The Future of Radiofrequency Ablation is Looking BETA

Short and Long Term Studies of Bimodal Electric Tissue Ablation (BETA) in a Porcine Model

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Thesis Abstract

Introduction

Radiofrequency ablation (RFA) is a popular method of treating unresectable liver tumours by the use of a high frequency, alternating electrical current that heats and destroys tumour cells. The size of the ablation is limited by localised charring of adjacent tissue that prevents further conduction of the radiofrequency current. In the clinical setting, this results in increased rates of local recurrence in tumours that are greater than 3 cm in diameter as multiple, overlapping ablations need to be performed to treat the one tumour.

To overcome this problem, a modified form of RFA called Bimodal Electric Tissue Ablation (BETA) has been created. BETA adds a direct electrical current to the alternating radiofrequency current, thus establishing its bimodal character. When direct currents are used in biological tissues, water is transferred from anode to cathode by a process called electro-osmosis. By attaching the cathode to the radiofrequency electrode, water is attracted to the area thus preventing tissue desiccation and charring.

The BETA circuit has been constructed and tested using a porcine model. The aims of the studies are to confirm that larger ablations can be produced with the BETA system and that it is safe to use in an animal model. Three studies have been performed to test these aims in porcine liver.

Methods

The first study was designed to compare sizes of the ablation produced between standard RFA and the BETA circuit. This was followed by a long-term study to assess associated changes to liver function and pathological changes within the liver as well as identifying

any other treatment related morbidity. The third study assessed the difference in ablation size and safety aspects when the positive electrode of the direct current circuitry was moved from small surface area under the skin to a large surface area on the skin.

Results

Ablations with significantly larger diameters are created with the BETA circuit using a multi-tine needle (49.55 mm versus 27.78 mm, $p<0.001$). This finding was confirmed in the third experiment using a straight needle (25 mm versus 15.33 mm, $p<0.001$). Ablations produced by the BETA circuit induce coagulative necrosis within the treated liver and the injury heals by fibrosis in a manner similar to other thermal therapies. Significant rises in some serum liver enzymes are seen within 24 hours of treatment but these return to normal within 4 days. An electrolytic type injury can be produced at the site of the positive electrode. By increasing the surface area of this electrode, the risk of tissue damage is decreased but ablations are significantly smaller (18 mm versus 25 mm, $p<0.001$).

Conclusions

The BETA circuit consistently produces significantly larger ablations than RFA. The treatment appears safe but positioning of the positive electrode of the direct current requires careful consideration. Injuries produced behave like other thermal therapies with coagulative necrosis followed by fibrotic healing. As larger ablations are consistently produced, it is hypothesised that with further refinements, tumours greater than 3 cm in diameter could be treated with lower rates of recurrence.

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Statement

I declare that this thesis contains no material which has been accepted for the award of any other degree in any university and that to the best of my knowledge and belief, contains no material previously written by another person, except where due reference is made in the text. I consent to this thesis being made available for photocopying and loan if applicable and if accepted for the award of the degree.

Christopher Dobbins

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Preface

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Chapter 1

Literature Review

1.1 Introduction

It has been well established that for patients with colorectal liver metastases or hepatocellular carcinomas, their best chance of long-term survival is with surgery. However, it is estimated that only approximately 15-25% of these patients are amenable to surgical intervention[1-6]. For this reason, there is a place for ablative treatments that can treat these unresectable liver tumours. The ideal ablation should be a single episode of energy delivered through one insertion of a solitary applicator that results in cure[7]. It should also be reliable, associated with minimal morbidity and be both time and cost effective.

1.2 Radiofrequency Ablation

Radiofrequency ablation (RFA) was first proposed as a potential ablative treatment for liver tumours in 1990 when animal studies performed by Rossi et al. from Italy and McGahan et al. from the United States were reported[8,9]. The initial clinical experiences with the technique were published by the Italian group in 1995[10,11]. Since that time, much research has been conducted into further refining the technique and expanding its uses. It has now become one of the most popular ablative techniques currently available for the treatment of liver tumours that are not suitable for surgical resection. Although initially considered a purely palliative procedure designed for local tumour control, confidence with its use has grown and now many authors are advocating it as a first line, potentially curative form of treatment of liver tumours[12-14].

However, RFA is in need of further refinement as it still contains many limitations before it can be accepted as a viable alternative to liver resection. The most profound limitation is the associated rate of local tumour recurrence that occurs after treatment with RFA. Although variable, the rate is always greater than that seen with liver resection. Local recurrence after treatment tends to occur early and represents a treatment failure. Steps need to be taken to understand why recurrences occur and how to overcome them.

1.2.1 Principles of Radiofrequency Ablation

Radiofrequency ablation involves the delivery to liver tumours of an alternating electrical current that alternates at radiofrequencies (range 10-3x10^9 kHz) to liver tumours[7]. Most systems available for clinical application make use of currents that lie within the medium radiofrequency range (400-500 kHz)[15]. This stimulates the movement of ions back and forth as they follow the sinusoidal waveform of the alternating electrical current[7] (**Figure 1.1**). This rapid movement of ions leads to the development of frictional heat[7]. Tumour cells are especially susceptible to increases in temperature and irreversible damage starts to occur above 42 degrees Celsius[16].

A radiofrequency generator is used to create the current and it is delivered to the tumour by means of a needle electrode that is placed directly into the tumour substance. Grounding pads are placed upon the legs of the patient to complete the electrical circuit and these provide a large surface area to prevent electrical burns to the skin (**Figure 1.1**). Although bipolar techniques have been described and used experimentally, most of the clinical experience reported has been with a monopolar set up that is similar to the one described by Ni and co-workers[7,17]. The monopolar set up of this technique is easier to apply percutaneously as it makes use of a solitary needle.

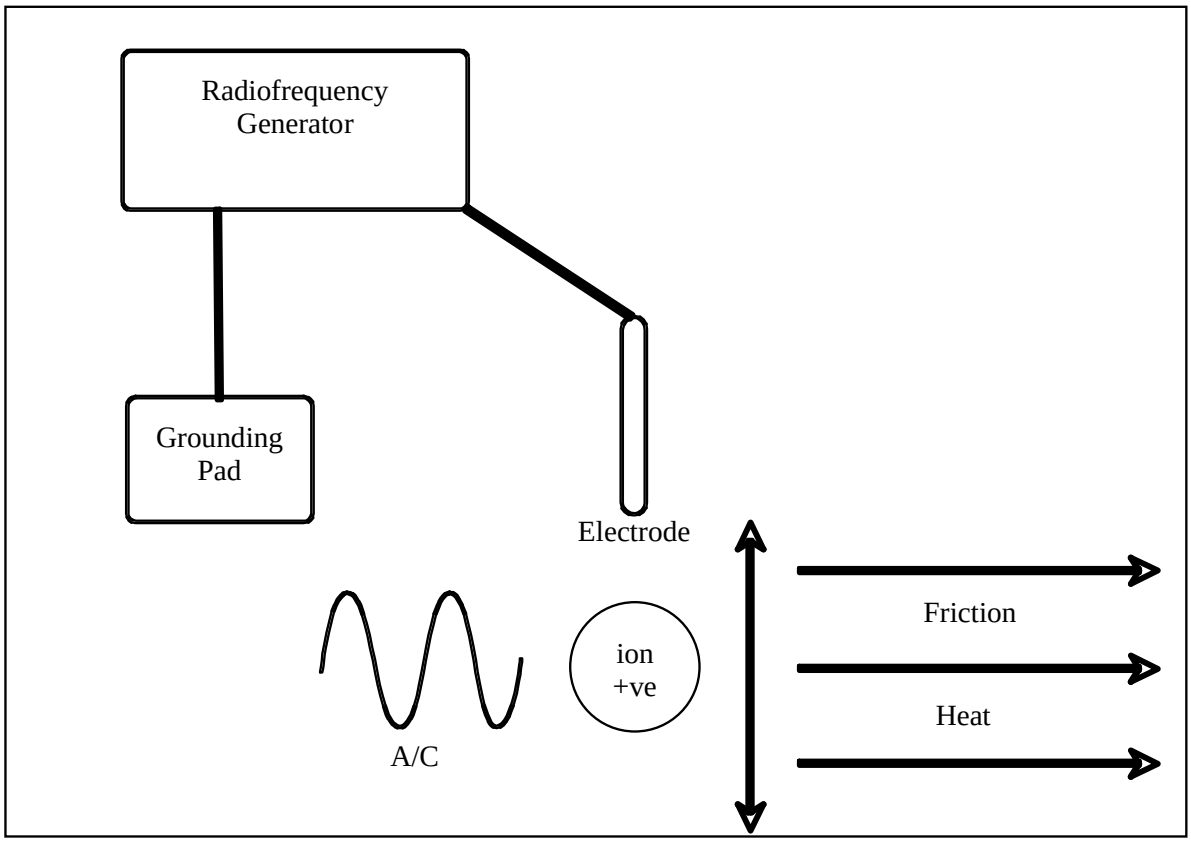

Figure 1.1

Diagrammatic representation of typical RFA setup that illustrates alternating current passing between electrode and grounding pad

As the sinusoidal waveform of the alternating current passes through tissue, ions move back and forward as they try to move towards (or away—depending upon polarity) the electrode leading to generation of friction and heat

As the tumour is heated, the treated tissues become desiccated and subsequently temperatures in this area around the electrode become very high. Consequently, the areas immediately adjacent to the needle become charred. The electrical resistance of the charred tissue rapidly increases to the point that it is no longer able to act as a conductor. At this

point, no further useful ablation can occur as the current flow ceases and ultimately the ablative process stops. This change usually occurs very quickly and is known as “roll off”.

1.2.2 Tissue Responses to Heat

As tissue temperature rises above 42 degrees Celsius, irreversible cell damage begins over the following 30-60 minutes. At these temperatures, inactivation of vital cell enzymes starts to occur[7,16]. As temperatures increase to 60 degrees Celsius, the time required to irreversible damage decreases exponentially. At 60 degrees Celsius, proteins that are an integral part of cellular and mitochondrial enzymes (as well as DNA and RNA constituents) become instantaneously denatured[7,16]. At 100 degrees Celsius, vaporisation of tissue water occurs and carbonisation and charring commence[7,16]. At 300 degrees Celsius, smoking and further carbonisation occurs and this further perpetuates the heat effect[7,16].

Vascular changes are also seen with increasing temperatures. At 44 degrees Celsius, an irreversible decrease in tumour blood flow is seen[7,16]. Complete destruction of tumour microvasculature occurs once temperatures rise above 60 degrees Celsius[7,16]. The ideal thermal ablation is thought to be between 60 and 100 degrees Celsius.

Heat related tissue injury is thought to occur in 2 distinct phases; a direct phase that is followed by an indirect phase[16]. The direct phase occurs during the heating process and the indirect phase refers to the ongoing tissue damage that occurs after removal of the heat source and tissue temperatures have returned to normal[16].

When examining liver tissue that has been treated with thermal therapy, 4 distinct zones are seen within the tissue[7]. At the site of the electrode is an application zone that demonstrates

complete effacement of cells on histological examination. There may be evidence of carbon deposition at this area. Surrounding this is the central zone or "white zone" that consists of damaged tissue undergoing thermal related coagulative necrosis[7]. In the initial stages, intact tissue architecture is seen but irreversible damage is present within cells[7]. Beyond this central zone is a transition zone or "red zone" that exhibits signs of haemorrhage but still contains viable tissue[7]. Peripheral to the transition zone is a reference zone that contains untouched, normal tissue[7]. The only areas that should be considered as completely non-viable are the application and central zones. These non-viable areas eventually heal by fibrosis that starts at the periphery and moves into the central areas. This process usually takes several months and leaves a small, residual fibrous scar[8,9].

1.2.3 Clinical Uses

In the clinical setting, RFA was initially performed percutaneously by radiologists using local anaesthesia and sedation. The tumour is localised with ultrasound and after infiltration with a suitable local anaesthetic, the needle is directed through the skin into the substance of the tumour. The ablation process is then commenced. Ultrasound is most commonly used to monitor progress by detecting the presence of echogenic bubbles that occur at the periphery of the ablated area. These bubbles develop as a result of intracellular water vaporisation. A CT scan is generally performed in the post-operative period to ensure that the tumour has been completely covered. Real time MRI and CT scanning have now been developed and are being used more frequently as an alternative to ultrasound.

Intra-operative RFA has also been used extensively and considerable experience with both laparoscopic RFA and RFA performed at laparotomy has been reported. There are advantages with the use of a surgical route. These include better visualisation of the

tumour; the risk of heat related damage to adjacent organs such as the hepatic flexure of the colon and the stomach can be minimised; and the improved sensitivity of intra-operative ultrasound[18]. Intra-operative RFA does have the disadvantage of associated increased morbidity related to surgery and anaesthesia[18].

1.2.4 Associated Morbidity and Mortality

Radiofrequency ablation has been shown to be a safe procedure with a mortality rate of less than 2%[19,20]. The most common causes of mortality are related to fulminant liver failure and to unrecognised post-procedural haemorrhage. Careful patient selection should reduce the risk of the former and judicious clinical observation should prevent the latter.

The most common complication is the development of a post-ablation syndrome (an influenza type illness of fever with general malaise, pain at the ablation site and nausea) that usually occurs after treatment and usually lasts for 3-5 days[21]. It occurs in approximately one third of patients and is directly related to the amount of tissue ablated[21]. Associated rates of major morbidity are less than 10%[22-24]. Bleeding complications such as sub-capsular haematomas have been reported and may require blood transfusion. Liver failure can occur in poorly selected patients. Thermal damage to organs abutting the liver – such as the hepatic flexure of the colon and the stomach – can occur, particularly when the percutaneous route is used. Liver abscesses can occur in ablation sites and relates to necrotic tissue being left in-situ[25]. The risk of developing a liver abscess is greater if a preceding biliary tract procedure has been performed[25]. Bile duct injuries and bile leakage can occur when ablation is performed in close proximity to the central hilar structures [26]. Skin burns at the site of the grounding pad have been described but the risk of this can be minimised by the use of multiple grounding pads[27].

There is a small risk of tumour seeding following treatment, particularly when a percutaneous route is used[28]. It has been recommended that the needle tract be ablated upon needle removal as this manoeuvre theoretically reduces the risk of needle tract seeding[29]. Risk of seeding appears to be increased when a percutaneous biopsy is performed prior to RFA treatment[29].

Experiments have been performed to see if RFA can cause an entity similar to cryoshock (see **Section 1.2.8.4 Cryotherapy**). Results indicate that this does not occur and no clinical case has yet been described[30,31]. It has been proposed that thermal therapies induce localised tissue ischaemia around the areas treated and this prevents the release of the responsible cytokines into the general blood stream[31].

1.2.5 Experience with Colorectal Liver Metastases

Colorectal cancer is one of the most common malignancies in western societies and is the second most common cause of cancer-related death in Australia[32]. Liver metastases are common and occur in approximately 50% of patients with this disease[33]. Those metastases found at initial presentation are termed “synchronous” while those appearing later are termed “metachronous”. Untreated, patients with colorectal liver metastases rarely live 5 years and the median duration of survival without any form of treatment is less than 12 months[33].

It is well established that patients have their best chance of long-term, disease-free survival when the tumour is surgically resected. Surgical resection can be performed as a formal anatomical resection based upon the vascular supply to the various segments of the liver[34]. Alternatively, for those tumours that are peripherally placed, a wedge resection can be

performed[34]. The most important oncological aspect of liver resection of any type is to ensure complete removal of the entire tumour with a 1 cm margin of normal tissue[35]. When this is achieved, the risk of local recurrence is small[35]. In carefully selected patients, 5-year survival rates range between 30 and 50%[34,36]. The most important clinical aspect of liver resection is to ensure that enough liver is left behind to ensure liver failure does not develop. In normal, healthy livers, this is at least 20% of the original volume; for those livers that have undergone chemotherapy, 30% of the original volume; and for patients with well compensated liver dysfunction, at least 40% residual volume is required[37].

Although surgical resection remains the current gold standard of treatment for colorectal liver metastases, only approximately 20% of patients are amenable to this form of treatment[36]. Factors such as bilobar distribution of multiple tumours, extrahepatic disease, underlying liver dysfunction and other co-morbidities can preclude patients from being a surgical candidate[36]. Neo-adjuvant chemotherapy with the use of agents such as 5-fluorouracil, irinotecan and oxaliplatin can occasionally shrink unresectable tumours thus making them resectable[38]. However, this occurs in only 15-30% of treated cases[39,40]. Chemotherapy is rarely curative but can increase survival duration and quality of life. With current chemotherapy regimes, the median rate of survival is approximately 20 months[41,42].

With the evidence that is currently available, patients with resectable disease should be offered surgical resection in the first instance. Although it is yet to be universally accepted, most clinicians who treat these malignancies consider ablation as being a local treatment of liver metastases that are not amenable to surgical resection[43]. This includes those patients with bilobar disease and those who are not fit for resection. Currently, colorectal liver metastases are a contraindication to liver transplant and as chemotherapy is rarely curative,

an appropriate and effective ablative technique for these tumours is needed[38]. The ablative therapy should follow surgical principles by completely destroying the tumour and a rim of normal tissue.

Many case series have been published with actuarial (estimated) survival rates calculated by the Kaplan-Meier method. 1-, 3- and 5-year survival rates calculated by these methods are in the range of 96-88%, 64-40% and 44-17% respectively[44-50]. The median survival duration is in the range of 32-52 months from time of diagnosis[44-47,51] (**Table 1.1**). Most of these reports are not compared with surgery. However, in a study comparing resection with combined resection/ablation and ablation alone, Abdalla et al. reported survival rates of approximately 90%, 42% and 21% at 1, 3 and 5 years respectively in 57 patients[52]. These authors found that patients who underwent surgical resection had a significantly higher survival than when treated with RFA alone or when RFA was combined with resection (65% versus 22% versus 36% respectively at 4 years).

Local recurrence rates for RFA are variable and have been high with rates recorded between 9 and 47% (**Table 1.1**). Local recurrences tend to occur with greater frequency in tumours that are greater than 3 cm in diameter with some reports showing local recurrence rates of greater than 50% [53]. These reports also show that the overall prognosis for these particular patients is worse than those with small tumours. A systematic review performed by Sutherland et al. in 2006 concluded RFA may be of modest benefit only to patients with colorectal liver metastases[54].

Study	Patients/ Tumours	Median Follow Up	1-year survival	3-year survival	5-year survival	Median Survival	Local Recurrence
Machi et al. 2006[44]	100 patients 507 tumours	24.5 months	90 %	42%	30.5%	48 months	20.5%
Sorensen et al. 2007[46]	102 patients 332 tumours	23.6 months	96%	64%	44%	52 months	N/A
Abitabile et al. 2007[47]	47 patients 147 tumours	33 months	88%	57%	21%	39 months	8.8%
Jakobs et al. 2006[48]	68 patients	21.4 months	N/A	68%	N/A	N/A	18%
Gillams and Lees 2005[50]	167 patients	N/A	91%	40%	17%	32 months	N/A
Berber et al. 2005[51]	135 patients N/A	1-52 months range	N/A	N/A	N/A	29 months	46%
Abdalla et al. 2004[52]	57 patients 110 tumours	21 months	80%	37%	22%	N/A	9%
Van Duijhoven et al. 2006[55]	87 patients 199 tumours	25 months	N/A	N/A	N/A	27.8 months	46.2%
Bleicher et al. 2003[56]	59 patients N/A	11 months	N/A	N/A	N/A	N/A	18.3%
Chen et al. 2005[23]	134 patients 333 tumours	Range 3-57 months	75.3%	25.3%	N/A	N/A	10.5%
Joosten et al. 2005[49]	28 patients 72 patients	25 months	93%	75%	N/A	N/A	14%
Solbiati et al. 2001[57]	109 patients 172 tumours	Range 6-52 months	N/A	33%	N/A	30 months	29.6%

Table 1.1

Recently published case series of RFA use for colorectal metastases with survival and local recurrence rates

(N/A – results not available)

The problem with most of the published reports on the use of RFA for colorectal liver metastases is that they are often in the form of poorly matched comparative studies or anecdotal case series. Most case series only report short to medium term data and although they appear to have reasonable estimates of long-term survival, this is often in the presence of local recurrence. There have been no randomised controlled trials comparing liver

resection with RFA to date. Such a study is very hard to justify as surgical resection is well established as a potentially curative procedure in carefully selected cases. The comparative studies of the 2 procedures that have been reported have always shown better results following surgical resection but as alluded to earlier, patients undergoing resection tend to have more favourable characteristics[58,59].

Somewhat surprisingly, there are yet to be any reports of studies that compare chemotherapy with chemotherapy plus local ablation. This type of study can be justified and would clarify whether there is additional significant benefit obtained by ablating these tumours. The study performed by Abdalla et al. indicated that the use of RFA had a survival advantage over chemotherapy alone. A phase 2 randomised controlled trial known as The CLOCC Trial has been initiated in Europe to compare treatment with RFA and chemotherapy versus chemotherapy alone and it has recently closed[60]. No data has yet been published or presented but the results will be valuable.

Current data indicate that the rate of local recurrence after treatment with RFA is variable and ranges between 9 and 47% (**Table 1.2**). Most recurrences occur within the first 12 months after treatment[55]. Local recurrence indicates that local treatment failure has occurred and in tumours larger than 3 cm in diameter, it appears to occur more frequently[53].

1.2.6 Experience with Hepatocellular Carcinomas

Hepatocellular carcinomas (HCCs) are primary tumours of the liver and are often associated with cirrhotic change[61]. These tumours develop in response to chronic liver damage and cirrhosis and are particularly related to chronic infection with hepatitis B and C viruses[61]. This tumour is the 5th most common cause of cancer-related death worldwide and is

particularly endemic in certain parts of South-East Asia and parts of Africa[61]. It is less common in western countries such as Australia and the United States of America and rates in these regions are low (3.97/100 000 and 2.4/100 000 respectively)[62,63]. However, the rates in Australia have doubled from 2.06 to 3.97/100 000 for men and 0.57 to 0.99/100 000 for women in the last 20 years[62]. Similar incidence rises have been seen in the United States of America, Japan, the United Kingdom and France[63]. Patients with untreated hepatocellular carcinomas have a very poor prognosis with a median survival of 3 months and less than 5% of patients survive for 5 years[63,64].

The optimal treatment of HCCs currently remains surgical. In selected cases, liver transplant can be performed and this has the advantage of not only removing the cancer but also providing the best treatment for their underlying liver dysfunction that may be associated with the disease. However, patients have to meet strict criteria before they can be considered a suitable transplant candidate[65,66]. The Milan criteria state the tumour must be less than 5 cm in diameter or, if multiple tumours are present, no more than 5 can be present with a maximal diameter of 3 cm. Patients meeting these criteria and undergoing transplantation have a 75% chance of survival at 5 years[66,67].

Surgical resection is also a potentially curative form of treatment. Resections are similar to those performed for colorectal liver metastases and take the form of formal anatomical resections or wedge resections of peripherally placed tumours. Once again, careful patient selection is mandatory and patients can only have minimal underlying liver dysfunction. Five-year survival rates have been reported between 35-50%[68-70].

The role of systemic chemotherapy for patients with HCCs appears limited. No survival advantage has been reported with any particular regime[71]. Likewise, Trans-Arterial Chemo-Embolisation (TACE), a technique that involves embolising the hepatic artery after administration of intra-arterial chemotherapy, only appears to be of modest benefit to patient survival[71]. The use of radio-labelled lipiodol has also been shown to be useful in arresting the progression of HCC but has not been shown to be curative[72].

Radiofrequency ablation has been used in 2 ways for patients with HCC. It has been used as a potentially curative, ablative treatment for unresectable tumours. It has also been used as an interim treatment for patients on liver transplant waiting lists[73-75].

As an ablative treatment, it sounds very attractive as these patients often have a degree of underlying liver dysfunction and RFA produces minimal damage to tissue outside the area treated. There have been only few long-term clinical reports published. Once again, current reports have been patient case series with heterogenous groupings of patients. No randomised controlled trial has been performed comparing RFA with liver resection or transplant. Comparative studies that have been reported have been poorly matched and are always in favour of surgery[70,76-78]. Despite this, 5-year actuarial survival rates of 28 to 58% have been reported, although this is often in the presence of recurrence[23,44,79-82]. These are comparable to surgical resection and would seem to indicate that RFA does have a genuine role in treatment. However, long-term data is lacking. Once again, local recurrence rates are variable and range between 10% and 46% (**Table 1.2**). Most recurrences occur within 12 months of treatment.

