International Journal of Hyperthermia

ISSN: 0265-6736 (Print) 1464-5157 (Online) Journal homepage: informahealthcare.com/journals/ihyt20

Magnetically mediated hyperthermia: current status and future directions

P. Moroz, S. K. Jones & B. N. Gray

To cite this article: P. Moroz, S. K. Jones & B. N. Gray (2002) Magnetically mediated hyperthermia: current status and future directions, International Journal of Hyperthermia, 18:4, 267-284, DOI: 10.1080/02656730110108785

To link to this article: https://doi.org/10.1080/02656730110108785

Published online: 09 Jul 2009.

INT. J. HYPERTHERMIA, 2002, VOL. 18, NO. 4, 267–284

Taylor & Francis
healthsciences

Magnetically mediated hyperthermia: current status and future directions

P. MOROZ†*, S. K. JONES‡ and B. N. GRAY‡

†Centre for Applied Cancer Studies, The University of Western Australia, Nedlands, 6000, Perth, Western Australia, Australia
‡Sirtex Medical Ltd, PO Box 760, North Ryde 1670, Sydney, New South Wales, Australia

(*Received 26 June 2001; revised 28 September 2001; accepted 15 October 2001*)

The use of hyperthermia in the treatment of cancers is appealing because, as a physical therapy, hyperthermia would have far fewer restrictive side effects than chemotherapy and radiotherapy, and it could be used in combination with these therapies. However, the currently available modalities of hyperthermia are often limited by their inability to selectively target tumour tissue and, hence, they carry a high risk of collateral organ damage or they deposit heat in a very localized manner which can result in under-treatment of a tumour. Magnetically mediated hyperthermia (MMH) has the potential to address these shortcomings. MMH consists of the localization of magnetic particles or seeds within tumour tissue followed by exposure to an externally applied alternating magnetic field to cause them to heat. Since this concept was introduced (over 40 years ago), MMH has evolved into four general sub-classes: arterial embolization hyperthermia (AEH), direct injection hyperthermia (DIH), intracellular hyperthermia (IH) and interstitial implant hyperthermia (IIH). It is the purpose of this article to review these four sub-classes in terms of experimental or clinical results, advantages, limitations and current status.

Key words: Magnetic, direct, hyperthermia, embolization, arterial, intracellular, interstitial.

1. Introduction

It is well established that sustained temperature above 42°C will cause necrosis of living cells[1–4]. It is thought that hyperthermia (42–46°C) alters the function of many structural and enzymatic proteins within cells, which in turn alters cell growth and differentiation and can induce apoptosis[5–9]. The prospect of using hyperthermia alone to treat tumours is appealing because hyperthermia is a physical treatment and so would have fewer side effects than chemotherapy or radiotherapy. This would allow for a greater number of repeated treatments without limitation from the accumulation of toxic side effects. Even mildly elevated temperatures are known to significantly potentiate the effects of radiotherapy[10–14] and chemotherapy[10, 13, 15].

The currently available modalities of hyperthermia are often limited by deficiencies in tumour targeting ability and the resultant tissue temperature distribution. These problems are highlighted by considering the results of clinical trials of the various modalities of hyperthermia in the treatment of liver cancers[16]. The techniques of radiofrequency capacitance hyperthermia (RFCH), phased arrays of microwave antennae (MH), whole body hyperthermia (WBH) and isolated hepatic perfusion hyperthermia (IHPH) are tissue non-specific and lack hepatic tumour

*To whom correspondence should be addressed. e-mail: pmoroz@cyllene.uwa.edu.au

International Journal of Hyperthermia ISSN 0265–6736 print/ISSN 1464–5157 online
http://www.tandf.co.uk/journals
DOI: 10.1080/0265673011010878 5

targeting ability. This limits the attainable tumour temperatures due to the risk of collateral organ damage. In the cases of WBH and IHPH, the uniformly elevated body and hepatic temperatures obtained result in an unacceptably high risk of death due to organ failure. The techniques of radiofrequency probes (RFP), microwave probes (MP), interstitial laser photocoagulation (ILP) and direct tumour injection of hot water, on the other hand, seem limited by their very specific deposition of heat and the need for accurate tumour localization and physical access to the tumour. As a result, tumours larger than 5 cm require probe repositioning due to the limited diffusion of heat through tissue and, in the case of RFP and MP, the characteristics of the electromagnetic field patterns. Furthermore, widespread miliary disease is difficult to treat, and tumour recurrence and the development of new disease are further problems with such localized techniques.

Magnetically mediated hyperthermia (MMH) is a largely experimental modality of hyperthermia that has the potential to address the targeting and tissue heat distribution shortcomings of other hyperthermia modalities. In broad terms, the technique consists of localizing magnetic particles or seeds within tumour tissue and then applying an external alternating magnetic field to cause them to heat by hysteresis loss, Neel relaxation or induced eddy currents. This heat then conducts into the surrounding cancerous tissue. The concept of MMH was first described by Gilchrist *et al.*[17] in 1957. Their idea was to treat lymphatic metastases of large bowel cancer with heat by allowing microscopic ferromagnetic particles to embolize in lymph nodes draining the primary cancer site and then applying an external alternating magnetic field to cause hysteretic heating of the particles. In this study, magnetite particles of diameter 0.02–0.1 μm were injected into the sub-serosa of the intestinal wall of dogs with the expectation that the particles would accumulate in regional lymph nodes. The regional lymph nodes were then dissected out and exposed to an alternating magnetic field of strength 200–240 Oersted (Oe). It was found that a concentration of 5 mg of magnetite per gram of lymph node tissue yielded a temperature increase of 14°C in 3 min. Two years later, the same group conducted an *in vivo* study using rabbits in which inguinal lymph nodes were successfully targeted with heat[18]. Total necrosis of the nodes was reported after 3 min of heating at 470 Oe. One further study by this group in 1965 showed that 30 min of heating at 50°C was necessary to kill all cells within a mesenteric lymph node of a dog *in vivo*[19]. This early work, based on lymphatic uptake of microscopic ferromagnetic particles, clearly proved that it was possible to selectively embolize and heat tissue *in vivo* using MMH.

Since the 1960s, MMH has developed along four general pathways, all having in common the deposition of ferromagnetic material within or adjacent to tumour tissue. This first step is then followed by exposure to an externally applied alternating magnetic field to cause heating of the ferromagnetic material and, hence, surrounding tumour tissue. The first broad sub-class of MMH is arterial embolization hyperthermia (AEH), in which the arterial supply of a tumour is used as a pathway to carry ferromagnetic particles into the tumour tissue followed by exposure to an alternating magnetic field. The second sub-class is direct injection hyperthermia (DIH), in which the tumour is directly injected with ferromagnetic particles before exposure to an alternating magnetic field. Another sub-class is that of intracellular hyperthermia (IH), in which the ferromagnetic particles are modified to facilitate their cellular uptake by the tumour. The heat then generated on exposure to an alternating magnetic field originates from within the tumour cell, as compared to

AEH and DI where the heat originates intravascularly and extracellularly, respectively. The fourth sub-class of MMH is interstitial implant hyperthermia (IIH), in which macroscopic ferromagnetic seeds are surgically implanted into the tumour tissue before exposure to an alternating magnetic field. This fourth sub-class is currently in clinical use in the treatment of prostatic, neurologic and various other tumour types. The objective of this study was to review each of these four sub-classes of MMH with regard to experimental or clinical results, advantages, limitations and current status.

