PRE CLINICAL AND CLINICAL DEVELOPMENT OF MICROWAVE ABLATION AS A TREATMENT FOR UNRESECTABLE COLORECTAL LIVER METASTASES

Thesis submitted for the degree of

Doctor of Medicine

At the University of Leicester

By

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University of Leicester

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STATEMENT OF ORIGINALITY

The Work on which this thesis is based is my own, independent work except where acknowledged

Production of the microwave and permittivity devices, investigation of field characteristics, supply of materials was by Dr Peter Clegg and Professor Cronin of the Medical Devices Technology Group, University of Bath. Experiments utilising this equipment were devised and carried out by the author although in many cases this required assistance from Dr Clegg in equipment operation.

Andy Strickland

June 2004

"What drugs will not cure, the knife will; what the knife will not cure, the cautery will; what the cautery will not cure must be considered incurable."

Hippocrates circa 460 BC

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PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS

Strickland AD, Clegg PJ, Cronin NJ, Swift B, Festing M, West KP, Robertson GSM, Lloyd. An experimental study of large volume ablation in the liver. British Journal of Surgery. 2002; 89 1003-8.

Swift B, Strickland AD, Clegg PJ, Cronin NJ, Wills J, West KP, Lloyd DM. The histological features of microwave coagulation therapy: an assessment of a new applicator design. International Journal of Experimental Pathology. 2003; 84 17-29.

Strickland AD, Clegg PJ, Cronin NJ, El Abassy M, Lloyd DM. Rapid microwave ablation of large hepatocellular carcinoma in the high risk patient. Asian Journal of Surgery 2005; 28 2, 151-3.

A.D.Strickland, F. Ahmad, DM Lloyd. Tumour Ablation: Principles and Practice. Chapter 17b: Microwave Ablation – Surgical Perspective. Pages 215-221. ISBN 0-387-95539-9 (2005).

Ahmad F, Strickland AD, Wright GM, Elabassy M, Kiruparan P, Bell PRF, Lloyd DM. Laparoscopic Microwave Tissue Ablation of Hepatic Metastasis from a Parathyroid Carcinoma. *European Journal of Surgical Oncology*. In Press.

Strickland AD, Robertson GSM, Lloyd DM. Ablative options for unresectable colorectal liver metastases a review. (Submitted)

Strickland AD, Sutton CD, Clegg PJ, Cronin NJ, Thomas A, Lloyd DM. Microwave ablation and anti-angiogenic therapy: New hope for previously untreatable metastatic

colorectal cancer of the liver. (Submitted)

Strickland AD, Lloyd DM Microwave ablation as a treatment for colorectal liver metastases. (Submitted)

P. Clegg, N. Cronin, A. Strickland, G. Robertson, A. Dennison, D. Berry, D. Lloyd.In Vivo Complex Permittivity Measurements of Normal Human Liver Parenchyma and Secondary Liver Metastases Over the Frequency Range 0.5 – 10 GHz. (submitted)

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Internet Publications

Strickland AD, Lloyd DM. The treatment of inoperable liver cancer: Non-surgical treatments. British Liver Trust Website (accepted for publication on the website)

Strickland AD, Lloyd DM.Liver cancer: Surgery. British Liver Trust Website (accepted for publication on the website)

PUBLISHED ABSTRACTS

"Single treatment large volume microwave ablation in liver is now achievable". Presented at World Congress of Digestive Surgery 2002. Asian Journal of Surgery 26 No. 1 Jan 2003 S90.

"Microwave penetration through human liver parenchyma and hepatic colorectal metastases". Presented at World Congress of Digestive Surgery 2002. Asian Journal of Surgery 26 No. 1 Jan 2003 S90.

"In-vivo complex permittivity measurements of human liver parenchyma and hepatic colorectal metastases". Presented at Association of Surgeons Dublin 2002. British Journal of Surgery. 2002; 89, sppl. 1, 53.

Microwave Liver Ablation – Early Clinical Results in the Treatment of Inoperable Colorectal Metastases with a New Applicator. Presented at 5th European IAHPB meeting Istanbul, May 2003.

"Microwave coagulation therapy for metastatic liver tumours from colorectal cancer: a clinical study". Abstract published: Journal of Pathology 2001; 192 (supplement) 3A.

PRESENTATIONS TO LEARNED SOCIETIES

"Microwave ablation for Liver tumours" was presented at the East Midlands Surgical Society 2001.

"Management of colorectal metastases: Surgery and ablative techniques" Institute of Cancer Study Day 2001

"Microwave ablation technical and development details" was presented to the Department of Hepatobiliary Surgery, University of Essen, Germany 2001

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Andrew Strickland, Peter J Clegg, Nigel J Cronin, Fateh Ahmad, David M Lloyd. Optimising Efficiency of Microwave Ablation in Normal Hepatic Parenchyma and Hepatic Colorectal Metastases: Selection of the Optimum Microwave Frequency.

American HepatoPancreatoBiliary Association (AHPBA) Annual Meeting, Florida, USA, 15-17th April 2005.

Ahmad F, Strickland AD, Clegg PJ, Cronin NJ, Lloyd DM. Successful large volume microwave tissue ablation of metastasis from parathyroid carcinoma. Association of Surgeons of Great Britain and Ireland Annual Meeting, Glasgow, UK, 13-15th April 2005

F. Ahmad, A. D. Strickland, D. M. Lloyd. Rapid large volume microwave tissue ablation (MTA) of liver tumours using a novel applicator, 50th Annual Meeting of the Society of Academic and Research Surgery (SARS), Newcastle, United Kingdom, 12-13th January 2005

F. Ahmad, A. D. Strickland, D. M. Lloyd. Rapid large volume microwave tissue ablation (MTA) of liver tumours using a novel applicator. Prizewinner. 4th International Meeting on Hepatocellular Carcinoma, Hong Kong, 14-16th December 2004

F. Ahmad, A. D. Strickland, D. M. Lloyd. Laparoscopic large volume, single insertion, microwave tissue ablation (MTA) of liver tumours using a novel applicator. 19th World Congress of the International Society for Digestive Surgery, Yokohama, Japan, 8-11th December 2004

F. Ahmad, A. D. Strickland, D. M. Lloyd. Rapid large volume microwave tissue ablation (MTA) of liver tumours using a novel applicator. Shortlisted for Prize. 19th

World Congress of the International Society for Digestive Surgery, Yokohama, Japan, 8-11th December 2004

Lloyd DM, Strickland AD, Clegg PJ, Cronin NJ, Elabassy M, Ahmad F. Rapid large volume microwave tissue ablation (MTA) of unresectable liver tumours using a novel applicator. 6th World Congress of the International HepatoPancreatoBiliary Association (IHPBA) Meeting, Washington, USA, 2-6th June 2004

Strickland A.D, Ahmad F, Lloyd D. M. Large volume microwave tissue ablation (MTA) of unresectable liver tumours using a novel applicator design. Prizewinner. East Midlands Surgical Society Annual Meeting, Rotherham, UK, 15th April 2004

DM Lloyd, F Ahmad, AD Strickland. Microwave Ablation for Liver Tumours.. 5th European Congress of the International HPB Association (IHPBA), Istanbul, Turkey, May 2003

Advances in Microwave Ablation of Liver Tumours. DM Lloyd, AD Strickland, F Ahmad, Clegg P, Cronin N. American Congress of Surgeons. Chicago, USA, October 2003

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CHAPTER 1

TREATMENT OF COLORECTAL LIVER METASTASES: A REVIEW OF CURRENTLY AVAILABLE ABLATIVE TECHNIQUES

1.1 INTRODUCTION

1.2 THERMAL TECHNIQUES: AN OVERVIEW

1.3 MICROWAVE

i) Equipment Advances

ii) Cytotoxicity of Radiofrequency

iii) Treatment Monitoring

iv) Complications

v) Results

vi) Conclusion

1.4 NON THERMAL TECHNIQUES

1.5 FUTURE DEVELOPMENTS

1.6 ABLATIVE TREATMENTS: SUMMARY

1.7 TABULAR COMPARISON OF ABLATIVE TECHNIQUES

1.1 INTRODUCTION

Colorectal cancer is the third most common cancer in the world. Since 1970 the number of patients diagnosed with this disease has increased year on year with 676,000 patients diagnosed in 1990 (Boyle 1997). Of this number up to 50% will go on to develop liver metastases (Levitan et al. 1990).

For patients with liver metastases of colorectal origin, hepatic resection is the only treatment which is curative with 5 year survival rates varying between 25 and 40 % (Fong et al. 1999, Scheele et al.1991). Patients who are unsuitable for surgery or do not wish to undergo an operation have a much bleaker prognosis as median survival for this group is less than 12 months (Stangl et al. 1994). Only 20% of patients with colorectal metastases are suitable for an operation due in part to the large amount of normal liver resected during surgery (Geoghegan et al. 1999). Bi-lobar distribution of metastases can be a contraindication to surgery, because the amount of hepatic parenchyma resected would leave a liver remnant of insufficient volume, resulting in hepatic failure.

Destruction or ablation of tumour tissue causing a minimal disturbance to the surrounding liver parenchyma is an attractive proposition for patients with conventionally unresectable disease and has led to considerable work in this field. Although a number of ablative techniques have undergone preliminary investigation there is no consensus regarding the most effective modality.

By definition ablative treatments leave volumes of dead tissue within the liver substance. This leads to a number of potential complications in particular, pleural effusions, abscess formation, and biliary or vascular damage are important. Local recurrence in ablated lesions is an important problem associated with some techniques as cell death is not predictable at the peripheries of an ablation. This is particularly common when tumours are adjacent to vascular structures which act as a heat sink. Many treatments are limited by the relatively small volume of ablation produced by a single insertion of the probe and multiple overlapping treatments are therefore required for the complete destruction of larger tumours. The significance of these problems varies between different ablative techniques, the details of which will be discussed.

The majority of ablative techniques rely on the insertion of a device (usually referred to as a probe or an applicator) into the tumour laparoscopically, percutaneously or at laparotomy. The device is commonly positioned using intra-operative ultrasound or CT/MRI guidance. Many treatments are monitored in real-time in an effort to ensure complete tumour ablation and prevent significant collateral damage. Ablation of the tumour tissue is brought about in a number of different methods but these techniques can be divided broadly into thermal and non-thermal treatments.

1.2 THERMAL TREATMENTS: AN OVERVIEW

Many different thermal treatments exist including cryotherapy, radiofrequency, laser, microwave and focussed ultrasound. Heat is a potent method of inducing cell death and is introduced into the liver from this variety of different energy sources (see Section 1.7 for a summary shown in tabular form). To date, the vast majority of patients who have undergone ablative treatments have been treated using thermal methods. In the interests of brevity, the alternative ablative techniques are not reviewed in detail although each is compared and contrasted in the table at the end of this chapter.

1.3 MICROWAVE

Microwaves form part of the electromagnetic spectrum causing heating due to the interaction between water molecules and the waves. This method of ablation has been popularised in Japan and other centres in the Far East. Treatment involves the percutaneous introduction of a thin electrode (applicator) into the tumours. Microwaves are released from the applicator forming a field around its tip. Direct heating of water molecules occurs in the microwave field produced around the applicator rather than by conduction from the probe itself (Tabuse 1998). Heating is not, therefore, purely reliant on conduction through the tissues and cytotoxic temperature increases are reached rapidly. Outside the microwave field, however, heating of tissue occurs by thermal conduction.

i) Equipment Advances

Initially microwave devices were used as an adjunct to surgery to help arrest bleeding from the spleen and liver (Tabuse and Katsumi 1981). It was later developed as an ablative treatment (Yamaue et al. 1984). Most microwave systems are able to produce a volume of ablation approximately 2cm in diameter and patients often undergo multiple insertions of the electrode at two or three different sessions to enable larger tumours to be ablated. To increase the size of the single treatment ablation volume different techniques have been employed. One of the most successful is the attenuation of the blood supply to the liver. The large volume of blood flowing through the liver's parenchyma was thought to significantly reduce the ablated volume (Shibata et al. 2000). This hypothesis was supported by the fact that occlusion of the portal vein or hepatic artery increased the ablated volume, while interrupting both vessels produced a still larger lesion (Shibata et al. 2000, Takamura et al. 2001). Pigs' livers were treated with microwave therapy with and without a Pringle manoeuvre with the finding that the diameter of the ablated area increased almost 100% (9.8mm to 18.8mm) and the volume approximately 400%. The technique was applied to humans and a similar effect was seen when tumours were treated. An increase of 26.9 to 41.1mm in ablation diameter was noted when a Pringle manoeuvre was utilised. Blood flow attenuation may have aided the increase in the proportion of tumours that were completely treated with blood flow interruption observed in the trial (Shibata et al. 2000). As the human

treatments were performed percutaneously, the interruption of the liver's blood supply was achieved using a balloon catheter introduced through the femoral vessels. This, however, does limit the degree to which this technique can be considered as minimally invasive and increases the expertise and time requirements. A newer generation of microwave technology has been developed using novel ceramics to allow more efficient radiation of microwave energy into tissue. It is this technology on which this thesis is based.

ii) Cytotoxicity of treatment

Microwave energy causes heating to tissue due to its effect on water molecules. Exposures are usually very short with most systems producing lesions within two to three minutes. The temperatures around the heating applicator reach in excess of 140°C and fall rapidly at increasing distances from the probe. As with many other heat dependent ablative techniques, an obvious blanched area is produced and the temperature at the interface between macroscopically normal and abnormal liver is approximately 62°C +/- 6°C (Dong et al. 1998). This implies that the macroscopic edge of the lesion may well not be the edge of the cellular effect as this temperature is achieved over a number of minutes.

Ablation size is limited as currently available microwave applicators are capable of producing 2.0cm diameter ellipsoids. The consequence of this ablation configuration is that many liver metastases require repeated

insertions of the probe or repeated treatment sessions (Shibata et al. 2000, Seki et al. 1999). Although microwaves produce intensely cytotoxic environments very quickly, the relatively small volume of ablation achieved in a single treatment limits its use as an ablative technique for small tumours. Experiments in rats with implanted colorectal liver tumours demonstrated the cytotoxic effect of the microwave as a potentially curative treatment (Chen et al. 1999). Confirmation of the cytotoxic effect came from biopsies of human tumours post treatment in which complete tumour destruction in 8 of the 19 patients investigated was demonstrated (Dong et al. 1998). The majority of work involving microwave ablation has taken place in Japan and other parts of the Far East; consequently most clinical research has been performed in patients with hepatocellular cancer.

iii) Monitoring of treatment

Monitoring of treatment is usually performed using ultrasound. Early studies investigating microwave treatment of obstructive prostate cancer demonstrated the microwave applicator was easily identified using trans-abdominal ultrasound (Harada et al. 1990). During the treatment the heated tissue became hyper-echoic in comparison to the surrounding normal tissue. The size of the hyper-echoic area enlarged over the course of the microwave treatment which lasted approximately 60 seconds. A similar pattern was seen when microwave ablation was used as a treatment for unresectable hepatocellular cancers (Seki et al. 1994) although this appearance changed to one of mixed hyper- and hypo-echogenicity within two weeks. This

mixed echo pattern remained constant for the period of follow up (11-33 months). The increase in echogenicity immediately following treatment allows clinicians to estimate the completeness of treatment.

With microwaves producing a relatively small volume of ablation, treatment of tumours 2cm in diameter and above required multiple insertions of the probe. The change in the echogenicity of the ablated region combined with the acoustic shadowing it produces can obscure the tumour margins (Takeuchi et al. 1998). In an attempt to overcome this difficulty, Takeuchi and colleagues used colour doppler imaging to assess the completeness of ablations (Takeuchi et al. 1998). This group found the changes generated by using colour doppler scanning after treatment correlated well with post-operative CT and MRI scans.

iv) Results of treatment

In common with many ablative treatments results of microwave treated colorectal liver metastases are short term and directly related to the size of tumour/s ablated. Encouraging results were found in the treatment of small (less than 2cm in diameter) hepatocellular cancers in which no local recurrence in the treated areas occurred in any of the 18 patients studied (follow up 11-33 months) (Seki et al. 1994). In a similar study investigating the effect of the microwave on colorectal metastases, 2 of 15 patients experienced recurrences in what were considered to be completely ablated lesions (follow up 9-37 months) (Seki et al. 1999). This study, however,

only included patients with colorectal metastases 3cm or less in diameter. Other work comparing results of ablation with and without hepatic blood flow attenuation found a 7% versus 45% respective local recurrence rate (Shibata et al. 2000). The difference in the rate of recurrence was ascribed to the larger volume of ablation achieved by reducing blood flow through the liver which reduced the heat sink effect of its vasculature (Shibata et al. 2000). A recent study randomised patients with resectable colorectal liver metastases to hepatic resection or microwave ablation. Patient survival at 3 years in these two groups was similar although it was lower in the microwave group 14% compared to 23% although the difference was not statistically significant (Shibata et al. 2000).

v) Complications of Treatment

The usual complications of ablative therapy such as abscess formation, post-procedure fever and right upper quadrant pain have been described for microwave treatment (Shibata et al. 2000, Seki et al. 1999). Microwave heating occurs rapidly especially within the field and therefore structures such as vessels may be at risk from such a technique. Due to their relatively low flow, biliary structures are at particular risk of damage. Shimada et al. suggested intra-biliary cooling with cold saline may help to prevent biliary damage. In patients with tumours near biliary structures treated in this manner, microwave coagulation did not damage these structures (Shimada et al. 1998).

Other more serious problems such as intra-peritoneal seeding have also been documented (Shimada et al. 1998). Peritoneal seedlings were described in two patients both of whom had tumours on the surface of their liver. It is not difficult to imagine if the microwave electrode was inserted a number of times into these areas, small pieces of malignant tissue could be dislodged into the peritoneal cavity. It may be that single insertion microwave applicators reduce or eliminate this risk.

Percutaneous microwave applicators have caused burns to the skin and subcutaneous tissue of some patients. This is due to reflection of the microwaves at the applicator tissue interface causing excessive heating of the applicator shaft following prolonged or high wattage treatments (Shimada et al. 2000, Dong et al. 1998).

vi) Conclusion

Microwave ablation uses the heating effect of an alternating electromagnetic wave on tissue water. Rapid ablation of small tumours has been achieved successfully, although results in larger tumours are not commonly reported. The small volume of cell death achieved using microwave ablation requires tumours over 2cm in diameter to undergo multiple insertions of the electrode at more than one treatment session. Most microwave systems allow percutaneous treatment avoiding the cost of laparotomy and expensive

in-patient hospitalisation. The difficulty in treating lesions 2cm and larger has limited the popularity of currently available microwave systems.

1.4 NON-THERMAL TECHNIQUES: AN OVERVIEW

Non-thermal ablation techniques rely on an alteration in the local chemical environment of the metastasis to cause tumour cell death. Fewer patients with colorectal metastases have been treated with these techniques although percutaneous ethanol injection (PEI) has long been recognised as an effective and cheap method of ablating small hepatocellular cancers. In contrast to some of the thermal techniques in which energy is radiated from an applicator tip in a field, both PEI and electrolysis rely on passive diffusion of chemicals to cause tumour cell death. Real-time monitoring of these techniques is also far more challenging.

1.5 FUTURE DEVELOPMENTS

Many problems exist with ablative techniques not least of which is the volume of ablation produced by a single treatment. The majority of technologies utilise overlapping treatments to completely destroy a tumour with the 0.5-1.0cm margin suggested by many clinicians. Much of the difficulty in treating larger tumours is due to the difficulty of introducing the power required to ablate all the required tissue. Newer applicators are improving the delivery of the ablative energy and therefore the volume of tissue treated though often at the cost of increasing the diameter of the probe

or applicator introduced. Larger diameter applicators can often only be used at open or laparoscopic surgery limiting the patient population that may benefit. Development of percutaneously introduced applicators capable of delivering high levels of ablative energy safely and without damaging the tissues they are introduced through remains one of the biggest challenges to be overcome.

Improvements in pre-operative imaging are likely to have a major impact not only on patient selection but also on treatment monitoring and assessment of ablation. In our experience, imaging often underestimates the number of metastatic deposits present in a particular patient although this has rarely impacted on the decision to ablate. Increasingly accurate pre-treatment imaging may allow a greater proportion of patients to undergo potentially curative, percutaneous treatments.

Confirmation of the completeness of the destruction of a given tumour is an important issue for all ablative techniques. Improvements in real-time imaging have been rapid and it is possible that trans-abdominal ultrasound may become superseded by MRI for the assessment of ablation in percutaneously delivered systems. The use of MRI scanning for not only placement but temperature and ablation monitoring is an exciting development although the costs involved in using such equipment may be prohibitive for many centres.

1.6 ABLATIVE TREATMENTS: SUMMARY

Although ablative therapies have undergone considerable development in recent years no consensus over the most efficacious exists. Many of these treatments have been associated with encouraging results but no randomised controlled trials exist. For widespread adoption of such techniques such trials are required and due to patient numbers these will need to be multicentre. The gold standard treatment for colorectal hepatic metastases is currently surgery, however it may not be long before trials of surgery versus ablative treatments for such patients become ethical. It is likely that this technology is here to stay and will play an increasingly significant role in the treatment of liver malignancies.

Technique	Insertion	Tumour Destruction	Real-time Monitoring	Ablation Diameter*	Complications/ Problems	Special Features/notes
Cryotherapy	At laparotomy, rarely percutaneously	Cooling causing intracellular ice & osmotic changes	Intra-operative Ultrasound	Large: 8cm	Cryoshock (multi-organ dysfunction syndrome), cracking and bleeding from ice ball, high local recurrence rate	Many patients treated, more long-term F/U data
Radio frequency	At laparotomy, laparoscopic or percutaneous	Heat via cellular oscillation from alternating current	Intra-operative or percutaneous ultrasound	Moderate: 4-5cm	Vessel damage from heat and skin damage from grounding pad heat.	Popular choice with clinicians due to reasonable ablation volume coupled with flexible insertion
Laser therapy	Mainly percutaneous	Heat from contact with a laser light source	Magnetic Resonance Imaging	Small: 2-3cm	Few complications noted, large tumours not treatable	Limited by small ablation volume costly equipment & technical expertise required
Microwave coagulation	At laparotomy or percutaneous	Heat from a radiated microwave field	Intra-operative Imaging	Small: 2 cm unless blood flow attenuated	Vessel damage and burns to skin from percutaneous treatments reported	Reflection of power at applicator/tissue interface reduces efficiency of system
Focussed Ultrasound	Extra-corporeal treatments	Heat induced through acoustic agitation of cells	Percutaneous ultrasound	Small: 2mm	Skin burns and liver lacerations reported. Problems producing complete ablations	Practical difficulties in achieving complete tumour ablations
Percutaneous Ethanol Injection	Percutaneous	Dehydration, denaturation of cell proteins	Percutaneous Ultrasound	Small: 2cm	Inability to cause complete tumour ablation	Now rarely used for ablation of colorectal metastases
Electrolysis	Laparotomy	pH changes around electrodes	No method available	Moderate: ~ 4cm	None reported. Long treatment times	Limited human data

1.7 Tabular Comparison of Ablative Techniques. * Following single insertion of device.

CHAPTER 2

AN INTRODUCTION TO MICROWAVE PHYSICS, WAVE THEORY, ELECTROMAGNETIC AND MICROWAVE APPLICATOR DESIGN

2.1 WAVE BASICS

i) **Definitions and characteristics of a wave**

ii) **Waveforms**

iii) **Mechanical and Electromagnetic waves**

iv) **Electromagnetic spectrum**

v **Characteristics of microwaves**

vi) **Production of microwaves**

2.2 MICROWAVE ENDOMETRIAL ABLATION (MEA)

i) **Microwave ovens**

ii) **Menorrhagia**

iii) **Effect of frequency on microwave penetration and development of the MEA applicator**

iv) **Development of a microwave applicator for the treatment of colorectal liver metastases**

2.1 WAVE BASICS

i) Definitions and characteristics of a wave

The definition of a wave is a disturbance which is periodic in time and space. In general, waves have a number of features:-

1. Exhibit no net transport of material
2. Transport energy
3. Have characteristic waveforms
4. Propagate at uniform waveform-independent speed
5. Have speeds dependent on medium

Using sound as an example waveform, these features can be explored to gain understanding as to how they apply to the electromagnetic waveform of a microwave. Sound waves certainly do not transport material although one can appreciate its energy transporting ability by standing near to a music speaker at high volume. The characteristic waveform of each wave is very important as it is this waveform that gives it its distinguishing feature. Using light to illustrate this point, each colour of light in the visible spectrum has that characteristic due to its wavelength; any change in this waveform will alter the colour interpreted by the observer. The waveform has no effect on the speed of transmission as there is no difference in the speed of sound travel in between high and low frequency sound (if this was the case music would be very disorientating and to be comprehensible it would have to be transmitted in a staggered manner according to the wavelengths of the different components).

Similarly the energy associated with the waveform does not affect the speed of transmission: high volume sound travels no more quickly than lower energy noise. The dependency of sound wave speed on the transmitting medium can be appreciated by comparing the speed of transmission through solid and gaseous mediums; for example, an on coming locomotive will always be heard through vibrations in the railway tracks before the atmosphere due to the difference in transmission speeds through the media (sound energy being transmitted more quickly through solid media).

ii) Waveforms

The illustrations show the usual manner in which a wave is displayed. A wavelength is illustrated in figure 2.1 and is represented by the symbol λ.

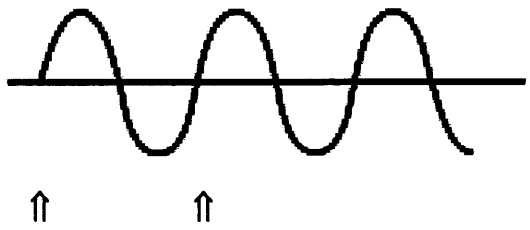

Figure 2.1. Typical graphic of a wave, the distance between the two arrowheads depict 1 wavelength (λ)

The wavelength of a signal does not, however, denote the amount of energy carried by that wave merely the interval between two similar phases of the wave. The amount of energy carried by a wave is shown by its amplitude. Amplitude is shown graphically by the maximum deviation from the mean. The following two figures illustrate the difference between two waves with the same wavelengths but differing amplitudes.

The wave on the left has a small deviation from the mean compared with that to the right. This indicates the wave to the right carries the greater energy. High volumes of sound will therefore have waves with high amplitudes compared to those at lower volumes.

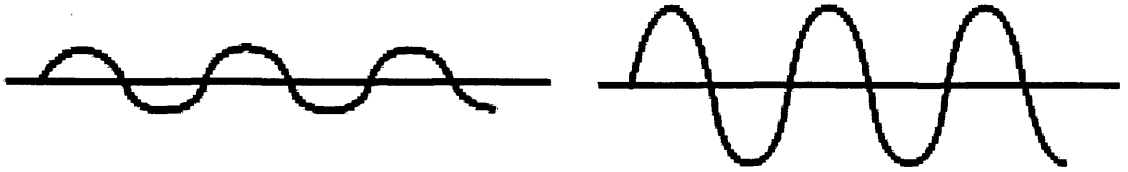

Figure 2.2. Comparison between two waves of equal wavelength but different amplitudes. The wave to the right of the figure shows far more deviation from the mean (line drawn through the middle of each waveform) indicating it carries a greater amount of energy.

iii) Mechanical and Electromagnetic waves.

A number of the elements of this chapter have been illustrated using analogies between microwaves and sound energy. Some significant differences, however, are found between these two sources not least of which are their transmission characteristics. Sound is not part of the electromagnetic spectrum as it is a mechanical wave thereby requiring matter for propogation of the sound wave. Electromagnetic waves have no such restrictions and as such can be propagated through a vacuum. Indeed electromagnetic waves may be propagated through a vacuum without any loss of energy contained in the wave. Electromagnetic waves are further distinguished from mechanical waves by the components from which they are made. All the waves in the electromagnetic spectrum are made up of a combination of a magnetic and electric portions. These both vary at time dependant intervals in a direction that is 90°

to the direction of the travel of the wave and to each other (see Figure 2.3).

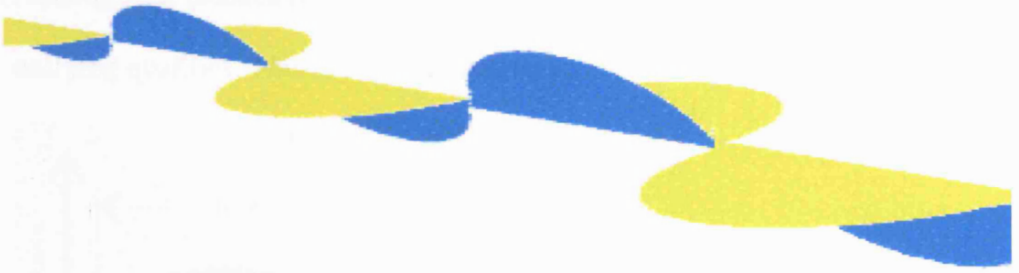

Figure 2.3. Graphical representation of an electromagnetic wave showing the electrical (blue) and magnetic (green). It can be seen that the electrical and magnetic portions of the wave act at 90 degrees to one another.

iv) Electromagnetic spectrum

The electromagnetic spectrum merely describes a range of electromagnetic waves (i.e. non-mechanical waves) which have wavelengths that vary from extremely short (gamma rays) to those that have very long wavelengths (radio waves). An example of the electromagnetic spectrum is seen below in Figure 2.4. From the schematic it can be appreciated that these waves have fundamentally different characteristics based upon their wavelength.

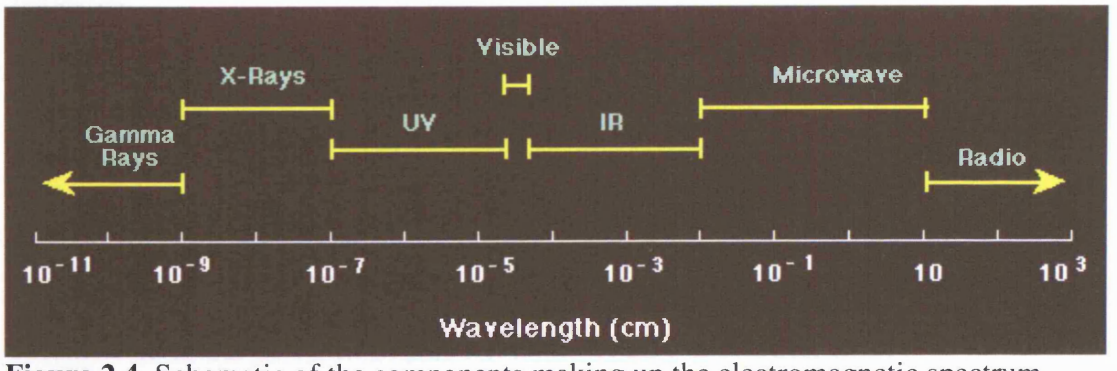

Figure 2.4. Schematic of the components making up the electromagnetic spectrum (EMS).

Although the characteristics of the spectrum vary considerably there are a number of unifying qualities. This is best summed up in the following Figure 2.5.

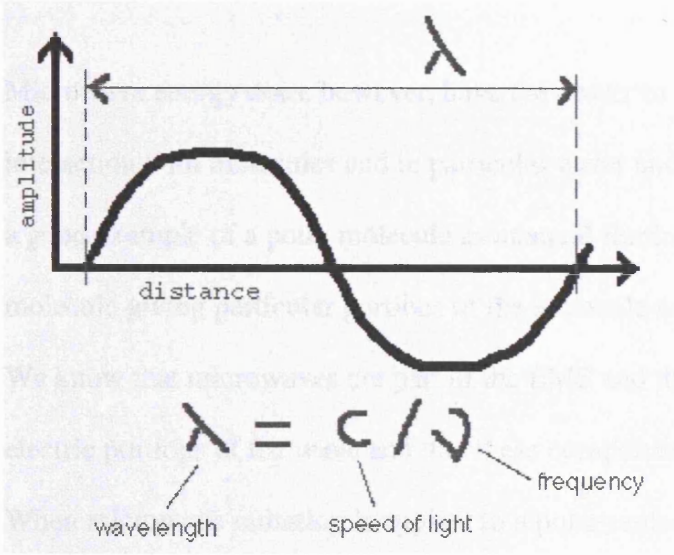

Figure 2.5. Unifying equation of the components of the EMS.

All the waves in the EMS are governed by this equation. The waves move through a vacuum at the speed of light and the other characteristics of the wave have precise mathematical relationships to one another. Electromagnetic waves are produced by the motion of electrically charged particles. These waves are also called "electromagnetic radiation" because they radiate from the electrically charged particles.

v) Characteristics of microwaves

Microwaves are part of the electromagnetic spectrum with wavelengths between 300 MHz (3x 10 to the power of 8) and 300 GHz (3x 10 to the power of 11). In terms of the EMS, the frequency of these waves is not particularly high. This is important as microwave radiation is **non ionising** and therefore does not possess the energy to

displace electrons from atoms in the way that x- and gamma rays are able to. This prevents microwaves from inducing the genetic mutations by ionising molecules within DNA.

Microwave energy does, however, have the power to induce frictional heating from its interaction with molecules and in particular water and other polar molecules. Water is a good example of a polar molecule as unequal sharing of electrons takes place in the molecule giving particular portions of the molecule net positive and negative charge. We know that microwaves are part of the EMS and therefore have magnetic and electric portions of the wave and that these components are continually fluctuating. When microwave radiation is applied to a polar molecule such as water, the molecule is continually rotating to align itself with the fluctuating microwave, this causes continuous rotation of the molecules and frictional heating.

vi) Production of microwaves

Microwaves are produced using a piece of equipment known as a magnetron. Essentially, a magnetic field is applied across two electrodes (an anode and a cathode). The anode has a cylindrical shape with fins projecting inwards and has the cathode shaped like a central pole within it. When a potential is applied across the system the cathode heats emitting electrons. These electrons are attracted to the positive anode and would pass in a straight line towards the anode except for the influence of the magnetic field. The magnetic field causes the electrons to be diverted taking an expanding circular orbit around the cathode eventually reaching the anode. It is this combined effect of the electric and magnetic field on the electrons emitted

from the cathode that produces microwaves. Magnetrons produce microwaves at a fixed frequency and this frequency is related to the configuration of the cathode and anode. Altering the space between the anode and cathode changes the frequency of the microwaves produced. A picture of a magnetron is shown in Figure 2.6 below.

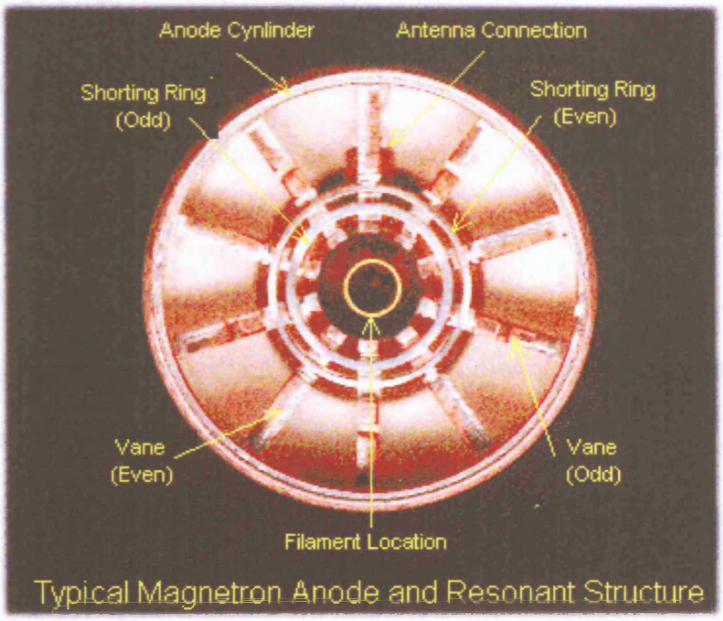

Figure 2.6. Schematic showing the internal structure of a microwave magnetron.

2.2 MICROWAVE ENDOMETRIAL ABLATION

i) Microwave ovens

In 1946 an engineer named Percy Spencer was investigating the production of microwaves using a new device called a magnetron for use in radar equipment. He noticed that following a session of work in the microwave laboratory a chocolate bar in his pocket had melted. Intrigued by this development he repeated the experiment with popcorn and an egg discovering the heating effect microwaves have on organic

tissues. Following this discovery, he developed the first microwave oven from which a multi-million pound industry has developed.

Microwave ovens have become popular due to their speedy and uniformity in their cooking. To appreciate the advantage microwave cooking has over conventional ovens one needs to understand the two principal methods of heating used by these two appliances. Conventional ovens use a high temperature into which the object to heated is placed. The object is then heated by conduction: heated air molecules collide with the outermost portion of the food and energy is equilibrated between the two molecules. In turn the food becomes heated as more peripheral molecules interact with molecules deeper into the structure. This means conventional ovens heat food from the outside into the food centre. This has a number of disadvantages not least is the fact that gradient between the temperature of the oven and food has to be relatively high or temperature increases in the food will be very slow. The second is that the temperature increase in the object to be heated is not uniform. It is often difficult to cook food from frozen in a conventional oven due to the slow heating of the centre of the foodstuff.

Microwave ovens heat food by the action of the microwaves on polar molecules (principally water) in the foodstuff. Microwaves have the ability to penetrate through tissues as and they do so interact with the molecules of the food giving up the wave's energy. This heating allows much of the tissue to be heated simultaneously rather than having to rely on conduction. This simultaneous heating effect due to wave penetration into tissue is the main reason why microwave ovens are able to cook quickly and evenly. This even and rapid heating of water molecules gives

microwaves considerable potential in the ablation of human tissue as discussed in Chapter 1. The initial microwave ablation work undertaken by the Medical Devices Technology Group (MDTG) at the University of Bath was in the treatment of menorrhagia.

ii) Menorrhagia

This is a very common condition which is characterised by heavy or prolonged menstrual blood loss. In many cases this can be treated without the need for surgery (or ablation) though it remains a troublesome symptom in a proportion of women. For patients with symptoms that were refractory to medical intervention, treatment required a hysterectomy (surgical excision of the uterus) to be performed with its attendant complications (Abbot and Garry 2002). The MDTG tackled this problem by ablating the uterine endometrium using microwaves delivered from a newly designed applicator, a technique known as Microwave Endometrial Ablation (MEA). This has become a very successful procedure and recently been approved by the FDA in America. A number of trials and publications have been produced as a result of this work (Hodgson et al 1999, Wallage et al 2003, Milligan et al 2002, Sharp et al 1995).

iii) Effect of frequency on microwave penetration and development of the MEA applicator

Very few adverse effects from MEA have been reported after many thousands of treatments. This is in part due to the care taken over the applicator design, operating power and frequency of the waves utilised in the treatment. Successful endometrial

ablation relies upon destruction of the endometrium without excessive damage to the myometrium, no microwave penetration into the pelvis and the maintenance of a cool applicator handle. With these conditions in mind a microwave applicator was developed. This used novel ceramic materials along with advances in knowledge of the microwave field radiation from this applicator to produce an effective treatment. Testing revealed that even after prolonged exposures, microwaves were demonstrated to be contained within the uterus with no penetration through the structure nor was a significant rise in applicator handle temperature detected. The following two diagrams help to explain how this was achieved.

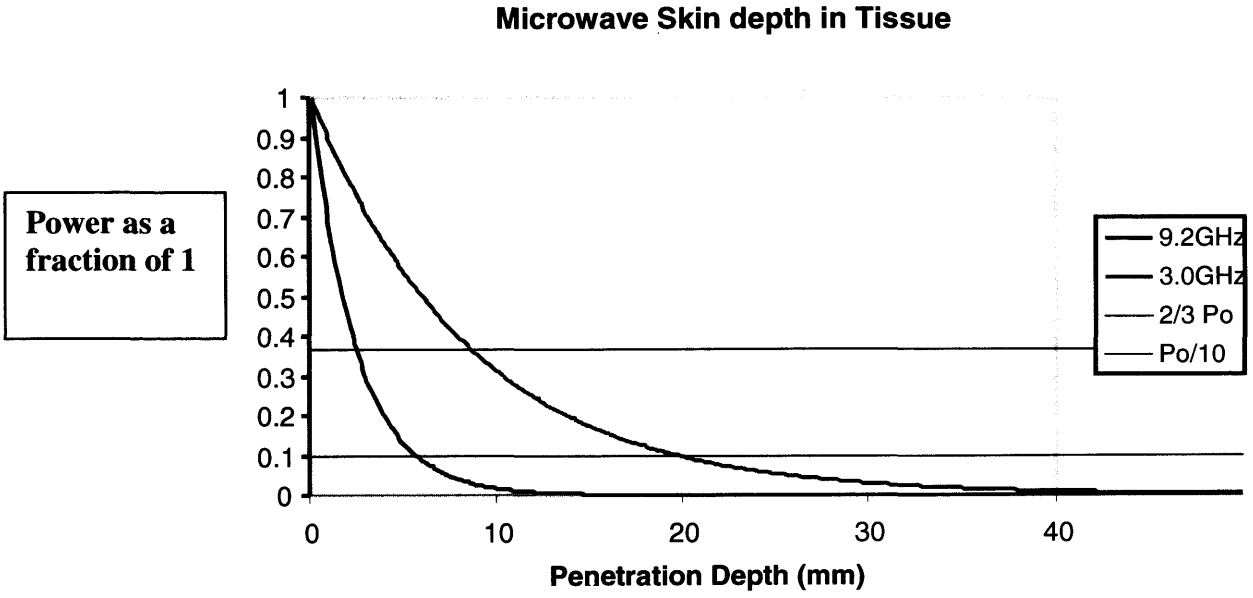

Figure 2.7. Graph showing the effect of frequency on penetration of microwaves through tissue. Microwave penetration through tissue is known as skin depth and is defined by the distance at which approximately 2/3 of the incident power has been dissipated (black line).

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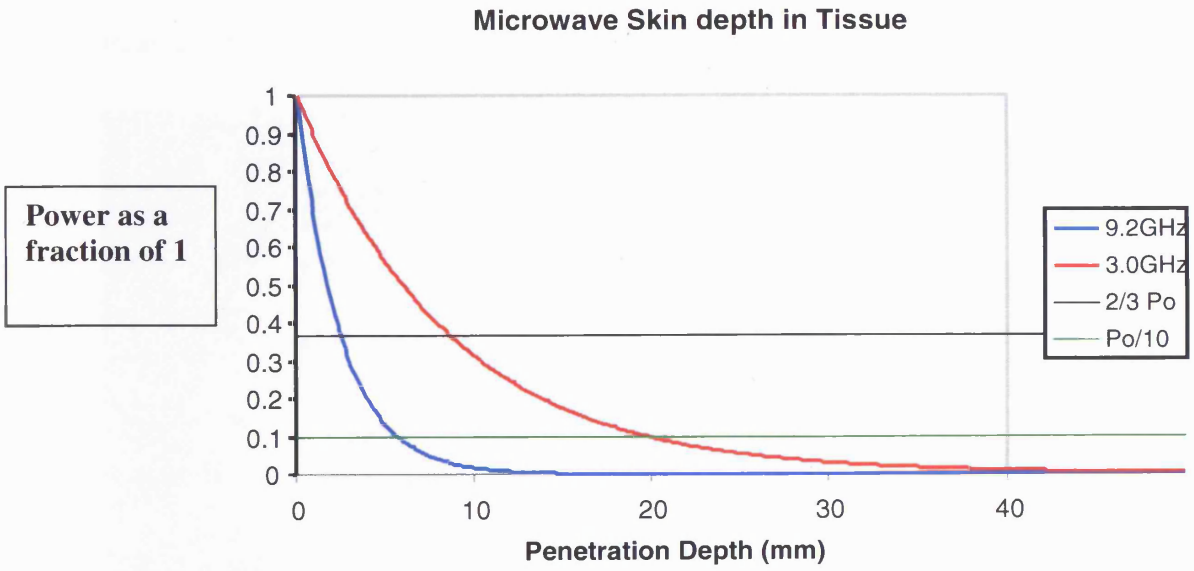


Figure 2.7. Graph showing the effect of frequency on penetration of microwaves through tissue. Microwave penetration through tissue is known as skin depth and is defined by the distance at which approximately 2/3 of the incident power has been dissipated (black line).

From the graph it can be appreciated why the frequency of 9.2GHz was selected for the MEA applicator. At this frequency, the vast majority of the energy associated with the microwave is deposited in the first few millimetres of the tissues into which it is radiated. This property induces a fairly ferocious field near the applicator but the intensity of the heating drops very rapidly with increasing distances from the applicator. This is ideal for use as an ablative technique in the context of endometrial ablation as an intense heating effect is required to adequately destroy the endometrium but this energy should only be dispensed within the uterus to prevent damage to adjacent pelvic structures. The microwave was tested on many ex-vivo specimens with a very sensitive microwave detector placed outside the uterus. No penetration of the specimens was found as predicted by the computer models. A treated specimen is shown in Figure 2.8.

Figure 2.8. Ex-vivo uterus (sectioned) following MEA testing. The white heated region can be seen to the right of the picture showing the extent of the heating and the

charred inner region of ablated tissue to the extreme right. It should be noted that the extent of the ablation does not reach through the organ.

The radiating and reflection characteristics of the MEA device were tested and modelled. Using a network analyser to estimate the electromagnetic characteristics of the uterus, MEA applicators were tested. The graph shows the reflection of the applicator over a relatively narrow range of microwave frequencies.

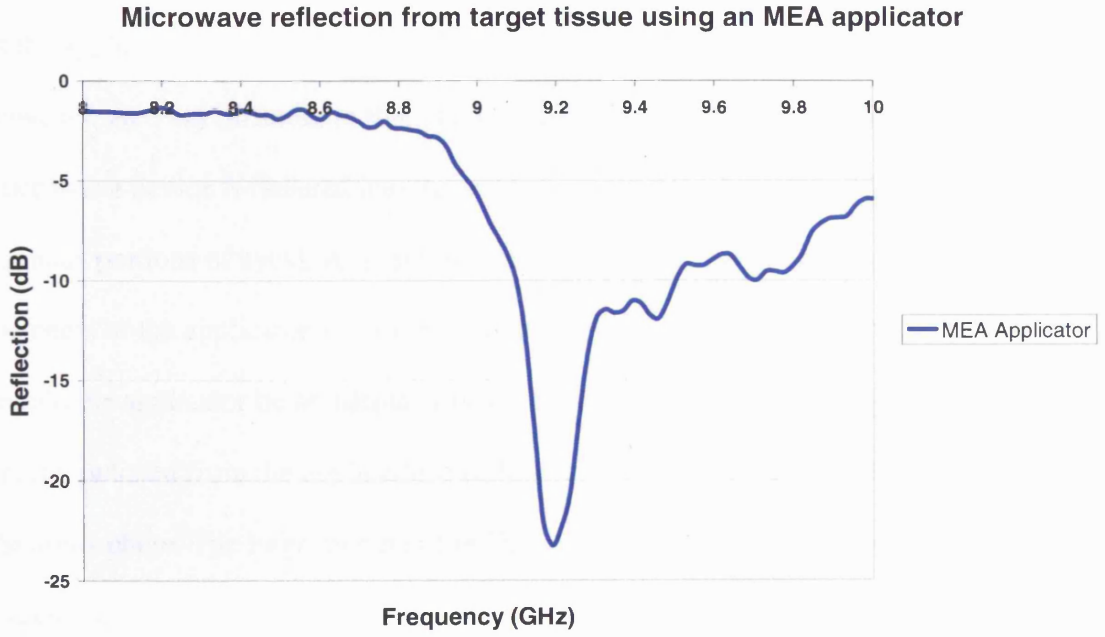


Figure 2.9. Reflection of microwaves from the applicator/endometrium interface. Reflection of microwaves is measured in decibels (dB) and importantly decibels are measured in a logarithmic manner (-10 dB is therefore approximately 10% reflection and –20 dB 1% reflection). Note that at the operating frequency of 9.2 GHz reflection is very small and implies very good match between the microwave applicator and the tissue into which it is radiating. This low reflection implies very efficient heating of

the target tissue will occur as the vast majority of the energy contained by the wave will be absorbed by the tissue.

Figure 2.9 only shows the reflection between uterine tissue and the MEA applicator. The design of the applicator ensured a good "match" between the applicator and the target tissue. A good match is one in which the majority of the radiated energy will enter the target from the applicator; a poor match will result in far more energy being reflected back into the applicator (more details on "matches", electromagnetics and the implications for the efficiency of microwave radiation are contained within Chapters 5 and 6). Figure 2.9 shows that very little of the incident energy is reflected at the applicator/endometrium junction. The electromagnetic properties of air, however, are very different to that of tissue and this is utilised as a safety feature (if a microwave device is radiated into the air there is potential for injury particularly to the aqueous portions of eyes). As mentioned previously, the match between the radiating elements of the applicator and air is very different. The result of this difference is that should the applicator be accidentally powered whilst held in the air, far more of the energy radiated from the applicator is reflected back into the device rather than into the atmosphere. The large amount of reflected energy triggers an internal safety circuit automatically terminating the treatment.

iv) Development of a microwave applicator for the treatment of colorectal liver metastases

The MEA applicator has been very successful in both experimental models and clinical trials for the treatment of menorrhagia. Due to funding issues the work in liver

tumour ablation was started with the MEA applicators. As mentioned previously, these applicators are not ideal for liver work as they operate at a high frequency and thereby have a relatively compact radiating field. This is important as all ablative systems are limited by the size of the lesion that it is possible to ablate in a single insertion of the applicator. By using the frequency of 9.2GHz the MEA system was already disadvantaged and this negated much of the superior radiating properties of the applicator. A second disadvantage was the low power at which these applicators worked: 30 Watts. Modifications to the powering magnetron did, however, allow increases in the output to 45 Watts. When the applicators were used in the ex-vivo setting the shape of the radiating portion of the direct launch MEA applicator was poorly designed for treating colorectal liver metastases (see figures 2.10 and 2.11 for pictures of an MEA applicator and close up of the radiating portion of the applicator). The applicator has a spherical, and therefore blunt end which is used as a safety feature for MEA to prevent perforation of the uterus. Colorectal metastases are hard, woody structures and the force required to place the applicator within the target treatment area was prohibitive.

