THE WINSTON CHURCHILL MEMORIAL TRUST OF AUSTRALIA

Report by Alexander E. Dunn

2013 Churchill Fellow

THE LESLIE (LES) J. FLEMING CHURCHILL FELLOWSHIP to study magnetic induction release of cancer drugs at the Royal Institution of Great Britain.

Signed: *Alexander Dunn* Dated: *11/04/2014*

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1 Introduction

The key to good prognosis for cancer patients is early diagnosis and treatment. The Cancer Institute estimates that if cancer is caught in Stage 1, patient survival rate can be as high as 84%, whereas if it is undetected until Stage 4, survival rate falls to 14%.[1] This is because as cancers develop and metastasise, the likelihood of successful treatment or surgery is severely reduced. Presently, one of the most common mechanisms of cancer treatment is the use of drugs - such as chemotherapy – which attack the cancerous cells. However, many of these modern drugs have adverse side effects when they are administered in their current systemic manner.

My project looks at the development of a novel nanomaterial that can address these problems. The purpose of the nanomaterial is to improve our ability to detect cancer in its early stages and subsequently treat the cancer. The nanomaterial can achieve this goal by acting as a Magnetic Resonance Imaging contrast agent and by exhibiting controllable drug delivery – a phenomenon that requires the nanomaterial to store the toxic cancer drugs in an inert state until release is desired at a specific time and location. Controllable drug delivery relies on the use of a stimulus that can be controlled by the administering doctor which initiates the release of these drugs from their inert state at the cancer site. Generally speaking, this nanomaterial consists of a polymer-nanoparticle conjugate. A polymer is a long chain organic molecule made up of many repeating subunits. A nanoparticle is a solid particle with a diameter between 1 and 100 nm.

In this field of research, the use of magnetic induction as a drug release stimulus is gaining much interest, and here lies the core of this project. Magnetic induction is a physical phenomenon in which a magnetic material releases heat in response to the presence of a high frequency alternating magnetic field. This phenomenon allows us to direct an alternating magnetic field to a specific part of the body (that is, the cancer site), and subsequently induce a temperature change within the magnetic nanoparticles located at that site in a desired timeframe. This temperature change can in turn be used to initiate release of the drugs, resulting in temporally and spatially controlled drug delivery. Ultimately, this allows us to target specific sites within the body without damaging healthy tissue.

Cancer is usually detected and characterised using medical imaging techniques such as Magnetic Resonance Imaging (MRI); the quality of the diagnosis is dependent upon the quality of the image. Introduction of a contrast agent that interacts with the physical phenomena of the imaging modality allows us to create a clearer image of the cancer tissue – MRI contrast agents are often associated with magnetic nanomaterials.

The Leslie (Les) J. Fleming Churchill Fellowship allowed me to travel to the United Kingdom and Germany to study magnetic induction for these biomedical applications. Most significantly this involved laboratory research at the Healthcare Biomagnetics Laboratory at the Royal Institution of Great Britain. This also involved attending conferences and meetings with various industry and academic experts in the field around the UK and Germany.

Acknowledgements

I would like to thank May Lim (University of New South Wales) and Cyrille Boyer (University of New South Wales) for their guidance throughout this project. I would like to thank members of the UCL Healthcare and Biomagnetic Laboratories, particularly Prof. Thanh Nguyen, Dr Paul Southern and Dr Stephen Nesbitt for facilitating my laboratory research. I am also grateful to my other collaborators (mentioned herein) for taking the time to meet with me during my travels. Finally the Winston Churchill Memorial Trust and the Fleming family for making this project possible.

2 Executive Summary

2.1 Author

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2.2 Highlights

- Undertook laboratory research at the University College London (UCL) Healthcare and Biomagnetic Laboratory (HBL) at the Royal Institution of Great Britain into the development of magnetic nanoparticles for cancer oriented controllable drug delivery (London, UK).
- Attended the SCI and RSC 2013 McBain Medal Award Meeting (Cambridge, UK).
- Presented project and findings to the UCL HBL research group (London, UK).
- Assisted in *in vivo* hyperthermia studies at the UCL Cancer Institute (London, UK).
- Visited the Berlin Ultrahigh Field Facility (Berlin, Germany).
- Met with a leading commercial nanotechnology company, *MagForce AG* (Berlin, Germany).
- Met with magnetic hyperthermia experts from *NanoTherics* (Staffordshire, UK).
- Presented findings at the International Conference on Nanotechnology in Medicine (NanoMED) 2014 (London, UK).

2.3 Recommendations

Recent advancements in polymer, nanoparticle and hardware design have vastly improved the feasibility of the use of magnetic induction as a stimulus for controllable drug delivery. I recommend the following:

- Wider implementation of the Intrinsic Loss Parameter (ILP) over the Specific Absorption Rate (SAR) by researchers in this field.
- Develop a more concrete understanding of the true limits of magnetic field strength and frequency of an AC magnetic field that can be withstood by humans.

To continue the development of my nanomaterial for controllable drug delivery, further optimization of the magnetic properties of the nanoparticle core is required. Thereafter the project is ready to proceed to *in vitro* cell studies.

2.4 Dissemination and Implementation

- My Churchill Fellowship allowed me to present some of my key research findings at the International Conference on Nanotechnology in Medicine (NanoMED) 2014 and to the UCL Healthcare and Biomagnetics Laboratory research group.
- Upon continuation of my project in Australia, findings will be disseminated in peer-reviewed journals and future conferences.
- The techniques, skills and knowledge that I have developed during my Fellowship (particularly laboratory work) will be directly implemented in to my future research efforts at the University of New South Wales.

3 Programme

25 November 2013 – 14 February 2014, United Kingdom

- Laboratory research at the University College London Healthcare and Biomagnetics Laboratories in the Royal Institution of Great Britain (London).

- Attended the Society of Chemical Industry (SCI) Colloid and Surface Chemistry Group and the Royal Society of Chemistry (RSC) Colloid and Interface Science Group McBain Medal Award Meeting 2013 (Cambridge).

- Presented project and findings to the UCL HBL research group (London, UK).

- Assisted in *in vivo* hyperthermia studies at the University College London (UCL) Cancer Institute for treatment of melanoma (London).

14 February – 20 February 2014, Germany

- Visited the Berlin Ultrahigh Field Facility (BUFF) in the Max-Delbrück-Center for Molecular Medicine (Berlin).

- Met with collaborators in a commercial nanotechnology company, *MagForce AG* (Berlin).

20 February 2013 – 28 February 2014, United Kingdom

- Met with *NanoTherics*, the manufacturer of the *magneTherm* nanoparticle hyperthermia testing system (Staffordshire).

- Attended and presented at the International Conference on Nanotechnology in Medicine at the Royal Free Hospital – University College London (London).

4 Main Body

4.1 Background

4.1.1 Drug Delivery

Drug delivery refers to the controlled release of therapeutic compounds at a specific site within the body in response to a stimulus that we can control. The literature has considered a wide range of stimuli that we can use for these biomedical applications. These stimuli include laser light, cancer tissue pH differentials and alternating magnetic fields.