Study	Patients	Median Follow Up	1-year survival	3-year survival	5-year survival	Median Survival	Local Recurrence
Vivarelli et al. 2004[78]	79 patients >79 tumours	15.6 +/- 11.7 months	78%	33%	N/A	N/A	15.2%
Raut et al. 2005[79]	194 patients 289 tumours	34.8 months	84.5%	68.1%	55.4%	N/A	5%
Ferrari et al. 2006[81]	40 patients 50 tumours	N/A Study from 2003-05	91.8%	59%	28.4%	N/A	17.5%
Choi et al. 2007[83]	570 patients 674 tumours	26 months	95.2%	69.5%	58%	77 months	10.9%
Yu et al. 2005[84]	30 patients 30 tumours	Mean 28.8 +/-9 months	N/A	N/A	N/A	N/A	46.6%
Machi et al. 2005[44]	65 patients 191 tumours	20 months	N/A	N/A	39.9%	40 months	16.9%
Chen et al. 2005[23]	204 patients 430 tumours	Range 3-57 months	84.6%	63.1%	N/A	N/A	7.9%
Hori et al. 2004[85]	69 patients 104 tumours	18.1 months	N/A	N/A	N/A	N/A	20.6%
Lencioni et al. 2005[86]	187 patients 240 tumours	24 months	97%	71%	48%	49 months	10%
Buscarini et al. 2001	88 patients 101 tumours	34 months	89%	62%	33%	48 months	13.6%

Table 1.2

Recent studies of RFA for HCC with estimated survival and local recurrence

(N/A – results not available)

Radiofrequency ablation has also been used as a bridging treatment to liver transplant[73-75]. Shortage of suitable donors for liver transplantation remains an ongoing problem and patients are often required to wait up to 12 months before transplant can be performed. In the absence of effective treatments, a considerable proportion of patients can become unsuitable transplant candidates as a result of disease progression. It is thought that if these

tumours can be successfully treated with RFA, then disease progression should be limited and patients can remain on transplant waiting lists[74,87].

The bridging treatment gives some insight into the efficacy of RFA in treating these tumours as the liver is harvested and sent for pathological examination. The results indicate that coagulative necrosis does occur in tumours and complete necrosis in tumours is present in between 55-85% of tumours[88-91]. This is often in contrast to complete radiological response. Small pockets of viable tumour cells were occasionally visualised. These would most likely have become clinically relevant if transplant had not occurred[88,90,91]. Once again, recurrence appears to occur in tumours greater than 3 cm in diameter[88-91].

1.2.7 Experience with Other Tumours

Most of the experience of RFA has been with HCCs and colorectal liver metastases. However, RFA has also been used for many other types of liver tumours.

There have been very few reports of RFA being used with cholangiocarcinoma, the other common type of tumour that arises primarily in the liver[92,93]. Zgodzinski et al. reported that a patient with an intrahepatic cholangiocarcinoma tumour was successfully treated with RFA and remained well for the following 2 years[92]. Slakey et al. reported on the successful use of RFA for a patient with an intrahepatic recurrence of cholangiocarcinoma following previous resection[93]. The authors state that the patient had remained well in the following 10 months. The reason that few cholangiocarcinomas have been treated is may be that these are invariably related to the biliary system and RFA could cause serious damage to biliary structures.

Radiofrequency ablation has been shown to be useful as a treatment of metastatic carcinoid tumours[94-97]. These tumours are generally slow growing and do not appear to behave as aggressively as HCCs and colorectal liver metastases. They can produce a well-documented syndrome that is characterised by diarrhoea, flushing and abdominal pain that relates to the secretion of neuroendocrine hormones such as somatostatin[95]. Radiofrequency ablation is useful in palliating these patients by reducing the active tumour cell load and so the amount of somatostatin that is released is also reduced[94,98-100]. Treatment of these tumours may also improve life expectancy if early diagnosis and treatment is instituted[96].

Radiofrequency ablation has been used for metastatic liver tumours associated with gastric, breast and pancreatic liver primaries[101]. If there is no evidence to suggest that resection of these metastatic liver tumours provides any long-term benefit, it is debatable whether any such good could be achieved by ablating these tumours with RFA. Although controversial, some authors have reported good long term results following resection in carefully selected patients with isolated liver metastases from breast cancer [102]. However, treatment of breast cancer metastases with RFA has been disappointing with very high rates of recurrent disease. Livraghi reported greater than 50% recurrence rates in 24 patients with liver metastatic breast cancer and results were not as good as surgery[103].

Good long term results can be achieved for certain metastatic sarcomas such as gastrointestinal stromal tumours, when surgical resection is combined with the chemotherapeutic agent, imatinib[104]. Results for these tumours treated with RFA are not as good with shorter disease free survival and overall survival when compared to surgery[104].

Radiofrequency ablation has also been used extensively and successfully for tumours situated outside the liver and in particular, in small renal cell cancers, osteoid osteomas, pancreas tumours, lung tumours and small breast cancers[105-112].

1.2.8 Radiofrequency Ablation comparison with other commonly used Ablative Therapies

Although the focus of this discussion is on RFA as a genuine treatment option for unresectable liver tumours, it is not the only ablative technology that is currently available. It is necessary to consider some of the other ablative techniques that are currently available in order to place RFA in correct perspective.

1.2.8.1 Microwave Coagulation Therapy

In many respects, Microwave Coagulation Therapy (MCT) is similar to RFA and is able to be performed by percutaneous or operative means. It is possibly the most frequently used alternative thermal ablative technique for treatment of liver tumours. It was first developed in the 1980s by Tabuse in Japan, primarily as a coagulation tool to assist in liver resection[113,114]. It was unable to establish itself as being particularly useful in this regard but it did lead to it being developed as an ablation device. It quickly became commercially available as a treatment option for unresectable tumours. As the procedure was developed in Japan, it has been most widely used throughout South-East Asia and therefore, most of the clinical experience with the technique comes from this region.

The basic principle of MCT is the delivery of microwave energy that results in heating and subsequent destruction of tumours[114]. To perform MCT, an electrode that is connected to a microwave generator is placed into the liver tumour[115]. Microwaves are emitted from the

electrode with a frequency of 2450 MHz[15,114,116]. The emitted microwaves cause intracellular water molecules to vibrate and rotate thus leading to the generation of heat and heat-related tissue damage and necrosis follows[15,114-116]. No grounding pads are required – this is the major difference between MCT and RFA[115].

The area of necrosis that is produced tends to have an elliptical shape. The maximal diameter in the short axis with this technique is about 2 cm. As a result, several applications need to be made to completely ablate all but the very smallest tumours. Recently, electrodes with multiple antennae have been developed that can be used to create larger volumes of necrosis[117]. These appear similar to the multi-tined RFA electrodes in that they can perform overlapping ablations that form a much larger necrotic area.

Microwave coagulation therapy is thought to have 2 advantages over RFA. It creates increases in tissue temperatures at a much higher rate than RFA and therefore, treatment time is considerably less[118]. It is also thought to be safer around blood vessels and is less susceptible to heat sink effects[118].

Studies have shown that as a treatment for colorectal liver metastases, the survival rates are 91.4%, 46.4% and 29% respectively at 1, 3 and 5 years for small lesions[119,120]. These are similar to those recorded for RFA. Survival rates for small treated HCCs are again similar to RFA and are 97%, 81% and 43% respectively at 1, 3 and 5 years for solitary lesions up to 3 cm in diameter[121 122,123 124,125].

Treatment with MCT results in greater than 80% complete necrosis rates in tumours less than 2 cm in diameter but is less effective in larger tumours. Reported complications are

similar to RFA with sub-capsular haematomas, bile leak, liver abscess and portal vein thrombosis being described[126].

Two groups have performed studies comparing MCT and RFA. One by Shibata et al. found that there were no significant differences in therapeutic effects and complication rates when looking at a total of 72 patients[127]. Only small numbers were studied and even though there was no significant difference, the trend would indicate that both therapeutic effects and complication rates favoured RFA. It can be concluded that a Type 1 statistical error may have been present. The number of treatment sessions required was significantly less for RFA. These findings were confirmed in 2 follow up studies that were performed by the same group[128,129]. Lu et al. performed a retrospective analysis of the 2 modalities and also found no significant difference in terms of recurrence rate, treatment efficacy and long term survivals[130].

1.2.8.2 Laser Induced Interstitial Thermotherapy

Laser Induced Interstitial Thermotherapy (LITT) was first proposed as a potential treatment for unresectable liver tumours in 1983[131]. The absorbed energy of the laser light is converted to heat and thermal damage results. A laser unit has 3 basic components; a power source, a lasing medium and reflecting mirrors[132]. The most commonly used laser is the neodymium: yttrium-aluminium-garnet laser (Nd:YAG) which produces a laser beam in the infra-red range[132]. This has been shown to give the greatest tissue penetration of the lasers that are currently available[132]. The laser light is transmitted from thin fibres into the tumour tissue where it is absorbed. This absorption of light energy creates heat and resultant thermal coagulation. The procedure can be performed percutaneously or with an open, surgical procedure and is typically performed under CT or MRI guidance[132].

Ablations produced using this method are generally up to 2 cm in diameter[132]. The size of the laser induced ablation is limited by localised tissue charring (in a mechanism similar to that found with RFA) which limits the further absorption of light[132]. The size constraint can be overcome by placing multiple fibres and beam-splitting techniques. It may also be overcome by the usage of cooled-tipped fibres to prevent tissue charring. The procedure can be performed percutaneously or by laparoscopy or laparotomy[132].

Laser induced interstitial thermotherapy is able to induce necrosis in greater than 90% of tumours. It is a relatively safe procedure with complications reported similar to other modalities. The mortality rate is less than 1% and the rate of major morbidity is reported at 16%[132]. Liver abscess, bile duct injury, pleural effusion, liver infarction and pleural effusions have all been reported following treatment with LITT[132].

This technique has been predominantly used for unresectable colorectal liver metastases. Vogl et al. reported survival rates for use with colorectal metastases in 603 patients over a ten year period of 94% at 1 year, 56% at 3 years and 37% at 5 years[133]. All tumours were less than 5 cm and fewer than 5 in number for each patient. No patient with evidence of extrahepatic spread was included in the study. This group also reported low recurrence rates with 1.9% recurrence at 6 months and 4.4% recurrence at 6 months for tumours greater 4 cm in size[133].

These outstanding results achieved by Vogl et al. have not been consistently replicated. Christophi et al. reported their experience of the use of LITT in 80 patients[134]. They reported a 5-year survival of 6.7% and were only able to achieve tumour response in

67%[134]. They recorded a local recurrence rate of 33%[134]. The authors attributed the poorer results to the type of electrode being used[134]. This was a bare fibre electrode which is prone to causing tissue charring[134]. They now use a diffusing fibre that allows for more homogeneous light emission. It is argued that this may reduce the chance of tissue charring and improve their results[134].

Experience with HCC treated with LITT appears to be much less than that of colorectal metastases in the English literature. A series of 74 patients treated for small HCC revealed survival rates of 99% at 1 year, 68% at 3 years and 15% at 5 years[135]. Local recurrence rates were recorded at 1.6% to 6%[135].

There is only one study directly comparing LITT with RFA[81]. Eighty one patients with small HCC were divided into 2 well-matched groups. Rates of tumour necrosis were superior with RFA and as a result, more treatment sessions were required for LITT. Long-term survival rates were also superior for patients with Child-Pugh A cirrhosis when treated with RFA.

1.2.8.3 High Intensity Focussed Ultrasound

High Intensity Focussed Ultrasound (HIFU) is an ablative technique that uses ultrasound waves. It is potentially non-invasive and can be employed extra corporeally. It was first advocated as a potential therapeutic technique in 1942 by Lynn and co-workers[136]. William Fry and his group performed the first experiments with this technique in 1950s by producing ablations within the brains of monkeys and cats[136]. His brother, Frank Fry, then introduced the procedure to the clinical realm where he used HIFU to treat patients with Parkinson's Disease by ablating certain regions within the brain[136]. It required a craniotomy as the skull

would cause widespread scattering of the ultrasonic waves and prevented a sharp focus being achieved. That necessity could not be avoided at that time. With only rudimentary imaging techniques available, the procedure could not be introduced into clinical practice. Interest in the technique has re-emerged as technology has improved and there are now several different types available and reports of experience in the use for treatment of prostate cancer, liver tumours and breast tumours are starting to emerge. Experimental work into treatment of renal and other tumours has also been performed.

Ultrasound waves have the property of being able to pass through the body without causing harm. This property has made ultrasound scanning a particularly useful diagnostic tool[137]. When the ultrasound beam has sufficient energy and is focussed, the energy can be transferred into the tissue causing heat with subsequent heat damage and necrosis[137 138].

To date, patients who have undergone HIFU treatment of liver tumours have had to undergo a general or spinal anaesthetic[137]. This is predominantly to allow the patient to be positioned and kept motionless for the duration of the treatment[137]. The patient is usually positioned either supine or with their right side upwards[137]. Ultrasound waves are unable to pass through gas filled organs (such as bowel and lungs) and considerable imaging and planning is undertaken. Often, the right lung requires ventilatory manipulation by partially collapsing the lung to prevent it from obscuring the beam. This manipulation also reduces the amount of movement the liver undergoes during the process of ventilation. Overlying ribs can prevent ultrasound transmission and some investigators have advocated rib resection to prevent this.

Although it has created considerable interest, actual reports of clinical experience using HIFU with liver tumours are limited. The largest experience is from China where Wu et al. have demonstrated its use in the treatment of HCC[139]. This group performed HIFU in 30 patients with malignant lesions in various sites that was followed by surgical resection within 2 weeks[139]. Macroscopically, the treated areas were quite distinct from normal tissue. Microscopic examination, there was evidence of coagulative necrosis and cell death within the tumour cells and extensive destruction to tumour blood vessels[139]. In a small non-randomised trial, this same group found that the combination of HIFU with TACE (Transcatheter arterial chemoembolisation) appeared to prolong life significantly when compared to TACE alone [140]. A pilot study using HIFU for patients with unresectable liver tumours has been performed in the United Kingdom but only 11 patients were enrolled [138]. Although it appears that they will be able to safely perform a clinical trial, no significant conclusions can be performed from this particular study.

In the absence of mortality and morbidity data, its safety cannot be assessed at this stage. Wu et al. did describe some patients with associated skin burns [139]. The length of the procedure is also a limiting factor as small tumours can take up to 3 hours to treat and large tumours up to 10 cm tend to take up to 5 hours to ablate fully[139].

As the procedure is still experimental, there are yet to be any comparative studies with RFA performed.

There have been no reports of long-term clinical trials using HIFU and it is premature to comment on its use in standard clinical practice for treatment of liver tumours.

1.2.8.4 Cryotherapy

Cryotherapy is a form of ablation that uses freezing and has been in use since the 1960s[141]. It is the oldest form of tumour ablation therapy of clinical significance. Its popularity has waxed and waned. It involves the use of a cryoprobe that is placed into the tissue and the probe is perfused with liquid nitrogen[141]. The probe temperature decreases to minus 196 degrees Celsius and an ice-ball forms within the tissue[141]. The ice-ball is then rapidly thawed[141]. This technique leads to the formation of intracellular crystals and the thawing process leads to cell disruption. The process can be performed under ultrasound guidance as the periphery of the ice-ball is easily seen on ultrasound[141]. The procedure is most commonly performed with a laparotomy and intra-operative ultrasound[141]. Laparoscopic and percutaneous techniques have been described but are not used commonly in clinical practice [142,143]. The cryoprobe is usually about 1 cm in diameter and consequently is difficult to use percutaneously.

The complication rate for cryotherapy is greater than the other techniques described with rates reported up to 40%. It is now less popular than other ablative techniques. Similar types of complications are seen with cryotherapy such as hepatic abscesses, hepatic failure, liver abscess, biliary fistulae and pleural effusions. More specific cryotherapy problems such as hypothermia are evident. Cryoshock is a common cause of cryotherapy-related mortality. Cryoshock leads to shock and multi-organ failure as a result of a massive release of the cytokines, TNF alpha, Il-1 and Il-6[144]3[1, 3]2. These are released in response to large numbers of free radicals that develop as a result of the extreme changes in temperature. It has been reported that cryoshock occurs in 1% of treatments and when it occurs, death is common (Seifert and Morris reported a mortality rate of 18%)[145]. Liver shearing is another

unique form of major morbidity where the frozen liver cracks with resultant haemorrhage upon thawing[141]. Overall mortality rates are greater than other forms of ablative therapy[146].

Cryotherapy has been used quite extensively for the treatment of colorectal liver metastases and reports of actuarial survival rates of 62-87%, 43-15% and 23-0% at 1, 3 and 5 years following treatment have been published[147-150]. Cryotherapy shares with all ablative techniques the problem of recurrence rate. Recurrence rates within the previously treated cryosites have been reported up to 25% within 22 months[150]. This is significantly greater than recurrence after surgical resection[147].

Although used extensively for treatment of colorectal liver metastases, only a few studies describing experience of cryotherapy with hepatocellular carcinoma have been published. Zhou et al. have a series of 234 patients who had a 5-year survival of 26.9 %. This was improved to 39% following addition of other forms of treatment such as hepatic artery ligation[151].

There are no comparative studies that directly compare the results of RFA with cryotherapy for liver tumour treatment. However, RFA has a considerable advantage over cryotherapy in having lower rates of morbidity and mortality.

1.2.8.5 Percutaneous Injection Therapy

Percutaneous injection therapy is the simplest and least expensive ablative procedure currently available and has now been in use for more than 20 years. The principle of Percutaneous injection therapy is to inject a substance that is toxic to the tumour cells into the tumour resulting in necrosis[152]. The most common agent injected is ethanol as it is

inexpensive and readily available. Percutaneous Ethanol Injection Therapy (PEIT) tends to have widespread use in Asia where the rate of hepatocellular carcinoma is highest and its inexpensive nature makes it an excellent treatment option[152,153].

Acetic acid has been used in a similar manner. It is as efficacious as ethanol and fewer treatment sessions are required but it is more expensive and less readily available than ethanol[154,155].

To perform the procedure, the lesion is localised by ultrasound examination. The lesion is then injected with alcohol until the tumour is saturated. Ultrasonography is able to follow the free flow of ethanol into the tumour[152,153]. The alcohol causes intracellular and extracellular dehydration and thrombosis of small surrounding vessels[152,153]. This leads to tissue ischaemia and ultimately, cell death and necrosis[152,153]. To completely ablate a tumour, multiple treatments are generally required. Usually 4 sessions are required to ablate lesions up to 2 cm in diameter and 6 sessions required to ablate lesions up to 3 cm in diameter[152,153]. Ease of repetition compensates for the need for multiple treatments.

Recurrence rates are quite high and must reflect the fact that incomplete tumour necrosis is not uncommon. Rates of recurrence have been reported up to 33% in 12 months for tumours up to 3 cm in size[156]. Although it is simple to use and easily repeatable, PEIT induces tumour necrosis in only around 80% of HCCs[152]. There is a tendency for these tumours to have septae within them that can prevent the diffusion of alcohol throughout the tumour[152]. Secondly, this form of ablation does not include a rim of normal tissue so small satellite lesions located outside of the main tumour mass will not be ablated[152]. The necrosis rates rapidly decline with tumours larger than 3 cm[152]. For solitary small HCCs in

patients with Child-Pugh A cirrhosis, the 5-year survival rates using ethanol injection has been found to approach that of liver resection[156].

Percutaneous ethanol injection therapy is far less successful in the treatment of other liver tumours. The explanation reflects the physical characteristic of the tumour and its environment. HCC tends to be a relatively soft tumour that is surrounded by hard, fibrotic liver parenchyma that may be cirrhotic[153]. Consequently the ethanol tends to remains within the tumour and is unable to permeate into the outer liver parenchyma. In contrast, other liver tumours (such as colorectal liver metastases) tend to be hard and lie within a soft parenchyma[157,158]. As ethanol is injected into these lesions, it tends to diffuse out into the surrounding parenchyma[157,158]. Therefore, its use is limited as it only produces necrosis rates around 50% and requires multiple injections of large quantities of ethanol to be successful[157,158]. For these reasons, PEIT is generally not considered a suitable treatment for liver tumours such as colorectal liver metastases.

Percutaneous injection therapy has been shown to be very safe and its safety profile has been well established. The most commonly reported complication is pain. This is usually managed with simple analgesics. Transient alcohol intoxication is also common but self-limiting[152]. Post-treatment fever as a result of tumour necrosis is well documented. Mortality rates have been described as less than 0.1%[152]. There is a small risk of needle tract seeding— about 1%.

There have been 2 reports comparing RFA with PEIT for treatment for HCC and one of these is a randomised controlled trial. Both reports have found that RFA is superior to PEIT in terms of number of treatment sessions required, rates of tumour necrosis and rates of

local recurrence[159-161]. Percutaneous ethanol injection therapy is the far cheaper modality however[161].

In conclusion, of all the ablative techniques that are currently available, RFA probably shows the most promise. Percutaneous ethanol injection therapy is limited to patients with HCCs and RFA is superior in terms of rates of tumour necrosis, number of treatment sessions required and recurrence rate[155,162,163]. It is able to be performed in less sessions and is more effective than MCT[129]. It is less morbid than cryotherapy[49,164]. It is easier to perform than LITT[165].

Radiofrequency ablation is supported by more published clinical and experimental work than most of the other modalities (PEIT may be the exception). Therefore, these other modalities could arguably, all be considered experimental.

1.2.9 Factors associated with local recurrence

Regardless of the tumour type, the local recurrence rate following treatment by RFA is variable and often high. Each recurrence should be considered a treatment failure.

1.2.9.1 Tumour size

The size of the tumour is the most significant factor associated with local tumour recurrence. This has been repeatedly in multiple published studies[53,55,85]. As tumours increase above 3 cm in size, they become more difficult to treat and local recurrence rates rise accordingly. Tumours less than 3 cm generally have a local recurrence rate of less than 15% but tumours greater than 5 cm in diameter have recurrence rate of 58%[53]. Many

studies have shown that the long term survival of patients with these larger tumours is significantly worse than those with smaller tumours[53].

With electrodes that are currently available commercially, the largest tumour that can be ablated with a 1 cm safety margin is approximately 3.5 cm in diameter[166]. Beyond this size, multiple ablations are generally required to cover each individual tumour. Inability by these methods to achieve total tumour necrosis is reflected in recurrence.

The 2 most common ploys for treating tumours with multiple overlapping ablations are the cluster form and the cylindrical form[115]. The cluster form involves performing multiple ablations directly adjacent to one another until the tumour is covered[115]. The cylindrical form involves performing multiple ablations in parallel straight lines until the entire tumour is covered[115]. This is the method generally recommended. Small satellite lesions often exist close to, but not attached to, the tumour and tumour spikes may get missed when multiple ablations are performed[167]. In a recently published meta-analysis by Mulier et al., failure to obtain a 1 cm margin around the tumour was associated with a significantly increased risk of local recurrence[53]. Normal surgical principle accepts a tumour clearance of at least 1 cm as appropriate[35]. However, the most likely cause of failure is incomplete necrosis and associated viable tumour cells within the non necrotic remnant[167,168]. These local recurrences often occur at the periphery of the tumour[167,168].

Ablation is usually performed with ultrasound guidance. When multiple ablations are performed, poor ultrasonic visualisation can occur as ablated areas can mask untreated areas[169]. As these areas do not get treated, incomplete ablation occurs. It has also become apparent that the degree of necrosis visualised does not always correlate with the actual

volume of tumour ablated[168,169]. To overcome this, newer sonic contrast agents are being used with some success[170-174]. Radiofrequency ablation performed under CT and MRI guidance has also been successfully used but is often not as widely available[175].

1.2.9.2 Tumour Position

In the context of RFA, the position of tumours has been shown in many studies to influence their rate of local recurrence. Sub-capsular tumours have been reported to have increased rates of local recurrence when a percutaneous route is used[176]. These tumours lie within 2 cm of the liver capsule. Percutaneous treatment of these tumours is painful[176]. These tumours are at risk of leading to tumour seeding when close to the parietal peritoneum and closely related structures are at increased risk of damage during the procedure. When a surgical approach is used, either by laparoscopy or laparotomy, many of these problems can be avoided.

Tumours that are located close to large blood vessels are also associated with an increased risk of local recurrence[55]. This includes those tumours that are located close to the liver hilum in segments 1, 4 and 5[55]. A well-described "heat sink" effect occurs in these areas. This is associated with a poorer quality ablation and higher rate of recurrence[55]. Local blood flow leads to cooling of the tissues adjacent to the vessel. This cooling leads to a decrease in the volume of coagulative necrosis that occurs in this area[168]. The shape of the ablation produced is distorted and is not the typical sphere or ellipse that would be expected[166,168].

Problems associated with the "heat sink" phenomenon can be counteracted by vascular occlusion. At laparotomy or laparoscopy, the portal vessels may be temporarily occluded (a

Pringle manoeuvre). When performing a percutaneous procedure, vascular occlusion can be achieved by placing a balloon catheter angiographically into either the hepatic artery or the portal vein. These manoeuvres have been shown to increase the size of the ablation and make the ablation more uniform and spherical in shape[168]. There appears to be a decreased rate of local recurrence following these manoeuvres[55].

1.2.9.3 Tumour Type, Differentiation and Number

It appears that less aggressive tumours such as neuroendocrine tumours have a lower risk of recurrence than metastases from colorectal cancer and HCCs[53]. However, different tumours have different properties and neuroendocrine tumours tend to have a slower rate of growth than these other more aggressive tumours[95]. It is possible that these less aggressive tumours may recur just as frequently but take a longer time to do so. Longer follow up may be required to demonstrate this. The risk of local recurrence appears to be no different between HCCs and colorectal liver metastases[53,177].

