EXPERIMENTAL USE OF NOVEL PULSE GENERATOR FOR IRREVERSIBLE ELECTROPORATION

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Abstract: Irreversible electroporation (IRE) is a relatively new technique for minimal invasive non-thermal ablation treatment of solid tumors. This article describes basics of IRE and available devices for this technique. The parameters of commercial NanoKnife IRE generator are verified and the unique experimental IRE generator developed at the Brno University of Technology is presented in this paper. Further, there is a comparison of thermal effects on a target tissue of commonly used radiofrequency ablation (RFA) and the novel IRE generator using an infrared camera. All experiments were performed *ex vivo* on a porcine liver. The difference in maximum temperature between the output powers used was 17.8 °C with RFA and 3 °C with IRE. This is a significant difference and shows the IRE's capability of liver tissue ablation without thermal damage. The new IRE generator is also capable of replacing NanoKnife because of its features and versatility.

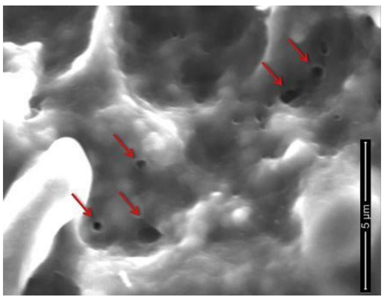
Keywords: Development, Irreversible Electroporation, Radiofrequency ablation, Infrared camera

1. INTRODUCTION

Minimally invasive treatment techniques are the current trend in medicine. This paper focuses on ablation techniques. Irreversible electroporation (IRE) is an efficient but not well-known technique with a number of advantages. Therefore, new experimental IRE generator, inspired by commercially available NanoKnife IRE generator, was developed. The thermal effects on the treated tissue of the new IRE generator and radiofrequency ablation device (RFA) are compared. Although it has some disadvantage, the new experimental IRE generator is capable of solving various medical indications.

This method uses cell membrane properties in its favor. Plasma membrane consists of a lipid bilayer with embedded K^+ and Na^+ channels ensuring regulated transmission of ions through the membrane between intracellular and extracellular liquids. Electrically, the membrane behaves like a capacitor in parallel with a high resistance resistor. If the cell is exposed to short high voltage electrical pulses, then the cell membrane charges up and nano-scale pores are formed in the membrane (see Figure 1a) [1].

a)



b)

Figure 1 a) Scanning electron microscopy image of multiple nano-sized pores (red arrows) on the cell membrane of swine liver after IRE ablation [3]. b) Photograph of the electroporated liver. Blood vessels and other sensitive collagen structures remain undamaged [2].

Thanks to these pores the membrane permeability to ions and macromolecules increases. This effect is a function of various pulse parameters as well as parameters of the cell. These are shape, length, amplitude, a number of pulses and intervals between them, but also cell type and cell size, membrane flexibility or permeability. Increasing intensity of electric field yields larger pores [1].
If the permeabilization is transient it is referred to reversible electroporation, and if it is permanent it is irreversible electroporation. Both phenomena have important application in biomedical technology. Reversible electroporation is used for fusion of cells, induction of drugs into cells or transdermal delivery of drugs (e.g. anti-cancer drugs) and genes. [3] Irreversible electroporation is used for sterilization of liquid media or grocery from micro-organisms but over the past 9 years, it has found its place in minimally invasive tissue ablation. A number of studies on its validity, safety and efficiency in *in vivo* animal and human models is performed all over the world. Moreover, it seems to be promising treatment method in other applications as well [4].

2. IRREVERSIBLE ELECTROPORATION

As mentioned above, irreversible electroporation (IRE) is novel non-thermal tissue ablation technique. It uses electrical energy to produce focused cell death which comes within seconds. This is an advantage of IRE in comparison with other tissue ablation modalities which use thermal energy instead. These methods include radiofrequency ablation (RFA), microwave ablation and cryoablation. Additional benefits of irreversible electroporation include shorter treatment time, ability to treat considerably larger lesions, sharp boundaries of ablated region, less damage to normal surrounding tissue and minimal dissipation of thermal energy. Furthermore, it is not negatively affected by local blood flow [4].