2. Arterial embolization hyperthermia

AEH is based on utilizing the arterial supply of a tumour to provide a pathway to enable selective embolization of the tumour with magnetic particles. The subsequent application of an external alternating magnetic field causes heating of the embolized particles by hysteresis or Neel relaxation. This modality of MMH has yet to be trialled in humans; however, the technique would seem well suited to the treatment of hepatic malignancies due to the nature of hepatic tumour blood supply. In this setting, it is envisaged that tumour embolization could be done via a gastroduodenal artery catheter attached to a subcutaneous infusaport, or under radiologic guidance employing a transfemoral catheter approach. The basis for this approach to the tumour is the fact that macroscopic liver tumours derive virtually all their blood supply from the hepatic arterial system, and so any substance infused into the arterial system will have the potential to preferentially target liver tumours[20–23]. This is also the basis for the currently used treatments of selective internal radiation therapy (SIRT), hepatic arterial chemotherapy (HAC) and transarterial chemoembolization (TACE). After verifying successful tumour embolization radiologically, the patient would be exposed to an externally applied alternating magnetic field which would generate high temperatures in the embolized particles and, hence, in the tumour tissue.

2.1. *Experimental results*

The earliest reports on AEH have typically used a dog kidney model to demonstrate the principle. In 1976, Rand *et al.*[24] impregnated liquid silicone with finely powdered iron oxide particles and injected the suspension transfemorally into the renal artery of dogs to simply embolize normal renal tissue. The eviscerated kidneys of the sacrificed animals were then exposed to a 570 Oe magnetic field alternating at 20 kHz. A heating rate of 12°C/min was observed in the medulla and a rate of 10°C/min was recorded in the renal cortex. The liquid silicone used by Rand *et al.* had earlier been used to occlude the blood supply of cerebral tumours[25, 26]. Although this study did not embolize tumour tissue, or generate therapeutic tissue temperatures *in vivo*, the general principle of AEH was demonstrated.

In a similar experiment, Sako *et al.*[27] arterially embolized a dog kidney with 5 mL of ferropolysaccharide containing 10% of 30 μm iron particles. Subsequent exposure to a 500 kHz magnetic field resulted in renal tissue temperatures of 42°C being recorded. However, a temperature of 41°C was also recorded in the subcutaneous tissue of the dog, suggesting that significant eddy current heating due to the magnetic field occurred in the superficial tissues. This finding highlights one of the potential problems of AEH, that being the heating of subcutaneous tissues at high field frequencies. Matsuki and Yanada[28] also explored AEH using the dog kidney model. In this study, 200 μm capsules containing 50 μm metal flakes (iron, phos-

phorus, chromium and carbon) capable of releasing cytotoxic agents were arterially embolized into the kidney of a dog, which was then exposed to a magnetic field of 7 kA/m alternating at 200 kHz. A tissue temperature of 43°C was recorded in the embolized kidney, while there was no increase in the temperature of a control kidney. This study did not report on temperatures in subcutaneous tissue or in skin, nor was post-treatment survival considered. However, the encouraging temperatures generated, along with the release of cytotoxic agents in the presence of such elevated temperatures, certainly warrants further investigation.

In 1994, Mitsumori *et al.*[29] tested dextran coated magnetite particles suspended in lipiodol or in a degradable starch microsphere. Earlier work had shown that such particles generated significant heat upon exposure to an alternating magnetic field[30]. The study reported an increase of over 12°C after 10 min of heating, *in vivo*, in a rabbit kidney following arterial embolization. Mitsumori *et al.* followed up this work with a study in 1996[31] in which four rabbits containing implanted hepatic VX2 carcinomas were arterially infused with dextran coated magnetite particles suspended in lipiodol and exposed to a 21.6 kA/m magnetic field alternating at 100 kHz. Tumour temperature increases of 0.6–1.4°C/min were recorded. The dextran coated particles used by Mitsumori *et al.* were 75 nm in diameter with a 7.4 nm magnetite core. At these sub-domain sizes, particles generate heat by Neel relaxation, in contrast to the larger multidomain particles used in most earlier work which generate heat by hysteresis loss effects[32, 33]. Suspensions that contain particles less than 100 nm in diameter, such as those used by Mitsumori *et al.*, are defined as *magnetic fluids*, and may be heated by weaker magnetic fields that are generally less likely to cause tissue eddy current heating or peripheral nerve and muscle stimulation. This may be an advantage over the use of larger multi-domain particles, which may require stronger field conditions for heating[32].

A recent study by Minamimura *et al.*[34] also employed magnetic fluids in the treatment of liver tumours in rats. In this study, the rats were arterially embolized with a dextran magnetite complex suspended in saline. On exposure to an alternating magnetic field, a heating rate of 0.2°C/min in tumour tissue and 0.18°C/min in normal liver tissue in six male Wister rats containing the Walker 256 carcinosarcoma was observed. Tumour temperatures of ~43°C were maintained for 30–40 min. The dose of arterially infused iron was ~20 mg of iron (magnetite) in a dextran coating. Three days after treatment, there had been a significant tumour response in the treated rats as compared to the control groups. Another recent study has also demonstrated a significant tumour response to AEH[35]. In this latter study, rabbits containing hepatic VX2 carcinomas were treated with AEH that consisted of arterial embolization with γ-Fe_2O_3 (150 nm) containing microspheres (32 μm diameter), followed by heating to over 42°C for 20 mins by exposure to a 45 kA/m magnetic field alternating at 53 kHz. Fourteen days after treatment, the mass of the treated tumours was significantly less than that of untreated controls, with large areas of tumour necrosis demonstrated histologically.

A study by Moroz *et al.*[36] reported that a tumour iron concentration of 2–3 mg/g was necessary to produce tumour heating rates of 0.5–1.0°C/min. In this study, 20 rabbits containing hepatic VX2 carcinomas received a hepatic arterial infusion of γ-Fe_2O_3 (100–200 nm diameter) suspended in lipiodol and were exposed to a magnetic field of 30 kA/m alternating at 53 kHz. The study reported a mean tumour to normal liver iron concentration ratio of 5.3 and tumour heating rates of up to 11.5 times greater than those in adjacent normal liver. Further work by this group employing

the same tumour model and infusion regimen as above has also shown that large hepatic tumours may be more amenable to treatment with AEH than small hepatic tumours[37]. In this study, the heating characteristics of 13 rabbits containing small tumours (less than 2.1g) were compared to those of 13 rabbits containing large tumours (2.1 g or more), the tumours of the two groups being matched retrospectively by iron concentration. On exposure to a magnetic field of 45 kA/m alternating at 53 kHz, large tumours heated at almost double the rate of small tumours (5.0°C/min compared to 2.8°C/min, $p = 0.006$). It was suggested that this result was due to the relatively better heat conduction and poorer cooling mechanisms within the necrotic regions of the larger tumours as compared to the smaller tumours. Further work is needed to confirm this finding, which may confer a significant advantage on AEH over other hyperthermic modalities in the treatment of hepatic malignancies.

2.2. *Advantages of AEH*

A potentially major advantage of AEH over existing modalities of hyperthermia is that of a more effective tissue temperature distribution. The poor venous drainage of most tumours, together with their chaotic vascular patterns, will favour the embolization of ferromagnetic particles within a tumour[38]. In this way, a gradient of ferromagnetic particles may be produced through the tumour and surrounding tissue, with the highest concentration of particles in the tumour. As a result, there would not be a sharp drop in temperature at the tumour edge, where micro-extensions of the tumour could elude hyperthermia. Theoretically, such sub-optimal heating could occur when hyperthermic techniques that produce very localized high temperatures such as lasers, radiofrequency probes or interstitial ferromagnetic implants are used. The use of radiofrequency probes and lasers is often limited to the treatment of liver tumours less than 5 cm in diameter as a result of the highly localized heat distribution that these modalities produce[16]. On the other hand, one study has suggested that AEH may overcome this limitation by virtue of the structure of larger tumours and the distribution of ferromagnetic particles within the tumour and surrounding normal liver tissue[37]. Nor would there be a uniformly high temperature throughout the organ containing the tumour due to the uneven particle distribution. A high uniform temperature within an organ is undesirable, as demonstrated by the high patient mortality following treatment of hepatic malignancies with whole body hyperthermia and isolated hepatic perfusion hyperthermia[39–41]. With respect to the potential treatment of hepatic malignancies, the arterial targeting mechanism is well established and is in clinical use currently for the treatment of hepatic malignancies in SIRT, HAC and TACE.

