Engineered cylindrical NiFe magnetic nanoparticles for cancer cell death

Wong, Shawn De Wei

2019

Wong, S. D. W. (2019). Engineered cylindrical NiFe magnetic nanoparticles for cancer cell death. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/81428>

<https://doi.org/10.32657/10356/81428>

**NANYANG
TECHNOLOGICAL
UNIVERSITY**

SINGAPORE

**Engineered Cylindrical NiFe Magnetic Nanoparticles
for Cancer Cell Death**

Wong De Wei Shawn

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

2019

Engineered Cylindrical NiFe Magnetic Nanoparticles for Cancer Cell Death

Wong De Wei Shawn

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

A thesis submitted to the Nanyang Technological
University in partial fulfilment of the requirement for the
degree of Doctor of Philosophy

2019

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research done by me except where otherwise stated in this thesis. The thesis work has not been submitted for a degree or professional qualification to any other university or institution. I declare that this thesis is written by myself and is free of plagiarism and of sufficient grammatical clarity to be examined. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

16/1/19

.....
Date

shawn

.....
Wong De Wei Shawn

Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it of sufficient grammatical clarity to be examined. To the best of my knowledge, the thesis is free of plagiarism and the research and writing are those of the candidate's except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

17 Jan 2019

.....
Date

.....
Assoc. Prof Lew Wen Siang

Authorship Attribution Statement

This thesis contains material from 6 paper(s) published in the following peer-reviewed journal(s) where I was the first and/or corresponding author.

Chapter 1 is published as D. W. Wong, M. C. Sekhar, W. L. Gan, I. Purnama, and W. S. Lew, Dynamics of three-dimensional helical domain wall in cylindrical nanowires, *Journal of Applied Physics* **117**, 17A747 (2015).

The contributions of the co-authors are as follows:

- M. C. Sekhar, W. L. Gan and I. Purnama assisted in the collection and provided guidance in the interpretation of the simulation data.
- The project was supervised by Prof W. S. Lew.
- All authors discussed the results and contributed to the manuscript.

Chapter 1 is published as D. W. Wong, I. Purnama, G. J. Lim, W. L. Gan, M. C. Sekhar and W. S. Lew, Current-induced three-dimensional domain wall propagation in cylindrical NiFe nanowires, *Journal of Applied Physics* **119**, 153902 (2016).

The contributions of the co-authors are as follows:

- I. Purnama, W. L. Gan and M. C. Sekhar assisted in the collection and provided guidance in the interpretation of the experimental and simulation data.
- G. J. Lim assisted in the fabrication of the anisotropic magnetoresistance measurement setup.
- The project was supervised by Prof W. S. Lew.
- All authors discussed the results and contributed to the manuscript.

Chapter 2 is published as W. L. Gan, M. C. Sekhar, D. W. Wong, I. Purnama, S. Y. Chiam, L. M. Wong and W. S. Lew, Multi-vortex states in magnetic nanoparticles, *Applied Physics Letters* **105**, 152405 (2014).

The contributions of the co-authors are as follows:

- I assisted in the fabrication and characterization of the magnetic nanoparticles.
- M. C. Sekhar and I. Purnama assisted in the collection and provided guidance in the interpretation of the experimental and simulation data.
- S. Y. Chiam and L. M. Wong conducted the transmission electron microscopy.
- The project was supervised by Prof W. S. Lew.
- All authors discussed the results and contributed to the manuscript.

Chapter 3 is published as D. W. Wong, W. L. Gan, Y. K. Teo and W. S. Lew, Heating efficiency of triple vortex state cylindrical magnetic nanoparticles. (Submitted)

The contributions of the co-authors are as follows:

- W. L. Gan assisted in the design of the magnetic field configurations and micromagnetic simulations.
- Y. K. Teo assisted in the *in vitro* cell viability experiments.
- The project was supervised by Prof W. S. Lew.
- All authors discussed the results and contributed to the manuscript.

Chapter 4 is published as D. W. Wong, W. L. Gan, N. Liu and W. S. Lew, Magneto-actuated cell apoptosis by biaxial pulsed magnetic field, *Scientific Reports* **7**, 10919 (2017).

The contributions of the co-authors are as follows:

- W. L. Gan assisted in the fabrication of the magnetic nanoparticles and magneto-actuated experiments.
- L. Ning assisted in the *in vitro* cell viability experiments.
- The project was supervised by Prof W. S. Lew.
- All authors discussed the results and contributed to the manuscript.

Chapter 5 is published as D. W. Wong, W. L. Gan, Y. K. Teo and W. S. Lew, Interplay of cell death signaling pathways mediated by alternating magnetic field gradient, *Cell Death Discovery* **4**, 49 (2018).

The contributions of the co-authors are as follows:

- W. L. Gan assisted in the design of the magnetic field configurations.
- Y. K. Teo assisted in the *in vitro* cell viability experiments.
- The project was supervised by Prof W. S. Lew.
- All authors discussed the results and contributed to the manuscript.

16/1/19

.....

Date

shawn

.....

Wong De Wei Shawn

Abstract

This thesis investigates the magnetization dynamics of cylindrical NiFe magnetic nanoparticles (MNPs), fabricated by template-assisted pulsed electrodeposition and differential chemical etching technique. Upon relaxation from a magnetically saturated state, a double vortex nucleation consisting of a clockwise and an anticlockwise vortex forms at the opposite ends of the MNP, which gradually extends and is connected via a third vortex core on its curved surface. Micromagnetic simulations revealed that the magnetization reversal occurs via the nucleation of the triple vortex state and abrupt splitting of the clockwise and anticlockwise vortices.

The application of different magnetic field configurations to control the MNPs can manipulate the pathways leading to cell death, playing a pivotal role in cancer treatment. In an alternating magnetic field, the magnetic hysteresis of the MNPs results in heat dissipation that causes necrosis of cancer cells. High aspect ratios MNPs were observed to form the triple vortex state, which displayed high heating efficiency as measured by the specific absorption rate.

For uniform magnetic fields, biaxial field configuration has been shown to be the most efficient magneto-actuated cell apoptosis method, which maximized the induced magnetic torque. Light transmissivity dynamics showed that MNPs under the biaxial field configuration have higher responsiveness over a wide range of frequencies as compared to uniaxial field configurations. The magneto-mechanical cell destruction efficacy was substantiated by a greater reduction in cell viability in *in vitro* experiments. High aspect ratios MNPs with the triple vortex state had increased low field susceptibility that translated to a larger magneto-mechanical actuated force, leading to higher efficacy in cell death.

For non-uniform magnetic fields, MNPs in a strong vertical magnetic field gradient were able to apply sufficient force on the cell to trigger the intracellular pathway for cell apoptosis, thus significantly reducing cell viability. In contrast, MNPs in an alternating magnetic field gradient can effectively rupture the cell membrane leading to higher lactate dehydrogenase leakage and lower cell viability, proving to be an effective induction of cell death via necrosis. The capability of the MNPs as both magnetic hyperthermia and magneto-actuation cell destruction agents is demonstrated by inducing different cell death signaling pathways, exemplifying the intricate interplay between apoptosis and necrosis.

Acknowledgements

I would like to extend my sincerest appreciation and gratitude to:

Prof. Lew Wen Siang for the guidance and support throughout my undergraduate and postgraduate research. All the journal publications and conferences that followed would be impossible without him.

Dr. Naganivetha Thiyagarajah, Mr. Yang Hyunwoo, Mr. Cong Hai and Dr. Alex See for their mentorship and support throughout the course of my research. Their endless encouragements and cheers spur me to work even harder each day.

Mr. Gan Weiliang for his never-ending feedback and criticism during the course of my research.

Finally, words cannot express my thankfulness to my parents for their support and understanding.

Table of Contents

Abstract.....	1
Acknowledgements.....	2
Chapter 1 – Introduction	6
1.1 Domains and Domain Walls	6
1.2 Domain Wall Motion	8
1.3 Hysteresis Loop	9
1.4 Micromagnetic Model.....	10
1.5 Micromagnetic Simulators.....	13
1.6 Domain Wall Stability	17
1.7 Three-dimensional Domain Wall.....	21
1.8 Domain Wall Motion	23
1.9 Anisotropic Magnetoresistance.....	27
1.10 Current-induced Domain Wall Driving	32
1.11 Domain Wall Pinning and Depinning Mechanism	35
1.12 Néel and Brown Relaxation Mechanisms.....	38
1.13 Magnetic Hyperthermia	40
1.14 Magneto-Mechanical Actuation.....	45
1.15 Cell Cycle.....	46
1.16 Programmed Cell Death.....	47
1.17 Cancer	48
1.18 Motivation.....	49
1.19 Organization of Thesis.....	51
Chapter 2 – Experimental Techniques	53
2.1 Chapter Introduction	53
2.2 Pulsed Electrodeposition.....	53
2.3 Thermal Evaporation	58
2.4 Magnetron Sputtering	60

2.5 Photolithography	62
2.6 Magnetoresistance Measurement.....	65
2.7 Scanning Electron Microscopy	67
2.8 Energy Dispersive X-ray Spectroscopy	69
2.9 Vibrating Sample Magnetometer	70
2.10 Micromagnetic Simulation Parameters.....	72
2.11 Cell Culture	73
2.12 Fluorescence Microscopy	75
2.13 Magnetic Hyperthermia Setup	78
Chapter 3 – Functionalization of Magnetic Nanoparticles for Magnetic Hyperthermia	79
3.1 Chapter Introduction	79
3.2 Fabrication of Cylindrical NiFe Magnetic Nanoparticles.....	80
3.3 Composition Control.....	83
3.4 Magnetization Reversal in Cylindrical Magnetic Nanoparticles	86
3.5 Biocompatible Surface Coating	92
3.6 Calorimetric Measurements	95
3.7 Chapter Summary	102
Chapter 4 – Magnetic Nanoparticles Actuated by Biaxial Pulsed Magnetic Field.....	103
4.1 Chapter Introduction	103
4.2 Light Transmissivity Dynamics	104
4.3 Biocompatibility	110
4.4 Magneto-Actuated Cell Apoptosis.....	113
4.5 Comparison of Magnetic Nanoparticles with Different Aspect Ratios	118
4.6 Chapter Summary	122
Chapter 5 – Interplay of Cell Death Signaling Pathway by Magnetic Field Gradient	124
5.1 Chapter Introduction	124
5.2 Uniform Magnetic Field	126
5.3 Non-Uniform Magnetic Field	131

5.4 Alternating Magnetic Field Gradient	133
5.5 Quantification of Cell Death.....	134
5.6 Quantification of Caspase-3/7 Activation.....	138
5.7 Quantification of Cell Membrane Damage.....	139
5.8 Chapter Summary	141
Chapter 6 – Conclusion & Future Works	143
6.1 Conclusion	143
6.2 Future Works	144
References.....	147
Publications.....	167
Conferences.....	168

Chapter 1 – Introduction

In recent years, magnetic nanoparticles (MNPs) have increasingly gained traction in biomedical fields as contrast agents for magnetic resonance imaging, biosensors and controlled drug delivery.¹⁻⁶ In addition, MNPs with engineered magnetic properties and high biocompatibility have been shown to be a promising candidate for cancer treatment. Magnetic hyperthermia is a well-established cancer treatment technique which employs the use of localized heating by the Néel relaxation of the MNPs under a high frequency alternating magnetic field, to induce cancer cell apoptosis and tumor regression.⁷⁻¹⁰ The magnetic hysteresis of the MNPs results in heating of tumor tissue to temperatures above 43 °C, thereby leading to cancer cell necrosis.^{11,12} In low frequency alternating magnetic fields, the heat generated is negligible, but the mechanical stress exerted on the cells is sufficient to achieve cell apoptosis.¹³⁻¹⁵ Magneto-mechanical actuation is achieved by the spatial rotation of the particles to align to the applied magnetic field by Brown relaxation, which produces a magnetic torque.¹⁶ In this thesis, the fabrication and capability of cylindrical NiFe MNPs, with multi-vortex states, was evaluated for magnetic hyperthermia and magneto-mechanical actuation to induce different cancer cell death signaling pathways.

1.1 Domains and Domain Walls

The concepts of domain hypothesis and molecular field were first introduced by P. Weiss in 1906.¹⁷ A magnet is divided into small volume fractions such as domains, which are effectively magnetized to the value of spontaneous magnetization. Each domain is oriented along a different easy axis, and thus the net sample magnetization is zero. When applying a magnetic field, the domains grow at the expense of other domains, thereby resulting in a net magnetization in the system. However, there was no direct experimental observation of domains in a real material until 1949. J. Williams, R. M. Bozorth, and W. Shockley published

their work on domains in single SiFe crystals, which was done at the Bell Telephone Laboratories.¹⁸

When a magnetic material is saturated along the easy axis, the exchange and anisotropy energies are kept at a minimum, while the magnetostatic energy becomes larger due to the demagnetizing field. If the magnetic material is divided into domains, this will result in a reduction of magnetostatic energy, while maintaining the anisotropy energy and exchange energy at a minimum energy state. Figure 1.1 (a) illustrates a uniaxial anisotropy of single crystal, which has uniform magnetization throughout the crystal and maximum demagnetization energy. As the crystal is divided into two or four anti-parallel spontaneous magnetization, this results in a great reduction in the demagnetization energy, as shown in Figure 1.1 (b-c). By forming a closed domain, the demagnetization energy can be further reduced. But the closure of the domains deviates from the easy axis direction, thus increases the anisotropy energy, as shown in Figure 1.1 (d-e). If the crystal is divided into n number of domains, the demagnetization energy reduces by $\sim 1/n$ times, but the existence of a domain wall (DW) between two adjacent magnetic domains results in a rise in DW energy. DWs have finite width, within which the transition from one domain to another is realized by means of a gradual rotation of the spin directions. The energy of DW arises from the spin energy needed to overcome the exchange energy and magnetic anisotropy energy to rotate. There is no infinite formation of DWs, but DW energy and demagnetization energy are minimized.

Figure 1.1 Illustrations of domains and domain walls

1.2 Domain Wall Motion

Spin transfer torque (STT) has allowed the control of the magnetization of ferromagnetic materials and also used to move DWs in magnetic nanowires by spin current pulses. The current injected into the ferromagnetic nanowires becomes spin-polarized due to the spin-dependent diffusive scattering.¹⁹⁻²² As the spin-polarized current passes through the DW, there is a transfer of spin angular momentum to the DW, whereby a torque is applied to the DW moment owing to the conservation of angular momentum. This results in a displacement of the DW, which is known as current-induced DW motion. Furthermore, this interaction is highly localized as it only occurs in the region where the spin current flows. This phenomenon was theorized by L. Berger and J. C. Slonczewski in 1996.^{23,24} Figure 1.2 illustrates the interaction of a spin polarized electron with the magnetization of a thin ferromagnetic material, which results in a change of the magnetization direction due to the STT effect.

Figure 1.2 Illustration of STT effect in ferromagnetic materials. The interaction between the incoming spin-polarized electron and ferromagnetic material changes the direction of the electron spin. The difference in spin polarization induces a torque on the magnetization of ferromagnetic material owing to the STT effect.

1.3 Hysteresis Loop

The magnetic properties of a material can be obtained from examining its characteristics shown in its hysteresis loop. A hysteresis loop displays the relationship of the induced magnetic flux density B at various field strength H , which is also known as the B – H loop. Figure 1.3 illustrates a typical hysteresis loop of ferromagnetic materials.

The hysteresis loop of a material is obtained by measuring the magnetic flux generated, while sweeping a magnetic field. A ferromagnetic material in its initial state is not magnetized and will follow the dotted line with increasing magnetic field strength H . At high H , most of the magnetic domains are aligned with the direction of the field and any further H increase will have no significant increase in magnetic flux B . Hence, it is said to be magnetically saturated. At this point, known as the saturation magnetization M_s of the material, all the magnetic dipoles within the material are aligned in the direction of H .

As H is reduced to zero ($H = 0$), it is observed that the magnetic flux does not return to zero. This is known as the point of retentivity, which indicates the remanent magnetization M_r in the material. As H reverses, the magnetic flux reduces to zero ($B = 0$). This is known as the point of coercivity on the loop H_c , which indicates where the overall magnetization of the material is zero. It is the amount of magnetic field needed to remove the remanent magnetization in the material, also known as the coercivity of the material. As H increases further in negative direction, the material will also be saturated at high H .

Next, decreasing H to zero will have the similar remanent magnetization equal to that obtained in the positive field direction. As H increases in the positive direction ($H > 0$), the magnetic flux again reduces to zero ($B = 0$). However, the loop does not return to the origin ($B = 0, H = 0$) because a certain amount of H is now required to remove the remanent

magnetization. Therefore, the loop follows a dissimilar path from saturation at high H , completing the hysteresis loop.

Figure 1.3 Illustration of the hysteresis loop of ferromagnetic materials.

1.4 Micromagnetic Model

The magnetization dynamics of ferromagnetic materials with various geometries was investigated using numerical methods, such as the finite difference to solve the Landau–Lifshitz–Gilbert–Slonczewski (LLGS) equation in three dimensions.²⁵⁻²⁷ These micromagnetic simulations provided insights to the magnetization configurations at the microscopic level, which prompted the correlation between analytical models and observations from experimental results. Landau–Lifshitz equation, named for Lev Landau and Evgeny Lifshitz, describes the precession of magnetization $\mathbf{M}$ in an effective magnetic field $\mathbf{H}_{eff}$ with damping α . The total energy of a system is described by:

$$E_{Total} = E_{Exchange} + E_{Anisotropy} + E_{Zeeman} + E_{Dipolar} = -\int \mu_0 \mathbf{H}_{eff}(\mathbf{r}) \cdot \mathbf{M}(\mathbf{r}) d^3r$$

where $\mathbf{H}_{eff} = -\frac{1}{\mu_0} \nabla_M E$.

The exchange energy is derived from the exchange interaction where individual magnetic moments try to align neighboring magnetic moments with themselves, given by:

$$E_{Exchange} = A \int |\nabla \mathbf{m}|^2 d^3 r$$

where A is the exchange coupling constant.²⁸

The anisotropy energy is the energy due to the combination of crystal structure and spin-orbit interaction, given by:

$$E_{Anisotropy} = \int -K m_z^2 d^3 r$$

where K is the anisotropy constant.²⁸ In this approximation, uniaxial anisotropy, the easy axis is the z -direction.

The Zeeman energy is the interaction energy between the magnetization $\mathbf{M}$ of the material with any external applied field, given by:

$$E_{Zeeman} = -\mu_0 \int \mathbf{M}(\mathbf{r}) \cdot \mathbf{H}(\mathbf{r}) d^3 r$$

where $\mathbf{H}(\mathbf{r})$ is the external applied field and μ_0 is the vacuum permeability.²⁸ The Zeeman energy is minimized when all the magnetic moments in the material aligns with the applied field.

The dipolar energy or demagnetizing energy is the resultant energy from the interaction of magnetic moments with each other, given by:

$$E_{Dipolar} = -\mu_0 \int \mathbf{H}_{de}(\mathbf{r}) \cdot \mathbf{M}(\mathbf{r}) d^3 r$$

where the demagnetizing field $\mathbf{H}_{de}(\mathbf{r})$ has components contributed from the divergence of magnetization within the volume and surface poles.²⁹

Landau–Lifshitz–Gilbert (LLG) equation:

$$\frac{d\mathbf{M}(\mathbf{r})}{dt} = -\gamma \mathbf{M}(\mathbf{r}) \times \mathbf{H}_{eff}(\mathbf{r}) - \frac{\bar{\alpha}}{M_s} \mathbf{M}(\mathbf{r}) \times (\mathbf{M}(\mathbf{r}) \times \mathbf{H}_{eff}(\mathbf{r}))$$

where $\gamma \mathbf{M}(\mathbf{r}) \times \mathbf{H}_{eff}(\mathbf{r})$ is the precession of $\mathbf{M}(\mathbf{r})$ in a local field $\mathbf{H}_{eff}(\mathbf{r})$ and

$\frac{\bar{\alpha}}{M_s} \mathbf{M}(\mathbf{r}) \times (\mathbf{M}(\mathbf{r}) \times \mathbf{H}_{eff}(\mathbf{r}))$ is the empirical damping term.

Landau–Lifshitz–Gilbert–Slonczewski (LLGS) equation with STT term:

$$\begin{aligned} \frac{d\mathbf{M}(\mathbf{r})}{dt} = & -\gamma \mathbf{M}(\mathbf{r}) \times \mathbf{H}_{eff}(\mathbf{r}) - \frac{\bar{\alpha}}{M_s} \mathbf{M}(\mathbf{r}) \times (\mathbf{M}(\mathbf{r}) \times \mathbf{H}_{eff}(\mathbf{r})) \\ & - (\mathbf{u} \cdot \nabla) \mathbf{M}(\mathbf{r}) + \frac{\beta}{M_s} \mathbf{M}(\mathbf{r}) \times (\mathbf{u} \cdot \nabla) \mathbf{M}(\mathbf{r}) \end{aligned}$$

where β is the non-adiabatic STT constant. The velocity $\mathbf{u}$ has amplitude of $\frac{JPg\mu_B}{2eM}$, Landè g-

factor g , Bohr magneton μ_B , electron charge e , polarization rate P and applied current

density J . For Permalloy NiFe, the factor $\frac{g\mu_B}{2eM}$ amounts to $7 \times 10^{-11} \text{ m}^3/\text{C}$. Figure 1.4 illustrates

the magnetization dynamics that describes the precessional motion of $\mathbf{M}$ about $\mathbf{H}_{eff}$, with a damping torque that tends to move $\mathbf{M}$ towards the direction of $\mathbf{H}_{eff}$.

Figure 1.4 Illustration of the Landau–Lifshitz–Gilbert–Slonczewski (LLGS) equation

1.5 Micromagnetic Simulators

1.5.1 Object Orientated Micro Magnetic Framework (OOMMF)

OOMMF is a portable and extensible public domain micromagnetic program by the Applied and Computational Mathematics Division of the Information Technology Laboratory at the National Institute of Standards and Technology US. The fully functional micromagnetic package also allows other software developers to expand its capability based on the OOMMF foundation. The main developers of OOMMF, M. Donahue and D. Porter (1999),³⁰ use the LLG form:

$$\frac{d\mathbf{M}(\mathbf{r},t)}{dt} = -|\bar{\gamma}|\mathbf{M}(\mathbf{r},t) \times \mathbf{H}_{eff}(\mathbf{M}(\mathbf{r},t)) - \frac{|\bar{\gamma}|\alpha}{M_s}\mathbf{M}(\mathbf{r},t) \times (\mathbf{M}(\mathbf{r},t) \times \mathbf{H}_{eff}(\mathbf{M}(\mathbf{r},t)))$$

where $\mathbf{M}$ is the magnetization of the material, $\mathbf{H}_{eff}$ is the effective magnetic field and α is the Landau Lifshitz damping parameter.

If one assumes $\gamma = (1 + \alpha^2)\bar{\gamma}$ and $\bar{\alpha} = |\bar{\gamma}|\alpha$, the LLG equation is mathematically equivalent to the Gilbert form, 1955:

$$\frac{d\mathbf{M}}{dt} = -|\gamma|\mathbf{M} \times \mathbf{H}_{eff} + \frac{\alpha}{M_s} \left(\mathbf{M} \times \frac{d\mathbf{M}}{dt} \right)$$

OOMMF is available at <http://math.nist.gov/oommf/>.

1.5.2 MuMax3

MuMax3 is an open-source, GPU-accelerated micromagnetic simulation program.³¹ It calculates the magnetization dynamics in ferromagnetic materials using a finite-difference discretization method, similar to OOMMF. The advantage of GPU acceleration is 1–2 orders of magnitude increase in speed, as compared to CPU-based micromagnetic simulations, i.e. OOMMF. MuMax3 is available at <http://mumax.github.io>.

1.5.3 Simulation Data

1. Iteration: The number of iteration of the ordinary differential equation solver.
2. Field Updates: The number of calculated effective field by the ordinary differential equation solver.
3. Simulation Time: The elapsed simulation time. [s]
4. Time Step: The elapsed simulation time of the most recent step taken by the ordinary differential equation solver. [s]
5. Step Size: The normalized value of most recent step taken by the ordinary differential equation solver.
6. B_x, B_y, B_z : The value of the x, y , and z direction of the nominal applied field. [T]
7. B : The value of the nominal applied field (< 0). [T]
8. $|\mathbf{m} \times \mathbf{h}|$: The max value of $|\mathbf{M} \times \mathbf{H}_{eff}|/M_s^2$ over all the spins.
9. $(M_x, M_y, M_z)/M_s$: The value of the x, y , and z direction of the average magnetization of the magnetic material.
10. Total Energy: The total average energy density for the magnetically active material. [J/m³]
11. Exchange Energy: The average energy density due to the exchange interactions. [J/m³]
12. Anisotropy Energy: The average energy density due to the crystalline and surface anisotropies. [J/m³]
13. Dipolar Energy: The average energy density due to the self-demagnetizing fields. [J/m³]
14. Zeeman Energy: The average energy density due to the interaction with the external applied field. [J/m³]

1.5.4 Discretization

Micromagnetics simulators use the finite difference method which requires the discretization or segmentation of the investigated geometry over a grid of identical volume and cuboidal shape cells.^{32,33} The atomic magnetic moments inside each individual cubic cell behave as a single particle, and hence each cell is considered to be homogeneously magnetized. This assumption is acceptable as the exchange interaction is largely responsible for the homogeneous alignment of the magnetic moment at the atomic level. A finer discretization mesh can be obtained by increasing the number of cells, i.e. using a smaller simulation cell size, which is more suitable for geometry with curved surfaces.³⁴

Figure 1.5 illustrates the impact of increasing the number of cells in a cylindrical geometry nanowire; a unit cell size of (a) $25 \times 25 \times 5$ nm, (b) $10 \times 10 \times 5$ nm (c) $5 \times 5 \times 5$ nm. If the simulation cell size is too large or the number of cells is too low, the simulation will be a poor representation of the shape in study. This can affect the influence of shape anisotropy on the magnetization, and hence greatly affect the micromagnetics. Figure 1.5 (a) shows the formation of multiple vortex domains due to the loss of shape anisotropy of the nanowire when the cell size is greater than the exchange length of the material.

Figure 1.5 Side view of the discretization on cylindrical geometry nanowire with increasing number of cells

1.5.5 Exchange Length

Exchange length is the length at which the magnetization in the material does not vary significantly as the exchange energy is the most dominant factor compared to the other energies.³⁵ Therefore, the number of cells in the micromagnetic simulations is determined by the exchange length of each individual material, such that no incorrect independent domains will be formed. The theoretical exchange length of a given material is calculated by:

$$\lambda_{ex} = \sqrt{\frac{2A}{\mu_0 M_s^2}}$$

where A is the exchange energy, μ_0 is the magnetic constant and M_s is the saturation magnetization of the material.^{36,37} The exchange length λ_{ex} gives the quantitative measure for the required grid resolution of the micromagnetic object in study. Table 1 shows a list of materials parameters for the common ferromagnetic materials used in the micromagnetic simulations.

Material	Exchange Energy, A [J/m]	Magnetization, M_s [A/m]	Anisotropy, K [J/m ³]	Exchange Length, λ_{ex} [nm]
Ni	9.0×10^{-12}	4.9×10^5	-5.7×10^3 (cubic)	7.72
Fe	2.1×10^{-13}	1.70×10^6	4.8×10^4 (cubic)	3.40
Co	3.0×10^{-13}	1.40×10^6	5.2×10^5 (uniaxial)	4.94
Ni ₅₀ Fe ₅₀	5.85×10^{-12}	1.11×10^6	0	2.76
Ni ₈₀ Fe ₂₀	1.30×10^{-13}	8.6×10^5	0	5.29

Table 1 Material parameters of some common ferromagnetic materials ^{36,37}

1.6 Domain Wall Stability

When a high current or magnetic field is applied, the profiles of DWs in planar nanowires will start to break down above the Walker limit.^{38,39} The unstable DW profile results in an oscillation of the instantaneous DW velocity and the abrupt drop in the average velocity, as shown in Figure 1.6. The reliable control of DW motion induced by magnetic field or current has been a major issue and technological challenge in magnetic nanowires.

Figure 1.6 Illustration of Walker breakdown phenomenon. The DW velocity is presented against applied magnetic field H and applied current density J in a nanowire.

A recent micromagnetic study on the current-induced DW dynamics in ferromagnetic cylindrical nanowires showed that the transverse DWs formed in cylindrical nanowires behave significantly different from DWs in planar nanowires.⁴⁰⁻⁴² In cylindrical nanowires, it has been predicted that the DW velocity is linearly dependent on the current density and the absence of Walker breakdown is the result of geometrical symmetry of the nanowires.