The procedure of the IRE treatment is following. Required number of needle electrodes is placed in the area of the solid tumor. Usually, the voltage of each pulse is between 1500 V and 4000 V, it is 100 μs long with 1 s between the pulses in series of 90 pulses. The nanopores are formed and the cell goes to apoptosis, it dies. Adjacent structures such as blood vessels or nerves remain undamaged (see Figure 1b). The area of dead cells is soon filled with new healthy cells whereas the tumor cells are not able to regenerate.

3. RADIOFREQUENCY ABLATION

Radiofrequency ablation technique (RFA) is the interventional procedure for cancer treatment. High-frequency alternating current is applied to the tissue via active electrodes and produces a frictional heating of the tissue. Unfortunately, the cell destruction in the affected area does not occur selectively. Moreover, flowing liquids, such as blood flowing in nearby blood vessels cool down the area and complicate the process of RFA. Another problem connected with blood vessels, biliary tract, vascular structures and other histologically similar tissue is their destruction by produced heat. Therefore, RFA is not the ideal method to treat tissues which are adjacent to these structures. [5]

4. EXPERIMENTS

Only one device performing IRE on animal models and humans is commercially available so far. It is called NanoKnife from AngioDynamics and it is used by medical research teams worldwide. Only one piece of this device is available in Czechia and even a rent is not affordable for lots of the research teams. Therefore, Department of Power Electrical and Electronic Engineering, BUT in cooperation with University Hospital Brno Bohunice has developed unique high-voltage pulse generator for IRE.

4.1. THE PARAMETERS VERIFICATION OF NANOKNIFE IRE GENERATOR

At first, the exact parameters of NanoKnife were measured to set the requirements for development of the new device. The current probe TCP0030 from Tektronix and voltage probe N2771A from

Agilent were used to measure the data (see Figure 2a). NanoKnife consists of a high-voltage power supply with a high-voltage storage capacitor and a transistor switch connecting the high voltage directly to electrodes. This configuration requires a safety clamping transistor which ensures short-cut of the output. This avoids delivering an uncontrolled width of the pulse (>100 μs) during control failure or damage of the main transistor switch. NanoKnife provides voltage up to 3 kV and current up to 50 A (see Figure 3a). It can use up to 6 electrodes, but usually, not more than 3 are used. Unfortunately, only disposable electrodes with special chip might be used. The price of this device in thousands of Euros is another downside. Pulses are nicely rectangular but their length is limited only by the charge on the high-voltage capacitor. [4], [5].

a) b)

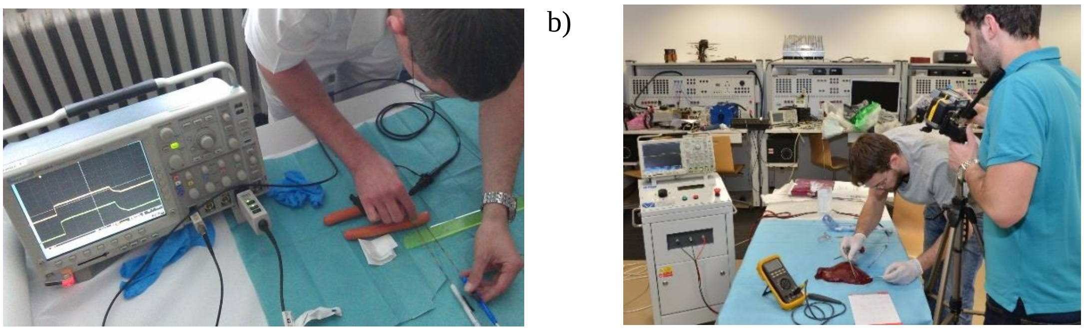


Figure 2 a) An acquisition of exact parameters from NanoKnife IRE generator, b) Photograph from the experiment with the novel balloon catheter described in Chapter 4.2.