Another potential advantage of AEH is the prospect of repeated hyperthermia treatments without the need for further dosing or corporeal invasion if the particles remain *in situ* after embolization. AEH could also be performed radiologically via a transfemoral catheter and could also be readily combined with chemotherapy or radiotherapy. In a transfemoral approach, selective segmental catheterization of the liver could be employed to spare as much normal tissue as possible. A further potential advantage of AEH could be that when true hyperthermic temperatures (42–46°C) are used, in contrast to thermoablative temperatures, the risk of a shock syndrome due to the sudden release of large amounts of necrotic tumour material would be reduced, as there would be no major inflammatory response since apoptosis is the major mechanism of cell death following hyperthermia.

2.3. *Limitations of AEH*

The discussion above shows there have only been a few studies in small animals involving tumour models. Particle distribution, clearance and toxicity remain largely unknown. Magnetic field tolerance and safety limits are unclear for the type of magnetic coils that would be needed in a human trial. Magnetic fields may themselves cause treatment limiting eddy current heating of skin and other tissues, especially if multidomain particles that require strong magnetic fields are used. Tumours without a good arterial supply would not be amenable to this form of treatment. Furthermore, since micrometastases do not have an established arterial supply they will not be directly targeted by AEH. Arterial embolization also carries the risk of embolization and subsequent ischaemic necrosis of normal tissues, a problem which could be worsened by subsequent heating. AEH may, therefore, not be suited to the treatment of tumours situated in organs with a single arterial supply. This potential problem highlights the need for pre-treatment radiological determination of particle distribution and sound thermometry during treatment to avoid excessive thermal damage to normal tissues.

However, some of these limitations may be overcome by the use of coated particles to optimise distribution and minimize toxicity, supraselective arterial catheterization to spare as much normal tissue as possible, coadministration of angiotensin II or adrenalin with the particles to cause extratumoural vasoconstriction to improve tumour targeting (blood vessels within hepatic tumours do not respond to these drugs, and so do not constrict) as is done in SIRT, and the use of radiological techniques to determine the tissue distribution of iron particles prior to heating. The use of neuromuscular blockers in a general anaesthetic could prevent peripheral nerve and skeletal muscle stimulation, and the use of magnetic fluids and more moderate field conditions could also avoid the problem of neuromuscular stimulation.

2.4. *Current status*

AEH has a sound theoretical basis, and may offer some significant advantages over existing hyperthermic techniques, particularly in the treatment of hepatic malignancies. Although small animal studies have produced some very encouraging results, they are few in number and many questions remain unanswered. There are many safety issues regarding particle clearance and toxicity that are unknown, as is the question of whether sufficient heat can be obtained from ferromagnetic particles at magnetic field strengths and frequencies that are tolerable in humans. Although a number of groups are making progress in these areas, a great deal of work is still needed before a trial of AEH could occur in a human subject.

3. Direct injection hyperthermia

DIH consists of suspending microscopic ferromagnetic particles in a carrier fluid then directly injecting the suspension into the tumour tissue, with the subsequent application of an alternating magnetic field to cause heating of the deposited particles. In contrast to AEH, where the heat originates from ferromagnetic particles within blood vessels, the heat generated in DIH originates from extracellularly deposited particles.

3.1. *Experimental results*

In addition to exploring AEH, Rand *et al.*[42, 43] have also investigated DIH. In this work, ferromagnetic particles were suspended in normal saline and directly injected into the renal pelvis of 24 experimental rabbits containing unilateral implanted renal VX2 carcinomas. On exposure to a magnetic field of 1000 Oe alternating at 2 kHz, tumour temperatures of 55°C were obtained. Histologic examination of the tumours 3 days after heating revealed complete destruction when constant temperatures above 50°C were maintained. A report by Luderer *et al.*[44] presented the results of treating mice containing implanted murine breast adenocarcinoma with a subcutaneous injection of 0.1 g of glass-ceramic coated magnetic particles adjacent to the tumour. The mice were then exposed to an alternating magnetic field for 2 h. Five days after treatment it was reported that 17 out of 33 treated mice had no tumours and that, after 100 days, four of these mice still had no tumour recurrence. Another study by Borrelli *et al.*[45] also produced encouraging tissue heating using very similar glass-ceramic coated magnetic particles.

Hase *et al.*[46, 47] combined direct tumour injection of ferromagnetic particles with tumour ischaemia via hepatic arterial embolization. In rabbits containing a hepatic VX2 carcinoma, the hepatic artery was embolized with gelatin sponge powder and the tumour was directly injected with 1–3 g of 100 μm pure iron particles suspended in a polysaccharide. The subjects were then exposed to a 500 kHz magnetic field for 15 min. The study reported tumour temperature increases of up to 7.1°C and normal liver temperature increases of less than 2.5°C. This study did not report tumour response or rabbit survival, but demonstrated a large tumour/normal tissue temperature differential and suggested that the combination of hyperthermia with arterial occlusion is worthwhile.

In 1993, Chan *et al.*[48] reported on the use of 3–15 nm colloidal magnetic iron oxide particles to heat tumours. In this study, cultured human lung cancer and breast cancer cells were placed in a medium containing the colloidal particles (1–3 mg/mL of iron). Upon exposure to radiofrequency magnetic fields (0.15–1.1 MHz, 8–12.5 kA/m), temperatures of 42–45°C were obtained. Significant exposure dependent cytotoxicity was reported. Further work by Chan *et al.*[49], using mice containing implanted subcutaneous hind-limb tumours reported a tumour/body differential of 6.5–8.5°C on exposure to a 7.15 kA/m magnetic field alternating at 0.85 MHz. The tumours were infiltrated with supraparamagnetic colloidal iron oxide particles by direct intratumoural injection. It was found that a tumour iron concentration of only 0.5–1.0 mg/g was needed to produce this differential heating.

As discussed above, Matsuki and Yanada[28] investigated AEH using 200 μm capsules containing 50 μm metal flakes (iron, phosphorus, chromium and carbon). In this same study, capsules were directly injected into a VX2 liver carcinoma of a rabbit followed by exposure to a magnetic field of 7 kA/m alternating at 200 kHz. Tissue temperatures of ~43°C were reported. In 1997, Jordan *et al.*[50] investigated the technique of direct tumoural injection of ferrite particles (dextran ferrofluid containing magnetite particles 3 nm in size, 1.5×10^{-2} mg ferrite per mm^3 of tumour tissue) into mammary carcinomas in mice. A steady state tumour temperature of 47°C for 30 min was obtained by exposure to a 6–12.5 kA/m magnetic field alternating at 520 kHz. The study reported generally good local control of the tumours, but found that some tumours continued to grow. It was suggested that an uneven particle distribution and, therefore, non-uniform tumour temperatures were the reason.

Investigation of more sophisticated delivery techniques was, therefore, recommended.

Shinkai *et al.*[51] employed a colloidal magnetite material dispersed in a carboxymethylcellulose solution to potentially improve the targeting of radiofrequency capacitative hyperthermia (RFCH). In this study, the authors directly injected the femur of a pig with their solution and then exposed the region to an 8 MHz radiofrequency capacitative heating device. It was found that a localized tissue temperature of 43°C was generated in 7 min of heating, while the temperature of control tissue was only 40°C at this time. The tumour targeting ability of RFCH has been a major limitation of the technique[16]. Shinkai *et al.* have proposed an interesting way in which to potentially reduce a major limitation of an existing treatment. Further investigation in an animal tumour model is certainly warranted.

