Xin Zhang *Editor*

Biological Effects of Static Magnetic Fields

Second Edition

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Abstract: With the development of magnet technology, the potential impacts of magnetic fields on human health have caused increasing concerns. On the other hand, although permanent magnets have been used in magnetotherapy a long time ago, their effectiveness has always been debated. This is partially due to the discrepancy in reported static magnetic field (SMF) bioeffects, as well as an incomplete understanding about the underlying physical and biological mechanisms. Fortunately, recent conceptual and technological advances have revealed that the previously reported inconsistencies are mainly caused by experimental setup variations, such as magnetic field type, strength, distribution, treatment procedure as well as magnetic properties of various biological samples. The purpose of this book is to review current scientific evidence and summarize the emerging topic about the effects of SMFs on biological samples ranging from single molecules, subcellular

compartments, cells to whole organisms. We will also summarize reported effects of SMFs on cancer, the immune and nervous system, bone and diabetes, etc. We realize that the ambiguities in this field can be resolved, or at least much reduced by combining advanced techniques and concepts in interdisciplinary research, as well as standardized double-blind experiments and data analysis. These will not only help clarify most dilemmas in this field, achieve a better understanding of the underlying mechanisms, but also enable future rational design and applications of SMFs in clinical diagnosis and therapy.

Preface

With the development of modern technology, people are exposed to various types of magnetic fields, which include static magnetic field (SMF), whose magnetic flux density and distribution do not change over time, as well as time-varying magnetic fields of different frequencies. The most commonly seen SMFs include the moderate SMFs generated by permanent magnets that exist everywhere, and higher SMFs generated by the core component of magnetic resonance imaging (MRI) machines for medical diagnosis (most clinical and preclinical MRIs are ~0.5–9.4 T). Accordingly, WHO (World Health Organization) and ICNIRP (International commission on nonionizing radiation protection) have published guidelines for the SMF exposure of human bodies to ensure that people are not overexposed. On the other hand, magnetic therapy, although not in the mainstream medicine, has been widely used by many people worldwide as alternative or supplementary treatments. The goal of this book is to summarize current scientific evidence for the biological effects of SMFs, including the observed phenomenon, their underlying mechanisms, as well as the study limitations. Although the current literature concerning bioeffects of magnetic fields is replete with reports that are often not reproducible by other independent labs, we found that it is mostly caused by magnetic field parameter and biological sample variations, as well as an insufficient understanding of this multidisciplinary and interdisciplinary area that involves biology, physics, chemistry, and engineering. Moreover, the potential experimenter bias may also be a nonnegligible factor. Therefore, we encourage people to not only perform double-blinded analysis in independent studies, but also clearly describe all the experimental details about the magnetic field, biological samples, and the experimental procedure which will be crucial for people to perform further subjective analysis and mechanistic investigations. It should be mentioned that we will not cover magnetic nanoparticle studies, which have promising therapeutic application potentials in medicine; we will focus on the externally applied SMFs on human and animal objects, as well as their magnetic properties, but not elaborate on the weak magnetic fields produced by the electric currents in our brain, heart, or muscle, which is another promising new area for medical applications. We try to cover most aspects of biological effects of

SMFs but also want to apologize for any missed research findings that have not been included in this book. Our goal is trying to provide people with an overview of the current understanding of the biological effects of SMFs and hope to encourage more scientists to get involved in this field so that we can get a clearer view in the near future.

Hefei, China Xin Zhang

Contents

Abbreviations

5-HIAA	5-Hydroxyindole acetic acid
5-HT	5-Hydroxytryptamine
$A_{2A}R$	Adenosine A2A receptor
AC	Alternating current
ACAN	Aggrecan
AChE	Acetylcholinesterase
AfD	Amphid neurons with finger-like ciliated endings
AIF	Apoptosis-inducing factor
ALT	Alanine aminotransferase
AMF	Alternating magnetic field
AMRi	Advanced Magnetic Research Institute
AMS	Anisotropy of magnetic susceptibility
APO	Apomorphine
AP-SMF	Alternating pole static magnetic field
APX	Ascorbate peroxidase
ASCs	Adipose-derived stem cells
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BMD	Bone mineral density
BMP	Bone morphogenetic protein
BP	Blood pressure
BRS	Baroreflex sensitivity
BSA	Bovine serum albumin
CaMKII	Calcium/calmodulin-dependent protein kinase II
CAP	Compound action potentials
CAT	Catalase
Cd	Cadmium
CDKA	Cyclin-dependent kinases A
CFU	Colony-forming units
CHO	Chinese hamster ovary

CL	Cardiolipins
Col2A1	Collagen type II alpha 1
CRF	Continuous radiofrequency
CRY	Cryptochrome
CSCs	Mesenchymal cancer stem cells
CSF	Colony-stimulating factor
CTA	Conditioned taste aversion
CTV	Cell tracking velocimeter
CycD	D-type cyclin
DHE	Dihydroethidium
DIY	Do-it-yourself
DLPFC	Dorsolateral prefrontal cortex
DMF	Dynamic magnetic field
DPN	Diabetic peripheral neuropathy
DPSCs	Dental pulp stem cells
DSB	Double-strand break
DW	Diabetic wound
ECG	Electrocardiogram
EEG	Electroencephalograph
EGFR	Epidermal growth factor receptor
ELF	Extremely low frequency
EMFs	Electromagnetic fields
EMT	Epithelial-mesenchymal transition
EPR	Electron paramagnetic resonance
ERK	Extracellular regulated protein kinases
ESR	Electron spin resonance
FAC	Ferric ammonium citrate
FAD	Flavin adenine dinucleotide
FDA	Food and Drug Administration
fMRI	Functional MRI
FTIR	Fourier-transform infrared G gauss
GABA	γ-Aminobutyric acid
GAG	Glycosaminoglycan
GAS	General adaptation syndrome
GCs	Granulosa cells
GERD	Gastroesophageal reflux disease
GFAP	Glial fibrillary acidic protein
GLUT2	Glucose transporters 2
GMF	Gradient magnetic field
GPx	Glutathione peroxidase
GR	Glutathione reductase
GT	Glutathione transferase
hASCs	Human adipose-derived mesenchymal stromal stem cells
hEBD	Human embryoid body-derived

HF	High frequency
HFD	High-fat diet
HFF	Human foreskin fibroblast
HiSMF	High static magnetic field
HMFs, HyMF	Hypomagnetic fields
HO	Hydrogen peroxide
HRP	Horseradish peroxidase
hUASMCs	Human umbilical artery smooth muscle cells
HUVECs	Human umbilical veins endothelial cells
Hz	Hertz
ICNIRP	International Commission on Non-Ionizing Radiation Protection
IFN	Interferon
IGRT	Image-guided radiotherapy
IL	Interleukin
IONs	Iron oxide nanoparticles
IRM	Isothermal remanent magnetization
ISCA1	Iron-sulfur-containing assembly protein
JNK	c-Jun N-terminal kinase
LC3	Light chain 3
LDH	Dehydrogenase
LF	Low frequency
LFS	Low-frequency sine wave
LPS	Lipopolysaccharide
MAP2	Microtubule-associated protein-2
MBMSCs	Mandibular bone marrow mesenchymal stem cells
MCC	Mandibular condylar chondrocyte
MCG	Magnetocardiogram
MD	Molecular dynamics
MDA	Malondialdehyde
MEG	Magnetoencephalography
metHb	Methemoglobin
MF	Magnetic field
MFEs	Magnetic field effects
MME	Magnetic molecular energizer
MNPs	Magnetic nanoparticles
MP	Membrane potential
MPG	Magnetoplethysmography
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MS	Multiple sclerosis
MSAD	Magnetic sphincter augmentation device
MSCs	Mesenchymal stem cells
MTB	Magnetotactic bacteria
NBT	Nitro blue tetrazolium

NCV	Nerve conduction velocity
NHMFL	National High Magnetic Field Laboratory
NK	Natural killer cell
NMDA	N-methyl D-aspartate
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NPCs	Neural progenitor cells
NRF2	Nuclear factor erythroid 2-related factor 2
NSCLC	Non-small cell lung cancer
OPCs	Oligodendrocyte precursor cells
OVX	Ovariectomized
PBMC	Peripheral blood mononuclear cell
PD	Parkinson's disease
PDL	Periodontal ligament width
PDMS	Polydimethylsiloxane
PEMF	Pulsed electromagnetic fields
PHA	Phytohemagglutinin
PI3K	Phosphoinositide 3-kinase
PMA	Phorbol 12-myristate 13-acetate
PMC	Puromycin
PMNs	Polymorphonuclear leukocytes
PNCA	Proliferating cell nuclear antigen
POD	Peroxidase
POP	Polyphenoloxidase
PRF	Pulsed radiofrequency field
PTB	Preterm birth
PyMT	Polyoma middle T oncoprotein
QSM	Quantitative susceptibility mapping
RBC	Red blood cell RH relative humidity
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RP	Radical pair
RPM	Radical pair mechanism
RTKs	Receptor tyrosine kinases
SAED	Selected area electron diffraction
SD	Stable domain
SHR	Spontaneously hypertensive rat
SMF	Static magnetic field
SNAP-25	Synaptosomal-associated protein 25
SNARE	Soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor
SOD	Superoxide dismutase
Sox2	SRY-box transcription factor 2
SQUID	Superconducting quantum interference device

STZ	Streptozocin
SVZ	Subventricular zone
T	Tesla
T1D/T1DM	Type 1 diabetes mellitus
T2D/T2DM	Type 2 diabetes mellitus
TERT	Telomerase reverse transcriptase
TLR4	Toll-like receptor-4
TMS	Transcranial magnetic stimulation
TNF	Tumor necrosis factor
TPT	Thermal pain threshold
tSMS	Transcranial static magnetic field stimulation
TTF	Tumor treating field
TVMF	Time-varying magnetic field
UV	Ultraviolet
VDCC	Voltage-dependent calcium channel
VEGF	Vascular endothelial growth factor
VSM	Vibrating sample magnetometer
VSMCs	Human vascular smooth muscle cells
WBC	White blood cell
WHO	World Health Organization
WM	White matter

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.

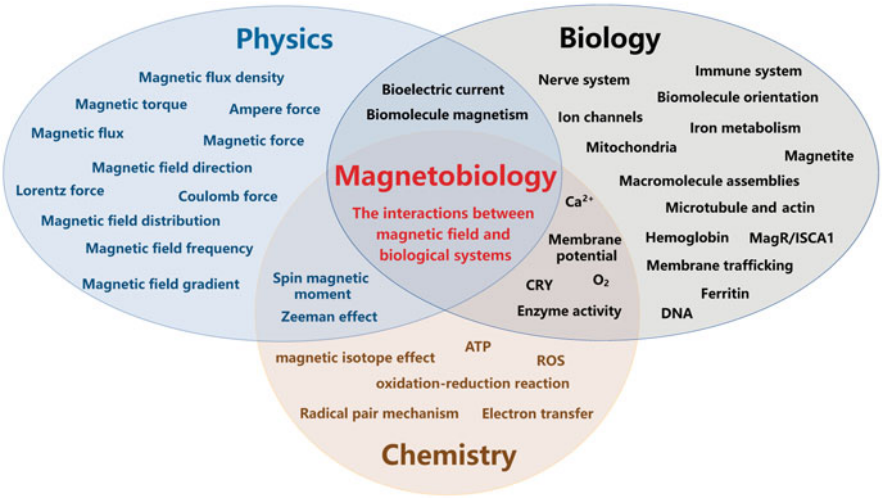

Fig. 1.1 Magnetobiology is an interdisciplinary research area

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

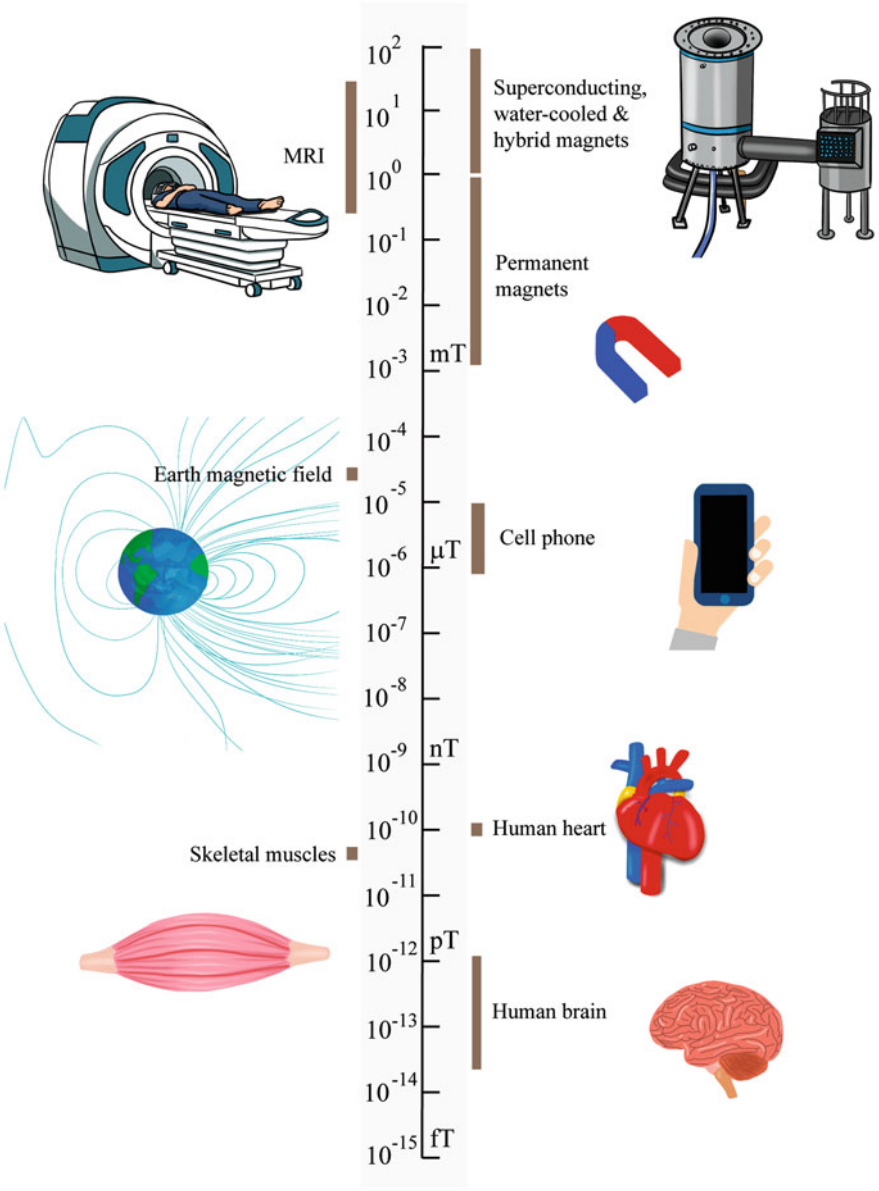

Fig. 1.2 Magnetic fields of different magnetic flux density. *T* tesla, *MRI* magnetic resonance imaging. (Illustration courtesy of Ding Joe Wang)

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

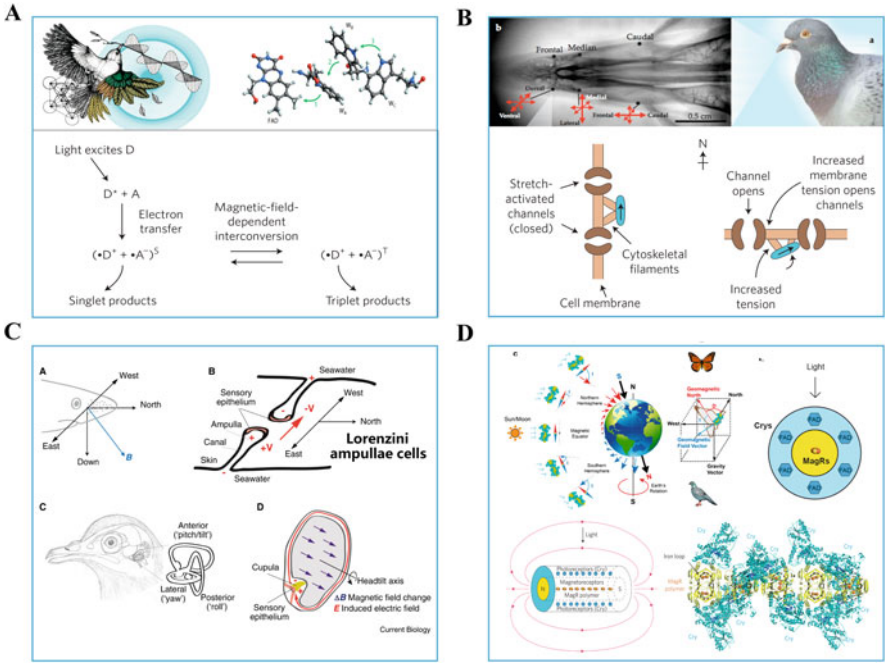

Fig. 1.3 Different hypotheses of magnetoreception. (**a**) The radical pair mechanism (RPM) hypothesis (Ritz et al. 2000; Ball 2011; Hore and Mouritsen 2016). (**b**) The magnetite hypothesis (Lohmann and Johnsen 2008; Lohmann 2016). (**c**) Electromagnetic induction hypothesis (Bellono et al. 2018; 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

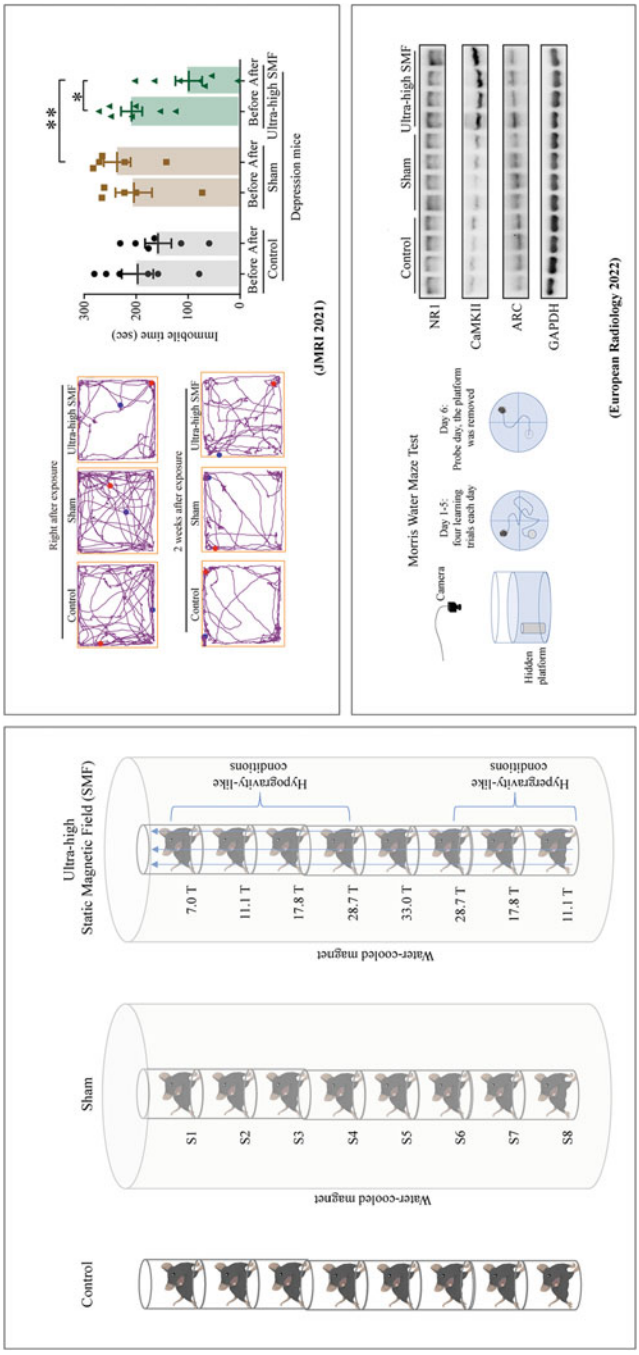

Fig. 1.4 Short-term ultra-high SMF exposure on healthy mice. [Figures are adapted with permission from (Lv et al. 2022; Khan et al. 2022). $*p < 0.05$, $**p < 0.01$]

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.

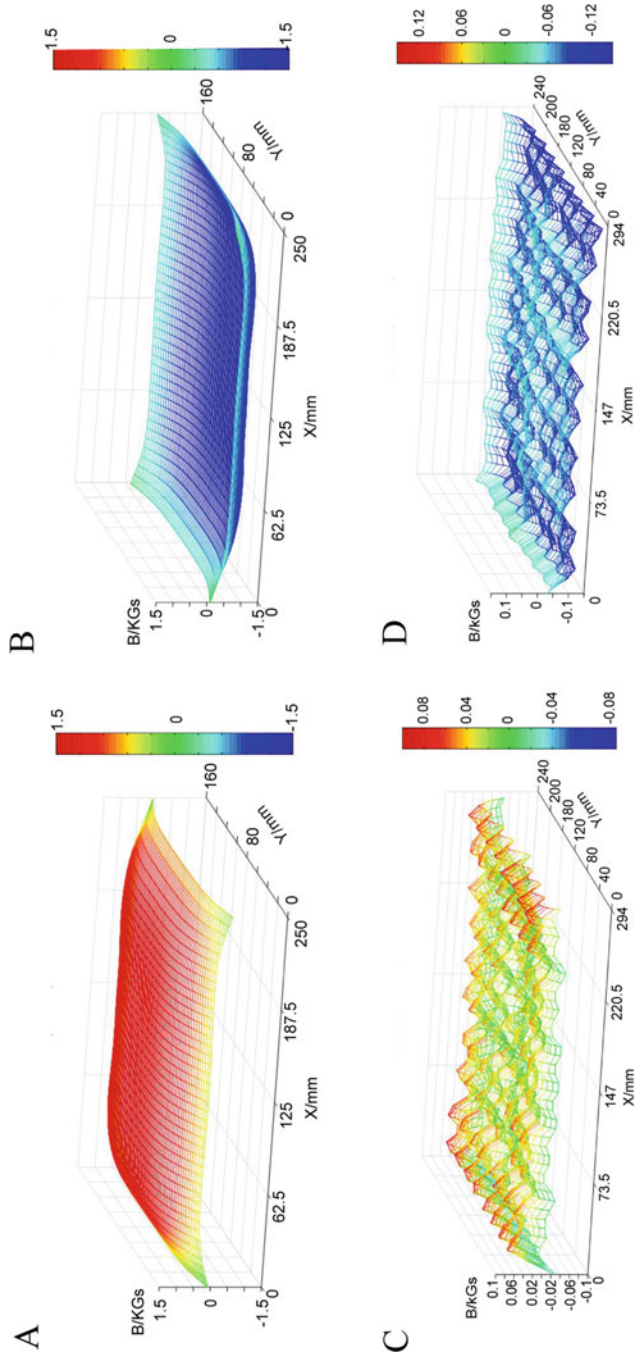

Fig. 1.5 Magnetic field distributions on the surface of 4 different permanent magnets. (**a**) Vertically upward direction with horizontal homogeneousness. (**b**) Vertically downward direction with horizontal homogeneousness. (**c**) Vertically upward direction with horizontal inhomogeneousness. (**d**)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

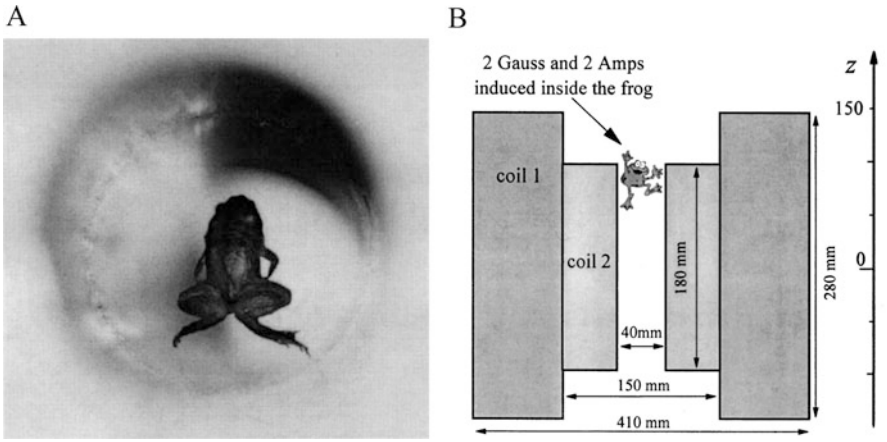

Fig. 1.6 The flying frog. (**a**) A small frog levitated in the stable zone within a 16 T magnet. (**b**) Illustration of the position of the frog within the magnet. [Reprinted with permission from (Geim and Simon 2000). Copyright © AIP Publishing LLC]

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

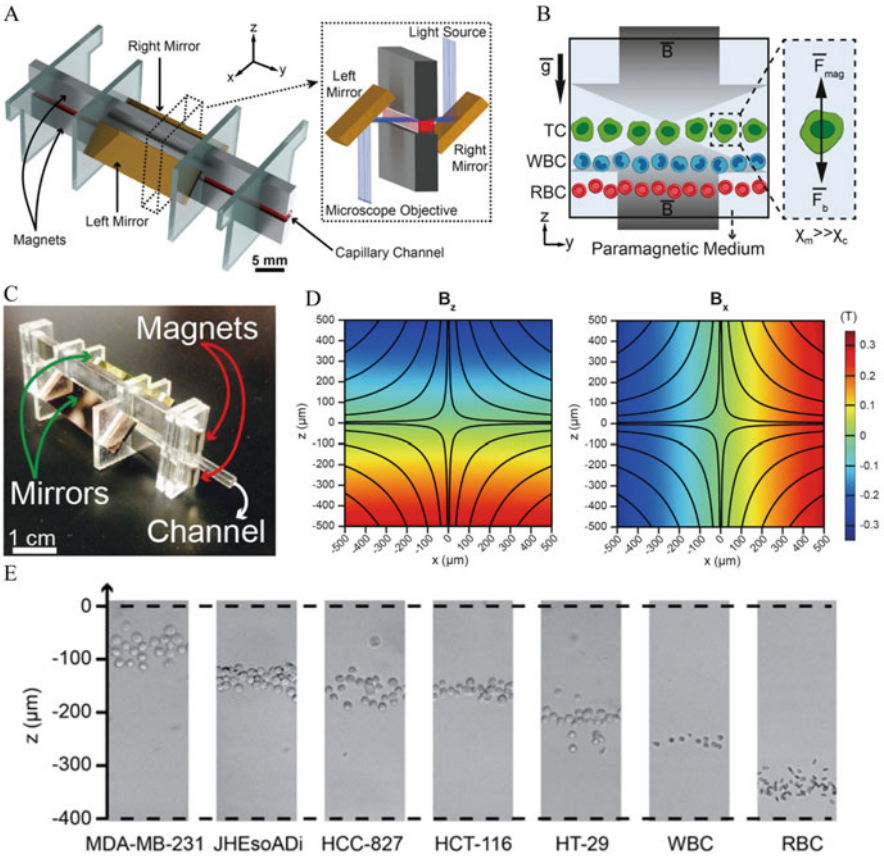

Fig. 1.7 Magnetic levitation of single cells using a densitometry platform, the MagDense cell density meter. (**a**) Illustration of the platform. (**b**) Final equilibrium height of cells in MagDense. Owing to the magnetic induction (B) and gravity (g), cells are levitated in the channel and are focused 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.

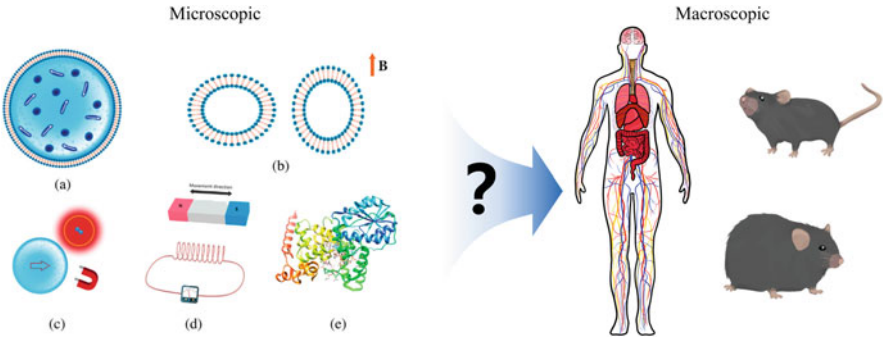

Fig. 1.8 The gap between microscopic mechanisms and macroscopic bioeffects. The left part was reprinted with permission from (Torbati et al. 2022). (The right part illustration courtesy of Shu-tong Maggie Wang and Ding Joe Wang)

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;

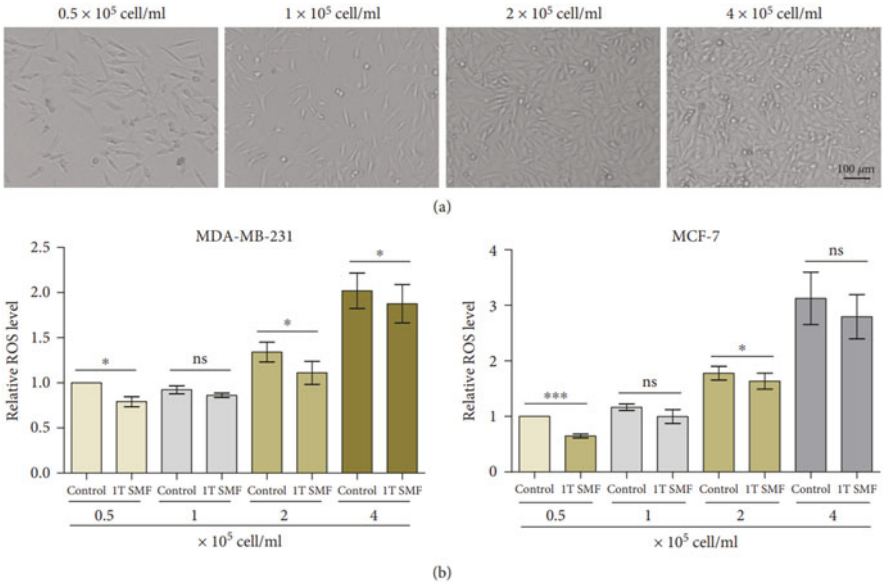

Fig. 1.9 Cells plated at different density have different ROS levels. Two types of breast cancer cell lines were plated at four different densities and subjected to an inhomogeneous SMF at 1 T (max). *ns* not significant; $*p < 0.05$; $***p < 0.005$. [Reprinted with permission from reference (Wang and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

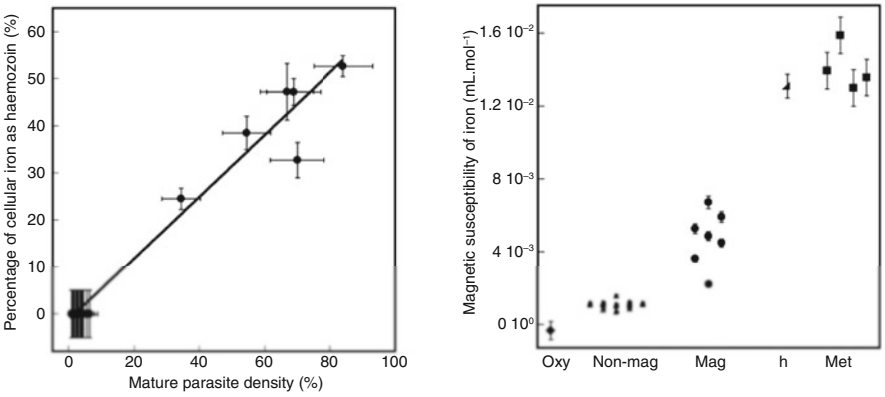

Fig. 1.10 Magnetic susceptibility of iron in malaria-infected red blood cells (RBCs). (Left) Percentage of cellular iron converted to hemozoin vs. mature parasite density. (Right) Scatter plot of the molar magnetic susceptibility of iron in standard samples of oxyhemoglobin (Oxy), hematin (h), methemoglobin (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

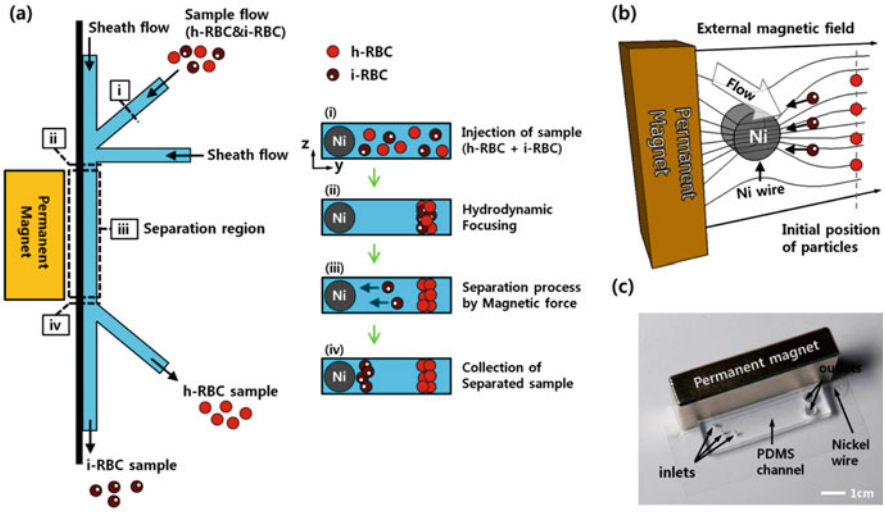

Fig. 1.11 Malaria-infected RBC separation using a high magnetic field gradient. (**a**) Schematic diagram of i-RBC (infected red blood cell) separation using the paramagnetic characteristics of hemozoin in i-RBCs. (**b**) Working principle of magnetophoretic separation with a ferromagnetic nickel wire 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

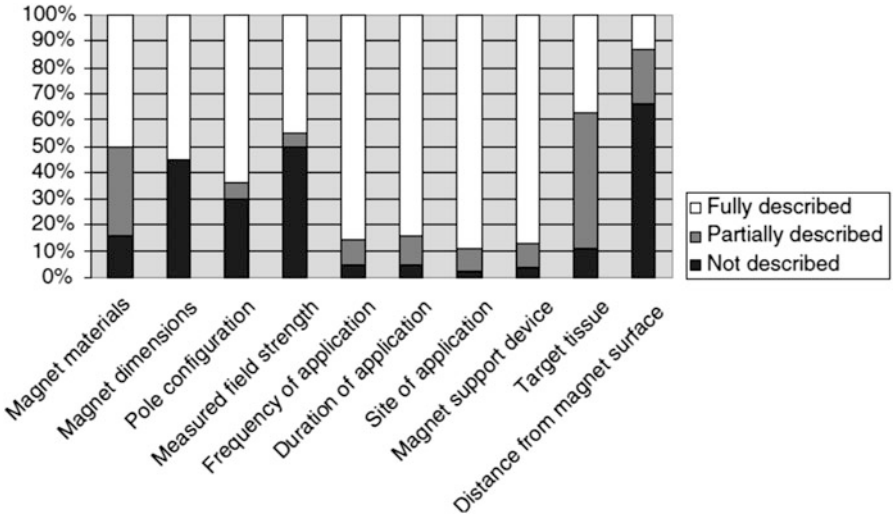

Fig. 1.12 Quality of reporting 10 SMF dosage and treatment parameters was assessed in 56 human studies. [Reprinted with permission from (Colbert et al. 2009). Copyright © 2007 The Authors (open access)]

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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Chapter 2
Static Magnetic Field Direction-Induced Differential Biological Effects

Biao Yu and Xin Zhang

Abstract Although lacking mechanistic explanations, different poles of permanent magnets could generate different effects on living organisms has been claimed decades ago, especially in the field of magnetotherapy. In recent years, several studies have confirmed that different magnetic field directions could indeed induce some differential effects in biological systems, including tumor inhibition, blood glucose level regulation, etc. However, it has been a neglected factor by most researchers in the past, which has led to many inconsistent experimental results in the literature. This chapter aims to systematically compare and summarize the biological effects induced by static magnetic fields of different directions. We also discuss about the possible mechanisms, which currently is still largely a mystery. We hope researchers in this field can pay attention to the static magnetic field directions so that they can clearly describe the field direction and/or distributions information in their studies, which will help clarify some confusions and reduce inconsistencies for future investigations.

Keywords Magnetic field (MF) · Static magnetic field (SMF) · Magnetic field direction · Magnetic pole

2.1 Introduction

On the one hand, the planet Earth itself can be seen as a big magnet with north and south poles, but how magnetoreceptive animals sense the Earth's magnetic field direction is still an open question. On the other hand, effects of magnetic field on human health have been noticed for a long time, which will be discussed in more details in Chaps. 8 and 15 of this book, but magnetic field direction-induced

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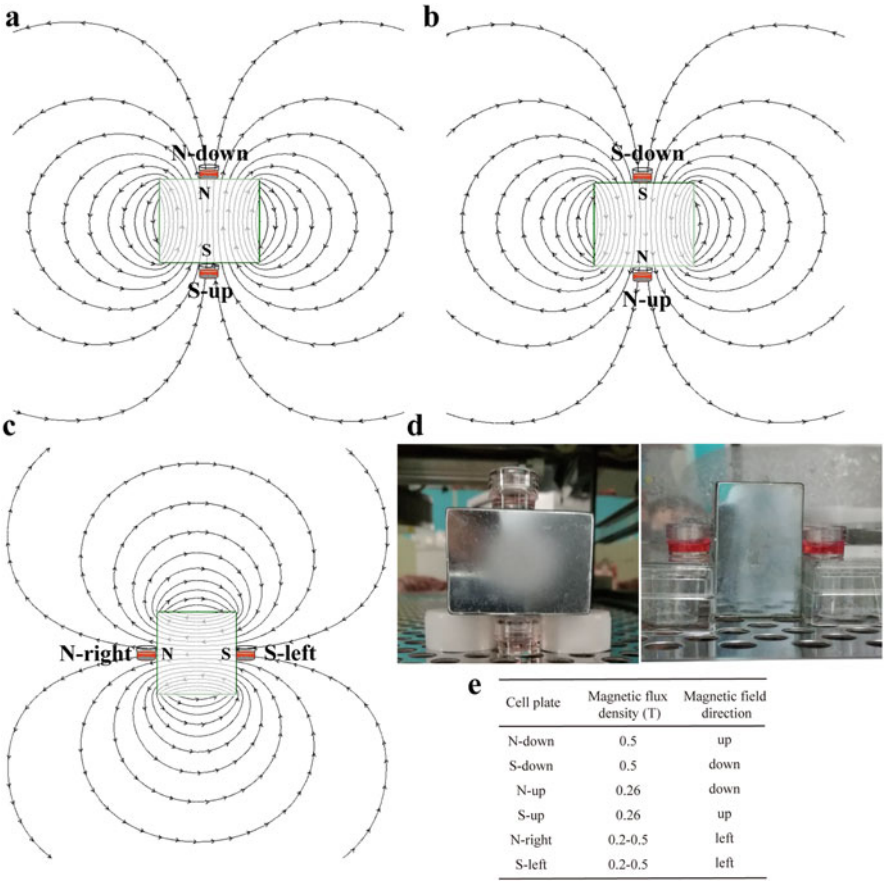
differential bioeffects have been neglected by most researchers in this field. However, the different poles of permanent magnets have been frequently brought up by people in magnetotherapy for their observed differences. However, the scientific experimental validations or theoretical explanations are both lacking until recent few years. In this chapter, we will summarize reported studies in the literature, which have demonstrated that static magnetic field (SMF) direction is indeed a very important reason that has caused many experimental inconsistencies in MF-induced bioeffects.

2.2 Magnetic Poles vs. Magnetic Field Directions

There are some reports in the magnetotherapy field stating that different poles of a permanent magnet would have differential effects on human bodies. The most famous claim was brought up by Dr. Albert Roy Davis and Walter C. Rawls Jr., who published a very interesting book "*Magnetism and its effects on the living systems*" in 1974. In this book, they claimed that the North (N) pole and South (S) pole of the magnet could have dramatically different effects on living systems. According to their book, the original finding was actually from an "earthworm incident" in 1936, in which the earthworms had eaten through the one side of the cardboard container near the S pole while the earthworms in the other container near N pole did not. The magnetic flux density was around 3000 Gauss (0.3 T) in this "earthworm incident." Further analysis revealed that the earthworms near the S pole were "one-third larger, longer in length and larger in diameter and were extremely active." In this book, they also described many interesting findings about the differential effects of N vs. S magnetic pole on biological processes, such as the ripen speed of green tomatoes, radish seed germination, small animals, as well as cancers. Overall, they think the N pole is the "negative energy pole," which arrests life growth and/or development, while the S pole is the "positive energy pole" that increases growth and development. Although their claims have not been scientifically proven, there are many other no-scientific reports supporting the Davis and Rawls's claims. However, since no illustration, picture or other data was provided in their book about these experiments, the relative locations of the earthworms or other samples they tested near the magnets are unclear. Not much experimental details are available either.

After reading this interesting but puzzling book, we have a lot of questions in mind. Does the magnetic pole really matter for living organisms? If it does, is it really because of the "magnetic pole" per se, or it is because of the magnetic field direction? Does the North or South pole magnet could generate the same effects when they were placed on the top vs. bottom, or at the side of the samples? Does MF direction affect some specific aspect of biological activities?

Apparently, to answer these questions, it is necessary to perform carefully designed and well controlled studies to test their claims. For example, in a previous study done by our group in 2018, we set up the experiments to expose cells to 0.2–0.5 T SMFs by facing different magnetic poles to the cells to provide different



e

Cell plate	Magnetic flux density (T)	Magnetic field direction
N-down	0.5	up
S-down	0.5	down
N-up	0.26	down
S-up	0.26	up
N-right	0.2-0.5	left
S-left	0.2-0.5	left

Fig. 2.1 Experimental setup to differentiate bioeffects of magnetic field direction vs. magnetic poles. (**a**–**c**) Illustrations of experimental setup. Black arrows indicate magnetic field direction. The SMF was provided by placing the cell culture plate on the center of a 6 cm × 5 cm × 3.5 cm neodymium permanent magnet (measured surface magnetic field intensity is 0.4–0.5 T), with the North (N) or South (S) pole facing up. The control group was placed with at least 30–40 cm away from the magnet with a measured magnetic field intensity background of 0.9 Gs, which was 5000-fold lower than the 0.5 T experimental groups. (**d**) Experimental setup. (**e**) Information about the magnetic field strength and field direction in each experimental condition. [Reprinted with permission from (Tian et al. 2018)]

MF directions, as well as the same MF direction, but the cells are facing different magnetic poles (Fig. 2.1). The surface magnetic flux density of the neodymium permanent magnets we used in the experiments is ~0.5 T, with the N or S pole facing different directions. The "N-down" means that the N pole is at the bottom of the sample. And the "S-up" means that the S pole is at the top of the sample. Therefore, the "N-down" and "S-up" both provided vertically upward SMF direction (Fig. 2.1a). The "N-up" and "S-down" both provided vertically downward SMF

direction (Fig. 2.1b). In addition, the "N-right" and "S-left" both provided horizontal MF directions but with different magnetic poles facing the samples (Fig. 2.1c), which also mimic most MRI machines in hospitals that provide horizontal MFs on patients. By setting up SMFs in these ways, multiple parameters could be tested side-by-side (Fig. 2.1d, e). We found that the cell numbers were reduced after 2-day treatment of upward direction SMFs, but not by downward direction SMFs in two lung cancer cell lines, A549 and PC9 cells (Tian et al. 2018). This confirms that different SMF directions indeed have distinct cellular effects. However, there was no difference for the "N-right" and "S-left," which indicates that the magnetic pole per se did not make a difference. Therefore, these results indicate that it is the magnetic field direction, but not the magnetic pole that generates differences on biological samples, at least for this type of cellular experiments.

2.3 Bioeffects Induced by Different Magnetic Poles/Field Directions

We did a thorough literature searching to answer the question about whether different magnetic poles/field directions can really induce different bioeffects. Most studies we found were done in the last 10 years, including the ones from our group. Here we summarize and analyze all published studies we can find that involved SMFs of different directions (or magnetic poles) at organism level (Table. 2.1) or cellular level (Table. 2.2). Although it is clear that magnetic field direction can often cause differential effects on some aspects of living organism, no explicit rules can be concluded at current stage yet. It should also be mentioned that we did not include SMF studies that used a single SMF direction in their study, or implanted magnets in animals in a specific way, which cannot provide side-by-side comparison between different magnetic poles/field directions. However, it is interesting that all studies we found about SMF directions are vertically upward vs. downward.

2.3.1 Bioeffects of Different Direction Static Magnetic Fields in Living Organisms

In recent few years, an increasing number of studies have been conducted to investigate whether and how SMFs directions can affect living organisms, but the results are not very consistent. We summarize and compare reported results on SMFs in upward and downward directions and classified them into: "upward ≠ downward" and "upward = downward" based on whether SMFs in upward and downward directions have similar bioeffect on the living organisms (Table. 2.1).

Among the 26 relevant studies, 14 of them revealed differences between upward and downward directions. Although the research subjects, magnetic field parameters

Table 2.1 Biological effects on living organisms caused by upward vs. downward SMFs

Species	Animals	SMF parameters	Magnetic field-induced effects				References
			Indicators	Upward	Downward	Effect	
Mouse	Nude mice bearing A549 tumor	9.4 T, 8 h/day, 11 days	A549 tumor growth	Decreased	No effect	Upward ≠ downward	Yang et al. (2021)
	Nude mice bearing GIST-T1 tumor	0.01–0.5 T, 6 h/day, 38 days	GIST-T1 tumor growth				Tian et al. (2018)
	HFD/STZ induced T2D mice	0.1 T, 24 h/day, 12 weeks	Blood glucose	Increased	Decreased		Yu et al. (2021)
	STZ induced T1D mice	0.1 T, 24 h/day, 9 weeks	Number of trabeculae of tibia	No effect	Increased		Unpublished data
			Blood glucose		Decreased		
	BALB/c mice fed with Lieber-DeCarli diets	0.1 T, 12 h/day, 6 weeks	Fatty liver, liver inflammation				Song et al. (2021)
	Swiss Webster mice	128 mT, 1 h/day, 5 days	Brain edema, spleen cellularity	Increased	No effect		Milovanovich et al. (2016)
			Liver inflammation	No effect	Decreased		
		16 mT, 28 days	Serum transferrin and iron	Increased	No effect		Djordjevich et al. (2012)
			Iron in brain and liver		Decreased		
		1 mT, 30 days	Zinc in spleen	Decreased	No effect		De Luka et al. (2016)
Rat	Huntington disease Wistar rats	0.32 T, 7 days	Behavior activity distance	No effect	Increased		Giorgetto et al. (2015)
	Spontaneously hypertensive rats	16 mT, 30 days	Anxious-like behavior		Decreased		Tasic et al. (2021)
			Lymphocytes count in blood, bone marrow erythrocytes count	Decreased	No effect		

(continued)

Table 2.1 (continued)

Species	Animals	SMF parameters	Magnetic field-induced effects				References
			Indicators	Upward	Downward	Effect	
			Bone marrow erythrocytes count	No effect	Increased		
			Systolic blood pressure variability in high frequency domains				Tasic et al. (2017)
			Heart rate, systolic blood pressure variability in low frequency domains		Decreased		
Insect	*Tenebrio*	50 mT, 24 h	Distance traveled, average speed	Increased			Todorovic et al. (2013)
			Locomotor activity	Decreased	Increased		
Plant	*Arabidopsis* young seedlings	600 mT, 4 days	Root growth	Increased	No effect		Jin et al. (2019)
Microorganisms	*Bacteroidetes, Firmicutes*	0.1 T, 24 h/day, 12 weeks	*Bacteroidetes* abundances	Decreased	Increased		Yu et al. (2021)
			Firmicutes abundances	Increased	Decreased		
Mouse	Swiss Webster mouse	128 mT, 5 days, 1 h/day	White blood cells, lymphocytes, spleen granulocytes	Decreased		Upward = downward	Milovanovich et al. (2016)
		16 mT, 28 days	Spleen granulocytes				Djordjevich et al. (2012)
		1 mT, 30 days	Copper and zinc in liver; copper in brain				De Luka et al. (2016)
	Mongolian gerbils	0.32 T, 4 days	Time spent in Rotarod test				Bertolino et al. (2013a)
			Density of the neurons in the CA1; in the number of M1 and striatal neurons	Increased			
			Wound healing	Increased			

	Diabetic db/db mouse	0.5 T, 24 h/day, 10 weeks				Feng et al. (2022)
	Thrombosis mouse	1.4 mT −46 mT, 7 days	Antithrombotic			San et al. (2001)
Rat	Wistar rats	0.16 T, 24 h/day, 5–15 days	Tissue repair			Bertolino et al. (2006)
	Spontaneously hypertensive rats	16 mT, 30 days	Platelets in peripheral blood, granulocytes in the spleen and bone marrow	Decreased		Tasic et al. (2021)
			Erythrocytes in the spleen	Increased		
			Heart and kidney morphological characteristics	No effect		
			Arterial blood pressure, systolic blood pressure variability in very low frequency domain	Decreased		Tasic et al. (2017)
			Baroreceptor reflex sensitivity	Increased		
	Parkinson's disease Wistar rats	0.32 T, 14 days	Time spent in Rotarod test; number of neurons	Increased		Bertolino et al. (2013b)
			Glial cells number	Decreased		
Microorganisms	*C. albicans* CAF2–1 cells	0.5 T, 24 h	*C. albicans* hyphal length			Sztafrowski et al. (2019)
Insect	*Paramecium caudatum*	200–220 mT, 96 h	Population growth	Decreased		Elahee and Poinapen (2006)

Table 2.2 Biological effects at cellular level caused by upward vs. downward SMFs

Species	Cell lines	SMF treatment parameters	Magnetic treatment-induced effects				References
			Indicators	Upward	Downward	Effect	
Rat	Rat skeletal muscle myoblasts (L6)	80 ± 5 mT, 3 days	Myogenic cell differentiation and hypertrophy	Increased	No effect	Upward ≠ downward	Coletti et al. (2007)
			Number of cells fused into multinucleated myotubes				
Human	Lung cancer cell line (A549)	9.4 T, 24 h	ROS and p53 level				Yang et al. (2021)
	Lung cancer cells (A549 and PC9), colon cancer (HCT116) and colorectal cancer (LoVo) cells	1 T, 8 h, 48 h	DNA synthesis, cell number	Decreased			Yang et al. (2020)
	Gastrointestinal stromal cancer (GIST-T1), lung cancer (PC9 and A549), colon cancer (HCT116) and breast cancer (MCF7) cells	0.2–1 T, 24 h	Cell number				Tian et al. (2018)
	Human hepatocyte cells HL7702	0.4 T, 24 h	ROS level	No effect	Decreased		Song et al. (2021)
Mouse	Mouse embryonic fibroblasts cells (NIH3T3)		Transwell cell migration and cell proliferation capacity		Increased		Feng et al. (2022)
			Cellular ROS level	Decreased	No effect		
	Mouse insulinoma cells (Min6)	0.14–0.5 T, 24 h		No effect	Decreased		

			Labile iron level and NRF2 expression				Yu et al. (2021)
			ROS level	Increased			
			Relative cell number	Decreased	Increased		
Human	Lung cancer cells (A549)	9.4 T, 24 h	DNA synthesis	Decreased		Upward = downward	Yang et al. (2021)
	Chronic myelogenous leukemia (HL60), promyelocytic leukemia (K562) cells	0.2–1 T, 24 h	Cell number				Tian et al. (2018)
	Human small airway epithelial (HSAEC2-KT and HSAEC30-KT), tracheal epithelial (HBEC30-KT), retinal pigment epithelial (RPE1) cells	0.2–0.5 T and/or 1 T, 24 h	Cell number	No effect			
	Human corneal epithelial cells	20 and 250 Gs; 25 and 1500 Gs, 10 h, 12 h, 24 h, 6–7 days	Vortex or whorl patterns	Increased			Dua et al. (1996)
Mouse	Chinese hamster ovary (CHO)	0.50 T, 0.26 T, 6 h	ATP level	No effect			Wang et al. (2018)
	Mouse embryonic fibroblasts (NIH3T3), mouse fibroblast (L929) cells	0.5 T, 24 h	NRF2 nuclear import	Decreased			Feng et al. (2022)

and the investigated experimental indicators are very diverse, which led to a large variation in these reports. However, it is interesting that there are multiple studies indicating that the upward direction SMFs might have more beneficial effects than the downward direction SMFs. For example, the upward direction SMF (0.01–0.5 T, 6 h/day, for 38 days) exposure inhibited GIST-T1 tumor growth in nude mice by 19.3% while the downward SMF did not produce significant effect (Tian et al. 2018). Moreover, Yang et al. found that the upward 9.4 T SMF for 88 h significantly inhibited A549 tumor growth (tumor growth inhibition = 41%), but not in the downward 9.4 T SMF (Yang et al. 2021). Furthermore, the upward SMF treatment significantly increased the distance traveled and average speed of *Tenebrio* (insects) (Todorovic et al. 2013) and increased plant root growth in *Arabidopsis* young seedlings (Jin et al. 2019).

In contrast, there are also some studies indicating that the downward direction SMFs improved the living organism status more significantly than in the upward direction (Table. 2.1). For example, three separate studies used a downward SMF of ~0.1 T to efficiently decrease blood glucose levels in T2D (Yu et al. 2021) and in T1D mice (unpublished data), as well as effectively alleviate alcohol-induced liver damage and lipid accumulation, and improve liver function (Song et al. 2021). Meanwhile, our group also found that a ~0.1 T downward SMF improved the multiple diabetic complications, but not in 0.1 T upward SMF (Yu et al. 2021) (unpublished data). More specifically, two studies in spontaneously hypertensive rats found that the anxious-like behavior (Tasic et al. 2021) and heart rate (Tasic et al. 2017) could be improved by downward direction of 16 mT SMF, but not upward SMF. There are also a few other studies that investigated the influences of different SMF directions on plants (Jin et al. 2019) and gut microbiota (Yu et al. 2021).

In the meantime, there are also 12 studies that show no significant difference between upward and downward direction SMFs. For example, 16 mT (Djordjevich et al. 2012) and 128 mT (Milovanovich et al. 2016) SMF exposures altered the hematological parameters and biological changes of Swiss Webster mice, but no difference was induced by different SMF directions. San et al. found that the thrombosis was significantly ameliorated in both upward and downward groups of BALB/c mice exposed to 1.4–46 mT SMFs (San et al. 2001).

The discrepancy about the different effects of SMFs direction on living organisms could be resulted from multiple aspects, including research subject, SMF devices that generated different magnetic flux density and distributions (Fig. 2.2), as well as experimental procedures, including exposure time and assay time-points. Further systematic studies are needed to get more in-depth information.

2.3.2 Bioeffects of Different SMF Directions at Cellular Level

Similar to living organism, the cellular studies of SMFs of different field directions also produced seemingly inconsistent results, which is reasonable to some extent because the cellular experiments have more variable parameters than that in vivo.

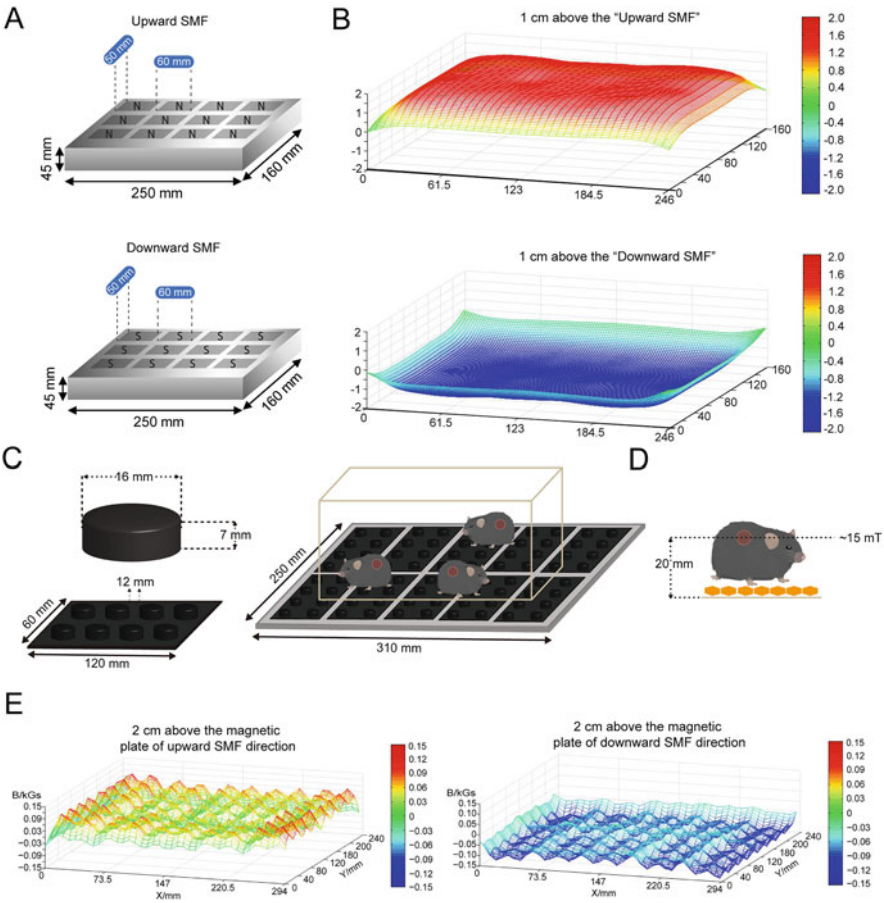


Fig. 2.2 Examples of static magnetic fields of different directions and distributions used on living organisms. (**a**) A diagram of the two magnetic plates that provide upward and downward SMFs and (**b**) the MF distribution 1 cm above the magnetic plates, where the mice bodies locate. Reprinted with permission from (Yu et al. 2021). (**c**) The device consists of ten plates. Each plate contains 8 cylindrical permanent magnets. Moreover, the magnets were placed next to each other with the same orientation. The whole cage was placed on the magnetic plate. (**d**) The magnetic field strength is ~15 mT at 2 cm above the magnetic plate, where the mice wound located. (**e**) Devices with different directions of magnetic plates and magnetic field intensities at the positions of the mice in each exposure condition are provided and measured. [Reprinted from (Feng et al. 2022). Open access]

Again, we summarize and compare reported results and categorize them into "upward ≠ downward" or "upward = downward" (Table. 2.2).

Among the 12 relevant studies, 7 of them showed differential effects while 5 of them did not. Among the 7 studies that showed differential effects, 4 of them showed that the upward direction SMF has more significant effects (Coletti et al. 2007; Tian

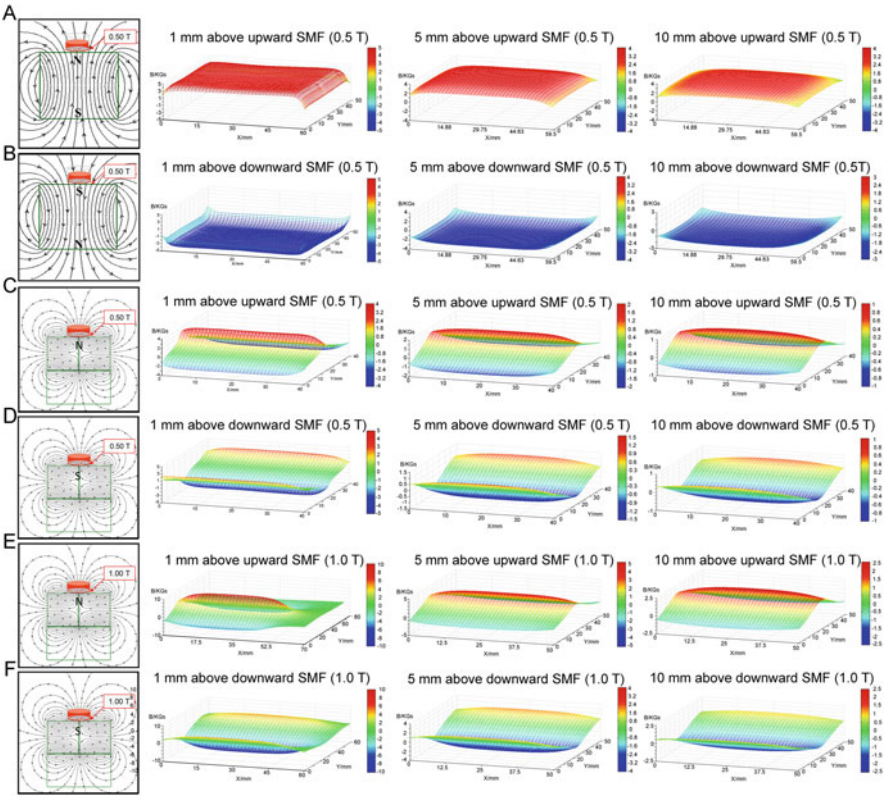


Fig. 2.3 Some commonly used permanent magnet-based magnetic devices in cellular studies. (**a**–**f**) Cell culture plates were placed at different direction magnet device. Black arrows indicate magnetic field direction. The SMF was provided by neodymium permanent magnet with upward and downward direction of 0.5 T magnet (**a**, **b**), 0.5 T magnet assembly (**c**, **d**), and 1.0 T magnet assembly (**e**, **f**) magnetic field intensity, with N or S pole upward (Tian et al. 2018; Wang et al. 2018). Magnetic field distribution in the cell exposure area, 1 mm, 5 mm, and 10 mm above the magnetic plates. Left part of the figures was adapted from the above-mentioned refs with permission. The magnetic flux distribution scans on the right were performed by a magnet analyzer (LakeShore 475 DSP Gaussmeter)

et al. 2018; Yang et al. 2020, 2021) and 3 of them reported that the downward direction has more significant effects (Song et al. 2021; Yu et al. 2021; Feng et al. 2022). As we have introduced in Chap. 1 of this book, cell type, cell density, and MF treatment time could all contribute to the differences in cellular experiments. Moreover, as we mentioned in Fig. 2.2, the SMF distributions on different SMF devices can be very different. For example, for SMFs provided by square shaped permanent magnet of the same size, the SMF direction and distributions can be very different (Fig. 2.3).

Therefore, as mentioned in Chap. 1 of this book, multiple details should be considered when performing these SMF experiments, including the sample distance from the magnet surface, SMF flux density distribution, material composition and dimension of the magnet, magnet polar configuration, and duration of magnet application, etc. For example, as shown in Fig. 2.3, the magnetic flux densities at 1 mm from the surface of the three type magnets were 4700–4980 Gs, 4190–4890 Gs, and 9630–10,330 Gs, respectively; at 5 mm from the surface of the magnets, the magnetic flux densities dropped to 3520–3890 Gs, 2180–2450 Gs, and 4370–5120 Gs, respectively; at 10 mm from the surface of the magnets, the magnetic flux densities further decreased to 2420–3090 Gs, 1050–1120 Gs, and 2120–2450 Gs. The differences in magnetic flux density and spatial SMF distribution arrangement resulted in significant differences in the values of magnetic flux density. Therefore, people in this field should start to accurately measure their SMFs by using a magnet analyzer, which can provide accurate 3D information about the magnetic flux density and distributions (Fig. 2.3).

2.4 Possible Mechanisms

As introduced in Chap. 1, various factors could result in these differential effects of SMF in vivo and in vitro, including SMF flux density, gradient, exposure time, cell type, etc. From above-mentioned animal and cellular studies about SMFs of different directions in this chapter, it is obvious that the field direction is also a key factor for some specific bioeffects. Although the mechanisms behind the observed results are still not completely understood, there are some studies have tried to address them and provided some important clues.

First of all, magnetic field can control the state of electrons, manipulate the unpaired electrons in free radicals, which provides a theoretical basis for cellular reactive oxygen species (ROS) regulation by SMF (Timmel et al. 1999; Ikeya and Woodward 2021). However, the exact effects of SMFs on cellular ROS levels are highly variable in different studies (Wang and Zhang 2017), which will be systematically summarize in Chap. 6. Although some studies proposed that changed cellular ROS formation can be affected by SMF directions (Sullivan et al. 2011; Djordjevich et al. 2012; De Luka et al. 2016; Milovanovich et al. 2016; Naarala et al. 2017; Tasic et al. 2017, 2021; Liu et al. 2019; Song et al. 2021; Yang et al. 2021; Yu et al. 2021), there is no physical explanation for this yet. This is probably due to the complexed ROS generating and clearing system in living organisms and cells.

Secondly, there is a difference between adherent cells vs. suspended cells in SMF direction-induced effects, probably due to shape anisotropy. Our previous study indicated that the upward or downward SMFs had a significant effect on the cell number in multiple types of adherent cells, while there seems to be no difference for suspended cells in the liquid cell culture medium (Tian et al. 2018). This is probably due to the fact that adherent cells are fixed in position so that they will have a shape anisotropy and directional preference. In contrast, suspended cells are round in shape

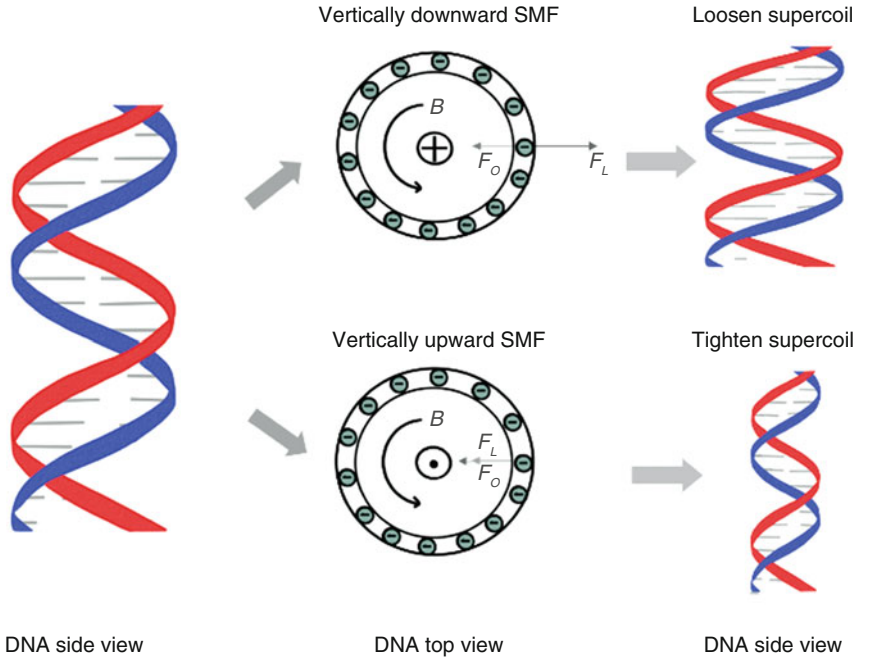


Fig. 2.4 Cranked DNA motion and the magnetic Lorentz forces. (Left and right) side view of DNA, (middle) top view of DNA cross section. For downward and upward SMFs, the Lorentz force (F_L) of negatively charged DNA has different directions. F_0 is an endogenous centripetal force determining DNA rotation. Arrows indicate rotation direction. [Illustration courtesy of Ding Joe Wang, based on reference (Yang et al. 2020). Open access]

and can rotate freely in the liquid medium, which make them independent of directions.

Thirdly, DNA synthesis has been shown to be differentially regulated by SMFs of different directions. Since DNA is negatively charged and undergoes fast rotation to get winding and unwinding during DNA replication in living cells, the externally applied SMF will affect the DNA movement through Lorentz force. We combined theoretical calculation and cellular experiments to show that the upward and downward SMFs have differential effects on the DNA rotation and supercoil in cells (Fig. 2.4) (Yang et al. 2020), which resulted in differential effects on DNA synthesis. Specifically, the upward moderate to high SMFs can inhibit DNA synthesis by Lorentz forces exerted on the negatively charged moving DNA (Yang et al. 2020, 2021). This theory is actually consistent with other results that have shown the cell proliferation inhibition effects of upward SMFs (Zhang et al. 2017; Tian et al. 2018; Wang and Zhang 2019; Yang et al. 2021).

Fourthly, there may be some link between these SMF direction-induced bioeffects with the earth magnetic field. It is known that the earth magnetic field is a quasi-SMF that is static for most of the time, but can be affected by solar storm.

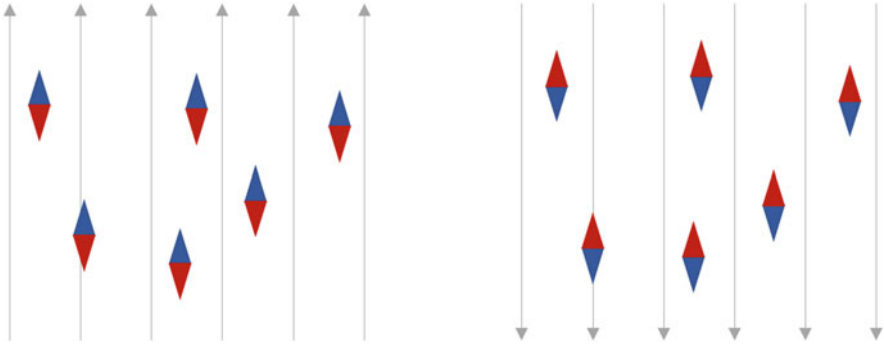


Fig. 2.5 A hypothesis for magnetic field direction-induced bioeffects. The small compass-like needle represents ferromagnetic or paramagnetic components in living organisms that could mediate magnetic field direction-induced bioeffects

The directions of earth magnetic field at the southern hemisphere and northern hemisphere are opposite. However, the earth magnetic field is much weaker than the SMFs used in currently reported studies. Whether and how these factors are linked are completely unknown.

Additionally, although the physical mechanisms about the differential bioeffects caused by SMFs of different directions are still lacking, there are some biological experimental evidences that revealed some potential aspects for people to invest in the future. For example, our study shows that the iron metabolism was differentially affected by the upward vs. downward SMFs in diabetic mice. We found that the ~100 mT SMF of a downward direction alone could improve pancreas function by regulating iron metabolism and ROS production. Meanwhile, the downward SMF, but not the upward SMF, markedly restored the *Bacteroidetes* population and reversed the iron complex outer membrane receptor gene reduction in the mice gut microbiota, and reduced iron deposition in the pancreas (Yu et al. 2021).

Lastly, although there is still no evidence yet, we hypothesis that there might be some ferromagnetic or paramagnetic components in cells that behave like tiny magnets, which will change direction when external SMF applies (Fig. 2.5). The orientation of these components will trigger differential downstream signal transduction pathways in cells and/or various cellular processes. However, since the biological system is very complicated, we currently have only very limited information about the magnetism of materials inside our bodies, which will be reviewed in the next chapter of this book. With the help of recently developed techniques, we will be able to get a better understanding of the magnetism of biomolecules, cells, and tissue in the near future, which is actually one of the main research focuses of our group.

2.5 Summary and Future Perspectives

From current experimental evidences collected, we can conclude that it is the magnetic field direction, but not the magnetic pole, that have induced differential effects. In fact, it is clear that multiple studies have demonstrated that field direction could influence the biological responses at both cellular and living organism level. It is known that research in the literature about magnetic field bioeffects is filled with experimental discrepancies. Here we show that this is not only due to the differences in biological sample types, magnetic field types, flux density, and gradient, but also field direction. We encourage people in this field accurately measure their SMFs using equipment such as a magnet analyzer, which can provide detailed and accurate 3D information about their magnetic device. Although the exact mechanisms about most of the SMF direction-induced bioeffect differences are still unclear, these results alert people that they should pay attention to the magnetic field direction used in their biological studies. Moreover, it is worth to mention that currently some researches related to magnetic therapy as well as the biological effect studies about MFs are not well described or properly controlled. There are multiple other factors that have led to the large variations in the clinical or research work about the SMFs. Meanwhile, these researches need replication and we hope we can make great advancement after we have the proper knowledge of the magnetic field and biological systems, which will improve the current status of magnetic therapy. It indicates a potential to use different magnetic field direction in the future development of SMFs as a new physical therapy modality.

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Chapter 3
Magnetic Properties of Biological Samples

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Abstract Magnetic properties of materials determine their response to the externally applied magnetic field. Although most living organisms, including human bodies, are diamagnetic as a whole, they have a very complexed composition. The purpose of this chapter is to summarize the known facts about the magnetic properties of biological samples, including the magnetic susceptibility, magnetic anisotropy of biomolecules (nucleic acid, proteins and lipids, etc.), organisms, tissues, and cells. Although there are still not enough data in this aspect, especially live biological samples in physiological conditions, current evidences already show that biological samples at different states show different magnetism. For example, the oxygenated red blood cells are diamagnetic while the deoxygenated red blood cells are paramagnetic, which are mainly due to their hemoglobin at different states and have been used in magnetic resonance imaging to diagnose different types of bleeding. The chain-like ferromagnetic magnetosome in magnetotactic bacteria is also the tool for their orientation in earth magnetic field. Therefore, systematic examination of magnetic properties of biological samples is not only essential to avoid ambiguities, complexities, and limitations to the interpretations of magnetic field-induced bioeffects, but also critical for the magnetic field-based technical development.

Keywords Magnetic field (MF) · Static magnetic field (SMF) · Magnetic property · Magnetic susceptibility · Magnetic moment · Anisotropy of magnetic susceptibility (AMS)

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3.1 Introduction

A large number of magnetic field-induced bioeffects have been reported, but the interpretations are often difficult, which require a better understanding of the underlying physics, chemistry, and biology mechanisms. Since all materials will respond to an externally applied magnetic field, either negligibly or strongly, depending on their magnetic properties, it is essential to nail down the exact magnetic properties of various biological samples. The goal of this chapter is to summarize the known facts of biological sample magnetic properties, which will provide a basis for the understanding of field-induced bioeffects as well as magnetic field and magnetism-related techniques.

There are a few physical quantities that are frequently used when describing the magnetic properties of biological samples, including magnetic moment, magnetization, magnetic susceptibility, and anisotropy (Table 3.1).

Just like other materials, biomaterials can be generally categorized into diamagnetic, paramagnetic, or ferromagnetic materials, according to their magnetic properties. Table 3.2 lists some representative diamagnetic, paramagnetic, and ferromagnetic substances in biological systems, which is the foundation to

Table 3.1 Physical quantities that are frequently used when describing the magnetic properties of biological samples

Physical quantities	Definition
Magnetic moment	A physical quantity representing the magnetic strength
Magnetization (M)	The macro-magnetic strength, which is the average magnetic moment per unit volume of substances
Magnetic susceptibility (χ)	χ is the degree to which a material can be magnetized in an external magnetic field, defined by the ratio of magnetization M to magnetic field strength H per unit volume of substances. For linear and isotropic substances, $M = \chi H$
Magnetic anisotropy, or anisotropy of magnetic susceptibility (AMS), or magnetic anisotropy	The difference in the magnetic susceptibility of a sample in multiple directions
Magnetic dipole moment	An elementary magnetic structure, such as a compass magnet, with north and south magnetic poles that experiences a torque in a uniform magnetic field
Magnetic remanence	The net magnetic dipole moment of a magnetic structure after the removal of an external magnetic field

Table 3.2 Representative diamagnetic, paramagnetic, or ferromagnetic substances in biological systems

Magnetism	Biological samples and related substances
Diamagnetic	Water, carboxyhemoglobin, oxyhemoglobin, normal tissues and most cells
Paramagnetic	O_2, ferrohemoglobin, deoxyhemoglobin, Fe-transferrin, some types of reactive oxygen species (ROS) including superoxide and hydroxyl radical
Ferromagnetic	Magnetosome, iron oxides including magnetite

understand many different phenomena such as field-induced bioeffects and field-related applications.

Diamagnetic materials have negative χ, and their internal magnetic field is opposite to the externally applied magnetic field. In fact, the weak basic characteristic of diamagnetic substances is a property of all materials, which is caused by the non-cooperative behavior of orbital electrons when exposed to the external magnetic field. There are paired electron spins in all diamagnetic materials. The paired spin magnetic moments offset each other without contributing to the overall magnetic moment, and the magnetic properties are only determined by the electric orbital motion. However, when exposed to the external magnetic field, their angular momentum and magnetic moments will change, and the magnetic moments can no longer completely offset each other, which will produce a net magnetization opposite to, or resisting, the external magnetic field. Therefore, the magnetic susceptibility is negative. Currently, it is well known that most components in living organisms are diamagnetic, including water, lipid, and most proteins.

Paramagnetic materials have positive χ, which is weakly magnetized along the direction of the externally applied magnetic field. Paramagnetic materials include some metals with free electrons, such as aluminum and platinum, as well as molecules with unpaired electrons. Although they can be attracted by magnets, but the attraction is very weak. Moreover, it should be mentioned that metal ions are not ferromagnetic in their physiological state. For instance, iron in our human bodies is mostly combined with hemoglobin in the blood, and some exists in ferritin in organs including liver, spleen, and brain. There is also a small amount of iron in the myoglobin of skeletal muscle.

Ferromagnetic materials usually have large χ. When placed in an external magnetic field, the ferromagnetic materials will be magnetized, which remain even after the external magnetic field has been removed. Currently, whether there are other ferromagnetic materials in living organisms except for magnetosome in magnetotactic bacteria and iron oxides in some cells are still unclear.

Water is the main component of most living organisms including human bodies. At 37 °C, the magnetic susceptibility of water is about -9.05×10^{-6} (SI) (Schenck 1996), which makes the living organisms weakly magnetic as a whole. Although we have ~40–50 mg/kg of iron in our bodies, it is mostly evenly spread so that the whole human body is still weakly diamagnetic. Nonetheless, since the metal ions, as well as other paramagnetic and ferromagnetic substances can concentrated in some organs and cells, which might result in different magnetism other than diamagnetism. It should be noted that although different biological tissues have different magnetic susceptibilities, the magnetic susceptibility of most tissues is about 20% higher or lower than that of water (Schenck 1996). Therefore, although the human body contains both iron and some other paramagnetic metal ions, it is overall diamagnetic.

Besides magnetic susceptibilities, since the intrinsic magnetic properties of biological samples, from biomolecules, cells, to tissues and organs, are determined not only by their compositions, such as paramagnetic substance content, but also by the substance distribution and the overall structures. Most biological samples are complex, non-uniform, and unsymmetric, so that most of them have anisotropy of

Table 3.3 Unit conversion of some commonly used magnetism physical quantity in magnetobiology

Quantity	Symbol	SI units	CGS units	Conversion
Magnetic field intensity	H	A/m	Oe	1 A/m = $4\pi/10^3$ Oe
Magnetic induction, magnetic flux density	B	T	Gs	1 T = 10^4 Gs
Magnetic moment	m	Am^2	emu	1 Am^2 = 10^3 emu
Magnetization (volume)	M_v	A/m	emu/cm^3	1 A/m = 10^{-3} emu/cm^3
Magnetization (mass)	M_m	Am^2/kg	emu/g	1 Am^2/kg = 1 emu/g
Magnetic susceptibility (volume)	χ_v	1	emu/cm^3 or $emu/(cm^3$ Oe)	1 = $1/4\pi$ emu/cm^3 or 1 = $1/4\pi$ $emu/(cm^3$ Oe)
Magnetic susceptibility (mass)	χ_m	m^3/kg	emu/g or emu/(g Oe)	1 m^3/kg = $10^3/4\pi$ emu/g or 1 m^3/kg = $10^3/4\pi$ emu/(g Oe)

magnetic susceptibility (AMS, or magnetic anisotropy), which is defined as the difference in the magnetic susceptibility of a sample in different directions. The magnetic anisotropy of biological samples is often assessed by measuring the sample's volume magnetic susceptibility in axial and radial directions, especially for rod-shaped samples. A difference in magnetic susceptibility values in the two directions is considered to indicate that the sample has magnetic anisotropy, and this difference is the magnetic anisotropy (Hong et al. 1971).

It should be mentioned that some researchers have used international system of units (SI) while others use Gaussian system of units/CGS units, which have caused a lot of inconvenience for people to compare between different studies. Here we list the commonly used physical quantities in this field to help clarify the confusions (Table 3.3). In this chapter, we try to unify the magnetic susceptibility data in the literature into SI units and include them in the tables (Tables 3.4, 3.5, and 3.6). However, due to the incomplete information in some reported studies, there are still some data that we cannot convert the unit.

3.2 Magnetic Properties of Biomolecules

First, we will introduce the reported results about the magnetic properties of biomolecules, including nucleic acid, proteins, lipids, as well as components in blood.

3.2.1 Nucleic Acid

Since Watson and Crick described the double helical structure of DNA, it has been at the center of bioscience and biotechnology. Besides its fundamental role in life science, the materials science of DNA has become an emerging interdisciplinary

Table 3.4 Reported magnetic susceptibility of proteins

Sample	Magnetic susceptibility χ		References
	Data in the literature	Converted to SI ($\times 10^{-9}$ m^3/kg)	
Globin	-0.53×10^{-6} cgs	−6.66	Havemann et al. (1962)
Oxy- and carbon-oxide hemoglobins	-0.54×10^{-6} cgs	−6.78	Havemann et al. (1962)
Oxy- and carbonmonoxyhemoglobins	$-(0.580 \pm 0.010) \times 10^{-6}$ cgs	-7.28 ± 0.001	Savicki et al. (1984)
Ferrohemoglobin	1191×10^{-6} cgs	0.93	Taylor and Coryell (1938)
Ferromyoglobin	$12{,}400 \times 10^{-6}$	9.16	Taylor (1939)
Ferrimyoglobin	$14{,}200 \times 10^{-6}$	10.49	Taylor (1939)
Ferrihemoglobin	2520×10^{-6}	1.97	Coryell et al. (1937)
Ferricytochrome c	2120×10^{-6} cgs emu	2.05	Boeri et al. (1953)
Cytochrome a (oxidized)	2400×10^{-6} cgs emu	0.30	Ehrenberg and Yonetani (1961)
Cytochrome a3 (oxidized)	7900×10^{-6} cgs emu	0.99	Ehrenberg and Yonetani (1961)
Fe-transferrin	$(15{,}700 \pm 500) \times 10^{-6}$ cgs	2.56 ± 0.08	Ehrenberg and Laurell (1955)
Chromatium iron protein, high potential (oxidized)	$(900 \pm 100) \times 10^{-6}$ cgs	0.023 ± 0.003	Ehrenberg and Kamen (1965)
Chromatium iron protein, high potential (reduced)	$(150 \pm 200) \times 10^{-6}$ cgs	0.004 ± 0.006	Ehrenberg and Kamen (1965)
Chromatium cytochrome c (oxidized)	$(3100 \pm 600) \times 10^{-6}$ cgs	2.99 ± 0.58	Ehrenberg and Kamen (1965)
Chromatium cytochrome c (reduced)	$(500 \pm 300) \times 10^{-6}$ cgs	0.48 ± 0.29	Ehrenberg and Kamen (1965)
Laccase A ($\chi_{Cu,ox}-\chi_{Cu,red}$)	$(570 \pm 60) \times 10^{-6}$ cgs emu	0.060 ± 0.006	Ehrenberg et al. (1962)
Ceruloplasmin I ($\chi_{Cu,ox}-\chi_{Cu,red}$)	$(439 \pm 80) \times 10^{-6}$ cgs emu	0.046 ± 0.002	Ehrenberg et al. (1962)

Table 3.5 Reported Magnetic susceptibility of porphyrins

Sample	Magnetic susceptibility χ		References
	Data in the literature	Converted to SI ($\times 10^{-6}$)	
Hemin	-1.2×10^{-6}cgs	−15.07	Sullivan et al. (1970)
Protoporphyrin IX dimethyl ester	-585×10^{-6}	−0.73	Eaton and Eaton (1980)
Mesoporphyrin IX dimethyl ester	-595×10^{-6}	−0.75	
Tetraphenylporphyrin (H2TPP)	-385×10^{-6}	−0.48	
Tetrakis (pivaloylphenyl) porphyrin	-690×10^{-6}	−0.87	

Table 3.6 Reported magnetic susceptibilities of tumor vs. non-tumor tissues

Sample	Magnetic susceptibility χ		References
	Data in the literature	Converted to SI ($\times 10^{-9}$ m^3/kg)	
Transplanted hepatoma (Morris No. 3683)	$-(0.688 \pm 0.0046) \times 10^{-6}$ emu/gm	-8.64 ± 0.058	Senftle and Thorpe (1961)
Liver tissue from tumor-bearing rat	$-(0.670 \pm 0.0012) \times 10^{-6}$ emu/gm	-8.42 ± 0.015	
Liver from normal control animals	$-(0.637 \pm 0.0059) \times 10^{-6}$ emu/gm	-8.00 ± 0.074	
S91 melanoma from mouse	0.151×10^{-6} cgs (77 K) -0.042×10^{-6} cgs (194 K) -0.147×10^{-6} cgs (294 K)	1.90 (77 K) −0.53 (194 K) −1.85 (294 K)	Mulay and Mulay (1967)
S91A melanoma from mouse	-0.0078×10^{-6} cgs (77 K) -0.100×10^{-6} cgs (194 K) -0.193×10^{-6} cgs (294 K)	−0.10 (77 K) −1.26 (194 K) −2.42 (294 K)	
Mouse leg muscle	-0.186×10^{-6} cgs (77 K) -0.186×110^{-6} cgs (194 K) -0.221×10^{-6} cgs (294 K)	−2.34 (77 K) −2.34 (194 K) −2.78 (294 K)	

research area in recent years (Kwon et al. 2009). However, the question as to whether DNA's magnetism is intrinsic or extrinsic remains controversy.

In 1961, Muller and his colleagues discovered the high-concentration unpaired electrons in phage (Mueller et al. 1961). As a consequence, the magnetic properties of DNA have attracted much attention. However, as it is difficult to exclude some impurities other than nucleic acid, such as some metal ions associated with nucleic acid, people failed to prove the magnetic properties were intrinsic to DNA (Kwon et al. 2009). In 1961, Walsh et al. stated that there might be evidence of the external origin of such magnetic properties. They detected spin signals of ferromagnetic electrons in some DNA samples, determined that iron (Fe) was the main source of such signals by means of X-ray fluorescence analysis, and observed the existence of ferromagnetic Fe_2O_3 by electron microscope (Walsh et al. 1961). After Nakamae et al. discovered the nonlinear paramagnetic behavior of B state of λ-DNA molecules at low temperature (Nakamae et al. 2005), Mizoguchi and his colleagues proposed that oxygen molecules are possible external source of this paramagnetism (Mizoguchi et al. 2006).

Besides iron, other factors also contribute to the unique magnetic property of DNA. In 2010, Omerzu et al. found a strong electron spin resonance (ESR) peak and a strong electron transfer in the freeze-dried Zn-DNA (Omerzu et al. 2010). Zn^{2+} itself is not paramagnetic, which means that the ESR signal of Zn-DNA comes from the unpaired electrons (Starikov 2003). Kwon et al. proposed that delocalized electrons exist in Zn-DNA because the cations of Zn^{2+} replace H^+ in hydrogen bonds of complementary bases in the double helix structure of DNA (Kwon et al. 2009). In terms of theoretical research, Starikov used the extended Hubbard Hamiltonian model to calculate the role of electron correlations in deoxyribonucleic acid

duplexes, and proposed that further research is needed to analyze the antiferromagnetism/superconductivity of DNA (Starikov 2003). Apalkov and Chakraborty directly calculated the almost linear temperature-dependence of magnetization of DNA, and they think the magnetization of DNA chains is due to the electron–electron and/or electron–vibration interactions (Apalkov and Chakraborty 2008).

A number of studies on the magnetic susceptibility of nucleic acids are being conducted along with investigations into the physical model of nucleic acids. In 2005, Nakamae et al. measured the magnetic susceptibility of DNA. According to their measurement, the magnetic susceptibility of λ-DNA is $-(0.63 \pm 0.1) \times 10^{-6}$ emu/g ($-7.91 \pm 1.26 \times 10^{-9}$ m^3/kg, SI) at a temperature higher than 100 K, in both A-DNA and B-DNA states. However, when the temperature is lower than 20 K, λ-DNA is paramagnetic in the state of B-DNA, and when the temperature reaches as low as 2 K, it resumes diamagnetic in the state of A-DNA. Moreover, the S-shaped M–H (magnetization–magnetic field) curve might be caused by the circular current along λ-DNA (Nakamae et al. 2005). In 2006, through detecting using the superconducting quantum interference device (SQUID), Lee et al. also found the S-shaped magnetization curve of dried fibrous salmon sperms A-DNA, proposing that the curve was a result of solenoid charge transmission along the spiral structure of DNA in the magnetic field (Lee et al. 2006). More information about the electromagnetic properties of DNA as a material can be found in the book (Kwon et al. 2009).

3.2.2 Protein

We summarized the reported magnetic susceptibility of proteins in Table 3.4, including both the original data in the literature in various units, as well as their converted values in SI units.

3.2.2.1 Hemoglobin and Myoglobin

When it comes to the magnetic properties of proteins, hemoglobin is the first protein that we should discuss since it has a special place in the study of the magnetism of biological materials due to its unique structure. Gamgee had previously proven in 1901 that oxyhemoglobin, carboxyhemoglobin, and methemoglobin are diamagnetic, which was later confirmed by other researchers (Gamgee 1901). Pauling and Coryell published two articles in PNAS on the magnetic properties of hemoglobin and related compounds, offering a complete description of their magnetism and structure, laying the foundations for further investigations (Pauling and Coryell 1936a, b). They proposed that oxyhemoglobin and carboxyhemoglobin are diamagnetic because they lack unpaired electrons, whereas deoxyhemoglobin (ferrohemoglobin) contains four unpaired electron pairs per iron atom and has a magnetic moment of 5.46 μ_B per heme. In the years thereafter, several investigations

into hemoglobin have been performed. The Gouy method was used by Taylor et al. to determine the magnetic moment of the cow ferrohemoglobin, which was (5.43 ± 0.015) μ_B (Taylor and Coryell 1938). When Havemann and colleagues discovered that the mass magnetic susceptibility of globin was -0.53×10^{-6} cgs (-6.66×10^{-9} m^3/kg, SI), confirming Pauling's hypothesis that globin is diamagnetic (Havemann et al. 1962). Savick and colleagues also investigated the magnetic susceptibility of oxy-and carbonmonoxyhemoglobins (Table 3.4) (Savicki et al. 1984). Taylor first reported the magnetic susceptibility of ferrimyoglobin, which he found to be the same as that of ferric hemoglobin. In myoglobin, where there is only one heme per molecule and hemoglobin can be well separated in solution, there is no interaction between the four hemes of hemoglobin (Taylor 1939). But this conclusion contradicts Pauling's previous suggestion (Pauling and Coryell 1936a). Then, with reference to the source of magnetic properties in hemoglobin and myoglobin, Weissbluth proposed that low-spin compounds (e.g., oxyhemoglobin) are not paramagnetic and they are entirely diamagnetic in low-spin derivatives (Weissbluth 1967).

Hemoglobin and myoglobin derivatives exhibit considerable changes in their magnetic properties when the R groups on the one side of the porphyrin–globin complex are replaced by other ions or molecules. However, although all of these hemoglobin and myoglobin derivatives include Fe element centers, there is also a distinction between high and low spin. For example, the hemoglobin F– and OH_2–derived derivatives are high-spin types, but the hemoglobin CN– and imidazole-derived derivatives have low-spin. Furthermore, Coryell et al. discovered that the magnetic susceptibility of ferrihemoglobin varied dramatically when the pH of the solution was altered (Coryell et al. 1937). In a study published in 1975, Anusiema discovered that 5% *t*-butanol had an influence on the magnetic susceptibility of ferrihemoglobin. These phenomena may be caused by the fact that the Fe atom in ferrihemoglobin is octahedrally located in solutions with different pH values than hemoglobin, causing a shift in its isotype via bond changes and proton loss; and that different pH environments can lead to changes in the position of the Fe atom relative to the porphyrin ring, resulting in changes in the high- and low-spin states. In addition to the fact that *t*-butanol might enhance the number of high-spin groups, it is possible that any of these factors may produce changes in the magnetic properties of ferrihemoglobin (Anusiem 1975).

3.2.2.2 Cytochromes

Cytochromes are heme-type compounds that are coupled with proteins and contribute to electron transport (Senftle and Hambright 1969). By measurement and calculation, Boeri et al. found that the magnetic susceptibility of cytochrome c was 2120×10^{-6} cgs emu (2.05×10^{-9} m^3/kg, SI) (Boeri et al. 1953). When Lumry et al. studied the magnetic properties of ferricytochrome c by altering the quantity of water bound by the protein, they discovered that the reduced state of ferrous ferricytochrome c was more less diamagnetic than the oxidized state, which was

consistent with previous findings. Furthermore, the magnetic susceptibility of the protein attached to water was the same as the magnetic susceptibility of unbound water. The dried form of ferricytochrome c was more paramagnetic than the dehydrated form. This difference may be due to the pairing of electrons as water alters some imidazole groups (Lumry et al. 1962). Ehrenberg and Yonetani hypothesized that the magnetic susceptibility of cytochrome a in the oxidized state is 2400×10^{-6} cgs emu (0.30×10^{-9} m^3/kg, SI), which was similar to that of cytochrome c, because both the oxidized and reduced forms are low-spin. When reduced, cytochrome a3 is a high-spin Fe(II) derivative, and the oxidized form has a magnetic susceptibility of 7900×10^{-6} cgs emu (0.99×10^{-9} m^3/kg, SI) (Ehrenberg and Yonetani 1961). A study conducted by Banci indicated that both diamagnetism and paramagnetism contributed to the magnetic susceptibility of cytochrome b5 when the magnetic field was measured in various orientations. The levels of magnetization observed in different orientations were also variable. As a result, they determined that in the paramagnetic heme-containing system, it is the metal ions and porphyrins that contribute to the magnetic susceptibility, with both of these contributing much more than the side chains of different aromatic groups and peptide chains. The magnetic anisotropy is induced by the structure of the heme, as well as other factors (Banci et al. 1998).

3.2.2.3 Ferritin, Fe-Transferrin, and Ferredoxin

Considering that the core element Fe is the primary source of the magnetic properties of hemoglobin, it is worthwhile to consider the magnetic properties of all proteins containing Fe atoms as well. Ferritin is an iron storage protein with a high iron concentration, and it is found in large quantities in the body. After conducting measurements, Granick discovered that the ferritin iron was in the ferric state, with a magnetic moment of 3.78 μ_B per g-atom of Fe (Granick and Michaelis 1942). Using NaOH, the iron in ferritin can be easily extracted, resulting in a precipitate of iron hydroxide that has a magnetic moment of 3.77 μ_B, which was the same as the same value of the magnetic moment of iron. Later, Rawlinson and Scutt compared ferric hydroxide with ferritin and discovered that iron in alkaline solutions had a low-spin state (Rawlinson and Scutt 1952). Allen and colleagues used AC-magnetic susceptometry to found that horse spleen ferritin and thalassemic human spleen ferritin were superparamagnetic (Allen et al. 2000).

Transferrin is an iron-transporting serum protein that possesses two specific metal binding sites. Ehrenberg et al. determined the paramagnetic susceptibility of the iron in Fe-transferrin at 20 °C to be $(15{,}700 \pm 500) \times 10^{-6}$ cgs ($2.56 \pm 0.08 \times 10^{-9}$ m^3/kg, SI). Based on the investigation of the chromium complexes and manganese and cobalt complexes of transferrin, Aisen et al. came to the conclusion that there is no evidence for exchange interaction between Fe^{3+} ions contained within the same protein (Aisen et al. 1969). Transferrin is a magnetic compound in which the contribution of orbital spin is almost completely ignored, and the high spin of Fe

ions is the primary explanation for its magnetic production (Ehrenberg and Laurell 1955).

Ferredoxin, found in *Clostridium pasteurianum*, is an iron–sulfur protein with a low redox potential that includes seven iron atoms and plays a significant part in the electron transfer process. The magnetic moment of ferredoxin was discovered by Poe et al. using nuclear magnetic resonance (NMR) techniques, and they came to the conclusion that there is substantial diamagnetic coupling between the iron atoms of ferredoxin, indicating that these iron atoms are relatively close together in terms of spatial proximity (Poe et al. 1970). It was discovered in 1965 by Ehrenberg and Kamen that the chromatium high-potential iron protein was diamagnetic Fe(II) in its reduced state and had a magnetization of 1.46 μ_B/iron in its oxidized state, indicating that there was a statistically significant difference in magnetization between the oxidized and reduced states (Ehrenberg and Kamen 1965). Blomstrom computed that the average magnetic moment per iron atom of ferredoxin for the five susceptibility measurements in solution is (1.96 ± 0.21) μ_B and came to the conclusion that all seven iron atoms in ferredoxin are Fe(III), and the Fe(II) in the reduced state has a low spin compared to the Fe(III) (Blomstrom et al. 1964).

3.2.2.4 Copper Proteins

Copper is an important transition metal in biology. The functions of copper proteins include simple electron transfer, substrate oxidation/oxygenation, and oxygen migration. Three common types of copper proteins have been defined based on their electron and magnetic properties. Type 1 (T1) copper proteins are also known as blue copper proteins; type 2 (T2) copper proteins do not possess a blue copper center and are usually found in oxidation/oxygenation enzymes (e.g., galactose oxidase); type 3 (T3) copper proteins have binuclear copper centers, each linked by three histidine. T3 copper proteins are involved in oxygen transport and activation (hemocyanin and tyrosinase). Both T1 and T2 copper are electron paramagnetic resonance (EPR) active in their oxidized state (Cu(II), 3d9, $S = 1/2$), T3 copper is EPR silent on oxidation, and all types of copper centers are in the reduced state (Cu(I), 3d10, $S = 0$) (Rich and Maréchal 2012). As early as 1958, Nakamura, after measuring and calculating the difference in magnetic susceptibility between apo-, oxidized, and reduced laccase, discovered that the magnetic susceptibility of copper in native laccase was 24×10^{-6}/g Cu (Nakamura 1958). Based on measurements and calculations, Ehrenberg et al. reported magnetic susceptibility difference ($\chi_{Cu,ox}$–$\chi_{Cu,red}$) of (570 ± 60) × 10^{-6} cgs emu (0.060 ± 0.006 × 10^{-9} m^3/kg, SI) for laccase A and (430 ± 80) × 10^{-6} cgs emu (0.046 ± 0.002 × 10^{-9} m^3/kg, SI) for ceruloplasmin I. They found that only approximately 40% of the copper was in the form of Cu(II) (Ehrenberg et al. 1962), which was consistent with Broman's ESR conclusion in 1962 (43–48%) (Broman et al. 1962). Aisen et al. measured and calculated the diamagnetic anisotropy of ceruloplasmin to be 7.1×10^{-7} cgs/cm^3 (8.92×10^{-6}, SI) and suggested that at least 40% of the copper in ceruloplasmin is

paramagnetic, supporting the notion that ceruloplasmin possesses seven tightly bound copper atoms per molecule, three of which are Cu(II) (Aisen et al. 1967).

3.2.2.5 Assembled Proteins

Aside from the influence of particular metal ions on electron transport, the magnetic susceptibility of protein molecules is closely related to the structure of the protein molecules. One of the most interesting for scientists is tubulin. Microtubules are macromolecular compounds found in the cytoplasm of all eukaryotic cells, and that they are assemblies made up of elongated protofilaments (Amos and Baker 1979). Protofilaments are fibrillar network structures formed by first tying together microtubule protein dimers (Amos and Klug 1974; Wickstead and Gull 2011). This ordered arrangement of the assembly structure is of great interest for the study of its magnetic properties. In 1982, Vassilev et al. observed parallel arrangement of microtubules in a magnetic field of 0.02 T, which is caused by the diamagnetic anisotropy of the tubulin molecule (Vassilev et al. 1982). Diamagnetic anisotropy is a result of the anisotropy of chemical bonds, which was more apparent in resonant structures such as aromatic groups, peptide bonds, or double and triple carbon bonds. As early as 1936, Pauling proposed the theory that aromatic ring-induced cyclic currents cause diamagnetic anisotropy in aromatic molecules (Pauling 1936). And aromatic amino acids, in which the same non-local ring currents are present, and non-aromatic molecules, despite the absence of local ring currents, have diamagnetic anisotropy consisting of the sum of the local anisotropy built between atoms (Maret and Dransfeld 1985). Pauling further calculated the diamagnetic anisotropy of the peptide bond to be -5.36×10^{-6} emu and indicated that the anisotropic magnetic susceptibility of the amino acid residues of each chain with an α-helical secondary structure is $(2.6 \pm 0.2) \times 10^{-6}$ per amino acid residue (Pauling 1979). Samulski and Tobolski successfully localized r-benzyl L-glutamate with a highly α-helical structure by a moderate intensity magnetic field (Samulski and Tobolsky 1971). When amino acids are organized in the α-helical form, all of the peptide bonds are situated in a plane parallel to the helical axis, and the total magnetization of this amino acid is derived from the sum of individual magnetizations along the main axis. And some studies have shown that tubulin dimers have a relatively high proportion of α-helices (circular dichroism tests have shown that 25% of amino acids contain α-helix structures) and that this secondary structure is oriented along the long axis (Ventilla et al. 1972; Lee et al. 1978). This theoretical foundation allowed Bras to predict the value of diamagnetic anisotropies of the tubulin dimers with a minimum value of 1.01×10^{-28} m^3, which was confirmed in 1998 (Bras et al. 1998). He subsequently confirmed a further discussion of the diamagnetic anisotropy of tubulin dimers in 2014, determining values of magnetic anisotropy of 3.7 and 4.5 $\times$ 10^{-27} J/T^2 (different calculation methods) and suggested that in addition to the α-helix, the β-sheet structure also makes a contribution (Bras et al. 2014). This method for determining the diamagnetic magnetization of tubulin dimers can be well applied to other proteins and macromolecular assemblies. It is believed that fibrin and

collagen have a similar assembled protein structure to tubulin, and that they are also magnetically orientated. In 1983, Freyssinet et al. found that the strong orientation of Fibrin occurs when it is aggregated in a strong magnetic field by observing the magnetically induced birefringence (Freyssinet et al. 1983). Torebett et al. used the same method to observe the self-assembly process of collagen and estimated the value of the anisotropy of magnetic susceptibility of collagen to be about -1×10^{-25} J/T^2 (Torbet and Ronziere 1984). The investigation of the magnetic properties of these assembled proteins not only provides theoretical support for the magnetic properties of cells and tissues in living organisms but also provides a new direction for the study of biomaterials with magnetic anisotropy.

3.2.3 Lipids

As early as in 1939, Lonsdale proposed that the presence of hydrocarbon chains contributes to the magnetic anisotropy in aliphatic compounds (Lonsdale 1939). These double or triple bonds constrain some electrons to occupy the planar orbitals of the atoms, thus generating the diamagnetic anisotropy if the chain is perpendicular to the SMF direction. In 1970, Chalazonitis reported the phenomenon that the rod-like outer segment of the frog retina can be aligned with the externally applied SMF direction (Chalazonitis et al. 1970). Then Hong et al. proposed that the diamagnetism of component molecules is involved in the orientation of biomembrane. They demonstrated that the sum of molecule–magnetic field interactions is sufficient to orient retinal rod cells, and the magnetic orientation of other membranous microstructures depends on their structure and morphology (Hong et al. 1971). In 1978, Boroske et al. measured and calculated the magnetic anisotropy of egg lecithin, determining a variation in magnetic susceptibility parallel and perpendicular to the lecithin molecule's long axis of $-(0.28 \pm 0.02) \times 10^{-8}$ cgs ($-3.52 \pm 0.25 \times 10^{-8}$, SI) at 23 °C (Boroske and Helfrich 1978). In 1984, Scholz et al. reported that the anisotropy of magnetic susceptibility of cylindrical vesicles was 1.7 times greater than that for egg lecithin. They ascribed the variation to the different concentrations of unsaturated acyl chains in the two samples (Scholz et al. 1984). In 1993, Azanza examined the magnetic properties of dried human erythrocyte membrane powder in a magnetic field of 5 T. In this study, they observed that the magnetic susceptibility of dried human red blood cell membranes was $-(4.59 \pm 0.15) \times 10^{-7}$ emu/g ($-5.77 \pm 0.19 \times 10^{-9}$ m^3/kg, SI) and the value of the anisotropy of magnetic susceptibility was $-(9.18 \pm 0.3) \times 10^{-7}$ emu/g ($-11.53 \pm 0.38 \times 10^{-9}$ m^3/kg, SI) (Azanza et al. 1993). The orientation changes of red blood cells and cylindrical lipid vesicles in SMFs (Fig. 3.1) (Boroske and Helfrich 1978; Higashi et al. 1993) will be discussed in more details in Chap. 6 of this book. In fact, there are also several other reports about the orientation of protein-free lipid bilayer structures in SMFs (Gaffney and McConnell 1974; Maret and Dransfeld 1977).

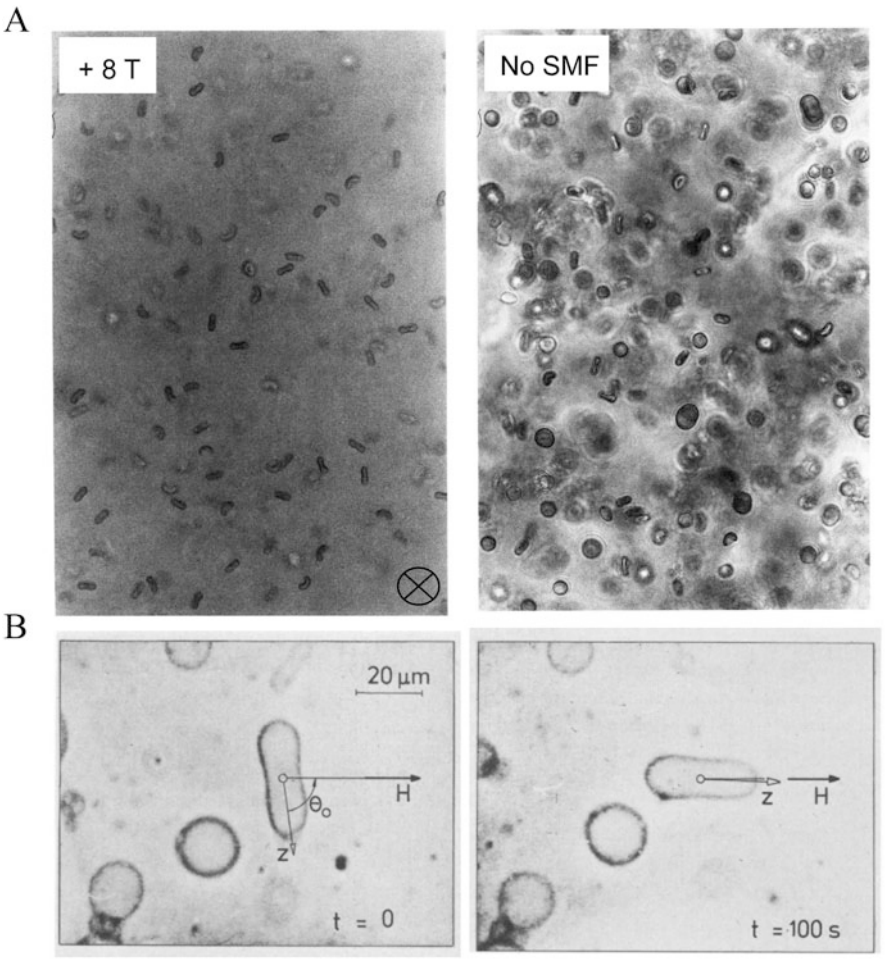

Fig. 3.1 Orientation changes in static magnetic field determined by lipid membrane in red blood cells and cylindrical lipid vesicles. (**a**) Red blood cells change orientation with or without an 8 T SMF. The field direction was normal to the paper. Reprinted with permission from (Higashi et al. 1993). (**b**) Cylindrical vesicles made from egg lecithin were exposed to a homogeneous field of 1.5 T parallel to the sample slides. [Reprinted with permission from (Boroske and Helfrich 1978)]

3.3 Blood and Relevant Chemical Compounds

In 2000, Sosnitsky et al. used the magneto-plethysmography (MPG) technology to detect that the magnetic susceptibility difference between human blood in vitro and water was 5×10^{-6}, which greatly differs from that of water (Sosnytskyy et al. 2000). In 2012, Jain applied MRI to measure the difference in volume magnetic susceptibility between fully oxygen-containing and fully deoxygenated blood in vitro, which was 0.273 ppm (cgs) (3.43×10^{-6}, SI) (Jain et al. 2012).