In this project, I have focused on the use of a technique called 'magnetic induction hyperthermia'. In the past, this technique has involved the use of magnetic fields to generate heat within a magnetic nanomaterial which in turn heats and destroys the diseased tissue[*].[2] Recently, however, researchers have proposed the use of magnetically induced heat to stimulate the release of therapeutic agents that are stored in an inert state in or on the nanoparticles.[3] By directing the magnetic field at the diseased tissue, we can ensure that the toxic drugs are released in a site specific manner. Therefore, the drugs only attack the diseased tissue and consequently we are able to minimize their hazardous side effects.

4.1.2 Physical Principles of Magnetic Induction Hyperthermia

When a magnetic nanomaterial is exposed to an alternating magnetic field, the nanoparticles can produce heat through three main mechanisms: eddy currents, hysteresis losses, and relaxation losses. The significance of each of these mechanisms is largely dependent upon the size of the material.

First, eddy currents are of most significance for larger magnetic or conductive materials. In this mechanism, the magnetic flux associated with the presence of an alternating magnetic field will result in the induction of small, swirling electric currents. Due to electrical resistance, these currents result in the generation of heat within the material. Eddy currents do not make a meaningful contribution to nanoparticle heating.

Second, hysteresis losses contribute a degree of heating within ferromagnetic materials. These materials naturally form 'magnetic domains' which are simply small regions of magnetic

[*] Generally speaking, at temperatures of 40-45 °C the diseased cells are simply damaged, however it has been shown that various anticancer drugs can be simultaneously introduced to improve the treatment efficacy. At temperatures >46 °C the cells experience thermoablation and are completely destroyed.

material that are aligned with the same magnetic moment. When exposed to an alternating magnetic field, the moment of these domains tend to rotate in alignment with the external field; this results in heat generation within the material. These hysteresis losses are not significant when small magnetic fields and/or small nanoparticles are used.

Finally, the most significant source of heat generation in magnetic induction hyperthermia is relaxational losses. When a ferromagnetic material is sufficiently small (< 100 nm) it may exist as a single magnetic domain, and exhibit a property known as superparamagnetism. When a superparamagnetic material is exposed to an alternating magnetic field it may produce heat through Brownian or Néel relaxational losses. Brownian relaxation occurs when the nanoparticles physically rotate within their supporting medium, resulting in the generation of heat. Néel relaxation losses, however, involves rapid change in the magnetization direction, also resulting in the generation of heat.

It is important to note that in many biological environments, the nanoparticles will adhere to the exterior of surrounding cell walls which limits their freedom to exhibit Brownian motion. This consequently reduces their ability to heat up in response to the magnetic field. To compensate for this loss in heating ability, higher nanoparticle concentrations are required. Concentrations on the order of tens to hundreds of mg/mL of iron have been reported.

Classical literature suggests that the overall result of magnetic nanoparticles being exposed to high frequency alternating magnetic fields is generation of a quantity of heat that is proportional to the frequency of the field.[4] This observation, however, has recently been questioned by researchers who have observed that at some frequencies, the heating capabilities of certain nanoparticles ‘resonate’ and exhibit notably better heating capabilities. There currently does not exist a generally accepted explanation for this observation.

4.1.3 Nanoparticle Design

It is well established that particles exhibiting superparamagnetic behavior are ideal for hyperthermia applications because of the ease with which they exhibit relaxational heat losses. It is possible to confer superparamagnetic properties to magnetic materials through reduction in particle size until the coercivity is reduced to zero. Particle size will depend on the synthesis methodology and reaction conditions.

Another area of concern for hyperthermia application is particle agglomeration. Agglomeration refers to the clumping, or aggregation, of the particles. In clinical applications, this can lead to health concerns associated with thrombosis. The main interest in agglomeration

comes from its impact on the nanoparticles' primary function: heating ability. Several research groups have reported that the heating properties of magnetic nanoparticles may be impacted by their agglomeration. There does not currently exist a reliable model or predictive mechanism for the specific impacts of agglomeration on particle heating. Typically, agglomeration is controlled through introduction of a stabilizing agent – usually an organic molecule, such as a polymer chain.

4.1.4 My Research Project

One of the major problems facing many polymer-nanoparticle conjugate designs is undesirable release of drugs prior to the stimulus being switched on. This is unattractive because it results in the drugs attacking healthy tissue, ultimately defeating the purpose of controllable drug delivery. The cause of this problem tends to be the reliance of many nanocomposite designs on Van de Waals forces as a means of storing the drugs within the polymer shell. The weak nature of these bonds results in a tendency for the drugs to uncontrollably 'leak' out of the nanocomposite.

Previously at UNSW, we have developed a system that aims to address this issue by examining the use of covalent bonds to store the drugs which will allow us to ensure that the drugs are attached to the nanocomposite in a reliable and sturdy way, subsequently preventing them from taking action until the bond is broken in response to the stimulus that the administering doctor can control. In this case, the heat from the magnetic induction stimulus encourages decomposition of the bond (equilibrium shifts left), subsequently releasing the drugs in their original form. Specifically, we are looking at the use of Schiff Base bonds (outlined in Figure 1). This mechanism relies on the reaction between an amine group present on many anti-cancer drugs and an aldehyde/ketone group present on my custom polymer.

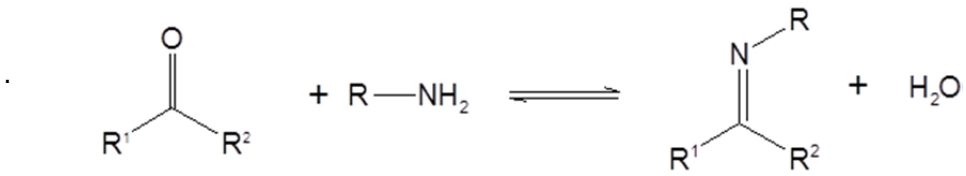

Figure 1: The Schiff Base bond formation mechanism. An amine group (present on many anti-cancer drugs) reacts with the C=O bond in an aldehyde or ketone (built in to my custom polymer) to create a C=N covalent bond. This linkage provides anchorage point for the drug (R-NH_2).

Another problem facing many polymer-nanoparticle composite designs is cancer-site specificity. Whilst most external stimuli (such as alternating magnetic fields) are relatively site-

specific (that is, they can be used to target a particular part of the body), the specificity may be improved by designing a system that also responds to cancer cell-specific properties. For example, healthy tissue is naturally buffered at physiological pH (~7.4) by the body's homeostatic mechanisms. It is well-established, however, that cancer cells tend to be lower in pH (acidic) due to poor lymphatic drainage.[5] Therefore, a system that releases drugs only under the acidic conditions of cancer cells can be said to exhibit passive-targeting properties. That is, even if the nanocomposite is erroneously exposed to the alternating magnetic field stimulus outside the cancer site, the drugs will still not be released. Conveniently, the Schiff Base bond tends to degrade at low pH by favouring the reverse reaction. Therefore, the Schiff base bonds can be used to ensure that the drugs are only released at the cancer site.

