprinted with permission from reference (Wang and Zhang 2019)]

Takahashi et al. 1996; Baba et al. 2001; Caceres-Cortes et al. 2001; Swat et al. 2009), as well as ROS levels (Fig. 1.9) (Wang and Zhang 2019). In fact, we also chose 6 other human cancer cell lines and found that for most of them, their cell number could be reduced by 1 T SMF when seeded at higher densities, but not at lower densities (Zhang et al. 2017c). This indicates that cell density could generally influence the impact of SMFs on human cancer cell lines.

Then we further tested a few other non-cancer cell lines and found that cell density could directly influence the effects of SMFs on their proliferation as well. In addition, the pattern is different in different kinds of cells. Although the mechanism is still not completely understood, our data revealed that EGFR and its downstream pathways might contribute to the cell type- and cell density-induced variations. However, as mentioned above, since cell density can have multiple effects on cells, such as calcium level (Carson et al. 1990) and signaling pathways, other factors are likely to be involved. For example, in 2004, Ogiue-Ikeda and Ueno found that although A7r5 cells (smooth muscle cells, spindle shaped) and GI-1 cells (human glioma cells, spindle shaped) could orient in an 8 T SMF. They concluded that the MF affected the cell division process, and only the proliferating cells at high density were oriented under the MF (Ogiue-Ikeda and Ueno 2004). However, it was interesting that the orientation did not occur when the cells were under the confluent condition at the start point of the MF exposure, when the cell density was too high.

Apparently, further analysis is needed to unravel the complete mechanisms of cell density-dependent variations in SMF-induced cellular effects. Before we have a clear understanding of the molecular mechanisms, people should always pay extra attention to the cell density in their own studies, as well as in literature reading.

1.3.3 Cell Status Influences the Cellular Effects of Static Magnetic Fields

Besides the cell type and density, cell status can also affect the cellular effects of SMFs. For example, in RBCs, the hemoglobin conditions can directly affect the magnetic properties of the whole cell. In normal RBCs, the hemoglobin is oxygenated and the cell is diamagnetic. In fact, they are slightly more diamagnetic than water because of the diamagnetic contribution of globin. However, when the cells were treated with isotonic sodium dithionite to make the hemoglobin in deoxygenated reduced state or treated with sodium nitrite to oxidize the hemoglobin (methemoglobin), the RBCs would become paramagnetic. Back in 1975, Melville et al. directly separated RBCs from whole blood using a 1.75 T SMF (Melville et al. 1975). In 1978, Owen used a 3.3 T SMF with high gradient to separate RBCs (Owen 1978). The paramagnetic methemoglobin containing RBCs could be separated from diamagnetic untreated RBCs as well as diamagnetic leukocytes (white blood cells, WBCs) (Owen 1978). In fact, "magnetophoresis" has been applied in RBC, called RBC magnetophoresis, which uses an applied magnetic field to characterize and separate the cells based on the intrinsic and extrinsic magnetic properties of biological macromolecules in these cells (Zborowski et al. 2003; Moore et al. 2013). In 2013, Moore et al. designed an open gradient magnetic RBC sorter and tested on label-free cell mixtures (Moore et al. 2013). They showed that in the open gradient magnetic RBC sorter, the oxygenated RBCs were pushed away from the magnet and the deoxygenated RBCs were attracted to the magnet. Moreover, the effect for the oxygenated RBC's was very weak and comparable to that of other non-RBC cells in the blood, which do not contain hemoglobin and could be considered as non-magnetic. They proposed that the quantitative measurements of RBC mobility in cell suspension were the basis for engineering design, analysis, and fabrication of a laboratory prototype magnetic RBC sorter built from commercially available, block permanent magnets to serve as a test bed for magnetic RBC separation experiments (Moore et al. 2013).