It is not clear whether the degree of tumour differentiation in HCC is associated with a variable risk of local recurrence. Yu et al. found a significant rise in local recurrence rates following treatment of high grade HCCs yet other studies have been unable to show a significant relationship[84,82,85]. Tumour markers such as alpha FP and CEA do not appear to have a significant impact upon the rate of local recurrence but may influence distant recurrence[84,178]. It is interesting to note that CEA levels tend to rise after the early treatment of colorectal liver metastases. It is postulated that this occurs because the antigen is released into the blood stream from the damaged tumour cells[178].

Although some studies have found an increased risk of local recurrence with greater than one tumour, this has not been a consistent finding[177].

1.2.9.4 Radiofrequency Ablation Delivery Technique

The manner in which RFA is performed impacts upon the rate of local recurrence. A large meta-analysis has shown that when patients are treated with a surgical approach, the local recurrence rates are significantly lower when compared to those treated percutaneously[53]. Furthermore, within the context of the surgical approach, laparotomy is superior to laparoscopy[53]. There are several possible explanations why a surgical route is superior to the percutaneous route:

- Arguably, a surgical approach allows the surgeon to more accurately assess the liver. This can be done by direct vision and palpation. Small lesions may be detected that would have otherwise been missed.

- Intra-operative ultrasound is superior to trans-cutaneous ultrasound. Using intra-operative ultrasound, factors that mask the image (such as the ribs, lungs and other gas filled organs) are no longer in the field that is being scanned[179].

- By taking a percutaneous route, the degree of electrode positioning may be limited and the ideal electrode position may not be possible[179]. Using a surgical approach, the operator has much greater scope to position the electrode.

- Vascular manipulation is better controlled when a surgical approach is used[179]. The patient's systemic blood pressure and central venous pressure are easy to

manipulate with the general anaesthetic. A Pringle manoeuvre is generally not difficult to perform during surgery. Patients are also completely immobilised during the procedure when under a general anaesthetic which makes performing the procedure easier.

The obvious disadvantage of a surgical approach is the higher morbidity rates that are associated with this method. However, short-term morbidity should not be a contraindication to treatment if cure is what can be achieved.

1.2.9.5 Electrode and Generator Type

There are 4 basic types of electrode currently available for commercial use. All function in quite different ways[7]. All have been devised to overcome the current limitations of RFA as related to localised charring and ablation size. *Radionics* have produced a "cooled tip" electrode that is a straight needle that is constantly perfused with saline. The electrode tip is kept therefore kept cool (as the name suggests) and is designed to prevent charring. The second type of needle is the "wet needle" which is a straight needle with perfusion holes that allows for isotonic or hypertonic solution to permeate into the surrounding tissues with a subsequent decrease in the degree of tissue charring. The third type is the multi-tined electrode as produced by *Radiotherapeutics* and *Radionics*. These electrodes have multiple small electrodes that are housed inside an insulated cannula. These tines are extended once the cannula has been placed into position. The tines all work concurrently thus producing overlapping ablative zones that fuse and form a single large ablation zone. The fourth variety is the cluster type that usually has 3 straight needles in close proximity and this works in a manner similar to the multi-tined electrodes.

As there have been very few clinical studies that compare these different electrodes, it is difficult to make valid conclusions about their efficacy. Van Duijnhoven et al. have found that the *Radiotherapeutics*-devised multi-tined electrode and *Radionics* cluster electrode had significantly higher local recurrence rates[55]. Although the reason for the poorer performance of these electrodes is not known, there is experimental evidence that incomplete fusion of the ablated areas surrounding the tines (thus producing clover shaped ablative zones) can occur[168]. This may be responsible for the increased recurrence rates for these electrodes as seen by Van Duijnhoven et al.[55,168].

Each type of radiofrequency generator has an inbuilt algorithm also intended to delay tissue charring[7]. These are in the form of feedback loops from tissue impedance levels that allow the generator to make adjustments to power output in an effort to delay the roll-off process[7]. Each type of generator is slightly different but no comparative work has yet been performed.

1.2.9.6 Other Factors Related to Local Recurrence

Other factors that have been shown to significantly increase the risk of local tumour recurrence are; physician experience with RFA, focal residual tumours and the rare event of mistargeting the tumour[180]. Glaibermann et al. have also found that ablation sizes are reduced when performed in cirrhotic livers so this needs to be considered when performing RFA in these patients.[181]

1.2.10 Overcoming Recurrence

When radiofrequency ablation first came into use, it was designed as a purely palliative procedure and in some circumstances was quite useful in this regard. In particular, those patients who had metastatic neuroendocrine tumours and were symptomatic from secretion of somatostatin gained good symptomatic control after RFA treatment[98-100].

As experience with the technique has grown, it has been increasingly used with a more curative intent. In this regard it has shown some promise with reasonable long-term actuarial survival rates being seen. However, while local tumour recurrence rates remain high, the concept of RFA being used with curative intent is unrealistic.

It is imperative that when assessing a liver tumour and the most appropriate approach for its management, a multi-disciplinary approach be used[182]. If treatment with curative intent is planned, then the approach that gives the greatest chance of cure should be utilised. Often this means that a surgical approach may be the preferred modality regardless of the morbidity risks.

The problems associated with tumour location have largely been overcome although tumours sited within the hilum are likely to always remain unsuitable for thermal techniques because of potential damage to hilar structures. The problems associated with subcapsular tumours can be dealt with by using a surgical approach. Heat sink effects can be overcome by either angiographic or surgical vascular occlusion.

The biggest challenge facing RFA is the characteristic size limitation and it is here that concentrated efforts should be made. If a tumour can be treated with a rim of normal tissue

by a single uniform ablation without having to resort to multiple areas of overlapping, then a better quality ablation could be achieved. This should lead to the tumour undergoing necrosis in a more uniform manner. It has been well established that, when localised charring occurs, ablation ceases. This "roll off" process is considered to be an integral part of the ablative process as it is thought to enhance necrosis[183]. If localised tissue charring can be prevented or at least delayed, then ablation should be able to continue for longer and this in turn should lead to a larger volume of necrotic tissue. This may be able to be achieved with the use of liver hydration. Although there is little evidence currently available, there has been one report and some experimental evidence to suggest that the so called "wet electrodes" may be superior to the others when considering local recurrence [55]. These wet electrodes can prevent or at least slow the desiccation that occurs and produce larger volumes of ablated tissue.

The power that can be delivered to the tissues is also dependent upon the state of hydration of the liver[184]. The amount of tissue that is able to be ablated drops off exponentially with increasing distance from the electrode[16]. A fine balance exists where low powers ablate little tissue but very large powers lead to the tissues charring more readily. This occurs as the tissue temperature rises around the electrode and charring of the tissue occurs thus leading to premature cessation of ablation. Slowing this charring process may allow much larger powers to be delivered and hence, much larger ablative zones to be created. By covering a tumour larger than 3 cm in diameter with a solitary ablation, it is hypothesised that its risk of local recurrence should be less.

1.2.11 Conclusion

Radiofrequency ablation shows promise as an effective treatment option for unresectable liver tumours. It has many aspects of the ideal ablation technique being minimally invasive, quick and safe but reliability of cure remains a problem. This is partly because there has been a lack of properly conducted trials in regards to survival benefits over chemotherapy and surgery and possibly due to inadequate pre-clinical, experimental testing.

The problems of long-term cure are particularly relevant to those tumours larger than 3 cm in size as rates of local recurrence remain high. By finding ways to increase ablation size, it is anticipated that if a single ablation could be performed to treat these larger tumours, uniform necrosis would be achieved and the rate of recurrence could be reduced. It is most likely that this could be achieved by finding ways to prevent or at least delay tissue charring that occurs around the electrode tip.

1.3 Electrolysis

Electrolysis, electrochemical therapy or direct current therapy is a novel and experimental technique for the treatment of tumours. Low level, direct electrical current is used to destroy tumours cells by passing the current between 2 electrodes of opposing polarity. It differs from other ablative techniques such as RFA as it employs a non-thermal technique of inducing tissue damage. Although not commonly described as a truly genuine ablative technique for unresectable liver tumours in the medical literature, it has been thoroughly investigated in experimental conditions and there is a large body of clinical experience from China. It also has the advantages of being easy to perform and is relatively inexpensive.

1.3.1 History

The concept of the use of direct current electricity for the treatment of tumours has been around for well over a century. It was initially considered a treatment to induce tumour regression by manipulating electrical fields and electrical properties of the tumours as opposed to destroying the tumour in its entirety. Perhaps the most famous example of the 19th century work was a report by Neftel in 1869 who treated a male patient with recurrent breast cancer with electrolysis[185]. This patient, who was a congressman and eminent member of American society, was not considered to be a suitable surgical candidate and so was treated with electrolytic therapy repeatedly for 12 months[185]. He apparently survived for 3 years before allegedly dying from an unrelated cause [185].

Although there were sporadic reports on the use of electrolytic therapy throughout the 19th century and the first half of the 20th century, it wasn't until the 1950s and 1960s that interest re-emerged in the technique. Humphrey and Seal in 1959 had shown that they could cause regression of sarcoma in a mouse model with the use of electrolysis [185,186]. Further animal

based studies followed that have confirmed the anti-tumour effects of electrolytic therapy. Similar results had been shown in other animal models such as rabbits, rats, hamsters and pigs using other tumour types such as melanoma, lung adenocarcinoma, hepatoma and fibrosarcoma models[187-192].

In 1976, Srinivasan reported the first case of electrolytic therapy use to completely ablate a tumour in a rat model[185]. Up until that time, electrolytic therapy was used to induce tumour regression as opposed to ablation and it was thought that the anti-cancer properties related to electric field effects. This was followed up by studies published in 1980 in which lung tissue using rabbit and pig models were destroyed by electrolytic ablation[193,194].

Perhaps the most influential work on the use of electrolytic therapy came from Bjorn Nordenstrom, a Swedish professor of radiology. He extensively investigated the use of electrical currents to treat tumours in the 1970s and 1980s. As part of his seminal work on biological closed electrical circuits, he used electrolytic therapy to treat 26 primary lung cancers in 20 patients[195]. All of these patients were deemed unsuitable for standard treatments that were available at the time. Of these 20 patients, he was successful in causing tumour regression in 12 of the 26 tumours and completely ablated a tumour in one patient[195]. Over the period (2 to 5 years) that he followed these patients, there was no progression of any "successfully" treated tumour[195].

1.3.2 The Chinese Experience

In 1987, Nordenstrom gave a series of lectures in China based upon his research with biological closed electric circuits that included the results of the 26 patients with lung cancer who were treated with electrolytic treatment. His work was very well received and as a

result of this, the technique was brought into clinical use throughout China where it was known as electrochemical therapy[192]. The use of electrochemical therapy rapidly expanded throughout China from 1987. In 1992, it was reported at the 1st Conference of The International Association for Biological Closed Electric Circuits held in Stockholm that greater than 4000 patients had been treated with electrochemical therapy in more than 800 hospitals[196]. The tumours treated were wide and varied and were a mix of benign and malignant, superficial and visceral[196]. In 1998, the 2nd Conference of The International Association for Biological Closed Electric Circuits was held in Beijing and further follow up data was presented from the Chinese experience. Over 10 000 patients had been treated in over 1 000 hospitals by this time with some interesting long term data[192].

The techniques employed to perform electrochemical therapy were similar to Nordenstrom's descriptions and platinum electrodes were commonly utilised. The anode was generally placed in the centre of the tumour and the cathode placed at the periphery of the tumour[196]. The electrodes were placed 3.0-3.5 cm apart. On occasion, multiple pairs of anodes and cathodes were used to treat the tumour and this depended upon the size of the tumour[196]. Superficial tumours were generally treated under direct vision. Visceral tumour electrodes were placed under X-ray, ultrasonic or CT guidance[196]. Voltages used were mostly in the range of 8-10 volts (V); currents in the range of 40-80 milliamperes (mA) and 100 coulombs (C) were usually delivered per cubic centimetre with delivery time for 25 C taking approximately 25 minutes[196].

While enormous numbers of patients have been treated with the technique, reports of the clinical experiences with the technique are few. Despite the claims that over 10 000 patients had been treated with electrochemical therapy up to 1998, data was reported in only 7642

malignant cases (3802 superficial and 3840 visceral)[192]. The treatment had been used with many different types of tumours but the most commonly treated malignant tumours were lung carcinomas (1113 patients), skin cancers including melanoma (1185 patients), breast cancers (644 patients) and liver cancers (961 patients)[192]. Of the 3802 patients with superficial malignant tumours, 80% of tumours demonstrated either a complete or partial response to the electrochemical therapy[192]. Seventy two per cent of the 3840 patients who had visceral tumours treated experienced either partial or complete response[192].

These reports from China can be criticised. Large numbers of patients were lost to follow up. Of those patients that were available to follow up, the data presented on the patients and the tumour characteristics are vague. Heterogeneous groups of tumours were treated in many different facilities and the treatments did not appear to be performed in a standardised manner. Appropriate evaluation in animal models prior to clinical use does not appear to have occurred. Statistical analysis is lacking and perhaps most importantly, the patients were not treated in clinical trials and therefore, the treatment was not compared with other more standard treatments (or even no treatment). However, all liver tumour-ablative technologies including RFA have been the subject of this kind of non-scientific approach to development and treatment to some extent.

From this large body of anecdotal evidence, it can probably be concluded that electrochemical treatment appears to be safe to perform in a clinical setting and that it was quite likely to be useful in the setting of unresectable tumours. The use of electrochemical therapy for the treatment of unresectable tumours certainly warranted further investigation, albeit, with a more scientific approach.

1.3.3 The University of Adelaide Experience

In 1997, the first report on the use of electrolysis (as it was now known) in a patient with colorectal liver metastases from a group at The University of Adelaide in Australia was published. This group was led by Professor Guy Maddern FRACS in collaboration with Mr Ashley Dennison FRCS from Leicester in the United Kingdom[197]. It was the intention of this group to systematically test and develop electrolytic treatment as a potential ablative therapy for unresectable colorectal liver metastases. This was conducted through a series of animal and clinical experiments that were published from the late 1990s to the mid 2000s.

Large animal studies to assess safety and morbidity confirmed the presence of coagulative necrosis that had healed by approximately 4 months[198]. Safety aspects were confirmed in animal models and there was never any evidence of major morbidity associated with the procedure[198,199]. In particular, there was no major haemorrhage, bile leak or bile duct injury or evidence of liver dysfunction.

Furthermore, there was no evidence that it produced any *SIRS* type reaction or any multi-organ failure in a manner similar to cryoshock, the cytokine mediated form of shock and multi-organ failure[200,201]. This was confirmed by both clinical observation and specific testing for the presence of the cytokines TNF alpha, IL-1 and IL-6 that are implicated in driving this form of shock[200,201]. It was postulated that because thrombosis of small vessels around the ablation site, this prevented the rapid release of these cytokines into the general circulation.

Most of the preceding work had been in the presence of normal liver tissue. Following the publication of 2 case reports, one with a patient with colorectal metastases and one with

HCC, it was confirmed liver tumours could potentially be successfully treated with the technique[197,202]. This was followed up by a study where 5 patients undergoing curative liver resection had one of their metastases treated with electrolysis prior to resection[203]. This study confirmed that these tumours could undergo complete necrosis and that it could be performed safely under general anaesthetic. No form of major morbidity was seen and in particular, no *SIRS* type reaction was observed in any patient post-operatively and no cardiac arrhythmia was seen.

A follow up study in 9 patients with unresectable colorectal liver metastases was performed with a median follow up time of 9 months[204]. The results of this study showed that complete radiological response was obtained in all patients. Two out of 9 patients developed local recurrence and 6 out of 9 developed distant recurrence. Four out of 9 developed extrahepatic spread and 3 out of 9 died during the follow up time as a result of disease progression.

The major advantage of electrolysis over other ablative techniques is that it has been shown that potentially, it could be safely used around blood vessels[205]. As it is a non-thermal technique, it does not have associated problems with heat sink[205]. At the doses of the technique that were used, it was shown that the necrosis can be limited by the blood vessel wall and therefore, the risk of haemorrhage is minimised[205].

There are 2 major limiting factors of the technique; the lack of real time radiological monitoring and delivery time required[206].

- Ultrasound was not useful to monitor electrolysis as the gas production that occurs at both electrodes made monitoring of the technique impossible[206]. As a result, all electrolytic ablations performed in this series of experiments were with a laparotomy and direct vision. The size of the tumours had to be accurately determined prior to treatment and the appropriate coulomb dose had to be calculated to ensure complete coverage of the tumour with a surrounding area of normal tissue. Potentially, this limitation could be overcome by use of a monitor probe that could accurately measure pH at the anode and cathode and this was successfully used in a large animal study[206]. This form of monitoring is invasive however and may be difficult to use in a percutaneous setting[206].

- The other major limiting factor of electrolysis for liver tumours was the time span that was required. Electrolysis is effective yet slow and these studies showed that for tumours from 5 to 30 mm in diameter, 42 to 210 minutes were required[203]. The report of the HCC patient had a treatment time of 288 minutes[202]. This limitation was problematic and did not lend itself to a simple solution. By increasing the settings of the electrolysis, there was the potential to induce heat effects. This would introduce a different therapeutic mechanism.

As these limitations could not be simply overcome, the use of electrolysis or electrochemical therapy for the treatment of liver tumours has remained an experimental technique in the western world.

1.3.4 Mechanism of Action

Electrolysis is quite different from other ablative therapies in that it has a non-thermal mode of action. Although a small temperature rise can occur with this treatment, it does not reach temperatures that will cause tissue damage. It is a technique whereby a low level direct electrical current is passed between 2 conducting electrodes of opposing polarity (positive and negative) that are placed in biological tissue. The electrodes are designated anode (+ve) and cathode (-ve). The biological tissue acts as an electrolyte solution that facilitates the movement of ions between the 2 electrodes.

1.3.5 Electrochemical Changes

When the current is commenced, a series of electrochemical changes occur at each electrode that ultimately results in tissue damage. This primarily occurs through the decomposition of water molecules and from oxidation and/or reduction of molecules dissolved within the water.

At the anode, water is broken down to form acidic hydrogen ions with oxygen being liberated as a gas. Consequently, the pH of the area falls to the vicinity of 1-2[185]. It has also been found that chloride ions become oxidised to form molecular chlorine and this can be liberated as a gas[185]. The metal electrodes become corroded during the process and form metal ions that move toward the negative cathode[185]. Water molecules tend to move from the anode to the cathode so the tissues around the anode tend to become desiccated[185].

Tissue injury at the anode occurs primarily as a result of the acidic environment that is created by the electrolytic process. As well as being given off as a gas, some of the chlorine that is formed can diffuse into tissues[185]. Chlorine is a powerful oxidant that can also

induce tissue damage[185]. One of the reactions that occur as a result of the presence of chlorine is the oxidation of haemoglobin to methaemoglobin[185]. The deposition of methaemoglobin onto the metal anode gives the anode its characteristic black colour at the end of the electrolytic procedure[185]. As a result of the acidic environment and the presence of chlorine, the volume of tissue necrosis tends to be greater at the anode than cathode.

The anode reactions are summarised below[185]:

Anode

$2\ Cl^- \rightarrow Cl_2 + 2e^-$

$M \rightarrow Mn^+ + ne^-$ (M is a metal, n is its valency)

$M_2O + 2OH^- \rightarrow 2MO + H_2O + 2e^-$

$2\ H_2O \rightarrow O_2 + 4H^+ + 4e^-$

At the cathode, water is converted to hydroxide ions with the liberation of hydrogen as a gas. The formation of hydroxide ions leads to a rise in tissue pH to approximately 9. These ions are a powerful reducer of substances and thus produce tissue injury[185]. Molecular hydrogen can also cause tissue destruction. Dissolution of the metal cathode material also occurs at the cathode with the presence metallic salts causing further tissue injury[185]. Metal oxides located within the region are further reduced by the presence of electrons attracted to the area and this results in the further production of hydroxide ions[185]. As the cathode reactions primarily involve the reduction of water molecules, water is constantly attracted into the region, thus making the region oedematous[185]. The effect of sodium hydroxide on haemoglobin can lead to the formation of haemochromogens which are also black in colour[185]. Due to the presence of water, this is usually considerably less intense in colour than that seen at the anode[185]. The cathode reactions are summarised below[185].

Cathode

$$2H_20 + 2e^- \rightarrow 2\ OH^- + H_2$$

$$2\ MO + H_2O + 2e^- \rightarrow 2M_2O + 2OH^-$$

$$O_2 + 2\ H_2O + 4e^- \rightarrow 4OH^-$$

Hydrogen gas is liberated from the cathode and chlorine and oxygen gas are liberated from the anode.

In conclusion, oxidative reactions occur at the anode that leads to the formation of a very acidic environment and the formation of molecular chlorine. Both the acidic environment and the diffusion of chlorine into tissue lead to extensive tissue damage. At the cathode, reduction type reactions tend to occur and hydroxide ions are formed. Hydrogen gas is also liberated and molecular hydrogen may play a small part in causing tissue injury. The greatest amount of tissue injury occurs at the anode and water moves from the anode to cathode, thus making the cathodic region oedematous.

1.3.6 Tissue Ischaemia

During the process of electrolysis, localised tissue ischaemia occurs as a result of microvascular thrombosis that occurs in the capillaries around the tumour[185]. This phenomenon particularly seems to occur in relation to the anode but also occur at the cathode[185]. This is thought to further augment the tissue damage that occurs as a result of electrolysis treatment.

However, when used close to large blood vessels, the risk of inducing thrombosis appears to be minimal. This has been confirmed experimentally when electrodes have been placed inside large calibre vessels. After the delivery of 100 C, only 1 out of 4 vessels developed thrombus[198]. The reason is thought to relate to continual blood flow removing pro-thrombotic constituents that present in the blood during electrolysis[198].

Furthermore, when electrolysis is performed near large blood vessels, the risk of damage to the blood vessel itself appears to be minimal as the electrical resistance of the blood vessels is much higher than that of normal parenchyma[192]. This results in current flowing along different paths rather than through the vessel as it follows the path of least resistance[192].

Electrolysis has the advantage over other thermal modes of ablation in that theoretically, it is a safe and potentially useful treatment of tumours located very close to large blood vessels. It causes neither damage to the vessel nor thrombosis within the vessel and being a non-thermal treatment, it is not susceptible to the cooling effects of the intact blood flow leading to impaired tissue necrosis as a result of heat sink[192].

1.3.7 Movement of Water

As alluded to in the earlier discussion on the electrochemical changes that occur at the electrode, water tends to move from anode to cathode resulting in tissue desiccation at the anode and tissue oedema at the cathode. This phenomenon has been widely recognised and reported and is seen at both macroscopic level and microscopic level.

When examining tissue that has been treated with electrolysis, one can appreciate obvious necrosis surrounding both electrodes. However, the tissue that is seen immediately adjacent

to the cathode shows swollen and oedematous cells that are being repelled from one another[207]. The tissue surrounding the anode is desiccated with small cells that are actively undergoing necrotic changes[207].

The mechanism for the transport of water in these circumstances is complex and is related to processes of electro-osmosis. Nordenstrom had identified 4 electro-osmotic water transport systems and some of these can explain the movement of water from anode to cathode in a closed electric circuit[195].

Nordenstrom describes Type 1 electro-osmosis or "fixed charge electro-osmosis" as a net movement of water that results from the electrostatic interaction of the di-electric matrix and aligned water molecules[195]. This form of electro-osmosis requires narrow interstitial channels or "capillaries" (as Nordenstrom describes them) with fixed charges. Most cells have a surplus of fixed negative charges on their surfaces and as water moves through the interstitial spaces thus approximating the movement through these "capillaries", movement from positive to negative electrode ensues[195]. The direction of water flow is dependent upon the polarity of surplus charge of the "capillaries". If positive, the water molecules move from negative to positive and if the surplus charge is negative, then water moves from positive to negative[195]. As the surplus charge is negative, the direction of flow is from positive to negative (**Figure 1.2**).

