DIPLOMA THESIS

Medizinische Universität Graz

THE ROLE OF IRREVERSIBLE ELECTROPORATION (IRE) AS AN ABLATION TECHNIQUE IN INTERVENTIONAL RADIOLOGY

submitted by

DI(FH) Gerhard Wernigg

to gain the academic degree

Doktor der gesamten Heilkunde
(Dr. med. univ.)

at the

Medical University of Graz
Clinical Department for General Diagnostic Radiology

supervised by

Univ.-Ass. Dr. med. univ. Thomas Wimmer
Univ.-Prof. Dr. med. univ. Michael Fuchsjaeger

Graz, 13/03/2016

STATUTORY DECLARATION

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Graz, 13/03/2016 DI(FH) Gerhard Wernigg *eh*

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr. Thomas Wimmer and Univ.-Prof. Dr. Michael Fuchsjäger and their colleagues at the clinical department of radiology in Graz for the opportunity to review such a relevant and fascinating topic for my diploma thesis. Through their guidance and several internships that I could experience at their clinic, they greatly affected me in my quest for my future career in radiology and nuclear medicine.

Special thanks go to my family that supported me after my decision to study medicine as my second career path being an engineer at the age of 26. My parents always stood behind me and my decisions and helped me in great extent through their mental and financial support throughout my studies.

Very special thanks also go to my girlfriend Elisabeth, who always believed in me and gave me new perspectives in life when I needed them the most. In the end it was her who brought me into medicine and changed my life for the better.

Further thanks go to Elisabeth's, as well as to my parents for taking care of our dog when we had no time to do so.

And last but not least I would like to thank my dear colleagues Elisabeth, Lisl, Malimmes and Bernhard for their companionship and help, that made the journey of the past six years so much more enjoyable.

ABSTRACT

The purpose of this thesis is to give a comprehensive overview of the current status of irreversible electroporation (IRE) in research and clinical practice as an ablation technique in interventional radiology.

Image-guided percutaneous treatment techniques are gaining importance in interventional oncology as minimally invasive therapeutic option for patients not eligible for surgical tumor resection, as salvage therapy and even as primary therapy for small tumors.

Irreversible electroporation, a relatively novel technique (introduced to clinical medicine in 2007), has certain benefits compared to well established thermal ablation techniques, but also possible drawbacks.

The aim of this work is to give an overview ranging from the basic physical principles to state-of-the-art implementation of irreversible electroporation in Interventional Radiology.

We furthermore want to deliver a thorough review of this technique based on current literature, including basic, translational science as well as clinical studies. In brief, we will also compare the outcomes of IRE to other ablation techniques and determine the fields in which it may be a superior choice of treatment. We will also try to give a future outlook on this fascinating matter.

Comparative and systematic literature review will constitute the base of this work.

TABLE OF CONTENTS

1 List of Abbreviations

ATP	Adenosine triphosphate
BCL-2	B-Cell lymphoma 2
CEUS	contrast enhanced ultrasound
CT	Computed Tomography
DC	Direct Current
DNA	Deoxyribonucleic acid
DSA	digital subtraction angiography
ECG	Electrocardiography
ER	Endoplasmic reticulum
FEM	Finite element method
HCC	Hepatocellular carcinoma
H-E	Hematoxylin-eosin
H-FIRE	High-frequency irreversible electroporation
IRE	Irreversible Electroporation
IVU	intravenous urography
LAC	locally advanced pancreatic adenocarcinoma
LAPC	locally advanced pancreatic cancer
LRFS	local recurrence free survival
MR, MRI, MRT	Magnetic Resonance Imageing / Tomography
MWA	Microwave ablation
NADH	Nicotinamide adenine dinucleotide
nsPEF	Nanosecond Pulsed Electric Field
NTIRE	Non Thermal Irreversible Electroporation
NVB	neurovascular bundle
PS	Phosphatdylserine
PTP	Permeability transition pore
RCC	renal cell carcinoma
RE	Reversible Electroporation
RECIST	Response Evaluation Criteria in Solid Tumors
RF, RFA	Radiofrequency ablation
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SEER	Surveillance, Epidemiology, and End Results Program
SPEF	Steep pulsed Electric Field
SR	Sacroplasmic reticulum
SRM	small renal mass
TIRE	Thermal irreversible electroporation
TNM	Tumor, Node, Metastasis Classification of Malignant Tumors
TRUS	transrectal ultrasound
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
UCLA	University of California, Los Angeles
US	Ultrasound
vWF	von Willebrand factor

2 LIST OF FIGURES

"The important thing is to not stop questioning. Curiosity has its own reason for existence. One cannot help but be in awe when he contemplates the mysteries of eternity, of life, of the marvelous structure of reality. It is enough if one tries merely to comprehend a little of this mystery each day."[2]

- Albert Einstein

3 INTRODUCTION

Oncology, the study of cancer, is one of the most rapidly evolving areas in modern medicine. Treatment of cancer has been a driving force in medicine ever since it was first described in what is the first known paper on the topic – the Edwin Smith Papyrus from about 3000 BC – an ancient Egyptian textbook of surgery. The writing says about the disease that "there is no treatment". [3]

Humanity and its technologies have come a long way since then. Oncology today is a conflux of the scientific achievements of countless doctors and scientists from sometimes most particular fields. It is the outcome of several hundred years of research in anatomy, physiology, chemistry, epidemiology, pathology, biotechnology, and many other related fields.

Each new development in one of the related fields refines the treatment approach of a specific cancer type. With the continual progression of cancer therapy, there have been introduced important advantages in treating and eliminating tumors, so that in fact, today there are effective treatments, but there is still room for improvement.

Classic tumor surgery often implies major interventions and long recovery periods for the patient. These are both detrimental for the patient as well as for the health care system, which is obliged to cut down on cost and the period of hospitalization. Thus, there is a high demand for less invasive forms of treatment that can precisely target specific tumors. [4]

Therapeutic methods such as chemical ablation, cryoablation and high temperature ablation have gained widespread attention and broad clinical acceptance for treating a wide range of tumor types and tissues. [5]

Irreversible electroporation (IRE) is a novel minimally invasive approach to tumor ablation, but instead of heating or freezing a tumor, it utilizes a process called *electropermeabilization* or *electroporation*.

Within just a few decades, electroporation has evolved from being an interesting idea to becoming a feasible treatment strategy. Electroporation is a platform technology and it is likely to improve in rapid speed. [6]

Electroporation is based on the ability of pulsed electric fields to change the permeability of cell membranes. This effect can be reversible (reversible electroporation, RE) or irreversible (irreversible electroporation, IRE).

Reversible electroporation is used as a way to make cells permeable to chemicals and large molecules, which otherwise would not be able to pass a cell membrane. This method is of great use as a clinical technique in chemotherapy (*electro chemotherapy*) to notably enhance treatment efficacy by channeling drugs into tumor cells much more efficiently.

Electroporation furthermore enables the transfer of other molecules into cells, e.g. genes. While viral vectors can have serious limitations in terms of immunogenicity and pathogenicity, *gene electrotransfer* is much safer, cheaper and easier to realize. [7]

Cells that are treated with RE are able to recover by closing the pores again after the procedure. This is enabled by applying low electric field strengths to the targeted cells.

Irreversible electroporation is attained through the application of stronger electric fields or longer periods of pulsation of the field and was initially an undesired effect of electroporation. It permanently opens cell membranes and forces cells into apoptosis (programmed cell death) or induces necrosis through impaired cellular homeostasis. IRE therefore has been discovered as a method of permanently destroying cancerous cells and today, it is used as a standalone ablation technique.

3.1 General and theoretical advantages of IRE

Operating without significant generation of heat and favoring apoptosis over of necrosis, IRE has certain benefits compared to more established thermal ablation techniques. [4]

The fundamental difference between IRE and other ablation techniques is its ability to create cell death without significant heat generation through ultra-short. Consequently, IRE avoids the problem of the heat/cold-sink effect which is inherent to thermal ablation techniques.

The heat/cold-sink effect has been a major obstacle for minimally invasive tumor ablation ever since thermal ablation found its way into clinical practice. It is evoked by the dissipation of thermal energy near large vessels in the ablation area. The blood flow through the vessel cools (heat-sink) or respectively heats (cold-sink) the tissue surrounding the vessel and thus causes incomplete ablation in perivascular tumor tissue, which may result in tumor recurrence.[4] With IRE there is no heat/cold-sink effect, since ablation is not a result of thermal energy being applied.

In contrast to thermal ablation techniques IRE also preserves vital structures such as nerves or vessels, glandular ducts etc. within the ablation zone, since IRE targets cell membranes and spares the extracellular matrix of tissues (e.g. collagen), which facilitates regeneration of healthy tissues. Ablation techniques that make use of thermal energy create an ablation area where cell death rate is subject to a gradient that lessens to the outer margin of the ablation area, while IRE happens wherever a certain electric field strength threshold is exceeded resulting in a sharper border of the ablation zone even on a microscopic layer. [8]

IRE has the theoretical benefit of creating complete cell death within an area, when sufficient electric field strengths are reached. Ablation zones demonstrate sharp margins and sharp demarcation on the order of only 1-2 cells between affected and spared areas, hence the trademarked clinically approved IRE device *Nano Knife®*.

While thermal ablation procedures cause coagulative necrosis of the ablation zone, the cells affected by IRE also show a significant fraction of apoptosis. Heat induced coagulative necrosis kills tumor cells, but also healthy tissue surrounding the tumor. Vital structures surrounding or embodied into tumor tissue are destroyed and the destruction of the ablation area leaves nothing but necrotic debris. This may result in a strong immune system response leading to inflammation and in some cases facilitates superinfection.

IRE on the contrary ideally induces apoptosis (to some extent also necrosis) within well-defined borders. Physiological programmed cell death is a controlled process of the human body and is well understood by its immune system. The immune system's response to this immune mediated cell death, which is a natural part of the cell cycle, is well programmed and induces phagocytes to clear up post-ablation detritus. This puts substantial less stress on the immune system, leads to faster regeneration of the organs and better recovery of the patient after the intervention.[4]

Real-time monitoring through image guidance (US, CT and MR) before, during and after treatment is a general advantage of this interventional radiologic procedures. Complications as well as outcome can be monitored throughout the procedure. Several studies have demonstrated that the IRE ablation zone size monitored by intra- and postprocedural US or CT corresponds well to histologic and gross pathological measurements.[8] This suggests a high accuracy of US and CT imaging in monitoring the ablation procedure and the use of imaging to follow-up the results of an ablation procedure.

Electric energy has another major benefit over mechanical energy (such as heat or cold temperature), it does not take much time to build up.

> *"A typical IRE procedure for a solid tumor, with a size of approximately 3 cm in diameter, uses 90 pulses with an ultra-short pulse length of 100 microseconds. A single IRE ablation session takes less than one minute. Therefore, even with three or four overlapping ablations, total IRE treatment time is under 5 minutes."*[4]

Shorter ablation times correlate with reduced anesthesia time, less complications, less pain for the patient, decreased costs and the opportunity to treat multiple lesions in one session.

Despite these and further advantages that will be described in this work, IRE raises new questions and interventional radiologists face new problems that need to be solved.

3.2 CHALLENGES OF IRE

Planning and calculating the distribution of electric fields in various and often inhomogeneous organic materials is still a challenging task and one of many research fields regarding this technology.

There is still plenty of ongoing research on the topic of the effects of strong electric fields on non-targeted organs and areas. Undesired muscle contractions during ablation, including the diaphragm, are an issue that usually is handled by paralytic agents to induce muscle relaxation.

Another problem that IRE faces is the occurrence of cardiac arrhythmias that can potentially be induced by electrical pulses, especially during the vulnerable period of the cardiac cycle, the atrial or ventricular systole. Since cardiac synchronization devices have been developed, which only produce pulses during the non-vulnerable period of the cardiac cycle, no further arrhythmias have been reported in clinical studies. [4]

One primary consideration behind muscle contractions and cardiac synchronization is, that for IRE to be performed, the patient needs to undergo general anesthesia. In contrast thermal ablation can be performed under conscious sedation, which avoids complications of general anesthesia. [9]

Equipment wise IRE necessitates neuromuscular and cardiac monitoring, while it does not require grounding pads, cooling systems or expensive gases used in other ablation techniques.

Irreversible Electroporation requires careful treatment planning, including a combination of imaging and accurate mathematical modeling to predict the extent of ablation zones. The mathematical model of the three dimensional electric field distribution in often heterogeneous tissue with different electrical conduction properties still needs a lot of experimenting to deduct the correct parameters for each tissue and tumor type. [9]

Optimal device settings are still being under discussion, as Quin et al.[9] found that recommended thresholds for IRE as described by the original report of Rubinsky et al.[10] can be much too low to effectively kill tumor cells. The assumption that tumor tissue, known to be much more resistant to apoptotic pathways, will have the same response to IRE pulses as normal tissue might present a major flaw. [11]

The vast spectrum of different tumor types and tissues, as well as many of the different possible applications of IRE, opened up a big new field for future research on this topic. Almost every day new publications about IRE are being announced.

This thesis will try to provide an overview on the whole topic of irreversible electroporation as well as to integrate some of the latest available in-depth knowledge about important recent publications.

4 HISTORY OF ELECTROPORATION

Irreversible electroporation has only been introduced to clinical medicine in the year 2007, but its history surprisingly reaches as far back as in the mid-18th century.

4.1 18TH AND 19TH CENTURY

In his research Nollet describes the formation of red spots on human skin in areas where electrical sparks have been applied. Whether these red spots are a direct consequence of the first known experimental application of IRE or evoked through thermal damage to the skin, is still being discussed today. [1]

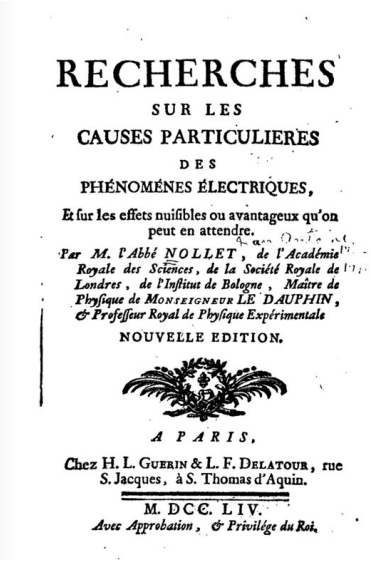
RECHERCHES
SUR LES
CAUSES PARTICULIERES
DES
PHÉNOMÈNES ÉLECTRIQUES,
Et sur les effets nuisibles ou avantageux qu'on peut en attendre.
Par M. l'Abbé NOLLET, de l'Académie Royale des Sciences, de la Société Royale de Londres, de l'Institut de Bologne, Maître de Physique de MONSEIGNEUR LE DAUPHIN, & Professeur Royal de Physique Expérimentale
NOUVELLE EDITION.
A PARIS,
Chez H. L. GUERIN & L. F. DELATOUR, rue S. Jacques, à S. Thomas d'Aquin.
M. DCC. LIV.
Avec Approbation, & Privilége du Roi.

Figure 1: 1754 – Nollet [1]

The idea that these red dots were the first observation of the effects of IRE on human tissue is being supported by the fact that erythemas are common in electroporation of skin and secondly, that static electric generators of that time were not able to cause any significant heating.

From 1780 Luigi Galvani observed what he later described as *animal electricity*. He observed the muscles of a dead frog placed on an iron plate to twitch when touching his spinal cord with a bronze hook. Alessandro Volta deducted from Galvani's discovery that two different metals in the same electrolyte create a direct current, that the frog could be replaced with a different electrolyte and thus developed the voltaic pile in 1800 – the first electrical battery. [12]

In 1802 J.W. Ritter recognized what is now called *Ritter's opening tetanus* which is defined as *"the tetanic contraction that occasionally occurs when a strong current, passing through a long stretch of nerve, is suddenly interrupted*". The phenomenon was not understood by that time, but in the 20th century, it essentially led to the understanding of what electroporation does and how it affects cell membranes. [13]

Throughout the 18th century, these revelations in physiology and electricity were deeply interconnected and the science of electrophysiology emerged.

In 1898 a study by G.W. Fuller added a new facet to the topic of electroporation and this might be the first observation of an irreversible electroporation event. The *"Report on the investigations into the purification of the Ohio river water at Louisville, Kentucky"* [14] described an experiment that showed that multiple high voltage discharges have a bactericidal effect on a water sample without any recognizable development of heat in the fluid. [9]

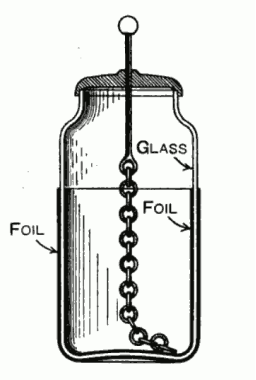

Figure 2: Leyden Jar [12]

In the late 19th century A.D Rockwell reported his observation that the red corpuscles in a Leyden jar changed their shape and lost their color after applying electrical discharges. [15]

The change in shape and color was most probably caused by irreversible electroporation of the blood cells affected by the flowing currents in this earliest form of a capacitator (then known as a *"condenser"*). The Leyden jar was a crucial tool for the investigation of electroporation of cells in suspension.

To summarize the history of electroporation before the 20th century, it can be said that certain important findings occurred that were fully understood in their right context much later. [12]

4.2 First half of the 20th century

A set of lectures by Jex-Blake in 1913 discussed the differences between the lethal effects of human made electricity and lightning. He stated that while man-made electricity in e.g. industrial accidents caused primarily thermal damage, injuries through lightning seemed to be of different origin. [16]

A very peculiar phenomenon occurring in humans who were struck by lightning is the appearance of so called *"Lichtenberg figures"* on the skin. These figures are thought to have the same origin like Nollet's red spots – irreversible electroporation of the skin. [12]

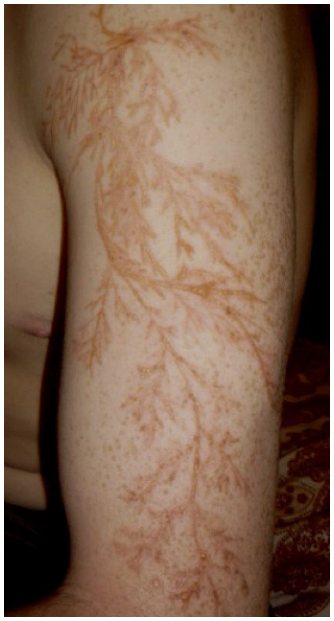

Figure 3: Lichtenberg Figure [1]

Another important mentioning in Jex-Blake's lectures was the recovery of animals from heart failure induced by the application of electric shocks. Today it is thought that defibrillation is significantly influenced by effects from electroporation occuring. [16]

During the 1920s and 30s G.M. McKinley performed a series of experiments on chick embryos and concluded that the damage caused by high frequency fields cannot solely be caused by thermal energy. His paper proposes that there are also other related mechanisms involved. [17]

H. Fricke's 1925 hypothesis for the cell membrane to be a dielectric layer opened the discussion for the event of a dielectric rupture phenomenon caused by electric fields. [18]

In 1951 A.L. Hodgkin made the significant connection and linked Ritter's opening tetanus to a breakdown of the insulating properties of cell membranes. This was about the first understanding of how the process of what today is called electroporation might actually work. [19]

In the mid-1900s a growing body of evidence also supported the notion that the application of electricity to biological tissues not only caused thermal damage, but that there must be another mechanism involved. The concept that cell membranes are dielectric layers that can break down under the application of electrical currents has found growing acceptance in the scientific community. [12]

4.3 1950 TO 1970

Till 1956 electric nerve stimulation has been researched for almost 180 years since Galvani's discovery of "animal electricity". Unsurprisingly, then the first systematic work on reversible and irreversible electroporation by B. Frankenhaeuser and L. Widén was done on nerves. The scientists found that nerves could be inactivated through the application of electric pulses at amplitudes up to ten times the normal threshold and pulse duration increased from under 1ms to more than 100ms. They published the study under the title *"Anode break excitation in desheathed frog nerve"* and described the phenomenon to be reversible to a fair extent. [20]

Stämpfli et al. confirmed this observation in a soon following paper entitled *"Membrane potential of a Ranvier Node measured after the electrical destruction of its membrane"* and commented that membranes recovered like an electrolytic condenser immediately after breaking down under one short pulse, while very strong pulses destroyed the membrane irreversibly. [21]

Since G.W. Fuller's study of the bactericidal effects of electroporation in 1898 the research on electroporation was carried out in parallel between biomedicine and the food processing technologies. In 1961 H. Doevenspeck reported about non-thermal ablation using electric pulses to break apart cellular components for the processing of animal meat. Doevenspeck's results showed that the outcome of his study was clearly provided by non-thermal effects on the cell membrane. [22]

Only six years later, in 1967 the team of Sale and Hamilton set the scientific basis for the field of irreversible electroporation with the release of three exceptional papers about the "bactericidal action of electric fields".

The first of the papers evaluated the non-thermal bactericidal effect of electric fields on microorganisms, namely bacteria and yeasts. The applied field currents ranged up to 25 kV/cm. The death of the organisms was not due to the products of electrolysis and the temperature rise in the suspension was too small to cause any lethal effects. Also, no changes in the pH of the suspension were of any significance. The sensitivity to electric fields of the various species also differed, so that the yeasts showed more sensitivity than the bacteria. [23]

The conclusion of this study was that the parameters for killing a cell, in order of importance, are the electrical field strength and secondly the time of exposure.

In the second study, the team tried to expose the exact mechanism by which the cells are being killed. The spectroscopy of the leakage of Eschericia coli cell content in the medium was the essential observation which led to the expertise, that the irreversible loss of the membrane's function as a barrier is what causes the cells to die. [24]

Electron microscopy was used to demonstrate this judgement, but a complete breakdown of the membrane could not be verified. This suggested that the damage affected areas that could not be identified.

