International Journal of Biomedical Science and Engineering
2022; 10(1): 1-11
http://www.sciencepublishinggroup.com/j/ijbse
doi: 10.11648/j.ijbse.20221001.11
ISSN: 2376-7227 (Print); ISSN: 2376-7235 (Online)

An In-vitro Method for Current Induced Ventricular Fibrillations

Michael Koch[1,*], Michael Bartonek[1], Jiri Silny[2], David Santer[3], Bruno Podesser[3,4]

[1]Eaton Industries, Vienna, Austria
[2]Femu, Rheinisch-Westfälische Technische Hochschule Aachen, Aachen, Germany
[3]Ludwig Boltzmann Institute for Cardiovascular Research, Ludwig Boltzmann Gesellschaft, Vienna, Austria
[4]Department of Biomedical Research, Medical University of Vienna, Vienna, Austria

Email address:
MichaelKoch@eaton.com (M. Koch)
[*]Corresponding author

To cite this article:
Michael Koch, Michael Bartonek, Jiri Silny, David Santer, Bruno Podesser. An In-vitro Method for Current Induced Ventricular Fibrillations. *International Journal of Biomedical Science and Engineering*. Vol. 10, No. 1, 2022, pp. 1-11. doi: 10.11648/j.ijbse.20221001.11

Received: November 29, 2021; **Accepted:** January 10, 2022; **Published:** January 18, 2022

Abstract: The motivation of the current study was to provide threshold values of rabbit hearts for ventricular fibrillation (vf) induced by electric current of 50Hz and to develop a flexible but robust experimental setup for stimulation experiments on the beating heart. The stimulation was performed in a way that the current flow through the heart is nearly homogeneous, similar to the situation of an electrical accident. In this way the work should also serve as a basis for future vf-related investigations, e.g. sine waves from 10Hz up to 10kHz, mixed signals, current pulses and pure DC. One of the main objectives was to avoid deviations of the data due to inappropriate experimental methods, such like direct contact of the heart tissue or interferences from the nervous system which one get from whole animal experiments. The work should additionally give the proper setup to gather the necessary data for transformations of animal data to human data. First an experimental procedure and setup for hearts of small animals that fulfills most important requirements for stimulation of the myocardium and the measurement of various heart parameters in a repeatable way was developed. In this way specific stimulation experiments were performed on rabbit hearts in an ejecting, blood-perfused isolated heart model to determine the threshold values for vf at 50Hz. Additional experiments to determine the electrical field inside and outside of the heart as well as the dependency of different stimuli modes (T-wave trigger, stimulation for several periods) have been conducted. In the verification with a frequency of 50Hz, current density of (7,3± 3,8)mA/cm^2 results as a mean threshold of ventricular fibrillation from 143 experiments. Finally a comparison to other research works in this field was performed to show the advantages and disadvantages of the respective approaches.

Keywords: Ventricular Fibrillation, Isolated Heart Model, Vf-threshold, Rabbit Hearts, Experimental Setup

1. Introduction

Ventricular fibrillation (vf) due to electric currents is one of the most dangerous fault scenarios, which can occur in an electrical distribution environment. Residual current devices (RCD) have been successfully introduced to the market for many decades to protect humans from such harmful events. One of the big issues in this field is related to the specification of the current-threshold for ventricular fibrillation at different harmonic frequencies and for arbitrary signals. Different aspects of ventricular fibrillation are research objectives of numerous studies in the last century. Those investigations of the threshold of vf used animals with different size, anatomy and physiology (e.g calfs, sheeps, pigs, dogs, rabbits, rats or guinea pigs) by In vitro or In situ approaches. In In vivo studies as described by Geddes, Ferris, Kouwenhoven, Kiselev, Dalziel, Knickerbocker and Jacobsen [8], [9], [12], [13], [14], [15], [16] a current of predominantly 50 or 60 Hz is injected between the fore and hind legs, the current at the beginning of fibrillation is defined as the threshold. In situ and In vitro experiments as described by Sugimoto, Knickerbocker, Roy, Hohnloser and Antoni [10], [15], [17], [18], [2] the current is directly applied into the heart by means of electrodes of different size and position. Therefore, the

current in the heart shows a very inhomogeneous distributions; the measured threshold depends on the arrangement and the size of the electrodes. Therefore, the reported thresholds of ventricular fibrillation, given in the literature, can only be hardly compared. Moreover the question arise, to what extend the individual thresholds correspond to a worst case situation.

The benefit of this project is the determination of stimulation thresholds and ventricular fibrillation at frequencies from 10Hz up to 10kHz in a homogeneous electric field without direct contact of the heart of a big number of animals (100 rabbits). Although the amount of electronics increased in the past 20 years, the research in this field decreased and so there are very less activity in this field. This also emphasize the importance for advanced investigations of the effects of current onto the human over a wide range of frequencies and also for different signals related to nowadays power-electronics driven signals.

The main objective of our research work is to develop a flexible experimental system for a working rabbit heart, with respect to electrical stimulation where we can, at a first step, investigate the vft from 10Hz up to 10kHz in a very reproducible way. Furthermore, the setup should provide a quasi-homogeneous electrical field which stimulates the heat without direct contact between electrodes and the heart, as in a real accidental situation. Additionally, we show the vft results from 100 rabbit heart over the mentioned frequency range. In a later work which is not part of this report, we work on the transformation of the data to the human heart and additionally on a whole body (finite difference) simulation to readjust a real accidental situation. To prepare this it is also necessary to determine the current amount of current which flows through the heart (and the residual amount which flows beside the heart in the supplement blood solution in which the heart works), which we have done by additional measurements as shown later. An additional important task for the later conversion to the human is the determination of threshold values with respect to the heart stimulation (this are data which can be used to compare to the human situation, due to the fact that these data are available from the human).

For the experiments rabbit hearts are used. The reason for this choice was the similarity of the rabbit heart from an electrophysiological point of view to the human one. It has a coronary topology similar to the human one as described by Galinanes [26] and also important properties regarding the electrical behavior which are relevant for the cardiac action potential properties as the ion currents and intracellular ion concentrations described by Nerbonne, Valentin and Hondeghem [27], [28], [29]. Furthermore, there are investigations which shows the similarity to the human heart with respect to contractile and diastolic behavior as written by Jung [30] and the mechano-electrical coupling as described by Quinn [31]. Additionally, the rabbit heart is closely related to the human heart regarding the so-called 'relative-size' as described by Panfilov [3]. In the paper from Panescu and Kroll [32] there was made a broad literature study about the complexity of the use of animal models for human safety consideration. The outcome also emphasizes the use of rabbit hearts. The basic system for the experiments was an working heart model which used an erythrocyte-enriched Krebs-Henseleit (KH) buffer as described by Podesser [6] and provides exellent oxygenation, leading to superior hemodynamic and metabolic performance.