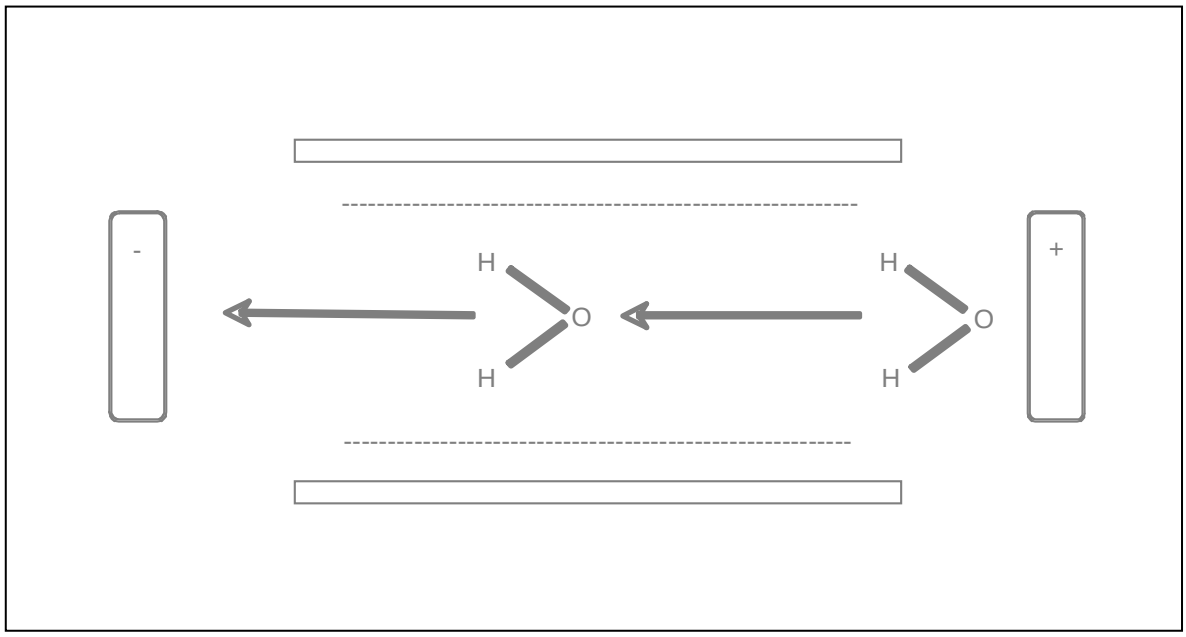

Figure 1.2

As there is a surplus of negative charge on the surface of most cells, as water molecules pass through interstitial tissues, the molecules become aligned and pass from positive to negative electrode.

Type 2 electro-osmosis results from the decomposition of water at the electrodes into hydrogen ions and hydroxide ions. These move away from their respective electrodes and eventually recombine to form new water molecules. In the in vivo setting, H^+ ions move more freely through tissue than OH^- ions due to the inherent electronegative charge of tissues. As a result, there is a net accumulation of water towards the cathode side of the circuit.

Type 3 electro-osmosis results from the fact that hydrogen ions (H^+) are able to bind with water molecules and can therefore carry water from anode to cathode. Anions (ie OH^-) are unable to bind water.

$$H^+ + H_2O \rightarrow H_3O^+$$

Type 4 electro-osmosis, also known as "field-induced osmosis", occurs close to the charged electrodes. The electrodes attract water molecules that become continuously electrolysed. As water is attracted to each electrode, it is unlikely that this form of electro-osmosis contributes to the net movement of water from one electrode to the other.

As a result of Types 1, 2 and 3 electro-osmosis, a net flux of water from anode to cathode occurs.

1.3.8 Other Considerations

Previous research had shown that the volume of necrosis that is produced by electrolysis is in a direct, linear relation to the amount of current that is passed through the tissue[192]. Therefore, discrete dosages of current could be delivered that would induce a predictable amount of tissue damage. This current dose is measured in coulombs (C) and is calculated by multiplying the amount of current passed (amperes) by the time it is passed (seconds). Most tumours required a dosage in the order of 1000 C to treat.

Electrode materials may be of importance. Some types of metals have a tendency to electrolyse easily, thus leading to preferential dissolution over the tissue effects. Some metals such as lead are clearly not suitable to be used as electrodes as salts that can be

formed can be toxic. Platinum has commonly been used for electrolytic experimental and clinical work[185]. It is relatively inert and the small volume of platinum salts that are produced may have antibacterial and antineoplastic properties[185]. The disadvantage of using platinum is that it can be brittle and is relatively expensive[185].

1.3.9 Clinical Experience with Liver Tumours

Apart from the material that was published by The University of Adelaide group, there have been very few reports that describe the use of electrolysis with liver tumours.

Using electrochemical therapy as a treatment for liver tumours, 2 short reports from China were published. Lao et al. reported on 50 patients with liver tumours treated with electrochemical therapy[208]. Most tumours were HCCs. The size of tumours treated ranged from 3.5 to 21 cm in diameter. All tumours were deemed unresectable. Complete response was seen in only 11 patients and 17 patients showed a partial response. The 12-month survival rate was calculated to be 69%. In a study of 74 patients, Wang Hua-Ling found a 12 month survival rate of 33.33% when patients with HCC were treated with electrochemical therapy alone[209]. This improved to 58.33% when electrochemical therapy was combined with TACE and this was statistically significant ($p<0.05$).

Of the 961 patients with liver tumours treated with electrochemical therapy that were reported at the 2nd Conference for The International Association for Biological Closed Electric Circuits in Shanghai in 1998, 69% of patients exhibited either complete or partial response to the treatment[192]. A 5-year survival rate of 15% had been calculated which compares favourably with the 5% 5-year survival rate that is seen in western countries[192]. However, this favourable result must be kept in context of a large proportion of patients

being lost to follow up. Since 1998, there does not appear to be any further follow up reports published on the use of electrochemical therapy use in China.

1.3.10 Conclusion

Electrolysis is a non-thermal form of ablative therapy that has been extensively investigated as a potential treatment for liver tumours. It involves passing a direct electrical current between 2 electrodes to cause electrochemical changes within the tissue that result in tissue damage. Electrolysis will also produce a shift in water from positive electrode to negative electrode. It is safe and well tolerated but is limited in the clinical setting by time constraints and an inability to be easily monitored by radiological means.

1.4 Bimodal Electric Tissue Ablation

From the previous discussions, it has been identified that an ablative technology that can effectively destroy tumours would have a role in the treatment of unresectable liver metastases and hepatocellular carcinomas. The treatment would be useful for those specific types of liver tumours that are confined to the liver without extrahepatic spread that are not amenable to surgical resection. Radiofrequency ablation shows promise in this regard and is able to be performed percutaneously or surgically, appears to be well tolerated by patients and is effective in causing tumour destruction. It is limited by size constraints that result from tissue desiccation and localised tissue charring which prevent the conduction of current. As a result, tumours larger than 3 cm in diameter are less effectively treated and the rate of local recurrence is higher.

Liver electrolysis has also shown promise as an ablative technology for treatment of liver tumours. It too could effectively cause tissue destruction and appears to be well tolerated by patients. Although predominantly used surgically, it could easily lend itself to a percutaneous route. Electrolytic doses can be used to produce defined volumes of tissue necrosis in a linear relationship. Electrolysis has the advantage of being useful around blood vessels and is not susceptible to the heat sink effects of other thermal ablation modalities. It produces movement of water from anode to cathode thus making tissues surrounding the cathode oedematous and the anode desiccated. It was limited by time constraints as it was a very slow process and there was no easy solution to overcome this.

As the fundamental problem of RFA size is based upon tissue desiccation and as electrolysis has a problem with time constraints but moves water from one electrode to the other, it is possible that by combining the 2 modalities, the inherent weaknesses of both modalities

could potentially be overcome. By joining the cathode of the direct current to the main radiofrequency current with the anode placed close by, potentially both currents could be run simultaneously with water being drawn into the tissues surrounding the electrode as they are being heated. As there are now 2 modalities working together, this concept has been termed “Bimodal Electric Tissue Ablation” (BETA) (**Figure 1.3).**

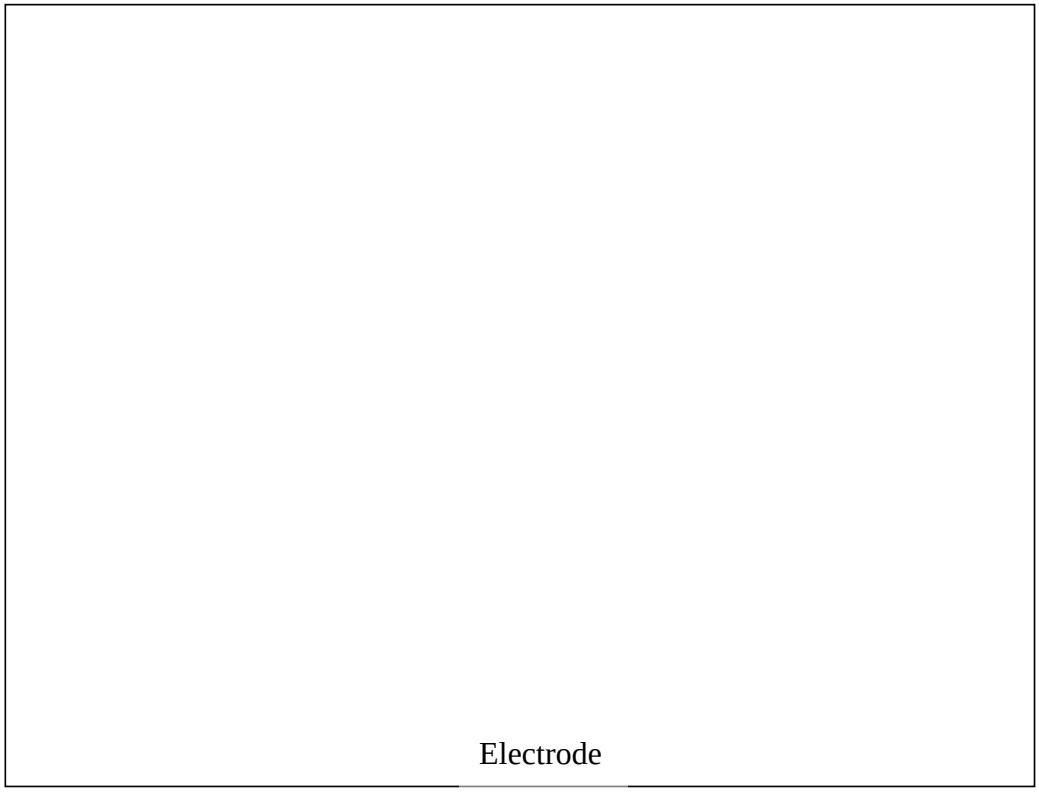

Figure 1.3

Diagrammatic representation of Bimodal Electric Tissue Ablation

On the left side is a standard radiofrequency set up. On the right side is the direct current set up with –ve electrode connected to the radiofrequency electrode. Water should then move from +ve electrode to the ablating electrode.

This combination would most likely result in a thermal-type ablative therapy. The main contribution of the electrolysis aspect of the bimodal technique would be to provide hydration to the area as the radiofrequency aspect heats the tissues. Electrolysis may make a small contribution to ablation but it would most likely be negligible, particularly as it would be the cathode that would be attached to the main electrode and the entire ablation technique would most likely be relatively rapid when compared to the process of electrolysis. In essence, the technique is a modified form of RFA. As it is thermal energy produced by radiofrequency waves that will create the ablation, the pathological process will be identical to that using standard RFA.

The aim of any modification to RFA is to be able to provide a superior treatment for tumours that are greater than 3 cm in diameter. In theory, the BETA technique should eventually be able to achieve this aim.

It is hypothesised that BETA will provide a treatment for these larger liver tumours superior to the standard forms of RFA. As water is drawn into the area being heated, tissue desiccation should be reduced and the time to subsequent tissue charring should be prolonged. This should result in a large volume of tissue necrosis. It is anticipated that in the clinical setting, this would result in larger tumours being covered with a single ablation without having to resort to the multiple overlapping techniques that are currently employed. A single ablation should produce necrosis in a more uniform manner with a smaller risk of incomplete necrosis. The end result of this may be a lower risk of localised recurrence after treatment. The chance of cure should therefore be greater.

Prior to testing this hypothesis in patients with liver tumours, a BETA circuit needs to be constructed and vigorously tested in experimental settings. One of the major criticisms of all ablative technologies is they are often rushed into clinical use prior to appropriate laboratory testing. It needs to be confirmed that an ablation performed by the BETA circuit is larger than an ablation performed with similar settings on a standard radiofrequency circuit. Furthermore, the safety aspects of the BETA circuit need to be confirmed. It has been well established that RFA and liver electrolysis are safe when used in situ in both experimental and clinical settings. It is therefore likely that the BETA procedure would also be safe to perform.

Ideally, the new circuit should be tested in a tumour model. Both HCC and colorectal liver metastases models are able to be reliably produced in small animals such as mice and rats. Unfortunately, there is no large animal liver tumour model available that is reliable enough to be used for an experiment such as this. Therefore, all experiments were performed in normal liver. This too has been a problem for all ablative technologies. However, once the safety aspects have been confirmed, then BETA could potentially be performed in the human setting, within the context of a clinical trial.

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Chapter 2

Study Aims and Hypotheses

Three experiments have been performed testing the BETA system in a porcine model.

2.1 Experiment 1

The aim of the first study is to compare sizes of the ablation produced in normal liver with the BETA system with those produced from a standard RFA system. The ablations performed by the radiofrequency system will act as controls. This study will also compare the pathological changes created by the 2 systems.

For this study, the following hypotheses have been made:

- By constructing a bimodal electric current system with the cathode placed at the main electrode of the radiofrequency circuit and the anode placed nearby, water will be attracted to this electrode.

- As water becomes attracted to the negative electrode, the amount of localised tissue charring will be reduced and therefore, the time for ablation will be able to continue for longer prior to “roll off”.

- If the time of treatment should be longer, this will result in a larger ablation being produced.

- ❖ The pathological changes in the treated liver will appear similar at a macroscopic and microscopic level when ablations produced with the BETA system are compared to the RFA controls.

2.2 Experiment 2

The aim of the second study is to assess the result of treatment with the BETA system over a longer term. This study will assess any morbidity associated with the procedure by way of direct observation of the animal, regular blood test analysis and autopsy. Pathological changes in the treated liver tissue will also be assessed over time to confirm that the injuries heal in an appropriate manner. The animals will be killed at different time points to ascertain how the injuries produced change over time.

The following hypotheses have been made:

- ❖ Injuries produced with the system will behave like other thermal therapies and undergo coagulative necrosis with timely healing by fibrosis.

- ❖ Treatment with the BETA system will be as safe as RFA.

2.3 Experiment 3

The third experiment has been performed as a result of the second experiment. Although it shall be discussed in greater detail in subsequent chapters, it was found that tissue damage occurs at the site of the positive electrode during the second experiment. It is the aim of this experiment to test the BETA system using a grounding pad placed upon the skin that is attached to the positive electrode. This shall be compared with ablations produced with the

previous set up where the positive electrode was connected to a scalpel blade placed under the skin and RFA controls. This study will also be able to confirm the findings of the previous 2 studies.

For this study it has been hypothesised that:

- By increasing the surface area of the positive electrode, the risk of inducing tissue damage will be reduced.

- The ablations produced will be of a similar size to those produced with the previous set up and should be larger than controls.

- The injuries produced will be similar at a macroscopic and microscopic level.

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Chapter 3

Materials and Methods

3.1.1 Materials

3.1.1 Radiofrequency Generator (**Figure 3.1**)

A *Boston Scientific RF 3000* radiofrequency generator was used for all experiments. This generator is available commercially and is commonly used in clinical practice. The generator can connect to one electrode and up to 4 grounding pads. Radiofrequency energy is simply delivered to tissues by manually selecting the power level (up to a maximum of 200 watts (W)) and starting the generator. Upon commencement of the ablation process, the generator makes continual measurements of time, power in watts and impedance (tissue resistance) in ohms (Ω). As the tissue desiccates, an increasing impedance level reflects the resultant tissue resistance. The generator has an inbuilt algorithm that reduces the amount of power being delivered to the tissues as the impedance rises until "roll off". At this point, the system shuts down attempting to direct any more radiofrequency energy into the tissues. In clinical settings with this machine, the process is usually started with the wattage set at 80 and increased by 10 W every 10 minutes until "roll off". The process is then usually repeated.

3.1.2 AC/DC Power Adaptor (**Figure 3.2**)

A *Maplin* AC/DC power adapter was used for all experiments. This particular power adaptor has two insulated wires that connect to an electrical plug and converts an alternating current to a low voltage direct current. This has been modified by having the plug removed and two alligator clips connected to the wires. These two wires act as positive (anode) and (cathode) electrodes. This particular adaptor has the ability to have the voltage manually adjusted from 3 volts (V) up to 12 V.

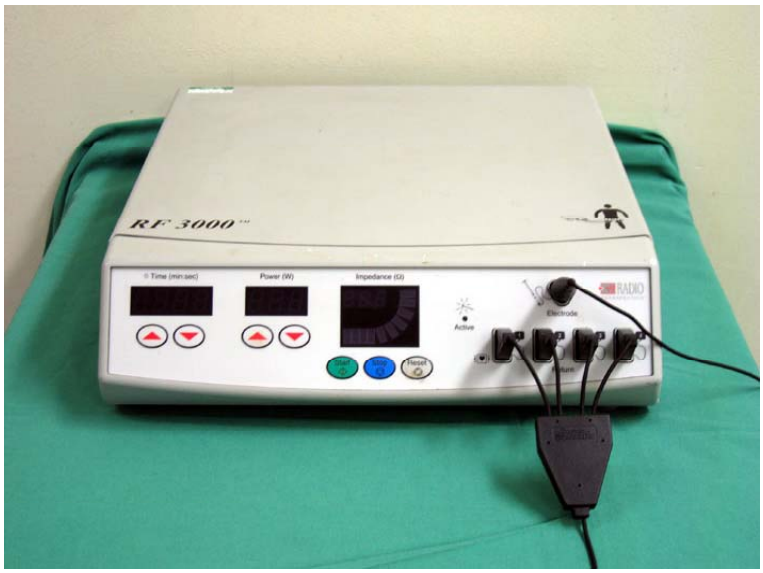

Figure 3.1 *Boston Scientific RF 3000 radiofrequency generator*

Figure 3.2 *Maplin AC/DC power adaptor*

3.1.3 Electrodes (Figure 3.3)

For the first experiment, a *Boston Scientific* 35 mm *LeVeen* multi-tine needle electrode was used. This particular electrode complements the *Boston Scientific* generators. It has an insulated needle that house 10 electrodes. Upon placement of the needle, the tines are

extended from the needle into the surrounding tissues in an umbrella formation. Upon completion of the ablative process, the tines are retracted and the needle is removed. In the clinical setting, these electrodes are considered disposable and are contraindicated from re-use. This particular electrode is designed to produce a spherical 35 mm diameter zone of necrosis.

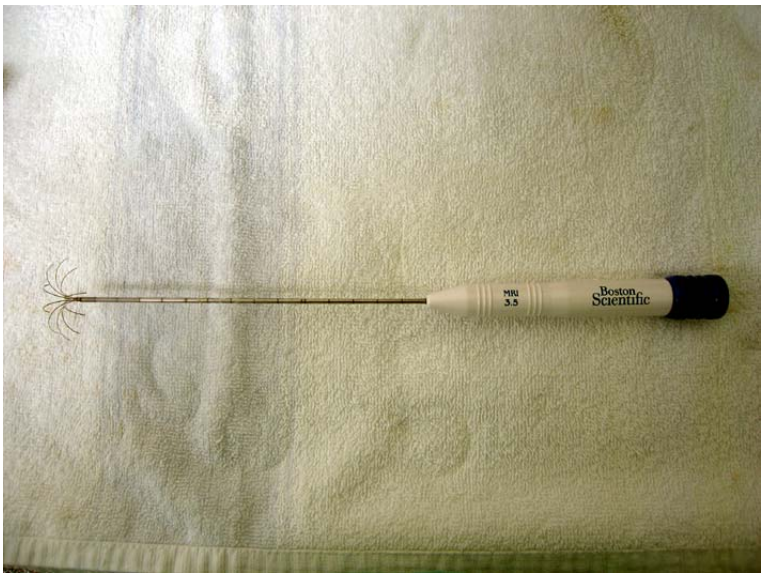

Figure 3.3

Boston Scientific 35 mm multi-tine needle with 10 tines (fully extended)

The 2 follow up studies used 16 gauge aluminium rods, 4 cm in length. These were prepared from a long piece of aluminium and worked with both currents, easily fit within the electrode wire of the radiofrequency generator and were inexpensive (**Figure 3.4**). A new electrode was used in each animal.

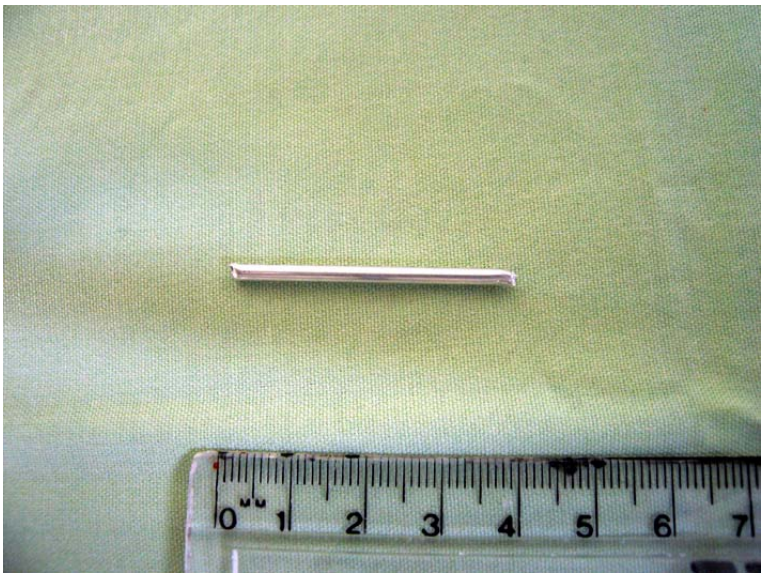

Figure 3.4

Example of 4 cm aluminium rod used in Experiment 2 and 3

3.1.4 Bimodal Electrical Tissue Ablation (BETA) Circuit (**Figure 3.5**)

The BETA circuit basically involves the addition of the direct electrical current to the alternating radiofrequency current. To achieve this, the 2 generators that were described in **Sections 3.1.1** and **3.1.2** were used. The wire that extends from the radiofrequency generator to the electrode to had some of its insulation removed. This was done to allow the 2 circuits to be united. The negative electrode of the AC/DC adaptor was connected to an inductor rated at 1 mH and this inductor was connected to the radiofrequency generator wire at the point where the insulated covering was removed. The inductor was used as this allows leakage of the direct current into the higher frequency alternating current but prevents back flow of the higher frequency current into the direct current circuit. The positive electrode of the direct current generator was attached to a scalpel blade and this was placed into the subcutaneous tissues of the animal to complete the direct current circuit. The radiofrequency circuit was completed by the addition of 2 grounding pads that were placed on the skin the animal. Two pads were used as skin burns could occur with the use

of only 1. This circuit described above was able to have either the direct current or the radiofrequency current running individually or simultaneously and the wattages and voltages could be manually set.

Figure 3.5

Bimodal Electric Tissue Ablation Circuitry

A–radiofrequency generator; B–AC/DC power adaptor; C–positive electrode of direct current connected to scalpel blade; D–negative electrode connected to inductor; E–connecting wire; F–electrode; G–Radiofrequency electrode wire; H–Wires connecting to grounding pads

3.1.5 Animals

Specific pathogen-free (SPF) female domestic white pigs were used for the study. These animals were used, as their livers are similar in size and anatomy to human livers.

Each pig was approximately 3 months old and weighed between 25 and 35 kg. The pigs were housed at The Queen Elizabeth Hospital animal house and each procedure was performed at this facility. If the pigs were to be kept alive post-operatively for more than 2 weeks, they were transferred to the Institute of Medical and Veterinary Science Gilles Plains campus as The Queen Elizabeth Hospital animal house was not suitable for long term housing.

All animals were admitted to the experimental facility at least 2 days prior to the experiment for a period of acclimatisation. The animals were housed in individual pens, maintained at 23+/-1 degree Celsius, at ambient humidity. Lighting was artificial, with a 12-hour on/off cycle. The air exchange rate and airflow speed complied with the Australian code of practice for the care and use of experimental animals. The pigs were fed and watered ad libitum (standard grower diet of 0.7 g of available lysine per megajoule of digestible energy, with a digestible energy content of 14MJ/kg). Water quality was suitable for human consumption. Pre-operatively, the pigs were fasted for 12 hours.

The animals used in the survival studies were monitored daily by research staff and animal house technicians. Clinical record sheets were used to record animal progress post-operatively. Each clinical record sheet was specifically designed for the particular experiment the animal was undertaking. If the animal exhibited any signs of distress, it was to be electively euthanased under general anaesthetic with a lethal dose of intravenous phenobarbitone.

The general conduct of each study conformed to the 'Code of Practice for the Care and Use of Animals for Scientific Purposes' (NHMRC/CSIRO/AAC 2004) and the SA Prevention of Cruelty to Animals Act 1985.

3.1.6 Grounding Pads (**Figure 3.6**)

Grounding plates produced by *ValleyLab* were used for all studies. These particular plates are placed on the skin after shaving of the area and have a gel plate to ensure a good connection to the skin. A new disposable plate was used for each experiment.