An *in vitro* study by Hilger *et al.*[52] examined the effect of magnetic thermoablation in cow muscle. In this study, 50–180 mg of magnetite particles (1 micron diameter) suspended in 0.3 mL of physiologic saline (Aldrich, Steinheim, Germany) containing 1% Tween 80 (Fluka, Buchs, Switzerland) were directly injected into prepared cylindrical cavities within samples of fresh cow muscle and exposed to a magnetic field of 6.5 kA/m alternating at 400 kHz. Temperature increases of up to 87°C were recorded within 15 mm from the particle deposits. It was suggested that lesions up to a volume of 0.131 cm^3 could be treated using a dose of 180 mg of particles. Although the author was investigating the technique for use in the ablation of muscle lesions and the cooling effects of blood flow were absent, the principle of generating localized tissue heat with magnetic particles and externally applied magnetic fields was again demonstrated. Hilger *et al.*[53] have also recently tested DIH on immunosuppressed mice containing implanted human breast adenocarcinoma, as a model of the potential treatment of human breast cancer. In this work, a ferrofluid containing 10 nm magnetite particles was directly injected into the tumours, which were then exposed to a 6.5 kA/m magnetic field alternating at 400 kHz. Mean tumour temperatures of 63°C were recorded after 2–3 min of heating and all tumours showed histologic evidence of necrosis.

3.2. *Advantages of DIH*

DIH would allow good control over the deposition of ferromagnetic particles, with a maximum of normal tissue spared. As a result, much higher localized concentrations of ferromagnetic particles could be achieved within tumours, with higher temperatures resulting. DIH does not depend on an arterial pathway to the tumour, so a wider range of tumour types would be amenable to treatment, and arterial catheterization and its consequent risks could be avoided. The technique could also be carried out percutaneously under radiological guidance and could be combined with radiotherapy and chemotherapy. If the injected particles were likely to remain *in situ* then repeated treatments would be possible without the need for further corporeal penetration.

3.3. *Limitations of DIH*

A prerequisite for DIH would be accurate tumour visualization and adequate access. Large or irregularly shaped tumours would need repeated injections and the deposition of large amounts of particles. The hyperthermia techniques that use radiofrequency probes, microwave probes and lasers are generally limited to the treatment of liver tumours 5 cm or less in diameter without repositioning[16]. It is

likely that DIH would also be limited by similar size constraints due to limitations on the diffusion of heat from the deposited particle mass within the tumour tissue. This limitation would be further magnified by the preservation of the cooling effects of tumour and surrounding blood flow, in contrast to AEH where the embolization of particles within the vascular supply of a tumour is likely to have some inhibitory effect on blood flow. Miliary disease would not be conducive to treatment due to the need for many injections. The very localized deposition of the particles would lead to only localized heating, which would increase the risk of not treating tumour micro-extensions or surrounding micro-metastases. The need for tumour penetration would also increase the risk of needle track implantation or local tumour spread. A further consideration is the possibility of particle migration following injection. As with AEH, tissue eddy current heating and neuromuscular stimulation by the magnetic field could be treatment limiting, although this would depend on the particles used (sub-domain vs multi-domain) and, hence, the required magnetic field conditions.

3.4. *Current status of DIH*

While there have been some promising results in small animal studies, as for AEH there remain many questions regarding particle clearance and toxicity, magnetic field safety and the efficacy of DIH in large animals or humans. DIH may allow better targeting and control of particle deposition than AEH, but it seems likely that the technique would suffer from the same limitations as other treatments that rely on direct tumour penetration such as interstitial ferromagnetic implants, radiofrequency probes, interstitial lasers and injection of hot water, without having any clear advantages over these techniques.

4. Intracellular hyperthermia

IH is a modality of MMH that employs more intricate magnetic particles. The particles usually have some form of coating with or without tumour specific antibodies and are delivered to the tumour by either arterial embolization or direct injection. The particles are then selectively ingested by the tumour cells, with minimal uptake by normal cells (differential endocytosis), although extracellular deposits of particles will invariably remain and make some contribution to the heating of the tumour and surrounding tissue. After particle ingestion, exposure to an alternating magnetic field results in heat being generated from within the tumour cells, with the expectation that normal cells (which ingest relatively fewer particles) are relatively protected from thermal damage. The ferromagnetic component of the particles used in IH is typically less than 100 nm in diameter (sub-domain) and, therefore, generates heat by Neel and Brownian relaxation.

4.1. *Experimental results*

In 1979, Gordon *et al.*[54] found that tumours may ingest very small magnetic iron oxide particles, which, if subsequently exposed to an alternating magnetic field, could generate intracellular hyperthermia in tumours. This contrasted with work by Gilchrist *et al.*[17], Medal *et al.*[18] and Rand *et al.*[24], where heat was generated extracellularly. The Gordon study was conducted on 26 Sprague Dawley rats containing implanted mammary tumours. Magnetic iron oxide (Fe_3O_4) particles (100 mg) of 26 or 60 Å diameter suspended in a sucrose solution were injected into a tail vein. Two days after injection, the rats were exposed to a magnetic field alternating at 450 kHz for 12 min. Tumour temperature increases of $\sim 8^{\circ}C$ were recorded. It was also

reported that there were no side effects or toxicities to the particles, and histologic evidence of tumour necrosis was presented. However, it is difficult to ascertain the precise role played by intracellular hyperthermia in this study. Although microscopic tissue analysis revealed that tumour cells had indeed taken up particles, substantial deposits of particles were also present outside tumour cells, in normal liver tissue and in other tissues such as spleen and kidney, suggesting that heat generated from extracellular particles may have contributed to the results.

In 1990, Suzuki *et al.*[55] tested the heating of liposomal ferromagnetic particles on VX2 carcinomas implanted in the thighs of rabbits. The particles consisted of ferrosoferric oxide (Fe_3O_4) coated with a liposomal membrane containing haematoporphyrin with neoplastic affinity. In one group of rabbits, 200 mg/kg of particles were injected into the femoral artery to allow embolization within the tumour, and the rabbits exposed to a magnetic field alternating at 400 kHz. After 10 min of heating, a temperature increase of 14.8°C was observed. However, temperature increases were also observed in control groups (femoral artery ligation only group and magnetic field exposure only group), suggesting that this apparatus may have caused significant tissue heating due to eddy currents. Furthermore, inspection of the tumours using microscopy showed iron particles not only within tumour cells, but also in blood vessels throughout the tumour, again suggesting that the results were not due to intracellular hyperthermia alone.

A significant development in IH occurred in 1995 when Suzuki *et al.*[56] described the preparation of magnetite particles labelled with tumour specific monoclonal antibodies, via polyethylene glycol with terminal carboxy or amino groups. Such particles were incubated with BM314 cells. Analysis of the cells after incubation determined that 90 pg of magnetite had been adsorbed per tumour cell, four times the amount adsorbed per control cell. The authors estimated that such an adsorption efficacy could heat a 1 cm^3 tumour to over 42°C in 30 s using a 45.5 kA/m magnetic field alternating at 240 kHz.

An *in vitro* study by Shinkai *et al.*[57] explored the technique of IH using rat glioma cells. In this study, cationic liposomes containing magnetite were found to be electrostatically attracted to the negatively charged glioma cells 10-fold more than neutral liposomes, which resulted in the increased intracellular deposition of magnetite within tumour cells. A maximum magnetite concentration of 55 pg/cell was obtained using the cationic liposomes, which was 10 times greater than the intracellular concentration attained using neutral liposomes. On exposure to an alternating magnetic field, tumour temperatures of over 43°C were reported. In 1999, Shinkai *et al.*[58] followed up this work with an *in vivo* study using F344 rats containing implanted femoral subcutaneous gliomas. In this study, magnetite cationic liposomes were injected subcutaneously into the tumours and the rats were then exposed to a 384 Oe magnetic field alternating at 118 kHz for 30 min. Tumour temperatures over 43°C were recorded and 90% of tumours were found to have regressed completely on histological examination. In 2001, Le *et al.*[59] reported successful tumour control using antibody fragment coated magnetoliposomes. This coating conferred a 7-times greater tumour affinity on the particles compared to those used by Shinkai *et al.*, as described above. In this study, the magnetoliposomes were directly injected into the centre of femoral gliomas in nude mice (5 mg magnetite per 1 mL, 260 μg of antibody fragment coated magnetoliposomes per gram of tumour). The mice were then exposed to a 118 kHz magnetic field of strength 30.6 kA/m (384 Oe) for 30 min

once a day for 3 days. Tumour temperatures of 43°C were obtained and after 14 days there was no increase in the volume of the tumours.