The third paper showed that the electric field strengths for inducing lysis of different organisms range from 3.1 kV/cm to 17 kV/cm while the equivalent induced transmembrane voltages only range from 0.7 V to 1.15 V. This observation led to the understanding that the external electric field induces *"conformational changes in the membrane structure resulting in the loss of its semipermeable properties".* [25]

By the end of the 1960s, it was widely accepted that electrical pulses have a permeabilizing effect on cell membranes and that this effect can lead to the loss of homeostasis in the cell which then leads to lysis of the cell. It was understood that this effect is non-thermal and that certain electrical field magnitudes can be tolerated and reversed, while others lead to irreversible and lethal damage. [9,12]

4.4 1970 TO 2000

The following 30 years of research in the field revolved around electroporation, mainly ruling out irreversible electroporation as an undesired side effect serving only as the upper limit to reversible electroporation.

U. Zimmermann and his group delivered first systematic data on the electrical parameters for electroporation and irreversible electroporation in cells. Testing human and bovine red blood cells surprisingly resulted in different asymptotic values for electroporation. This suggested different irreversible electroporation thresholds in different cell types. [26]

The first to propose that the permeabilization of cell membranes using electroporation works through the formation of pores were K. Kinosita and T. Tsong in the year 1977. The team was able to show that the size of the pores can be varied and that these pores eventually reseal. [27] In their osmotic mass transfer experiments of blood cells, they were also able to show, that the process of resealing also largely depends on temperature. *"At 37 °C the treated membrane rapidly regains its impermeability to cations, whereas, at 3 °C the cells remain highly permeable even after 20h."* [28] This observation adds another parameter to electroporation and the potential ability and achieve cell states close to IRE, but with the ability to reseal.

With cell fusion and the introduction of genes into cells two major discoveries were made in the year 1982, bringing electroporation into the limelight of modern medicine. [29,30]

By the end of the 1980s, research in electroporation begins to switch from bare cells to tissue pursues the following three important directions.

M. Okino, H. Mohri and Orlowski et al. independently described the use of reversible permeabilization of cell membranes as an effective way to introduce cytotoxic agents into malignant cells. [31,32]

Prausnitz et al., who were influenced by a paper of Powell et al. demonstrating the reversible electroporation of frog skin, discovered in 1989 that transdermal drug delivery was significantly increased through the incorporation of electroporation. [33]

From 1987 Lee et al. investigated induced trauma through electrical discharge and proved that 1 to 30 discharges of 1 ms and 150 V/cm separated by 5s did produce a temperature rise of less than 0.1 °C and caused irreversible damage. The dissected rat muscles that were used in this experiment showed a decrease in electrical resistance following each

pulse. This important finding suggests that the conductivity of the affected tissue can be a measure of tissue damage. Longer application times of up to 1 s were found to increase tissue temperature for 10 to 20 °C and was therefore considered to injure cells in both ways, thermally and non-thermally. [34]

In the last decade of the 20th century reversible electroporation evolved into a feasible method to enhance drug delivery into cells, while irreversible electroporation still represented the undesirable effect.

Titomirov et al. were the first to report a successful introduction of plasmid DNA into a living tissue in 1991. In the same year L.M. Mir published his work on the facilitation of anticancer drugs into malignant cells forming the term electrochemotherapy. It is today a well-established application of reversible electroporation and widely accepted as a treatment option for cancer patients. [35]

Skin electroporation was established in 1993 for transdermal drug delivery. [33]

Two independent papers published in the late 90s were able to prove that cell death induced by electroporation was not only caused by necrosis, but also through apoptosis. Both papers found chromosomal DNA fragmentation via in vitro experiments that are a clear sign of late apoptosis. [36,37]

J. Piñero et al. were the first to suggest irreversible electroporation as a possible tumor treatment option by stating *"The possibility of killing tumor cells by electroporation, as a variant of electrotherapy, constitutes, in our opinion, a promising procedure in cancer therapy, avoiding the undesirable side effects normally derived from treatment with cytotoxic drugs."* [36]

A *vascular lock* phenomenon was first described in 1998 and defined as blood flow being blocked in the area where the electric field is applied. The resulting ischemia is being thought of as a beneficial effect in the context of tumor treatment. [38]

4.5 2000 TO 2008

Nanosecond Pulsed Electric Field (nsPEF) was an area of research formed by Schoenbach et al. in 1997 that tried to prove that ultra-high voltage pulses of only nanoseconds could electroporate internal cell structures without the disturbance of the cell membrane. However, more recent studies on the matter demonstrated that this is not the case and the cell membrane is affected in the same way as internal structures. nsPEF is still worth mentioning because it has been found out that this method after all, can induce apoptosis and is even partially capable of inhibiting tumor growth. [39]

Yao et al. followed the idea of nsPEF and put their efforts in creating an IRE ablation method to *"destroy both nucleus and membrane"* in 2004 which they called *Steep Pulsed Electric Field (SPEF).* Their procedure combined the supposed intracellular effects from *"high-frequency components"* with the *"low-frequency components"* that were known to affect the outer membrane of cells. They succeeded in killing tumor cells and delayed tumor growth by applying SPEF pulses of a fast rising edge (~ 200 ns) followed by a slow exponential decay ($\tau \sim 200\ \mu s$). [40]

Considering that the low amplitude they used and that the rise time of 200 ns today is commonly used in electroporation A. Ivorra and B. Rubinsky believe that the observed effects do *"not differ from the effects of conventional IRE"* and that therefore Yao et al. were, despite unconsciously, the *"first ones to obtain evidence of the capabilities of IRE as a tissue ablation method for tumors"*. [12]

In 2004 R. Davalos and B. Rubinsky filed a US patent application recognizing IRE as an ablation method that, in contrast to existing thermal ablation methods, can easily be applied in highly perfused areas e.g. areas in the proximity of blood vessels. They stated that IRE can be designed in a way to avoid thermal damages from the electric pulses and that this feature would improve post-treatment healing. The team referred to their procedure as non-thermal irreversible electroporation (NTIRE) – which is the type of IRE that is performed today. [41]

Much NTIRE research was done subsequently, establishing possible treatment strategies for human hepatocarcinoma cells in vitro. Experiments on rodents and pigs followed and showed, that while tumor cells could be destroyed, large blood vessels remained intact.

J. Edd and R. Davalos performed IRE on 14 pigs using ultrasound guided 18 gauge stainless needles and came to the following conclusions. All of the pigs survived the treatment. Generalized muscle contraction occurred, but could be treated with Pancuronium. Ultrasound examination of the ablation zone presented hypoechoic areas directly following the ablation, but permuted into a uniform hyperechoic space after 24 hours. The histological examination of the livers demonstrated the ablation area continuously necrotic with sharp demarcation between the ablated zones and the supposedly physiological tissue. Macroscopic assessment showed large vessels not affected by the procedure. [27]

Similar research was performed by Rubinsky et al. producing further interesting results. In a series of studies, the researchers showed that the connective tissue of vessels stayed intact, while the number of vascular smooth muscle cells was decreased when performing IRE on the carotid arteries. They suggested that IRE could be a way to treat atherosclerotic processes. [42]

Collaborating with Onik et al, the Rubinsky group also experimented with the ablation of prostate tissue in vitro and could show that structures such as urethra, bladder, rectum, vessels and nerves could be preserved during the ablation, even though they were included in the areas of the high electric field application. [10]

Lee et al. reported similar findings as Rubinsky while inserting electrodes percutaneously into pig livers. Apoptotic processes could be demonstrated as well as sharp transition zones between ablated and normal tissue. The researchers also showed that real-time monitoring was possible during the process. [8]

Al-Sakere et al. studied the immune response following IRE and concluded that an immune response is not necessary to successfully ablate tumors by IRE and IRE could therefore be a feasible treatment option for immunodepressed cancer patients. [43]

J. Edd and R. Davalos analyzed mathematical computer modeling to predict the shape and extent of lesions created by IRE. Their task was to evaluate thresholds for specific sorts of tissues in experiments and incorporate their findings into computer models to predict the distribution of a field's magnitude in a tissue according to electrode configurations and applied voltages. [44]

In 2007 Lavee et al. studied the potential of IRE as an ablation method for the treatment of atrial fibrillation as an alternative to thermal ablation methods. They also reported that the demarcation zones were clear and sharp. The treated areas manifested electrical isolation and the authors concluded that IRE is *"a new and exciting modality to perform atrial ablation, which holds the potential of providing very swift, precise, and complete transmurality with no local heating effects."* [45]

It is finally worth mentioning the work of Tekle et al., who demonstrated the exposure of phosphatdylserine (PS) to the outer surface of cell membranes when affected by electroporation. PS is an important component of phospholipid membranes and is used as a key element in the cell cycle to signal apoptosis to macrophages engulfing the cells. The authors refer to these cells as *apoptosis-mimetic* and consider this phenomenon to facilitate the process of non-inflammatory phagocytosis altogether. This also supports the findings about the preservation of tissue scaffolding and helps to explain the rapid recovery of tissues after an IRE treatment. [46]

The first high voltage pulse generator using single-use disposable electrodes and therefore representing the first commercial system approved for clinical irreversible electroporation was produced in 2008.

Since 2008 IRE new areas of application for IRE are developed at a rapid pace and IRE has been established as a standalone ablation technique. As a new treatment option IRE opened a large new field for research and today it is ready to compete with well-established thermal ablation techniques and able to prove its capabilities in this process. [12]

5 Biochemistry and Physics of Electroporation

In order to understand irreversible electroporation and to efficiently use it in the treatment of patients, a certain understanding of cellular physiology, knowledge about electric fields and to some extent the comprehension of the calculations and mathematical models in use is required.

This chapter will provide insight into these biotechnological fields.

5.1 Cellular Physiology

The cellular membrane is a chemical and electrical barrier that separates the cell interior from its surroundings. It is a bi-lipid layer that acts as a regulatory barrier permeable only to very small molecules like water, oxygen and carbon dioxide and to a very small degree to polar compounds of hydrophobic molecules (e.g. amino acids, DNA, RNA, carbohydrates, proteins and ions). These molecules are either transported into the cell via endocytosis or through membrane proteins that allow a controlled carriage through pores and channels or by active transport. The transmembrane transport of essential ions like Na^+, Ka^+, Ca^{2+} and Cl^- is largely controlled by the cell with ion pumps and exchangers. These enable the cell to build up chemical and electrical gradients.

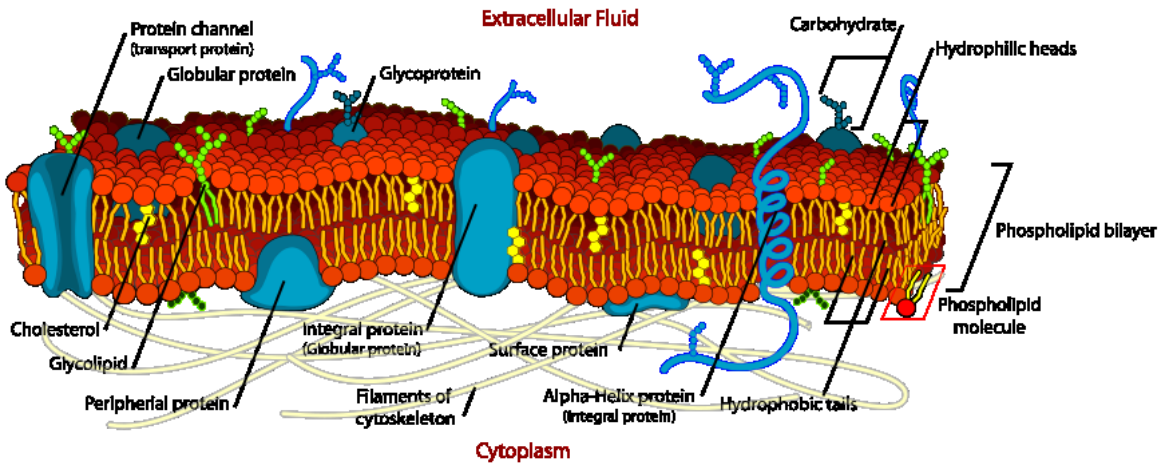

Figure 4: A detailed diagram of the cell membrane [47]

Under physiological conditions the cell keeps chemical gradients for Na^+ and K^+ across the cell membrane to facilitate the membrane potential that is vital to many cellular functions.

The majority of cellular reactions is controlled by the Ca^{2+} signaling system and the concentration of free Ca^{2+} in the cytoplasm is under strict control. It is kept very low (~0.1 µM) inside the cell, while the outside concentrations of free Ca^{2+} are multifold higher (~1.3 mM). High levels of intracellular ATP entertain the different active ports that maintain these gradients and sustain the energy-dependent cellular processes which the cell integrity depends on.

Depending on the strength and/or time period electric fields are applied to a cell membrane it either temporarily (electroporation) or irreversibly (IRE) increases the permeability to molecules and ions by supposedly forming so called *nanopores*. [48]

For nanopore-formation it takes at least an electric field of ≥ 0.5 V/nm according to Lee, Edward W., Wong.[48] In their work *"Electron Microscopic Demonstration and Evaluation of IRE induced nanopores"* they reference a molecular dynamics simulation made by M. Tarek stating that the formation of pores happens in two steps:

1. After electric field application water molecules line up in single file and penetrate the hydrophobic center of the bilayer lipid membrane.
2. The water channels grow in length and diameter, and expand into water filled pores. At that point the lipid head groups move from the membrane-water interface to the middle of the bilayer and the pore stabilizes.

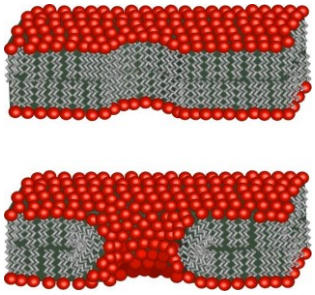

Figure 5 - Pore Schematic [22]

Tarek furthermore proposes that the formation of these pores develops faster with the application of higher electric fields and that this process happens within nanoseconds. [49]

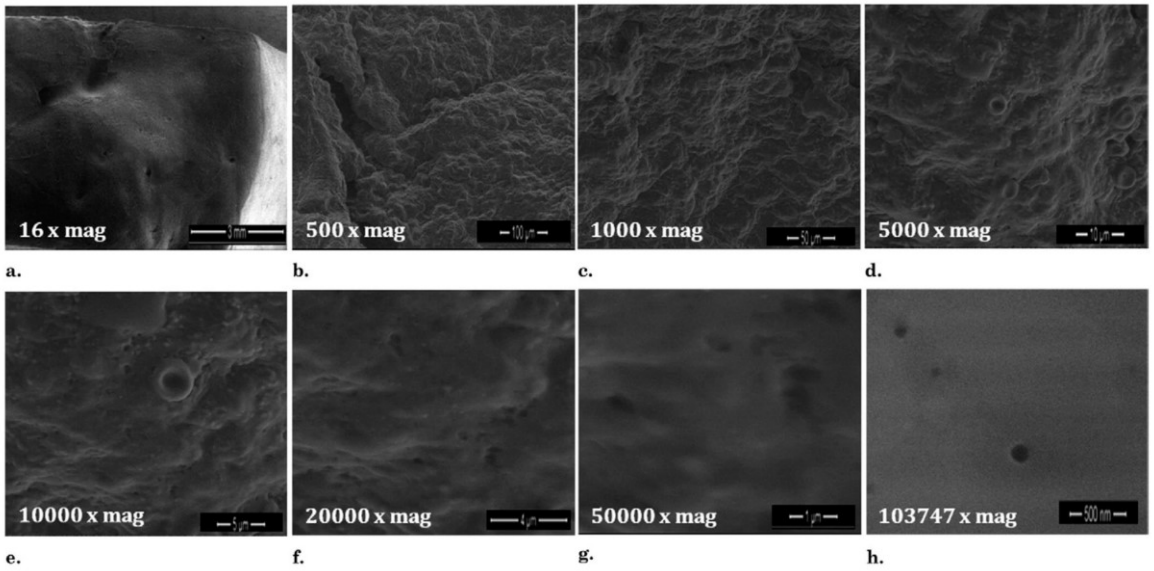

Figure 6: Series of SEM images of IRE-ablated rabbit hepatocytes with increasing magnification [48]

These openings in the cell membrane lead to a rundown of the Na^+ and K^+ gradients and the loss of the cell's control over Ca^{2+} levels due to an immense influx of Ca^{2+} from the extracellular matrix.

The cell tries to compensate by investing much of its energy in the form of ATP into maintaining the transmembrane ion gradients using the Ca^{2+}, Na^+ and K^+-ATPases. These efforts quickly drain the cell's energy sources and this adds up to the direct loss of ATP out of the cell. Caused by the damage of the cell membrane the production of reactive oxygen species (ROS) increases rapidly and manifoldly, leading to further membrane injury and protein denaturation.

These developments pose potential threats to a cell's viability and can activate pathological signals that lead to necrotic or apoptotic cell death.

Depending on the parameters of electroporation and the duration of the permeabilization the cell may be able to recover before control of Ca^{2+} level is lost and reseal, or otherwise die.

Cell membranes are believed to be able to recover completely on given conditions. While pores from electroporation form in microseconds it takes minutes to reseal them, depending on electrical parameters and temperature. Variables for resealing, are the availability of sufficient ATP for the Na^+, K^+ ATPase to regain membrane potential and for the Ca^{2+} ATPase to shift enough of the free intracellular Ca^{2+} out of the cell or into the storages of the sarcoplasmic or endoplasmic reticulum (SR or ER) or the mitochondria. If the cell manages to reseal the membrane and reestablish ion homeostasis, it can survive and the electroporation would be reversible.

In cases where the cell does not manage to reestablish ion homeostasis, the formed pores become permanent and therefore the cell is permeabilized indefinitely. The subsequent Ca^{2+} overload and the ROS production being set in motion lead to a secondary breakdown of the membrane in a vicious cycle. This cycle results in the loss and depletion of all the ATP resources, the breakdown of the membrane and eventually in cell death either via apoptosis (a controlled process requiring ATP) or by necrosis.

Survival or death of a cell largely depends on the balance between degradative mechanisms (largely influenced by Ca^{2+} and ROS) and membrane repair mechanisms. Due to the loss of proteins, enzymes, coenzymes, RNA and important metabolites, used for the recycling of ATP, permeabilization of the membrane is perilous to the cell. Thus, most

cells are very apt at membrane repair having effective emergency response mechanisms to confront leakage. [50]

Besides the SR and the ER mitochondria also offer a valuable capacity for Ca^{2+} and thereby play a significant role in shaping and buffering Ca^{2+} signaling. While a small uptake of Ca^{2+} can have a positive effect on oxidative phosphorylation larger uptakes can result in a Ca^{2+} overload that results in an opening of the permeability transition pore (PTP). The widespread opening of mitochondrial PTPs due to a Ca^{2+} overload is basically a cells final step towards apoptosis or necrosis, constituting a point of no return and therefore resulting in irreversible electroporation.

It is important to understand that the threshold for the survival of cells varies with the kind and state of the used tissue, as well as the type of application (in vitro, ex vivo, in vivo).

Whether IRE causes apoptosis or necrosis depends on the availability of ATP during the process. Cells committed to apoptosis but lacking ATP to achieve it die by necrosis.

Apoptosis is the programmed cell death that is organized in a controlled manner, turning the cell into encapsulated apoptotic bodies that are usually removed by macrophages without a trace and without triggering further inflammatory reactions. On the contrary, necrosis is resulting in cell lysis and an uncontrolled release of cellular components triggering inflammatory reactions.

The morphological features that allow to differentiate apoptosis from necrosis only depict the final status. There are several different pathways that lead to cell death, all following their own rules and mechanisms. Because of the large expenditure of ATP caused by electroporation the researchers Hanne Gissel, Raphael C. Lee, and Julie Gehl suggest that it is more likely that death evoked by electroporation follows a necrotic pathway. [6]

The reports on the pathways that lead to cell death are in fact somewhat contradictory. Newer studies have found that apoptosis in cells exposed to IRE can occur even without the formation of nanopores, through the release of intracellular calcium from the ER and mitochondria releasing cytochrome c that initiates apoptosis. The development of ROS in the cell may induce oxidative stress-mediated apoptosis as well. [9]

5.2 THE ELECTRIC FIELD

It is important for physicians as well as for researchers to develop a basic understanding about the nature of electric fields in order to make good use of electroporation. According to F. Mahmood in fact, *"the clinician's level of comprehension of this technology should match that of the established treatment technologies, such as external beam radiation therapy in cancer irradiation."*[6]

5.2.1 Basic Theory and Definitions

Electric force is described by **Coulomb's law** and is one of the four fundamental interactions known to physics. It emerges from the presence of charges and acts between charges only. The force **F** between two point charges is directly proportional to the product of the charges and inversely proportional to the square of the distance between them.[51]

$$\mathrm{F} = K \cdot \frac{q_1 q_2}{r^2} \hat{r}$$

K is Coulomb's constant, q_1 and q_2 are the point charges, r is the distance and "r-hat" is a unit vector pointing from q_2 to q_1. Two charges that have the same sign act repulsively while those with different signs attract each other.

The principle of superposition is applied whenever there are more than two point charges present. It states that the force on any charge in the system is the sum of the Coulomb forces from each of the other charges. Applying these two principles of electrostatics it is possible to solve, in theory, any problem of electrode configurations in electroporation.

The **electric field E** is a quantity defined as the electric force per charge **F**/q. It is understood as a force per charge applied at a point without charge. Its SI units are newtons per coulomb (N/C), or more conveniently, volts per meter (V/m). A particle of the charge *q* in the electric field is subject to a force **F** = *q* * **E**, and therefore **E** = **F** / *q*.