By combining the alternating magnetic field and pH stimuli, we are able to create a drug carrier that allows us to achieve high spatial and temporal control over the drug release mechanism. High spatial control from the cancer-specific acidic conditions, and temporal control from the magnetic induction stimulus that we can switch on and off at will. An outline of the mechanism is included in Figure 2.

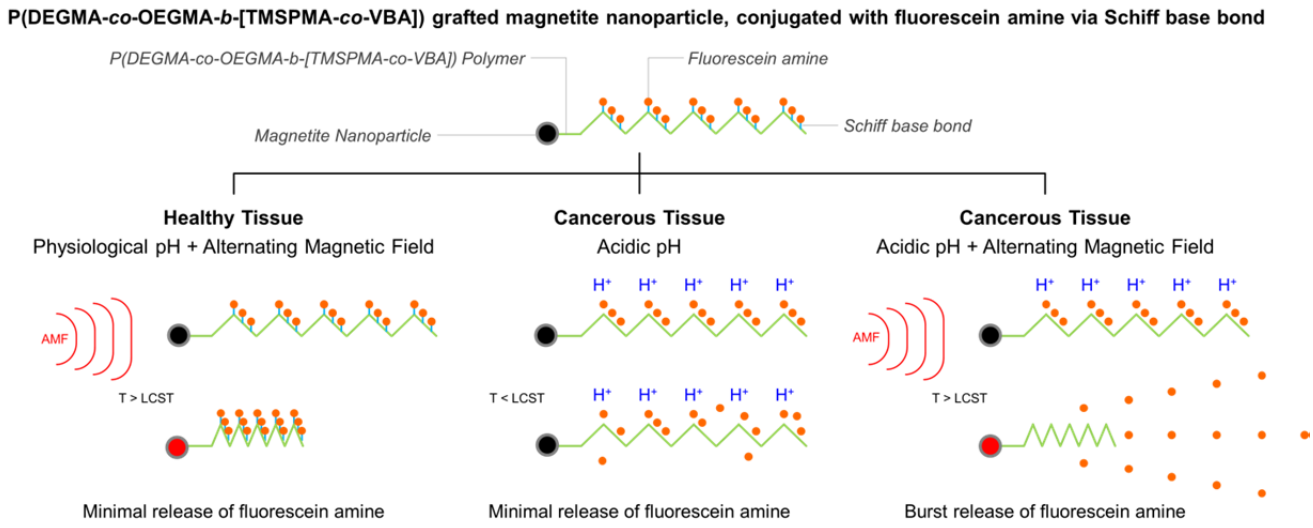

Figure 2: Overview of the nanoparticle-polymer composite's drug release mechanism. In this study I used a model molecule, fluorescein amine, in place of a cancer drug. A) in healthy tissue (pH 7.4), application of an alternating magnetic field causes the particle to heat up above the lower critical solution temperature (LCST) of the polymer, resulting in a contraction of the polymer chains, with a minimal release of the therapeutic compounds due to only partial hydrolysis of the Schiff base bonds. B) in cancerous tissue (pH ~ 5.5), an acidic environment causes a slow hydrolysis of Schiff base bonds, again resulting in minimal release of the model therapeutic compound. C) Application of alternating magnetic field (AMF) in acidic environment (such cancerous tissue) achieves a synergistic effect whereby a rapid hydrolysis of Schiff base bonds is observed due to the increase in temperature and low pH, resulting in a 'burst' release of the model therapeutic compound. Image not to scale. Reproduced from Dunn et al. (2014) with permission from The Royal Society of Chemistry[6].

4.2 General Findings

4.2.1 The Magnetic Alternating Current Hyperthermia System

One of the key reasons for travelling to the UK was to make use of the HBL patented Magnetic Alternating Current Hyperthermia (MACH) system. This is a custom made magnetic induction machine that is specifically designed to assess the heating capabilities, and hence effectiveness, of nanoparticles for biomedical applications. It achieves this by offering an alternating magnetic field with highly tunable field strength, and tunable frequency in the 'safe' range (see section 4.2.2) for use in biomedical applications. The MACH system also has replaceable coil fitting which allows the user to change the coil design (coil diameter, tube diameter, pitch, shape turn density etc.). This allows the user to test a range of conditions to optimize their particles for a wide range of applications.

The system is fitted with an optical temperature probe which measures subtle changes in temperature of a sample and subsequently records this data using *TrueTemp* software. This allows the user to measure the rate of change of temperature within the sample due to the presence of the alternating magnetic field.

In this project, I used a standard 6 turn copper coil with the specifications outlined in Table 1. When a 24 A current was passing through this coil, a field strength of 4.5 kA/m is produced. As with any conducting material, heat is generated during operation which may be undesirably transferred to the sample, producing a false temperature increase. To avoid this problem, the hollow copper coil has been fitted with a continuously flowing coolant which dissipates any heat generated within the coil during operation. Additionally, the inner lining of the coil is stuffed with insulating foam to prevent any heat transfer from the copper coil to the sample.

Table 1: Specifications for 6 turn induction copper coil.

Tube diameter (mm)	5
Pitch (mm)	5.5
Coil inner diameter (mm)	20
Insulating material	Foam
Insulator thickness (mm)	5
Turn density (/m)	180