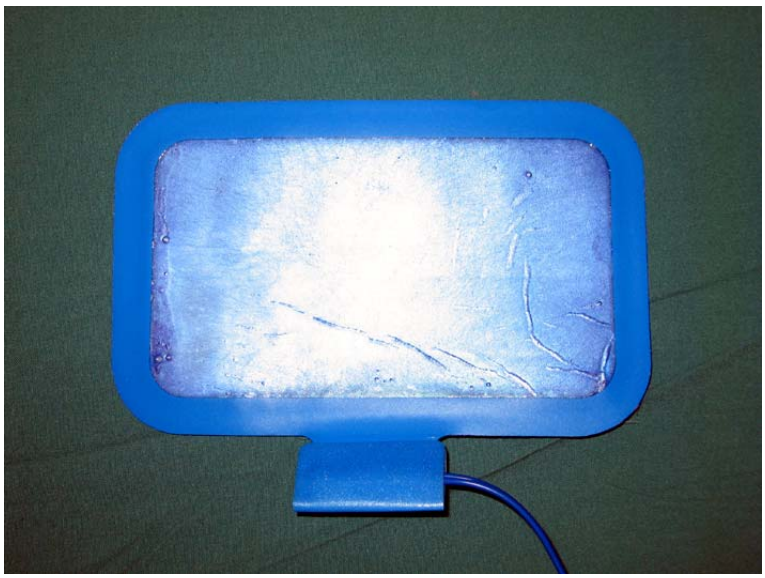

Figure 3.6

ValleyLab grounding pad showing gel plate that attaches to skin

Used in all experiments and acted as positive electrode in Experiment 3

3.2 Experimental Methods

3.2.1 Experiment 1

The primary aim of the first experiment was to compare the diameters of ablations produced by the BETA circuit with RFA controls.

This studied was given ethical approval from the University of Adelaide Animal Ethics Committee and the combined Institute of Medical and Veterinary Science/ The Queen Elizabeth Hospital animal ethics committees.

This was a non-survival study and the general conduct of the study conformed to the 'Code of Practice for the Care and Use of Animals for Scientific Purposes' (NHMRC/CSIRO/AAC 2004) and the SA Prevention of Cruelty to Animals Act 1985.

Anaesthesia

All procedures were performed under general anaesthetic that was administered by an experienced animal house technician. Pigs were completely fasted of food for 12 hours prior to commencement of anaesthesia. Every pig was sedated with intramuscular ketamine (10 mg/kg) mixed with xylasine (1mg/kg). One per cent inhalational halothane was used to induce and maintain anaesthesia. An endotracheal tube was placed to secure the pig's airway once anaesthesia was induced. An endotracheal temperature probe was placed inside the endotracheal tube to monitor core temperature of the animal. The animal was placed upon a warming pad in the base of its cradle to assist in temperature homeostasis. A lingual oxygen saturation probe was used. ECG monitoring was utilised throughout the procedure and two leads were placed above the left and right front legs and one below the

left front leg. Readings of heart rate, temperature, oxygen saturations, end-tidal carbon dioxide levels and cardiac rhythm were recorded throughout the procedure. The pig received normal saline solution intravenously by a cannulated ear vein placed after anaesthesia was established. At the end of the procedure, the animal was killed by an intravenous injection of phenobarbitone whilst still under anaesthesia and the liver was harvested.

Ablation Procedure

Grounding pads connected to the *RF 3000* generator were placed upon the legs of the animal. A midline laparotomy incision was made from the xiphisternum to the umbilicus. The stomach, spleen and bowel were protected by use of moist gauze packs. The ligaments that attach to the liver at its periphery were divided to allow full mobility of the organ.

All RFA controls and ablations produced by the BETA circuit were performed in the left lateral lobe of the liver or the large median lobe of the liver. The median lobe can be further subdivided into the left and right medial lobes. The right lateral lobe was not used as it is situated quite posteriorly in the animal and is difficult to access.

To perform a control ablation, the electrode was placed within the liver tissue and the tines were fully extended. The liver was checked to ensure that no tine had passed through to the other side and to ensure that no other organ was at risk of damage. The starting wattage was set at 80 W. Power and impedance values were recorded every 30 seconds from commencement until completion of the ablative procedure. Completion was deemed to have occurred when the wattage value fell below 5 W and/or the impedance value rose to greater than 700 Ω on 2 consecutive readings. If 25 minutes had occurred since the

commencement of the ablation process, the procedure was electively terminated. At this point, the generator was stopped and the electrode removed. Haemostasis was secured prior to the commencement of the next ablation (by direct pressure to the ablated area for 5-10 minutes). Surgical diathermy to the electrode site was utilised if direct pressure failed. No direct current was used during this part of the procedure.

The BETA technique was performed in 2 stages. A 15-minute period of treatment with direct current alone was performed. This was followed by use of both circuits simultaneously. To achieve an ablation with the BETA circuit, a scalpel blade connected to the positive electrode was placed into the subcutaneous tissues of the laparotomy wound. The negative electrode was connected to the radiofrequency electrode wire in a manner described in **Section 3.1.4**. The electrode was placed into the liver parenchyma and the tines fully extended. The liver was checked to ensure safe placement of the probe in a manner similar to the controls. The AC/DC adaptor was set at 9 V and commenced for 15 minutes. The presence of a direct current was confirmed by the observation of bubbling from around the electrode and objective measurement of the current by use of an ammeter/voltmeter.

Upon the completion of the first phase of BETA treatment, the radiofrequency circuit was started at 80 W and both the direct current and radiofrequency circuits were run simultaneously. Measurements for power (watts) and impedance (ohms-Ω) were recorded every 30 seconds from commencement until the ablation process was deemed to have finished as measured by 2 consecutive power readings of less than 5 W and/or impedance values of greater than 700 Ω.

Up to 4 non-overlapping controls were performed in the left lateral lobe of the liver, depending upon space. Readings were taken for wattage and impedance every 30 seconds until roll off. Following this, up to 4 non-overlapping ablations with the BETA circuit were performed in the larger median lobe of the liver. Recordings were taken every 30 seconds for impedance and wattage.

Ablations were repeatedly performed until it was felt that no further ablation could be performed without encroaching upon other ablative zones already created.

Ablation Assessment

After death, the liver was harvested and the treated areas were dissected from the liver mass and divided along their greatest diameter. Two experimenters measured the diameters and the mean result was recorded. Photographs were taken of each ablation. The ablation zones were then placed in 10% buffered formalin for a period of 2 weeks. They were then prepared for histopathological examination with haematoxylin and eosin staining.

Comparison

Ablation zone diameters and time durations of the 2 groups were compared. The macroscopic and histologic appearances were recorded and compared.

Statistics

Data for size of the ablative zones was analysed using a linear mixed model fitted using REML (restricted maximum likelihood) variance analysis, treating the ablation methods as fixed effects and pigs as random effects. Analysis was carried out using the GenStat computer program. Statistical significance was set at $p<0.05$.

3.2.2 Experiment 2

The aim of the second experiment is to perform a long-term safety and morbidity study and to confirm that timely healing of the ablated areas occurs.

This study was a survival study and was given ethical approval from the University of Adelaide Animal Ethics Committee and the combined Institute of Medical and Veterinary Science/ The Queen Elizabeth Hospital animal ethics committees. The general conduct of the study conformed to the 'Code of Practice for the Care and Use of Animals for Scientific Purposes' (NHMRC/CSIRO/AAC 2004) and the SA Prevention of Cruelty to Animals Act 1985.

Anaesthesia

Each pig underwent a general anaesthetic with anaesthesia provided by an experienced animal house technician. Pigs were completely fasted of food for 12 hours beforehand. Every pig was sedated with an intramuscular ketamine (10 mg/kg) mixed with xylasine (1mg/kg). Each pig was then weighed and transferred to the operating theatre. One per cent inhalational halothane mixed in oxygen was used to induce and maintain anaesthesia in the animal. An endotracheal tube was placed to secure the pig's airway once anaesthesia was established. An endotracheal temperature probe was placed inside the endotracheal tube to monitor core temperature of the animal. The animal was placed upon a warming pad in the base of its cradle to assist in temperature homeostasis. A lingual oxygen saturation probe was used. ECG monitoring was utilised throughout the procedure and two leads were placed above the left and right front legs and one below the left front leg. Readings of heart rate, temperature, oxygen saturations, end-tidal carbon dioxide levels and cardiac rhythm were

recorded throughout the procedure. The pig received normal saline solution through an intravenous line placed in the right external carotid vein throughout the course of the anaesthetic.

At the end of the procedure, anaesthesia was ceased and the pig was supplied with high flow oxygen and ventilatory support until self-ventilation recommenced. An intravenous injection of an opioid analgesic, buprenorphine (0.01 mg/kg) was provided and a single, prophylactic dose of procaine penicillin (15 mg/kg) was administered. Once self-ventilation recommenced, the endotracheal tube was removed and the pig was returned to an individualised, warm pen and was closely watched for signs of distress for a period of 4 hours or until it was awake and able to stand. Food was supplied so the pig was able to recommence eating as soon as it wished.

After completion of ablations in the first 6 pigs, the main anaesthetic agent used was changed from halothane to isoflurane (1.5% mixed with high flow oxygen) and this was used for the remainder of all anaesthestics. This occurred as halothane was no longer available for use and isoflurane is recognised as being a safer anaesthetic than halothane.

The first pig to be anaesthetised with isoflurane developed a marked tachycardic whilst under anaesthesia and the procedure had to be abandoned prior to any ablation being performed. After discussion and review by supporting veterinary staff, the use of xylasine as part of sedation was identified as a potential source of cardiac instability and was removed from all subsequent anaesthetics.

For any other anaesthetic not related to the ablation procedure (for example, the draining of wound abscesses), a similar protocol was used but the endotracheal temperature probe and heating pad were omitted. These anaesthetics were invariably very short and any problems associated with temperature control were unlikely.

Intravenous Line Procedure

Prior to commencing ablation, a 14 gauge, sterile intravenous line was surgically inserted. The line was placed into the right external jugular vein of every pig. Enough length was used to ensure that the line could be secured up to the back of the neck.

The skin was prepared with an iodine-based antiseptic solution. An incision was made over the region of the sternocleidomastoid muscle and the external jugular vein was identified. It was tied off with 4/0 silk and the line was inserted and secured with two 4/0 silk ties. Blood was aspirated from the line and then flushed with 10 mL normal saline to ensure patency. Blood samples were taken for analysis after discarding the first 20 mL of blood. These blood samples were used as the "pre-operative bloods". The wound was closed in 2 layers with a continuous absorbable 3/0 suture.

The intravenous line was secured to the skin with interrupted 1/0 nylon sutures up to the back of the neck and further secured with sterilised plastic tape. The line was used to administer intravenous saline throughout the procedure. The line was left in position at the end of the procedure for a period of 7 days to assist in the required blood sampling. The line was flushed twice daily with heparinized saline to maintain patency. It was monitored daily for signs of infection. If infection was suspected, it was removed forthwith under general anaesthetic.

Ablation Procedure

The ablation procedure was commenced after placement of the intravenous line. The abdomen was prepared with an iodine-based antiseptic solution (Betadine). Grounding pads connected to the *RF 3000* generator were placed upon the legs of the animal. Full aseptic precautions were taken and all surgical instruments were sterile.

A midline laparotomy incision was made from the xiphisternum to the umbilicus. The stomach, spleen and bowel were protected by use of moist gauze packs. The peripheral ligaments that attach to the liver were divided to allow full mobility of the organ.

Six ablations in total were performed in the left lateral lobe of the liver and the large median lobe of the liver. The median lobe can be further subdivided into the left and right medial lobes. The right lateral lobe was not used for the performance of any ablation as it is located posteriorly in the animal and is difficult to access.

The BETA technique was performed in 2 stages. Firstly, a 5-minute period of treatment with direct current alone was performed. This was followed by use of both circuits simultaneously with the radiofrequency circuit set at 20 W. To perform an ablation with the BETA technique, a scalpel blade connected to the positive electrode was placed into the subcutaneous tissues of the laparotomy wound. The negative electrode was connected to the radiofrequency electrode wire in a manner described in **Section 3.1.4**. The probe was placed into the liver parenchyma and the liver was checked to ensure safe placement of the probe in a manner similar to the controls. The AC/DC adaptor was set at 9 V and activated for 5 minutes. The presence of a direct current was confirmed by the observation of

bubbling from around the electrode and objective measurement of the current by use of an ammeter.

Upon the completion of the first phase of BETA treatment, the direct current was switched off and the ECG trace was checked for a normal cardiac rhythm, as considerable electrical interference to the ECG trace would occur. Once a normal rhythm was confirmed, both circuits were commenced simultaneously (9 V DC and 20 W AC). Measurements for power (watts) and impedance (ohms- Ω) were recorded every 30 seconds from commencement until the ablation process was deemed to have finished as measured by 2 consecutive power readings of less than 5 W and/or impedance values of greater than 700 Ω.

Upon completion of each ablation, haemostasis was secured. This was performed by manner of direct pressure for a period of 5-10 minutes. If bleeding continued, surgical diathermy was used at the probe site.

Upon completion of the ablation procedure, all surgical packs were removed and the abdomen closed. The abdominal muscles were closed with a continuous absorbable 1/0 suture reinforced with interrupted sutures. The skin was closed with an absorbable subcuticular suture.

Study protocol

Eight pigs were to be used for this study. Two pigs were killed after 48 hours of the procedure, 2 were killed after 2 weeks, 2 were killed after 2 months and 2 were killed after 4 months. This allowed serial assessment of pathological changes and morbidity.

Blood test analysis included complete blood picture [haemoglobin (Hb) and white cell count (WCC)], serum electrolytes (values of sodium, potassium, chloride, urea and creatinine), liver function tests [serum values of bilirubin, gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), albumin and the international normalised ratio (INR)] and C-reactive protein (CRP). These were performed pre-operatively and at 24 hours, 48 hours, 4 days, 7 days, 2 weeks, 1 month, 2 months and 4 months depending on the survival of the animal. All post-operative blood samples were compared to the pre-operative value that acted as a control. All blood test analyses where performed in a routine fashion by the Division of Biochemistry and Division of Haematology at the Institute of Medical and Veterinary Service located at The Queen Elizabeth Hospital.

Each pig was weighed just prior to death. The pigs were killed under general anaesthetic, after blood sampling, by a lethal dose of intravenous phenobarbitone. The surgical wounds were then inspected and an autopsy restricted to the chest and abdominal cavity was performed. Any abnormalities that were detected were recorded. The liver was harvested, inspected and any abnormalities found recorded. The necrotic ablation zones were dissected and the greatest diameter measured. Each ablative zone was examined macroscopically and photographed. Sections were then taken for histopathological examination. The tissue was fixed in 10% buffered formalin for 2 weeks and then processed in a routine fashion. Paraffin sections were prepared using haematoxylin and eosin staining. All abnormalities and histopathological changes over time were reported.

Statistics

The blood test results have been analysed using the Wilcoxon matched pairs signed rank test. Statistical significance was set at $p<0.05$. The ablation sizes were compared using 2-tailed Student's t-test with statistical significance set at $p<0.05$.

3.2.3 Experiment 3

The aim of the third study was to compare the effect of increasing the surface area of the area connected to the positive electrode to the set up with the scalpel blade as described in Experiments 1 and 2. This study was performed as it was found in the second experiment that an electrolytic type reaction occurred at this electrode causing skin damage. Details of this effect will be discussed further in the results and discussion sections on Experiment 2. It is hypothesised that by increasing the surface area of the positive electrode, the risk of damage done to the skin should be greatly reduced.

This study was a survival study and was given ethical approval from the University of Adelaide Animal Ethics Committee and the combined Institute of Medical and Veterinary Science/ The Queen Elizabeth Hospital animal ethics committees. The general conduct of the study conformed to the 'Code of Practice for the Care and Use of Animals for Scientific Purposes' (NHMRC/CSIRO/AAC 2004) and the SA Prevention of Cruelty to Animals Act 1985.

Anaesthesia

Anaesthetic details are similar to those described in Experiment 2. Pigs were completely fasted of food for 12 hours prior to commencement of anaesthesia. Every pig was sedated

with intramuscular ketamine (10 mg/kg). Each pig was then weighed and transferred to the operating theatre. One and a half per cent isoflurane mixed in oxygen was used to induce and maintain anaesthesia. An endotracheal tube was placed to secure the pig's airway once anaesthesia was established. An endotracheal temperature probe was placed inside the endotracheal tube to monitor core temperature of the animal. The animal was placed upon a warming pad in the base of its cradle to assist in temperature homeostasis. A lingual oxygen saturation monitor was used. ECG monitoring was utilised throughout the procedure and leads were placed above the left and right front legs and below the left leg. Readings of heart rate, temperature, oxygen saturations, end-tidal carbon dioxide levels and cardiac rhythm were recorded throughout the procedure. The pig received normal saline solution intravenously by a surgically placed intravenous line throughout the course of the anaesthetic.

At the end of the procedure, anaesthesia was ceased and the pig was supplied with high flow oxygen and ventilatory support until self-ventilation recommenced. An intravenous injection of an opioid analgesic, buprenorphine (0.01 mg/kg) was provided and a single, prophylactic dose of procaine penicillin (15 mg/kg) was administered. Once self-ventilation recommenced, the endotracheal tube was removed and the pig was returned to an individualised, warm pen and was closely watched for signs of distress for a period of 4 hours or until it was awake and able to stand. Food was supplied so the pig was able to recommence eating as soon as it wished.

Study Protocol

Upon commencement of the general anaesthetic, a surgically placed intravenous line was placed in the right external jugular vein in an identical fashion as that described for

Experiment 2. After preparing the abdomen with an iodine-based sterilising solution, a midline abdominal incision was made from xiphisternum to umbilicus. The liver was mobilised by dividing the ligaments at the lateral borders of the liver. All other surrounding organs were protected and packed away with moist gauze packs. All ablations were performed by use of a 4 cm straight aluminium rod that was connected to a *Boston Scientific RF 3000* generator. Full aseptic precautions were undertaken during this procedure and all equipment used was sterilised in an autoclave prior to the procedure.

Six ablations were performed in each liver. Two RFA controls were performed with power set at 80 W. Two ablations with the BETA circuit were performed with the positive electrode connected to a scalpel blade placed under the skin at the superior edge of the laparotomy wound. Two ablations with the BETA circuit were then performed with the positive electrode connected to a *ValleyLab* grounding pad placed on the skin adjacent to the area of the liver. Photographs of both positive electrode sites were performed prior to commencement of the ablations.

The BETA procedure involved the direct current running at 9 V for 15 minutes and then both currents running simultaneously with power set at 80 W. This starting power was used in an attempt to reach "roll off" early and minimise the size of the tissue injury induced by the positive electrode connected to the scalpel blade. Readings of power in watts (W) and impedance in ohms (Ω) were recorded every 30 seconds until "roll off" occurred. This was deemed to have occurred when either power fell to less than 5 W and/or impedance rose to greater than 700 Ω on 2 consecutive readings. Haemostasis for each ablation was ensured prior to commencing the subsequent ablation.

Upon completion of the ablation procedure, the abdomen was closed with a 1/0 dissolvable continuous stitch and the skin was closed with 3/0 subcuticular suture.

At 48 hours, the animal was killed under anaesthesia by injection of phenobarbitone. Prior to death, photographs were taken of the areas of the positive electrode placement and compared to pre-ablation electrode placement. After death, full thickness skin biopsies were taken from both regions of the positive electrode and placed in 10% buffered formalin. A skin biopsy from an unrelated area was also taken to act as a control and placed in 10% buffered formalin.

Following this, the liver was harvested and ablation zones were resected in their entirety. The maximum cross sectional diameter was measured between the red zones of the ablative zone and compared. The macroscopic appearance of each specimen was noted and photographs were taken of each specimen. Each resected ablation was fixed in 10% buffered formalin for a period of 2 weeks.

Paraffin slides were prepared in a routine fashion and stained with haematoxylin and eosin. All slides were subjected to histological examination.

Statistics

The data for ablation diameter size was analysed by analysis of variance using pigs as blocks and assuming the two measurements per pig are duplicate measurements. Statistical significance was set at $p<0.05$.

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Chapter 4

Results

4.1 Experiment 1

Four female domestic SPF pigs were used for this study. From these pigs, 14 RFA controls and 12 BETA circuit ablations were produced. Four RFA controls and 2 BETA circuit ablations were produced in the first pig, 3 RFA controls and 3 BETA circuit ablations were produced in the second pig, 4 RFA controls and 4 BETA circuit ablations were produced in the third pig. Three RFA controls and 3 BETA circuit ablations were produced in the 4th pig. The large BETA zones in the first pig took up considerable room and no further ablations were possible in the liver of this particular pig without risk of overlapping.

4.1.1 Time of Procedure

The controls continued for a mean time of 249 seconds. The BETA circuit ablations were pre-treated for 15 minutes with direct current only and then would continue for a mean time of 1115 seconds with the combination of RFA and direct current running simultaneously. On 4 occasions, the BETA procedure did not "roll off" after 25 minutes and at this point, were electively terminated. One control did not "roll off".

4.1.2 Ablation Zone Size (Table 4.1)

The mean diameter of controls was 27.78 mm (SE 3.37). The BETA zones were significantly larger with a mean diameter of 49.55 mm (SE 3.46), $p<0.001$. No animals or individual ablations were excluded from statistical analysis.

	Control size (time)	**BETA size** (time)
Pig 1		
Ablation 1	25 mm (120 secs)	70 mm (1500 secs)
Ablation 2	30 mm (120 secs)	55 mm (1500 secs)
Ablation 3	30 mm (90 secs)	
Ablation 4	32 mm (150 secs)	
Pig 2		
Ablation1	25 mm (180 secs)	62 mm (1500 secs)
Ablation 2	30 mm (420 secs)	60 mm (900 secs)
Ablation 3	38 mm (270 secs)	50 mm (900 secs)
Pig 3		
Ablation 1	22 mm (90 secs)	43 mm (930 secs)
Ablation 2	23 mm (120 secs)	44 mm (930 secs)
Ablation 3	28 mm (90 secs)	50 mm (930 secs)
Ablation 4	30 mm (60 secs)	30 mm (930 secs)
Pig 4		
Ablation 1	32 mm (120 secs)	43 mm (930 secs)
Ablation 2	30 mm (1500 secs)	33 mm (1500 secs)
Ablation 3	23 mm (150 secs)	45 mm (930 secs)
Mean Size	27.78 +/- SE 3.37mm (mean time 249 secs)	49.52 +/- SE 3.62 mm, $p<0.001$ (mean time 1115 secs)

Table 4.1

Maximal diameter of ablation zone (mm) and associated procedure times (seconds) of all ablations

4.1.3 Other Findings

During the BETA procedure, the area surrounding the tissue being ablated would become congested and would secrete fluid from the liver surface (**Figure 4.1**). After treatment with the BETA procedure, the electrode would slip out of the parenchyma without resistance.

After treatment with standard techniques, some residual liver tissue would still be adherent to the tines of the needle electrode. The tines had a tendency to be cleaner upon removal from the liver tissue following treatment with the modified circuit. This phenomenon is

attributed to area immediately adjacent to the tines of the electrode becoming a fluid-filled cavity and the tines being relatively inert.

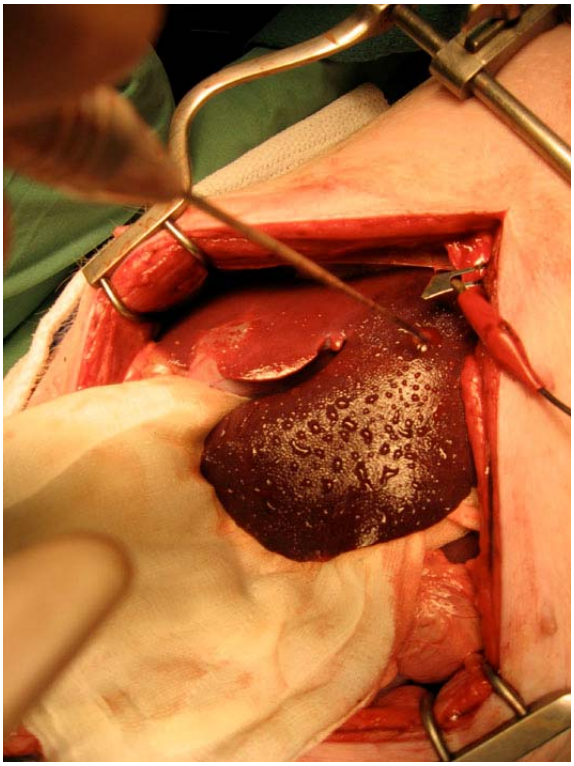


Figure 4.1

Photograph taken during the BETA treatment with both circuits running simultaneously

Note that this part of the liver has become congested and is secreting fluid from the surface.

4.1.4 Morphology (**Figure 4.2**)

The BETA technique produced ablation zones that looked morphologically similar to the RFA controls but the corresponding zones were larger. At the centre of each ablation was a cavitated area with dark tissue evident. This was far more pronounced in the BETA zones than the controls. Surrounding this was a pale region. A red rim was seen beyond this and represents the “red zone”. The red zone separates the ablated tissue from normal liver parenchyma.