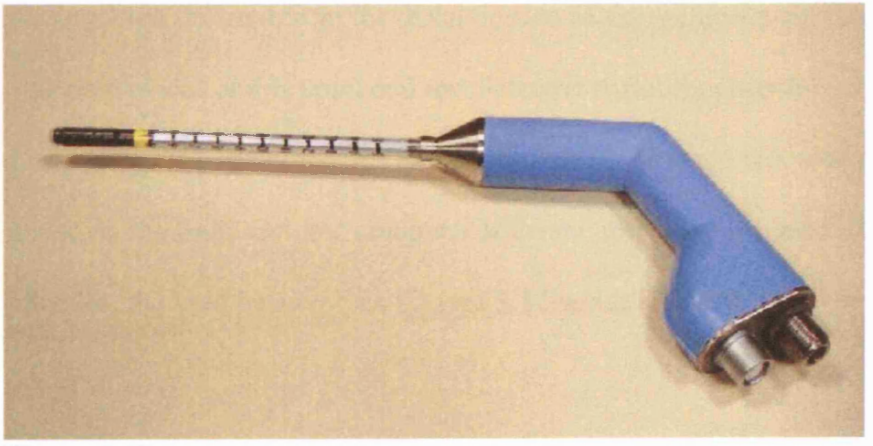


Figure 2.10.

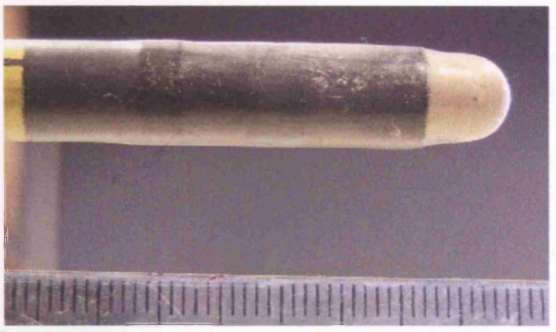
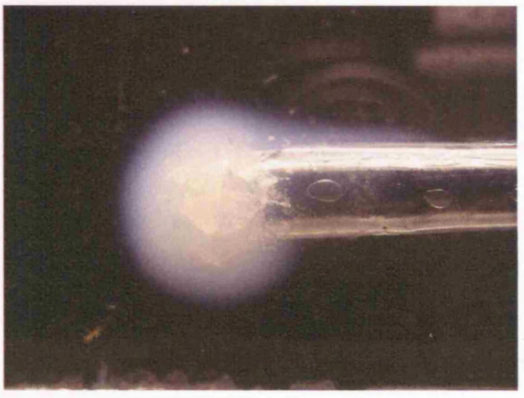
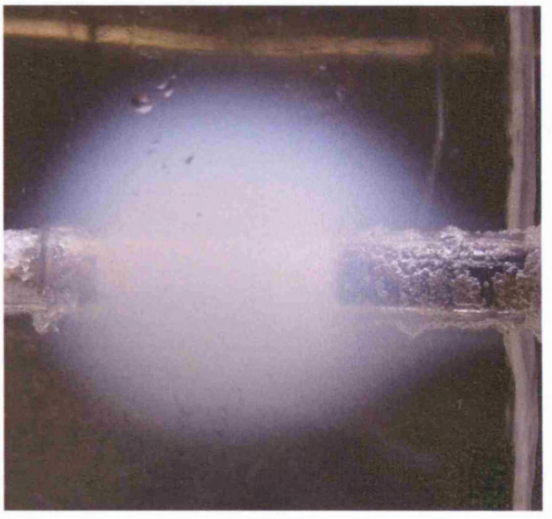


Figure 2.11.

Figures 2.10 and 2.11. Figure 2.10 shows a Direct Launch MEA™ applicator. The graduated portions on the antenna are centimetres giving clinicians guidance to the penetration of the applicator. Figure 2.11 shows a close up of the spherical end of the MEA applicator with a millimetre scale. It is here that that the microwave field is radiated from in a spherical fashion. It was thereby termed a direct launch applicator due to this feature.

In an attempt to counter the problem of difficult penetration of the applicator into hepatic colorectal metastases, the applicator was machined with a cutting tip although this immediately altered the microwave field characteristics of the device. The field was now not radiated from the distal portion of the applicator as a metal cutting tip was now placed at this point and the dielectric radiating ceramic was placed more proximally. The effects of this alteration were noted when the microwave fields were tested on the finite element computer software and using temperature sensitive gels to visualise the field heating (see Figures 2.12 and 2.13 on the following page).





Figures 2.12 and 2.13. Figure 2.12 (to the left) shows a direct launch MEA applicator radiating into PAG temperature sensitive gel. The spherical field radiated from the end of the applicator can be appreciated. Figure 2.13 to the right shows a modified MEA applicator in which the cutting tip has been introduced. It can be appreciated that the field is centred more proximally along the applicator shaft rather than being emitted from the distal cutting tip.

All new radiating microwave devices had the radiated field modelled using predictive finite element computer software until a design that was suitable was found. Applicators were subjected to testing in a tissue phantom. The phantom used was polyacrylamide gel (PAG) incorporating a colour changing surfactant agent (Adekanol, Asahi Denka Kogyo K. K., Tokyo, Japan) which is activated at a temperature dependent upon the particular grade of surfactant used. Polyacrylamide is transparent until the activation temperature is reached and this allows direct observation of the heat distribution in a phantom closely simulating soft tissue (Bini

1984). PAG is composed of 70% water by volume and simulates the water content and density of soft tissue. This allows the comparison between the predicted field and the region of heating that is actually induced by the applicator during treatment.

Initial work centred on the possibility of modifying the MEA applicators so they could be used in the liver ablation work. Unfortunately, even after the cutting tip modification, this proved to be an unsustainable hope fairly quickly. From Chapter 1 it can be seen that previously available microwave applicators have considerable drawbacks as a method of liver tumour ablation and this is mainly due to the small volume of ablation that can be achieved with a single insertion of the microwave probe. To be able to ablate larger volumes of tissue the applicator will have to deliver greater amounts of energy than had been used previously with MEA. When tested at higher powers the applicators often failed due to combustion of the plastic coating around the applicator and breakdown of the radiating ceramic. It was felt that this was a result of the very intense fields around the applicator due to the high frequency used (it should be remembered that the original operating frequency was 9.2GHz and that the skin depth at this frequency was 5mm). Modification of the MEA applicators was therefore abandoned for work on a 2.45GHz applicator using new, recently formulated ceramics. The result of this work was the 2.45 GHz interstitial microwave applicator seen below. Much of the work in this thesis is based on the testing and development of this applicator (see Figures 2.14 and 2.15).

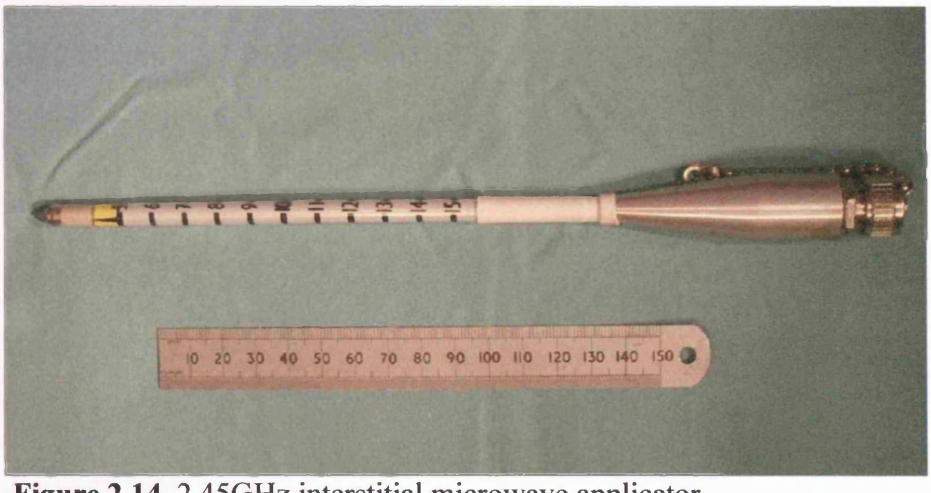


Figure 2.14. 2.45GHz interstitial microwave applicator.

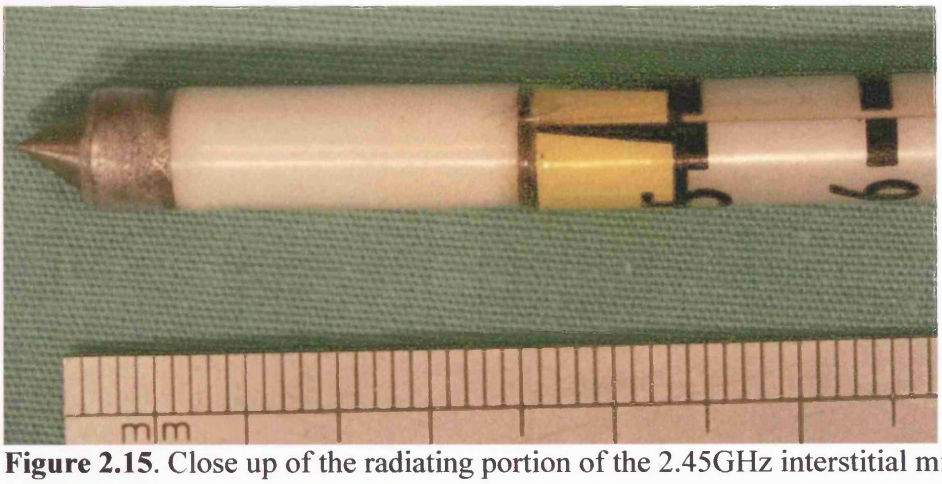


Figure 2.15. Close up of the radiating portion of the 2.45GHz interstitial microwave applicator.

CHAPTER 3

MICROWAVE LESION PREDICTABILITY: GROSS LESION PRODUCTION, TOLERANCE AND LESION EVOLUTION.

3.1 INTRODUCTION

3.2 AIMS

3.3 METHODS

i) **Lesion reproducibility following the application of similar energy doses.**

ii) **Effect of escalating volumes of ablation**

iii) **Large volume microwave ablation and the production of a dose response curve**

iv) **Statistical analysis**

3.4 RESULTS

i) **Lesion reproducibility**

ii) **Lesion evolution in the small animal model**

iii) **Treatment tolerance in the small animal model**

iv) **Tolerance of escalating ablation volumes in the small animal model**

v) **Large volume microwave ablation and lesion evolution in the large animal model**

vi) **Production of a dose response curve in the large animal model**

vii) **Treatment tolerance in the large animal model**

□

3.5 DISCUSSION

i) **Lesion reproducibility**

ii) **Lesion evolution**

iii) **Treatment tolerance and escalation of ablation volumes**

iv) **Large volume microwave ablation**

v) **Dose response curve**

3.6 CONCLUSION

3.1 INTRODUCTION

Ideally, any ablative technique has a number of requirements. Firstly, the energy applied to the tumour needs to induce tumour cell death bringing about destruction of the metastasis. Secondly, the lesion volumes induced by the energy source should be reproducible when similar treatment parameters are applied. Thirdly, control over the volume of ablation induced is required to allow treatment of lesions of differing sizes with the minimum disruption to normal liver parenchyma and its vessels. Lastly, the lesions induced by the ablative mechanism need to be tolerated by the organism into which they are placed. Succinctly, the ablative mechanism is required to produce predictable effects in terms of the ablation volume induced at particular power levels.

The cytotoxicity of microwave ablation has been examined previously in both animal models and human treatments with encouraging results (Chen et al 1999, Seki et al 1999, Shibata et al 2000). However little data exists regarding the reproducibility of microwave lesions with particular reference to the volume of ablation produced. This is likely to be a function of the limitations of previous microwave equipment namely its inability to produce lesions in excess of 2cm (Shibata et al 2000). With the new microwave equipment developed by the Medical Devices Technology Group the reproducibility of lesion volumes become an important issue.

Patients with unresectable hepatic metastases almost by definition have multiple tumours (unless the patient presents with a single, very awkwardly placed, central lesion). The ability to predict the effect of multiple ablations on the organism into which the ablations are placed has obvious implications though again little data

addressing this issue are available. The effect of larger ablation volumes is not fully understood and appears to vary according to the ablative mechanism. Cryotherapy in particular has been associated with a multi-organ dysfunction syndrome known as cryoshock following ablation of larger volumes of tissue (Weaver et al 1995, Seifert and Morris 1999). No data exists on the effect of increasing the load of ablated tissue following microwave treatment on an organism.

To allow treatment of malignancies of different sizes, the microwave equipment needs to be capable of producing differing volumes of ablation. For safe and effective treatment of a given metastases prior knowledge of the ablation volume produced following a given set of parameters is required. Ideally a dose response curve generated from previous experimentation will allow accurate and effective ablations to be performed. No such data exists with currently available microwave equipment due to its inherent technical problems.

3.2 AIMS

The aim of this chapter is to answer the question of reproducibility of microwave lesions when the same parameters in terms of energy and duration of treatment are applied both within the same and different animals. It also investigates the effect of time on lesion evolution and the clinical effects of microwave ablation on the animal as a whole.

The aims of this second part of the study experiment were threefold: Firstly, to test the new microwave equipment's ability to produce volumes of ablation in normal porcine

liver in a reproducible manner. Secondly, to investigate lesion evolution, producing a dose response curve and lastly to investigate the tolerance and safety of such a procedure in the animal model. Porcine livers were chosen for the model in this second portion of the study due to the similarities to human liver both in terms of size and physiology (Mizrahi et al. 1996).

3.3 METHODS

i) Lesion reproducibility following the application of similar energy doses.

All animal experimentation was conducted in accordance with the Operation of Animals (Scientific Procedures) Act 1986 after authorisation from the University of Leicester Ethical Committee and Home Office. Lesion reproducibility was investigated using a small animal model (rats). A total of 45 male, Wistar rats over 350g were randomly assigned into groups consisting of 5 animals.

All animals were anaesthetised using inhalational anaesthetic and subcutaneous injections of normal saline and opiate analgesia were administered prior to performing a laparotomy. At operation the liver was exposed and mobilised to allow placement of the microwave applicator. The lobes for treatment were selected and the microwave applicator placed centrally within them and a 12 Watt treatment for 20 seconds was administered to two lobes. The microwave applicator was then withdrawn and the abdominal wound repaired.

Rats receiving two microwave ablations were divided into 6 groups who were killed at increasing intervals from the treatment: 0, 6, 24 and 48 hours , 7 and 30 days. A control group of 5 animals receiving laparotomy, liver mobilisation and insertion of the applicator but no microwave treatment were killed after 7 days.

ii) Effect of escalating volumes of ablation

Two further groups with 5 animals in each were used to investigate the clinical effect of larger volumes of microwave ablation. Each group underwent anaesthesia and surgery as described previously although each animal received three or four ablations. The effect of this on the animals was assessed by observation with reference to disturbance to normal behaviour patterns and the animals weight.

iii) Large volume microwave ablation and the production of a dose response curve

Fourteen large white, female pigs (body weight 45-50kg) were anaesthetised and underwent insertion of a tunnelled central venous catheter, allowing easy administration of fluids and analgesics.

Each animal underwent a laparotomy to allow good exposure of the liver. Once adequately exposed, the UBMDT microwave applicator was inserted into the hepatic parenchyma and a three-minute treatment was applied. To reproduce the conditions in which it is believed the microwave will be used and to assist in the production of a dose response curve, pigs underwent multiple treatments which in some cases

included resection of a liver lobe. The range of powers selected were 36, 50, 100, 150 or 200 Watts with each pig receiving a combination of treatments. Adjacent liver lobes, viscera and other organs were protected by placement of moist surgical packs. A microwave detector was used in all operations to ensure the personnel present did not receive microwave dosages in excess of EEC regulations.

The microwave system used included a generator (ETM 200 SC Travelling Wave Tube Amplifier, ETM Electromagnetic Inc., 35451 Dumbarton Court, Newark, CA 94560, USA driven by a Hewlett Packard 8350B Sweep Oscillator, Agilent Technologies UK Ltd., Eskdale Road, Winnersh Triangle, Wokingham, Berkshire, RG41 5DZ, UK) producing microwaves at a frequency of 2.45GHz with variable power available from 0-250 Watts. Microwaves were transmitted along a cable to the hand held applicator, the Figure 3.1 below shows the set up of the microwave equipment.

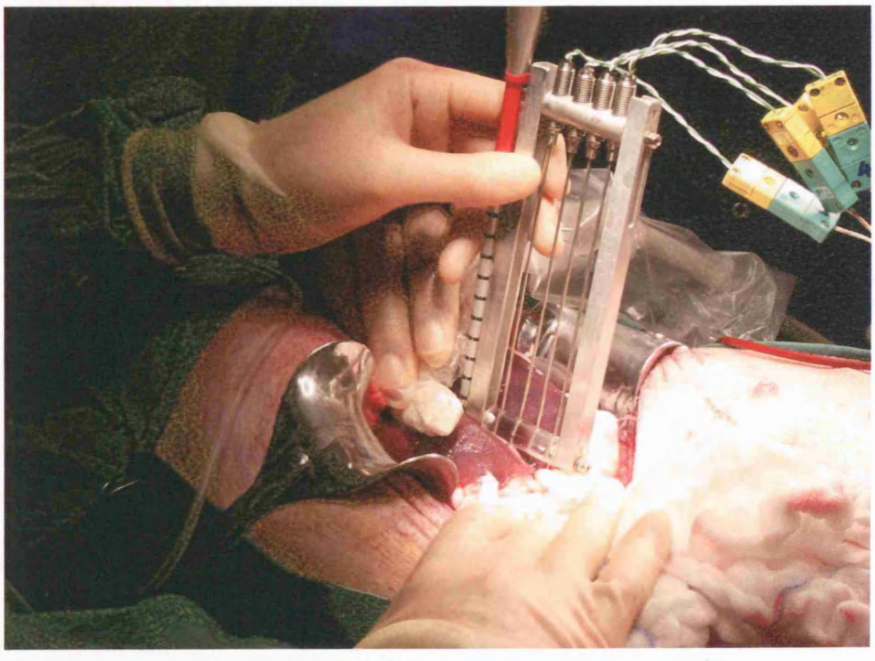


Figure 3.1: The microwave applicator (red sleeve) is being placed into a piece of porcine liver parenchyma. The aluminium jig seen contains thermocouples used for

recording temperatures at increasing distances from the microwave applicator. An intraoperative ultrasound T-probe can also be seen.

Following microwave ablation treatment, the abdominal wall was closed and the skin sutured. Local anaesthetic was injected into the wound and long acting opiates were administered intravenously for at least three days. Blood samples were taken from the central venous catheter for the first week. Lesion evolution was examined over time with animals sacrificed at increasing intervals from 0 hours to 60 days. The time points investigated were 0, 1, 7, 30 and 60 days.

All animals were killed using a Home Office approved method and the laparotomy wound reopened. The liver was harvested and the ablations observed and the diameter recorded. Following measurement, the ablations were placed in standard histological fixative (formalin). The peritoneal cavity of each animal was investigated for signs of damage and in addition to the ablated lobes a portion of liver distant to the treatments was harvested. A portion of lung, spleen and kidney was also harvested and placed into the histological fixative. Subsequent histological investigation of the ablations, untreated liver and other organs was carried out and is the subject of the next chapter.

iv Statistical analysis

The rat work used a Mann-Whitney analysis to investigate any differences between the groups. This was possible as the rat work was much more standardised in comparison to the porcine investigations.

For the porcine work a general linear model analysis of variance was used to compare lesion diameters at different time points and power levels, followed by Tukey's test to compare lesion diameters at different time points corrected for power levels. Linear and quadratic regression analysis was used to predict lesion diameter for any given power level. Ninety percent prediction bands were used so that, using the prediction band, there would be a 95% probability that the lesion diameter in an individual would exceed a specified diameter for any given power level. A significance level of p=0.05 was used. All calculations were performed using the MINITAB statistical package (Minitab Inc., 3081 Enterprise Drive, State College, PA 16801-3008, USA).

3.4 RESULTS

i) Lesion reproducibility in the small animal model

The microwave lesions produced in rats were achieved with great care to ensure each treatment was produced using the same conditions. The microwave lesions produced proved to be very reproducible in terms of macroscopic size both intra and inter animal (see Tables 3.1 and 3.2 next page). At no time period was a significant difference noted in the lesions size produced in lobe A or B and variation within a group was usually less than 1mm.

Increasing the number of lesions and therefore the overall dose of microwaves did not produce a significant difference in the macroscopic lesion size.

ii) Lesion evolution in the small animal model (see later in chapter for photographs)

Following the induction of a microwave lesion a circular lesion the thickness of the liver lobe was produced. Initially, four obvious macroscopic zones were produced; an inner area of darkened often quite charred region immediately adjacent to the microwave applicator surrounded by a blanched zone. The blanched zone was surrounded by a rim of darker tissue which appeared to be a zone of haemorrhage later confirmed on microscopy Figure 3.2. The haemorrhagic zone was the most peripheral region of macroscopic change induced by the microwave as surrounding this was the last zone that of macroscopically normal liver. The inner, charred area of tissue and the blanched region were considered dead and the diameter of this was measured to calculate the size of the induced lesion. In the most distal portion of liver considerable a wedge shaped region of what was considered to be venous congestion was seen.

After six hours the lesion had undergone considerable change as the blanched region has extended into what was previously the haemorrhagic zone. Even after this short period of time the macroscopic extent of the lesion was very distinct and therefore the demarcation between treated and untreated liver was obvious. As a consequence of the extension of the blanched zone, lesion sizes produced at time zero were smaller than at 6hours but statistically not significantly so (probably due to relatively small sample sizes). Similarly, the wedge shaped region of venous congestion was noted to have undergone blanching and that this region had become more distinct. It was considered that this area was likely to be due to infarction of the liver due to its

position being most distal to the blood supply. It was unclear whether this was due to venous congestion or infarction of is inflow.

At time periods of 1, 2 and 7 days no significant alteration of lesion diameter was noted. Lesions were very distinct from the surrounding normal liver and had developed a ringed appearance from the extension of the lesion. The distal wedge infarct became similarly distinct from the surrounding normal hepatic parenchyma.

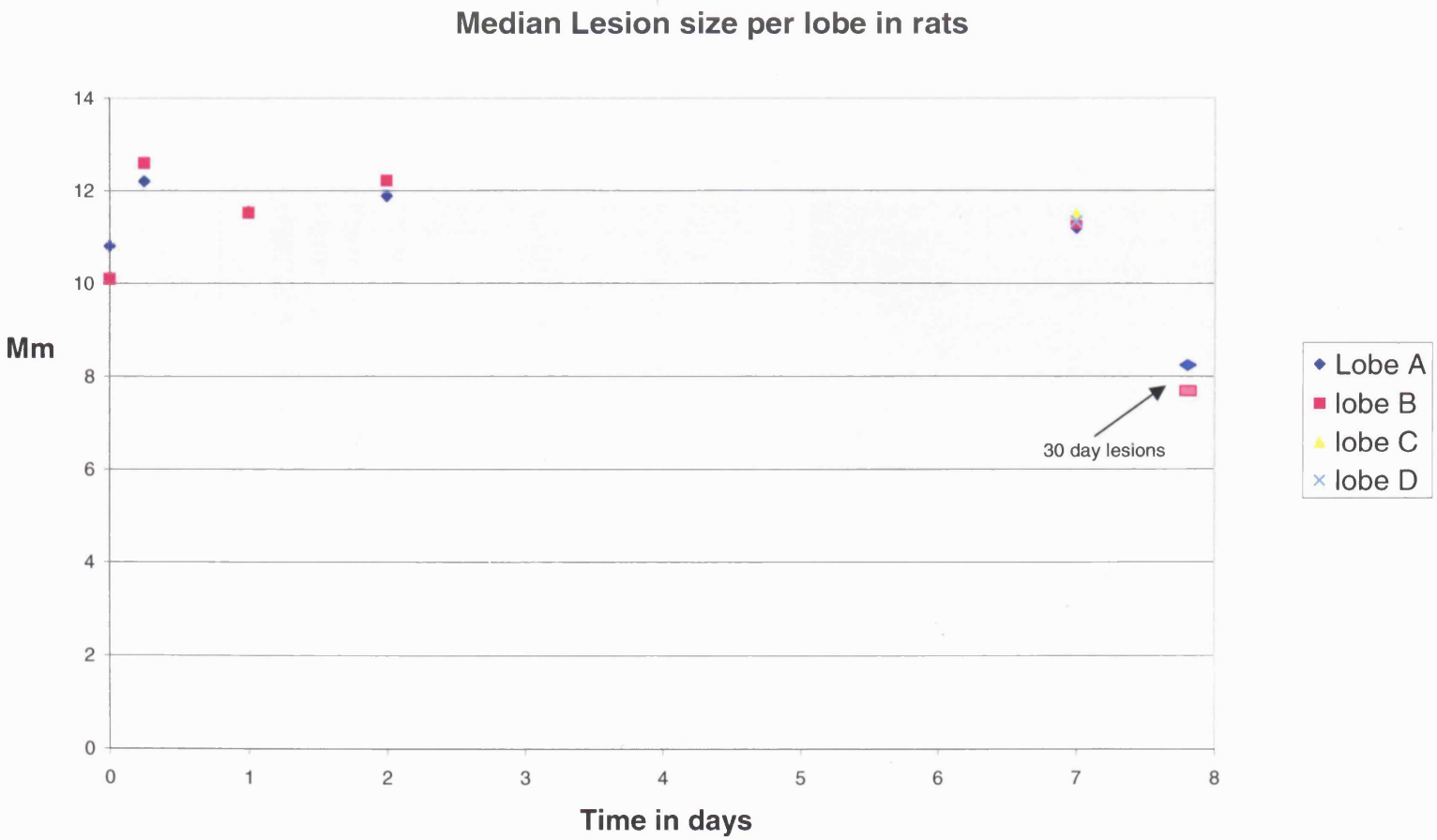
By 30 days however, (Figure 3.5) a reduction in the median lesion diameter had occurred and this was significant compared to the median lesion sizes at 6hrs (<0.01), 24 and 48 (both <0.05 Dunn's multiple comparisons test). In most rats the distal infarct had been resorbed and macroscopically normal liver parenchyma replaced the periphery of what was previously the microwave lesion. Control animals had no macroscopic lesion within the liver at cull (7 days)

Table 3.1: Macroscopic lesion size (in mm) for rats with 2 microwave lesions

	0 hours		6 hours		24 hours		48 hours		7 days		30 days	
Rat	lobe A	Lobe B	Lobe A	Lobe B	Lobe A	Lobe B	Lobe A	Lobe B	Lobe A	Lobe B		
1	11.2	10.5	13.3	13.0	12.0	12.0	11.3	12.0	11.0	10.6	7.6	4.2
2	10.5	8.1	12	12.6	13.0	13.0	12.3	11.8	11.7	11.3	8.3	8.2
3	12.1	9.6	12.2	12.5	11.3	12.0	14.1	12.1	11.0	11.0	9.1	7.4
4	10.7	10.7	12.0	11.8	9.2	8.6	12.2	12.2	10.5	10.5	7.2	7.2
5	9.2	9.7	11.5	13.1	12.2	12.0	9.5	13.0	9.8	11.6	8.4	8.3
6	11.0	11										
Median	10.5	9.9	12.2	12.6	11.5	11.5	11.9	12.0	11.0	11.0	8.0	7.1

Table 3.2: Macroscopic lesion size (in mm) for rats with 3 and 4 microwave lesions (survival time: 1 week)

	3 Lesion Group (1 week)			4 Lesion Group (1 week)			
Rat	Lobe A	Lobe B	Lobe C	Lobe A	Lobe B	Lobe C	Lobe D
1	12.0	13.0	12.0		11.2	11.5	13.0
2	12.0	12.0	14.0	11.0	11.0	11.0	11.2
3	12.0	10.0	12.0	11.0	10.0	11.0	10.0
4	11.0	11.5	11.0	10.0	9.8	12.0	11.0
5	12.1	12.2	14.0	12.0	13.7	12.0	11.5
Median	11.8	11.7	12.6	11.0	11.1	11.5	11.3



Graph 3.1. Median lesion size in the treated lobes at differing time intervals. Note the groups with 3 and 4 lesions killed at day 7 have such close measurements the symbols overlap.

Lesion evolution in the small animal model

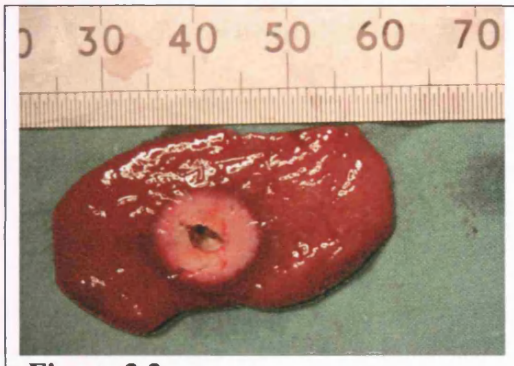


Figure 3.2

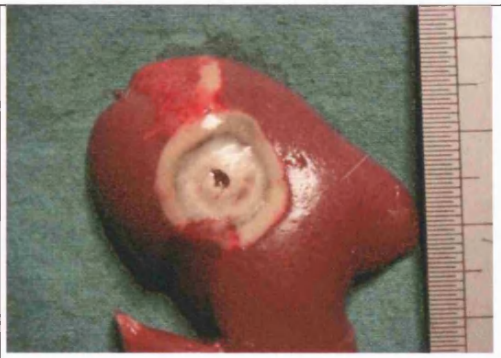


Figure 3.3

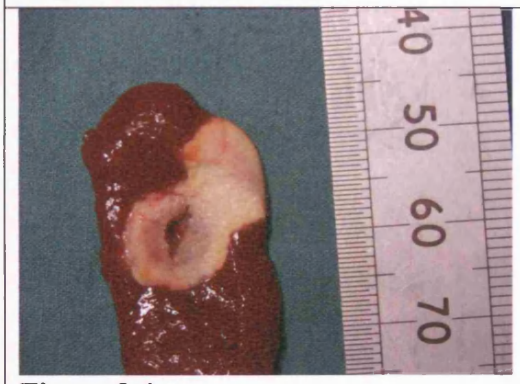


Figure 3.4

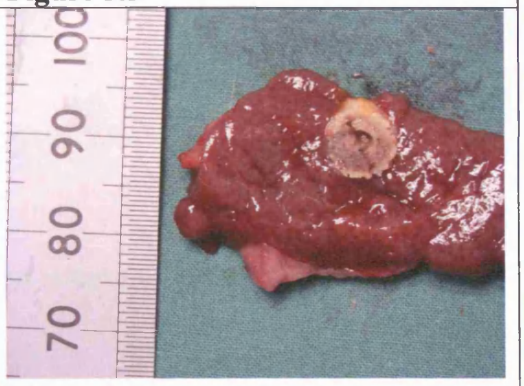


Figure 3.5

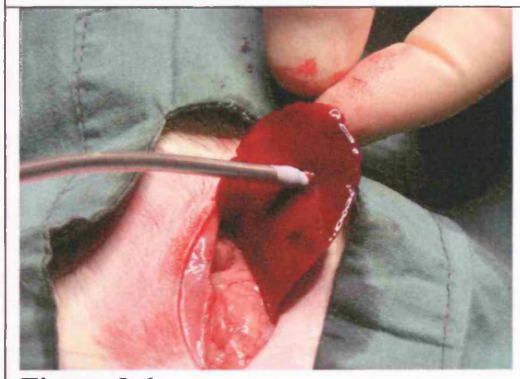


Figure 3.6

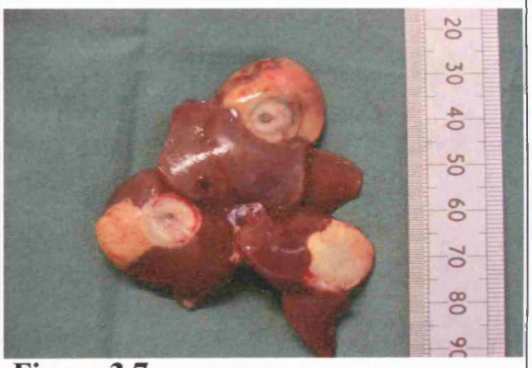


Figure 3.7

Figure 3.2: 0 days
Figure 3.3: 1 days
Figure 3.4: 7 days

Figure 3.5: 30 days
Figure 3.6: Set up of microwave
Figure 3.7: Multi Lesion liver
(ruler scales are in centimetres)

vi) Treatment tolerance in the small animal model

Overall microwave lesions were tolerated well by the animals. After 2-3 hours post surgery almost all rats were mobile and feeding. No obvious differences in behaviour were noted between the rats undergoing the control laparotomy and those with microwave treatments. At all culls no damage to liver lobes, other internal organs or the peritoneal cavity was obvious macroscopically. No animals in any of the groups were culled early for microwave-induced complications.

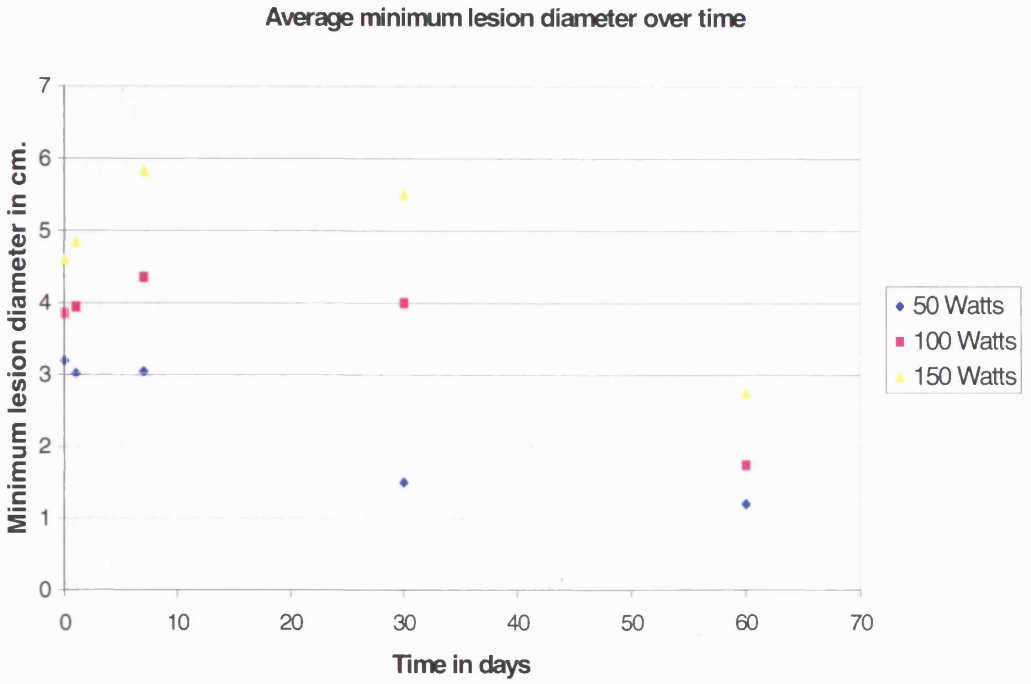
vii) Tolerance of escalating ablation volumes in the small animal model

Two groups of rats were subjected to 3 and 4 microwave treatments and culled after 1 week. Behaviour of these animals was subjectively no different to that observed in any of the other treatment of control groups. One animal in the 3 lesion group died from a possible microwave complication as at post mortem it was found that the animal had a small gastric perforation. This was either the result of a stress ulcer or contact with a liver lesion that had been replaced into the abdomen whilst still hot. The stomach was not examined histologically. None of the 4 lesion treatment group died prior to their scheduled cull. Again no damage to distal portions of liver or other organs was seen at post mortem. All animals within this group were either regaining lost weight or had put on weight at cull.

viii) Large volume microwave ablation and lesion evolution in the large animal model

The UBMDTG microwave equipment was able to produce large volume ablations in each of the livers treated. No bleeding was evident from the applicator cavity and the inner region of the lesion appeared desiccated. The majority of lesions produced were spherical and therefore circular on sectioning. At all time points, the liver adjacent to the lesions was macroscopically and microscopically normal.

The characteristics and sizes of the lesions changed over the course of the experiment. Immediately following treatment, a spherical volume of ablation was produced. On sectioning, a central blanched region with a surrounding zone, possibly haemorrhagic in nature, was seen. Within the blanched region, small areas of cavitation were noted, being especially numerous nearer the centre of the lesion. These were interpreted as a consequence of steam/bubble formation. Microscopy revealed damaged hepatocytes within the blanched region particularly towards the lesion centre. The surrounding darker halo revealed an abundance of erythrocytes although the hepatocytes showed no signs of heat damage. Lesion diameters varied according to the power applied with larger diameters being produced by treatments using higher powers. The mean lesion diameter for each power at each time interval investigated is shown graphically in Graph 3.2.



Graph 3.2. Average minimum lesion diameter evolution.

After 24 hours, the peripheries of most lesions were well demarcated from the surrounding hepatic tissue. There was no significant difference between lesion diameters at 0hrs, 24hrs, 7, or 30 days (>0.05), which averaged 4.0 (SE 0.23), 3.9 (SE 0.15), 4.4 (SE 0.17) and 3.5 (SE 0.31) cm respectively (corrected for dose level), but a significant ($p<0.05$) decrease in diameter to 1.7 (SE 0.22) cm at 60 days using Tukey's post-hoc comparisons. At twenty- four hours the blanched area extended into the area previously occupied by the darker halo, and this was confirmed microscopically. In a number of cases, the extension of the lesion was not entirely uniform with the greatest increases being into the areas of hepatic parenchyma distal to the vascular supply. The material within the lesion confines was not identifiable as normal liver parenchyma and had become a mass of paler, amorphous tissue.

After one week, the ablated volumes were well demarcated from the surrounding tissue with the lesion being surrounded by a thin lighter coloured rim. Microscopy revealed necrotic cells within the lesion the limit of which was defined by the presence of a fibrous capsule corresponding to the lighter coloured rim seen macroscopically. After 30 and 60 days the macroscopic and microscopic appearance of each lesion had not altered from that seen at one week. By 60 days, however, the mean lesion diameter had decreased significantly to less than half the diameter seen at preceding intervals ($p<0.05$). One 50 Watt lesion had decreased in size dramatically leaving only an irregular scar. The following page illustrates the lesion evolution.

Lesion Evolution in the large animal model

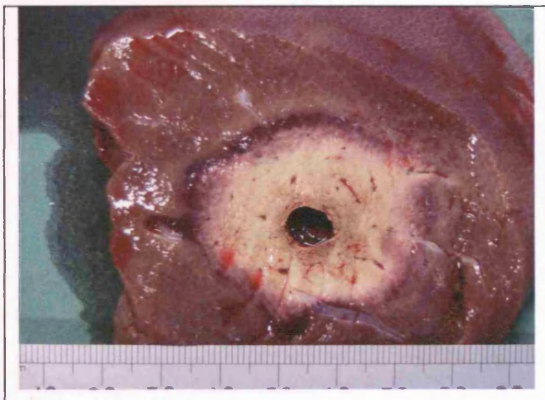


Figure 3.8

Figure 3.9

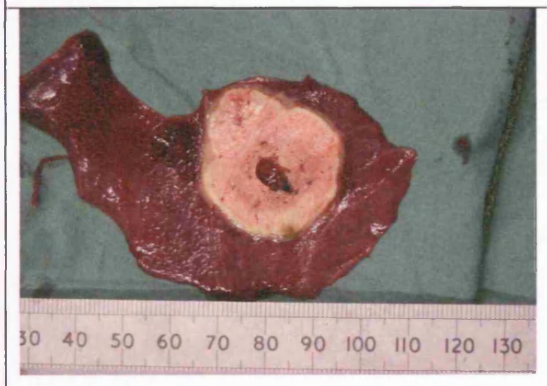


Figure 3.10

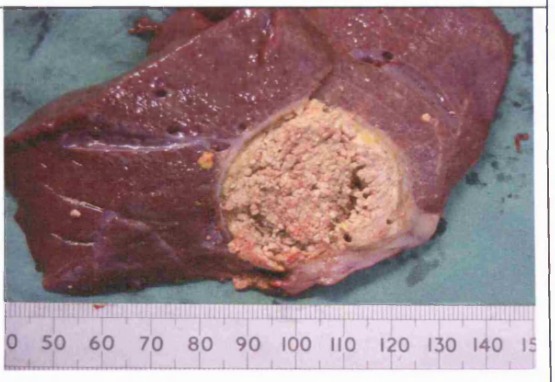


Figure 3.11

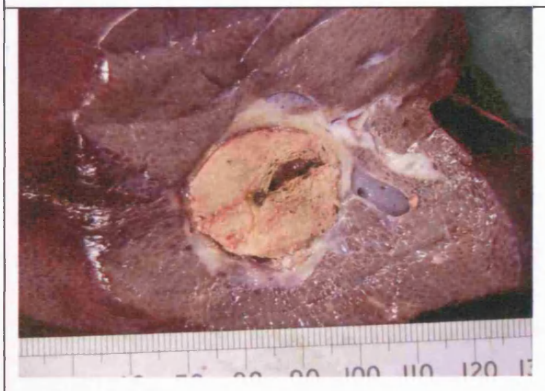


Figure 3.12

Figure 3.8: 100 Watts at time zero

Figure 3.9: 150 Watts at 1 day

Figure 3.10: 50 Watts at 7 days

Figure 3.11: 100 Watts at 30 days

Figure 3.12: 100 Watts at 60 days

(all ruler scales are in centimetres)

vi) Production of a dose response curve in the large animal model

As there were no significant differences in lesion diameters up to 30 days, all data (excluding the diameters at 60 days) were used for the dose response analysis. Regression analysis showed a highly significant ($p<0.0005$) linear dose response relationship over power settings from 36 to 200 Watts, with 77% of the variation in lesion diameter being accounted for by variation in power. The middle line in Figure 3.13 shows the estimated mean lesion diameter as a function of power (solid line). Figure 3.13 also shows the prediction interval of individual lesions (broken line) at a given Wattage following a 3 minute treatment. For instance, to produce a volume of ablation 4cm. in diameter, a 3 minute treatment with 150 Watts has a 95% chance of achieving this with a 5% chance that the lesion size produced will be greater than 6cm in diameter.

vii) Treatment tolerance in the large animal model

Animals tolerated the procedure extremely well. No evidence of infections, haemorrhage or bile leaks were noted in any of the lesions. Most pigs were mobile and feeding within six hours of the procedure, none exhibited clinical signs of a Systemic Inflammatory Response Syndrome in terms of lethargy dyspnoea or reluctance to feed (personal correspondence with Professor WC Chapman). All pigs scheduled to survive one week or longer gained weight and did not exhibit any signs of stress. No animal suffered microwave related complications.

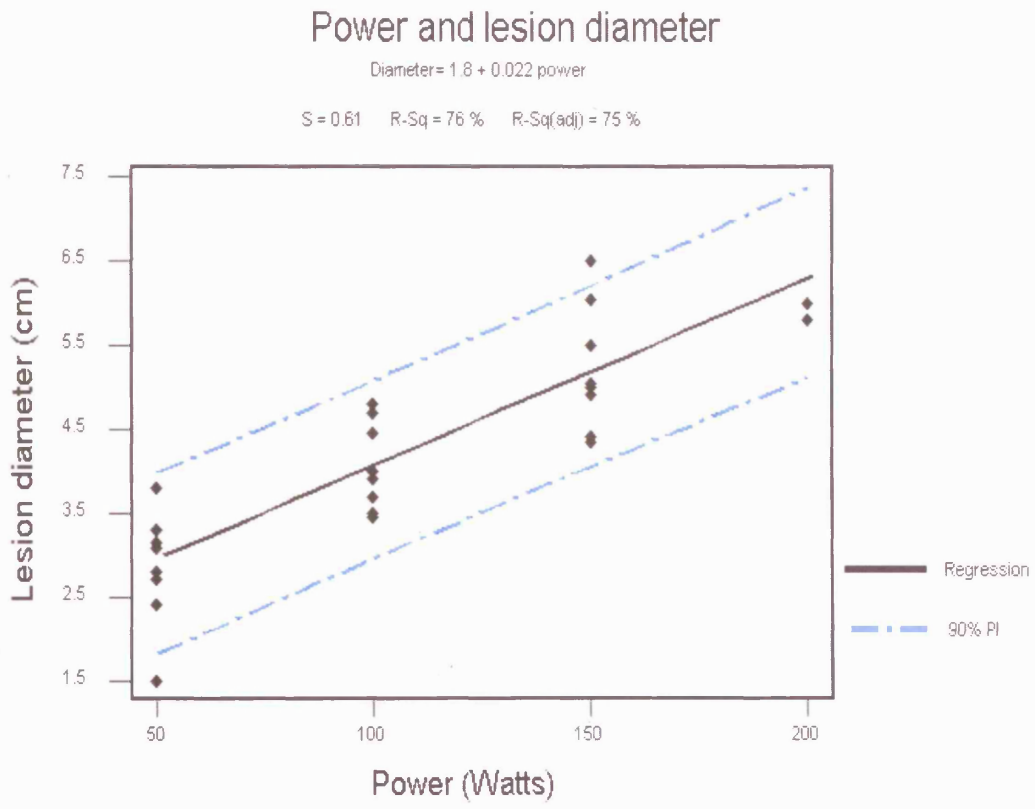


Figure 3.13: Regression of lesion minimum diameter on microwave power for all lesions up to and including 30 days post operation. The point at the 50 Watt power level with a lesion diameter of 1.5cm was taken at 30 days and may have begun to shrink.

Note that the dashed lines show the 90% prediction interval for individual lesions so that 95% of lesions are expected to have diameters above the lower dashed line. The solid line represents the best estimate of mean diameter for a given power level.

3.3 DISCUSSION

i) Lesion reproducibility

Microwave lesions proved to be very reproducible in the small animal model with no difference being obvious in lesion size produced in two different lobes. Increasing the number of ablations had no apparent effect on the lesion size. All lesions where full thickness discs within the liver as the radiating portion of the microwave was a four millimetres in length and the microwave field radiated from it was spherical. All lesions were placed within the central portion of the liver although more peripheral portions may not have produced such regular lesions. A more variable effect is illustrated in Figures 3.3, 3.4 and 3.7 in which the distal infarct produced by the microwave lesion can be seen to involve differing volumes parenchyma. The volume of this infarct was not measured formally but observation suggested the variation of its volume was considerable. Although unproven this may reflect the ability of rat hepatic parenchyma to shunt blood in collaterals around the lesion induced. Human liver is not lobed to such a degree and therefore the potential for collateral supply of areas distal top any ablative lesion is increased. Indeed, distal infarcts produced by ablative techniques has not been reported.

ii) Lesion evolution

In both animal models the immediate findings of an inner blanched area surrounded by an outer, darker rim are in accordance with previous microwave macroscopic findings (Chen et al 1999, Shibata et al 2000, Kato et al 1996). This outer zone is a haemorrhagic region with the blanched zone and eventual lesion extending into this area. This lesion enlargement occurs as hepatocytes within this outer region experience smaller thermal doses and undergo death more slowly. The combination of thermal stressing in conjunction with ischaemia from the ruptured capillaries causes the cell death in this region seen at twenty four hours. Demarcation of the lesions was evident (especially from twenty four hours onwards) suggesting hepatocytes may have a definitive temperature at which irreparable damage occurs.

iii) Treatment tolerance and escalation of ablation volumes

Both animal models appeared to tolerate microwave ablation of liver parenchyma well. Escalation of the microwave dose was studied more closely in the rat model due to the greater number available. Rats tolerated 4 lesions within the liver without significant morbidity and no mortality. This is encouraging as microwave ablations for treatment of human conditions are likely to be multiple. Alternative ablative treatments have experienced difficult not only with complete ablations of large lesions within the liver, but also in terms of the production of a systemic inflammatory response type syndrome manifesting itself as a multi-organ dysfunction syndrome (cryoshock) commonly causing death (Seifert and Morris 1999, Weaver et al 1995).