Generally the electric field is a function of space and time (**E** = **E** (x,y,z,t)). To make life easier in electroporation, considering that a system's response time is much smaller than the duration of the exposure to an electric field, it can be assumed to be in a steady state (**E** = **E** (x,y,z)) which cancels out the time factor. The polarization of a cell membrane takes about 1 µs with a typical voltage pulse duration of 0.1 – 10ms in clinical applications

of electroporation. Furthermore, the pulse generator keeps a constant potential difference across electrodes so that electrostatic conditions can be assumed.

With forces attracting or repelling each other at different magnitudes depending on their charge and the space between them the electric field is defined as a ***vector field*** that is generated by electric charges or time-varying magnetic fields.

The relationship between electric and magnetic fields and the mathematical basis for electrostatics in general are described in the **Maxwell equations**. They should be mentioned in the context of electric fields, but detailing on this topic would go beyond the scope of this diploma thesis.

The **electric potential** φ is defined as *"the amount of work needed to move a unit charge from a reference point to a specific point against an electric field"*. [52] and is measured in joules per coulomb (= volts). The electric potential is assumed to be zero at infinity. The difference between the electric potentials of two points in space is the electric voltage.

In theory, the calculation of an electric field is simple and follows the laws of electrostatics (Maxwell equations). In the reality of the electroporation of biological tissues, many more variables have to be factored into this calculation. According to F. Mahmood medical doctors should not concern themselves with how the electric field is calculated; rather, they should be able to interpret the numbers of a calculation and to a certain extent be able to point out parameters that could alter the electric field from one clinical situation to another. [6]

From this practical point of view, medical doctors do not require many more concepts from electromagnetics, but should give attention to the more concrete issues of the electric field.

The electric field is called a field, because it can be specified at every point in space and it can take on different values at these different points in space. But while, for example, temperature is known to be a scalar field, that takes on different scalar values like for example 10°C on the outside of my window and 24 °C inside of it, electric fields are made up of vectors. That means that in addition to a bare number, the electric field also has a direction at every point in space. This is known as a vector field **E**, while the size of each Vector is denoted as |**E**| or *E* and defines the electric field strength.

In clinical situations the parameters of significance are the electric field strength, but also the orientation of the electric vectors. Why is that so?

Biological tissues like muscle, skin or the parenchyma of organs etc. are **anisotropic**, which makes them sensitive to the direction of electric vectors.

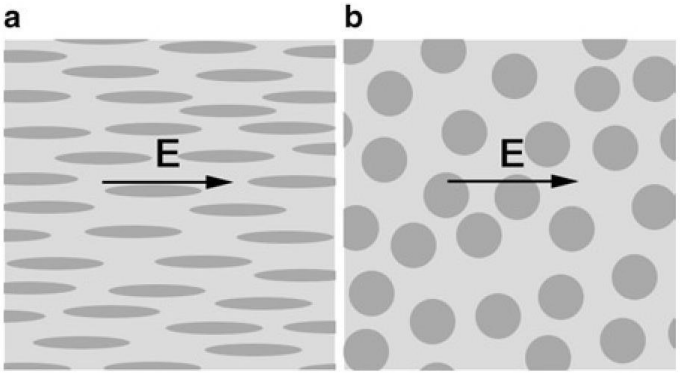

Figure 7: Anisotropy of cells [6]

Fig. 7 (a) is an artistic representation of anisotropic tissue exposed to a uniform electric field. The elliptic shapes resemble the cells. The vector of the electric field affects the induced electric field in the cell membranes, while in (b) the direction of the electric field vector makes no difference as opposed to the cell bodies of circular shape.

The **induced transmembrane voltage** ($\Delta\Psi_{m)}$ for spherical cells is defined by

$$\Delta\Psi_m = 1.5\, ER \cos\theta,$$

where E is the electric field in the region where the cell is situated, R is the cell radius, 1.5 is the form factor (f)and θ is the angle measured from the center of the cell with respect to the direction of the field. $\Delta\Psi_m$ only lasts for the duration of the exposure to the induced electric field. This equation resembles spherical cells in a direct current (DC) homogeneous field as shown in fig. 5 (b).

When in suspension, many cells have spherical shapes, but for cells in tissue this is mostly not the case. The most useful approximation for cells in a tissue are cylinders (e.g. muscle cells, axons), oblate spheroids (e.g. erythrocytes), and prolate spheroids (e.g. bacilli).

For typical tissue eligible for electroporation an approximation between a sphere and a cylinder makes sense, altering the equation for a cylinder to

$$\Delta\Psi_m = 2\, ER \cos\theta,$$

where $f = 2$ (opposed to 1.5 for a sphere). In summary, this means that the electrical field "sees" different geometries from different perspectives in figure 7.a, but not in situation 7.b. One should keep in mind that in theoretical treatments, cells are usually considered as spheres, while in reality this tends not to be the case.

When characterizing the electric field of an electrode system, it is common to use V/m to express the nominal electric field strength. This is, in fact, a close approximation of the reality in cases where for example, two plate electrodes with a fixed inter-plate distance are used, for example 100 V difference between the plates and 1cm would equal a nominal field strength of 100 V/cm.

Switching from plate electrodes to pins or needles with the same inter-electrode distance of 1cm and voltage difference of 100 V the voltage to meter ratio of 100 V/cm is still in use, but we have no idea what the actual electric field strength distribution looks like in the region between the pins, or where "100 V/cm" really is.

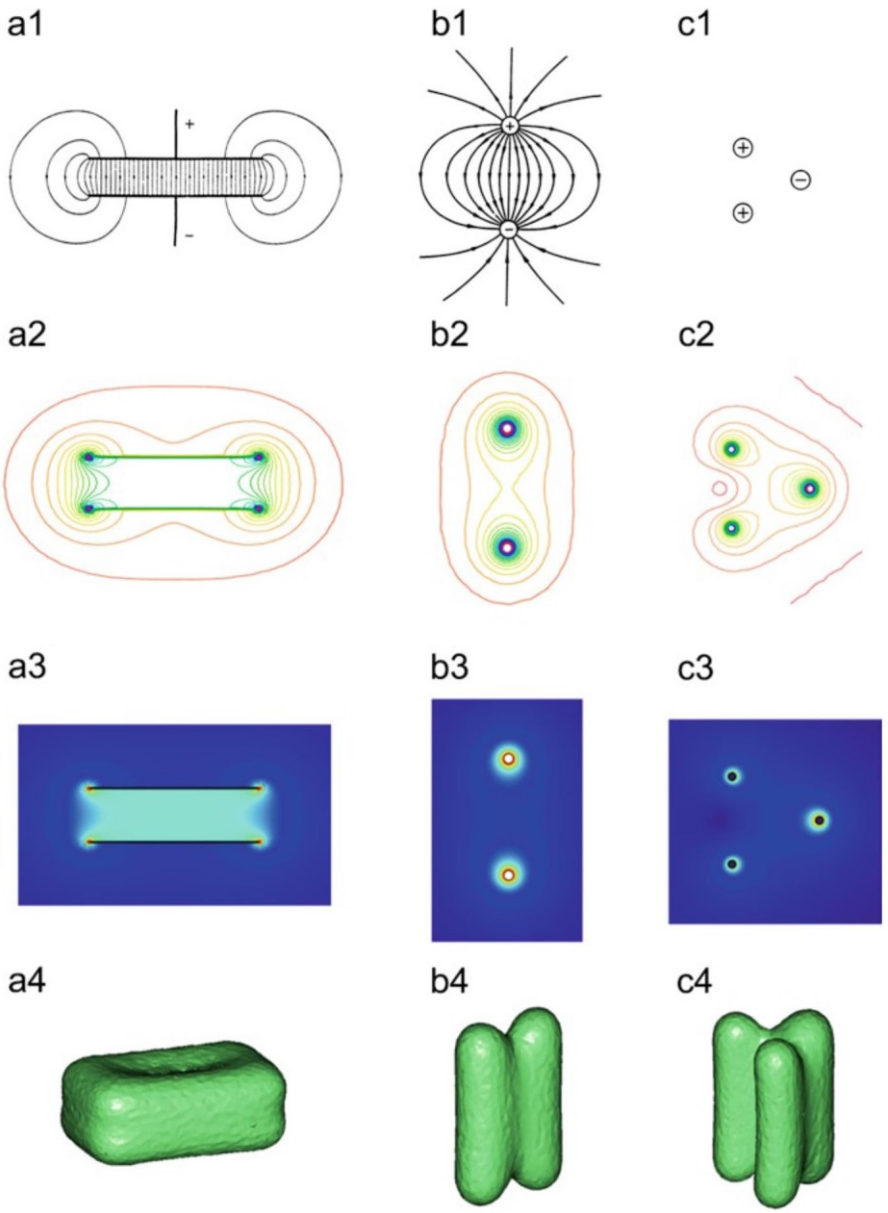

Figure 8: Electrode Configuration - Field Distribution [6]

Figure 8 displays four different types of representation of the electric fields by three different electrode configurations. (a1, b1) field line plots, (a2, b2, c2) contour plots, (a3, b3, c3) slice plots, (a4, b4, c4) iso-surface plots.

For the understanding of a given electric field distribution, usually more than one type of data illustration is needed.

In cases like fig. 8c1 "V/m" is not self-explanatory, it is not even obvious what is meant by the fraction "V/m" here. To make an approximation of "V/m" in this case, a symmetry plan and voltage encoding of the electrodes has to be performed.

To avoid misinterpretation, it is advised to state the nominal *voltage* of the electrodes and provide concise geometrical data on the electrode configuration, instead of stating an electric field strength.

On the other hand, in studies where the same electrode configuration is used with different voltage conditions "V/m" on the other hand can be very handy. [6]

5.2.2 Electric Field Parameters

The most important electric field parameters for electroporation are the pulse generator the electrode geometry and the tissue.

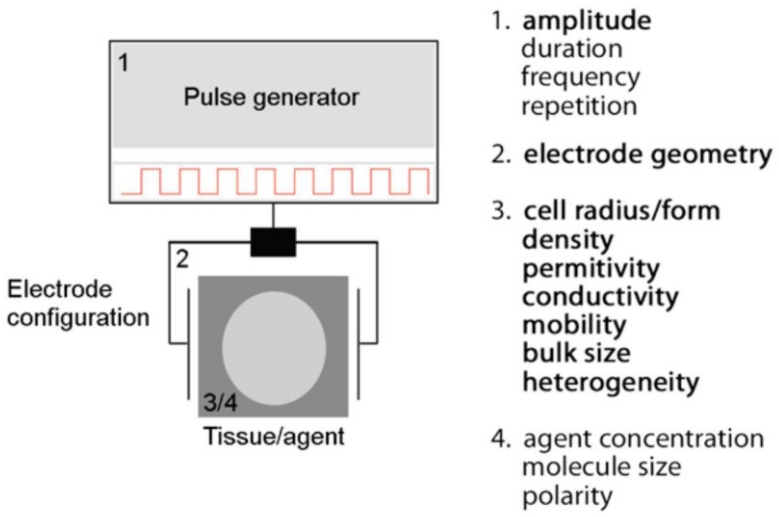

Figure 9: Electric Field Parameters in Electroporation [6]

Figure 9 gives a schematic overview of the most relevant parameters in electroporation, the ones in bold letters alter the electric field and are therefore directly affecting the level of induced permeability of the cell membrane.

The pulse generator is the most well-understood and easiest way to control the electric field strength by simply adjusting the applied voltage. The electric field strength is directly linked to the applied voltage. Changes in voltage will always result in a linear response of the electric field strength. E.g. adjusting the voltage from 100 V/m to 200 V/m will always double the field strength as a result. Therefore

$$E' = EU' / U,$$

where E' is the new electric field strength and U' is the new applied voltage, opposed to E being the old electric field strength and U being the old applied voltage.

Considering **electrode configuration,** the relations between size, shape and geometry of the electrodes are hard to determine. Regardless, the laws of electromagnetics facilitate us with the following truth; multiplying all dimensions of an electrode configuration, including the voltage by the same factor, the electric field is rescaled based on the used factor. By that means, that electrode configurations can be quite easily scaled.

$$E' = E\, d / d',$$

E is the old and E' is the new electric field strength and d is the old and d' is the new plate distance (in an example of infinite parallel plate electrodes). For pin electrodes, again, the principle of superposition has to be applied in the calculations. [6]

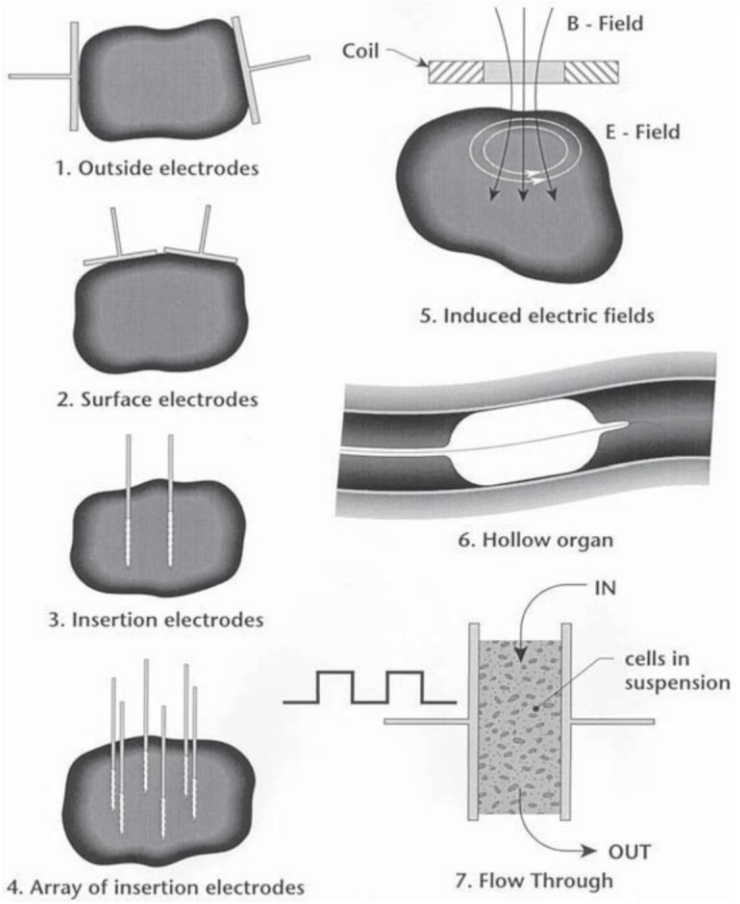

Figure 10: Basic electrode configurations. [53]

Figure 10 displays different electrode configurations, including hollow organ electrodes in the form of catheters (fig. 10.6). Flow through systems (fig. 10.7) could be used to treat body fluids. Other electrode configurations are more common. Figure 10.4 shows the most commonly used electrode configuration in electroporation; an electrode array with multiple electrodes that are usually placed surrounding the targeted tumor tissue. [53]

The number of electrodes used as well as their polarization plays a significant role in the outcome of any electroporation procedure.

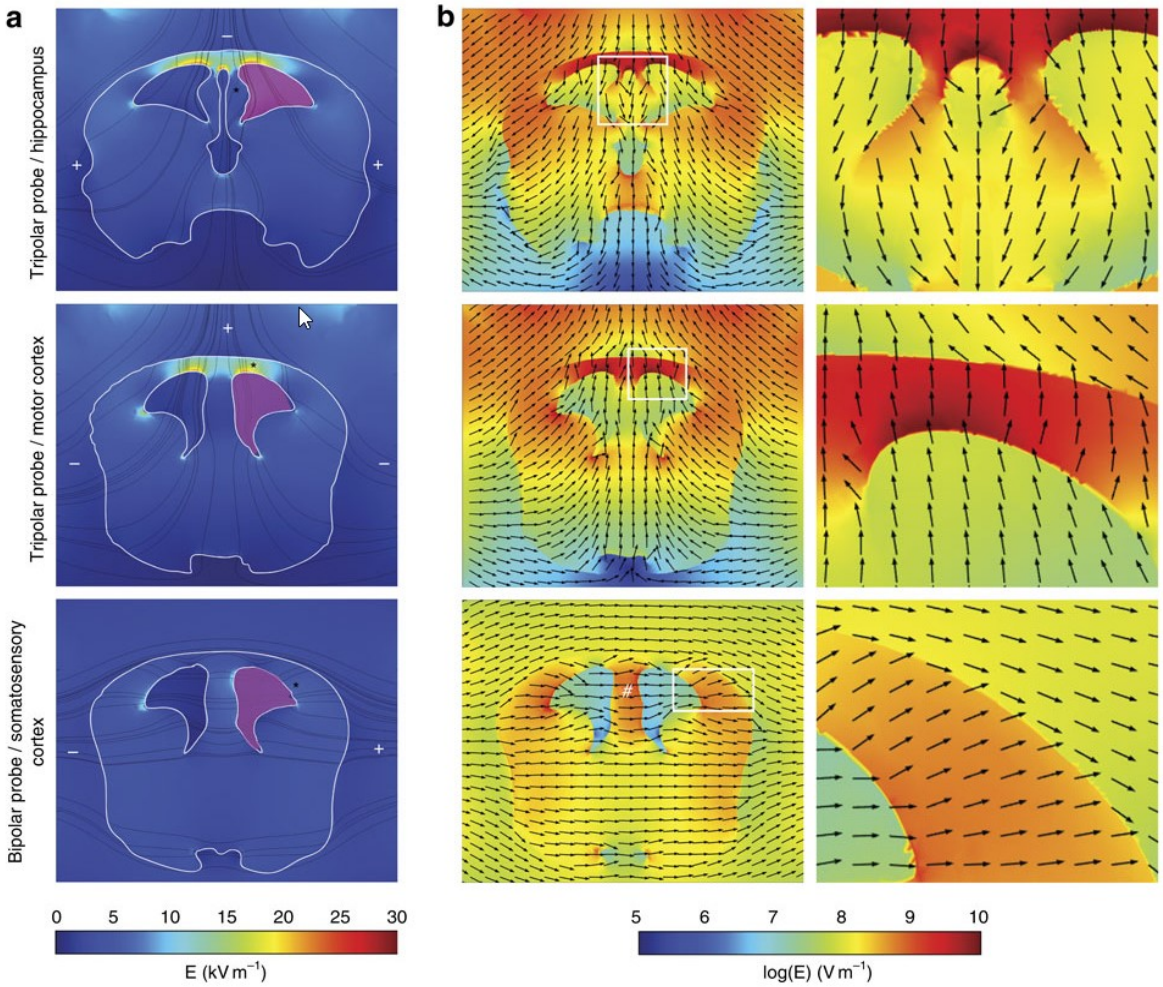

Figure 11: Effects of electrode configuration on electric field intensity in brain tissue; [54]

In figure 11.a stream lines for the electric field are indicated in black, minus and plus indicate electrode position and polarity, the electric field intensity is color coded. Figure 11.b shows the electric field intensity on a logarithmic colored scale along with the field direction (black arrows). [54]

The first two rows in figure 11 demonstrate the different outcome of electroporation with the same electrode placement, but different polarity. Switching the polarity between different pairs of needles notably enhances the coverage in the electroporated area. [53]

To calculate electrode configurations for real life applications, it is best practice to incorporate them into physical computer models using commercial packages like COMSOL Multiphysics. The mathematical approach of the finite element method (FEM) will be discussed in the next chapter (3.2.3). [6]

Probably the most demanding parameter in the calculation of the electric field distribution for electroporation is **the tissue** of the target region. The correct evaluation and calculation of biological tissues, be it in physiological conditions or in pathological conditions, is a very challenging task in the planning of the electric field.

The morphology and heterogeneity, as well as local changes in cellularity have tremendous impacts on the electric field, cause perturbation and are able to either reduce or increase local field strength. Varying cell shape, size, density and conductivity greatly affect the induced transmembrane electric field.

Due to the relatively short pulses used in electroporation the mobility of cells is of no significant relevance. The shifts in electric conductivity between different types of tissue and the size of the bulk target volume are also important parameters and affect the electric field to a great extent.

As with the configuration of electrodes, numerical computer calculations are needed to approximate the electric field distribution accordingly to the many tissue types that can be involved in treatment situations. Uncertainty about the conductivities and thicknesses of the targeted tissues remain a problem as to what values should be put into these calculations. There is very little data to work with on the electrical properties of biological tissues and its intrinsic characteristics to both be a conductor as well as an insulator in a given situation does complicate the process even further. [6]

A. Ivorra and B. Rubinsky have published an interesting paper addressing the problem of heterogeneity of tissues in 2007. The researchers investigated the use of electrolytic and non-electrolytic gels to generate the precise electrical fields, which are required for controlling electroporation in irregular tissues. They illustrated their applications using finite element computer simulations and demonstrated the feasibility of their concepts by irreversibly electroporating a rat liver.

The team came to the conclusion that matched conductivity gels could possibly solve a few problems regarding irregularly shaped tissues (e.g. superficial hard tumors). They also suggested a new type of electrode; an injectable electrode consisting of highly conductive gels that could be used for the electroporation of hollow structures. [55]

5.2.3 Calculations and Models

The ultimate goal of treatment planning in irreversible electroporation is to determine the optimal modeling for the electrode position and number, electric field amplitude, pulse duration, number and frequency to non-thermally ablate only the targeted tissue. [56]

While electropermeabilization, aiming for reversible electroporation, is a threshold phenomenon with a lower and an upper end well within close limits, irreversible electroporation has more leeway in terms of upper limits. Still thresholds for initial permeabilization need to be determined in experiments and vary greatly between different cells and tissues.

In vitro electroporation of cells in suspension is the easiest task of mathematical modeling. The cells are separate with comparably large distances between them. The electric field that is applied is homogenous and uniform in direction and magnitude. Therefore, the mathematical model that is used to calculate the electric field intensity for in vitro electroporation is the one of an ideal capacitor ($E = U / d$).