As early as in 1901, Gamgee found that the magnetic susceptibility of hemoglobin and other hemochrome derivatives was negative, while that of protoheme and ferroheme was positive (Gamgee 1901). The magnetic properties of hemoglobin or its derivative compounds were decided by the state of iron. Globin is diamagnetic, and the magnetic properties difference of hemoglobin are determined by heme, a compound composed of iron and porphyrin. Therefore, although the porphyrin core is diamagnetic, the magnetic properties of different metalloporphyrins are obviously different. Heme with Fe(II) as the central ion is known as ferroheme, while heme-containing Fe(III) is called ferriheme. If Fe(III) is connected to a hydroxyl ion, the heme is called iron hydroxide heme; the compound composed of Fe(III) and chlorine is called ferric chloride heme. When these four hemes are combined with globin, they form reduced hemoglobin and methemoglobin, respectively. These two types of hemoglobin can react with other ions or molecules to form a large number of hemoglobin derivatives. For instance, myoglobin is a well-known example (Pauling and Coryell 1936a). In 1980, Eaton et al. measured the magnetic properties of four porphyrin compounds and found that their magnetic susceptibilities were all negative (Eaton and Eaton 1980). Moreover, Chane et al. conducted X-ray diffraction on various porphyrins, metalloporphyrins, and hemins, and found that porphyrin cores are not always planar, and metal ions are not always located in the plane of four pyrrole nitrogen atoms. The magnetic properties of these ions are different fundamentally because of their different types, spatial positions, and chemical states (Chance et al. 1966).

Some studies have shown that dipyridine hemochrome, dicyanide hemochrome, and CO-pyridine hemochrome are all diamagnetic (Pauling and Coryell 1936a; Wang et al. 1958), while the magnetic moment of ferroheme in NaOH ranges from 4.83 to 5.02 μ_B, and that of fixed hemin is within the range of 5.81–5.97 μ_B (Pauling and Coryell 1936a; Schoffa and Scheler 1957; Havemann et al. 1962; Hambright et al. 1968). Moreover, the magnetic susceptibility tensor of heme porphyrins is mostly axial, and this central axis is perpendicular to the plane of heme, indicating that heme is anisotropic and heme-containing substances would exhibit magnetic anisotropy (Banci et al. 1998).

3.4 Magnetic Properties of Organisms, Tissues, and Cells

3.4.1 Unicellular Organisms

As early as in 1936, Bauer and Raskin proposed that the diamagnetic properties of *Saccharomyces cerevisiae* and some bacteria, such as *Escherichia coli* and proteus, increase by 4% after death. They speculated that these unicellular organisms were in an active paramagnetic state when they were alive, but after death, the diamagnetic properties would increase due to the loss of paramagnetic components (Bauer and Raskin 1936). In 1964, Sugiura and Koga studied the magnetic susceptibility of the *Saccharomyces cerevisiae* during dormancy, metabolizing and heat treatment. They

found that the diamagnetic properties of *Saccharomyces cerevisiae* cells only increased by 0.3% after death, and they also estimated and calculated the magnetic susceptibility of yeasts using the Gouy technique, which show that the magnetic susceptibility of yeasts floating in water was -0.733×10^{-6} cgs (-9.21×10^{-6}, SI) and that of water was -0.720×10^{-6} cgs (-9.04×10^{-6}, SI) (Sugiura and KOGA 1964a, b).

When it comes to the magnetic properties of unicellular organisms, we have to mention magnetotactic bacteria (MTB), which have characteristic compass-like magnetosomes in their bodies and can orient and migrate along the geomagnetic field lines and have been extensively studied (Bazylinski and Frankel 2004; Bazylinski and Williams 2006; Faivre and Schuler 2008; Lefevre et al. 2011). MTB were firstly reported in 1975 by Blakemore for its magnetotaxis phenomenon (Blakemore 1975), and the ultrastructural details of magnetosome were then described in 1980 (Balkwill et al. 1980). In most magnetotactic bacteria, the magnetosomes are arranged in chains, which make the total magnetic dipole moment of the bacteria determined by the sum of the permanent magnetic dipole moment of the individual magnetosome particles (Bazylinski and Frankel 2004).

In 2007, Melnik developed an instrument called cell tracking velocimeter (CTV) to detect the magneto-optical mobility of atrophic bacillus (formerly known as bacillus globigii), *Bacillus thuringiensis,* and bacillus cereus. It was found that all bacterial strains showed the peak value of element Mn and relatively high average magnetic mobility after sporulation. They believed that the high level inherent magnetic susceptibility reflected by magnetic mobility was mostly likely caused by paramagnetic element Mn (Melnik et al. 2007). Zhou et al. further explored the intrinsic magnetic properties of spores in *Bacillus megaterium*, *Bacillus cereus,* and *Bacillus subtilis*. They measured the samples with the SQUID and found that the average magnetic moment of spores in *Bacillus cereus* was 5.1 μ_B (Mn content 2.3×10^{22} kg^{-1}), which was relatively low, while that of *Bacillus megaterium* and *Bacillus subtilis* were 5.9 μ_B (Mn content 1.55×10^{22} kg^{-1}) and 5.5 μ_B (Mn content 4.2×10^{22} kg^{-1}). It is obvious that the Mn content was inconsistent with the magnetic moment. Although Mn was the main reason for the paramagnetic properties of spores in *Bacillus subtilis*, the author proposed that it is 3^+ rather than 2^+. Their conclusion is that the paramagnetic properties of bacillus spores are caused by different chemical states of the element Mn, and the magnetic susceptibility can be changed by varying the Mn content in the culture medium (Zhou et al. 2018).

3.4.2 Tissues

3.4.2.1 Normal Tissues

As early as 1967, Bauman et al. clarified the relationship between iron compounds and the magnetic susceptibility of hepatic tissues. Through measuring the magnetic susceptibility of hepatic tissues without iron protein and hemosiderin, they found

that, each gram of ferritin–hemosiderin (storage iron) would be expected to increase the magnetic susceptibility of a human liver by about 0.08×10^{-6} emu (1.00×10^{-6}, SI) per cubic centimeter (Bauman and Harris 1967). In 2021, Klohs et al. used the vibrating sample magnetometer (VSM) to measure the magnetic susceptibility and water content of fresh and chemically fixed mouse tissues (Klohs and Hirt 2021). They found that all samples show mass susceptibilities between -0.068 and -1.929×10^{-8} m^3/kg (SI), compared to -9.338×10^{-9} m^3/kg (SI) of double distilled water. Moreover, they found that, the cardiac tissues feature stronger diamagnetic properties compared with other tissue samples. Besides, the magnetic susceptibility of chemically fixed cardiac tissues is generally smaller than that of fresh tissue. It is the chemical fixation, but not the water content, that affected the diamagnetic properties of the samples. However, when it comes to the other organs and tissues, it is unknown why there is no significant difference between fresh vs. fixed tissues in terms of magnetic susceptibility. It is interesting that fixed tissue showed no dependence of susceptibility with temperature, whereas fresh tissue does, indicating the presence of paramagnetic components (Klohs and Hirt 2021).

Most studies of about normal tissue magnetic properties focused on the brain. For example, in 1992, Kirschvink et al. reported the existence of ferromagnetic substances in cerebral tissues (Kirschvink et al. 1992). In 2015, Kopani et al. show that among all the areas of human brain, the areas related to motor function (Globus pallidus, putamen, and substantia nigra) have the highest iron concentration. Iron in the human brain mainly exists in the form of ferritin, hemosiderin (decomposition product of ferritin), and other biomineralized oxides such as hematite (Fe_2O_3), magnetite (Fe_3O_4), and maghemite (γ-Fe_2O_3) (Kopani et al. 2015). In 2017, Kopáni et al. proposed that the content of diamagnetic oxyhemoglobin and paramagnetic deoxyhemoglobin also contributed to the magnetic susceptibility of cerebral tissues, but the contribution was determined by the content of these substances (Kopáni et al. 2017). In 2018, Hametner et al. clarified the direct correlation between the magnetic susceptibility and iron content of cerebral tissues by measuring quantitative susceptibility mapping (QSM) of human cerebral tissues, which further spelled out the influence of iron content in cerebral tissues on the magnetic susceptibility of cerebral tissues (Hametner et al. 2018).

Gelderen et al. used the torque balance to measure the distribution of resonance frequency around WM fiber bundle of the human brain, so as to study and calculate the anisotropy of magnetic susceptibilities in WM. They found that the susceptibility of WM in the central nervous system was decided by the direction of WM relative to the magnetic field, and such anisotropy would produce a tiny magnetic torque directly proportional to the volume of the fiber bundle. The quantitative results showed that the anisotropic magnetic susceptibility of WM ranged from 13.6 to 19.2 ppb (13.6×10^{-6} to 19.2×10^{-6}, SI). Based on the above results, the resonance frequency of MRI depends on the direction of brain microstructures relative to the main magnetic field, and the orientation of these microstructures affects the resonance frequency of WM to some extent (van Gelderen et al. 2015).

It is interesting but puzzling that although nearly all studies show that biological tissues are diamagnetic, there is one study that detected paramagnetism for all the tissues (Sant'Ovaia et al. 2015). They also reported that the samples obtained in females showed lower values of magnetic susceptibility than those resected from males and the samples collected from the lungs of smokers have higher values of magnetic susceptibility.

For the magnetic anisotropy, most studies also focused on the brain (Svennerholm et al. 1992; Luo et al. 2014). For example, it was shown that the myelin sheath could affect the anisotropic magnetic susceptibilities of cerebral tissues. According to Luo et al., the variation of the MR frequency of white matter (WM) is dependent by the symmetrical distribution of magnetic substances in cells rather than the average magnetic susceptibility of tissues. They used the rat optic nerve which contain longitudinally arranged myelin sheath and neurofilament tissues as models, and measured its magnetic susceptibility using the deviation of MR resonance frequency. The results showed that the magnetic susceptibility of water was -9.035 ppm (-9.035×10^{-6}, SI), there was a difference of $-(0.116 \pm 0.010)$ ppm ($-0.116 \pm 0.010 \times 10^{-6}$, SI) between the volume magnetic susceptibilities of the optic nerve and water, and the difference between the longitudinal magnetic susceptibilities of optic nerve was $-(0.043 \pm 0.009)$ ppm ($-0.043 \pm 0.009 \times 10^{-6}$, SI) (Luo et al. 2014).

Besides, organs such as kidneys, heart, and connective tissues are also characterized by magnetic anisotropy. Their anisotropy is different from that of myelin sheath in cerebral tissues. For example, the kidney has ordered tubular structure, basal membrane, and renal epithelial cells, which are all potential sources of anisotropic magnetic susceptibility. Through the susceptibility tensor imaging, it is found that magnetic anisotropy of tubular was more diamagnetic when tubules were aligned with the magnetic field, and it was more paramagnetic when tubules were aligned orthogonal to the magnetic field (Xie et al. 2015). With fibrous protein known as collagen, connective tissues have a more orderly structure. As mentioned earlier, collagen fibrils have planes of peptide groups parallel to the helical axis, and their net magnetic susceptibility is the highest in the direction parallel to the fibril axis. Such a tendency is contrary to the anisotropy of magnetic susceptibilities observed in bones and myocardial fibers (Dibb et al. 2017).

3.4.2.2 Tumor Tissues

In 1961, Senftle and Thorpe measured the magnetic susceptibility of hepatic tissues in rats with implanted hepatoma and that of normal hepatic tissues, and found that the hepatoma was more diamagnetic while normal rats' liver tissues were less diamagnetic (Senftle and Thorpe 1961) (Table 3.6). They also processed the samples at the liquid nitrogen temperature and measured the magnetic susceptibilities of water and different tissues within the temperature range of 77–263 K. Their results showed that the magnetic susceptibilities of the three types of tissues varied

drastically within the temperature range of 130–140 K, and below 150 K (Senftle and Thorpe 1961).

Mulay et al. studied the magnetic susceptibilities of Cloudman S91 melanoma, S91A melanoma (without melanin), and normal mouse tissues and found that their magnetic susceptibilities are tissue and temperature-dependent. They pointed out that such a result was caused by magnetic substances in different tissues, such as free radicals or paramagnetic ions. Their ESR results confirmed the existence of free radicals and paramagnetic ions in S91 melanoma (Mulay and Mulay 1967).

Abnormal expression of iron and ferritin can be observed in many types of cancers. Brem et al. measured the magnetization of human meningioma tissue and human non-tumor hippocampus tissue. The diamagnetic signal meningioma tissue (2.14×10^{-5} Am^2/kg) was much larger than that of hippocampus tissue (0.22×10^{-5} Am^2/kg) (Brem et al. 2006).

3.4.3 Cells

3.4.3.1 Blood Cells

The most well-studied cellular magnetisms are about red blood cells (RBCs). Based on the theory proposed by Pauling et al. for the paramagnetic deoxyhemoglobin and paramagnetic methemoglobin, Xue et al. got the magnetic susceptibilities of RBCs at three different state by theoretical calculation, CTV and SQUID-MPMS (Xue et al. 2019) (Table 3.7). The magnetism difference between RBCs is mainly determined by the paramagnetic deoxyhemoglobin and methemoglobin, and the main contribution is made by the iron atom in heme group, which has been mentioned before. The

Table 3.7 Reported magnetic susceptibility of cells

Cells	Magnetic susceptibility χ (SI)	References
Oxy red blood cells	$-(9.19 \pm 0.47) \times 10^{-6}$ (Magnetophoresis) $-(9.73 \pm 1.34) \times 10^{-6}$ (SQUID-MPMS) -9.23×10^{-6} (theory)	Xue et al. (2019)
Deoxy red blood cells	$-(6.39 \pm 1.1) \times 10^{-6}$ (Magnetophoresis) $-(7.34 \pm 1.17) \times 10^{-6}$ (SQUID-MPMS) -5.72×10^{-6} (theory)	
Met red blood cells	$-(6.46 \pm 0.88) \times 10^{-6}$ (Magnetophoresis) $-(6.02 \pm 1.1) \times 10^{-6}$ (SQUID-MPMS) -5.27×10^{-6} (theory)	
HeLa cells	-0.515×10^{-6}	Kashevskii et al. (2006)
CNE-2Z cells (cytoplasm)	$(9.888 \pm 0.6) \times 10^{-9}$ m^3/kg	Tao et al. (2020)
CNE-2Zcells (nucleus)	$-(6.813 \pm 0.003) \times 10^{-9}$ m^3/kg	

magnetically induced velocity of the RBCs, the magnetophoretic mobility, depends on both the oxygenation or oxidation state of hemoglobin iron and the quantity of hemoglobin iron within each RBC (Jin et al. 2011). Moreover, the magnetophoresis of RBCs based on their magnetic susceptibility has been developed. The malarial parasite digests hemoglobin to leave high-spin oxidized heme products which are paramagnetic, but normal low-spin oxyhemoglobin, which is diamagnetic. Consequently, it should be possible to separate parasitized erythrocytes from normal oxygenated ones which will selectively separate the cells which have such a high-spin form of hemoglobin and can be magnetically concentrated in the blood of infected patients (Paul et al. 1981). Meanwhile, cell magnetophoresis may also prove to be capable of separating nucleated RBCs from cell mixtures and identifying different levels of methemoglobin based on the different oxidative susceptibilities of the intracellular environment (Zborowski et al. 2003).

Many studies on the magnetic properties of cells are based on the magnetic properties of RBCs due to their unique composition and biconcave disk shape. Covered by the cell membrane composed of lipid bilayers, RBCs orient in the magnetic field (Fig. 3.1). However, the magnetic orientation of solidified RBCs was opposite to that of normal RBCs, and their biconcave discoid shape was perpendicular to the SMF direction. For this phenomenon, they held that the average ratio of cell membrane of RBCs to hemoglobin was about 1:70, and the orientation of the hemoglobin in the magnetic field was determined by the order of hemoglobin when it was solidified. The spin state of hemoglobin decides its paramagnetic and diamagnetic anisotropy. They measured the anisotropic (van Gelderen et al. 2015) magnetic susceptibilities of immobilized RBCs in a high-spin state and a low-spin state, which were 2×10^7 D_B/cell and 5×10^6 D_B/cell, respectively. According to the results, the orientation of the solidified RBCs was determined by the diamagnetic anisotropy of some hemoglobin in the cells, and the RBCs in a high-spin state had a greater magnetic anisotropy because of their stronger paramagnetic anisotropy (Takeuchi et al. 1995).

Yamagishi et al. compared the diamagnetic orientation of platelets and RBCs in a high SMF. Through measuring the magnetic anisotropy of RBCs and platelets parallel to and perpendicular to the SMF, they obtained the magnetic anisotropy of RBCs ($\Delta\chi = 8.3 \times 10^6$ D_B/cell) and platelets ($\Delta\chi = 1.2 \times 10^7$ D_B/cell) (Table 3.8). They also showed that microtubules can be aligned very well in SMF, which played a critical role in the magnetic anisotropy of RBCs (Yamagashi et al. 1992). Moreover, two recent studies also investigate the magnetism of monocyte, another type of

Table 3.8 Reported magnetic anisotropy of cells

Cells	Anisotropy of magnetic susceptibility $\Delta\chi$	References
Red blood cells (high-spin)	2×10^7 D_B/cell	Takeuchi et al. (1995)
Red blood cells (low-spin)	5×10^6 D_B/cell	
Red blood cells	8.3×10^6 D_B/cell	Yamagashi et al. (1992)
Platelets	1.2×10^7 D_B/cell	

blood cells. Kim et al. used the CTV to analyze the magnetic characteristics of human monocytes, plasma platelets, oxygen-containing erythrocytes, and methemoglobin erythrocytes, concluding that monocytes had the highest magnetic mobility and that the average magnetic mobility of monocytes was 7.8 times faster than that of methemoglobin erythrocytes. In addition, positive magnetic velocity was also observed in some plasma samples, indicating that platelets may contain iron. This result shows that monocytes and platelets are possibly paramagnetic (Kim et al. 2019; Gómez-Pastora et al. 2021).

3.4.3.2 Cancer Cells

Inspired by the magnetic separation technique, in 2006, Kashevskii et al. examined the magnetic susceptibility of HeLa cancer cells by detecting their trajectory in the magnetic field and comparing it with that of RBCs. They found that the cells' diamagnetic susceptibility increased with the increase of their diameter, which they think is because the nucleus and cytoplasm have different magnetic properties. Their measurement shows that the diamagnetic susceptibility of HeLa tumor cells is $-(0.5136–0.5179) \times 10^{-6}$ (different tumor cell diameter), while the diamagnetic susceptibility of red blood cells varied from -0.731×10^{-6} (with full oxygenated hemoglobin) to -0.573×10^{-6} (with reduced hemoglobin) (Kashevskii et al. 2006).

Also in 2006, Han et al. successfully separated human breast cancer cell lines (MCF-7, MDA-MB-231 and MDA-MB-435) using the magnetophoresis microseparator of the paramagnetic capture (PMC) mode (Fig. 3.2). After measuring the micro-electrical impedance of these human breast cancer cell lines and comparing it with that of the normal human breast tissue cell line MCF-10A, it was found that normal cell lines and cancer cell lines had significantly different micro-electrical impedance spectra. Hence, cells can be identified and classified according to different pathological stages of human breast cancer cell lines, which verifies different magnetic properties of these cells to some extent (Han et al. 2006).

In 2020, our laboratory measured the magnetic susceptibility of human nasopharyngeal carcinoma CNE-2Z cells by SQUID-MPMS3. It was found that the M–H curve of CNE-2Z cells was paramagnetic at low temperatures, which indicated the existence of some paramagnetic components. We further examined the magnetic susceptibilities of cytoplasm and nucleus of CNE-2Z cells and found that the mass magnetic susceptibilities of cytoplasm and nucleus were $(9.888 \pm 0.6) \times 10^{-9}$ m^3/kg (SI) and $-(6.813 \pm 0.003) \times 10^{-9}$ m^3/kg (SI), respectively. This result shows that the cytoplasm of CNE-2Z cells is paramagnetic, which is probably caused by mitochondria and free radicals. However, the nucleus is diamagnetic, because it mainly contains DNA, nucleoprotein, and lipids (Tao et al. 2020).

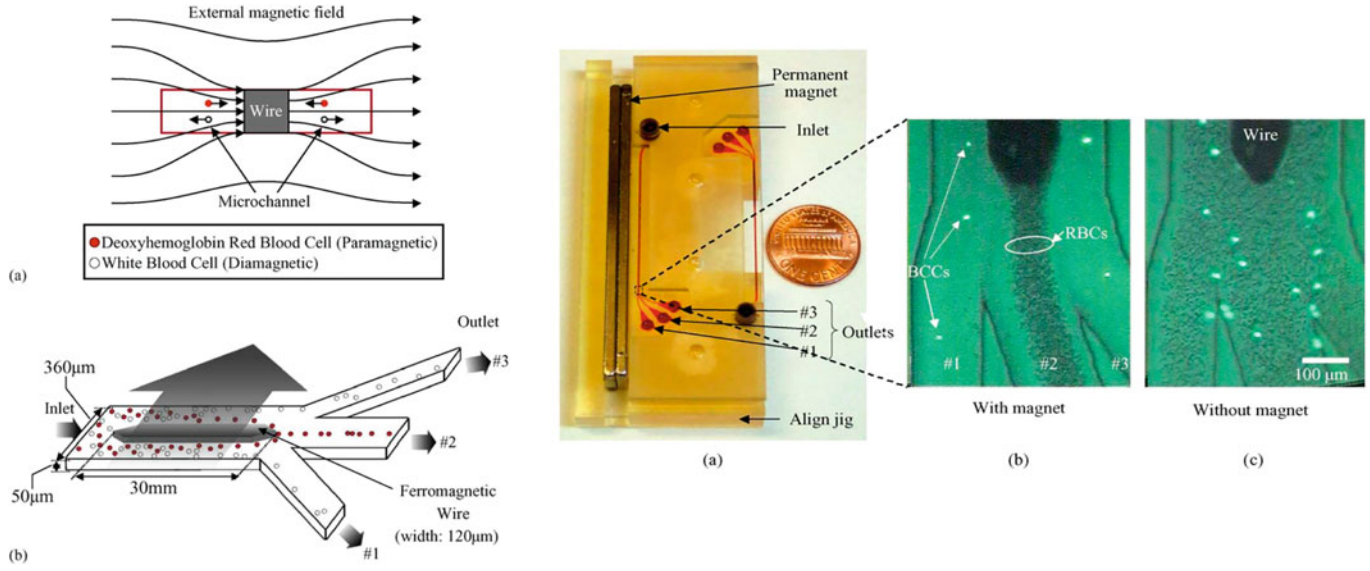

Fig. 3.2 Paramagnetic capture mode (PMC) microseparator to isolate breast cancer cells from blood. Left: Illustrations of the PMC microseparator. (**a**) Cross-sectional view of the microchannel and (**b**) perspective view of the microchannel that has one inlet and three outlets. Right: (**a**) Top view of the fabricated PMC microseparator. Fluorescently probed BCCs passing through the microchannel of the PMC microseparator at (**b**) an average flow velocity of 0.05 mm/s with an external magnetic flux of 0.2 T, and (**c**) an average flow velocity of 0.05 mm/s without the external magnetic flux. [Reprinted with permission from reference (Han et al. 2006)]

3.5 Conclusion

The magnetic properties of biological samples are critical for their responses to externally applied magnetic fields, which are determined by their composition, structure, and a variety of other factors. Currently, the exact magnetic properties of most biological samples are still unclear, especially at their physiological and pathological conditions. Future works are strongly encouraged to systematically and accurately measure the magnetic properties of biological samples at molecule, cellular, and tissue levels, which are essential for the development of magnetic field-based research, diagnosis, and therapeutic techniques.

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Chapter 4
Molecular Mechanisms for Electromagnetic Field Biosensing

Kris Dammen-Brower, Avi Sardana, and Kevin J. Yarema

Abstract Almost all types of life that have been appropriately investigated have shown some indication of biological response to magnetic fields. An alluring application of this ever-increasing amount of information describing how biological systems sense magnetic fields and transduce this information into physiological response is to treat human disease. Toward that goal, this chapter summarizes electromagnetic biosensing in a diverse set of organisms across several phyla, and discusses how the underlying mechanisms apply or do not apply to humans.

Keywords Static magnetic fields (SMFs) · Biological magnetoreceptors · Magnetic field biosensing · Magnetite · Cryptochromes · Chemical magnetosensing

4.1 Introduction

This chapter explores the biological basis for therapeutic effects of electromagnetic fields (EMFs) in people, with a focus on static magnetic fields (SMFs; basic definitions of magnetism are briefly reviewed in Sect. 4.1 of this chapter). At present, there is no clear and widely accepted mechanism by which SMFs benefit human health (Driessen et al. 2020); indeed, there is considerable skepticism in the mainstream media (as well as in certain parts of the scientific literature) whether SMFs have any effect at all. For example, a purported lack of beneficial effects sometimes is deduced from studies that EMFs (in general) have negligible detrimental effects. To illustrate, although there are occasional reports to the contrary (Carles et al. 2020), the bulk of the evidence indicates that living in proximity to high voltage electrical power lines does not increase the risk of cancer (Ahlbom et al. 2001; Anonymous 2002; Schüz 2011; Crespi et al. 2016; Amoon et al. 2022). To some,

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this lack of harm also implies that EMF exposure likely has negligible beneficial effects.

On the other hand, it is well established that a wide range of living organisms—ranging from bacteria, mollusks, crustaceans, insects, fish, amphibians, reptiles, birds, and mammals (Wiltschko and Wiltschko 2012; Todorović et al. 2020)—use the earth's relatively weak magnetic field (i.e., geomagnetism) for orientation, navigation, and additional purposes covered in Sect. 4.2 of this chapter. As will become evident from this information, the mechanisms that many species use for magnetoreception are highly specialized and do not apply directly to humans. In some cases, however, the underlying molecular basis of magnetic sensing relies on broad mechanisms observed across phyla, thereby providing a conceptual basis for how human cells, tissues, and organs can respond to SMFs. If nothing else, precedent from non-mammalian systems provides a starting point for investigation of magnetic field sensing in humans. One example lies in ongoing efforts to establish the presence and activity of magnetite in people (Sect. 4.3.1.2). For context, magnetite (discussed in Sect. 4.3) was first described in prokaryotes half a century ago and now is regarded as a "well-known" biosensor of magnetic fields.

Although much has been discovered about magnetoreception, many aspects of magnetic field biosensing remain poorly understood and their basic mechanisms are unknown. This is particularly true for humans, where the very topic of whether people have the ability to sense magnetic fields, much less respond to them, remains controversial. Here, in Sect. 4.4 of this chapter, we provide an overview of sensing mechanisms found elsewhere in nature that may apply to humans and detail speculation of "novel" ways that human cells, tissues, and organs sense and respond to magnetic fields.

4.2 Magnetism, Basic Definitions

This section briefly introduces basic concepts and definitions of magnetism related to biological systems; a more detailed description of magnetic phenomena is provided elsewhere in this book, in introductory physics textbooks, or fairly reliable internet sources such as Wikipedia. The information presented here in Sect. 4.1 is not meant to be comprehensive, but to provide a sufficient basis for understanding the subsequent sections of this chapter without the need to refer to outside material.

4.2.1 Ferromagnetism, Paramagnetism, and Diamagnetism

Ferromagnetism is "everyday" magnetism; for example, permanent magnets (such as ubiquitous refrigerator magnets or removable car bumper stickers) are ferromagnetic. A ferromagnetic substance becomes magnetized when exposed to a magnetic field and retains this feature "permanently" after removal from the field. As a caveat,

ferromagnetism is not strictly permanent because field strength often wanes over time, and can be affected (i.e., field direction can be reversed) by exposure to a subsequently applied field or diminished by heating the magnet. Nevertheless, field strengths of ferromagnets can be remarkably stable over long periods of time in the absence of high temperatures or counteracting magnetic fields. As a second nuance, although the term "ferro" implicitly suggests that ferromagnets contain iron, several other metals have ferromagnetic properties including most alloys of nickel and cobalt as well as several rare earth metals (neodymium is a well-known example). A final feature, important for biological magnetosensing, is that these metals are not inherently magnetic without the proper atomic-level organization. For example, iron in solution or in prevalent biological contexts (e.g., when it is complexed with hemoglobin in erythrocytes) is not ferromagnetic. Instead, constituent atoms and molecular structures (e.g., iron oxides in the case of ferromagnets) must be organized into distinct crystalline structures to be magnetic. Such structures can occur naturally as lodestone (iron ore) in the mineral world and, in specialized situations in the biological realm, as magnetite.

Paramagnetism is a dynamic phenomenon; paramagnetic substances "become magnetic" while exposed to a magnetic field, but the induced effect rapidly decays upon loss of the primary magnetic field as thermal motion quickly randomizes the spin orientations of the constituent atoms. Examples of paramagnetic substances include free electrons found in metals and unpaired electrons found in many biological molecules. Indeed, in biology, many proteins are complexed with metals that have unpaired electrons, leading to the development of the commonly used electron paramagnetic resonance (EPR) spectroscopies (Bertini et al. 2012; Sahu and Lorigan 2020). In addition to exploiting the natural paramagnetism of biological molecules, efforts are underway to create ultramagnetic cells by endowing them with chelated paramagnetic iron (Ramesh et al. 2018). Similarly, paramagnetic chemical probes are becoming increasingly useful for studying biological macromolecules (Miao et al. 2022).

Diamagnetism is a property of all materials that describes the formation of an induced magnetic field in the direction opposite to an externally applied field; in other words, the induced field attempts to repel the applied field (note that this is opposite to paramagnetism where the induced field is attracted to, and aligns with, the external field). Molecules found in biological systems ranging from water to bioorganic macromolecules are typically only very weakly diamagnetic, resulting in diamagnetism overshadowed by external fields or by surrounding paramagnetic or ferromagnetic entities. Despite eliciting weaker responses in biological molecules than paramagnetism, diamagnetic effects on biological systems can be dramatic because diamagnetic materials can be stably floated in magnetic fields. As a result frogs and mice have been levitated using strong magnetic fields (Valles Jr et al. 1997; Liu et al. 2010), making for visually effective demonstrations of their diamagnetic properties.

4.2.2 Field Types and Strengths

Life evolved in the presence of the Earth's magnetic field (i.e., "geomagnetism"); the geomagnetic field (GMF) fluctuates in direction and strength over time and space. Currently, the GMF has a magnitude at the Earth's surface that ranges from 25 to 65 microteslas (μT; or 0.25 to 0.65 gauss [1 T equals 10,000 guass]). This field is considered to be "weak" insofar as it cannot be detected by humans during their everyday activities in meaningful or noticeable ways without specialized instruments. To provide context for magnetic field strengths: the human brain emits a much weaker magnetic field (~0.1–1 picoT) while cardiac pacemakers produce fields about an order of magnitude stronger than GMFs (~500 μT); a refrigerator magnet is yet another order of magnitude stronger (~5 mT); a device custom-built to treat cultured human cells (Fig. 4.1) provides another ~2 order jump in field strength (~0.25 T); another order of magnitude increase in strength (to ~1–3 T) represents typical stereo loudspeaker fields as well as MRI exposure; and finally, a 17 T field represents the strength needed to famously levitate a frog (Valles Jr et al. 1997). For the purposes of this chapter's discussion, magnetic fields in the range of geomagnetism are termed "weak." For higher strength fields, fields below 1 T are considered to be "moderate" strength and those above 1 T are considered to be "strong" (most therapeutic magnetic treatments fall in the moderate strength category). Finally, although many electromagnetic fields (EMFs) involve a time-varying component, this chapter primarily focus on time invariant, i.e., "static" magnetic fields (which, as mentioned, are called "SMFs"). In the scientific literature, it is sometimes assumed that EMFs with frequencies of less than 100 Hz have biological effects similar or identical to SMFs (Markov 2014; Lohmann et al. 2022). However, in the current chapter, we exclusively focus on studies without a time-varying component.

4.3 Overview of Magnetoreception in Various Organisms

The ability to sense magnetic fields—or "magnetoreception"—has evolved across almost all phyla of mobile organisms starting with ancient magnetotactic bacteria that exploit the GMF to move up or down in the water column and nematodes that also use magnetic fields to move vertically in the soil (Clites and Pierce 2017; Lin et al. 2020; Diego-Rasilla and Phillips 2021). Other animals including amphibians, butterflies, birds, and even mammals use the Earth's magnetic field for navigation during long distance migrations (Blanco et al. 2022; Lohmann et al. 2022). A sampling of such magnetosensing organisms is provided below, along with brief mechanistic insights. This information is not intended to be comprehensive but is instead meant to provide an overview of known and postulated molecular mechanisms found throughout nature as a prelude to a more detailed description of the three "well known" modes of magnetic sensing (magnetite, chemical, and inductive) in Sect. 4.3 of this chapter.

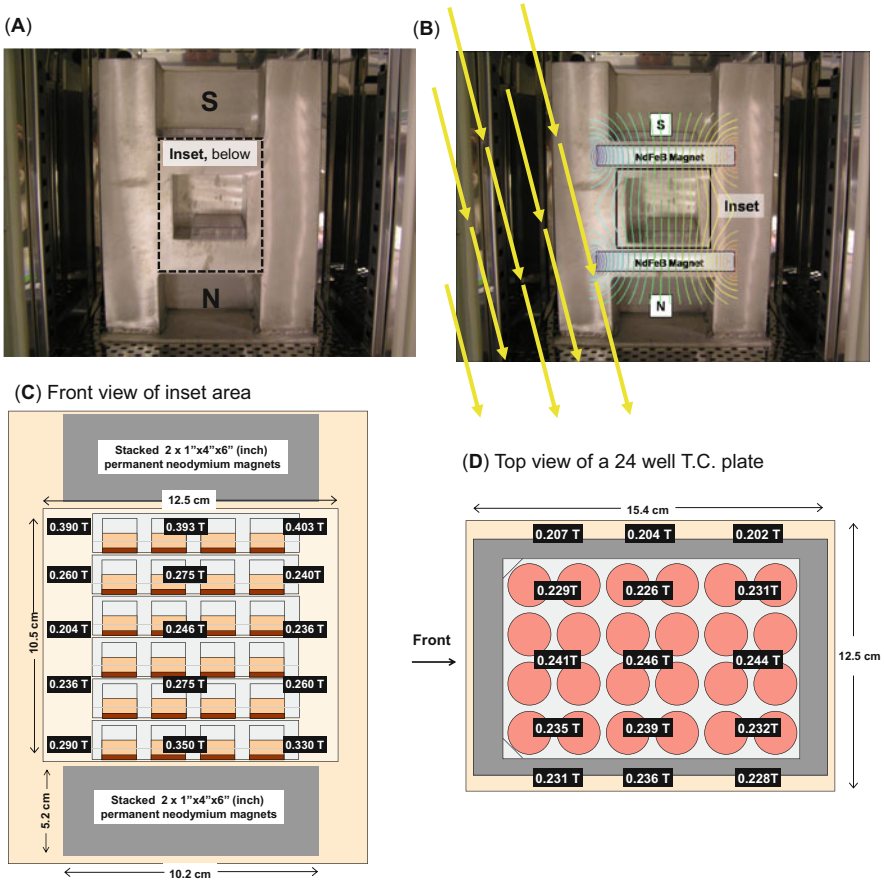

Fig. 4.1 Device used to treat cells with "therapeutic" strength SMFs. (**a**) The device is shown inside a standard-sized cell culture incubator with the position of permanent neodymium alloy magnets indicated with the "**S**" and "**N**." (**b**) The magnet-applied magnetic field lines are shown (colored lines) with an approximate representation of the GMF based on the positioning of the incubator in Baltimore, Maryland, USA indicated with the yellow arrows. (**c**) A front view of the inset from Panel (**a**) is shown as a front view of 6 stacked 24 well tissue culture (T.C.) plates in the central area of the magnetic treatment device between the two neodymium magnets. Representative measurements of field strength are shown, as measured at "cell level," indicated in dark red, orange indicates cell culture media. (**d**) A top view of a plate in the "inset" area (**a**, **c**) shows the dimensions and relative field strength at various positions on a standard 24-well cell culture plate located in one of the two center plates (i.e., the third and fourth plates, stacked vertically). When used for biological studies, only the four centered (from top to bottom) plates were typically used (the bottom plate remained empty) resulting in cells being exposed to SMF strengths ranging from 0.23 to 0.28 T. [Reprinted with permission from (Wang et al. 2009, 2010). Open access]

4.3.1 Bacteria

Magnetotactic bacteria were first described by Salvatore Bellini in a monograph published in 1963 (Bellini 1963) with a seminal peer-reviewed report by Robert Blakemore published 12 years later (Blakemore 1975), culminating finally in a detailed review in the *Annual Review of Microbiology* published in 1982 (Blakemore 1982). These bacteria contain "permanent" magnets in the form of nano-sized (e.g., of an average size of ~420 Å) cuboidal- to octahedral-shaped iron grains that allow them to orient themselves with the geomagnetic (0.25–0.65 gauss) or applied magnetic fields. Upon orientation, which is entirely passive in nature (e.g., even dead magnetotactic bacteria become aligned with an applied magnetic field), living bacteria actively swim along the field in a predominantly northward direction for bacteria harvested from the Northern Hemisphere and in a southward direction for bacteria from the southern hemisphere (Blakemore 1982).

After half a century, investigation of magnetotactic bacteria remains robust with ever-increasing understanding of the integration of the iron grains into higher order structures such as "magnetosomes"; comprehension of the biosynthetic machinery for these structures; and insights into the dynamic control of these microorganism's iron-processing physiology in changing environments (Araujo et al. 2016). A recent report by Lin and coauthors constructs a scenario where the initial biomineralization of intracellular iron nanoparticles across bacterial phyla occurred as a mechanism to cope with stress associated with reactive oxygen species (ROS) and ultraviolet (UV) radiation before the Earth gained a substantial ozone layer. The resulting iron-based biomolecules were proposed to be later co-opted for magnetoreception (Lin et al. 2020). In particular, the iron nanoparticles evolved the ability to form chains of several magnetosomes to create a magnetic moment along a cell's motility axis, facilitating parallel orientation with the Earth's magnetic field (Monteil and Lefevre 2020). Finally, proteins have been identified that produce scaffolds for magnetosome formation in magnetic-sensing bacterium; MamY is one membrane-bound protein involved in this process that works with two organizing proteins (MamK and MamJ) that actively organize magnetosomes (Toro-Nahuelpan et al. 2019). Although not directly related to human health or magnetic therapy in people, magnetotactic bacteria illustrate how even very "primitive" organisms have the ability to exploit magnetic sensing to enhance survival and gain an evolutionary advantage over competing species.

4.3.2 Plants

A review article published in 2014 noted that plants have several means of detecting geomagnetic fields, which affect their growth and development (Maffei 2014). However, at the time, the biochemical mechanisms were poorly defined. In the past few years, several mechanistic studies have been published to fill this void,

building on early studies in the field that posited that magnetosensitive responses were best explained by the cryptochrome-based radical pair mechanism (Dhiman and Galland 2018). Indeed, this mechanism is becoming increasingly well established, being supported by several studies that show the magnetic responses are co-dependent on the presence or absence of light (Agliassa et al. 2018; Pooam et al. 2019). These studies, however, have extended our understanding of plant-based magnetoreception beyond cryptochromes. For example, in one study, expression of the CAB-protein (chlorophyll *a*, *b*-binding protein) of *Arabidopsis* responded to 188 μT even in the absence of cryptochromes 1 and 2, indicating that this plant species had additional magnetosensing mechanisms. Nevertheless, cryptochrome sensing was still involved because the magnetic field effects were substantially enhanced under blue light, both negatively and positively (Dhiman and Galland 2018).

Arabidopsis responds to magnetic fields even under conditions of pulsed, blue light exposure, suggesting that radical pair formation by static magnetic fields "cannot act at a reaction step in the cryptochrome photocycle because these radical-pair intermediates (Trp°/FADH° or Tyr°/FADH°) have reported lifetimes only in the millisecond time scale" (Pooam et al. 2019). Ultimately, this study concludes that short-lived, transient reactions associated with flavin photoreduction cannot transduce magnetic field exposure; rather, the radical pairs impacted by the SMF must be formed during an extended period consistent with reoxidation of the flavin. This process is light-independent, requires molecular oxygen (Müller and Ahmad 2011), and involves the formation of ROS, leading to the conclusion that, while cryptochromes are magnetosensing, they themselves are not the actual magnetic sensors (Agliassa et al. 2018; Vanderstraeten et al. 2018; Pooam et al. 2019). Static magnetic fields may only preface flavin reoxidation, and third-party cellular factors might be involved. Although the mechanism for magnetosensing in plants remains incompletely defined, practical applications for SMF in plant biology are being reported. For example, SMFs promote seed germination and improve growth rigor in passion fruit plants (Menegatti et al. 2019).

4.3.3 Invertebrates

As described above, single-celled organisms such as magnetotactic bacteria have a remarkable ability to exploit magnetoreception-based sensing for directional movement. We next turn to more complex creatures to illustrate how a diversity of life forms—using added biochemical strategies—have the ability to sense and respond to magnetic fields.

4.3.3.1 Nematodes

The soil-dwelling nematode worm *Caenorhabditis elegans* is well studied in a laboratory setting and constitutes a facile model for investigation of "simple" multicellular organisms. For example, all of this creature's neurons are mapped, allowing for exquisitely sensitive investigation of brain function at the molecular and genetic levels. To complement and extend laboratory study of these nematodes, Vidal-Gadea and coauthors reported fascinating magnetoreception in "wild" *C. elegans* whose populations were isolated from different sites across the globe. These groups migrated at angles to an applied magnetic field that optimized vertical translation in their native soil, with northern- and southern-hemisphere worms displaying opposite migratory preferences (Vidal-Gadea et al. 2015). In these experiments, magnetotaxis was traced to genes expressed in the "amphid neurons with finger-like ciliated endings" (AfD) that previously had been implicated in thermosensation (Mori 1999). The specific genes involved in magnetotaxis in these cells include: two independent mutant alleles of ttx-1, important for AfD differentiation; the triple mutant lacking guanylyl cyclases, gcy-23, gcy-8, and gcy-18, which together are critical for AfD function; and two independent mutant alleles of each tax-4 and tax-2 genes that encode subunits of a cGMP-gated ion channel implicated in stimuli transduction in sensory neurons (Vidal-Gadea et al. 2015).

Additional factors that contribute to nematode magnetotaxis have been unraveled in follow-up studies (Vidal-Gadea et al. 2018; Bainbridge et al. 2019). In particular, temperature, humidity, and carbon dioxide levels were examined and discounted as possible alternative explanations for the worms' apparent responses to magnetic fields. Confirmation and new insights into biological mechanism were gained by showing that AfD neurons' calcium release profiles responded to alterations in magnetic field strength or orientation, and that the nematodes' ability to align with magnetic fields was lost as they entered a starvation state (i.e., no food intake for 30 min) (Vidal-Gadea et al. 2018). At the organism level, the orientation of nematodes while changing direction under the influence of a magnetic field was examined. It was found that nematodes prefer acute turns, aligning their bodies with the magnetic field before determining their ultimate migratory route. Interestingly, this process is similar to how periodic bodily alignments in birds underlie their "compass calibration with magnetic fields" (Bainbridge et al. 2019). There remain unexplained aspects of nematode magnetotaxis as well. For example, the worms have an odd preference for a leftward arcing trajectory toward the north magnetic pole (Vidal-Gadea et al. 2018).

4.3.3.2 Mollusks and Crustaceans

It has been known for over three decades that the marine mollusk *Tritonia diomedea* has an ability for geomagnetic orientation (Lohmann and Willows 1987) which,

similar to nematodes, has been traced to specific neurons. In a series of studies beginning in the late 1980s, Lohmann and colleagues reported that perturbation of geomagnetic strength magnetic fields change electrical activity in a single neuron (left pedal 5, LPd5) (Lohmann et al. 1991). Subsequent experiments identified four such neurons including LPd5, LPd6, RPd5, and RPd6 (Wang et al. 2004). These neurons fired an increased number of action potentials when the horizontal component of the ambient magnetic field was rotated. This response disappeared when all nerves emerging from the brain were cut, suggesting a peripheral locus for the geomagnetic transducer (Popescu and Willows 1999) and leading to speculation that magnetic biosensors that affect brain function (in general) "could be in the big toe, or anywhere" (Hand 2016).

In addition to mollusks, crustaceans constitute another prominent category of sea creatures that respond to GMFs as exemplified by the spiny lobster (Lohmann and Ernst 2014). As Lohmann and Ersnt explain, spiny lobsters have a magnetic compass of the polarity type, similar to salmon and mole rats, that determines north using the horizontal component of the geomagnetic field (Lohmann and Ernst 2014) [another type of magnetic compass found in nature is the inclination compass used by birds, insects, amphibians, and reptiles such as sea turtles which defines "poleward" as the direction where the angle between the magnetic field vector and gravity vector is the smallest (Vácha et al. 2008; Wiltschko et al. 2021)]. To date, similar to mollusks and nematodes, where the actual molecular-level biosensor remains unknown, magnetoreceptors in crustaceans have yet to be definitively identified. One possibility is that magnetite nanoparticles ~50 nm in diameter similar to those found in magnetotactic bacteria act as the receptors. Evidence in support of this idea includes higher than background levels of magnetic material in shrimp and barnacles that respond to geomagnetism; significantly, these species experience disorientation upon demagnetization of these putative magnetite-based receptors (Buskirk and O'Brien Jr. 2013) and can also have their preferred orientation deflected by re-magnetization of the putative magnetite particles in a different direction (Lohmann and Ernst 2014). A recent study found that ~10% of genes expressed in the central nervous system of spiny lobsters (*Panulirus argus*), a species of magnetically sensitive invertebrates, were differentially expressed in response to a magnetic pulse known to alter magnetic orientation behavior (Ernst et al. 2020). This study found that many of the altered genes encode proteins linked to iron regulation and oxidative stress consistent with the impact of a magnetic pulse on magnetite-based magnetoreceptors, but the authors caution that numerous of the affected genes have no known role in magnetotaxis.

Besides magnetite, a biosensing option in water-dwelling organisms is electromagnetic induction (as discussed in more detail in Sect. 4.3.3 below), which occurs when electrically conductive material moves through a magnetic field in any direction not parallel to the field (seawater is particularly conducive for transmission of electrical currents). As a result, positively and negatively charged particles move to the opposite sides of the object resulting in a voltage that depends on the velocity of the object relative to the magnetic field. In summary, because the Earth's magnetic field is a particularly pervasive cue in environments they inhabit, it is not surprising

that at least some crustaceans have evolved the ability to sense this field (Lohmann and Ernst 2014).

4.3.3.3 Insects

Crustaceans are arthropods, a phylum shared with insects; consequently it is not surprising that insects also provide numerous examples of magnetoreception and magnetotaxis including ants, bees, moths, and butterflies (de Oliveira et al. 2010; Dreyer et al. 2018; Wan et al. 2021). Magnetoreception has been particularly well studied in bees, no doubt because of their agricultural importance as pollinators. This role critically depends on their direction finding ability and innate compass (Vale and Acosta-Avalos 2021), which allows them to find and "remember" the location of food sources over distances up to five kilometers away. An early study showed that bees have magnetic remanence consistent with the presence of magnetite (Gould et al. 1978); subsequent electron paramagnetic resonance (EPR) imaging of honeybees showed magnetite was primarily located in the insects' abdomens (El-Jaick et al. 2001; Lambinet et al. 2017; Shaw et al. 2018).

Despite decades of study, the precise mechanism of magnetoreception in bees remains controversial. For example, bumblebees have iron-based granules exhibiting magnetic character located not only in their abdomens, as found in honeybees, but also at peripheral sites on their wings and heads (Jandacka et al. 2015). In addition to iron-based sensing, titanium appears to be utilized for magnetoreception in some species of Hymenoptera such as the migratory ant *Pachycondyla marginata* (Wajnberg et al. 2017; Fleischmann et al. 2020), thereby providing new models for how magnetite-based direction finding could work in these insects. Some evidence suggests that honeybees may have a dual sensing system that includes photochemical reactions (Válková and Vácha 2012; Fleischmann et al. 2018). Other studies have downplayed complementary mechanisms based on evidence that honeybee magnetoreception works in the total dark, where the requisite initiating photochemical reactions are not possible (Liang et al. 2016) (the radical pairs mechanism discussed in detail in Sect. 4.3.2 explains the requirement for light for chemical magnetoreception). Support for dual-sensor magnetoreception in insects is provided by vertebrates—in particular several species of birds—that appear to rely on both magnetite and chemical magnetoreception for direction finding and migration over very long distances. Another possibility is that bees and ants have both types of sensors that work independently, with chemical magnetosensing acting as a backup mechanism (Dovey et al. 2013; Fleischmann et al. 2020).

4.3.4 Vertebrates

4.3.4.1 Overview

The discussion up to now covered several "ancient" organisms that have unique biological abilities that often do not carry over to more advanced phyla such as vertebrates (for example, although magnetite appears in more advanced animals, the specialized arrangement of the iron crystals into magnetosomes observed in bacteria has not been found above the prokaryotic level). It is clear, however, that magnetoreception is found in many higher organisms including vertebrates and, at least in some cases, detection of the magnetic fields relies on non-magnetite-based biosensors. For example, several types of fish—exemplified by sharks—have specialized electrical sensing organs that are thought to also provide magnetoreception through induction, as discussed in Sect. 4.3.3. Other species, such as salmon, use magnetic fields for navigation over vast distances in the open ocean and for returning to the precise site of their birth to procreate, requiring the correct choice between multiple river junctions as they move upstream. In addition, many amphibians and reptiles have the ability to detect magnetic fields, but these examples will not be described further. Instead, we will briefly cover fish (Sect. 4.3.4.2) before moving onto birds (Sect. 4.3.4.3), where chemical magnetoreception (Sect. 4.3.2) has become well established. We will then cover mammals (Sect. 4.3.4.4) who share many biological similarities with humans and thus provide a reasonable scientific foundation to explain how magnetic fields can influence biological responses and work therapeutically in people.

4.3.4.2 Fish

Fish was one of the first groups of animals studied with respect to magnetoreception because of their extensive migratory patterns that depend of GMFs (Formicki et al. 2019). Early studies connected the ability of elasmobranchs (rays, skates, sharks, etc.) to both detect electrical fields and the magnetic field of the Earth, orienting themselves with these fields in the ocean (Murray 1960). There were soon many studies that established that multiple fish species were capable of magnetoreception (Fommel and McCleave 1973; Quinn 1980; Quinn and Brannon 1982; Chew and Brown 1989; Ogura et al. 1992; Paulin 1995). The primary receptors that detect and respond to magnetic cues in fish have yet to be unambiguously established (Anderson et al. 2017). Magnetite, however, has been widely assumed to be involved (Kirschvink et al. 2001; Naisbett-Jones et al. 2020). That said, without any currently known receptors or synaptic connections, it remains unclear how this information is processed by the fish. An interesting magnetosensing mechanism proposed for fish involves the inactivation of calcium dependent, intestinal proteases by hypomagnetic field-induced decreases in calpain activity in crucian carp (*Carassius carassius*) and common carp (*Cyprinus carpio*). Yet another provocative

idea is that fish host commensal magnetic-sensing bacteria benefiting from a hospitable living environment which provide the host magnetoreception ability (Natan and Vortman 2017; Boggs 2020).

4.3.4.3 Birds

Magnetoreception has been described in many birds (Wiltschko and Wiltschko 2019)—and indeed may be ubiquitous across the avian world—including remarkable examples such as the arctic tern that literally navigates from one end of the globe to the other. Although traveling shorter distances, the homing pigeon exemplifies precision direction finding ability that uses magnetic cues; mechanistically, a pigeon's homing ability was first reported to depend on magnetite-based receptors in the beak (Wiltschko and Wiltschko 2013) or the inner ear, which function in a light-independent manner (Nimpf et al. 2019). These magnetoreceptors, however, only record magnetic intensity and as such, are just one component of a bird's multifactorial navigation mapping ability. Increasing evidence suggests that birds, reminiscent of bees where a dual sensing system for GMFs has been proposed, use both magnetite and photoreceptors; as a caveat, not all studies have found evidence for magnetite-based magnetoreception in pigeons (Malkemper et al. 2019).

The light-sensing ability of birds has been linked to cryptochrome proteins, whose underlying chemistry is described in more detail in Sect. 4.3.2. Briefly here, these proteins have long been known to participate in circadian rhythms when located *in the nuclei* of certain retinal cells. Bolte and coworkers discovered forms of cryptochrome (Cry1a and Cry1b) *in the cytosol* of retinal cells in migratory birds (e.g., European robins and homing pigeons) that depend on both light and magnetic fields for direction finding (Bolte et al. 2016). The unique cytosolic localization of these cryptochromes suggests that they are not involved in circadian rhythms; instead, their non-nuclear localization implicates their involvement in photosensing-based magnetoreception. In recent years, cryptochrome 4a (Cry4a) has been increasingly linked to a combined light-sensing magnetoreception mechanism in birds (Günther et al. 2018; Pinzon-Rodriguez et al. 2018). Interestingly, Cry4a contains 4 radical-pair states, compared to 3 normally found in other organisms like plants, leading to the hypothesis that the fourth pair evolved to initiate magnetic signaling by interaction with a nearby tyrosine residue (Wong et al. 2021).

Overall, magnetic sensing in birds has three main characteristics, as summarized by Wiltschko and Wiltschko (2019). First, as already mentioned, it is an inclination compass that does not distinguish between north and south, instead it recognizes poleward field lines that run downward and equatorward fields where lines run upward. Second, avian magnetoreception is narrowly tuned to the intensity of the ambient magnetic fields; higher or lower intensities cause disorientation. Finally, it requires wavelengths of light ranging from ultraviolet to ~565 nm (green light); disorientation results from higher wavelengths.

4.3.4.4 Mammals

The elucidation of magnetoreception in mammals has lagged behind other types of organisms such as bacteria and birds where much is now known (even though mysteries remain). Nevertheless, even a decade ago, there were intriguing pieces of evidence supporting magnetic field sensing in mammals (Begall et al. 2014). This preliminary evidence suggested that mammals, human's closest evolutionary relatives, *do* respond to magnetic fields in several ways. In particular, magnetoreception studies have suggested that mammals can utilize GMFs for homing and direction finding by exploiting certain magnetotactic abilities documented across other phyla. Briefly, these studies showed that cetaceans can migrate thousands of kilometers based on magnetic cues (Granger et al. 2020); moles rats have brain neurons sensitive to magnetic stimuli that affect nest building orientation (Němec et al. 2001; Caspar et al. 2020); other rodents displaced hundreds of meters (or more) from their homes can return successfully based in part on magnetic homing; bats preferentially build nests aligned to magnetic fields and have similar roosting preferences attributed to magnetic-sensing components of the cornea (Lindecke et al. 2021); and cattle, sheep, deer and even dogs preferentially (for reasons otherwise unclear) align their bodies along N–S magnetic axes (Begall et al. 2014).

Indications are emerging that mammals can exploit geomagnetism for reasons beyond direction finding and homing. For example, the success of red foxes in hunting mice is correlated with the alignment of the direction of jumping attacks with GMFs when the fox's vision is obscured by snow or high vegetation (Červený et al. 2011). Relevant to the ultimate objective of this chapter and book—which is the evaluation of magnetic field therapy in humans—mice (presumably when safe from being hunted from red foxes) experience changes in stress-induced analgesia (the inability to feel pain) dependent on SMF exposure (Betancur et al. 1994). Subsequent studies showed that shielding of the ambient magnetic fields to produce hypomagnetic fields (HMFs) reduces stress-induced analgesia in rodents (Choleris et al. 2002; Prato et al. 2005). These and other similar studies have established that exposure, or in other cases a lack of exposure, to magnetic fields can affect mammalian biology in biomedically relevant ways. Importantly, in theory these effects be complemented, augmented, and amplified by using stronger field strengths of specialized treatment devices available in clinical settings.

Overall, rodent-based studies over the past several years provide a foundation for the idea that magnetic therapy is viable in mammals even at low field strengths. One of the most compelling medical indications is for pain management. For example, a study where toothaches were induced in mice showed that exposure to SMF resulted in a lower "mouse grimace score" that was connected biochemically to reduced expression of P2X3 receptors implicated in the generation of pathological pain (Zhu et al. 2017). More broadly, approximately two thirds of studies show positive analgesic effects of SMFs (Fan et al. 2021). At the cellular level, SMF exposure enhanced wound healing in murine cells in vitro in a scratch assay (Ebrahimdamavandi and Mobasheri 2019). SMF also promotes wound healing

in vivo; for example, in one study elevated epithelialization and revascularization were observed in diabetic mice (Shang et al. 2019), and a modest improvement in respiratory rate and other aspects of lung health were observed in mice subject to radiation damage to the lungs (Rubinstein et al. 2018). There are even indications that SMF treatment elicits anti-cancer responses in mice by decreasing telomerase expression and cell migration (Fan et al. 2020), or in combination with immunotherapeutics such as cetuximab (Gellrich et al. 2018). Another interesting study built on prior evidence that bone reformation is enhanced by SMFs by examining mice subjected to hindlimb unloading and reloading (Yang et al. 2021b). Finally, there is even evidence that intracranially SMF-treated mice show decreased amyloid plaque accumulation in a mouse model of Alzheimer's disease (Lin et al. 2021) and exposure to SMF can ameliorate type 2 diabetes (Carter et al. 2020).

4.4 Types of Biological Magnetoreceptors

The overview of magnetoreception provided above in Sect. 4.2 highlighted two major molecular-level mechanisms underlying magnetic field detection across several classes of diverse organisms. The first, and most prevalent, is the exploitation of magnetite across many types of life for direction finding and additional biological responses (magnetite is discussed further in Sect. 4.3.1). Evidence is also consolidating behind the chemistry-based radical pairs mechanism (RPM) as a second modality for magnetoreception. There have been various biological iterations of the RPM in which cryptochrome proteins are putatively utilized by organisms ranging from bumblebees to birds as part of their magnetic compass, and even to mice where pain sensing was found close to 30 years ago to be modulated by both light and magnetic field exposure (Betancur et al. 1994). Cryptochromes and other chemistry-based possibilities for magnetoreception are discussed further in Sect. 4.3.2. Finally, a third more specialized mode of magnetic field detection, electrical induction, is covered in Sect. 4.3.3.

4.4.1 Magnetite

4.4.1.1 Structure and Biosynthesis in Prokaryotes

Magnetite can be considered to be the original biological magnetoreceptor. The "original" designation is based both on evolutionary history with magnetite present in early-evolving life forms such as bacteria and unicellular algae (Lefèvre and Bazylinski 2013), and because it was the first magnetic biosensor discovered and characterized by modern science, being linked to behavioral responses in living organisms for half a century or more (Bellini 1963; Blakemore 1975). Magnetite is common in the abiotic mineral world, comprising a major source of iron ore;

chemically, magnetite is crystalline iron oxide (Fe_3O_4), a ferromagnetic crystal form which becomes a permanent magnet after exposure to an applied magnetic field. In bacteria, individual magnetite particle sizes range from 35 to 120 nm with a particle size distribution much narrower than possible using chemical synthetic methods (Kahani and Yagini 2014); the size range of prokaryote-made magnetite is consistent with single-domain crystals that can be as small as 20 nm or as large as 100 nm (Mirabello et al. 2016). In magnetotactic bacteria, individual magnetite crystals are arranged into "magnetosomes," which are aggregates (usually linear chains) of ~20 magnetite crystals aligned along the long axis of the cell. Each magnetite crystal is surrounded by a membrane and is connected to the cell wall through cytoskeletal filaments (Mirabello et al. 2016; Toro-Nahuelpan et al. 2019). Magnetosome biosynthesis in prokaryotes, which involves the formation of these unique mineralized organelles, is increasingly being unraveled. It is now known to require many genes that initiate nucleation and participate in the growth of the crystals. These genes are organized in operons located in what is known as the "magnetosome island" (Arakaki et al. 2008; Murat et al. 2010; Lower and Bazylinski 2013; Mirabello et al. 2016; Ben-Shimon and Zarivach 2021).

4.4.1.2 Distribution and Function in Higher Organisms Including Humans

Magnetite has been discovered and studied across many species; it has now been detected in crustaceans, insects, birds, salmon, sea turtles, and other animals (even mammals such as cattle) that can orient themselves with respect to the Earth's magnetic field. Indeed, a recent examination of 13 eukaryotes found 11 magnetosome gene homologs universally present, leading to speculation that magnetite biomineralization represents an example of deep homology across eukaryotic life (Bellinger et al. 2022). In practice, the presence of magnetite in people has been reported in the human brain (Kirschvink et al. 1992; Gilder et al. 2018; Khan and Cohen 2018; Wang et al. 2019) as well as in the heart, spleen, and liver (Grassi-Schultheiss et al. 1997; Schultheiss-Grassi et al. 1999). Magnetite isolated from higher animals typically exists as single-domain crystals similar to those found as chains in magnetosomes in magnetotactic bacteria (Johnsen and Lohmann 2008). The origin and source of magnetite in higher organisms such as people remain unclear because counterparts to biosynthetic and structure-organizing genes in bacteria [Mms5, Mms6, Mms7 (MamD), Mms13 (MamC), MamF, ManG, and MmsF (Mirabello et al. 2016)] do not seem to be present in eukaryotes. Nevertheless, spontaneous chemical crystallization of magnetite, while resulting in different size distributions and shapes than found in bacteria, can remain active for magnetoreception (Kahani and Yagini 2014; Leão et al. 2020).

Mechanistically, there are several postulated ways for magnetite crystals to transduce geomagnetic field information to the nervous or other organ systems. These mechanisms are guided by lessons learned from bacteria, where each magnetite crystal is surrounded by several proteins and a membrane connected to the cell

wall through cytoskeletal filaments capable of force transduction (Mirabello et al. 2016; Ben-Shimon and Zarivach 2021). This molecular arrangement allows torque to be transmitted from the magnetosome to other parts of a cell via the cytoskeleton when the crystalline magnetite nanoparticles attempt to rotate to align with the GMF or another external field. In higher organisms, if a similar system was in place, force could be transduced to secondary receptors (such as stretch receptors, hair cells, or mechanoreceptors); another possibility is that the rotation of intracellular magnetite crystals might directly or indirectly open ion channels (Cadiou and McNaughton 2010).

Indirect evidence for physical connections between magnetite and the cytoskeleton comes from the aforementioned studies where shrimp and barnacles that experience disorientation upon demagnetization (Buskirk and O'Brien Jr. 2013) can have their preferred orientation deflected by re-magnetization of the putative magnetite particles in a different direction (Lohmann and Ernst 2014). If magnetite crystals could freely rotate, they would quickly adopt random orientations inconsistent with these effects, which require all (or at least some) of the magnetite in the organism's body remain aligned in a certain way. Accordingly, magnetite presumably must be tethered to larger biomacromolecules, such as the cytoskeleton (which plays a dual role in both immobilizing the magnetite crystals and transducing force when the nanoparticles attempt to rotate to maintain alignment with the GMF or other magnetic field). Figure 4.2 conceptually illustrates the tethering of magnetite to the cytoskeleton and force transduction to membrane components while Cadiou and McNaughton present a detailed description of how this type of force transduction hypothetically functions in eukaryotic cells (Cadiou and McNaughton 2010).

4.4.2 Chemical Magnetosensing

4.4.2.1 Background: The Chemical Basis of the Radical Pair Mechanism (RPM)

Chemical reactions that proceed through radical intermediates can be influenced by magnetic field effects (MFEs) that alter reaction rate, yield, or product distribution (Rodgers 2009); the "radical pair mechanism" (RPM) underlies these effects. An RPM-influenced reaction begins when a ground-state precursor species (e.g., "A and B") are excited to produce two singlet radicals, i.e., a spin-correlated radical pair (RP); the singlet RP electrons can then undergo a spin-selective reaction to produce the singlet product (Fig. 4.3). However, if coherent evolution of the spin state converts singlet RPs to triplet RPs on a similar (or faster) time scales as singlet product formation, the triplet product can be formed, resulting in either different reaction kinetics or product composition for the chemical reaction.

The role of magnetism comes into play when S → T (singlet to triplet) conversion and the reverse T → S conversion of the spin-correlated RP are driven by magnetic interactions. Remarkably, even a weak applied magnetic field, with much smaller

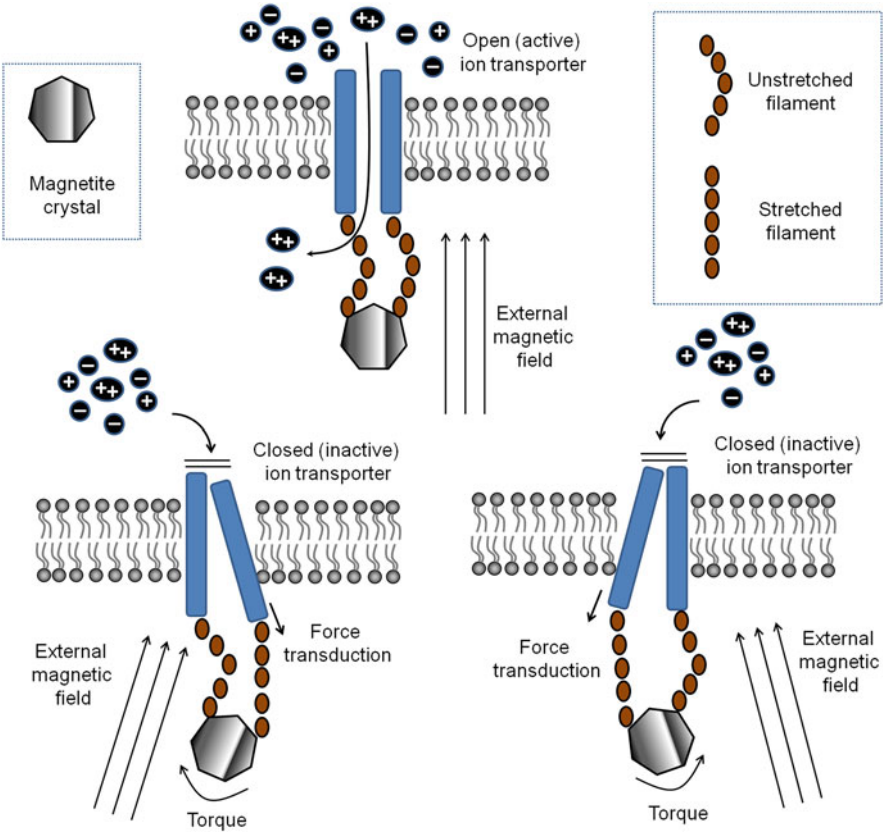

Fig. 4.2 Conceptualization of magnetite-based force transduction. (**Top**) An ion channel in the "open" conformation is shown along with connections to an intracellular magnetite particle via unstretched filaments; under these conditions the magnetic field of the magnetite is aligned with an externally applied field. (**Bottom**) Upon misalignment of the magnetite and the external field, the magnetite turns in an attempt to re-align with the external field resulting in the generation of torque that can stretch the filaments and, in the process, transduce force to membrane elements (in this depiction, the ion channel becomes distorted and subsequently experiences changes in activity)

effects on the reactants than factors such as thermal motion at physiological temperature, can profoundly influence product formation in an RPM reaction. A simplifying analogy provided by Rodgers to describe the influence of an external magnetic field is to consider a train approaching a railway switch (Rodgers 2009). The train is being propelled by a locomotive, which requires considerable energy, but the final destination (i.e., the composition of the reaction products) and time it takes to reach it (i.e., the reaction kinetics) completely depend on the expenditure of a small amount of energy (e.g., an amount that can be provided by a single person in a few seconds of effort) to change a junction switch in the track from one route to

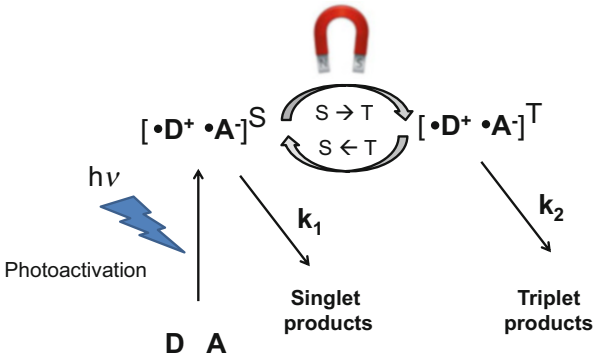

Fig. 4.3 Diagram outlining the radical pair mechanism (RPM). A spin-coupled radical pair is generated (by photoactivation in magnetoreception) resulting in a donor molecule (**D**) transferring an electron to an acceptor molecule (**A**). An external magnetic field affects interconversion between singlet (S) and triplet states (T) of the radical pair; typically, the presence of an applied field increases the transient abundance of the triplet state resulting in more rapid production of triplet products (i.e., k_2 compared to k_1 increases upon application of an external force, or in the case of GMF sensing, appropriate alignment of the field with the reacting spin-coupled radical pair). [Illustration based on (Ritz et al. 2000; Rodgers 2009), and Wikipedia]

another destination. This relatively tiny force is equivalent to the role that magnetic fields—even weak GMFs—can play in determining the outcome of RPM reactions.

4.4.2.2 The RPM in Magnetic Field Biosensing

Based on the above explanation, RPM reactions provide a second biological transducer for weak SMFs (after magnetite) such as GMFs. The proposed mechanism requires the production of initiating free radical intermediates. In purely chemical systems, appropriate radical-inducing catalysts could be introduced into the system for this purpose. In biological systems, where such catalysts do not play a role, the production of the initiating radical pair (RP) is generally believed to require the absorption of a photon (i.e., from visible light). Accordingly, the receptors involved in such sensing need to be located on or within a few hundreds of microns of the surface of an organism where ambient light could penetrate. Most logically, these receptors would be located in the eye, which is already optimized for photosensing; based on this reasoning, leading candidates for magnetoreception are cryptochrome proteins (Karki et al. 2021). Ritz and coauthors outlined how cryptochromes could function magnetoreception in the year 2000 (Ritz et al. 2000). They described how, upon exposure to blue light, these proteins transfer an electron to flavin adenine dinucleotide (FAD) resulting in both the protein and the flavonoid having unpaired electrons—i.e., the "radical pair" required for an RPM reaction (Kavet and Brain 2021). It should be noted that the exact RPM reactants remain ambiguous. It is generally thought that in addition to the flavin, the other radical pair is one of three

tryptophan residues on the cryptochrome protein. However, it has also been speculated that the other reactant might be ascorbic acid rather than a tryptophan (Lee et al. 2014). Nevertheless, an RPM reaction "activates" cryptochrome proteins for chemical magnetosensing, often in conjunction with magnetite-based mechanisms as discussed above.

Cryptochrome reactions depend on continuous photoexcitation (Kattnig et al. 2016), explaining the requirement for both light and the presence of a magnetic field—i.e., the "dual sensing" mechanisms mentioned above for bees, birds, and mice. In an RPM reaction, the presence, absence, relative strength, and orientation of the magnetic field affects the length of time cryptochrome remains activated due to the correlated spins and bearing of the two unpaired electrons being dependent on said field (Ritz et al. 2000). In turn, activation of cryptochrome affects the light sensitivity of retinal neurons, meaning that a bird (or a bee) can see the color phase shift caused by the magnetic field (Ritz et al. 2000). In practice, the dependence of dual sensing mechanisms on continuous photoexcitation can be used empirically to deconvolute magnetosensing behavior. For example, bees are known to have the biochemical machinery for RPM magnetoreception, but their ability to utilize their magnetic compass in the total dark indicates that magnetic direction finding ability can function solely via a magnetite mechanism (Liang et al. 2016).

4.4.3 Electromagnetic Induction

4.4.3.1 Biological Precedent for Induction: The Ampullae of Lorenzini

Sharks, stingrays, and certain cartilaginous fish have electroreceptive organs known as ampullae of Lorenzini that can detect changes to electric potential; these specialized structures allow these sea creatures detect direct electric currents in water and help sense the weak electric fields of prey and predators (Murray 1960). The ampullae of Lorenzini also allow sharks (and other animals with these physiological structures) to detect even very weak magnetic fields (Meyer et al. 2005). This ability results from the phenomenon where the movement of electrically conductive material through a magnetic field in any direction other than parallel to the field lines results in the migration of positively and negatively charged particles to opposite sides of the object (Roth 2012). As a result, a voltage is generated that depends on the velocity of the object's motion relative to the magnetic field. From a physics perspective, this phenomenon is known as the "Hall effect." It states that a magnetic field exerts force on a moving ionic current and, as a result, a magnetic field perpendicular to the flow of an electric current will exert force to deflect and separate the charged ions.

Specialized biological systems are able to detect and respond to the mismatch in electrical charge potential as the host organism moves through misaligned SMFs. In the past few years, glycosaminoglycan (GAG) structures and other polysaccharides have been implicated as prime candidates for transducing electric currents induced

by the Hall effect in the ampullae of Lorenzini. In particular, the hydrated keratin sulfate jelly-like material found in this organ is the highest naturally occurring proton conducting substance (Josberger et al. 2016; Selberg et al. 2019). Similarly, the polysaccharide chitin is widely distributed in the series of gel-filled canals that comprise the ampullae of Lorenzini in chondrichthyan fish, and is proposed to play a similar electrosensing role (Phillips et al. 2020). Interestingly, mucin-like, glycan-rich macromolecules in various mammalian species such as the Guiana dolphin and egg-laying mammals such as the duck-billed platypus and echidna are proposed to play a role in magneto-electro-location (although the composition of their mucin-rich glands is yet to be fully characterized) similar to the ampullae of Lorenzini (Melrose 2019).

4.4.3.2 The "Hall Effect"—Relevance Beyond Specialized Electroreceptive Organs?

The Hall effect has been—at least on the internet—used to explain the effects of magnetic fields in biological settings in some clearly misguided ways. For example, one claim is that electrons (being regarded as "charged particles") orbiting the nucleus of an atom (i.e., they are presumed to be moving in space) are propelled to higher velocities, thereby enhancing chemical reactivity. In reality, applied magnetic fields only influence chemical reactivity through electron spin effects via the specialized RPM reactions described above in Sect. 4.3.2. Another common misconception is that the Hall effect can be used to explain changes to blood flow observed upon magnetic field exposure. While it is true that blood does contain copious amounts of charged (e.g., sodium and chloride ions) and paramagnetic (e.g., hemoglobin in certain oxidation states) entities, the physical forces generated by the Hall effect are dwarfed (orders of magnitude smaller) compared to the kinetic energy associated with blood flow (which is generated through the mechanical action of the heart) and the thermal motion of biomolecules at body temperature. As a result, the idea that electromagnetic induction plays a role (outside of specialized ampullae of Lorenzini) in transducing magnetic field exposure into biological response is often met with disbelief.

4.5 Mechanisms for Static Magnetic Field Effects on Human Biology

Now that we have outlined biosensors found in the natural biological world for magnetoreception, we will revisit each of them in the context of human biology and provide a synopsis whether they plausibly play a role in magnetotherapy. As will be described in Sect. 4.5.1, the established magnetosensors do not provide a satisfying

explanation for responses observed in humans, spurring speculation in Sect. 4.4.2 about "other" possibilities.

4.5.1 "Established" Biosensors/Magnetoreceptors

4.5.1.1 Magnetite

Over the past 30 years, there have been periodic reports of magnetic iron (i.e., magnetite) in the human body with some of these studies being debunked because of possible contamination (Hand 2016). For context, many of these studies came from the same era when aluminum "contamination" from cooking utensils and containers was (in retrospect implausibly) linked to plaques associated with Alzheimer's disease (Savory et al. 1996). Other reports of magnetite in humans, however, have remained plausible. One such study was published in the Proceedings of the National Academy (USA) that reported detailed parameters about magnetite-like iron assemblages in the human brain (Kirschvink et al. 1992). These crystal structures resembled magnetite from magnetotactic bacteria and fish and were present at minimum levels of five million single-domain crystals per gram for most types of brain tissues. Certain regions of the brain (e.g., pia- and dura-derived samples) had ~20-fold higher levels; further, the magnetite occurred in clumps of 50–100 crystals. The magnetite nanoparticles distributed (or based on the numbers outlined in the next section, a better description might be "sparsely scattered") through neuronal and astroglial membranes have been proposed to play roles in perception, transduction, and storage of information that arrives to the neocortex (Banaclocha et al. 2010).

To provide context for these findings, 1 g of brain tissue has roughly one billion cells. Accordingly, if the magnetite clumps were intracellular, only about one in 500 to one in 20,000 cells—depending on the exact size of the clumps and which part of the brain was under analysis—could contain a magnetite clump. If the clumps were extracellular [which is not consistent with the proposed role of magnetite-based force/signal transduction in eukaryotic cells, as outlined in Fig. 4.2 (Cadiou and McNaughton 2010)], additional cells could be directly impacted by, or interact with, the magnetite. Either way, based on the reported amount of magnetite, only a relatively fraction of brain cells could be involved in magnetoreception through a magnetite-based mechanism.

Another comparison is that honeybees have approximately 10^8 magnetite crystals in their bodies (Kirschvink and Gould 1981); based on a mass of ~100 mg, a bee has ~10^9 (one billion) copies of magnetite per gram or about 200-fold more on a mass basis. While it is at least theoretically plausible that only a minor subset of human neural cells might be involved in magnetosensing, the search for these cells constitutes a veritable "needle in the haystack" scenario. In the three decades, since this PNAS report was published (Kirschvink et al. 1992), magnetoreception via magnetite in the human brain remains unproven. Showing remarkable persistence,

however, the lead author of the PNAS study, Joe Kirschvink, has continued to pursue the possibility of magnetic sensing in the human brain and was featured in a Science (the magazine) news article describing how he and his colleagues are embarking on the next generation of studies to pursue the elusive goal of obtaining "definitive" proof for magnetotherapy or other magnetic field effects in humans (Hand 2016).

4.5.1.2 Chemical Magnetoreception Via Cryptochromes

As just mentioned, efforts to confirm that magnetoreception exists in humans continue with exciting new initiatives being planned (Hand 2016; Chae et al. 2019). It should be noted that in addition to the decades old hypothesis that the human brain (and other tissue) contains magnetite, humans may have a dual sensing system similar to bees, birds, and mice based on cryptochromes. Two complementary lines of evidence support this idea. First, geomagnetic fields can influence the light sensitivity of the human visual system (Thoss et al. 2000, 2002), evoking cryptochrome-based systems found in other species. Second, a biochemical foundation for this hypothesis is falling into place. In particular, humans express two cryptochromes (hCRY1 and hCRY2) in their eyes showing that—at least in theory—humans have the biochemical machinery for chemical magnetoreception (up to now, these proteins have primarily been linked to circadian rhythms). Foley and coworkers performed a critical experiment in support of this hypothesis by taking a transgenic cross-species approach to show that hCRY2, which is heavily expressed in the retina, can function as a magnetic field sensor in the magnetoreception system of *Drosophila* in a light-dependent manner (Foley et al. 2011). Although this result showed that hCRY2 has the molecular capability to function as a light-sensitive magnetosensor, it must be emphasized that as-of-yet there is no firm evidence that hCRY proteins function in magnetosensing roles in humans or even in other mammals such as dogs and apes that exhibit certain GMF-sensing abilities and, perhaps entirely coincidentally, express cryptochromes in the retina (Nießner et al. 2016). In concert with biochemical evidence that humans have light-sensing magnetoreception machinery, there are reports that people can respond behaviorally. For example, one study showed that food-deprived men (but not women) used geomagnetic cues for orientation (Chae et al. 2019).

4.5.1.3 Induction: Revisiting the Effects of SMFs on Red Blood Cells

The idea that magnetic fields can influence blood flow and cardiovascular circulation is pervasive. As mentioned earlier, an often-mentioned but fallacious scientific basis for this premise is that an applied magnetic field has inductive effects on iron-laden red blood cells (RBCs) and influences the overall circulation of the blood. On the one hand, this idea is reasonable considering that RBCs typically constitute 40% or more of the volume of blood. Accordingly, if magnetic field-associated induction really

was biologically significant, the overall circulation of the blood easily could be affected. In reality, any inductive "Hall effect" force is too weak to measurably affect blood constituents. Secondly, there is considerable confusion and misinformation that iron in RBCs is "magnetic." Clearly it is not ferromagnetic because it is not organized in crystalline magnetite form. Iron in RBCs, however, is paramagnetic, which can have diagnostic value with certain caveats. For example, a paper from 1961 titled "Problems in the Measurement of Blood Flow by Magnetic Induction" (Wyatt 1961) reported technical issues that bedeviled already then 20 year old efforts to exploit the electromagnetic fields generated by movement of paramagnetic iron found in RBCs to measure blood circulation.

In the intervening years, the study of iron in RBCs under the influence of magnetic fields has become increasingly sophisticated. For example, a 2003 publication by Zborowski et al. (2003) outlined the magnetophoretic mobility of different populations of deoxy and oxygenated erythrocytes (i.e., RBCs). This study showed that, with the development of a new technology, cell tracking velocimetry, it was possible to measure the migration velocity of deoxygenated and metHb-containing erythrocytes exposed to a magnetic field of 1.4 T (i.e. a, MRI-strength field). In this study, erythrocytes with 100% deoxygenated hemoglobin had a magnetophoretic mobility of 3.86×10^{-6} mm^3 s/kg compared to 3.66×10^{-6} mm^3 s/kg for erythrocytes containing 100% metHb; in other words, both of these forms of hemoglobin displayed paramagnetic properties. By comparison, oxygenated erythrocytes had magnetophoretic mobilities ranging from -0.2×10^{-6} mm^3 s/kg to $+0.30 \times 10^{-6}$, indicating that these cells were primarily diamagnetic (Zborowski et al. 2003). The detection and analysis of these properties have matured since 2003, allowing dielectrophoretic and magnetophoretic methods to now be used for diagnosis of medical conditions such as malaria parasite-infected red blood cells (Kasetsirikul et al. 2016).

Although RBCs can now be studied and leveraged for diagnostic medical tests by exploiting their magnetic properties, it is less clear that externally applied magnetic fields have legitimate therapeutic effects on blood circulation, as is often claimed by vendors of "magnetic therapy" products. In particular, the impact of applied magnetic fields on unpaired electrons in contexts other than RPM reactions (as discussed above) is negligible under weak (i.e., geomagnetic strength) fields, and even strong (i.e., 1.3–3 T MRI-strength fields) have negligible "chemical" effects. Indeed, a comparison the impact of lower field strengths from ~6 to 40 mT showed negligible, inconsistent, and variable effects on platelet and RBC counts, hemoglobin, hematocrit, and other blood-related parameters in rats (Mustafa et al. 2020). The lack of any such clear effect is evidenced in rather lax regulatory oversight by agencies including the US FDA, which allows "wellness" magnetic field devices to be marketed to "treat" almost any type of ailment because safety is not an issue (Anonymous 2015). As a caveat, the word "treat" in the previous sentence is not completely accurate in a medical sense because the FDA specifically prohibits claims of therapeutic efficacy against any particular disease condition for magnetic field generating devices.

4.5.2 "Other" Human Biosensors

4.5.2.1 Human Cells Appear to Have Additional Magnetosensing Capacity

Evidence exists that humans can respond to magnetic fields. For example, magnetic fields influence the geomagnetic field sensitivity of our eyes (Thoss and Bartsch 2007). This evidence remains controversial because (apart from unscientific internet claims) a lack of clear evidence exists to explain how the three "canonical" modes of magnetoreception (magnetite, chemical magnetosensing, and induction) function in humans. In particular, even the first two modes required for visual geomagnetic perception in other species remain largely mysterious in humans. In part, progress is slow because many experiments performed with lower species (e.g., dissection of brains in living nematodes to uncover specific neurons involved in magnetoreception) cannot be performed in humans. In a way, ethical (commonsense, really) considerations that prevent such experimentation in humans have been a blessing in disguise, forcing researchers to use cell lines a surrogate for in vivo testing. These studies have led to the discovery of responses to magnetic fields at the cell level that do not involve any of the three "established" modes of magnetoreception. For example, immobilized cells maintained in dark incubators with unchanging SMFs are not expected to exhibit chemical magnetosensing (because they're kept in the dark) or induction (because they do not move); similarly, there is no plausible mechanism for the presence of magnetite in these cells. To briefly illustrate this point, we next mention both an outside example based on HMF exposure (Sect. 4.5.2.2) and work from this author's lab using moderate strength SMFs (Sect. 4.5.2.3).

4.5.2.2 HMF Effects on Cell Behavior Are Mediated by the Cytoskeleton

Recently, Mo and coauthors found that HMFs repress expressions of genes associated with cell migration and cytoskeleton assembly in human neuroblastoma cells grown in cell culture conditions that were not plausibly subject to any of the magnetite, chemical magnetoreception, or induction mechanisms (Mo et al. 2016). Going beyond analysis of gene expression, they showed that HMF modulated "whole cell" behaviors in SH-SY5Y cells including control of cell morphology, adhesion, and motility, tracing these changes to the actin cytoskeleton. This study suggested that the elimination of the geomagnetic field affects the assembly of the motility-related actin cytoskeleton, and implicates F-actin as a target of HMF exposure and positions it as a potential novel mediator of GMF sensation (Mo et al. 2016). In a more recent study, the cytoskeleton of osteocytes was altered by SMF exposure along with changes to cellular morphology, function-related protein expression, cytokine secretion, and iron metabolism (Yang et al. 2021a);

as a caveat, these multifaceted responses required exposure to a rarely available 16 T magnetic field.

4.5.2.3 SMF Effects on Lipid Membranes and Downstream Signaling

The author's laboratory has published two studies (Wang et al. 2009, 2010) that implicate biological membranes as the "biosensor" for magnetic fields in the apparent absence of canonical chemical (i.e., cryptochrome-mediated) or magnetite mechanisms. These studies were based on literature reports that SMFs alter the biophysical properties of lipids (Braganza et al. 1984) and by extension, higher order structures such as lipid bilayers (Rosen 2003b; De Nicola et al. 2006; Nuccitelli et al. 2006). Based on these studies, we postulated that biological membranes were a plausible "biosensor" for magnetoreception in cell culture investigations where magnetite was absent and the cells involved had no obvious light-sensing ability. Further, based on the threshold of ~0.2 T reportedly required for SMFs to have an impact on biological membranes (Braganza et al. 1984), we undertook two studies where cells were treated with 0.23–0.28 T SMFs (the variation is due to different placement of tissue culture plates in the incubator device, *see* Fig. 4.1).

In one study, in part spurred by clinical efforts to use ~0.3 T SMFs to treat Parkinson's disease (PD), we monitored the impact of similar magnetic fields on the adenosine A_{2A} receptor ($A_{2A}R$) in the PC12 rat adrenal pheochromocytoma cell line that displays metabolic features of PD (Wang et al. 2010). We found that SMF reproduced several responses elicited by ZM241385, a selective $A_{2A}R$ antagonist, including altered calcium flux, increased ATP levels, reduced cAMP levels, reduced nitric oxide production, reduced p44/42 MAPK phosphorylation, inhibited proliferation, and reduced iron uptake. Biological responses to ZM241385 result from direct binding to $A_{2A}R$. By contrast, SMF—not being a conventional small molecule pharmacological agent—must elicit cellular responses through a fundamentally different mode of action. A plausible mechanism, outlined in cartoon form in Fig. 4.4, is that ~0.25 T SMF exposure directly alters the biophysical properties of lipid bilayers, which in turn rapidly modulates ion channel activity (Rosen 2003a) and thereby perturbs the intra-and extracellular levels of Ca^{2+} levels (Wang et al. 2009, 2010).

4.5.2.4 Lipid Membrane-Based Mechanisms Can (Speculatively) Account for Biphasic Kinetic Responses to Constant Magnetic Field Exposure

In a separate study, human embryoid body-derived (hEBD) LVEC cells were treated with the ~0.25 T fields for time periods of 15, 30, and 60 min, 2, 4, and 8 h, and finally up to 7 days (Wang et al. 2009). Software analysis of gene expression obtained by Affymetrix mRNA profiling of these cells showed that nine signaling

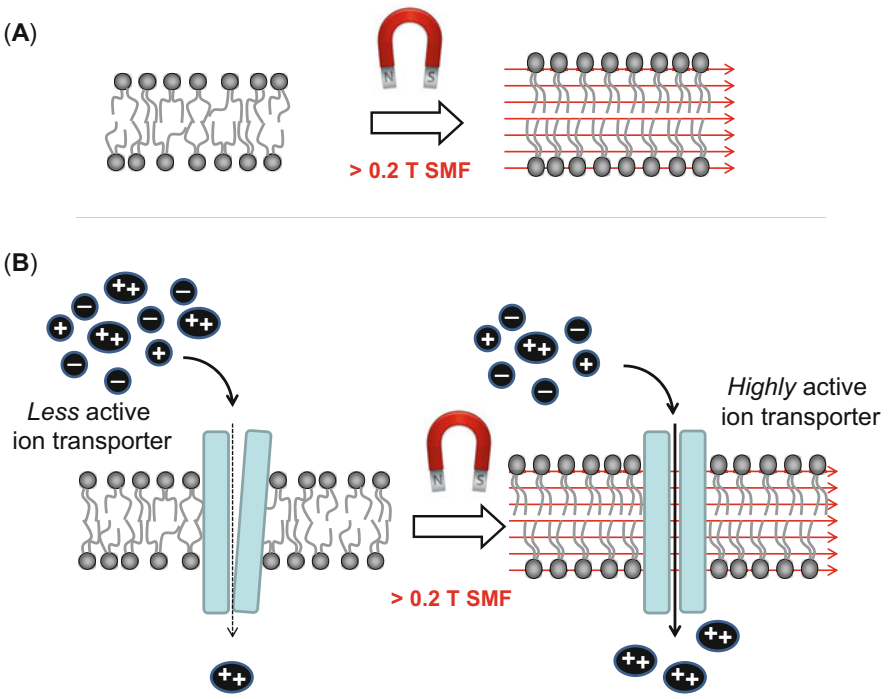

Fig. 4.4 Proposed mechanism for direct effects of SMF on biological membranes. (**a**) Based on literature reports of SMF effects on lipids (Braganza et al. 1984), we propose that field strengths of >0.2 T impose superdiamagnetic organization on lipid bilayers. (**b**) Extending this concept to biological membranes (i.e., lipid bilayers with embedded proteins such as the cartoon of an ion channel as shown), we found that calcium ion flux rapidly responds to ~0.25 T fields (Wang et al. 2009, 2010). This response can be explained by allosteric regulation of ion channel activity by the relative membrane organization and biophysical properties in presence and absence of the external SMF. This response is conceptually similar to a variation of the magnetite-based mechanism shown in Fig. 4.2 where ion channel activity is not modulated by direct action on the channel (as shown in that figure) but instead results from magnetite's action on *cis* elements in a membrane that—upon perturbing membrane structure or organization—have an effect on proximally located ion channels [this mechanism is described in detail elsewhere (Cadiou and McNaughton 2010)]. [Reprinted from (Wang et al. 2009, 2010), open access]

networks responded to SMF; of these, detailed biochemical validation was performed for the network linked to the inflammatory cytokine interleukin 6 (IL-6). We found a biphasic response to SMF exposure (Fig. 4.5) where short-term (<4 h) activation of IL-6 mRNA expression occurred with coordinated up-regulation of toll-like receptor-4 (TLR4) and ST3GAL5, phosphorylation of p38, and calcium efflux. Interestingly, the initial multifaceted up-regulation of IL-6 mRNA was already being attenuated by 24 h but actual production of secreted IL-6 did not peak until day 2 after which it dropped to sub-steady levels by day 4.

A biochemical mechanism—outlined in Fig. 4.6—for the biphasic kinetic response that puzzlingly occurred in the constant presence of a steady SMF can be

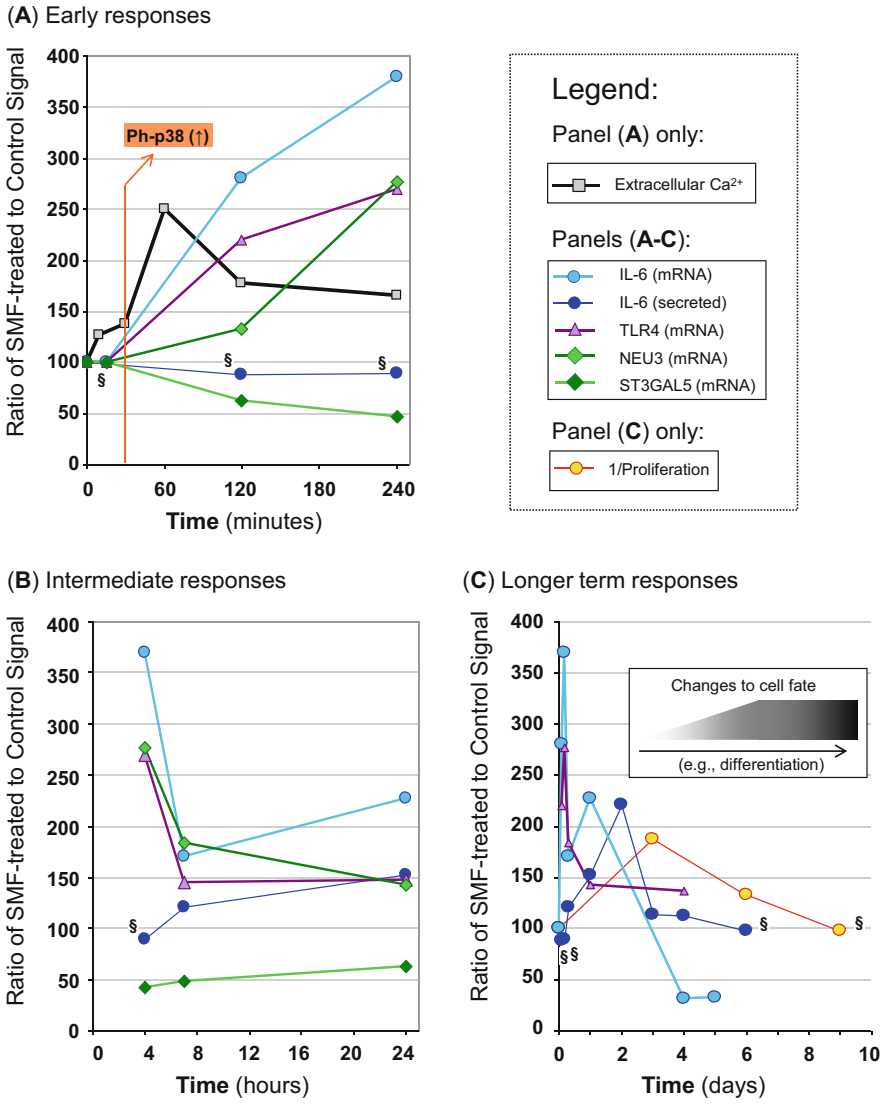

Fig. 4.5 Timeline of SMF-induced, IL-6 associated responses in hEBD LVEC cells. (**a**) Early responses that occur within 4 h of the start of continuous SMF exposure include p38 phosphorylation by 30 min, calcium flux, and the other parameters denoted in panel (**b**), which shows intermediate responses that occur over the first day. Finally, (**c**) shows longer term responses over the first week or so of SMF exposure. Data is shown for $n \geq 3$ independent experiments and $p < 0.05$ for all data except for that indicated by "§" where $p > 0.05$ (these data were analyzed by SD but error bars are omitted from these graphs for clarity). All data shown—except for the proliferation data in Panel (**c**) that gives the reciprocal relationship for cell proliferation—compares SMF exposed to control cells with a value of 100 as a baseline. [Reprinted from (Wang et al. 2009), open access]

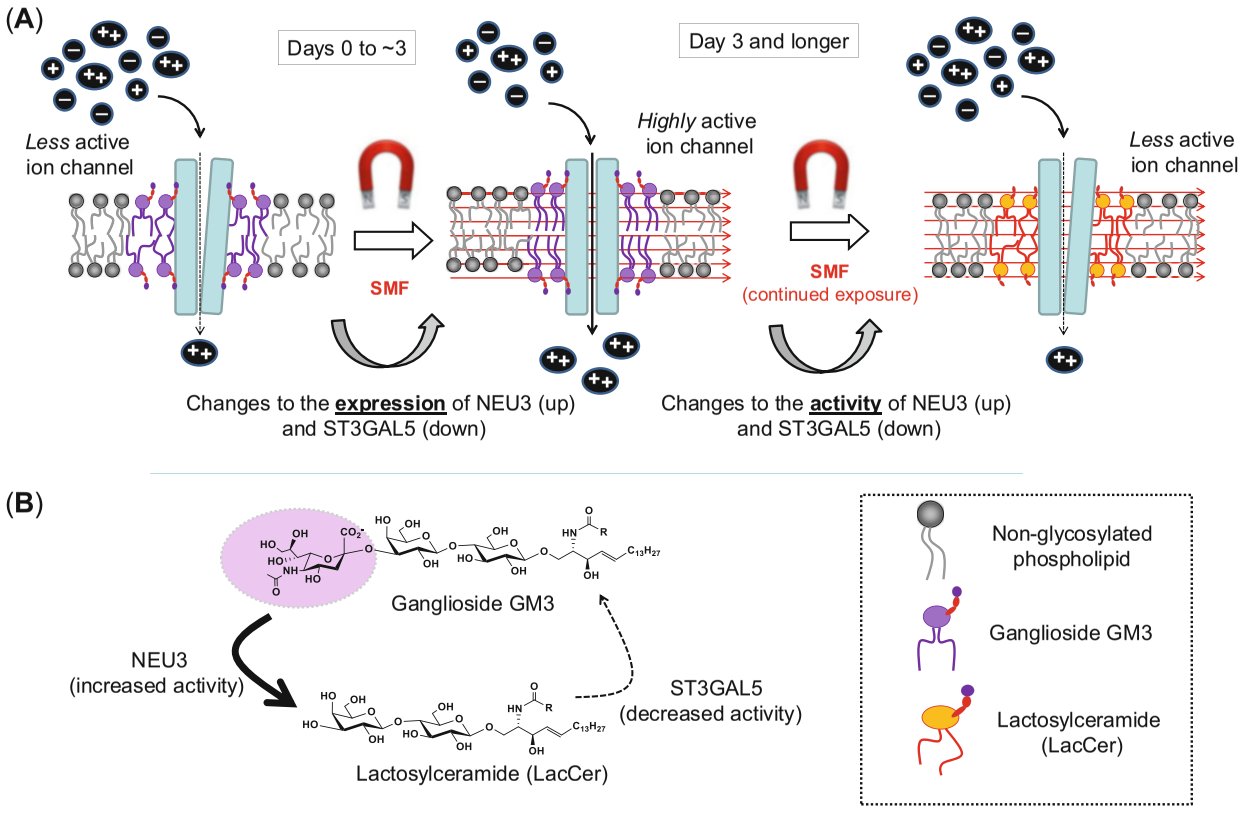

Fig. 4.6 Proposed mechanism for biphasic response to constant SMF exposure. (**a**) This proposal builds on the mechanism shown in Fig. 4.4 with the added provision that SMF exposure alone cannot fully convert low (or inactive) ion channels to a highly active form. Instead, the added contribution of ganglioside GM3 is required; for context GM3 is a prominent constituent of lipid rafts that surround membrane proteins and modulate their activity; this concept is explained in detail in Hakomori's "glycosynapse" publications (Hakomori 2002, 2004; Toledo et al. 2004). As a consequence, over the initial period of SMF treatment

(e.g., Days 0–3), SMF exposure and GM3's impact combine to convert ion channels from low to high activity state. During this time, however (as shown in Fig. 4.5), changes to the expression of NEU3 and ST3GAL5 lead to a later onset reduction in GM3 abundance (as shown in Panel **b**); in particular, GM3 becomes depleted after 2–3 days of SMF exposure when the newly made NEU3 becomes active. As proposed in the far right section of Panel (**a**), SMF alone (i.e., in the absence of GM3) is not sufficient to maintain lipid conformation or biophysical properties to support "highly active" ion channel flux. (**b**) Biochemical details of NEU3 (a sialidase that removes sialic acid from ganglioside) and ST3GAL5 (the sialyltransferase that converts LacCer to GM3) are shown. The increased activity NEU3 converts GM3 to LacCer, which cannot be replenished at normal rates because of the concomitant decrease in ST3GAL3

postulated based on the increased expression of NEU3 and the decreased expression ST3GAL5 at early time points. These enzymes work together to reduce levels of cell surface-displayed sialic acid, including that found on ganglioside GM3. Specifically, NEU3 is a sialidase that removes the sialic acid from GM3, thereby generating LacCer; at the same time loss of the biosynthetic enzyme ST3GAL5 prevents regeneration of GM3 (as well as other gangliosides such as GM1). The net effect of this functionally coordinated response is diminution of cell surface levels of GM3, which we previously showed can affect cell surface signaling (Wang et al. 2006) and others have shown affect gangliosides' ability to modulate calcium ion activity (Carlson et al. 1994). Accordingly, we speculate that SMF exposure immediately affects calcium ion channel activity through changes to the bulk biophysical properties of the surrounding membranes. This sets in motion a series of events that ultimately counteract the impact of SMF. In other words, the initial stimuli presented by SMF exposure is counteracted by longer term (also SMF-induced) loss of GM3 (i.e., GM3 ultimately proves to be a stronger mediator of the responses studied than SMF), which ultimately attenuates and in fact reverses IL-6 production over longer exposure periods.

4.5.2.5 Lipid Membranes as a Magnetic Field Biosensor—Revisiting Earlier Evidence

In addition to the speculative mechanism just presented, we briefly revisit magnetic sensing in nematodes (Sect. 4.3.3.1), where specific neurons have been identified to be responsive to the GMF. Early studies, consistent with findings in mollusks and crustaceans (Sect. 4.3.3.2), suggested that the actual biosensor was peripheral to the neurons found to respond to magnetic fields. A more recent study, however, provides convincing evidence that the neurons themselves have magnetic-sensing ability of activated calcium flux and activation in the absence of synaptic input (Vidal-Gadea et al. 2015). This information is consistent with our cell-based findings where SMF exposure of human neural-like cells appeared to directly interact with membranes to trigger downstream response. A counterpoint to this hypothesis, however, is that the nematode study monitored GMF-strength magnetic fields, which are much weaker than the ~0.2 T fields previously described as necessary for direct "magnetic field sensing" by changes to the biophysical properties of membranes; indeed, nematodes have biogenetic magnetite (Cranfield et al. 2004). To conclude, membranes—in and of themselves—may provide added modes of magnetic field biosensing not detected up to now. Similar to many aspects of "magnetobiology," confirmation of this possibility provides exciting future research opportunities.

4.6 Concluding Comments

This chapter revisits, albeit briefly, magnetic field biosensing abilities found across many diverse organisms and attempts to apply the information that has been compiled over the past half century or so to prospects for "magnetotherapy" in humans (this concept is extended in Chap. 15). As covered above, "Nature" has evolved two well-established modes of magnetoreception (magnetite and cryptochrome RPM mechanisms) as well as a more specialized inductive mechanism exemplified by certain fish that have "ampullae of Lorenzini" sensing organs. As summarized in Sect. 4.5, these mechanisms do not provide a fully compelling explanation for effects of magnetic field exposure in human cells, resulting in speculative models (in part based on this author's previous research) where moderate strength SMFs directly modulate the biophysical properties of biological membranes with profound consequences on downstream signaling pathways, gene expression, and ultimately cell fate.

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Chapter 5
Controlling Cell Membrane Potential with Static Nonuniform Magnetic Fields

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Abstract The coordinated activity of a myriad of ion channels in a cell is a spectacular biological and physical phenomenon. Understanding mechanisms governing the ion channel gating and setting membrane potentials is key to developing targeted therapeutic strategies using non-contact magnetic stimulations. In this study, we demonstrate theoretically that ion channel activity can be controlled by a static gradient magnetic field. The analysis revealed that specific ion membrane channels can be turned off and on by remotely applying a high-gradient magnetic field, thus modulating the cell membrane potential. The suggested model and mechanisms provide a general framework for identifying possible hidden mechanisms of biomagnetic effects associated with modulation of ion channel activity by high-gradient static magnetic fields.