Animal studies used to replicate the condition ablated approximately 33% of the liver inducing death in approximately 45% of the animals treated (Blackwell et al 1999, Chapman et al 2000). Animals in the 4 ablation group had approximately 25% of the liver ablated excluding the distal infarcts. Morbidity in this group was minimal and mortality was zero. Although the volume of liver ablation was not as great as previous cryoshock studies, the lack of post operative sequelae in this group is encouraging.

iv) Production of large volume microwave lesions

The findings show the UBMDTG microwave equipment is able to produce large volume ablations of hepatic parenchyma from 3 to over 6cm in diameter very rapidly i.e. within three minutes. Lesion production was controllable and predictable with the majority being spherical. Lesion geometry mimicked the microwave field which radiated in an almost spherical fashion.

The new design of the applicator allowed increased penetration of the microwave field through the tissue throughout the treatment cycle. Increasing radiating efficiency resulted in a far greater proportion of the power being transmitted into the tissue rather than being dissipated in heating the applicator. This allowed the use of higher powers than has previously been possible with alternative microwave devices. Such devices are unable to utilise such high power settings due to reflection at the applicator/tissue interface during treatment (Tabuse 1998, Tucker De Sanctis et al 1998) . The combination of increased radiation efficiency and higher powers being utilised, resulted in large ablated volumes of hepatic parenchyma being induced.

Lesion size is determined by the radius from the applicator at which a cytotoxic temperature is reached. Since cytotoxicity is a function of time at temperature as well as the absolute temperature (Seifert et al 1998), lesion size is a function of time with all other parameters being equal. However, if the temperature does not reach a certain critical level, cell death will not be achieved regardless of the exposure time. Currently no other ablative technique is able to deliver such large volumes of ablation in such a short time span. Indeed, of the few ablative techniques that can produce such large ablation diameters with a single applicator insertion, the time required to do so is much greater and in some cases associated with significant morbidity (Seifert et al 1998, Bagia et al 1998, Cozzi et al 1994, Weaver et al 1995).

v) Dose response curve

At all time points, the microwave equipment behaved in a predictable, dose dependent manner as illustrated in Figure 3.13. This is important as safe and predictable treatment of liver tumours requires knowledge of the degree of tissue destruction and healing which occurs following ablation. The line of best fit does not go through the origin (Figure 3.13) as there appears to be a non-linear relationship between lesion diameter and power at lower and possibly higher power levels. The dose response curve does show some individual variation in the diameter although the majority of this is likely to be due to biological factors such as blood flow and liver lobe size. Large volume microwave ablation of liver parenchyma was not associated with significant morbidity or mortality in this model. In contrast to published experience with cryotherapy, no cracking or haemorrhage was noted either during or following the microwave procedure (Seifert and Morris 1999). Secondly, as the microwave

applicator is a single cylinder with no other moving parts, it does not require the placement of numerous energy sources or have the potential problems associated with retraction of multiple arrays (Germer al 1997, Seki et al 2000) .

3.6 CONCLUSION

As a technique, microwave ablation appears to be highly reproducible in terms of the macroscopic lesion size induced under similar conditions. Both animals models tolerated induction of both multiple and large lesions with little apparent morbidity. Lesions evolved in a manner that was predictable and in a similar manner in both models.

The large animal model highlighted the ability of newly designed microwave applicators to produce large volume microwave lesions in a dose dependant fashion. No other microwave system is capable of similar performance. This microwave equipment has potential to alter treatment of patients with unresectable colorectal liver metastases or hepatocellular carcinomas. The new applicator however, is 6.8mm in diameter will initially require placement at laparotomy although a 5mm flexible laparoscopic applicator is being developed. Work is also in progress on a new 2.4mm applicator suitable for percutaneous use. This will require the use of further ceramics at the applicator tip and computer modelling of the microwave field. Should this percutaneous applicator become available it may allow the treatment of high risk patients by avoiding a laparotomy.

CHAPTER 4

MICROWAVE LESION PREDICTABILITY 2: HISTOLOGICAL AND ULTRA-STRUCTURAL ASSESSMENT OF LESION PRODUCTION AND EVOLUTION

4.1 INTRODUCTION

(i) Importance of histology in an ablative setting

(ii) Basic liver histology

4.2 AIMS

4.3 METHODS

(i) Small animal model

(ii) Large animal model

(iii) Human ex-vivo experiments

(iv) Human in-vivo experiments

4.4 RESULTS

(i) Small and Large animal model

(ii) Human ex-vivo and in-vivo

4.5 DISCUSSION

4.6 CONCLUSION

4.1 INTRODUCTION

i) Importance of histology in an ablative setting

Ablative techniques require consistency not only macroscopically in terms of the ablation volume produced by a given set of parameters but also microscopically. Introduction of a technique such as microwave ablation requires knowledge of its local effects in the ablated region and the adjacent tissue, cells in the same organ but distant to the treatment volume and other organs (i.e. any systemic effect the technique may have). Previous chapters have described the effect of larger volume ablations when produced by alternative ablative techniques such as cryotherapy in which a multi-system disorder known as cryoshock has been induced (Seifert and Morris 1999, Weaver et al. 1995). Little has been published on the effect of microwave ablation in animal models and these works concentrate on the local effect of the procedure (Kato et al. 1996, Chen et al. 1999). No work on large volume ablations and their potential for damage of cells within the same organ but distant to the treatment area and multi-organ damage has been published.

Microwave ablation has potential to causes significant damage to regions of the liver outside the intended target. The liver is comprised of a network of interconnecting vessels for both blood and bile with the potential to allow the passage of hot fluid along these channels causing damage along their paths. This could lead to disastrous consequences particularly in the biliary tract where damage to the larger bile ducts results in structures and obstruction to the outflow of bile. It is well known in human patients that damage to the biliary tree for instance during a laparoscopic cholecystectomy can lead to many problems requiring additional operative procedures and in some cases death. Bile ducts are at greater risk of damage when compared to vascular structures as the flow of material

along the latter vessels is much greater and may act as a heat sink protecting the vessel's integrity.

Microscopic damage of liver parenchyma outside the region of treatment may have significant implications for patients with impaired liver function. Individuals with hepatocellular carcinoma and liver cirrhosis form a large group of patients who may benefit from locally destructive techniques. Evidence of tissue destruction outside the intended treatment area may have important consequences (in terms of inducing hepatic failure) in these patients where liver function is already impaired.

Ablative techniques by definition produce regions of dead tissue which, following infection of this region, may induce the formation of an abscess. Hepatic abscesses can present a major problem to the clinician with the patient requiring at least a percutaneous drainage procedure. For patients with pre-existing malignancy and its consequences in terms of malnutrition and a general deterioration in health, an abscess following ablation could have serious consequences.

ii) Basic Liver Histology

The liver is the large organ contained in the upper abdomen largely concealed by the lower ribs and costal cartilages. It consists of a solid organ mass enclosed in a connective tissue capsule (Glisson's capsule), which is penetrated by a number of vascular and biliary structures. The liver is unusual in that it has both an arterial and venous supply from the hepatic artery and portal circulation respectively. It is therefore an extremely vascular organ that consumes 25% of the resting cardiac output.

Much of the liver's function is that of biosynthesis and it is able to perform this with the products of digestion that are delivered to it from the portal system. The oxygen delivered to the hepatocytes from the hepatic arteries however, supports this intense metabolic activity. Hepatocytes therefore, require good access to both oxygenated arterial blood and the products of digestion from the portal circulation. The functional unit of the liver is know as a lobule and combines both vascular prerequisites with a method of excreting waste products i.e. the biliary system see Figure 4.1 below.

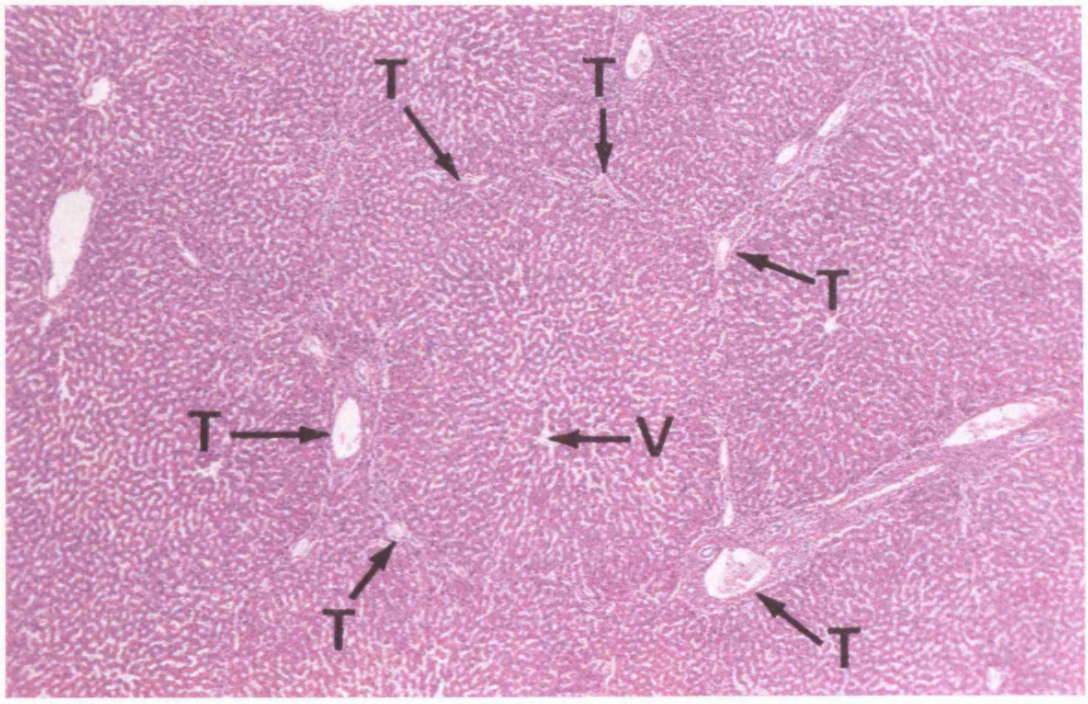


Figure 4.1. Normal porcine liver

The hexagonal, lobular structure is easily seen in the figure and comprises of a mass of hepatocytes enclosed is a connective tissue boundary. Within the connective tissue boundary are the portal tracts (T) containing each of the three vessel types: i.e. branches of the portal vein, hepatic artery and biliary ducts. At the centre of the lobule is the central vein (V) draining the blood from the lobule. Between the hepatocytes are pathways for the blood to pass called sinusoids allowing the hepatocytes maximal the contact with blood allowing easy and efficient exchange of metabolites. Nutrient rich blood from the portal

tracts percolates through the lobules towards the central vein where it eventually passes into a hepatic vein and on into the systemic circulation.

The structure of the liver has consequences for ablative techniques, not least the cooling, heat sink effect of so much blood. Techniques which rely on the conduction of energy through the tissue to cause cell destructive effect will be relatively inefficient as at the periphery of the lesion heat/cold/chemicals are rapidly transported away by the vasculature. The second point regarding the physical properties of the liver is that it has a very high water content estimated at 75% (Duck 1990). Water is very poor conductor of heat and has a relatively high specific heat capacity (the amount of energy required to increase the measured unit of a substance by 1°C). The microwave has a significant advantage compared to techniques based on conduction as heating occurs rapidly within the field radiated from the microwave applicator tip. This speedy heating lessens the losses of temperature due to vascular heat sink effects.

4.2 AIMS

This chapter seeks to answer a number of questions related to microwave ablation. How sharp is the delineation between the ablated region and the normal tissue? Are the volumes of ablation complete or are they islands of viable cells (particularly around the blood vessels acting as heat sinks), which could cause local recurrences in cancers. How is the damaged expressed within the liver and how does the liver accommodate such an injury? What is the histological evolution of the lesions? Is there evidence of effect outside the lesion within the same organ or evidence of effect outside the organ i.e. a systemic effect? Does increasing the ablated volume cause a recognisable systemic effect? Do rat and pig livers show similar features of damage and evolution of the lesions? Do human livers show similar features and can the animal results be extrapolated into human treatments?

4.3 METHODS

(i) Small animal model

The rats were divided into 9 groups of 5. All rats underwent a midline laparotomy and insertion of the needle microwave probe into two liver lobes. The first 6 groups underwent two ablations and were culled at different time points (0hrs, 6hrs, 24hrs, 48hrs, 1 week and 30 days). Group 7 was assigned as the controls receiving two insertions of the microwave probe into the liver but no microwaves. Groups 8 and 9 had three and four ablations placed into the liver and were culled at 1 week, a time point that was considered long enough to allow any detrimental effects of the larger volume of ablated liver to manifest themselves. Microwave ablations were controlled by an attendant Physicist (PC), each treatment being 20 seconds in duration at a frequency of 9.2 GHz and a power of 13.3 Watts. To prevent collateral tissue damage, wet surgical gauze was placed over adjacent liver lobes and the abdominal wall. All rats were monitored closely in their post-operative phase with food and water available at all times.

Immediately after their culling the lesions in each rat were inspected and measured using callipers. Each lesion, together with the normal liver, spleen, kidney and lung, were preserved in 10% formaldehyde solution.

(ii) Large animal model

Fourteen Female, Large White pigs (body weight 45-50 kg) were fasted overnight and sedated using an intra-muscular injection of Stressnil 5ml/kg (Janssen Animal Health), once local anaesthetic EMLA cream had been applied to the ears. Intravenous access was

achieved and propofol (6mg/kg) administered. The animal was transferred to the operating theatre, intubated and anaesthesia was introduced and maintained using gaseous isoflurane in a closed circuit. The animal's skin was prepared with standard surgical antiseptics and an upper midline laparotomy performed. The liver was mobilised and lobes for microwave treatment selected. All treatments lasted 180 seconds with a power setting ranging from 36-200 Watts at a frequency of 2.45 GHz. Once all the ablations had been performed the animals were given intravenous opiate buprenorphine, (Rickett and Colman Products Ltd), and the abdominal wall and skin were sutured. Local anaesthetic was injected into the wound prior to the ceasation of anaesthesia. All animals were closely observed during the post-operative period with analgesia administered for at least 3 days.

To allow the investigation of lesion evolution pigs were culled at increasing intervals. These intervals were 0 hours (2 pigs), 24 hours (4 pigs), 1 week (4 pigs), 30 Days (2 pigs) and 60 days (2 pigs). At post mortem each lesion was identified, measured and placed into 10% formaline solution. Lung, spleen, kidney and normal liver were also harvested and placed in fixative for one week

(iii) Human ex-vivo experiments

Immediately following a liver resection for metastatic colorectal adenocarcinoma, a 1cm incision was made with a scalpel in the liver capsule. The microwave applicator was inserted for a distance of 3cm, either into normal liver parenchyma or a metastatic tumour. Microwave power at the applicator was preset at the magnetron unit to 30 Watts (W) and electrical match was qualitatively monitored throughout treatment. The magnetron operated at a fixed frequency of 9.2Ghz and power was applied for periods ranging between 30 and 180 seconds. The applicator was removed and the tissue sectioned parallel to the capsular surface and the applicator cavity was identified. The area of blanching was

measured at its greatest dimension and 6mm biopsy cores were taken at increasing distances extending radially from the probe cavity in several specimens. The biopsies were bisected and half placed in 4% buffered glutaraldehyde solution for transmission electron microscopy, whilst the second half was frozen in liquid nitrogen for enzyme histochemistry, to demonstrate the activities of glucose-6-phosphatase, phospho-fructokinase and acid phosphatase. The remaining tissue was fixed for 24 – 48 hours in 10% aqueous formaldehyde solution. Consent was obtained from the patients.

(iv) Human in-vivo experiments

At laparotomy, the applicator was introduced under surgical sterile conditions into both normal liver parenchyma and metastatic tumour deposits. The procedure was monitored by intra-operative ultrasound. The hepatic vasculature was temporarily occluded by external compression (the Pringle manoeuvre) in one specimen to assess the overall effect on the burn radius produced. The specimens were then immediately excised and the tissues were processed in an identical manner to the ex-vivo specimens.

Tissue blocks were selected from each specimen type within the trials and transferred to a Shandon Hypercenter™ XP automated processor. A Leica Jung Autostainer XL stained paraffin-embedded sections with Mayers Haematoxylin and Eosin. Selected 4μm sections were also stained with Periodic Acid Schiff (PAS). The stained sections were assessed on an Olympus BX40 microscope with polarising facilities. Portions of tissue from the ablated lesions at increasing intervals from the microwave applicator were also investigated ultrastructurally with an electron microscope (ultrastructural work courtesy of Dr Ben Swift Registrar in Histopathology).

4.4 RESULTS

(i) Animal Models (Large and Small)

0 Hours (Pig and Rat)

Macroscopically the lesions from both species were very similar in appearance. The lesions were circular when sectioned with a central cavity representing the site of applicator insertion. Two distinct areas were seen, the first composed of blanched tissue adjacent to the cavity, the second as a darker rim surrounding the first.

Microscopically the architecture of the liver was altered with the formation of a clearly defined applicator cavity that appeared lined by parenchymal cells that had undergone coagulative necrosis with minimal carbonisation. The hepatocytes within the central area showed evidence of thermal insult with nuclear hyperchromatism and chromatin "smearing". An area of sinusoidal dilatation into which haemorrhage had occurred bound the second zone (Figures 3 and 4). It would appear that the liver plates had collapsed inwards towards the source of the thermal energy. Beyond this zone, the parenchyma appeared normal.

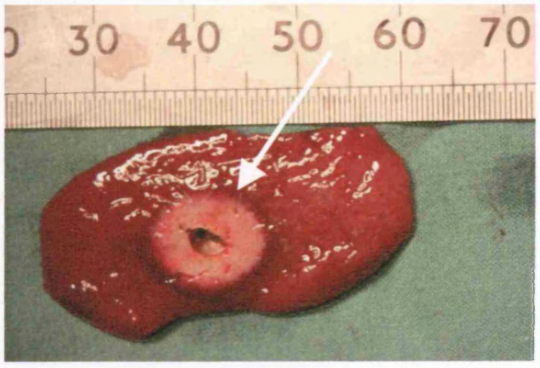


Figure 4.2. Rat lesion at time zero. Arrowed region corresponds to region of sinusoidal dilatation and haemorrhage seen in Figure 4.3.

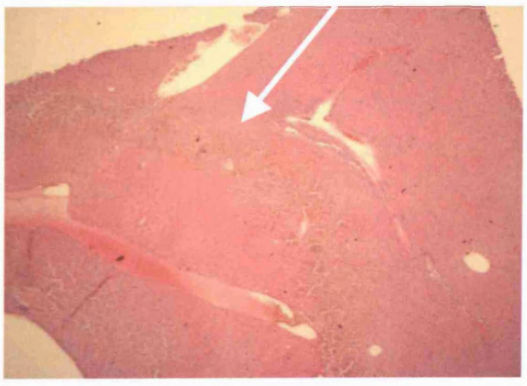


Figure 4.3. Low power microscopic rat lesion at time zero. The rim of haemorrhage (arrowed) can be easily seen.

Examination of portal triads also revealed evidence of thermal damage. The bile duct epithelium and the vascular endothelium showed evidence of thermal damage, being more prominent centrally and less evident distally. The portal connective tissue also showed temperature-related damage. The normal staining pattern was altered such that on standard H&E preparations the connective tissue developed a distinct basophilic discolouration centrally. Viewing the specimens under polarised light visually demonstrates the degree of thermal damage. The normal birefringent properties of collagen is lost entirely close to the applicator cavity and altered in thermally damaged tissue, where it is seen to develop a reddish-orange hue distinct from its normal yellow-white colouration. This appears to be a sensitive marker of thermal damage, to such an extent that portal triads distal from the burn cavity are shown to possess evidence of damage in the absence of identifiable cytological changes within the surrounding parenchyma.

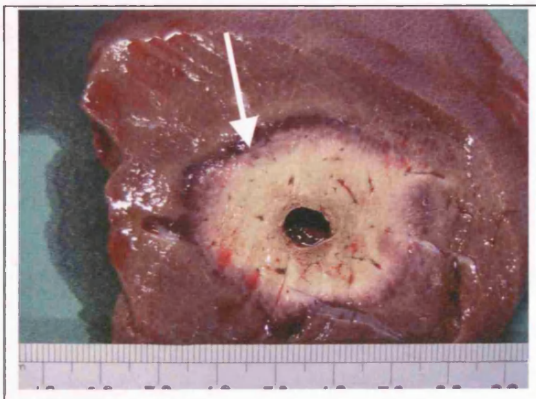


Figure 4.4. Porcine lesion at time zero. Note the similarity in the lesion edges between the two species

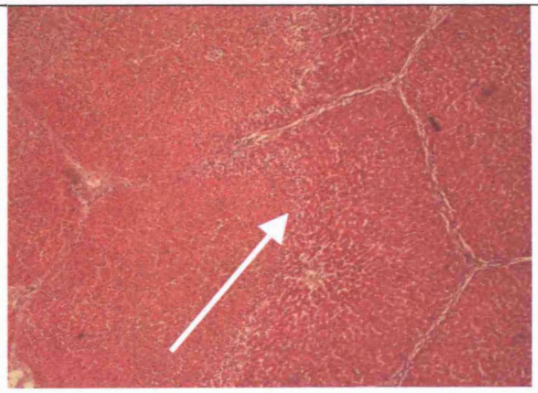


Figure 4.5. Porcine lesion at moderate power showing haemorrhagic edge of lesion (arrowed)

6 Hours Post Treatment (Rat only)

Six hours following treatment, the configuration of the lesion had changed. The haemorrhagic zone was much lighter in colour, the appearance being that of the blanched lesion extending into the haemorrhagic zone, resulting in an increase in the size of the overall lesion. A second obvious feature present in a number of subjects was a wedge-

shaped area of infarction extending from the microwave lesion to the edge of the lobe. Due to its position at the most distal point to the proximal blood supply it was assumed that this was as a result of ischaemia.

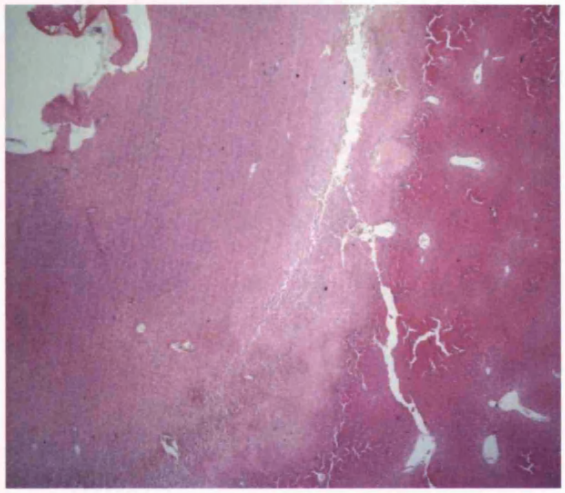


Figure 4.6. Extension of the ablated lesion into the region of haemorrhage. The boundary between the two is very distinct.

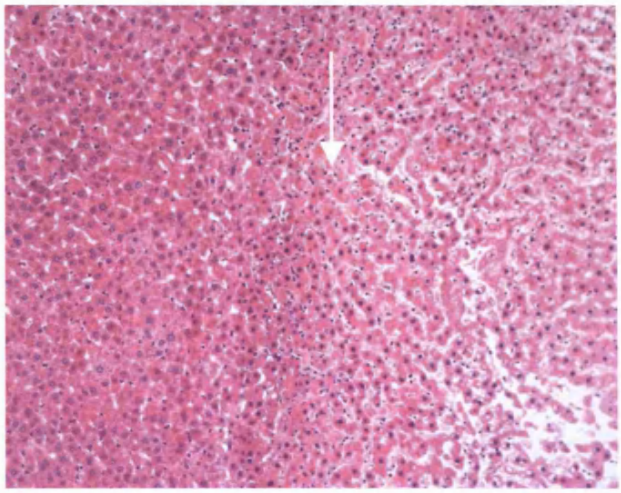


Figure 4.7. Moderate power micrograph showing the lesion boundary (arrowed). Note the obvious distinction between normal looking cells (left) and the abnormal necrotic cells in the lesion (right).

Low power examination revealed extension of the lesion to the outer limits of the haemorrhagic zone. At higher power numerous apoptotic bodies were seen at the outer margin of the ablation which, again, was easily identified. A few scattered neutrophils were seen at the lesions periphery and margination of these cells was also observed in some blood vessels within the surrounding unaffected liver.

24 Hours Post Treatment (Rat and Pig)

One-day post treatment the lesions were very similar to those seen at 6 hours. The microwave-induced lesion possessed obvious inner and outer zones, the latter produced by the extension of the ablation into the haemorrhagic zone. The distal wedge infarct was also well demarcated from the surrounding tissue in many of the rat subjects although the size

varied between lesions. Several of the lesions within porcine subjects began to degenerate centrally forming amorphous granular material.

In contrast to the macroscopic findings considerable microscopic differences were seen between the 6 and 24 hr lesions. The lesion edges were again well demarcated with a dense neutrophilic infiltrate present at the periphery extending into the ablated tissue. Numerous apoptotic bodies were seen in this area, as was further evidence of granulocyte margination. Frank necrosis was now observed in many of the cells near the edge of lesion where karyorrhexis had reduced the cells to "ghostlike" remnants of hepatocytes. Occasional fibroblasts were also seen proliferating between normal and ablated liver, arising from within portal triads or surrounding central veins. Cells nearer the lesion centre, however, did not show any features of necrosis despite receiving a greater thermal insult than those placed more peripherally. Little or no inflammation was seen in the surrounding parenchyma. Figure 4.8 shows the 24 hour appearances of the lesion edge in both animal models. It can be seen that the histological patterns seen in each model are similar.

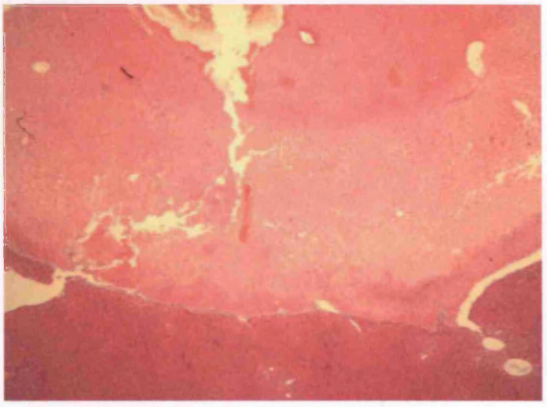


Figure 4.8. 24 hour low power rat lesion showing the sharp delineation between normal liver and the ablation.

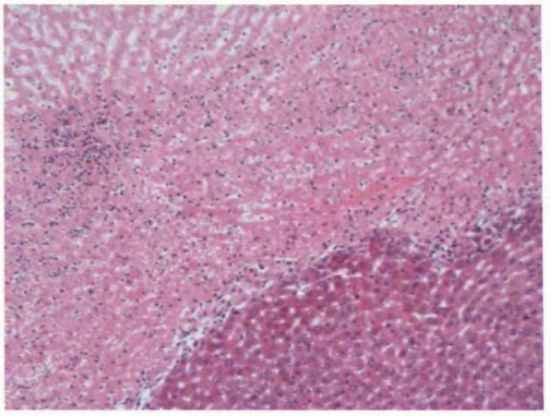


Figure 4.9. The "ghost like", empty cells in the ablated region are surrounded by a neutrophil infiltrate. Note the sharp cut off between normal liver and the lesion

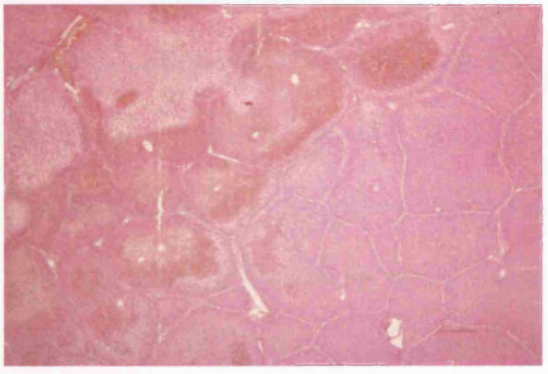


Figure 4.10. 24 Hour low power porcine lesion. Again the delineation between the ablation and normal liver is noticeable.

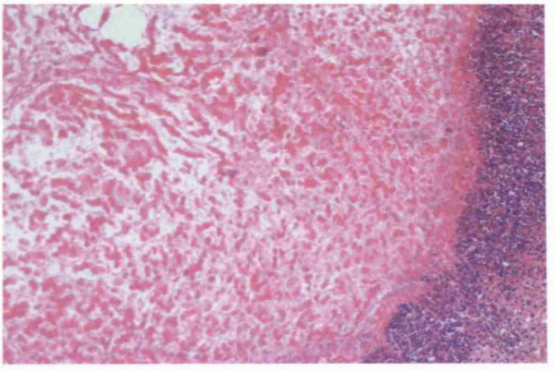


Figure 4.11. The intense neutrophil reaction to the induced lesion can be seen along with obviously dead cells within the ablation.

48 Hours (Rats only)

Little change from the previous findings was seen with the naked eye at this time. Neutrophil infiltration into the ablation had progressed further by 48 hours. At the lesion boundary the most striking feature was the presence of islands of fibroblasts extending and proliferating along the lesion boundary, appearing to encapsulate the lesion. The contrast between the heavily staining nucleated cells at the lesion centre and those frankly necrotic, anuclear cells towards the lesion periphery was also particularly marked.

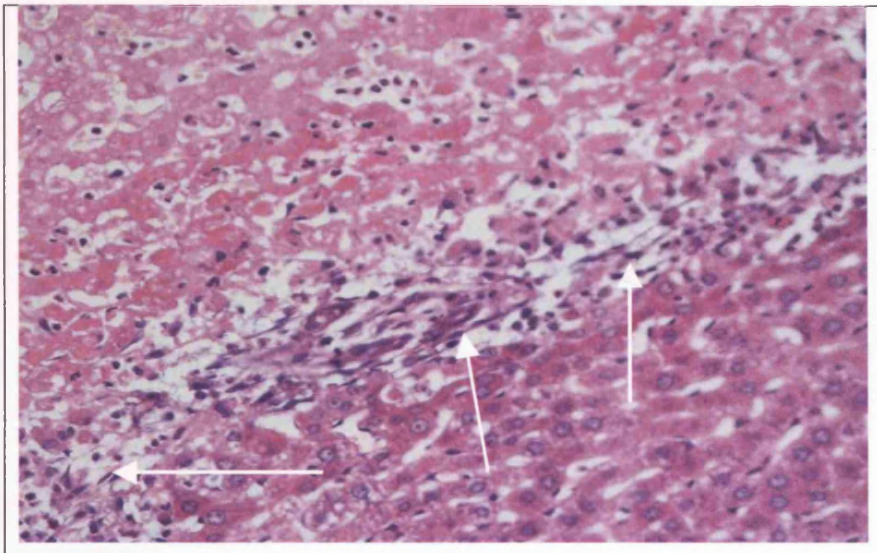


Figure 4.12. The lesion edge at 48 hours in a rat shows the appearance of fibroblasts (arrowed) at the lesions periphery. This is the beginning of the lesions encapsulation.

1 Week (Pigs and Rats)

Seven-day-old lesions were very well demarcated from the surrounding liver parenchyma. The lesion consisted of amorphous cream material around which an obvious yellowish boundary approximately one millimetre in diameter was seen. No evidence of infection was seen in any of the lesions investigated. The remaining liver and other abdominal organs appeared normal including those organs harvested from rat groups 8 and 9 in whom three and four ablations had been placed.

In the interval between 24 hours and a week a prominent fibrous capsule had developed between the lesion and the surrounding liver parenchyma due to the fibroblast proliferation. Bile duct regeneration was identified within the fibrous capsule and the neutrophil infiltrate was no longer evident, although some macrophages and multinucleate giant cells were now present in the periphery. The centre of the lesion remained nucleated, however, some evidence of necrosis was now seen.

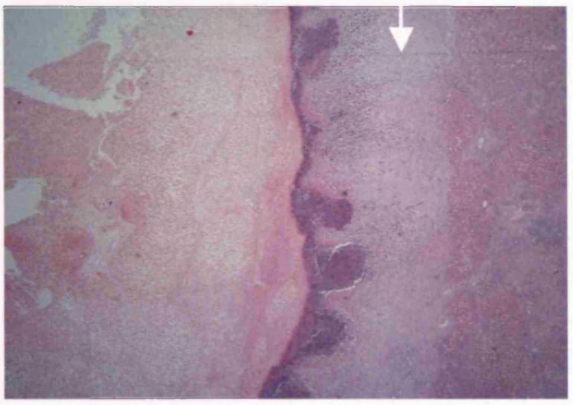


Figure 4.13. Porcine lesion at one week. The capsule (arrowed) is seen between the normal liver (extreme right) and the dark line of neutrophils.

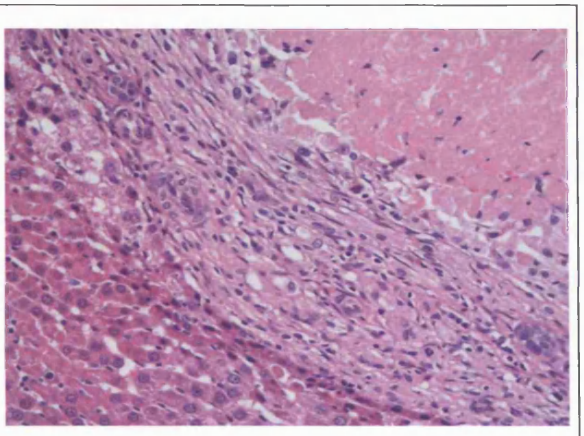


Figure 4.14. Rat lesion at one week. The capsule is well demonstrated lying between the necrotic ablation (top right) and the normal liver parenchyma (bottom left).

1 Month (Pig and Rat)

Most striking of all was the reduction in size of the wedge infarction and the overall lesion. In many of the rats the wedge infarct had been completely reabsorbed although a reduction in size of this area was seen in all animals. The microwave-induced lesion was reduced in the majority of cases by 50%, compared with the time 0 lesions, with the central cavity containing amorphous granular debris. In some cases the omentum had become adherent to the lesion surface although no other organs were found to be adherent to the liver, nor was there any evidence of rupture of the lesion.

The capsule seen at one month was of greater diameter. Many areas of bile duct reduplication were present in this capsule. Interestingly, even after this considerable length of time, the cells near the centre of the lesion had not yet undergone significant karyolysis or karyorrhexis. A large decrease in the volume of lesional cells was seen and often only a thin band separated the capsule from the more central nucleated cells. Surrounding the lesion were histologically normal hepatocytes.

A new feature noted in the fibrous capsule and in the larger lesions was the presence of lymphoid follicles, from which a light infiltrate of lymphocytes was seen to enter the normal hepatic parenchyma though no evidence of active hepatitis was identified.

60 Days (Pig only)

Two months following the microwave treatment the lesions had decreased in size dramatically. In one pig both the 50 and 100 Watt lesions had shrunk down to small irregular scars. The 150 Watt lesion still remained recognisable although this also had undergone a dramatic reduction in size. Encapsulating these areas was a white fibrous band similar to that described previously.

All lesions showed reduction in the volume of necrotic material in the lesion centres, with loss of the frankly necrotic material. The inflammatory cells had now reached the inner zone of cells in which the nuclei were present. Lymphoid follicles were present in the fibrous capsule and to some extent in the adjacent normal liver. Examination of the liver over a centimetre away revealed no abnormalities. The common bile ducts from these pigs were also examined, though no abnormalities were found.

ii) Human Ex-vivo and In-vivo

Following microwave ablation of normal parenchyma a sharp demarcation was evident between treated and untreated tissue, however tumour ablation produced similar macroscopic and microscopic results.

At 30 Watts the zone of thermal damage was seen macroscopically as a spherical area of blanching similar to those described in the animal models. The evolution was studied using an intra-operative ultrasound probe with real time scanning correlating with the macroscopic pathological findings. The formation of many echogenic lesions within the parenchyma, or tumour, in close proximity to the applicator was revealed, a process that appeared to coincide with the release of hot gases and fluid from the cavity.

Both normal liver parenchyma and metastatic tumour would appear to demonstrate a similar zonal pattern of thermal damage. As described above, a zone of coagulative necrosis approximately 3-4mm in width is formed centrally, with loss of the normal architecture and the development of cystic spaces. Adjacent to this central area is a zone composed of hepatocytes with a compact sinusoidal architecture, showing identifiable nuclear damage with nuclear hyperchromatism and chromatin 'smearing'. The liver plates showed evidence of collapse, with trabeculae greater than two cells thick in areas and containing portal triads demonstrating the identifiable changes highlighted above. This zone is demarcated by the evidence of frank coagulation proximally and sinusoidal expansion distally, the latter indicating the burn radius. Beyond this point the hepatocytes would appear to be cytologically and architecturally normal suggesting no evidence of thermal damage.

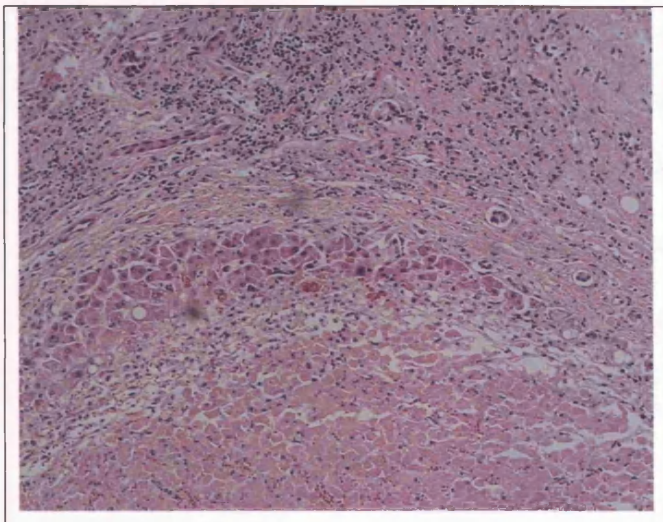


Figure 4.15. Post ablation section of human liver following microwave ablation (This patient underwent ablation of an HCC in cirrhotic liver)

The central veins and portal triads also show changes within the birefringent properties of collagen, but no obvious structural damage was identified.

The activity of specific hepatic enzymes within ablated parenchyma, as shown by immunohistochemistry, showed a sharp demarcation between the normal and the reduced, or absent, enzyme function within tissue that had undergone thermal damage. Transmission electron microscopy revealed the presence of ultrastructural damage in close proximity to the applicator cavity, with thermal destruction of cells within zone 1 and an associated disruption of cell organelles (predominantly mitochondria, due to their relative abundance within hepatocytes). Damage was also noted around bile canaliculi within this zone. Ultra-structural examination of cell organelles showed no damage beyond 10mm from the edge of the cavity, correlating with the macroscopic burn radius.

4.5 DISCUSSION

The small and large animal models mimic each other both in terms of the hepatic injury sustained, and how it is contained and eventually heals. For large volume microwave therapy to become an accepted treatment for inoperable liver tumours it needs to produce

lesions in a predictable, safe and efficacious manner. Lesions produced in both the rats and pigs were well tolerated and post operatively the animals showed little or no signs of distress. The macroscopic appearance of an area of spherical blanching suggests that these changes are due to the desiccating effects of heat generated by the application of microwave energy. The effects are such that the trabecular architecture of normal hepatocytes begins to collapse inwards, towards the probe cavity, as the tissue is treated. Thus, sinusoids are compressed and narrowed centrally, and at the edge of the thermal damage the sinusoids are widened. This confirms the findings in previous studies by Kato et al. 1996. These features are not as well defined within ablated tumour deposits, either in-vivo or ex-vivo, possibly due to the lack of a formal, well-defined architecture allowing such observations. The architecture of normal liver is altered proximally, with the resultant coagulative necrosis evident and the subsequent formation of a well-defined cavity. The surrounding debris is acellular and carbonised in areas, due to the evolution of high temperatures. Within this coagulated area, the resulting material is seen to develop a 'lace-work' appearance. This would be consistent with the ultra-sonographical findings of echo formation immediately adjacent to the applicator and would represents the generation of hot gas-filled pockets, assumed to contain steam. Kato et al. 1996 also described similar appearances within rabbit livers, with the tissue adjacent to the applicator vaporising. Magnetic resonance imaging at regular intervals post-treatment showed the development of a cystic structure, as fluid accumulated in the newly developed cavity. These findings were also confirmed by Matsukawa et al. 1997.

The distinct alteration in the birefringent nature of the collagen suggests that thermal damage has occurred, even in the absence of recognisable changes within the surrounding tissue. These findings would appear to confirm previous studies, and would suggest that temperatures have reached in excess of 60-70 degrees centigrade. Changes in birefringence has been shown to act as a marker for thermal damage and has been studied previously

(Thomsen et al. 1989) and was considered an indication of thermal damage at low temperatures. Collagen fibres are polymers composed of helical chains and micro-fibrils, stabilised by horizontal molecular cross-links (Ramachandran and Ramakrishnan 1976). Denaturation produces birefringent changes due to the uncoiling of the helical structure of collagen and loss of the inter-bridging molecular bonds (Schober et al. 1986). The colour changes may also suggest an altered uptake of the stains used in processing. The damage to portal triads can be accounted for by the dissipation of heat via fluid within the biliary or vascular systems. Heat generated locally by the microwave applicator appears to be diverted away from the central cavity by blood and bile, heated to abnormal temperatures. The resultant effect is of thermal damage to the endothelium, or epithelium, within these vessels and denaturation of the surrounding connective tissue (Thomsen et al. 1989). This intra-vascular dissipation of heat energy may also account for the irregular extension of the burn radius beyond the spherical blanching observed during intra-operative ultra-sound scanning. Delicate finger-like projections were seen to infiltrate the parenchyma for short distances, a finding also previously reported (Matsukawa et al. 1997). Macroscopically however, this was not a noticeable phenomenon and significant extra-lesional damage to the surrounding hepatic parenchyma from the tracking of hot fluids along vessels was not found. Interestingly, despite the presence of large amounts of necrotic material no animals from either species exhibited signs of abscess formation within the lesions.

One concern regarding large volume microwave treatment is the confinement of the effect, with particular emphasis being placed on the possibility of damage to nearby vascular or biliary vessels from hot fluid being forced along them during lesion production. Whilst evidence of such "tracking" was identified ultra-sonographically and histologically, no long-term consequences were identified in any of the one or two month survivors.

The difference between the appearances of the inner and outer zones of the lesion appeared somewhat paradoxical. The inner zone cells although receiving a higher dose of microwaves and therefore thermal damage remained nucleated even 60 days after the procedure whereas the outer burn zone cells underwent rapid karyolysis. One explanation may be the microwave effect itself. Examination of the treated liver parenchyma following a prolonged period of fixation in 4% formaldehyde solution also revealed the extent of this thermal damage. Glycogen is soluble within formaldehyde solution and, following long fixation periods, will leach out of the specimen, an artefact known as 'polarisation' (Culling 1963). Microwaves have long been advocated as a fast fixation method for human tissues, thus following treatment of the parenchyma, the ablated area retains its glycogen content, presumably due to the rapid fixation and alteration of the hepatocytes plasma membranes (Boon and Kok 1989). Staining with Periodic-acid Schiff reagent confirms the zone of fixation, and would appear to correlate well with the macroscopic findings. Studies have suggested that the appropriate temperature for fixation of human liver to be in excess of 50 degrees, and possibly optimal at 70 degrees centigrade (Leong et al.1985). Therefore, these findings, combined with the presence of collagen denaturation, would suggest that such temperatures have been reached, and is well within the required 42.5°C needed for efficient ablation (Matsukawa et al. 1997). It would be unlikely that any cell could survive such a thermal insult and it is predicted that the cells within this radius would undergo autolysis subsequently

Many authors have voiced concern over the ability of ablative therapies to treat 3-4cm lesions adequately and safely (Dodd et al. 2000,Shimada et al. 1998). The reasons for the failure of ablative treatments to treat larger lesions may be due to inaccurate placements of the applicator (Curley et al.2000), the effect of nearby vascular structures (Ravikumar et al.

1991) or the possibility of heterogenous treatment volumes (Jiao 1999). Our group suggests that multiple insertions of a probe to cover the entire volume of a tumour using whatever ablative technique risks the formation of islands of untreated tumour cells from which local recurrences may arise. The ability to treat large lesions, rapidly with a single insertion of the applicator is therefore a highly desirable property, especially when it is considered that many patients with inoperable liver tumours are elderly. Therefore treatments which require a prolonged laparotomy are not desirable. Cryotherapy can produce large volumes of ablation (Seifert et al. 1998) although it is associated with significant dose-dependent side effects (Weaver et al. 1995, Cozzi et al. 1994, Stewart et al. 1995). Electrolysis, is not currently a percutaneous technique and the time required to produce a four centimetre sphere of ablation is often many hours (Robertson et al. 1998). The currently available microwave equipment is able to producing lesions up to 2cm in diameter relatively quickly (Shibata et al. 2000). Attempts to increase the volume of ablation produced with a single insertion have previously been unsuccessful unless the hepatic blood flow is attenuated. Not only does this require complex cannulation of the vascular tree, it increases the invasiveness of the procedure.

The microwave equipment designed and produced by the Medical Devices Technology Group from Bath University is able to produce large volumes of ablation safely in both rat and pig liver. Both species were able to tolerate the lesions produced, suffered no vascular or biliary complications healing the lesions after forming a protective capsule. Importantly, no islands of viable cells were seen in the ablated volume. The lesions produced in the human showed identical features. Although they were too hot to touch up to the periphery, there was little identifiable evidence of damage beyond the zone of nuclear smearing, in keeping with those observed within our animal trials. The absence of microscopic changes was also identified by Matsukawa et al., who reported that evidence of damage was only seen in animal trials 3 days post-ablation (Matsukawa et al. 1997). Therefore, it is

suggested that both the macroscopic burn radius and ultra-sonographical diameter would represent the minimal zone of treatment. Using the fact that both the small and large animal model exhibited very similar histological patterns following microwave treatment it is not unreasonable to expect human livers to behave in an identical manner. Although the lesion diameters were reduced compared with the porcine experiments this can be accounted for by the lower power and higher frequency applied. The results from these animal trials outlined within this paper demonstrate the safety of using the frequency of 2.45GHz and power up to 150 Watts to induce multiple ablations of hepatic tissues with this microwave equipment. As such, this will allow us to adequately treat lesions up to 6cm in diameter, hitherto unparalleled volumes, with a single insertion of the applicator over an extremely short time period (180 seconds). This represents a significant advance in surgical management.

Further research and development within this field aims to include the measurement of alterations in the levels of tumour-related proteins and indicators of tissue damage. The future aim is for the development of applicators allowing effective treatment either laparoscopically or percutaneously under ultra-sound guidance. This would require the design of an applicator narrower in width, but able to produce a large ablative burn. This would require alteration in the physical properties of the probe tip, and is the subject of on-going research. A potential hazard associated with all microwave modalities is reflected power and this is measured in decibels (dB) with the Microsulis Research Group using – 10dB as an arbitrary acceptance limit for an applicator. Minimising the percentage of reflected power is essential to ensure that the majority of power applied heats the tissue, prevents damage to the microwave generator and diminishes heating of the applicator shaft, which could result in burns incurred by the patient or the operator; both unacceptable risks.

It is hoped that future developments in staining and processing techniques within the laboratory, including the possibility of staining with vital dyes, combined with the commencement of further human trials, will aid in the confirmation of the extent of lethal cell damage. The continued research within this field should yield interesting results, both in terms of histological findings and in improved patient outcomes

4.6 CONCLUSIONS

Histologically, the effect of the microwave appeared very reproducible and predictable. The treatment induced a lesion that was sharply defined, containing no islands of viable cells and did not appear to be significantly impaired by vessels acting as heat sinks. The liver and animal appeared to cope well with the microwave insult, inducing the formation of a dense fibrous capsule that may well have prevented infection of the lesions and abscess formation. Lesions appeared to evolve in a predictable manner and in a way that was similar for both animal species. The initial results in humans showed similar features to those in the animal models and therefore it may be assumed that any microwave ablations placed in a patient would show similar lesion edge characteristics and evolve in a similar manner to those described here. No histologically demonstrable systemic effect was noted even in the groups of rats in whom 3 and 4 ablations were placed despite between 25 and 30% of the liver being ablated. This is encouraging as previous studies investigating cryoshock in different animal models were all able to demonstrate lung inflammation. Results from these investigations are encouraging and indicate that microwave ablation may be an effective ablative treatment and also well tolerated in patients with unresectable colorectal liver metastases.

CHAPTER 5

MICROWAVE LESION PREDICTABILITY 3: MICROWAVE PENETRATION THROUGH COLORECTAL METASTASES AND NORMAL LIVER PARENCHYMA.

5.1 INTRODUCTION

i) Why is microwave penetration important?

ii) Microwave penetration through tissues: electromagnetic theory

iii) Skin Depth calculation

iv) Complex permittivity

5.2 AIMS

5.3 MATERIALS AND METHODS

i) Calibration of equipment

ii) Clinical measurement technique

5.4 RESULTS

i) Calibration of equipment

ii) Clinical measurements in normal liver parenchyma and colorectal metastases

iii) Statistical analysis

iv) Calculation of microwave penetration (skin depth)

Why is microwave energy consistently absorbed more quickly by colorectal metastases than normal liver parenchyma?

5.5 DISCUSSION

5.6 CONCLUSIONS

PREDICTABILITY 3: MICROWAVE PENETRATION THROUGH TUMOUR TISSUE AND NORMAL LIVER PARENCHYMA.