The threshold of vf can only be investigated in animals. General applicable results can be gain In vitro investigations on perfused isolated hearts. The Working heart- and Langendorff - perfused rabbit heart meets the requirements for our target well and is one of the most studied models for vf as described by Panfilov [3]. The excitability and contractibility of the isolated heart should remain stable during the investigation. For this reason the ventricle pressure, the atrial flow, the aortic flow and the ECC are continuously monitored and stimulation thresholds are repeatedly measured during each experiment by application of electric pulses of different duration. Furthermore the application of current for stimulation and release of vf should take in to account worst case conditions.

Studies from Furman, Chen and Roy [20], [21], [22] have shown, that the thresholds of stimulation and vf are affected by the size of electrodes on the heart. Consequently a homogeneous current flow over the heart (where the electrode diameter is bigger than the heart diameter and the electrodes have no direct contact to the myocardium (Figure 2) should result in thresholds which reflects the situation of an electrical accident in the best way. It is clear that the current distribution could not be homogeneous in the heart, due to the anatomy of the organ. Moreover, statistics about electrical accidents as described by Kieback (e.g. [23]) demonstrate a highest lethality rate for current flow between hands and foots. Therefore, a longitudinal orientation of the current in the heart was implemented in this study. The experimental set-up allowed an application of currents of different shapes, amplitudes and frequencies.

Basic simulations on a rectangular myocardial tissue have been performed to gain a qualitative picture of the excitation pattern in the case of reentry processes. A monodomain model (diffusion-reaction system) with intracellular FitzHugh-Nagumo (FHN) coupling was implemented in the following manner: the stimulation variable $u(x,t)$ was calculated by finite difference method for 2'nd order spatial derivatives and $v(x,t)$ by explicit Euler integration. Model parameters were taken from the papers of Rogers and Pertsov [4], [5]. In this way we were able to investigate the situation at different kind of electric stimulations.

$$\frac{\partial u(x,t)}{\partial t} = (c_1 u(x,t)(u(x,t)-a)(1-u(x,t)) - c_2 u(x,t)v(x,t) + i_{ext} \quad (1)$$

$$\frac{\partial v(x,t)}{\partial t} = b\,((u(x,t) - d\,v(x,t)) \quad (2)$$

with von-Neumann boundary condition

$$\frac{\partial u(x)}{\partial n} = g(x), g(x) = 0\ \forall x \in \partial\Omega \quad (3)$$

and numerical stability condition (h...grid size, C... constant from FHN parameters) $dt \leq \frac{h^2}{2C}$.

In Figure 1 a simulation result on the action potential of a rectangular piece of myocardial tissue is displayed. The black area shows the repolarized tissue, the yellow parts the action potential wave fronts and the red parts exhibit the tissues in the refractory condition. Regular action potential waves (i.e. the regular heart beat) propagate as a vertical line from left to right over time. During this process a longitudinal current stimulus was applied for a short time duration (some ms), representing an additional disturbing stimulus. In this figure 1 can see the resulting splitting of waves of the action potential and some reentry waves, which sustain this non-synchronous behavior of the modeled myocard tissue. From the resulting patterns for vf one can refer to the concept of an effective heart size, which shows a close relation of the rabbit and the human heart with respect to the patterns of vf as described by Panfilov [3].

Figure 1. *Resulting pattern of a longitudial current stimulation.*

2. Rabbit Hearts and Their Preparation

Adult male inbred White New Zealand rabbits with an average weight of $(2905 \pm 260)g$ (means$\pm$ STD) and $(8,3 \pm 0,7)g$ heart weight (wet condition) were used in the experiments. All experimental animal studies were approved by the Ethical Committee, Medical University of Vienna, and the Ministry of Science, Republic of Austria. Animals are cared for in accordance with the 'Guide for the Care and use of Laboratory Animals'.

2.1. Working-heart Model

For the experiments a dedicated working heart model with an erythrocyte-enriched Krebs-Henseleit (KH) buffer (pH: $7,4 \pm 0,04$ at $37,5°C$) was used. The composition of the Krebs buffer (in mmol per liter) was as follows: NaCl 118; KCl 4,7; $CaCl_2$ 2,5; $MgSO_4$ 1,2; NaEDTA 0,5; $NaHCO_3$ 25; and glucose 11,1. Additional 2,5 IU/l insulin and $2g/l$ bovine albumin were added to prevent edema. This buffer was also the base for the erythrocyte suspension. Bovine blood was taken from the jugular vein. Thereafter, erythrocyte concentrates were prepared by washing blood four times 10 min with physiological NaCl at 3000 rounds/min. By this procedure most of the leuko- and thrombocytes as well as the serum and the heparin were washed out. Finally, $0{,}2mg/dl$ gentamycin were added to prevent bacterial growth. On the very day the experiment was conducted, the concentrate was resuspended and washed three times with oxygenated KH-buffer before use. The final hematocrit value was 30%. The isolated heart system used is a modified working heart model Type 830K (H. Sachs Elektronik). The oxygenation of the erythrocyte suspension was provided by a dialyzer (Centrysystem 400HG, Cobe Laboratories, Inc, USA) using 20% O_2, 75% N_2, and 5% CO_2 for the erythrocyte suspension and 95% O_2 and 5% CO_2 for the crystalloid perfusate. The oxygenated suspension was transported via a filter (Pall-Ultipor-Blood Transfusion Filter 40 μm, Pall Biomedizin) by a light-barrier-controlled hose pump to the reservoir. Erythrocyte suspension was recirculated with a total volume of 280ml. Coronary effuse was collected from a tube that was inserted into the pulmonary artery. For further information about the working heart model see Podesser [6], [7].

2.2. Experimental Frame

To achieve reproducible conditions a frame system which includes the heart with the buffer solution, was developed. The inner setup one can see in Figure 2. The stimulus electrodes (copper), used to apply the electric current signal and to deliver the defibrillation pulse, were positioned above and below the heart within the frame. Embedded into this experimental frame, an electric field sensor (green), which measured the voltage (1V/cm), was inserted. The ECG electrodes (light blue) were mounted on one of the cannulas (yellow and dark blue) and had always the same distance to each other. A temperature sensor, which was used to control the temperature at the beginning of the experiment $(37,5 \pm 0,5)°C$ measured the solutions temperature. To improve the temperature stability, the frame has a double wall design, filled with water of controlled temperature.