DW motion in a planar nanowire at zero applied fields is given by:

$$\dot{\phi} = \frac{\alpha}{(1+\alpha^2)} \left(\frac{\beta-\alpha}{\alpha} \frac{u}{\Delta} - \frac{\gamma MN}{2} \sin 2\phi \right)$$

$$\dot{q} = \frac{1}{(1+\alpha^2)} \left((1+\alpha\beta)u + \frac{\Delta\gamma MN}{2} \sin 2\phi \right)$$

where ϕ is the magnetization angle of the DW, q is the position of the DW, Gilbert damping α , non-adiabatic STT constant β , gyromagnetic ratio γ , anisotropy constant N , magnetization M , and Δ is the DW width.

In the low current density regime, below Walker breakdown, the DW is tilted at a fixed angle during its motion, and hence:

$$\dot{\phi} = 0$$

$$\sin 2\phi = \left(\frac{\beta-\alpha}{\alpha} \right) \frac{2}{\Delta\gamma MN_z} u$$

$$\dot{q} = \left(\frac{\beta}{\alpha} \right) u$$

The model shows that the speed of the DWs in the planar nanowire in the low current density regime, below the Walker breakdown limit, is linearly proportional to both the applied current density as well as the ratio between α and β . At high current densities, above the Walker breakdown limit, the DW changes its shape continuously via repetitious nucleation of anti-vortices within itself.

In contrast, R. Wieser et al. mentioned that the cylindrical shape of transverse DWs in cylindrical nanowires leads to a shape invariance of the DW.⁴³ In the absence of applied field ($H = 0$) and demagnetization field ($N = 0$), the magnetization dynamics of the DW becomes:

$$\dot{\phi} = \frac{(\beta - \alpha) u}{(1 + \alpha^2) \Delta}$$

$$\dot{q} = \frac{(1 + \alpha \beta) u}{(1 + \alpha^2)}$$

These results show that the DWs in cylindrical nanowire rotate with a constant angular velocity and travel at a constant speed that is linearly dependent on u . From the above equations, the comparison between the velocities of DWs in planar and cylindrical nanowires can be inferred. M. Yan et al. further elaborated on the dynamics of the DW in cylindrical nanowire: they attributed the absence of Walker breakdown to the fact that the DW retains its shape as it propagates along the nanowire.⁴⁰ Hence, the DW profile remains stable and is allowed to rotate within the cylindrical nanowire.^{40,43}

In cylindrical nanowires, the type of DW formed is dependent on the diameters of the cylindrical nanowires and the exchange length of the material.⁴⁴⁻⁴⁷ In thin cylindrical nanowires, the magnetization is homogeneous throughout the nanowire radial cross section due to higher energetic cost of the exchange energy, which prevents the spins from curling around the nanowire long axis. The nanowire can be considered as a one-dimensional system. As the diameter of the nanowire increases, the nanowire gradually converts into a three-dimensional system and the spins are allowed to curl around the nanowire axis. Therefore, when the exchange length of the given material is larger than the nanowire diameter, a transverse DW is observed, whereas at a larger diameter, a vortex DW is observed, as shown in Figure 1.7 (a) and (b), respectively.

Figure 1.7 The magnetization configurations of cylindrical nanowires showing (a) transverse domain wall and (b) vortex domain wall.⁴⁴ In this system, the equilibrium magnetization is aligned with its long axis due to shape and crystalline anisotropy, and a head-to-head DW is initialized and allowed to relax until a stable state is reached.

The three-dimensional DWs in cylindrical magnetic nanowires have been imaged by several methods, such as X-ray magnetic circular dichroism photoemission electron microscopy, magnetic force microscopy and high resolution electron holography (Figure 1.8).⁴⁸⁻⁵¹ The magnetization reversal process in cylindrical nanowires with different diameters is unique and involves the nucleation, depinning, and propagation of DWs. Thus, understanding the magnetization configuration and dynamics of cylindrical nanowires is complicated due to the three-dimensional nature of their spin configurations.

Figure 1.8 High resolution electron holography imaging of a DW structure in cylindrical nanowire.⁵¹ A magnetic field perpendicular to the nanowire axis was applied before imaging to nucleate transverse DW.

1.7 Three-dimensional Domain Wall

In Figure 1.9, the helical DW is a three-dimensional transition region between magnetizations with clockwise and anticlockwise vortex orientations.⁵² The nanowire was initially saturated in the direction parallel to the nanowire long axis. At relaxation state, a three-dimensional vortex magnetization with anticlockwise and clockwise orientations is formed at the opposite ends of the nanowire, as shown in the cross sectional view in Figure 1.9 (a) and (c), respectively.⁵² The two vortices gradually extend towards the center of the cylindrical nanowire and are eventually connected via the helical DW, as shown in Figure 1.9 (b). The helical DW is in a stable state for a 350-nm-diameter nanowire.

Figure 1.9 The magnetization configurations of a cylindrical NiFe nanowire with a diameter of 350 nm. Cross sectional view of the three-dimensional magnetization configurations showing anticlockwise (a) and clockwise (c) vortex orientations at the ends of the nanowire, and the two vortices are connected *via* a gradual rotation of magnetization at the nanowire center, thus forming the helical DW (b).

From the discretized magnetization $\mathbf{M}(\mathbf{r}_j)$ of the cylindrical nanowire, the demagnetization field $\mathbf{H}(\mathbf{r}_i)$ is expressed as

$$\mathbf{H}(\mathbf{r}_i) = -\sum_j \tilde{\mathbf{N}}(\mathbf{r}_i - \mathbf{r}_j) \mathbf{M}(\mathbf{r}_j)$$

where $\mathbf{N}$ is the demagnetization tensor at each point in the nanowire.⁵³ The stray field $\mathbf{H}(\mathbf{r}_i)$ created by the helical DW can then be calculated by applying the convolution theorem:

$$\mathbf{H}(\mathbf{r}_i) = -\mathcal{F}^{-1} \left[\mathcal{F}(\tilde{\mathbf{N}}) \cdot \mathcal{F}(\mathbf{M}) \right]$$

Figure 1.10 shows the calculated stray field profile along the long axis of the nanowire and at a distance of 10 nm from the nanowire surface. At the helical DW, the calculated peak stray field is approximately 1.8×10^5 A/m.

Figure 1.10 The extracted perpendicular stray field component of the nanowire is plotted against the long axis of the nanowire, indicating a strong stray field in the perpendicular direction from the helical DW.

1.8 Domain Wall Motion

A vortex is displaced by the application of a spin-polarized current predominantly through a gyrotropic force.⁵⁴ For vortex DW in a nanowire, the DW position is fixed with the vortex displaced towards one edge. When a spin-polarized current is applied, the DW moves in the direction of the conduction electron flow due to the localized momentum transfer from the STT effect, while the vortex experience a gyrotropic force to determine the equilibrium lateral vortex displacement.

Figure 1.11 shows the average DW velocity in a 350-nm-diameter cylindrical nanowire as a function of current density, which is determined by analyzing the time taken by the DW to travel from $x = 0$ nm to $x = 800$ nm. A minimum current density (J_i) for the DW to overcome its intrinsic pinning is found to be 5.0×10^{11} A/m², whereby the DW remains static with a current density that is lower than J_i .⁵² The DW motion can be categorized into two distinct regions: low and high current density regimes. When a low current density is applied, i.e. $5.0 \sim 6.6 \times 10^{11}$ A/m², a sharp slope of the DW velocity curve was observed, which is similar to the pre-breakdown regime in Walker's model.⁵⁵ At high current densities, i.e. $> 6.6 \times 10^{11}$ A/m², the DW velocity increases near linearly thereafter without any discernable limit.

Figure 1.11 The average DW velocity in a 350-nm-diameter cylindrical nanowire with respect to the applied current density. Inset shows the close up data of the DW velocity for the range of applied current density $4.3 \sim 7.1 \times 10^{11}$ A/m².

In Figure 1.12 (a) and (b), the cross sections of the helical DW in motion are presented at successive moments in time with low and high current densities. At low current densities, the helical DW is observed to rotate about the nanowire axis as it propagates in the nanowire.⁵² At high current densities, this rotation is found to be non-existent while the DW profile remains stable.

Figure 1.12 Illustration of the current-induced DW motion. The cross sections indicate the orientation of the DW at successive moments in time. The yellow arrow illustrates the orientation of the DW (a) with a rotational motion at low current density ($5.0 \times 10^{11} \text{ A/m}^2 < J \leq 6.6 \times 10^{11} \text{ A/m}^2$) and (b) without a rotation at high current density ($J > 6.6 \times 10^{11} \text{ A/m}^2$).

This behavior is further illustrated in the DW displacement as a function of time, which shows that the instantaneous velocity of the DW becomes more uniform as the current density is increased, as shown in Figure 1.13. The non-uniform DW velocity at low current density can be attributed to the loss in the energy due to its rotation, which deters the DW from Walker breakdown.⁴³ At high current densities, the DW moves linearly with an increasing velocity due to the disappearance of the DW rotation. However, there is a sudden drop in the slope of the DW velocity curve, *i.e.* decrease in DW mobility owing to the suppressed Walker breakdown, as shown in the inset of Figure 1.11.⁵⁵ The DW profile is also observed to be stable even up to $5.0 \times 10^{12} \text{ A/m}^2$.

Figure 1.13 The DW displacement with respect to time, for $J = 5.0 \times 10^{11} \text{ A/m}^2$, $6.6 \times 10^{11} \text{ A/m}^2$ and $21.9 \times 10^{11} \text{ A/m}^2$ indicating the linear and non-linear motion of the DW.

1.9 Anisotropic Magnetoresistance

The Stoner–Wohlfarth model was formulated for coherent magnetization rotation in small ellipsoidal shaped ferromagnetic particles with size smaller than the single domain limit.²⁸ However, there was a mismatch between the analytical model and experimental results for high aspect ratio MNPs. Therefore, Aharoni et al. evaluated analytically the curling reversal mode in ferromagnetic prolate spheroids based on its cylindrical symmetry property.²⁸ In addition, they derived the correlation between the coercivity and nucleation field of high aspect ratio ferromagnetic MNPs for coherent rotation and curling modes.

This analytical model only predicted the reversal modes without any simplifications related to its functional form and magnetostatic fields. The first micromagnetic simulations corrected this model by clarifying that reversal in nanowires occurs neither by coherent magnetization rotation nor curling reversal mode, but by DWs nucleation at the ends of the nanowires, and subsequent depinning and propagation along its long axis. The types of DWs formed are dependent on the diameters of the cylindrical nanowires and the exchange length of the material.⁴⁴ When the exchange length of the given material is larger than the nanowire diameter, a transverse DW is observed, whereas at a larger diameter, a vortex DW is observed.

Hence, the understanding of DWs nucleation and propagation is essential to investigate the magnetization reversal processes of magnetic materials. Two-dimensional DWs in planar nanowires have been intensively studied, where the spin distribution and reversal modes are largely determined by shape anisotropy. Few studies have been done on nanowires with cylindrical geometry, which introduce complexities in the types of DWs nucleated and their propagation behavior under applied fields and current.

The magnetization reversal in a single cylindrical NiFe nanowire or MNP, which occurs via the nucleation of a third vortex core and abrupt splitting of the clockwise and anticlockwise vortices, is investigated by anisotropic magnetoresistance measurements (AMR). The micromagnetic simulations have shown that for nanowires and MNPs with $l > 300$ nm, the DWs dynamics and stability are consistent and representative.

To fabricate the single nanowire device for electrical characterizations, the nanowires were drop casted on Si/SiO₂ substrates, after which lithography was used to form the patterned contact pads. A clean interface was obtained by reverse-sputtering the substrate before the deposition of the adhesion layer (5 nm of Cr) and the metal film (350 nm of Au). The SEM image of the device along with the schematic of the AMR measurement is shown in Figure 1.14. All measurements were performed with a low reading bias current density $J = 1.03 \times 10^9$ A/m² to avoid joule heating or electron migration.

Figure 1.14 SEM image of a 350-nm-diameter cylindrical nanowire and set of Au contacts, with schematics of AMR measurement setup.

The magnetoresistance ΔR of single cylindrical NiFe nanowire was measured at 300 K. The nanowire was first magnetized and saturated along the axis of interest with an external field of 3 kOe. The samples were then rotated with respect to the direction of applied magnetic field H to obtain the angular dependence of the magnetoresistance. Figure 1.15 shows the magnetoresistance curves at two different angles ($\theta = 0^\circ$ and 45°) between the applied magnetic field H and the nanowire long axis. The magnetoresistance of the nanowire measured during the field up-sweep (from -265 Oe to $+265$ Oe) and down-sweep (from $+265$ Oe to -265 Oe) is shown by the black and red solid lines, respectively. The magnetoresistance exhibited jumps at low fields (~ 64 Oe) for both parallel ($\theta = 0^\circ$) and oblique ($\theta = 45^\circ$) applied magnetic fields. The measured magnetoresistance at $\theta = 45^\circ$ was also found to decrease more slowly than that of the parallel magnetic field ($\theta = 0^\circ$) in a field range of between -110 Oe to $+100$ Oe. Whereas at $\theta = 90^\circ$ the magnetoresistance and micromagnetic simulations did not show any sudden jumps up to $H = 250$ Oe, as shown in Figure 1.16 and Figure 1.17.

Figure 1.15 AMR for a single cylindrical nanowire at $\theta = 0^\circ$ magnetic field. The AMR shows the magnetization reversal in a single cylindrical NiFe nanowire, which occurs via the nucleation of the third vortex core and abrupt splitting of the clockwise and anticlockwise vortices.

Figure 1.16 Magnetization configurations of the nanowire at various perpendicular magnetic field strengths from -250 Oe to +250 Oe. The arrows illustrate the direction of magnetization and the colors red and blue represents the clockwise and anticlockwise vortices, respectively.

Figure 1.17 AMR for a single cylindrical nanowire at $\theta = 90^\circ$ magnetic field.

Figure 1.18 shows the extracted up-sweep magnetic field AMR from Figure 1.15 and its correlation to the micromagnetic simulations, will be further discussed in subsequent chapters. The measurements of ΔR in a series of experiments in which the nanowire is initially saturated ($\Delta R = 0$), as compared to the remanence state, is shown in Figure 1.19.

Figure 1.18 Up-sweep magnetic field AMR at $\theta = 0^\circ$ applied magnetic field.

Figure 1.19 The measurements of ΔR in a series of experiments in which the nanowire is initially saturated ($\Delta R=0$) and the third vortex is subsequently nucleated by an applied field.

1.10 Current-induced Domain Wall Driving

The current-induced DW motion was examined by introducing a pulsed current generator into the AMR measurement setup, as shown in the inset of Figure 1.20. Initially, the third vortex core was nucleated at the center of the nanowire by an up-sweep magnetic field along its long axis. Subsequently, 10-ns-long current pulses of different polarities were applied, while the resistance of the nanowire was measured under a bias current of $J = 1.03 \times 10^9$ A/m². The rise and fall time of the current pulse were 300 ps. Figure 1.20 shows that the resistance of the nanowire drops drastically after the application of a pulse current.

Figure 1.20 AMR showing DW motion by the injection of a pulsed current. Inset shows the SEM image of a cylindrical nanowire and set of Au contacts, with schematics of AMR measurement setup.

Figure 1.21 shows the magnetization configurations of the nanowire, as revealed by micromagnetic simulations, before and after the application of pulse current. The results show that the current-induced magnetization configuration of the nanowire, where the vortex core is driven to the end of the nanowire, differed from the field-induced dynamics that shows the abrupt splitting of the vortices. As the vortex core is pushed out by the injected current pulse, a single vortex magnetization configuration is formed throughout the nanowire. The magnetization state (II) as shown by the micromagnetic simulation is expected to have a lower magnetoresistance, as the magnetization configuration within the nanowire is more uniform, as compared to the initial state (I).

Figure 1.21 Magnetization configurations of the cylindrical nanowire corresponding to the states before and after DW driving. The arrows illustrate the direction of magnetization and the colors red and blue represent the clockwise and anticlockwise vortices, respectively.

Figure 1.22 shows the probability (P) at which a drop in resistance was observed against the applied current density for various pulse durations (2 ns, 5 ns, and 10 ns). $P = 1$ means that the vortex core is always driven to the end of the nanowire by the current pulse. To obtain the probability, each measurement was repeated 50 times. The results show that, at applied current densities $J > 4.66 \times 10^{12} \text{ A/m}^2$, there was a gradual increase in probability from 0 to 1 for the 5 ns and 10 ns pulse durations. The probability for the 2 ns pulse duration remained relatively low, even with increasing current densities. This minimum current density needed to overcome

the intrinsic pinning of the vortex domain is found to be $J_{th} = 4.66 \times 10^{12} \text{ A/m}^2$. In Chapter 1.7, the calculated minimum current density (J_i) for the vortex domain to overcome its intrinsic pinning in a smooth cylindrical nanowire was found to be $J_i = 5.0 \times 10^{11} \text{ A/m}^2$. The higher minimum current density ($J_{th} > J_i$) can be attributed to the lower mobility arising from the surface roughness, which contributes to the viscous damping of the DW motion.^{56,57} It is then possible to obtain the vortex domain velocity as a function of the applied current density by noting the lowest current at which $P = 1$. In the experimental results, the vortex domain velocity was found to be $\sim 158 \text{ m/s}$ for a 10 ns pulse at $J_{10 \text{ ns}} = 5.17 \times 10^{12} \text{ A/m}^2$ and $\sim 317 \text{ m/s}$ for a 5 ns pulse at $J_{5 \text{ ns}} = 5.33 \times 10^{12} \text{ A/m}^2$.

Figure 1.22 The driving probability of the DW as a function of current density for a range of pulsed durations (2 ns, 5 ns and 10 ns).

1.11 Domain Wall Pinning and Depinning Mechanism

In micromagnetic simulations, the DW pinning strength of the diameter modulation is found to be largely dependent on the modulation depth.⁵² Hence, the pinning of DW in cylindrical nanowires was investigated by introducing diameter modulations along the nanowires. In diameter-modulated cylindrical nanowires, the point within the nanowire where the diameters differ, acts as a pinning site. Figure 1.23 shows the SEM image of the device along with the schematic for AMR measurement. The magnetoresistance jump due to the abrupt switching of the magnetization was observed to occur at a higher field of $H \approx +125$ Oe as compared to an unmodulated nanowire.

Figure 1.23 AMR for a diameter-modulated cylindrical nanowire at $\theta = 0^\circ$ magnetic field and SEM image of a diameter-modulated cylindrical nanowire and set of Au contacts, with schematics of AMR measurement setup.

This magnetization configuration in the diameter-modulated cylindrical nanowires was also examined by micromagnetic simulations, as shown in Figure 1.24. Initially, the diameter-modulated nanowire was saturated in the $-x$ direction parallel to the nanowire long axis (I). The clockwise and anticlockwise vortices started to move towards the thicker region of the nanowire, which similarly led to a reduction in the parallel magnetization component and caused a drop in magnetoresistance (II). The vortex domain was then formed in the thicker region of the nanowire at $H = 0$ Oe (III). As the magnetic field direction was reversed, the vortex domain was annihilated. Next, the abrupt splitting of the two vortices was also observed, which led to an abrupt increase in the parallel magnetization component and also in the magnetoresistance (IV-V). As the nanowire began to saturate in the $+x$ direction, the magnetoresistance of the nanowire returned to its initial value (VI).

Figure 1.24 Magnetization configurations of the nanowire at various magnetic field strengths. The arrows illustrate the direction of magnetization and the colors red and blue represent the clockwise and anticlockwise vortices, respectively.

In addition, micromagnetic simulation results revealed that the switching field of the cylindrical nanowire increased when the difference in the diameter of the nanowire Δd was larger, as shown in Figure 1.25. The increase in the switching field showed that the pinning strength of the modulation increased with increasing Δd and this phenomenon was also observed in planar nanowires.⁵⁸

Figure 1.25 The plot of switching field against $\theta = 0^\circ$ applied magnetic field obtained by micromagnetic simulations.

1.12 Néel and Brown Relaxation Mechanisms

The applications for MNPs have been extensively researched in biomedical fields, such as magnetic hyperthermia,⁵⁹⁻⁶² magneto-mechanical cell destruction,⁶³⁻⁶⁶ magnetic resonance imaging¹⁻³ and drug delivery,⁴⁻⁶ to compensate for the drawbacks of current diagnosis and therapy methods. The greatest advantage of MNPs is that they can be remotely controlled by an external magnetic field. The resultant magnetic response can be in the form of heat dissipation or magnetic torque, which is dependent on the applied magnetic field configuration and magnetization dynamics of the MNPs.⁶⁷

However, different biomedical applications require specific rotation mechanism in diverse magnetic field configurations. Bio-sensors for cancer bio-markers use magnetic spectroscopy of the MNPs Brownian motion to quantitatively measure the bound fraction and relaxation times of the MNPs within seconds.⁶⁸⁻⁷⁰ In magnetic particle imaging for quantification of MNPs concentrations, it predominantly requires Néel relaxation of the MNPs, while Brownian relaxation, caused by MNPs size distributions, should be minimized.⁷¹ The two mechanisms that exist for the MNPs relaxation processes are Néel and Brownian relaxation, which results in either heat dissipation or spatial rotation of the MNPs. Néel relaxation is correlated to the re-orientation of the MNP magnetic moment to the magnetic field, while Brownian relaxation is correlated to the spatial rotation of the MNP, as shown in Figure 1.26.⁷²⁻⁷⁴

Figure 1.26 Illustration of Néel and Brownian relaxation.

Néel (t_N) relaxation time in the absence of magnetic fields is given by:

$$t_N = t_0 e^{\frac{KV}{k_B T}}$$

Brownian (t_B) relaxation time in the absence of magnetic fields is given by:

$$t_B = \frac{3\eta V_H}{k_B T}$$

where η is the viscosity coefficient, inverse attempt frequency t_0 , magnetic anisotropy constant K , volume of MNPs V , Boltzmann constant k_B ($1.38 \times 10^{-23} \text{ JK}^{-1}$), temperature T and hydrodynamic volume of the MNPs V_H . In principle, the faster mechanism dominates, but both Néel and Brownian mechanisms can occur simultaneously, coupled through heat dissipation and magnetic torque.⁷⁵ The Brownian relaxation causes the heating mechanism of MNPs to be sensitive to η , whereas Néel relaxation is not affected by η . When both Néel and Brownian mechanisms occur simultaneously, the effective relaxation time (t_{eff}) is given by:

$$\frac{1}{t_{eff}} = \frac{1}{t_B} + \frac{1}{t_N}$$

In smaller MNPs, the dominant mechanism is Néel relaxation, while for larger MNPs it is Brownian relaxation. In Néel relaxation, the MNP magnetization changes direction as a result of the reconfiguration of its magnetic moment and is dependent on the MNP size and the temperature. While in Brownian relaxation, the MNPs undergo a spatial rotation and are dependent on external conditions, such as viscosity and chemical binding. The sensitivity of the Brownian relaxation is sufficient for temperature, viscosity and chemical binding measurements.⁷⁶⁻⁷⁸ It is important to understand the contributions of these magnetic relaxation mechanisms in order to tune and adapt the design of the MNPs to obtain the optimal heat generation for magnetic hyperthermia or magnetic torque for magneto-actuated cell death.

1.13 Magnetic Hyperthermia

A well-established method for treating cancerous tumors is magnetic hyperthermia, which uses localized heat generated by the relaxation mechanism of the MNPs in a high frequency alternating magnetic field, thereby triggering cancer cell apoptosis and tumor regression.⁷⁻¹⁰ The magnetic hysteresis of the MNPs result in heat dissipation that induces a rise in temperature to a range of 41–43 °C. Exposing tumor tissue to temperatures above 43 °C causes necrosis of cancer cells.^{11,12}

1.13.1 Hysteresis Losses

In an alternating magnetic field, the heat dissipated by the MNPs in one magnetic field cycle equals the area of the hysteresis loop A , given by:

$$A = \int_{-H_{\max}}^{+H_{\max}} \mu_0 M(H) dH$$

where $M(H)$ is the magnetization of MNPs, in an alternating magnetic field with frequency f and amplitude $\mu_0 H_{\max}$.⁷⁹⁻⁸¹ To maintain a low MNPs dose and short treatment duration in magnetic hyperthermia, the MNPs heating efficiency must be maximized. The measurement of MNPs heating performance is referred to as specific absorption rate (SAR), which is given by the heat dissipated per unit of mass of MNPs (Wg^{-1}):

$$\text{SAR} = \frac{A f}{\rho}$$

where ρ is the density of MNPs.

The density ρ of a $\text{Ni}_{1-x}\text{Fe}_x$ alloy can be obtained as function of composition, which is given by:

$$\rho_{\text{Ni}_{1-x}\text{Fe}_x} = \rho_{\text{Ni}} \frac{1 - \left(1 - \frac{M_{\text{Fe}}}{M_{\text{Ni}}}\right)x}{1 - \left(1 - \frac{M_{\text{Fe}} \rho_{\text{Ni}}}{M_{\text{Ni}} \rho_{\text{Fe}}}\right)x}$$

where ρ_{Ni} and M_{Ni} is the density and atomic weight of Ni, and ρ_{Fe} and M_{Fe} is the density and atomic weight of Fe.⁸²

1.13.2 Linear Response Theory

Linear response theory (LRT) describes the dynamic response of an assembly of MNPs using the Néel and Brown relaxation time which assumes a linear response of the MNPs to the magnetic field.⁸³⁻⁸⁸ The alternating magnetic field is given by:

$$H(t) = H_{\max} \cos(\omega t)$$

where ω is the angular frequency of the magnetic field. The Néel–Brown relaxation time is given by:

$$t = t_0 \exp\left(\frac{K_{\text{eff}} V}{k_B T}\right)$$

The hysteresis area for MNPs with random orientations is given by:

$$A = \frac{\pi \mu_0^2 H_{\max}^2 M_s^2 V}{3 k_B T} \frac{\omega t}{1 + \omega^2 t^2}$$

where μ_0 is the permeability of the vacuum, k_B is the Boltzmann constant, M_s is the saturation magnetization for the bulk material, V is the volume of the MNPs, T is the absolute temperature, K_{eff} is the effective uniaxial anisotropy constant, and t_0 is the frequency factor of the Néel and Brown relaxation time (10^{-9} – 10^{-12} s). LRT is valid for MNPs with strong

anisotropy under the application low magnetic fields, when $\xi = \frac{\mu_0 H_{\max} M_s V}{k_B T} < 1$. The LRT is still valid at the transition region between superparamagnetic ($\omega t < 1$) and ferromagnetism ($\omega t > 1$), where the hysteresis loop area is at a maximum for small amplitude magnetic fields.

1.13.3 Stoner–Wohlfarth Theory

Stoner–Wohlfarth model based theories (SWMBTs) omit thermal activation, which is justified at temperature $T = 0$ or infinite magnetic field frequency $f \rightarrow \infty$. The hysteresis area for MNPs with random orientations is given by:

$$A = 2 \mu_0 H_c M_s$$

where H_c is the coercive field. For finite frequencies f and temperatures $T \neq 0$, the hysteresis area is given by:

$$A(T, f) = 2 \mu_0 H_c(T, f) M_s$$

The analytical formula for a temperature-dependent H_c based on an approximation of the measurement time t_m , with anisotropy field $\mu_0 H_k = 2K_{eff}/M_s$, is given by:

$$\mu_0 H_c = 0.48 \mu_0 H_k \left(1 - \left(\frac{k_B T}{K_{eff} V} \left(\ln \frac{t_m}{t_0} \right) \right)^{\frac{3}{4}} \right)$$

SWMBTs are more suitable for modelling the heat dissipation for randomly orientated MNPs that are far from the superparamagnetic and ferromagnetic transition region.

As the size of MNPs increases, incoherent reversal modes causes a reduction in H_c , making LRT and SWMBTs invalid. The limit of validity of LRT is at $\xi < 1$, when M varies linearly with H and $\text{SAR} \propto H^2$. When $\xi > 1$, SWMBTs is valid for $\mu_0 H_{\max} > 2\mu_0 H_c$ and H_c independent of MNP volume. Lastly, MNPs with large sizes allow the formation of magnetic vortex or multi-domains and DWs. In such cases, the magnetization reversal process occurs by DWs nucleation and propagation, as described by Rayleigh model and $\text{SAR} \propto H^3$ (Figure 1.27).^{88,89}

Figure 1.27 Illustration of the analytical models to describe the heat dissipation of MNPs, as a function of their size and magnetic phase (superparamagnetic or ferromagnetic regime).^{88,89}

1.13.4 Eddy Current

In an alternating magnetic field, there will be an induced voltage in the material placed within the field, which results in formation of circular currents, i.e. eddy currents. By Lenz's law, the eddy current produces a magnetic field to oppose the changing magnetic field, and hence reacting back to the original magnetic field source.