Keywords Cell responses to magnetic fields · Membrane potential · Ion channels · Magnetogenetics

5.1 Introduction

Membrane potential (MP) is the difference in electric potentials between the inside and the outside of a cell, which in excitable and non-excitable cells usually varies in a wide range from −3 to −100 mV. The ability of cells to regulate their MPs is critical to many processes, including regulation of cell volume, cell cycle, sensing, DNA synthesis, differentiation, proliferation, muscle contraction, transmitting

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signals, cancer progression, and wound healing (Yang and Brackenbury 2013; Levin 2014, 2020; Abdul Kadir et al. 2018). Although it is much known how membrane potential and bioelectrical signals control cell behavior, many mysteries remain, e.g., how undifferentiated and cancer cells can maintain a low membrane potential, allowing them to be mitotically active and highly plastic. In contrast, mature, terminally differentiated, and quiescent cells are prone to hyperpolarization and usually do not undergo mitosis (Fig. 5.1). Another physical parameter governing cell life is membrane rigidity. Importantly, both these parameters (MP and membrane rigidity) are not independent: the cell membrane rigidity is proportional to the square of the membrane voltage (Zablotskii et al. 2016b). Thus, for cells with a high membrane potential (Fig. 5.1), the electrostatic contribution to membrane bending rigidity (Delorme et al. 2007) is sufficiently large, and therefore the force necessary to deform a cell membrane is enhanced in comparison with cells having low membrane voltage (depolarized membrane). Hence, by tuning MPs, one can also control cell rigidity, which might be important for cancer cells that have paradoxically small membrane potentials allowing them to be very plastic and invasive (Zablotskii et al. 2018). Taken together, these facts may lead to the thought that the functions and fate of cells are mainly determined by the magnitude of the membrane potential. On the other hand, it is known that the whole-cell machinery and cell fate can be dramatically affected by static magnetic fields. There are several examples. Exposure of macrophages to a non-uniform magnetic field causes extreme elongation of macrophages and has a profound effect on their molecular components and organelles (Wosik et al. 2018). A gradient magnetic field affects adipogenic differentiation of mesenchymal stem cells by the transmission of mechanical stress from the membrane to the cytoskeleton, resulting in F-actin remodeling and subsequent down-regulation of adipogenic genes (Zablotskii et al. 2014a). Magnetic fields can guide the differentiation of stem cells into specific cell types by coordinating the mechanical and electric cell properties via magnetomechanical stress arising in cells (Zablotskii et al. 2014b, 2016a). Moreover, high static magnetic fields can alter the geometry of the early cell cleavages of *Xenopus laevis* eggs (Denegre et al. 1998) and change orientation and morphology of mitotic spindles in human cells (Zhang et al. 2017). A moderate static MF can dysregulate DNA replication (Yang et al. 2020).

Since the membrane potential correlates with mitosis, DNA synthesis, cell cycle progression, and overall proliferation (Cone 1971; Binggeli and Weinstein 1986), the ability to control MPs with a magnetic field may represent a new tool for cell therapy in various diseases. Thus, the remote and noninvasive control of living cells using magnetic fields is an attractive perspective for many researchers working in biology and medicine.

The mechanisms governing the cell MP are extensive, and ion channel-dependent regulation of MP plays a central role among them. Here we suggest the theoretical framework of regulating of ion channel expression and controlling MP with static gradient magnetic fields.

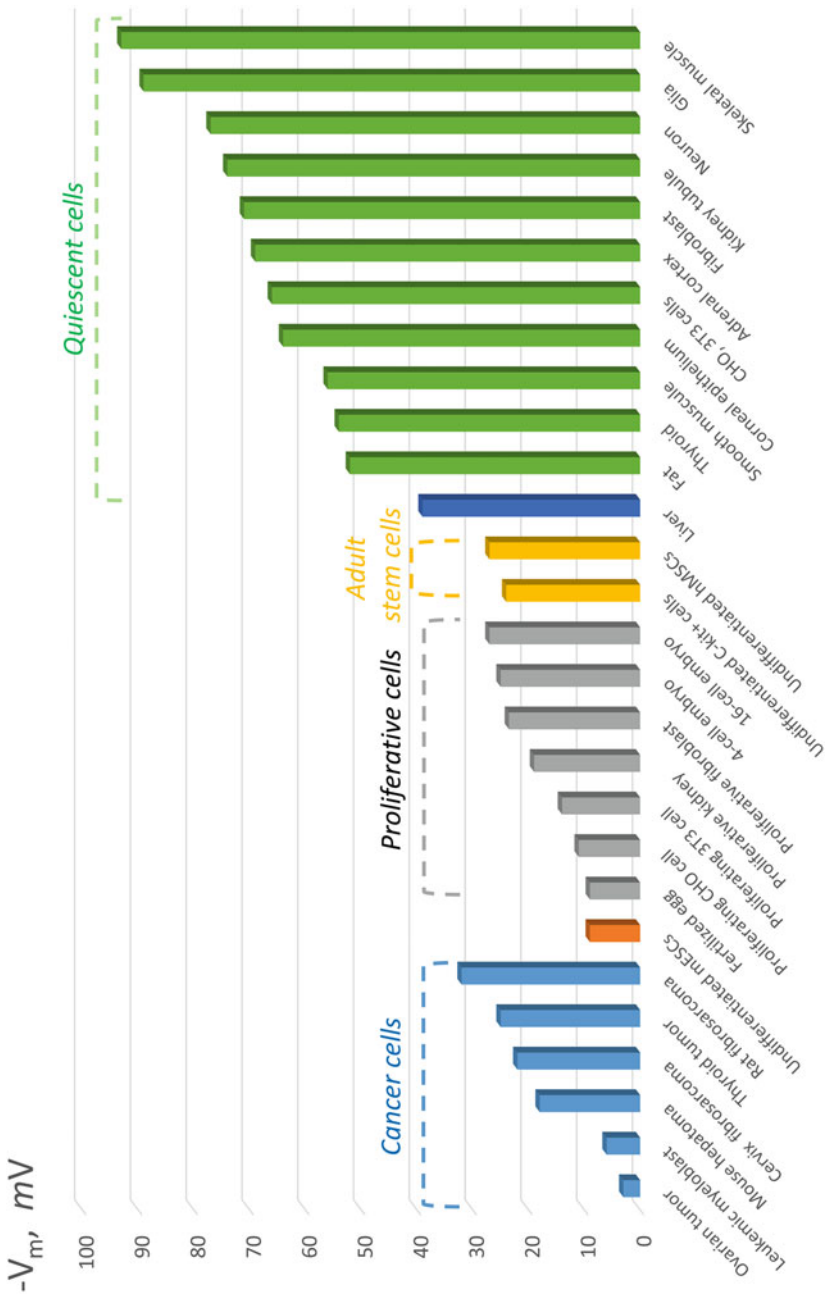

Fig. 5.1 Membrane potentials (in mV) for different cell types. [The MP values were taken from (Binggeli and Weinstein 1986; Levin and Stevenson 2012) and references therein]

5.2 Magnetic Forces

In general, the magnetic forces are central to cell responses to an applied magnetic field (MF).

There are three types of magnetic forces acting on moving ions, cells, and subcellular components: (1) the Lorentz force, which is proportional to the ion velocity and charge; (2) magnetic gradient forces, $F_{\nabla B} \propto \nabla B^2$ (where B is the magnetic induction and ∇ is the differential operator nabla) (Hinds et al. 2001; Zablotskii et al. 2016a); and (3) the concentration-gradient magnetic force, $F_{\nabla n} \propto B^2 \nabla n$ (where ∇n is the gradient of the concentration of the diamagnetic or paramagnetic species) (Waskaas 1993; Leventis and Gao 2002; Bund and Kuehnlein 2005; Dunne et al. 2011; Svendsen and Waskaas 2020).

It should be emphasized here that a cell is an electrical unit, in which the electrical (Coulomb) forces control many intracellular processes. In cells, the electrical forces often dominate the magnetic forces. For example, the Lorentz force acting on moving intracellular ions could be comparable to the intracellular Coulomb forces in magnetic fields with an induction larger than 10^6 T (Zablotskii et al. 2018). In cell systems, the magnetic forces can be sufficient in magnitude to compete with the electric forces either in spatially inhomogeneous magnetic fields with extremely large magnetic gradients (Zablotskii et al. 2016a, b, 2018; Barbic 2019) or in uniform MFs with high magnetic field strength (Zhang et al. 2017). Thus, in such magnetic fields, the magnetic forces can compete and interfere with electrical forces and thereby change the cell functionality. For example, in living tissues and cells, a static magnetic field with the gradient of the order of 1000 T/m generates the magnetic gradient forces with the same volume density as that of gravity, $f_g = \rho g \approx 10^4$ Nm^{-3} (Zablotskii et al. 2018) (here ρ is the cell mass density and g is the acceleration of free fall). In cells, depending on the magnetic susceptibilities of cell organelles and gradient value, the magnetic gradient forces reach (10–100) pN (Zablotskii et al. 2016a) and therefore can alter the cell machinery. Here, we employ analytical models to investigate how the cell membrane potential changes in high-gradient static magnetic fields.

Many molecules and ions that determine the membrane potential have small magnetic moments mainly due to nuclear spins and hence they are subjected to the magnetic gradient forces. However, despite small values of these magnetic moments, a high-gradient magnetic field can act on crossing cell membrane ions with a relatively large force. These magnetic gradient forces can either assist or oppose ion movement through the membrane. The magnetic force is given by

$$\vec{F} = p_{\mathrm{m}} \frac{\mathrm{d}\vec{B}}{\mathrm{d}l}, \tag{5.1}$$

where p_{m} is the magnetic dipole moment of the ion, $\boldsymbol{B}$ is the magnetic induction vector. Of note, in Eq. (5.1), the derivative is taken with respect to direction $\boldsymbol{l}$, which is parallel to the magnetic dipole moment of an ion, $\boldsymbol{l}//\boldsymbol{p}_{\mathbf{m}}$. In a magnetic field, the ion

energy and torque acting on magnetic dipole are $E = -(\boldsymbol{p}_\mathbf{m}\boldsymbol{B})$ and $\boldsymbol{T} = \boldsymbol{p}_\mathbf{m} \times \boldsymbol{B}$, accordingly.

Being exerted on ions crossing the cell membrane, the magnetic gradient force is capable of changing MP. Indeed, the activity of membrane ion channels regulates the membrane potential by setting the ion diffusion flux balance: $j_\mathrm{D} + j_\mathrm{E} = 0$, where j_D is the diffusion flux, j_E is the ion flux driven by the electric potential gradient across the membrane. In a high-gradient magnetic field, the magnetic gradient forces (Eq. 5.1) create a magnetically driven ion flux, j_M, which is added to the diffusion and electric fluxes.

Below, we derive the explicit dependence of the resting membrane potential on the magnetic gradient value.

5.3 Resting Cell Membrane Potential in a Gradient Magnetic Field: Generalized Nernst Equation

As mentioned above, in a static gradient MF, the magnetic gradient forces create an ion flux through the cell membrane, which competes with ion fluxes determined by the gradients of electric potential and ion concentration. Thus, in gradient MFs, three ion fluxes set the equilibrium membrane potential, keeping the total flux equal to zero: $j_\mathrm{D} + j_\mathrm{E} + j_\mathrm{M} = 0$, as depicted in Fig. 5.2.

Let us consider the Nernst equilibrium potential in the presence of a gradient magnetic field. Considering ions' substance as a dilute solute, for points inside a cell, one can write the chemical potential of the solute as

$$\mu_i = kT \ln(n_i) + ze\varphi_i - p_\mathrm{m} B_i, \tag{5.2}$$

where φ_i is the electric potential inside a cell, e is the electron charge, z is the ion valence ($z = +1$ for a positive, univalent ion), k is the Boltzmann constant, B_i is the magnetic field induction inside the cell, n_i is the intracellular ion concentration, and T is the absolute temperature. Of note, the chemical potential of a dilute solute with no electric and magnetic fields is $\mu = kT \ln(n) + \psi$ (Landau et al. 1995), where ψ is a function of the pressure and temperature. In Eq. (5.2), the last two terms represent the electrostatic and magnetic energies of an ion, accordingly.

For points outside a cell, the solute chemical potential is

$$\mu_0 = kT \ln(n_0) + ze\varphi_0 - p_\mathrm{m} B_0, \tag{5.3}$$

where n_0 is the ion concentration outside the cell, φ_0 and B_0 are the electric potential and magnetic field induction outside the cell. From the equality of the solute chemical potentials, $\mu_i = \mu_0$, which is the phase equilibria condition, one can arrive at

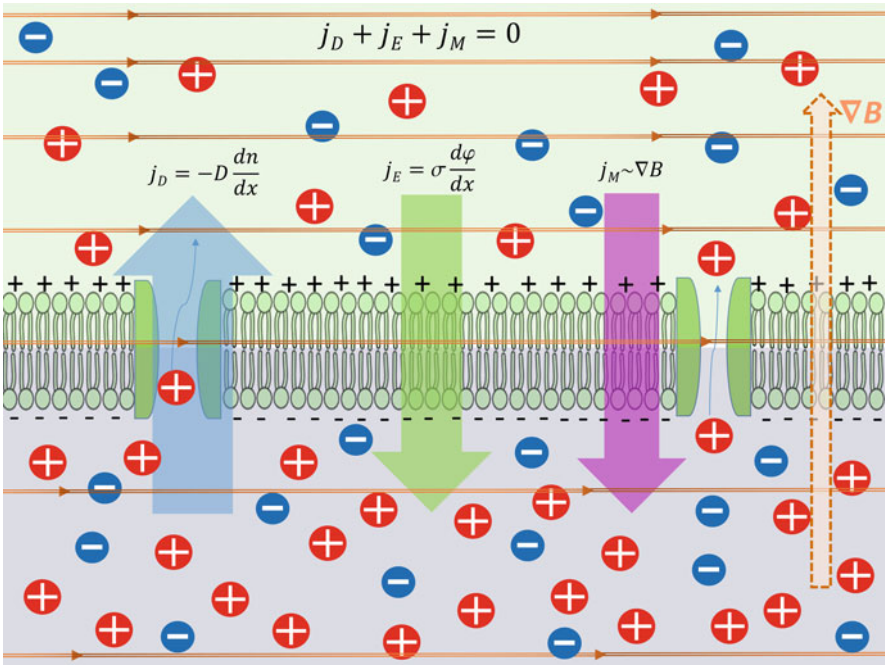

Fig. 5.2 In the presence of a gradient magnetic field, three ion fluxes set the equilibrium membrane potential: j_D is the diffusion flux, j_E is the ion flux driven by the gradient of the electric potential across the membrane, and j_M is the flux due to the magnetic gradient forces. In equilibrium, $j_D + j_E + j_M = 0$. The orange parallel lines represent the magnetic field lines. [Reprinted with permission from (Zablotskii et al. 2021)]

$$ze(\varphi_i - \varphi_0) - p_m(B_i - B_0) = kT \ln\left(\frac{n_0}{n_i}\right). \tag{5.4}$$

In Eq. (5.4), on the left, we denote the differences as: $(\varphi_i - \varphi_0) = V_m$ and $(B_i - B_0) = GL$, where $G = |dB/dr|$ is the magnetic flux density gradient and L is the half of the mean cell size. Finally, from Eq. (5.4) we get the equilibrium cell membrane potential as a function of the magnetic field gradient:

$$V_m = \frac{kT}{ze} \ln\left(\frac{n_0}{n_i}\right) \pm \frac{p_m}{ze} GL. \tag{5.5}$$

By inserting the Faraday constant $F = eN_A$ and the gas constant $R = kN_A$ we obtain a generalized Nernst equation in the form (Zablotskii et al. 2016b)

$$V_{\mathrm{m}} = \frac{RT}{zF} \ln\left(\frac{n_0}{n_i}\right) \pm \frac{p_{\mathrm{m}}}{z\mathrm{e}} GL. \tag{5.6}$$

Note, the sign of the second term on the right could be positive or negative, depending on the direction of the magnetic field gradient. In the limiting case of the absence of the MF, the Eq. (5.5) turns into the classical Nernst equation:

$$V_{\mathrm{m}} = \frac{RT}{zF} \ln\left(\frac{n_0}{n_i}\right), \tag{5.7}$$

which is used to calculate the resting membrane potential when the membrane is permeable to a single ion: K^+, Ca^{2+}, Na^+, or Cl^-.

It is curious, though, that starting with completely different laws, the first law of thermodynamics and $PV = RT$, we can end up with the same kind of the equation (Eq. 5.6) (P is the pressure and V is the volume). The derivation goes as follows.

Let us consider ions in a volume, V as an ideal gas in a gradient static magnetic field. The pressure of a gas of ions is $P = RT/V$. Now we calculate the work, A which one mole of the ionic gas performs when it expands from volume V_1 to V_2 at $T = \text{const}$ and $N = \text{const}$:

$$A = \int_{V_1}^{V_2} P\mathrm{d}V = \int_{V_1}^{V_2} \frac{RT}{V}\mathrm{d}V = RT \ln\left(\frac{V_2}{V_1}\right) = RT \ln\left(\frac{n_i}{n_0}\right). \tag{5.8}$$

The work of the gas Eq. (5.8) goes for an increment of the internal energy of the system $\Delta U = \Delta U_{\mathrm{el}} + \Delta U_{\mathrm{mag}}$: $\Delta U_{\mathrm{el}} = zeN_A V_{\mathrm{m}}$—the electrostatic ions energy, and $\Delta U_{\mathrm{mag}} = -N_A p_{\mathrm{m}} GL$—the magnetic energy. Then, using the first law of the thermodynamics, $0 = \Delta U + A$ (of note, the heat supplied to the system, $Q = 0$) one can directly arrive at the Eq. (5.6).

Thus, a gradient magnetic field can change the cell MP as prescribed by Eq. (5.6). The important question here is: how large can these changes for the experimentally attainable laboratory magnetic fields be? As it follows from Eqs. (5.6) and (5.7), the relative change of the resting MP caused by a gradient MF is

$$\frac{\Delta V_{\mathrm{m}}}{V_{\mathrm{m}}} = \frac{p_{\mathrm{m}} GL/ze}{RT \ln\left(n_0/n_i\right)/zF} = \frac{p_{\mathrm{m}} GL}{kT \ln\left(n_0/n_i\right)}. \tag{5.9}$$

From Eq. (5.9), we estimate the critical gradient value, G_{cr} for which the ratio $\Delta V_{\mathrm{m}}/V_{\mathrm{m}}$ is unity. This imply that an application of static MF with the critical gradient makes 100% changes of the MP. For estimations of the critical gradient, we use the following ion concentrations for a mammalian neuron: $[K^+]_{\mathrm{out}} = 3$ mM, $[K^+]_{\mathrm{in}} = 140$ mM, $[Na^+]_{\mathrm{out}} = 145$ mM, $[Na^+]_{\mathrm{in}} = 18$ mM, $[Cl^-]_{\mathrm{out}} = 120$ mM, $[Cl^-]_{\mathrm{in}} = 7$ mM [40] (we denote the concentrations of a specific ion inside and outsides of the cell as $[\mathrm{Ion}]_{\mathrm{in}}$ and $[\mathrm{Ion}]_{\mathrm{out}}$ throughout, unless specified otherwise).

The magnetic moments of these ions are very small and are on the same order of magnitude as the nuclear magneton, $\mu_n = 5.05 \times 10^{-27}$ J/T: $p_{Na+} = 2.22\mu_n$ (sodium-23), $p_{K+} = 0.39\mu_n$ (potassium-39), $p_{Cl-} = 0.821\mu_n$ (chloride-35), and $p_{Ca2+} = 0$ (calcium-40). Among these ions, Na^+ has the largest magnetic moment and Ca^{2+} has zero electronic and nuclear magnetic moments. So, a magnetic gradient field does not affect Ca^{2+} contribution to the MP. For the above listed ion concentrations and magnetic moments, the estimations from Eq. (5.9) give G_{cr} of the order of (10^{10}–10^{11}) T/m. However, the currently reachable magnetic gradient values are of the order of (10^6–10^7) T/m (Dempsey et al. 2014).

Thus, it is unlikely that a static MF with currently reachable values of the gradient can cause a significant change of the resting membrane potential of excitable cells. Nevertheless, this is possible for cells with low values ($V_m < 10$ mV) of the membrane potential: cancer cells, proliferative cells, and undifferentiated mESCs (see Fig. 5.1). It is paradoxical that highly differentiated tumor cells (human hepatocellular carcinomas: Tong, HepG2, Hep3B, PLC/PRF/5, Mahlavu, and HA22T) have low membrane potentials (Binggeli et al. 1994). Now we estimate the critical gradient for cells with low MPs. In the Nernst equation (Eq. 5.7), the numerical value of the prefactor is $(RT/zF) = 25.2$ mV (for $z = 1$ and $T = 300$ K). This implies that for cells with $V_m = 3$–6 mV such as ovarian tumor and leukemic myeloblast (Fig. 5.1), the value of $\ln(n_0/n_i) = 0.12$–0.24. For these cells, the critical gradient value which makes 100% changes of the MP is $(4–8) \times 10^9$ T/m as estimated from Eq. (5.9). Importantly, the above made estimations of the critical gradient value assumed 100% changes of the resting MP. However, only a few percent of changes in the MP can lead to significant changes of the whole-cell machinery, especially during the development of an organism. Thus, for cells having low MPs a magnetic field with the magnetic gradient of the order of (10^7–10^8) T/m (the experimentally attainable laboratory magnetic gradients) is capable of changing MP by a few percent. Magnetic systems generating magnetic fields with gradients on the order of 10^9 T/m would allow for significant alteration of the membrane potential in accordance with predictions based on Eq. (5.6). However, the question arises where such gradient fields are achievable.

Surprisingly, static MFs with the large enough spatial gradients can be generated in a laboratory at the microscale and nanoscale utilizing micro- and nano-magnets. Below, we describe magnetic systems that allow us to achieve MFs with extremely large gradients.

5.4 Smallest Magnets Generate the Highest Magnetic Gradients

An approach to reach the highest magnetic gradient with the smallest magnets is based on the fact that the magnetic gradient value benefits greatly from scale reduction (Cugat et al. 2003; Zablotskii et al. 2010, 2013). Therefore, micro- and

nano-magnets can generate high-gradient magnetic fields. For example, in the vicinity of a magnetic nanostructure, the magnetic field gradient can be large enough, up to 10^7 T/m (Dumas-Bouchiat et al. 2010; Zanini et al. 2011, 2012; Osman et al. 2012, 2013; Zablotskii et al. 2016b). On the surface of a 8 nm diameter ferritin particle, the magnetic gradient value is 4.9×10^8 T/m (Barbic 2019).

Other systems, generating high-gradient magnetic fields on the nanoscale length, are analytically examined below.

5.4.1 Magnetic Nanoparticles

We consider a single domain magnetic nanoparticle (MNP) of the radius R_0 with a magnetic moment $p_m = M_s V$ (where M_s and V are the MNP saturation magnetization and volume). The magnetic induction value and its radial gradient at the axis which coincides with the magnetic moment direction are

$$B = \frac{2\mu_0 M_s R_0^3}{3r^3} \tag{5.10}$$

and

$$\frac{dB}{dr} = \frac{2\mu_0 M_s R_0^3}{r^4}. \tag{5.11}$$

Near the MNP surface, the modulus of the radial magnetic gradient is $\frac{dB}{dr} = \frac{2\mu_0 M_s}{R_0}$ for $r = R_0$, as follows from Eq. (5.11). Of note, the tangential component of B is two times smaller than that of the axial. Thus, near a MNP, the magnetic gradient value obeys: $\nabla B \approx \mu_0 M_s / r$, where r is the characteristic length scale of a specific task.

5.4.2 Magnetized Slabs

The stray field distribution generated by a uniformly magnetized slab was calculated elsewhere (Joseph and Schloman 1965; Hubert and Schäfer 1998; Thiaville et al. 1998; Zablotskii et al. 2010). A practical interest represents the magnetic field distribution near the edge of a long uniformly magnetized slab. Here, the magnetic field gradient obeys (Samofalov et al. 2013)

$$|\nabla_n B| = \frac{\sqrt{2}\mu_0 M_r}{\pi x}, \tag{5.12}$$

where x is the distance to the slab edge, $\boldsymbol{n}$ is an arbitrary unit vector directed from the slab edge to the point where the field gradient is calculated, μ_0 is the vacuum permeability, and M_r is the slab remanent magnetization. Importantly, in Eq. (5.12) the modulus of the magnetic field gradient does not depend on the direction of vector $\boldsymbol{n}$ if $x \gg a$, where a is the half width of the slab. From Eq. (5.12) it follows that when approaching the edge of the slab ($x \to 0$), the magnetic gradient increases and goes to infinity. For the distance $x = 1$ μm and $\mu_0 M_r \approx 1.2$ T (which is the remanent magnetization of commercially available NdFeB magnets), an estimation made from Eq. (5.12) gives a large enough value of the magnetic field gradient, $|\nabla B| \approx 5 \times 10^5$ T/m.

5.4.3 Axially Magnetized Cylinder with a Hole

Here, we show that a cylindrical magnet with an axial hole generates a high-gradient MF just above the hole. The magnetic field and its gradient distributions around a cylindrical magnet of the radius R with a hole of radius r were calculated analytically in Zablotskii et al. (2016b). In the limiting case of a hole with the smallest radius, when $r \to 0$, above the hole at its axis, the axial component of the magnetic induction logarithmically depends on the distance, z, from the magnet top (Samofalov et al. 2013):

$$B_z = 2\pi\mu_0 M_r \ln\left(\frac{2R}{z}\right). \tag{5.13}$$

From Eq. (5.13) for the axial component of the field gradient, one can arrive at

$$\frac{dB_z}{dz} = \frac{2\pi\mu_0 M_r}{z}. \tag{5.14}$$

For a single parabolic-shaped magnetic pole used in magnetic tweezers, the magnetic gradient is given by a similar formula (de Vries et al. 2005)

$$\frac{dB_z}{dz} = \frac{\mu_0 M_r}{z}, \tag{5.15}$$

where z is the distance from the magnet pole. From Eq. (5.15), an estimate shows that for a parabolic-shaped magnetic pole of size 1 μm, the gradient is 3×10^6 T/m at 100 nm from the tip (de Vries et al. 2005). Thus, in the above considered magnetic systems, the magnetic gradient increases dramatically when approaching the magnet edges or hole.

To summarize, at the nanoscale length, there are no principal physical limitations to reach magnetic fields with a high magnetic gradient. In the next section, we will

show how the magnetic nanostructures can be used to control the cell membrane potential with gradient magnetic fields.

5.5 Controlling Membrane Potential with MNPs Bound with Ion Channels

First, we consider a chain of magnetic nanoparticles (MNPs) placed on a cell membrane which can create spatially modulated magnetic flux distributions with a sufficient gradient (Fig. 5.3). For the shown chains of four MNPs with parallel and perpendicular orientation of the magnetic moments with respect to the membrane surface, the magnetic gradient force acts in direction parallel to the membrane, as it is illustrated in Fig. 5.3a–d. In practice, magnetic nanoparticles can be retained and accumulated on the cell membrane with different uptake inhibitors, e.g., see Lunov et al. (2011). In organisms, biogenic and non-biogenic magnetic nanoparticles and their conglomerates can form chains on the cell membrane due to self-organization process (Gorobets et al. 2022).

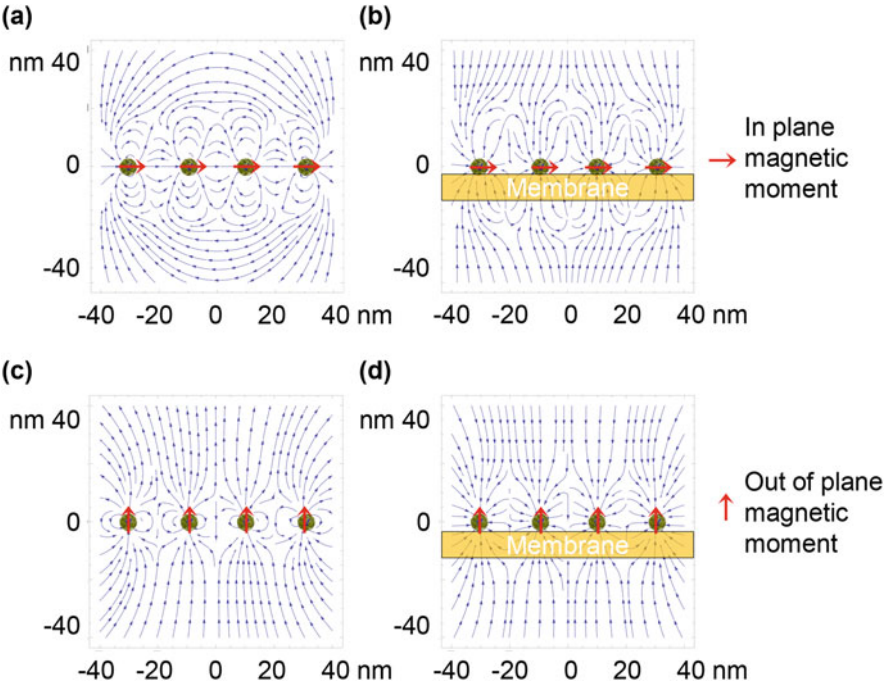

Fig. 5.3 Vector fields of the magnetic induction (**a**, **c**) and magnetic gradient (**b**, **d**) in the vicinity of four magnetic nanoparticles magnetized parallel and perpendicular to the membrane surface. In (**b**, **d**) arrows indicate the directions of the magnetic gradient forces. [Reprinted from (Zablotskii et al. 2016b). Open access]

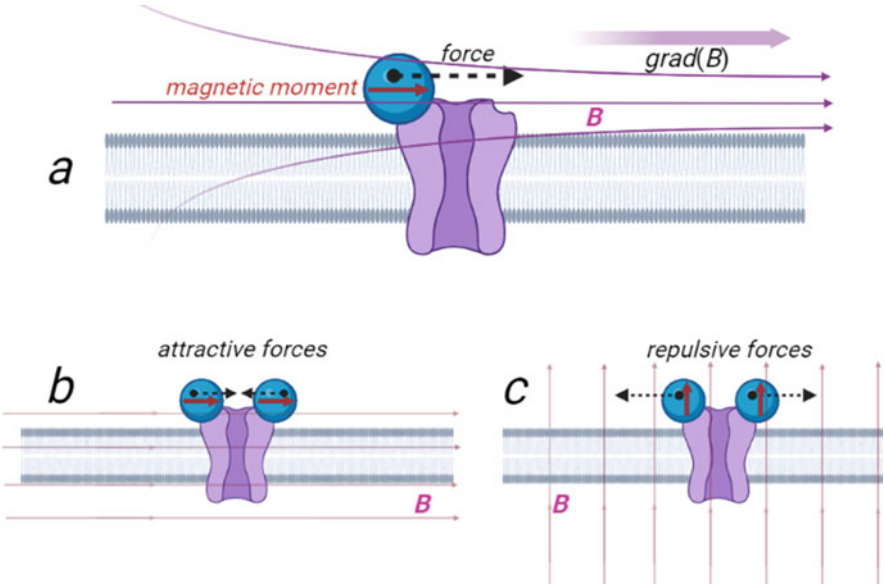

Fig. 5.4 Ion channel in the cell membrane experiences the magnetic force from a magnetic nanoparticle and the externally applied gradient magnetic field. Upon activation of a gradient magnetic field source, the ion channel is forced close (**a**). Channel closing and opening by the interaction of two magnetic nanoparticles bound to the channel body in the presence of a uniform magnetic field (**b**, **c**)

The magnetic gradient forces localized near the cell membrane may affect cell functions in two main ways: (1) direct changing the resting membrane potential, as predicted in Eq. (5.6) and (2) locally disturbing the channel gating mechanism resulting in a modulation of the resting and action MPs. The former can occur locally as a consequence of a very high field gradient, as given in Eq. (5.11). The second mechanism is more effective when a magnetic nanoparticle is directly bound to a membrane channel, especially to mechanosensitive or ligand-gated channels. Below we consider this mechanism in detail. There are two possible cases: one magnetic nanoparticle bound to a channel in combination with a source of high-gradient magnetic field (Fig. 5.4a) and two interacting magnetic nanoparticles bound to a channel (Fig. 5.4b, c).

First, we consider a magnetite (Fe_3O_4) nanoparticle with $M_s = 510$ kAm^{-1} and $R_0 = 5$ nm, coupled to a channel domain in the presence of an MF with gradient parallel to the membrane (Fig. 5.4a). In such a case, the force acting on the particle is also parallel to the membrane (Fig. 5.4a). If this force is comparable with the forces driving the channel opening/closing, it disturbs the expression of the channel. For example, upon activation of a gradient magnetic field source, the ion channel is forced close (Fig. 5.4a). The magnetic moment of the magnetite particle is $p_m = 4\pi R_0{}^3 M_s/3 \approx 2.67 \times 10^{-19}$ Am^2. It is interesting that a superparamagnetic 8 nm diameter ferritin particle has the magnetic moment of the same order, $p_m = 2.1$

$\times 10^{-19}$ Am^2 (Barbic 2019). Thus, in an MF with gradient $|\nabla B| \approx 3.7 \times 10^8$ T/m a 5 nm magnetite particle experiences the force of the order of 100 pN, which is sufficient to close the channel (Fig. 5.4a).

Second, we consider a case when two magnetite or ferritin nanoparticles coupled to the body of a mechanosensitive ion channel (Fig. 5.4b, c). In this case, one particle is in a high-gradient magnetic field generated by the second nanoparticle. An estimate made from Eq. (5.15) gives the gradient value $|\nabla B_r| \approx 2.6 \times 10^8$ T/m in the vicinity of an MNP. In an MF with this gradient value, the magnetite nanoparticle is subjected to the force $f = p_m \nabla B \approx 68$ pN.

Thus, a large magnetic field gradient from one particle acting on the second particle results in the magnetic gradient force of the order 100 pN. Similar estimates for the magnitude of the magnetic force between two ferritin particles were obtained in Barbic (2019).

Thus, there is the possibility to control the expression of mechanosensitive channels with an external MF and MNPs with the magnetic moments of the order (2–3) $\times 10^{-19}$ Am^2 coupled to the channel body (Fig. 5.4b, c). Figure 5.4 illustrates the concept of magnetically controlled channel expression with (a) one MNP in a gradient external MF and (b) two MNPs bound to membrane receptors in an external homogeneous MF. Considering the second mechanism (two MNPs in an MF) an intriguing approach to channel gating can be proposed: use a uniform rotating moderate magnetic field to periodically switch ion channels between open and closed states. Indeed, the particle moment saturates at relatively low magnetic fields (~1 Tesla), and therefore upon changing the field direction, the nanoparticle magnetic moments will be oriented either parallel (Fig. 5.4b) or perpendicular to the cell membrane (Fig. 5.4c). In the first, the interparticle attraction closes the channel, while in the second, the interparticle repulsion makes the channel open.

Another mechanism of magnetic control of mechanosensitive ion channel with ~1 μm magnetic particles bound to the integrin receptors in combination with a high-gradient magnetic field was suggested in Dobson (2008). Here the magnetic particles are pulled toward the field gradient, deforming the cell membrane, and activating adjacent mechanosensitive ion channels.

It is remarkable that the magnetic control of the channel expression with magnetite nanoparticles allows us to drive the cell membrane potential. The possible mechanism is described as follows.

The membrane potential depends on each one of the permeabilities and ions concentrations. The three major ions, K^+, Na^+, and Cl^- are differentially distributed across the cell membrane at rest set the membrane potential (V_m) using passive ion channels. The influence of each of ions is determined using the Goldman equation (also known as Goldman–Hodgkin–Katz equation) (Goldman 1943; Hodgkin and Katz 1949), which is similar in form to the Nernst Equation, but incorporates permeability to Na^+ and Cl^-:

$$V_m = \frac{RT}{F} \ln \left[\frac{P_K[K^+]_{out} + P_{Na}[Na^+]_{out} + P_{Cl}[Cl^-]_{in}}{P_K[K^+]_{in} + P_{Na}[Na^+]_{in} + P_{Cl}[Cl^-]_{out}} \right], \tag{5.16}$$

where P_{ion} is the permeability for that ion, $[ion]_{out}$ is the extracellular concentration of that ion (in moles/m^3), and $[ion]_{in}$ is the intracellular concentration of that ion. In Eq. (5.16), the permeability of the channel may depend on the magnetic field gradient through the probability of opening the channel. It is believed that a cell may have thousands of ion channels, and the probability of any of them being open (at any given time) is typically in the range of a few to a few tens of percent (Sachs 1994; Zabel et al. 1996). Magnetic gradient forces impose mechanical stress on the plasma membrane and channel body.

Assuming two-state Boltzmann statistics, the probability that the channel is in the open state is given by Reeves et al. (2008)

$$W_{open} = \left(1 + \mathrm{Exp}\left(\frac{\Delta G}{kT}\right)\right)^{-1}, \tag{5.17}$$

where ΔG is the total free energy difference between closed and open states. We define $\Delta G = G_{open} - G_{closed}$, the total free energy difference between closed and open states. In the presence of a gradient MF, $\Delta G = \Delta G_{elec} + \Delta G_{prot} + \Delta G_{memb} + \Delta G_{mag}$ where the terms represent the change in electrostatic gating energy, internal protein conformation free energy, membrane-deformation free energy, and magnetic energy, respectively.

Let us consider the magnetic channel gating mechanism based on two iron loaded ferritin particles or two magnetite particles bound to a channel (Fig. 5.4). Since these two particles can attract or repulse each with the force $F_{mag} = p_m \nabla B$, which could be large as 100 pN, one can neglect the first three contributions in ΔG, and therefore write $\Delta G \approx \Delta G_{mag} = F_{mag} r_c = p_m r_c \nabla B$, where r_c is the channel radius. Indeed, in this case, the estimated magnetic gradient forces is about 100 pN, while the Coulomb forces driving the channel gating are about 10 pN (Wu et al. 2016). So, we can neglect the electrostatic and membrane elastic energies with respect to the magnetic energy contribution. The ion permeability is proportional to the probability (Eq. 5.17) of the channel opening. Thus, the ion permeability as a function of the magnetic gradient can be approximated as

$$P(\nabla B) = 2P_0 \left(1 + \mathrm{Exp}\left(\frac{p_m r \nabla B}{kT}\right)\right)^{-1}, \tag{5.18}$$

where P_0 is the ion permeability in the absence of an MF. There are two limiting cases for Eq. (5.18); when $\nabla B = 0$, the permeability is $P(0) = P_0$, while for $\nabla B \to \infty$, the permeability goes to zero meaning the closed channel state.

Let us now analyze how the membrane potential changes with the permeability dependent of the magnetic gradient (Eq. 5.18). Imaging that MNPs bound to bodies of only one type of ion channels: K^+, Na^+, or Cl^-. Next, we sequentially substitute

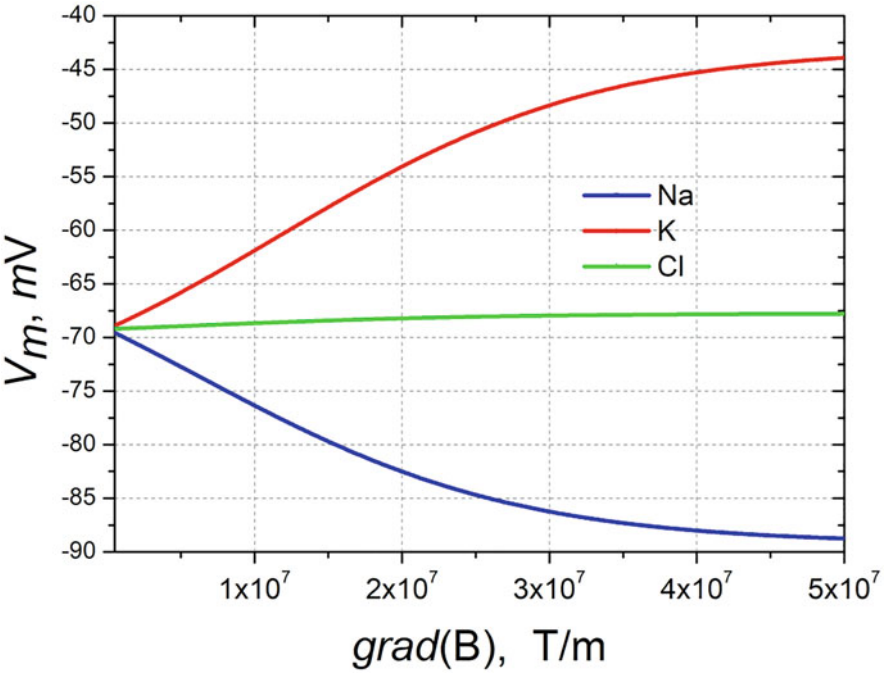

Fig. 5.5 Resting membrane potentials (in mV) in mammalian neuron (at $T = 27$ °C) as a function of the magnetic gradient (in T/m) as calculated from Eqs. (5.16) and (5.18). In an MF with zero magnetic gradient or in the absence of an MF, the channel permeability ratio is $P_K(0):P_{Na}(0):P_{Cl}(0) = 1:0.05:0.45$. The curves represent MP changes with the magnetic gradient value due to gradual closing (decreasing channel permeability given by Eq. 5.18) one of the three ion channels: K^+—red, Na^+—blue, and Cl^-—green

dependence Eq. (5.18) into Eq. (5.16), for one of the three ions (K^+, Na^+, or Cl^-), keeping the permeabilities of the remaining ions constant.

In Fig. 5.5, for a mammalian neuron, we plot the resting membrane potentials vs ∇B ss calculated from Eqs. (5.16) and (5.18). For the calculations of the resting MP, the relative zero-field ($B = 0$ or $\nabla B = 0$) permeabilities for a typical neuron at rest, $P_K:P_{Na}:P_{Cl} = 1:0.05:0.45$, were used. In a mammalian neuron, the ion concentrations used in the calculations are: $[K^+]_{out} = 3$ mM, $[K^+]_{in} = 140$ mM, $[Na^+]_{out} = 145$ mM, $[Na^+]_{in} = 18$ mM, $[Cl^-]_{out} = 120$ mM, and $[Cl^-]_{in} = 7$ mM (McCormick 2014).

As can be seen from Fig. 5.5, relatively subtle differences in ion channel expression make cells with vastly different membrane potential values. The most significant changes (up to 20–25% in an MF with gradient 5×10^7 T/m) in the resting MP undergo when the MF gradually closes Na^+ or K^+ channels (the blue and red curves in Fig. 5.5), while the blocking Cl^- channels change the MP in a few percent only (green curve in Fig. 5.5). Importantly, blocking potassium channels makes the

membrane depolarized, while blocking sodium channels leads to the membrane hyperpolarization.

Thus, we presented the proof-of-concept mechanism for remote magnetic control of the cellular MP and showed the possibility of a selective decrease of the channel expression by externally applied gradient magnetic fields.

Static homogeneous magnetic fields can also affect the diffusion of biological particles through the Lorentz force and hypothetically change the membrane potential. However, the results presented in Kinouchi et al. (1988) show that in solution, the Lorentz force can suppress the diffusion of univalent ions (e.g., Na^+, K^+, and Cl^-), but the threshold magnetic field is extremely high, approximately 5.7×10^6 T. Another possibility of controlling the MP with static homogeneous MFs through the concentration-gradient magnetic forces acting on the ions was theoretically examined in Zablotskii et al. (2021). This method also requires the use of a high uniform MF with the magnetic induction of the order of (10^2–10^3) T/m, not currently available in laboratory. On the contrary, a low-frequency (1–10 Hz) spatially modulated magnetic field with an amplitude of 70 mT is capable of changing the magnetic field up to 8 mV in skeletal muscle cells (Rubio Ayala et al. 2018).

5.6 Conclusions

Understanding the processes underlying cell responses to high magnetic fields is a fundamental challenge of ever-increasing interest, not only because it is a central problem of magnetobiology, but also because it is the goal of many therapeutic strategies using magnetic fields. In this context, we show that molecular intracellular processes associated with changes in the cell membrane potential can be controlled by a static gradient MF. We derived analytically the exact equations for the cell membrane potentials in the presence of a static gradient magnetic field. We showed that an application of a gradient MF may change the ion channel expression and ion flux balance resulting in changes of the cell membrane potential. Schematic illustrations of the mechanisms of the MF effects on ion diffusion through the cell membrane and channel gating are shown in Figs. 5.2 and 5.4.

To a large extent, a high gradient magnetic field on the cell membrane, in combination with magnetite (or ferritin) magnetic nanoparticles bound to specific ion channels could dramatically affect the cellular MPs and thereby change cell phenotype. We have suggested a new possible mechanism of ion channels periodical switching on and off with two magnetic nanoparticles seeded onto a channel body in the presence of a uniform moderate magnetic field rotating with a certain frequency.

The possibility of the remote control of the channel expression and cell membrane potentials by a gradient magnetic field without using various types of channel blocking agents seems to be a very intriguing direction in cell therapy and nanomedicine.

Ultimately, to address the most demanding challenges in medicine and biology utilizing magnetic fields, it is necessary to answer the question: what are the

cause-effect relationships between magnetically induced changing cell membrane potential and whole-cell machinery?

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Chapter 6
Impact of Static Magnetic Fields on Cells

Xinmiao Ji and Xin Zhang

Abstract As the basic unit of living organisms, the cell is where the macroscopic phenomenon meets the microscopic mechanisms. The focus of this chapter is on current evidence of SMFs on human cells and some animal cells, with a special focus on the factors that contributed to the seemingly inconsistent experimental results in the literature. We summarize cellular effects of static magnetic fields (SMFs), including cell orientation, proliferation, microtubule and cell division, actin, viability, attachment/adhesion, morphology, migration, membrane, cell cycle, DNA, reactive oxygen species (ROS), adenosine triphosphate (ATP) as well as calcium. Although it is obvious that for each aspect, the experimental results are highly variable, there are some effects that have clear physical explanations and confirmed phenomenon. For example, magnetic properties of the cells and their subcellular structures are determined by their compositions and structures, which will directly affect their orientation in high SMFs. However, there are still many unanswered questions. For example, the effects of SMFs on cellular ROS have been reported by numerous studies, but the effects are highly variable in different magnetic settings and sample types and there are still not clear physical explanations. Although the upscaling of the mechanisms from cells to tissues and living organisms is still a huge challenge, given the essential roles of cells in various living organisms, they are no doubt the central hub for researchers in this field to unravel the underlying mechanism and explore the future application of various SMFs.

Keywords Magnetic field (MF) · Static magnetic field (SMF) · Cell type · Cell density · Red blood cell (RBC) · Orientation · Microtubule · Calcium

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6.1 Introduction

Just like temperature and pressure, magnetic field is an important physical tool that could impact multiple objects and processes. Although there are numerous reports about the SMF bioeffects, their results are highly variable. However, as we have discussed in Chaps. 1 and 2, the seemingly inconsistent observations are mostly due to the different SMF parameters, such as different types of magnetic fields (static or time-varying, pulsed or noise), magnetic fields with various flux densities (weak, moderate, or strong) or frequencies (extremely low frequency, low frequency, or radiofrequency), as well as biological sample types, which can all lead to diverse and sometimes even completely opposite results.

In addition, as we have discussed in Chaps. 3–5, cells are filled with various cellular contents and biomolecules of different magnetic properties that will respond to the MF differently. For example, it has been shown that the peptide bonds united into organized structures, such as α-helix, which confers proteins diamagnetic anisotropy (Pauling 1979). Organized polymers, such as microtubules that are composed of well-organized tubulin, are also demonstrated to have strong diamagnetic anisotropy and could be aligned in the presence of magnetic fields (Vassilev et al. 1982; Bras et al. 1998, 2014). In fact, it has been found that even the dissolved oxygen in water could be modulated by high SMFs (Ueno and Harada 1982; Ueno et al. 1994, 1995). The effects of SMFs on cells have been reviewed and discussed previously (Adair 2000; Dini and Abbro 2005; Miyakoshi 2005, 2006; Ueno 2012; Albuquerque et al. 2016). Recently, Torbati et al. published a very comprehensive review about the coupling of mechanical deformation and electromagnetic fields in biological cells (Torbati et al. 2022). In this review, besides electric field, they also summarized and discussed the major mechanisms governing the interaction for MFs with cellular functions (Fig. 6.1), which proposed that deformation mediated interaction is likely to be one of the primary mechanisms governing the impact of magnetic fields on cellular function. This provides a very important point of view that once the MFs are first translated into mechanical deformation in the cell and cell membrane, which in turn may trigger an electrical response via mechanisms such as tension-activated ion channels. Consequently, cellular mechanical signal can affect multiple aspects of cellular behaviors, including cell proliferation, endocytosis, etc. Chap. 5 of this book also provided a detailed in-depth analysis for SMF-induced membrane changes, with special focus on ion channels, membrane potential, and gradient SMFs.

Here in this chapter, our goal is to provide an overview for the current evidence of SMF effects on cells, with a special focus on the differential cellular effects reported in previous studies, which seems contradictory in many aspects. We try to analyze the reasons that have caused these inconsistencies. Here we mainly discuss about human cells and some animal cells, while cells of plants, bacteria, and other organisms will be discussed in Chap. 7.

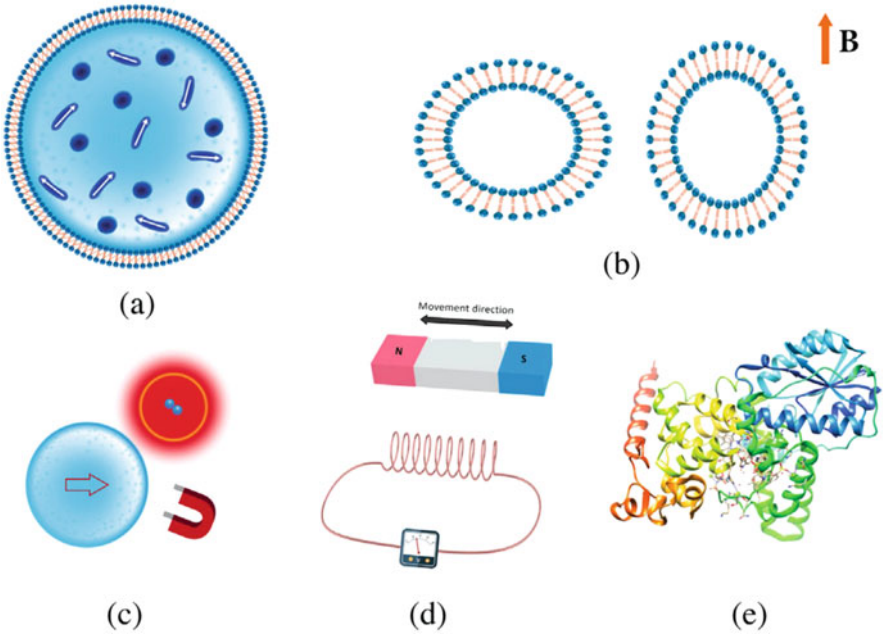

Fig. 6.1 Major mechanisms governing the interaction for magnetic fields with cellular functions. (**a**) In the presence of magnetic particles, their interaction with an applied magnetic field could conceivably activate sensory mechanisms. The cell's magnetic susceptibility may become different than the ambient medium, leading to a noticeable magnetic Maxwell stress. (**b**) Anisotropic diamagnetism of cell membrane, which means that the magnetic susceptibility of a biological cell membrane is anisotropic, and its in-plane component differs from its out-of-plane value, which causes deformation. Physically, the deformation proceeds due to the attempt by the lipid molecules to reorient under the action of an applied magnetic field such that the vesicle then stretches parallel to the field. (**c**) For nonhomogeneous magnetic fields, a force proportional to the magnetic field gradient is developed, i.e., $B \times \nabla B$. (**d**) In the phenomenon of magnetic induction, an electric current is generated due to the temporal variation of the magnetic field. Alternatively, this also occurs when a charged object moves in a magnetic field. (**e**) Magnetic fields can in principle alter chemical reactions and have been proposed to impact free-radical recombination rates. [Reprinted with permission from (Torbati et al. 2022)]

6.2 Cellular Effects of Static Magnetic Fields

SMFs could induce multiple cellular effects depending on the magnetic field itself as well as the cells examined. Here we will mainly discuss some cellular effects that have been reported by multiple independent studies, such as SMF-induced changes in cell orientation, proliferation, microtubule and cell division, actin, viability, attachment/adhesion, morphology, migration, cell membrane, cell cycle, DNA, intracellular reactive oxygen species (ROS), and calcium. Our focus here is mainly on human cells.

6.2.1 Cell Orientation

The orientation changes of biomolecules and cells are one of the most well studied aspects of SMF bioeffects. The magnetic properties of biological samples have been discussed in Chap. 3. It has been proved that when objects with high magnetic susceptibility anisotropy are exposed to strong SMFs, they will change their orientations. There are multiple examples for cells align themselves in parallel to the SMF direction. Among them, the best studied example was erythrocytes (red blood cells, RBCs). The first reported RBC orientation change induced by SMF was in 1965 by Murayama, who found that sickled RBCs were oriented perpendicular to a 0.35 T SMF (Murayama 1965). It is interesting that in 1993, a work carried out by Higashi et al. showed that normal RBCs were also aligned by an 8 T SMF but the orientation direction was different from what Murayama has observed (Higashi et al. 1993). Their results showed that normal RBCs oriented with their disk planes parallel to the field direction (Fig. 6.2). In 1995, they reported that the cell membrane components, including the transmembrane proteins and lipid bilayers, were the major reasons for RBC alignment in 8 T SMF (Higashi et al. 1995). In addition, they found that the paramagnetism of membrane-bound hemoglobin contributes significantly to this orientation (Takeuchi et al. 1995; Higashi et al. 1996). These results clearly demonstrate that cells can be oriented by strong SMFs and the effects depend on the molecular components of the cell. Besides RBCs, more components in the blood stream have also been studied, such as platelets (Yamagishi et al. 1992; Higashi et al. 1997) and fibrinogen (Torbet et al. 1981; Yamagishi et al. 1990; Iwasaka et al. 1994).

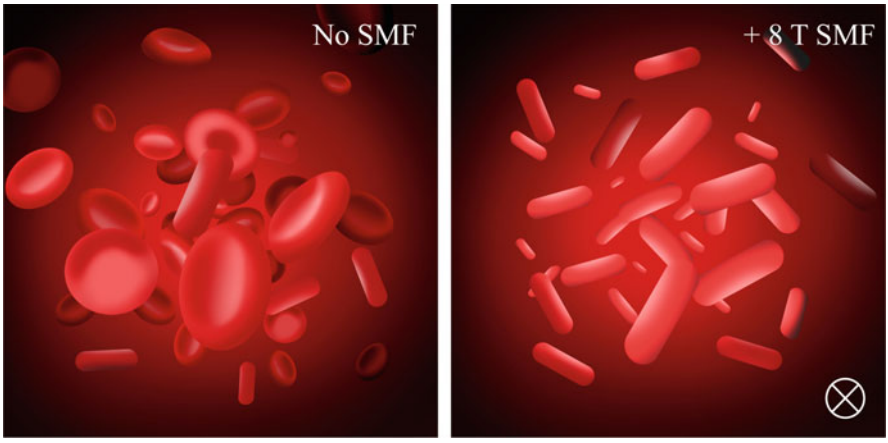

Fig. 6.2 Red blood cells were aligned by an 8 T static magnetic field. Left: red blood cells in control condition, with no SMF. Right: red blood cells in an 8 T SMF. The field direction was normal to the paper. [Illustration courtesy of Shu-tong Maggie Wang and Ding Joe Wang, based on experimental results from reference (Higashi et al. 1993)]

Moreover, some other cells like osteoblast cells, smooth muscle cells, and Schwann cells could also be aligned in parallel to the direction of the strong magnetic fields when they are exposed for a prolonged period. In 2000 and 2002, Kotani et al. found that osteoblast cells were oriented in parallel to the field direction by an 8 T SMF and the bone formation was significantly stimulated to grow along the direction of the magnetic field (Kotani et al. 2000, 2002). In 2001, Umeno et al. found that smooth muscle cell was aligned along the magnetic field direction after they were exposed to an 8 T SMF for 3 days (Umeno et al. 2001). In 2003, Iwasaka et al. found that the 14 T SMF aligned smooth muscle cell assemblies and the cell colonies were extended along the field direction (Iwasaka et al. 2003). Eguchi et al. found that Schwann cells were also oriented in parallel to the 8 T SMF after 60 h exposure (Eguchi et al. 2003). They used linearly polarized light and observed changes in the intracellular macromolecule behavior in 8 T and 14 T SMFs (Iwasaka and Ueno 2003a, b). In 2005, they also examined the actin cytoskeleton in Schwann cells and found that actin fibers were oriented in the direction of 8 T SMF (Eguchi and Ueno 2005). More interestingly, the Schwann cells did not orient in the 8 T SMF when an inhibitor of small GTPase (guanosine triphosphatase) Rho-associated kinase was added, which indicated that the SMF-induced Schwann cell orientation was dependent on Rho-regulated actin fibers (Eguchi and Ueno 2005). In 2007, Coletti et al. found that 80 mT SMF-induced myogenic L6 cells to align in parallel bundles, an orientation conserved throughout differentiation. They proposed that SMF-enhanced parallel orientation of myotubes was relevant to tissue engineering of a highly organized tissue such as skeletal muscle (Coletti et al. 2007).

In the meantime, there are also multiple examples showing that cells could align in perpendicular to the direction of the magnetic fields, such as the bull sperm. The orientation of bull sperm was examined by a few studies, which actually showed stronger alignment effects than RBCs and platelets. The bull sperm cell has a head that mainly contains diamagnetic cell membrane and DNA. It also has a long tail with microtubules inside. In 2001, Emura et al. found that the orientation of bull sperm cells could be affected by SMFs in an MF strength-dependent manner (Emura et al. 2001). They found that the bull sperm could reach 100% alignment perpendicular to the direction of the MF at just below 1 T (Emura et al. 2001). In 2003, Emura et al. showed that the whole bull sperm and the sperm heads were orientated perpendicular to 1.7 T SMF while the paramecium cilia were aligned in parallel to 8 T SMF (Emura et al. 2003). It was interesting that the sperm tail is theoretically predicted to be in parallel with the field direction due to the diamagnetic anisotropy of microtubules, which will be discussed later. But why the whole sperm is aligned in perpendicular to the field direction is still unclear. It is possible that the sperm head has a stronger diamagnetic anisotropy, which dominates the whole sperm.

Another example of cell orientation in perpendicular to the direction of the magnetic field is neurite outgrowth. In 2008, Kim et al. showed that the application of 0.12 T SMF for 3–5 days could be used to modulate the orientation and direction of neurite formation in cultured human neuronal SH-SY5Y cells and PC12 cells (Kim et al. 2008). It is interesting that they found the neurites perpendicular to the SMF had long, thin, and straight appearance while the neurites in parallel to the SMF

direction had “thickened or beaded” dystrophic appearance. More importantly, they not only found the neurites tended to orient perpendicular to the direction of SMF, the direction can also be changed after the SMF direction has changed (Kim et al. 2008).

From evidences mentioned above, it is clear that SMF-induced cell orientation is cell type-dependent. Actually, Ogiue-Ikeda and Ueno compared three different cell lines, including the smooth muscle A7r5 cells, human glioma GI-1 cells, and human kidney HFK293 cells for their orientation changes under 8 T for 60-h exposure. They found that while the smooth muscle A7r5 cells and the human glioma GI-1 cells aligned along the field direction, the human kidney HEK293 cells were not aligned (Ogiue-Ikeda and Ueno 2004). They proposed that this was probably due to their different cell shapes because both A7r5 and GI-1 cells were spindle shaped while HEK293 cells were polygonal shaped. In addition, the orientation of adherent cells such as osteoblasts, smooth muscle cells, and Schwann cells in strong SMFs usually took a few days while floating/suspended cells such as RBCs exhibited a diamagnetic torque rotation in only a few seconds under SMFs of the same flux density. This also implies that when our human bodies are exposed to externally applied SMFs, the orientation of free circulating blood cells would be affected more readily compared to other types of cells.

Table 6.1 summarizes some reported studies about the orientation of cells in SMFs (Table 6.1). It is apparent that other than cell types, the SMF-induced cell orientation change is largely dependent on the MF intensity. The reported that cell orientation changes were all achieved in SMFs of at least 80 mT, and actually most of them were done in ultra-strong magnets, such as in 8 T SMF. Therefore, it is not surprising when Gioia et al. investigated the effect of chronic exposure to a 2 mT SMF on in vitro cultured swine granulosa cells (GCs) and did not observe cell orientation changes (Gioia et al. 2013). In addition, the cell type is an important factor because most cells do not have strong structure characteristics like sperm cell, nor RBCs.

Besides the orientation change of cells themselves in magnetic fields, cells can also be oriented by moderate and strong SMFs when they are embedded in collagen, a macromolecule that has strong diamagnetic anisotropy (Torbet and Ronziere 1984). In 1993, it was found that human foreskin fibroblasts embedded in collagen gel were oriented by 4.0 and 4.7 T SMFs (Guido and Tranquillo 1993). Human glioblastoma A172 cells embedded in collagen gels, but not A172 cells alone, oriented perpendicular to the field direction of 10 T SMF (Hirose et al. 2003). Therefore, the orientation for cells embedded in collagen is largely due to the diamagnetic anisotropy of collagen fibers, which orient in perpendicular direction of SMF. Another example was provided in 2000 by Kotani et al., who found that osteoblast cells themselves were oriented in parallel to the field direction by an 8 T SMF, but the mixture of osteoblast cells and collagen oriented perpendicular to the magnetic fields (Kotani et al. 2000). This is interesting and promising because the stimulation of bone formation to an intended direction using a combination of strong SMF and potent osteogenic agents could possibly lead to a clinically viable treatment of bone fractures and defects. In addition, in 2003, Eguchi et al. found that

Table 6.1 Static magnetic field-induced cell orientation in different studies

Cells examined	SMF flux density	To the SMF direction	References
Human mesenchymal stem cells derived from human newborn cords	18 mT	Parallel	Sadri et al. (2017)
Myogenic cell line L6 cells	80 mT		Coletti et al. (2007)
Paramecium cilia	8 T		Emura et al. (2003)
Normal erythrocytes			Higashi et al. (1993)
Osteoblast cells			Kotani et al. (2000)
Smooth muscle cells			Umeno et al. (2001)
Smooth muscle A7r5 cells and human glioma GI-1 cells			Ogiue-Ikeda and Ueno (2004)
Schwann cells			Eguchi et al. (2003)
Actin cytoskeleton in Schwann cells			Eguchi and Ueno (2005)
Smooth muscle cells			Umeno and Ueno (2003)
Smooth muscle cells	14 T		Umeno and Ueno (2003)
Smooth muscle cell colonies			Iwasaka et al. (2003)
Bone marrow-derived stromal cells of rats	0.12 T	Perpendicular	Okada et al. (2021)
Neurite growth of human neuronal SH-SY5Y cells and PC12 cells	0.12 T		Kim et al. (2008)
Sickled erythrocytes	0.35 T		Murayama (1965)
Bull sperm	~0.5–1.7 T		Emura et al. (2001)
Peritoneal macrophages	1.24 T		Wosik et al. (2018)
Whole bull sperm and bull sperm heads	1.7 T		Emura et al. (2003)
Osteoblast cells mixed with collagen	8 T		Kotani et al. (2000)
Schwann cells mixed with collagen			Eguchi et al. (2003)
Human glioblastoma A172 cells embedded in collagen gels	10 T		Hirose et al. (2003)
Cultured swine granulosa cells (GCs)	2 mT	No change	Gioia et al. (2013)

(continued)

Table 6.1 (continued)

Cells examined	SMF flux density	To the SMF direction	References
Schwann cells treated with an inhibitor of small GTPase Rho-associated kinase	8 T		Eguchi and Ueno (2005)
Human kidney HEK293 cells			Ogiue-Ikeda and Ueno (2004)
Human glioblastoma A172 cells	10 T		Hirose et al. (2003)

Schwann cells themselves oriented in parallel to the 8 T SMF after 60-h exposure but when they were embedded in collagen, they were aligned in perpendicular to the field direction (Eguchi et al. 2003). These data all showed that the collagen has a strong alignment effect on cells embedded in SMFs.

The shapes of most mammalian somatic cells are symmetric and surrounded by and attached to their extracellular matrix and neighboring cells. Therefore, they are less likely to have strong alignment effects in SMFs like sperm cells or RBCs in weak to moderate SMFs. However, the SMF-induced orientation effects can potentially affect their cell division and subsequently tissue development. In addition, it was very promising that Kotani et al. found that an 8 T SMF could cause osteoblasts to orient in parallel to the magnetic field and stimulate bone formation along the field direction. This implies that people may be able to apply SMFs in clinical treatment such as bone disorders. In fact, the orientation effects of RBCs might also provide some insights to help understanding the working mechanism of some magnetic therapy products. Continued efforts are encouraged to investigate more on blood cells, muscles, neurons, bones and sperms, as well as their potential medical applications in the future.

6.2.2 Cell Proliferation/Growth

Not surprisingly, the effect of SMFs on cell proliferation is also cell type-dependent. We summarize some reported studies about the SMF-induced cell proliferation/growth changes (Table 6.2).

Multiple evidence showed that SMFs could inhibit cell proliferation. For example, Malinin et al. exposed mouse fibroblast L-929 cells and human fetal lung fibroblast WI-38 cells to 0.5 T SMF for 4–8 h after they were frozen in liquid nitrogen and found that the subsequent cell growth was significantly inhibited (Malinin et al. 1976). In 1999, Pacini et al. examined the effects of 0.2 T SMF in human breast cancer cells and found that 0.2 T not only reduced cell proliferation but also enhanced the vitamin D anti-proliferative effect (Pacini et al. 1999b). In 2003, Pacini et al. examined human skin fibroblasts for their effects in 0.2 T SMF generated by a magnetic resonance tomography and found that the cell proliferation was reduced (Pacini et al. 2003). In 2008, Hsieh et al. found that 3 T SMF inhibited

Table 6.2 Static magnetic field-induced cell proliferation/growth changes in different studies

Cells examined	SMF flux density	Cell proliferation/ growth	References
Planarian regeneration model	200 μT	Inhibited	Van Huizen et al. (2019)
HT-1080 fibrosarcoma cells	600 μT		Gurhan et al. (2021)
Swine granulosa cells (GCs)	2 mT		Gioia et al. (2013)
Chondrocyte isolated from Wistar rats	2 mT		Escobar et al. (2020)
Human umbilical artery smooth muscle cells (hUASMCs)	5 mT		Li et al. (2012)
Human glioblastoma cell line (A172)	5 mT		Ashta et al. (2020)
Human nephroblastoma cell line G401 and human neuroblastoma cell line CHLA255	5.1 mT		Yuan et al. (2018b)
Human mesenchymal stem cells derived from human newborn cords	18 mT		Sadri et al. (2017)
Human breast adenocarcinoma cell line (MCF-7) and human foreskin fibroblast (HFF) cells	5/10/15/20 mT		Hajipour Verdom et al. (2018)
4 T1 breast cancer cells	150 mT		Fan et al. (2020)
Human breast cancer cells	0.2 T		Pacini et al. (1999b)
Human skin fibroblasts	0.2 T		Pacini et al. (2003)
Adipose-derived stem cells (ASCs)	0.5 T		Wang et al. (2016)
Multiple cancer cell lines	1 T		Zhang et al. (2017c)
Human chondrocytes	3 T		Hsieh et al. (2008)
Jurkat cells	4.75 T		Aldinucci et al. (2003b)
Human nasopharyngeal carcinoma CNE-2Z and colon cancer HCT116 cells	1 and 9 T		Zhang et al. (2015, 2016)
Osteosarcoma cell lines MNNG/HOS, U-2 OS, and MG63	12 T		Wang et al. (2022)
Human umbilical vein endothelial cells	60 or 120 μT	Promoted	Naarala et al. (2017)
Human umbilical endothelial cells	60 and 120 μT		Martino et al. (2010)
HT-1080 fibrosarcoma cells	200/300/ 400 μT		Gurhan et al. (2021)
Human dental pulp stem cells	1 mT		

(continued)

Table 6.2 (continued)

Cells examined	SMF flux density	Cell proliferation/ growth	References
			Zheng et al. (2018)
Mesenchymal stem cells	20 mT		Alipour et al. (2022)
Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs)	21.6 mT		Hamid et al. (2022)
Olfactory ensheathing cells (OECs)	70 mT		Elyasigorji et al. (2022)
Osteoblast cells (MG-63)	72–144 mT		Yuan et al. (2018a)
The human umbilical cord-derived MSCs	0.14 T		Wu et al. (2022)
Mouse breast cancer cell line 4 T1	0.15 T		Fan et al. (2020)
Bone marrow stem cells	0.2 T		Chuo et al. (2013)
Mandibular bone marrow mesenchymal stem cells (MBMSCs) in the MBMSC/mandibular condylar chondrocyte (MCC) coculture system	0.280 T		Zhang et al. (2021)
Dental pulp stem cell proliferation	0.4 T		Lew et al. (2018)
Human adipose-derived mesenchymal stromal stem cells (hASCs)	0.5 T		Maredziak et al. (2017)
Human chondrocytes	0.6 T		Stolfa et al. (2007)
Human normal lung cells	1 T		Zhang et al. (2017c)
Murine osteoblastic cell line MC3T3-E1	16 T		Yang et al. (2018)
Chondrocyte isolated from Wistar rats	1 mT	No change	Escobar et al. (2020)
Mouse neuroblastoma cell line N2a	5.1 mT		Yuan et al. (2018b)
Stromal vascular fraction (SVF) cells (isolated from healthy donors)	50 mT		Filippi et al. (2019)
Murine osteoblastic cell line MC3T3-E1	0.2–0.4 T and 500 nT		Yang et al. (2018)
Myotube cell	0.08 T		Coletti et al. (2007)
Dental pulp cells	0.29 T		Hsu and Chang (2010)
Hematopoietic stem cells	1.5 and 3 T		Iachininoto et al. (2016)

(continued)

Table 6.2 (continued)

Cells examined	SMF flux density	Cell proliferation/ growth	References
Human malignant melanoma cells and the normal human cells	4.7 T		Short et al. (1992)
Normal and PHA-activated peripheral blood mononuclear cells (PBMC)	4.75 T		Aldinucci et al. (2003b)
Unstimulated mononuclear blood cells	7 T		Reddig et al. (2015)
Chinese hamster ovary (CHO)	9 T		Zhang et al. (2016)
Bacterial strain *Shewanella oneidensis* MR-1	14.1 T		Gao et al. (2005)

human chondrocytes growth in vitro and affected recovery of damaged knee cartilage in vivo in the pig model. They also mentioned that these results may be specific to the parameters used in this study and may not apply to other situations, field strengths, forms of cartilage injury, or animal species (Hsieh et al. 2008). In 2012, Li et al. found that the proliferation of human umbilical artery smooth muscle cells (hUASMCs) was significantly decreased after 5 mT SMF exposure for 48 h compared with the non-treated group (Li et al. 2012). In 2013, Mo et al. showed that magnetic shielding increased human neuroblastoma SH-SY5Y cell proliferation (Mo et al. 2013), which indicated that the geomagnetic field may have an inhibitory effect on SH-SY5Y neuroblastoma cell proliferation. In 2013, Gioia et al. investigated the effect of a 2 mT SMF on GCs and found that the doubling time was significantly reduced ($p < 0.05$) in exposed samples after 72 h of culture (Gioia et al. 2013). In 2016, Wang et al. exposed adipose-derived stem cells (ASCs) to 0.5 T SMF for 7 days and found that the cell proliferation was inhibited (Wang et al. 2016). We found that 1 T and 9 T SMFs could inhibit the proliferation of human nasopharyngeal carcinoma CNE-2Z and colon cancer HCT116 cells (Zhang et al. 2015, 2016).

There are also some studies showing that SMFs could promote proliferation of some cell types, such as bone marrow cells, stem cells as well as endothelia cells. For example, Martino et al. found that 60 and 120 μT SMFs increased the cell proliferation of human umbilical vein endothelial cell (Martino et al. 2010). In 2013, Chuo et al. found that a 0.2 T SMF increased the proliferation of bone marrow stem cells (Chuo et al. 2013). In 2007, Stolfa et al. used MTT assay to study the effect of 0.6 T SMF on human chondrocytes and found that the MTT reading was increased by 0.6 T SMF (Stolfa et al. 2007), which was probably due to the increased cell proliferation and/or cell viability or metabolic activity. Maredziak et al. found that 0.5 T SMF increased the proliferation rate of human adipose-derived mesenchymal stromal stem cells (hASCs) via activation of the phosphoinositide 3-kinase/Akt (PI3K/Akt) signaling pathway (Maredziak et al. 2017). Recently, Wu et al. reported that exposure to SMFs of 140 mT (Max) causes membrane depolarization

transduced by T-type voltage-gated calcium channels into second-messenger cascades that regulate downstream gene expression, which increase human mesenchymal stem cells (MSCs) proliferation (Wu et al. 2022).

However, there are also some studies shown that cell proliferation was not affected by SMFs. For example, in 1992 Short et al. found that 4.7 T SMF treatment did not affect cell number of either human malignant melanoma cells or the normal human cells (Short et al. 1992). In 2005, using a nuclear magnetic resonance (NMR) spectrometer, Gao et al. found that even 14.1 T SMF exposure for 12 h did not affect cell growth of bacterial strain *Shewanella oneidensis* MR-1 (Gao et al. 2005). In 2007, Coletti et al. found that 80 mT SMF did not affect myotube cell proliferation (Coletti et al. 2007). In 2010, Hsu and Chang found that 0.29 T SMF did not affect the cell proliferation of dental pulp cells (Hsu and Chang 2010). In 2015, Reddig et al. found that exposure of unstimulated mononuclear blood cells to 7 T SMF alone or in combination with varying gradient magnetic fields and pulsed radiofrequency fields did not affect cell proliferation (Reddig et al. 2015). Iachininoto et al. investigated the effects of 1.5 T and 3 T gradient SMFs for their effects on hematopoietic stem cells and found that the cell proliferation was not affected (Iachininoto et al. 2016).

Moreover, there are some studies that have compared different cell types. For example, in 2003 Aldinucci et al. tested the effects of combining a 4.75 T SMF and a pulsed electromagnetic field (EMF) of 0.7 mT generated by an NMR apparatus. They found that the 4.75 T SMF did not affect cell proliferation in both normal and PHA-activated peripheral blood mononuclear cells (PBMC), but significantly reduced proliferation in Jurkat leukemia cells (Aldinucci et al. 2003b). We found that 1–9 T SMFs inhibited CNE-2Z and HCT116 cancer cells but not the Chinese hamster ovary (CHO) cells (Zhang et al. 2016). In addition, we found that the EGFR/Akt/mTOR signaling pathway, which was upregulated in many cancers, was involved in SMF-induced cancer cell proliferation inhibition (Zhang et al. 2015, 2016). In addition, as we have mentioned before, SMF-induced effects on cell proliferation were not only cell type-dependent, but also dependent on SMF flux density as well as cell density. More investigations are needed to unravel additional mechanisms and specific effects of a given SMF on a specific cell type.

6.2.3 Microtubule and Cell Division

Purified microtubules have been known for a long time to be a target of SMFs as well as electric fields, which align along the magnetic field and electric field direction due to diamagnetic anisotropy of tubulin dimers (Vassilev et al. 1982; Bras et al. 1998, 2014; Minoura and Muto 2006; Wang et al. 2008). It was also shown that tubulin assembly in vitro was disordered by a 10–100 nT hypogeomagnetic field (HGMF; magnetic fields $<$200 nT) (Wang et al. 2008). These studies demonstrated that microtubules could be affected by SMFs in vitro, but the effects of SMFs on microtubules in cells were less reported. In 2005, Valiron et al. showed that the

microtubule and actin cytoskeleton could be affected by 7–17 T ultra-high SMFs in some cell types during interphase (Valiron et al. 2005). In 2013, Gioia observed actin and alpha-tubulin cytoskeleton modifications in swine granulosa cells after 3 days exposure to a 2 mT SMF (Gioia et al. 2013). However, this effect seems to be cell type- and/or exposure time-dependent because our group did not observe obvious microtubule abnormalities in CNE-2Z or RPE1 interphase cells when we exposed them to 1 T SMF for 3 days or 27 T ultra-strong SMF for 4 h (Zhang et al. 2017b).

Microtubule is a key component for mitotic spindle, which is mainly composed of microtubules and chromosomes and is the fundamental machinery for cell division. However, information about the mitotic spindles in SMFs was not provided in the above-mentioned studies. In contrast, time-varying magnetic fields and electric fields have been shown to be able to affect mitotic spindle and cell division. For example, in 1999, Zhao et al. found that a small physiological electric field could orient cultured human corneal epithelial cells through affecting cell division (Zhao et al. 1999). In 2011, Schrader et al. observed spindle disturbances in human-hamster hybrid (A(L)) cells induced by the electrical component of the mobile communication frequency range signal (Schrader et al. 2011). However, for time-varying magnetic fields, people need to distinguish the effects caused by the magnetic fields per se or the thermal effect. In 2011, Ballardin et al. found that 2.45 GHz microwaves could disrupt spindle assembly (inducing multipolar spindles) in Chinese hamster V-79 cells, which was not due to the thermal effects (Ballardin et al. 2011). In contrast, in 2013, Samsonov and Popov found that exposure to 94 GHz radiation increased the rate of microtubule assembly and that effect was actually caused by the thermal effect (Samsonov and Popov 2013). The thermal effect in Samsonov and Popov's study is likely due to the high frequency compared to Ballardin et al.'s study. Moreover, there is a well-known electromagnetic approach called tumor treating fields (TTF, TTFields) that use low-intensity (1–3 V/cm) and intermediate-frequency (100–300 kHz) alternating electric fields to treat cancers such as glioblastoma. The mechanism has been proved to be mainly through disturbing mitotic spindle formation (Kirson et al. 2004; Pless and Weinberg 2011; Davies et al. 2013). TTFields destroy cells within the process of mitosis via apoptosis and have no effect on non-dividing cells (Pless and Weinberg 2011). In fact, the U.S. Food and Drug Administration has approved this technology for use in glioblastoma (Davis 2013).

We previously found that mitotic spindles could be affected by SMFs (Luo et al. 2016). Our results show that 1 T SMF treatment for 7 days could increase the abnormal mitotic spindles and mitotic index (% of cells in mitosis) in HeLa cells, which is likely due to the effect of SMF on microtubules. In addition, this phenotype is also time-dependent because when cells were treated for shorter time, the effects were not obvious. Although 1 T SMF did not affect the overall cell cycle distribution, it could delay the mitotic exit using synchronization experiment (Luo et al. 2016), which will be discussed in the cell cycle section later in this chapter.

Since purified microtubules can be aligned by SMFs, we predict that the spindle orientation could also be affected, which is a critical determining factor for cell division orientation. In fact, back in 1998, Denegre et al. found that 16.7 T large

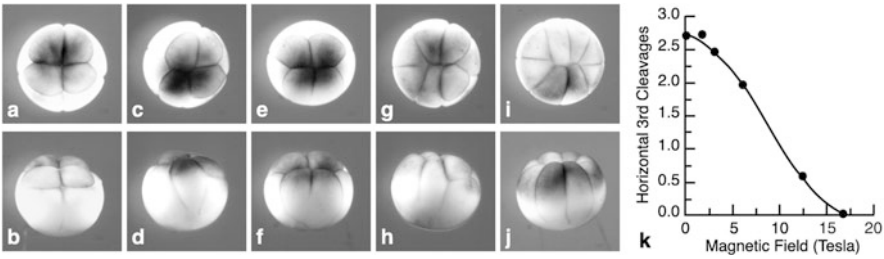

Fig. 6.3 Third cleavage in an animal-vegetal (AV)-parallel static magnetic field. Top (**a**, **c**, **e**, **g** and **i**) and side (**b**, **d**, **f**, **h** and **j**) views of eight-cell embryos from an AV-parallel field, showing the classes of third cleavage reorientation. For the side view, the embryo in the top view was rotated with the animal pole away from the viewer. The numbers of horizontal cleavages depicted are four (normal; **a**, **b**), three (**c**, **d**), two (**e**, **f**), one (**g**, **h**), and zero (**i**, **j**). (**k**) The average number of horizontal third cleavages per embryo as a function of field strength. [Reprinted with permission from (Denegre et al. 1998). Copyright © 1998, National Academy of Sciences, USA]

gradient ultra-high SMF could affect the division orientation of Xenopus eggs (Fig.6.3) (Denegre et al. 1998). In 2006, Eguchi et al. showed that 8 T SMF could also change the cleavage plan formation in frog embryo division (Eguchi et al. 2006). It was proposed that SMFs may affect the orientation of astral microtubules and/or spindles, which was theoretically proven later by Valles (2002), but no experimental evidence has been reported. In 2012, Mo et al. found that hypogeomagnetic field (HGMF; magnetic fields <200 nT) could cause a decrease in horizontal third cleavage furrows and abnormal morphogenesis in Xenopus embryos (Mo et al. 2012). In addition, they used immunofluorescence staining of tubulin to show the reorientation of the spindle of four-cell stage blastomeres. Their results indicated that a brief (2-h) exposure to HGMF was sufficient to interfere with the development of Xenopus embryos at cleavage stages. Also, the mitotic spindle could be an early sensor to the deprivation of the geomagnetic field, which provided a clue to the molecular mechanism underlying the morphological and other changes observed in the developing and/or developed embryos (Mo et al. 2012).

In the meantime, although it was shown that the microtubule and actin cytoskeleton in interphase cells could be affected by 7–17 T ultra-high SMFs in some cell types (Valiron et al. 2005), information about the mitotic spindle in ultra-high SMFs was not provided. Using human nasopharyngeal cancer CNE-2Z cells and human retinal pigment epithelial RPE1 cells, we found that the spindle orientation could be altered by a 27 T ultra-high SMF. More interestingly, we found that the spindle orientation was determined by both microtubules and chromosomes (Zhang et al. 2017b) (Fig. 6.4). High SMF-induced spindle orientation and morphology changes are recoverable for the non-cancer RPE1 cells, but not for the CNE-2Z cancer cells, which caused cancer cell growth arrest.

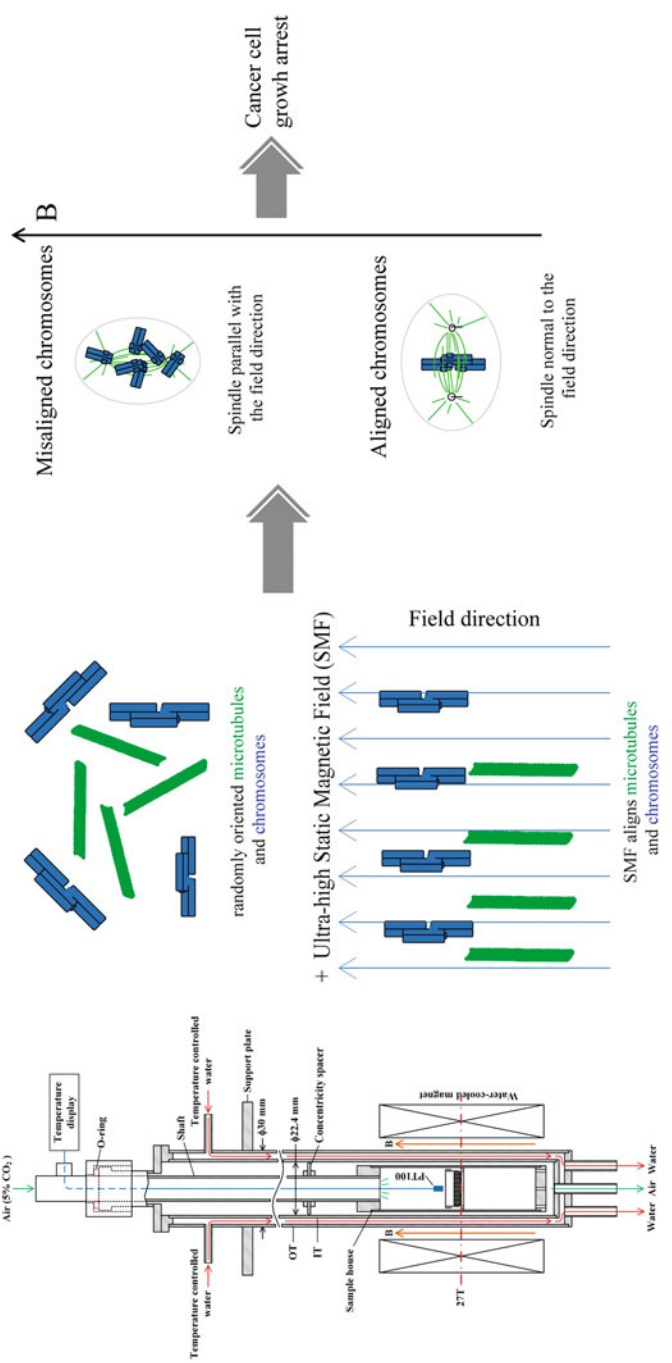

Fig. 6.4 Mitotic spindle orientation changes in high static magnetic fields are determined by the balance between microtubules and chromosomes. A water-cooled magnet (WM4 in the High Magnetic Field Facility of Chinese Academy of Sciences) and a specialized cell incubation system were used to provide a homogeneous 27 T SMF on cells. [Figures are adapted from (Zhang et al. 2017b), open access]

6.2.4 Actin

Besides microtubules, the actin cytoskeleton has also been reported to be affected by SMFs in some cell types. For example, Mo et al. showed that in the absence of the geomagnetic field (GMF), the so-called hypomagnetic field (HMF) environment, the adhesion and migration of human neuroblastoma SH-SY5Y cells were inhibited, which were accompanied with a reduction in cellular F-actin amount and disordered kinetics of actin assembly in vitro (Mo et al. 2016). These results indicated that elimination of the GMF affected assembly of the motility-related actin cytoskeleton and suggested that F-actin was a target of HMF exposure and probably a mediator of GMF sensation (Mo et al. 2016).

Although whether actin could serve as a mediator of GMF sensation still needs to be further confirmed, there are multiple other studies have shown that actin could be affected in cells by SMFs. The most striking and convincing data was provided in 2005 by Eguchi and Ueno (2005), which was briefly mentioned in the cell orientation section above. They examined the actin cytoskeleton in 8 T ultra-high SMF-treated Schwann cells and found that actin fibers were oriented in the direction of the magnetic field. However, when the Schwann cells were treated with an inhibitor of small GTPase Rho-associated kinase, which disrupted actin fibers, the orientation phenotype induced by 8 T SMF no longer existed. This indicated that the SMF-induced Schwann cell orientation was dependent on Rho-regulated actin fibers (Eguchi and Ueno 2005). Therefore, their data directly showed that the Rho-regulated actin fibers were involved in SMF-induced cell orientation, at least in Schwann cell. Another example for SMF-induced actin alteration was in 2007 by Coletti et al. who used myogenic cell line L6 and found that 80 mT SMF promoted myogenic cell alignment and differentiation (Coletti et al. 2007), which was also introduced in the previous cell orientation section (Table 6.1). More specifically, they observed increased accumulation of actin and myosin as well as formation of large multinucleated myotubes, which was derived from increased cell fusion efficiency, but not cell proliferation (Coletti et al. 2007). In addition, a few other studies also showed SMF-induced actin alterations. For example, in 2009, Dini et al. found that 72-h of 6 mT SMF exposure caused human leukemia U937 cell F-actin modification (Dini et al. 2009). In 2013, Gioia found actin cytoskeleton modifications in swine granulosa cells after 3 days exposure to a 2 mT SMF (Gioia et al. 2013). Lew et al. found that 0.4 T SMF could increase the fluorescence intensity of the F-actin (Lew et al. 2018).

There are also some studies that reported the unchanged actin in SMF-treated cells. For example, in 2005, Bodega et al. examined primary cultures of astroglial cells for their responses to 1 mT sinusoidal, static, or combined magnetic field for various timepoints and did not observe any significant changes on actin (Bodega et al. 2005). In my opinion, their magnetic flux density in their study might be too low to induce actin alteration. We examined multiple human cancer cells, such as human nasopharyngeal cancer CNE-2Z and colon cancer HCT116 cells, for their responses to 1 T SMF for 2–3 days and did not observe any significant changes on

actin (data not shown). However, the cells we examined are different from above-mentioned cell types that have actin alterations upon SMF exposure, such as neuroblastoma cells, Schwann cells, and myogenic cell. These cells may have different actin regulation network than the cancer cell lines we examined. From the above-mentioned studies, it is likely that actin cytoskeleton in cells respond to SMFs in a cell type- and magnetic field flux density-dependent way, which will need more systematic investigations.

6.2.5 Cell Viability

So far, most studies showed that SMFs had minimum effects on cell viability. For example, in 1992, Short et al. found that 4.7 T SMF treatment did not affect cell viability in both human malignant melanoma cells and normal human fibroblast cells (Short et al. 1992). In 2003, Pacini et al. found that 0.2 T SMF could affect the cell morphology and proliferation but not the cell viability of human skin fibroblasts (Pacini et al. 2003). In 2009, Dini et al. reported that 72-h exposure of 6 mT SMF did not affect cell viability in human leukemia U937 cells (Dini et al. 2009). In 2013, Gioia et al. investigated the effect of chronic exposure to a 2 mT SMF on in vitro cultured swine granulosa cells (GCs) and found that the SMF exposure did not affect the cell viability (Gioia et al. 2013). In 2016, Romeo et al. examined human fetal lung fibroblasts MRC-5 exposed to 370 mT SMF and found that the cell viability was not affected (Romeo et al. 2016). We examined 1 T SMF-induced effects on cell viability in 15 different cell lines, including human cancer cell lines CNE-2Z, A431 and A549, non-cancer cell line 293 T as well as CHO cells, etc. In fact, we checked four different cell densities and found that the cell viability was not obviously changed by 1 T SMF in any of these cell types (Zhang et al. 2017c). These studies, including more than 20 different cell types, showed that SMFs do not have obviously effect on cell viability.

However, there are a few studies indicate that SMFs could increase apoptosis in some cell types. In 2005, Chionna et al. reported that 6 mT SMF-induced apoptosis in Hep G2 cells in a time-dependent manner. The apoptosis was almost negligible at the beginning of experiment but increased to about 20% after 24-h of continuous exposure (Chionna et al. 2005). In 2006, Tenuzzo et al. found that 6 mT SMF could promote apoptosis in T hybridoma 3DO cells, human liver cancer Hep G2 cells, and rat thyroid FRTL cells, but not human lymphocytes, mice thymocytes, human histiocytic lymphoma or human cervical cancer HeLa cells (Tenuzzo et al. 2006). In 2008, Hsieh et al. found that 3 T SMF-induced human chondrocytes apoptosis through p53, p21, p27, and Bax protein expression (Hsieh et al. 2008). In 2016, Wang et al. exposed adipose-derived stem cells (ASCs) to 0.5 T SMF for 7 days and found that the cell viability was inhibited (Wang et al. 2016).

It is interesting and puzzling that when SMFs are combined with some other treatments, they have been shown to have totally opposite effects. For example, in 2001, Tofani et al. found that when 3 mT SMF was combined with 3 mT 50 Hz time-

varying magnetic fields, the apoptosis of human colon carcinoma WiDr and breast cancer MCF-7 cells were increased, while the MRC-5 cells were not affected (Tofani et al. 2001). In 2006, Ghibelli et al. found that exposure to SMFs of NMR (1 T) could increase damage-induced apoptosis in tumor cells of hematopoietic origin, but not mononuclear white blood cells, showing that NMR may increase the differential cytotoxicity of antitumor drugs on tumor vs. normal cells (Ghibelli et al. 2006). These studies show that SMF could promote the apoptosis effects of time-varying magnetic fields or antitumor drugs. However, there are also evidences showing that SMF could protect some cells from apoptosis. For example, in 1999, Fanelli et al. showed that 0.3–60 mT SMFs could reduce cell apoptosis induced by damaging agents such as etoposide (VP16) and puromycin (PMC) (Fanelli et al. 1999). It was also interesting that although Tenuzzo et al. found that 6 mT SMF could promote apoptosis in T hybridoma 3DO cells, human liver cancer Hep G2 cells, and rat thyroid FRTL cells, when the SMF was combined with apoptotic inducing drugs, such as cycloheximide and puromycin, it had a protective effect because the majority of cells could be rescued from apoptosis, except for 3DO (Tenuzzo et al. 2006).

Therefore, the effect of SMFs on cell apoptosis is magnetic field intensity, treatment time, and most importantly, cell type-dependent. In most reported cases, the cell viability was not affected by SMFs. However, there were also a few reports indicating that some cells could be affected. In addition, SMFs could have combinational or antagonistic effects when they are combined with other treatments, such as time-varying magnetic fields or different cell damaging agents. Further investigations are strongly needed to unravel the underlying mechanisms.

6.2.6 Cell Attachment/Adhesion

There are several studies showing that the cell attachment could be affected by SMFs. For example, in 2011, Sullivan et al. exposed the cells directly to SMFs right after seeding with an exposure time of 18 h and found that WI-38 (human fetal lung fibroblast cells) attachment was significantly reduced by 35–120 mT SMFs (Sullivan et al. 2011). In 2012, Li et al. exposed human umbilical artery smooth muscle cells (hUASMCs) to 5 mT SMF for 48-h and found that the cell adhesion was obviously decreased (Li et al. 2012). In 2014, Wang et al. found that moderate SMFs of 0.26–0.33 T could reduce human breast cancer MCF-7 cell attachment (Wang et al. 2014).

Although these results indicate that cell attachment/adhesion may be affected by SMFs, the consensus result is still lacking. In most cases, SMFs seem to inhibit the cell attachment/adhesion, there are also opposite evidences. For example, Mo et al. found that shielding of the geomagnetic field also inhibited cell adhesion and migration accompanied with a reduction in cellular F-actin amount in human neuroblastoma SH-SY5Y cells (Mo et al. 2016). This indicates that in the absence of SMF, the cell attachment could also be reduced. Moreover, in our own experience, the cell attachment/adhesion of most cells was not affected by moderate SMFs.

Not surprisingly, the SMF-induced changes in cell attachment also seemed to be cell type-dependent. In 1992, Short et al. tested both human malignant melanoma cells and the normal human cells and found that the malignant melanoma cells had reduced attachment to the tissue culture surface while the normal fibroblasts were not affected by the 4.7 T SMF (Short et al. 1992). Wang et al. found that although human breast cancer MCF-7 cell attachment was reduced by moderate SMFs of 0.26–0.33 T, the HeLa cell attachment was not affected (Wang et al. 2014). In addition to the different cell types, the experimental procedure, such as the timing of SMF exposure before or after the cells have been attached to the cell culture plates, is also likely to be a key factor that influences the experimental outcomes. Moreover, we found that the supporting substrate, such as the cell culture plate and the coverslip, can also influence the experimental results about cell attachment/adhesion. Therefore, more researches are certainly needed to examine the exact effects of SMFs on cell attachment/adhesion, as well as their consequences in vivo.

6.2.7 Cell Morphology

Multiple studies have shown that the cell shape can be altered by SMFs. In 2003, Pacini et al. found that the morphology of human skin fibroblast cells was modified by 0.2 T SMF (Pacini et al. 2003). In the same year, Iwasaka et al. found that 14 T SMF affected the morphology of smooth muscle cell assemblies, and the shapes of the cell colonies extended along the direction of the magnetic flux (Iwasaka et al. 2003). Chinonna et al. also reported time-dependent cell shape and membrane microvilli changes in human histiocytic lymphoma U937 cells and human lymphocytes by a 6 mT SMF (Chionna et al. 2003). In 2005, Chionna et al. found that Hep G2 cells exposed to 6 mT SMF for 24 h were elongated with many irregular microvilli randomly distributed on the cell surface, as well as a less flat shape due to partial detachment from the culture dishes. In addition, cytoskeleton was also modified in a time-dependent manner (Chionna et al. 2005). In 2009, Dini et al. found that 72 h of 6 mT SMF caused human leukemia U937 cell shape change and F-actin modification, appearance of membrane roughness and large blebs and impaired expression of specific macrophagic markers on the cell surface (Dini et al. 2009). It was also interesting that although the cell growth was inhibited, the average cell size of *rat* pituitary adenoma GH3 cells was increased by prolonged exposure to 0.5 T SMF (Rosen and Chastney 2009). In 2013, Gioia found cell length and thickness changes, as well as actin and alpha-tubulin cytoskeleton modifications in swine granulosa cells after 3 days exposure to a 2 mT SMF (Gioia et al. 2013). Mo et al. found that magnetic shielding made the human neuroblastoma SH-SY5Y cells smaller in size and more round in shape, which was likely due to the disordered kinetics of actin assembly (Mo et al. 2016).

Not surprisingly, there are also many studies that did not observe cell morphology changes after SMF exposure. For example, in 1992, Sato et al. found that there were no cell shape changes in HeLa cells after 1.5 T SMF exposure for 96 h (Sato et al.

1992). In 2003, Iwasaka et al. found that no distinct changes in cell morphology in smooth muscle cells including cell membrane components occurred during the 3-h exposure to 8 T magnetic field (Iwasaka and Ueno 2003b). In 2005, Bodega et al. examined primary cultures of astroglial cells for their responses to 1 mT sinusoidal, static, or combined magnetic fields for various timepoints and did not observe any significant changes on actin (Bodega et al. 2005). Again, the cell type may play a very important role in the SMF-induced cell morphology changes. For example, in 1999, Pacini et al. found that a 0.2 T magnetic field-induced obvious morphology change in human neuronal FNC-B4 cell but did not affect mouse leukemia or human breast carcinoma cells (Pacini et al. 1999a).

In addition, multiple other factors could also determine whether people can observe cell morphology changes after SMF exposure, such as magnetic flux density and exposure time, as well as detection techniques and experimental setup. There are two studies that both used freezing and SMF but the experimental results are totally different. The first one was in 1976, Malinin et al. exposed mouse fibroblast L-929 cells and human fetal lung fibroblast WI-38 cells to 0.5 T SMF for 4–8 h after they were frozen and found that the cell morphology was significantly changed after they were thawed and cultured for 1–5 weeks (Malinin et al. 1976). In contrast, in 2013, Lin et al. found that when 0.4 or 0.8 T SMFs were used during the slow cooling procedures of RBCs, the survival rates of frozen-thawed RBCs were increased and there was no morphological changes (Lin et al. 2013). The mechanisms of the SMF + freezing-induced cell growth and/or morphological changes between these two studies are still unknown, which could be due to the SMF + freezing procedure differences, or cell type differences. More studies are needed to test more cells in both procedures to reveal the underlying mechanisms.

6.2.8 Cell Migration

There are some studies showing that SMFs could affect cell migration. On the one hand, studies show that cell migration can be inhibited by SMFs. For example, back in 1990, Papatheofanis found that 0.1 T SMF could inhibit cell migration of human polymorphonuclear leukocytes (PMNs) (Papatheofanis 1990). In 2012, Li et al. found that 5 mT SMF treatment for 48 h inhibited human umbilical artery smooth muscle cells (hUASMCs) migration (Li et al. 2012). In 2021, our group found that an upward direction gradient SMF provided by a NdFeB permanent magnet (Fig. 6.5a, b) can increase the cellular ROS level of ovarian cancer HO8910 and SKOV3 cells and inhibit their migration. In contrast, the normal human ovarian cells (IOSE386) migration was not affected (Fig. 6.5c–e) (Song et al. 2021). These results show that the SMF effects on cell migration are also cell type-dependent. We further performed RNA sequencing and found that these moderate SMFs increased the oxidative stress level and reduced the stemness of ovarian cancer cells. Consistently, the expressions of stemness-related genes were significantly decreased, including hyaluronan receptor (CD44), SRY-box transcription factor 2 (Sox2), and cell myc proto-oncogene

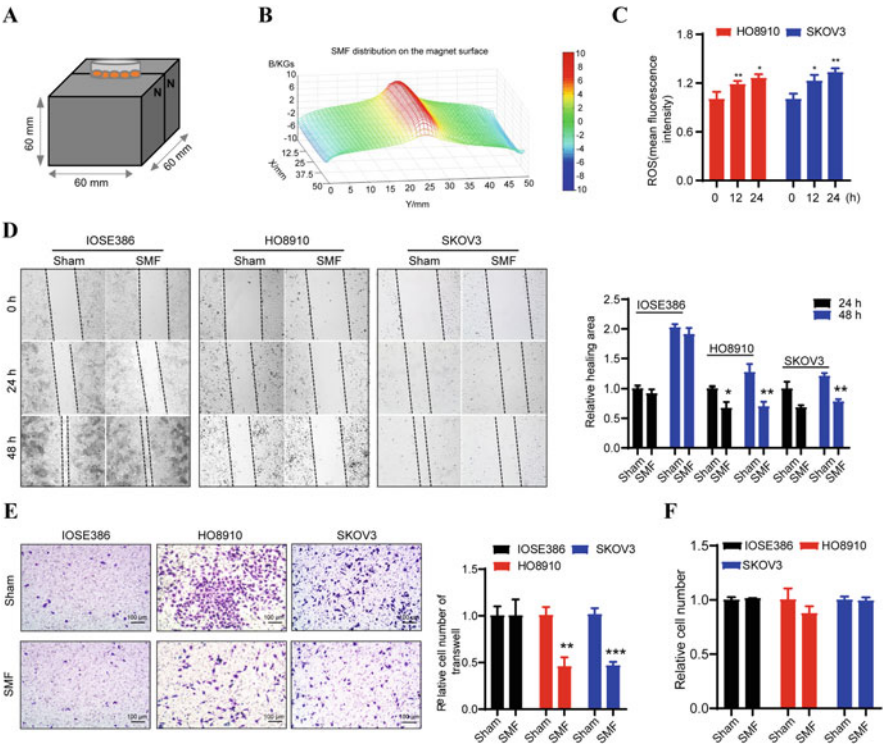

Fig. 6.5 Moderate static magnetic fields increase ovarian cancer cell ROS levels and inhibit cell migration. (**a**) Illustration of cells exposed to a moderate SMF provided by a permanent magnet. (**b**) Magnetic field distribution on the magnet surface was measured by a magnet analyzer. The SMF range in the cell culture dish area is 0.1–0.5 T. (**c**) ROS levels of HO8910 and SKOV3 cells exposed to the moderate SMF at different time points and one-way analysis of variance (ANOVA) with Bonferroni correction for comparison between three groups. (**d**) Wound healing assays of IOSE386, HO8910, and SKOV3 cells exposed to moderate SMF. Quantification of the relative healing area is shown on the right. Comparisons were made between two groups by Student's *t* test. (**e**) Transwell invasion assays of IOSE386, HO8910 and SKOV3 cells treated with or without 20 μM H_2O_2. Quantification of the invasive cells is shown on the right. Comparisons were made between two groups by Student's *t* test. (**f**) Relative cell numbers of IOSE386, HO8910, and SKOV3 cells exposed to moderate SMF for 24 h. Comparisons were made between the experimental group and the sham control group by Student's *t* test. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. [Reprinted from (Song et al. 2021), open access]

protein (C-myc). The ovarian cancer metastasis in mice was also inhibited (Song et al. 2021).

On the other hand, there are also studies indicate that SMFs can increase cell migration. For example, in 2016, Mo et al. found that in the absence of the geomagnetic field, the human neuroblastoma cell migration was inhibited accompanied with a reduction in cellular F-actin amount (Mo et al. 2016). This indicates that geomagnetic field may be important for cell migration. Recently, by NIH3T3 cellular experiments in vitro and diabetic wound healing experiments in vivo, we

show that high glucose-induced impairments in cell migration can be improved by moderate SMF treatment, which makes them a potential tool to improved diabetic wound healing (Feng et al. 2022). However, the relevant studies about SMFs and cell migration are too few to find some clues about the magnetic parameter and cell types.

It should be mentioned that there are many studies using gradient SMFs to separate different cell populations based on their different migration ability, which is called magnetophoresis. Based on the measured magnetic moments of hemoglobin and the relatively high hemoglobin concentration of human RBCs, the differential migration of RBCs was possible if exposed to a high gradient SMF. For example, in 2003, Zborowski et al. used a mean magnetic field of 1.40 T and a mean gradient of 0.131 T/mm to separate deoxygenated and methemoglobin (metHb)-containing RBCs (Zborowski et al. 2003). The existence of unpaired electrons in the four heme groups of deoxy and metHb gives them paramagnetic properties, which is very different from the diamagnetic property of oxyhemoglobin. Zborowski et al. showed that the magnetophoretic mobility for erythrocytes with 100% deoxygenated hemoglobin and for erythrocytes containing 100% metHb were similar, while oxygenated erythrocytes were diamagnetic (Zborowski et al. 2003). Magnetophoresis could provide a way to characterize and separate cells based on magnetic properties of biological macromolecules in cells (Zborowski et al. 2003). In fact, this technique has been used in both malaria detection and infected erythrocyte separation. Although many other techniques are also available, magnetophoretic is very promising because of their high specificity for malaria parasite-infected RBCs (Kasetsirikul et al. 2016).

There are also some studies using gradient SMFs to "guide" cell migration. For example, in 2013, Zablotskii et al. showed that SMF gradient could assist cell migration to those areas with the strongest magnetic field gradient, thereby allowing the buildup of tunable interconnected stem cell networks, which is an elegant route for tissue engineering and regenerative medicine (Zablotskii et al. 2013).

6.2.9 Stem Cell Differentiation

Stem cell is probably one of the most susceptible cell types that are responsive to MFs. In fact, there have been multiple studies that have investigated the effect of SMFs on stem cells, such as dental pulp stem cells (DPSCs), bone marrow stromal cells (BMSCs), human adipose-derived stem cells (hASCs), etc., which have been previously discussed in some reviews (Sadri et al. 2017; Marycz et al. 2018; Ho et al. 2019).

In recent few years, there are more studies that have reported the promotion effects of SMFs on stem cells. For example, it was shown that a 0.4 T SMF can enhance dental pulp stem cell proliferation by activating the p38 mitogen-activated protein kinase pathway as its putative mechanism (Lew et al. 2018). In 2019, using the planarian regeneration model, Van Huizen et al. found that weak magnetic fields

(WMFs) of <1 mT altered stem cell proliferation and subsequent differentiation via changes in ROS accumulation and downstream heat shock protein 70 (Hsp70) expression, indicating that by adjusting SMF strength, SMFs can increase or decrease new tissue formation in vivo (Van Huizen et al. 2019). In 2021, Zhang et al. investigated the effect of moderate SMF on the chondrogenesis and proliferation of mandibular bone marrow mesenchymal stem cells (MBMSCs) in the MBMSC/mandibular condylar chondrocyte (MCC) coculture system. They found that the proliferation of MBMSCs was significantly enhanced in the experimental group with MBMSCs cocultured with MCCs under SMF stimulation relative to controls. Glycosaminoglycan (GAG) content was increased, and SOX9, collagen type II alpha 1 (COL2A1), and aggrecan (ACAN) were also increased at the mRNA and protein levels. This indicates the potential of moderate SMF in repairing condylar cartilage defects in medicine (Zhang et al. 2021). Recently, Wu et al. found that ~100 mT SMF regulates T-type calcium ion channels and mediates mesenchymal stem cells proliferation (Wu et al. 2022). There are also two studies that have investigated the SMF effects on cancer cell stemness (Zhao et al. 2021; Song et al. 2022), which will be discussed in Chap. 9 of this book.

6.2.10 Cell Membrane

The cell membrane itself is dielectric and plays important roles in cellular responses to external stimuli, especially for electromagnetic fields. As we have mentioned in the introduction, SMF could affect cellular function via membrane, which has been reviewed from a physical point of view, focusing on deformation (Torbati et al. 2022), and gradient SMF-induced membrane changes involving ion channels and membrane potential (Chap. 5 of this book).