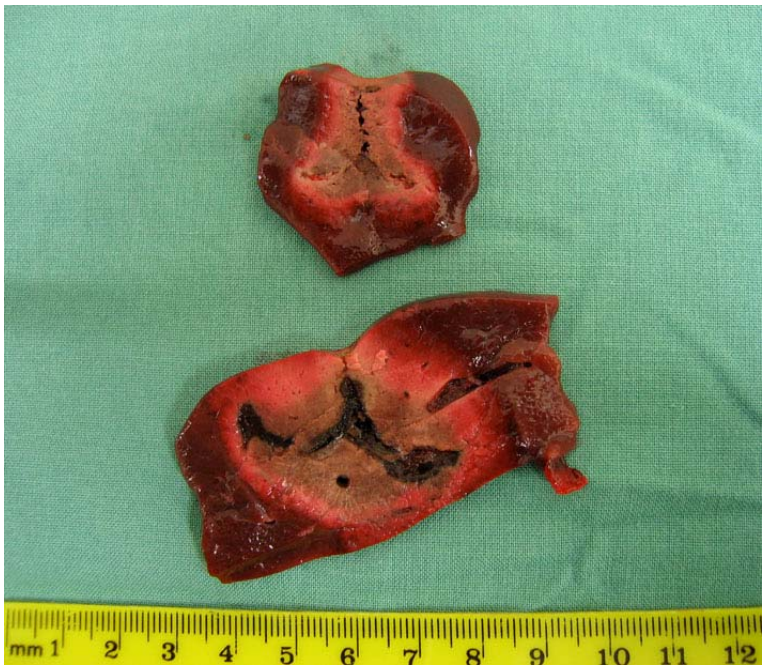


Figure 4.2

Photograph illustrating morphological similarities of RFA control (top) and BETA (bottom) Although similar, each corresponding zone is greater in size in the BETA zone. Note the dark tissue surrounding the central cavity, the only major difference seen between the two techniques.

4.1.5 Histology (**Figure 4.3**)

Four distinct zones were seen on histological examination of both controls and the BETA zones. The innermost area (that is tissue directly adjacent to the tines of the needle electrode) of both techniques showed extreme heat affected tissue destruction. This tissue was non-viable. However, subtle differences were apparent. The RFA controls showed extensive cell shrinkage with dark pyknotic nuclei and disrupted cytoplasmic borders (**Figure 4.3**).

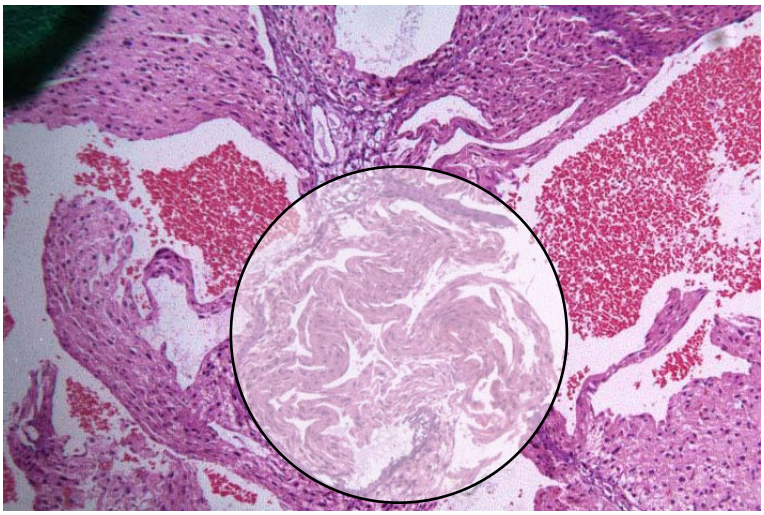


Figure 4.3

Application zone of RFA control (H and E section – 4x magnification)

Note small cells with blurring of cytoplasm and small dark nuclei (circled).

This tissue is non-viable.

The BETA zones also showed pyknotic nuclei and loss of cellular detail but exhibited marked cell swelling. This appearance is most likely a result of oedema that would correspond to water being drawn into the region (**Figure 4.4**).

Surrounding this was an area of degeneration, with cell swelling, fading of nuclei and blurring of cytoplasmic boundaries. Both controls and BETA zones looked similar. Beyond this was a zone of congestion where there were large numbers of erythrocytes beyond this region, normal tissue was seen. All corresponding areas were larger in the BETA zones when compared to controls (**Figure 4.5**).

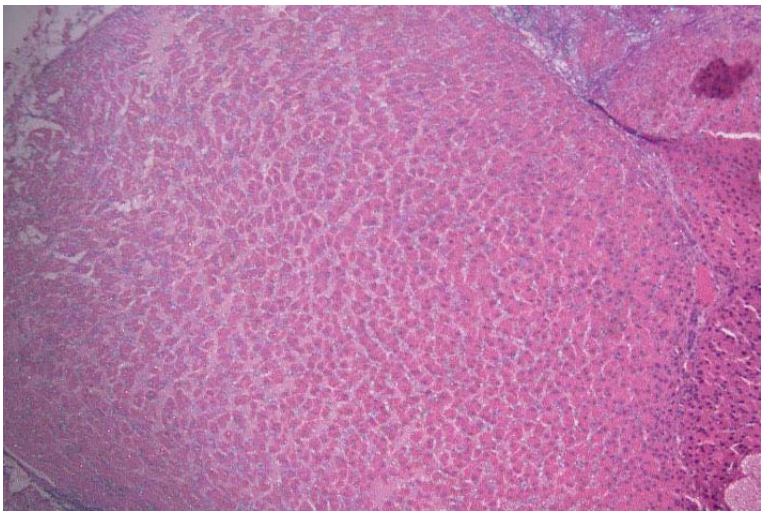


Figure 4.4

Central application zone of BETA ablation

These cells are swollen and oedematous in comparison to controls. The cytoplasm is pale and nuclei are dark. There is evidence of oedema in the interstitial spaces.

(H and E section – 4x magnification)

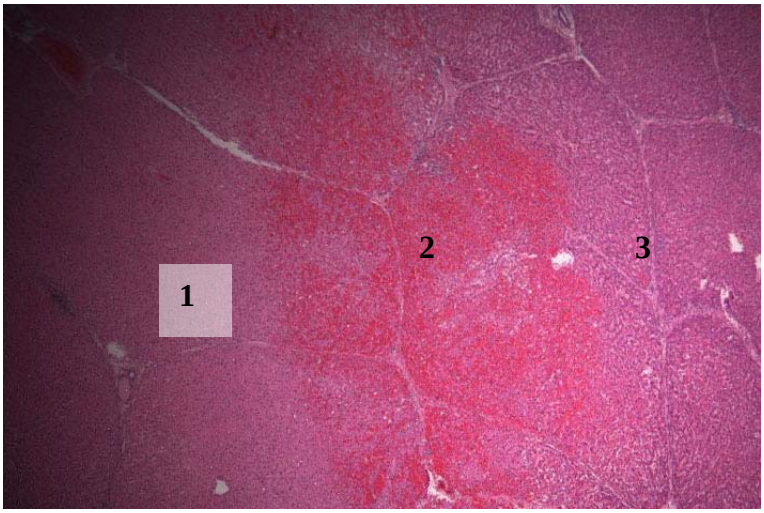


Figure 4.5

Control area of degeneration (1) surrounded by a red rim (2)

The red rim contains abundant erythrocytes indicating haemorrhage.

Beyond red zone is normal liver parenchyma (3). (H and E section – 4x magnification)

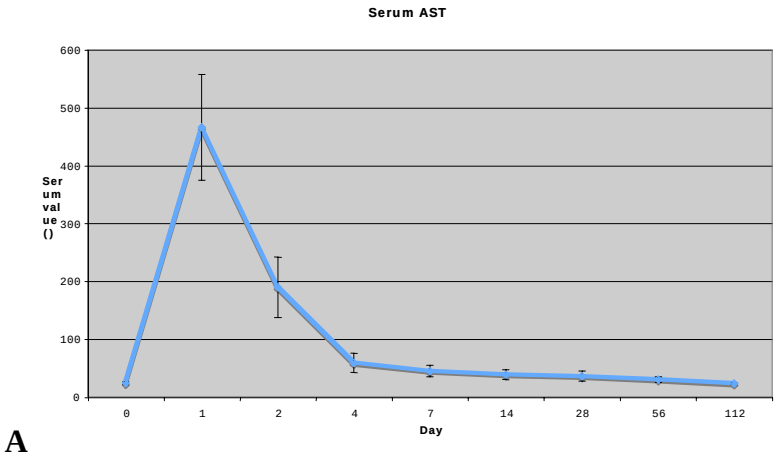
4.2 Experiment 2

Ten pigs in total were used for this experiment. The first 2 pigs died under anaesthetic during the course of the procedure. An autopsy was performed in both animals that concentrated on the chest and abdominal cavity. This revealed abnormal appearances in both lung bases. A biopsy was taken which revealed a form of non-suppurative pneumonia and therefore, it was considered that these 2 animals were unfit for anaesthesia. After discussing the problems with both ethics committees, these 2 pigs were replaced. No further intra-operative problems was observed and post-operatively, all pigs recovered well and no animal had to be euthanased prematurely.

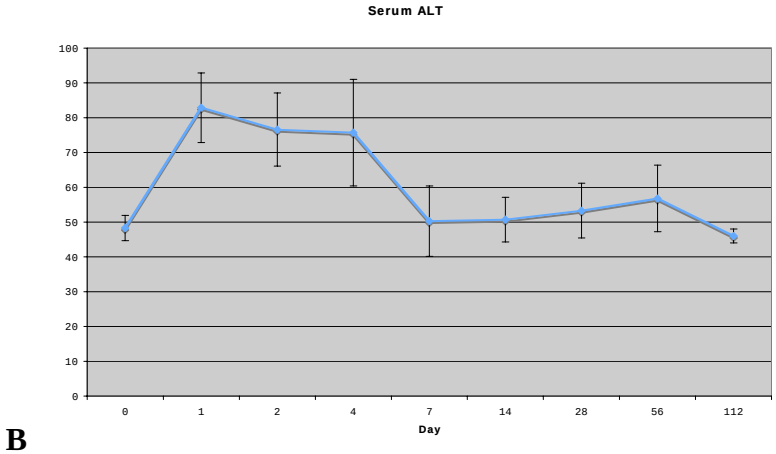
4.2.1 Blood Results (Table 4.2)

Liver Function

When compared to the pre-operative levels, significant levels of AST were seen in the first post-operative day ($p=0.03$). These levels remained significantly elevated until day 4 after the procedure. After 4 days, there was no significant change when compared to baseline. In comparison, ALT did have an appearance of elevation on the first post-operative day but this did not reach statistical significance ($p=0.35$) (**Figure 4.6 A, B**).



A



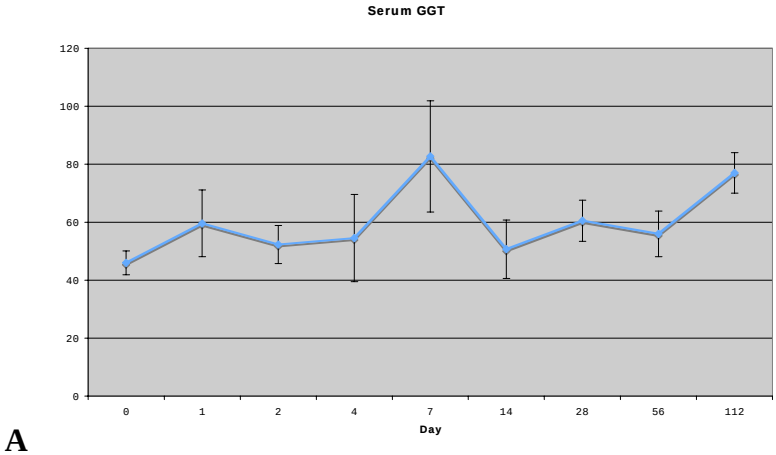
B

Figure 4.6

***A**–Graph showing relationship of serum AST over time (error bars represent SE)*

***B**–Graph showing serum ALT over time (error bars represent SE)*

The levels of GGT and ALP did not exhibit any significant change in the post-operative period (p=0.5 and 1.0 respectively). The bilirubin level did not show a significant change on the first post-operative day or any subsequent day (p=0.41). Both the serum albumin and INR level did not show any significant change in the post-operative period (p=1) (**Figure 4.7A, B, C).**



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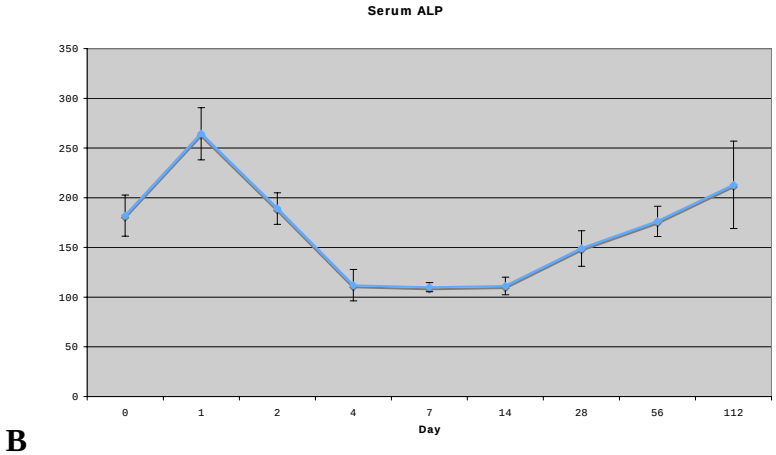

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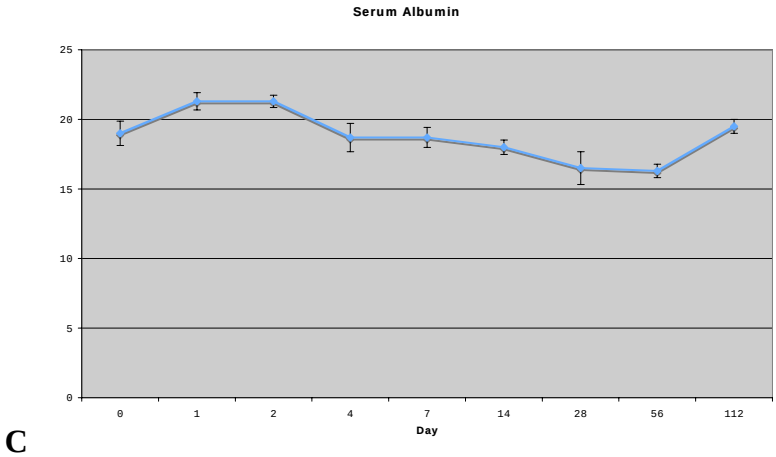


C

Figure 4.7

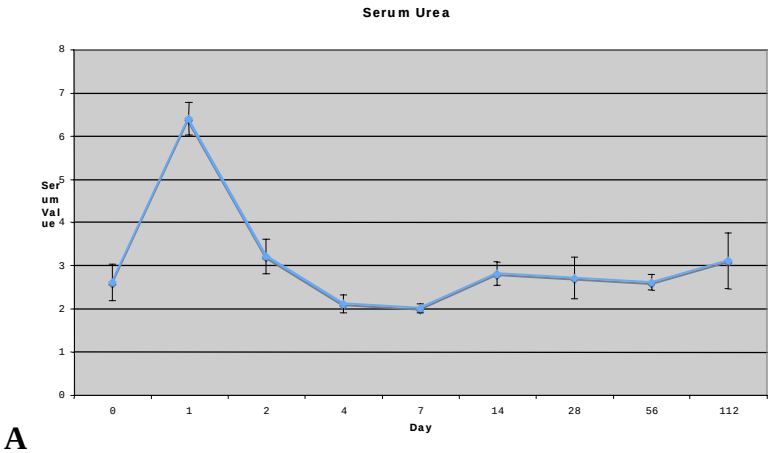
***A**–Graph showing serum GGT over time (error bars represent SE)*

***B**–Graph showing serum ALP over time (error bars represent SE)*

***C**–Graph showing serum albumin over time (error bars represent SE)*

Renal Function

When compared to baseline, a significantly elevated serum urea was seen on the first post-operative day (p=0.045). This returned to normal on the second post-operative day. Serum creatinine levels did not show significant change in the post-operative period (p=1) (**Figure 4.8A,B).**



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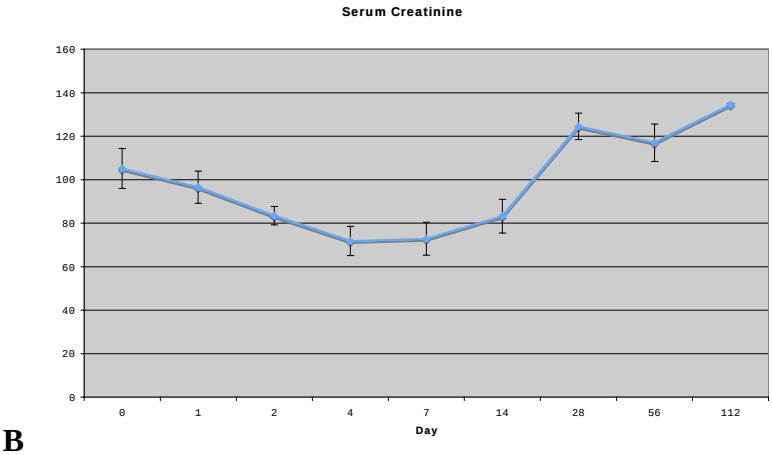


B

Figure 4.8

***A**–Graph showing serum urea over time (error bars represent SE)*

***B**–Graph showing serum creatinine over time (error bars represent SE)*

Other Blood results

Serum Hb and WCC did not exhibit any significant change in the post-operative period (p=1.0 for both Hb and WCC). C-reactive protein levels showed a significant elevation on the first post-operative day (p=0.027) (**Figure 4.9**). These remained significantly elevated for until day 4 when it returned to normal.

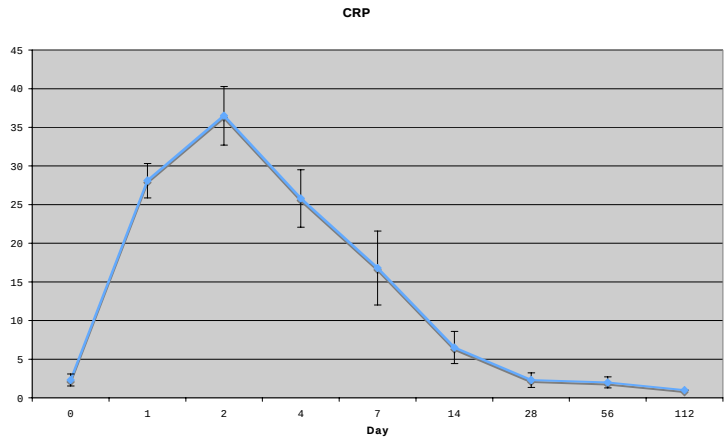


Figure 4.9

Graph showing serum CRP over time (error bars represent SE)

	Pre-Op	Day 1 Post-op	Day 2 Post-op	Day4 Post-op	Day 7 Post-op
Urea	2.612 +/- SE 0.42	6.39 +/- SE 0.38 (p=0.045)	3.22 +/- SE 0.40 (p=1)	2.12 +/- SE 0.21 (p=1)	1.95 +/- SE 0.11 (p=1)
Creatinine	105.13 +/- SE 9.16	96.5 +/- SE 7.39 (p=1)	83.38 +/- SE 4.24 (p=1)	71.83 +/- SE 6.76 (p=0.62)	72.83 +/- SE 7.52 (p=0.68)
Albumin	19.0 +/- SE 0.87	21.25 +/- SE 0.62 (p=1)	21.25 +/- SE 0.45 (p=1)	18.67 +/- SE 1.02 (p=1)	18.67 +/- SE 0.72 (p=1)
Bilirubin	3.0 +/- SE 0.19	4.65+/- SE 0.50 (p=0.41)	3.625 +/- SE 0.26 (p=1)	3.33 +/- SE 0.21 (p=1)	3.17 +/- SE 0.17 (p=1)
ALP	182.0 +/- SE 20.62	264.4 +/- SE 26.3 (p=0.50)	189.1 +/- SE 15.75 (p=1)	112.0 +/- SE 15.85 (p=0.50)	110.0 +/- SE 4.65 (p=0.08)
GGT	46.0 +/- SE 4.1	59.6 +/- SE 11.49 (p=1.0)	52.3 +/- SE 6.59 (p=1.0)	54.5 +/- SE 15.04 (p=1.0)	82.7 +/- SE 19.18 (p=0.668)
ALT	48.3 +/- SE 3.61	82.9 +/- SE 10.01 (p=0.35)	76.6 +/- SE 10.51 (p=1.0)	75.7 +/- SE 15.31 (p=1.0)	50.3 +/- SE 10.11 (p=1.0)
AST	24.25 +/- SE 2.426	466.25 +/- SE 91.02 (p=0.03)	189.5 +/- SE 51.97 (p=0.03)	58.83 +/- SE 16.6 (p=0.78)	45.0 +/- SE 9.774 (p=0.30)
LD	625.7 +/- SE 36	2688.4 +/- SE 382 (p=0.02)	1967.7 +/- 374 (p=0.02)	1236.2 +/- 223.9 (p=0.16)	1013 +/- 172.25 (p=0.16)
CRP	2.25 +/- SE 0.77	28.13+/- SE 2.23 (p=0.027)	36.5 +/- SE 3.8 (p=0.027)	25.83 +/- SE 3.72 (p=0.061)	16.83 +/- SE 4.78 (p=0.089)
Hb	92.6 +/- SE 10.37	94.1 +/- SE 10.98 (p=1.0)	96.9 +/- SE 11.76 (p=1.0)	87.4 +/- SE 14.61 (p=1.0)	78.6 +/- SE 12.09 (p=1.0)
WCC	9.37 +/- SE 5.35	8.63 +/- SE 4.08 (p=1.0)	8.14 +/- SE 3.77 (p=1.0)	8.24 +/- SE 6.29 (p=1.0)	9.05 +/- SE 7.17 (p=1.0)
INR	0.99 +/- SE 0.03	1.12 +/- SE 0.09 (p=1.0)	1.02 +/- SE 0.04 (p=1.0)	0.95 +/- SE 0.05 (p=1.0)	1.01 +/- SE 0.03 (p=1.0)

Table 4.2

Mean serum values of blood tests +/- standard error over first 7 days

Significance set at $p<0.05$ (p values recorded beneath mean serum values)

4.2.2 Morbidity and Pathology Results—2 Day Pigs

Both pigs remained well post-operatively. Both pigs had good appetites and were mobilising on the first post-operative day. Upon post mortem examination, there was evidence of a skin injury at the site of the positive electrode (**Figure 4.10**). In one pig, this area was biopsied and subjected to histopathological examination with paraffin slides prepared with haemoatoxylin and eosin staining. Sections confirmed the presence of full thickness skin necrosis. The rest of the wound remained intact without any evidence of infection. Inside the abdomen, there was no evidence of any haemorrhage or leakage of bile into the intra-abdominal cavity. There were some fine fibrinous type adhesions surrounding the liver. The gallbladder, stomach, small bowel, colon, pancreas and spleen all appeared normal.

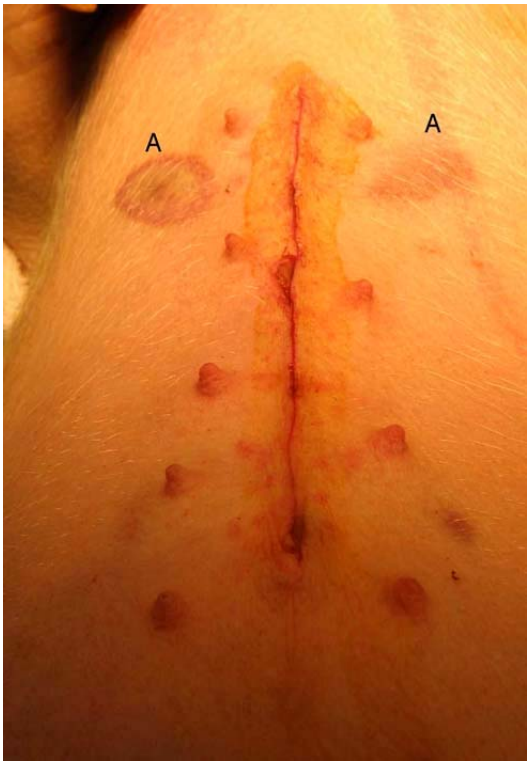


Figure 4.10

Photograph taken in the immediate post-operative period of the surgical wound

Areas marked **A** *illustrate skin injury at position of scalpel blade placed under skin.*

On dissection of the liver, the ablation zones measured a mean diameter of 30 mm. The site of ablation was characterised by a circumscribed zone of pallor that was well delineated from the adjacent normal liver. A pink, hyperaemic rim of tissue surrounded this zone of pallor. In the centre of the ablation site, a darkened linear depression was present which corresponded to the electrode probe placement and associated tissue cauterisation (**Figure 4.11**).

