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Cardiac Ablation via Electroporation

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Abstract—**Thermal-based ablation for the treatment of arrhythmias is known to cause issues (e.g. heat loss due to blood perfusion, mechanical damage of the tissue from excessive heat, etc.) that hamper the success of the treatment. A novel technique termed "electroporation" is a process that leads to pore formation in cell membranes. These pores may cause cellular death without inducing negative thermal effects. We successfully developed a system, tools, and methodology to operate this new ablation technique. Preliminary in vivo acute animal studies (ovine) suggest distinct lesion morphology. High transmurality success rates also suggest the possibility of applying this new ablation modality to cardiac ablation. A long term study confirming lesion durability is necessary to warrant the successful adoption of this technique.**

I. Introduction

Despite the popularity of radio frequency (RF) ablation in cardiac arrhythmia treatment, creating a lesion epicardially without inducing negative effects continues to present a challenge. High rates of blood perfusion in some areas further prevent the success of ablation due to heat loss [1]. Attempts to overcome this challenge often involve increasing energy delivery to dangerous levels, resulting in the occurrence of safety issues. Tissue perforations and thermal coagulation of blood are common findings during thermal-based ablation [2].

Electroporation is a process that increases the permeability of the cell membrane through an application of short high-voltage electrical pulses [3]. The underlying mechanism of electroporation is not yet clearly understood. However, it is widely believed that the electric field changes the electrochemical potential across the cell membrane and induces instabilities in the polarized cell membrane lipid bilayer [4]. The unstable membrane then undergoes transient changes in its shape, creating sub-micron sized aqueous pathways through the membrane [5].

Depending on the amount of energy applied (amplitude, pulse duration, number of pulses, and frequency), electroporation can result in the opening of the cell membrane either temporarily or permanently [6]. When the membrane is permanently opened, the cell undergoes necrosis [7]. Once the pores are formed in the membrane, free ion exchange takes place through these pores, resulting in disruption and death of the cell. Therefore, this biological ablation is considered to overcome limitations of thermal-based ablation with the added benefit of short ablation time.

The purposes of this study were to: 1) develop systems and methods for electroporation, and 2) evaluate feasibility of its use in cardiac tissue. Specifically, it was our interest to determine if this ablation method can create transmural lesions without encountering safety issues.

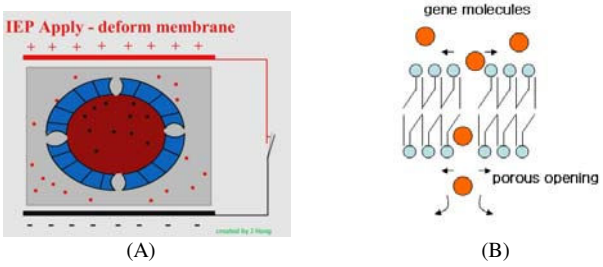

Fig. 1: A schematic drawing of electroporation effect (A), and opening of the lipid bilayer (B). Creating pores in the membrane enables ions to pass through.

II. Methods and System Development

A. Device Development

Two types of devices were developed for delivery of electroporation energy. The clamp device was for isolating pulmonary veins and vena cavae by clamping around tubular structures, and the linear probe device was for making epicardial lesions on the heart.

The clamp was a modification of Medtronic Cardioblate BP2 RF ablation device (Model 60831, Medtronic, Inc., Minneapolis, MN). This device had bipolar electrode jaws that were shortened to 5.3 cm from the original 7.0 cm. Electrodes were first made from 1 mm diameter stainless steel wire. The appearance of thermal surface burns at higher energy levels prompted us to look at higher heat capacity, heat conduction, and higher electrical conductivity materials for the electrodes. A new set of prototypes was built with 1 mm diameter solid gold wire or 1 mm diameter lower cost gold-plated stainless steel wire.