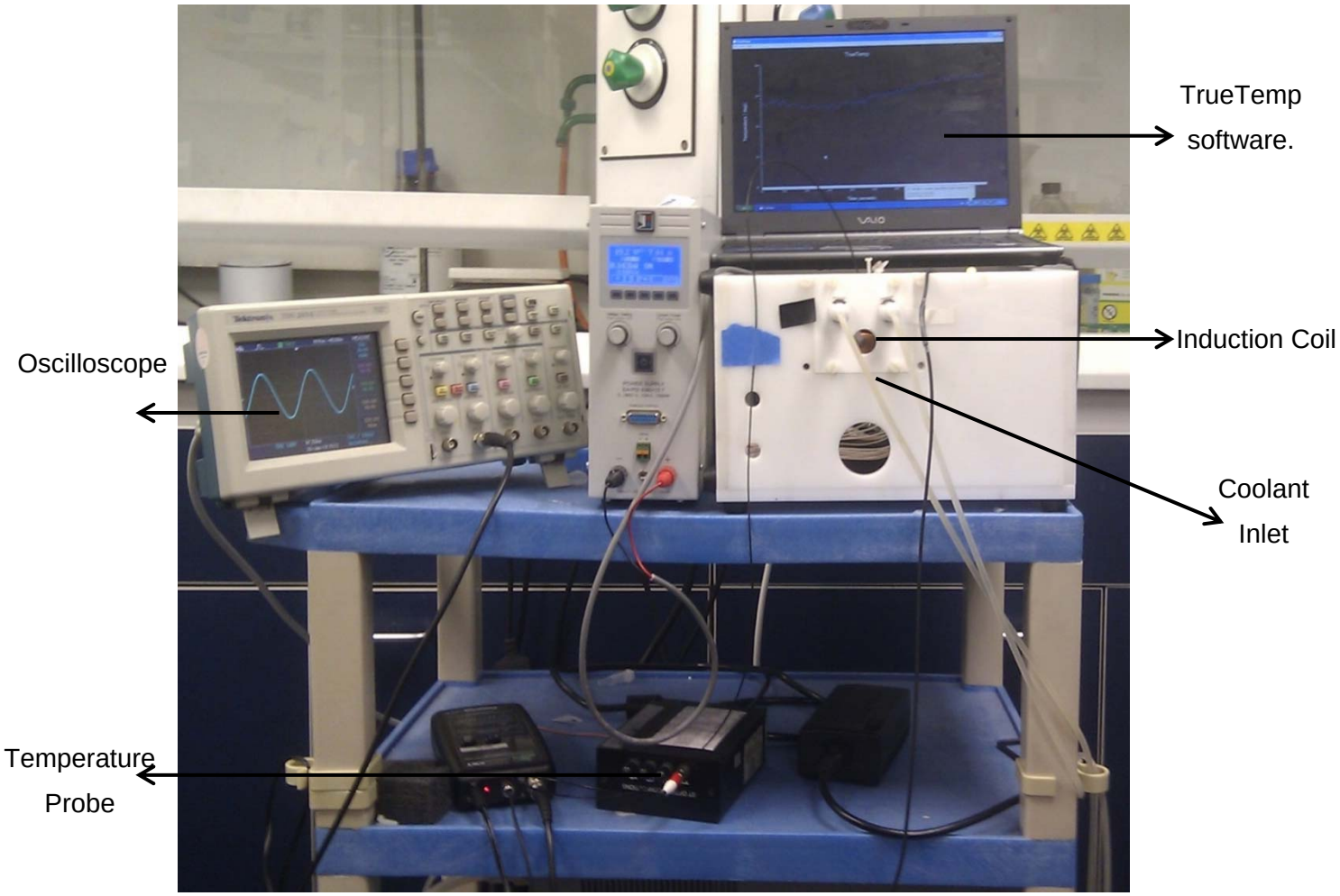

Figure 3: The custom made Magnetic Alternating Current Hyperthermia (MACH) System.

4.2.2 Ideal Conditions for Magnetic Hyperthermia

Despite notable amounts of research in to the use of alternating magnetic fields for biomedical applications, there is surprising uncertainty about the implications of the use of this technology on the human body and what constitutes a 'safe' exposure limit. This is a cause of some concern because of stigma associated with electrical conduction created by the flow of charges within nervous tissue triggered by high strength magnetic fields and the generation of eddy currents within tissue. During my fellowship, I discussed this issue with a number of groups.

It is well known that eddy currents form within human tissue causing a temperature rise within the body which is interpreted as tangible discomfort by the patient.[7] It has been noted that the magnitude of the eddy currents, and hence discomfort experienced by the patient, is proportional to the radius of the body part exposed to the field; for example, the human torso experiences greater eddy current formation than a human arm. This patient discomfiture limits the range of usable magnetic field strengths for hyperthermia applications. Therefore, to achieve additional heating above these limits, nanoparticle concentration must be increased, or nanoparticle design improved. Researchers at the Berlin Ultrahigh Field Facility (see section 4.4.3) noted that rapid movements in high strength static fields caused dizziness and the patient to see 'flashing lights'[†].

Based on these observations, for most human applications researchers have focused on field strengths of less than 5 kA/m, and frequencies below 1 MHz.[2] However, due to a lack of significant evidence, the validity of these limits are somewhat disputed. One generally accepted ruling is that the impacts of the frequency and field strength on the human body are interdependent. Very few reliable, systematic studies of the interdependent impacts of frequency and field strength on various parts of the human body have been conducted. Consequently, increasing the field strength is at the expense of the frequency, which must be lowered such that the product of the two is below a constant value: $H.f = 4.85 \times 10^8$.[8]

Other groups, such as *MagForce AG* and *Nanotherics*, argue that the frequency should be kept as low as possible (<200 kHz). This recommendation is based on the observation that tissue heating within the body increases with frequency squared, whereas nanoparticles usually only heat linearly with frequency. Therefore, as frequency increases the return of nanoparticle heating is countered by greater negative impacts on the patient's body. This limitation is problematic as this places significant limits on the maximum achievable heat loss due to Brownian relaxation which is dependent on higher frequencies for heat generation – particularly in viscous fluids, such as blood.

It is clear that further work in elucidating the impacts of alternating magnetic fields on the human body would be beneficial for this field of research. Whilst some *in vivo* studies have been completed using the mild conditions outlined in the previous paragraph, the efficacy of this treatment mechanism could be improved through development of a greater understanding of this relationship.

[†] This is likely due to magnetic field induced electric current formation within the retinal nerve.

4.2.3 Specific Absorption Rate vs Intrinsic Loss Parameter

When reporting the heating capabilities of a nanoparticle agent in the literature, it has become the norm to report the Specific Absorption Rate (SAR) in W/g, defined by Equation 1.

$$SAR = \frac{dT}{dt}.\frac{c}{m_{Fe}} \quad (1)$$

Where $\frac{dT}{dt}$ is the rate of change of temperature of the bulk solution, *c* is the heat capacity of the fluid (usually water) (in J/g.K) and m_{Fe} is the mass of iron in the fluid. The SAR value outlines the general heating capabilities of the agent when exposed to an arbitrary alternating magnetic field. The usefulness of this parameter is limited by its independence from meaningful heating conditions (specifically, magnetic field strength and frequency). This means that SAR values are not comparable between laboratories using different hyperthermia setups with different frequency and field strength settings.

To circumvent this, researchers at the UCL HBL in London have proposed widespread use of an Intrinsic Loss Parameter (ILP), defined by Equation 2.

$$ILP = \frac{SAR}{fH^2} \quad (2)$$

Where *SAR* is the Specific Absorption Rate (Equation 1), *f* is the frequency of the alternating magnetic field (in Hz) and *H* is the magnetic field strength (in A/m).[4] This parameter will allow more universal comparison of the heating capabilities of nanoparticles for these applications.[‡] Maximising a nanoparticle's ILP is desirable as it reduces the concentration of nanoparticles required to generate sufficient heat for achieve therapeutic temperatures and therefore minimizes the quantity of foreign material introduced into the patient's body.

Recall the discussion about the ideal conditions for hyperthermia in section 4.2.2. When considering the ideal frequency and magnetic field strength for nanoparticle heating, one must take note of how sacrificing field for frequency (or vice versa) may impact the ILP value of their system.