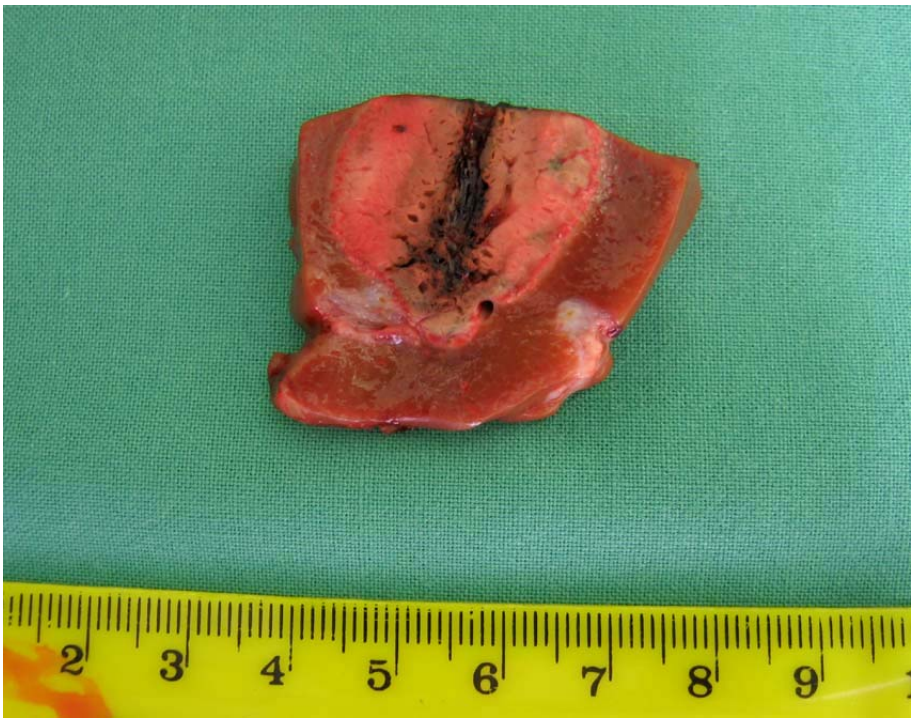


Figure 4.11

Macroscopic view of a 2-day ablation

A pale area surrounds a central dark depression.

A pink rim on the periphery separates pale area from normal looking liver.

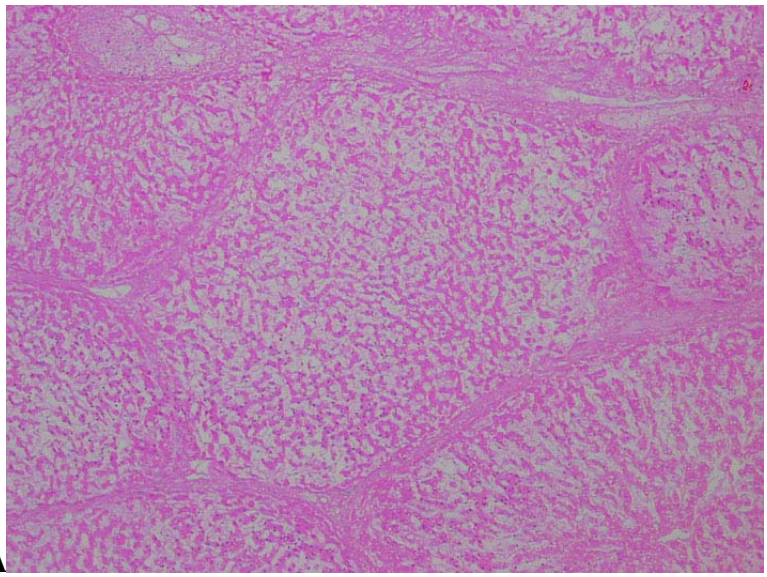
On histopathologic examination, there were three zones identified.

- The site of the electrode probe placement showed tissue cauterisation, with complete loss of hepatocytes and bile ducts together with total effacement of lobular structure.

- The surrounding tissue, corresponding to the pale tan zone seen macroscopically, showed established coagulative necrosis of hepatic lobules (**Figure 4.12A**). At the edge of this infarct a rim of reactive fibroblasts and proliferating bile ductules was evident.

- Peripheral to this, there was a rim of hepatocyte parenchyma showing evidence of ischaemic damage, characterised by centrilobular necrosis (**Figure 4.12B**).

A



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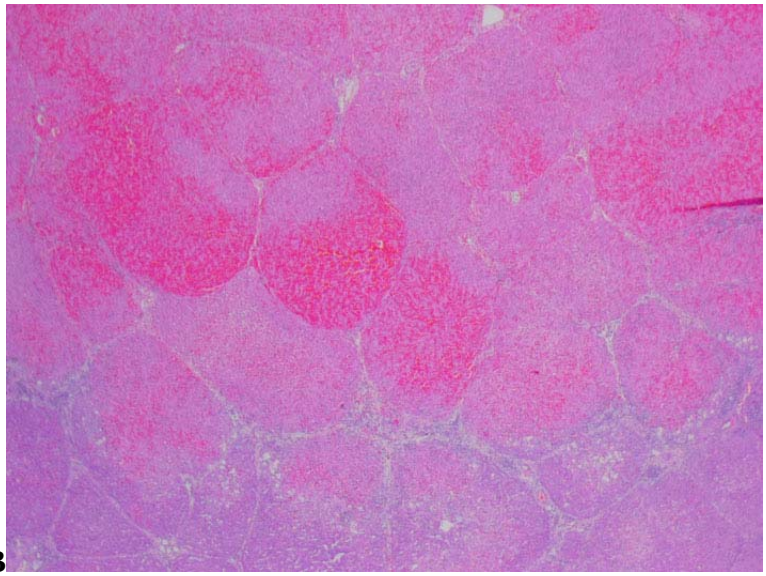


Figure 4.12

A*–Low power view from central pale area showing cells undergoing coagulative necrosis (H and E section – 4x magnification)*

B*–Rim of hepatocyte parenchyma showing evidence of ischaemic damage, characterised by centrilobular necrosis (H and E section – 4x magnification)*

4.2.3 Morbidity and Pathology Results—2 Week Pigs

During the 2-week period following treatment, both animals remained well and maintained good appetite and hydration. Each animal was able to mobilise without discomfort on the second post-operative day and both pigs gained approximately 3 kg in weight during this time.

On post mortem examination, the sites of the positive electrode appeared to be hard and blackened with underlying swelling consistent with an eschar type material. The rest of the wound appeared normal. On reopening of the wound, discharge of purulent material from these areas of swelling was evident consistent with the presence of an associated abscess in both animals (**Figure 4.13**).

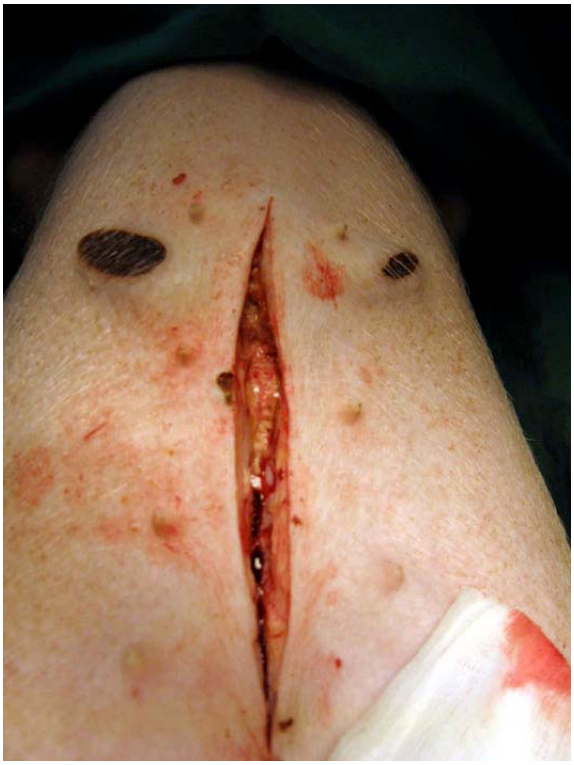


Figure 4.13

Surgical wound at 2 weeks

Note dark eschar type appearance of skin at position of positive electrode. Upon reopening wound, discharge of purulent material from the swellings underneath was evident.

On examination of the intra-abdominal cavity, there was no evidence of intra-abdominal collections, bile leakage or haemorrhage. The gallbladder, stomach, small bowel, colon and spleen all appeared to be normal. The liver was surrounded by fine adhesions. Apart from the necrotic areas produced by the ablative process, the liver appeared essentially normal.

All ablation zones appeared similar macroscopically and microscopically. The ablations were a mean diameter of 25 mm and were firm in texture. Macroscopically, each ablation zone was characterised by a well-circumscribed, pale-tan area that appeared to be surrounded by a fibrous capsule. A centrally located linear groove was identified, corresponding to the site of the electrode (**Figure 4.14**).

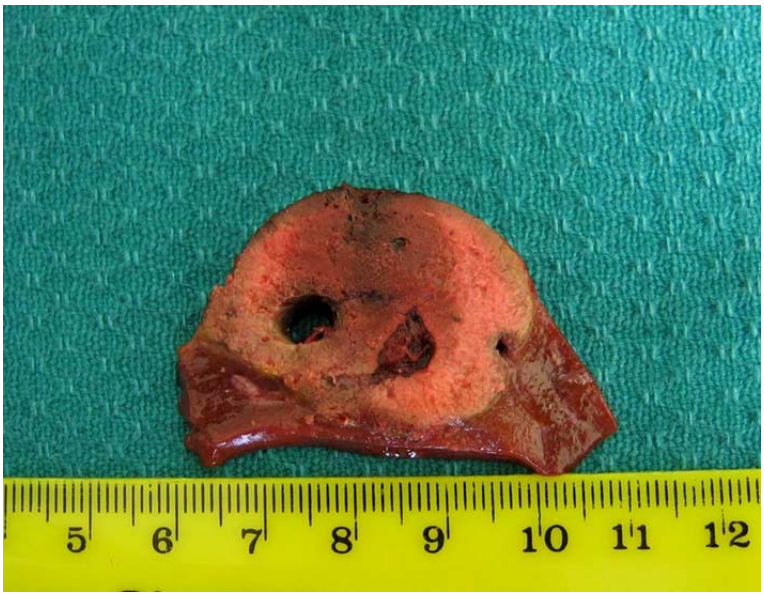


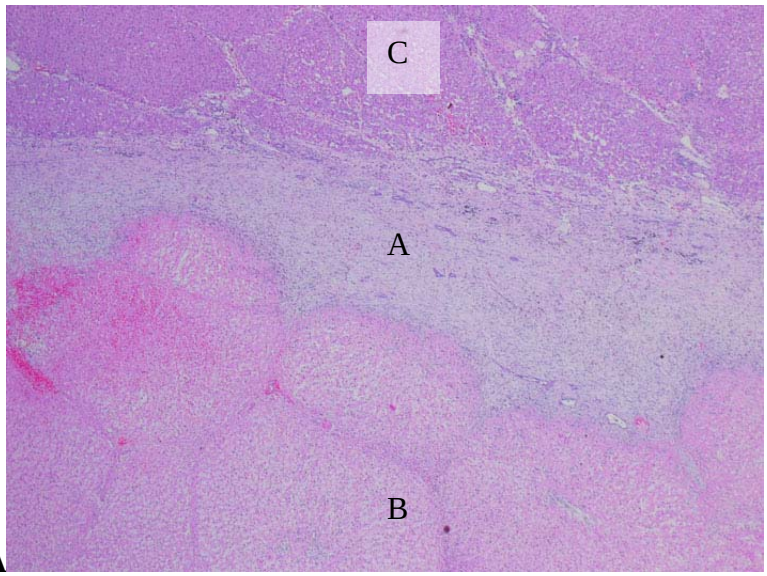
Figure 4.14

Macroscopic view of ablation at 2 weeks

Visible on periphery is a thin fibrous layer separating normal liver tissue from abnormal pale and material. Two vessels are evident within the ablated area and these vessels are completely surrounded by necrotic tissue.

Histological examination revealed an established fibrous reaction at the periphery of the necrotic area that was associated with a variably intense mononuclear inflammatory cell infiltrate (**Figure 4.15A**). Foreign body type giant cells could be seen ingesting the necrotic debris (**Figure 4.15B**).

A



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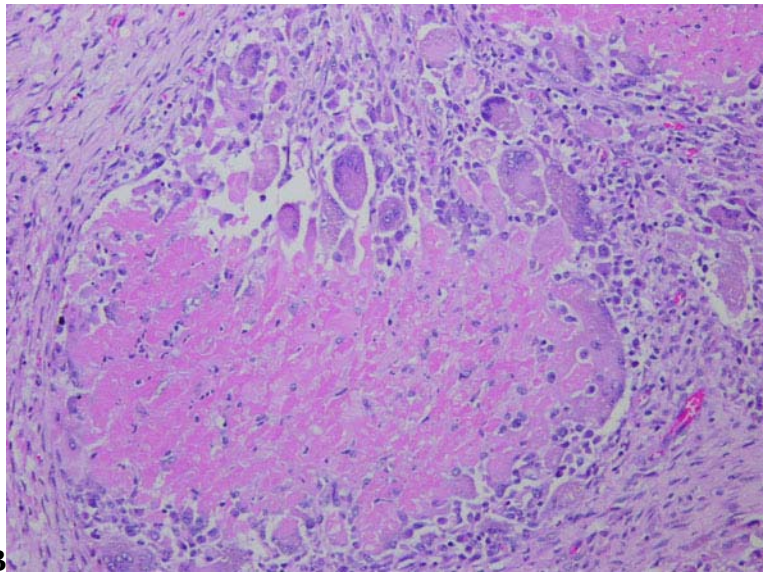


Figure 4.15

***A**–Fibrous layer (A) separating normal liver (B) from tissue undergoing coagulative necrosis (C)*

(H and E section – 4x magnification)

***B**–High power view of area of necrotic material being ingested by giant cells*

(H and E section – 10x magnification)

4.2.3 Morbidity and Pathology Results—2 Month Pigs

Both pigs remained well in the 2 months after treatment. Both pigs gained 40 kg in weight and retained very healthy appetites. At the site of the previous positive electrode, both pigs developed a walled off abscess. Despite this, the pigs had remained systemically well. At the time of removal of the intravenous line, the abscesses were incised and drained. These healed without further complication.

On post mortem examination, the wounds had healed without evidence of infection. There were no intra-abdominal collections of any kind present. The gallbladder, stomach, small bowel, colon and spleen appeared normal. There were dense adhesions surrounding the liver. The liver appeared normal except for the presence of soft, well-circumscribed nodules consistent with the previously performed ablations.

Macroscopic examination of the ablative zones revealed a well-circumscribed area of necrosis with a thick fibrous capsule. The contents of the ablative zone showed tissue fragmentation into 2-3 mm nodules. The previously noted central groove had disappeared. All ablation zones appeared similar. The surrounding liver appeared macroscopically normal (**Figure 4.16**).

On histological examination, the liver showed established scar tissue and gradual reorganisation of the necrotic area. There is a thick fibrous capsule at the periphery of the

ablated tissue and broad bands of fibrous tissue extending into the necrotic zones. Areas of patchy dystrophic calcification were also apparent. All ablations appeared similar on histological examination (**Figure 4.17**).

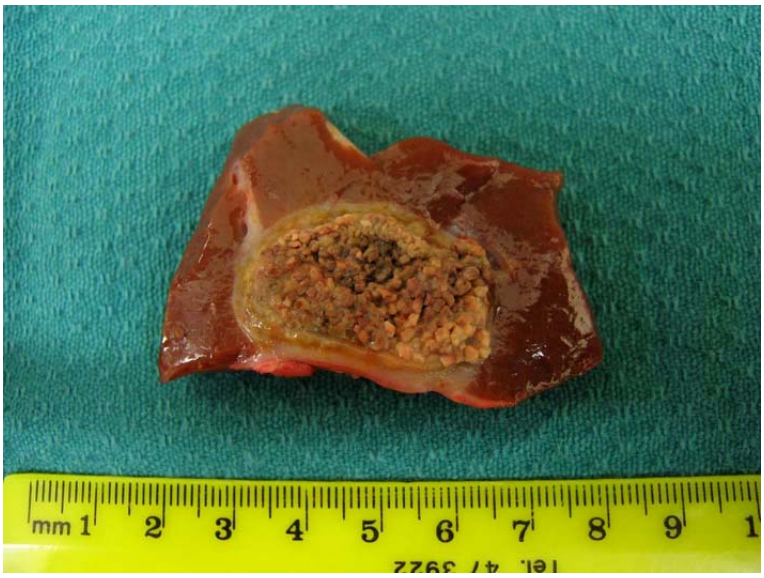


Figure 4.16

Macroscopic view of 2-month ablation

Note thick fibrous layer separating normal looking liver from central necrotic fragments.

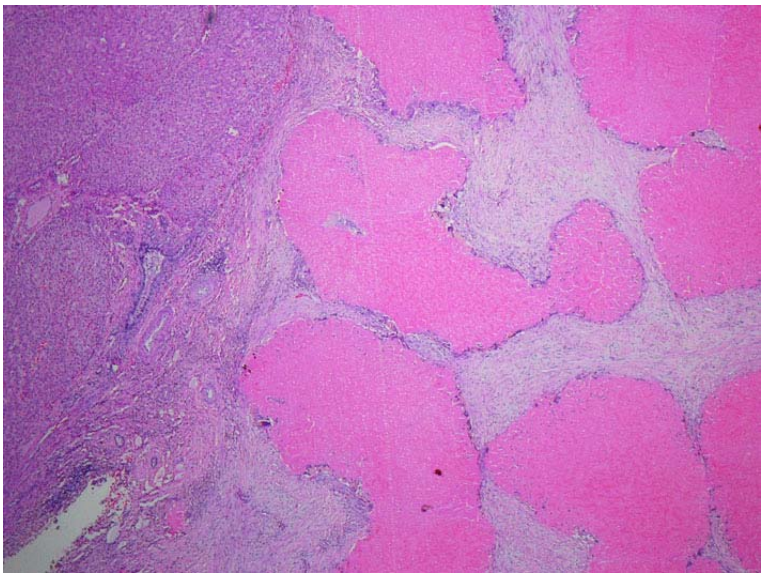


Figure 4.17

Low power view of periphery of ablated area at 2 months

Fibrous tissue is now extending into central area and surrounds small islands of necrotic tissue. (H and E section – 4x magnification)

4.2.5 Morbidity and Pathology Results—4 Month Pigs

Both pigs in this group gained approximately 60 kg in weight post-operatively. Each remained well throughout the 4 months and was able to mobilise without problems and maintained a vigorous appetite. In a manner that was similar to the 2-month pigs, each developed a wound abscess in the early post-operative period at the site of the positive electrode that required incision and drainage under anaesthesia. This was performed at the same time as the removal of the intravenous line. The wounds healed without further complication following this.

Upon post mortem examination, the wound was well healed. There was no evidence of intra-abdominal collections of any sort. The stomach, gallbladder, spleen, small and large bowel were essentially normal. The liver was surrounded by dense adhesions and was considerably larger in size than that seen in the other pigs. The ablations were considerably smaller than those seen in the other pigs.

On macroscopic examination, the ablation sites appeared as small 5 mm diameter fibrous lesions with small amounts of associated necrotic debris. The surrounding liver appeared essentially normal (**Figure 4.18**).

By four months, the fibrous scar tissue had undergone extensive remodelling and the size of the scar had contracted substantially. The neighbouring hepatic parenchyma showed evidence of regeneration (**Figure 4.19**).

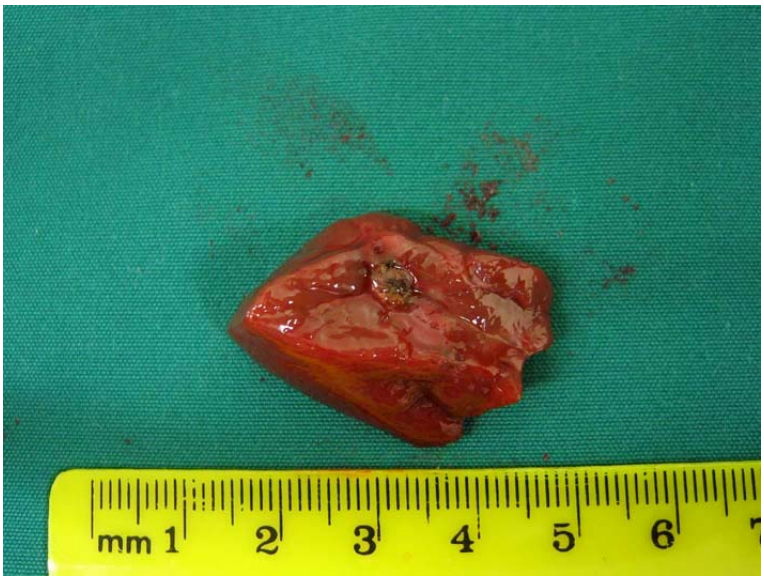


Figure 4.18

Macroscopic view of ablated area at 4 months

It is now much smaller in appearance and is associated with only a small amount of necrotic material.

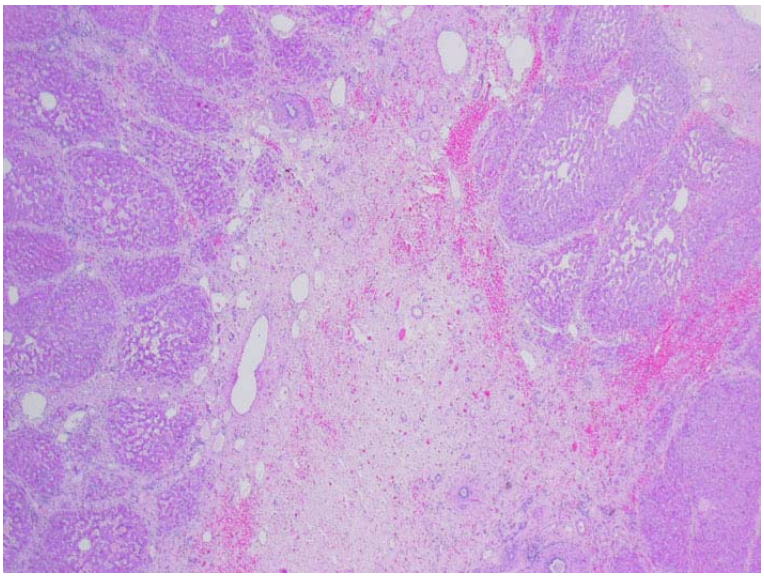


Figure 4.19

Low power view of ablated area at 4 months showing small contracted, mature fibrous scar

(H and E section – 4x magnification)

4.3 Experiment 3

Six pigs were used for this experiment. All animals recovered well after surgery and no animal had to be euthanased early.

4.3.1 Procedure Findings

During the ablation procedure, vigorous bubbling around the electrode was seen with both BETA circuits and this was not seen with the RFA controls. This bubbling corresponded to the release of hydrogen gas that occurs as part of the electrolytic process at the negative electrode. The bubbling associated with the scalpel blade ablation always appeared to be more vigorous than the grounding pad.

The time to roll off was longest following the scalpel blade ablation with a mean time of 101.5 seconds. The mean time to roll off for the grounding pad was 75 seconds. The mean time to roll off for controls was 30 seconds.

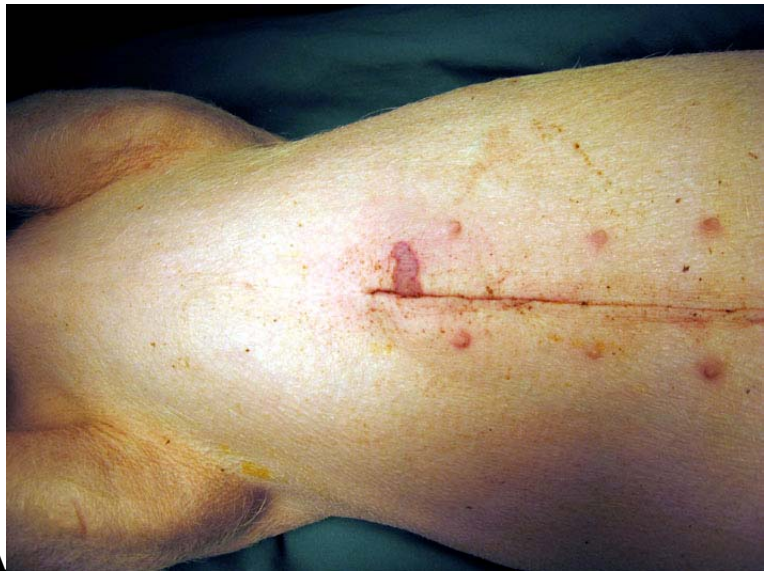
In the immediate post-operative period all animals had an area of discolouration at the site of the scalpel blade that indicating that skin damage had occurred. Three animals showed some mild erythema at the site of the grounding pad. The other three appeared normal.

The scalpel blade appeared black and corroded on examination at the end of the procedure. The grounding pad looked essentially unchanged.

4.3.2 Autopsy Findings

At 48 hours, the scalpel blade site remained discoloured and had a purple–black appearance (**Figure 4.20A**). The macroscopic appearance of the skin in the area of the grounding pad associated with the pad looked essentially normal (**Figure 4.20B**).