In fact, high SMF-induced membrane alignment change is one of the best studied effects on biomolecules. The cell membrane mainly consists of phospholipids and embedded proteins, and the phospholipids of a cell membrane are orderly arranged in a double layer, called lipid bilayers. Due to the diamagnetic anisotropy of phospholipid molecules in the lipid bilayer (Braganza et al. 1984; Helfrich 1973), the phospholipid molecules would align or reorient in the high SMFs, which consequently affect the bulk biophysical properties of the cell membrane. In fact, the RBC orientation changes mentioned earlier are one of the best examples illustrating the action of SMF on cell membrane to affect cellular behaviors (Fig. 6.2). Moreover, in a more simplified model, the lipid vesicles made from egg lecithin are shown to be able to completely align parallelly to an external 1.5 T SMF in seconds (Fig. 6.6) (Boroske and Helfrich 1978). These studies demonstrate that the origin of magnetic alignment of nonspherical vesicles is the interaction of the magnetically anisotropic bilayer with the externally applied SMFs.

Later, multiple studies have shown that the cell membrane permeability can be increased by SMFs. For example, in 2011, Liu et al. used Atomic Force Microscope (AFM) to reveal that a 9 mT SMF could increase the number and size of the holes on

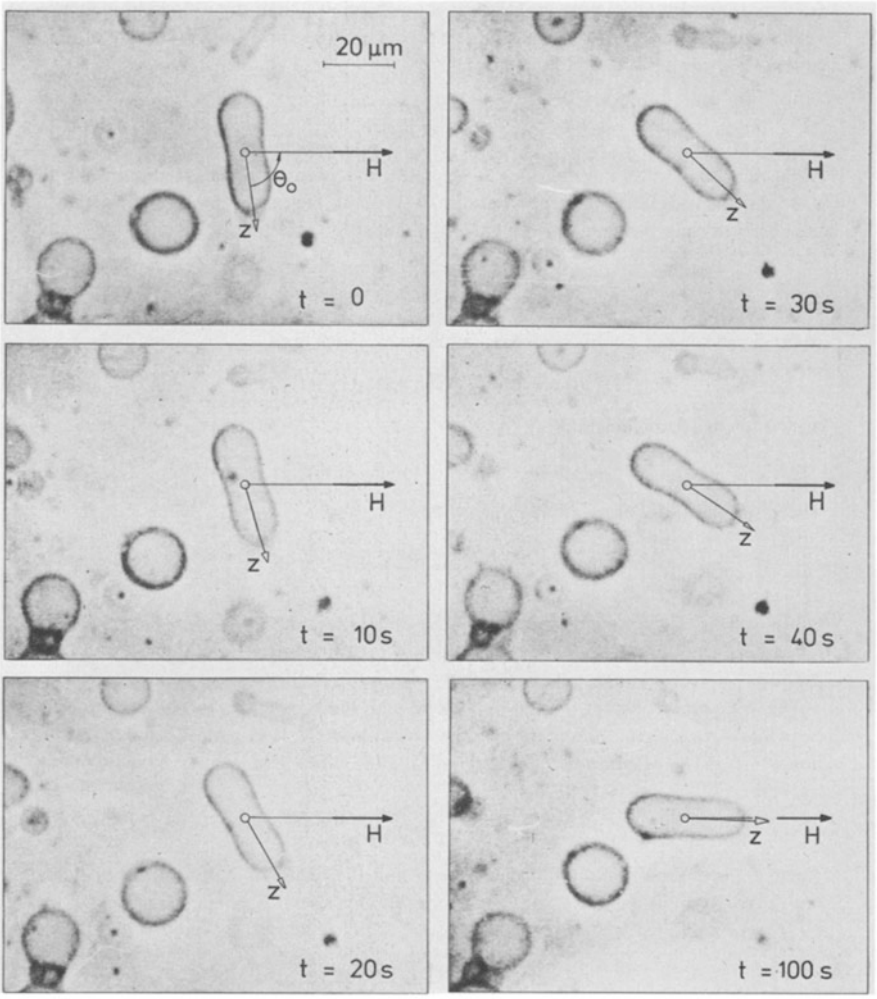

Fig. 6.6 Alignments of a cylindrical lipid vesicle in a static magnetic field. To measure the field-induced alignment of cylindrical vesicles made from egg lecithin, a homogeneous field of 1.5 T was applied parallel to the sample slides. Simultaneously, the sample was observed under a phase contrast microscope, with the optical axis being normal to the slides. The vesicle movements, translational and rotational were recorded. The vesicles could be moved parallel and perpendicular to the magnetic field and rotated around the microscope axis so that the initial angle of orientation made with the field was variable. [Reprinted with permission from (Boroske and Helfrich 1978)]

the cell membrane of K562 cells, which may increase the membrane permeability and the flow of the anticancer drugs (Liu et al. 2011). In 2012, Bajpai et al. found that 0.1 T SMF could suppress both gram positive (*S. epidermidis*) and gram negative bacteria (*E. coli*) growth, which was likely due to SMF-induced cell membrane damages (Bajpai et al. 2012). There are also multiple studies indicated that SMFs

could increase the membrane rigidity in cells. For example, in 2013, Lin et al. found that a 0.8 T SMF decreased membrane fluidity and enhanced erythrocyte membrane stability to resist dehydration damage caused by slow cooling procedures (Lin et al. 2013). They found that the SMF coupled with the slow cooling procedure increased the survival rates of frozen-thawed erythrocytes without obvious cellular damage. Therefore they proposed that the SMFs increased the biophysical stability of the cell membrane, which reduced dehydration damage to the erythrocyte membrane during the slow cooling procedure (Lin et al. 2013). In 2015, Hsieh et al. showed that dental pulp cells (DPCs) treated with a 0.4 T SMF had a higher tolerance to lipopolysaccharide (LPS)-induced inflammatory response when compared to untreated controls. They suggested that 0.4 T SMF attenuates LPS-induced inflammatory response to DPCs by changing cell membrane stability/rigidity (Hsieh et al. 2015). Lew et al. used 0.4 T SMF to treat dental pulp stem cells (DPSCs) and suggested that the cell membranes of the DPSCs were affected to influence intracellular calcium (Lew et al. 2018).

The effects of SMFs on cell membrane are also cell type-dependent. In 2006, Nuccitelli et al. showed that 6 mT SMF exposure for 5 min affected cell membrane potential differently in various cell types. Specifically, the 6 mT SMF caused depolarization in Jurkat cells but hyperpolarization in U937 cells (Nuccitelli et al. 2006). In addition, high resolution imaging techniques like AFM or electron microscopy are also important to reveal the SMF-induced cell membrane changes, which have been used in multiple studies to reveal the membrane changes or membrane associated protein changes caused by SMFs (Jia et al. 2007; Liu et al. 2011; Wang et al. 2014). In contrast, low resolution imaging techniques are less likely to unravel the membrane changes. In 2010, Wang et al. used an illustration to show the potential mechanism of SMFs on cell membrane, some of the associated receptor and channel proteins, as well as the downstream effectors (Wang et al. 2010). In addition, since membrane dynamics changes can affect the activity of membrane embedded proteins, SMFs may also affect some of the membrane associated proteins, such as mechanosensitive ion channels or other embedded proteins (Petrov and Martinac 2007; Wang et al. 2010).

Since SMF-induced membrane bending not only affects ion channels, but also leads to the generation of electrical fields via flexoelectricity, the effects of SMFs on cell membrane could potentially affect a large number of cellular processes, which are still underexplored. It is possible that some of the SMF-induced effects on nervous system (Chap. 13), ROS and calcium changes that will be discussed later in this chapter, are all related to the SMF-induced membrane deformation, which should be investigated in more details in the future.

6.2.11 Cell Cycle

There are a few studies indicating that SMFs may be able to affect cell cycle in some types of cells or at specific conditions. For example, in 2010, Chen et al. found that

8.8 mT SMF increased the G2/M phase and decreased G1 and S phases in K562 cells (Chen et al. 2010). In 2013, Mo et al. showed that magnetic shielding promoted cell cycle progression in the G1 phase of SH-SY5Y cells (Mo et al. 2013). We found that 1 T SMF could cause a mitotic arrest to reduce cell number in synchronized HeLa cells (Luo et al. 2016).

On the other hand, most other studies found that the cell cycle was not affected by SMFs. For example, in 2010, Hsu and Chang found that 0.29 T SMF did not affect the cell cycle of dental pulp cells (Hsu and Chang 2010). Also in 2010, Sarvestani et al. investigated the effects of a 15 mT SMF on cell cycle progression in rat bone marrow stem cells (BMSCs) and did not find any cell cycle changes (Sarvestani et al. 2010). We analyzed multiple cell types seeded at different cell densities for the effects of 1 T SMF (Zhang et al. 2017c). For all the cell lines we tested, 1 T SMF exposure for 2 days did not significantly affect the cell cycle. In addition, we exposed human colon cancer HCT116 cells and human nasopharyngeal cancer CNE-2Z cells to 9 T SMF for 3 days (Zhang et al. 2016), or exposed CNE-2Z cells to an ultra-high 27 T SMF for 4 h and did not observe obvious cell cycle changes (Zhang et al. 2017b).

However, the effect of SMFs on cell cycle is likely to be cell type-dependent, just like most other SMF-induced cellular effects. In 2010, Zhao et al. found that 13 T SMF had no obvious effect on the cell cycle distribution in both CHO cells and DNA double-strand break repair-deficient mutant XRS-5 cells, but decreased the G0/G1 phase and increased S phase cell percentage in human primary skin AG1522 cells (Zhao et al. 2010). This indicates that maybe SMFs have more effects on cell cycles in primary cells than immortalized cells. In addition, the specific cell cycle changes in SMF-induced are different in reported studies (Chen et al. 2010; Zhao et al. 2010). Therefore, further investigations are needed to examine more cell types and/or experimental conditions for the exact effect of SMFs on cell cycle.

Although most results so far showed that SMFs did not change the overall cell cycle distribution of a given cell population, we found that prolonged exposure (7 days) to 1 T SMF could increase the abnormal spindle percentage and the mitotic index in HeLa cells (Luo et al. 2016). Moreover, we found that the duration of mitosis was increased by 1 T SMF. Using cell synchronization experiment, we found that 1 T SMF could delay cells exiting from mitosis. In the absence of 1 T SMF, most of the double thymidine synchronized cells exit from mitosis 12 h after thymidine release. However, there were a significantly increased number of HeLa cells staying in mitosis in the presence of 1 T SMF.

6.2.12 DNA

Due to the public health concerns about the power lines, mobile phones, and cancer, DNA integrity is frequently studied in pulsed MFs (McCann et al. 1993; Cridland et al. 1996; Olsson et al. 2001; Zhou et al. 2002; Williams et al. 2006; Ruiz-Gomez et al. 2010). As early as 1984, Liboff et al. show that DNA synthesis in cells could be

increased by time-varying MFs (Liboff et al. 1984). Although so far there are still not enough evidences to confirm the harmful mutagenesis effects of these time-varying MFs on human bodies, more researches are still needed since people have increased exposure to various time-varying magnetic fields nowadays.

In contrast, SMF-induced DNA damage and mutation are relatively less revealed. In 2004, Takashima et al. used somatic mutation and recombination test system in DNA repair-proficient and -deficient strains of *Drosophila melanogaster* to test strong SMFs for their possible effects on DNA damage and mutation in flies. They found that 2, 5, or 14 T fields exposure for 24 h caused a statistically significant enhancement in somatic recombination frequency in the postreplication repair-deficient flies, whereas the frequency remained unchanged in the nucleotide excision repair-deficient flies and in the DNA repair-proficient flies after exposure. In addition, they found that exposure to high magnetic fields induces somatic recombination in Drosophila and that the dose-response relationship is not linear (Takashima et al. 2004). Other than this work in flies, most other studies revealed that SMFs do not cause DNA damage or mutation. For example, in 2015, Reddig et al. found that exposure of unstimulated human mononuclear blood cells to 7 T SMF alone or combined with varying gradient magnetic fields and pulsed radiofrequency fields did not induce DNA double-strand breaks (Reddig et al. 2015). In 2016, Romeo et al. examined human fetal lung fibroblasts MRC-5 exposed to 370 mT SMF and found that the DNA integrity was not affected (Romeo et al. 2016). Wang et al. exposed adipose-derived stem cells (ASCs) to 0.5 T SMF for 7 days and did not observe DNA integrity changes (Wang et al. 2016). Therefore, these studies did not reveal the direct DNA damage. Interestingly, in 2014, Teodori et al. found that the DNA damage in primary glioblastoma cells caused by X-ray irradiation could be prevented by an 80 mT SMF exposure, which is likely due to the SMF-induced protection effect on mitochondria membrane potential (Teodori et al. 2014). So 80 mT SMF might have a protective role in X-ray-induced DNA damage. However, it was also shown that combining 10 T SMF with X-ray-irradiation could promote the micronucleus formation, although the 10 T SMF itself does not have any effects on micronucleus formation (Nakahara et al. 2002). The available evidences so far about SMF-induced DNA damage and mutation are still not sufficient to a solid conclusion. Most studies revealed that SMFs do not cause DNA damage or mutation in human cells. However, more investigations are encouraged to examine different cell types and magnetic field intensities to help us to achieve a more complete understanding on this issue.

Besides DNA damage that we discussed above, the alignment of DNA in the presence of magnetic field was also studied. It was reported that the DNA chain can be aligned by strong SMFs because of its relative large diamagnetic anisotropy (Maret et al. 1975), which is mainly due to their stacked aromatic bases. In addition, it has been theoretically predicted that the highly compacted mitotic chromosome arms can generate electromagnetic fields along the chromosome arm direction (Zhao and Zhan 2012) and chromosomes should be able to be fully aligned by SMFs of around 1.4 T (Maret 1990). In addition, Andrews et al. showed that the isolated mitotic chromosomes can be aligned by an electric field (Andrews et al. 1980). We

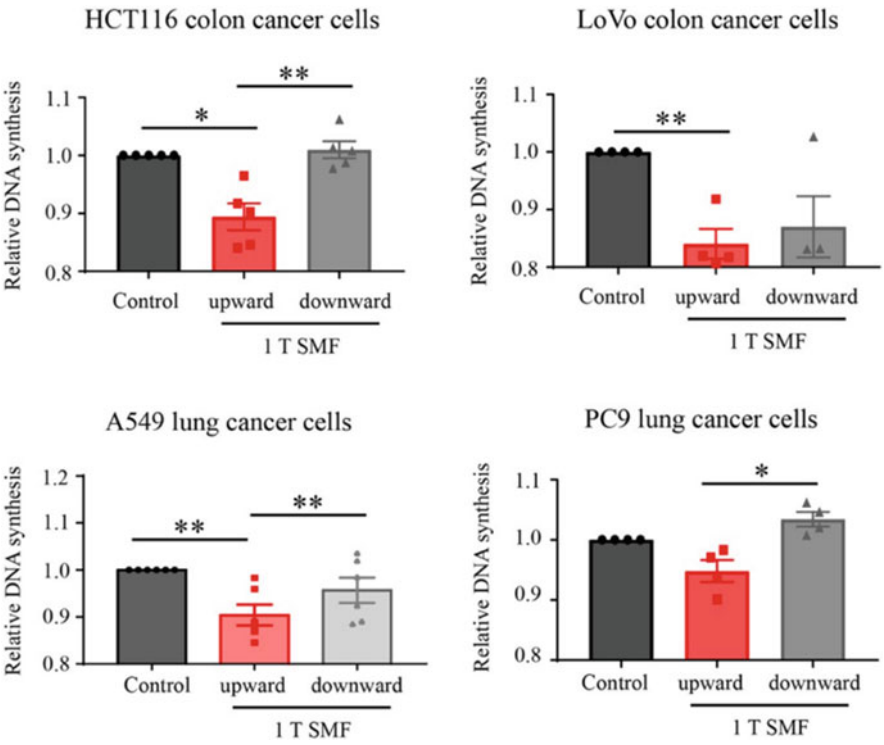

Fig. 6.7 DNA synthesis is decreased by 1 T upward but not downward magnetic field. Inhomogeneous SMFs were generated by permanent magnets. *$p < 0.05$, **$p < 0.01$. [Reprinted from (Yang et al. 2020), open access]

found that a 27 T ultra-high SMF could affect the mitotic spindle orientation in human cells, in which chromosomes played important roles (Zhang et al. 2017b).

Moreover, the nature of intertwined double-strand DNA determines that the DNA has to rotate in cells (Keszthelyi et al. 2016). Since DNA is negatively charged and undergoes fast rotation during replication in living cells, we predict that its movement will be affected by Lorentz force, especially in high SMFs. Combined theoretical calculation and cellular experiments, we show that moderate to high SMFs can directly inhibit DNA synthesis/replication (Yang et al. 2020). We used two colon cancer and two lung cancer cell lines to detect the SMF effect on DNA synthesis, which was determined by BrdU incorporation. We observed that the DNA replication was decreased by about 5–15% by upward direction SMF in four different cell lines, while downward direction SMF did not generate such effect (Fig. 6.7). The differential effects of SMFs of different directions on DNA synthesis have been discussed in Chap. 2 of this book.

6.2.13 Intracellular Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) are highly active radicals, ions, and molecules that have a single unpaired electron in their outer shell of electrons. ROS include free oxygen radicals ($\cdot O_2^-$, ·OH, NO·, etc.) and non-radical ROS (H_2O_2, N_2O_2, ROOH, HOCl, etc.). It is well known that low levels of ROS can act as intracellular signaling messengers that oxidize protein thiol groups, modify protein structure and functions while higher levels of ROS could nonspecifically attack proteins, lipids, and DNA to disrupt normal cellular processes (Liou and Storz 2010; Shi et al. 2014). There are also multiple studies showing that the elevated ROS levels in cancer cells compared to normal cells could contribute to the cancer progression (Gao et al. 2007). However, there are also some studies indicating that excessive oxidant stress slows cancer cell proliferation, threatens their survival and therapeutic interventions to further increase the oxidant stress level in newly formed tumor cells, which is likely to make them prone to death (Schumacker 2006, 2015; Trachootham et al. 2006).

ROS level change after SMF treatment is probably the most frequently reported phenomenon in the field of magnetobiology. We have previously reviewed the literature about ROS changes by various magnetic fields in 2017 (Wang and Zhang 2017). However, in the past few years, there are a large number of new studies that have reported the effects of SMFs on ROS levels. We categorize the reported studies according to their effects, including ROS level elevation (Tables 6.3 and 6.6), reduction (Table 6.4), or no change (Tables 6.5).

However, there is no rules we can find in these studies yet. For example, for hypomagnetic fields, some studies showed ROS elevation (Fu et al. 2016), some showed reduction (Politanski et al. 2013), and some showed no difference (Politanski et al. 2013; Van Huizen et al. 2019). These variations could be due to the cell type, magnetic flux density, or even timepoint differences. For example, Sullivan et al. showed that the oxidant production increased 37% in WI-38 cells exposed to SMF (230–250 mT) during the first 18 h after seeding, but no change was observed after a prolonged 5-day exposure (Sullivan et al. 2011), which indicates that the SMF-induced ROS elevation is time-dependent. Moreover, ROS was known to be different in different cell types, as well as different cell densities (Limoli et al. 2004; Wang and Zhang 2019). Furthermore, we recently found that gradient moderate SMFs can regulate the oxidative stress and inhibit metastasis in the ovarian cancer cells (Song et al. 2021).

6.2.14 Adenosine Triphosphate (ATP)

Whether SMFs could affect the enzymatic ATP synthesis in vitro has been debated. In 2008, Buchachenko and Kuznetsov reported magnetic interactions on the rate of enzymatic synthesis of ATP in vitro (Buchachenko and Kuznetsov 2008). They found that the ATP synthesis can be significantly increased by 55 and 80 mT SMFs

Table 6.3 SMF increases the total ROS level of various cell lines

Species	Samples	SMF flux density	Treatment time	Assays	ROS levels	References
Human	SH-SY5Y neuronal-like cells	2.2 mT	1 d	DCFH-DA	Increase	Calabro et al. (2013)
	U937 (monocyte tumor cells)	0.6 mT	2 h			De Nicola et al. (2006)
	MCF-7 breast cancer cell, HFF foreskin fibroblasts	10 mT	1/2 d			Hajipour Verdom et al. (2018)
	HeLa (cervical cancer cell line)		1/2 d		Further increase cisplatin-induced ROS elevation	Kamalipooya et al. (2017)
	Peripheral blood neutrophils	60 mT (S pole)	45 min	Dihydrorhodamine (DHR 123)	Increase	Poniedzialek et al. (2013)
	Embryonic lung fibroblast WI-38 cell	232–252 mT	18 h	DCFH-DA		Sullivan et al. (2011)
	THP-1 (monocytic leukemia cells)	1.2 T	1 d	Carboxy-H_2DCF-DA		Zablotskii et al. (2014)
	Human-hamster hybrid A (L) cells, mitochondria-deficient ρ(0) A(L) cells, double-strand break repair-deficient (XRS-5) cells	8.5 T	3 h			Zhao et al. (2011)
Rodent	Primary mouse skeletal muscle cells	<3 μT	3 d			Fu et al. (2016)
	Mouse embryonic stem cell-derived Flk-1$^+$ cardiac progenitor cells	0.2–5 mT	<3 min			Bekhite et al. (2013)
	5-day-old embryoid bodies grown from embryonic stem cells	1/10 mT	8 h			Bekhite et al. (2010)
	NCTC 1469 (normal mouse liver cell line)	0.4 T	1/24/48/72 h	Carboxy-H_2DCF-DA		Bae et al. (2011)

	Rat lymphocyte	5 mT	15 min/1/2 h	DCFH-DA	Increase	Politanski et al. (2013)
			15 min/2 h		Increase ROS levels in X-ray treated group	
Canidae	C2 (Canine mastocytoma tumor cells)	0.5 T	3 d		Increase nanomaterial-induced ROS elevation	Marycz et al. (2017)

Table 6.4 SMF reduces the total ROS level of various species

Species	Samples	SMF flux density	Treatment	Assays	ROS levels	References
Human	Neuroblastoma SH-SY5Y cells	Hypomagnetic field (<0.2 μT)	12/24/36/48/60 h	DCFH-DA	Reduce	Zhang et al. (2017a)
	Peripheral blood neutrophils	60 mT (N and S poles)	15 min	Dihydrorhodamine (DHR 123)		Poniedzialek et al. (2013)
	A549 bronchial epithelial cells	389 mT	30 min	DCFH-DA		Csillag et al. (2014)
	ASCs (adipose-derived mesenchymal stromal stem cells)	0.5 T	3 d		Reduce nanomaterial-induced ROS elevation	Marycz et al. (2017)
	MCF-7/MDA-MB-231 (breast cancer cell line)	1 T	1/2 d		Reduce	Wang and Zhang (2019)
	U251 (brain glioblastoma), GIST-T1 (gastrointestinal stromal tumor), HCT116 (colon epithelial carcinoma), CNE-2Z (nasopharyngeal cancer), HepG2 (hepatocellular carcinoma), EJ1 (bladder cancer), RPE1 (retina epithelial), HSAEC-30KT (normal lung cell line)		1 d			
Rat	Rat lymphocytes	0 mT (by 50 mT MFs opposite to the geomagnetic field)	2 h		Reduce the ROS level of X-ray treatment group	Politanski et al. (2013)
	C6 (rat brain glial)	1 T	1 d		Reduce	Zhang et al. (2017a)
Invertebrate	Planarians	200 μT (applied SMF after shielding geomagnetic field by a MagShield box)	3 d	Carboxy-H_2DCF-DA		Van Huizen et al. (2019)

Table 6.5 SMF has no significant effect on total ROS level

Species	Samples	SMF flux density	Treatment	Assays	ROS levels	References
Human	Neuroblastoma SH-SY5Y cells	Hypomagnetic field (<0.2 μT)	4/6 h	DCFH-DA	No change	Zhang et al. (2017a)
	Peripheral blood neutrophils	60 mT	30 min (S and N poles)	Dihydrorhodamine (DHR 123)		Poniedzialek et al. (2013)
			45 min (N pole)			
	Lung fibroblast cells (WI-38)	230–250 mT	5 d	DCFH-DA		Sullivan et al. (2011)
	Fetal lung fibroblast MRC-5 cells	370 mT	1 h/d, 4 d			Romeo et al. (2016)
	Adipose-derived mesenchymal stromal stem cells (ASCs)	0.5 T	3 d			Marycz et al. (2017)
	293 T (kidney epithelial), HeLa (cervix epithelial adenocarcinoma), PC3 (prostate adenocarcinoma), normal lung cell line (HSAEC-2KT/HBEC-30KT)	1 T	1 d			Wang and Zhang (2019)
Rodent	Rat lymphocytes	0 mT (50 mT MFs directed opposite to the geomagnetic field)	15 min/ 1 h/2 h			Politanski et al. (2013)
			15 min/ 1 h		Does not affect ROS of X-ray group	
		5 mT	1 h			
	NIH-3T3 (mouse embryo fibroblast), differentiated PC-12 (rat pheochromocytoma), CHO (Chinese hamster ovary)	1 T	1 d		No change	Wang and Zhang (2019)
Canidae	C2 (canine mastocytoma tumor cells)	0.5 T	3 d			Marycz et al. (2017)
Bacterium	*E. coli* bacteria	100 mT	30 min	Cell ROX dye		Bajpai et al. (2014)

Table 6.6 Effect of static magnetic field on specific ROS

ROS types	Species	Samples	SMF flux density	Treatment	Assays	ROS levels	References
H_2O_2	Plant	*Vicia faba* L. (shoot)	30 mT	8 d, 8 h/d	Trichloracetic acid extraction method	Increase	Haghighat et al. (2014)
		Soybean seeds (embryo/ hypocotyl)	150/200 mT	1 h	Photo-absorption method of titanium–hydroperoxide complex		Shine et al. (2012)
		Cucumber seeds	200 mT				Bhardwaj et al. (2012)
	Human	Neuroblastoma SH-SY5Y cells	<0.2 μT (hypomagnetic field)	36 h	H_2O_2 test kit	Decrease	Zhang et al. (2017a)
		Fibrosarcoma HT1080	0.2–2 μT (shielding of the geomagnetic field)	6/12/24 h	HRP-AUR (horseradish peroxidase-Amplex UltraRed) dye		Martino and Castello (2011)
	Bovine	Pulmonary artery endothelial cells (PAEC)		8/24 h			
	Plant	Maize (leaf)	100 mT	2 h	Photo-absorption method of titanium–hydroperoxide complex		Anand et al. (2012)
			200 mT	1 h			
		Mungbean (leaf)	600 mT	–	Peroxidase-coupled assay		Chen et al. (2011)
		Mung bean (leaf/ root)				Reduce the H_2O_2 level of cadmium treatment group	
	Human	Neuroblastoma SH-SY5Y cells	<0.2 μT (hypomagnetic field)	6/12/24/ 48 h	H_2O_2 test kit	No change	Zhang et al. (2017a)
		Pancreatic AsPC-1 cancer cells	0.2–2 μT (shielding of the geomagnetic field)	12/24 h	HRP-AUR fluorometric assay		Martino and Castello (2011)

	Plant	Shallot leaves	7 mT	8/12/17 d	Trichloracetic acid extraction method		Cakmak et al. (2012)
		Vicia faba L. (root)	30 mT	8 d, 8 h/d			Haghighat et al. (2014)
		Mung bean (root)	600 mT	–	Peroxidase-coupled assay		Chen et al. (2011)
$\cdot O_2^-$	Human	Neuroblastoma SH-SY5Y cells	31.7–232.0 mT	1 d	NBT	Increase	Vergallo et al. (2014)
	Plant	Soybean seeds (embryo/ hypocotyl)	150/200 mT	1 h	EPR spectroscopy (PBN is used to trap $\cdot O_2$–)/XTT-colorimetric assay		Shine et al. (2012)
		Cucumber seeds	200 mT		XTT-colorimetric assay		Bhardwaj et al. (2012)
	Rat	Female rat primary macrophages	<12 μT	6 m	NBT (nitro blue tetrazolium)	Reduce	Roman and Tombarkiewicz (2009)
	Plant	Maize (leaf)	100 mT	2 h	EPR spectroscopy (PBN is used to trap $\cdot O_2$–)		Shine and Guruprasad (2012)
			200 mT	1 h			
		Soybean seeds (leaf)	150/200 mT				Baby et al. (2011)
		Mung bean (root)	600 mT	–	Hydroxylamine oxidation method		Chen et al. (2011)
		Mung bean (leaf/ root)				Reduce cadmium-induced $\cdot O_2$– elevation	
		Winter wheat (leaf)				MF alone reduces. $\cdot O_2$– level, reduce Cd or Pb—induced $\cdot O_2$– elevation	Chen et al. (2017)
	Human	Neuroblastoma SH-SY5Y cells	<0.2 μT (hypomagnetic field)	6/12/24/ 36/48 h	DHE (dihydroethidium)	No change	Zhang et al. (2017a)

(continued)

Table 6.6 (continued)

ROS types	Species	Samples	SMF flux density	Treatment	Assays	ROS levels	References
	Rat	Male rat primary macrophages	<12 μT (shielding of the geomagnetic field)	6 mT	NBT		Roman and Tombarkiewicz (2009)
	Plant	Mung bean (leaf)	600 mT	–	Hydroxylamine oxidation method		Chen et al. (2011)
·OH	Plant	Soybean seeds (embryo/ hypocotyl)	150/200 mT	1 h	EPR spectroscopy (POBN is used to trap ·OH)	Increase	Shine et al. (2012)
		Maize (leaf)	100 mT	2 h	EPR spectroscopy (DMPO is used to trap ·OH)	No change	Shine and Guruprasad (2012)
			200 mT	1 h			

in the presence of $^{25}Mg^{2+}$. However, later studies by Crotty et al. failed to reproduce their results (Crotty et al. 2012) and the reason was still unclear (Hore 2012). Although the magnetic flux densities in these two studies were almost identical, the experimental details about the magnetic field setup were provided by Crotty et al. but not by Buchachenko and Kuznetsov. In addition, it is also possible that the difference was due to the fact that these two groups have used different sources of proteins. Buchachenko and Kuznetsov used a monomeric creatine kinase isozyme from snake venom, whereas Crotty et al. used dimeric creatine kinase. To our point of view, the above-mentioned factors about both the magnetic fields and the protein itself could potentially produce seemingly inconsistent results. Therefore, more investigations are encouraged to address this question.

Besides the in vitro catalytic studies, there are also some cellular works showing that the ATP level in cells could be affected by SMFs. However, the exact effects also seem to be case dependent. Back in 1995, Itegin et al. found that chronically applied SMF of 0.02 T had differential effects on various ATPase. The mean activities of Na^+–K^+ ATPase and Ca^{2+} ATPase were significantly increased by SMF but that of Mg^{2+} ATPase was non-significantly reduced (Itegin et al. 1995). It is possible that different cells have different ATPase network so that their responses to SMFs could be dissimilar. In 2010, Wang et al. tested moderate SMF (~0.25 T) on PC12 cells (derived from a pheochromocytoma of the rat adrenal medulla) and found that the ATP level was moderately, but statistically significantly increased (Wang et al. 2010). There was another study by Kurzeja et al. that also reported ATP level increase induced by SMF, although it was done in the presence of fluoride. In 2013, Kurzeja et al. found that moderate SMFs (0.4, 0.6, and 0.7 T) could rescue fluoride-induced ATP decrease in fibroblasts. In addition, the effect was magnetic flux density-dependent, in which 0.7 T SMF produced more significant effects than 0.4 and 0.6 T SMFs (Kurzeja et al. 2013).

There were also some studies showing that the cellular ATP level could be reduced by SMFs in a magnetic flux density- and cell type-dependent manner. For example, in 2011, Zhao et al. used 8.5 T strong homogeneous SMF to test its effects in three cell lines, including human-hamster hybrid A(L) cells, mitochondria-deficient [$\rho(0)$ A(L)] cells, and double-strand break (DSB) repair-deficient (XRS-5) cells. They found that SMF-induced ATP content change was magnetic flux density, time, as well as cell type-dependent (Zhao et al. 2011). Moreover, their results indicated that the 8.5 T SMF-induced cellular ATP decrease was partially mediated by mitochondria and the DNA DSB repair process because the ATP level in wild type A(L) cells could recover 12–24 h after SMF exposure but the mitochondria-deficient or double-strand break repair-deficient (XRS-5) cells could not (Zhao et al. 2011). In 2018, our group used rat adrenal PC12 cells to compare SMFs of different flux densities for their effects on ATP. Our results show that although 0.26 or 0.50 T SMFs did not affect ATP, 1 T and 9 T SMFs affected ATP level differently and time-dependently. Moreover, SMF-induced ATP level fluctuations are correlated with mitochondrial membrane potential changes (Wang et al. 2018).

6.2.15 Calcium

Calcium plays important roles in a number of biological systems, especially in signal transduction cascades. The magnetic field-induced calcium changes in cells are mostly studied in time-varying magnetic fields (Walleczek and Budinger 1992; Barbier et al. 1996; Tonini et al. 2001; Zhou et al. 2002; Fassina et al. 2006; Yan et al. 2010) and was found to be dependent on cell status and magnetic flux density (Walleczek and Budinger 1992) as well as other magnetic field parameters (Carson et al. 1990). There are multiple studies showing that the calcium level was increased by 50–60 Hz magnetic fields (Barbier et al. 1996; Tonini et al. 2001; Fassina et al. 2006).

Similar to time-varying magnetic fields, there are also many studies show that the calcium level was increased by SMFs. For example, in 1998, Flipo et al. examined the in vitro effects of 0.025–0.15 T SMFs on the cellular immune parameters of the C57BL/6 murine macrophages, spleen lymphocytes, and thymic cells (Flipo et al. 1998). Exposure to the SMF for 24 h resulted in increased intracellular Ca^{2+} level in macrophages and increased Ca^{2+} influx in concanavalin A-stimulated lymphocytes (Flipo et al. 1998). In 2006, Tenuzzo et al. showed that 6 mT SMF could increase the calcium level in multiple cell lines (Tenuzzo et al. 2006). Prina-Mello et al. exposed rat cortical neurons to SMF of 0.75 T for 1 h and observed increased calcium level (Prina-Mello et al. 2006). In 2009, Dini et al. found that 6 mT SMF could cause significant increase in calcium level in human leukemia U937 cells (Dini et al. 2009). In 2010, Wang et al. found that 0.23–0.28 T SMF could increase extracellular calcium level in rat adrenal pheochromocytoma PC12 cells (Wang et al. 2010). In addition, they found that SMFs could antagonize CGS21680-induced calcium reduction, which was similar to the effect of a selective A(2A)R antagonist ZM241385 (Wang et al. 2010). In the same year, Hsu and Chang also found that 0.29 T SMF in combination with Dex/β-GP significantly increased the extracellular calcium concentration at the early stage, followed by obvious calcium deposits later, which may contribute to the accelerated osteogenic differentiation and mineralization of dental pulp cells (DPCs) (Hsu and Chang 2010). In 2014, Surma et al. found that weak SMFs increased the intracellular calcium and accelerated the development of skeletal muscle cells from newborn Wistar rats in primary culture (Surma et al. 2014). In the same year, Bernabo et al. showed that a 2 mT SMF could cause a reversible cell membrane depolarization wave (of about 1 min), which induced intracellular calcium increase and mitochondrial activity decrease in vital granulosa cells (Bernabo et al. 2014).

In the meantime, there are also some studies showing that the intracellular calcium was not affected by SMFs. For example, in 1986, Bellossi exposed neonatal isolated chick brains to SMFs of 0.2–0.9 T and did not observe calcium efflux changes (Bellossi 1986). Papatheofanis et al. exposed mice to 1 T SMF for 30 min/day for 10 days and did not observe calcium alteration (Papatheofanis and Papatheofanis 1989). In 1990, Calson et al. found that 0.15 T SMF did not affect the cytosolic calcium level in HL-60 cells (Carson et al. 1990). In 1992, Yost and

Liburdy combined extremely low frequency (ELF) time-varying magnetic fields with SMFs and examined their effects on calcium signaling in the lymphocyte (Yost and Liburdy 1992). Their results showed that a 1 h exposure of thymic lymphocytes to a 16 Hz, 42.1 μT magnetic field combined with a colinear SMF of 23.4 μT inhibited calcium influx in mitogen-activated cells but not resting lymphocytes. However, it was interesting that either the time-varying magnetic fields or the SMF alone did not have such effects (Yost and Liburdy 1992). In 2008, Belton et al. found that application of 1, 10, or 100 mT SMF did not affect the calcium response to ATP in HL-60 cells (Belton et al. 2008). In 2009, Belton et al. and Rozanski et al. depleted GSH in HL-60 cells and then examined their responses to 0.1 T SMF and did not observe obvious calcium changes (Belton et al. 2009; Rozanski et al. 2009).

So far as we know, there are only a few studies that have reported the inhibition effect of SMFs on calcium. In 1992, Yost and Liburdy found that a combination of 16 Hz, 42.1 μT time-varying magnetic fields with 23.4 μT SMF could decrease calcium level in thymic lymphocytes (Yost and Liburdy 1992). In 1996, Rosen et al. found that a 120 mT SMF caused a minor reduction in the peak calcium current amplitude and shift in the current–voltage relationship in cultured GH3 cells (Rosen 1996). In 2012, Li et al. found that 5 mT SMF could decrease cytosolic free calcium concentration in human vascular smooth muscle cells (VSMCs) (Li et al. 2012).

There are also many indirect evidences showing that calcium is involved in SMF-induced cellular effects. For example, in 1990, a study using human polymorphonuclear leukocytes (PMNs) showed that 0.1 T SMF could induce degranulation and cell migration inhibition, which could be prevented by pretreatment of calcium channel antagonists diltiazem, nifedipine, and verapamil in dose-dependent manner (Papatheofanis 1990). In 2005, Okano and Ohkuno found that neck exposure to 180 mT (B(max)) SMF alone for 5–8 weeks significantly suppressed or retarded the development of hypertension together with increased baroreflex sensitivity (BRS) in SMF group. Their results indicated that SMF may increase the L-type voltage-gated calcium channel blocker nicardipine-induced hypotension by more effectively antagonizing the Ca^{2+} influx through the calcium channels compared with the nicardipine injection (NIC) treatment alone (Okano and Ohkubo 2005). In 2006, Ghibelli et al. found that 1 T SMF could potentiate the cytotoxic effects of puromycin and VP16, which could be prevented by calcium chelating agents EGTA and BAPTA-AM as well as the calcium channel blocker nifedipine (Ghibelli et al. 2006). In 2008, Yeh et al. found that 8 mT SMF increased the efficacy of synaptic-transmission in crayfish tail-flip escape circuit in a calcium-dependent way (Yeh et al. 2008). Also in 2008, Morris et al. used pharmacological agents for L-type calcium channel to show that SMF-induced anti-edema effect may work through the L-type calcium channels in vascular smooth muscle cells (Morris and Skalak 2008).

The differential effects of SMF-induced calcium changes are likely due to multiple reasons, such as cell types, magnetic flux density as well as incubation time. There are multiple studies indicating that different cell types have differential calcium changes when exposed to SMFs. In 1999, Fanelli et al. found that the calcium level in different cell types responded to 6 mT SMF differently, which seemed to be correlated to the SMF-induced anti-apoptotic effect (Fanelli et al.

1999). They further found that both the protective and potentiating effects of 6 mT and 1 T SMFs in drug-treated cells were mediated by the Ca^{2+} influx from the extracellular medium, which only happened in some cell types (Fanelli et al. 1999; Ghibelli et al. 2006). In 2003, Aldinucci et al. tested the effects of combining a 4.75 T SMF and a pulsed EMF of 0.7 mT generated by an NMR apparatus for 1 h. They found that in Jurkat leukemia cells the calcium level was reduced significantly after exposure (Aldinucci et al. 2003b) but in normal or in phytohemagglutinin (PHA) challenged lymphocytes the calcium level was increased (Aldinucci et al. 2003a). In addition, the SMF-induced calcium changes are also magnetic flux density-dependent. In 2006, Ghibelli et al. proposed that both the anti-apoptotic effect of a 6 mT SMF and the potentiating effect of a 1 T SMF were mediated by calcium influx (Ghibelli et al. 2006). In 2014, Zhang et al. examined multiple mineral elements for MC3T3-E1 cells during osteoblast mineralization when they were exposed to 500 nT, control geomagnetic field, 0.2 T, and 16 T SMFs. They found that the calcium level was decreased by 500 nT and 0.2 T SMFs but increased by the 16 T SMF (Zhang et al. 2014). This magnetic flux density-induced difference may have contributed to some of the inconsistencies in the literature, in addition to the cell type-induced variations. Moreover, the SMF-induced calcium changes are also likely to be time-dependent. In 2005, Chionna et al. found that Hep G2 cells exposed to 6 mT SMF had increased calcium level in a time-dependent manner and it reached the highest level at 4 h (Chionna et al. 2005). Table 6.7 summarizes the calcium changes induced by SMFs in the literature (Table 6.7).

Since calcium plays crucial roles in cellular processes such as cell proliferation as well as apoptosis, it is not surprising that different magnetic flux densities could cause differential effects on calcium levels in various cell types, which lead to totally diverse cellular effects. In addition, there are also several studies that reported some signal transduction pathway changes, which are probably due to, or at least partially due to, the SMF-induced calcium modulation. For example, in 2012, Li et al. found that 5 mT SMF could influence the proliferation, migration, and adhesion of human umbilical artery smooth muscle cells (hUASMCs) by inhibiting the clustering of integrin β1, decreasing cytosolic free calcium concentration, and inactivating FAK (Li et al. 2012). We previously found that 1 T SMF could inhibit human CNE-2Z cancer cell proliferation, which was related to the EGFR-Akt-mTOR pathways (Zhang et al. 2015, 2016). As mentioned earlier in this chapter, we found that EGFR and its downstream pathways likely contribute to the cell type- and cell plating density-induced variations in SMF-induced cell proliferation changes (Zhang et al. 2017c). In fact, the kinase activity of EGFR protein itself could be directly inhibited by SMFs (Zhang et al. 2016), which will be further discussed in Chap. 9. Lew et al. used 0.4 T SMF to treat dental pulp stem cells and found that the cell proliferation rate was increased. Their results indicated that 0.4 T SMF affected the cellular membranes of the DPSCs and activated intracellular calcium ions, which may activate p38 MAPK signaling to reorganize the cytoskeleton and increase cell proliferation of the DPSCs (Lew et al. 2018). Moreover, Maredziak et al. showed that 0.5 T SMF increased the proliferation rate of human adipose-derived

Table 6.7 Static magnetic field-induced calcium changes in different studies

Sample information	SMF intensity	Calcium level	References
HT-1080 human fibrosarcoma cells	200/300/400/500/600 μT	Increase	Gurhan et al. (2021)
Skeletal muscle cells from newborn Wistar rats in primary culture	60–400 μT		Surma et al. (2014)
The human embryonic kidney cell line HEK 293	1 mT		Bertagna et al. (2022)
Vital granulosa cells	2 mT		Bernabo et al. (2014)
Multiple cell lines	6 mT		Tenuzzo et al. (2006)
Human leukemia U937 cells	6 mT		Dini et al. (2009)
BMSCs (isolated from the bone marrow of Sprague–Dawley rats)	10 mT and 50 mT		He et al. (2021)
Macrophages	0.025–0.15 T		Flipo et al. (1998)
Rat adrenal pheochromocytoma PC12 cells	0.23–0.28 T		Wang et al. (2010)
Dental pulp cells DPCs	0.29 T in combination with Dex/β-GP		Hsu and Chang (2010)
Human oligodendrocytes precursor cells (OPCs)	300 mT		Prasad et al. (2017)
Rat cortical neurons	0.75 T		Prina-Mello et al. (2006)
Murine osteoblastic cell line MC3T3-E1	16 T		Yang et al. (2018)
Thymic lymphocytes	23.4 μT	No change	Yost and Liburdy (1992)
HL-60 cells	0.1 T		Belton et al. (2009) and Rozanski et al. (2009)
HL-60 cells	0.15 T		Carson et al. (1990)
HepG2	0.5 T		Chen et al. (2018)
Neonatal isolated chick brains	0.2–0.9 T		Bellossi (1986)
Thymic lymphocytes	16 Hz, 42.1 μT time-varying MFs + 23.4 μT SMF	Decrease	Yost and Liburdy (1992)
Human umbilical artery smooth muscle cells (hUASMCs)	5 mT		Li et al. (2012)
GH3 cells	120 mT		Rosen (1996)
Murine osteoblastic cell line MC3T3-E1	0.2 T and 500 nT		Yang et al. (2018)

mesenchymal stromal stem cells via activation of the phosphoinositide 3-kinase/Akt (PI3K/Akt) signaling pathway (Maredziak et al. 2017).

6.3 Conclusion

Since the human body is composed of various cells, which are filled with various components that can respond to the magnetic fields, most studies in the bioeffects of magnetic fields are carried out at cellular level. The parameters of the magnetic fields as well as the cells examined both have enormous impact on the experimental outcomes. So far, most cellular effects of SMFs are largely dependent on magnetic field types, flux density, cell types, as well as other factors mentioned in Chap. 1. The cellular effects not only include the above-mentioned aspects such as cell orientation, proliferation, calcium level changes, but also some other aspects that are relatively less studied and not included in this chapter, such as gene expression, mitochondria, and immune system. It is obvious that further investigations are needed to get a more complete understanding of the cellular effects of SMFs. Overall, most cellular effects of SMFs are relative mild, except for the orientation changes in strong SMFs. In our own lab, to get unbiased and reproducible results throughout our studies, we always have at least two researchers to conduct the same sets of experiments independently and gathered their results together for data analysis. More importantly, people should know that the cellular effects of SMFs are influenced by various factors and parameters of magnetic field and the cells, as well as the experimental procedure, such as incubation time and magnetic field direction. In addition, the absence of magnetic field effects in some experiments contrasted with the positive findings reported by other investigators. These discrepancies may be attributable to an inadequate detection capacity of instrument or techniques. Therefore, people should not only carefully record and analyze all experimental factors, but also try to take advantages of the advanced modern technologies to get a more comprehensive understanding of the cellular effects of SMFs.

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Chapter 7
Impact of SMFs on Microorganisms, Plants, and Animals

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Abstract Static magnetic field (SMF) exists in nature widely and plays an essential role in the biological evolution. Due to the rapid development of superconducting technology, the intensities of SMFs used for medical and academic research purposes have steadily increased in recent years. This chapter presents an overview on the biological effects induced by SMFs with intensities ranging from mT to several Teslas (T). The effects of SMFs on microorganisms are divided into six sections, including cellular growth and viability, morphological and biochemical modifications, genotoxicity, gene and protein expression, magnetosome formation sensing magnetic field, and application of SMFs on antibiotic resistance, fermentation, and wastewater treatment. The effects of SMFs on plants are divided into six sections, including germination, growth, gravitropism, photosynthesis, redox status, and cryptochromes (CRYs) sensing magnetic field. The effects of SMFs on animals are divided into seven sections, including *Caenorhabditis elegans*, insects, *Helix pomatia*, aquatic animals, *Xenopus laevis*, mice and rats, and magnetic sensing protein in animals. This chapter will be very helpful for better understanding the biological responses to SMFs in different species and their underlying mechanisms.

Keywords Static magnetic fields (SMFs) · Microorganisms · Plants · Animals · Biological effects

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7.1 Introduction

Static magnetic field (SMF) is a ubiquitous environmental factor for all living organisms during the evolution process. A variety of organisms including bacteria, algae, snails, planaria, honeybee, salmon, lobsters, salamanders, homing pigeons, robins, mice, and possibly humans have been demonstrated the ability to sense magnetic fields (MFs) for orientation in navigation, migration, homing, escaping, and nest building (Qin et al. 2016). Although the biophysical mechanisms of magnetoreception are poorly understood, three main hypothesis for magnetosensing have been proposed: (1) magnetic induction, which can only be applied to marine creatures, owing to the high conductivity of salt water; (2) the magnetite hypothesis that proposes a process mediated by crystals of permanently magnetic material (magnetite) with an evolutionary genetics hypothesis for magnetite formation; (3) the radical pair mechanism (RPM) which relies on a chemical reaction involving specialized photoreceptors (Fedele et al. 2014; Bellinger et al. 2022). However, the recently proposed MagR/Cry-based biocompass model combines the concepts of ferrimagnetism and the involvement of Cry in magnetoreception, which have also attracted a lot of attention (Qin et al. 2016).

Since the Industrial Revolution in the 1850s, human-made sources of SMFs have become inevitable environmental factors for organisms on Earth. In particular, the development of electromagnets in the nineteenth century and superconducting magnets in the mid-twentieth century has greatly increased the risk of the exposure of organisms to higher magnetic fields. Acute and chronic exposure of organisms to SMFs, which are often ten and more times greater than geomagnetic fields, have been investigated for decades. However, the exact mechanisms underlying the influence of SMFs on living systems are still largely unknown, and until now there is no unique theory about magnetic field–organism interaction. In this review, we limit our discussion on the evidence of the biological response of SMFs with the intensities ranging from a few mT to several Teslas (T) on microorganisms, plants, and animals and explore recent results on the investigation of magnetoreception in these organisms.

7.2 SMFs on Microorganisms

7.2.1 SMFs on Cellular Growth and Viability

The influence of magnetic fields with various flux densities on the growth rate and viability of microbes has been investigated in bacteria, yeast, and plant pathogenic fungi. Under low and moderate magnetic fields, the inhibition on the growth of microorganisms has been reported in various bacteria species. Bajpai et al. (2012) showed that a SMF of 100 mT suppressed the growth of both gram-positive (*S. epidermidis*) and gram-negative bacteria (*Escherichia coli*, *E. coli*), which was

related to the cellular membrane damage. Fan et al. (2018) discussed the effect of long-term exposure to a moderate SMF on *Enterococcus faecalis* and showed that the cellular proliferation of *Enterococcus faecalis* (*E. faecalis*) was inhibited by a SMF of 170 mT with 120 h exposure. The moderate SMFs in the range from 50 to 500 mT on the growth of *Streptococcus pyogenes* (*S. pyogenes*) was investigated by Morrow et al. (2007) and was found that the growth inhibition was observed up to 300 mT, but an increase in growth rate when cells were exposed to 500 mT. Although a SMF of 300 mT had no influence on the growth of *E. coli* in nutrient rich Luria Bertani (LB) medium, it increased bacterial cell culture density during late growth in diluted LB (Potenza et al. 2004). El May et al. (2009) also reported that a SMF of 200 mT failed to alter cellular growth but induced a decrease of colony-forming units (CFU) between 3 and 6 h followed by an increase from 6 to 9 h. Ben Mouhoub et al. (2018) showed that 57 mT SMF improved the viability of *Salmonella Hadar* compared with the control group. Under high magnetic fields, Kazuhiro et al. (1997) reported that the cellular growth of *Bacillus subtilis* MI113 and genetically transformed *B. subtilis* MI113 (pC112) was significantly increased by exposure to homogeneous 7 T and inhomogeneous 5.2–6.1 T magnetic fields. Moreover, a SMF of 5.2–6.1 T promoted survival rate of *E. coli* B cells of stationary phase, which the CFU number and the amount of S factor encoded by the *rpoS* gene were much higher than that under a geomagnetic field (Horiuchi et al. 2001). These observations suggest that SMFs are not always negative to the growth of microorganism, which are closely related to the intensities of SMFs, types of bacteria, and exposure manners.

The combined effects of SMF and other environmental factors on the growth of microorganisms are largely unknown. Ji et al. (2009) showed that a SMF of 450 mT inhibited the growth and even killed *E. coli*, in which the inhibitory effect was increased with temperature. Masahiro et al. (2000) compared the effect of SMF exposure up to 100 mT on the culture of *Streptococcus mutans* (*S. aureus*) and *E. coli* grown in aerobic and anaerobic conditions. They found that the bacterial growth was inhibited by the SMF in anaerobic conditions, but remained unaffected when the SMF was applied in aerobic conditions, indicating that oxygen played an inhibitory effect for the magnetic field. Letuta and Berdinskiy (2019) found that the concurrent treatment of isotope ^{67}Zn and 25–35 mT SMF increased the colony formation ability and growth rate constant of *E. coli* by 2–4 times compared with non-magnetic zinc isotopes ^{64,66}Zn.

There are few studies on the growth and sporulation of phytopathogenic microscopic fungi under the static magnetic fields. Nagy and Fischl (2004) showed that the applied magnetic fields with flux intensities ranging from 0.1 to 1 mT decreased the growth of phytopathogenic fungi colonies and the number of *Fusarium oxysporum* conidia, while the number of the developed conidia of *Alternaria alternata* and *Curvularia inaequalis* was increased. Maria Cristina et al. (2003) and Jan et al. (2007) provided further evidence on the growth depression of fungi exposed to SMF. A 1.5–2 times faster growth rate was found in *Aspergillus niger* exposed to a static B-field varying from 40 to 80 T than in sham controls and the B-field exposure could have an effect on the biodegradability of materials by enhancing the growth rate and

the aggressiveness of the fungus. However, Ruiz-Gómez et al. (2004) reported that magnetic fields had no effect on fungal growth.

In yeast, Lucielen Oliveira et al. (2010) showed that a SMF of 25 mT resulted in an increase of glutathione content and biomass in *Saccharomyces cerevisiae* (*S. cerevisiae*). Muniz et al. (2007) reported that the biomass (g/L) increment of *S. cerevisiae* DAUFPE-1012 was 2.5 times greater in cultures exposed to 220 mT SMF as compared with non-exposed cultures. Kthiri et al. (2019) furtherly reported that under the treatment of 250 mT SMF, the growth and viability of *Saccharomyces cerevisiae* and colony formation decreased significantly after 6 h, but increased from 6 to 9 h. In contrast, Malko et al. (1994) reported that yeast cells subjected to a static MF of 1.5 T over the course of seven cell divisions displayed growth rates similar to unexposed cells, indicating that moderate SMF had minimal effect on the growth of yeast. With the intensity increase of SMFs, Masakazu et al. (2004) found that gradient magnetic fields of 14 T exhibited the decelerated growth in a liquid–gas mixture system.

7.2.2 SMF on Morphological and Biochemical Modifications

The morphological study of SMF treated cells using transmission electron microscope (TEM) revealed that bacterial cell wall was ruptured by SMF exposure (Ji et al. 2009). Quiñones-Peña et al. (2017) reported that the prototype of *E. coli:* strain-EPEC E2348/69 exposed to 107 mT SMF reduced its aggregation and altered the adhesion pattern, which was related to the expression of its BFP cilia. A SMF of 200 mT significantly altered the phospholipid proportions in *Salmonella typhimurium* (*S. typhimurium*) wild type and *dam* mutant strain, which the most affected were those of the acidic phospholipids, cardiolipins (CL) (Mouadh et al. 2012). Egami et al. (2010) investigated the effect of SMFs on the budding of *S. typhimurium* and found that the size of budding yeast cells and the budding angle were affected by a SMF of 2.93 T. In homogeneous magnetic field, the budding direction of daughter yeast cells was mainly oriented in the direction of magnetic field B; in contrast, in inhomogeneous magnetic field, the daughter yeast cells tended to bud along the axis of capillary flow in regions where the magnetic gradient was high.

Microorganisms as models for analyzing fundamental metabolic responses to magnetic fields have great advantages, as they represent simple unicellular organisms. Letuta (2020) found that the maximum concentration of ATP was generated under the action of the magnetic isotope ^{25}Mg and an electro SMF of 70–90 mT. The composition of membrane lipids in *S. typhimurium* was disturbed by 200 mT SMF, which the bacteria tried to change SFA, UFA, and CFA, and hydroxyl FA levels to maintain membrane fluidity, while the UFAs/SFAs ratio of *Salmonella* reached equilibrium after 9 h of exposure (Ramla et al. 2017). Similarly, Mihoub et al. (2012) showed that a SMF of 200 mT significantly affected the lipid proportions in membrane, leading to an unusual accumulation of the acidic phospholipids

cardiolipins, with a significant increase of membrane cyclic fatty acids and a meaningful increase of the total unsaturated fatty acids to total saturated fatty acids ratios of the exposed cells. Tang et al. (2019) exposed *Flavobacterium* m1–14 to 100 mT SMF for 0, 24, 48, 72, or 120 h, respectively, and found that the length of the cells increased significantly by SMF treatment. Compared with the control group, after 24 h, 48 h, 72 h, and 120 h of 100 mT SMF treatment, the length of the cell increased by 123%, 258%, 70.1%, and 31.2%, respectively; among them, the cells treated by the magnetic field for 48 h were more elongated (Fig. 7.1). The inhibition of mycelia growth by a SMF of 300 mT was accompanied by morphological and biochemical changes, and Ca^{2+}-dependent signal transduction pathways were involved in conidia germination (Maria Cristina et al. 2003). The patterns of metabolites released from *S. pyogenes* exposed to different magnetic flux intensities ranging from 50 to 500 mT were significantly altered (Morrow et al. 2007). A SMF of 250–300 mT elicited the maximal release of the majority of metabolites. Hu et al. (2009) reported that the composition and conformation of nucleic acid, protein, and fatty acid of *E. coli* were altered by 10 T SMF, which were reflected by the changes of spectral region of Fourier-transform infrared (FTIR) spectroscopy combined with cluster analysis. She et al. (2009) further found that 3.46–9.92% of the disorder coils in the secondary structures of protein were altered into α-helices by 10 T SMF; in contrast, 10 T SMF had little influence on *Staphylococcus aureus* (*S. aureus*).

7.2.3 SMF on Genotoxicity

In living organisms, the production of free radicals has the potential to interact with DNA and plays an important role both in the aging process and environmental stress related adverse effects. Exposure of cells to 300 mT SMF significantly reduced the yield of 8-hydroxyguanine in extracted DNA compared to controls, suggesting some possible antioxidant protection to *S. pyogenes* at this field strength (Morrow et al. 2007). Carlioz and Touati (1986) showed an induction of the expression of a *soxS::lacZ* fusion gene following strong SMF exposure. Fan et al. (2018) confirmed that *Enterococcus faecalis* (*E. faecalis*) could induce a stress response by upregulating the expression of *dnaK* gene and the expression of virulence genes *efaA* and *ace* under the treatment of SMF. Righi et al. (2020) exposed irradiated *Deinococcus radiodurans* (*D. radiodurans*) cells to SMF and found that their cell viability was improved, which might be due to the improvement of the efficiency of DNA fragment recombination by SMF exposure.

The direct evidence on the genotoxicity of SMFs is limited and controversial. Mahdi et al. (1994) exposed various mutant strains of *E. coli* to a homogeneous SMF of either 500 mT or 3 T. No evidence of increased DNA damage was detected in SMF-sexposed *E. coli*, even with bacterial strains disabled for DNA repair. Masateru et al. (1999) performed a bacterial mutation assay to determine the mutagenic potential of SMF. No mutagenic effects were detected in four uvrB strains of *S. typhimurium* (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA.

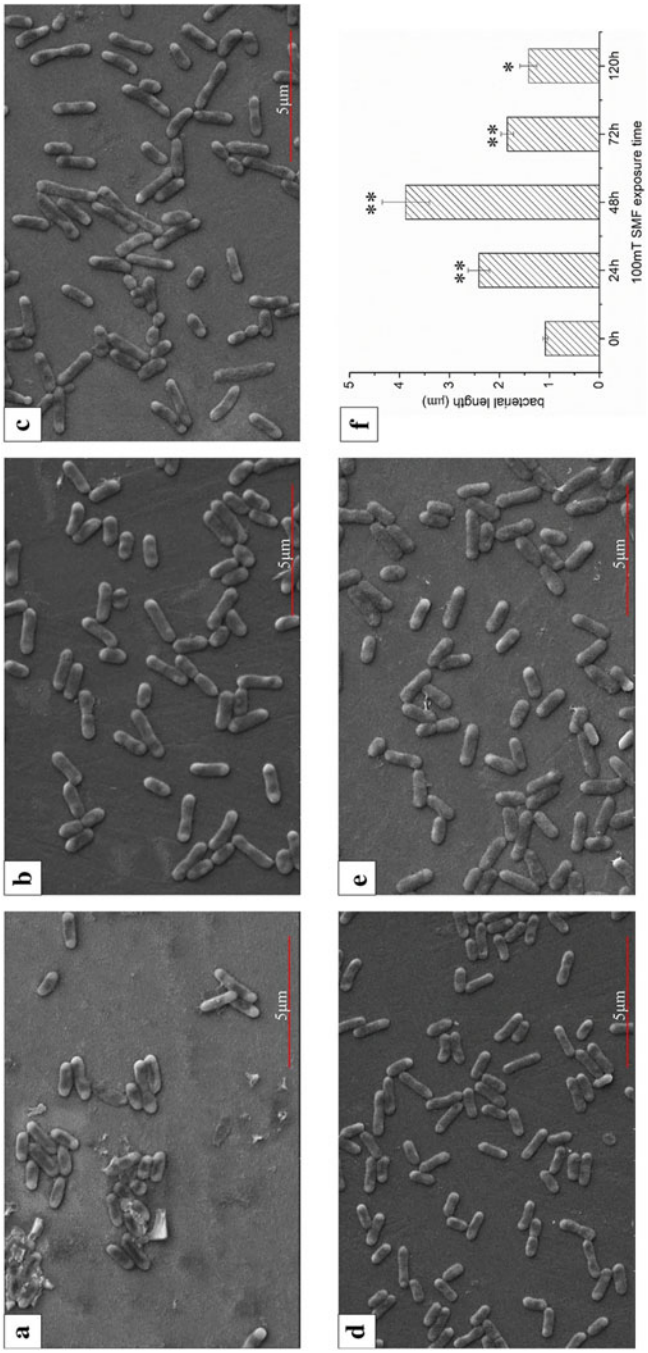

Fig. 7.1 Scanning electron micrographs of the cellular morphology following different treatments. (**a**) Untreated bacteria; (**b**) exposed to 100 mT SMF for 24 h; (**c**) exposed to 100 mT SMF for 48 h; (**d**) exposed to 100 mT SMF for 72 h; (**e**) exposed to 100 mT SMF for 120 h; (**f**) length of bacteria in different exposure times. [Reprinted with permission from (Tang et al. 2019)]

Schreiber et al. (2001) also reported that exposures to a SMF of 7.2 T did not show any alteration in the number of *His*$^+$ revertants in *Salmonella* mutagenicity test. Yoshie et al. (2012) reported that no statistically significant differences in the mutation frequency in thymine synthesis genes were observed between SMF-exposed cells and unexposed cells at any of the applied magnetic flux intensities. SMFs up to 13 T caused neither mutagenicity nor co-mutagenicity in the superoxide dismutase (SOD)-deficient *E. coli* strain QC774 or in its parental strain GC4468, suggesting that exposure to high SMFs did not affect the behavior of superoxide in these microorganisms. However, the modification of chromatin conformation was reported in *E. coli* cells by Belyaev et al. (1994). Zhang et al. (2003) showed a dose–response relationship between the magnetic flux intensity (5 and 9 T SMF) and an increase in mutation frequency in the SOD-deficient *E. coli* strain QC774.

7.2.4 SMF on Gene and Protein Expression

Differential gene expression is a critical event, common to all biological systems, allowing the accurate response under normal conditions and adaptation to various environmental stresses including magnetic fields. Tsuchiya et al. (1999) reported that inhomogeneous magnetic fields ranging from 5.2 to 6.1 T enhanced the transcription of the *rpoS* gene in *E. coli*. Three cDNAs were found to be expressed only in *E. coli* exposed to 300 mT SMF, whereas one cDNA was more expressed in the controls (Potenza et al. 2004). El May et al. (2009) found that the expression level of the 16S rRNA mRNA in *Salmonella Hadar* (*S. Hadar*) remained stable during the exposure of 200 mT SMF, while mRNAs of *rpoA*, *katN*, and *dnaK* genes were over-expressed following 10 h of SMF exposure. Ikehata et al. (2003) reported that a slight decrease in the expression of genes related to respiration was observed in the budding yeast, *Saccharomyces cerevisiae* (*S. cerevisiae*), exposed to 14 T SMF, whereas no changes were observed with field strengths <5 T. Although 14.1 T SMF caused little effects on cell growth of *Shewanella oneidensis* (*S. onedensis*) MR-1, apparent changes at transcriptional levels were detected in exposed cells, in which 21 genes were upregulated while other 44 genes were downregulated (Gao et al. 2005). In contrast, Potenza et al. (2012) reported that no differences were observed in gene expression in *Tuber borchii* mycelium after exposure to SMF, and only the activities of glucose 6-phosphate dehydrogenase and hexokinase were increased. These results indicated that the effects of the magnetic fields on the expression of genes are variable and dependent on parameters applied as well as the cell type.

Protein is the essential unit for biological activities in cells and their functions are determined by the sequence of amino acid including primary and tertiary structures of protein. Snoussi et al. (2012) investigated the effect of 200 mT SMF on the outer membrane protein pattern in *S. Hadar*. They found that a total of 11 proteins displaying more than a twofold change were differentially expressed in exposed cells, among which 7 were upregulated and 4 downregulated. The proteomic

analysis provided a further overview of potentially important cytosolic proteins, in which a total of 35 proteins displaying more than a twofold change were differentially expressed in exposed cells, among which 25 were upregulated and 10 were downregulated. The stress response to a SMF of 200 mT was essentially set up to avoid oxidative damages, with the overexpression of proteins directly involved in oxidative stress response and metabolic switches to counteract oxidative stress (Snoussi et al. 2016).

7.2.5 *Magnetosome Formation Sensing Magnetic Fields*

Microbial magnetosomes represent a special category of intracellular organelles that are synthesized by magnetotactic bacteria (MTB). As a group of Gram-negative aquatic prokaryotes, MTB had a broad range of morphological types, including vibrioid, coccoid, rod, and spirillum. They used the magnetosomes to sense and modify their orientation according to the magnetic field (Moisescu et al. 2014). Magnetosomes comprised magnetic iron-bearing inorganic crystals enveloped by an organic membrane (Staniland et al. 2007). The membrane of magnetosomes contained a unique set of proteins that were thought to direct the biomineralization of magnetite crystals and magnetosome chain formation and regulation (Komeili et al. 2004). Forty-eight proteins were identified as magnetosome-specific proteins in *Magnetospirillum magneticum* (*M. magneticum*) AMB-1, and at least 13 proteins were potentially involved in the formation of magnetosomes, which were encoded by the *mam* and *mms* genes (Matsunaga et al. 2005). Among the genes known to be essential for magnetosome formation, *magA*, *mms6*, *mamA,* and *mms13* were involved in iron uptake (Chikashi et al. 1995; Grünberg et al. 2001), synthesis of magnetite crystals of a uniform size and narrow size distribution with a cubo-octahedral morphology (Amemiya et al. 2007), magnetosome assembly (Komeili et al. 2004), and formation of magnetosomes, respectively. The superior crystalline and magnetic properties of magnetosomes have been attracting much interest in studying biomineralization and medical applications such as drug delivery, magnetic resonance imaging, and array-based assaying (Yoshino and Matsunaga 2006; Matsunaga et al. 2007; Barber-Zucker et al. 2016).

Wang et al. (2008) found that exposure to hypomagnetic field less than 500 nT restrained the growth of *M. magneticum* strain AMB-1 during the stationary phase, but increased the percentage of bacteria that contained mature SD magnetosomes in their exponential growth phase. The average size of magnetic particles in cells exposed to hypomagnetic field was larger (>50 nm) and they contained a larger proportion (57%) of SD particles compared to those grown in the geomagnetic field only. 200 mT SMF could impair the cellular growth and raise Cmag values of the cultures (Wang et al. 2009). The number of magnetic particles per cell and the linearity of magnetosome chain were affected by SMF exposure. Moreover, the expression of *mamA*, *mms13*, *magA* genes was upregulated by SMF. Blondeau et al. (2018) explored the effect of magnetotactic bacterium AMB-1 magnetosome chain

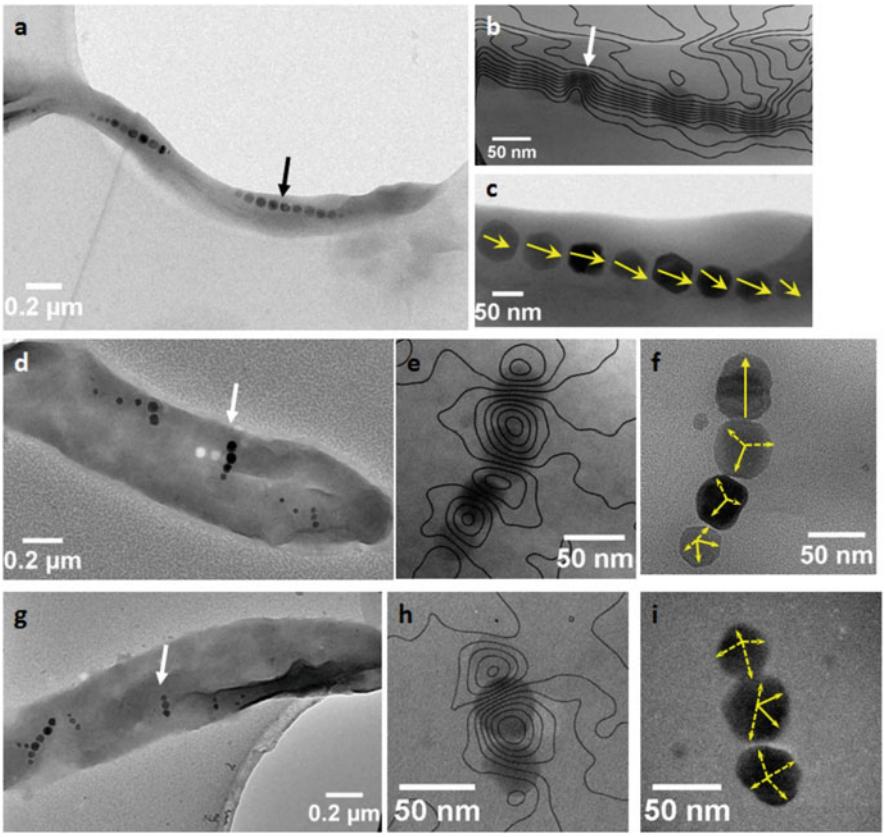

Fig. 7.2 Electron microscopy observations of magnetic and crystallographic orientations in magnetosomes. (**a**) TEM image of an AMB-1 cell in suspension observed after 7 days of incubation in absence of a magnetic field (black arrow shows the selected chain for off-axis image), (**b**) corresponding magnetic phase contours of magnetosome chains determined by off-axis EH (**d**, **g**) TEM images of encapsulated AMB-1 bacteria observed after 7 days of incubation in presence of a magnetic field (white arrows show the selected chains for off-axis images), (**e**, **h**) corresponding magnetic phase contours of magnetosomes chains determined by off-axis EH, and (**c**, **f**, **i**) corresponding HRTEM images with ⟨111⟩ directions determined by using Selected Area Electron Diffraction (SAED) and materialized by yellow bars. [Reprinted from (Blondeau et al. 2018), open access]

alignment under conditions of limited external magnetic field. The bacteria were under a silica matrix, and some bacteria exposed to a field of 80 mT exhibited several magnetic lines. The chains of magnetosomes were arranged parallel to each other but offset relative to the longitudinal axis of the bacteria as shown in Fig. 7.2.

7.2.6 Application of Static Magnetic Fields on Antibiotic Resistance, Fermentation, and Wastewater Treatment

The application of SMF of 0.5 ± 2 mT significantly enhanced the activity of the antibiotic gentamicin against *Pseudomonas aeruginosa* (Benson et al. 1994). Stansell et al. (2001) found that exposure of *E. coli* to SMF of 4.5 mT significantly increased its antibiotic resistance. Tagourti et al. (2010) showed that exposure to a 200 mT SMF increased the efficiency of gentamicin against *S. Hadar*, but did not affect the diameter of the inhibition zone of some other antibiotics actives on Enterobacteria: penicillin, oxacillin, cephalotin, neomycin, amikacin, tetracycline, erythromycin, spiramycin, chloramphenicol, nalidixic acid, and vancomycin. However, Grosman et al. (1992) reported that a SMF of 0.5 ± 4.0 T had no significant influence on the growth of two strains of *E. coli* or *S. aureus* after exposure time of 30 ± 120 min, nor were there any effects on sensitivity to several antibiotics.

The influence of SMF on fermentation process has been investigated in biomass, and enzyme activity (Motta et al. 2001). da Motta et al. (2004) showed that exposure to 220 mT SMF significantly increased the biomass (g/L) of *S. cerevisiae* strain by 2.5-fold and the concentration of ethanol by 3.4-fold as compared with SMF non-exposed cultures. Glucose consumption was higher in magnetized cultures, which was correlated to the ethanol yield. Invertase is an enzyme (b-fructofuranosidase, EC 3.2.1.26) used to produce noncrystallizable sugar syrup from sucrose. Taskin et al. (2013) showed that the maximum invertase activity and biomass concentration were achieved with the spores exposed to 5 mT SMF.

Enhancement of biochemical processes by SMF has been applied in biological wastewater treatment. SMF had a positive effect on activated sludge biomass growth and dehydrogenase activity, which was similar to the observation in *p*-nitroaniline removal with activated sludge (Niu et al. 2014). Low and moderate SFMs could enhance the activities and growth of nitrite-oxidizing bacteria, increasing the removal of organic pollutants from wastewater (Jia et al. 2018). The effect of SMF exposure on the biodegradation rate of a mixture of pollutants was investigated by three strains including *Pseudomonas stutzeri LBR* (KC157911), *Cupriavidus metallidurans LBJ* (KU659610), and *Rhodococcus equi LBB* (KU743870) isolated and identified near Bizerte, Tunisia. Mansouri et al. (2019) applied 200 mT to these three strains and found there was an increase by 20% in the growth of the exposed bacterial population compared to controls, and 98% of biodegradation of DDT and 90% for BaP after 30 days of follow-up. The efficiency of phenol biodegradation was greatly increased by 30% under moderate SMFs (Kriklavova et al. 2014). Krzemieniewski et al. (2003) reported that a SMF of 400–600 mT stimulated the conditioning of wastewater sludge. A significant 30% increase in maximum nitrogen removal rate and an approximate 1/4 saving in cultivation time were achieved by using a SMF of 60 mT, indicating that the magnetic field was useful and reliable for fast start-up of anammox process (Liu et al. 2008). In algal-bacterial symbiotic system, Tu et al. (2015) reported that SMF stimulated both algal growth and oxygen production, suggesting that magnetic field could reduce the energy consumption

required for aeration during the degradation of organic matter in municipal wastewater. Although SMFs have shown interesting potential in biodegradation of wastewater, there are some negative results. Mateescu et al. (2011) showed that SMFs of 500 and 620 mT produced an atypical growth in the fungus that was characterized by less and swollen, bombastic colonies which did not spread on the entire surface of the culture medium. Jasmina et al. (2012) reported that SMF ($B = 17$ mT) negatively influenced the growth of *E. coli* and *Pseudomonas putida* that were commonly found in wastewater treatment plants, but positively influenced enzymatic activity.

In addition to the application in wastewater biological treatment, SMFs also have broad application prospects for decolorization and de-oiling. In terms of decolorization reactions, Shao et al. (2019) studied the decolorization effect of marine microbial communities on azo dyes under SMF and found that the decolorization, chemical oxygen demand (COD) removal, and detoxification efficiency were higher at 45.3 mT SMF. Tan et al. (2020) found that the SMF and the salt-tolerant yeast *Candida tropicalis* SYF-1 co-enhanced SBR (named MSF-SBR) had higher and more stable ARB (acidity) under high salt and continuous operating conditions Red B processing efficiency (Shao et al. 2019). Ren et al. (2018) studied the effect of SMF on the high-efficiency oil-removing bacteria Acinetobacter B11, and the results showed that under a low-intensity magnetic field of 15–35 mT, the permeability of the cell membrane was increased and superoxide disproportionation was improved. Enzyme (SOD) activity effectively enhanced the lipid degradation performance of bacteria.

7.3 SMF on Plants

7.3.1 SMF on Germination

Magnetic seed treatment is one of the physical presowing seed treatments that have been reported to enhance the germination of crop plants. The rate and percentage of germination were increased by low and moderate SMFs in barley seed, rice (*Oryza sativa* L.) seeds, chickpea (*Cicer arietinum L.*) seeds, sunflower seeds, bean seeds, wheat seeds, okra (*Abelmoschus esculentus* cv. *Sapz pari*), garden pea (*Pisum sativum L.* cv. *climax*), mung beans seeds, onion seeds (*c.v. Giza Red*), and cumin seeds. However, there are few reports on negative results of germination stimulated by moderate SMFs. The effects of SMFs at various intensities and exposure periods on the germination of different plants were summarized in Table 7.1.

The coeffects of SMFs with other factors on germination have been investigated to obtain higher germination. Poinapen et al. (2013) investigated the magnetic flux intensity, together with exposure time, seed orientation (North and South polarity), and relative humidity (RH) in tomato (*Solanum lycopersicum L.*) var. MST/32 seeds. They found that higher germination (~11.0%) was observed in magnetically exposed seeds than in non-exposed ones, suggesting a significant effect of non-uniform SMFs on seed performance with respect to RH, and more pronounced

Table 7.1 The effect of SMF on the germination of plant species

Plant species	SMF exposure	Biological effect	References
Barley seeds	125 mT for 1, 10, 20, and 60 min, 24 h, and chronic exposure	Increasing in length and weight	Martinez et al. (2000)
Rice (*Oryza sativa L.*) seeds	150 mT, 250 mT for chronically and 20 min	Increased the rate and percentage of germination	Carbonell et al. (2000)
Chickpea (*Cicer arietinum L.*)	0–500 mT for 1–4 h	Enhanced seed germination, speed of germination, seedling length, and seedling dry weight	Vashisth and Nagarajan (2008)
Sunflower seeds	0–250 mT for 1–4 h	Increased the speed of germination, seedling length, and seedling dry weight	Ananta and Shantha (2010)
Bean and wheat seeds	4 mT, 7 mT for 7 days	Promoted the germination ratios	Cakmak et al. (2010)
Okra (*Abelmoschus esculentus cv. Sapz pari*)	99 mT for 3 and 11 min	Increased the germination, growth, and yield	Naz et al. (2012)
Seeds of garden pea (*Pisum sativum L. cv. Climax*)	60 mT, 120 mT, and 180 mT for 5, 10, and 15 min	Enhanced the germination parameters	Muhammad et al. (2012)
Mung beans seeds	0.07, 0.12, 0.17 and 0.21 T for 20 min	Improved the germination	Tarlochan and Pandey (2015)
Onion seeds (*c.v. Giza Red*)	30 or 60 mT	Increased all germination and seedling growth characters	Hozayn et al. (2015)
Cumin seeds	150 and 500 mT	Improved germination	Vashisth and Joshi (2017)
Seeds of wheat (*Triticum aestivum L. cv. Kavir*)	30 mT for 4 days, 5 h/day	Did not affect germination percent of the seeds, but increased the speed of germination and vigor index II	Payez et al. (2013)
Rice (*Oryza sativa*) seeds	125 or 250 mT for 1 min, 10 min, 20 min, 1 h, 24 h, or chronic exposure	Reduced the germination time	Florez et al. (2004)

effects were observed during seed imbibition rather than during later developmental stages. Jovicic-Petrovic et al. (2021) found that the synergistic effect of *B. amyloliquefaciens D5 ARV* and 90 mT exposure increased the germination rate of white mustard (*Sinapis alba L.*) by 53.20%.

The mechanism of SMF on germination is not very clear. Bahadir et al. (2018) reported that 125 mT SMF treatment improved the germination of *Lathyrus chrysanthus Boiss* by breaking dormancy. Raipuria et al. (2021) showed that 200 mT SMF promoted nitric oxide via nitric oxide synthase to ameliorate the

UV-B stress during germination of soybean seedlings. Kataria et al. (2020) reported the role of nitric oxide (NO) at 200 mT SMF induced seed germination and early growth characteristics of soybean (*Glycine max*) seedlings under salt stress and found that pretreatment of seeds with 200 mT SMF positively stimulated the germination and then promoted the seedling growth.

7.3.2 SMF on Growth

The effects of SMFs on growth have been well studied in various seeds of crop, vegetable, and fruit. Extremely low magnetic field at 47 ± 5 μT promoted the maize seedling growth (Hajnorouzi et al. 2011). Besides, Vashisth and Nagarajan (2010) found that under the same conditions, seedlings of sunflower showed higher seedling dry weight, root length, root surface area, and root volume; moreover, in germinating seeds, enzyme activities of amylase, dehydrogenase, and protease were significantly higher in treated seeds than controls as shown in Fig. 7.3. The beneficial effects of low SMFs on the growth have been well investigated in potato plantlets, barley seeds, soybean, corn, *Zea mays*, pea, and radish seedlings as shown in Table 7.2.

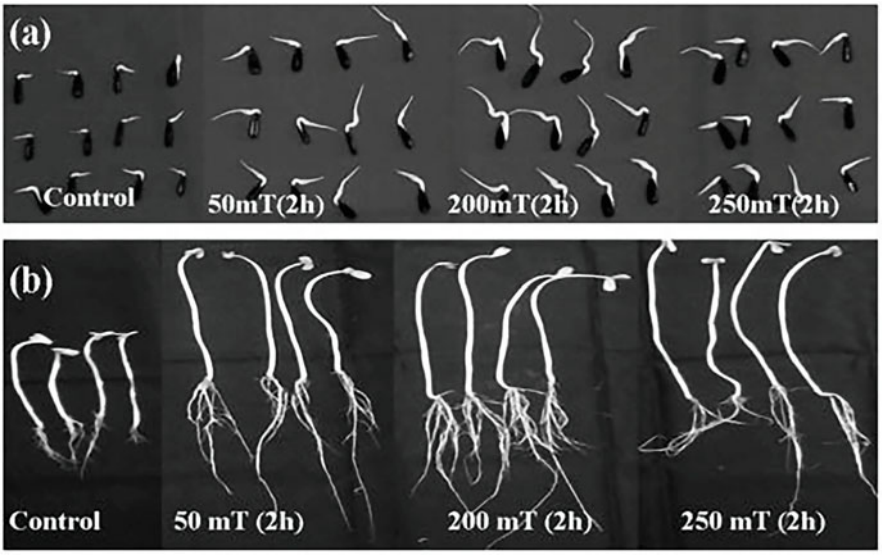

Fig. 7.3 Effect of pre-germination exposure of sunflower seeds on (**a**) speed of germination and (**b**) seedling vigor. [Reprinted with permission from (Vashisth and Nagarajan 2010)]

Table 7.2 The effect of SMF on the growth of plant species

Plant species	SMF exposure	Biological effect	References
Maize seedling	47 ± 5 μT, 4 days	Promoted the maize seedling growth	Hajnorouzi et al. (2011)
Potato plantlets	4 mT, 20 days	Had beneficial effects on the growth promotion and enhancement of CO_2	Iimoto et al. (1996)
Barley seeds	125 mT, 1, 10, 20, and 60 min, 24 h, and chronic exposure	Stimulated the first stages of growth and increases in length and weight	Martinez et al. (2000)
Soybean	200 mT SMF, 1 h	Enhanced the soybean plant height, area of third trifoliate leaves, width of the midrib and minor vein	Fatima et al. (2021b)
Corn	125 or 250 mT, 10 days	Grew higher and heavier than control	Florez et al. (2007)
Zea mays	50 mT, 0.25, 0.5, 1 h	Increased the root length, radicle length, and protein percentage	Subber et al. (2012)
Pea	125 or 250 mT SMF, 1, 10 and 20 min, 1 and 24 h and continuous exposure	Longer and heavier than the corresponding control	Carbonell et al. (2011)
Seedlings of sunflower	200 mT, 2 h	Improved the growth and yield of the sunflower	Vashisth et al. (2021)
Tomato	100 mT, 10 min and at 170 mT for 3 min	The mean fruit weight, the fruit yield per plant, the fruit yield per area, and the equatorial diameter of fruits were increased	De Souza et al. (2006)
Lettuce plants	0.44 T, 0.77 T and 1 T, 1, 2, and 3 h	Increased the growth and biomass production	Latef et al. (2020)

7.3.3 SMF on Gravitropism

Gravitropism is the most conspicuous response to the gravitational force in plants, which plays an essential role in maintaining the spatial orientation of seedlings and stable balance of massive plants. The ability of plants to sense gravity is largely attributed to starch-filled amyloplasts, which is a long-lived response throughout the entire life. Kuznetsov and Hasenstein (1996) reported that high-gradient magnetic fields (HGMFs) induced intracellular magnetophoresis of amyloplasts. The shoots of *lazy-2* mutant of tomato (*Lycopersicon esculentum Mill., cv. Ailsa Craig*) exhibited negative gravitropism in the dark, but responded positively gravitropically in red light. The induced magnetophoretic curvature showed that *lazy-2* mutants perceived the displacement of amyloplasts in a similar manner than wild type and the high MF did not affect the graviresponse mechanism (Hasenstein and Kuznetsov 1999). Weise et al. (2000) reported that *Arabidopsis* stems positioned in a high-gradient magnetic field (HGMF) on a rotating clinostat showed the lack of apical curvature after basal amyloplast displacement, indicating that gravity perception in the base

was not transmitted to the apex. Jin et al. (2019) reported that root growth was significantly enhanced by SMFs in an intensity and magnetic direction dependent way, which was mediated by CRY and auxin signaling pathways in Arabidopsis. Hasenstein et al. (2013) examined the movement of starch grains of corn, wheat, and potato (*Solanum tuberosum*) in suspension during parabolic flights and found that magnetic gradients were able to move diamagnetic compounds under weightless or microgravity conditions and serve as directional stimulus during seed germination in low-gravity environments. Yano et al. (2001) reported that the primary roots of radish (*Raphanus sativus L.*) seedlings responded tropically to the 13–68 mT SMF with the tropism appearing to be negative and the roots responded significantly to the south pole of the magnet.

7.3.4 SMF on Photosynthesis

The effects of SMF on the photosynthesis have been investigated in various plants including soybean, corn, *Lemna minor*, and lettuce. Shine et al. (2011) reported that presowing magnetic treatment could improve biomass accumulation in soybean. Polyphasic chlorophyll *a* fluorescence transient from magnetically treated soybean plants gave a higher fluorescence yield. Baghel et al. (2016) provided further evidence that polyphasic chlorophyll *a* fluorescence (OJIP) transient from magnetically treated plants gave a higher fluorescence yield at J–I–P phase. Moreover, nitrate reductase activity, PIABS, photosynthetic pigments, and net rate of photosynthesis were also higher in plants that emerged from soybean seeds exposed to 200 mT SMF. In corn plants, Anand et al. (2012) reported that SMFs of 100 and 200 mT increased the photosynthesis, stomatal conductance, and chlorophyll content. The pretreatment of seeds of two corn cultivars with different magnetic treatments significantly alleviated the drought-induced adverse effects on growth by improving chlorophyll, photochemical quenching, and non-photochemical quenching (Javed et al. 2011). Jan et al. (2015) found that the reduced geomagnetic field (GMF) significantly stimulated growth rate of the total frond area in the magnetically treated *Lemna minor* plants, while the enhanced GMF pointed toward inhibition of growth rate in exposed plants in comparison to control, but the difference was not statistically significant. All photosynthetic pigments in lettuce seeds (*Lactuca sativa var. capitata L.*) were induced markedly under 0.44 T, 0.77 T, and 1 T SMF, especially chlorophyll *a* (Latef et al. 2020).

There are few studies on the coeffects of SMFs and other environmental factors on photosynthesis. Kataria et al. (2021) reported that 200 mT SMF pretreatment enhanced photosynthetic performance in soybean under supplemental ultraviolet-B radiation. Fatima et al. (2021a) found that 200 mT SMF pretreatment caused enhancement of leaf growth along with photosynthesis even under the presence of ambient UV-B stress. Moreover, pretreatment with 50–300 mT SMF increased water uptake by the midrib of soybean (*Glycine max, variety JS-335*), which in turn led to an increase in photosynthesis and stomatal conductance (Fatima et al. 2017). In

addition, Jovanić and Sarvan (2004) reported that SMF induced significant changes in bean leaf fluorescence spectra and temperature, which the fluorescence intensity ratio (FIR) and change of leaf temperature βT were increased with the increase of MF intensity.

7.3.5 SMF on Redox Status

The uncoupling of free radicals including reactive oxygen/nitrogen species (ROS/RNS) is involved in the underlying mechanism of SMF induced oxidative stress in plants. The activities of free radical scavenging enzymes, including catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione transferase (GT), peroxidase (POD), ascorbate peroxidase (APX), and polyphenol oxidase (POP), have been well documented to be altered by SMF exposure in various plants, including pea, radish (*Raphanus sativus*), *Leymus chinensis*, soybean, cucumber (*Cucumis sativus*), broad bean, corn, parsley (*Petroselinum crispum*), and wheat (Regoli et al. 2005; Baby et al. 2011; Jouni et al. 2012). Mohammadi et al. (2018) found that 0.2 mT SMF increased the contents of nitric oxide (NO), hydrogen peroxide (HO), and salicylic acid (SA) in tobacco cells (*Nicotiana tabacum cv. Barley 21*), and suggested that a signaling pathway activated by SMF starting from accumulation of NO and HO, then increased the cyclic nucleotides and subsequent decreased the cyclin-dependent kinases A (CDKA) and D-type cyclin (CycD). Cakmak et al. (2012) reported that SMF of 7 mT increased lipid peroxidation and H_2O_2 levels in shallot (*Allium ascalonicum*) leaves. Jouni et al. (2012) found that treatment of plants with 15 mT SMF caused accumulation of reactive oxygen species (ROS), lowered the antioxidant defense system, and increased the peroxidation of membrane lipids in broad bean (*Vicia faba L.*). Shokrollahi et al. (2018) found that 20 mT SMF decreased ferrous and HO contents, content and activity of ferritin and catalase in soybean plants, but the opposite responses were observed under 30 mT treatments. Shine et al. (2012) showed that SMFs of 150 and 200 mT enhanced production of ROS mediated by cell wall peroxidase, while the increase in the cytosolic peroxidase activity indicated that this antioxidant enzyme had a vital role in scavenging the increased H_2O_2 produced in seedlings from the magnetically treated soybean seeds. In mung bean seedlings treated with 600 mT SMF followed by cadmium stress, Chen et al. (2011) found that the concentration of malondialdehyde, H_2O_2, and O^- were decreased, while the NO concentration and NOS activity were increased compared to cadmium stress alone, indicating that MF compensates for the toxicological effects of cadmium exposure were related to NO signal.

7.3.6 Cryptochromes Sensing Magnetic Field

Cryptochromes (CRYs) are flavoproteins that direct a diverse array of developmental processes in response to blue light in plants (Yu et al. 2010). CRY has been suggested to be a potential magnetoreceptor for light-initiated electron transfer chemistry which might be magnetically sensitive to virtue of the radical pair mechanism (Evans and Davidson 2013; Hore and Mouritsen 2016). Geomagnetic field (GMF) has been hypothesized to affect the redox balance of cryptochromes and the related signaling state (Vanderstraeten et al. 2015); however, the influence of strong SMF on the function of CRYs is still largely unexplored.

Three CRYs, CRY1, CRY2, and CRY3 are encoded in *Arabidopsis* genome (Lin and Todo 2005). CRY1 and CRY2 function as major blue light receptors regulating blue light induced de-etiolation, photoperiodic flowering, and circadian clock (Liu et al. 2016). Xu et al. (2014) found SMF of 500 μT modified the function of CRYs. The blue light-dependent phosphorylations of CRY1 and CRY2 were enhanced in *Arabidopsis* seedlings grown in a 500 μT MF, whereas the near-null MF weakened the blue light-dependent phosphorylation of CRY2 but not CRY1; in the darkness, dephosphorylations of CRY1 and CRY2 were slowed down in the 500 μT MF, whereas dephosphorylations of CRY1 and CRY2 were accelerated in the near-null MF. According to the calculation of radical pair mechanism in a relatively realistic model of the radical pair system in *Arabidopsis* CRY1, Solov'yov et al. (2007) showed that 500 μT MF could increase the signaling activity of cryptochrome by up to 10%, suggesting that the function of CRYs was affected by magnetic field. Pooam et al. (2019) investigated the response of Arabidopsis CRY1 in vivo to 500 μT SMF using both plant growth and light-dependent phosphorylation as an assay, then they found that the magnetically sensitive reaction step in the cryptochrome photocycle must occur during flavin reoxidation, and likely involved the formation of ROS. Ahmad et al. (2007) reported that 500 μT MF enhanced the blue light-dependent inhibition of hypocotyl growth of *Arabidopsis*. Hypocotyl growth of *Arabidopsis* mutants lacking CRYs was unaffected by the increase of magnetic intensity, while cryptochrome-dependent responses, such as blue light-dependent anthocyanin accumulation and blue light-dependent degradation of CRY2 protein, were enhanced at the higher magnetic intensity. However, with experimental conditions chosen to match Ahmad's study, Harris et al. (2009) found that in no case consistent, statistically significant MF responses were detected.

CRYs evolved from photolyases are conserved across many different species. In addition to plants, the expression of CRYs has been detected in migratory birds and the eyes of mammals, which were putative sites for magnetoreceptors in vertebrates, and there was no evidence for intracellular magnetite in putative vertebrate magnetoreceptors identified by magnetic screening (Möller et al. 2004; Nießner et al. 2013; Edelman et al. 2015). In animals, CRYs also functioned as circadian photoreceptors in the *Drosophila* brain, mediating the light resetting of the 24 h clock; but in vertebrates, the CRYs acted as the main negative regulators for the circadian feedback loop, due to the difference in light sensing (Yoshii et al. 2009;

Fedele et al. 2014). Non-Drosophila insects can also encode CRY1 and CRY2, but CRY1 retain their light-sensing properties, whereas the CRY2s act as vertebrate-like negative regulators. Marley et al. (2014) reported that MF exposure coupled with CRY photoactivation during embryogenesis was sufficient to produce heightened seizure susceptibility in resultant *Drosophila* third instar (L3) larvae. Giachello et al. (2016) provided evidence that exposure to a MF of 100 mT was sufficient to potentiate the ability of light-activated cryptochrome to increase neuronal action potential firing, indicating that the activity of cryptochrome was sensitive to an external MF that was capable of modifying animal behavior.

7.4 SMF on Animals

7.4.1 SMF on Caenorhabditis elegans

Caenorhabditis elegans (*C. elegans*) is a small free-living nematode that has been widely utilized to address fundamental questions of developmental biology, neurobiology, and behavioral biology. *C. elegans* is similar to higher eukaryotes in many molecular and cellular pathways (Kaletta and Hengartner 2006) and offers unique advantages, including the ease of maintenance, small size, short life cycle, genetic manipulability, stereotypical development, and high-throughput capability. As about 50% of its genes have human homologs, *C. elegans* based assays are increasingly used to evaluate potential toxicity of different stressors in humans and mechanisms of toxicity by physical and chemical exposures (Kazazian Jr. 2004; Dengg and van Meel 2004; Rajini et al. 2008; Sprando et al. 2009; Boyd et al. 2010).

Recent evidence has shown that the *C. elegans* oriented to the earth's magnetic field during vertical burrowing migrations neuron pair (Vidal-Gadea et al. 2015). A pair of neurons called the AFD neurons, which carry information about temperature and chemical stimuli from the environment, were critical for magnetic navigation in *C. elegans*. The further investigation showed the unique spatiotemporal trajectories of magnetotactic processes in *C. elegans* under different external conditions including temporal, spatial, and environmental factors. They found that the magnetic orientation of these "small worm" might be stronger under dry conditions (<50% RH) (Bainbridge et al. 2020). Using worms with mutations at some of the genes expressed in the AFD neurons and a calcium sensitive protein, it was found that the tax-4 gene, which encoded an ion channel protein similar to a photoreceptor found in the retina of human eyes, was required for magnetotaxis (Rankin and Lin 2015). These data represented a significant advance in our understanding of the neurobiology underlying how organisms navigate using the Earth's magnetic field. Recently, Cheng et al. (2022) found that exposure *C. elegans* to 0.5 T and 1 T SMFs greatly decreased the avoidance behavior of the pathogenic *Pseudomonas aeruginosa*. The total serotonin level was significantly increased by exposure to 0.5 T and 1 T SMF; in contrast, SMFs had few effects on other three neurotransmitters including choline, γ-aminobutyric acid (GABA), dopamine as shown in Fig. 7.4. These data indicated

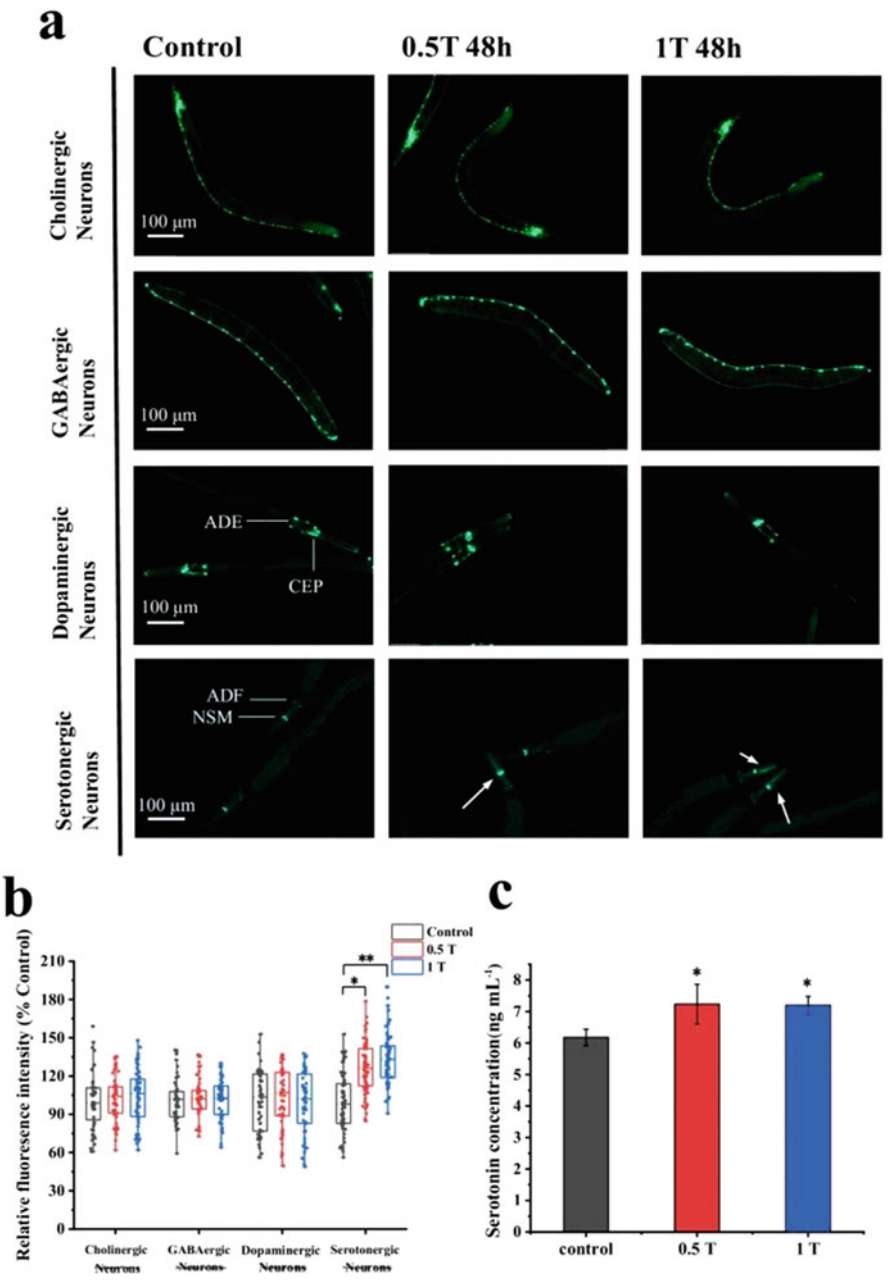

Fig. 7.4 Effects of 0.5 T and 1 T SMFs exposure for 48 h on neurons and neurotransmitters of *C. elegans*. (**a**) Fluorescence imaging of each neurotransmitter neurons; from top to bottom are cholinergic neurons, GABAergic neurons, dopaminergic neurons, and serotonergic neurons. (**b**) Analysis of fluorescence intensity of four neurotransmitter systems ($n \geq 30$ nematodes/group). (**c**) Serotonin concentrations after long-term exposure to SMF. [Reprinted with permission from (Cheng et al. 2022)]

that moderate-intensity SMFs induced neurobehavioral disorder might be modulated by serotonin in *C. elegans*.

The biological effects of SMFs on *C. elegans* have been focused on the development, aging process, behavior, and global gene expression. Hung et al. (2010) reported that treatment with 200 mT SMF reduced the development time from the L2 to the L3 stage by 20%, from L3 to L4 by 23%, and from L4 to young adult by 31%. With SMF treatment, the average lifespan was reduced from 31 to 24 days in wild-type nematodes. The upregulation of *lim-7*, *clk-1*, *daf-2*, *unc-3,* and *age-1* by SMF treatment was verified by quantitative real-time PCR; in contrast, lifespan analyses showed that SMF treatment had no effect on *let-7*, *unc-3*, and *age-1* mutants, indicating that the induction of gene expression by SMFs was selective and dose-dependent. Lee et al. (2012) showed that long-term and low-dosage exposure to 200 mT SMF was capable of inducing an apoptosis-mediated behavioral decline in nematodes. 26 differentially expressed genes including apoptosis, oxidative stress, and cancer-related genes were identified, indicating that a global molecular response to SMF exposure occurred. Mutations in genes involved in major apoptotic pathways, that is, *ced-3*, *ced-4*, and *ced-9*, abolished this SMF-induced behavioral decline. Kimura et al. (2008) reported that genes involved in motor activity, actin binding, cell adhesion, and cuticles were transiently and specifically induced by 3 or 5 T SMF exposure in *C. elegans*. Several genes encoding apoptotic cell-death activators and secreted surface proteins were upregulated by ionizing radiation, instead of SMFs. Exposure to 3 or 5 T SMFs did not induce DNA double-strand breaks or germline cell apoptosis during meiosis. However, we found that 8.5 T SMFs resulted in a time-dependent lifespan decrease and alteration of development rate and stages in *C. elegans*. Germ cell apoptosis dramatically increased upon exposure to 8.5 T SMF in worms via core apoptotic machinery, which could be prevented by concurrent treatment with a free radical scavenger, dimethyl sulfoxide (Wang et al. 2015). Yang et al. (2022) further explored the biological effects of 10 T SMF on sperms and their offspring in *him-5* male mutants of *C. elegans* and found that sperms were sensitive targets of high SMFs as shown in Figs. 7.5 and 7.6. Although 10 T SMF had little effect on the morphology of sperms, the size of unactivated sperms and the function of sperms were modified by SMF exposure, leading to diminish the reproductive capacity of *him-5* male worms. These observations provided interesting information regarding the adverse effects of high SMFs on the reproductive function of *C. elegans* and their offspring, which could improve our understanding of the fundamental aspects of high SMFs on biological system.