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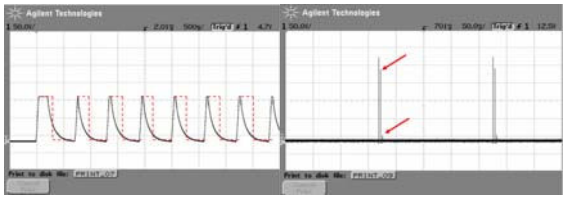
The linear probe device was a suction-assisted device with linear electrodes. Early versions of the devices had relatively shallow suction pods and a wide, 6mm electrode spacing. The level of suction was typically kept at 350 mmHg. The pod was designed to have a 2.5 mm electrode spacing and a 5 mm deep suction cup. To reduce the amount of thin-walled tissue that was drawn into the probe, a new pod was designed that reduced the depth to 3.5 mm.

Fig. 2: Bipolar clamp device (left) and linear surface probe devices (right) with gold plated stainless steel electrodes.

B. Generators

1) Medtronic Research Defibrillator 2960

During early stage of this study, an external defibrillator was used for delivery of high voltage pulses. This system (Medtronic Research Defibrillator 2960) was capable of charging its 750 µF (micro-farad) storage capacitor up to 900V, (delivering a maximum 780V and 40A in short pulses). The pulse parameters (pulse width, interval, number of pulses, and burst frequency) can be customized using a computer program written in LabVIEW (National Instruments, Austin, TX). However, voltage ramping effects (decrease in voltage over time) and irregular pulse shapes resulted from the limited size of the capacitor and system resources hampered proper evaluation of pulse parameters and associated effects on tissue. Long manual charging time between the pulse trains (approximately 5 to 7 seconds) made it also impossible to evaluate overall ablation time.



(a) (b)

Fig. 3: Distorted waveforms of Research Defibrillator 2960. (a) red line indicates its intended pulse shape, (b) multiple pulses shows severe voltage drop and skipping pulse (intended to be 10 pulses for each series).

2) Prototype High Voltage Generator System

A new high voltage generator system was internally designed and built. This circuit consisted of 4 IGBTs in an H-bridge configuration and was triggered by an Agilent 33220 function generator through independent optocouplers which isolated high voltage from a high energy capacitor which was continuously charged using a DC power supply (Kepco model APH 1000M). A computer running a LabVIEW software application provided the capability to program arbitrary burst pulses in various frequencies on the function generator via USB.

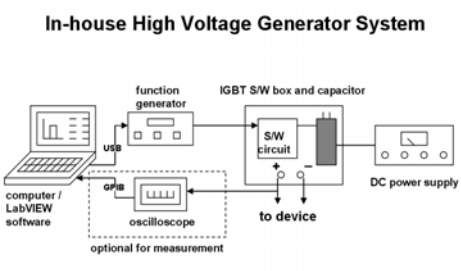


Fig. 4: High voltage generator system

Three or five trains of pulses were delivered at a frequency between 1 and 5 Hz. A single train consisted of 10 to 40 pulses (typical pulse width ranged from 100 to 400 µsec with less than 50% duty cycle).

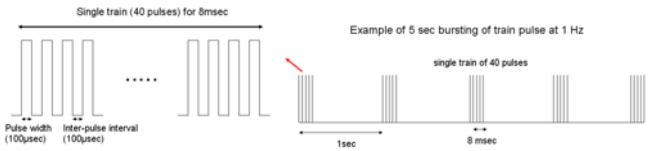


Fig. 5: An example of 5 sec delivery of trains. (a) Single train is composed of 40 pulses (b) each train is delivered every second (1 Hz).

C. Animal Preparation

A total of four sheep were tested in this study. Sheep were cared for according to the Medtronic Physiological Research Laboratories (PRL) Standard Operating Procedures and The Guide for Care and Use of Laboratory Animals. The ovine model was chosen because sheep are able to withstand relatively long surgical procedure times. Sheep were given morphine and were induced with a short-acting barbiturate or short-acting hypnotic. Isoflurane was used to maintain the sheep at an appropriate plane of anesthesia.

D. Surgical Procedure

A left or right thoracotomy was performed to access the heart. A jugular venotomy was performed to allow access for an electrophysiologic (EP) diagnostic catheter for electrical measurements. A carotid arteriotomy was performed to place an arterial pressure line to monitor the hemodynamic state of the animal. Alternatively, a peripheral pressure catheter was placed for pressure monitoring. Anti-arrhythmics were administered if necessary. Heparinization was administered before termination to prevent blood coagulation.