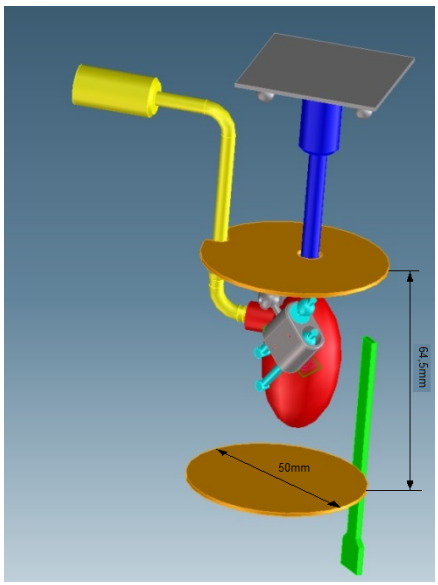

Figure 2. *Position of the sensors and the stimulation electrodes.*

3. Experimental Setup and Measuring Procedure

3.1. Experimental Setup

The main part of the experimental set-up is a PXI-System from National Instruments. Electric current for stimulations and defibrillations of the hearts are injected between two circular copper electrodes (orange in Figure 2) positioned more than 2cm above and below the heart. For monitoring and data collection a LabView program was developed which monitors the ECG-, left ventricular pressure- and left aortic flow signal, furthermore the temperature, the stimulus current signal, the electrode-voltage, the electrical field sensor signal and a trigger signal which details the start of the stimulation within the ECG. To follow the left ventricular pressure a micro-tip catheter (Millar SPR-407, Millar, USA) is inserted through the aorta inside of the left ventricle and measured by a Hellige measuring system, connected to the PXI system. The aortic flow is sensed at the connection from the working heart model to the heart by an electromagnetic flow probe (flowmeter Narcomatic RT-500, Narco-Biosystems, USA) and feed into a transit time flow meter module (TTFM 700) from Harvard Apparatus (HA) which is connected to the PXI system.

The ECG is captured by Ag-AgCl-electrodes, which are mechanical connected at the inflow cannula and positioned by a fixed frame on the heart surface. The signal is feed into an EGC amplifier (ECGA, Harvard Apparatus) and a R-Wave Trigger module (RWT, Harvard Apparatus). The output of the R-wave trigger is feed into a pulse delay system (TCP110 from TTi), capable of shifting the (delayed) pulse over the whole EGC-trace. The TTL signal output is used as a PFI0-interrupt signal for the function generator card in the PXI system to generate a trigger pulse for the start of the stimulus, enabling a stimulus to start at any point in the time along the ECG signal (e.g. at the T-wave). For the measurement of the electric field an isolated PCB with two conductive pads at a distance of 10mm for an easy access to the electric field vector is deployed. This signal is feed directly into the PXI system by a DAQ card (NI PXI-6251). The electric current flowing through experimental frame with the heart inside is measured by a Tektronix current sensor (TCPA300, TPC312) and also feed into the PXI system. The temperature is measured by a Fluke temperature module (80TK) connected to the PXI-system. The heart is stimulated by an arbitrary function generator module (NI PXI-5441). The start is triggered by the pre-adjusted trigger signal (as mentioned above) and is amplified by a current amplifier (PA2202X from Rohrer, Germany). To prevent DC-offsets an adjustable DC-control in the software for the stimulus and also a hardware offset in the amplifier are used. This offset is checked throughout the whole experiment. For higher frequencies ($> 1kHz$) an additional current amplifier (Hero Power PFL2250, $150V_{eff}/20A_{eff}/150kHz_{3dB}$) is used.

For the defibrillation a simple monophasic defibrillator (RC circuit, max 30J) module is connected to the stimulus electrodes. The stimulus is generated by two LabView applications which generates threshold pulses and sine wave stimuli triggered by the delayed R-wave trigger signal. Signal processing and statistical analysis are done with Matlab.

3.2. Testing Procedure

3.2.1. Heart Preparation and System Setup

Before excising the heart, the perfusion system was filled with the buffer solution and tempered at $37,5°C$. After this, a conductivity measurement of the buffer solution was performed at 1kHz, 100mA (again at the end of the experimental procedure) and also the output of the field sensors. This was done by the internal sensor in the frame and also by an external sensor, to check the proper functionality. All rabbits were anesthetized using 5mg/kg of Ketamine-HCI and 20mg/kg Propofol via the ear vein. Heparin (300IU/kg) was administered to prevent coagulation. The beating heart was quickly excised (weight, diameter and perimeter were determined) and immediately fixed on the already perfused aortic cannula of the perfusion apparatus (see Figure 3). Hearts were beating spontaneously and did not need any pacing during the experiment. The diameter and extend of each heart (in the transversal plane) is measured and listed. After the hearts were introduced they stayed for 15 minutes in Langendorff mode. During this time left-ventricular pressure, aortic flow, coronary flow and heart beat were measured each 5 min. Afterward the system was switched to working heart mode and a blood gas analysis was performed. During this time the different vital parameter were again documented each 5 min. After finishing the system setup (ECG electrodes, pressure sensor and stimulus electrodes are placed) vital parameters were captured each 15 min. Furthermore the coronary flow (ml/min) was calculated by subtracting measured aortic flow from left atrial flow. If these parameters were within the expected range (i.e. temperature $37,5°C$, heart beat >150 b/min, atrial flow >90 ml/min), the experiments were started.