5.1 INTRODUCTION

i) Why is microwave penetration important?

All ablative techniques have been tested in large animal models (usually porcine) to investigate lesion volumes and tolerance of the technique (Eggstein et al, 2003, Chapman et al 2000, Chang et al 2002, Chinn et al 2001, Strickland et al 2002, Goldberg et al 1998, Dachmann et al 1990, Wang et al 1997, Wemyss-Holden et al 2002, Wemyss-Holden et al 2000). Unfortunately, no experimental colorectal cancer cell line is available and therefore experiments are performed on animals with normal liver parenchyma. As a result of this it can be argued that the effect on tumour tissue of any given ablative system is unknown and that any dose response curve produced invalid. This chapter explores the relationship between microwave penetration through normal liver parenchyma and hepatic colorectal metastases.

Many alternative ablative techniques rely on conduction of heat (cryotherapy, laser, radiofrequency) or passive diffusion of chemicals (electrolysis, percutaneous ethanol injection or P.E.I.). Conduction is not only an inefficient means for the transference of heat through tissues with a high water content such as liver, but also due to the high hepatic blood flow, the risk of inefficient ablation is higher due to a heat sink effect (Mulier et al 2003, Burdio et al 2003, Ravikumar et al 1991) .

The microwave equipment used in these experiments produces a field of microwaves from the radiating tip of the equipment a schematic of which is seen in Figure 1.

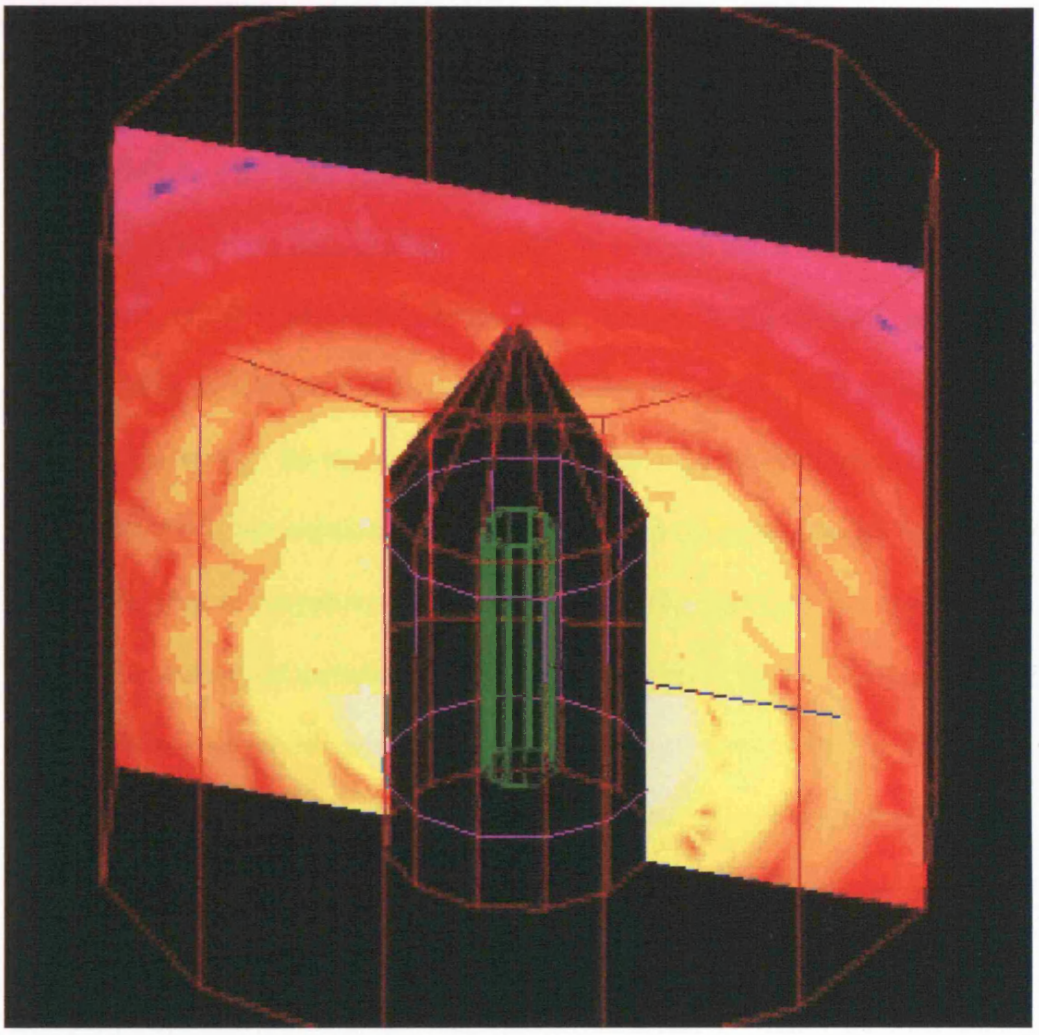


Figure 1. Schematic of the microwave field (in 2D) produced from the newly designed applicator (central black object). Different colours represent the amplitude (intensity) of the field at any given point from the radiating elements of the applicator. The fact that the most intense (white/yellow) portion of the field is not confined to a space immediately adjacent to the applicator suggests that good radiation into the tissue will occur and that reflection is minimal. The colours ranging from white to pink indicate field amplitude (field strength) at individual points in the field. White

nearest the applicator indicates the higher values decreasing to lower amplitudes with the lowest shown on this schematic being pink.

Lesions produced in the large animal model in Chapter 3 were produced over 180 seconds which is far more rapid than any alternative technique. Such a rapid ablation allows comparatively little time for conduction to occur and it is therefore likely that the majority of the heating is due to the direct interaction between the microwaves and water molecules within the treated tissue. It follows therefore, that penetration of microwaves through the tissue is of great importance in the production of large lesions since it is the interaction between waves and tissue that causes the heating. A large difference in the penetration of microwaves through normal liver parenchyma and hepatic colorectal metastases may significantly alter the lesion size achievable in tumour tissue. Any difference in the microwave penetration of tissues will affect the volume of the field produced within the tissue and therefore the volume in which direct field heating occurs and ultimately lesion size.

If microwave penetration through colorectal metastases is significantly different to that of normal liver parenchyma then this has considerable consequences upon the microwave applicator designs. Efficient radiation of microwave energy into the tissue relies on knowledge of reflection phenomena at the applicator tissue interface and any difference in the electromagnetic properties of tumour tissue may affect the radiating efficiency of the applicator and therefore its performance in tumour tissue. No data exists regarding the penetration of microwaves through these tissue types in either the ex or in-vivo setting.

ii) Microwave penetration through tissues: electromagnetic theory

As microwaves pass through tissues the wave gives up energy as it interacts with polar molecules. In human tissue the most prevalent polar molecule is water and throughout the microwave spectrum of frequencies the interaction between the waves and this molecule is strong. Microwave penetration through tissue is described in terms of "skin depth" which is the point in the tissue at which the wave has given up a mathematically defined proportion of its energy designated δ. In essence this expression defines the point at which the wave has given up approximately 67% (or 2/3) of its energy. Skin depth is therefore quoted as a distance and in this context is usually millimetres see Figure 2 below.

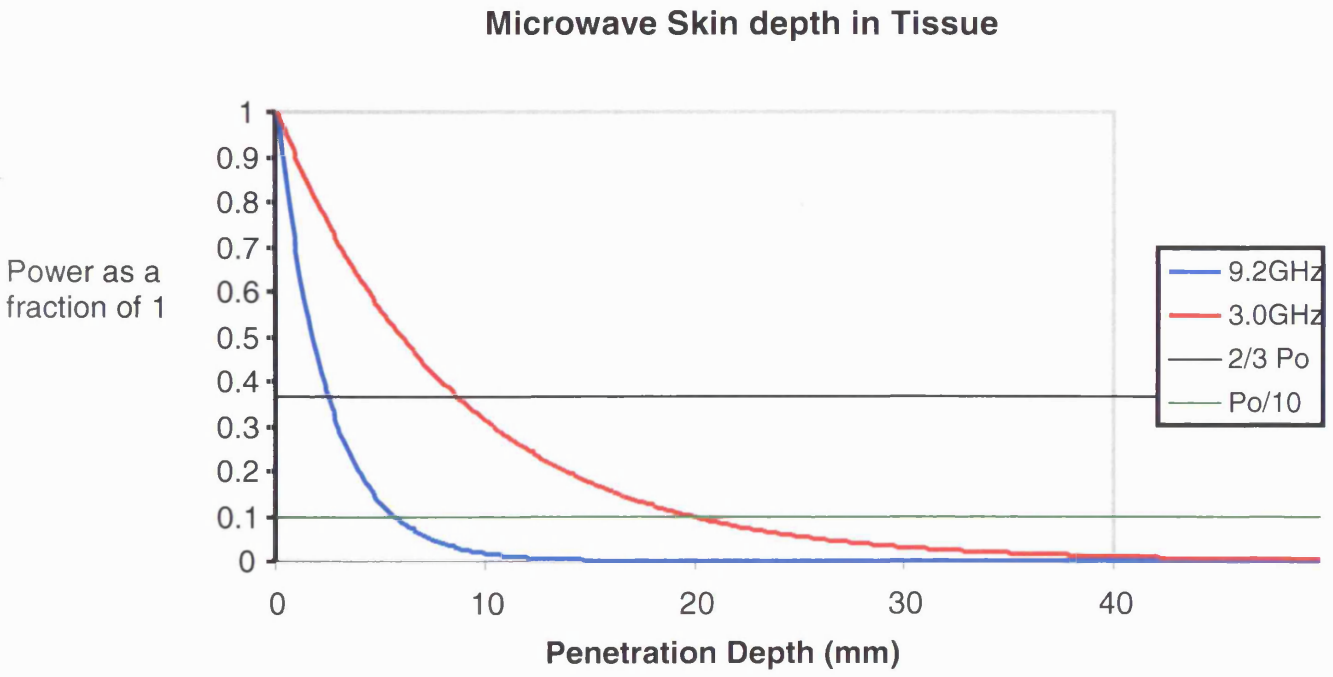


Figure 2. Skin depth (black line 2/3 Po where Po is the initial or incident power i.e. the point at which the wave has lost approximately 2/3 of its power or amplitude known as the skin depth) calculation for normal liver at 9.2 and 3.0 GHz. It is

important to note that with all other parameters equal, frequency has a profound effect on microwave penetration through tissue. (these figures were based on the **predicted** permittivity values for normal liver).

Microwaves are part of the electromagnetic spectrum and as such are governed by wave theory widely investigated by physicists for many years. The interaction of waves with different media has been studied for many years as the laws that govern these interactions have been utilised in many different ways. The formulae which have been produced to enable the calculation of skin depth follow. The formulae are annotated to allow the reader easier understanding of the process. Reduction in the energy of the wave is expressed as a decrease in its amplitude.

iii) Skin Depth: Calculation

Equation (1)

$$\delta = \frac{1}{\alpha}$$

Skin Depth

expression for the decrease in amplitude of wave per metre (attenuation coefficient)

The attenuation factor α is calculated by the following equation.

Equation (2)

$$\alpha = \omega . \sqrt{\frac{\mu..\varepsilon .}{2} \left[\sqrt{1+\left(\frac{\sigma}{\omega.\varepsilon}\right)^2} - 1 \right]}$$

Where: ω = angular frequency or put more simply is the product of :-

2 x pi x frequency of the microwave signal used i.e.

$\omega = 2.\pi.f$ (where f is the microwave frequency investigated in Hertz)

$\sigma =$ conductivity (this is the reciprocal of resistivity which a measure of resistance per unit length)

$\mu =$ permeability of free space (is a magnetic quantity which would be of importance in magnetic materials but is unimportant in organic tissues as these are not generally magnetic and therefore assumed to be 1 in all these equations).

$\varepsilon = \varepsilon_r . \varepsilon_0$

ε_r = relative permittivity this is the multiplying factor for the permittivity of free space and is a dimensionless quantity (eg. this may be 3 and therefore the permittivity of a particular tissue is x3 the value of permittivity of free space)

ε_0 = Permittivity of a vacuum. It should be remembered that a true vacuum allows an electromagnetic signal to pass an infinite distance without that signal losing energy to the vacuum. The permittivity of any substance through which an electromagnetic wave is radiated is compared to that of a vacuum.

By measuring the complex permittivity of human liver parenchyma and colorectal liver metastases we can determine the value of episilon and therefore solve equation (2) by determining α and from this equation (1) determining the skin depth and therefore microwave penetration of these two tissues.

iv) Complex Permittivity

Permittivity is a number which describes the relative ease with which a wave can penetrate a substance. The higher the value of the number, the greater the resistance of the tissue to the propogation of the wave through it. Permittivity in this context is the mathematical description of the interaction of the wave and the material through

which it passes. It is known as complex permittivity because the value is a complex number i.e. it is made up of two parts. For instance, the velocity of a body travelling in a straight line is also a complex number. Velocity is similar to complex permittivity as it has two components namely magnitude **and** direction. A body moving at a constant speed in a straight line will have a constant velocity however a body moving in a circular orbit at a constant speed has an ever changing velocity as at no point in its orbit does its direction remain constant. In the case of complex permittivity, the number is described as having a "**real**" part which describes the dielectric constant of the material and an "**imaginary**" part which describes the total energy of losses experienced by the wave as it passes through the material. Together these two components give all the information it is possible to know about the propogation of a wave of certain frequency through a material. Once a material's complex permittivity is known, the skin depth can be calculated and, therefore, the penetration of microwaves through a tissue can be calculated.

5.2 AIMS

The aims of these experiments were to measure the complex permittivity of normal human liver parenchyma and hepatic colorectal metastases, to investigate if these measurements varied between individuals, if so by how much and whether any difference between the two tissue types was reproducible. From these measurements the microwave penetration through the tissues could be calculated and its effect on applicator performance assessed.

5.3 MATERIALS AND METHODS

The previous equations allow the calculation of the penetration of microwaves at different frequencies to be calculated. To allow this the permittivity of normal human liver parenchyma and colorectal metastases needed to be measured. Measurement of the complex permittivity of certain materials has been undertaken in a number of fields but its use in the treatment of human disease is novel.

Complex permittivity of a material over a range of microwave frequencies can be calculated from the magnitude and phase change of the reflected wave of a wave following its radiation into the substance to be measured (see Figure 3). Built from published literature (Land and Campbell 1992), a nickel coated, open-ended coaxial probe was placed into the hepatic parenchyma or tumour to be measured (Figure 4). A microwave signal over a range of frequencies of 1 to 10 GHz was passed into the tissue. The reflected wave was passed into a Vector Network Analyser (VNA) where, using computer software, the complex permittivity of each substance was computed (Figure 5). A minimum of 5 readings were taken from each tissue type measured.

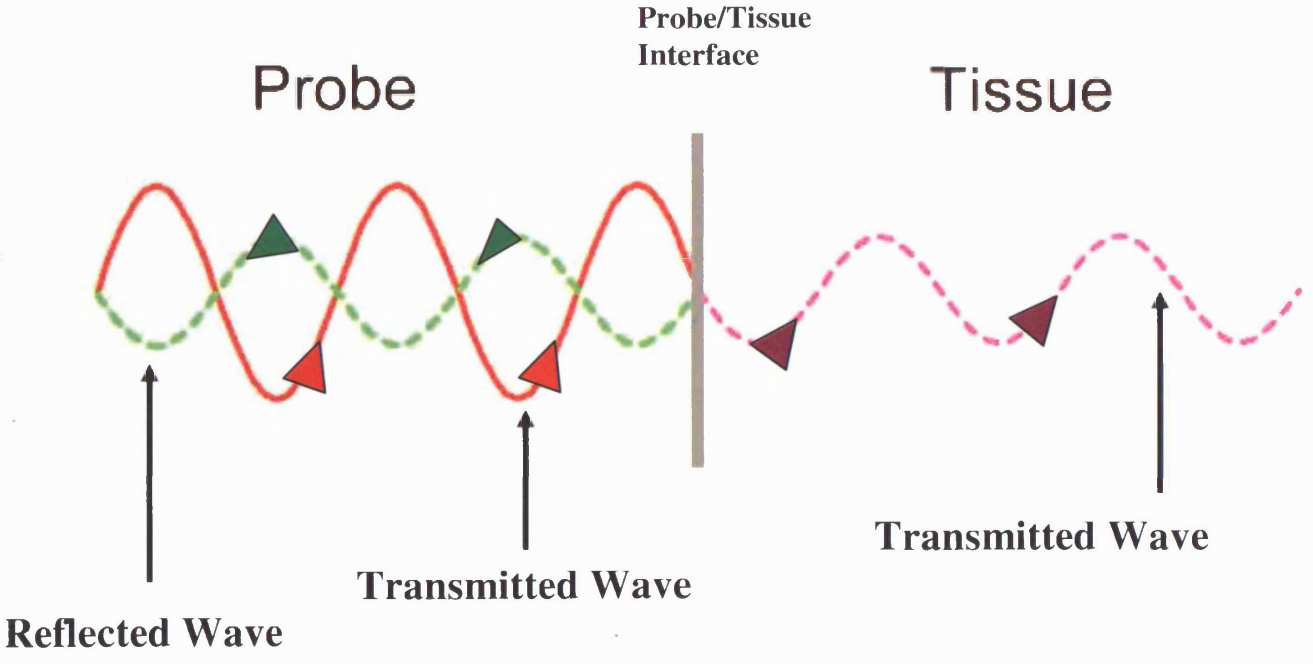


Figure 3. Schematic showing the method of data aquisition used by the permittivity probe when placed into tissue. Red line is original wave transmitted along the coaxial probe and out into the tissue. Purple line is transmitted wave into the tissue and passing through it. Green line is reflected from probe tissue interface back into the coaxial probe. Grey line is interface between the coaxial probe and tissue. Phase change of a wave can be seen from the diagram. In this example the phase of the original (red) wave has been changed 180 degrees (green).

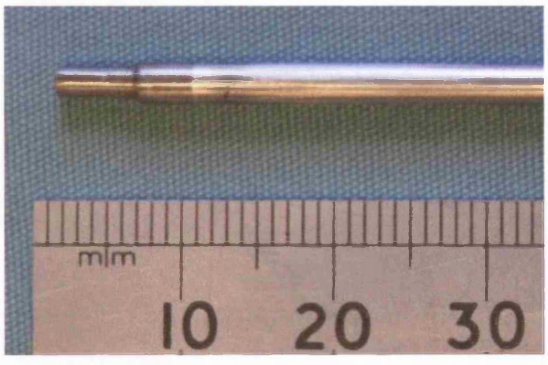


Figure 4. The open-ended coaxial probe used for taking permittivity readings.

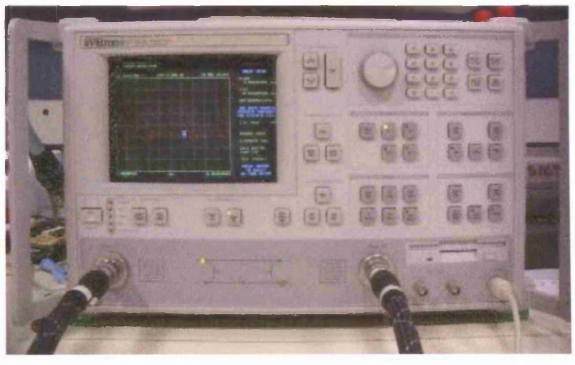


Figure 5. The Vector Network Analyser.

i) Calibration of the equipment

Currently no in-vivo permittivity data over a microwave frequency range exists and it was therefore essential to ensure that the data gathered was accurate as there is no comparable data. Prior to each reading, the system was calibrated against a set of four standards of known complex permittivity followed by a confirmatory measurement of a saline solution of known complex permittivity, close to the expected value of the test materials. The complex permittivities of the four calibration standards were selected to give a wide spread of values inclusive of the range to be measured in

practice and were, a short circuit (made from pure indium), water, ethanol and methanol. The solutions used for the calibration of the equipment were extremely pure (known as AnalaR® grade) in an effort to maximise the accuracy. The confirmatory test solution was selected as 0.1N saline as this was expected to be an approximation to the likely measured values of complex permittivity in organic tissues (the complex permittivity of 0.1N saline is also known) (Buckley and Maryott 1958). When good agreement was obtained between the known and measured complex permittivity of the 0.1N saline test solution the in vivo tissue measurements were commenced (see figure 6 for a typical calibration chart). Successful calibration was achieved prior to obtaining readings from each subject and as can be seen from figure 6 agreement between the calculated and measured value was extremely close.

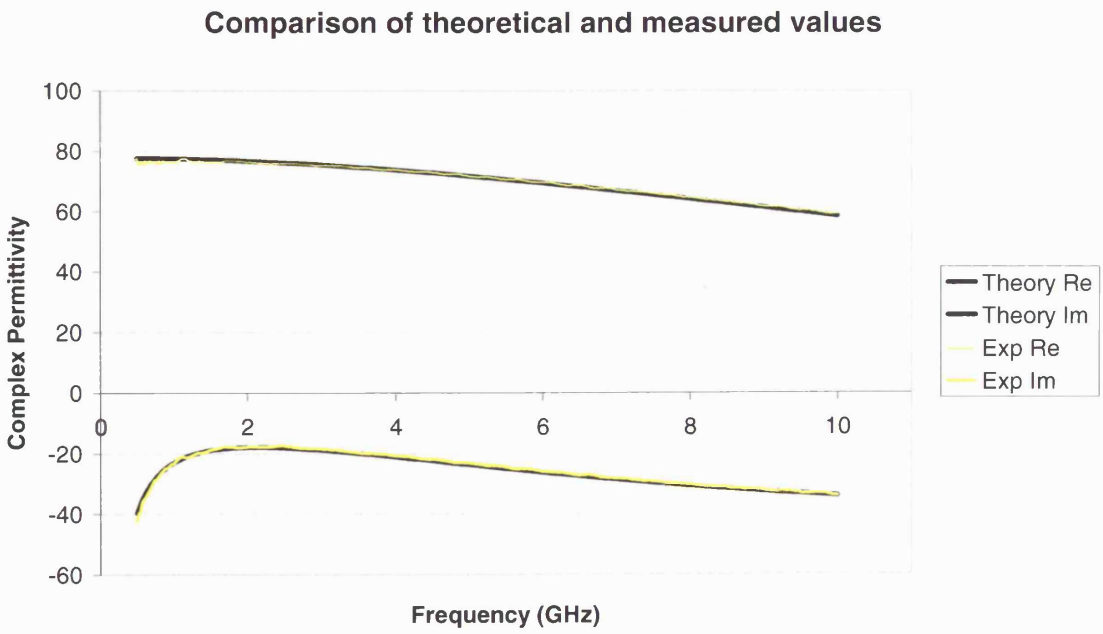


Figure. 6. Agreement between measured and theoretical complex permittivity over microwave frequencies of 0.5-10 Gigahertz of 0.1% saline solution.

Interpretation of the graph: The X axis is the range of which the microwave frequencies were tested i.e. 0.5 to 10 GHz with complex permittivity on the Y axis. The real portion of complex permittivity is the upper, positive portion of the graph and highlights the ability of the substance to take up energy from the wave (in this case the energy is expressed as heat). The greater the real portion of complex permittivity the better the substance being measured is at taking up energy from the wave. Using this graph it can be seen that this property of the substance being measured is dependant upon the frequency of the microwave applied i.e. from the graph for saline is better able to take up energy from the wave at lower frequencies than higher. The negative portion of the graph relates to the imaginary portion of complex permittivity and defines the total energy losses experienced by that wave as it travels through the substance being measured. The more negative the value of the imaginary portion of complex permittivity the greater the losses experienced by the wave. Again in this example the imaginary portion of complex permittivity is frequency dependant as it can be seen from the graph energy loss per unit length of tissue is much less at 2GHz than 10GHz.

Close agreement between the known and measured values were of great importance as this implied that readings being taken were in fact a true representation of the tissues characteristics. The equipment in fact, showed excellent agreement between the known and measured values with better than 1% error found. As scant information regarding the electromagnetic characteristics of tissue is available it would be difficult to cross-reference the values.

ii) Clinical Measurement Technique

Eleven metastases in the livers of nine patients were measured using the sterilised, open-ended coaxial probe. Prior to any readings the system was set up and calibrated until accurate readings were produced for the 01.N saline solution. The coaxial sensor was sterilised using ethylene oxide to prevent it being damaged by autoclaving. At laparotomy, the probe was placed into the patient's liver parenchyma at five different points. Good apposition between the tissue and the sensor was ensured and the readings taken. The sensor was wiped with alcohol swabs between each reading and following the acquisition of normal liver parenchyma data it was placed into the colorectal metastasis/es. Each metastasis was measured five times and the mean reading calculated.

5.4 RESULTS

i) Calibration of the equipment

The system was successfully calibrated prior to each set of readings. The agreement between the known and measured permittivity reading for the 0.1N saline was extremely close (<1% error) at each session the equipment was used. The extreme sensitivity of the equipment was highlighted at a session in which the measured result for the 0.1N saline and the known values was consistently different. Despite repeated checks of the equipment no fault could be found. It was realised that the discrepancy was due to the unusually low temperature of the calibrating solutions used. At most sessions the readings were taken some hours after the equipment had arrived in the theatre complex and therefore the temperature of the solutions had equilibrated with

the theatre's. On this particular morning the equipment had been taken straight from the car where it had sat in a cold car overnight. The lower temperature of the solutions was enough to significantly alter the reading obtained for the 0.1N saline test solution. Following equilibration of the solutions to the ambient temperature the usual accurate reading was obtained.

ii) Clinical measurements in normal liver parenchyma and colorectal metastases

Complex permittivity was measured at 401 data points over the microwave frequency range of 0.5 - 10 GHz in approximately 3 seconds. Five readings were taken from each tissue type and the results averaged. Readings obtained from normal liver were very consistent. Readings obtained from a single patients liver varied only a small degree as did those obtained from different patients. Results from colorectal metastases again showed little variation between different lesions within the same liver but also little variation was noted between patients. A difference was however noted between the results obtained from normal liver parenchyma and colorectal metastases. A typical result of complex permittivity measurements in normal hepatic parenchyma and colorectal metastasis is shown in figure 7. The graph shows both the real and imaginary values of complex permittivity over the range of microwave frequencies tested.

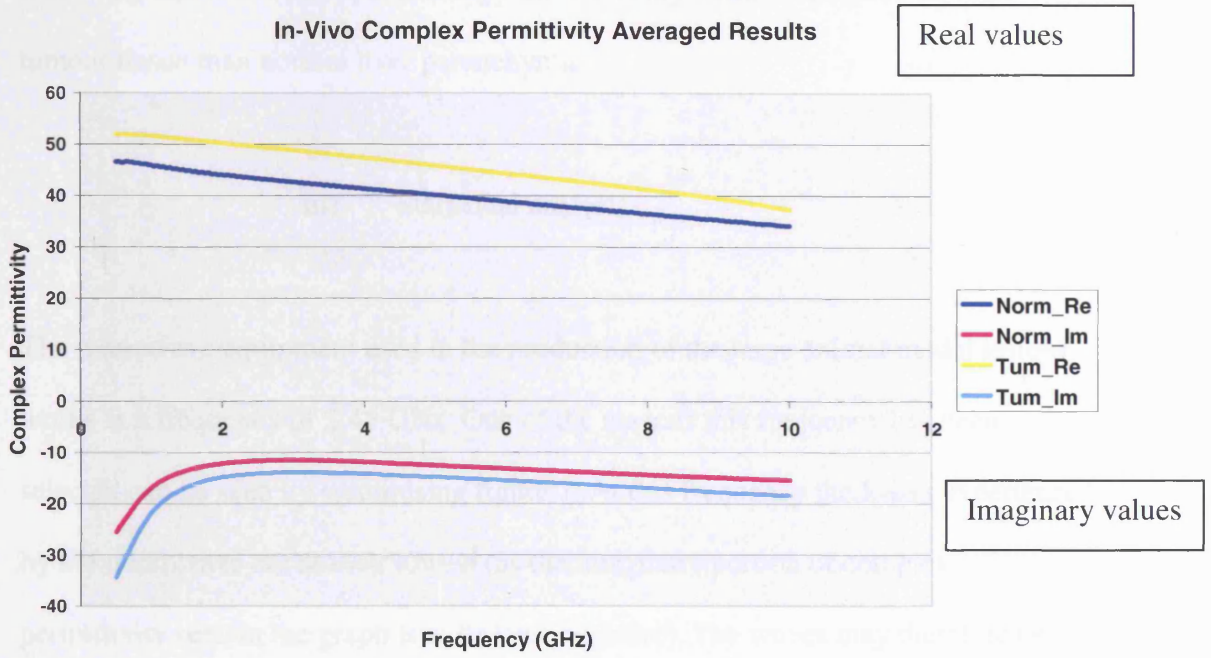


Figure 7. Comparison of the complex permittivity of normal hepatic parenchyma and colorectal liver metastases.

To aid interpretation of the graph, it is helpful to be reminded of the manner in which the graph should be interpreted. The real portion of the graph (positive values) is an indication of the tissue's ability to take up energy from the wave, a higher reading indicating a greater amount of energy being absorbed by the material. From the graph it can be seen that across the frequencies tested, the tumour tissue was able to store/absorb a greater amount of energy when compared to the normal hepatic parenchyma. This result was consistent for all patients tested.

The imaginary (negative) values describe the total energy losses experienced by the wave. More negative values indicate greater losses per unit length of tissue. Again, over the complete range of frequencies tested a reproducible difference was noted. Tumour tissue had a consistently more negative value across the frequencies tested

indicating that microwaves lose energy more quickly when propagated through tumour tissue than normal liver parenchyma.

iii) Statistical analysis

The microwave equipment used in the production of the large animal model lesions works at a frequency of 2.45 GHz. One of the reasons this frequency has been selected can be seen by scrutinising figure 7. At this frequency the losses experienced by the microwave are at their lowest (as the imaginary portion of complex permittivity seen in the graph is at its least negative). The waves may therefore be radiated over a greater distance allowing the production of larger lesions. This is the reason that commercial microwave ovens use this frequency for cooking, as it is the most efficient.

The values of the real and imaginary portions of complex permittivity at the frequency of 2.45GHz were compared (see figures 8 and 9). Results were analysed using a Wilcoxson rank test paired analysis to assess whether the difference noted in the values was significant. A value of $p< 0.05$ was designated to be statistically significant. Highly significant differences ($p< 0.009$) were found to exist between tumour tissue and the normal hepatic parenchyma for both the real and imaginary portions of complex permittivity indicating that microwaves do interact between the two materials in a different manner.

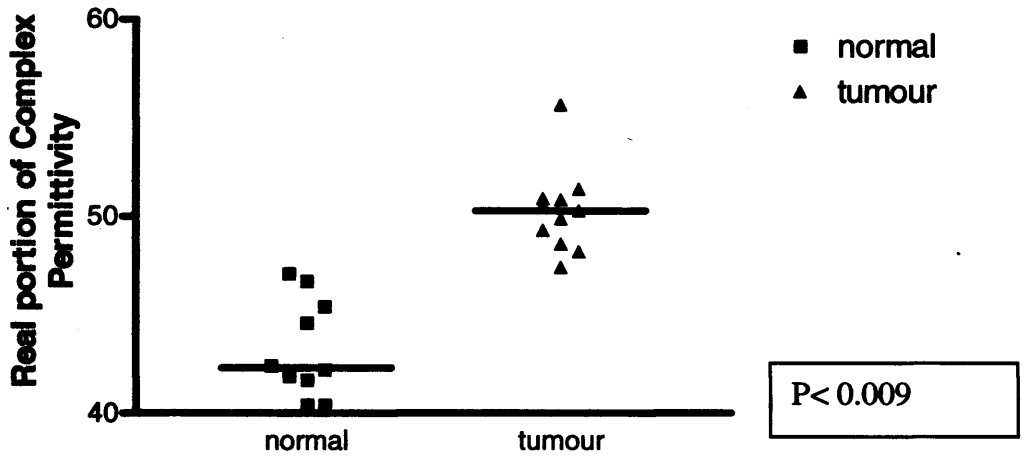


Figure 8. Real portion of complex permittivity at the microwave frequency of 2.45GHz. A highly significant difference is seen between the two tissue types at this microwave frequency indicating tumour tissue takes/stores more microwave energy than normal liver parenchyma.

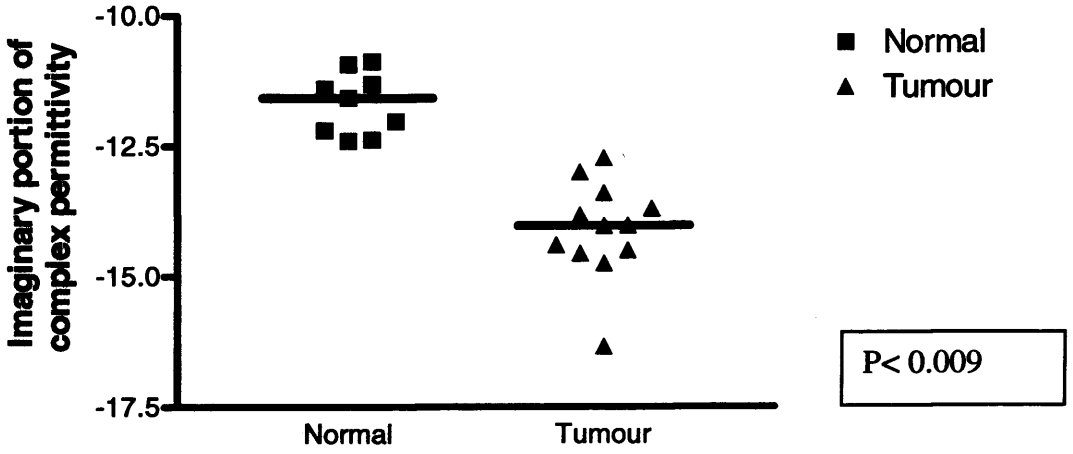


Figure 9. Again this shows a highly significant difference in the imaginary portion (total losses experienced by the wave) of complex permittivity. At this frequency microwaves loose their energy more rapidly when passing through tumour tissue.

iv) Calculation of microwave penetration (skin depth)

Using the values obtained for the complex permittivity of normal human parenchyma and colorectal liver metastases it was possible to solve equations (1) and (2) producing skin depth data for the tissues investigated (see figure 10). From the graph of figure 10 it can be seen that skin depth and therefore microwave penetration is greater in normal liver parenchyma than hepatic colorectal metastases. This was noted in **all** the patients investigated. The difference in skin depth was highly significant as, using a Wilcoxson Rank Paired Analysis test a "p" value of < 0.009 was obtained. The mean skin depth for both tissue types has been calculated and plotted graphically (see figure 10). Although there is a highly significant difference between the skin depth in the two tissue types the actual difference in penetration of microwaves between the two is small, 2.54mm.

Microwave Skin Depth at 2.45GHz of normal liver parenchyma and colorectal metastases

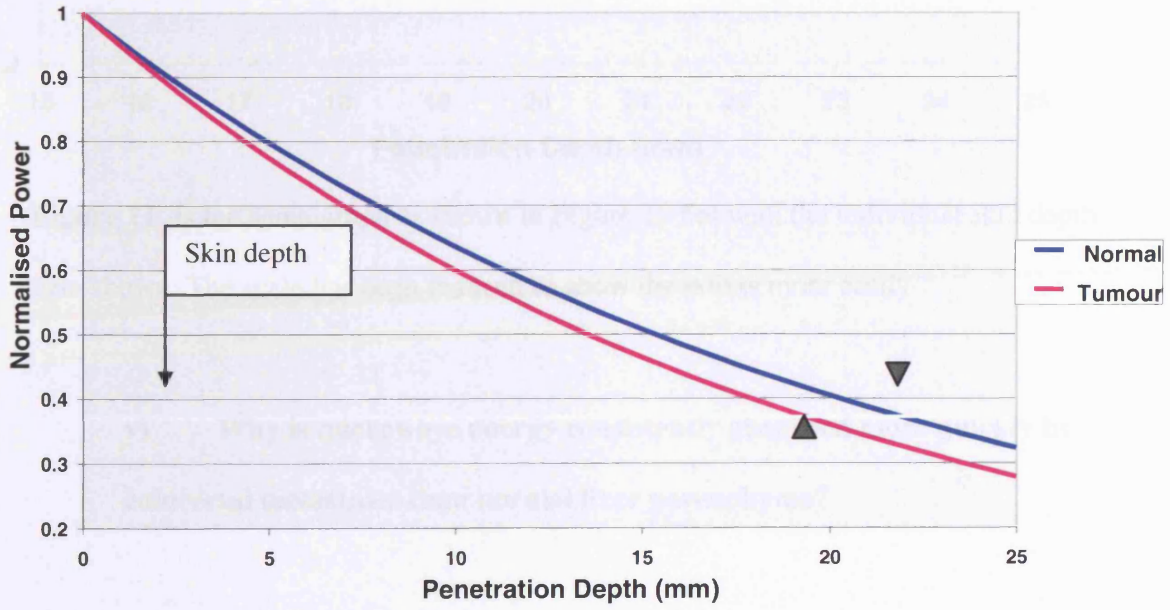


Figure 10. Microwave Skin Depth in human liver parenchyma and hepatic colorectal metastases. The X axis is in millimetres and the Y axis is a fraction of the energy of the wave. The white line is the mathematically defined point at which skin depth is calculated (i.e. the distance that the wave has to pass through the tissue to lose 67% of its original energy). The two arrowheads indicate the increased skin depth in normal liver parenchyma when compared to that of the tumour tissue (2.5mm).

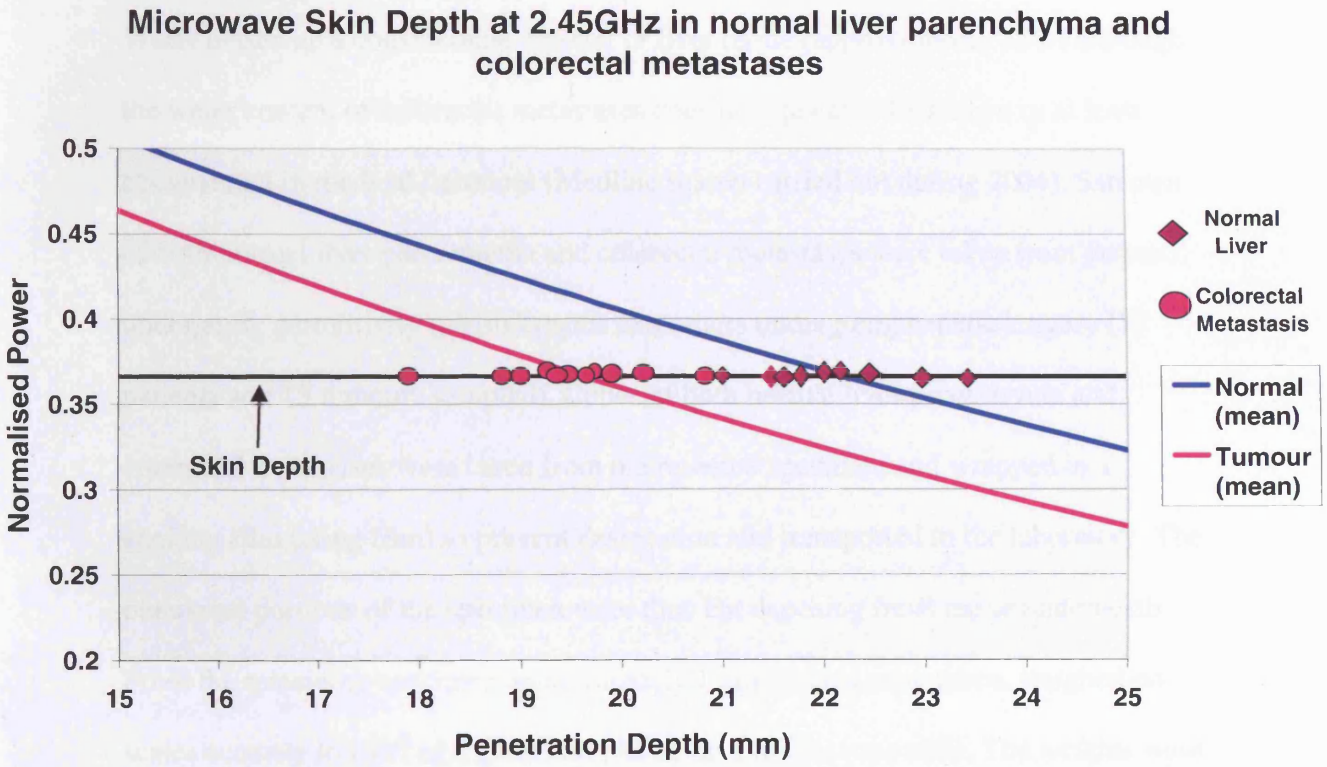


Figure 11. Is the same graph as shown in Figure 10 but with the individual skin depth data shown. The scale has been reduced to show the points more easily.

v) Why is microwave energy consistently absorbed more quickly by colorectal metastases than normal liver parenchyma?

No previous information regarding microwave energy propagation through normal liver parenchyma and colorectal metastases is available. Microwaves are, however, known to strongly interact with polar molecules.Water molecules are highly polar due to the electronically asymmetrical bonds between the negatively charged oxygen atom and the positively charged hydrogen molecules. The constantly oscillating microwave interacts with the molecule causing it to rotate generating friction between the particles expressed as heat.

Water makes up a considerable amount of liver tissue (approximately 75%) although the water content of colorectal metastases does not appear to be known or at least documented in medical literature (Medline search carried out during 2004). Samples of both normal liver parenchyma and colorectal metastases were taken from patients undergoing permittivity measurements and others undergoing hepatic surgery (12 patients and 13 tumours sampled). Cubes of both normal liver parenchyma and colorectal metastases were taken from the resected specimen and wrapped in a cooking film (cling film) to prevent desiccation and transported to the laboratory. The outermost portions of the specimen were then cut exposing fresh tissue underneath. From the remaining specimen approximately 1 gram chips were taken, weighed on scales accurate to 100^{th} of a gram and placed on a microscope slide. The weights were recorded and slides numbered. Approximately 10 chips from normal liver and colorectal metastases (or as many as the size of tissue cube permitted) were taken and the samples placed in an oven at approximately 45-50°C for 5 days. Initial samples were weighed on the fifth day and placed back into the oven for a further 3 days to investigate if the dry weight had not been achieved in the initial incubation.

Results from this additional work revealed that 5 days at 45-50°C was enough to desiccate the tissue to its dry weight. All the samples lost a significant amount of weight. 95 samples from tumour tissue and 100 samples from normal liver parenchyma were investigated from a total of 12 patients. The weight loss of each chip was recorded and the average for that tissue type taken. The table below shows the average weight loss by desiccation expressed as a percentage of the initial wet weight. Medians for all the samples are shown in bold at the bottom of the table.

Normal liver parenchyma	Colorectal metastasis	% weight loss difference
74	80	6
73	81	8
71	83	12
68	77	9
70	79	9
75	78	3
69	83	14
72	85	13
69	80	11
61	80	19
70	81	11
68	80	12
Median 70	**80**	**11**

Table 1. The table shows the percentage weight of normal liver and hepatic colorectal metastases made up by water calculated following desiccation. The last column shows the difference in the percentage of water by weight between the two tissue types. It can be seen from the table that in all samples the weight loss was

greater in tumour indicating the water content of this tissue is higher than that of normal liver parenchyma.

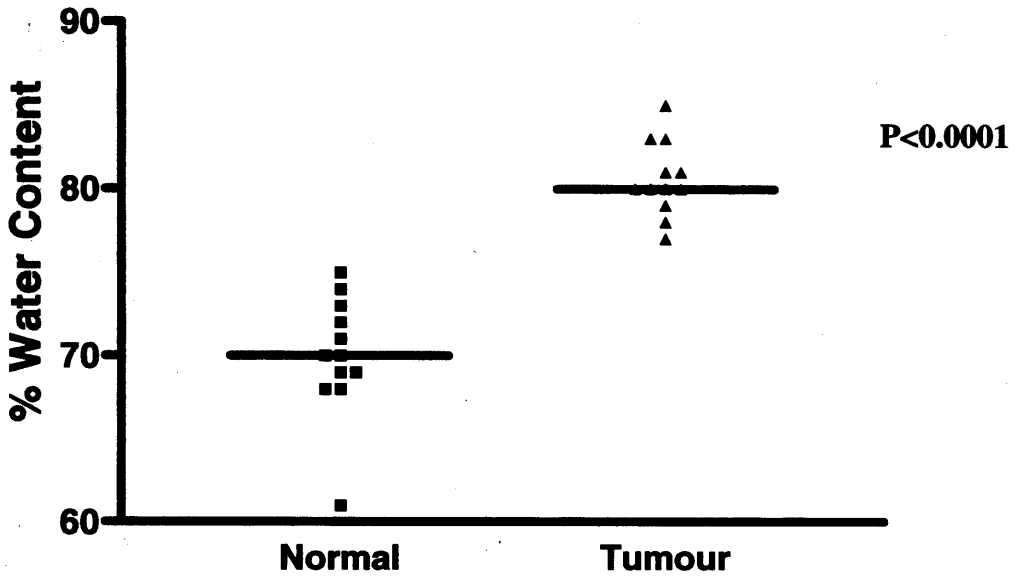


Figure 12. Using a Mann-Whitney U test a highly significant difference is observed in the water content of normal hepatic parenchyma and colorectal metastases.

5.5 DISCUSSION

Using the co-axial probe, the vector network analyser and the appropriate computer software it was possible to obtain complex permittivity data in nine patients with eleven metastases. The data is likely to be accurate as firstly, the equipment was accurately calibrated prior to its use in the subjects and secondly results were very reproducible with little variation between the two tissues measured.

Measurements of complex permittivity for each tissue were very reproducible with little variance. A small but highly statistically significant difference was seen in the

skin depth of the two materials indicating the interaction between the microwaves and measured tissues was detectably different. Tumour tissue exhibited a consistently smaller skin depth indicating that microwaves give up more energy per unit length of tissue than normal human liver parenchyma. The difference of 2.54mm in skin depth is, however, a relatively small difference in real terms. It should be remembered that the skin depth measurement will represent the radius of any microwave field and therefore this difference in wave penetration represents a 5mm diameter smaller field in tumour compared to that in the normal liver parenchyma. The results are from a small series of patients and as such can only act as a guide as to the difference in microwave penetration of these two tissues. The results, however, are very statistically significant and no overlap between the two sets of readings is seen. This does suggest that there is in fact a reproducible difference in the electromagnetic properties of the two tissues.

Chapter 3 investigated the ability of the 2.45GHz microwave equipment to induce lesions in a dose dependant manner. A criticism of this work is that the dose response curve was produced in normal liver parenchyma and not tumour tissue. Any difference to the previously generated dose response curve using tumour tissue would be due to a number of factors and not entirely because of discrepancy in microwave propagation through the two tissue types. These results may be advantageous for microwave ablation of colorectal liver metastases as these results suggest the microwave field will be more concentrated within the tumour as it is able to penetrate this tissue less effectively. The wave will therefore give up the energy carried by the wave within this volume leaving the normal, surrounding, liver parenchyma comparatively unaffected.

Accurate in-vivo complex permittivity measurements in normal liver parenchyma and hepatic colorectal metastases have not previously been available. Any attempts at modelling microwave fields radiated from applicators have been based on estimated values. As accurate values of the complex permittivity of colorectal liver metastases have now been acquired, this will allow their use in finite element computer models. Such models allow predictions of the radiated field from a particular applicator design to be tested giving information on the distance it is radiated, its intensity at certain regions and the degree of reflected power to be estimated. Figure 1 shows a still picture of such a finite element computer prediction used to produce one of the microwave applicators used in the large animal model experiment. The information gained from this work can be used to test different applicator designs. A smaller diameter probe which could have a laparoscopic or percutaneous use could be designed without the need for building and testing of a prototype. This work also goes some way to answering the question regarding the likelihood that a previously unknown quality of tumour tissue was preventing large volume ablation by the applicators designed in the Far East. This work does reveal a difference in the microwave penetration through the two tissue types but it is not large and does not account for the discrepancy in the ablation volumes produced by the Japanese applicators when compared to the interstitial applicator being research in this thesis. The following figure shows why it was unlikely that any physical property of tumour tissue was likely to account for the poor results achieved by the Japanese microwave applicators when treating tumours.