[‡] It is important to note that the ILP relies on low field approximations and is therefore not appropriate at all field strengths. Readers are directed to the paper by Kallumadil et al. (2009) for further details.

4.3 Laboratory Work - Royal Institution of Great Britain

4.3.1 Project Background

Between various meetings, conferences and general research, I spent some time doing laboratory based work in the University College London Healthcare and Biomagnetics Laboratories at the Royal Institution of Great Britain. In this project I was looking at the development of a nanomaterial that shows simultaneous potential as an MRI contrast agent and an instrument for magnetic hyperthermia based controllable drug delivery.

The main focus of my research at the Royal Institution was on the development and optimization of the magnetic properties of nanoparticles that can be utilised in this project. The HBL research group has several experts who focus on the development of magnetic nanoparticles for these applications, and their laboratory is equipped with the necessary apparatus for advanced hyperthermia studies.

Herein, I give a brief overview of my findings relating to this project. Detailed results have been presented at various conferences, and will be published in peer-reviewed journals.

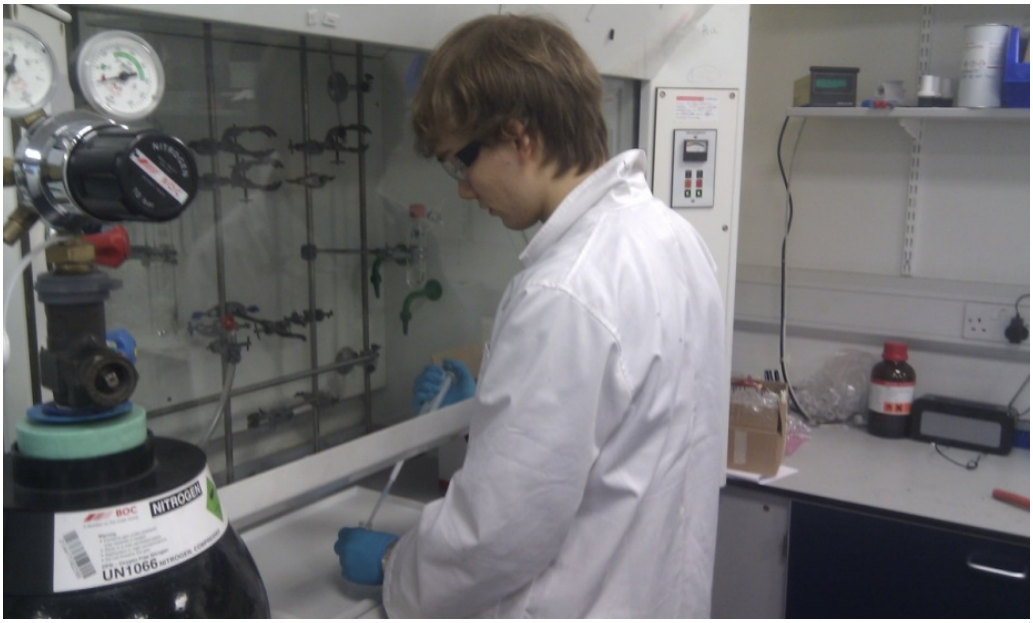

Figure 4: A photo of me doing laboratory work at the HBL, Royal Institution of Great Britain (London, United Kingdom).

4.3.2 Nanoparticle Synthesis

Iron oxide is commonly used in magnetic hyperthermia because it has well understood synthesis methods, strong magnetic properties and is relatively non-toxic and hence biocompatible. Additionally it has FDA approval and is therefore easier to use in clinical applications. In this project, I synthesised and tested several nanoparticle designs in order to assess their efficacy for these applications: 1) acicular, silica coated magnetite from reduction of a hematite intermediary, 2) cubic, silica coated magnetite, and 3) spherical magnetite by coprecipitation of Fe(II) and Fe(III). Whilst all of these particles were in the magnetite iron-oxide phase, they had different shapes, sizes and coatings. The acicular (or ellipsoidal) morphology was chosen as it has been reported that these structures exhibit greater bio-circulation[9] and cellular uptake[10] (as opposed to spherical nanoparticles).

The composition of each of these nanoparticles was confirmed using X-Ray diffraction crystallography (XRD) (results shown in Figure 5). The nanoparticle morphology was confirmed using Transmission Electron Microscopy (TEM) (results shown in Figure 5). The nanoparticle hydrodynamic diameter and agglomeration was assessed using Dynamic Light Scattering (DLS) analysis (results not shown).