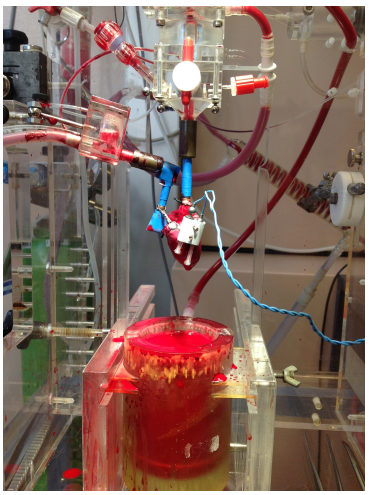

Figure 3. *Mounted heart in the Working Heart Model.*

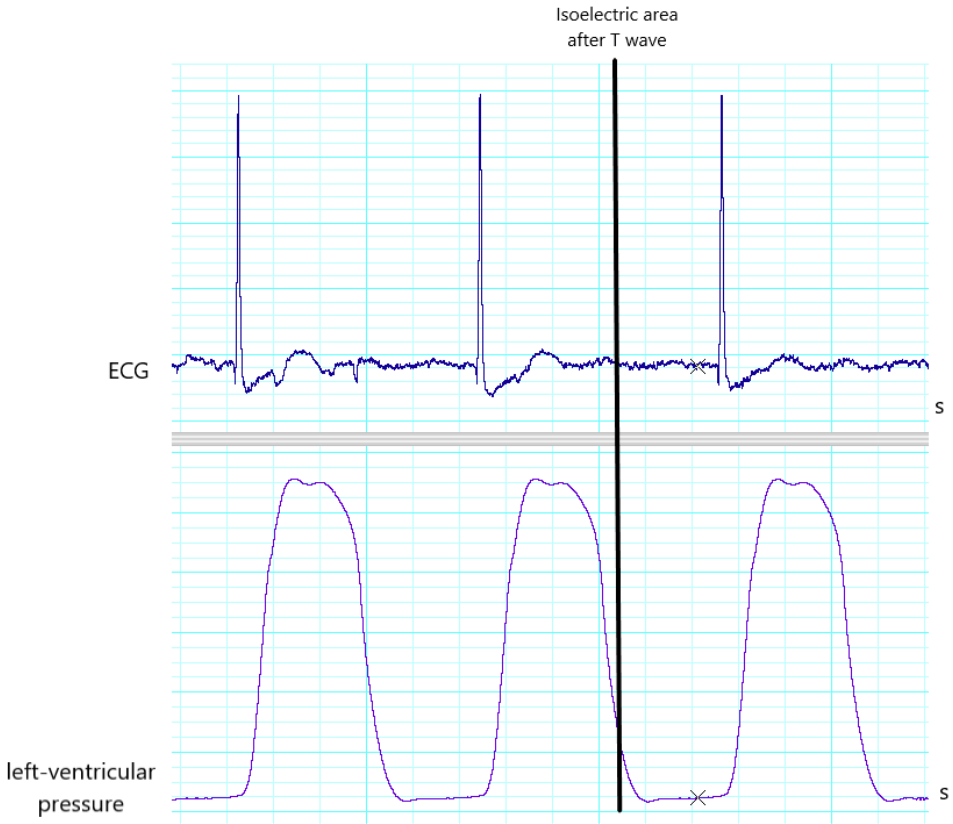

Figure 4. *Stimulation in the isoelectric phase after T-wave.*

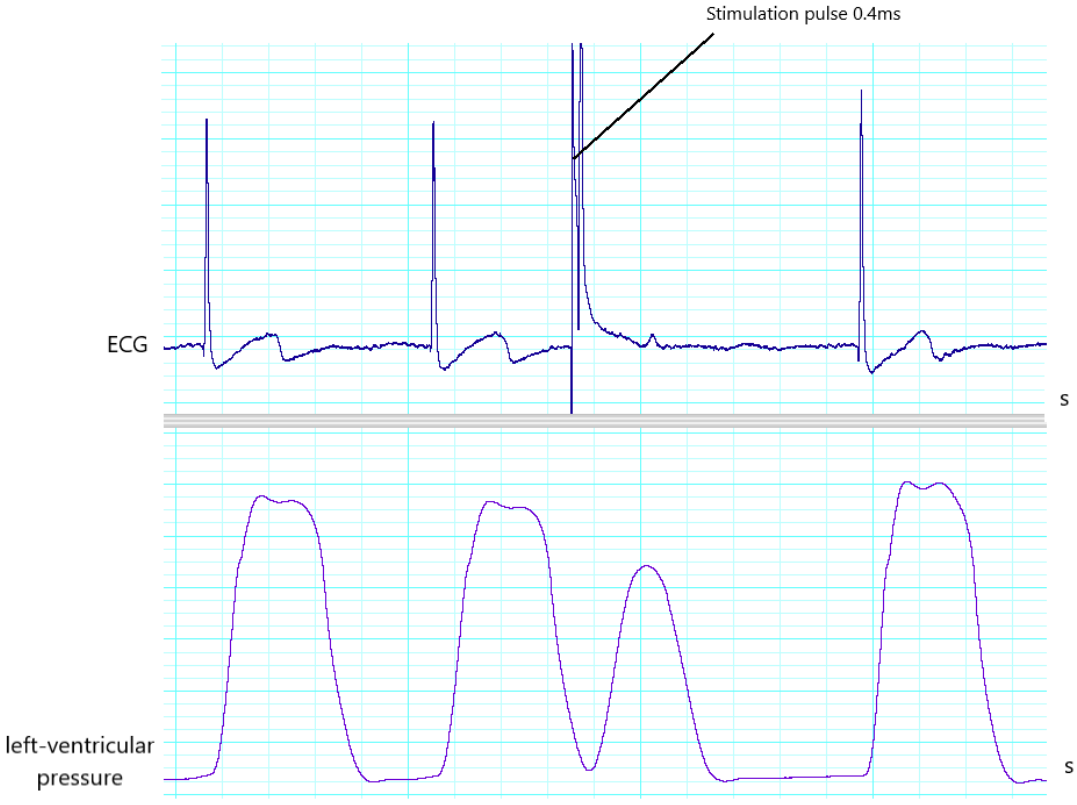

Figure 5. *Stimulated contraction.*

These measurements served as a calibration for the heart excitability. Furthermore these experimental results were used for the conversion of the measured fibrillation values at rabbit heart level to the human heart (this is the motivation for these explicit measurements but the translation to humans is not part of this paper), due to the knowledge of these values at the human from pacemaker therapy. The experiments started with a series of rectangular pulses (with durations of 0.2ms, 0.4ms and 0.8ms resp.) placed between the end of the T-wave and the begin of the P-wave to determine the stimulation thresholds (see Figure 4). This is done by the help of the relation between the ECG and the left ventricular pressure signal (see also the well known Wiggers diagram). We trigger to the R-wave of the ECG and release a delayed trigger signal to the input trigger of the pulse generator. The delay time is determined at the beginning of the experiment and depends on the specific properties of the heart under test. Due to the continuous monitoring of the ECG and left ventricular pressure signal we can avoid a misinterpretation of the result, like a to early trigger event or a stimulation into the T-wave.