In *ex vivo* electroporation experiments usually one type of tissue is involved and pulses are delivered by plate or needle electrodes of various shapes (see fig.9). Additionally, the biological tissue consists of distinct cells of varying shapes and types of organization. Their plasma membrane conductivity is relatively low, compared to the surrounding extracellular matrix. The resulting electric fields can no longer be considered homogenous.

Adding up to complexity of the mathematical model needed to calculate the electric field intensity and distribution in this case is the circumstance, that during electroporation the conductivity of the plasma membrane is increased. This substantially changes the electric phenomena inside the tissue.

As mentioned at the end of the last chapter the *in vivo* electroporation of biological tissue is a delicate affair. Organs are usually composed of different tissues and cells with a great diversity of geometry, dimensions and structure. This adds an even higher degree of inhomogeneity which is further exceeded by the irregular structure of neoplastic tissue.

That being said, mathematical modeling and calculation of electric field distribution is a relatively simple tool for the analysis and explanation of experimental results. The influence of electric parameters and organ characteristics can be evaluated. For these models being adequate, however, they must be rigorously validated with exact measurements of electrical parameters in vivo.

Mathematical models can be used to simulate different electroporation configurations, but they cannot replace the experimental work that is needed to determine all of the influencing factors. Mathematical modeling can greatly help us to understand the processes of electroporation and our experimental results. It can accelerate the acquisition of knowledge in this field and lower the costs for new experiments. It cannot replace scientific experimentation. [57]

Finite Element Method – FEM

The finite element method is a numerical technique used to simulate processes in physics that would otherwise be near impossible to calculate. The idea is to break down complex structures with different physical properties to a level that is much less complex. In the case of IRE this could be an organ like the liver, which is broken down into lobes, then smaller lobes and even further into cells. An electric field distribution cannot be solved for the liver as a whole complex structure. However, it can be solved for a liver cell.

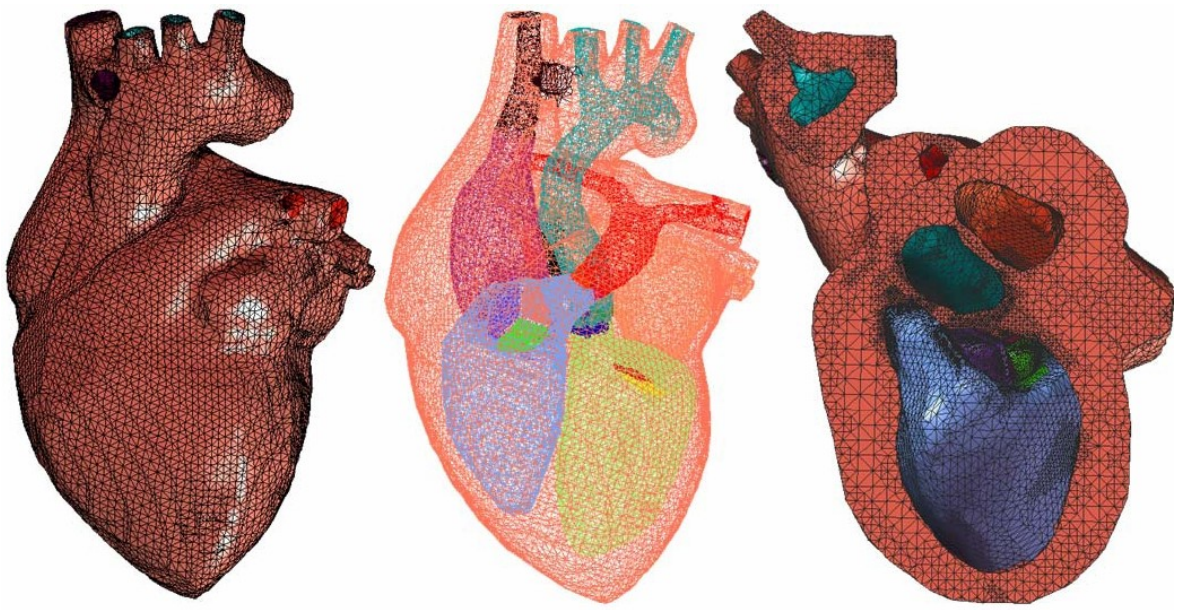

Figure 12: 3D finite element mesh of a human heart [58]

The overall pattern of the elements is referred to as "the finite element mesh". The accuracy of the calculation depends on the number of elements in the mesh. It should be an approximation that is fine enough to give adequate accuracy, but within a reasonable computing time. The mesh should resemble the elements of the real biological system that is simulated.

The elements are then assigned with their properties that should resemble their real world physical properties like electrical conductivity or resistance or their position in space or their behavioral changes during electropermeabilization in the case of electroporation. The properties are usually deducted from experimentation and their validity is essential for the calculation to produce accurate results.

When applying an electric field to the system the goal would be to get to a mathematical equation that describes the whole system and its reaction. To get to this equation the behavior of every unique element and changes in every defined property (nodes) of this element will be expressed in a function. The resulting functions for this element form a matrix, which is a starting point for a series of steps based on the fundamental laws of physics (mainly electromagnetism in this case; see chapter 3.2.1).

These laws form several system equations that will be applied to the element. The new matrix will then resemble e.g. a conductivity matrix for the element, which can then be worked with as a whole e.g. by applying an electric field vector upon it. The processes are carried out for every element in the mesh to get a conductivity matrix for each one of them.

The next step is to combine all the individual matrices into one large matrix representing the conductivity of the whole system. This is achieved by merging and reducing the matrices of the elements through solving the equations of one matrix and substituting the result into the matrix of the next element.

This process is repeated for as long as until all matrices are resolved and a single solution is left. This result can then be used like a key working backwards to solve all the equations of the system and obtain the properties (e.g. electric conductivity) of all elements. The corresponding electric field intensities can then be easily calculated for the whole system.

In relation to IRE it is worth mentioning, that the very first paper on NTIRE was a theoretical study that used FEM to predict nonthermal tissue ablation volumes by pulsed electric fields. [27,56]

It is very important for the mathematical model to be validated by comparison of the measured results and the calculated values. A good model is characterized by a high correlation between the results of the calculation and the measurement.

The objective of mathematical modeling regarding electroporation is to make in vivo electroporation more efficient in ablating targeted tissues, while minimizing it in the surrounding tissues. Thus, mathematical models can help to minimize side effects and improve the safety of electroporation. They can furthermore be very useful in the transfer of knowledge from experimental work into clinical practice. [57]

6 Fields of Application in Medicine

Many clinical applications of electroporation are well established today. Electroporation is used to enhance the delivery of drugs in chemotherapy. Furthermore, it enables molecules like oligonucleotides, ions, dyes and radioactive tracers to enter cells that would otherwise be inaccessible to them. There are applications for electroporation of the skin to enhance transdermal transport of large compounds, such as lidocaine, insulin, heparin and vaccines. Gene electrotransfer is heavily researched and a promising application for potential new treatments. [9]

Irreversible electroporation on the other hand has led a shadowy existence until only a few years ago. It was considered only to be an undesired adverse effect of electroporation. A lot of research on electroporation was done to minimize the effects of IRE and find the accurate thresholds to avoid this effect.

About a decade ago a few scientists began to realize the potential of IRE and to research on it as a possible standalone ablation technique (see chapter 2.5). The ability to induce nonthermal cell death and target cell membranes is the main motivation to use IRE in tumor ablation. Sparring of tissue scaffolds, avoiding the heat-sink effect and achieving sharp demarcation zones within the ablated tissue makes IRE unique among the available ablation modalities. [9]

This chapter will showcase IRE's current capabilities and provide an insight on the status quo of IRE in the context of clinical applications and studies.

6.1 Liver

Literature suggests that liver lesions may present the optimal target for IRE ablation. [9]

Hepatocellular carcinoma (HCC) is the third leading cause of cancer related mortality worldwide and primary and secondary (metastatic) liver tumors are an increasing global health problem. While systemic treatment options of HCC remain limited, surgical and locally ablative therapies offer improved survival outcomes for both, primary and metastatic tumors. The liver is a common site for metastases, especially from colorectal carcinoma. Minimally invasive therapies have over the past years matured to proper treatment options and are part of todays practice guidelines. [59]

The effective treatment of liver tumors without causing major trauma ensures better post-operative recovery and much shorter hospitalization times compared to classical tumor surgery. IRE represents the newest technology in this field and is currently proving its feasibility compared to the well-established thermal ablation methods. The suggested advantages are given by the absence of thermal effects and side effects due to thermal ablation.

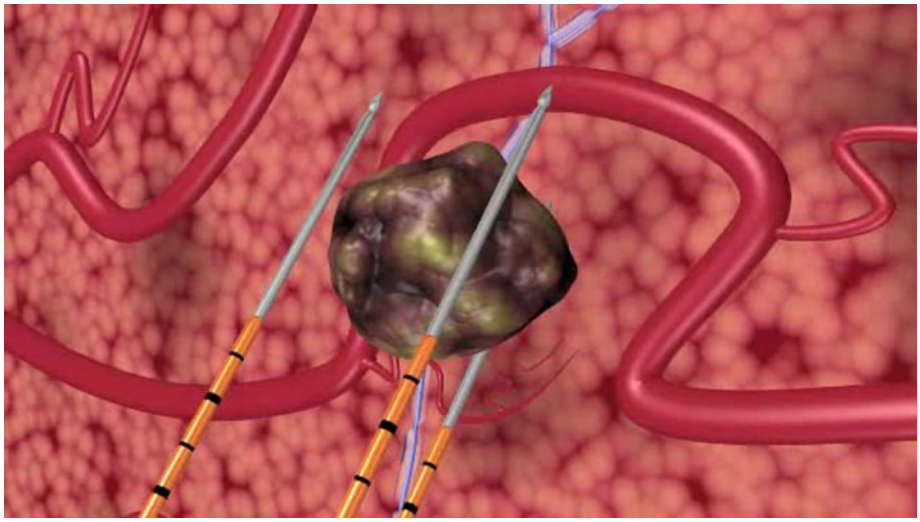

Figure 13: Schematic of IRE – vascular structures and nerves adjacent to tumor [60]

The ablation of liver tumors can be a very difficult task adjacent to vascular structures. These structures can be spared using carefully planned IRE treatment which can therefore be safely performed on liver lesions. [9]

Lee et al. reported the effectiveness of IRE in hepatic tissue ablation and evaluated the radiologic-pathologic correlation of the IRE-induced cell death in 2010. They performed 55 ultrasound (US) guided ablations on 16 Yorkshire pigs and imaged the results with US, magnetic resonance (MR) and computer tomography (CT). The mean diameter of the ablation zones was 33.5 mm ± 3 and the mean total procedure time per ablation was 6.9 minutes. They had no complications in any of the 16 animals. The ablation zones were well characterized in all of the imaging modalities and real-time monitoring was feasible with US. The immunohistochemical evaluation was performed with hematoxylin-eosin (H-E), Von Kossa and von Willebrand factor (vWF) staining and confirmed complete cell death within a sharply demarcated treatment area. Involvement of apoptotic cell death was shown by positive staining of apoptotic markers (TUNEL, BCL-2 oncoprotein). Peri-ablative structures like blood vessels and bile ducts as well as adjacent nonablated tissues were preserved. The team concluded that IRE is a *"fast, safe and potent ablative method, causing complete tissue death by means of apoptosis".* [8]

Another in vivo experiment on the porcine liver was performed by Ben-David et al. in 2012. The objective was to determine the best parameters for IRE to achieve the largest target zones of coagulation for two monopolar 18-gauge electrodes. The influencing variables that were specified were the number of electrical pulses (n = 20-90), the length of pulses (20-100 µs), generator voltage (2250-3000 V), inter-electrode spacing (1.5-2.5 cm), and a varying length of active electrode exposure (1.0-3.0 cm). Four sets of experiments have been performed in 25 in vivo pig livers with different settings for the five mentioned variables.

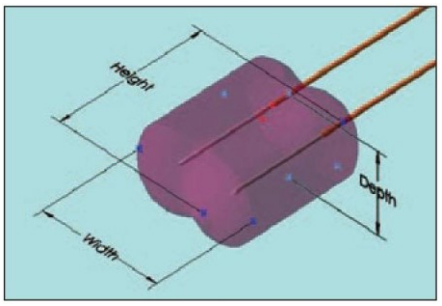

Figure 14: Measurements [32]

In the first experiment the effects of pulse width and number was examined simultaneously. Parameter combinations of at least 50 pulses of not less than 50 microseconds showed no statistical difference in the size of the resulting coagulation. The ablation zones were not influenced in their size or shape by the presence of blood vessels. Blood vessels and bile ducts remained intact while the ablated zones showed severe macroscopic and microscopic changes. Treatments of 20 pulses of 20 µs resulted in significantly smaller ablation zone width, while their depth (see fig. 14) did not alter. Increasing pulse width or number over 70 caused audible popping during the procedure and resulted in white ablative coagulation around the electrodes.

In the second experiment different generator voltages were tested (2250, 2650 and 3000 V, n=70 at 70 µs) and compared at a 1.5cm spacing between electrodes. Increased voltages yielded in significantly increased ablation width and depth (see fig. 14).

In the third experiment different inter-electrode spacings were tested (1.5, 2.0 and 2.5 cm, n=90 at 100 µs). By increasing the space between the electrodes from 1.5 to 2.0 cm the width of the ablation zone (see fig. 14) was increased. Further increasing to 2.5 cm resulted in either hourglass-shaped or even two noncontiguous ablation zones.

In the last experiment the effect of electrode exposure was evaluated for 1.0, 1.5, 2.0, 2.5 and 3.0 cm active electrode lengths (n=30 at 70 µs, 1.5 cm spacing). Increase in electrode exposure from 1-3 cm showed a linear correlation to an increased ablation zone height (see fig. 14).

The scientists concluded that optimal treatment of tumors with IRE is a consequence of careful planning of the tested parameters to achieve the optimal threshold to ablate the

tumor tissue effectively without harming adjacent vital structures. They suggest the use of closely spaced multiple electrodes and note that the multiple variable parameters in IRE require characterization of the role they play in defining the final area of ablation. [61]

It is important noting, that these experiments were performed on porcine livers under physiological conditions and that electric field distribution may differ in pathologically influenced tissues. Laufer et al. found in ex vivo experiments that the conductivity of the tumor tissue in hepatic tumors is *much higher* than that of normal liver tissue. [62]

To further investigate the effects of IRE in pathological tissues Guo et al. studied IRE in a rat model of HCC. 30 Sprague-Dawley rats were inflicted with HCC and divided into treatment and control groups. Treatment groups were treated with eight 100-µs 2,500-V pulses to ablate the targeted tumor tissue.

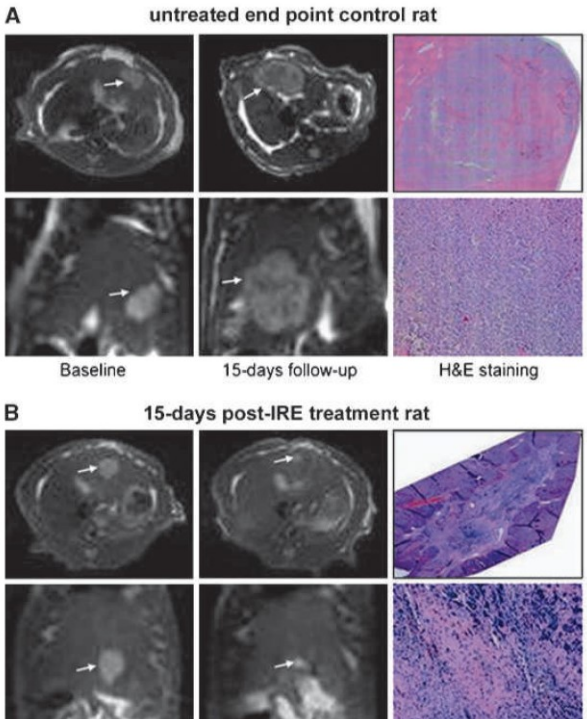

Figure 15: MRI and corresponding H&E images of control rats (A) and treated rats (B) [34]

MRT scans were performed 15 days after treatment and showed significant tumor size reductions (see fig. .15). Pathological assessment confirmed extensive tumor necrosis and full regression in 9 out of 10 treated rats 7 to 15 days after treatment. The researchers came to the conclusion that IRE *"can be an effective strategy for targeted ablation of liver tumors".* [63]

In 2012 Kingham et al. evaluated the safety and short-term outcomes of IRE to ablate perivascular malignant liver tumors. They reviewed the outcomes and complications of 28 patients with 65 tumors treated with IRE when resection or thermal ablation was not indicated due to tumor location. 22 Patients were treated via an open approach while 6 were treated percutaneously. The tumors were sized within a range of 0.5 to 5 cm with a median tumor size of 1 cm and 25 of them were located <1cm from a major hepatic vein and 16 were <1 cm from a major portal pedicle. There were no treatment-associated mortalities and the overall morbidity were 3%. After 6 months one patient showed persistent disease (1.9%) and three tumors recurred locally (5,7%).

Kingham et al. found IRE to be a safe treatment for perivascular malignant hepatic tumors and liver malignancies and suggested that longer follow-up would be necessary to determine long-term efficacy. They also noted, that indications for using IRE to treat liver tumors were not yet clearly defined, but due to the low short term failure rate in their study, studying the efficacy of IRE in a greater number of patients would be reasonable.[64]

Cannon et al. came to similar conclusions in 2013, when they studied the safety and efficacy of IRE for hepatic tumors in proximity to vital structures in a clinical setting. They observed treatment related complications and local recurrence free survival (LRFS) of their patients over a 2-year period. 44 patients were undergoing 48 IRE procedures, 20 colorectal, 14 hepatocellular, and 10 other metastases. 39 patients were treated percutaneously, 5 in an open approach.

The majority of the patients (72%) had received and failed at least one other therapeutic approach prior to being treated with IRE. All (100%) of the performed treatments showed initial technical success. Five patients faced complications, but all resolved within 30 days. LRFS was 97.4% at 3 months, 94.6% at 6 months and 59.5% at 12 months with a trend toward higher recurrence rates for tumors over 4 cm in diameter. The researchers judged IRE to be a safe treatment for hepatic tumors in proximity to vital structures and proposed further prospective evaluation to determine the optimal effectiveness of IRE in relation to size and technique for IRE of the liver. [65]

About 70% of liver metastases are surgically unresectable due to their anatomic location, limited functional liver reserve, or the presence of comorbidities. The 5-year survival rate for untreated metastatic liver disease is < 1%. Percutaneous thermal ablation techniques have raised the 5-year survival rate up to around 50% for HCC and to 25%-46% for colorectal metastases. [66]

Nonetheless, thermal treatment approaches have their limits with tumors in close proximity to vital structures like bile ducts or major vessels. IRE may present a significant advancement in treating previously untreatable patients or augment the outcome of patients previously treated with other techniques.

In 2014 Silk et al. assessed biliary complications after IRE of hepatic tumors that were located < 1 cm from major bile ducts. In contrast to the research done by Kingham or Cannon, the team, especially emphasized on the examination of the safety of IRE for larger, more centrally located liver tumors in very close proximity to major bile ducts; locations that are usually contraindicated for thermal ablation.

Within one and a half years 22 hepatic metastases in 11 patients were treated in 15 ablation sessions and reviewed with CT-scans before and after treatment. The median tumor size treated was 3.0 cm (as opposed to 1.0 cm in Kingham's paper) and tumors adjacent to the common, left or right hepatic duct.

A few patients presented with transient abnormal post-ablative laboratory values of bilirubin and alkaline phosphatase. In one case the abnormal values persisted due to a local tumor recurrence that was obstructing bile ducts. In a second patient the persisting values were caused by a local tumor progression at the porta hepatis. This patient required stent placement in the bile duct. Both of these patient's conditions were classified to be secondary to tumor progression, and not caused by bile duct injury.

Temporary increases of bilirubin and alkaline phosphatase post treatment were linked secondary to the damage of hepatocytes, leaky blood vessels secondary to endothelial damage resulting in edema, and to increased extracellular pressure that could cause temporary stasis in the bile ducts. Furthermore, electroporation in areas bordering the ablation zone, as well as irreversible electroporation cause hepatocytes to leak bilirubin and alkaline phosphatase, adding up to the other factors. However, the scientists mention that the abnormal laboratory values may have also been caused by factors related to the previous treatment history of the patients that was not covered in this work, due to the retrospective design of the study.