Based on these results the new IRE generator was designed. At a first sight, it might look same as NanoKnife IRE system but its working principle and internal construction are in fact completely different. It works as follows. The line voltage is converted to variable mid-voltage DC-link with a storage capacitor. The high-voltage transformer is switched by mid-voltage IGBT transistor and the diode rectifier is connected to its output. The pulse transformer on the output principally does not allow transfer of a pulse longer than 150 μs, because of a core saturation. Even though this layout increases a complexity of the power stage, it brings a superior safety of operation for the patient as well as for the doctor especially in a case of failure.

a)

b)

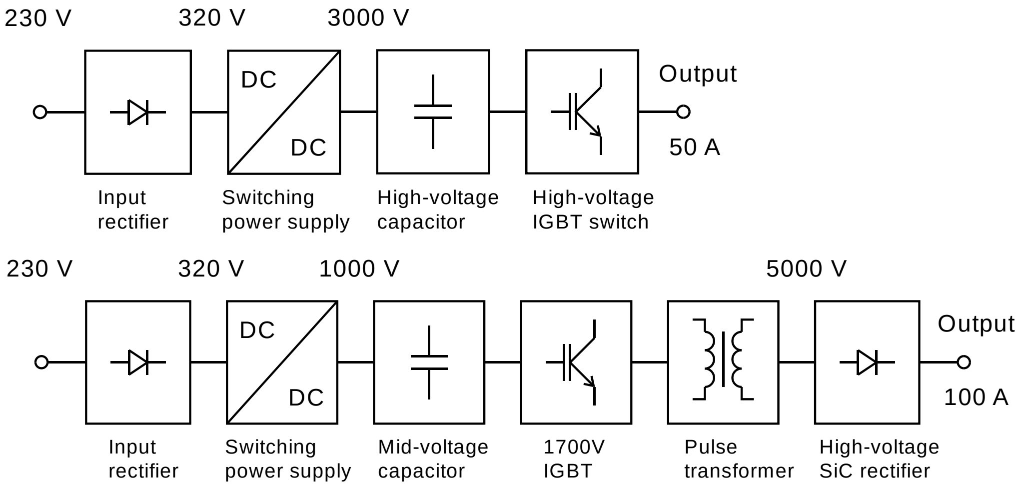


Figure 3 a) A block diagram of NanoKnife and b) new BUT IRE generator.

The newly developed device is capable of 5 kV and 100 A (see Figure 3b) and its price is significantly lower than NanoKnife. It is well suited for experimental purposes because of its greater range of output parameters and ability to connect a broad spectrum of electrode types (needles and catheters). At once up to 3 electrodes might be used. As mentioned earlier, the length of the pulse is restricted but it is not perfectly rectangular. However, this fact does not have any effect on the functionality of the device. The BUT generator contains relatively heavy iron-core transformer (approximately 15 kg). This might be disadvantageous for the transportation of the device [5].

4.2. THE THERMOGRAPHIC MEASUREMENTS DURING IRE AND RFA

In this experiment, radiofrequency ablation and irreversible electroporation were compared. The measurements were performed *ex vivo* on a porcine liver at room temperature. All thermograms were recorded using an infrared camera (FLIR B200 with a sensitive thermocouple probe). The camera was at a distance of 1 m from the sample (see Figure 2b). The temperature of the tissue was measured during the experiments as well as immediately after the ablation procedure inside the liver tissue [5].

RFA 1500, RITA Medical Systems was used for the experiment. RFA was performed in a bipolar mode. A Habib EndoHPB catheter with a pair of active electrodes was inserted into the liver tissue. The catheter consisted of 2 ring electrodes 8 mm apart with a distal electrode 5 mm from the leading edge. Power settings of 5 W and 10 W were used for different time periods (up to 90 s). Nominal values of the RFA generator are either 150 W in 25-100 Ω, 460 kHz in power and temperature control mode or 25 W in 10-999 Ω, 460 kHz in track ablation mode [5].