The amplitude would be increased until a stimulated contraction occurred, sensed very well by the pressure signal in the left ventricular (see Figure 5). This is the so-called stimulus threshold value. It is known that the Rheobase of the atria is higher than that of the ventricle. If we increase the amplitude until a reaction occurs it is most probable that we trigger a contraction of the left ventricle. The observation of the left ventricular pressure signal shows also the contraction of the whole myocardium, therefore we can also exclude muscle twitches. Furthermore the vital parameters are also monitored and stored. From these measurements we get a strength-duration relationship as described by Lapicque [25] for the excitability of the myocard.

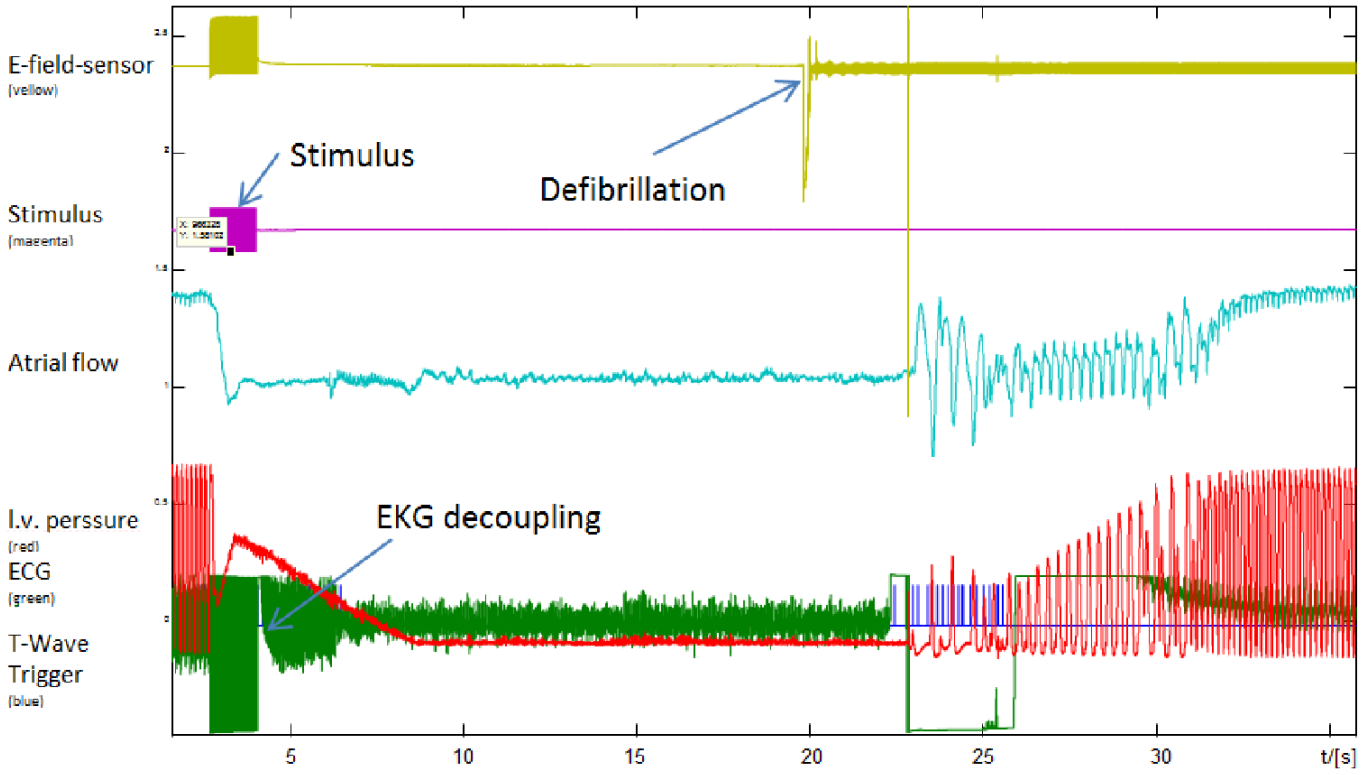

Figure 6. *Representative experimental tracing from stimulation to defibrillation*

3.2.2. Fibrillation and Defibrillation Process

The vf experiments always started at 50Hz, followed by the predefined frequencies according to the test protocol. Finally the 50Hz experiment was repeated, followed by a measurement of the stimulus threshold values. To determine the vf threshold a current of a distinct frequency and amplitude was applied (before the P-wave) for 3 seconds, followed by a pause of 20-90 seconds. If no vf was detected the amplitude was increased by 3mA in the next stimulation step. If vf occurred, two cases had to be distinguished. First sustained vf (if vf lasts for more than 5 seconds; this value was derived from the fact that an ICD would fire if the device detects failing ECG cycles for more than 10 ECG periods) or vf with spontaneous remission. If vf was detected the defibrillator was connected to the stimulus electrodes and a shock-signal was applied. Usually 1-3 shocks were necessary to terminate the vf. After a period of approx. 5 minutes, if all the parameters were in the expected range, the fibrillation experiment started again. In a standard experimental plan 5-7 vf experiments were performed on a single rabbit. The specific condition whether the experiments were continued was the shape of the ECG, the atrial flow, the ventricular pressure and also the calculated

coronary flow. In Figure 6 the upper (yellow) trace shows the output of the electrical field sensor. The second (magenta) trace shows the stimulus signal. The next trace is the atrial flow signal. Below one can see the left ventricular pressure signal (red), which is one of the most important signals because it also delivers information during the current stimulation and is also strongly related to the ECG signal (below, green line). The blue trace shows the T-wave trigger signal, to check that the stimulation was started at the intended moment. In the figure we see a complete stimulation experiment. At the beginning of the timeline, there is a normal heart action followed by a 3 second electric current stimulation. During this time vf is initiated. One can see especially the effect on the pressure signal which decreases due to the vf. After a certain time the defibrillation pulse was set which can be seen very well on the E-field sensor signal. The small spike after the defibrillation pulse originates from the recoupling of the ECG- and stimulation-source as an EMC interference. Usually it takes a short time (e.g. 2s) before the heartbeat starts again and the full heart action is restored.