A



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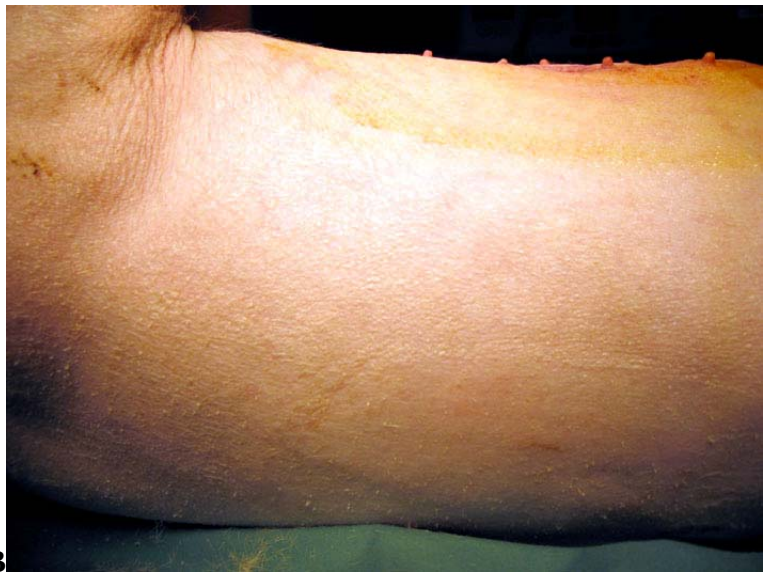

Figure 4.20

Photographic appearance of positive electrode sites upon completion of the procedure

***A**–Anterior abdominal wall at site of scalpel blade with obvious associated injury*

***B**–Area of grounding pad placement that looks normal*

Apart from the positive electrode sites, all wounds remained essentially clean and dry without evidence of infection. The macroscopic appearance of the skin at the grounding pad site looked normal in all animals. At autopsy, examination of the abdominal cavity did not show any significant abnormality. There was no sign of any collections, haematomas or leakage of bile. The stomach, small bowel, spleen colon and gallbladder all appeared normal in all animals.

On histopathological examination of the skin sites, the area of the scalpel blade showed evidence of a full thickness burn type injury (**Figure 4.21**). The area from the grounding pad looked similar to the control skin and did not exhibit any evidence of necrosis or tissue injury (**Figure 4.22**).

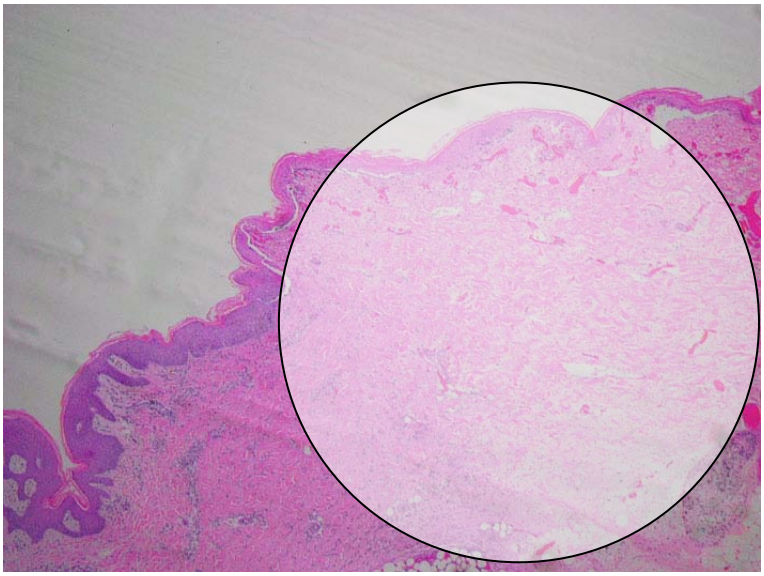

Figure 4.21

Low power view confirming area of necrosis (circled) that appears well demarcated from normal surrounding skin (H and E section – 4x magnification)

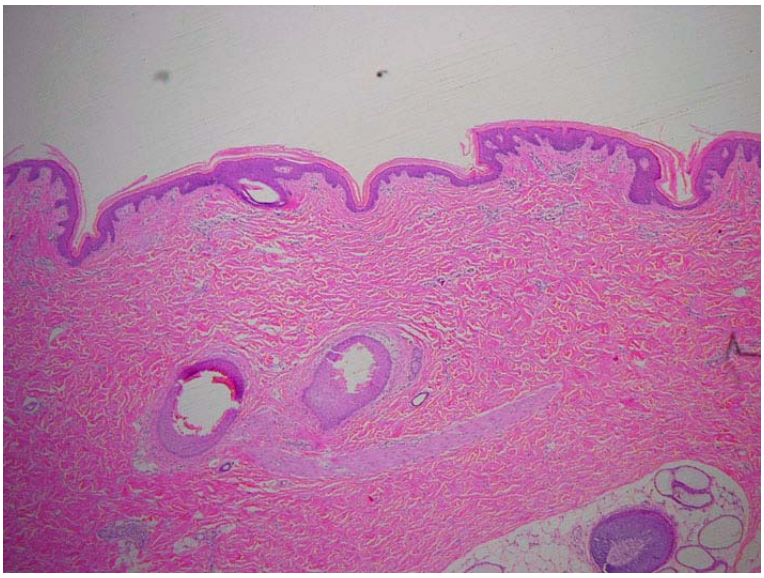

Figure 4.22

Low power view of skin from grounding pad site that looks essentially normal

Control skin looks similar to this. (H and E section – 4x magnification)

4.3.3 Ablation Findings

All ablations looked macroscopically and microscopically similar. Four zones were seen within each ablation. A central, charred, black groove was surrounded by a pale area of tissue. This tissue was undergoing coagulative necrosis on histopathological examination. Surrounding the pale area was a hyperaemic rim that showed haemorrhage, necrosis and an early ingrowth of fibroblasts on microscopic examination. Beyond the haemorrhagic rim was tissue that looked macroscopically and microscopically normal.

The mean transverse diameter of the scalpel blade ablations was largest with diameter of 25 mm (SE 1.078 mm). These were significantly larger than both the grounding pad ablations and the controls ($p<0.001$). The grounding pad ablations were significantly larger than the controls ($p<0.001$) but smaller than the scalpel blade ablations ($p<0.001$) (**Table 4.3**).

Treatment	Mean (mm)	SE (mean)
Control	15.33	0.577
Pad	18	1.238
Scalpel	25	1.078

Table 4.3 Ablation size

($p<0.001$ between all groups)

****

Chapter 5

Conclusions

5.1 Size Comparison—BETA versus RFA

The ultimate aim of the BETA system is to consistently produce a larger ablation than standard radiofrequency ablation. It has been well established that after treatment with RFA, the risk of local recurrence is much greater when the tumour is greater than 3 cm in diameter. This may be a result of the inadequacy of the overlapping treatment techniques that are employed when treating these larger tumours. If a single ablation that completely covers these larger tumours can be produced without the need for overlapping, the risk of local recurrence may be reduced.

It has been shown that when an ablation is produced in normal liver using the BETA system, the ablation zones produced are significantly larger than RFA controls using the same settings. This was demonstrated in Experiment 1 and further confirmed in Experiment 3. It is postulated that this is a result of the target liver becoming less desiccated thus leading to a delay in charring of the tissues and allowing the procedure to continue for longer. This hydrating effect was demonstrated by the manner in which the liver becomes congested and oedematous and secreted fluid from its surface. The lack of adherence of the surrounding tissue when removing the multi-tine electrode and the findings on histological examination support the postulate that water has been drawn into the tissues surrounding the needle. The increased time that the ablation process is able to continue is ultimately the determinant for the significantly larger ablation zones.

It has also been shown that the BETA system can be applied to a commercially available needle electrode (using the *LeVeen* multi-tine electrode). It is anticipated that the BETA system can be used with other commercially available needle electrodes. The electrode used for this particular experiment is designed to produce a 35 mm diameter ablation and this electrode can treat a 2 cm diameter tumour in its entirety with one application. The controls were smaller than this but previous reports have shown that the size of the ablation zone is often smaller in vivo than that claimed possible by the manufacturer[166]. The diameter of necrosis produced with the BETA system was considerably larger than 35 mm (mean 49.55 mm) and potentially a 3 cm tumour could have been treated with this system with one application. This particular electrode had not been designed to treat tumours this size.

The first 3 hypotheses for the first experiment were as follows:

- By constructing a bimodal electric current system with the cathode placed at the main electrode of the radiofrequency circuit and the anode placed nearby, water will be attracted to this electrode.

- As water is attracted to the cathode, localised tissue charring will be reduced and therefore, the ablation time prior to “roll off” will be greater.

- If the treatment time is longer, this will result in a larger ablation being produced.

The results that have been obtained from the first experiment and confirmed in the third experiment allow all 3 hypotheses to be accepted.

5.2 Pathological Changes—Short Term

The fourth hypothesis of the first experiment was that when ablations produced with the BETA system are compared to the RFA controls the macroscopic and microscopic appearances would be similar.

The results of the first experiment allow this hypothesis to be accepted. The pathological changes noted match the descriptions of thermal ablations reported in the literature. There is a central application zone surrounding a pale area or "white zone". Beyond this is a red rim of haemorrhagic tissue also known as the "red zone". The liver tissue beyond the red zone looked macroscopically normal. The corresponding zones produced by the BETA circuit are consistently larger than those produced by the radiofrequency circuit.

There were some subtle differences between the 2 modalities that warrant further discussion. Within the application zone (the area closest to the electrode) of the BETA produced ablation zone, there appeared to be an increased amount of dark colouration when compared to radiofrequency controls. There are 2 possible explanations for this:

- It may be related to an increase in the amount of carbon deposition as a result of tissue charring following a long ablation time during the BETA treatment. This explanation is not favoured, as "roll off" did not occur on 4 separate occasions during the BETA treatment. With no "roll off" being observed, tissue charring

could not have developed and consequently carbon deposition would not have occurred.

- It is more likely that the dark colouration was a result of the electrolytic changes related to the direct current that occur at this electrode. It is known that at the cathode, sodium hydroxide action upon haemoglobin can be lead to the formation of haemochromagens as a result of reduction reactions[185] . These haemochromogens are usually a dark brown-black colour[185]. It is argued that the presence of these haemochromagens that cause the discolouration that is seen at the electrodes [185]. At the anode, chlorine action can lead to the formation of methaemoglobin that is also black in colour and gives the electrode its characteristic dark colour. With water accumulation at the cathodic area, dilution of the colouring agent occurs. Therefore, the intensity of the colouration is less than that at the anodic area. In the scenario of the BETA procedure, water is being accumulated but at the same time is also being heated. It is likely that some of the water will have been converted to steam due to the heating effect and the liquid water accumulation would not have been as great. As a result, the dilution effect on the haemochromagens will have been diminished and the degree of colouring consequently becomes more obvious.

When comparing the 2 modalities at the microscopic level, there were again many similarities and a few subtle differences observed. Four distinct areas were seen on histological examination of both controls and the BETA zones and these corresponded to the macroscopic appearances. The innermost zones (that corresponds to the tissue situated directly adjacent to the needle electrode) both showed extreme heat affected tissue

destruction. This tissue was non-viable in both settings. Nevertheless, differences between the 2 modalities were noted. The controls showed extensive cell shrinkage with dark pyknotic nuclei and disrupted cytoplasmic borders. The BETA zones also showed pyknotic nuclei and loss of cellular detail. The BETA zone, however, exhibited marked cell swelling. This appearance is most likely a result of oedema that would correspond to water being drawn into the region. This appearance appears to match the description of cathodic application zones that are seen at the cathode of an electrolytic ablation. In these cathodic areas, the cells are oedematous with pyknotic nuclei[207]. In contrast, the anodic region has small, desiccated cells as a result of tissue dehydration[207].

Surrounding this innermost application zone was an area of degeneration, with cell swelling, fading of nuclei and blurring of cytoplasmic boundaries. This zone corresponded to the "white zone" seen macroscopically. Both controls and BETA zones looked similar. Beyond this was an area of congestion where there were large numbers of erythrocytes present that corresponded with the "red zone" seen macroscopically. Beyond the red zone, normal looking liver parenchyma was seen. All corresponding zones were larger in the BETA zones when compared to controls.

The subtle changes observed suggest that water had been actively drawn into the area. They also indicate that the thermal effects are not the only mechanism causing tissue injury at this stage. It would appear that some electrolytic related tissue injury is occurring as well. As there had been 15 minutes of "hydration" with the use of direct current alone, this is not altogether surprising.

The fourth hypothesis of the first experiment that the ablations should have similar macroscopic and microscopic appearance can be accepted. However, some subtle variations are seen. These variations are unlikely to impact significantly upon the long-term performance of the BETA system.

5.3 Pathological Changes—Long term

The first hypothesis of the Experiment 2 was that injuries produced with the system should behave like other thermal therapies and undergo coagulative necrosis with timely healing by fibrosis. This was to confirm that these injuries heal in a predictable and appropriate manner.

The injuries produced by the BETA circuit were found to undergo coagulative necrosis in the treated area. The injuries heal by the process of fibrosis and heal from the periphery towards the centre. These results are consistent with injuries produced by RFA and other thermal therapies such as MCT and LITT[7,9,16,210]. Injuries produced by electrolysis also produce coagulative necrosis with healing by fibrosis that heal from the periphery inwards[198]. It takes approximately 4 months for the healing process to be complete. This again is consistent with the other ablative technologies.

Therefore, the hypothesis that injuries should induce coagulative necrosis with timely healing by fibrosis has been confirmed.

5.4 BETA—Related Morbidity

The second hypothesis of the second experiment was that treatment with the BETA system is as safe as RFA.

5.4.1 Blood Results

The significant rise in the levels of some of the liver enzymes is quite predictable and is related to the injury produced by the ablative process. The significant rise in serum urea is consistent with some dehydration of the animal. This is not necessarily a direct result of the BETA procedure but more likely related to the major surgical intervention as a whole. Serum creatinine levels did not exhibit significant change. Therefore, these results confirm that a degree of self-limiting, altered renal function has occurred. This emphasises the importance of adequate intravenous fluid therapy during and after the BETA procedure. The post-operative rise in CRP levels indicates that an inflammatory process has commenced which was confirmed on histological examination with inflammatory cells seen on the periphery of the 2-day ablation zone. Although transient rises in serum liver enzymes were seen, the fact that the INR level, bilirubin levels and protein levels did not exhibit significant change indicates that the liver was still able to function normally despite the injuries produced. The presence of stable haemoglobin and white cell counts indicate there was no major haemorrhage, nor infective process associated with the treatment given. These findings are supported by the fact that daily observation of the animals did not detect any serious abnormal responses and the post mortem examination findings.

Other animal studies performed using RFA and electrolysis has shown similar results when blood analysis has been undertaken[8,207]. These findings also confirm that the injuries

produced behave in a predictable manner and no unexpected morbidity has occurred. The hypothesis that BETA is as safe as RFA has partly been confirmed by these results.

5.4.2 Associated Morbidity

During the second experiment, the first 2 pigs died under anaesthesia. It is thought that this was not as a direct result of the ablative process but related to anaesthetic complications. Both animals had compromised respiratory systems that were confirmed at autopsy. The changes seen within the lungs were not an acute problem and would have been present pre-operatively. As a result, these 2 animals were clearly not fit for a long anaesthetic as both had a pre-existing compromised respiratory reserve that would have affected their performance under anaesthesia. Once replaced, no further problems were encountered during the course of this experiment. No such problem was experienced in either the first or third experiment.

Excluding these 2 animals, it would appear that the animals tolerate the BETA procedure under anaesthesia well. It is important to note that no cardiac arrhythmias were observed. This is expected as the current produced by the radiofrequency circuit is at a frequency too high to affect the cardiac conducting system. Electrolysis has been extensively tested in animals and humans. Although it had been shown to induce cardiac arrhythmias in very small animals (such as mice) when placed directly over the heart, the low levels of current used have never been reported to induce arrhythmia in larger animals or humans.

In previous studies, both electrolysis and RFA have been shown to be relatively safe with acceptable rates of morbidity and mortality. The research on the use of electrolysis at The University of Adelaide did not record any significant treatment-related morbidity in either

animal or human studies. The only complications of note were wound-related in several of the studies using pigs. The numbers of animals and people treated from this group has always been relatively small and therefore, it would be expected that some treatment-related complications might still be seen.

It has been well established that standard RFA is associated with a mortality rate of between 1-2% and a major morbidity risk of around 10%[19,20,22]. The most common complications are bleeding, liver dysfunction, bile leaks, liver abscesses, skin burns at grounding pad sites and damage to other organs[19,20,22]. Although none of these complications were seen, all could potentially occur as a result of treatment with the BETA procedure as it is very similar to standard RFA. From the results of the second experiment, it would appear that BETA is not associated with more complications than standard RFA–apart from a problem related to the positive electrode site.

Although the BETA procedure is safe and behaves in a predictable manner similar to other techniques, injury occurring at the positive electrode site is a concern. There are 2 possible explanations:

- Firstly, there may be some leakage from the radiofrequency circuit into the direct current circuit thus inducing a thermal injury at the positive electrode site in a manner similar to what occurs at the main electrode site. A small electrical inductor was used within the circuit to prevent this leakage from occurring but it is quite possible that some leakage may have occurred. A new inductor was used in every pig but it is also possible that by the end of the procedure, it may have worn and was no longer functional.

- The second reason that may explain the skin injury at this site is as a direct result of the electrolytic process that occurs at the positive electrode. This explanation would seem to be the more plausible one. Previous experiments with electrolytic ablations had shown that the greatest volume of necrosis occurs at the site of the positive electrode[211]. At this electrode, hydrochloric acid, chlorine gas and oxygen are produced[211]. The hydrochloric acid and chlorine induce tissue injury at this site. The electrode at this site typically turns black as a result of chlorination. It had been observed that the scalpel blade was always black in appearance upon removal after use and that indicates that an electrolytic process has been occurring in this area.

Therefore, when considering the blood results and the morbidity results, we can accept the hypothesis that BETA is as safe as RFA. However, the positive electrode requires further consideration. The placement of a scalpel blade in the subcutaneous tissues is not a suitable option in any human therapeutic situation. The scalpel had been used in this site as it was shown to consistently work when performing the previous direct comparison study.

The adverse findings related to the positive electrode led to the third experiment being performed. If the safety hypothesis was to be accepted unequivocally, this had to be addressed. For the third experiment, the following hypotheses had been made:

- By increasing the surface area of the positive electrode, the risk of inducing tissue damage will be reduced.

- ❖ The ablations produced will be of a similar size to those produced with the previous set up and should be larger than controls.

- ❖ The ablations produced will appear similar at the macroscopic and microscopic level.

The injury produced at the scalpel blade site appeared to resemble a full-thickness burn type injury. This almost certainly represents an electrochemical burn as the presence of a blackened, corroded scalpel blade after treatment confirms a vigorous electrolytic ablation had occurred. The mechanism of this occurrence has been previously discussed. This was not seen with the grounding pad set up. The 2 forms of BETA resulted in similar appearances of tissue injury at the macroscopic and microscopic level. Therefore, the first and third hypotheses could be accepted.

Both methods of performing BETA resulted in significantly larger ablations being created than with standard RFA controls alone. This correlates with the results from the first experiment that showed BETA could consistently perform larger ablations than RFA. Despite this, the scalpel blade proved to consistently produce larger ablations than the grounding pad. Therefore, the second hypothesis of this experiment must be rejected. The most likely reason is the electrolytic reactions were more vigorous with the scalpel blade set up. The outer skin of the pig is very thick and is most likely somewhat resistant to the conduction of electrical currents. As resistance was most likely greater, the flow of current between the 2 polarities would have been reduced, the degree of electrochemical change that occurred at each electrode would have been reduced and this may have restricted the

amount of water that would accumulate at the electrode. As a result, tissue desiccation and charring occur sooner and smaller ablations are produced.

The fact that the BETA circuit still works with this slightly altered arrangement, albeit less effectively, suggests that it provides a satisfactory solution. It means that significantly larger ablations can still be produced without the associated skin injury. This set up is also more suitable to a clinical setting as it means potentially, a larger ablation could be produced with a solitary needle. Ideally, in the clinical setting, the procedure would be able to be performed either percutaneously or laparoscopically. The use of a single needle would then be desirable. This set up would provide the simplest solution. Further refinements to electrode design where both polarities are present within the one electrode may provide a more satisfactory solution. A bipolar technique may also overcome this problem but would require the use of two needles.

The hypothesis that the BETA technique is as safe as RFA has now been proven in principle. However, the positioning of the positive electrode requires careful consideration. Tissue damage related to electrochemical change at the positive electrode can and does still occur. It would appear that although most of the tissue damage occurs with the radiofrequency circuit, the direct current circuit is not without hazard as originally assumed.

5.5 Study Limitations

Although results that show considerable promise have been obtained from this series of experiments, there are some limitations to the research that require discussion. All of this research has been performed in normal liver. There are no large animal tumour models currently available that are reliable enough for research of this nature. There has been some

work into developing ways of inducing liver tumours in large animal models but this poses ethical and logistical issues[212]. This limitation has been an issue with all ablation technology research and may explain the failure to identify the problems associated with local recurrence prior to its introduction into clinical practice.

No electrolytic ablation control was utilised for these experiments and the presence of these may have clarified the subtle changes that were seen. The reason for not making use of electrolytic controls is because the electrolytic process is considerably slower than the thermal effects of RFA and it was thought that the electrolytic effects would therefore be negligible. It would appear that although this may still be the case, electrolysis might take some part in the ablative process as well as the thermal effects. If these experiments were to be repeated, it may be beneficial to perform electrolytic controls as well as the RFA controls.

The other limitation of this research has been the use of a radiofrequency generator from a single manufacturer. All machines have inbuilt algorithms that are unique to each manufacturer that are designed to try and maximise ablation size. There is a possibility that the results obtained from this series of experiments are related to bypassing the inbuilt algorithm of the *Boston Scientific RF 3000* generator. As electrolytic changes had been seen during the ablation process and autopsy findings, the chance of this would be small. Ideally, all experiments would be performed using a machine with no inbuilt algorithm but there is no such machine commercially available currently.

It is important to note that when using multi-tine needles, the manufacturers recommend that the ablation procedure should be performed in 2 stages; one with the tines partially

deployed and a second ablation with the tines fully deployed. It is thought that this enhances necrosis in the treated tissue[7]. This advice was not followed during the first experiment as it would have introduced too many variables and is difficult to standardise.

It is also worth noting that *Boston Scientific* recommends that clinicians using their generator adhere to an ablation schedule. The schedule incorporates an algorithm of progressively increasing the wattage depending upon the recordings for impedance. For research purposes, however, this algorithm also introduces too many variables and would also be inappropriate in the context of these experiments.

It should be noted that the ablation sizes produced in the second and third experiments were considerably smaller than those of the first experiment. This was done in the second experiment to allow multiple ablations in each liver, thus reducing the number of animals required for the experiment. The third experiment had smaller ablations performed as it was a survival study and as the problems with the positive electrode had been identified, the size of tissue injury associated with the positive electrode had to be limited. The assumption then is that the results can be extrapolated to larger ablations. Ideally, these assumptions should be confirmed with further study.

5.6 Suggested Areas of Further Study

Prior to the use of BETA in human subjects, some further large animal, in vivo experiments are recommended. Firstly, a device that has all of the circuitry fully integrated would be ideal. It should be constructed without inbuilt programming or algorithms and should be able to be manually programmed. This would remove the limitation of each manufacturer's

specifications and programming. It would be worth repeating the first experiment with such a device.

It is known that superior ablations can be achieved with RFA when a manoeuvre to occlude blood flow through the liver is implemented[168]. This is particularly useful around blood vessels where a "heat sink" effect is known to occur. It would be worth testing BETA with and without this manoeuvre, around large blood vessels.

Both electrolysis and RFA have been assessed for the potential to cause cryoshock and there is no evidence that either causes this kind of multi-organ failure. Therefore, it would be surprising if BETA could be associated with this major complication. However, it would be worth repeating this kind of experiment with the BETA circuitry to confirm that it does not cause a similar entity and further confirm its safety.

Radiofrequency ablation has also been used for unresectable renal cell cancers, osteoid osteomas and lung metastases. Electrolysis has been experimented with in pancreas and lung. It may be worth testing BETA in these organs.

In practice, long, time-consuming ablations are undesirable. Further optimisation studies where different RFA wattages are used may help to shorten the time required for bimodal ablation. It may also be possible that the period of hydration with the direct electrical current is not required and could be omitted.

5.7 Concluding Remarks

Bimodal Electric Tissue Ablation is a form of thermal therapy that combines the alternating, high frequency electrical current with a low level direct electrical current. By employing the water-attracting properties of the cathode of the direct current, significantly larger ablations can be produced when compared to RFA controls. The mechanism of tissue injury is predominantly from the radiofrequency component although electrochemical changes associated with the electrodes may play a small role in ablation.

The BETA system appears to be relatively safe in comparison with RFA using in vivo studies. However, the placement of the positive electrode needs consideration as it can potentially induce tissue injury. This is most likely as a result of electrochemical changes that can occur at this electrode. It may be possible to overcome this limitation by increasing the surface area of the positive electrode such as with a grounding pad placed upon the skin. However, this diminishes the ablation size.

By producing a larger sized ablation, BETA has the potential to treat larger, unresectable tumours with reduced local recurrence. This is because a single application of energy can be employed to treat tumours greater than 3 cm in diameter rather than performing multiple overlapping ablations as currently occurs with RFA.

It has been proposed that the ideal ablation should be a single episode of energy delivered by a solitary applicator insertion resulting in cure[7]. It should also be reliable, associated with minimal morbidity and be both time and cost effective. It would appear that BETA has the potential to fulfil these goals.

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