During the past decade or so, Jordan *et al.*[60–63] have produced several reports on IH. After evaluating ferromagnetic particles and their potential role in magnetic fluid hyperthermia in 1993[60], Jordan *et al.*[61] showed that human colonic adenocarcinoma cells accumulated up to 1.1 pg of ferrite per cell when the cells were cultured in a medium containing ferrite particles. However, on exposure to an alternating magnetic field, which led to the generation of temperatures of over 43°C, there was no improved cytotoxicity over that of water bath exposure at 43–45°C. The authors suggested that this could be due to the intracellular digestion of the particle coating and recommended improvements in this area for future work. More recently, Jordan has investigated the cellular uptake and biological effect of both dextran and aminosilan coated magnetic particles[62]. In this study, normal human cell types (fibroblasts and neurons) and carcinomas (colonic adenocarcinoma and glioma), were cultured in separate mediums, each containing the differently coated ferrite particles and exposed to an alternating magnetic field. Increased cell uptake of iron (up to 400 pg/cell following culture of glioma cells with aminosilan coated particles, and up to 500 pg/cell following culture of fibroblasts with dextran coated particles) and a greater cell kill compared to a water bath were reported. In a recent article, Jordan *et al.*[63] describe, in some detail, the apparatus and possible treatment schedules for the treatment of human neurologic and prostatic tumours via magnetic fluid intracellular hyperthermia. The outcomes of such trials are eagerly awaited.

4.2. *Advantages of IH*

IH has all the potential advantages that accompany AEH and DIH, depending upon the route of delivery. In addition, IH has the potential to focus the heat generated by the particles to within the tumour cells, and so possibly improving treatment efficacy. As has been discussed, extracellular particle deposits are likely to contribute to the effect, so a controlled *in vivo* study would be worthwhile to elucidate the contributions of intracellular and extracellular hyperthermia in this setting. Another advantage of IH arises from the fact that the particles used are virtually always sub-domain (i.e. magnetic fluids), and so the required magnetic fields may be more moderate and, hence, safer than the stronger fields required when multi-domain particles are used in AEH and DIH.

4.3. *Limitations of IH*

The question of the relative contributions of intracellular and extracellular heating needs further evaluation in a live tumour model. For IH to be effective, the large majority of tumour cells would need to ingest a sufficient quantity of particles to ensure adequate heating throughout the tumour, and those cells that did not ingest particles would need sufficient exposure to the heat generated by extracellular particle deposits to cause necrosis. If an arterial embolization delivery technique is used, it is questionable whether this could be achieved in very large or poorly vascularized tumours, especially those with semi-necrotic cores that may harbour viable tumour cells, since it may simply not be possible to deliver particles to those cells, as is the case with AEH. On the other hand, as with DIH, a direct injection approach may result in a sub-optimal particle distribution, and under-treatment.

4.4. *Current status of IH*

IH carries with it all the advantages and limitations of AEH and DIH, but in addition has the exciting potential to significantly improve the focus of hyperthermia within a tumour by virtue of the more intricate particles used. The results of small animal studies on IH have been very promising. Although IH, like AEH and DIH, is still in the pre-clinical evaluation stages, a human trial appears relatively close, as it seems that most of the required technology has been developed.

5. Interstitial implant hyperthermia

IIH consists of the implantation of ferromagnetic rods or seeds directly into tumour tissue followed by exposure to an external alternating magnetic field. The seeds are typically of the order of 1 mm in diameter and 1–7 cm in length. Various alloys including Ni-Cu, Fe-Pt and Pd-Co have been used, with some incorporating a coating to prevent corrosion. The seeds are implanted percutaneously under radiologic guidance or at laparotomy in regular arrays spaced 1 cm from each other within tumour tissue. The seeds are manufactured to have a desired Curie point to prevent local tissue overheating and reduce the need for invasive tissue thermometry. Heat is generated within the ferromagnetic seeds upon exposure to an alternating magnetic field by a different mechanism to those described above. The magnetic field induces eddy currents in the seeds, which produce heat due to resistance to the electrical current. Magnetic field frequencies of 10–100 kHz are typically used so as to avoid neuromuscular stimulation and to minimize tissue eddy current heating. In a clinical setting, heating is repeated for three or four sessions. In addition to implanted seeds, microwave antennae with cables have also been used to generate interstitial hyperthermia[64, 65].

The technique of using ferromagnetic thermoseeds to ablate tissue was first described by Burton *et al.*[66] for the potential treatment of brain tumours. Following this report, a wealth of experimental data has emerged during the 1980s and 1990s. As a result, this modality of MMH has evolved into clinical use for a variety of human tumours. These clinical results will be reviewed along with the most recent experimental developments.

5.1. *Clinical and experimental results*

Cerebral malignancies have been investigated as candidates for IIH. In 1990, Kida *et al.*[67] implanted 1.8 mm diameter, 15–20 mm long Fe-Pt alloy seeds with a Curie point of 68–69°C into the brain metastases of seven patients. Hyperthermia treatment consisted of a tissue temperature of 44–46°C for 30–60 min two-to-three times a week on exposure to a 240 kHz magnetic field. In six patients, external beam radiotherapy was added. Two complete responses were reported, along with one partial response. A larger study by Kobayashi *et al.*[68] reported a response rate of 35% among 23 patients with brain tumours after using thermoseeds with a Curie point of 68°C. This latter study employed a magnetic field of 1.65 kA/m alternating at 240 kHz. In another study on brain tumours by Stea *et al.*[69], 28 patients with supratentorial gliomas were implanted with ferromagnetic seeds to obtain a tissue temperature of ~42°C upon exposure to an alternating magnetic field. The median survival of this group was 20.6 months. Another study by Stea *et al.*[70] compared the outcome of 25 patients with supratentorial gliomas who were treated with thermoseed hyperthermia and external beam radiotherapy with that of 37 such patients who were treated with external beam radiation alone. The study reported that the risk of

death was approximately halved in the group that had hyperthermia added to the treatment regime.

Extracranial tumours have also been treated with IIH. Mack *et al.*[71] used the technique to treat 44 patients with head and neck, pelvic and chest wall tumours. Fourteen gauge cannulae were inserted into the tumours before being loaded with Ni-Si seeds of Curie points ranging from 50–80°C. The patients were also treated with ^{192}Ir (median radiation dose of 88.7 Gray). A magnetic field of 1.5–2.0 kA/m alternating at 80–100 kHz was used and a mean maximum tumour temperature of 43.7°C was obtained. The study reported a complete response rate of 61% and a partial response rate of 31.7%. Although the response rate was impressive, almost 50% of the patients experienced some form of toxicity. However, the author considered this acceptable in view of the high response rate. In 1996, Tohnai *et al.*[72] treated eight patients with oral cavity cancers with pre-operative chemotherapy and Fe-Pt seeds (Curie point = 68°C) for 45 min once a week. The study reported a 100% histologic response rate.

IIH has also been trialled experimentally on bone and ocular tumours. Akagi *et al.*[73] inserted a Kirschner wire into the medullary cavity of a rabbit tibia containing an implanted VX2 carcinoma and showed that therapeutic temperatures could be obtained. Takegami *et al.*[74] developed a bone cement with the potential to treat bone malignancies. The cement contained magnetite powder that actually generated heat by hysteresis loss rather than through induced eddy currents. In this study, the cement was implanted into rabbit and human cadaver tibiae, then exposed to a magnetic field of 300 Oe alternating at 100 kHz. Temperatures of 50–60°C were obtained under these conditions.