3.2.3. Intra-/extracardial Measurements

One of the major challenges in the experiments was the determination of the effective electric current-flow through the rabbit heart. To assess the electrical field inside the rabbit heart, a field sensor, equal to the extracardial electrical field sensor which was embedded into the experimental frame, was used. The setup was changed to the Langendorff mode and the field sensor was inserted into the right ventricle. A current of 100mA (in addition 10mA and 50mA) at frequencies ranging from 10Hz to 100kHz were applied to the heart. The electric field was determined by measuring the voltage of the sensors (intra- and also extracardial). In addition the measured voltage at the stimulation electrodes was used to determine the range of the conductivity of the experimental setting. For the different effective current calculations the conductivity of the experimental setup was measured (buffer solution inside the frame and also the buffer solution within the heart in the frame). This was done by applying a current onto the stimulus electrodes and voltage measurements. Especially the bilayer effect on the electrode-electrolyte interface was taken into account.

The reason for doing this in Langendorff mode was the kind of the mechanical implementation for the working heart mode. In Langendorff mode the field sensor can be inserted into the right ventricle. One of the variabilities in this setting was the fact that only the left ventricle was working, whereas the right ventricle was not as well supplied with blood compared to the left one. Another problem was the natural movement of the heart and the fact that the inside of the frame is optically not accessible during the measurements which led to some variations with respect to the sensor position. Due to the different amount of blood in the ventricle there was a difference between the systolic and diastolic state of the heart. For these reasons 10 dedicated experiments of this kind have been conducted to get a more accurate picture of the situation, together with additional different experimental settings for the determination of the effective current density inside the heart.

3.2.4. Statistics

Results were given with additional mean and SD. All statistical tests are done in MATLAB.

4. Results and Discussion

4.1. Results for 50Hz Stimulation

One of the studies key result is the validity of the experimental setup especially for 50 Hz, which is capable to systematically monitor and generate reproducing and reasonable data as we could show in several vf experiments. A further benefit is the high degree of flexibility due to the concept of an adjustable frame and a very flexible software model. Because of this concept modifications are not only quite quickly applied but also very robust in their results and reproducibility.

In the following we list the raw data (only extreme outliers are removed) of the performed experiments (heart in a frame with support solution as described above). Further calculation for the heart values (where we rule out the additional effects due to the experimental setup) are part of ongoing work and would go beyond the scope of this paper. Stimulation thresholds of the heart, are measured in 70 rabbit hearts and 143 experiments.

The ventricular fibrillation thresholds are measured with 50Hz sinusoidal current packets of a duration of 3s. They are determined in 70 isolated rabbit hearts and 116 experiments. The mean fibrillation thresholds (see Table 5) have a factor of 2,24 results for the ratio between the current density for vf and for stimulation threshold of the myocard with 0,8 ms cathodic pulses. Due to the fact that these values are for the whole experimental setting (frame with the heart inside, $A = 20cm^2$) it does not make sense to calculate current density values, in contrast to the pure heart values later.

Table 1. *Stimulation thresholds in [mean, SD].*

Pulse duration $[ms]$	**0,2**	**0,4**	**0,8**
Current $[mA]$	235,68 $\pm$ 51,67	150,6 $\pm$ 31,92	99 $\pm$ 21

Table 2. *Ventricular fibrillation thresholds in [mean, SD] (median).*

Stimulus (sine, 50Hz)	**3$[s]$**
Current $[mA]$	198,5 $\pm$ 69,7

To get more to the direction of the pure heart values of current and current density we made measurements which showed that the values of the electrical fields in- and outside the heart (E_{in} and E_{ex}) are in a similar range (Table 3). This was done by placing an electric field sensor inside the right ventricle to measure the internl electrical field in the heart and one electrical field sensor outside of the heart (the sensor was

placed at the frame wall in the same height like the internal sensor, which is shown in Figure 2.

It is also reasonable that due to the different situations during systole and diastole (different amount of blood in the ventricles) there are differences in the values of the electrical field inside the heart. This should be further investigated by additional measurements and an improved setup (e.g. for polarized impedance on the stimulation electrodes). Nevertheless it reflects the situation in a real (living) heart.

Table 3. *Intra-extracardial measurements at 50Hz.*

Nr.	**56**	**57**	**58**	**59**	**62**	**mean**	**SD**
E_{in}/E_{ex}	1,114	1,285	1,185	1,013	1,21	1,192	0,028

From this and the determination that the average transversal area of the rabbit hearts is $(5.47 + / - 0.75)cm^2$, we get the stimulation and vf thresholds given as heart current resp. current density in Tables 4 and 5. It should be mentioned that we give an averaged density value over the whole heart although the current densities in the heart are not homogeneous. We do this to compare our values with other ones. It should serve as a guideline to evaluate our results.

Table 4. *Stimulation thresholds in [mean, SD].*

Pulse duration [ms]	**0,2**	**0,4**	**0,8**
Current [mA]	43,54 $\pm$ 9,8	26,4 $\pm$ 6	18,6 $\pm$ 4
Current density [mA/cm^2]	7,96 $\pm$ 1,8	4,83 $\pm$ 1,1	3,4 $\pm$ 0,74

Table 5. *Ventricular fibrillation thresholds in [mean, SD].*

Stimulus (sine, 50Hz))	**3[s]**
Current [mA]	40 $\pm$ 20
Current density [mA/cm^2]	7,3 $\pm$ 3,8

Corresponding reports from Furman, Chen and Roy [20], [21], [22] have shown a decrease of the stimulation threshold resp. vf thresholds in the heart after application of current stimulation via electrodes with lager surface. Similar behavior is confirmed by Chen [21] for the fibrillation threshold in dogs.

Thus, the application of an approximately homogeneously distributed current over the whole experimental frame in which the heart acts promise us to determine the lowest thresholds for stimulation and vf in our experiments. Because of the knowledge of the total current in the heart (due to the intra-extracardial measurements) and the size of cross-section through individual rabbit hearts, perpendicular to the current flow, we have calculated a mean current density within the rabbit hearts. In the literature, the minimal stimulation thresholds are given with $2,3mA/cm^2$ (2ms pulse via $0,87cm^2$ catheter electrode in right ventricle) by [20] and $1,8mA/cm^2$ (2ms pulses, $0,28cm^2$ implanted myocardium electrode) by [21]. These values are well comparable with a stimulation threshold of $3,26mA/cm^2$ for 0,8ms pulses, resulting from this study.