The overall results of this study agree with the previously published observations that IRE did not cause any major complications related to blood vessel or bile duct damage. It adds to the existing literature, that IRE may be a safe treatment option also for larger central tumors close to hepatic veins or the portal pedicle. The authors suggest that further studies with extended follow-up periods are necessary to establish the safety profile of IRE in this setting. [66]

A thorough assessment of IRE risk factors in the treatment of liver malignancies was conducted by Dollinger et al. in 2015. 85 IRE procedures on 114 malignant liver tumors (52 being primary, 62 secondary) not suitable for thermal ablation or resection were investigated. No death related to IRE occurred. The complications included 7.1% (6/85) major complications and 18.8% (16/85) minor complications, according to the standardized grading system of the Society of Interventional Radiology. Postablative abscess presented the most frequent major complication (4.7%, 4/85) and was seen mostly in patients with bilioenteric anastomosis, which was also identified as a general risk factor for major complications during IRE treatment of the liver. Minor complications mainly included hemorrhage, supposedly caused by the lack of cauterization of the needle tracts, and portal vein branch thrombosis, which is believed to be caused by the portal vein narrowing with reduced blood flow. The latter is a previously described complication of IRE ablation. [67,68]

The study rated IRE ablation of malignant liver tumors as a relatively low-risk procedure, but emphasized the increased risk of post-ablative abscess formation in patients with bilioenteric anastomosis. [68]

In a case study of a 68-year-old female with colon cancer and synchronous bilateral unresectable liver metastases, Schoellhammer et al. reported IRE to make the unresectable resectable. In an interdisciplinary treatment procedure IRE was used for margin ablation of the tumor, leaving the hepatic veins intact, while removing the microscopic tumorous margin around these vital structures. The patient was alive and without recurrent disease 30 months after resection. [69]

Sugimoto et al. evaluated the safety and short-term outcome of Japanese patients with HCC treated with IRE. Five out of six tumors (86%) were successfully treated, with no local recurrence after almost a year. They suggested that image guided percutaneous IRE is well tolerated by patients and can achieve satisfactory local disease control, especially for small HCCs. [70]

The current literature on IRE ablation of liver tumors suggests a promising outlook. IRE has certain advantages over thermal treatments, especially in the proximity of temperature-sensitive structures such as blood vessels and bile-ducts. Incomplete tissue ablation adjacent to major hepatic vessels due to the heat-sink effect is not an issue with IRE. It therefore may overcome most of the problems raised with thermal ablation. In conclusion IRE offers a treatment option for patients with focal liver tumors that are not eligible for surgical resection or thermal ablation. [68]

6.2 KIDNEY

Due to advances in medical imaging the detection rate of small renal masses up to 4 cm in diameter has shown a steady increase in the past two decades. While nephron sparing surgery is considered to be the gold standard and primary therapy for SRMs, focal therapies such as radiofrequency ablation (RFA) or cryoablation are available for patients with poor surgical outlook or multiple tumors.

Focal thermal therapies in kidneys face the same problems as in other organs. The vicinity of vital structures such as blood vessels, nerves, the renal collecting system and ureter as well as neighboring organs (such as the suprarenal glands or intestines) restricts the application of thermal ablation modalities. The heat-sink effect is especially important due to the high perfusion rate of this organ and can impair the thermal ablation outcome in the vicinity of large vessels and the renal collecting system. IRE therefore presents a highly desirable alternative for the treatment of tumors in a well perfused, vital organ like the kidney. [71]

While irreversible electroporation of tumors of the liver has already gained a respectable amount of clinical acceptance, IRE of the kidney until recently has mostly been studied in animal models and is still in an earlier stage of development. One early human trial of IRE was performed in advanced malignancies in kidneys (besides livers and lungs) but showed a considerable failure rate with kidney and lung tumors. [9]

Thomson et al. investigated the safety of IRE in humans for about one year from November 2008. The Australian scientists performed IRE on 69 tumors not responsive to alternative treatment in 38 volunteers. IRE of the kidney was performed ten times in seven patients.

One patient developed partial ureteric obstruction and increased creatinine levels following IRE of a renal lesion. The ureteric stricture had to be treated with a double-J ureteric stent. Two patients presented with transient hematuria caused by the punctuation of the renal pelvis with an 18-gauge electrode. One patient had an electrode tip inserted into the inferior portion of the left adrenal gland and consecutively suffered from hypertension. For two following months the patient described severe postural hypotension. Two of the seven patients required a second IRE procedure. CT-follow up after three months confirmed ablation in five of the seven patients.

Albeit facing several complications the researchers found the IRE safety profile to favorably compared with those of thermal ablation devices and prognosticated for IRE to have a significant impact on cancer management. [72]

Another early work on IRE of renal cell carcinoma (RCC) done by Pech et al. in 2011 focused mainly on cardiac arrhythmias and the safety of ablating RCC tissue using IRE. Treatment was performed in six patients undergoing general anesthesia and cardiac synchronization of the IRE pulses. Aside from one case of a supraventricular extrasystole no adverse effects were encountered.

The study suggested the potential advantages of IRE as opposed to thermal ablation methods, including the absence of the heat-sink-effect, possible conservation of important structures, avoidance of shrinkage of the renal pelvis or necrosis in this area. The renal tissue was immediately surgically removed after IRE was performed. The cells showed a mismatch between plasma and the nuclear volume, but no dead cells were found in the specimens. IRE of the kidney seemed to offer a feasible and safe technique to treat patients with kidney tumors. [73]

Since 2010 a mentionable amount of research has been performed in animal models.

Tracy et al. evaluated IRE on renal parenchyma and the renal collecting system in a porcine model in 2010. Eight female Yorkshire pigs were treated with laparoscopic ablations using either monopolar or bipolar IRE. The pig's kidneys were collected between 10 minutes and 14 days for macroscopic and histological analysis. 24 ablations in total have been performed and all pigs survived the procedure without complications.

The lesions were observed to be initially hemorrhagic, then decreased progressively in size to small white scars over the 14-day-period. The histologic analysis, which included NADH staining for cellular viability, showed diffuse tubular desquamation, eosinophilia and nuclear pyknosis, with absence of cellular viability in the samples taken immediately after IRE.

Specimens taken after 7 days presented with diffuse cellular necrosis with early peripheral granulation changes. 14 days after the treatment, marked tissue granulation, chronic inflammation and dystrophic calcification with early fibrosis and cellular contraction occurred. Urothelial injuries showed signs of repair 14 days after IRE treatment. [74]

To study IRE as a nephron-sparing therapy to reduce the risk of renal failure, Wendler et al. made the first radiological approach to examine the acute dynamic vascular perfusion monitored by digital subtraction angiography (DSA) during an NTIRE procedure performed on the porcine kidney in 2011. Additionally, high-resolution X-ray as used in mammography was performed before, during and after NTIRE of three isolated porcine ex vivo kidneys.

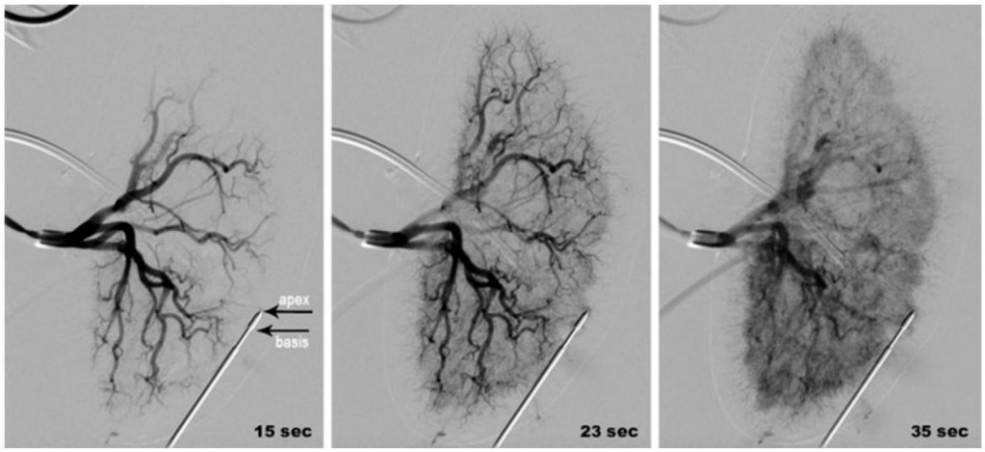

Figure 16: DSA while performing NTIRE [75]

Figure 16: The DSA showed no relevant changes, such as extravasations, perfusion gaps, open areas, accumulations, or stasis in the renal parenchyma during the NTIRE procedure.

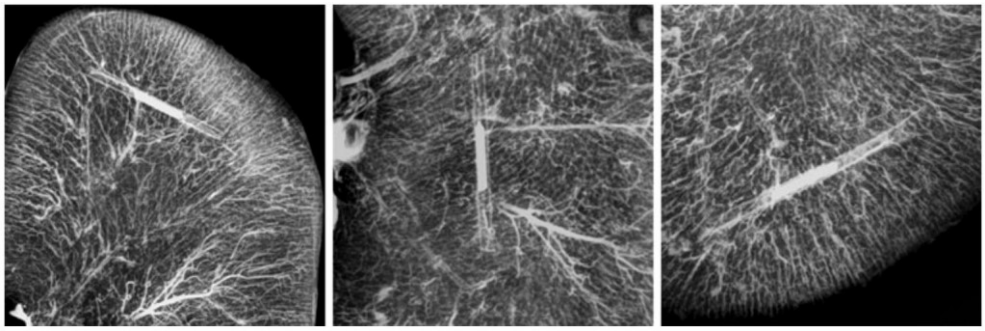

Figure 17: high-resolution X-ray imaging in low-kV exposure [75]

Figure 17: Angiography during NTIRE and high-res X-ray could easily be correlated, marking the NTIRE puncture channels per metal marker. In the marked ablation zones no extravasation and no disruption of the terminal vascular bed of renal cortical parenchyma was observed.

The results of both radiologic approaches showed no acute vascular destruction of the renal parenchyma and no dysfunction of the kidney perfusion. The researchers concluded that NTIRE offers an ablation method without collateral vascular damage, as opposed to thermal ablation methods. [75]

In the same year the team also studied NTIRE effects on the renal urine-collecting system using intravenous urography (IVU), urinary cytology as well as histology and MRI.

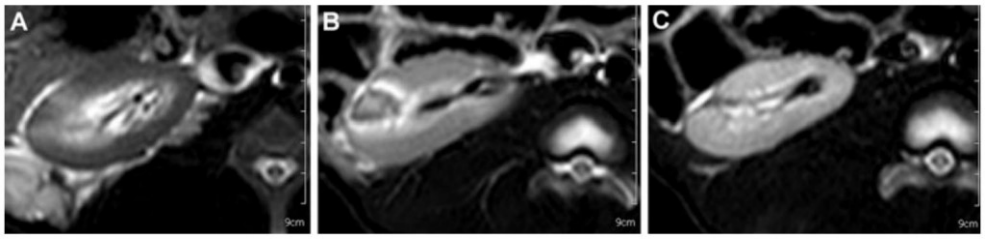

Figure 18: MRI of the center part of the right kidney at 30 min (a), 7 days (b), a) d 28 days after NTIRE (c)[76]

They performed 8 NTIRE ablations in three male swine. 7 days after the procedure MRI and histology demonstrated localized necrosis. 28 days after NTIRE the renal parenchyma presented with complete destruction and localized scarification, while the urine-collecting system was preserved and showed urothelial regeneration. IVU and MRI showed normal morphology of the renal calyxes, pelvis and ureter without any alterations. The team suggested that NTIRE could be used as a targeted ablation method of centrally located renal masses. [76]

In 2013 Olweny et al. investigated a novel generator capable of alternately delivering NTIRE and TIRE at about 90°C which was supposed to increase the versatility of the device. All 24 ablations were performed in 12 pigs and showed no adverse effects during or after the procedure.

The study compared the outcome of NTIRE vs TIRE ablation with this device and came to the conclusion, that both were safe, but, for obvious reasons, NTIRE's advantages over thermal ablation methods was lost when TIRE was applied. The NTIRE lesions were significantly smaller and had less inflammation than those caused by TIRE. TIRE also caused more significant and permanent damage to the collecting system and should thus be reserved for ablation of peripheral lesions. [77]

Thermal energy can also be generated by conventional IRE and even cause thermal injury, based on the tissue type, probe exposure lengths, pulse lengths and proximity to metal. Dunki-Jacobs et al. therefore suggest in their paper from 2014 to be especially aware of this when placing the probes in proximity to critical structures and to carefully choose appropriate settings for probe exposure length and pulse length to prevent thermal injury. [78]

A few other studies conducted in animal models all came to the same conclusion; that IRE of the kidneys and the renal system may present a safe and feasible new treatment option. They stated the known advantages of non-thermal ablation over thermal ablation and emphasized on IRE's ability to preserve vital structures in well perfused organs like the kidneys. [79–81]

Wagstaff et al. provided an interesting study protocol for IRE of the kidney in 2015. In the quest for further advancement of renal tumor treatment with IRE the researchers suggested a prospective, human, in-vivo study with two major objectives.

The primary objective was to study the safety and efficacy of IRE ablation for renal masses. The secondary objective was to find a validated imaging modality for follow-up.

Ten patients with solid enhancing masses, who were candidates for radical nephrectomy, underwent IRE treatment four weeks prior to their nephrectomy. MRI and CEUS (contrast-enhanced ultrasound) imaging were performed at baseline, one week and four weeks post IRE. Pathological examination was performed after radical nephrectomy to evaluate IRE ablation success.

The researchers aimed to test two-needle electrode configurations with constant device settings to produce comparable results. To limit radiation exposure for the patients the follow-up imaging modalities were chosen to be MRI and CEUS. The IRE procedure itself was CT guided and exposed the patients to an estimated 32 mSv of ionizing radiation. CT follow-up was found to add too much radiation exposure for the patients.

They also suggested the short follow-up period of only 4 weeks to be a limiting factor for the study, but considered further delay of the final treatment unethical.

This trial was considered to provide essential knowledge on IRE of renal masses, and may guide future research on this promising ablative technique. [71]

Considering that ablation therapy for small renal masses is gaining importance in clinical practice and even proved equivalent in outcome compared to surgical resection for thermal techniques such as RFA and microwave ablation, it will be fascinating to see where protocols like the one presented by Wagstaff et al. will take irreversible electroporation of kidney tumors in the course of the next few years.

6.3 PANCREAS

Beyond liver and kidney tumors, IRE has also been applied in the treatment of locally advanced unresectable pancreatic cancer. [9]

Pancreatic cancer is the fourth leading cause of cancer deaths in the US. It has the highest mortality rate of up to 90% upon all known cancer types. The statistics of pancreatic cancer in the US show that the number of incidences of new cases and the numbers of death is almost the same. [82]

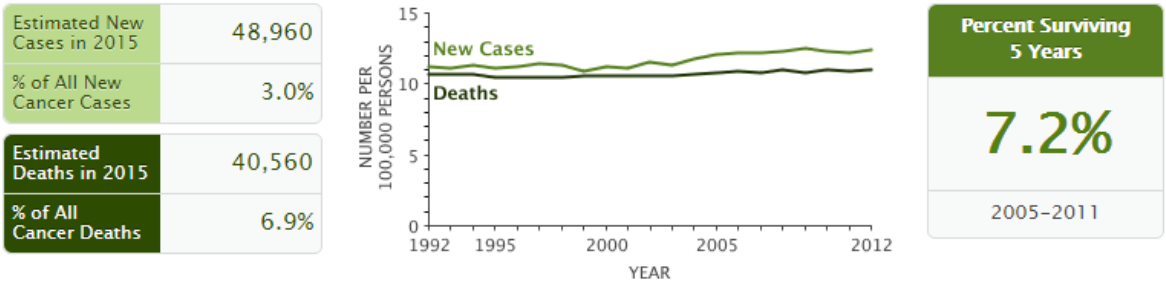

Figure 19: Cancer of the Pancreas - SEER Stat Fact Sheets [83]

The diagram in Figure 19 also shows that for the past 20 years a progression in the curative treatment of pancreatic cancer has been almost nonexistent. Despite a combination of surgical resection and various systemic chemotherapies the disease still has a very poor prognosis, as the 5-year survival rate of 7.2% clearly indicates. The prognosis also limits aggressive surgical resection. The median survival time after resection has been reported to be 10.6 months. [84]

Early research on the treatment of pancreatic cancer with IRE was done in animal models, mainly in swine. A pilot study done by Charpentier et al. in 2010 experimented with different parameters of IRE in the treatment of the pancreas of four domestic female swine, using two monopolar probes spaced 9-15 mm apart and the application of 90 1500 V/cm pulses to the tissue.

Ablation was successful in three out of four pigs. Probe spacing of 15mm in the fourth pig at the same pulse duration, amplitude and frequency showed no signs of ablation after two weeks. It was concluded that the wider probe spacing at this relatively low voltage did not suffice to produce irreversible electroporation, but resulted in reversible electroporation and therefore did not result in cell death. The researchers concluded that IRE appears to be a safe method for pancreatic tissue ablation. [85]

Another early work on IRE of the pancreas was done by a team of researchers in Louisville, Kentucky in 2011. In their report the scientists mention the limits of thermal tumor ablation techniques due to pancreatitis or damage to major vascular structures and suggest IRE to possibly solve these problems. The main objectives of their study were to investigate the optimal settings for IRE of pancreatic tissue and assess the safety of the procedure in the porcine model. Six female Yorkshire x Landrace swine were treated with IRE.

One treatment was unsuccessful due to inability to achieve a stable current using a 3-cm length, 2-cm spaced monopolar probe at 3000 V. Another ablation attempt failed in another swine with too closely spaced (1.5 cm) monopolar probes at 25 pulses of 2300 V. An additional pig's treatment initially failed at 18 pulses of 3000 V at a 2 cm spacing. It was then successfully ablated in a second attempt with 90 pulses at 2000 V.

No immediate complications or cardiac arrhythmias occurred during the experiments. All animals recovered with no pancreatic necrosis, no ascites and only mild adhesions. Transient increases in amylase and lipase was recognized until day three post-IRE. Pathologic analysis after 72 hours, 7 days, and 14 days post-IRE revealed ablation defects with significant destruction of the pancreatic tissue with preserved vascular structures.

The researchers could successfully demonstrate that IRE of the pancreas performed at an optimal voltage is well tolerated while preserving vascular structures and with a rapid resolution of pancreatic inflammation. [86]

Research done in mouse models in Barcelona, Spain in 2012 found that IRE increased the mouse survival rate, modulated the pathology and that the mice recovered quickly. [87]

Bagla and Papadouris reported one case of successful IRE treatment of an unresectable stage III (T4N0M0) pancreatic adenocarcinoma in a 78-year old male in 2012. Six months follow up was done with MRI and cancer antigen 19-9 serology and showed no signs of disease progression or recurrence. The researchers suggested that IRE may be a promising additive therapy to chemotherapy and radiation in patients with advanced pancreatic cancer. [88]

One of the researchers of the mentioned porcine study that was done in Louisville, Kentucky in 2011 is Robert C.G. Martin II. He also took part in the study of IRE's effects on the porcine liver, pancreas and kidney and retroperitoneal tissue that was mentioned chapter 4.2 of this work. [78]

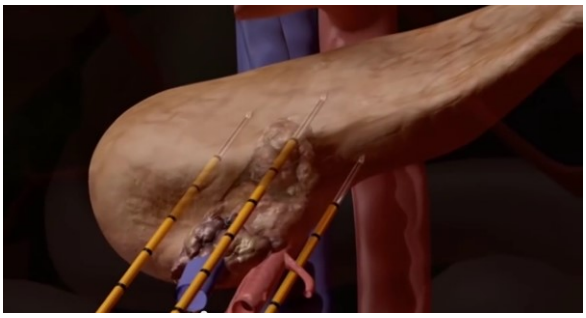

Figure 20: IRE treatment of pancreas [89]

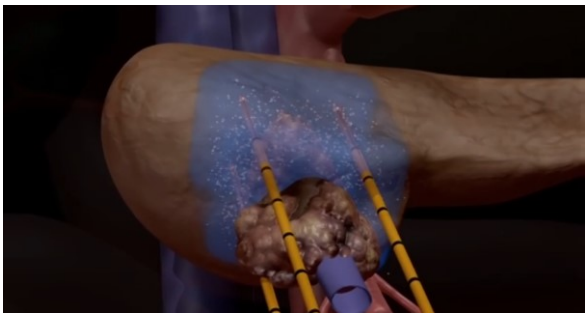

Figure 21: IRE treatment of Pancreas[89]

In 2012 Martin et al. published a prospective multi-institutional pilot study about patients that underwent IRE for locally advanced pancreatic cancer between December 2009 and March 2011 that evaluated the safety and efficacy of IRE in these patients. 27 patients were evaluated for 90-day morbidity, mortality and local disease control. 19 patients had in situ IRE performed, the other eight patients underwent margin accentuation with IRE in combination with surgical resection.

IRE was successfully performed in all of the patients (100%). All patients showed transient elevation of their amylase and lipase levels in the first three days post-IRE. In follow-up no pancreatitis was found. One mortality happened during the 90-day trial period.

The team evaluated IRE for the ablation of locally advanced adenocarcinoma to be a safe and feasible primary local treatment in otherwise unresectable pancreatic cancer, but stated that this therapy is still in the very early evaluation phase of its use and efficacy. [90]

The same team published a prospective study in 2013 with 54 patients with locally advanced pancreatic adenocarcinoma (LAC) to evaluate their overall survival after IRE treatment. In a comparison of IRE with standard therapy, there was improvement in the local progression-free survival of 14 vs. 6 months, $p = 0.01$, distant progression-free survival of 15 vs. 9 months, $p = 0.02$, and overall survival of 20 vs. 13 months, $p = 0.03$.

IRE ablation therefore was found to offer greater local palliation and potential improved overall survival compared with the standard radiation-chemotherapy treatments. [91]

D. Kwon joined R. Martins team for a prospective study from July 2010 to January 2013 that was published in 2014. The aim was to further investigate IRE's capabilities in the multimodal therapeutic context. A total of 48 patients with locally advanced pancreatic cancer (LAPC) underwent pancreatectomy with IRE margin accentuation of the superior mesenteric artery and/or the anterior margin of the aorta.