Another well studied example of cells with different magnetic property is malaria-infected RBCs. Researchers have utilized malaria byproduct, hemozoin, to study and separate malaria-infected RBCs in a magnetic field gradient (Paul et al. 1981; Moore et al. 2006; Hackett et al. 2009; Kasetsirikul et al. 2016). During intraerythrocytic maturation, malaria trophozoites could digest up to 80% of cellular hemoglobin, which accumulates toxic heme. To prevent heme iron from participating in cell-damaging reactions, the parasite polymerizes beta-hematin dimers to

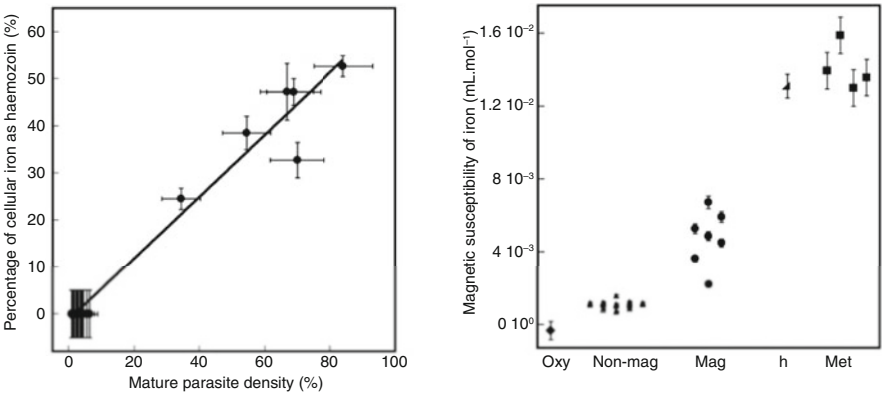

Fig. 1.10 Magnetic susceptibility of iron in malaria-infected red blood cells (RBCs). (Left) Percentage of cellular iron converted to hemozoin vs. mature parasite density. (Right) Scatter plot of the molar magnetic susceptibility of iron in standard samples of oxyhemoglobin (Oxy), hematin (h), methemoglobin (Met), and for magnetic (Mag) and non-magnetic (Non-mag) fractions of malaria-infected red cell cultures. [Reprinted with permission from (Hackett et al. 2009). Open access. Copyright © 2008 Elsevier B.V.]

synthesize insoluble hemozoin crystals. In the process, the heme is converted to a high-spin ferriheme, whose magnetic properties were studied a long time ago (Pauling and Coryell 1936). In fact, in 2006, Moore et al. used magnetophoretic cell motion analysis to provide direct evidence for a graduated increase of live cell magnetic susceptibility with developing blood-stage parasites, which is compatible with hemozoin increase (Moore et al. 2006). In 2009, Hackett et al. experimentally determined the source of the cellular magnetic susceptibility during parasite growth. They found that the parasites converted approximately 60% of host cell hemoglobin to hemozoin and this product was the primary source of the increase in cell magnetic susceptibility (Fig. 1.10). While the magnetic susceptibility of uninfected cells was similar to water, the magnetically enriched parasitized cells have higher magnetic susceptibility (Hackett et al. 2009). Therefore, the magnetic fields with gradient could be used in malaria diagnosis and malaria-infected RBC separation (Paul et al. 1981; Kasetsirikul et al. 2016).

Magnetic fractionation of erythrocytes infected with malaria has also been used in enrichment of infected cells from parasite cultures and separation of infected cells from uninfected cells in biological and epidemiological research, as well as clinical diagnosis. In 2010, Karl et al. used high gradient magnetic fractionation columns to quantitatively characterize the magnetic fractionation process. They found that the infected cells had approximately 350 times higher magnetic binding affinity to the column matrix compared to the uninfected cells (Karl et al. 2010). In addition, the distribution of captured parasite developmental stages shifted to mature stages as the number of infected cells in the initial samples and flow rate increased (Karl et al. 2010). Furthermore, in 2013, Nam et al. used permanent magnets and ferromagnetic wire to make a polydimethylsiloxane (PDMS) microfluidic channel integrated with a

