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# Integration of a semi-automatic *in-vitro* RFA procedure into an experimental setup

DOI 10.1515/cdbme-2016-0020

**Abstract:** Radiofrequency ablation (RFA) is a standard clinical procedure for treating many cardiac arrhythmias. In order to increase the success rate of this treatment, the evaluation of lesion development with the help of intracardiac electrogram (EGM) criteria has to be improved further. We are investigating *in-vitro* the electrophysiological characteristics of cardiac tissue by using fluorescence-optical and electrical techniques. In this project, it is intended to create ablation lesions under defined conditions in rat atria or ventricle and to determine the electrical activity in the myocardium surrounding these lesions less than 1 s after the ablation. Therefore, we developed a semi-automatic RFA procedure, which was integrated into an existing experimental setup. Firstly, a controllable protection circuit board was designed to galvanically isolate the sensitive amplifiers for measuring extracellular potentials during the ablation. Secondly, a real-time system was implemented to control and to autonomously monitor the RFA procedure. We verified each component as well as the different sequences of the RFA procedure. In conclusion, the expanded setup will be used in future *in-vitro* experiments to determine new EGM criteria to assess lesion formation during the RFA procedure.

**Keywords:** ablation lesion; extracellular potentials; *in-vitro* experiments; radiofrequency ablation.

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## 1 Introduction

Atrial fibrillation is one of the most frequent cardiac arrhythmias in the western world and radiofrequency ablation is a standard clinical procedure for treating this arrhythmia. According to Shurrab et al., the recurrence rate of an arrhythmia is around 35–40 [1]. In order to increase the success rate, a specific knowledge about the transmuralities as well as the geometry of an ablation lesion is necessary. To date, several control methods exist to evaluate lesion formation, such as late gadolinium magnetic resonance imaging [2] or ultrasound [3]. Unfortunately, none of these methods offer robust criteria to assess ablation lesions. *In-vitro* experiments allow a unique chance to investigate cardiac electrophysiology of vital myocardium under controlled conditions. Thiagalingam et al. used a force-sensing catheter to evaluate the importance of catheter contact force in a porcine *ex-vivo* model [4]. Moreover, Otomo et al. investigated the (EGM)-based criteria to assess single point ablation lesions in a porcine model and demonstrated a high relation between changes of intracardiac EGMs and lesion transmuralities [5]. However, the observational design of this study was limited. Therefore, further studies are required to determine the changes of EGMs due to lesion development under defined conditions. We established an *in-vitro* setup to measure the electrophysiological characteristics of rat myocardium by using simultaneously fluorescence-optical mapping and electrical techniques. In this study, we present the integration of a semi-automatic RFA procedure into the *in-vitro* setup. Therefore, it will be feasible to create ablation lesions and to determine the electrical activity of cardiac tissue immediately under defined conditions.

## 2 Methods

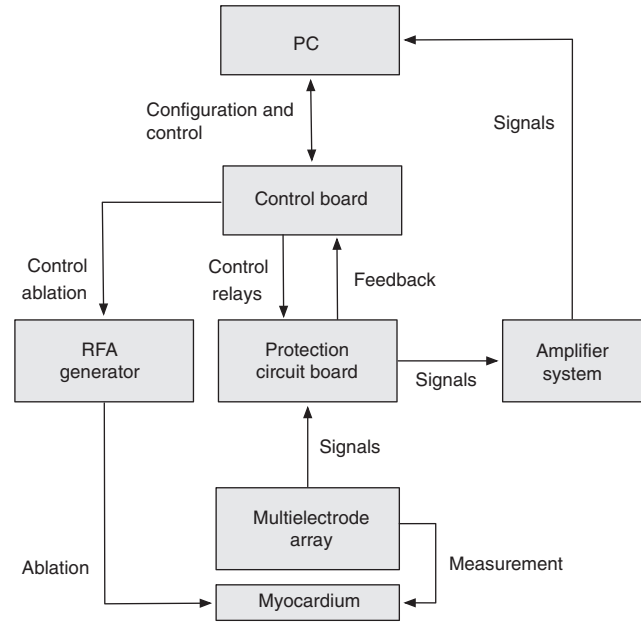
### 2.1 System overview

All animal experiments were approved by the local committee for animal welfare (35-9185.81/G-61/12). For defined