7.4.2 SMF on Insects

Magnetic fields have been shown to affect the orientation, oviposition development, fecundity, and behaviors for a wide variety of insects. The insect eggs have advantages in magnetic exposure for a large number of eggs which can be placed into the magnet at the same time. The SMF at 4.5 mT had no effect on egg lying, but

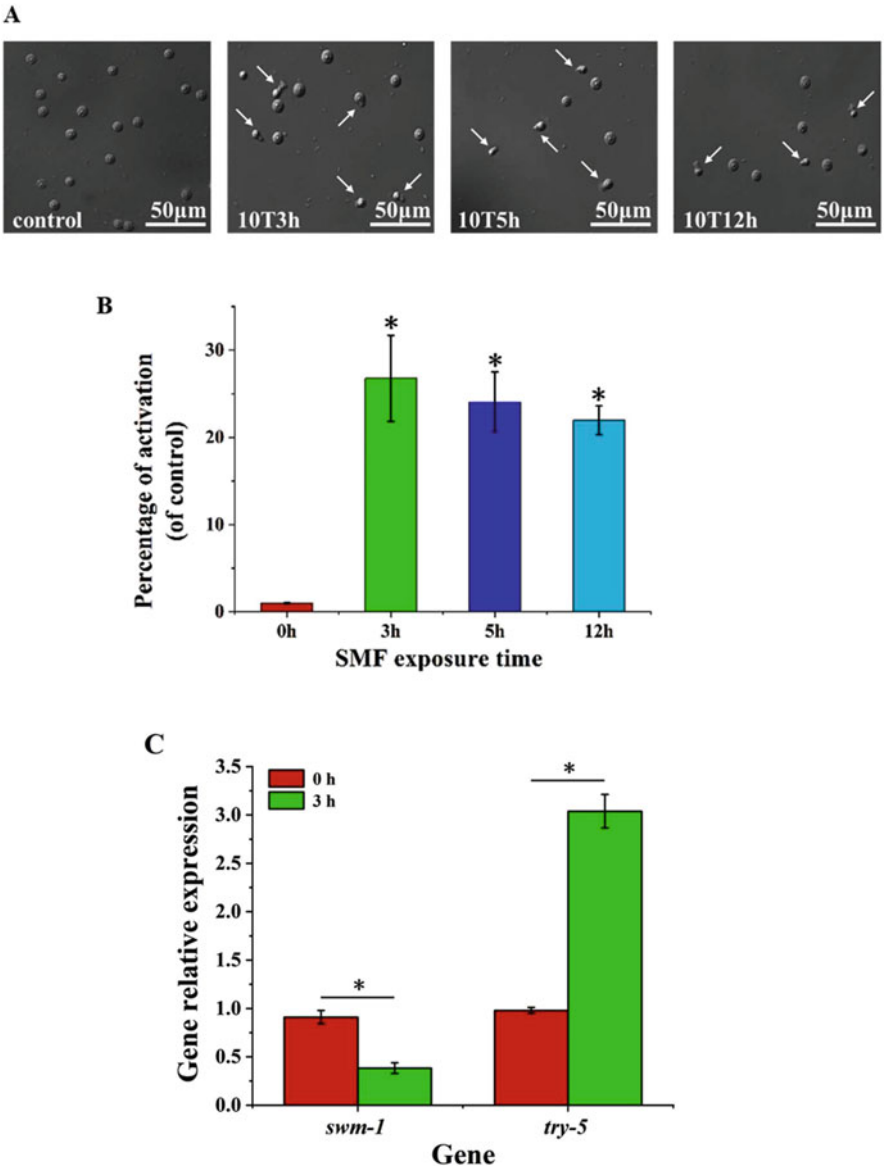

Fig. 7.5 10 T SMF accelerated the activation of sperms. (**a**) The male *him-5* mutants were exposed to 10 T SMF, and the premature activation of sperm was measured. White arrows represent activated sperm (pseudopodia). (**b**) The percentage of premature activation of sperm with 10 T SMF exposure. (**c**) Relative mRNA expression of *swm-1*and *try-5* genes in male *him-5* mutants with 10 T SMF exposure. Data were pooled from three independent experiments. Error bars indicate ± SEM; $*p < 0.05$, compared with the control group. Scale bars, 50 μm. [Reprinted with permission from (Yang et al. 2022)]

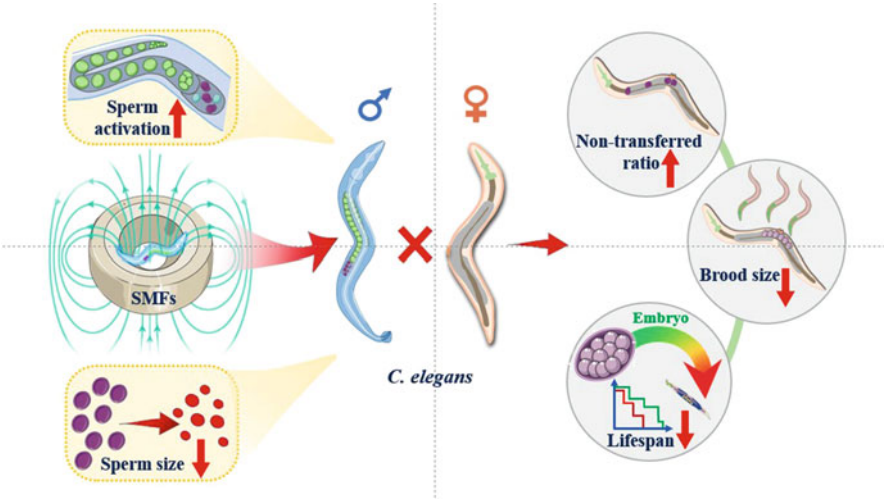

Fig. 7.6 Graphical abstract. Effects of 10 T static magnetic field on the function of sperms and their offspring in *Caenorhabditis elegans.* [Reprinted with permission from (Yang et al. 2022)]

increased mortality of eggs, larvae, and pupa, and diminished adult viability in *Drosophila* (Ramirez et al. 1983). Decreased hatching rate after exposure to a weak SMF during early embryogenesis was also obtained in *D. melanogaster* and *Heliothis virescens* (tobacco budworm) (Ho et al. 1992; Pan 1996). A significant increase of *Hylotrupes bajulus* viability and larval mass was reported after exposure to a SMF of 98 mT (Rauš Balind et al. 2009). The SMF of 60 mT reduced the embryonic and post-embryonic development and induced weaker viability in two different species, *Drosophila melanogaster* and *Drosophila hydei* (Savić et al. 2011). Todorovic et al. (2019) found that chronic exposure to 110 mT SMF significantly decreased the gut mass and the activity of glutathione reductase (GR) and glutathione S-transferase (GST) as compared to the control in *Blaptica dubia* (*B. dubia*). They further reported that 110 mT SMF decreased nymph body mass and glycogen content in the fat body but increased all examined parameters of locomotion, indicating that *B. dubia* nymphs were sensitive to SMF exposure (Todorovic et al. 2020). Oak and beech populations of *Drosophila subobscura* had longer development time, and lower viability was observed in N and S groups of 2.4 T SMF, which was mediated by oxidative stress (Todorović et al. 2015). Apparent hatching delay of strong magnetic fields was observed in mosquito eggs in the center of 9.4 and 14.1 T magnets (Pan and Liu 2004).

In insects, the neuroendocrine system is a main regulator of all aspects of life processes, such as development and behavior, and the detection and activity of an external magnetic field may be transmitted by the neuroendocrine system (Blanchard and Blackman 1994; Gilbert et al. 1996). A SMF of 375 mT caused the disturbance of development and survival of pupae of the honeybee and *Tenebrio molitor*, yellow mealworm (Prolic and Jovanovic 1986; Prolić and Nenadović 1995). The

morphometric parameters of the A1 and A2′ neurosecretory neurons of the protocerebrum as well as the morphometric parameters of the *corpora allata* were changed by a SMF of 320 mT (Perić-Mataruga et al. 2008). However, SMF of 50 mT did not effect on pupa-adult development dynamic of two examine *Tenebrio* species, but modulated their motor behavior (Todorović et al. 2013).

The antennal lobe of *Drosophila* provides an ideal intact neural network model to investigate neural circuit function (Ng et al. 2002). Yang et al. (2011) found that a SMF of 3.0 T modilated the rhythmic spontaneous activities of large LNs and correlated activity of ipsilateral pairs of large LN/LN in *Drosophila* antennal lobe, indicating that *Drosophila* could be an ideal intact neural circuit model to evaluate the effects of magnetic field stimulations.

Mutagenic effects of a static magnetic field were investigated by increasing mutation rate in population of *Drosophila* exposed to magnetic field 10–12 times greater than geomagnetic one (Giorgi et al. 1992). Exposure to 2, 5, or 14 T fields caused a statistically significant enhancement in somatic recombination frequency in the postreplication repair-deficient flies, whereas the frequency of somatic recombination remained unchanged in the nucleotide excision repair-deficient flies and in DNA repair-proficient flies after exposure (Takashima et al. 2004).

7.4.3 SMF on Helix pomatia

Helix pomatia possesses simple nerve system and displays simple behavioral repertoire. Single identified neurons have been documented as a good experimental model for the relatively large size, easy manipulation, consistent position on the surface of the ganglia, and consistent type of synaptic connections. Nikolić et al. (2008) reported that the magnetic field of 2.7 mT intensity caused changes in the amplitude and duration of action potential of the Br neuron in subesophageal ganglia of the garden snail *Helix pomatia*, whereas the 10 mT magnetic field changed the resting potential, amplitude spike, firing frequency, and duration of action potential of the Br neuron. Moreover, significant increase of the activity of Na^+/K^+-ATPase and the expression of its α-subunit in nervous system were observed in *Helix pomatia* exposed to 10 mT SMF (Nikolić et al. 2013). With single, 30-min long, and whole body exposed to 147 mT, Hernádi and László (2014) reported that SMF exposure mediated peripheral thermal nociceptive threshold by affecting the serotonerg as well as the opioiderg system.

7.4.4 SMF on Aquatic Animals

Sea urchins are the only invertebrates with the same development patterns as mammals. Moreover, the gametes of sea urchins can be obtained easily, the eggs and early embryos are transparent, and the early development of embryos is highly

synchronous. A SMF of 30 mT delayed the onset of mitosis in two species of sea urchins, *Lytechinus pictus* and *Strongylocentrotus purpuratus*. There was an eight-fold increase in the incidence of exogastrulation in *Lytechinus pictus* embryos exposed to SMFs, while magnetic fields had no effects on species *Strongylocentrotus purpuratus* embryos (Levin and Ernst 1997). Exposure of fertilized eggs of *Echinometra mathaei* to 30, 40, and 50 mT of magnetic fields delayed the onset of early cleavage division and significantly decreased the cleaved cells for exposed embryos. As the increase of intensity of the magnetic fields, earlier appearances of abnormalities were observed (Sakhnini and Dairi 2004).

The interaction among neurons in escape circuit of crayfish has been well studied. As the lateral giant (LG) neuron was easy to access for electrophysiological study, Ye et al. (2004) found that exposure to SMF at 4.74–43.45 mT increased the amplitude of action potential (AP) in LG depending upon both the intensity of field and duration of field exposure, which was mediated by the increasing level of intracellular Ca^{2+} in the LG. The excitatory postsynaptic potential (EPSP) produced via electrical and chemical synapses in the lateral giant neuron was enhanced after 30 min of SMF exposure (8.08 mT). Perfusion of field-exposed crayfish bath solution or preloading of Ca^{2+} chelator and intracellular Ca^{2+} release blocker failed to observe the SMF-induced enhancement on EPSP (Yeh et al. 2008).

As an increasingly important model species in genetic and neurobehavioral studies, zebrafish (*Danio rerio*) is an excellent organism for better understanding the biological mechanism of SMFs. Using a fast, fully automated assay system relying on negative reinforcement, Shcherbakov et al. (2005) recorded statistically highly significant reactions to weak magnetic field changes in Mozambique tilapia, a fish migrating regularly between freshwater and the sea, and non-migratory zebrafish. Takebe et al. (2012) found that zebrafish responded to a magnetic field as weak as the geomagnetic field by bidirectional orientation with group-specific preferences regardless of close kinships. SMFs with density from 4.7 to 11.7 T profoundly disturbed the orientation and locomotion behaviors of adult zebrafish, and the independence of these effects from other sensory modalities suggested that they were mediated by the vestibular system as shown in Fig. 7.7 (Ward et al. 2014). In addition, the SMFs could be disrupting metabolism and immunity of the Caspian kutum fry during acute and subacute exposures (Loghmannia et al. 2015). Ge et al. (2019) showed that 9.0 T SMF exposure had no effect on the survival and overall development of zebrafish embryos, but slowed down the development speed of the whole animal. They surmised that microtubule and spindle positioning were perturbed under such high SMF.

7.4.5 *SMF on* Xenopus laevis

Xenopus embryos are thought to be a useful tool for studying vertebrate development, and gene expression for their embryogenesis is rapid and completed outside of the female. The hatching rate of embryos of the frog *Rana pipiens* subjected to the

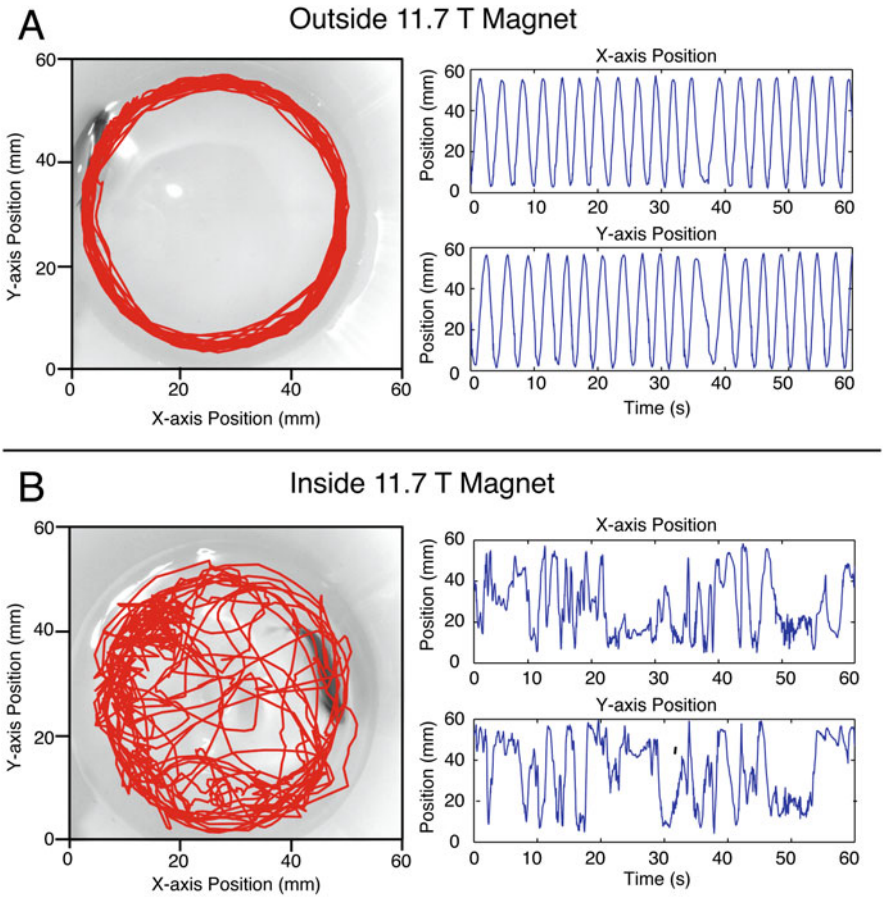

Fig. 7.7 Adult zebrafish behavior outside and inside of an 11.7 T vertical magnetic field. Tracing of adult zebrafish path in visible green light during 1 min prior to magnetic field entry (**a**) and during 1 min inside the magnet (**b**). *X*- and *Y*-position coordinates are displayed as a function of time. Upon entry into the magnet, fish swimming becomes erratic, with frequent rolling, tight circling and increased swimming velocity. [Reprinted from (Ward et al. 2014), open access]

field of a 1 T permanent magnet was found to be reduced (Neurath 1968). Ueno et al. (1984) investigated embryos of African clawed toads exposed to 1 T magnetic field and found that the magnetic field exerted no harmful or modifying effects on gastrulation and neurulation; however, exposed embryos occasionally developed into tadpoles with reduced pigmentation, axial anomalies, or microcephaly. Compared to the first and the second cleavage, the third cleavage was the most susceptible to reorientation in strong SMFs. Exposure to SMF at 16.7 T altered the direction of the third cleavage furrow from its normal horizontal type to the perpendicular type, which was confirmed by embryos exposed to 8 T (Denegre et al. 1998; Eguchi et al. 2006). These results indicated that SMFs might act directly on the microtubules of

the mitotic apparatus to cause distortion of the third cleavage furrow. Kawakami et al. (2006) found that a SMF of 11–15 T significantly retarded normal development and induced microcephaly, two heads, abnormal cement glands, and multiple malformations. Moreover, the gene expression of *Xotx2* (an important regulator of fore and midbrain morphogenesis) and *Xag1* (essential for cement gland formation) was greatly suppressed by strong SMF. Mietchen et al. (2005) investigated the morphology of fertilizable *Xenopus laevis* eggs with and without jelly coat that were subjected to a SMF of up to 9.4 T and found that no effect was observed when the jelly layers of the eggs were left intact, indicating the action of magnetic fields might involve cortical pigments or associated cytoskeletal structures normally held in place by the jelly layers.

The effects of SMF exposure on nerve conduction were investigated in frog sciatic nerves. A significant increase in the nerve conduction velocity (NCV) of compound action potentials (CAP) in sciatic nerves was observed by exposure to a uniform SMF of 1.16 T. Edelman et al. (1979) observed a significant increase in the amplitude of CAP in frog sciatic nerves when a uniform SMF of 385 or 600 mT was applied perpendicular to the axis of the nerve fibers. Although NCV of CAP was not affected by the 8 T SMF, Eguchi et al. (2003) reported that under SMF exposure an optimal time interval existed in the relative refractory period (1.0–1.1 ms) during which some ions move dynamically through specific ion channels. Satow et al. (2001) found that 0.65 T SMF increased excitability in bullfrog sartorius muscle during the recovery period in a conditioning-test stimulation paradigm. With the exposure of in vitro frog sciatic nerve fibers to moderate-intensity gradient SMF up to 0.7 T, Okano et al. (2012) found the values of the nerve conduction velocity of C fibers were significantly reduced by Bmax of 0.7 T SMF but not by 0.21 T SMF, relative to the unexposed control. Although the mechanistic reasons for this decrease have yet to be clarified, SMF could affect the behavior of some types of ion channels associated with C fibers.

7.4.6 SMF on Mice and Rats

7.4.6.1 SMF on Bone Growth, Healing, and Loss

SMF has been considered as a physical therapy on bone health maintenance and bone disorders treatment for it can enhance bone fracture healing and bone formation by osteoblast both in vivo and in vitro (Trock 2000; Miyakoshi 2005; Saunders 2005; Wang et al. 2011). Zhang et al. (2018a) found that 4 mT SMF could inhibit the structural deterioration of trabecular and cortical bone and reduce mechanical strength in T1DM rats. They compared the microstructure and mechanical properties of mouse bone under either hypomagnetic field (HyMF, 500 nT) or moderate SMF (MMF, 0.2 T) and found that exposure to MMF for 4 weeks had a significant effect on bone biology mechanical properties but bone microarchitecture was not affected, whereas HyMF significantly inhibited mouse growth and bone elasticity (Zhang

et al. 2018b). Shan et al. (2021) reported that the tooth movement speed was significantly faster and the periodontal ligament (PDL) width was significantly increased under a SMF of 20–204 mT. 2–4 T SMFs improved bone microstructure and strength by stimulating bone formation and inhibiting bone resorption (Yang et al. 2021).

With implantation of magnetized rods into the middle diaphysis of rat femurs to generate SMF, Yan et al. (1998) found that the femurs adjacent to magnetized specimens had significantly higher bone mineral density (BMD) and calcium content than those adjacent to the unmagnetized specimen. The significantly reduced BMD in this ischemic bone model could be prevented by long-term SMF exposure of 3 weeks (Xu et al. 2001). The SMF accelerated not only the bone neoformation but also the integration of the bone grafts (Puricelli et al. 2009; Leesungbok et al. 2013). Kotani et al. (2002) showed that high SMF of 8 T stimulated ectopic bone formation in and around subcutaneously implanted bone morphogenetic protein (BMP) 2-containing pellets in mice, in which the orientation of bone formation was parallel to the magnetic field. Using ovariectomized (OVX) rat model to represent the clinical features of bone loss, Xu et al. (2010) observed that SMF significantly increased the BMD of osteoporotic lumbar vertebrate without affecting the E2 (17-b-estradiol) levels of serum compared with sham control. Taniguchi et al. (2004) examined the effect of the whole-body exposure to SMF on bone formation and found that SMF could contribute to the relief of pain induced by adjuvant arthritis and BMD was also accelerated significantly. However, with the same SMF exposure device, Taniguchi and Kanai (2007) reported that SMF did inhibit the bone loss of tibia in OVX rats to some extent, but its BMD was still much lower than normal rats, which might be due to the enhanced locomotor activity.

7.4.6.2 SMF on Cardiovascular System

Blood Pressure and Blood Flow

SMF in the mT range has been reported to modulate circulatory hemodynamics and/or arterial blood pressure (BP) and baroreflex sensitivity (BRS). Okano and Ohkubo (2003, 2005, 2006) found that whole-body exposure to SMF suppressed spontaneously hypertensive rats (SHR), which was mediated by nitric oxide (NO) pathway, Ca^{2+} channel, and hormonal regulatory systems. With the calculation of the hematological characteristics, Tasic et al. (2021) found that SMFs with different orientations had adverse effects on the hematological indicators of spontaneously hypertensive rats, but their cardiac and renal morphological features were not affected. Li et al. (2020) found that 20–150 mT SMF had antithrombotic effects in constructed rat and mouse thrombosis models, indicating a non-invasive prevention and treatment way for clot-related diseases.

It is well known that surface temperature and cutaneous blood flow are closely parallel to each other. Ichioka et al. (2003) reported that the whole body of anesthetized rats exposed to 8 T SMF was associated with reduced skin blood flow and

temperature, which could be recovered after removal of the animal from the magnet. Both increases and decreases in skin and rectal temperatures were observed in mice exposed to SMFs with intensities ranging from 0.4 to 8 T. In contrast to these observations, no evidence was found for a change in body temperature of rodents exposed to strong homogeneous or gradient magnetic fields (Tenforde 1986).

Cardiac Function

Blood flow in an applied magnetic field gives rise to induce voltages in the aorta and other major arteries of the central circulatory system that can be observed as superimposed electrical signals in the electrocardiogram (ECG). The largest magnetically induced voltage occurs during pulsatile blood flow into the aorta and results in an increased signal at the location of the T-wave in the ECG. A marked increased T-wave in the ECG records was observed in squirrel monkeys during the exposure to stationary fields of 2–7 T and rabbits exposed to 1 T SMF (Beischer and Knepton Jr. 1964; Togawa et al. 1967). Similar observation was reported by Gaffey and Tenforde (1981) that a field strength dependent increase in the amplitude of the T-wave signal in the rat ECG was revealed during exposure to homogeneous stationary magnetic fields of 2 T, which might be due to a superimposed electrical potential generated by aortic blood flow in the presence of a stationary magnetic field. The exposure of rats to a SMF of 128 mT decreased the activities of glutathione peroxidase (GPx) and the CuZn superoxide dismutase (CuZn-SOD) in rat cardiac muscle (Amara et al. 2009).

Hematological Parameters

The effects of SMFs on hematological parameters have been studied in rats at the intensity of 128 mT. Amara et al. (2006b) reported that a SMF of 128 mT significantly decreased the growth rates, but increased the plasmatic total protein levels, hemoglobin, red blood cells, white blood cells, platelet number, and the activities of lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in male Wistar rats; in contrast, the glucose concentration was unaffected. Milovanovich et al. (2016) showed that both upward- and downward-oriented SMF of 128 mT caused a reduction in the amount of total white blood cells (WBC). Chater et al. (2006) found that subacute exposure to a SMF of 128 mT stimulated biosynthesis of plasma corticosterone and metallothionein activities in female rats, while increased blood glucose and decreased insulin release, leading to a diabetic-like state in pregnant rats. Elferchichi et al. (2016) showed an impaired glucose homeostasis and a deregulated lipid metabolism after SMF exposure in adult rats. But, they noticed that a SMF of 128 mT induced a pseudoanemia status with increased monocarboxylate transporters (MCT4) and glucose transporter 4 (Glut4). Atef et al. (1995) investigated changes of hemoglobin (Hb)'s characteristics in Swiss mice using hundreds of mT for 10 min and found that the rate of Hb oxidative

reaction was declined by 350–400 mT. However, Djordjevich et al. (2012) found that differently oriented SMF of 16 mT did not alter hemoglobin and hematocrit, although the upward and downward fields caused statistically significant higher levels of serum transferrin.

In addition, the supplementation with vitamin D corrected and restored glycemia and insulinemia in SMF-exposed rats (Lahbib et al. 2015). Selenium (Se) improved adverse oxidative stress in blood induced by SMF, whereas zinc supplementation could prevent toxic effects of SMFs probably by its antioxidant proprieties (Ghodbane et al. 2011).

7.4.6.3 SMF on Digestive System

The effects of SMFs on digestive system are largely unknown and most of studies mainly focus on the intensity of 128 mT. A SMF of 128 mT increased total GSH levels and the activity of superoxide dismutase (SOD) and catalase (CAT) in rat liver and hepatocyte apoptosis through a caspase-independent pathway involving mitochondrial apoptosis-inducing factor (AIF), which was restored by Se and vitamin E supplementations (Ghodbane et al. 2015). Amara et al. (2009) found that exposure of rats to a SMF of 128 mT increased the 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) concentration in kidney.

7.4.6.4 SMF on Endocrine System

The influence of SMFs on endocrine system has been linked to their function, such as insulin, pineal gland, and testis. Jing et al. (2010) found that 180 mT SMF exposure could significantly accelerate the diabetic wound (DW) closure process and enhance the wound tensile strength (TS); however, 180 mT local SMF exposure had slight effect on insulin secretion or pancreatic cells of diabetic rats (Rosmalen et al. 2002). Under the neodymium permanent magnets, Feng et al. (2022) found that SMFs promoted diabetic mice wound healing by suppressing oxidative stress. Elferchichi et al. (2011) showed that the metabolic alterations following exposure to a SMF of moderate intensity could trigger the development of a pre-diabetic state. Exposure to a SMF of 128 mT induced an increase in plasma glucose level and a decrease in plasma insulin concentration in rats, which could be corrected by vitamin D supplementation (Lahbib et al. 2010, 2015). Moreover, β cell insulin content, the expression of glucose transporter GLUT2 and islet area were lower in SMF-exposed group compared to control. Tang et al. (2021) found that moderate-intensity SMFs could cause the abnormalities of glucose metabolism in rats' brain in an intensity-dependent way, which was closely related to anxiety behavior as shown in Fig. 7.8. However, László et al. (2011) showed that daily SMF exposure repeated for several weeks was protective against the development of high blood glucose level in diabetic mice. Li et al. (2020) also reported that moderate intensity of SMFs, 400 mT and 600 mT, had the protective effects on diabetic mice. Yu et al. (2021) further found

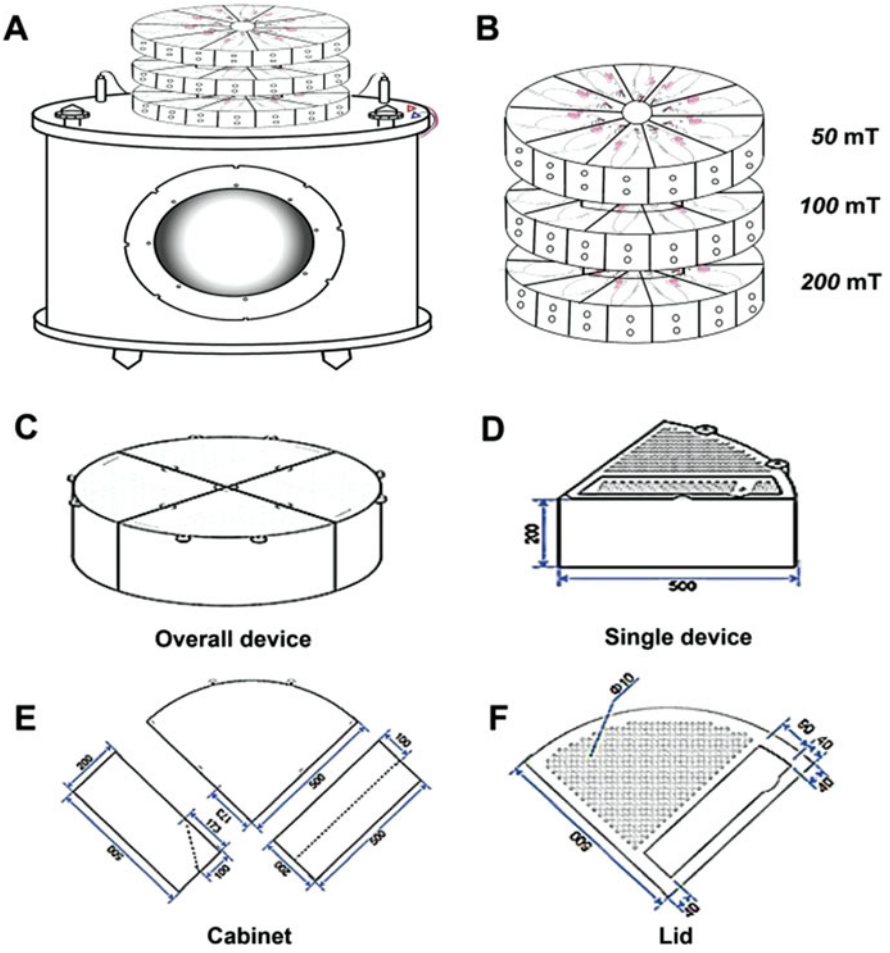

Fig. 7.8 Schematic diagram of static magnetic field exposure. (**a**) Whole body of the rats was exposed using the superconducting magnet exposure source. (**b**) Organic squirrel cages developed by our laboratory according to the MF distribution map of the superconducting magnet. (**c**) Overall device of organic squirrel cage. (**d**) Single device of organic squirrel cage. (**e**) Cabinet of organic squirrel cage. (**f**) Lid of organic squirrel cage. [Reprinted with permission from (Tang et al. 2021)]

that downward 100 mT SMF could reduce the occurrence of hyperglycemia, fatty liver, weight gain, and tissue damage effectively, while upward SMF cannot. Both weak static fields (800 G) for periods between 12 h and 8 days and a 7-Tesla MRI magnet for 45 min had slight effect on nighttime pineal, serum melatonin levels, 5-hydroxytryptamine (5-HT), and 5-hydroxyindole acetic acid (5-HIAA) in exposed rats (Kroeker et al. 1996). Abdelmelek et al. (2006) reported that a SMF of 128 mT induced an increase in norepinephrine content in rat gastrocnemius muscle.

7.4.6.5 SMF on Lymphatic System

Bellossi (1986) showed that the lifetime was prolonged significantly by uniform SMFs of 600 or 800 mT in female AKR mice, which developed spontaneous lymphoblastic leukemia. Yang et al. (2009) observed that SMFs of 200–400 mT prolonged the average lifetime of mice bearing L1210 leukemia cells and increased the spleen and thymus index in normal mice. Milovanovich et al. (2016) reported that a SMF of 128 mT caused a reduction in the amount of lymphocytes in serum and a decrease of granulocytes in the spleen, kidney inflammation, a specific redistribution of pro-inflammatory cells in blood and various organs. De Luka et al. (2016) showed that a SMF of 1 mT reduced the content of zinc in mouse spleen, while copper amount remained unchanged.

7.4.6.6 SMF on Nervous System

The nervous system, including brain, spiral cord, and neurons, is important target of magnetic fields. SMF exposure had a strong modulation effect on cellular hydration in different tissues of rats including brain tissue. Krištofiková et al. (2005) showed functional teratogenic risks of the alterations in the orientation of 140 mT SMF for postnatal brain development and functional specialization of both hippocampi in rats. The whole-body SMF exposure and local SMF exposure on the spine resulted in practically identical ear thicknesses and significant effects of the SMF might involve a lower spinal response to the SMF exposure, and showed that local SMF exposure on the spine affected ear thickness, indicating that the place of local SMF action may be in the lower spinal region (Kiss et al. 2015). Dincic et al. (2018) reported increased synaptosome ATPase activities in rat synaptosomes exposed to 1 mT SMF. Veliks et al. (2004) investigated the influence of 100 mT SMF on autonomic nervous system in rat brain by evaluating heart rate and rhythmicity and found that the effectiveness of SMF in large measure depended on both functional peculiarities and functional activities of brain autonomic centers. Yakir-Blumkin et al. (2020) implanted a small magnetic sheet into the rat skull, which an average magnetic field intensity of 4.3 mT in the subventricular zone (SVZ) and 12.9 mT in the endothelial layer, and found that low-intensity SMF exposure enhanced the proliferation of SVZ cells in young adult rats and DCX-expressing new cells in the neocortical area.

Behavioral effects are essential response of nervous system function. Exposure to 128 mT SMF not only altered emotional behavior of rats in the plus maze and long-term spatial memory, but also led to cognitive impairments or at least to substantial attention disorders in the Morris water maze (Ammari et al. 2008). Saeedi Goraghani et al. (2019) found that simultaneous exposure to 5 mT SMF increased the neurobehavioral effects of MK-801, *N*-methyl D-aspartate (NMDA) receptor blocker, in male Wistar rats. Maaroufi et al. (2013) showed that SMF exposure had no massive effect but affected long-term spatial memory. Weiss et al. (1992)

confirmed that acute behavioral and neural effects on rats became apparent at 4 T in a simple T-maze study. A 30 min exposure of rats to a 9.4 T superconducting magnet induced tight circling locomotor activity, conditioned taste aversion (CTA), and the express of c-Fos in specific vestibular and visceral nuclei within the brainstem (Nolte et al. 1998; Snyder et al. 2000). They extended the studies on the relationship of rat behavior and SMF of 7 or 14 T and found that depressed drinking, more circling, and less rearing actions were observed in SMF-exposed group, while CTA was acquired in a short time, and the direction of circling was dependent on the orientation of SMF to rats as shown in Fig. 7.9 (Houpt et al. 2007, 2012). The behavior response of magnetic field exposure was abolished by chemical labyrinthectomy, suggesting that the vestibular apparatus of the intact inner ear is the locus of magnetic field interaction (Houpt et al. 2007; Cason et al. 2009). Tkac et al. (2021) found that 16.4 T SMF induced long-term impairment of the vestibular system in mice, while 10.5 T SMF exposure had no effect.

Magnetic therapy as a non-contact, non-invasive, and cheap physiotherapeutic method has been used for analgesic modulation. Gyires et al. (2008) reported that acute exposure of mice to 2–754 mT SMF resulted in an opioid-mediated analgesic action in the writhing test in the mouse. Exposure of mice to both inhomogeneous (3–477 mT) and homogeneous (145 mT) SMF generated an analgesic effect toward visceral pain elicited by chemically induced pain (Kiss et al. 2013). Zhu et al. (2017) found that the orofacial pain levels of mice in the environment of 20–204 mT SMF could be reduced and significantly downregulated P2X3 receptors of trigeminal ganglion (TG) in mice during experimental tooth movement.

Using EEG detection, Rivadulla et al. (2018) found that 0.5 T SMF treatment for 1–2 h could reduce epileptiform activity in anesthetized rats and monkeys. Antal and László (2009) found that inhomogeneous subchronic SMF could prohibit the increased sensitivity of mice to mechanical stimuli in neuralgia in mice, which was in consistent with the pain suppression by SMF of clinical magnetic resonance order. With rat model of Huntington disease, the static magnetic field north and south promoted a distinct behavioral profile and morphological preservation after 7 days of lesion with quinolinic acid associated with apomorphine (APO) (Giorgetto et al. 2015). Lv et al. (2022) found that 7 T SMF exposure for 8 h attenuated the depressive state of depressed mice, including reducing the immobility time of the tail suspension test and increasing sucrose preference. Brain tissue analysis showed that 11.1–33.0 T and 7 T SMF can increase oxytocin by 164.65% and 36.03%, respectively, promoting the increase of *c-Fos* level in the hippocampus by 14.79%. However, Sekino et al. (2006) reported that a SMF of 8 T upregulated the action potentials of nerve C fiber, which enhanced pain feeling in rats for the C fiber is functioned as pain transmitter.

7.4.6.7 SMF on Reproduction and Development

The adverse effects of SMF on aspects of spermatogenesis, organogenesis, or even ontogenesis in humans have cause great concern in recent years. Embryonic

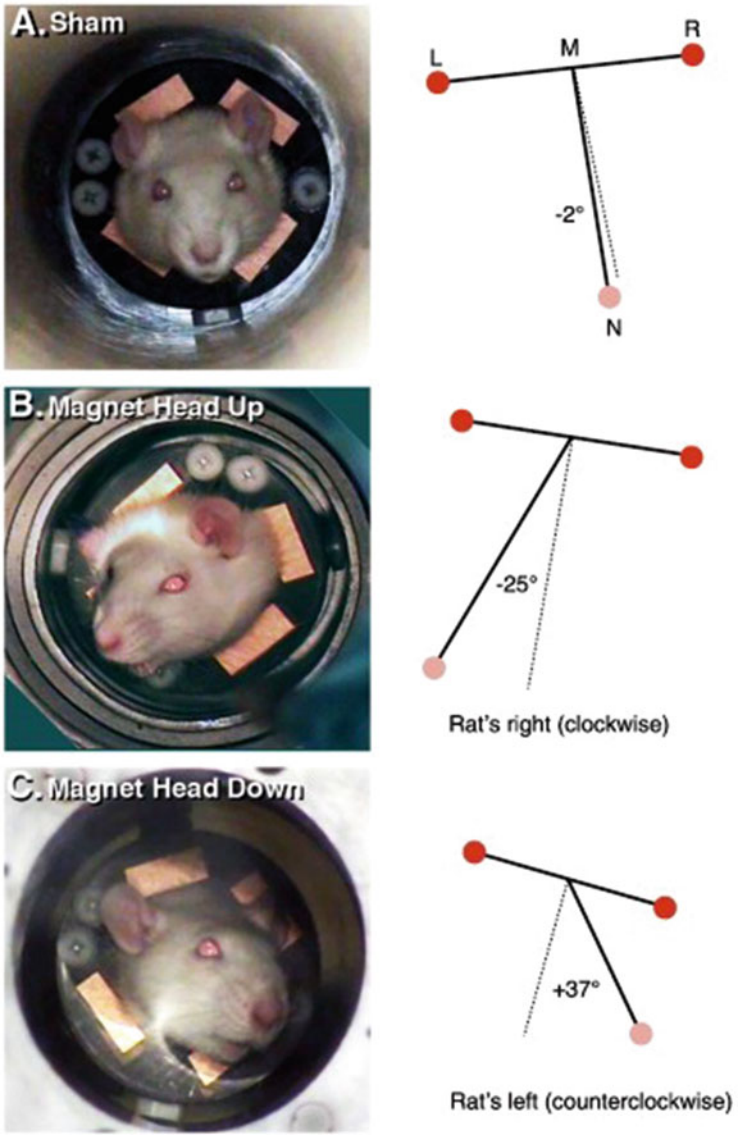

Fig. 7.9 Examples of rats during (**a**) sham exposure, (**b**) 14.1 T magnetic field with head up, and (**c**) 14.1 T magnetic field with head down. Panels on the left are frames from the video recording. Panels on the right demonstrate the quantification of head tilt calculated as the angle from the nose (N) to the midpoint (M) between the position of the left eye (L) and right eye (R). A deviation from the perpendicular toward the rat's right was assigned a negative angle (**a**), while a deviation toward the rat's left was assigned a positive angle (**c**). [Reprinted with permission from (Houpt et al. 2012)]

development is a highly sensitive process to SMFs. Many researchers have explored the biological effects of SMF exposure with different magnetic field intensities and

different exposure methods on mice and their embryos. The exposure modes were mainly intermittent short-term and continuous long-term exposure, and studies found that different steady-state magnetic field parameters and exposure methods have different effects on the organism as shown in Table 7.3.

Table 7.3 SMF on reproduction and development

Species	SMF exposure	Biological effect	References
Mice	1.5 T for 30 min	Slight changes in spermatogenesis and embryogenesis	Narra et al. (1996)
Rat	128 mT, 1 h/day for 30 days	No influences on spermatogenesis in rat testis, the testosterone concentration reduced, and oxidative stress increased	Amara et al. (2006a)
Male and female adult rats	9.4 T SMF for 10 weeks	No adverse biologic effects in male and female adult rats or their progeny	High et al. (2000)
Pregnant mice	7 T SMF 30 min/day for 18.5 days	No any obvious effect on mice' diverse behaviors like locomotion, exploration, spatial learning	Hoyer et al. (2012)
Mice	500–700 mT for single, short-term or continuous, long-term exposure	No significant differences	Tablado et al. (2000)
Mice	20 mT, 30 min/day 3 times/week, 2 weeks	A decrease in sperm count, motility and daily sperm production with marked testicular histopathological changes	Ramadan et al. (2002)
Mice	4.7 T SMF from day 7.5 to 9.5	Had no significant effects on pregnant outbred mice and fetal development	Okazaki et al. (2001)
Mice	2.8–476.7 mT for 40 min/day	The fetal development and the delivery were normal	László and Pórszász (2011)
Rats	30 mT exposure from day 1 to 20	Decrease in the number of live fetuses per litter in rats	Mevissen et al. (1994)
Mouse fetuses	400 mT for 60 min a day	Obvious teratogenic influence on fetal development	Saito et al. (2006)
Mice	1.5 T and 7 T for 75 min/day	Had no adverse effect on duration of pregnancy, litter size, number of live births, or birth weight, and did not lead to teratogenic effects	Zahedi et al. (2014)
Mice	7 T for entire prenatal development	Decreased the embryonic weight and developmental retardation	Zaun et al. (2014)
Mouse	60 mT SMF for 20 min	Increased the cleavage rate of embryos	Baniasadi et al. (2021)

7.4.7 *Magnetic Sensing Protein in Animals*

Many animals have evolved to sense the direction of the geomagnetic field for orientation, navigation, and migration over long distances. The blue light receptor CRYs that could form radical pairs after exposure to blue light were suggested to be a magnetoreceptor based on the proposition that radical pairs were involved in the magnetoreception. CRYs are expressed not only in plant, but also in newts, fruit flies, birds, and the eyes of mammals (Möller et al. 2004; Nießner et al. 2013). Gegear et al. (2008) reported that *cry* mutants of *Drosophila melanogaster* showed neither naive nor a magnetic field, while the wild-type flies showed significant naive and trained responses to the magnetic field. Expression of monarch butterfly (*Danaus plexippus*) cryptochrome gene in *Drosophila cry* mutants rescued the responses to the magnetic field (Gegear et al. 2010). Marley et al. (2014) reported that MF exposure coupled with CRY photoactivation during embryogenesis was sufficient to produce heightened seizure susceptibility in resultant *Drosophila* third instar (L3) larvae. Giachello et al. (2016) provided new evidence that exposure to MF of 100 mT was sufficient to potentiate the ability of light-activated CRY to increase neuronal action potential firing, indicating that the activity of CRY was sensitive to an external MF that was capable of modifying animal behavior. CRYs also function as circadian photoreceptors in the *Drosophila* brain, mediating the light resetting of the 24 h clock, but in vertebrates, the CRYs act as the main negative regulators for the circadian feedback loop, due to the difference in light sensing (Yoshii et al. 2009; Fedele et al. 2014). Non-Drosophila insects encode CRY1 and CRY2, but CRY1 retains their light-sensing properties, whereas the CRY2s act as vertebrate-like negative regulators.

In order to investigate a possible interaction between CRY4 and the iron-sulfur-containing assembly protein (ISCA1) from European robin (*Erithacus rubecula*), CRY4 has recently been proposed to be relevant for magnetic field sensing. Kimø et al. (2018) reported that the ISCA1 complex and CRY4 were capable of binding; however, the peculiarities of this binding argue strongly against ISCA1 as relevant for magnetoreception. In the fruit fly, CRY plays a light-independent role as "assembling" protein in the rhabdomeres of the compound eyes (Schlichting et al. 2018). Schleicher et al. (2017) demonstrated that photo-induced electron transfer reactions in *Drosophila melanogaster* cryptochrome were indeed influenced by magnetic fields of a few millitesla. Günther et al. (2018) sequenced night-migratory European robin (*Erithacus rubecula*) Cry4 from the retina and predicted the currently unresolved structure of the erCry4 protein, which suggested that erCry4 should bind Flavin. They also found that Cry1a, Cry1b, and Cry2 mRNA displayed robust circadian oscillation patterns, whereas Cry4 showed only a weak circadian oscillation. CRYs are sensing magnetic fields in insects as well as in humans. Nohr et al. (2017) presented compelling evidence for an extended electron transfer cascade in the *Drosophila* cryptochrome and identified W394 as a key residue for flavin photoreduction and formation of a spin-correlated radical pair with a sufficient lifetime for high-sensitivity magnetic field sensing. Xu et al. (2021) found that the

photochemistry of cryptochrome 4 (CRY4) from the night-migratory European robin (*Erithacus rubecula*) was magnetically sensitive in vitro, and more so than CRY4 from two non-migratory bird species, chicken (*Gallus gallus*) and pigeon (*Columba livia*). Site-specific mutations of ErCRY4 revealed the roles of four successive flavin-tryptophan radical pairs in generating magnetic field effects and in stabilizing potential signaling states in a way that could enable sensing and signaling functions to be independently optimized in night-migratory birds. Wan et al. (2021) reported that monarchs responded to a reversal of the inclination of the Earth's magnetic field in an UV-A/blue light and CRY1, but not CRY2, dependent manner, and further demonstrated that both antennae and eyes, which expressed CRY1, were magnetosensory organs.

7.5 Conclusion and Perspectives

SMFs are constant fields, which do not change in intensity or direction over time. There are four SMF parameters relevant for the interaction with a biological system: target tissue(s), magnet characteristics, magnet support device, and dosing regimen. Although the interaction of SMFs with living organisms is a rapidly growing field of investigation, many inconsistencies and seemingly contradictory observations exist. These inconsistencies in the literature are linked to the lack of appropriate systematic approaches to isolate the bioeffects of the treatment relative to other factors including geomagnetic field, the use of different exposure systems, different biological model systems, and the lack of uniformity in culture conditions.

With rapid development of superconducting technology, the magnetic flux density of SMFs used for medical and academic research purposes has steadily increased. Exposure to several Tesla (T) or higher from magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) instruments has become common in pursuit of higher resolution and sensitivity, and human and animal studies have been performed at up to 9.4 and 21.1 T, respectively. In the meanwhile, strong SMFs may also be generated by thermonuclear reactors, magnetohydrodynamic systems, and superconducting generators. The facilities equipped with bubble chambers, particle accelerators, superconducting spectrometers, and isotope devices with high magnetic flux density separation units may have areas around these. However, data on living organisms from exposure to strong SMFs have not been sufficient to evaluate these potential ecosystem risks and explore the function of magnetoreception.

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Chapter 8
Static Magnetic Fields on Human Bodies

Xin Zhang

Abstract With the development of modern technologies, people have increased exposure to various types of electromagnetic fields, including static magnetic field (SMF). Accordingly, World Health Organization and international commission on non-ionizing radiation protection have also publish guidelines for the safety application of magnetic fields on human bodies. This chapter summarizes the study results of SMF effect on human bodies, as well as some magnetic field applications in medicine (magnetomedicine). It not only includes some commonly seen SMFs, such as the weak Earth magnetic field that we are all exposed to, but also moderate to ultra-high field generated by magnetic resonance imaging scanners in the hospitals. Magnetic surgery, magnetoencephalography, and magnetocardiogram, which have been used in clinics, are also briefly introduced. SMF-based magnetic therapies are also discussed, which have a long-debated history and still lack of systematic mechanics investigations and sufficient double-blinded, randomized and placebo-controlled human studies. Based on the research progresses in the last few decades, we predict that magnetomedicine will have a great potential in the near future.

Keywords Magnetic field (MF) · Static magnetic field (SMF) · Earth magnetic field · Geomagnetic field (GMF) · Magnetic resonance imaging (MRI) · Magnetic therapy

8.1 Introduction

From a simplified view, the human body is mainly composed of weak diamagnetic materials, including water, most proteins, and lipids. The term diamagnetic means that the substance repels with the externally applied magnetic field (MF). In an

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externally applied magnetic field, the electron motions in diamagnetic molecules make small changes, which generate weak magnetic fields in the opposite direction to the external MFs. Although the diamagnetic properties of most living organisms are very weak, since the repulsive force is proportional to the product of the MF intensity and the field gradient, the forces can be amplified by ultra-strong magnetic field. For example, the most famous case is the "flying frogs" a few decades years ago. People put small diamagnetic objects such as water drops, flowers, grasshoppers, and small frogs in the 16 T ultra-strong static magnetic field (SMF) produced by a vertical electromagnet and levitated those small objects. Theoretically, the human body could also be levitated if we have a vertically oriented, large-sized high-field magnet.

Due to the fast development of technologies, people have increased exposure to different kinds of electromagnetic fields (EMFs) nowadays. Most EMFs are time-varying magnetic fields (also called dynamic magnetic fields), such as 50–60 Hz power line EMFs as well as radiofrequency EMFs emitted by cell phones and microwaves. Therefore, these EMFs have attracted paramount interests. There are many reviews and books about this topic and we will not discuss about the details here. The focus of our book is SMFs, which have non-changing magnetic fields over a certain period of time (0 Hz). The most common SMFs that people are exposed include the weak but ubiquitous Earth magnetic field/geomagnetic field (GMF) (~0.5 Gauss, ~50 μT). In the meantime, people can also be exposed to magnetic resonance in imaging (MRI) scanners in the hospitals (most of them are between 0.5 and 3 T), as well as permanent magnets of various magnetic intensities that some people may use as alternative medicine for some chronic medical conditions such as chronic pain relief, as well as small magnets that are frequently used in household items such as refrigerators, toys, and accessories. Moreover, with the development of ultra-high field MRI machines, people have increasing exposure to high SMFs, which unsurprisingly raised new concerns. Therefore, the effects of SMFs and their effects on human bodies certainly require more research to get a better understanding.

From the safety point of view, since the public are always concerned about various EMFs, (World Health Organization) WHO initiated the International EMF project to assess health and environmental effects of exposure to static and time-varying electric and MFs. More information can be found at the WHO website: https://www.who.int/health-topics/electromagnetic-fields, or the international commission on non-ionizing radiation protection (ICNIRP) website: https://www.icnirp.org/. It should be mentioned that the ICNIRP updates their guidelines for radiofrequency magnetic fields from 100 kHz to 300 GHz (https://www.icnirp.org/en/frequencies/radiofrequency) very frequently, about every 2 years. As for now, Aug 2022, the last updated radiofrequency magnetic fields guideline was in 2020. In contrast, the most updated guideline for SMFs was published in 2009 and has not been updated since then (https://www.icnirp.org/en/frequencies/static-magnetic-fields-0-hz). One of the most important reasons for this is that SMFs are much safer than EMFs.

Table 8.1 Limits of exposure to SMF set by ICNIRP (international commission on non-ionizing radiation protection)

	Exposure characteristics	Magnetic flux density
Occupational[a]	Exposure of head and of trunk	2 T
	Exposure of limbs[b]	8 T
General public[c]	Exposure of any part of the body	400 mT

ICNIRP recommends that these limits should be viewed operationally as spatial peak exposure limits

[a]For specific work applications, exposure up to 8 T can be justified, if the environment is controlled and appropriate work practices are implemented to control movement-induced effects

[b]Not enough information is available on which to base exposure limits beyond 8 T

[c]Because of potential indirect adverse effects, ICNIRP recognizes that practical policies need to be implemented to prevent inadvertent harmful exposure of persons with implanted electronic medical devices and implants containing ferromagnetic material, and dangers from flying objects, which can lead to much lower restriction levels such as 0.5 mT. This table and its annotation are from the ICNIRP guideline for SMF (Ziegelberger and International Commission on Non-Ionizing Radiation Protection 2009)

WHO and ICNIRP have set the upper limit for SMF exposures for both public and occupational exposures. According to the last guideline published by ICNIRP in 2009, the upper limit for the public exposure is 400 mT and occupational exposure is 2 T/8 T (Table 8.1). The limit of exposure for general public of 400 mT was calculated by applying a reduction factor of 5–2 T, which has been proved to have no demonstrated robust effect on animals (Gaffey and Tenforde 1983; Tenforde 2005) or humans. The exposure of SMFs above 8 T requires approval of the research protocol by an Institutional Review Board as well as the informed consent of the subjects.

Although there are also some countries that have a stricter standard, such as Bahrain, Republic of Korea, and Iran, the ICNIRP guideline published in 2019 is still the basis for most countries to set their standards, especially for the occupational exposure, as shown on the WHO website (Table 8.2).

8.2 Earth Magnetic Field/Geomagnetic Field (GMF)

As mentioned above, the most common SMF that all people are exposed to is the Earth magnetic field/GMF, which is around 0.5 Gauss/50 μT (0.3–0.6 Gauss, depending on locations). GMF is much weaker compared to other types of SMF exposure but it is present everywhere and is exceptionally important to the living organism on Earth. It is now known that the Earth can create a region around the planet, called the magnetosphere. It is believed that planets without an intact global magnetic field are subject to atmospheric stripping by the solar wind. For example, people think that Mars does not have a global magnetic field so that the solar wind has contributed to the loss of water and the erosion of Mars' atmosphere. In contrast, the Earth has its magnetic field (magnetosphere), which protects our whole planet

Table 8.2 Exposure limits of SMF in different countries

Country	Magnetic flux density	
	Public	Workers
Bahrain	40 mT	0.2 T
Republic of Korea		
Iran		0.2 T/2 T/5 T
Denmark		2 T
Hungary		
Israel		
Switzerland		
Austria		2 T/8 T
Cyprus		
Greece		
Finland		
Sweden		
United Kingdom of Great Britain and Northern Ireland		
Netherlands	400 mT/0.5 mT	
Croatia	400 mT	2 T
Singapore		
New Zealand		2 T/8 T
Norway		
Germany	400 mT/500 mT	
Argentina	N/A	2 T/60 mT
Belgium		2 T/8 T
Bulgaria		
France		
Ireland		
Italy		
USA		

Information is from WHO website, which was last updated on June 20, 2018. For more detailed information, please check out at: https://www.who.int/data/gho/data/indicators/indicator-details/GHO/magnetic-flux-density-(microt)
N/A not applicable

from harmful solar and cosmic particle radiation, as well as erosion of the atmosphere by the solar wind (Fig. 8.1). More information about the magnetosphere can be found at the NASA website (https://www.nasa.gov/magnetosphere).

It is well known that birds, bees, turtles, and some other animals are shown to sense GMF for direction during migration and there are many studies about GMF and animal magnetoception. There are also some other animal behaviors that were reported to be correlated to GMF. For example, people found some interesting but enigmatic phenomena that dogs like to align their bodies along the Earth magnetic field when they excrete (defecation and urination) (Hart et al. 2013). More information about the SMF effects on microorganisms, plants, and animals will be discussed

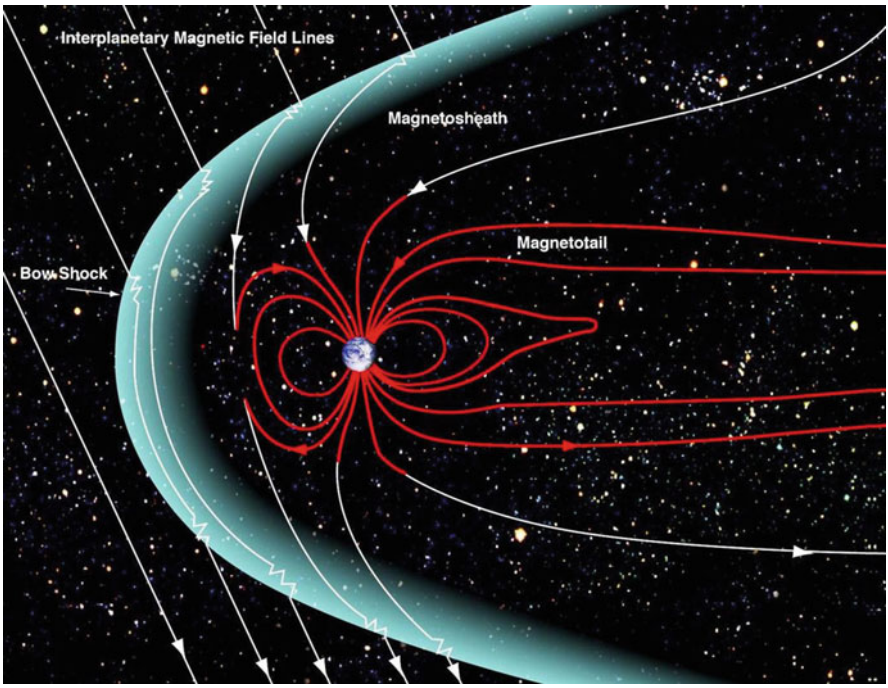

Fig. 8.1 Earth's magnetosphere. The shape of the Earth's magnetosphere is directly affected by solar wind (the sun is on the left). The image was from the NASA website (https://www.nasa.gov/mission_pages/sunearth/multimedia/magnetosphere.html). [Credit: NASA/Goddard/Aaron Kaase. Therefore, for a longer period of time, the Earth magnetic field/GMF is not strictly static, or as static as permanent magnets]

in Chaps. 7 and 13. Although the progress in this particular field is vast in the past few years, more efforts are still needed to unravel the exact and detailed mechanisms to explain various animal behaviors in SMFs, especially the weak GMF.

Whether humans can sense GMF has always been debated. It is interesting that there are a few new studies in recent few years indicating that humans can sense Earth MF (Chae et al. 2019, 2022; Wang et al. 2019). In 2019, Wang et al. reported that the Earth-strength MFs can produce strong, specific, and repeatable effects on human brainwave activity in the electroencephalography (EEG) alpha-band (8–13 Hz), and they propose the mechanism to be related to ferromagnetic transduction element, such as biologically precipitated crystals of magnetite (Fe_3O_4) (Wang et al. 2019). On the other hand, Chae et al. also studied human magnetoreception and stated that starved men have better magnetoreception ability than women (Chae et al. 2019). Recently, they indicated that a magnetic field resonance mechanism mediates light-dependent magnetic orientation in men (Chae et al. 2022). Apparently, this field still remains blurred and we are still far away from understanding the nature of it.

In the meantime, there are multiple studies indicating that GMF could affect other aspects of human. For example, Thoss and Bartsch indicated that the GMF could actually affect human visual system (Thoss and Bartsch 2003, 2007) although the mechanism is not completely understood. Burch et al. indicated that the GMF can affect melatonin secretion (Burch et al. 2008), which is a possible mechanism for the neurological and cardiovascular effects of altered GMF. In addition, Lipnicki et al. showed that there may even be some association between GMF activity with dream bizarreness (Lipnicki 2009). However, there are also some reports that reported negative results. For example, in 2002, Sastre et al. examined the effects of controlled changes in the GMF on 50 human volunteers for electroencephalogram (EEG) and did not find any obvious correlation (Sastre et al. 2002). Since different aspects were measured in these individual studies, they are not exactly comparable.

On the other hand, there are also some evidences showing that in the absence of GMF, frequently referred to hypomagnetic field (HMF), the gene expression, cell proliferation, migration, and adhesion of some human cancer cells could all be affected (Martino and Castello 2011; Mo et al. 2013, 2014, 2016). For example, Mo et al. did multiple studies about the effects of HMF on human SH-SY5Y neuroblastoma cells. In 2013, they showed that continuous HMF exposure significantly increases the proliferation of human SH-SY5Y neuroblastoma cells by promoting cell cycle progression (Mo et al. 2013); in 2014, they compared the transcriptome profiles of SH-SY5Y cells exposed to either the HMF or the GMF and found multiple genes are differentially expressed, including MAPK1 and CRY2 (Mo et al. 2014). In 2016, they found that in HMF, SH-SY5Y cells have reduced F-actin cytoskeleton as well as reduced adhesion and migration (Mo et al. 2016). In addition, HMF was also found to reduce the reactive oxygen species (ROS) level in human pancreatic AsPC-1 cancer cell line and bovine pulmonary artery endothelial cells (PAEC) (Martino and Castello 2011), which is consistent with some studies reporting that SMFs could increase ROS in some cancer cells. In addition, they also did some studies in *Xenopus laevis* (African clawed frog) and found that HMF could cause a decrease in horizontal third cleavage furrows and abnormal morphogenesis in Xenopus embryos (Mo et al. 2012). Their results indicate that a 2-h brief exposure to HMF is sufficient to interfere with the development of Xenopus embryos at cleavage stages. Although this study was done in frogs, the impact of HMF on mitotic spindle and cell division could also be potentially comparable in other organisms, including humans.

In fact, to make things even more complicated, we need to keep in mind that the GMF is not strictly static. It is part of a dynamic, interconnected system that responds to solar, planetary, and interstellar conditions. Therefore, it is not surprising that the GMF around us would have slight fluctuations during day vs. night, winter vs. summer, and also depend on whether there are sporadically occurred solar winds. In fact, it has been reported that the GMF disturbances and/or solar radiation are correlated with suicide/depression in Japan, Taiwan, Finland, and Australia (Partonen et al. 2004; Berk et al. 2006; Tada et al. 2014; Nishimura et al. 2020). Therefore, no matter whether or not humans can sense the GMF for direction like some migrating or homing animals do, current available evidences indicate that

our bodies are indeed affected, or more accurately, protected by the Earth magnetic field. More investigations are encouraged to get a more comprehensive understanding on this topic.

8.3 Time-Varying Magnetic Fields and Their Clinical Applications

Although the focus of this book and this chapter are SMFs, here I want to briefly introduce the time-varying magnetic fields and their clinical applications because their successful development in clinics may shed light on the future progress of SMFs in clinics.

8.3.1 Magnetoencephalography and Magnetocardiogram

As mentioned in the beginning of this chapter, the human body is mainly composed of weak diamagnetic materials, such as water, proteins, and lipids. However, our bodies also generate currents that produce small magnetic fields (Cohen et al. 1980). Neurons in our brain, nerve cells, and muscle fibers are all excitable cells that can generate currents when they are activated. Consequently, relevant instruments were also developed to measure these electric activities. For example, electrocardiogram (ECG) measures the electrical activity of the heart, and electroencephalogram (EEG) measures the electrical activity of the brain, both of which have been widely used in clinic.

Magnetic fields produced by the human body have been measured, which are actually very weak (10^{-10} to 10^{-5} gauss). It is well accepted that the human brain can be divided into multiple areas, and each of them is responsible for different aspects of behavior. The accurate and efficient connectivity between these areas is critical for normal function of a healthy brain. Although a single neuron could only produce very weak current, it can be amplified when the neurons are clustered and aligned together and excited simultaneously. In this case, the neurons can produce magnetic fields that are strong enough to be detected using superconducting quantum interference devices (SQUIDs) (Zimmerman et al. 1970; Hamalainen et al. 1993). Weak alternating magnetic fields outside the human scalp, produced by alpha-rhythm currents, were demonstrated. The fields near the scalp are about 1×10^{-9} gauss (peak to peak) (Cohen 1968). Magnetoencephalography (MEG) is a noninvasive sophisticated technique that captures the magnetic fields generated by synchronized intraneuronal electrical activity, which yields rich information on the spatial, spectral, and temporal signatures of human brain function. It is capable of imaging electrophysiological brain activity with good (~5 mm) spatial resolution and excellent (~1 ms) temporal resolution and provides significant value in

elucidating the neural dynamics of the human connectome in health and disease (O'Neill et al. 2015). There are many very useful reviews and research articles for MEGs showing that neuroimaging methods like MEG represent an outstanding approach to better understand the mechanisms of both normal and abnormal brain functions (Brookes et al. 2011; He et al. 2011; Pizzella et al. 2014; Kida et al. 2015; O'Neill et al. 2015; Pang and Snead 2016; Stefan and Trinka 2017; Baillet 2017). Similarly, magnetocardiogram (MCG) measures the magnetic fields of the heart, which is a complementary or alternative tool for noninvasive detection of coronary artery disease (Kandori et al. 2010; Wu et al. 2013).

In addition, MEG appears to be more sensitive than EEG and can provide additional and different information compared to EEG (Cohen 1972). MEG is useful for functional neurosurgery and connectivity analyses. Since MEG could offer additional insights not possible by MRI when used to study complex network function, people are combining MEG (which has high temporal resolution) with functional MRI (fMRI), which has high spatial resolution, to provide more information on human brain function (Hall et al. 2014). In particular, MEG is most widely applied to the study of epilepsy, a brain disorder that causes people to have seizures (Kim et al. 2016; Pang and Snead 2016). In addition, simultaneous MEG/EEG recording and analysis could provide complimentary information and better detection sensitivity for tracing primary epileptic activity (Hunold et al. 2016; Stefan and Trinka 2017). Moreover, for chronic neurological disorders such as epilepsy, functional connectivity detected through hemodynamic and electromagnetic techniques help to identify the interactions between epileptic activity and physiological networks at different scales. fMRI and EEG/MEG functional connectivity can help in localizing important drivers of epileptic activity and can also help in predicting postsurgical outcome (Pittau and Vulliemoz 2015). In recent few years, with the help of quantum sensors, people are able to develop MEG into a helmet-like wearable device, which does not rely on superconducting technology and allows the free and natural movement of the subjects or patients during scanning (Boto et al. 2018).

8.3.2 Transcranial Magnetic Stimulation

First of all, the magnetic fields in transcranial magnetic stimulation (TMS) are pulsed magnetic fields, but not static magnetic field. TMS is an electromagnetic method that uses a “coil” placed near the head to stimulate small regions of the brain and is used to diagnose or treat multiple diseases such as stroke and depression. It is the best-known magnetic field-related therapeutical instrument that are applied in clinics world widely. In fact, TMS is currently covered by some health insurance in the United States to treat diseases like depression. Some of their applications may be inspirational for people to study SMFs, especially for their applications in the nervous system. There are many reviews that are helpful for people to get more information on this topic (Hallett 2007; Rossi et al. 2009; Pitcher et al. 2021).

8.4 Static Magnetic Fields and Their Clinical Applications

Besides the weak GMF of ~50 μT, nowadays people have more chances to get exposed to much stronger SMFs. On the one hand, MRI scanners are used in the hospitals all over the world, which is the best application of high magnetic field in human health. On the other hand, there are also some SMF-based magnetotherapy products that are available in many countries and mostly used by people by themselves, which will be discussed in more detail by Dr. Kevin Yarema in Chap. 15 of this book.

8.4.1 Magnetic Resonance Imaging (MRI)

MRI has a superior soft-tissue contrast compared to other radiological imaging methods, which makes it a powerful tool in many physiological and functional applications. Currently, the SMF of most MRI scanners in hospitals is 0.5–3 T, which is around 10,000–60,000 times higher than the GMF. This is exceptionally stronger than the GMF or other permanent magnets that people can easily get access to. However, MRI is considered to be a very safe diagnosis technique, as long as the operation follows the basic guidelines. For example, people with pacemakers should not use MRI because the pacemakers may be reprogrammed or turned off by the MFs of MRI. People with some other implants, such as ferrous intra-cranial vascular clips, should also avoid MRI because the strong SMF of MRI may cause possible movement of the implants. Cell phones and credit cards may be damaged by the MFs so that they should also be kept out of the MRI room. It is well recognized that for the regular exposure to the MRI, there are some commonly experienced symptoms including nausea and headaches, which are all reversible (Heilmaier et al. 2011). This will be discussed in more details in Chap. 13 of this book.

In the meantime, since high SMF field can help providing enhanced sensitivity, higher resolution as well as decreased acquisition time, MRI machines with higher magnetic field strength are already developed. For example, the 7 T MRI can obviously provide much more information than the 3 T or 1.5 T MRIs (Fig. 8.2). In the meanwhile, people are continuously investigating on building MRI machines with ultra-high magnetic fields. Beside the clinical studies on 9.4 T MRIs, the 10.5 T MRI was also tested on humans (Grant et al. 2020). This pilot study found that the subjects' cognitive performance was not compromised at isocenter while their eye movements increased. In addition, they experienced small changes in vital signs but no field-induced increase in blood pressure. None of the effects was identified as compromising subject safety. In the meantime, animal studies have been carried out on much higher field MRIs. For example, in 2010, Schepkin et al. tested mouse and rat brains using a 21.1 T MRI, the highest field MRI to date, at the National High Magnetic Field Laboratory (NHMFL) in the United States. They were able to achieve imaging resolution of 50 μM, which is much higher than the lower field

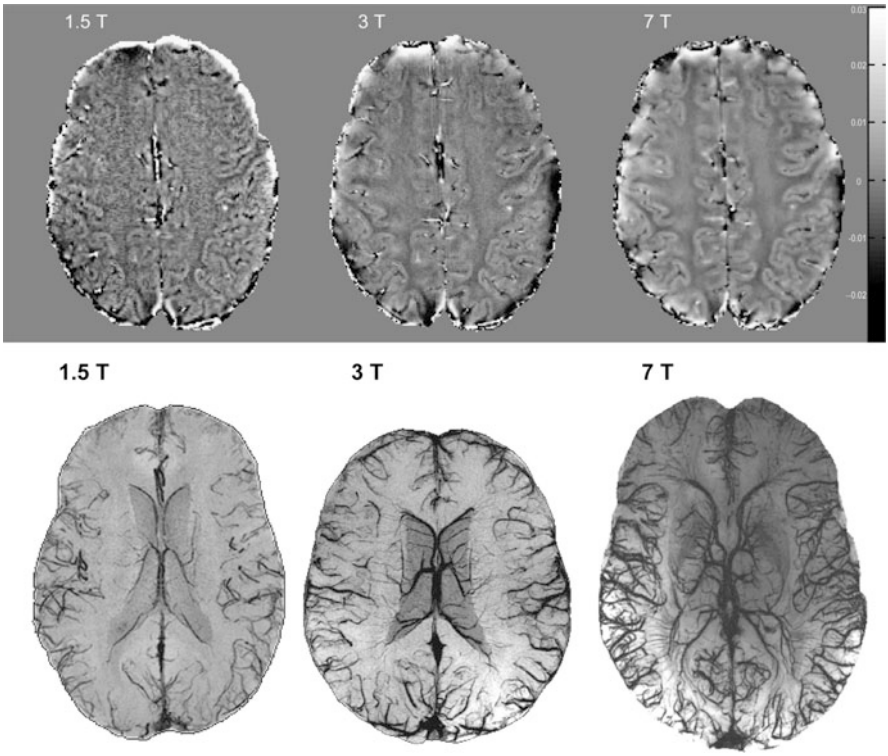

Fig. 8.2 Higher field MRIs have improved resolution. Up: Phase images at 1.5 T, 3 T, and 7 T normalized by field strength and echo time with an isotropic resolution of 0.8 mm. Reprinted with permission from (Zhong et al. 2008). Bottom: Three SWI minimum intensity projections (mIPs) at 1.5 T, 3 T, and 7 T with resolutions of 0.7 × 0.7 × 1.0 mm^3, 0.5 × 0.5 × 1.0 mm^3, and 0.5 × 0.5 × 0.5 mm^3 (Monti et al. 2017). [Reprinted with permission from (Ladd et al. 2018)]

MRIs. In addition, they also compared 21.1 T MRI to 9.4 MRI and found that the 21.1 T MRI can provide much more detailed features about the tissues and blood vessels in the rodent brain (Schepkin et al. 2010). This showed the promising future of developing similar MRI for human.

Since our knowledge of the biological effects of SMFs will guide us for future increase in field strength for MRI to benefit medical diagnosis, more studies are definitely needed to investigate the biological effects of ultra-high SMFs, which are necessary for the future application of ultra-high field MRI machines on humans. In recent few years, there are multiple studies that were performed on this purpose. For example, in 2021, Wang et al. reported a study to address the effects of 28-day long-term exposure to high SMFs of up to 12 T on healthy male C57BL/6 mice (Wang et al. 2021). They found some alterations in the Mg, Fe, Zn, Ca, and Cu content in mice, but did not reveal any detrimental effects. In addition, our group has performed a series of animal studies to investigate the safety issues of SMFs above 20 T (Tian et al. 2018, 2019, 2021; Lv et al. 2022; Khan et al. 2022). In 2018, we first reported a

pilot study of 3.7–24.5 T SMFs 9-h exposure on tumor-bearing nude mice and found overall good biosafety on except for some moderate liver impairment (Tian et al. 2018). Then we reduced the exposure time to 1–2 h and used healthy C57BL/6 mice for our next few studies. We found that 3.5–23.0 T SMF exposure for 2 h did not show obvious harmful effects on healthy mice, including food and water consumption, blood glucose levels, blood routine, blood biochemistry, as well as organ weight and HE stains (Tian et al. 2019). In a later study, we further increased the field to 33.0 T and reduced the exposure time to 1 h, which is closer to the clinical MRI exposure time, and did not show significant changes for most physiological indicators in the healthy C57BL/6 mice (Tian et al. 2021). In addition, behavior tests were also performed to examine the potential neurological effects of 3.5–33.0 T SMF treatment for 1–2 h on healthy C57BL/6 mice. Surprisingly, we found that this high-field SMF treatment could improve the mental state and spatial memory of these mice (Lv et al. 2022; Khan et al. 2022), which was further confirmed by physiological and behavior tests with CUS (chronic unpredictable stress) depression mice that were treated with 7 T SMF for 8 h (Lv et al. 2022). These preliminary studies not only provide useful safety information for the development of ultra-high MRI, but may also indicate that high SMFs have the potential to be developed as anti-depression treatment modalities in the future.

It should be noted that although current MRI machines in the hospitals are considered to be safe, the long-term consequences of repeated exposure and their potential beneficial effects on human bodies are still incomplete identified. In addition, obvious advantages of ultra-high field MRI machines encourage people to design ultra-high MRIs for technical benefits. This also calls for attention for necessary studies for the accompanied safety issues. More efforts are needed to help establish guidelines for occupational staff and patient exposures to higher field SMFs.

8.4.2 Magnetic Surgery

As early as in 1957, Equen et al. have reported the retrieval of foreign bodies in the esophagus, stomach, and duodenum by using magnets (Equen et al. 1957). However, the application of magnets in clinics were not much progressed, until in the past two decades, an increased amount of interests and progresses were made, especially in the GI (gastrointestinal) tract (Cantillon-Murphy et al. 2015). For now, magnetic surgery, which is to apply magnetic fields in surgical procedures, has been developed into multiple surgical areas, especially in gastrointestinal surgery, which provides a minimally invasive surgery choice that benefits various procedures (Diaz et al. 2019). Doctors in the field of magnetic surgery have reached some consensus, aiming to reduce surgical trauma, improve the exposure of the surgical field and the surgical operability (Lv et al. 2019; Bai et al. 2022).

For now, most magnetic surgery can also be called as magnet-assisted surgery, which uses permanent magnets to perform minimally invasive surgery (Fig. 8.3).

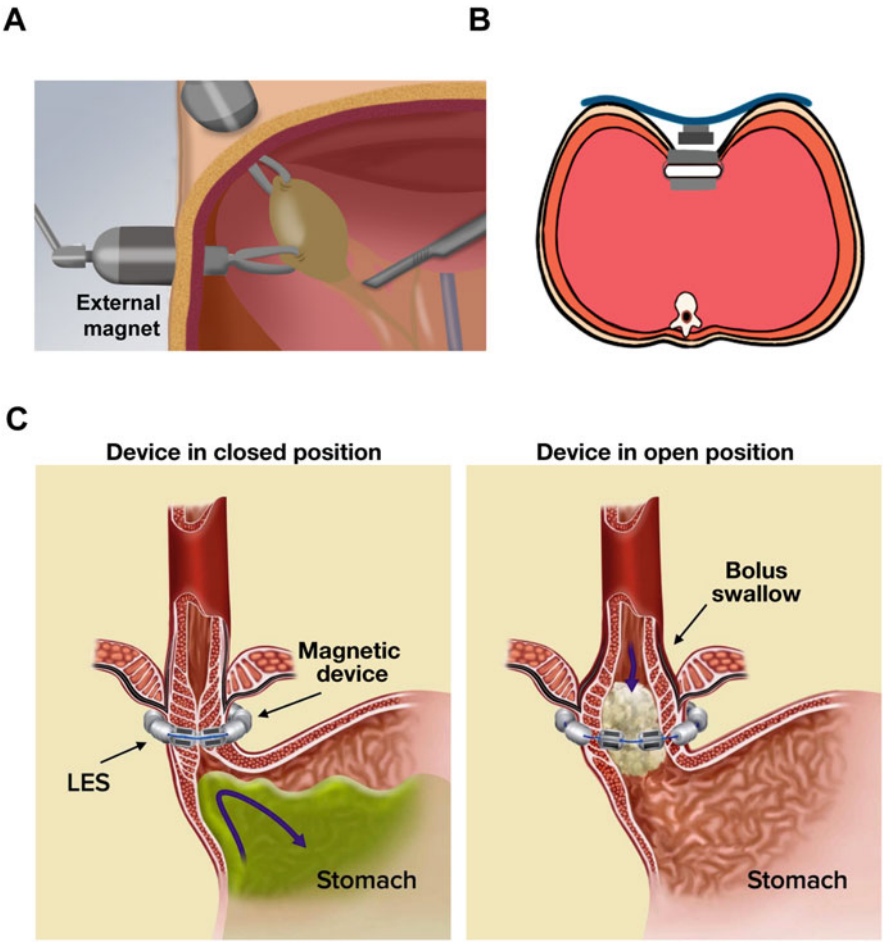

Fig. 8.3 Two types of magnetic surgery, one temporally uses magnets during the surgical procedure, and one places magnets in the human bodies for years. (**a**) Temporarily used magnets during the surgical procedures to provide better anchorage. (**b**) A novel minimally invasive magnetic procedure used to correct pectus excavatum. Both illustrations courtesy of Ding Joe Wang. (**c**) The magnetic sphincter augmentation for the treatment of gastroesophageal reflux disease, in which the magnetic rings can be placed in the patient's bodies for years. [Reprinted with permission from (Ganz et al. 2016)]

Magnets have been used for tissue retraction, anchoring, mobilization, and anastomosis. It should be noted that the progresses of magnetic surgery in the last few decades were mainly boosted by the development of magnetic materials, especially neodymium magnet, which can provide strong enough magnetic force to enable the doctors to design various novel surgical procedures. For example, there is already a magnetic surgical system that has been approved by Food and Drug Administration (FDA), called the Levita™ Magnetic Surgical System, to be used on laparoscopic

cholecystectomy. It has been shown that routine use of this system may facilitate a reduction in the total number of laparoscopic trocars used, leading to less tissue trauma and improved cosmesis (Haskins et al. 2018). There was also a retrospective review of consecutive patients who underwent magnetic-assisted liver retraction during primary or revisional laparoscopic bariatric surgery at the Duke Center for Metabolic and Weight Loss Surgery between October 2016 and August 2017. It is clear that the magnetic-assisted liver retraction is a novel approach that allows a safe, reproducible, incision-less technique for unconstrained, port-less intra-abdominal mobilization, which enhances surgical exposure while decreasing the number of abdominal incisions (Davis et al. 2019). It has also been shown that magnetic liver retraction in bariatric surgery is associated with decreased postoperative pain scores, decreased hospital length of stay, and increased operating supply costs (Welsh et al. 2021).

Besides the magnets that are used temporally during the surgical procedure, there are also cases that the magnets are placed inside the human bodies for long term, to correct pectus excavatum (sunken chest) (Harrison et al. 2007, 2010, 2012; Jamshidi and Harrison 2007; Graves et al. 2017), or gastroesophageal reflux disease (GERD) (Bonavina et al. 2013; Lipham et al. 2015; Ganz et al. 2016). For example, magnetic sphincter augmentation (Fig. 8.3), an FDA-approved procedure that involves placing a magnetic device over the lower end of the esophagus, near the sphincter, has been proved to be an effective and safe surgical method for the treatment of GERD (Bonavina et al. 2013; Lipham et al. 2015; Ganz et al. 2016). Ganz et al. performed a prospective study of the safety and efficacy of a magnetic sphincter augmentation device in 100 adults with GERD for 6 months or more, at 14 centers in the United States and the Netherlands. Eighty-five subjects were followed up for 5 years. They found that augmentation of the lower esophageal sphincter with a magnetic sphincter provides significant and sustained control of reflux, with minimal side effects or complications, which validate the long-term safety and efficacy of the magnetic sphincter augmentation device for patients with GERD (Ganz et al. 2016).

8.4.3 Magnetic Therapy Using SMFs

Looking back into history, magnetic therapy has been debated for thousands of years and there were multiple rounds of up and downs (Basford 2001). It is interesting that the lack of solid scientific explanation for the working mechanism of magnetic field on human bodies does not really prevent people from using magnets at their own wish. Although it is never a mainline medicine, there are still many people currently using magnetic therapy as an alternative and complementary treatment for some chronic diseases, such as arthritis, wound healing, and analgesic therapy (pain relief). Every year, the magnetic therapy products have billions of dollars in sales worldwide. In fact, this is mostly because many people using magnetic therapy do find themselves benefiting from them. For example, there are some magnetic therapy products on amazon.com. Some of these products have thousands of positive

Table 8.3 Moderate static magnetic fields reduced pain level in post-polio patients

Pretreatment and posttreatment pain scores			
	Active magnetic device ($n = 29$)	Inactive device ($n = 21$)	Significance
Pretreatment pain score	9.6 ± 0.7	9.5 ± 0.8	ns
Posttreatment pain score	4.4 ± 3.1	8.4 ± 1.8	$p < 0.0001$
Change in score	5.2 ± 3.2	1.1 ± 1.6	$p < 0.0001$

Proportion of subjects reporting pain improvement by magnetic activity of the treatment device		
	Active magnetic device	Inactive device
Pain improved	$n = 22$ (76%)	$n = 4$ (19%)
Pain not improved	$n = 7$ (24%)	$n = 17$ (81%)

The top table shows that the pain score is efficiently reduced by active magnetic device. The bottom table shows that the % of patients that have effective pain relief is much higher in the active magnetic device group. Both tables were based on results from reference (Vallbona et al. 1997)
NS no significance

comments claiming that they could alleviate the pain and discomfort, especially the magnet bracelets that have some relatively stronger magnets embedded. By browsing the magnetic therapy products on the market, it is not surprising that the magnetic bracelets that received good reviews usually have their magnetic flux densities clearly labeled and most of them are within the range of hundreds to thousands of gausses (0.01–1 T).

Despite the fact that magnetic therapy has a long history, it is still not well accepted by the mainstream medicine. In some cases, it is even considered to be pseudoscience. The doubts are mainly due to the lack of consistency and scientific explanations (as discussed in Chap. 1). There are many efforts that have been devoted to trying to resolve this issue and some of them did provide positive results. For example, in 1997, Vallbona et al. conducted a well-controlled study on 50 post-polio patients and found that the 300–500 Gauss (0.03–0.05 T) SMFs (active magnetic device) significantly reduced the patient pain level from 9.6 to 4.4 ($p < 0.0001$) on a 10-point scale (Vallbona et al. 1997) (Table 8.3, top). It is interesting that the sham-exposure system that maximally mimics the magnetic device (inactive device) also had some placebo effects and reduced the patient pain level from 9.5 to 8.4. However, it is obvious that the pain level change in the SMF-treated group is fivefold more efficient than the placebo-device group (5.2 vs. 1.1, $p < 0.0001$). In addition, 76% of the patients in the active magnetic device group reported much reduced pain while the placebo-device group only have 19% patient (Vallbona et al. 1997) (Table 8.3, bottom). This study was done with proper controls, which provided people with convinced evidences that SMFs could indeed have beneficial effects on pain relief.

Another two scientifical studies in the field of magnetic therapy were performed by Alfano et al. and Juhasz et al. In 2001, Alfano et al. did a randomized, placebo-controlled, 6-month trial conducted from 1997 through 1998 on people with

fibromyalgia (Alfano et al. 2001). In addition to sham controls, they compared a group of people that were exposed to sleep pads with magnets that provided low uniform SMF of negative polarity (Functional Pad A) with a group exposed to sleep pads with magnets that varied both spatially and in polarity (Functional Pad B). In fact, they did find that the Functional Pad A had the most significant effects and both Functional Pad A and B groups showed improvements in functional status, pain intensity level, tender point count, and tender point intensity after 6 months of treatment, but they did not differ significantly from changes in the control groups (Alfano et al. 2001). Therefore, although this study showed that the magnetic sleep pads had the potential to work, the effects were not statistically significant. I think the major reason for the lack of efficiency in their study might be the magnetic field strength, which is too low (below 1 mT). Increasing the magnetic field strength to hundred to thousand gauss might work. However, scientific studies are needed to be done to prove this. Moreover, in 2014, Juhász et al. did a randomized, self- and placebo-controlled, double-blind, pilot study included 16 patients diagnosed with erosive gastritis. They used inhomogeneous SMF-exposure intervention at the lower sternal region over the stomach with peak-to-peak magnetic induction of 3 mT and 30 mT/m gradient at the target site. They did find clinically and statistically significant beneficial effect of the SMF- over sham-exposure on the erosive gastritis symptoms. The average effect of inhibition was 56% ($p = 0.001$). This indicates that inhomogeneous SMF could be a potential alternative or complementary method for erosive gastritis (Juhasz et al. 2014). It is interesting that their magnetic field intensity seems much lower than most other studies that have positive results.

Current evidences show that magnetic field strength is a key issue for potential magnetic therapy applications. Overall, it is believed that magnetic fields with too weak strength are not enough to produce enough energy. As mentioned above, the permanent magnets most people used for magnetic therapy have been proved to be effective ranging from hundreds to thousands of gausses. For example, in 2002, Brown et al. showed that 0.05 T SMF for 4 weeks could reduce chronic pelvic pain in patients (Brown et al. 2002). In 2011, Kovacs-Balint et al. did a research on 15 young healthy human volunteers and found that an inhomogeneous 0.33 T (Bmax) SMF exposure for 30 min could increase the thermal pain threshold (TPT) (Kovacs-Balint et al. 2011). However, it is possible, and very likely, that different symptoms have different requirements for the magnetic field intensity, as well as other magnetic field parameters.

For example, Richmond et al. compared a magnetic wrist strap with (1502–2365 gauss), a demagnetized (<20 gauss) wrist strap, an attenuated (250–350 gauss) magnetic wrist strap, and a copper bracelet. Their results show that wearing a magnetic wrist strap or a copper bracelet did not appear to have any meaningful therapeutic effect, beyond that of a placebo, for alleviating symptoms and combating disease activity in rheumatoid arthritis (Richmond et al. 2013). For now, we are not sure about the reason for this lack of efficacy, however, as mentioned in Chap. 1, magnetic field parameters and multiple other factors have led to the large variations in the clinical or research work about the SMFs. For example, although lacking scientific mechanistic foundations so far, it is interesting that there are multiple

Table 8.4 The north and south magnetic poles are claimed to have different "healing effects" by some magnetic therapy manufactures and therapists

North pole-"negative"	South pole-"positive"
Inhibits relieves pain	Excites increases pain
Reduces inflammation	Increases inflammation
Produces an alkaline effect	Produces an acid effect
Reduces symptoms	Intensifies symptoms
Fights infections	Promotes microorganisms
Supports healing	Inhibits healing
Reduces fluid retention	Increases fluid retention
Increases cellular oxygen	Decreases tissue oxygen
Encourages deep restorative sleep	Stimulates wakefulness
Produces a bright mental effect	Has an over productive effect
Reduces fatty deposits	Encourages fatty deposits
Establishes healing polarity	Polarity of an injury site
Stimulates melatonin production	Stimulates body function
Normalizes natural alkaline pH	

It is still not very clear whether these are real, but different magnetic field directions DO generate some differences. Although from the scientific point of view, there is no explanation for this yet, I do not exclude the possibility that these claims, or at least some of them, might be true. More scientific studies are strongly encouraged to explore this question

claims about the differential effects of the two different magnetic poles on human bodies (Table 8.4). In fact, there are two recent papers observed differential effects of different magnetic field directions (De Luka et al. 2016; Milovanovich et al. 2016). Although more research is strongly needed to confirm their results, I think people should pay attention to the magnetic poles or directions when they investigate the biological effects of magnet fields in the laboratory, or simply want to try some magnetic therapy products.

The differential effects of the magnetic field direction and north/south poles need to be further confirmed by more scientific researches and ultimately to provide clear scientific explanations. For now, I myself are not clear why two different poles can make any differences because there is no physical difference between the north and south pole of the magnet, at least from our current scientific knowledge. However, it is possible that some unknown mechanism indeed exists to explain these observations. Moreover, since it has already been shown that magnet could levitate single cells when the magnetic field is upward to balance the gravity (Durmus et al. 2015), it makes more sense to me if it is the magnetic field direction that makes the differences that people observed. More interestingly, Durmus et al. demonstrated that each cell type (i.e., cancer, blood, bacteria, and yeast) has a characteristic levitation profile, and they have identified unique differences in levitation and density blueprints between breast, esophageal, colorectal, and non-small cell lung cancer cell lines, as well as heterogeneity within these seemingly homogenous cell populations (Durmus et al. 2015). This indicates that various cell types in the human

body might respond totally differently to the magnetic fields. More researches are needed to confirm this.

8.5 Discussion

It is worth to mention that currently many researches related to magnetic therapy as well as the biological effect studies about magnetic fields are not well described or properly controlled. In 2008 and 2009, Colbert et al. wrote two important and comprehensive reviews (Colbert et al. 2008, 2009), which stated that *"Complete descriptions of the SMF dose that was applied to human participants are notably lacking in the majority of SMF therapy studies published to date. Without knowing the SMF dose that was delivered to the target tissue, we cannot draw meaningful inferences from clinical trial results. As research on SMF therapy progresses, engineers, physicists and clinicians need to continue to work together to optimize SMF dosage and treatment parameters for each clinical condition. Future publication of SMF studies should include an explicit assessment of the SMF dosage and treatment parameters outlined in this review, so as to be able to replicate previous studies, validly assess outcomes and make objective, scientific comparisons between studies."* The parameters they outlined include the magnet materials, magnet dimensions, pole configuration, magnetic flux density, frequency of application, duration of application, site of application, magnet support device, target tissue, distance from magnet surface, which all have great potential to directly affect the outcomes (Colbert et al. 2008, 2009) (Table 8.5). Many related researches need replication and we hope we can make great advancement after we have the proper knowledge of the magnetic field and biological systems, which will not only be helpful for WHO to assess any possible health consequences, but also improve the current status of magnetic therapy, which definitely needs much more rigorous experimentation. In fact, FDA has already approved the use of TTF (tumor treating fields), which

Table 8.5 10 essential static magnetic field dosing parameters

	Static magnetic field dosing parameters
1	Target tissue(s)
2	Site of magnet application
3	Distance of magnet surface from target tissue(s)
4	Magnetic field strength
5	Material composition of permanent magnet
6	Magnet dimensions: size, shape, and volume
7	Magnet polar configuration
8	Magnet support device
9	Frequency of magnet application
10	Duration of magnet application

Adapted from reference (Colbert et al. 2008). We recommend that people should all follow these standards when reporting their results

delivers low-intensity, intermediate-frequency (100–300 kHz), alternating electric fields to treat newly diagnosed and recurrent glioblastoma, which works by disrupting cancer cell division, with no significant damage to normal non-dividing cells (Kirson et al. 2004; Pless and Weinberg 2011; Davies et al. 2013). Although TTF is a type of electromagnetic field therapy using low-intensity electrical fields, not SMFs, it may shed light on the SMF investigations for their potential clinical usage.

8.6 Conclusion

Since human body itself is an electromagnetic object, it is not surprising that the magnetic fields can produce some effects on us. However, the electrochemical processes within the human bodies are very complicated and still remain incompletely understood. Therefore, the actual physical effects of magnetic fields on human bodies will still need continuous efforts to achieve a complete understanding. In the meantime, magnetic therapy may be an alternative or complementary method in the clinical use, especially in cases when conventional therapy options are unavailable. In addition, whether the magnetic therapy works does not depend on our understanding for its underlying biological mechanisms. As Dr. Basford said in his review (Basford 2001) *"An electric or magnetic therapy is first discovered by the populace, resisted by the medical establishment, and then discarded—only to arise again in the future in a slightly different form. Although sophistication has increased, this pattern is likely to continue into the future until clear treatment benefits and, one hopes, a convincing mechanism of action are established."* Currently, what we should do is to try our best to unravel the mysteries so that we can maximize the benefit we can get from these nature powers. In the meantime, we should alert people that there are numerous unreliable websites or products about magnetic therapy. We believe that with the increasing efforts to use legitimate and scientifically backed methods in the field of magnetic field research, we will gain more mechanistic insights to facilitate the clinical application of SMFs and make magnetic therapy scientifically respectable.

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Chapter 9
Potential Applications of Static Magnetic Fields in Cancer Treatment

Xin Zhang

Abstract This chapter lists current evidence (from molecular level, cellular level, animal level to patient level) and some potential mechanisms for the effects of static magnetic field on cancer inhibition. The direct impacts of static magnetic fields on cancer cells are summarised, including cancer cell proliferation, division, migration and invasion, as well as cancer cell stemness. Moreover, static magnetic field s can also affect microcirculation and angiogenesis, and regulate immune system to inhibit cancer in vivo. Furthermore, the prospective applications of static magnetic field alone or in combination with chemotherapy drugs, time-varying magnetic fields as well as radiotherapy in cancer treatment are reviewed. The potential mechanisms and factors that contributed to the inconsistencies are also discussed. These evidences demonstrate that static magnetic fields have a great potential to be used as a physical tool to inhibit cancer, but further investigations are still needed to optimize the static magnetic field parameters and exposure procedures, as well as combinational therapy modalities.

Keywords Magnetic field (MF) · Static magnetic field (SMF) · Cancer cell · Alternative treatment · Combined therapy

9.1 Introduction

The advances in tumor treating fields (TTFs) electric therapy, which has been approved by the Food and Drug Administration (FDA) to be used on recurrent and newly diagnosed glioblastoma in 2011 and 2015, respectively, provided a great example to illustrate the advantages of physical modality in cancer treatment. However, although magnetic therapy using SMF has been used by some people as

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alternative treatment on multiple chronic diseases for years, the scientific foundation is still lacking. As we have introduced in previous chapters, many studies have investigated the biological effects of static magnetic fields (SMFs), with results that depended on multiple factors including SMF parameters, biological sample and experimental procedure differences. In particular, the difference in cell types made a significant impact. A large number of reports show that cancer cells and some specific cell types, including stem cells, embryonic or neuronal cells, are more susceptible to SMFs, while most other non-cancer cells are much less affected.

Here we would like to focus on the impacts of SMFs on cancer. It is well known that cancer cells are different from normal cells in various aspects. For example, multiple types of cancers proliferate in response to signalling from oncoproteins such as EGFR (epidermal growth factor receptor) and we found that SMF can affect EGFR orientation to reduce its activity as well as related pathways to inhibit some cancer cell proliferation (Zhang et al. 2015, 2016). Moreover, most cancer cells are at a more active dividing state compared to normal cells. We found that moderate and strong SMFs can interfere with microtubules so that the cell division can be affected (Zhang et al. 2017a). The metastatic behaviours and stemness of cancer cells are also drastically different from non-cancer cells. We recently found that moderate SMF could inhibit ovarian cancer cell migration, invasion and stemness, while having a negligible effect on the non-cancer ovarian cells (Song et al. 2021). However, Zhao et al. reported that the osteosarcoma stem cells metastasis in mice was promoted by moderate SMFs of tilted and gradient direction (Zhao et al. 2021). In addition, the cancer microcirculation/angiogenesis and immune responses in vivo are also different from normal tissues. Here I summarize the SMFs effects on cancer in mice studies (Table 9.1), which indicates that higher field SMF, longer treatment time, and vertically upward direction seem to be positively correlated with the anti-cancer efficacy. For example, Zhu et al. found that 0.6 T SMF treatment for 2–3 months efficiently inhibited cancer growth in transgenic polyoma middle T oncoprotein (PyMT) mice by ~60–70%, but 0.3 T did not have this effect (Zhu et al. 2020). Our group found that for the same SMF flux density, the upward SMFs could inhibit cancer growth in mice while the downward SMFs could not (Tian et al. 2018; Yang et al. 2021). Moreover, for the upward direction 9.4 T SMF treatment, a 200-h treatment can inhibit cancer growth by 62.88% (Tian et al. 2022) while 88-h can inhibit cancer growth by 44.7% (Yang et al. 2021), although we did not compare the same types of cancer side-by-side.

In this chapter, I will first introduce the studies about the direct SMF effects on cancer cells in vitro and in vivo, including cancer cell proliferation, division, migration and invasion, as well as stemness. Then from the in vivo point of view, the contributions of SMF effect on microcirculation/angiogenesis and immune regulation are also discussed, which is followed by the combination of SMFs with other treatments, including chemodrugs, time-varying magnetic fields, etc.

Table 9.1 The cancer inhibition effects of static magnetic fields on mice are related to magnetic flux density, direction, treatment time

Mice	SMF flux density and direction		Treatment time	Effects on cancer	References
Transgenic polyoma middle T oncoprotein (PyMT) mice	0.3 T[a]	Vertically upward	Continuously for 2–3 month		Zhu et al. (2020)
Female BALB/c (nu/nu) mice bearing GIST-T1 tumor	0.4–0.5[b]	Vertically downward	Continuously for 38 days	No effect	Tian et al. (2018)
Male BALB/c (nu/nu) mice bearing A549 tumor	9.4 T[c]		88 h in total (8 h/day, 11 times, every other day)		Yang et al. (2021)
Female BALB/c (nu/nu) mice bearing GIST-T1 tumor	0.4–0.5[b]	Vertically upward	Continuously for 38 days	19.3% Inhibition on tumor growth	Tian et al. (2018)
Female BALB/c (nu/nu) mice injected with ovarian cancer SKOV3 cells	0.5[d]		Continuously for 42 days	~40% Decrease on metastasis	Song et al. (2021)
Male BALB/c (nu/nu) mice bearing A549 tumor	9.4 T[c]		88 h in total (8 h/day, 11 times, every other day)	44.7% Inhibition on tumor growth	Yang et al. (2021)
Transgenic polyoma middle T oncoprotein (PyMT) mice	0.6 T[a]		Continuously for 2–3 month	~60–70% Inhibition on tumor growth	Zhu et al. (2020)
Female BALB/c (nu/nu) mice bearing GIST-T1 tumor	9.4 T[c]		200 h in total (10 h/day, daily)	62.88% Inhibition on tumor growth	Tian et al. (2022)
K7M2 osteosarcoma stem cells injected to the proximal tibia of anesthetized male BALB/c mice	0.2–0.4[e]	Tilted direction	Continuously for 2 weeks	Increase metastasis (not statistically significant)	Zhao et al. (2021)

h hour

[a]Max SMF flux density measured at the surface of small neodymium permanent magnet cubes, horizontally inhomogeneous

[b]Max SMF flux density measured at the surface of big neodymium permanent magnet plate, horizontally homogeneous

[c]Homogeneous SMF provided by superconducting magnet

[d]Inhomogeneous SMF provided by the inside bore of superconducting magnet (off the center)

[e]Inhomogeneous SMF provided by the outside of superconducting magnet

9.2 Direct Effects of Static Magnetic Fields on Cancer Cells In Vitro and In Vivo

9.2.1 Static Magnetic Fields Could Inhibit Some Cancer Cell Proliferation

As introduced in previous chapters, the exact cellular effects of SMFs on cells are largely dependent on cell types so that there is no consensus effect of SMF on various kinds of cells. For example, Sullivan et al. examined the effect of 35–120 mT SMFs on four different types of cells and found that the effects varied greatly among them (Sullivan et al. 2011). However, among different cell types, the cell growth/proliferation inhibition effects of SMF on cancer cells are much more consistent compared to other cell types.

Multiple studies have shown that SMFs could inhibit cancer cell growth while having a minimal effect on non-cancer cells. Although in each individual study, the cell types examined were very limited, we can see a clear trend that SMFs tend to inhibit cancer cells but not non-cancer cells. For example, in 1996, Rayman et al. showed that cell growth of a few cancer cell lines could be inhibited by 7 T SMF (Raylman et al. 1996). Later, a few studies used both cancer and non-cancer cells and found that they respond to the SMFs differentially. For example, in 2003, Aldinucci et al. found that 4.75 T SMF did not affect human peripheral blood mononuclear cells (PBMC) but inhibited Jurkat leukemia cell proliferation (Aldinucci et al. 2003b). In 2006, Ghibelli et al. showed that 1 T SMF could increase the chemotherapy-induced apoptosis in human tumor U937 monocytes but not mononuclear white blood cells (Ghibelli et al. 2006). In 2011, Tatarov et al. tested the effect of 100 mT SMF on mice bearing metastatic mouse breast tumor EpH4-MEK-Bcl2 cells. They found that exposure of the mice to magnetic fields for 3 or 6 h, but not 1 h, daily for as long as 4 weeks suppressed tumor growth (Tatarov et al. 2011). Their study not only indicated that the moderate SMF could inhibit mouse breast cancer growth, but also showed that the inhibition was directly correlated to the SMF exposure time (Tatarov et al. 2011). In 2015, Zafari et al. investigated the effects of SMF (5, 10, 20 and 30 mT) for 24–96 h on the viability of the human cervical cancer HeLa cells and fibroblast cells. They found that the increase of SMF intensity and incubation time increased cell death percent and proliferation rate in HeLa cells more obviously compared to fibroblast cells (Zafari et al. 2015).

There are some mechanistic studies that have explored the differential effects of SMFs on cancer vs. non-cancer cell proliferation. For example, many types of cancer cells proliferate in response to signalling from Receptor Tyrosine Kinases (RTKs), and the effect of magnetic fields (MFs) on EGFR phosphorylation has been investigated in several studies (Jia et al. 2007; Sun et al. 2008, 2013). It was shown that both 0.4 mT 50 Hz low frequency and 2 μT 1.8 GHz radiofrequency time-varying MFs increased EGFR phosphorylation. However, it was very interesting that this effect could be reversed by incoherent (“noise”) MFs of the same MF intensities (Sun et al. 2008, 2013). These results not only demonstrate that EGFR is a molecular

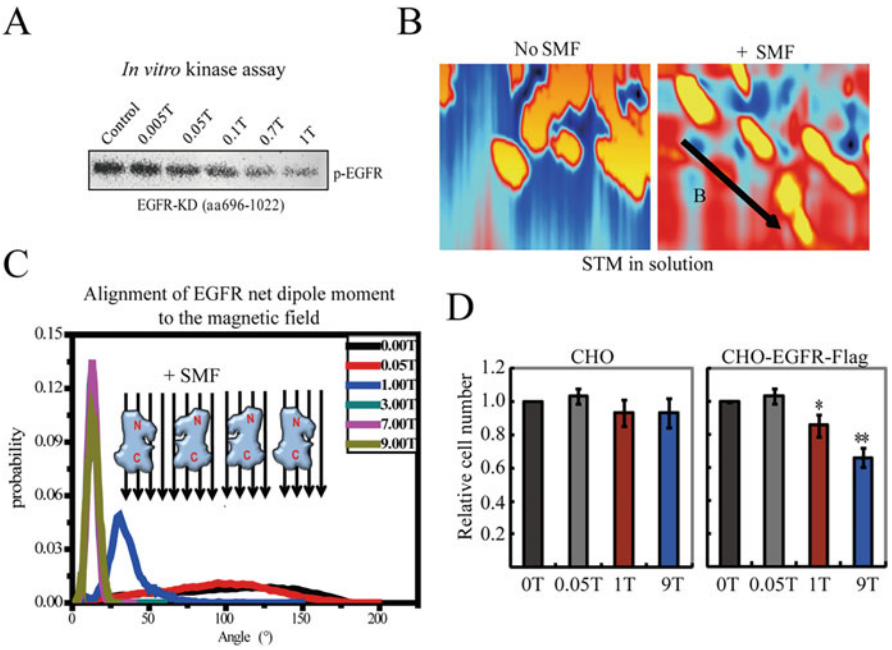

Fig. 9.1 Static magnetic fields inhibit EGFR activity by changing its orientation to inhibit cell proliferation. (**a**) In vitro kinase assays show that moderate SMFs could inhibit EGFR kinase domain autophosphorylation. Western blot of phosphor-EGFR was shown. SMFs of 0.005–1 T were tested. Incubation time was 10 min. (**b**) Liquid-phase scanning tunnelling microscopy (STM) shows that a 0.4 T SMF could change EGFR kinase domain orientation. (**c**) Computer-based calculation shows that the probability of the EGFR kinase domain net dipole moment aligns with SMF field direction in a MF flux density-dependent manner. (**d**) The cell number of CHO cells was not affected by 0.05, 1, or 9 T SMF while the cell number of CHO cells overexpressing EGFR-Flag was significantly reduced by 1 T and 9 T SMFs. Incubation time was 3 days. $^{*}p < 0.05$; $^{**}p < 0.01$. [Figures were adapted from reference (Zhang et al. 2016). Copyright © 2016 Impact Journals, LLC. Open access]

target for MFs, but also show that the different types of MFs have differential effects on EGFR activities. In 2016, our group tested SMF effects on EGFR and found that moderate and strong SMFs could actually inhibit EGFR activity both in vitro and in cells in a MF flux density-dependent way (Zhang et al. 2016) (Fig. 9.1a). We further explored the underlying mechanism using scanning tunnelling microscopy (STM) (Fig. 9.1b) and molecular dynamics (MD) simulation (Fig. 9.1c). We found that SMF could affect the orientation of EGFR kinase domain, which interfered with the normal interaction between EGFR monomers to inhibit their activation. In addition, although the CHO (Chinese hamster ovary) cell number was not affected by 0.05 T, 1 T or 9 T SMFs, EGFR transfected CHO cells became responsive to SMFs and were effectively inhibited by 1 T and 9 T SMFs (Fig. 9.1d). This indicates that EGFR is at least one of the key factors that contribute to SMF-induced cancer cell inhibition.

Table 9.2 Systematic analysis of 15 different cell lines revealed that both cell type and cell density influenced the 1 T SMF induced effects on cells

	Cell line names	Cell line information	Effects of 1 T SMF on cell number	
			High density	Low density
Human solid cancer	CNE-2Z	Nasopharyngeal cancer	Reduction	Increase
	HCT116	Colon cancer		No effect
	A431	Skin cancer		
	A549	Lung cancer		
	MCF7	Breast cancer		Increase
	PC3	Prostate cancer		No effect
	EJ1	Bladder cancer	No effect	Increase
Human non-cancer	HSAEC2-KT	Normal lung	Increase	Increase
	HSAEC30-KT			No effect
	HBEC30-KT			Increase
	RPE1	Retinal pigment epithelial	No effect	No effect
	293T	Embryonic kidney		
Rodent	CHO	Chinese hamster ovary	No effect	No effect
	CHO-EGFR	Chinese hamster ovary, transfected with EGFR-flag	Reduction	Increase
	NIH-3T3	Mouse embryo fibroblast		No effect

7 Human solid cancer cell lines, 5 human non-cancer cell lines as well as 3 rodent cell lines were included. Cells were plated 1 day ahead for attachment to the culture plate before they were exposed to 1 T SMF for another 2 days. 4–5 × 10^5 cells were plated in the "high densities" group so that the cells were confluent at the end of experiments. 0.5 × 10^5 cells were plated in the "low densities" group so that the cells were around half confluent at the end of experiments. Experiments were repeated for 3–4 times by two independent researchers. [Results were from reference (Zhang et al. 2017b).]

As mentioned above, most studies have only tested one or very few cell types, which prevented people from getting a comprehensive view of the cellular effects of SMF on different kinds of cells. Therefore, our group side-by-side compared 15 different cell lines, including 12 human (7 cancer cell lines and 5 non-cancer cell lines) and 3 rodent cell lines for their responses to 1 T inhomogeneous SMF provided by a permanent magnet. We found that SMF not only affect cell proliferation in a cell type-dependent manner, the cell density also played indispensable roles (Table 9.2) (Zhang et al. 2017b). For example, the growth of A549 lung cancer cells was inhibited by 1 T SMF when they were seeded at a high density but the growth of normal lung cells was promoted (Table 9.2).

We further analysed their EGFR-mTOR-Akt pathway and found that the A549 lung cancer and HSAEC2-KT non-cancer lung cells have dramatically different EGFR-mTOR-Akt pathway expression and activation (Fig. 9.2) (Zhang et al. 2017b). The EGFR expression and phosphorylation levels are much higher in A549 lung cancer cells than in HSAEC2-KT normal lung cells. The mTOR and AKT expression and phosphorylation levels are also significantly higher in A549

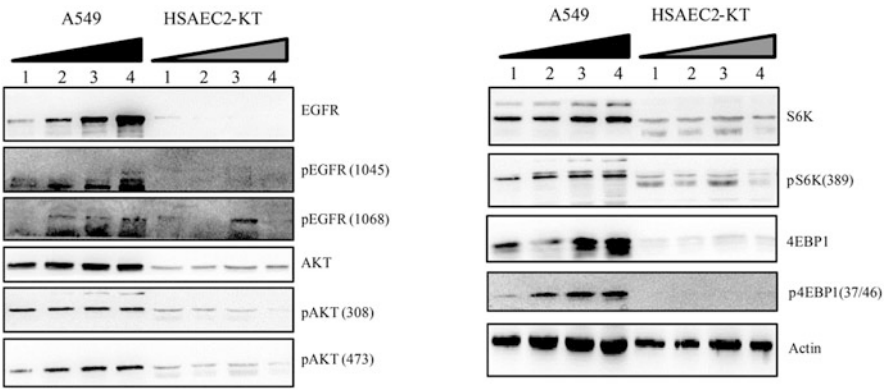

Fig. 9.2 Human lung cancer A549 and normal lung HSAEC2-KT cells have differential EGFR-Akt-mTOR pathway expression and phosphorylation. Human lung cancer A549 and normal lung cells HSAEC2-KT cells were plated at four different cell densities 1 day ahead before they were harvested for Western Blot. "1" indicates the lowest cell density. "4" indicates the highest cell density. [Reprinted from Ref. (Zhang et al. 2017b). Copyright © 2016 Impact Journals, LLC. Open access]

lung cancer cells. These results, combined with the EGFR studies mentioned above, demonstrate that EGFR-mTOR-Akt pathway is likely to be one of the key factors that contribute to the cell type differences in SMF-induced cell proliferation changes. In addition, it should be mentioned that the cell density also affected the A549 lung cancer cells and normal lung cells HSAEC2-KT in different pattern (Fig. 9.2). For example, the EGFR and 4EBP1 expression and phosphorylation level were increased in higher cell density compared to lower cell density in A549 lung cancer cells but not in HSAEC2-KT normal lung cells. These results indicate that EGFR-mTOR-Akt pathway may be a key factor that contributes to both cell type- and cell density-dependent SMF effects.