Ablations were performed epicardially using the bipolar clamp or linear surface probe devices at the following locations: left or right pulmonary vein (PVs), left atrial appendage (LAA), right atrial appendage (RAA), superior and inferior vena-cavae (SVC and IVC), right ventricular outflow

tract (RVOT), left ventricle (LV) free-wall, and right atrial free-wall. Additionally, ablations were performed on the esophagus to evaluate damage to this muscle. Ablations were marked using nickel-titanium surgical U-clips as lesions were not expected to be visible during surgery. At least one RF ablation was performed on an appendage using a Medtronic Cardioblate BP2 system as a control.

Following all ablations, the animal was paced to check for exit block and bipolar electrograms (EGMs) were recorded to check for entrance block. In order to evaluate the ability to make atrial free wall lesions, the linear probe device was used to make lesion lines around an "island" of tissue.

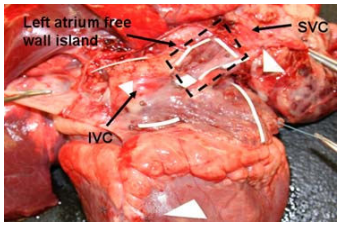


Fig. 6: Right atrial free-wall island is made between superior vena cava (SVC) and inferior vena cava (IVC) using linear probe device. SVC and IVC lesions were made using the clamp device.

E. Data Collection

ECG, bipolar electrograms, and arterial pressures were displayed on a Prucka electrophysiological workstation (CardioLab), used to monitor the animal's health, and saved for subsequent analysis. Ablation parameters/settings were recorded along with location of the ablation.

F. Necropsy and Histopathology

Sheep underwent a necropsy that was focused on the heart and the esophagus. Tissue samples were collected in 10% neutral buffered formalin. Trimmed tissue samples were paraffin embedded and hematoxylin-eosin stained and Masson's Trichrome stained sections were generated. Pathology objectives were 1) identification of presence of a cardiac lesions caused by energy delivery, 2) description of type and extent of tissue change with assessment of cardiomyocytes, interstitium, blood vessels, and endocardial thrombus deposition, 3) identification of evidence of particular lesion characteristics in a changing tissue environment (e.g. if ample epicardial fat tissue is nearby), and 4) assessment of lesion transmurality of lesions in representative cross sections.

III. Results And Discussion

A. Electroporation lesions

Lesion evaluations for transmurality from the bipolar clamp device are shown in Table 1. SVC and IVC lesions were 100% transmural with any/all methods of lesion evaluation. Non-transmural lesions from the RAA and PVs were due to existence of fat content on the area. On the LAA, there was one case that exit block did not agree with other methods. There was one case that histology did not agree with other methods on the RAA.

	RAA (n=7)	LAA (n=6)	PVs (n=6)	SVC (n=7)	IVC (n=7)
Entrance block	86%	100%	84%	100%	100%
Exit block	86%	84%	84%	100%	100%
Histology	71%	100%	84%	100%	100%

Table 1. Results of lesion evaluation from bipolar clamp ablation. Percents of transmural lesions are shown.

The linear probe device was evaluated by creation of free-wall isolated "islands" of tissue. This was a continual learning process in terms of how to best design the linear probe device as well as where to best ablate on the right atrial-intercaval free-wall region to create an isolated region of tissue.

The original intent was to create a pair of parallel lines, followed by verification that pacing could be performed from between the lines and a pacing lead implanted. Then, the end-cap lesions would be applied to form a closed "box" of lesions around the pacing site that could be evaluated chronically. However, when the parallel probe lesions were placed too close to each other (2cm), they eliminated all conduction between the two lines. We found that the lesions needed to be separated by a minimum distance of 3 cm. This may have been due to local circumferential myofiber orientation or perhaps the probe effects covered a larger area than intended. We confirmed isolation of conduction in two animals with this method

B. Histopathology

1) Cardiac lesions

The electroporated lesions were in general not visible grossly but could be detected via histology on lesion cross-sections with a maturation period in the animal prior to sacrifice as short as one hour. Histomorphologically, the lesions were well demarcated from the unaffected tissue and displayed edema or hemorrhage in the interstitium, contraction band formation or myofiber breakup with cell swelling of cardiomyocytes, and loss of of the native myofiber birefringence under polarized light. This was distinct from the lesion characteristics of RF hyperthermic-induced ablations where contraction bands were restricted to the outer rim of the lesion and cardiomyocytic coagulation necrosis was present in the center. The induced inflammatory reaction within these acute ablations was minimal. Collagen denaturation that resembled thermogenic damage was noted in some of the lesions suggesting possible thermal effects when higher settings of energy were applied.