This loss of magnetic energy is dissipated as heat in the material and the eddy current loss is given by:

$$P_{\text{eddy}} \propto H_{\text{max}}^2 \times f^2 r^2$$

where f is the frequency, r is radial distance and H is the magnetic field strength.⁹⁰ The eddy currents loss is only significant in materials above the centimeter scale, and thus negligible for MNPs in magnetic hyperthermia.^{85,91} However, the high frequency magnetic fields can induce eddy currents that cause undesirable temperature rise in healthy cells or tissues. The critical value of $H_{\text{max}} \times f = 4.85 \times 10^8 \text{ Am}^{-1}\text{s}^{-1}$ was obtained for the thorax,⁹⁰ $H_{\text{max}} \times f = 5 \times 10^8 \text{ Am}^{-1}\text{s}^{-1}$ for prostate cancer⁹² and $H_{\text{max}} \times f = 15\text{--}18 \times 10^8 \text{ Am}^{-1}\text{s}^{-1}$ for glioblastoma.^{67,93,94}

1.13.5 Specific Absorption Rate

The efficiency of heat dissipation of MNPs can be experimentally measured in terms of SAR, which is the energy dissipated per unit of mass of MNPs (Wg^{-1}), and is given by:

$$\text{SAR} = C \frac{\Delta T}{\Delta t} \frac{1}{m_{\text{MNP}}}$$

where C is the specific heat of the medium ($C_{\text{water}} = 4.18 \text{ Jg}^{-1}\text{°C}^{-1}$), initial slope of the time against temperature graph $\Delta T/\Delta t$ and mass of the MNPs m_{MNP} . However, SAR values in Wg^{-1} is not fully representative of the heating efficiency of MNPs as heat dissipation is also influenced by frequency (f) and magnetic field strength (H). Therefore, effective specific absorption rate or intrinsic loss power (ILP) is used to characterize the MNPs heating efficiency, given by:

$$\text{ILP} = \frac{\text{SAR}}{H^2 f}$$

1.14 Magneto-Mechanical Actuation

The growing interest in MNPs is due to their use for fighting cancers in a targeted manner. Although a majority of cancers (> 85%) are localized initially, modern treatments often resort to chemotherapy, with the systemic injection of toxic drugs affecting healthy organs and tissues.⁶² Not only does this approach result in a poor therapeutic index (1–2%), but there are also severe secondary effects on the patients, which often includes adversely affecting vital functions. Such drawbacks greatly limit the efficacy of such treatments by constraining their frequency and the amount of administered drugs. Furthermore, the biggest drawbacks of magnetic hyperthermia are the invasiveness and low targeting accuracy of specific cancerous areas with homogenous heat generation and a high frequency and amplitude magnetic field.^{95,96}

As such, the concept of magneto-actuated cell death has been proposed, where micro-discs under an alternating magnetic field of ~100 Hz can exert sufficient mechanical force to trigger programmed cell death or apoptosis.¹³⁻¹⁵ The magneto-mechanical cell destruction technique is a non-invasive and highly targeted treatment with high specificity and efficiency. It is accomplished by the spatial rotation of the MNPs to align to the magnetic field by Brownian relaxation, which subsequently produces a magnetic torque on the cancer cells (Figure 1.28).^{7,16} This mechanical stress exerted on the cells can cause mechanical disruption or compromise the integrity of the cell membrane, thereby inducing necrosis.⁹⁷⁻⁹⁹ It has also been established in *in vitro* cell destruction experiments that spin-vortex mediated stimulus by MNPs was sufficient for the initiation of programmed cell death.^{63,100,101}

Figure 1.28 Illustration of magneto-mechanical cell destruction in uniform magnetic fields

1.15 Cell Cycle

Cell division occurs by an orderly sequence of events which involves the duplication of cell content, following which the cell then divides into two genetically identical daughter cells. All cells abide closely by this cycle to ensure accurate duplication of genetic information essential for the continued growth and reproduction of living things.

The observation of individual cells is difficult in an intact mammalian body. Thus, the cell biology studies on mammalian cell cycle utilizes isolated cells from normal tissues or tumors and cultured in microtiter plates, i.e. flat plates with multiple wells that functions as small test tubes, supplemented with the essential nutrients needed for growth. However, normal mammalian cells that are grown outside their natural environment (under controlled conditions), cannot divide indefinitely as their lifespan is genetically determined and limited. Human fibroblasts cells have a Hayflick limit of ~25-40 divisions and normal human fetal cell population ~60-80 divisions due to replicative cell senescence or biological ageing.¹⁰² Some mammalian cells can undergo mutations such that they evade normal cellular senescence and proliferate indefinitely *in vitro*, becoming immortal cell lines. Immortal cell lines are widely and commonly used for cell biology studies as they serve as an indefinite supply of genetically homogeneous cells (Figure 1.29).

Figure 1.29 Bright-field optical microscopy image of mammalian cells (HeLa cells) proliferation in culture medium.

1.16 Programmed Cell Death

Mammalian cells are particularly valuable for studying the molecular control of cell proliferation in multicellular organisms. Research has not only focused on the understanding of the normal controls of cell numbers in healthy tissues, but also in the loss of these controls in cancer cells. The number of cells in a multicellular organism is highly regulated by the control of the rate of cell division and cell death. Cells, which are no longer needed, commit suicide by an intracellular death process, known as programmed cell death or apoptosis.

Apoptosis is a form of programmed cell death defined by morphological characteristics changes such as membrane blebbing, reduction of cell volume and formation of apoptotic bodies.¹⁰³⁻¹⁰⁵ It is vital for normal development, homeostasis and functioning of the immune system, and anti-inflammatory reactions. Necrosis is a form of unprogrammed cell death arising from external perturbations with the release of intracellular contents after cell membrane damage, which results in inflammation.¹⁰⁶⁻¹⁰⁸ Multiple cell death pathways can be observed simultaneously in cell cultures or tissues exposed to different types of MNPs and magnetic field configurations.

The highly conserved intracellular mechanism responsible for apoptosis is mediated by a family of protease enzymes, known as Caspases. The functions of Caspases in programmed cell death was first discovered and characterized in 1993.¹⁰⁹ Each Caspase is equipped with a cysteine in its active site that nucleophilically attacks and cleaves downstream target proteins at specific aspartic acids leading to a signaling cascade that results in apoptosis.¹¹⁰⁻¹¹² The activation of Caspases ensures that the components in every cell are dismantled in a controlled process, such that cell death is initiated with minimal effect on neighboring tissues and allowing apoptotic bodies to be recycled by other cells.

1.17 Cancer

The cells of a multicellular organism operate as a single system committed to collaboration, reproducing by cellular duplication and division. To function as a single system, the cells send, receive, and interpret specific sets of signals that control the behavior of individual cells. Each cell acts appropriately – rest, divide, differentiate or undergo apoptosis – to sustain the multicellular organism. Cells are constantly subjected to stresses, such as deoxyribonucleic acid (DNA) damage that can cause mutations in the genome. As such, a robust system of regulation and surveillance is required to ensure that these damaged cells are not allowed to proliferate.¹¹³⁻¹¹⁶ Evidently, the most dangerous mutation is one that gives a cell the selective advantage to survive and proliferate in an uncontrolled manner. These mutations often implicate genes involved in the regulation of cell growth, division and death.¹¹⁷

Gain-of-function mutations in proto-oncogenes that govern cell proliferation convert these genes into potent oncogenes capable of constitutively stimulating cell division.¹¹⁷ Tumor suppressor genes function to arrest cell cycle to allow time for DNA repair, or if needed induce apoptosis to prevent the further propagation of errors.¹¹⁸⁻¹²¹ Mutations that result in non-functional tumor suppressor genes allow cell cycle to proceed unchecked, leading to the accumulated mutations. Subsequent cycles of mutation, competition, and natural selection occurring within the population of somatic cells enable these cells to acquire additional phenotypes, leading to the development of cancer.¹²²⁻¹²⁴ Cancer cells are characterized by two heritable properties; unrestricted cell division and the ability to invade healthy cells or tissues. When the cell proliferation is out of control, it will lead to the growth of a tumor. The tumor eventually gains metastatic properties as it breaks through the basement membrane, detaches and spreads to other parts of the body, as shown in Figure 1.30.

Figure 1.30 Illustration of a malignant (cancerous) tumor.

The HeLa cell line is one of the oldest and most commonly used in cell biology research. The cell line originated from an individual named Henrietta Lacks, who had treatment for cervical cancer at Johns Hopkins Hospital in 1951.¹²⁵ HeLa is an immortal cell line and a common model system for mammalian cell study due to its remarkably durable and prolific nature. This rapid and perpetual division of the HeLa cells makes them an ideal model for large scale *in vitro* cancer research treatment testing.

1.18 Motivation

In Singapore, about 37 people are diagnosed with cancer each day, according to Singapore Cancer Registry.¹²⁶ The World Health Organization's International Agency for Research on Cancer approximates an annual of 9.6 million cancer-related death and 18.1 million newly diagnosed cancer cases worldwide in 2018.¹²⁷ The most prominent treatment methods are surgery, radiotherapy and chemotherapy. The surgical removal of the primary tumor is usually the preferred method, however, some deep-rooted tumors are inoperable and inaccessible or have ill-defined borders. Hence, radiotherapy and chemotherapy are employed with, or instead of, surgery.

The disadvantages of radiotherapy are possible damage to surrounding healthy tissues and the inability to target tumors that are hidden in the imaging scans as these are excluded in the 3D radiation planning models. In addition, the incapability to destroy all cancer cells in the tumor or to relieve mass effect in certain parts of the body, e.g. brain, renders radiotherapy even less effective. The biggest drawback of chemotherapy is the development of treatment-related side effects, which include gastrointestinal, musculoskeletal or constitutional symptoms. Furthermore, the dosage of chemotherapeutic agents is limited by the threshold of the patient, and hence the treatment dose may be insufficient to effectively treat all malignant cells. For these reasons, it is crucial to develop improved or alternative techniques to overcome the drawbacks of current cancer treatment methods.

Engineered nanoparticles for improved cancer therapy and diagnosis is an exciting and viable concept. Recently, experiments with rodent models have shown that nanoparticle-based therapy wiped out the original targeted breast cancer tumors and metastases in other parts of the body.¹²⁸ However, the current nanoparticles research focuses on the design of the nanoparticles and demonstration of the effectiveness of *in vitro* cancer treatment. Therefore, there is a lack of the fundamental interactions of nanoparticles with biological systems from the animal model to the human level, resulting in poor clinical translation. Herein, the proposal to overcome this fundamental limitation is by combining the use of magneto-mechanical actuation and thermic responses of the cylindrical NiFe MNPs in an applied magnetic field on HeLa cells *in vitro*.

MNPs' magnetic properties are directly correlated to its size, structure and morphology, therefore the choice of fabrication is fundamentally crucial. The template-assisted electrodeposition and differential chemical etching technique allows for good control over the size and compositions of MNPs. The main advantage of this synthesis process is high scalability and ease of production for large quantities of MNPs. Furthermore, MNPs made of

soft magnetic materials, with closed spin-vortex magnetization and high saturation magnetization, do not require strong magnetic fields for their manipulations.

1.19 Organization of Thesis

The introduction chapter has given a brief overview of the magnetization dynamics and configurations of DWs in cylindrical magnetic nanostructures and its applications in cancer treatment. This included a conceptual overview of the Néel and Brown relaxation mechanisms involved in magnetic hyperthermia and magneto-mechanical actuation to induce cancer cell death.

The second chapter describes the experimental techniques for the fabrication and characterization of magnetic nanostructures. The cell culture protocols and imaging for HeLa cell work *in vitro* are also elaborated.

The third chapter describes the magnetization reversal process in cylindrical NiFe MNPs fabricated by the template-assisted pulsed electrodeposition with differential chemical etching, and optimized for various dimensions and compositions. The heating efficiency of the MNPs was obtained by time-dependent calorimetric measurements and compared to the theoretical models and the static hysteresis derived from micromagnetic simulations of NiFe MNPs and VSM measurements.

The fourth chapter describes a highly efficient magneto-actuated cancer cell apoptosis method using a biaxial pulsed magnetic field configuration that maximizes the induced magnetic torque. Its effectiveness was evaluated by light transmissivity dynamics of MNPs and reduction in the HeLa cell viability. MNPs, with different aspect ratios, can form different number of vortex states which affect the magnetic susceptibility and torque experienced by the MNPs, hence impacting their efficacy in inducing programmed cancer cell death.

The fifth chapter describes a method to induce cell necrosis using an alternating magnetic field gradient configuration, which oscillates the MNPs with a force sufficient to mechanically rupture the cell membrane. The Acridine Orange and Ethidium Bromide fluorescence dual staining reveals an increase in necrotic cell population coupled with higher lactate dehydrogenase leakage and greater reduction in cell viability, indicating cell necrosis.

The final chapter concludes this thesis and suggests possible future work.

Chapter 2 – Experimental Techniques

2.1 Chapter Introduction

Materials are the foundation of experimental magnetism and the potential applications depend on their magnetic and structural properties. Most magnetic materials studied in research laboratories are synthetically made, often by a complex fabrication process. Although there have been huge advances in the synthesis of magnetic nano-materials for biomedical applications, only few cases, such as magnetic resonance imaging contrast agents, have reached clinical practice. Hence, the efficiency and biocompatibility of magnetic nanoparticles (MNPs) remains to be investigated for other applications such as magnetic hyperthermia treatment, magneto-mechanical actuation, tissue regeneration or controlled drug delivery. In this chapter, the fabrication and characterization methods of the magnetic thin films and nanostructures are presented. By leveraging on these well-established fabrication methods, the template-assisted pulsed electrodeposition with differential chemical etching was developed and optimized for various dimensions and compositions of MNPs. In addition, the cell biology processes for *in vitro* applications of the MNPs are also presented.

2.2 Pulsed Electrodeposition

Electrodeposition involves the deposition of a metal or metallic alloy onto a conducting surface through electrolysis in a well-formulated electrolyte, which consists of an aqueous solution of a simple salt or complex salt.^{129,130} The electrodeposition circuit is made up of the anode (positive electrode), the cathode (negative electrode), a saturated calomel electrode (reference electrode), the electrolyte bath and a current source. During electrolysis, the reduction reaction occurs at the cathode, while the oxidation reaction takes place at the anode. The conducting substrate is attached to the cathode for the material X (Ni or Fe) to be deposited.

For an insoluble anode, the reactions are described by:

Faraday's law states that the amount of material deposited at the cathode is proportional to the amount of total coulombs passed through the electrochemical cell, given by:

$$Q_c = \int I \partial t$$

$$Z_c = \frac{M_w}{n F}$$

$$W = \int \frac{I \partial t M_w}{n F}$$

where Q_c is the total coulombs passed, Z_c is the electrochemical equivalent of the material, I is the current applied over time t , M_w is the molar mass of the material, n is the number of electrons and F is the Faraday constant. Herein, demonstrates a magnetic cylindrical nanowires/nanostructures fabrication technique which incorporates pulsed electrodeposition nanowires growth and differential chemical etching to produce compositionally modulated or diameter-modulated cylindrical nanostructures. The VersaSTAT 3 electrodeposition system from Princeton Applied Research setup is shown in Figure 2.1.

Figure 2.1 Princeton Applied Research VersaSTAT 3 electrodeposition system.

The electrolyte consists of 0.5M NiSO₄, 0.01M FeSO₄ and 0.5M H₃BO₃. Alternating pulses of high potential V_H and low potential V_L were applied to grow the compositionally modulated Ni_xFe_{1-x} nanowires, as shown in Figure 2.2. As the reduction potential of Ni²⁺ ions is higher than Fe²⁺ ions, the electrodeposition at V_H will favour Ni deposition. Therefore, a Ni-rich layer is formed within the anodized aluminum oxide (AAO) membrane when V_H is applied, while a Fe-rich layer is formed when V_L is applied. By alternating the potential between V_H and V_L , compositionally modulated nanowires will then be formed within the AAO membrane. This phenomenon can be attributed to anomalous co-deposition behaviour of NiFe alloys, which favours the deposition of less noble metal Fe.

The side reaction of metal reduction involving the evolution of H₂, gives rise to the effect of anomalous co-deposition.¹³¹⁻¹³³ This side reaction process depletes protons and increases the concentration of OH⁻ in the electrolyte. The surge in OH⁻ leads to the formation and adsorption of metal mono-hydroxide ions or metal hydroxides X(OH)⁺ during the deposition process, which is described by:

where X refers to Ni and Fe atoms.

Figure 2.2 Graph of electrodeposition potential (V) vs elapsed time (s).

The AAO templates are Whatman® Anodisc inorganic filter membrane obtained from Sigma-Aldrich and Nanoporous AAO from InRedox. The removal of AAO is typically carried out by the application of phosphoric acid.¹³⁴⁻¹³⁷ However, phosphoric acid will have secondary reactions with the nanowires, resulting in various oxidation products which will affect the magnetic properties and the size of the nanowires. Therefore, the AAO template was dissolved in NaOH to release the NiFe nanowires.

The nanowires were then thoroughly rinsed with deionized water to be de-alkalized. Subsequently, the nanowires were partially etched with dilute HNO_3 to form the cylindrical nanostructures or completely etched to form MNPs. The observed diameter modulations along the length of the nanostructures are due to the differential etching rate between different compositions of the NiFe nanostructures. The diameter of the modulations will decrease with further etching until only the desired modulation depth is obtained. The process flow of the pulsed electrodeposition and differential chemical etching is shown in Figure 2.3.

The advantages of using a combination of template-assisted electrodeposition and differential chemical etching are; good control over magnetic cylindrical nanostructures

composition by adjusting the electrolyte composition and deposition potential, and the high aspect ratio that is not achievable by the conventional lithographic processes employed to grow cylindrical magnetic nanostructures with high yield. Common magnetic cylindrical nanowires made of Ni,¹³⁸⁻¹⁴⁰ Fe,^{141,142} NiFe,^{143,144} CoNi,¹⁴⁵⁻¹⁴⁷ CoFe^{148,149} and CoNiFe¹⁵⁰⁻¹⁵² have been fabricated for optics or field emission applications.

Figure 2.3 Schematic illustrations of the pulsed electrodeposition and differential chemical etching process flow. (a) Pulsed electrodeposition setup, (b) compositionally modulated nanowires formed within AAO template, (c) nanowires etched with dilute HNO_3 and (d) partially etched nanowires forming diameter-modulated nanostructures.

2.3 Thermal Evaporation

Physical vapor deposition (PVD) is the most common method of thin film deposition, where the film is formed by direct atom transfer from the source to the substrate in a gas phase.^{153,154} In evaporation, the source can be heated up to 400–1000 °C during the deposition process. The deposition chamber is evacuated to a high vacuum (10^{-7} Torr) or ultrahigh vacuum ($<10^{-9}$ Torr) to allow atoms from the source to arrive at the substrate with minimal collisions with other gas atoms in the chamber, and hence films with high purity are produced.¹⁵⁵

In e-beam evaporation, the desired material is loaded into a molybdenum or tungsten crucible, where it is heated to high temperatures by using a focused beam of electrons. This causes the source material to melt and evaporate. The hot source material travels to the substrate and condenses back to the solid state. The thermal evaporation Edwards Auto 306 e-beam evaporation system was used to deposit a layer of aluminum onto the AAO template, before the pulsed electrodeposition process, to ensure good conductivity.

Figure 2.4 Edwards Auto 306 e-beam evaporation system.

Figure 2.5 Schematic illustration of e-beam evaporation.

AAO templates consist of high density arrays of uniform and parallel pores formed in a hexagonal-close-packed structure. Due to the highly-ordered pore structures, AAOs have been utilized extensively as templates in nanotechnology fabrication. The AAO template is prepared by the anodization of aluminum in the electrolytes under appropriate electrochemical conditions. The pore size of the AAO template can be controlled with great precision. Template-assisted electrodeposition allows fabrication of high aspect ratio nanostructures in a variety of metals.¹⁵⁶ The diameter d of the cylindrical nanostructure is determined by the pore size of the AAO templates (150–350 nm). The length l is dependent on the total deposition duration and limited by the AAO template thickness.

Figure 2.6 SEM image of AAO templates from (a) the top view and (b) the side view.

2.4 Magnetron Sputtering

Magnetron sputtering is another PVD method that utilizes magnetically-confined plasma initiated near the surface of the target material.¹⁵⁷ The surface of the target material is bombarded by positively-charged ions from the plasma, and atoms of the target material are sputtered out and deposited onto the substrate to form thin films. The deposition chamber is evacuated to high vacuum (10^{-5} to 10^{-7} Torr) and an inert gas (typically Ar or Xe) is flowed into chamber. The use of a magnetic field behind the cathode to trap the electrons results in an increase in deposition rates and higher density plasma generation at lower pressures. Hence, this also reduces the background gas interference in the thin film growth and energy losses in the sputtered atoms through gas collisions. This PVD method is commonly chosen for deposition of metallic or insulating films with highly specific electrical or optical requirements.

Figure 2.7 AJA 8-target UHV magnetron sputtering deposition system.

Figure 2.8 Schematic illustration of magnetron sputtering deposition.

Sputter yield S is the rate at which surface atoms are removed by ion bombardment, and given by the mean number of atoms removed from the target surface per incident ion:

$$S = \frac{\text{no. of atoms removed}}{\text{no. of incident ions}} = 10^5 \frac{W}{At}$$

where W is the weight loss of the target, A is the atomic number of the target material, t is the sputtering time and I is the ion current.¹⁵⁸ The yield is influenced by the energy of incident ions, material of the target, angle of incident ions and crystal structure of the target. Figure 2.9 illustrates the variation in sputtering yield S with incident ion energy. A threshold energy exists in the low energy region, while S reaches a maximum in the high energy region.¹⁵⁹⁻¹⁶¹

Figure 2.9 Schematic illustration of magnetron sputtering deposition.

2.5 Photolithography

Photolithography is an optical method for pattern transfer onto a substrate by using sophisticated optics to expose a desired pattern onto a substrate coated with photosensitive chemicals.¹⁶² Photoresists are light-sensitive compounds and categorized into two types: positive and negative resists. For positive photoresist, the exposed areas on the resist are removed after developing, which gives smaller features, superior resolution, but has higher cost and poor adhesion. For negative photoresist, the unexposed areas are removed after developing, which gives better adhesion, but are unable to produce sub-micron features. The photoresist consisting of a diazonaphthoquinone (DQ) and a novolac resin (N). The weakly bonded N_2 in the DQ undergoes a photolytic decomposition when exposed to light into an unstable ketocarbene and N_2 , where the addition of UV will free the N_2 and create a highly reactive C site. Wolff re-arrangement occurs; ketocarbene rearranges to a more stable ketene, which is very hygroscopic. When H_2O is added, the C double bond is replaced with a C single bond and an OH group, producing carboxylic acid (COOH) which is soluble in the developer.

Substrate preparation – Silicon wafers with an oxidized layer of SiO_2 were chosen for electrical isolation. After which, the substrates were immersed in acetone for 30 mins and then in isopropyl alcohol for 30 mins, while being sonicated. Visual inspection of the substrate surface was done to ensure that the contamination on the surface was kept to a minimum and the entire cleaning procedure was repeated if necessary. Next, the template-assisted electrodeposited cylindrical NiFe nanostructures were drop casted onto the substrate, and ready for photolithography of contact pads.

Spin coating photoresist – The substrate was placed onto the vacuum chuck of the spin coated. The AZ5214E photoresist (AZ Electronic Materials USA Corp.) was pipetted onto the substrate to cover entire substrate surface. The optimized spin coating recipe were spin rate 2000 rpm and spin duration 45 s.

Soft bake of photoresist – The resist coated substrate is transferred onto the hot plate for 120 s at 110 °C. The time duration of soft baking is very crucial as the most of the solvents are removed and the photoresist becomes photosensitive or image-able only after soft baking.

Figure 2.10 Ultrasonic cleaner and SCS spin coater.

UV exposure – The substrate is placed on the Suss MicroTec MJB4 mask aligner to be exposed for 15 s at 6 mWcm^{-2} , using the Align & expose, soft contact mode. In the soft contact mode, the photomask and substrate are brought in contact. The structural resolution is better than in proximity exposure mode. The vacuum securing the substrate onto the chuck is maintained during exposure and the only force to press the substrate against the photomask is applied during the vertical movement of the stage.

Figure 2.11 Suss MicroTec MJB4 mask aligner.

Developing and inspection – The exposed substrate was immersed in the developer AZ400K (AZ Electronic Materials USA Corp.) diluted 1:4 in deionized water for 10 s, then rinsed thoroughly with deionized water for 30 s to stop the developing process. The duration of developing is also critical as under- or over-developed patterns may occur, and hence the immersion duration was adjusted accordingly. The photolithography process was carried out in a cleanroom to reduce the risk of contamination.

Thin film deposition – A clean interface was obtained by reverse-sputtering the substrate before the deposition of the adhesion layer of 5 nm Cr and the contact material of 350 nm Au in the AJA 8-target UHV magnetron sputtering deposition system. Au is the most suitable material for contact pads due to its low resistance and anti-corrosive properties.

Lift off process – The substrate is immersed into a lift-off solvent and agitated gently. Inspection on the optical microscope is needed to ensure lift off is completed and repeated if necessary. The photolithography and deposition of the electrical contacts process is illustrated in Figure 2.12.

Figure 2.12 Illustration of the electrical contacts fabrication process flow.

2.6 Magnetoresistance Measurement

Although magnetic imaging techniques have been widely used to determine the DW position and orientations, it is an inconvenient and impractical method of DW detection.¹⁶³⁻¹⁶⁵ Therefore, the alternative is measuring DW resistance or magnetoresistance, which has been proven to be an effective way in differentiating the spin configurations in magnetic nanostructures.¹⁶⁶⁻¹⁶⁹ Conventionally, the electrical contacts of the cylindrical nanowires, kept inside the AAO template, are made by sputtering an Au contact layer after the electrodeposition to make parallel contacts with multiple nanowires.¹⁷⁰⁻¹⁷² Hence, the magnetoresistance measurement results are based on multiple cylindrical nanowires. Herein, demonstrates a method to measure a single cylindrical nanostructure by releasing the nanowires or nanostructures from the AAO template, which are drop casted on a Si/SiO₂ substrate, followed by photolithographic patterning and deposition of Au contact pads onto the nanostructures.¹⁷³

The probe station utilized an optical microscope and micro-manipulators to precisely position the thin needle-like probes onto the contact pads of the device. It was fitted with six long tungsten probe with 5 μm tip with a 45° bend near probing end; a pair for voltage and resistance measurement, a pair for measuring current and a pair for pulsed driving current. The external magnetic field was applied by an electromagnetic coil located underneath the probe station with metal arms extensions. A gauss meter measured the magnetic field strength and exported the data to the workstation. The probe station is constructed on a vibration isolation table that floats on a cushion of compressed gas ensuring a vibration-free electrical measurement.

The magnetic field up-sweep and down-sweep was applied to the single cylindrical NiFe nanowires, while the magnetoresistance of the nanowire is measured by a Keithley 2400 sourcemeter. The samples were then rotated with respect to the direction of applied magnetic field H to obtain the angular dependence of the magnetoresistance. For current-induced DW

motion, Picosecond Pulse Labs 10,300B current generator was used to inject pulsed current into the magnetoresistance measurement setup.

Figure 2.13 Probe station setup for angular magnetoresistance measurement and SEM image of a 350-nm-diameter cylindrical nanowire and set of Au contacts, with schematics of AMR measurement setup.

2.7 Scanning Electron Microscopy

The scanning electron microscope (SEM) operates a high energy electron beam in a raster pattern, with wavelength shorter than light, enabling the observation of structures at ~ 1 nm resolutions. JEOL JSM-6700F utilizes a field emission gun as the electron source, in combination with electromagnetic lenses to focus (objective lens) and control the size (condenser lens) of the electron beam, to achieve high resolution observation and analysis of surface topography. The SEM-imaged region emits a variety of signals upon interaction with the electron beam, i.e. secondary electrons, backscattered electrons and characteristic X-rays. The subtle variation in the surface roughness of the sample affects the angle of incident electrons, which causes a change in intensity of the secondary electron detection, and is used to generate the SEM image.

Figure 2.14 JEOL JSM-6700F field emission scanning electron microscope.

Figure 2.15 (a) shows the SEM image of the cylindrical NiFe nanowires fabricated by pulsed electrodeposition. Figure 2.15 (b-d) shows the SEM images of the cylindrical NiFe nanostructures after partial etching by HNO_3 .

Figure 2.15 SEM image of (a) unmodulated and (b-d) modulated cylindrical NiFe nanostructures.

2.8 Energy Dispersive X-ray Spectroscopy

JEOL JSM-6700F is also equipped with a lithium-drifted silicon, solid-state device and a multi-channel spectrum analyzer for elemental analysis, in conjunction with SEM. The energy dispersive X-ray spectrometer (EDX) allows the accurate and non-destructive elemental analysis of the sample by the detection of X-rays. When the inner shell electron is excited by the incident electron beam, it is removed and creates a vacancy, which is filled by an outer shell electron. This difference in energy between the inner and outer electrons releases X-rays, which is measured by the EDX spectrometer. The X-ray energies are characteristic of the element from which it was emitted. The spectrum of the energies of X-rays versus counts is evaluated to obtain the elemental composition of the sample. Capital Roman letter (*K*, *L* or *M*) indicates the shell containing the inner vacancy and Greek letter (α , β) specifies the group which the X-ray line belongs.