Besides RTK pathway, the SMF effect on DNA synthesis is also an important step in cell proliferation, which has been introduced in Chap. 6. Using BrdU incorporation assay to measure DNA synthesis rates, we first found that 1 T moderate SMF could inhibit DNA synthesis in colon cancer HCT116 and LoVo, and lung cancer PC9 and A549 cells (Yang et al. 2020), but 0.5 T SMF has no effects on DNA synthesis (Yang et al. 2021). Then we used higher field SMF provided by a superconducting magnet, and found that DNA synthesis was significantly decreased by both upward (14.3%, $p < 0.01$) and downward (18.6%, $p < 0.01$) 9.4 T SMFs after 24 h (Fig. 9.3a). We also used Western blot analysis to examine the level of TOP2α (DNA topoisomerase II Alpha), which functions to bring the higher order compaction of chromatin to form condensed mitotic chromosomes during G2-M transition. Our results show that TOP2α was decreased in both upward and downward 9.4 T SMF-treated cells (Fig. 9.3b). The DNA synthesis inhibition by SMFs is likely due to the DNA supercoil changes through Lorenz forces on the negatively charged DNA in motion. More specifically, we have previously proposed that the

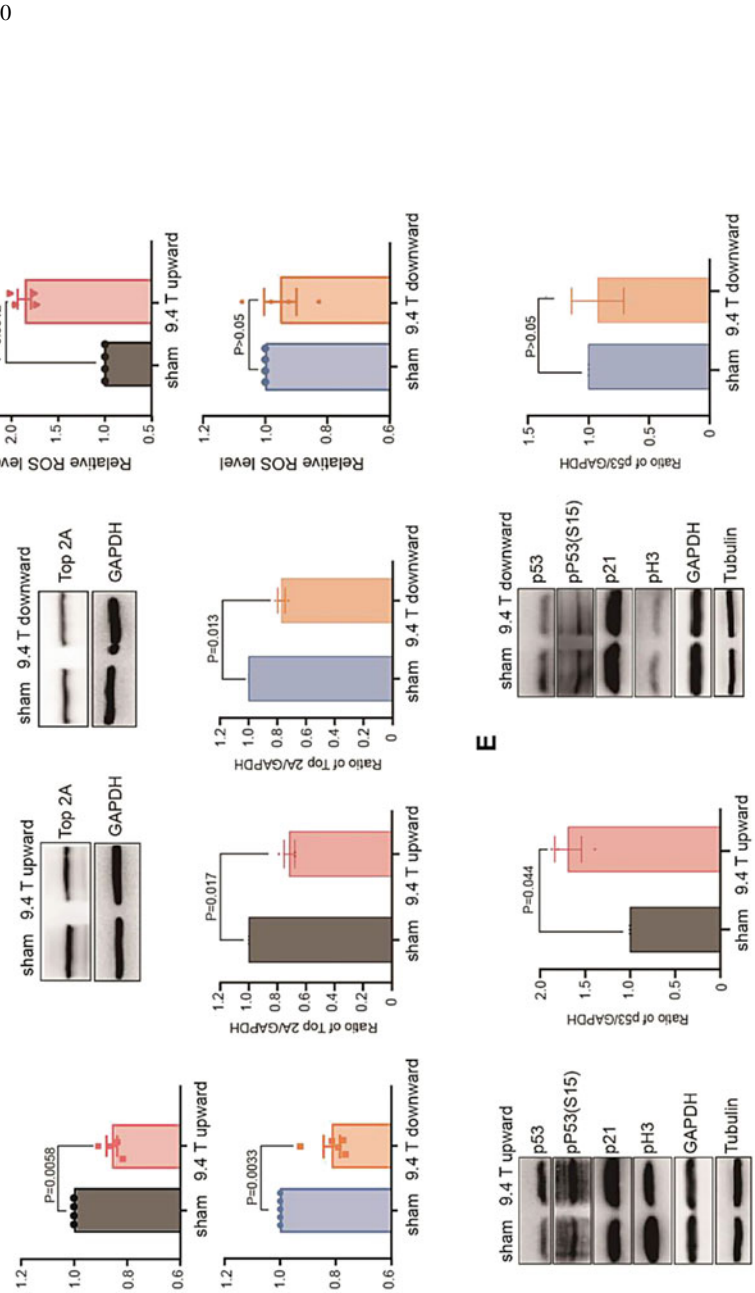

Fig. 9.3 High field 9.4 T SMFs inhibit DNA synthesis and regulate p53 in a SMF direction-dependent manner. (**a**) 9.4 T SMFs obviously inhibited the DNA replication of cells. (**b**) The level of TOP2α treated with 9.4 T SMFs analyzed by Western blots and quantified by ImageJ software. (**c**) Upward 9.4 T SMF significantly increased the ROS levels of A549, but the downward SMF did not. (**d**) Representative Western blots shows the level of phosphorylated P53 (S15) and P53 in the cells exposed with upward 9.4 T SMF were dramatically increased. (**e**) Representative Western blots shows the level of phosphorylated P53 (S15) and P53 in the cells exposed with downward 9.4 T SMF and statistical analysis for P53. [Reprinted from reference (Yang et al. 2021). Open access]

upward SMF could cause tightened DNA supercoils while the downward SMF causes loosen supercoils (Yang et al. 2020). Interestingly, we found that the upward 9.4 T SMF significantly increased reactive oxygen species (ROS) level (Fig. 9.3c), while the downward 9.4 T SMF did not (Fig. 9.3c). It is well known that ROS play central roles in multiple cellular processes, including triggering P53 activation, a key tumor suppressor. In fact, our data showed that the upward 9.4 T SMF could activate and upregulate P53 (Fig. 9.3d), but the downward 9.4 T SMF had no such effect (Fig. 9.3e), which is consistent with the ROS level changes. It is possible that the tightened DNA supercoils caused by Lorenz forces in upward 9.4 T SMF is a key step to boost ROS level, which consequently activates P53 and further inhibits DNA replication and cell proliferation.

To further confirm the results we got in vitro, we examined the tumor tissues of the mice treated with or without 9.4 T SMF for the tumor suppressor P53 and the proliferation marker Ki-67. It is obvious that the P53 level was significantly increased by the upward 9.4 T SMF, but not downward 9.4 T SMF (Fig. 9.4a, b). Moreover, the Ki-67 level was significantly decreased by the upward 9.4 T SMF, but not much by the downward 9.4 T SMF. These are consistent with our findings that 9.4 T upward SMF could inhibit A549 lung cancer cell growth both in vitro and in vivo. Therefore, although both the upward and downward 9.4 T SMF could inhibit DNA synthesis in vitro, only the upward 9.4 T SMF significantly increased ROS and P53 levels, decreased mitotic index and caused G2 cell arrest, which collectively lead to tumor growth inhibition in tumor bearing mice (Fig. 9.4c).

However, it should be mentioned that there are also a few studies showing that SMFs could promote cancer cell proliferation. For example, we previously found that moderate SMFs can inhibit cancer cell proliferation when they are plated at high density, but can also increase some cancer cell numbers when they are plated at low density (Table 9.2) (Zhang et al. 2017b). It is a pity that we were not aware of the importance in SMF direction in this study at that time, so the SMF direction information was missing. In addition, Fan et al. show that ~150 mT SMF treatment accelerated 4 T1 breast cancer cell proliferation. However, they also showed that SMF treatment shortened the telomere length, decreased telomerase activity, and inhibited the expression of the cancer-specific marker telomerase reverse transcriptase (TERT) (Fan et al. 2020). However, the SMF direction and cell density information are both missing, so we cannot exclude the possibility of the direction- and cell plating density-induced effects. More research is needed to test various SMF conditions and cancer cells to get more complete information.

9.2.2 Static Magnetic Fields and Cancer Cell Division

Besides cell proliferation, there are other cellular components that play indispensable roles in SMF-induced cancer inhibition, such as cell division. Since cell division is a key step that leads to tumor growth, perturbations that disrupt or interfere with cell

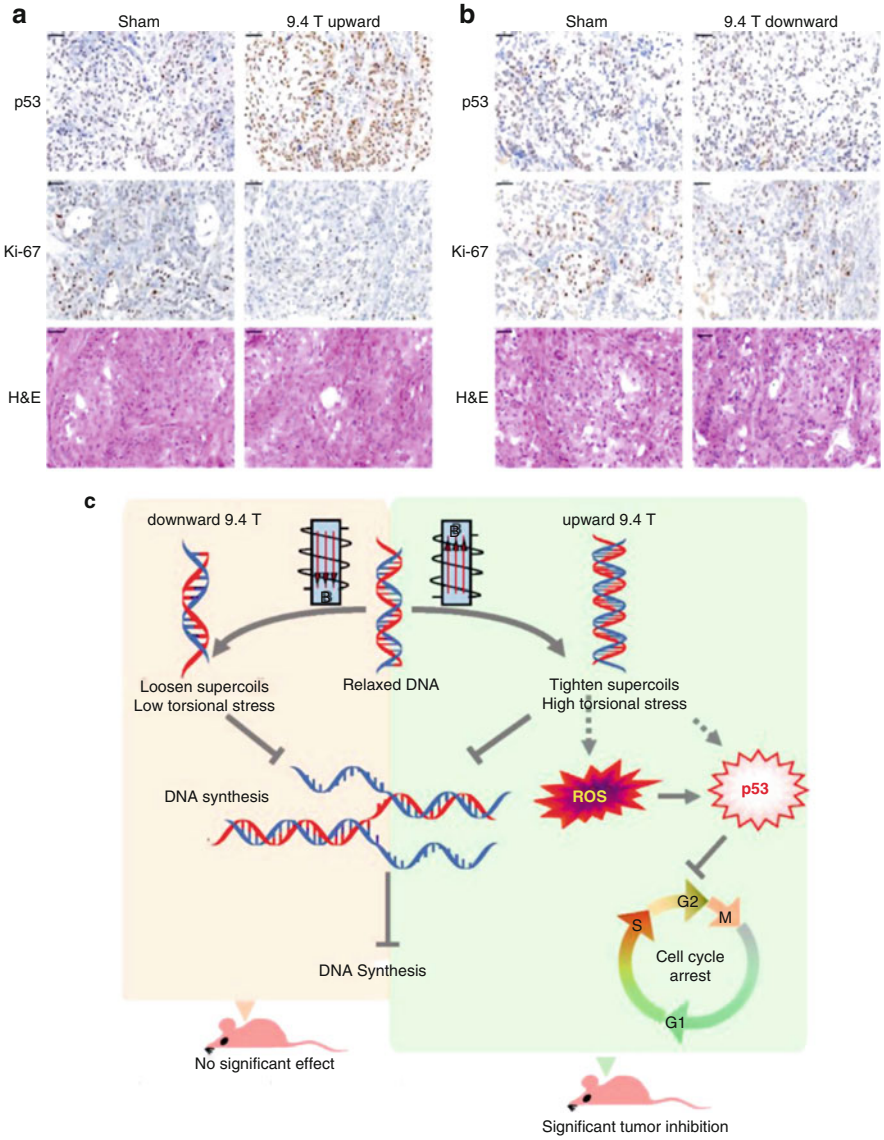

Fig. 9.4 9.4 T SMF increased P53 level and decreased Ki-67 level in mice tumor tissues. Representative images of P53 and Ki-67 immunohistochemistry staining or HE staining of sham, (**a**) upward 9.4 T SMF or (**b**) downward 9.4 T SMF treated mice tumor tissues. Scale bar: 50 μm. (**c**) The model of 9.4 T magnetic fields influence the cell number of A549 lung cancer cells. [Reprinted from reference (Yang et al. 2021). Open access]

division could inhibit tumor growth. In fact, there are multiple chemodrugs that target cell division, such as Taxol. In addition, the most well studied electromagnetic therapy in cancer treatment, the TTF, also target cell division.

The key structure that controls the whole cell division process is the mitotic spindle, which is mainly composed of microtubules. It is well known that microtubules can be affected by SMFs and recent evidences showed that cell division could also be affected by SMFs, which was discussed in Chap. 6. In 2017, we reported that the SMF-induced spindle orientation and morphology changes are due to the combined alignment effects of both microtubules and chromosomes in the magnetic field (Fig. 9.5). Application of the magnetic field parallel to the coverslip allowed us to discriminate torques on chromatin vs. microtubules, and in this case, it appears that torques on well aligned chromatin dominated, aligning spindles preferentially with their microtubules normal to the field, and their metaphase plate parallel to the field. More importantly, although high-field SMFs can change the spindle

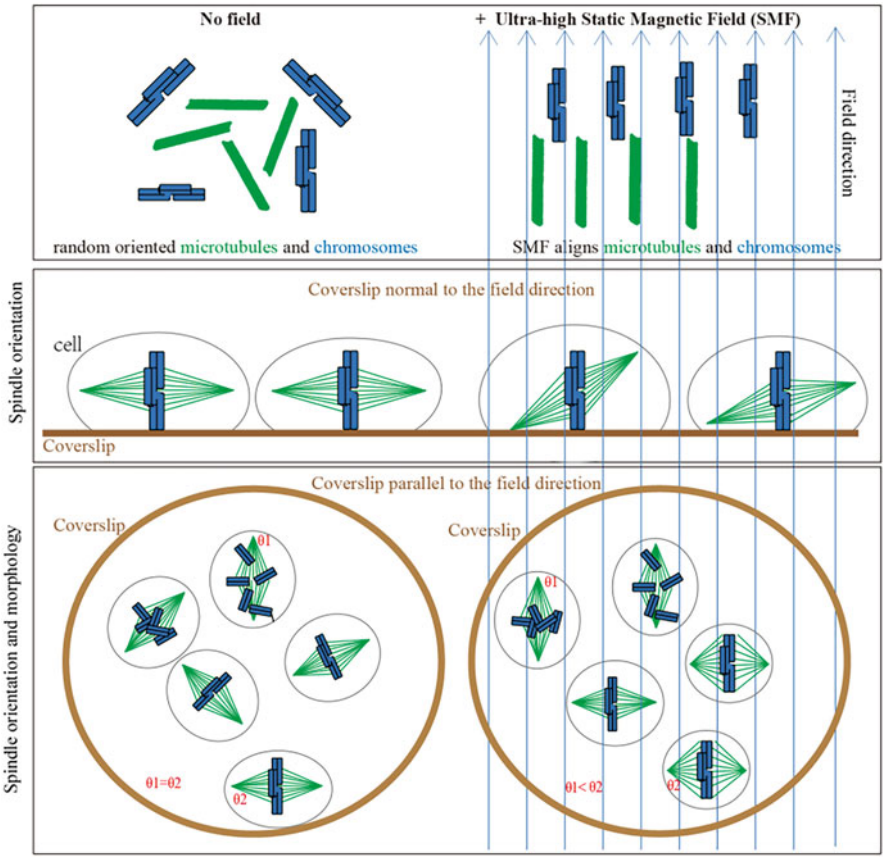

Fig. 9.5 Models show that ultra-high static magnetic fields align microtubules and chromosomes to change spindle orientation and morphology. Blue upward arrows show the magnetic field direction. Cells were plated on coverslips, which were placed in the ultra-high magnetic field either normal to or in parallel with the field direction. '1' measures the pole angle of metaphase spindles in parallel to the magnetic field/gravity direction and '2' measures the pole angle of metaphase spindles normal to the magnetic field/gravity direction. [Reprinted reference (Zhang et al. 2017a). Open access]

orientation and morphology in both cancer and non-cancer cells, we found that the non-cancer cells can recover after the cells were taken out of SMFs. However, the cancer cells do not have a recovery ability, and their growth will be halted even after they are taken out of the SMF.

9.2.3 Static Magnetic Fields and Cancer Metastasis

Metastasis is the leading cause of cancer patient death, which involves cancer cell migration and invasion and is regulated by multiple factors. As far as we know, there are only three studies that have investigated on the SMF effects on cancer cell migration/invasion and/or cancer metastasis. In 2020, Fan et al. reported that a moderate SMF of ~150 mT can inhibit 4 T1 breast cancer cell migration (Fan et al. 2020), but they did not perform animal experiments. In 2021, our group found that gradient moderate SMFs (~0.5 T) provided by a superconducting magnet or permanent magnet can increase ROS level and inhibit ovarian cancer cell migration, invasion (Fig. 9.6), and inhibit ovarian cancer metastasis in mice (Fig. 9.7) (Song et al. 2021). However, also in 2021, using a titled direction gradient SMF provided by a superconducting magnet (Fig. 9.8), Shang's group reported a metastasis promoting effects on osteosarcoma (Zhao et al. 2021).

9.2.4 Static Magnetic Fields and Cancer Cell Stemness

There have been multiple studies that have investigated the effects of SMFs on stem cells, such as dental pulp stem cells (DPSCs), bone marrow stromal cells (BMSCs), human adipose-derived stem cells (hASCs), etc., which have been introduced in Chap. 6 of this book, and in some reviews (Sadri et al. 2017; Marycz et al. 2018; Ho et al. 2019). However, the effect of SMFs on cancer cell stemness was not reported until recently, which reported opposite effects of moderate SMF on cancer stemness and metastasis (Song et al. 2021; Zhao et al. 2021).

The report from our group using vertically upward direction SMFs of ~0.5 T provided by either a permanent magnet, or a superconducting magnet (Figs. 9.6a and 9.7), showed that these SMFs can increase ROS levels in ovarian cancer cells and inhibited their stemness and metastasis (Song et al. 2021). It is known that ROS could affect the epithelial-mesenchymal transition (EMT), promote the transition of mesenchymal cancer stem cells (CSCs) into epithelial CSCs and then bulk cells (Fig. 9.9a). We exposed SKOV3 cells to inhomogeneous moderate SMFs provided by permanent magnets (0.1–0.5 T) for 24 h and used real-time PCR to find out that the stemness-related genes were significantly downregulated by SMF treatment, including SRY-box transcription factor 2 (Sox2), Nanog, cell myc proto-oncogene protein (C-myc), hyaluronan receptor (CD44), and CD133 (Fig. 9.9b). Moreover,

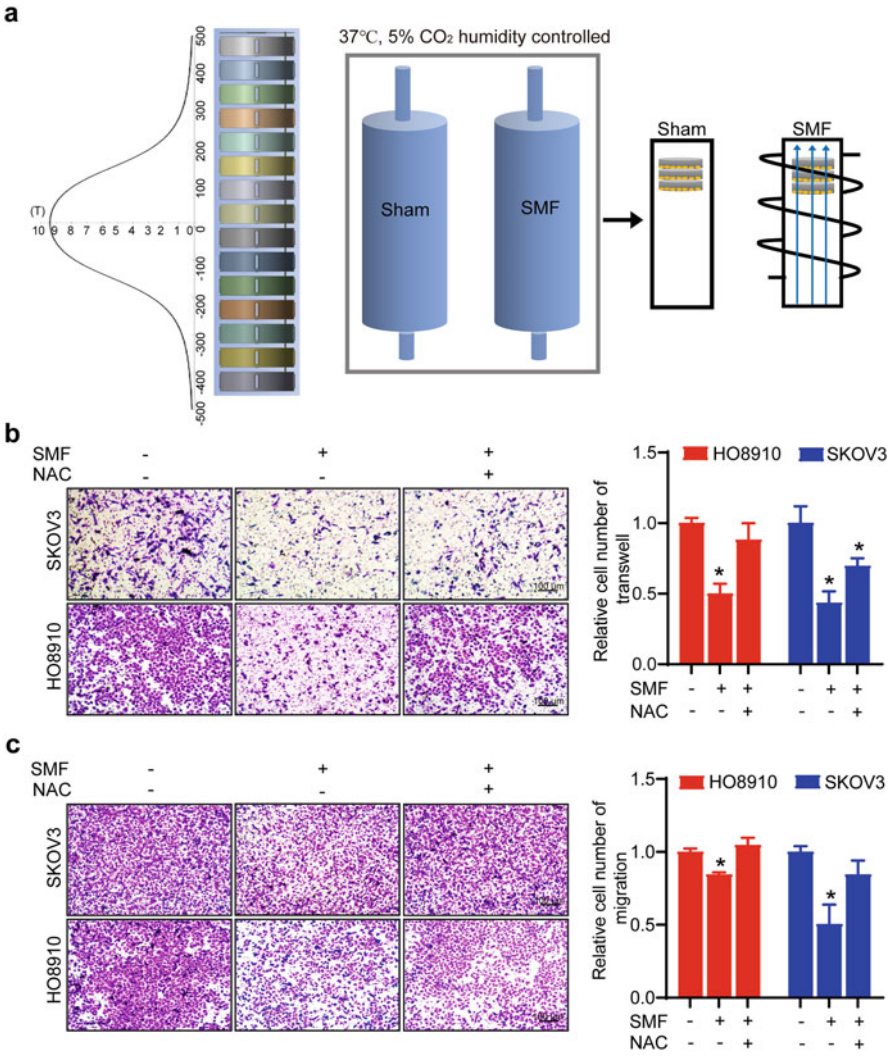

Fig. 9.6 Moderate SMFs inhibit ovarian cancer invasion in a ROS-dependent manner. (**a**) Cells were placed in the upper part of the superconducting magnet, where the SMF is about 0.5 T. (**b**) Transwell invasion assays and (**c**) migration assays of SKOV3 and HO8910 ovarian cancer cells in the absence or presence of SMF and/or NAC. $*p < 0.05$. [Reprinted from reference (Song et al. 2021). Open access]

the cell morphology of SKOV3 cells changed from mesenchymal-like states to epithelial-like states after SMF exposure (Fig. 9.9c). Furthermore, we exposed the HO8910 and SKOV3 cells to SMF for 12 days and detected their sphere-forming ability. The number and size of OC cell spheres were obviously decreased by SMF

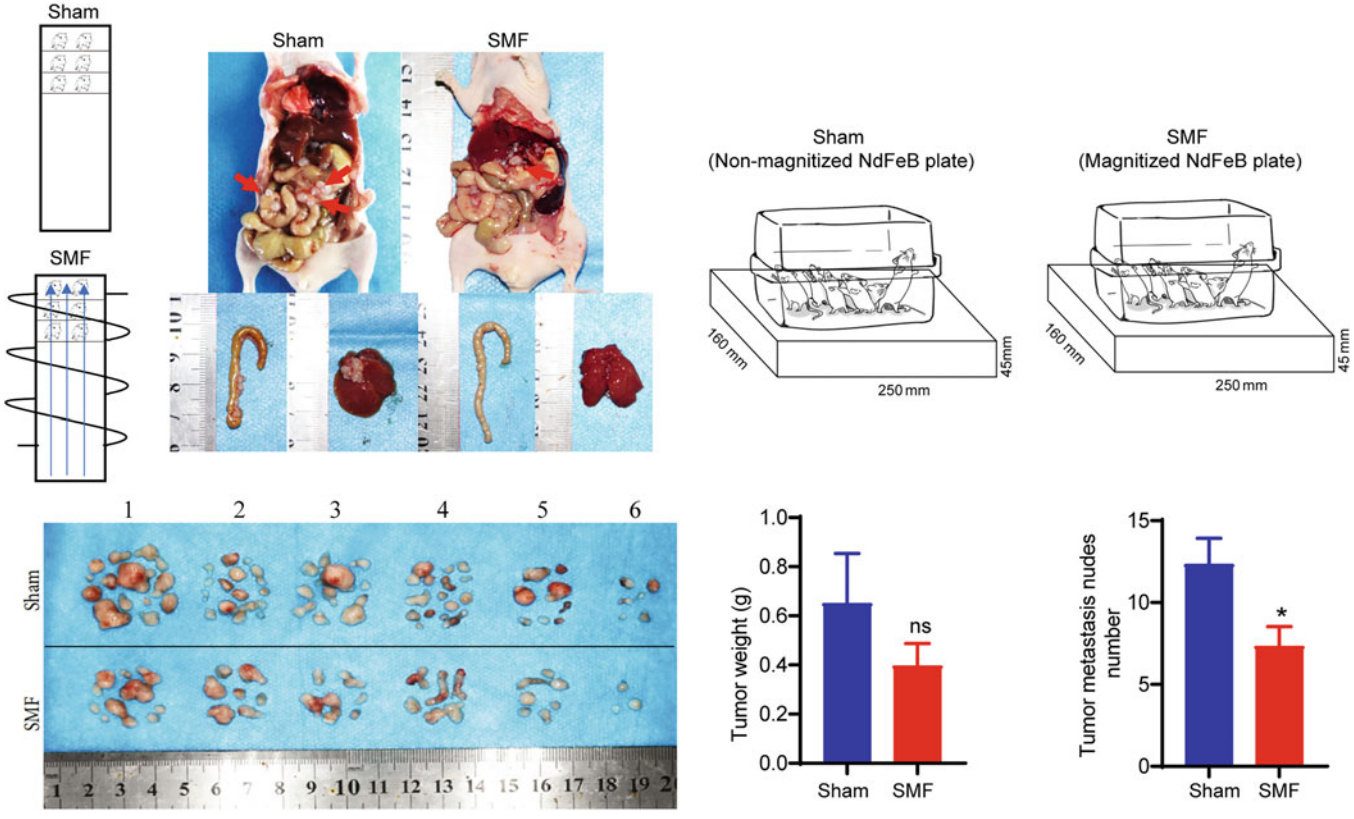

Fig. 9.7 Moderate SMFs inhibit ovarian cancer metastasis in mice. Mice bearing ovarian cancer were exposed to moderate SMFs using a superconducting magnet (10 h/day and 7 days/week) or a permanent magnet plate (continuously 6 weeks). Mice were examined for metastasis at the end of the experiment. *$p < 0.05$; *ns* not significant. [Reprinted from reference (Song et al. 2021). Open access]

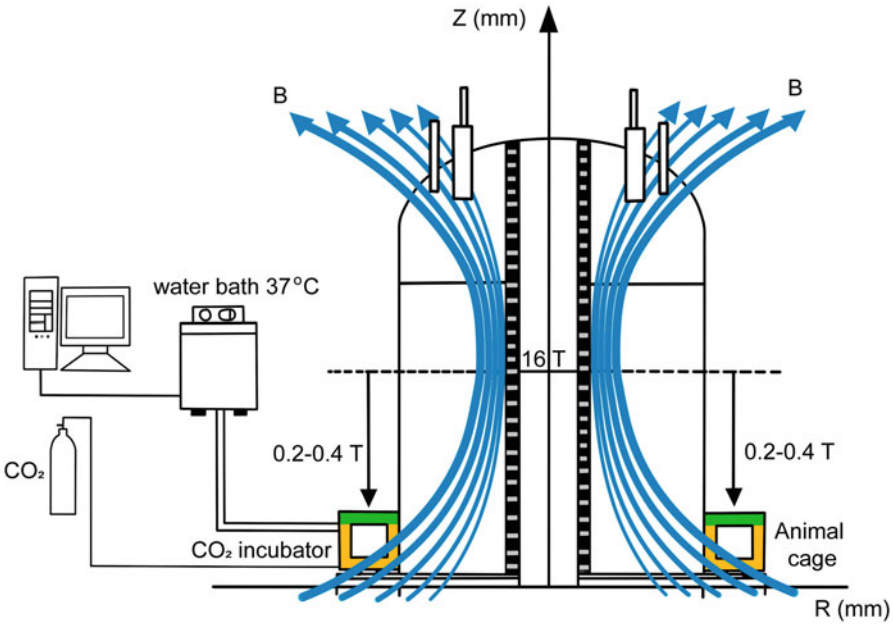

Fig. 9.8 Scheme of the exposure system that provides a 0.2–0.4 T SMF by a 16 T superconducting magnet. [Illustration courtesy of Ding Joe Wang, based on reference (Zhao et al. 2021)]

(Fig. 9.9d) (Song et al. 2021). These data suggested that ovarian cancer stemness was significantly reduced by this moderate SMF treatment.

In contrast, another study from the Shang's group reported that a tilted gradient SMF provided by a superconducting magnet can also increase the ROS levels in osteosarcoma stem cell, but promoted their stemness (Zhao et al. 2021). It is interesting that two independent studies both performed cellular and animal experiments about moderate SMFs on cancer cell stemness, but got opposite effects. There are multiple possible reasons: (1) They used different cell lines, Song et al. used SKOV3 and HO8910 ovarian cancer cells while Zhao et al. used osteosarcoma stem cells. The cells in the bone system are very susceptible to SMF treatment, which will be discussed in Chap. 11. The magnetic directions were different. Song et al. used vertically upward SMFs while Zhao et al. used a tilted SMF. Although the mechanisms for SMF direction-induced bioeffects are still unclear, the differences have been extensively discussed previously discussed in Chap. 2. Obviously, we cannot get any conclusions about SMF and their effects on cancer cell stemness yet at this time point. However, given the importance of cancer stem cells in cancer development, people should perform more studies to get a better understanding on this topic.

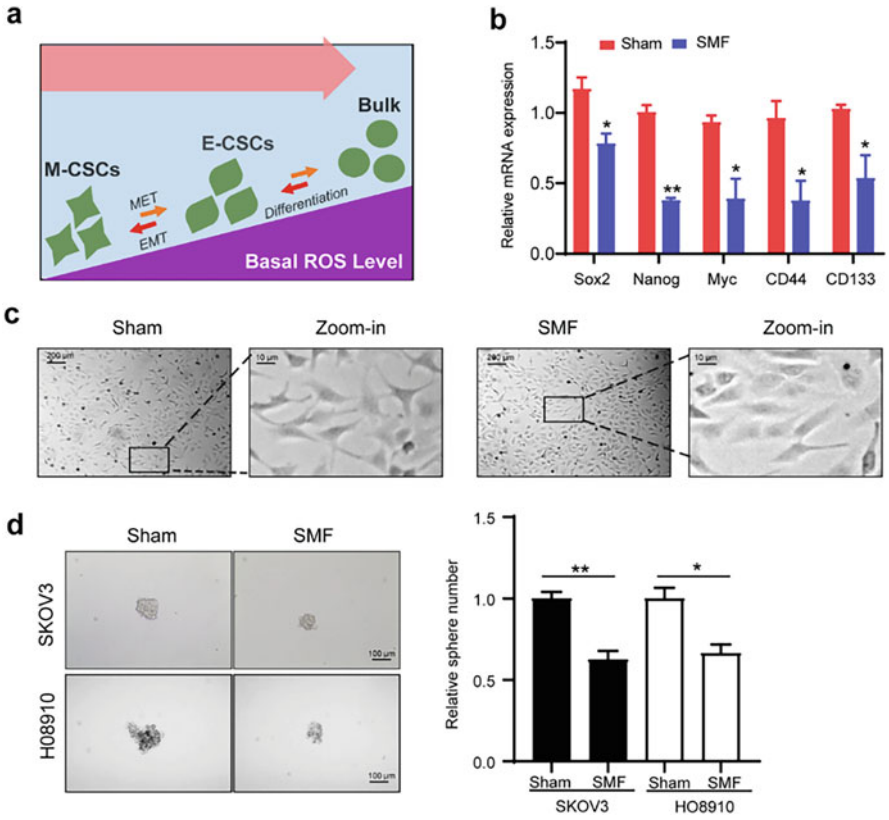

Fig. 9.9 Moderate SMFs reduce ovarian cancer stemness. (**a**) Illustration of the effects of ROS level on CSCs. (**b**) The relative mRNA expressions of stemness genes were measured by qPCR. (**c**) Representative bright-field images of SKOV3 cells exposed to Sham or moderate SMF for 24 h. (**d**) The sphere number and size were measured in SKOV3 and HO8910 cells treated with SMF for 12 days. All comparisons were made between the experimental group and the Sham control group by Student's *t* test. $*p < 0.05$ and $**p < 0.01$. [Reprinted from reference (Song et al. 2021). Open access]

9.3 Static Magnetic Fields and Tumor Microcirculation and Angiogenesis

The above-mentioned effects of SMFs are directly on cancer cells, including cancer cell proliferation, division, migration and invasion, as well as cancer cell stemness. In fact, there are a few studies indicating that moderate SMFs could inhibit angiogenesis and tumor microcirculation to inhibit cancer growth in vivo. For example, in 2008, Strieth et al. examined the effects of SMF (<600 mT) on A-Mel-3 tumors growing in dorsal skinfold chamber preparations of Syrian Golden hamsters. They found that short-time exposure to SMF (~150 mT) resulted in a significant reduction

of red blood cell velocity (vRBC) and segmental blood flow in tumor microvessels (Strieth et al. 2008). At 587 mT, a reversible reduction of vRBC and a reduction of functional vessel density were observed. In addition, they found that prolongation of the exposure time from 1 min to up to 3 h had a more significant result. Moreover, SMFs not only reduced blood flow in tumor vessels but also activated and increased the adherence of platelets (Strieth et al. 2008). In 2009, Strelczyk et al. further evaluated the effects of prolonged exposure to SMFs on tumor angiogenesis and growth. They found that 586 mT SMF exposure for 3 h could inhibit both tumor angiogenesis and growth (Strelczyk et al. 2009). Detailed analysis revealed that the functional vessel density, vessel diameters and vRBC in tumors were all reduced by SMFs. In addition, they also observed increased edema after SMF exposure, which indicated that SMFs might increase tumor microvessel leakiness. In 2014, their group did some further analysis and found that the 587 mT SMF did increase the tumor microvessel permeability significantly in A-Mel-3-tumor-bearing hamsters (Gellrich et al. 2014) (Fig. 9.10). It was interesting but not surprising that the functional tumor microvessels, labeled by FITC-dextran, were much decreased after SMF exposure, especially after the repeated SMF exposure, which was likely due to the inhibited tumor angiogenesis. Nevertheless, it was obvious that both SMF single exposure and repeated exposure increased the blood vessel leakiness and the repeated SMF exposure had stronger effects. In addition, the authors propose that the increased microvessel permeability was likely the reason for the improved anti-tumor efficacy of SMFs in combination with paclitaxel (Fig. 9.10) (Gellrich et al. 2014).

An independent group also reported the effects of SMF on angiogenesis. In 2009, Wang et al. investigated the effects of the gradient SMF (0.2–0.4 T, 2.09 T/m, exposure time 1–11 days) on angiogenesis in the human umbilical veins endothelial cells (HUVECs) as well as two in vivo models, a chick chorioallantoic membrane (CAM) and a Matrigel plug (Wang et al. 2009). Their results showed that the HUVECs proliferation was significantly inhibited after 24-h exposure. In addition, the two in vivo models both showed decreased angiogenesis after 7 or 11 days of exposure (Wang et al. 2009). Although this study was not carried out in a tumor-related model, it showed the inhibition effect of moderate SMFs on angiogenesis, which was consistent with the results reported by Strieth and co-workers (Strieth et al. 2008; Strelczyk et al. 2009). Moreover, our group analyzed the lung cancer A549 cell formed tumor tissue in mice that were exposed to 9.4 T SMFs for 88 h. We stained them with CD31, a blood vessel marker, and counted the vessel numbers in each group of mice. We found that the vessel numbers are reduced in both upward and downward SMF groups (Fig. 9.11) (unpublished data), which indicates that the inhibition effects of SMF on angiogenesis is not MF direction-dependent.

Taken together, these studies showed that moderate to high SMFs have the ability to reduce angiogenesis in some animal models, which implied their potential for tumor growth inhibition in vivo. Additional research is needed to ascertain this effect, such as the effects of other magnetic field intensities as well as more types of tumor models.

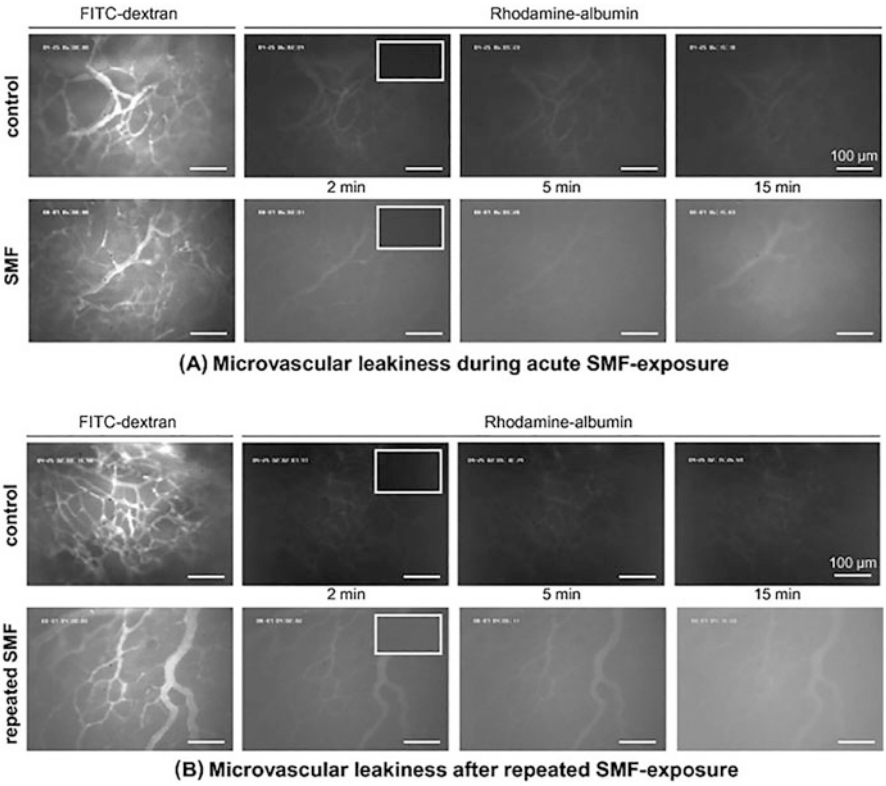

Fig. 9.10 A 587 mT SMF exposure induces intratumoral microvascular leakiness in A-Mel-3-tumor-bearing hamsters. On day 10 after tumor cell implantation representative ROIs (regions of interest) were chosen after FITC-dextran administration, highlighting functional tumor microvessels, before rhodamine-labeled albumin was given intravenously. In control groups, there was a continuous slight increase of fluorescent albumin in the extravascular compartment but the increase was stronger after SMF exposure. (**a**) In vivo fluorescence microscopy for analysis of microvascular leakiness during SMF-exposure. Animals were exposed to the sham control or the SMF of 587 mT during the whole in vivo assessment of microvascular permeability on day 10. (**b**) In vivo fluorescence microscopy of animals that have been repeatedly exposed to SMF of 587 mT for 3 h on day 5, 7, 9 after tumor implantation. The intratumoral microvascular leakiness was stronger in animals after repeated exposure to SMF even with regard to the obviously rather low functional vessel density. [Reprinted with permission from reference (Gellrich et al. 2014). Copyright © 2013 Elsevier Ireland Ltd.]

9.4 Static Magnetic Fields Inhibit Cancer Through Immune Regulation

It has been demonstrated that immune status in humans and in mouse models affects the risk of cancer development in an etiology-dependent manner (Reiche et al. 2004; de Visser et al. 2006). Genetic elimination or depletion of immune cells alters cancer progression in experimental models. Activation of anti-tumour adaptive immune

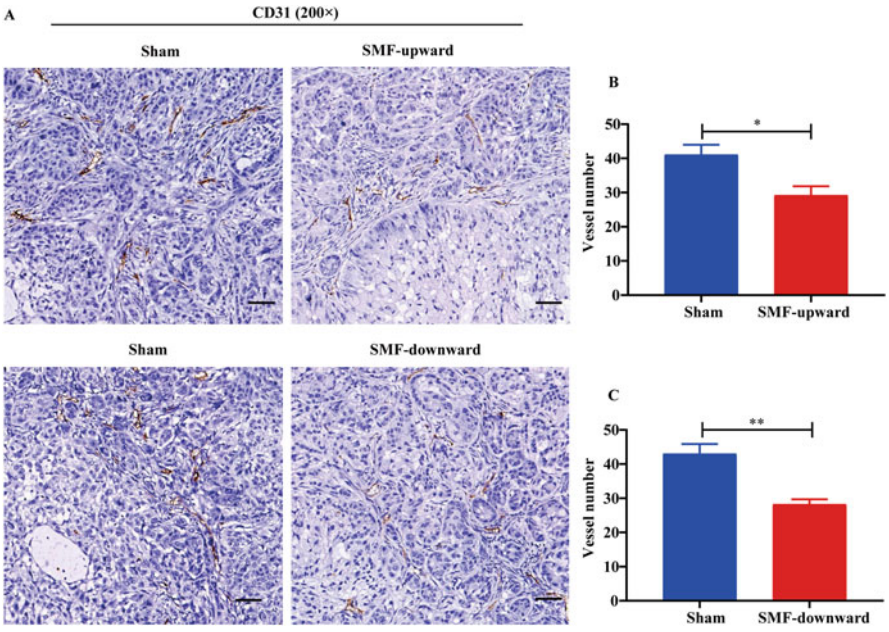

Fig. 9.11 Both upward and downward 9.4 T homogeneous SMFs reduce the vessel number in lung cancer tissues. (**a**) The tumor tissues were stained for a blood vessel marker, CD31. (**b, c**) The vessel numbers were counted from 6 independent views from the tissues. Data represent means ± SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Our group unpublished work

responses can suppress tumour growth. There are some reports about time-varying MFs regulating immune system to inhibit cancer. However, although several studies have shown that SMFs can affect immune systems, which are summarized in Chap. 12 of this book, there are currently only two studies that have addressed the effect of SMF on immune system and their regulation on cancer (Lin et al. 2019; Zhu et al. 2020).

In 2020, Zhu et al. reported a comprehensive study showing that exposure to moderate SMFs (Max magnetic flux density at the surface of the magnetic cubes is at 0.6 T) led to increased granule and cytokine secretion as well as ATP production and mitochondrial respiration from CD8+ T cells (Zhu et al. 2020). These effects were inhibited by knocking down the Uqcrb and Ndufs6 genes of the mitochondrial respiratory chain, whose transcriptions were regulated by candidate magnetoreceptor genes Isca1 and Cry1/Cry2. SMF exposure also promoted CD8+ T cell granule and cytokine secretion and repressed tumor growth in vivo. SMFs enhanced CD8+ T cell cytotoxicity, and the adoptive transfer into tumor-bearing mice resulted in significantly enhanced antitumor effects (Fig. 9.12). Their study suggests that moderate SMFs enhance CD8+ T cell cytotoxicity by promoting mitochondrial respiration and promoted the antitumor function of CD8+ T cells.

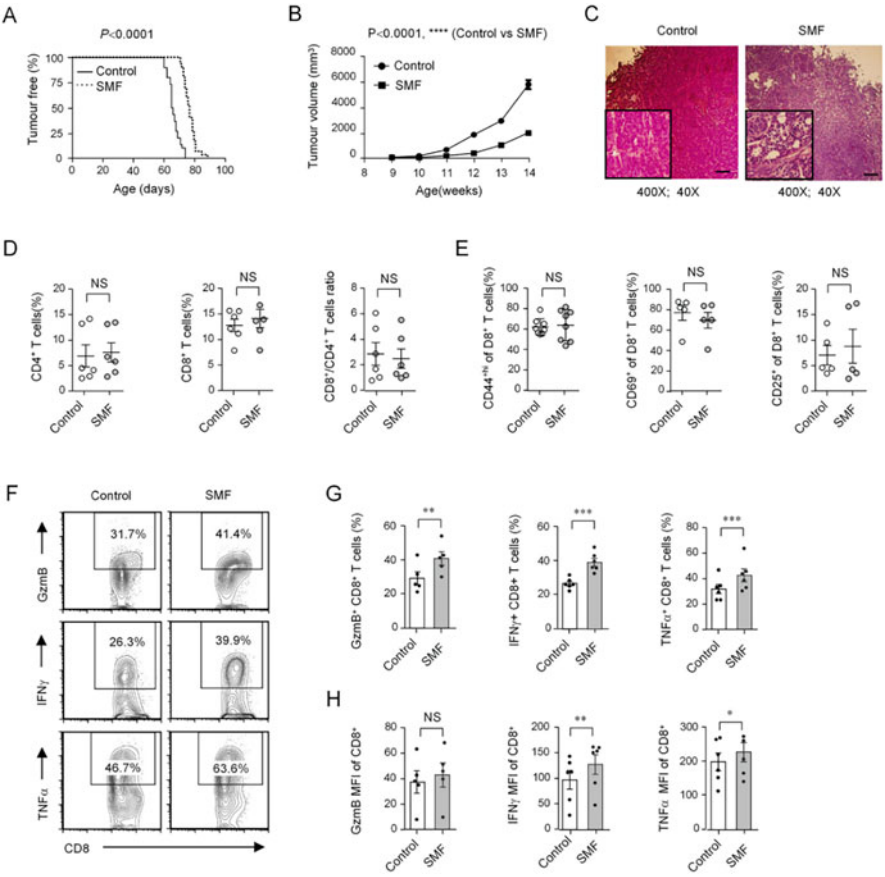

Fig. 9.12 Moderate SMFs promote the antitumor response of CD8+ T cells in vivo. PyMT mice were exposed to magnetic plates made of small magnetic cubes (surface Max 0.6 T), N pole upward facing the mice. Tumor onset (**a**) and tumor growth (**b**) of PyMT mice were monitored. (**c**) HE-stained mammary tumor sections from PyMT mice (scale bars 200 μm). (**d**) % Statistics for CD4+, CD8+ T cells, and the CD8+/CD4+ T cell ratio among tumor-infiltrating T cells in PyMT mice, (**e**) % Statistics for the expression of CD69, CD44 and CD25 in tumor-infiltrating CD8+ T cells in PyMT mice, and (**f**) cytokine/granule production of tumor-infiltrating CD8+ T cells in PyMT mice as analyzed by flow cytometry. (**g**, **h**) Percentage (**g**) and MFI (**h**) statistics for the expression of GzmB, IFNγ and TNFα in tumor-infiltrating CD8+ T cells in PyMT mice as analyzed by flow cytometry. Data were analyzed by log-rank test (**a**), two-way ANOVA (**b**), or Student's *t* test (**d**, **e**, **g**) (*NS* no significance, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$). Error bars indicate the SEM. [Figure and legend are adapted from reference (Zhu et al. 2020). Open access]

In fact, in 2019, Lin et al. have explored the potential for enhancing the killing ability of NK cells by co-culturing the NK cells with K562 leukemia cells under a 0.4 T SMF (Lin et al. 2019). They found that the viability and killing activity of the NK92-MI cells were significantly increased by the 0.4 T SMF. Although their study

was only performed at the cellular level, and they did not test them in animals, these results indicate the great potential of moderate SMFs to boost NK cells to inhibit cancer. Moreover, it should be mentioned that in Zhu et al's study, the 0.3 T SMF did not generate such effects (Zhu et al. 2020), which is consistent with our previously mentioned point that SMF strength is a critical factor in the SMF effects on cancer inhibition.

9.5 Static Magnetic Fields in Combination with Other Treatments

9.5.1 Static Magnetic Fields in Combination with Chemodrugs

There are a large number of researches studied the combinational effects of SMF with chemotherapy drugs, and most of them used moderate SMFs (Table 9.3). Multiple studies have achieved enhanced anti-tumor efficacy compared to SMF or chemodrugs alone. For example, in 2014, Gellrich et al. found that a 587 mT SMF could significantly increase the anti-tumor efficiency of paclitaxel chemotherapy in A-Mel-3-tumor-bearing hamsters because the 587 mT SMF inhibited tumor angiogenesis and inceased tumor microvessel permeability significantly (Gellrich et al. 2014). Our group also found that 1 T moderate intensity SMF could increase the antitumor efficacy of mTOR inhibitors, EGFR inhibitors, Akt inhibitors, as well as Taxol and 5-Fu (Zhang et al. 2015; Luo et al. 2016). In addition, chemotherapy drug adriamycin had an enhanced inhibition effect on the growth of leukemic cells K562 and transplanted mammary tumors in mice when it was combined with moderate intensity SMFs of 110 mT or 8.8 mT, respectively (Gray et al. 2000; Hao et al. 2011). In 2006, Ghibelli et al. showed that 1 T SMF increased apoptosis induced by anti-tumor drugs in human tumor U937 monocytes but not mononuclear white blood cells (Ghibelli et al. 2006).

It was proposed that the cell membrane permeability can be increased by SMFs to allow more drugs to enter cells (Tofani et al. 2003; Liu et al. 2011; Gellrich et al. 2014). This is an appealing explanation because it can explain the combined effects of SMFs and chemodrugs. It is also explainable because SMFs were shown to affect lipids. However, it is puzzling that SMFs have variable effects when combined with chemotherapy drugs (Table 9.3), which indicates that the combinational effects of SMFs with chemodrugs may be drug-specific and/or cell type-specific.

However, it should be mentioned that the current experimental results about combination of SMFs with Cisplatin are not completely consistent. Although we and Vergallo et al. found that SMFs did not increase the efficacy of Cisplatin, there are also some other evidences showing opposite results. For example, it was shown that SMFs could increase the antitumor effects of Cisplatin in mice bearing Lewis lung carcinoma (Tofani et al. 2003) and leukemic cells K562 (Chen et al. 2010). This

Table 9.3 A table to show the variable effects about combination of SMFs with different chemodrugs and cytotoxic drugs for their effects in different cells

Cell line/animal model information	Chemodrug	SMF	Drug efficacy	References
Mice bearing Lewis lung carcinoma	Cisplatin	3 mT	Increase	Tofani et al. (2003)
T hybridoma 3DO cells	Cycloheximide, puromycin	6 mT		Tenuzzo et al. (2006)
Leukemic cells K562	Cisplatin	8.8 mT		Chen et al. (2010)
Transplanted mammary tumors in mice	Adriamycin			Hao et al. (2011)
Leukemic cells K562	Paclitaxel			Sun et al. (2012b)
	Adriamycin	110 mT		Gray et al. (2000)
	Vincristine	500 mT		Our unpublished data
A-Mel-3-tumor-bearing hamsters	Paclitaxel	587 mT		Gellrich et al. (2014)
Human cancer cells, CNE-2Z and HCT116	mTOR inhibitors	1 T		Zhang et al. (2015)
	EGFR inhibitor afatinib			Zhang et al. (2016)
Human cancer cells, CNE-2Z, MCF-7, HeLa and HCT116	Taxol and 5-Fu			Luo et al. (2016)
Leukemic cell line HL-60	Mixture of 5-Fu, cisplatin, doxorubicin and vincristine			Sabo et al. (2002)
Human tumor U937 monocytes	Puromycin, etoposide, hydrogen peroxide			Ghibelli et al. (2006)
Human cancer CNE-2Z cells	AKT inhibitors (MK2206, BEZ-235)			Zhang et al. (2017b)
HCT116 and LoVo colon cancer cells	Topotecan			Yang et al. (2020)
Human gastrointestinal stromal tumor GIST-T1 cells	Imatinib mesylate	9.4 T		Tian et al. (2022)
B16 melanotic melanoma	Cyclophosphamide	3 mT	No effect	Tofani et al. (2003)
Normal human monocytes, lymphocytes and tumor Jurkat cells	Puromycin	6 mT and 1 T		Ghibelli et al. (2006)
Lymphocyte, thymocytes, U937, HepG2, HeLa, FRTL-5	Cycloheximide, Puromycin	6 mT	Reduced	Tenuzzo et al. (2006)
Human tumor U937 monocytes	Puromycin			Ghibelli et al. (2006)
Human neuroblastoma SH-SY5Y cells	Cisplatin	31.7–232 mT		Vergallo et al. (2014)
Human cancer cells, CNE-2Z, MCF-7, HeLa and HCT116		1 T		Luo et al. (2016)

is probably due to the different magnetic intensities in independent studies or cell type differences. Both of these factors could directly influence the magnetic effects as we have discussed earlier. More specifically, studies reported that SMFs of 1–10 mT could increase the antitumor efficacy of Cisplatin (Tofani et al. 2003; Chen et al. 2010) but in ours (Luo et al. 2016) and Vergallo et al.'s studies (Vergallo et al. 2014), we both used stronger magnetic fields (31.7–232 mT in Vergallo et al.'s study and 1 T in our study). Maybe lower magnetic field intensity could increase the Cisplatin efficacy while higher magnetic field intensity has the opposite functions. The exact effects and mechanisms of combining SMFs with Cisplatin in different cells need to be further investigated.

In fact, there are some studies indicated that both MF intensity and cell type could influence the effect of SMF in combination with drugs. For example, in 1999, Fanelli et al. found that SMFs with different intensities starting from 6 gauss could decrease the extent of cell death by apoptosis induced by several agents in different human cell systems via modulation of Ca^{2+} influx, and this effect was MF intensity-dependent (Fanelli et al. 1999). This directly showed that the MF intensity could influence the effect of SMFs with drugs. For cell type induced difference, in 2003, Aldinucci et al. tested a few different cell types for the effects of combining a 4.75 T SMF and a pulsed EMF of 0.7 mT generated by an NMR apparatus for 1 h. They found that in T cell leukemia Jurkat cells the calcium level was reduced significantly after exposure (Aldinucci et al. 2003b) but in normal or in PHA challenged lymphocytes the calcium level was increased (Aldinucci et al. 2003a). Moreover, in 2006, Ghibelli et al. compared two different MF intensities (1 T vs. 6 mT), four different cell lines (two cancer cell lines, human leukemic monocyte lymphoma U937 cells and T cell leukemia Jurkat cells as well as two types of normal cells, human monocytes and lymphocytes) (Ghibelli et al. 2006). It was not surprising that neither the 1 T nor the 6 mT SMF induced apoptosis in all four types of cells. However, it is interesting that 1 T SMF increased puromycin (PMC)-induced apoptosis in U937 cells, but not in other three cell types (Ghibelli et al. 2006). In addition, unlike 1 T SMF, the 6 mT SMF did not increase the PMC-induced apoptosis in any of the cells. In contrast, it reduced the PMC-induced apoptosis in U937 cells (Ghibelli et al. 2006). Moreover, Tenuzzo et al. used 6 mT SMF and apoptosis-inducing agents to compare their effects on multiple types of cells and found that SMF interfered with apoptosis in a cell type- and exposure time-dependent manner (Tenuzzo et al. 2006). In addition, we have reported that 1 T SMF could increase the efficacy of some chemodrugs (5-Fu, Taxol) in multiple human solid cancer cell lines, such as breast cancer MCF-7, colon cancer HCT116, nasophageal cancer CNE-2Z cells but only at some drug concentrations (Luo et al. 2016). Therefore, MF intensity, cell type, drug concentration, and even exposure time, could all influence the combinational effect of SMF with drugs.

Moreover, although most studies have used moderate SMFs, we recently reported that 9.4 T high-field SMF can also increase the efficacy of the chemotherapy drug imatinib mesylate. More importantly, it also ameliorates chemodrug-induced toxicity and depression in mice (Fig. 9.13) (Tian et al. 2022). We compared the anti-tumor effects of 9.4 T SMF with or without imatinib mesylate on BALB/c (Nu/Nu) mice

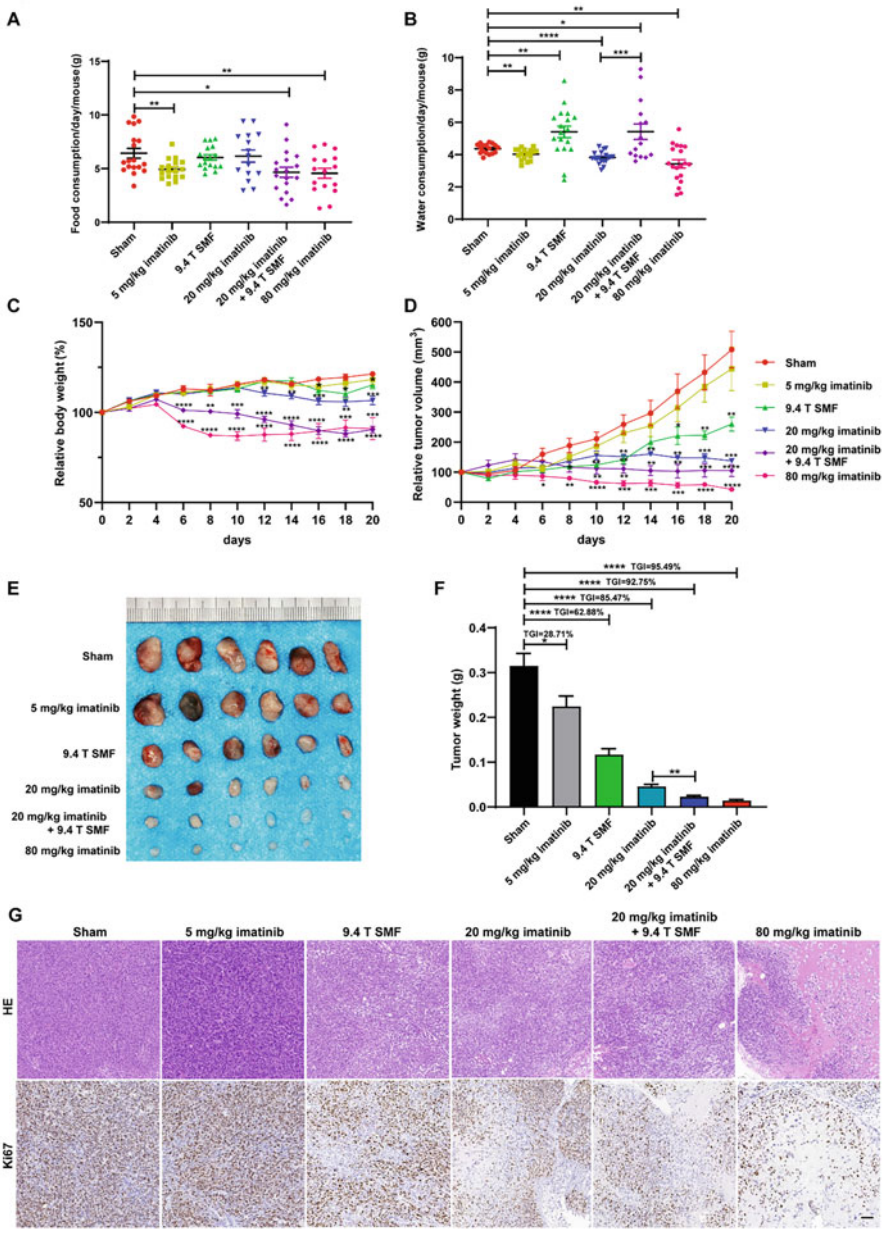

Fig. 9.13 9.4 T SMF inhibits GIST-T1 tumor growth and increases the efficacy of imatinib mesylate. Food (**a**) and water (**b**) consumption, the relative body weight (**c**) and tumor volume (**d**) were measured every 2 days. Tumor (**e**) and their weight (**f**) were measured at the end of the experiment. (**g**) HE and Ki67 staining of the tumor tissues. Scale bar: 50 μm. Data are presented as the mean ± SEM. For those that have statistical significance, we label them as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$. [Reprinted with permission from (Tian et al. 2022)]

bearing gastrointestinal stromal tumor (GIST-T1) cells. We found that the tumor growth was inhibited up to 62.88% when treated with 9.4 T SMF alone for 200 h. More importantly, 9.4 T SMF combined with 20 mg/kg imatinib mesylate can result in 92.75% tumor suppression, which is close to the anti-tumor effect of high dose (80 mg/kg) imatinib. However, 80 mg/kg imatinib caused severe side effects, including significantly reduced gain of body weight, abnormal liver function and depressive behaviors in mice. In contrast, 9.4 T SMF treatment significantly reduced these side effects, especially for the depressive behaviors. Thus, our results demonstrate that 9.4 T SMF not only has anti-tumor effects on its own, but also could improve the anti-tumor effect of imatinib mesylate, reduce its toxicity and improve the mice mental health, which unraveled the great clinical potentials of high SMF in future applications.

Therefore, it is clear that although in most cases, SMFs could increase the efficacy of chemodrugs, there are also some studies showed different results (Table 9.3). These differential effects could be caused by cell type, field intensity as well as drug differences, etc. Consequently, the strategy of combining SMFs of different intensities with various chemodrugs in different cancer cells also needs to be further investigated.

9.5.2 *Static Magnetic Fields in Combination with Time-Varying Magnetic Fields*

There are multiple studies showing that SMFs combined with time-varying magnetic fields could inhibit cancer cell growth (Tofani 2015) (Table 9.4). For example, Tofani et al. have made series progresses on the combination of SMF and 50 Hz time-varying MF. In 2001, Tofani et al. showed that 3 mT SMF combined with 50 Hz time varying MF could induce more apoptosis in cells compared to SMF or

Table 9.4 A table to summarize current literatures about combination of SMFs with time-varying MFs for their effects in different cells

Cell line/animal model information	50 Hz time-varying MFs	SMF	Anti-cancer effects	References
Cultured astroglial cells	1 mT	1 mT	No effect	Bodega et al. (2005)
Human colon WiDr and breast MCF-7 adenocarcinoma	3 mT	3 mT	Increase apoptosis	Tofani et al. (2001)
MRC-5 embryonal lung fibroblast			No effect	
Nude mice with WiDr cells	5 mT	5.5 mT	Increased survival time	Tofani et al. (2002)
Neuroblastoma and nephroblastoma cells	5.1 mT	5.1 mT	Decreased proliferation and increased apoptosis	Yuan et al. (2018)

the 50 Hz time varying MF alone (Tofani et al. 2001). In addition, it was interesting that apoptosis only occurred in the two transformed cell lines (WiDr human colon adenocarcinoma and MCF-7 human breast adenocarcinoma) but not the nontransformed cell line (MRC-5 embryonal lung fibroblast). They also tested them in nude mice xenografted with WiDr cells and exposed them for 70 min/day, 5 days/week, to ≤5 mT SMF in combination with time-varying MF for 4 weeks and found that the tumor was significantly inhibited (up to 50%) (Tofani et al. 2001). In 2002, they further tested the effects of 5.5 mT SMF in combination with 50 Hz time varying MF and found that the survival time of nude mice with WiDr cells was increased by 31% when the mice was exposed to magnetic fields for 70 min/day for 4 weeks (Tofani et al. 2002). When the mice were exposed to the magnetic fields for 4 consecutive weeks, significant inhibition of tumor growth (40%) together with a decrement in tumor cell mitotic index and proliferative activity were observed. In addition, they also found a significant increase in apoptosis together with a reduction in immunoreactive p53 expression (Tofani et al. 2002). These works indicate that SMF + 50 Hz time-varying MF of above 3 mT may have anti-cancer potentials. In contrast, lower MF intensity, such as 1 mT SMF did not induce cell apoptosis as 3, 10 or 30 mT SMFs did (Tofani et al. 2001). Actually, their results could potentially explain why Bodega et al. did not observe any changes when they exposed cultured astroglial cells to a combined 1 mT SMF with sinusoidal 50 Hz time-varying MF for 11 days (Bodega et al. 2005), which might due to the low magnetic field strength.

To our knowledge, all reported studies used the combination of milli-Tesla SMFs (1–10 mT) with 50 Hz time-varying MF of similar MF intensity (Table 9.4). The combination effects of SMFs with higher magnetic field intensity and/or in combination with time-varying MFs of other frequencies besides 50 Hz have not been reported. Whether the currently reported cancer inhibition effects of milli-Tesla SMFs with 50 Hz time-varying MF can also be applied to other magnetic field parameters, such as different magnetic field intensity or frequency, is still unknown. In addition, since the three cell lines Tofani et al. tested showed different responses to the combinational treatment of SMF+ time-varying MF (increased apoptosis in two cancer cells lines WiDr and MCF-7 but not non-cancer cell line MRC-5), it is likely that the effects are also cell type-dependent. Whether other cancer cell types can also be inhibited by SMF+ time-varying MF still need more investigations.

9.5.3 Static Magnetic Fields in Combination with Radiotherapy

Radiation therapy (radiotherapy) is commonly used in cancer treatment. It uses high-energy radiation to kill cancer cells and reduce tumor size. Currently, the most commonly used types of radiation are X-rays. In some cases, gamma rays and charged particles are also used for cancer treatment. In recent years, image-guided

radiotherapy (IGRT) has greatly improved the precision and accuracy of radiotherapy, which takes advantage of modern imaging techniques such as ultrasound, X-ray and CT (computed tomography) scan. The information provided by these imaging techniques before and during radiotherapy treatment not only shows the size, shape and position of the tumor itself, the surrounding tissues and bones, but also allows instant correction for positioning deviations and thereby improves the precision of daily radiotherapy fractions. Although CT scan is mostly used in current IGRT, MRI-guided radiotherapy is attracting increasing attention. It is well known that MRI gives superior soft tissue contrast and more importantly, MRI could offer the advantage of providing IGRT without delivering an additional radiation dose to the patients compared to CT or X-ray imaging. Currently, multiple groups are building or starting to test MRI-guided radiotherapy.

Along with the introduction of MRI-guided radiotherapy, the potential effects of SMFs on ionizing radiation have become increasingly important. However, the accompanied lab studies about the combinational effects of SMF and radiation is lacking. Although there are some evidences showing that the effects of ionizing radiation on cells could be strengthened by Time-varying MFs, such as 50 Hz magnetic fields (Francisco et al. 2013), the studies about SMFs in combination with radiotherapy are much less. So far there are only a few studies that have investigated the combinational effects of SMFs with ionizing radiation and most of these studies indicated that SMFs might be able to increase the effectiveness of radiotherapy (Table 9.5). For example, in 2002, Nakahara found that although 10 T SMF itself had no effect on CHO-K1 cell growth, cell cycle distribution, or micronucleus frequency, they could cause an increase in the micronucleus formation induced by 4 Gy X-rays (Nakahara et al. 2002). In 2010, Sarvestani et al. investigated the effects of a 15 mT SMF alone for 5 h or 0.5 Gy X-ray +15 mT SMF sequential exposures (first X-ray and then SMF for 5 h) on cell cycle progression in rat bone marrow stem cells (BMSC). They did not find any cell cycle changes in SMF alone treated cells but found that 15 mT SMF exposure could further increase the G2/M cell percentage induced by 0.5 Gy X-ray (Sarvestani et al. 2010). In 2014, Teodori et al. investigated the genotoxic effect of 80 mT SMF, both alone and in combination with X-ray irradiation, on primary glioblastoma cells. Their results showed that exposure of cells to 5 Gy of X-ray irradiation alone led to extensive DNA damage, which was significantly reduced by 80 mT SMF (Teodori et al. 2014). The DNA damage promotion effect of 10 T SMF in CHO-K1 cells (Nakahara et al. 2002) and the DNA damage reduction effect of 80 mT SMF in primary glioblastoma cells (Teodori et al. 2014) seem to be controversial. However, this difference could be due to the cell type or magnetic field intensity difference. In 2013, Politanski et al. investigated the combined effect of X-ray radiation and SMFs on ROS in lymphocytes from male albino Wistar rats. Their results indicated that 5 mT SMF increased the ROS changes induced by 3 Gy X-ray radiation while "0 mT" (50 μT magnetic field induction opposite to the geomagnetic field) always showed opposite effects compared to 5 mT SMF (Politanski et al. 2013). This indicated that different magnetic field intensity could directly influence its effect on radiation-induced effects. More researches are needed to get a complete understanding about different

Table 9.5 A table to summarize current literatures about combination of SMFs with different doses of X-ray radiation for their combined effects in different cell types

Samples	Irradiation	SMFs	Specific SMF effects compared to irradiation alone	SMF effects on irradiation cytotoxicity	References
Primary glioblastoma cells	5 Gy X-ray	80 mT	Reduced DNA damage	Decrease	Teodori et al. (2014)
Chinese hamster ovary CHO-K1 cells	1, 2 Gy X-ray	10 T	No effect	No change	Nakahara et al. (2002)
TK6 human lymphoblastoid cells	1–4 Gy 6 MV photons	1 T	No effect on clonogenicity of TK6 cells		Yudhistiara et al. (2019)
Human leukocytes	4 Gy of (60) Co-γ irradiation	Homogeneous and inhomogeneous SMF before irradiation; homogeneous SMF after irradiation. 159 mT	No effect on DNA repair		Kubinyi et al. (2010)
Human head/neck cancer and lung cancer cells	2, 4, 6 Gy X-ray	1.5 T	No effect on radioresponsiveness		Wang et al. (2016)
MDA-MB-231 and MCF-7 human breast cancer cells	4, 6, 8, 10 Gy X-ray	1 T	1 T SMF reduced X-ray-induced ROS elevation, but not prevent X-ray-induced cell number reduction or cell death increase		Wang and Zhang (2019)
Human leukocytes	4 Gy of (60) Co-γ irradiation	Inhomogeneous 159 mT SMF after irradiation	Decrease the DNA repair	Increase	Kubinyi et al. (2010)
Chinese hamster ovary CHO-K1 cells	4 Gy X-ray	10 T	Increased micronucleus		Nakahara et al. (2002)

magnetic field intensities, especially around the range of MRI scanners, and their effects on radiation-induced effects on different cell types. Other types of radiation, such as gamma radiation, should also be investigated.

9.6 Patient Studies

It is interesting and promising that time-varying electromagnetic fields have been shown to be effective in multiple studies at the patient level and were introduced as a novel cancer treatment modality. The most famous example was the tumor treating fields (TTF, or TTFields) therapy, which delivers low-intensity, intermediate-frequency (100–300 kHz), alternating electric fields that cause apoptosis or cell death by inducing mitotic catastrophe and can effectively inhibit the growth of a variety of human and rodent tumor cell lines, with no significant damage to normal non-dividing cells (Kirson et al. 2004; Pless and Weinberg 2011; Davies et al. 2013). In addition, Barbault et al. examined patients with various types of cancer using a noninvasive biofeedback method to identify "tumor-specific frequencies" (Barbault et al. 2009). They implied that cancer-related frequencies appeared to be tumor-specific and treatment with tumor-specific frequencies was feasible, well tolerated and may have biological efficacy in patients with advanced cancer (Barbault et al. 2009). Recently, Kim et al. used TTF to study the metastatic potential of U87 and U373 glioblastoma cell lines and found that TTF affected NF-κB, MAPK and PI3K/AKT signalling pathways as well as downregulated VEGF, HIF1α and matrix metalloproteinases 2 and 9, which indicated that TTF could be a promising novel anti-invasion and anti-angiogenesis therapeutic strategy for glioblastoma patients (Kim et al. 2016). More importantly, studies reported that treating recurrent glioblastoma patients with TTF improved overall survival (OS) and there was no unexpected adverse effects (De Bonis et al. 2012; Rulseh et al. 2012). Due to these clinical outcomes, TTF was approved by the FDA as an alternative to the standard treatment for patients with recurrent and newly diagnosed glioblastoma.

In contrast, although a large number of in vitro and in vivo studies indicated the anticancer potentials of SMFs, there is only a very small amount of data concerning their application in clinical cancer treatment so far. In 2003, Salvatore et al. found that there was no increase in the severity of chemotherapy toxicity as measured by white blood cell count and platelet count in the participants exposed to SMF (Salvatore et al. 2003). In 2004, Ronchetto et al. examined 11 patients with "heavily pretreated" advanced cancer in a pilot study with different SMF exposure and found that the magnetic fields can be safely administrated according to their exposure schedules (Ronchetto et al. 2004). Although these studies indicated the safety of SMFs at patient level, the effectiveness of these SMFs on cancer inhibition is still lacking, which still needs to be proved. In fact, there are some clinical studies reported in some Chinese journals about the successful application of SMFs on some cancer treatments, which have been reviewed by Dr. Zhou, although also written in Chinese (Zhou 2000). In these studies, it seems that applying permanent

magnets either alone or in combination with time-varying MF or radiotherapy could have positive effects in cancer inhibition, and the effects are correlated with the magnetic field intensities. More specifically, it was shown that the SMF of 0.2 T and above had anti-cancer effects but SMFs below 0.1 T did not. To my point of view, although these studies do not really meet the criteria of scientific investigations, they appear promising. However, more double blinded, well controlled clinical investigations are needed to confirm their claims.

In the meantime, it is interesting and promising that there are also some positive findings for magnetic devices that use permanent magnets, but spin them at low speed, called extremely low-frequency magnetic fields (Wang et al. 2011; Sun et al. 2012a; Nie et al. 2013a, b). For example, in 2012, Sun et al. investigated the effects of 420 r/min, 0.4 T magnetic fields on the survival and palliation of general symptoms in 13 advanced non-small cell lung cancer (NSCLC) patients (Sun et al. 2012a). The patients were treated for 2 h/day, 5 days/week for 6–10 weeks. While the median survival of the advanced NSCLC patients receiving supportive care was 4 months, their "spinning magnetic device" could prolong the median survival to 6 months, which was increased by 50%. Although 6 months median survival was still shorter than that of patients receiving chemotherapy (Cisplatin, 9.1 months; Carboplatin, 8.4 months), the magnetic field-treated patients had no severe toxicity or side-effects. More importantly, the 1-year survival rate was 31.7%, which was much higher than patients only receiving supportive care (15%) and comparable to patients receiving chemotherapy (Cisplatin, 37%; Carboplatin, 34%). In the meantime, the magnetic fields treated patients had improved physical conditions and alleviated symptoms in general (Sun et al. 2012a). In fact, the effect of this type of machine has also been proved to be effective on advanced cancer patients (Yang et al. 2018) as well as in cancer cells and mice models (Wang et al. 2011; Nie et al. 2013a, b). Meanwhile, there are also other unofficial reports claiming that spinning magnets could be used as alternative treatments for patients. Therefore, it is a promising field to explore but apparently these reported studies are still at a very preliminary stage. In fact, an important criticism of these human case reports is the lack of control subjects. Therefore, more rigorous, well controlled and double-blinded clinical trials are strongly needed to prove the effectiveness of SMFs in cancer treatment. The magnetic field parameters, such as the field strength, fixed or spinning, exposure schedule and cancer types should all be tested.

9.7 Discussion

The mechanisms of the differential responses of cancer vs. non-cancer cells to SMFs still remain partially understood. However, SMF-induced microtubule interference is a broad impact on most dividing cells. Moreover, cancer and non-cancer cells have been shown to respond differentially to cell cycle perturbations. For example, it has been reported that the human non-transformed cells and cancer cells have significant survival difference in response to the microtubule drugs treatment (Brito and Rieder

2009). Brito and Rieder found that both nocodazole and Taxol, two microtubule poisons, could kill much more HeLa and U2OS cancer cells than the non-cancer RPE1 cells. Specifically, 5 nM of Taxol, which is approximately the clinical concentration for chemotherapy, could kill 93% of HeLa cells and 46% of U2OS cells but only killed 1% of RPE1 cells (Brito and Rieder 2009). In addition, different types of cancer cells also have differential responses to microtubule drugs (Tang et al. 2013). Moreover, the depletion of plk1 (polo-like kinase), which is a vital regulator in multiple cellular processes, especially in cell cycle progression, caused significant cell proliferation and cell cycle abnormalities in human cervical cancer HeLa cells, but not the non-cancer RPE1 or MCF10A breast cells (Liu et al. 2006). Therefore, targeting microtubules or cell cycle could generate different effects on cancer vs. non-cancer cells or in different types of cancer cells.

Meanwhile, we should keep in mind that although EGFR and cell division are important, they are definitely not the only reasons that can explain the differences between SMF-induced differential effects among various cell types. Other factors are also likely involved. For example, Short et al. showed that 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), which indicated that the cell attachment was differentially affected by SMF in cancer vs. non-cancer cells. Moreover, other aspects should also be carefully investigated, such as cell metabolism, mitochondria functions, ROS (reactive oxygen species) responses and ATP level, which could all be affected differentially in cancer vs. normal cells. Our group is currently working on these topics and we expect to have a much better understanding on this issue in the near future.

9.8 Conclusion

Cancer is a heterogeneous disease and its complexity has hindered the development of effective and safe treatments. The studies listed in this chapter greatly helped us to understand some of the mechanisms that SMFs affect cancer cells and their potential applications in cancer treatment in the future. We only discussed about membrane receptor EGFR, cell division and microcirculation here, but it is likely that other aspects are also involved in SMF-induced cancer inhibition, such as ion channels, ROS, the immune system as well as metabolism. Moreover, current cellular studies and animal models of SMF effects on cancers are variable in reproducibility, and further systematic studies of different treatment parameters would be definitely beneficial. In the meantime, while some mechanisms of action have been proposed, their substantiation is needed. Although more research should be conducted to demonstrate its safety and efficacy, current experimental results indicate that SMF is relatively safe. Understanding and exploiting the potential application of SMFs would be an essential aspect of adjuvant therapies targeting conventional treatment-resistant tumor in the future.

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Chapter 10
Effects of Static Magnetic Fields on Diabetes and Its Complications

Chuanlin Feng, Biao Yu, and Xin Zhang

Abstract Diabetes, a metabolic chronic disease characterized by hyperglycemia, has dire consequences for health and well-being if left uncontrolled. In recent years, there are some studies about the effects of static magnetic fields (SMFs) on diabetes and its complications, but the reported effects are highly inconsistent, especially for glycemia levels. The aim of this chapter is to compare and analyze reported effects of multiple parameter SMFs on glycemia and insulin levels, as well as diabetic complications. It is interesting that although the reported effects of SMFs on glycemia and insulin levels are variable due to the differences in SMF parameters and experimental subjects, SMFs have consistently shown beneficial effects on diabetic complications including wound healing. Mechanistic studies indicate that SMFs may play an important role in insulin secretion by affecting membrane proteins, hormone levels, and reactive oxygen species. This not only contributes to a better understanding of SMF effects on diabetes and its complications, but also lays the foundation for more systematic and in-depth studies to develop potential applications of SMFs in the clinical setting of diabetes in the future.

Keywords Magnetic field (MF) · Static magnetic field (SMF) · Glycemia · Insulin · Diabetes · Diabetic complications · Mechanisms

10.1 Introduction

Diabetes mellitus is a serious chronic condition with hyperglycemia, mostly because the body cannot generate enough insulin or cannot efficiently utilize insulin. There are two main types of diabetes, including type 1 diabetes mellitus (T1D, or T1DM) and type 2 diabetes mellitus (T2D, or T2DM). But there are also some specific forms

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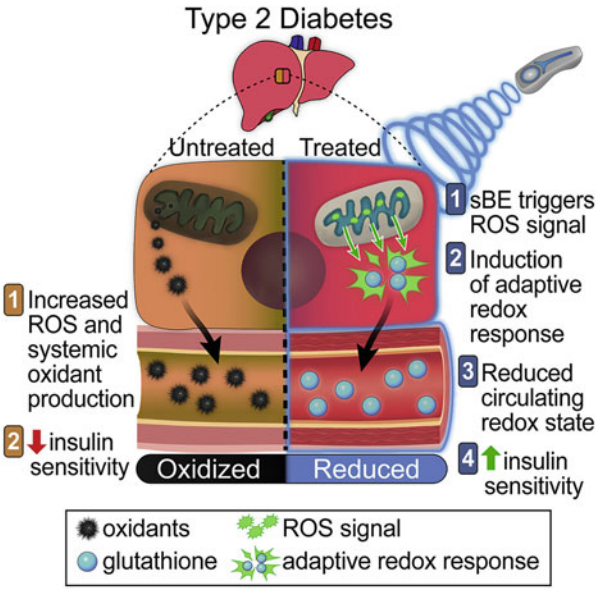

Fig. 10.1 A combined static magnetic field and static electric field can alleviate T2D. [Reprinted from reference with permission (Carter et al. 2020).]

of diabetes mellitus, for example, diabetes that occur during pregnancy, or mediated by drugs or chemicals, viral infections, etc. (Forbes and Cooper 2013; Magliano et al. 2021). Among the various reasons for triggering diabetes, the main causes include autoimmune destruction of pancreatic islet cells, insulin resistance, and insufficient insulin secretion (American Diabetes Association 2010). Besides hyperglycemia, diabetes can also cause a series of complications, including dysfunctions in the kidney, retina, cardiovascular system, neurons, and liver, which are the major causes of morbidity and mortality in diabetic patients (Morrish et al. 2001; Demir et al. 2021).

In recent years, there are multiple studies that have reported the effects of magnetic fields on diabetes and its complications. For example, Carter et al. performed multiple mice experiments to demonstrate that a combined static magnetic field (SMF) and static electric field can effectively improve glycemia, insulin resistance, and glucose intolerance in T2D (Carter et al. 2020) (Fig. 10.1). Our group compared four types of moderate SMFs, with different SMF flux, directions, and distributions, and found that a ~100 mT vertically downward direction SMF could effectively alleviate the development of hyperglycemia, fatty liver, and weight gain in T2D (Yu et al. 2021) (Fig. 10.2). Both of these two studies have showed beneficial effects on T2D and both of them have pointed out that the oxidative stress regulation plays an essential role. In this chapter, we will focus on the effects and mechanisms of various types of SMF treatments on glycemia, diabetes and its complications.

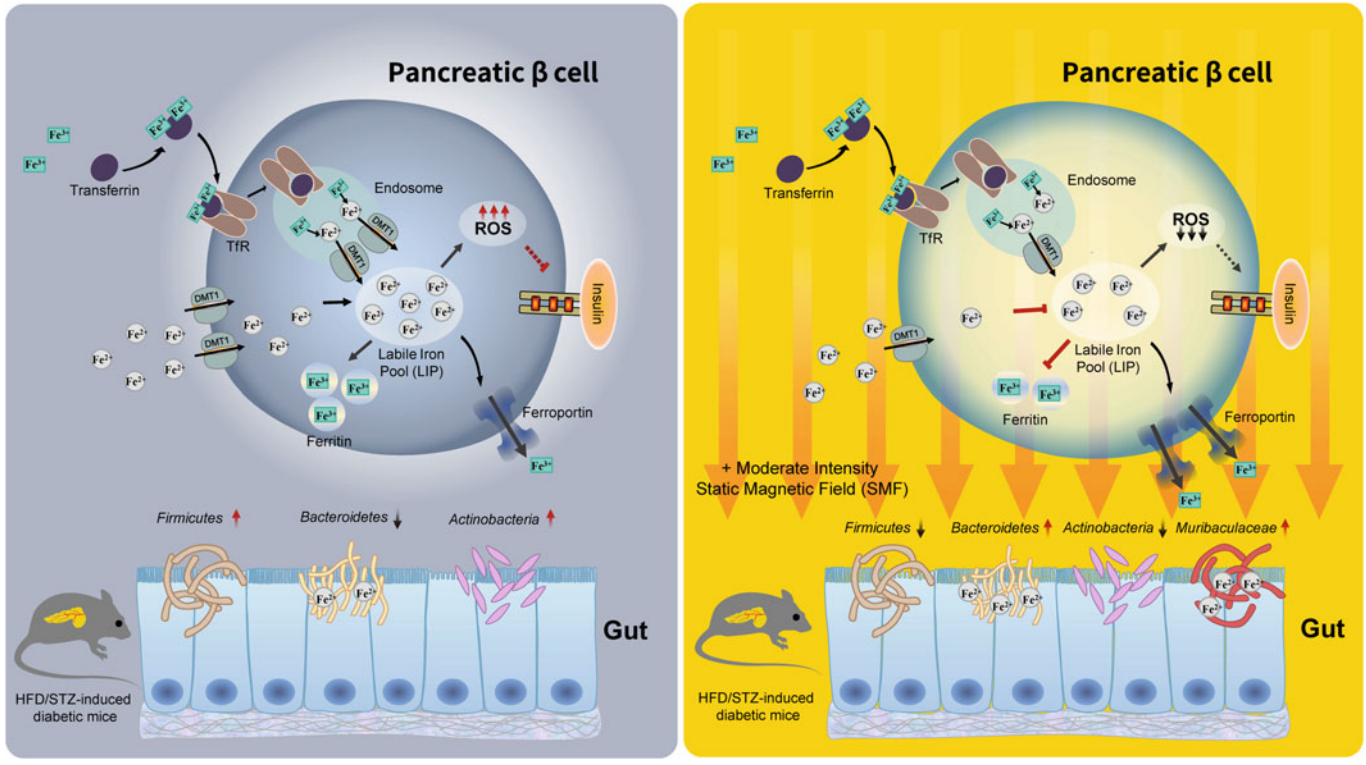

Fig. 10.2 A ~100 mT downward direction static magnetic field improves iron metabolism and prevents high-fat diet/streptozotocin-induced T2D. [Reprinted from reference with permission (Yu et al. 2021).]

10.2 Effects of Static Magnetic Fields on Glycemia Levels in Diabetic Animals

Currently, the effects of various SMFs on glycemia, the key indicator for diabetes diagnosis, in diabetic model animals are still inconsistent (Table 10.1), which is largely due to the SMF parameter differences in different experiments. Some studies have reported that SMF can raise glycemia levels. For example, Carter et al. reported that 3 mT horizontal SMF exposure for 7 h/day for consecutive 25 days significantly increased glycemia (Carter et al. 2020). Conversely, studies have also reported that SMF can decreased glycemia levels. Li et al. reported that alternating pole SMFs (400 and 600 mT) exposure for 24 h can also induce glycemia reduction (Li et al. 2020). In addition, there are also studies that found no effect of SMF on glycemia. For example, our group found no statistically changes in glycemia levels in db/db mice after exposure to ~15 mT inhomogeneous SMF (Feng et al. 2022). Zhang et al. used a 4 mT equipment to treat with diabetic rats for 16 weeks and did not observe significant changes in glycemia levels either (Zhang et al. 2018). We found that T2D mice treated with ~100 mT upward direction SMF for consecutive 12 weeks increased the glycemia level, while the downward SMF decreased glycemia (Yu et al. 2021). These demonstrate that SMF parameter, especially SMF direction, is critical for the SMF effects on glycemia.

10.3 Effects of Static Magnetic Fields on Insulin Levels in Diabetic Animals

Generally speaking, increased insulin levels usually correspond to the decreased glycemia. However, it is not always the case because insulin resistance is another important feature of diabetes, which results in reduced sensitivity of the body to insulin. As far as we know, there are only three studies that have reported the effects of SMFs on insulin levels in diabetic mice, and their results are also variable (Table 10.2). However, Carter et al. found that although the combined static magnetic and electric fields decreased insulin secretion, they still can decrease glycemia by increasing the insulin sensitivity in mice (Carter et al. 2020). Therefore, people are recommended to also measure the insulin sensitivity in their studies to get a more comprehensive understanding of how SMFs affect the glucose metabolism. In our study, we found that the ~100 mT downward direction SMF not only increased the insulin levels, but also improved the insulin sensitivity in high-fat diet (HFD)/streptozotocin (STZ)-induced T2D mice (Yu et al. 2021).

Table 10.1 Effects of static magnetic fields on glycemia levels in diabetic animals

Species	Induction modality	SMF parameters	Exposure time	Glycemia levels	Other effects	References
C57BL/6J mice	HFD induction for 4–8 weeks	3 mT horizontal direction	7 h/day, 25 days	Increased	Elevated glycemia and worsened glucose tolerance	Carter et al. (2020)
	HFD induction for 6 weeks, then intraperitoneal injection of 45 mg kg^{-1} STZ for 3 consecutive days	~100 mT upward direction	24 h/day, 12 weeks		Significantly reduced glucose clearance, exacerbates hepatocyte steatosis, increases iron storage	Yu et al. (2021)
CD1 mice	Intraperitoneal injection of STZ at a dose of 100, 150, 200 mg kg^{-1}	2.8–476.7 mT	30 min/day, 6 weeks	Decreased	N/A	László et al. (2011)
C57BL/6J mice	HFD induction for 6 weeks, then intraperitoneal injection of 45 mg kg^{-1} STZ for 3 consecutive days	~100 mT downward direction	24 h/day, 12 weeks		Reduced hyperglycemia, body weight, tissue damage in T2D mice; regulated iron metabolism, improved oxidative stress and viability of pancreatic islet cells	Yu et al. (2021)
ICR mice	HFD induction for 2 weeks, then intraperitoneal injection of 80 mg kg^{-1} STZ for 3 consecutive days	400 mT, 600 mT, alternating pole	24 h/day, 60 days		Reduced glycemia, improved glucose tolerance, improved lipid metabolism, increased insulin secretion	Li et al. (2020)
Sprague-Dawley rats	Intravenous injection of STZ at a dose of 50 mg kg^{-1}	4 mT	2 h/day, 16 weeks	No change	N/A	Zhang et al. (2018)
db/db mice	Spontaneous type	~15 mT	24 h/day, 10 weeks			Feng et al. (2022)
Sprague-Dawley rats	Intraperitoneal injection of STZ at a dose of 60 mg kg^{-1}	180 mT	24 h/day, 5–19 days			Jing et al. (2010)
ICR mice	HFD induction for 2 weeks, then intraperitoneal injection of 80 mg kg^{-1} STZ for 3 consecutive days	200 mT	24 h/day, 60 days			Li et al. (2020)

(continued)

Table 10.1 (continued)

Species	Induction modality	SMF parameters	Exposure time	Glycemia levels	Other effects	References
Wistar rats	Subcutaneous injection of STZ at a dose of 65 mg kg^{-1}	230 mT	24 h/day, 7–21 days			Zhao et al. (2017)
C57BL/6J mice	HFD induction for 6 weeks, then intraperitoneal injection of 45 mg kg^{-1} STZ for 3 consecutive days	400 mT, 600 mT, alternating pole	24 h/day, 12 weeks			Yu et al. (2021)

Table 10.2 Effects of static magnetic fields on insulin levels in diabetic animals

Species	Induction modality	SMFs parameters	Exposure time	Insulin levels	Other effects	References
C57BL/6J mice	HFD induction for 6 weeks, then intraperitoneal injection of 45 mg kg^{-1} STZ for 3 consecutive days	~100 mT downward direction	24 h/day, 12 weeks	Increased	Downward SMF improved pancreatic function by regulating iron metabolism, reactive oxygen species (ROS) production, and gut microbiota, increased the area of the pancreatic islets and improved the insulin sensitivity	Yu et al. (2021)
ICR mice	HFD induction for 2 weeks, then intraperitoneal injection of 80 mg kg^{-1} STZ for 3 consecutive days	200 mT, 600 mT, alternating pole	24 h/day, 60 days		600 mT alternating pole SMF slightly increased the number of cells in the islets	Li et al. (2020)
Sprague-Dawley rats	Intravenous injection of STZ at a dose of 50 mg kg^{-1}	4 mT	2 h/day, 16 weeks	No change	N/A	Zhang et al. (2018)
C57BL/6J mice	HFD induction for 6 weeks, then intraperitoneal injection of 45 mg kg^{-1} STZ for 3 consecutive days	~100 mT upward direction	24 h/day, 12 weeks		Upward SMF decreased the insulin sensitivity	Yu et al. (2021)
ICR mice	HFD induction for 2 weeks, then intraperitoneal injection of 80 mg kg^{-1} STZ for 3 consecutive days	400 mT alternating pole	24 h/day, 60 days		400 mT alternating pole SMF slightly increased the number of cells in the islets	Li et al. (2020)

10.4 Effects of Static Magnetic Fields on Diabetic Complications

The hyperglycemia of diabetes produces glucotoxicity that cause damage to the macrovasculature system (cardiovascular disease), microvasculature system (diabetic nephropathy, diabetic retinopathy, and neuropathy), and other tissues (diabetic bone, diabetic foot, and diabetic encephalopathy), resulting in various complications (Ceriello 2005; Cole and Florez 2020).

Diabetes significantly impairs bone formation, reduces the mechanical strength of bone, and ultimately leads to osteoporosis (Hofbauer et al. 2022). It also accelerates the degeneration of skeletal structures (Rabe et al. 2021), makes diabetic patients more prone to fractures (Janghorbani et al. 2007; Wang et al. 2019) and difficult to heal after fractures (Retzepi and Donos 2010), which make the mortality rate due to fractures significantly higher than that of the non-diabetic population (Gulcelik et al. 2011). In 2018, Zhang et al. showed that a 4 mT SMF treatment (2 h/day, 16 weeks) can improve bone stiffness, increase the expression of osteogenesis-related genes, and improve symptoms associated with diabetic osteoarthropathy (Zhang et al. 2018). Although it is the only report so far that has investigated on the SMF effects on diabetes osteoarthropathy (Zhang et al. 2018) as far as we know, there are actually a large number of studies demonstrated that SMFs can exhibit positive effects on the skeletal system of non-diabetic animals, which has been reviewed (Zhang et al. 2014) and discussed in the Chap. 11 of this book. Moreover, our group found that a ~100 mT downward direction SMF increased the number of trabecular osteoblasts in the tibia of T1D mice, but not in 0.5 T upward SMF (unpublished data).

Moreover, it should be noted that at least 50% of diabetic patients suffer from diabetic neuropathy, a set of clinical syndromes caused by damage to the peripheral and autonomic nervous systems, which causes allodynia, spontaneous pain, burning, and numbness (Feldman et al. 2019). Similar to the above-mentioned effect of SMFs on bone, there are also many studies of SMFs on nervous system in non-diabetic animals, which will be discussed in Chap. 13 of this book. However, there are only two studies so far that have investigated the effects of SMFs on diabetic neuropathy and the results are still inconclusive. László et al. examined the STZ-induced CD1 mice treated with 2.8–476.7 mT inhomogeneous SMF for 0.5 h/day for 6 weeks and found no significant effect (László et al. 2011). However, Weintraub et al. found that shoe insole of 45 mT alternating pole SMF (24 h/day, 4 months) can play a mitigating role in patient feet with symptoms associated with diabetic neuropathy (Weintraub et al. 2003).

Lastly, it is well known that one of the most prevalent complications in diabetic patients is diabetic wounds (Bowling et al. 2015), which are usually hard to heal and can lead to infection, amputation, and even death (Falanga 2005; Lavery et al. 2010; Lipsky et al. 2012). It is interesting that although various SMFs have inconsistent effects on glycemia, insulin levels, and diabetic neuropathy, all four reported studies of SMFs on wound healing in diabetic mice we got from the literature showed very consistently positive effects (Table 10.3). In fact, in 2021, Lv et al. have reviewed

Table 10.3 Static magnetic fields accelerate diabetic wound healing in all four reported studies

Species	Induction modality	SMFs parameters	Exposure time	Specific results	References
db/db mice	Spontaneous type	~15 mT	24 h/day, 22 days	Facilitated wound closure and re-epithelialization, reduced necrotic areas of wound tissue, increased collagen fibers, improved cell viability and migration, reduced cell death, significantly reduced nuclear factor erythroid 2-related factor 2 levels, and decreased intracellular oxidative stress	Feng et al. (2022)
Sprague-Dawley rats	Intraperitoneal injection of STZ at a dose of 60 mg kg^{-1}	180 mT	24 h/day, 5–19 days	Inflammatory cell counts and necrosis levels were significantly reduced. Healing rate was significantly increased, and the total healing time was shortened. Collagen deposition and wound tensile strength were substantially increased	Jing et al. (2010)
Wistar rats	Subcutaneous injection of STZ at a dose of 65 mg kg^{-1}	230 mT	24 h/day, 7–21 days	Wound area reduction rate was significantly accelerated. Total wound healing time was reduced. Wound tissue strength and stress levels were significantly enhanced	Zhao et al. (2017)
db/db mice	Spontaneous type	600 mT	24 h/day, 14 days	Accelerated wound healing, promoted re-epithelialization, revascularization, and inflammation regression, and upregulated anti-inflammatory gene expression	Shang et al. (2019)

about the effects of multiple types of magnetic fields, including time-varying magnetic fields, on diabetic wounds, which show that all types of magnetic fields have positive effects in promoting diabetic wound healing, according to the literature (Lv et al. 2021). This is interesting and promising, but the reasons for this phenomenon are varied and still unclear.

10.5 Effects of Static Magnetic Fields on Glycemia and Insulin Levels in Cells and Non-Diabetic Animals

Besides the studies of SMFs on diabetic animals, there are actually quite a few studies performed on non-diabetic animals (Table 10.4). Similar to that of diabetic animals, the results in non-diabetic animals are also inconsistent. However, it is interesting that there are no studies reporting decreased glycemia levels in non-diabetic animals so far. Gorczynska et al. found that blood glucose in Wistar rats can be elevated by 1 mT and 10 mT SMFs (Gorczynska and Wegrzynowicz 1991). Meanwhile, several works by Lahbib et al. also found that 128 mT SMF increased glycemia levels in Wistar rats (Lahbib et al. 2010, 2015a, b). In addition, some studies have also shown no effect of SMF on glycemia levels. Currently, we cannot make an accurate conclusion or explanation because of the differences in mice strains, SMF parameters, and SMF treatment methods.

Moreover, insulin levels were also investigated in many studies in cells and non-diabetic mice (Table 10.5). We found that treatment of INS-1 cells with 400 mT SMF for more than 6 h can increase insulin expression and secretion (Mao et al. 2015, 2017), and the exposure of INS-1 cells with 6 T SMF for 1 h can also increase insulin secretion (Sakurai et al. 2009). Interestingly, studying the effects of SMFs on islet cells isolated from Sprague-Dawley rats, Hayek et al. found that the SMF increases insulin levels in a magnetic flux density-dependent manner at magnetic flux density of 0.1–1 mT and lower initial glucose concentrations (5.4 mmol/L) (Hayek et al. 1984). In contrast, these effects were not significant at higher (16.7 mmol/L) initial glucose concentration conditions (Hayek et al. 1984). From the above results, we speculate that the influence of SMFs on insulin is related to the magnetic flux density, exposure time, and the initial glucose level.

10.6 Analysis of Inconsistent Effects of Static Magnetic Fields on Glycemia or Insulin

It is obvious that SMFs have generated very variable effects on most aspects of diabetes and complications, except for the diabetic wound healing. We think there are multiple factors that contribute to these inconsistencies, which are discussed below.

First of all, the major factor is the SMF parameters, including distributions (direction and gradient, etc.) and flux densities generated by the different devices (Fig. 10.3), especially the SMF direction. Our group has previously reported on the SMF direction-induced differential bioeffects (Tian et al. 2018; Yang et al. 2020, 2021) and has also systematically summarized them in Chap. 2 of this book. Moreover, we have side-by-side compared four different SMF settings and different exposure times on HFD/STZ-induced T2D mice. We found that different magnetic flux densities, distributions, directions, and treatment time could produce totally

Table 10.4 Effects of static magnetic fields on glycemia levels in non-diabetic animals

Species	SMF parameters	Exposure time	Glycemia levels	Other effects	References
Wistar rats	1 mT, 10 mT	1 h/day, 10 days	Increased	Elevated levels of growth hormone, thyrotropin, thyroid hormone, cortisol, and glucagon. Decreased insulin levels	Gorczynska and Wegrzynowicz (1991)
	128 mT upward direction	1 h/day, 15 days		Elevated glycemia, lactated glycerol, cholesterol, and phospholipids. Decreased plasma insulin levels. Significantly decreased glycogen levels in quadriceps and liver tissue	Elferchichi et al. (2010)
				Decreased body weight, liver weight, lactate, cholesterol, phospholipids, serum insulin, and triglyceride levels	Elferchichi et al. (2011)
		1 h/day, 5 and/or 15 days		Significantly elevated plasma levels of glycerol, cholesterol, phospholipids, serum insulin, and lactate. Decreased liver glycogen levels	Lahbib et al. (2010)
		1 h/day, 5 days		Reduced islet area and lack of glucose transporters 2 (GLUT2) expression in the outer membrane of islet cells	Lahbib et al. (2015a)
				Decreased plasma insulin levels	Lahbib et al. (2015b)
		1 h/day, 13 days		Increased hematocrit and hemoglobin concentration. Increased aspartate aminotransferase and lactate dehydrogenase activity. Decreased plasma insulin levels	Chater et al. (2006)
		1 h/day, 10 days		Elevated platelet and hemoglobin levels. Increased aspartate aminotransferase and lactate dehydrogenase activity	Sihem et al. (2006)
BALB/c mice	– $2.9 \sim +2.9 \times 10^{-6}$ T	24 h/day, 30 days	No change	N/A	Hashish et al. (2008)
	50 mT	10 h/day, 25 days			Abbasi et al. (2007)

Table 10.5 Effects of static magnetic fields on insulin levels in cells and non-diabetic animals

Species	SMF parameters	Exposure time	Insulin level	Other effects	References
INS-1 cells	400 mT	12–72 h	Increased	Upregulates the expression of pancreatic-specific transcriptional factors and vesicular secretory proteins. Enhances insulin gene promoter activity and enhances insulin gene expression	Mao et al. (2017)
	400 mT	6–18 h		Increased insulin gene expression	Mao et al. (2015)
	6 T horizontal direction	1 h			Sakurai et al. (2009)
Wistar rats	1 mT, 10 mT	1 h/day, 10 days	Decreased	Increased levels of glucagon, growth hormone, thyrotropin, thyroxine, cortisol	Gorczynska and Wegrzynowicz (1991)
	128 mT upward direction	1 h/day, 13 days		Elevated platelet and hemoglobin levels. Increased aspartate aminotransferase and lactate dehydrogenase activity	Chater et al. (2006)
		1 h/day, 15 days		Elevated glycemia, lactated glycerol, cholesterol, and phospholipids. Decreased plasma insulin levels. Significantly decreased glycogen levels in quadriceps and liver tissue	Elferchichi et al. (2010)
				Decreased body weight, liver weight, lactate, cholesterol, phospholipids, and triglyceride levels	Elferchichi et al. (2011)
				Significantly elevated plasma	Lahbib et al. (2010)

(continued)

Table 10.5 (continued)

Species	SMF parameters	Exposure time	Insulin level	Other effects	References
		1 h/day, 5 and/or 15 days		levels of glycerol, cholesterol, phospholipids, and lactate. Decreased liver glycogen levels	
		1 h/day, 5 days		Reduced islet area and lack of GLUT2 expression in the outer membrane of islet cells	Lahbib et al. (2015a)
				N/A	Lahbib et al. (2015b)
Isolated pancreatic islet cells from Sprague-Dawley pregnant rats	0.1–1 mT	48 h	Insulin levels depend on initial glucose concentration and magnetic flux density	Under high glucose condition, insulin release was inhibited. Under low glucose concentration conditions, SMF can increase insulin levels in a flux density-dependent manner	Hayek et al. (1984)

differential effects on glycemia (Yu et al. 2021). More specifically, we found that neither the 400 mT, 600 mT alternating pole SMFs (Figs. 10.3a, b), nor the ~100 mT upward direction SMF (Fig. 10.3c) reduced blood glucose levels, while the ~100 mT downward direction SMF (Fig. 10.3d) could reduce blood glucose. Furthermore, most of studies showing elevated glycemia levels and reduced insulin levels in non-diabetic animals have used a 128 mT SMF exposure system (Fig. 10.3g) by the Lake Shore electromagnets device manufactured by Lake Shore Cryotronics, Inc. (Tables 10.4 and 10.5). Interestingly, the direction of SMF generated by their device is vertically upward, which reinforce our hypothesis that the upward direction SMF has a tendency to increase glycemia. Moreover, the magnetic flux density also matters because Hayek et al. found that the release of insulin is dose-dependent with magnetic flux density (Hayek et al. 1984).

Secondly, the biological sample differences contributed to the experimental inconsistencies. This point has also been brought up and reviewed in Chaps. 1 and 3 of this book. As far as we know from Tables 10.1 and 10.2, several types of diabetic animal models have been used to evaluate the effects of SMFs on glycemia or insulin. Some studies use chemical-induced diabetic models, for example, STZ or alloxan, while others use genetic diabetic animals of different strains. For example,

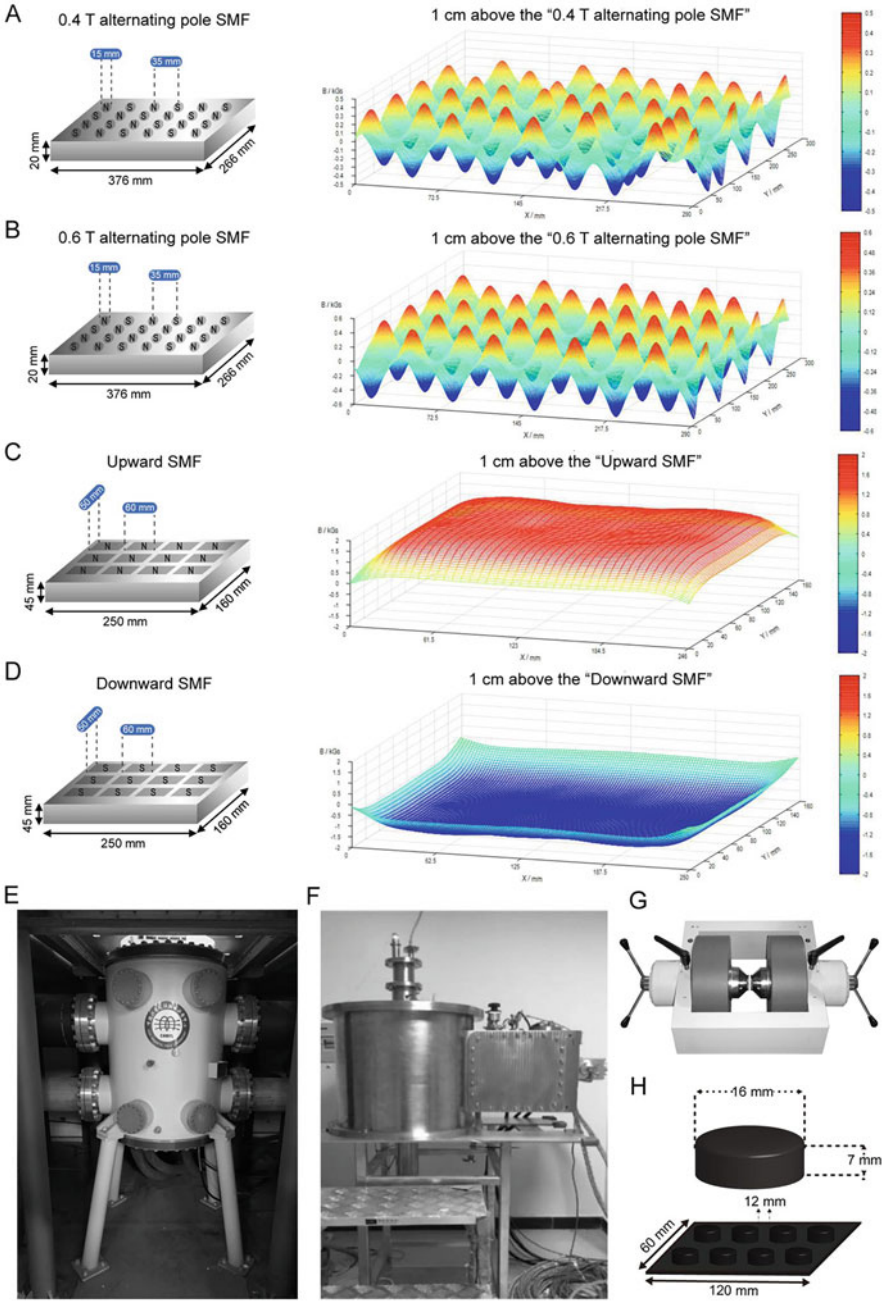

Fig. 10.3 Examples of apparatus that have different static magnetic field settings. (**a**, **b**) Experimental setup and magnetic field distribution for mice exposed to 0.4 T and 0.6 T inhomogeneous SMFs provided by alternating pole magnets (Yu et al. 2021); (**c**, **d**) Experimental setup and magnetic field distribution for mice exposed to upward and downward quasi-uniform SMFs (Yu et al. 2021); Figures were adapted from (Yu et al. 2021), open access. (**e**) A water-cooled magnet (water-cooled magnet #4) in the Chinese High Magnetic Field Laboratory that can provide

by analyzing the results of Yu et al. and Li et al. we found that they used the same magnetic field parameters (alternating pole SMFs of 400 mT and 600 mT), but the glycemia level of T2D mice was different (Li et al. 2020; Yu et al. 2021). We speculate that the mice strain and the modeling methods of the diabetic mice are also important factors. Yu et al. used C57BL/6J mice, whereas Li et al. used ICR mice. And Yu et al. used high-fat chow to feed mice for 6 weeks and then injected 45 mg kg^{-1} of STZ, while Li et al. used high-fat chow to feed mice for 2 weeks and then injected 80 mg kg^{-1} of STZ. In addition, our recent studies found that SMFs also have different effects on glycemia in mild and severe forms of type 1 diabetes (unpublished data). Therefore, since there are multiple diabetes subtypes, and the same type of diabetes also varies based on severity, the exact effects of SMFs are also different.