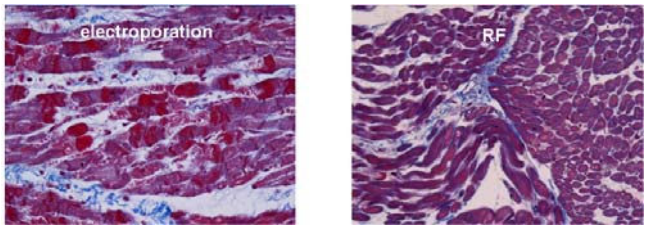


Fig 7: Contraction band necrosis, cardiomyocte swelling and breakup after electroporation, and coagulation necrosis of cardiomyofibers after RF energy delivery.

In electroporation lesions, intralesional veins and to a lesser extent arteries in showed occasionally endothelial denudation. In contrast, RF-induced ablations showed consistent and severe blood vessels damage. Lesions created on the RVOT indicated that the linear probe device could create lesions that were up to 4 mm in depth.

2) Esophageal lesions

Esophageal lesions from electroporation energy were characterized by myocyte and interstitial damage practically identical to the cardiac lesions. The lesions were restricted to the muscle layer; the luminal epithelial layer and the delicate Lamina muscularis mucosae –a small rim of smooth muscle cells beneath the epithelial basal layer- were left without pathological changes. In contrast, the RF ablations compressed the esophageal wall and destroyed the epithelial and muscular layers, and the adventitia.

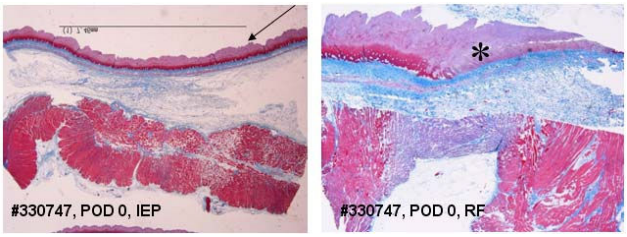


Fig. 8: Comparison of esophageal lesions induced by electroporation ablation (left) and RF ablation (right). Note the intactness of the epithelial layer (arrow) when IEP was applied. The same layer is necrotic after RF energy delivery (*).

C. Arcing

During ablation with the clamp device, we often experienced arcing, which limited the full delivery of power. These devices were initially fitted with mechanical compression limiting features near the tips of the jaws to act as closure stops to prevent the possibility of the electrodes touching and shorting together. It was noted that arcing often occurred near these stops so it was suspected that fluids on the stops were serving as a lower impedance path for arcs to follow. Later devices incorporated stops that were built into the proximal part of the jaws. This eliminated one source of arcing. However, due to continued arcing issues, most ablations performed using the clamp device used sections of thin walled silicone tubing to cover any exposed electrode during energy deliveries in an attempt to prevent arcing. Arcs sometimes followed a path through the interface between the edge of the silicone tubing and the tissue.

D. Study Limitations

During this initial feasibility study, we used various energy settings and several different types of electrodes, creating challenges for comparison of ablation results. Moreover, the use of two different types of generators hampered the fair comparison of lesions even further. Nevertheless, this study shows that it is possible to apply the electroporative ablation modality to cardiac tissue.

IV. Conclusion

In this study we successfully developed devices and a system for use in ablation using an electroporation mechanism. Acute animal studies indicated that the lesions created using electroporation were morphologically distinct from RF-induced lesions. High transmurality success rates suggest the possibility of using this new ablation modality in cardiac applications. In addition, the esophageal ablation results showing targeted muscle cell necrosis while keeping the epithelial surfaces is promising for prevention of serious complications during posterior left atrial ablations.
A long term study confirming lesion durability needs to be completed as a follow-up to warrant the successful adoption of this technique.

Acknowledgment

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