measurement conditions, the rat myocardium is positioned in a tissue bath with heated Krebs-Henseleit solution, which allows the nutrition and oxygenation of the preparation for several hours. Fluorescence-optical mapping is performed from the bottom side of the tissue bath by using a voltage-sensitive dye (di-4-ANEPPS). A self-developed multielectrode array is used to measure the extracellular potentials of rat myocardium surrounding the acute ablation lesion. The electrical recordings are digitized with a data acquisition system from National Instruments and are analyzed by a custom written software in MATLAB 8.1 (The MathWorks Inc., Natick, MA, USA). For the *in-vitro* RFA procedure, we are using the electrosurgical unit MD1 (Micromed, Wurmlingen, Germany) with low power settings as well as manual control by a foot switch. The ablation electrode (diameter of 0.3 mm) is placed perpendicular onto the surface of the myocardium to create punctiform ablation lesions. We developed a controllable protection circuit board (CPCB) to ensure that no residual currents from the ablation electrode will damage the sensitive measurement equipment during the ablation. Moreover, the electrosurgical unit as well as the CPCB are controlled by a real-time system. This monitors the reproducible RFA procedure, which consists of three different sequences. In a first step, the measurement electrodes have to be disconnected safely from the amplifier system. Secondly, the electrosurgical unit is switched on by the real-time system to create lesions with fixed times and power settings. In a last step, the measurement electrodes are reconnected with the amplifier system to measure the electrical activity after the RFA procedure without a large time delay. Figure 1 shows the relationship between each component and their functional relation, which are explained in detail in the following sections.

## 2.2 Controllable protection circuit board

The self-developed CPCB represents a main component of the *in-vitro* RFA procedure and has to fulfill the following requirements: high electrical isolation, fast switching rates, and small (negligible) influence on signal quality. Therefore, we used the electromechanical relay IM26GR (Axicom, Germany) to ensure the galvanic separation of each measurement channel (surge capability up to 2500 Vrms between open contacts). The switching operation of the relays is controlled by an external TTL signal with switching times less than 5 ms. In order to minimize electrical interference during the sensitive measurement phase, the relays are only active in the second sequence of the RFA procedure. Moreover, the control board (CB)



**Figure 1:** Overview of the components and their functional relationship for the *in-vitro* RFA procedure.

monitors the switching state of each relay by using a monitoring voltage. After the successful switching of all relays (monitoring voltage grounded) the RFA procedure will be continued. The electrical components are powered by an external battery to reduce powerline noise.

## 2.3 Control board

The main component of the CB is the microcontroller ATxmega128A1U (Atmel Corporation, San Jose, CA, USA), which controls the different sequences during the RFA procedure and monitors the state of the CPCB as well as of the electrosurgical unit. We configured the controller with a self-developed C-program to ensure real-time behavior and to process user input. Before the RFA procedure, the microcontroller is programmed by a custom written software in MATLAB 8.1. Hereby, the user will specify the switching times of the relays and the ablation time. Furthermore, we used two controllable relays KT12-1A-40L (Meder, Germany) to connect the electrosurgical unit and the ablation electrode during the ablation process. This will reduce additional interfering signals on the ablation electrode during the measurement phase. After the successful configuration of the microcontroller and the manual start of the RFA procedure, the CB switches the relays on the CPCB. Afterwards, the relays on the CB are switched on and the electrosurgical unit is activated for a defined ablation time. In the last phase, the electrosurgical

unit is switched off, the relays of the CB as well as the CPCB are reset and the electrical measurement is started.

## 2.4 Signal acquisition

For data acquisition, we used two portable 4-channel precision measurement systems, which were developed at the Institute of Biophysics of the Medical University of Graz. The acquired signals are amplified by a factor of 100 and filtered with an active 4th-order Bessel antialiasing filter (cutoff frequency: 20 kHz). The signals are simultaneously digitized with 100 ksps and 16-bit resolution (NI9215, National Instruments Germany GmbH, Munich, Germany).