According to Roy [24] result from pig experiments under application of 60Hz current (for 5s) via disk electrodes (area for $5cm^2$) which are attached on the chest (sagittal direction of the current vector) vf thresholds occurred at $15\pm1,4mA/cm^2$. Our result of $7,3mA/cm^2$ at 50Hz current fit very well due to the fact that we have a longitudial current vector and which is also in contrast to Roy [24] not outside the body (no skin, lungs, etc in between). Also this situation reflects the problem of comparison due to different experimental methods.

A further possibility to compare our results with literature offers the ratio between the vf threshold at 50Hz current and stimulation threshold of the heart with cathodic pulses. Sugimoto [10] calculated a ratio of 1,1 (5s 50Hz and 10ms pulse) and Hohnloser [18] presented a relation of 2,0 (1s 50Hz and 1s pulse) for this ratio. Our investigations yield (3s 50Hz and 0,8ms pulse) a factor of 2,24, which coincide well with the available literature. Previous literature offers only little possibility for the verification of our results, due to the big differences in the current flow distributions in the heart and individual experiments.

If we compare our results to the famous papers from Geddes and Ferris [8, 9], where in experiments on different animals (in contrast to our experiments as an whole body experiment; 15 rabbits) also a longitudinal current vector over the heart was performed, we get the values:

Table 6. *Comparison of ventricular fibrillation thresholds.*

Author	**Current path**	**Current [mA_{RMS}]**	**duration [s]**	**Frequency [Hz]**
Our results	longitudial	$\approx$ 40	3	50
Geddes	left fore leg - left hind leg	$\approx$ 30	5	60
Geddes	right fore leg - left hind leg	$\approx$ 40	5	60
Ferris	right fore leg - left hind leg	$\approx$ 20-50	3	60

Out of this we also see that our experimental result are compareable. This is important dur to the fact, that the experimental method fit and can extended now to frequencies $\neq 50Hz$.

4.2. Differences to Other Experiments

Experiments on isolated hearts, where the electrodes are sewed onto the heart tissue or where the electrodes have a direct galvanic contact (often endocardial catheters are

*used)*as described by Sugimoto, Lubbe, Roy and Hohnloser [10, 11, 17, 18]. Here we have an impact of the heart tissue and additionally a direct galvanic contact of the stimulus electrodes onto the heart, which leads to virtual electrode effects of the myocardium and a quite inhomogeneous field flow.

Experiments on isolated hearts, where the electrodes are sewed on the heart tissue and the heart itself was modified (e.g. distraction of the atria or the sine node was eliminated) as described by Antoni [2]. We have again virtual electrode effects and a modified (injured) heart.

Whole body experiments. In the work from Geddes, Knickerbocker, Jacobsen and Roy [8], [15], [16], [24] we have three additional effects to consider. First, we can not measure only the effects on the heart, because the body and especially the brainstem (vagus nerve) can influence the heartbeat. Second, the heart values are not available and so we have especially for the conversion to the human situation to do a recalculation from the whole body to the heart and afterwards to the human situation, so the calculation path is longer and therefore we have an additional source of inaccuracy. Third, we have to sustain an anesthesia for the time of the experiments. The necessary substances have usually an effect on the circulatory system.

Furthermore it is important to use a heart which is big enough to sustain vf and that the action potential, especially the refractory phase, is similar to the human situation. Also the cell structure (e.g. the potassium channels) should be comparable to the human and the anatomy (e.g. are the Purkinje fibers are transmutual or not) of the heart should be also taken into consideration. This was as mentioned before the reason for the use of rabbit hearts. An additional problem is that experiments are done often with only 5-10 animals, which is an also a source of inaccuracy due to the big scattering of measurements in biological systems. Most of the isolated heart experiment where done with a Langendorff model by the use of a Krebs-Henseleit solution, whereas our experiments are done with the more realistic working heart model and use of an erythrocyte enriched KH buffer solution which guarantees a better oxygen transport. So in our experiments we used 100 rabbits with an erythrocyte enriched KH buffer, where no direct galvanic contact to the myocardium and no manipulation or injury of the heard happened, furthermore there are no effects from anesthesia and also no virtual electrodes effects present.

5. Conclusion

In contrast to the existing view of vf-thresholds, which is a result of different experimental setups at different frequency ranges, different ways of stimulation, different methods of contacting the heart to the current and also different kind (often only a small amount) of animals, this work should be serve as a basis to get at the end a closed overview over the whole frequency range with respect to vf by a standardized method of a big amount of measurements. Because we have shown that the results in our setup correlate to famous past works, we can continue to further nowadays topics, which arises from the increasing amount of power-electronics in all areas from household applications and e-mobility to industrial plants. One of the major parts in the research work was the flexible setup, especially with respect to the heart frame and therefore to the opportunity to justify different (spatial) current vectors through the heart. Furthermore, due to the experimental process, we are also able to measure additional parameters such like stimulation thresholds to be able to improve the transformation of animal data to human data. An additional task is the improvement of simple but meaningful mathematical model and simulation models which can be realized by a 'hardware (heart)-in-the-loop' process, where theoretical (or simulation) results are proved by recurring working heart experiments under the different boundary conditions. Due to the experimental procedure and the setup its is possible to do a huge amount of experiments which also improves the statistical expressiveness of the measured data.

Furthermore due to the fact that we are able to measure several heart parameters (such like aortic flow, atrial flow, left ventricular pressure, coronary flow, electric fields inside and outside of the heart, electric heart activity (ECG) at different locations) during a current stimulation (with waveforms from DC up to 100kHz, mixed signals, pulses and current signals which are measured on an e.g. power-electronic circuit, with different current vectors in the heart) at different conditions (temperature, influence of drugs, changing oxygen density, different mechanical heart loads), we believe that this setup can provide deeper insights to electrophysiological processes, especially in the context of accidental current effects on the heart.

Acknowledgements

The project was funded by the FFG (Oesterreichische Forschungsfoerderungsgesellschaft). Project Number 841285. The authors would like to thank Milat Inci from LBI for his strenuous efforts always to meet our requirements and for his conscientious work at the experiments.

References

[1] Biegelmeier, G, Lee, W. R., *New considerations of ventricular fibrillation for a. c. shocks at 50-60Hz*, IEE Proc. Vol. 127 (1980) Nr. 2 S103-110.

[2] Weirich J, Hohnloser S, Antoni H., *Factors determining the susceptibility of the isolated guinea pig heart to ventricular fibrillation induced by sinusoidal alternating current at frequencies from 1 to 1000 Hz.*, Basic Res Cardiol. 1983 Nov-Dec; 78 (6): 604-16.