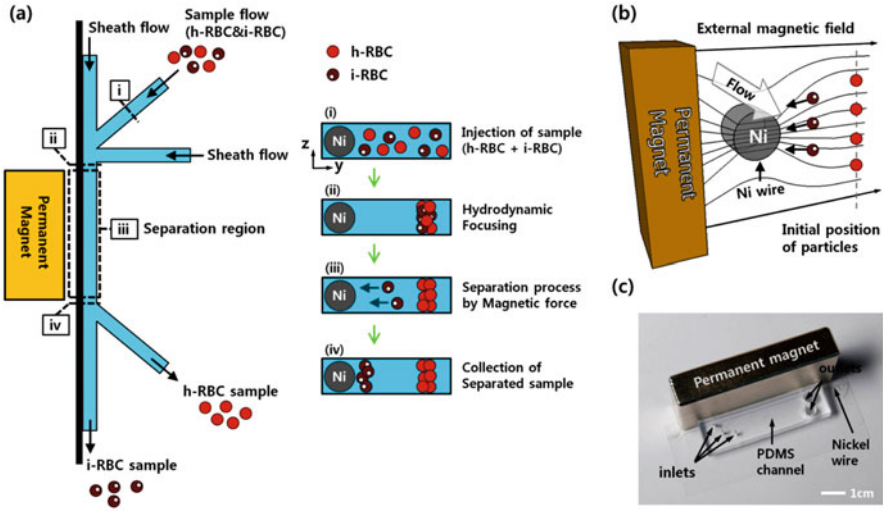

Fig. 1.11 Malaria-infected RBC separation using a high magnetic field gradient. (**a**) Schematic diagram of i-RBC (infected red blood cell) separation using the paramagnetic characteristics of hemozoin in i-RBCs. (**b**) Working principle of magnetophoretic separation with a ferromagnetic nickel wire in an external magnetic field. (**c**) Photograph of the permanent magnet for applying an external magnetic field in the microchannel and a microfluidic device consisting of the PDMS microchannel and a nickel wire. [Reprinted with permission from (Nam et al. 2013). Copyright © 2013, American Chemical Society]

ferromagnetic wire fixed on a glass slide to separate infected RBCs in various developmental stages (Fig. 1.11). Late-stage infected RBCs were separated with a recovery rate of around 98.3%. Early-stage infected RBCs had been difficult to separate due to their low paramagnetic characteristics but can also be successfully separated with a recovery rate of 73%. Therefore, it could provide a potential tool for malarial-related studies (Nam et al. 2013).

Besides the cell status mentioned above, the cell lifespan or cell age can also influence SMF-induced cellular effects. In 2011, Sullivan et al. found that various points during the lifespan of fetal human lung fibroblast WI-38 cells affected the cellular responses to moderate intensity SMF (Sullivan et al. 2011). SMF exposure decreased cell attachment by less than 10% in younger cultures (population doubling level 29) but can decrease cell attachment by more than 60% in older cultures (population doubling level 53). In 2004, Ogiue-Ikeda and Ueno found that the smooth muscle A7r5 cells could be aligned along an 8 T magnetic field direction only when the cells were actively proliferating at a higher density (Ogiue-Ikeda and Ueno 2004). In addition, in 2014 Surma et al. also found that fully differentiated myotubes at late stages of development were less sensitive to weak SMF and myotubes at the stage when electromechanical coupling was forming dramatically reduced the contraction frequency during the first minute's weak SMF exposure (Surma et al. 2014). These results demonstrate that even for the same cell type and same SMF exposure, the cellular effects could be influenced by their status, such as

lifespan. The underlying mechanisms are still unknown and need to be further investigated.

1.4 Other Factors Contributing to the Lack of Consistencies in Bioeffect Studies of Magnetic Fields

The above-mentioned parameters, including magnetic flux density, cell types, cell plating density, and cell status, are just a few examples that directly influence the cellular effects of SMFs. It is very likely that other aspects of cell status also contribute to the differential effects of SMF on cells. There are multiple other factors that complicate the situation, such as magnetic field exposure time, direction, gradient, etc. Interested readers can look into our Chap. 1 for more information. In the meantime, we recommend researcher in this field to provide as detailed information as possible about their experimental setup as well as the biological samples, which will help us to understand better of the cellular effects of SMFs. Further investigations at both cellular and molecular levels are needed to get a comprehensive understanding.