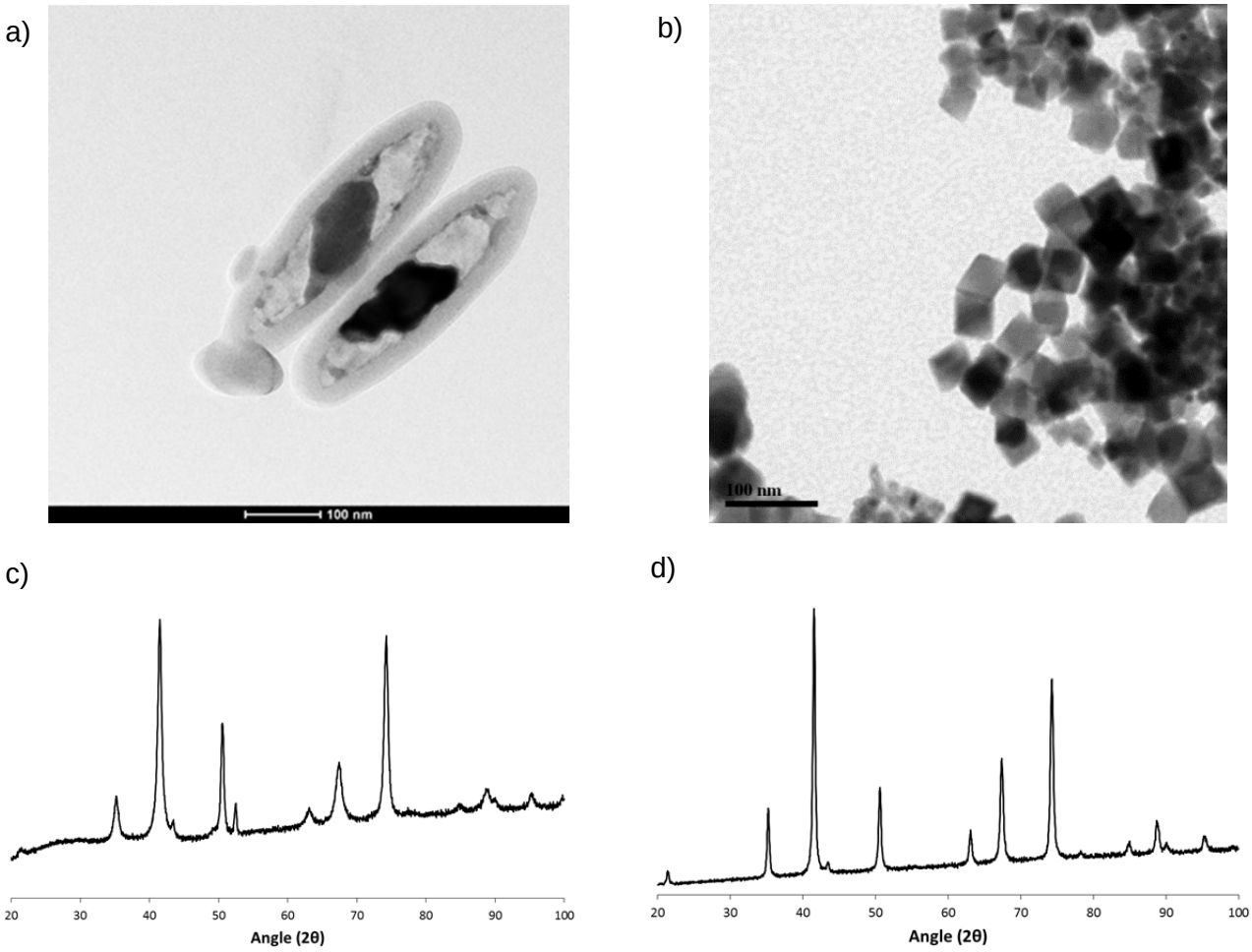

Figure 5: Transmission Electron Microscope (TEM) images and X-Ray Diffraction (XRD) spectra of magnetic nanoparticles. a) TEM image of acicular, silica coated magnetite nanoparticles. b) TEM image of cubic magnetite nanoparticles. c) XRD spectrum for acicular, silica coated magnetite nanoparticles. d) XRD spectrum for cubic magnetite nanoparticles.

4.3.3 Magnetic and Heating Properties

The effectiveness of the agent is dependent of the quantity and ease with which drugs are released from the agent. This is directly related to the heating properties of the nanoparticle core which is in turn dependent upon its magnetic properties.

It was noted that the cubic magnetite was a more efficient heater than the silica coated, acicular magnetite (see Figure 7). The key cause of this was attributed to the reduction in the nanoparticle hysteresis. Figure 7a (acicular, silica coated magnetite) shows a larger hysteresis than Figure 7c (cubic magnetite), and this corresponds to a deecrease in heating efficiency. The impact of this hysteresis on heating efficiency is largely to do with energy loss associated with overcoming the larger coercive field in the hysteretic particles. This is particularly problematic in these applications because relatively small magnetic field strengths are used; if these field strengths are comparable in magnitude to the coercive field of the nanoparticles, then the majority of the heating potential is expended in overcoming the coercive field. As discussed in section 4.2.2, for these applications, field strengths of 5 kA/m (63 Oe) are typical, therefore nanoparticles that exhibit hysteresis above 63 Oe are likely to exhibit significantly limited heating properties. From this we can conclude that it is likely that the heating properties of this agent can be improved by further reducing the magnetic hysteresis. The simplest method of achieving this is by reducing nanoparticle size through modification of the synthesis methodology. This is a point of further investigation upon my return to Australia.

It is important to note, however, that whilst the temperature of the bulk solution gives us an idea of the heating properties of the nanoparticles, we are more interested in the surface temperature of the nanoparticles. This is because the drugs are stored at the surface of the nanoparticles, and therefore it is the temperature of the surface that will initiate the decomposition of the covalent, drug storage Schiff Base bond. I have confirmed this hypothesis by comparing the drug release capabilities of this agent when heated by magnetic induction (heat generated within the nanoparticle, creating a high surface temperature), and when heated by an oil bath (constant temperature throughout the entire solution). In other words, the agent may still be effective at achieving drug release, even if the temperature of the bulk solution is relatively low.

Typically the surface will be significantly higher in temperature than the bulk solution due heat loss and dissipation within the system. Presently, there does not exist an effective mechanism of measuring the surface temperature of nanoparticles. Several investigations are currently being undertaken to shed light on this topic.[11]

Another observation about the various nanoparticle designs is the impact of a silica coating on the heating capabilities of the agent. It was observed that samples coated with silica were less efficient heaters than those without the silica coating. This is likely due to two reasons: 1) the mass of the silica contributes to the weight of the nanoparticles, and hence reduces the mass of iron per gram of particles, and 2) the silica coating acts as an insulating layer that reduces the surface temperature of the particles, and hence reduces the drug release capability of the agent. Therefore, the net functionality of the silica coating needs to be called in to question. Whilst it allows us to modify nanoparticle shape and improves drug storage capacity, it inhibits the heating and hence drug release capability of the agent.

Each of these observations have been reflected in the M-H and magnetic induction heating curves of the samples in Figure 7.

A qualitative examination of the magnetic properties of the nanoparticles is shown in Figure 6. A strong, rare earth magnet has been used to separate the magnetic nanoparticles from suspension. The interaction between the nanoparticles and the permanent magnet is strong enough to overcome the weight of the vial.

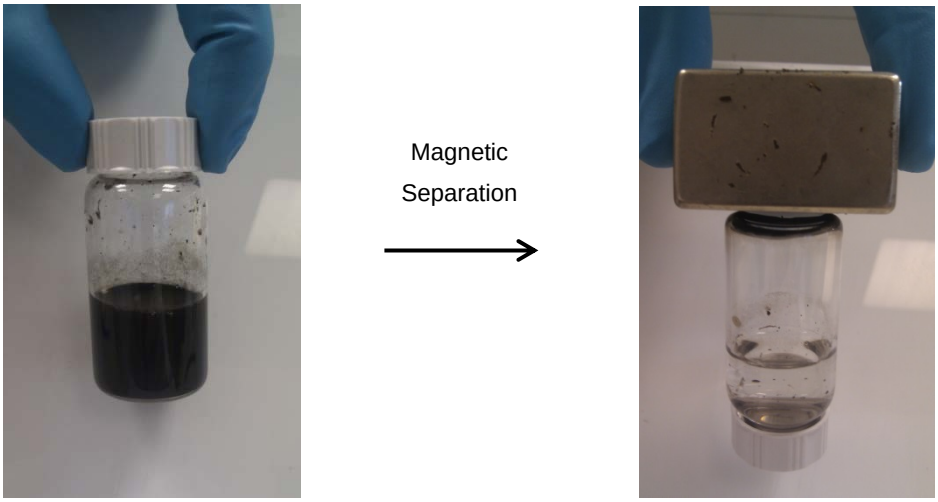

Figure 6: Demonstration of the nanocomposites' responsiveness to an external magnetic field.

The next phase in this project would be to continue optimization of this nanoparticle design to improve heating capabilities. Following that, I shall look at drug release studies with the new, improved nanoparticle cores.