The third factor is the SMF treatment method, including the duration of exposure, whether to use pretreatment. It has been shown that exposure time is a key factor that contributes to the differential effects of magnetic fields on biological samples. We exposed diabetic mice to SMF for different time points and found that the effects on glycemia are time dependent. After 8 weeks SMF exposure, the glycemia of diabetic mice was not reduced, but after 9 weeks SMF exposure, the glycemia of diabetic mice was significantly reduced compared with the sham control group (Yu et al. 2021). According to László et al. and Li et al. we also found different effects of SMFs exposure time on glycemia (László et al. 2011; Li et al. 2020). In addition, SMF pretreatment may also be an important factor contributing to differences in experimental results. We pretreated the mice with SMF for 6 weeks before they were induced for T2D, whereas Li et al. treated the mice with SMF after they were induced for T2D, which may have contributed to the difference in their results. Finally, whole-body and targeted exposure were also categorized as SMF treatment method, which could also be a potential factor for inconsistencies. From Tables 10.1, 10.2, 10.4, and 10.5, although there is no report using targeted exposure in experiments, the possibility that researchers will not use targeted exposure in the future cannot be ruled out. And we advocate that the effects of SMFs on specific organs, such as the pancreas and liver, should also be explored to discover the specific biological effects of SMFs on specific organs.

Therefore, in order to promote the standardization of related research, we recommend that investigators should carefully design their experiments and accurately describe the experimental details. This includes but not limited to the relevant parameters of the magnetic fields in the experiment (the distance of magnet surface from tissue, exposure time, magnetic flux density, direction, and distribution), and treatment procedure. Besides the basic parameters including body weight, diet, and

Fig. 10.3 (continued) vertical SMFs up to 27.5 T; (**f**) A superconducting magnet in Xin Zhang lab that can provide vertical SMF up to 10 T; (**g**) The Lake Shore device (picture was from the public website: https://www.lakeshore.com/products/categories/overview/discontinued-products/discontinued-products/em4-em7-electromagnets); (**h**) Magnetic plate contains 8 cylindrical permanent magnets of 0.5 T. [Figure was adapted from (Feng et al. 2022), open access]

glycemic change profile in diabetic mice, other assays are also recommended, such as insulin levels and sensitivity, bone mineral density, and angiogenesis markers. The animal sex, age, species, and other key factors should also be clearly recorded.

10.7 Potential Mechanisms for the Effects of Static Magnetic Fields on Glycemia or Insulin

Some preliminary investigations of the potential mechanisms underlying the effects of SMFs on glycemia and insulin have been performed (Fig. 10.4). For example, it was shown that pancreatic islet β-cells can release insulin to reduce glycemia, and SMFs may affect transcription factors and transport channels in pancreatic islet β-cells to regulate insulin secretion (Gorczynska and Wegrzynowicz 1991; Lahbib et al. 2015a; Mao et al. 2017). Some other mechanisms have been proposed, such as iron metabolism, norepinephrine, insulin conformation, cell membrane conformation.

However, it should be mentioned that although these mechanistic study results are listed in Fig. 10.4, it is clear that there is still no consensus model so far. Moreover, most of them are hypothesis-based, and the direct molecular evidence, or more importantly, the physical mechanism is still lacking. In addition, due to differences in the SMF parameters, treatments, and subjects used in these studies, the mechanisms by which SMFs affect glycemia and insulin levels are very diverse. Therefore, in the future, we should systematically study their mechanism and focus more on a biophysical perspective.

10.8 Conclusion

In conclusion, although the regulation of glycemia and insulin levels by SMFs is inconclusive so far due to the SMFs parameter and biological sample difference, it is clear that multiple SMFs treatment modalities have shown significant beneficial effects on diabetic complications, especially the consistently improving effects on diabetic wound healing. In addition, based on current experimental evidences, we have also revealed some clues to optimize SMF parameters to achieve better anti-diabetic effects, including SMF flux density, direction, and distribution. We believe that more systematic and in-depth investigations will definitely help us to unravel the detailed mechanisms of SMF regulation on diabetes and its complications, both biologically and physically, so that we can eventually take the best advantages of SMFs and apply them in the clinical treatment of diabetes.

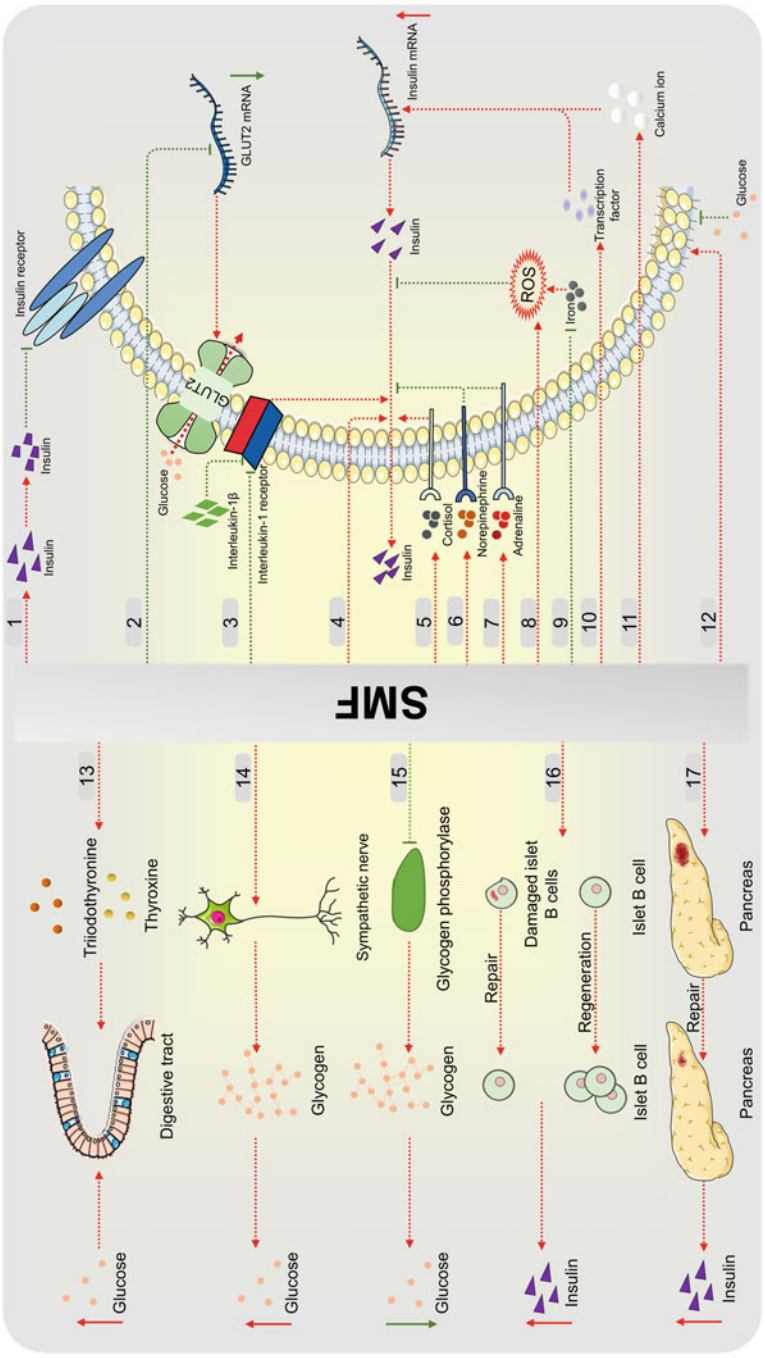

Fig. 10.4 Potential mechanisms for the static magnetic field effects on glycemia or insulin proposed by reported studies. **1.** SMF exposure may change the conformation of insulin, reducing its affinity for the insulin receptor and impairing its function (Elferchichi et al. 2011). **2.** After SMF exposure, GLUT2 expression in pancreatic islet β-cells is reduced, which prevents pancreatic islet β-cells from sensing extracellular glucose concentrations, resulting in cells being unable to release insulin (Lahbib et al. 2015a). **3.** SMF exposure can evoke an effect resembling interleukin-1 receptor antagonist, which may be equivalent to

Fig. 10.4 (continued) insulin intake reducing interleukin-1β level, suppressing the recruitment of innate immune cells, and thus diminishing glycemia (László et al. 2011). **4.** The soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors protein complex can help insulin transport out of cells, and exposure to SMF promotes mRNA expression of synaptosomal-associated protein 25 and synaptotagmin 1, components of the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors protein complex, to facilitate insulin release (Mao et al. 2017). **5.** SMF promotes insulin release by increasing cortisol levels (Gorczynska and Wegrzynowicz 1991). **6.** SMF inhibits insulin release and increases glycemia by raising norepinephrine levels (Abdelmelek et al. 2006; Elferchichi et al. 2011). **7.** SMF elevates adrenaline levels (stimulate pancreatic islet B cell α receptors) to decrease insulin release and promote glycemia elevation (Gorczynska and Wegrzynowicz 1991). **8.** SMF increases intracellular ROS levels to reduce insulin secretion (Elferchichi et al. 2010). **9.** SMF restores the abundance of iron complex outer membrane receptor genes in gut microbiota, thus probably allowing dietary iron to enter microbes, reducing iron storage in cells, decreasing oxidative stress caused by excess iron accumulation, and finally restoring insulin secretion (Yu et al. 2021). **10.** SMF promotes insulin gene expression by inducing the expression of multiple transcription factors that bind to the promoter regions of insulin genes (Mao et al. 2017). **11.** SMF contributes to insulin-related mRNA expression and insulin secretion by increasing intracellular calcium concentration (Sakurai et al. 2009). **12.** SMF of defined intensity can change the lipid layer of the cell into the nematic phase, thus constituting a barrier to the diffusive movement of glucose, which is not beneficial to glucose transport to the interior of the cell (Gorczynska and Wegrzynowicz 1991). **13.** SMF elevates thyroxine and triiodothyronine levels (enhance the absorption of glucose in the digestive tract) to induce hyperglycemia (Gorczynska and Wegrzynowicz 1991). **14.** SMF reduces insulin levels by inducing sympathetic hyperactivity (Lahbib et al. 2010). **15.** SMF decreases glycemia by reducing the activity of glycogen phosphorylase and diminishing glycogen breakdown in the liver (Li et al. 2020). **16.** SMF can reduce glycemia by promoting the regeneration and repair of pancreatic islet B cells, protecting pancreatic islet cells, and improving insulin secretion (Li et al. 2020). **17.** SMF can repair the injury of the pancreas and improve the function of pancreatic islet cells to promote insulin secretion (Li et al. 2020). The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license

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Chapter 11
Impacts of Static Magnetic Field on Bone Health

Huanhuan Lv, Jiancheng Yang, and Yanru Xue

Abstract This chapter summarizes the impacts of static magnetic field (SMF) on bone health. The first part is about the impacts of SMF on the biological behavior of bone mesenchymal stem cells (BMSCs), osteoblasts, bone marrow macrophages (BMMs) and osteoclasts, the possible application of SMF combined with magnetic nanomaterials on osteoblasts, and the impacts of SMF on postmenopausal osteoporosis and diabetic osteoporosis. The second part is about the impacts of hypomagnetic field (HyMF) on osteoblasts and osteoclasts, and the mechanism of HyMF on the recovery of microgravity-induced bone loss. The third part is about the impacts of SMF on osteosarcoma, osteosarcoma stem cells and the impacts of the combination of SMF and chemical drugs on osteosarcoma. Based on the researches, the possible mechanism of the effects of SMF on bone health is related with the regulation on iron metabolism. This chapter provides the theoretical and experimental basis for the ideal of developing the magnetic therapy equipment which acting as the adjuvant therapy on bone diseases in the future.

Keywords Static magnetic field · Hypomagnetic field · Bone health · Osteoporosis · Osteosarcoma · Bone metabolism

11.1 Introduction

The maintenance of healthy bone tissues requires continuous bone remodeling, which includes osteoclast-mediated dissolution and resorption of old or damaged bone and osteoblast-mediated new bone formation. Magnetic fields have unique ability to penetrate and act directly on bone tissues. Since Bassett et al. firstly reported that magnetic field can effectively accelerate fracture healing in 1970, a

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large number of studies have shown that magnetic fields exhibit therapeutic effect on various bone diseases (Andrew et al. 1974; Zhang et al. 2017c). In 1979, magnetic field was approved by Food and Drug Administration (FDA) for the adjuvant treatment of clinical bone diseases such as osteoporosis and osteoarthritis.

Static magnetic field (SMF), with constant magnetic field strength and direction, has a long history of basic and clinical research in bone biology. It can be divided into four classes based on magnetic field strength, including hypomagnetic field (<5 μT), weak magnetic field (5 μT–1 mT), moderate magnetic field (1 mT–1 T), and high magnetic field (>1 T) (Zhang et al. 2017c). Numerous studies have shown that SMF can prevent and treat osteoporosis or promote fracture healing and bone regeneration.

11.2 Impacts of Static Magnetic Fields on Osteoporosis

Osteoporosis is a pathological bone loss because of a disbalance in bone remodeling where bone resorption mediated by osteoclast exceeds bone formation mediated by osteoblast resulting in low bone mineral density (BMD), microarchitectural deterioration of bone, and even fragility fractures (Shoback et al. 2020). Numerous studies have shown that SMFs can promote the proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) and osteoblasts, while inhibiting the formation of osteoclasts and bone resorption, thereby preventing osteoporosis.

11.2.1 Impacts of Static Magnetic Fields on Bone Mesenchymal Stem Cells and Osteoblasts

Osteoblasts are mainly differentiated from bone mesenchymal stem cells (BMSCs) and perform bone forming functions in bone. The process of bone formation includes osteoblastic proliferation, differentiation, maturation, and secretion of various matrix proteins, and finally mineralization of these matrix proteins to form new bone. The effects of SMF on BMSCs and osteoblasts revealed in recent years are summarized in Table 11.1.

Numerous studies have reported the effect of SMFs on the proliferation of osteoblast and BMSCs, but different studies have yielded different results. Rat cranial osteoblasts and osteoblast cell lines UMR106 and ROS17/2.8 were treated with 160 mT SMF, and the proliferation of these cells was found to be unaffected by the SMF (Yamamoto et al. 2003). On the other hand, the proliferation rate of MG63 and MC3T3-E1 osteoblasts was inhibited under SMF, but the cell cycle process was not affected (Chiu et al. 2007; Cunha et al. 2013). The cell proliferation of rat BMSCs was also inhibited by 15 mT SMF (Javani et al. 2013). However, a study showed that 15 mT SMF can promote the proliferation of human BMSCs (Kim et al.

Table 11.1 The effects of static magnetic fields on BMSCs and osteoblasts

Cell models	Instruments	SMF strength	Exposure time	Results	References
Human BMSCs	$Nd_2Fe_{14}B$ disc magnet	15 and 50 mT	Continuous for 3, 7, and 14 days	Enhancing osteoblastic proliferation, ALP activity, calcium content, and bone nodule formation	Kim et al. (2015)
Rat BMSCs	Self-made SMF generated by electrified solenoid	15 mT	Continuous for 24, 48, 72, and 96 h	Inhibiting cellular proliferation	Javani et al. (2013)
Mice BMSCs	$Nd_2Fe_{14}B$ magnets	0.2, 0.4, and 0.6 T	Continuous for 14 days	Inhibiting adipogenic differentiation; promoting osteoblastic differentiation in an intensity-dependent manner	Chen et al. (2020)
Human DPSCs	Self-made SMF generated by electrified solenoid	1, 2, and 4 mT	Continuous for 2, 4, 6, 8, 10, 12, 16, and 20 days	Promoting osteoblastic proliferation and differentiation in 1 mT; no changes in 2 and 4 mT	Zheng et al. (2018)
Primary osteoblast of rat	$Nd_2Fe_{14}B$ magnets	160 mT	Continuous for 1 and 14 days	No change in cellular proliferation, increasing the formation of bone nodules, the calcium content, and ALP activity	Yamamoto et al. (2003)
Rat DPSCs	$Nd_2Fe_{14}B$ magnets	290 mT	Continuous for 7 and 14 days	Enhancing ALP activity and calcium deposition	Hsu and Chang (2010)
MG63 human osteoblast-like cells	$Nd_2Fe_{14}B$ magnets	0.1, 0.25, and 0.4 T	Continuous for 24, 48, and 72 h	Inhibiting cellular proliferation, increasing ALP activity and extracellular matrix release	Chiu et al. (2007)
MG63 cells	MagnetoFACTOR-24 (Chemicell, Germany)	320 mT	Continuous for 1, 3, and 7 days	Reducing cell number, increasing osteocalcin secretion at 3rd day and decreasing osteocalcin secretion at 7th day	Cunha et al. (2013)
MG63 cells	$Nd_2Fe_{14}B$ magnets	0.4 T	Continuous for 12, 24, 48, and 72 h	Increasing osteoblast differentiation	Huang et al. (2006)
Mouse osteoblastic MC3T3-E1 cells	Superconducting magnet (Oxford Instruments, Oxon, UK)	8 T	Continuous for 60 h	No effect on cellular proliferation, enhancing ALP activity and bone nodule formation	Kotani et al. (2002)

(continued)

Table 11.1 (continued)

Cell models	Instruments	SMF strength	Exposure time	Results	References
MC3T3-E1 cells	Superconducting magnet (JASTEC, Kobe, Japan)	16 T	Continuous for 2 and 8 days	Increasing cellular proliferation, ALP activity, calcium content, and bone nodule formation	Yang et al. (2018b)
MC3T3-E1 cells	Self-made permanent magnet	2 T	Continuous for 14 days	Increasing osteoblast differentiation	Yang et al. (2021a)

2015). Zheng et al. (2018) showed the proliferation of human dental pulp stem cells (DPSCs) can be increased by 1 mT SMF, but not affected by 2 and 4 mT. In addition to moderate-SMF, some studies have also focused on the effect of high-SMF of >1 T on osteoblast proliferation. There was no significant change in the number of MC3T3-E1 cells exposed to a SMF at 8 T (Kotani et al. 2002), but 16 T exposure promoted osteoblast proliferation (Yang et al. 2018b). The possible reason for the difference in osteoblastic proliferation under SMFs is caused by the inconsistent inoculum density of cells. Indeed, it was shown that the effect of 0.4 T SMF on the proliferation of MG63 cells was dependent on the initial cell density (Huang et al. 2006). The 1 T SMF produced three different effects (no effect, promotion, and inhibition) on cells with different inoculum densities.

Although the proliferative response of BMSCs and osteoblasts is inconsistent to different SMFs, osteogenetic differentiation can be promoted by SMF with different intensities. 15 mT SMF increased alkaline phosphatase (ALP) activity, calcium release, and mineralized nodule formation in human BMSCs in a time-dependent manner and upregulated the expression of osteogenic marker genes (Kim et al. 2015). BMSCs have the potential to differentiate into multiple cell types, including adipocytes and osteoblasts. Decreased osteogenic differentiation and increased adipogenic differentiation of BMSCs might lead to osteoporosis. Recently, Chen et al. (2020) demonstrated that SMFs of 0.2–0.6 T promoted the osteoblastic differentiation but inhibited their adipogenic differentiation of mice BMSCs in an intensity-dependent manner. SMF at 1 and 290 mT significantly promoted the osteogenetic differentiation and mineralization of dental pulp stem cells (DPSCs) (Zheng et al. 2018; Hsu and Chang 2010). Consistent with the results in BMSCs and DPSCs, numerous studies have shown that SMF also promotes the differentiation and mineralization of primary osteoblasts and osteoblast cell lines. For example, 160 mT SMF enhanced the formation of mineralization nodules, the calcium content, and ALP activity in primary osteoblasts from rat (Yamamoto et al. 2003); osteoblast differentiation was facilitated by 0.4 T SMF in MG63 human osteoblast-like cells (Chiu et al. 2007; Huang et al. 2006); osteoblastic differentiation was promoted by 2 T SMF in mouse osteoblastic MC3T3-E1 cells (Yang et al. 2021a); continuous 16 T SMF exposure for 8 days promoted the ALP activity and mineralized nodules formation in MC3T3-E1 cells (Yang et al. 2018b); 14 days and 21 days in culture after SMF exposure for 60 h, the differentiation and matrix synthesis of cultured MC3T3-E1 cell was enhanced (Kotani et al. 2002).

11.2.2 Impacts of Static Magnetic Fields Combined with Magnetic Nanomaterials on Bone Mesenchymal Stem Cells and Osteoblasts

In recent years, with the rapid development of nanomaterials, the application of magnetic nanomaterials has been gradually promoted. Magnetic nanomaterials are

Table 11.2 The effects of static magnetic fields combined with magnetic nanomaterials on BMSCs and osteoblasts

Cell models	SMF strength	Nanoparticles	Results	References
Human DPSCs	35 ± 5 mT	ION-incorporated calcium phosphate cement scaffold	Yielding greater ALP activity, increased expressions of osteogenic marker genes, and more calcium nodules	Xia et al. (2019)
Mouse BMSCs	20–120 mT	γ-Fe_2O_3 nanoparticles	Promoting the osteogenic differentiation of BMSCs	Sun et al. (2014)
Rat BMSCs	1 T	IONs-loaded bovine serum albumin	Increasing the uptake of nanoparticles and the osteogenic differentiation in BMSCs	Jiang et al. (2016)
MC3T3-E1 cells	100 mT	Poly(L-lactide)/Fe_3O_4 nanofibers	Enhancing the proliferation and osteogenic differentiation of MC3T3-E1 cells	Cai et al. (2015)
MC3T3-E1 cells	100 mT	Mineralized collagen coated IONs	Promoting osteoblastic differentiation	Zhuang et al. (2018)
MC3T3-E1 cells	70–80 mT	IONs modified by oleic acid and poly (lactide-*co*-glycolide)	Improving cell attachment and osteogenic differentiation	Hao et al. (2019)
MC3T3-E1 cells	200 mT	α-Fe_2O_3/γ-Fe_2O_3 nanocomposite	Enhancing osteogenic differentiation	Marycz et al. (2020)
MC3T3-E1 cells	200 mT	$CoFe_2O_4$/P (VDF-TrFE) nanocomposite coatings	Promoting the expression of osteogenic gene makers	Tang et al. (2020a)
MC3T3-E1 cells	200 mT	Zinc ferrite ($ZnFe_2O_4$) coating	Improving the expression of osteogenic differentiation-related genes	Tang et al. (2020b)

generally made of iron oxide nanoparticles (IONs) compounded with other materials. IONs have been used as a nanomaterial because of their high specific surface area, easy surface modification, and good biocompatibility, and have been applied in biomedical fields such as magnetic resonance imaging, tissue engineering, magnetic drug targeting, and gene therapy (Dadfar et al. 2019). However, IONs can also alter some biological functions of cells, such as IONs can promote osteoblast differentiation and bone formation (Wang et al. 2016).

Due to the superparamagnetic property of IONs, exterior magnetic field can alter their physicochemical properties, and most applications of magnetic nanomaterials are related to their specific magnetic characteristics. Therefore, the application of single magnetic nanomaterials to study osteogenic differentiation is gradually decreasing, and many studies have attempted to combine SMF with magnetic nanomaterials, and these approaches have better effects than the action of a single magnetic field or magnetic nanomaterials (Table 11.2).

Xia et al. (2019) found that the addition of SMF significantly enhanced ALP activity, the expressions of osteogenic marker genes, and the formation of calcium nodules in human DPSCs treated with ION-incorporated calcium phosphate cement scaffold. IONs-loaded bovine serum albumin (BSA) (Fe_3O_4/BSA) particles were prepared by Jiang et al. (2016), external SMF exposure could elevate the uptake of Fe_3O_4/BSA and significantly enhance the osteogenic differentiation of BMSCs, as evidenced by increased ALP activity, calcium deposition, and expressions of collagen type I and osteocalcin at both mRNA and protein levels. Treatment of MC3T3-E1 cells with Poly(L-lactide) coated iron nanoparticles and exposure to an additional 100 mT of SMF showed a more significant promotion effect on osteoblast proliferation and differentiation than the treatment of single iron nanoparticles (Cai et al. 2015). Zhuang et al. (2018) revealed that 100 mT SMF enhanced osteoblastic differentiation of MC3T3-E1 cells treated with iron oxide nanoparticle/mineralized collagen coatings. Hao et al. (2019) demonstrated that cell attachment and osteoblastic differentiation were markedly improved by the IONs modified by oleic acid and poly (lactide-*co*-glycolide) in the presence of an external SMF. Furthermore, the expression of piezo-type mechanosensitive ion channel component 1 (Piezo1), a key receptor for sensing mechanical stimuli, was upregulated, implied that the synergistically enhanced osteoblast differentiation was caused by the mechanical stimuli. Marycz et al. (2020) composited the α-Fe_2O_3/γ-Fe_2O_3 nanocomposite and loaded them on MC3T3-E1 cells, found that osteogenic differentiation was enhanced in the presence of a 200 mT SMF. Moreover, Marycz et al. (2020) also found that osteogenic differentiation can be significant promoted by thermoplastic polyurethane and poly(lactic acid) polymer doped IONs in adipose-derived mesenchymal stem cells (ASCs) under 200 mT SMF. Tang et al. (2020a, b) demonstrated that the combination of $CoFe_2O_4$/P(VDF-TrFE) nanocomposite coatings or zinc ferrite ($ZnFe_2O_4$) coatings and 200 mT SMF could significantly upregulate the expression level of osteoblastic differentiation-related genes. In conclusion, a certain intensity of SMF combined with different forms of magnetic nanomaterials can synergistically promote the differentiation of osteoblasts.

11.2.3 Impacts of Static Magnetic Fields on Bone Marrow Macrophages and Osteoclasts

Osteoclasts are mainly differentiated from bone marrow macrophages (BMMs) and perform bone resorption functions in bone. In normal physiological conditions, osteoclast can remove old or damaged bone and induce osteoblasts to form new bone. In contrast to osteoblasts, there are limited reports on the effect of SMF on osteoclast differentiation. Kim et al. (2018) systematically investigated the effect of 15 mT of SMF on the differentiation of BMMs to osteoclasts. The results showed that SMF inhibited osteoclast formation and decreased the activity of tartrate-resistant acid phosphatase (TRAP) and bone resorption activity. The inhibition of

osteoclast differentiation and bone resorption function was also found when BMMs were cultured in conditioned medium after SMF treatment of osteoblasts. Zhang et al. (2017b) found that SMF at 0.2 T promoted pre-osteoclast Raw 264.7 cells to highly express almost all osteoclast-forming genes, leading to differentiation and formation of osteoclasts, while 16 T had a significant inhibitory effect, which was the opposite of its effect on osteoclasts. This regulatory effect of SMF on osteoclasts may be related to the regulation of nitric oxide production and iron metabolism in osteoclasts by SMF (Zhang et al. 2017c; Dong et al. 2019). Recently, a study showed osteoclastogenesis can be enhanced by 2 T SMF in vivo and in vitro (Yang et al. 2021b).

11.2.4 Impacts of Static Magnetic Fields on Postmenopausal Osteoporosis

Postmenopausal osteoporosis is the most common primary osteoporosis, and ovariectomy (OVX) is the most mature and recognized animal model for studying postmenopausal osteoporosis. A samarium-iron-nitrogen magnetic material with a magnetic strength of 180 mT was implanted in the right side of the L3 spinous process of the lumbar spine of OVX rats, and after 6 weeks of exposure, the BMD of the lumbar vertebrae near the magnetic material was significantly higher than that of rats in the sham-operated (Sham) and OVX groups (Xu et al. 2011). Another group of OVX rats was exposed to SMF in the whole body at 30–200 mT for 12 weeks, and the BMD and bone area of the rats exposed to the magnetic material were significantly higher than those of the non-magnetized OVX rats (Taniguchi and Kanai 2007). Yang et al. (2021b) exposed OVX mice to 0.2–0.4 T and 0.6 T SMF for 4 weeks and found that SMFs prevented the reduction in bone density, the deterioration in trabecular and cortical bone microarchitecture, and the weakness in bone mechanical properties caused by OVX. Moreover, bone histochemical analysis revealed that osteoclast formation was decreased in cancellous bone and cortical bone, and osteoblast formation was increased in trabecular bone by SMF. In conclusion, these results demonstrate that SMF ca alleviated OVX-induced bone loss effectively and imply that SMF may become a potential biophysical treatment modality for postmenopausal osteoporosis. However, further clinical trials are needed to confirm the osteoporosis-preventing effect of SMFs.

11.2.5 Impacts of Static Magnetic Fields on Diabetic Osteoporosis

Diabetic osteoporosis is one of the common chronic complications of people with diabetes, which belongs to the secondary osteoporosis, mainly manifested as the

decrease of bone mass, the increase of fragility, and the reduction of bone microstructure (Marin et al. 2018; Nilsson et al. 2017). A growing number of studies have shown that people with diabetes have poor control of blood glucose will cause bone metabolism disorders, eventually lead to diabetic osteoporosis. At present, it is generally believed that type 1 diabetes leads to decrease in BMD, while the situation of type 2 diabtes is relatively complicated, and BMD may decrease, remain unchanged, or even increase (Vestergaard 2007).

Magnetic fields including both SMF and dynamic magnetic field have been shown to ameliorate the complications of diabetes mellitus (Choi et al. 2018; Li et al. 2016; Mert et al. 2020; Zhao et al. 2017; Lv et al. 2021). Recently, the study by Yu et al. showed that 0.4 T or 0.6 T SMF vertically ground down could prevent high-fat diet / streptozocin (HFD/STZ)-induced diabetes in mice and improve the adverse physiological state like high blood glucose level in diabetic mice (Yu et al. 2021). Carter et al. found that short-term exposure of mice with type 2 diabetes to the compound field of electrostatic field and SMF could enhance insulin sensitivity and improve insulin resistance by regulating the redox environment of the whole body of mice (Carter et al. 2020).

There are several researches about the dynamic magnetic fields on diabetic osteoporosis (Zhou et al. 2015; Jing et al. 2011; Cai et al. 2018). As to the effects of SMF on diabetic osteoporosis, there are only one report. Zhang et al. showed that after being exposed to a 4 mT SMF for 16 weeks could inhibit the structural damage of trabecular bone and the reduction of bone mechanical properties of STZ-induced diabetic rats (Zhang et al. 2018). In addition, Zhang et al. also found that SMF increased serum osteocalcin content, promoted bone mineral deposition, and increased the number of osteoblasts in the bone of diabetic rats (Zhang et al. 2018). The above researches may indicate that SMF could prevent diabetes-induced skeletal health problems, including the deterioration of microstructure, compromised mechanical strength, and altered bone metabolism.

11.3 Impacts of Hypomagnetic Field on Bone Metabolism

A magnetic field that is much lower than geomagnetic field (GMF) is usually called hypomagnetic field (HyMF, or HMF). The lack of a natural HyMF on Earth, and researchers need special equipment to set up HyMF environment (Zhang et al. 2021). During long-distance space missions, astronauts will be exposed to an environment with HyMF (Belyavskaya 2004). Numerous studies have reported that HyMF causes a variety of injuries and diseases at the molecular, cellular, animal, and clinical levels. Current research showed that HyMF had effects on the central nervous system, blood system, brain cognition, and embryonic development (Fu et al. 2016b; Jia et al. 2011; Mo et al. 2012). The HyMF environment has significant inhibitory effects on cytoskeleton assembly (Mo et al. 2016), cell proliferation (Mo et al. 2013), and embryonic development (Osipenko et al. 2008).

HyMF is closely related to the metabolism of skeletal system. Zhang et al. found that exposure of pre-osteoclast RAW264.7 cells in HyMF for 4 days significantly

promoted osteoclastic differentiation and bone resorption activities (Zhang et al. 2017b), which was partly due to reduced nitric oxide (NO) production and NO synthase activity (Van't Hof and Ralston 2001; Zhang et al. 2017a). Furthermore, after exposing the osteoblastic MC3T3-E1 cells to HyMF for 8 days, the matrix mineralization was restrained, and calcified nodules and calcium deposition were significantly reduced (Yang et al. 2018b). HyMF inhibited the proliferation and adhesion capacity of satellite muscle cells isolated from rats (Eldashev et al. 2011). Fu et al. found that HyMF exposure for 3 days reduced the skeletal muscle cell viability and mitochondrial activity (Fu et al. 2016a).

HyMF aggravated bone loss induced by hindlimb unloading (HLU) in rats and mice (Jia et al. 2014; Yang et al. 2018a). In addition, iron overload contributed to the inhibitory effects of HyMF on the recovery of microgravity-induced bone loss. Xue et al. found that HyMF inhibits the recovery of microgravity-induced bone loss, probably by suppressing the elevated iron levels' return to physiological level. The study showed that mechanical unloading resulted in bone loss maybe through inducing the increase in iron levels of the bone, liver, and serum. Following reloading, the changes in iron metabolism-related protein expression, the increases in iron levels, and the damages in bone physiology induced by mechanical unloading were recovered to normal condition under GMF environment. However, these changes were not recovered in reloaded mice under HyMF. The iron chelator deferoxamine mesylate (DFO) decreased the iron content in the bone, liver, and spleen and significantly reversed unloading-induced bone loss under HyMF reloading (Xue et al. 2020). These findings help better understand the role of GMF in the recovery of microgravity-induced bone loss and provide a new insight into for the treatment of astronauts' bone loss after spaceflight.

11.4 Impacts of Static Magnetic Fields on Osteosarcoma

Osteosarcoma is the most common primary malignant tumor of bone, and its incidence accounts for about 12% of primary bone tumors (Kansara et al. 2014; Bielack et al. 2009). Osteosarcoma mainly occurs in adolescents under 20 years old, accounting for about 90% of the incidence (Botter et al. 2014). At present, surgical treatment combined with chemotherapy is currently the most effective treatment strategy for osteosarcoma (Moore and Luu 2014; Harrison et al. 2018). However, due to tumor metastasis and local recurrence, the 5-year survival rate of patients with osteosarcoma has not significantly changed in the past few decades. Therefore, finding and developing new treatments for osteosarcoma are very important for the patients with osteosarcoma.

The impacts of SMF on tumor are affected by many factors. The maximum magnetic exposure intensity of the limbs is much higher than that of the trunk and head under the current safety exposure standards for SMF exposure. Moreover, the main incidence area of osteosarcoma is located in the long bones of the extremities, and there are no large blood vessels and organs in the limbs. Therefore,

osteosarcoma is suitable for the local exposure to SMF, and SMF may be developed as a potential therapy for bone tumor.

11.4.1 Impacts of Static Magnetic Fields on Osteosarcoma

Studies have shown that a SMF of 0.618 mT inhibited the proliferation of MG63 osteosarcoma cells (Cohly et al. 2003). Herea et al. reported a kind of magnetothermal therapy based on alternating magnetic fields and nanoparticles that effectively inhibited osteosarcoma cells (Herea et al. 2018). 12 T high-intensity static magnetic field (HiSMF) could induce cell cycle arrest and suppress the proliferation of MNNG/HOS and U-2 OS human osteosarcoma cells by causing the excessive accumulation of intracellular free iron and reactive oxygen species (ROS). Meanwhile, 12 T HiSMF could enhance the cytotoxicity of cisplatin and sorafenib in osteosarcoma cells. 1–2 T HiSMF inhibited the progression of osteosarcoma both in vivo and in vitro. However, after exposure to 1–2 T HiSMF, the iron content in osteosarcoma was significantly reduced compared with the control group (Unpublished data). Altogether, the biological effect of the SMF on osteosarcoma is still unclear.

11.4.2 Impacts of Static Magnetic Fields on Osteosarcoma Stem Cells

Cancer stem cells (CSCs) play a key role in cancer metastasis and recurrence. Prolonged exposure to SMF of 0.2–0.4 T induced the proliferation and tumorsphere formation in K7M2 and MG63 OSCs. Moreover, SMF promoted the release of ferrous iron (Fe^{2+}) and provoked ROS generation in OSCs. Interestingly, 0.2–0.4 T SMF evidently triggered the autophagic degradation of ferritin, which is characterized by the activation of microtubule-associated protein 1 light chain 3 (LC3) and nuclear receptor co-activator 4 (NCOA4) and downregulation of ferritin heavy chain 1 (FTH1) in OSCs. SMF exposure promoted the self-renewal ability of OSCs via autophagic degradation of ferritin, implying that ferritinophagy may be a potential molecular target for cancer (Zhao et al. 2021).

11.4.3 Impacts of Static Magnetic Fields in Combination with Chemical Drugs on Osteosarcoma

Dihydroartemisinin, a classical antimalarial drug, exhibits strong anti-tumor effect. Dihydroartemisinin alone inhibited the activity and proliferation of human

osteosarcoma cell and induced cell death (Shen et al. 2020). When dihydroartemisinin combined with SMF of 16 T did not show any significant effects on the cell viability of osteosarcoma cell lines including MG-63, U2OS, 143B, and MNNG HOS (Unpublished data).

Metformin is used for treating diabetes mellitus and has also attracted much attention due to the anti-tumor effects. Metformin inhibited the proliferation of osteosarcoma cells and osteosarcoma stem cells and induced the apoptosis or autophagy of osteosarcoma cells dependent on the concentration (Zhao et al. 2019). Metformin combined with indicated concentration of ferric ammonium citrate (FAC) induced endoplasmic reticulum stress-mediated apoptosis in osteosarcoma. Exposure to 1.5 T SMF could concentrate FAC in the tumor tissue of osteosarcoma-bearing mice. Meanwhile, metformin combined with FAC inhibited the growth of osteosarcoma in tumor-bearing mice under 1.5 T SMF (Unpublished data).

High-dose ascorbate acts as an adjuvant therapy for cancer with safety and tolerability and to a certain extent enhances the sensitivity of tumor cells to chemotherapy drugs. High-dose ascorbate had no toxic effect on osteosarcoma but combined with cisplatin synergistically inhibited the growth of osteosarcoma cells (Zhou et al. 2020). After intravenous injection or intratumoral injection of superparamagnetic iron oxide nanoparticle Feraheme, the iron content in tumor site significantly increased with external SMF of 1 T. Increasing the iron content in the tumor site by SMF exposure can enhance the inhibitory effect of high-dose ascorbate on osteosarcoma (Unpublished data).

11.5 Conclusion

In summary, these findings suggested that SMF affects the bone cells, osteoporosis and osteosarcoma, and the possible mechanism involved in the regulation of SMF on biological behavior of bone tissues is related to iron metabolism. However, there are still great disagreement on the effects of SMF on bone health. The understanding of the biological function and potential mechanism of SMF on bone cells and skeletal system, provides a theoretical basis to develop the magnetic therapy equipment which is used for adjuvant therapy on bone diseases in the future.

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Chapter 12
Effects of Static Magnetic Fields on the Immune System

Xinyu Wang and Xin Zhang

Abstract Immune system is the fundamental part of human health, which is closely related to various physiological and pathological conditions. Currently, there are some preliminary studies that have reported the effects of static magnetic fields (SMFs) on the immune system, at cellular, animal, and human levels. Here in this chapter, we summarize their experimental results, which show that SMF exposure can affect immune organs, immune cells, and cytokines, either positively or negatively. There are also a few reports showing that SMFs could affect immune system through local application of magnets at the brain, which indicates the critical roles of the central nervous system. Although the regulation of nervous system by SMFs is still an underexplored research area, these current evidences already show their promising potentials, which deserves more systematic and in-depth investigations. Future studies are encouraged to focus on comparing various magnetic flux densities, gradients, and treatment procedures, as well as different aspects of the immune system.

Keywords Static magnetic fields · Immune organs · Immune cells · Cytokines

12.1 Introduction

As we all know, the immune system plays a key role in maintaining the health of living organisms. It has a large and complex network of immune organs, immune cells, and cytokines, which not only serves as the organism's tissue system for executing immune responses and immune activities, but also plays an essential role in practically all aspects of human health (Parkin and Cohen 2001). When a

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foreign pathogenic microorganism attacks a host, two separate but interconnected branches, nonspecific/innate immune responses and specific/adaptive immune responses, will be activated (Fig. 12.1). These two systems work closely to protect organisms from infection and injury (Tomar and De 2014). The innate immune response, particularly macrophages and neutrophils, which can execute phagocytic tasks, works as the first line of defense against pathogenic invasion, which is seen in even the simplest animals. Adaptive immunity is the hallmark of the immune system of higher animals. This response consists of antigen-specific reactions through T lymphocytes and B lymphocytes. B cells produce antibodies that are specific for up to $\sim 10^{18}$ unique targets, whereas T cells can produce $\sim 10^{13}$ different receptors (Parkin and Cohen 2001). Moreover, current studies suggest that the immune system is not a fully autonomous system. For example, the central nervous system can also regulate the immune function of the body through releasing neurotransmitters, neuropeptides, and other substances (Kenney and Ganta 2014).

SMFs can have various biological effects that can influence multiple aspects of human health and disease, such as cancer, diabetes, the bone system, and nervous system, which are discussed in the other chapters of this book. Here we focus on the immune system and summarize the relevant researches to provide a starting point for more in-depth research in the future.

12.2 Effects of Static Magnetic Fields on Immune Organs

Immune organs, such as the thymus, spleen, and lymph nodes, are the sites where immune cells are produced, developed, matured, and settled. There are multiple studies showing that SMFs can affect immune organs, most of which were performed by exposing the whole animal to SMFs, except for one in vitro study (Table 12.1). Most studies showed that SMFs could affect the cell numbers in the spleen and bone marrow. However, since researchers have used different MF flux intensities and directions, as well as different model animals and treatment time, it is currently hard to draw an explicit conclusion of the exact effect about SMFs on immune organs.

Several in vivo studies showing that SMFs could affect the weight or cell count of immune organs, but do not produce pathological changes in these immune organs. Most of these studies were performed in healthy mice. For example, Djordjevich et al. exposed male Swiss-Webster mice to 16 mT SMFs of different directions for 28 days and observed increased total cell number and lymphocyte number, a decrease of granulocytes number in the spleens of SMF-treated groups (Djordjevich et al. 2012). Similar results were observed later by Milovanovich and his colleagues, who found a significantly increased total spleen cell count and decreased granulocyte count but no significant pathological changes in the spleen tissue of SMF-treated male Swiss-Webster mice (Milovanovich et al. 2016). In 2019, Wang et al. exposed healthy C57BL/6 mice to high SMFs at 2–12 T for 28 days and did not find significant pathological changes in the spleen (Wang et al. 2019). Also, Tsuji et al

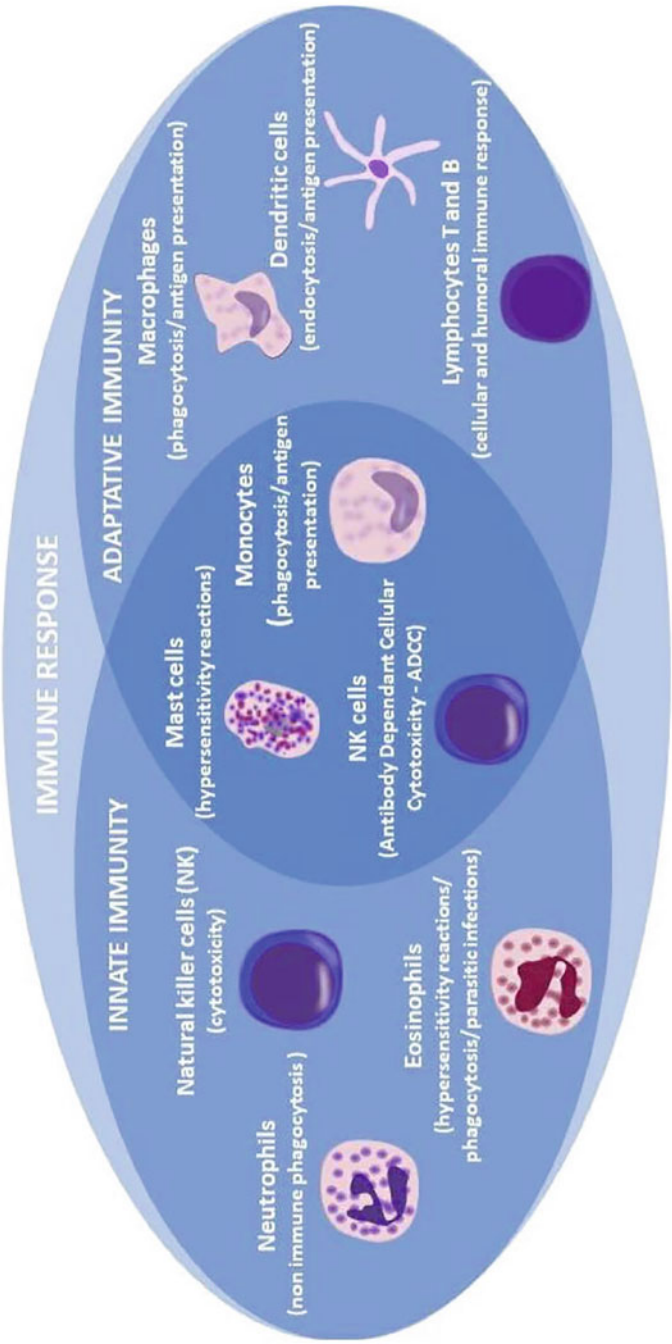

Fig. 12.1 Types of immunity and representative immune system cells. [Reprinted with permission from (Piszczek et al. 2021)]

Table 12.1 Effects of SMFs on immune organs

Subjects			SMF			Effects	References
			Flux density	Exposure time	Direction		
In vitro	Spleen explants		0.2 mT	N/A	N/A	Proliferation rate of splenocytes originating from the mesoderm↑	Ivanova et al. (2018)
In vivo	Healthy animal model	Male Swiss-Webster mice	16 mT	28 days	Upward	Total spleen cells↑**; spleen lymphocytes↑*; spleen granulocytes↓**	Djordjevich et al. (2012)
					Downward	Total spleen cells↑**; spleen lymphocytes↑**; spleen granulocytes↓**	
		Male Wistar rats	60 mT	21 days	Posterior to the frontoparietal suture	Thymus weight↑*	Janković et al. (1993a)
				25 days	Posterior occipitoparietal suture	Thymus weight↑	Janković et al. (1993b)
		Male Swiss-Webster mice	128 mT	1 h/day, 5 days	Upward	Total spleen cells↑**; spleen granulocytes↓**; normal spleen pathology slides	Milovanovich et al. (2016)
					Downward	Spleen granulocytes↓**	
		Male C57BL/6 mice	2–4 T, 6–8 T, 1 0–12 T	28 days	N/A	No significant pathological changes in the spleen	Wang et al. (2019)
		BALB/C mice	5 T	24 h, 48 h		No significant change in spleen weight	Tsuji et al. (1996)
		Male C57BL/6 mice	3.5–23 T	2 h	Upward	13.5 T spleen weight↑**; no significant pathological changes in the spleen	Tian et al. (2019)
			7.0–33 T	1 h			

						33.0 T spleen weight↓**; no significant pathological changes in the spleen	Tian et al. (2021)
	Disease animal mode	Male spontaneously hypertensive rats (SHR)	16 mT	30 days		Total spleen cells↑; spleen erythrocytes↑; spleen granulocytes↓; total bone marrow cells↓; bone marrow erythrocytes↑; bone marrow granulocyte↓	Tasic et al. (2021)
				30 days	Downward	Total spleen cells↑; spleen erythrocytes↑; spleen granulocytes↓; total bone marrow cells↑; bone marrow granulocytes↓; bone marrow lymphocytes↓	
		Female AKR mice	0.4 T, 0.6 T, 0.8 T	2 h/day, 5 days/week, until death	N/A	No statistically significant difference in spleen weight; 400 mT thymus weight↑; 600 mT, 800 mT thymus weight↓*	Bellossi et al. (1988)
			Inhomogeneous, 900 mT	0.5–2 h/day, 5 days/week, until death		No significant difference in spleen or thymus weight	

h hour

*$p < 0.05$; **$p < 0.01$, ***$p < 0.001$, no asterisks mean no statistical significance

exposed healthy BALB/C mice to high SMF at 5 T for 24 h, 48 h and did not find significant changes in spleen weight (Tsuji et al. 1996). However, when our group exposed C57BL/6 mice to SMFs of higher densities (3.5–33 T) and shorter time (1–2 h), we found an increase in spleen weight in the 13.5 T-treated mice and a decrease in spleen weight in the 33.0 T group. But there was no significant pathological change in their spleen tissue (Tian et al. 2019, 2021). The reasons for the spleen weight changes are not clear yet.

Besides the exposure of whole mice to SMFs, in 1993, Janković et al. had implanted 60 mT micromagnets into the rat brain behind the frontoparietal suture and behind the occipitoparietal suture. They found an increase in thymus weight occurred after 21 and 25 days of treatment, respectively (Janković et al. 1993a, b). There are also some studies performed on disease animal models, including spontaneously hypertensive rats (SHR) and leukemia-prone AKR mice (Bellossi et al. 1986; Tasic et al. 2021). Tasic et al. found that exposure of male SHR rats to 16 mT SMF of different directions for 30 days resulted in different changes in total bone marrow cells while similar changes in total spleen cells, spleen erythrocytes, and spleen granulocytes (Tasic et al. 2021). Bellossi et al. exposed leukemia-prone AKR mice to uniform vs. inhomogeneous MFs and found no significant change in spleen weight in either group, but increased thymus weight in mice exposed to 600 mT and 800 mT uniform MFs (Bellossi et al. 1988).

The in vitro study by Ivanova et al. shows that a 0.2 mT SMF can increase the proliferation rate of splenocytes originating from the mesoderm (Ivanova et al. 2018), which could potentially explain the changed spleen size in other in vivo studies. However, the exact effects of different SMFs on various types of spleen cell proliferation are still not clear. The differential effects of SMFs with different directions and their underlying mechanisms also need further investigations.

12.3 Effects of Static Magnetic Fields on Immune Cells

Immune cells are mainly divided into two types: innate immune cells and acquired immune cells (Fig. 12.1). When the body is invaded by pathogens, innate immune cells are the first line of the body defense, which will respond rapidly and non-specifically. In contrast, acquired immune cells can target and destroy the invading pathogens in a specific way (McComb et al. 2019). It has been reported that SMFs can produce a variety of effects on immune cells, which are related to the SMF direction and flux density, the types of immune cells, and the exposure procedure (Table 12.2).

As we can see in Table 12.2, there are more studies performed on the innate immune cells than acquired immune cells. Upon exposure to moderate SMFs, the innate immune cells tend to get more activated and initiate the inflammatory responses in mice and human. For example, the phagocytic capacity of C57BL/6 mice macrophages and the phagocytic index of Raw 264.7 macrophages were decreased after SMF-treated (Flipo et al. 1998; Dini and Panzarini 2010), the

Table 12.2 Effects of SMFs on immune cells

Immune cells		SMFs	Exposure time	Effects	References
Macrophages	C57BL/6 mice macrophages	0.8–1.4 mT	24 h	Ca^{2+}↑*; phagocytic capacity ↓	Flipo et al. (1998)
	Raw 264.7 macrophages	6 mT	N/A	Phagocytic index↓*; phagocytic rate↓*;	Dini and Panzarini (2010)
	Human macrophages	476 mT, inhomogeneous	24 h	Pro-inflammatory cytokine release↓*	Vergallo et al. (2013)
	Db/db mice macrophages	0.6 T, upward	24 h/day, 14 days	Anti-inflammatory gene expression↑*; wound healing↑	Shang et al. (2019)
Neutrophils	Rat peritoneal neutrophils	2.5, 20 mT	400–2000 s	ROS↑	Noda et al. (2000)
	Healthy human neutrophils	60 mT downward inhomogeneous	15/30/45 min	ROS at 15 min↓**; ROS at 45 min↑*; no change at 30 min	Poniedzialek et al. (2013)
		60 mT upward inhomogeneous		ROS at 15 min↓**; ROS at 45 min↑; no change at 30 min	
NK cell	Human NK92-MI	0.4 T, upward	72 h	Cell viability↑; antitumor↑	Lin et al. (2019)
Lymphocytes	Human lymphocytes	6 mT+ apoptogenic treatment	24 h	Both freshly and aged lymphocytes apoptosis↓	Tenuzzo et al. (2009)
		6 mT		Aged lymphocytes spontaneous apoptosis↓	
	Rat lymphocytes	7 mT + $FeCl_2$	3 h	Apoptosis↑**; necrosis↑*	Jajte et al. (2002)
T cells	Human CD4+ T cell	10 T + PHA	3 h	Apoptosis↑***	Onodera et al. (2003)
	Human CD8+ T cell				
	C57BL/6 mice CD8+ T cell	0.3 T	24, 48, 72 h	ATP↑** Antitumor function↑	Zhu et al. (2020)

h hour

*$p < 0.05$; **$p < 0.01$, ***$p < 0.001$, no asterisk means no statistical significance

pro-inflammatory cytokine of human macrophages was decreased, and the anti-inflammatory gene expression of db/db mice macrophages was increased after SMF-treated (Vergallo et al. 2013; Shang et al. 2019). Exposure of granulocytes from healthy human blood and rat peritoneal neutrophils to moderate SMF resulted in the production of reactive oxygen species (ROS), which is found to be associated with the exposure time and SMF direction (Noda et al. 2000; Poniedzialek et al. 2013). Natural killer cells (NK cells) are found to be more cytotoxic under 0.4 T SMF (Lin et al. 2019). The few studies related to the effects of SMFs on acquired immune cells were mostly performed under additional stimulation. For example, the apoptosis of lymphocytes was more pronounced under $FeCl_2$/phytohaemagglutinin (PHA) stimulation (Jajte et al. 2002; Onodera et al. 2003).

12.3.1 Effects of Static Magnetic Fields on Macrophages

Macrophages are one of the essential innate immune cells in the body, which are mainly involved in immune defense and inflammatory regulation (Parkin and Cohen 2001). Several studies have shown that SMFs can affect the phagocytic function of macrophages. For example, in 1998, it was reported that macrophages isolated from C57BL/6 mice exposed to a SMF of 0.8–1.4 mT for 24 h resulted in an increase in Ca^{2+} level and a concomitant decrease in their phagocytic capacity (Flipo et al. 1998). In 2010, Dini et al. noted that 6 mT SMF decreased the phagocytic index and phagocytic rate of macrophages. They also found that compared to early stages of macrophage differentiation, the SMF had a greater effect on macrophages at later stages of differentiation (Dini and Panzarini 2010). In order to gain insight into the mechanisms by which SMFs affect macrophage function, some researchers have focused on the release of inflammatory factors from macrophages and the expression levels of genes associated with inflammation. For example, in 2013, Vergallo et al. exposed macrophages that were cultured in vitro to a 476 mT inhomogeneous SMF for 24 h and found that the release of pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-8 (IL-8), and transforming growth factor-α (TNF-α) was inhibited (Vergallo et al. 2013). In 2019, Shang et al. found that an inhomogeneous SMF (alternating N/S pole of magnets of 0.6 T max at the surface) could upregulate anti-inflammatory gene expression in macrophages, promoting macrophage migration, polarization toward M2 and wound healing, and ultimately inflammation resolution (Shang et al. 2019). These results suggest that the SMF may affect the inflammatory function of macrophages through gene expression.

12.3.2 Effects of Static Magnetic Fields on Neutrophils

Neutrophils are also called polymorphonuclear leukocytes. When a pathogen invades, neutrophils accumulate at the site of infection under the action of

chemotactic factors and kill the pathogen through phagocytosis and degranulation. To kill the pathogen entrapped inside the vacuole, neutrophils produce and release high quantities of antibacterial peptides, proteases, and ROS. The robust ROS production is also called "the respiratory burst" (El-Benna et al. 2016; Liew and Kubes 2019). As far as we know, there are two reports about SMFs and neutrophils, which both focused on the ROS levels changes. For example, in 2000, Noda et al. exposed rat peritoneal neutrophils to SMFs of 2.5 mT and 20 mT for 400–2000 s and observed enhanced ROS levels during the respiratory burst in a time-dependent manner (Noda et al. 2000). In 2013, Poniedziałek et al. exposed peripheral blood neutrophils from blood samples of healthy individuals to inhomogeneous SMFs for 15, 30, or 45 min while treated them with a respiratory burst stimulant phorbol 12-myristate 13-acetate (PMA) that induces oxidative burst in neutrophils (Poniedzialek et al. 2013), they found that exposure of PMA-stimulated and unstimulated cells to a SMF for 15 min resulted in ROS levels decrease, while extending the incubation period to 45 min resulted in ROS levels increase. However, there was no significant difference in ROS levels after 30 min of incubation (Poniedzialek et al. 2013). Although these two studies got different results, they both showed that the SMF effects on ROS are exposure time-dependent. This is similar to the inconsistent effects on ROS levels in other systems, which is discussed in Chap. 6 of this book.

12.3.3 Effects of Static Magnetic Fields on Lymphocytes

Lymphocytes are at the heart of the immune response. According to their origin, morphological structure, surface markers, and immune function, lymphocytes can be divided into three types: T cells, B cells, and NK cells (Larosa and Orange 2008). T cells are responsible for clearing intracellular pathogens and tumors (Larosa and Orange 2008). B cells provide humoral immunity against extracellular pathogens through antibody production (Larosa and Orange 2008). NK cells play an important role in innate immunity and respond to cytotoxicity and cytokine release (Chen et al. 2020).

According to the differentiated antigens on their surface, T cells can be divided into two major subpopulations, CD4+ vs. CD8+ T cells (McComb et al. 2019). Janković and his colleagues implanted 60 mT micromagnets into the brains of rats and revealed increased CD4+ T cell number and decreased CD8+ T cell number (Janković et al. 1991, 1993a) PHA, an initiator of mitosis in cultures of peripheral lymphocytes, can promote mitotic transformation of peripheral blood lymphocytes into different types of lymphocytes (Pisciotta et al. 1967). Onodera et al. used peripheral blood mononuclear cells (PBMCs) extracted from healthy human bodies, including lymphocytes and monocytes, and stimulated them with PHA along with SMF treatment. They found that a 10 T SMF exposure reduced the viability of PHA-activated CD4+ and CD8+ T cell subpopulations, although the 10 T SMF

alone did not affect the viability of either CD4+ or CD8+ T cells (Onodera et al. 2003). Zhu et al. isolated CD8+ T cells from the splenocytes of C57BL/6 mice and exposed CD8+ T cells to 0.3 T SMF. RNA-Seq-based transcriptome analysis found that *Uqcrb* and *Ndufs6* genes related to mitochondrial respiratory electron transport

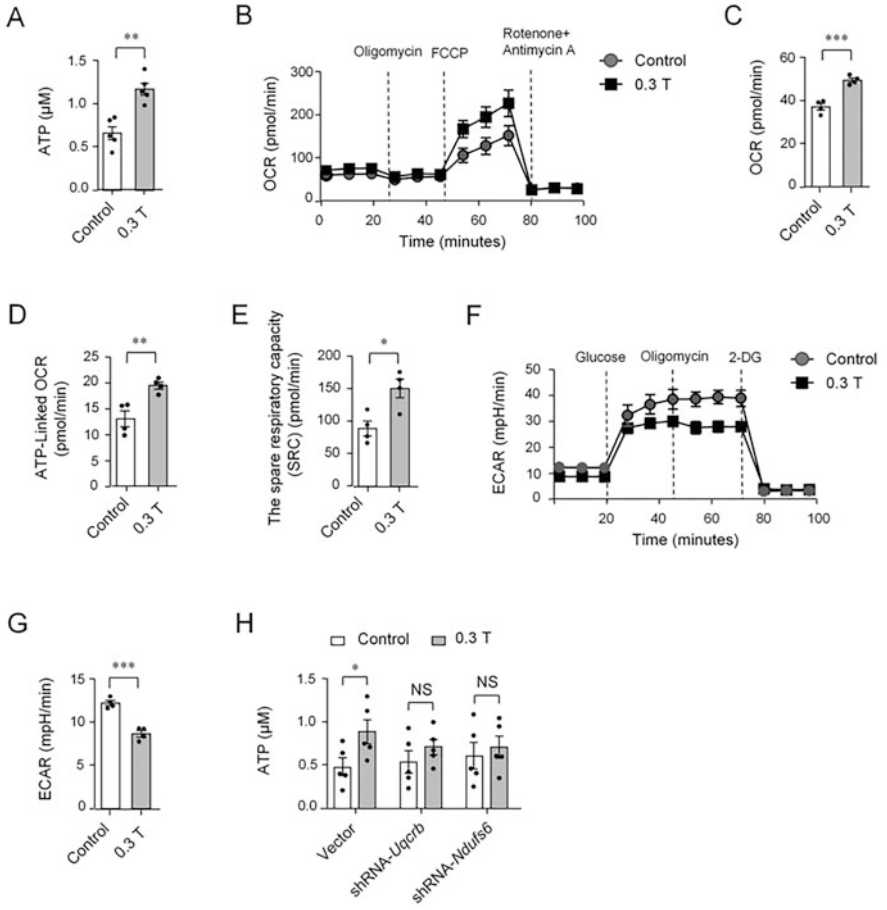

Fig. 12.2 Moderate SMFs improve ATP production and mitochondrial respiration of CD8+ T cells. (**a**) The relative intracellular ATP concentration was measured in CD8+ T cells stimulated with anti-CD3 and anti-CD28 antibodies for 72 h (n=5). (**b**) OCR of stimulated CD8+ T cells at baseline and in response to oligomycin, FCCP, and rotenone with antimycin as detected by the Seahorse MitoStress assay. (**c**) Baseline OCR of stimulated CD8+ T cells (n=4). (**d**) ATP-linked OCR (baseline OCR minus the OCR in the presence of oligomycin) of stimulated CD8+ T cells (n=4). (**e**) The spare respiratory capacity (SRC) of stimulated CD8+ T cells (n=4). (**f**) ECAR of stimulated CD8+ T cells at baseline and in response to glucose, oligomycin, and 2-DG as detected by the Seahorse MitoStress assay. (**g**) Baseline ECAR of stimulated CD8+ T cells (n=4). (**h**) ATP concentration of knockdown CD8+ T cells compared with that in cells transfected with vectors in the presence or absence of magnets (n=5). NS, no significance, *p<0.05, **p<0.01, ***p<0.001 [Reprinted from (Zhu et al. 2020), open access]

chain were upregulated in SMF-treated CD8+ T cells. At the same time, the level of intracellular ATP was increased in SMF-treated CD8+ T cells (Fig. 12.2). They believed that SMF promotes the transcription of *Uqcrb* and *Ndufs6* via candidate magnetoreceptor genes and designed experiments to prove it. And they also found that 0.3 T SMF exposure enhanced CD8+ T cell cytotoxicity and promoted the antitumor function of CD8+ T cells in vivo (Zhu et al. 2020).

As far as we know, there is no reported study about the effect of SMF on B cells yet, and there is only one study that investigated the effects of SMF on NK cells. In 2019, Lin et al. exposed NK92-MI cell lines to a 0.4 T SMF. They assessed the NK cell viability by MTT assay and tested the signaling cascades with inhibitors of DAG/IP3, STAT3, ERK, JNK, and p38 pathway. It was found that a 0.4 T SMF significantly increased the viability of NK92-MI cells by activating multiple MAPK signaling pathways (ERK, JNK, and p38-MAPK), which in turn increased their ability to kill K562 tumor cells (Lin et al. 2019). This suggests that SMF can increase NK cytotoxicity and viability, which might boost the anti-cancer capacity of NK cells.

There are also some researchers directly isolate lymphocytes from human or rat blood when they examine the effect of SMFs, without differentiate the specific types. For example, in 2009, Tenuzzo et al. found that a significant decrement of the apoptotic rate in both freshly isolated and aged lymphocytes when cells were challenged with apoptogenic treatment under 6 mT SMF. Also, when SMF was applied during the aging of lymphocytes (5 days of culture), the rate of spontaneous apoptosis was lowered by SMF treatment. The investigation of the gene expression in freshly isolated and in culture-aged human lymphocytes indicates that SMF exposure for up to 24 h increased bax and p53, and decreased hsp70 and bcl-2 (Tenuzzo et al. 2009). In 2002, Jajta et al. found that when SMFs were combined with drugs, more pronounced apoptosis occurred. For example, Jajte et al. exposed isolated rat lymphocytes to a 7 mT SMF for 3 h and found no significant change in the percentage of apoptotic or necrotic cells. However, when they were exposed to a combination of a 7 mT SMF and $FeCl_2$, the percentage of apoptotic and necrotic cells was significantly increased (Jajte et al. 2002).

12.4 Effects of Static Magnetic Fields on Cytokines

Cytokines, a class of bioactive molecules, are synthesized and secreted by activated immune cells, stromal cells (e.g., vascular endothelial cells, fibroblasts, epithelial cells, etc.), and certain tumor cells after stimulus (Tomar and De 2014). They can be divided into interleukins (IL), interferons (IFN), tumor necrosis factor (TNF), colony stimulating factors (CSF), etc. They have various functions such as regulating the growth of specific tissues and defending against viruses (Dembic 2015). Several studies have explored the effects of SMF on cytokines at the animal and cellular levels (Table 12.3).

Table 12.3 Effects of SMFs on cytokines

Subjects		MFs	Exposure time	Effects	References
Animal level	Healthy male BALB/c mice	30–150 μT SMF + 100 and 200 nT AMF	2 h/day, 14 days	TNF-α, IFN-γ, IL-2 and IL-3↑*	Novoselova et al. (2019)
	Diabetes mellitus Sprague-Dawley rats	4 mT	2 h/day, 8 weeks	VEGF, TGF-β1, TNF-α, and IL-6↓*	Chu et al. (2017)
	Female BALB/C mice with SP2/0 tumor	80 mT	2 h/day, 9 days	TNF↑**	Wu et al. (2000)
Cellular level	Human macrophages	Inhomogeneous, 476 mT	24 h	IL-6, IL-8 and TNF-α↓**	Vergallo et al. (2013)
	Human lymphocytes			IL-6↓*, IL-10↑*	
	C57BL/6 mice CD8+ T cell	0.3 T, upward	24, 48, 72 h	24 h, 48 h no changes; 72 h INF-γ and TNF-α↑***	Zhu et al. (2020)
		0.6 T, upward		24 h, 48 h no changes; 72 h INF-γ↑***; TNF-α↑*	
	Human CD4+ T cell	0.5 mT, 0.5 T	2 h	IFN-γ↓*	Salerno et al. (2006)

h hour
*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, no asterisk means no statistical significance

At animal levels, the cytokine changes are variable in different experiments using different MF setting and animal models. For example, Novoselova et al. investigated the effects of combined 30–150 μT SMFs and 100 or 200 nT alternating MFs (AMF) on cytokine production in healthy BALB/C male mice and observed tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-2 (IL-2), and interleukin-3 (IL-3) levels increase, as well as cytokine aggregation in plasma (Novoselova et al. 2019). Chu et al. exposed diabetic rats to 4 mT SMF and found that the cytokines vascular endothelial growth factor (VEGF), TGF-β1, TNF-α, and IL-6 were significantly reduced in serum (Chu et al. 2017). Wu et al. showed that an 80 mT SMF could increase TNF level, inhibit tumor growth, and improve the mice immune function (Wu et al. 2000).

At the cellular level, the results are also variable in different experimental settings. For example, Vergallo et al. found that a 476 mT inhomogeneous SMF not only inhibited the release of the pro-inflammatory factors IL-6, IL-8, and TNF-α from macrophages, but also inhibited the release of IL-6 and promoted the release of the anti-inflammatory factor IL-10 from lymphocytes (Vergallo et al. 2013). Zhu

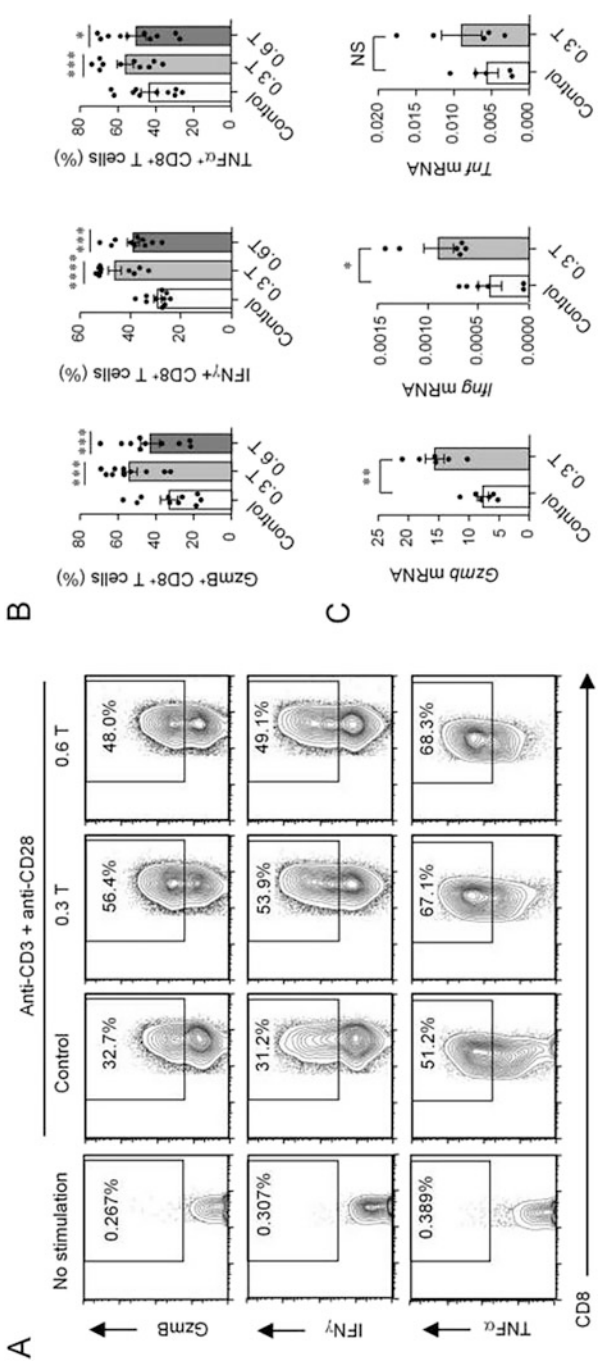

Fig. 12.3 Moderate SMFs enhance CD8+ T cell granule and cytokine secretion at 72 h stimulation. (**a**) Cytokine/granule production of stimulated mouse CD8+ T cells analyzed by flow cytometry. Cell samples were stimulated with anti-CD3 and anti-CD28 antibodies in the presence of 0.3 T or 0.6 T permanent magnets, and control cells were treated without magnets. Cell samples with no stimulation were used to show the baseline of cytokine secretion. (**b**) Percentage statistics for the expression of *GzmB*, IFN-γ and TNFα of CD8+ T cells stimulated for 72 h (B, n = 10). (**c**) Relative transcriptional levels of *Gmzb*, *Tnfa*, and *Ifng* in 0.3 T SMF-treated and control CD8+ T cells (n = 6). The cell samples were stimulated with anti-CD3 and anti-CD28 antibodies for 72 h. All the relative transcription levels of target genes were normalized to β-actin. NS, no significance, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. [Reprinted from (Zhu et al. 2020), open access]

et al. found that CD8+ T cells increased the secretion of IFN-γ and TNF-α after exposure to 0.3 T and 0.6 T SMFs for more than 72 h (Zhu et al. 2020) (Fig. 12.3). Salerno et al. exposed human CD4+ T cells to MFs generated by magnetic resonance units (0.5 T) and double cylindrical coils (0.5 mT) for 2 h and observed a reduction of IFN-γ level (Salerno et al. 2006).

12.5 Static Magnetic Fields May Be Able to Regulate the Immune Function Through Central Nervous System

As early as 1987, it was reported that neuroendocrine influences of the central nervous system modulating immune function, and there are also feedbacks from the immune system to the brain, indicating the central nervous system and immune

Table 12.4 Three studies reported that implanted magnets on the skull could positively affect rat immune systems

Subjects	60 mT SMF[a], N pole facing the skull			Effects		References
	Location		Time			
Female Wistar rats	Anterior to the frontoparietal suture		34 days	CD4+/CD8+ ratio↑	Antibody potency: occipital > frontal and parietal exposure	Janković et al. (1991)
	Posterior to the frontoparietal suture			CD4+/CD8+ ratio↑**		
	Posterior occipitoparietal suture			CD4+/CD8+ ratio↑		
Male Wistar rats	Pineal gland removed	Posterior occipitoparietal suture	25 days	Antibody levels similar to control		Janković et al. (1993b)
	Pineal gland not removed			Thymus weight↑ Antibody levels↑*		
	Locus coeruleus damage	Posterior to the frontoparietal suture	21 days	Thymus weight, CD4+/CD8+ ratio and antibody levels similar to control		Janković et al. (1993a)
	Locus coeruleus undamaged			Thymus weight, CD4+/CD8+ ratio and antibody levels↑*		

*$p < 0.05$; **$p < 0.01$

[a]These three studies used the same type of magnets implanted in mice. It was described that "Micromagnetic beads of convex 'N' polarity and flat 'S' polarity, 5.4 mm in diameter and 2.7 mm thick, of 60 mT (600 Gauss) influx density, and of magnetic field's influence of about 9 mm in depth and 8 mm in width from the axle of the magnet were employed in this study" (Janković et al. 1993a)

system interaction (Solomon 1987). It has been reported by Janković et al. in three studies that magnets of 60 mT implanted on the rat skull may be able to promote the immune function by acting on central nervous system, such as the locus coeruleus and the pineal gland (Table 12.4). In all three studies, they implanted the N pole of the magnet facing the skull. First, in 1991, Janković et al. implanted two magnets bilaterally into the skull anterior to the frontoparietal suture (frontal brain exposure), posterior to the frontoparietal suture (parietal brain exposure), and posterior to the occipitoparietal suture (occipital brain exposure), with the N pole facing the skull, and treated the rats for 34 days. They observed an increase in the cellular ratio of CD4+/CD8+ T cells, higher antibody potency, and enhanced overall immunity to humoral and cell-mediated immune responses (Janković et al. 1991). It is interesting that when the magnets were implanted on the skull occipital region, which is close to vicinity of the pineal gland, the highest immune response was obtained. Next, in 1993, they removed the rat pineal gland and implanted two magnets facing the skull symmetrically behind the occipitoparietal suture and found that the antibody levels in rats with pineal gland excised and implanted with micromagnets are similar to those of control rats, whereas rats with pineal gland unexcised and implanted with micromagnets had increased antibody levels and increased thymus weight (Janković et al. 1993b). This indicates the involvement of pineal gland in this magnet-induced immune regulation. In the same year, Janković et al. also compared rats implanted with two magnets symmetrically behind the frontoparietal suture and found that the cellular ratio of CD4+/CD8+ T cells and antibody levels in rats with damaged locus coeruleus are similar to controls, whereas rats with undamaged locus coeruleus had increased CD4+/CD8+ T cell ratios and antibody levels, and increased thymus weight (Janković et al. 1993a). This indicates the involvement of locus coeruleus in this regulation.

The pineal gland is the part of brain that secretes melatonin (Sapède and Cau 2013). It was reported that one of the most significant melatonin's pleiotropic effects is the regulation of the immune system (Carrillo-Vico et al. 2005). Although MFs have been reported to reduce nocturnal pineal melatonin secretion (Welker et al. 1983), it is unclear whether melatonin plays a key role when the magnets were implanted on the skull occipital region, which requires more experiments to prove. The locus coeruleus is the main site for the synthesis of norepinephrine in the brain (Schwarz and Luo 2015), which is a messenger from the brain to the immune system (Kohm and Sanders 2000). Although there is no research on the effect of MFs on the synthesis of hormones in the locus coeruleus yet, we speculate that MFs may affect the synthesis of hormones in the locus coeruleus and affect the immune status of the body. Although all the above-mentioned points are just speculations for now, which need to be further explored, the three reported studies by Janković et al. proposed an interesting and appealing possibility to regulate the immune system by applying SMF at the central nervous system.

12.6 Conclusion

From the limited reports mentioned above, we can see that SMFs could generate impacts on some aspects of the immune system, including the cell numbers in immune organs, macrophage function, ROS released by neutrophils, lymphocyte apoptosis, NK cell cytotoxicity and cytokines levels, etc. It is interesting that a few studies indicate that moderate SMF may be able to promote the immune response more toward the anti-inflammatory direction by regulating macrophages, increase the cancer-killing capacity of T cells and NK cells, as well as modulating the immune system through distally applied magnet on the skull. Moreover, it seems that moderate to strong SMFs may affect the apoptosis of aged or drug-treated, affecting dividing, but not nondividing immune cells, which reveals the potential to explore the combination of SMFs with other treatment methods. However, it is apparent that there are still too few studies about SMFs on immune system for us to draw any explicit conclusions. Our chapter here is just to provide a starting point for people that are interested in this aspect. How SMFs of different parameters, including from weak to high SMFs with low to high gradient, can affect the detailed immune responses, including different subpopulations of T, B, and NK cells are still unclear. Whether SMFs can affect some autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, and urticaria are unknown either. Therefore, people are encouraged to perform more well controlled double-blinded experiments to unravel the potential applications of SMF on immune system, which will undoubtably provide the basis for multiple physiological and pathological conditions of human health in the future.

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Chapter 13
Biological Effects of Static Magnetic Fields on the Nervous System

Yue Lv and Xin Zhang

Abstract The applications of transcranial magnetic stimulation (TMS) and magnetoencephalography (MEG) have demonstrated the interconnected relationship between time-varying magnetic fields (MFs) and the nervous system. Moreover, in recent years, dozens of studies have shown that static magnetic fields (SMFs) could also influence the nervous system of animals and humans, either positively or negatively. For example, some studies have shown that SMFs of certain parameters could have some analgesic effects, while high-field magnetic resonance imaging (MRI) could induce transient dizziness, nausea, and vertigo in some people. However, the specific effects of SMFs on the nervous systems have not been systematically explored or reviewed due to the diversity of magnetic parameters, research objects, and detection standards. This chapter focuses on the SMF effects on the nervous system at cellular, animal, and human levels, which will help to understand the influence of SMFs on the nervous system, and lay a foundation for promoting the development of high-field MRI and the potential application of SMFs in nervous system diseases.

Keywords Magnetic field (MF) · Static magnetic field (SMF) · Nervous system · Animal behaviors · Magnetic resonance imaging (MRI)

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13.1 Introduction

The nervous system in our human bodies affects every aspect of us, not only including our conscious movement and thinking, but also the unconscious breathing and digestion. Most vertebrates, including birds, reptiles, and mammals, have both a central nervous system (the brain and spinal cord) and a peripheral nervous system (the cranial, spinal, and splanchnic nerves).

Nervous systems are composed of two types of cells, nerve cells and glial cells. Nerve cells are also called neurons, which are individual specialized cells that serve as the basic building blocks and structural unit of the nervous system. They can be divided into motor neurons (receive signals from the brain and spinal cord and transmit them to relevant organs) and sensory neurons (receive signals from the sensory organs and transmit them to the brain and spinal cord), depending on their function. Glial cells include multiple cell types, such as astrocytes, oligodendrocytes, microglia, ependymal cells, and radial glia in the central nervous system, as well as Schwann cells and satellite cells in the peripheral nervous system. Glial cells do not transmit signals themselves, but can provide supporting functions for the neurons.

From the structural and functional point of view, nerve is an enclosed bundle of axons in the peripheral nervous system that communicates with the central nervous system. There are three types of nerves: afferent (transmit signals from the sensory organs to the central nervous system), efferent (transmit signals from the central nervous system to muscles and glands), and mixed nerves (transmits signals between the two). It should be pointed out that there are two terms in the nervous system that seem to be confusing, neuronal cells and neural cells. Neural cells not only include neuronal cells, but also include glia cells.

There are many intertwining links between the nervous system with magnetic field (MF). For example, magnetoencephalography (MEG), a noninvasive technique that captures the MFs generated by synchronized intraneuronal electrical activity of human brain, can provide unique information about the electrophysiological brain activity (Baillet 2017; Stefan and Trinka 2017). Transcranial magnetic stimulation (TMS), an electromagnetic technique stimulates small regions of the brain to diagnose or treat multiple neuronal diseases, such as stroke and depression (Hallett 2007; Rossi et al. 2009; Pitcher et al. 2021). Besides, magnetic resonance imaging (MRI) is a safe and widely used noninvasive diagnostic technique, but can cause some transient neuronal side effects on patient, such as dizziness, nausea, and vertigo (Heilmaier et al. 2011). Moreover, it is very interesting that multiple studies have reported the correlation of suicide with geomagnetic field disturbance caused by solar storm (Partonen et al. 2004; Berk et al. 2006; Nishimura et al. 2020).

Although the above-mentioned cases are not exactly caused by static magnetic fields (SMFs), which has a constant magnetic field over a certain period of time, they have demonstrated multiple entangled connections between the nervous systems with magnetic fields. In this chapter, we will summarize the SMF effects on the nervous system, aiming to recapitulate the known facts and provide a starting point for future exploration.

13.2 Effects of Static Magnetic Fields on Neural Cells

It should be noted that neural cells not only include neuronal cells, but also include glia cells. The effects of SMF on neural cells are variable, which can be divided into three categories: positive effects, no obvious effects, and negative effects (Table 13.1).

13.2.1 Some Static Magnetic Fields Can Promote Neural Cell Functions

As early as 1999, Pacini et al. reported that the normal human neuronal cell line FNC-B4 underwent significant changes in cell morphology after exposure to a SMF generated by a 200 mT MRI for 15 min, revealing branching neurites with synaptic features. The emergence of branching neurites and increased synaptic connections are considered hallmarks of neurological transformation, suggesting SMFs contribute to neuronal differentiation and enhance neuronal plasticity (Pacini et al. 1999). In 2009, a study exposed neonatal rats to a 100 mT SMF for 12 days and found the SMF increased expression of Mash1 in isolated neural precursor cells in the brain's neocortex and hippocampus. The mRNA expression of activated neurogenic genes such as Math1 and Math3 can promote differentiation of neural precursor cells into neurons (Nakamichi et al. 2009). In 2010, Wang et al. found that 250 mT SMF could affect the adenosine A_{2A} receptor ($A_{2A}R$) involved in the pathogenesis of Parkinson's disease (PD) to produce a similar effect as $A_{2A}R$-selective antagonist named ZM241385, a potential nondopaminergic PD drug, which offset some of the PD-related endpoints exacerbated by the $A_{2A}R$ agonist CGS21680 (Wang et al. 2010).

As reported in 2017, Prasad et al. exposed human oligodendrocyte precursor cells (OPCs) to a 300 mT SMF for 2 h a day for 2 weeks, and found that it promoted the differentiation of OPCs by enhancing its myelination capability and the secretion of nerve-influencing factors (BDNF, NT3) and increasing intracellular calcium influx and the gene expression of L-type channel subunits-CaV1.2 and CaV1.3 (Prasad et al. 2017). In 2019, Shih-Yin Ho et al. found that mice exposed to 500 mT MFs for 7 days had a significant increase in the number of neurosphere formation in mice neural progenitor cells (NPCs), accompanied by increased expression of Sox2 and Cyclin B. Furthermore, SMF promoted mice NPCs differentiation toward neuronal lineage and displayed a significant increase in degrees of morphological and electrophysiological maturity (Ho et al. 2019).

The positive effect was also observed in studies of MF effects on non-mammalian neurons. In 2008, after exposing *Tenebrio pupa* to 320 mT SMF for 8 days, it was discovered that the morphological parameters of the forebrain A1 and A2 neuroendocrine cells had been significantly changed (Peric-Mataruga et al. 2008). Similarly, Nikolic et al. exposed Br neurons in the lower esophageal compound ganglia of

Table 13.1 Effects of static magnetic fields on neural cells

Objects	SMF		Effects on nervous system		References
	Intensity	Time	Specific effects		
Br neurons of the snail inferior esophageal nerve complex	2.7 mT, 10 mT	15 min	Enhanced action potential amplitude, shortened spike time	Positive	Nikolic et al. (2008)
The lateral LG neurons of the crayfish tail	8.08 mT	30 min	Increased action potentials, excitatory synaptic potentials, and synaptic transmission efficiency		Yeh et al. (2008)
The neural precursor cells of the Wistar rat	100 mT	12 days	Decreased NPCs self-proliferation and astrocyte differentiation, increased neuronal differentiation, Mash1, Math1, and Math3 expression		Nakamichi et al. (2009)
Human neuronal cell line FNC-B4	200 mT	15 min	Promoted neuron differentiation and synapse formation		Pacini et al. (1999)
PC12 cells	250 mT	6 h	Produced a similar effect as $A_{2A}R$-selective antagonist		Wang et al. (2010)
Human oligodendrocyte precursor cell	300 mT	2 h/day, 2 weeks	Increased cell differentiation, BDNF, NT3, Ca^{2+} influx, the gene expression of L-type channel subunits-CaV1.2 and CaV1.3		Prasad et al. (2017)
Tenebrio pupa forebrain neurosecretory neurons	320 mT	8 days	Cells and nuclei of neurons enlarged as morphology changes		Peric-Mataruga et al. (2008)
The neural precursor cells of the ICR mice	500 mT	7 days	Increased neurosphere number, cyclin B/Sox2 expression, promoted neural lineage differentiation and neuronal maturation		Ho et al. (2019)
Rat astrocytes	1 mT	1 h	No significant effect on morphology, proliferation, or expression of heat shock proteins and actin	No obvious effect	Bodega et al. (2005)
Rat neocortex and hippocampal astrocytes	100 mT	7 days	No significant effect of cell viability, GFAP or PNCA expression		Hirai and Yoneda (2004)
Frog's sciatic nerve fibers	0.21 T 2.6 T/m	6 h	No significant effect on nerve conduction		Okano et al. (2012)

(continued)

Table 13.1 (continued)

Objects	SMF		Effects on nervous system		References
	Intensity	Time	Specific effects		
Sciatic nerve of the rat	1 T	12 h/day, 4 weeks	No significant effect on sciatic nerve regeneration		Cordeiro et al. (1989)
Motor neurons in chicken embryos	1.5 T	6 h	No significant effect on proliferation and migration in motor neuron cells		Yip et al. (1994)
Astrocytes in the rat spinal cord	2.1 T	2 h, 72 h	No significant effect on astrocyte morphology or activity		Khodarahmi et al. (2010)
Neural stem cells in the DG subgranular cell layer of mouse hippocampus	<5 μT	8 weeks	Inhibition of adult neural stem cell proliferation	Negative	Zhang et al. (2021)
SD rat cerebral cortex astrocytes	0.5 mT	6 days	Increased astrocyte apoptosis and necrosis		Buemi et al. (2001)
Spinal cord of adult Guinea pig	500 mT	10 min	Reduced compound action potential, unchanged response latency		Coots et al. (2004)
Frog's sciatic nerve fibers	0.7 T 6.47 T/m	4–6 h	Inhibition of nerve conduction		Okano et al. (2012)
Large interneurons in the antennal lobe of *Drosophila melanogaster*	3 T	8 h	Reduced the amplitude and frequency of neuronal action potentials and the average frequency of spontaneous extracellular activity		Yang et al. (2011)
Embryonic mice	15 T	30 min	Death of neurons in the hippocampus and inhibition of neuronal differentiation in the remaining cells		Valiron et al. (2005)

h hour, *min* minute

Roman snails to 2.7 and 10 mT SMFs for 15 min and found that they both enhanced the amplitude of action potentials of Br neurons, shortened the duration of action potential spikes (Nikolic et al. 2008). Moreover, the 10 mT SMF also altered their resting membrane potential. Next, they used 10 mT SMF to treat Br neurons for 15 min and found significantly increased expression of the sodium–potassium pump alpha subunit in the plasma membrane of neurons and potassium pump activity (Nikolic et al. 2013). Additionally, Yeh et al. showed that the action potentials and excitatory postsynaptic potentials are enhanced in lateral giant neurons of the isolated ganglion of crayfish exposed to a 8.08 mT SMF for 30 min (Yeh et al. 2008).

13.2.2 Some Static Magnetic Fields Have No Obvious Effect on Neural Cells

As early as 1989, Cordeiro et al. exposed 44 injured rat sciatic nerves to a 1 mT SMF for 12 h per day for 4 consecutive weeks and did not find significant effect of the MF on sciatic nerve regeneration (Cordeiro et al. 1989). In 1994, it was found that a 1500 mT SMF exposure for 6 h did not affect the proliferation and migration of lateral motor neurons in chick embryos (Yip et al. 1994). In 2004, Hirai and Yoneda found no significant changes in the survival rate of astrocytes in the rat neocortex and hippocampus after exposure to a 100 mT SMF for 7 days. However, there were significant changes in the expression of glial fibrillary acidic protein (GFAP) and proliferating cell nuclear antigen (PNCA), along with neuronal marker protein microtubule associated protein-2 (MAP 2) (Hirai and Yoneda 2004). Moreover, Khodarahmi et al. found that the exposure to a 2.1 T SMF for 2 h or 72 h had no significant effect on the morphology and activity of in situ astrocytes in the rat spinal cord either (Khodarahmi et al. 2010).

13.2.3 Some SMFs Inhibit Neural Cell Functions

In 2001, a study reported that after rat astrocytes were subjected to a weak SMF of 0.5 mT for 6 days, there was an increase in apoptosis and necrosis (Buemi et al. 2001). In 2004, Coots et al. reported significantly reduced compound action potential amplitudes in the spinal cord of adult guinea pigs after their spines were exposed to a 500 mT SMF for 10 min (Coots et al. 2004). In 2005, Valiron et al. showed that exposure to a SMF of exceeding 15 T for 30 min or longer resulted in neuronal death in the hippocampus of embryonic mice and interfered with neural differentiation of the remaining cells (Valiron et al. 2005). In 2011, Yang et al. exposed local large interneurons in the antennal lobe of *Drosophila melanogaster* to a 3 T SMF for 8 h and found it interfered with the spontaneous neural activity of the neurons, including a reduction in the amplitude and frequency of action potentials, a reduction in the average frequency of extracellular spontaneous activity (Yang et al. 2011). In 2012, it was found that after 6 h exposure of gradient SMF of 0.2–0.7 T, the conduction velocity of C fibers in the isolated sciatic nerve of adult male African clawed frogs was inhibited by 0.7 T SMF, but not 0.21 T SMF, which provided a basis for the analgesic effect of moderate SMFs (Okano et al. 2012).

13.3 Effects of Static Magnetic Fields on Animal Behaviors

Besides the investigations of SMF on neural cells, there are also many studies that have observed animal behaviors because an individual's behavior is mostly controlled by the nervous system.

13.3.1 The Behavioral Effects of SMFs Exposure on Rodents

Rodents are extensively used by researchers to study the behavioral effects of SMFs. The behavioral abilities that are commonly observed and evaluated include balance ability, social behavior, exploration behavior, activity ability, anxiety-like behavior, depression-like behavior, and behavior that shows spatial learning and memory ability, as well as pain-related behaviors.

13.3.1.1 Balance Ability

The balance ability is mainly reflected in behaviors such as running, walking, and turning, which are affected by factors such as motor coordination, vestibular function, and muscle strength. Researchers found that after exposure to a SMF of 3.5–23.0 T for 2 h, C57BL/6 mice's balance was temporarily impaired, and the time to reach the designated position on the balance beam was significantly prolonged (Khan et al. 2022). In a study by Tkac et al. mice were exposed to 16.4 T and 10.5 T SMFs, and three balance beams with different size and shape were used to investigate their balance ability. They found that the foot slips of mice in the 16.4 T SMF exposure group were significantly different from the control group on 15 mm and 8 mm square balance beam, but not on the 17 mm diameter round beams. Mice exposed to 10.5 T SMF had no significant difference in balance beam test results across all three specifications compared to control mice. Moreover, the researchers also studied the balance abilities of the mice in the SMF "motion" group that entered and exited the 16.4 T magnet 20 times in 2 min. The 15 mm square balance beam and 17 mm round balance beam tests were significantly longer than the control group, but the 8 mm square balance beam test results were not significantly changed (Tkac et al. 2021). Therefore, the strength of the SMF, the way the animals are exposed, as well as the instruments of the test can all influence the test results.

Balance ability is also considered to be an indicator of vestibular organ function. When the vestibular system is impaired or disturbed, dizziness or imbalance in the body occurs, increasing the risk of falls and injuries (Agrawal et al. 2009). The vestibular system has been shown to be susceptible to MF disturbances, leading to behavioral changes in animals. For example, Houpt et al. placed adult Sprague-Dawley rats in a superconducting magnet with a 14.1 T SMF in the center and a gradient of 50 T/m and found that the SMFs inhibited rearing behavior and induced conditioned taste aversion, and 30 min of continuous exposure to the SMF also led to circling behavior in rats (Houpt et al. 2011). It is also consistent with clinical observations of dizziness and altered taste in MRI subjects. Further research found that the angle formed by the animal's head and the direction of the MF could affect their circling behavior. If the rostral axis of the mouse is in the same (0°) or opposite (180°) direction as the MF, the mice exhibit obvious counterclockwise or clockwise circle behaviors. This change in animal behavior may be the result of a response of the vestibular nervous system to strong SMFs (Houpt et al. 2013) and also regulated

by estradiol and accompanied by upregulation of c-Fos levels in brainstem regions closely related to vestibular function (Houpt et al. 2007). Moreover, this response is blocked by chemical labyrinthectomy, showing that the integrity of the vestibular system of the inner ear is important for this MF response (Cason et al. 2009).

13.3.1.2 Social Behaviors

In addition, animal social behaviors are a series of complex and important social interactions between individuals. Social cognition is an important component of social behavior. At the same time, social interaction and social cognition are essential to maintaining the structure and stability of social communication within animal groups (Berry and Bronson 1992). When social behavior is impaired, individuals are at risk for many neurological disorders, such as depression, bipolar disorder, schizophrenia, autism spectrum disorder, and obsessive-compulsive disorder (Battle 2013). In 2013, Kiss et al. investigated the effect of 145 mT homogeneous and 3–477 mT inhomogeneous SMF on social behavior in pain model mice by using a special magnet device half full of rubidium magnets. They found that the SMF group mice had a reduced pain level and boosted sociability (Kiss et al. 2013). In our recent research, we used a three-chambered social test and found that the social novel index of healthy mice was significantly increased after exposure to a 11.0–33.0 T SMF (Fig. 13.1), which indicated that the SMF significantly improved mice social abilities (Lv et al. 2021). Similarly, 9.4 T SMF had a positive effect of sociality on imatinib-treated mice with the social index significantly increased (Tian et al. 2022).

13.3.1.3 Anxiety and Depression Levels

Exploration behavior is an innate behavior for many species primarily for searching food and shelter. In rodents, higher levels of anxiety were often associated with less self-exploration and more depression-like behaviors. In 2008, Ammari et al. found increased anxiety levels of rats exposed to a SMF of 128 mT for 1 h/day for 5 days (Ammari et al. 2008). Laszlo et al. found that there were no statistically significant differences on anxiety levels between SMF-exposed mice (30 min, 2–754 mT) and the control group mice (Laszlo et al. 2009). According to a recent study, after 30 days of continuous exposure to a SMF with an average gradient of 10 mT/cm, the anxiety levels were lower in male rats of spontaneously hypertensive model exposed to the SMF than in the control group. Moreover, the effect of the downward direction SMF was more significant than that of the upward direction (Tasic et al. 2021). Besides, Shuo et al. found that the anxiety levels of Wistar rats were increased after exposing to 200 mT SMF continuously for 15 days (1 h/day) with abnormality of glucose metabolism (downregulation of HK1 and PFK1) and pathological changes in the brain (pyknosis, edema of neurons, and slight widening of the perivascular space) (Shuo et al. 2021). Our study showed that 11.0–33.0 T SMF

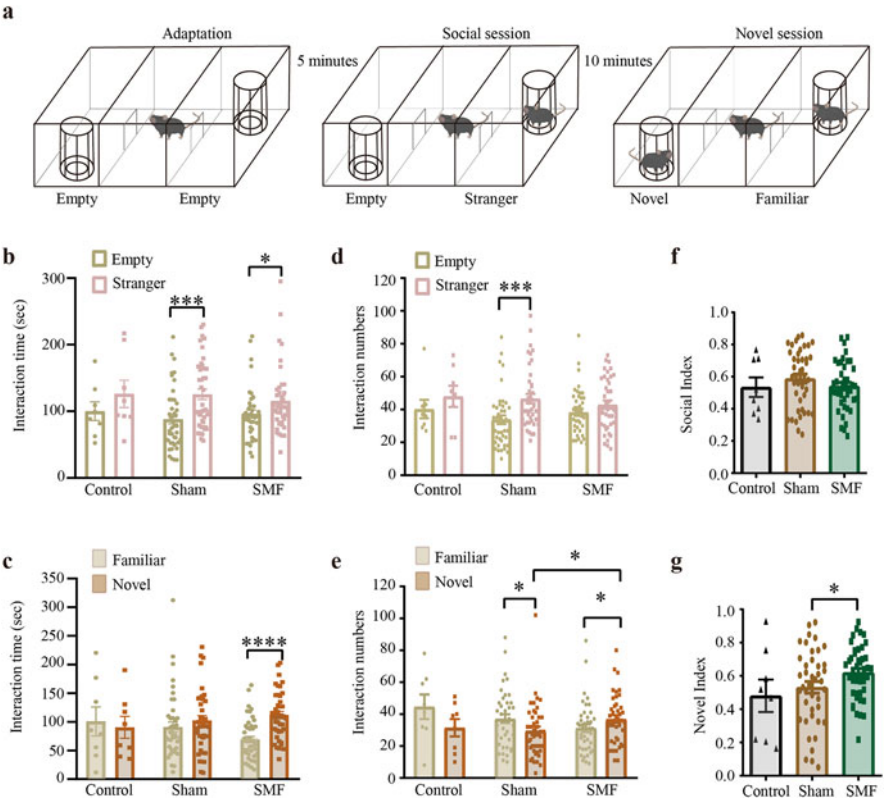

Fig. 13.1 Ultra-high SMF improved social ability for novelty. (**a**) Illustration of three-chambered social test device. (**b**, **d**) Interaction time and numbers with strange mouse and empty cage in social session. (**c**, **e**) Interaction time and numbers with familiar mouse and novel juvenile mouse in novel session. (**f**, **g**) Social index in social session and novel index in novel session, respectively. [Reprinted with permission from (Lv et al. 2021)]

exposure decreased the anxiety-like and depression-like behaviors of healthy C57BL/6 mice (Lv et al. 2021). Our group also have found that 9.4 T SMF enhances the anti-anxiety and anti-depression levels and exploratory activities in imatinib mesylate treated tumor-bearing mice (Tian et al. 2022). It seems that the specific effects of SMF on rodents' anxiety, depression, and exploration behavior were influenced by a variety of factors, including SMF parameters, exposure time, and experimental subjects.

13.3.1.4 Spatial Learning and Memory

The ability to learn and memorize spatial information is an essential survival skill for almost all animals. Morris water maze test is frequently used for measuring the

learning and spatial memory of rodents. Ammari et al. found that 128 mT SMF exposure resulted in poor performance in the Morris water maze test in rats, indicating spatial memory impairment (Ammari et al. 2008). Similarly, Tkac et al. found a significantly longer escape latency in the water maze after 4 weeks of chronic exposure to a 16.4 T SMF than in the control group. But the 10.5 T SMF did not result in any significant changes in the Morris water maze test (Tkac et al. 2021). However, the study of Khan et al. showed that the mice in the SMF-exposed group had a much shorter escape latency compared with the mice in the sham exposure group, which was accompanied by an elevated expression of calcium/calmodulin-dependent protein kinase II (CaMKII) (Khan et al. 2022). Our study also showed that 11.0–33.0 T SMF can improve the performance of healthy C57BL/6 mice in Morris water maze test, which suggested that high SMF can improve the learning and spatial memory of mice (Lv et al. 2021).

13.3.1.5 Pain-Related Behaviors

Besides the above-mentioned behavioral tests that are frequently used to monitor the nervous function of rodents, there are some other behavioral tests for pain levels (Fan et al. 2021). For example, writhing experiment is widely used to test the pain level and analgesic effect of drugs or other treatments in rodents. Gyires et al. reported that the analgesic effect of 1.6 mT and 0.16 T/m inhomogeneous SMF can work as effective as opioids in 0.6% acetic acid-induced writhing experiment in mice (Gyires et al. 2008). In addition, directed mouth wiping behavior can be used as a reliable measure of pain after tooth movement in experimental rats (Yang et al. 2009). Zhu et al. found that SMF can reduce pain levels in mice trigeminal ganglion and downregulate P2X3 receptors, which play important roles in the development and maintenance of tooth movement pain (Zhu et al. 2017).

13.3.2 The Behavioral Effects of Static Magnetic Field Exposure on Zebrafish

Zebrafish is also a good model organism that has been used by researchers to study the effects of SMFs on their behavior. For example, in 2014, Ward et al. exposed adult zebrafish to 4.7 T SMF in the horizontal direction and 11.7 T SMF in the vertical direction for 2 min, and found increased swimming speed, frequent circling, tumbling, diving, and other behaviors, independent of visual and lateral line hair cell function (Ward et al. 2014). In 2016, Pais-Roldan et al. found that exposure to 14 T SMF for 2 h induced fusion of the otoliths in zebrafish larvae. Consequently, this altered the larvae's swimming behaviors, including reduced activity, rotational movements, and inability to maintain normal swimming posture. This finding suggests that otolith fusion directly affects the larvae's swimming ability and

balance. SMF can lead to otolith fusion in zebrafish, which also provides a new idea for finding magnetoreceptors in vertebrates (Pais-Roldan et al. 2016). Ge et al. reported that free swimming of zebrafish larvae was not affected by a 9.4 T SMF exposure for 24 h, except for finer visual functions such as delayed response development. However, this developmental delay of zebrafish larvae disappeared after 1 day of returning to a normal environment (Fig. 13.2) (Ge et al. 2019).

13.3.3 The Behavioral Effects of Static Magnetic Field Exposure on Other Animals

Besides rodents and zebrafish, researchers have also studied the behavior of other animals in SMF. For example, Rosen and Lubowsky found that after 50 s of 0.12 T SMF exposure, the amplitude and variability of visual evoked responses in adult cats significantly decreased, indicating a marked decrease in striatal cortex excitability (Rosen and Lubowsky 1987). Using 0.5 T neodymium magnets, Aguila et al. reported the effects of SMF on the nervous systems of awake macaques and cats under anesthesia with transcranial static magnetic field stimulation (tSMS). They found that tSMS reduces cortical excitability in macaques and decreased neuronal responses in cats by reversibly altering cortical perception and neuronal activity (Aguila et al. 2016).