## 2.5 Test phase

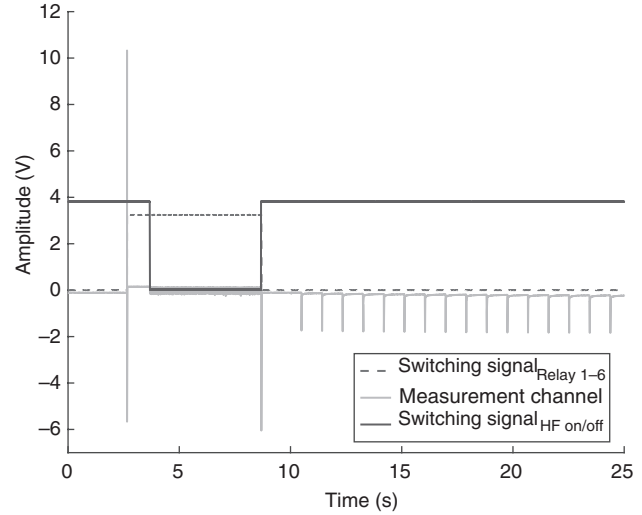
The above-mentioned components had to be integrated into the experimental setup. In this test phase, the complete RFA procedure and the interaction between the different components were examined carefully. For this purpose, conductive saline solution was used instead of living rat myocardium. We placed the multielectrode array, the ablation electrode, and a stimulus electrode in the tissue bath, which was filled with saline solution. A rectangular shaped current pulse with a duration of 1 ms was generated by a constant current stimulus isolator A365 (World Precision Instruments, Sarasota, FL, USA) to simulate the electrical activity of myocardium. The output power of the electro-surgical unit was set to 2 W. Noise power  $P_{\text{noise}}$  of the acquired signal was determined as

$$P_{\text{noise}} = \frac{f_s}{N} \cdot \sum_{n=1}^N (u(n) - \mu_{u(n)})^2, \quad (1)$$

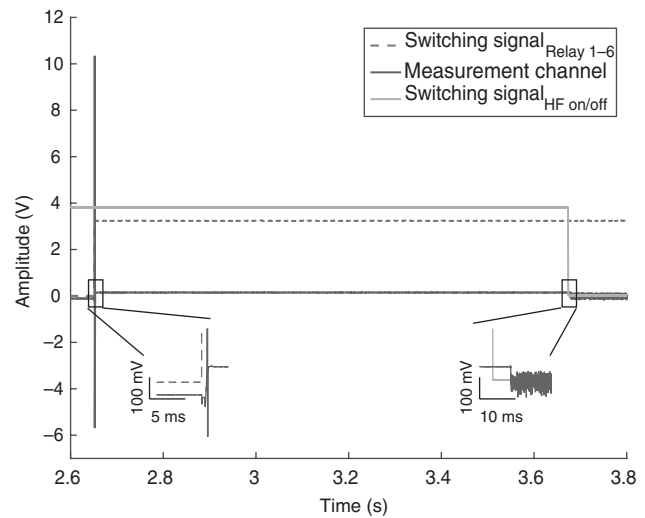
where  $n$  are the samples of the considered interval of noise,  $f_s$  is the sample rate (100 kHz),  $u(n)$  is the signal, and  $\mu_{u(n)}$  is the mean value of the signal  $u(n)$ .

## 3 Results

A complete RFA procedure with the different switching states of the relays on the CPCB, the ablation process and the electrical measurements are shown in Figure 2. The switching of the relays caused some ripple and two clear peaks in the measured signals. The stimuli started around 2 s after the ablation. Figure 3 shows the second sequence of the RFA procedure before the ablation. After the successful switching of the relays (active high), the switching signal of the electro-surgical unit was set (active low). The CB detected the switching of the relays via the monitoring

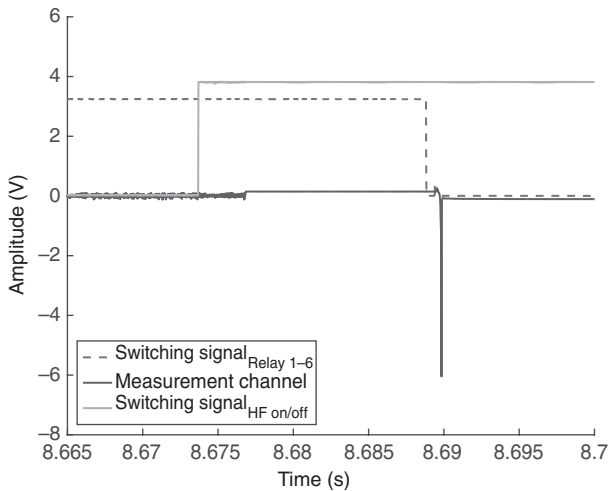


**Figure 2:** Electrical recording, switching signal for the relays (Relay 1–6) on the CPCB (active low), and switching signal for the electro-surgical unit (active high) during the complete RFA procedure.