Figure 2.16 JEOL energy dispersive X-ray spectrometer.

2.9 Vibrating Sample Magnetometer

A vibrating sample magnetometer (VSM) is an instrument for measuring the properties of a magnetic material invented by S. Foner at Lincoln Laboratory MIT in 1955.¹⁷⁴ By Faraday's law of induction, the magnitude of magnetic moment of the material, and is given by:

$$\nabla \times \mathbf{E} = \frac{\partial \mathbf{B}}{\partial t}$$

Faraday's law states that a changing magnetic field within a loop will give rise to an induced current, which is due to a force or voltage within that circuit. The electromotive force generated in the pick-up coils is relative to the magnetization of the material. It is also dependent on the alignment of the magnetic moment relative to the coils.

The voltage is an integral of electric field E along the coil and depends on the rate of change of induction B ,^{174,175} which is given by:

$$U = \oint \mathbf{E} d\mathbf{l} = \iint \frac{\partial \mathbf{B}}{\partial t} d\mathbf{S}$$

The pick-up coils are usually static, while the sample holder vibrates in the changing magnetic field. To reduce interference of external sources of magnetic fields, a pair of pick-up coils is implemented such that external field contributions that are added to one coil can be subtracted from the other coil. The VSM uses the electromagnetic induction and lock-in principle of measurement. The lock-in principle of measurement allows the accurate measurement of signals in the presence of noise.^{174,175} Using the trigonometric identity:

$$\cos(\theta)\cos(\phi) = \frac{1}{2}\cos(\theta - \phi) + \frac{1}{2}\cos(\theta + \phi)$$

Assuming that the sample vibrates with frequency ω giving the signal of that frequency and amplitude A in pick-up coils due to magnetic induction. That signal is multiplied with the signal

frequency ω_l from the generator that is vibrating the sample, hence a phase difference of φ .

Using the trigonometric identity:

$$A \cos(\omega t) B \cos(\omega_l t + \varphi) = \frac{1}{2} AB \cos(\omega t - \omega_l t - \varphi) + \frac{1}{2} AB \cos(\omega t + \omega_l t + \varphi)$$

Using the same generator, so $\omega = \omega_l$:

$$\frac{1}{2} AB \cos(\omega t - \omega_l t - \varphi) + \frac{1}{2} AB \cos(\omega t + \omega_l t + \varphi) = \frac{1}{2} AB \cos(\varphi) + \frac{1}{2} AB \cos(2\omega t + \varphi)$$

The fast varying component $\frac{1}{2} AB \cos(2\omega t + \varphi)$ can be filtered out using a low pass-filter,

which only remains the constant voltage $\frac{1}{2} AB \cos(\varphi)$ that is proportional to the sample signal

and is at maximum if the phase difference is π . The signal from the pick-up coils have interference from external sources of magnetic fields and are given by:

$$S_{coil} = \sum_i A_i \cos(\omega_i t + \varphi_i)$$

Multiply by the reference signal from the generator:

$$S_{coil} B \cos(\omega_r t + \varphi) = \sum_i \left(\frac{1}{2} A_i B \cos(\omega_i t - \omega_r t - \varphi_i) + \frac{1}{2} A_i B \cos(\omega_i t + \omega_r t + \varphi_i) \right)$$

The signals with a reference frequency $(\omega_i t - \omega_r t)$ contribute to the constant voltage received from the mixer. Hence, by resolving the contributions from the external sources of magnetic fields, the signal from the sample can be obtained. The configurations of coils usually employ a symmetric even number of coils and dependent on the experiment, signal-to-noise ratio, convenience, and magnetic field symmetry considerations. The conventional configurations for which the axis of vibration is perpendicular to the magnetic field, is defined as the transverse geometry, as shown in Figure 2.17.

Figure 2.17 LakeShore 8600 Series vibrating sample magnetometer with schematics of the pick-up coils configuration and axis of vibration perpendicular to the magnetic field.

2.10 Micromagnetic Simulation Parameters

The magnetization dynamics of the magnetic nanowires or nanostructures in the various magnetic fields configurations were studied by means of micromagnetic simulation programs, OOMMF and MuMax3.^{30,31} The material parameters used for $\text{Ni}_{80}\text{Fe}_{20}$; saturation magnetization (M_s) of 860×10^3 A/m, exchange stiffness constant (A_{ex}) of 1.3×10^{-11} J/m, magneto-crystalline anisotropy (k) of 0, Gilbert damping constant (α) of 0.005 and non-adiabatic constant (β) between 0.04–0.15.^{52,176-180} A cell size of $5 \text{ nm} \times 5 \text{ nm} \times 5 \text{ nm}$ was used for all simulations, which is sufficiently small as compared to the exchange length.

2.11 Cell Culture

HeLa cells were grown in T-75 flask as a monolayer in Dulbecco's Modified Eagle's medium supplemented with 4.5 g/L glucose, 2 mM L-glutamine, 10% fetal bovine serum and 1% penicillin/streptomycin.^{181,182} The cell lines were incubated in a humidified atmosphere at 37 °C, 5% CO₂. The cell cultures were passaged at ~80% confluence using Trypsin-EDTA to detach the cells from the T-75 flask, and then pelleted by centrifugation at 2000 rpm for 5 min. Next, the supernatant was extracted and the cells pellets were re-suspended in cell culture medium. Countess automated cell counter and Trypan Blue (TB) was used to count the HeLa cells in suspension and the cells were seeded at 1×10^4 cells/well in a 96-well microtiter plate. All reagents were purchased from Thermo Scientific™. All experiments were done in quadruplicates. The results were represented as the mean $\pm$ standard deviation (SD). Statistical significance was analyzed using one-way analysis of variance (ANOVA) with OriginPro, OriginLab. A *p* value of < 0.05 was considered to be statistically significant.

Figure 2.18 HERMLE Labortechnik Z326K centrifuge

Figure 2.19 Invitrogen Countess™ Automated Cell Counter measures the total count, live count, dead count, and mean diameter of cells.

A typical animal cell is very small (10-20 μm in diameter), colorless and translucent as the cell is made up of 70% water by weight.¹⁸³ Therefore, the cell in its natural state is almost invisible under an optical microscope, as shown in Figure 2.20. This led to the discovery and development of cell dye and stains to provide sufficient contrast to help identify the location of specific proteins and targets of interest within the cell. The cells were stained with 0.4% TB to assess cell viability using the dye exclusion test, before seeding into microtiter plates. TB is a vital stain that selectively dye non-viable or dead cells with an intense blue.

Figure 2.20 Optical microscopy image of HeLa cells with no dye or stains.

2.12 Fluorescence Microscopy

Fluorescence is the absorption and subsequent re-emission of light by organic and inorganic objects. The re-emission process occurs almost simultaneously with the excitation process due to the very short delay of $< 10^{-8}$ s between the absorption and emission of photons. The concept of fluorescence was first described by G. Stokes in 1852, where he observed the emission of red light from the mineral fluorspar by ultraviolet excitation.¹⁸⁴ He also found that the emitted fluorescence is at a longer wavelength as compared to the excitation light. In 1930s, fluorescent dyes were introduced as a stain for tissue components, bacteria, and other pathogens to aid in biological investigations. Fluorescent dyes are detection reagents made from non-protein molecules that only absorb specific wavelengths of light and re-emit desired wavelengths of light. The use of multiple fluorescent dyes allows the simultaneous identification of individual components in a cell with a high degree of specificity. Hence, fluorescence microscopy has become an indispensable tool for cell biology.

The fluorescence microscope works by illumination of the sample with a specific wavelength of light, and the subsequent separation of the re-emitted light or fluorescence from the strong excitation light. The emitted light enters the detector or the digital camera system revealing the resultant fluorescent structures, superimposed with a high contrast dark background. The quality of the image is highly dependent on the background darkness as the excitation light is many orders of magnitude greater than the emission light.

Figure 2.21 Nikon Eclipse Ti-S inverted microscope.

Figure 2.21 shows the illustration of the working principle of fluorescence microscopy. The light source is usually a xenon or mercury burner located at the back of the microscope. The excitation light travels into the excitation filter of the microscope, where only the desired wavelength of light passes through, filtering all other unwanted wavelengths of light. At the dichromatic beam-splitting mirror, light with shorter wavelengths is efficiently reflected, while light with longer wavelengths passes through. The dichromatic mirror is fixed at 45° with respect to the incoming excitation light and reflects the light at 90° directly through the objective lens to illuminate the sample. The fluorescence emission light from the sample is focused by the image forming function of the objective lens. Since the re-emitted light has longer wavelengths than the excitation light, it passes through the dichromatic mirror and towards the detector or the digital camera system.

Cell death is typically classified into two broad categories: apoptosis and necrosis. As a rule, classification of cell death in a given model should always include morphological examination coupled with at least one of the other assays.¹⁸⁵ Fluorescence microscopy with

differential uptake of fluorescent DNA binding dyes (Acridine Orange and Ethidium Bromide (AO/EB) dual staining, Caspase-3/7 activation and lactate dehydrogenase leakage) are methods of choice for their simplicity, rapidity, and accuracy.

AO permeates both live and dead cells staining the nucleus green, while EB only stains the nucleus of cells with compromised membrane integrity red.¹⁸⁶⁻¹⁸⁹ CellEvent™ Caspase-3/7 Green Detection Reagent enables fluorescent detection of caspase 3/7 activation. The reagent contains a four amino acid peptide (DEVD) conjugated to a nucleic acid binding dye that inhibits the ability of the dye to bind to DNA. After the activation of caspase 3/7 in apoptotic cells, the dye is cleaved and binds to DNA, producing a green fluorescent emission.¹⁹⁰⁻¹⁹³ Lactate dehydrogenase (LDH) is an enzyme present in the cytosolic compartment of cells.¹⁹⁴⁻¹⁹⁶ Membrane damage that causes cytosolic leakage will result in the release of LDH into the surrounding cell culture medium. LDH catalyses the reduction of nicotinamide adenine dinucleotide (NADH) through the oxidation of lactate to pyruvate. A red coloured formazan salt is formed from the reduction of tetrazolium salts by NADH. The amount of formazan formation can be quantified spectrophotometrically.

2.13 Magnetic Hyperthermia Setup

SAR was experimentally obtained from time-dependent calorimetric measurements by placing the NiFe MNPs in an alternating magnetic field generated by the hyperthermia equipment, SPG-10AB-II high frequency induction heater, as schematically illustrated in Figure 2.22. MNPs in aqueous suspension with concentrations of 0.05–0.1 mg/ml were poured into a falcon tube. An optical fiber temperature probe was immersed into the falcon tube, which was insulated by Styrofoam and surrounded by the induction coils. The temperature of the coils were maintained at 28.0 ± 0.5 °C by a water recirculating chiller. The initial temperature of the suspension was maintained at 28.0 ± 0.5 °C for 1 min to eliminate any heat contributions from the induction coils. A fixed frequency of 360 kHz and alternating magnetic field range of 15.9 to 47.8 kAm⁻¹ was applied, within the criterion for clinical magnetic hyperthermia.

Figure 2.22 Magnetic hyperthermia system, SPG-10AB-II high frequency induction heater.

Chapter 3 – Functionalization of Magnetic Nanoparticles for Magnetic Hyperthermia

3.1 Chapter Introduction

Template-assisted pulsed electrodeposition with differential chemical etching is a simple and inexpensive fabrication method to produce MNPs of various composition, Ni, Fe or Co. $\text{Ni}_{80}\text{Fe}_{20}$, Permalloy, is a ferromagnetic material that displays exceptional magnetic properties such as high permeability, low coercivity, near zero magnetostriction and distinct anisotropic magnetoresistance. In cylindrical NiFe MNPs, a triple vortex state is formed, in which clockwise and anticlockwise vortices are connected at the center of the nanowire via a third vortex core, resulting in a three-dimensional magnetization configuration.

In this chapter, the precise control of the MNPs composition and length is demonstrated by tuning the electrodeposition potential and pulse duration, while the diameter is defined by the template pore size. The magnetization reversal mechanism, which occurs via the nucleation of a third vortex core and abrupt splitting of the clockwise and anticlockwise vortices, was investigated by anisotropic magnetoresistance measurements and micromagnetic simulations. In addition, the theoretical heat dissipation from the MNPs for magnetic hyperthermia applications was calculated from the simulations of hysteresis loops and vibrating sample magnetometer measurements. Using time-dependent calorimetric measurements, the specific absorption rate and intrinsic loss power of the MNPs was determined and compared with the analytical calculations.

3.2 Fabrication of Cylindrical NiFe Magnetic Nanoparticles

Compositionally modulated cylindrical NiFe nanowires were grown using the AAO template-assisted pulsed electrodeposition technique at room temperature (25 °C) in an electrolyte consisting of 0.5M NiSO₄, 0.01M FeSO₄ and 0.5M H₃BO₃.¹⁹⁷⁻¹⁹⁹ The AAO template was dissolved in 1M NaOH solution and the released nanowires were thoroughly rinsed with deionized water to de-alkalize. Subsequently, the Fe-rich regions in the nanowires were completely etched away in dilute HNO₃ to form the MNPs, as shown in Figure 3.1.¹⁹⁷

Figure 3.1 Schematic illustration of the pulsed electrodeposition and differential chemical etching process flow.

Associated intellectual property:

“Fabrication of Magnetic Nanoparticles by Electrodeposition and Differential Chemical Etching Techniques”, W. L. Gan, M. Chandra Sekhar, D. W. Wong, and W. S. Lew Technology Disclosure, NTU ref: TD/083/14 (2013).

The diameter of the MNPs ($d = 350$ nm) was determined by the AAO template pore size, while the length ($l = 75$ nm, 200 nm, 500 nm) was controlled by the pulse durations. An increase in the high potential pulse V_H duration of 2 s to 40 s results in an increased in l from 75 nm to 500 nm. The dimensions of the MNPs were characterized by SEM images, as shown in Figure 3.2 and 3.3.

Figure 3.2 SEM image of cylindrical NiFe MNPs fabricated via template-assisted pulsed electrodeposition and differential chemical etching technique.

Figure 3.3 The graph of MNP length l vs high potential pulse V_H duration.

Transmission electron microscopy (TEM) and selective area electron diffraction pattern (SAED) indicate a polycrystalline structure, as shown in Figure 3.4. The SAED pattern from the MNPs reveals a face-centered cubic (fcc) crystalline structure and the TEM image shows a lattice fringe spacing of 2.32 Å, corresponding to the (111) planes of fcc Ni-Fe lattice. The lattice spacing of the MNPs is smaller than bulk NiFe is attributed to the intrinsic stresses caused by the crystal imperfection and lattice mismatch between MNPs and AAO templates.^{131,200} By Debye–Scherrer’s relationship, the crystallite particle size can be calculated from the broadness of the diffraction peak, and is given by:

$$\beta = \frac{K \lambda}{L \cos \theta}$$

where L is the full width at half maximum (FWHM), λ is the wavelength of X-ray, K is the Scherrer constant and θ is the Bragg angle. From Figure 3.16 X-ray diffraction (XRD) pattern, the crystallite size is calculated to be ~11 nm. This corresponds to reported literature on NiFe or Ni nanostructures and nanowires with negligible or very small magneto-crystalline anisotropy (k).¹³¹ The crystal quality of MNPs is dependent on the electrodeposition conditions and can be modified by controlling the direction of crystal growth, which can affect the magnetic properties of the MNPs.

Figure 3.4 (a) TEM and (b) SAED image of MNP showing polycrystalline structure.

3.3 Composition Control

The anomalous co-deposition phenomenon has been observed for the deposition of Fe in NiFe thin films and nanowires with a dependency on the applied potential, as shown in Figure 3.5. In the deposition of Fe group metals, the less noble metal deposits preferentially as compared to the more noble ones due to anomalous co-deposition effect.²⁰¹ At lower potentials such as -0.9V , the proportion of Fe in the deposited alloy can almost be equal to the amount of Ni despite Fe ions being much scarcer than Ni ions.²⁰² However, with the increase of negative potential from -0.9 V to -1.4 V , the effect of anomalous co-deposition peaks at -1.3V and subsequently reduces till the co-deposition of NiFe alloys becomes non-anomalous.²⁰³

Figure 3.5 The graph of high potential pulse V_H vs Fe% in our NiFe thin film and nanowires.

The composition of the fabricated cylindrical NiFe MNPs is controlled by the electrodeposition potential V_H or the electrolyte composition. To show the large degree of control in the MNPs composition, various compositions of MNPs have fabricated ($\text{Ni}_{88}\text{Fe}_{12}$, $\text{Ni}_{76}\text{Fe}_{24}$, $\text{Ni}_{52}\text{Fe}_{48}$ and $\text{Ni}_{36}\text{Fe}_{64}$) and verified by EDX measurements, as shown in Figure 3.6. The EDX spectrometer displays the energies of X-rays (1–20 keV) against the number of

counts. The position of an energy peak in the spectrum is used to identify the element and the area under the peak is proportional to the number of atoms of the element. The qualitative analysis or identification of elements in the spectrum is usually achieved using manufacturer's software.^{204,205}

Figure 3.6 EDX spectrum for $\text{Ni}_{88}\text{Fe}_{12}$, $\text{Ni}_{76}\text{Fe}_{24}$, $\text{Ni}_{52}\text{Fe}_{48}$ and $\text{Ni}_{36}\text{Fe}_{64}$.

Figure 3.7 shows the normalised hysteresis loop obtained by VSM measurements for the NiFe MNPs with various composition. The magnetic field is increased to a value that is sufficient to overcome the effective magnetic anisotropy such that the magnetization reaches saturation.

Figure 3.7 Normalized hysteresis loops of NiFe MNPs ($d = 350$ nm and $l = 500$ nm) for $\text{Ni}_{88}\text{Fe}_{12}$, $\text{Ni}_{76}\text{Fe}_{24}$, $\text{Ni}_{52}\text{Fe}_{48}$ and $\text{Ni}_{36}\text{Fe}_{64}$ measured in the in-plane and out-of-plane directions.

The squareness ratio SQR is a basic measurement of how square the hysteresis loop is, given by:

$$\text{SQR} = \frac{M_r}{M_s}$$

The values of coercivity H_c , and squareness $\text{SQR} = M_r/M_s$ for in-plane and out-of-plane applied magnetic fields are tabulated in Table 2. In general, the trend of in-plane H_c is higher than the out-of-plane H_c for Ni-rich MNPs ($\text{Ni}_{88}\text{Fe}_{12}$, $\text{Ni}_{76}\text{Fe}_{24}$ and $\text{Ni}_{52}\text{Fe}_{48}$), but reversed for Fe-rich MNPs ($\text{Ni}_{36}\text{Fe}_{64}$), which is in agreement with the previous studies on anomalous co-deposition of NiFe nanowires.²⁰³

Ni:Fe ratio	In-plane H_c (Oe)	Out-of-plane H_c (Oe)	In-plane SQR	Out-of-plane SQR
Ni ₈₈ Fe ₁₂	84.5	33.8	0.060	0.054
Ni ₇₆ Fe ₂₄	62.7	54.0	0.023	0.027
Ni ₅₂ Fe ₄₈	68.6	51.4	0.048	0.049
Ni ₃₆ Fe ₆₄	80.7	91.1	0.064	0.054

Table 2 Coercivity H_c , and squareness $SQR = M_r/M_s$ values of of NiFe MNPs for Ni₈₈Fe₁₂, Ni₇₆Fe₂₄, Ni₅₂Fe₄₈ and Ni₃₆Fe₆₄ measured in the in-plane and out-of-plane directions.

3.4 Magnetization Reversal in Cylindrical Magnetic Nanoparticles

In micromagnetic simulations, the MNP with diameter (d) of 350 nm and length (l) of 500 nm was first saturated in the $-x$ direction parallel to its long axis (I), as shown in Figure 3.8. As the applied magnetic field was reduced, a clockwise and an anticlockwise vortex were nucleated at the ends of the MNP, which started to move toward the center of the MNP, leading to a gradual reduction of the parallel magnetization component that caused a drop in the magnetoresistance (II). At 0 Oe (III), a third vortex core was found to be nucleated when the two vortices met at the center of the MNP. As the magnetic field direction was reversed, the third vortex core was annihilated at $H \approx -10$ Oe. Next, an abrupt splitting of the two vortices was observed, which led to a sharp increase in the parallel magnetization component and also the magnetoresistance (IV-V). The magnetoresistance of the MNP returned to its initial value (VI) as the MNP began to saturate in the $+x$ direction.

Figure 3.8 Magnetization configurations of the MNP at various magnetic field strengths from -250 Oe to +250 Oe. The arrows illustrate the direction of magnetization and the colors red and blue represent the clockwise and anticlockwise vortices, respectively.

The hysteresis loops give detailed information on the magnetization states of the MNPs and its reversal mechanism under an applied magnetic field. Initially, the MNP was saturated by applying a large magnetic field along the MNP long axis and then relaxed. The simulations show that the MNPs have two distinct reversal processes with respect to an out-of-plane field depending on their length (l).

At lengths $l < 100$ nm, only a single vortex nucleation was observed, characterized by a curling magnetization found in single domain circular nano-magnets.^{206,207} The interaction between the magnetostatic energy and exchange energy favors an in-plane, closed flux domain structure – the single vortex state. For lengths $100 < l < 300$ nm, a double vortex nucleation occurred, consisting of a pair of anti-clockwise and clockwise vortex cores at the opposite ends of the MNPs. However, the annihilation of one vortex occurs as the magnetic field decreases,

resulting in a single vortex state. The hysteresis loop for a MNP with $l = 200$ nm and $d = 150$ –350 nm is shown in Figure 3.9.

As l increases to more than 300 nm, the annihilation of vortex at low fields was absent, whereas a third vortex core was nucleated on the curved surface of the MNP. In Figure 3.10, the hysteresis loop for a MNP, with $l = 500$ nm and $d = 150$ –350 nm, illustrates the reversal process from double vortex to triple vortex state. At sufficiently low magnetic fields, the triple vortex state is formed, which is a stable magnetization configuration.

Figure 3.9 The hysteresis loop of MNPs with $d = 150$ –350 nm and $l = 200$ nm.

Figure 3.10 The hysteresis loop of MNPs with $d = 150\text{--}350$ nm and $l = 500$ nm.

The exchange energy, demagnetization or dipolar energy and Zeeman energy contributions to the total energy of the MNP are plotted as a function of applied magnetic field H along the MNP long axis in Figure 3.11 to 3.14, respectively. At large magnetic fields, the Zeeman energy contribution predominates and the spins are essentially aligned in the magnetic field direction. This parallel arrangement of the spins to the field minimizes the exchange energy contribution to the total magnetic energy. As the applied magnetic field is reduced, a clockwise and an anticlockwise vortices are nucleated at the ends of the MNP, which start to move toward the centre of the MNP, leading to a gradual reduction of the parallel magnetization component that causes a drop in the Zeeman contribution, while other contributions become increasingly significant. The magnetization of the MNP tries to minimise the stray field, and thus demagnetization energy is minimal. At sufficiently low magnetic fields, the triple vortex state is formed, which is a stable magnetization configuration, with total energy kept at a minimum, as shown in Figure 3.10. As the magnetic field reverses, the sharp drop in exchange energy corresponds to the abrupt splitting of the two vortices.

Figure 3.11 The plot of exchange energy against applied magnetic field H .

Figure 3.12 The plot of dipolar energy against applied magnetic field H .

Figure 3.13 The plot of Zeeman energy against applied magnetic field H .

Figure 3.14 The plot of total energy against applied magnetic field H .

3.5 Biocompatible Surface Coating

NiFe MNPs tend to aggregate due to the effects of strong dipole interactions between neighboring MNPs. Therefore, the surface modification of the MNPs using biocompatible and biodegradable polymer, such as chitosan,²⁰⁸⁻²¹⁰ polyvinyl alcohol,²¹¹⁻²¹³ oleic acid,²¹⁴⁻²¹⁶ dextran^{217,218} and, most commonly polyethylene glycol (PEG),²¹⁹⁻²²⁴ has been proposed. PEG is a hydrophilic polymer that has been widely used for improving blood circulation of liposomes and MNPs.²²⁵⁻²²⁸ To disperse the cylindrical NiFe MNPs into water, a biocompatible 5000 g mol⁻¹ PEG was used as a stabilizer.²²⁹ The SEM image shows the formation of an oxide shell around the MNPs, shown in Figure 3.15. This oxide shell prevents oxidation of the magnetic materials in the MNPs. Previous research works on FeCo MNPs and Fe MNPs have shown severe oxidation from just exposure to the atmosphere.^{229,230}

Figure 3.15 SEM image of NiFe MNPs with PEG coating.

The X-ray diffraction (XRD) pattern peak was mainly indexed at the (111) crystal planes which corresponds to the face-centered cubic (fcc) structure of bulk NiFe, as shown in Figure 3.16. This indicates that the MNPs were electrodeposited with a preferred orientation of (111), which is also evident in NiFe nanowires fabricated by electrodeposition or sputtering.^{131,231} In addition, there was an absence of diffraction peaks corresponding to spinel oxide ((NiFe)₃O₄), which results from the formation of oxide phases due to the high concentration of Fe.²³² The high crystallinity of the NiFe MNPs led to negligible surface spin canting, and hence retained the high saturation magnetization and small coercivity of the MNPs.^{233,234} Further characterizations for the PEG-coated NiFe MNPs were conducted using EDX measurement. As shown in Figure 3.17, mainly of Ni and Fe elements were detected, with the presence of a small percent of O element, an indication of the oxide shell formed around the MNPs.

Figure 3.16 XRD pattern for NiFe MNPs with PEG coating.

Figure 3.17 EDX pattern for NiFe MNPs with and without PEG coating.

From Figure 3.18, the cell viability of the HeLa cells exposed to uncoated and PEG-coated NiFe MNPs after 24 hours are 82.2% and 82.6%, respectively. After 48 hours, the cell viabilities decreased slightly to 79.9% and 82.1%, displaying slightly higher biocompatibility for PEG-coated MNPs. The cytotoxicity of the cylindrical NiFe MNPs to HeLa cells are comparable to other commercially available ferromagnetic NPs used in magnetic hyperthermia research.²³⁵ During the duration of < 4 hours, the cellular metabolic activity in cell controls with nanoparticles did not change drastically in comparison with cells controls without nanoparticles. The magnetic nanoparticles revealed insignificant cytotoxic effects to cells and they remained ~100% viable relative to control cells under 4 hours. Therefore, the effects of endocytosis on the intracellular cytotoxicity can be kept at a minimum during the duration of magnetic hyperthermia treatments. Our optical microscopy observation did not show any major cell morphology changes within 4 hours.

Figure 3.18 Cell viability with displayed images of HeLa cells after incubation with MNPs, over a period of 0–48 hr ($\pm$ SD, $p < 0.05$).

3.6 Calorimetric Measurements

The NiFe MNPs, with $l = 500$ nm and $d = 350$ nm, were exposed to an alternating magnetic field of 15.9 to 47.8 kAm^{-1} (200 to 600 Oe) at a frequency of 360 kHz, and the temperature-time curve is displayed in Figure 3.19. As characterized by the SAR equation, the SAR values were calculated to be 427 Wg^{-1} , 1054 Wg^{-1} and 1742 Wg^{-1} , for 15.9 kAm^{-1} , 31.9 kAm^{-1} and 47.8 kAm^{-1} , respectively. As predicted, the larger the magnetic field strength, the greater the SAR value, i.e. the SAR value was proportional to the magnetic field strength. Therefore, an additional parameter ILP was obtained to give a better evaluation of the heating efficiency of the MNPs for magnetic hyperthermia. As characterized by the ILP equation, the ILP values were calculated to be 4.69 $\text{nHm}^2\text{kg}^{-1}$, 2.88 $\text{nHm}^2\text{kg}^{-1}$ and 2.12 $\text{nHm}^2\text{kg}^{-1}$, for 15.9 kAm^{-1} , 31.9 kAm^{-1} and 47.8 kAm^{-1} at 360 kHz, respectively.

Figure 3.19 Temperature-time curves of 0.05 mg/ml NiFe MNPs, with $l = 500$ nm and $d = 350$ nm, measured in aqueous suspensions. The field frequency was 360 kHz, while the magnetic field strength increased from 15.9 kAm^{-1} , 31.9 kAm^{-1} and 47.8 kAm^{-1} (200 Oe to 600 Oe).