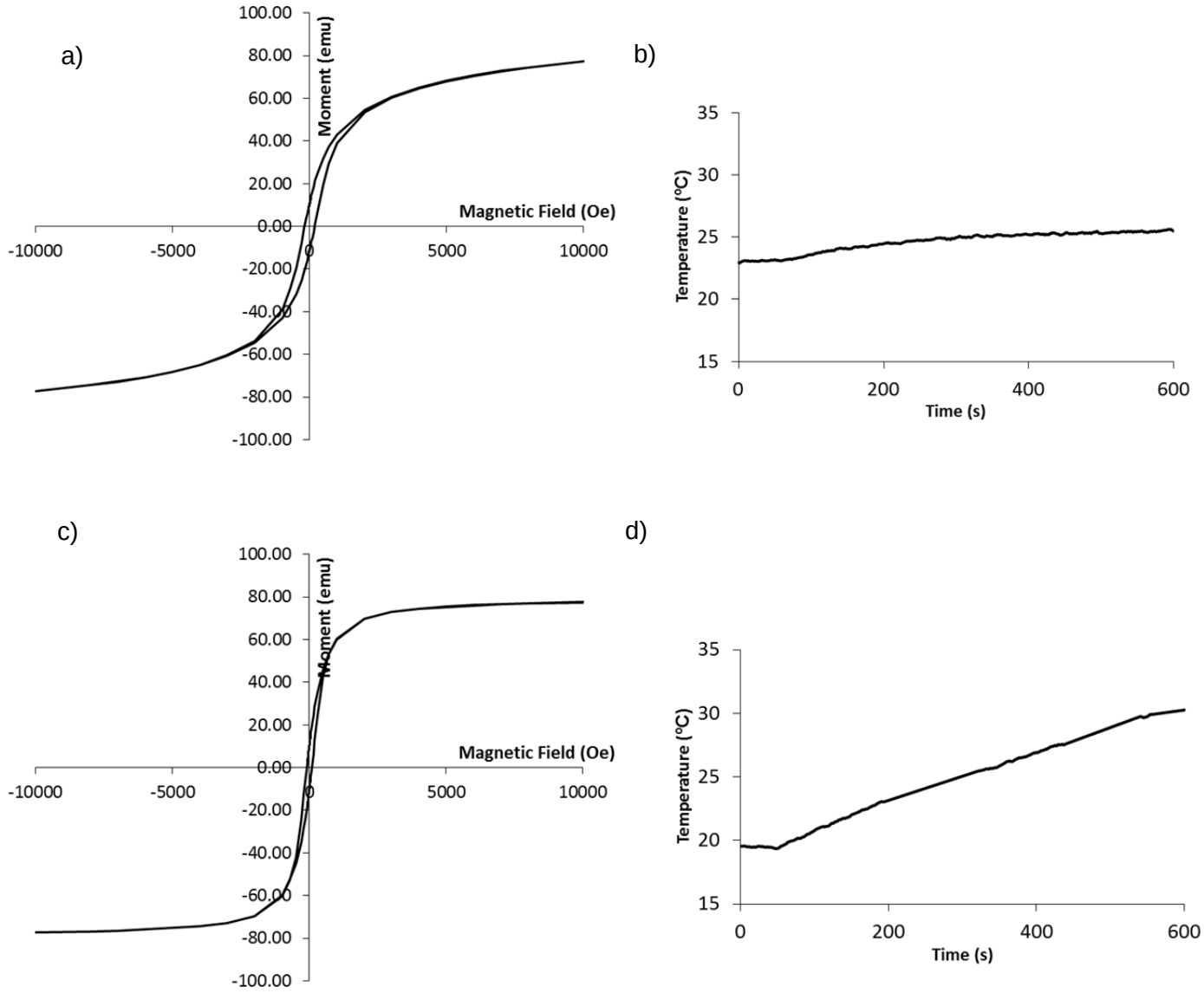

Figure 7: M-H (300 K) and magnetic induction heating curves of magnetic nanoparticles (f = 900 kHz, field strength = 13.5 kA/m). a) M-H diagram for acicular, silica coated magnetite nanoparticles (coercive field = 225 Oe)[§]. b) Heating curve for acicular, silica coated magnetite nanoparticles (5 mg/mL, 10 min). c) M-H diagram for cubic magnetite nanoparticles (coercive field = 79 Oe). d) Heating curve for cubic magnetite nanoparticles (5 mg/mL, 10 min).

[§] Note that the acicular, silica coated magnetite nanoparticles approach saturation more gradually. This is due to the presence of the diamagnetic silica coating.

4.3.4 In Vivo Studies

Whilst at the UCL HBL, I was given the opportunity to assist in one of their *in vivo* magnetic induction hyperthermia studies. These studies looked at the treatment of melanoma in mice by heating the tumours using magnetic nanoparticles to induce apoptosis within the cancerous cells. In these studies magnetic nanoparticles (~56 mg/mL of iron) were directly injected in to melanoma tumours in mice at a volume proportional to the tumour size. The mice were subsequently exposed to an alternating magnetic field of ~5 kA/m and 900 kHz for 30 min resulting in a surface temperature of ~45 °C. Results of these studies are to be published in coming months.

4.4 Notable Meetings and Conferences

4.4.1 UNSW-HBL Collaboration (London, UK)

The University College London Healthcare Biomagnetics and Nanomaterials Laboratories (HBL) research group is based at the Royal Institution of Great Britain. One of the key research interests of this group is the development of magnetic nanoparticles for biomedical applications. As a result of the time that I spent with HBL, I was able to develop an ongoing mutually beneficial collaborative relationship with some of the group's researchers. We hope to combine the expertise with the research groups at UNSW with those of HBL to further develop and optimise the design of our nanomaterial. Initially, this project will focus on the integration of UNSW's functional polymers and HBL's novel nanoparticles to develop a nanomaterial that is highly responsive to alternating magnetic fields for controllable drug delivery. By acting as the point of contact between these two groups, I hope to use this initial collaborative project to inspire a long term relationship may develop in to a range of future projects in this field.

4.4.2 McBain Medal Award Meeting 2013 (Cambridge, UK)

Each year the Society of Chemical Industry (SCI) Colloid and Surface Chemistry Group and the Royal Society of Chemistry (RSC) Colloid and Interface Science Group award the McBain Medal to a young scientist who has made a significant contribution to colloid and interface science. In 2013, Dr Oren Scherman (University of Cambridge) was awarded the McBain Medal. In recognition of this award, several prominent researchers from the UK presented their recent work in dynamic and controlled self-assembly at interfaces. Among the work presented were some novel developments in the field of drug delivery. Most of the work that was covered was fundamental research which will likely be used to develop novel and advanced drug delivery technologies. Among these novel advancements were the use of AFM and STM microscopy as means of imaging and studying chemical bonds, with potential use for drug storage technologies; molecule trapping within honeycomb structures;[12] and hydrogels for drug delivery.