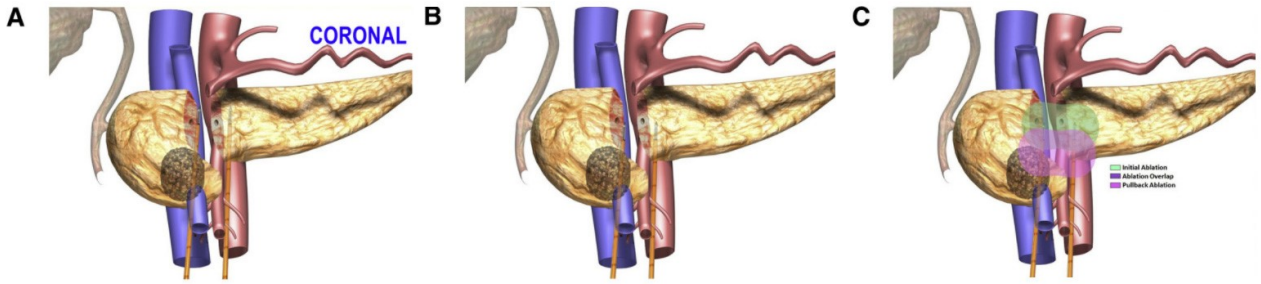

Figure 22: Pancreatectomy with IRE margin accentuation[92]

Figure 22 (A) displays coronal images of the technique for margin augmentation during pancreatectomy using IRE in an LAPC. Subsequent probe pullback is demonstrated in (B). Ablation areas that result from IRE under subsequent pullback is shown in (C).

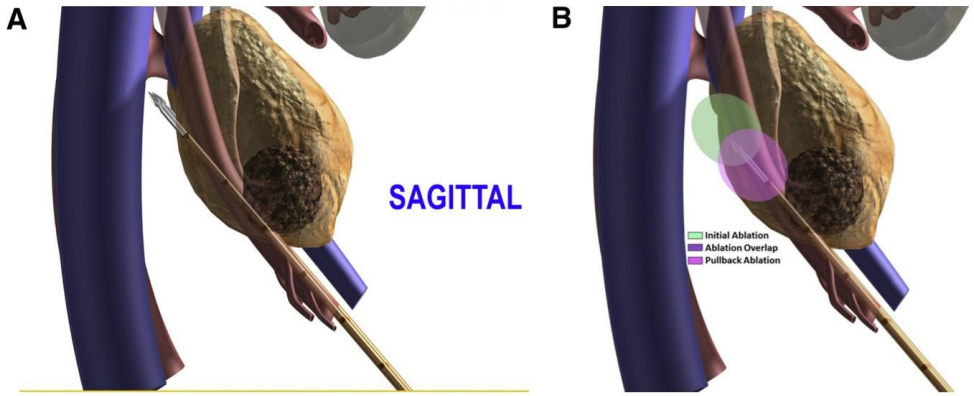

Figure 23: Sagittal images of IRE margin accentuation[92]

Intraoperative IRE was delivered successfully in all of the patients in an average of 12 minutes (see figures 22 & 23). 44 adverse events were detected in 18 patients during the 90-day follow-up, 5 of which were possibly linked to IRE. According to RECIST criteria no local recurrence was found at the 90-day follow-up.

More than half of the patients could proceed with their adjuvant radiation therapy and chemotherapy within a median time of 2.4 months. No deaths were reported during the 90-day span. 28 patients (58%) developed recurrence at 24 months, the median overall survival time was 22 months and progression-free survival was 11 months.

The study suggests that IRE for margin accentuation results in similar, if not better, outcomes in comparison with other treatment modalities. The low local recurrence rate exceeded expectations in the long-term follow-up and more effective treatment could be achieved through a continuous optimization in multimodal therapy concepts. [92]

In 2015 Martin et al. released a major paper on the treatment of 200 LAPC stage III patients with irreversible electroporation. It is aimed to demonstrate the efficacy of IRE treatment as a part of a multimodal treatment of LAPC.

The patients were treated and monitored in a multicenter, prospective institutional review board-approved registry from July 2010 to October 2014. Of the 200 patients with LAPC 150 underwent IRE alone, 50 had pancreatic resection and IRE margin enhancement performed. All patients were treated with induction chemotherapy and about half received chemo-radiation therapy for a median of 6 months before IRE.

In the follow-up with a median of 29 months 6 patients (3%) have experienced local recurrence. The median overall survival was 24.9 months within a range from 4.9 to 85 months.

The researchers therefore concluded, that the addition of IRE to conventional multimodal therapy results in a substantially prolonged survival and that ablative control of the primary tumor may prolong survival. [93]

Despite all the potential advantages brought to pancreatic cancer patients by IRE, Månsson et al. report about a major caveat.

In their case report they warn about severe complications with IRE performed in the presence of a metallic stent. They report that many pancreatic cancer patients have a metallic stent in the bile duct. According to the manufacturers of IRE equipment contraindications for IRE ablation include ablation in the vicinity of devices with metallic parts.

The doctors cite the case of a 72-year-old woman who had received a self-expanding metallic stent in the common bile duct due to a biliary obstruction. The patient was initially excluded from IRE treatment due to the presence of the stent, but 5 months later she was accepted for IRE treatment of her unresectable LAPC in a private clinic.

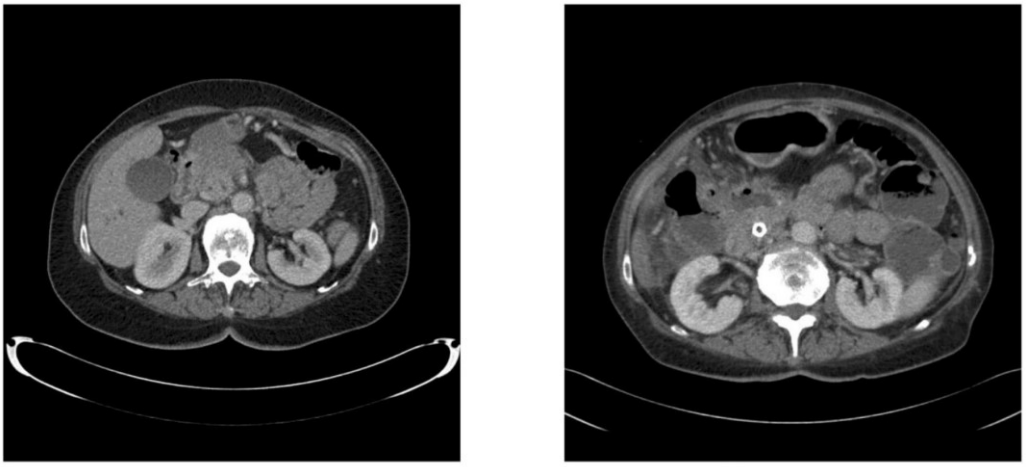

Figure 24: CT scan pre and one month post IRE ablation [94]

One month after treatment the patient reported continued diarrhea and increasing abdominal pain. CT revealed a small abscess (as seen in the right image of figure 24). Without any signs of peritonitis, she was treated with antibiotics and then sent home. Eight weeks post-IRE treatment she still had extensive diarrhea and showed both clinical and laboratory signs of infection. The diarrhea was treated with a laparoscopic loop sigmoidostomy. Two days later she went into hypovolemic shock, presented with a distended abdomen and underwent emergency laparotomy. An extensive, well organized abscess was found behind the transverse colon and both the duodenum as well as the transverse colon were found to be perforated in close proximity to the stent. Several attempts to repair the damages during the following 20 days failed and the patient eventually died.

The authors concluded that IRE treatment in the vicinity of the stent in the bile duct may have caused perforation of the duodenum and transverse colon, and bleeding from a branch of the superior mesenteric artery which in the end led to her death. [94] Since the perforation appeared delayed it remains unclear whether this complication is directly related to the IRE procedure or may be a result of prolonged inflammation, abscess or even tumor invasion.

In conclusion IRE may establish its role in improving the outcome in multimodal treatment of pancreatic cancer and offers a treatment option in palliative settings where surgical treatment is restricted due to local tumor invasion in blood vessels and nerves.

6.4 PROSTATE

Equally to many other fields in oncology, focal minimally invasive therapy is gaining interest in the treatment of prostate cancer. Cryoablation shows excellent cancer control rates and extremely low morbidity ever since Onik et al. introduced the concept of focal cryotherapy in 2002. [95]

However, cryoablation faces some of the typical problems linked to thermal ablation procedures. Areas bordering ablation zones show variable damage after cryoablation and injuries to adjacent structures such as the urethra, the rectum and the neurovascular bundle (NVB) are possible complications. Multiple freeze thaw cycles may be required which prolongs procedure times significantly. Furthermore, cryoablation catheters are quite complex and require liquid nitrogen or argon gases and is consequently rather expensive. Widespread acceptance of cryoablation in prostate cancer was therefore limited by these factors. [96]

These disadvantages of cryoablation may be overcome by theoretical strengths of irreversible electroporation and have consequently led to the first trials of IRE in prostates in animal models and to the first application of IRE ever performed in humans. [9]

Onik, Mikus and Rubinsky published a paper on IRE's implications for prostate ablation in 2007. They treated the prostates of six dogs with US-guided trans-rectally or percutaneously placed IRE probes.

The researchers found that the lesions in the prostate caused by IRE had unique characteristics compared to thermal lesions and found sharp margins in the transition zone between complete necrosis and normal surrounding tissue. Also they observed rapid resolution of the lesions and marked shrinkage within 2 weeks.

Surrounding structures such as vessels, nerves, the urethra and the rectum were unaffected by the IRE treatment. The concluding thoughts were, that the characteristics of IRE could be very advantageous in a clinical setting and could potentially benefit the outcomes of prostate ablation. [97]

The groups first human clinical trial was performed shortly later in 16 male patients with localized prostate cancer and a major concern in keeping continence and potency.

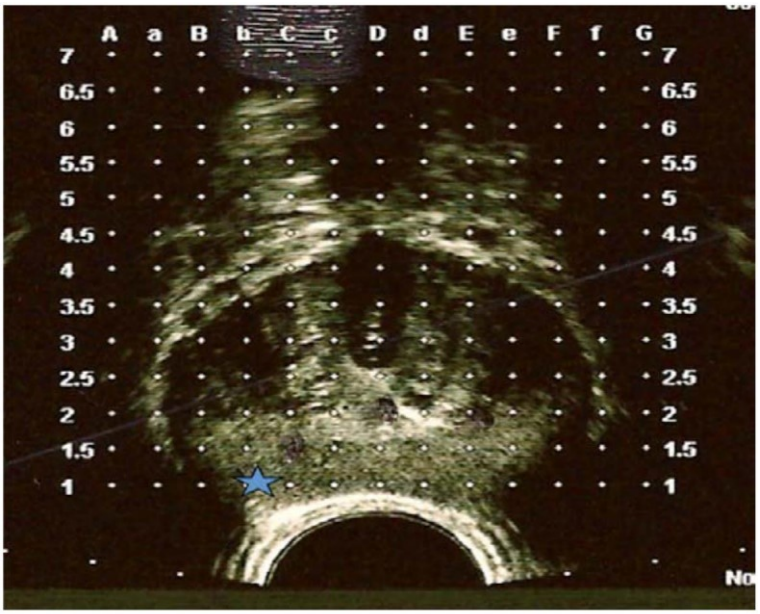

Figure 25: US of the brachytherapy grid overlay used during the 3D Prostate Mapping Biopsy [98]

After performing meticulous transperienal mapping biopsy (see fig. 25) patients were placed in the dorsal lithotomy position. The coordinates of the tumors that were defined by the mapping biopsy were then used to place four 18-gauge IRE electrodes percutaneously under TRUS guidance through the perineum to surround the tumor.

Then 90 pulses of 70-100 µs at 1500 volts in a 100 ms interval were delivered between node pairs in a bipolar manner until all the permutations for the 4-probe array were covered. In cases where cancer was adjacent to the NVB (see blue star in fig. 25) the NVB was included in the ablation area. Patient follow-up was performed three weeks post treatment via transperienal US guided biopsies.

All patients in the trial tolerated the procedure well. All patients were continent immediately after the procedure and potency was preserved in all cases where it was given before treatment. No complications occurred during the IRE ablations. Post-operative biopsies showed no evidence of cancer in 15 patients.

One patient was successfully treated with focal cryosurgery after Gleason 6 cancer was found outside the treated area. HE staining proved all epithelial elements to be gone. Vascular elements and nerve bundles were preserved.

The researchers found many of the advantages seen in the animal model confirmed in this first patient experience and were pleased with the results and the dosimetry of the IRE protocol. Furthermore, they were impressed with the speed of IRE compared with

thermal ablation. They suggested that despite all their patients were treated in a focal manner, IRE could as well be used to treat the whole prostate gland and, as it was the first patient experience of IRE altogether, they could well imagine to extend IRE treatment to other organs. They found IRE particularly suited to focal therapy of prostate cancer. [98]

In the context of these findings, it appears remarkable, that not much research on prostate ablation with IRE has been published following this initial success story.

One more multicenter prospective human in vivo pilot study considering the safety and efficacy of irreversible electroporation for the ablation of prostate cancer was published by Van den Bos et al. in 2014. [99]

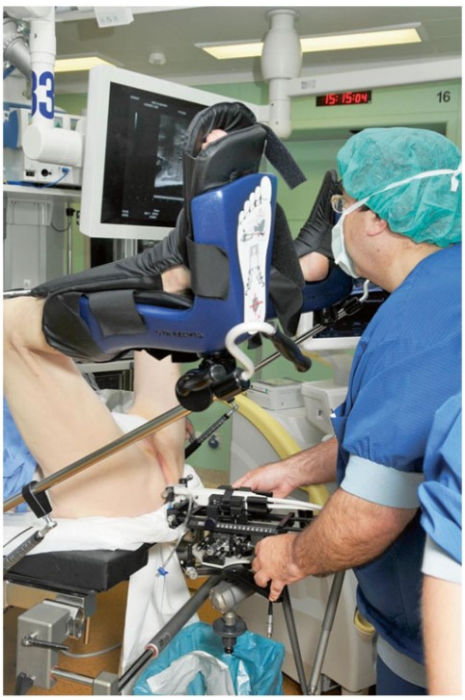

Figure 26: Extended lithotomy position with ultrasound inserted [70]

An overview article on the topic was released by Van den Bos in the magazine Interventional Urology in early 2016.

It stated IRE would be a promising focal therapy for the treatment of primary or recurrent prostate cancer and suggested that because of the appealing benefits such as the avoidance of long term urinary complaints and the preservation of sexual function, IRE would deserve further investigation in this context. [100]

6.5 LUNG

In May 2010 Dupuy et al. published their study of IRE in a swine lung model. Nine pigs underwent 15 percutaneous irreversible electroporation treatments under fluoroscopic guidance. The IRE electrodes were placed in the central and middle third of the right mid and lower lobes in all animals. Post-IRE X-rays were taken to evaluate eventual occurrences of pneumothorax. The pigs were sacrificed either 2 or 4 weeks after treatment and underwent high-resolution CT scanning and X-ray 1 hour before sacrifice. The lungs then were investigated in macro- and microscopic pathology.

There were no reports of significant adverse events. Pathological examination revealed focal areas of diffuse alveolar damage, fibrosis and inflammatory infiltration within the boundaries of the interlobular septae. The blood vessels and bronchioles within the ablation areas were completely spared.

IRE was concluded to be a safe and effective ablation method in the swine lung model. [101]

A study performed by Deodhar et al. that was also done in a porcine model came to similar conclusions and proposed IRE to be a "potential alternative to thermal ablative modalities". [101]

In 2011 Thomson et al. released a human trial with 38 volunteers with advanced malignancy of the liver, kidney or lung that were unresponsive to alternative treatment and signed in for IRE treatment. In total 69 separate tumors were treated with complete target tumor ablation achieved and verified by CT in 46 cases (66%).

Most treatment failures occurred in renal and lung tumors. Lung tumors were treated in three patients and none of them had a satisfactory tumor response. It turned out that it was not possible to completely treat the entire lesions with IRE. Two out of three lung ablations developed a pneumothorax but did not require specific treatment and resolved spontaneously. [72]

Overall human trials did not provide enough evidence, that IRE may be a suited ablation method for lung nodules in humans. The main technical issue appears to be the great conductivity difference between airspace and solid tissue and the necessity for exact probe placement. Treatment of perihilar or mediastinal tumors in close proximity to major vessels may still be an IRE indication in selected cases.

6.6 BRAIN

Although studies in animal models were published, IRE of the human brain has not yet been performed. Irreversible electroporation of the canine brain was done by Paulo A. Garcia who works on the topic since 2009 and has published several papers during the past years.

In a pilot study in 2009 he and his team conducted the first experiments on canine brains based on the findings of Davalos, Mir and Rubinsky. Numerical modeling was also used in the study to calculate the electric field distribution. As opposed to other organs increased brain pressure (BP) through IRE induced edema had to be treated with mannitol to lower BP to physiological levels. Neurosonography and MRI was performed 24 hours post-IRE and confirmed clearly demarcated ablation zones. Postoperative recovery was good with the canine subject not suffering from any neurologic disabilities and eating within 10 hours of the IRE procedure. IRE has been found to be safe for the treatment of canine brain tissue. [102]

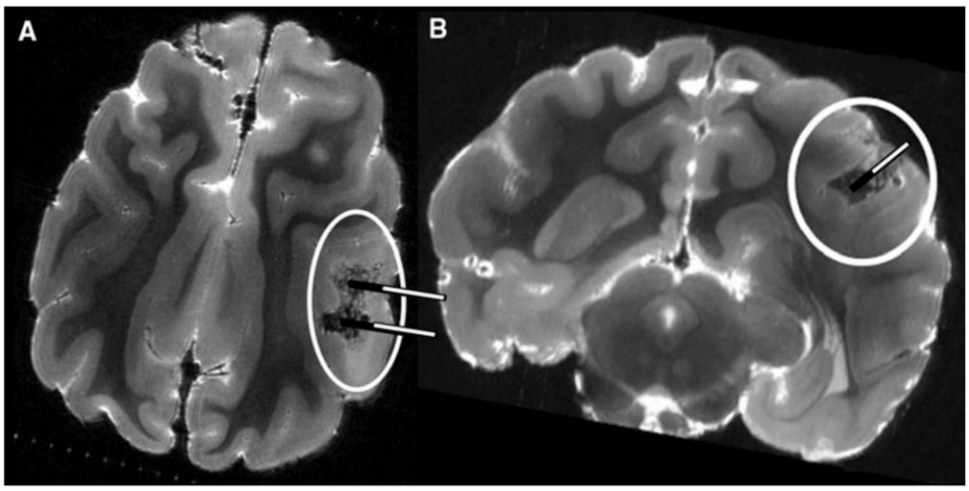

Figure 27: ex vivo high-resolution 7.0 T MRI of NTIRE treated brain [103]

Further studies were published by Garcia et al. in 2010, 2011 and 2014, the latest an in vitro study on human brain cells. [104]

The canine in vivo experiments were aimed at determining the suitable parameters of electroporation of brain tissue for effective treatment planning. Conductivities of brain tissue were deducted and used in numerical simulations that also tried to incorporate temperature related changes in conductivity. Correlation between models and experiments was a target to aid planning for the application of NTIRE therapies in the treatment of brain cancer. [103,105]

The effects of IRE on a malignant glioma was researched in a canine subject. [106]

6.7 BREAST

The amount of research that has been done on the effects of IRE on breast tissue is limited. Only two publications were found during the research phase of this thesis.

The first study was conducted in 2009 by Neal and Davalos and evaluated IRE as a possible alternative minimally invasive treatment for breast cancer. The study was set up in two steps. In the first step in vitro experiments were performed on human mammary carcinoma cells to determine a baseline electric field threshold to induce IRE.

A cell shape factor of $\lambda = 1.5$ (as used for spherical cells – see chapter 3.2) at a 12 µm cell diameter corresponded to 1.8 V transmembrane voltage in a 1000 V/cm electric field. This potential is well above the 1 V found to typically induce IRE. The parameters of 80 square wave 1000 V/cm 100 µs pulses at a rate of 1 pulse per second were found to be sufficient to ablate the 95% of the cells in suspension. The chosen threshold was found to be sufficient for the design of the in vivo treatment protocols.

In the second step these findings and numbers from in vitro experimentation were incorporated into a three dimensional numerical model based on FEM (see chapter 3.2.1). The shape and size of the simulated tumor mass were chosen to be a sphere of 1 cm in diameter based on what was found to be the most common phenotype in newly diagnosed breast cancers (50% of the newly diagnosed breast cancers according to the used literature).

The model simulated the use of two monopolar electrodes showing many unique treatment characteristics of the heterogeneous tissue volumes. IRE was found to be capable of fully treating the targeted heterogeneous tissues without inducing any thermal effects significant enough to cause thermal damage, which was considered to decrease the likelihood of significant scarring.

These facts were found to prove that IRE could provide improved treatment outcome while minimizing aesthetic effects. It was also supposed that rapid lesion resolution would enable healthy cells to repopulate the ablated areas, making IRE an advantageous treatment modality for breast cancer and other localized pathologies. [107]

A second study that was released by Neal et al. in 2010 was built upon the insights gained in the first study and was the first in vivo study on the topic. Human mammary tumors were orthotopically implanted in female Nu/Nu mice and then ablated with a 1000 V/cm electric field applied via a single needle electrode. All mice tolerated the treatment well and no signs of pain were noted during the study.

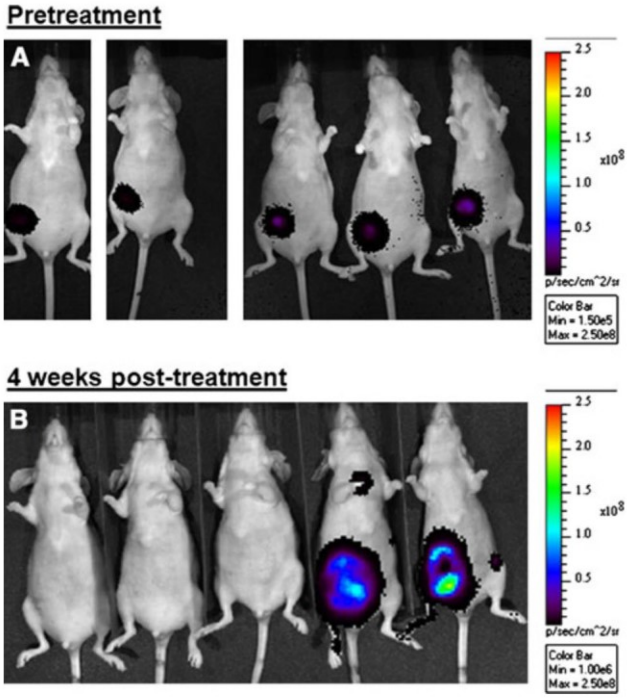

Figure 28: Bioluminescence of IRE-treated tumors [78]

Tumor regression was observed in 5 out of 7 MDA-MB231 human mammary tumors.