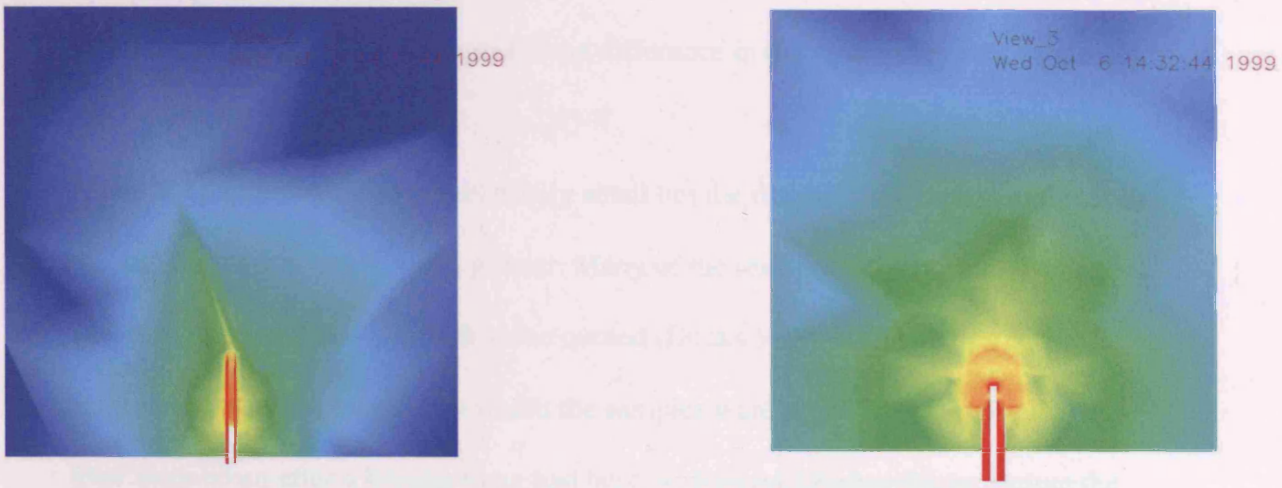


Figure 12. Finite element computer predictions of the microwave field emitted from a "Far East" microwave applicator (left) and that of the recently designed interstitial applicator from the Medical Devices Technology Group. The images are to scale with the width of each image representing 6cm. The obvious difference between the two field representations is the superior radiation of the microwave field by the Bath applicator. It should be stressed that these images have been produced using the data obtained prior to any treatment. As the Far East applicator is known to suffer increasingly poor field radiation properties as treatment progresses it is likely the difference in the radiated fields would only increase during a treatment.

The results of the desiccation experiments appear to largely explain the difference in microwave propagation through tumour and normal liver parenchyma. This experiment also shows the proportion of water within tumour tissue was higher than normal liver parenchyma. This was predicted by the permittivity results but was unexpected when the physical properties of colorectal metastasis are examined. Metastases are hard almost woody in texture and it was a surprise when the desiccation experiment revealed that the water content of this tissue was higher than

the much softer liver parenchyma. It is likely that the water content of these tissues is the overriding factor that accounts for the difference in the microwave propagation.

Again the patient numbers are relatively small but the results show each sample taken the tumour's water content was greater. Many of the results for the normal liver show values that are less than the 75% value quoted (Ducks 1990) although this is accounted for by the method by which the samples were acquired. The samples of liver were taken after a hepatectomy had been performed. During this procedure the blood supply to the liver is interrupted and the substance of the liver cut over a 30 to 60 minute period. During this time the tissue is drying in the theatre air and this coupled with the transport time from one hospital to the laboratory may account for the lower water contents in some samples. As each both tissue types would have been subjected to similar conditions they acted as their own controls. Any discrepancy between previously quoted water content figures for liver parenchyma and those is unimportant as the prime purpose of the experiment was to investigate the difference between the two.

5.6 CONCLUSIONS

As Wei states in his paper Biological applications of a technique for broadband complex permittivity measurements, "Accurate measurements of dielectric properties of biological substances are essential for both fundamental studies and biological applications, such as microwave hyperthermia" (Wei 1992). Accurate, in-vivo, complex permittivity measurements have now been taken for normal liver parenchyma and colorectal metastases. This allows accurate data to be used in

computer models aimed at testing different microwave applicator designs without having to produce physical prototypes. The difference in the measured properties of the two tissues is likely to be largely accounted for by the difference in water content of the two tissues. This information however is limited by the fact that the properties of the tissue change almost immediately following the initiation of a microwave ablation. The complex permittivity of the tissue will change as it is heated and desiccated by the treatment making the data pre-treatment almost obsolete. The next chapter aims to answer the question how the electromagnetic properties of tissue change with a microwave treatment and whether they do so in a predictable manner and how this affects the performance of the new applicators.

CHAPTER 6

MICROWAVE LESION PREDICTABILITY 4: WATER CONTENT, COMPLEX PERMITTIVITY AND THE EFFECT OF MICROWAVE ABLATION TREATMENT ON WAVE PENETRATION THROUGH TISSUE

6.1 INTRODUCTION

i) **Discrepancies in theoretical and actual performance of previous microwave applicators following analysis of the permittivity and microwave penetration results from the previous chapters**

ii) **Indirect evidence of microwave field extension as a result of the ablation process**

6.2 AIMS

6.3 MATERIALS AND METHODS

i) **Ex-vivo bovine liver**

ii) **Ex-vivo human liver parenchyma and colorectal liver metastases**

6.4 RESULTS

i) **Ex-vivo bovine liver: Permittivity readings**

ii) **Ex-vivo bovine liver: Desiccation results**

iii) **Ex-vivo human liver parenchyma and colorectal liver metastases: Permittivity Readings**

i) **Ex-vivo human liver parenchyma and colorectal liver metastases: Desiccation results**

6.5 DISCUSSION

6.6 CONCLUSIONS

PREDICTABILITY 4: WATER CONTENT, COMPLEX PERMITTIVITY AND THE EFFECT OF MICROWAVE ABLATION TREATMENT ON WAVE PENETRATION THROUGH TISSUE

6.1 INTRODUCTION

i) Discrepancies in theoretical and actual performance of previous microwave applicators following analysis of the permittivity and microwave penetration results from the previous chapters

The previous chapter dealt with the finite differences in microwave penetration through two tissue types by measuring their complex permittivity. The experiments suggested that at 2.45GHz approximately 2/3 of the wave's energy had been given up at a radius of 2cm (i.e. the skin depth) whichever tissue the microwave was being radiated into. Theoretically, this should make the production of lesions larger than 4cm in diameter difficult however ablations up to 6.5cm in diameter were produced in 180 seconds in the large animal model.

As previously stated, other ablative techniques that rely on thermal conduction or passive diffusion require much greater periods of time to produce lesions approaching 5cm in diameter (over 720 seconds with RF [if at all possible with RF] and 30-40 minutes in the case of cryotherapy) (Ruers et al. 2001). Although thermal conduction is likely to account for some of the peripheral ablation volume of a microwave lesion, it is unlikely to account for a large amount of this due to the short time the microwave was radiated into the tissue and the poor conductive properties of tissue. A second

reason for the prolonged treatment times in these systems reliant upon conduction is the thermal gradient generated. Heat will pass more quickly from one point to another if the gradient between the two regions is steep. The microwave generates temperatures of well over 100°C near the applicator shaft and compared to radiofrequency (RF) probes which have temperatures near the probe of only around 80°C (Rossi et al. 1993). This relatively low temperature is necessary for RF treatments as it prevents charring and the increasing impedance associated with this (Livraghi et al 1997). The temperature gradients induced by microwave treatment are therefore are much steeper than with RF thereby increasing the speed with which any conductive heating occurs with the former treatment modality.

The results of the previous chapter provide an interesting conundrum as Japanese microwave researchers have recorded the fact that the match (the efficiency with which microwave energy is transferred from the applicator to the tissue to be treated) is initially very good (Tabuse 1998, Shibata et al 2000). From the previous chapter it has been demonstrated that the skin depth of microwaves radiated at 2.45GHz is approximately 20mm in tumour tissue (it should be remembered that this is the *radius* of any radiated microwave field). The microwaves used in microwave ablation devices will have the same properties and therefore the measurements taken will apply equally to the devices constructed in the Far East as they do for those constructed in the UK. Chapter 1 reviewed previous microwave technology and highlighted the fact that previous microwave technologies were only able to produce volumes of ablation with a *diameter* of 2cm (Takamura et al 2001). If the initial match is as close as claimed why is there this large discrepancy in the theoretically predicted ablation volume and what is achieved? The explanation for this discrepancy is the

rapid drop off in the match between the microwave applicator and the tissue into which it is being radiated. As heating is initiated, the tissue into which the microwaves are radiated changes rapidly and with it the electromagnetic properties. As the tissue is heated the match between applicator and tissue is no longer as good and deteriorates rapidly with a considerable proportion (up to 40%) of the applied energy being reflected at the tissue applicator interface (Tabuse 1998, Shibata et al 2000). It was previously thought that this inevitable change in the electromagnetic properties of the treated tissue would cause a similarly inevitable reduction in the radiating efficiency in any microwave ablation device.

Heating of tissue will reduce the water content of the tissues especially if it is intense as is the case with ablative type heating. This, however, does provide an interesting paradox as tissue which has become water depleted should provide less "resistance" to the passage of microwaves bearing in mind that the principal reason for the decay of microwave energy is in the interaction with water. If a tissue is water depleted there are fewer water molecules for the waves to interact with and therefore the wave will pass through the tissue giving up less of its energy.

ii) Indirect evidence of microwave field extension as a result of the ablation process

Evidence of increasing microwave penetration through tissue during an ablation was found during work using the 9.2GHz microwave applicator. Chapter 2 describes the technique of endometrial ablation for menorrhagia refractory to medical treatment. The microwave applicator for this used a frequency of 9.2GHz which has a skin depth

in liver of 5mm. The result of using high frequency microwaves is that the vast majority of the wave's energy is given up in the first few millimetres of tissue it encounters. This is very useful in the context of endometrial ablation where destruction of the endometrium is required without damage to the myometrium and microwave penetration into the pelvis. The microwave field in this application is very concentrated and consequently the field is relatively fierce causing rapid heating of the target tissue. This form of applicator is the antithesis of what is required for ablation in the context of colorectal metastases which requires much larger volumes of ablation.

Early work on the ablation project used a 9.2GHz microwave applicator with a magnetron (equipment used to generate microwaves at a fixed Wattage and frequency) that was altered to increase the power available to 45 Watts (endometrial ablation is performed using 30Watts). At this time experiments investigating the heating of liver parenchyma were being undertaken. The microwave applicator was placed into the tissue and temperature monitoring devices (the thermocouples used in the porcine work Chapter 3) placed at increasing intervals. A data acquisition unit and computer again as per the porcine experiments monitored the change in temperature.

What is important is the properties of the thermocouples themselves due to their metallic structure. Metal within a microwave field is heated preferentially compared to other structures as the waves induce rapid electron fluxes within the metal (in a similar manner to a wire being passed through a magnetic field generating an electric current i.e. the basis of a dynamo). This is known as direct field heating and occurs much more rapidly than an increase in temperature caused by conduction.

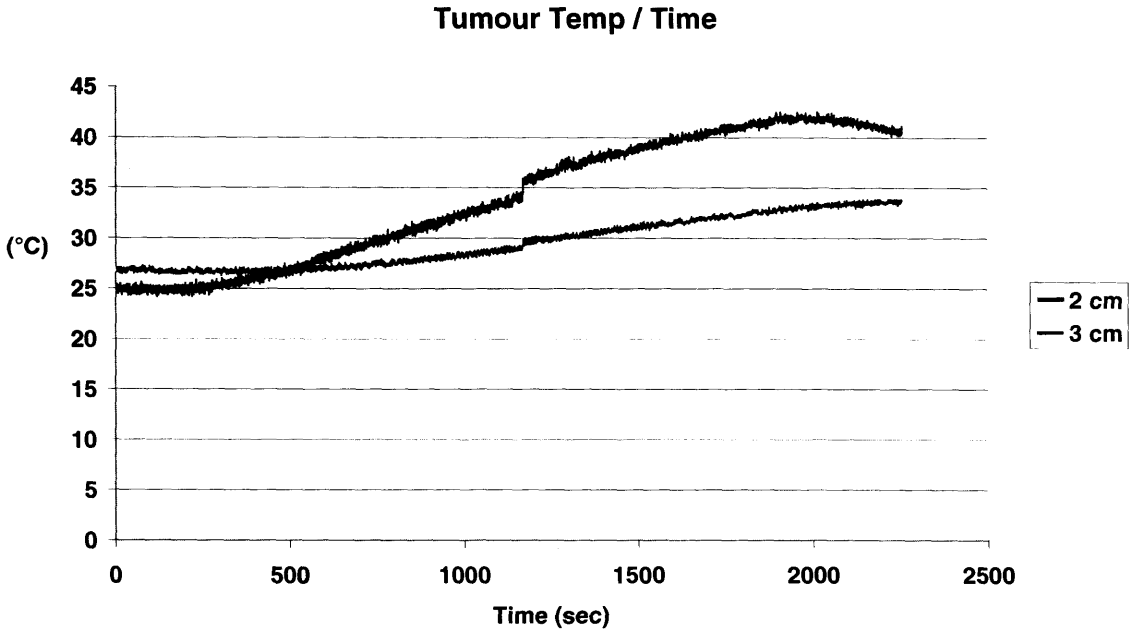


Figure 1. Increase in temperature at 2 and 3 cm intervals from the microwave applicator following treatment of ex-vivo liver with a 9.2GHz microwave applicator using 30 Watts.

The above graph shows the effect on a 9.2GHz applicator using 30Watts placed into human liver parenchyma. Thermocouples were placed at 2 and 3cm intervals from the applicator. It can be seen that the rise in temperature is relatively slow and is accounted for by the fact that the field is only reaching approximately 5mm into the tissue. The temperature rise is therefore accounted for by thermal conduction through the liver. Although the field may have penetrated further through the tissue the shallow incline indicates that it did not reach 2cm and therefore the first temperature sensor. The rate of temperature increase due to direct field heating is illustrated in Figure 2.

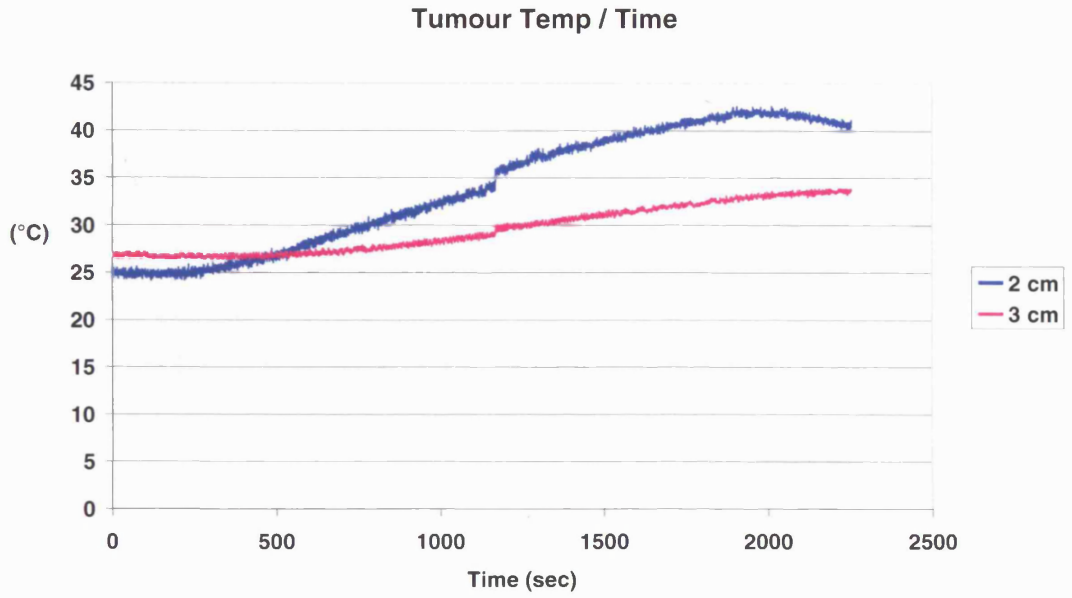


Figure 1. Increase in temperature at 2 and 3 cm intervals from the microwave applicator following treatment of ex-vivo liver with a 9.2GHz microwave applicator using 30 Watts.

The above graph shows the effect on a 9.2GHz applicator using 30Watts placed into human liver parenchyma. Thermocouples were placed at 2 and 3cm intervals from the applicator. It can be seen that the rise in temperature is relatively slow and is accounted for by the fact that the field is only reaching approximately 5mm into the tissue. The temperature rise is therefore accounted for by thermal conduction through the liver. Although the field may have penetrated further through the tissue the shallow incline indicates that it did not reach 2cm and therefore the first temperature sensor. The rate of temperature increase due to direct field heating is illustrated in Figure 2.

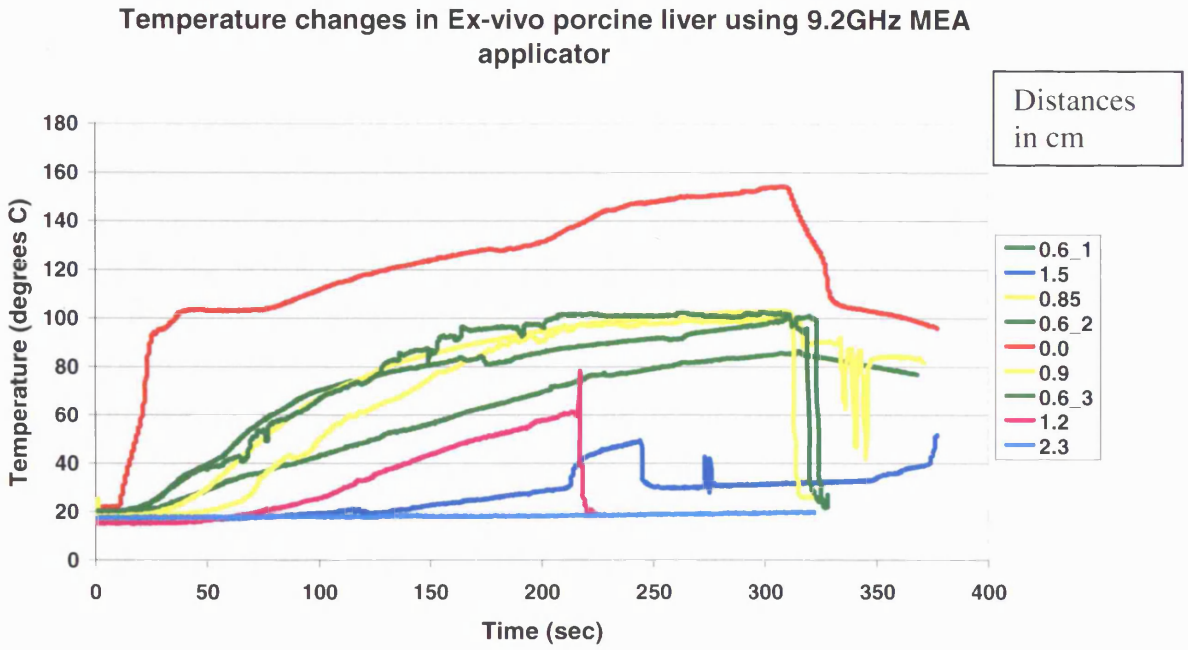


Figure 2. Increase in temperature at different intervals from the applicator (in cm) as measured by metallic thermocouples. The steep gradient (representing rapid heating) of the red line at 0cm from the applicator is of interest as this illustrates the effect of direct field heating on the thermocouple.

This second graph illustrates the increase in temperature in a piece of liver following the insertion of the 9.2GHz microwave applicator. What is of interest is the red line which represents the increase in temperature attributable to direct field heating of thermocouple placed adjacent to the microwave applicator. Here the steep gradient of the line is due to the direct effect of the microwave field on the thermocouple. It is known that the skin depth of the tissue is 5mm so adjacent to the applicator the field will be strong. In this particular experiment the ablation duration was 300 seconds. The steep decline in the temperature of the 0mm thermocouple is seen after the power is switched off and the thermocouple equilibrates with the surrounding tissue. The difference in the gradients of these 2 graphs illustrates the great difference in the rate of heating by thermal conduction through the liver and that from the microwave field.

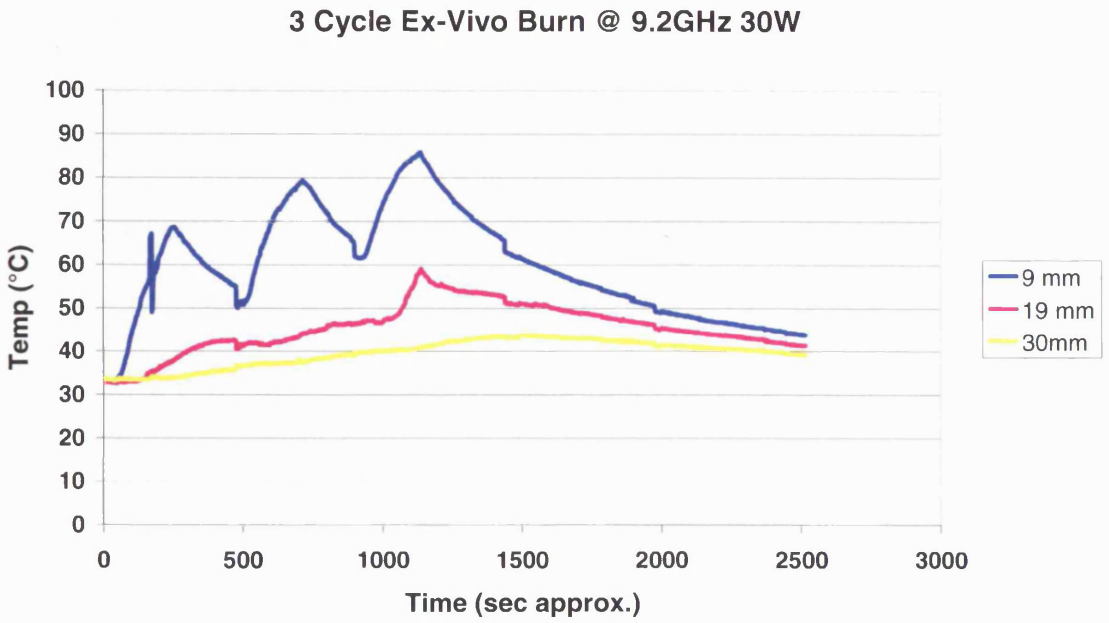


Figure 3. Temperature profiles of thermocouples placed at 9, 19 and 30 millimetres. In this experiment the ablation was "pulsed" with 3 minutes of ablation followed by three minutes with the power cut. In this experiment the applicator was placed in a piece of recently (minutes) resected liver and thermocouples were placed at 9, 19 and 30mm away from the applicator. The microwaves were switched on and off in 3 minute cycles with the temperature probes continuing to acquire data during the whole time. What is of interest is the fact that the 9mm sensor has a high rate of temperature increase and fall off suggesting it is within the microwave field. After approximately 1200 seconds the temperature reading for the second sensor at 19mm began to climb at a high rate with a gradient very similar to that of the 9mm sensor. There was no other reason for the 19mm sensor to begin reading these high readings unless it was experiencing direct heating from the microwave field (one should remember that field penetration of microwaves at 9.2GHz is approximately 5mm). This was indirect evidence that the microwave ablation treatment does in fact alter the tissue into which it was placed to a large enough degree to allow significant increases

in the distance the field can penetrate. This is an exciting finding as it suggested that microwave treatment of liver tumours at a lower frequency, with increased power, could potentially induce large enough volumes of ablations to treat all but the largest hepatic tumours. It was then necessary to prove that this increase in temperature had indeed come about due to the change in permittivity of the tissue treated. This increase in the penetration of the microwave field has not been investigated previously.

6.2 AIMS

This chapter attempts to answer two questions, firstly, how does the microwave ablation treatment itself affect the permittivity of the tissue the applicator is placed in and secondly to what degree.

6.3 MATERIALS AND METHODS

The method of calculating complex permittivity was presented in the previous chapter. The same equipment and technique (including calibration) for data acquisition was used in this work as that in the previous chapter. The effect of the ablation was analysed using ex-vivo butcher's bovine liver and subsequently human liver parenchyma and colorectal metastases.

i) Ex-vivo bovine liver

Bovine liver was placed into a dish and the microwave probe introduced and held by a retort stand clamp (see Figure 4). An ablation was induced at a set Wattage for 3 minutes. The lesion induced was then sectioned perpendicularly to the microwave applicator shaft and the applicator removed. The lesion was allowed to cool to room temperature and the permittivity readings could then be taken. The lesions were similar in appearance to those shown in Chapter 3 having a distinctly zonal appearance. The permittivity probe was used and readings taken from the lesion epicentre immediately adjacent to the applicator cavity extending radially towards the macroscopically unaffected liver parenchyma. The tip of the permittivity probe is approximately 2mm in diameter and therefore readings were taken approximately every 2-2.5mm. Placing it in a clamp ensured good apposition between the tissue and the endplate of the permittivity sensor. Again 401 data points were taken over a range of microwave frequencies (0.5 to 10GHz). A second set of readings were taken from the opposite side from those taken initially.

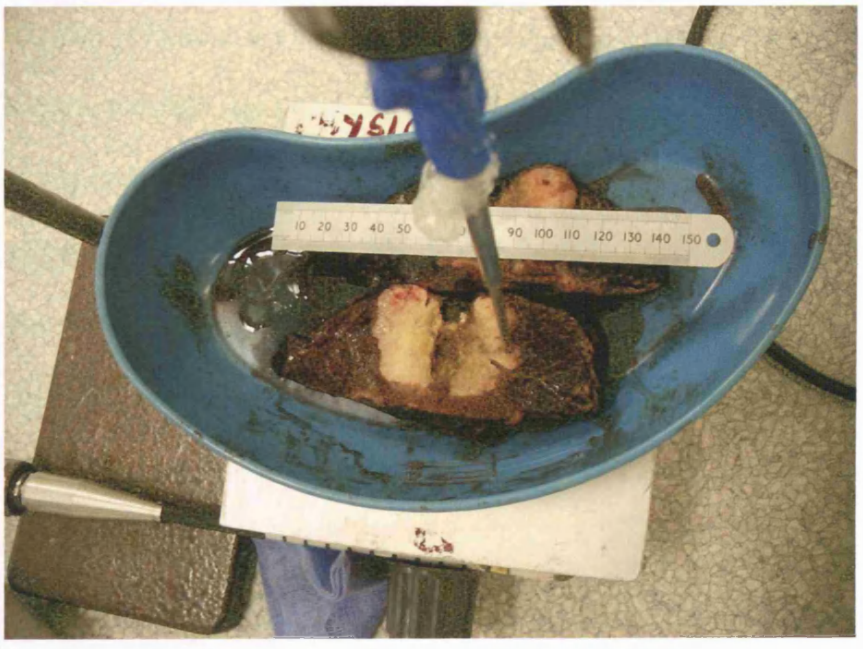


Figure 4. Figure shows the set up for a "perm burn perm" procedure. The permittivity of the tissue was taken prior to any microwaves. After an appropriate microwave

treatment the permittivity of the tissue was taken at intervals from the burn epicentre. The figure shows the permittivity probe being held onto a colorectal metastasis (whiter material) following a microwave treatment when obtaining permittivity data. The burn epicentre can clearly be seen as the darker region cylindrical in shape mimicking the shape of the microwave applicator.

After the readings were taken, chips of the treated tissue were taken and desiccated. The chips were taken from the two regions which were analysed by the permittivity probe. The chips were not of exact sizes as the degree of water loss in the tissue compared to its neighbours was important.

ii) Ex-vivo human colorectal liver metastasis

A similar method was employed for the human tissue. Unlike the previous chapter the readings were all taken using ex-vivo tissue samples. Following a liver resection the portion of liver removed was taken and the microwave applicator placed into the centre of a large metastasis. An ablation was then performed, the duration of which being decided upon by the size of the lesion (previously estimated by intra-operative ultrasound or the CT scan). The duration and power of the ablation was determined by the observers to replicate what would be required to completely treat the lesion had this been a therapeutic procedure. Much of the decision making process was determined by the results of the in-vivo pig work and the dose response curve subsequently generated.

The permittivity readings and water desiccation experiments were performed as described above.

6.4 RESULTS

i) Ex-vivo bovine liver: Permittivity readings

Complex permittivity was measured at 401 data points over the microwave frequency range of 0.5 - 10 GHz in approximately 3 seconds. The results are again expressed graphically in Figure 5 below.

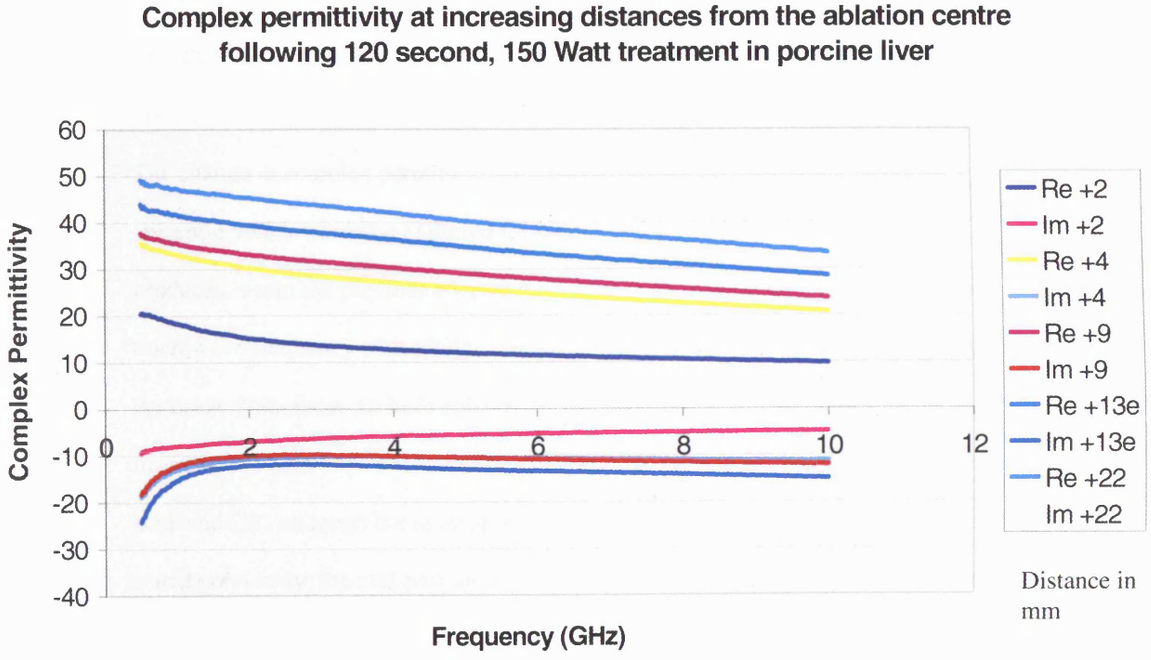


Figure 5. Shows the real and imaginary readings taken after a 120 second microwave treatment in porcine liver at a power of 150 Watts at increasing intervals from the burn epicentre. Re +2 indicates the real portion of permittivity at a distance of 2mm from the burn epicentre, Im +2 represents the imaginary portion of complex permittivity reading obtained at a distance of 2mm from the burn epicentre. Other figures following the Re (real) or Im (Imaginary) designate the distance from the burn

epicentre. Re +13e and Im +13e represent the real and imaginary portions of complex permittivity respectively taken at what was seen to be the macroscopic burn edge. The distance of +22 was macroscopically normal liver parenchyma and thought to be representative of untreated tissue.

Figure 5 is a good example of the results obtained from these experiments. Good calibration was achieved prior to obtaining the measurements and therefore it was considered that these readings represented accurate data. From the graph it can be seen that microwave treatment induces significant change in both real and imaginary portions of complex permittivity across the range of microwave frequencies tested. The change in complex permittivity is very large particularly closer to the burn epicentre which would be expected following visual inspection of the lesions produced. From the previous chapter the real portion of complex permittivity for a microwave frequency of 2.45GHz is approximately 45. At 2.45 GHz the reading for the tissue 2mm from the burn epicentre is 14.5 and at 4mm it is 29. Even at the macroscopic burn edge a lower reading for the real portion of complex permittivity is achieved (38) although the reading returns to normal (45) at a distance of 22mm. As stated previously, the real portion of complex permittivity is a measurement of the ability of a substance to take up and store energy, something that polar molecules are able to do well. The results suggest that the tissue treated has either undergone change that prevents the uptake of energy or that it has lost some of its polar molecules and hence its ability to store energy.

The imaginary portion of complex permittivity is similarly affected by the treatment. Here it can be seen that again, across all microwave frequencies tested, a pronounced

change in the value for imaginary complex permittivity is encountered especially at regions nearer the lesion epicentre. Areas nearer the lesion epicentre have a less negative value that that measured in normal liver parenchyma in the previous chapter. The negativity of the imaginary portion of complex permittivity increases with increasing distances from the microwave applicator. Again as stated previously, imaginary portion of complex permittivity refers to the total losses per unit distance experienced by the wave as it passes through the substance being investigated. The results show that the microwaves treatment causes far less energy to be lost in the tissue nearer the applicator allowing the waves to penetrate further, thereby increasing the skin depth. In summary, the microwave treatment had appreciably affected the tissue treated in terms of its electromagnetic properties and that this effect was proportional to the distance from the applicator. Following a microwave treatment, the tissue near to the lesion epicentre had become far more "transparent" to microwaves in that the waves were able to penetrate this region far more easily giving up a smaller proportion of their energy. Using the analogy of light, the tissue close in to the applicator has changed from say darkened glass used for sunglasses through which light barely penetrates to clear glass through which light travels losing almost no energy. This change in the permittivity of near tissue may in part account for the penetration of the field through greater volumes of tissue and the ability of the microwave to produce the large lesions seen in Chapter 3. The experiment was repeated using 100 Watts with similar results (see Figure 6), i.e. the resistance to microwaves as measured by the complex permittivity was reduced by treatment and that the effect decreased with increasing distance from the applicator. Again this is novel work, which has not until now been undertaken and certainly not published. Figure 6 shows the changes in complex permittivity induced by a 100 Watt

microwave ablation. It can be seen that the results are very similar to that of the 150 Watt lesion.

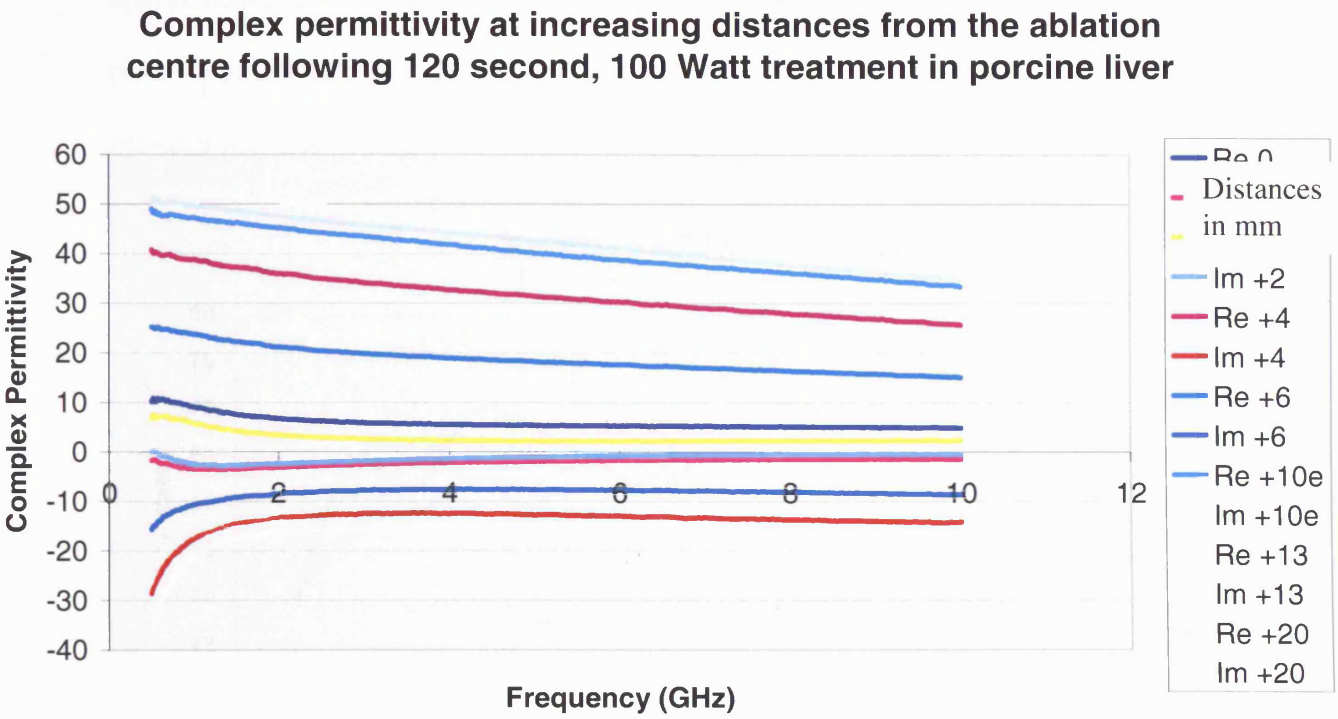


Figure 6. This figure should be interpreted in the same manner as Figure 5. The complex permittivity of ex-vivo bovine liver has been measured following a 100 Watt microwave ablation. "Re" and "Im" are abbreviations for real and imaginary portions of complex permittivity with the figure representing the distance from the applicator that the measurements were taken. Again "e" stands for the macroscopic burn edge. Overall a very similar trend can be seen in that the ablation reduces the overall complex permittivity of the tissue after treatment and that this change is proportional to the distance from the applicator.

ii) Ex-vivo bovine liver: Desiccation results

The regions that were used for the measurements were taken and sectioned into pieces approximately 2mm in diameter. The chips of tissue were desiccated allowing the percentage of water by weight to be calculated. Figure 7 below shows the relationship between water content and distance from the burn epicentre.

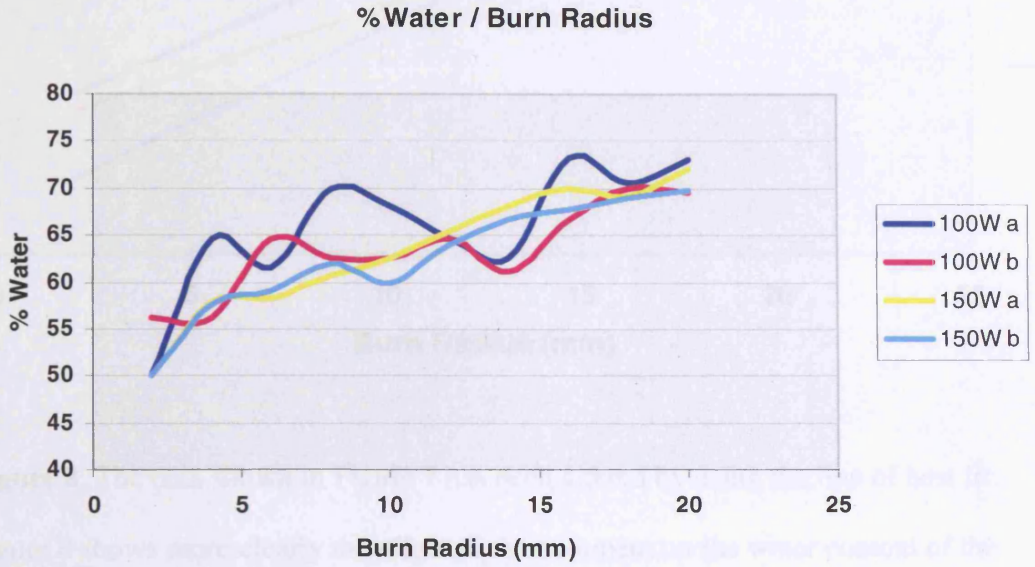


Figure 7. This graph shows the change in the percentage of the tissue's weight made up by water following a 100 Watt and a 150 Watt treatment in ex-vivo bovine liver parenchyma. It can be seen that for all lesions the inner region of the ablation has lost more water when compared to more distant regions and that this general trend is applicable to all the different burns. The figures "a" and "b" refer to the two regions from which the permittivity measurements were taken and subsequently the water content data.

The results are more easily interpreted when the line of best fit is used as shown in Figure 8.

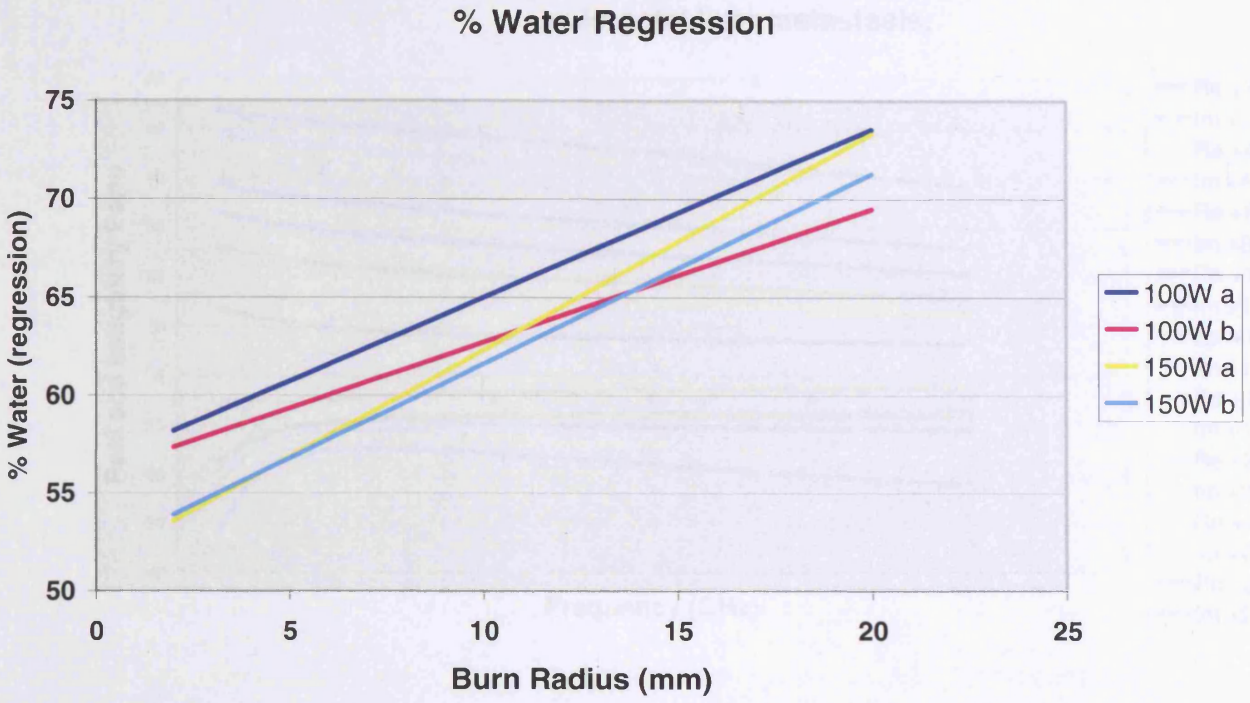


Figure 8. The data shown in Figure 7 has been altered by using the line of best fit. Figure 8 shows more clearly the effect of the treatment on the water content of the lesion at different intervals from the probe. Each of the treatments using the same power have very similar gradients although as would expected the gradients differ between the two powers. The 150 Watt ablations show a steeper gradient because the larger amount of energy delivered has increased the degree of desiccation of the inner burn region.

iii) Human colorectal metastases results: Permittivity readings

Two patients with large colorectal metastases were investigated following a microwave treatment. Again a similar scenario to what was seen in the ex-vivo porcine liver was noted with human colorectal liver metastases.

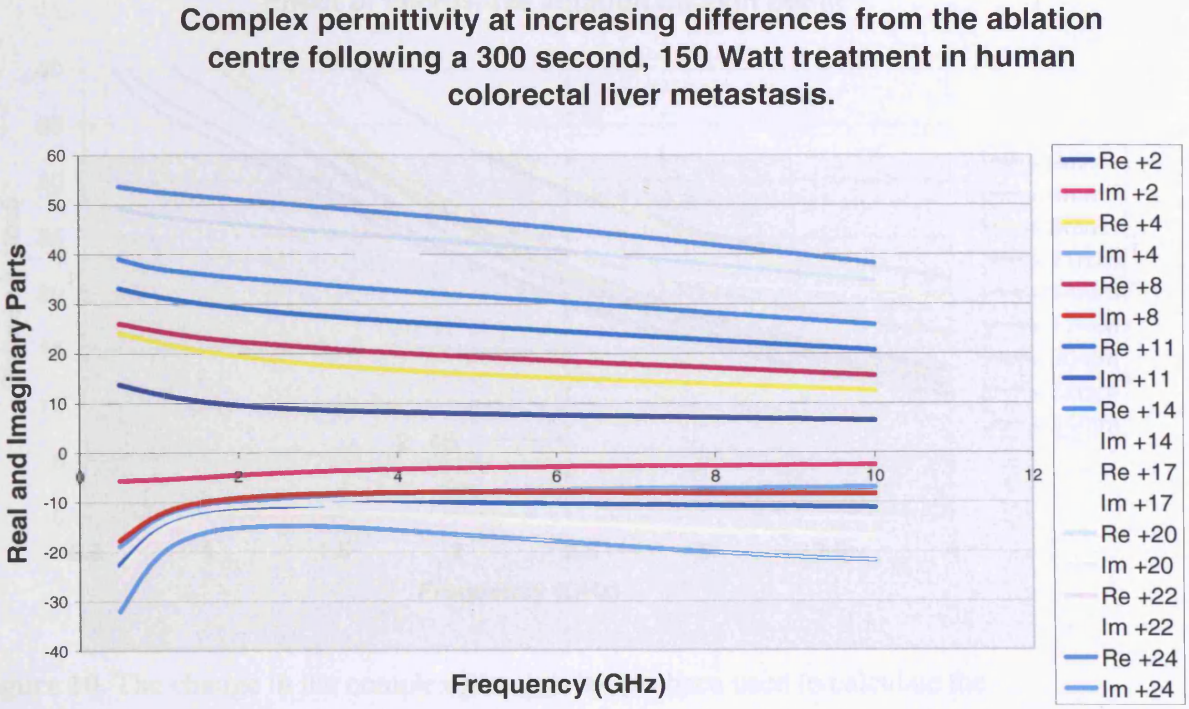


Figure 9. The change in complex permittivity in a human colorectal metastasis following a microwave ablation treatment of 300 seconds at 150 Watts. This graph should be interpreted along the lines of Figure 5. The results show very similar results to those in Figure 5 in that both the real and imaginary portions of complex permittivity have been affected by the treatment in a manner that is that proportional to the distance from the applicator.

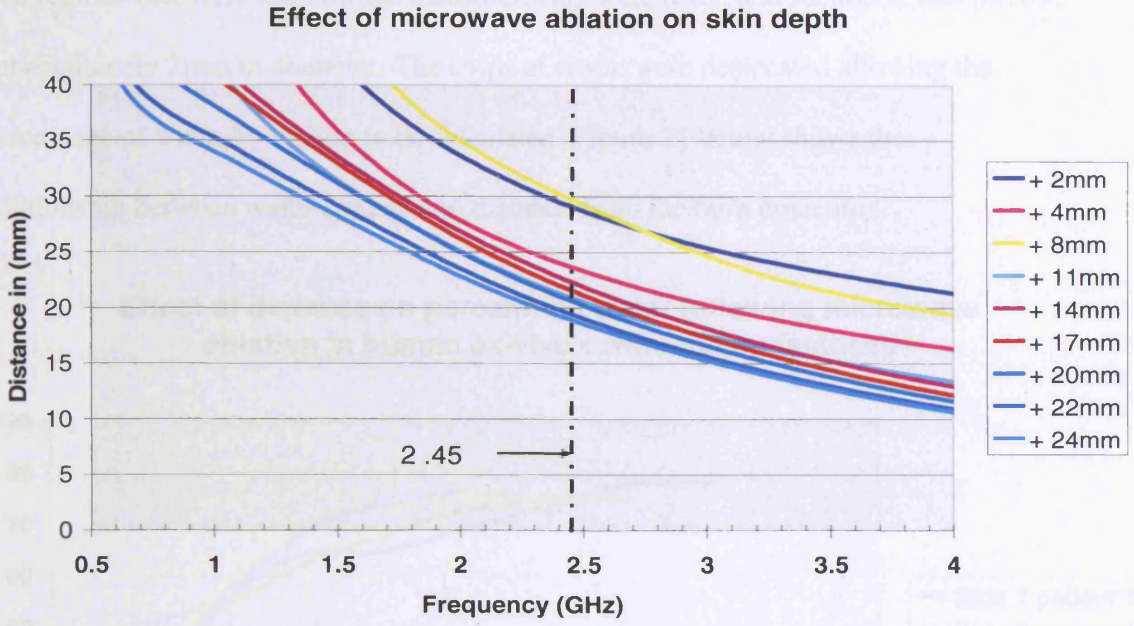


Figure 10. The change in the complex permittivity has been used to calculate the change in skin depth at the distances taken. The dashed arrow on the graph shows the skin depth at the important frequency of 2.45GHz. It should be remembered that Chapter 5 calculated the skin depth of a 2.45GHz microwave in colorectal metastases to be approximately 19mm. From the graph it can be seen that the skin depth has increased markedly and that again as seen in the results for complex permittivity the effect is dependant on the distance from the applicator.

These results confirm the suspicion that the treatment alters the tissue in a manner that is potentially advantageous when considering the treatment of larger tumours. The skin depth has been increased by over 10mm (thereby increasing any ablation diameter by 20mm) in regions that are near to the applicator.

iv) Human liver and tumour results: Desiccation results

The regions that were used for the measurements were taken and sectioned into pieces approximately 2mm in diameter. The chips of tissue were desiccated allowing the percentage of water by weight to be calculated. Figure 11 below shows the relationship between water content and distance from the burn epicentre.