Recent work on experimental ocular tumours has been promising. Steeves *et al.*[75] implanted ferromagnetic seeds in sub-retinal ocular melanomas of rabbits and treated the subjects with various combinations of radiation and 46–47°C temperatures for 1 h. The study found that hyperthermia should be given during irradiation. Murray *et al.*[76] also found a synergistic effect between radiation and IIH. In this study, a transgenic murine model of retinoblastoma was treated with external beam radiotherapy, IIH or a combination of the two treatments. The study reported a 100% cure rate among tumours that had been treated with hyperthermia at 54°C, and also reported a large reduction in the dose of radiation necessary in combination with hyperthermia to achieve the same therapeutic effect as radiotherapy alone. A year later, the same authors showed that there was no diffuse ocular toxicity in a study on normal rabbit ocular tissue following IIH[77].

Prostatic cancers are another type of malignancy that have been successfully treated with IIH. In 1997, Loening and Tucker[78], in conjunction with Ablation Technologies (San Diego, CA), conducted a phase I trial on 10 patients with prostatic carcinoma. These patients had prior radiotherapy or surgery. Up to 40 Pd-Co ferroseeds were implanted into the prostate and the patients underwent weekly treatments without anaesthesia. The treatment was well tolerated and without complications. Further investigation into IIH by this group trialled Pd-Co seeds of 1 mm diameter and 14 mm in length in arrays 1 cm apart and 5 mm from the prostatic capsule edge in 15 patients with prostatic cancer[79]. Histologic examination of the prostate 90 days after treatment revealed necrosis up to the capsule, with very little necrosis beyond the capsule. The only side effects were urinary retention, pelvic pressure, urge to urinate and tingling in the thighs and penis.

5.2. *Advantages of IIH*

The ferromagnetic seeds used in IIH are thermally self-regulating, so there is a reduced need for invasive thermometry, and the safety level of the technique is increased because very high temperatures, and thus tissue burning and charring, are avoided. The rapid initial heating of the seeds results in a rapid attainment of steady state temperature so that total treatment time is reduced. Biocompatible seeds can be permanently left *in situ* for repeated treatments. IIH spares almost all normal tissue, because the seeds are implanted into the tumour tissue under direct vision. Implantation can be done under radiological guidance, so that open surgery can be avoided. IIH can also be used in conjunction with other treatment modalities such as radiotherapy and chemotherapy. The technique has been demonstrated to work in a wide variety of human tumour types *in vivo*.

5.3. *Limitations of IIH*

Thermoseed migration may be a problem, as may corrosion, although the latter can be avoided by suitable choice of material or gold plating. Nickel may cause contact allergies, and coating may detract from the heating. The seeds may interfere with future radiologic investigations such as MRI. It may be difficult to completely treat irregular shaped tumours, and some tumours may be difficult to access. There are also the complications of the implantation procedure and the risk of infection. If thermoablative temperatures are obtained, then there is the risk of a shock syndrome induced by the sudden large production of necrotic tumour material. IIH may be limited by heat diffusion through surrounding tissue as the procedure produces only a very localized point source of heat. If a tumour has a high blood flow, or the surrounding blood flow is high, then cooling may significantly dampen the temperatures attained. Without extensive thermometry, it cannot be certain that an entire tumour attained therapeutic temperatures. Non-uniform tumour temperatures may be a problem and cold spots may result. Also, the ferromagnetic seeds must be aligned in the direction of the applied magnetic field, which may be difficult to achieve in some cases.

5.4. *Current status of IIH*

Of the four classes of MMH, IIH is the only one that has been trialled clinically. This relatively new technique has produced some very good response rates and is generally safe. Although IIH may suffer similar drawbacks to other direct tumour penetration techniques of hyperthermia, IIH has the advantage that after the implantation, subsequent treatments are non-invasive. Although the technique should still be regarded as experimental, the promising results warrant further investigation. The outcomes of further trials with this modality are awaited with interest.

6. Conclusion

The concept of MMH was first introduced over 40 years ago. Although the technique has a sound theoretical basis, for many years progress was slow and greatly limited by the available technologies of the day. Technological advances over the past decade have resulted in more extensive studies and in the development of more intricate techniques. IIH is currently the most advanced modality of MMH, and has been used to treat human tumours with some success for several years. IH involving the use of magnetic fluids containing sub-domain sized magnetic particles that generate heat via Neel relaxation seems poised for a human trial in the near

future. Although AEH and DIH based on hysteretic heating of multidomain ferromagnetic particles in human subjects is not yet on the horizon, a number of research groups are making progress in these areas. The coming decade promises to be a fascinating one as the results of work in these areas emerges to determine whether or not MMH will fulfil its promised potential.

References

1. Cavaliere R, Ciocatto EC, Gionanella BC, Heidelberger C, Johnson RO, Margottini M, Mondovi B, Moricca G, Rossi-Fanelli A. Selective heat sensitivity of cancer cells. Biochemical and clinical studies. *Cancer* 1967; 20: 1351–81.
2. Robinson JE, Wizenberg MJ, McCready WA. Combined hyperthermia and radiation, an alternative to heavy particle therapy for reduced oxygen enhancement ratios. *Nature* 1974; 251: 521–2.
3. Steeves RA. Hyperthermia in cancer therapy: where are we today and where are we going? *Bull NY Acad Med* 1992; 68: 342–50.
4. Dewey W. Arrhenius relationships from the molecule and cell to the clinic. *Int J Hyperthermia* 1994; 10: 457–83.
5. Harmon BV, Takano YS, Winterford CM, Gobe GC. The role of apoptosis in the response of cells and tumours to mild hyperthermia. *Int J Radiat Biol* 1991; 59: 489–501.
6. Sellins KS, Cohen JJ. Hyperthermia and induced apoptosis in thymocytes. *Radiat Res* 1991; 126: 88–95.
7. Fairbairn JJ, Khan MW, Ward KJ, Loveridge BW, Fairbairn DW, O'Neill LK. Induction of apoptotic cell DNA fragmentation in human cells after treatment with hyperthermia. *Cancer Lett* 1995; 89: 183–8.
8. Bertone V, Barni S, Silvotti MG, Freitas I, Mathe G, Pontiggia P. Hyperthermic effects on the human metastatic liver: a TEM study. *Anticancer Res* 1997; 17: 4713–6.
9. Christophi C, Winkworth A, Muralihdaran V, Evans P. The treatment of malignancy by hyperthermia. *Surg Oncol* 1999; 7: 83–90.
10. Brady LW. Hyperthermia: the development of management fees. In: Steeves RA and Paliwel BR eds. *A Categorical Course in Radiation Therapy: Hyperthermia*. Illinois: Radiological Society of North America, 1987: 179–82.
11. Herman TS, Teicher BA, Jochelson M, Clark J, Svensson G, Coleman CN. Rationale for use of local hyperthermia with radiation and selected anticancer drugs in locally advanced human malignancies. *Int J Hyperthermia* 1988; 4: 143–58.
12. Overgaard J. The current and potential role of hyperthermia in radiotherapy. *Int J Radiat Oncol Biol Phys* 1989; 16: 535–549.
13. Anderson RL, Kapp DS. Hyperthermia in cancer therapy: current status. *Med J Australia* 1990; 152: 310–5.
14. Overgaard J, Gonzalez DG, Hulshof M, Arcangeli G, Dahl O, Mella O, Bentzen SM. Randomised trial of hyperthermia as adjuvant to radiotherapy for recurrent or metastatic malignant melanoma. *Lancet* 1995; 345: 540–3.
15. Engelhardt R. Hyperthermia and drugs. *Recent Re Cancer Res* 1987; 104: 136–204.
16. Moroz P, Jones SK, Gray BN. The status of hyperthermia in the treatment of advanced liver cancer. *J Surg Oncol* 2001; 77: 259–69.
17. Gilchrist RK, Medal R, Shorey WD, Hanselman RC, Parrott JC, Taylor CB. Selective inductive heating of lymph nodes. *Ann Surgy* 1957; 146: 596–606.
18. Medal R, Shorey WD, Gilchrist RK, Barker W, Hanselman R. Controlled radiofrequency generator for production of localized heat in intact animal. *Arch Surg* 1959; 79: 427–431.
19. Gilchrist RK, Shorey WD, Russell C, Hanselman C, Depeyster FA, Yang J, Medal R. Effects of electromagnetic heating on internal viscera: a preliminary to the treatment of human tumours. *Ann Surg* 1965; 161: 890–5.
20. Breedis C, Young G. The blood supply of neoplasms in the liver. *Am J Pathol* 1954; 30: 969–77.
21. Stribley KV, Gray BN, Chimel R, Heggie JCP, Bennett RC. Internal radiotherapy for hepatic metastases; the blood supply of hepatic metastases. *J Surg Res* 1982; 33: 25–32.
22. Archer S, Gray BN. The vascularization of small liver metastases. *Br J Surg* 1989; 76: 545–8.