4.4.3 Berlin Ultrahigh Field Facility (Berlin, Germany)

The Berlin Ultrahigh Field Facility (B.U.F.F.) is a research facility in the Max Delbrück Center for Molecular Medicine. B.U.F.F. focusses on the development of magnetic resonance (MR) technologies for biomedical applications. Perhaps of most interest to my project was their Hybrid

Radiofrequency Applicator for Magnetic Resonance Imaging and RF induced hyperthermia.[13] This technology uses novel techniques to induce localized temperature changes within the body by carefully aligning radio wave peaks. Using this technique, BUFF has shown that they can achieve a temperature increase of ~ 10 °C with a locality of ~3 cm.

This technique has several advantages. Primarily, it removes the need for magnetic nanoparticles to induce heat within the body. Whilst magnetic nanoparticles have been used for several biomedical applications, there still exists a stigma about their biocompatibility; specifically, the body's ability to remove the particles. It is possible that this new RF technique can non-invasively induce a sufficiently high temperature change to facilitate drug delivery. This technique also allows simultaneous MR imaging, allowing the administering doctor to create an almost real time image of the targeted site whilst inducing the temperature change. Additionally, by using a technique known as MR Thermography (MRTh), they are able to use the same machine to simultaneously measure and subsequently control the local temperature change within the body.

I have developed a collaborative relationship with B.U.F.F., endeavouring to combine my drug delivery mechanism with their MR-RF heating system for localised controllable drug delivery.

4.4.4 MagForce AG (Berlin, Germany)

MagForce AG is one of the world's leading companies in the development of nanotechnology for cancer treatment. Perhaps their most notable work is in the development of the *NanoTherm*^{TM} therapy system, which received approval for use on humans in the EU in 2010. This system consists of an aqueous nanoparticle suspension named *NanoTherm AS1*^{TM}, which is injected directly in to the tumour site. These nanoparticles are then heated using the *NanoActivator*^{TM} unit, the first magnetic inductor approved for use on humans. The system as a whole is controlled using the *NanoPlan*^{TM} simulation software.

MagForce AG has completed initial human trials on the efficacy of its *NanoTherm*^{TM} system, which combines thermoablation, hyperthermia and radiotherapy for the treatment of the glioblastoma, a notoriously aggressive brain cancer. Conventional treatment of glioblastoma relies on surgery; however, even in the cases where the surgery is successful, there is a strong tendency for the cancer to relapse. However, in the hyperthermia system used by *MagForce AG* the nanoparticles are retained within the tumour for an extended period of time and can therefore be used for multiple treatments. Therefore this system can be used to treat recurrent tumours.

From their studies, *MagForce AG* has shown a marked increase in the average survival time of patients suffering from this terminal disease.

Each treatment has three phases: planning, installation and heating. First, the glioblastoma is mapped using magnetic resonance imaging; from this map, the surgeon is able to drill a small hole in the skull and subsequently drill small holes in the tumour. Up to 0.3 mL of *NanoTherm AS1*^{TM} (112 mg/mL of iron) is then injected per cm. Next, a catheter and a temperature probe are inserted into the tumour which are used to monitor the treatment. The patient is then treated through exposure to the magnetic field using the *NanoActivator*^{TM} controlled by the *NanoPlan*^{TM} system, which relies on feedback from the temperature probe.

The *NanoTherm AS1*^{TM} consists of magnetic (magnetite) nanoparticles (~14 nm diameter) that are stabilized using an aminosilane. An interesting property of these nanoparticles is that their stability breaks down upon exposure to the saline environment of the human body, causing the nanoparticles to agglomerate when they are injected. This agglomeration discourages particle dispersion, subsequently concentrating the particles in the targeted site. This agglomeration process means that the nanoparticles are retained within the tumour site for an indefinite period of time, allowing the administering doctor to expose the patient to several one hour treatments over a period of weeks. Fortunately, the magnetic properties of the nanoparticles are unaffected by this agglomeration process, and are still able to exhibit significant heating.

The *Nanoactivator*^{TM} system is the world's first magnetic inductor designed for use in human biomedical applications. This system operates at 100 kHz and 4-18 kA/m. The temperature within the tumour is measured using a temperature probe during the first treatment, and the field strength is subsequently modified using the *NanoPlan*^{TM}. At the *Nanoactivator*^{TM}'s maximum operating conditions, patients have been known to complain about high temperatures on the surface of their skin; this is likely due to tissue eddy current formation.

MagForce is currently considering an expansion in to the field of controllable drug delivery. I have established a tentative collaborative relationship regarding this investigation.

4.4.5 NanoTherics (Staffordshire, United Kingdom)

NanoTherics is one of the world's key providers of academic and laboratory based AC field heating machines for cancer treatment applications. *Nanotherics*' main product is their versatile *magneTherm* hyperthermia system which is specifically designed for biomedical applications. It is

important to be able to test a wide variety of frequencies when assessing the efficacy of the heating properties of a nanoparticle agent because of the observed 'resonant' heating associated with some specific frequencies (see section 4.1.2). This system has been adopted by more than 50 research groups globally.

4.4.6 NanoMED: International Conference on Nanotechnology in Medicine

NanoMED is an annual, international conference held in London that focuses on the use of nanotechnology in medicine. Over a period of three days, the conference covers a wide range of topics including tissue engineering, imaging, biosensors, and drug delivery. At this conference, I was given the opportunity to present a paper "Polymer functionalised nanoparticles for imaging and alternating magnetic field induced drug delivery" which covered some of my findings during my fellowship. *NanoMED* attendees were also given the opportunity to visit Prof. Alexander Seifalian's laboratory (University College London).

Figure 8: Photo of my presentation at the annual NanoMED *conference at the Royal Free Hospital, London.*

5 Conclusions and Recommendations

The use of magnetic induction shows promise as a stimulus for controllable drug delivery in cancer treatment. The magnetic induction machines are sufficiently advanced so as to facilitate this process, and recent advances in nanoparticle and polymer design show promise for these systems. From my time abroad, my recommendations are as follows:

- Further studies to elucidate the ideal frequency and magnetic field strength for hyperthermia applications in the human body are required.
- Research groups should consider implementing the Intrinsic Loss Parameter in future work to allow ease of comparability between studies in different laboratories.
- Further investigation of non-invasive localized heating mechanisms that do not rely on nanoparticles (such as RF heating) may improve the efficacy of controllable drug delivery.

The next phase in my research project is to consider *in vitro* cell studies to assess the efficacy of this nanoparticle system on various cancer cell lines.

Some of the key findings from my fellowship have been disseminated at various talks and conferences. During my time at the Royal Institution, I presented results from my project to the HBL Research group. As part of my attendance at the 2014 NanoMED conference in London, I presented my project as both a seminar and poster. Further, I have developed an ongoing relationship with some of the researchers at the Royal Institution, B.U.F.F. and MagForce AG and hope that this can be used to facilitate further collaboration. All of the techniques and skills that I have developed will be shared with researchers at UNSW and applied in future research endeavours. I intend to assist in the development of future projects in a consultative manner. Finally, as this project progresses all key findings, including those associated with my work abroad, will be published appropriately in peer reviewed journals.

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