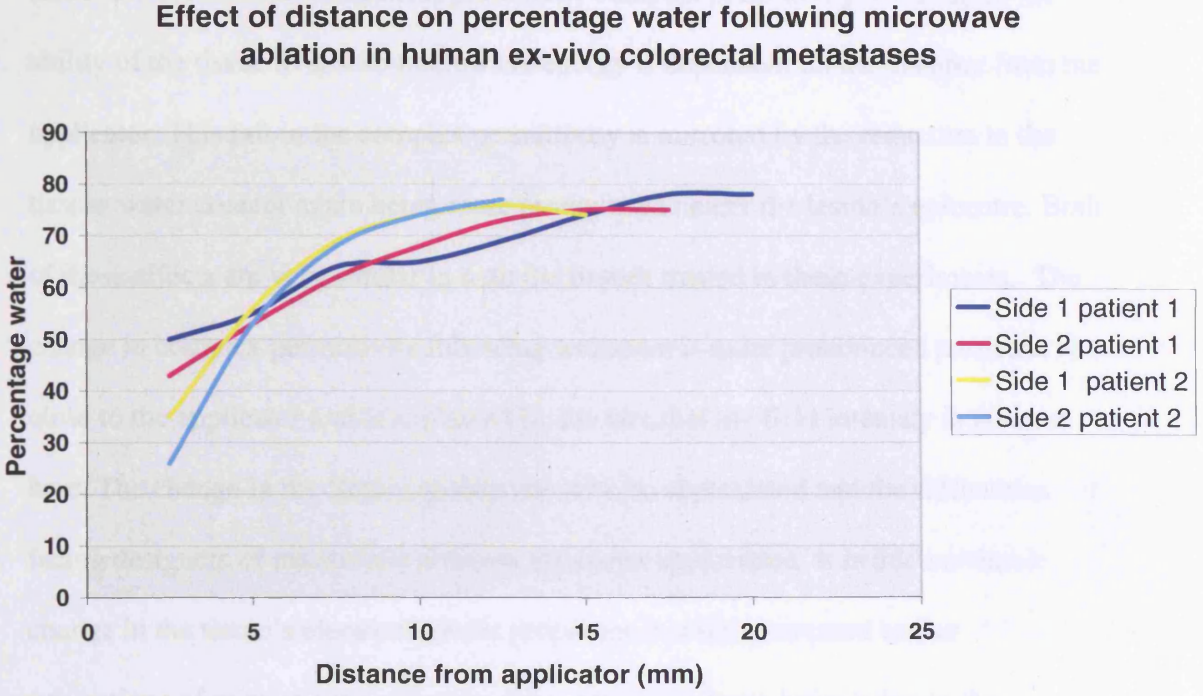


Figure 11. The graph shows the change in the water content of the chips of tumour by weight at increasing distances from the applicator. It should be noted that the distances quoted are not necessary accurate as the measurements were not taken at specific points. What is important is the trend of much greater desiccation occurring nearer the ablation epicentre.

6.5 DISCUSSION

Microwave ablation does appear to induce significant change in the electromagnetic properties of the treated tissue in a predictable manner. The heat generated particularly near to the applicator causes a substantial drop in the real and imaginary (the latter becomes less negative) portions of complex permittivity. This fall in the ability of the tissue to absorb microwave energy is dependent on the distance from the applicator. This fall in the complex permittivity is mirrored by the reduction in the tissues water content again being more pronounced nearer the lesion's epicentre. Both of these effects are very similar in both the tissues treated in these experiments. The change in complex permittivity following treatment is quite pronounced particularly close to the applicator and is explained by the fact that the field intensity is greatest here. The change in the tissues quality can now be appreciated and the difficulties facing designers of microwave ablation apparatus appreciated. It is this inevitable change in the tissue's electromagnetic properties that has prevented earlier generations of microwave applicators from producing large lesions due to the increased proportion of power that was reflected at the applicator/tissue interface.

Much of the criticism of this work would be the small numbers of experiments performed not giving much reproducibility to the results. This is mainly due to the significant logistical and monetary difficulties involved in taking these measurements. Almost all the equipment had to be transported from Bath to Leicester to take these measurements. The equipment (particularly the network analyser) was essential to work continuing at the University of Bath Medical Devices Technology Group and therefore the work was carried out at weekends. Both this and the need for large

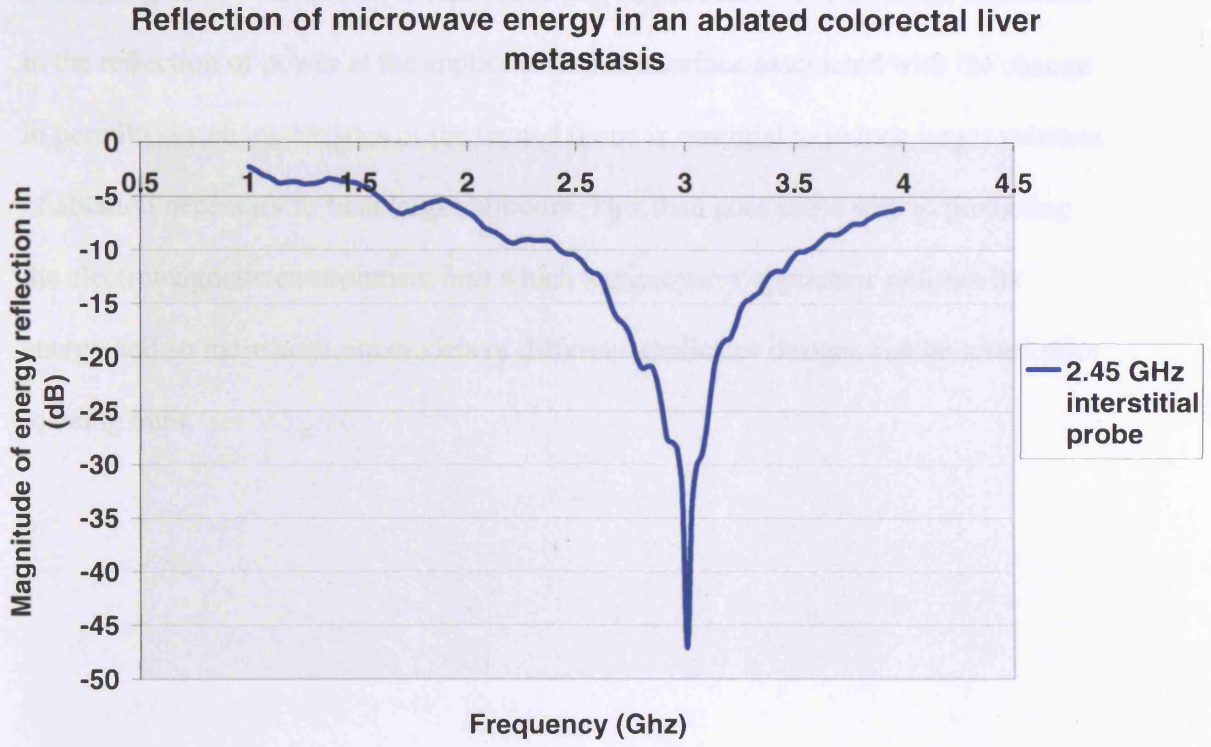
metastases to be resected to allow a microwave treatment followed by permittivity measurements reduced the number of opportunities to take these measurements

A second area for criticism would be the lack of in-vivo post ablation permittivity work. One of the main reasons for the ex-vivo technique is a limitation particular to the probe used to make permittivity readings. In order for this to work efficiently, the tip has to be very flat (to within a few microns). The material used in this probe is not thermostable and with heating would expand and extrude through the end of the coaxial cable. As a result of this property the permittivity probe had to be sterilised using ethylene dioxide to prevent damage to the smooth surface from the expansion and contraction that would have occurred following autoclaving. This instability in the face of widely varying temperatures is the reason that the lesions were all allowed to cool prior to the acquisition of the permittivity readings. Temperature does have an impact on tissue permittivity (Colpitts 1993) and therefore the results obtained in these experiments are not the entire picture but do however provide a useful guide to the change in the tissue characteristics following an ablation. The ideal scenario would be to obtain in-vivo, real-time permittivity readings in human liver parenchyma and colorectal metastases during an ablative treatment. Work on a thermostable, non-metallic permittivity probe is in progress and it will be interesting to compare the results obtained by the two different techniques.

Testing of the 2.45GHz microwave applicator has already demonstrated its ability to produce large lesions when higher powers are used (Chapter 3). It was theorised that the reason this particular microwave device was successful in terms of the ablation diameter possible in comparison to previously used applicators was the ability to

retain the match throughout the ablation cycle. This was partly due to observations from the microwave power output which measures the reflected wave energy in Watts as the ablation progresses. This figure starts out as negligible but even during a 150 Watt ablation less than 5% of the power appears to be reflected. This observation can now be tested using the data obtained from these experiments. The permittivity data from the tissue adjacent to the applicator i.e. the tissue that undergone the greatest heating and desiccation was used to model the performance of the applicator. Theoretically this should produce the worst match possible due as this tissue has undergone the greatest change from its initial state. The finite element model showed even at this extreme the match between the applicator and tissue remained very good and consequently the amount of reflected energy remained small. Again this data obtained from tumour tissue following an ablation is useful in testing different applicators using the finite element computer model. As the data for a liver metastasis has been acquired it can be used to assess the magnitude of the power reflection at the applicator tissue interface. Figure 11 below is an example of such a diagram. The complex permittivity data for the metastasis has been used to formulate the prediction of the reflection at the described boundary. It can be seen that at 2.45GHz the reflection is in the order of –10 dB approximately 10%. Initially the applicators were "tuned" so the reflection at the applicator tissue interface was less than 1% (-20dB) when treatment started. This data highlighted the fact that the match changes with the treatment and the curve shown below shifted to the right and therefore the match between applicator and tissue was decaying (causing greater reflection of energy). Using this data the applicators were changed show that the match between the applicator and tumour at time zero i.e. initiation of treatment was not as close as previously. This was undertaken to allow for the tissue changes that occur during

treatment and instead of the match becoming worse during treatment it in fact became better as the curve shown below shifted.



6.6 CONCLUSION

Results from these experiments provide evidence that a significant change in the electromagnetic properties of tissues undergoing a microwave ablation occur with treatment. These changes are most significant near to the applicator itself where the field is at its most intense but reduce in their magnitude at increasing intervals from the applicator. Results in ex-vivo porcine liver, and human tissue are very similar.

The data obtained show a clear effect in terms of the electromagnetic properties following a microwave ablation. The results show that microwave induced desiccation of the tissue allows the radiated waves to penetrate further into the tissue heating a greater volume and in turn producing larger volumes of ablation. Reduction in the reflection of power at the applicator/tissue interface associated with the change in permittivity characteristics of the treated tissue is essential to induce larger volumes of ablation necessary to treat larger tumours. This data goes some way to predicting the electromagnetic environment into which a microwave applicator radiates its energy and so more accurate models of different applicator designs can be tested prior to being built.

CHAPTER 7

MICROWAVE LESION PREDICTABILITY 5: REAL-TIME IMAGING OF MICROWAVE ABLATION USING INTRA-OPERATIVE ULTRASOUND AND THERMAL MONITORING DEVICES.

7.1 INTRODUCTION

i) Current use of ultrasound in hepatobiliary surgery

ii) Intra-operative ultrasound: its use in currently available ablative techniques

7.2 AIMS

7.3 MATERIALS AND METHODS

i) Intraoperative ultrasound in the ex-vivo setting

ii) Temperature monitoring in the ex-vivo setting

iii) Intra-operative ultrasound in the in-vivo setting

iv) Temperature monitoring in the in-vivo setting

7.4 STATISTICS

7.5 RESULTS

i) Intra-operative ultrasound in the ex-vivo setting

ii) Temperature monitoring in the ex-vivo setting

iii) Intra-operative ultrasound in the in-vivo setting

iv) Temperature monitoring in the in-vivo setting

7.6 DISCUSSION

7.7 CONCLUSIONS

MICROWAVE LESION PREDICTABILITY 5: REAL-TIME IMAGING OF MICROWAVE ABLATION USING INTRA-OPERATIVE ULTRASOUND AND THERMAL MONITORING DEVICES.

7.1 INTRODUCTION

i) Current use of ultrasound in hepatobiliary surgery

Ultrasound is cheap, convenient and uses non-ionising radiation to image the liver and surrounding structures. It is, however, an operator dependent modality and the accuracy of any reports generated from such an investigation are directly proportional to the skill of the operator. A significant advance was made with the introduction of the intra-operative ultrasound in which a sterile probe is placed directly onto the liver surface allowing hepatic parenchyma, its vessels and metastases to be visualised. The relationship between the hepatic vessels, bile ducts and metastases is important as this can dictate the "resectability" of an individual's disease (Machi et al. 1991).

Liver metastases are also assessed using cross-sectional imaging modalities such as CT and MRI. All extra-corporeal modalities are limited in the diagnosis of small <0.5-1 cm metastases as the definition of the technique cannot distinguish between normal parenchyma and tumour tissue. All techniques currently understage hepatic malignancies such that a combination of pre-operative imaging, intra-operative visualisation and handling of the liver is performed to assess tumour burden. Intra-operative ultrasound (IOUS) is now regarded as the most efficacious method to detect and assess the topography of liver metastases and is used routinely in many centres (Nicoli et al. 2004, Heriot AG et al. 2004, Zacherl L et al. 2002. A number of studies have compared IOUS with pre-operative cross sectional imaging with IOUS

consistently being regarded as the most sensitive and specific (Soyer et al. 1992, Armour et al. 1993 and Paul et al. 1994). In two of these studies IOUS was thought to have sensitivities and specificities of around 95%. Radiology is a very fast moving specialty and many improvements in the resolution of all imaging modalities continue. Assessment of hepatic malignancies using intra-operative ultrasound is practised widely around the world and will continue until pre-operative imaging techniques become even more sensitive and specific.

ii) Intra-operative ultrasound: its use in currently available ablative techniques

It is unsurprising given the standard use of IOUS in liver surgery that many ablative techniques use this means as a method of imaging the ablation technique in real-time. IOUS is particularly useful when cryotherapy ablation is being used. The change in the consistency of the tissue as it is frozen into an iceball allows very accurate imaging of the edge of the ablative effect (Ravikumar et al. 1987, Charnley et al. 1989, Rivoire et al. 1996 and Lam et al. 1995). Accurate imaging is important as ablative techniques require similar tumour clearances as resectional surgery. Many authors feel that the minimum clearance that should be obtained is 5mm with some having evidence that a minimum of 1cm is mandatory (Henne-Bruns D et al. 1993). Radiofrequency also uses IOUS as a form of monitoring and to aid placement of the probes within the metastases (Curley et al. 1999).

Ablative techniques require accurate placement of the delivery probe within the metastasis and the ability to monitor the progression of the ablation to ensure the completion of treatment along with the preservation of important structures such as

the main bile ducts and blood vessels. The ability to perform this function accurately would be an obvious benefit to an ablation system and particularly beneficial in patients with multiple metastases or in those with tumours in cirrhotic livers. The microwave system that has been used for this research consists of an applicator made from coaxial metal with a ceramic radiating portion onto which a steel cutting tip is positioned. It is important to remember that the cutting tip does not constitute any portion of the radiating device and therefore needs to be placed greater than half way through any tumour.

Previous microwave ablation devices use IOUS for the placement and monitoring of ablations although lesion monitoring is of secondary importance. Previous portions of this thesis have described the very limited (but very reproducible) ablation volume that is induced by this technology and the much larger volumes induced by the newly developed microwave. To ablate tumours 3cm or larger previous microwave devices have required multiple insertions to ensure complete destruction of the lesion. The IOUS is used here in an attempt to place the probe accurately thereby producing overlapping ablations without leaving islands of viable tumour cells. This is both a time consuming and technically challenging process (Buscarini and Rossi 1997, Curley et al. 2000, Cha et al 2000). A modality that shows the progression of the lesion and gives an accurate estimation of an ablation size would be an important development.

7.2 AIMS

This aim of this study was to assess the ability of intra-operative ultrasound (IOUS) to image and measure the zone of ablation produced by this equipment in ex-vivo bovine liver and also in-vivo porcine hepatic parenchyma. The second aim was to investigate lesion temperature distribution in both these two tissue types.

7.3 MATERIALS AND METHODS

i) Intra-operative ultrasound in the ex-vivo setting

The object of this experiment was to observe and characterise the ultrasound appearances after the induction of a microwave lesion and to examine the correlation between the macroscopic ablation diameter and that as measured by ultrasound in bovine liver. Equipment used in this study included a microwave amplifier (ETM 200 SC Travelling Wave Tube Amplifier, ETM Electromagnetic Inc., 35451 Dumbarton Court, Newark, CA 94560, USA driven by a Hewlett Packard 8350B Sweep Oscillator, Agilent Technologies UK Ltd., Eskdale Road, Winnersh Triangle, Wokingham, Berkshire, RG41 5DZ, UK) which produces microwaves at a frequency of 2.45GHz with variable power available from 0-250 Watts. Microwaves are transmitted along a cable to the hand held applicator (probe) designed by the UBMDTG from which the waves are radiated. Intra-operative ultrasound data was gathered using an Aloka Echo Camera SSD-500 ultrasound with a 5MHz "T" probe.

The microwave applicator was placed vertically into the bovine liver and maintained in this position by means of a retort stand. Following application of ultrasound gel, a 5MHz ultrasound "T" probe was placed perpendicularly to the shaft of the applicator and the microwave treatment was initiated. The ultrasound image (frozen and captured on an attached thermal printer) was compared to the sectioned lesion immediately following treatment. To assess the ability of real-time ultrasound to monitor lesion production, progressively longer microwave treatments were undertaken. Treatment times were increased by thirty seconds to a maximum of three minutes (180 seconds) at powers of 50 and 100 Watts and the ultrasound image captured at 30 second intervals. Following the cessation of power, the liver was sectioned and the maximum ablation diameter was recorded. The extent of the ablation was determined as the maximum diameter of the blanched region produced by the treatment. Comparisons were made between the IOUS image produced (maximum ablation diameters being determined from the scans by a Consultant Radiologist BM, PMR or ME) and the maximum ablation diameters as measured by the author and PC.

ii) Temperature monitoring in the ex-vivo setting

The ex-vivo acquisition of temperature data was collected using Radio Spares K type thermocouples, (PO Box 99, Corby, Northhants N17 9RS UK.) and an Anville Instruments Series 410 data acquisition system, Laser House, Doman Road, Camberley Surrey GU15 3DF UK through which thermal data was transferred to a computer for storage. In all experimentation a microwave monitor was employed to ensure that personnel were not exposed to excessive radiation dosages. The purposes

of these experiments were to assess how effective this equipment was at thermal data acquisition, how best it should be achieved in the in-vivo experiments and the maximum temperatures that could be expected in the tissue to allow calibration of the temperature monitoring equipment.

iii) Intra-operative ultrasound in the in-vivo setting

The same equipment as that used in the ex-vivo IOUS experiments was used for this work although the operators were different. The ex-vivo ultrasonography work was mainly carried out by the author although the in-vivo work the images were acquired by a Consultant Radiologist. Again images were acquired every 30 seconds and printed using the same laser equipment. The details of the animals used, ablation details and cull intervals have been documented in Chapter 3.

iv) Temperature monitoring in the in-vivo setting

In both experiments thermal data was collected using thermocouples implanted into the hepatic parenchyma at increasing intervals. The ex-vivo experiments made attempts at temperature measurement using a set of thermocouples held in the jaws of a clamp. This proved to be a poor method for collecting this data due to movement of the thermocouples and consequent irregular temperature measurements. To eliminate the movement errors, an aluminium jig which held the thermocouples at 1cm intervals was constructed (Figure 1). The jig was placed adjacent to the microwave applicator and the ultrasound used to measure the distance from the applicator of the nearest thermocouple. Once this was established, the distance of each subsequent

thermocouple could then be calculated. Thermal data was collected immediately prior to the treatment, during the ablation and for some minutes following the treatment allowing the rates of cooling at different intervals to be assessed.

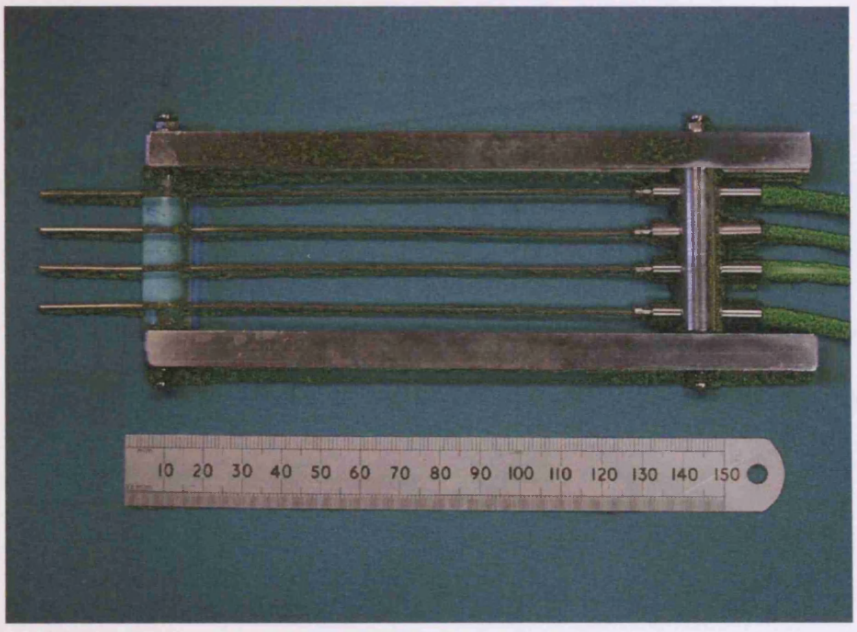


Figure 1. Aluminium jig constructed to house the thermocouples used for real-time temperature measurements. The thermocouples can be seen protruding from the left edge of the jig and could be buried into the hepatic parenchyma. The wires leading out from each thermocouple entered the data acquisition unit. The interval between each thermocouple is 1cm.

7.4 RESULTS

i) Intra-operative ultrasound in the ex-vivo setting

The microwave applicator was clearly imaged when placed into the butchers liver parenchyma. On the application of power, a highly echogenic area was produced

extending out from the applicator. This echogenic area increased in size as the treatment progressed although the degree of enlargement was less pronounced nearer the end of the treatment. Following the cessation of power, very little change in the ultrasound image was noted (i.e. the hyperechoic lesion seen on the image did not fade significantly).

Sectioning of the liver revealed an inner dark area immediately adjacent to the applicator surrounded by an obvious blanched lesion. The lesion edge was considered to be the border between the blanched area and the surrounding macroscopically normal liver. Comparison of the ultrasound image diameter with the sectioned lesion diameter revealed a significant difference in the two at almost every time point. Ultrasound consistently overestimated the macroscopic lesion size (see table 1) with the exception of the early 50 watt images.

Power in Watts

Time in Seconds	50	100	150
0	0	0	0
30	-10	+11	
60	-4	+8	
90	+6	+12	
120	+12	+15	
150	+8	+17	
180	+9	+19	+14

Table 1. Ultrasound prediction of lesion size compared to macroscopic measurement

expressed as +/- millimetres for ex-vivo liver. Positive values indicate overestimation and negative values underestimation of the lesion size by the ultrasound when compared to the maximum macroscopic diameter of the ablated lesion on sectioning of the treated tissue.

ii) Temperature monitoring in the ex-vivo setting

Much of this work was undertaken to investigate the most practicable method for thermal data acquisition in the in-vivo setting although a number of experiments were undertaken at different Wattages and times. The experiments showed that thermal data acquisition was possible from this system.

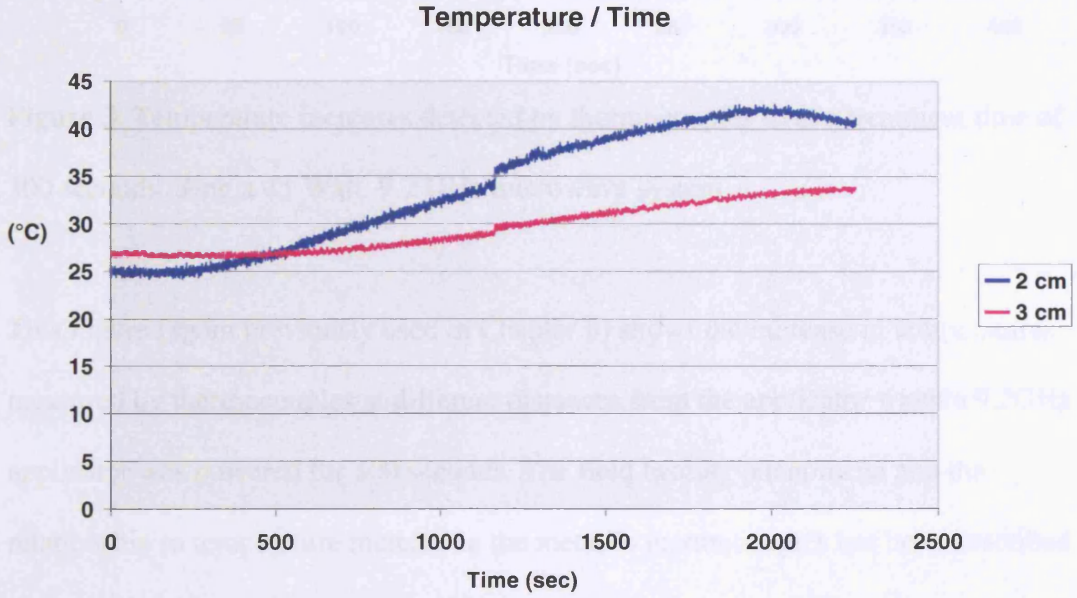


Figure 2. Increase in temperature in hepatic parenchyma following a microwave ablation of 40 Watts using the 9.2GHz microwave system. A steady rise in temperature can be seen in both thermocouples although the rate of temperature increase is greater in the thermocouple nearer the ablation centre.

In all experiments, thermocouples nearer the applicator showed faster rates of heating and acquired higher overall temperatures. This is unsurprising when one considers the field deterioration discussed in Chapters 6 and 7.

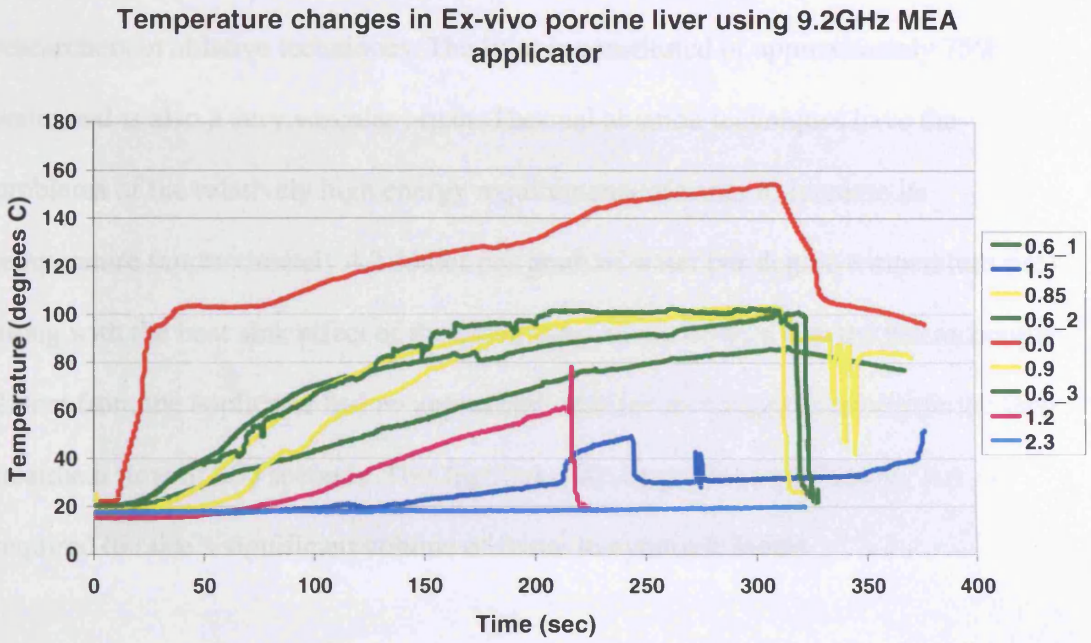


Figure 3. Temperature increases detected by thermocouples over a treatment time of 300 seconds using a 45 Watt, 9.2 GHz microwave system.

This Figure (again previously used in Chapter 6) shows the increase in temperatures measured by thermocouples at different distances from the applicator when a 9.2GHz applicator was powered for 300 seconds. The field heating phenomena and the relationship to temperature increase in the metallic thermocouples has been described in Chapter 6 however other interesting properties can be noted. The rapid rise in the thermocouple placed at 0mm from the applicator (red) is likely to be due to field heating but this plateaus out at just over 100°C for approximately 60 seconds. This is likely to be due to the fact that at this temperature the energy is being used to vaporise the water molecules. Later in the burn the temperature begins to rise suggesting that

that at this region immediately adjacent to the applicator the tissue is almost completely desiccated. Again thermocouples placed at greater intervals from the ablation centre show a much lower rate of temperature gain and also maximum temperature achieved. The graph also illustrates part of the problem that has faced researchers in ablative techniques. The liver is constituted of approximately 75% water and is also a very vascular organ. Thermal ablation techniques have the problems of the relatively high energy requirements of water to increase its temperature (approximately 4.2 Joules per gram of water per degree temperature rise) along with the heat sink effect of the blood flow. It can be seen that the thermocouple 2.3cm from the applicator had no appreciable rise in this temperature despite the long treatment time of 300 seconds. This highlights the large amount of energy that is required to raise a significant volume of tissue to cytotoxic levels.

iii) Intra-operative ultrasound in the in-vivo setting

The applicator was again easily imaged under IOUS in the in-vivo liver parenchyma. Following the application of power, the image was initially unchanged although after a few seconds, the area immediately adjacent to the tip became hypoechoic. Immediately following this period of hypoechoity came a sudden expansion in the lesion size in which the signal changed to one of high echogenicity. The outline of this change was not initially regular and passage of bubbles along what were assumed to be vessels was noted. This passage of bubbles occurred throughout the treatment cycle. The transition from lower to higher echogenicity occurred more quickly following the application of higher powers.

With each power setting the area of high echogenicity increased in size around the applicator tip as the treatment progressed and became termed the "thermal cloud". Once this thermal cloud was initiated, the applicator became difficult to visualise and an acoustic shadow was cast over the tissue that this cloud occupied (see Figure 4). The maximum extent of the thermal cloud was reached at the end of the treatment though its characteristics changed following the cessation of power. At the end of the treatment the ablation was represented by an oval shaped, highly echogenic image. Following the cessation of power the edges of the ablation became far less echogenic until they were represented by a hypoechogenic edge (Figure 5). At greater intervals, the majority of the ablation became hypoechogenic with a small, central portion of the image remaining highly echoic though the edge of the lesion remained clear.

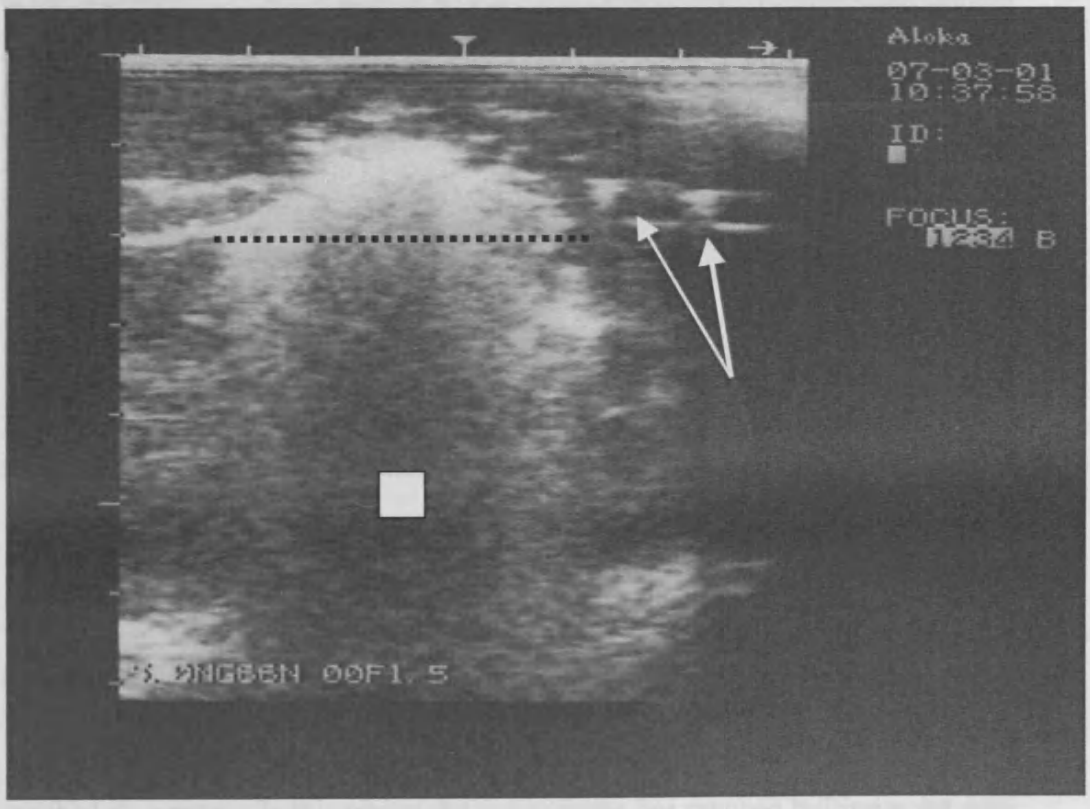


Figure 4 : Real time image of a 100 Watt lesion during the 3 minute ablation period.

Two thermocouples (arrowed) yet to be engulfed by the thermal cloud (black dotted line) can be seen to the right of the cloud. The acoustic shadow (white square placed in the centre of the dark acoustic shadow) cast by the ablation can be clearly seen, the microwave applicator is obscured by the shadow. The scale at the top of the ultrasound picture is in centimetres. When the acoustic shadow is observed it is easy to appreciate how difficult accurate placement of say another laser or RF probe would be. This highlights the advantage of a single placement technique that this microwave system allows.

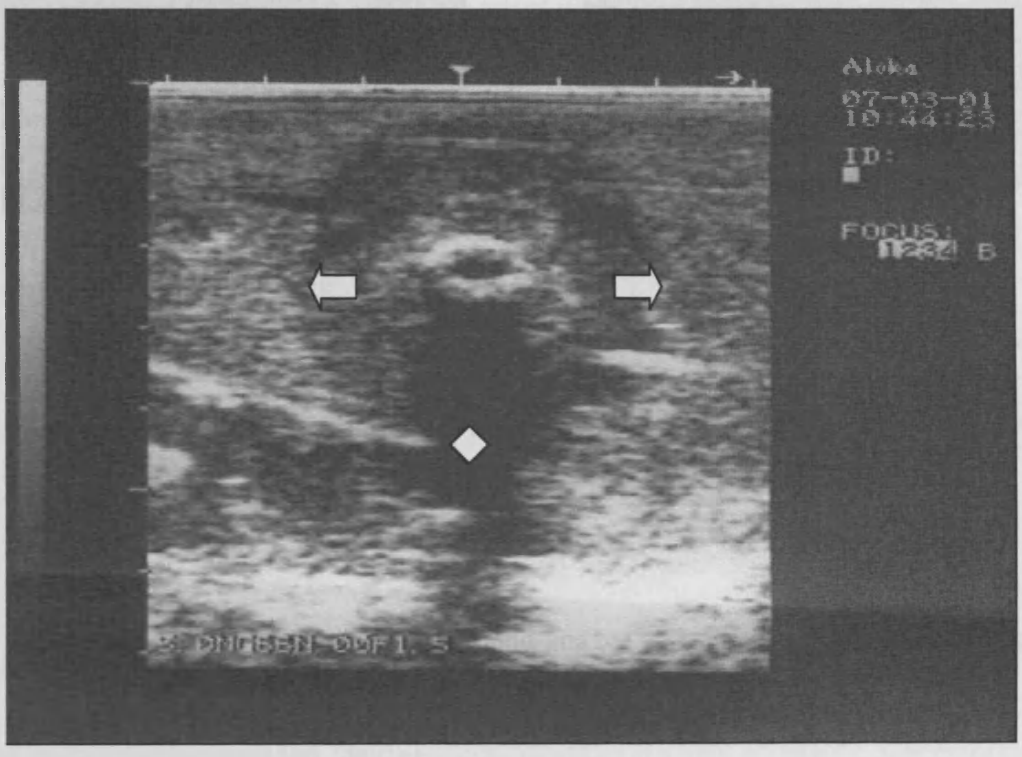
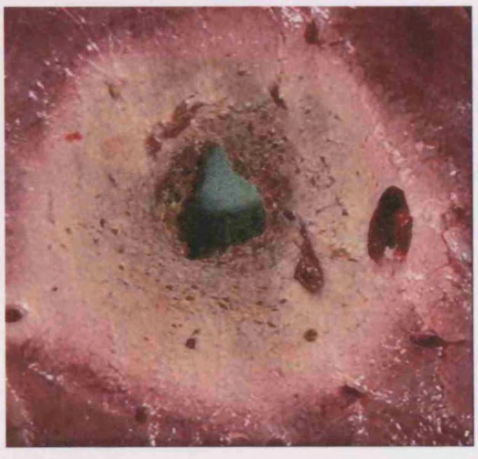
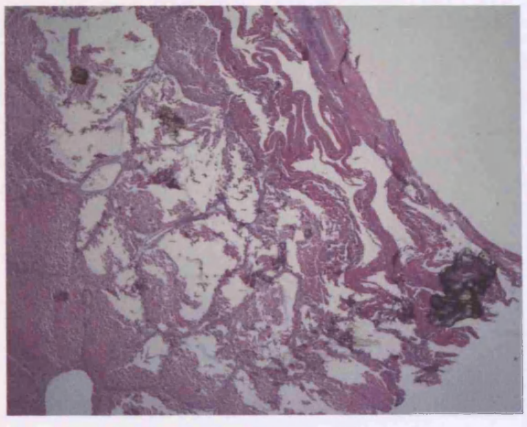


Figure 5. IOUS image following 3 minutes of power off and with the microwave applicator removed. The edge of the lesion has become hypoechoic (arrowheads) though some high signal echoes remain in the centre of the ablation; these are likely to represent the charred region at the centre of the ablation where the temperature has reached its greatest. In this region the temperature has reached boiling point with consequent steam and bubble production causing disruption of the tissue leaving a

honeycombed appearance, the air remaining in this region causes the bright echoes seen at the centre of the image, in essence the echoes show a ring into which the applicator had been placed (see Figures 6 and 7). It can be seen that the acoustic shadow has reduced in magnitude but a significant shadow has remained in the tissue (the white diamond sits in the centre of this region).





Figures 6 and 7. The honey comb appearance of the inner ablation region immediately adjacent to the microwave applicator can be seen macro and microscopically. Air in these regions accounts for the bright echoes seen in the post procedure ultrasound image of Figure 5.

The diameter of the ablation on ultrasound images was dose dependant in that higher powers induced larger images. Little difference between the magnitude of the maximum extent of the highly echogenic thermal cloud and the later hypoechogenic lesion edge was noted. The degree to which the ultrasound predicted the lesion size was partially dependant on the length of survival of the animal following the microwave treatment.

Table 2 shows the relationship between the diameter of the image produced by the IOUS compared to that seen macroscopically. It can be seen that at time zero the ultrasound overestimates the lesion seen macroscopically (as the majority of figures are positive) usually by a few millimetres. After twenty four hours and 1 week This has changed to a large extent in that very few positive figures are now seen with the majority now being negative. At greater intervals, the ultrasound estimation of the lesion at operation became less accurate. By 60 days, none of the measured lesions were as large as that predicted by ultrasound although those produced with higher Wattages were more accurate.

Power in Watts

Cull in days	36	50	100	150	200
0	+7 +7	+2 +4	-4 +11 +4 0 -3 -1 +4	-1 +4 +11 +9	
1		-3 +1 -1	-14 +1 -1	-7 +5 -6	-15

7		-6 -1	-15 -6	-8 -8	
30		+14	+2	-5	
60		+33	+30 +2	+22 +12	

Table 2. Ultrasound prediction of lesion size compared to macroscopic measurement expressed as +/- millimetres for in-vivo porcine liver. Again positive values indicate overestimation of the lesion by the IOUS and negative values underestimation.

iv) Temperature monitoring in the in-vivo setting

Thermocouples produced an obvious image when visualised using the IOUS and calculation of the distance of the initial thermocouple could be measured. In all cases, temperatures recorded from the thermocouples nearer the applicator rose more quickly and reached higher absolute values than those at greater distances. The maximum temperature reached by each thermocouple occurred at predictably different intervals. The monitor nearest the applicator reached maximum temperature within 10 seconds of the cessation of power. All other thermocouples reached their maximum temperature readings after a greater period of time following the cessation of power. Distance from the applicator determined the time after power off that the maximum temperature was reached. Thermocouples further from the burn centre attained their maximum temperatures more slowly than those nearer the applicator, this occurred in a sequential manner.

Temperature profiles were often smooth although movement of the jig caused some variations within the trace (see figure 7). Obtaining correlation between the temperature traces and ultrasound images was difficult due to the variability of values obtained from thermal monitoring. The nearest thermocouple often reached a value approaching 100°C following which it plateaued until the point at which power was halted. None of the thermocouples placed at a distance of 2cm or greater reached 100°C.

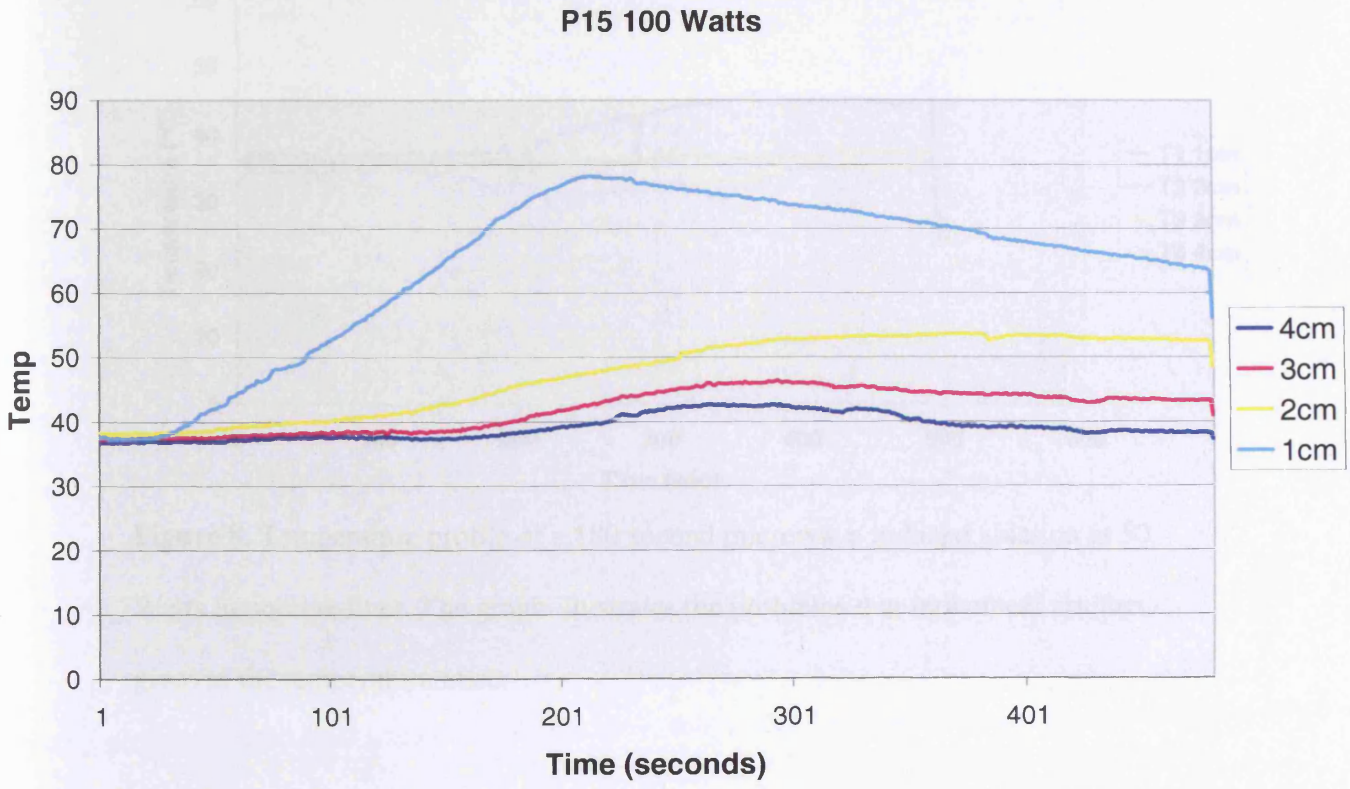


Figure 7. Temperature readings obtained from the thermocouples mounted within the aluminium jig during a 100 Watt ablation. This was a good, smooth trace although many of the traces obtained from the ablations were not of the quality of this.

One of the interesting features of the temperature monitoring in the porcine model was the effect of the microwave field or indeed the lack of it. Chapter 6 noted a field effect as shown by the temperature profile of a thermocouple placed outside the limits of the microwave field. Following treatment, the inner region of the tissue became desiccated and the field penetrated a greater distance through the tissue causing the temperature profile of the thermocouple to change. The above figure does not show any such changes in the temperature profiles.

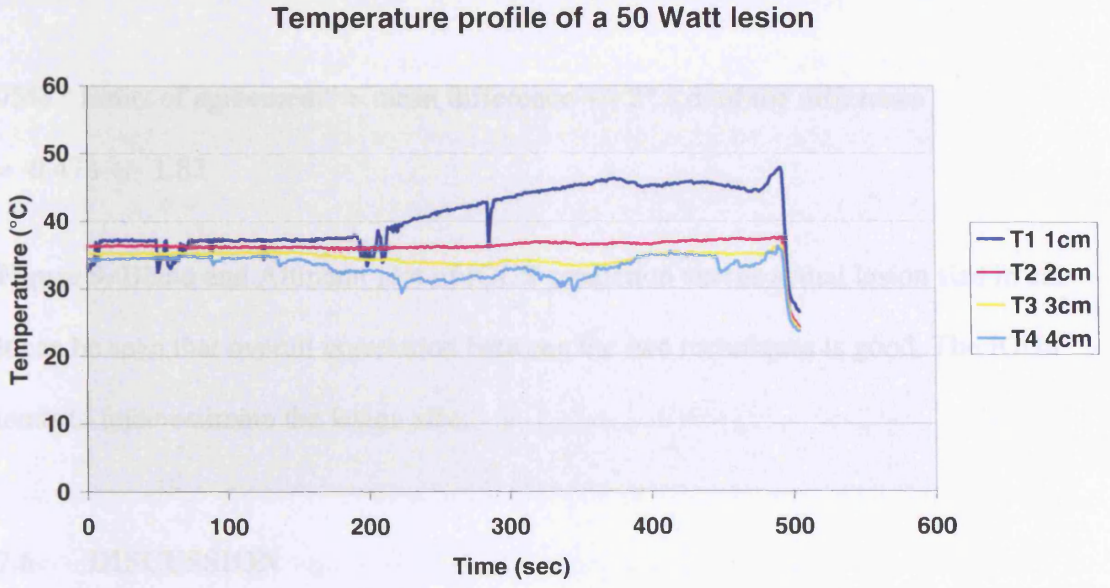
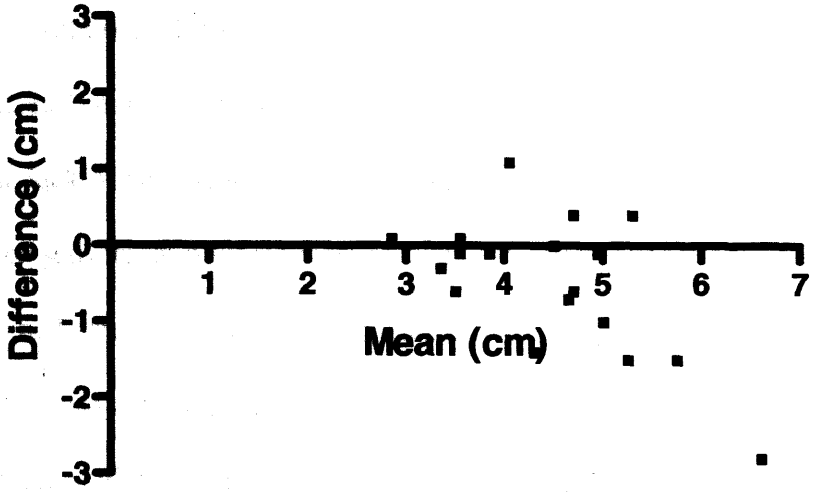


Figure 8. Temperature profile of a 180 second microwave induced ablation at 50 Watts in porcine liver. The graph illustrates the problems that movement artefact gives to the temperature trace.