13.4 Effects of Static Magnetic Fields on the Nervous System in Humans

13.4.1 MRI-Related Studies

Currently, high-field SMFs have been demonstrated to transiently affect the human nervous system and cause neurological symptoms or influence behavior. Subjects often experience some discomfort following the MRI examination, such as transient but severe vertigo, nystagmus, metallic taste, and tingling in and around the machine (Heilmaier et al. 2011). It has been proposed that the vertigo, nystagmus, and metallic taste are related to the interference of SMF with vestibular system, and the tingling sensation is related to the electric fields in nerves and muscles by rapid changes in gradient MFs (De Wilde et al. 2005; Kim and Kim 2017). Additionally, these symptoms are also worsened with higher field of MF, so individuals in 7 T MRI may experience more discomfort than in 1.5 T MRI (Hoff et al. 2019). Staff near the MRI machine also reported transient vertigo and balance problems (Walker et al. 2020). In 2013, 41 healthy subjects underwent extensive neuropsychological testing, such as memory, hand-eye coordination, and attention following MRI at different MF strengths (1.5 T, 3.0 T, and 7.0 T). During the examination, although

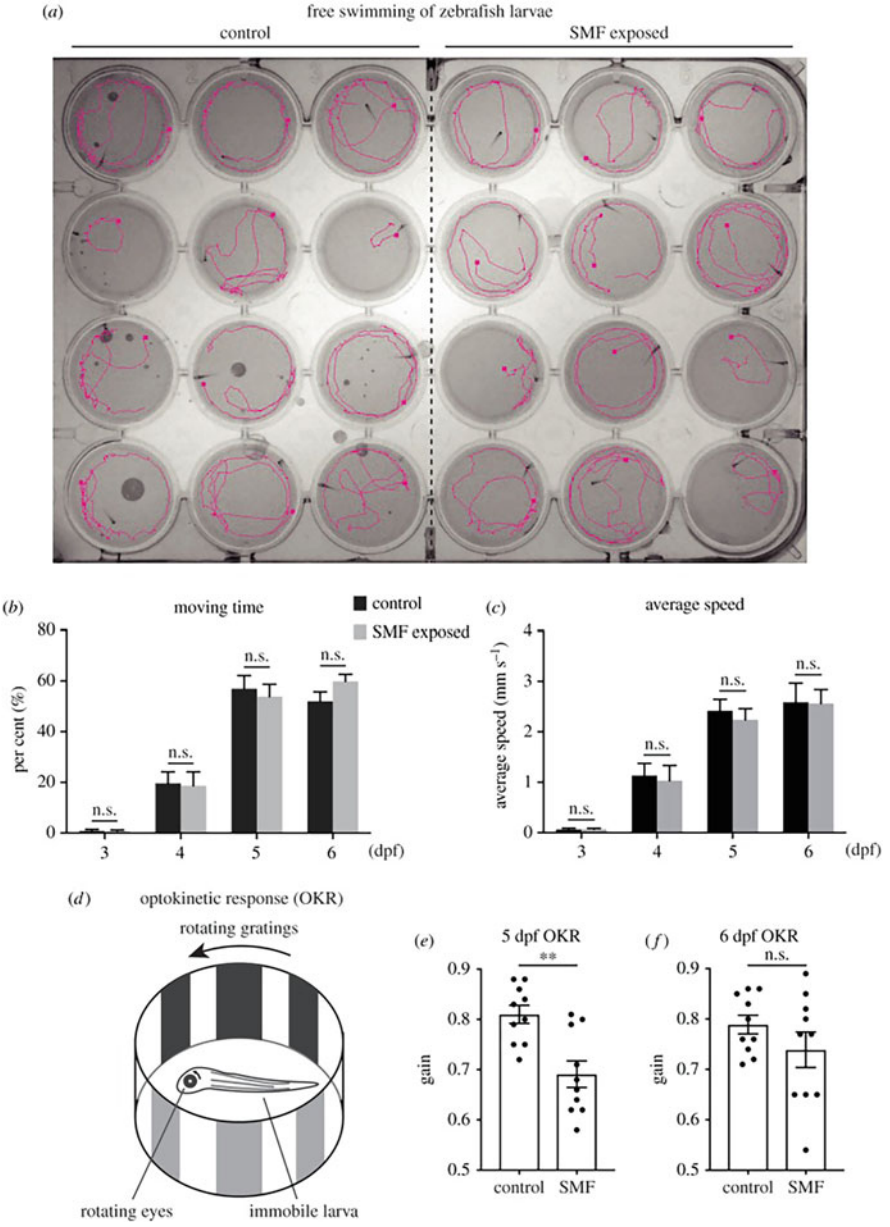

Fig. 13.2 Behavioral effects of 9.4 T SMF on zebrafish. (**a**) The red line represents the zebrafish larvae motion in 1 min; (**b**, **c**) the average swimming time and speed of zebrafish in the free-swimming experiment, respectively; (**d**) schematic diagram of the eye movement experiment in zebrafish; (**e**) the results of the eye movement experiment in zebrafish larvae on the fifth and sixth day after fertilization. [Reprinted from (Ge et al. 2019), open access]

subjects experienced transient symptoms such as dizziness, nystagmus, phosphenes, and tinnitus, none of the three types of MRIs caused severe impairment to the subjects' cognitive functions (Heinrich et al. 2013). It is interesting that when researchers compared 10 healthy and 2 patients lacking labyrinthine function in SMF generated by 3 T and 7 T MRI for their eye movement and stinging, they found that all healthy subjects experienced intense nystagmus in MRI, but not the patients with no labyrinthine function. This demonstrates the importance of the labyrinth for SMF-induced nystagmus. It also showed that nystagmus intensity was not only proportional to MF intensity, but also related to MF direction and subject's head orientation (Roberts et al. 2011).

At present, the safety studies of high-field MRI show that SMFs generated by high-field MRI are relatively safe for human nervous system. The international safety standard of SMF exposures is introduced in Chap. 8 of this book, Tables 8.1 and 8.2, which set the up limit of SMF exposure to 8 T. Moreover, researchers found that exposure to a SMF of 9.4 T generated by MRI did not significantly affect vital signs or cognitive performance in healthy volunteers (Atkinson et al. 2007). In 2020, a study found that cognitive functions like fatigue, executive ability, and working memory were not significantly impacted by 10.5 T high-field MRI, except for eye movement responses and metallic taste (Fig. 13.3). Among them, metallic taste is obviously related to field intensity, which increases with the increase of field intensity, and vertigo sensation has a weak trend with field intensity. However, it is interesting that lightheadedness, nervousness, double vision, warm/cold feeling were all reduced with increased field strengths (Fig. 13.4) (Grant et al. 2020).

Moreover, for pregnant women and newborns, a Canadian clinical report found no significant increase in congenital developmental abnormalities among pregnant women undergoing MRI in the first 3 months of pregnancy (Ray et al. 2016). For ultra-high-field MRI, Budinger and Bird pointed out that there are no foreseeable

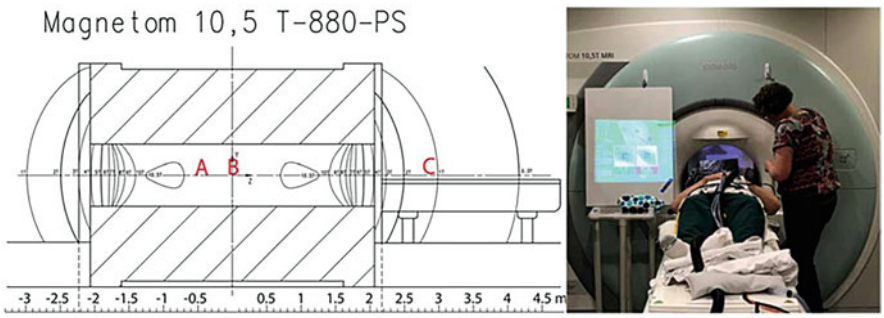

Fig. 13.3 Field plot for 10.5 T system (left) and photograph of one subject prior to an imaging study (right). The field plot shows the contour line of 1–10 T; the isocenter is at 10.498 T, and the small bubbles labeled 10.5 T are above 10.5 T. For body studies, the subject's head was approximately at position A; for head studies, the subject's head was in the center of the position B; supine physiological monitoring was also done at "home," with the table out; the subject's head was located approximately at position C exposed to about 1 T. [Reprinted with permission from (Grant et al. 2020)]

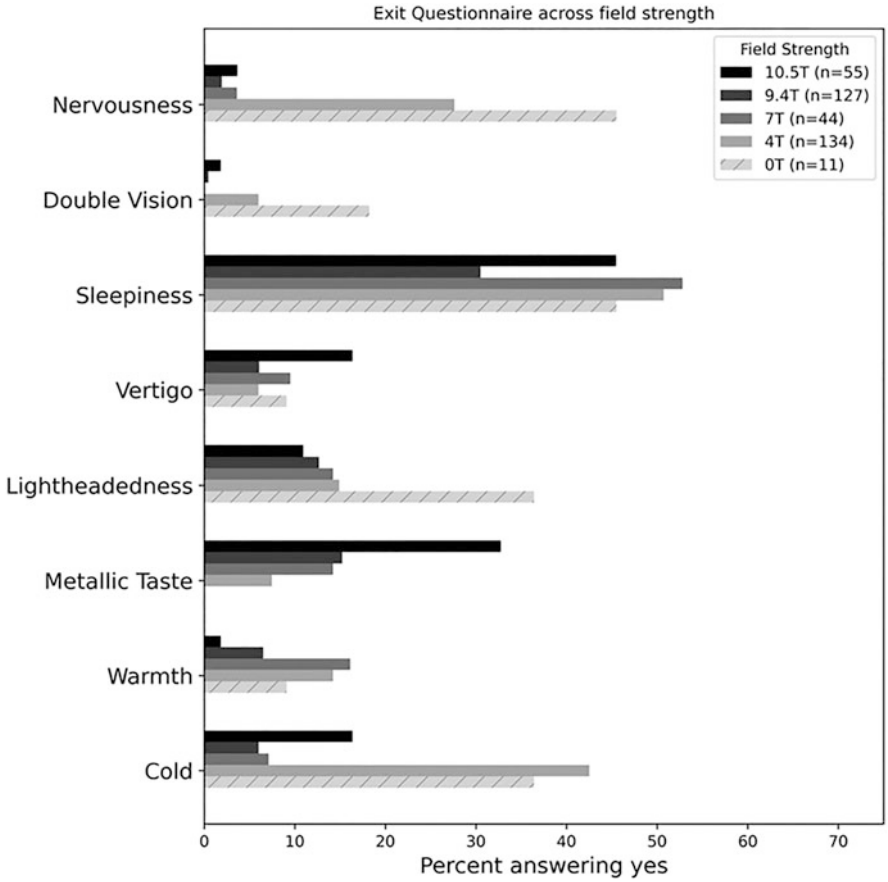

Fig. 13.4 Exit questionnaire results from the facility for 0 T (ramped down 4 T), 4 T, 7 T, 9.4 T, and 10.5 T. [Reprinted with permission from (Grant et al. 2020)]

barriers to brain MRI and MRS (magnetic resonance spectroscopy) in the field below 20 T, both in terms of technology and human safety (Budinger and Bird 2018).

13.4.2 Other Studies of Static Magnetic Field Effects on Human Nervous Systems

Besides MRI, there are also some other studies that have examined the SMF effects on the nervous systems. For example, there are some human studies that have investigated the effects of SMFs on pain, which have been reviewed by our group in 2021 (Fan et al. 2021). We did a meta-analysis of seven trials that assessed the analgesic effect of SMF by pain score (Fig. 13.5). The pooled estimate of the effect

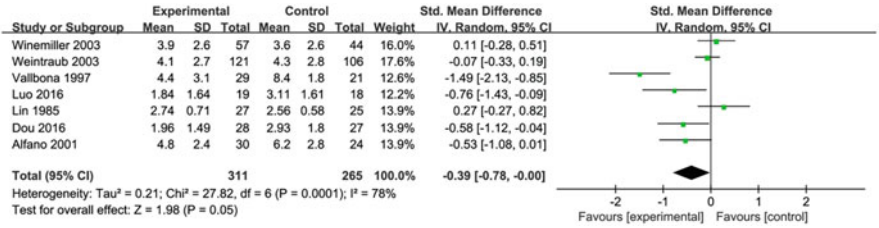

Fig. 13.5 Forest plot of the analgesic effects of static magnetic fields. The magnetic flux densities in these studies range from~0.02 to 0.4 T. [Reprinted with permission from (Fan et al. 2021)]

between SMF treatment and the placebo control had marginal significance, which suggested that SMF treatment does have moderate pain relief effect. Moreover, we recently found that increased MF flux density can directly and significantly increase the analgesic effect on multiple mice pain models (unpublished data).

Researchers have also attempted to explore the effects of SMFs on the human nervous system using tSMS. For example, in 2011, Oliviero et al. recorded single-pulse TMS-evoked motor potentials in the motor cortex of 11 awake subjects before and after 10 min of tSMS. They found an average 25% reduction in motor cortex excitability that persisted for several minutes after the end of tSMS, which was related to field strength (Oliviero et al. 2011). People found that tSMS reduces motor cortex excitability in the human anterior central cortex (Dileone et al. 2018) and can transiently alter the intracortical inhibitory system (Nojima et al. 2015). Moreover, it has been reported that moderate SMF could regulate immune system through central nervous system (Janković et al. 1991, 1993a, b), which has been discussed in details in Chap. 12 of this book.

13.5 Discussion

Currently, there are some studies that have tried to unravel how SMFs affect the nervous system. Among them, the most well studied are the SMF-induced nystagmus and vertigo through vestibular system. Magnetic vestibular stimulation is generated by the interaction of MFs with ionic currents naturally occurring in the lymphatic fluid inside the labyrinth. Roberts et al. described this in more detail. They suggest that the MF generates Lorentz forces that push against the apex of the semicircular canals, resulting in nystagmus, and emphasize the dual role of endolymph in the transmission of ionic current and fluid pressure. At the same time, the cup-like vestibular organ acts as a pressure sensor, allowing MFs to cause nystagmus and vertigo (Fig. 13.6) (Roberts et al. 2011). Researchers believe the vestibular structure and function have great significance in the study of the nervous system and MF responses of animals and humans (Houpt et al. 2007; Cason et al. 2009).

At the cellular level, the results are variable. For example, it has been demonstrated that extracellular regulated protein kinases (ERK) and c-Jun N-terminal

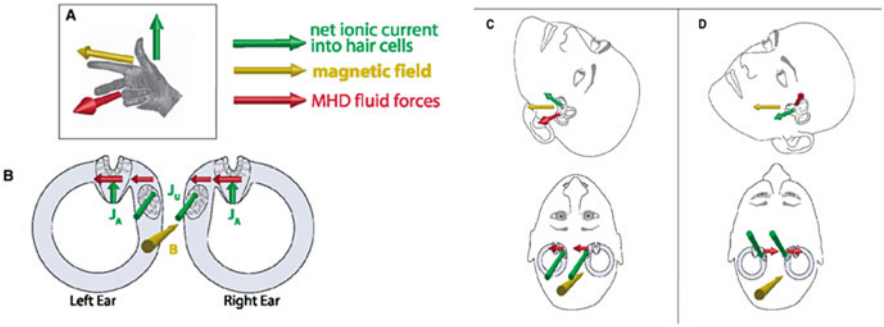

Fig. 13.6 Geometric models using Lorentz forces. (**a**) Right-hand rule relationship between current (green), MF (yellow), and resulting Lorentz force (red). (**b**) Two-dimensional view of the lateral canals, ampulla, and utricle, through the top of the head (vertical canals not shown), head pitching position, resulting Lorentz force to the left (same direction as in Panel **c**). The sign of the utricular force contribution depends on the pitch position of the head in the MF, as shown in Figs. **c** and **d**. (**c**) Two-dimensional view of the same head pitch position (utricle current vector pointing slightly upward), and the resulting Lorentz force is applied to the left side of the body. (**d**) Head pitch down (utricle current vector pointing slightly down), utricle cell Lorentz force to the right. [Reprinted from (Roberts et al. 2011), open access]

kinase (JNK) were significantly activated, respectively, in the differentiation activity and stress responses of rat cortical neurons in 5 T SMF exposure of 1 h (Prina-Mello et al. 2006). It is possible that a deeper cause is MF-induced changes in resting membrane potential, a micro-scale magnetofluidic effect. A SMF also affects intracellular Ca^{2+} concentrations, suggesting this mechanism involves voltage-dependent Ca^{2+} channels. A study reported that crayfish LG neurons' action potential amplitudes were increased by regulating the intracellular Ca^{2+} concentration in a 4.74–43.45 mT SMF (Ye et al. 2004). The excitatory postsynaptic potential in LG neurons was enhanced after 30 min exposure to an 8.08 mT O-shaped magnet. This conclusion is confirmed by the fact that neither the MF-treated crayfish electrolyte nor the pre-addition of Ca^{2+} chelators and intracellular Ca^{2+} release blockers can produce the same effect (Yeh et al. 2008).

It is possible that the orientation of phospholipids in membrane, microtubules, and actin in SMF due to diamagnetic anisotropy contributed to at least some of the observed effects. For example, Pall et al. observed changes in voltage-dependent calcium channel (VDCC) activity, intracellular calcium, and membrane depolarization after SMF exposure (Pall 2013). Eguchi et al. found that Schwann cells were arranged parallel to a high SMF of 8 T after being exposed to it for 60 h. The same arrangement of actin backbone happened in Schwann cells, and this cellular organization was inhibited by small molecules named guanosine triphosphatases Rho protein-related kinases (Eguchi et al. 2003).

Although there are currently very limited mechanistic studies about the analgesic effects of SMFs, some reports have indicated the involvement of membrane receptors as well as electrical transduction (Fig. 13.7). For example, in 2012, Okano et al. examined in vitro frog sciatic nerve fibers and found that 0.7 T SMF could reduce the

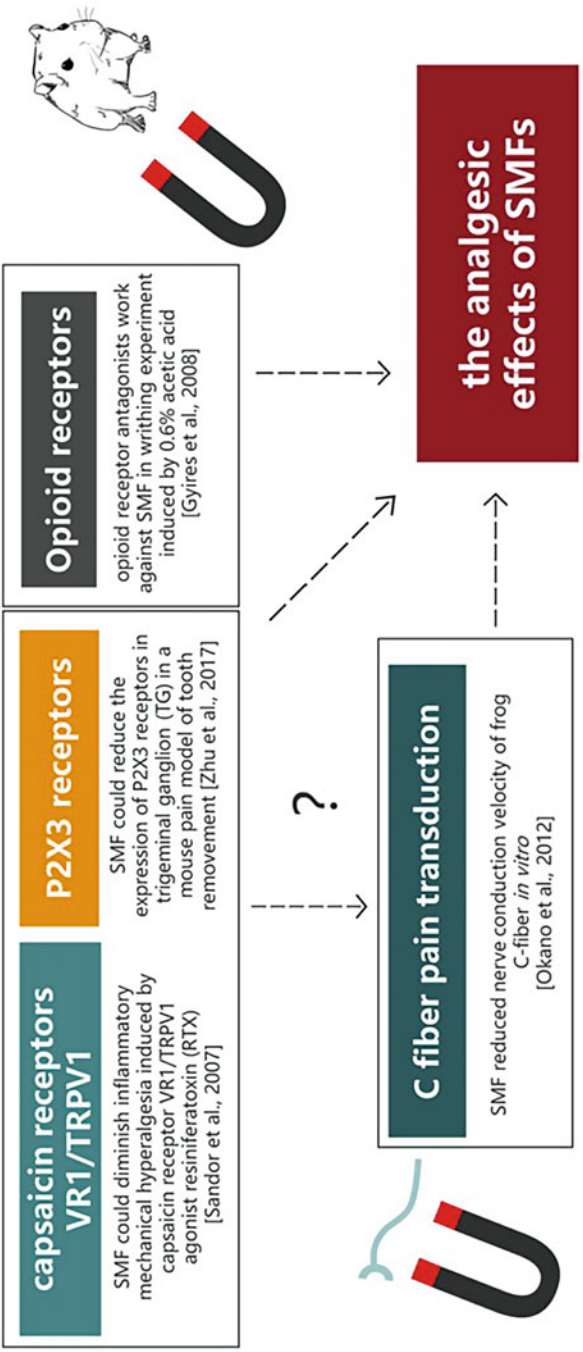

Fig. 13.7 Potential mechanisms of the analgesic effects of static magnetic fields. [Reprinted with permission from (Fan et al. 2021)]

nerve conduction velocity of C fibers by 5% (Okano et al. 2012). The authors speculated that the cell membrane and ion channels might be affected. Although no direct molecular-level experimental evidence was provided, it is interesting that the other three studies trying to address the analgesic mechanism of SMFs also pointed to membrane proteins, using either some membrane receptor agonist, antagonist, or membrane receptor expression level itself (Fan et al. 2021).

13.6 Conclusion

The influences of SMFs on the nervous system have attracted increasing attention, but differences in exposed subject, experimental conditions, including SMF parameters (SMF strength, direction, gradient, exposure time, etc.) and research tools have prevented us from drawing unambiguous conclusions about their exact effects. Although high-field SMFs could transiently interfere with the vestibular organs and cause some unpleasant but reversible feelings, there are also quite a few positive effects of moderate or high-field SMFs that have been reported, such as analgesic effects, memory, and mental state improvement, including anti-depression. Therefore, besides discovering the underlying mechanism, people should perform more investigations to optimize SMF conditions so that we can safely use them in medical diagnosis as well as treatment in the future.

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Chapter 14
The Biological Effects of Long-Term Static Magnetic Field Exposure

Hanxiao Chen and Xin Zhang

Abstract Although in most cases, people are exposed to static magnetic fields (SMFs) for just a short period of time, there are increasing situations where long-term exposure becomes inevitable, including magnets implanted in patients, magnetic therapy, and occupational exposure of magnetic resonance imaging staff. Consequently, the potential beneficial and/or harmful effects of such exposure, as well as its underlying mechanism, have triggered research endeavors. In this chapter, we have collected reported experimental data on animals and humans that were subjected to SMFs for more than 2 weeks, either continuously or intermittently. In animal models, it is found that long-term exposure to moderate SMFs can influence multiple aspects, including blood pressure and glucose regulation, the relief of pain, the promotion of bone formation, etc. Differences between continuous vs. intermittent exposure, human experimental results vs. epidemiological studies are discussed. Although most animal and human studies so far have suggested little/no risk of long-term exposure, or even beneficial effects for most moderate SMFs, there are still some exclusions that need attention. More research is still needed to comprehensively assess the exact long-term biological effects of various SMFs on different physiological and pathological conditions before we can make the best use of them.

Keywords Static magnetic fields (SMFs) · Long-term exposure · Biological effects · Continuous and intermittent exposure · Implanted and non-implanted magnets

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14.1 Introduction

Magnetic fields can be divided into different types depending on their parameters. A constant magnetic field, which does not change in magnetic flux density or direction over a certain period of time, is called static magnetic field (SMF). For example, the earth is surrounded by quasi-SMFs of 25 μT (tesla) and 65 μT, which are static for a certain period of time, but can also be affected by solar wind. Aside from this, there are many applications of SMFs such as the core part of magnetic resonance imaging (MRI) machines, the nuclear magnetic resonance (NMR) spectrometer, and the MagLev trains. Due to the increased exposure to SMFs in the last few decades, the interaction between SMF and organisms has become a rapidly developing research area.

Up to now, researchers have identified several biophysical mechanisms of SMF in organisms, including electrodynamic interactions with ionic conduction currents, the orientation of magnetically anisotropic structures in uniform fields, the translational force exerted on a paramagnetic or ferromagnetic substance placed in a magnetic field gradient, and modification of chemical reactions (Maret and Dransfeld 1977; World Health Organization 2006; Torbati et al. 2022). Although the theories are relatively straightforward, due to the complexity of the biological systems and the variability of magnetic fields in independent studies, the interpretations of the various experimental observations have been very complicated and inconsistent, which was discussed in Chap. 1 of this book.

Currently, there are largely two groups of people that could have long-term and/or repeated SMF exposures. One group includes workers in MRI examinations in hospitals, as well as in magnet factories, who are occupationally exposed to magnetic fields. The other group includes people who use magnetic fields to alleviate disease symptoms or improve health. For example, a magnet can be implanted on the sternum and is paired with an external magnetic brace to treat patients with pectus excavatum (Jamshidi and Harrison 2007) or implanted around the distal esophagus in patients with gastroesophageal reflux disease (GERD) (Bortolotti 2021) (Fig. 14.1), both of which fall in the category of magnetic surgery. There are also many people who use SMF-based magnetic mattress and bracelet, etc. Therefore, it is important to find out the exact long-term biological effects of magnetic fields and their potential actions on human bodies.

Here we have collected recent studies of long-term SMF exposure (over a period of 2 weeks or longer, continuously or intermittently) in animals and humans, with a special focus on the detailed magnetic field parameters, which has been proved to be very critical in the previous chapters of this book. We analyze their results in the hope of providing better understandings of the long-term biological effects of SMF on living organisms so that we can take the best advantage of them in the future.

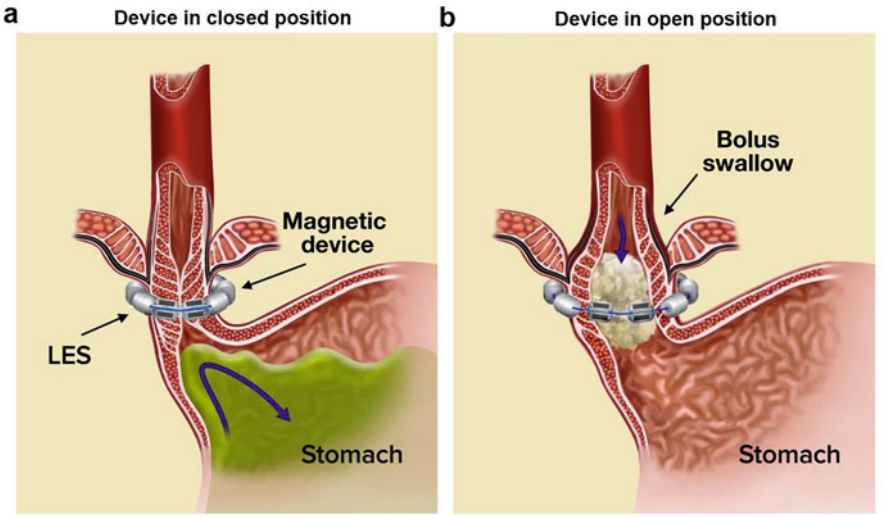

Fig. 14.1 Magnetic sphincter augmentation device that has been used on human bodies for years. (LINX Reflux Management System, Torax Medical, Shoreview, MN, USA) (**a**) Device in closed position; (**b**) device in open position. [Reprinted with permission from (Ganz et al. 2016). Copyright © 2015 The AGA Institute]

14.2 Animal Studies

In this review, we screened studies that were exposed to SMFs for longer than 2 weeks, which are further classified into continuous (SMF exposure 24 h/day for over 2 weeks) and intermittent (SMF exposure for several minutes or hours a day for over 2 weeks) exposure. Most relevant animal studies used rodents, while other animal models, such as zebrafish, medaka fish, and marine benthic animals, were also used.

14.2.1 Continuous Exposure

In this type of experiment, animals are exposed to SMFs 24 h/day for more than 2 weeks, either non-implanted or implanted.

14.2.1.1 Non-implanted

Non-implanted refers to the situations that the magnetic devices were not placed into the animal or human bodies. The magnetic devices, either permanent magnets or electromagnets, are placed outside of the animal or human bodies so that the SMF

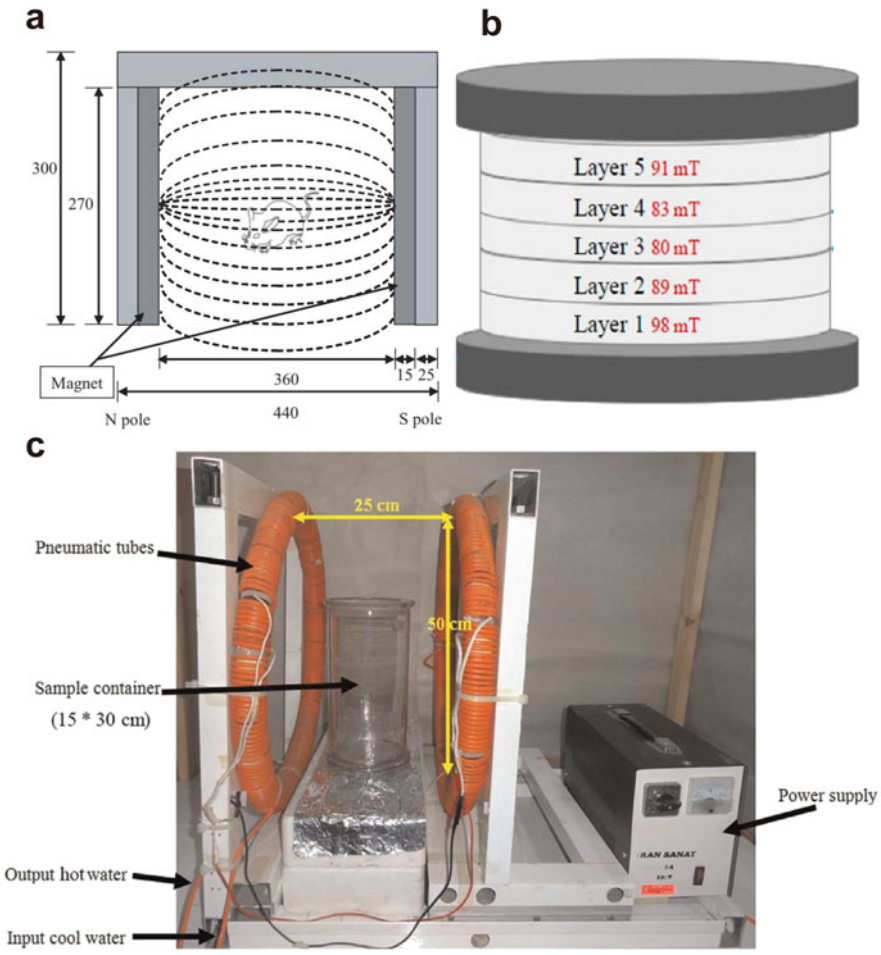

Fig. 14.2 Examples of SMF exposure set-ups for non-implanted SMF studies. Two permanent magnets were placed on opposite sides of (**a**) the mice cage (Taniguchi and Kanai 2007). Copyright © 2006 The Authors (open access); and (**b**) dishes with the fish embryos (Sun et al. 2019). Copyright © 2019 The Authors (open access); (**c**) device used to produce electromagnetic fields. [Reprinted with permission from (Loghmannia et al. 2015). Copyright © 2014 Elsevier Inc.]

can penetrate the whole body or the specific target area (Fig. 14.2). This is actually the most common way to perform magnetobiology studies. The results of continuous long-term SMF exposure by non-implanted magnet on animals are summarized, including the influence on reproductive system, blood pressure, pain relief, etc. (Table 14.1).

From Table 14.1, we can see that there are multiple studies about the reproductive system. In fact, there has always been a concern about the influence of environmental conditions on the reproductive system because it is much more sensitive and

Table 14.1 Continuous long-term SMF exposure (non-implanted) on animals

Subjects	Magnet type	Flux density	Time	Effects	Classification	References
Mussels M. edulis	Electromagnet	0.0037 T	3 months	No changes in gonad index and condition index	Reproductive system	Bochert and Zettler (2004)
Medaka fish	Permanent magnet	~0.1 T	15 days	No impacts on embryo development		Sun et al. (2019)
Albino mice		0.5–0.7 T	Gestation to birth	No changes in the body or testis-epididymis weight gain of pups		Tablado et al. (2000)
Albino mice		0.7 T	35 days	No changes in testicular or epididymal weights		Tablado et al. (1996)
				No changes in sperm head size but increase sperm head abnormality		Tablado et al. (1998)
Spontaneously hypertensive rats		0.005 T	12 weeks	Suppress and delay blood pressure elevation	Blood pressure regulation	Okano et al. (2005a)
Wistar-Kyoto rats		0.025 T		Reverse the reserpine-induced symptoms of reduced blood pressure		Okano et al. (2005b)
Spontaneously hypertensive rats		0.01 T, 0.025 T		Suppress and retard the development of hypertension		Okano and Ohkubo (2003)
Wistar rats		0.012 T	10 weeks	Depress sympathetic agonists-induced hypertension and hemodynamics changes		Okano and Ohkubo (2007)
Spontaneously hypertensive rats		0.016 T	30 days	Reduce arterial blood pressure, enhance baroreceptor reflex sensitivity		Tasić et al. (2017)
Sprague-Dawley rats		0.03 T (range 0.02–0.08 T)	12 weeks	The pain relief effects increased with activity and BMD	Pain relief	Taniguchi et al. (2004)
			4 weeks	Improve blood flow and reactive speed response, relief pain		Kanai and Taniguchi (2012)
Wistar rats			12 weeks	Inhibit the reduction in bone mineral density	Skeleton system	Taniguchi and Kanai (2007)

(continued)

Table 14.1 (continued)

Subjects	Magnet type	Flux density	Time	Effects	Classification	References
C57BL/6J mice		0.6 T	21 days	Reduce all-trans retinoic acid-induced bone loss		Chen et al. (2020)
			3 months	Reduce dexamethasone-stimulated osteoporosis		
Wistar albino rats		0.001 T	50 days	Increase ATPases, AChE activities, and MDA level	Synaptosomes	Dinčić et al. (2018)
BKS-Lepr^{db}/J mice		0.015 T	10 weeks	Promote the diabetic wound healing process	Diabetic complications	Feng et al. (2022)
C57BL/6J mice		0.1 T	12 weeks	Prevent high blood glucose, weight gain, fatty liver		Yu et al. (2021)
Sprague-Dawley rats		0.18 T	19 days	Promote the diabetic wound healing process and strengthen the wound tensile strength		Jing et al. (2010)
C57BL/6 mice	Superconducting magnet	2–12 T	28 days	No differences in physiological indexes	A safety study	Wang et al. (2019)

vulnerable to external stimuli compared with other systems. A research about marine benthic animals demonstrated that when *Mussels M. edulis* was kept in a 3.7 mT SMF for 3 months during their reproductive period in spring, the gonad index and condition index revealed no significant differences from the control group (Bochert and Zettler 2004). The embryo development in medaka fish in vivo with long-term SMF exposure did not reveal any impact on embryo development with 15-day exposure of up to ~100 mT (Sun et al. 2019). Tablado et al. exposed mice to a 0.7 T SMF for 35 days, and no changes were observed in their testicular or epididymal weights, and the size of sperm heads was also unaffected (Tablado et al. 1996, 1998). However, an increase in percentage of sperm head abnormality (lack of hook) was observed (Tablado et al. 1998). Tablado et al. also showed that the exposure of pregnant mice to a 0.5–0.7 T SMF did not change the body or testis-epididymis weight gain in pups (Tablado et al. 2000). Although not much abnormalities have been reported in this aspect, since the number of relevant studies is too limited, we still need more investigations to make sure the exact influence of long-term SMF exposure on reproductive system. We have also published a review about the SMF effect on reproductive system, including various exposure conditions (Song et al. 2022).

There are also several studies that have explored the effects of SMFs in blood pressure regulation. In 2003, Okano et al. found that 3.0–10.0 mT or 8.0–25.0 mT SMF exposure for 12 weeks can suppress and retard the development of hypertension in spontaneously hypertensive rats (Okano and Ohkubo 2003). In addition, lower field of 5 mT produced the same effect of reducing blood pressure but 1 mT did not have such effect (Okano et al. 2005a). This conclusion was confirmed in 2017 by Tasić et al. (2017). Besides, it was shown that a loop-shaped flexible rubber magnet adjusted to the neck region of a rat with intraperitoneal phenylephrine and dobutamine for 10 weeks can significantly depressed agonist-induced hypertension (Okano and Ohkubo 2007). However, it is interesting that Okano et al. have compared the effect of a 25 mT SMF on normotensive (having normal blood pressure) vs. hypotensive rats. They found that the 25 mT SMF did not cause any cardiovascular changes during an exposure period of 3 months (Okano et al. 2005b) but can significantly inhibit the reserpine-induced hypotension (Okano et al. 2005b). These indicate that SMFs may not affect normotensive animals, but could affect blood pressure in pathological conditions. It is very interesting and also puzzling that SMFs seem to be able to "properly" regulate blood pressure in these animals, by raising or lowering blood pressure to bring it back to the normal level. However, it should be mentioned that many of these studies were performed by the same group of researchers. Therefore, more research is needed to unravel these intriguing regulation effects of SMFs on blood pressure regulation.

Other aspects of SMF influences were also investigated, including pain relief, skeleton system, wound healing, and other diabetic complications. For example, adjuvant arthritis rats exposed to 30 mT SMF for 12 weeks not only had a pain relief effect, but also increased bone mineral density (BMD) (Taniguchi et al. 2004). Using the same experiment conditions, Taniguchi et al. found that the ovariectomized (OVX)-induced BMD reduction could also be inhibited by SMF treatment,

indicating its potential to be used to reduce menopausal symptoms in postmenopausal women (Taniguchi and Kanai 2007). Chen et al. proposed that the magnetic fields influence bone formation by affecting the differentiation of bone marrow mesenchymal stem cells (Chen et al. 2020). And the pain relief is probably due to the improvement of blood flow induced by SMF (Kanai and Taniguchi 2012). The chronic SMF exposure can also increase ATPases, AChE (acetylcholinesterase) activities, and MDA (malondialdehyde) level in rat synaptosomes (Dinčić et al. 2018). Moreover, it has also been shown that the long-term SMF treatment can have positive effects on diabetic wound healing and other diabetic complications (Jing et al. 2010; Yu et al. 2021; Feng et al. 2022).

It should be pointed out that most long-term SMF exposure studies have used SMFs of <1 T, which is mainly because of experimental setup limitations. However, there is one study that has addressed the biological effects of high SMF (2–12 T) exposure on mice continuously for 28 days. They used a large bore, superconducting magnet to perform this study. The results showed that there were no differences in the body weight, organ coefficients, or histomorphology of major organs in mice after exposure (Wang et al. 2019), which provides essential biosafety information for the future development of high-field SMFs in medicine.

14.2.1.2 Implanted

With the development of magnetic surgery technology, long-term magnet implantation has been shown to be useful in treating multiple diseases, such as pectus excavatum (Jamshidi and Harrison 2007; Bortolotti 2021), gastroesophageal reflux disease (Bortolotti 2021), etc. Moreover, numerous studies have reported the positive effects of moderate SMF on bone system, immune system, and the nervous system, which has been discussed in Chaps. 11, 12, and 13 of this book. It is therefore necessary to explore the safety and biological effects of long-term magnet implantation so that we can take the best advantage of the SMF in medicine in the future.

There are multiple studies using implanted magnets to examine their effects on skeleton system (Table 14.2). In 1998, Yan et al. implanted tapered rods with magnetization in bilateral femurs of rats and measured their BMD and bone calcium content 12 weeks after implantation, which revealed that the values increased compared with unmagnetized group (Yan et al. 1998). The same SMF intensity but with a treatment time of 21 days also leads to an improved osteogenesis (Nagai et al. 2000). A small disc magnet (max. 180 mT) implanted to OVX rats for 6 weeks statistically significantly increased BMD value and improved clinical effect on osteoporotic lumbar vertebrae (Xu et al. 2011) (Fig. 14.3). Some researchers think that the improved collateral circulation and blood circulation are the root cause of promoting bone formation. Ischemic rats whose femoral artery was ligated had reduced BMD and weight, and these can be reversed at the third week post-implantation of 180 mT magnets (Xu et al. 2001).

Table 14.2 Continuous long-term SMF exposure (implanted permanent magnets) on animals

Subjects	SMF flux density	Period of time	Effects	Classification	References
Wistar rats	0.01–0.017 T	7 days	Increase width of the periodontal ligament and root resorption	Orthodontic tooth movement	Tengku et al. (2000)
		14 days	No differences in tooth movement		
	0.06 T	14 days	Increase the number of CD4+ lymphocytes	Immune response	Janković et al. (1991)
		24 days	Increase the size of the thymus, hemagglutinin titer, and the number of CD4+ lymphocytes		
		34 days			
		25 days	Increase the number of plaque-forming cells and hemagglutinin titer in normal and pinealectomized rats, the former is more obvious		Janković et al. (1993b)
Rats		21 days	Potentiate immune responses in normal rats, and abrogate the immunosuppression induced by destruction of the locus ceruleus		Janković et al. (1993a)
Wistar rats	0.16 T	3–7 weeks	Increase vasomotion amplitude	Hemodynamics and vasoconstriction	Xu et al. (2013)
Spontaneously hypertensive rats	0.18 T	8 weeks	Increase nicardipine-induced hypotension		Okano and Ohkubo (2005)
		6 weeks	Enhance nicardipine-induced hypotension		Okano and Ohkubo (2006)
Wistar rats		12 weeks	Increase BMD and bone calcium content	Skeleton system	Yan et al. (1998)
		21 days	Improve osteogenesis in vivo		Nagai et al. (2000)
		6 weeks	Increase BMD value, clinical effect on osteoporotic lumbar vertebrae		Xu et al. (2011)
		3 weeks	Increase BMD and weight		Xu et al. (2001)
			Increase BMD and collateral circulation		Xu et al. (2007)

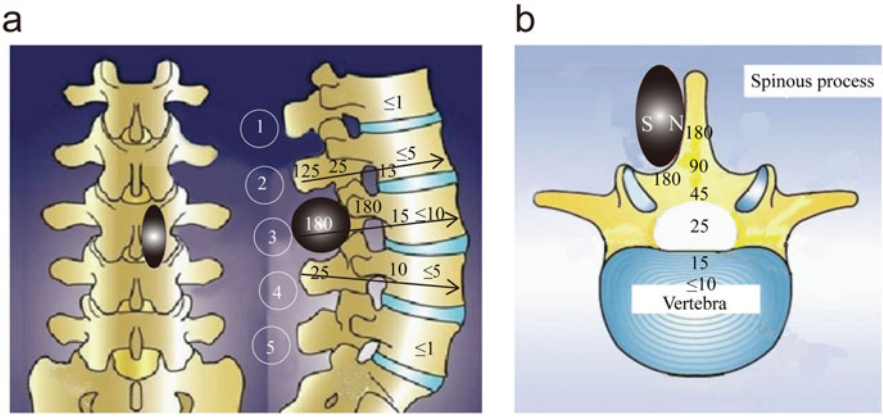

Fig. 14.3 Examples of SMF exposure with implanted magnets. Implanted magnet in lumbar vertebrae (**a**) and its spatial distribution (**b**). [Reprinted from (Xu et al. 2011), open access]

Studies have also indicated that SMFs can affect hemodynamics. In 2005, Okano et al. investigated the combined effects of a moderate SMF and nicardipine and found that the SMF induced a significant increase in the nicardipine-induced hypotension (Okano and Ohkubo 2005). Their subsequent research shows that the SMF may enhance nicardipine-induced hypotension by antagonizing the Ca^{2+} influx more effectively through the Ca^{2+} channels, or due to the upregulation of inducible nitric oxide (NO) synthase (Okano and Ohkubo 2006). Since blood vessel ingrowth is a pre-requisite for bone formation, a magnetized rod implantation for 3–7 weeks was shown to increase not only hemodynamics but also vasomotion (Xu et al. 2013). Therefore, although the studies are still very limited, these current results suggest that magnetic rod implantation may increase bone mineral density by altering hemodynamics, Ca^{2+} influx, and vasoconstriction. It is not clear why the permanent magnets in these studies, regardless of the rod or disk-like shape, all had a maximum magnetic field density of ~180 mT. We think it was probably the maximum flux density they can get at that time, being limited by the magnet size. More studies with different magnetic field conditions are encouraged for validation and/or improvement, which seems to be a promising future development for the application of SMFs, especially permanent magnets, in medicine.

There are also some researches about magnetic fields and immune response using implanted ways, which have been discussed in more details in Chap. 12 of this book. It should be mentioned that, theoretically, the movement of animals in SMFs can generate electrical currents leading to more bioeffects (Crozier et al. 2007). However, we did not find significant difference between the non-implanted and the implanted experiments, which may be due to the fact that SMF in most of these studies is not strong enough, and/or the animals are not actively moving.

14.2.2 Intermittent Exposure

Since intermittent SMF exposure over a period of time is more feasible in reality than continuous exposure, many studies have been carried out this way (Table 14.3). People have used different types of SMF devices in their research, including regular electromagnet and permanent magnet for lower SMF intensities, as well as superconducting and water-cooled magnets for higher SMF intensities.

The experiments with permanent magnets used magnetic flux densities of ~0.5 T. László et al. found that a max. 476 mT SMF is useful for chronic pain. They found a 30 min daily magnetic treatment for 2 weeks did not prevent the development of mechanical allodynia but can inhibit the increased sensitivity in neuropathic pain (Antal and László 2009). Besides, exposure for 6 weeks in the same experimental conditions significantly reduces plasma glucose level as compared to control in diabetic mice (László et al. 2010). They also demonstrated that daily 40-min whole body exposure to SMF prevented lipopolysaccharide (LPS)-induced preterm birth (PTB) in mice (László and Pórszász 2011). Tian et al. used permanent magnets with max. surface intensity of 0.5 T with upward direction, 6 h a day for 38 days, which inhibited GIST-T1 tumor growth in nude mice by 19.3% (Tian et al. 2018). No adverse effects were found in these studies.

For electromagnet-produced SMFs of varying strength, the effects are more diverse. It was shown that a 4 mT SMF exposure for 16 weeks (2 h/day) prevented bone architectural deterioration and strength reduction in type 1 diabetic rats (Zhang et al. 2018). And 2 h/day 5 mT SMF exposure for 14 days had no damage to noise-induced hearing loss. The author proposed that although SMFs promoted the reactive oxygen species (ROS) level in the first, they also accelerate antioxidative enzymes activation later. This combined actions finally caused negligible changes in hearing loss (Politański et al. 2010). The oxidative stress in rat cortex brain and hippocampus also increased under the combined effect of SMF and cadmium (Cd) (Amara et al. 2011). Moreover, although a 128 mT SMF exposure had no effect on epididymal sperm count, spermatozoa motility, or genital organ weight after 30-day exposure (Amara et al. 2006a), zebrafish exposed to 2.5, 5, 7.5 mT had increased levels of cortisol and decreased sex hormone concentrations (Sedigh et al. 2019). Therefore, as we have discussed recently, more research is needed on the effects of electromagnets on the reproductive system (Song et al. 2022). Moreover, the effects of SMFs on hematological parameters are also inconsistent. For both 128 mT SMFs, Amara et al. found that subacute exposure (1 h/day, 5 days) did not change hematological parameters but 30-day consecutive exposure significantly increased hemoglobin, red blood cells, white blood cells, and platelet number (Amara et al. 2006b). While Elferchichi et al. found that SMF 1 h/day for 15 consecutive days decreased red blood cell count, hemoglobin, and hematocrit values (Elferchichi et al. 2016).

The effects of SMFs generated by MRI were also different. Pregnant mice were exposed at the bore entrance (1.5 T and 7 T, 75 min/day, 18 days) during the entire period of pregnancy, and no effect was observed with pregnancy rate,

Table 14.3 Intermittent exposure

Subjects	Magnet	SMF flux density	Period of time	Effects	References
Sprague-Dawley rats	Electromagnet	0.004 T	2 h/day, 16 weeks	Prevent bone architectural deterioration and strength reduction in T1DM rats	Zhang et al. (2018)
C57BL/6 mice		0.005 T	8 h on the first day, then 2 h/day, 14 days	No damage to hearing loss	Politański et al. (2010)
Zebrafish		0.0025, 0.005, 0.0075 T	1 h/day, 3 weeks	Decrease the concentration of the sex hormones	Sedigh et al. (2019)
Wistar rats		0.128 T	1 h/day, 30 days	Increase hemoglobin, red blood cells, white blood cells, and platelet number	Amara et al. (2006b)
			1 h/day, 15 days	Decrease red blood cell count, hemoglobin, and hematocrit values	Elferchichi et al. (2016)
			1 h/day, 30 days	No effect on epididymal sperm count, spermatozoa motility, and genital organ weight	Amara et al. (2006a)
				Increase oxidative stress in rat cortex brain and hippocampus	Amara et al. (2011)
Balb/c mice	Permanent magnet	Max. 0.4767 T	30 min/day, 2 weeks	Inhibit the increased sensitivity in neuropathic pain	Antal and László (2009)
CD1 mice			30 min/day, 6 weeks	Reduce blood glucose level	László et al. (2010)
C57BL/6 mice			40 min/day, 17 days	Prolongs induced preterm birth (PTB)	László and Pórszász (2011)
BALB/c mice		0.4–0.5 T	6 h/day, 38 days	Inhibit GIST-T1 tumor growth	Tian et al. (2018)
Wistar albino rats	MRI-generated SMF	50 cm from the bore opening of 1.5 T MRI	12 h/day, 8 weeks	Deteriorate bone microstructure and vitamin D metabolism	Gungor et al. (2015)
C57Bl/6J mice		1.5 and 7 T	75 min/day, 18 days	No deleterious effect on offspring	Zahedi et al. (2014)
		7 T		No changes in emotional behavior, spatial or emotional learning	Hoyer et al. (2012)
C57BL/6 mice		16.4 T	3 h/day, 2 times a week, 4 weeks	Impair the vestibular system	Tkáč et al. (2021)
			3 h/day, 2 times a week, 8 weeks		

h hour, *min* minute

malformations, sex distribution, or postpartum death of offspring (Zahedi et al. 2014), neither in emotional behavior, spatial or emotional learning (Hoyer et al. 2012). However, there are also some adverse biological effects. Chronically exposed to 16.4 T SMFs (3 h/day, 2 times a week) for 4 weeks and 8 weeks both result in impairment of the vestibular system in mice (Tkáč et al. 2021). And the night period exposure (12 h/day, 8 weeks) in the position that 50 cm from the bore opening of the magnet in 1.5 T MRI devices (about 200 mT) deteriorates bone microstructure and vitamin D metabolism, for the mean cortical thickness, the mean trabecular wall thickness, number of trabeculae per 1 mm^2, and the mean vitamin D level were lower in SMF exposure group (Gungor et al. 2015).

14.3 Human Studies

Because of experimental limitation, ethical restriction and regulations, there are only a few studies available on human SMF long-term exposure (Table 14.4), including orthodontic tooth movement and pain relief, both of which showed no harmful, and even beneficial effects. For example, Bondemark et al. have studied the effects of SMF on human dental pulp and gums. First in 1995, they found that the first maxillary premolar and adjacent gingival tissue exposed to a bonded magnet with a max. magnetic flux density of 0.09 T did not cause any histologically detectable changes in human pulp or gums after 8-week exposure in seven individuals (Bondemark et al. 1995). In 1998, they bonded magnets with slightly higher intensities to the buccal surface of the upper premolars of eight subjects for 9 months and found SMFs did not influence human buccal mucosa (Bondemark et al. 1998). In 2003, Weintraub et al. randomly assigned 375 patients with II or III stage of diabetic peripheral neuropathy (DPN) into the experimental group wearing continuous magnetized insoles (45 mT) for 4 months. Their results showed that the magnetized insoles can reduce numbness, tingling, and exercise-induced foot pain (Weintraub et al. 2003). However, other researchers evaluated 11 subjects with vertebral deformity and back pain and found that repeated 30-min local exposure (10 times a week) to non-uniform SMF has no clinically significant effect on pain perception (Mészáros et al. 2013).

In fact, for the long-term exposure of SMF on human bodies, one of the best examples is magnetic sphincter augmentation device (MSAD), an implantable device that is used in treating gastroesophageal reflux disease (Fig. 14.1) (Ganz et al. 2016). It has been used world widely. Besides its clinical benefits for effectively treating GERD, there are also several studies conducted on the safety of this type of treatment. For example, a survey in 100 patients during a 6-year period showed that MSAD provides safe and long-term reduction of esophageal acid exposure and substantial symptom improvement (Bonavina et al. 2013). Another safety analysis of the first 1000 patients treated with MSAD also confirms the safety of this device and the implantation technique itself (Lipham et al. 2015). Moreover, a study in 85 subjects that have been implanted with this magnetic device reported no

Table 14.4 Laboratory studies on humans using permanent magnets

Subjects	SMF flux density	Exposure time	Effects	References
The premolar and adjacent gingival tissue in seven individuals	0.01–0.09 T	8 weeks, continuously	No histologically detectable changes in human pulp and gums	Bondemark et al. (1995)
The buccal surface of maxillary premolars in eight individuals	0.08–0.14 T	9 months, continuously	No increase in keratinization or other signs of surface abnormalities	Bondemark et al. (1998)
Feet of patients with diabetic peripheral neuropathy (DPN)	0.045 T	4 months, magnetized insoles, intermittently	Reduce numbness, tingling, and exercise-induced foot pain	Weintraub et al. (2003)
Patients with vertebral deformities and back pain	0.192 T	30 min/week, 10 weeks, intermittently	No clinical effect on pain	Mészáros et al. (2013)
100 patients with GERD	N/A	Median implant duration was 3 years (range 378 days–6 years)	Reduce distal esophageal acid exposure, improve sustained symptom and had no substantial or new safety issues	Bonavina et al. (2013)
1000 patients with GERD	N/A	Median implant duration was 274 days	No intraoperative complications, no device migrations or malfunctions	Lipham et al. (2015)
85 patients with GERD	N/A	5 years	No device erosions, migrations, or malfunctions and improve the anti-reflux barrier	Ganz et al. (2016)

new safety risks in 5 years and it works efficiently in improving the anti-reflux barrier (Ganz et al. 2016).

14.4 Epidemiological Studies

Although most animal and human studies showed no effects, or even beneficial effects of long-term SMF exposure, it is interesting and worrisome that some research in the form of questionnaires indicates some potential risks (Table 14.5). For example, a survey on the relationship between MRI-generated SMF exposure and hypertension shows that the occurrence of hypertension may be related to SMF exposure (Bongers et al. 2018). Schaap et al. also observed a positive correlation between the magnetic field strength of MRI scanner and the reported symptoms

Table 14.5 Epidemiological studies with occupational exposure to MRI

Research objects	SMF flux density	Effects	References
361 employees of 14 clinical and research MRI facilities	1.5 T, 3.0 T and 7.0 T	Observe a positive association between scanner strength and reported symptoms, such as vertigo	Schaap et al. (2014)
Male workers of an MRI-manufacturing facility	Cumulative SMF exposure ≥7.4 K tesla minutes	The occurrence of hypertension may be related to SMF exposure	Bongers et al. (2018)
120 MRI personnel	As high as 0.5 T	Had a higher proportion of symptoms such as headaches, sleep problems, palpitations, fatigue, and attention problems	Ghadimi-Moghadam et al. (2018)

(mainly vertigo) among the workers using MRI scanners of 1.5 T, 3.0 T, and 7.0 T (Schaap et al. 2014). Ghadimi et al. designed a questionnaire to collect information from 120 MRI personnel, the study showed increased frequencies of adverse effects in MRI workers, who had a higher proportion of symptoms, such as headaches, sleep problems, palpitations, fatigue, and attention problems than control group (Ghadimi-Moghadam et al. 2018). These surveys indicate that occupational exposure to SMFs might have some correlations to the appearance of health problems, and magnetic flux density seems to be a main influencing factor compared with exposure time. However, these studies did not consider other confounding variables including environmental contaminants, as well as the potential bias of the MRI workers.

14.5 Discussions

We have summarized the reported studies of long-term SMF effects by the exposure method. It is interesting that there are some differences between continuous exposure and intermittent exposure. Continuous SMFs exposure mostly showed either negligible or even beneficial effects while the results of intermittent exposure are highly variable. We think there are mainly two reasons.

Firstly, due to the limitations of experimental set up, most continuous SMF exposure experiments have used permanent magnets. However, the intermittent exposure experiments have used various magnets. It is interesting that the adverse effects are usually correlated with electromagnets, but not permanent magnets. Considering the fact that electromagnetic devices may cause additional heat, noise and weak electric field, it is difficult so far to determine whether some of the reported adverse effects were generated by these confounders. Also due to the limitations of experimental setup, most continuous SMF exposure experiments have used moderate SMF while the SMFs of intermittent exposure are highly variable. It is not surprising that higher SMF intensity could generate more effects compared to lower field.

Secondly, we hypothesize that maybe the general adaptation syndrome (GAS) is involved. It has been shown that the intensity of an organism's response to a stressful stimulus fluctuates with time, which was described as GAS. The stimulus occurs only once in continuous exposure, but in intermittent exposure the stimulations occur repeatedly, which may make the biological system very difficult to return to homeostasis. We propose this hypothesis because we found it interesting that even using the same type of magnetic field device and same magnetic field intensity, it was shown that the effects of continuous and intermittent exposure to alternating magnetic fields are also different. A study showed that the intermittent electromagnetic fields (1 min ON/OFF cycles, repeated 10 times every 2 h, 6 times/day during 48 h) in combination with NO increased cell death, but the continuous exposure (48 h) in combination with NO did not induce significant increase in cell death (Boland et al. 2002). In 1993, researchers studied the influence of 45-Hz magnetic fields on the brain functions. Ten volunteers were exposed to a continuous field and ten received an intermittent exposure (1 s ON/OFF cycles) for 1 h. Most of the changes in the measurements of electroencephalograph (EEG) were observed after intermittent exposure. Continuous exposure with the same amplitude and frequency produced no significant changes (Lyskov et al. 1993).

For human studies, it is interesting that although current experimental results showed no adverse effects, the epidemiological studies using questionnaires for MRI workers have reported the appearance of hypertension, headaches, sleep problems, and other health problems. We think there are at least four reasons. First of all, the magnetic field in MRI is higher than most experimental studies, and MRI workers standing by the machine are exposed to gradient SMFs. These both could cause more significantly effects. Secondly, most MRI workers take the survey have worked with the MRI machines for years so that the exposure time is much longer. Thirdly, since MRI workers are repeatedly and intermittently exposed to magnetic fields, the general adaptation syndrome that we mentioned above may contribute to the symptoms reported. Last but not the least, the questionnaires cannot exclude psychological factors.

However, it should be mentioned that although reported studies showed that most long-term SMF treatment did not cause serious harmful effects to animals or humans, we still need to pay extra attention and perform a lot more investigation. In fact, we recently found that even moderate SMF of some specific parameter generated by permanent magnets may also produce harmful effects at some special conditions. For example, we recently found that the health condition of mice that have consumed a large amount of alcohol (heavy drinking) was deteriorated by weeks of continuously exposure to upward SMFs of ~0.1 T with magnetic flux of $\sim 4.5 \times 10^{-3}$ Wb provided by permanent magnet plate, but not by the downward direction (Song et al. Our lab unpublished data). In contrast, when using healthy mice and the same sets of SMF devices, their health conditions are not harmed even after years of continuous exposure. In fact, their health conditions are even improved (Fan et al. Our lab unpublished data). The health conditions of mice drinking lower amount of alcohol were also improved by these SMF devices. Moreover, as mentioned before, the SMF effects on mice with different blood pressure level before

exposure are totally different. Therefore, the subject's status is a very important factor that determines the SMF exposure consequences. Moreover, it was reported that 0.7 T SMF exposure for 35 days could cause sperm heads abnormality, which should also cause some attention and more investigations (Tablado et al. 1998).

14.6 Conclusion

In this chapter, we have reviewed the biological effects of animals and humans that are exposed to SMFs for over 2 weeks, either continuously or intermittently. Most studies were carried out in animals, which indicate that long-term moderate SMF exposure could positively function in pain relief, bone formation promotion, blood pressure, and blood glucose regulation. Although the reported studies for humans are not very abundant, current studies focused on moderate SMFs, which seem to have some positive effects too. However, epidemiological studies, most of which used questionnaires, indicate potential mild negative effects although the influence of psychological factors was not ruled out. More double-blinded studies are encouraged to investigate the effects of long-term exposure, which will help to promote the safe application of SMFs in health and medicine.

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Chapter 15
Prospects, Pitfalls, and Opportunities for Human Static Magnetic Field Therapy

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Abstract This chapter provides an overview of the prospects of using electromagnetic fields (EMFs), with a specific focus on static magnetic fields, for treatment of human disease. The information provided covers the underlying basis for widespread skepticism surrounding "magnetotherapy"—which in part is deserved based on overinflated claims by its practitioners over the past two centuries (or even longer). On the other hand, a compelling scientific foundation is in place to propel nascent efforts to use magnetotherapy from a questionable niche medical practice into the mainstream; a goal of this chapter is to provide a summary of this information using specific (but non-comprehensive) examples of human ailments that are expected (based on current information) to benefit from magnetic field treatment.

Keywords Magnetic field therapy · Magnetotherapy · Static magnetic fields (SMFs)

15.1 Introduction

Therapies that involve exposure to electromagnetic fields (EMFs) date back to the inception of practical methods to harness and exploit magnetism and electricity. Anecdotal folklore suggests that the subset of these therapies using time-invariant (i.e., static) magnetic fields (SMFs) extend back two or even 3000 years [perhaps even to 1000 BC (Mourino 1991)], when "lodestones" were thought to have the ability to draw disease out of a person's body (Zyss 2008; Palermo 2015). Jumping forward, by the early sixteenth century (AD) the Swiss physician Paracelsus was using magnets to treat epilepsy, diarrhea, and hemorrhage, and in the mid eighteenth century, the Austrian doctor Franz Mesmer had opened a healing salon in Paris to treat the untoward effects of the body's innate "animal magnetism" (Mourino 1991). With the advent of electricity as a power source, EMFs were added to the healing

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repertoire and were being used to assist bone healing as early as the mid-nineteenth century, with definitive literature reports verifying efficacy appearing in the 1970s (Bassett et al. 1974a, b).

Since World War II, magnetic field therapy (usually referred to as "magnetotherapy" in this chapter) has flourished across the globe—albeit unevenly with various levels of acceptance in different countries—with an estimated two million recipients each year (Markov 2009). Magnetotherapy has many attractive features, including its relatively low cost compared to many current treatment modalities, its typically noninvasive nature, and its established safety record (with obvious exceptions, such as individuals with medical device implants such as pacemakers or insulin pumps). On the other hand, magnetotherapy has a long-standing reputation for quackery. To give one example of the origins of this reputation, by the late nineteenth century, Thatcher's Chicago Magnetic Company (a mail order outfit) claimed that "magnetism properly applied will cure every curable disease no matter what the cause" (Macklis 1993).

Today, similar overblown rhetoric from some quarters continues to obscure the valid scientific underpinnings of magnetotherapy. In part, magnetotherapy remains controversial because its opponents persist in making polarized blanket statements that categorically reject the possibility of beneficial health effects, while many proponents of magnetotherapy promise miracle cures for long lists of disparate ailments. The reality almost certainly lies between these extremes, and the purpose of this chapter is to provide an overview of what is currently known about human magnetic field therapy, what is not known, and what needs to be known (and done) to move this field forward.

15.2 Overview of Electromagnetic Field (EMF) Treatment Modalities

Although somewhat arbitrary, EMF therapeutic modalities are generally categorized in five categories as outlined by Markov (though some classification schemes give six categories) in an excellent synopsis of the influence of magnetic fields on human health (Markov 2014). These categories are briefly discussed below.

15.2.1 Low-Frequency Sine Waves

Low-frequency sine wave (LFS) electromagnetic fields are based on predominant commercially supplied electricity sources, which are 60 Hz in North American and generally 50 Hz in Europe and Asia (Markov 2014). One use of LFS is as an alternative to high-frequency fields in deep brain stimulation for the treatment of epilepsy (Goodman 2005; Goodman et al. 2005). Another potential application is for

the treatment of cancer (Blackman 2012); more broadly, efforts are underway to use diverse frequencies of EMFs including SMFs to treat cancer (Zimmerman et al. 2012).

15.2.2 Pulsed Electromagnetic Fields (PEMFs)

Pulsed electromagnetic fields (PEMFs) are low-frequency fields with specific wave shapes and amplitudes (Markov 2014). PEMF treatment was introduced clinically in the 1970s by Bassett and colleagues, who used a specific biphasic low-frequency signal for bone healing, particularly for the treatment of delayed fractures (Bassett et al. 1974a, b). Although reports continue to appear questioning the efficacy of PEMF therapy (Rose and Bryan-Frankson 2008), transcranial magnetic stimulation devices have been approved by the US Food and Drug Administration (FDA) for patients not responsive to chemical antidepressants (Martiny et al. 2010; Anonymous 2011). In addition, there are a profusion of PEMF devices that are sold and marketed as FDA-registered "wellness devices"; these products, however, are not permitted to claim efficacy for treating disease (Anonymous 2015).

There are, nevertheless, several studies that do indicate various forms of therapeutic effectiveness for PEMF beyond solely bone healing and the treatment of depression. A study in 2019 by Elshiwi and coworkers indicated that PEMF can improve the clinical outcomes of physical therapy when used alongside it as a treatment for lower back pain (Elshiwi et al. 2019). PEMF also has shown therapeutic potential for the treatment of rheumatoid arthritis and other diseases characterized by chronic inflammation and immune dysfunction (Ross et al. 2019). Finally, recent studies concluded that PEMF has a pro-osteogenic and pro-chondrogenic effect on mesenchymal stem cells, and thus could be used in the field of regenerative medicine to improve grafting and tissue repair (Varani et al. 2021).

15.2.3 Pulsed Radiofrequency Fields (PRFs)

Pulsed radiofrequency field (PRF) therapy refers to a technique where radio frequency oscillations are generated at a defined rate of pulses per second with frequencies ranging from 1.0×10^4 to 3.0×10^{11} Hz. Therapeutically, PRFs offer an alternative to continuous radiofrequency (CRF) therapy, which has been used since the 1970s and offers the advantage of pain control without tissue destruction (Byrd and Mackey 2008). These therapies typically utilize frequencies between 300 and 750 kHz, are now delivered to precise locations in the body by catheter, and as mentioned, are used in two primary modalities: in continuous mode, these devices are designed to produce deep heat, while in pulsed (non-thermal) mode, which uses short (e.g., 20 ms) high-voltage bursts followed by a longer (e.g., 480 ms) silent phase to allow for heat dissipation, they are used for soft tissue

stimulation (Markov 2014). Thermal PRF (i.e., CFR) therapy delivers high current focally to ablate the tissue of interest (e.g., a tumor or cardiac tissues that trigger arrhythmias) by heating to temperatures of 60–80 °C, resulting in focal tissue destruction (Byrd and Mackey 2008).

It remains controversial whether nonthermal PRF truly avoids biological effects due to heating; for example, although temperatures stay at or below 42 °C minimizing cell death or tissue destruction, heat shock response nonetheless could be triggered. Resolving this ambiguity will ultimately be necessary to fully define the biochemical mechanism of therapeutic responses associated with PRF therapy. Despite uncertainty over mechanism (and even efficacy), PRF is being used to treat a growing list of indications which are typically oriented toward amelioration of pain, including axial pain, radicular pain, facial pain, inguinal pain and orchialgia, and miscellaneous pain syndromes (Byrd and Mackey 2008).

15.2.4 Transcranial Magnetic/Electric Stimulation (TMS)

Transcranial magnetic stimulation (TMS) involves applying very short magnetic pulses of up to 8 Tesla to selected portions of the brain (Markov 2014). During TMS, a magnetic field generator is placed in proximity to the head of the person receiving the treatment (Groppa et al. 2012). The coil produces electric currents in the region of the brain just under the coil through electromagnetic induction. TMS can be used to diagnose connections between the brain and a muscle to evaluate damage from several indications, including stroke, multiple sclerosis, amyotrophic lateral sclerosis, movement disorders, motor neuron disease, and injuries (Groppa et al. 2012).

Therapeutically, TMS has been evaluated for conditions such as movement disorders, stroke, amyotrophic lateral sclerosis, multiple sclerosis, epilepsy, consciousness disorders, tinnitus, depression, anxiety disorders, obsessive-compulsive disorder, schizophrenia, craving/addiction, and motor conversion (Lefaucheur et al. 2014). In particular, more recent findings continue to support the therapeutic benefit of TMS as an option for otherwise treatment-resistant depression (Garnaat et al. 2018) and depression in adolescents (Croarkin and MacMaster 2018). Additionally, a 2019 study by Philip and coworkers indicated that one form of TMS, intermittent theta-burst stimulation (iTBS), is likely to be effective for the treatment of post-traumatic stress disorder (Philip et al. 2019). As of 2020, TMS has been approved as a therapeutic method for the treatment of two psychiatric disorders: major depressive disorder and obsessive-compulsive disorder, with approval by the FDA in 2008 and 2018, respectively (Iglesias 2020).

In a 2020 review, Lefaucheur and coauthors concluded that there is sufficient evidence to accept “definite efficacy” for the analgesic effect of high-frequency (HF) TMS of the primary motor cortex (M1) contralateral to the pain, the antidepressant effect of HF-TMS of the left dorsolateral prefrontal cortex (DLPFC), and LF-TMS of contralesional M1 in post-acute stroke. “Probable efficacy” is proposed for several indications, including but not limited to the antidepressant effect of

low-frequency (LF) TMS of the right DLPFC (as well as the left for Parkinson's disease patients specifically), HF-TMS of the left DLPFC for the treatment of fibromyalgia pain, HF-TMS of bilateral M1 regions for motor impairment, and iTBS to treat spasticity in multiple sclerosis patients. Finally, TMS achieves "possible efficacy" in a number of indications, including LF-TMS of the left temporoparietal cortex for auditory-verbal hallucinations and of the auditory cortex for chronic tinnitus (Lefaucheur et al. 2014).

15.2.5 Static/Permanent Magnetic Fields (SMF)

Static magnetic fields—that is, time-invariant magnetic fields—are a feature of various permanent magnets; alternatively, they can be generated by passing direct current (DC) through a coil (Markov 2014). These fields are the primary focus of this book, with detailed description of the underlying physics provided elsewhere; in this chapter, SMFs will be discussed based on their field strengths with Sect. 15.3.1 covering weak fields in the range of the Earth's magnetic field (<0.65 gauss or ~65 μT). Section 15.3.2 will discuss the *absence* of these fields, which by default make a convincing case that humans can detect and (subconsciously) respond to weak magnetic fields. Finally, Sect. 15.3.3 will provide an overview of the therapeutic use of more powerful moderate strength fields that range up to ~1 T (one Tesla or 10,000 gauss). Strong fields above one Tesla are rarely used in magnetotherapy per se, but people are exposed to these field strengths during magnetic resonance imaging (MRI), generally without any discernible impact on health.

15.2.6 "Non-therapeutic" Electromagnetic Field (EMF) Exposure Allays Safety Concerns

Over the past century or so, humans have been increasingly subject to inadvertent exposure from man-made EMFs. For example, the rise of metal industries, welding processes, and certain electrified train systems in the late nineteenth centuries resulted in significant exposure for workers and even bystanders to SMFs; in 1921, Drinker and Thomson asked the question "Does the magnetic field constitute an industrial hazard?" and concluded that it didn't (Hartwig et al. 2009). Over the years as new "EMF"-based threats have emerged (Tucker and Schmitt 1978), such as living under high-voltage power lines or the ubiquitous adoption of cell phones, which have raised fears of childhood and brain cancers, have been met with detailed scrutiny that have ruled out clear-cut evidence of harm. Ultimately meta-analysis of many such studies has cast doubt on the idea that EMF exposure causes any measurable detriment to human health, acting as a helpful baseline for establishing the safety of magnetotherapy. On the other hand, the (general) lack of deleterious

effect of EMFs has also been used to cast doubt on whether beneficial effects are possible as well, based on the assumption that these fields likely have *no* meaningful impact on human health; a substantial portion of this chapter either directly or indirectly addresses this fallacy.

15.3 Biomedical Effects of Static Magnetic Field Therapies Categorized by Field Strength

15.3.1 "DIY" Treatments with Low to Moderate Strength Static Magnetic Fields Are Widespread But Unproven

The largest segment of extant "magnetic therapies" falls into the do-it-yourself (DIY) category, where individuals use various types of permanent magnets that provide continuous SMF exposure. This modality of magnetotherapy is used to treat a wide range of ailments, with a quick internet search (conducted in January, 2017, but similar results have been obtained for at least 20 years) including magnetic bedding pads, magnets embedded in pillows, magnetic shoe insoles, magnetic back belts, magnetic leg and arm supports, magnetic bracelets, magnetic finger and toe rings, and multipurpose magnetic pads that can be customized to wear on virtually any part of the body. Note that no specific weblinks are provided here for several reasons. First, any particular commercial link is apt to be quickly out of date; second, this publication wishes to avoid the appearance of endorsing any particular product; and finally, to spur any interested reader to perform their own search for "magnetic therapy products" (or similar terms). Such a search will almost certainly provide—above and beyond many sites selling these products—numerous links running the gamut from "debunking" the entire idea of magnetotherapy and mocking consumers for falling for a billion-dollar "scam"—reportedly a conservative value for annual sales of these products, which was reported almost 20 years ago (Weintraub 1999)—to enthusiastic endorsements for efficacy against a broad gamut of human diseases; increasingly, products are coming available to treat one's pets as well.

Intuition alone makes a powerful case that many DIY magnetotherapy efforts are likely misguided and minimally effective. Even if the magnets used are "high-quality" (e.g., constructed from latest neodymium-based alloys) as advertised, with field strengths reported in the range of tens to hundreds gauss (i.e., up two to three orders of magnitude stronger than the Earth's magnetic field), one key issue is that magnets themselves are NOT therapeutic. This point is discussed by Markov (2009) who describes how the term "magnetic therapy" is a misnomer. Instead, he emphasizes that the therapeutic effects of magnets emanate from the *fields* they generate and the subsequent interaction of these fields with the target tissue or organ in a person (note that the use of the "magnetotherapy" in this chapter implicitly denotes magnetic *field* therapy). In this regard, it is critical to note that field strength decreases exponentially with distance from the surface of a permanent magnet (for

example, by ~2 orders of magnitude in only a few millimeters for magnets in the range of hundreds of gauss), and therefore, field strength is negligible in deep tissue that would need to be penetrated to have an effect on many of the conditions purportedly treated with magnetotherapy.

One example of this pitfall is provided by a report where commercial magnetic wraps had no effect on blood circulation in horses (Steyn et al. 2000) or pain perception in people (Kuipers et al. 2007), which—because the field strengths used did not penetrate effectively into tissue to the depth where the target vessels or nerves were located—were not surprising results. More trivially, but still important, magnets placed in clothing or otherwise attached via wrappings that surround the body provide inconsistent magnetic field exposure to the intended target tissue if the clothing or wrapping is loose or not applied and worn consistently from day to day. An illustration of this point is that the field strength of a 500 gauss magnet can be as little as 1 gauss only 1 or 2 cm away from the magnet's surface. As a result, determination of dose—a key parameter in determining medical efficacy—is typically impossible to determine with any degree of accuracy in DIY magnetotherapy (Markov 2009).

15.3.2 Hypomagnetic Fields (HMF)—Evidence for Magnetotherapy by Default?

Interestingly, the impact of weak to moderately strong SMF on human health perhaps has been demonstrated most convincingly by default; that is, by observing the effects of the absence of geomagnetic-strength magnetic fields. These studies have exploited a century of efforts to develop materials designed to shield sensitive equipment from magnetic fields, such as submarine telegraph cables, electric power transformers, cathode ray tubes, and magnetic phonograph cartridges. To achieve the required shielding, "mu-metals" have been developed that have a representative composition of ~77% nickel, 16% iron, 5% copper, and 2% chromium or molybdenum (Jiles 1998). In essence, a mu-metal is a high-permeability alloy that does not block magnetic fields per se, but instead provides a path for the magnetic field lines to go around the area intended to be shielded. Details on magnetic field shielding are largely beyond the scope of this discussion, but more information can be found online (e.g., in technical documents provided by vendors of magnetic shield products such as http://www.magnetic-shield.com/pdf/how_do_magnetic_shields_work.pdf). For this discussion, the key point is that products exist that can effectively shield objects from ambient magnetic fields that, for practical purposes, can isolate a research subject from a background (generally the Earth's) magnetic field. Geomagnetic field shielding produces what has come to be known as "hypomagnetic fields" (HMFs).

In the past few years, a provocative set of experimental results have emerged, indicating that HMF has numerous biological and biomedical effects across

species—including humans. For example, long-term HMF exposure is associated with embryonic malformation in insects (Wan et al. 2014), amphibians [e.g., newts (Asashima et al. 1991) and frogs (Mo et al. 2012)], and rodents [e.g., mice (Fesenko et al. 2010)], as well as abnormal DNA methylation in murine embryonic stem cells (Baek et al. 2019). Additional effects of HMF have been described in rodents, including inhibition of stress-induced analgesia (Prato et al. 2005), decreased noradrenaline release (Choleris et al. 2002; Zhang et al. 2007), and impairment of learning and neurogenesis in the hippocampus (Zhang et al. 2021). Learning defects have been described in birds (Xu et al. 2003) and *Drosophila* (Zhang et al. 2004). Finally, the negative impact of HMF has been reported to extend to humans; these effects have often and most convincingly been deduced from space flight, where the geomagnetic field is negligible in strength because it is generally not practical to confine a person to an artificially shielded HMF area. These studies have shown HMF effects in humans that include perturbed circadian rhythms (Wever 1970; Bliss and Heppner 1976) and weakened cognitive function (Binhi and Sarimov 2009).

The generally deleterious effects of HMF across several biological processes in many species, including the still-speculative but nevertheless plausible observations in people, have strengthened the case that weak magnetic fields *do* have legitimate biomedical relevance. For example, it appears that GMFs keep us healthy and contribute to normal physiology. Extrapolating from these observations, it has been hypothesized that because a lack of magnetic fields is harmful, field strengths stronger than the Earth's magnetic field might exacerbate and extend the beneficial impact of GMF exposure. A parallel drawn from pharmacology is that many natural drugs, such as aspirin or the antioxidant resveratrol, must be consumed at much higher levels to have a medical effect than a person can reasonably obtain from natural consumption (Scott et al. 2012). Similarly, arguments have been made—abutted with claims that humans evolved when the Earth's magnetic field was as much as an order of magnitude stronger than it is today—the earth's magnetic field is constantly waxing and waning, and even reverses polarity on a millions-of-years time scale (Mori et al. 2013), an event associated with mass extinctions (Lipowski and Lipowska 2006)—that to achieve maximum benefits from magnetotherapy, stronger magnetic fields should be used.

15.3.3 Stronger Magnetic Fields—Impacts on Human Health

15.3.3.1 Moderate Strength Static Magnetic Field Therapy

The benefits (or necessity) of using stronger than GMF-strength fields for human therapy have spurred efforts to use static magnetic fields much stronger than afforded naturally by today's geomagnetic fields. In some cases, these strategies involve "DIY" efforts with magnets in the tens to hundreds of milli-Tesla range, but, as discussed above, these efforts are likely ineffective for treatments that require deep penetration of tissue. As an alternative, medical devices, often from Europe, that

create stronger electromagnetic fields have been marketed. The US FDA generally permits these for "general wellness" (Anonymous 2015) while prohibiting claims for efficacy for treatment of any specific medical indication.

In some cases, proponents of magnetic therapy are pursuing more rigorous evidence of efficacy. One example is provided by continuing efforts of Joe Kirschvink and colleagues to demonstrate that humans are affected by externally applied magnetic field in ways that are medically relevant (Hand 2016). Another example of moving forward with therapeutic intervention is provided by the *Advanced Magnetic Research Institute* (AMRi) that has developed a "Magnetic Molecular Energizer™" (MME) device (Bonlie 2001) capable of producing SMFs of 0.3–0.5 T that completely penetrate the human body in an ~20 cm radius (Fig. 15.1). Based on the assumption that the "biosensor" for magnetic reception is located directly in the diseased or damaged tissue, a patient is positioned with the magnetic field centered on the affected area. Double-blind clinical trials seemingly showed efficacy against lower back pain (ClinicalTrials.gov Identifier: NCT00325377) and possibly against symptoms of diabetic neuropathy (ClinicalTrials.gov Identifier: NCT00134524). The results of these studies, however, were difficult to interpret because positive outcomes were not statistically different from placebo-treated patients, who also experienced marked improvement (Dean Bonlie, personal communication). These clinical studies illustrate two recurring themes in efforts to establish clinical efficacy for magnetotherapy; first, therapeutic effectiveness is most well established for pain perception (the subject of these tests) and second, the placebo effect is often overwhelming in magnetotherapy; both of these points are further elaborated in Sect. 15.5.3, below.

15.3.3.2 Higher Strength Static Magnetic Field Exposure

Strong fields above 1 Tesla are rarely used in magnetotherapy per se, but people are routinely exposed to field strengths of 1.3 (and now up to 3) T during magnetic resonance imaging (MRI). As of 2016, over 150 million people have undergone MRI procedures, with ~ten million undergoing examination each year (Anonymous 2016). Overall, it is accepted that MRI has little, if any, discernible impact on health, either beneficial or deleterious (Schenck 2000). Based on this apparent lack of response, SMFs are generally regarded to be safe by regulatory agencies such as the US Food and Drug Administration (FDA) (Anonymous 2015). Upon comprehensive review of the literature available described the in vivo and ex vivo effects of SMFs, Hartwig and coauthors confirmed that >1 T SMFs that accompany MRI are rarely harmful (Hartwig et al. 2009), with the possible exception of inconclusive reports where exposure led to acute neurobehavioral effects, such as eye-hand coordination speed and visual and auditory working memory problems (De Vocht et al. 2006) and a non-statistically significant increase in spontaneous abortions in MRI workers (Evans et al. 1993). It should be noted that these reports dealt with MRI workers and, no doubt based on warnings raised by these speculative studies,

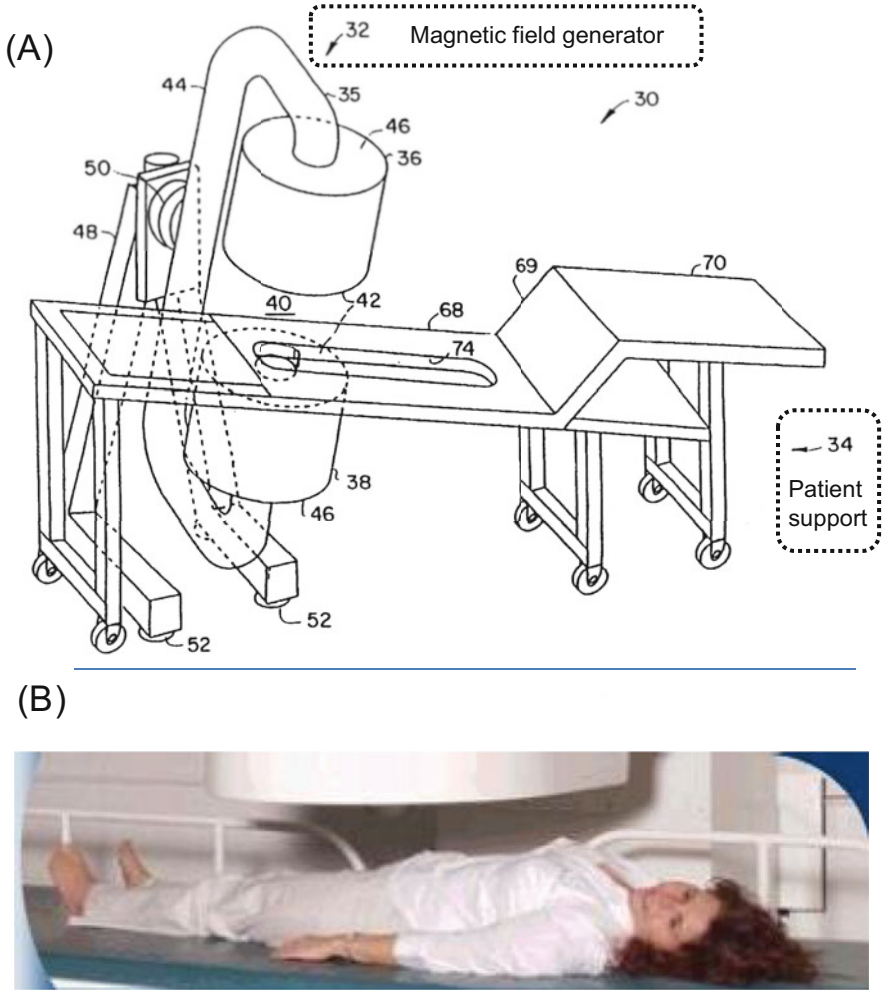

Fig. 15.1 The Molecular Magnetic Energizer™ (MME) device and illustration of a patient during treatment. (**a**) The MME [as illustrated in U.S. Patent documents (Bonlie 2001)] consists of two major elements: a magnetic field generator (32) for producing a treating magnetic field and a patient support (34) for positioning a patient within the magnetic field. The magnetic field generator consists of a magnetic circuit (35) having an upper electromagnet (36) and a lower electromagnet (38) separated by a gap (40) on their adjacent pole faces (42) and connected by a C-shaped core (44) (or "C-core") on their opposing poles (46). In the embodiment shown, C-core has a circular cross-section with an 8-inch (20.3 cm) diameter. The electromagnets are wired in parallel with a power supply to create magnetic fields of the same sense. For example, the positive pole of the upper electromagnet 36 would face the negative pole of the lower electromagnet 38 (or vice versa). (**b**) A patient is shown positioned in the MME device in a supine position; it should be noted that the magnetic field generator apparatus can be rotated and otherwise adjusted via parts 48, 50, and 52 to accommodate patients who prefer to be treated in other positions, for example, lying on their side. (Image from public website: http://www.amri-intl.com/)

safety standards have been tightened and follow-up and continuing problems have not been reported.

15.4 Prospects for Therapeutic Areas

Magnetotherapy has been applied to almost any imaginable human ailment. For example, MedicineNet (http://www.medicinenet.com/script/main/art.asp?articlekey=22961) summarizes conditions claimed to be diagnosed or treated using magnetic field therapy (largely through the "DIY" methods mentioned above) to include arthritis, cancer, circulatory disorders, diabetic neuropathy, fibromyalgia, HIV/AIDS, immune dysfunction, infection, inflammation, insomnia, multiple sclerosis, muscle pain, neuropathy, pain, rheumatoid arthritis, sciatica, and stress, as well as to increase energy and prolong life. The above-mentioned AMRi Corporation, which utilizes stronger strength SMF therapy, is investigating the treatment of ailments that range from spinal cord injury, brain injury, stroke impairment, multiple sclerosis, muscular dystrophy, cerebral palsy, Parkinson's disease, Alzheimer's disease, congestive heart failure, to orthopedic conditions involving bone and joint repair. As described in Sect. 15.5 below, many find it implausible that a "one size fits all" treatment could be effective against so many indications, and this doubt in part contributes to disbelief in therapeutic efficacy for magnetic field exposure. However, as discussed next, pain perception, blood flow, and effects on the cardiovascular system, as well as the impact on stem cells and cells found in the neurological system, provide a compelling scientific basis for beneficial effects of SMFs that, if carefully and rigorously translated to the clinic, hold legitimate promise for human therapy.

15.4.1 Pain Perception

A substantial body of evidence has accumulated showing that exposure to EMFs affects pain sensitivity (nociception) and pain inhibition (analgesia); in particular, acute exposure to various EMFs has been shown to inhibit analgesia in many studies (Del Seppia et al. 2007). In some studies, however, depending on the duration, intensity, frequency, and repeated nature of EMF exposure, increased analgesia has actually been observed (Del Seppia et al. 2007). While many of these studies—conducted in diverse organisms ranging from snails to mice to people—have involved time-varying fields, there is also substantial evidence that SMFs can affect pain perception. These findings have most convincingly come from HMF studies where mice apparently detect and respond to the absence of the ambient geomagnetic field.

In a pioneering study, mice experienced a maximum analgesic response after 4–6 days of exposure (Prato et al. 2005). Follow-up studies showed a more complex

biphasic response, where geomagnetic shielding for 1 h per day for 10 consecutive days initially decreased the pain threshold over the first 2 days, followed by a sharp increase peaking by the fifth day, with a return to pre-exposure values within 8 days (Del Seppia et al. 2007). Interestingly, the kinetics of this response roughly mirror an in vitro cell-based assay response to moderate strength SMF (Wang et al. 2009) described in more detail below in Sect. 15.4.3. It was found more recently that the application of transcranial static magnetic field stimulation (tSMS) to the primary motor cortex (M1) and primary somatosensory cortex (S1) of humans may affect cortical processing of pain, making this a possible noninvasive method for the treatment of chronic pain alongside other standard-of-care treatments (Kirimoto et al. 2018).

15.4.2 Blood Flow/Vascularization

As discussed in more detail in Chap. 4, beneficial effects of magnetotherapy in humans often have been attributed to improved blood flow. Although many of the Internet claims in this regard are nonsensical—for example, the idea that a magnetic field attracts the iron in the blood is based on the misconception that hemoglobin is ferromagnetic. Instead, iron in oxygenated blood is diamagnetic, which means there is a real but almost negligible force repelling the blood; on the other hand, deoxygenated blood is paramagnetic, which means there will be a similarly almost negligible force attracting the blood (Zborowski et al. 2003). Either way, these effects are dwarfed by thermal motion and the ambient flow of the blood (as discussed in more detail in Chap. 4).

Nevertheless, there is evidence—although inconclusive because of many conflicting or inconclusive studies—that magnetic fields can legitimately modulate blood flow in humans (or other mammals). As an aside, some negative results can be accounted for by the trivial explanation that the magnetic fields used were not strong enough to penetrate deeply into the tissue where the target blood vessels were used. One example with horses was mentioned above (Steyn et al. 2000). Similarly, a study using 500 gauss (0.05 T) fields to measure blood flow in the forearms of healthy young men was equally ineffective (Martel et al. 2002); this is not surprising because field strength would be two to three orders of magnitude lower at the location of the targeted blood vessels embedded in tissue. A ~tenfold larger field (4042 gauss, or ~0.4 T), by contrast, *did* statistically affect blood flow in treated fingers (Mayrovitz and Groseclose 2005); interestingly, this effect was actually a reduction in blood flow, converse to what is generally thought to be therapeutically beneficial.

A set of studies in rabbits using similar strength fields (i.e., ~0.18–0.25 T) also showed legitimate effects of SMFs on blood flow (Xu et al. 1998; Okano and Ohkubo 2001; Gmitrov et al. 2002). These three studies demonstrated a biphasic response of blood flow where exposure enhanced vasodilation when the vessels were

vasoconstricted and enhanced vasoconstriction in vessels that were vasodilated; in other words, the SMFs appeared to work to maintain circulatory homeostasis and "normalize" vascular function. A conceptually similar normalization effect was observed in mice where the impact of surgical intervention that would otherwise cause luminal diameter expansion in vascular networks was abrogated by continual exposure to SMFs over 4–7 days (Morris and Skalak 2007). Together, these studies suggest that while SMF exposure does have an interesting effect on blood flow, it likely is not mediated through magnetic or inductive effects on iron containing molecules (hemoglobin) or cells (RBCs) per se.

Instead, therapeutic effects on blood flow are likely mediated by "non-canonical" mechanisms (i.e., not magnetite, chemomagnetic sensing, or inductive mechanisms, which are the three molecular mechanisms found throughout nature in many diverse organisms, as discussed in detail in Chap. 4). Another interesting feature of these studies is that field strengths of greater than ~0.1 T (1000 gauss) were needed for efficacy; as mentioned, the simple explanation is that weaker field strengths could not penetrate deeply enough into tissue to reach the intended site of action (i.e., the blood vessels themselves). Another explanation (again as discussed in more detail in Chap. 4) is that field strengths of ~0.2 T or higher can alter the biophysical properties of lipid assemblies (Braganza et al. 1984). As a result, the properties of lipid biolayers (i.e., biological membranes) are affected in ways that putatively explain many phenomena observed in magnetotherapy. For example, changes in ion flux could reasonably be explained by allosteric changes to ion channels brought about changes to the biophysical properties of membranes, rather than the less plausible explanation that SMF directly affects the movement of ions (i.e., through an inductive or "Hall effect," which has sometimes been postulated to explain the mechanism of magnetotherapy). Similarly, changes to signal pathway activity can be explained by the effects of magnetic field exposure on the biophysical properties of membranes, as discussed below for neural cells. Both of these topics are discussed in the next section in the context of studies performed in the author's laboratory.

In addition to affecting blood flow, some studies have shown that SMF can play a role in angiogenesis and improvement of vascularization, generally in combination with a nanocomposite scaffold. For example, a 2016 study indicated that murine osteoblasts stimulated by a combination of SMF and magnetic nanoparticle scaffolding promoted angiogenesis in endothelial cells, predominantly indicated by the expression of angiogenesis-related genes and the formation of capillary tubes (Yun et al. 2016). Another, later, study showed that tissues were vascularized more quickly in cell-containing nanocomposite hydrogels when those hydrogels were exposed to SMF, suggesting that SMF has a vasculogenic effect on engineered bone grafts (Filippi et al. 2019). Even more recently, it was found that exosomes derived from bone mesenchymal stem cells stimulated with magnetic nanoparticles and SMF can promote angiogenesis and improve wound healing (Wu et al. 2020, 2021).

15.4.3 Evidence for Treatment of Neurological Disease and Neural Regeneration

In a study that was inspired by the need to find a scientific basis for coalescing evidence that magnetic field therapy may be a viable treatment option for neurological ailments via moderate strength fields (i.e., 0.1–1 Tesla), we treated the PC12 rat adrenal pheochromocytoma cell line with ~0.25 T SMFs. PC12 cells display metabolic features of Parkinson's disease (PD) (Blum et al. 2000; Meng et al. 2007), such as possessing intracellular substrates for dopamine (DA) synthesis, metabolism, and transport, and they also abundantly express adenosine A_{2A} receptors (e.g., $A_{2A}R$) implicated in PD (Kobayashi et al. 1998). In these studies, we showed that SMF treatment reproduced several responses elicited by ZM241385, a selective $A_{2A}R$ antagonist; SMF exposure also counteracted several PD-relevant endpoints exacerbated by $A_{2A}R$ agonist CGS21680 in a manner similar to ZM241385 (Wang et al. 2010). These results raise the intriguing hypothesis that SMF can reproduce the effects of a promising class of non-dopaminergic PD drugs (i.e., ZM241385 and analogues) in a noninvasive manner, and more broadly, holds potential for ameliorating additional neurological disorders such as Alzheimer's and Huntington's diseases through modulation of $A_{2A}R$ (Takahashi et al. 2008).

In a second study from the author's laboratory, SMF-mediated responses associated with transient interleukin-6 (IL-6) signaling in human embryonic cells [the hEBD LVEC line (Shamblott et al. 2001)] translated into changes observable at the whole cell level (Wang et al. 2009). The responses observed in these cells began very rapidly after SMF exposure began, first observed within 15–30 min in increased transcription of mRNA for IL-6, with actual secretion of this pro-inflammatory cytokine increasing for the next 2–4 days.

Because IL-6 guides differentiation of neural stem cells primarily to astrocytes (Taga and Fukuda 2006)—which is generally a medically unwanted outcome because hyperproliferation of this cell type leads to scar formation rather than regeneration—we investigated whether evidence of astrocytogenesis was seen in SMF-treated cells. Interestingly, responses consistent with astrocyte differentiation (i.e., slowed proliferation and morphological changes) expected from IL-6 exposure were not seen; neither were biochemical markers of astrocyte differentiation (Fig. 15.2a). Instead, markers found in neurons (Fig. 15.2b) and oligodendrocytes were manifest (Fig. 15.2c, d), indicating that the other pathways modulated by SMFs [nine other signaling pathways besides IL-6 were affected by SMF exposure in this study (Wang et al. 2009)] tuned—and in fact reversed—the usual, and most often unwanted, pro-inflammatory activity of IL-6. Ultimately, if oligodendrocyte formation can be promoted in vivo by SMF treatment without concomitant scar-forming astrocyte enhancement, this capability could lead to noninvasive therapies for conditions such as multiple sclerosis (MS) that are linked to oligodendrocyte pathologies.

Other studies outside the author's laboratory have also investigated the role of SMF in the treatment of neurological disease, as well as in neural growth and regeneration. For example, it has been found that moderate-intensity SMF promotes

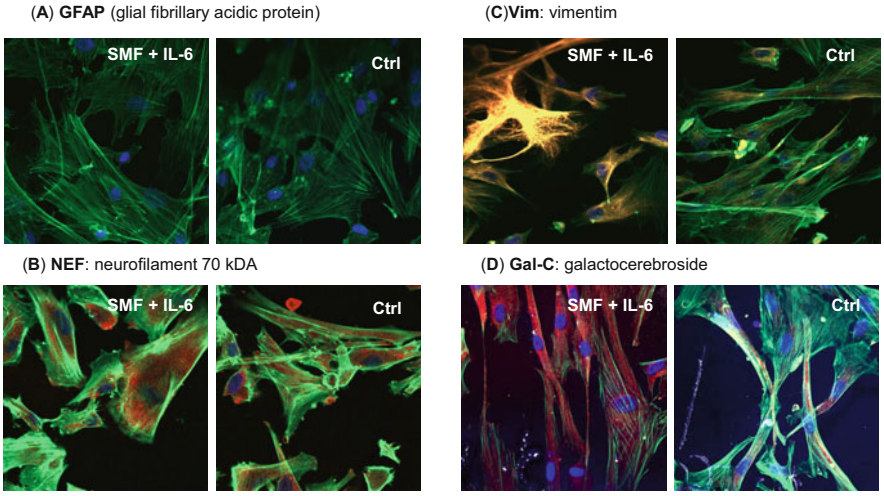

Fig. 15.2 SMF treatment reverses astrocyte differentiation in hEBD LVEC (human embryonic) cells. In these experiments, cells were treated with 4.0 ng/mL IL-6 and exposed to SMFs (control cells received neither stimuli) and the monolayers were co-stained with Oregon Green 488 phalloidin to visualize actin, the nuclear dye DAPI (blue), and one of the following markers (red). In Panel (**a**), the GFAP astrocyte marker was absent from both the control and treated cells (IL-6 treatment alone causes up-regulation, not shown). Panel (**b**) shows the neuron marker NEF. Panels (**c**) and (**d**) show expression of the pre-oligodendrocyte markers (**c**) Vim and (**d**) Gal-C, respectively, upon combined IL-6 and SMF treatment. Images were obtained by confocal microscopy, using identical exposure settings for each set of photographs. [Reprinted from (Wang et al. 2009), open access]

the differentiation of oligodendrocyte precursor cells into oligodendrocytes and the secretion of neurotrophic factors (Prasad et al. 2017), as well as increasing neurosphere formation and proliferation of neural progenitor cells (Ho et al. 2019). However, SMF in this same intensity range can also have a negative impact on astrocytes, adversely affecting their viability and decreasing their mitochondrial function (da Costa et al. 2021). Examples of research into SMF specifically for the treatment of neurological disorders include a 2018 study by Rivadulla and coauthors that demonstrated a reduction in epileptiform cortical activity in both a rat and monkey (Rivadulla et al. 2018), and a slightly earlier study by Dileone et al. that indicates that SMF modulates cortical activity in a dopamine-dependent manner in Parkinson's disease patients (Dileone et al. 2017).

15.4.4 Stem Cells

One of the most notable recent developments in the study of biological SMF application is the usage of SMF for stem cell engineering, in which SMF is used as a method of regulating stem cell fate. This emerging application for SMF has

particularly been used for chondrogenic, osteogenic, and adipogenic stem cells, and it has been speculated that SMF can be used to induce synthesis and secretion of extracellular microvesicles from stem cells for regenerative medicine as well (Marycz et al. 2018). For example, it has been shown that bone mesenchymal stem cells stimulated with magnetic nanoparticles and SMF can produce exosomes that promote both osteogenesis and angiogenesis (Wu et al. 2021). Human mesenchymal stem cell proliferation, alignment, and expression of stemness marker genes can also be affected by SMFs of up to 24 mT, indicating that SMF can be used as a tool to induce differentiation (Sadri et al. 2017). Adipose-derived stem cells, meanwhile, have been studied for the purpose of improving cardiac regeneration. Stem cells have been preloaded with superparamagnetic iron oxide particles and exposed to SMF, resulting in increased retention of cardiac cells and improved recovery of heart function (Wang et al. 2016).

Some other forms of stem cell research involving SMFs have been conducted that do not involve the utilization of chondrogenic, osteogenic, or adipogenic stem cells. For example, moderate-intensity SMF has been found to improve proliferative activity in neural progenitor cells in mice, as well as increasing neurosphere formation, as mentioned above in Sect. 15.3.3 (Prasad et al. 2017). Stem cell-related applications of SMF are just beginning to emerge, but they are already showing promise in the field of regenerative medicine for multiple different cell types.

15.4.5 Other Therapeutic Areas for Static Magnetic Field

There are a variety of other, less prominent therapeutic applications that have been researched for SMF as well. SMF has been studied for use in the treatment of Type II diabetes and other redox-related metabolic diseases (Carter et al. 2020), the investigation of effects on radiotherapeutic endpoints when combined with ionizing radiation (Mohajer 2019), and understanding the impact of magnetic fields on orthodontic movement of teeth and regeneration of oral tissue (Lew et al. 2021), among others. It is possible that, in the future, other applications of SMF therapy will become prominent research topics, such as SMF application to stem cell research (Sect. 15.4.4).

15.5 Pitfalls with SMF Clinical Studies and Acceptance of Magnetotherapy

15.5.1 Hyperbolic and Ambiguous Claims vs. Outright Rejection of Magnetotherapy

It can be a daunting task to precisely match treatment parameters to various pathological indications even for long-standing medicines. For example, it has taken a

century to understand how to fully exploit aspirin as a medicine; indeed, some aspects of this drug remain poorly understood. For example, at a pharmacological level, the need for esterase processing of aspirin is not fully elucidated (Lavis 2008). However, much is known about some aspects of aspirin, including the impact of administration duration on dosage effectiveness, as well as how it appears to reduce the risk of cardiovascular disease. On the other hand, no evidence exists that aspirin is effective against many other conditions, for example, pancreatic cancer or neurological disorders such as Alzheimer's disease. Aspirin is again used here to illustrate pitfalls—and lessons to be learned—for magnetic field therapy. Aspirin, if tested against a wrong medical indication—or at the wrong dose or duration—could easily be shown to have no effect, but this does not mean that it has no benefit for other ailments. Similarly, magnetic field therapy should not be considered to be debunked if a certain treatment modality shows no effect against a certain ailment; indeed, to the contrary, careful compiling of conditions that do *not* work could be extremely helpful in guiding treatments toward diseases and other ailments where the magnetotherapy *does* work.

Unfortunately, the efficacy of magnetic therapy has been clouded by ambiguity that results in large part from study design, as illustrated by a review of over 50 studies approximately a decade ago (Colbert et al. 2007, 2009). In these studies, only two provided sufficiently detailed experimental protocols to actually reproduce the work; although a more recent systematically analyzed compilation of studies does not appear to be available, anecdotal perusal of the literature over the past decade suggests that the problem of incomplete reporting of experimental conditions persists up to today. As Markov forcefully editorializes, until parameters used in magnetic field therapy—starting at a very basic terminology level to overcome confusion over semantic differences between "magnetic therapy" and "magnetic field therapy" (i.e., magnets themselves have no therapeutic effect but the fields they produce do)—magnetotherapy is apt to remain marginalized and not fully accepted by the mainstream scientific and medical communities (Markov 2009). Indeed, Markov (and his colleagues) have been trying to educate about these issues for at least two decades, and in that vein, has proposed a set of parameters that must be considered and clearly defined; these endpoints are discussed next in Sect. 15.5.2.

15.5.2 Parameters Necessary to be Controlled in Magnetotherapy

The variety of commercially available EMF devices—often with poorly characterized and sometimes misrepresented field strength specifications—makes it difficult to compare the physical and engineering characteristics of any particular device used in any reported study, thus providing significant obstacles for analysis of clinical efficacy. Markov outlines a set of parameters that must be controlled, defined, and reported to be able to evaluate magnetotherapy outcomes (Markov 2009); these are:

- **Type of field.**
- **Intensity or induction.**
- **Spatial gradient (dB/dx).**
- **Localization.**
- **Time of exposure.**
- **Depth of penetration.**
- *Temporary change (dB/dt).*
- *Frequency.*
- *Pulse shape.*
- *Component (electric or magnetic).*

An attraction of SMF therapy is that the latter four parameters (indicated in *italics*) are not in play, thereby simplifying evaluation of this therapeutic modality, and in theory, increasing the reproducibility of the studies.

15.5.3 The Placebo Effect

As already alluded to above, pain response was the only medical outcome where magnetic fields unambiguously had a beneficial therapeutic effect, based on the bulk of the literature reviewed by Del Seppia and coauthors a decade ago (Del Seppia et al. 2007). Many of the relevant studies were performed in animals, often rodents, where there presumably is no placebo effect, but in humans, the placebo response cannot be discounted so easily. Indeed, difficulties in establishing benefits of magnetic therapy result in part from designing experiments that account for the placebo effect. For example, a study from 1978 describes "the extreme cleverness with which perceptive individuals unintentionally used subtle auxiliary clues to develop impressive records of apparent magnetic field detection" (Tucker and Schmitt 1978). Of course, in many cases, not even "extreme cleverness" is necessary for a test subject to figure out whether they are part of the placebo control arm of a study, because real magnets have a propensity to attract loose magnetically susceptible objects such as paper clips.

As discussed earlier, evidence suggests that deep-penetrating SMFs of at least 0.2 T are required to affect the biophysical properties of membranes (Braganza et al. 1984) implicated in therapeutic responses in humans at the cell level (Wang et al. 2009, 2010). The only plausible way to deliver these fields in a deeply penetrating manner is to use electrical coils to generate the required moderate strength (e.g., 0.3–0.5 T) magnetic fields. One example of such an instrument is the MME device (Fig. 15.1) developed by AMRi (Bonlie 2001), which requires seven miles of copper coils situated above and below a patient (the entire apparatus is close two stories in height). In theory, pitfalls that befall efforts to conduct controlled clinical trials using DIY-type wearable magnets (such as attracting or not attracting) loose paperclips during everyday activities can be avoided by strictly monitoring the treatment environment. In reality, however, when in operation, electricity running through

the device needed to generate the SMFs creates a perceptible humming noise, making it obvious whether or whether or not actual treatment is underway. As a result, control subjects in double-blind clinical studies (ClinicalTrials.gov Identifier: NCT00325377 and NCT00134524) were subject to record MME device noise. Interestingly—and perhaps unsurprisingly—a large placebo effect was observed in these studies that plausibly can be explained by the belief of control subjects that they were undergoing legitimate SMF exposure.

The placebo effect—evidenced by sham-treated test subjects experiencing improvement to long-standing conditions (lower back pain and diabetic neuropathy) that were not responsive to conventional medical treatment at rates comparable to SMF-treated individuals—illustrates the growing realization that placebo treatment is not equivalent to "no treatment." Briefly, placebo effect depends on belief in the effectiveness of the treatment; in fact, the opposite "nocebo" effect has been proposed where a patient who disbelieves in a treatment may experience a worsening of symptoms (Kennedy 1961). Of note, "belief" is a rather ambiguous concept, but can theoretically be converted into physiological modulation through opioid neurotransmitters whose endogenous production is controlled by the brain.

The placebo effect can be powerful, with attempts to objectively measure its contribution to medical intervention overall ranging from 30 to 40% of overall observed effects of a medicine. The impact of the placebo effect varies among treatment modalities and disease conditions, with one of the stronger responses reported for the effects of antitussive medicines in patients with acute upper respiratory tract infections. In these patients, 85% of the reduction in coughing was linked to the placebo effect, whereas only 15% was linked to the actual physiological effects of the pharmacological agents (Eccles 2002). It appears that the placebo effect might be equally pervasive and influential in response to SMF treatments and, in a lesson being learned from psychiatry (Horgan 2013), the field should consider embracing—rather than being embarrassed by—this aspect of magnetotherapy.

15.6 Concluding Comments

This chapter describes various modes of EMF therapy, with the main focus on SMFs. Up to now, this therapeutic modality has both shown promise and been downplayed, in part due to over-enthusiastic claims by its practitioners. Accordingly, strict guidelines have been proposed to maintain "quality control" when patients are being treated with magnetotherapy in efforts to rigorously establish efficacy against specific medical indications, several of which are mentioned and described in some detail (e.g., pain perception and management, blood flow and vascularization, neurological regeneration, and stem cell differentiation in Sect. 15.4).

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