Next, NiFe MNPs, with $d = 350$ nm and $l = 100\text{--}500$ nm, were exposed to an alternating magnetic field of 47.8 kAm^{-1} (600 Oe) at a frequency of 360 kHz, and the temperature-time curve is displayed in Figure 3.20. As characterized by the SAR equation, the SAR values were calculated to be 409 Wg^{-1} , 618 Wg^{-1} and 1742 Wg^{-1} , for $l = 100$ nm, 200 nm and 500 nm at 47.8 kAm^{-1} and 360 kHz, respectively. As characterized by the ILP equation, the ILP values were calculated to be $0.50 \text{ nHm}^2\text{kg}^{-1}$, $0.75 \text{ nHm}^2\text{kg}^{-1}$ and $2.12 \text{ nHm}^2\text{kg}^{-1}$ for $l = 100$ nm, 200 nm and 500 nm at 47.8 kAm^{-1} and 360 kHz, respectively.

Figure 3.20 Temperature-time curves of 0.05mg/ml NiFe MNPs, with $d = 350$ nm, measured in aqueous suspensions. The magnetic field strength of 47.8 kAm^{-1} (600 Oe) and frequency of 360 kHz, while the MNP length l increased from 100 nm to 500 nm.

It was clear that MNPs with $l = 500$ nm had far greater heating efficiency than MNPs with $l = 100$ nm and 200 nm, leading to a more significant temperature rise. The highest SAR value of MNPs with $l = 500$ nm was 1742 Wg^{-1} at 47.8 kAm^{-1} and 360 kHz. For comparison, the SAR values for magnetic field of 15.9 to 31.9 kAm^{-1} (200 to 400 Oe) at a frequency of 360 kHz and MNPs with $d = 350$ nm and $l = 100$ –500 nm were tabulated in Figure 3.21. Under the same conditions, the SAR and ILP values of MNPs with $l = 500$ nm were four magnitudes higher than those with MNPs of smaller l . From micromagnetic simulations, it was observed that as l increases to > 300 nm, the magnetization reversal process of the MNP changed from a double vortex state to triple vortex state. At $l < 100$ nm, only a single vortex nucleation was observed, and at $100 < l < 300$ nm, a double vortex nucleation occurred. The remanent magnetization M_r of the MNP was higher for the triple vortex state as compared to the single and double vortex state.

Figure 3.21 Tabulated SAR values for NiFe MNPs, with $l = 100\text{--}500$ nm and $d = 350$ nm under a magnetic field strength of 15.9 to 47.8 kAm $^{-1}$ (200 Oe to 600 Oe) and frequency of 360 kHz.

For single domain MNPs, the theoretical model to calculate the dynamic hysteresis loop has been proposed by Carrey et al.⁸⁸ For multi-domains MNPs, the use of micromagnetic simulations to obtain static hysteresis loop for calculation was reasonable for MNPs with large sizes, above the critical size for superparamagnetism, as the switching time of the magnetization is in the order of 10^{-9} s. Since the switching time of magnetic hyperthermia is in the order $\sim 10^{-6}$ s, the large MNPs is able to keep up with the alternating magnetic field. The area of hysteresis loops from micromagnetic simulations of NiFe MNPs and VSM measurements was used to theoretically calculate the SAR values and tabulated in Figure 3.22.

The SAR values of MNPs with $l = 100$ nm and 200 nm displayed a small SAR value at low magnetic fields below H_c and sharply increased till it reaches a saturation at high magnetic fields, which is characteristic of the ferromagnetic regime. In contrast, the magnetic field dependence of the SAR value of $l = 500$ nm MNPs, with the triple vortex state, followed a non-linear relationship with SAR values that were ~ 6 times greater. The high remanent

magnetization M_r of the triple vortex state in the $l = 500$ nm MNPs was evident in the non-zero SAR values at low magnetic fields. The comparison between calorimetric measurements (Figure 3.21) and analytical calculations (Figure 3.22) indicated a qualitative and quantitative agreement on the features of MNPs in the ferromagnetic regime, displaying small SAR values at low magnetic fields and saturation at high magnetic fields which was correlated to the H_c of the MNPs.

Figure 3.22 Theoretical SAR values for NiFe MNPs, with $l = 100$ – 500 nm and $d = 350$ nm.

The heat dissipation of NiFe MNPs with triple vortex states were compared for $d = 150$ – 350 nm, under an alternating magnetic field of 47.8 kAm^{-1} (600 Oe) at a frequency of 360 kHz , and the temperature-time curve is displayed in Figure 3.23. The SAR and ILP values were calculated to be 1785 Wg^{-1} , 2073 Wg^{-1} and 2750 Wg^{-1} , and $2.17 \text{ nHm}^2\text{kg}^{-1}$, $2.52 \text{ nHm}^2\text{kg}^{-1}$ and $3.34 \text{ nHm}^2\text{kg}^{-1}$, for $d = 350$ nm, 250 nm and 150 nm, respectively. The MNPs with $d = 150$ nm and 250 nm were able to reach the optimal therapeutic temperature of 43°C in 4.92 mins and 7.45 mins at concentration of 0.1 mg/ml . After 10 mins, $d = 350$ nm MNPs were only able to reach the highest temperature of 40°C . Comparing MNPs with different aspect ratios, it was found that the heating efficiency of $d = 150$ nm MNPs was 1.54 times greater than $d = 350$ nm

MNPs. This was because MNPs with $d = 150$ nm possessed the highest low field susceptibility and remanent magnetization. Therefore, the SAR value was closely correlated to the magnetization reversal process of the MNPs with both variations in l and d .

Figure 3.23 Temperature-time curves of 0.1 mg/ml NiFe MNPs, with $l = 500$ nm, measured in aqueous suspensions. The magnetic field strength of 47.8 kAm^{-1} (600 Oe) and frequency of 360 kHz, while the MNP length d increased from 150 nm to 350 nm.

From Figure 3.10, it can be observed that the area of hysteresis A evolves significantly with the diameter of the MNP. Therefore, the SAR value of the $d = 150$ nm MNPs increases so rapidly and saturates at a maximum SAR value of 6263 Wg^{-1} . The analytical calculations showed that the MNPs with higher aspect ratios has higher hysteresis losses, resulting in higher theoretical SAR values, as shown in Figure 3.24. The comparison between calorimetric measurements (Figure 3.23) and analytical calculations (Figure 3.24) were in good qualitative agreement, but there were quantitative disagreements in the values of hysteresis losses.

Figure 3.24 Theoretical SAR values for NiFe MNPs, with $d = 150\text{--}350$ nm and $l = 500$ nm.

The mismatch between the experimental and theoretical values arose from the NiFe MNPs being non-superparamagnetic and possesses non-negligible remanent magnetization, leading to unwanted agglomeration due to strong magnetic dipole interactions between neighboring MNPs.^{236,237} Since hydrodynamic volume of MNPs is a component governing the Brownian motion, the extent of aggregation of the MNPs will determine the dominating relaxation mechanism, i.e. Néel or Brownian relaxation. Hence, an aggregated group of MNPs versus a single free MNP will greatly differ in SAR values. Furthermore, an alternating magnetic field can induce nano-columns or nano-chains formation which exhibit dissimilar Brownian relaxation mechanism, and hence accounted for the discrepancy between the experimental and theoretical values.²³⁸⁻²⁴⁰

3.7 Chapter Summary

The high SAR values displayed by the cylindrical NiFe MNPs, comparable with iron oxide MNPs (IOMNPs) and superparamagnetic iron oxide nanoparticles (SPIONs),^{79,241} demonstrates the capability of these MNPs in heat dissipation under an alternating magnetic field. MNPs with the triple vortex state had far greater heating efficiency than MNPs with double or single vortex state, which have a SAR value of four times greater, attributed to the high M_r of the MNPs in the triple vortex state. Comparing MNPs with different aspect ratios, it was observed that the heating efficiency of $d = 150$ nm MNPs was 1.54 times greater than $d = 350$ nm MNPs due to a larger M_r and low field susceptibility. Both calorimetric measurements and micromagnetic simulations showed the correlation between the magnetization reversal process and the higher hysteresis losses from $d = 150$ nm MNPs, resulting in higher experimental and theoretical SAR values.

However, the relatively large size of MNPs allowed for their accumulation in tumors via the enhanced permeability and retention (EPR) effect.²⁴²⁻²⁴⁴ This passive and non-selective process was a consequence of a leaky and underdeveloped tumor vascular system that causes large particles to gather at the tumor. These particles exited the circulation due to the leaky vascular system, but remained at the tumor site due to inefficient draining into the lymphatic system. Therefore, for the usage of these NiFe MNPs for clinical applications, the fine-tuning of the biocompatible PEG surface coating is needed. Such improvement will allow the MNPs to disperse better and reduce the unwanted agglomeration due to strong magnetic dipole interactions. Nevertheless, the easy control of the sizes of the NiFe MNPs and their magnetic properties indicated great potential for *in vivo* magnetic hyperthermia cancer therapy trials.

Chapter 4 – Magnetic Nanoparticles Actuated by Biaxial Pulsed Magnetic Field

4.1 Chapter Introduction

MNPs can be remotely controlled or manipulated by an external applied magnetic field, unlike any other nanomaterials. The resulting magnetic response of the MNPs can be in the form of a mechanical force or heat generation, governed by the magnetic field configuration, magnetic moment and magnetic susceptibility of the MNPs.⁶⁷ In the previous chapter, magnetic hyperthermia requires the use of a high frequency alternating magnetic field in the kHz–MHz range to induce a localized heat generation that induces cancer cell death at a critical temperature of above 43 °C.⁷⁻¹⁰ However, its invasiveness and great difficulty in targeting a specific cancerous area with homogenous heat generation poses a fundamental problem, in addition to the requirement of high frequency and strong magnetic fields.^{95,96}

Recently, it has been demonstrated that magnetic microdiscs, under an alternating magnetic field of less than 100 Hz, can exert sufficient mechanical force to achieve cell apoptosis.¹³⁻¹⁵ In contrast to achieving tumor regression through the effects of hyperthermia, the magneto-mechanical cell actuation method provides a non-invasive and targeted treatment method with high specificity and efficiency. Magneto-mechanical actuation is achieved by the spatial rotation of the particles to align to the applied magnetic field by Brown relaxation, which produces a magnetic torque.^{7,16} Most experimental work uses superparamagnetic particles, with zero remanent magnetization and low saturation magnetization values, to avoid agglomeration occurring due to magnetic stray fields. Therefore, the use of soft magnetic materials, with closed spin-vortex magnetization and high magnetization of saturation, eliminates the need of high magnetic fields for manipulation of these particles. Although the efficacy of relatively

large microdiscs with a single vortex state is well-understood,¹³⁻¹⁵ there is little research work on multi-vortex MNP *in vitro* magneto-actuated cancer cell apoptosis effects.

In this chapter, a biaxial pulsed magnetic field configuration was shown to be a highly efficient magneto-actuated cancer cell apoptosis method. Its effectiveness was demonstrated in the light transmissivity dynamics of MNPs and in the decrease in the viability of HeLa cells. Micromagnetic simulations showed that the MNPs, with different aspect ratios, can form triple vortex states that increases the magnetic susceptibility and torque experienced by the MNPs. These results were further validated by their efficacy in inducing programmed cancer cell death.

4.2 Light Transmissivity Dynamics

The application of a magnetic field parallel to the laser beam aligns the long axis of the MNPs collinear to the optical axis of the beam, leading to a high amount of light transmitted through the suspension of MNPs, as shown in Figure 4.1. On the other hand, the application of a magnetic field perpendicular to the laser beam decreases the amount of transmitted light, as the MNPs long axis was facing and blocking the laser beam. I_{max} corresponds to the maximum light intensity measured, while I_{min} was the minimum light intensity measured due to the misalignment of the MNPs caused by Brownian motion.^{13,197} The change in transmitted light intensity ($\Delta I = I_{max} - I_{min}$) was measured over a range of frequencies. The frequency response of ΔI is representative of the MNP responsiveness to the applied magnetic field, which in turn is determined by the torque experienced by the MNP, and subsequently the resultant mechanical force exerted on the cells.

Figure 4.1 Illustrations of the light intensity maximum I_{max} and minimum I_{min} for the light transmissivity measurements.

The magnetic torque experienced by MNPs that aligns its net magnetic moments to the applied magnetic field was experimentally observed through the light transmissivity measurements of a suspension of MNPs, with diameter (d) of 150 nm and length (l) of 500 nm. In this experiment, a quadrupole electromagnet was employed to apply an external magnetic field in two orthogonal directions. A laser and photodiode measure the light intensity transmitted through a suspension of MNPs in deionized water (DI) under different applied magnetic field configurations. The viscosities and refraction indices of DI water and DMEM + 10% FBS were 0.88 cP and 0.94 cP, 1.33 and 1.345, respectively.²⁴⁵ Both values were comparable and show consistent light transmissivity results.

Figure 4.2 and 4.3 illustrates the different applied magnetic field configurations. In uniaxial AC and DC pulsed magnetic field configuration, the magnetic field of 10 Oe was applied in a single direction using two opposite coils with AC or DC pulses. It is important to note that such a small amplitude magnetic field is insufficient to change the magnetization

states of MNPs, but enough to control the orientations of the MNPs. While in biaxial pulsed magnetic field configuration, the magnetic field was pulsed in two orthogonal directions.

Figure 4.2 Illustrations of applied magnetic field configurations.

Figure 4.3 The normalized transmitted light intensity for 1 Hz under the different applied magnetic field configurations; uniaxial DC pulsed (red), uniaxial AC pulsed (black) and biaxial pulsed magnetic fields (blue).

Magneto-mechanical cell actuation relies on two mechanisms to induce a force on the cell walls to induce cell apoptosis. The first of which is the Brown relaxation which describes the rotation of the MNPs to the direction of the applied field due to an induced magnetic torque. The Brown relaxation time t_B characterizes the speed of rotation and was found to be 24 ms in our experiment by measuring the time taken for the light intensity to reach $0.9I_{max}$ from $0.1I_{max}$. The second mechanism is the random rotational Brownian motion which introduces disorder in the alignment MNPs after the magnetic field is removed.⁷ However, this rotation is slower, with the rotational Brownian motion time t_R measured to be 400 ms.

The MNPs were initially magnetized and saturated by applying a large magnetic field of 1 kOe. Subsequently, the light transmissivity dynamics measurements for uniaxial AC pulsed (black), uniaxial DC pulsed (red) and biaxial pulsed magnetic fields (blue) were measured over a range of frequencies (1–30 Hz), as shown in Figure 4.4, 4.5 and 4.6. The change in transmitted light intensity $\Delta I = I_{max} - I_{min}$ at each frequency was subsequently tabulated in Figure 4.7.

Figure 4.4 The change in amplitude for increasing frequencies (1–30 Hz) for uniaxial AC pulsed magnetic field.

Figure 4.5 The change in amplitude for increasing frequencies (1–30 Hz) for uniaxial DC pulsed magnetic field.

Figure 4.6 The change in amplitude for increasing frequencies (1–30 Hz) for biaxial pulsed magnetic field.

In uniaxial field configurations, the frequency response was limited by the large t_R as shown in Figure 4.7, where the oscillation amplitude of the uniaxial AC and DC pulsed magnetic fields shows a sharp decrease with increasing frequency. In contrast, the biaxial pulsed field shows a high ΔI of 94.9–99.7% at low frequencies of 1–5 Hz. The application of magnetic field in orthogonal directions has two advantages. Firstly, it was no longer limited by the large t_R as the need for MNP misalignment due to the random rotational Brownian motion was eliminated.²⁴⁶ This resulted in the diminishing value of ΔI being less substantial at higher frequencies. Secondly, a biaxial field configuration also ensures that the magnetic field was applied at a larger angle to the MNPs, hence maximizing the magnetic torque. The light transmissivity dynamics demonstrates that the biaxial field configuration can solicit a faster and stronger magnetic response from the MNPs across a wide range of frequencies.

Figure 4.7 The change in transmitted light intensity ΔI measured over a range of frequencies under the different applied magnetic field configurations; uniaxial DC pulsed (red), uniaxial AC pulsed (black) and biaxial pulsed magnetic fields (blue).

4.3 Biocompatibility

Endocytosis is a type of active transport that enables the uptake of macromolecules and particles from the surrounding medium into a cell.²⁴⁷ The plasma membrane of the cell surrounds the targeted particle forming a pocket, which then buds off inside the cell to form a newly created intracellular vesicle with the ingested particle, as shown in Figure 4.8. When the HeLa cells were exposed to the MNPs, some MNPs were internalized to the cells due to endocytosis, and accumulated in the digestive vacuoles. This process causes the cell to overload and leads to induced cell death.^{248,249}

Figure 4.8 Illustration of endocytosis of MNPs.

The cell viability was determined using PrestoBlue, a permeable resazurin-based cell viability reagent, which uses the reducing power of viable cells to quantitatively measure cell proliferation. PrestoBlue assay kit was performed according to the manufacturer's protocol. To obtain the biocompatibility of the MNPs, a concentration of 0.1 mg/ml was incubated with the HeLa cells over a period of 0–48 hours. Subsequently, the cells were washed and incubated with the PrestoBlue reagent at 37°C and 5% CO₂ for 2 hours. The cell viability was determined

using Synergy H4 Hybrid Multi-Mode Microplate Reader and the absorbance readings for each well at 570 nm and 600 nm were recorded. The cell viability was expressed as a percentage relative to the cells unexposed to MNPs. Each experiment was performed in quadruplicate sets of experimental and control assays in a 96-well microtiter plate. The results were represented as the mean $\pm$ standard deviation (SD). Statistical significance was analyzed using one-way analysis of variance (ANOVA) with OriginPro, OriginLab. A p value of < 0.05 was considered to be statistically significant.

From Figure 4.9, the survival rates of HeLa cells exposed to $d = 150$ nm, 200 nm and 350 nm MNPs were 88.1%, 83.9% and 83.3%, respectively, displaying high biocompatibility. After 48 hours, the cell survival percentages drop slightly to 83.4%, 83.2% and 82.5%. These results revealed that the $d = 150$ nm MNPs have a higher survival rate as compared to the other MNPs ($d = 250$ nm and 350 nm). The cytotoxicity rates of the MNPs were comparable to other commercially available ferromagnetic nanoparticles used for hyperthermia research with HeLa cells at similar concentrations.²³⁵

Figure 4.9 Cell viability of HeLa cells after incubation with MNPs ($d = 150$ nm, 200 nm 350 nm), over a period of 0–48 hours ($\pm$ SD, $p < 0.05$).

The decrease in cell viability after incubation for 48 hours was due to the MNPs being endocytosed by the HeLa cells due to weak cell adhesive interactions between the MNPs and cell membrane. The fluorescence microscopy imaging of the HeLa cells incubated with MNPs for 48 hours without any magnetic field treatment has verified the above phenomenon of endocytosis. The cells were observed to be smaller and less spread with greater stimulated formation of lamellipodia and filopodia, as shown in Figure 4.10. When the MNPs are endocytosed or has lost contact with the cell surface, they are rendered ineffective for the magnetic field treatments. In addition, the cells are at an increased risk of cytotoxicity from the MNPs and effects of overloading. Surface functionalizing of MNPs with specific attachment can be explored to help prevent the MNPs from detaching from the cell surface and remain attached to cell surface receptors to reduce endocytosis and prolonged stimulation.

Figure 4.10 Fluorescence microscopy image of HeLa cells and NiFe MNPs incubated for 48 hours without magnetic field treatment.

4.4 Magneto-Actuated Cell Apoptosis

The efficiency of the various magnetic field configurations were investigated in *in vitro* cell destruction experiments. A magnetic field of 140 ± 10 Oe was applied to the suspension of HeLa cells with a concentration of 0.1 mg/ml of MNPs, for a range of frequencies between 1–20 Hz over a period of 10 mins each. During the experiments, the temperature of cell medium was monitored by an infrared thermometer and kept at 23.0 ± 0.5 °C, ensuring the contribution of magnetic hyperthermia was negligible. From Figure 4.11, the cell viability after the application of uniaxial AC pulsed magnetic field was at $> 90\%$ for all frequencies, which indicates low cell death induced by magneto-mechanical effects. This was reflective of the light transmissivity results in Figure 4.7, where the MNP was unable completely rotate to align to the field direction, resulting in a smaller magnetic torque experienced by the MNP, and hence a weaker mechanical force exerted on the cells. The cell viability also shows an increase in average effectiveness of 6.6% for uniaxial AC to DC pulsed magnetic field. However, at frequencies above 10 Hz, the DC pulsed magnetic field becomes increasingly ineffective in rotating the MNPs by Brown relaxation ($\Delta I \approx 0\%$), which resulted in falling toxicity, as shown in Figure 4.11.

The biaxial pulsed magnetic field actuated a larger magnetic torque on the MNP that led to an increase in the effects of *in vitro* magneto-actuated cell apoptosis. Although a steady decline in the effectiveness of cell destruction with increasing frequencies was observed, the effects of magneto-actuated induced cell death was still apparent at high frequencies. The lower responsiveness of MNPs to the magnetic field demonstrates that a smaller magnetic torque was generated. Therefore, reinforcing that lower frequencies were more effective for magneto-actuated cell apoptosis, with the biaxial pulsed magnetic field showing an increase in average efficacy of 17.9% and 11.3% over the uniaxial AC and DC pulsed magnetic field, respectively.

Figure 4.11 Cell viability of HeLa cells after magneto-actuated cell apoptosis treatment for different applied magnetic field configurations; uniaxial DC pulsed (red), uniaxial AC pulsed (black) and biaxial pulsed magnetic fields (blue) ($\pm$ SD, $p < 0.05$).

Ferromagnetic microdiscs have been shown to activate mechanosensitive ion channels mediated by mechanical stimuli, which is able to initiate cell apoptosis and programmed cell death.¹³ The activation of mechanosensitive channels, due to cell membrane stretching, leads to the increase of intracellular calcium.^{250,251} This prolonged exposure to high concentration of intracellular calcium triggers cell apoptosis.²⁵²⁻²⁵⁴ After the application of the biaxial pulsed magnetic field, the cell death was analyzed by light and fluorescence microscopy.

Optical images show drastic changes in the morphology of the HeLa cells structures. The control cells maintain an elongated cell structure, whereas the cells exposed to the applied magnetic field showed a more circular and rounded-off cell structure, as shown in Figure 4.12 (a) and (b), respectively. This was an apparent cell fractionation and redistribution of nucleic material.¹³ These observed effects were characteristic of apoptosis via genomic DNA fragmentation. The percentage of dead cells was obtained by using haemocytometry and

Trypan Blue (TB) solution. TB dye exclusion test is routinely used as a cell stain to assess cell viability, where only the non-viable cells are permeable to the dye.

Figure 4.12 *In vitro* cell apoptosis by biaxial pulsed magnetic field. Optical microscopy images of HeLa cells and NiFe MNPs (a) no field control and (b) with biaxial pulsed magnetic field treatment at 1 Hz. The cells were stained with Ethidium Bromide (EB) to observe apoptotic changes.

The HeLa cells before and after the magnetic field treatment were stained with Ethidium Bromide (EB), which is a common dye used for DNA and RNA detection, to observe the cell death pathway. EB enters only non-viable cells with damaged membrane and fluoresce with an orange colour, after binding to DNA.^{255,256} The fluorescence images were captured on the Nikon Eclipse Ti-S inverted microscope equipped with DAPI-FITC-TRITC filter at an excitation maximum of 300 nm and 520 nm and an emission maximum of 600 nm. A minimum of 500 cells were counted in each group to obtain a quantitative measure of cell apoptosis at each frequency between 1–20 Hz. In the absence of an applied magnetic field, the control cells with MNPs have shown to be non-cytotoxic, as revealed in Figure 4.13 (a). While the HeLa cells after the magneto-actuated treatment exhibit signs of early apoptosis as indicated by bright nuclear staining with EB, as shown in Figure 4.13 (b).

Figure 4.13 *In vitro* cell apoptosis by biaxial pulsed magnetic field. Fluorescence microscopy images of HeLa cells and NiFe MNPs (a) no field control and (b) with biaxial pulsed magnetic field treatment at 1 Hz. The cells were stained with Ethidium Bromide (EB) to observe apoptotic changes.

The quantified data from TB-stained and EB-positive fluorescent cells for frequencies between 1–20 Hz are presented in Figure 4.14. A larger number of apoptotic cells and dead cells were observed at low frequencies of 1–5 Hz. At higher frequencies, the number of apoptotic cells and dead cells decreased, but still showed signs of apoptosis. The TB staining of 49.1% at 1 Hz (12.6% for control) indicates that the MNPs in the presence of the biaxial magnetic field can induce sufficient mechanical force to compromise the integrity of the cell membrane. It was observed that some cells had change in morphology without EB staining, which can be attributed to necrotic cell death caused by a strong mechanical disruption to the cell structure.¹⁴ Hence, Figure 4.14 shows an overall higher percentage of dead cells count from the TB dye test as compared to the percentage of apoptotic cells.

Figure 4.14 The quantified data from TB-stained and EB-positive fluorescent cells for frequencies between 1–20 Hz ($\pm$ SD, $p < 0.05$).

4.5 Comparison of Magnetic Nanoparticles with Different Aspect Ratios

The low field magnetic susceptibility (χ) was obtained from micromagnetic simulated MNP hysteresis loops as a function of l and d for both single vortex state (dashed lines) and triple vortex state (solid lines), and tabulated in Figure 4.15. The triple vortex state is a frustrated state that was found to be stable in ferromagnetic MNPs over a wide range of material parameters.¹⁹⁷ To obtain the single vortex state in MNPs with $l > 300$ nm, a clockwise bias field was initially applied to favor the formation of a single vortex magnetization in the MNPs. An increase in low field susceptibility as the magnetization state of the MNP switches from a single to triple vortex state at $l > 300$ nm was observed.

Figure 4.15 The low field magnetic susceptibility (χ) was obtained as a function of MNP $d = 150\text{--}350$ nm and $l = 50\text{--}500$ nm for both single vortex state (dashed lines) and triple vortex state (solid lines).

Similarly, the remanent magnetization of the MNP was higher for the triple vortex state as compared to the single vortex state, as tabulated in Figure 4.16. This increase was most significant for $d = 150$ nm MNPs, in contrast to ferromagnetic discs where a decrease in diameter or increase in length leads to a reduction in susceptibility.^{257,258}

Figure 4.16 The magnetization M_r/M_s was obtained as a function of MNP $d = 150$ – 350 nm and $l = 50$ – 500 nm for both single vortex state (dashed lines) and triple vortex state (solid lines).

The magnetic torque exerted on a MNP by the applied magnetic field is given by:

$$\tau = m \times \mu_0 H = \mu_0 M_r V H \sin\theta$$

where M_r is the magnetic moment of the MNP at remanence, H is the applied field, μ_0 is the magnetic permeability ($\mu_0 = 4\pi \times 10^{-7} \text{ Hm}^{-1}$), and V is the volume of the MNP. The angle θ is the angle between the applied magnetic field and plane of the MNP, which gives maximum torque when the field is at 90° .

In a uniform magnetic field, the force acting on the HeLa cells can be approximated by calculating the force exerted at the edge of the MNP:

$$F = \frac{2\tau}{l}$$

In Figure 4.17, the F/H graph showed that a $d = 150$ nm MNP was able to induce a significantly greater force as compared to the other MNPs. At 140 Oe, the force for $d = 150$ nm, 250 nm and 350 nm MNPs were obtained to be 88.9 pN, 27.0 pN and 19.1 pN, respectively. Although a force of ~ 100 pN is needed to rupture the cell membrane physically,²⁵⁹⁻²⁶¹ only 0.5 pN is needed to activate ion channels leading to cell apoptosis.^{262,263}

Figure 4.17 The force exerted by MNPs with respect to applied magnetic field for $d = 150$ –350 nm and $l = 50$ –500 nm.

The effects of magneto-actuated cell apoptosis were compared for MNPs of different aspect ratios ($d = 150$ nm, 250 nm and 350 nm), with triple vortex states, under the biaxial pulsed magnetic field. Similar trends of decreasing effectiveness at higher frequencies, with highest cell death recorded at 1 Hz, as shown in Figure 4.18. A drop of 12% effectiveness in

cell viability was also observed at all frequencies for both $d = 200$ nm and 350 nm MNPs as compared to $d = 150$ nm MNPs. This result was supported by micromagnetic simulations which showed that $d = 150$ nm MNPs were able to exert a force that was more than four times larger than MNPs with $d > 200$ nm.

Figure 4.18 The comparison of cell viability of HeLa cells after magneto-mechanical cell destruction treatment using MNPs with $d = 150$ – 350 nm and $l = 500$ nm ($\pm$ SD, $p < 0.05$).