As mentioned above, despite the numerous scientific research and non-scientific case reports about the magnetic effects on living organisms, the magnetic field effects on biological systems are still looked upon with doubts and suspicion by many scientists outside of the field, as well as by the mainstream medical community. This is largely due to a lack of consensus on the biological effects in general that are backed up by solid scientific evidences and explanations. We have to admit that the countless scientific research or non-scientific case reports are enriched with many seemingly contradictory results, which make many people confused and hence become suspicious, including myself a few years ago. Then we carefully analyzed the evidence in the literature about the biological effects of magnetic fields to try to view them collectively in a scientific way. We found that most of these inconsistencies can be explained by the different parameters of either the magnetic fields or the biological samples people used in individual studies. For example, the magnetic field parameters mentioned above in this chapter all contribute to the differential effects, such as the types of magnetic fields, the field intensities and frequencies, the homogeneity and directions of the MFs, the magnetic poles, and the exposure time. More importantly, we found that the biological samples people examined directly affect the magnetic effects. For example, both cell types and cell densities have direct impact on the effects of 1 T SMF on cells (Zhang et al. 2017c). The Shang group compared the effects of 500 nT, 0.2 T, 16 T on osteoblast MC3T3-E1 cells (Zhang et al. 2014b), as well as pre-osteoclast Raw264.7 cells (Zhang et al. 2016) and found that the osteoblast and osteoclast cells responded totally oppositely to these SMFs. Both hypo and moderate magnetic fields reduced osteoblast differentiation but promoted osteoclast differentiation, formation, and resorption. In contrast, 16 T SMF increased osteoblast differentiation inhibited osteoclast differentiation. They

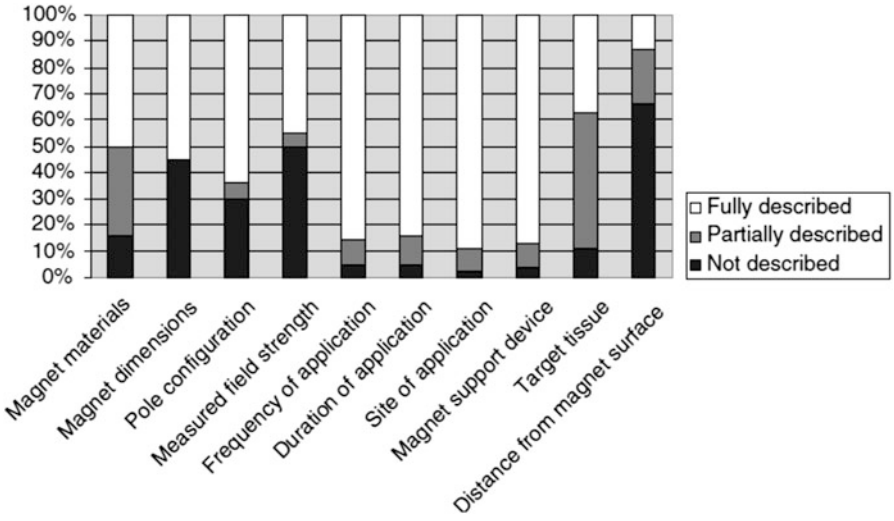

Fig. 1.12 Quality of reporting 10 SMF dosage and treatment parameters was assessed in 56 human studies. [Reprinted with permission from (Colbert et al. 2009). Copyright © 2007 The Authors (open access)]

also wrote a particular review to systematically summarize the effects of SMFs on bone that is worth to look into (Zhang et al. 2014a). More surprisingly, some people (including ourselves) found that even cell passage number could affect the experimental results.

It should also be mentioned that, theoretically, if two magnetic devices both provide SMFs of same parameters, including flux density, gradient, and distribution, there should be no differences between them, or their effects on biological systems. However, by analyzing the differential effects in the literature about SMF-induced effects on reproductive development, we found that different types of magnetic devices often cause differential bioeffects (Song et al. 2022). Specifically, it seems that some electromagnetic fields may have induced bioeffects because of nonnegligible gradient, heat effect, and minor 50/60 Hz ripple, which are much reduced in superconducting magnets. The heat effect and minor 50/60 Hz ripple can be completely avoided by permanent magnets.