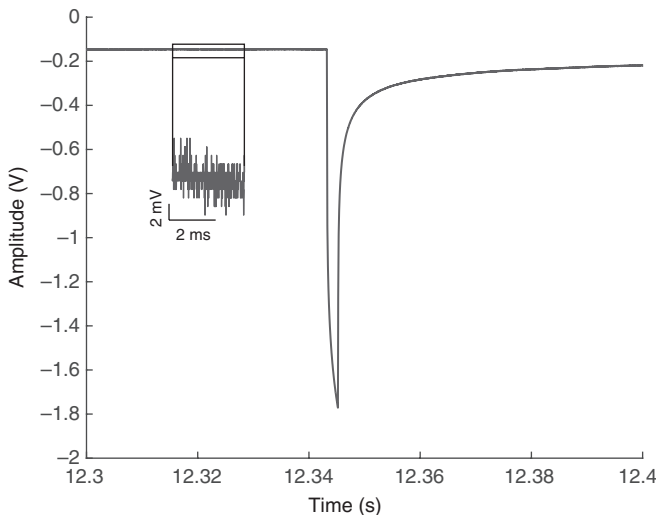


**Figure 3:** Switching signals for the relays (Relay 1–6) on the CPCB (active low) and for the electro-surgical unit (active high) at the beginning of the ablation. Insets present the time delays after the switching of the relays (around 1 ms; left) and the electro-surgical unit (around 5 ms; right).

voltage (not shown in Figure 3) and high-frequency current was supplied to the ablation electrode after a short time delay (around 5 ms). Interfering signals were acquired during the ablation, although the electrodes were separated from the amplifier system. After the ablation, the switching signal of each relay was reset (see Figure 4). The delay of the electro-surgical unit was around 3 ms. Figure 5 presents the recorded stimulus signal after the ablation. According to equation (1), the noise power was calculated



**Figure 4:** Switching signals for the (Relay 1–6) on the CPCB (active low), and switching signal for the electrosurgical unit (active high) at the end of the ablation. The time delay of the electrosurgical unit was around 3 ms.



**Figure 5:** Measured stimulus signal of the stimulus generator. Inset illustrates the signal noise used for noise calculations.

to  $0.0391 V^2$ , which amounted in a signal-to-noise ratio (SNR) of 65.47 (18.16 dB).

## 4 Discussion

We developed a semi-automatic RFA procedure to create ablation lesions on living myocardium. For this purpose, a configurable real-time system controls and monitors independently the RFA procedure. In the test phase, the individual sequences of the RFA procedure were validated successfully. The sequential switching and the switching

times of the relays as well as of the electrosurgical unit were verified. The CPCB, which was based on electromechanical relays, securely protected the sensitive amplifier system. A maximum ablation power of 8 W verified the dielectric strength of the CPCB. Additionally, we included a safety delay (waiting time) of 15 ms after the ablation to ensure the guaranteed switching of the electromechanical relays. Short-term ripples as well as sharp peaks occurred in the acquired signal due to switching processes of the relays. However, these irregularities are within the configurable waiting times and will not influence the subsequent electrical measurements. The determined SNR demonstrated that the CPCB did not strongly increase interfering signals. The irregular shape of the measured stimulus signal was probably caused by capacitive effects of the Ag/AgCl electrodes. The delay between the activation of the MD1 by the CB and the effective start of the ablation could be induced by an internal response time of the electrosurgical unit. A similar time delay was seen when this device was switched off. Therefore, it has to be regarded for further measurements that the relays of the CPCB should not switch during this time period. The safety requirements of the whole setup were also tested. Therefore, we investigated the failure behavior of the real-time system, which was less than 1 ms, as well as the clear separation between the ablation electrode and the electrosurgical unit during the active state of the relays. In conclusion, the presented *in-vitro* RFA procedure allows the creation of acute ablation lesions with varying geometry and transmuralty under reproducible conditions. Furthermore, it is feasible to determine electrophysiological changes of myocardium surrounding the ablation lesion after approximately 20 ms.

**Acknowledgment:** The authors would like to thank Kurt Feichtinger and Manfred Schroll for their support.

### Author's Statement

Research funding: Stefan Pollnow is supported by a scholarship of the Karlsruhe School of Optics and Photonics (KSOP). Conflict of interest: Authors state no conflict of interest. Material and Methods: Informed consent: Informed consent is not applicable. Ethical approval: The conducted research is not related to either human or animals use.

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