4.6 Chapter Summary

The proposed cell death mechanisms for uniaxial AC pulsed (black), uniaxial DC pulsed (red) and biaxial pulsed magnetic fields (blue) were summarized in Figure 4.19. In uniform magnetic fields, MNPs will experience a magnetic torque that aligns its net magnetic moments to the field direction by Brown relaxation, which translates into a force onto the cell. In uniaxial DC field, the misalignment of MNPs was due to random Brownian motion, as shown in Figure 4.19 (a). In uniaxial AC field, the MNPs were continuously made to re-align to the new field directions $+B$ and $-B$, which translate into a force exerted onto the cell, as shown in Figure 4.19 (b). In the case of biaxial field, the MNP misalignment is not dependent on random rotational Brownian motion and the orthogonal directional fields enables the magnetic field to be applied at a larger angle to the MNPs, hence maximizing the magnetic torque and force onto the cell, as shown in Figure 4.19 (c). In all magnetic field configurations, this spatial rotation of the MNPs exerts a force onto the cell, activating mechanosensitive ion channels, triggering cell apoptosis.

It has been demonstrated that the biaxial pulsed magnetic field actuated a larger magnetic torque on the MNP that resulted in an increase *in vitro* magneto-actuated cell apoptosis. This was attributed to the orthogonal directional fields that eliminates the reliance on rotational Brownian motion. The TB-stained and EB-positive fluorescent cells have shown the integrity of the cell membrane being compromised and the initiation of programmed cell death via apoptosis. Comparing MNPs of different aspect ratios, it was found that the $d = 150$ nm MNPs possessed the highest low field susceptibility and remanent magnetization, thus allowing a larger force to be exerted on the cells. This was seen in the cell viability test where the $d = 150$ nm MNPs produced 12% higher cell death.

Figure 4.19 Schemes of the proposed cell death mechanisms for different applied magnetic field configurations; uniaxial DC pulsed (red), uniaxial AC pulsed (black) and biaxial pulsed magnetic fields (blue).

Chapter 5 – Interplay of Cell Death Signaling Pathway by Magnetic Field Gradient

5.1 Chapter Introduction

When exposed to different types of MNPs and magnetic field configurations, multiple cell death pathways, such as apoptosis and necrosis, can be observed simultaneously in cell cultures or tissues. Apoptosis is a form of programmed cell death characterized by morphological features such as reduction of cell volume, membrane blebbing and formation of apoptotic bodies.^{103,104} It is vital for normal development, homeostasis and functioning of the immune system, and anti-inflammatory reactions. Necrosis is a form of unprogrammed cell death arising from external perturbations with the release of intracellular contents after cell membrane damage, causing inflammation. In the previous chapter, in the case of low frequency uniform magnetic fields, the heat generated by the MNPs was negligible, but the mechanical stress exerted on the cells was only sufficient for the initiation of programmed cell death, apoptosis.

In this chapter, the force exerted onto the HeLa cells by MNPs with different aspect ratios under both uniform and non-uniform magnetic fields configurations was examined. The applied magnetic field generates a magnetic torque on the MNPs, which in turns exerts a force onto the HeLa cells inducing apoptosis or necrosis. Fluorescence microscopy with Acridine Orange and Ethidium Bromide (AO/EB) dual staining attributed the cell death mechanism to be predominantly apoptosis by the uniform magnetic field or field gradient. In an alternating field gradient, the MNPs oscillates with a force sufficient to mechanically rupture the cell membrane. The fluorescence dual staining reveals an increase in necrotic cell population coupled with higher lactate dehydrogenase leakage and greater reduction in cell viability, indicating cell necrosis.

A pair of electromagnetic coils were employed to create a vertically oriented magnetic field with two configurations; uniform magnetic field (i.e. zero gradient) and non-uniform magnetic field with a vertical magnetic field gradient, as shown in Figure 5.1 (a) and (b). An infrared thermometer was used to remotely monitor the cell culture medium and kept at 23.0 ± 0.5 °C, eliminating any contributions from magnetic hyperthermia.

Figure 5.1 Experimental setup of the electromagnetic coils employed to produce a vertically oriented magnetic field with two configurations; uniform magnetic field (i.e. zero gradient) and non-uniform magnetic field with a vertical magnetic field gradient.

5.2 Uniform Magnetic Field

In a uniform magnetic field, the MNP experiences a magnetic torque that rotates it so that its net magnetic moments are aligned to the field direction. The relationship between the magnetic torque and the applied magnetic field is given by:

$$|\boldsymbol{\tau}| = |\mathbf{m} \times \mathbf{B}| = MVB \sin\theta$$

where $\mathbf{m}$ is magnetic moment and M is the magnetization of the MNP in the applied magnetic field $\mathbf{B}$, the volume V of the MNP is given as $\pi(d/2)^2 l$, and θ is the angle between the long axis of the MNP and $\mathbf{B}$. The magnitude of the force acting on the HeLa cells can be obtained by calculating the magnetic torque exerted on the edge of the MNP:

$$F = \frac{2|\boldsymbol{\tau}|}{l} = M \frac{\pi d^2}{2} B \sin\theta$$

The angular dependence of $\mathbf{M}$ was obtained by applying a rotating constant magnitude uniform magnetic field $\mathbf{B}$ with respect to MNP long axis from $\theta = 0$ to 180° . The MNPs were first magnetically saturated by applying a strong magnetic field of 0.1 T. Upon relaxation, an anticlockwise vortex and a clockwise vortex form at the ends of the MNP, and are connected by a third vortex nucleated at the center of the MNP.^{101,197} A weak magnetic field of $B = 0.005$ – 0.025 T was applied, which is inadequate to influence the magnetization configurations of the MNPs, but sufficient for inducing the spatial rotation of the MNPs. This weak field induces a small gyration of the third vortex core about its equilibrium position, with respect to the field direction, as shown in Figure 5.2.

Figure 5.2 MNP magnetization configuration in a weak applied magnetic field from $\theta = 0^\circ$ to 180° , showing the rotation of the third vortex.

The magnetization M/M_s in an applied magnetic field ($B = 0.005\text{--}0.025$ T) with angular dependence ($\theta = 0\text{--}180^\circ$) for MNPs with diameters of $d = 150, 250$ and 350 nm were plotted individually in Figure 5.3, 5.4 and 5.5, respectively. As the weak magnetic field rotates from 0° to 180° , M was observed to increase and saturate when the field was perpendicular ($\theta = 90^\circ$), from which the maximum torque can be obtained.

Figure 5.3 MNP ($d = 150$ nm) magnetization M/M_s in an applied magnetic field ($B = 0.005\text{--}0.025$ T) with angular dependence ($\theta = 0\text{--}180^\circ$).

Figure 5.4 MNP ($d = 250$ nm) magnetization M/M_s in an applied magnetic field ($B = 0.005$ – 0.025 T) with angular dependence ($\theta = 0$ – 180°).

Figure 5.5 MNP ($d = 350$ nm) magnetization M/M_s in an applied magnetic field ($B = 0.005$ – 0.025 T) with angular dependence ($\theta = 0$ – 180°).

The force (F) exerted by the MNPs with respect to applied magnetic field strength (B) for various field angles (θ) shows the maximum force for MNPs with diameters $d = 150$, 250 and 350 nm to be 0.041 pN, 0.11 pN and 0.20 pN, respectively (Figure 5.6). While the physical rupture of the cell membrane requires a force of ~ 100 pN,²⁵⁹⁻²⁶¹ a force of 0.5 pN is sufficient for the activation of mechanosensitive ion channels mediated by mechanical stimuli, leading to apoptosis.^{262,263} To observe the cellular response to the MNPs in an uniform magnetic field, the HeLa cells were exposed to a similar amplitude field of $B = 0.005\text{--}0.025$ T, with a concentration of 0.1 mg/ml of MNPs, for a period of 10 mins each.

Figure 5.6 Magnitude of force exerted by MNPs ($d = 150$, 200 and 350 nm), calculated by the torque exerted on the edge of the MNP, with respect to the angle θ and magnetic field strength $B = 0.005\text{--}0.025$ T.

The cell viability was assessed using the PrestoBlue assay kit, a fluorescent indicator of cell proliferation. The HeLa cells were incubated with PrestoBlue reagent at 37 $^\circ\text{C}$, 5% CO_2 for 2 hours after the magnetic field treatment. The Tecan Infinite M200 PRO Microplate Reader

was used to measure the absorbance values at 570 nm and 600 nm. Each experiment was performed in quadruplicate sets of experimental and control assays in a 96-well microtiter plate. The results were represented as the mean $\pm$ SD. Statistical significance was analyzed using one-way ANOVA and a p value of < 0.05 was considered to be statistically significant.

The cell viabilities were observed to decrease gradually with increasing field strength, and was slightly lower in cells with low aspect ratio MNPs, as shown in Figure 5.7. The results from this assay demonstrate that the magnetic field strength and MNP aspect ratios has no immediate adverse effect, with cell viability greater than 80%. In the absence of magnetic field ($B = 0$ T), the cytotoxicity of the MNPs had minimal effect on cell viability, which was consistent with the known cytotoxicity rates of NiFe MNPs used in hyperthermia research with HeLa cells.²³⁵

Figure 5.7 Cell viability of HeLa cells after uniform magnetic field treatment of $B = 0.005$ – 0.025 T.

5.3 Non-Uniform Magnetic Field

By reversing the polarity of one electromagnetic coil, a non-uniform magnetic field with a field gradient (FG) was obtained, as shown in Figure 5.8. In the FG configuration, the magnetic field was applied in a single direction using DC pulses. A Hirst gauss meter was used to measure the magnetic field strength between a pole gap of 1 cm, with a resolution of 1 Oe, that corresponds to the 96-well microtiter plate with a well depth of 1.03 cm.

Figure 5.8 The plot of a non-uniform magnetic field with a field gradient of $\nabla B = 4.98 - 23.3$ T/m.

In a non-uniform magnetic field, a translational force acts on the MNPs, which is proportional to ∇B . The force acting on the MNPs is given by:

$$F = (\mathbf{m} \cdot \nabla) \mathbf{B} = M \frac{\pi d^2 l}{4} \nabla B$$

The maximum force for $d = 150, 250$ and 350 nm MNPs were calculated to be 0.18 pN, 0.49 pN and 0.97 pN, respectively (Figure 5.9).

Figure 5.9 Magnitude of force acting on MNPs ($d = 150, 200$ and 350 nm), proportional to the magnetic field gradient ∇B .

The cell viability also exhibits similar trends after the FG treatment ($\nabla B = 0\text{--}23.3$ T/m). A significant reduction in cell viability was observed after the HeLa cells were treated with low aspect ratio MNPs at larger gradients, $\nabla B > 12.4$ T/m, as shown in Figure 5.10. The micromagnetic simulations shows that only the low aspect ratios MNPs were able to deliver a force surpassing the limit required to trigger cell apoptosis, which was also substantiated in a 20% increase in efficacy in reduction of cell viability as compared to high aspect ratios MNPs. Previous work has showed that the maximum magnetic torque generated by soft magnetic materials has an upper boundary that is influenced by body geometry and saturation magnetization of the material, independent on the magnitude of the applied magnetic field.²⁶⁴ In contrast, the magnetic torque induced in an unsaturated magnetic material is reliant on the magnitude of the applied field. After reaching magnetic saturation, the magnetic field should be applied at an optimal angle in order to produce the maximum torque, but not by simply

increasing the magnitude of applied field. Therefore, the force exerted by the magnetic torque is limited by an upper boundary, while a greater translational magnetic force can be generated by larger directional derivatives in the applied magnetic field.

Figure 5.10 Cell viability of HeLa cells after non-uniform magnetic field treatment of $\nabla B = 0$ –23.3 T/m ($\pm$ SD, $p < 0.05$).

5.4 Alternating Magnetic Field Gradient

In the alternating magnetic field gradient (AFG), the polarity of the electromagnetic coils was constantly reversed by AC pulses, which inverses the direction of the magnetic field gradient periodically. Thus, the MNPs undergo a lateral back and forth oscillation with a dependency on the frequency and magnitude of the AC pulses. The alternating force induces movement of the MNPs in two opposite directions, downwards into the cells and upwards away from the cells. To examine the effectiveness of the treatments, the HeLa cells were exposed to both FG and AFG configurations for a range of frequencies between 0.17–3.33 Hz over a period of 10 mins each.

5.5 Quantification of Cell Death

The cells were stained with AO/EB for the quantification of live, apoptotic and necrotic cell populations in the control and treated groups. AO is a cell-permeant nucleic acid binding dye that stains both viable and non-viable cells and emits green fluorescence. EB is a sensitive fluorescent dye that only stains cells with damaged membranes and emits red fluorescence. This fluorescence distinction between live, apoptotic and necrotic cells allows AO/EB dual staining to be a qualitative and quantitative evaluation of the cell proliferation and cell death effects in our treatments.¹⁸⁶⁻¹⁸⁹ The cells were incubated with 20 µg/ml AO and 20 µg/ml EB fluorescent dyes at 37 °C, 5% CO₂ for 30 mins, and analyzed under a Nikon Eclipse Ti-S inverted microscope equipped with DAPI-FITC-TRITC filter. A minimum of 300 cells were counted in each well to obtain the ratio between apoptotic and necrotic cells at each frequency between 0.17–3.33 Hz and reported as a percentage of the total number of cell counted. Each experiment was performed in quadruplicate sets of experimental and control assays in a 96-well microtiter plate. The results were represented as the mean $\pm$ SD. Statistical significance was analyzed using one-way ANOVA and a *p* value of < 0.05 was considered to be statistically significant.

Fluorescence microscopy images enabled the detection of changes in cell morphology by the differential uptake of the fluorescent DNA binding dyes. HeLa cells exposed to FG show orange apoptotic cells with fragmented chromatin, cell blebbing and the formation of apoptotic bodies, which implies that the FG configuration predominantly induces apoptosis (Figures 5.11 (a-b)). In contrast, the majority of HeLa cells exposed to AFG have ruptured nuclear and plasma membranes, exhibiting less defined cellular outlines (Figures 5.11 (c-d)). In addition, a uniformly red nucleus with organized structure was observed, indicating necrosis.

Figure 5.11 Optical (left) and fluorescence (right) microscopy images of HeLa with AO/EB dual staining. (a, b) Treatment with magnetic field gradient (FG) shows membrane blebbing, fragmented chromatin and formation of apoptotic body, indicating apoptosis. (c, d) Treatment with alternating magnetic field gradient (AFG) shows an unapparent outline of the cells, rupture of both nuclear and plasma membranes, indicating necrosis.

The quantified data from AO/EB dual staining are presented in Figure 5.12 (a-b). In the case of FG, the percentage of apoptotic cells population saturates with increasing frequencies. The highest percentage of apoptotic cells occurred at 3.33 Hz with 47% of the HeLa cell population. Switching to AFG, the percentage of necrotic cells increased near linearly as the frequency increases, while the percentage of apoptotic cells remains low at <15%. The highest percentage of necrotic cells (58%) was observed after the AFG treatment at 3.33 Hz, with the largest number of oscillation cycles. The *in vitro* study demonstrates that the cell membrane can be effectively disrupted by the lateral oscillations of the MNPs. In comparison, AFG was shown to be more effective at inducing HeLa cell death via largely necrosis.

Figure 5.12 The quantification cell populations in (a) magnetic field gradient (FG) and (b) alternating magnetic field gradient (AFG) groups. The results were displayed as the mean percentage (%) of live, apoptotic and necrotic cell populations ($\pm$ SD, $p < 0.05$).

The cell viability of both magnetic field gradient configurations were determined using the PrestoBlue assay to further investigate the cell death mechanisms. The HeLa cells were incubated with PrestoBlue reagent at 37 °C, 5% CO₂ for 2 hours after the magnetic field treatment. The Tecan Infinite M200 PRO Microplate Reader was used to measure the

absorbance values at 570 nm and 600 nm. Each experiment was performed in quadruplicate sets of experimental and control assays in a 96-well microtiter plate. The results were represented as the mean $\pm$ SD. Statistical significance was analyzed using one-way ANOVA and a p value of < 0.05 was considered to be statistically significant.

The results showed that FG and AFG led to a maximum reduction of 25% and 51% in cell viability, respectively (Figure 5.13). For FG, the cell viability remains relatively constant even when frequency was increased, reflecting a similar trend as the percentage of live cells from AO/EB dual staining. In contrast, AFG showed a more significant decrease in cell viability at all frequencies.

Figure 5.13 Cell viability of HeLa cells after magnetic field gradient (FG) and alternating magnetic field gradient (AFG) treatment for a range of frequencies between 0.17–3.33 Hz ($\pm$ SD, $p < 0.05$).

5.6 Quantification of Caspase-3/7 Activation

Caspase-3/7 are downstream executioner caspases associated with the apoptotic pathway that are responsible for apoptotic chromatin condensation and DNA fragmentation.¹⁹⁰ The activation of caspase-3/7 is thus a hallmark characteristic of apoptosis. The activity of caspase-3/7 in the HeLa cells were measured by CellEvent™ Caspase-3/7 Green Detection Reagent, a nucleic acid binding fluorescent dye. In apoptotic cells with activated caspase-3/7, the DEVD peptide is cleaved, which allows the dye to bind to DNA and emits bright green fluorescence, as shown in Figure 5.14.¹⁹¹⁻¹⁹³ The reagent was diluted into phosphate-buffered saline with 5% fetal bovine serum to a final concentration of 5 μ M. The cell culture medium was removed and the cells were incubated with 100 μ L of diluted reagent at 37 °C, 5% CO₂ for 30 mins. The Tecan Infinite M200 PRO Microplate Reader was used to measure the fluorescence signal at the absorption and emission values of 502 nm and 530 nm, respectively. Each experiment was performed in quadruplicate sets of experimental and control assays in a 96-well microtiter plate. The results were represented as the mean $\pm$ SD. Statistical significance was analyzed using one-way ANOVA and a *p* value of < 0.05 was considered to be statistically significant.

Figure 5.14 Fluorescence microscopy images of caspase-3/7 activated cells showing active caspase in bright green fluorescence.

The percentage of caspase-3/7 activated cells was substantially increased in FG from 9% to 53% at 3.33 Hz, a clear indication of induced apoptosis, as shown in Figure 5.15. The treatment with AFG showed minimal caspase-3/7 activity at all frequencies (< 15%). Therefore, the activation of caspases-3/7 provided further evidence for the induction of apoptosis in response to the treatment with FG.

Figure 5.15 Percentage of caspase-3/7 activated cells after magnetic field gradient (FG) and alternating magnetic field gradient (AFG) treatment for a range of frequencies between 0.17–3.33 Hz ($\pm$ SD, $p < 0.05$).

5.7 Quantification of Cell Membrane Damage

When the cell membrane integrity is compromised or damaged, lactate dehydrogenase (LDH), a cytosolic enzyme present in most cells, is released into the surrounding cell culture medium.^{194–196} The leakage of LDH into the cell culture medium from damaged cells is quantitatively measured by Pierce LDH cytotoxicity assay kit. After the magnetic field treatment, the cell culture supernatant was transferred to a new microplate and mixed with the reaction mixture reagent. After the microplate was incubated at room temperature for 30 mins, the reactions were stopped by adding the stop solution. The LDH activity was determined by

spectrophotometric absorbance at 490 nm. Each experiment was performed in quadruplicate sets of experimental and control assays in a 96-well microtiter plate. The results were represented as the mean $\pm$ SD. Statistical significance was analyzed using one-way ANOVA and a p value of < 0.05 was considered to be statistically significant.

The inherent linearity of the Pierce LDH cytotoxicity assay kit allows it to accurately enumerate the number of necrotic cells in the cell culture medium.¹⁹⁴ The treatment with AFG reveals a continuous rise in LDH levels for increasing frequencies, while the treatment with FG showed insignificant changes in LDH levels at all frequencies ($< 21\%$), as shown in Figure 5.16. At 3.33 Hz, the LDH release was 381% higher than the untreated control cells. This was in accordance with the AO/EB dual staining results, which shows that the percentage of necrotic cells increases only for the treatment with AFG. A 24 hour exposure to MNPs, without any magnetic field treatments, showed no significant increase in extracellular LDH in the cell culture medium. This ensures that the observed extracellular LDH was not due to stimulated LDH secretion by exposure to the MNPs, but due to the loss of cell membrane integrity.²⁶⁵

Figure 5.16 Lactate dehydrogenase (LDH) leakage from the loss of cell membrane integrity in HeLa cells ($\pm$ SD, $p < 0.05$).

5.8 Chapter Summary

The proposed cell death mechanisms for the uniform and non-uniform magnetic fields were summarized in Figure 5.17. In a uniform magnetic field, the MNP experiences a torque that aligns its net magnetic moments to the field direction by Brown relaxation, as shown in Figure 5.17 (a). After the magnetic field was removed, random rotation Brownian motion causes random change in the orientation of MNPs. The uniform magnetic field configuration utilizes the spatial rotation of MNP, which exerts a force onto the cell, activating mechanosensitive ion channels, leading to cell apoptosis.¹³ The activation of mechanosensitive ion channels as a result of cell membrane stretching, leads to the increase in intracellular calcium.^{250,251} This prolonged exposure to high concentration of intracellular calcium triggers cell apoptosis.²⁵²⁻²⁵⁴

In non-uniform magnetic fields, there is a translational force acting on the MNPs which is proportional to the magnetic field gradient. For FG, the MNPs experience a force by the one-dimensional linear magnetic field gradient into the cells. The force exerted by the MNPs on the cell is sufficient for mechanical stimuli mediated cell apoptosis by the activation of mechanosensitive ion channels, but is insufficient to physically rupture the cell membrane, as shown in Figure 5.17 (b). In contrast, AFG oscillates the MNPs laterally to induce mechanical damage to the cell membrane, leading to necrosis, as shown in Figure 5.17 (c).

Figure 5.17 Schemes of the proposed cell death mechanisms for uniform and non-uniform magnetic fields. (a) Uniform magnetic field configuration utilizes the spatial rotation of MNPs to exert a force onto the cell, activating mechanosensitive ion channels, leading to cell apoptosis. (b) In magnetic field gradient (FG), MNPs experience a force by the one-dimensional linear field gradient downwards into the cells, only sufficient for mechanical stimuli mediated cell apoptosis by the activation of mechanosensitive ion channels. (c) In alternating magnetic field gradient (AFG), the lateral oscillation of MNPs can mechanically rupture the cell membranes, triggering necrosis.

Chapter 6 – Conclusion & Future Works

6.1 Conclusion

Cylindrical NiFe MNPs were fabricated using the template-assisted pulsed electrodeposition technique combined with differential chemical etching. Micromagnetic simulations predicted some intriguing dynamics properties of the triple vortex state in MNPs, which were confirmed experimentally by AMR measurements. The magnetization reversal of the MNP occurs via the nucleation of an anticlockwise and a clockwise vortex at the ends of the MNP connected by a third vortex at the center of the MNP, and is followed by the abrupt splitting of the clockwise and anticlockwise vortices.

High SAR values have been reported for the NiFe MNPs, with tunable size and composition, demonstrating high efficiency in heat generation for magnetic hyperthermia when exposed to an alternating magnetic field. Using time-dependent calorimetric measurements, the specific absorption rate and intrinsic loss power of the MNPs were determined and compared with the analytical calculations from micromagnetic simulations and vibrating sample magnetometer measurements. This comparison indicated a qualitative and quantitative agreement on the features of ferromagnetic MNPs, which displayed small SAR values at low magnetic fields and saturation at high magnetic fields that is correlated to the coercive field of the MNPs. A discrepancy on the amplitude of the SAR values between experiments and theory arose from the non-superparamagnetic nature of the MNPs and non-negligible remanent magnetization, which led to unwanted agglomeration due to strong magnetic dipole interactions between neighboring MNPs.

Consequently, the ability to control the initiation of cell apoptosis or necrosis by magneto-actuation of MNPs was demonstrated. A larger magnetic torque can be actuated on the MNP with a biaxial pulsed magnetic field configuration, which led an increase *in vitro*

magneto-actuated cell apoptosis. This was attributed to the orthogonal directional fields that eliminate the reliance on rotational Brownian motion. Comparing MNPs with different aspect ratios, it was found that the high aspect ratio MNPs possessed the highest low field susceptibility and remanent magnetization, thus allowing a larger force to be exerted on the cells and translated into a 12% increase in cell death. In a uniform magnetic field or FG, the force exerted by MNPs on the cells is sufficient to induce cell apoptosis. By introducing an AFG, the force exerted from oscillations of the MNPs was sufficient to physically rupture the cell membrane, leading to necrosis. The LDH activity in the cell culture medium began to increase in parallel to the increase in necrotic cell populations measured by AO/EB dual staining. Hence, this remote magneto-actuation approach is a non-invasive and highly effective treatment method that can inhibit cancer cell proliferation by inducing apoptosis or necrosis.

6.2 Future Works

In recent years, nano-medicine and controlled drug delivery using MNPs have undergone extensive development and progress. The early works on nanoparticles as drug carriers in the 1980s and early 1990s involved liposomes, polymeric micelles, drug-polymer conjugates, polyion complexes and degradable nanoparticles. By the 2000s, nano-medicine and controlled drug delivery have become the pinnacle of biomedical research that focuses on advanced MNPs therapeutics in the $d = \sim 10\text{--}100$ nm range, while receiving great marketing approval and entering clinical trials.^{65,266,267} Early efforts used high frequency magnetic fields as a remote actuation tool, and the focus was on using magnetic hyperthermia to release drugs from heat-sensitive MNPs for cancer treatments. Recently, there has been a shift in focus to magneto-mechanical actuation of MNPs observed in low frequency magnetic fields and in the absence of heat generation. Cho et al. showed that biofunctionalized MNPs can target specific cancer cells, and the magnetic fields can induce cell apoptosis in a remote and non-invasive manner, regardless of the depth of the cells in the body.²⁶⁸

Further demonstrating that the MNPs from this research can achieve magneto-actuation *in vivo* (using small animal model organism) can support the realization of remote magneto-actuation in biological and clinical applications. This will involve developing a biocompatible surface coating that is biofunctionalized with biomolecules that can target specific cell surface receptors.²⁶⁹ By binding the MNPs to cell surfaces, it offers a potential mean to remotely manipulate and control cellular functions on targeted and specific cells. The external magnetic fields, outside the body, can penetrate deep into the body to exert a magnetic force or torque on the MNPs attached to cell surface receptors, as shown in Figure 6.1. Such magneto-actuation of cell surface receptors open the avenue for a diverse range of biomedical applications from tumor targeting to tissue engineering and regenerative medicine to differentiation of stem cells,^{270,271} and control of cellular calcium signaling.²⁷²

An *in vitro* study on colon cancer cells has demonstrated cell apoptosis signaling via a magneto-actuation of surface receptors in a low magnetic field gradient.²⁶⁸ The biofunctionalized MNPs were attached with antibodies that targets cell surface receptors DR4, a death receptor that triggers apoptosis when the clustering of many receptors on the cell surface occurs. The magnetization of the MNPs was induced by the magnetic field and the inter-particle attraction causes the clustering of the DR4 receptors activating a biochemical response signaling the cell apoptosis pathway, inducing colon cancer cell death. This phenomenon was further demonstrated *in vivo* in zebrafish embryos injected with biofunctionalized MNPs coated with biomolecules targeting the apoptosis OTR receptor. This new direction offers exciting opportunities to the research areas of nano-medicine and controlled drug delivery. However, its future success heavily relies on a thorough and cross-disciplinary understanding of the biological, chemical and physical phenomenon governing the behavior of biofunctionalized MNPs in various magnetic field configurations and cellular environment.

Uniform Magnetic Field

Non-Uniform Magnetic Field

Figure 6.1: Illustration of magneto-actuation of cell surface receptors (yellow) in uniform magnetic field (i.e. zero gradient) and non-uniform magnetic field with a vertical magnetic field gradient.