Bioluminescence imaging was used to tag and visualize the cancerous cells from the tumors. The image in Fig. 28a was taken 1 h prior to treatment and shows that all tumors had approximately the same size. Fig. 28b was taken 4 weeks post-IRE and shows complete ablation without regression in the first three mice, while the two that did not regress continued to grow and spread.

Histological examination was used to confirm the findings from bioluminescence imaging and provide further information on why two of the tumors continued to grow. The tumors that did not regress were found to be the largest tumors in the range and that they were not sufficiently covered by the electric field and therefore exceeded the treatment region.

The used 1000 V/cm therefore have been found to present the minimum threshold for in vivo ablation of breast cancer. It was also suggested that an additional resection margin typically used in surgical resection of at least 0.5 cm should also be incorporated into the modelling of the ablation zones for IRE.

Based on these results the researchers concluded IRE may be able to successfully treat breast cancer tissue in the rodent model and could present an advantageous alternative to surgical resection for breast conserving therapy. [108]

6.8 Further Applications

The applications described above present the major areas in which IRE is used as a treatment method today, but IRE and the research surrounding this technique is not limited to these fields.

Besides the medical uses IRE is heavily used and researched in the context of food preservation. As already mentioned in the history chapter of this work (see chapter 2) IRE is used in this field for almost fifty years to deactivate microorganisms with only minimal product damage. Nonetheless this technology is still under development for possible new fields of application within this domain. [109]

In medicine IRE is being researched for several other treatment options including the ablation of vascular smooth muscle cells [110] (blood vessels), nerves [111,112], uveal melanoma [113] (eyes), soft tissue tumors [114] and even for myocardial ablation [115].

The field is continually progressing and reinventing itself over time with new applications discovered on a regular basis.

7 COMPARISON OF ABLATION TECHNIQUES

This chapter provides a brief overview of the most important minimally invasive ablation methods that are currently in clinical use and what sets IRE apart.

Chemical, thermal and non-thermal ablation modalities will be discussed.

Chemical ablation today is still used in developing regions of the world as it is cheap and can be effective. Instillation of ethanol or acetic acid has been studied for the longest clinical follow-up periods among all percutaneous ablation techniques, especially as a treatment modality for HCC in patients with cirrhosis.

Ethanol instillation works through two mechanisms. The first is immediate dehydration of the cytoplasm, protein denaturation and subsequent coagulation necrosis. The second mechanism is necrosis of the vascular endothelium, consequent vascular thrombosis and ultimately ischemic tissue necrosis.

However, chemical ablation largely depends on the tissue composition of a tumor and uniform diffusion of the applied agent is hard to achieve. Thus, in developed countries, chemical ablation has been largely replaced with focal thermal ablation and lately IRE. [5]

Thermal ablation therapies focus on destroying tumor tissue by applying thermal energy, either heating (hyperthermic ablation) or cooling (cryoablation), to a tissue to induce irreversible cellular damage. Cytotoxic temperatures are needed for an adequate destruction of the entire tumor including a certain ablative margin.

Cryoablation is in widespread clinical use today as a therapy for focal primary renal tumors and treatment of bone tumors and metastases. Current cryoablation devices use the Joule-Thomson effect to create freeze and thaw cycles in the ablation zone.

The Joule-Thomson effect describes the change in temperature under the influence of changing compression of a gas. Argon is one example of a gas that cools down to temperatures as low as -140°C during expansion. Systems, working with nitrogen at its critical point are an alternative currently under development.

Hyperthermic ablation produces irreversible cellular damage at temperatures above 50°C through protein coagulation. Hyperthermic ablation methods currently in use include RF ablation, microwave ablation, laser ablation and ultrasonic ablation.

Radiofrequency ablation (RFA) presents the best studied, clinically relevant percutaneous ablation modality today. RFA uses the Joule effect to rapidly heat a targeted tissue around an electrode. Tissues near to the electrode are heated most effectively due to the high current density in this area, while peripheral areas receive heat mainly by thermal conduction.

Tissue dehydration and water vaporization in the targeted tissue dramatically increase impedance in the circuit and is thus used as an indicator for effective treatment during the procedure.

Most RF ablation systems operate in a monopolar mode while using different types of electrodes that are chosen depending on the target tissue. Ground pads are attached to a large surface of the skin to provide for electric current flow without skin burns.

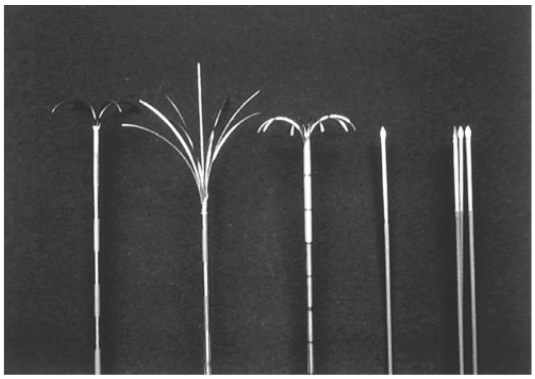

Figure 29: RF electrode designs [86]

The electrode designs reach from single internally cooled electrodes to whole clusters of electrodes as well as expandable electrode systems that deploy several smaller electrodes from a single needle shaft and create either umbrella- or star-shaped arrays and thereby distribute heat more effectively in the surrounding tissues than single tip electrodes.

RF ablation is widely in use in the ablation of metastases (especially in the liver) or in the treatment of cardiac arrhythmias caused by abnormal electrical pathways.

Microwave Ablation uses rotating dipoles to apply electromagnetic energy in the range from 300 MHz to 300 GHz to tissues and thereby heat them. The applied electromagnetic energy (typically operated at either 915 MHz or 2.45 GHz) forces molecules with an intrinsic dipole moment (e.g. water) to continuously realign with the applied field. The increasing kinetic energy then elevates the local tissue temperature.

A potential advantage of microwaves over other thermal ablation methods are their ability to readily penetrate through biologic materials irrespective of their electrical conductivity.

Microwave power can produce extremely high temperatures (>150°C), can be operated through multiple antennas simultaneously without the need for ground pads and heats tissue more efficiently than RF energy.

On the downside microwave energy is inherently more difficult to distribute than RF energy and the devices tend to be a lot bulkier than the ones used in RF. Cable heating can also present a mentionable drawback.

Microwave ablation found wide clinical acceptance and is mostly used for the ablation of liver tumors.

Laser ablation is rarely used for thermal tumor ablation and is only rarely adopted across Europe. Lasers are an efficient and precise energy source for tissue heating and are widely accepted in medicine e.g. for treatment of the skin or corrective procedures in the eye. Advantages of lasers in interstitial tumor ablation include MRI suitable optical applicators that are small in diameter and thus eliminate artifacts on CT or MRI images.

The problem with using lasers for interstitial ablation is that light is scattered and absorbed rapidly by most body tissues and has therefore limited penetration capabilities. Lasers create only small ablation zones of 1-2 cm in diameter. Furthermore, charred and desiccated tissues cannot be overcome or penetrated by laser light. High-power lasers also require cooling of the used fibers to avoid skin burns, increasing the optical applicators diameter and thereby squanders the benefit of a small applicator size.

HIFU – Clinically used for uterus myoma and liver ablation (especially in Asia). Advantages: Non-invasive & transdermal (like diagnostic ultrasound). Ablation zone can be customized (from small voxels). Disadvantages: MRI necessary (MRI thermal measurement and planning). Acoustic window and coupling (skin) necessary. Slow.

Thermal ablation in general has its limits considering that heterogeneity of heating throughout a targeted volume is more often the rule than an exception.

Apart from the heterogenic distribution of temperature in heterogenic tissues no sharp demarcation can be achieved in the transitional zones. Temperatures in transitional areas of ablation show gradual changes, which leads to a margin in the transition zone for cells to be either ablated or survive the process.

Thermal damage is mostly necrotic damage and therefore results in local and systemic inflammatory reactions in order to dismantle all the necrotic debris. [116]

The described heat-sink effect (see chapter 1.) is a potential disadvantage of thermal ablation techniques. Thermal ablation in the proximity of large vessels is impaired by the dissipation of thermal energy thus resulting in incomplete ablation in perivascular tumor tissue. This can result in incomplete ablation and in tumor recurrence. [5]

Non-thermal ablation – IRE

Irreversible electroporation has been thoroughly discussed in this diploma thesis. To summarize the most striking benefits:

- Targeting the cell membrane and preserving extracellular structures
- Thus preservation of vital structures within IRE-ablated zone
- No heat/cold-sink effect
- Complete ablation with well-demarcated margins
- Apoptotic cell death
- Real time monitoring
- Short ablation times
- Shorter recovery periods [4]

IRE is the youngest technology in the field of minimally invasive ablation therapies and has certain advantages over well-established ablation methods. The issues that arise with the use of this fairly new technology are subject of the next chapter.

8 ISSUES AND PROBLEMS OF IRE

Several drawbacks of irreversible electroporation were already discussed throughout this work.

IRE has proven that it can be a potent ablation technique, but it is also a challenging piece of technology that requires thorough understanding of its principles, its indications and its contraindications (see the case report at the end of chapter 4.3).

It is also a very young technology that still needs extensive research and clinical testing in all of its fields of application. Many of these fields still lack data from human trials and many of the explored ablation settings and thresholds stem from animal models or even from in vivo experiments.

To differentiate the known drawbacks a little further it is helpful to subdivide the occurrences on one side into general problems that are owed to the nature of the ablation method itself and on the other side into possible complications that may occur in certain clinical contexts.

General problems that lie within the technology of IRE include the following topics:

- General anesthesia and presence of an anesthesiologist always required
- Triggering of muscle contractions (can be reduced by muscle relaxation)
- Triggering of cardiac arrhythmia (can be avoided by cardiac gating)
- Experienced operator is required
- Requirement of careful treatment planning and modeling
- Exact placement of multiple parallel electrodes is required (caging of the tumor)
- Ablation may be incomplete due to electric field inconsistencies
- Lack of data supporting preference over other techniques
- Lack of data for long term outcomes
- Thus IRE is still a salvage and experimental niche application and established techniques should be preferred if available and possible
- Thermal damage possible when used at intensities too high for the targeted tissue

Problems that may occur in several clinical contexts:

- Transient damage to nerves
- Possible cell and vessel leakage (mostly transient) leading to edema and enzyme leakage (liver and pancreas)
- Thrombosis and vascular occlusion was reported in some cases
- Abscesses (reported in patients with bilioenteric anastomosis)
- Complications in the presence of metallic devices / stents
- No coagulation at insertion sites may result in bleeding (although needles have higher gauge than thermal devices=thinner)

These were the main issues that could be identified during the extensive literature study that has been performed for this thesis. The following compilation was performed to organize these issues in a comprehensive way.

General problems that lie within the technology of IRE

Muscle contractions. Excitable tissues like muscles and nerves respond to electric stimulations with contractions inflicting pain. Electric field thresholds for muscle contraction is two orders of magnitude lower than that for electroporation. Muscle contractions during electroporation can result in the movement of the electrodes and therefore in inducing electric fields in surprisingly large volumes of non-targeted tissue. Thus, the ablation outcome can be influenced greatly by the occurrence of muscle contractions. Three mechanisms can provoke a muscle twitch during electroporation; first, a multiple involuntary spinal reflex response, second, direct motor-neuron electrical stimulation in the region of electrode contact and third, from direct electrical stimulation of denerved muscles. The muscle contractions can be dealt with in several ways. Careful treatment planning and electrode design and arrangement can help to prevent muscle contraction. [117]

Further, the electric field parameters like wave forms, pulse length and/or frequency can be modified in a way to inhibit muscle contraction. High-frequency irreversible electroporation (H-FIRE) has been shown to produce ablative lesions at 250 kHz or 500 kHz similar to the ones produced with IRE, but without the need for paralytic agents. [118]

Cardiac arrhythmia during IRE was first seen in a human trial conducted in 2009 when 38 volunteers with different tumor types were subjected to IRE under general anesthesia. It was one of the first human trials of IRE ever conducted and thus faced many problems during the procedure. The cardiac arrhythmias occurred in four of the first patients

treated with IRE. The procedure needed to be aborted in two patients and the arrhythmia was resolving immediately after ablation abortion. One patient suffered from transient supraventricular tachycardia after undergoing IRE treatment, but it resolved without treatment. One patient developed atrial fibrillation that required cardioversion after IRE treatment. Subsequent IRE treatments were performed under cardiac synchronization. [72]

In 2011 ECG synchronization was further investigated by Deodhar et al. in an animal model. Swine lung and myocardium was irreversibly electroporated in 11 pigs. Fatal events occurred with only unsynchronized IRE and at less than 1.7cm from the heart, while no major events were seen with ECG synchronized IRE. [119]

Cardiac synchronization may prevent IRE induced arrhythmia, but while Deodhar's study showed no adverse effects under synchronization, Sugimoto et al. warned about possible synchronization failures during IRE and advised to perform IRE near the heart only under extraordinary caution. [120]

No adjustability during a pulse train is owed to the short application time of IRE. 90 pulses are often applied within less than two minutes. This leaves a very small timeframe to let observations during the procedure influence parameters of the complex electric field distribution. It is simply not feasible to adjust any of the parameters during an IRE session. For the same reasons monitoring during electroporation is an issue. Therefore, extra careful treatment planning and modeling is required prior to performing IRE. [5]

Thermal damage in IRE was reported in experiments on the porcine liver and pancreas by Dunki et al. but was largely attributed to inappropriate electrode arrangements and unsuitable IRE parameters in use. [78]

Faroja et al. conducted a study entirely devoted to the topic. In *"Irreversible Electroporation Ablation: Is all the Damage Nonthermal?"* the team tried to determine whether high-dose IRE induces thermal damage in physiological liver tissue.

They found that temperature rose in all of their experiments above a 34°C baseline and correlated linearly with IRE's energy dose which they defined as the product of voltage and the number of pulses (oddly they didn't include pulse time in this definition).

Mean temperatures as high as 86°C ± 3 have been observed for 270 pulses at 2500 V. For 90 or more pulses they recorded 50°C or more at the same applied voltage. The team concluded that IRE can in some conditions induce thermal "white zone" coagulation and therefore induce thermal necrosis in ablated tissues. [121]

These findings were reviewed by Silk et al. in 2014 and found not to be applicable to clinically accepted IRE treatment parameters due to the protocols used in the study. They considered the used device settings as not suitable for clinical practice (e.g. unusual high pulse repetitions and voltage settings). [66]

Problems in their clinical context

Transient damage to nerves was investigated by two different teams in 2011. One study was conducted in Xi'an, China by Li et al. in a rodent model. A sequence of 10 100 µs square pulses of 3800 V/cm was applied to rat sciatic nerves at a treatment length of 10 mm. Follow-up observed the rats for 10 weeks. Findings showed that the nerves treated with IRE could fully recover after 7 weeks. The scientists concluded that nerve preservation during IRE treatment is possible for nerves involved in malignant tumors while tumor tissue could be completely ablated. [112]

Another study that was conducted almost in parallel was performed by Schoellnast et al. to investigate the effects of IRE on nerves in a pig model. The setup resembled the ablation protocol used in the prostate in which an NVB may be ablated twice (worst case scenario). Ablation therefore was done with ninety 70 µs pulses at 1500 V/cm. Histology revealed all nerves exhibiting an axon injury with intact endoneurium and perineurium architecture. A comparison group treated with RFA revealed acute coagulative necrosis including irreversibly damaged perineurium and endoneurium. IRE was found to damage nerves, but preserved the endoneurium architecture and therefore has a potential for axonal regeneration. [122]

In a second study two years later the same team published the delayed effects on nerve regeneration. It could be demonstrated that morphological regeneration took place within a two months' time period. Functional recovery, however, was variable and it was stated that in reference to the Chinese study in rodents, because of possible differences in recovery times between small non-myelinated, non-sheath nerves compared to the sciatic nerve, complete functional recovery of the nerves might have taken longer and therefore may exceed this time period. [123]

Nonetheless, these experiments suggest that nerve damage caused by IRE treatment may be of transient character and that full recovery of nerves post-IRE is possible.

Possible blood vessel leakage caused by IRE was reported in a paper published by Silk et al. and described the formation of edema consequently following the leaky endothelial damage in vessels, both in and surrounding a treatment area. [66]

Abscesses in patients with bilioenteric anastomosis was reported in a study investigating adverse effects of IRE in liver malignancies and therefore classified as a risk factor for IRE in these patients. Minor complications by the way, mainly consisted of hemorrhage and portal vein branch thrombosis. [68]

Severe complications in the presence of a metallic stents were reported in a single case following IRE treatment of pancreatic cancer in close proximity to a metallic bile duct stent. The case is described in detail in chapter 4.3. [94]

A lack of coagulation at the insertion sites of IRE probes may result in bleeding, according to an overview work about the advances of minimally invasive tumor treatments published in 2011. [5]

9 Future Outlook and Conclusion

Current research in irreversible electroporation largely revolves around the determination of optimal treatment parameters for certain tissues. Clinical trials are to some degree still rare compared to experiments conducted in animal models. Patient numbers treated with IRE are still small.

Further in vivo human studies will be needed to produce more data resulting in the development of better mathematical models that may improve the ablation technique and make it an easier and more accessible treatment option for clinicians.

In addition to improved usability and the need for sufficient empirical data from human trials, the future success of IRE will also depend on the availability of recommendations and guidelines from major associations of several clinical specialties. These guidelines should offer a wide range of information regarding certified indications for IRE treatment as well as clear instructions for the application process and the recommended parameters.

It would also be advantageous for scientists researching IRE to be able to connect with peers via platforms that are dedicated to IRE as a collaborative research project.

Interdisciplinary collaboration between various specialties such as interventional radiologist and oncologists, surgeons, etc. will be crucial to advance this technology in the context of multimodal oncologic treatment.

The combination of IRE with other ablation or treatment modalities, for example the more traditional oncologic approaches, is presumably another topic for lots of research to come. Interdisciplinary approaches are often fruitful and beneficial for the development of new treatment approaches – IRE has already proven to be no exception.

Irreversible electroporation will most certainly mature and complement, in some cases maybe supersede ablation techniques that are currently in use.

10 REFERENCES

1. Nollet JA. Recherches sur les causes particulieres des phénoménes électriques. Paris, 1754.

2. Puckett D. Advice Greatest Quotes - Quick, Short, Medium Or Long Quotes. Find The Perfect Advice Quotations For All Occasions - Spicing Up Letters, Speeches, And Everyday Conversations: Emereo Publishing, 2016. ISBN: 9781489157973. Available from: https://books.google.at/books?id=Z36WCwAAQBAJ.

3. American Cancer Society. The History of Cancer 2014.

4. Lee EW, Thai S, Kee ST. Irreversible electroporation: a novel image-guided cancer therapy. Gut Liver 2010;4 Suppl 1:S99-S104.

5. Ahmed M, Brace CL, Lee FT, Goldberg SN. Principles of and advances in percutaneous ablation. Radiology 2011;258:351–69.

6. Stephen Kee, Julie Gehl, Edward W. Lee. Clinical Aspects of Electroporation.

7. Marshall E. CLINICAL TRIALS, Gene Therapy Death Prompts Review of Adenovirus Vector. Science 1999;286:2244–5.

8. Lee EW, Chen C, Prieto VE, Dry SM, Loh CT, Kee ST. Advanced hepatic ablation technique for creating complete cell death: irreversible electroporation. Radiology 2010;255:426–33.

9. Deipolyi AR, Golberg A, Yarmush ML, Arellano RS, Oklu R. Irreversible electroporation: evolution of a laboratory technique in interventional oncology. Diagn Interv Radiol 2014;20:147–54.

10. Rubinsky B, Onik G, Mikus P. Irreversible electroporation: a new ablation modality--clinical implications. Technology in Cancer Research & Treatment 2007;6:37–48.

11. Silk M, Tahour D, Srimathveeravalli G, Solomon SB, Thornton RH. The state of irreversible electroporation in interventional oncology. Semin Intervent Radiol 2014;31:111–7.

12. Ivorra A, Rubinsky B. Historical Review of Irreversible Electroporation in Medicine. In: Nagel J, Rubinsky B, eds. Irreversible Electroporation. Berlin, Heidelberg: Springer Berlin Heidelberg, 2010:1–21. (Series in Biomedical Engineering). ISBN: 978-3-642-05419-8.

13. Noad HM. Lectures on electricity, comprising glavanism, magnetism, electro-magnetism, magneto- and thermo- electricity, and electro-physiology. 3rd ed. London: G. Knight and Sons, 1849.

14. Fuller GW. Report on the investigations into the purification of the Ohio River water : at Louisville, Kentucky, made to the president and directors of the Louisville water

company. New York: D. Van Nostrand Company, 1898. Available from: http://worldcatlibraries.org/wcpa/oclc/1122074.

15. Rockwell AD. The medical and surgical uses of electricity, including the X-ray, Finsen light, vibratory therapeutics, and high-frequency currents,. New York: E. B. Treat & company, 1903. Available from: http://worldcatlibraries.org/wcpa/oclc/14810826.