7.5 STATISTICAL METHODS

The predictive value of the IOUS and compared to the actual lesion diameter was measured using a Bland and Altmann plot



95% " limits of agreement" = mean difference +/- 2* s.d. of the difference

= -0.478 +/- 1.82

Figure 9. Bland and Altmann plot of IOUS prediction versus actual lesion size in cm. It can be seen that overall correlation between the two techniques is good. The IOUS tends to underestimate the lesion size.

7.6 DISCUSSION

In all the different forms of liver investigated, the microwave applicator was easily imaged and the ablation produced could be visualised. Microwave ablations produced an initial increase in the echogenicity of the liver around the applicator tip and that this "thermal cloud" increased in size with the duration of power applied. It is considered that the increase in echogenicity was the consequence of cavitation and steam/bubble formation in the heated tissue an assumption that is not unreasonable given the macro and microscopic appearances of the ablation post procedure.

Microwave ablations performed in ex-vivo porcine liver produced images that were, on measurement, far in excess of the macroscopic lesion induced with the exception of the early images in the 50 Watt ablations. This was considered to be a consequence of the lack of perfusion and blood within the vessels of the tissue. As heating progressed, steam bubbles were forced along vessels or tissue planes where they remained causing distortion of the ultrasound image. In perfused tissue, real-time IOUS detects the passage of such elements along vessels where they condense due to the cooling effect of the vessel contents and no longer distort the ultrasound image. The image produced in freshly culled liver was similar to that seen in in-vivo liver and very representative of the macroscopic lesion in terms of lesion diameter. It is likely that this difference was due to the presence of blood filled patent vessels in the recently culled tissue.

In-vivo IOUS was able to image the progression of the ablation. The image changed in a number of predictable manners. After the initiation of power, the image became hypoechoic around the applicator tip as the innermost tissue was heated. When the water in the innermost tissue is heated the image changed rapidly to a highly echogenic one as steam and bubbles were formed (once a critical temperature likely to be around 100°C had been reached) and channelled along the vasculature of the tissue. As the ablation progressed the highly echogenic area or thermal cloud increased in size as tissue and fluid further from the applicator was heated. The maximum diameter reached was achieved at cessation of power whereupon the quality of the image changed again. The hyperechoic boundary of the lesion faded leaving a hyperechoic edge as steam/bubble formation ceased and condensation occurred.

Initial in-vivo ultrasound predictions appear to exceed the lesion size as measured macroscopically at time zero whilst appearing to be less than the measured sections at 1 and 7 days. This is likely to be accounted for by the increase in lesion size seen following microwave treatment. This discrepancy is likely to be due to the lesion expansion that is seen after time zero and documented in chapters 3 and 4 where the extent of the ablation moves into the haemorrhagic regions surrounding the initial lesions at time zero. Again this is in keeping with previously documented microwave, radiofrequency and other ablation technique studies. The ablation at time zero consists of a blanched area surrounded by a haemorrhagic zone. At twenty four hours and longer, the hepatocytes within the haemorrhagic zone die and therefore the ablated volume and consequently its diameter increases. It is thus likely that this phenomenon accounts for the change in the predictive value of the ultrasound. At greater intervals, the IOUS prediction appears to be less accurate although this is likely to be due to the regeneration of normal liver and the consequential shrinkage of the ablations. The in-vivo work highlights the ability of ultrasound to image the progression of the ablation in real-time and that the diameter of the image following a treatment is at least that of an ablation at one day to a week.

Treatment with the microwave produced what has been termed a thermal cloud. This image caused an acoustic shadow which lasted throughout and after the treatment. This shadow may have significant implications for the use of ablative treatment in the clinical setting. Many of the thermal ablation techniques use either intraoperative and transabdominal ultrasound to monitor lesion progression in an attempt to ensure complete lesion ablation and also for the reinsertion of the probe to treat a further

portion of a tumour. Almost all ablative technologies have difficulty in treating lesions 3cm or greater in diameter. If an ablative technology is able to produce a lesion 3cm in diameter, then 7 insertions are required to completely ablate a 3cm tumour with an acceptable ablation margin around its edge. The experiments revealed the acoustic shadow produced by the treatment is not inconsiderable and it is easy to imaging how difficult it would be to place an applicator accurately once 3 to 4 ablations have been introduced with their attendant acoustic shadows. The fact that many large tumours have been treated with technology that produces small volume ablations is a testament to the skill of the operators. The fact that this microwave is able to produce large volume ablations, rapidly, following a single insertion into the tissue and that it's progress may be monitored in real-time is obviously of great potential benefit.

The relationship between the ultrasound predicted ablation diameter and what is actually documented following sectioning of the lesions at certain time intervals is obviously of great importance when the technique is used in human treatments. IOUS is widely available in hospitals and particularly in Hepatobiliary units where expertise is often considerable. It is now known from this study that if the thermal cloud covers and extends slightly beyond any tumour that is to be treated it is very likely that it will be completely ablated.

The ex-vivo temperature experiments showed that real-time data could be gained during microwave ablations. The experiments revealed however that the acquisition of such data is not without its difficulties. A method for holding the thermocouples had to be devised and constructed as the position and relationship to the microwave

applicator could not be guaranteed. The aluminium jig was constructed to overcome these problems although this in itself was quite unwieldy and would be difficult to use in the clinical situation.

Using the thermocouple jig the temperature at different intervals from the microwave applicator could be monitored in real time. Maximum temperatures are reached by the more peripheral thermocouples well after the time at which power was halted due to the conduction of heat from the centre of the lesion, where the majority of the microwave's energy had been deposited. Unlike alternative microwave systems, increasing the applied power induced higher temperatures within the lesions and greater temperatures at greater distances from the applicator due to the efficient radiation of the microwave power from the applicator into the tissue. It is likely that temperature monitoring proved to be difficult variable due to a number of biological factors that are difficult to control for. Such factors include the size of the liver lobes, proximity of vasculature and the cold saline swabs placed around the lobe to the applicator and the degree of contact between the thermocouple and the tissue in which it was placed. It is not difficult to imagine how contact between tissue and the thermocouples could be compromised when one looks at the way the data for the ablations was achieved. The operators hand held the microwave and the aluminium jig whilst the ultrasonographer held the IOUS T probe. Movement of various hands was unavoidable especially when the lobe being treated became quite warm!

The temperature profiles shown in this chapter suggest that no field heating effect was present. This is implied as the temperature profiles for the innermost thermocouples was not very steep whilst treatment was taking place and that the

temperature drop off following the cessation of power was not marked. From chapter 5 it is known that microwave penetration through tissue is over 20 mm for a microwave frequency of 2.45 GHz and therefore a field effect should have been noted. This is even stranger especially in light of the results from chapter 6 in which microwave penetration through tissue is seen to extend further with treatment (if such ex-vivo data can be extrapolated to the in-vivo setting). The explanation is likely to be due to the field density of the two different microwave frequencies used in the experiments. The initial experiments used the high microwave frequency of 9.2GHz. We know from Chapter 2 that at this frequency the skin depth of the waves is 5mm i.e. that within 5mm approximately 2/3 of the wave's energy has been expended. At 2.45 GHz the skin depth is much greater and it is due to this difference that the explanation may be derived. The small skin depth of the 9.2 GHz wave means that at any point within the first 5 mm from the applicator the field is very intense and therefore the field density is very dense and its effects quite ferocious. At 2.45 Ghz the skin depth is much greater and the microwave field is therefore spread over a much greater volume reducing the field density. As the treatment progresses the microwave tissue penetration is increased stretching the field over a much greater volume further reducing the field density. It is likely that this reduction in field density goes a long way to explaining the lack of "field heating" effects on the thermocouples.

7.7 CONCLUSIONS

This study highlights the potential of IOUS for the real-time monitoring and prediction of ablation diameters following microwave ablation. In-vivo thermal

monitoring is possible although it produces results that are often variable even following similarly powered ablations. The use of real-time temperature monitoring may well be to ensure cytotoxic temperatures have been reached at the tumour margins rather than for continuous data acquisition.

CHAPTER 8

HUMAN TREATMENTS USING MICROWAVE ABLATION DEVICE FOR THE TREATMENT OF UNRESECTABLE LIVER METASTASES

8.1 INTRODUCTION

8.2 EX AND IN-VIVO HUMAN WORK

i) Post resection treatments

ii) Pre resection treatments

8.3 PILOT STUDY

i) Pilot Study patient inclusion and exclusion criteria, aims and follow up cross sectional imaging

ii) Individual patients details, treatments and postoperative recoveries

iii) Example pre and post operative cross sectional scans

8.4 HUMANITARIAN TREATMENTS

i) Humanitarian patients inclusion and exclusion criteria

ii) Individual patient details, treatments and postoperative recoveries

iii) Laparoscopic treatments

iv) Cumulative results from the humanitarian treatments

v) Example pre and postoperative cross sectional scans

8.5 DISCUSSION OF HUMAN TREATMENTS

i) **Pilot study**

ii) **Assessment of microwave technology**

iii) **Lessons learnt**

iv) **Future objectives for human treatments**

8.6 CONCLUSIONS

8.1 INTRODUCTION

Microwave ablation as a treatment for liver tumours is not a new idea and has been widely practised in the Far East for approximately two decades (Yamaue et al.1984). Its acceptance as a useful intervention has remained confined to a number of enthusiasts using a multi-insertional approach for the treatment of tumours 2cm in diameter or greater (Shibata et al 2000). The novel microwave system examined in this work has many advantages not least of which is the ability to induce larger volumes of ablation effectively, rapidly and with few complications in the animal models tested. The computer and animal modelling work described previously is theoretically promising yet the microwave had not been used in human patients until now.

8.2 EX AND IN-VIVO HUMAN WORK

i) Post resection treatments

Initial work in human patients used liver that had been recently removed in a standard surgical procedure. A right hemi-hepatectomy is a commonly undertaken hepatic resection in which a relatively large portion of liver tissue is excised. Liver from patients undergoing such a procedure allowed the microwave applicator to be tested in human tissue. Initial work was performed with the 9.2 GHz equipment as this was readily available due to the previously endometrial ablation work carried out and developed previously. As mentioned in Chapter two it became immediately apparent that the 9.2 GHz applicator would have to be modified with the introduction of a

cutting tip as the previously rounded end of the endometrial probe did not allow easy placement within the relatively firm hepatic colorectal metastases.

The applicator was duly modified and 3 patients underwent post resection ablations of both tumour deposits and normal liver parenchyma. Some of the data has been presented in the chapters investigating complex permittivity of normal liver parenchyma and colorectal metastases.

ii) Pre-resection treatments

The microwave equipment was also tested in patients with liver tumours prior to their resection. The microwave applicator was placed under IOUS into the tumour deposit and power was applied in 3 patients. No change in physiological parameters such as pulse and blood pressure were identified on the intra-operative anaesthetic monitors. Using this evidence and the work performed in animal models together with the fact that microwave treatments have been performed in the Far East for many years, it was felt that progression to human treatments would be reasonable.

8.3 THE PILOT STUDY

i) Pilot Study Patient inclusion and exclusion criteria, aims and follow up cross sectional imaging

An application to the Local Ethics Committee was made to gain permission for the treatment of patients with unresectable liver metastases using the modified 9.2Ghz

MEA applicator. Approval for the pilot study was granted according to strict inclusion/exclusion criteria. The MEA equipment and the procedure itself have an extremely good safety record with many thousands of treatments performed each year (Downes and O'Donovan 2000, Cooper et al. 2005). Patients suitable for treatment were identified at the Multi-Disciplinary Team meeting at which all individuals with colorectal and hepatobiliary cancers are discussed. Patients had the procedure explained to them by the operating Consultant and Research Fellow in the out patient clinic.

Inclusion criteria

- Unresectable liver metastases of colorectal origin, fewer than 10 in number
- Medically fit to a withstand general anaesthesia and laparotomy
- Less than 80 years of age
- No evidence of extra-hepatic malignant disease
- Metastases had to be definable i.e. not diffuse disease throughout the liver

Aims

The aims of the pilot study were to assess the short-term safety of the procedure and the effect of the ablation on the disease at 3 months. Disease modification was to be assessed using cross sectional imaging at 6 weeks and 3 months.

Safety Committee

In addition to the above measures a monitoring committee made up of an Oncological Surgeon (not directly involved with the project), an Oncologist and a Statistician was

formed to oversee the safety of the work. At the completion of the pilot study the results were presented to this committee for its scrutiny, advice and comments.

Other Measures

The Ethics committee required the provision of a palliative care counselling service along with the standard patient and General Practitioner information sheets. All patients had the treatment discussed with them prior to the procedure by both the Consultant Surgeon performing the ablation and Research Fellow involved.

ii) Individual patient details, treatments and post operative recoveries

The details of each patient, their presentation, operation details, post operative recovery and longer term outcomes are presented.

- **Patient 1**: 79 yr old male

History and presentation

Feb 2000 underwent excision of rectal cancer with prolonged hospital stay due to the development of renal failure post operatively. Follow up imaging of the liver identified 5 lesions requiring an extended right hemi-hepatectomy for complete excision. It was felt that this patient would not tolerate such a procedure as he was frail, (extended right hemi-hepatectomy in the elderly is not a well tolerated procedure Seyama et al. 2003) although he may withstand an ablation.

Operation and ablation details

At operation in April 2001 8 lesions were found. A resection of the 2 largest lesions was performed along with ablation for the remaining tumours. Lesion size ranged from 5mm to 20mm.

Lesion No.	Size (mm)	Segment	Freq. (GHz)	Applicator	Pringle	Power (Watt)	Time (sec)
1/2	31/34	3/4	n/a	resected	yes	n/a	n/a
3	4.5	2	9.2	6.4mm vol	yes	45	60
4/5	5/5	4/8	9.2	6.4mm vol	no	45	180
6	16	5/6	9.2	6.4mm vol	no	45	120
7	20	7/8	9.2	5.0mm vol	yes	45	120
8	7	5/6	9.2	5.0mm vol	yes	45	120

Postoperative recovery (in patient days: 66)

This patient had a prolonged postoperative stay due to a bile leak from the resection margin (proven by CT) but no complications appeared to be attributable to the microwave.

Longer-term outcome

Cross sectional scans at 6 weeks, 3 months and 9 months postoperatively showed no evidence of tumour recurrence. He died 11 months post-procedure at home from pneumonia.

- **Patient 2**: 79 yr old female

History and presentation

This lady presented with large transverse colon cancer with 4 synchronous, bilobar liver metastases noted on imaging. The patient was considered too frail for major liver resection or chemotherapy and therefore a combined colonic resection and ablation was considered to be a sensible approach minimising the number of anaesthetics the patient underwent. She had previously suffered from breast cancer. It was decided that she should undergo a colonic resection and ablation of the liver metastases at the time of her colonic resection preventing two operations.

Operation and ablation details

A combined colonic and hepatic ablation performed. MLA details as follows.

Lesion No.	Size (mm)	Segment	Freq. (GHz)	Applicator	Pringle	Power (Watt)	Time (sec)
1	10	2	9.2	5.0mm vol	no	45	120
2	18	3	9.2	5.0mm vol	no	45	120
3	20	8/1	9.2	5.0mm vol	no	45	180
4	19	4	9.2	5.0mm vol	no	45	180

Postoperative recovery (in-patient days: 11)

The postoperative recovery was unremarkable.

Longer-term outcome

Scans at 6 weeks and 3 months showed no tumour recurrence although the imaging at 7 months revealed 2 new hepatic lesions noted distant to the previous MLA treatments. A new ovarian primary was also noted in the pelvis this prevented any further intervention. She died 11 months following treatment.

- **Patient 3**: 73 yr old female

History and presentation

This female presented with a large rectal cancer and synchronous liver metastases. She was on the waiting list for cardiac surgery due to her ischaemic heart disease but it was felt (by the Cardiothoracic surgeon) that her cancer should be treated initially before cardiac revascularisation could be considered. It was felt that ablation would be less of an insult to her than hepatectomy and therefore a combined colorectal and ablation was performed obviating the need for two operations.

Operation and ablation details

A combined colorectal resection and hepatic ablation was performed.

Lesion No.	Size (mm)	Segment	Freq. (GHz)	Applicator	Pringle	Power (Watt)	Time (sec)
1	11.5	3	9.2	5.0mm vol	no	45	120
2	24	4a	9.2	6.4mm vol	no	45	240
2	24	4a	9.2	6.4mm vol	no	45	150
3	27	4b	9.2	6.4mm vol	no	45	180
3	27	4b	9.2	5.0mm vol	no	45	120
4	15	4/5	9.2	5.0mm vol	no	45	120

Postoperative recovery (in-patient days: 6)

The initial postoperative recovery was unremarkable however this woman suffered a myocardial infarct (confirmed at post mortem) on day 6 and died. The pathologist found nothing abnormal with respects to the liver except areas of confined ablation. The family declined examination of the liver tissue for the purposes research into the microwave's effectiveness. It was felt that the microwave treatment had not contributed to this patient's death.

- **Patient 4**: 59 yr old female

History and presentation

This woman underwent a right hemicolectomy in Oct 1999 for Dukes B colonic carcinoma. She was given not chemotherapy post operatively due to her moderately severe renal failure. In 2001 two hepatic metastases noted on cross sectional imaging whereupon she was treated with 5 Fluorouracil and folinic acid.

Operation and ablation details

Initially this woman was to undergo standard liver resection. at laparotomy 4 additional liver lesions were noted and therefore a right hemi-hepatectomy and ablation of the left lobe lesions was performed.

Lesion No.	Size (mm)	Segment	Freq. (GHz)	Applicator	Pringle	Power (Watt)	Time (sec)
1	5	1	9.2	5.0mm vol	no	45	120
2	8	3	9.2	10mm flat	no	45	120

Postoperative recovery (in-patient days: 12)

She made an unremarkable postoperative recovery.

Longer-term outcome

All subsequent scans have not revealed any recurrent tumour (now >3yrs post surgery).

- **Patient 5**: 73 yr old male

History and presentation

This man presented with carcinoma of the right colon and bi-lobar liver metastases on imaging. This patient had significant co-morbidity including ischaemic heart disease, hypertension and rheumatoid arthritis for which he was taking immunosuppressant drugs.

Operation and ablation details

He was considered too frail to undergo a staged procedure (i.e. bowel resection followed by hepatectomy) and therefore a combined colonic resection and ablation of the liver metastases was performed.

Lesion No.	Size (mm)	Segment	Freq. (GHz)	Applicator	Pringle	Power (Watt)	Time (sec)
1	10	8	9.2	6.4mm vol	no	45	120
2	10	4	9.2	6.4mm vol	no	45	120
3	30	2	9.2	6.4mm vol	no	45	240
3	30	2	9.2	6.4mm vol	no	45	120
4	20	2	9.2	6.4mm vol	no	45	120
5	25	4/5	9.2	6.4mm vol	no	45	300
6	15	4	9.2	6.4mm vol	no	45	120
7	6	4	9.2	12mm flat	no	45	90
8	3	6	9.2	12mm flat	no	45	60

Postoperative recovery (in-patient days: 14)

Post operative recovery was unremarkable.

Longer-term outcome

Thirty-five days post procedure the patient was readmitted with a gram-negative septicaemia secondary to a urinary tract infection. Despite medical treatment the patient died of overwhelming sepsis. Liver function as determined by routine liver function tests and coagulation times was normal on the samples taken on admission to hospital and no indication that the sepsis was related to the liver was found.

- **Patient 6**: 69 yr old male

History and presentation

This man underwent a sigmoid colectomy in Jan 2001 but developed liver metastases whilst receiving chemotherapy (5 FU and folinic acid) in May 2001. He was commenced on Oxaliplatin in June 2001 and referred to the HPB Unit. Imaging revealed bi-lobar metastases.

Ablation details:

Lesion No.	Size (mm)	Segment	Freq. (GHz)	Applicator	Pringle	Power (Watt)	Time (sec)
1	54	8/4	9.2	6.4mm vol	no	45	540
1	54	8/4	9.2	6.4mm vol	no	45	120
1	54	8/4	9.2	6.4mm vol	no	45	120
1	54	8/4	9.2	6.4mm vol	no	45	120
2	15	4	9.2	6.4mm vol	no	45	120
3	15	4	9.2	6.4mm vol	no	45	60
4	8	4	9.2	6.4mm vol	no	45	120
5	8	8	9.2	10mm flat	no	45	120
6	2	2	9.2	10mm flat	no	45	120

Postoperative recovery (in-patient days: 7)

He made an unremarkable postoperative recovery.

Longer-term outcome

Complete ablation of the treated lesions was seen at initial 6 week scan though the 3 month imaging revealed new lesions distant to the previous ablations. These new lesions were not considered resectable and he was re-referred back for consideration of further chemotherapy. He died 19 months post procedure.

- **Patient 7**: 58 yr old male

History and presentation

This man was admitted with an obstructing carcinoma of the descending colon which was resected. Per-operatively he was noted to have bi-lobar hepatic metastases and commenced on chemotherapy. Imaging revealed 9 lesions within the liver.

Operation and ablation details

At operation eight lesions were ablated and one in segment 3 was resected.

Lesion No.	Size (mm)	Segment	Freq. (GHz)	Applicator	Pringle	Power (Watt)	Time (sec)
1	15	4/5	9.2	5.0mm vol	no	45	120
2	15	2	9.2	10mm flat	no	45	350
3	8	45	9.2	10mm flat	no	45	120
4	10	8	9.2	10mm flat	no	45	120
5	6	4/8	9.2	10mm flat	no	45	60
6	8	3	9.2	10mm flat	no	45	120
7	5	3	9.2	10mm flat	no	45	60
8	12	4/5	9.2	6.4mm vol	no	45	240

Postoperative recovery (in-patient days: 7)

He made an unremarkable recovery.

Longer-term outcome

After two clear CT scans he developed new liver deposits at six months. These new lesions appeared to be separate from the previously ablated lesions. He is currently alive 3 years post procedure.

- **Patient 8**: 69 year old female

History and presentation

This woman underwent a right hemicolectomy in April 2001 and was noted to have bi-lobar, synchronous liver metastases. She was treated with oxaliplatin chemotherapy and cross sectional imaging revealed 6 lesions in a bi-lobar distribution. It is likely that this patient did benefit from the procedure as she recovered well from the surgery and was back to her previous activities quickly post operatively. As this lady had progressive disease despite receiving second line chemotherapy an attempted curative ablation seemed reasonable to both the clinicians and patients.

Operation and ablation details

At operation 3 additional lesions to those seen on the imaging were found and all were ablated.

Lesion No.	Size (mm)	Segment	Freq. (GHz)	Applicator	Pringle	Power (Watt)	Time (sec)
1	20	7	9.2	5.0mm vol	no	45	180
2	15	8	9.2	5.0mm vol	no	45	210
3	12	5	9.2	10mm flat	no	45	120
4	20	6	9.2	10mm flat	no	45	120
5	15	6	9.2	10mm flat	no	45	120
6	20	7	9.2	10mm flat	no	45	120
7	25	7	9.2	10mm flat	no	45	120

8	8	3	9.2	10mm flat	no	45	60
9	8	3	9.2	5.0mm vol	no	45	60
10	12	5	9.2	5.0mm vol	no	45	75
11	20	6	9.2	5.0mm vol	no	45	60
12	15	6	9.2	5.0mm vol	no	45	60
13	25	7	9.2	5.0mm vol	no	45	60
14	20	7	9.2	5.0mm vol	no	45	60

(the table above shows that some of these lesions were treated with the flat probe in addition to the spike applicator and therefore appear twice).

Postoperative recovery (in patient days: 14

Unremarkable.

Longer-term outcome

Initial cross sectional images showed completely treated lesions although at 5 months new lesions were noted in the liver. These were not in a position that could be ablated or resected, she died 18 months post procedure.

- **Patient 9:** 64 yr old female In patient stay: 11 days

History and presentation

This lady underwent a sigmoid resection for an obstructing carcinoma with bilobar, synchronous metastases noted per-operatively. She was treated with chemotherapy and referred to Leicester for further assessment. Cross sectional imaging revealed bilobar metastases.

Operation and ablation details

At operation it was found that (Unusually) 3 of the 4 deposits were situated in the left lobe and only 1 in the right. The patient underwent a left hemi-hepatectomy and an ablation of the right liver deposit.

Lesion No.	Size (mm)	Segment	Freq. (GHz)	Applicator	Pringle	Power (Watt)	Time (sec)
1	20	8	9.2	6.4mm	no	45	240

Postoperative recovery (in patient days: 11)

Unremarkable.

Longer-term outcome

No further liver lesions have been detected on any of her follow up scans and she remains well.

- **Patient 10**: 68 year old female

History and presentation

This lady had a colonic tumour resected in 2002 and subsequently developed bi-lobar liver metastases on follow up cross sectional imaging.

Operation and ablation details

She underwent a right hemi-hepatectomy and an ablation of the single deposit in the left lobe.

Lesion No.	Size (mm)	Segment	Freq. (GHz)	Applicator	Pringle	Power (Watt)	Time (sec)
1	20	2/3	9.2	5.0mm vol	no	45	180

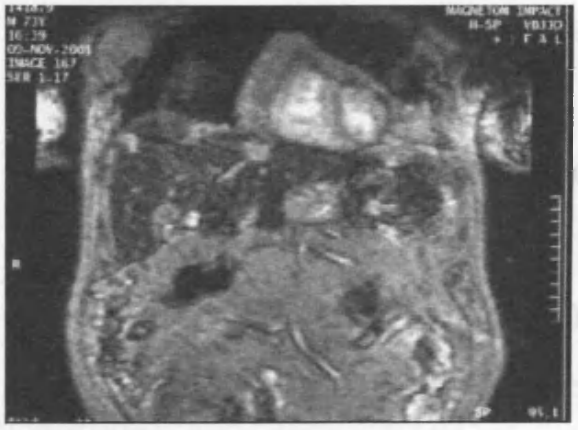
Postoperative recovery (in patient stay 10 days)

Unremarkable.

Longer-term outcome

Post-operative cross sectional imaging to date has revealed no new liver lesions although pelvic recurrence has been noted, she died 26/10/03, 19 months post procedure.

iii) Example pre and postoperative cross sectional scans



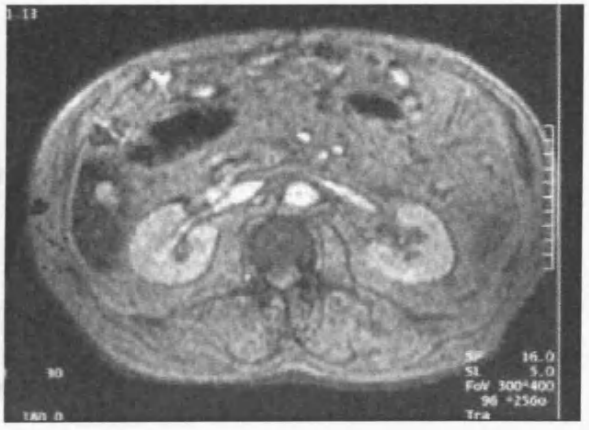


Figure 1. MRI scan illustrating a patient with bi-lobar liver metastases. This patient was treated by a left lateral segmentectomy and ablation of tumours in the right lobe.

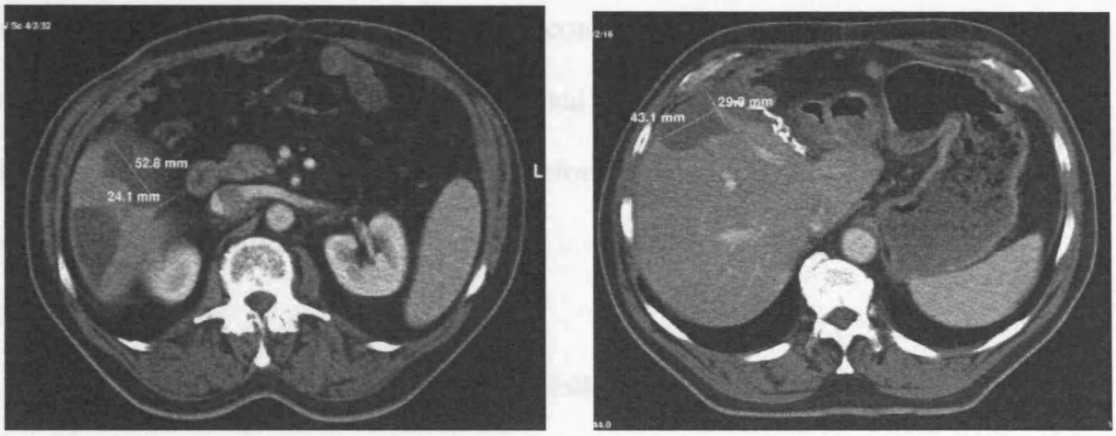


Figure 2. Post operative CT scan of the same patient 3 months post procedure. The sharp margins from the ablation can be seen interspersed between normal liver, the clips from the resection margin can also be seen.

8.4 HUMANITARIAN TREATMENTS

i) Humanitarian patients inclusion and exclusion criteria

A number of patients referred to the Upper GI/Hepatobiliary MDT meeting did not fit the criteria for the pilot study and these cases were considered on their individual merits. In some cases the patients with tumours too large for treatment by the 9.2GHz frequency equipment were not included in the Pilot Study for fear of incomplete

ablation of the lesion/s. Another group of patients who did not fit into the criteria stipulated for the Pilot Study were those patients suffering from HCCs and associated liver cirrhosis not suitable for resection. Although these tumours make up a very small proportion of liver tumours in the UK a small number of cases were identified at the MDT meeting. A selected number of such humanitarian cases are outlined below. The majority of patients treated were done so with an intention to cure although this was not always the case if palliation or symptom control (particularly carcinoid patients) was considered the primary objective. Approval from the Medicines and Healthcare products Regulatory Agency was obtained before each treatment as was individual consent from each patient.

ii) Individual patient details, treatments and post-operative recoveries

- **Patient 1:** 75 yr old male with HCC and liver cirrhosis In patient stay: 14 days

History and presentation

This retired General Practitioner presented with vague right upper quadrant pain. An ultrasound of the area revealed a mass lesion in the right lobe of his liver. Both CT and MRI imaging revealed a 6.5-7cm lesion occupying segment 4/8 high up in the dome of the right lobe. This finding, in combination with a significantly raised alpha-fetoprotein level of 5814 Ku/L (normal 0-9), strongly suggested he had a primary liver tumour. Pre-operative blood tests indicated some degree of

hepatic dysfunction (possibly cirrhosis) although his clotting (INR) and albumin were within normal levels. A right hemi-hepatectomy was planned yet the use of the microwave equipment was discussed with the patient and family.

Operation and ablation details

During surgery on 10/5/01 it was evident that the liver was cirrhotic, a degree of portal hypertension was noted and some ascites was present. The liver was mobilised and the tumour was found to be invading the diaphragm. The tumour was freed from the diaphragm (a portion of which was resected) and an attempt at surgical removal of the large liver tumour was made. During this manoeuvre the patient began to bleed significantly because of the portal hypertension. The patient became hypotensive and required aggressive fluid and blood product resuscitation. Further surgical dissection was halted whilst fresh frozen plasma was administered to improve blood clotting. At this point it was declared unsafe to proceed with a formal hemi-hepatectomy or even a segmental excision because of the unstable nature of the patient.

An MLA treatment was planned using the 2.45GHz system. Temperature probes were placed approximately 5mm outside the tumour periphery and the microwave probe placed slightly eccentrically within the tumour mass. A two-minute MLA treatment at 150 Watts was initiated and a second two-minute treatment performed by inserting the probe through a different aspect of the tumour. The ablation was monitored in real time using temperature sensors rather than IOUS. Following these treatments no bleeding was noted from the cavities made by the

applicator. Some bleeding was noted from the much smaller cavities made by the temperature sensors which was controlled using sutures.

Lesion No.	Size (mm)	Segment	Freq. (GHz)	Applicator	Pringle	Power (Watt)	Time (sec)
1	65	4/8	2.45	6.4mm vol	No	150	240

Postoperative recovery (in-patient days 18)

He recovered well though required aspiration of intra-pleural fluid.

Longer-term outcome

At follow-up, multiple scans have not been able to demonstrate viable tumour in the area of ablation. Furthermore, his alpha-fetoprotein tumour marker levels fell dramatically (levels of 60 were documented) giving supporting evidence that the tumour had been successfully ablated by the microwave ablation. No hepatic recurrence of the tumour was found although he did develop pulmonary and cerebral metastases. He died 03/08/2003, 27 months post procedure.

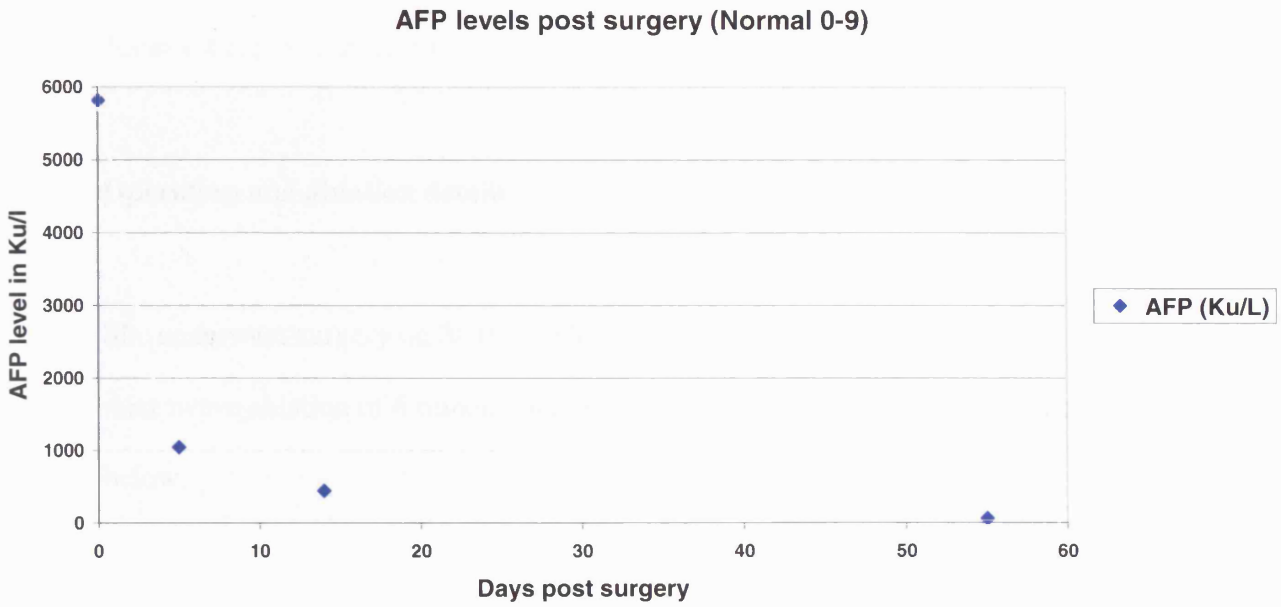


Figure 3. Alphafetoprotein levels following microwave ablation of the HCC.

- **Patient 2:** 37 yr old female - colorectal liver metastases.

History and presentation

This woman, a practising Jehovah's Witness, presented with an abdominal mass subsequently found to be a transverse colon cancer. She underwent a right hemi colectomy where it was found that she had peritoneal disease and extensive liver metastases. The hepatic disease was bilobar and precluded standard liver resection. She was given chemotherapy Irinotecan and an experimental angiogenesis inhibitor PTK 787. Some reduction in disease volume was achieved and she was considered for liver resection and MLA treatment. Her CT scans

demonstrated a mass of tumour in the left lobe (segments 2 and 3) measuring 10cm x 4 cm. and at least five or six within the right lobe.

Operation and ablation details

She underwent surgery on 3/10/01. This involved a left hepatectomy and microwave ablation of 6 tumours within the right lobe. The details are shown below.

Lesion No.	Size (mm)	Segment	Freq. (GHz)	Applicator	Pringle	Power (Watt)	Time (sec)
1	4	5	9.2	10mm flat	no	45	60
2	15	4b	9.2	5.0mm vol	no	45	180
3	35	4a	2.45	6.4mm vol	no	150	145
3	35	4a	2.45	20mm flat	no	100	90
4	20	7/8	9.2	6.4mm vol	no	45	150
5	62	5/6/7/8	2.45	20mm flat	no	100	180
5	62	5/6/7/8	2.45	20mm flat	no	150	240

Postoperative recovery (in-patient days: 7)

She did well following surgery and suffered no complications.

Longer term outcome

Scans to date (July 04) have shown no hepatic disease although she has now developed pulmonary metastases but is currently still alive over 3 years post procedure and receiving palliative chemotherapy.

- **Patient 3:** 59 year old male

History and Presentation

This man had bilobar deposits of carcinoid tumour (greater than 20 lesions) and suffered from significant carcinoid syndrome symptoms such as diarrhoea and flushing. It was decided that as his symptoms were so debilitating that debulking of his tumours using multiple ablations would be a sensible treatment.

Operation and ablation details

At open operation a left lateral bisegmentectomy was performed with over 20 microwave ablations though many of these were performed with the surgface applicator.

Postoperative recovery (in-patient stay 8 days)

Unremarkable

Longer-term outcome

Following his treatment this man has no carcinoid symptoms, opening his bowels once to twice a day and has even felt well enough to run a marathon recently.

iii) Laparoscopic treatments

To date three patients have been treated laparoscopically, all with encouraging results. The first patient was unusual as the lesion that was treated was a hepatic metastasis from a parathyroid carcinoma. The patient had underdone a parathyroidectomy and lymphadenectomy yet this radical resection had not reduced the serum calcium. Cross sectional imaging revealed a 4cm lesion in segment 8 of the liver. To ensure this abnormality was responsible for the elevated serum calcium, a transjugular hepatic vein blood sample was extracted and the level of PTH was compared to that of peripheral blood. The levels of PTH were significantly higher in the blood from the hepatic vein and therefore it was concluded that ablation of this lesion would be beneficial. Unlike hepatic colorectal metastases, such metastases are rare and data regarding the potential curability of a hepatic resection is very scant. It was therefore decided that a minimally invasive approach to the treatment of this lesion would be the most appropriate. A laparoscopic ablation under direct vision monitored in real time and with laparoscopic IOUS was performed. No complications were noted following this treatment and the patient was discharged 3 days post-operatively with normal serum calcium. No hepatic recurrence has been noted and the patient's serum calcium has remained within normal limits. She remains well with no evidence of hepatic recurrence 18 months post procedure.

Two other patients both with benign tumours have been treated laparoscopically. One patient presented with a large haemangioma and the second with an adenoma (both lesions were 3cm in diameter). Both of these patients suffered from right upper quadrant pain. Despite investigations, no other pathology was found which could have accounted for their symptoms. As these were essentially benign conditions the patients were offered either standard, open liver resection or laparoscopic ablation.

Both patients opted for the minimally invasive treatment option despite being informed of its experimental nature. Patients were treated under general anaesthesia and a standard pneumoperitoneum achieved via a 10mm umbilical port. Two further 10mm ports for the laparoscopic ultrasound and microwave applicator were employed for the treatment. Both patients were discharged on the 2nd post-operative day and no complications were noted. Subsequent cross sectional imaging has shown complete ablation of the presenting lesions in both patients. The right upper quadrant pain experienced by both these patients has resolved.

iv) Example pre and post operative scans

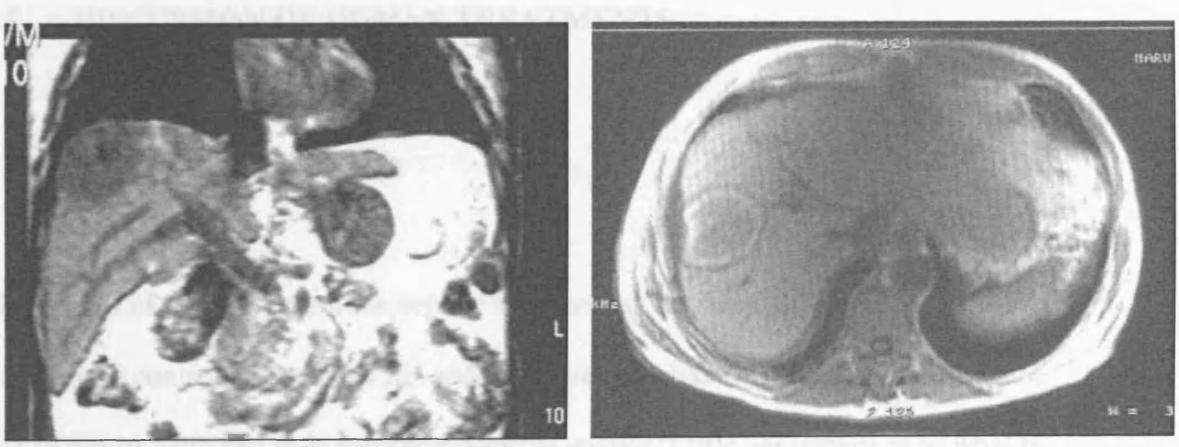


Figure 4. Patient with a single, large hepatocellular cancer which was ablated using the 2.45 GHz equipment. The preoperative MRI (left) shows the lesion in segment 8/4. The post-operative MRI scan (right) shows the lesion 12 months post ablation. No evidence of local or organ recurrence can be seen.

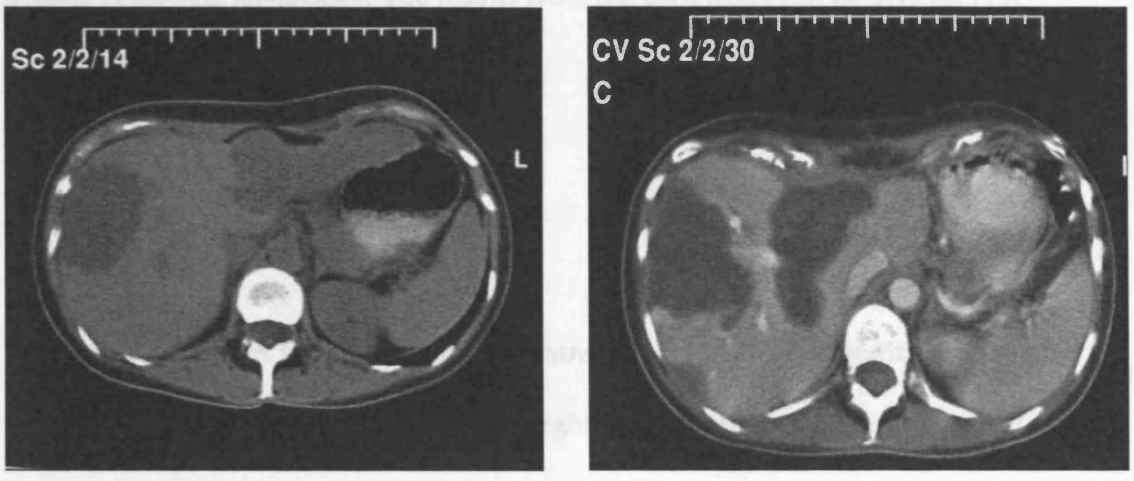


Figure 5. Patient with multiple, large, bi-lobar metastases seen on the pre-operative CT (left) was treated with a left lateral bisegmentectomy and multiple ablations in the right lobe. The post-operative CT (right) is seen at 3 months post procedure and shows complete ablation of the lesions with no hepatic recurrence noted.

8.5 DISCUSSION OF HUMAN TREATMENTS

i) Pilot study

The benefit of liver resection for hepatic tumours has not been subjected to randomised controlled trials but nevertheless resection has become the gold standard due to results achieved following comparative studies. Little agreement as to what is potentially resectable disease exists as hepatic surgery is a rapidly evolving specialty. When advances in chemotherapeutic agents are taken into consideration a definition of what is a surgically resectable disease is difficult to define. The Pilot Study was not a randomised, controlled study but simply sought to investigate the short-term effects

of the microwave as a technique in terms of safety, patient recovery and its effect on hepatic colorectal metastases. The results from the pilot study can be divided into those with negative and positive features.

Negative features

The negative features from the study are centred around patient mortality and tumour recurrence. Of the ten pilot study patients eight had an entirely unremarkable post operative recovery, the remaining two patients suffering unwanted complications are now discussed. The first patient developed a biliary leak from the resected margin of a metastasis treated surgically. This was not a microwave complication as both CT scanning and ERCP confirmed the leak to be from the resected margin of a metastasis treated by standard surgical resection rather than an ablation site. The patient was relatively frail and had previously endured a relatively long post operative recovery following his colorectal operation which was complicated by renal failure. A bile leak is a well known complication of liver surgery which is usually treated conservatively but occasionally requires insertion of a stent into the CBD to aid cessation.

The second patient with a post operative complication was patient 3 who suffered a fatal myocardial infarction, confirmed at post mortem, following surgery. Until this event her post operative recovery was entirely unremarkable. This patient had a combined procedure to prevent her having to undergo two anaesthetics. She was known to suffer from ischaemic heart disease and was on the waiting list for coronary artery bypass grafting. After her diagnosis of rectal cancer and liver metastases the cardiac surgeons postponed her surgery until the colorectal carcinoma was treated. It

is likely that this was a major contributing factor in the poor outcome from surgery in this lady.

One further patient (Pilot patient 5) was readmitted to hospital five weeks post procedure with gram-negative septicaemia secondary to a urinary tract infection. As mentioned in his information summary he underwent 8 ablations and made an otherwise unremarkable postoperative recovery. He was a patient who was suffering from rheumatoid arthritis and was consequently taking immunosuppressant drugs which would have increased his susceptibility to sepsis. This man was also catheterised during his procedure and at his operation it was noted that the bladder was involved with either tumour or peri-tumoural inflammation and a cuff of bladder was removed. The combination of these factors may have increased the risk of urinary sepsis.

The other negative finding with the pilot study is the number of patients with subsequent tumour development either in the liver or at other locations. Only 1 of the first ten patients is completely tumour free at 3 years although it should be pointed out that her operation would not have been possible without the ability to ablate some of her lesions. Of the ten pilot patients six developed further tumours either within the liver or around the region of the initial colorectal resection a result in line with previous studies of alternative ablative technologies. Recurrences were not seen in the ablated regions even when large tumours had been ablated but in areas of liver previously not thought to contain tumour. The new microwave technology performed far more effectively than modalities technologies particularly when large tumours were treated. Clearly the microwave is not designed for use in tumours that are not

macroscopically visible and the technology itself cannot be criticised for inadequately treating these patients.

Many positives can be taken from this project, not least is the performance of the technology in the treatment of the liver metastases. The microwave equipment appeared to treat the lesions adequately without causing excessive damage to the liver parenchyma, hepatic vessels or biliary structures. The majority of patients were discharged from hospital following short in patient stays and no significant complications from the ablated tissue were noted in particular there were no abscesses associated with the ablations.

Following the procedures none of the patients exhibited signs of a systemic disturbance similar to that described following large ablations using cryotherapy. Certainly, almost all patients exhibited a transient temperature and a short lived (days) rise in hepatic transaminases thought to be the result of damage to hepatic tissue surrounding the regions of tumour ablation. All ablative techniques describe a transient rise in transaminases levels due to hepatic cell destruction. Little attention was paid to this in this project as many patients underwent concomitant liver resections (which also produce a rise in transaminases levels). The largest ablations saw rises of up to 12 times normal for AST and ALT within the first 48 hours post procedure but these levels had halved by day five. No disturbance in cardiac, respiratory or renal systems was noted either intra-operatively or following the surgery (except as described previously in the case of patient 3). No damage to adjacent structures or organs from the microwave ablations was noted in any of the patients.

In all cases which received postoperative cross sectional imaging at 6 weeks, no instances of incomplete tumour ablation was found. The lesions produced were spherical in appearance and sharply defined within the surrounding hepatic parenchyma mirroring the results from the previous animal studies. No detectable damage to vascular or biliary structures was noted either at the time of operation or on subsequent scans.

ii) Humanitarian treatments

The microwave equipment was used to treat many patients with very different pathology. In almost all cases the 2.45GHz microwave applicator was used to ablate the tumours. In terms of patient recovery and length of in-patient stay the results were very similar to that found in the pilot study and in all but a very few cases postoperative recovery was unremarkable. Those treated laparoscopically experienced a far shorter in patient stay (1-3 days) compared to patients in whom the treatment was performed at open surgery (shortest in patient stay 7 days).

Over 40 patients with more than 200 tumours have now been treated using the 2.45GHz microwave technology. Follow up of these patients is shorter than that of the pilot study and therefore only short-term data are available although similarly encouraging.

This thesis was designed to record the pre-clinical and clinical development of microwave ablation as a treatment for patients with unresectable liver tumours and

therefore detailed descriptions of the ongoing humanitarian trial is outside the scope of this work.

iii) Assessment of microwave technology

Overall the microwave technology has functioned in the predicted manner. Magnetrons, the source of the microwaves, are well known to be robust and reliable. Prior to this thesis however it was the applicators which were the relatively untested portion of the equipment. The vast majority of the safety and technical issues were addressed in the bench testing of the device. The single greatest advance was the change in design of the applicator from the 9.2GHz MEA applicator to the 2.45GHz hepatic interstitial applicator. Essentially, the radiating material was changed to a very robust ceramic that had recently been engineered by Southbank University. This allowed more efficient radiation of microwaves for a far greater period of time. Bench testing of altered MEA probes had lead to failures of the applicators with the radiating devices disintegrating with the intensely hostile environment created at the applicator/tissue interface (it should be remembered that a large proportion of the energy in a 9.2GHz applicator is dispensed very close to the applicator). The second big advance was the use of an intensely heat resistant bonding material to attach the cutting tip to the radiating ceramic.