References

- 1 Lévy, M. *et al.* Degradability of superparamagnetic nanoparticles in a model of intracellular environment: follow-up of magnetic, structural and chemical properties. *Nanotechnology* **21**, 395103 (2010).
- 2 Elias, A. & Tsourkas, A. Imaging circulating cells and lymphoid tissues with iron oxide nanoparticles. *ASH Education Program Book* **2009**, 720-726 (2009).
- 3 Vitaliano, G. *et al.* S262. Novel Targeted Clathrin-Based Superparamagnetic Iron Oxide Nanoparticles for CNS Magnetic Resonance Imaging of Dopamine Transporters. *Biological Psychiatry* **83**, S450 (2018).
- 4 Tietze, R. *et al.* Magnetic nanoparticle-based drug delivery for cancer therapy. *Biochem Biophys Res Commun* **468**, 463-470 (2015).
- 5 Chertok, B. *et al.* Iron oxide nanoparticles as a drug delivery vehicle for MRI monitored magnetic targeting of brain tumors. *Biomaterials* **29**, 487-496 (2008).
- 6 Inozemtseva, O. A. *et al.* in *Nanotechnology and Biosensors* (eds Dimitrios P. Nikolelis & Georgia-Paraskevi Nikoleli) 175-192 (Elsevier, 2018).
- 7 Golovin, Y. I. *et al.* Towards nanomedicines of the future: Remote magneto-mechanical actuation of nanomedicines by alternating magnetic fields. *J Control Release* **219**, 43-60 (2015).
- 8 Ling, Y. *et al.* Highly efficient magnetic hyperthermia ablation of tumors using injectable polymethylmethacrylate–Fe₃O₄. *RSC Adv.* **7**, 2913-2918 (2017).
- 9 Zhang, W. *et al.* Novel nanoparticles with Cr³⁺ substituted ferrite for self-regulating temperature hyperthermia. *Nanoscale* **9**, 13929-13937 (2017).
- 10 Cabrera, D. *et al.* Unraveling viscosity effects on the hysteresis losses of magnetic nanocubes. *Nanoscale* **9**, 5094-5101 (2017).
- 11 Kobayashi, T. Cancer hyperthermia using magnetic nanoparticles. *Biotechnology Journal* **6**, 1342-1347 (2011).
- 12 Goldstein, L. S., Dewhirst, M. W., Repacholi, M. & Kheifets, L. Summary, conclusions and recommendations: adverse temperature levels in the human body. *Int J Hyperthermia* **19**, 373-384 (2003).
- 13 Kim, D. H. *et al.* Biofunctionalized magnetic-vortex microdiscs for targeted cancer-cell destruction. *Nat Mater* **9**, 165-171, doi:10.1038/nmat2591 (2010).
- 14 Cheng, Y. *et al.* Rotating magnetic field induced oscillation of magnetic particles for in vivo mechanical destruction of malignant glioma. *J Control Release* **223**, 75-84 (2016).

- 15 Martinez-Banderas, A. I. *et al.* Functionalized magnetic nanowires for chemical and magneto-mechanical induction of cancer cell death. *Sci Rep* **6**, 35786 (2016).
- 16 Fung, A. O., Kapadia, V., Pierstorff, E., Ho, D. & Chen, Y. Induction of Cell Death by Magnetic Actuation of Nickel Nanowires Internalized by Fibroblasts. *The Journal of Physical Chemistry C* **112**, 15085-15088 (2008).
- 17 Weiss, P. La variation du ferromagnétisme avec la température. *Comptes Rendus* **143**, 1136-1149 (1906).
- 18 Williams, H., Bozorth, R. & Shockley, W. Magnetic domain patterns on single crystals of silicon iron. *Physical review* **75**, 155 (1949).
- 19 Parkin, S. *et al.* Magnetically engineered spintronic sensors and memory. *Proceedings of the IEEE* **91**, 661-680, doi:10.1109/JPROC.2003.811807 (2003).
- 20 Tatara, G. & Kohno, H. Theory of current-driven domain wall motion: Spin transfer versus momentum transfer. *Physical review letters* **92**, 086601 (2004).
- 21 Zhang, S. & Li, Z. Roles of nonequilibrium conduction electrons on the magnetization dynamics of ferromagnets. *Physical Review Letters* **93**, 127204 (2004).
- 22 Barnes, S. & Maekawa, S. Current-spin coupling for ferromagnetic domain walls in fine wires. *Physical review letters* **95**, 107204 (2005).
- 23 Slonczewski, J. C. Current-driven excitation of magnetic multilayers. *Journal of Magnetism and Magnetic Materials* **159**, L1-L7 (1996).
- 24 Berger, L. Emission of spin waves by a magnetic multilayer traversed by a current. *Physical Review B* **54**, 9353 (1996).
- 25 Brown Jr, W. *Micromagnetics*. (Wiley, 1963).
- 26 Ivanov, Y. P. & Chubykalo-Fesenko, O. in *Magnetic Nano- and Microwires* (ed Manuel Vázquez) 423-448 (Woodhead Publishing, 2015).
- 27 Coey, J. M. D. *Magnetism and Magnetic Materials*. (Cambridge University Press, 2010).
- 28 Aharoni, A. *Introduction to the Theory of Ferromagnetism*. Vol. 109 (Clarendon Press, 2000).
- 29 O'Handley, R. C. *Modern Magnetic Materials: Principles and Applications*. (Wiley, 1999).
- 30 Donahue, M. J. & Porter, D. G. Interagency Report NISTIR 6376. *National Institute of Standards and Technology, Gaithersburg, MD* (1999).
- 31 Vansteenkiste, A. *et al.* The design and verification of MuMax3. *AIP Advances* **4**, 107133 (2014).

- 32 Miltat, J. E. & Donahue, M. J. Numerical micromagnetics: Finite difference methods. *Handbook of magnetism and advanced magnetic materials* **2**, 14-15 (2007).
- 33 Fidler, J. & Schrefl, T. Micromagnetic modelling-the current state of the art. *Journal of Physics D: Applied Physics* **33**, R135 (2000).
- 34 Rave, W., Ramstöck, K. & Hubert, A. Corners and nucleation in micromagnetics1. *Journal of magnetism and magnetic materials* **183**, 329-333 (1998).
- 35 Abo, G. S. *et al.* Definition of magnetic exchange length. *IEEE Transactions on Magnetics* **49**, 4937-4939 (2013).
- 36 Seberino, C. & Bertram, H. N. Concise, efficient three-dimensional fast multipole method for micromagnetics. *IEEE transactions on magnetics* **37**, 1078-1086 (2001).
- 37 Manfred, F. *Micromagnetism and the microstructure of ferromagnetic solids*. (Cambridge university press, 2003).
- 38 Thiaville, A., Nakatani, Y., Miltat, J. & Suzuki, Y. Micromagnetic understanding of current-driven domain wall motion in patterned nanowires. *Europhysics Letters (EPL)* **69**, 990-996, doi:10.1209/epl/i2004-10452-6 (2005).
- 39 Mougín, A., Cormier, M., Adam, J. P., Metaxas, P. J. & Ferré, J. Domain wall mobility, stability and Walker breakdown in magnetic nanowires. *Europhysics Letters (EPL)* **78**, 57007, doi:10.1209/0295-5075/78/57007 (2007).
- 40 Yan, M., Kákay, A., Gliga, S. & Hertel, R. Beating the Walker Limit with Massless Domain Walls in Cylindrical Nanowires. *Physical Review Letters* **104** (2010).
- 41 Wieser, R., Vedmedenko, E. Y., Weinberger, P. & Wiesendanger, R. Current-driven domain wall motion in cylindrical nanowires. *Physical Review B* **82**, 144430 (2010).
- 42 Ivanov, Y. P., Chuvilin, A., Lopatin, S. & Kosel, J. Modulated magnetic nanowires for controlling domain wall motion: toward 3D magnetic memories. *ACS nano* **10**, 5326-5332 (2016).
- 43 Wieser, R., Vedmedenko, E. Y., Weinberger, P. & Wiesendanger, R. Current-driven domain wall motion in cylindrical nanowires. *Physical Review B* **82** (2010).
- 44 Wieser, R., Nowak, U. & Usadel, K. D. Domain wall mobility in nanowires: Transverse versus vortex walls. *Physical Review B* **69** (2004).
- 45 Hertel, R. & Kirschner, J. Magnetization reversal dynamics in nickel nanowires. *Physica B: Condensed Matter* **343**, 206-210 (2004).
- 46 Hertel, R. Computational micromagnetism of magnetization processes in nickel nanowires. *Journal of Magnetism and Magnetic Materials* **249**, 251-256 (2002).

- 47 Forster, H. *et al.* Micromagnetic simulation of domain wall motion in magnetic nanowires. *Journal of Magnetism and Magnetic Materials* **249**, 181-186 (2002).
- 48 Da Col, S. *et al.* Observation of Bloch-point domain walls in cylindrical magnetic nanowires. *Physical Review B* **89**, 180405 (2014).
- 49 Chandra Sekhar, M. *et al.* Helical domain walls in constricted cylindrical NiFe nanowires. *Applied Physics Letters* **101**, 152406 (2012).
- 50 Da Col, S. *et al.* Nucleation, imaging, and motion of magnetic domain walls in cylindrical nanowires. *Applied Physics Letters* **109**, 062406 (2016).
- 51 Biziere, N. *et al.* Imaging the fine structure of a magnetic domain wall in a Ni nanocylinder. *Nano letters* **13**, 2053-2057 (2013).
- 52 Wong, D. W., Chandra Sekhar, M., Gan, W. L., Purnama, I. & Lew, W. S. Dynamics of three-dimensional helical domain wall in cylindrical NiFe nanowires. *Journal of Applied Physics* **117**, 17A747 (2015).
- 53 Abert, C., Selke, G., Kruger, B. & Drews, A. A Fast Finite-Difference Method for Micromagnetics Using the Magnetic Scalar Potential. *IEEE Transactions on Magnetics* **48**, 1105-1109 (2012).
- 54 Beach, G. S. D., Tsoi, M. & Erskine, J. L. Current-induced domain wall motion. *Journal of Magnetism and Magnetic Materials* **320**, 1272-1281 (2008).
- 55 Yan, M., Andreas, C., Kákay, A., García-Sánchez, F. & Hertel, R. Fast domain wall dynamics in magnetic nanotubes: Suppression of Walker breakdown and Cherenkov-like spin wave emission. *Applied physics letters* **99**, 122505 (2011).
- 56 Dolocan, V. O. Domain wall pinning and interaction in rough cylindrical nanowires. *Applied Physics Letters* **105**, 162401 (2014).
- 57 Beach, G. S., Nistor, C., Knutson, C., Tsoi, M. & Erskine, J. L. Dynamics of field-driven domain-wall propagation in ferromagnetic nanowires. *Nat Mater* **4**, 741-744 (2005).
- 58 Akerman, J., Muñoz, M., Maicas, M. & Prieto, J. L. Stochastic nature of the domain wall depinning in permalloy magnetic nanowires. *Physical Review B* **82** (2010).
- 59 Yang, Y. *et al.* Orientation Mediated Enhancement on Magnetic Hyperthermia of Fe₃O₄Nanodisc. *Advanced Functional Materials* **25**, 812-820 (2015).
- 60 Yang, Y. *et al.* Synthesis of nonstoichiometric zinc ferrite nanoparticles with extraordinary room temperature magnetism and their diverse applications. *Journal of Materials Chemistry C* **1**, 2875 (2013).

- 61 Bellizzi, G. & Bucci, O. M. in *Emerging Electromagnetic Technologies for Brain Diseases Diagnostics, Monitoring and Therapy* (eds Lorenzo Crocco, Irene Karanasiou, Michael L. James, & Raquel Cruz Conceição) 129-191 (Springer International Publishing, 2018).
- 62 Thiesen, B. & Jordan, A. Clinical applications of magnetic nanoparticles for hyperthermia. *International Journal of Hyperthermia* **24**, 467-474 (2008).
- 63 Kim, D.-H. *et al.* Biofunctionalized magnetic-vortex microdiscs for targeted cancer-cell destruction. *Nature materials* **9**, 165-171 (2010).
- 64 Chiriac, H. *et al.* Fe-Cr-Nb-B ferromagnetic particles with shape anisotropy for cancer cell destruction by magneto-mechanical actuation. *Scientific reports* **8**, 11538 (2018).
- 65 Golovin, Y. I. *et al.* Towards nanomedicines of the future: Remote magneto-mechanical actuation of nanomedicines by alternating magnetic fields. *Journal of Controlled Release* **219**, 43-60 (2015).
- 66 Martínez-Banderas, A. I. *et al.* Functionalized magnetic nanowires for chemical and magneto-mechanical induction of cancer cell death. *Scientific reports* **6**, 35786 (2016).
- 67 Maier-Hauff, K. *et al.* Efficacy and safety of intratumoral thermotherapy using magnetic iron-oxide nanoparticles combined with external beam radiotherapy on patients with recurrent glioblastoma multiforme. *Journal of neuro-oncology* **103**, 317-324 (2011).
- 68 Zhang, X. *et al.* Molecular sensing with magnetic nanoparticles using magnetic spectroscopy of nanoparticle Brownian motion. *Biosensors and Bioelectronics* **50**, 441-446 (2013).
- 69 Weaver, J. B. & Kuehlert, E. Measurement of magnetic nanoparticle relaxation time. *Medical physics* **39**, 2765-2770 (2012).
- 70 Rauwerdink, A. M., Giustini, A. J. & Weaver, J. B. Simultaneous quantification of multiple magnetic nanoparticles. *Nanotechnology* **21**, 455101 (2010).
- 71 Reeves, D. B. & Weaver, J. B. Combined Neel and Brown rotational Langevin dynamics in magnetic particle imaging, sensing, and therapy. *Appl Phys Lett* **107**, 223106 (2015).
- 72 Dieckhoff, J., Eberbeck, D., Schilling, M. & Ludwig, F. Magnetic-field dependence of Brownian and Néel relaxation times. *Journal of Applied Physics* **119**, 043903 (2016).
- 73 Reeves, D. B. & Weaver, J. B. Combined Néel and Brown rotational Langevin dynamics in magnetic particle imaging, sensing, and therapy. *Applied physics letters* **107**, 223106 (2015).

- 74 Weaver, J. B. *et al.* Quantification of magnetic nanoparticles with low frequency magnetic fields: compensating for relaxation effects. *Nanotechnology* **24**, 325502 (2013).
- 75 Mamiya, H. & Jeyadevan, B. Hyperthermic effects of dissipative structures of magnetic nanoparticles in large alternating magnetic fields. *Scientific reports* **1**, 157 (2011).
- 76 Adam, M. R., Eric, W. H. & John, B. W. Nanoparticle temperature estimation in combined ac and dc magnetic fields. *Physics in Medicine & Biology* **54**, L51 (2009).
- 77 Rauwerdink, A. M. & Weaver, J. B. Viscous effects on nanoparticle magnetization harmonics. *Journal of Magnetism and Magnetic Materials* **322**, 609-613 (2010).
- 78 Rauwerdink, A. M. & Weaver, J. B. Measurement of molecular binding using the Brownian motion of magnetic nanoparticle probes. *Applied Physics Letters* **96**, 033702 (2010).
- 79 Hervault, A. & Thanh, N. T. K. Magnetic nanoparticle-based therapeutic agents for thermo-chemotherapy treatment of cancer. *Nanoscale* **6**, 11553-11573 (2014).
- 80 Shaterabadi, Z., Nabiyouni, G. & Soleymani, M. Physics responsible for heating efficiency and self-controlled temperature rise of magnetic nanoparticles in magnetic hyperthermia therapy. *Progress in biophysics and molecular biology* (2017).
- 81 Delavari, H., Hosseini, H. R. M. & Wolff, M. Modeling of self-controlling hyperthermia based on nickel alloy ferrofluids: Proposition of new nanoparticles. *Journal of Magnetism and Magnetic Materials* **335**, 59-63 (2013).
- 82 Chen, Y., Bandeira, I., Rowe, D. & Min, G. An exact density formula for substitutional solid solution alloys. *Journal of materials science letters* **13**, 1051-1053 (1994).
- 83 Verde, E. L., Landi, G. T., Gomes, J. d. A., Sousa, M. H. & Bakuzis, A. F. Magnetic hyperthermia investigation of cobalt ferrite nanoparticles: Comparison between experiment, linear response theory, and dynamic hysteresis simulations. *Journal of Applied Physics* **111**, 123902 (2012).
- 84 Hergt, R. *et al.* Physical limits of hyperthermia using magnetite fine particles. *IEEE Transactions on magnetics* **34**, 3745-3754 (1998).
- 85 Rosensweig, R. E. Heating magnetic fluid with alternating magnetic field. *Journal of magnetism and magnetic materials* **252**, 370-374 (2002).
- 86 Maenosono, S. & Saita, S. Theoretical assessment of FePt nanoparticles as heating elements for magnetic hyperthermia. *IEEE transactions on magnetics* (2006).
- 87 Sawyer, C. A. *et al.* Modeling of temperature profile during magnetic thermotherapy for cancer treatment. *Journal of Applied Physics* **105**, 07B320 (2009).

- 88 Carrey, J., Mehdaoui, B. & Respaud, M. Simple models for dynamic hysteresis loop calculations of magnetic single-domain nanoparticles: Application to magnetic hyperthermia optimization. *Journal of Applied Physics* **109**, 083921 (2011).
- 89 Cobianchi, M. *et al.* Experimental determination of the frequency and field dependence of Specific Loss Power in Magnetic Fluid Hyperthermia. *Journal of Magnetism and Magnetic Materials* **444**, 154-160 (2017).
- 90 Atkinson, W. J., Brezovich, I. A. & Chakraborty, D. P. Usable frequencies in hyperthermia with thermal seeds. *IEEE Transactions on Biomedical Engineering*, 70-75 (1984).
- 91 Deatsch, A. E. & Evans, B. A. Heating efficiency in magnetic nanoparticle hyperthermia. *Journal of Magnetism and Magnetic Materials* **354**, 163-172 (2014).
- 92 Johannsen, M. *et al.* Clinical hyperthermia of prostate cancer using magnetic nanoparticles: presentation of a new interstitial technique. *International journal of hyperthermia* **21**, 637-647 (2005).
- 93 van Landeghem, F. K. *et al.* Post-mortem studies in glioblastoma patients treated with thermotherapy using magnetic nanoparticles. *Biomaterials* **30**, 52-57 (2009).
- 94 Maier-Hauff, K. *et al.* Intracranial thermotherapy using magnetic nanoparticles combined with external beam radiotherapy: results of a feasibility study on patients with glioblastoma multiforme. *Journal of neuro-oncology* **81**, 53-60 (2007).
- 95 Goya, G., Grazu, V. & Ibarra, M. Magnetic nanoparticles for cancer therapy. *Current Nanoscience* **4**, 1-16 (2008).
- 96 Pankhurst, Q. A., Connolly, J., Jones, S. K. & Dobson, J. Applications of magnetic nanoparticles in biomedicine. *Journal of physics D: Applied physics* **36**, R167 (2003).
- 97 Liu, D., Wang, L., Wang, Z. & Cuschieri, A. Magnetoporation and magnetolysis of cancer cells via carbon nanotubes induced by rotating magnetic fields. *Nano Lett* **12**, 5117-5121 (2012).
- 98 Wang, B. *et al.* Necrosis of HepG2 cancer cells induced by the vibration of magnetic particles. *Journal of Magnetism and Magnetic Materials* **344**, 193-201 (2013).
- 99 Bouchlaka, M. N. *et al.* Mechanical disruption of tumors by iron particles and magnetic field application results in increased anti-tumor immune responses. *PLoS One* **7**, e48049 (2012).
- 100 Leulmi, S. *et al.* Triggering the apoptosis of targeted human renal cancer cells by the vibration of anisotropic magnetic particles attached to the cell membrane. *Nanoscale* **7**, 15904-15914 (2015).

- 101 Wong, D. W., Gan, W. L., Liu, N. & Lew, W. S. Magneto-actuated cell apoptosis by biaxial pulsed magnetic field. *Scientific Reports* **7**, 10919 (2017).
- 102 Campisi, J. The biology of replicative senescence. *European journal of cancer* **33**, 703-709 (1997).
- 103 Fink, S. L. & Cookson, B. T. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infect Immun* **73**, 1907-1916 (2005).
- 104 Susan, E. Apoptosis: A Review of Programmed Cell Death. *Toxicologic Pathology* **35**, 495-516 (2007).
- 105 Elmore, S. Apoptosis: a review of programmed cell death. *Toxicologic pathology* **35**, 495-516 (2007).
- 106 Proskuryakov, S. Y., Konoplyannikov, A. G. & Gabai, V. L. Necrosis: a specific form of programmed cell death? *Experimental cell research* **283**, 1-16 (2003).
- 107 Ouyang, L. *et al.* Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. *Cell proliferation* **45**, 487-498 (2012).
- 108 Kitanaka, C. & Kuchino, Y. Caspase-independent programmed cell death with necrotic morphology. *Cell death and differentiation* **6**, 508 (1999).
- 109 Miura, M., Zhu, H., Rotello, R., Hartwig, E. A. & Yuan, J. Induction of apoptosis in fibroblasts by IL-1 β -converting enzyme, a mammalian homolog of the *C. elegans* cell death gene *ced-3*. *Cell* **75**, 653-660 (1993).
- 110 Ekert, P. G., Silke, J. & Vaux, D. L. Caspase inhibitors. *Cell Death And Differentiation* **6**, 1081 (1999).
- 111 Li, H. & Yuan, J. Deciphering the pathways of life and death. *Current opinion in cell biology* **11**, 261-266 (1999).
- 112 Nicholson, D. W. & Thornberry, N. A. Caspases: killer proteases. *Trends in biochemical sciences* **22**, 299-306 (1997).
- 113 Elledge, S. J. Cell cycle checkpoints: preventing an identity crisis. *Science* **274**, 1664-1672 (1996).
- 114 Sherr, C. J. Cancer cell cycles. *Science* **274**, 1672-1677 (1996).
- 115 Lengauer, C., Kinzler, K. W. & Vogelstein, B. Genetic instabilities in human cancers. *Nature* **396**, 643 (1998).
- 116 Zhou, B.-B. S. & Elledge, S. J. The DNA damage response: putting checkpoints in perspective. *Nature* **408**, 433 (2000).
- 117 Croce, C. M. Oncogenes and cancer. *New England Journal of Medicine* **358**, 502-511 (2008).

- 118 Ozaki, T. & Nakagawara, A. Role of p53 in cell death and human cancers. *Cancers* **3**, 994-1013 (2011).
- 119 Miyashita, T. & Reed, J. C. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* **80**, 293-300 (1995).
- 120 Vogelstein, B., Lane, D. & Levine, A. J. Surfing the p53 network. *Nature* **408**, 307 (2000).
- 121 Bunz, F. *et al.* Disruption of p53 in human cancer cells alters the responses to therapeutic agents. *The Journal of clinical investigation* **104**, 263-269 (1999).
- 122 Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *cell* **144**, 646-674 (2011).
- 123 Reya, T., Morrison, S. J., Clarke, M. F. & Weissman, I. L. Stem cells, cancer, and cancer stem cells. *nature* **414**, 105 (2001).
- 124 Kim, N. W. *et al.* Specific association of human telomerase activity with immortal cells and cancer. *Science* **266**, 2011-2015 (1994).
- 125 Skloot, R. & Turpin, B. The immortal life of Henrietta Lacks. (2010).
- 126 Annual Registry Report. (Singapore Cancer Registry, 2018).
- 127 Stewart, B. W. & Wild, C. World Cancer Report 2014. Lyon, France: International Agency for Research on Cancer. *World Health Organization*, 630 (2014).
- 128 Duan, X. *et al.* Photodynamic Therapy Mediated by Nontoxic Core–Shell Nanoparticles Synergizes with Immune Checkpoint Blockade To Elicit Antitumor Immunity and Antimetastatic Effect on Breast Cancer. *Journal of the American Chemical Society* **138**, 16686-16695 (2016).
- 129 Schlesinger, M. & Paunovic, M. *Modern electroplating*. Vol. 55 (John Wiley & Sons, 2011).
- 130 Dennis, J. *et al.* Woodhead Publishing Series in Metals and Surface Engineering. *Handbook of Smart Coatings for Materials Protection* (2014).
- 131 Salem, M. S. *et al.* Magnetic characterization of nickel-rich NiFe nanowires grown by pulsed electrodeposition. *Journal of Materials Chemistry* **22**, 8549-8557 (2012).
- 132 Matulis, J. & Sližys, R. On some characteristics of cathodic processes in nickel electrodeposition. *Electrochimica Acta* **9**, 1177-1188 (1964).
- 133 Grimmett, D. L., Schwartz, M. & Nobe, K. Pulsed Electrodeposition of Iron-Nickel Alloys. *Journal of the Electrochemical Society* **137**, 3414-3418 (1990).
- 134 Stępniewski, W. J. & Salerno, M. 321-357 (One Central Press: Manchester, UK, 2014).

- 135 Zaraska, L., Kurowska, E., Sulka, G. D., Senyk, I. & Jaskula, M. The effect of anode surface area on nanoporous oxide formation during anodizing of low purity aluminum (AA1050 alloy). *Journal of Solid State Electrochemistry* **18**, 361-368 (2014).
- 136 Sulka, G. D. Highly ordered anodic porous alumina formation by self-organized anodizing. *Nanostructured materials in electrochemistry* **1**, 1-116 (2008).
- 137 Sulka, G. D. & Stępniewski, W. J. Structural features of self-organized nanopore arrays formed by anodization of aluminum in oxalic acid at relatively high temperatures. *Electrochimica Acta* **54**, 3683-3691 (2009).
- 138 Nielsch, K., Müller, F., Li, A. P. & Gösele, U. Uniform nickel deposition into ordered alumina pores by pulsed electrodeposition. *Advanced Materials* **12**, 582-586 (2000).
- 139 Encinas-Oropesa, A., Demand, M., Piraux, L., Huynen, I. & Ebels, U. Dipolar interactions in arrays of nickel nanowires studied by ferromagnetic resonance. *Physical Review B* **63**, 104415 (2001).
- 140 Yin, A., Li, J., Jian, W., Bennett, A. & Xu, J. Fabrication of highly ordered metallic nanowire arrays by electrodeposition. *Applied Physics Letters* **79**, 1039-1041 (2001).
- 141 Haehnel, V. *et al.* Towards smooth and pure iron nanowires grown by electrodeposition in self-organized alumina membranes. *Acta Materialia* **58**, 2330-2337 (2010).
- 142 Borissov, D., Isik-Uppenkamp, S. & Rohwerder, M. Fabrication of Iron Nanowire Arrays by Electrodeposition into Porous Alumina. *The Journal of Physical Chemistry C* **113**, 3133-3138 (2009).
- 143 Atalay, F. E., Kaya, H., Atalay, S. & Tari, S. Influences of deposition time and pH on magnetic NiFe nanowires fabrication. *Journal of Alloys and Compounds* **469**, 458-463 (2009).
- 144 Rheem, Y., Yoo, B., Koo, B. K. & Myung, N. V. Electrochemical synthesis of compositionally modulated NiFe nanowires. *physica status solidi (a)* **204**, 4021-4024 (2007).
- 145 Tang, X.-T., Wang, G.-C. & Shima, M. Magnetic layer thickness dependence of magnetization reversal in electrodeposited CoNi/Cu multilayer nanowires. *Journal of Magnetism and Magnetic Materials* **309**, 188-196 (2007).
- 146 Vega, V. *et al.* Tuning the magnetic anisotropy of Co–Ni nanowires: comparison between single nanowires and nanowire arrays in hard-anodic aluminum oxide membranes. *Nanotechnology* **23**, 465709 (2012).

- 147 Rosa, W. d. O. d., Vivas, L. G., Pirota, K. R., Asenjo, A. & Vázquez, M. Influence of aspect ratio and anisotropy distribution in ordered CoNi nanowire arrays. *Journal of Magnetism and Magnetic Materials* **324**, 3679-3682 (2012).
- 148 Esmaily, A., Venkatesan, M., Razavian, A. S. & Coey, J. Diameter-modulated ferromagnetic CoFe nanowires. *Journal of Applied Physics* **113**, 17A327 (2013).
- 149 Fodor, P. S., Tsoi, G. M. & Wenger, L. E. Zero magnetization states in electrodeposited Co_{0.45}Fe_{0.55} nanowire arrays. *Journal of applied physics* **93**, 7035-7037 (2003).
- 150 Qin, J. *et al.* Differences in the magnetic properties of Co, Fe, and Ni 250– 300 nm wide nanowires electrodeposited in amorphous anodized alumina templates. *Chemistry of materials* **17**, 1829-1834 (2005).
- 151 Atalay, S., Kaya, H., Atalay, F. & Aydogmus, E. Magnetoimpedance effects in a CoNiFe nanowire array. *Journal of Alloys and Compounds* **561**, 71-75 (2013).
- 152 Atalay, F. *et al.* The effect of back electrode on the formation of electrodeposited CoNiFe magnetic nanotubes and nanowires. *Applied Surface Science* **256**, 2414-2418 (2010).
- 153 Li, P. & Chu, P. in *Thin Film Coatings for Biomaterials and Biomedical Applications* 3-28 (Elsevier, 2016).
- 154 Mattox, D. M. *Handbook of physical vapor deposition (PVD) processing*. (William Andrew, 2010).
- 155 Hung, L., Zheng, L. & Blanton, T. Epitaxial growth of MgO on (100) GaAs using ultrahigh vacuum electron-beam evaporation. *Applied Physics Letters* **60**, 3129-3131 (1992).
- 156 Sulka, G. D. Highly ordered anodic porous alumina formation by self-organized anodizing. *Nanostructured materials in electrochemistry*, 1-116 (2008).
- 157 Wasa, K. in *Handbook of Sputtering Technology (Second Edition)* 41-75 (Elsevier, 2012).
- 158 McClanahan, E. D. & Laegreid, N. in *Sputtering by Particle Bombardment III: Characteristics of Sputtered Particles, Technical Applications* (eds Rainer Behrisch & Klaus Wittmaack) 339-377 (Springer Berlin Heidelberg, 1991).
- 159 Stuart, R. & Wehner, G. Sputtering yields at very low bombarding ion energies. *Journal of Applied Physics* **33**, 2345-2352 (1962).
- 160 Holland, L. Vacuum deposition of thin films. *London: Chapman & Hall, 1970* (1970).
- 161 Wehner, G. K. Controlled sputtering of metals by low-energy Hg ions. *Physical Review* **102**, 690 (1956).