23. Archer S, Gray BN. Comparison of portal vein chemotherapy with hepatic arterial chemotherapy in the treatment of liver micrometastases. *Am J Surg* 1990; 159: 325–9.
24. Rand RW, Snyder M, Elliot D, Snow H. Selective radiofrequency heating of ferrosilicone occluded tissue. *Bull LA Neurol Soc* 1976; 41: 154–9.
25. Mosso JA, Rand RW. Ferromagnetic silicone vascular occlusion. *Ann Surg* 1973; 178: 663–8.
26. Snyder M, Rand RW. Utilization of barium impregnated ferromagnetic silicone in vascular occlusion. *Bull LA Neurol Soc* 1975; 40: 145–52.
27. Sako M, Morita M, Ohtsuki S, Adachi S, Watanabe H, Hasegawa M, Shimizu T, Iwasawa Y, Okuda K, Fujii M. Studies on selective embolohyperthermic therapy of tumours by using ferromagnetic particles. *J Jap Soc Cancer Ther* 1984; 19: 2168–71.
28. Matsuki H, Yanada T. Temperature sensitive amorphous magnetic flakes for intratissue hyperthermia. *Mat Sci Eng* 1994; A181/A182: 1366–8.
29. Mitsumori M, Hiraoka M, Shibata T, Okuna Y, Masunaga S, Koishi M, Okajima K, Nagata Y, Nishimura Y, Abe M, Ohura K, Hasegawa M, Nagae H, Ebisawa Y. Development of intra-arterial hyperthermia using a dextran magnetite complex. *Intl J Hyperthermia* 1994; 10: 785–93.
30. Tazawa K, Takemori S, Yamashita I, Kato H, Kasagi T, Saito T, Yamamoto K, Katsuyama S, Maeda M, Honda T, Kimura E, Fujimaki M, eds. Intracellular hyperthermia by fixated submicron particle exciting in inductive field of 500 kHz. In: *Hyperthermic Oncology*. London: Taylor & Francis, 1988; 869–70.
31. Mitsumori M, Hiraoka M, Shibata T, Okuno Y, Nagata Y, Nishimura Y, Abe M, Hasegawa M, Nagae H, Ebisawa Y. Targeted hyperthermia using dextran magnetite complex: a new treatment modality for liver tumours. *Hepato-Gastroenterol* 1996; 43: 1431–7.
32. Jordan A, Wust P, Scholz R, Faehling H, Krause J, Felix R. Magnetic fluid hyperthermia. In: Hafeli U, Schutt W, Teller J, Zborowski M, eds. *Scientific and Clinical Applications of Magnetic Carriers*. New York: Plenum Press, 1997; 569–95.
33. Fannin PC, Charles SW. Measurement of the Neel relaxation of magnetic particles in the frequency range 1 kHz to 160 MHz. *J Phys D: App Phys* 1991; 24: 76–7.
34. Minamimura T, Sato H, Kasaoka S, Saito T, Ishizawa S, Takemori S, Tazawa K, Tsukada K. Tumour regression by inductive hyperthermia combined with hepatic embolization using dextran magnetite incorporated microspheres in rats. *Int J Oncol* 2000; 16: 1153–8.
35. Jones SK, Winter J, Gray BN. Treatment of experimental rabbit liver tumours by selectively targeted hyperthermia. *Int J Hyperthermia* 2001; in press.
36. Moroz P, Jones SK, Winter J, Gray BN. Targeting liver tumours with hyperthermia: Ferromagnetic embolization in a rabbit liver tumour model. *J Surg Oncol* 2001; 78: 22–29.
37. Moroz P, Jones SK, Gray BN. The effect of tumour size on ferromagnetic embolization hyperthermia in a rabbit liver tumour model. *Int J Hyperthermia* 2001; in press.
38. Vaupel P. Tumour blood flow. In: Molls M, Vaupel P, eds. *Blood Perfusion and Microenvironment of Human Tumours*. Berlin: Springer-Verlag, 2000; 41–5.
39. Hafstrom LR, Holmberg SB, Naredi PL, Lindner PG, Bengtsson A, Tidebrant G, Schersten TS. Isolated hyperthermic liver perfusion with chemotherapy for liver malignancy. *Surg Oncol* 1994; 3: 103–8.
40. De Vries MR, Rinkes IH, Van De Velde CJ, Wiggers T, Tollenaar RA, Kuppen PJ, Vahrmeijer AL, Eggermont AM. Isolated hepatic perfusion with tumour necrosis factor alpha and melphalan: experimental studies in pigs and phase I data from humans. *Recent Results Cancer Res* 1998; 147: 107–19.
41. Hammerschmidt S, Wahn H, Langmann P, Scheppach W. Acute liver failure after whole body hyperthermia treatment. *German Med Weekly Rev* 1999; 124: 1379–82.
42. Rand RW, Snow HD, Elliott DG, Snyder M. Thermomagnetic surgery for cancer. *App Biochem Biotech* 1981; 6: 265–72.
43. Rand RW, Snow HD, Brown WJ. Thermomagnetic surgery for cancer. *J Surg Res* 1982; 33: 177–83.
44. Luderer AA, Borrelli NF, Panzarino JN, Mansfield GR, Hess DM, Brown JL, Barnett EH. Glass ceramic mediated, magnetic field induced localized hyperthermia: response of a murine mammary carcinoma. *Radiat Res* 1983; 94: 190–8.