For the IRE was used the newly designed device and unique balloon catheter with 3 electrodes on the external surface. Each electrode was 1 mm wide and 10 mm long. They were 120° apart from each other on the balloon of 8 mm in diameter. The catheter was inserted into the liver tissue approximately 1 cm below the surface and filled with physiological saline solution. This assures good contact between the tissue and the electrodes. A sequence of 50 pulses 100 μs for all three combinations of active electrode pairs was applied. That means 150 pulses for the whole electroporation experiment. The two tested output voltages were 1,500 V and 2,500 V. The electric current was 4 A and 10 A respectively. This means that the average power was 0.6 W in the case of 1.5 kV and 2.5 W with 2.5 kV (then the peak power was 6 kW and 25 kW respectively) [5].

The experiment with the same setting was performed using two needle electrodes from NanoKnife IRE generator. The electrodes were 1 mm in diameter, with 10 mm long active part. The distance between the electrodes was 15 mm. This is their standard configuration. With the changing distance, the effect of IRE will change as well. Different values of current and voltage were tested. The further experiments were carried out on 14 domestic swine (50-60 kg) *in vivo*. IRE in bile duct was done using the balloon catheter with electrodes.

5. RESULTS AND DISCUSSION

The exact parameters of NanoKnife were verified. The measured data showed that the maximum voltage is really 3 kV and the maximum current is 50 A. The construction solution of NanoKnife was studied and based on the observation a new device was designed and constructed. It disposes of the maximum of 5 kV and 100 A which greatly expands the possibilities for experimental use. All tests were carried out on *ex vivo* porcine liver.

The IRE experiments with the needle electrodes in the standard configuration, mentioned above, revealed really interesting findings. If the peak power during the pulse is between 5 kW and 8 kW the thermal damage is imperceptible and macroscopically not visible. However, histological results show some minor changes. The changes of the tissue by the peak power of 15 kW are visible by the naked eye. We can say that 15 kW is approximately the limit between IRE and thermal damage of the tissue. If the peak power is e.g. 25 kW the tissue is thermally damaged and it turns white. It is an emergence of coagulation necrosis and protein denaturation. These findings go hand in hand with results from the thermographic measurements of IRE and RFA liver ablation experiment. The peak output powers of IRE were 6 kW and 25 kW. The maximum temperature was 20°C with 1.5 kV (6 kW) and 23°C with 2.5 kV (25 kW) which means a difference of 3°C. Histological changes of liver tissue were visible only in the case of the higher voltage.

In order of the thermal effect comparison between IRE and RFA, the average power values of IRE are important (0.6 W and 2.6 W). The power is delivered continuously during the RFA procedure. It

means the tissue was exposed to the power of 5 W and 10 W. At a first sight, this is a significant difference of the power applied to the tissue. The maximum temperature at 5 W was 37.7°C and with 10 W it was 55.5°C which is a difference of 17.8°C [5].
The thermograms showed that the temperature in the area of RFA application is less homogenous. Moreover, the sensitive structures adjacent to ablated area are not affected by IRE. In comparison, visible thermal changes of the tissue were observed in the case of RFA application. It must be considered, that heating effect is influenced by the cooling effect of circulating fluids, such as blood. This is advantageous in the case of IRE but works against the RFA where the temperature increase is required [5].
The trials on *in vivo* porcine liver were performed with interesting interim results which are still being processed.

6. CONCLUSION

Based on the measurements with NanoKnife and its inner construction solution, new IRE generator was developed and tested on the *ex vivo* porcine liver. All experiments and measurements proved that the newly developed BUT IRE generator is capable of replacing expensive NanoKnife IRE system. Its suitability for liver tissue ablation was proved. The new IRE generator does not heat up the tissue as much as commonly used RFA which is greatly advantageous.
Future research will be focused on development of novel AC (alternating current) IRE generator for cardiac ablation and treatment of arrhythmias, and H-FIRE (High-frequency irreversible electroporation) generator as well as improvement of presented balloon catheter and development of new pulse applicators. Various IRE generators have potential use in oncology, intervention radiology, cardiology or aesthetic surgery.

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