16. Jex-Blake AJ, Royal College of Physicians of London. Death by electric currents and by lightning : the Goulstonian lectures for 1913 delivered before the Royal College of Physicians of London. London: Printed at the Office of the British Medical Association, 1913. Available from: http://worldcatlibraries.org/wcpa/oclc/36125663.

17. G.M. McKinley. Short electric wave radiation in biology in Biological effects of radiation. Duggar, B.M. (ed.). New York: McGraw-Hill Book Co., 1936.

18. Fricke H. A Mathematical Treatment of the Electric Conductivity and Capacity of Disperse Systems ii. The Capacity of a Suspension of Conducting Spheroids Surrounded by a Non-Conducting Membrane for a Current of Low Frequency. Phys. Rev. 1925;26:678.

19. Hodgkin A. L. The iconic basis of electral activity in nerve and muscle. Biological Reviews 1951;26:339–409.

20. Frankenhaeuser B, Widén L. Anode break excitation in desheathed frog nerve. The Journal of Physiology 1956;131:243–7. (http://onlinelibrary.wiley.com/doi/10.1113/jphysiol.1956.sp005459/pdf).

21. Stämpfli R, Willi M. Membrane potential of a ranvier node measured after electrical destruction of its membrane. Experientia;13:297–8.

22. Doevenspeck H. Influencing cells and cell walls by electrostatic impulses. Fleischwirtshaft;1961:986–7.

23. Sale A, Hamilton WA. Effects of high electric fields on microorganisms: I. Killing of bacteria and yeasts. Biochimica et Biophysica Acta (BBA) - General Subjects 1967;148:781–8.

24. Hamilton WA, Sale A. Effects of high electric fields on microorganisms: II. Mechanism of action of the lethal effect. Biochimica et Biophysica Acta (BBA) - General Subjects 1967;148:789–800.

25. Sale A. Effects of high electric fields on micro-organismsIII. Lysis of erythrocytes and protoplasts. Biochimica et Biophysica Acta (BBA) - Biomembranes 1968;163:37–43.

26. Zimmermann U, Pilwat G, Riemann F. Dielectric Breakdown of Cell Membranes. Biophys J 1974;14:881–99.

27. Davalos RV, Mir LM, Rubinsky B. Tissue Ablation with Irreversible Electroporation. Ann Biomed Eng 2005;33:223–31.

28. KINOSITA K, TSONG TY. Formation and resealing of pores of controlled sizes in human erythrocyte membrane. Nature 1977;268:438–41.

29. Zimmermann U. Electric field-mediated fusion and related electrical phenomena. Biochim Biophys Acta 1982;694:227–77.

30. Neumann E, Schaefer-Ridder M, Wang Y, Hofschneider PH. Gene transfer into mouse lyoma cells by electroporation in high electric fields. EMBO Journal 1982;1.

31. Okino M, Mohri H. Okino, M. & Mohri, H. Effects of a high-voltage electrical impulse and an anticancer drug on in vivo growing tumors. Jpn J Cancer Res 78: 1319-1321. Japanese journal of cancer research: Gann 1988;78:1319–21.

32. Orlowski S. Transient electropermeabilization of cells in culture. Increase of the cytotoxicity of anticancer drugs. Biochemical pharmacology 1988;37:4727–33. (http://worldcatlibraries.org/wcpa/oclc/115465898).

33. Prausnitz M. Electroporation of mammalian skin: a mechanism to enhance transdermal drug delivery. Proceedings of the National Academy of Sciences of the United States of America 1993;90:10504–8. (http://worldcatlibraries.org/wcpa/oclc/121758302).

34. Lee R. Electrical injury mechanisms: electrical breakdown of cell membranes. Plastic and reconstructive surgery 1987;80:672–9. (http://worldcatlibraries.org/wcpa/oclc/115905397).

35. Titomirov A. In vivo electroporation and stable transformation of skin cells of newborn mice by plasmid DNA. Biochim Biophys Acta 1991;1088:131–4. (http://worldcatlibraries.org/wcpa/oclc/119255706).

36. Piñero J, López-Baena M, Ortiz T, Cortés F. Apoptotic and necrotic cell death are both induced by electroporation in HL60 human promyeloid leukaemia cells. Apoptosis 1997;2:330–6.

37. Hofmann F, Ohnimus H, Scheller C, Strupp W, Zimmermann U, Jassoy C. Electric Field Pulses Can Induce Apoptosis. J. Membrane Biol.;169:103–9.

38. Gehl J. Vascular reactions to in vivo electroporation: characterization and consequences for drug and gene delivery. Biochim Biophys Acta 2002;1569:51–8. (http://worldcatlibraries.org/wcpa/oclc/121299739).

39. Schoenbach KH, Peterkin FE, Alden RW, Beebe SJ. The effect of pulsed electric fields on biological cells: experiments and applications. IEEE Transactions on Plasma Science 1997;25:284–92.

40. Chenguo Y. Experimental studies on Killing and inhibiting effects of steep pulsed electric field (SPEF) to target cancer cell and solid tumor. IEEE Transactions on Plasma Science 2004;32:1626. (http://worldcatlibraries.org/wcpa/oclc/4798774172).

41. Patent US20070043345 - Tissue ablation with irreversible electroporation, 2007. (Accessed February 26, 2016, at http://www.google.co.ve/patents/US20070043345).

42. Edd JF, Horowitz L, Davalos RV, Mir LM, Rubinsky B. In vivo results of a new focal tissue ablation technique: irreversible electroporation. IEEE Trans Biomed Eng 2006;53:1409–15.

43. Al-Sakere B, Bernat C, Andre F, et al. A study of the immunological response to tumor ablation with irreversible electroporation. Technology in Cancer Research & Treatment 2007;6:301–6.

44. Edd D. Mathematical modeling of irreversible electroporation for treatment planning. Technology in Cancer Research & Treatment 2007;6:275–86. (http://worldcatlibraries.org/wcpa/oclc/162196471).

45. Lavee J. A Novel Nonthermal Energy Source for Surgical Epicardial Atrial Ablation: Irreversible Electroporation. The Heart Surgery Forum 2007;10:E162-E167. (http://worldcatlibraries.org/wcpa/oclc/4639524856).

46. Tekle E. Phagocytic clearance of electric field induced 'apoptosis-mimetic' cells. Biochem Biophys Res Commun 2008;376:256–60. (http://worldcatlibraries.org/wcpa/oclc/5899874591).

47. Wikipedia. Cell membrane - Wikipedia, the free encyclopedia, 2016. (Accessed February 22, 2016, at https://en.wikipedia.org/w/index.php?oldid=691698787).

48. Lee EW, Wong D, Prikhodko SV, et al. Electron microscopic demonstration and evaluation of irreversible electroporation-induced nanopores on hepatocyte membranes. J Vasc Interv Radiol 2012;23:107–13.

49. Tarek M. Membrane electroporation: a molecular dynamics simulation. Biophys J 2005;88:4045–53.

50. Han R, Campbell KP. Dysferlin and muscle membrane repair. Curr Opin Cell Biol 2007;19:409–16.

51. Wikipedia. Electric charge - Wikipedia, the free encyclopedia, 2016. (Accessed February 23, 2016, at https://en.wikipedia.org/w/index.php?oldid=704676625).

52. Encyclopedia Britannica. Physics / Electric Potential, 2014. (Accessed February 21, 2016, at http://www.britannica.com/science/electric-potential).

53. Hofmann GA. Instrumentation and electrodes for in vivo electroporation. Methods Mol Med 2000;37:37–61.

54. dal Maschio M, Ghezzi D, Bony G, et al. High-performance and site-directed in utero electroporation by a triple-electrode probe. Nat Commun 2012;3:960.

55. Ivorra A, Rubinsky B. Electric field modulation in tissue electroporation with electrolytic and non-electrolytic additives. Bioelectrochemistry 2007;70:551–60.

56. Golberg A, Yarmush ML. Nonthermal irreversible electroporation: fundamentals, applications, and challenges. IEEE Trans Biomed Eng 2013;60:707–14.

57. Dejan Semrov DM. Numerical Modeling for In Vivo Electroporation. Methods Mol Med;(Vol.37: Electrically Mediated Delivery of Molecules to Cells):63–81.

58. Zhang Y, Bajaj C, Sohn B. 3D finite element meshing from imaging data. Computer Methods in Applied Mechanics and Engineering 2005;194:5083–106.

59. David Li , Josephine Kang, Benjamin J. Golas, Vincent W. Yeung, David C. Madoff. Minimally invasive local therapies for liver cancer. Cancer Biol Med 2014;2014:217–36. (Accessed February 26, 2016).

60. Article - Clinic for Prostate Therapy Heidelberg. (Accessed February 26, 2016, at http://www.prostata-therapie.de/en/press/article/view/elektrisches-feld-zerstoert-gezielt-prostata-krebs/).

61. Ben-David E, Appelbaum L, Sosna J, Nissenbaum I, Goldberg SN. Characterization of irreversible electroporation ablation in in vivo porcine liver. AJR Am J Roentgenol 2012;198:W62-8.

62. Laufer S, Ivorra A, Reuter VE, Rubinsky B, Solomon SB. Electrical impedance characterization of normal and cancerous human hepatic tissue. Physiol Meas 2010;31:995–1009.

63. Guo Y, Zhang Y, Klein R, et al. Irreversible electroporation therapy in the liver: longitudinal efficacy studies in a rat model of hepatocellular carcinoma. Cancer Res 2010;70:1555–63.

64. Kingham TP, Karkar AM, D'Angelica MI, et al. Ablation of perivascular hepatic malignant tumors with irreversible electroporation. J Am Coll Surg 2012;215:379–87.

65. Cannon R, Ellis S, Hayes D, Narayanan G, Martin RCG. Safety and early efficacy of irreversible electroporation for hepatic tumors in proximity to vital structures. J Surg Oncol 2013;107:544–9.

66. Silk MT, Wimmer T, Lee KS, et al. Percutaneous ablation of peribiliary tumors with irreversible electroporation. J Vasc Interv Radiol 2014;25:112–8.

67. Dollinger M, Müller-Wille R, Zeman F, et al. Irreversible Electroporation of Malignant Hepatic Tumors - Alterations in Venous Structures at Subacute Follow-Up and Evolution at Mid-Term Follow-Up. PLoS ONE 2015;10:e0135773.

68. Dollinger M, Beyer LP, Haimerl M, et al. Adverse effects of irreversible electroporation of liver malignancies under CT fluoroscopic guidance: a single-center experience. Diagn Interv Radiol 2015.

69. Schoellhammer HF, Goldner B, Merchant SJ, Kessler J, Fong Y, Gagandeep S. Colorectal liver metastases: making the unresectable resectable using irreversible electroporation for microscopic positive margins - a case report. BMC Cancer 2015;15:271.

70. Sugimoto K, Moriyasu F, Kobayashi Y, et al. Irreversible electroporation for nonthermal tumor ablation in patients with hepatocellular carcinoma: initial clinical experience in Japan. Jpn J Radiol 2015;33:424–32.

71. Wagstaff PGK, Bruin DM de, Zondervan PJ, et al. The efficacy and safety of irreversible electroporation for the ablation of renal masses: a prospective, human, in-vivo study protocol. BMC Cancer 2015;15:165.

72. Thomson KR, Cheung W, Ellis SJ, et al. Investigation of the safety of irreversible electroporation in humans. J Vasc Interv Radiol 2011;22:611–21.

73. Pech M, Janitzky A, Wendler JJ, et al. Irreversible Electroporation of Renal Cell Carcinoma, A First-in-Man Phase I Clinical Study. Cardiovasc Intervent Radiol 2011;34:132–8.

74. Tracy CR, Kabbani W, Cadeddu JA. Irreversible electroporation (IRE): a novel method for renal tissue ablation. BJU Int 2011;107:1982–7.

75. Wendler JJ, Pech M, Blaschke S, et al. Angiography in the isolated perfused kidney: radiological evaluation of vascular protection in tissue ablation by nonthermal irreversible electroporation. Cardiovasc Intervent Radiol 2012;35:383–90.

76. Wendler JJ, Pech M, Porsch M, et al. Urinary tract effects after multifocal nonthermal irreversible electroporation of the kidney: acute and chronic monitoring by magnetic resonance imaging, intravenous urography and urinary cytology. Cardiovasc Intervent Radiol 2012;35:921–6.

77. Olweny EO, Kapur P, Tan YK, Park SK, Adibi M, Cadeddu JA. Irreversible electroporation: evaluation of nonthermal and thermal ablative capabilities in the porcine kidney. Urology 2013;81:679–84.

78. Dunki-Jacobs EM, Philips P, Martin RCG. Evaluation of thermal injury to liver, pancreas and kidney during irreversible electroporation in an in vivo experimental model. Br J Surg 2014;101:1113–21.

79. Deodhar A, Monette S, Single GW, et al. Renal tissue ablation with irreversible electroporation: preliminary results in a porcine model. Urology 2011;77:754–60.

80. Sommer CM, Fritz S, Wachter MF, et al. Irreversible electroporation of the pig kidney with involvement of the renal pelvis: technical aspects, clinical outcome, and three-dimensional CT rendering for assessment of the treatment zone. J Vasc Interv Radiol 2013;24:1888–97.

81. Wendler JJ, Porsch M, Hühne S, et al. Short- and mid-term effects of irreversible electroporation on normal renal tissue: an animal model. Cardiovasc Intervent Radiol 2013;36:512–20.

82. Irreversible Electroporation, A New Hope for the Pancreatic Cancer Patients. J Nanomedine Biotherapeutic Discov 2015;05.

83. Cancer of the Pancreas - SEER Stat Fact Sheets, 2015. (Accessed March 3, 2016, at http://seer.cancer.gov/statfacts/html/pancreas.html).

84. Robert C. G. Martin, II. Irreversible electroporation of stage 3 locally advanced pancreatic cancer: optimal technique and outcomes 2015.

85. Charpentier KP, Wolf F, Noble L, Winn B, Resnick M, Dupuy DE. Irreversible electroporation of the pancreas in swine: a pilot study. HPB (Oxford) 2010;12:348–51.

86. Bower M, Sherwood L, Li Y, Martin R. Irreversible electroporation of the pancreas: definitive local therapy without systemic effects. J Surg Oncol 2011;104:22–8.

87. José A, Sobrevals L, Ivorra A, Fillat C. Irreversible electroporation shows efficacy against pancreatic carcinoma without systemic toxicity in mouse models. Cancer Lett 2012;317:16–23.

88. Bagla S, Papadouris D. Percutaneous irreversible electroporation of surgically unresectable pancreatic cancer: a case report. J Vasc Interv Radiol 2012;23:142–5.

89. Newsplex Now. New Study Shows IRE Treatment Significantly Improves Remission Rates for Pancreatic Cancer, 2015. (Accessed February 26, 2016, at https://www.youtube.com/watch?v=TGKvQUFQK4U).

90. Martin RCG, McFarland K, Ellis S, Velanovich V. Irreversible electroporation therapy in the management of locally advanced pancreatic adenocarcinoma. J Am Coll Surg 2012;215:361–9.

91. Martin RCG, McFarland K, Ellis S, Velanovich V. Irreversible electroporation in locally advanced pancreatic cancer: potential improved overall survival. Ann Surg Oncol 2013;20 Suppl 3:S443-9.

92. Kwon D, McFarland K, Velanovich V, Martin RC. Borderline and locally advanced pancreatic adenocarcinoma margin accentuation with intraoperative irreversible electroporation. Surgery 2014;156:910–22.

93. Martin RCG, Kwon D, Chalikonda S, et al. Treatment of 200 Locally Advanced (Stage III) Pancreatic Adenocarcinoma Patients With Irreversible Electroporation: Safety and Efficacy. Ann Surg 2015;262:486–94.

94. Månsson C, Nilsson A, Karlson B. Severe complications with irreversible electroporation of the pancreas in the presence of a metallic stent: a warning of a procedure that never should be performed. Acta Radiol Short Rep 2014;3:2047981614556409.

95. Onik G, Narayan P, Vaughan D, Dineen M, Brunelle R. Focal "nerve-sparing" cryosurgery for treatment of primary prostate cancer: A new approach to preserving potency. Urology 2002;60:109–14.

96. Nagel J, Rubinsky B, eds. Irreversible Electroporation. Berlin, Heidelberg: Springer Berlin Heidelberg, 2010. (Series in Biomedical Engineering). ISBN: 978-3-642-05419-8.

97. Gary Onik, Paul Mikus, Boris Rubinsky. IRE Implications for Prostate Ablation 2007.

98. Nagel J, Rubinsky B, eds. Irreversible Electroporation. Berlin, Heidelberg: Springer Berlin Heidelberg, 2010. (Series in Biomedical Engineering). ISBN: 978-3-642-05419-8.

99. van den Bos W, Bruin DM de, Muller BG, et al. The safety and efficacy of irreversible electroporation for the ablation of prostate cancer: a multicentre prospective human in vivo pilot study protocol. BMJ Open 2014;4:e006382.

100. W. van de Bos, De la Rosette,Jean J. M. C. H. IRE for Prostate Cancer. In: Rastinehad AR, Siegel DN, Pinto PA, Wood BJ, eds. Interventional Urology. Cham: Springer International Publishing, 2016:161–3. ISBN: 978-3-319-23463-2.

101. Dupuy DE, Aswad B, Ng T. Irreversible Electroporation in a Swine Lung Model. Cardiovasc Intervent Radiol 2011;34:391–5.

102. Garcia PA, Rossmeisl JH, Robertson J, Ellis TL, Davalos RV. Pilot study of irreversible electroporation for intracranial surgery. In: 2009 Annual International Conference of the IEEE Engineering in Medicine and Biology Society. p. 6513–6.

103. Garcia PA, Rossmeisl JH, Neal RE, et al. Intracranial Nonthermal Irreversible Electroporation, In Vivo Analysis. J Membrane Biol 2010;236:127–36.

104. Neal RE, Rossmeisl JH, D'Alfonso V, et al. In vitro and numerical support for combinatorial irreversible electroporation and electrochemotherapy glioma treatment. Ann Biomed Eng 2014;42:475–87.

105. Thomas L. Ellis, Paulo A. Garcia. Nonthermal irreversible electroporation for intracranial surgical applications, Laboratory Investigation. J Neurosurg 2011;2011:681–8.

106. Garcia. Non-Thermal Irreversible Electroporation (N-TIRE) and Adjuvant Fractionated Radiotherapeutic Multimodal Therapy for Intracranial Malignant Glioma in a Canine Patient. Technol Cancer Res Treat 2011.

107. Neal RE, Davalos RV. The feasibility of irreversible electroporation for the treatment of breast cancer and other heterogeneous systems. Ann Biomed Eng 2009;37:2615–25.

108. Neal RE, Singh R, Hatcher HC, Kock ND, Torti SV, Davalos RV. Treatment of breast cancer through the application of irreversible electroporation using a novel minimally invasive single needle electrode. Breast Cancer Res Treat 2010;123:295–301.

109. Golberg A, Fischer J, Rubinsky B. The Use of Irreversible Electroporation in Food Preservation, 2009. (https://www.researchgate.net/profile/Boris_Rubinsky/publication/225856016_The_Use_of_Irreversible_Electroporation_in_Food_Preservation/links/0912f511a9a93d2322000000.pdf).

110. Maor E, Ivorra A, Mitchell JJ, Rubinsky B. Vascular smooth muscle cells ablation with endovascular nonthermal irreversible electroporation. J Vasc Interv Radiol 2010;21:1708–15.

111. Friedland S, Triadafilopoulos G. A novel device for ablation of abnormal esophageal mucosa (with video). Gastrointest Endosc 2011;74:182–8.

112. Li W, Fan Q, Ji Z, Qiu X, Li Z. The effects of irreversible electroporation (IRE) on nerves. PLoS ONE 2011;6:e18831.

113. Mandel Y, Laufer S, Belkin M, Rubinsky B, Pe'er J, Frenkel S. Irreversible electroporation of human primary uveal melanoma in enucleated eyes. PLoS ONE 2013;8:e71789.

114. Martin RCG, Philips P, Ellis S, Hayes D, Bagla S. Irreversible electroporation of unresectable soft tissue tumors with vascular invasion: effective palliation. BMC Cancer 2014;14:540.

115. Maor E, Kain D, Zager Y, Leor J. myocardial ablation and decellularization by irreversible electroporation, a novel non-thermal non-pharmacological biophysical approach. Journal of the American College of Cardiology 2013;61:E1832.

116. Al-Sakere. A Study of the Immunological Response to Tumor followin IRE 2007.

117. Goldberg R. IRE considering muscle activation. Technology in Cancer Research & Treatment 2012;2012:189–201.

118. Arena CB, Sano MB, Rossmeisl JH, et al. High-frequency irreversible electroporation (H-FIRE) for non-thermal ablation without muscle contraction. Biomed Eng Online 2011;10:102.

119. Deodhar A, Dickfeld T, Single GW, et al. Irreversible electroporation near the heart: ventricular arrhythmias can be prevented with ECG synchronization. AJR Am J Roentgenol 2011;196:W330-5.

120. Sugimoto K, Moriyasu F, Takeuchi H, et al. Case study to assess the safety of irreversible electroporation near the heart. Springerplus 2015;4:74.

121. Faroja M, Ahmed M, Appelbaum L, et al. Irreversible electroporation ablation: is all the damage nonthermal? Radiology 2013;266:462–70.

122. Schoellnast H, Monette S, Ezell PC, et al. Acute and subacute effects of irreversible electroporation on nerves: experimental study in a pig model. Radiology 2011;260:421–7.

123. Schoellnast H, Monette S, Ezell PC, et al. The delayed effects of irreversible electroporation ablation on nerves. Eur Radiol 2013;23:375–80.