In 2009, Colbert et al. wrote a comprehensive review "Static Magnetic Field Therapy: A Critical Review of Treatment Parameters" (Colbert et al. 2009). Their purpose was to summarize SMF studies involving the application of permanent magnets in humans. In this review, they critically evaluated the reporting quality of 10 essential SMF dosing and treatment parameters and proposed a set of criteria for reporting SMF treatment parameters in future clinical trials (Fig. 1.12). They reviewed 56 studies about magnetic therapy, in which 42 studies were done in patient populations and 14 studies were done in healthy volunteers. As we have discussed in earlier part of this Chapter, the magnetic field parameters greatly influence their effects on biological systems. However, by analyzing 10 magnetic

Table 1.1 Information that should be included when reporting the biological effects of SMFs

Biological samples	Static magnetic fields	Application
Species	Magnet materials	Frequency of application
Tissue	Magnetic device types	Duration of application
Cell type	Pole configuration	Timing of experiments (AM vs. PM)[a]
Cell density	Magnetic field distribution (including direction)	Site of application
Culture condition	Magnetic field flux	Magnet support device
	Magnetic field gradient	Sham condition
		Distance from magnet surface

[a]The day vs. night might also be a potential factor that could influence the biological effects of SMFs because of the circadian clocks and the earth magnetic field fluctuations

field related parameters in these studies, including the magnet materials, magnet dimensions, pole configuration, measure field strength, frequency of application, duration of application, site of application, magnet support device, target tissue, distance from magnet surface, and found that 61% of the studies failed to provide enough experimental details about the SMF parameters to permit protocol replication by other investigators.

Moreover, there are also some other factors contributing to these differences, such as instrument and technical sensitivities, which have been greatly improved in the past few decades. Nowadays, people have much advanced instruments and techniques, which should enable more findings that were not detectable before. The absence of magnetic field effects in some studies may simply due to the technical limitations and/or inadequate control of experimental conditions. We should take advantage of the modern technologies to answer related questions. For example, we recently used liquid-phase scanning tunneling microscopy (STM) to get high resolution single molecular images of proteins (Wang et al. 2016) and combined with biochemistry, cell biology as well as molecular dynamics simulation to reveal that moderate and strong SMFs could change EGFR orientation to inhibit its activation and some cancer cell growth (Zhang et al. 2016).

At the same time, we should keep all relevant factors in mind, such as magnetic field type and intensity, cell type, and density when we do our own research and analyze the relevant literature. Apparently, the lack of sufficiently detailed description of SMF parameters greatly prevented people from getting consensus conclusions from these studies. We strongly encourage people in the field of magnetic field studies to clearly state their experimental details in their own research (Table 1.1). This will help us reduce the diversity and contradictions in this field and also help us to correctly understand the mechanism of the biological effects caused by the magnetic field.

Last but not least, it should be realized that the field of electromagnetic fields on biological systems is filled with experimental results that cannot be reproduced by

other labs. Other than the previous mentioned factors in this chapter, the experimenter bias is almost always sub-conscious, but is considered to be a significant contributing factor to the problems of reproducibility in this area of science. Therefore, to remove experimenter bias and thus meet the gold standard for assessing the effects of magnetic fields on biological systems, the person analyzing the data shouldn't be aware of the exposure conditions. In another word, blinded analysis should be performed. Moreover, to get unbiased and reproducible results, our group have always tried to minimize experimental variations by doing the same sets of experiments for more than three times by at least two different researchers. They performed the experiments independently, and their results were pooled together for blinded analysis.

1.5 Conclusion

Since the human body itself is an electromagnetic object, it is not surprising that the magnetic fields could produce some effects. There are indeed many convincing experimental evidences as well as theoretical explanations about the effects of magnetic field on some biomolecules, such as the cytoskeleton microtubules, membrane, as well as some proteins (will be discussed in Chaps. 3–6). In the meantime, most studies in the literature on the biological and health effects of magnetic fields had been inconclusive or contradictory, which was largely due to the various parameters used in individual studies, including the magnet fields themselves, samples examined, as well as the experimental setup. It seems that there is a large gap between atom/molecular level and cell/tissue/organism level that people need to fill in to correctly and scientifically understand the biological effects of magnetic field. For now, experimental and theoretical studies are both at a very preliminary stage. To help us get a more complete understanding of the biological effects of magnetic fields and their underlying mechanisms, more systematic, well controlled and fully described experimental details are strongly encouraged. Furthermore, increased collaborations between scientists in physics, biology, and chemists are necessary to make substantial progresses in this emerging field.

Ethics The frog research studies in this chapter had their ethics approved. For Okano et al. 2012, it was stated that "the animal experiments were carried out with the approval of the Animal Ethics Committee of Chiba University (Chiba, Japan)."

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