The pilot study did however use modified 9.2GHz applicators to ablate hepatic colorectal metastases and other liver tumours. During the pilot study no failures of either the applicators or the microwave equipment were recorded. None of the applicators or other pieces of microwave equipment had developed faults or were

shown to fail in any way. The disadvantage of using the 9.2GHz applicators are highlighted when the ablation details of pilot study patient 6 are studied. The large lesion that was discovered and ablated in segment 8/4 required a considerable period of treatment and placement in more than one site to achieve a complete treatment. Lesions this size were too large to treat with this low power, high frequency system.

Microwave leakage, i.e. treatment of tissue other than the target was a concern of the LREC such that a microwave detector was used with each ablation. This piece of equipment is usually used to ensure microwave oven safety and that no leakage of energy is present before sale to the consumer and consequently it is very sensitive. Concern over microwave leakage is very pertinent as extraneous waves have the ability to heat and damage the aqueous humours of the eye impairing vision. Recently concerns have also been expressed over the safety of mobile telephones which use microwaves for communication (Lonn et al. 2004, Kundi et al. 2004). No leakage of waves was detected in any of the ablations. This was reassuring and in conjunction with the automatic trip switch built into the system (caused by the difference in the complex permittivity of air and tissue and the consequent increase in microwave reflection back into the system when radiated into the air) allowed the treatments to occur without exposing the surgical team to microwave radiation. The microwave equipment did cause damage in two patients but was really as a result of human error; the tip was left in contact with the skin after an ablation causing a mild burn, a small 1cm blister, in two cases.

The 2.45GHz applicator used in the Humanitarian work was also tested on ex-vivo tissue, and used for the majority of this thesis. A single failure of the applicator was

recorded during the animal work although this was attributed to fatigue in the casing of the ceramic which perished during a high power ablation. This casing has been superseded by designing the applicator without the need for such a component, no further applicator failures have been documented since.

The 2.45GHz system was able to ablate the large tumours suggested by the results from the animal trials and appeared to do so in a manner that was predictable and safe. The travelling wave tube (the microwave device which is able to emit a range of microwave frequencies prior to their amplification in terms of wattage) allows the reflected energy to be measured during a treatment. As predicted by the computer models very little of the energy radiated from the applicator was reflected; at no point during a treatment with up to 150 Watts was the reflected energy above 10 Watts. Compared to previously available applicators this is a far smaller percentage of energy that is wasted by the microwave system and accounts for the far superior performance in terms of ablative potential.

One of the disadvantages of the 2.45GHz system is that the radiating tip (the white area in Figure 15, Chapter 2 is quite long). The consequence of this is that the clinician needs to be treating a tumour of sufficient size to cover the length of the radiating element of the applicator. Smaller tumours do not cover the entire radiating tip which is a potential safety hazard of this system especially when higher wattages are being used.

iv) Lessons Learnt

One of the major lessons learned was the need for a skilled IOUS operator to aid identification of tumours and accurate placement of the applicator within a lesion. This was particularly so for the patients with colorectal liver metastases as by definition these patients had multiple deposits in the liver. Over half the patients who came to surgery had a greater number of metastases within the liver than was suggested by the cross sectional imaging beforehand. This has obvious ramifications not only in terms of curability but the type of procedure attempted.

Unsurprisingly, patients with pre-existing cardiac co-morbidity had a poorer outcome than those with no such pre-existing disease. Neither of the two patients in whom a cardiac cause of death was identified had any discernable complication induced by the microwave procedure itself. In both of these patients a combined colorectal and hepatic operation was performed. Subsequent examination of the literature revealed some evidence that the clinicians using cryotherapy for similar treatments had found that patients undergoing combined procedures had higher mortality and morbidity rates (Cheung and Morris 2000). Since the results of the Pilot Study were examined no combined hepatic and colorectal procedures have been performed with a consequent reduction in perioperative morbidity.

The vast majority of patients have tolerated microwave ablation of their liver tumours very well with no obvious deleterious systemic effect. Most patients however developed a pyrexia during the postoperative phase and this was attributed to the effect of the presence of volumes of dead or dying tumour/liver within the ablated regions. The resections margins of liver that was excised surgically could also have

contributed to this. No infective organism was cultured from specimens taken and the pyrexia settled spontaneously after a few days.

All patients treated exhibited a rise in serum transaminases used in routine liver function assessment. This rise was transient and settled to normal limits within a few days. Again this effect was attributed to the effect of the treatment on normal liver cells affected by the treatment.

v) Future Objectives for human treatments

The microwave had shown itself to have potential in the field of hepatic tumour ablation. What is required is treatment of patients with longer term follow up for the role of microwave ablation to become further defined. A number of different centres within the UK have now begun using the microwave with the aim of evaluating its performance upon their patients. Future possible roles for the microwave are discussed more fully in the following chapter.

vi) Problems with trials of ablation in liver surgery

The field of liver surgery has expanded markedly in recent years with more patients than ever before being treated for liver tumours. The advent of ablation has further increased patient numbers undergoing hepatic procedures although no consensus on the most efficacious exists. No randomised data exists investigating the outcome of chemotherapy versus chemotherapy and ablation. Much of the difficulty in constructing such trials is that numbers of patients within each liver unit are

comparatively small and there is no widespread agreement on what is a potentially resectable distribution of metastases and what is not. This makes design of multicentre trials difficult if the baseline parameters are not agreed.

Chemotherapy is another confounding feature for work such as this with new agents such as Oxaliplatin and the as yet unlicensed Angiogenesis inhibitor used in patient 2 of the humanitarian study are being more widely used. Again the timing of the use of such agents is not uniform with some patients receiving them and some not. Certainly the patients outlined in the above studies are not uniform in terms of their treatment and it is therefore difficult to assess the impact of ablation on survival.

8.6 CONCLUSIONS

The microwave system developed by this group has shown itself to work in a predictable manner in ex-vivo and in-vivo animal experiments. The data obtained from this work has been utilised to allow the treatment of patients with hitherto incurable liver metastases. This new system has behaved in a manner that was entirely predicted by the previous experiments. Although follow up is relatively short the results from this work indicate that this technique is likely to have a role to play in the treatment of hepatic metastases. With advances in chemotherapy ablation may become the preferred choice of treatment for patients with liver tumours particularly if this can be achieved using minimally invasive techniques.

CHAPTER 9

DISCUSSION OF CURRENT WORK, FUTURE RESEARCH NECESSARY AND OTHER POSSIBLE APPLICATIONS OF MICROWAVE TISSUE ABLATION (MTA)

9.1 DISCUSSION OF WORK COMPLETED

i) **Animal modelling and lesion predictability**

ii) **Histological predictability**

iii) **Electromagnetic characterisation of liver parenchyma and colorectal metastases and the effect of treatment on these characteristics**

iv) **Real time imaging of microwave ablation**

v) **Human treatments**

9.2 FUTURE RESEARCH NECESSARY

i) **Microwave equipment**

ii) **Real-time imaging of microwave lesions**

iii) **Systemic effects of MTA**

iv) **Human treatments**

9.3 OTHER POSSIBILE APPLICATIONS FOR MICROWAVE TISSUE ABLATION

i) **Introduction**

ii) **Colorectal cancer**

iii) **Oesophageal cancer**

iv) **Varicose veins**

v) **Psoriasis**

9.4 CONCLUSION

9.1 DISCUSSION OF WORK COMPLETED

i) Animal modelling and lesion predictability

The use of living models for this work was necessary and very useful in the context of working this technique up into a viable treatment for patients with hitherto incurable liver malignancies. Both models used allowed the predictability of the treatment to be assessed both in terms of reproducibility when conditions were repeated and when a parameter (in this case power) was changed to produce a dose response curve. The small and large animal models showed that the lesions produced when treatment parameters were identical were very similar (particularly in the small animal model). The porcine group allowed the predictability of lesions induced by differing powers to be investigated. A dose response curve was produced- something that was not possible with previous microwave equipment.

The models further allowed predictability in terms of response to treatment to be ascertained and how this response evolved with time. Animals generally recovered very quickly from the operative and ablative insult with no long term sequelae demonstrated in either group. Animals that underwent multiple ablations (mimicking the likely use of the technique in humans with colorectal metastases) showed no increased morbidity or mortality.

ii) Histological predictability

The large and small animal models along with the "ablate and resect" human patients revealed a pattern of histological features common to all three species investigated. The method with which the ablated liver was surrounded by inflammatory cells and subsequently contained by fibrotic tissue occurred in a similar method in both groups It has so far not been possible to obtain tissue from the human subjects who have undergone ablative treatment. A second important point in terms of histological predictability was the central lesion produced by the treatment. Alternative ablative modalities require multiple treatments to induce large volume ablations and the poor results associated with such a methodology are likely to be the result of islands of viable, untreated cells. It was important to show this new microwave system induced complete cell death in the target area in even the very large ablations. In none of the 500 or so slides examined no viable cells were identified until the fibrous or inflammatory boundary was reached- implying that the ablated volume would be a completely dead region following treatment.

Histological investigation of kidney, lung, spleen and portions of liver parenchyma distant to the ablated region did not show any morphological damage or evidence of increased inflammatory infiltrates in any of the animal groups investigated. This is important when one considers the inflammatory infiltrates seen in the lungs particularly in animal models of cryoshock (Chapman et al. 2000).

iii) Electromagnetic characterisation of liver parenchyma and colorectal metastases and the effect of treatment on these characteristics

The work carried out to characterise the electromagnetic characteristics of liver parenchyma and colorectal liver metastases was something that had been attempted previously but not in the in-vivo setting. The data obtained from this was invaluable in determining whether previous poor performance by microwave ablation devices was due to a large difference in the electromagnetic characteristics of the two tissues or from physical or design- induced, characteristics of the microwave applicators. Results from this work were to allow accurate modelling parameters for the field produced by different ceramics and applicator configurations without the need to build prototypes of the newer generation of microwave applicators from Bath University. Lastly, the data from this allowed an estimation of the applicator performance throughout the treatment cycle to be made and to confirm the proposed correlation with tissue water content.

The data obtained from these experiments was likely to be very accurate as calibration of the equipment against known standards was performed prior to each set of readings taken. The equipment was also tested by determining the complex permittivity against a known standard and only when an accurate set of readings were obtained was the measuring probe used to take readings from the tissue. The graphs in Chapter 5 show how very accurate this equipment was.

Data from the tissue samples was very reproducible, showing little variation although a distinct but relatively small difference was detected between the two tissue types. The effect of this was that the poor performance of previous microwave applicators could not be ascribed to the difference in complex permittivity between the two tissue types but to the applicator design. It also allowed a large degree of confidence in that the performance of the applicators in colorectal liver metastases would be very similar to that measured in normal liver parenchyma, and that the dose response curves generated in the porcine model would be broadly applicable to human treatments. This indeed appeared to be so when the lesions produced in patients were measured using cross sectional imaging.

The electromagnetic work also investigated the effect of treatment on the complex permittivity of the target tissue and subsequently the new system's ability to radiate microwaves efficiently into the target tissue. The work showed that microwave ablation caused a significant change in the complex permittivity of the target tissue and that the change induced was proportional to the distance from the applicator. The Physicists at Bath University also used the data to investigate the effect the changes in permittivity had on the performance of the 2.45 GHz applicator recently designed.

iv) Real-time imaging

The ability to measure the real-time effects of an ablative device would be very useful. Cryotherapy produces a very sharply defined edge to the iceball and this can be viewed with high accuracy during intra-operative ultrasound monitoring. Unfortunately, cryotherapy is associated with very poor outcomes for moderate to

large tumours in terms of completeness of ablation. Although it has been reported that the edge of the iceball overlaps the tumour by 0.5- 1cm, the results in terms of tumour recurrence have been poor (Seifert and Morris 1999).

The microwave system investigated two forms of real-time monitoring: intra-operative ultrasound (IOUS) and temperature monitoring at intervals from the applicator. The IOUS was a very useful tool for monitoring of the ablation and was able to predict the lesion size with a reasonably good degree of accuracy. In addition to its use in real-time imaging IOUS was an invaluable tool for lesion detection (as a number of occult lesions were found in a number of patients) at the time of operation as well as applicator placement within the tumour.

Real-time temperature monitoring was not as successful at predicting lesion size and it was not very reproducible in the animal model. It is likely that this was due to sporadically poor contact between the tissue and the thermocouples. Temperature monitoring has developed into a useful tool for human ablations as a "flying lead" is now used. Previous suggestions have mentioned that 50-55°C is a cytotoxic temperature if the cell remains at this temperature for a few seconds (Dong et al.1998). During treatment ablations the "flying lead" which is a thermocouple linked to a digital temperature recorder is placed at the periphery of the tumour or ablation to monitor the temperatures at that site. This is a second way in which the ablation can be monitored and tumour destruction ensured. It is particularly useful if the tumour is irregular in morphology or at the periphery where heat losses from convection and radiation are at the greatest.

v) Human treatments

To date, the microwave equipment has been used in over 50 patients in the UK and Malaysia with encouraging results. Due to the relatively short time that microwave ablation has been used as a therapeutic tool no long-term results are yet available. The pilot study highlighted the fact that the microwave can be used to treat multiple metastases without any obvious deleterious effect to the patient. The majority of patients had a relatively short in-patient stay which was comparable to that following a liver resection. Three patients have undergone a laparoscopic treatment with an average post-operative stay of less than 2 days.

Patients underwent cross sectional imaging of the liver at between 4 and 6 weeks following the treatment. The images revealed areas of "punched out" liver where the ablation had taken place. None of these enhanced following the addition of an intra-venous contrast indicating that no detectable blood flow was entering these regions and that any tissue there was dead. None of these early CT scans gave any indication that any of the lesions had been incompletely treated. This was very significant particularly as some of the lesions treated were large (>6cm in diameter).

None of the pilot study or any of the patients treated on humanitarian grounds (as the latter did not fit the criteria for the pilot study) showed evidence of a systemic effect except one patient in whom a large HCC in a cirrhotic liver was treated. She developed some breathlessness post-operatively which required diuretic therapy.

Some of the pilot study patients died in the postoperative period although these patients were known cardiopaths with ischaemic heart disease. Post mortem examination confirmed that they had died of myocardial infarctions which had been suspected clinically. The vast majority of patients in whom adverse events were recognised were either known to have moderate to severe pre-existing morbidity and /or had a combined colonic resection and hepatic ablation +/- resection. It has been reported that in colorectal cancer (treated with cryotherapy) patients who underwent excision of the primary at the same time as the hepatic ablation had a higher morbidity and mortality rate than those with staged interventions (Cheung and Morris 2000). The use of staged interventions (i.e. hepatic treatment at a separate time from the colorectal excision) along with the exclusion of patients with very significant cardiac morbidity and the mandatory use of IOUS for detection of occult lesions were significant learning points during this work. Overall, microwave treatment of unresectable liver tumours has met with encouraging short-term results.

9.2 FUTURE RESEARCH NECESSARY

i) Microwave equipment

The current 2.45GHz applicator has a diameter of 6.7mm and therefore cannot be thought of as a potentially percutaneous ablation device. Microwave ablation is likely to become an increasingly attractive method for treating liver metastases if equipment allows the full range of open, laparoscopic and percutaneous treatments. Work has already been completed on a 5mm applicator (ideal for use through a 5mm laparoscopic port) although the much more difficult task of designing an applicator

with a diameter suitable for percutaneous work is underway. Much of the problem with narrower applicators is the ability of the cable to deliver enough energy without itself being heated and the fact that the applicator is likely to be much more brittle than its predecessors. The physical difficulties of producing a microwave applicator 2.5mm in diameter with good ablative potential in terms of ablation volume are considerable.

Further work is needed with regard to the changes in complex permittivity of the target tissue during a treatment. As mentioned in Chapter 6, the work presented in this thesis shows the effect after a treatment on the complex permittivity of tissue. This is only a guide to the effect of the treatment as it is taken when the tissue has cooled to room temperature and is therefore a static measurement. What is of great importance is the real-time change in the electromagnetic properties of the tissue as the treatment progresses as this affects the radiating efficiency of the device. This knowledge is likely to enable better designs to be developed particularly when every small increase in efficiency is essential (i.e. the development of limited diameter/percutaneous applicators).

ii) Systemic effects of MTA

Chapter 1 mentions a syndrome which has been termed "Cryoshock". This describes a condition in which a patient suffers a fatal exaggeration in the inflammatory response induced following a large volume hepatic tumour ablation with cryoablation. Levels of inflammatory markers have been greatly raised in patients undergoing cryoablation leading to the suspicion that this is an exaggerated SIRS-type response (Seifert and

Morris 1999, Weaver et al. 1995) Until the development of this new microwave equipment, it has not really been possible to induce large volume ablations within the liver without using cryotherapy ablation. It is unclear therefore that although an equivalent syndrome to cryoshock has not been described following a thermal treatment, this may just be a consequence of the smaller ablation volumes possible with previous equipment. The newly designed microwave equipment is now capable of producing volumes of ablation equivalent or greater than that possible with cryoablation although it is unknown whether a fatal inflammatory response would be induced. What is also not known is the extent to which microwave ablation induces the inflammatory system and whether it is volume dependent and directly proportional to the volume of tissue ablated.

Work is now in progress in Leicester as a follow up to this thesis in which the systemic effect of ablation induced by a number of different ablative techniques (and surgical resection) is investigated, compared and contrasted. The effect on different organs will be investigated using histology, immunohistochemistry and cytokine assays following different volumes of hepatic ablation in a rat model. The cytokine release in humans will be assessed and the relationship between volume of ablation and inflammatory cytokine induction investigated. It would be very interesting to reveal whether the grossly elevated levels of inflammatory cytokines seen even with relatively small volumes of cryoablation are reproduced in microwave treatments.

iii) Human treatments

The area in which the microwave ablation has the greatest potential is in the treatment of human patients who are currently considered resectable. This would however also be the most controversial. Liver resection is currently the only potentially curative option for the treatment of patients with malignant liver tumour/s (mainly colorectal metastases in the UK). With improving standards of surgery, anatomical knowledge of the liver, anaesthesia and post-operative care, 30-day mortality is low and for a single colorectal metastasis 5-year survival is in the order of 60% (Fong et al 1999). Exposure of the liver has changed little in this time and still requires a long transverse incision across the entire upper abdomen (a "rooftop" incision) often requiring extension superiorly into the xiphisternum. This incision causes considerable post-operative pain and usually requires epidural analgesia. The operation is followed by a stay in the High Dependency Unit and usually 10 days to 2 weeks in hospital.

Ablative treatments, however, may shortly challenge the philosophy of liver surgery for "operable disease" particularly as they may be administered via laparoscopic or even percutaneous means. Traditionally, ablative techniques have lacked the ability to deliver treatment volumes great enough to treat all but the smallest metastases. Treatment of larger tumours, using any of the many different ablative modalities have been associated with consistently poor outcomes, particularly, with regard to incomplete ablation. The results from the microwave work are encouraging especially with respect to the treatment of larger metastases. None of the larger metastases (defined by ourselves as 4cm in diameter or greater) have suffered from an incomplete ablation as indicated by subsequent cross sectional imaging.

To date the MTA equipment has been used in a laparoscopic setting very successfullyin 3 patients. Results obtained from the patients with larger tumours are very encouraging and it is a very appealing prospect to have the ability to treat these lesions quickly, effectively and safely. The potential for percutaneous or laparoscopic treatment of liver metastases in patients with operable disease has many potential advantages not least being the avoidance of the rooftop incision, the relatively long postoperative hospital stay and attendant cost implications. The treatment of operable tumours in this way is very controversial and is likely to be met with considerable resistance from surgeons as formal resection is considered the gold standard. With the advent of laparoscopic ultrasound, now used routinely in many hospitals to assess the operability of liver metastases, one would have the ability to ensure as much as is possible that no lesions would be missed before ablating the disease in addition to providing a method to monitor the ablation process in real-time. Whilst this technique is plausible the benefits of such a technique would have to be assessed using a randomised controlled trial. It is likely that such work would have to be performed in a number of centres to acquire satisfactory patient numbers.

9.3 OTHER POSSIBILE APPLICATIONS FOR MICROWAVE TISSUE ABLATION

i) Introduction

Microwave treatment particularly in an ablative or tissue destructive sense has very attractive theoretical advantages over other ablation devices. Firstly, there is a high energy exchange between microwaves and water molecules throughout the entire

microwave frequency range. The strength of this interaction allows very efficient transfer of energy from that associated with an electromagnetic wave into molecular kinetic energy which is expressed as heat (see Chapter 2). Water constitutes a very high proportion of the majority of soft tissues and as shown in Chapter 5 certainly some cancers. This again implies microwave treatment would be useful in the treatment of soft tissues. Using high frequency systems, microwave energy would be rapidly absorbed or "soaked up" by the tissue to be treated, causing relatively intense heating of the target tissue without collateral damage.

The second advantage of microwave energy in this context is its flexibility in terms of tissue penetration. The obvious example from this thesis is demonstrated in Chapter 5 in which the tissue penetration of microwaves in liver parenchyma and colorectal metastases was investigated over a range of microwave frequencies. This work demonstrated that in this particular tissue low frequencies of 2.45GHz had a skin depth which was almost five times as great as that of the higher frequency of 9.2GHz (22mm compared to 5mm respectively). Flexibility in tissue penetration has obvious advantages as the microwave frequency can be adjusted according to the desired penetration required. This particular aspect is described in further detail in the following section.

A further advantage of microwave treatment for ablation is the use of special microwave radiating ceramics. These ceramics are almost loss free i.e. almost none of the wave's energy is lost when radiated through the material similar to that for electromagnetic waves of the visible spectrum passing through a piece of glass. A second role for the ceramics is to provide a "match" to the tissue into which the

microwaves are to be radiated. Although no data exists as to the electromagnetic properties of colorectal cancer it is likely that the water content is likely to be similar to that of normal colon or colorectal liver metastases and considerably different to air. If the microwave device was not in contact with the target tissue then the match between the ceramic and the medium into which the microwaves were being radiated would be far from optimum causing increased reflection of energy. This reflected microwave energy can be detected and measured. This system is in use already in the microwave generators available from Microsulis PLC and is used as a safety device to prevent radiation of microwaves into air. In the subsequently proposed scenarios for which microwave treatment may have a role to play this would also be a useful mechanism to ensure contact between the applicator and the target tissue.

ii) Colorectal cancer

Colorectal cancer is a very common disease both in the UK and worldwide. The incidence has been increasing year on year since the 1970s with approximately one million cases being diagnosed yearly (Boyle 1997). In the UK and Western world in general an increasingly large number of elderly or infirm patients are being diagnosed with the condition. Surgery is the only curative treatment but in a number of cases patients are clinically unsuitable or unwilling to undergo a formal laparotomy and resection of the affected portion of colon or rectum. It is in this group that microwave treatment of the colorectal primary may become a useful therapeutic measure.

Obstruction of the colon or rectum by the primary remains a problem for untreated colorectal cancers thereby requiring operative resection or stenting. Stenting is often not an option in a number of tumours especially if they are not stenosing, relatively soft or friable. Stenting is currently expensive and requires the expertise of a number of different specialists. A form of local control for the colorectal primary, whilst not curative, may be useful to prevent obstruction and unpleasant symptoms such as diarrhoea and rectal bleeding. Such techniques are available for the rectum in the form of TART (Trans-Anal Resection of Tumour) but local treatment of colonic neoplasms is more difficult.

Currently, ablation of colonic primaries is carried out using argon lasers delivered via an endoscope. Argon lasers have similar problems to those documented in Chapter 1 i.e. that tissue penetration, powers and destructive effect are all rather low Muralidharan et al. 2001, Germer et al 1998, Gillams et al. 2000, Vogl et al. 1999). A safe and effective system in which energy could be delivered to the target tissue without penetrating the bowel wall would be a useful tool. Microwave ablation of colonic primaries is distinctly possible and has been attempted in the rectum previously (Oshiumi et al. 1984, Li et al. 1984) Microwave energy could be conveyed to an endoscopically delivered ablation device easily as all that is required would be a piece of coaxial cable-something that is considerably more robust that the optical fibres required to deliver laser energy. A suitable dielectric would used to delivered a field of microwaves probably is a straight line out from the applicator so the user could "paint" the area to be treated. A microwave device such as this would be useful for the treatment of bleeding tumours or possibly other conditions such as angiodysplasia, sessile polyps or even bleeding duodenal ulcers.

For this technique to become a reality the electromagnetic properties of colorectal cancer would have to be measured. In a similar manner to that described in this thesis, the complex permittivity of colon, rectum and the carcinomas could be measured. Using this data along with the physical constraints of an applicator that would be required to fit within an endoscope, a finite element modelling computer program could dictate the most suitable ceramics and the subsequent field produced. Using the data gained from this, prototype applicators could be built and tested.

iii) Oesophageal Cancer

Carcinoma of the oesophagus is another example of a common and often difficult disease to treat. Patients commonly present with dysphagia although the disease is commonly well advanced, and often incurable, before the patient presents due to the remarkable capacity of the oesophagus to propel food boluses into the stomach (Nash and Gerdes 2002). Dysphagia is a very unpleasant symptom and currently patients considered incurable undergo balloon dilatation of the tumour along with stent insertions. Balloon dilatation is a method that requires repeated sessions and is unpleasant for the patient (Metcalfe and Steger 2004. Stents are also prone to displacement and become blocked with food debris (Metcalfe and Steger 2004). Endoscopically delivered microwave ablation treatments may allow more prolonged and effective relief from the symptoms minimising the risk of perforating the oesophagus. The electromagnetic properties of oesophagus and its carcinoma would need to be obtained to allow design of the optimum performing applicators.

iv) Varicose Veins

Varicose veins are distended, tortuous and lengthened veins most commonly found in the lower limbs and affect the great and short saphenous systems. This condition affects approximately 40% of the population, and in addition to being unsightly, varicose veins have the potential to cause problems which include phlebitis, cutaneous ulceration, lipodermatosclerosis and haemorrhage. Standard treatment for varicose veins has been surgical excision or ligation of a defective valve although this has been limited by recent guidelines from the National Institute for Clinical Excellence. Surgery involves the stripping of the vein to the level of the knee and avulsion of the veins below that point. Many patients have severe pain following the treatment and are uncomfortable for weeks.

Minimally invasive treatments for varicose veins are in their infancy and again microwave coagulation would be an attractive alternative to surgery. Research into this treatment is ongoing with a microwave applicator being inserted into the great saphenous vein at the ankle and passed up to the level of the sapheno-femoral junction in the upper thigh. The microwaves are then emitted from the tip of the applicator causing endothelial damage to the vein eventually causing it to sclerose.

v) Psoriasis

This is chronic, remitting condition of the skin affecting approximately 1% of the population of the UK in which scaly, red or silvery patches form on the elbows, forearms, knees, legs and scalp. The condition is characterised by an overgrowth of

the epidermis with a shortening of the cell cycle time and an increase in the number of proliferative cells. Current treatments seek to destroy the abnormally proliferative epidermal cells either by topical creams, drugs or ultraviolet light. Microwave therapy using an applicator emitting waves with a high frequency and therefore very limited tissue penetration may be a useful intervention in controlling the scaly regions. Using a high frequency system energy would be deposited into the epidermal layer without burning the deeper strata of the skin. Hyperthermia at between 42 and 45°C causes a slow-down in cell replication. The aim of the microwaves is to induce these therapeutic temperatures in a targeted way, ie just the epidermis, with the aim of slowing the replication. This application is currently under development by the Medical Devices Technology group at the University of Bath.

9.4 CONCLUSION

The aim of this thesis was to investigate the use of a new generation of microwave equipment and to assess whether the lesions produced were predictable in terms of their size, their effect on the organism and their histological characteristics and whether the ablated lesions could be imaged in real-time. Furthermore, part of this work was to examine whether this system could be applied as a therapeutic procedure for patients with unresectable liver metastases.

Work from the animal models indicated that microwave ablation is a very predictable technique and that lesion diameter, and therefore volume, can be accurately predicted. Using certain parameters of power/time a dose response curve was formulated. Furthermore, lesions were produced rapidly and were well tolerated by all organisms

into which they were placed. The volume of ablation induced was far in excess of alternative treatments indicating that some tumours may be ablated following a single insertion of the applicator.

Histologically, the induced ablations followed a pattern of being surrounded by inflammatory cells subsequent formation of a fibrous capsule. All the cells within the ablated volume exhibited morphological indications of cell death with no islands of viable cells being found. Importantly, no histological features of systemic damage were noted in any of the organs or portions of distant liver harvested.

Almost all the experiments performed using the microwave were done so using normal liver parenchyma and so it was unclear whether the results obtained would be applicable to human colorectal liver metastases. Chapters 5 and 6 examined the differences in the electromagnetic environment (known as the complex permittivity of a tissue) of normal liver parenchyma and colorectal metastases in the in-vivo situation. Little intra-tissue variation in complex permittivity was found in both normal and tumour tissue. However, a small but highly statistically significant difference was noted between the complex permittivities of two tissue types. This difference was attributed to the water content of the tissues and although highly significant statistically the actual effect in terms of lesion generation was found to be small. In clinical terms there would be little discernable difference in treating normal liver or colorectal metastases in terms of lesion generation. Lastly, it was proven that design flaws in previous microwave equipment was responsible for the poor performance of these applicators and that even with the change in the electromagnetic

environment induced by treatment this new generation of equipment continued to perform well.

The IOUS system highlighted the fact that the microwave system could be imaged in real-time although results were not so impressive using continuous thermal monitoring. IOUS was a useful in predicting lesion size following an ablation whereas thermal monitoring was useful to ensure cell death would occur at a certain distance from the applicator.

The microwave system was used for the treatment of patients with unresectable tumours with encouraging short-term results. This novel microwave system appears to have considerable potential in terms of ablation for hepatic tumours and may go on to be a very viable alternative to resection of operable tumours. A number of other areas appear to lend themselves very readily to treatment using microwave based technology.

BIBLIOGRAPHY

Abbott JA, Garry R. The surgical management of menorrhagia. Hum Reprod Update. 2002 Jan-Feb;8(1):68-78.

Armour FR, Babineau T, Bleday R, Seele G. Laparoscopy/thoracoscopy for staging: 1. Staging endoscopy in surgical oncology. *Semin Surg Oncol* 1993; **9**: 51-55

Bagia JS, Perera DS, Morris DL. Renal impairment in hepatic cryotherapy. *Cryobiology*. 1998 Jun; **36** (4):263-7.

Bini MG. The Polyacrylamide as a Phantom Material for Electromagnetic Hyperthermia Studies. *IEEE Transactions on Biomedical Engineering* 1984; **BME-31**: 317-22.

Blackwell TS, Debelak JP, Venkatakrishnan A, Schot DJ, Harley DH, Pinson CW et al. Acute lung injury after hepatic cryoablation: correlation with NF-kappa B activation and cytokine production. *Surgery* 1999; **126**: 518-26.

Boon ME, Kok LP. *Microwave cookbook for pathology.* 1989, 3rd Edn., Leiden Colomb Press.

Boyle P. Global Burden of Cancer. *Lancet* 1997; **353**; Suppl. II 23-26.

Buckley F, Maryott A. Tables of dielectric dispersion data for pure liquids and dilute solutions. *National Bureau of Standards*1958, Circular 589 . 11/.

Burdio F, Guemes A, Burdio JM, Navarro A, Sousa R, Castiella T, Cruz I, Burzaco O, Lozano R. Bipolar saline-enhanced electrode for radiofrequency ablation: results of experimental study of in vivo porcine liver. *Radiology.* 2003; **229** (2): 447-56.

Buscarini L, Rossi S. Technology for radiofrequency thermal ablation of liver tumours. *Sem Laparosc Surg* 1997; **4**, 96-101.

Cha CH, Lee FT Jr, Gurney JM, Markhardt BK, Warner TF, Kelcz F *et al.* CT versus sonography for monitoring radiofrequency ablation in a porcine liver. *AJR* 2000; **175**: 705-11.

Chang CK, Hendy MP, Smith JM, Recht MH, Welling RE. Radiofrequency ablation of the porcine liver with complete hepatic vascular occlusion. *Ann Surg Oncol.* 2002; **9** (6): 594-8.

Chapman WC, Debelak JP, Blackwell TS, Gainer KA, Christman JW, Pinson CW *et al.* Hepatic cryoablation-induced acute lung injury: pulmonary hemodynamic and permeability effects in a sheep model. *Arch Surg* 2000; **135**: 667-72.

Charnley RM, Doran J, Morris DL. Cryotherapy for liver metastases: a new approach. *Br J Surg* 1989; **76**: 1040-1.

Chen HH, Cooper A, Taylor I, Johnson CD. Effect of a microwave coagulator on implanted liver neoplasms in rats. *Dig Surg* 1999; **16**: 140-4.

Cheung D, Morris DL. Synchronous hepatic cryotherapy and resection of colonic primary is a high risk procedure. *HPB Surg*. 2000; **11** (6): 379-82.

Chinn SB, Lee FT Jr, Kennedy GD, Chinn C, Johnson CD, Winter TC 3rd, Warner TF, Mahvi DM. Effect of vascular occlusion on radiofrequency ablation of the liver: results in a porcine model. *AJR Am J Roentgenol.* 2001; **176** (3): 789-95.

Colpitts B. Temperature sensitivity of coaxial probe complex permittivity measurements: experimental approach. IEEE Trasactions on Microwave Theory and Techniques 1993; **41** [2], 229-233. 2/.

Cooper KG, Bain C, Lawrie L, Parkin DE. A randomized comparison of microwave endometrial ablation with transcervical resection of endometrium; follow up at a minimum of five years. *BJOG* 2005; **112** (4): 470-5.

Cozzi PJ, Stewart GJ, Morris DL. Thrombocytopenia after hepatic cryotherapy for colorectal metastases: correlates with hepatocellular injury. *World J Surg* 1994; **18**: 774-6.

Culling CFA. *Handbook of histological techniques.* 1963; London: Butterworths publishing, p244.

Curley SA, Izzo F, Ellis LM, Nicolas VJ, Vallone P. Radiofrequency ablation of hepatocellular cancer in 110 patients with cirrhosis. *Ann Surg* 2000; **232**: 381-91.

Dachman AH, McGehee JA, Beam TE, Burris JA, Powell DA. US-guided percutaneous laser ablation of liver tissue in a chronic pig model. *Radiology.* 1990; **176** (1): 129-33.

Dodd GD, Soulen MC, Kane RA, Livraghi T, Lees,W.R, Yamashita Y *et al.* Minimally invasive treatment of malignant hepatic tumors: at the threshold of a major breakthrough. *Radiographics* 2000; **20**: 9-27.

Dong BW, Liang P, Yu XL, Zeng XQ, Wang PJ, Su L *et al.* Sonographically guided microwave coagulation treatment of liver cancer: an experimental and clinical study. *AJR* 1998; **171**: 449-54.

Downes E, O'Donovan P. Microwave endometrial ablation in the management of menorrhagia: current status. *Curr Opin Obstet Gynecol.* 2000; **12** (4): 293-6.

Duck F. Physical properties of tissue. Academic Press, London . 1990.

Eggstein S, Neeff H, Szarzynski M, Jungraithmayr W, Haberstroh J, Kirste G, Schmitt-Graeff A, Farthmann EH. Hepatic cryotherapy involving the vena cava. Experimental study in a pig liver model. *Eur Surg Res.* 2003 Mar-Apr; **35** (2): 67-74.

Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer, analysis of 1001 consecutive cases. *Ann Surg* 1999; **230**, 309-321.

Geoghegan JG, Scheele J. Treatment of colorectal liver metastases. [Review] [94 refs]. *Br J Surg* 1999; **86**: 158-69.

Germer CT, Albrecht D, Roggan A, Isbert C, Buhr HJ. Experimental study of laparoscopic laser-induced thermotherapy for liver tumours. *Brit J Surg* 1997; **84**: 317-20.

Germer CT, Roggan A Ritz JP et al. Optical properties of native and coagulated human liver tissue and metastasis in the near infra red spectrum. *Lasers Surg Med* 1998; **23**, 194-203.

Gillams AR, Lees WR. Survival after percutaneous, image guided, thermal ablation of hepatic metastases from colorectal cancer. *Dis Colon Rectum* 2000; **43**, 656-661.

Goldberg SN, Hahn PF, Halpern EF, Fogle RM, Gazelle GS. Radio-frequency tissue ablation: effect of pharmacologic modulation of blood flow on coagulation diameter. *Radiology.* 1998; **209** (3): 761-7.

Harada T, Kigure T, Kumazaki T, Etori K, Satoh Y, Tsuchida S. Intraoperative real-time ultrasonic scanning for microwave coagulation of the prostate. *Urologic Radiol* 1990; **12**, 45-49.

Henne-Bruns D, Vogel I, Schroder S, Schreiber HW, Kremer B. Resektion von Lebermetastasen colorectaler carcinome. Chirurg 1993; 64: 283-289.

Heriot AG, Reynolds J, Marks CG, Karanjia N. Hepatic resection for colorectal metastases- a national perspective. *Ann R Coll Engl.* 2004; 86 (6): 420-4.

Hodgson DA, Feldberg IB, Sharp N, Cronin N, Evans M Hirschowitz L. Microwave endometrial ablation: development, clinical trials and outcomes at three years. Br J Obstet Gynaecol. 1999 Jul;**106** (7):684-94.

Jiao LR. Percutaneous radiofrequency thermal ablation for liver tumours. *Lancet* 1999; **354**: 427-8.

Kato T, Sato Y, Hamazoe R. Effects of microwave tissue coagulation on the livers of normal rabbits; a comparison of findings of image and analysis and histopathological examination. *Br J Radiol* 1996; **69**: 515-521.

Kundi M, Mild K, Hardell L, Mattsson MO. Mobile telephone and cancer- a review of epidemiological evidence. *J Toxicol Environ Health B Crit Rev.* 2004; **7** (5): 351-84.

Lam CM, Shimi SM, Cuschieri A. Ultrasonographic characterization of hepatic cryolesions. An ex vivo study. *Arch Surg* 1995; **130**: 1068-72.

Land D, Campbell A. A quick accurate method for measuring the microwave dielectric properties of small tissue samples. *Physics in Medicine & Biology* 1992; **37** (1): 183-192.

Leong AS-Y, Daynon ME, Milios J, Microwave irradiation as a form of fixation for light and electron microscopy. J. Pathol; 146, 313-321.

Levitan N, Hughes KS. Management of non-resectable liver metastases from colorectal cancer. *Oncology* 1990; **4**, 77-84.

Li DJ, Luk KH, Jiang HB, Chou CK, Hwang GZ. Design and thermometry of an intracavitary microwave applicator suitable for treatment of some vaginal and rectal cancers. *Int J Radiat Oncol Biol Phys*. 1984; **10** (11): 2155-62.

Livraghi T, Goldberg SN, Monti F, Bizzini A, Lazzaroni S, Meloni F *et al.* Saline-enhanced radio-frequency tissue ablation in the treatment of liver metastases. *Radiology* 1997; **202**: 205-10.

Lonn S, Ahlbom A, Hall P, Feychting M. Mobile phone use and the risk of acoustic neuroma. *Epidemiology* 2004; **15** (6): 653-9.

Machi J, Isomoto H, Kurhiji T, et al. Accuracy of intraoperative ultrasound in diagnosing liver metastases from colorectal cancer: evaluation with post-operative follow-up results. *World Journal of Surgery* 1991; **15**: 551-557.

Matsukawa T, Yamashita Y, Arkawa A, Yoshimatsu S, Murakami R, Nishiharu T, Takahashi M. Percutaneous microwave coagulation therapy: An experimental study. *Radiation Med.* 1997; **15**, 217-222.

Metcalfe MJ, Steger AC, Leslie A. Benign complications of expandable metal stents used in the palliation of oesophageal carcinoma: two case reports. *Br J Radiol.* 2004; **77** (915): 245-7.

Milligan MP, Etokowo G, Kanumuru S, Mannifold N Microwave endometrial ablation: patients' experiences in the first 3 months following treatment. J Obstet Gynaecol. 2002; **22**(2):201-4.

Mizrahi SS, Jones JW Jr, Bentley FR. A facilitated technique for hepatectomy of porcine liver. *J Invest Surg* 1996; **9**: 393-8.

Mulier S, Ni Y, Miao Y, Rosiere A, Khoury A, Marchal G, Michel L. Size and geometry of hepatic radiofrequency lesions. *Eur J Surg Oncol.* 2003; **29** (10): 867-78.

Muralidharan V, Christophi C. Interstitial laser thermotherapy in the treatment of colorectal liver metastases. [Review] [143 refs]. *J Surg Oncol* 2001; **76**: 73-81.

Nash Cl, Gerdes H. Methods of palliation of oesophageal and gastric cancer. *Surg Oncol Clin N Am.* 2002; **11** (2): 459-83.

Nicoli N, Casaril A, Mangiante G, Ciola M, Hilal MA, Marchiori L. Surgical treatment for liver metastases from colorectal carcinoma: results of 228 patients. *Hepatogastroenterology* 2004; 51 (60): 1810-4.

Oshiumi Y, Chou T, Saku M, Murakami J, Kajiwara T, Yoshikai T. Intrarectal hyperthermia of circumferential rectal cancer. Report of two cases. *Radiat Med.* 1984; **2** (4): 265-9.

Paul MA, Mulder LS, Cuesta MA, Sikkenk AC, Lyesen GK, Meijer S. Impact of ultrasonography on treatment strategy for colorectal cancer. *Br J Surg.* 1994; **81**: 991-7.

Ramachandran GN, Ramakrishnan C. Molecular structure in: *Biochemistry of Collagen.* Ramachandran GN and Reddi AH., eds. 1976 New York: Plenum Publishers, 45-84.

Ravikumar TS, Kane R, Cady B, Jenkins RL, McDermott W, Onik G *et al.* Hepatic cryosurgery with intraoperative ultrasound monitoring for metastatic colon carcinoma. *Arch Surg* 1987; **122**: 403-9.

Ravikumar TS, Steele GJ, Kane R, King V. Experimental and clinical observations on hepatic cryosurgery for colorectal metastases. *Cancer Res* 1991; **51**: 6323-7.

Rivoire ML et al. Hepatic cryosurgery precision: evaluation of ultrasonography, thermometry, and impedancemetry in a pig model. *J Surg Oncol* 1996; **61**: 242-8.

Robertson GS, Wemyss-Holden SA, Dennison AR, Hall PM, Baxter P, Maddern GJ. Experimental study of electrolysis-induced hepatic necrosis. *Br J Surg* 1998; **85**: 1212-6.

Rossi S, Fornari F Buscarini L. Percutaneous ultra-sound guided radiofrequency electrocautery for the treatment of hepatocellular carcinoma. *J Intervent Radiol* 1993; **8**, 97-103.

Ruers TJM, Joosten J Jager GJ Wobbes T. Long-term results of treating hepatic colorectal metastases with cryosurgery. *Br J Surg* 2001; **88**, 844-849

Scheele J, Stangl R, Altendorf-Hofmann A, Gall FP. Indicators of prognosis after hepatic resection for colorectal secondaries. *Surgery* 1991; **110**, 13-29.

Schober R, Ulrich F, Sander T, Durselen H, Hessel S. Laser induced alteration of collagen substructure allows microsurgical tissue welding. *Science* 1986; **232**, 1421-1422.

Seifert JK, Junginger T, Morris DL. A collective review of the world literature on hepatic cryotherapy. [Review] [129 refs]. *J R Coll Surg Edinb* 1998; **43**: 141-54.

Seifert JK, Morris DL. World survey on the complications of hepatic and prostate cryotherapy. *World J Surg* 1999; **23**: 109-13.

Seki T, Wakabayashi M, Nakagawa T, Itho T, Shiro T, Kunieda K *et al.* Ultrasonically guided percutaneous microwave coagulation therapy for small hepatocellular carcinoma. *Cancer* 1994; **74**: 817-25.

Seki T, Wakabayashi M, Nakagawa T, Imamura M, Tamai T, Nishimura A *et al.* Percutaneous microwave coagulation therapy for solitary metastatic liver tumors from colorectal cancer: a pilot clinical study. *Am J Gastroenterol* 1999; **94**: 322-7.

Sharp NC, Cronin N, Feldberg I, Evans M, Hodgson D, Ellis S. Microwaves for menorrhagia: a new fast technique for endometrial ablation. *Lancet.* 1995; **346** (8981):1003-4.

Shibata T, Murakami T, Ogata N. Percutaneous microwave coagulation therapy for patients with primary and metastatic hepatic tumors during interruption of hepatic blood flow. *Cancer* 2000; **88**: 302-11.

Shibata T, Niinobu T, Ogata N, Takami M. Microwave coagulation therapy for multiple hepatic metastases from colorectal carcinoma. *Cancer* 2000; **89**: 276-84.

Shimada S, Hirota M, Beppu T, Matsuda T, Hayashi N, Tashima S *et al.* Complications and management of microwave coagulation therapy for primary and metastatic liver tumors. *Surg Today* 1998; **28**: 1130-7.

Soyer P, Levesque M, Elias D, Zeitoun G, Roche A. Preoperative assessment of respectability of hepatic metastases from colonic carcinoma: CT portography vs sonography and dynamic CT. *AJR* 1992; **159**: 741-744.

Stangl R, Altendorf-Hofmann A. Factors influencing the natural history of colorectal liver metastases. *Lancet* 1994; **343**, 1405-1410.

Stewart GJ, Preketes A, Horton M, Ross WB, Morris DL. Hepatic cryotherapy: double-freeze cycles achieve greater hepatocellular injury in man. *Cryobiology* 1995; **32**: 215-9

Strickland AD, Clegg PJ, Cronin NJ, Swift B, Festing M, West KP *et al.* An experimental study of large volume microwave ablation in the liver. *Brit J Surg* 2002; **89**, 1003-1008.

Tabuse K, Katsumi M. Application of a microwave tissue coagulator to hepatic surgery the hemostatic effects on spontaneous rupture of hepatoma and tumor necrosis. *Nippon Geka Hokan - Archiv fur Japanische Chirurgie* 1981; **50**: 571-9.

Tabuse K. Basic knowledge of a microwave tissue coagulator and its clinical applications. *J of HPB Surg* 1998; **5**: 165-72.

Takamura M, Murakima T, Shibata T, Takeshi I, Niinobu T, Kawata S *et al.* Microwave coagulation therapy with interruption of hepatic blood in-or outflow: An experimental study. *J Vasc Interv Radiol* 2001; **12**, 619-622.

Takeuchi H, Tamura R, Baba T, Kawashima T, Fukazawa T, Yunoki Y *et al.* Real-time evaluation of the effectiveness of microwave coagulation therapy for hepatocellular carcinoma using color Doppler imaging. *Acta Medica Okayama* 1998; **52**: 255-60.

Thomsen S, Pearce JA, Cheong WF. *IEEE Transactions Biomed Engineering* 1989; **36**, 1174-1175.

Tucker De Sanctis J, Goldberg SN, Mueller PR. Percutaneous treatment of hepatic neoplasms: A review of current techniques. *Cardiovasc Intervent Radiol* 1998; **21**: 273-96.

Vogl TJ, Mack MG, Muller PK, Straub R, Engelmann K, Eichler K. Interventional MR: interstitial therapy. [Review] [28 refs]. *Eur Radiol* 1999; **9**: 1479-87.

Wallage S, Cooper KG, Graham WJ, Parkin DE. A randomised trial comparing local versus general anaesthesia for microwave endometrial ablation. *BJOG*. 2003 Sep;**110** (9.):799-807.

Wang ZB, Wu F, Wang ZL, Zhang Z, Zou JZ, Liu C, Liu YG, Cheng G, Du YH, He ZC, Gu ML, Wang ZG, Feng R. Targeted damage effects of high intensity focused ultrasound (HIFU) on liver tissues of Guizhou Province miniswine. *Ultrason Sonochem*. 1997; **4** (2): 181-2.

Weaver ML, Atkinson D, Zemel R. Hepatic cryosurgery in treating colorectal metastases. *Cancer* 1995; **76**: 210-4

Wemyss-Holden SA, Robertson GS, Hall PD, Dennison AR, Maddern GJ. Electrolytic treatment of colorectal liver tumour deposits in a rat model: a technique with potential for patients with unresectable liver tumours. *Diges Dis* 2000; **18**: 50-7.

Wemyss-Holden SA, Dennison AR, Finch GJ, Hall Pd Pde L, Maddern GJ. Electrolytic ablation as an adjunct to liver resection: experimental studies of predictability and safety. *Br J Surg.* 2002; **89** (5): 579-85.

Yamaue H, Katsumi M, Tabuse K, Aoyama O, Noguchi H, Egawa H *et al.* [Experimental studies of the incidence of metastases following the microwave coagulation therapy for malignant tumor]. [Japanese]. *Nippon Geka Hokan - Archiv fur Japanische Chirurgie* 1984; **53**: 662-6.

Zacherl J, Scheuba C, Imhof M, Zacherl M, Langle F, Pokieser P et al. Current value of intraoperative sonography during surgery for hepatic neoplasms. *World J Surg.* 2002; **26** (5): 550-4.

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