[3] Alexander V. Panfilov, *Is heart size a factor in ventricular fibrillation? Or how close are rabbit and human hearts?* Heart Rhythm 3 (7): 862-4 Â· August 2006.

[4] Rogers, *A collocation-Galerkin finite element model of cardiac action potential propagation*.

[5] Pertsov, *Spiral waves of excitation underlie reentrant activity in isolated cardiac muscle*.

[6] B. Podesser et al., *The erythrocyte-perfused "working heart" model: Hemodynamic and metabolic performance in comparison to crystalloid perfused hearts*, J Pharmacol Toxicol 41 (1999) 9-15.

[7] B. Podesser et al., *New developments in the isolated working heart: a comparison of neonatal, immature, and adult rabbits after sixty minutes of ischemia in respect to hemodynamic and biochemical parameters.*, J Pharmacol Toxicol 1993 Dec; 30 (4): 189-96.

[8] Geddes LA, Cabler P, Moore AG, Rosborough J, Tacker WA.*Threshold 60-Hz current required for ventricular fibrillation in subjects of various body weights*, IEEE Trans Biomed Eng. 1973 Nov; 20 (6): 465-8.

[9] Ferris, L. P., King, B. G., Spence, P. W. and Williams, H. B., *Effect of Electric Shock on the Heart*, Bell System Technical Journal, (1936) 15: 455-468. doi: 10.1002/j.1538-7305.1936.tb03562.x.

[10] Sugimoto, T., Schaal, S. F., Wallace, A. G, *Factors Determining Vulnerability to Ventricular Fibrillation Induced by 60-CPS Alternating Current*, Circulation Research, (1967) doi: 10.1161/01RES.21.5.601.

[11] Lubbe, W. F., Bricknell, O. L., and Marzagao, C., *Ventricular fibrillation threshold and vulnerable period in the isolated perfused rat heart*, Cardiovascular Research, (1975) 9: 613-620.

[12] Kouwenhoven, W. B., Knickerbocker, G. G., Chestnut, R. W., Milnor, W. R. and Sass, D. J. *Ac shocks of varying parameters affecting the heart*, AIEE Trans. Communication and Electronics, (May 1959), 78, 163-169.

[13] Kiselev, A. P. *Threshold values of safe current at mains frequency. Problems of electrical equipment, electrical supply and electrical measurements (in Russian)*, Sbornik MIIT, (1963), 171, 47-58.

[14] Dalziel, C. F. and Lee, W. R. *Reevaluation of lethal electric currents*, Transactions of Institute of Electrical and Electronics Engineers, (1968).

[15] Knickerbocker et al., *Fibrillating parameters of direct and alternating (20Hz) currents separately and in combination-an experimental study*, IEEE Trans. Comm. COM-21 (9): 1015-1027, (1973).

[16] Jacobsen et al. *Experimentelle Untersuchungen an Schweinen zur Frage der Mortalität durch sinusförmige, phasengeschnittene sowie gleichgerichtete elektrische Ströme*, Biomed. Technik 20: 99-107, (1975).

[17] Roy et al. *Intercardiac catheter fibrillation thresholds as a function of the duration of 60-Hz currents and electrode area*, IEEE Trans. Bioded. Eng. BME-24 (5): 430-435, (1977).

[18] Hohnloser et al. *Influence of direct current on the electrical activity on the heart and on its susceptibility to ventricular fibrillation*, Basic Res. Cardiol. 77: 237-249, (1982).

[19] Antoni *Pathophysiological basis of ventricular fibrillation*, In J. F. Bridges, G. L. Ford, I. A. Sherman and U. Vainberg (eds.), Comprehensive Human Physiology. Springer- Verlag, Berlin: 1825-1842, (1985).

[20] Furman et al. *Endocardial electrical threshold of human cardiac response as a function of electrode surface area*, Digest 7th Int. Conf. Med. and Biol. Eng. Stockholm, Sweden Aug. 14-19, p. 71., (1967).

[21] Chen et al. *Relationship between pacemaker fibrillation thresholds and electrode area*, Med. Instrum. 9 (4): 165-170, (1975).

[22] Roy *Summary of cardiac fibrillation thresholds for 60-Hz currents and voltages applied directly to the heart*, Med. Biol. Eng. Comput. 18: 657-659, (1980).

[23] Kieback *Stromunfälle, Herzkammerflimmern und Letalität: Statistische Auswertungen von Kenndaten zu Stromunfällen und ihrer Letalität zur Beurteilung des Grenzrisikos für das Auftreten von Herzkammerflimmern beim Menschen*, (2009).

[24] Roy et al. *60Hz ventricular fibrillation thresholds for large-surface-area electrodes*, Med. Biol. Eng. Comput. 24: 471-474, (1986).

[25] Lapicque L (1907), *J Physiol Pathol Generale 9: 567-578*, Figure 3.

[26] Galinanes, M., Hearse, D. J.,*Species differences in susceptibility to ischemic injury and responsiveness to myocardial protection.* Cardioscience 1990, 1 (2): 127-43.

[27] Nerbonne, J. M., *Molecular basis of functional voltage-gated K+ channel diversity in the mammalian myocardium.* J Physiol 2000, 525 2: 285-98.

[28] Valentin, J. P., Hoffmann, P., De Clerck, F., Hammond, T. G., Hondeghem, L., *Review of the predictive value of the Langendorff heart model (Screenit system) in assessing the proarrhythmic potential of drugs.* J Pharmacol Toxicol Methods 2004, 49: 171-181.

[29] Hondeghem, L. M., *Disturbances of cardiac wavelength and repolarization precede Torsade de Pointes and ventricular fibrillation in Langendorff perfused rabbit hearts.*Prog Biophys Mol Biol 2016; 121 (1): 3-10.

[30] Jung, B., Odening, K. E., Dall'Armellina, E., Foell, D., Menza, M., Markl, M., Schneider, J. E., *A quantitative comparison of regional myocardial motion in mice, rabbits and humans using in-vivo phase contrast CMR.* J Cardiovasc Magn Reson 2012, 14: 87.

[31] Quinn, T. A., Kohl, P., Ravens, U., *Cardiac mechano-electric coupling research: fifty years of progress and scientific innovation.* Prog Biophys Mol Biol 2014; 115 (2-3): 71-5.

[32] Panescu, Kroll, Brave, *Limitations of animal electrical cardiac safety models* Annual Int. Conf Proc IEEE Eng Med Biol Soc. 2014.