- 162 Albuquerque, N. M. *Photolithography : a manual*. (Tamarind Institute, c2002., 2002).
- 163 Hayward, T. *et al*. Direct imaging of domain-wall interactions in Ni 80 Fe 20 planar nanowires. *Physical Review B* **81**, 020410 (2010).
- 164 Park, H. S., Baskin, J. S. & Zewail, A. H. 4D Lorentz electron microscopy imaging: magnetic domain wall nucleation, reversal, and wave velocity. *Nano letters* **10**, 3796-3803 (2010).
- 165 Meier, G. *et al*. Direct imaging of stochastic domain-wall motion driven by nanosecond current pulses. *Physical review letters* **98**, 187202 (2007).
- 166 Nguyen, V. *et al*. Detection of domain-wall position and magnetization reversal in nanostructures using the magnon contribution to the resistivity. *Physical review letters* **107**, 136605 (2011).
- 167 Nguyen, V. *et al*. Magnon magnetoresistance of NiFe nanowires: Size dependence and domain wall detection. *Applied Physics Letters* **99**, 262504 (2011).
- 168 Ebels, U., Radulescu, A., Henry, Y., Piraux, L. & Ounadjela, K. Spin accumulation and domain wall magnetoresistance in 35 nm Co wires. *Physical review letters* **84**, 983 (2000).
- 169 Hayashi, M. *et al*. Influence of current on field-driven domain wall motion in permalloy nanowires from time resolved measurements of anisotropic magnetoresistance. *Physical review letters* **96**, 197207 (2006).
- 170 Ohgai, T. *et al*. Template synthesis and magnetoresistance property of Ni and Co single nanowires electrodeposited into nanopores with a wide range of aspect ratios. *Journal of Physics D: Applied Physics* **36**, 3109 (2003).
- 171 Evans, P., Yi, G. & Schwarzacher, W. Current perpendicular to plane giant magnetoresistance of multilayered nanowires electrodeposited in anodic aluminum oxide membranes. *Applied Physics Letters* **76**, 481-483 (2000).
- 172 Piraux, L. *et al*. Giant magnetoresistance in magnetic multilayered nanowires. *Applied Physics Letters* **65**, 2484-2486 (1994).
- 173 Wong, D. *et al*. Current-induced three-dimensional domain wall propagation in cylindrical NiFe nanowires. *Journal of Applied Physics* **119**, 153902 (2016).
- 174 Smith, D. O. Development of a Vibrating-Coil Magnetometer. *Review of Scientific Instruments* **27**, 261-268 (1956).
- 175 Zieba, A. & Foner, S. Detection coil, sensitivity function, and sample geometry effects for vibrating sample magnetometers. *Review of Scientific Instruments* **53**, 1344-1354 (1982).

- 176 Thomas, L. *et al.* Oscillatory dependence of current-driven magnetic domain wall motion on current pulse length. *Nature* **443**, 197-200 (2006).
- 177 Schneider, M. L., Gerrits, T., Kos, A. B. & Silva, T. J. Gyromagnetic damping and the role of spin-wave generation in pulsed inductive microwave magnetometry. *Applied Physics Letters* **87**, 072509 (2005).
- 178 Eltschka, M. *et al.* Nonadiabatic Spin Torque Investigated Using Thermally Activated Magnetic Domain Wall Dynamics. *Physical Review Letters* **105** (2010).
- 179 Heyne, L. *et al.* Direct Determination of Large Spin-Torque Nonadiabaticity in Vortex Core Dynamics. *Physical Review Letters* **105** (2010).
- 180 Pollard, S. D., Huang, L., Buchanan, K. S., Arena, D. A. & Zhu, Y. Direct dynamic imaging of non-adiabatic spin torque effects. *Nat Commun* **3**, 1028 (2012).
- 181 Freshney, R. I. *Culture of animal cells: a manual of basic technique and specialized applications*. (John Wiley & Sons, 2015).
- 182 Lindl, T. & Steubing, R. *Atlas of living cell cultures*. (John Wiley & Sons, 2013).
- 183 Alberts, B. *et al.* Molecular Biology of the Cell. (2002).
- 184 Stokes, G. G. XXX. On the change of refrangibility of light. *Philosophical Transactions of the Royal Society of London* **142**, 463-562 (1852).
- 185 Renvoizé, C., Biola, A., Pallardy, M. & Bréard, J. Apoptosis: Identification of dying cells. *Cell Biology and Toxicology* **14**, 111-120, doi:10.1023/a:1007429904664 (1998).
- 186 Galluzzi, L. *et al.* Guidelines for the use and interpretation of assays for monitoring cell death in higher eukaryotes. *Cell death and differentiation* **16**, 1093 (2009).
- 187 Habel, N. *et al.* Zinc chelation: a metallothionein 2A's mechanism of action involved in osteosarcoma cell death and chemotherapy resistance. *Cell death & disease* **4**, e874 (2013).
- 188 Hong, J. & Wu, J. Induction of apoptotic death in cells via Bad gene expression by infectious pancreatic necrosis virus infection. *Cell death and differentiation* **9**, 113 (2002).
- 189 Bezabeh, T., Mowat, M., Jarolim, L., Greenberg, A. & Smith, I. Detection of drug-induced apoptosis and necrosis in human cervical carcinoma cells using ¹H NMR spectroscopy. *Cell death and differentiation* **8**, 219 (2001).
- 190 Porter, A. G. & Jänicke, R. U. Emerging roles of caspase-3 in apoptosis. *Cell death and differentiation* **6**, 99 (1999).
- 191 Gelles, J. D. & Chipuk, J. E. Robust high-throughput kinetic analysis of apoptosis with real-time high-content live-cell imaging. *Cell death & disease* **8**, e2758 (2017).

- 192 Kiefmann, M. *et al.* IDH3 mediates apoptosis of alveolar epithelial cells type 2 due to mitochondrial Ca²⁺ uptake during hypocapnia. *Cell death & disease* **8**, e3005 (2017).
- 193 Huang, T.-C., Lee, J.-F. & Chen, J.-Y. Pardaxin, an antimicrobial peptide, triggers caspase-dependent and ROS-mediated apoptosis in HT-1080 cells. *Marine drugs* **9**, 1995-2009 (2011).
- 194 Chan, F. K., Moriwaki, K. & De Rosa, M. J. Detection of necrosis by release of lactate dehydrogenase activity. *Methods Mol Biol* **979**, 65-70 (2013).
- 195 Nair, B. G. *et al.* Aptamer conjugated magnetic nanoparticles as nanosurgeons. *Nanotechnology* **21**, 455102 (2010).
- 196 Xie, X., Wang, S. S., Wong, T. C. S. & Fung, M. C. Genistein promotes cell death of ethanol-stressed HeLa cells through the continuation of apoptosis or secondary necrosis. *Cancer cell international* **13**, 63 (2013).
- 197 Gan, W. L. *et al.* Multi-vortex states in magnetic nanoparticles. *Applied Physics Letters* **105**, 152405 (2014).
- 198 Salem, M. S. *et al.* Magnetic properties of cylindrical diameter modulated Ni₈₀Fe₂₀ nanowires: interaction and coercive fields. *Nanoscale* **5**, 3941-3947 (2013).
- 199 Pereira, A., Palma, J. L., Vazquez, M., Denardin, J. C. & Escrig, J. A soft/hard magnetic nanostructure based on multisegmented CoNi nanowires. *Phys Chem Chem Phys* **17**, 5033-5038 (2015).
- 200 Qin, W., Chen, Z., Huang, P. & Zhuang, Y. Crystal lattice expansion of nanocrystalline materials. *Journal of alloys and compounds* **292**, 230-232 (1999).
- 201 Matlosz, M. Competitive Adsorption Effects in the Electrodeposition of Iron-Nickel Alloys. *Journal of the Electrochemical Society* **140**, 2272-2279 (1993).
- 202 Llavona, Á., Pérez, L., Sánchez, M. C. & de Manuel, V. Enhancement of anomalous codeposition in the synthesis of Fe–Ni alloys in nanopores. *Electrochimica Acta* **106**, 392-397 (2013).
- 203 Dragos, O., Chiriac, H., Lupu, N., Grigoras, M. & Tabakovic, I. Anomalous codeposition of fcc NiFe nanowires with 5–55% Fe and their morphology, crystal structure and magnetic properties. *Journal of The Electrochemical Society* **163**, D83-D94 (2016).
- 204 Scimeca, M., Bischetti, S., Lamsira, H. K., Bonfiglio, R. & Bonanno, E. Energy Dispersive X-ray (EDX) microanalysis: A powerful tool in biomedical research and diagnosis. *European journal of histochemistry: EJH* **62** (2018).

- 205 Morgan, A. J. X-ray microanalysis in electron microscopy for biologists. *Royal Microscopical Society Microscopy Handbook* **5**, 1-79 (1985).
- 206 Cowburn, R., Koltsov, D., Adeyeye, A., Welland, M. & Tricker, D. Single-domain circular nanomagnets. *Physical Review Letters* **83**, 1042 (1999).
- 207 Van Waeyenberge, B. *et al.* Magnetic vortex core reversal by excitation with short bursts of an alternating field. *Nature* **444**, 461-464 (2006).
- 208 Luo, L. *et al.* Hydrogen peroxide biosensor based on horseradish peroxidase immobilized on chitosan-wrapped NiFe₂O₄ nanoparticles. *Microchimica Acta* **174**, 55-61 (2011).
- 209 Luo, L. *et al.* Amperometric glucose biosensor based on NiFe₂O₄ nanoparticles and chitosan. *Sensors and Actuators B: Chemical* **145**, 293-298 (2010).
- 210 Zhou, S. *et al.* Development of multifunctional folate-poly (ethylene glycol)-chitosan-coated Fe₃O₄ nanoparticles for biomedical applications. *Macromolecular Research* **22**, 58-66 (2014).
- 211 Rana, S., Gallo, A., Srivastava, R. & Misra, R. On the suitability of nanocrystalline ferrites as a magnetic carrier for drug delivery: functionalization, conjugation and drug release kinetics. *Acta Biomaterialia* **3**, 233-242 (2007).
- 212 Sivakumar, P., Ramesh, R., Ramanand, A., Ponnusamy, S. & Muthamizhchelvan, C. Synthesis and characterization of nickel ferrite magnetic nanoparticles. *Materials Research Bulletin* **46**, 2208-2211 (2011).
- 213 Sivakumar, P., Ramesh, R., Ramanand, A., Ponnusamy, S. & Muthamizhchelvan, C. Preparation of sheet like polycrystalline NiFe₂O₄ nanostructure with PVA matrices and their properties. *Materials Letters* **65**, 1438-1440 (2011).
- 214 Tomitaka, A., Koshi, T., Hatsugai, S., Yamada, T. & Takemura, Y. Magnetic characterization of surface-coated magnetic nanoparticles for biomedical application. *Journal of magnetism and magnetic materials* **323**, 1398-1403 (2011).
- 215 Kodama, R. H., Berkowitz, A. E., McNiff Jr, E. & Foner, S. Surface spin disorder in NiFe₂O₄ nanoparticles. *Physical Review Letters* **77**, 394 (1996).
- 216 Maaz, K. *et al.* Synthesis and magnetic characterization of nickel ferrite nanoparticles prepared by co-precipitation route. *Journal of Magnetism and Magnetic Materials* **321**, 1838-1842 (2009).
- 217 Ayala, V., Herrera, A. P., Latorre-Esteves, M., Torres-Lugo, M. & Rinaldi, C. Effect of surface charge on the colloidal stability and in vitro uptake of carboxymethyl

- dextran-coated iron oxide nanoparticles. *Journal of nanoparticle research* **15**, 1874 (2013).
- 218 Khot, V., Salunkhe, A., Thorat, N., Ningthoujam, R. & Pawar, S. Induction heating studies of dextran coated MgFe_2O_4 nanoparticles for magnetic hyperthermia. *Dalton Transactions* **42**, 1249-1258 (2013).
- 219 Phadatare, M., Khot, V., Salunkhe, A., Thorat, N. & Pawar, S. Studies on polyethylene glycol coating on NiFe_2O_4 nanoparticles for biomedical applications. *Journal of Magnetism and Magnetic Materials* **324**, 770-772 (2012).
- 220 Islam, M. N., Abbas, M. & Kim, C. Synthesis of monodisperse and high moment nickel-iron (NiFe) nanoparticles using modified polyol process. *Current Applied Physics* **13**, 2010-2013 (2013).
- 221 Sertkol, M., Köseoğlu, Y., Baykal, A., Kavas, H. & Başaran, A. Synthesis and magnetic characterization of $\text{Zn}_{0.6}\text{Ni}_{0.4}\text{Fe}_2\text{O}_4$ nanoparticles via a polyethylene glycol-assisted hydrothermal route. *Journal of Magnetism and Magnetic Materials* **321**, 157-162 (2009).
- 222 Liu, X. L. *et al.* Optimization of surface coating on Fe_3O_4 nanoparticles for high performance magnetic hyperthermia agents. *Journal of Materials Chemistry* **22**, 8235-8244 (2012).
- 223 Lee, D. H. *et al.* Enhanced cellular uptake of silica-coated magnetite nanoparticles compared with peg-coated ones in stem cells. *Journal of nanoscience and nanotechnology* **15**, 5512-5519 (2015).
- 224 Jang, D.-H. *et al.* Induced Heat Property of Polyethyleneglycol-Coated Iron Oxide Nanoparticles with Dispersion Stability for Hyperthermia. *Journal of nanoscience and nanotechnology* **13**, 6098-6102 (2013).
- 225 Yu, W. W. *et al.* Forming biocompatible and nonaggregated nanocrystals in water using amphiphilic polymers. *Journal of the American Chemical Society* **129**, 2871-2879 (2007).
- 226 Sperling, R. A., Pellegrino, T., Li, J. K., Chang, W. H. & Parak, W. J. Electrophoretic separation of nanoparticles with a discrete number of functional groups. *Advanced Functional Materials* **16**, 943-948 (2006).
- 227 Torchilin, V. P. Recent advances with liposomes as pharmaceutical carriers. *Nature reviews Drug discovery* **4**, 145 (2005).
- 228 Dubertret, B. *et al.* In vivo imaging of quantum dots encapsulated in phospholipid micelles. *Science* **298**, 1759-1762 (2002).

- 229 Abbas, M. *et al.* One-pot synthesis of high magnetization air-stable FeCo nanoparticles by modified polyol method. *Materials Letters* **91**, 326-329 (2013).
- 230 Kura, H., Takahashi, M. & Ogawa, T. Synthesis of Monodisperse Iron Nanoparticles with a High Saturation Magnetization Using an Fe (CO) x - Oleylamine Reacted Precursor. *The Journal of Physical Chemistry C* **114**, 5835-5838 (2010).
- 231 Nahrwold, G. *et al.* Structural, magnetic, and transport properties of Permalloy for spintronic experiments. *Journal of Applied Physics* **108**, 013907 (2010).
- 232 Chen, Y., Luo, X., Yue, G.-H., Luo, X. & Peng, D.-L. Synthesis of iron–nickel nanoparticles via a nonaqueous organometallic route. *Materials Chemistry and Physics* **113**, 412-416 (2009).
- 233 Abbas, M. *et al.* Size-controlled high magnetization CoFe₂O₄ nanospheres and nanocubes using rapid one-pot sonochemical technique. *Ceramics international* **40**, 3269-3276 (2014).
- 234 Abbas, M., Islam, M. N., Rao, B. P., Abdel-Hamed, M. & Kim, C. Facile one-pot chemical approach for synthesis of monodisperse chain-like superparamagnetic maghemite (γ -Fe₂O₃) nanoparticles. *Journal of Industrial and Engineering Chemistry* **31**, 43-46 (2015).
- 235 Tomitaka, A., Hirukawa, A., Yamada, T., Morishita, S. & Takemura, Y. Biocompatibility of various ferrite nanoparticles evaluated by in vitro cytotoxicity assays using HeLa cells. *Journal of Magnetism and Magnetic Materials* **321**, 1482-1484 (2009).
- 236 Mehdaoui, B. *et al.* Optimal size of nanoparticles for magnetic hyperthermia: a combined theoretical and experimental study. *Advanced Functional Materials* **21**, 4573-4581 (2011).
- 237 Serantes, D. *et al.* Influence of dipolar interactions on hyperthermia properties of ferromagnetic particles. *Journal of Applied Physics* **108**, 073918 (2010).
- 238 Klokkenburg, M. *et al.* In situ imaging of field-induced hexagonal columns in magnetite ferrofluids. *Physical review letters* **97**, 185702 (2006).
- 239 Liu, C.-M. *et al.* Magnetic nanochains of metal formed by assembly of small nanoparticles. *Chemical communications*, 2726-2727 (2004).
- 240 Mehdaoui, B. *et al.* Large specific absorption rates in the magnetic hyperthermia properties of metallic iron nanocubes. *Journal of Magnetism and Magnetic Materials* **322**, L49-L52 (2010).

- 241 Iacovita, C. *et al.* Polyethylene glycol-mediated synthesis of cubic iron oxide nanoparticles with high heating power. *Nanoscale research letters* **10**, 391 (2015).
- 242 Petros, R. A. & DeSimone, J. M. Strategies in the design of nanoparticles for therapeutic applications. *Nature reviews Drug discovery* **9**, 615 (2010).
- 243 Maeda, H., Greish, K. & Fang, J. in *Polymer therapeutics II* 103-121 (Springer, 2006).
- 244 Matsumura, Y. & Maeda, H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer research* **46**, 6387-6392 (1986).
- 245 Fröhlich, E. *et al.* Comparison of two in vitro systems to assess cellular effects of nanoparticles-containing aerosols. *Toxicology in Vitro* **27-360**, 409-417 (2013).
- 246 Vitol, E. *et al.* Optical transmission modulation by disk-shaped ferromagnetic particles. *Journal of Applied Physics* **111**, 07A945 (2012).
- 247 Cooper, G. (USA: Sinauer Associates, 2000).
- 248 Gupta, A. K. & Gupta, M. Cytotoxicity suppression and cellular uptake enhancement of surface modified magnetic nanoparticles. *Biomaterials* **26**, 1565-1573 (2005).
- 249 Gupta, A. K. & Curtis, A. S. Lactoferrin and ceruloplasmin derivatized superparamagnetic iron oxide nanoparticles for targeting cell surface receptors. *Biomaterials* **25**, 3029-3040 (2004).
- 250 Sigurdson, W., Ruknudin, A. & Sachs, F. Calcium imaging of mechanically induced fluxes in tissue-cultured chick heart: role of stretch-activated ion channels. *American Journal of Physiology-Heart and Circulatory Physiology* **262**, H1110-H1115 (1992).
- 251 Xian-Cheng, Y. & Sachs, F. Block of Stretch Activated Ion Channels in *Xenopus* Oocytes by Gadolinium and Calcium Ions. *Science* **243**, 1068 (1989).
- 252 Clapham, D. E. Calcium signaling. *Cell* **131**, 1047-1058 (2007).
- 253 Mattson, M. P. & Chan, S. L. Calcium orchestrates apoptosis. *Nature cell biology* **5**, 1041-1043 (2003).
- 254 Boehning, D. *et al.* Cytochrome c binds to inositol (1, 4, 5) trisphosphate receptors, amplifying calcium-dependent apoptosis. *Nature cell biology* **5**, 1051 (2003).
- 255 Baskić, D., Popović, S., Ristić, P. & Arsenijević, N. N. Analysis of cycloheximide-induced apoptosis in human leukocytes: fluorescence microscopy using annexin V/propidium iodide versus acridin orange/ethidium bromide. *Cell biology international* **30**, 924-932 (2006).

- 256 Vitol, E. A., Novosad, V. & Rozhkova, E. A. Multifunctional ferromagnetic disks for modulating cell function. *IEEE transactions on magnetics* **48**, 3269-3274 (2012).
- 257 Guslienko, K. Y., Novosad, V., Otani, Y., Shima, H. & Fukamichi, K. Field evolution of magnetic vortex state in ferromagnetic disks. *Applied Physics Letters* **78**, 3848-3850 (2001).
- 258 Leulmi, S. *et al.* Comparison of dispersion and actuation properties of vortex and synthetic antiferromagnetic particles for biotechnological applications. *Applied Physics Letters* **103**, 132412 (2013).
- 259 Sen, S., Subramanian, S. & Discher, D. E. Indentation and adhesive probing of a cell membrane with AFM: theoretical model and experiments. *Biophys J* **89**, 3203-3213 (2005).
- 260 Muller, D. J., Helenius, J., Alsteens, D. & Dufrene, Y. F. Force probing surfaces of living cells to molecular resolution. *Nat Chem Biol* **5**, 383-390 (2009).
- 261 Afrin, R., Yamada, T. & Ikai, A. Analysis of force curves obtained on the live cell membrane using chemically modified AFM probes. *Ultramicroscopy* **100**, 187-195 (2004).
- 262 Baumgarten, C. M. Origin of mechanotransduction: stretch-activated ion channels. (2013).
- 263 Powers, R. J. *et al.* The local forces acting on the mechanotransduction channel in hair cell stereocilia. *Biophys J* **106**, 2519-2528 (2014).
- 264 Abbott, J. J., Ergeneman, O., Kummer, M. P., Hirt, A. M. & Nelson, B. J. Modeling magnetic torque and force for controlled manipulation of soft-magnetic bodies. *IEEE Transactions on Robotics* **23**, 1247-1252 (2007).
- 265 Xu, Y. *et al.* Exposure to TiO₂ nanoparticles increases Staphylococcus aureus infection of HeLa cells. *Journal of nanobiotechnology* **14**, 34 (2016).
- 266 Williams, H. D. *et al.* Strategies to address low drug solubility in discovery and development. *Pharmacological reviews* **65**, 315-499 (2013).
- 267 Bamrungsap, S. *et al.* Nanotechnology in therapeutics: a focus on nanoparticles as a drug delivery system. *Nanomedicine* **7**, 1253-1271 (2012).
- 268 Cho, M. H. *et al.* A magnetic switch for the control of cell death signalling in in vitro and in vivo systems. *Nature Materials* **11**, 1038 (2012).
- 269 Dobson, J. Remote control of cellular behaviour with magnetic nanoparticles. *Nature Nanotechnology* **3**, 139 (2008).

- 270 Dobson, J., Cartmell, S. H., Keramane, A. & El Haj, A. J. Principles and design of a novel magnetic force mechanical conditioning bioreactor for tissue engineering, stem cell conditioning, and dynamic in vitro screening. *IEEE transactions on nanobioscience* **5**, 173-177 (2006).
- 271 Kanczler, J. M. *et al.* Controlled differentiation of human bone marrow stromal cells using magnetic nanoparticle technology. *Tissue engineering Part A* **16**, 3241-3250 (2010).
- 272 Mannix, R. J. *et al.* Nanomagnetic actuation of receptor-mediated signal transduction. *Nature nanotechnology* **3**, 36 (2008).

Publications

D. W. Wong, W. L. Gan, Y. K. Teo and W. S. Lew, Interplay of cell death signaling pathways mediated by alternating magnetic field gradient, *Cell Death Discovery* **4**, 49 (2018).

D. W. Wong, W. L. Gan, N. Liu and W. S. Lew, Magneto-actuated cell apoptosis by biaxial pulsed magnetic field, *Scientific Reports* **7**, 10919 (2017).

D. W. Wong, I. Purnama, G. J. Lim, W. L. Gan, M. C. Sekhar and W. S. Lew, Current-induced three-dimensional domain wall propagation in cylindrical NiFe nanowires, *Journal of Applied Physics* **119**, 153902 (2016).

D. W. Wong, M. Chandra Sekhar, W. L. Gan, I. Purnama, and W. S. Lew, Dynamics of three-dimensional helical domain wall in cylindrical nanowires, *Journal of Applied Physics* **117**, 17A747 (2015).

W. L. Gan, M. Chandra Sekhar, D. W. Wong, I. Purnama, S. Y. Chiam, L. M. Wong and W. S. Lew, Multi-vortex states in magnetic nanoparticles, *Applied Physics Letters* **105**, 152405 (2014).

I. Purnama, W. L. Gan, D. W. Wong, and W. S. Lew. Fast current-induced Skyrmion motion in guided track, *Scientific Reports* **5**, 10620 (2015).

Conferences

D. W. Wong, W. L. Gan, Y. K. Teo, and W. S. Lew, Programmable cell death mediated by configurable magnetic fields, *Institute of Physics Meeting*, Singapore (2018).

D. W. Wong, W. L. Gan, and W. S. Lew, Diminished interference of stray field in free layer switching in perpendicular magnetic tunnel junction, *9th International Conference on Materials for Advanced Technologies*, Singapore (2017).

D. W. Wong, W. L. Gan, N. Liu and W. S. Lew, In vitro magneto-mechanical cancer cell destruction efficacy under biaxial DC pulsed magnetic field, *IEEE International Magnetism Conference*, Dublin, Ireland (2017).

D. W. Wong, N. Thiagarajah, H. Yang, S. Ye, H. Cong, A. See and W. S. Lew, Symmetrical free layer switching in magnetic tunnel junctions with perpendicular anisotropy, *GlobalFoundries TECH Forum*, Singapore (2017).

N. Thiagarajah, H. Yang, D. W. Wong, S. Ye, H. Cong, A. See, Reactive Ion etching for STT-MRAM device patterning: Approach to gain interconnect margin, *GlobalFoundries TECH Forum*, Singapore (2017).

D. W. Wong, N. Thiagarajah, H. Yang, S. Ye, H. Cong, A. See and W. S. Lew, Stray field interference of free layer switching in perpendicular magnetic anisotropy magnetic tunnel junction, *IEEE Magnetism Symposium*, Singapore (2016).

D. W. Wong, N. Liu, Y. Yang, W. L. Gan, C. B. Tan, J. Ding and W. S. Lew, Multi-vortex magnetic nanoparticles: A twin-functionalized agent for magnetomechanical cancer-cell destruction and hyperthermia, *International Conference on Magnetism*, Barcelona, Spain (2015).

D. W. Wong, Y. Yang, W. L. Gan, N. Liu, I. Purnama, M. Chandra Sekhar, S. Y. Wong, J. Ding and W. S. Lew, Magnetic nanoparticles for magnetomechanical cell destruction and magnetic hyperthermia agents, *IEEE International Magnetism Conference*, Beijing (2015).

I. Purnama, W. L. Gan, D. W. Wong, W. S. Lew, Guiding current-induced Skyrmion motion, *The 9th International Conference on Computational Physics*, Singapore (2015).

D. W. Wong, M. Chandra Sekhar, W. L. Gan, I. Purnama and W. S. Lew, Dynamics of helical domain wall in cylindrical NiFe nanowires, *59th Magnetism and Magnetic Materials Conference*, Honolulu, Hawaii (2014).

D. W. Wong, W. L. Gan, Y. Yang, M. Chandra Sekhar, I. Purnama, J. Ding and W. S. Lew, Magnetic Nanoparticles for Cancer Treatment, *50th Anniversary IEEE Magnetics Symposium*, Singapore (2014).

D. W. Wong, M. Chandra Sekhar, I. S. Kerk and W. S. Lew, Magnetoresistance of diameter-modulated cylindrical magnetic nanostructures, *IEEE International Magnetics Conference*, Dresden, Germany (2014).

D. W. Wong, M. Chandra Sekhar, W. L. Gan, I. Purnama and W. S. Lew, Helical domain wall dynamics in cylindrical nanowires for 3D-data storage, *The 8th Joint Meeting of Chinese Physicists Worldwide*, Singapore (2014).

W. L. Gan, D. W. Wong, Y. Yang, M. Chandra Sekhar, I. Purnama, J. Ding and W. S. Lew, Magnetic nanoparticles with triple-vortex state, *59th Magnetism and Magnetic Materials Conference*, Honolulu, Hawaii (2014).

W. L. Gan, D. W. Wong, I. S. Kerk, and W.S. Lew, 2D transport of superparamagnetic microbeads on a ferromagnetic hexagonal nanolattice, *The 8th Joint Meeting of Chinese Physicists Worldwide*, Singapore (2014).

W. L. Gan, I. Purnama, D. W. Wong, W. S. Lew, Current-driven Skyrmion dynamics in a memory device, *59th Magnetism and Magnetic Materials Conference*, Honolulu, Hawaii (2014).

W. L. Gan, I. Purnama, D. W. Wong, C. I. Ang, W. S. Lew, High Speed Transport of 1D Skyrmion Gas, *50th Anniversary IEEE Magnetics Symposium*, Singapore (2014).