45. Borrelli NF, Luderer AA, Panzarino JN. Hysteresis heating for the treatment of tumours. *Phys Med Biol* 1984; 29: 487–94.
46. Hase M, Sako M, Fujii M, Ueda E, Nagae T, Shimizu T, Hirota S, Kono M. Experimental study of embolohyperthermia for the treatment of liver tumours by induction heating to ferromagnetic particles injected into tumour tissue. *Nippon Acta Radiol* 1989; 49: 1171–3.
47. Hase M, Sako M, Hirota S. Experimental study of ferromagnetic induction heating combined with hepatic arterial embolization for the treatment of liver tumours. *Nippon Igaku Hoshasen Gakkai Zasshi* 1990; 50: 1402–14.
48. Chan DCF, Kirpotin DB, Bunn PA. Synthesis and evaluation of colloidal magnetic iron oxides for the site specific radiofrequency induced hyperthermia of cancer. *J Magn Magn Mat* 1993; 122: 374–8.
49. Chan DCF, Kiroptin DB, Bunn PA. Physical chemistry and *in vivo* tissue heating properties of colloidal magnetic iron oxides with increased power absorption rates. In: Hafeli U, Schutt W, Teller J, Zborowski M, eds. *Scientific and Clinical Applications of Magnetic Carriers*. New York: Plenum Press, 1997; 607–18.
50. Jordan A, Scholz R, Wust P, Fahling H, Krause J, Wlodarczyk W, Sander B, Vogl TH, Felix R. Effects of magnetic fluid hyperthermia on C3H mammary carcinoma *in vivo*. *Int J Hyperthermia* 1997; 13: 587–605.
51. Shinkai M, Yanase M, Suzuki M, Honda H, Wakabayashi T, Yoshida J, Kobayashi T. Intracellular hyperthermia for cancer using magnetite cationic liposomes. *J Magn Magn Mat* 1999; 194: 176–84.
52. Hilger I, Hergt R, Kaiser WA. Effects of magnetic thermoablation in muscle tissue using iron oxide particles, an *in vitro* study. *Invest Radiol* 2000; 35, 170–9.
53. Hilger I, Andra W, Hergt R, Hiergeist R, Schubert H, Kaiser WA. Electromagnetic heating of breast tumours in interventional radiology: *in vitro* and *in vivo* studies in human cadavers and mice. *Radiol* 2001; 218: 570–5.
54. Gordon RT, Hines JR, Gordon D. Intracellular hyperthermia: a biophysical approach to cancer treatment via intracellular temperature and biophysical alterations. *Med Hypo* 1979; 5: 83–102.
55. Suzuki S, Arai K, Koike T, Oguchi K. Studies on liposomal ferromagnetic particles and a technique of high frequency inductive heating *in vivo* studies of rabbits. *J Jap Soc Cancer Ther* 1990; 25: 2649–58.
56. Suzuki M, Shinkai M, Kamihira M, Kobayashi T. Preparation and characteristics of magnetite labelled antibody with the use of polyethylene glycol derivatives. *Biotechnol Appl Biochem* 1995; 21: 335–45.
57. Shinkai M, Yanase M, Honda H, Wakabayashi T, Yoshida J, Kobayashi T. Intracellular hyperthermia for cancer using magnetite cationic liposomes: *in vitro* study. *Jap J Cancer Res* 1996; 87: 1179–83.
58. Shinkai M, Yanase M, Suzuki M, Honda H, Wakabayashi T, Yoshida J, Kobayashi T. Intracellular hyperthermia for cancer using magnetite cationic liposomes. *J Magn Magn Mat* 1999; 194: 176–84.
59. Le B, Shinkai M, Kitade T, Honda H, Yoshida J, Wakabayashi T, Kobayashi T. Preparation of tumour specific magnetolipiosomes and their application for hyperthermia. *J Chem Eng Jap* 2001; 34: 66–72.
60. Jordan A, Wust P, Fahling H, John W, Hinz A, Felix R. Inductive heating of ferromagnetic particles and magnetic fluids: physical evaluation of their potential for hyperthermia. *Int J Hyperthermia* 1993; 9: 51–68.
61. Jordan A, Wust P, Scholz R, Tesche B, Faehling H, Mitrovics T, Vogl T, Cervos-Navarro J, Felix R. Cellular uptake of magnetic fluid particles and their effects on human adenocarcinoma cells exposed to AC magnetic fields *in vitro*. *Int J Hyperthermia* 1996; 12: 705–22.
62. Jordan A, Scholz R, Wust P, Schirra H, Schiestel T, Schmidt H, Felix R. Endocytosis of dextran and silan coated magnetite nanoparticles and the effect of intracellular hyperthermia on human mammary carcinoma cells *in vitro*. *J Magn Magn Mat* 1999; 194: 185–96.
63. Jordan A, Scholz R, Klaus MH, Johannsen M, Wust P, Nadobny J, Schirra H, Schmidt H, Deger S, Loening S, Lanksch W, Felix R. Presentation of a new magnetic field therapy

system for the treatment of human solid tumours with magnetic fluid hyperthermia. *J Magn Magn Mat* 2001; 225: 118–26.
64. Nakajima T, Roberts DW, Ryan TP, Hoopes PJ, Coughlin CT, Trembly BS, Strohbehn JW. Pattern of response to interstitial hyperthermia and brachytherapy for malignant intracranial tumour: a CT analysis. *Int J Hyperthermia* 1993; 9: 491–502
65. Emami B, Scott C, Perez C, Asbell S, Swift P, Grigsby P, Montesano A, Rubin P, Curran W, Delrowe J, Arastu H, Fu K, Moros E. Phase III study of interstitial thermoradiotherapy compared with interstitial radiotherapy alone in the treatment of recurrent or persistent human tumours: a prospectively controlled randomised study by the radiation therapy oncology group. *Int J Radiat Oncol Biol Phys* 1996; 34: 1097–104.
66. Burton AL, Hill M, Walker AE. The RF thermoseed—a thermally self-regulating implant for the production of brain lesions. *IEEE Trans Biomed Eng* 1971; 18: 104.
67. Kida Y, Ishiguri H, Ichimi K, Kobayashi T. Hyperthermia of metastatic brain tumour with implant heating system: a preliminary clinical result. *Neurol Surg* 1990; 18: 521–6.
68. Kobayashi T, Kida Y, Tanaka T, Hattori K, Matsui M, Amemiya Y. Interstitial hyperthermia of malignant brain tumours by implant heating system: clinical experience. *J Neuro-Oncol* 1991; 10: 153–63.
69. Stea B, Kittleson J, Cassady JR, Hamilton A, Guthkelch N, Lulu B, Obbens E, Rossman K, Shapiro W. Treatment of malignant gliomas with interstitial irradiation and hyperthermia. *Int J Radiat Oncol Biol Phys* 1992; 24: 657–67.
70. Stea B, Rossman K, Kittleson J, Shetter A, Hamilton A, Cassady JR. Interstitial irradiation versus interstitial thermoradiotherapy for supratentorial malignant gliomas: a comparative survival analysis. *Int J Radiat Oncol Biol Phys* 1994; 30: 591–600.
71. Mack CF, Stea B, Kittleson JM, Shimm DS, Sneed PK, Phillips TL, Swift PS, Luk K, Stauffer PR, Chan KW, Steeves R, Cassady JR, Cetas T. Interstitial thermoradiotherapy with ferromagnetic implants for locally advanced and recurrent neoplasms. *Int J Radiat Oncol Biol Phys* 1993; 27: 109–15.
72. Tohnai I, Goto Y, Hayashi Y, Ueda M, Kobayashi T, Matsui M. Preoperative thermochemotherapy of oral cancer using magnetic induction hyperthermia. *Int J Hyperthermia* 1996; 12: 37–47.
73. Agaki M, Tsuboyama T, Ikenaga M, Matsusue Y, Hiraoka M, Nakamura T. Antitumour effects of localized hyperthermia on an experimental bone tumour using an intramedullary nail. *Int J Hyperthermia* 1997; 13: 387–400.
74. Takegami K, Tetsuya S, Hiroki W, Jun S, Takashi Y, Seiichi M, Takehiro S, Atsumasa U. New ferromagnetic bone cement for local hyperthermia. *J Biomed Mater Res* 1998; 43: 210–4.
75. Steeves RA, Tompkins DT, Nash RN, Blair JR, Gentry LL, Bhudatt PR, Murray TG, William ME. Thermoradiotherapy of intraocular tumours in an animal model: concurrent versus sequential brachytherapy and ferromagnetic hyperthermia. *Int J Radiat Oncol Biol Phys* 1995; 33: 659–62.
76. Murray TG, Obrien JM, Steeves RA, Smith BJ, Albert DM, Cicciarelli N, Markoe AM, Tompkins DT, Windle JJ. Radiation therapy and ferromagnetic hyperthermia in the treatment of murine transgenic retinoblastoma. *Arch Opthalmol* 1996; 114: 1376–81.
77. Murray TG, Steeves RA, Gentry L, Bresnick G, Boldt HC, Mieler WF, Tompkins D. Ferromagnetic hyperthermia: functional and histopathologic effects on normal rabbit ocular tissue. *Int J Hyperthermia* 1997; 13: 423–36.
78. Loening SA, Tucker RD. Treatment of prostate cancer by thermal ablation using interstitial temperature self-regulating seeds. *Poster Presentation, 15th World Congress on Endourology* 1997; http://ablationtech.com/poster.htm.
79. Tucker RD. The principle of induction heating for the thermal ablation of tissue in medical applications 2000; http://ablationtech.com/abinductheat.htm.